

2012-06-19

# An Investigation of the Nutritional Condition of Low-Latitude Fish Larvae: Growth, Transport, and Implications for Population Connectivity

Martha J. Hauff

University of Miami, marthah13@yahoo.com

Follow this and additional works at: [https://scholarlyrepository.miami.edu/oa\\_dissertations](https://scholarlyrepository.miami.edu/oa_dissertations)

---

## Recommended Citation

Hauff, Martha J., "An Investigation of the Nutritional Condition of Low-Latitude Fish Larvae: Growth, Transport, and Implications for Population Connectivity" (2012). *Open Access Dissertations*. 804.  
[https://scholarlyrepository.miami.edu/oa\\_dissertations/804](https://scholarlyrepository.miami.edu/oa_dissertations/804)

This Open access is brought to you for free and open access by the Electronic Theses and Dissertations at Scholarly Repository. It has been accepted for inclusion in Open Access Dissertations by an authorized administrator of Scholarly Repository. For more information, please contact [repository.library@miami.edu](mailto:repository.library@miami.edu).



UNIVERSITY OF MIAMI

AN INVESTIGATION OF THE NUTRITIONAL CONDITION OF LOW-LATITUDE  
FISH LARVAE: GROWTH, TRANSPORT, AND IMPLICATIONS FOR  
POPULATION CONNECTIVITY

By

Martha J. Hauff

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 2012

©2012  
Martha J. Hauff  
All Rights Reserved

UNIVERSITY OF MIAMI

A dissertation submitted in partial fulfillment of  
the requirements for the degree of  
Doctor of Philosophy

AN INVESTIGATION OF THE NUTRITIONAL CONDITION OF LOW-LATITUDE  
FISH LARVAE: GROWTH, TRANSPORT, AND IMPLICATIONS FOR  
POPULATION CONNECTIVITY

Martha J. Hauff

Approved:

\_\_\_\_\_  
Robert K. Cowen, Ph.D.  
Professor of Marine Biology and Fisheries

\_\_\_\_\_  
Dean of the Graduate School

\_\_\_\_\_  
Su Sponaugle, Ph.D.  
Professor of Marine Biology and Fisheries

\_\_\_\_\_  
Andrew Bakun, Ph.D.  
Professor of Marine Biology and  
Fisheries

\_\_\_\_\_  
M. Danielle McDonald, Ph.D.  
Assistant Professor of Marine Biology  
and Fisheries

\_\_\_\_\_  
G. Joan Holt, Ph.D.  
Professor of Marine Science  
University of Texas

HAUFF, MARTHA J.

(Ph.D., Marine Biology and Fisheries)

An Investigation of the Nutritional Condition  
of Low-Latitude Fish Larvae: Growth, Transport,  
and Implications for Population Connectivity

(June 2012)

Abstract of a dissertation at the University of Miami.

Dissertation supervised by Professor Robert K. Cowen.

No. of pages in text. (159)

Coral reef fishes are typically characterized by a protracted pelagic larval dispersal period, which creates the potential for connectivity of geographically discrete populations. The degree to which connectivity occurs is affected not only by whether larvae are transported from one reef to another, but also by whether they reach a settlement site in adequate nutritional condition to survive the juvenile period and beyond. It is possible that larvae with different dispersal trajectories (i.e. those that are retained close to shore as compared to those that travel great distances) may differ in their condition levels, and thereby, the extent to which they serve to replenish local populations. Condition levels during larval life, and their relationship to environmental factors, are thus important determinants of regional demography and patterns and scales of population connectivity. In the work presented here, larval fish condition was measured using two different indices: RNA/DNA ratios (R/Ds) and otolith-derived growth measurements. R/Ds are utilized frequently in studies of temperate larval fish ecology, but have only rarely been applied to investigations of low-latitude taxa. The sensitivity of the R/D to variations in prey availability in a tropical/subtropical context was assessed in a laboratory feeding experiment in which larval cobia were subjected to full and reduced (20%) rations. R/Ds were found to respond to reductions of prey

availability, and this response was on par with analogous decreases in larval otolith growth. Having established that the R/D can reflect changes in larval food supply in warm water species, the index was used in concert with otolith size and growth to assess the condition of coral reef fish larvae collected in and around the Florida Keys Reef Tract. When nearshore (likely locally retained) and offshore (broadly dispersing) larvae were compared, it was found that, for three of four species examined, nearshore larvae exhibited faster growth and higher R/Ds as compared to their offshore counterparts. An examination of the changes in the distributions of individual condition levels with age (coupled with measurements of larval fish prey availability) indicated that the observed differences in mean condition were likely due to predation-related selective loss of the lowest condition larvae in nearshore waters. To identify possible molecular correlates of larval survival and condition, single nucleotide polymorphisms (SNPs) were genotyped in nearshore and offshore-collected larvae of a common Caribbean reef fish, the bluehead wrasse. Results revealed multiple loci that were likely under selection due to association with condition-related traits, and these loci may therefore be relevant to future investigations into gene-mediated physiological determinants of condition. As a whole this dissertation sheds light on both environmental and genetic components of larval coral reef fish condition, and it thereby contributes to our understanding of the processes that govern population connectivity, as well as our ability to manage and protect coral reef resources in a rapidly changing environment.

## ACKNOWLEDGEMENTS

As an aspiring graduate student I wrote to Dr. Robert Cowen to express my interest in larval fish ecology and my keen desire to work in his lab. He took me on and, in doing so, he turned my lifelong ambitions for a career in marine biology into reality. I will always be indebted to him for having opened up that door for me, and I thank him for having faith in my abilities and providing me with the guidance and encouragement I needed to bring this work to completion.

In addition to Professor Cowen, Professors Su Sponaugle, Danielle McDonald, Andy Bakun and Joan Holt all served on my committee, and I am grateful for it. They were wonderful mentors, and they each contributed something unique to my graduate experience. It was Dr. Sponaugle's formidable body of work that laid the intellectual foundation for much of the research presented here, and she attended to my manuscripts with the time and care that I could have only expected from a major advisor. Dr. McDonald welcomed me into her lab, and offered space to work and much needed equipment. But perhaps more importantly, she provided me with inspiration, and I look up to her as a young, confident female scientist just beginning her career and really making her mark in her field. Dr. Bakun has provided me with inspiration of a different sort; on many occasions he has posed incisive questions that have helped me to look at my own research, and tenets of larval ecology in general, in a new light. I feel that my graduate education and my approach to thinking about scientific problems have benefitted from my interactions with him. Dr. Holt agreed to serve on my committee



never having met me. When I first contacted her, I was aware of her widely respected expertise in the field of larval physiological condition, but I did not know how kind and helpful she would be. The tutorials I received on a visit to her lab made it possible for me to carry out the research plan I had envisioned, and her offer to take me on an impromptu birding expedition (it was, after all, April in South Texas) made my trip particularly memorable. (I got my painted bunting!)

During my time at RSMAS, I was lucky enough to have some additional academic mentors, who were under no obligation to help me, but contributed their time and thought regardless. Dr. Doug Crawford was a valuable resource. His advice helped me to overcome many hurdles in terms of both analytical techniques and data interpretation and I am thankful for the stimulating and entertaining conversations that we shared. Dr. Margie Oleksiak came to be like a second advisor to me. She provided me with technical and intellectual advice, and kind encouragement. Her confidence in my abilities as a scientist have made a real and lasting change to the way I view myself and my potential, and I can not thank her enough. Dr. Claire Paris was one of my first friends at RSMAS. I have long-since looked to her as a role model, and I have turned to her for both personal and professional support throughout my time in Miami. Moreover, her skills as a modeler are in high demand, and I feel fortunate to have had the opportunity to collaborate with her and benefit from her singular expertise.

The field and analytical work comprised in this dissertation would have been impossible without the assistance of many, many lab mates, a few of whom were

particularly indispensable: Cedric Guigand the renaissance man of the Cowen Lab, made the field-work possible. He built and designed sampling equipment, planned and orchestrated research cruises, and worked tirelessly to facilitate data collection, and I am happy to have him as a dear friend, not just a colleague. Laura Parsons was, for a number of months, my right hand man. Her impeccable work ethic and attention to detail made it possible for me to rely on her for assistance with laboratory data collection that was integral to this dissertation. I couldn't have finished everything without her help. She also provided a welcome distraction from the drudgery, and our coffee breaks and cycling banter were often the highlight of my workday.

There still more folks worthy of special mention. Kristen Walter and Evan D'Alessandro helped with otolith reading. Katie Shulzitski and I collaborated extensively on assembling and maintaining the large database generated from our fieldwork. Larval rearing experiments were done in collaboration with Sean Bignami, who built the rearing setup. Captain Shawn Lake and the crew of the R/V F.G. Walton Smith helped to make our field expeditions a success. Field collection and/or microscopy assistance were provided by Carie Bickson, Katie Shulzitski, Klaus Huebert, Amy Exum, Lisa Gundlach, Steve Trbovich, Tom Murphy, Becky Howe, Eric Buck, Jason Downing, and Casey Gioia, among others.

The members of the Cowen lab, past and present, offered support, friendship, and fun, and I thank them sincerely. They are: Adam Greer, Jessica Luo, Johnathan Kool, Dave Richardson, Cedric Guigand, Joel Llopiz, Klaus Huebert, Kristen Walter, Katie Shulzitski, Kirsten Grorud-Colvert, Claire Paris, Evan D'Alessandro, Tauna Rankin,

Michelle Paddack, Sean Bignami, Esther Goldstein and Jess Watson, and Laura Parsons.

Finally, the individuals most deserving of the credit for anything I have ever accomplished or will accomplish in the future are...

My Uncle Lee, who first inspired me to “be curious”.

My family, the Hauffs, Brian, Jeannie, Nellie, who have been the best, most perfect imperfect family I could ever want. I love them and I thank them for loving me; it makes me who I am and helps me do the things I’m not sure I can do.

My husband, Dr. Joel Llopiz. His contributions to my science and my life cannot be put into mere words. Nor can my love and appreciation.

Thank you, all.

## TABLE OF CONTENTS

	Page
LIST OF TABLES .....	ix
LIST OF FIGURES .....	x
Chapter	
1 GENERAL INTRODUCTION AND RATIONALE .....	1
Population connectivity .....	2
Larval fish condition .....	3
The intersection of connectivity and condition.....	4
Means of investigating condition.....	6
2 RNA/DNA RATIOS IN A LOW-LATITUDE LARVAL FISH: EFFECTS OF REDUCED FOOD AVAILABILITY AND IMPLICATIONS FOR THE TEMPERATURE-RNA/DNA RATIO RELATIONSHIP .....	11
Background .....	11
Materials and methods .....	15
Larval rearing.....	15
Laboratory processing.....	17
Otolith analysis .....	17
RNA and DNA quantification .....	19
Taxonomic and temperature-based RNA/DNA ratio comparisons .....	20
Statistical analysis.....	21
Results.....	22
Size and growth.....	22
RNA/DNA ratios .....	23
The RNA/DNA ratio across taxa and temperatures.....	24
Discussion .....	25
Size and growth.....	26
RNA/DNA ratios .....	27
The RNA/DNA ratio across taxa and temperatures.....	30
3 NUTRITIONAL CONDITION OF CORAL REEF FISH LARVAE VARIES WITH DISPERSAL HISTORY: PREY, PREDATORS, AND POPULATION CONNECTIVITY .....	41
Background .....	42
Methods.....	47
Field sampling.....	47
Laboratory processing.....	49
RNA and DNA quantification .....	50
Otolith microstructure analysis.....	51

Molecular identification of larvae.....	53
Prey availability analysis .....	54
Statistical analysis.....	55
Results.....	58
Mean condition in nearshore vs. offshore larvae .....	58
Daily growth and size-at-age .....	59
Cumulative probability distribution functions .....	59
Prey availability .....	60
Discussion.....	61
Prey availability and dispersal pathways .....	62
Selective mortality in the nearshore environment .....	64
Species specificity of the dispersal history-condition relationship.....	65
Effects of magnitude and type of predation.....	66
Offshore as larval habitat.....	67
Conclusions.....	68
4 SINGLE-NUCLEOTIDE POLYMORPHISMS (SNPS) SHED LIGHT ON CONDITION IN CORAL REEF FISH LARVAE.....	88
Background.....	89
Methods.....	92
Study species.....	92
Study site.....	92
Larval collections.....	93
Juvenile collections.....	95
Laboratory processing of larvae.....	95
Condition analyses .....	96
SNP discovery and validation.....	101
SNP genotyping .....	102
Statistical analysis.....	103
Results.....	105
Modeled larval origins .....	105
Population structure .....	106
SNP-trait associations .....	107
Discussion.....	108
Evidence of selection .....	108
Population structure .....	112
Application of the biophysical model.....	117
SNPs with geography.....	118
Conclusions.....	119
5 SUMMARY AND CONCLUSIONS .....	130
LITERATURE CITED.....	138

## LIST OF TABLES

Table 2.1. Comparison of standardized RNA/DNA ratios and weight-specific growth rates of 14 different species of fish larvae from a range of temperatures .....	33
Table 3.1. Least-squares regressions of otolith radius with SL, and Pearson's correlation coefficients of residuals from the otolith radius-at-age and SL-at-age relationships .....	73
Table 3.2. Sample sizes and significance levels from REML analyses of mean recent growth and mean RNA/DNA ratio differences between nearshore and offshore groups .....	74
Table 3.3. Significance levels from pairwise REML analyses of differences in daily growth and size-at-age between nearshore and offshore larvae at distinct points in larval life .....	75
Table 4.1. Natal origins of 269 <i>T. bifasciatum</i> larvae based on 100 replicate runs of the CMS .....	122
Table 4.2. SNPs of interest: 14 outlier SNPs whose $F_{ST}$ values fell above the 99 <sup>th</sup> percentile of a simulated, neutral-behaving data set in one (or more) of three condition-related trait association tests .....	123

## LIST OF FIGURES

Figure 2.1. Mean SL of cobia larvae sampled from full ration and reduced ration feeding treatments .....	34
Figure 2.2. Mean growth and size-at-age of full ration and reduced ration cobia larvae sampled at 5, 10 and 14 dph .....	35
Figure 2.3. Mean R/D of cobia larvae sampled from full ration and reduced ration feeding treatments at 10 and 14 days post hatch .....	36
Figure 2.4. Cumulative probability distribution functions (CDFs) of R/D in relation to SL for larvae reared under full ration and reduced ration feeding conditions .....	37
Figure 2.5. Box plot showing the variability (i.e. scatter) of R/Ds of individual larvae from Full Ration and Reduced Ration treatments with increasing age .....	38
Figure 2.6. Least-squares regression of standardized RNA/DNA ratio (sR/D) with temperature .....	39
Figure 2.7. Proportions of larvae from both Full Ration and Reduced Ration treatment groups in each of three size classes .....	40
Figure 3.1. Map of the study region, which includes the Florida Keys reef tract and potential upstream sources of larvae flowing in from the Yucatán Peninsula or the West Florida shelf .....	76
Figure 3.2. Two potential mechanisms for an increase in the lowest observed larval condition levels (i.e. the 10 <sup>th</sup> percentile) with increasing larval age.....	77
Figure 3.3. Mean recent growth of larvae collected at nearshore stations and offshore stations for four species of coral reef fish .....	78
Figure 3.4. Mean RNA/DNA ratio of larvae collected at nearshore stations and offshore stations for 4 species of coral reef fish .....	79
Figure 3.5. Mean daily growth for larvae collected at nearshore stations and offshore stations on cruises 1 and 2 .....	80
Figure 3.6. Mean size-at-age for larvae collected at nearshore stations and offshore stations during cruises 1 and 2 .....	81
Figure 3.7. Cumulative probability distribution functions (CDFs) of recent growth and RNA/DNA ratio in relation to larval age .....	82

Figure 3.8. 10 <sup>th</sup> percentiles of nearshore and offshore groups plotted together with 95% confidence intervals generated from 1,000 bootstrapped randomizations of the data .....	83
Figure 3.9. 90 <sup>th</sup> percentiles of nearshore and offshore groups plotted together with 95% confidence intervals generated from 1,000 bootstrapped randomizations of the data.....	84
Figure 3.10. Nearshore vs. offshore larval fish prey availabilities in biomass/m <sup>3</sup> for the three specific prey types consumed by <i>T. bifasciatum</i> and <i>X. novacula</i> .....	85
Figure 3.11. Least-squares regressions of condition vs. prey availability (biomass/m <sup>3</sup> ) for recent growth and RNA/DNA ratio .....	86
Figure 3.12. Proportional depth distributions of all four species summed over all cruises .....	87
Figure 4.1. Large map of the sampling scheme with an inset map of the greater study region .....	124
Figure 4.2. Modeled dispersal pathways generated by the CMS for two examples of 30 day old larvae collected nearshore in the middle Florida Keys and offshore in the Florida Current.....	125
Figure 4.3. Population structure of <i>T. bifasciatum</i> with two clusters as determined by STRUCTURE .....	126
Figure 4.4. Empirical $F_{ST}$ values as a function of expected heterozygosity for comparisons of the initial (i.e. younger) pool of larvae vs. surviving larvae, fast growing vs. slow growing larvae, and high RNA/DNA ratio vs. low RNA/DNA ratio larvae .....	127
Figure 4.5. $F_{ST}$ between larvae with natal origins in the Florida Keys Reef Tract and those originating near to or upstream of the Yucatán peninsula, as inferred from biophysical model output .....	128
Figure 4.6. Boxplot of $F_{ST}$ values form a pairwise comparison of larvae collected from nearshore and offshore water masses.....	129



## Chapter 1. General introduction and rationale

*We were curious. Our curiosity was not limited, but as wide and horizonless as that of Darwin or Agassiz or Linnaeus or Pliny. We wanted to see everything our eyes would accommodate, to think what we could, and, out of our seeing and thinking, to build some kind of structure in modeled imitation of the observed reality. We knew that what we would see and record and construct would be warped, as all knowledge patterns are warped, first, by the collective pressure and stream of our time and race, second by the thrust of our individual personalities. But knowing this, we might not fall into too many holes—we might maintain some balance between our warp and the separate thing, the external reality. The oneness of these two might take its contribution from both.*

-John Steinbeck, *The Log From the Sea of Cortez*, 1951

It has been said that the ocean is the last great frontier—that we know more of the surface of the moon than of the bottom of the sea. We strap on masks and SCUBA tanks, or tow sensors, nets or even cameras through the water just to catch a glimpse of the world below. Through an accumulation of glimpses, afforded by the advent of new technologies and the original thoughts of scientists climbing upon each other's shoulders, our picture of life in the oceans is becoming increasingly clear. Still, there is much left to learn.

The gaps in our knowledge become especially pronounced when we venture away from the relative familiarity of the coastal habitat into the three-dimensional vastness of open water. The pelagic environment, which seems so far removed from the estuary, the tide pool, and the coral reef, is the medium through which almost all demersal marine animals must pass. For species that are benthic-oriented as adults, such as coral reef fishes, pelagic dispersal of the earliest life stages is the norm.

### ***Population connectivity***

Among marine metazoans, coral reef fishes exhibit relatively long larval dispersal periods; depending on the taxon, reef fish larvae may spend from a few days to a few months in the plankton before settling back to the benthos. Generally, the survival advantages conferred by this life history strategy are thought to lie not in the actual transport of individuals away from natal origins, but rather in the possibility that the pelagic environment constitutes a more favorable larval habitat (Strathmann et al. 2002). For example, compared to the reef, the pelagic realm may offer density-independent feeding opportunities for the numerous siblings that result from a single spawning event. In addition, the diffuse nature of the oceanic environment is likely correlated with reduced exposure to predators and the disruption of parasite and disease cycles (Strathmann 1974, Johannes 1978, Strathmann et al. 2002, Strathmann 2007). Still, the protracted larval phase of reef fishes also creates the potential for long distance dispersal and, by association, extensive population connectivity (i.e. exchange of individuals among spatially discrete populations). This raises the question: to what extent and on what spatial scales does population connectivity actually occur? Answering this question (which has direct relevance to population demography and management) is a central goal of marine ecology today (Mora & Sale 2002, Cowen et al. 2007, Cowen & Sponaugle 2009).

For many years, tropical reef fish communities were thought to be mostly open with widespread exchange of larval propagules and a decoupling of local reproductive output and juvenile recruitment (Sale 1978, Roughgarden et al. 1985, Roberts 1997).

However, recent research has indicated that reef fish larvae can be active, competent swimmers with acute sensory and orientation capabilities (Fisher 2005, Lecchini et al. 2005, Simpson et al. 2005, Leis 2006, Montgomery et al. 2006), and both spawning adults and their offspring have been shown to exhibit behavior that promotes return of larvae to natal reefs (Paris & Cowen 2004, Gerlach et al. 2007, Leis et al. 2007a, Dixon et al. 2008, Karnauskas et al. 2011). These observations, coupled with the existence of physical features that might facilitate local retention (Limouzy-Paris et al. 1997, Cowen 2002, Sponaugle et al. 2002, Sponaugle et al. 2005), suggest that ecologically relevant levels of dispersal may be occurring on much smaller spatial scales than originally thought (Jones et al. 1999, Cowen et al. 2000, Swearer et al. 2002, Paris & Cowen 2004, Jones et al. 2005, Cowen et al. 2006, Almany et al. 2007, Buston et al. 2012).

### ***Larval fish condition***

While the larval phase represents a mere fraction of an individual fish's overall lifespan, mortality during this period is extremely high (McGurk 1986) and also extremely variable (Houde 1987)—so much so that larval dynamics are thought to be one of the major drivers of fish recruitment levels and population demography. In 1914, Johan Hjort first brought this concept to light, surmising that the temporal inconsistencies observed in fisheries yields were most likely a function of whether a critical period of larval life—the point at which the yolk sac was expended and exogenous feeding commenced—coincided with adequate prey availability. Now, almost a century later, the importance of the larval phase to fish population demography and, ultimately, species persistence has been widely acknowledged and extensively explored. Hjort's "critical

period hypothesis” has been expanded upon by researchers who have proposed and demonstrated that: 1) larval survival throughout the pelagic duration is contingent upon the phenology of high productivity phytoplankton blooms and the following peaks in secondary production (“match-mismatch” model, Cushing 1975), 2) better larval nutritional condition (often measured in terms of growth) can result in better prey detection and predator evasion abilities (Pepin 1991, Fuiman and Magurran 1994, Pepin et al. 2003, Dower et al. 2009 ), and 3) faster growth results in larger sizes-at-age and quicker passage through the larval stage, both of which lead to lower levels of total predation mortality (Cushing 1975, Anderson 1988, Pepin 1991, Leggett & DeBlois 1994, Hare & Cowen 1997, Shoji & Tanaka 2006, Plaza & Ishida 2008).

### ***The intersection of connectivity and condition***

Clearly, feeding success in the plankton and the associated condition benefits are critical to larval survival, but the effects are not limited to larval life. Advantages associated with enhanced larval condition can carry over into the juvenile phase, resulting in differential survivorship among individuals with disparate larval growth histories (e.g. Sogard 1997, Pechenik et al. 1998) . This is especially true for coral reef fishes, whose populations have been shown to undergo severe bottlenecks whereby newly settled juveniles transitioning to the space- and resource-limited reef are subject to high levels of starvation and predation (Sale & Ferrell 1988, Doherty et al. 2004). Strong evidence suggests that such intense mortality may selectively remove fish of lower nutritional condition, and individuals with slower larval growth rates or smaller (or, in some cases, larger) sizes at settlement are often less likely to survive to reproduce (Searcy &

Sponaugle 2001, Bergenius et al. 2002, Shima & Findlay 2002, Hoey & McCormick 2004, Meekan et al. 2006, Sponaugle & Grorud-Covert 2006). The effects of larval condition on post-larval survivorship are particularly germane to issues of reef fish population connectivity since connectivity is governed not only by whether larvae are physically transported (either actively or passively) from one reef to another, but also by whether they reach a settlement site in adequate nutritional condition to successfully recruit and effectively contribute to a population (Pineda et al. 2007, Hamilton 2008, Cowen & Sponaugle 2009, Shima & Swearer 2009). With this in mind, the work presented here seeks to investigate whether larval coral reef fish with contrasting dispersal trajectories (i.e. local retention vs. long distance dispersal) may exhibit different levels of condition and might, therefore, differ in their survival potential, ultimately influencing patterns of population connectivity.

Larvae may encounter markedly different habitats depending on whether they are retained nearshore or transported far offshore. On average, those residing closer to shore should encounter higher levels of productivity and, subsequently, higher prey abundances (Denman & Powell 1984, Leichter et al. 1998, Olson 2001, Leichter et al. 2003). But, higher productivity could entail a tradeoff, as it may also support elevated concentrations of predators (Johannes 1978). Farther offshore, waters are, on the whole, highly oligotrophic, but dynamic physical processes, including frontal convergence or divergence and submesoscale eddies, can create patches of favorable habitat. Upwelling-driven nutrient enrichment and downwelling-induced accumulation of swimming or buoyant plankters can both enhance larval prey availability, though these features tend to be short lived (Bakun 1996, Olson 2002, Bakun 2006, Munk 2007). Ephemeral

patchiness of larval fish prey may also occur on even finer scales in association with thin layers and stratification of the water column (Cowles et al. 1998, Lough & Broughton 2007, Young et al. 2009). The temporal and spatial frequencies of areas of enrichment in the heterogeneous oceanic environment are still poorly resolved. Whether the average fish larva is able to exploit such features, and whether the features are sufficient to sustain optimal levels of larval condition (i.e. growth), should both have a significant impact on the degree of effective exchange that occurs between two geographically disparate populations. We address this issue by measuring and comparing the distributions of individual condition levels in larvae with nearshore and offshore dispersal trajectories.

#### ***Means of investigating condition***

Because of the importance of nutritional condition to larval and juvenile survival, assessing larval condition has long been a priority for fisheries biologists (reviewed in Ferron and Leggett 1994). Morphometrics, which are perhaps the most intuitive approach to measuring condition, have been effectively used to analyze and compare adult fish. However, they have limited applicability in larval studies due to shrinkage effects and general damage incurred by the larvae from handling and preservation (Theilacker 1980, McGurk 1985, Jennings 1991). Histological studies can offer accurate and timely insights into changes in cellular quality, and thus condition. Yet such analyses are somewhat subjective, lack definitive quantifiability and, like morphometrics, can be affected by handling and preservation damage (O'Connell and Paloma 1981).

The aragonitic otoliths (ear stones) of teleost fishes are of singular value in assessing larval condition, for they contain a permanent record of an individual's growth

rate throughout larval life (Brothers et al. 1976, Victor 1982). In 1971, Panella first discovered that the continued accretion of aragonite results in daily deposition of concentric increments of alternating density. It has been repeatedly demonstrated for a variety of taxa that the width of each otolith increment typically corresponds proportionally to larval somatic growth, and the relationship between feeding and otolith growth is strong enough that increment widths can be confidently used as an indicator of nutritional status (Houde 1978, Campana 1990, Hare & Cowen 1995, Puvanendran & Brown 1999). The archival nature of otoliths makes them uniquely valuable in the study of fish age and growth (Sponaugle 2010), and analyses of otolith microstructure can be successfully applied to larval fish ecology and recruitment questions (e.g. Searcy and Sponaugle 2001). In the research presented in this dissertation, otolith-derived growth rates were one of two metrics utilized to compare growth (and thus condition) of coral reef fish with different dispersal histories.

A wide variety of biochemical indices have also been used as measures of larval condition, including digestive and metabolic enzyme activities; total protein, carbohydrate, or lipid contents; fatty acids; and carbon:nitrogen ratios. Of the many biochemical assays that have been proposed, this dissertation focuses on the RNA/DNA ratio. The premise of this assay is that, while the amount of DNA per diploid cell is relatively constant, the amount of RNA per cell (which is composed primarily of ribosomal RNA) varies with increases or decreases in protein synthesis as mediated by the ribosomes (Buckley 1984, Clemmesen 1988, Clemmesen 1996). In some laboratory studies, decreases in RNA/DNA ratios have been observed within as little as 24 hours of food deprivation (Buckley 1979, 1984, Wright & Martin 1985, Raae et al. 1988, Richard

et al. 1991, Clemmesen & Doan 1996, Rooker & Holt 1996, Catalán et al. 2007) making them particularly good reflections of a larva's very recent growth status. Over the past two decades, use of the RNA/DNA ratio has become commonplace in investigations of high latitude fish larvae. Moreover, the development of an intercalibration technique has allowed for the comparison of RNA/DNA ratios across studies and among taxa (Caldarone et al. 2006). However, the use of this index has been almost absent from the tropical and sub-tropical literature (but see Westerman and Holt 1988, 1994, Rossi-Wongtschowski et al. 2003, Tanaka et al. 2007). Previous work has shown that the relationship of the RNA/DNA ratio to temperature-induced changes in larval growth could be confounded by the fact that, for some species, increased growth at high temperatures may occur in the form of hyperplasia (an increase in cell numbers rather than cell size), which would not require a higher concentration of RNA per cell (Malzahn et al. 2003). Furthermore, temperature-dependent elevation of growth rates could result from increases in ribosomal activity, rather than quantity, which might also result in a partial decoupling of the RNA/DNA–growth relationship (Caldarone 2005, Buckley et al. 2008). By measuring RNA/DNA ratios in laboratory-reared and wild-caught low-latitude fish larvae, the work presented here helps to tease apart the factors that affect RNA/DNA ratios and their relationship to temperature. It also seeks to investigate the potential value of using the RNA/DNA assay in concert with the longer-term picture afforded by otolith microstructure, with an eye towards providing a better characterization of the physiological responses associated with variations in larval condition.

The methods described above offer valuable information as to larval condition status, shedding light on the relationship between environment and growth. Yet, they do



not necessarily account for the specific adaptive physiological mechanisms at play. Recent advances in high throughput molecular sequencing enable large-scale genotyping of non-model organisms with high speed and accuracy at relatively low costs (Oleksiak 2010). Thus, it is possible to investigate molecular correlates of larval growth and/or condition. Specifically, these new technological developments can be used in the unbiased, untargeted discovery of thousands of potentially informative single nucleotide polymorphisms (SNPs) across entire genomes (Brumfield et al. 2003). These SNPs can then be sequenced in individual larvae to determine whether particular alleles might be associated with variations in larval fish condition. Outside of the biomedical literature, there are, to date, only a few examples of the identification of SNPs in association with phenotypic traits (Tao & Boulding 2003, Namroud et al. 2008, Renaut et al. 2011). The work presented here constitutes one of the first examples of the ecological applicability of genome-wide SNP studies. Moreover, it highlights the fact that an individual larva's condition and its likelihood of survival are determined not only by its environment but also by the interaction of the environment with the larva's inherent disposition, as determined by its genes (Baldwin 1896, Blackburn & Schneider 1994).

In an effort to explore the many facets of larval condition and the associated implications for coral reef fish population connectivity, the work presented in this dissertation takes several different tacks. Chapter Two uses a controlled laboratory experiment to investigate the specific nature of the RNA/DNA ratio, a condition index that is almost ubiquitous in temperate larval fish research but very rarely applied in tropical contexts. This chapter pays special attention to the potential utility of the index at low latitudes and its relationship to temperature. In Chapter Three, the effects of

environment and, specifically, dispersal trajectory on larval coral reef fish condition are explored, using field-collected data, and the implications that this work has for population connectivity are discussed. Finally, molecular correlates of condition are identified in Chapter Four, demonstrating the influence of genes on larval growth and underscoring the importance of gene-by-environment interactions in determining larval condition and survival.

## **Chapter 2. RNA/DNA ratios in a low-latitude larval fish: effects of reduced food availability and implications for the temperature-RNA/DNA ratio relationship**

RNA/DNA ratios of laboratory-reared cobia larvae were examined under *ad libitum* and reduced ration feeding conditions to assess the sensitivity of the RNA/DNA ratio to fluctuations in food availability. Larvae subjected to reduced food concentrations exhibited significantly lower RNA/DNA ratios than their fully fed counterparts, and ration-related differences in RNA/DNA ratio were mirrored by analogous differences in growth, as indicated by otolith microstructure. An among-feeding treatment investigation of the variability of the RNA/DNA ratio with standard length showed that food-limited larvae exhibited a distinct reduction in RNA/DNA ratio variability at large sizes. This reduced variability was likely related to slow growth of poor condition larvae, and the associated underrepresentation of low RNA/DNA ratio individuals in the largest size classes. Comparison of RNA/DNA ratios obtained for fully fed cobia larvae with those of other larval fish species from a diverse array of thermal habitats revealed a negative correlation between RNA/DNA ratio and temperature. The findings presented here represent one of only a few reports on RNA/DNA ratios in tropical or sub-tropical taxa, and they provide an important point of reference for future studies of condition in low-latitude larval fishes.

### **BACKGROUND**

The early life stages of marine fishes are characterized by extremely high and variable mortality rates (McGurk 1986), and even slight fluctuations in larval

survivorship (driven largely by starvation and predation) can have profound effects on overall population dynamics (Houde 1987). Suboptimal feeding not only increases a larva's susceptibility to starvation (Hjort 1914, May 1974, Cushing 1975, Lasker 1978), it also negatively affects growth, potentially resulting in greater risk of predation due to factors such as decreased size-at-age, prolonged pelagic duration, and deteriorated escape response (Blaxter 1986, Bailey & Houde 1989, Houde 1989b, Hare & Cowen 1997, Takasuka et al. 2003, Skajaa et al. 2004). Furthermore, events during the larval period can have implications for subsequent ontogenetic stages, as condition during larval life is often associated with carryover effects that greatly influence survival in the larval-juvenile transition and beyond (Searcy & Sponaugle 2001, Shima & Findlay 2002, Hoey & McCormick 2004, Grorud-Colvert & Sponaugle 2006, Hamilton 2008, Grorud-Colvert & Sponaugle 2011, Lonnstedt & McCormick 2011). Given the significance of early life history in determining overall recruitment levels, it is important to have accurate and broadly applicable measures of larval condition. Such indices facilitate a clearer understanding of the population fluxes observed in economically important fisheries species, as well as other non-commercial taxa that are no less integral to ecosystem structure and function.

Various approaches have been taken to evaluate different aspects of larval fish condition (reviewed in Ferron and Leggett 1994, Suthers 1998). Otolith microstructure analyses are particularly informative in that they can yield life-long records of age-specific somatic growth rates (Pannella 1971, Campana 1990, Hare & Cowen 1995, Sponaugle & Pinkard 2004, Sponaugle et al. 2010). Consequently, they are used widely and productively in studies involving growth (i.e. condition) appraisal (Sponaugle 2010).

Still, otolith-based techniques are time and labor intensive, and otoliths may not always manifest changes in condition as rapidly as some biochemical measures (Wright & Martin 1985, Molony & Choat 1990, Rossi-Wongtschowski et al. 2003, Dänhardt et al. 2007). Among biochemical indices, the RNA/DNA ratio (R/D) has been used increasingly over the past three decades as a reliable gauge of recent larval growth (Clemmesen 1996, Buckley et al. 1999, Chícharo & Chícharo 2008). The premise of using R/D as an index of condition is that, while the amount of DNA per diploid cell remains constant, the amount of RNA is variable. Overall quantities of cellular RNA, comprised primarily of ribosomal RNA, fluctuate with variations in ribosomal protein synthesis, so depressed growth (e.g. resulting from nutritional stress) is accompanied by decreased quantities of RNA, and thus lower R/D.

Extensive laboratory calibrations of R/Ds have been conducted for larvae from multiple fish taxa and under different feeding regimes. Research has shown that values decrease significantly within a short period of time (as quickly as 1-2 d) when larvae are deprived of adequate food supplies (Buckley 1979, 1984, Wright & Martin 1985, Raae et al. 1988, Richard et al. 1991, Clemmesen & Doan 1996, Rooker & Holt 1996, Catalán et al. 2007). Knowledge garnered from laboratory experiments can be subsequently applied to field investigations, and has been used to estimate growth rates, and determine levels of starvation of larvae *in situ* (Buckley & Lough 1987, Grønkjær et al. 1997, Rooker et al. 1997, Kimura et al. 2000, Chícharo et al. 2003, Voss et al. 2006, Tanaka et al. 2008), as well as to compare relative condition of larvae in different locations (Höök et al. 2007, Lee et al. 2007, Kanstinger & Peck 2009).

It has been suggested that, among taxa, larvae with warmer habitat ranges might exhibit relatively lower R/Ds than those with cold-water distributions (Goolish et al. 1984, Malzahn et al. 2007, Buckley et al. 2008), however such a pattern is yet to be empirically demonstrated (but see Buckley et al. 2008). There is sound physiological reasoning behind the hypothesis that R/Ds might be lower in tropical and sub-tropical larvae: protein growth is associated with ribosome quantity, but it is also a function of ribosomal activity (Henshaw et al. 1971, Smith 1981, Lied et al. 1983). It is possible, then, that fishes inhabiting colder environments may have evolved to compensate for temperature-related low ribosomal activity with higher ribosome quantity. Conversely, warm water taxa likely experience relatively greater ribosomal efficiency, and thus require fewer ribosomes (and less RNA) to attain necessary levels of growth. The relationship between temperature and R/D has been demonstrated within a great many individual species (Buckley 1982, Mathers et al. 1993, Suneetha et al. 1999, Caldarone et al. 2003, Caldarone 2005), and, while temperature-related differences *among* species have also been addressed (Buckley et al. 2008, Díaz et al. 2009), data on warm water larvae are notably sparse. In fact, laboratory-based studies of larval R/D have been published for only three low-latitude species (Westerman & Holt 1988, 1994, Rossi-Wongtschowski et al. 2003, Tanaka et al. 2007).

In light of this information gap, we investigated the effect of food availability on R/Ds in the larvae of the cobia (*Rachycentron canadum*), an easily cultured, fast growing fish that is distributed nearly circumglobally in the tropics and sub-tropics, and spawns at temperatures of ca. 25-30 °C (Ditty & Shaw 1992). Because feeding incidences for larvae in low-latitude regions are often high (Llopiz & Cowen 2009, Llopiz et al. 2010), a

feeding experiment completely depriving larvae of food may be less ecologically relevant than one in which larvae are exposed to reduced food concentrations. (Moreover, if food were withheld entirely, the elevated metabolic rates that are observed at such high temperatures could result in complete starvation mortality so rapid it would inhibit our ability to measure condition changes; Houde 1989a.) Thus, the overall objective of this study was to assess the sensitivity of cobia R/Ds to the potentially subtle changes in growth that might result from reduced food availability, not complete food deprivation. For reference, we evaluated the food-related response of R/D with concomitant changes in otolith-derived growth rates. Additionally, the patterns of distribution of individual R/Ds with size were documented in order to examine the effects of nutritional stress on condition-related population composition and variance. Finally, the nature of R/Ds in tropical larvae relative to those in temperate taxa was explored by comparing data obtained for cobia with published values for a variety of other species measured in previous studies across a broad spectrum of temperatures.

## **MATERIALS AND METHODS**

### ***Larval rearing***

Newly hatched cobia larvae were obtained from the University of Miami Experimental Hatchery in late May of 2011. The broodstock consisted of nine wild-caught individuals (male:female ratio unknown), and all larvae were derived from a single spawning event naturally induced by temperature and photoperiod. At 2 d post-hatch (dph), larvae were stocked in six replicate 400 l conical-bottom rearing tanks at an average density of ca. 10 larvae l<sup>-1</sup>. Tanks were supplied continuously with 1 µm filtered

seawater, and the water turnover rate in each tank was maintained at approximately 360% d<sup>-1</sup>. According to twice-daily measurements taken throughout the experiment, temperature ranged from 27.2-28.3 °C, with a mean of 27.8 °C. Among-tank temperature variation at any given time was negligible (<0.5 °C), as all tanks were immersed in a common water bath. Because the rearing system was located outside, larvae experienced natural light cycles (15 h light: 9 h dark).

For 2 d following stocking, food was supplied uniformly to all six tanks, allowing larvae to transition to exogenous feeding before being subjected to dietary stress. At 5 dph, each tank was assigned to one of two treatment groups, full ration (FR) or reduced ration (RR), each consisting of three replicate tanks. In FR treatments, food was administered at levels consistent with *ad libitum* feeding regimens in standard cohoia larviculture protocol (Benetti et al. 2008), while in RR treatments, larvae were provided with ca. 20% of what FR larvae received. From 5-9 dph, larvae in the FR treatments were pulse fed rotifers (*Branchionus plicatilis*) at a density of 3-4 rotifers ml<sup>-1</sup> 3 x d<sup>-1</sup>, while larvae in RR treatments were fed only 1 rotifer ml<sup>-1</sup> 2 x d<sup>-1</sup>. On days 10 and 11 post-hatch, larvae began to be transitioned off of rotifers and onto artemia (*Artemia franciscana*) nauplii. As artemia were added to the larval diet in increasing quantities, rotifer supply was gradually reduced. Throughout this weaning period, RR larvae continued to receive a 20% relative ration. By 12 dph, larvae had been fully transitioned off of rotifers and were fed only artemia nauplii (0.17 ml<sup>-1</sup> 3 x d<sup>-1</sup> for FR larvae and 0.05 ml<sup>-1</sup> 2 x d<sup>-1</sup> for RR larvae).

To assess growth and condition over the course of the experiment, 9-12 larvae were sampled from each of the six tanks at three different time points: 5 dph (at the



outset of the experiment immediately prior to implementation of FR and RR feeding treatments), 10 dph, and 14 dph. Upon removal from rearing tanks, live larvae were placed in individual 1.5 ml cryovials and immediately flash frozen in liquid nitrogen, then stored at  $-80^{\circ}\text{C}$  for later analysis. Larvae were always sampled at dawn before the first feeding of the day to ensure that biochemical assays would not be confounded by larval gut contents.

### ***Laboratory processing***

Frozen samples were thawed one at a time in preparation for biochemical and otolith analysis. For each larva, standard length (SL) was measured to the nearest 0.01 mm using a Leica MZ12 stereomicroscope equipped with a stage micrometer. The larva's head was then removed with a microscalpel and preserved in 95% EtOH until otoliths could be extracted. The remaining trunk was immediately homogenized in 150  $\mu\text{l}$  ice-cold 1M NaCl and stored at  $-80^{\circ}\text{C}$  for subsequent nucleic acid quantification.

### ***Otolith analysis***

Otoliths were used to investigate daily growth and size-at-age of larvae reared in FR and RR treatments. As the hatch time of larvae in this experiment was known, it was possible to validate the daily deposition of increments by simply comparing number of increments with actual larval age. For all larvae sampled at 5 dph, the number of otolith increments was equal to larval age (i.e. 5). In older larvae (10 and 14 dph), the innermost region of the otolith was obscured and the location of the first increment tended to be indiscernible, thus the number of visible increments was usually equal to larval age minus

1. Given these observations, it can be assumed that—at least over the age range evaluated in this study—increments are indeed laid down on a daily basis and, for otoliths from 10 and 14 dph larvae, the first visible increment typically corresponds to day 2 post-hatch. Enumerations and calculations of increment widths were all carried out using sagittal otoliths, which were larger and had clearer increments relative to lapilli. Sagittae were dissected out according to standard practice, cleaned, placed on a glass slide in a drop of high viscosity immersion oil, and allowed to clear for 7-10 days to facilitate reading (Sponaugle 2009). After clearing, one sagitta from each larva was randomly selected and examined at 400× magnification using a Leica DMLB oil-immersion microscope outfitted with a polarized filter (which enhanced the visibility and contrast of otolith features). Microscope images were transmitted to a computer via a Dage MTI video camera and captured with a framegrabber. They were then analyzed using the program Image Pro Plus 7.0 (Media Cybernetics); increments along the longest axis of the otolith from the core to the edge were digitally marked and counted, and resulting increment widths and increment radii (distances from the otolith core to each individual increment) were calculated. All otoliths were read blind with respect to feeding treatment. If increment count did not agree with known larval age, otoliths were re-read. If the second read did not agree with the known age of an individual (which usually occurred when otoliths were extremely cloudy or obscured and/or increments were particularly faint), the otolith was discarded and that individual's other sagitta was used instead. In the cases where neither of the two sagittae from an individual was readable, that individual was excluded from analysis ( $n = 3$ ). Least-squares regression showed a significant positive relationship between otolith radius and SL ( $r^2 = 0.90$ ,  $p < 0.0001$ ), and the residuals of

SL-at-age and otolith radius-at-age regressions were also positively correlated (*Pearson's correlation coefficient* = 0.788,  $p < 0.0001$ ). These findings confirm that otolith growth can be used as a proxy for somatic growth (Hare and Cowen 1995), and thus an index of larval condition.

### ***RNA and DNA quantification***

Concentrations of RNA and DNA in each sample homogenate were measured using a fluorometric microplate assay following the methods of Westerman and Holt (1988), with the exception that the fluorophore SYBR Green II (SGII, Molecular Probes) was used in place of Ethidium Bromide. Briefly, homogenates were treated with SGII, and fluorescence levels in each sample were read using a Tecan GENios plate reader (ex: 485 nm, em: 535 nm). These initial fluorescence measurements corresponded to total quantities of nucleic acids (RNA and DNA combined). To measure DNA alone, RNA was selectively digested with RNase (Sigma R-4875) and fluorescence was read again. Fluorescence attributable to RNA could then be determined by calculating the differences between the first and second measurements. RNA and DNA standard curves were generated from fluorescence measurements of known quantities of RNA (type III from baker's yeast, Sigma R-6750) and DNA (type I from calf thymus, Sigma D-1501) that had been treated with SGII. These standard curves were used to calculate the concentrations of RNA and DNA in each cobia sample based on fluorescence levels measured as described above.

### ***Taxonomic and temperature-based RNA/DNA ratio comparisons***

Historically, among-study comparisons of R/Ds have been impeded by high variability in measurements from one lab to the next, likely stemming from differences in the protocols, equipment, and reagents used at different facilities (Caldarone & Buckley 1991, McGurk & Kusser 1992, Clemmesen 1993, Canino & Caldarone 1995). However, inter-laboratory inconsistencies can be largely accounted for by standardizing data to a common ratio of the slope of the DNA standard curve to the slope of the RNA standard curve (Berdalet et al. 2005, Caldarone et al. 2006). In the present study the mean ‘DNA standard slope:RNA standard slope’ ratio was 10.63. In order to compare the R/Ds of coxia larvae measured here with those of other taxa, our data, along with measurements from 17 other studies for which DNA slope:RNA slope ratios had been published (Table 2.1), were all standardized to a common slope ratio of 2.4 (an intermediate value that has precedence in the literature; Buckley et al. 2008, Diaz et al. 2011) . The studies that were included in this comparison (which comprised 14 different species across a broad thermal gradient) consisted of both field sampling and laboratory rearing experiments, with the requirements that (1) larvae reared in the laboratory had been provided *ad libitum* (or equivalent) food supply, (2) for field investigations, if more than one habitat or time point was sampled, data were taken from the group with the highest mean condition, and (3) the temperature (or temperature range) that the larvae had experienced in the lab or in the field was reported. Knowing the temperatures to which larvae had been exposed facilitated greater understanding of the relationship between R/D and environment. Moreover, it allowed for the estimation of daily somatic growth according to the following multispecies model (Buckley et al. 2008):

$$G = 0.0145T + 0.0044T \times (sR/D) - 0.078$$

where G = weight specific growth rate ( $d^{-1}$ ), T = temperature ( $^{\circ}C$ ), and sR/D = standardized R/D.

### *Statistical analysis*

To examine the relationship between food availability and condition in larval cobia, multiple condition indices (R/Ds, SLs, and otolith-derived growth traits) were compared among larvae in FR and RR feeding treatments. Each feeding treatment included three replicate rearing tanks, so differences among treatments were analyzed using nested ANOVA with tank included as a nested effect (SYSTAT 11.0, Wilkinson 1992). Tests of SLs and R/Ds among FR and RR treatments were carried out for datasets from each of the 3 sampling time points (5, 10, and 14 dph), which all satisfied assumptions of equal variance and normal distribution. Otolith increment radii and increment widths at distinct points in larval life were also compared across feeding treatments; for both of these traits, separate nested ANOVAs were performed at increments 8, 10, and 12 (representing ages 8, 10, and 12 dph). Again, variances were equal and distributions were normal and, since only one measurement per fish was used in each test, the assumption of independence of data points required for ANOVA was met, precluding the need for repeated measures techniques.

In addition to the parametric, age-specific analyses described above, non-parametric methods were employed to more specifically explore the effect of reduced food availability on R/Ds and their distributions over the complete larval size range investigated. Because the relationship between R/D and SL was heteroscedastic, and thus

difficult to define in terms of SL, a local density estimator approach (Pepin et al. 1999) was applied to predict the change in variability of R/D with size in both FR and RR feeding treatments. In this analysis, kernel smoothing was used to estimate the cumulative probability distribution functions (CDFs) of R/Ds as a function of SL and neighboring R/D observations. To assess differences between the CDFs of FR and RR larvae, the 10<sup>th</sup>, 50<sup>th</sup>, and 90<sup>th</sup> percentiles, as well as the distance between the 10<sup>th</sup> and 90<sup>th</sup> percentiles (referred to hereafter as scatter), were analyzed. Five hundred randomizations of each of the two data sets (FR and RR) were synthesized through Monte Carlo simulation, and the percentiles and scatters of the original data were compared to those of the randomizations. An original CDF at a given SL was significantly different from average if the scatter was greater or less than 97.5% of the synthetic scatter values at that SL (Pepin et al. 1999).

It was also important to evaluate the effects of feeding treatment on the distributions of individual R/Ds in relation to larval age, not just size. Larvae were collected at three discrete ages (5, 10, and 14 dph) rather than continuously over the age range, thus it was not appropriate to apply a local density estimator CDF analysis, so standard non-parametric box plots were used to compare the scatter between the 10<sup>th</sup> and 90<sup>th</sup> percentiles of R/Ds in each age group.

## **RESULTS**

### ***Size and growth***

A total of 182 cobia larvae were sampled, with SLs ranging from 3.6 mm in the smallest 5 dph larvae to 12.0 mm in the largest 14 dph larvae. At the outset of the

experiment (5 dph), SLs were similar among tanks designated as FR and RR (overall mean = 4.51 mm), but by 14 dph (after 8 d of reduced food availability) larvae in RR feeding treatments had significantly smaller SLs than their FR counterparts (Fig. 2.1). Growth throughout larval life was investigated in greater detail using otolith increment widths and increment radii as proxies for daily growth rates and sizes-at-age, respectively. Both growth rates and sizes-at-age differed among feeding treatments from ca. 8 dph onward (Fig. 2.2). Pairwise comparisons of FR and RR groups at increments 8, 10, and 12 indicated that RR larvae had significantly slower growth than FR larvae at increments 8 and 10, and significantly smaller size-at-age at all three points tested (Fig. 2.2).

### ***RNA/DNA ratios***

R/D values of larvae sampled throughout the study ranged from 0.9-9.2. The lowest R/Ds were observed at 5 dph (mean = 2.76). Thereafter, values increased continually with age in both FR and RR treatments (Fig. 2.3). At both 10 dph and 14 dph, R/Ds in RR larvae were significantly lower than those in FR larvae (Fig. 2.3).

Non-parametric analyses of R/Ds revealed further differences among treatments. According to CDFs of R/D relative to SL, the 90<sup>th</sup> percentile of R/Ds in FR larvae increased steadily with size until reaching an asymptote of approximately 7.8 at ca. 6 mm SL (Fig. 2.4a). In RR larvae, the 90<sup>th</sup> percentile of R/D ratios also appeared to reach a maximum of around 7.8 at ca. 6 mm SL, but after that point, it decreased slightly with increasing SL (Fig. 2.4b).

The patterns of variance of R/D values with SL were notably distinct in FR and RR feeding treatments, as evidenced by both visual inspection of the variance and measurements of scatter (Figs. 2.4a, b, c, and d). In the smallest size classes, CDF-derived scatter was similar for FR and RR larvae, but the RR scatter values were slightly higher at intermediate SLs and much lower at the largest SLs compared to FR values. While the scatter of R/Ds in FR larvae was 3 or higher across the range of SLs sampled, that of the RR treatment fell notably to  $< 1$  in the larger size classes, reflecting decreased R/D variance. The observed scatter was significantly higher than average in the middle size classes of both FR and RR CDFs, but only the RR CDF showed significantly lower than average R/D scatter in the larger size classes (between 9 and 10 mm SL, Figs. 2.4e and 2.4f).

When the variability of individual R/Ds was examined in relation to size, no between-treatment differences were apparent. Estimates of scatter were similar among FR and RR feeding treatments at all three age groups sampled (5, 10, and 14 dph; Fig. 2.5).

### ***The RNA/DNA ratio across taxa and temperatures***

The R/Ds of FR (i.e. high food availability) cobia larvae measured in this experiment were compared to published reports of R/Ds in similarly well-fed laboratory-reared larvae as well as field-collected larvae of a variety of other taxa. The 14 different species included in the comparison experienced a wide array of temperatures, from 3.0 °C (Atlantic cod) to 29.4 °C (bluefin tuna). Standardization of raw R/Ds from all studies yielded standardized R/D (sR/D) values ranging from 0.58 to 7.15. The mean sR/D of FR



cobia larvae sampled throughout this experiment was 0.92, constituting the second lowest mean sR/D observed among all studies surveyed (Table 2.1). Overall, sR/D generally decreased with increasing water temperature, and a least-squares regression of log transformed mean sR/D with mean temperature showed a significant negative correlation between the two ( $r^2 = 0.323$ ,  $p < 0.01$ ; Fig. 2.6). Calculations of  $G d^{-1}$  based on mean temperatures and sR/D values of the various taxa examined indicated that, despite having low sR/Ds, larvae at the highest temperatures (including cobia) likely exhibit high rates of growth relative to larvae sampled at low temperatures (Table 1).

## DISCUSSION

The research presented here demonstrates that reduced food concentrations result in a decrease in mean larval cobia growth, and growth effects are, in turn, reflected in R/Ds. These findings indicate that the R/D index effectively captures changes in condition, even in cases where food supply is merely reduced, not completely withheld. According to our data, food availability not only influences mean condition of a given larval population, it also impacts patterns of variability of individual larval condition levels with size; careful analysis of these patterns provides important information relevant to the processes of growth and mortality. Clearly, the R/D can serve as a valuable tool in assessing larval condition, and comparison of R/Ds across species allowed for an investigation of the nature of the R/D and its dependence on temperature. Our analysis reveals a negative correlation between temperature and R/D, likely attributable to thermal effects on biochemical reaction rates.

### ***Size and growth***

According to otolith microstructure, food-limited larvae were smaller and slower growing than fully fed larvae of the same age after ca. 2 d of reduced food availability. Between-feeding treatment differences in both size-at-age and growth rate were highly significant at 8 and 10 dph and, while differences appeared to be less significant at 12 dph, it is worth noting that sample sizes at the oldest ages were reduced. (Larvae sampled at 5 and 10 dph were not old enough to provide any data for those analyses.)

Corroborating our otolith-based findings, SL measurements revealed that food-limited larvae were significantly shorter at a given age as compared to fully fed larvae. Still, between-treatment differences in SL were not as substantial as expected. In some cases, nutritionally compromised larvae have been shown to exhibit only slight declines in lengthwise growth rates (perhaps due to a dependence of skeletal elongation on ontogeny; Anderson and Gutreuter 1983), but they suffer more pronounced decreases in growth rate in terms of body area, body depth, or weight as they stop storing or begin catabolizing energetic reserves (Wyatt 1972). Just such a mechanism is likely behind the observation of Hare and Cowen (1995) that, in bluefish larvae, otolith radius is more closely related to body area than to SL. Neither dry weight nor body area was obtained in the present experiment, but it is likely that, in cobia too, otolith growth is linked more tightly to a multidimensional metric of somatic growth (i.e. body area or weight) than to SL alone. This would account for the somewhat weak relationship between SL and food level despite the robust correlation of food level and otolith growth.

### ***RNA/DNA ratios***

The ration-related differences observed in larval cobia growth were mirrored by analogous differences in R/Ds: larvae receiving reduced rations exhibited lower mean R/Ds at both 10 and 14 dph. Previous studies have shown that complete starvation usually elicits a clear response in larval fish R/D within 1-2 d (Buckley 1979, Wright & Martin 1985, Raae et al. 1988, Rooker & Holt 1996, Catalán et al. 2007) but partial ration experiments, have produced mixed results. Neither Canino (1997) nor Caldarone (2005) saw any significant differences in R/Ds among full ration and reduced ration feeding treatments in walleye pollock or haddock, respectively. Likewise, in an analysis of larval herring, Houlihan et al. (1995) found no effect of reduced food concentration on an alternative measure of RNA content, the ratio of RNA to total protein. However, according to Buckley et al. (1984), R/Ds of larval sand lance declined significantly when concentrations of prey were reduced by 50 or 80%, and a mesocosm study of Atlantic cod showed that different prey concentrations corresponded to disparate R/Ds (Clemmesen et al. 2003). In larval cobia, the observed differences in R/Ds induced by a decrease of food availability (rather than complete food deprivation) suggest that the R/D is sensitive to relatively subtle changes in nutrition and growth.

The effects of food concentration on larval cobia R/Ds are discernable not only in mean values, but in size-specific distribution patterns as well, as evidenced by the differences in CDFs among feeding treatments. In FR larvae, for example, the 90<sup>th</sup> percentile R/D reached a maximum at ca. 7 mm SL, and remained roughly constant with increasing size. Conversely, the 90<sup>th</sup> percentile in the RR feeding treatment reached a comparable maximum value at approximately the same SL, but as SL increased beyond

that point, the 90<sup>th</sup> percentile appeared to decline. This finding suggests that, when food supply is limited, growth of all larvae in a population is curtailed, and even the highest condition individuals are unable to achieve optimal R/Ds.

Another important outcome of the CDF analysis was the observation that, in the RR treatment, R/D scatter (a measure of variability) was distinctly contracted at larger sizes—such an effect was not observed in the distribution of FR larvae. Field-based investigations of R/D variability have shown comparable reductions in scatter in the largest larval size classes. Most notably, Pepin et al. (1999) found a similar pattern for six different larval fish species collected from Conception Bay, Newfoundland. There are multiple mechanisms that could account for decreased variability in condition with increasing size, but the most probable explanation is that differential mortality of larvae with different survival probabilities (i.e. different levels of condition) results in selective removal of low condition larvae from the population over time, causing an increase in the minimal condition level (i.e. the 10<sup>th</sup> percentile) and a decrease in condition variability (Pepin et al. 1999). Selective predation is likely a key driver in this *in situ* process, but what about laboratory rearing experiments in which predation is non-existent? An alternative cause of distinctly low scatter values in the largest larvae is based on potential inconsistencies in growth coincident with varying levels of condition. If low R/D larvae are growing slowly, they may never attain the largest sizes, in which case, the largest size classes would be restricted to the fish with the highest R/Ds. Accordingly, variability in condition in large larvae would be particularly low.

In order to determine whether the low R/D variability observed in large RR coibia larvae was more dependent on differential mortality of poor condition individuals or

retardation of their growth, we conducted an age-specific analysis of scatter to compare to our size-specific observations. At 14 dph (the oldest age observed in this experiment) the R/D scatter of RR larvae was actually larger than that of FR larvae. This contrasts with our sized-based findings in which RR larvae show a decrease in R/D scatter at the longest SLs. The high variability in R/Ds of RR larvae in the oldest age class presumably comprised both high-condition, larger fish as well as poorly fed (and potentially dying) smaller ones. Broad scatter of condition values in larvae of a common age implies that condition-specific mortality alone is not responsible for the between-treatment differences in R/D variability with size. Decreased growth rates resulting from food limitation are likely contributing to the low scatter observed at large size classes in the RR CDF. An inspection of the size distributions of larvae in FR and RR treatments supported this conclusion, revealing that RR larvae were more abundant in smaller size classes and far less abundant in the largest size classes as compared to those in FR treatments (Fig. 2.7). Clearly, as previous studies have also illustrated (Pepin et al. 1999, Pepin 2004, Voss et al. 2006, Dower et al. 2009), examining the variability in larval condition, and not just the means, can provide important insight into the processes governing condition and mortality during early life.

As described above, prior investigations into the effect of partial ration reduction on R/Ds have yielded inconsistent results, but until now, such studies have been performed only for temperate dwelling species. The high responsiveness of cobia R/Ds (evaluated here as both mean values and non-parametric probability distributions) to a reduction in food concentration is likely a function of the high temperatures that characterize larval cobia habitat, and the fast growth and elevated metabolic demands

inherent in such warm environments. It stands to reason, then, that larval R/Ds in other tropical and sub-tropical fish larvae are similarly sensitive to fluctuations in feeding environment and condition. This level of sensitivity is particularly important when considering that the role of the larval stage in shaping populations is based not only on survival rates during the larval period, but also on relatively slight differences in larval condition that might lead to increased or decreased probability of survival in later life stages. The potential capacity of the R/D to detect relatively subtle condition differences in low-latitude larvae makes this index particularly relevant to studies of coral reef fish population connectivity. Assessing the differences in condition of larvae from diverse origins, or with contrasting dispersal histories (and thus distinct environmental experiences) can provide valuable information as to which adult habitats serve as the sources of the larvae and, consequently, the juveniles that have a high probability of surviving to contribute to a population. Identifying these locations is, of course, crucial to reef management and conservation efforts.

### ***The RNA/DNA ratio across taxa and temperatures***

Compared to all other taxa examined, fully fed cobia larvae exhibited low sR/Ds. Nevertheless, values observed for cobia were in line with those of other tropical and sub-tropical species (Garcia et al. 2006, Faria et al. 2011a). Moreover, sR/Ds obtained for cobia larvae resulted in a calculated value of G that puts these larvae on par with fast growing, warm water larval taxa, consistent with the quick maturation and rapid growth for which cobia are known (Chou et al. 2001). It has been proposed that larvae inhabiting cooler environments might have higher R/Ds because temperature driven depression of

ribosome activity would necessitate increases in ribosome quantity (Goolish et al. 1984). To address this issue, Buckley et al. (2008) compared sR/D in the larvae of seven different species, but no correlation between temperature and sR/D was observed. By supplementing the comparison made by Buckley et al. with results from new laboratory rearing experiments (including the present study) and additional data from eligible field based investigations, we were able to detect a significant trend of decreasing sR/D with increasing environmental temperature.

Despite best efforts to standardize the data included in this comparison, there remains a substantial amount of variability evident in the sR/D-temperature relationship. Such variability—likely stemming from the slight differences in sizes, ages, and experimental procedures used in each study—is unavoidable in a synthesis of this nature. Still, the relationship between sR/D and temperature was robust to these inconsistencies.

Although the inclusion of field-sampled larvae could raise questions as to comparability, these additional data provide valuable information, especially given the paucity of laboratory experiments published for low-latitude species. One might expect mean R/Ds observed in the laboratory under *ad libitum* feeding regimes to exceed those observed in field collected samples, but R/Ds of wild-caught larvae are often similar to (Grønkjær et al. 1997) or greater than (Buckley et al. 1984, Clemmesen & Evans 2001) R/Ds obtained for reared fish. This is likely due to the fact that a laboratory setup cannot perfectly replicate the combined physical, chemical, and biological conditions of the natural environment to which larvae are adapted. Prey type, in particular might play a role, as natural prey items are generally more calorically rich than the prey provided in rearing experiments (Holm et al. 1991). Additionally, as a result of predation pressure in

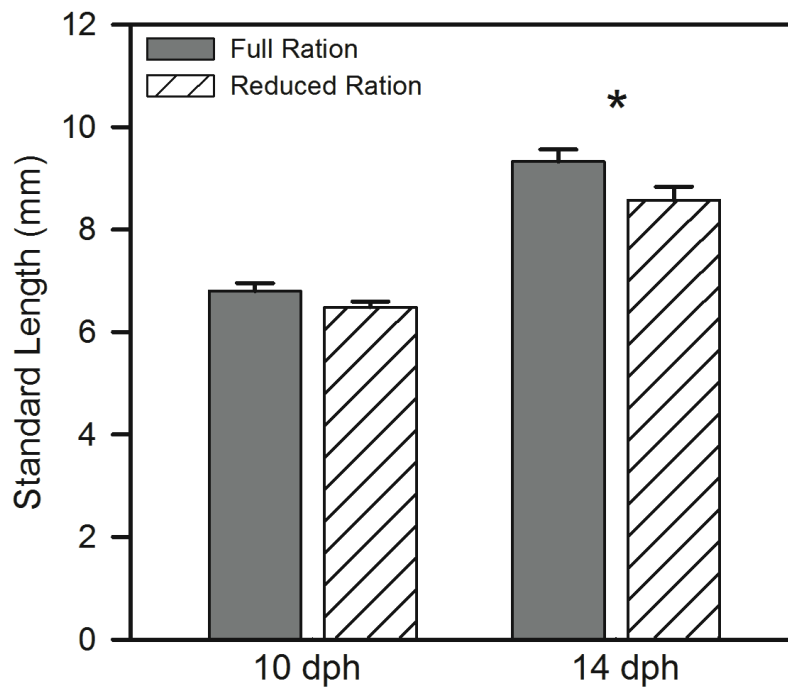
the field, it is possible (as discussed earlier) that the selective removal of poor condition individuals leaves higher proportions of the “fittest” larvae, and results in elevated field R/Ds that are on par with or slightly higher than those observed in rearing experiments. In examining the temperature-dependent distribution of the field-derived data points relative to laboratory-derived data, it is apparent that the two types of studies generate similar patterns. The mutual corroboration of field and laboratory-based observations verifies the appropriateness of analyzing all investigations together.

By comparing data from a variety of larvae representing different species and diverse thermal distributions, it was possible to analyze the condition-R/D relationship over a broad range of temperatures. Nucleic acid indices like R/Ds lend themselves to inter-taxon comparison because of the potential universality of the fundamental biochemistry underlying metabolism and growth. Still, further investigation into the species specificity of these mechanisms is required. In light of the substantial changes in climate that are projected to occur in coming years (Trenberth et al. 2007), understanding the effects of temperature on ecological and physiological processes is critical, and describing the behavior of R/Ds in low-latitude species will likely be important in studying and predicting early life history processes in the future. The results obtained in this laboratory-based investigation of larval cobia R/Ds under full ration and reduced ration feeding regimes help to ameliorate the relative shortage of published data on R/Ds in low-latitude species. They also serve to demonstrate that the R/D can be used as a tool to examine and elucidate the processes governing mortality and condition during the larval stage and beyond.

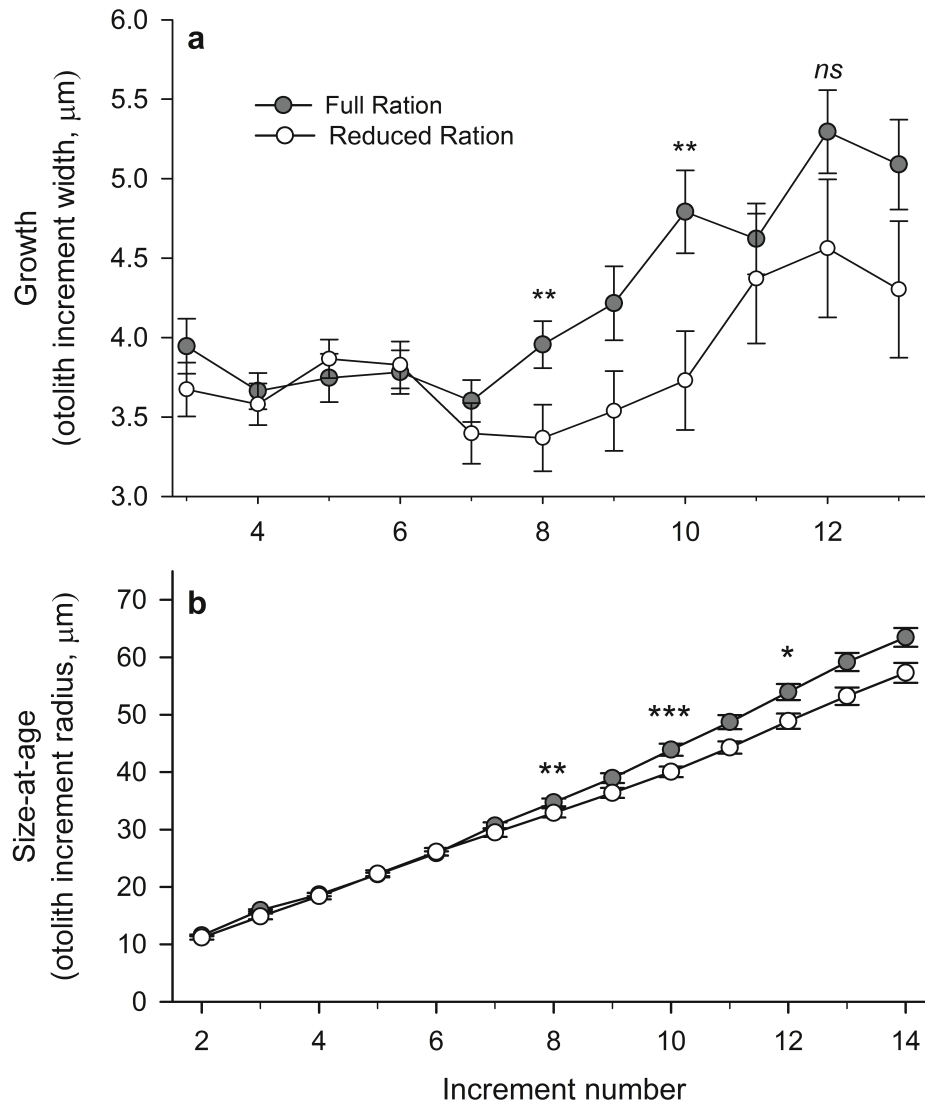


**Table 2.1:** Comparison of standardized RNA/DNA ratios (sR/D) and weight-specific growth rates ( $G d^{-1}$ ) of 14 different species of fish larvae from a range of temperatures. Data from the present study on cobia larvae are highlighted in grey. All other datasets were sourced from the primary literature. Some studies reported only SL or age, but not both; *n.r.* indicates where data were not reported. † identifies studies included in a previous investigation of the relationship between RNA/DNA ratios and temperature by Buckley et al. (2008).

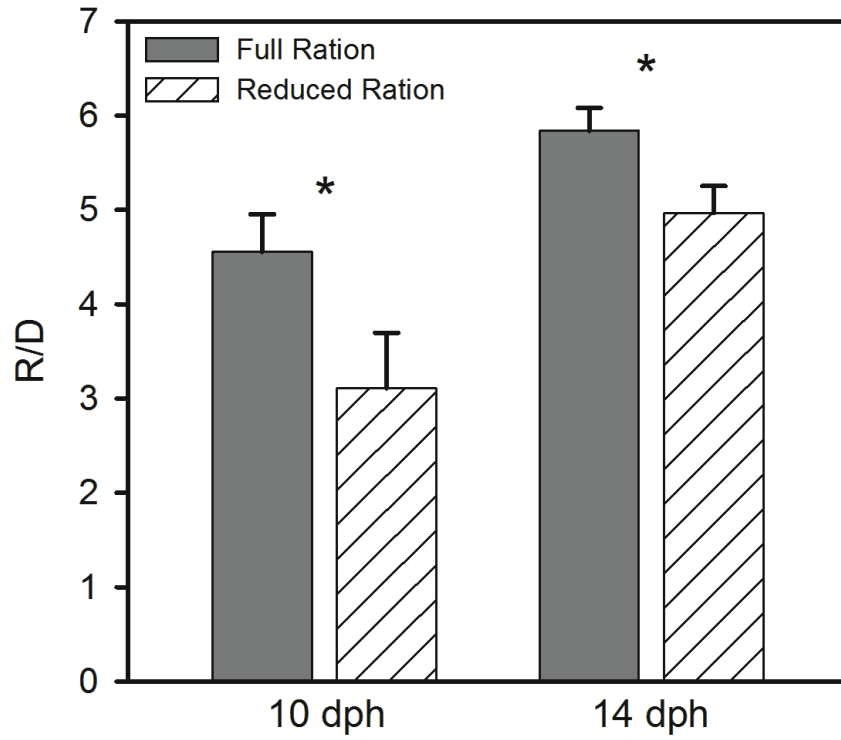
Reference	Common Name (Species)	Field or Lab	Age (d)	Standar d Length (mm)	Temp. (°C)	Mean sR/D	Mean G d <sup>-1</sup>
Garcia et al. 2006	bluefin tuna ( <i>Thunnus thynnus</i> )	Field	2–19	3.5–8.5	23.5–29.4	1.89	0.60
Garcia et al. 2006	albacore tuna ( <i>Thunnus alalunga</i> )	Field	2–19	2.9–6.8	23.5–29.4	1.67	0.57
Westerman and Holt 1994†	red drum ( <i>Sciaenops ocellatus</i> )	Lab	10–14	<i>n.r.</i>	28	2.25	0.67
present study	cobia ( <i>Rachycentron canadum</i> )	Lab	5–14	4.5–9.5	28	0.92	0.51
Rossi-Wongtchowski et al. 2003†	Brazilian sardine ( <i>Sardinella brasiliensis</i> )	Lab	8–12	<i>n.r.</i>	21.3–25.1	2.72	0.61
Faria et al. 2011b	Senegalese sole ( <i>Solea senegalensis</i> )	Lab	8–14	3.5–7.5	21	0.58	0.35
Díaz, E. et al. 2008	anchovy ( <i>Engraulis encrasicolus</i> )	Field	<i>n.r.</i>	7.5–11.4	15.8–17	2.17	0.39
Díaz, M.V, et al. 2011	Argentine anchovy ( <i>Engraulis anchoita</i> )	Field	<i>n.r.</i>	4.5–5.0	14.1–17.7	4.74	0.56
Díaz, E. et al. 2011	sardine ( <i>Sardina pilchardus</i> )	Field	<i>n.r.</i>	7.5–11.4	13.3–15.8	2.29	0.35
Chícharo 1997	sardine ( <i>Sardina pilchardus</i> )	Field expt.	<i>n.r.</i>	4.5–7.0	15.3	1.74	0.33
Clemmesen 1994	herring ( <i>Clupea herengus</i> )	Lab	4–13	<i>n.r.</i>	12–16.7	2.78	0.38
Skajaa et al. 2003†	Norwegian cod ( <i>Gadus morhua</i> )	Lab	8–21	4.7–6.0	10	3.22	0.28
Lee et al. 2007	dab ( <i>Limanda limanda</i> )	Field	<i>n.r.</i>	7.5–11	8.2–10.5	7.15	0.19
Suneetha et al. 1999	herring ( <i>Clupea herengus</i> )	Lab	<i>n.r.</i>	10–12	11	2.18	0.26
			<i>n.r.</i>	8–12	8	3.11	0.22
			<i>n.r.</i>	10–12	5	3.00	0.14
Gronkjaer et a. 1997†	Baltic cod ( <i>Gadus morhua</i> )	Lab	10–12	<i>n.r.</i>	8	3.35	0.23
St. John et al. 2001	North Sea cod ( <i>Gadus morhua</i> )	Lab	10–12	<i>n.r.</i>	8	2.78	0.21
Canino 1997†	walleye pollock ( <i>Theragra chalcogramma</i> )	Lab	10–16	<i>n.r.</i>	6	2.82	0.16
			7–19		10	2.51	0.25
Caldarone et al. 2005†	haddock ( <i>Melanogrammus aeglefinus</i> )	Lab	8–18	<i>n.r.</i>	8	2.67	0.21
			9–16		6	2.51	0.15
					9	2.89	0.24
Caldarone et al. 2003†	Atlantic cod ( <i>Gadus morhua</i> )	Lab	6–14	<i>n.r.</i>	6	3.23	0.17
					3	3.89	0.09



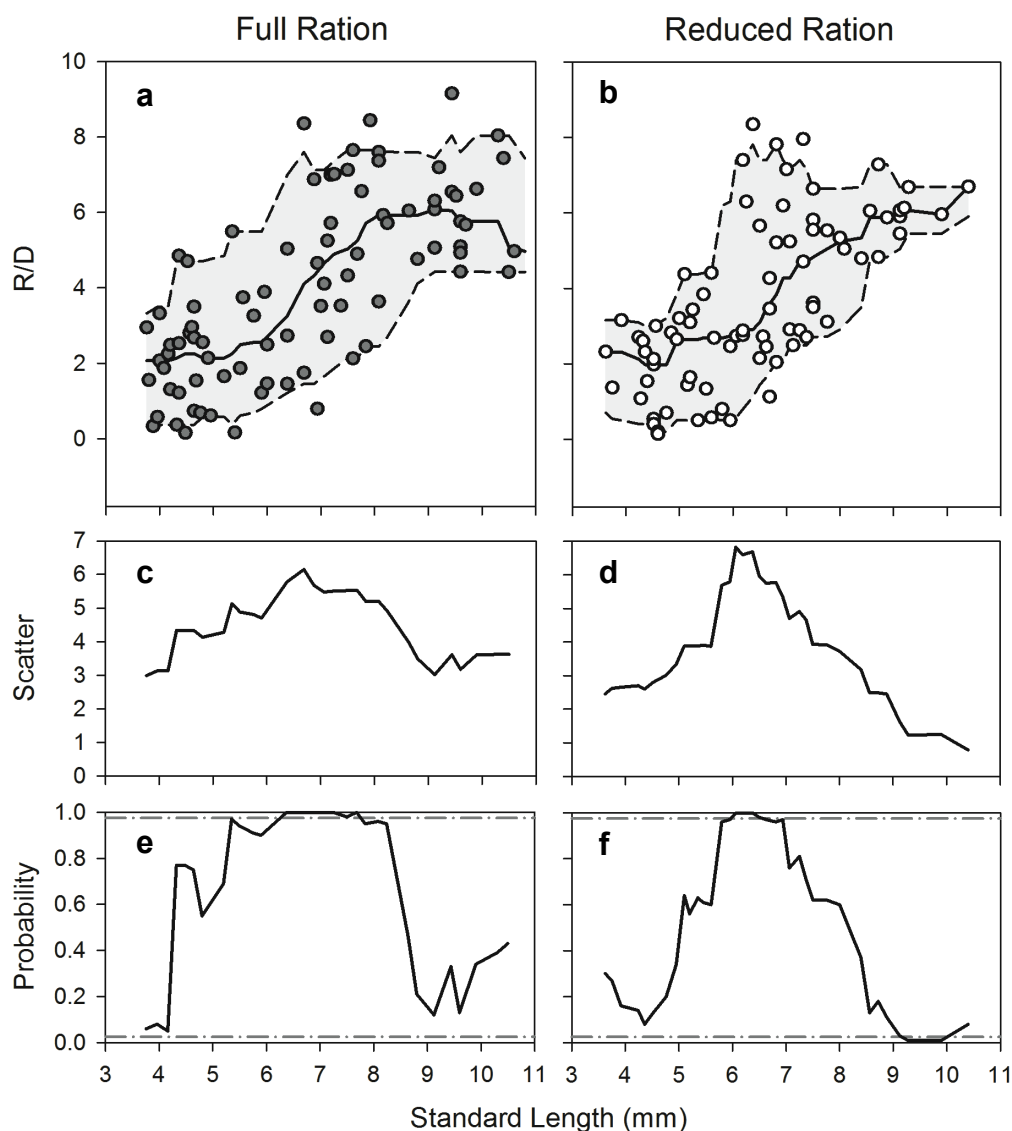
**Fig. 2.1:** Mean SL ( $\pm$ SE) of cobia larvae sampled from full ration and reduced ration feeding treatments at 10 and 14 days post hatch. Asterisk indicates significant difference at  $p < 0.05$  according to nested ANOVA. 10 dph:  $n = 63$ , 14 dph:  $n = 58$ .



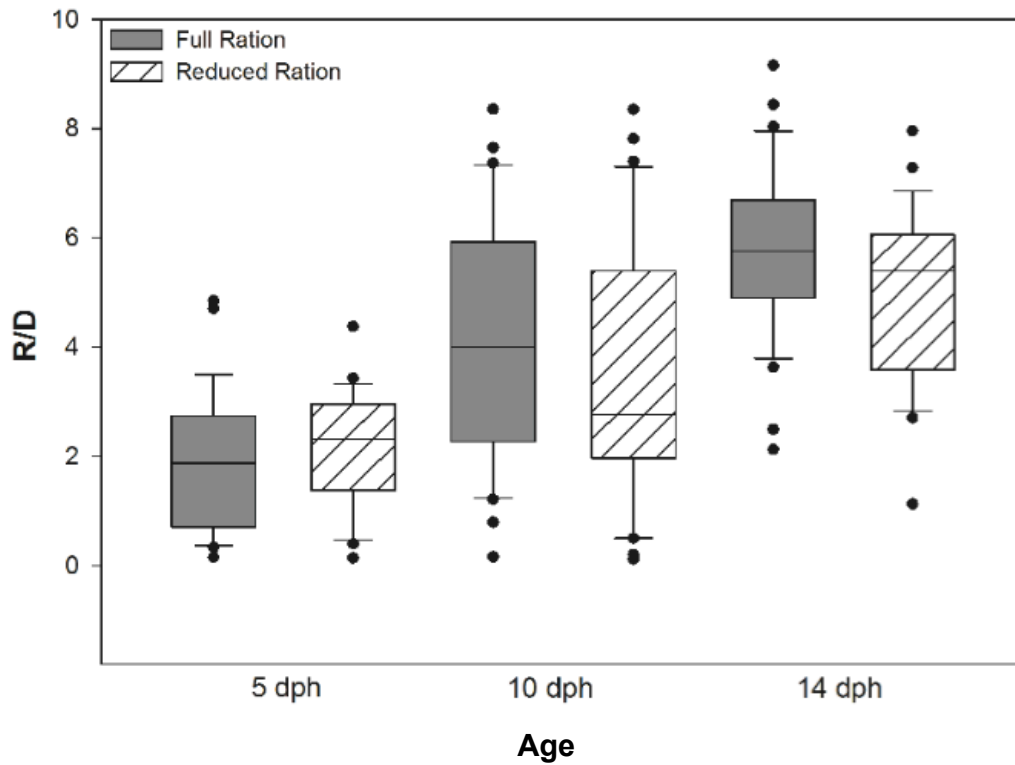
**Fig. 2.2:** Mean (a) growth ( $\pm\text{SE}$ ) and (b) size-at-age ( $\pm\text{SE}$ ) of full ration ( $n = 86$ ) and reduced ration ( $n = 81$ ) cobia larvae sampled at 5, 10 and 14 dph. Mean growth (otolith increment width) and mean size-at-age (otolith radius) were compared at three points: days 8, 10, and 12 of larval life. Asterisks indicate statistically significant differences according to nested ANOVA (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , *ns*: not significant).



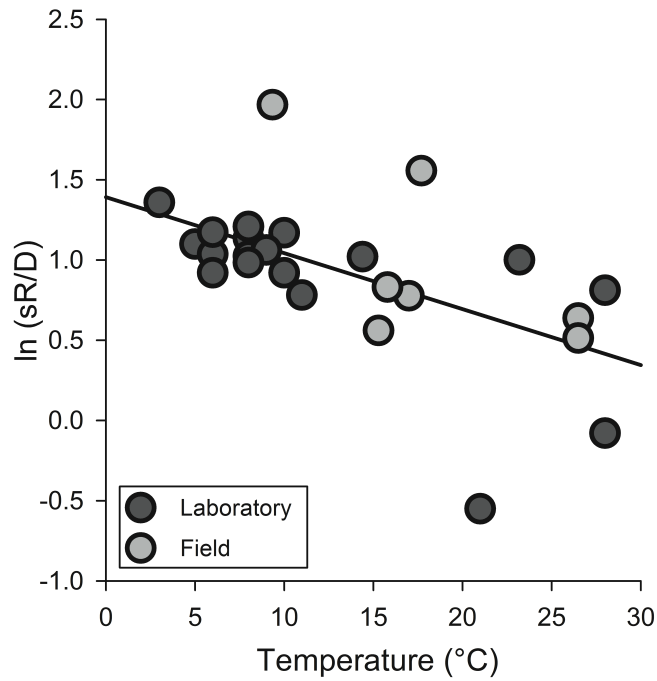
**Fig. 2.3:** Mean R/D ( $\pm$ SE) of cobia larvae sampled from full ration and reduced ration feeding treatments at 10 and 14 days post hatch. Asterisks indicate significant difference at  $p < 0.05$  according to nested ANOVA. 10 dph:  $n = 63$ , 14 dph:  $n = 58$ .



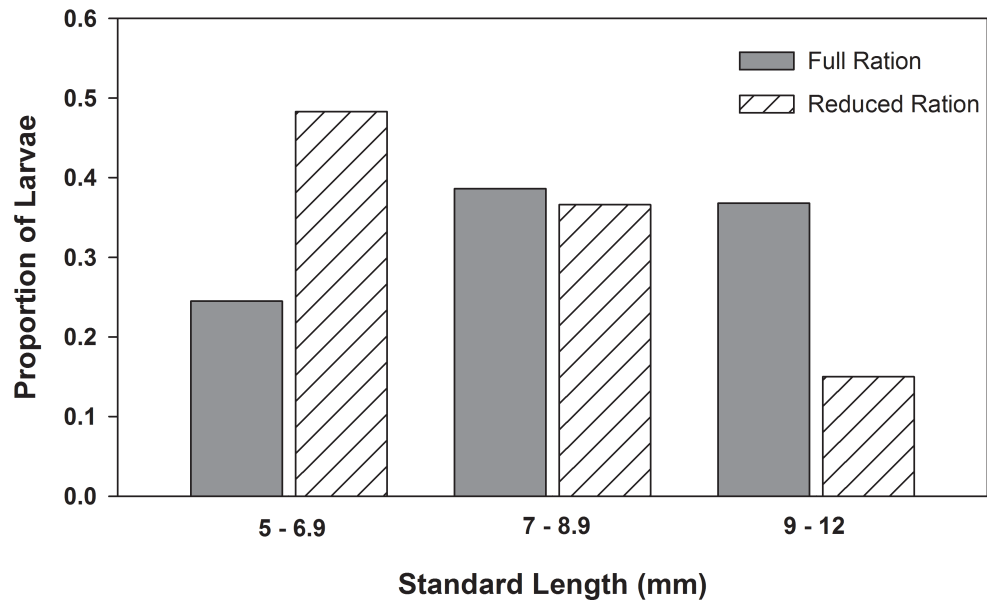
**Fig. 2.4:** Cumulative probability distribution functions (CDFs) of R/D in relation to SL for larvae reared under (a) full ration and (b) reduced ration feeding conditions (full ration:  $n = 86$ ; reduced ration:  $n = 81$ ). Solid lines in a and b indicate medians, and dashed lines above and below medians indicate 90<sup>th</sup> and 10<sup>th</sup> percentiles according to the CDFs. Circles represent individual raw R/D values. R/D scatter (i.e. the distance between the 10<sup>th</sup> and 90<sup>th</sup> percentiles) corresponds to the shaded areas in a and b, and is plotted against SL for (c) full ration and (d) reduced ration larvae. The probabilities of occurrence of the scatter values reported in c and d were calculated based on Monte Carlo simulation of 500 randomizations of the actual data. Probabilities for full ration (e) and reduced ration (f) datasets are plotted against SL. Dashed grey lines in e and f indicate thresholds of statistical significance; where probabilities are greater than 0.975 or less than 0.025, scatter is significantly higher or lower than average relative to the 500 synthetic data sets.



**Fig. 2.5:** Box plot showing the variability (i.e. scatter) of R/Ds of individual larvae from Full Ration (gray) and Reduced Ration (hatched) treatments with increasing age (5, 10, and 14 dph). Boxes encompass from the 25<sup>th</sup> to the 75<sup>th</sup> percentiles of observed R/D values, horizontal lines represent the medians, and whiskers extend from the 10<sup>th</sup> to the 90<sup>th</sup> percentiles (and are analogous to the 10<sup>th</sup> and 90<sup>th</sup> percentiles shown in the SL-based CDF plot in Fig. 2.4, above).



**Fig. 2.6:** Least-squares regression of standardized RNA/DNA ratio (sR/D) with temperature ( $y = -0.035x + 1.392$ ,  $r^2 = 0.321$ ,  $p = 0.003$ ). Values are separate means of 26 species-specific datasets derived from 17 published studies of well-fed laboratory-reared larvae and field-caught larvae (Table 2.1). Where temperatures for a given study varied, mean temperature was used.



**Fig. 2.7:** Proportions of larvae from both Full Ration (gray) and Reduced Ration (hatched) treatment groups in each of three size classes.



### **Chapter 3. Nutritional condition of coral reef fish larvae varies with dispersal history: prey, predators, and population connectivity**

The degree of connectivity that occurs among coral reef fish populations is determined not only by whether larvae can physically travel from one geographically discrete population to another, but also by whether they reach a settlement site in adequate nutritional condition to survive the juvenile phase and ultimately reproduce. Well-documented carryover effects between life stages mean that larvae exhibiting different levels of condition in the plankton may differ in their post-settlement mortality. Thus, to better understand the relative potential for local retention and long-distance dispersal to contribute to reef fish population maintenance, it is necessary to examine the variation in larval condition with dispersal trajectory. To this end, we undertook three cruises in the summers of 2007 and 2008, collecting ichthyoplankton and environmental data (MOCNESS and CTD) at 90 stations on cross-shelf transects along the Florida Keys reef tract, and in the Loop Current upstream of the Florida Keys. MOCNESS tows at each station yielded larvae from a broad range of coral reef fish taxa and, for a subset of larvae identifiable to species (bluehead wrasse, pearly razorfish, bluelip parrotfish, and great barracuda), RNA/DNA ratios and otolith-derived growth rates were obtained. These indices were used to evaluate the condition of individual larvae collected across distinct water masses and, while results varied among species, data indicated that, for three of the four taxa (bluehead wrasse, bluelip parrotfish, and pearly razorfish), larvae collected closer to shore exhibited significantly higher condition as compared to larvae collected offshore. These among-region differences could not be explained by enhanced feeding due to increased environmental prey availability, as the preferred prey items of the larvae

were actually less abundant at nearshore sampling stations. An examination of the distributions of individual larval condition levels with age indicated that the observed nearshore-offshore differences are instead a result of dissimilar levels of selective mortality occurring between the two regions.

## **BACKGROUND**

Like most benthic-oriented marine species, coral reef fishes exist as spatially explicit populations interconnected by the exchange of pelagic larvae. This exchange of individuals, known as population connectivity, can be critical in shaping local demography, sustaining species, and determining evolutionary pathways (Botsford et al. 2001, Armsworth 2002, Hanski & Gaggiotti 2004, Hastings & Botsford 2006). Thus, defining the scales over which connectivity occurs is one of the central goals of marine ecology today (Mora & Sale 2002, Cowen & Sponaugle 2009).

In recent years, views of marine population connectivity have shifted: while reef fish were once thought to be relatively panmictic, with recruitment decoupled from local production (Roughgarden et al. 1985, Roberts 1997), research now suggests that larval behavior and orientation (Lecchini et al. 2005, Simpson et al. 2005, Gerlach et al. 2007), and the utilization of physical retention mechanisms by larvae and spawning adults (Paris & Cowen 2004, Sponaugle et al. 2005, Karnauskas et al. 2011) all contribute to decreased dispersal distances. Local retention may therefore be more important than long distance dispersal in maintaining populations (Cowen et al. 2000, Swearer et al. 2002, Jones et al. 2005, Cowen et al. 2006, Almany et al. 2007). Still, patterns of connectivity are likely to vary widely with taxon, time, and space (Bradbury et al. 2008, Hogan et al. 2012) and, owing largely to the difficulty of tracking tiny, diffuse larvae in the open ocean (Thorrold

et al. 2002), the relative contributions of local and foreign larvae to overall recruitment are poorly resolved for most populations.

Biophysical modeling studies (Cowen et al. 2006, Paris et al. 2007, Werner et al. 2007) and genetic parentage analyses (Planes et al. 2009, Hedgecock 2010, Saenz-Agudelo et al. 2011) have advanced the field significantly, shedding light on transport processes and mapping out patterns of retention. However, the degree of connectivity that occurs among populations depends not only on whether larvae can physically travel (either passively or actively) from one reef to another, but also on whether they arrive at the settlement site in adequate nutritional condition to survive to reproduction, and thus effectively contribute to the population (Pineda et al. 2007, Hamilton et al. 2008, Cowen & Sponaugle 2009). During the pelagic phase, condition may be compromised if larvae encounter suboptimal feeding environments. Reductions in growth due to limited prey availability can result in decreased size-at-age and prolonged PLD (Houde 1989b, Leggett & Deblois 1994, Hare & Cowen 1997, Skajaa et al. 2004, Bochdansky et al. 2008). These effects not only undermine survivorship during the larval phase (Blaxter 1986, Bailey & Houde 1989, Takasuka et al. 2003), they also influence an individual's probability of survival in later life stages (Sogard 1997, Pechenik et al. 1998, Searcy & Sponaugle 2001, Gagliano et al. 2007). Early juvenile life has long been recognized as a severe population bottleneck for coral reef fishes whereby new recruits are subject to high levels of predation and starvation (Sale & Ferrell 1988, Doherty et al. 2004). This mortality pressure has been shown to selectively affect fish of lower larval condition. For example, individuals that grow more slowly as larvae or settle at smaller sizes often have

lower juvenile survivorship (Searcy & Sponaugle 2001, Bergenius et al. 2002, Vigliola & Meekan 2002, McCormick & Hoey 2004, Rankin & Sponaugle 2011).

Historically, a wide variety of indices have been used to measure larval fish condition (Ferron & Leggett 1994), but otolith-derived measurements are the most ubiquitous (Sponaugle 2010). Otolith size is positively correlated with somatic size and, in the vast majority of larval fishes, otolith increments are laid down on a daily basis, providing recoverable records of growth rates, and thus condition, throughout larval life (Pannella 1971, Campana 1990, Hare & Cowen 1995, Searcy & Sponaugle 2001).

While otolith-based analyses are singular in their utility, the time and effort they require can be limiting. In temperate larval fish research, the RNA/DNA ratio (R/D) has been used increasingly as an efficient and accurate measure of condition that can accommodate greater sample sizes with less effort (Clemmesen & Doan 1996, Buckley et al. 1999, Chícharo & Chícharo 2008). Moreover, some evidence has shown that the R/D index may reflect the influence of environment on physiology more rapidly than other measures, manifesting the effects of environmental changes within 24 hours (Buckley 1979, 1984, Wright & Martin 1985, Raae et al. 1988, Richard et al. 1991, Clemmesen & Doan 1996, Rooker & Holt 1996, Catalán et al. 2007). The premise of this assay is that, while the amount of DNA per diploid cell is constant, the amount of RNA (which consists primarily of ribosomal RNA) fluctuates with ribosomally mediated growth. To date, R/Ds have been used only once for field-based condition studies of low latitude larval fish (Garcia et al. 2006), and they have never been applied to studies of tropical reef fish larvae. We therefore measured both otolith-based traits and R/D to gain a multifaceted measure of larval reef fish condition, and also to assess the relative

performance of the R/D index in detecting growth differences of larval coral reef fish *in situ*.

To investigate the effects of different dispersal trajectories on the aforementioned measures of condition (otolith growth and R/D), larvae were sampled from nearshore and offshore water masses along and upstream of the Florida Keys reef tract (FKRT, Fig. 3.1). The dominant physical feature in this region is the Florida Current (FC), a western boundary current with speeds up to  $2 \text{ m s}^{-1}$ . The FC is fed by the Loop Current (LC), which is, in turn, fed by Caribbean waters that travel up through the Yucatán Channel and into the Gulf of Mexico. The LC can protrude to varying degrees into the Gulf of Mexico but, when pinched off, it flows eastward from the mouth of the Yucatán Channel directly into the Straits of Florida (SOF).

As the LC approaches the Florida shelf, the interaction of frontal meanders with bathymetry results in the shedding of mesoscale and submesoscale eddies, which are propagated with the dominant flow northeastward between the fringing reef and the FC boundary (Lee et al. 1995, Fratantoni et al. 1998, Lee and Williams 1999). The frequent presence of these eddies represents a likely retention mechanism by which locally-spawned larvae may remain close to their natal reef (Limouzy-Paris et al. 1997, Sponaugle et al. 2005) and benefit from the enhanced productivity that the eddies provide (Hitchcock et al. 2005). Yet larvae in this region may also be sourced from distant upstream populations. Flow of the LC from the Yucatán could result in delivery of larvae from the reefs of Caribbean Mexico and southward to the FKRT, and biophysical modeling efforts have shown the possibility of such connectivity occurring (Cowen et al. 2006). The deep mesophotic reefs that dot the 100-m isobath of the West Florida shelf

constitute another component of the regional metapopulation (Slattery et al. 2011); these understudied reefs are often bathed in the flow of the extended LC, and their larvae could be delivered downstream. Additionally, coral reefs surrounding the island of Cuba, due south of the FKRT, also represent a potential source of larval subsidy to the FKRT, although the strong, fast flow of the LC through the SOF presents a significant barrier to dispersal, and cross-current larval transport is thus relatively unlikely.

In the dynamic physical environment of the LC/FC region, both local retention and long distance dispersal could be contributing to the maintenance of reef fish populations. Depending on their dispersal pathways, larvae may encounter markedly different conditions. Productivity in the coastal waters adjacent to the FKRT is generally thought to be elevated (Leichter et al. 1998, Olson 2001, Leichter et al. 2003), which could translate into increased prey availability (but also potentially higher predator abundance) closer to shore (Denman & Powell 1984). Consequently, the FKRT affords the opportunity to collect and compare larvae with different dispersal trajectories, different natal origins, and, potentially, different levels of condition.

Given that larval condition—not just larval survival—can have significant implications for population connectivity, the primary objective of this study was to investigate whether larvae with contrasting dispersal histories (i.e. local retention vs. long distance transport) differ in their nutritional condition. Additionally we sought to resolve the mechanisms behind any such differences. This latter component of the investigation is of particular importance because there are other factors, in addition to food availability, that might affect mean condition levels, and these factors could vary depending on dispersal pathway. Most notably, high levels of selective mortality (e.g. predation) and

the disproportionate removal of low condition individuals from a group of larvae could inflate mean condition values independent of prey availability. If we are to assess whether and how different dispersal trajectories affect overall patterns of connectivity, it is crucial for us to understand how the larval experience, in terms of both feeding *and* predation, might differ between locally retained and long distance dispersing larvae.

## **METHODS**

### ***Field sampling***

Samples were collected over three cruises during the summers of 2007 and 2008 (Cruise 1: 29 May-14 June, 2007; Cruise 2: 30 July-13 August, 2007; Cruise 3: 17 June-2 July, 2008). All three surveys employed the same general sampling scheme, and included four paired, cross-shelf transects along the FKRT and one paired transect upstream of the Keys (Fig. 3.1). Each cross-shelf transect consisted of nearshore stations, which spanned from inside the barrier reef out to the mixed frontal region at the edge of the FC, as well as offshore stations in the oceanic waters of the FC core. All stations in the upstream transects were located far offshore, intersecting the LC before it entered the Florida Straits. Because of the variability in the location of the LC/FC with time, the exact geographical coordinates of the offshore and upstream stations shifted from cruise to cruise. The positions of these stations were determined while underway based on prevailing flow patterns, as inferred from near real-time satellite-derived ocean color imagery (IMARS, University of South Florida), sea surface altimetry data (CCAR, University of Colorado), and shipboard acoustic Doppler current profiler (ADCP) data. The five paired transects described above constituted the basic framework of each survey,

and additional sampling was conducted as time allowed. Specifically, supplemental collections on cruises 1 and 2 (47 stations total) were made in a zig-zag pattern, alternately covering nearshore and offshore locations along the length of the FKRT (Fig. 3.1).

At most stations, ichthyoplankton were collected using a paired multiple opening closing net and environmental sensing system (MOCNESS), which consisted of a 4 m<sup>2</sup> MOCNESS and a 1 m<sup>2</sup> MOCNESS joined together, fitted with 1 mm and 150 µm mesh nets, respectively (Guigand et al. 2005). This paired design allowed for simultaneous collection of fish larvae and their smaller zooplankton prey. A built-in flowmeter provided a record of volume filtered. Nets were towed obliquely at ca. 1.5 ms<sup>-1</sup>, sampling discrete 20 m depth bins from 80 m to the surface (except when bottom depth <80 m required omission of the deepest bins). Sampling time for each depth bin ranged from 5-8 minutes. At a subset of nearshore stations that were too shallow for MOCNESS deployment, collections were made using a double frame net (a 2 m<sup>2</sup> opening with 1 mm mesh net adjoined to a 0.5 m<sup>2</sup> opening with 150 µm mesh net). The frame net was towed horizontally 3 m below the surface at a speed of 1.5 ms<sup>-1</sup>. Tow durations were ca. 10 minutes, and volume-filtered data were obtained using an attached flowmeter.

Because one of the objectives of this study was to measure R/D of fish larvae, and because RNA degrades rapidly, only larvae from the shallowest nets (i.e. larvae that had been collected within 15-20 min.) were preserved for condition analysis. This included samples from the 0-20 and 20-40 m MOCNESS nets, as well as the frame net. When these nets were retrieved, contents from the cod-ends were rinsed with ice-cold filtered seawater and immediately sorted in frozen glass trays. Reef fish larvae (provisionally



identified to lowest possible taxonomic level) were flash frozen in liquid nitrogen for later analysis, and the remaining contents of each sorted sample were fixed in 95% EtOH. All plankton samples not sorted onboard the ship (i.e. the deeper depth bins) were transferred directly to 95% EtOH.

In order to characterize the larval habitat, environmental data were recorded throughout the study region. Sensors on the MOCNESS measured temperature, salinity, oxygen and fluorescence during each tow, and CTD casts conducted at most stations (as bottom depth permitted) yielded complete water column profiles.

### ***Laboratory processing***

Of the many taxa of reef-associated larvae collected, four were relatively abundant throughout the study region and identifiable to the species level, and were thus suitable for condition analysis. These four species included two wrasses (*Thalassoma bifasciatum* and *Xyrichtys novacula*), one parrotfish (*Cryptotomus roseus*), and the great barracuda (*Sphyraena barracuda*). *T. bifasciatum*, *C. roseus*, and *S. barracuda* could all be identified to the species level morphologically. *X. novacula* could be identified to the genus level morphologically, but molecular analysis was required to confirm species identity (see below). Identification of larvae in the field had been cursory, so a more careful inspection of each individual was required before condition analyses could be conducted. Morphological identification necessitated a relatively long processing time at room temperature, posing a threat to RNA integrity. To prevent sample degradation, larvae were thawed in RNAlater-ice (Invitrogen), a proprietary solution that preserves cellular RNA in frozen tissue samples by deactivating RNases. Once stabilized, thawed

larvae were identified to the lowest possible taxonomic level. Standard length (SL) was measured to the nearest 0.01 mm using a Leica MZ12 stereomicroscope equipped with a stage micrometer, and the larva's head was excised with a microscalpel and preserved in 95% EtOH until otoliths could be extracted later. The viscera were also removed in order to prevent potential bias of R/D measurements by gut contents. The remaining trunk was immediately homogenized in 150  $\mu$ L ice-cold 1M NaCl and the homogenate was stored at  $-80^{\circ}\text{C}$  for subsequent nucleic acid quantification.

### ***RNA and DNA quantification***

Concentrations of RNA and DNA in each sample homogenate were measured using a fluorometric microplate assay following the methods of Westerman and Holt (1988), with the exception that the fluorophore SYBR Green II (SGII, Molecular Probes) was used in place of Ethidium Bromide. Briefly, larval homogenates were treated with SGII, and fluorescence levels in each sample were read using a Tecan GENios plate reader (ex: 485 nm, em: 535 nm). Initial fluorescence measurements corresponded to total quantities of nucleic acids (RNA and DNA combined). To measure the fluorescence attributable to DNA alone, RNA was selectively digested with RNase (Sigma, R-4875) and fluorescence was read again. The relative fluorescence of RNA could then be determined by calculating the differences between the first and second measurements. Standard curves for RNA and DNA were generated from fluorescence measurements of serial dilutions of known quantities of type III RNA from baker's yeast (Sigma, R-6750) and type I DNA from calf thymus (Sigma, D-1501) that had been treated with SGII. The resulting standard curves were used to convert relative fluorescence to actual

concentrations of RNA and DNA, and allowed for calculation of the ratio of RNA to DNA in each fish larva. This assay was effective at determining nucleic acid concentrations in the vast majority of larvae, but for some of the smallest individuals, autofluorescence of the fluorophore overwhelmed the signal of RNA, precluding accurate measurement. Any such individuals were excluded from analysis. Additionally, fish that appeared to have been substantially damaged or degraded during the collection process were also excluded, as the RNA concentration in such specimens might have been affected.

### ***Otolith microstructure analysis***

Otoliths were examined to investigate daily growth and size-at-age of larvae collected from nearshore and offshore water masses. For this analysis, a subset of larvae of each species was randomly selected from the samples in which the larvae occurred, with the fraction of individuals taken from each sample roughly proportional to the total number of individuals in that sample. In two of the species analyzed in this study (*T. bifasciatum* and *S. barracuda*), otolith increments are reported to be deposited on a daily basis (Victor 1982, D'Alessandro et al. 2011). For the remaining two taxa (*C. roseus* and *X. novacula*) daily increment formation can be assumed based on studies of closely related species (Victor 1982, Hare & Cowen 1991, Lou & Moltschaniwskyj 1992). Enumeration of daily increments and calculations of increment widths were carried out using the sagittal otoliths of *T. bifasciatum*, *X. novacula*, and *C. roseus*, and the lapillar otoliths of *S. barracuda*. Sagittae and lapilli were dissected out according to standard practice and cleaned of debris (Sponaugle 2009). Sagittae were placed on a glass slide in

a drop of high viscosity immersion oil, and allowed to clear for 7-10 days to facilitate reading (Sponaugle & Cowen 1997), but the lapilli of *S. barracuda* were less clear and had to be sectioned. Sectioning was accomplished by mounting each lapillus on a glass slide with crystal-bond thermoplastic glue and polishing down to the otolith core with fine grain (P2000) silicon-carbide abrasive paper (Nihonkenshi Co.).

After clearing or sectioning was complete, one otolith from each larva was randomly selected and examined at 400× (or in the case of *S. barracuda* lapilli, 1000×) magnification using a Leica DMLB oil-immersion microscope outfitted with a polarized filter (which helped to enhance visibility of otolith features). Microscope images were fed to a computer via a Dage MTI video camera. Focus and contrast were optimized for otolith readability, and images were captured with a frame grabber. Using image analysis software, increments along the longest axis of the otolith from the core to the edge were digitally marked and counted, and resulting increment widths and increment radii (distances from the otolith core to each individual increment) were calculated. Otoliths were read blind with respect to collection time, collection location, and SL. Initially each otolith was read twice. If the increment counts from the first and second reads were within 5% of each other, one of those two reads was randomly chosen as the final read. When the difference between the first and second reads was >5%, a third read was performed. If the third read was within 5% of either of the first two reads, the final read was randomly selected from one of the two closest reads; otherwise the otolith was excluded from the analysis (Sponaugle 2009). Most reads were carried out using the image analysis program Image Pro Plus 7.0 (Media Cybernetics), but due to limitations on software access, second and third reads for some otoliths were performed with the

open source image analysis program, ImageJ (<http://rsb.info.nih.gov/ij>, Abramoff et al. 2004). The pixel settings in ImageJ were calibrated according to those of Image Pro Plus (i.e. a given number of pixels corresponded the same length in each program), and a comparison of otolith reads performed using the two different programs verified that the type of software used for image analysis did not affect results.

When otolith measurements were complete and final reads had been obtained, least-squares regression showed significant positive relationships between otolith radius and SL for all four taxa. Moreover, the residuals of SL-at-age and otolith radius-at-age regressions were also positively correlated (Table 3.1). These findings confirmed that, in the species analyzed here, otolith growth is a suitable proxy for somatic growth, and could be used as index of larval condition (Hare & Cowen 1995).

### ***Molecular identification of larvae***

Three different species of the genus *Xyrichtys* inhabit the study area (Richards 2006) and although members of this genus are noticeably distinct from other genera, congeners are morphologically indistinguishable. In order to identify *Xyrichtys* larvae to the species level, a 655 bp fragment of the Cytochrome Oxidase I gene (COI) was sequenced in a subsample of individuals randomly selected from across all cruises and stations (n = 517). Source material for genetic analysis consisted of eyeballs harvested from each larva. Isolation and purification of DNA, PCR amplification, PCR product purification, and sequencing were all carried out according to Richardson et al. (2007) and D'Alessandro et al. (2010). DNA was isolated using a Genfind kit (Agencourt), and then purified using a Sephadex G-50-based protocol. Amplification of the target region

was carried out with two *Xyrichtys*-specific primers (Xyr-F1: ATAGTGGGCACAGCCCTAAGC, Xyr-R1: TGGTAAAGAATTGGGTACCTCC), which were designed from multiple sequence alignments of *Xyrichtys* COI sequences publically available on GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). Additionally, an internal (nested) primer was designed for use during sequencing (Xyr-F2: CCTTCTTGAGACGACCAAA). Of the 517 larvae identified, 503 (97.4%) were *Xyrichtys novacula*. The other two species of this genus, *X. martinicensis* and *X. splendens*, constituted 8 (1.4%) and 6 (1.2%) of larvae, respectively. The 14 non-*novacula* individuals were spread among 13 different stations representing both nearshore and offshore locations, and a mixture of all cruises. This finding allayed concerns regarding the potential for patchy distributions of the less common species. Given the overwhelming dominance of *X. novacula* across cruises and regions, sequencing of remaining individuals was deemed unnecessary and, for the purposes of analysis, all *Xyrichtys* larvae were classified as *X. novacula*.

### ***Prey availability analysis***

To investigate the relationship between prey availability and larval condition, zooplankton species composition and abundance were examined in 200 EtOH-preserved plankton samples from the 0-20 m and 20-40 m 1 m<sup>2</sup> MOCNESS nets. Seven different types of zooplankters make up the majority of mesozooplankton in the study area, and are known to be the dominant components of the diets of coral reef fish larvae diet in the Florida Current region (Llopiz & Cowen 2009). These seven prey types, which include appendicularians, calanoid copepods, harpacticoid copepods, and four different genera of

cyclopoid copepods (*Oncaea*, *Oithona*, *Corycaeus*, and *Farranula*), were targeted for analysis. Enumeration of individuals in each net was accomplished using a subsampling method following Llopiz and Cowen (2008). Individual counts per sample were standardized by volume filtered and converted to biomass using taxon-specific averages of individual dry weights from the literature (Chisholm & Roff 1990, Webber & Roff 1995, Hopcroft et al. 1998). Total biomass of all seven prey types was calculated for each net, however, both *T. bifasciatum* and *X. novacula* are known to consume three taxa of copepods (harpacticoids, *Oncaea*, and *Farranula*) almost exclusively (Llopiz & Cowen 2009). Thus, to best describe the prey environment relevant to *T. bifasciatum* and *X. novacula* in particular, separate biomass calculations of harpacticoids, *Oncaea*, and *Farranula* were also computed.

### ***Statistical analysis***

To examine the relationship between larval nutritional condition and dispersal history, R/D ratios and recent growth (mean width of the last three complete otolith increments) were compared among larvae collected from two different water masses: nearshore and offshore. Both R/D ratios and growth rates varied with ontogeny, so in order to compare condition in larvae of different ages, it was necessary to remove any ontogenetic effects. This was accomplished by regressing each of the two condition measures against larval age (as obtained from otolith analyses). Residuals obtained from the R/D-age and recent growth-age regressions served as age-independent measures of R/D and recent growth, respectively.

Potential differences in both mean R/D and mean recent growth between water masses (nearshore and offshore) were analyzed using a restricted maximum likelihood (REML) nested mixed-effects model (SYSTAT 13.0, Wilkinson 1992). This statistically conservative technique is particularly suitable for multiple-stage oceanographic field sampling; it allows for grouping of samples across cruises and corrects for the lack of independence of data points that are obtained from the same net, or nested within the same station or cruise (Picquelle & Mier 2011). Also included in the model were the covariates temperature and salinity, set as random effects to account for the potential influence of environment on the relationship between larval condition and water mass.

In addition to the comprehensive condition comparisons described above, age-specific analyses of growth were conducted. Otolith increment radii and increment widths at distinct points in larval life were compared across water masses. For both of these traits, separate REML analyses were performed for every fifth day of larval life, beginning at increment 15.

Comparing the mean condition of nearshore and offshore larvae is useful, but it does not fully reveal how the distribution of individual condition levels might change with age, or, importantly, how the shape of that distribution over the full larval age range might vary between nearshore and offshore environments. To address these latter two components of the data, and to shed light on the causal mechanisms that may be operating, a local density estimator (LDE) approach following Pepin et al. (1999) was applied. This method uses kernel smoothing to estimate cumulative probability distribution functions (CDFs) of all observed individual condition values (R/D or recent growth, in this case) based on a covariate (i.e. larval age) and neighboring values. CDFs



of both R/D with age and recent growth with age were generated for larvae collected from different water masses. The differences between nearshore and offshore CDFs were evaluated by plotting the 10<sup>th</sup>, 50<sup>th</sup> and 90<sup>th</sup> percentiles of each distribution. The location of the 10<sup>th</sup> percentile is particularly informative because it traces the condition level of the lowest condition larvae in the distribution. If low condition individuals are selectively lost from the population, the 10<sup>th</sup> percentile will increase, shifting upward with increasing age (Fig. 3.2a). It is possible that a similar pattern of an increasing 10<sup>th</sup> percentile could result if, given a diversity of condition levels at an initial age (i.e. hatching), all larvae regardless of condition, converge on a high condition level over time (Fig. 3.2b), yet, such a scenario is improbable, ecologically speaking (Pepin 2004). Thus we assume here that an increase in the 10<sup>th</sup> percentiles represents selective mortality at work, and by comparing 10<sup>th</sup> percentiles it is possible to assess whether the degree of selective mortality differs with contrasting dispersal trajectories. The 90<sup>th</sup> percentiles of a distribution can also provide important information: because they represent the highest condition fish in a given distribution, a comparison of 90<sup>th</sup> percentiles reveals whether the maximum attainable condition level differs between nearshore- and offshore-collected larvae. If prey is limiting in one environment compared to the other, we would expect to see a significantly lower 90<sup>th</sup> percentile, as the larvae in a prey-poor habitat would presumably be unable to attain the same upper threshold of condition levels that would be allowed by a prey-rich environment. To statistically determine whether a particular quantile (either the 10<sup>th</sup> or the 90<sup>th</sup>) differed significantly among nearshore and offshore CDFs, 95% confidence intervals (CIs) about that quantile were estimated from 1,000 randomizations of the data (R boot package, Canty and Ripley 2012). Two quantiles (e.g.

the 10<sup>th</sup> percentile of the nearshore CDF and the 10<sup>th</sup> percentile of the offshore CDF) were said to be significantly different from each other if their CIs did not overlap.

To put any condition related findings into context, comparisons were made between the relative availability of larval fish prey at nearshore and offshore stations. Once again, an REML model was used, this time to test for significant differences in the densities of the dominant prey types across water masses, while accounting for any potential bias of cruise, station, or net. Finally, least squares regressions of individual larval condition measures (R/D and recent growth) with ambient prey availabilities were carried out to determine whether any significant correlation might exist.

## RESULTS

### *Mean condition in nearshore vs. offshore larvae*

With data from all cruises combined together, mean recent growth was observed to be significantly faster nearshore than offshore for three of the four study species (*T. bifasciatum*, *C. roseus*, and *X. novacula*; Fig. 3.3, Table 3.2). Mean R/D was significantly higher nearshore for one species, *T. bifasciatum*, but among-water mass R/D differences were not significant for other taxa (*C. roseus*, *X. novacula* or *S. barracuda*, Fig. 3.4, Table 3.2). Depending on whether sufficient sample sizes were available, condition measures in each species were also examined separately for each cruise. Nearshore recent growth was significantly faster than offshore recent growth in four of nine cruise-species combinations, and similar but non-significant trends were evident in two others. R/Ds were higher in the nearshore than in the offshore for five cruise-species combinations, and in three cases those differences were significant. On no occasion was recent growth

or R/D ever found to be significantly greater in offshore larvae than nearshore larvae (Figs. 3.3 and 3.4, Table 3.2).

### ***Daily growth and size-at-age***

Growth throughout larval life was investigated in greater detail by plotting the mean growth trajectories of both nearshore and offshore larvae with age. Pairwise, among-water mass comparisons of specific increment widths and increment radii (proxies for daily growth and sizes-at-age, respectively) showed that, for *T. bifasciatum* and *C. roseus*, daily growth was consistently higher at nearshore stations, and this higher growth translated into larger sizes-at-age (Figs. 3.5 and 3.6, Table 3.3). The same was true for *X. novacula* larvae from cruise 1, but in cruise 2, the relationship between growth and water mass broke down (Figs. 3.5 and 3.6, Table 3.3). For *S. barracuda* no differences between larvae collected nearshore and those collected offshore were evident.

### ***Cumulative probability distribution functions***

When CDFs of individual condition levels were constructed for *T. bifasciatum* (Figs. 3.7a-d), the nearshore plots of recent growth and R/D both featured gradual increases in the 10<sup>th</sup> percentiles with age. Such increases were not observed in the CDFs of offshore conspecifics. In fact, in offshore *T. bifasciatum*, the 10<sup>th</sup> percentiles actually decreased slightly with age in both the recent growth and R/D CDFs. When the 10<sup>th</sup> percentiles of the nearshore and offshore CDFs were plotted together, separation of the 95% CIs (from ca. 27 dph onward in the recent growth CDF and ca. 24 dph onward in the R/D CDF) indicated that the minimal condition level observed at older age classes was

significantly greater nearshore than offshore (Figs. 3.8a and 3.8b). Similar patterns were evident in the recent growth CDFs of both *C. roseus* (Figs. 3.7e-f) and *X. novacula* (Figs. 3.7i-j), with significant differences between the 10<sup>th</sup> percentiles of nearshore and offshore distributions at older ages (Figs. 3.8c and e). However, for R/D, the 10th percentiles of the nearshore and offshore groups of these two species were not significantly different (Figs. 3.8d and f). Of the four reef fish investigated, *S. barracuda* was the only species for which, irrespective of condition measure (recent growth or R/D), no nearshore-offshore differences in the 10<sup>th</sup> percentile were observed (Figs. 3.8g and h).

With regard to the 90<sup>th</sup> percentiles of the CDFs, visual inspection did not reveal any clear differences between nearshore and offshore distributions, and when nearshore and offshore 90<sup>th</sup> percentiles were plotted together, the 95% CIs consistently overlapped (Fig. 3.9), indicating that the upper boundaries of the condition distributions were not significantly different between water masses for either condition index in any species.

### ***Prey availability***

For all seven larval fish prey types combined, prey densities at nearshore and offshore stations did not differ (REML:  $p = 0.303$ ). When the three specific prey types favored by *T. bifasciatum* and *X. novacula* were analyzed as a sub-group, the density of these organisms was found to be higher offshore than nearshore (REML:  $p = 0.004$ , Fig. 3.10). This trend was consistent when each of these three prey types was considered independently (Fig. 3.10), and was statistically significant (REML) for both *Farranula* ( $p = 0.019$ ) and *Oncaea* ( $p = 0.020$ ).

When the direct relationship between prey availability and condition was investigated, no correlation was observed; least squares regressions were calculated for all three species that had exhibited significant nearshore-offshore differences in condition (Fig. 3.11), but neither of the two condition indices measured (recent growth or R/D) was related to prey availability in any of the species tested.

## **DISCUSSION**

The results from this study show that, in some coral reef fish larvae, mean condition levels are strongly influenced by larval dispersal trajectory. Larvae collected from nearshore stations exhibited higher condition than those collected offshore, and this pattern held for three different species of reef fish using two different measures of growth-related condition. Previous studies that have shown dispersal-related differences in condition generally attribute their findings to variations in prey availability associated with dispersal pathways (e.g. Hamilton et al. 2008, Shima & Swearer 2009). The potential for differential predation-driven selective mortality to affect mean condition levels in larvae with contrasting dispersal histories has been discussed in the literature (Sponaugle et al. 2009, Shima & Swearer 2010), but not tested. Our data empirically show that among-water mass differences in selective mortality, but not overall prey abundance, underlie the observed significant differences in mean larval condition between larvae with contrasting dispersal histories, thus highlighting the important role that predation plays in shaping larval condition distributions.

### ***Prey availability and dispersal pathways***

Given the general tendency for coastal waters to be enriched relative to the open ocean (Denman & Powell 1984), it is conceivable that productivity and, thus, larval food supply would be higher in the nearshore, and that spatial variation in prey availability might be responsible for the observed nearshore-offshore differences in condition.

However, contrary to expectations, the overall biomass of available larval reef fish prey was actually lower nearshore. Moreover, there was no correlation between prey biomass and larval condition on an individual level. Thus, our results cannot be explained by simple differences in total food abundance.

The relationship between prey availability and larval condition was further explored by examining the probability distributions of individual condition values with larval age. If larvae in the nearshore were experiencing feeding-related augmentation of growth, we would expect to see greater maximal condition levels among those larvae—the fittest fish in the nearshore would be of higher condition than the fittest fish offshore. Yet, we did not see such a pattern. According to our data, the maximal condition levels (i.e. 90<sup>th</sup> percentiles) in the CDFs of nearshore larvae were statistically indistinguishable from those of offshore larvae in all species. This similarity of the 90<sup>th</sup> percentiles among water masses provides support for the prey biomass-based finding that food levels in the environment are not likely to be driving the nearshore-offshore mean condition differences observed here.

By comparison, other research *has* shown linkages between prey availability and larval condition. In a previous investigation of *T. bifasciatum* across the northern SOF (adjacent to the current study site), Sponaugle et al. (2009) reported significant

correlations between cross-straits variation in prey abundance and larval gut fullness, and between gut fullness and growth. The area surveyed by Sponaugle et al. did not include nearshore stations (as defined herein), but it comprised a number of stations that extended well past the FC into the oligotrophic waters in the far eastern SOF. Sponaugle and colleagues may have witnessed a greater range of prey availability, spanning both sides of a theoretical prey abundance threshold, above which increasing prey availability does not significantly affect growth, but below which larval condition is increasingly compromised. Examples of such thresholds have been reported for other larval fish (e.g. Betsill & Van de Avyle 1997), and it is possible that, in the study region sampled here, prey availability may have been above such a threshold (even at offshore stations), precluding the detection of prey-related effects on condition.

Another potential explanation for the decoupling of prey availability and larval condition in the present study exists. Fine-scale heterogeneity of zooplankton distributions, i.e. patchiness, has been well documented (Cowles et al. 1998, Lough & Broughton 2007, Young et al. 2009), yet most oceanographic plankton sampling often occurs on much larger spatial scales than those relevant to individual fish larvae (Pepin 2004). In an environment where prey are highly aggregated in small patches, a larva with limited motility may have a relatively low probability of encountering food, but any such mismatch between larva and prey would be indiscernible in net samples that average prey concentrations over 10s to 100s of cubic meters. As such, we acknowledge that the absence of a prey-condition relationship observed here could be a function of our inability to compare larval fish with their actual prey ambit.

### *Selective mortality in the nearshore environment*

While we did not find prey-related differences in condition between nearshore and offshore habitats, we did see clear and significant differences in selective mortality. An upward shift in the 10<sup>th</sup> percentiles of condition with age in nearshore relative to offshore CDFs was observed, providing evidence of disproportionate, selective loss of slow growing or low R/D fish from the nearshore distribution. Removal of the poorest condition larvae inflated the mean condition level at nearshore stations, resulting in higher mean condition nearshore than offshore despite the fact that the maximum condition levels (90<sup>th</sup> percentiles) did not vary between the two groups. Between-water mass differences in mean condition can therefore be ascribed to dissimilar levels of selective mortality (likely stemming from asymmetrical predation) associated with different dispersal trajectories.

In evaluating the CDFs of larval condition with age, it is interesting to note that, at hatching, nearshore and offshore groups start out with similar condition distributions. This is not unexpected when considering that at very young larval ages, if there is variability in condition (i.e. as conferred by parental investment), even the highest condition individuals are extremely vulnerable to predation. In the earliest stages of larval life, there is little variation in swimming capability (Leis et al. 2007b) and predator avoidance behavior (Miklosi et al. 1997, Killen et al. 2007, Ohata et al. 2011), thus predation on this age class may not be highly selective (Killen et al. 2007). As larvae mature, however, a range of condition levels in a population may manifest themselves as differing degrees of adeptness at detecting, avoiding, and escaping from predators (Skajaa et al. 2004). With increasing age, there is the introduction of trait variation on



which selective mortality (likely in the form of predation) can act, and in nearshore larvae we see the increase in the CDF 10<sup>th</sup> percentile with age representative of greater selective mortality among older larvae.

### ***Species specificity of the dispersal history-condition relationship***

If food were the driving force behind the condition differences observed here, one might expect *S. barracuda* larvae to show nearshore-offshore condition patterns similar to those of other larvae, as this species should also be vulnerable to sub-optimal prey availability. However, unlike *T. bifasciatum*, *C. roseus*, and *X. novacula*, *S. barracuda* larvae did not exhibit higher condition in nearshore water masses. The data even suggest that, although the trends were not significant, mean *S. barracuda* condition may have been slightly higher offshore. Nearshore mean condition level does not seem to be regulated by selective mortality in *S. barracuda*, as it appears to be in other species. This is not entirely surprising considering that *S. barracuda* are notably distinct from the other three taxa examined here. They have comparatively short PLDs and are precocial; they grow and develop quickly and are likely to be strong swimmers from early in ontogeny. Additionally, they represent one of only a few taxa that are piscivorous (i.e. consume other larval fishes) as larvae, and they exhibit distinctly different vertical distributions, primarily occupying the shallowest portions of the water column, which most larval reef fish tend to avoid (Fig. 3.12; D'Alessandro et al. 2011). These traits could result in less exposure and/or susceptibility of *S. barracuda* larvae to predators, and an associated reduction in the level of selective mortality observed.

### ***Effects of magnitude and type of predation***

The difference in the degree of selective mortality operating in nearshore and offshore distributions of *T. bifasciatum*, *C. roseus*, and *X. novacula* larvae begs the question of how predator fields may differ between nearshore and offshore water masses. Can we relate our observations to the abundance of predators? The suite of organisms in this speciose sub-tropical study region that could potentially prey upon larval reef fishes encompasses a wide variety of taxa, sizes, and life history stages. The effort needed to characterize the entire larval predator field to which fish larvae may be exposed was well beyond the scope of this study, and the task has eluded most previous research efforts, with the exception of some studies related to sampling pelagic juvenile fishes (e.g. Tanabe 2001). Furthermore, even if one group of predators may appear to play a significant role in larval fish predation mortality, without sampling the entire suite of predators, we do not know the relative proportion of larval mortality that can be ascribed to specific predator types, and no such reports are available in the literature.

We do know, however, that one component of the predator field consists of piscivorous larval fishes, including larval tunas, billfishes, and barracudas (Llopiz & Cowen 2008, Llopiz et al. 2010, D'Alessandro et al. 2011). Because larval piscivores were collected concurrently with the larval reef fish that we were targeting, we have information on their abundances (Shulzitski et al. in prep). When the delta densities (frequency of occurrence  $\times$  concentration when present, Serafy et al. 2007) of piscivorous larval fish were compared, we saw no significant differences between nearshore and offshore stations (REML of delta density:  $p > 0.05$ ). In fact, piscivorous taxa were slightly more abundant offshore. Yet piscivorous larval fishes may make up a small

component of the entire predator field and, unfortunately, we have no direct measurements of any other predator types.

Predator abundance is certainly important in shaping of selective mortality, but, research has shown that predator type (e.g. filter feeders vs. raptorial, etc.) can also affect the level and type of pressure exerted on a prey population (Bailey & Houde 1989). Interestingly, some predator species have been shown to be less selective than others when feeding on larval fish (Takasuka et al. 2004, Robert et al. 2010), thus different taxa of predators could result in variability in the observed patterns of selection. Similarly, the behavior of predators can also influence the degree to which mortality is or is not selective. Aggregation by predators, for example, may result in less selective mortality because even the highest condition fish larvae are likely to be eaten when they encounter rare but large and dense groups of predators (Torgersen 2007). Given the lack of empirical data on larval fish predation, we cannot speculate as to whether predator abundance, identity, or behavior (or a mix of all three) underlies the differences in observed selective mortality between nearshore and offshore water masses, but we can be confident that larvae with different dispersal histories likely encountered disparate predator regimes.

### ***Offshore as larval habitat***

As discussed above, prey differences between nearshore and offshore water masses did not appear to be of sufficient magnitude to elicit an observable effect on condition. This implies that the quality of offshore habitat surveyed here was adequate to sustain larval growth, without apparent detriment to condition distributions. In many

research contexts, offshore, open ocean environments are often grouped together, yet we know that, in the open ocean, complex and dynamic biophysical interactions can result in substantial spatial and temporal heterogeneity (Bakun 2006). The physical dynamics of the LC likely generate higher levels of productivity relative to other oceanic habitats, partly due to enrichment and/or convergence that might exist when geostrophic imbalance is created by curvature in the current; upwelling of nutrients and increased productivity resulting from cyclonic curvatures and accumulation of prey at downwelling convergence zones generated from anticyclonic curvatures could both act to enhance the larval fish feeding environment (Bakun 1996, Olson 2002, Bakun 2006). Additionally, cyclonic, cold-core, frontal eddies derived from meanders on the left side of the LC (where larvae from the Yucatán and the West Florida shelf would most likely be entrained) can result in nutrient enrichment in the upper water column, elevated productivity, and subsequent increased larval fish prey availability (Anderson & Robinson 2001, Biggs & Ressler 2001). Still such features are likely to be ephemeral, and more direct research on the LC as larval reef fish habitat could shed further light on the magnitude, as well as the temporal and spatial dynamics (e.g. patchiness) of enhanced prey availability in this region.

### ***Conclusions***

In the present study, we provide empirical observations of the changes in the distributions of condition levels with age. Coupled with the finding that overall prey biomass is not responsible for nearshore-offshore condition differences, these data provide a missing link, offering strong evidence that predation-driven selective mortality

differs depending on a larva's dispersal trajectory, and is higher near shore. Thus, while larvae remaining close to shore may or may not benefit from enhanced food availability, there is likely a tradeoff involved whereby the advantages of local retention come with an associated cost of increased exposure to predation.

This is not the first study to document an effect of nearshore vs. offshore dispersal history on larval condition in a connectivity context. Shima and Swearer (2009, 2010), for example, demonstrated that temperate reef fish (common triplefin) larvae developing in nearshore harbor waters exhibited higher mean condition than those that spent the PLD on the neighboring open coast, but, while the potential role of elevated nearshore predation in generating mean condition differences was acknowledged, the existence of such a mechanism was not established.

Hamilton et al. (2008) similarly found that, in the waters around St. Croix, *T. bifasciatum* that had likely developed offshore during early and intermediate larval life grew, on average, more slowly than their nearshore counterparts. However, the authors ascribed this difference to sparse prey availability farther from shore, and argued that offshore larvae were therefore subject to increased levels of selective mortality. This contrasts with the findings of the present study, in which direct measurement of selective mortality in both nearshore and offshore environments revealed clear selection for high condition larvae in nearshore waters, and no such selectivity offshore. We therefore offer an alternative interpretation of Hamilton et al.'s results; it is possible that, as we observed in and around the Florida Keys, increased levels of selective predation close to shore led to disproportionate removal of slower growing, smaller larvae in nearshore water masses. This would be consistent with Hamilton et al.'s findings that 1) average growth was

relatively low while larvae were residing offshore—contrary to what would be expected in a highly selective environment—and 2) mean growth of offshore dispersing larvae increased around the time they would have been reentering coastal waters (and experiencing increased exposure to size- or growth-selective predators). Thus, intense selective mortality nearshore may have played a role in shaping the observed condition distributions. These two potential selection mechanisms (nearshore selection for high condition larvae due to differential predation, and offshore selection for high condition larvae with better survival abilities when prey are limited) are not mutually exclusive, and could have operated in concert to generate the pre- and post-settlement patterns of survival that Hamilton et al.'s study revealed. Additionally, differences in the biophysical regimes that surround St. Croix and the FKRT could result in among region variability in the nature of selection most important in determining dispersal related patterns of condition.

Cohort tracking studies (e.g. Sponaugle et al. 2006, Hamilton et al. 2008, Rankin and Sponaugle 2011) are often required to detect selective mortality in populations over time (but see Hawn et al. 2005, Sponaugle et al. 2010, 2011). The CDF analysis used here represents a unique non-parametric tool that can provide evidence of the changes in trait distributions with increasing age from individuals with different hatch dates collected over broad temporal ranges (Pepin et al. 1999, Pepin 2004). Importantly, it also accounts for within sample ranges of variability. In the present study, CDFs highlight the potential danger of using mean values to make assumptions about survival probabilities of a group: high variability of condition may result in a lower overall mean, but must not be mistaken for low population-wide probability of survival or settlement success.

Offshore larvae in this study, for example, exhibited lower mean condition as a whole, but a substantial proportion of those individuals exhibited the same high condition levels as nearshore larvae. Thus, it would be erroneous to ascribe low survival probabilities to all individuals in the group based on the mean. This has great relevance as we move toward incorporating the differential effects of dispersal history on condition into our estimations of ultimate population connectivity.

The likely importance of both food availability and predation type in determining condition levels in a population draws attention to the need for information on the feeding environment and predator fields that may be encountered by locally retained and long distance dispersing larvae. The fact that larvae collected from offshore FC and LC locations in this study did not exhibit obvious food related reductions in growth relative to their nearshore counterparts, and the observation that larval prey abundances at offshore stations were equal to or greater than those at nearshore stations, both suggest that long distance dispersers in this study were not subject to disproportionate levels of nutritional stress (though potential prey patchiness offshore must be considered). On the whole, this implies that the LC (or parts of it) may represent a suitable larval habitat that can support relatively far-reaching larval exchange. Thus, all dispersal pathways may not be created equal and long distance transport is not necessarily synonymous with poor larval habitat. When two geographically distant populations are connected by a corridor of favorable habitat (in terms of striking the delicate balance between having enough food to eat but also avoiding predators), then the degree of connectivity between them may be greater than that between more closely situated populations separated by less productive waters.

Dispersal-mediated larval condition has recently been identified as an important factor in determining patterns of reef fish connectivity (e.g. Hamilton et al. 2008, Cowen & Sponaugle 2009). Our work shows that, while dispersal trajectory does indeed impact mean larval condition, it may do so in unexpected ways. Given the relationship between larval dispersal history and selective mortality, future research should address whether differing levels of selection during larval life might be balanced or reversed by selective pressures at and after settlement. Such information, combined with an improved understanding of the prey and predator fields inherent to different dispersal pathways (especially on the fine spatial scales relevant to individual larvae) will help us to better estimate, model, and predict the potential effects of transport-related differences in selective mortality on population connectivity. Predictions like these will be crucial to resource managers as they seek to preserve coral reef communities in the face of mounting anthropogenic threats.



**Table 3.1:** Least-squares regressions of otolith radius with SL, and Pearson's correlation coefficients of residuals from the otolith radius-at-age and SL-at-age relationships. Results confirm the appropriateness of using otolith size as a proxy for somatic size in each of the 4 study species.

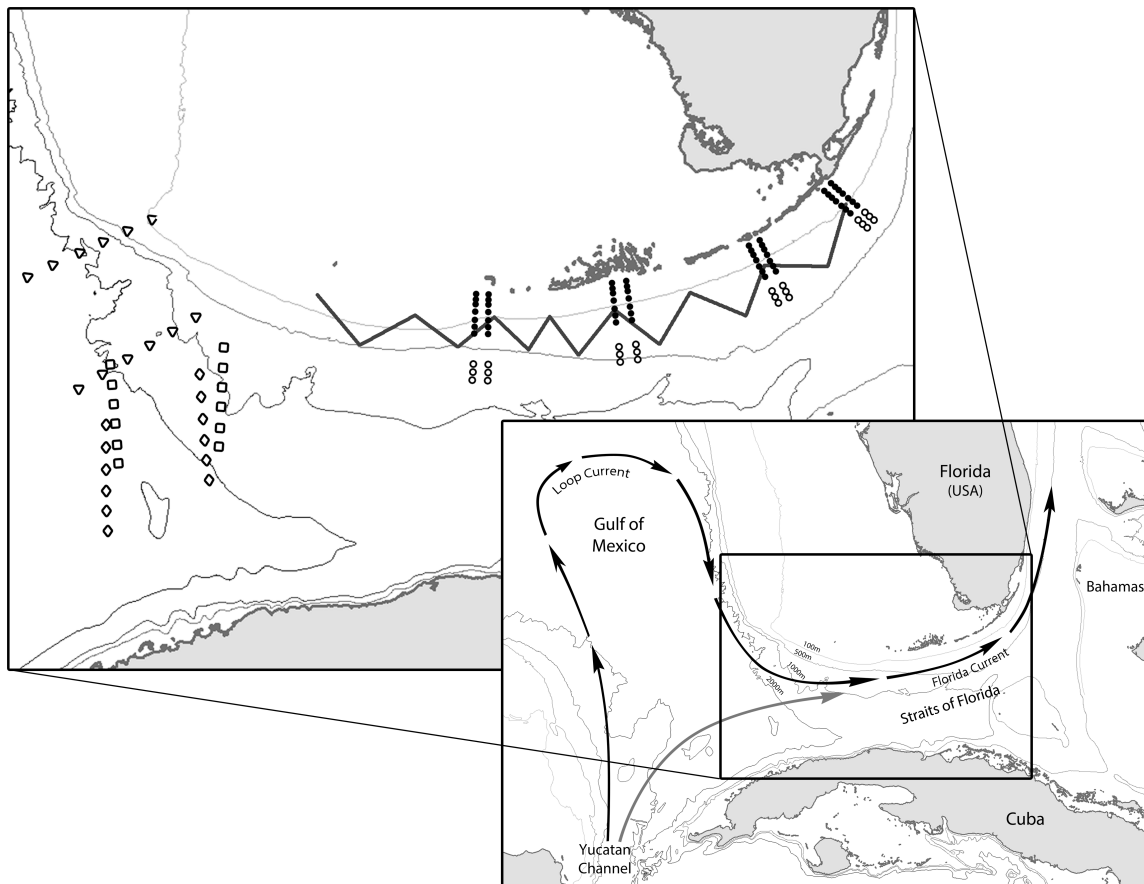
	<b>Least Squares Regression</b>		<b>Pearson's Correlation</b>	
	$r^2$	p	r	p
<i>Thalassoma bifasciatum</i>	0.92	<0.001	0.69	<0.001
<i>Cryptotomus roseus</i>	0.77	<0.001	0.66	<0.001
<i>Xyrichtys novacula</i>	0.91	<0.001	0.28	<0.001
<i>Sphyraena barracuda</i>	0.91	<0.001	0.57	<0.001

**Table 3.2:** Sample sizes and significance levels (p values) from REML analyses of mean recent growth (left) and mean R/D (right) differences between nearshore and offshore groups.  $n_t$ : total sample size of nearshore and offshore combined,  $n_n$ : sample size of nearshore individuals,  $n_o$ : sample size of offshore individuals. Where differences were significant, the trend is indicated.

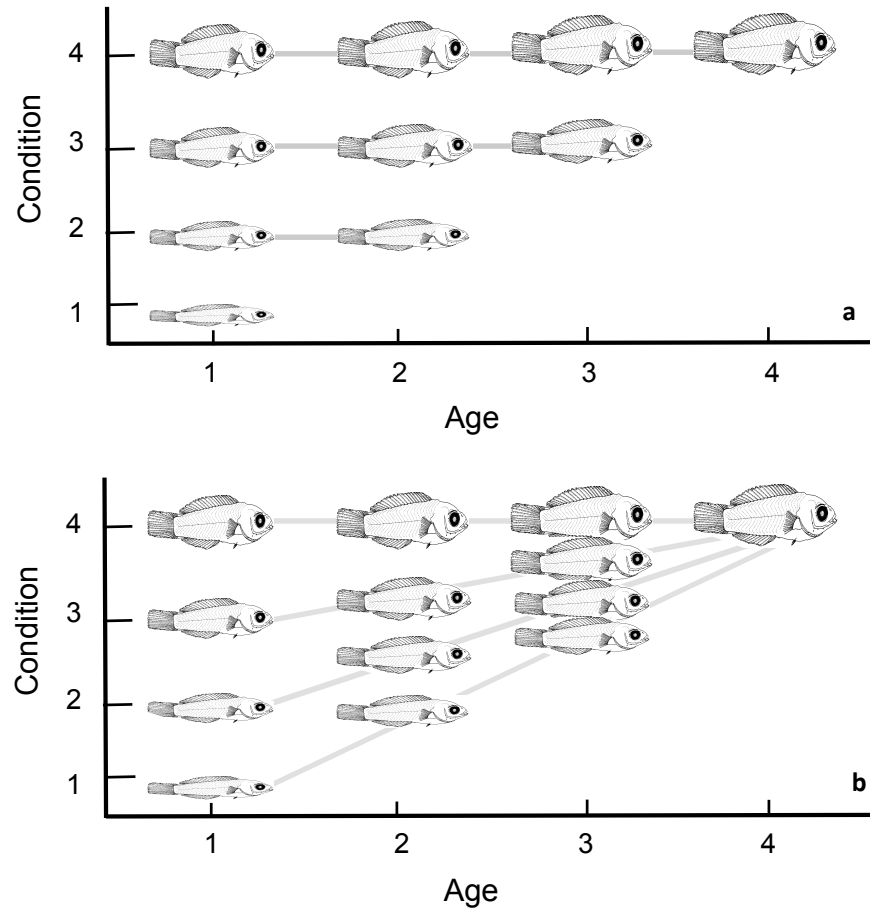
	Cruise	Recent Growth					RNA/DNA				
		$n_t$	$n_n$	$n_o$	p	Trend	$n_t$	$n_n$	$n_o$	p	Trend
<i>Thalassoma bifasciatum</i>	All Combined	461	62	399	<b>0.010</b>	Near>Off	426	60	366	<b>0.001</b>	Near>Off
	Cruise 1	278	49	229	<b>0.012</b>	Near>Off	271	48	223	<b>0.012</b>	Near>Off
	Cruise 2	122	10	112	0.518	–	111	9	102	0.369	–
	Cruise 3	61	3	58	–	–	44	3	41	–	–
<i>Cryptotomus roseus</i>	All Combined	330	151	179	<b>&lt;0.001</b>	Near>Off	231	149	82	0.118	–
	Cruise 1	6	4	2	–	–	6	4	2	–	–
	Cruise 2	249	145	104	<b>&lt;0.001</b>	Near>Off	225	145	80	0.108	–
	Cruise 3	75	2	73	–	–	–	–	–	–	–
<i>Xyrichtys novacula</i>	All Combined	359	198	161	<b>0.002</b>	Near>Off	354	195	159	0.139	–
	Cruise 1	62	22	40	<b>0.006</b>	Near>Off	61	22	39	<b>0.008</b>	Near>Off
	Cruise 2	202	96	106	<b>0.009</b>	Near>Off	201	96	105	0.826	–
	Cruise 3	95	80	15	0.215	–	92	77	15	<b>0.035</b>	Near>Off
<i>Sphyraena barracuda</i>	All Combined	167	80	87	0.326	–	167	80	87	0.709	–
	Cruise 1	35	24	11	1.0	–	34	23	11	0.416	–
	Cruise 2	75	27	48	0.660	–	75	27	48	0.261	–
	Cruise 3	56	28	28	0.546	–	56	28	28	0.346	–

**Table 3.3:** Significance levels (p values) from pairwise REML analyses of differences in daily growth (top) and size-at-age (bottom) between nearshore and offshore larvae at distinct points in larval life (days 15, 20, 25, and 30). Significant p values shown in bold. Sample sizes for each species and cruise are consistent with those shown in Table 2.

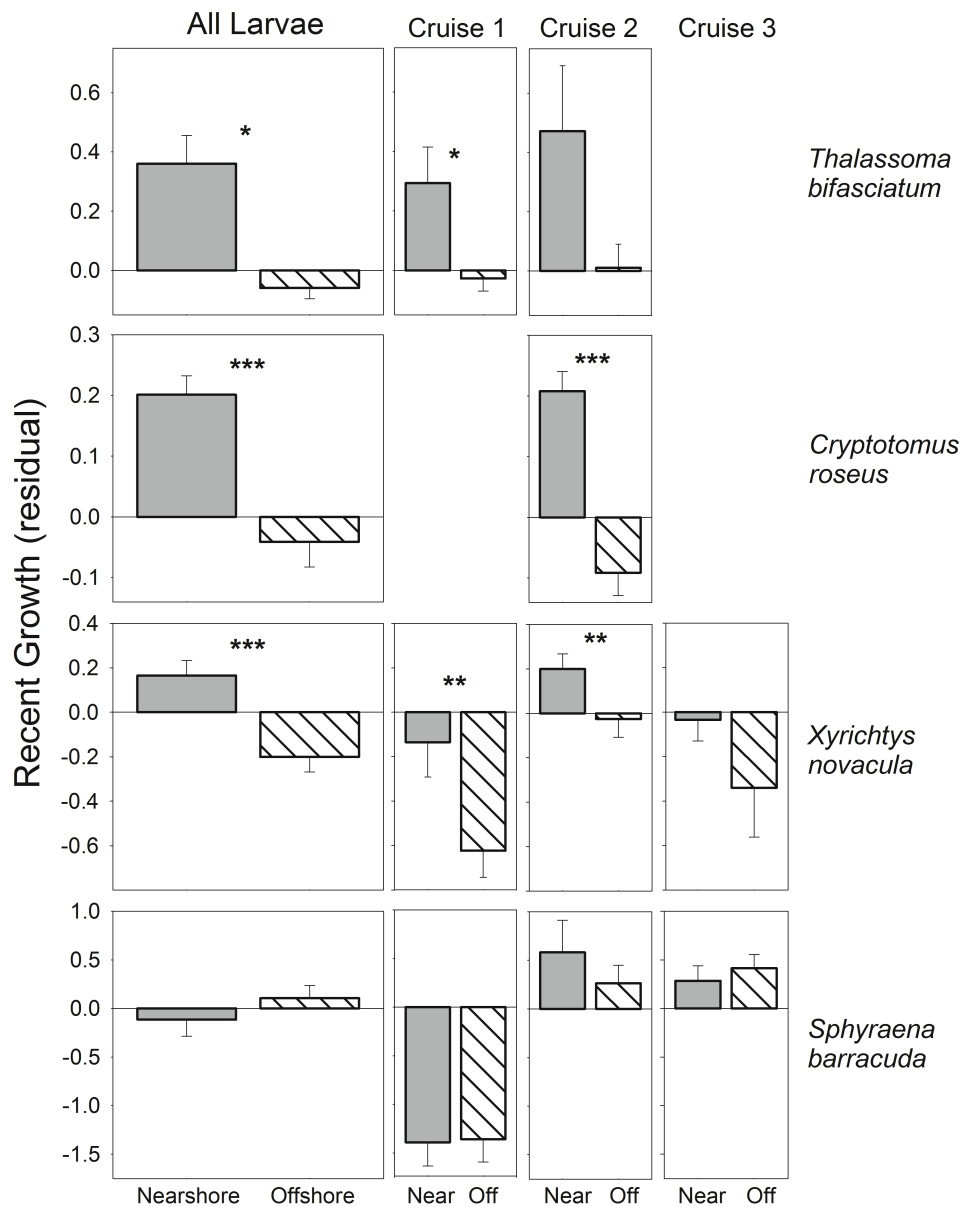
Daily Growth								
	Cruise 1				Cruise 2			
	15	20	25	30	15	20	25	30
<i>Thalassoma bifasciatum</i>	0.31	0.28	<b>0.012</b>	<b>0.010</b>	0.28	0.36	<b>0.006</b>	–
<i>Cryptotomus roseus</i>	–	–	–	–	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<i>Xyrichtys novacula</i>	0.33	0.80	<b>0.013</b>	<b>0.006</b>	0.65	<b>0.025</b>	0.31	0.88
<i>Sphyraena barracuda</i>	0.30	0.22	0.76	–	0.32	0.71	0.42	–
Size-at-age								
	Cruise 1				Cruise 2			
	15	20	25	30	15	20	25	30
<i>Thalassoma bifasciatum</i>	0.16	0.12	0.20	<b>0.030</b>	<b>0.045</b>	<b>0.041</b>	<b>0.007</b>	–
<i>Cryptotomus roseus</i>	–	–	–	–	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<i>Xyrichtys novacula</i>	0.59	0.32	0.27	0.067	0.94	0.75	0.30	0.88
<i>Sphyraena barracuda</i>	0.68	0.79	0.92	–	0.78	0.21	0.42	–



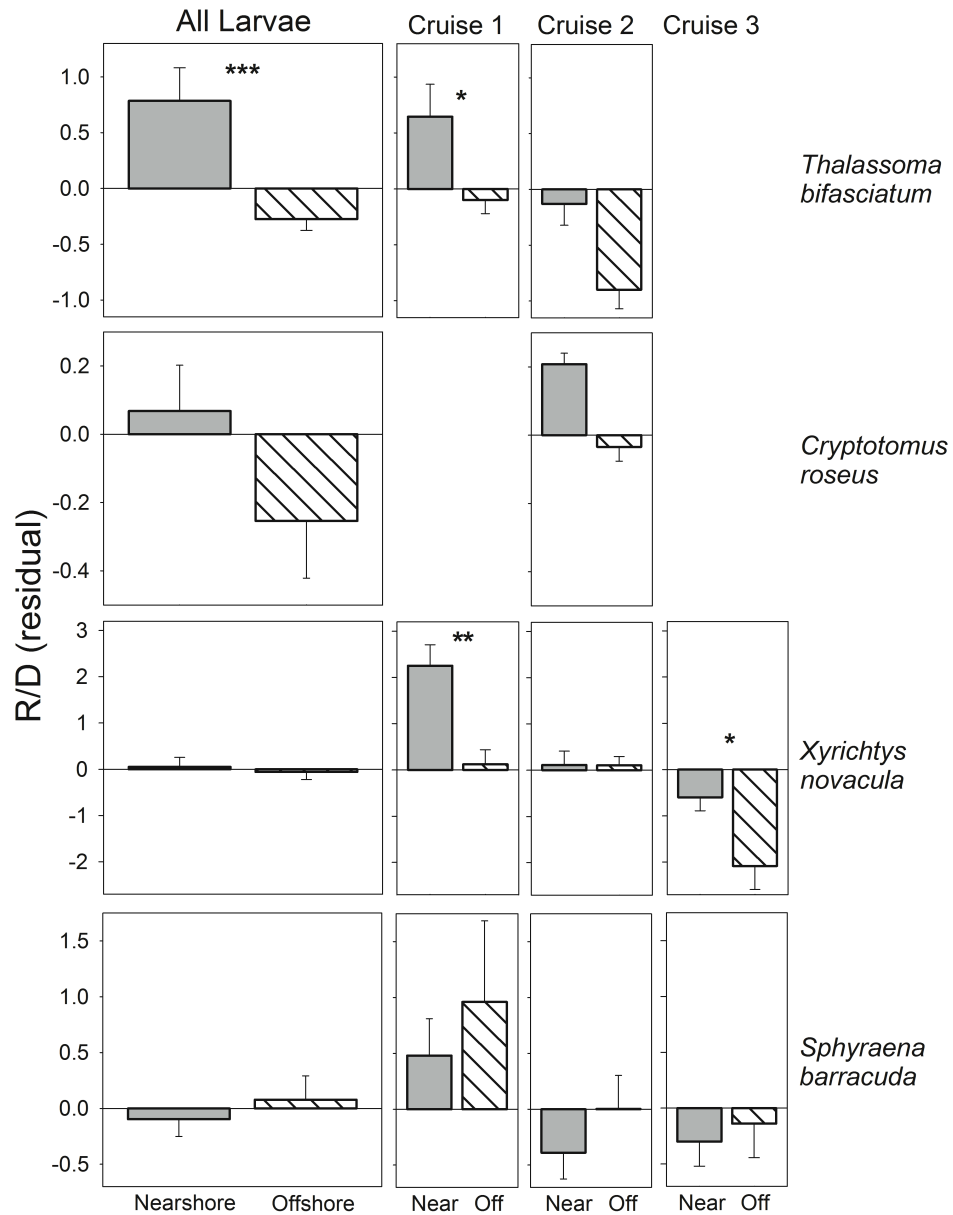
**Fig. 3.1:** Map of the study region, which includes the Florida Keys reef tract and potential upstream sources of larvae flowing in from the Yucatán Peninsula or the West Florida shelf. The physical oceanography of the region is dominated by the Loop Current, which flows up from the Yucatán Channel and protrudes to varying degrees into the Gulf of Mexico before feeding into the Straits of Florida to form the Florida Current. Black arrows show a representative pathway for the Loop Current/Florida current. Gray arrow shows an alternative trajectory of the Loop Current observed when the meander is pinched off. Sampling scheme is shown in the expanded map (upper). Closed symbols represent nearshore stations, open symbols represent offshore stations. Cross-shelf transects are depicted with circles. The locations of the upstream transects varied with cruise depending on the location of the loop current; upstream stations from cruises 1, 2 and 3 are represented with triangles, squares and diamonds, respectively. Dark grey zig-zag line shows cruise track when sampling supplemental stations.



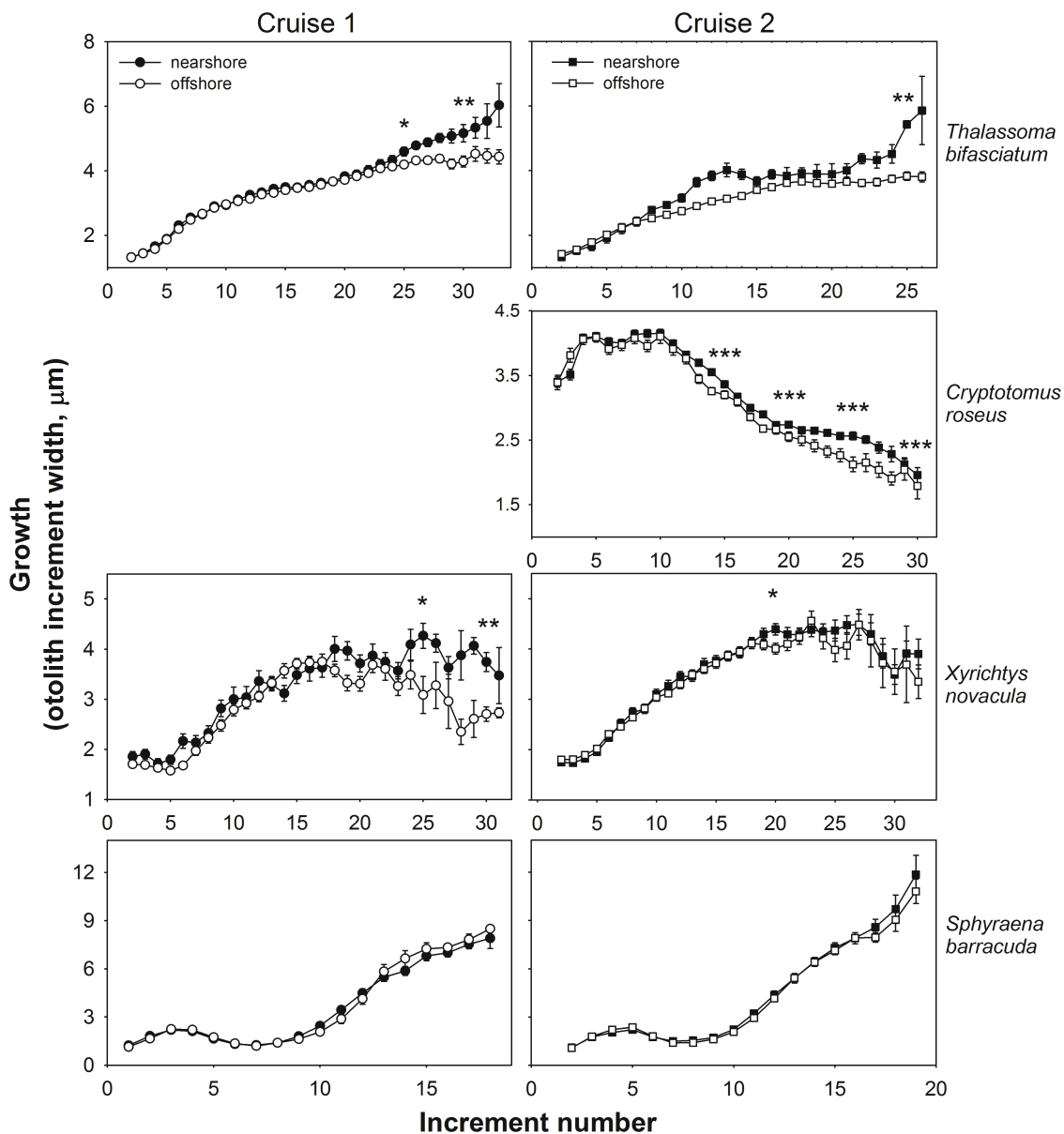
**Fig. 3.2:** Two potential mechanisms for an increase in the lowest observed larval condition levels (i.e. the 10<sup>th</sup> percentile) with increasing larval age. In scenario a, there is selective loss of poor condition larvae from the distribution. In scenario b (less realistic) all larvae attain high condition levels regardless of initial condition.



**Fig. 3.3:** Mean recent growth (average otolith increment width of last three full days of larval life)  $\pm$ SE of larvae collected at nearshore stations (grey) and offshore stations (hatched) for four species of coral reef fish. The far left column shows trends when larvae from all cruises were combined. If sample sizes were sufficient, separate cruises were analyzed individually. Sample sizes and significance levels are shown in Table 2.

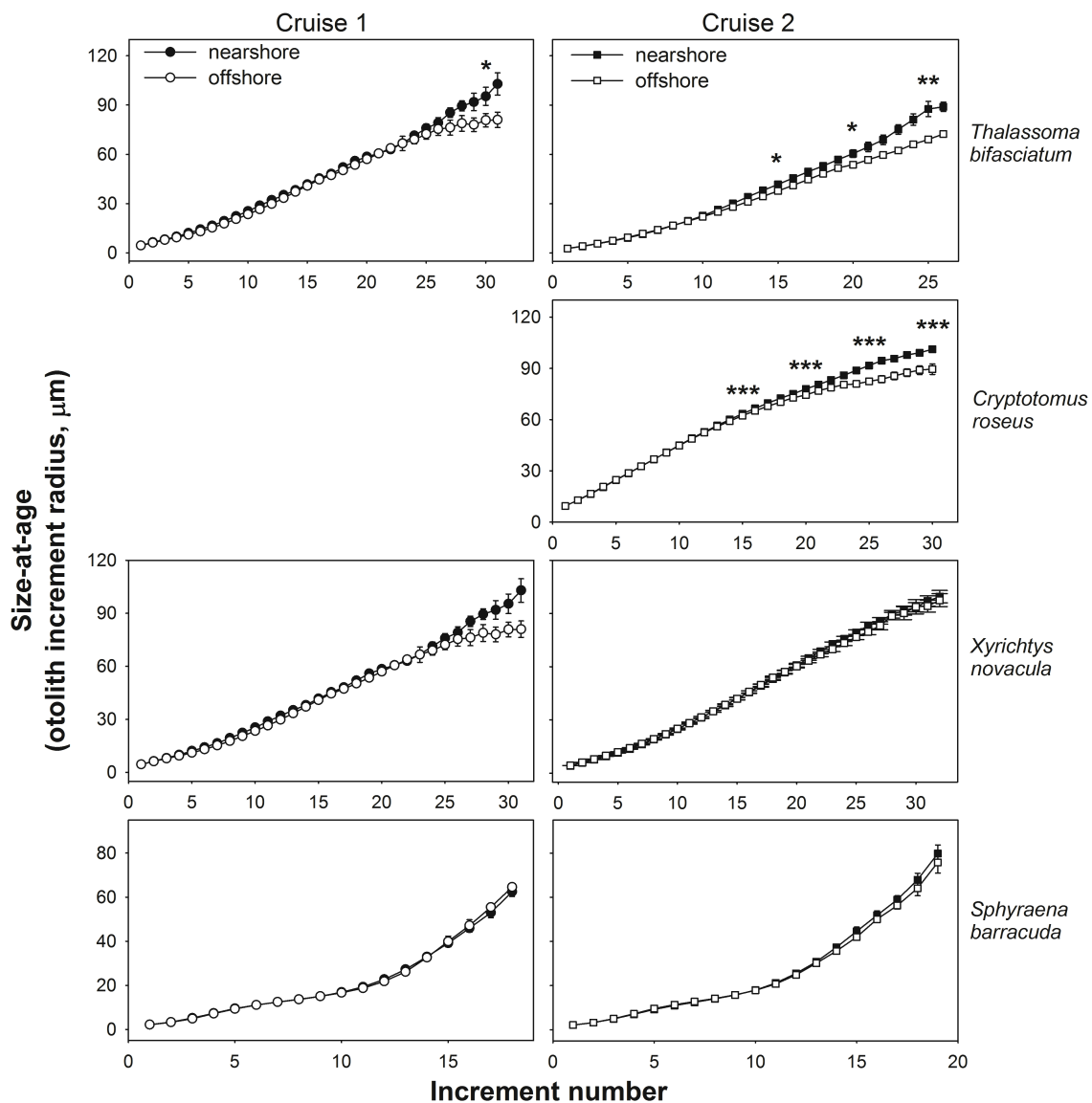


**Fig. 3.4:** Mean R/D ( $\pm$ SE) of larvae collected at nearshore stations (grey) and offshore stations (hatched) for 4 species of coral reef fish. The far left column shows trends when larvae from all cruises were combined. If sample sizes were sufficient, separate cruises were analyzed individually. Sample sizes and significance levels are shown in Table 2.

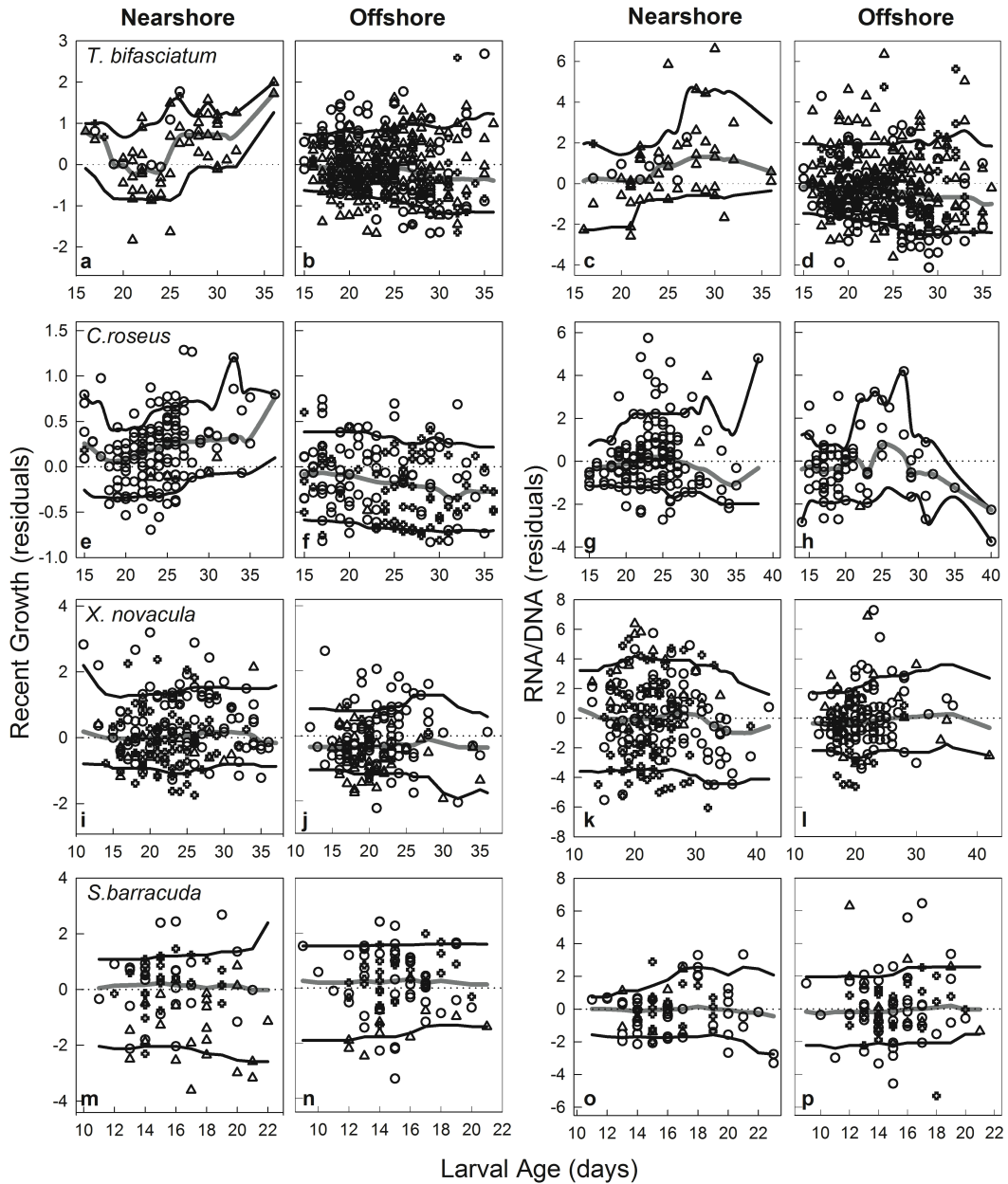


**Fig. 3.5:** Mean daily growth ( $\pm$ SE) for larvae collected at nearshore stations (solid symbols) and offshore stations (open symbols) on cruises 1 and 2 (except sample size of *C. roseus* was insufficient for cruise 1). Separate nearshore vs. offshore comparisons were made at distinct points in larval life, every 5<sup>th</sup> day beginning at 15dph (e.g. increments 15, 20, 25 and 30). Significance levels are shown in Table 3.3. Sample sizes are consistent with those used for REML analysis of recent growth, shown in Table 3.2.

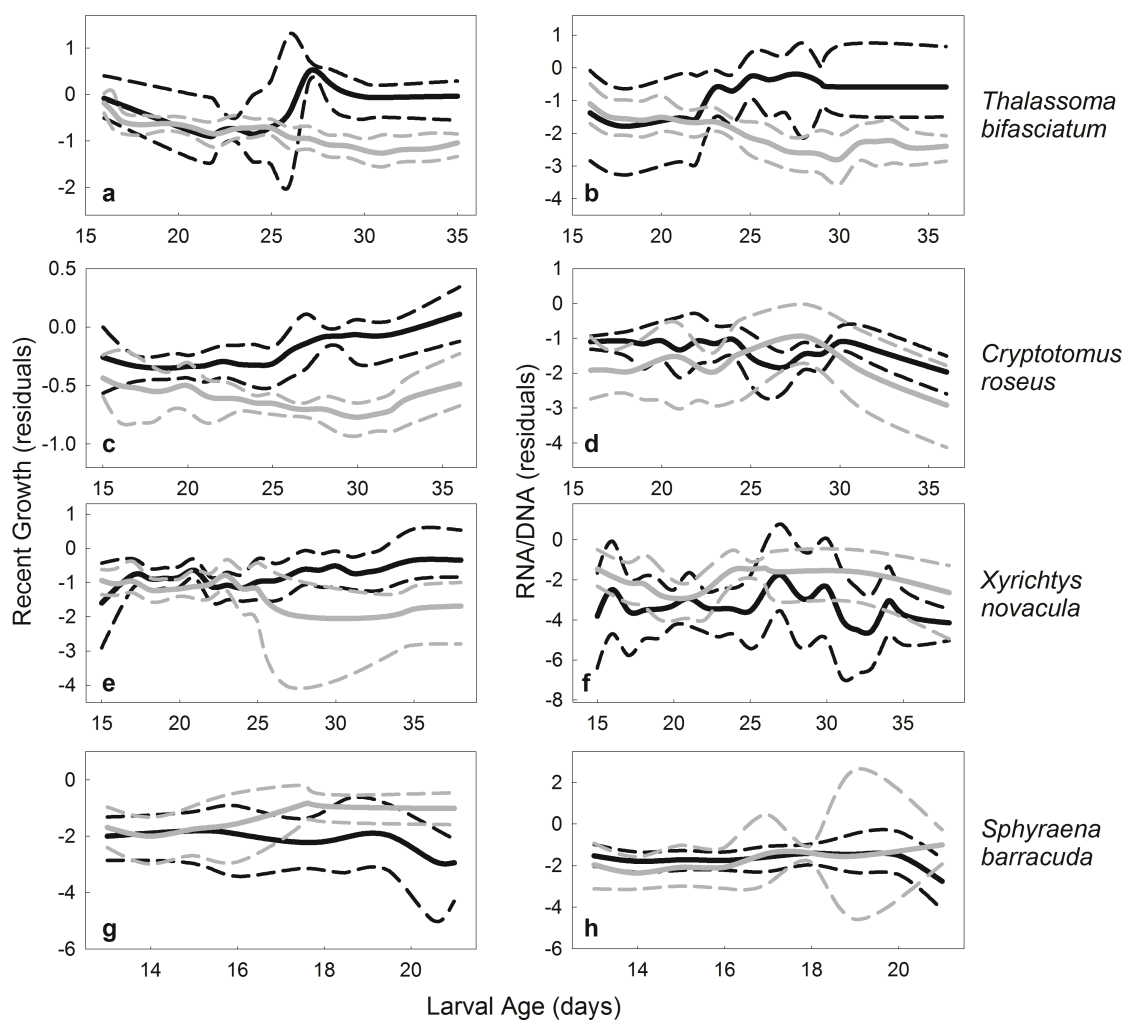




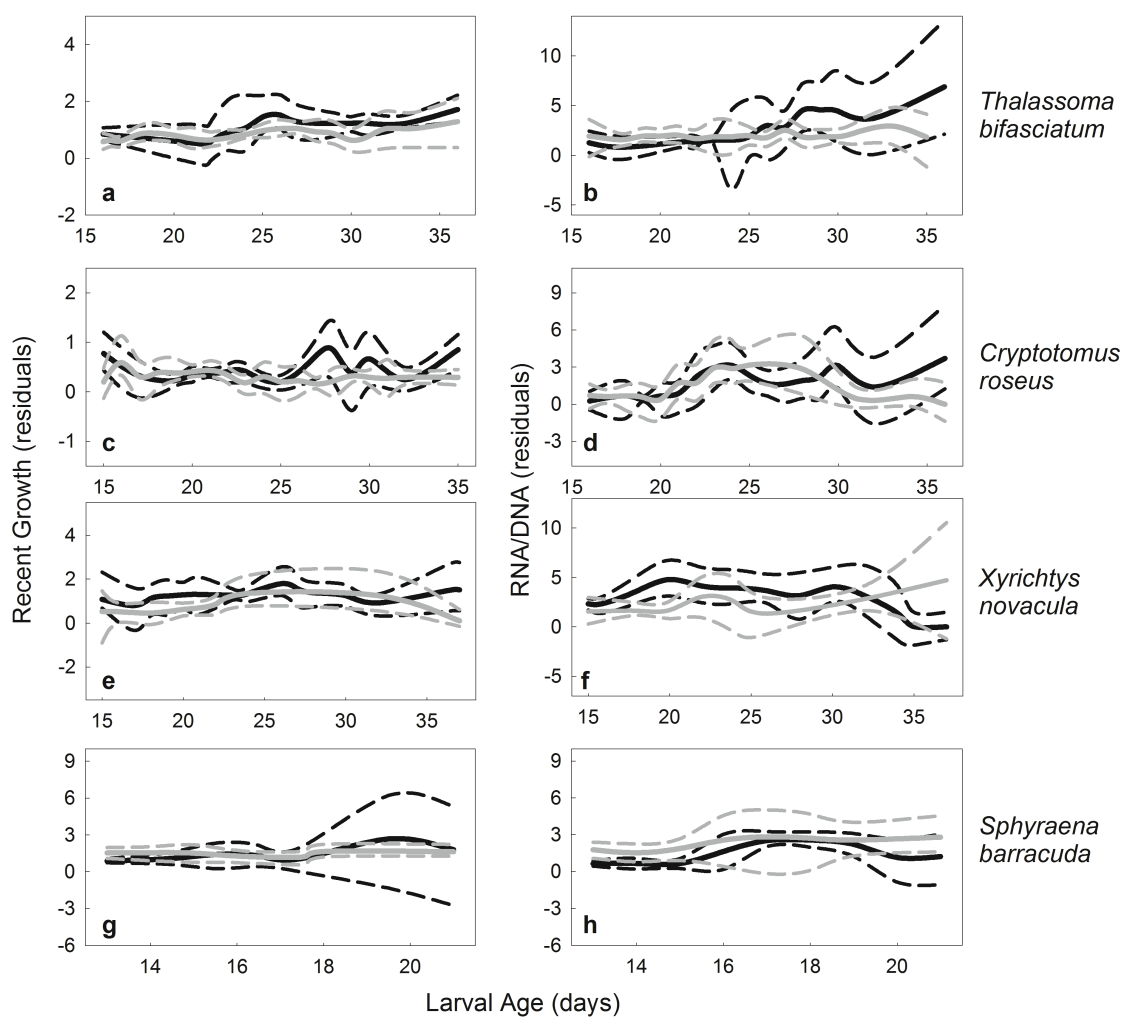
**Fig. 3.6:** Mean size-at-age ( $\pm$ SE) for larvae collected at nearshore stations (solid symbols) and offshore stations (open symbols) on cruises 1 and 2 (except sample size of *C. roseus* was insufficient for cruise 1). Separate nearshore vs. offshore comparisons were made at distinct points in larval life, every 5<sup>th</sup> day beginning at 15dph (e.g. increments 15, 20, 25 and 30). Significance levels are shown in Table 3.3. Sample sizes are consistent with those used for REML analysis of recent growth, shown in Table 3.2.



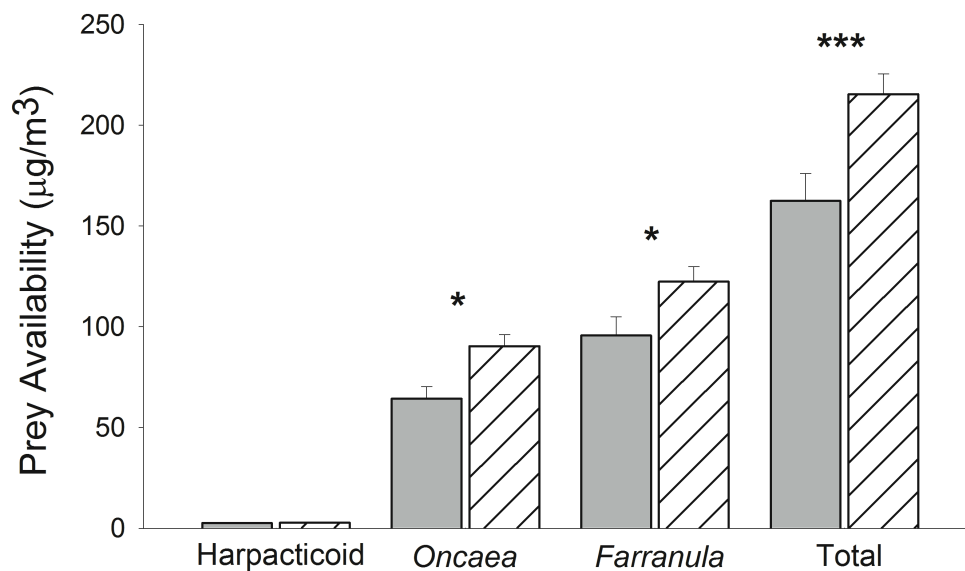
**Fig. 3.7:** Cumulative probability distribution functions (CDFs) of recent growth (left) and R/D (right) in relation to larval age for *T. bifasciatum* (a-d), *C. roseus* (e-h), *X. novacula* (i-l), and *S. barracuda* (m-p). Upper and lower solid black lines indicate the 90<sup>th</sup> and 10<sup>th</sup> percentiles of the distributions, respectively. Solid grey lines indicate medians. Individuals from all cruises are shown together; triangles: cruise 1, circles: cruise 2, crosses: cruise 3.



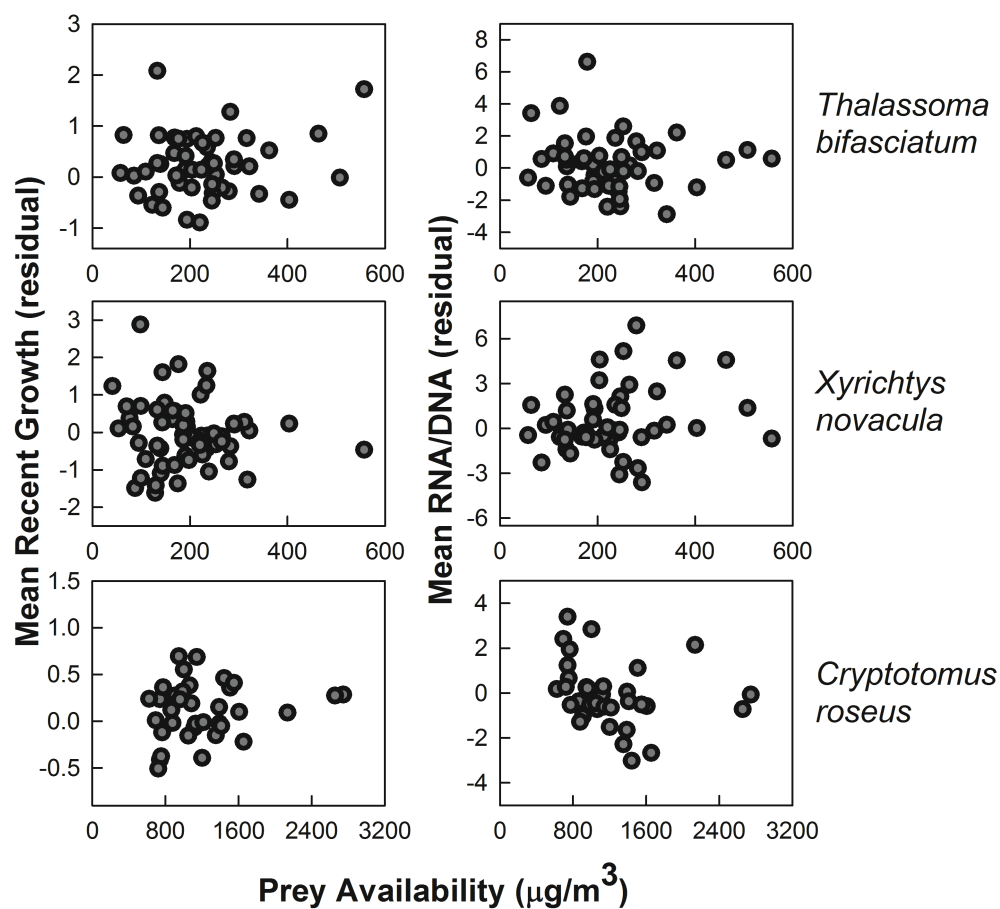
**Fig. 3.8:** 10<sup>th</sup> percentiles of nearshore and offshore groups from the CDFs shown in Fig. 3.7 plotted together with 95% confidence intervals (CIs) generated from 1,000 bootstrapped randomizations of the data. Recent growth is on the left and R/D is on the right. Solid lines show the 10<sup>th</sup> percentiles. Upper and lower dashed lines show the upper and lower CIs. Nearshore is shown in black, offshore is shown in gray. Differences between nearshore and offshore are statistically significant ( $p < 0.05$ ) where CIs do not overlap.



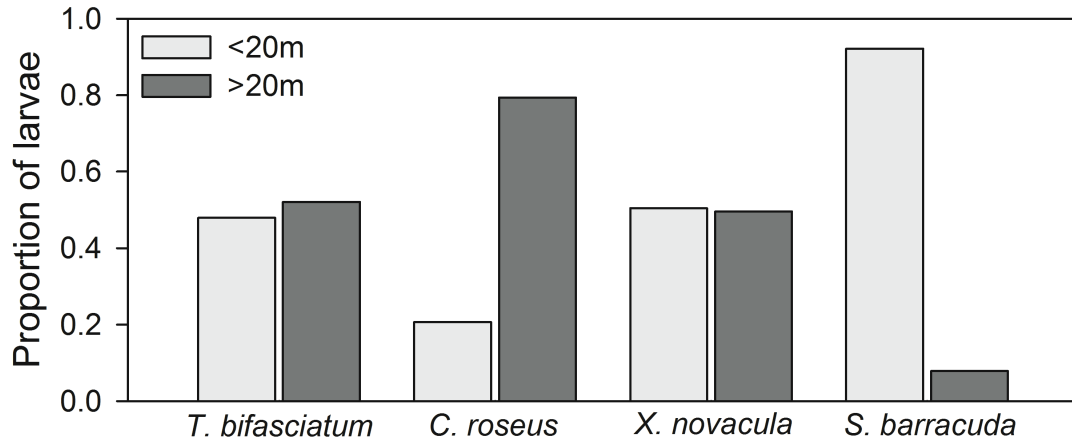
**Fig. 3.9:** 90<sup>th</sup> percentiles of nearshore and offshore groups from the CDFs shown in Fig. 3.7 plotted together with 95% confidence intervals (CIs) generated from 1,000 bootstrapped randomizations of the data. Recent growth is on the left and R/D is on the right. Solid lines show the 90<sup>th</sup> percentiles. Upper and lower dashed lines show the upper and lower CIs. Nearshore is shown in black, offshore is shown in gray. Differences between nearshore and offshore are statistically significant ( $p < 0.05$ ) where CIs do not overlap.



**Fig. 3.10:** Nearshore (gray) vs. offshore (hatched) larval fish prey availabilities ( $\pm$ SE) in biomass/m<sup>3</sup> for the three specific prey types consumed by *T. bifasciatum* and *X. novacula*. 'Total' bars show all three prey types combined. Prey density was significantly lower nearshore than offshore for *Oncaea* (REML:  $p = 0.020$ ), *Farranula* (REML:  $p = 0.019$ ) and all three prey types combined (REML:  $p = 0.004$ ).



**Fig. 3.11:** Least-squares regressions of condition vs. prey availability (biomass/ $\text{m}^3$ ) for recent growth (left) and R/D (right). For *T. bifasciatum* and *X. novacula*, totals of the 3 prey types favored by those species were used. For *C. roseus*, the diet is less specific, so totals of all 7 larval fish prey types were used. Regression lines not shown because no relationships were significant ( $r^2$  values ranged from 0.008 to 0.058,  $p$  for all regressions was  $>0.05$ ).



**Fig. 3.12:** Proportional depth distributions of all four species summed over all cruises. Light grey bars show proportion of larvae of a given species collected in the shallow depth bins (<20 m). Dark grey bars show proportion of larvae of a given species collected in deep depth bins (>20 m).

## **Chapter 4. Single-nucleotide polymorphisms (SNPs) shed light on condition in coral reef fish larvae**

Growth-related condition of coral reef fish larvae affects their survivorship during the pelagic larval phase, and can also carry over, influencing survival probability in subsequent life stages. Thus, larval condition has the potential to impact reef fish population demography, connectivity and, ultimately, persistence. As a step towards investigating the genetic and physiological mechanisms underlying larval condition, we sought to identify single nucleotide polymorphisms (SNPs) that might be associated with one (or more) of three condition-related traits (growth, RNA/DNA ratio, and duration of survival) in the larvae of an abundant Caribbean reef fish, the bluehead wrasse. High-throughput sequencing of an unbiased pool of individuals yielded over 56,000 SNPs. By using a random subset of those SNPs ( $n = 313$ ) to genotype larvae collected from locations along and upstream of the Florida Keys reef tract, we were able to identify 14 different loci that significantly co-varied with specific traits. If these results are extrapolated to the genome level then 4.5% of SNPs may be under selection, or else linked to genes under selection, potentially due to their association with the traits in question. The distributions of condition-associated SNPs were examined relative to both Bayesian-based population structure and geography (which were, incidentally, unrelated to each other). Biophysical modeling was also employed to estimate the original spawning locations of larvae collected from the plankton. Yet, SNP distributions were not correlated with either population clusters or modeled larval origins. This is not surprising given the lack of spatial differentiation that has been observed among populations of *T. bifasciatum*, both in this and previous work.



## **BACKGROUND**

Single nucleotide polymorphisms (SNPs) constitute by far the largest source of variation in most genomes (Collins et al. 1998), and thanks to the recent development and streamlining of next-generation sequencing (Schlotterer 2004, Rosenblum & Novembre 2007, Van Tassell et al. 2008), among-individual and among-population patterns in this variation can be readily explored. The utility of SNPs in both evolutionary and ecological inquiries has been gaining traction, largely because genome-wide SNP data sets provide robust, multi-locus coverage that translates into increased analytical power (Brumfield et al. 2003). This has proven useful not only in elucidating genetic lineages, but also in detecting loci that may be actively undergoing selection (Seddon et al. 2005, Rosenblum & Novembre 2007, Bradbury et al. 2010, Willing et al. 2010, Freamo et al. 2011). Furthermore, the mechanisms behind potential directional selection can be explored, as it is possible to identify probable associations between individual SNPs and specific, adaptively relevant traits. Examples of such studies in wild, non-human populations are rare as of yet, but whole genome scans have identified individual SNPs associated with temperature adaptation in pine trees (Namroud et al. 2008), pollution resistance in minnows (Williams & Oleksiak 2011) and growth in whitefish (Renaut et al. 2011). In the present study, we utilized SNP markers to look for specific genes (i.e. quantitative trait loci, or QTLs) that might be correlated with growth-related condition and survivorship in the larvae of a Caribbean coral reef fish.

Like most benthic-oriented marine animals, coral reef fishes exhibit a bi-partite lifecycle and early ontogeny is characterized by a pelagic larval dispersal period, during which there is the potential for the exchange of individuals among geographically

disparate populations. A fish's condition during larval life (often measured as its growth rate) can have a profound influence on its survival throughout the pelagic phase, for slowly growing larvae may be disproportionately susceptible to both starvation (Hjort 1914, May 1974, Cushing 1975, Lasker 1978, Dower et al. 2009), and predation (Blaxter 1986, Bailey & Houde 1989, Houde 1989b, Hare & Cowen 1997, Takasuka et al. 2003, Skajaa et al. 2004). Moreover, as a result of latent effects (*sensu* Pechenik 2006) , condition during the larval period can be strongly linked to survival probability in subsequent life stages since larvae that grow faster or are larger at a given age often survive preferentially after settling onto the reef (e.g. Searcy and Sponaugle 2001, Shima and Findlay 2002, Hoey and McCormick 2004, Hamilton 2008, Lonnstedt and McCormick 2011) .

Clearly, variability in larval growth is important in determining overall fish recruitment levels, but, more specifically, it is of great consequence for a central topic in marine ecology today, population connectivity (Cowen et al. 2007, Cowen & Sponaugle 2009). Recent research has shown that larvae with contrasting dispersal trajectories may vary substantially in their condition levels (Hamilton et al. 2008, Sponaugle et al. 2009, Shima & Swearer 2010), and may therefore contribute asymmetrically to the reproductive pool of a particular local population (Cowen & Sponaugle 2009). If, for example, foreign, long-distance dispersing larvae exhibit comparatively poor condition before or at the time of settlement, then the post-settlement survival probability of those individuals may be compromised (again, as a result of latent effects), thereby limiting the amount of demographic subsidy provided by distant populations. The degree of realized population

connectivity (which requires foreign larvae to survive to reproduction, *sensu* Pineda et al. 2002) would thus be reduced.

Given the relevance of growth-related condition to larval ecology in general, and patterns of population connectivity in particular, there is a need for a more complete understanding of how and why individual growth rates vary with time and space. The factors affecting growth necessarily include both environmental forces, and an individual's inherent capacity to respond to those forces as determined by its genes (Baldwin 1896, Blackburn & Schneider 1994, Hutchings 2011). Thus, the present study focused on potential genetic correlates of larval condition as a means of providing insight into some of the molecular and physiological mechanisms underlying variability in growth and survival. We put these findings into an ecological context by examining the population structure of *T. bifasciatum* within our study region and by considering whether the location from which a larva originated had any bearing on its SNP distributions. Because the actual origins of larvae sampled from the pelagic environment are unknown, we applied a biophysical modeling technique to hindcast larval dispersal histories. This allowed us to look for links between particular spawning sites and inherent condition potential (as indicated by selectively important SNPs) that might make larvae from one site or another either more or less likely to survive to settle to a given population.

## METHODS

### *Study species*

The bluehead wrasse (*Thalassoma bifasciatum*) occurs reliably throughout the reefs of the Caribbean. Adults generally spawn on a daily basis (Warner et al. 1975, Sponaugle & Cowen 1997) and, while the pelagic larval duration can be highly variable, larvae tend to spend *ca.* 45-49 days in the plankton before settling back to the reef (Victor 1986, Sponaugle & Cowen 1997). Temporal recruitment patterns in this species often consist of periodic settlement pulses associated with the lunar cycle (Sponaugle & Cowen 1997, Sponaugle & Pinkard 2004). As is true for most fish, the aragonitic otoliths (ear stones) of *T. bifasciatum* larvae provide a valuable record of age and growth, as they consist of concentric rings, or increments, that are deposited on a daily basis (Pannella 1971, Victor 1982). Conveniently, otolith microstructure analyses of *T. bifasciatum* larvae have shown that the width of each increment generally corresponds to the rate of somatic growth for a given day, and, similarly, the radius of the otolith is a proxy for larval size (Searcy & Sponaugle 2000, Sponaugle et al. 2011).

### *Study site*

Samples were collected from the nearshore and offshore water masses along and upstream of the Florida Keys reef tract (FKRT, Fig. 4.1). The degree to which the *T. bifasciatum* population in the FKRT may be subsidized by foreign larvae is likely a function of the dynamic nature of the physics in this region. The Yucatán Current flows up from the Caribbean, through the Yucatán Channel, and into the Gulf of Mexico. There it becomes the Loop Current (LC), which can protrude to varying degrees into the Gulf of

Mexico and, when pinched off, flows eastward directly into the Straits of Florida (SOF). In the SOF, the LC turns into the Florida Current (FC), and flow rates in this geographically constrained region can be as high as  $2 \text{ m s}^{-1}$ .

As the LC approaches the Florida shelf, and the current's frontal meanders interact with the bathymetry, mesoscale and sub-mesoscale eddies frequently result. These eddies propagate along the front, moving with the dominant flow northeastward between the fringing reef and the FC boundary (Lee et al. 1995, Fratantoni et al. 1998, Lee and Williams 1999). The frequent passage of these eddies represents a likely retention mechanism by which locally spawned larvae may remain close to their natal reef (Limouzy-Paris et al. 1997, Sponaugle et al. 2005). Yet there is also the potential for influx of larvae from distant sources, as flow of the LC from the Yucatán could result in delivery of larvae from the reefs of Caribbean Mexico. The deep (*ca.* 100 m) mesospheric reefs of the West Florida shelf may also represent a source population of reef fish larvae (Slattery et al. 2011), and their proximity to the LC makes it physically feasible for them to be delivered downstream to the FKRT. Additionally, the fish spawned on the coral reefs surrounding the island of Cuba have the potential to be transported to the FKRT, though the strong, fast flow of the LC through the SOF presents a significant barrier to dispersal, and cross-SOF larval transport is relatively unlikely.

### ***Larval collections***

*T. bifasciatum* larvae were collected during three replicate research cruises conducted in June and August of 2007 and July of 2008. Each of the three surveys included cross-shelf transects of nearshore stations (comprising reef-adjacent waters and

the mixed frontal region at the edge of the FC) as well as offshore stations in the oceanic waters of the FC core. Additionally, upstream transects were positioned to intersect the LC far offshore before it entered the SOF and approached the FKRT (Fig. 4.1). Because of the variability in the location of the LC/FC with time, the exact geographical coordinates of the offshore and upstream stations shifted from cruise to cruise. The positions of these stations were determined while underway based on prevailing flow patterns as inferred from the following information sources: 1) near real-time satellite-derived ocean color imagery (IMARS, University of South Florida), 2) sea surface altimetry data (CCAR, University of Colorado), 3) shipboard acoustic Doppler current profiler (ADCP) data, and 4) a composite oceanographic forecast derived from a variety of data (including MODIS and MERIS satellite infrared and ocean color data) and compiled by ROFFS Ocean Forecasting Service (M. Roffer).

At most stations, larval *T. bifasciatum* were collected using a multiple opening closing net and environmental sampling system (MOCNESS) with a 4 m<sup>2</sup> opening and 1 mm mesh nets. All nets were towed obliquely at *ca.* 1.5 ms<sup>-1</sup>, sampling discrete 20 m depth bins from 80 m to the surface (except when bottom depth <80 m required omission of the deepest bins). Sampling time for each depth bin ranged from 5-8 minutes. At a subset of nearshore stations that were too shallow for MOCNESS deployment, collections were made using a frame net (2 m<sup>2</sup> opening with 1 mm mesh net). The frame net was towed horizontally 3 m below the surface at a speed of 1.5 ms<sup>-1</sup>. Tow durations for this net were *ca.* 10 minutes.

In order to carry out SNP discovery and RNA/DNA ratio (R/D) analysis (see below) it was necessary preserve the RNA concentrations in individual larvae. Because

RNA degrades rapidly at ambient temperatures due to inherent RNase activity, only larvae that could be collected and preserved within *ca.* 30 minutes were suitable for molecular and condition analyses, thus fish for this study came exclusively from the 0-20 and 20-40 m MOCNESS nets and the shallow water frame net, which could be recovered quickly after sampling. When these nets were retrieved, contents from the cod-ends were rinsed with ice-cold filtered seawater and immediately sorted in frozen glass trays. Reef fish (including *T. bifasciatum*) were provisionally identified to the lowest possible taxonomic level and flash frozen in liquid nitrogen until the completion of the cruise, at which point they were transferred from liquid nitrogen to a -80° C deep freezer.

### ***Juvenile collections***

To increase the sample size for population structure analysis, and to gain insight into the factors related to observed SNP variability, some juvenile *T. bifasciatum* were included in the investigation. Juveniles were sampled from the reefs of the FKRT coincident with ship-based pelagic larval collections in the summers of 2007 and 2008. Benthic sampling was conducted at two locations along the upper FKRT (Pickles Reef and Sand Island Reef) and two locations along the lower FKRT (American Shoals and Looe Key; Fig. 4.1). Juveniles were collected by SCUBA divers using dip nets and the anesthetic quinaldine (Sigma), and were stored in 95% EtOH until the time of analysis.

### ***Laboratory processing of larvae***

To maintain RNA integrity, frozen larvae were thawed in RNA*later*-ice (Invitrogen), a proprietary solution that preserves cellular RNA in frozen tissue samples

by deactivating RNases. Taxonomic identities of thawed larvae were confirmed based on morphologic characters, and standard lengths (SLs) were measured to the nearest 0.01 mm using a Leica MZ12 stereomicroscope equipped with a stage micrometer. From a subset of individuals randomly sampled across all *T. bifasciatum* larvae, one or two eyeballs and/or the lower jaw were removed and returned to RNAlater for subsequent RNA extraction and SNP discovery (see below). Each larva's head was then excised with a microscalpel and preserved in 95% EtOH so that otoliths could be dissected out and analyzed at a later date. The viscera were also removed in order to prevent potential bias of R/D measurements by gut contents. The remaining trunk was immediately homogenized in 150  $\mu$ L ice-cold 1M NaCl and the homogenate was stored at -80°C for later use in nucleic acid quantification.

### ***Condition analyses***

As indices of individual larval condition, we used otolith-derived recent growth rates (i.e. mean otolith growth per day in  $\mu$ m), which serve as proxy for somatic growth rates (Pannella 1971, Searcy & Sponaugle 2000), as well as R/Ds. The use of R/D as a growth and condition index is based on the fact that, while the amount of DNA per diploid remains constant, the amount of RNA (composed primarily of ribosomal RNA) varies with ribosomally-mediated protein synthesis (Clemmesen 1996, Buckley et al. 1999). Thus R/Ds reflect biochemical growth processes with very little lag time.

**RNA and DNA quantification:** Total concentrations of nucleic acids in each larval sample homogenate were measured using a fluorometric microplate assay (after Westerman & Holt 1998) using the fluorophore SYBR Green II (SGII, Molecular



Probes). Larval homogenates were treated with SGII, and fluorescence levels in each sample were read using a Tecan GENios plate reader (ex: 485 nm, em: 535 nm). Initial fluorescence reads corresponded to total quantities of RNA and DNA combined. To measure the fluorescence attributable to DNA alone, RNA was selectively digested with RNase (Sigma, R-4875) and fluorescence was read again. The relative fluorescence of RNA could then be determined by calculating the difference between the first and second measurements. Standard curves relating fluorescence to RNA and DNA were generated by measuring the fluorescence levels of serial dilutions of known quantities of RNA and DNA standards (type III RNA from baker's yeast, Sigma R-6750; type I DNA from calf thymus, Sigma D-1501) that had been treated with SGII. Fluorescence readings could then be converted to relative to actual concentrations of RNA and DNA, allowing for calculation of the RNA to DNA ratio in each fish larva. This assay was effective at determining nucleic acid concentrations for the vast majority of *T. bifasciatum* larvae, but those <3 mm SL were excluded because autofluorescence of the fluorophore may have overwhelmed the subtle signal of RNA in such small individuals. Additionally, if fish appeared to have been substantially damaged or degraded during the collection process they were excluded from the analysis, as the RNA concentration in such specimens might have been affected.

**Otolith microstructure analysis:** *T. bifasciatum* otoliths were examined so that larval ages in days and daily otolith growth rates could be obtained. Otolith microstructure was analyzed in a subset of larvae randomly selected from the samples in which they occurred, with the fraction of individuals taken from each sample roughly proportional to the total number of individuals in that sample. Enumeration of daily

increments and calculations of increment widths were carried out using the sagittal otoliths, which were dissected and processed according to standard protocol (Sponaugle 2009). Sagittae were placed on a glass slide in a drop of high viscosity immersion oil and allowed to clear for 7-10 days to facilitate reading (Sponaugle 2009).

After clearing, one otolith from each larva was randomly selected and examined at 400× magnification using a Leica DMLB oil-immersion microscope outfitted with a polarized filter (which helped to enhance visibility of otolith features). Microscope images were fed to a computer via a Dage MTI video camera. Focus and contrast were optimized for otolith readability, and images were captured with a frame grabber. Using image analysis software (Image Pro Plus 7.0, Media Cybernetics), increments along the longest axis of the otolith from the core to the edge were digitally marked and counted, and resulting increment widths and increment radii (distances from the otolith core to each individual increment) were calculated. Otoliths were read blind with respect to collection time, collection location and SL. Initially each otolith was read twice. If the increment counts from the first and second reads were within 5% of each other, one of those two reads was randomly chosen as the final read. When the difference between the first and second reads was >5%, a third read was performed. If the third read was within 5% of either of the first two reads, the final read was randomly selected from one of the two closest reads; otherwise the otolith was excluded from the analysis(Sponaugle 2009).

When otolith measurements were complete and final reads had been obtained, least-squares regression showed highly significant positive relationships between otolith radius and SL ( $r^2 = 0.92$ ,  $p < 0.001$ ), and the residuals of SL-at-age and otolith radius-at-age regressions were also positively correlated (Pearson's Correlation Coefficient:  $R =$

0.69,  $p < 0.001$ ). These findings confirmed that, for the *T. bifasciatum* larvae collected in this study, otolith growth would serve as a suitable proxy for somatic growth and could be used as index of larval condition (Hare & Cowen 1995).

### ***Hindcasting larval origin***

A novel adaptation of the coupled biophysical Connectivity Modeling System (CMS; C. Paris, U. of Miami/RSMAS) was used to backtrack trajectories of actual sampled larvae and to thereby obtain an estimate of the natal origin of each individual. The CMS makes predictions of larval dispersal by incorporating bathymetry, physical forcing and generalized organismal behavior parameters into an off-line particle-tracking Lagrangian individual-based model (IBM). In this study, the vertical velocity fields were derived from four nested ocean circulation simulations: Global HYCOM (Hybrid Coordinate Ocean Model), GOM (Gulf of Mexico) HYCOM, F-KeyS (Florida Keys) HYCOM (Kang et al. 2008, Kourafalou & Kang in press), and Bahamas ROMS (Regional Ocean Modeling System), with grid scales ranging from 1/12 to 1/100 of a degree. Movement at each 6-hr time step also included a vertical diffusivity function as well as sub-grid stochastic displacement as determined by a Markov process. (For details of the model design see Cowen et al. 2006 online supplementary materials: [www.sciencemag.org/content/311/5760/522/suppl/DC1](http://www.sciencemag.org/content/311/5760/522/suppl/DC1), Paris et al. 2007, and Sponaugle et al. 2012)

Hindcast dispersal trajectories were estimated for *T. bifasciatum* larvae collected on cruises 1 and 2 (June and August of 2007). The initiation point of the simulation of a given larva was based on the actual location and depth at which the larva was collected,

and each run modeled the flow field using archived data corresponding to the time window during which that larva was dispersing. The duration of dispersal for each simulation was set to larval age in days (as determined from otolith analyses described above), thus the endpoint of the reverse model trajectory represented the larva's assumed location at age 0 (i.e. at spawning). To gain probabilistic estimates of larval origin, 100 repeat runs were carried out for each individual, allowing for a determination of the most likely location in which a given larva was born. Larvae were categorized as having originated from one of four broad potential habitats: 1) The FKRT, 2) the Yucatán Peninsula (and farther upstream in the Caribbean), 3) the West Florida Shelf, and 4) Cuba. Spatial envelopes were designated around each of these potential habitats (Fig. 4.2), and an individual's birthplace was assigned according to the habitat envelope that contained the largest proportion of that fish's 100 model endpoints (i.e. origins). If no single habitat contained at least 20% of the endpoints from a given larva's 100 replicate runs, or if the difference in the proportion of endpoints between the two most likely habitats differed by <10%, then that larva could not be assigned an origin and was excluded from model-related analyses.

This assignment system may seem less stringent than one would find in studies that model forward, open-ended dispersal (e.g. Paris et al. 2005, Cowen et al. 2006, Butler et al. 2011, Sponaugle et al. 2012), which require simulated particles of appropriate age to pass within small distances of actual reef habitat in order to constitute successful settlement. There is good reason for this: in forward projections, larvae may or may not survive dispersal and may or may not encounter settlement habitat, but, because we are estimating reverse pathways of real, sampled larvae, we know conclusively that

each larva survived dispersal up to the point of collection, and must have originated somewhere. The task, then, is simply to use the available information to select the most likely point of origin from a limited number of suitable benthic habitat sites. Excluding a larva from analysis because the simulation predicts a point of origin slightly farther away from an actual habitat than one would expect in real life disregards valuable information provided by the model about the most likely general direction of dispersal. On the other hand, we may make erroneous assumptions if we are too cavalier in assigning fish to spawning locations that are only weakly supported by the model output. The protocol used here attempts to balance these considerations, maximizing the amount of information gained without corrupting it.

### ***SNP discovery and validation***

To avoid ascertainment bias, RNA for SNP identification was isolated from a random assortment of larvae collected across two years and three different cruises. Total RNA was isolated from pooled eyes and snouts of 365 fish using a guanidinium thiocyanate buffer (Chomczynski & Sacchi 1987) followed by purification using the Qiagen RNeasy Mini kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's protocols. Prior to sequencing, ribosomal RNA molecules were selectively removed to allow greater sequencing of less abundant transcripts (RiboMinus, Life Technologies). Transcripts were sequenced using one Illumina lane of paired-end, 2x60 bp reads at Cofactor Genomics (St. Louis, MO). Construction of the sequencing library, sequencing, and identification of putative SNPs was performed by Cofactor Genomics. Essentially, the Oases transcriptome assembler

(<http://www.ebi.ac.uk/~zerbino/oases/>) was used to assemble a reference sequence from the transcripts, and then the transcript reads were aligned to the reference sequence for polymorphism identification.

### ***SNP genotyping***

Genomic DNAs for individual genotyping were extracted from the lower jaw and/or tail tissues of 180 larval and 196 juvenile *T. bifasciatum* using a modified version of the technique described by Aljanabi and Martinez (1997). Genotyping utilized Sequenom MassARRAY technology. Multiplex assays designed using MassARRAY Assay Design Software (Sequenom, San Diego, CA, USA) targeted 394 coding and non-coding SNPs that had been previously screened for quality, coverage ( $\geq 10 \times$  on each polymorphism), and sufficiently long flanking regions ( $\geq 70$ bp on each side). If multiple SNPs were proximal to one another (i.e.  $< 70$  base pairs apart), one SNP was chosen and the other(s) was translated into a degenerate nucleotide (e.g., K = G or T). Reaction conditions were performed by iPLEX chemistry as recommended by Sequenom across 13 plates at the University of Miami's Hussman Institute of Human Genomics. SNP genotypes were called using the Sequenom System Typer 4.0 Analysis package. This software uses a three-parameter model to calculate the significance of each potential genotype. Based on the relative significance, a final genotype is called and assigned a putative confidence level (conservative, moderate, aggressive, or user call). Non-calls (e.g. low probability, bad spectrum) were also noted.

### ***Statistical analysis***

To examine the relationship between larval nutritional condition and individual SNP loci, R/Ds and recent growth measurements (mean width of the last three complete otolith increments) were obtained as described above. Both R/D and recent growth varied with ontogeny, so in order to compare condition in larvae of different ages it was necessary to remove any ontogenetic effects. This was accomplished by regressing each of the two condition measures against larval age (as obtained from otolith analyses). Residuals of the R/D-age and recent growth-age regressions served as age-independent measures of R/D and recent growth, respectively.

Genome-wide association tests were conducted to identify any SNP loci that might be significantly associated with condition-related traits, and therefore under selection. One technique that has been successfully used to detect associations between individual loci and particular traits is an  $F_{ST}$ -based method whereby, for each SNP, the level of allelic variation among trait-defined groups is compared to that within groups (Beaumont & Nichols 1996). Under selective neutrality, the stochastic processes of genetic drift and gene flow will theoretically affect all loci similarly, resulting in uniform allelic divergence across the genome. Conversely, if a particular SNP is under selection (or linked to a gene under selection) in one group compared to another, then the allelic diversity (or  $F_{ST}$ ) is likely to be much higher among groups than within groups (Lewontin & Krakauer 1973). With this in mind, the analysis of molecular variance (AMOVA) function in Arlequin v.3.5.1 (Excoffier & Lischer 2010) was used to calculate pairwise  $F_{ST}$  values for each SNP between groups defined by contrasting condition-related

phenotypes. These groups included larvae with faster recent growth vs. those with slower recent growth, larvae with high R/D vs. those with low R/D, and finally, “initial” larvae (representing an initial pool, <20 days old, with limited history of exposure substantial selective pressure) vs. “survivor” larvae (that had survived >30 days in the plankton). For each pair of groups, the program FDIST2 (Beaumont & Nichols 1996) was used to generate 20,000 simulated loci based on the average heterozygosity of the empirical data as calculated by Arlequin. The simulated data sets could be assumed to have neutral  $F_{ST}$  distributions among groups, thus empirical  $F_{ST}$  values were plotted with the 99<sup>th</sup> percentiles of simulated values, and any loci that fell above the 99<sup>th</sup> percentile were considered to be outliers and potentially under selection associated with the condition trait being examined. Once outlier loci were identified, we performed a chi-squared test to determine whether the number of outlier loci shared among different condition-related traits was greater than would be expected at random. For this test, the expected number of shared loci was calculated as the product of the percentage of outliers associated with the first trait, the percentage of outliers associated with the second trait, and the total number of loci surveyed (Campbell & Bernatchez 2004).

Once trait-associated SNPs had been identified, we sought to determine whether they might be disproportionately distributed among larvae with different modeled larval origins. Thus, we measured locus-specific pairwise  $F_{ST}$ , between individuals assigned to the Yucatán spawning region and those assigned to the FKRT spawning region, and compared them to a simulated neutral distribution. A Wilcoxon rank-sum test was also applied to look specifically at whether the pairwise  $F_{ST}$ s between the FKRT-born and



Yucatán-born larvae would be higher in loci associated with condition-related traits than in the larger pool of non-trait-associated (neutral behaving) loci.

The population structure of *T. bifasciatum* within the study region was examined in order to investigate potential stratification and put the existence of any significant outlier SNP loci into context. We used a Bayesian clustering approach as implemented by the program STRUCTURE (v. 2.3, Pritchard et al. 2000), which estimates shared population ancestry of individuals based solely on their genotypes (with no *a priori* expectations) assuming Hardy–Weinberg equilibrium and linkage equilibrium in ancestral populations. Repeat runs of the model were carried out, each with a different value for the number of predefined clusters (K). Ks of 1-10 were all tested. The program used a Monte Carlo Markov Chain (MCMC) with burn in of  $10^5$  iterations followed by  $10^5$  MCMC iterations for clustering inference to obtain the probability value of a given K. The most likely number of clusters in the population was determined by comparing the relative probability values of each K (Pritchard et al. 2000), and by assessing the rate of change of the natural log of the probability of the data between successive Ks (Evanno et al. 2005). Once the number of clusters was established, Distruct v.1.1 (Rosenberg 2004) was used to generate bar plots to depict each individual's proportional cluster membership.

## RESULTS

### *Modeled larval origins*

Coupled biophysical modeling was used to estimate probabilistic dispersal pathways of 269 different individuals—252 of which could be assigned likely points of

origin based on the criteria outlined above (Table 4.1). For 180 of these larvae, corresponding SNP genotypes had also been obtained. The model output indicated that the majority of larvae originated near to or upstream of the Yucatán peninsula, and that the second most common larval origin was the FKRT. When model-derived larval origins were compared with the locations from which larvae were collected, it was found that larvae sampled from nearshore FKRT waters were more likely than offshore-sampled larvae to have originated locally (i.e. in the FKRT). Conversely, offshore-collected larvae were more likely to have been spawned at distant locations (Yucatán and beyond) as compared to their nearshore counterparts. However, modeled larval origins could not always be predicted by location of larval collection (Table 4.1).

### ***Population structure***

Repeat runs of STRUCTURE with different possible values of K provided strong evidence for the existence of two distinct genetic clusters within the regional population studied here ( $\text{Pr}(K) = 1.0$ ). Yet these clusters could not be explained by either geography or time. Groups of larvae collected nearshore and offshore, as well as juveniles sampled from the benthic habitat of the FKRT were all observed to contain similar proportions of individuals from each cluster (referred to henceforth as red and blue, Fig. 4.3a). Temporal distributions were also explored and, on the whole, neither red nor blue clusters were clearly associated with a given month or year (Fig. 4.3b). Plotting the clusters in the context of model-derived natal origins revealed that groups of fish from different probable spawning locations did not differ in their cluster compositions (Fig. 4.3c), and the two clusters were represented nearly equally within each spatially explicit larval

origin group (Fig. 4.3d). Findings were similar with regard to larval collection location.

To determine the proportions of genetic variation partitioned among and within red and blue groups, an AMOVA comparison was made between larvae that had  $\geq 90\%$  identity with the red cluster and those that had  $\geq 90\%$  identity with the blue cluster. The vast majority of the variation (97.65%) was distributed within clusters, while only a small amount (2.35%) was distributed among clusters. The  $F_{ST}$  between red and blue clusters was 0.0236 ( $p < 0.001$ ).

### ***SNP-trait associations***

Simulation based trait association tests assessed the patterns of SNP frequencies within and among groups of larvae characterized by distinct condition-related traits. Pairwise  $F_{STS}$  of individual SNPs were calculated between initial larvae and survivors, slow and fast growing larvae, and between high R/D and low R/D larvae. In the initial vs. surviving larvae comparison, seven loci fell above the 99<sup>th</sup> percentile of the modeled neutral distribution and were identified as outliers (Fig. 4.4a), and in the growth rate comparison seven outliers were also detected (Fig. 4.4b), while in the R/D comparison, three such outliers were observed (Fig. 4.4c). Over all tests, 14 SNPs (out of a total of 313 that had been sequenced) were outliers relative to the neutral expectation, and were therefore implicated as being under selection (or linked to other genes under selection) due to association with traits that affect larval growth and/or survival (Table 4.2). A subset of three SNPs showed association with more than one condition-related trait, significantly exceeding the number of shared loci that would be expected at random. One outlier locus (GPx) was shared between initial vs. survivor and high vs. low R/D

comparisons (Chi-squared:  $p < 0.001$ ), and two loci (LPP2 and PSMB1) were outliers in both initial vs. survivor and fast vs. slow growth comparisons (Chi-squared:  $p < 0.01$ ). To ascertain the functions of the 14 trait-associated loci, a BLAST search ( $e \leq 0.01$ ) was performed, revealing that 11 were located in known protein-coding genes, while the remaining four had not been annotated (Table 4.2).

When pairwise  $F_{ST}$ s were calculated between larvae likely born in the FKRT (according to the biophysical model) and those likely coming from the Yucatán peninsula region, no SNP  $F_{ST}$  values fell above the 99<sup>th</sup> percentile of the expected neutral distribution (Fig 4.5a), meaning no outlier loci were detected. Furthermore, according to a Wilcoxon rank-sum test between condition-associated loci and all other loci, there was no evidence that SNPs associated with adaptive traits showed more among-larval origin variability (i.e. higher  $F_{ST}$ ) than did all other non-condition-associated loci ( $p > 0.05$ , Wilcoxon rank-sum test, Fig. 4.5b). The same test was conducted for pairwise  $F_{ST}$  among larvae collected from nearshore and offshore water masses, and again the  $F_{ST}$  values of SNPs associated with condition related traits were not any more variable between water masses than the  $F_{ST}$ s of neutral behaving SNPs ( $p > 0.05$ , Wilcoxon rank-sum test, Fig. 4.6).

## **DISCUSSION**

### ***Evidence of selection***

A substantial amount of research has recently been conducted applying genome scans to identify outlier loci under selection (reviewed in Nosil et al. 2009). Yet, the work presented here represents one of only a few ecological studies (including Tao &

Boulding 2003, Namroud et al. 2008 and Renaut et al. 2011) that have used SNP markers to single out loci associated with specific phenotypic traits. From a random, genome-wide assortment of 313 SNPs, we were able to detect 14 different outliers that deviated from the neutral expectation. Thus, 4.5% of the SNPs examined in *T. bifasciatum* are potentially under selection due to an association with larval condition or survival.

The proportion of non-neutral loci that we observed is in line with what has been reported for levels of divergence among groups of individuals experiencing extremely dissimilar degrees or types of selective pressure. For example, Williams and Oleksiak (2011) studied killifish populations in highly polluted super-fund sites and, using the  $F_{ST}$  summary statistics method applied in the present study, they found 2.3-8.5% of all loci to be non-neutral in a comparison of polluted sites and clean reference populations. Similarly, Willing et al. (2010), using the same analytical approach, obtained a value of 3.9% when comparing guppy populations from three segregated river drainages characterized by very different predator regimes. That our percentages of non-neutral SNPs are in the same range as those from such distinctly selective scenarios speaks to the high intensity of selective pressure experienced by coral reef fish larvae in the pelagic realm. Mortality during the larval stage is intense, as must necessarily be the case for organisms as fecund as most coral reef fishes (McGurk 1986, Houde 2002). These high rates of mortality are often assumed to be synonymous with high selectivity, disproportionately favoring fish larvae with elevated growth or condition (Pepin et al. 1999), and our findings are in accordance with such an assumption.

Of the 14 QTLs identified in this study, 10 were located on known coding genes according to a BLAST search. Among the products of these genes are essential metabolic

proteins, such as NADH and the Cytochrome bc1 complex (key components in oxidative phosphorylation), and LDH (a limiting enzyme of anaerobic respiration). These proteins are critical to the production of ATP required for growth and activity, and should thus have substantial influence on larval abilities to evade predators, capture prey, migrate to favorable habitat, etc. The Glutathione Peroxidase (GPx) gene was associated with both larval R/D and duration of survival. The antioxidant activity of GPx has been well documented (Brigelius-Flohé et al. 2002), and it may be especially important to the protection of easily oxidized polyunsaturated fatty acids (PUFAs), which, in the field of aquaculture, are generally acknowledged as being necessary to successful larval fish development (Tocher 2010). Interestingly, the relationship between GPx and larval fish growth has been previously investigated; non-significant trends of increased GPx concentrations with faster larval growth were reported for cod larvae (Hamre et al. 2008), and an upregulation of GPx gene expression coincident with increased growth was observed in larvae of an abundant Mediterranean fish, the common dentex (Bermejo-Nogales et al. 2007). Like GPx, Proteasome subunit beta type-1A (PSMB1) was also shared between two different traits; this locus was an outlier in both recent growth and survival duration comparisons. Generally, proteasomes are ubiquitous within the cell, likely performing a variety of catalytic functions and affecting protein turnover (Rivett 1993). Furthermore, it has been established that the PSMB1 gene is tightly physically linked with the gene that codes for the TATA binding transcription factor (Trachtulec et al. 1997), thus the detection of PSMB1 as an outlier in this study may be related to processes of gene expression. Another proteasome gene, Proteasome subunit alpha type 7 (PSMA7) was also identified as an outlier locus. It too codes for a multicatalytic protein,

but it also plays an critical role in regulating the hypoxia-inducible factor-1a, an important transcription factor for cellular responses to oxygen tension (Cho et al. 2001). This locus is intriguing because hypoxia tolerance may be especially important for survival in coral reef fish larvae, which, because they inhabit warm, low-latitude waters, have high weight-specific oxygen demands relative to temperate taxa (Rombough 1988, Houde 1989a). This high oxygen demand, combined with the gas-exchange limitations of individuals with cutaneous respiration and/or still developing gills (Rombough 1988, Pelster 2008), as well as the high activity requirements placed on individuals that must a) feed frequently in order to balance the high metabolism inherent in warm environments (Houde 1989a), and b) locate scarce settlement habitat in order to survive, means that tropical reef fish larvae may be prone to periodic oxygen deficits.

When considering the functions of trait-associated loci, it must be noted that the gene on which each outlier SNP is located may not necessarily be the gene undergoing selection. If any loci are strongly linked to adaptive genes, they will still exhibit the signature of selection (i.e. distinctly high among-group  $F_{ST}$  relative to other loci), even if they, themselves, are not adaptive. The discussion of the trait-associated loci presented above is therefore intended as an exploration of the potential mechanisms behind any observed selection, not an exhaustive summary of mechanistic processes. To more precisely determine the reasons underlying particular SNPs' non-neutral divergence among condition-defined groups, future studies should address whether each SNP is a synonymous or non-synonymous mutation and, if the mutation is synonymous, whether it might still affect mRNA splicing, translation, or stability or whether it is simply linked to a separate causative genetic polymorphism.

It should be acknowledged that the three different tests carried out for the three different traits (recent growth, R/D, and survival duration) were not necessarily independent of each other because the samples were drawn from the same pool of individuals, and thus some individuals appeared repeatedly in more than one pairwise comparison. However, the composition of samples did vary from test to test, and each test investigated a different hypothesis, and a different component of the data. This is supported by the fact that, while three loci (21%) were associated with more than one of the traits being examined, the remaining 11 loci were each unique to one of the three traits. The analytical design applied here was necessary in order to investigate the different manifestations of larval condition independently. This is important, as all genes may not be similarly related to the different traits being examined. Genes associated with recent growth rates, for example, may not be related to R/Ds, because growth can sometimes be mediated by ribosomal activity rather than ribosomal quantity (Buckley et al. 2008). Similarly, genes relevant to the duration of larval survival may not always be related to recent growth or R/D ratio, especially in cases where the survival ability conferred by growth is less significant than that conferred by some other larval attribute (e.g. swimming speed, sensory perception, or camouflage). By testing for SNP associations with respect to all three different condition-related traits and examining where traits intersect and where they do not, we are able to better understand the various facets of larval growth and survival.



### ***Population structure***

Using a Bayesian approach, as implemented in the program STRUCTURE, we detected two distinct genetic clusters within the larvae sampled. However, visualizations of the data according to both larval collection location and modeled probabilistic larval origin indicated that the patterns observed were not associated with geography. That the *T. bifasciatum* larvae surveyed here failed to show spatial genetic divergence is not surprising. Lacson (1992) compared allozymes among *T. bifasciatum* populations from Puerto Rico and Jamaica and found no sign of genetic divergence. Investigating structure on a much larger scale, Shulman and Bermingham (1995) used mitochondrial DNA (mtDNA) and restriction fragment length polymorphisms (RFLPs) to compare *T. bifasciatum* (and other reef fish species) among six sites across the Caribbean, but they, too, found no evidence of genetic differentiation. In an investigation by Purcell et al. (2006) examining *T. bifasciatum* microsatellites over both space (14 different sites) and time (spanning a two-year period), an absence of genetic structure was again reported, and, finally, Haney and colleagues (2007), who took a more targeted approach, used microsatellites and the mitochondrial control region to compare the genetic composition in a population exhibiting high levels of self-recruitment (St. Croix, USVI) with that of other populations throughout the Caribbean. Still, no evidence of differentiation was detected. In the present study, we sampled only a small portion of the entire *T. bifasciatum* range, yet, the increased genomic coverage afforded by SNPs, can provide high resolution of potential genetic lineages, and might therefore be expected to capture genetic divergence not reflected in other markers (Brumfield et al. 2003). Indeed, SNPs revealed genetic structure that had been previously unrecognized, but this structure was

not spatially distributed.

The possible explanations for the heterogeneous clustering of the genetic background observed here are many. First, it must be stated that a large proportion of the individuals used for demographic analysis were not taken from a known benthic habitat, as would be the case in a standard population genetics study. Rather they were sampled from the pelagic environment (albeit, often within very close proximity to the FKRT). Therefore, they could theoretically (if not reasonably) have been derived from any four or more distinct source populations. Using the CMS, we were able to infer likely larval origins, and thus assign larvae to geographically discrete spawning populations, but these classifications are not definitive. We did see complete admixture of red and blue clusters in benthic juveniles of the FKRT. Similar admixture of clusters was observed in larvae collected near to the FKRT, as well as those collected far up stream in the LC, which, because of the nature of the physical environment, almost certainly came from non-FKRT habitats. These findings give us no reason to suspect that the divergence of the two observed lineages is a product of geography. Nonetheless, to confirm this categorically, adult *T. bifasciatum* would have to be sampled from benthic habitats, ideally over a much wider range than was considered here. It is conceivable that spatial segregation elsewhere in the Caribbean has generated the two distinct, but previously undetected clusters, and that both of these clusters mix together in the LC/FC region, but such a scenario seems unparsimonious, especially in light of the relatively intense scrutiny that the genome of this species had been subject to, and the high levels of gene flow that have been documented.

It is perhaps more probable that the red and blue clusters identified by STRUCTURE are not a function of ongoing drift or adaptation, but are instead a signature of historical evolutionary processes. In a study of two coral reef fishes in the Great Barrier Reef (*Plectropomus maculatus* and *Lutjanus carponotatus*), the presence, in both species, of two or more genetically distinct clades that were evenly distributed among spatially separate populations was hypothesized to be the result of historical habitat contractions, and subsequent expansions driven by intermittent glaciation (Evans et al. 2009). Over the course of *T. bifasciatum*'s evolutionary history, glaciation events (the most recent of which was the Younger Dryas ca. 12,000 years ago, Broecker & Denton 1990) may have significantly affected both adult distributions and patterns of larval dispersal. At the glacial maximum in the Pleistocene, coral reef habitat in the Caribbean was greatly reduced (likely more than an order of magnitude, Shulman and Bermingham 1995), as most of the continental shelf habitat would have been eliminated due to a decrease in sea level of up to 130 m (Rezak et al. 1985). Consequently, it is possible that sub-populations of *T. bifasciatum* were historically segregated, allowing for divergence either through genetic drift or through differential levels of selection among populations, and that, when the ice receded, recolonization of intermediate habitat and restoration of Caribbean-wide circulation patterns led to renewed mixing and population expansion. Based on mtDNA *T. bifasciatum* (along with other Caribbean and Indo-Pacific reef fish populations), appear to have an excess of gene diversity relative to an infinite-allele model (Haney et al. 2007). This trend is consistent with population history that includes a weak population bottleneck followed by expansion (Grant & Bowen 1998), and therefore supports the hypothesis that the clusters reported here are

historically driven.

An additional hypothesis for the existence of non-spatial genetic structure exists. Given that ongoing (contemporary) allopatry seems unlikely based on the data, there remains the possibility of sympatric divergence. The phenomenon of assortive mating could theoretically explain the fact that both red and blue clusters co-occur throughout the sampled range. The notably high diversity of coral reef fish species that exist within the framework of the reef habitat is a testament to the potential for sympatric, selection-driven adaptation to occur, and the coral reef literature is full of examples of differentiation and/or speciation of coexisting populations. In a coral dwelling goby of the Great Barrier Reef, differential individual preference for specific habitat types (i.e. species of branching corals) resulted in micro-scale spatial segregation within the reef and increased frequency of mating among fish with similar coral host preferences, ultimately leading to morphological differentiation (Munday et al. 2004). A similar example can be taken from the Caribbean, where some Hamlets of the genus *Hypoplectrus* (Serranidae) exhibit aggressive mimicry, and have evolved coloration similar to that of non-predatory reef fishes, presumably in order to increase success in approaching and attacking prey. The adaptation of different color morphs (and associated assortive mating) has led to speciation, with different species distinguished by their coloration (Puebla et al. 2007). These examples constitute just two representative mechanisms by which clustering of genetic material can occur in the absence of spatial segregation. They represent the outcome of many generations of cumulative genetic divergence. It is possible that the subtle population clustering observed in *T. bifasciatum* is the product of some incipient sympatric adaptation process, which may or may not ever

come to fruition. Establishing more concretely whether the structure detected here is the result of historical or ongoing processes is beyond the reach of the present study, but it could be investigated in the future, for example, by using allele frequencies and inter-allelic variation to estimate the ages of the specific polymorphic loci most responsible for cluster divergence (Slatkin & Rannala 2000).

### *Application of the biophysical model*

This work represents the first time that the CMS (or indeed any biophysical model, to our knowledge) has been used to track larval dispersal in reverse. Our findings demonstrate that the model can be applied to larval hindcasting, and can thereby provide a hypothetical context in which to examine a variety of ecological and evolutionary questions. As it was employed here, the CMS incorporated empirically-derived biological parameters, such as age and duration of dispersal, as well as stochastic sub-grid scale movement and vertical diffusivity, and in the future, hindcast runs of the model can continue to be parameterized as data on larval reef fish behavior, including ontogenetic vertical migration (e.g. Irisson et al. 2010), larval swimming capacity (e.g. Leis et al. 2011) and orientation abilities relative to environmental cues (e.g. Irisson et al. 2009) are becoming increasingly available.

Using the technique outlined in the methods above, we classified approximately 94% of the larvae into probable larval origin categories, and the majority of larvae (71%) were predicted to have come from the in and around (or upstream of) the reefs of the Yucatan Peninsula. This is plausible in light of the dominant flow of the LC/FC within the study region. At face value, the model output reported here might appear to differ

somewhat from the large body of work that has emerged over the last decade, indicating that local retention of larvae could be more important than long distance dispersal in subsidizing populations (discussed in Cowen and Sponaugle 2009) . However in this investigation, we would expect to see very few larvae with a far downstream (FKRT) larval origins, because most of the larvae included in the model were collected from the lower (western) FKRT transects, or points farther west (and thus farther upstream), and so larvae originating in the upper (eastern) FKRT would have had to migrate against the dominant regional flow (see Yeung et al. 2001 for a discussion of regional counter currents). Also, the larvae whose trajectories were modeled here came from the pelagic environment, not the reef proper, so the high proportions of Yucatan-born larvae could be, but are not necessarily, a reflection of what would be observed if our modeling efforts were focused only on the dispersal pathways successful FKRT settlers. Such an investigation is not undertaken here, as it would be tangential to the fundamental motivating questions of the present study: whether specific SNPs might be associated with condition, and whether any condition-associated SNPs might be related to geographical population structure and/or dispersal.

***SNPs with Geography: Patterns relating to collection location and likely larval origin***

Based on the fact that the genetic structure of *T. bifasciatum* within the study region was evenly mixed in space, we would not expect condition-related SNPs to be asymmetrically apportioned among either collection locations, or modeled larval origins unless selective pressure differed from one location to the next. We have no *a priori* reason to assume that selection on larvae would differ solely based on natal origin, Two

complementary analyses between the most distant spawning sites (the FKRT and the Yucatan region; illustrated in Fig. 5) supported the lack of differential levels of selection by showing that, 1) between the FKRT and the Yucatan, none of the 313 SNPs examined differed significantly in their allelic variability (as indicated by the fact that no outlier  $F_{ST}$ s were detected), and 2) the 14 SNPs identified as being adaptively important based on larval condition were not any more divergent in an among spawning site comparison than were all other (adaptively neutral) loci.

We also sought to determine whether trait-associated SNPs might be correlated with either nearshore or offshore larval habitat (based on larval collection location). In this case we do have an *a priori* assumption that the degree or type of selective pressure on larval *T. bifasciatum* varies among nearshore and offshore water masses, likely due to differences in selective predation (Chap. 3, this dissertation). Thus one might expect the SNPs that were singled out for their association with condition to differ among groups. However this was not the case. The 14 adaptively important SNPs and the remaining neutral loci were showed similar levels of allelic variability among mater masses (Fig. 6), suggesting that the selective mortality described elsewhere in this dissertation may not have been related to the condition-related SNPs identified here.

### ***Conclusions***

This research presented here used an  $F_{ST}$ -based coalescent simulation method to identify specific SNPs that can be considered candidates for natural selection based on their observed associations with condition-related traits. While this method is frequently used, a wide variety of trait association techniques exist (e.g. Bayesian analyses, tests of

minor allele frequency), and future work could reanalyze the same data set to ascertain whether our results might be corroborated using alternative but complementary approaches. Regardless, this study provides a good jumping off point for more targeted examination of the molecular and physiological mechanisms driving condition variability in larval coral reef fishes.

While a significant proportion of the overall pool of SNPs showed signatures of selection, no loci had yet been driven to fixation. In addition the identities adaptively important loci differed depending on the trait being examined (i.e. recent growth, R/D, or duration of survival). Both of these observations are likely reflecting the substantial heterogeneity of the larval environment in both space and time, as well as the potentially conflicting demands of different ontogenetic life stages (Gagliano et al. 2007). Research investigating fluctuations in larval and juvenile *T. bifasciatum* survivorship with temperature has shown that larval traits such as growth rate and size-at-age, may confer different levels of survival probability depending on ambient conditions. Most notably, at cold temperatures larvae that settled at higher condition level were more likely to survive on the reef, yet this pattern was reversed at the highest water temperatures (Grorud-Colvert & Sponaugle 2011). Different, but equally complex patterns of temperature-mediated variability in selection have been reported in other reef fish species (e.g. bicolor damselfish, Rankin and Sponaugle 2011). Additionally the shifts in selective pressure on growth rates between the larval and the juvenile periods have been shown to result in balancing selection, maintaining phenotypic diversity in the early life stages of the Ambon damselfish (Gagliano et al. 2007). These examples highlight only a few of the ways in which heterogeneity in the larval milieu (be it spatial, temporal or ontogenetic)



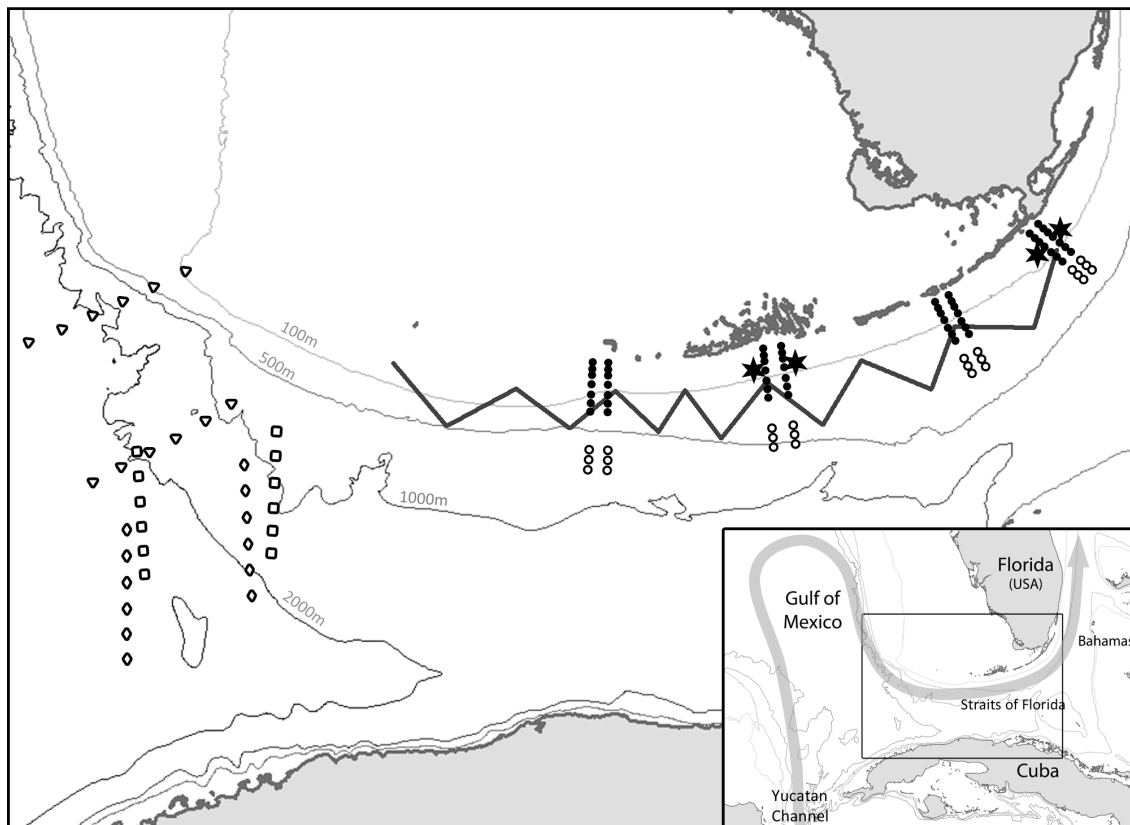
leads to distinct variability in the nature of selection on a given trait. Thus, while physiological mechanisms governing larval condition can be investigated by identifying instances of selection on particular genes, the interaction of those genes with an ever-changing environment result in complexities in larval condition and survival that are just beginning to be understood.

**Table 4.1:** Natal origins of 269 *T. bifasciatum* larvae based on 100 replicate runs of the CMS. Included is a comparison of the proportion of individuals assigned to each larval origin based on the location from which they were sampled (nearshore or offshore).

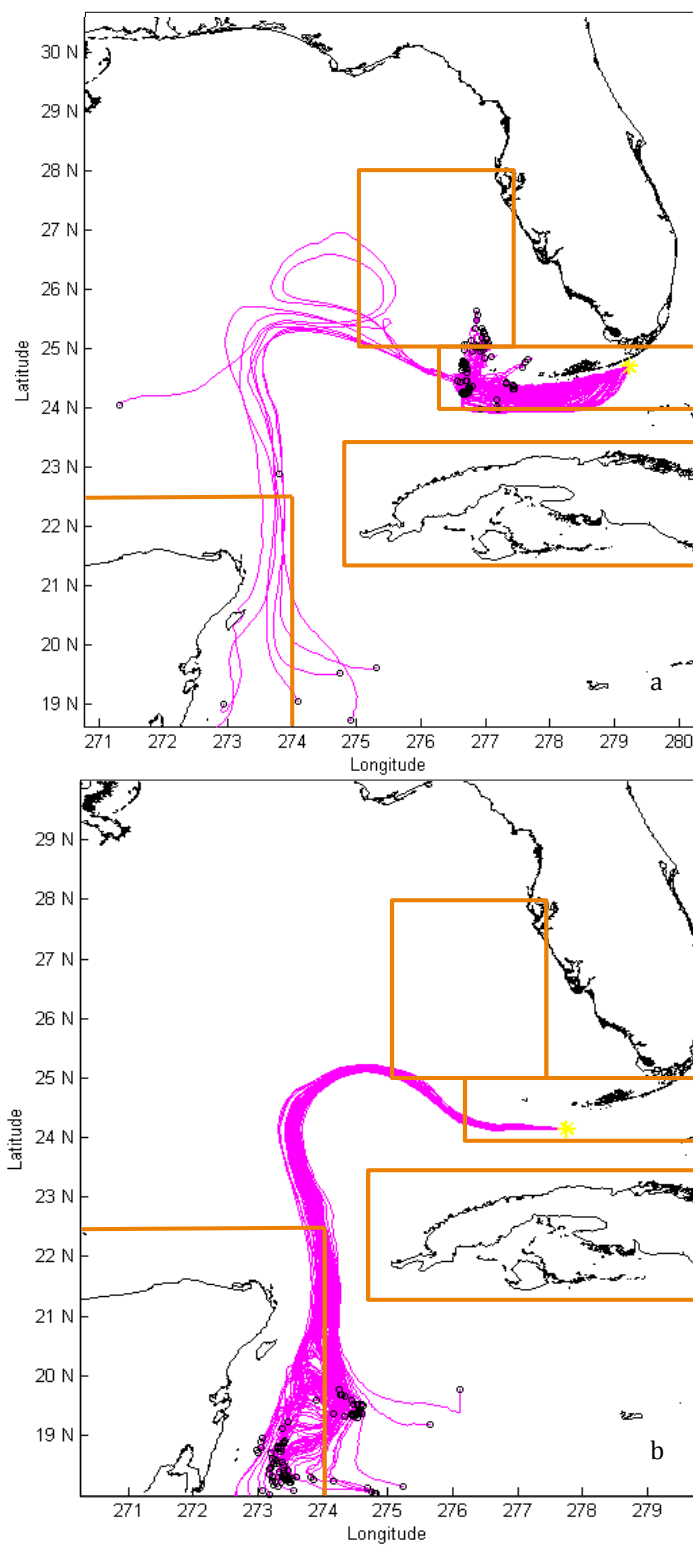
<b>Probabilistic Larval Origin</b>	<b>n Nearshore</b>	<b>Proportion Nearshore</b>	<b>n Offshore</b>	<b>Proportion Offshore</b>	<b>n Total</b>	<b>Proportion of Total</b>
Florida Keys Reef Tract	13	0.28	31	0.14	44	0.16
West Florida Shelf	1	0.02	19	0.09	17	0.06
Western Cuba	6	0.13	19	0.09	25	0.09
Yucatán Peninsula (or further upstream)	25	0.53	141	0.63	166	0.71
No Classification	2	0.04	12	0.05	17	0.06

**Table 4.2:** SNPs of interest: 14 outlier SNPs whose  $F_{ST}$  values fell above the 99<sup>th</sup> percentile of a simulated, neutral-behaving data set in one (or more) of three condition-related trait association tests. A BLAST search revealed that 10 of these loci were located on known protein coding genes, in which case the protein function is listed.

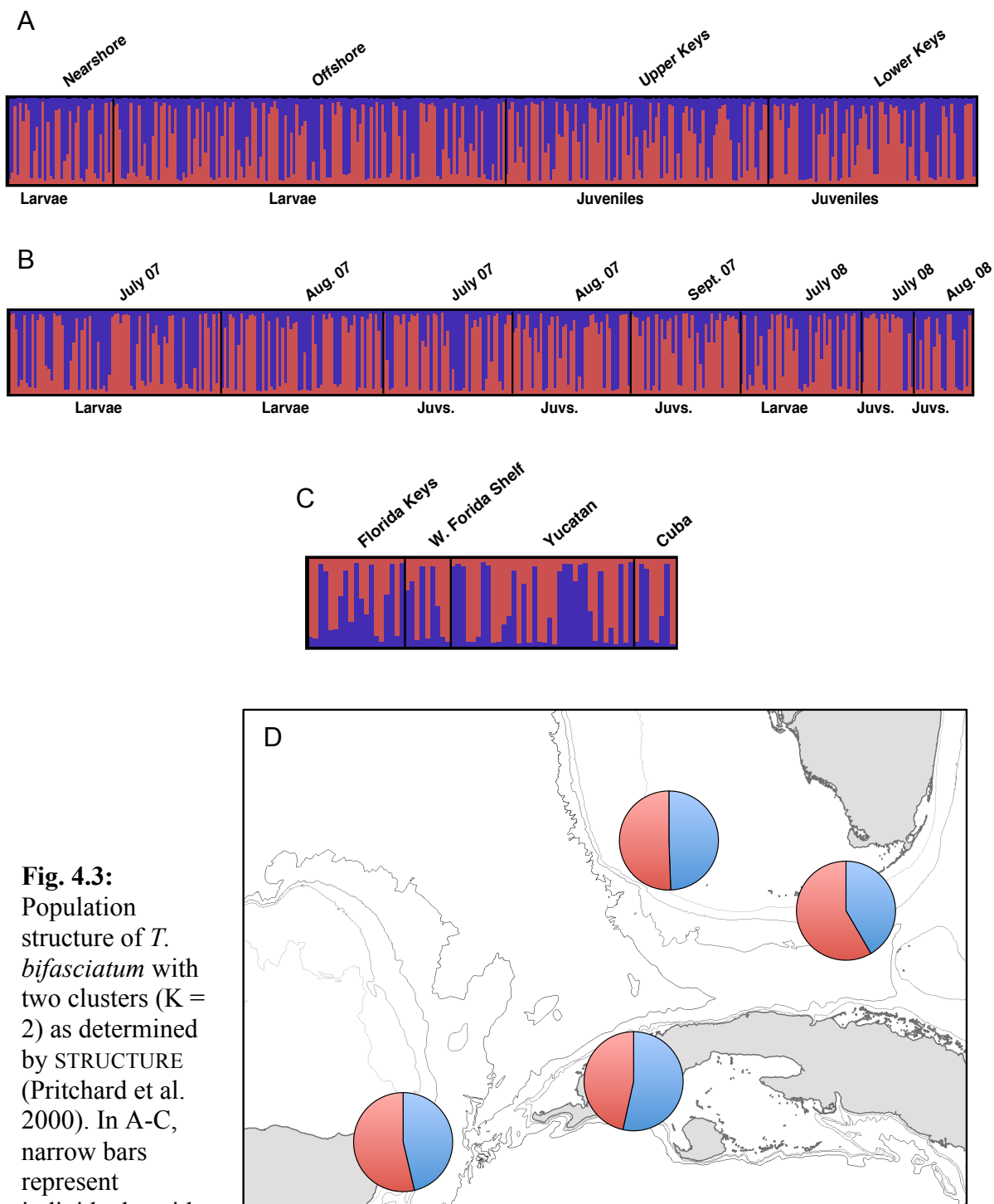
Locus	Protein Function	Trait Association		
		Init. vs. Surv.	Fast G vs. Slow G	High R/D vs. Low R/D
<i>Proteasome subunit beta type-1A (PSMB1)</i>	Multicatalytic proteinase complex	X	X	
<i>Lipid phosphate phosphohydrolase 2 (LPP2)</i>	Regulates cell signaling by modifying the concentrations of lipid phosphates versus their dephosphorylated products	X	X	
<i>Glutathione peroxidase (GPx)</i>	Prevents oxidative damage of polyunsaturated fatty acids (PUFAs).	X		X
<i>Proteasome subunit alpha type 7 (PSMA7)</i>	Multicatalytic proteinase complex		X	
<i>Cytochrome bc1 complex - subunit 9 (CytBC1-9)</i>	Third complex in the electron transport chain. (biochemical generation of ATP via oxidative phosphorylation)	X		
<i>Transmembrane 9 superfamily member 3 (TM9SF3)</i>	Chaperones insertion of proteins into and translocation of proteins across the inner mitochondrial membrane.	X		
<i>Mitochondrial import inner membrane translocase subunit 9 (TIM9)</i>	Chaperones insertion of proteins into and translocation of proteins across the inner mitochondrial membrane.			X
<i>L-lactate dehydrogenase (LDH)</i>	Glycolysis: interconversion of pyruvate and lactate	X		
<i>Collagen type I alpha 3 (COL1A3)</i>	Collagen production			X
<i>NADH dehydrogenase subunit 5 (NADH5)</i>	First complex in the electron transport chain. (Generates the electrochemical potential to produce ATP)		X	
<i>Loc 35501</i>	Not annotated but high similarity to igf2p3 (insulin-like growth factor 2 mRNA-binding protein 3)		X	
<i>Loc 15726</i>	Not annotated	X		
<i>Loc 11248</i>	Not annotated		X	
<i>Loc 5061</i>	Not annotated		X	



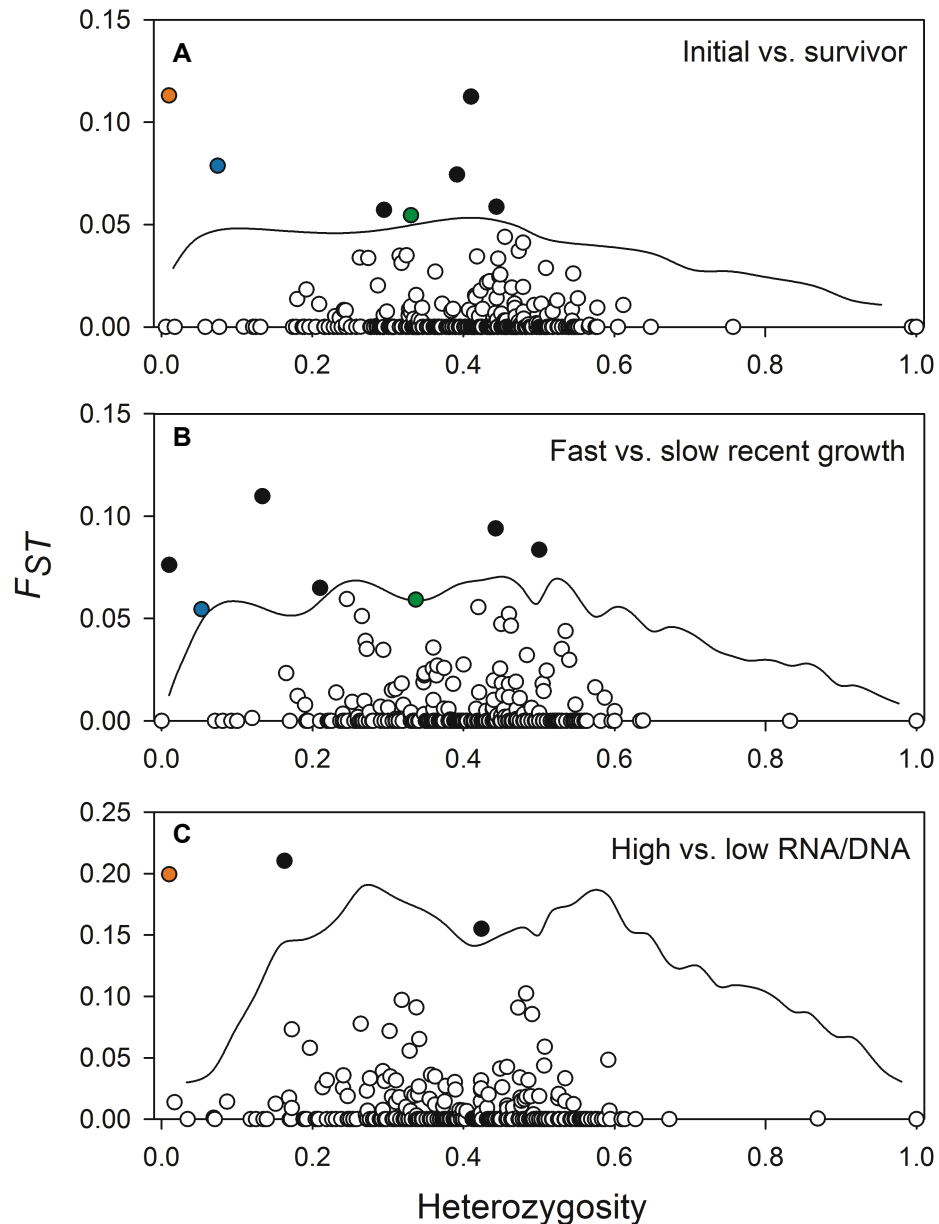
**Fig. 4.1:** Large map of the sampling scheme with an inset map of the greater study region, which includes the Florida Keys reef tract and potential upstream sources of larvae flowing from Caribbean Mexico and the Yucatán Peninsula via the Yucatán Channel. The physical oceanography of the region is dominated by the Loop Current (represented with a thick grey band in the inset), which protrudes to varying degrees into the Gulf of Mexico before feeding into the Straits of Florida to form the Florida Current. Closed symbols in the sampling scheme represent nearshore stations and open symbols represent offshore stations. Cross-shelf transects are depicted with circles. The location of the upstream transects varied with cruise depending on the behavior of the loop current; upstream stations from cruises 1, 2 and 3 are represented with triangles, squares and diamonds, respectively. The dark grey zig-zag line shows the cruise track when sampling supplemental stations as time allowed. Benthic juvenile collection locations in the upper and lower FKRT are depicted with stars.



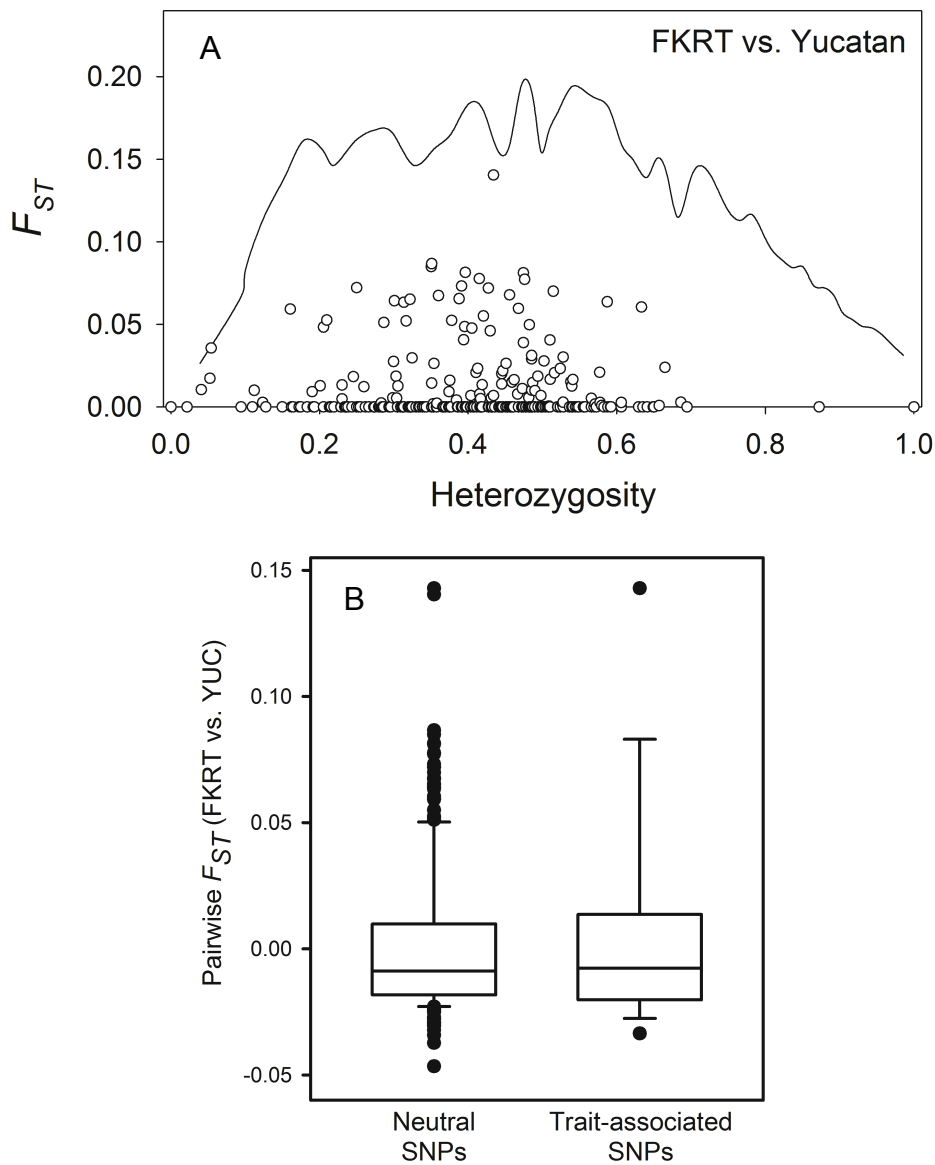
**Fig. 4.2:** Modeled dispersal pathways generated by the CMS for two examples of 30 day old larvae collected a) nearshore in the middle Florida Keys, and b) offshore in the Florida Current. Each figure shows 100 replicate runs for a single larva. In each panel, exact collection location (the point at which runs were initiated) is shown with a yellow star. Model endpoints at age = 0 dph (indicative of larval origins, are marked with black circles). Classification boundaries for assignment of larvae to natal populations based on endpoint locations are shown in orange.



**Fig. 4.3:** Population structure of *T. bifasciatum* with two clusters ( $K = 2$ ) as determined by STRUCTURE (Pritchard et al. 2000). In A-C, narrow bars represent individuals, with colors (red and blue) depicting their proportional identities with the red and blue clusters, respectively. Thin black lines separate putative groups. Individuals are plotted by A) collection location, B) time (month and year), and C) likely natal origin based on 100 repeat runs of the Connectivity Modeling System. D) Each of the four potential larval origin regions with their respective cluster compositions.

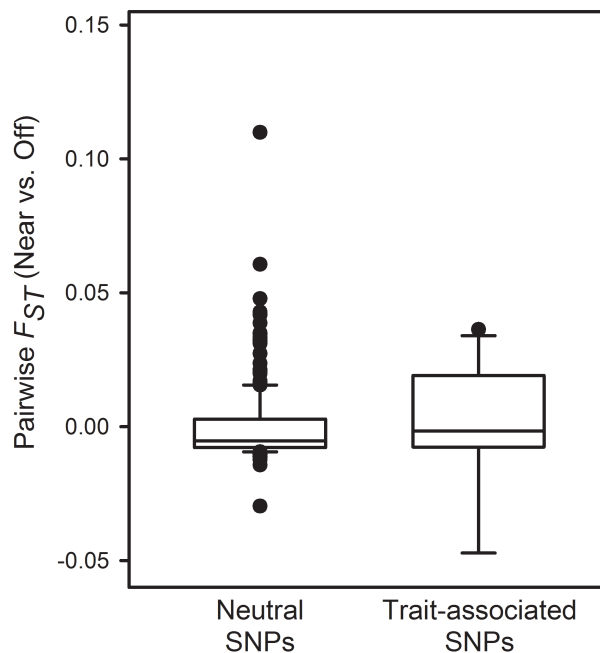


**Fig. 4.4:** Empirical  $F_{ST}$  values as a function of expected heterozygosity for comparisons of A) the initial (i.e. younger) pool of larvae vs. surviving larvae, B) fast growing vs. slow growing larvae, and C) high R/D vs. low R/D larvae. Solid lines indicate the 99<sup>th</sup> percentiles of an expected neutral distribution estimated using the summary-statistic method of Beaumont and Nichols (1996). SNPs conforming to the neutral expectation are shown with open circles. The 14 non-neutral outlier loci that fall above the simulated 99<sup>th</sup> percentiles are shown with filled circles. Colored circles indicate loci that were outliers in more than one condition related comparison; orange = *GPx*, blue = *LPP2*, green = *PSMB1*.



**Fig. 4.5:**  $F_{ST}$  between larvae with natal origins in the Florida Keys Reef Tract (FKRT) and those originating near to or upstream of the Yucatán peninsula, as inferred from biophysical model output. A) Locus by locus  $F_{ST}$  between the two natal origins plotted with the 99<sup>th</sup> percentile (black line) of a simulated neutral distribution. B) Boxplot of  $F_{ST}$  values for SNPs found to be significantly associated with condition-related traits (outlier SNPs) compared with those of all other (i.e. neutral behaving) loci. Boxes show the 25<sup>th</sup> to the 75<sup>th</sup> percentiles around the median, with whiskers extending to the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Values falling beyond these percentiles are also shown.





**Fig. 4.6:** Boxplot of  $F_{ST}$  values from a pairwise comparison of larvae collected from nearshore and offshore water masses.  $F_{ST}$  values of SNPs found to be significantly associated with condition-related traits (outlier SNPs) are shown relative to those of all other (i.e. neutral behaving) loci. Boxes show the 25<sup>th</sup> to the 75<sup>th</sup> percentiles around the median, with whiskers extending to the 90<sup>th</sup> percentiles. Individual outliers are also shown.

## CHAPTER 5. Summary and Conclusions

The research presented here explores variability in larval fish condition as evidenced by biochemical, otolith-based, and molecular correlates. This work focuses primarily on the relationship between a larva's condition and its environment, especially as determined by dispersal trajectory. Any correlations between larval dispersal pathways and condition levels could have profound implications for overall patterns and scales of coral reef fish population connectivity, so the results presented herein have the potential to inform habitat management and coral reef preservation strategies. In addition, they lay the groundwork for promising avenues of future connectivity-related research.

In Chapter Two of this dissertation, the behavior and applicability of a particular biochemical condition index, the RNA/DNA ratio, were explored. Larvae of a fast growing, circumtropically-distributed fish, the cobia (*Rachycentron canadum*), were reared in the laboratory, allowing us to examine the relationship of RNA/DNA ratios to feeding and growth in a low-latitude context. While otolith-derived growth rates are perhaps the most commonly employed tool in assessing larval condition, RNA/DNA ratios can be obtained more quickly and easily, and have therefore been used increasingly over the past two decades in temperate larval fish research (e.g. Clemmesen 1996, Chícharo 1997, Clemmesen et al. 2003, Caldarone et al. 2006, Voss et al. 2006, Malzahn et al. 2007, Buckley et al. 2008, Chícharo & Chícharo 2008, Faria et al. 2011a). The RNA/DNA ratio has only rarely been applied to questions relating to tropical/sub-tropical larval fish ecology (but see Westerman & Holt 1988, Westerman & Holt 1994, Rooker et al. 1997, Tanaka et al. 2008), and studies that measure RNA/DNA ratios in conjunction with otolith growth are also scarce (Clemmesen and Doan 1996, Rossi-Wongtchowski et

al. 2003). With this in mind, we sought to evaluate the response of nucleic acids to feeding stress in a high temperature regime and to compare this response to similarly induced changes in daily otolith growth.

Based on feeding experiments, larvae subjected to reduced food availability exhibited significant decreases in mean RNA/DNA ratio, consistent with an analogous decrease in otolith growth rates. To gain a better understanding of the effects of feeding on the distribution of condition levels across populations, cumulative distribution functions of individual RNA/DNA ratios with standard length (SL) were evaluated, and comparisons were made between larvae from well-fed and food-limited treatments. It was found that in food-limited larvae the variability of RNA/DNA ratios decreased at larger sizes. Specifically, the minimal condition level of the larvae in the largest size classes was higher in food-limited treatments as compared to full-ration treatments. The observation of increased minimal condition levels with size in low prey availability scenarios could be interpreted in one (or both) of two ways: 1) it is possible that the lowest condition individuals were selectively lost from the population, or 2) larvae with the lowest condition may have been shorter, causing them to be overrepresented in the smaller size classes and underrepresented in the larger size classes. Because there were no predators in this experimental setup (and because larval cobia are not cannibalistic), it is likely that the absence of low condition levels at larger size classes mostly reflects slow growth of the poorest condition larvae and the concentration of low condition individuals in smaller size classes. These results draw attention to the limitations of summary statistics (i.e. group-wide means) and their inability to account for variations on the

individual level. In field-based research, such variations are likely to have important ecological implications.

Using an intercalibration technique devised by Calderone and colleagues (2006), it was possible to compare the RNA/DNA ratios measured in cobia with those of larvae from both field collections and laboratory experiments. The fish included in this comparison represented a wide variety of taxa and a range of thermal habitats. It had previously been hypothesized that, because of the effects of temperature on enzyme activity, larvae in warmer environments would exhibit lower RNA/DNA ratios—presumably the higher activity of ribosomes in warm waters should reduce the number of ribosomes required to maintain a given rate of protein synthesis (Calderone 2005, Buckley et al. 2008). Perhaps due to the scarcity of data for high-temperature (i.e. tropical) taxa, no RNA/DNA–temperature correlation had previously been observed. But, when we compiled all available published larval fish RNA/DNA ratios (including many recent contributions) and included the values obtained here for cobia, we saw a clear and significant reduction in RNA/DNA ratio with temperature. This observation may be pertinent to future condition-related research, especially as larvae are forced to adapt to a changing climate and warming seas.

Given its demonstrated utility in assessing the feeding-related growth of low-latitude taxa, the RNA/DNA index was used to analyze four different species of wild-caught coral reef fish larvae from the waters surrounding the reefs of the Florida Keys. In the work presented in Chapter Three, RNA/DNA ratios as well as otolith-derived growth rates were measured in reef fish larvae collected from nearshore and offshore sampling stations. The results revealed that, for most taxa, average condition was higher among

larvae retained closer to shore. At face value, this seems consistent with the observation-based hypothesis that higher productivity in coastal waters might translate into a more favorable feeding environment (Denman & Powell 1984, Olson 2001, Leichter et al. 2003). However, larval prey availability was examined, and prey items were actually more abundant at offshore stations than nearshore stations. An inspection of the age-specific distributions of individual condition levels (measured as both recent otolith growth rates and RNA/DNA ratios) revealed that maximum condition levels were comparable in nearshore and offshore habitats. The mean condition differences that had been observed were due solely to the fact that the lowest condition levels in nearshore larvae were higher than those in offshore larvae. Thus, it seems probable, that low condition larvae had been selectively lost from the nearshore population, inflating the mean condition in that region.

This selective loss, coupled with evidence that total prey abundances do not appear to have been driving any among water mass condition differences, suggests that the selective mortality observed in nearshore larvae is a function of spatial variation in predation. A number of studies have reported higher mean condition in nearshore waters (Chen & Chiu 2003, Hamilton 2008, Shima & Swearer 2009), and it is often speculated that selective predation may be operating. However, this is the first report (to our knowledge) that demonstrates selective mortality on fish larvae in nearshore (vs. offshore) habitats and, furthermore, provides evidence that the selective mortality is predation-driven.

The observations made in Chapter Three reveal interesting patterns, but they also raise many questions, thereby suggesting many potentially fruitful areas of future

research. It was surprising that prey availability was not observed to have an effect on larval growth. There are two potential explanations for this. First, it is possible that we are measuring prey availability on the wrong scales. It has been shown that the zooplankters that constitute larval fish prey can often be heterogeneously distributed in micro-scale patches. Thus, small pockets of food could be separated by prey-depauperate waters. The degree to which larval fishes might be able to exploit spatially discrete prey fields is largely unexplored. Predictions of a larva's range of perception based on altricial temperate taxa (e.g. Pepin 2004) suggest that the scales on which typical oceanographic sampling is conducted may be too large to detect variability that would be relevant to individual larvae. But low-latitude species, by comparison, have been shown to have remarkable swimming abilities and large fields of perception resulting from well-developed sensory capacities (Simpson et al. 2005, Leis 2006, Gerlach et al. 2007). Even the youngest reef fish larvae of the genus *Thalassoma* can maintain sustained swim speeds of ca.  $7.5 \text{ cm s}^{-1}$  (Leis et al. 2011). In other words, these early stage larvae could potentially travel more than a kilometer within 4 hours. Is it possible, then, that some larvae may be well suited to finding and exploiting patchily distributed prey in open ocean environments? This question might be answered with *in situ* approaches.

Observations using new imaging technologies (Cowen & Guigand 2008, McClatchie et al. 2012) could help to describe micro-scale distributions of larval prey in nearshore and offshore habitats, while studies of larval behavior in the water column (e.g. Leis et al. 2006, Irisson et al. 2009) might provide more realistic estimates of feeding capabilities.

In addition to scale-related sampling bias, there is another potential explanation as to why larval condition was not affected by prey abundances in this investigation.

Perhaps environmental prey availabilities were consistently high in both nearshore and offshore water masses. There could be a threshold level of prey availability below which growth is compromised, but above which growth is asymptotic and unaffected by continued increases in prey supply. A threshold of this nature has been observed in threadfin shad (Betsill & Van den Avyle 1997). This explanation would imply that the offshore stations surveyed in this study provided relatively high quality feeding environments. In other research, prey–growth correlations have been documented (Sponaugle et al. 2009), yet in such cases the range of prey availabilities surveyed may have been greater and/or the regions investigated may have comprised more prey-scarce habitats as compared to the Loop Current and Florida Current stations sampled here. Laboratory rearing of reef fish larvae (which has for most non-pomacentrid species proven to be challenging) could allow for more detailed description of the physiological responses to a range of ration levels, and, again, *in situ* examination of the habitat could help to describe variations in prey availability.

In Chapters Two and Three, RNA/DNA ratios and otolith growth rates provided important information as to the effects of the environment on larval condition, yet they did not address the specific physiological mechanisms at play. To this end, the final data chapter of this dissertation used a genome wide scan to identify single nucleotide polymorphisms (SNPs) in the bluehead wrasse, *Thalassoma bifasciatum*, and then genotyped those SNPs to determine whether particular loci might be associated with condition-related traits. Fourteen different SNPs were found to be correlated with variability in growth rates, RNA/DNA ratios, duration of larval survival, or some combination of the three. These fourteen SNPs were, therefore, implicated as being

adaptively important. Additional work is needed to determine whether the condition-associated SNPs were themselves causative, or whether they were simply linked to other causative loci. Furthermore, once the causative loci are identified, the proteins coded for can be investigated, and the potential mechanisms by which they regulate larval condition can be characterized.

It is important to note that adaptively important SNPs were not observed to be disproportionately associated with particular spawning locations. (This finding is based on the estimation of likely natal origins for larvae collected throughout the study area, whose reverse dispersal trajectories were simulated using a coupled biophysical model.) We therefore have no reason to believe that larvae from one location or another might have inherently better or worse condition. Our finding is consistent with the fact that neither this nor any other study of population structure of *T. bifasciatum* (Shulman & Bermingham 1995, Purcell et al. 2006, Haney et al. 2007) have ever identified any geographically-based genetic divergence within the species. Curiously, two distinct genetic clusters were observed, but these clusters were in no way correlated with either time or space. In the future, the factors underlying this observed divergence could be examined more thoroughly by investigating whether they may be the remnants of historical population segregation. A determination of allele age could be applied to estimate the time at which these two lineages originated. Additionally, a careful analysis of specific SNPs or traits associated with the respective clusters could help to determine the degree to which one cluster or the other might represent a more adaptive genotype.

The completion of this body of work comes at a critical time. Anthropogenic stressors including, but not limited to, overfishing and climate change are threatening the



continued existence of coral reef communities as we know them. Elucidating patterns of coral reef fish population connectivity and how such patterns are affected by larval condition is essential to ongoing efforts to manage, protect, and thus hopefully preserve coral reefs and their inhabitants. In the simplest sense, the goal of this dissertation was to contribute to the larger body of related work that is increasing our understanding of life in the oceans and what sustains it. Overall, I hope that an increased understanding might engender a general appreciation for the intricacy of natural processes, and, through a combination of understanding, appreciation, and perhaps even awe, we humans might be better equipped with the knowledge of how to best conserve natural resources, and simultaneously driven by a desire to do so.

*'Let us go,' we said, 'into the Sea of Cortez, realizing that we become forever a part of it; that our rubber boots slogging through a flat of eelgrass, that the rocks we turn over in a tide pool, make us truly and permanently a factor in the ecology of the region. We shall take something away from it, but we shall leave something too.' And if we seem as small factor in a huge pattern, nevertheless it is of relative importance. We take a tiny colony of soft corals from a rock in a little water world. And that isn't terribly important to the tide pool. Fifty miles away the Japanese shrimp boats are dredging with overlapping scoops, bringing up tons of shrimps, rapidly destroying the species so that it may never come back, and with the species destroying the ecological balance of the whole region. That isn't very important in the world. And thousands of miles away the great bombs are falling and the stars are not moved thereby. None of it is important or all of it is.*

-John Steinbeck, *The Log From the Sea of Cortez*, 1951

## LITERATURE CITED

- Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research* 25:4692-4693
- Almany GR, Berumen ML, Thorrold SR, Planes S, Jones GP (2007) Local replenishment of coral reef fish populations in a marine reserve. *Science* 316:742-744
- Anderson JT (1988) A review of size dependent survival during pre-recruit stages of fishes in relation to recruitment. *Journal of Northwest Atlantic Fishery Science* 8:55-66
- Anderson LA, Robinson AR (2001) Physical and biological modeling in the Gulf Stream region Part II. Physical and biological processes. *Deep-Sea Res Part I-Oceanogr Res Pap* 48:1139-1168
- Anderson RO, Gutreuter SJ (1983) Length, weight and associated structural indices. In: Nielsen LA, Johnson DL (eds) *Fisheries Techniques*. American Fisheries Society, Bethesda, MD
- Armstrong PR (2002) Recruitment limitation, population regulation, and larval connectivity in reef fish metapopulations. *Ecology* 83:1092-1104
- Bailey KM, Houde ED (1989) Predation on eggs and larvae of marine fishes and the recruitment problem. *Adv Mar Biol* 25:1-83
- Bakun A (1996) *Patterns in the Ocean: Ocean Processes and Marine Population Dynamics*, California Sea Grant, La Jolla
- Bakun A (2006) Fronts and eddies as key structures in the habitat of marine fish larvae: opportunity, adaptive response and competitive advantage. *Sci Mar* 70:105-122
- Baldwin JM (1896) A New Factor in Evolution. *The American Naturalist* 30:441-451
- Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. *P Roy Soc Lond B Bio* 263:1619-1626
- Benetti DD, Orhun MR, Sardenberg B, O'Hanlon B, Welch A, Hoenig R, Zink I, Rivera JA, Denlinger B, Bacoat D, Palmer K, Cavalin F (2008) Advances in hatchery and grow-out technology of cobia *Rachycentron canadum* (Linnaeus). *Aquac Res* 39:701-711
- Berdalet E, Roldan C, Olivar MP, Lysnes K (2005) Quantifying RNA and DNA in planktonic organisms with SYBR Green II and nucleases. Part A. Optimisation of the assay. *Sci Mar* 69:1-16
- Bergenius MAJ, Meekan MG, Robertson DR, McCormick MI (2002) Larval growth predicts the recruitment success of a coral reef fish. *Oecologia* 131:521-525

- Bermejo-Nogales A, Saera-Vila A, Calduch-Giner JA, Navarro JC, Sitja-Bobadilla A, Perez-Sanchez J (2007) Differential metabolic and gene expression profile of juvenile common dentex (*Dentex dentex* L.) and gilthead sea bream (*Sparus aurata* L.) in relation to redox homeostasis. *Aquaculture* 267:213-224
- Betsill RK, Van den Avyle MJ (1997) Effect of temperature and zooplankton abundance on growth and survival of larval threadfin shad. *Transactions of the American Fisheries Society* 126:999-1011
- Biggs DC, Ressler PH (2001) Distribution and abundance of phytoplankton, zooplankton, ichthyoplankton, and micronekton in the deepwater Gulf of Mexico. *Gulf of Mexico Science* 19:7-29
- Blackburn DG, Schneider CW (1994) Old Wine in a New Bottle: Evolution by Another Name. *Journal of Theoretical Biology* 171:233-337
- Blaxter J (1986) Development of sense organs and behaviour of teleost larvae with special reference to feeding and predator avoidance. *Trans Am Fish Soc* 115:98-114
- Bochdansky AB, Gronkjaer P, Pepin P, Leggett WC (2008) Food limitation in larval fish: ontogenetic variation in feeding scope and its potential effect on survival. *Marine Ecology Progress Series* 367:239-248
- Botsford LW, Hastings A, Gaines SD (2001) Dependence of sustainability on the configuration of marine reserves and larval dispersal distance. *Ecology Letters* 4:144-150
- Bradbury IR, Hubert S, Higgins B, Borza T, Bowman S, Paterson IG, Snelgrove PVR, Morris CJ, Gregory RS, Hardie DC, Hutchings JA, Ruzzante DE, Taggart CT, Bentzen P (2010) Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in response to temperature. *Proc R Soc B-Biol Sci* 277:3725-3734
- Bradbury IR, Laurel B, Snelgrove PVR, Bentzen P, Campana SE (2008) Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. *Proc R Soc B-Biol Sci* 275:1803-1809
- Brigelius-Flohé R, Wingler K, Müller C (2002) Estimation of individual types of glutathione peroxidases. *Methods in Enzymology* 347:101-112
- Broecker WS, Denton GH (1990) What drives glacial cycles? *Scientific American* 262:48-56
- Brothers EB, Mathews CP, Lasker R (1976) Daily growth increments in otoliths from larval and adult fishes. *Fish B-Noaa* 74:1-8

- Brumfield RT, Beerli P, Nickerson DA, Edwards SV (2003) The utility of single nucleotide polymorphisms in inferences of population history. *Trends in Ecology & Evolution* 18:249-256
- Buckley L (1982) Effect of temperature on growth and chemical composition of larval winter flounder (*Pseudopleuronectes americanus*). *Marine Ecology Progress Series* 8:181-186
- Buckley L, Caldarone E, Ong T-L (1999) RNA-DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. *Hydrobiologia* 401:265-277
- Buckley LJ (1979) Relationships Between RNA–DNA Ratio, Prey Density, and Growth Rate in Atlantic Cod (*Gadus morhua*) Larvae. *Journal of the Fisheries Research Board of Canada* 36:1497-1502
- Buckley LJ (1984) RNA-DNA ratio: an index of larval fish growth in the sea. *Marine Biology* 80:291-298
- Buckley LJ, Caldarone EM, Clemmesen C (2008) Multi-species larval fish growth model based on temperature and fluorometrically derived RNA/DNA ratios: results from a meta-analysis. *Marine Ecology Progress Series* 371:221-232
- Buckley LJ, Lough RG (1987) Recent growth, biochemical-composition, and prey field of larval haddock (*Melanogrammus aeglefinus*) and atlantic cod (*Gadus morhua*) on Georges Bank. *Can J Fish Aquat Sci* 44:14-25
- Buckley LJ, Turner SI, Halavik TA, Smigielski AS, Drew SM, Laurence GC (1984) Effects of temperature and food availability on growth, survival, and RNA-DNA ratio of larval sand lance (*Ammodytes americanus*). *Marine Ecology Progress Series* 15:91-97
- Buston PM, Jones GP, Planes S, Thorrold SR (2012) Probability of successful larval dispersal declines fivefold over 1 km in a coral reef fish. *Proc R Soc B-Biol Sci* 279:1883-1888
- Butler MJ, Paris CB, Goldstein JS, Matsuda H, Cowen RK (2011) Behavior constrains the dispersal of long-lived spiny lobster larvae. *Marine Ecology Progress Series* 422:223-237
- Caldarone EM (2005) Estimating growth in haddock larvae *Melanogrammus aeglefinus* from RNA : DNA ratios and water temperature. *Marine Ecology Progress Series* 293:241-252
- Caldarone EM, Buckley LJ (1991) Quantitation of DNA and RNA in crude tissue extracts by flow injection analysis. *Analytical Biochemistry* 199:137-141

- Caldarone EM, Clemmesen CM, Berdalet E, Miller TJ, Folkvord A, Holt GJ, Olivar MP, Suthers IM (2006) Intercalibration of four spectrofluorometric protocols for measuring RNA/DNA ratios in larval and juvenile fish. *Limnol Oceanogr Meth* 4:153-163
- Caldarone EM, Onge-Burns JMS, Buckley LJ (2003) Relationship of RNA/DNA ratio and temperature to growth in larvae of atlantic cod *Gadus morhua*. *Marine Ecology Progress Series* 262:229-240
- Campana SE (1990) How reliable are growth back-calculations based on otoliths? *Can J Fish Aquat Sci* 47:2219-2227
- Campbell D, Bernatchez L (2004) Generic scan using AFLP markers as a means to assess the role of directional selection in the divergence of sympatric whitefish ecotypes. *Mol Biol Evol* 21:945-956
- Canino MF (1997) Nucleic acid contents and growth of first-feeding walleye pollock larvae in response to prey densities typical of sub-Arctic ecosystems. *J Fish Biol* 51:41-52
- Canino MF, Caldarone EM (1995) Modification and comparison of 2 fluorometric techniques for determining nucleic-acid contents of fish larvae. *Fish B-Noaa* 93:158-165
- Canty A, Ripley B (2012) Boot: Bootstrap R (S-Plus) Function In: R
- Catalán IA, Berdalet E, Olivar MP, Roldán C (2007) Response of muscle-based biochemical condition indices to short-term variations in food availability in post-flexion reared sea bass *Dicentrarchus labrax* (L.) larvae. *J Fish Biol* 70:391-405
- Chen CS, Chiu TS (2003) Early life history traits of Japanese anchovy in the northeastern waters of Taiwan, with reference to larval transport. *Zool Stud* 42:248-257
- Chícharo MA (1997) Starvation percentages in field caught *Sardina pilchardus* larvae off southern Portugal. *Sci Mar* 61:507-516
- Chícharo MA, Chícharo L (2008) RNA:DNA ratio and other nucleic acid derived indices in marine ecology. *Int J Mol Sci* 9:1453-1471
- Chícharo MA, Esteves E, Santos AMP, dos Santos A, Peliz A, Ré P (2003) Are sardine larvae caught off northern Portugal in winter starving? An approach examining nutritional conditions. *Marine Ecology Progress Series* 257:303-309
- Chisholm L, Roff J (1990) Size-weight relationships and biomass of tropical neritic copepods off Kingston, Jamaica. *Marine Biology* 106:71-77

- Cho S, Choi Y-J, Kim J-M, Jeong S-T, Kim J-H, Kim S-H, Ryu S-E (2001) Binding and regulation of HIF-1 $\alpha$  by a subunit of the proteasome complex, PSMA7. FEBS Letters 498:62-66
- Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry 162:155-159
- Chou R-L, Su M-S, Chen H-Y (2001) Optimal dietary protein and lipid levels for juvenile coho (*Rachycentron canadum*). Aquaculture 193:81-89
- Clemmesen C (1988) A RNA and DNA fluorescence technique to evaluate the nutritional condition of individual marine fish larvae. Meeresforschung 32:134-143
- Clemmesen C (1993) Improvements in the Fluorometric-Determination of the RNA and DNA Content of Individual Marine Fish Larvae. Marine Ecology Progress Series 100:177-183
- Clemmesen C (1994) The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. Marine Biology 118:377-382
- Clemmesen C, Buhler V, Carvalho G, Case R, Evans G, Hauser L, Hutchinson WF, Kjesbu OS, Mempel H, Moksness E, Otteraa H, Paulsen H, Thorsen A, Svaasand T (2003) Variability in condition and growth of Atlantic cod larvae and juveniles reared in mesocosms: environmental and maternal effects. J Fish Biol 62:706-723
- Clemmesen C, Doan T (1996) Does otolith structure reflect the nutritional condition of a fish larva? Comparison of otolith structure and biochemical index (RNA/DNA ratio) determined on cod larvae. Marine Ecology Progress Series 138:33-39
- Clemmesen C, Evans G Whatever happened to the likely larvae? In. Proc 25th Annual Larval Fish Conference
- Clemmesen CM (1996) Importance and limits of RNA:DNA ratios as a measure of nutritional condition in fish larvae. In: Watanabe Y, Yamashita Y, Oozek Y (eds) Survival Strategies in Early Life Stages of Marine Resources. A.A. Balkema, Rotterdam
- Collins FS, Brooks LD, Chakravarti A (1998) A DNA polymorphism discovery resource for research on human genetic variation Genome Research 8:1229-1231
- Cowen RK (2002) Oceanographic Influences on Larval Dispersal and Retention and Their Consequences for Population Connectivity In: Coral Reef Fishes. Academic Press, London, New York
- Cowen RK, Gawarkiewicz G, Pineda J, Thorrold SR, Werner FE (2007) Population connectivity in marine systems: an overview. Oceanography 20:14-21

- Cowen RK, Guigand CM (2008) In situ ichthyoplankton imaging system (ISIIS): system design and preliminary results. *Limnol Oceanogr Meth* 6:126-132
- Cowen RK, Lwiza KMM, Sponaugle S, Paris CB, Olson DB (2000) Connectivity of marine populations: Open or closed? *Science* 287:857-859
- Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. *Science* 311:522-527
- Cowen RK, Sponaugle S (2009) Larval Dispersal and Marine Population Connectivity. *Annu Rev Mar Sci* 1:443-466
- Cowles R, Desiderio A, M C (1998) Small-scale planktonic structure: persistence and trophic consequences. *Oceanography* 11:4-9
- Cushing DH (1975) *Marine ecology and fisheries*, Cambridge University Press, London
- D'Alessandro EK, Sponaugle S, Llopiz JK, Cowen RK (2011) Larval ecology of the great barracuda, *Sphyraena barracuda*, and other sphyraenids in the Straits of Florida. *Marine Biology* 158:2625-2638
- D'Alessandro EK, Sponaugle S, Serafy JE (2010) Larval ecology of a suite of snappers (family: Lutjanidae) in the Straits of Florida, western Atlantic Ocean. *Marine Ecology Progress Series* 410:159-175
- Dänhardt A, Peck MA, Clemmesen C, Temming A (2007) Depth-dependent nutritional condition of sprat *Sprattus sprattus* larvae in the central Bornholm Basin, Baltic Sea. *Marine Ecology Progress Series* 341:217-228
- Denman KL, Powell TM (1984) Effects of physical processes on planktonic ecosystems in the coastal ocean. *Oceanogr Mar Biol Ann Rev* 22
- Díaz E, JM T, F V (2009) Biochemical composition and somatic growth of pelagic larvae of three fish species from the Bay of Biscay. *Marine Ecology Progress Series* 382:173-183
- Díaz E, Txurruka J, Villate F (2011) Growth maximization in early sardine larvae: a metabolic approach. *Marine Biology* 158:1135-1148
- Díaz E, Txurruka JM, Villate F (2008) Biochemical composition and condition in anchovy larvae *Engraulis encrasicolus* during growth. *Marine Ecology Progress Series* 361:227-238
- Diaz MV, Pájaro M, Olivar MP, Martos P, Macchi GJ (2011) Nutritional condition of Argentine anchovy *Engraulis anchoita* larvae in connection with nursery ground properties. *Fisheries Research* 109:330-341

- Ditty JG, Shaw RF (1992) Larval development, distribution, and ecology of cobia *Rachycentron canadum* (Family: Rachycentridae) in the northern Gulf of Mexico. *Fish B-Noaa* 90:668-677
- Dixson DL, Jones GP, Munday PL, Planes S, Pratchett MS, Srinivasan M, Syms C, Thorrold SR (2008) Coral reef fish smell leaves to find island homes. *Proceedings of the Royal Society B-Biological Sciences* 275:2831-2839
- Doherty PJ, Dufour V, Galzin R, Hixon MA, Meekan MG, Planes S (2004) High mortality during settlement is a population bottleneck for a tropical surgeonfish. *Ecology* 85:2422-2428
- Dower JF, Pepin P, Kim GC (2009) Covariation in feeding success, size-at-age and growth in larval radiated shanny (*Ulvaria subbifurcata*): insights based on individuals. *J Plankton Res* 31:235-247
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Mol Ecol* 14:2611-2620
- Evans RD, van Herwerden L, Russ GR, Frisch AJ (2009) Strong genetic but not spatial subdivision of two reef fish species targeted by fishers on the Great Barrier Reef. *Fisheries Research* 102:16
- Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10:564-567
- Faria AM, Chicharo MA, Goncalves EJ (2011a) Effects of starvation on swimming performance and body condition of pre-settlement *Sparus aurata* larvae. *Aquat Biol* 12:281-289
- Faria AM, Muha T, Morote E, ChÍcharo MA (2011b) Influence of starvation on the critical swimming behaviour of the Senegalese sole (*Solea senegalensis*) and its relationship with RNA/DNA ratios during ontogeny. *Sci Mar* 75:87-94
- Ferron A, Leggett WC (1994) An Appraisal of Condition Measures for Marine Fish Larvae. In: *Advances in Marine Biology*, Vol 30, Book 30
- Fisher R (2005) Swimming speeds of larval coral reef fishes: impacts on self-recruitment and dispersal. *Marine Ecology Progress Series* 285:223-232
- Freamo H, O'Reilly P, Berg PR, Lien S, Boulding EG (2011) Outlier SNPs show more genetic structure between two Bay of Fundy metapopulations of Atlantic salmon than do neutral SNPs. *Mol Ecol Resour* 11:254-267
- Fuiman LA, Batty RS (1994) Susceptibility of atlantic herring and plaice larvae to predation by juvenile cod and herring at 2 constant temperatures. *J Fish Biol* 44:23-34



- Gagliano M, McCormick MI, Meekan MG (2007) Survival against the odds: ontogenetic changes in selective pressure mediate growth-mortality trade-offs in a marine fish. *Proc R Soc B-Biol Sci* 274:1575-1582
- Garcia A, Cortes D, Ramirez T, Fehri-Bedoui R, Alemany F, Rodriguez JM, Carpena A, Alvarez JP (2006) First data on growth and nucleic acid and protein content of field-captured Mediterranean bluefin (*Thunnus thynnus*) and albacore (*Thunnus alalunga*) tuna larvae: a comparative study. *Sci Mar* 70:67-78
- Gerlach G, Atema J, Kingsford MJ, Black KP, Miller-Sims V (2007) Smelling home can prevent dispersal of reef fish larvae. *Proc Natl Acad Sci U S A* 104:858-863
- Goolish EM, Barron MG, Adelman IR (1984) Thermoacclimatory response of nucleic-acid and protein-content of carp muscle-tissue - influence of growth-rate and relationship to glycine uptake by scales. *Can J Zool* 62:2164-2170
- Grant WS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. *Journal of Heredity* 89:415-426
- GrønkJær P, Clemmesen C, St. John M (1997) Nutritional condition and vertical distribution of Baltic cod larvae. *J Fish Biol* 51:352-369
- Grorud-Colvert K, Sponaugle S (2006) Influence of condition on behavior and survival potential of a newly settled coral reef fish, the bluehead wrasse *Thalassoma bifasciatum*. *Marine Ecology Progress Series* 327:279-288
- Grorud-Colvert K, Sponaugle S (2011) Variability in water temperature affects trait-mediated survival of a newly settled coral reef fish. *Oecologia* 165:675-686
- Guigand C, Cowen R, Llopiz J, Richardson D (2005) A coupled asymmetrical multiple opening closing net with environmental sampling system. *Marine Technology Society Journal* 39:22-24
- Hamilton S (2008) Larval history influences post-metamorphic condition in a coral-reef fish. *Oecologia* 158:449-461
- Hamilton SL, Regetz J, Warner RR (2008) Postsettlement survival linked to larval life in a marine fish. *Proc Natl Acad Sci U S A* 105:1561-1566
- Hamre K, Mollan TA, Sæle Ø, Erstad B (2008) Rotifers enriched with iodine and selenium increase survival in Atlantic cod (*Gadus morhua*) larvae. *Aquaculture* 284:190-195
- Haney RA, Silliman BR, Rand DM (2007) A multi-locus assessment of connectivity and historical demography in the bluehead wrasse (*Thalassoma bifasciatum*). *Heredity* 98:294-302

- Hanski I, Gaggiotti OE (2004) Ecology, Genetics and Evolution of Meta-populations, Elsevier Academic Press, San Diego
- Hare JA, Cowen RK (1991) Expatriation of *Xyrichtys novacula* (pisces, labridae) larvae - evidence of rapid cross-slope exchange. J Mar Res 49:801-823
- Hare JA, Cowen RK (1995) Effect of age, growth-rate, and ontogeny on the otolith size - fish size relationship in bluefish, *Pomatomus saltatrix*, and the implications for back-calculation of size in fish early-life history stages. Can J Fish Aquat Sci 52:1909-1922
- Hare JA, Cowen RK (1997) Size, growth, development, and survival of the planktonic larvae of *Pomatomus saltatrix* (Pisces: Pomatomidae). Ecology 78:2415-2431
- Hastings A, Botsford LW (2006) Persistence of spatial populations depends on returning home. Proc Natl Acad Sci U S A 103:6067-6072
- Hawn AT, Martin GB, Sandin SA, Hare JA (2005) Early juvenile mortality in the coral reef fish *Chromis cyanea* (Pomacentridae): The growth-mortality hypothesis revisited. Bulletin of Marine Science 77:309-318
- Hedgcock D (2010) Determining parentage and relatedness from genetic markers sheds light on patterns of marine larval dispersal. Mol Ecol 19:845-847
- Henshaw EC, Hirsch CA, Morton BE, Hiatt HH (1971) Control of protein synthesis in mammalian tissues through changes in ribosome activity. J Biol Chem 246:436-&
- Hitchcock GL, Lee TN, Ortner PB, Cummings S, Kelble C, Williams E (2005) Property fields in a Tortugas Eddy in the southern straits of Florida. Deep Sea Research Part I: Oceanographic Research Papers 52:2195-2213
- Hjort J (1914) Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. Rapp Proc Reun Con Inter l'Expl Mer 20:1-228
- Hoey AS, McCormick MI (2004) Selective predation for low body condition at the larval-juvenile transition of a coral reef fish. Oecologia 139:23-29
- Hogan JD, Thiessen RJ, Sale PF, Heath DD (2012) Local retention, dispersal and fluctuating connectivity among populations of a coral reef fish. Oecologia 168:61-71
- Holm J, Svaasand T, Wennevik V (1991) Handbook of cod culture: brood stock and fry production, Institute of Marine Research, Bergen
- Höök TO, Gorokhova E, Hansson S (2007) RNA:DNA ratios of Baltic Sea herring larvae and copepods in embayment and open sea habitats. Estuarine, Coastal and Shelf Science 76:29-35

- Hopcroft RR, Roff JC, Lombard D (1998) Production of tropical copepods in Kingston Harbour, Jamaica: the importance of small species. *Marine Biology* 130:593-604
- Houde E (1987) Fish early life dynamics and recruitment variability. *Amer Fish Soc Sym* 2:17-29
- Houde ED (1978) Critical Food Concentrations for Larvae of 3 Species of Sub-Tropical Marine Fishes. *Bulletin of Marine Science* 28:395-411
- Houde ED (1989a) Comparative growth, mortality, and energetics of marine fish larvae - temperature and implied latitudinal effects. *Fish B-Noaa* 87:471-495
- Houde ED (1989b) Subtleties and episodes in the early life of fishes. *J Fish Biol* 35:29-38
- Houde ED (2002) Mortality. In: Fuiman LA, Werner RG (eds) *Fishery science: The unique contributions of early life stages*. Blackwell Publishing, Oxford
- Houlihan DF, Pedersen BH, Steffensen JF, Brechin J (1995) Protein synthesis, growth and energetics in larval herring (*Clupea harengus*) at different feeding regimes. *Fish Physiology and Biochemistry* 14:195-208
- Hutchings JA (2011) Old wine in new bottles: reaction norms in salmonid fishes. *Heredity* 106:421-437
- Irisson JO, Guigand C, Paris CB (2009) Detection and quantification of marine larvae orientation in the pelagic environment. *Limnol Oceanogr Meth* 7:664-672
- Johannes RE (1978) Reproductive strategies of coastal marine fishes in the tropics. *Environmental Biology of Fishes* 3:65-84
- Jones GP, Milicich MJ, Emslie MJ, Lunow C (1999) Self-recruitment in a coral reef fish population. *Nature* 402:802-804
- Jones GP, Planes S, Thorrold SR (2005) Coral Reef Fish Larvae Settle Close to Home. *Current Biology* 15:1314-1318
- Kanstinger P, Peck MA (2009) Co-occurrence of European sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*) and sprat (*Sprattus sprattus*) larvae in southern North Sea habitats: Abundance, distribution and biochemical-based condition. *Sci Mar* 73:141-152
- Karnauskas M, Cherubin LM, Paris CB (2011) Adaptive Significance of the Formation of Multi-Species Fish Spawning Aggregations near Submerged Capes. *PLoS One* 6
- Killen SS, Gamperl AK, Brown JA (2007) Ontogeny of predator-sensitive foraging and routine metabolism in larval shorthorn sculpin, *Myoxocephalus scorpius*. *Marine Biology* 152:1249-1261

- Kimura R, Watanabe Y, Zenitani H (2000) Nutritional condition of first-feeding larvae of Japanese sardine in the coastal and oceanic waters along the Kuroshio Current. ICES Journal of Marine Science: Journal du Conseil 57:240-248
- Lacson JM (1992) Minimal genetic-variation among samples of 6 species of coral-reef fishes collected at la-parguera, puerto-rico, and discovery bay, jamaica. Marine Biology 112:327-331
- Lasker R (1978) The relation between oceanographic conditions and larval anchovy food in the California Current: identification of factors contributing to recruitment failure. Rapp P-V Reun Cons Int Explor Mer 173:212-230
- Lecchini D, Shima J, Banaigs B, Galzin R (2005) Larval sensory abilities and mechanisms of habitat selection of a coral reef fish during settlement. Oecologia 143:326-334
- Lee O, Danilowicz BS, Nash RDM (2007) Small-scale variability in growth and condition of dab *Limanda limanda* (L.) larvae in relation to an Irish Sea tidal-mixing front off the west coast of the Isle of Man. J Fish Biol 71:1056-1068
- Leggett WC, Deblois E (1994) Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? Netherlands Journal of Sea Research 32:119-134
- Leichter JJ, Shellenbarger G, Genovese SJ, Wing SR (1998) Breaking internal waves on a Florida (USA) coral reef: a plankton pump at work? Marine Ecology Progress Series 166:83-97
- Leichter JJ, Stewart HL, Miller SL (2003) Episodic nutrient transport to Florida coral reefs. Limnology and Oceanography 48:1394-1407
- Leis J, Hay A, Trnski T (2006) In situ ontogeny of behaviour in pelagic larvae of three temperate, marine, demersal fishes. Marine Biology 148:655-669
- Leis J, Wright K, Johnson R (2007a) Behaviour that influences dispersal and connectivity in the small, young larvae of a reef fish. Marine Biology 153:103-117
- Leis JM (2006) Are larvae of demersal fishes plankton or nekton? In: Southward AJ, Sims DW (eds) Advances in Marine Biology, Vol 51, Book 51
- Leis JM, Hay AC, Gaither MR (2011) Swimming ability and its rapid decrease at settlement in wrasse larvae (Teleostei: Labridae). Marine Biology 158:1239-1246
- Leis JM, Hay AC, Lockett MM, Chen JP, Fang LS (2007b) Ontogeny of swimming speed in larvae of pelagic-spawning, tropical, marine fishes. Marine Ecology Progress Series 349:255-267

- Lewontin RC, Krakauer J (1973) Distribution of gene frequency as a test of theory of selective neutrality of polymorphisms. *Genetics* 74:175-195
- Lied E, Rosenlund G, Lund B, von der Decken A (1983) Effect of starvation and refeeding on in vitro protein synthesis in white trunk muscle of atlantic cod (*Gadus morhua*). *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* 76:777-781
- Limouzy-Paris CB, Graber HC, Jones DL, Ropke AW, Richards WJ (1997) Translocation of larval coral reef fishes via sub-mesoscale spin-off eddies from the Florida current. *Bulletin of Marine Science* 60:966-983
- Llopiz JK, Cowen RK (2008) Precocious, selective and successful feeding of larval billfishes in the oceanic Straits of Florida. *Marine Ecology Progress Series* 358:231-244
- Llopiz JK, Cowen RK (2009) Variability in the trophic role of coral reef fish larvae in the oceanic plankton. *Marine Ecology Progress Series* 381:259-272
- Llopiz JK, Richardson DE, Shiroza A, Smith SL, Cowen RK (2010) Distinctions in the diets and distributions of larval tunas and the important role of appendicularians. *Journal Limnology and Oceanography* 55:983-996
- Lonnstedt OM, McCormick MI (2011) Growth history and intrinsic factors influence risk assessment at a critical life transition for a fish. *Coral Reefs* 30:805-812
- Lou DC, Moltshaniwskyj NA (1992) Daily otolith increments in juvenile tropical parrotfishes and surgeonfishes. *Australian Journal of Marine and Freshwater Research* 43:973-981
- Lough RG, Broughton EA (2007) Development of micro-scale frequency distributions of plankton for inclusion in foraging models of larval fish, results from a Video Plankton Recorder. *Journal of Plankton Research* 29:7-17
- Malzahn AM, Clemmesen C, Rosenthal H (2003) Temperature effects on growth and nucleic acids in laboratory-reared larval coregonid fish. *Marine Ecology Progress Series* 259:285-293
- Malzahn AM, Clemmesen C, Wiltshire KH, Laakmann S, Boersma M (2007) Comparative nutritional condition of larval dab *Limanda limanda* and lesser sandeel *Ammodytes marinus* in a highly variable environment. *Marine Ecology Progress Series* 334:205-212
- Mathers EM, Houlihan DF, McCarthy ID, Burren LJ (1993) Rates of growth and protein synthesis correlated with nucleic-acid content in fry of rainbow trout, *Oncorhynchus mykiss* - effects of age and temperature. *J Fish Biol* 43:245-263

- May RC (1974) Larval mortality in marine fishes and the critical period concept. In: Blaxter JHS (ed) The early life history of fish. Springer-Verlag, New York
- McClatchie S, Cowen R, Nieto K, Greer A, Luo JY, Guigand C, Demer D, Griffith D, Rudnick D (2012) Resolution of fine biological structure including small narcomedusae across a front in the Southern California Bight. *J Geophys Res-Oceans* 117
- McCormick MI, Hoey AS (2004) Larval growth history determines juvenile growth and survival in a tropical marine fish. *Oikos* 106:225-242
- McGurk MD (1986) Natural mortality of marine pelagic fish eggs and larvae - role of spatial patchiness. *Marine Ecology Progress Series* 34:227-242
- McGurk MD, Kusser WC (1992) Comparison of 3 methods of measuring RNA and DNA concentrations of individual Pacific herring, *Clupea pallasii*, larvae. *Can J Fish Aquat Sci* 49:967-974
- Meekan MG, Vigliola L, Hansen A, Doherty PJ, Halford A, Carleton JH (2006) Bigger is better: size-selective mortality throughout the life history of a fast-growing clupeid, *Spratelloides gracilis*. *Marine Ecology Progress Series* 317:237-244
- Miklosi A, Pongracz P, Csanyi V (1997) The ontogeny of antipredator behaviour in paradise fish larvae (*Macropodus opercularis*) .2. The response to chemical stimuli of heterospecific fishes. *Behaviour* 134:391-413
- Molony BW, Choat JH (1990) Otolith increment widths and somatic growth rate: the presence of a time-lag. *J Fish Biol* 37:541-551
- Montgomery JC, Jeffs A, Simpson SD, Meekan M, Tindle C (2006) Sound as an orientation cue for the pelagic larvae of reef fishes and decapod crustaceans. In: Southward AJ, Sims DW (eds) *Advances in Marine Biology*, Vol 51, Book 51
- Mora C, Sale PF (2002) Are populations of coral reef fish open or closed? *Trends in Ecology & Evolution* 17:422-428
- Munday PL, van Herwerden L, Dudgeon CL (2004) Evidence for sympatric speciation by host shift in the sea. *Current Biology* 14:1498-1504
- Munk P (2007) Cross-frontal variation in growth rate and prey availability of larval North Sea cod *Gadus morhua*. *Marine Ecology Progress Series* 334:225-235
- Namroud MC, Beaulieu J, Juge N, Laroche J, Bousquet J (2008) Scanning the genome for gene single nucleotide polymorphisms involved in adaptive population differentiation in white spruce. *Mol Ecol* 17:3599-3613
- Nosil P, Funk DJ, Ortiz-Barrientos D (2009) Divergent selection and heterogeneous genomic divergence. *Mol Ecol* 18:375-402

- Ohata R, Masuda R, Yamashita Y (2011) Ontogeny of antipredator performance in hatchery-reared Japanese anchovy *Engraulis japonicus* larvae exposed to visual or tactile predators in relation to turbidity. *J Fish Biol* 79:2007-2018
- Oleksiak MF (2010) Genomic approaches with natural fish populations. *J Fish Biol* 76:1067-1093
- Olson DB (2001) Biophysical dynamics of western transition zones: a preliminary synthesis. *Fisheries Oceanography*:133-150
- Olson DB (2002) *Biophysical dynamics of ocean fronts*, John Wiley & Sons
- Pannella G (1971) Fish otoliths: daily growth layers and periodical patterns. *Science* 173:1124-1127
- Paris CB, Cherubin LM, Cowen RK (2007) Surfing, spinning, or diving from reef to reef: effects on population connectivity. *Mar Ecol-Prog Ser* 347:285-300
- Paris CB, Cowen RK (2004) Direct evidence of a biophysical retention mechanism for coral reef fish larvae. *Limnol Oceanogr* 49:1964-1979
- Paris CB, Cowen RK, Claro R, Lindeman KC (2005) Larval transport pathways from Cuban snapper (*Lutjanidae*) spawning aggregations based on biophysical modeling. *Marine Ecology Progress Series* 296:93-106
- Pechenik JA (2006) Larval experience and latent effects - metamorphosis is not a new beginning. *Integr Comp Biol* 46:323-333
- Pechenik JA, Wendt DE, Jarrett JN (1998) Metamorphosis Is Not a New Beginning. *BioScience* 48:901-910
- Pelster B (2008) Gas exchange. In: Finn RN, Kapoor BG (eds) *Fish Larval Physiology*. Science Publishers, Enfield
- Pepin P (1991) Effect of Temperature and Size on Development, Mortality, and Survival Rates of the Pelagic Early Life History Stages of Marine Fish. *Can J Fish Aquat Sci* 48:503-518
- Pepin P (2004) Early life history studies of prey – predator interactions: quantifying the stochastic individual responses to environmental variability. *Can J Fish Aquat Sci* 61:659-671
- Pepin P, Dower JF, Davidson FJM (2003) A spatially explicit study of prey-predator interactions in larval fish: assessing the influence of food and predator abundance on larval growth and survival. *Fisheries Oceanography* 12:19-33

- Pepin P, Evans GT, Shears TH (1999) Patterns of RNA/DNA ratios in larval fish and their relationship to survival in the field. *ICES Journal of Marine Science* 56:697-706
- Picquelle S, Mier KL (2011) A practical guide to statistical methods for comparing means from two-stage sampling. *Fisheries Research* 107:1
- Pineda J, Hare JA, Sponaugle S (2007) Larval Transport and Dispersal in the Coastal Ocean and Consequences for Population Connectivity. *Oceanography* 20:22-39
- Planes S, Jones GP, Thorrold SR (2009) Larval dispersal connects fish populations in a network of marine protected areas. *Proceedings of the National Academy of Sciences* 106:5693-5697
- Plaza G, Ishida M (2008) The growth-mortality relationship in larval cohorts of *Sardinops melanostictus*, revealed by using two new approaches to analyse longitudinal data from otoliths. *J Fish Biol* 73:1531-1553
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of Population Structure Using Multilocus Genotype Data. *Genetics* 155:945-959
- Puebla O, Bermingham E, Guichard F, Whiteman E (2007) Colour pattern as a single trait driving speciation in *Hypoplectrus* coral reef fishes? *Proc R Soc B-Biol Sci* 274:1265-1271
- Purcell JFH, Cowen RK, Hughes CR, Williams DA (2006) Weak genetic structure indicates strong dispersal limits: a tale of two coral reef fish. *Proc R Soc B-Biol Sci* 273:1483-1490
- Puvanendran V, Brown JA (1999) Foraging, growth and survival of Atlantic cod larvae reared in different prey concentrations. *Aquaculture* 175:77-92
- Raae AJ, Opstad I, Kvenseth P, Walther BT (1988) RNA, DNA and protein during early development in feeding and starved cod (*Gadus morhua* L.) larvae. *Aquaculture* 73:247-259
- Rankin TL, Sponaugle S (2011) Temperature Influences Selective Mortality during the Early Life Stages of a Coral Reef Fish. *PLoS One* 6:e16814
- Renaut S, Nolte AW, Bernatchez L (2010) Mining transcriptome sequences towards identifying adaptive single nucleotide polymorphisms in lake whitefish species pairs (*Coregonus* spp. Salmonidae). *Mol Ecol* 19:115-131
- Renaut S, Nolte AW, Rogers SM, Derome N, Bernatchez L (2011) SNP signatures of selection on standing genetic variation and their association with adaptive phenotypes along gradients of ecological speciation in lake whitefish species pairs (*Coregonus* spp.). *Mol Ecol* 20:545-559



- Rezak R, Bright TJ, McGrail DW (1985) Reefs and banks of the northwestern Gulf of Mexico, Wiley, New York
- Richard P, Bergeron JP, Boulhic M, Galois R, Personleruyet J (1991) Effect of starvation on rna, dna and protein-content of laboratory-reared larvae and juveniles of *Solea solea*. Marine Ecology Progress Series 72:69-77
- Richards WJ (ed) (2006) Early Stages of Atlantic Fishes: An Identification Guide for the Western Central North Atlantic, CRC Press, Boca Raton, FL
- Richardson DE, Vanwye JD, Exum AM, Cowen RK, Crawford DL (2007) High-throughput species identification: from DNA isolation to bioinformatics. Molecular Ecology Notes 7:199-207
- Rivett AJ (1993) Proteasomes - multicatalytic proteinase complexes. Biochemical Journal 291:1-10
- Robert D, Takasuka A, Nakatsuka S, Kubota H, Oozeki Y, Nishida H, Fortier L (2010) Predation dynamics of mackerel on larval and juvenile anchovy: is capture success linked to prey condition? Fisheries Science 76:183-188
- Roberts CM (1997) Connectivity and management of Caribbean coral reefs. Science 278:1454-1457
- Rombough P (1988) Respiratory gas exchange, aerobic metabolism, and the effects of hypoxia during early life. In: Hoar W, Randall D (eds) Fish Physiology, The Physiology of Developing Fish, Book XI. Academic Press, San Diego New York
- Rooker JR, Holt GJ (1996) Application of RNA: DNA ratios to evaluate the condition and growth of larval and juvenile red drum (*Sciaenops ocellatus*). Marine and Freshwater Research 47:283-290
- Rooker JR, Holt GJ, Holt SA (1997) Condition of larval and juvenile red drum (*Sciaenops ocellatus*) from estuarine nursery habitats. Marine Biology 127:387-394
- Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. Molecular Ecology Notes 4:137-138
- Rosenblum EB, Novembre J (2007) Ascertainment bias in spatially structured populations: A case study in the eastern fence lizard. Journal of Heredity 98:331-336
- Rossi-Wongtschowski CLD, Clemmesen C, Ueberschar B, Dias JF (2003) Larval condition and growth of *Sardinella brasiliensis* (Steindachner, 1879): preliminary results from laboratory studies. Sci Mar 67:13-23

- Roughgarden J, Iwasa Y, Baxter C (1985) Demographic-theory for an open marine population with space-limited recruitment. *Ecology* 66:54-67
- Saenz-Agudelo P, Jones GP, Thorrold SR, Planes S (2011) Connectivity dominates larval replenishment in a coastal reef fish metapopulation. *Proc R Soc B-Biol Sci* 278:2954-2961
- Sale PF (1978) Reef fishes and other vertebrates: a comparison of social structures. In: Reese ES, Lighter FJ (eds) *Contrasts in Behaviour*. Wiley-Interscience, New York
- Sale PF, Ferrell DJ (1988) Early survivorship of juvenile coral-reef fishes. *Coral Reefs* 7:117-124
- Schlotterer C (2004) The evolution of molecular markers - just a matter of fashion? *Nat Rev Genet* 5:63-69
- Searcy SP, Sponaugle S (2000) Variable larval growth in a coral reef fish. *Marine Ecology Progress Series* 206:213-226
- Searcy SP, Sponaugle S (2001) Selective mortality during the larval-juvenile transition in two coral reef fishes. *Ecology* 82:2452-2470
- Seddon JM, Parker HG, Ostrander EA, Ellegren H (2005) SNPs in ecological and conservation studies: a test in the Scandinavian wolf population. *Mol Ecol* 14:503-511
- Serafy JE, Valle M, Faunce CH, Luo J (2007) Species-specific patterns of fish abundance and size along a subtropical mangrove shoreline: an application of the delta approach. *Bull Mar Sci* 80:609-623
- Shima JS, Findlay AM (2002) Pelagic larval growth rate impacts benthic settlement and survival of a temperate reef fish. *Marine Ecology Progress Series* 235:303-309
- Shima JS, Swearer SE (2009) Larval quality is shaped by matrix effects: implications for connectivity in a marine metapopulation. *Ecology* 90:1255-1267
- Shima JS, Swearer SE (2010) The legacy of dispersal: larval experience shapes persistence later in the life of a reef fish. *J Anim Ecol* 79:1308-1314
- Shoji J, Tanaka M (2006) Growth-selective survival in piscivorous larvae of Japanese Spanish mackerel *Scomberomorus niphonius*: early selection and significance of ichthyoplankton prey supply. *Marine Ecology Progress Series* 321:245-254
- Shulman MJ, Bermingham E (1995) Early-life histories, ocean currents, and the population-genetics of caribbean reef fishes. *Evolution* 49:897-910
- Simpson SD, Meekan M, Montgomery J, McCauley R, Jeffs A (2005) Homeward bound. *Science* 308:221-221

- Skajaa K FA, Folkvord A (2004) Swimming, feeding and predator avoidance in cod larvae (*Gadus morhua* L.): trade-offs between hunger and predation risk. In: Browman HI SA (ed). Proc The big fish bang Proc 26th Annu Larval Fish Conf
- Skajaa K, Ferno A, Folkvord A (2004) Ontogenetic- and condition-related effects of starvation on responsiveness in herring larvae (*Clupea harengus* L.) during repeated attacks by a model predator. *J Exp Mar Biol Ecol* 312:253-269
- Slatkin M, Rannala B (2000) Estimating allele age. *Annual Review of Genomics and Human Genetics* 1:225-249
- Slattery M, Lesser MP, Brazeau D, Stokes MD, Leichter JJ (2011) Connectivity and stability of mesophotic coral reefs. *Journal of Experimental Marine Biology and Ecology* 408:32-41
- Smith MAK (1981) Estimation of growth potential by measurement of tissue protein synthetic rates in feeding and fasting rainbow trout, *Salmo gairdnerii* Richardson. *J Fish Biol* 19:213-220
- Sogard SM (1997) Size-selective mortality in the juvenile stage of teleost fishes: A review. *Bulletin of Marine Science* 60:1129-1157
- Sponaugle S (2009) Daily otolith increments in the early stages of tropical fishes. In: Green B, Mapstone B, Carlos G, Begg G (eds) *Gathering information from otoliths of tropical fishes Methods and Technologies in Fish Biology and Fisheries*. Springer.
- Sponaugle S (2010) Otolith microstructure reveals ecological and oceanographic processes important to ecosystem-based management. *Environmental Biology of Fishes* 89:221-238
- Sponaugle S, Boulay JN, Rankin TL (2011) Growth- and size-selective mortality in pelagic larvae of a common reef fish. *Aquat Biol* 13:263-273
- Sponaugle S, Cowen RK (1997) Early Life History Traits and Recruitment Patterns of Caribbean Wrasses (Labridae). *Ecol Monogr* 67:177-202
- Sponaugle S, Cowen RK, Shanks A, Morgan SG, Leis JM, Pineda JS, Boehlert GW, Kingsford MJ, Lindeman KC, Grimes C, Munro JL (2002) Predicting self-recruitment in marine populations: Biophysical correlates and mechanisms. *Bulletin of Marine Science* 70:341-375
- Sponaugle S, Grorud-Colvert K, Pinkard D (2006) Temperature-mediated variation in early life history traits and recruitment success of the coral reef fish *Thalassoma bifasciatum* in the Florida Keys. *Marine Ecology Progress Series* 308:1-15
- Sponaugle S, Grorud-Covort K (2006) Environmental variability, early life-history traits, and survival of new coral reef fish recruits. *Integr Comp Biol* 46:623-633

- Sponaugle S, Lee T, Kourafalou V, Pinkard D (2005) Florida current frontal eddies and the settlement of coral reef fishes. *Limnology and Oceanography* 50:1033-1048
- Sponaugle S, Llopiz JK, Havel LN, Rankin TL (2009) Spatial variation in larval growth and gut fullness in a coral reef fish. *Marine Ecology Progress Series* 383:239-249
- Sponaugle S, Paris C, Walter K, Kourafalou V, DiAlessandro E (2012) Observed and modeled larval settlement of a reef fish to the Florida Keys. *Marine Ecology Progress Series* 453:201-212
- Sponaugle S, Pinkard DR (2004) Impact of variable pelagic environments on natural larval growth and recruitment of the reef fish *Thalassoma bifasciatum*. *J Fish Biol* 64:34-54
- Sponaugle S, Walter KD, Denit KL, Llopiz JK, Cowen RK (2010) Variation in pelagic larval growth of Atlantic billfishes: the role of prey composition and selective mortality. *Marine Biology* 157:839-849
- St. John MA, Clemmesen C, Lund T, Koster T (2001) Diatom production in the marine environment: implications for larval fish growth and condition. *Ices Journal of Marine Science* 58:1106-1113
- Strathmann R (1974) Spread of sibling larvae of sedentary marine invertebrates. *American Naturalist* 108:29-44
- Strathmann RR (2007) Three functionally distinct kinds of pelagic development. *Bulletin of Marine Science* 81:167-179
- Strathmann RR, Hughes TP, Kuris AM, Lindeman KC, Morgan SG, Pandolfi JM, Warner RR (2002) Evolution of local recruitment and its consequences for marine populations. *Bulletin of Marine Science* 70:377-396
- Suneetha KB, Folkvord A, Johannessen A (1999) Responsiveness of selected condition measures of herring, *Clupea harengus*, larvae to starvation in relation to ontogeny and temperature. *Environmental Biology of Fishes* 54:191-204
- Suthers IM (1998) Bigger? Fatter? Or is faster growth better? Considerations on condition in larval and juvenile coral-reef fish. *Austral Ecol* 23:265-273
- Swearer SE, Shima JS, Hellberg ME, Thorrold SR, Jones GP, Robertson DR, Morgan SG, Selkoe KA, Ruiz GM, Warner RR (2002) Evidence of self-recruitment in demersal marine populations. *Bulletin of Marine Science* 70:251-271
- Takasuka A, Aoki I, Mitani I (2003) Evidence of growth-selective predation on larval Japanese anchovy *Engraulis japonicus* in Sagami Bay. *Marine Ecology Progress Series* 252:223-238

- Takasuka A, Oozeki Y, Kimura R, Kubota H, Aoki I (2004) Growth-selective predation hypothesis revisited for larval anchovy in offshore waters: cannibalism by juveniles versus predation by skipjack tunas. *Marine Ecology Progress Series* 278:297-302
- Tanabe T (2001) Feeding habits of skipjack tuna *Katsuwonus pelamis* and other tuna *Thunnus* spp. juveniles in the tropical western Pacific. *Fisheries Science* 67:563-570
- Tanaka Y, Gwak W-S, Tanaka M, Sawada Y, Okada T, Miyashita S, Kumai H (2007) Ontogenetic changes in RNA, DNA and protein contents of laboratory-reared Pacific bluefin tuna *Thunnus orientalis*. *Fisheries Science* 73:378-384
- Tanaka Y, Satoh K, Yamada H, Takebe T, Nikaido H, Shiozawa S (2008) Assessment of the nutritional status of field-caught larval Pacific bluefin tuna by RNA/DNA ratio based on a starvation experiment of hatchery-reared fish. *J Exp Mar Biol Ecol* 354:56-64
- Tao WJ, Boulding EG (2003) Associations between single nucleotide polymorphisms in candidate genes and growth rate in Arctic charr (*Salvelinus alpinus* L.). *Heredity* 91
- Thorrold SR, Jones GP, Hellberg ME, Burton RS, Swearer SE, Neigel JE, Morgan SG, Warner RR (2002) Quantifying larval retention and connectivity in marine populations with artificial and natural markers. *Bulletin of Marine Science* 70:291-308
- Tocher DR (2010) Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquac Res* 41:717-732
- Torgersen T (2007) Aggregated predators and vulnerability-independent mortality of prey. *Can J Fish Aquat Sci* 64:941-955
- Trachtulec Z, Hamvas RMJ, Forejt J, Lehrach HR, Vincek V, Klein J (1997) Linkage of TATA-binding protein and proteasome subunit C5 genes in mice and humans reveals synteny conserved between mammals and invertebrates. *Genomics* 44:1-7
- Trenberth K, Jones P, Ambenje P, Bojariu R, Easterling D, Klein Tank A, Parker D, Rahimzadeh F, Renwick J, Rusticicci M, Soden B, Zhai P (2007) Observations: surface and atmospheric climate change. In: Solomon S, Qin D, Manning M, Z C, Marquis M, Averyt K, Tignor M, Miller H (eds) *Climate change 2007: the physical science basis Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge and New York

- Van Tassell CP, Smith TPL, Matukumalli LK, Taylor JF, Schnabel RD, Lawley CT, Haudenschild CD, Moore SS, Warren WC, Sonstegard TS (2008) SNP discovery and allele frequency estimation by deep sequencing of reduced representation libraries. *Nat Methods* 5:247-252
- Victor BC (1982) Daily otolith increments and recruitment in 2 coral-reef wrasses, *Thalassoma bifasciatum* and *Halichoeres bivittatus*. *Marine Biology* 71:203-208
- Victor BC (1986) Delayed metamorphosis with reduced larval growth in a coral-reef fish (*Thalassoma bifasciatum*). *Can J Fish Aquat Sci* 43:1208-1213
- Vigliola L, Meekan MG (2002) Size at hatching and planktonic growth determine post-settlement survivorship of a coral reef fish. *Oecologia* 131:89-93
- Voss R, Clemmesen C, Baumann H, Hinrichsen HH (2006) Baltic sprat larvae: coupling food availability, larval condition and survival. *Marine Ecology Progress Series* 308:243-254
- Warner RR, Robertson DR, Leigh EG (1975) Sex change and sexual selection. *Science* 190:633-638
- Webber MK, Roff JC (1995) Annual biomass and production of the oceanic copepod community off Discovery Bay, Jamaica. *Marine Biology* 123:481-495
- Werner FE, Cowen RK, Paris CB (2007) Coupled Biological and Physical Models Present Capabilities and Necessary Developments for Future Studies of Population Connectivity. *Oceanography* 20:54-69
- Westerman ME, Holt GJ (1988) The RNA-DNA ratio: Measurement of nucleic acids in larval *Sciaenops ocellatus*. *Contributions in Marine Science* 30S:117-124
- Westerman ME, Holt GJ (1994) RNA-DNA ratio during the critical period and early larval growth of the red drum *Sciaenops ocellatus*. *Marine Biology* 121:1-9
- Wilkinson L (1992) SYSTAT: the system for statistics, Vol, Evanston, IL
- Williams LM, Oleksiak MF (2011) Ecologically and evolutionarily important SNPS identified in natural populations. *Mol Biol Evol* 28:1817-1826
- Willing EM, Bentzen P, van Oosterhout C, Hoffmann M, Cable J, Breden F, Weigel D, Dreyer C (2010) Genome-wide single nucleotide polymorphisms reveal population history and adaptive divergence in wild guppies. *Mol Ecol* 19:968-984
- Wright DA, Martin FD (1985) The effect of starvation on rna-dna ratios and growth of larval striped bass, *Morone saxatilis*. *J Fish Biol* 27:479-485
- Wyatt T (1972) Some effects of food density on the growth and behaviour of plaice larvae. *Marine Biology* 14:210-216

- Yeung C, Jones DL, Criales MM, Jackson TL, Richards WJ (2001) Influence of coastal eddies and counter-currents on the influx of spiny lobster, *Panulirus argus*, postlarvae into Florida Bay. *Mar Freshw Res* 52:1217-1232
- Young KV, Dower JF, Pepin P (2009) A hierarchical analysis of the spatial distribution of larval fish prey. *Journal of Plankton Research* 31:687-700