Comparative Demography of a Coral Reef Fish: Vertical and Horizontal Spatial Variability in Bicolor Damselfish (Stegastes partitus) Traits, Survival, and Reproduction

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COMPARATIVE DEMOGRAPHY OF A CORAL REEF FISH: VERTICAL AND HORIZONTAL SPATIAL VARIABILITY IN BICOLOR DAMSELFISH (STEGASTES PARTITUS) TRAITS, SURVIVAL, AND REPRODUCTION

By

Esther Diane Goldstein

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Coral reef fish populations are composed of subpopulations across a range of depths and geographic locations that are connected through larval dispersal. As reefs decline, coral reef organisms may become dependent up habitats at the periphery of their distributions. The objectives of this dissertation were to determine the influence of habitat variability on population demographics, reproduction, survival, and the consequences to population connectivity of a common coral reef fish (bicolor damselfish: Stegastes partitus) across the Florida Shelf and the depth distribution of the species. Assessing demographic plasticity and habitat suitability for a model species across depth strata (SS: shallow shelf <10 m and DS: deep shelf 20-30 m in the Florida Keys, and MPR: mesophotic 60-70 m at Pulley Ridge) provides insight into the potential for DS and mesophotic reefs (30-150 m) to be sources of larvae and coral reef refuges. Otolith-derived traits of young fish revealed that higher productivity in the lower Florida Keys compared to the upper Florida Keys led to faster growth after only 4 days post-settlement to the reef. Habitat variation continued to affect fish throughout their life cycle, indicated by a shift toward slower growth rates and larger asymptotic sizes based on otolith-derived
ages, and populations dominated by older and larger individuals in deeper habitats. Per capita reproductive investment in spawning batch, measured as gonado-somatic index (GSI), batch fecundity, and oocyte area, was highest in MPR habitats, followed by SS, then DS. Assessment of the drivers of demographic variation suggested that low reproductive investment in DS habitats was the result of high predator densities, risk-avoidance behavior, and decreased access to planktonic food. Higher C:N ratio (lipid content), higher trophic position, and longevity, traits associated with MPR habitats, were linked with high GSI, whereas in shallower habitats there was a trade-off between fast growth and low reproductive investment per spawning batch. The consequences of habitat suitability to population connectivity were assessed based on total subpopulation egg production using depth-specific demographic parameters, population densities, and habitat availability. Fish on SS and mid-shelf (10-20 m) reefs in the Florida Keys had the highest subpopulation reproductive output, whereas fish in DS habitats had the lowest egg production. MPR fish had the lowest population densities but high habitat availability, resulting in an estimate of 14% of the total egg production from the study region sourced from Pulley Ridge. Coral reef fish exhibit a broad range of demographic plasticity, and as a result, mesophotic coral reefs are suitable environments for reef fish, with long-lived individuals and high reproductive investment per spawning batch. Mesophotic reefs are potential refuges for reef fish that can provide high condition larvae, but may be less resilient than shallower habitats due to their dependence upon long-lived individuals for population persistence. Incorporation of such spatially explicit demographic plasticity in the assessment of habitat suitability and resilience should inform and enhance the effectiveness of management strategies.
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Chapter 1: General introduction

Background

In recent decades, coral reef ecosystems worldwide have been declining at an alarming rate, and future projections predict that this pattern will continue (Gardner et al. 2003, Pandolfi et al. 2003, Mumby et al. 2006, Munday et al. 2008, IPCC 2013). The deleterious effects of climate change, atypical temperature fluctuations, and anthropogenic and natural stressors on coral reef habitats have been thoroughly studied; however, the impacts of habitat degradation on coral reef fish populations are less clear (Jones et al. 2004). If reefs continue to degrade and populations of coral reef fish are restricted to healthy reef habitats for survival, spatial variation in habitat quality may be an important component of assessing the future of coral ecosystems. Environmental parameters that vary across geographic regions and depths can affect coral reef habitat quality and population demographics of coral reef fish (Ruttenberg et al. 2005, Paddack et al. 2009). There is evidence that deeper and mesophotic coral reefs may serve as refuges that are buffered from storms, temperature fluctuations, and anthropogenic stresses that heavily impact shallower reefs (Bongaerts et al. 2010). Coral reefs across broad depth distributions share many of the same coral and fish taxa (Kahng et al. 2010), yet there are inherent differences in habitat parameters associated with depth that likely influence reef fish populations (Lesser et al. 2009, Kahng et al. 2010). Mesophotic reefs are potentially marginal environments at the periphery of the depth distribution of many coral reef fishes. Therefore, the refuge function of deeper reef habitats to supply larvae to degraded shallow reefs may be limited by habitat quality and suitability for coral reef
fishes. Comparing fish populations across geographic regions and depths provides insight into demographic plasticity and environmental constraints across both vertical and horizontal spatial scales, thus providing information about spatial variation in habitat suitability and habitat quality, and the efficacy of mesophotic coral ecosystems as reef refuges.

The majority of coral reef fishes have a bipartite life cycle, whereby reef associated adults spawn pelagic larvae that typically remain in the plankton for ~30-45 d (Lester and Ruttenberg 2005) before they metamorphose and return to the reef environment. As a result, larvae have the potential to settle in variable habitats with a range of biotic and abiotic environmental parameters that can influence traits and survival throughout ontogeny. Oceanographic conditions such as eddy-induced upwelling can affect productivity and food availability (Hitchcock et al. 2005) and lead to faster larval growth (Shulzitski et al. 2015). Temperature that affects energetics, growth rates, body size, and reproduction (Donelson et al. 2010, Trip et al. 2014) can vary across depths (Lesser et al. 2009) and geographic regions as a result of latitudinal clines (Houde 1989) and variable oceanographic conditions such as upwelling and tidal bores (Leichter et al. 1996). Spatial variation in fish communities and predator densities affect density dependent processes, competition, and mortality (Booth 1995, Forrester 1995, Hixon and Carr 1997) that can be mediated by habitat type and structure (Booth and Wellington 1998, Holbrook et al. 2000). These processes can have compounding effects throughout the life cycle of an individual starting from settlement to the reef and continuing through to maturation, influencing lifetime growth, size, and reproductive output.
For organisms with bipartite life cycles, settlement to the reef is a critical period of high mortality (Almany and Webster 2006). Food availability, population densities, and predation may differ among settlement habitats and influence growth, competition, and survival for young fish (Booth 1995, Hixon and Carr 1997, Connell 1998, Booth and Hixon 1999). Predation is often trait-mediated and linked to high mortality for individuals with unfavorable traits such as lower condition or small body size that increase the risk of gape-limited predation (Anderson 1988, Hare and Cowen 1997). Early life history traits associated with the pelagic larval stage, such as larval growth rates and size- and condition-at-settlement, can carry over to affect post-settlement survival (Searcy and Sponaugle 2001, Nemeth 2005, Sponaugle and Grorud-Colvert 2006, Hamilton et al. 2008, Shima and Swearer 2009, Fuiman et al. 2010, Rankin and Sponaugle 2011, Smith and Shima 2011, D’Alessandro et al. 2013, Shima et al. 2015), and the direction or degree of selective mortality can be mediated by the environment (Grorud-Colvert and Sponaugle 2010, Rankin and Sponaugle 2011). Therefore, spatial variation in settlement habitat across regions and depths creates the potential for local patterns in the degree and direction of selective mortality (Conover et al. 2009, Fuiman et al. 2010). The combination of environmental parameters such as food availability and local trait adaptations can influence population size and population connectivity among spatially separated habitats through post-settlement mortality of individuals and differential survival rates of newly settled fish.

After settlement to the reef environment, coral reef fishes often remain in close proximity to a settlement habitat, and are subject to the constraints of the habitat through their life cycle. Many of the same processes that influence traits and survivorship of
recruits and juveniles also affect adult individuals, such as food availability, temperature, density, and predation risk, and may have lifetime consequences associated with energetic trade-offs, growth, size, and reproduction (Donelson et al. 2010, Forrester et al. 2011, Brooker et al. 2013). Survival is a key parameter associated with juvenile fish, whereas fitness and reproduction are equally as important for adult individuals.

Maximizing reproductive contributions to future generations is a balance between energetic investment in multiple growth and reproduction processes that can be regulated by temperature, food availability, and predation risk (Heino and Kaitala 1999). Individual reproductive output and demographic rates interact with population dynamics such as density, age structure, and sex ratios to influence population level reproductive output. In the context of population connectivity, habitat suitability can affect population demographics and energetic trade-offs that determine reproductive output, larval supply, and source-sink dynamics.

Habitat quality, which affects reproduction and population connectivity, is the result of both habitat suitability for individual organisms and factors that affect population size, such as population density and the amount of available habitat (Gibson 1994). Habitat suitability can be influenced by benthic habitat preference (Holbrook et al. 2000), predation risk, competition, food quality and availability (Brooker et al. 2013), and behavioral energetic trade-offs (Chiba et al. 2007, Pavlova et al. 2010, Grol et al. 2010, Schrandt and Lema 2011). Potential responses to habitat suitability for coral reef fish include growth rate, longevity, condition, maternal reproductive investment, and fitness (Chambers and Leggett 1996, Heino and Kaitala 1999, Booth and Hixon 1999, Donelson et al. 2008). Population demographics and reproductive output at the level of
the individual combined with population size and the amount of suitability habitat, provide insight into the importance and potential larval contribution of a habitat and subpopulation to the larger metapopulation.

The amount of suitable habitat for coral reef fish may continue to decline in shallow reef environments (Jones et al. 2004), whereas expansive mesophotic coral reefs with high coral cover (Locker et al. 2010, Kahng et al. 2010) may be more resilient. Additionally, high fecundity (Holstein et al. 2015) and population connectivity between deep and shallow corals (Van Oppen et al. 2011, Serrano et al. 2014) are consistent with the refuge function of mesophotic reefs for corals. Recently, new potential threats such as lionfish invasions in the Caribbean (Lesser and Slattery 2011) and declines in coral cover (Menza et al. 2007) have been observed on mesophotic reefs, indicating that the stability of deeper reefs may be threatened. In combination, the impacts of habitat variability on early life history traits and survival, fish demographic rates, population structure and density, and total available habitat provides insight into causes and consequences of environmental variability across regions and depths, demographic plasticity of coral reef fish, habitat quality, and potential larval supply from mesophotic coral reef habitats.

**Study Species**

*Stegastes partitus* (bicolor damselfish) is a common demersal reef fish found throughout the Florida Keys, the Bahamas, and the Caribbean (Emery 1973). Adults and juveniles are territorial, with high site fidelity, a small home range (Johnson and Hixon 2010), and a reported longevity of ~7 years (Caldow and Wellington 2003). Juvenile bicolor damselfish are zooplanktivorous and also forage on benthic and planktonic algae (Emery 1973), whereas adult diets are primarily composed of zooplankton with some
planktonic algae (Booth and Hixon 1999). Demersal eggs are spawned year-round with peak spawning in summer ~1-7 d after the full moon (Schmale 1981, Robertson et al. 1988, Sponaugle et al. 2012). Males defend nests that often include multiple clutches from many female mates, and embryonic development is ~3.5 d (Knapp and Warner 1991). After hatching, planktonic larvae have a pelagic larval duration (PLD) of 22-32 days (Sponaugle et al. 2012). Settlement to the reef peaks during the new and 3rd quarter moons during which time larvae enter reef habitats and metamorphose into demersal juveniles (Sponaugle and Cowen 1996, D’Alessandro et al. 2007).

The bicolor damselfish is an ideal model study species because it is abundant, widely distributed, and has a broad depth range (Garcia-Sais 2010). In addition, the life-history traits of this species are conducive to studies of multiple life stages. Late-stage larvae are attracted to light traps for collection, juveniles settle at large sizes (~10 mm), and adults maintain territories that can be easily located for repeated observations.

The bicolor damselfish has been used as a model species in a number of previous studies, but few have addressed lifetime effects of large-scale horizontal and vertical spatial variability on traits, selective mortality, demographics, and reproduction. During the early life stages, bicolor damselfish experiences selective mortality favoring larger sizes at settlement (Rankin and Sponaugle 2011) in accordance with the growth-mortality hypothesis that larger individuals will preferentially survive (Anderson 1988). Yet survival is higher for fish with slower growth rates during the first 6 days after settlement (Rankin and Sponaugle 2011). Larval traits can also affect microhabitat preferences (Nemeth 2005), and settlement substrates with varying degrees of protection from predators affect juvenile survival (Nemeth 1998, Rankin and Sponaugle 2014). Adult S.
*partitus* demography varies across small spatial scales between backreef and forereef environments where increased competition and predation risk leads to faster growth, increased mortality, and higher reproductive output on forereefs (Figueira et al. 2008). Fish density, a spatially variable biological parameter, can also differentially affect size-selective mortality, depending on ontogeny, with selection for small sizes early in life, and larger sizes later in life stages (Johnson and Hixon 2010). Additionally, experimental studies have shown that food availability may affect multiple life stages and processes including survival (Booth and Hixon 1999) and adult reproductive output (Samhouri 2009). Despite the wealth of data on bicolor damselfish, the majority of studies that have utilized bicolor damselfish as a model species have not addressed large scale (e.g., Florida Shelf-wide) or depth-related patterns in traits, survival, demographics, reproduction, or population size. Moreover, this study explores the consequences of spatially variable environmental and biological factors on patterns of fish population demography, population connectivity, and mesophotic coral reef refuges.

**Study Site**

This study took place across the Florida Shelf at shallow shelf (SS: <10 m) and deep shelf (DS: 20-30 m) sites in the Florida Keys and mesophotic coral reef sites (MPR: 60-70 m) at Pulley Ridge on the west Florida Shelf. The Florida Keys reef tract is the only coral barrier reef system in the continental United States (Ogden et al. 1994). It is located seaward of the Florida Keys at the SE tip of the Florida Peninsula and spans ~300 km. Pulley Ridge is a mesophotic hermatypic coral reef on the west Florida Shelf that stretches ~100 km with ~30 km of known coral reef habitat at the southern portion of the ridge in depths of ~60-75 m (Jarrett et al. 2005, Locker et al. 2010). Pulley Ridge and the
Florida Keys are connected by the Loop Current that forms in the Gulf of Mexico and is fed by the Yucatan Current. The Loop Current feeds into the Florida Current that continues past the Florida Keys, and then exits the Straits of Florida to become the Gulf Stream.

The study region is influenced by dynamic oceanographic conditions. Cyclonic cold-core eddies that form along the boundary of the Loop Current are one of the dominant mesoscale features in the Florida Keys, and often move slowly through the lower Florida Keys (up to ~100 day residence time) and speed up through the spatially constrained Straits of Florida in the upper Florida Keys. Eddies are associated with delivery and advection of plankton and fish larvae to reefs habitats (Sponaugle et al. 2005, D’Alessandro et al. 2007) and upwelling of nutrient rich water that increases productivity (Hitchcock et al. 2005). Across vertical spatial scales, breaking internal waves and tidal bores deliver cooler nutrient-rich water to deeper reefs more frequently, and for longer duration, than shallower reefs (Leichter et al. 1996, 1998, 2014). Coral reefs at Pulley Ridge are primarily influenced by the Loop Current that delivers warm, clear, oligotrophic water that promotes coral growth in the low light environment at the boundary of hermatypic coral reef depth distributions (Jarrett et al. 2005).

Objectives

The overarching objective of this study was to determine causes and patterns of spatial variation in population dynamics and demographics across vertical and horizontal spatial scales throughout the life-cycle of a reef organism. A secondary goal was to assess the potential refuge function of deep shelf and mesophotic coral reef habitats to support populations of a small demersal coral reef fish and supply larvae to degraded shallow
reefs. In Chapter 2, to assess the effects of habitat variation on early life history traits (ELHTs: larval growth, PLD, settlement size, juvenile growth) and selective mortality, I compared ELHTs of four cohorts bicolor damselfish, and mortality rates of 14 cohorts, across horizontal spatial scales in the upper and lower Florida Keys. I hypothesized that the lower Florida Keys would have higher productivity, and therefore higher food availability for planktivores, as a result of eddy upwelling. To evaluate the subregional variation in nearshore environments, I compared 17 years of chlorophyll a data from the region. In Chapter 3, I assessed population demographics across a vertical scale encompassing three depths. I measured *S. partitus* recruitment, population density, size and age structure, adult growth, maximum size, and reproductive investment in shallow shelf reefs (0-10 m) and deep shelf reefs (20-30 m) of the lower Florida Keys and mesophotic reefs of Pulley Ridge (60-70 m). I conducted transect surveys to quantify differences in recruit densities and population densities across depth strata. Population age structure, individual growth rates, and maximum asymptotic size were assessed using otolith-derived ages. Comparisons of reproductive investment between depth strata were quantified using a combination of histological slides of fish ovaries, and calculations of batch fecundity. In Chapter 4, I evaluated habitat suitability across depth strata by comparing predator densities and benthic habitat composition using transect surveys, behavioral time allocation, energy allocation, and differences in fish diet and feeding. Differences in *S. partitus* diet and feeding were evaluated using a combination of gut content analysis and $\delta^{15}$N and $\delta^{13}$C stable isotopes. To address the repercussions of habitat suitability on the refuge function of mesophotic reefs, I assessed the impacts of energetic trade-offs on lipid content and fitness of individuals. The refuge function of
mesophotic reefs is contingent upon larval supply, therefore in Chapter 5 I assessed bicolor damselfish population distributions and quantified total potential reproductive output for the entire study region, across depths and subregions. Distributions, densities, and subregional population sizes of bicolor damselfish were calculated using visual survey and benthic habitat data in the Florida Keys, available from NOAA (National Atmospheric and Oceanic Administration), and ROV transect data at Pulley Ridge. Depth stratified demographic parameters from previous work (Chapter 3) were applied to subpopulation size estimates to calculate potential subregional reproductive output, and the estimated proportion total larvae that is sourced from Pulley Ridge. In combination, these studies evaluate the range of demographic plasticity and environmental influences on population dynamics of a coral reef fish throughout its life cycle, across an expansive geographic region, and the known depth distribution of the species. The results provide insight into coral reef fish population resilience and the potential refuge function of mesophotic coral reef habitats.
Chapter 2. Subregional differences in productivity lead to spatial patterns of growth and survivorship in a coral reef fish

Summary

For organisms with bipartite lifecycles, the ontogenetic shift from the pelagic larval environment to the benthic coral reef environment is a critical transition that is often associated with high mortality rates. Spatial variation in environmental parameters and settlement habitats has the potential to mediate fish traits and selective mortality. In this study, I examined subregional spatial differences in the early life history traits (ELHTs) of four cohorts of the bicolor damselfish *Stegastes partitus* in the upper and lower Florida Keys, USA. Larval ELHTs (larval growth, pelagic larval duration, settlement size) and patterns of larval trait selective mortality were consistent between the upper and lower Florida Keys. However, for juveniles, growth during the first four days post-settlement was significantly higher in the lower Florida Keys compared to the upper Florida Keys, and likely linked to high productivity of upwelled water associated with the retention of mesoscale eddies in the subregion. Mortality rates were also higher in the lower Florida Keys for fish up to three weeks post-settlement. Results of this study demonstrate that variable oceanography and settlement habitat across subregional spatial scales can affect fish traits and the composition of young fish soon after settlement to the coral reef environment.

Background

For a coral reef fish, successful settlement and recruitment to the reef requires survival through multiple life stages and environments. During the planktonic larval stage, oceanographic features and other aspects of the larval environment affect survival,
selective mortality, and the condition of larvae prior to settlement (Botsford et al. 1994, Sponaugle et al. 2011). Subsequently, fish undergo a critical ontogenetic shift from the pelagic environment to the benthic reef environment. This transition often coincides with high mortality rates (Sogard 1997) associated with changes in competition, predation, and food availability.

Variability in environmental parameters can lead to population level spatial patterns in early life history traits. Productivity and food availability are key habitat parameters that fluctuate throughout marine ecosystems, and often have a direct relationship to fish growth and condition (Jones 1986, Levin et al. 1997, Booth and Hixon 1999). In the reef environment, fish population density can also differ spatially and temporally based on local habitat (Holbrook et al. 2000, Brokovich et al. 2006) and magnitude of recruitment events (Sale et al. 1984, Holbrook and Schmitt 2003). For territorial fish species, population densities may alter growth rates by influencing food availability and energetic investment in defensive behaviors (Forrester 1990, Johnson 2008). Interactions among habitat parameters can also influence individual fish traits and potentially affect fish populations at larger subregional and regional spatial scales.

High mortality associated with the transition from the pelagic environment to the benthic reef environment creates the potential for trait-mediated selective processes that alter fish survival and spatial patterns in fish traits (Sponaugle and Grorud-Colvert 2006, Rankin and Sponaugle 2011, Di Franco et al. 2013). Fish population density can affect selective mortality by impacting the predation risk associated with risky aggressive behavior (Johnson 2008) and competition for shelter and food (Hixon et al. 2012). Additionally, post-settlement survival and fitness in the reef environment are often linked
to larval traits including growth rate, condition, and size at settlement through “carry-over” effects (Searcy and Sponaugle 2001, McCormick and Hoey 2004, Sponaugle and Grorud-Colvert 2006, Hamilton et al. 2008, Shima and Swearer 2010, Smith and Shima 2011). Extrinsic habitat variation in food availability, population density, and predation pressure in the reef environment can determine the extent to which particular larval traits confer a selective advantage (Smith and Shima 2011). Spatial variation in selection can lead to local trait adaptations, regulate connectivity among subpopulations, and influence realized population connectivity, or the individuals that settle and survive to reproduce (Figueira 2009, Clarke et al. 2010, Carson et al. 2011, Shima et al. 2015).

The lower and upper Florida Keys (LK, UK) are dynamic environments composed of transient oceanographic features and multiple habitats that likely support fish populations with different traits. The orientation change from east-west in the LK to north-south in the UK affects the physical environment of the two locations. Westward alongshore winds and currents are common in LK, in comparison to characteristic onshore winds in the UK (Lee et al. 1992). Mesoscale eddies, with horizontal scales typically less than 100 km, are a dominant feature in the Straits of Florida that often propagate offshore of the LK and upwell nutrient rich water that increases primary and secondary productivity and food availability for marine organisms (Lee et al. 1994, Hitchcock et al. 2005), and influence larval transport and delivery to reefs (Sponaugle et al. 2005, D’Alessandro et al. 2007). These eddies propagate slowly along the Florida Current front in the LK, but then speed up and shear apart through the UK, forming submesoscale eddies, with diameters on the scale of tens of kilometers, that propagate northward along the front (Lee et al. 1992, 1994, Fratantoni et al. 1998). The supply of
settlement stage larvae and subsequent recruitment are consistently higher in the LK compared to the UK and this is not explained by oceanography, suggesting that other factors such as reproductive output, growth, larval condition, or survival are responsible for the observed patterns (Sponaugle et al. 2012). The distinct environments and oceanography of the UK and LK appear to affect fish early life history traits and selection, and thus have the potential to restrict or promote subregional population connectivity in the Florida Keys.

For this study, I focused on the critical period of metamorphosis from the pelagic larval stage to the reef associated juvenile stage to assess whether subregional environmental differences in the UK and LK impact early life history traits (ELHTs: larval growth, pelagic larval duration, settlement size, post-settlement growth) and survival for a common coral reef fish. I hypothesized that higher productivity, driven by eddy upwelling, leads to faster post-settlement growth of fish in the LK. However, higher densities of new recruits to the reef environment in the LK may also increase competition and selective mortality related to ELHTs. Spatial patterns of fish traits and survival in concert with subregional patterns of productivity may provide insight into the effects of habitat variation on post-settlement fitness of individuals in local fish populations, realized population connectivity, and the degree to which subregional environmental variability affects population dynamics for coral reef fish.

**Methods**

**Study Species**

*Stegastes partitus* (bicolor damselfish) is a common demersal reef fish found throughout the Florida Keys, the Bahamas, and the Caribbean Sea (Emery 1973). Adults
and juveniles are territorial, with high site fidelity and a small home range (Johnson and Hixon 2010). Demersal eggs are spawned year-round with peak spawning during the summer ~3 d after the full moon (Schmale 1981, Robertson et al. 1988). Peak reef settlement is between the third quarter and the new moons, during which time larvae settle to reef habitats and metamorphose into demersal juveniles (Sponaugle and Cowen 1996, D’Alessandro et al. 2007). Following reef settlement, bicolor damselfish undergo an ontogenetic diet shift from a juvenile diet that consists primarily of benthic algae, to an adult diet of zooplankton and some benthic and planktonic algae (Emery 1973, Booth and Hixon 1999), making it an ideal study species to assess the impacts of subregional productivity on reef fish.

**Subregional patterns of productivity**

Chlorophyll a data, used as a proxy for productivity, were obtained from the SERC-FIU Water Quality Monitoring Network for the years 1995-2011. Water quality samples were collected seasonally, so data time points were restricted to months in which samples were collected in both the upper and lower Florida Keys to avoid confounding subregional differences in chlorophyll a with seasonal variation. For the analysis, chlorophyll a values were averaged across samples years and seasons at each of the 87 sample sites in the Florida Keys. After verifying that the assumptions of the kriging model were met, chlorophyll a data were interpolated using ordinary kriging in ArcGIS using the semivariogram method to fit the best model.

**Field collections**

Fish collections occurred from June-August in 2007 and June-July in 2008 at two replicate coral reef sites in each of the UK and LK (Fig. 2.1). Light traps were deployed
to intercept late-stage larvae as they settled to the reef (Sponaugle and Cowen 1996, Rankin and Sponaugle 2011) during 15-d time periods of peak settlement encompassing the new and third quarter lunar phases (Sponaugle and Cowen 1996, D’Alessandro et al. 2007). At each site, up to four replicate light traps were deployed 1 m below the surface and 50 m apart at sunset and then collected on the next day at sunrise. Following the appearance of late-stage larvae in the light traps, juvenile fish up to three weeks post-settlement were counted and collected weekly by divers along 15 5x1 meter transects at each of the sites in the UK and LK.

Otolith analysis

Four juvenile cohorts of S. partitus collected during June and July of 2007 and 2008 were aged using otolith microstructure analysis. The standard length of each fish was measured to the nearest 0.01 mm using a Leica MZ12 dissecting microscope and Image Pro Plus 7.0 image analysis software (Media Cybernetics). Left and right lapilli were extracted from each fish and stored in immersion oil, and one otolith per fish was chosen for microstructure analysis based on clarity and ease of reading. Daily otolith growth increments and settlement marks were enumerated using a Leica DMLB microscope at 400x magnification, a digital camera, and Image-Pro Plus software (Sponaugle 2009). Each otolith was read up to three times, and fish were included in analyses if a minimum of two otolith reads were within a 5% margin of error. The final otolith read used in the analysis was then selected randomly from the replicate reads.

Early life history traits and selective mortality

Otolith increments were used as a proxy for somatic growth after verifying a significant relationship between fish standard length and otolith radius for pooled sites
and cohorts ($p<0.001, r^2=0.87$). Each cohort was defined using a 14-d back-calculated settlement window. Otolith-derived traits were not linearly related to fish age, therefore, fish were divided into three age groups to assess trait-based selective mortality. Age groups were defined as (1) settlers collected in light traps on the night of settlement, (2) recruits that were 1-10 days old post-settlement, and (3) juveniles that were 11-20 days old post-settlement. Daily otolith-derived growth corresponding to the pelagic larval stage was averaged across two larval growth periods from 1-10 days and 11-20 days post-hatch. Post-settlement growth was averaged during the first 4 days following settlement to the reef. The analysis included only fish that were $>4$ days post-settlement, and limitations in the number of older fish available resulted in slightly smaller sample sizes for older age groups. Because temperature has a significant influence on *S. partitus* growth (Rankin and Sponaugle 2011), we obtained daily satellite derived sea surface temperature for the study period from NOAA Coast Watch (daytime Aqua-MODIS, NASA) to compare the water temperatures experienced by each cohort.

For all analyses, fish from the two replicate sites in each subregion were combined to correspond to the subregional scale of productivity data. Subregional differences in otolith-derived traits and selective mortality were compared using two-factor General Linear Mixed Models with cohort as a random factor and subregion and age group as fixed factors (R Statistical Software, lme4 package). Models were simplified if interaction terms were not significant, and Tukey post-hoc tests were used when significant differences emerged (R Statistical Software, multcomp package).
Population density and mortality

To obtain fish density and comparative mortality estimates in the UK and LK, weekly fish surveys and collections of fish up to 2 cm total length, along 15 5x1 meter transects, from June-August 2007 and June-July 2008 were used to track 14 separate S. partitus cohorts through time. Total average density of S. partitus up to three weeks post-settlement in each subregion was calculated using total counts of fish from weekly survey data. I used size frequency distributions to calculate relative mortality between the subregions. Using the least squares regression relationship between otolith-derived age and standard length from the four primary study cohorts (June and July 2007 and 2008, y=0.28x+10.65, r²=0.73, p<0.0001) I converted the standard lengths of all fish collected from transects to post-settlement age. A correction factor that incorporated zero-count fish surveys in which no fish were observed (or collected) was applied to cohort age-based fish abundance calculations to avoid over-estimating fish abundances. Calculated age-based fish abundances were averaged across all fish cohorts from June-August 2007 and June-July 2008. The decline in fish abundance with age was used to estimate mortality rates with the catch curve method. This method uses a linearized exponential mortality equation:

\[ \ln(N_t) = a + Mt \]

where \( N_t \) is the fish abundance at time \( t \), \( a \) is the intercept, \( M \) is the natural mortality rate, and \( t \) is time (Pauly 1984, Beverton and Holt 1993). The method assumes that there are no temporal trends in recruitment magnitude, and that levels of recruitment fluctuate around a mean value. However, the methods is robust to some variation in recruitment
magnitude (Meekan et al. 2001). The slope of the linear regression line, mortality rate, was compared between subregions using ANCOVA (R Statistical Software version 3.1.2).

**Results**

A total of 766 *Stegastes partitus* were used in the analysis after excluding 232 fish that were either outside of the 14-day cohort settlement window, or had otolith reads did not meet the 5% margin of error criteria (Table 2.1). Ordinary kriging interpolation predictions using averaged chlorophyll a data as a proxy for productivity (root mean square=0.06) revealed that long-term primary productivity was higher in the LK compared to the UK during a time period that included the study cohorts (Fig. 2.1). Subregional water temperatures, averaged from the first hatch date through the final collection date of all fish cohorts were within 0.5°C during each cohort sample month, and there was <2°C temperature range across the entire study period (Table 2.1). Based on the narrow temperature range throughout the study period, all cohorts were combined for analyses, but cohort was included as a random factor in the analyses.

*Fish traits and selective mortality*

There were no subregional differences in ELHTs associated with the pelagic larval stage, (larval growth days 1-10 and 11-20 post-hatch, pelagic larval duration (PLD), or settlement size; Table 2.2, Fig. 2.2a-c). However, there was evidence of consistent selective mortality related to some pelagic ELHTs in both the UK and the LK. For recruits in both subregions there was selection for faster growth during days 1-10 post-hatch. This selection was reversed for juveniles such that selection favored fish with slower growth during days 1-10 in the pelagic environment (Table 2.2, Fig. 2.2a). Selective mortality related to growth over days 11-20 post-hatch was not evident (Table
2.2). In both subregions, there was also consistently higher survival of individuals with longer PLDs and larger settlement sizes (Table 2.2, Fig. 2.2b, c). Daily post-settlement growth, averaged over the first 4 days after settlement to the reef, was faster in the LK compare to UK, but there was no evidence of selective mortality (Table 2.2, Fig. 2.2d).

**Population density and mortality**

Densities of fish up to 3 weeks post-settlement were higher, on average, in the LK compared to the UK for the 14 study cohorts (t=5.21, p<0.0001, Fig. 2.3). Mortality estimates using the natural log of the change in fish abundance with age in each subregion were higher in the LK than the UK (ANCOVA, p<0.001). The slope of the mortality regression in the LK was significant (p=0.007), with a mortality estimate of 0.02, whereas in the UK, abundances did not decrease with age precipitously to generate a significant slope and thus mortality estimate (Fig. 2.4).

**Discussion**

The Straits of Florida is a dynamic environment, and cyclonic eddies that upwell nutrient rich water, and potentially increase plankton availability for planktivorous fish, have longer residence times in the LK than the UK (Lee et al. 1994, Hitchcock et al. 2005). Chlorophyll a levels, linked to productivity, were higher in the LK compared to the UK, consistent with the hypothesis that subregional differences in oceanography lead to higher productivity, and potentially higher food availability for planktivores in the LK.

**Cohort early life history traits**

Bicolor damselfish that settled to reefs in the UK and LK had similar pelagic early life history traits with respect to larval growth, pelagic larval duration, and settlement size.
The absence of spatial patterns in ELHTs despite persistent subregional differences in the oceanographic environments, suggests that the S. partitus late stage larvae collected in the UK and LK experienced comparable pelagic larval environments, or they were robust to environmental variability. The location of larvae throughout the pelagic larval stage was unknown for this study; however, there is evidence that bicolor damselfish populations are well-mixed throughout the Bahamas (Christie et al. 2010) and the Caribbean Sea (Purcell et al. 2009), thus it is unlikely that larvae collected in the UK and LK remained in one subregion of the Florida Keys throughout larval development. The ELHTs of larvae that develop in similar water masses are expected to be analogous (Hamilton et al. 2008, Shima and Swearer 2009). However, in the Straits of Florida, S. partitus was the only one of five larval reef fish species that did not demonstrate faster recent growth in mesoscale eddies compared to those collected outside of eddies (Shulzitski et al. 2015), suggesting that larval growth may not be tightly coupled to productivity in this species.

Despite arriving to the reefs of the UK and LK with similar pelagic larval traits, there were subregional differences in growth after fish settled to the reef environment. Bicolor damselfish in the LK grew 4% faster during the first four days post-settlement than fish in the UK. Although this value is small, differences in growth were apparent after only 4 days in the reef environment despite potential physiological lags related to assimilation efficiency between feeding and growth (Elliott and Persson 1978, Armstrong et al. 2013), or carry-over from the larval stage (Shima and Swearer 2010, Smith and Shima 2011, D’Alessandro et al. 2013). Mesoscale eddies and higher productivity in the LK compared to the UK may create conditions that are favorable to faster growth for S.
*S. partitus* that recently settled to the reef environment. Cyclonic eddies have been linked to favorable conditions for fish larvae in the Straits of Florida (Hitchcock et al. 2005) and higher food availability for anchovies off the coast of Japan (Nakata 2000). Faster growth in the LK can also provide a survival advantage for young fish by minimizing the window of time when fish are most vulnerable to predation (Miller et al. 1988, Bailey and Houde 1989, Meekan and Fortier 1996), potentially mitigating high mortality associated with settlement to the reef.

**Population density and mortality**

In addition to subregional differences in growth, aged-based density and mortality calculations revealed higher recruit densities coupled with higher daily mortality of *S. partitus* in the LK. Comparatively, mortality estimates revealed higher morality in the LK than the UK indicated by a significant declining slope in the age-frequency analysis in the LK, whereas in the UK, a negative slope was not detected. These results are corroborated by a concurrent study that demonstrated a consistently higher supply of late-stage larvae to the LK, and a larger subregional difference in the magnitude of late-stage larvae compared to subregional differences in juvenile densities (Sponaugle et al. 2012), suggesting higher levels of mortality in the LK. Despite these higher levels of mortality, densities of *S. partitus* up to 3 weeks post-settlement were higher in the LK.

**Selective mortality**

Daily mortality rates were measurable in the LK and undetectable in the UK, suggesting higher mortality in the LK, but the direction of selective mortality relative to pelagic ELHTs was consistent across subregions. For young *S. partitus* in the reef environment, fish that grew more slowly during the first 10 days as larvae experienced
higher mortality than fish that grew quickly as larvae. However, this pattern changed for older fish in the reef environment, toward higher mortality for fish that grew quickly as larvae. Nonlinear selection and ontogentic shifts in selective mortality in other fish species have been attributed to a bet-hedging strategy and the benefit of maintaining genetic variability in larval growth rates (Gagliano et al. 2007, Shima et al. 2015). The composition and size of predators may also influence the degree to which mortality is selective (McCormick and Hoey 2004).

Selection for longer PLDs and larger settlement sizes was consistent in both the UK and the LK, in concert with the “bigger is better” component of the Growth Mortality Hypothesis as a means of reducing susceptibility to predation (Anderson 1988, Rankin and Sponaugle 2011). Selective mortality is primarily the result of predation (Sogard 1997, Hixon and Jones 2005), but can be influenced by competition, and water temperature, and can vary among species (Sponaugle and Grorud-Colvert 2006, Rankin and Sponaugle 2011). Similarity in the direction of selective mortality across the Florida Keys reflects the strength of selection for larger settlement sizes and longer PLDs for this species. Regardless of location, directional selection is consistent despite subregional environmental variation in productivity, post-settlement growth, and mortality.

Post-settlement environments in the upper and lower Florida Keys did not result in local adaptations or local larval trait-based selective mortality. This is contrary to other studies that report habitat-mediated differences in “carry-over effects” of larval traits on post-settlement survival at the microhabitat scale (Smith and Shima 2011), and differences in selection based on experimentally altered predation pressure (McCormick and Hoey 2004). This study addresses subregional impacts on selective mortality, so it is
possible that processes that vary within subregions including predation and microhabitat could affect selective mortality, but the impacts do not scale up to subregional patterns. Since patterns of selective mortality are consistent across the UK and LK in this study, there is no evidence that population connectivity between the upper and lower Florida Keys is restricted by differences in direction of ELHT selective mortality at the subregional spatial scale.

Conclusions

Our results revealed subregional patterns in productivity, post-settlement growth, and mortality of *Stegastes partitus*. Newly settled fish in the LK grew more quickly than fish in the UK despite higher juvenile densities that could increase competition, suggesting that food is not a limiting factor in the LK. Faster post-settlement growth in the LK was not a result of selective mortality differences between subregions. The lack of selective mortality related to post-settlement growth in this study was in contrast to a previous study showing higher survival linked to slower post-settlement growth across 13 cohorts of *S. partitus* collected in the UK (Rankin and Sponaugle 2011). Potential explanations for the difference in results include: (1) I averaged growth during the first 4 rather than the first 6 days post-settlement, and selection on post-settlement growth may require more days to be apparent than the present study allowed based on fish ages; or (2) more cohorts may be required for small but consistent differences in traits to become significant.

Subregional differences in primary productivity and oceanography in the UK and the LK likely drive differences in bicolor damselfish growth and mortality. The LK had higher productivity, higher densities of young fish, and faster post-settlement growth
rates than the UK. However, mortality rates for fish in the LK were also higher, potentially as a result of competition or predation (Miller et al. 1988). These patterns illustrate the consequences of subregional differences in settlement habitat for fish larvae. On the population level, faster post-settlement growth and higher densities of young fish, despite higher mortality are likely a result of higher productivity and cyclic replenishment of larvae during settlement pulses. Subregional differences in growth and mortality were detectable after just a few days in the reef environment. The impacts of habitat variation on fish populations have the potential to compound as fish mature to affect condition, body size, reproduction, population demographics, and population connectivity.

In sum, our results demonstrate that reef fish populations can be influenced by the interplay between dynamic oceanography and spatially variable reef-based processes. The feeding methods of planktivorous fishes may link oceanographic environments and benthic reef habitats, enabling the transfer of pelagic nutrients from frequent eddy upwelling and other transient oceanographic processes to directly influence the traits and abundance of demersal reef organisms.
Table 2.1. Summary of bicolour damselfish cohorts used in otolith analyses. Cohort name refers the settlement month and year of each cohort. Subregion refers to the upper Florida Keys (UK) and lower Florida Keys (LK) and N is the number of fish of each age group used in the analyses.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Subregion</th>
<th>Water temperature °C</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Settlers</td>
</tr>
<tr>
<td>June 2007</td>
<td>UK</td>
<td>27.13</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>LK</td>
<td>27.57</td>
<td>29</td>
</tr>
<tr>
<td>July 2007</td>
<td>UK</td>
<td>28.66</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>LK</td>
<td>29.01</td>
<td>29</td>
</tr>
<tr>
<td>June 2008</td>
<td>UK</td>
<td>27.78</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>LK</td>
<td>27.97</td>
<td>59</td>
</tr>
<tr>
<td>July 2008</td>
<td>UK</td>
<td>28.87</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>LK</td>
<td>29.09</td>
<td>57</td>
</tr>
</tbody>
</table>
Table 2.2. Results of General Linear Mixed Models for otolith-derived traits with subregion (UK and LK) and age level (S=settlers, R=recruits, J=juveniles) as fixed factors, and cohort as a random factor. Non-significance in denoted as “ns” and numbers in Subregion and Age level columns are p-values. Multiple pairwise comparisons are results of Tukey HSD, and inequality values are means ±SE.

<table>
<thead>
<tr>
<th>Subregion</th>
<th>Age level</th>
<th>Pairwise comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval growth days 1-10</td>
<td>ns</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>J &lt; S &lt; R</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0 ±0.05 &lt; 5.2 ±0.04 &lt; 5.3 ±0.04</td>
</tr>
<tr>
<td>Larval growth days 11-20</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>PLD</td>
<td>ns</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S &lt; J</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29.9 ±0.17 &lt; 30.5 ±0.19</td>
</tr>
<tr>
<td>Settlement size</td>
<td>ns</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S &lt; R &amp; S &lt; J</td>
</tr>
<tr>
<td></td>
<td></td>
<td>225.4 ±1.05 &lt; 234.0 ±1.16 &amp; 225.4 ±1.05 &lt; 235.0 ±1.27</td>
</tr>
<tr>
<td>Post-settlement growth</td>
<td>&lt;0.01</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LK &gt; UK</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.3 ±0.05 &lt; 5.1 ±0.06</td>
</tr>
</tbody>
</table>
Figure 2.1. Ordinary kriging interpolation of chlorophyll a in the Florida Keys. Values were averaged at each sample site from 1995-2011. Bicolor damselfish samples were collected from the lower Florida Keys at American Shoal (24°31.241, -81°31.041) and Looe Key (24°32.528, -81°24.891). Samples from the upper Florida Keys were collected from Pickles Reef (24°59.17, -80°24.94) and Sand Island (25°01.109, -80°22.044). Black outlines show the delineation of upper and lower Florida Keys subregions; no fish were sampled in the Middle Keys. Chlorophyll a data were provided by the SERC-FIU Water Quality Monitoring Network which is supported by EPA Agreement #X994621-94-0 and NOAA Agreement #NA09NOS4260253.
Figure 2.2. Significant differences in mean otolith-derived early life history traits among age groups (settlers, recruits, juveniles) of *S. partitus* in the upper Florida Keys (UK) and lower Florida Keys (LK), reflecting selective mortality. Traits are mean (a) larval growth during days 1-10 post-hatch, (b) pelagic larval duration, (c) settlement size, and (d) mean daily growth during the first 4 days post-settlement. Letters denote significant differences. Subregions and age groups are plotted separately for visual purposes even when there was no significant effect.
Figure 2.3. Mean density of bicolor damselfish up to three weeks post-settlement for 14 cohorts of *S. partitus* tracked in 2007 and 2008 in the upper (UK) and lower (LK) Florida Keys.
Figure 2.4. The relationship between the natural log of the mean abundance of young bicolor damselfish and age, based on otolith-derived length-age relationships for 14 cohorts of *S. partitus* tracked in 2007 and 2008 in the upper (UK) and lower (LK) Florida Keys. The slope of the regression equation corresponds to an estimated mortality rate. The regression equation and $r^2$ value is included for significant relationships.
Chapter 3. Refugia and resilience: Variable population demographics and reproduction of a coral reef fish across vertical spatial scales

Summary

With the degradation of shallow coral reefs, mesophotic coral ecosystems (30-150 m), at the depth boundary of many coral reef organisms, may be important refuges that are buffered from anthropogenic impacts, storms, and irregular temperature fluctuations. However, depth-driven differences in coral reef habitats can affect fish population demographics, and may have consequences for population resilience, persistence, and connectivity. I compared recruitment, population density and structure, size, growth, and reproductive output of the bicolor damselfish (*Stegastes partitus*) across shallow shelf (<10 m) and deep shelf (20-30 m) reefs in the Florida Keys, and mesophotic reefs (60-70 m) on the west Florida Shelf. Population densities and recruitment of juveniles decreased with depth, and population size and age distributions shifted toward older and larger individuals as depth increased. Otolith-derived ages indicated that bicolor damselfish found on mesophotic reefs reached larger asymptotic sizes and had longer lifespans than fish found in shallower habitats. Based on measurements of oocyte area and batch fecundity estimates using oocyte counts, mesophotic reef fish also had higher reproductive investment. Results of this study indicate that by supporting populations of large, long-lived individuals, mesophotic reef environments may be important sources of larvae for reef fish populations and valuable refuges for coral reef fishes.
Background

For coral reef fish populations, demographic parameters such as growth, body size, mortality, and reproduction are often linked to variation in environmental parameters across geographic regions or gradients such as latitudinal clines (Houde 1989, Schultz and Conover 1997, Billerbeck et al. 2000, Trip et al. 2014). Depth distributions of demersal coral reef fish can also impact population demographics at small spatial scales as a result of rapid changes in faunal assemblages, light, temperature, and oceanography from shallow reef environments to mesophotic depths (30-150 m, Lesser et al. 2009). Demographic patterns of coral reef fish populations across depths can have significant effects on population dynamics and reproductive output, and therefore, potential population connectivity across the depth distribution of a species.

In the dynamic coral reef environment, oceanographic features can affect fish populations by modifying the physical and biological parameters of a habitat. Eddies are associated with delivery of larvae and planktonic organisms to reef habitats, or advection of larvae away from coral reefs (Limouzy-Paris et al. 1997, Sponaugle et al. 2005, D’Alessandro et al. 2007), with spatial and temporal consequences to local fish population size, density, and age distributions (Sale et al. 1984, Armsworth 2002). Oceanographic features that upwell cold nutrient rich water, and increase food availability, can affect fish growth rates in pelagic environments (Meekan et al. 2003, Shulzitski et al. 2015), as well as growth and reproduction in benthic reef habitats (Ruttenberg et al. 2005, Chapter 2). The interplay between environmental factors such as population density, temperature, and productivity, governs population demographics, growth, and reproduction of coral reef fishes.
In addition to the effects of oceanography, temperature, and food limitation, post-settlement habitat availability, quality, and selective mortality can have substantial impacts on population structure and demographics. The availability of preferred microhabitat varies spatially, and influences fish density and population age and size structure, particularly if shelter accessibility is an important component of predator avoidance (Nemeth 1998, Holbrook et al. 2000). For young fishes, post-settlement selective mortality and the frequency of traits within a population can vary based on food availability, temperature, and location (Chapter 2, Sponaugle and Grorud-Colvert 2006, Rankin and Sponaugle 2011, Block and Steele 2014). For older fish, high population densities and predation may increase life-long selection for superior competitors, slow-growing individuals with large asymptotic body sizes (Johnson and Hixon 2010). Differences in habitat characteristics can also limit connectivity between populations by influencing selection for local trait adaptations (Marshall et al. 2010b). Post-settlement habitat characteristics mediate interactions between larval supply and mortality to influence local patch size, population dynamics (Holbrook and Schmitt 2003), and population demographics.

Trade-offs between growth and reproduction are a fundamental aspect of life-history theory that can also be habitat dependent. For a single species, trade-offs can vary between individual organisms or subpopulations, and lead to variation in demographics and reproduction. Fitness trade-offs between growth and reproduction are influenced by resource availability and mortality risk (Heino and Kaitala 1999). For example, it may be optimal to minimize reproduction when predation risk is high (Pavlova et al. 2010). For territorial species, population density can also alter energetic investment in growth
(Forrester 1990) and reproduction (Thresher 1983, Samhouri 2009). Combinations of local environment and community dynamics drive spatially variable energetic investment in costly behavioral and physiological processes.

Reef fish demographic studies typically focus on shallow water populations, however, as reef habitats decline worldwide, it is increasingly valuable to understand the resilience and stability of deeper and potentially marginal coral reef ecosystems.

Mesophotic coral ecosystems (~30-150 m deep) may be important refuges for coral reef organisms that are likely connected to shallower reef habitats through larval dispersal (Van Oppen et al. 2011, Serrano et al. 2014). Mesophotic reefs are potentially buffered from climate change, temperature fluctuations, and anthropogenic disturbances, and may be naturally protected sources of larvae for degraded coral reefs (Lesser et al. 2009, Kahng et al. 2010, Slattery et al. 2011).

Oceanographic features, the physical environment, and habitat quality and availability for reef fish can vary across vertical spatial scales. Deeper reefs are frequently exposed to cold water temperatures that are often associated with slower growth rates (Meekan et al. 2003) and larger asymptotic body sizes (Van Voorhies 1996). In the Florida Keys and islands in the Indo-Pacific, internal waves and tidal bores decrease water temperatures, and supplement food for planktivorous organisms through frequent delivery of high productivity water in reef habitats down to 30-40 m deep compared to shallower reefs (Leichter et al. 1998, 2003, 2014, Wolanski et al. 2004). For reef fishes, physiological state including growth, reproductive potential, and energy stores can be associated with depth gradients (Hoey et al. 2007). Population age and size structure are also affected by depth limitations or differences in vertical distributions of
suitable habitat for juvenile and adult individuals (Brokovich et al. 2006). Depth-driven differences in habitat and environment can affect population demographics, reproductive investment, larval condition (Berkeley et al. 2004, Marshall et al. 2010a), and larval supply. To better understand the potential refugia value of deep and mesophotic reefs, it is necessary to quantify depth-related variation in population demographics and reproduction.

In this study I compared population demographics and reproductive output of a common coral reef fish, across three depth strata that encompass the depth distribution of the study species (5-70 m) to assess the refuge function of mesophotic reefs for coral reef fish populations. I focused on four fundamental demographic parameters that likely affect population dynamics, larval connectivity, and recruitment between spatially separated subpopulations: (1) population density, (2) size and age distributions, (3) individual growth and asymptotic size, and (4) reproductive investment. Assessing depth-driven fish population demographics and reproduction is a step toward evaluating the role of deeper coral reef habitats as refuges, and the prospective benefits of additional protection for mesophotic coral habitats that may be essential for the persistence of coral reef ecosystems.

Methods

Study design

Location - The Florida Shelf reef tract encompasses coral reef habitats across a range of depths, from Pulley Ridge on the west Florida shelf in the Gulf of Mexico to the Florida Keys (Fig. 3.1). This study took place during the summer months of 2012-2015 across three depth strata on the Florida Shelf that are oceanographically connected via the Loop
Current that forms in the Gulf of Mexico and feeds into the Florida Current (Lee et al. 1994). Shallow shelf (SS, <10 m deep) and deep shelf (DS, 20-30 m deep) sites were located at two replicate reefs, American Shoal and Looe Key, in the lower Florida Keys (Fig 3.1). Pulley Ridge is a mesophotic coral ecosystem that stretches >200 km along the west Florida Platform with known hermatypic coral reef benthic habitat spanning ~32 km along the ridge (Jarrett et al. 2005). The primary mesophotic reef (MPR, 60-70 m deep) study location was at the southern portion of Pulley Ridge that is reported to have the highest coral percent cover (Fig. 3.1, Jarrett et al. 2005).

**Study species** - The bicolor damselfish, *Stegastes paritus*, is a common demersal coral reef fish with a broad depth range (Garcia-Sais 2010, Kahng et al. 2010), high site fidelity, and a small territorial home range (Johnson and Hixon 2010). Demersal eggs are spawned year-round with peak spawning in summer ~3 d after the full moon (Schmale 1981, Robertson et al. 1988). Nests are defended by males during ~3.5 d of embryonic development (Knapp and Warner 1991), and after hatching, planktonic larvae have a mean pelagic larval duration of 30 days (Sponaugle and Cowen 1996). Settlement to the reef peaks in the summer months during the new and 3rd quarter moons during which time larvae enter reef habitats and metamorphose into demersal juveniles (Sponaugle and Cowen 1996, D’Alessandro et al. 2007).

**Field collections** - Temperature was recorded at each depth strata throughout the study period from 2012-2014 (10 minute intervals using Onset Hobo data loggers at SS and DS sites; 60 minute intervals using an ADCP at MPR depths). During the summers of 2012-2013 (July-September 2012 and July-August 2013) divers surveyed recruits along 15 5 m x 1 m transects at each SS and DS site during monthly 7-d periods of peak settlement.
following the 3\textsuperscript{rd} quarter moon. All bicolor damselfish recruits <2 cm total length (TL) were counted to quantify settlement magnitude for monthly fish cohorts. Additionally, 25 m x 2 m transect bicolor damselfish surveys were completed by SCUBA divers at SS (n=39 transects) and DS (n=39 transects) sites. At MPR sites in the summers of 2012 and 2013, a total of 99 ROV (UNCW \textit{Super Phantom S2}) video transects were recorded during daylight hours, each covering a 100 m distance with a 5 m width field of view. ROV transects were scored to quantify \textit{S. partitus} densities and benthic habitat. Detailed methods are described in Reed et al. (2014).

Bicolor damselfish were collected by divers using hand nets quinaldine anesthetic. For accurate quantification of population age structure, SCUBA divers collected every \textit{S. partitus} along a subset of 25 m x 2 m transects in the Florida Keys (SS n=5, DS n=7), and technical divers randomly collected bicolor damselfish that were encountered at Pulley Ridge. Targeted fish collections aimed to encompass the largest and smallest individuals found in each depth strata to represent the entire span of size and age distributions. All bicolor damselfish were measured to the nearest 0.01 mm using digital calipers, and the sex of each fish was determined visually following dissection. SS and DS fish were weighed (wet) to the nearest 0.01 g and stored frozen at -80°C. Bicolor damselfish from MPR were immediately measured and frozen in liquid nitrogen for later storage at -80°C. During peak spawning, 1-7 d after the full moon, a subset of MPR fish were carefully dissected to extract ovaries for preservation in 10% phosphate buffered formalin, and the bodies were frozen. To account for any loss of body weight from the preservation process, weights were converted to fresh weight using the formula:

\[ \text{Weight}_{\text{wet}} = 0.04 + 1.06 \times \text{Weight}_{\text{frozen}}, \] from Thorstad et al. (2007) that incorporates loss of
liquid from the thawing. Formalin preserved ovary weights were converted to fresh weight following Hunter et al. (1985) and added to body weights to obtain total body weight. However, results of statistical analyses were equivalent with and without weight conversions. During peak spawning, SS and DS fish ovaries were dissected and preserved in 10% phosphate buffered formalin. Ovaries from all depth strata were fixed in 10% phosphate buffered formalin and then transferred to 70% ethanol for storage.

**Laboratory processing**

*Otolith ageing* - Otoliths were removed from fish, and one sagitta was randomly selected from each individual and imbedded in crystal-bond thermoplastic glue on a glass microscope slide. The sagitta was polished to a thin transverse section that included the primordium. Otoliths were digitally photographed at 100x magnification using a Leica DMLB microscope and an Infinity 2 digital camera, and annual otolith increments, validated by Caldow and Wellington (2003), were enumerated. Otoliths were aged by the same reader with a minimum of 1 wk between replicate reads. If two of three reads did not match, the otolith was not used in the analysis. If the fish was <1 year old, a second digital image was photographed using an oil immersion lens at 400x magnification so that daily otolith increments could be enumerated. As bicolor damselfish age, daily otolith increments become increasingly more difficult to discern, so young fish were only included in the analysis if they were less < 75 d post settlement or < 25 mm standard length (SL). Daily otolith increments were enumerated twice, once along each of the two longest axes of growth, and ages were rounded to the nearest 10\textsuperscript{th} of a year for analysis. If
the two reads did not round to the same age, then the otolith was excluded from the analysis. A total of 17 fish, out of a total of 189, were excluded from the analyses based on aging criteria.

Reproduction - Reproductive investment was measured using multiple metrics that focused on female fecundity and investment in oocytes based on previous evidence that bicolor damselfish are not mate limited, and density dependent processes affect female mating frequency more than males (Samhouri 2009). An instantaneous relative measure of reproductive potential, gonado-somatic index (GSI) was calculated using the ratio of fish ovary weight to body weight * 100, with a larger GSI indicating higher reproductive investment and reproductive potential. GSI can be influenced by multiple factors that affect ovary weight, such as ovary maturity and stage, oocyte size, recent spawning, or reabsorption of oocytes. Therefore, additional metrics of reproductive potential were also measured.

Bicolor damselfish have asynchronous ovary development with indeterminate fecundity, consequently, fecundity was calculated using an estimate of batch fecundity, or the number of oocytes released per spawning (Murua et al. 2003). To quantify batch fecundity, total oocyte counts for each ovary were calculated using the gravimetric method (Hunter et al. 1985) and then refined using the proportion of late-stage oocytes in each ovary calculated from histological slides (Hunter and Goldberg 1980). Whole ovaries were weighed to the nearest 0.001 mg following preservation in 10% phosphate buffered formalin and transfer to 70% ethanol. Histological sections of ovaries were cut at 5 µm widths and stained with haematoxylin and eosin. For all histological sections, digital photographs were taken at 10x magnification using a Leica DMLB microscope.
and an Infinity 2 digital camera. Subsequent image analysis was performed using Image Pro Plus 7.0 image analysis software (Media Cybernetics).

Differences in oocyte size distributions between ovary lobes and location along the ovary are uncommon in teleosts (West 1990), however, to verify that oocyte development was similar throughout the ovary; anterior, middle, and posterior transverse histological sections were made for a subset of whole fish ovaries (n=8). Ovary sections were photographed, oocytes were staged, and the longest and shortest diameters were measured for each oocyte that intersected a transect line through the longest axis of the histological section. Longest and shortest oocyte diameters were averaged and ovary lobe (left or right) and position (anterior, middle, posterior) were compared using a repeated measures linear mixed effect model with individual fish as the repeated measure (R statistical software, nlme package). No differences were found in oocyte diameter between left and right ovary lobes (p > 0.05) or position (p > 0.05). Therefore, for the remainder of the ovaries that were used in the analysis of batch fecundity, left and right ovary lobes were separated, one lobe was randomly selected for gravimetric ovary counts, and the other was used for histological sectioning.

Gravimetric oocyte counts were quantified by weighing three replicate subsamples from each fish ovary taken from the anterior, middle, and posterior of the ovary (Murua et al. 2003). All oocytes in each subsample were counted, excluding the primary growth phases (chromatin nucleolar and perinucleolar, West 1990). Oocyte counts mg\(^{-1}\) of weight were averaged between the three subsamples for each fish and then extrapolated to the total weight of the ovary to obtain estimates of total yolked oocyte counts for each fish.
To calculate batch fecundity, the gravimetric counts of total yolked oocytes were refined using histological sections for each ovary. Due to low sample sizes of fish with hydrated oocytes, batch fecundity calculations included females with tertiary yolk stage and migratory nucleus stage oocytes. Similar studies using the Mediterranean sardine (*Sardina pilchardus sardine*, Ganias et al. 2004) and a tropical Lutjanidae (*Lutjanus carponotatus*, Evans et al. 2008) found that including females with tertiary yolk stage and migratory nucleus stage oocytes in batch fecundity calculations yielded similar results to only hydrated oocyte fecundity calculations. Late-stage oocytes (tertiary yolk and migratory nucleus stages) could not be distinguished visually from secondary yolk oocytes using whole oocytes, so oocytes were staged histologically. Using image analysis software, a grid overlay was applied to a digital image of each histological section and all oocytes located at the intersection of the grid lines were staged following West (1990). Eight oocyte stages, in order from least to most developed, were identified: chromatin nucleolar (CN), perinucleolar (PN), cortical alveolar (CA), primary yolk (PY), secondary yolk (SY), tertiary yolk (TY), migratory nucleus (MNS), and hydrated oocytes (HO). To quantify only late-stage oocytes in batch fecundity calculations for each fish, the ratio of late-stage oocytes to total oocyte counts, excluding primary growth phases, from the histological sections was applied to the counts of whole oocytes from the gravimetric method. Batch fecundity calculations excluded ovaries with evidence of atresia or post-ovulatory follicles to avoid underestimating fecundity (Hunter et al. 1985, West 1990). If the nucleus was visible in the digital image of the histological section, then the area of the oocyte was calculated by outlining the oocyte circumference using Image Pro Plus software.
Total reproductive investment incorporates energetic investment at the level of individual oocytes, batch size, and frequency of spawning. The first two metrics were calculated for individual fish, whereas, a relative estimate of spawning frequency for each depth strata was calculated using a combination of the proportion of female fish with post-ovulatory follicles, or evidence of recent spawning, based on histological slides, and a temperature dependent post-ovulatory follicle reabsorption rate. Follicle reabsorption rates for bicolor damselfish were based on the study by Ganias et al. (2007) that showed that *Sardina pilchardus* post-ovulatory follicles were no longer visible after 80 hrs post-spawning in an average water temperature of 15.6°C and reabsorption increased by 3% for each 1°C increase in water temperature. Depth-specific mean temperatures were calculated for the study period of peak spawning for bicolor damselfish to calculate a reabsorption rate using the relationship described by Ganias et al. (2007).

**Data analysis**

*Temperature* - Temperature time series were plotted visually to compare general patterns among depth strata. To discern temperature fluctuations on smaller time scales, daily minimum were subtracted from daily maximum temperatures at each strata, and values were plotted as boxplots.

*Fish density and population structure* - Bicolor damselfish recruit densities calculated from transects for each cohort ($N_{\text{transects}}=15$ per cohort) were averaged for each sample site (American Shoal and Looe Key) within each depth strata so that site, rather than transect, was treated as a replicate for analysis. SS and DS recruit densities were compared using Two-way ANOVA with depth and cohort as factors, and a Tukey post-hoc test. Bicolor damselfish densities from 25 m x 2 m visual transects at SS and DS
habitats were based on stratified random visual surveys in reef habitats, whereas ROV transects from MPR included all benthic habitats. Live biota percent cover, assumed to be reef, at MPR was 57.09% in the survey area (Reed et al. 2014). Therefore, to make the datasets comparable, ROV data used for analysis included only surveys conducted during daylight and excluded 56 transects out of a total of 99 in which no bicolor damselfish were observed, with the assumption that these environments were not reef habitats. Therefore ~43% of the surveys were assumed to be reef habitat, which is approximately the same scale of coral cover estimated from ROV surveys (~57%). Even after excluding surveys with no bicolor damselfish observations, ROV fish counts may be conservative estimates of population densities at MPR depths compared to visual surveys (Andaloro et al. 2013, Laidig et al. 2013). Total bicolor damselfish densities between depths were compared using Kruskal-Wallis tests and post-hoc Dunn’s test with Bonferroni corrections.

To compare population size and age frequency distributions, SS and DS distributions included random collections in which all fish were measured and aged, and MPR distributions included fish that were selected randomly from collections. Frequency distributions were compared between strata using 2-sample Kolmogorov-Smirnov tests with Bonferroni corrections for multiple comparisons.

Size and age - Differences in bicolor damselfish growth among depth strata were evaluated using fish ages obtained from otolith analyses. Individual fish for otolith aging were selected from both random and targeted collections to include the entire span of ages and sizes within each population. Males and females were analyzed separately but
because the sex of immature fish could not be determined, the same young individuals were used for both male and female growth curves.

Age and size relationships were modeled using the Von Bertalanffy (VB) growth model with the formula: $L_t = L_\infty (1 - e^{-k(t-t_0)})$. The parameters were defined as: $L_t =$ standard length at time $t$, $L_\infty =$ theoretical asymptotic length, $k =$ growth coefficient that determines the rate at which length approaches $L_\infty$ (based on the steepness of the curve), $t =$ fish age in years, and $t_0 =$ hypothetical age at length 0. Growth curves were compared using likelihood ratio methods following Kimura (1980). Models were fit with nonlinear least squares, and model parameters were compared among populations using likelihood ratios with a general model that incorporated individual parameters for each population, and four sub models, each with a shared VB parameter. Parameter differences were depicted graphically using bivariate 95% confidence ellipses around parameter estimates of $k$ and $L_\infty$. The oldest fish from the MPR strata (n=3) were treated as outliers for model fitting because the method is sensitive to differences in age distributions among populations (Kimura 1980).

Reproduction - Size at maturity of female fish was calculated using SL (mm) and the presence of visible ovaries following dissection. Female size at maturity in each depth strata was calculated with logistic regression using a general linear model with binomial error terms and a logit link. GSI was compared among strata with one-way ANOVA after verifying that the assumptions of the model were met. Batch fecundity differences were assessed using ANCOVA with fish body weight as a covariate. To compare oocyte diameter among depth strata, average oocyte diameters for each oocyte stage were first calculated for individual fish to mitigate the effects of individual variation in oocyte
diameter. To avoid comparisons of oocyte area between oocyte stages, an ANCOVA model was fit for each oocyte stage with fish body weight as a covariate, p-values were Bonferroni corrected for multiple comparisons, and values were plotted as adjusted batch fecundity using ANCOVA relationships for each model. Final sample sizes of bicolor damselfish for each analysis differed based on the goal and criteria of the method (Table 3.1), and all analyses were implemented using R version 3.1.2.

Results

Water temperature was inversely related to depth, with the coldest temperatures in MPR habitats (Fig 3.2a). Seasonal water temperature fluctuations were more pronounced at SS and DS strata compared to MPR (Fig 3.2a). Prominent sub-seasonal temperature fluctuations occurred at MPR depths, particularly during the summer of 2013 (Fig. 3.2a), whereas the DS strata had the greatest daily temperature fluctuations, and MPR the smallest (Fig 3.2b).

Fish density and population structure

Bicolor damselfish recruit density during peak settlement was variable both temporally and spatially across strata. There was a significant difference between SS and DS in the Florida Keys, with higher recruitment in SS habitats. However, recruitment also differed between cohorts, and there was a significant interaction between cohort and stratum (Table 3.2). SS habitats showed greater variability among cohorts with a large settlement pulse in August 2012 that was significantly greater than all other SS cohorts (Tukey post-hoc tests: p < 0.05; Fig. 3.3). In comparison, DS habitats had lower, but
more consistent bicolor damselfish recruit densities (Table 3.2, Fig. 3.3) with no significant pairwise differences between cohort recruit densities (Tukey post-hoc tests: \( p > 0.05 \)).

Bicolor damselfish population densities decreased with depth (Fig. 3.4, Table 3.2). Total population densities were highest in SS strata, followed by \( \sim50\% \) decrease in median density with each consecutive depth strata, and a narrower range of densities observed as depth increased (Fig. 3.4, Table 3.2). Size and age frequency distributions indicated that SS and DS populations were skewed toward smaller and younger individuals with an overall pattern of declining frequency of individuals as size and age increased (Fig. 3.5a, b). In contrast, MPR size and age distributions were significantly different than SS and DS (Table 3.2) and more closely followed a Gaussian distribution (Fig. 3.5). MPR habitats had very few small, young fish, and distributions were shifted toward larger and older individuals compared to SS and DS (Fig. 3.5). SS and DS distributions were not significantly different (Table 3.2), however, DS habitats did have fish in a larger size class than SS (Fig. 3.5a), consistent with a shift in size distributions towards larger fish as depth increases. In all depth strata, males dominated the largest size classes, particularly in DS habitats, where the oldest age group was primarily composed of males (Fig. 3.5b).

**Size and age**

Von Bertalanffy growth curve comparisons indicated a pattern of slower growth (\( k \)) and larger asymptotic size (\( L_\infty \)) as depth increased for both males and females (Figs. 3.6 and 3.7; Table 3.3). Growth differences between male and female bicolor damselfish were not directly compared because growth parameters may have been influenced by
similar early growth trajectories of young fish within strata. However, despite these limitations, the pattern of smaller asymptotic size for female fish compared to males was consistent across all depth strata (Fig. 3.7). All likelihood ratio pairwise comparisons between depth strata were significant, with MPR growth patterns being the most distinctive with significant differences in all model parameters (Tables 3.2 & 3.3). Male bicolor damselfish from SS and DS differed in overall growth trajectories, however, differences in the growth models were primarily related to maximum asymptotic size, and the growth rate parameter alone (k) was not significantly different (Table 3.2). For female fish comparisons between SS and DS, the full model incorporating all growth parameters (k, \( L_\infty \), \( t_0 \)) was significantly different. However, the most biologically meaningful growth parameters, growth rate and asymptotic size, were not significantly different individually (Table 3.2, Fig. 3.7).

Reproduction

Based on logistic regression results, female bicolor damselfish had a 55% probability of maturity at 40 mm SL, and a 95% probability at 44.5 mm SL at all depth strata (Table 3.2). Temperature dependent follicle reabsorption rates and proportion of females spawning suggested a greater frequency of spawning for SS fish. The proportion of females with post-ovulatory follicles, or females that recently spawned, was highest in SS habitats, and similar between DS and MPR habitats (Table 3.4). Using temperature dependent follicle reabsorption rates, post-ovulatory follicles for SS and DS ovaries reflected recent spawning within 2 days of collection compared to 3 days for MPR fish (Table 3.4).
Mean GSI did not follow a consistent trend with depth but differed significantly between all depth strata (Fig. 3.8a). MPR had considerably larger GSI than both SS and DS, indicating higher reproductive investment (Table 3.2, Fig. 3.8a). The relationship between batch fecundity and depth stratum followed a similar pattern to GSI, although the only significant difference was higher batch fecundity, adjusted for body weight, in MPR habitats compared to the DS strata (Table 3.2, Fig. 3.8b). Differences in oocyte area, adjusted for fish body weight, also indicated larger oocyte area in MPR habitats compared to SS and DS. The MPR strata had significantly larger oocyte area in four of eight oocyte stages that were measured. Differences were not significant for the primary oocyte growth stages (CN and PN) or the two latest stages of oocyte development (MNS and HO) prior to spawning (Table 3.2, Fig. 3.9).

**Discussion**

In coral reef ecosystems, the environment can change rapidly along depth gradients and have impacts on coral reef fish populations. Demographic and reproductive patterns of bicolor damselfish revealed ecological and environmental differences and constraints between depth strata. Depth related fish demographic variation also elucidates potential causative factors that drive population dynamics across depths, and provides insight into population connectivity across vertical spatial scales and the refuge function of mesophotic reef habitats.

Fundamental population demographic parameters including population density, age frequency, and size structure are influenced by the magnitude of larval settlement, post-settlement growth, and longevity (Forrester 1995, Schmitt and Holbrook 1999a, 1999b). Shallow shelf and deep shelf strata in the Florida Keys are likely to experience
temporally coupled oceanographic features, such as the passage of mesoscale and sub-mesoscale eddies that can deliver pulses of larvae to reefs (Sponaugle et al. 2005). Despite the potential for temporally coupled oceanography, the magnitude and variability of larval settlement differed across depths, with significantly lower recruitment in DS habitats, suggesting that factors other than geography influence fish settlement magnitude. Recruitment was also temporally more variable in SS habitats compared to low but consistent monthly recruitment in DS habitats, implying that oceanographic conditions that bring pulses of larvae to reefs in the Florida Keys, may have a greater impact on SS habitats than deeper reefs. An ideal settlement depth can be determined by costs associated with the post-settlement depth range and preference of a species, or differences in habitat suitability (Srinivasan 2003, Brokovich et al. 2006). Alternatively, the distributions of settlement stage larvae within the water column (Lecchini et al. 2013), or the strength or source of settlement cues (Huijbers et al. 2012, Igulu et al. 2013, Piercy et al. 2014) may differ across strata leading to variation in recruitment magnitude and lower recruit densities in deeper habitats.

Post-settlement processes and mortality in the reef environment can significantly influence fish population size and age structure (Shulman and Ogden 1987). As depth increased, population densities of bicolor damselfish consistently decreased. Differences in bicolor damselfish densities across depth strata have the potential to affect population dynamics by influencing density-dependent competition and mortality (Hixon and Carr 1997, Hixon and Jones 2005). Densities decreased with depth, whereas, population age and size frequency distributions were roughly similar between the two depth strata in the Florida Keys. Age distributions had a more prominent decline in frequency from age 1 to
2 in SS compared to DS habitats, consistent with lower recruitment of young fish as depth increased, but potentially higher mortality in SS habitats.

Density, age, and size frequency distributions revealed that MPR habitats were unique in supporting significantly lower population densities and fewer young bicolor damselfish in comparison to the other strata. These data suggest that population replenishment of young fish may be an important factor that maintains high population densities in shallow habitats. The lack of young fish in MPR habitats could be driven by either recruitment or mortality, or a combination of the two factors. Evidence of lower recruitment of young fish in DS compare to SS strata and the consistent shift from populations dominated by young fish toward larger and older fish as depth increases, indicates that MPR habitats likely experienced very low recruitment of bicolor damselfish. Age-frequency distributions showed the highest frequency of individuals less than 1 yr old in SS and DS habitats, indicating high recruitment and consistent influx of young fish. In comparison, age distributions in MPR habitats peaked for older age groups, ~4 yrs old, suggesting infrequent settlement and recruitment. Few studies have quantified fish recruitment magnitude to mesophotic habitats, although there is evidence to suggest lower recruitment for many fish species on deeper reefs (Srinivasan 2003), and fish abundance generally decreases with depth (Garcia-Sais 2010). Age distributions also demonstrate that longevity of *S. partitus* is higher at mesophotic depths, with maximum fish ages of 13 yrs in MPR habitats, 6 yrs in DS, and 5 yrs in SS habitats.

Bicolor damselfish growth trajectories and reproductive output differed across strata, revealing that population differences were not restricted to larval supply and mortality, but that post-settlement environmental parameters and habitat constraints also
had significant sub-lethal impacts on individual fish. Male and female bicolor damselfish
grew slower and reached larger asymptotic sizes as depth increased. This pattern of
growth across depths is in accordance with the ecological and physiological effects of
Water temperatures decreased as depth increased, however, differences in temperature
between MPR habitats and shallower depth strata were greater in the summer than in winter. Adult growth was affected by annual water temperatures, whereas, recruitment
and reproduction both occur in the summer months when temperatures were the most
divergent. Summer temperature differences across strata have the potential to affect two
crucial processes in the life cycle of a reef fish that occur in the summer months: critical
early post-settlement growth when mortality is high, and reproduction. Slower growth for
young fish is generally associated with a protracted time period of high predation risk
(Anderson 1988). However, for bicolor damselfish, temperature dependent growth has
fitness consequences during the first week post-settlement, with higher survival for fish
with larger settlement size and slower post-settlement growth (Rankin and Sponaugle
2011). Differences in asymptotic body size across depths can have significant
consequences related to per capita reproductive output that scales isometrically to body
mass in teleost fish (Wooton 1990), however, colder temperatures may slow oocyte
development (Yoneda et al. 2014). In combination, slow growth and large asymptotic
sizes in mesophotic habitats may have beneficial ramifications related to survival and
high per capita reproductive output, but colder temperatures may reduce the frequency of
spawning.
Even after accounting for body size, gonado-somatic index, oocyte area, and batch fecundity were greater in mesophotic habitats, suggesting that factors other than size affect reproductive investment. The process of reproduction is influenced by the physical environment and complicated by the interactions between body size, batch size, oocyte area, and frequency of spawning. Reproduction in teleosts is sensitive to thermal regimes, often with optimal temperature ranges that maximize reproductive output (Ruttenberg et al. 2005, Pankhurst and Munday 2011). Colder temperatures in mesophotic habitats coincided with larger oocyte area, which is consistent with the effects of temperature on oocyte size in other fish species (Laptikhovhovsky 2006, Donelson et al. 2010, Yoneda et al. 2014). However, colder water temperature may also slow oocyte developmental and incubation period (Ganias et al. 2007, Pankhurst and Munday 2011), suggesting that MPR fish likely spawn less frequently than fish in SS and DS habitats, leading to trade-offs between spawning frequency and offspring provisioning (Maddams and McCormick 2012). Colder water temperatures and a lower proportion of females spawning in mesophotic habitats indicates that individual spawning frequency related to both oocyte development and mating frequency were likely lowest in MPR habitats and highest in SS habitats, suggesting a potential equilibrium between reproductive investment in spawning batch and frequency of spawning.

Oocyte area for mid-developmental stage oocytes was significantly different among strata, whereas the earliest and latest stages were not. The lack of significant differences when comparing primary oocyte growth stages can potentially be explained by the fact that early developmental stages are prior to maternal investment in yolk formation (West 1990). Whereas, the two latest stage oocytes that were not significantly
different had smaller sample sizes compared to other stages, and HO oocytes had irregular shapes, due to the histological sectioning process, making area measurements less precise. Larger oocyte area indicates higher maternal investment, larger larval size at hatching, better condition larvae, and a higher likelihood of survival in the pelagic environment (Marshall et al. 2010a, Donelson et al. 2010). Therefore, bicolor damselfish in mesophotic habitats may spawn less frequently but likely produce better condition larvae than fish on shallower reefs.

In addition to oocyte area, batch fecundity, which has been linked to differences in food availability rather than temperature (Yoneda et al. 2014), was higher in mesophotic habitats. Patterns of sub-seasonal temperature fluctuations suggest different oceanographic processes may influence reef habitats across depths such as upwelling, and shifts and meanders in the prevailing currents. Pulley Ridge had the largest sub-seasonal shifts in temperature, and the oceanography of the southwest Florida Shelf at Pulley Ridge is primarily influenced by the meandering Loop current that brings clear, low nutrient water onto the Florida Shelf, creating conditions conducive to coral growth (Jarrett et al. 2005). Deep shelf reefs in the Florida Keys had the highest daily fluctuations in temperature, likely associated with semidiurnal delivery of cold nutrient rich water by tidal bores (Leichter et al. 2003, 2014). Near the Florida Shelf, the deep chlorophyll maximum is located at approximately the same depth as MPR sites, 60-70 m, and upwelling and tidal bores regularly bring nutrient rich water onto reefs at the same depth as the DS strata (Lesser et al. 2009). Oceanographic processes imply higher nutrients and food availability as depth increases, corroborated by a higher prevalence of zooplanktivorous fish (Brokovich et al. 2008) and a shift from coral to sponge-dominated
habitat (Lesser et al. 2009) on mesophotic reefs. The hypothesis of higher food availability or quality with increasing depth is supported in mesophotic habitats where the combination of higher batch fecundity and larger oocyte area cannot be entirely explained by fish body size or temperature. However, deep shelf habitats had the lowest GSI and batch fecundity, suggesting that food availability may not be the only driver of reproduction, and that behavioral or physiological processes related to energy and time allocation that vary across depths (Chapter 4) also likely affect reproduction.

Bicolor damselfish exhibited trade-offs between growth, reproduction, and longevity. Fish in high population density SS habitats demonstrated a trade-off of fast growth but small body size, moderate batch fecundity and oocyte area, and likely frequent spawning. In contrast, bicolor damselfish in low-density MPR habitats had slower growth, but batch fecundity was consistently higher than expected based on body size alone, oocytes were larger, spawning was less frequent, and individuals were long-lived. The two life history strategies suggest trade-offs to maximize reproduction in differing habitats, and have the potential to ultimately result in similar lifetime reproductive output. Total fecundity and reproductive investment are a combination of energetic investment in individual offspring (oocyte area), batch size, and also frequency of spawning. In SS habitats, fish have shorter lifespans (maximum ~6 yrs) and potentially spawn more frequently (based on temperature, Yoneda et al. 2014), but smaller oocyte area suggests lower condition larvae. In mesophotic habitats fish have longer lifespans (maximum ~13 yrs), spawning is likely less frequent, but batch size and oocyte area are greater. For long-lived individuals, less frequent spawning, but large batch size and higher condition larvae may be a preferential strategy to maximize offspring survivorship.
and lifetime reproductive output. Whereas, individuals with short lifespans may spawn more frequently to maximize lifetime reproduction, at the expense of smaller batch sizes and lower condition larvae. In the Florida Keys, faster growth, larger asymptotic size, and high reproduction have been reported for *S. partitus* in forereef habitats with high mortality risk compared to back reef regions with lower predation (Figueira et al. 2008). While there are some parallel demographic patterns with our study, discrepancies in the inshore-offshore patterns compared to differences across depths may be due to greater differences in temperature and food quality or availability, or parameters that vary more consistently, across vertical rather than horizontal gradients. Compared to SS and MPR habitats, DS populations of *S. partitus* had lower growth rate, reproductive output, and longevity, suggesting that some factors that influence demographics such as predation risk and competition, may not vary linearly with depth gradients.

**Conclusions**

The examination of bicolor damselfish population demographics and reproduction uncovered predictable and explicable patterns across depth strata. Shallow shelf populations were highly dynamic with robust population densities, fast individual growth rates, small asymptotic sizes, moderate levels of reproductive investment per spawning batch, and short lives. Deep shelf habitats appeared to be limiting, with lower population densities than SS habitats, moderate individual growth and asymptotic size, and low reproductive investment. Mesophotic depths had less dynamic populations, with low densities, slower individual growth, large asymptotic sizes, high reproductive investment in oocyte area, high fecundity, likely lower frequency of spawning, and increased longevity (Table 3.5). Population demographic patterns were driven by a number of
environmental parameters that varied consistently across depths, but the relationships were not universally monotonic. Density differences were likely linked to larval supply, recruitment, and mortality, whereas growth followed a consistent pattern with depth indicating that growth and size were likely influenced by parameters that varied monotonically with depth, such as temperature. Population density, growth, size, and age patterns varied consistently with depth. Reproductive investment in individual larvae and batch size were highest in the deepest habitat (MPR), followed by the shallowest habitat (SS), indicating that the environmental parameters driving reproductive investment do not vary monotonically with depth or body size. Lower reproduction in DS may be partially explained by reduced food availability, but other biological processes that may not have a linear relationship with depth such as energy and time allocation, or predator densities, may also play a role.

The observed depth-related patterns in demography and reproduction of S. partitus have major implications for the role of deep and mesophotic habitats as coral reef refuges. Deep shelf habitats in the Florida Keys were marginal environments for small demersal reef fish, with low population densities and low per capita batch fecundity. These results indicate that DS habitats likely play a comparably small role in larval production and supply of bicolor damselfish. In contrast, mesophotic reef environments had lower densities, but large, long-lived individuals that spawned less frequently but had high batch fecundity and potentially high condition larvae. Mesophotic reef populations are composed of BOFFFFs (Big Old Fat Fecund Female Fish) that are large, old individuals that produce large batches of offspring and higher condition larvae, and therefore are an important component of fish populations that may disproportionally
contribute larvae to the metapopulation (Marshall et al. 2010a, Hixon et al. 2013). However, lower population density, distributions skewed toward older individuals, and very few young fish suggests that MPR fish populations may be less resilient to environmental disturbances and perturbations as a result of lower population abundances and replenishment rates compared to shallower habitats. Mesophotic reef fish populations appear to exhibit a “storage effect” in which the persistence of the population is dependent upon long-lived individuals during years of infrequent or low recruitment (Warner and Chesson 1985).

Mesophotic coral reef habitats are coral reef fish population repositories that are also potential refugia that can supply high condition larvae to degraded reef habitats. Other studies have shown high reproductive potential for coral species found at mesophotic depths (Holstein et al. 2015), indicating that mesophotic habitats are thriving coral reef ecosystems and important refuges for diverse coral reef organisms. However, potentially low population replenishment and dependence upon long-lived individuals, combined with lionfish invasions that are significantly affecting deeper habitats (Lesser and Slattery 2011), indicate that mesophotic coral reefs in the western Atlantic and Gulf of Mexico may be fragile ecosystems that merit additional research and protection to retain populations of fecund, long-lived individuals and their capabilities as coral reef ecosystem refuges and likely sources of high condition offspring and high larval production.
Table 3.1. Sample sizes of bicolor damselfish (*Stegastes partitus*) from collections and for analyses from shallow shelf (SS), deep shelf (DS), and mesophotic (MPR) depth strata. Transects indicate the number of SS and DS visual surveys by SCUBA divers, and MPR transects indicate the number of ROV surveys including only transects in which bicolor damselfish were observed. Randomly collected fish are fish collected along transects at SS and DS sites in which every fish along the transect was measured and aged, and MPR randomly collected fish include all fish collected by divers that were aged and measured. All other numbers correspond to counts of individual fish.

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>DS</th>
<th>MPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transects</td>
<td>41</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>Randomly collected fish</td>
<td>45</td>
<td>46</td>
<td>51</td>
</tr>
<tr>
<td>Aged fish</td>
<td>66</td>
<td>69</td>
<td>37</td>
</tr>
<tr>
<td>GSI index</td>
<td>48</td>
<td>27</td>
<td>12</td>
</tr>
<tr>
<td>Batch fecundity and oocyte area</td>
<td>17</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>
Table 3.2. Results of all statistical analyses comparing *Stegastes partitus* demography among depth strata (SS=Florida Keys shallow shelf, DS=Florida Keys deep shelf, MPR=Mesophotic Pulley Ridge). Significant differences (p<0.05) are depicted in bold, non-significant results are denoted by ns, and dashes represent comparisons that were not made. P-values were Bonferroni corrected for size and age distributions and oocyte area analyses. Differences in VB (Von Bertalanffy) growth models are presented for model parameters ($L_\infty$ and k) and the full model comparisons with unique values for each parameter (Model: $L_\infty$, k, and $t_0$) between the populations being compared. For oocyte area comparisons, only significant differences are listed. Oocyte stages are listed in order from earliest to latest stage oocytes (CA=cortical alveolar, PY=primary yolk, SY=secondary yolk, TY=tertiary yolk).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>SS vs DS</th>
<th>SS vs MPR</th>
<th>DS vs MPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruit density (ANOVA)</td>
<td>SS&gt;DS</td>
<td>ns</td>
<td>-</td>
</tr>
<tr>
<td>Cohort Strata*cohort</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All bicolor damselfish density</td>
<td>SS&gt;DS</td>
<td>SS&gt;MPR</td>
<td>DS&gt;MPR</td>
</tr>
<tr>
<td>(Kruskal-Wallis and Dunn's)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size distributions (KS test)</td>
<td>ns</td>
<td>SS≠MPR</td>
<td>DS≠MPR</td>
</tr>
<tr>
<td>Age distributions (KS test)</td>
<td>ns</td>
<td>SS≠MPR</td>
<td>DS≠MPR</td>
</tr>
<tr>
<td>VB male growth (likelihood ratio)</td>
<td>$L_\infty$</td>
<td>$L_\infty$</td>
<td>$L_\infty$</td>
</tr>
<tr>
<td></td>
<td>k</td>
<td>k</td>
<td>k</td>
</tr>
<tr>
<td></td>
<td>Model</td>
<td>Model</td>
<td>Model</td>
</tr>
<tr>
<td>VB female growth (likelihood ratio)</td>
<td>$L_\infty$</td>
<td>$L_\infty$</td>
<td>$L_\infty$</td>
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<tr>
<td></td>
<td>k</td>
<td>k</td>
<td>k</td>
</tr>
<tr>
<td></td>
<td>Model</td>
<td>Model</td>
<td>Model</td>
</tr>
<tr>
<td>Size at maturity (Logistic regression)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>GSI (ANOVA and Tukey)</td>
<td>SS&gt;DS</td>
<td>SS&lt;MPR</td>
<td>DS&lt;MPR</td>
</tr>
<tr>
<td>Batch fecundity (ANCOVA and Tukey)</td>
<td>ns</td>
<td>ns</td>
<td>DS&lt;MPR</td>
</tr>
<tr>
<td>Oocyte area (ANCOVA and Tukey)</td>
<td>ns</td>
<td>CA SS&lt;MPR</td>
<td>CA DS&lt;MPR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PY SS&lt;MPR</td>
<td>PY DS&lt;MPR</td>
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<tr>
<td></td>
<td></td>
<td>SY SS&lt;MPR</td>
<td>SY DS&lt;MPR</td>
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<tr>
<td></td>
<td></td>
<td>TY SS&lt;MPR</td>
<td>TY DS&lt;MPR</td>
</tr>
</tbody>
</table>
Table 3.3. Von Bertalanffy growth parameters and standard error from nonlinear least squares analyses for male and female bicolor damselfish (*Stegastes partitus*) from shallow shelf (SS), deep shelf (DS), and mesophotic (MPR) depth strata. Parameters are $L_\infty$ (maximum asymptotic standard length in mm), $k$ (growth parameter that corresponds with growth rate), and $t_0$ (hypothetical age at length 0).

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$L_\infty$</td>
<td>$k$</td>
</tr>
<tr>
<td>SS</td>
<td>51.88 ±0.96</td>
<td>1.51 ±0.20</td>
</tr>
<tr>
<td>DS</td>
<td>56.85 ±1.10</td>
<td>1.07 ±0.10</td>
</tr>
<tr>
<td>MPR</td>
<td>70.91 ±3.36</td>
<td>0.49 ±0.11</td>
</tr>
</tbody>
</table>
Table 3.4. Proportion of females spawning and calculated frequency of spawning from shallow shelf (SS), deep shelf (DS), and mesophotic (MPR) depth strata. Total\(_N\) is the number of females collected during peak spawning. The proportion of females spawning was determined using the presence of post-ovulatory follicles in fish ovaries as an indicator of recent spawning. Follicle reabsorption rates are the calculated number of hours post-spawn that post ovulatory follicles are visible in the ovary, and were calculated based on the temperature dependent relationship described by Gania et al. (2007) using depth specific temperatures from each strata during the study time period.

<table>
<thead>
<tr>
<th>Strata</th>
<th>Total(_N)</th>
<th>Proportion spawning</th>
<th>Temperature (°C)</th>
<th>Follicle reabsorption (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>42</td>
<td>0.46</td>
<td>31.80</td>
<td>41.0</td>
</tr>
<tr>
<td>DS</td>
<td>21</td>
<td>0.3</td>
<td>28.87</td>
<td>47.9</td>
</tr>
<tr>
<td>MPR</td>
<td>15</td>
<td>0.33</td>
<td>20.93</td>
<td>67.2</td>
</tr>
</tbody>
</table>
Table 3.5. Simplified summary of demographic parameter comparisons between shallow shelf (SS), deep shelf (DS), and mesophotic reef (MPR) depth strata. High, mid, and low refer to qualitative values of parameters. Reproductive investment is based on individual clutches rather than lifetime reproduction, and includes a general grouping of gonado-somatic index (GSI), batch fecundity, and oocyte area. Spawning frequency includes proportion of females spawning and calculated temperature dependent follicle reabsorption rates.

<table>
<thead>
<tr>
<th>Demographic parameter</th>
<th>High</th>
<th>Mid</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruit density</td>
<td>SS</td>
<td>DS</td>
<td>-</td>
</tr>
<tr>
<td>Total fish density</td>
<td>SS</td>
<td>DS</td>
<td>MPR</td>
</tr>
<tr>
<td>Growth rate</td>
<td>SS</td>
<td>DS</td>
<td>MPR</td>
</tr>
<tr>
<td>Asymptotic size</td>
<td>MPR</td>
<td>DS</td>
<td>SS</td>
</tr>
<tr>
<td>Reproductive investment</td>
<td>MPR</td>
<td>SS</td>
<td>DS</td>
</tr>
<tr>
<td>Spawning frequency</td>
<td>SS</td>
<td>DS</td>
<td>MPR</td>
</tr>
</tbody>
</table>
Figure 3.1. Map of study site locations and strata. All work at replicate shallow shelf and deep shelf sites was within a 75 m radius centralized GPS coordinates located at American Shoal shallow shelf (24.52732, -81.49848), Looe Key shallow shelf (24.54066, -81.43655), American Shoal deep shelf (24.52345, -81.49738), and Looe Key deep shelf (24.53951, -81.42265). Mesophotic Pulley Ridge collections took place within the black outlined polygon region labeled PR. Light gray depth contours are 5 m intervals from 0-10 m depths, dark gray are 10 m contours from 20 m (labeled on the map) to 100 m, and black starting at 400 m (labeled on map) are 400 m intervals.
Figure 3.2. (a) Temperature time-series and (b) difference between daily maximum and minimum temperature from Florida Keys shallow shelf (SS) and deep shelf (DS), and mesophotic Pulley Ridge (MPR) depth strata during the study period.
Figure 3.3. Results of monthly recruitment surveys at shallow shelf (SS) and deep shelf (DS) strata in the Florida Keys during the years 2012 and 2013. Barplots are means and standard errors. There was a significant interaction between cohort and depth strata, but significant differences (denoted with an asterisk) between strata are only depicted if the pairwise comparison was between the same monthly cohort.
Figure 3.4. Boxplot of *Stegastes partitus* densities from visual transect surveys at shallow shelf (SS) and deep shelf (DS) strata, and ROV surveys at mesophotic reefs (MPR). The central tendency line is the median value, and the bottom and top of the box correspond to the 25\textsuperscript{th} and 75\textsuperscript{th} quartiles of the data, respectively. The whiskers include values that are within 1.5 times the inter-quartile range (distance between the 25\textsuperscript{th} and 75\textsuperscript{th} quartiles), and outliers are plotted as points. All comparisons between strata were significant.
Figure 3.5. Frequency distributions of *Stegastes partitus* (a) standard length (mm) and (b) otolith-derived age from fish collected along transects at shallow shelf and deep shelf strata, and a random selection of fish from the mesophotic strata. Immature fish are depicted in light gray, females in darker gray, and males in black. Mesophotic size and age distributions were significantly different than shallow shelf and deep shelf distributions.
Figure 3.6. Von Bertalanffy growth model fit for (a) male and (b) female bicolor damselfish using otolith-derived age and standard length (SL) data from Florida Keys shallow shelf (SS) and deep shelf (DS), and mesophotic Pulley Ridge (MPR) depth strata. *Stegastes partitus* up to 6 yrs old were included in model fits based on the maximum age at SS and DS strata, thus, curves only extend to 6 yrs. Mesophotic reef fish >6 yrs old are plotted, but were treated as outliers in model fitting.
Figure 3.7. Bivariate 95% confidence ellipses for Von Bertalanffy growth model parameters ($L_\infty$ and $k$) for male and female bicolor damselfish (*Stegastes partitus*) from Florida Keys shallow shelf (SS) and deep shelf (DS), and mesophotic Pulley Ridge (MPR) depth strata.
Figure 3.8. Female bicolor damselfish (*Stegastes partitus*) (a) gonado-somatic index (GSI) and (b) batch fecundity for Florida Keys shallow shelf (SS) and deep shelf (DS) strata, and mesophotic Pulley Ridge (MPR) depth strata. GSI is the ratio of ovary weight to body weight * 100. Adjusted batch fecundity is the mean batch fecundity standardized to fish body weight using the relationship between fish body weight and batch fecundity in ANCOVA. Error bars are standard error. Letters indicate significant differences.
Figure 3.9. Average adjusted bicolor damselfish (*Stegastes partitus*) oocyte area from Florida Keys shallow shelf (SS) and deep shelf (DS), and mesophotic Pulley Ridge (MPR) strata. Panels correspond to oocyte stages (early stage on the left to late stage on the right: CN=chromatin nucleolar, PN=perinucleolar, CA=cortical alveolar, PY=primary yolk, SY=secondary yolk, TY=tertiary yolk, MNS=migratory nucleus, HO=hydrated oocyte). Oocyte areas were adjusted based on the relationship between fish body weight and oocyte area from ANCOVA relationships. Bars are mean oocyte area and error bars are standard error. A separate ANCOVA was performed for each oocyte stage for a total of eight comparisons, accordingly, p-values were Bonferroni corrected. Asterisks indicate significant differences.
Chapter 4. Habitat suitability across vertical spatial scales affects behavior, diet, and fitness of a demersal coral reef fish

Summary

Recent deterioration of shallow water coral reefs as a result of anthropogenic stressors, climate change, and natural disturbances suggests concomitant declines in habitat quality for many coral reef organisms. Mesophotic reef habitats (30-150 m deep) may be buffered from environmental stressors, thereby serving as refuges for coral reef fishes and as sources of larval supply for degraded reefs. However, mesophotic reefs are at the extreme depth distribution of many coral reef fish, implying decreased habitat suitability with depth. This study assessed habitat suitability of coral reefs across a broad depth distribution for a common demersal reef fish (*Stegastes partitus*: bicolor damselfish). Depth strata across the Florida Shelf ranged from shallow shelf (SS <10 m) and deep shelf (DS >20-30 m) habitats in the Florida Keys to mesophotic depths (MPR >60-70 m) at Pulley Ridge on the west Florida Shelf. Results indicate that environmental parameters and associated biological responses in bicolor damselfish differ across depths, but do not have a consistent relationship with depth. Behavioral analysis of energy allocation in SS and DS habitats revealed a higher frequency of “risky” behaviors in SS habitats which have lower predator densities. Accordingly, SS fish also had fuller guts than fish from other strata. General diet composition was similar across depths, however, there were subtle differences in stomach content counts of some prey categories. Stable isotope analysis revealed that fish in MPR habitats had the highest trophic position, a broad diet niche, and the widest range of nutrient sources. As a result, MPR fish had
significantly higher C:N ratios, linked to lipid content. In combination, high trophic position, high C:N ratio, and slow growth correlated with higher gonado-somatic index, used as a proxy for fitness, revealed a strong relationship between environmental variables and high fitness of bicolor damselfish in mesophotic habitats. These results suggest that MPR reefs are suitable, high quality habitats for a common demersal reef fish and may be important refuges for coral reef ecosystems as shallow reefs decline.

**Background**

Habitat quality for coral reef fish populations is determined by factors that affect population size including population density and the spatial extent of the habitat, in conjunction with habitat suitability that can have subtle but significant effects on population dynamics. For coral reef fishes, habitat suitability can significantly impact fish condition and energy availability for costly biological processes such as growth and reproduction. As a result, fitness trade-offs to maximize reproductive contributions to future generations can be influenced by spatially variable environmental parameters including resource availability and predation risk (Heino and Kaitala 1999). For coral reef fishes with pelagic larvae, habitat suitability can determine biological traits such as behavior, condition, and energy allocation toward fundamental processes that affect fitness and population connectivity by influencing larval contributions from source populations.

Variables that affect habitat suitability are often species specific, however for demersal reef fish, key parameters include predation, shelter, food availability, and food quality (Forrester 1990, Nemeth 1998, Holbrook et al. 2000). The biological responses of reef fishes to habitat suitability can be equally diverse, and encompass behavioral
adaptations and demographic parameters that can interact or have cascading effects on condition and fitness (Sala-Bozano and Mariani 2011). For example, local predator and competitor densities often vary spatially and influence fitness of individuals (Steele 1999, Holbrook et al. 2000, Figueira and Lyman 2007). In environments with high predation pressure, it may be optimal to minimize risky mating behavior, at the expense of reproductive output (Kozlowski and Teriokhin 1999, Ghalambor et al. 2004, Pavlova et al. 2010). Resource availability and predation risk can dictate energy allocation and trade-offs between metabolic processes. Accordingly, food availability, nutritional content, variation in food sources, and diet composition can affect fish condition, growth, and reproduction (Forrester 1990, Heino and Kaitala 1999). An organism with limited access to food may lack the surplus energy required to invest in multiple processes, and instead allocate energy between tissue repair and maintenance, growth, reproduction, or external energetic requirements such as defense and foraging (Rangeley and Godin 1992).

While numerous studies have addressed the impacts of habitat quality on demersal reef fish populations (Holbrook et al. 2000, Almany 2004, Schrandt and Lema 2011, Brooker et al. 2013, 2013, Boström-Einarsson et al. 2014), few studies have assessed spatial variation in habitat suitability across the depth distribution of species, particularly at the lower boundaries of hermatypic coral reef habitats (Srinivasan 2003, Brokovich et al. 2006, Hoey et al. 2007). As shallower reefs decline (Gardner et al. 2003), mesophotic hermatypic coral reef ecosystems, found at depths between 30-150 m, may be refuges for coral reef fishes and sources of larval supply (Lesser et al. 2009, Bongaerts et al. 2010). Mesophotic reefs are conceivably marginal environments at the extreme depth range of many coral reef organisms, suggesting potential constraints for fish populations.
Coral reef habitat and communities extend into mesophotic depths, however, depth limitations and preferred depth distributions of coral reef fish may be driven by habitat suitability and quality (Srinivasan 2003, Brokovich et al. 2006, Hoey et al. 2007).

The overarching objective of this study was to assess habitat suitability of coral reef ecosystems across a broad depth range for a model demersal coral reef fish (bicolor damselfish, *Stegastes partitus*) to provide insight in the potential refuge function of mesophotic coral reef ecosystems. Secondary questions to address the overall goal of the study assessed: (1) differences in habitat and predation risk across depths, (2) the influence of habitat variability on time allocation and behavioral trade-offs, (3) diet composition and food availability across depths, (4) the effects of habitat on fish condition, and (5) trade-offs associated with biological responses to environmental variables that vary across depths and ultimately affect reproduction and fitness.

**Methods**

This study took place during the summer months (June-September) from 2012-2015 in reef habitats that are oceanographically connected via by the Loop Current that forms in the Gulf of Mexico and feeds into the Florida Current in the Straits of Florida (Lee et al. 1992). Sampling sites were distributed across the Florida Shelf in habitats that ranged from multiple coral reef sites at mesophotic depths (MPR: 60-70 m) at Pulley Ridge on the west Florida Shelf, to replicate deep shelf (DS: >20-30 m) and shallow shelf (SS: <10 m) coral reef sites at Looe Key and American Shoal in the Florida Keys (Fig. 4.1). The study species, the bicolor damselfish (*Stegastes partitus*), is a common territorial demersal reef fish with a small home range. Young bicolor damselfish diets are composed of benthic algae and plankton, until fish undergo an ontogenetic diet shift to a
diet composed primarily of plankton (Emery 1973). Peak spawning occurs during the summer months ~1-7 days after the full moon, during which time demersal eggs and nests are guarded by males (Schmale 1981). Bicolor damselfish behavior is thoroughly documented, and includes identifiable feeding, aggression, courtship, and reproductive behaviors (Myrberg 1972).

Bicolor damselfish were collected by divers using hand nets and quinaldine. Samples from SS and DS sites were stored on ice until they could be transported to the lab and frozen. At Pulley Ridge bicolor damselfish were frozen in liquid nitrogen, and fish from all strata were stored frozen at -80°C. Standard lengths (SL) were measured to the nearest 0.01 mm, and body weights to the nearest 0.01 g. All SL were measured using fresh individuals, and fish from the Florida Keys were wet weighed, whereas wet weights of fish collections from Pulley Ridge could not be obtained. The majority of weight loss from freezing is associated with water loss during the thawing process, and PR fish were weighed when only partially thawed. However, as a precaution, to account for any loss of weight from the preservation process, frozen body weights were converted to fresh weight using the formula \( \text{Weight}_{\text{wet}} = 0.04 + 1.06 \times \text{Weight}_{\text{frozen}} \) (Thorstad et al. 2007). During peak spawning, a subset of MPR fish were carefully dissected prior freezing to extract ovaries for preservation in 10% phosphate buffered formalin, therefore, ovary weights to the nearest 0.01 g were converted to fresh weight following Hunter et al. (1985), and added to body weights to obtain total body weight. Overall results of statistical analyses were equivalent with and without weight conversions. All SS and DS females were dissected during peak spawning and fish ovaries were wet weighed to the nearest 0.01 g. Individuals >30 mm SL were used for all analyses.
**Fish density and benthic habitat**

In the Florida Keys at SS and DS sites, 25 m x 2 m transect visual surveys were conducted by SCUBA divers by swimming the length of a transect to identify mobile fish species, and count all individuals, and a second swim along the transect to count and identify small or cryptic individuals. Benthic habitat was quantified along a subset of transects \((N_{SS} = 21, N_{DS} = 38)\) using the point intercept method with 25 cm intervals. Predefined habitat categories were: CC = crustose coralline algae, CY = cyanobacteria, LC = live coral, DC = dead and bleached coral, MA = macroalgae, O = other (primarily silt), RB = rubble, SA = sand, SC = soft coral, SI = sessile invertebrate, and TA = turf algae. At every meter interval, the maximum vertical relief within a 1-m\(^2\) grid was estimated using height category ranges of <25, >25-50, >50-75, >75-100, >100-150, >150-200, and >200 cm. Fish densities at Pulley Ridge were analyzed using data from 99 ROV (UNCW Super Phantom S2) transects from the summers of 2012 and 2013. Each 100 m transect had an estimated 5 m width field of view and was conducted during daylight hours. Detailed ROV methods are described in Reed et al. (2014).

Predator densities were calculated using visual transects in the Florida Keys and ROV surveys at Pulley Ridge. Species were classified as predators following Nemeth (1998) and Randall (1967). When data were not available for potential predators, categories were assigned based on diet information at the family level, and data from FishBase.org (Froese and Pauly 2015). Data from ROV footage generally underestimates fish densities in comparison to visual surveys (Andaloro et al. 2013, Laidig et al. 2013), and ROV transects included all benthic habitat types at Pulley Ridge, whereas diver surveys were only conducted on reef habitat. Of the total number of ROV surveys, ~68%
included at least one predator observation. This value was similar to the reported live biota cover of 57.09% at Pulley Ridge (Reed et al. 2014), therefore, ROV surveys for analysis included only transects in which fish were observed, with the assumption that those transects were reef habitat. Predator densities were compared among depth strata using Kruskal–Wallis tests with Dunn’s post-hoc comparisons.

Benthic habitat data from SS and DS transects were analyzed using a Correspondence Analysis (CA), with counts of habitat type for each transect. Similar to Principle Component Analysis (PCA), CA is more appropriate for frequency or count datasets with many zeros (Legendre & Legendre 2012). For the CA interpretation, each transect point in two-dimensional space is most associated with, or corresponds to, the habitat categories nearest to the point. Therefore, habitat categories near SS transects are most associated with SS strata, and those near DS transects are most associated with DS.

Maximum vertical relief was included in the analysis as a supplemental quantitative variable with numeric vertical categories as ranks from lowest to highest relief.

Behavior

Bicolor damselfish behavior was quantified at SS and DS strata with digital cameras that were each positioned on the substrate at the beginning of a dive, and aimed at an individual fish from a distance of ~0.5 m. During each recording, a meter stick with markings every 15 cm, was held in front of the camera at multiple distances to estimate the vertical and horizontal field of view and for an estimation of fish swimming distance. Cameras were left undisturbed for the remainder of the dive to allow the fish to acclimate and resume normal behavior until the cameras were collected at the end of the dive. Fish behaviors typically differ throughout the day, particularly in the early morning and at
night (Myrberg 1972), therefore, cameras were deployed between 1000 -1600 to ensure that all recordings were comparable. For each video, adult fish (>40 mm SL) were selected randomly at the start of the dive, generally the first adults encountered once the diver arrived at the study site, and up to four cameras were deployed during a single dive.

A total of 21 SS and 19 DS behavioral videos were analyzed by a single individual in the laboratory. Quantification of fish behavior began 30 s after the diver was out the frame to ensure undisturbed fish behavior, and the total clip time that was quantified was 5 min of continuous footage for each fish. Seven behavior categories were quantified based on Myrberg (1972): shelter, out of view, plankton bites, benthic bites, vertical swim, aggression, and courtship. Shelter included time in the shelter, hiding, and evasive downward swimming motions. Out of view included swimming beyond the camera view or behind an object, and was generally associated with swimming long horizontal distances. Plankton bites were feeding motions in the water column as opposed to benthic bites. Vertical swim was an estimated maximum vertical distance above the benthos that the fish attained during the video, using ~7.5 cm distance intervals. All videos used for analysis had a field of view that encompassed the vertical swimming range of the fish. Aggression combined conspecific and interspecific chasing, circling, and nipping. Courtship behaviors included a dipping motion, swimming in a tilted position, nudging, and leading or following a conspecific. Shelter, aggression, and courtship were quantified as the amount of time dedicated to each activity divided by the total amount of time that the fish was in view during the 5-min video. Out of view was
quantified as the amount of time that the fish was not in view divided by the total 5 min.

*Plankton bites* and *benthic bites* were counts of individual bites per minute that the fish was in view.

Behavioral data were analyzed with Principle Component Analysis (PCA) to compare behavior among depth strata and individuals. PCA was performed using a correlation matrix of standardized variables. All data were transformed with a Box-Cox transformation and standardized before analysis. *Vertical swim* was a semi-quantitative variable, however, PCA is robust to variation in the precision of the dataset, and correlations for ranked variables are equivalent to Spearman’s rank correlation (Legendre and Legendre 2012). The second Principle Component (PC2) showed the most separation of SS and DS strata, therefore, individual fish PC2 scores were used for Hierarchical Clustering with Euclidian distance and Ward’s clustering method. The Hierarchical tree was cut at a Euclidean distance of 1.5 to obtain two clusters.

**Gut contents**

Bicolor damselfish for gut content analysis were >30 mm SL to reduce the effect of ontogenetic diet shifts, and all fish were collected between 0830 and 1700 to minimize diet variation as a result of diurnal fluctuations in prey assemblages and availability. Stomachs were dissected immediately after thawing, and stomach contents were carefully extracted. The total wet weight of the stomach contents was recorded to the nearest 0.001 mg, and a subsample of ~1/3 the total wet weight, was separated and weighed for diet identification. Stomach contents from the subsample were identified to the lowest taxonomic level possible and counted. Algae, *Trichodesmium* sp., and unidentifiable items could not be counted, therefore, dry weights were obtained using five broad gut
content categories: algae (benthic and phytoplankton), animal (included unidentifiable crustaceans that were likely copepods), copepods, *Trichodesmium* sp., and unidentified items that were primarily partially digested material with a mucous texture. Stomach contents were dried at 50°C until a stable weight was maintained (~48 hrs) and then weighed to nearest 0.001 mg.

Gut contents were analyzed at multiple levels, from broad comparisons of gut fullness to individual content identifications. Gut fullness was calculated using the wet weight of stomach contents and then compared among depth strata using ANCOVA with fish body weight as the covariate, and Tukey post-hoc comparisons. Stomach content dried weights were assumed to be proportional to the total stomach content, therefore, category dried weights from subsamples were multiplied by the ratio of the subsample and total gut content wet weights. Extrapolated prey item category dry weights were compared among depth strata using Two-way ANCOVA with extrapolated total gut content dry weight as a covariate, and strata and prey category as fixed factors. The full model included all interaction terms, and the model was simplified by sequentially removing terms that were not significant.

Diet composition of ingested prey item counts were compared using PCA. To account for differences in total prey items consumed, PCA was performed using the correlation matrix of standardized prey item counts. The dataset was Hellinger transformed before analysis and excluded 11 rare taxa with <20 total observations (amphipod, cnidarian, echinoderm, fish, foraminifera, mite, mollusc, nematode, Platyhelminthes, sipunculid, tunicate) for a total of nine common prey items in the analysis: Appendicularia, Annelida, calanoid copepods, copepods, Crustacea, Ostracoda,
cyclopoid copepods (the category included some poecillid copepods), and harpacticoid copepods, and eggs. Hierarchical Clustering analysis was performed using PC2 scores with Euclidian distance and Ward’s clustering method. The Hierarchical tree was cut at a Euclidean distance of 1.0 to obtain three clusters.

**Stable Isotopes**

Frozen fish muscle tissue for individuals >30 mm SL was dried at 50°C until a stable weight was maintained. Dried tissue was ground into a powder and 0.5-1.5 mg of tissue from each fish was weighed in tin capsules for analysis. Benthic algae samples, a mixture of non-calcareous turf algae and *Dictyota* sp., were collected during the summer months from SS sites (3 replicates from 2013), DS sites (2 replicates from 2013 and one from 2014), and MPR strata (3 replicates from 2013) and stored frozen at -20°C. All epiphytes were removed from algae before drying at 50°C, and 1-2 mg of dried algae tissue was placed in tin capsules. At SS sites, copepods were collected using diver-towed nets during 2013 and 2014 and stored frozen in water from the site at -20°C. To obtain enough sample material, multiple net tows from each summer collection were combined, and copepods were sorted from the sample, dried, and 0.5-1.5 mg of tissue were weighed for analysis. Divers were unable to tow nets at mesophotic depths due to the constraints of technical diving, consequently, copepods from all depth strata were sorted from five light trap collection replicates from each strata: two from 2012 and three from 2013 at SS and DS sites, and two from 2013 and three from 2014 at MPR sites, and stored in 95% ethanol. Ethanol storage can affect isotope values, however, the effects are likely to be minimal and uniform (Arrington and Winemiller 2002), and all copepod light trap samples were preserved using the same methods, making the values comparable (Araújo
et al. 2007). Additionally, copepod isotope values were analyzed primarily to assess $\delta^{15}$N values for trophic level calculations, and there is evidence that while ethanol preservation can affect $\delta^{13}$C, it does not affect $\delta^{15}$N in fish tissue (Kelly et al. 2006). The $\delta^{13}$C and $\delta^{15}$N of the samples were analyzed at the University of Miami using mass spectrometry on a Costech EA 4010 connected to a Thermo Delta V Advantage. Isotope values of $\delta^{13}$C and $\delta^{15}$N are reported using conventional delta notation, and values are relative to Vienna Pee Dee Belemnite (V-PDB) and atmospheric N$_2$, respectively, with $\pm$0.1 ‰ typical precision measurements of the standards.

Isotope $\delta^{13}$C values from fish muscle tissue were corrected for high lipid content for all analyses based average C:N ratios >3.5, using the formula presented by Post et al. (2007)

$$\delta^{13}\text{C}_{\text{normalized}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times \text{C:N}$$

Isotope biplots of mean ±SE of bicolor damselfish, copepod (light traps), benthic algae, and small copepods (net tows) from SS, DS, and MPR strata were plotted in comparison to organisms from the literature for general comparison of isotopes values across studies, and to verify estimates of trophic position of bicolor damselfish and copepods in the present study. Trophic position of bicolor damselfish was calculated using the formula from Post et al. (2002)

$$\text{Trophic position} = \lambda + (\delta^{15}\text{N}_{\text{secondary consumer}} - \delta^{15}\text{N}_{\text{base}}) / \Delta_n$$

Where $\lambda$ is the trophic position of the lower trophic organism that is used to obtain $\delta^{15}\text{N}_{\text{base}}$, starting with a value of 1 for primary producers, and $\Delta_n$ is the $\delta^{15}$N enrichment for each trophic level. For this study $\delta^{15}\text{N}_{\text{base}}$ was calculated for each strata using the mean isotope value of copepods collected in light traps. The trophic position of copepods
was estimated as 2.5, rather than a primary consumer, based on the sample composition of primarily large copepods, enriched $\delta^{15}N$ of light trap collections compared to smaller copepods from net tows (see Results), and the omnivorous diet of many copepods (Kleppel et al. 1996). A $\Delta_n$ value of 3.5 was used for trophic enrichment (Post 2002). The simple formula to estimate trophic position was selected, in preference to a two-source model that combines pelagic and benthic food webs, based on low benthic bites rates from video analysis (mean = 1.1 bites/min), and minimal benthic algae in gut contents (see Results). Trophic position was calculated for individual fish, and compared between strata using ANOVA and Tukey post-hoc tests.

Isotope niche width and diet breadth were estimated with $\delta^{15}N$ and $\delta^{13}C$ values of individuals in each depth strata. The isotopic diet niche width was assessed by calculating the spatial extent of $\delta^{15}N$ and $\delta^{13}C$ in biplot space using the area of a convex hull encompassing all data points for each depth strata. The range of $\delta^{15}N$ values and $\delta^{13}C$ values also provided estimates of the trophic length of the population and the variability of basal resources, respectively (Layman et al. 2007). Additionally, SIBER (Stable Isotope Bayesian Ellipses in R, Jackson et al. 2011) metrics were calculated because they are more robust to the effects of sample size on niche width. Standard ellipse areas encompassing the central 40% of the data were estimated for each depth strata by Bayesian inference, based on a bivariate normal distribution, and with a correction for small sample sizes. Uncertainty in ellipse area estimates for comparison among strata was calculated with $10^4$ posterior draws of the standard ellipse area to generate probabilities. All isotope niche width calculations were performed using the library SIAR (Stable Isotope Analysis in R; Parnell et al. 2010) in R statistical software.
Lipid content and energy allocation

Differences in lipid content of fish muscle tissue among strata were compared using the ratio of carbon to nitrogen percent weight in tissue (C:N) from stable isotope analyses. C:N ratios can be used as a proxy for condition because lipids are depleted in δ¹³C relative to other tissue types, resulting in a positive linear relationship between C:N and lipid content (Post et al. 2007). C:N ratios were analyzed using Two-way ANOVA with depth strata and sex as factors, and with Tukey post-hoc tests. The model was simplified by systematically removing non-significant terms. A measure of energetic investment in reproduction, gonado-somatic index (GSI), was calculated as the ratio of ovary weight to body weight * 100 for females that were collected during peak spawning following the full moon. (Chapter 3).

All study variables were measured on a subset of samples, therefore, to assess the influence of depth and individual fish lipid content, diet, and traits on reproduction and fitness, data from this study was combined with otolith-derived ages and growth rates from previous work by Goldstein (Chapter 3). Growth rate (k) was calculated using the Von Bertalanffy (VB) growth model formula: \( L_t = L_\infty (1 - e^{-kt}) \). Where \( L_t \) = standard length at time t, \( L_\infty \) = theoretical asymptotic length, \( k \) = growth coefficient that determines the rate at which length approaches \( L_\infty \) (based on the steepness of the curve), \( t \) = fish age in years, and \( t_0 \) = hypothetical age at length 0. To calculate a growth rate parameter for each fish (k), the SL of each fish \( (L_t) \) was combined with values of t, depth specific \( L_\infty \), and \( t_0 \) from Goldstein (Chapter 3). A PCA analysis using trophic position, C:N ratio, age, gut fullness, and VB growth was performed. Age and VB growth were correlated as a result of calculations of k, however, both variables were used for the
analysis because depth-specific VB formulas resulted in slightly different relationships between age and growth among strata. Reproductive investment during one spawning bout, was used as a proxy for fitness and was determined using gonado-somatic index (GSI) that provides an instantaneous measure of reproductive investment. The influence of the combination of variables was assessed in relation to GSI by calculating the least squares linear regression relationship of the individual fish values of the first principle component scores and GSI.

All data were analyzed with R version 3.1.2, multivariate analyses were performed using the FactoMineR package, and meaningful components were determined based on Kaiser-Guttman criterion (Legendre and Legendre 2012).

Results

Fish density and benthic habitat

Predator densities did not follow a consistent pattern with depth: the highest densities occurred in the DS strata, followed by SS, then MPR (Table 4.1, Fig. 4.2). Benthic habitats and maximum vertical relief also differed between SS and DS strata. Depth strata differentiated along the primary CA axis that comprised 42.4% of the variability in the dataset (Fig. 4.3). SS strata can be primarily characterized by a higher prevalence of macroalgae and cyanobacteria compared to crustose coralline algae, other (primarily silt), and live coral in DS strata (Table 4.2, Fig. 4.3). Sessile invertebrates were marginally associated with DS strata, and soft coral and turf algae with SS. Variation in the dataset along the secondary CA axis, that encompassed 13.3% of the variability in data, was primarily driven by rubble, and to a lesser degree sand, and dead coral (Table 4.2, Fig. 4.3). These three substrates were not common in either strata, although rubble
and dead coral were found in DS habitats more frequently, and sand was more common in SS compared to DS. Maximum vertical relief, the supplemental variable, showed higher maximum vertical relief, or rugosity, at DS reefs (Fig. 4.3), with a median range of 100-150 cm compared to 25-50 cm in SS habitats.

Behavior

Behavioral analysis of video data using PCA showed separation of SS and DS fish behaviors along PC2 (explaining 21.5% of the variation in the dataset) and minimal separation along PC1, that explained 31.5% of the variation in the dataset (Fig. 4.4). The behaviors that correlated most strongly with PC1 were fish that dedicated time to benthic bites, courtship, and shelter compared to time out of view and plankton bites. PC2, which showed the most differentiation between SS and DS sites, was most strongly correlated with vertical swim, aggression, shelter, and courtship (Fig. 4.4, Table 4.2). Clustering analysis along PC2 verified the separation of SS and DS behaviors along the axis. Cluster 1 was composed primarily of SS fish (14 out of 21 SS fish) that swam long vertical distances, were aggressive, and dedicated a large portion of time to courtship. Cluster 2 included the majority of DS fish (16 out of 19 DS fish) that did not swim long vertical distances, were less aggressive, sheltered more often, and to a lesser degree dedicated less time to courtship behavior (Fig. 4.4).

Gut contents

Bicolor damselfish in SS habitats had fuller guts than fish from DS and MPR strata, but this difference was only significant between SS and DS fish (Table 4.1, Fig. 4.5). Broad prey category diet composition did not differ significantly between depth strata (Table 4.1, Fig. 4.6). The majority of the dried weight of identifiable items in fish
guts was composed of *Trichodesmium* sp., followed by animals (many of which were likely copepods that could not be identified), copepods, and algae (Fig. 4.6). Interestingly, there was an interaction in the ANCOVA model between total dried gut content weight and prey category that was primarily driven by a faster increase in the ratio of *Trichodesmium* sp. in gut contents as total gut content weight increased (Table 4.1, Fig 4.7).

The diet composition of fish based on individual prey items along PC1, that explained 24% of the variation in the dataset, primarily described fish with a diet composed of many copepods from all taxonomic groups and ostracods, in comparison to fish that ate few of these prey categories (Fig. 4.8, Table 4.2). Depth strata differences in diet were negligibly explained by PC1, whereas PC2 that encompassed 16.1% of the variation in fish diets, was more closely linked with separation among depth strata (Fig. 4.8). There were significant correlations between PC2 and the ingestion of Appendicularia, and a strong negative correlation with Annelida (Table 4.2). Hierarchical clustering using PC2 scores showed some separation of diet composition between depth strata. Cluster 1 was composed of primarily MPR fish with 11 MPR, 5 SS, and 3 DS fish. Cluster 2 was a mixture of strata, but the majority were from SS (9) and MPR (8) strata, compared to 5 DS. Cluster 3 was primarily DS fish with 10 individuals, 2 SS and 1 MPR (Fig. 4.8). Diet composition between DS and MPR habitats had minor overlap along PC2, and SS diets were intermediate.

*Stable Isotopes*

Comparison of mean δ\(^{13}\)C and δ\(^{15}\)N of bicolor damselfish, copepods, small copepods (from net tows), and benthic algae showed the expected increase in δ\(^{15}\)N as
trophic level increased (Fig. 4.9). $\delta^{15}$N of algae samples differed minimally between depths, although MPR algae were slightly more enriched than the other strata. In contrast, mean $\delta^{13}$C differed more substantially between strata, with a pattern of depleted $\delta^{13}$C as depth increased. Copepod isotope values followed the same pattern as algae, although both $\delta^{15}$N and $\delta^{13}$C values were more similar across depth strata, and small copepods from diver-towed nets were less enriched in $\delta^{15}$N than larger copepods from light trap collections (Fig. 4.9). Isotope values of bicolor damselfish at each depth strata followed the patterns seen in copepods and algae, with depletion of $\delta^{13}$C with increasing depth. However, the difference in $\delta^{15}$N values in fish muscle tissue across strata showed enrichment with increasing depth (Fig. 4.9). Comparing isotope values from our study to a greater spatial and temporal context from the literature revealed additional information about isotope sources and variation in $\delta^{15}$N and $\delta^{13}$C (Table 4.3). *Trichodesmium* sp., a planktonic diazotroph, had extremely depleted $\delta^{15}$N values compared to other photosynthetic organisms including benthic turf algae and macroalgae. Algae from SS reefs in the Florida Keys had values similar to algae in our study, but were slightly more enriched in $\delta^{15}$N. Compared to shallower habitats, deep macroalgae from the Florida Keys showed depletion of $\delta^{13}$C to a greater degree than the differences seen in algae samples from this study (Fig. 4.9). Compared to other fish species, bicolor damselfish in the Florida Keys had similar $\delta^{13}$C values, but were less enriched in $\delta^{15}$N than herbivorous dusky damselfish, similar to ocean surgeonfish, and more enriched than Scaridae (Fig. 4.9, Table 4.3).

Comparisons of trophic position among strata, incorporating baseline differences in $\delta^{15}$N, revealed a significantly higher trophic position at MPR depths (Table 4.1) and a
monotonic positive increase in trophic position with depth (Fig. 4.10). Biplots of δ\textsuperscript{13}C and δ\textsuperscript{15}N values showed the broadest width of δ\textsuperscript{13}C values at MPR strata, and a similar span of δ\textsuperscript{15}N values between strata (Fig. 4.11). Convex hull area increased with depth (SS = 1.70, DS = 2.14, MPR = 4.10; Fig. 4.11). Similarly, standard ellipse area increased with depth, and Bayesian credible intervals that incorporate uncertainty into area calculations, indicated that MPR fish had the largest standard ellipse area, with 93.4% probability that MPR>SS, 93.3% that MPR>DS, and 61.0% that DS>SS (Table 4.1, Fig. 4.11).

**Lipid content and energy allocation**

Bicolor damselfish lipid content in muscle tissue, a proxy for condition, was measured using C:N ratios and showed significantly higher C:N ratios in MPR fish compared to SS and DS (Fig. 4.12, Table 4.1). Combining all measurements of individual fish variables (trophic position, C:N ratio, gut fullness, age and VB growth from Chapter 3) revealed correlations between variables and energy allocation. The primary PCA axis encompassed 69% of the variability in the dataset and was separated based on C:N ratio, trophic position, and age compared to fast growth rate and full guts (Table 4.2 Fig. 4.13a). Age and VB growth were negatively correlated as a result of the biological relationship between age and growth, and a numerical relationship because the calculation of VB growth relies on age. Trophic position and gut fullness were also negatively correlated (Table 4.3, Fig. 4.13a). MPR fish had generally higher PC1 scores, DS fish were in the mid-range, and SS fish had the lowest PC1 scores (Fig. 4.13a). Higher PC1 scores indicate higher trophic position, higher C:N ratio (lipid content), older fish, lower gut fullness, and slower growth. PC2 explained 18.8% of the variation and was only
significantly correlated with gut fullness (Table 4.2, Fig. 4.13a). Therefore, the relationship between PC1 scores, which explained the majority of the variation in the dataset, and GSI was assessed using linear regression. GSI had a significant positive relationship with PC1 (Fig. 4.13b, Table 4.1), and explained 40% of the variation in the dataset (P = 0.01, r² = 0.40).

**Discussion**

Depth-driven habitat variability impacts reef fish behavior, feeding, and diet composition, and has repercussions on condition and energy allocation toward costly biological processes such as reproduction. Differences in habitat related population demographics at small spatial scales have been identified for reef fish (Ruttenberg et al. 2005, Figueira et al. 2008, Chapter 3), Yet, predictable and explicable variation across the depth distribution of a species, and the consequences to fish population dynamics, remain elusive (Hoey et al. 2007). Assessing diet and energetic behavioral trade-offs, and the implication of energy allocation toward reproductive output of reef fishes in mesophotic environments, provides insight into habitat suitability and quality of potential deep reef refuges. Results of this study indicate that the combination of behavior, diet, and condition influences energetic investment in reproduction for bicolor damselfish across their distribution, and that mesophotic reefs provide a valuable habitat for planktivorous reef fish.

Behavioral time allocation of bicolor damselfish in the Florida Keys differed across depths, likely as a result of predation risk. Bicolor damselfish densities were consistently lower with increasing depths (Chapter 3), whereas, predator densities peaked in DS habitats. Differences in damselfish behaviors among depth strata primarily
separated along a secondary PCA axis that was related to “risky” behaviors, including maximum vertical swimming distance, aggression, and courtship. The primary PCA axis showed a broader separation between general time usage near a territory for maintenance and benthic feeding (benthic bites), compared to time away from the territory (out of view and plankton bites). Inherently “risky” behaviors such as swimming high in the water column, aggression, and courtship behavior, that are linked to increased predation risk (Myrberg 1972, Figueira and Lyman 2007, Pavlova et al. 2010), occurred in higher frequencies in SS habitats. In contrast to SS fish, individuals in DS habitats sheltered often, and were frequently outside of the field view. Time out of the camera view was generally the result of swimming long horizontal distances, thus, indicating that DS fish traveled long distances but remained near the benthos, in close proximity to shelters. For small demersal reef fish, available shelter is necessary for predator evasion and survival (Nemeth 1998, Steele 1999), thus shelter accessibility is likely more important in DS habitats with high predator densities. Shelter selection is influenced by body size and substrate morphology (Hixon and Beets 1993, Brooker et al. 2013), which differed between DS and SS strata. Although soft coral was common in SS habitats and may be useful as shelter for some species, this is likely not effective for damselfish (Kerry and Bellwood 2011). In DS strata, with high predator densities, higher maximum vertical relief implies higher reef complexity and shelter availability compared to SS strata that also had lower predator densities.

For bicolor damselfish, planktivorous feeding and courtship require time in the water column, when fish are most susceptible to predation (Myrberg 1972). Environmentally mediated trade-offs between predation risk, foraging, and reproduction
are common for teleosts (Holbrook and Schmitt 1988, Figueira and Lyman 2007, Pavlova et al. 2010) and characterize a balance between growth, survival, and reproduction to maximize lifetime fitness. Additionally, correlations between aggression and courtship have been observed in other studies and vary in conjunction with stress hormones and habitat heterogeneity (Schrandt and Lema 2011). Environmental stressors also increase synthesis and secretion of neuropeptides that are linked with suppression of feeding behaviors and food intake (Schrandt and Lema 2011), suggesting that higher stress as a result of predation risk in DS habitats may have additional condition and fitness ramifications beyond energy and time allocation. Behavioral time allocation data indicate that lower predator densities in SS habitats led to riskier behaviors and increased time dedicated to feeding and courtship activities, all of which have implications for fish condition, reproduction, and fitness.

Bicolor damselfish exhibited differences in gut fullness and fine scale diet across depths, but similar broad diet composition. Food quality and consumption is determined by food availability, behavior, and selectivity, and influences energetic surplus available for biological processes (Brooker et al. 2013). After accounting for differences in body weight, SS reef fish had significantly fuller guts than fish from deeper habitats. For SS individuals, increased risky vertical swimming, and behaviors that affect access to food in the water column, likely increased food intake in comparison to fish in DS habitats. While behavioral data are not available for MPR fish, gut fullness in mesophotic environments with low predator densities, was intermediate between SS and DS strata.

For planktivorous fish, diet can also be influenced by the availability and composition of plankton communities, which are influenced by multiple processes
including regional oceanography (Govoni et al. 2010) and diel vertical migration (Heidelberg et al. 2010). Mesophotic reefs at Pulley Ridge are primarily affected by the Loop Current that forms in the Gulf of Mexico and brings warm oligotrophic water, conducive to coral reef growth, to mesophotic depths (Jarrett et al. 2005). In the Florida Keys, eddies that propagate northward through the Straits of Florida are predominant features that upwell nutrient rich water and deliver pulses of plankton to reef habitats (Fratantoni et al. 1998, Hitchcock et al. 2005, Sponaugle et al. 2005, D’Alessandro et al. 2007). Eddies are associated with higher food availability, faster growth for larval fish (Shulzitski et al. 2015), and regional productivity differences that affect juvenile fish growth rates after settlement to the reef (Chapter 2). SS and DS strata in the Florida Keys are also differentially affected by breaking internal waves, or tidal bores, that deliver cold, nutrient rich water and plankton to deeper reefs more frequently, and for longer duration, than shallower reefs (Leichter et al. 1998, 2003, 2014). As a result, food availability for planktivorous organisms may differ across geographic locations, and in the Florida Keys, this is likely higher in deeper reef habitats. However, lower gut fullness in DS habitats despite expectations for higher plankton availability indicates that feeding for DS fish may be limited by behavioral predator avoidance and environmental stressors.

Although broad diet composition was roughly similar among depths, finer scale diet analysis revealed differences among strata. DS and MPR fish had the most substantial difference in diet that was largely the result of higher ingestion of appendicularians by mesophotic fish compared to ingestion of annelids, and to a lesser degree, calanoid copepods by DS fish. SS fish diets were intermediate, although ingestion of appendicularians was also more common among SS fish than DS. Diet variation
between depth strata implies differences in zooplankton community composition. Interestingly, appendicularians have not been identified in larval *Stegastes* spp. stomach contents (Llopiz and Cowen 2009), or adult bicolor damselfish (Johnson 1980). However, both appendicularians and copepods are common components of fish diets and have a similar nutritional content by size (Purcell et al. 2005). Appendicularians serve as a reliable and nutritionally rich food source in oligotrophic systems (Llopiz et al. 2010), indicating that MPR fish may capitalize on an available, abundant, high quality food source. In DS habitats with frequent tidal bore delivery of zooplankton, the available zooplankton community may differ, or fish feeding or prey selectivity may be constrained by predator avoidance behaviors (i.e. the need to swim close to the benthos).

Across all depths, bicolor damselfish diets included *Trichodesmium* sp., a diazotrophic filamentous cyanobacteria that constituted the majority of the gut contents. Ingestion of high quantities of *Trichodesmium* sp., approximately half of the total dried weight of gut contents, was unexpected for a primarily zooplanktivorous fish, although there is evidence of similar diet patterns in some Pomacentridae studies (Emery 1973, Johnson 1980). Consumption of *Trichodesmium* sp. by a demersal reef fish provides a direct nutrient pathway from atmospheric \( \text{N}_2 \) fixation to the reef environment (Carpenter and Romans 1991, McMahonlh et al. 2013), however, the quality, energetic content, and digestibility of *Trichodesmium* sp. for reef fish is unclear. Copepods are often associated with *Trichodesmium* sp. rafts (O’Neil and Roman 1994, Renate Eberl 2007), suggesting that it may be secondarily ingested by fish while they target copepods. Interestingly, there was an interaction between total gut content dried weight and prey category that was the result of increases in some diet categories, particularly *Trichodesmium* sp., as gut content
weight increased. Therefore, fish with fuller guts also consumed a higher proportion of *Trichodesmium* sp. compared to other prey items. SS fish that had significantly fuller guts, may obtain disproportionately lower additional energy from increased food consumption if *Trichodesmium* sp. is a lower quality food compared to copepods and other animals. The hypothesis that *Trichodesmium* sp. is assimilated, but is a low quality food source, is reinforced by depleted $\delta^{15}$N in muscle tissue from SS fish that typically had fuller guts, and thus consumed more *Trichodesmium* sp., that has depleted $\delta^{15}$N values. Nutritional value and availability of food sources elicits trade-offs between growth and reproductive output (Heino and Kaitala 1999, Samhouri 2009), and directly impacts fitness. The plasticity of *S. partitus* diets likely contributes to their ability to live across a broad depth range, but depth-driven differences in food quality and availability have further implications related to condition and fitness.

Gut content analysis provides insight into short-term diet composition, whereas stable isotope analysis contributes information on long-term feeding habits, nutrient source pools, trophic level, and the breadth of diet among depth strata. General patterns of algae, copepod, and bicolor damselfish $\delta^{13}$C and $\delta^{15}$N signatures revealed minimal enrichment of $\delta^{15}$N as depth increased, and substantial depletion of $\delta^{13}$C. $\delta^{15}$N enrichment is generally associated with increases in trophic level, clearly demonstrated by the differences in algae, copepod, and fish isotope values. The depleted $\delta^{15}$N of bicolor damselfish compared to dusky damselfish is consistent with a planktivorous diet and high consumption of *Trichodesmium* sp. by bicolor damselfish in comparison to fish with diets based on benthic food webs that are not linked to nitrogen fixation (Lamb-Wozniak 2007). $\delta^{13}$C changes minimally with trophic level and is therefore associated with nutrient
source pools (Post 2002). Isoscapes in the Florida Keys vary from inshore to offshore and across depths. $\delta^{13}C$ values of particulate organic matter become depleted from inshore to offshore environments (Lamb and Swart 2008), consistent with our results from SS to MPR. Deep water and upwelled $\delta^{15}N$ sources are enriched in comparison to surface waters as a result of $N_2$ fixation by diazotrophs, such as *Trichodesmium* sp. in shallow water. In the Florida Keys, the influence of upwelling and surface mixing are linked to isotope values of algae across depths, however, our results do not show the degree of $\delta^{15}N$ variation expected based on previous studies (Leichter et al. 2007).

Isotope analysis of both trophic position and diet breadth are dependent upon a thorough knowledge of baseline isotope values that can vary substantially spatially and temporally (Layman et al. 2011). Baseline isotope values are the result of a mixture of nutrient source pools that vary as a result of oceanographic forcing of nutrient uptake (Stokes et al. 2011), suggesting that some variation in the range of $\delta^{15}N$ and $\delta^{13}C$ values is expected. The present study utilized copepods as baseline values for trophic level calculations and incorporated multiple sampling time points and years to address temporal variability, and gut content analysis provided support that bicolor damselfish rely very little on benthic food sources. Additionally, supplemental benthic algae collections and net tows of copepods in SS strata confirmed isotope patterns of baseline copepods and bicolor damselfish.

Despite similarities between broad-scale diet compositions, isotope diet niche area was larger, and trophic position was unexpectedly higher, for bicolor damselfish in MPR strata compared to SS and DS fish. Total isotopic niche area is the result of the range of $\delta^{15}N$, associated with the breadth of diet with respect to trophic level, and $\delta^{13}C$ that is
linked to nutrient source pools (Layman et al. 2007, Jackson et al. 2011). The ranges of δ¹⁵N in fish muscle tissue were similar among strata, and larger isotope niche area in MPR habitats was primarily the result of a broader range of δ¹³C. Additionally, SS and DS strata had greater spatial overlap of isotope niche area, indicating a greater similarity in resource use and source pools (Hammerschlag-Peyer et al. 2011). This difference in carbon isotope width and lack of niche overlap between MPR and habitats in the Florida Keys implies a varied and unique nutrient source pool for fish in MPR habitats, and the probable effects of dynamic oceanography on delivery of nutrients and planktonic food sources to mesophotic depths at Pulley Ridge. This variable oceanographic environment is also reflected in greater sub-seasonal temperature variation at MPR compared to SS and DS strata (Chapter 3).

Abundance and composition of zooplankton along with selective feeding by damselfish affects diet and trophic levels and has implications for the condition and fitness of individuals. Trophic level of bicolor damselfish increased monotonically with depth despite similarities in broad-scale diet composition, but remained within the expected range for zooplanktivorous fish (Sommer et al. 2002). In comparison to SS fish, with the lowest trophic level, bicolor damselfish in MPR habitats ingested slightly more copepods, which have higher δ¹⁵N than other food sources, and marginally less Trichodesmium sp., that has a substantially lower δ¹⁵N than other food items (Montoya et al. 2002). Combined, these subtle differences in diet are consistent with a change in trophic level. The basis for the lower trophic level of DS fish is less clear, although fine-scale diet analysis did reveal the greatest difference in prey type between DS and MPR
strata, and DS fish may experienced reduced access to high value prey as a result of predator constrained vertical foraging height.

Lipid content, measured as C:N ratio, followed the same pattern as trophic level, with highest lipid content in mesophotic fish. In contrast, bicolor damselfish in DS habitats that exhibited less risky behavior and low gut fullness, had the same lipid content as SS fish. Gut fullness was greatest for SS fish, that also had low C:N ratios, indicating that differences in diet breadth and composition may have a greater impact on condition than gut fullness. Gut fullness was positively correlated with an increased ratio of *Trichodesmium* sp., suggesting that despite ingesting additional food, increased percent diet composition of *Trichodesmium* sp. may affect C:N ratios in SS fish. Diet quality and nutrient content can increase fish condition (Malzahn et al. 2007), suggesting that higher C:N ratios of fish in MPR habitats are likely the consequence of a higher quality diet.

The relationship between diet, age, and growth revealed that the combination of these processes had a significant impact on energy allocation to reproduction. Particularly, short-term reproductive investment, measured as GSI, had a positive relationship with trophic level, C:N ratio, and age, all traits associated with MPR fish populations that are composed of old, large, long-lived individuals with high reproductive investment in oocytes and high batch fecundity (Chapter 3). In contrast, reproductive investment was negatively correlated with gut fullness and fast growth, indicating a trade-off between growth and reproduction. The negative relationship between gut fullness and GSI is counterintuitive, however, there are several possible explanations for this pattern. A higher proportion of lower quality food in fuller guts or lower digestibility of food sources, associated with high consumption of *Trichodesmium* sp. may explain the
disconnect between gut fullness and GSI. Bicolor damselfish are also smaller and younger in SS habitats (Chapter 3), therefore, fish with full guts in SS habitats may allocate the majority of their energy to growth. Finally, reproductive investment is a combination of (1) fecundity and investment in individual oocytes, processes that are captured by GSI, and (2) frequency of spawning that cannot be measured by GSI (Maddams and McCormick 2012). Water temperatures are warmer in SS habitats (Chapter 3) and may increase metabolic activity and spawning frequency (Yoneda et al. 2014), explaining the discrepancy between high frequency of courtship behavior, high gut fullness, and low GSI for SS fish.

In MPR habitats where fish are older and larger (Chapter 3), have a higher trophic level, and higher lipid content, excess energy is available for high reproductive investment in spawning batches. Whereas in SS habitats with younger individuals (Chapter 3) and lower trophic levels, energy is allocated toward growth and feeding, indicated by higher gut fullness and more time spent in energetically expensive behaviors. Condition and fitness are often correlated in teleosts (Polivka 2011), and the combination of results indicate that habitat variation related to depth strata was linked to higher condition and higher reproductive investment in individual egg clutches for bicolor damselfish in MPR habitats.

Conclusions

This study highlights the compounding effects of habitat variation across depths on fitness for coral reef fish. Bicolor damselfish in MPR strata showed the greatest differences in diet, trophic position, and lipid content, whereas fish in DS and SS habitats had similar C:N ratios and trophic positions. Despite some similarities between depth
strata in the Florida Keys, fish in DS habitats exhibited less risky behaviors, likely the result of higher predator densities, and consequently had reduced gut fullness, and as seen previously, lower batch fecundity and investment in individual oocytes (Chapter 3).

Ultimately, the combination of biological responses to depth-driven environmental variability can have significant effects on energy allocation and fitness, and can scale-up to affect population dynamics and connectivity across depth strata by modifying reproductive output.

Mesophotic reef environments are at the farthest range of depth distributions for many coral reef taxa (Kahng et al. 2010), yet these marginal habitats may function as coral reef refuges that can supply larvae to declining shallow reefs (Bongaerts et al. 2010). Energetic trade-offs did not follow a consistent pattern with depth, and lower condition and reduced energy allocation to reproduction in DS habitats indicates a significant role of predators and diet in shaping habitat quality for small demersal reef fish. In SS habitats, fish with high gut fullness but low lipid content indicate the importance of food quality and energetic trade offs between fast growth and reproduction (Chapter 3). Zooplantivorous demersal reef fish in mesophotic habitats experience lower predator densities, feed at a higher trophic level on potentially higher quality food, attain higher lipid content, and grow more slowly in cool waters, resulting in excess energy that is available for reproductive investment. Mesophotic reef environments provide high quality habitat for planktivorous coral reef fish that has implications for the refugia function of deeper reefs to maintain populations of reef organisms with the ability to supply high population level reproductive output to sustain coral reef ecosystems.
Table 4.1. Results of statistical analyses comparing strata (SS=shallow shelf, DS=deep shelf, MPR=mesophotic). Significant comparisons (p<0.05) are in bold and sample sizes are listed for each test. C:N is the ratio of mass of carbon to nitrogen in fish muscle tissue, and GSI is gonado-somatic index. See Table 4.2 for a description of PC1 (principle component 1).

<table>
<thead>
<tr>
<th>Test</th>
<th>Significance</th>
<th>Sample size (SS, DS, MPR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predator density</td>
<td>DS&gt;SS&gt;MPR</td>
<td>41, 38, 67</td>
</tr>
<tr>
<td>Kruskal-Wallis and Dunn's</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gut fullness</td>
<td>SS&gt;DS</td>
<td>19, 26, 29</td>
</tr>
<tr>
<td>ANCOVA and Tukey HSD</td>
<td>MPR=SS &amp; DS</td>
<td></td>
</tr>
<tr>
<td>Diet category dry weights</td>
<td>Strata: SS=DS=MPR</td>
<td>16, 20, 20</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Prey category</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prey category*Total gut content dry weight</td>
<td></td>
</tr>
<tr>
<td>Trophic position</td>
<td>MPR&gt;SS &amp; DS</td>
<td>17, 17, 21</td>
</tr>
<tr>
<td>ANOVA and Tukey HSD</td>
<td>SS=DS</td>
<td></td>
</tr>
<tr>
<td>Isotope convex hull area</td>
<td>SS=1.70</td>
<td>17, 17, 21</td>
</tr>
<tr>
<td></td>
<td>DS=2.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MPR=4.10</td>
<td></td>
</tr>
<tr>
<td>Isotope Bayesian ellipse area</td>
<td>SS=1.06, SS&lt;DS 61.0%</td>
<td>17, 17, 21</td>
</tr>
<tr>
<td>10^6 bootstraps</td>
<td>DS=1.16, DS&lt;MPR 93.3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MPR=1.91, MPR&gt;SS 93.4%</td>
<td></td>
</tr>
<tr>
<td>C:N ratio</td>
<td>MPR&gt;SS &amp; DS</td>
<td>17, 17, 21</td>
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<tr>
<td>ANOVA and Tukey HSD</td>
<td>SS=DS</td>
<td></td>
</tr>
<tr>
<td>GSI vs. Fish variables (PC1)</td>
<td>GSI=3.7*PC1+4.1</td>
<td>SS+DS+MPR=13</td>
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<tr>
<td>Least squares linear regression</td>
<td>R^2=0.40</td>
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</table>
Table 4.2. Factor loadings (raw scores) for benthic habitat Correspondence Analysis (CA), and behavior, diet, and biological response Principle Component Analyses (PCA). Factor loadings that were strongly associated with habitat strata or variation along axis 1 or axis 2 are listed for CA analyses. For PCA, variable correlations that are significantly different from 0 are listed.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Axis 1</th>
<th>Axis 2</th>
<th>Correlation PC1</th>
<th>Correlation PC2</th>
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<tr>
<td>Benthic habitat CA</td>
<td></td>
<td></td>
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<tr>
<td>Macroalgae</td>
<td>2.41</td>
<td>1.22</td>
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<td>-</td>
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<td>Cyanobacteria</td>
<td>1.64</td>
<td>1.63</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
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<td>Live coral</td>
<td>-1.02</td>
<td>0.20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Other (primarily silt)</td>
<td>-1.5</td>
<td>-0.91</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Behavior PCA</td>
<td></td>
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<td></td>
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<tr>
<td>Plankton bites</td>
<td>0.47</td>
<td>-0.18</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Out of view</td>
<td>0.45</td>
<td>0.02</td>
<td>0.67</td>
<td>-0.84</td>
</tr>
<tr>
<td>Vertical swim</td>
<td>-0.02</td>
<td>-0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggression</td>
<td>-0.24</td>
<td>-0.49</td>
<td>-0.36</td>
<td>-0.60</td>
</tr>
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<td>-0.40</td>
<td>-0.33</td>
<td>-0.59</td>
<td>-0.40</td>
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<td>Benthic bites</td>
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<td>0.06</td>
<td>-0.60</td>
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<td>-0.64</td>
<td>0.47</td>
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<tr>
<td>Diet PCA</td>
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<td></td>
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<td>Appendicularia</td>
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<td>0.50</td>
<td>0.80</td>
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<tr>
<td>Annelida</td>
<td>0.22</td>
<td>-0.65</td>
<td>0.32</td>
<td>-0.78</td>
</tr>
<tr>
<td>Calanoid copepod</td>
<td>-0.24</td>
<td>-0.14</td>
<td>-0.35</td>
<td></td>
</tr>
<tr>
<td>Ostracoda</td>
<td>-0.26</td>
<td>0.03</td>
<td>-0.38</td>
<td></td>
</tr>
<tr>
<td>Cyclopoid copepod</td>
<td>-0.48</td>
<td>0.10</td>
<td>-0.70</td>
<td></td>
</tr>
<tr>
<td>Harpacticoid copepod</td>
<td>-0.45</td>
<td>0.29</td>
<td>-0.67</td>
<td>0.34</td>
</tr>
<tr>
<td>Copepod</td>
<td>-0.51</td>
<td>-0.13</td>
<td>-0.75</td>
<td></td>
</tr>
<tr>
<td>Biological response PCA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C:N ratio</td>
<td>0.48</td>
<td>-0.07</td>
<td>0.89</td>
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</tr>
<tr>
<td>Trophic position</td>
<td>0.46</td>
<td>0.46</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.45</td>
<td>-0.47</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>Gut fullness</td>
<td>-0.41</td>
<td>-0.59</td>
<td>-0.76</td>
<td>-0.57</td>
</tr>
<tr>
<td>Von Bertalanffy growth rate (k)</td>
<td>-0.44</td>
<td>0.47</td>
<td>-0.81</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.3. Stable isotope results and meta-analysis information obtained from the literature. Information includes depth strata of collection, study species, sample size, diet of the study organism, and the literature reference and study location.

<table>
<thead>
<tr>
<th>Strata</th>
<th>Sample</th>
<th>Sample size</th>
<th>Diet</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>Dusky damselfish <em>Stegastes dorsopunicans</em></td>
<td>7</td>
<td>herbivorous</td>
<td>Lamb-Wozniak 2007 Upper Florida Keys</td>
</tr>
<tr>
<td>SS</td>
<td>Ocean surgeonfish <em>Acanthurus bahianus</em></td>
<td>42</td>
<td>herbivorous</td>
<td>Lamb-Wozniak 2007 Upper Florida Keys</td>
</tr>
<tr>
<td>SS</td>
<td>Scaridae juvenile <em>Sparisoma viride</em> and <em>Scarus croisensus</em></td>
<td>44</td>
<td>herbivorous</td>
<td>Lamb-Wozniak 2007 Upper Florida Keys</td>
</tr>
<tr>
<td>SS</td>
<td>Scaridae initial <em>Sparisoma viride</em> and <em>Scarus croisensus</em></td>
<td>115</td>
<td>herbivorous/calcium carbonate</td>
<td>Lamb-Wozniak 2007 Upper Florida Keys</td>
</tr>
<tr>
<td>SS</td>
<td>Scaridae terminal <em>Sparisoma viride</em> and <em>Scarus croisensus</em></td>
<td>106</td>
<td>herbivorous/calcium carbonate</td>
<td>Lamb-Wozniak 2007 Upper Florida Keys</td>
</tr>
<tr>
<td>surface</td>
<td>POM</td>
<td>79</td>
<td>-</td>
<td>Lamb and Swart 2008 Lower Florida Keys</td>
</tr>
<tr>
<td>SS</td>
<td>Turf algae</td>
<td>20</td>
<td>-</td>
<td>Lamb et al. 2012 Upper Florida Keys</td>
</tr>
<tr>
<td>SS</td>
<td>Sponge</td>
<td>155</td>
<td>-</td>
<td>Lamb et al. 2012 Upper Florida Keys</td>
</tr>
<tr>
<td>SS</td>
<td>Shallow macroalgae <em>Codium isthmocladum</em></td>
<td>mean of min and max</td>
<td>-</td>
<td>Leichter et al. 2003 Middle Florida Keys</td>
</tr>
<tr>
<td>DS</td>
<td>Deep macroalgae <em>Codium isthmocladum</em></td>
<td>mean of mix and max</td>
<td>-</td>
<td>Leichter et al. 2003 Middle Florida Keys</td>
</tr>
<tr>
<td>surface</td>
<td><em>Trichodesmium 1</em></td>
<td>20</td>
<td>-</td>
<td>Montoya et al. 2002 Spring season Subtropical Atlantic</td>
</tr>
<tr>
<td>surface</td>
<td><em>Trichodesmium 2</em></td>
<td>36</td>
<td>-</td>
<td>Montoya et al. 2002 Fall season Subtropical Atlantic</td>
</tr>
</tbody>
</table>
Figure 4.1. Map of the study site locations. All work at replicate shallow shelf and deep shelf sites was within a 75 m radius of the centralized GPS coordinates located at American Shoal shallow shelf (24.52732, -81.49848), Looe Key shallow shelf (24.54066, -81.43655), American Shoal deep shelf (24.52345, -81.49738), and Looe Key deep shelf (24.53951, -81.42265). The black diamond designates Looe Key shallow shelf and deep shelf sites, and the open diamond is American Shoal shallow shelf and deep shelf. Pulley Ridge collections took place within the black outlined polygon region labeled PR. Light gray depth contours are 5 m intervals from 0-10 m, dark gray are 10 m contours from 20 m (labeled on the map) to 100 m, and contours beginning at 400 m (labeled on map) are 400 m intervals.

http://www.fgdl.org/metadata/fgdc_html/bathym.fgdc.htm
Figure 4.2. Boxplot of predator densities at shallow shelf (SS), deep shelf (DS), and mesophotic (MPR) depth strata on the Florida Shelf. The central tendency line is the median, the upper and lower hinges are 3rd and 1st quartiles, respectively, the upper and lower whiskers are within 1.5 times the inter-quartile range between the 1st and 3rd quartile, and the points are outliers. Predator densities were significantly different between all depth strata (p<0.05).
Figure 4.3. Correspondence analysis (plotted with scaling 1 to preserve Euclidan distance among objects) of point intersect benthic habitat types at SS (shallow shelf) and DS (deep shelf) strata. Maximum vertical relief, depicted as a blue arrow, was numerically ranked and was included as a supplemental variable in the analysis. Benthic habitat types correspond to: CC=crustose coralline, CY=cyanobacteria, DC=dead coral, LC=live coral, MA=macroalgae, O=other (mostly silt), RB=rubble, SA=sand, SC=soft coral, SI=sessile invertebrate, and TA=turf algae. Location of habitat labels can be interpreted similarly to arrows in PCA analyses. Points are individual transects.
Figure 4.4. Principle component analysis (plotted with scaling 1 to preserve Euclidian distance among objects) of fish behaviors at SS (shallow shelf) and DS (deep shelf) strata showing principle component 1 (PC1) that explained 31.5% of the variation in the data and principle component 2 (PC2) that explained 21.5% of the variation in behavior. Shelter, Aggression, and Courtship were calculated as time spent in the activity divided by total time when the fish was in view. Benthic bites and Plankton bites were number of bites divided by total time that the fish was in view. Time out of view was calculated as time out of view divided by the total time of the video. Clusters were determined using hierarchical clustering and Ward's method based on Euclidean distance of PC2 loadings. Groups were cut at a Euclidian distance of 1.5. Depth strata are identified by color, and hierarchical clusters by shape.
Figure 4.5. Adjusted average (± SE) gut fullness of bicolor damselfish ≥30 mm standard length, at shallow shelf (SS), deep shelf (DS), and mesophotic (MPR) depth strata, calculated using ANCOVA relationships between body weight and gut content weight. Different letters designate significant differences.
Figure 4.6. Adjusted mean dry weight of gut content categories (± SE) for bicolor damselfish at shallow shelf (SS), deep shelf (DS), and mesophotic (MPR) depth strata, calculated using the Two-way ANCOVA relationship with total gut content dried weight as the covariate, and prey category and Strata as factors. There were no significant differences between depth strata and a significant interaction between total gut content dried weight and prey category.
Figure 4.7. Relationship between total gut content dried weight and prey category dry weights to demonstrate the significant interaction between the two terms in the ANCOVA model. Total dried weights were extrapolated from subsample dried weights using the ratio of subsample wet weight to total gut content wet weight.
Figure 4.8. Principle component analysis (PCA) with scaling 1 (preserves Euclidean distance among objects) of individual bicolor damselfish gut content items excluding rare taxa with <20 observations for shallow shelf (SS), deep shelf (DS), and mesophotic (MPR) depth strata. PCA loading are plotted for principle component 1 (PC1) that explained 24% of the variation in the data and principle component 2 (PC2) that explained 16.1% of the variation. Counts of organisms were divided by total gut content wet weight, and values were Hellinger transformed. Clusters were determined using hierarchical clustering of PC2 loadings using Euclidian distance and Ward's method. Clusters were cut at a Euclidean distance of 1.0 to obtain 3 clusters. Organisms are Appendicularia (App), Annelida (Ann), calanoid copepod (Cal), copepod (Cop), Crustacea (Crust), Ostracoda (Ost), cyclopoid copepod (Cyc), harpacticoid copepods (Harp), egg from unknown sources (Eggs). Strata are identified by color and clusters by shape.
Figure 4.9. Mean (±SE) of δ¹³C and δ¹⁵N isotope values of bicolor damselfish, copepods, and benthic algae collected from shallow shelf (SS), deep shelf (DS), and mesophotic (MPR) habitats and values from the literature. Samples from this study use a consistent color scheme, and bicolor damselfish data points are circled, benthic algae are triangles, and copepods are diamonds, for ease of interpretation. Copepods collected using light traps were primarily large calanoid species, and were stored in 95% ethanol, whereas, "SS copepod small" were collected using diver-towed nets, included copepods of smaller sizes, and were stored frozen. Values obtained from the literature are indicated by asterisks, and sources are provide in Table 4.3. *Trichodesmium* δ¹³C values are not presented, indicated by the x-axis break and dashed line.
Figure 4.10. Mean (±SE) trophic position of bicolor damselfish >30 mm standard length at each depth strata (SS = shallow shelf, DS = deep shelf, MPR = mesophotic). Trophic position was calculated using δ$^{15}$N values of fish as higher consumers and copepods as baseline values, with an estimated trophic level of 2.5 for copepods, and δ$^{15}$N enrichment of 3.5 per trophic level. Significant differences (p<0.05) are depicted with an asterisk.
Figure 4.11. Stable isotope $\delta^{13}C$ and $\delta^{15}N$ biplot showing quantification of bicolor damselfish isotope niche space in each depth strata SS (shallow shelf), DS (deep shelf), MPR (mesophotic). Dotted lines are outlines of isotope convex hull area (total area: SS=1.70, DS=2.14, MPR=4.10). Standard ellipses based on bivariate normal distributions, encompassing ~40% of the data, were calculated using Bayesian methods in SIBER (Stable Isotope Bayesian Ellipses in R), with a correction for small sample sizes. Ellipse area calculations with $10^4$ bootstraps were SS = 1.06, DS = 1.16, and MPR = 1.91. Comparisons with Bayesian credible intervals between strata showed probabilities of: 61.0% that SS < DS, 93.3% that DS < MPR, and 93.4% that SS < MPR.
Figure 4.12. Comparison of bicolor damselfish mean (±SE) C:N ratios between depth strata as a proxy for lipid content. Different letters correspond to significant differences. There were no significant differences found between immature, male, and female fish, so the model was simplified to compare only strata.
Figure 4.13. Relationship between GSI (gonado-somatic index) and individual fish variables including trophic position, C:N ratio, age, gut fullness, and VB growth (Von Bertalanffy growth rate coefficient, k). Fish variables were condensed into (a) a single principle component (PC1) variable that explained 69% of the variation in the data set using principle component analysis, plotted with scaling 2 that preserves correlations among variables. (b) GSI regressed against PC1 (raw scores). Line is significant linear regression ($p = 0.01$, $R^2 = 0.40$, equation: $y=1.6x+4.1$). Depth strata (SS=shallow shelf, DS=deep shelf, MPR=mesophotic) are indicated by the grey color scale.
Chapter 5: Habitat availability and depth-driven population demographics regulate regional reproductive output of a common coral reef fish

Summary

Metapopulations of coral reef organisms often span large geographic regions and broad depth ranges. Consequently, habitat suitability and availability differs among subpopulations, creating spatially variable population demographics, and differences in subpopulation size and reproductive output. Mesophotic coral ecosystems at the extreme depth range of photosynthetic coral reefs (~30-150 m) are buffered from anthropogenic stressors and climate change and thus may be refuges for coral reef organisms. However, the degree of connectivity via larval supply from mesophotic reef habitats is restricted by population size and reproductive output of organisms in potentially marginal habitats at the edge of their depth distributions. This study incorporated data on population density, benthic habitat, and depth-stratified population demographics to assess bicolor damselfish population density, population size, and reproductive output from a broad geographic region encompassing the known depth distribution of the species: Florida Keys (0-35 m depths) and mesophotic reefs (~60-100 m) at Pulley Ridge on the west Florida Shelf. Results indicated population densities, population sizes, and reproductive output peaked at mid-depths (10-20 m) in the Florida Keys and declined as depth increased. Estimates of population reproductive output were considerably affected by differences in population demographics across depths, including fish size, sex ratios, and probability and frequency of spawning. Despite low population densities on mesophotic reefs, the potentially expansive reef area at Pulley Ridge resulted in an estimated subpopulation size that composed ~20% of the entire population in the study area including Pulley Ridge and the
Florida Keys, and ~14% of the total reproductive output. These results indicate that mesophotic reefs may be important larval source populations, and that habitat availability and depth-driven population demographics and dynamics have substantial effects on reproductive output and potential larval supply from reefs across geographic regions and depths.

**Background**

For demersal coral reef fishes with pelagic larvae, the magnitude of larval contribution to a metapopulation can differ among spatially distinct subpopulations. The degree of connectivity among subpopulations is dictated by source-sink dynamics and a combination of both larval supply and post-settlement survival (Robertson et al. 1988, Schmitt and Holbrook 1996) that are linked to the life cycle of individual organisms. Reproductive output restricts the upper limit of potential larval supply, which is then modified by growth and survival of planktonic larvae, delivery and settlement to the reef environment, and post-settlement processes, to determine population connectivity (Cowen and Sponaugle 2009).

At the scale of the subpopulation, population demographics and habitat suitability and availability can influence population densities, distributions, and reproductive output. Optimal habitats with high food and shelter availability, and low predation, can support high densities of fish and large population sizes (Hixon et al. 2012) with higher condition individuals (Jones 1986, Booth and Hixon 1999, Munday 2001). Habitat variability can differentially affect juvenile and adult fish distributions (Dahlgren and Eggleston 2000, Kimirei et al. 2015), and can affect the growth and size of individual fish. Population age structure and size distributions can result in subpopulation differences in reproductive

Population reproductive output is dependent on fish density and abundance, however, distributions and patterns of density and abundance also can vary temporally and spatially as a result of dynamic environmental processes. Oceanographic features that bring pulses of larvae to, or advect larvae away from, settlement habitats affect variation in settlement magnitude to reef habitats (D’Alessandro et al. 2007, Hogan et al. 2012), creating temporal and spatial heterogeneity in fish population distributions. Short-term peaks in fish densities can only be detected from fine-scale temporal sampling, and may ultimately have a minimal effect on population sizes. For example, the bicolor damselfish, S. partitus, has higher larval settlement in the lower Florida Keys compared to the upper Florida Keys, but this coincides with higher mortality rates (Chapter 2). This pattern suggests that the magnitude of subregional differences in population densities peaks during settlement pulses, but that high recruitment alone cannot predict long-term population sizes.

This study focused on bicolor damselfish populations across an extensive depth range of photosynthetic coral reef ecosystems, and a broad geographic area spanning from the reefs in the Florida Keys (0-35 m) to mesophotic depths (defined as ~30-150 m) on the west Florida Shelf. The Florida Keys encompasses a large geographic region with a diversity of reef habitats (Lidz et al. 2003), transient oceanographic features (D’Alessandro et al. 2007), and spatial variations in faunal assemblages (Jones and Thompson 1978, Maliao et al. 2008, Kellison et al. 2012) that have the potential to affect
temporal and spatial patterns of fish distributions and population dynamics. Additionally, deep shelf (>20 m-35 m) and mesophotic reef environments have received little attention to date, but may be important refuges for coral reef organisms (Brokovich et al. 2008, Lesser et al. 2009, Kahng et al. 2010, Slattery et al. 2011). For example, the ‘deep reef refugia hypothesis’ proposes that deeper reefs that are buffered from storms and temperature fluctuations may provide a refuge for coral reef organisms (Glynn 1996, Bongaerts et al. 2010). By safeguarding coral reef organisms, naturally protected deep shelf and mesophotic reefs may serve a function similar to marine reserves in supplying larvae from healthy source reefs. Mesophotic coral ecosystems share many of the same coral and fish taxa that are found on shallower reefs (Kahng et al. 2010), suggesting that they may be connected through larval dispersal. Vertical connectivity between coral populations (Van Oppen et al. 2011, Serrano et al. 2014), and high population fecundity of mesophotic corals (Holstein et al. 2015) recently have been identified, but limited data are available for fish populations on mesophotic reefs. Additionally, the refuge function of deep shelf and mesophotic reefs may be threatened by species invasions and other stressors that can disrupt coral reef systems and fish communities (Lesser and Slattery 2011).

An understanding of reef fish populations across multiple spatial scales would contribute to our ability to evaluate the resilience of coral reef communities, the potential recovery of stressed reefs through reproduction and larval dispersal, and to design marine reserves to encompass regions of high reproductive output that are potential larval source locations. The goal of this study was to identify spatial patterns of bicolor damselfish (Stegastes partitus) population distributions, and incorporate depth stratification and
regional variation in population demographics to determine spatially expansive estimates of fish population reproductive output. The overarching goal was to provide insight into spatially variable population dynamics and empirical data critical to connectivity modeling efforts applicable to marine spatial planning.

Methods

Study location

The Florida Current forms as the Loop Current flows out of the Gulf of Mexico into the Florida Straits. This major western boundary current connects the coral reefs distributed around the Florida Peninsula (Fig. 5.1). The Florida Keys reef tract is located at the SE tip of the Florida Peninsula and stretches for ~300 km. Pulley Ridge, a mesophotic hermatypic coral reef, is located at depths of ~60-100 m along the west Florida Platform. Coral reef habitat of Pulley Ridge is most prevalent in the southern portion of the ridge with estimates of near 60% of the benthic habitat occupied by live biota (Reed et al. 2014). This southern region includes a NOAA (National Oceanographic and Atmospheric Administration) designated Habitat Area of Particular Concern (HAPC) with known scleractinian coral and fish habitat (Fig. 5.1; Cross et al. 2005, Reed et al. 2014). Recent reports indicate that the region to the west of the Pulley Ridge HAPC also has hard bottom habitat occupied by coral reef fishes (Reed et al. 2014).

Study overview

Numerous datasets and sources were used to assess depth distributions, population densities, and reproductive output of bicolor damselfish. The structure of the study sequentially layered and refined analyses using each dataset to calculate depth-
stratified and regional reproductive output. The base datasets included benthic habitat data and spatially explicit density distributions obtained from visual surveys in the Florida Keys, and ROV fish and benthic habitat surveys at Pulley Ridge. These datasets were used to obtain population densities and probability of occurrence of bicolor damselfish in reef habitats across depths and regions. To modify densities to include only female fish, and incorporate empirical depth-stratified size distributions, we applied ratios and size distributions obtained from random fish collections from discrete depth strata (shallow shelf <10m, deep shelf 20-30 m, and mesophotic 60-70 m) to population densities. Fecundity is size dependent, thus, the regression relationship between fish size and batch fecundity was used to convert depth-specific densities and size distributions into estimates of reproductive output per unit area. Reproductive output estimates were further refined using fish collections by calculating the proportion of female fish that spawn from each depth bin, and included a temperature dependent spawning rate to estimate weekly reproduction. The final results incorporated population density data, depth-specific demographics, and benthic habitat to estimate depth-stratified weekly reproductive output for the entire reef habitat of the Florida Keys and Pulley Ridge mesophotic reef habitat.

Fish abundance datasets

*Florida Keys* - Visual survey data for a 12-yr time period from 2000-2011 (NOAA/NMFS Southeast Fisheries Science Center) were used to assess bicolor damselfish population densities and distributions throughout the Florida Keys. Data were collected during the summer months using a probabilistic sampling approach by dividing the Florida Keys Reef Tract into primary sampling units (PSUs) of 200 m x 200 m grid
cells. PSUs were randomly selected to conduct fish count surveys by SCUBA divers within a visual cylinder with a 7.5 m radius. If visibility was < 7.5 m, the radius of the observation cylinder was modified to 4 m and if visibility was < 4 m, the survey was not included in the analysis. For each fish species observed, divers recorded the count, minimum, median, and maximum estimated fish size by calibrating observations using a ruler attached to a rod, and triangular fish length distributions were calculated using length estimates (Bohnsack and Bannerot 1986, Smith et al. 2011). Detailed visual survey methods are described by Bohnsack and Bannerot (1986) and Smith et al. (2011).

**Pulley Ridge** - Bicolor damselfish densities from mesophotic reefs (60.3-94.5 m depth) at Pulley Ridge were calculated using 99 ROV transect surveys (UNCW *Super Phantom S2*) conducted within the HAPC during daylight hours in the summer months of 2012 and 2013. Sample locations were randomly selected by overlaying the reef habitat at Pulley Ridge with 28, 1 km x 1 km, random blocks. Five independent 100 m length ROV transects, with 100 m distance between transects, were conducted within each selected sampling block. Bicolor damselfish densities were calculated using a linear transect distance of 100 m and a 5 m width field of view. Additional ROV survey details are described in Reed et al. (2014).

**Spatial and temporal distributions**

**Florida Key** - To address temporal changes in fish distributions, the 12 yrs of visual survey data were separated into 3-yr time periods (2000-2002, 2003-2005, 2006-2008, 2009-2011) for analyses. Estimated length of bicolor damselfish observed during visual surveys were used to roughly separate fish counts into juvenile (≤5 cm total length, TL) and adult (>5 cm TL) age categories based on a ~50% maturity of female fish at 5 cm TL
(Chapter 3). Analyses included only benthic habitat categories in which bicolor damselfish were present, designated as rubble, patch reef, and reef. The reef habitat category included a combination of three different reef habitats with varying degrees of rugosity and complexity: continuous low relief, spur and groove low relief, and spur and groove high relief (Table 5.1). Bicolor damselfish juvenile and adult densities for each survey were calculated using the area of the visual survey cylinder, and all surveys within the same PSU were averaged to obtain an average PSU fish density m\(^{-2}\), and then extrapolated to total PSU fish density (fish 0.04 km\(^{2}\)).

The influence of depth and location on bicolor damselfish density in the Florida Keys was assessed using quantile regression (R statistical software, quantreg package). This method does not have the normality and variance assumptions of ordinary least-squares regression, and is ideal for data distributions with unequal variance where relationships between predictor variables and observations may not be consistent for all quantiles. Additionally, regression of the maximum quantile (90\(^{th}\) for this study) provides insight about the upper threshold of population densities with respect to limiting factors and constraints on population densities, whereas the median (50\(^{th}\)) quantile provides a measure of central tendency (Cade and Noon 2003). Neither latitude nor longitude accurately represents sample location along the curved Florida Keys reef tract, therefore, the sample location in the Florida Keys was linearized by fitting a polynomial regression curve to the reef tract (R\(^{2}\) = 0.96). Sample location was described by calculating the arc length from the PSU observation farthest west in the lower Florida Keys, to the fitted value of the sample location along the curve (R statistical software, pracma package). A location of 0 corresponds to the farthest west sample location, and the value increases as
the sample location approaches the upper Florida Keys. Some benthic habitat types were restricted to particular depths, confounding habitat-stratified depth analysis, so the quantile regression analyses included rubble, patch reef, and reef habitats, addressing differences in fish density with respect to only depth and location, and not habitat. The 90th quantile relationship between depth, location, and density was assessed separately for juvenile and adult fish. The 50th quantile regression relationship was assessed only for adult fish for later predictions of female density distributions and reproductive output. The relationship between depth and density was often not monotonic, so where there was curvature to the data distribution, a polynomial depth term was included in the quantile regression models. All model selection procedures, except for adult 90th quantile from 2000-2002, started with the full model including a squared term for depth and an interaction between depth and location. The full model was sequentially simplified for a total of 6 candidate models. For the adult 90th quantile model from 2000-2002, the full model did not include a squared term for depth based on visual inspection of the data.

For reproductive output calculations, analysis focused on the time period from 2009-2011 because it had the broadest sampling depth range, most consistent coverage of the Florida Keys, and was closest in time to the field-collected reproduction data (Chapter 3). Quantile regression relationships excluded locations in which no fish were observed; therefore, logistic regression was applied to address frequency of occurrence of bicolor damselfish from 2009-2011. A separate logistic regression analysis was performed for each habitat to assess potential differences in bicolor damselfish frequency of occurrence in different habitats, as well as the influence of depth and location on the presence of bicolor damselfish juveniles and adults. The full logistic model for probability of
occurrence in each habitat type included an interaction between depth and location, and the model was sequentially simplified to obtain four candidate models for comparison. The best-fit quantile regression and logistic regression models were determined based on Akaike information criterion \((AIC, \text{Akaike 1973})\). For model selection, models with delta AIC values <2 were considered (Burnham and Anderson 2002), and AIC weights were compared among competing models.

**Density predictions**

*Florida Keys* - Predictions of female bicolor damselfish densities for the spatial extent of reef tract in the Florida Keys were made using 2009-2011 median quantile regression results. Sex ratios could not be obtained from the visual survey data, so depth-specific sex ratios were calculated using transect collections from deep shelf (>20-30 m) and shallow shelf (0-10 m) reefs the lower Florida Keys from June-Aug 2013-2014 in which every fish observed along a 25 x 2 m transect was collected and sexed (Chapter 3). Transects were not conducted on mid-shelf reefs (10-20 m), therefore, shallow transect data were applied to the mid-shelf visual survey counts. This choice was based on the existence of an inflection point in the relationship between population density and depth at ~17-20 m, suggesting a habitat shift within that depth range.

Benthic habitat information associated with visual surveys for the Florida Keys included depth, location, and habitat information for the entire Florida Keys. Therefore, median density predictions for female bicolor damselfish were made for each PSU across the Florida Keys using the median quantile regression relationship. Bootstrapped median quantile regression 95% prediction intervals were calculated for each prediction. Density predictions throughout the Florida Keys were then modified using the probability of
occurrence from the logistic regressions and the proportion of mature female fish from depth-specific sex ratios from random collections to obtain an estimate of female bicolor damselfish counts in the Florida Keys. Based on low probability of occurrence of adult bicolor damselfish in rubble habitats in the Florida Keys, and the lack of rubble at Pulley Ridge, density predictions were not made for rubble habitats.

For regional and depth strata calculations, PSU predictions were extrapolated to total habitat area in each subregion and depth strata based on benthic habitat maps. Depth strata were defined as shallow shelf (0<10 m), mid-shelf (10<20 m), and deep shelf (20≤35 m). Bicolor damselfish predictions per unit area (km²) for each habitat type were calculated by dividing total population densities by the total habitat area of the subregion and depth strata. Regional error bars were based on extrapolated prediction intervals for density predictions and do not reflect compounded error associated with additional calculations.

*Pulley Ridge* - Bicolor damselfish ROV 100 m x 5 m transect density data from Pulley Ridge were less spatially and temporally extensive than the Florida Keys visual survey data. ROV sampling methods at Pulley Ridge have the potential to underestimate population densities in comparison to visual surveys (Andaloro et al. 2013). However, visual survey and ROV bicolor damselfish density data were determined to be comparable after ROV transects were modified to include only reef habitat, by comparing 2012-2013 ROV density data and 7 yrs of visual survey data from an overlapping spatial area in the Dry Tortugas (Wilcoxon signed-rank test, p>0.05). The assumption was made that ROV surveys underestimated bicolor damselfish densities because they included all habitats at Pulley Ridge, whereas visual survey data only included reef habitat. The
Pulley Ridge ROV dataset could not be separated into juvenile and adult fish because fish sizes were not estimated, therefore, densities were calculated using total fish counts. Median bicolor damselfish densities using ROV transects excluded transects with no fish observations. The number of fish m$^{-2}$ was extrapolated to fish km$^{-2}$ to match the spatial scale of visual survey calculations. The sample areas for fish presence and absence were comparable between Pulley Ridge and the Florida Keys. In the Florida Keys, presence or absence were calculated using average SCUBA diver observations within an approximate visual area of 177 m$^2$. The number of diver observations within each PSU ranged from 1-6 with an average of 2.6, indicating that the probability of occurrence in the Florida Keys, was based on ~458 m$^2$ area, compared to a 500 m$^2$ sample area at Pulley Ridge. Therefore, a probability of occurrence of bicolor damselfish of 0.43 was also incorporated into median density and total population calculations for Pulley Ridge.

To obtain female fish densities, median densities were adjusted using the ratio of females to the total number of bicolor damselfish collected during the summer of 2013 from Pulley Ridge. Fish were collected haphazardly, so sex and size distributions from mesophotic reefs were likely representative of the population, with 43% females. Densities in reef habitats were calculated by dividing total ROV density values by the reported 57.09% cover of live biota at Pulley Ridge (Reed et al. 2014), which was assumed to be reef. To illustrate the range of density values at Pulley Ridge, minimum and maximum confidence bounds for the number of females km$^{-2}$ were calculated using the same methods described above, but using 10$^{th}$ and 90$^{th}$ quantiles rather than median densities.
Total area of reef habitat was calculated using various estimates of the spatial extent of Pulley Ridge reported in the literature (Cross et al. 2005, Jarrett et al. 2005, Locker et al. 2010, Reed et al. 2014). The minimum estimate of total reef coverage used for analysis was the approximate area of the HAPC (350 km$^2$) with 57% coral cover, or ~200 km$^2$. The mid-range estimate was the total 350 km$^2$ extent of the HAPC with 100% potential fish habitat. The maximum estimate was calculated including potential reef area outside the HAPC, or 30 km x 25 km with assumed 57% coral cover, or ~428 km$^2$. The presence of patch reefs at Pulley Ridge is unknown, therefore, all reef habitat was assumed to be continuous. To calculate total area estimates of female bicolor damselfish at Pulley Ridge, median values of the number of females km$^{-2}$ were multiplied by minimum, mid-range, and maximum estimates of total reef area.

*Population demographics*

*Size distributions* - Fecundity has a positive linear relationship with fish size, therefore precise size distributions were incorporated into reproductive output calculations. Size observations from visual data were coarse, so additional data from fish transect collections at shallow (<10 m) and deep (20-30 m) shelf sites in the lower Florida Keys, and from mesophotic reefs (60-70 m) at Pulley Ridge from June-August 2013 and 2014 were used to refine size distributions of visual survey density data. All randomly collected mature female fish from shallow shelf, deep shelf, and mesophotic reefs were measured to the nearest 0.1 mm, and standard length (SL) size distributions were applied to density calculations. The size distributions of mature female fish for each depth strata were smoothed using a kernel density smoother, and the area under the curve was calculated to obtain proportions of the total female fish that were within 1 mm size bins.
(R statistical software, pracma package). The depth-specific size proportions were applied to the predicted counts of female bicolor damselfish, based on visual and ROV surveys, within each PSU in the FKS and each 1-km² observation for Pulley Ridge.

**Reproduction** - The relationship between SL and batch fecundity for bicolor damselfish (batch fecundity = 265.02*SL-5233.73, R²=0.39, adapted from Chapter 3) was used to calculate potential reproductive output for each fish observed, and then extrapolated to population reproduction for a given unit of area. The population fecundity values were adjusted using calculations of the number of female fish spawning in each depth strata during peak reproduction, based on the proportion of female fish collected that had ovaries with post-ovulatory follicles that are evidence of recent spawning (shallow shelf: 0.46, deep shelf: 0.30, mesophotic: 0.33; Chapter 3). The re-absorption of post-ovulatory follicles is temperature dependent, so mean temperatures from each depth strata (Onset Hobo temperature sensors for the shallow and deep shelf reefs in the Florida Keys, and ADCP for Pulley Ridge) during the time period of the fish collections (July and August 2014: shallow shelf=31.8°C, deep shelf = 28.87°C; July 2013: mesophotic = 20.93°C) were used to calculate the rate of post-ovulatory follicle re-absorption via the relationship described by Ganias et al. (2007). The combination of the proportion of the population spawning and rate of reabsorption of post-ovulatory follicles provided an estimate of the number of fish spawning and frequency of spawning. Bicolor damselfish spawn at dawn, and all post-ovulatory follicles were calculated to be re-absorbed within 72 hrs (shallow shelf = 41.0 hrs, deep shelf = 47.9 hrs, mesophotic = 67.2 hrs, Chapter 3). All fish collections were after dawn, therefore, presence of post-ovulatory follicles indicated spawning within the last 2 d on shallow and deep shelf reefs, and within the last 3 d on
mesophotic reefs. The proportion of spawning females was applied to fish distributions using the same depth strata categories as the size distributions (shallow shelf calculations were applied to 1-20 m, and deep shelf calculations were applied to >20-35 m). For bicolor damselfish, reproduction peaks during ~7 d following the full moon in the summer months (Schmale 1981, Robertson et al. 1988). Weekly reproductive output during peak reproduction was calculated using the combination of female fish density, depth-specific size distributions, and the depth-specific frequency of spawning using proportion of females spawning and follicle reabsorption rates. Regional reproductive output was extrapolated to strata and subregion estimates based on total reef area using the same methods described for female fish density calculations. Error bars represent error associated with density calculations and do not reflect any compounded error from additional calculations and extrapolations. All analyses were implemented using R version 3.1.2 (R Development Core Team).

Results

Spatial and temporal distributions

Quantile regression results revealed that while there was some variability in fish distributions between time periods, there were predominant temporal consistencies in the relationship between fish density, depth, and location. For all time periods, the best-fit 90th quantile model for juvenile fish densities, which can be interpreted as the maximum threshold for fish densities, included a squared term for depth, describing an increase in maximum population densities with depth, until an inflection point at ~17-20 m, followed by a decrease (Fig 5.2a). Densities peaked at mid depths, suggesting that maximum densities were limited at the shallowest and deepest locations in the Florida Keys (Table
5.2, Fig 5.2a). Juvenile 90\textsuperscript{th} quantile regressions also differed with respect to location along the Florida Keys, with a general pattern of maximum densities increasing from the LK to the UK (Table 5.2, Fig 5.2b). However, for juvenile fish, the 90\textsuperscript{th} quantile relationship also showed an interaction between depth and location for all time periods except 2006-2008 (Table 5.2), indicating that the relationship between juvenile fish maximum densities and depth differed along the Florida Keys, but maintained the general pattern of density limitation at the shallowest and deepest depths.

For adult fish from 2009-2011, the focal years for reproduction analyses, there was a general pattern of higher maximum densities in the UK compared LK (Fig. 5.2d). The negative parabolic relationship between density and depth was also consistent from 2006-2008 and 2009-2011. However, 2000-2002 and 2003-2005 showed a monotonic relationship between maximum adult fish density and depth. There was an interaction between depth and location for all time periods except for 2006-2008, indicating the relationship between population density and depth varies along the Florida Keys, but generally follows a negative parabolic relationship (Table 5.2).

The median quantile regression, a measure of central tendency, for adult bicolor damselfish densities showed a less consistent relationship between density, depth, and location than the 90\textsuperscript{th} quantile, indicating that while maximum threshold fish densities were restricted to particular depths and locations, median values were less influenced by these factors (Table 5.2, Fig 5.2 c-d). The relationship between depth and density was consistent along the Florida Keys for all time periods except 2006-2008, where an interaction term was included in the best-fit model (Table 5.2). Fish density had a parabolic relationship with depth from 2006-2008 and 2009-2011 with peaks densities at
mid-depths, and had monotonic relationship during 2003-2005. During the time period from 2000-2003, there was no effect of depth or location on median adult fish densities (Table 5.2).

Logistic regression results from 2009-2011 showed a high probability of juvenile fish occurrence in reef habitats for all depths (Fig. 5.3a). The relationship between probability of occurrence and depth for juveniles was negative in rubble habitats and positive in patch reef habitats. However, rubble was primarily restricted to shallower water, as indicated by broad 95% prediction intervals as depth increased (Table 5.3, Fig. 5.3a). Probability of adult occurrence on reef habitats increased with depth (Fig. 5.3b), whereas, on patch reefs, probability of occurrence across depths differed along the Florida Keys, with a significant interaction between depth and location (Table 5.3). Similar to rubble habitats, 95% prediction error bands increased for patch reefs as depth increased, as a result of fewer patch reefs in deeper habitats (Fig. 5.3b).

For density prediction calculations, the mean proportion of mature fish that were female from shallow shelf reefs was 57%, 52% from deep shelf reefs, and 43% of the total population from mesophotic habitats (Table 5.4). In reef environments, the overall relationship between the predicted median number of female bicolor damselfish km$^{-2}$ and depth was consistent across regions in the Florida Keys. Female densities were lowest in the shallowest habitats, peaked at mid-depths, and then declined slightly in the deepest habitats (Fig. 5.4a). Median predicted densities of female bicolor damselfish in mid and deep shelf patch reef habitats were higher than shallow shelf patch reefs in the MK and UK, but this pattern was not present in the LK, with negligible numbers of fish on patch reefs (Fig 5.4b).
At Pulley Ridge, bicolor damselfish median density from ROV transects, excluding transects with no fish observations, was 0.004 fish m$^{-2}$, or 4000 fish km$^{-2}$, with 43% females in the population, or 1720 females km$^{-2}$. Probability of occurrence in an ROV transect data was 0.43, indicating that this species was less common at Pulley Ridge compared to reef habitats in the Florida Keys, where juveniles had a close to 100% predicted probability of occurrence (Fig. 5.3a). Incorporating probability of occurrence, the estimated number of females at Pulley Ridge dropped to 739.6 females km$^{-2}$.

However, Pulley Ridge ROV transects included all habitat, and not just reef environments, so total densities, estimated by dividing the number of female fish (739.6 females km$^{-2}$), by the percent cover of live biota in ROV transects (57%), resulted in an estimated median density of 1297.544 females km$^{-2}$ in reef habitats (Fig. 5.4a). Prediction intervals could not be calculated at Pulley Ridge, therefore, 10$^\text{th}$ and 90$^\text{th}$ quantiles representing the entire range of densities were calculated, and consequently show a larger interval than 95% prediction intervals in the Florida Keys. Median female fish densities were substantially lower at Pulley Ridge compared all depth strata in the Florida Keys (Fig. 5.4a).

The amount of available habitat differed considerably between depths and regions and had substantial effects of subpopulation sizes. The total area of patch reefs was considerably lower than reef habitat, leading to smaller predicted total numbers of adult female fish (Fig. 5.5b). In the Florida Keys, available reef habitat peaked at mid-shelf reefs, where median female fish densities were also highest, leading to the highest predicted fish population sizes. The most pronounced difference between depths was apparent in the LK, where the total number of female fish was considerably higher on
mid-shelf reefs compared to both shallow and deep shelf reefs (Fig. 5.5a). In the MK and UK, shallow shelf and mid-shelf median numbers of females were similar, however, deep shelf habitats had lower predicted female population sizes. Although available habitat was not mapped as thoroughly at Pulley Ridge as in the Keys, Pulley Ridge bicolor damselfish populations have the potential to include numbers of female fish on approximately the same scale as individual subregions in the Keys (Fig. 5.5a). Summing all strata and subregions in the Keys provided a total Keys-wide female bicolor damselfish population in reef habitats of 1,686,172. Considering low and high estimates of reef area at Pulley Ridge, the total number of female bicolor damselfish ranges between 259,269 – 555,576, or approximately a quarter of the number of female fish over the entire extent of reef habitat in the Keys.

**Population demographics and reproduction**

Population demographic calculations were made for only reef habitat based on the large spatial extent and high numbers of bicolor damselfish in reef habitats compared to rubble (Fig. 5.3b) and patch reefs (Fig. 5.5b). Female fish population size distributions shifted from smaller sizes in shallower habitats, to a higher frequency of larger size fish as depth increased (Fig. 5.6). Predicted female fish reproduction in the FK based on median quantile regression analyses, incorporating depth-specific size distributions to calculate reproductive output, echoed the negative parabolic relationship between fish density and depth (Fig. 5.7). Depth-specific temperatures during the study period combined with the proportion of fish with post-ovulatory follicles produced differences in the proportion of spawning females and frequency of spawning across depths. At depths < 20 m, 46% of the population reproduced every 2 d, between 20-30 m depths,
30% of the population reproduced every 2 d, and between 60-70 m, 33% of the population reproduced every 3 d (Chapter 3). The sharp decline in weekly reproduction at 20 m (Fig. 5.7) was a direct result of the assumptions of the analysis and lack of continuous probability of reproduction data. Differences in the proportion of spawning females had a large impact on predicted weekly egg production, substantially decreasing reproductive output on deep shelf reefs (Fig. 5.7).

Predicted weekly egg production for each km$^2$ of reef habitat, grouped by subregion and depth strata, indicated consistently higher reproduction in mid-shelf habitats, and lower levels of weekly reproduction in shallow and deep shelf habitats (Fig. 5.8). Based on median weekly reproduction calculations, mesophotic habitats at Pulley Ridge had the lowest egg production per unit area compared to all other depth strata (Fig. 5.8).

On a regional spatial scale, reproductive output followed the same depth strata pattern as female density distributions. Reproduction peaked at mid-depths as a result of available reef habitat, particularly in the LK (Fig. 5.9). Median values of total potential reproductive output at Pulley Ridge were on the same scale as subregions and depth strata in the Florida Keys, however, summed together, populations in the Keys potentially produce 5-11 times more eggs during weekly peak reproduction periods in the summer months compared to Pulley Ridge. Of the total number of bicolor damselfish eggs produced over the entire region, 14% are potentially sourced from Pulley Ridge populations.
Discussion

I compiled data from several sources to assess spatially explicit depth-stratified population size and potential reproductive output of the bicolor damselfish across a large geographic area, and the entire known depth distribution of the species. The results provide information about population density, size, and reproductive output of a coral reef fish from their ostensibly preferred depths to potentially marginal habitats at the extreme depth range of coral reef ecosystems. Additionally, I assessed the relative effects of population density and habitat availability on bicolor damselfish population size and reproduction across an expansive study area. Estimates of population size and reproductive output provide insight into the influence of spatially variable subpopulation dynamics on potential population connectivity, and capacity of deep shelf and mesophotic reefs to function as refuges by supplying larvae to degraded shallow reef habitats (Lesser et al. 2009, Bongaerts et al. 2010).

Depth and population distributions in the Florida Keys

Bicolor damselfish population density distributions had underlying temporal and spatial patterns across regions and depths: fish densities were lowest at the extreme ranges of their depth distribution, and reached maximum densities at mid-depths. This difference in maximum population density across depth strata could be the result of multiple ecological processes such as differences in recruitment (Jones 1990, Forrester 1995) or density dependence related to predation or habitat (Forrester et al. 2008, Hixon et al. 2012). Such depth restrictions are common in coral reef fishes and can be regulated by a variety of factors including settlement to the reef environment, and survival and growth trade-offs throughout ontogeny (Srinivasan 2003). Depth limitations at the tails of
the distribution were evident for both juvenile and adult age groups indicating that habitat suitability, such as differences in preferred substrate complexity or food (Almany 2004, Grol et al. 2010), may not vary sufficiently across depths to differentially affect density patterns based of fish size and age. In the Florida Keys, maximum fish densities were limited by both depth and location, whereas median population densities were less variable and more spatially uniform throughout depths and subregions. Median and maximum peak densities suggest that mid-shelf reefs have the potential to support dense populations of demersal reef fish in comparison to both shallow and deep shelf habitats.

Geographic differences in fish densities did not always have a consistent relationship with depth along the Florida Keys. The interaction term between depth and location for juvenile and adult fish distributions over most time periods suggests that the relationship between depth and density changes along the Florida Keys, and that there is some temporal variation in distributions. Bicolor damselfish are very common in the Florida Keys, however, there is evidence of higher recruitment, coupled with higher mortality of young fish, in the LK compared to the UK (Sponaugle et al. 2012, Chapter 2). Such temporal and spatial variability in regional densities is expected given the variable nature of larval settlement and recruitment (Sponaugle et al. 2005, D’Alessandro et al. 2007).

The analysis of probability of occurrence revealed differences between juvenile and adult distributions that were not detectable based on densities alone. In reef habitats, juvenile fish were consistently present, whereas adult bicolor damselfish occurrence varied across depths. Age categories were based solely on visual estimations of fish size, therefore, juvenile fish may have a higher frequency of occurrence as a result of a larger
size category grouping. Occurrence of adults increased with depth in reef habitats, but maximum densities were lower on deeper reefs for most of the study time periods. Other studies have found that individual damselfish are less aggregated in continuous habitats compared to patchier, less spatially consistent, reef habitats (Levin et al. 2000). Bicolor damselfish may aggregate at high population densities in the most favorable, or most complex, reef habitats in shallower environments, and have a more dispersed distribution in deeper reef habitats that are more uniform in complexity. Clustered or patchy fish distributions lead to a lower frequency of occurrence if the scale of sampling is sufficient to capture the variation. In deeper habitats that are dominated by spur and groove low relief (Table 5.1), fish distributions may be more uniform with a high frequency of occurrence, but do not reach the high fish densities observed in more variable shallow habitats.

Bicolor damselfish frequency of occurrence also differed between depth and age group on patch reef and rubble. The high frequency of occurrence in the deepest range of patch reefs in our study may be the result of the low numbers of patch reefs deeper than ~15 m in the Florida Keys, or the result of settlement patterns to isolated habitats. For some reef fish species, settlement to patch reefs is higher than to continuous reefs as depth increases (Srinivasan 2003), and may be the result of the effects of habitat configuration on settlement cues (Morton and Shima 2013). Shallower patch reefs in the Florida Keys are also situated landward of the continuous reef tract, therefore, the concentration of settlement stage larvae in the water column may be diluted near shallower patch reefs, resulting in lower settlement. In rubble habitats, the frequency of occurrence of adult bicolor damselfish was consistently low, whereas juvenile fish were
often found in rubble habitats that were primarily restricted to shallow water depths. Differences in adult and juvenile frequency of occurrence were likely driven by microhabitat preference. Bicolor damselfish that have recently settled to benthic habitats are found in high densities in rubble (Nemeth 2005) that provides extensive shelter availability from predation for small fish (Nemeth 1998), but deficient shelter space for larger individuals. Although this dataset cannot distinguish differences in fish settlement and early post-settlement survival, these results indicate that rubble is an important shallow water habitat for young fish that is not available in deeper reef environments.

**Regional population distributions of female bicolor damselfish**

The combination of population densities, frequency of occurrence, and sex ratios of bicolor damselfish led to broad-scale distributions of female fish across regions and depth strata. Regional patterns mirrored the shape of the continuous density distributions across depths, with the highest densities found at mid-depths. These analyses incorporated frequency of occurrence and population density, ultimately revealing that higher probability of occurrence in deep shelf habitats buffered the impacts of lower fish densities per unit area. The relationship between female density and depth at Pulley Ridge is unique because the majority of the fish at Pulley Ridge were individuals of reproductive size and age, with mature females comprising 43% of the total fish population. The median value of female bicolor damselfish densities at Pulley Ridge indicated that female fish densities were substantially lower than densities in the Florida Keys. If Pulley Ridge and the Keys are considered continuous with respect to depth, then the results follow the negative parabolic relationship between density and depth, with the lowest population densities at mesophotic depths.
The amount of available reef habitat for bicolor damselfish populations varied considerably between depths and regions. Throughout the Keys, the total subpopulation number of female bicolor damselfish was primarily driven by differences in available habitat. Notably, deep shelf habitats had the smallest total population sizes. In the Keys, reef habitat declines between 30-35 m until a secondary outlier reef is reached at ~45 m (Lidz et al. 2003), limiting the total area of deep shelf habitat available to demersal reef fish. Mid-shelf reefs supported the highest densities of fish and provided the most extensive reef habitat, resulting in the highest population sizes. Patch reef fish populations reached densities that were comparable to other reef habitats, but the total area of patch reefs is minimal in the Keys, with negligible numbers of female bicolor damselfish compared to continuous reefs.

Mesophotic reefs at Pulley Ridge likely encompass a large expanse of benthic habitat for reef fish. Pulley Ridge is less thoroughly mapped than the Keys, however, recent ROV surveys confirm that reef habitat at Pulley Ridge extends to a flat basin and secondary ridge to the west of the ridge crest (Jarrett et al. 2005, Reed et al. 2014). Based on the estimates of total reef area, the population size of female bicolor damselfish at Pulley Ridge is substantial, with an estimated range of 15-33% of the total number of female fish found in the entire Florida Keys. Large population sizes suggest that, despite low population densities, Pulley Ridge is an important reef habitat for demersal coral reef fish.

**Reproduction**

Potential reproductive output, measured as egg production or population fecundity, is the result of a combination of population size, or spawning stock biomass, and
frequency of spawning. Fine-scale differences in fish populations, such as body size, can also mediate reproductive output. The positive relationship between fish body size and fecundity is well documented (Hixon et al. 2013) and observed in bicolor damselfish (Chapter 3). Larger parental body size also provides more subtle advantages to offspring including increased condition and survival in the planktonic environment (Berkeley et al. 2004, Marshall et al. 2010a). Female fish batch fecundity and oocyte area increased with depth in our study (Chapter 3) as well as body size, indicating that for each spawning bout, females from deeper habitats produce more larvae per capita, of higher condition, than shallower habitats. However, slower oocyte development related to colder water temperatures (Ganias et al. 2007, Yoneda et al. 2014) and empirical evidence of lower proportion of spawning females was incorporated into our analysis, decreasing egg production on mesophotic reefs by reducing proportion of the population spawning and the frequency of spawning. Although the decrease in the proportion of spawning females presumably changes more continuously with depth, the indication of lower reproductive output in deeper habitats is likely accurate despite the assumptions associated with mid-shelf reproduction calculations.

Reproductive output was driven by patterns of female fish densities across depth strata, but additional depth-related differences in the spawning population, spawning frequency, and fecundity significantly altered the underlying patterns. Mid-shelf peaks in reproductive output were even more striking than differences in female densities. Despite larger body sizes creating the potential for increased per capita reproductive output, fewer female fish spawned on deep shelf and mesophotic reefs based on post-ovulatory follicles in histological slides (Chapter 3), therefore, reproductive output was proportionally lower
than shallower reefs, in comparison to differences in population sizes. Median predicted reproductive output per unit area was markedly lower at Pulley Ridge than in the Keys, but total egg production was only 0.09-0.19 times lower than total egg production in the Keys. As depth increased, temperature dependent spawning frequency and lower spawning proportion of the population (Chapter 3) had a greater impact on reproductive output than larger body size. Total regional and depth strata egg production followed the underlying pattern of density distributions, but despite larger body size as depth increased, there were notable declines in egg production in deep shelf habitats and mesophotic habitats compared to shallower habitats.

**Conclusions**

Our results illustrate patterns of population distributions and reproductive output across depths and regions on the Florida Shelf. Mid-shelf reefs supported the highest densities of bicolor damselfish, had the highest area of suitable habitat, and as a consequence, were the source of the highest reproductive output in the Keys. While total population size and reproductive output at Pulley Ridge were lower than the Keys, mesophotic reefs supported ~20% of the female bicolor damselfish across the entire study region, primarily as a result of expansive reef habitat. Subpopulations of bicolor damselfish in potentially marginal habitats at Pulley Ridge may be an important source of larval supply for the Florida Keys and the Dry Tortugas. The size of bicolor damselfish increased as depth increased, and Pulley Ridge populations were dominated by large adult fish, indicating that, while total reproductive output was lower in deeper habitats in
comparison to shallower reefs, deeper fish had longer lifespans, and may represent a
long-lived, stable population of fecund female fish producing high condition larvae
(Chapter 3).

Spatial patterns of egg production were driven by variation in population density,
but additional biological parameters influenced the scale of reproductive output. Spatial
variability in demographic parameters and frequency of spawning, calculated based on
the proportion of the population that was spawning and temperature dependent
reabsorption of post-ovulatory follicles, considerably affected total population
reproductive output. The use of spawning stock biomass alone as a predictor of larval
supply and recruitment to fish populations is often debated (Hare 2014), and our results
reveal that population demographics are an important component of reproductive output
and larval supply. In addition to reproduction and larval supply, population connectivity
is also influenced by oceanographic currents, delivery of pelagic larvae to settlement
habitats (Limouzy-Paris et al. 1997, Sponaugle et al. 2005, Pineda et al. 2007), the effects
of high larval mortality (Leggett and Deblois 1994), and differential post-settlement
survival (Grorud-Colvert and Sponaugle 2010, Johnson et al. 2014). The magnitude of
spawning or egg production can be decoupled from subsequent settlement magnitude
(Robertson et al. 1988), but there is evidence that production and recruitment can be
linked when distance or isolation of source and settlement habitats are taken into account
(Cowen 2002, Saenz-Agudelo et al. 2015). Ultimately, source-sink dynamics are driven
by a balance of larval supply and fish recruitment. Reproductive output is one parameter
that can help refine models of population connectivity as an estimate of potential, rather
than absolute, larval supply from subpopulations.
Although not unexpected, the total area of available habitat had a large influence on potential regional egg production. Marine spatial planning for coral reef fish with relatively uniform spatial distributions can concentrate on areas with high population densities; however, targeting such hotspots likely cannot compensate for protecting a larger total reef area. These results highlight the need for detailed benthic habitat maps to allow predictions of demersal fish populations, particularly for understudied mesophotic reef ecosystems. Pulley Ridge and other mesophotic coral reef ecosystems have the potential to provide extensive reef habitat for fish populations on the same scale as their shallow water counterparts.

Our results highlight the importance of regional and habitat based analyses for estimating and predicting population sizes, and the effects of spatially variable demographics, density, and reproductive patterns on total population egg production. Biophysical modeling efforts that incorporate depth and habitat-stratified reproductive output with physical oceanographic processes to examine population connectivity can help to identify source and sink population dynamics. Results of this study provide one of the first geographically expansive estimates of fish population size and reproductive output across the entire depth distribution of a species. These estimates demonstrate that mesophotic reef habitats, at the extreme depth range of coral reef ecosystems, can support large reef fish populations that may also be important sources of larvae for shallow reef habitats.
Table 5.1. Comparison of the percent cover of reef habitat categories across depth strata in the Florida Keys. Categories are listed from left to right from least to most complex reef type.

<table>
<thead>
<tr>
<th>Strata</th>
<th>Continuous low relief</th>
<th>Spur and groove low relief</th>
<th>Spur and groove high relief</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shallow shelf</td>
<td>68.6%</td>
<td>23.6%</td>
<td>7.8%</td>
</tr>
<tr>
<td>Mid-shelf</td>
<td>21.6%</td>
<td>76.9%</td>
<td>1.5%</td>
</tr>
<tr>
<td>Deep shelf</td>
<td>11.6%</td>
<td>87.4%</td>
<td>1%</td>
</tr>
</tbody>
</table>
Table 5.2. Quantile regression results for the 90\textsuperscript{th} (0.9) quantile relationship between fish density (fish 0.04 km\textsuperscript{-2}), depth (D), and location (L) for four separate time periods for juvenile and adult bicolor damselfish, and median (0.5) quantile for adult fish for each time period. The full model included a polynomial term for depth (D\textsuperscript{2}) if the relationship between density and depth was not monotonic, and the interaction between depth and location (D*L). The table shows the best-fit models selected using AIC criterion. The Akaike weight (w) comparing the selected model to the set of candidate models is presented.

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Age Group</th>
<th>Quantile</th>
<th>Model</th>
<th>w</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000-2002</td>
<td>Juvenile</td>
<td>0.9</td>
<td>D*L+D\textsuperscript{2}+ε</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>0.9</td>
<td>D*L+ε</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>0.5</td>
<td>ns</td>
<td>0.37</td>
</tr>
<tr>
<td>2003-2005</td>
<td>Juvenile</td>
<td>0.9</td>
<td>D*L+D\textsuperscript{2}+ε</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>0.9</td>
<td>D*L+ε</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>0.5</td>
<td>D+ε</td>
<td>0.35</td>
</tr>
<tr>
<td>2006-2008</td>
<td>Juvenile</td>
<td>0.9</td>
<td>D+L+D\textsuperscript{2}+ε</td>
<td>0.56</td>
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<tr>
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<td>D+L+D\textsuperscript{2}+ε</td>
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</tr>
<tr>
<td></td>
<td>Adult</td>
<td>0.5</td>
<td>D*L+D\textsuperscript{2}+ε</td>
<td>0.40</td>
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<tr>
<td>2009-2011</td>
<td>Juvenile</td>
<td>0.9</td>
<td>D*L+D\textsuperscript{2}+ε</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>0.9</td>
<td>D*L+D\textsuperscript{2}+ε</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>0.5</td>
<td>D+D\textsuperscript{2}+ε</td>
<td>0.49</td>
</tr>
</tbody>
</table>
Table 5.3. Results of logistic regression, using GLM with a binomial distribution, for 2009-2011 juvenile and adult bicolor damselfish presence and absence in the Florida Keys. The table shows the best-fit models selected using AIC criterion. The full model included an interaction between depth and location (D*L). The Akaike weight ($w$) comparing the selected model to the set of candidate models is presented. Model parameters are depth (D), and linearized location (L), and $\varepsilon$ is the error term.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Model</th>
<th>$w$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>Reef</td>
<td>D+L+(\varepsilon)</td>
</tr>
<tr>
<td></td>
<td>Patch</td>
<td>D*L+ (\varepsilon)</td>
</tr>
<tr>
<td></td>
<td>Rubble</td>
<td>L+ (\varepsilon)</td>
</tr>
<tr>
<td>Juvenile</td>
<td>Reef</td>
<td>D+L+ (\varepsilon)</td>
</tr>
<tr>
<td></td>
<td>Patch</td>
<td>D+ (\varepsilon)</td>
</tr>
<tr>
<td></td>
<td>Rubble</td>
<td>D+ (\varepsilon)</td>
</tr>
</tbody>
</table>
Table 5.4. The proportion female bicolor damselfish from random fish collections in shallow shelf (SS), deep shelf (DS), and mesophotic (MPR) depth strata. Collection$_n$ corresponds to the number of transect replicates in SS and DS strata, and the number of collection dives in MPR strata. Sample size is the total number of adult fish collected from SS and DS and total number of fish from MPR. Total proportion of females was calculated using the sum of all collections. Mean proportion and standard deviations (SD) were calculated using collections as replicates, and mean values were used for additional analyses.

<table>
<thead>
<tr>
<th></th>
<th>Collection$_n$</th>
<th>Sample size</th>
<th>Total proportion females</th>
<th>Mean proportion females ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>11</td>
<td>30</td>
<td>0.57</td>
<td>0.57 ±0.30</td>
</tr>
<tr>
<td>DS</td>
<td>10</td>
<td>24</td>
<td>0.54</td>
<td>0.52 ±0.31</td>
</tr>
<tr>
<td>MPR</td>
<td>6</td>
<td>35</td>
<td>0.43</td>
<td>0.43 ±0.34</td>
</tr>
</tbody>
</table>
Figure 5.1. Map of the Florida Shelf study region designating subregions (PR=Pulley Ridge, LK=lower Florida Keys, MK=middle Florida Keys, UK=upper Florida Keys), benthic habitat in the Florida Keys, and probable reef habitat at Pulley Ridge. The rectangle polygon outline at Pulley Ridge designates upper estimates of potential reef habitat (25 km x 30 km) and the blue polygon represents the NOAA designated Habitat Area of Particular Concern (HAPC) with known scleractinian coral and fish habitat. Bathymetry contours are color-coded from light to dark gray designating 1 m contours from 0-10 m depths, 10 m contours from 20-100 m, and 400 m contours for >100 m, respectively. Depth contours at 20 m and 400 m are labeled for reference.
Figure 5.2. Representation of quantile regression relationships between depth, location, and bicolor damselfish density (fish 0.04 km\(^{-2}\)) for 2009-2011 for juveniles (a & b) and adults (c & d). Both median (0.5) and 90\(^{th}\) (0.9) quantiles are depicted for adult fish because median quantile relationships were used to predict median fish densities. For plots (a, c) that focus on depth, location was held constant for model predictions at the average value (1.17 units). For plots (b, d) that focus on location, depth was held constant for model predictions at 15 m. Error bands are bootstrapped 95% prediction intervals for each model. For example, for the median quantile, the prediction interval can be interpreted as 95% of the median quantile observations fall within the error band. The habitat associated with each fish density observation is designated by shape, and the quantile is designated by line type.
Figure 5.3. Predicted logistic regression relation between bicolor damselfish presence and depth for (a) juvenile and (b) adult fish in the Florida Keys from 2009-2011. Location was held constant at the mean value of 1.17 units for the graphical model depiction. Error bands are 95% prediction intervals. Line type corresponds to benthic habitat. Juvenile fish are predicted to be present at all reef locations.
Figure 5.4. Predicted density of bicolor damselfish during the time period from 2009-2011 at Pulley Ridge (PR) and the Florida Keys for (a) continuous reef and (b) patch reef environments within depth strata and subregion (LK=lower Florida Keys, MK=middle Florida Key, UK=upper Florida Keys, PR=Pulley Ridge). Depth strata were defined as: shallow shelf = <10 m, mid-shelf = 10 - <20 m, deep shelf = 20 - ≤35 m, mesophotic = 60.3-94.5 m). For the Florida Keys, error bars were calculated from prediction intervals of the median quantile regression model for adult bicolor damselfish. The dotted line separating PR from the Florida Keys designates differences in the methods used for calculations in each region. For PR the values were calculated for the median fish density for a unit of area in PR, and confidence intervals are 10th and 90th quantiles.
Figure 5.5. Predicted total number of bicolor damselfish females at Pulley Ridge (PR) and in the Florida Keys for (a) continuous reef and (b) patch reef environments within depth strata and subregion for 2009-2011 (LK=lower Florida Keys, MK=middle Florida Key, UK=upper Florida Keys, and PR=Pulley Ridge). Depth strata were defined as: shallow shelf = <10 m, mid-shelf = 10 - <20 m, deep shelf = 20 – 20m, and mesophotic = 60.3-94.5 m. For the Florida Keys, error bars are derived from prediction intervals of the median quantile regression model for adult bicolor damselfish. The dotted line separating PR from the Florida Keys designates differences in the methods used for calculations in each region. For PR, the median bicolor damselfish density was used to estimate the female fish population using an estimated reef area of 350 km² (area of the HAPC=Habitat Areas of Particular Concern) and the error bars represent a minimum estimate assuming 57% reef habitat within the HAPC, and a maximum estimate using 57% reef habitat within an upper estimate of reef area at PR (30 km x 25 km with 57% reef habitat).
Figure 5.6. Kernel density size (standard length) distributions of adult bicolor damselfish females from shallow shelf (also used for mid-shelf distributions), and deep shelf in the Florida Keys, and mesophotic reef collections from Pulley Ridge.
Figure 5.7. Predicted bicolor damselfish weekly reproductive output in relation to depth in the Florida Keys and Pulley Ridge in 2009-2011. Error bands were derived from bootstrapped 95% prediction intervals of median quantile regression predictions, and then modified based on probability of occurrence, and probability of reproduction. The decrease in reproduction at 20 m is a result of differences in the probability of reproduction across depths (<20 m: 46% of the population reproduces every 2 d, and ≥20 m: 30% of the population reproduces every 2 d). Weekly reproduction estimates also incorporated differences in fecundity based on fish size distributions across depth strata.
Figure 5.8. Predicted bicolor damselfish weekly reproductive output (number of eggs km$^{-2}$) across depth strata and regions from 2009-2011. For the Florida Keys (lower, middle, and upper Keys; LK, MK, UK), error bars were derived from bootstrapped 95% prediction intervals of median quantile regression predictions, and then modified based on probability of occurrence and probability of reproduction. Depth strata were defined as: shallow shelf = <10 m, mid-shelf = 10 - <20 m, deep shelf = 20 - ≤35 m, mesophotic = 60.3-94.5 m). The dotted line separating Pulley Ridge (PR) from the Florida Keys designates differences in the methods used for calculations in each region. For PR, values were derived based upon the median observed fish density, probability of occurrence, and probability of reproduction. The error bars were calculated from the 10$^{th}$ and 90$^{th}$ quantiles of calculated reproductive output estimates.
Figure 5.9. Predicted bicolor damselfish weekly reproductive output for the total area of available reef habitat across depth strata and subregions from 2009-2011. For the Florida Keys (LK, MK, UK), error bars were derived from bootstrapped 95% prediction intervals of 50th quantile regression predictions, and then modified based on probability of occurrence and probability of reproduction. Depth strata were defined as: shallow shelf = <10 m, mid-shelf = 10 - <20 m, deep shelf = 20 - ≤35 m, mesophotic = 60.3-94.5 m). The dotted line separating Pulley Ridge (PR) from the Florida Keys is to designate that the methods used for calculations were different in each region. For PR, values were derived based upon the median observed fish density, probability of occurrence, and probability of reproduction. The value is based on of 350 km² (approximate area of the HAPC=Habitat Areas of Particular Concern) and the error bars are based on a conservative estimate of 200 km² (area of the HAPC assuming 57% reef habitat) and an upper estimate using a reef area of 428 km² (estimates of the extent of reef outside the HAPC, ~750 km², assuming 57% reef habitat).
Chapter 6. Summary and conclusions

Collectively, this dissertation addressed the influence of spatially variable habitats on coral reef fish traits, survival, and reproduction from the scale of the individual to larger scale population demographics and reproductive output. This work assessed the extent of demographic plasticity of bicolor damselfish across their depth distribution, and the environmental drivers that influence habitat quality for fishes. Multiple environmental processes such as food availability, diet composition, predator densities, temperature, and benthic habitat affected population demographics and habitat suitability across vertical and horizontal spatial scales. Patterns of population demographics and habitat quality have implications for understanding subtle impacts of habitat loss that may ultimately affect reef ecosystems by altering population structure, abundance, and larval supply, with cascading effects on reef communities and diversity. This information provides insight into the habitat quality of mesophotic reefs located at the periphery of coral reef depth distributions, and the potential for mesophotic coral reefs to act as refuges for coral reef fishes by supplying larvae to degraded shallow reefs. The results highlight the need for spatially extensive studies of marine organisms to assess demographic plasticity and habitat quality, understand the role of ecosystem refuges, the function of marine reserves, and to predict coral reef resilience.

Spatial variation in early life history traits and selective mortality

Environmental variation in post-settlement habitat affected fish traits shortly after settlement to the reef environment. Higher productivity as a result of more frequent eddy
upwelling in the lower Florida Keys led to faster growth for juvenile bicolor damselfish during the first 4-d post-settlement to the reef. Faster juvenile growth in the lower Florida Keys was observed for fish cohorts that arrived to reefs in each subregion with the same larval traits (larval growth rate, pelagic larval duration, settlement size), and no evidence of selective mortality on post-settlement growth in either subregion (Chapter 2). These results suggest that differences in habitat can affect fish traits soon after settlement, during a critical time period associated with high mortality as fish transition from pelagic to benthic environments (McCormick 1998, Searcy and Sponaugle 2001, Hoey and McCormick 2004).

Patterns of growth and mortality in the Florida Keys indicate the effects of larval supply, food limitation, and predation on regulating growth, mortality, and population density of bicolor damselfish. The lower Florida Keys had higher settlement (Sponaugle et al. 2012), higher recruit densities, and faster juvenile growth compared to the upper Florida Keys. In comparison, primary productivity was lower in the upper Florida Keys, and juvenile fish grew more slowly. Faster juvenile growth despite higher densities of young fish, indicates that post-settlement growth was not limited by food availability or competition in the lower Florida Keys (Chapter 2).

Food availability is often linked to growth (Jones 1986, Booth and Hixon 1999), and the effects of food and resource limitations on growth can be mediated by competition and density dependent processes (Forrester 1990, Munday 2001, Hixon and Jones 2005). For planktivorous organisms in the upper and lower Florida Keys, food availability was likely higher in the lower Florida Keys, thus supporting higher densities of bicolor damselfish with faster growth. Mortality was also higher in the in the lower
Florida Keys, despite faster growth, suggesting that mortality was not linked to food availability, and instead may be influenced by differences in predation between the two subregions. For juvenile fish, population density and mortality are primarily influenced by predation (Connell 1998, Almany and Webster 2006), but can also be mediated by density-dependent processes (Hixon and Jones 2005, Figueira et al. 2008). High mortality and higher density of recruits in the lower Florida Keys, despite faster growth, is consistent with the relationship between food availability and growth, and the influence of density-dependent predation on mortality.

Although differences in juvenile growth rates between fish in the upper and lower Florida Keys were relatively small, faster growth was observed after only 4-d post-settlement, indicating a rapid response to habitat variability that may continue to affect fish as they mature. Faster growth is generally linked to higher prey availability (Pepin et al. 2015), survivorship, and condition for most reef fish (Meekan and Fortier 1996, Grorud-Colvert and Sponaugle 2010, D’Alessandro et al. 2013). Indeed, fish that are larger at settlement preferentially survive (Rankin and Sponaugle 2011). However, in contrast to the growth-mortality hypothesis (Hare and Cowen 1997), survival is higher for fishes with slow growth during the first 6-d on the reef the upper Florida Keys (Rankin and Sponaugle 2011). Subregional mortality differences in the Florida Keys are likely linked to density-dependent predation, however, lower mortality in a region with slower post-settlement growth is also consistent with the results of Rankin and Sponaugle (2011) that indicated higher survival for slower growing individuals.

The negative repercussions of faster growth may weaken after the first few critical days on the reef, and fast growth of older fish is often associated with higher condition
and survival (Booth and Hixon 1999, Johnson 2008, Meekan et al. 2010). In combination, the lower Florida Keys is a subregion of high food availability, high settlement, high mortality, but ultimately high population densities of bicolor damselfish that exhibit faster growth than the upper Florida Keys (Chapter 2). The results of this study provide an example of the effects of spatial variation in food availability and mortality on regulating population density, traits of reef fish, and the consequences of differential settlement habitats on growth and survival.

**Comparative demography across vertical spatial scales**

The range of demographic plasticity exhibited by coral reef fish across small and large-scale geographic distributions has been addressed in a number of studies (Gust et al. 2002, Ruttenberg et al. 2005, Figueira et al. 2008, Paddack et al. 2009, Berumen et al. 2011, Carter et al. 2014), yet population demographics across the vertical distribution of coral reef fishes has received far less attention (Srinivasan 2003, Hoey et al. 2007). Environmental variability such as temperature, wave action, prevailing currents, and food availability can differ over short vertical distances (Lesser et al. 2009) and affect growth, size, and reproduction. As shallow reefs decline (Pandolfi et al. 2003), coral reef organisms may become increasingly dependent on potentially marginal habitats at the periphery of their distributions. Understanding the effects of depth on population demographics such as age and size distributions, growth, and fish fecundity is essential to assessing the potential refuge function of deeper reef habitats to safeguard coral reef organisms and supply larvae to degraded reefs.

Our results revealed that population demographics varied across the depth distribution of bicolor damselfish, yet some parameters did not have a linear relationship
with depth. Recruit density and population density consistently declined with depth.

Similarly, population age and size structure shifted from young fish in the shallowest habitat to a population dominated by long-lived, large individuals in mesophotic habitats (Chapter 3). Fish density patterns and population structure indicated higher population replenishment of young fish in shallower habitats, and lower replenishment with increasing depth. Bicolor damselfish had longer lifespans, but also grew more slowly and reached larger asymptotic sizes in deeper habitats with colder water temperatures, consistent with the physiological relationship between temperature and growth (Van Voorhies 1996, Edeline et al. 2013). Differences in reproductive investment did not have a monotonic relationship with depth, with mid-level reproductive investment in the shallowest habitats and peak values in mesophotic habitats (Chapter 3). Batch fecundity and oocyte area were the highest in mesophotic environments, while spawning frequency was likely lower, indicating the highest reproductive investment in spawning batch, and potentially the highest condition larvae as a result of high maternal investment (Chambers and Leggett 1996, Donelson et al. 2008, Chapter 3).

Bicolor damselfish populations on mesophotic reefs are composed of few young fish and many large, long-lived individuals, or BOFFFFs (Big Old Fat Fecund Female Fish), with high reproductive investment, and likely higher condition larvae and disproportionate larval contributions to the metapopulation (Hixon et al. 2014). However, recruitment rates decreased with increasing depth, indicating that mesophotic habitats are an example of the “storage effect”, in which population persistence is dependent upon longer-lived individuals during periods of low recruitment (Chesson 1984, Hixon et al. 2014). Mesophotic reef fish populations have the capacity to supply high condition larvae
and have higher per capita batch fecundity, but may be less resilient than populations in shallower habitats that have higher population replenishment, and potentially the ability to quickly recover from environmental disturbances.

**Habitat suitability of coral reefs across depths**

Patterns of population demographics varied non-linearly with depth, with the lowest reproductive investment in mid-range habitats (Chapter 3), indicating that environmental parameters that are not strictly linked with depth affect fish reproductive investment, and therefore, habitat suitability. Physical parameters such as light and temperature are primarily depth-driven, and oceanographic conditions including upwelling and tidal bores can also be predictably linked with depth (Leichter et al. 2003, Lesser et al. 2009), however, other processes may be de-coupled from depth distributions. For example, shifts in the prevailing currents and eddy upwelling can affect food availability and growth for reef fishes (Lee et al. 1992, Hitchcock et al. 2005, Shulzitski et al. 2015), and fish community structure and predator densities may not change uniformly with depth (Brokovich et al. 2006). Therefore, to determine habitat suitability and the drivers of variation in population demographics across depths, we assessed the influence of predator densities, behavioral time allocation, diet, trophic level, and tissue lipid content on energetic trade-offs between growth and reproductive investment (Chapter 4).

The results of this study provided insight into the impact of environmental parameters on energetic trade-offs for bicolor damselfish across depths. Predator densities peaked in deep shelf habitats where reproductive investment was low, and fish spent more time in “risky” behaviors in shallow shelf habitats where there were fewer
predators. Risky behaviors included aggression, courtship, and swimming far from the benthos, all behaviors that increase risk of predation by requiring time in the water column away from shelter (Myrberg 1972, Catano et al. 2014). Time in the water column also increases access to planktonic food sources, indicating that predator-induced cautious behaviors of deep shelf fish may limit access to food. As a result of access to planktonic food, shallow shelf fish had fuller guts than fish from deeper strata, however, lipid content (C:N ratio) and trophic level were highest for mesophotic fish. These patterns indicate that high quality diets in mesophotic habitats had a greater impact on lipid content than gut fullness. Fish in mesophotic habitats also had the broadest nutrient source pool of the three depth strata, likely as a result of dynamic oceanography that was also indicated by sub-seasonal temperature variability in the region (Chapter 3).

In combination, the biological responses of bicolor damselfish to environmental habitat variability across depths indicated a trade-off between growth and reproductive investment. Individual reproductive investment was highest in mesophotic habitats where fish were old, grew slowly, had a higher trophic position, and higher lipid content that indicated surplus energy available for reproduction (Chapter 3). In shallower habitats, fish were younger, grew more quickly, had lower trophic positions, lower lipid content, and lower reproductive investment. Despite lower population densities in mesophotic habitats (Chapter 3), mesophotic reef environments provide suitable habitat with high quality food and low predator densities for long-lived adult bicolor damselfish. Suitable habitat allows individuals to invest energy in high reproductive investment in spawning batches and high condition larvae (Chapter 3). In contrast, shallower reef environments, particularly deep shelf reefs, are habitats with high predation risk and higher investment
in growth than reproduction based on gonado-somatic index (Chapter 4). Predation risk is increased by activities that require time in the water column such as feeding and courtship, and faster growth is a common tactic to avoid gape-limited predation (Anderson 1988). In predator-rich deep shelf habitats, “cautious” behaviors and faster growth (in comparison to mesophotic habitats) may reduce predation, however, energy allocation toward growth and time allocation away from courtship behaviors both minimize reproduction. Lower reproductive investment in shallow shelf habitats was linked to high gut fullness and growth, signifying a trade-off between growth and reproductive investment in batch size and larval condition, but also the importance of diet quality rather than quantity for energy availability and reproduction. Results of this study indicate that energy allocation toward reproduction differed across depths and was driven by habitat suitability related to predation risk, diet composition and food quality, and energetic trade-offs between growth and reproduction.

*Estimated larval supply from subregions and depth strata*

The value and quality of a habitat is species specific and dependent upon the suitability and quantity of available habitat (Gibson 1994, Harborne et al. 2011). Therefore, understanding the role of mesophotic coral reef habitats as refuges for a demersal coral reef fish requires that mesophotic reefs (1) support a large population of fish, and (2) supply larvae to shallower reef habitats. Assessing these parameters incorporates the effects of habitat suitability (Chapter 4) on depth-stratified population demographics (Chapter 3), habitat availability, fish subpopulation size, which is a combination of fish density and reef area, and the potential larval supply that is sourced from the habitat.
Using a combination of data sources from the Florida Keys and Pulley Ridge that included benthic habitat data, reef fish visual surveys, ROV surveys, and depth-stratified population demographic parameters (Chapter 3), I found that mid-shelf reefs in the Florida Keys had the highest abundance of bicolor damselfish throughout the study region, and the highest predicted egg production. Fish densities peaked at mid-depths (~17-20 m) and declined as depth increased (Chapter 5). Population abundance in each subregion in the Florida Keys and Pulley Ridge also followed a negative parabolic relationship with depth. Despite the correlation between larger body size and fecundity (Hixon et. al. 2013, Chapter 3), larger body sizes with increasing depth (Chapter 3) did not compensate for lower population densities or abundance. Therefore, predicted reproductive output per unit area declined with depth (Chapter 5). After incorporating the total area of reef habitat in each subregion and depth strata, the subpopulation of female fish at Pulley Ridge was estimated as ~20% of the regional population, yet only produced ~14% of the total predicted eggs for the entire study region (Chapter 5). This is due to the combined effects of low population density, lower proportion of females that were spawning, and reduced depth-specific spawning frequency that was temperature dependent (Ganias et al. 2007, Yoneda et al. 2014) at mesophotic reefs. Population densities and reproduction in mesophotic reef environments were lower than shallower habitats, therefore, the success of mesophotic reefs as refugia for coral reef fish is dependent upon the amount of available habitat.

**Conclusions**

Environmental variability in coral reef habitats can impact traits and survival of coral reef fishes throughout their life cycle, starting from critical survival after settlement
to the reef, and continue to have lifetime effects on growth, size, condition, reproduction, and fitness. The results of this dissertation scaled from the effects of variation in subregional productivity on daily growth, to the range of demographic plasticity across the depth distribution of a coral reef fish. This work indicates that population level variation and processes are influenced by compounding, yet subtle, differences in energetic trade-offs and diet that interact to affect traits of individuals, and finally augment to population level variation. Differences in behavior and diet between depth strata were subtle, followed by individual fish parameters such as growth, size, and reproduction. Subpopulation size, density, and total reproductive output were the result of multiple interacting processes at the largest spatial scale, and were markedly different across depth strata. The compounding effects of subtle processes on larger scale patterns reveal the sensitivity of coral reef organisms and coral reef fish populations to habitat variability and suitability.

Habitat-mediated traits and energetic trade-offs can scale-up to subpopulation demographic patterns that significantly influence population connectivity through post-settlement selection for favorable traits (Clarke et al. 2010, Fuiman et al. 2010), or by modifying reproductive output and larval supply (Cowen and Sponaugle 2009). Understanding spatial patterns and the drivers of habitat suitability and demographic parameters of coral reef fishes is increasingly important as fish communities change as a result of harvesting and other anthropogenic pressures (Mumby et al. 2006, Ruttenberg et al. 2011), and as coral reef habitats decline through habitat degradation (Pandolfi et al. 2003). As a consequence of coral reef decline, organisms that require reef habitats for survival may become dependent upon environments at the periphery of their distributions.
The results of this study indicate that, for a model species, environmental parameters that vary across depth and geographic distributions significantly affect demersal coral reef fish population demographics and have consequences related to habitat suitability.

The range of demographic plasticity across depths indicates that at the level of the individual fish, demersal coral reef fish can find suitable habitat even at the periphery of their depth distribution. At the population level, there was evidence that mesophotic reefs have low population densities, are sustained by long-lived individuals, and the populations are composed of few young fish suggesting low population replenishment. In contrast, expansive reef area indicates that mesophotic reefs are valuable habitats for long-lived, fecund coral reef fish that may be important for the persistence of coral reef ecosystems. Mesophotic coral reef habitats have the capacity to function as coral reef refuges with high reproductive output, but fish population size is limited by available habitat and population replenishment. These restrictions indicate potential ecosystem fragility, and that fish populations in mesophotic habitats may be less resilient than shallower coral reef populations.

Mesophotic coral reefs are valuable ecosystems for multiple coral reef organisms (Brokovich et al. 2006, Kahng et al. 2010, Holstein et al. 2015) with unique population demographics. The vulnerability of bicolor damselfish subpopulations in mesophotic habitats, indicated by density and age structure, suggests that even for a common coral reef fish with a broad depth distribution, subpopulation persistence may be dependent upon habitat management and preservation. The results of this work reveal the importance of assessing demographic plasticity and habitat suitability at the scale of the individual fish such as growth and fecundity, and at population level including population
replenishment and densities, for effective management and assessment of coral reef ecosystem resilience. If shallow reefs continue to decline, the survival of coral reefs may be contingent upon the persistence of remnant populations of coral reef organisms in marginal habitats. Demographic plasticity of bicolor damselfish demonstrates the adaptability of coral reef fishes across a range of habitat quality, the utility in preserving marginal coral reef habitats, and indicates that for demersal reef fish, peripheral populations may be capable of seeding and supporting coral reef ecosystems across broad geographic and depth distributions.
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