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Behaviour, recruitment and dispersal of coral reef fish larvae: Insight into the larval life-stage.

James Derek Hogan
University of Windsor

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by

James Derek Hogan

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Abstract

Despite the importance of larvae to the ecology of reef fishes, the ecology of the larval-stage itself is still relatively poorly understood because of the difficulty of tracking and observing very small fishes in the oceans' waters. Advances in the last few years have increased our understanding of the ecology of reef fish larvae, with a focus on the dispersal of larvae and connectivity between benthic adult populations. The goal of this dissertation is to gain insight into the life history of larval reef fishes. I have used a variety of techniques to investigate the biology and ecology of these animals as it pertains to their dispersal and recruitment to reef populations. I used recruitment monitoring surveys to investigate the occurrence of synchrony (or coherence) in recruitment (Chapter 2). I measured the spatial scale of recruitment coherence to infer larval patch size in the pelagic environment. I used an otolith microchemical assay to determine if larvae recruiting in coherent pulses experienced similar environments during their larval lives, inferring that larval patches were coherent throughout larval development and may have originated from similar source populations (Chapter 3). I used a different otolith microchemistry assay to directly measure the dispersal of larvae between reefs (Chapter 4). I measured the swimming performances of larvae of Caribbean reef fish species and used a specially designed swimming flume to examine the contribution of swimming and drifting to the displacement of larvae in a simulated pelagic environment (Chapters 5 and 6). I discuss the results of these studies in the context of the factors that likely cause variation in dispersal and recruitment. Finally, I suggest how to focus future research on the ecology of reef fish larvae.

Co-authorship

This thesis was written as a series of manuscripts, and as such, several people have been critical to the development of this thesis. Their contributions have been acknowledged in the form of co-authorships on these papers. In many of the chapters I use the first person plural wording as a form of acknowledgement of the contributions of these co-authors. In all these chapters I was the primary developer of the ideas, analyst of the data and writer of the manuscripts, however, various contributions were made by others, I acknowledge their specific contributions below:

Peter Sale: Peter has been important in the development of ideas throughout the thesis, in data analysis and for masterminding the ECONAR project from which all of the data for these chapters was generated.

Rebecca Fisher: Rebecca has been important in many aspects to the development of this thesis. She closely advised in the statistical analysis of the recruitment data from chapter 1, she was important in the development of the foundational ideas for chapter 2, she helped in the collection and analysis of the larval swimming data from chapter 4.

Brian Fryer: Brian has been important in the general analysis of the otolith chemical data and training a field biologist to use sophisticated machinery (i.e. not made out of PVC). Brian's geochemical expertise was also helpful with the analysis of the otolith chemical data in chapter 2.

Camilo Mora: Camilo was a very helpful colleague during his tenure in the Sale lab. From my first day of field work on Camilo helped me to generate ideas about the thesis, and the general direction of my research. We collaborated on chapter 5 of this thesis, and Camilo input his effort (and frustration) in the fieldwork as well as in the analysis and writing of the manuscript. Camilo also helped with the generation of ideas for chapter 1. Camilo was also important in the collection of recruitment data from 2002 through 2003.

Paul Chittaro: Paul was very helpful and accommodating helping me to learn how to use the ICP-MS, and teaching me how to polish otoliths. He helped with a lot of the field collections for all chapters and helped with the generation of ideas for chapter 3.

Cormac Nolan: Cormac is a colleague whom I worked alongside during the field seasons in 2003, 2004 and 2005. Cormac contributed immensely to the collection and identification of larval reef fishes that were used for experiments in chapter 4. He also helped in the collection of recruitment data and fish samples.

Paolo Usseglio: Paolo is a field colleague who helped greatly with the fieldwork for all chapters (except chapter 5) in all years of this study. Paolo also contributed to the data analysis in most chapters, and in chapter 2 particularly.

Dedication

This thesis is dedicated to my family

for all their love and support.

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Statement of Originality

I declare that all the content in this thesis is original, and that all the research related to it is a product of my own imagination, and that any ideas or quotations from the work of other people, published or otherwise, are fully acknowledged in accordance with the standard referencing practices of the discipline.

Table of Contents

List of Tables

List of Figures

List of Appendices

Chapter 1: General Introduction

The origins of ecology as a science began with the application of experimental and mathematical methods to investigate the factors that affect the abundance and distribution of organisms (Kingsland, 1991). One of the earliest contributions to ecological ideas was Charles Darwin's *On the Origin of Species*, which described the all-important struggle for existence by organisms. His ideas were influenced by the work of a Utopian philosopher named Thomas Robert Malthus, in particular his *Essay on Population* (1798). Malthus' thesis was that populations tend toward overproduction and if unchecked would increase until they exceeded the means of subsistence. Malthus also recognized that population checks did exist, such as starvation and disease, and that these checks were more important at higher population densities. Darwin applied Malthus' idea to natural populations to propose his theory of natural selection, where population over-production would lead to intense competition and increased mortality, and would drive the selection of fitter individuals, and ultimately affect the abundance and distribution of individuals of a species (Kingsland, 1991). The idea that competition regulates populations became a fundamental part of the study of ecology (Kingsland, 1991), and it was either explicitly or implicitly included in many studies on the populations of animals (e.g. Volterra, 1926; Nicholson and Bailey, 1935). As ecology evolved studies have focused on other factors that likely ultimately affect the abundance and distribution of a species, factors such as recruitment, the replenishment of populations usually by the addition of young individuals (Doherty and Williams, 1988).

The ecology of marine organisms lagged behind that of terrestrial organisms, which are much more accessible and amenable to study. However, with the advent of SCUBA, scientists began studying the ecology of marine organisms, including coral reef

fishes. Like their terrestrial colleagues, marine ecologists developed hypotheses to explain how reef fish populations are regulated. Following the lead of their terrestrial colleagues, the earliest reef fish studies assumed that competition among adult fishes on the reef was an important factor in ultimately explaining the abundance and distribution of a species (Jones, 1991). One model proposed to explain the regulation of reef fish populations suggested that that there was a surplus of individuals supplied to replenish populations (the replenishment of populations is also referred to as recruitment). As a result competition for resources on reefs was intense and therefore that competition led to population regulation (e.g. Smith and Tyler, 1972). A departure from this classical ecological principle was proposed by Sale (1977), who maintained that there was a surplus of individuals supplied to replenish populations but recognized that stochastic recruitment of fish larvae may be more important than post recruitment competition between adult forms for influencing the abundance and distribution of species.

The most recent idea proposed to explain population regulation in reef fishes is the recruitment-limitation hypothesis. This idea proposed by Doherty (1983) and Victor (1983) suggests that supply of individuals was not in surplus and therefore populations are regulated primarily by stochastic recruitment of larval fishes, and that competition between adults on the reef had a minimal effect on the population regulation.

This 180-degree shift in thinking about population regulation coincided with substantial growth in knowledge about the pelagic larvae of reef fishes that replenish the reef populations. Most reef fishes have a pelagic larval life-stage that differs markedly from the adult life-stage in terms of habitat, food, morphology and behaviour (Leis, 1991). The duration of the pelagic larval life-stage varies greatly among taxa; some larval

durations are as short as 8 days and others longer than 100 days (Victor, 1986; Thresher et al. 1989; Wellington and Victor, 1989). While the larval duration in many cases is only a small fraction of the life span of an individual, the larval life stage of reef fishes is thought to be as important or perhaps more important than the adult and juvenile stages in determining the abundance and distribution of these species (Doherty and Williams, 1988; Doherty, 2002).

The adults of most reef fishes are relatively sedentary in relation to their potentially highly dispersive pelagic larvae (Sale, 1980). While most adult reef fishes are obliged to stay on or very near a coral reef, the pelagic larvae of these species are thought to have the ability to disperse 10s to 100s of kilometers from their site of spawning (Cowen, 2002; Cowen et al. 2006). In the pelagic environment, larval fishes are subjected to the pressures of finding food in a vast oceanic environment; they are subjected to transport by ocean currents; they are subjected to predation; and eventually, some larvae will return to a reef to complete their life cycle, an act called settlement. Coral reef fishes are highly fecund compared to most vertebrate species. Egg production estimates range from 10,000 to over 1,000,000 eggs per female per year depending on the species (Sale, 1980). The vast majority of the mortality that occurs in a population of reef fishes occurs prior to settlement, during the larval stage. Estimates of larval mortality are on the order of 99% (Leis, 1991; Jones, 1991).

The abundance and distribution of reef fish larvae are highly stochastic because of broad dispersal and high mortality and reef fish populations are often regulated by recruitment of pelagic larvae (Doherty and Williams 1988; Doherty, 2002). This suggests that the larval life stage of reef fishes is of the utmost importance to the ecology of these

species. Dispersal is probably the most important life-history trait of reef fish larvae in the ecology of coral reef fishes. Dispersal is defined here as the movement of individuals away from the reef at which they were spawned. For the purposes of this thesis only dispersal occurring during the larval stage of life is discussed. It is important to the ecology of many species because dispersal links separate local populations, replenishing them from various sources, and reduces extinction risk caused by local disturbances (Barlow, 1981), local disturbance such as over-fishing. Connectivity between local populations through dispersal will affect the resilience of populations to disturbances, where populations completely closed to dispersal (i.e. replenished only from within own population) will be most susceptible to disturbance, and populations open to dispersal (i.e. receive much replenishment from other local populations) will be more resilient to disturbances. Despite its importance, little is known about the biology and ecology of the dispersive stage of the reef fish life cycle because larvae are minute and patchily distributed in the vast pelagic environment (Leis, 1991) and science is only beginning to understand the biology of these animals.

Reef fish larvae were initially thought to disperse passively through the pelagic environment at the whim of ocean currents (Leis, 1991). However, recent research has shown that reef fish larvae are capable swimmers (Stobutzki and Bellwood, 1994; Leis and Carson-Ewart, 1997; Fisher and Bellwood, 2003), with the ability to actively move vertically through the water column (Paris and Cowen, 2004; see review by Sponaugle et al., 2002), and possibly with the ability to navigate using sensory mechanisms (Stobutzki and Bellwood, 1998; Tolimieri et al., 2000; Atema et al., 2002). Reef fish larvae are also known to congregate in patches in the pelagic environment either by active behaviour or

by collection in hydrodynamic features (Victor, 1984; Paris and Cowen, 2004). Genetic studies suggest that reef fish larvae disperse smaller distances than expected based on their larval durations and oceanographic processes alone (Planes, 2002). These studies are supported by recent otolith tagging studies that have shown that there may be quite a lot of self-recruitment occurring (i.e. larvae returning to the population from which they were spawned) in reef fish populations (Jones et al., 1999; Swearer et al., 1999).

It is the goal of this thesis to generate insight into the still poorly understood larval stage of the reef fish life cycle. I use a variety of techniques including recruitment monitoring, behavioural assays, and natural otolith markers to investigate the biology and ecology of reef fish larvae as it pertains to their dispersal and eventual recruitment to reef populations. The general questions that I ask throughout the thesis pertain to where larvae come from, where they go to, and how they get there.

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Chapter 2: The Spatial Scale of Recruitment Coherence of Coral Reef Fishes of the Western Caribbean

INTRODUCTION

The idea that the scale of observation will affect the observed variation in the structure of populations and communities has been developed over the last 25 years (Schneider, 2001). Ecologists now understand that systems will show "characteristic" patterns and variations on a range of scales, and these patterns and variations are dependent on how fine or coarse the scale of observation (Levin, 1992). As a result, hypotheses pinpointing causal factors explaining observed patterns and variation are therefore linked to the scale of observation for that study. In order to determine the true underlying causes of pattern and variation in population or community structure, ecologists must conduct their observations on a range of spatial and temporal scales.

In terms of the dynamics of fish populations, recruitment has been shown to be an important factor in determining the monthly (McFarland et al., 1985) and seasonal fluctuations (Ochi, 1985; Pitcher, 1988; Robertson et al., 1988) in population size and the strength of year classes (Doherty and Fowler, 1994; Russ et al., 1996). Furthermore, recruitment has been shown to play a role in meta-population ecology of fishes where sites sharing recruitment pulses may be as far apart as 500-plus kilometers (Myers et al. 1997). The scale at which recruitment is observed will undoubtedly affect the patterns that are observed and the processes that are anticipated to define those patterns (i.e. Pitcher, 1988).

Studies have shown that there is a large amount of variation in recruitment on very small spatial scales (i.e.: Luckhurst and Luckhurst, 1977; Doherty, 1983; Victor 1984, Sale et al., 2005). Many species show habitat preferences (Sale and Dybdahl, 1975; Doherty and Williams, 1988; Booth and Wellington, 1998) and some studies have shown

that settlement habitat can have an effect on post-settlement growth and survival (See review by Booth and Wellington, 1998; also Eckert, 1987); this may explain why patches separated by less than 1 km will show markedly different magnitudes of recruitment (Doherty, 1991). However, despite the differences in magnitude at small scales, recruitment has been shown to be temporally coherent (synchronous) among sites at these scales, indicating that the processes that drive the success of a cohort (defined as a group of individuals of the same species arriving to the same reef at the same time) of larvae are occurring on scales of 1 km or greater (Doherty, 1991).

Many mechanisms have been proposed to explain spatial variability in recruitment. At scales of tens to hundreds of kilometers, large-scale currents and behavioural responses to those currents can affect where larval fishes are being transported. In addition, the length of time spent in the pelagic larval life-phase will affect how long individual larvae are subjected to physical transport processes (Sponaugle and Cowen, 1997). Furthermore, environmental factors such as weather and climate can affect recruitment strength at large scales (Myers et al, 1997). Among sites, the interplay between local current fields and larval behaviours like vertical migration, horizontal swimming and habitat selection will influence the spatial variability of recruitment (Sponaugle and Cowen, 1997; Paris and Cowen, 2004). Among-site variation in spawning and proximity to larval source populations might contribute to recruitment variation between sites, and site-specific exposure to wind and storms may also influence settlement and recruitment patterns on this scale (Sponaugle and Cowen, 1997). In order to pinpoint which factors are most important for influencing recruitment of any given species, I must first understand the scale at which recruitment is most variable.

Coherence in recruitment to fish populations, that is synchrony between sites in the magnitude of recruitment, has been shown to occur on a variety of spatial scales. Pitcher (1988) found that the recruitment of one tropical reef fish (*Pomacentrus* sp.) was coherent at replicates within a site spaced 15 m apart as well as between sites spaced >500 m apart, but not coherent between reefs separated by 25 km or between reefs in two geographic regions spaced ~1200 km apart. Victor (1984) found that larvae of the reef fish *Thalassoma bifasciatum* were settling simultaneously at sites up to 46 km apart. Myers et al. (1997) found that the spatial scale of recruitment coherence depended on whether the fish species was marine, freshwater or anadromous. They concluded that the spatial scale of recruitment coherence in temperate marine species was typically 500 km compared to freshwater species for which coherence occurred at scales less than 50 km, with anadromous species falling somewhere in the middle. In this study I examine the spatial scales of recruitment coherence for 13 species of coral reef fishes in the Mesoamerican Barrier Reef System in the Western Caribbean. I compare my results to previously published findings and I discuss my results in terms of the factors that likely cause variation in recruitment for these species.

MATERIALS AND METHODS

Data Collection

This study was conducted at three locations in the Mesoamerican Barrier Reef region of the Caribbean, including Turneffe Atoll in Belize (TA), Banco Chinchorro in

Mexico (BC) and Isla Roatan in Honduras (RO) (See Figure 2.1). Within each location there were three to seven sites, spaced approximately 10 km apart, that were censused for recruitment on a bi-weekly basis during the summer months (between May and September) of 2002 through 2004. Recruitment surveys were conducted immediately following the new and full moons, surveys were conducted concurrently at all three locations and completed within seven days following the new or full moon.

Recruitment data were collected on 13 species of reef fishes: one surgeonfish species; *Acanthurus bahianus*, one basslet species; *Gramma loreto*, four damselfishes; *Chromis cyanea, Stegastes planifrons, Stegastes diencaeus* and *Stegastes partitus*, four wrasses; *Halichoeres garnoti, Halichoeres pictus, Halichoeres maculipinna* and *Thalassoma bifasciatum*, and three parrotfishes; *Sparisoma aurefrenatum, Sparisoma atomarium* and *Sparisoma viride*. These species were selected based on the ease of their field identification, and because of their relative abundance and ubiquity on Caribbean coral reefs. Only the smallest size classes of each species were recorded as newly recruited juvenile individuals. For most species that size was ≤ 2.0 cm total length, except *A. bahianus* for which the smallest size class was ≤ 3.5 cm on account of the large size of the larval forms of this species, and the Sparisoma species for which the smallest size classes I considered were between 1.5 cm through to 2.5 cm. I excluded the smallest size class of these fishes (i.e. 1.0 – 1.49 cm) because it was difficult to find the small, cryptic juveniles of these species; as a result estimates of abundance of this size class were unreliable.

At each site recruitment was censused using 16 1 X 30 m transects, 8 of which were located at a deep position within a site (depth range 9 to 11 m), and 8 of which were

located at a shallow position within a site (depth range 6 to 7.5 m). Within each position transects were placed haphazardly, parallel to the reef crest, with a distance of 2 to 3 m between transects. The length of each transect was measured using a 30 m tape-measure that was reeled out as the divers counted the fishes, and the width of each transect was measured with a 1 m wide PVC T-bar that each diver held whist swimming. To account for observer bias in the estimates of recruitment, a series of calibrating and cross validation surveys including all observers were conducted at the beginning of each summer.

Habitat data were collected at each site, at both depths, in the summer of 2002 using 12 1 X 30 m transects, 6 of which were located in the deep position and 6 located in the shallow position. Within each position, the transects were haphazardly placed parallel to the reef crest with a distance of 2 – 3 m between transects. Up to 13 substratum categories were recorded every 25 cm using the point-intercept method (Porter, 1972). Habitat categories included rock, rubble, turf algae, foliose algae, calcareous algae, encrusting algae, branching corals, encrusting corals, foliose corals, massive corals, gorgonians, millepore corals, sand, erect sponges and encrusting sponges. Spatial complexity (rugosity) of the reef along each transect was measured by draping a brass chain along the substrate closely following the contours of the substrate every 10 m along the transect (Risk, 1972). The ratio of chain distance to linear distance gave a measure of how rugose the substrate was at a particular 10 m section of reef.

Data analysis

Recruitment coherency analysis

CALCULATING PAIR-WISE CORRELATIONS AND THE SPATIAL SCALE OF RECRUITMENT PULSES

I define recruitment coherency as the covariance in recruitment, through time, between pairs of recruitment survey sites, and coherency is measured by correlation coefficients. For example, a correlation coefficient of 1.00 between a site-pair indicates perfect coherence in recruitment between the two sites. Alternatively, a correlation coefficient of 0.00 between a site-pair indicates no coherence in recruitment between the two sites (i.e.: recruitment processes are de-coupled between the sites).

All recruitment data were log (x + 1) transformed prior to analysis because recruitment data are typically a log-normal distribution (McCune and Grace 2002) and species abundances on a transect are often zeros. For each species, individual pair-wise correlation coefficients were calculated between each pair of recruitment survey sites, using each census period as a replicate (i.e.: each correlation is between recruitment counts at two sites, through time). Because of their close proximity, the shallow and deep positions within each site were pooled. There was some variation in the number of times that individual sites were censused, in part due to weather conditions and also due to logistical constraints. To increase confidence in coherency estimates, correlation coefficients were calculated only for site-pairs that were concurrently sampled a minimum of 6 times (i.e.: concurrent sampling for at least 6 census periods over 3 years).

Site-pairs were assigned a correlation coefficient of zero in the case where a species was absent from one of the two sites at all concurrent census periods. Site-pairs where both sites were lacking any recruits of a species at all concurrent census periods were left as blank data cells. An alternative would be to give these sites a coefficient of 1, seeing as they both share a temporal pattern of no recruitment. However, this strategy leads to a bias towards very high coherence values for rare species therefore I rejected this idea.

EXAMINING AND REMOVING THE POTENTIAL EFFECT OF HABITAT.

The 13 habitat characteristics were converted to a proportional occurrence on each transect and averaged across all six transects within each position (shallow and deep) at each site. Pair-wise correlations were performed between the average recruitment of each species (across all census periods) at each site and the average proportional occurrence of the 13 habitat characteristics at each site, treating both deep and shallow habitat variables separately. Habitat data were not pooled between deep and shallow positions because averaging the two positions may yield habitat characteristics that are not representative of the actual habitat characteristics that exist at either position. Pooling the positional habitat data will also smooth over potentially important habitat characteristics that may have a strong influence on recruitment but may only occur in either deep or shallow habitats. This method basically treats deep and shallow habitat characteristics as independent habitat variables, thereby producing a total of 26 habitat variables per site.

First, the raw habitat variables were regressed against the average log-abundance of the total number of recruits at each site. This was done in order to ascertain which

habitat variables were most important to each species and to ensure that the maximum amount of habitat information relevant to each species was included in the analysis. Significant correlations were found between at least 4 of the habitat variables and the average log-abundance per site for all 13 species of fishes.

Habitat variables were then standardized to values between 1 and 0 and then weighted according to the R^2 from the regression between the raw habitat and the average log-abundance of each species (equation 1):

$$(1)\ \mathbf{swHv_{ijk} = ((Hv_{jk} - minHv_{1\text{-}jk}) / (maxHv_{1\text{-}jk} - minHv_{1\text{-}jk}))^* R^2_{ij,}}$$

where $swHv_j$ is the standardized weighted habitat variable j, Hv_{jk} is the raw value for habitat variable j at any site k, $minHv_{1\text{-}jk}$ is the minimum value recorded for any of the habitat variables 1 – j at any site 1 – k, $maxHv_{1\text{-}jk}$ is the maximum value recorded for any habitat variable 1 – j at any site 1 – k, and R^2_{ij} is the coefficient of determination from the regression between average log-abundance of the total number of recruits at each site for species I and each habitat variable 1 – j.

In order to examine and remove the potential confounding effects of habitat similarity on recruitment coherency among sites, the standardized weighted habitat similarity between site-pairs was quantified using a Manhattan city-block distance (the distance between two points measured along axes at right angles; McCune and Grace, 2002) measured, and subsequently regressed against the R^2 values from the recruitment correlations between sites.

CORRELATION BY DISTANCE AND THE SPATIAL SCALE OF RECRUITMENT COHERENCY

Pair-wise correlations of recruitment between sites were plotted as a function of the distance between sites. Linear regression was used to test whether there was a linear trend in recruitment coherence with increasing distance between site-pairs. Also, following Myers et al. (1997), a simple estimate of the spatial scale of recruitment coherence was calculated as the distance over which the pair-wise correlation between recruitment time series is reduced by a factor of e^{-1}, referred to as the e-folding distance (v) (equation 2):

$$\rho(d) = \rho_0 e^{-d/v}, \quad (2)$$

where ρ_0 is the correlation between two sites at distance of d = 0 (in this case, at the transect level), d = the distance between the two sites, and v = the e-folding distance. The average coherence at the transect level was determined empirically by sequentially averaging larger and larger numbers of transects at each site, calculating the correlation coefficient r (coherence) between the resulting averages and then fitting a regression equation to calculate the predicted r for an average of all 16 transects.

Life History Analysis

Life history data on the species of interest were collected from literature sources in order to test the hypothesis that species that have similar life history characteristics will also have similar patterns (or scales) of recruitment coherence (determined by the e-folding distance). Life history data were found for ten of the 13 species of interest (See Table 2.1 for life history data). I used hierarchical, polythetic, agglomerative cluster

analysis to group species based on their life history characters. I used Ward's method for amalgamation of groups based on a Euclidean distance matrix. Ward's method was used because it is a space conserving method, which minimizes increases in error sums of squares (McCune and Grace, 2002). I used Euclidean distance for clustering groups because this distance measure works best with Ward's method (McCune and Grace, 2002). The cluster tree was trimmed at 3 groups with linkage distances of less than 45 to provide a compromise between having too few groups and too many groups, providing an interpretable summary of similarities between species (Figure 2.2).

Discriminant Function Analysis (DFA) was used to determine which life history characters best separated the three distinct groups. A forward stepwise approach was taken for the inclusion of variables in the model. A classification procedure was used to determine how well the function could predict ownership to the three groups as determined by the cluster analysis. Analysis of variance was used to test whether there were significant differences in the e-folding distance between groups.

RESULTS

Recruitment of all monitored species to the sites in this study was highly variable in both space and time (Appendix 2.1). Visual analysis revealed no spatial pattern in recruitment for any species that could be gleaned from visual analysis. The stochasticity of recruitment seemed compounded in rare species (Appendix 2.1).

The exponential fit with distance to the decay of the pair-wise correlations among all pairs of sites for all species shows that the e-folding distance is quite variable between

species (Figure 2.3; Table 2.2). The e-folding distances ranged from 268 km for the species *Sparisoma aurofrenatum* to as little as 7.5 km for the species *Acanthurus bahianus* (Table 2.2). There does not appear to be any phylogenetic influence on the e-folding distance, that is to say, that membership to any particular family or genus does not explain the observed variation in e-folding distance among species ($ANOVA_{(family)}$: df = 4, F = 0.56, p = 0.70; $ANOVA_{(genus)}$: df = 6, F = 0.28, p = 0.92).

Cluster analysis revealed three distinct groups of species based on similarities in life history characteristics (Figure 2.2). The variables included in the discriminant function model that best explained group separation were maximum larval duration, hatching length and spawning mode (demersal vs. broadcast), however, only maximum larval duration significantly contributed to the model (Wilks' Lambda = 0.33; $p < 0.001$). This DFA model accurately classified all species into their appropriate life history groups making it a robust model for explaining group membership (Table 2.3). Analysis of variance revealed no significant differences in the e-folding distance between species groups based on life history characters (d.f. = 2; F = 0.07; p = 0.94).

Despite the fact that the e-folding distance was generally small (below 50 km) for most species, the variation in e-folding distance was quite large (Table 2.2). This is due to the fact that site-pair coherency can be quite low at very small distances in some species and can be quite high even at distances of separation greater than 250 km in some species (Figure 2.3, Appendix 2.1). In fact, some species (e.g. *C. cyanea* and *G. loreto*) show very high coherence in recruitment between sites in Banco Chinchorro, Mexico and Isla Roatan, Honduras (Figure 2.3, Appendix 2). Four species (*S. atomarium, S. viride, S.*

partitus and *T. bifasciatum*) showed consistent, linear declines in coherency with distance (Figure 2.3, Table 2.4).

DISCUSSION

The e-folding distance I calculated is a measure of the correlation scale of recruitment, here called coherence (Myers et al., 1997). The spatial scale of recruitment coherence was highly variable among species, more than 35-times greater for the species with the highest compared to the species with the lowest e-folding distance. I measured e-folding distance for 13 species of fishes from five taxonomic families. It might be expected that species that are closely related will have similar patterns of recruitment, and possibly similar e-folding scales. However, the variability in e-folding distance could not be accounted for by phylogenetic relationships between the species.

It has been suggested that coral reef fish populations are largely non-equilibrial systems, where density-dependent processes do not solely, or even primarily, regulate population sizes (see review by Doherty and Williams, 1988; Doherty, 2002). It is most likely that population dynamics are influenced by both density-dependent and density-independent processes, where density-dependent processes will be most important at the extremes of density (Doherty, 2002). In this case, often the number of fishes recruiting to a population will determine the strength of a cohort of fishes (Doherty and Flowler, 1994; Russ et al., 1996) and density-dependent mortality will act to dampen very strong cohorts (Doherty, 2002). If density-independent processes dominate and pulses in recruitment are not masked by post-settlement mortality then patterns of recruitment can be used to retrospectively estimate the abundance and distribution of larval fishes prior to settlement

(Doherty and Williams, 1988). I can speculate that factors intrinsic to the ecology of the larval forms of a species (life history traits) might be important for determining that species' abundance and distribution. Furthermore, by investigating the scale at which recruitment coherence occurs it may be possible to estimate larval patch size for a species (Victor 1984) and, by extension, indicate the scale at which processes are acting on these larval patches to cause variation in recruitment.

The scale of coherence for most species here is greater than or equal to the distance between nearest site-pairs, suggesting that in general, the factors that affect recruitment are not site specific. The scale of coherence for ten out of 13 species studied here was between 10 and 50 km suggesting that meso-scale processes are most likely to determine the success of a given larval patch(es) more so than events occurring on larger scales such as lunar or tidal cues events which are also known to be linked with recruitment (Doherty, 1991). Such meso-scale phenomena may include localized storm events, meso-scale hydrodynamic features like eddies and tidal flows, and the coincidence of larval fish patches and the zooplankton patches that they feed on.

Of the remaining species in this study, the spatial scale of recruitment coherence was very large, 140 km and 268 km, for two species, *Halichoeres garnoti* and *Sparisoma aurofrenatum* respectively. Regional scale processes are most likely to cause variations in recruitment of these species. Such processes might include large-scale storm events (i.e. hurricanes) and far-field currents, which may play a role in the transport of these larvae. It is unlikely that the larvae of these species are existing in high-density larval patches at these spatial scales, however, the diffuse larvae may be settling simultaneously by use of cues, such as lunar phases or regional scale tidal cues (see review by Doherty, 1991).

Conversely, for one species, *Acanthurus bahianus*, the spatial scale of recruitment coherence was very small, only 7.5 km, smaller than the separation between individual sites in this study. This suggests that whatever factors affect the variation in recruitment for this species, they are occurring on this small, within site scale. *A. bahianus* showed a high level of spatial patchiness in recruitment, and recruits of this species were recorded in only eight out of sixteen study sites in three years of monitoring (Appendix 2.1). Recruitment of this species was temporally incoherent between most site-pairs, where even neighbouring sites were completely decoupled from each other (Figure 2.3, Appendix 2.1). This suggests, for this species, that a high level of habitat selection may be occurring at settlement. *A. bahianus* larvae are very large at metamorphosis (Table 2.1), they have very strong swimming abilities nearing settlement (Fisher et al., 2005; Hogan et al., in press), and they have the ability to delay settlement (Bergenius et al., 2002). Settlement-stage *A. bahianus* larvae are very competent and it is possible that their recruits have specific habitat requirements and that they actively select very specific settlement sites.

Early life history traits can have a profound influence on the survival of fishes to the point of replenishment. Traits that affect fishes' dispersal, their ability to acquire food, or to avoid predators in the pelagic environment will ultimately affect the abundance and distribution of recruits of these fishes (Cowen and Sponaugle, 1997). Here I grouped species based on some life-history traits, and compared species groups for variation in the spatial scale of recruitment coherence. I grouped species using traits such as the length, and range of the pelagic larval duration, the size of larvae of the species at hatching, the size of the species at settlement and the mode of reproduction (whether the

eggs are broadcast into the pelagic realm or whether eggs are held in a nest until hatching). All of these traits might be expected to affect the dispersal potential, and the scale of recruitment coherence, of these species in one way or another.

Discriminant function analysis indicated that the group separation among species, based on life-history characters, was best explained by the species' maximum larval duration. However, the variation in the spatial scale of recruitment coherence was not explained by grouping species based on their life-history characteristics. There are some traits that I have not considered here that could be important for determining abundance and distribution of pelagic larvae of these species. I did not consider any behavioural traits like horizontal swimming ability nor vertical migration, both of which could affect the coherence of larval patches (Paris and Cowen, 2004) and determine a species' ability to avoid predators and acquire food resources. My results suggest that whatever causes a species' specific scale of recruitment coherence may be something that is inherent to the individual species and I cannot easily generalize these trends by looking to phylogenetic or life-history relationships among species.

Within species variation in e-folding distance was quite high (Table 2.2) in this study. This is attributed to low levels of coherence between some site-pairs that are near each other, and high levels of coherence between sites that are spaced far apart (Figure 2.3). Despite the high variation, the general trend was still toward a decline in coherence with distance, as demonstrated by the e-folding scale, and in some species there was even a negative linear relationship between site-pair distance and coherence (Table 2.4). The within species variation in coherence might be explained by far-field currents linking distant sites through larval dispersal (i.e. sites at Isla Roatan and Banco Chinchorro

and/or vice versa), by some residual habitat requirements/preferences of the species that prevent near sites being linked by recruitment, and potentially by synchrony to environmental cues (Sponaugle and Cowen, 1997). Whatever the case may be, it appears that the high variation in e-folding distance is a universal phenomenon among these species.

This is not the first study to investigate recruitment coherence nor to suggest that coherence can be used infer larval patch size of coral reef fishes. Victor (1984) used the spatial scale of coherence in settlement to infer patch size of larval *Thalassoma bifasciatum* in Panama. He found that temporally coherent settlement pulses arrived at reefs separated by 46 km. In this study, I found that the spatial scale of recruitment coherence for *Thalassoma bifasciatum* was 45 km, which corresponds quite closely with the results of Victors' study. A study by Paris and Cowen (2004) measured patch size of *Stegastes partitus* larvae off the west coast of Barbados via plankton tows. They measured the larval patches to be between 5.5 and 6.5 km in size along the north-south axis. In this study the scale of coherence of *S. partitus* was determined to be 24 km. However, recruitment coherence is only an estimate of larval patch size and not a true measure (Paris and Cowen 2004), and Victor (1984) found that the *T. bifasciatum* larvae that were settling synchronously in Panama were not all the same age, indicating that there was mixing of cohorts in the plankton. Therefore, the spatial scale of recruitment coherence may be a better indicator of the size of mixed larval patches and of the scale at which factors affect larval patches, rather than an actual estimate of individual patch size itself.

Using commercial catch-at-age data to reconstruct population sizes of recruits of their species, Myers et al. (1997) examined the spatial scale of variability in recruitment for marine, anadromous and freshwater fish species from temperate regions. They found a distinct pattern where recruitment coherence of temperate marine species was typically on a very large scale (500 km) and recruitment of freshwater species was coherent at much smaller scales (50 km). The e-folding distances that I report here for tropical marine fishes is most similar to the freshwater fishes of Myers et al. It must be noted that the scale at which Myers et al.'s (1997) study of recruitment coherence was conducted was larger than the scale of this study and the distances between stocks in their study were larger than the distances between sites here, so we may not expect the two studies to be equivalent.

Here I report the spatial scale of coherence in recruitment for 13 species of Caribbean reef fishes. I suggest that the scale of coherence can be used to estimate the spatial dimensions of pelagic processes that affect the patterns of replenishment of reef fish populations (also see Doherty and Williams, 1988). I conclude that further research needs to be done to determine what factors can explain the scales of recruitment coherence. In most cases here, I expect meso-scale pelagic processes to be the most important determinants of recruitment variability, however, for some species regional scale processes can be more important. These factors may be environmental or other intrinsic properties of the species. Lastly, the scale of recruitment coherence might indicate the scale at which sites are linked by the supply of larvae from source populations. While I recognize that coherent recruitment pulses do not necessarily represent genetically distinct larval cohorts (Victor, 1984; Paris and Cowen 2002) it is

possible that these multi-cohort larval patches come from similar upstream source locations. In fact, recent studies suggest that a large proportion of recruits of *Stegastes partitus*, during their larval phase, dispersed 30 km or less from their natal reef (Chittaro, 2005; Mora, 2004; Chapter 4 of this volume). A distance which corresponds quite closely with the spatial scale of coherence for this species (24 km). Further investigation is required to determine whether the spatial scale of coherence actually does represent cohorts coming from the same source populations.

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Table 2.1: Life history characteristics for 10 species of reef fishes from the Caribbean. HL is hatching length, SS is size at settlement, PLD mean is the average pelagic larval duration in days, PLD min is the minimum pelagic larval duration in days, PLD max is the maximum pelagic larval duration in days and PLD range is the difference between the PLD max and PLD min.

Family/Genus/Species	Spawning mode	HL (mm)	SS (cm)	PLD mean	PLD min	PLD max	PLD Range	References
Acanthuridae								
Acanthurus bahianus	Broadcast	1.70	2.7	52.3	42.0	68.0	26.0	Thresher, 1984; Robertson, 1992
Labridae								
Halachoeres garnoti	Broadcast	1.70	1.12	25.4	21.3	30.8	9.5	Sponaugle and Cowen, 1997; Wellington and Robertson, 2001
Halachoeres maculipinna	Broadcast	1.70	1.06	27.9	25.5	33.5	8.0	Sponaugle and Cowen, 1997; Wellington and Robertson, 2001
Halachoeres pictus	Broadcast	1.70	0.92	26.5	23.0	30.5	7.5	Sponaugle and Cowen, 1997
Thalassoma bifasciatum	Broadcast	1.60	0.92	51.4	40.0	74.0	34.0	Caselle, 1997
Scaridae								
Sparisoma viride	Broadcast	1.50	0.90	39.9	32.0	51.0	19.0	Koltes, 1993; Sponaugle and Cowen, 1996; Tolimieri, 1998.
Pomacentridae								
Chromis cyanea	Demersal	2.10	0.80	32.5	29	40.0	11.0	Thresher and Brothers, 1989; Wellington and Victor, 1989
Stegastes partitus	Demersal	2.16	1.14	30.0	26.7	30.0	3.3	Thresher and Brothers, 1989; Wellington and Victor, 1989; Robertson, 1992; Wellington and Robertson, 2001
Stegastes diencaeus	Demersal	2.38	1.04	27.3	21.7	27.3	5.6	Thresher and Brothers, 1989; Wellington and Victor, 1989; Wellington and Robertson, 2001; JD Hogan unpubl. data.
Stegastes planifrons	Demersal	2.42	1.08	28.3	22.0	28.3	6.3	Thresher and Brothers, 1989; Wellington and Victor, 1989; Wellington and Robertson, 2001; JD Hogan unpubl. data.

Table 2.2: e-folding distances, or the scale of recruitment coherence, for 13 species of Caribbean reef fishes.

Species	n	df	e-folding distance in km (± SE)	Sums of Squares	P-value
Acanthuridae					
Acanthurus bahianus	65	64	7.5 (16.5)	2.77	0.03
Grammatidae					
Gramma loreto	94	93	21 (64)	19.62	< 0.01
Labridae					
Halichoeres garnoti	94	93	140 (392)	20.16	< 0.01
Halichoeres maculipinna	94	93	29 (94)	18.16	< 0.01
Halichoeres pictus	94	93	12 (31)	12.72	0.01
Thallassoma bifasciatum	94	93	45 (217)	19.09	< 0.01
Scaridae					
Sparisoma atomarium	94	93	14.5 (49)	11.44	< 0.01
Sparisoma aurofrenatum	94	93	268 (2304)	32.16	< 0.01
Sparisoma viride	94	93	19.5 (55.5)	13.62	< 0.01
Pomacentridae					
Stegastes diencaeus	94	93	15 (49)	14.65	< 0.01
Stegastes partitus	94	93	24 (84)	21.42	< 0.01
Stegastes planifrons	94	93	11 (28)	9.88	0.01
Chromis cyanea	94	93	23 (87)	20.98	< 0.01

Table 2.3: Classification of group membership based on a discriminant function derived from the relationships between ten species of reef fishes in which similarities in life-history characters were the basis for group membership. Species associated with each group can be found in Figure 2.2.

	Group1	Group2	Group3	Percent Correct
Group1	2	0	0	100
Group2	0	2	0	100
Group3	0	0	6	100
Total	2	2	6	100

Table 2.4: Slopes of the lines from regressions testing for linear trends in recruitment coherence for each species with increasing distance between site pairs. Those in bold font indicate slopes that are significantly different from zero at $\alpha = 0.05$.

Species	Slope	p-value
Acanthurus bahianus	-0.06	0.62
Chromis cyanea	-0.02	0.81
Gramma loreto	0.16	0.13
Halachoeres garnoti	-0.19	0.07
Halachoeres maculipinna	-0.19	0.07
Halachoeres pictus	-0.12	0.24
Sparisoma atomarium	**-0.50**	**< 0.01**
Sparisoma aurofrenatum	-0.01	0.99
Sparisoma viride	**-0.25**	**0.02**
Stegastes diencaeus	-0.16	0.13
Stegastes partitus	**-0.32**	**< 0.01**
Stegastes planifrons	-0.06	0.58
Thalassoma bifasciatum	**-0.48**	**< 0.01**

Figure 2.1: Map of the Mesoamerican Barrier Reef System with sixteen study sites overlaid. Sites labeled BC are at Banco Chinchorro, Mexico; sites labeled TA are at Turneffe Atoll, Belize; sites labeled RO are at Isla Roatan, Honduras.

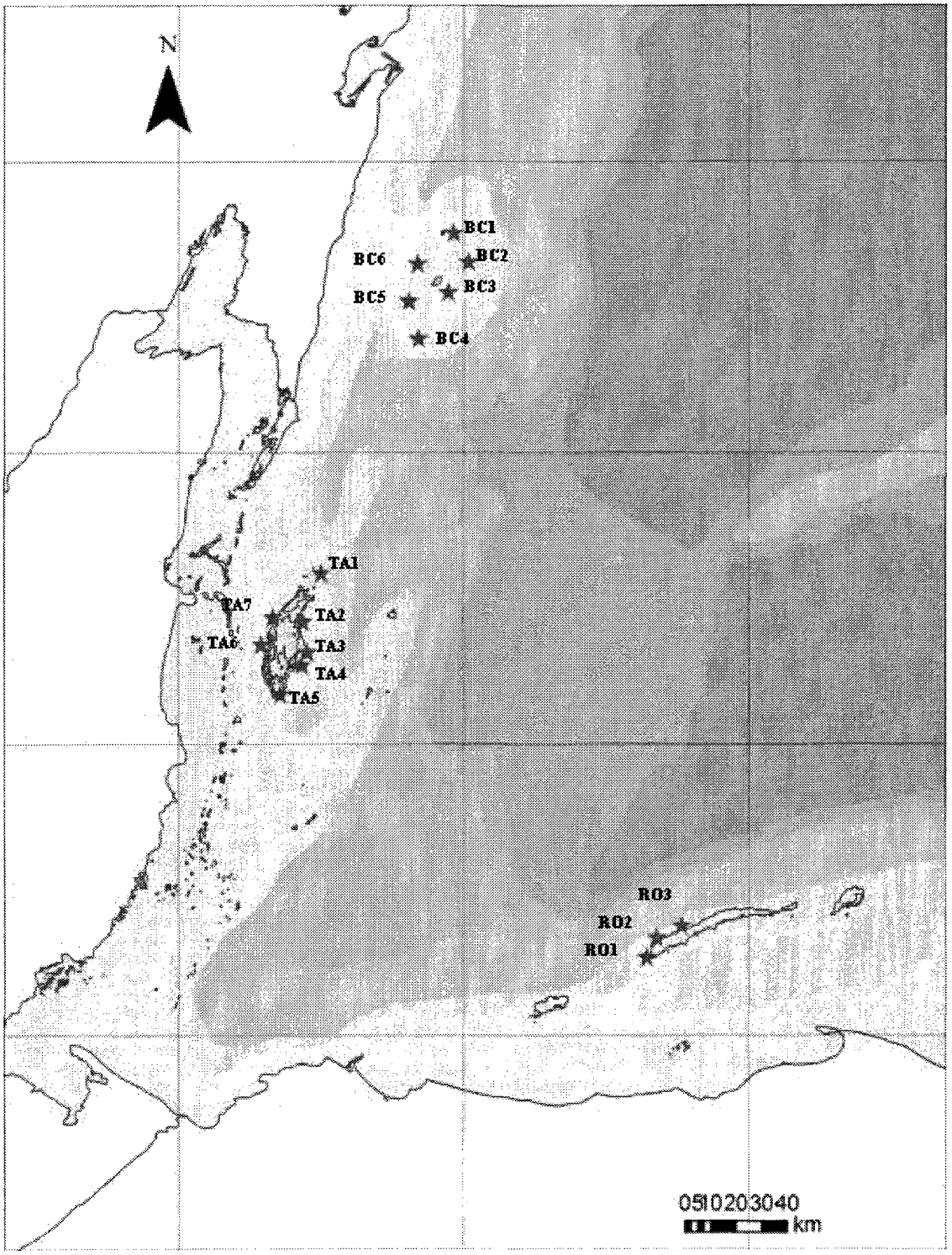


Figure 2.2: Cluster-tree defining membership of species to 3 distinct groups as distinguished by life-history characters. Groups are defined here by linkage distances greater than 20 and less than 50.

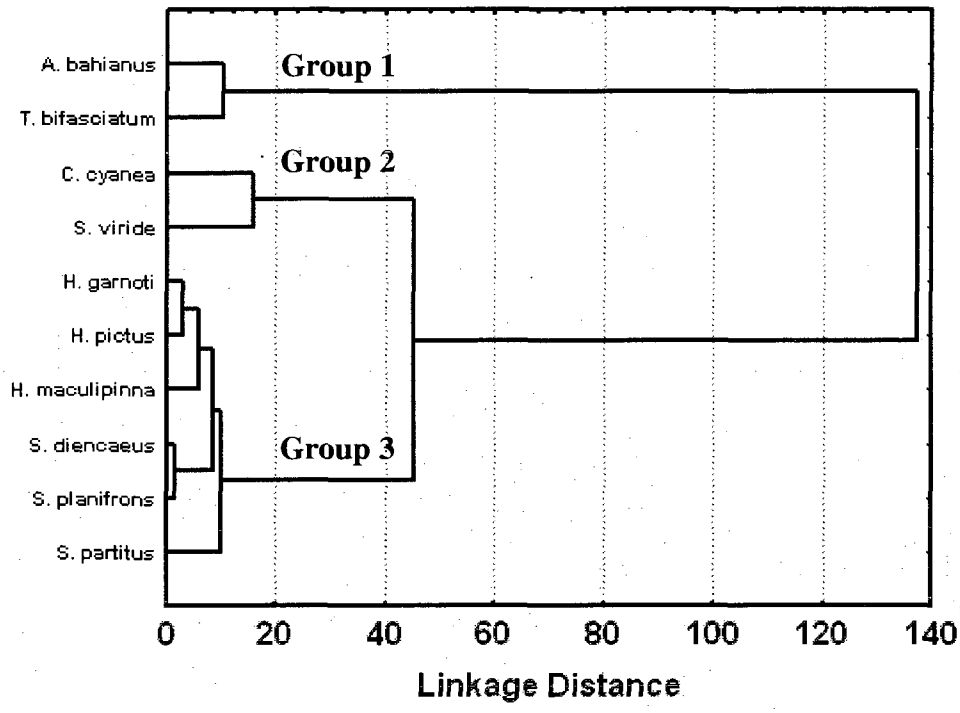
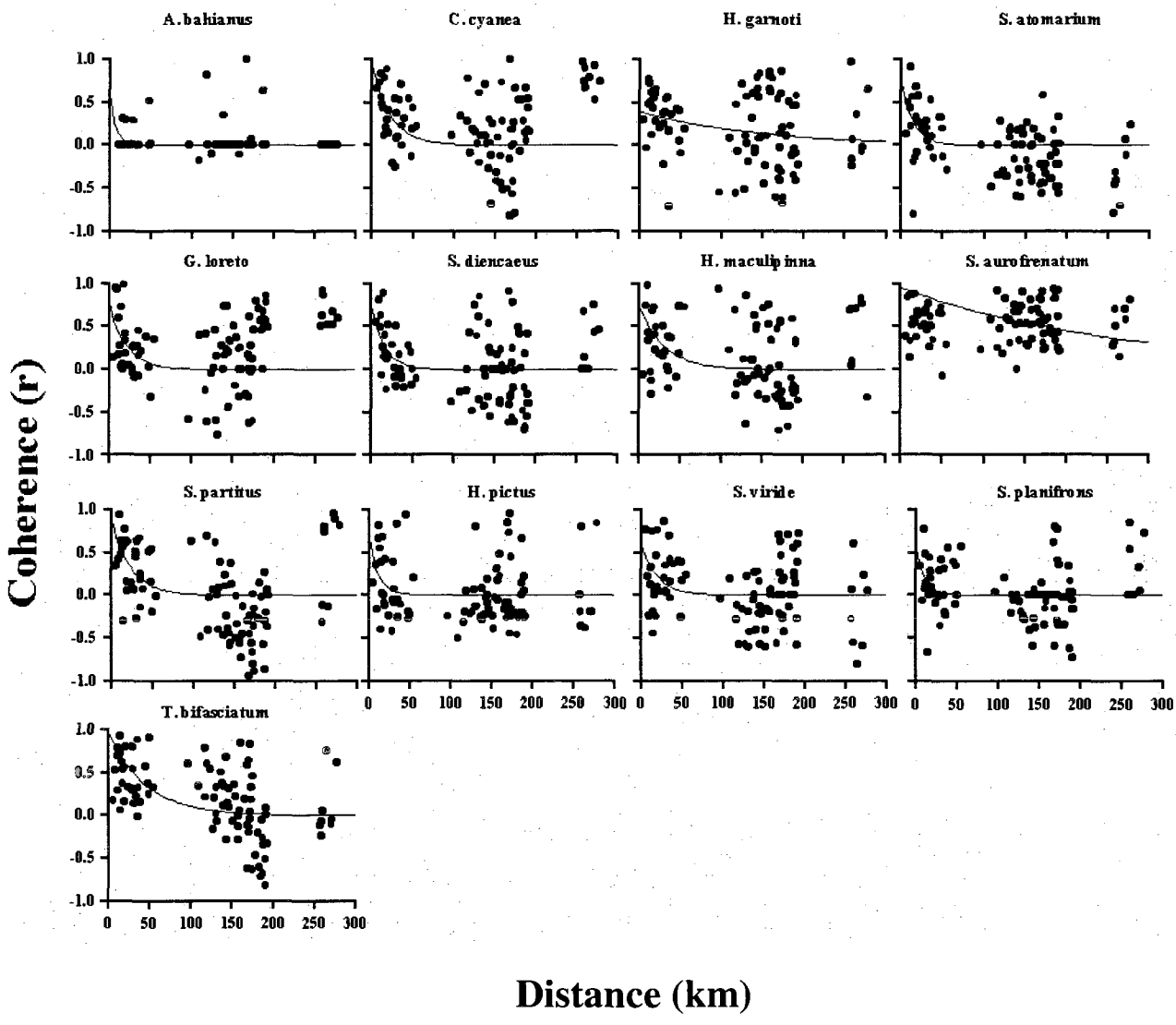
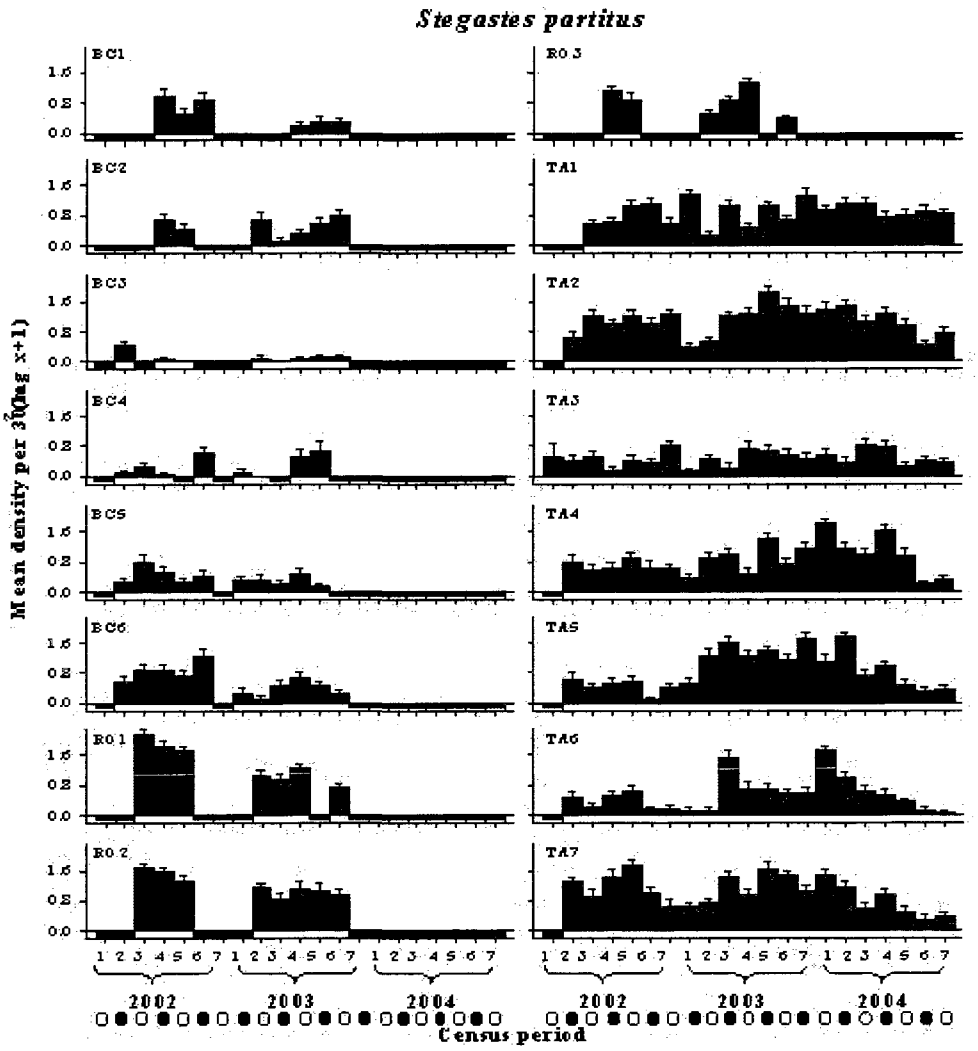


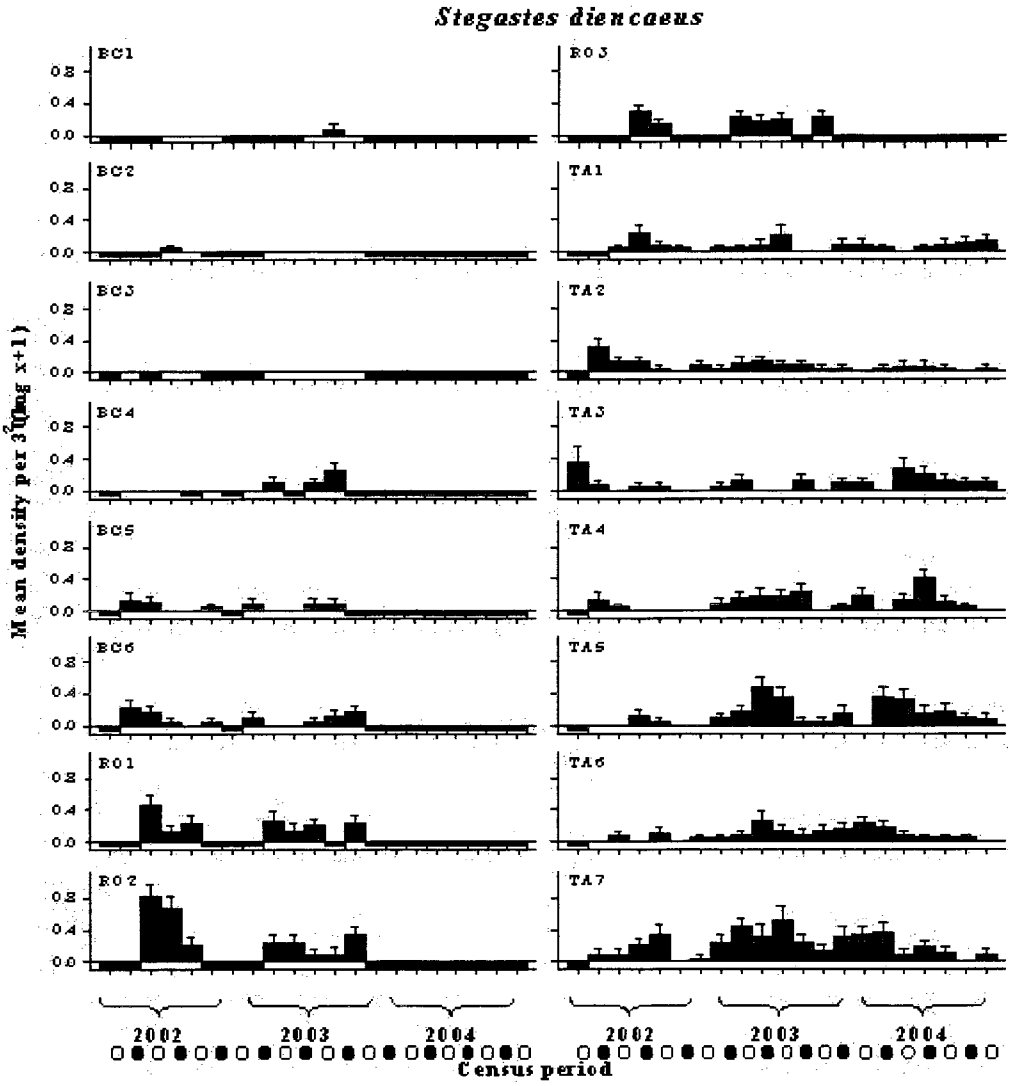
Figure 2.3: Correlation of recruitment between pairs of stocks versus distance between stocks for 13 Caribbean reef fish species.



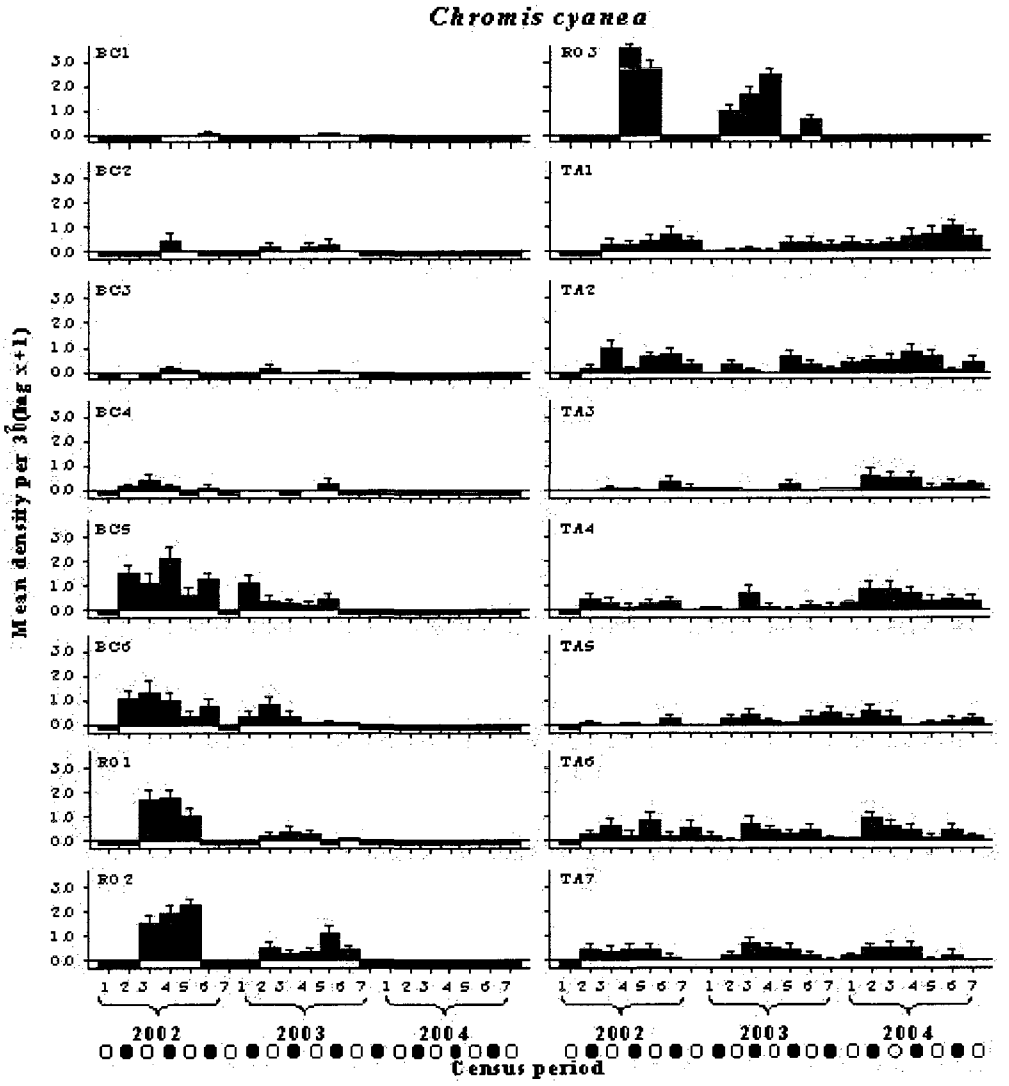
Appendix 2.1a: Density of recruits of *Stegastes partitus* at 16 sites in the MBRS over 3 years of sampling (2002 – 2004). Numbers on the x-axis represent census periods. Open circles on the x-axis represent full moons, filled circles represent new moons.



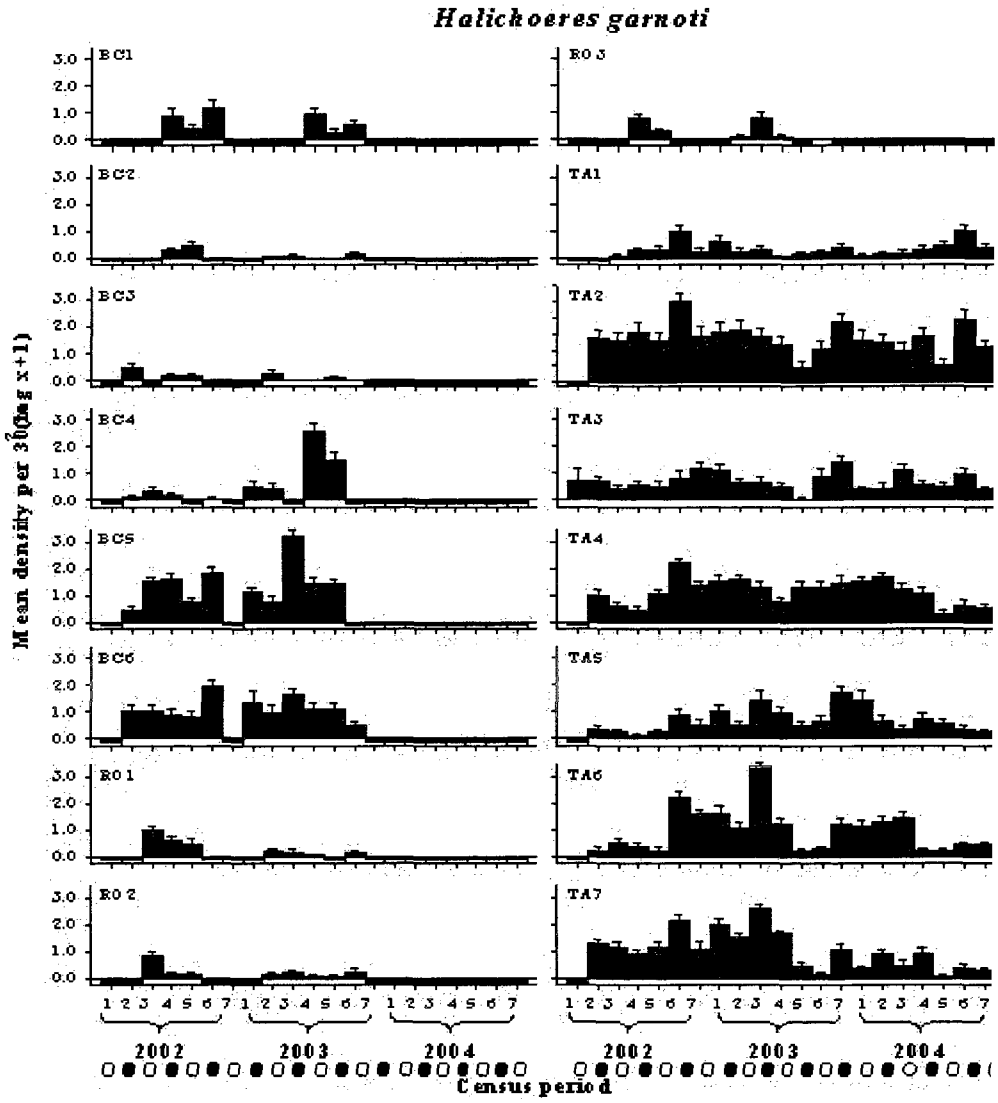
Appendix 2.1b: Density of recruits of *Stegastes diencaeus* at 16 sites in the MBRS over 3 years of sampling (2002 – 2004). Numbers on the x-axis represent census periods. Open circles on the x-axis represent full moons, filled circles represent new moons.



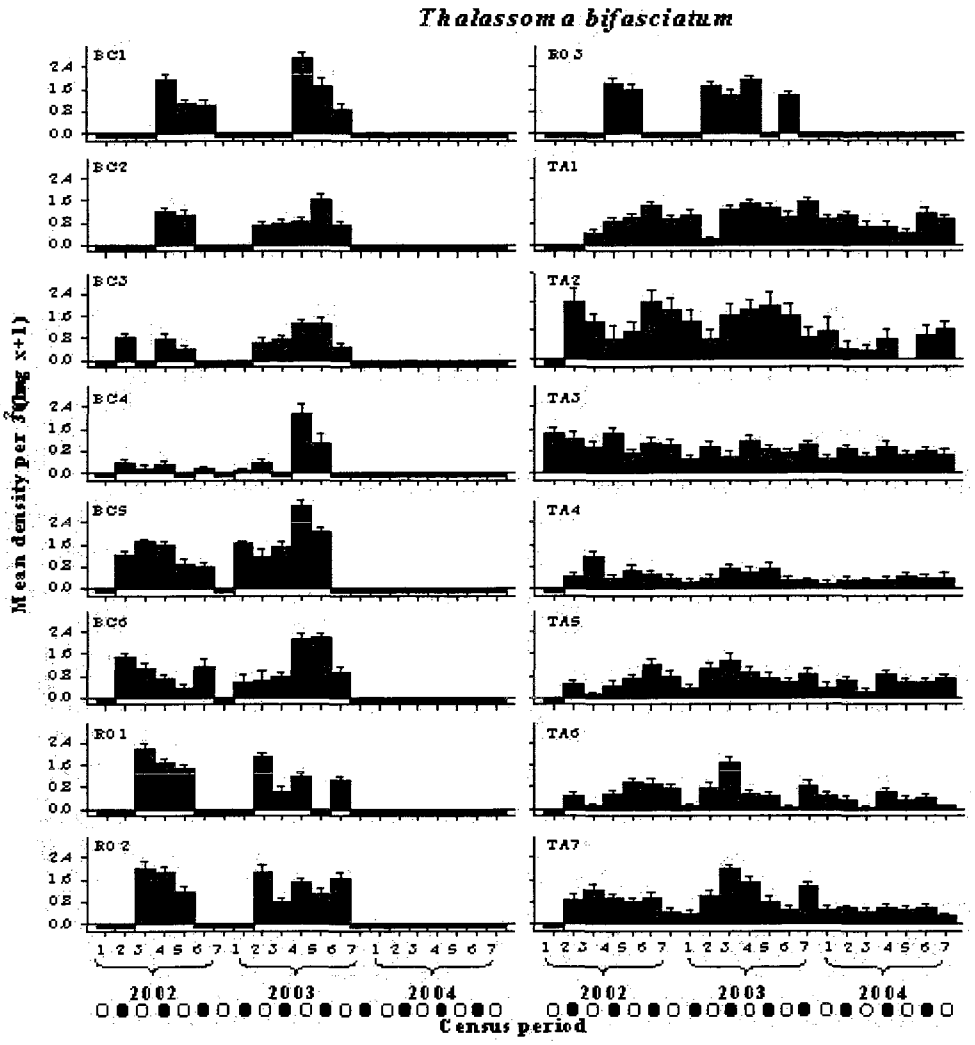
Appendix 2.1c: Density of recruits of *Chromis cyanea* at 16 sites in the MBRS over 3 years of sampling (2002 – 2004). Numbers on the x-axis represent census periods. Open circles on the x-axis represent full moons, filled circles represent new moons.



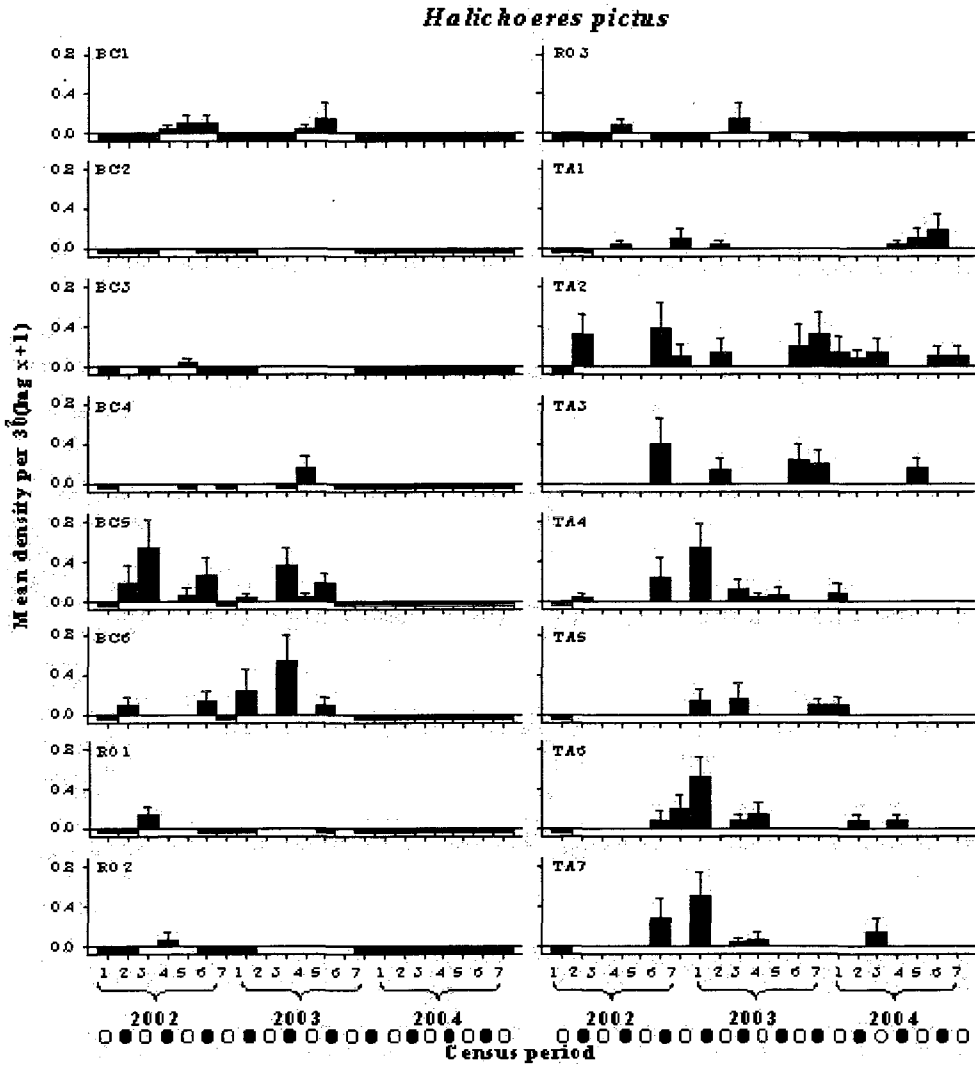
Appendix 2.1d: Density of recruits of *Halichoeres garnoti* at 16 sites in the MBRS over 3 years of sampling (2002 – 2004). Numbers on the x-axis represent census periods. Open circles on the x-axis represent full moons, filled circles represent new moons.



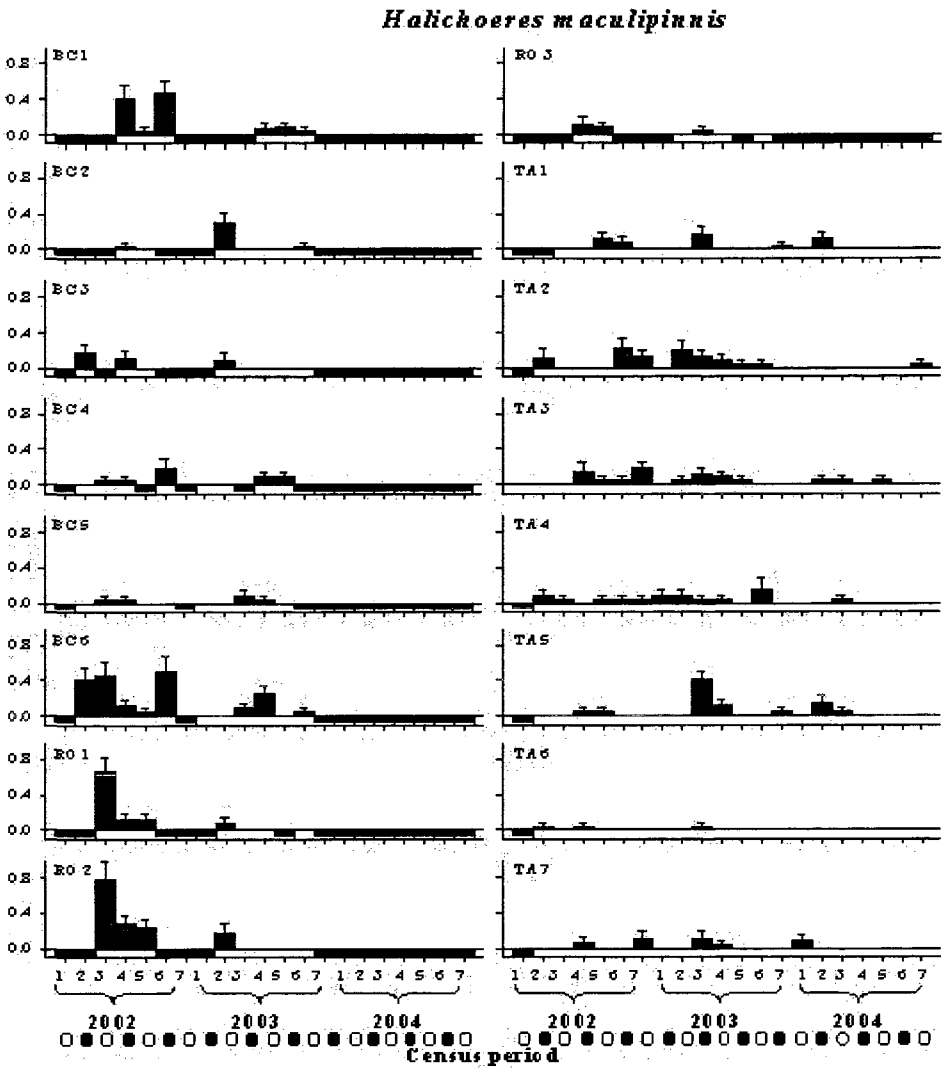
Appendix 2.1e: Density of recruits of *Thalassoma bifasciatum* at 16 sites in the MBRS over 3 years of sampling (2002 – 2004). Numbers on the x-axis represent census periods. Open circles on the x-axis represent full moons, filled circles represent new moons.



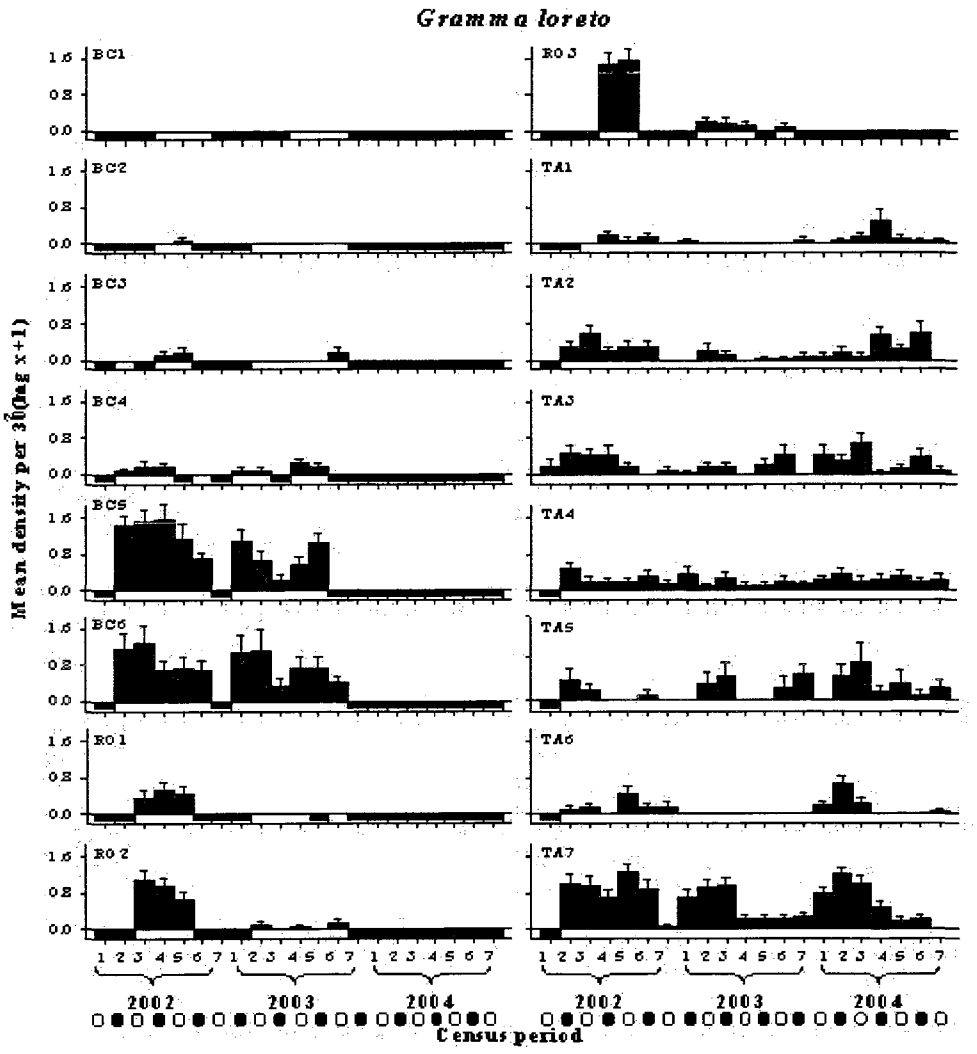
Appendix 2.1f: Density of recruits of *Halichoeres pictus* at 16 sites in the MBRS over 3 years of sampling (2002 – 2004). Numbers on the x-axis represent census periods. Open circles on the x-axis represent full moons, filled circles represent new moons.



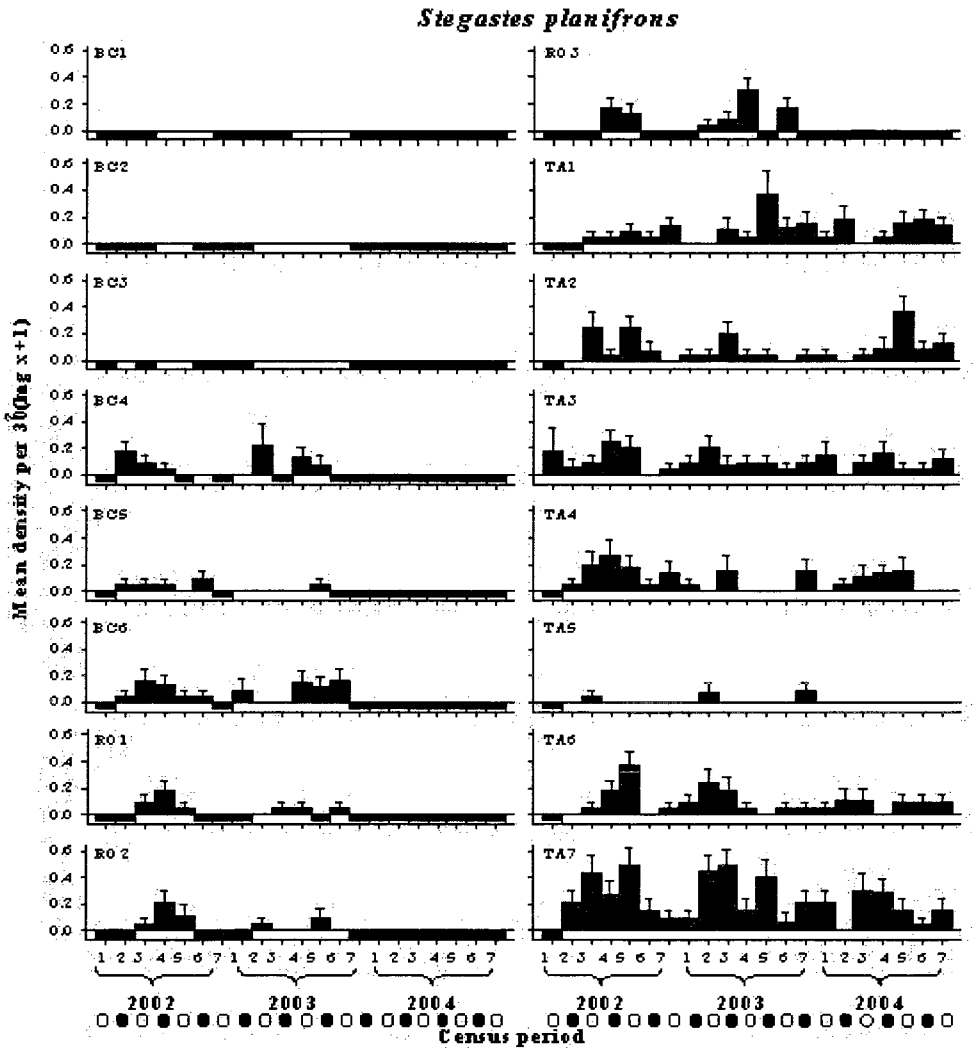
Appendix 2.1g: Density of recruits of *Halichoeres maculipinna* at 16 sites in the MBRS over 3 years of sampling (2002 – 2004). Numbers on the x-axis represent census periods. Open circles on the x-axis represent full moons, filled circles represent new moons.



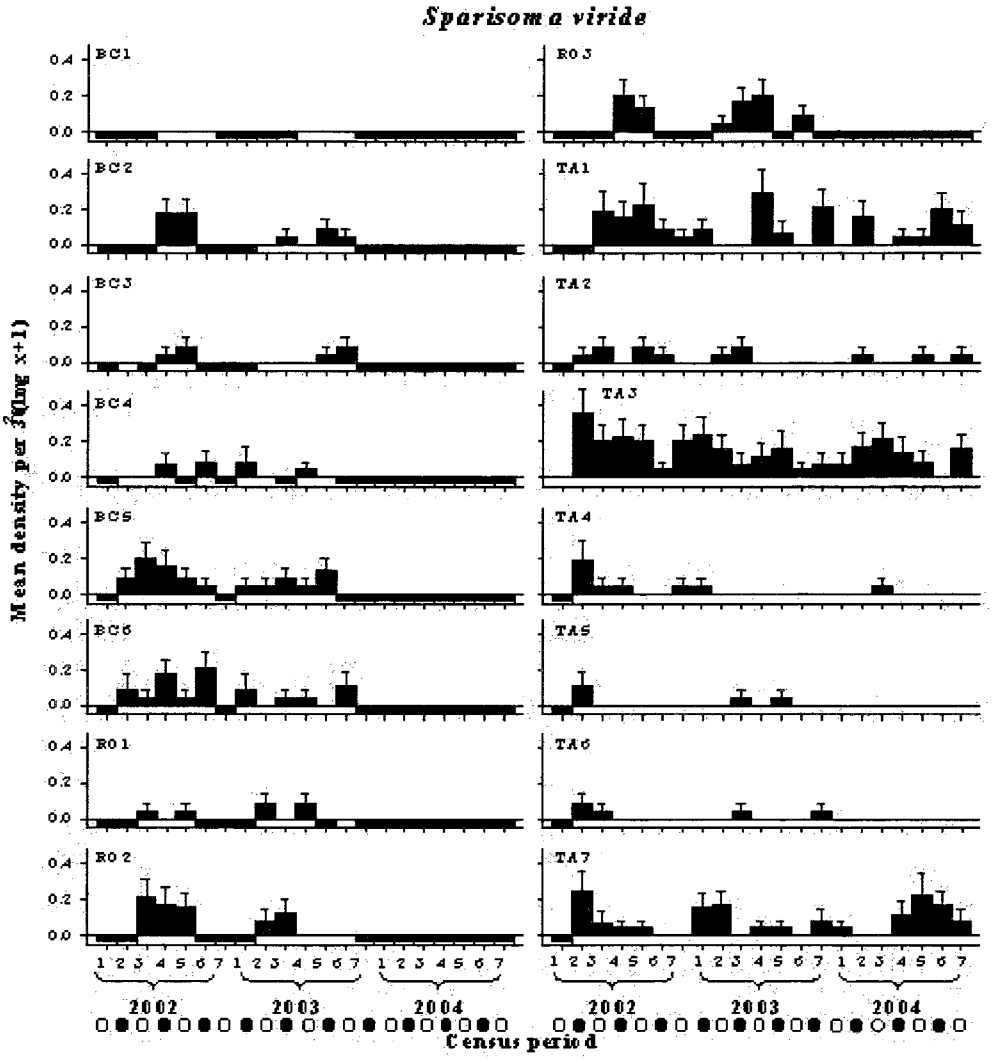
Appendix 2.1h: Density of recruits of *Gramma loreto* at 16 sites in the MBRS over 3 years of sampling (2002 – 2004). Numbers on the x-axis represent census periods. Open circles on the x-axis represent full moons, filled circles represent new moons.



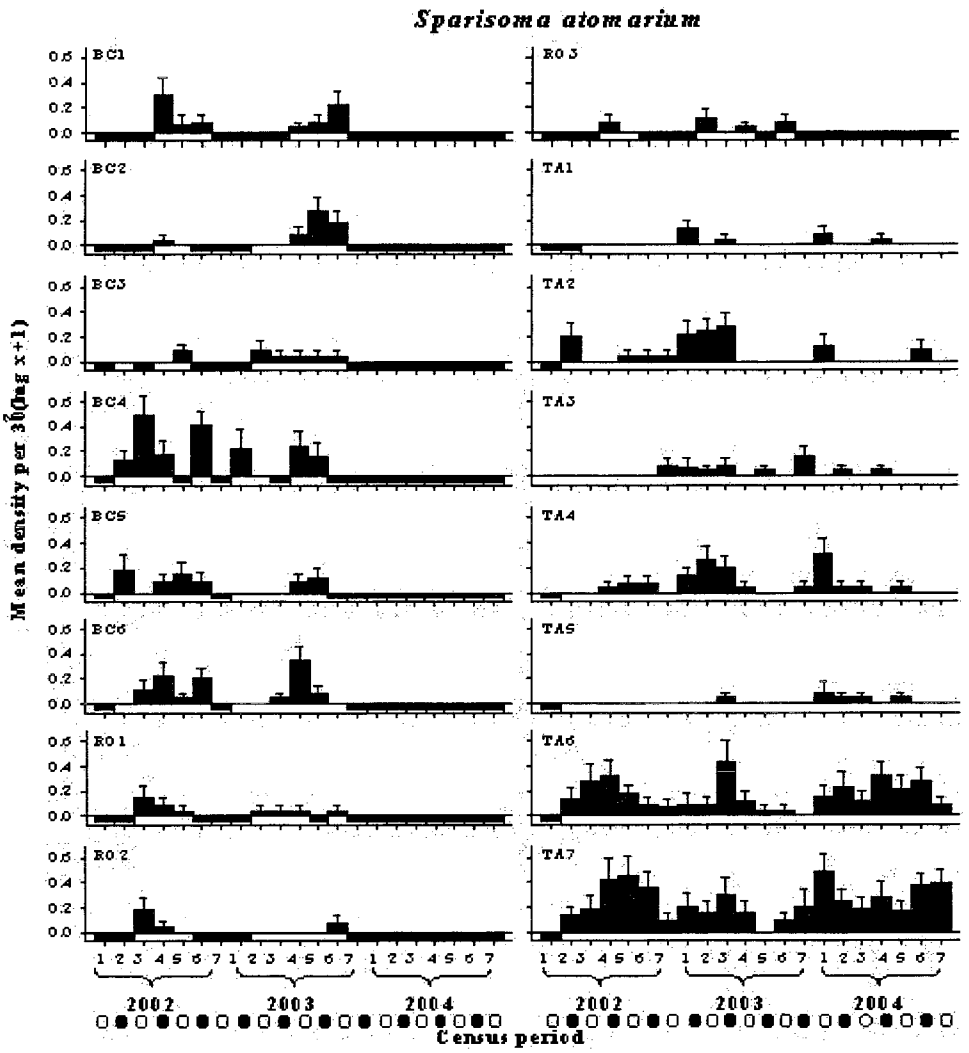
Appendix 2.1i: Density of recruits of *Stegastes planifrons* at 16 sites in the MBRS over 3 years of sampling (2002 – 2004). Numbers on the x-axis represent census periods. Open circles on the x-axis represent full moons, filled circles represent new moons.



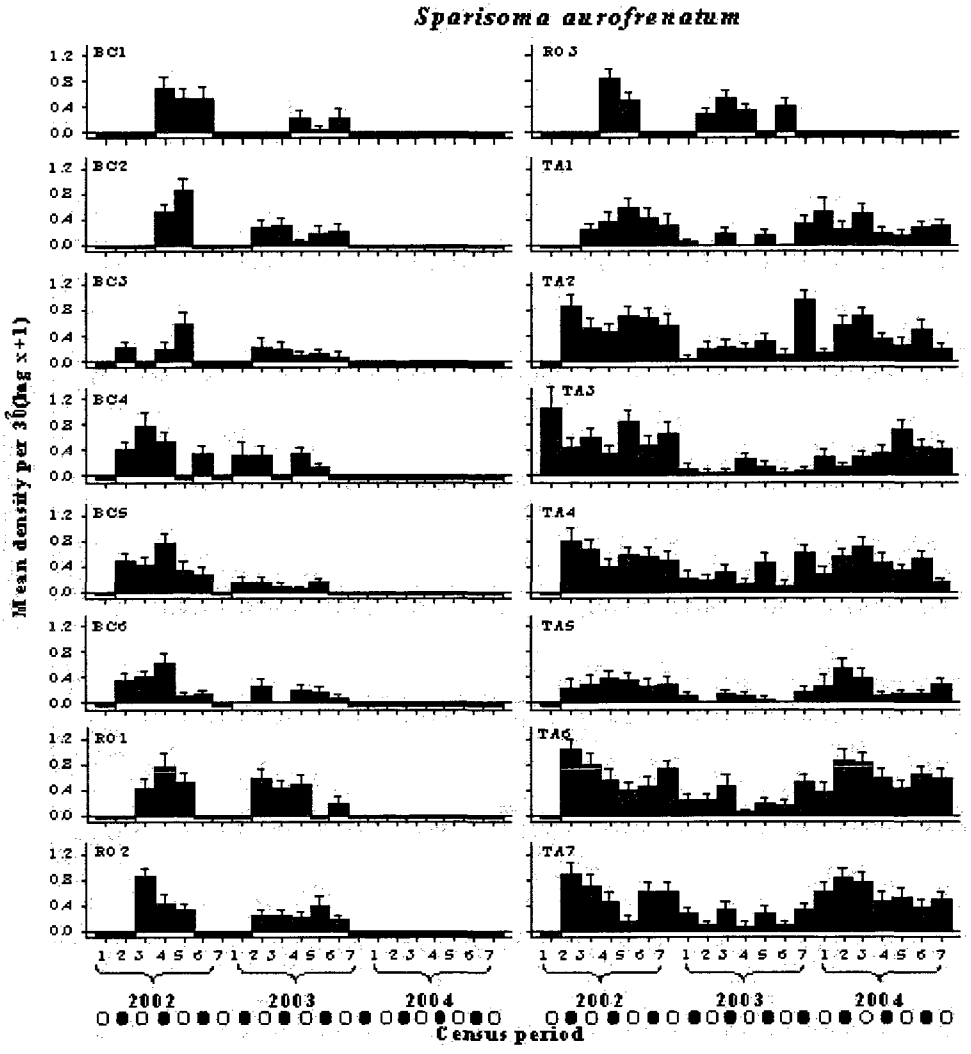
Appendix 2.1j: Density of recruits of *Sparisoma viride* at 16 sites in the MBRS over 3 years of sampling (2002 – 2004). Numbers on the x-axis represent census periods. Open circles on the x-axis represent full moons, filled circles represent new moons.



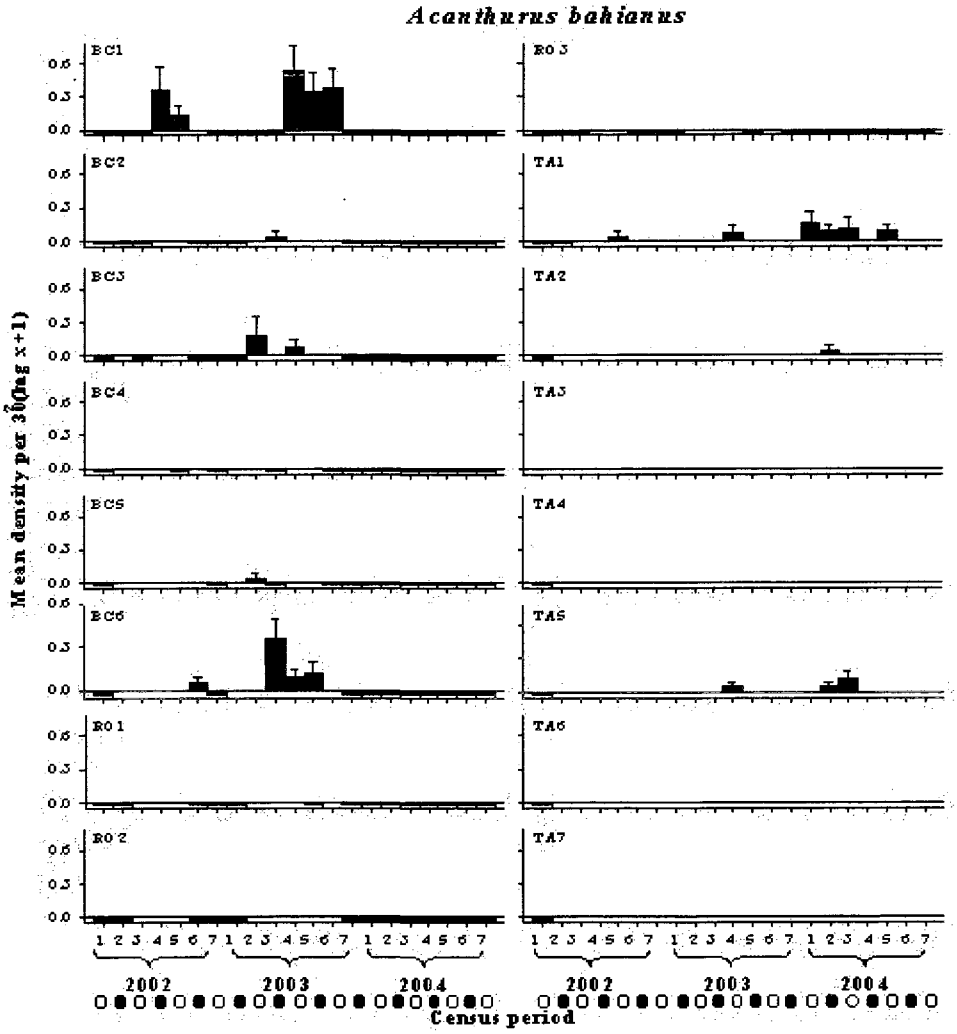
Appendix 2.1k: Density of recruits of *Sparisoma atomarium* at 16 sites in the MBRS over 3 years of sampling (2002 – 2004). Numbers on the x-axis represent census periods. Open circles on the x-axis represent full moons, filled circles represent new moons.



Appendix 2.11: Density of recruits of *Sparisoma aurofrenatum* at 16 sites in the MBRS over 3 years of sampling (2002 – 2004). Numbers on the x-axis represent census periods. Open circles on the x-axis represent full moons, filled circles represent new moons.



Appendix 2.1m: Density of recruits of *Acanthurus bahianus* at 16 sites in the MBRS over 3 years of sampling (2002 – 2004). Numbers on the x-axis represent census periods. Open circles on the x-axis represent full moons, filled circles represent new moons.



Chapter 3: Using recruitment coherence and otolith microchemistry data to infer dispersal movements and patch dynamics of a reef fish, *Stegastes partitus*, throughout the larval period

INTRODUCTION

Nearly all reef fishes have pelagic larvae and these larvae are minute and are potentially highly dispersive (Leis, 1991). Because of pelagic dispersal, larvae are likely to recruit elsewhere besides the reef at which they were spawned, causing a decoupling of local production and local recruitment (Roughgarden et al., 1988; Caley et al., 1996). Little is known about the ecology of larval reef fishes because they are difficult to track in the vast ocean environment (Leis, 1991). It is thought that larvae, once spawned, are transported away from the reef environment into an offshore environment and then return to a reef nearing the end of their larval development (Leis, 1991). Most studies of the distribution of larvae are conducted very near shore with towed nets and/or light traps but no single method is adequate to sample the full range of sizes and developmental stages (Leis, 1991). Larvae are known to exist in coherent patches, at least in the vicinity of reefs (Victor, 1984; Williams, 1986; Paris and Cowen, 2004; also see Chapter 2 of this volume). One way of estimating the size of these larval patches is by monitoring recruitment at various spatial scales and investigating the spatial scale of synchrony (or coherence) in the amount and timing of recruitment (Victor, 1984; Chapter 2 of this volume). What is poorly understood is the distribution of the youngest larval forms, and even more rare are studies that track larval movements throughout the entire larval period. The question becomes, do reef fish larvae disperse in coherent patches for a significant portion of their larval lives?

Recently there has been an increased interest in understanding the early life-history of reef fish larvae because of the importance of the larval stage for dispersal and population connectivity, the understanding of which is vitally important for proper conservation and management (Roberts, 1997). A variety of techniques have been developed in order to track movements of larvae, including artificial tagging (Jones et al., 1999; Jones et al., 2005; Thorrold et al., 2006); natural genetic markers (Shulman, 1998; Planes, 2002; Taylor and Hellberg, 2003); and natural otolith markers (Swearer et al., 1999; Patterson et al., 1999; Patterson et al., 2004).

Otolith chemical assays are a relatively new method of tracking fish movements. The geochemical signals that are incorporated into the otoliths can be used as a natural tag, and it has been shown that the concentrations of some elements reflect the composition of the water masses that fishes experience (Campana, 1999; Milton and Chenery, 2001; Elsdon and Gillanders, 2003; Elsdon and Gillanders, 2005a). Trace element distributions in the oceans surface waters reflect three things; the strength of source inputs; the amount of removal of each element via various mechanisms; and water circulation patterns (Chester, 1990). Understanding how these mechanisms act can allow us to speculate about the environments a fish likely encountered and compare one fish to other fishes. In general, ocean surface waters are richer in trace elements near shore versus in the open ocean, primarily because inputs from fluvial, terrigenous and atmospheric sources are greater (Chester, 1990). One major advantage of otolith chemical analysis is that the otolith acts as a "flight recorder", a time series of environmental history of a fishes' life. With the advancement of sampling techniques, such as laser

ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), I can investigate the movement of larval fishes throughout the entire larval period.

The objective of this study was to determine if the larvae of the damselfish *Stegastes partitus* occupied different water masses (environments) during the larval period. To do this I used LA-ICP-MS to measure the otolith chemistry across the entire larval period, subdivided into several developmental periods. I also wanted to determine if larval fishes dispersed in coherent patches throughout the larval period. To do so I compared a measure of otolith chemical similarity with a measure of recruitment similarity (coherence). Using otolith chemistry to evaluate the environments experienced by larval fishes and relating their environmental experiences to recruitment pathways will provide a better understanding of the source of larval supply to a reef population.

MATERIALS AND METHODS

Data Collection

STUDY LOCATION AND RECRUITMENT SURVEYS

This study was conducted at two locations in the Mesoamerican Barrier Reef region of the Caribbean, Turneffe Atoll in Belize (TA) and Banco Chinchorro in Mexico (BC). There were three sites within the BC location and four sites in TA, sites were spaced a minimum of 10 km apart (See Figure 3.1). The recruitment of juveniles of the reef fish *Stegastes partitus* was censused at these sites on a bi-weekly basis during the

summer months (from May to September) of 2002 through 2004. Recruitment surveys were conducted, by teams of divers on SCUBA, immediately following the new and full moons of a given month. Surveys were conducted concurrently at both locations and completed within seven days following the new or full moon. New recruits were defined as any individuals that were ≤ 2.0 cm total length as estimated visually by divers. At each site, recruitment was censused using 16, 1 X 30 m transects, 8 of which were located at a deep position within a site (depth range 9 to 11 m), and 8 of which were located at a shallow position within a site (depth range 6 to 7.5 m). Within each position transects were placed haphazardly, parallel to the reef crest, with a distance of 2 to 3 m between transects. The length of each transect was measured using a 30 m tape-measure that was reeled out as the divers counted the fishes, and the width of each transect was measured with a 1 m wide PVC T-bar that each diver held whilst swimming. To account for observer bias in the estimates of recruitment, a series of calibrating and cross validation surveys were conducted at the beginning of each summer including all observers.

FISH COLLECTION AND OTOLITH PREPARATION

Newly recruited juveniles of *S. partitus* were collected from each of the study sites during the routine recruitment census dives. Recruits were collected by divers on SCUBA using hand nets and a clove oil mixture (9:1; 70% isopropanol:pure clove oil). Collections were primarily made at the deep position within sites on the shallow fore-reef at approximately 10 m depth. For the purposes of this study I focus on the collections of fish from seven sites (four at Turneffe Atoll and three at Banco Chinchorro) from four census periods in 2002 and 2003 (See Table 3.1 for details of sites and sampling periods).

After collection, fish samples were preserved in 95% ethanol until the otoliths could be removed. Sagittal otoliths were removed from the fish, cleaned of any tissue using distilled water and a fine bristled paintbrush, embedded in Crystal Bond® glue, and polished in a transverse section using various lapping films from 30 μm to 9 μm grain size (Precision Surfaces International PSI-1630S-11, PSI-1612-11, PSI-1609-11). Otolith samples were mounted on petrographic slides (up to 18 otoliths per slide) for analysis in the ICPMS. The samples were cleaned in a class 100 clean room, placed in a milliQ water bath and sonicated for 10 minutes, and then triple rinsed with milliQ water. After cleaning, each slide was allowed to dry overnight in a laminar flow HEPA-filtered fume hood.

Data analysis

RECRUITMENT COHERENCE ANALYSIS

Recruitment coherence is measured here as the difference between two recruitment survey sites in the number of recruits received at a given time (note: this is different than the measure of coherence in chapter 2). The mean number of recruits, averaged over all transects, was calculated for each site at each census period. All recruitment data were log (x + 1) transformed prior to analysis because recruitment data is typically a lognormal distribution (McCune and Grace 2002) and species abundances on a transect are often zeros. Recruitment data were normalized by dividing each value of recruitment for any given site (e.g. site x at census period y) by the maximum number of recruits received by that site over all census periods. Normalization relativizes

recruitment between sites allowing the calculation of residual recruitment simply by taking the difference between two sites. Residuals (a measure of recruitment coherence) were calculated for all possible site-pairs by subtracting the normalized mean log-recruitment of one site by the normalized mean log-recruitment of all other sites and this was repeated for each individual census period. These calculations yielded a value of coherence between zero and one, where 0 indicates total coherence between two sites at a given time and 1 indicates complete incoherence between two sites at a given time. For the purposes of clarity and interpretability, the residuals were inverted (by subtracting them by 1) such that complete coherence is a value of 1 and no coherence is a value of 0.

OTOLITH MICROCHEMICAL ANALYSIS

Otoliths were chemically analysed at the Great Lakes Institute for Environmental Research, University of Windsor, using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). A Thermo Elemental X7 ICP-MS was operated with argon used as the carrier gas from the laser-sampling cell. The laser sampling system is a purpose-built system (Fryer et al., 1995) based on a non-homogenized, high power, frequency-quadrupled (266 nm) Nd:YAG (neodymium-doped yttrium aluminum garnet) laser. Frequency was set at 20 Hz, flash lamp power varied between 1.18 and 1.25 kV, pinhole beam constrictors of 1.0 - 1.5 mm were used to increase the spatial resolution of the laser sampling, and a half-wave plate was used to reduce the power of the beam and increase spatial resolution of the laser sampling. Based on these settings, the beam was calculated to be 28 - 31 μm in width. The beam was focused onto the sample using an

Olympus® BX-51 petrographic microscope and an Optics for Research® 266 nm 10X objective lens.

LA-ICP-MS data acquisition lasted 160 seconds with 60 seconds of instrument and gas background counts prior to the start of each ablation. Transects from the otolith core to edge were enabled by the use of an automated microscope stage, moving at a speed of approximately 7 $\mu m\ s^{-1}$. A trace element-doped glass standard (National Institute of Standards and Technology, NIST, 610), a homogeneous glass that is spiked with a range of elements in known concentrations, was analysed in duplicate at the beginning and the end of each sample batch (approximately 18 otoliths) to correct for instrument drift. Calcium was used as an internal standard to compensate for the signal variation caused by differences, between otoliths, in the volume of ablated material.

In total, 11 isotopes representing 9 elements were analysed by the LA-ICP-MS (See Table 3.2 for list of elements) and chemical concentrations, in parts per million, were calculated using Plasma Lab© software (Thermo Electron, 2003). Six multi-elemental signals were selected from each otolith transect between the core and the settlement mark as determined by visual analysis during ablation. The first signal represented the otolith material near the core of the otolith henceforth called the "Core" signal. The remaining five signals were chosen by taking equal developmental periods along the otolith transect from the end of the core signal until the settlement mark.

Each signal was comprised of average concentrations of each element, the amount of otolith material used to calculate each signal varied on a fish-by-fish basis as follows: The core signals were averaged from the amount of otolith material that represented growth from the otolith primordium up until the point in time where the yolk sac was

absorbed by the larvae. It has been suggested that otolith chemistry may be influenced by the maternal yolk added to the eggs (Brophy et al., 2004; Ruttenberg et al., 2005; Chittaro et al., 2006), therefore, I included otolith material in the core signal up until the yolk sac stage to isolate any effects on otolith chemistry from maternal inputs. The size of the otolith at yolk sac absorption was calculated by taking the average size of an otolith of a late-stage embryo and adding to that the amount of otolith growth, in the transverse plane, equivalent to 3 days growth post-hatch (the time until yolk-sac absorption). Otoliths of late-stage embryos of *S. partitus* are approximately 20 μm in diameter (Chittaro et al., 2006), equivalent to approximately 3.5 days growth in the egg. After hatching, yolk sac absorption takes approximately 3 days for pomacentrids (Thresher 1984). Post-hatching growth of otoliths in the transverse plane (from the core along the longest growth axis) is approximately 7 μm for the first three days of growth post-hatch (Wilson and Meekan, 2002). Therefore I calculated the size of the otolith, post-yolk sac absorption, to be approximately 27 μm in size. The remaining five signals were averaged from material from five equal sized portions of the otolith between the end of the core signal up until the settlement mark. These signals represent 5 equal developmental periods in the life of each fish and are hereafter referred to as "Beginning", "Early", "Middle", "Late" and "End" signals in developmental order from the end of the core signal up until the settlement mark.

STATISITCAL ANALYSIS

After signal selection, elements that met the following two criteria were included in statistical analyses; 1) concentrations of NIST 610 samples were determined with

satisfactory precision (i.e. coefficient of variation less than 10%); and 2) concentrations of elements were greater than detection limit for more than 85% of otoliths analysed. Data for elements that met these two criteria were Ln(x)+1 transformed to improve normality for multivariate statistical analyses.

We wanted to test whether otolith chemistry varied across an otolith transect at different developmental periods in the larval life, describing changes in the concentration of each element with increasing fish age. Furthermore, I wanted to test for differences between fishes caught at different sites (during different census periods) in terms of the otolith chemistry, taking into account differences across all developmental periods. In order to do this, repeated measures analysis of variance was used. This approach required that adjacent periods along the otolith transects (through the larval period) be equidistant, this was the impetus for sampling otolith chemistry at five equal developmental periods (See Above). Periods must be made equidistant to reduce possible error between periods; however, the core signals were not equal in size to the other five developmental periods. The core signal represents a distinct developmental (and chemical) period in the life of a fish and treating it differently from the other five developmental periods likely reduced error.

Univariate repeated measures ANOVA was first used to test the above hypotheses. Univariate RM-ANOVA assumes sphericity in the data, a spherical matrix has equal variances and covariances equal to zero (Zar, 1999). Mauchly's criterion was used to test the hypothesis of sphericity of orthogonal components. Mauchly's sphericity test examines the form of the common covariance matrix. The common covariance matrix of the within-subject variables (i.e. developmental periods) must be spherical, or

the F tests and associated p values for the univariate approach of testing within-subjects hypotheses are invalid, increasing the probability of Type I error (Zar, 1999). The hypothesis of sphericity was rejected here for all elements, therefore, I apply a multivariate RM-ANOVA approach that does not depend upon the assumption of sphericity (Zar, 1999). Since all elements and isotopes were examined at the same time (i.e. multiple dependent variables) the design can be best described as a doubly-multivariate approach (Statsoft Inc., 2001).

The repeated measures ANOVA provides tests of a number of hypotheses, however, I was interested in two specific terms in the model: the age term and the age x census period x site interaction. The age term tests for variability in otolith chemistry across the otolith transect, while the age x census period x site interaction tests for differences in otolith chemistry across the otolith transect between sites, within census periods. In the event that there was a significant age x census period x site interaction, I wanted to test for similarities in otolith chemistry between individual sites (within census periods).

To explore the site-pair relationships in across-transect otolith chemistry, a series of cluster analyses were performed. Cluster analysis was used here instead of typical paired comparisons associated with ANOVAs (e.g. Tukey's HSD *post hoc* test) because the number of paired comparisons in this case was extreme. Performing multiple *post hoc* tests increases the chances of Type I error, there are corrections that can be made to reduce Type I error (e.g. Bonferroni correction) however considering the large number of paired comparisons being performed here, any correction will greatly increase the chance of committing a Type II error.

Before performing cluster analyses all elemental concentrations were normalized to the maximum value for each individual element by developmental period within each census period. For example, concentrations of ^{25}Mg in the otolith core were normalized to the maximum value of ^{25}Mg in all core signals from all fishes from census period X, and so on. Normalization was done so that each element was weighted equally when performing cluster analysis, thereby removing biases of a few or a single element. Also, ^{138}Ba was removed from cluster analyses. Both Ba isotopes are very similar in their mean concentrations and variance in the otoliths, occurring in an almost 1:1 ratio (See Table 3.2), and univariate results from the RM-ANOVA indicated that these two isotopes behave similarly across an otolith transect. Thus, including both Ba isotopes would be pseudo-replication, and may cause unrealistic group clustering toward similarities in Ba concentrations due to the double weighting of Ba isotopes.

Three different iterations of cluster analysis were used to explore site-pair relationships in otolith chemistry. First, cluster analyses were performed using all elements across all ages (developmental periods) unweighted. Second, cluster analyses were performed using all elements across all ages but the concentrations were weighted such that later ages (i.e. closer to the settlement mark) were given a higher weighting than earlier ages (i.e. closer to the core). Age categories were weighted in a linear fashion as follows: core signals were multiplied by a factor of 1, the elemental concentrations in each successive age category were multiplied by the previous factor plus one. For example, the Beginning category was multiplied by a factor of 2, the "early" category was multiplied by a factor of 3, and so on until the End category, which was multiplied by a factor of 6. The weighting was done in recognition of the fact that fishes collected

from the same site are more likely to be similar in chemistry in the later portion of the otolith when these fishes are nearing their site of settlement. Third and last of all, cluster analyses were performed using all elements from only the End age category. This analysis was performed because of the expectation that fishes that settled to the same site likely had more similar chemistry in the portion of the larval period just before settlement.

Four separate cluster analyses were performed for each iteration, one for each census period, in recognition of the fact that otolith chemistry likely varied in a temporal way (Gillanders, 2002; Elsdon and Gillanders, 2005b; Chittaro, 2005) and thus to treat each census period separately. For these analyses, all individual fishes, identified by membership to one of the 7 sites, were input to see how these fishes clustered together. For each cluster analysis, I used Ward's method for amalgamation of groups based on a Euclidean distance matrix. The cluster analyses were used to group sites that were similar in terms of their otolith chemistry. In order to assess site-pair groupings, each cluster tree was analyzed separately and arbitrary groups were decided upon. Groups were defined by a tradeoff of two things with the goal of increasing interpretability; (1) few enough groups so that relationships between sites could be seen (i.e. not more groups than sites); and (2) enough groups such that one group did not contain most of the individual fishes in it. In all cases, cluster trees could be divided into 3 – 5 groups (e.g. Figure 3.2). To determine site membership to any given cluster group, individuals collected from a given site must be present in a cluster group in a greater proportion than that expected by random chance. For example, in census period 2002-5, there were 10 individuals analysed from site BC2. This represents 17% of the total number of fishes sampled

during this census period. Individuals from site BC2 were present in two of the cluster groups from census period 2002-5, 6 individuals clustered in group 1 and 4 individuals in group 4. However, the proportion of individuals from site BC2 in group 4 was less than that expected at random, that is, there were 32 fishes clustered in group 4, four of which were from site BC2, or 12.5% of all group 4 fishes. Since fishes from BC2 were in a lower proportion than that expected at random (i.e. 17%) the site BC2 was not considered a member of group 4. Site-pair relationships were ascertained in this manner for all census periods for each of the three iterations.

Each separate cluster analysis from each iteration yielded a Euclidean distance matrix. Each Euclidean distance is a measure of otolith chemical similarity between two individual fishes, the smaller the distance, the more similar two fishes are. I summarized the Euclidean distances between groups by averaging the individual Euclidean distances at the site-pair level. These site-pair measures of chemical similarity were then compared with site-pair measures of similarity in recruitment (i.e. recruitment coherence). I used regression analysis to test for a relationship between chemical similarity across the otolith transect and recruitment coherence (or synchrony). The expectation is that fishes that recruited synchronously at different sites show a similar pattern in cross-otolith chemistry, suggesting that these fishes experienced similar pelagic environments during their larval lives.

RESULTS

Of the original 11 isotopes analysed by LA-ICP-MS, six isotopes satisfied the two criteria for element selection mentioned above. These isotopes include ^{25}Mg, ^{43}Ca, ^{86}Sr, ^{137}Ba, ^{138}Ba and ^{208}Pb. Five of these isotopes (all except ^{43}Ca) were retained for multivariate analyses. Repeated measures multivariate analysis of variance showed there was a significant age effect, that is, that the five isotopes showed significant variation across the otolith transect from the core to the settlement mark averaged over all fish, sites and census periods (Table 3.3). When plotted univariately, each element shows different patterns of variation across the otolith transect (Figure 3.3). The isotope ^{25}Mg is present in relatively high concentrations in the core, there is an initial decrease in concentration after the core period and then this isotope tends to increase until the middle of the larval period, then decreases again closer to the time of settlement. Concentrations of ^{86}Sr are relatively low in the core and concentrations increase until the middle of the larval period and then decline approaching settlement. The two Ba isotopes show almost identical patterns of variation across the otolith transect. Both isotopes are present in high concentrations in the core, and decrease sharply thereafter until the middle of the larval period. In the later portion of the larval period, both isotopes increase in concentration until settlement. The isotope ^{208}Pb is also found in relatively high concentrations in the core and decreases gradually with increasing larval age up until the middle of the larval period and then increases slightly nearing settlement.

All other effects tested by the model were significant except the census period by site interaction term (Table 3.3). This result suggests that the overall average otolith

chemistry did not differ between sites within census periods (i.e. when not taking into account changes in chemistry across the otolith transects). However, it must be noted that the p-value is approaching the $\alpha = 0.05$ significance level (Table 3.3).

Finally, there was a significant age by census period by site interaction effect (Table 3.3). This effect tested the hypothesis that elemental trajectories across the otolith transect differed between sites within census periods, a significant result indicates that there were significantly different elemental trajectories between sites within census periods. In general, sites tended to be most different from each other in terms of otolith chemistry at the beginning ("core" and "beginning" signals) of the larval period and also near the end of the larval period (Figures 3.4 – 3.8). There was also quite a bit more within-site variation in the core and end signals (Figures 3.4 – 3.8). Most notably, concentrations of ^{25}Mg were highly variable in the core and beginning regions both between sites and within-sites (Figure 3.3 and Figure 3.4).

To explore site pair relationships, in terms of otolith element concentrations across otolith transects I investigated the outputs from the three iterations of the cluster analyses described in the methods, the results of which are summarized in Table 3.4. Individual fishes that were collected at the same site did not necessarily cluster together, within-site clustering was somewhat variable and in some cases fishes from a single site were represented in a number of cluster groups in the same cluster tree (Table 3.4). However, there were many cases where the individuals collected at the same site (in the same census period) clustered quite well together, for example, site TA4 from census period 2002-4 in iteration 1; 75% of individuals clustered in group 1. I assessed the level of within-site clustering by counting the number of times individuals from a given site

were clustered in a single cluster group. If a majority (50% or more) of individuals from a given site clustered in a single group, this was considered a high level of within-site clustering. I also analysed whether the three cluster iterations would concur with each other regarding within-site groupings. If a site did show good within-site clustering, then this would be evident from all three cluster iterations. I found that sites TA7, TA4, BC2 and BC5 had high levels of within site clustering, verified by all three iterations, for at least one census period (Table 3.5). Sites TA7, BC2 and BC5 showed consistent patterns of within-site clustering across census periods (Table 3.5). The remaining sites (BC6, TA2 and TA6) showed patterns of poor within-site clustering and is some cases this was consistent across census periods (e.g. TA2; Table 3.5).

The tendency of individual site-pairs to cluster together is summarized in Table 3.6. This table summarizes all site-pair groupings over all census periods and from all three iterations of clustering. What stands out from these analyses is that sites BC2 and BC6 tended to group frequently (67% of the time) with each other and the other Banco Chinchorro site, also they tended to group with one site on the windward side of Turneffe Atoll (TA2). Site BC5 also tended to group more with other BC sites than with TA sites. Site TA2 grouped often (67 – 83% of the time) with BC2 and BC6 in Mexico and with TA4 and TA6 in Belize. The remaining sites at Turneffe Atoll grouped with other sites within the atoll between 42 and 58% of the time. In general, there was a significant difference in the frequency of site-pair clustering between sites within the same location (atolls) versus site-pairs in different locations ($t = 2.07$; $df = 19$; $p = 0.05$).

We analysed the congruence in site-pair groupings between the three different iterations of cluster analyses by calculating the proportion of times within each census

period that the different methods agreed on site-pair groupings. The iteration using all unweighted age categories agreed with the site groupings of the iteration using weighted age categories 63% of the time (Table 3.7). This was the highest level of congruence among all four possible combinations of comparison (Table 3.7). All three iterations only agreed with each other 33% of the time.

Lastly I explored the relationship between site-pair otolith chemical similarity and site-pair coherence in recruitment. Recruitment coherence varied widely across the four census periods, from a value as low as 0.19 to a value as high as 0.98 (Figure 3.9). Regression analysis was performed separately for each iteration using only the Euclidean distances from the cluster analyses within each iteration. There was no significant relationship between otolith chemical similarity and recruitment coherence from the iteration using all age categories unweighted ($r^2 = 0.01$; $F_{(1,82)} = 0.80$; $p = 0.37$) nor for the iteration using the end age category only ($r^2 = 0.03$; $F_{(1,82)} = 1.20$; $p = 0.27$). There was a significant relationship between otolith chemical similarity and recruitment coherence from the iteration using all age categories weighted toward the end of the larval period ($r^2 = 0.09$; $F_{(1,82)} = 8.08$; $p = 0.005$). As Euclidean distance decreased, recruitment coherence tended to increase, suggesting that synchrony in the timing and magnitude of recruitment was related, to some extent, to similarity in otolith chemistry across the otolith transects.

DISCUSSION

We show here that there was significant variation in otolith elemental composition across otolith transects. I can identify two main patterns of variation in

otolith chemistry across otolith transects with the four elements examined. First, both ^{25}Mg and ^{86}Sr showed patterns of increasing concentrations until the middle of the larval period and decreasing concentrations thereafter until the end of the larval period (Figure 3.3). Second, both Ba isotopes and Pb tended to be in the highest concentrations early in larval life, dropping off rapidly with increasing age and then increasing again closer to the end of the larval period. The pattern of change in concentration of Pb was less drastic than the Ba isotopes, however, the general pattern is similar.

Concentrations of Mg, ^{137}Ba, ^{138}Ba and Pb were found to be relatively high in the core region of the otoliths and the core concentrations tended to be more variable than other regions of the otolith, especially for Mg (Figures 3.3 – 3.8). Elevated levels of trace elements in the cores of otoliths from a variety of species have recently been demonstrated for a number of elements including Mn, Ba, Mg, Zn, Sn, Ce and Pb (Brophy et al., 2004; Ruttenberg et al., 2005; Chittaro et al. 2006). The only element measured here that did not appear to occur in elevated concentrations in the otolith cores was ^{86}Sr, this too is consistent with previous findings (Ruttenberg et al., 2005; Chittaro et al. 2006).

Elemental concentrations in otoliths are widely believed to be records of the environment a fish has occupied over time (Campana, 1999). The concentrations of certain elements in the otoliths are known to be directly or indirectly related to the concentration of these elements in the water surrounding the fish (Campana, 1999; Milton and Chenery, 2001; Elsdon and Gillanders, 2003). These elements include, but are not limited to, Sr, Ba, Cu, Zn, Mn and Pb. Sampling elemental concentrations across

otolith transects will allow us to track the environmental history of fishes throughout the entire larval period.

The fact that Sr and Mg show similar patterns of variation across the otolith transect is not surprising. Both are conservative elements and major constituents of salinity thus their profiles reflect salinity (Chester 1990, Campana, 1999). The profiles of these two elements; low in the beginning, highest in the middle and low at the end of the larval life; point to the onshore-offshore-onshore movement of fish larvae. Generally speaking, near-shore waters tend to be less saline than open-ocean waters because of freshwater inputs like rivers, or run-off from shore (Chester, 1990). Here I see low concentrations of Mg and Sr in the early stages of the larval period before advection or dispersal and again at the end of the larval period when fishes are returning to the reef to settle.

The two barium isotopes tell a similar story. Barium has what is known as a nutrient-type profile, where it is generally depleted in surface waters, but enriches with increasing water depths. However, nutrient type elements can be brought back to the surface by oceanographic features such as upwellings when deep-water currents reach islands and shelves (Chester, 1990). It is known that Ba concentrations are relatively high in near-coast environments compared to open-ocean environments (Shen and Stanford, 1990). It has also been shown that barium concentrations in otoliths of reef fishes are highest in the portion of the otolith related to on-reef signatures (Patterson et al., 2005). Here I see that barium concentrations are highest early in the larval period and again late in the larval period, suggesting onshore-offshore-onshore movement of larvae. Lead concentrations also show a somewhat similar profile to the barium isotopes. Lead is

known to exist in the highest concentrations in surface waters, and is thought to be primarily input by anthropogenic sources; therefore, it is generally more abundant near shore. The profiles of lead in the otoliths examined here also support the general onshore-offshore-onshore movement of fish larvae.

This study demonstrates that fishes caught from several sites (during four census periods) differed in their otolith chemistry across the larval period, suggesting that fishes from different sites experienced different environments during the larval period (Table 3.3). First of all, cluster analyses revealed that fishes collected at the same site might have experienced variable environments during the larval period. Only in rare cases were all the fish from a single site clustered together in a single cluster group. In almost all cases across all the census periods at least some fishes, collected from the same site, had different cross-otolith chemical signatures. However, fishes from some sites did tend to cluster well together in certain census periods, that is, a majority (50% or more) of the fishes from one site clustered in the same group. Also, some sites clustered well consistently through time (e.g. BC2, BC5, TA4 and TA7; Table 3.5), while fishes from other sites did not cluster together consistently through time (e.g. BC6, TA2 and TA6; Table 3.5). These results suggest that the majority of the larvae that recruited to some of the study sites experienced the same environment during the larval period, while other sites received recruits that experienced various larval environments. Furthermore, some sites (Like TA7) consistently received a majority of larvae that experienced a similar larval environment. This hints at the possibility that a site is seeded by larvae coming from the same source population(s) consistently through time.

Also, certain site-pairs tended to cluster together in certain census periods (Table 3.4) and some site-pairs clustered consistently across census periods (Table 3.6). Site-pairs that were within the same location tended to cluster more frequently together than site-pairs between locations. For example, Banco Chinchorro sites clustered together a moderately high proportion of the time and Turneffe Atoll sites clustered together a moderate proportion of the time (Table 3.6). This suggests that larvae recruiting to the two atolls tended to experience more different environments during the larval period. Previous studies have demonstrated that fishes from particular populations experienced different environments during their larval lives. Thorrold et al. (1997) showed that Atlantic croaker larvae from two river populations occupied different coastal environments in their early life-history. Using chemical signals averaged over the otolith, Swearer et al. (1999) determined that certain fishes recruiting to St. Croix had experienced different larval environments, some showing evidence of near-shore retention (high element concentrations), others showing signatures consistent with dispersal through offshore waters. Sandin et al. (2005) found similar results to Swearer et al. (1999) using LA-ICP-MS to sample chemistry across otolith transects. Finally, Patterson et al. (2005) showed significant variation in across-otolith elemental signatures and they concluded that fishes at a particular reef had originated from multiple source populations and dispersed through different water masses during their larval lives.

In order to investigate whether site-pairs received larvae that experienced similar environments during the larval period (i.e. they spent a portion of their larval lives in a similar water mass) I compared site-pair similarity in otolith chemistry with site-pair similarity in recruitment (coherence). Coherence in recruitment is a measure of similarity

in the magnitude and timing of recruitment. The spatial scale at which recruitment coherence occurs can be interpreted to be an estimate of larval patch size (Victor, 1984; Chapter 2 of this volume). That means that two sites that are coherent in recruitment are possibly receiving larvae that dispersed in the same patch during some part of their larval lives. If this were the case, it might be expect that fishes recruiting to coherent sites had similar otolith chemical trajectories through the larval period, if so there may be a relationship between recruitment coherence and otolith chemistry through the larval period.

We took the Euclidean distances from the three iterations of cluster analyses as a measure of site-pair similarity in otolith chemistry. The three iterations were; (1) including all developmental periods; (2) including all developmental periods given greater weighting to the later portion of larval life and; (3) including only the "end" developmental period. Of these three iterations only the Euclidean distances from the weighted iteration showed any significant relationship with recruitment coherence (Figure 3.9). These results suggest that chemical similarity in the latter portion of the larval life is important for explaining some variation in coherence but a single signal from the end of the larval life is not sufficient on its own to explain any variation in coherence. More importantly, this suggests that fishes that recruited to different sites at the same time experienced more similar environments throughout the larval period. This suggests that these fish perhaps traveled together in a coherent patch through a similar water mass throughout a portion of their larval lives at least greater than the very last portion of the larval period.

In conclusion, this study has demonstrated that otolith elemental chemistry can elucidate events occurring in the larval life-stage of reef fishes. In general, fishes tended to show an onshore-offshore-onshore movement indicative of advection or dispersal away from reefs early in the larval period and then returning to a reef to settle. I have shown that larvae settling to reef populations may come from multiple sources, however, consistency in the otolith chemistry at some sites suggests a single source of larvae. Finally I have shown that site-pairs do receive larvae that dispersed in similar water masses during the larval period, future studies should focus on determining whether larvae recruiting in pulses to sites coherent in recruitment are in fact traveling in coherent patches and attempting to trace the origination of these patches to test whether larvae are originating from the same source populations.

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Table 3.1: Description of the sampling design subdivided by country, region and sites within regions. Sample sizes per sampling date are provided for each site. Sampling periods correspond to the following start dates: 2002-4: 9 July 2002; 2002-5: 22 July 2002; 2003-3: 30 June 2003; 2003-5: 30 July 2003.

Country	Region	Site	Latitude (decimal degrees)	Longitude (decimal degrees)	Sampling periods	# of fish collected
Belize	Turneffe Atoll (TA)	TA2	18.92161	-87.61786	2002-4, 2002-5, 2003-3. 2003-5	11, 9, 11, 13
		TA4	17.27357	-87.81123	2002-4, 2002-5, 2003-3. 2003-5	4, 3, 12, 10
		TA6	17.34370	-87.95487	2002-4, 2002-5, 2003-3. 2003-5	12, 6, 11, 9
		TA7	18.83585	-87.64640	2002-4, 2002-5, 2003-3. 2003-5	11, 11, 9, 9
Mexico	Banco Chinchorro (BC)	BC2	18.65757	-87.22820	2002-4, 2002-5, 2003-3. 2003-5	2, 10, 8, 6
		BC5	17.27356	-87.81123	2002-4, 2002-5, 2003-3. 2003-5	11, 9, 12, 4
		BC6	18.65133	-87.40492	2002-4, 2002-5, 2003-3. 2003-5	11, 10, 13, 13

Table 3.2: List of all elements sampled by LA-ICP-MS including: the average concentration of each element over all samples; the average concentration of each element from NIST 610 standards; the average detection limit of each element over all samples and; the percent of samples above detection limits for each element.

Element (isotope)	Average CV of NIST 610	% of samples > detection limit	Average detection limit by sample in ppm (±SD)	Overall average conc. in ppm (±SD)
Mg (25)	2.28	99.8	4.78 (1.19)	20.95 (21.98)
Ca (43)	1.39	100	363.89 (167.03)	407000 (19000)
Ca (44)	N/A	N/A	N/A	N/A
Mn (55)	2.24	78.5	0.35 (0.29)	0.93 (0.76)
Zn (66)	5.00	58.7	0.43 (0.43)	1.30 (4.09)
Sr (86)	2.46	100	4.75 (1.57)	2600 (250)
Sn (120)	3.77	38.9	0.37 (0.07)	0.65 (0.26)
Ba (137)	4.61	99.9	0.68 (0.34)	3.43 (2.46)
Ba (138)	2.36	100	0.05 (0.02)	3.42 (2.43)
Ce (140)	3.73	67.2	0.02 (0.01)	0.11 (0.36)
Pb (208)	5.38	98.3	0.02 (0.01)	0.11 (0.18)

Table 3.3: Results of the RM-MANOVA of the trajectories of five isotopes representing 4 elements across otolith transects of larval *Stegastes partitus* collected at 7 sites within the Mesoamerican Barrier Reef System.

Effect	df	Wilks' Lambda	p-value
Age	15	0.17	< 0.0001
Census period	30	0.82	< 0.0001
Site	90	0.75	0.0002
Census period x Site	25	0.63	0.0750
Age x Census period	75	0.50	< 0.0001
Age x Site	150	0.41	0.0056
Age x Census period x Site	450	0.10	0.0036

Table 3.4: Summary of the results of the three iterations of cluster analyses. Numbers represent percentages of fishes from a single site (columns) present in a given cluster group (rows). The table summarizes the results of four cluster analyses (corresponding to each sampling period) for each iteration. Asterisks indicate the significant contribution of a given site to a particular cluster group based on the presence of fishes from that site in a cluster group at a greater proportion than that expected by random chance (see methods for details).

Sampling Period Iteration 1	Group	Group N	BC2	BC5	BC6	TA2	TA4	TA6	TA7
2002-4	1	11	50*	0	0	27*	75*	25*	9
	2	17	0	64*	27	27	25	25	0
	3	22	50*	18	45*	36	0	17	73*
	4	12	0	18	27*	9	0	33*	18
2002-5	1	13	50*	22	30*	11	33*	17	0
	2	18	10	11	20	56*	33	67*	36*
	3	15	40*	33*	40*	22	0	17	9
	4	12	0	33*	10	11	33*	0	55*
2003-3	1	24	63*	50*	8	18	42*	27	22
	2	13	25*	17	46*	18*	8	0	0
	3	23	12	17	15	55*	33*	27	56*
	4	16	0	16	31*	9	17	45*	22
2003-5	1	16	17	25	46*	46*	0	0	22
	2	14	0	25*	31*	15	10	22	44*
	3	19	33*	50*	15	23	40*	44*	22
	4	11	33*	0	8	15	40*	11	11
	5	4	17*	0	0	0	0	22*	0
Iteration 2	Group	Group N	BC2	BC5	BC6	TA2	TA4	TA6	TA7
2002-4	1	20	100*	18	27	36*	0	25	55*
	2	22	0	55*	36*	27	25	42*	27
	3	7	0	9	0	18*	25*	17	9
	4	13	0	18	36*	18	50*	17	9
2002-5	1	15	60*	0	10	44*	33	33*	9
	2	13	30*	33*	50*	0	33*	17	0
	3	19	10	33	10	44*	33	17	64*
	4	11	0	33*	30*	11	0	33*	18
2003-3	1	24	25	33	31	18	33	55*	22
	2	42	38	50	54	64*	67*	45	67*
	3	10	38*	17*	15*	18*	0	0	11
2003-5	1	21	33	25	23	31	70*	22	22
	2	11	17	0	15	23*	10	44*	0
	3	13	33*	75*	23*	23*	0	11	11
	4	19	17	0	38*	23	20	22	67*

Iteration 3	Group	Group N	BC2	BC5	BC6	TA2	TA4	TA6	TA7
2002-4	1	19	50*	45*	55*	18	50*	25	9
	2	24	50*	27	18	45*	0	42*	73*
	3	19	0	27	36*	36*	50*	33*	18
2002-5	1	14	60*	11	20	33*	0	17	9
	2	9	0	0	20*	22*	33*	33*	18*
	3	3	0	33*	0	0	0	0	0
	4	32	40	56	60*	44	66*	50	73*
2003-3	1	16	25*	75*	15	27*	0	0	0
	2	23	13	0	23	18	50*	54*	56*
	3	25	13	8	46*	36*	42*	45*	33
	4	12	50*	17	15	18*	8	0	11
2003-5	1	23	50*	0	46*	38*	60*	11	22
	2	16	17	0	15	23	30*	33*	44*
	3	17	17	25	23	38*	10	44*	22
	4	8	17	75*	15*	0	0	11	11

Table 3.5: Summary of the within-site clustering for each site at each sampling period. Table values correspond to the number of cluster iterations that agreed upon high within-site clustering. High within-site clustering was defined as a majority of individuals (50% or more) from a single site clustered in the same group. Values of 3 indicate that all three cluster iterations agreed on high within-site clustering.

Sampling Period	BC2	BC5	BC6	TA2	TA4	TA6	TA7
2002-4	3	2	1	0	3	0	3
2002-5	3	1	2	1	1	2	3
2003-3	2	3	1	2	2	2	3
2003-5	1	3	0	0	2	0	1

Table 3.6: Summary of site-pair clustering pooled over all sampling periods and all three cluster iterations. Proportions are the proportion of times a given site-pair was clustered in the same cluster group.

Site Pair	# of CPs together	# of CPs in total	Proportion
BC2-BC5	8	12	0.67
BC2-BC6	8	12	0.67
BC2-TA2	10	12	0.83
BC2-TA4	7	12	0.58
BC2-TA6	4	12	0.33
BC2-TA7	3	12	0.25
BC5-BC6	8	12	0.67
BC5-TA2	3	12	0.25
BC5-TA4	5	12	0.42
BC5-TA6	3	12	0.25
BC5-TA7	2	12	0.17
BC6-TA2	8	12	0.67
BC6-TA4	7	12	0.58
BC6-TA6	7	12	0.58
BC6-TA7	4	12	0.33
TA2-TA4	8	12	0.67
TA2-TA6	8	12	0.67
TA2-TA7	7	12	0.58
TA4-TA6	6	12	0.50
TA4-TA7	6	12	0.50
TA6-TA7	5	12	0.42

Table 3.7: Summary of the congruence between the three cluster iterations in terms of site-pair groupings broken down by sampling period and averaged across all sampling periods.

Iterations	2002-4	2002-5	2003-3	2003-5	Average
Unweighted x Weighted	0.57	0.67	0.76	0.52	0.63
Unweighted x End only	0.62	0.33	0.57	0.38	0.48
Weighted x End only	0.62	0.43	0.62	0.62	0.57
Unweighted x Weighted x End only	0.43	0.14	0.48	0.29	0.33

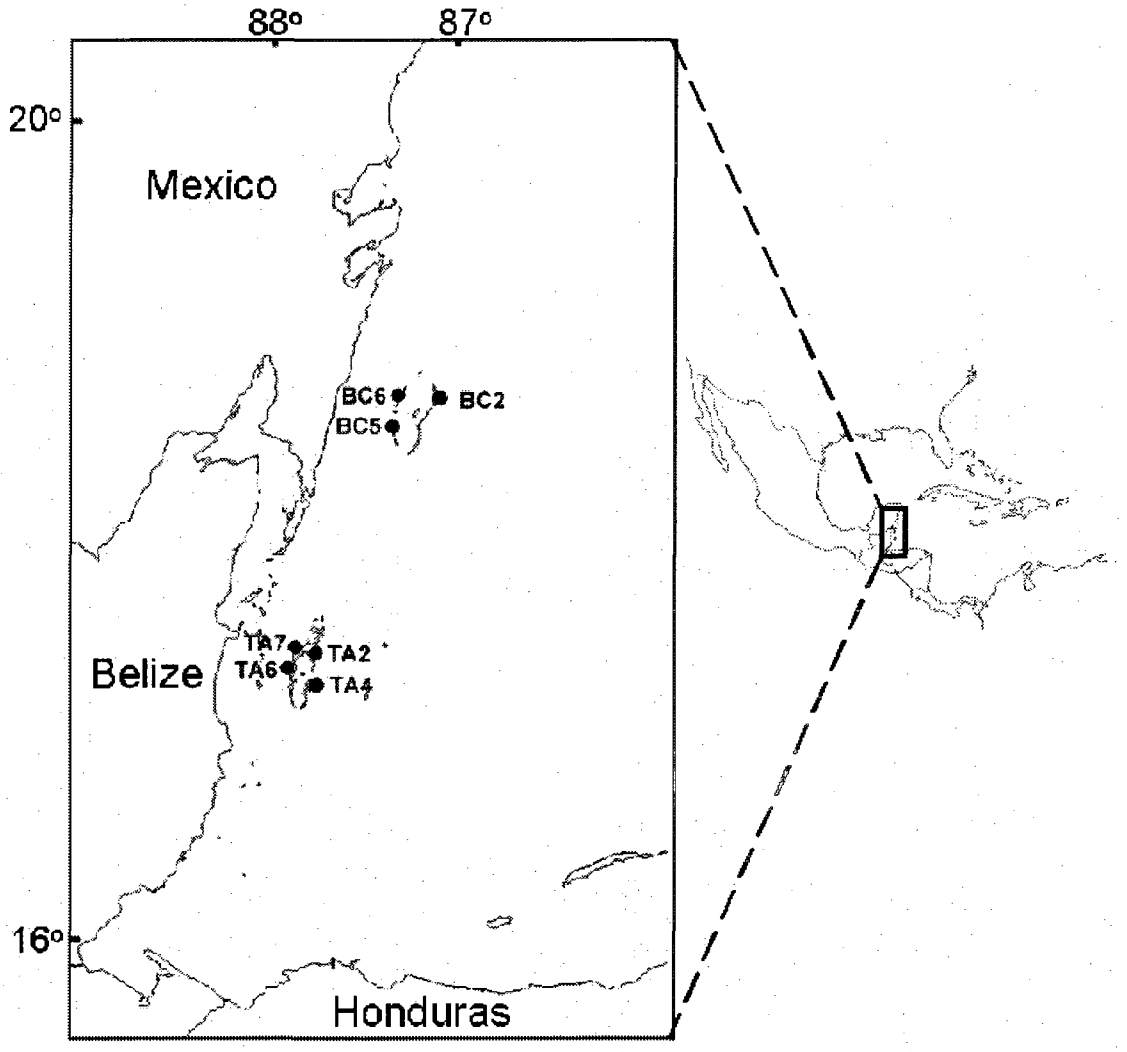


Figure 3.1: Map of the Mesoamerican Barrier Reef System including the locations of 7 study sites. Inset is a map of the Western Caribbean for reference and scale.

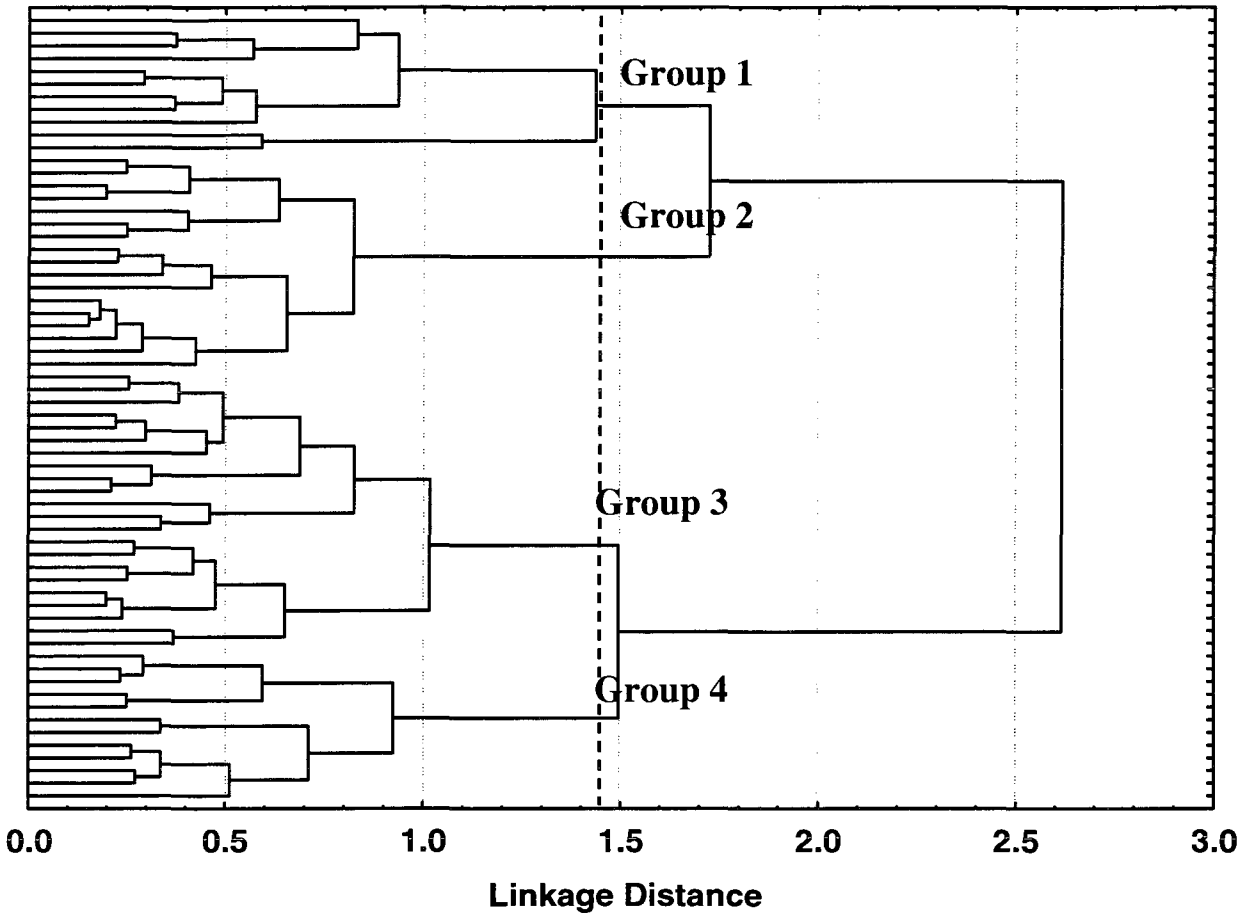


Figure 3.2: An example cluster tree from the first cluster analysis iteration including otolith chemical data from all developmental periods, unweighted. This example represents the clustering of fishes collected during sampling period 2002-4. The dashed line indicates the arbitrary group cutoff point limiting the number of groups to four in this example.

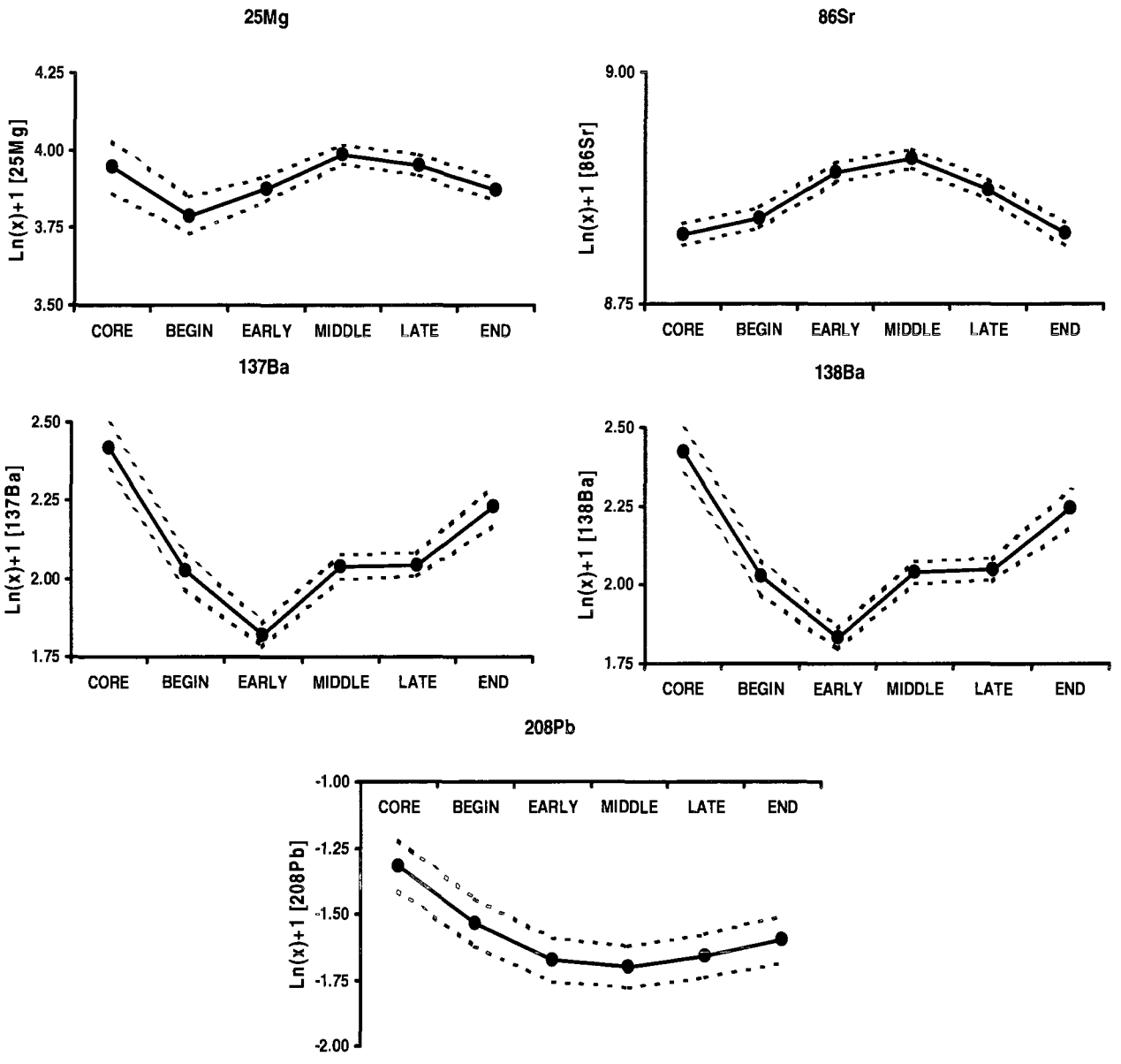


Figure 3.3: Average Ln (x)+1 concentrations of five isotopes across the six developmental periods. Concentrations are averaged over all fish from all sites and all sampling periods. The dashed lines indicate the 95% confidence limits around the mean concentrations for each developmental period.

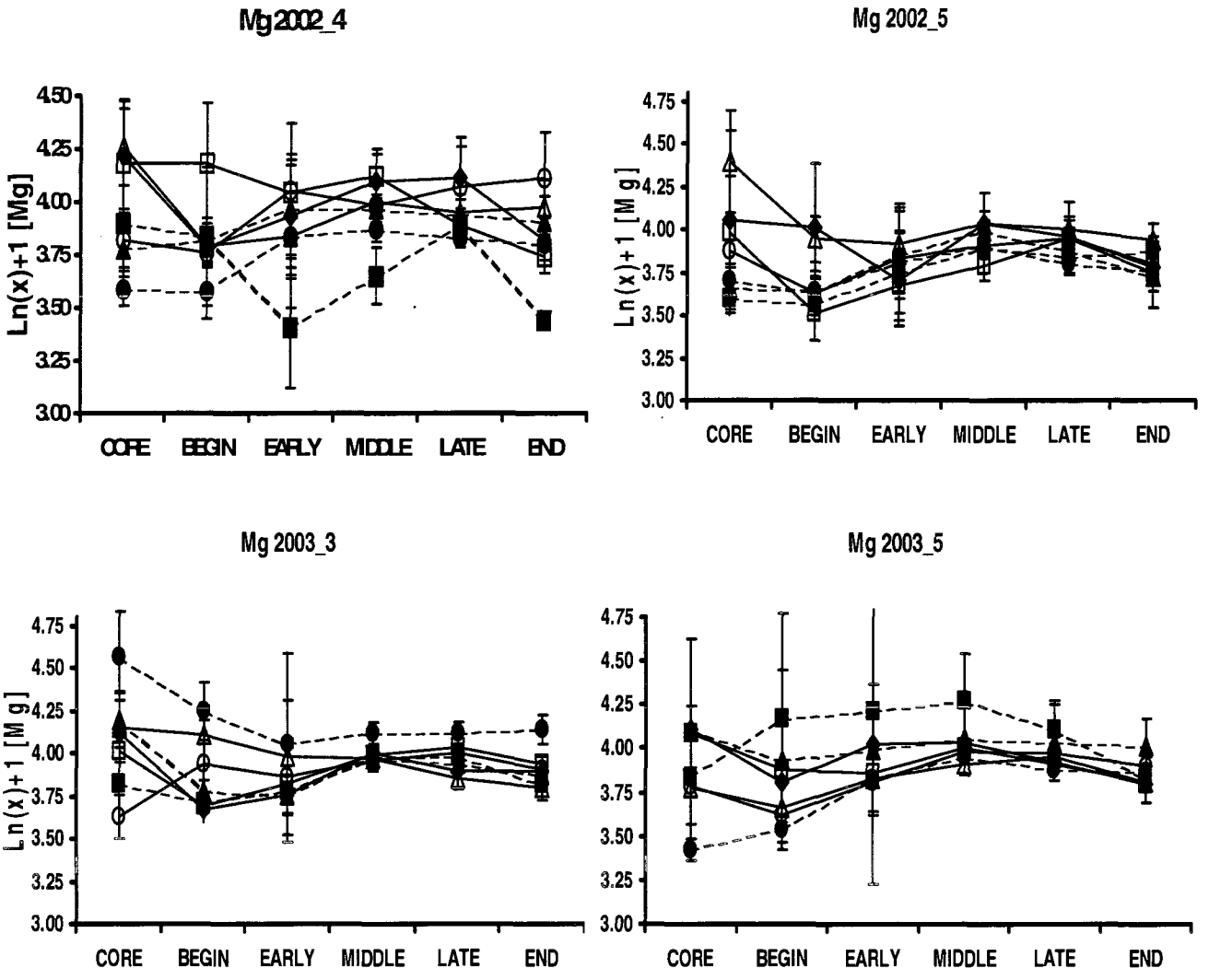


Figure 3.4: Average Ln(x)+1 concentrations of ^{25}Mg in the otoliths of fishes from the seven study sites across developmental periods. Error bars indicate standard errors. Solid lines indicate sites from Turneffe Atoll and dashed lines indicate sites from Banco Chinchorro. Shaded squares are site BC2; shaded triangles are BC5; shaded circles are BC6; open triangles are TA2; open squares are TA4; shaded diamonds are TA6 and; open circles are TA7.

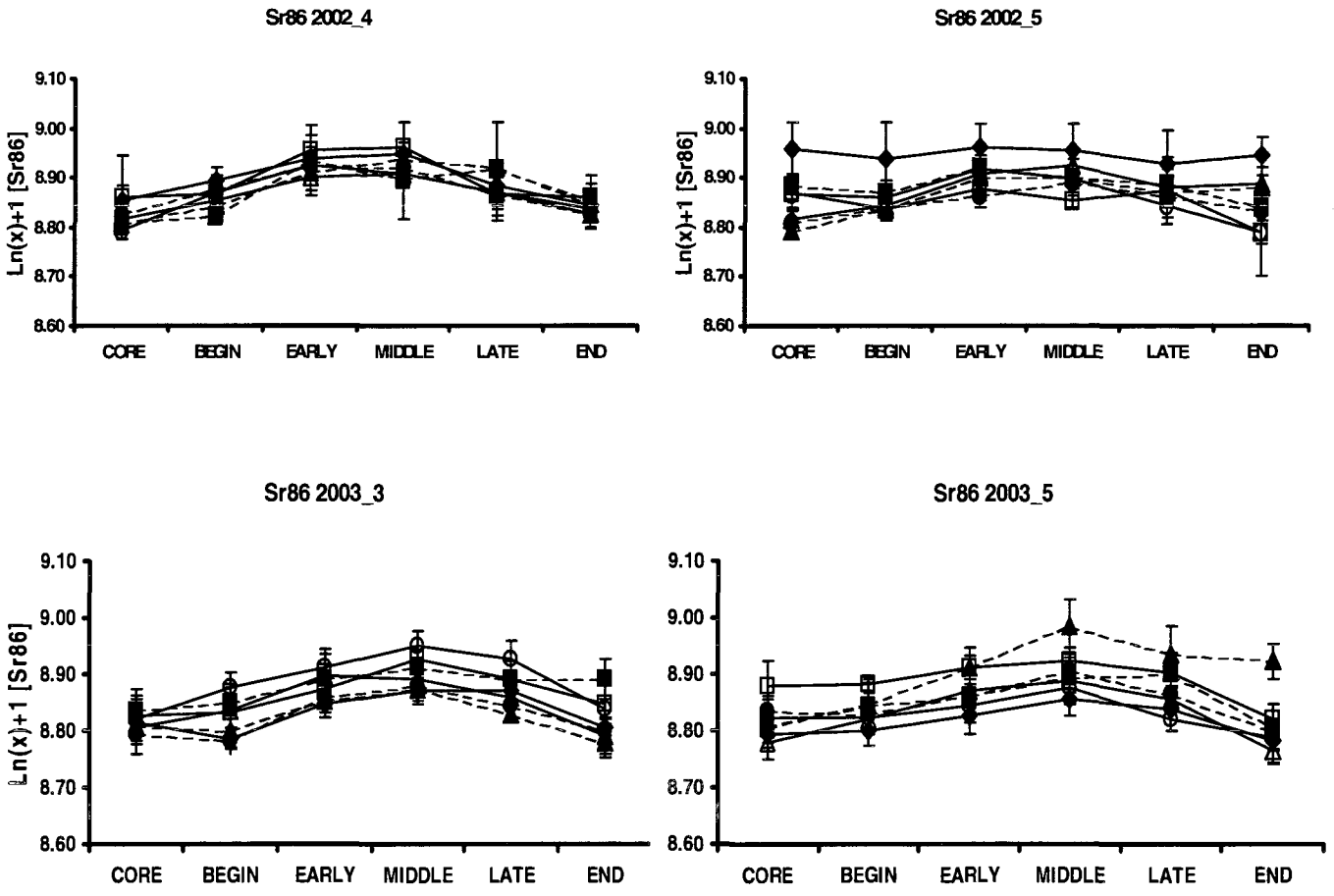


Figure 3.5: Average Ln(x)+1 concentrations of ^{86}Sr in the otoliths of fishes from the seven study sites across developmental periods. Error bars indicate standard errors. Solid lines indicate sites from Turneffe Atoll and dashed lines indicate sites from Banco Chinchorro. Shaded squares are site BC2; shaded triangles are BC5; shaded circles are BC6; open triangles are TA2; open squares are TA4; shaded diamonds are TA6 and; open circles are TA7.

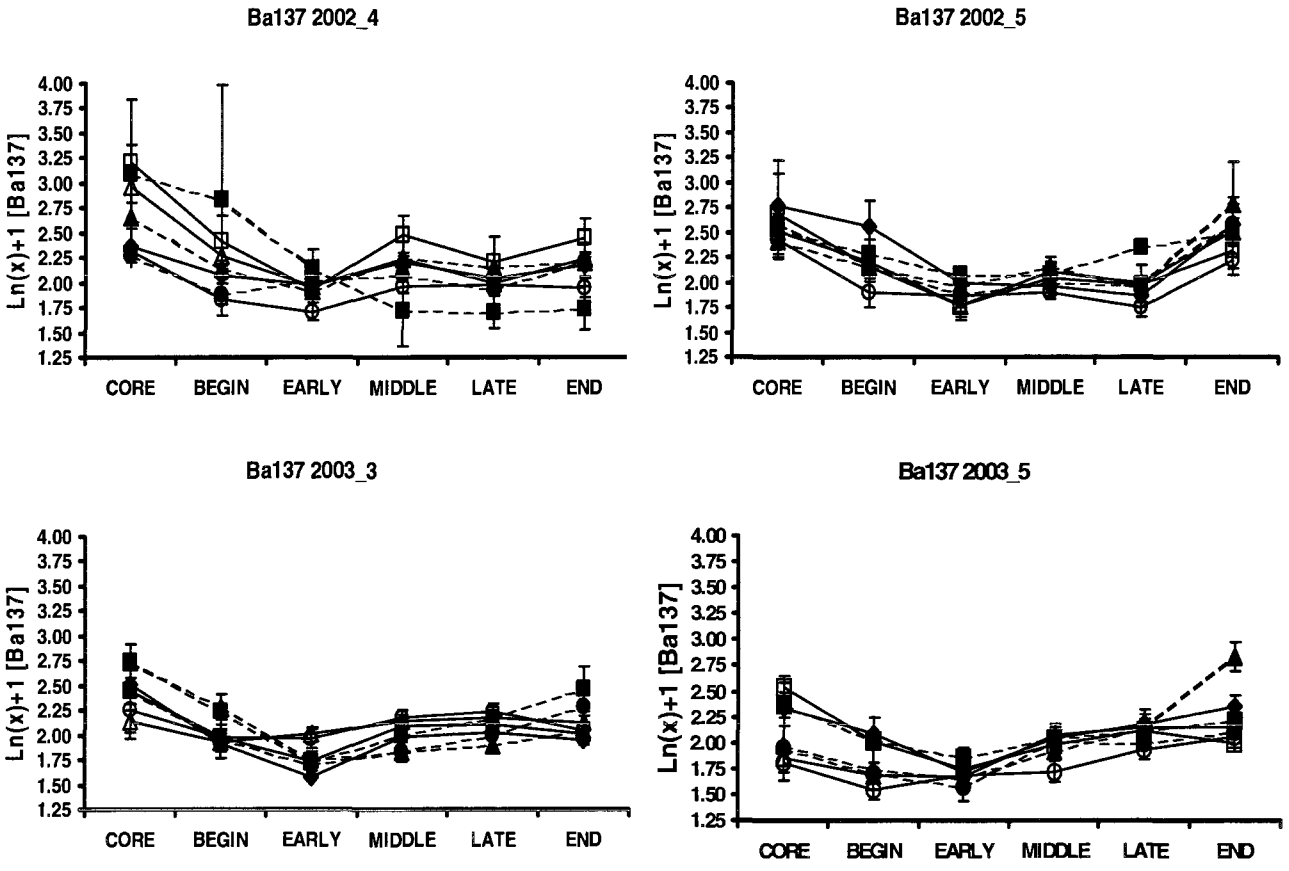


Figure 3.6: Average Ln(x)+1 concentrations of ^{137}Ba in the otoliths of fishes from the seven study sites across developmental periods. Error bars indicate standard errors. Solid lines indicate sites from Turneffe Atoll and dashed lines indicate sites from Banco Chinchorro. Shaded squares are site BC2; shaded triangles are BC5; shaded circles are BC6; open triangles are TA2; open squares are TA4; shaded diamonds are TA6 and; open circles are TA7.

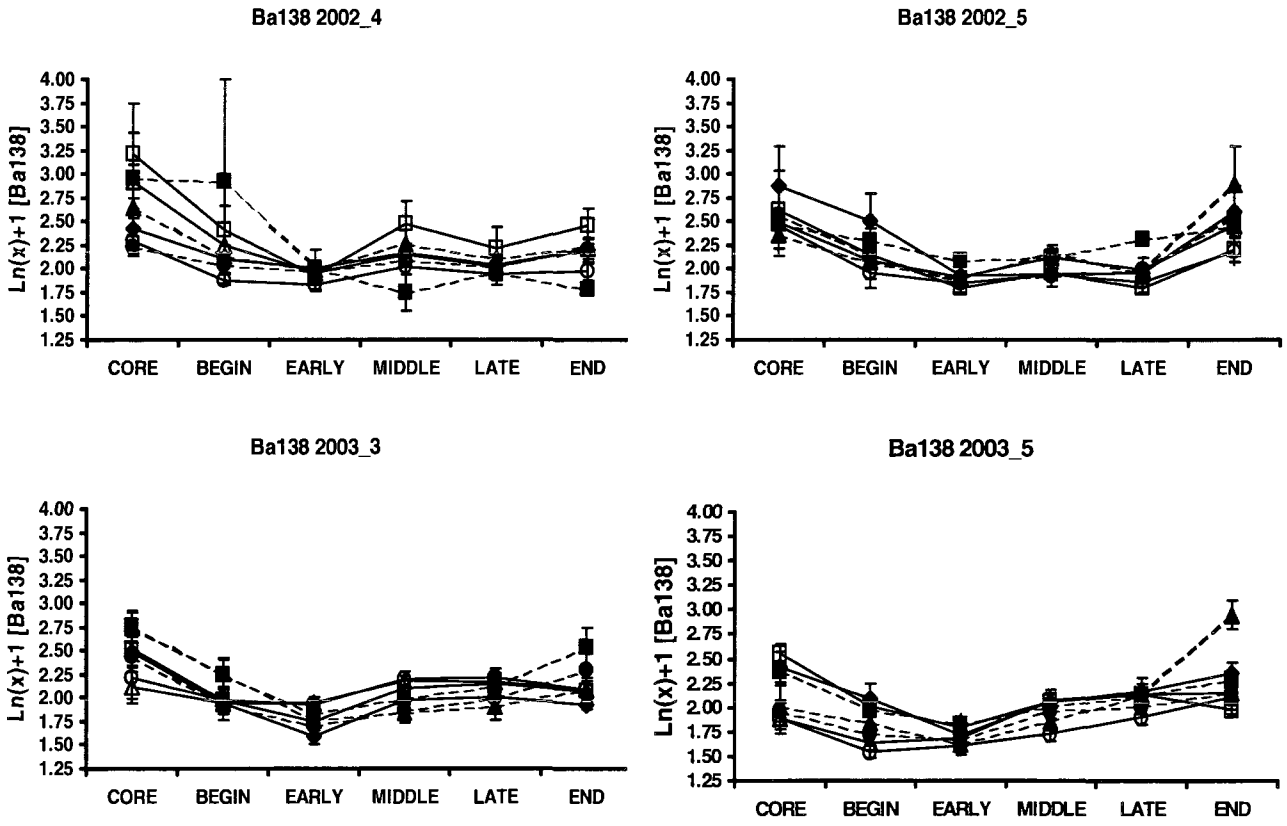


Figure 3.7: Average Ln(x)+1 concentrations of ^{138}Ba in the otoliths of fishes from the seven study sites across developmental periods. Error bars indicate standard errors. Solid lines indicate sites from Turneffe Atoll and dashed lines indicate sites from Banco Chinchorro. Shaded squares are site BC2; shaded triangles are BC5; shaded circles are BC6; open triangles are TA2; open squares are TA4; shaded diamonds are TA6 and; open circles are TA7.

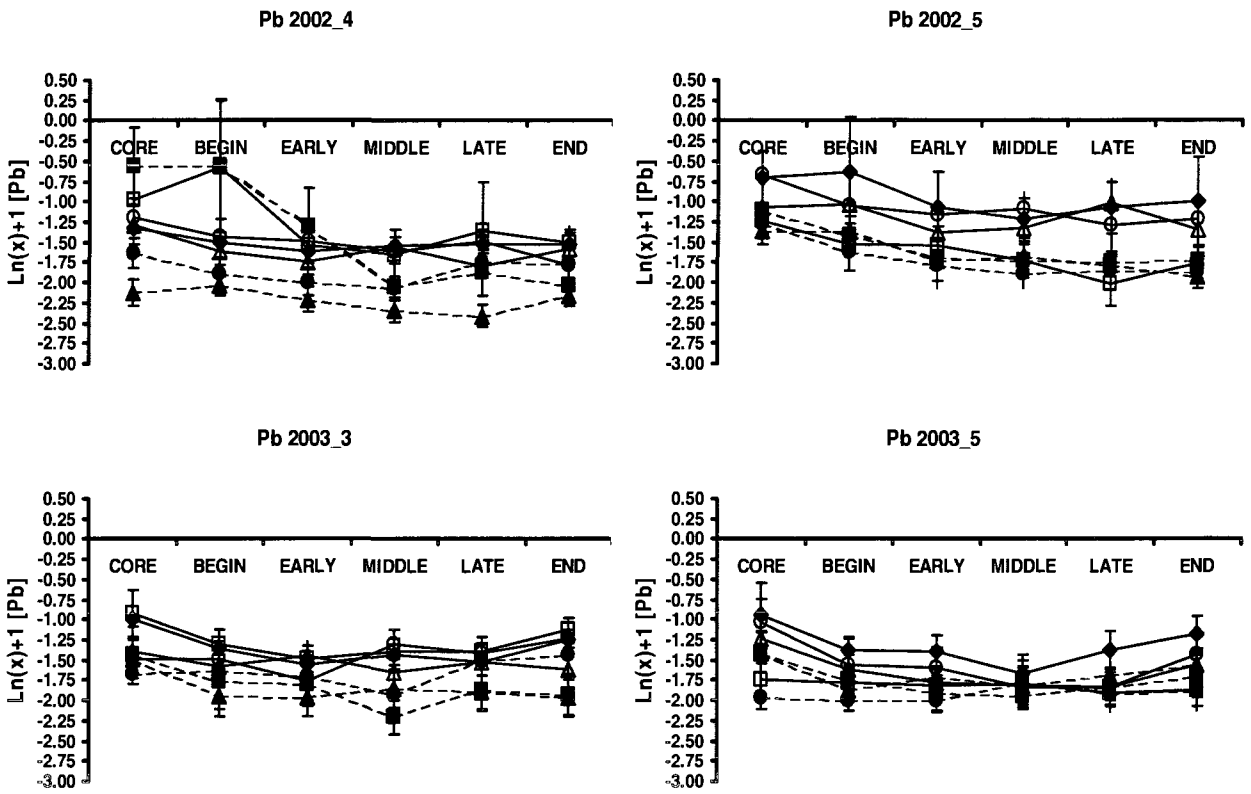
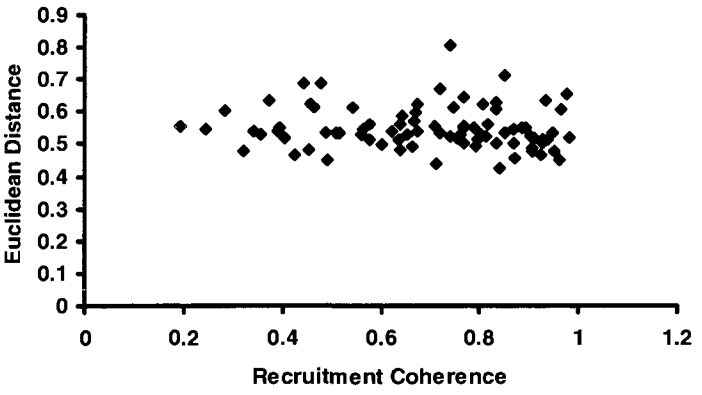
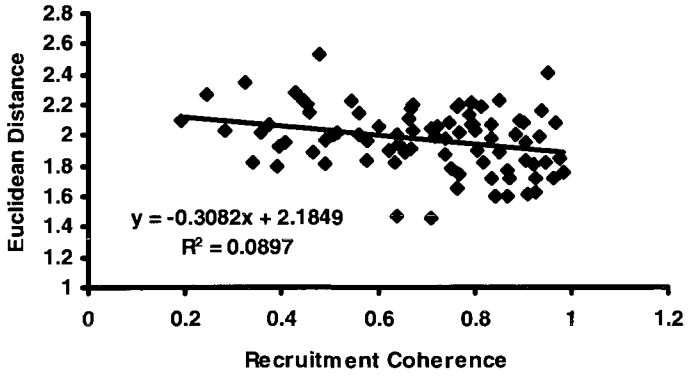


Figure 3.8: Average Ln(x)+1 concentrations of ^{208}Pb in the otoliths of fishes from the seven study sites across developmental periods. Error bars indicate standard errors. Solid lines indicate sites from Turneffe Atoll and dashed lines indicate sites from Banco Chinchorro. Shaded squares are site BC2; shaded triangles are BC5; shaded circles are BC6; open triangles are TA2; open squares are TA4; shaded diamonds are TA6 and; open circles are TA7.

a)



b)



c)

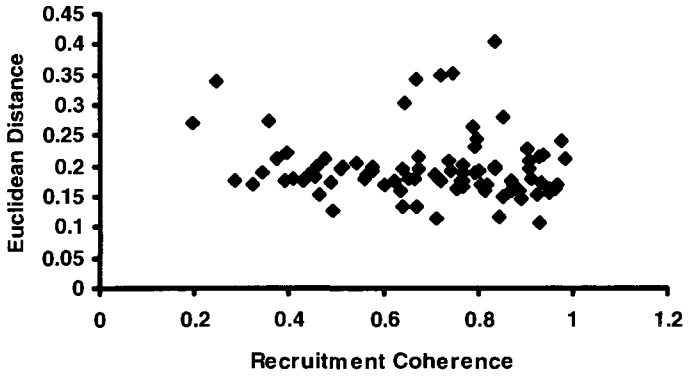


Figure 3.9: The relationship between recruitment coherence and Euclidean distance. Euclidean distances were obtained from the cluster analyses using each of the three cluster iterations; a) is the Euclidean distances from the iteration using all age categories unweighted ($r^2 = 0.01$; n.s.); b) is the Euclidean distances from the iteration using all age categories weighted late and; c) is the Euclidean distances from the iteration using only the "end" age category ($r^2 = 0.01$; n.s.). Note the difference in the scales of the y-axes.

Chapter 4: Spatial variation in otolith microchemistry across the Mesoamerican Barrier Reef System and investigating the spatial scale of dispersal of the reef fish *Stegastes partitus*.

INTRODUCTION

Almost all reef fishes have a pelagic larval life-stage and individuals will spend as few as nine days up to as many as 100 days or more in the pelagic environment away from the reefs that sustain the adult life-stages of these species (Leis, 1991). By comparison to the adult and juvenile life-stages of reef fishes, larval stages are potentially much more dispersive, being subjected to ocean currents whilst in the pelagic environment and having generally less developed sensory and swimming capabilities. This, coupled with a high level of mortality in the larval stage (Leis, 1991) highlights the importance of this life-stage in determining the distribution and abundance of most reef fishes.

Despite the importance of larvae to the ecology of reef fishes, the ecology of the larval-stage itself is still relatively poorly understood because of the difficulty of tracking and observing very small fishes in the oceans waters (Leis and McCormick, 2002; Mora and Sale, 2002). Advances in the science in the last few years have increased our understanding of the ecology of reef fish larvae, with a focus on the dispersal of larvae and connectivity among benthic adult populations (Stobutzki and Bellwood, 1997; Roberts, 1997; Jones et al., 1999; Swearer et al., 1999; Cowen et al., 2000; Armsworth et al., 2001; Mora and Sale, 2002; Fisher and Bellwood, 2003). The focus of research has been on dispersal of larvae because "larval exchange among populations (through dispersal) is vital to the study of marine population dynamics, management of fisheries stocks, and the design of marine reserves" (Cowen et al., 2000).

Researchers have come up with many different ways to estimate and/or track dispersal of larvae using both artificial and natural tags (see review by Thorrold et al.,

2002) including artificially tagged otoliths (Jones et al., 1999; Jones et al., 2005), natural genetic markers (e.g. Taylor and Hellberg, 2003) and natural otolith microchemical markers (e.g. Swearer et al, 1999). Recently the focus has been on developing techniques to analyse natural tags for studying dispersal because artificial tagging and recapturing of artificially tagged individuals is costly and time consuming (Thorrold et al., 2002). This paper focuses on the use of natural otolith microchemical markers, and associated techniques, to investigate the movements of larval fishes.

The use of microelemental signals in otoliths as natural tags for investigating movement of individuals relies on the deposition of trace elements into the otoliths from environmental sources. Otoliths are composed 97% of calcium carbonate with less than 1% being other inorganic trace impurities (Campana, 1999). These other trace elements are incorporated into the otolith matrix via uptake into the blood, passed onto the endolymph (inner ear fluid) and from there are included into the otolith matrix. It has been demonstrated that many elements that are incorporated into the otoliths are originally absorbed through the gills from the water bathing the fish (Farrell and Campana, 1996; Milton and Chenery, 2001; Elsdon and Gillanders, 2003) and it has been suggested that diet contributes little to otolith chemistry at concentrations found in the wild (Hoff and Fuiman, 1995; Milton and Chenery, 2001). Furthermore, otoliths are not metabolically active, that is, they are not subject to resorption meaning they are a stable record of the chemical environments experienced by the fish (Campana, 1999).

If concentrations of trace elements vary spatially in the waters, it is possible that individual fishes in different populations at different sites will be tagged naturally by bathing in these waters. This premise has been the basis for many studies tracking the

movement of fishes. Otolith microchemical analysis has been used successfully to track movements of individuals of fish species from ocean habitats to estuarine or bay nursery habitats and vice versa (Thorrold et al., 1997; Thorrold et al., 1998; Forrester and Swearer, 2002; Gillanders, 2002a; Gillanders, 2002b); to track the movements of large oceanic fishes roving over large areas (Arai et al., 2005); to track movements of coral reef fishes between reef and mangrove habitats (Chittaro et al., 2004); and to track dispersal movements of reef fish larvae (Swearer et al., 1999; Patterson et al., 2005; Sandin et al., 2005; Chittaro 2005).

One technique that is commonly used to track larval fish dispersal is by comparing chemical signatures from the core of the otoliths of the dispersed fishes to some reference population chemical signals, usually from the edges of otoliths from fishes of these reference populations. If a young fish's core chemistry matched with a reference signal then it was assumed that young fish was spawned at that reference population. Recent studies have shown that the chemistry of the otolith core does not reflect the environmental chemistry of the site the fish was spawned at, rather it appears to reflect either physiological factors or otolith structural differences, associated with embryonic development with the yolk-sac, that primarily determine the chemistry of the otolith at or around the core (Brophy et al., 2004; Ruttenberg et al., 2005; Chittaro et al., 2006).

In this study I attempt to back-track pelagic larval dispersal movements of juveniles of the coral reef fish *Stegastes partitus* among sites in the Mesoamerican barrier reef system (MBRS) in the western Caribbean. I will first determine if there is sufficient spatial variation in the concentration of trace elements in the otolith edges of juvenile

fishes from reference populations. If sufficient spatial variation exists for some elements I will attempt to classify recently dispersed individuals to these reference populations within the MBRS by using a near-natal otolith chemical signature collected from the portion of the otolith just after the embryonic yolk-sac was absorbed, to avoid the confounding chemistry associated with embryonic development.

MATERIALS AND METHODS

STUDY SPECIES

The species used for this study was the bicolor damselfish, *Stegastes partitus* (Poey, 1868) (Class Actinopterygii, Order Perciformes, Family Pomacentridae). *Stegastes partitus* is a relatively small species of reef fish (maximum total length of 10 cm), common to coral reefs of the Caribbean (Humann, 1989). Adults of this species defend small, permanent feeding territories vigorously (Myrberg, 1972). Females will spawn a clutch of approximately 2000 eggs on demersal substrata, (i.e. dead pieces of coral) within the males' territory (Cole and Sadovy, 1995). Males will defend the eggs until they hatch as larvae approximately 3.5 days after spawning (Cole and Sadovy, 1995). Larvae of *S. partitus* are nektonic (Leis, 2006; Chapters 5 and 6 of this volume) and undergo a pelagic life-stage that lasts between 24 and 40 days (Wellington and Victor, 1989; Wilson and Meekan, 2002; Hogan, unpubl. data), after which time they settle to a reef, where they stake out a feeding territory indefinitely (Myrberg, 1972).

S. partitus was chosen as a suitable organism for this study because they are relatively abundant and have a broad distribution in the Caribbean. The site-attached

nature of the species makes them relatively easy to collect on SCUBA (for otolith samples). The fact that individuals show site fidelity is beneficial for otolith microchemical analysis because one can be sure that the chemical make up of a fish's otolith is a record of the elemental history of the place where the fish was captured, assuming that otolith chemistry is related to, or influenced by, chemistry of the environment surrounding the fish (Farrell and Campana, 1996; Milton and Chenery, 2001; Elsdon and Gillanders, 2003).

FISH COLLECTION AND OTOLITH PREPARATION

Newly recruited juveniles of *S. partitus* (defined as any individual ≤ 2.0 cm) were collected from sites within the Mesoamerican Barrier Reef System (MBRS) during two distinct sampling periods in 2003. The first sampling period (SP1) was conducted from 16 – 21 July, and the second sampling period (SP2) was conducted approximately one month later from 12 – 25 August. The sampling for this study was done as part of a larger project, called ECONAR, that was ongoing in the MBRS, the goal of which was to census recruitment and make collections of individuals of a variety of species for analyses of connectivity among reefs in this region. Sites at Turneffe Atoll, Belize (TA) and Banco Chinchorro, Mexico (BC) were sampled bi-weekly during the summer of 2003. Included in the bi-weekly sampling regime were six sites from which individuals were collected during SP1 and the same six sites sampled in SP2 (see Table 4.1 for details of sites and sampling periods). During SP2 an additional 12 sites were sampled along the Belizean (BBR) and Mexican (MBR) barrier reefs. Collections made at these 12 sites were a one-off sampling, not to be repeated by the ECONAR sampling. For

logistical reasons only a subset of the ECONAR sites could be included in these analyses. The sampling periods were conducted during the week following the full moons of these months. Recruits were collected by divers on SCUBA using hand nets and a clove oil mixture (9:1; 70% isopropanol:pure clove oil) from six sites in Belize and Mexico during SP1 and from all 18 sites in the four regions (TA, BC, BBR and MBR) in Belize and Mexico during SP2 (see Table 4.1 and Figure 4.1). Each site was approximately 1 km^2 in size and sampling dives were done on the shallow fore-reef at approximately 10 m depth.

Fish samples were preserved in 95% ethanol until the otoliths could be removed. Sagittal otoliths were removed from the fish, cleaned of any tissue using distilled water and a fine bristled paintbrush, embedded in Crystal Bond® glue, and polished in a transverse section using various lapping films from 30 μm to 9 μm grain size (Precision Surfaces International PSI-1630S-11, PSI-1612-11, PSI-1609-11). Otolith samples were mounted on petrographic slides (up to 18 otoliths per slide) for analysis in the ICPMS. The samples were cleaned in a class 100 clean room, placed in a milliQ water bath and sonicated for 10 minutes, then triple rinsed with milliQ water. After cleaning, each slide was allowed to dry overnight in a laminar flow HEPA-filtered fume hood.

MICROCHEMICAL ANALYSIS

Otoliths were chemically analysed at the Great Lakes Institute for Environmental Research, University of Windsor, using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). A Thermo Elemental X7 ICP-MS was operated with argon used as the carrier gas from the laser-sampling cell. The laser sampling system is a purpose-built system (Fryer et al., 1995) based on a non-homogenized, high power,

frequency-quadrupled (266 nm) Nd:YAG (neodymium-doped yttrium aluminum garnet) laser. Frequency was set at 20 Hz, flash lamp power was 1.25 kV, a 1.5 mm pinhole beam constrictor was used to increase the spatial resolution of the laser sampling, and a half-wave plate was used to reduce the power of the beam and increase spatial resolution of the laser sampling. Based on these settings, the beam was calculated to be 23 - 27 μm in width. The beam was focused onto the sample using an Olympus® BX-51 petrographic microscope and an Optics for Research® 266 nm 10X objective lens.

LA-ICP-MS data acquisition lasted 160 seconds with 60 seconds of instrument and gas background counts prior to the start of each ablation. Transects from the otolith core to edge were enabled by the use of an automated microscope stage, moving at a speed of approximately 5 μm s^{-1}. A trace element-doped glass standard (National Institute of Standards and Technology, NIST, 610), a homogeneous glass that is spiked with a range of elements in known concentrations, was analysed in duplicate at the beginning and the end of each sample batch (approximately 18 otoliths) to correct for instrument drift. Calcium was used as an internal standard to compensate for the signal variation caused by differences, among otoliths, in the volume of ablated material.

In total, 12 elements and an additional 3 isotopes of these elements were analysed by the LA-ICP-MS (See Table 4.2 for list of elements) and chemical concentrations, in parts per million, were calculated using Plasma Lab© software (Thermo Electron, 2003). Two multi-elemental signals were selected from each otolith, one representing the otolith material near the core of the otolith henceforth called the "near-natal" signal and one representing material ablated near the edge of the otolith, a signal representing the site of collection, henceforth, called the "edge" signal. Each signal was comprised of average

concentrations of each element, the amount of otolith material used to calculate each near-natal and edge signal varied on a fish-by-fish basis as follows: the edge signals were averaged from the amount of otolith material that was ablated after the laser track had passed the settlement mark (as noted visually during the ablation experiment) to the edge of the otolith before the laser track crossed into the mounting medium. The near-natal signals were averaged from the amount of otolith material that was equivalent to 5 days of otolith growth after yolk sac absorption by the larvae. The signal was taken post-yolk sac absorption to eliminate any effects on otolith chemistry from the maternal yolk added to the eggs (Brophy et al., 2004; Ruttenberg et al., 2005; Chittaro et al., 2006).

The size of the otolith at yolk sac absorption was calculated by taking the average size of an otolith of a late-stage embryo and adding to that the amount of otolith growth, in the transverse plane, equivalent to 3 days growth post-hatch (the time until yolk-sac absorption). Otoliths of late-stage embryos of *S. partitus* are approximately 20 μm in diameter (Chittaro et al., 2006), equivalent to approximately 3.5 days growth in the egg. After hatching, yolk sac absorption takes approximately 3 days for pomacentrids (Thresher 1984). Post-hatching growth of otoliths in the transverse plane (from the core along the longest growth axis) is approximately 7 μm for the first three days of growth post-hatch (Meekan and Wilson, 2002). Therefore I calculated the size of the otolith, post-yolk sac absorption, to be approximately 27 μm in size. The near-natal signal was averaged from material from 5 days of growth after the yolk-sac was absorbed. This length of time (5 days) was chosen based on the length of time that a majority of particles are retained around reefs in Barbados and Belize (Paris and Cowen, 2004; Tang et al.,

2006). Therefore, the near-natal signals are likely to represent a chemical signal that is representative of an area near the site of spawning.

Statistical Analyses

After signal selection, elements that met the following two criteria were included in statistical analyses: 1) concentrations of NIST 610 samples were determined with satisfactory precision (i.e. coefficient of variation less than 10%); and 2) concentrations of elements were greater than detection limit for more than 85% of otoliths analysed. Despite meeting these two criteria, one element, cerium, was removed from statistical analyses because the detection limits in some cases for this element were zero, meaning I had no meaningful way to judge the precision of otolith measurements in these trials. Data for elements that met these two criteria were Ln(x) transformed to improve normality for multivariate statistical analyses.

Spatial variation in otolith microchemistry throughout the MBRS

One-way multivariate analyses of variance were used to investigate whether otolith chemistry varied significantly across all sites within the MBRS region. The otolith edge microchemical signals were used for these analyses since the edge signals are the best representation of site-specific chemical concentrations. I conducted two separate analyses to test for spatial variation, one for all 18 study sites sampled during SP2 and one for the six sites sampled in SP1, the latter was done to investigate spatial variation in chemistry among the sites that will be used to investigate dispersal movements of individuals within the MBRS. For the first analysis, a nested MANOVA design was used

to test for regional scale spatial variation in otolith chemistry as well as to test for differences across sites within regions. If significant differences in otolith chemistry were found among regions or sites within regions, discriminant function analysis was performed, using those elements with variable concentrations, to test whether there was sufficient chemical variation to discriminate among regions or sites at this large spatial scale. Canonical analyses were performed on the elemental data and the mean (± 95% confidence limits) canonical scores for each site from SP1 and for each region or site within region from SP2 were used to visualize the relationships among groups in terms of otolith chemistry. In both analyses Tukey's honestly significant difference multiple comparisons test (for unequal sample sizes) was used to test for differences among groups.

Dispersal of individuals within the MBRS

To investigate the dispersal movements of individual fishes within the MBRS, the otolith edge chemistries were used to create a chemical map of the MBRS. Multivariate analysis of variance was used to test for significant differences among sites based on otolith edge chemistry. If it was found that there were significant differences in otolith chemistry among sites, then a forward-stepwise discriminant function analysis (DFA) and canonical analysis was performed. The canonical analysis produced canonical scores that were used to visualize the relationships among sites in terms of otolith chemistry. The discriminant function analysis produced classification functions for classifying individuals to sites of natal origin. There are two steps to the DFA procedure: first, discriminant functions are created based on edge signals of otoliths from fish collected at

the "baseline" populations (sampled during SP1), for which the sites of collection are known; second, these same discriminant functions are used to estimate the site of natal origin for individual fish, using each fish's near-natal otolith signal. These fish were collected at the "mixed stock" populations from around the MBRS approximately one month later, during SP2. Collections from the mixed stock populations were made one month after the collections of the baseline populations because Chittaro (2005) showed that otolith chemistry was temporally instable at the scale of 2 weeks, therefore one cannot compare chemical signals from near-natal and edge portions of the otoliths unless the near-natal and edge portions were deposited around the same time. The pelagic larval duration of *S. partitus* is between 26 to 40 days long; therefore fishes spawned during SP1 of this study would be recruiting to reefs around one month later (i.e. SP2) and therefore the near-natal and edge signals, of the mixed-stock and baseline populations respectively, would have been deposited at approximately the same time.

It was assumed that each "baseline population" would contribute equally to the "mixed stocks", therefore, the *a priori* probability (an input statistic required by DFA) was made equal for each baseline population. From the DFA, a classification matrix and partial Wilks' Lambda statistics were determined. The former indicates the percent of fish that were correctly classified to the site from which they were known to have been collected, and the latter indicates the unique contribution of an element to the discriminatory power of the model (Statsoft Inc., 2001). The classification functions were used to estimate the natal sites for individuals from the mixed stock dataset. To assess the reliability of the classifications of mixed stock individuals made by the DFA, posterior probabilities were determined from Mahalanobis distances (the distance of each case to

the mean centroid of the nearest group; McCune and Grace, 2002). The posterior probabilities represent the probability of a fish belonging to the site to which it was classified. A greater posterior probability means a higher confidence in the assignment of an individual to a particular natal site. To ensure a high level of confidence in the estimates of dispersal, only those individuals from the mixed stock that were assigned to a natal site with greater than 90% posterior probability were considered.

RESULTS

Of the original 15 elements and isotopes analysed by the LA-ICP-MS, eight elements and an additional 2 isotopes were included in multivariate analyses (^{25}Mg, ^{43}Ca, ^{55}Mn, ^{57}Fe, ^{66}Zn, ^{86}Sr, ^{88}Sr, ^{137}Ba, ^{138}Ba, ^{208}Pb) based on the two criteria for element selection mentioned above.

Spatial variation in otolith microchemistry throughout the MBRS

Spatial variation throughout the entire MBRS (the mixed stocks)

A multivariate analysis of variance with a nested design revealed that there were significant differences in the multivariate elemental edge signatures between the 4 regions, TA, BC, BBR and MBR (Wilks' Lambda $_{(region)} = 0.425$; $F = 4.5$; $p < 0.001$) and sites within regions (Wilks' Lambda $_{site(region)} = 0.165$; $F = 2.0$; $p < 0.001$) from SP2 (mixed stocks). Univariate results showed that eight of the ten elements and isotopes

(^{25}Mg, ^{55}Mn, ^{66}Zn, ^{86}Sr, ^{88}Sr, ^{137}Ba, ^{138}Ba and ^{208}Pb) differed significantly between regions, sites within regions or both (Table 4.3).

Four of these eight elements (Mg, Zn, Ba and Pb) were variable at the regional scale (Table 4.3). Turneffe Atoll seemed to be the most different of all the regions in terms of the log-concentrations of elements that varied on a regional scale (Figure 4.2). Tukey's HSD multiple comparisons test showed that Turneffe Atoll had higher levels of ^{25}Mg, ^{66}Zn and ^{208}Pb than the other regions, and lower concentrations of ^{137}Ba, ^{138}Ba (Figure 4.2). Overall, the two barrier reef regions (BBR and MBR) were more similar to each other in otolith chemistry than any other regions (Figure 4.2). They did not differ significantly from each other in the concentrations of any of the five elements that varied on a regional basis. Canonical analysis revealed that 97% of the variation in otolith chemistry was explained by the first two canonical factors. Canonical factor 1 was most strongly associated with low concentrations of ^{138}Ba and high concentrations of ^{25}Mg; canonical factor 2 was associated weekly with low concentrations of ^{208}Pb (Figure 4.3). Canonical analysis revealed that there was good separation between regions based on the scores in the first canonical factor, however there was significant overlap in the canonical scores between the two barrier reef regions (Figure 4.3). Forward step-wise discriminant function analysis of fish from the four regions showed significant discrimination between regions (Chi-square = 78.70; df = 9; $p < 0.001$). This discriminant function was able to predict membership to regions with a moderate degree of accuracy (37 – 63% accuracy, Table 4.4).

Four elements (Mn, Sr, Ba and Pb) varied significantly among sites within regions (Table 4.3). Tukey's HSD multiple comparisons test showed that five of the 18 sites in

this analysis seemed to drive most of the variation in otolith chemistry at the site level, TA4, TA5, BC6, MBR1 and MBR12 varied significantly from multiple other sites in the log-concentrations of ^{137}Ba and ^{138}Ba (Figure 4.4 d and e). Furthermore, TA5 was the only site to vary significantly from any other sites in the concentrations of ^{86}Sr and ^{88}Sr (Figure 4.4 b and c). Another site, TA4 was the only site to vary significantly from other sites in its log-concentration of ^{208}Pb (Figure 4.4 f). Finally, BC2 and MBR1 varied significantly from multiple other sites in the log-concentration of ^{55}Mn, and site BC6 significantly varied in its log-concentration of ^{55}Mn from these two sites but not any other sites (Figure 4.4 a). Canonical analysis revealed 69% of the variation in otolith chemistry was explained by the first two canonical factors. Canonical factor 1 was most strongly associated with high concentrations of ^{88}Sr and with low concentrations of ^{138}Ba; canonical factor 2 was most strongly associated with high concentrations of ^{88}Sr and with low concentrations of ^{55}Mn and ^{86}Sr. Canonical analysis also revealed that there was significant overlap in the canonical scores between sites within the four regions (Figure 4.5). Forward step-wise discriminant function analysis of fish from these 18 sites showed significant discrimination between sites (Chi-square = 268.28; df = 85; $p < 0.001$). This discriminant function was able to predict membership to sites within regions with a low to moderate degree of accuracy (11 – 75% accuracy, Table 4.5).

Spatial variation in the baseline populations

A multivariate analysis of variance revealed that there were significant differences between the six sites from SP1 (baseline populations) in the concentrations of multivariate elemental edge signatures (Wilks' Lambda = 0.21; F = 1.6; p = 0.02). Univariate results showed that four of the eight elements (^{25}Mg, ^{55}Mn, ^{66}Zn and ^{208}Pb)

differed significantly between sites (Table 4.6). Tukey's HSD multiple comparisons test showed that site TA6 was the most different from other sites in the concentrations of ^{55}Mn, ^{66}Zn and ^{208}Pb (Figure 4.6 b,c and d). Site TA6 had significantly lower log-concentrations of these elements than sites BC2 and TA5 (Mn), BC2 (Zn) and BC2 and BC6 (Pb) respectively (Figure 4.6 b,c and d). The only other site-pair difference in otolith chemistry was between TA4 and TA5 in which case TA4 had a significantly lower log-concentration of ^{25}Mg than its neighbour site TA5. Canonical analysis revealed 82% of the variation in otolith chemistry was explained by the first two canonical factors. Canonical factor 1 was most strongly associated with high concentrations of ^{55}Mn; canonical factor 2 was most strongly associated with high concentrations of ^{25}Mg and low concentrations of ^{66}Zn. Canonical analysis also revealed that there was significant overlap in the canonical scores between sites in both canonical factors 1 and 2, however, site TA6 was the most different from all other sites in terms of its score in canonical factor 1 (Figure 4.7).

Dispersal of individuals within the MBRS

Forward-stepwise discriminant function analysis of fish from the baseline populations showed significant discrimination between sites (Chi-square = 49.76; df = 20; p = 0.0002). This discriminant function was able to predict membership to sites in the baseline population with a low to moderate degree of accuracy (17 – 68% accuracy, Table 4.7). The site with the best discrimination was TA6 and the site with the worst discrimination was TA1. The most important elements for discriminating between sites

were ^{55}Mn and ^{66}Zn (Wilks' Lambda = 0.48; p = 0.03 and Wilks' Lambda = 0.48; p = 0.02 respectively).

The contribution of fish spawned at the six baseline populations to the mixed stocks across the MBRS was estimated at 5.3% (9 out of 169) of the individuals collected from mixed stock populations (during SP2). Based on their "near-natal" chemistry, these 9 fish were classified to one of the six baseline populations with > 90% posterior probability. Specifically, site TA6, on the leeward side of Turneffe Atoll, was estimated to have seeded the majority of the individuals (7 of 9 or 78%) that could be assigned with high confidence (Figure 4.8). Three sites, BC2, TA1 and TA4, were estimated to have seeded no individuals with high confidence. All but one individual was estimated to have dispersed from sites on the atolls (Banco Chinchorro or Turneffe) to sites on the Belizean or Mexican barrier reefs (Figure 4.8). The estimated linear distance of dispersal from a fish's natal site was, on average 160 km (± 137 km; SD), with 5 of 9 individuals dispersing more than 100 km from their estimated natal sites (Figure 4.9).

DISCUSSION

Spatial variation in otolith chemistry throughout the MBRS

We show that there was significant variability between sites in the MBRS in terms of multi-elemental otolith edge signals. At a regional level, there was sufficient chemical variation to be able to discriminate moderately well between regions (41 – 75% assignment accuracy; Table 4.3 and Table 4.4). Overall, Turneffe Atoll had the most distinct otolith chemistry and the two barrier reef regions, delineated only by the arbitrary

political border between Belize and Mexico, were the most similar regions (Figure 4.2 and 4.3). At the site level, there was significant variation across all sites within regions. There was, in some cases, extensive overlap in the chemical signatures between sites, which led to moderately poor discriminatory power (11 – 67% assignment accuracy, Table 4.5). Fishes from some sites could be moderately accurately assigned, however, the bulk of the spatial variation at this scale appears to be driven by only a few outstanding sites (5 of 18 sites or 27%), while the majority of sites overlap significantly in their otolith chemistries (Figures 4.4 and 4.5).

Many studies have detected sufficient variation in otolith chemistry to distinguish between sites on a range of spatial scales (Thorrold et al., 1997; Patterson et al., 1999; Forrester and Swearer, 2002; Gillanders, 2002; Chittaro et al., 2004; Patterson et al., 2005). Initial studies that showed chemical variation in otoliths were conducted in estuarine and coastal systems where anthropogenic and terrigenous inputs of trace elements will influence water chemistry, and ultimately otolith chemistry (Forrester and Swearer, 2002), and are overall characterized by more spatial variation in water chemistry than are open ocean environments or isolated reefs and islands (Thorrold et al., 2002). In this study, the sites sampled were all tropical coral reefs some of which, those at Turneffe Atoll and Banco Chinchorro, are fairly remote locations with low density human populations and are located between 30 and 50 km from the mainland Caribbean. In some coastal habitats, bays and estuaries may promote the retention of water and may be relatively stable in their physico-chemical features (Forrester and Swearer, 2002). A barrier reef, like that in Mesoamerica, is much more likely to have dynamic physico-chemical features because the reefs do not represent discrete units of water (Patterson et

al., 2005), and chemical signatures in these systems may only be stable at broader spatial scales. This has been shown to be the case in some studies conducted on tropical coral reefs (Patterson et al., 1999; Patterson et al., 2005), where nearby reefs in a study system are likely sharing the same water masses (Patterson et al., 2005). The results of this study suggest that the regional scale might be the most appropriate scale to investigate dispersal movements of individuals among coral reefs using otolith edge chemical signatures to discriminate between regions.

Dispersal of individuals within the MBRS

The six sites sampled during sampling period one (baseline populations for the dispersal analysis) varied significantly from one another in their otolith edge chemistry (Table 4.6). There was, in some cases, overlap in the chemical signatures between sites (Figures 4.6 and 4.7), which led to poor discriminatory power, such as the case for TA1. However, some sites did show sufficient chemical uniqueness to be discriminated reasonably well, for example sites TA4 and TA6 (Table 4.7, Figures 4.6 and 4.7).

Using the near-natal elemental signatures from *S. partitus* juveniles from the mixed stocks around the MBRS, and comparing these near-natal signatures to otolith edge signatures from the baseline populations I found that several individuals collected around the MBRS were predicted to have originated from one of the six baseline sites at Turneffe Atoll or Banco Chinchorro. In all, nine individuals (5.3% of those sampled) that were spawned during or around sampling period 1 were collected at one of 18 mixed stock sites approximately one month later. The majority of these individuals, for which the posterior probability of assignment was > 90%, were predicted to have come from

one site at Turneffe Atoll (TA6). Not surprisingly, this site also had the highest confidence in assignment of fishes known to have been from that site (Table 4.7), and this site was the most distinct of the six baseline populations in its otolith edge chemistry (Figures 4.6 and 4.7). It is possible that other fishes collected from the mixed stocks in sampling period two were spawned from one of these six baseline populations, however, the confidence in the assignment of these individuals was less than ninety percent. There is some level of uncertainty in the assignment of fishes to natal sites since all possible source populations were not sampled (Campana, 2005). In fact, for studies conducted on reefs in systems like the MBRS, it may be impossible to sample all possible source populations. I attempted to reduce the error in the assignments by only considering fishes for which the posterior probability of assignment was greater than 90%. While this doesn't completely eliminate the possibility of assignment error due to the uncertainty of unsampled populations, it does constrain the error whereby error can only occur if an unsampled site is very similar in otolith chemistry to one of the baseline populations for which the chemistry is known.

In general, fishes tended to disperse northward and westward from the atolls toward the barrier reef (Figure 4.8). This general direction of dispersal is consistent with predictions from a hydrodynamic model produced by Tang et al. (2006) which models mean surface current circulation in the MBRS and in particular the Belizean Shelf. However, some fishes did not disperse in this direction (Figure 4.8) suggesting that larvae may take advantage of weaker, potentially retentive subsurface currents through vertical migration. The frequency distribution of dispersal distances of these nine recruits shows that there were two peaks in dispersal frequency, with one peak (44%) of individuals

settling to sites 60 km or less from their predicted natal site. The other peak in the frequency distribution was at distances greater than 300 km from the predicted natal reef, thirty three percent, or three individuals dispersed this distance. Long distance dispersal of some individuals is also consistent with the predictions of the hydrodynamic model produced by Tang et al. (2006). They show that surface currents are strongest in August (same month as this study), the retention of near-surface passive particles is lowest in this month and that particles can be carried north-westward away from Turneffe Atoll toward the Mexican barrier reef in a time frame relevant to the dispersal of *S. partitus* larvae (Tang et al., 2006).

Two other studies have investigated dispersal of *S. partitus* within the MBRS, one using otolith microchemistry to track dispersal, the other using genetic techniques. Chittaro (2005) used otolith microchemistry to investigate dispersal of *S. partitus* between sites within Turneffe Atoll during six sampling periods in 2002 and 2003. The most frequent distance of dispersal in Chittaro's study was approximately 20 km from the natal site, and some individuals dispersed the full length of the atoll. In some sampling periods, Chittaro (2005) found that site TA6 (the same site as in this study) contributed recruits to other sites around Turneffe, and in one sampling period (June 30 – July 6; two weeks prior to sampling period 1 of this study) the site TA6 contributed 72% of all fishes determined to have dispersed between sites in Turneffe Atoll during that sampling period. Another study by Mora (2004) used microsatellite DNA markers to investigate the spatial scale of larval dispersal of *S. partitus* within the MBRS. In his study, Mora sampled fish from all of the sites included in the present study, and then some, the individuals he assessed would have been spawned around the middle of July 2003 and recruited to reefs

in the MBRS around the middle of August 2003 (i.e. the same time period as this study). Mora (2004) found that dispersal distance was bimodal, where the first peak of dispersal (about 45% of individuals) was 60 km or less, and the second peak in dispersal distance (about 40% of individuals) was around 180 km (Figure 4.10). He found that some fishes dispersed greater than 300 km, but the proportion of individuals dispersing this distance was around 5% (Mora, 2004). Mora did not find that site TA6 in Turneffe Atoll seeded any other sites with recruits.

Results of these two studies showed some similarities to the present study, which was conducted in the same system, and in the case of Mora's study, at the same sites at the same time as this study. Here I show that dispersal distance was bimodal, where a moderate proportion of individuals dispersed distances 60 km or less, and another one-third of individuals dispersed great distances, over 300 km. The genetic data of Mora (2004) painted a similar picture, where a large proportion of individuals dispersed relatively short distances, and some were found to disperse distances over 300 km. The dispersal distances predicted in this study fit within and spanned across the frequency distribution of dispersal distances that were predicted by Mora (2004), suggesting some level of congruence between the two techniques. Chittaro (2005) found that between 6 and 35% of individuals dispersed between sites within Turneffe Atoll, suggesting a moderate amount of larval retention at the atoll. The results of this study showed that approximately 13% of individuals that were spawned at Turneffe Atoll dispersed to a site within Turneffe Atoll. There is growing evidence that larval retention has a significant influence on the spatial scale of population replenishment in reef fishes (Jones et al., 1999; Swearer et al., 1999; Cowen et al., 2000; Jones et al., 2005). The results of the

present study, and those of Mora (2004) and Chittaro (2005) are consistent with this idea, because despite strong advective currents in the region with the potential to disperse larvae long distances (Tang et al., 2006) a moderate proportion of larvae are recruiting to populations close to their natal reef.

However, it must be noted that the results of the studies by Mora and Chittaro may be somewhat unreliable. In the case of Chittaro's microchemistry analysis, the natal signatures of the dispersed fish were taken from otolith core samples, shown to be an unreliable estimator of natal site, at least for some elements (Brophy et al., 2004; Ruttenberg et al., 2005; Chittaro et al., 2006). The results of Mora's genetic study were weakened by the fact that the genetic signals he used to characterize each baseline populations were based on a sample of 10 individuals from each population, this sample size limits the ability to resolve genetic differences among populations (Ruzzante, 1998). Despite these caveats, there does seem to be some degree of congruence among the three studies. It appears that a consistent theme among the three studies is that a moderate proportion of individuals disperse less than or equal to 60 km from their natal sites. Furthermore, this study and Mora's genetic study seem to concur that dispersal of *S. partitus* larvae can occur over very large distances (up to and exceeding 300 km; See Figure 4.10). However, it must be restated that a moderate proportion of dispersal does appear to occur on smaller spatial scales (< 60 km) for *S. partitus*, and therefore, this is the scale that is likely most relevant to metapopulation ecology and fisheries management.

It is apparent that the larvae of *S. partitus* are capable of long distance dispersal, at least in the Mesoamerican reef system. In order to be very confident in the assignment

of individuals using techniques such as discriminant function analysis, it would be ideal to sample as many populations as possible that are likely to contribute individuals to the mixed stock populations. For *S. partitus* in the MBRS, sites as distant as 300+ kilometers could be potential source populations, seeding each other's reefs. This suggests that in order to ensure high levels of confidence in assignment of individuals to these populations, a large-scale sampling of multiple reefs throughout the entire system is necessary. I showed in this study that otolith microchemistry was most variable between regions within the MBRS and that there was some extent of mixing of water masses between individual sites within the regions. Therefore, the best course of action to determine the dispersal, and ultimately connectivity, in the MBRS using otolith microchemistry would be to sample a large number of sites within the system but to pool sites within regions and then estimate connectivity between the regions.

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Table 4.1: Description of the sampling design subdivided by country, region and sites within regions. Sampling dates for each site are provided, SP1 occurred between 16 July and 21 July 2003, SP2 occurred between 12 August and 25 August 2003.

Country	Region	Site	Latitude (decimal degrees)	Longitude (decimal degrees)	Sampling periods	# of fish collected
Belize	Turneffe Atoll (TA)	TA1	17.58825	-87.74385	SP1,SP2	6, 5
		TA4	17.27357	-87.81123	SP1,SP2	10, 10
		TA5	17.17323	-87.88743	SP1,SP2	10, 12
		TA6	17.34370	-87.95487	SP1,SP2	11, 10
	Barrier Reef (BBR)	BBR1	16.89583	-88.06250	SP2	8
		BBR4	17.24277	-88.04395	SP2	9
		BBR5	17.34723	-88.03238	SP2	10
		BBR8	17.64190	-88.02182	SP2	10
		BBR9	17.70000	-88.01000	SP2	10
		BBR10	17.78417	-88.00167	SP2	9
Mexico	Banco Chinchorro (BC)	BC2	18.65757	-87.22820	SP1,SP2	9, 7
		BC6	18.65133	-87.40492	SP1,SP2	10, 12
	Barrier Reef (MBR)	MBR1	18.22777	-87.82687	SP2	10
		MBR3	18.39822	-87.76787	SP2	9
		MBR6	18.66643	-87.71568	SP2	9
		MBR10	19.00497	-87.57893	SP2	10
		MBR12	19.17965	-87.52970	SP2	10
		TULUM	20.20280	-87.43530	SP2	9

Table 4.2: List of all elements sampled by LA-ICP-MS including: the average concentration of each element over all samples; the average concentration of each element from NIST 610 standards; the average detection limit of each element over all samples and; the percent of samples above detection limits for each element. Data for ^{44}Ca are not available because this isotope was used to standardize all other isotopes.

Element (isotope)	Average CV of NIST 610 (±SD)	% of samples > detection limit	Average detection limit by sample (±SD)	Overall average conc. (±SD)
Li (7)	5.7 (3.2)	39.3	0.92 (0.28)	1.85 (1.00)
Mg (25)	2.2 (0.9)	100.0	1.45 (0.49)	15.85 (7.81)
Ca (43)	1.2 (0.5)	100.0	124.83 (31.09)	396763.48 (25223.21)
Ca (44)	N/A	N/A	N/A	N/A
Mn (55)	2.4 (1.2)	95.0	0.15 (0.08)	0.79 (0.54)
Fe (57)	4.6 (3.3)	97.6	35.06 (11.74)	120.40 (44.72)
Zn (66)	6.8 (4.0)	85.7	0.39 (0.17)	1.37 (1.62)
Rb (85)	5.6 (2.7)	6.5	0.23 (0.07)	0.24 (0.11)
Sr (86)	4.2 (2.2)	100.0	10.86 (7.18)	2673.12 (457.20)
Sr (88)	1.6 (0.8)	100.0	0.06 (0.03)	2819.96 (467.48)
Sn (120)	5.4 (3.1)	51.3	0.54 (0.18)	0.68 (0.24)
Ba (137)	2.2 (1.2)	99.7	0.26 (0.11)	6.85 (9.28)
Ba (138)	2.2 (0.7)	100.0	0.03 (0.01)	6.75 (9.27)
Ce (140)	3.5 (1.9)	89.2	0.01 (0.01)	0.13 (0.29)
Pb (208)	5.5 (3.0)	91.1	0.03 (0.04)	0.15 (0.18)

Table 4.3: Univariate results of the nested MANOVA testing for differences in isotope concentrations between regions and sites nested within regions. Those results in bold indicate statistically significant differences among regions and/or sites within regions.

		^{25}Mg				^{43}Ca				**^{55}Mn**				^{57}Fe			
Effect	df	**SS**	**MS**	**F**	**p**	SS	MS	F	p	**SS**	**MS**	**F**	**P**	SS	MS	F	p
Region	3	**0.89**	**0.30**	**6.88**	**<0.001**	0.00	0.00	0.00	0.99	1.11	0.37	1.89	0.13	0.29	0.10	0.66	0.58
Site(Region)	14	1.03	0.07	1.70	0.06	0.05	0.00	1.00	0.18	**13.46**	**0.79**	**4.03**	**<0.001**	3.07	0.22	1.51	0.11
Error	145	6.24	0.04			0.37	0.00			28.48	0.20			20.99	0.15		
Total	162	8.16				0.42				41.94				24.54			

		^{66}Zn				**^{86}Sr**				**^{88}Sr**				**^{137}Ba**			
Effect	Df	**SS**	**MS**	**F**	**p**	**SS**	**MS**	**F**	**p**	**SS**	**MS**	**F**	**P**	**SS**	**MS**	**F**	**p**
Region		**5.76**	**1.92**	**4.19**	**0.01**	0.12	0.04	1.6	0.19	0.10	0.03	1.5	0.21	**13.41**	**4.47**	**12.73**	**<0.001**
Site(Region)	17	7.86	0.56	1.23	0.26	**0.91**	**0.07**	**2.70**	**0.002**	**0.68**	**0.05**	**2.20**	**0.01**	**20.67**	**1.48**	**4.21**	**<0.001**
Error	145	66.42	0.46			3.50	0.02			3.22	0.02			50.92	0.35		
Total	162	80.71				4.54				4.00				91.57			

		^{138}Ba				**^{208}Pb**			
Effect	df	**SS**	**MS**	**F**	**p**	**SS**	**MS**	**F**	**p**
Region		**13.16**	**4.39**	**13.61**	**<0.001**	**17.10**	**5.70**	**10.38**	**<0.001**
Site(Region)	17	**18.45**	**1.33**	**4.13**	**<0.001**	**14.80**	**1.06**	**1.93**	**0.03**
Error	145	46.72	0.32			79.64	0.55		
Total	162	84.89				112.85			

Table 4.4: Classification matrix from discriminant function analysis predicting membership of fishes to one of the four regions in the MBRS. Rows are the observed classifications, and columns are the predicted classifications. Region codes are the same as Table 4.1.

Region	Percent correctly classified	BBR	BC	MBR	TA
BBR	36.8	**21**	7	15	14
BC	63.2	3	**12**	1	3
MBR	37.5	15	11	**21**	9
TA	58.1	6	4	3	**18**
Total	44.2	45	34	40	44

Table 4.5: Classification matrix from discriminant function analysis predicting membership of fishes of known site membership to one of the 18 sites in the MBRS. Site codes are the same as Table 4.1.

Site	N	Number Correctly Classified	Percent Correctly Classified
BBR1	8	1	12.5
BBR4	9	1	11.1
BBR5	10	4	40.0
BBR8	10	3	30.0
BBR9	10	2	20.0
BBR10	10	2	20.0
TA1	5	1	20.0
TA4	9	5	55.5
TA5	9	2	22.2
TA6	8	2	25.0
MBR1	10	3	30.0
MBR3	9	1	11.1
MBR6	9	3	33.3
MBR10	9	2	22.2
MBR12	10	2	20.0
TULUM	9	1	11.1
BC2	7	3	42.9
BC6	12	9	75
Total	163	47	28.8

DFA statistics: Wilks' Lambda = 0.17; $F_{119, 916} = 3.60$; $p < 0.001$.

Table 4.6: Univariate results of the MANOVA testing for differences in isotope concentrations between baseline population sites. Those results in bold indicate statistically significant differences among sites.

		^{25}Mg				^{43}Ca				^{55}Mn				^{57}Fe			
Effect	df	**SS**	**MS**	**F**	**p**	SS	MS	F	p	**SS**	**MS**	**F**	**P**	SS	MS	F	p
Site	5	**1.09**	**0.21**	**2.72**	**0.03**	0.02	0.01	1.00	0.36	**4.23**	**0.85**	**4.62**	**0.001**	0.51	0.10	0.86	0.51
Error	50	**4.00**	**0.08**			0.19	0.01			**9.15**	**0.18**			5.94	0.12		
Total	55	**5.08**				0.21				**13.38**				6.45			

		^{66}Zn				^{86}Sr				^{88}Sr				^{137}Ba			
Effect	df	**SS**	**MS**	**F**	**p**	SS	MS	F	p	SS	MS	F	P	SS	MS	F	p
Site	5	**9.32**	**1.86**	**3.40**	**0.01**	0.19	0.04	1.3	0.27	0.20	0.04	1.6	0.17	1.21	0.24	0.43	0.83
Error	50	**27.42**	**0.55**			1.42	0.03			1.23	0.03			28.3	0.57		
Total	55	**36.75**				1.60				1.43				29.53			

		^{138}Ba				^{208}Pb			
Effect	df	SS	MS	F	p	**SS**	**MS**	**F**	**p**
Site	5	0.63	0.13	0.24	0.94	**15.54**	**3.11**	**4.21**	**0.003**
Error	50	25.96	0.52			**36.88**	**0.74**		
Total	55	26.60				**52.42**			

Table 4.7: Classification matrix from discriminant function analysis predicting membership of fishes from the baseline populations to the sites from the baseline populations to which they belong. Rows are the observed classifications, and columns are the predicted classifications. Site codes are the same as Table 4.1.

Site	% correct	BC2	BC6	TA1	TA4	TA5	TA6
BC2	44.44	4	0	0	2	3	0
BC6	40.00	1	4	3	0	0	2
TA1	16.67	1	1	1	1	2	0
TA4	50.00	0	2	0	5	0	3
TA5	40.00	2	1	3	0	4	0
TA6	67.67	0	1	0	2	1	7
Total	44.64	8	9	7	10	10	12

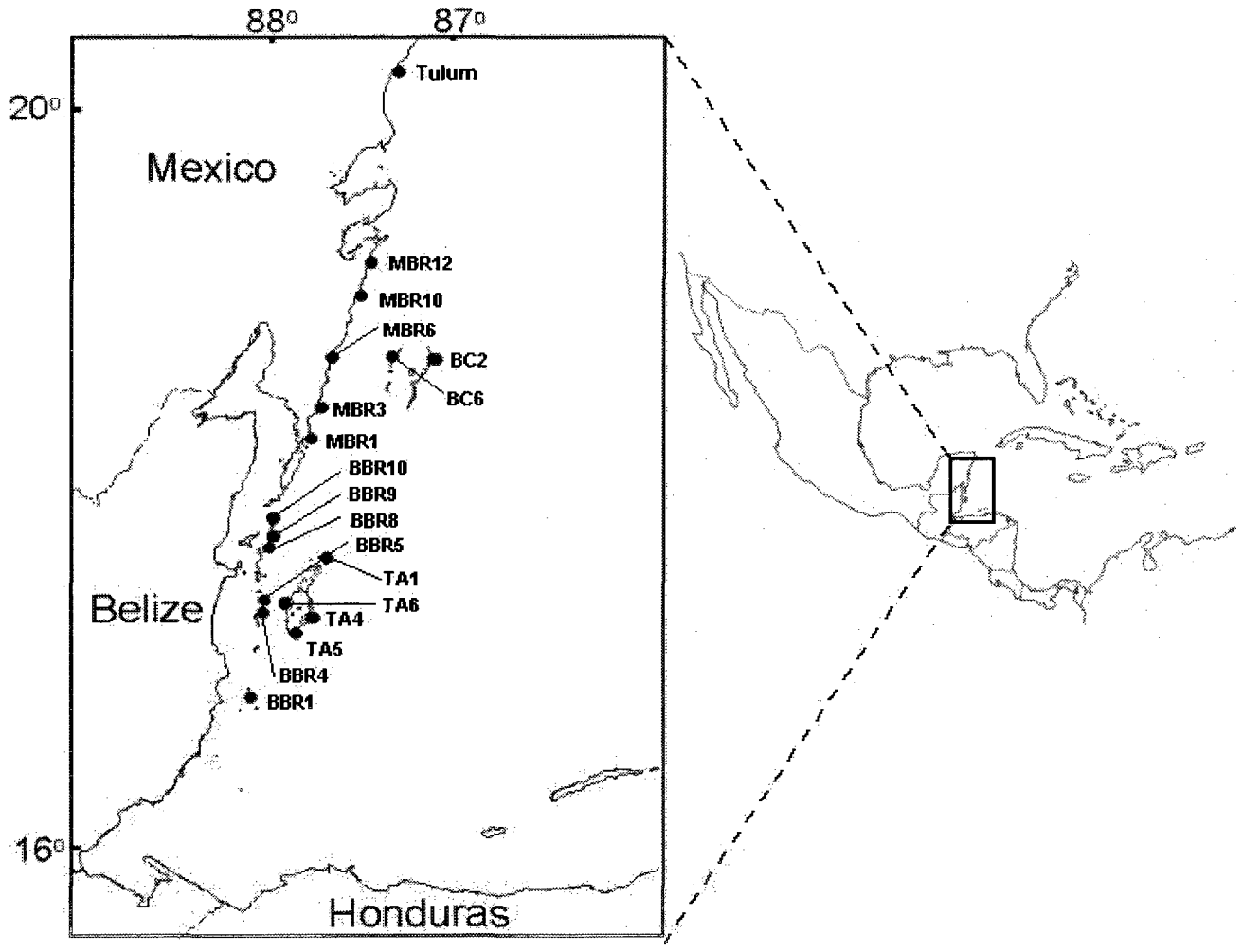


Figure 4.1: Map of the Mesoamerican Barrier Reef System including the locations of all 18 study sites. Inset is a map of the Western Caribbean for reference and scale.

Figure 4.2: Mean concentrations of Mg, Zn, ^{137}Ba, ^{138}Ba and Pb from the otoliths of fishes from the four regions in the mixed stock dataset. Error bars are standard deviations. Letters correspond to groupings; sites that are not significantly different share letters.

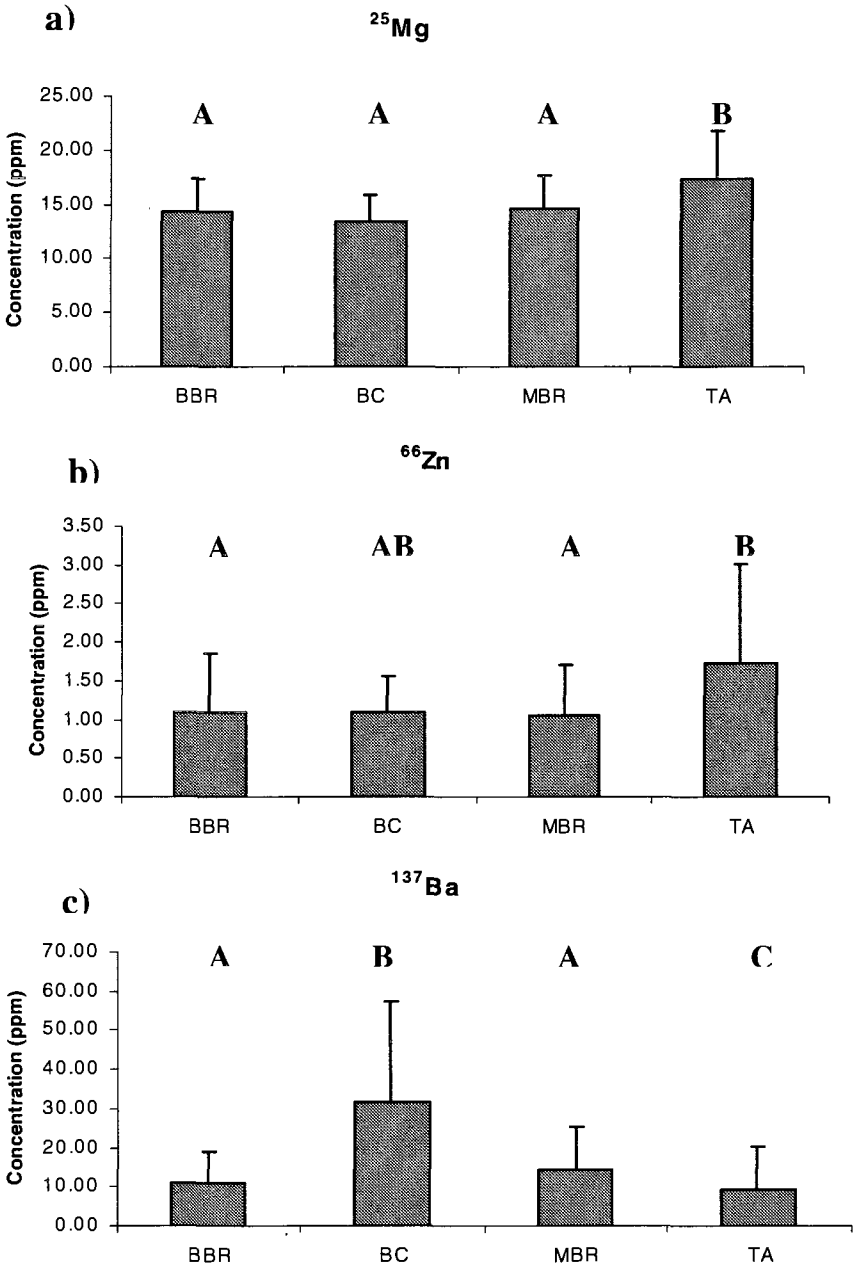
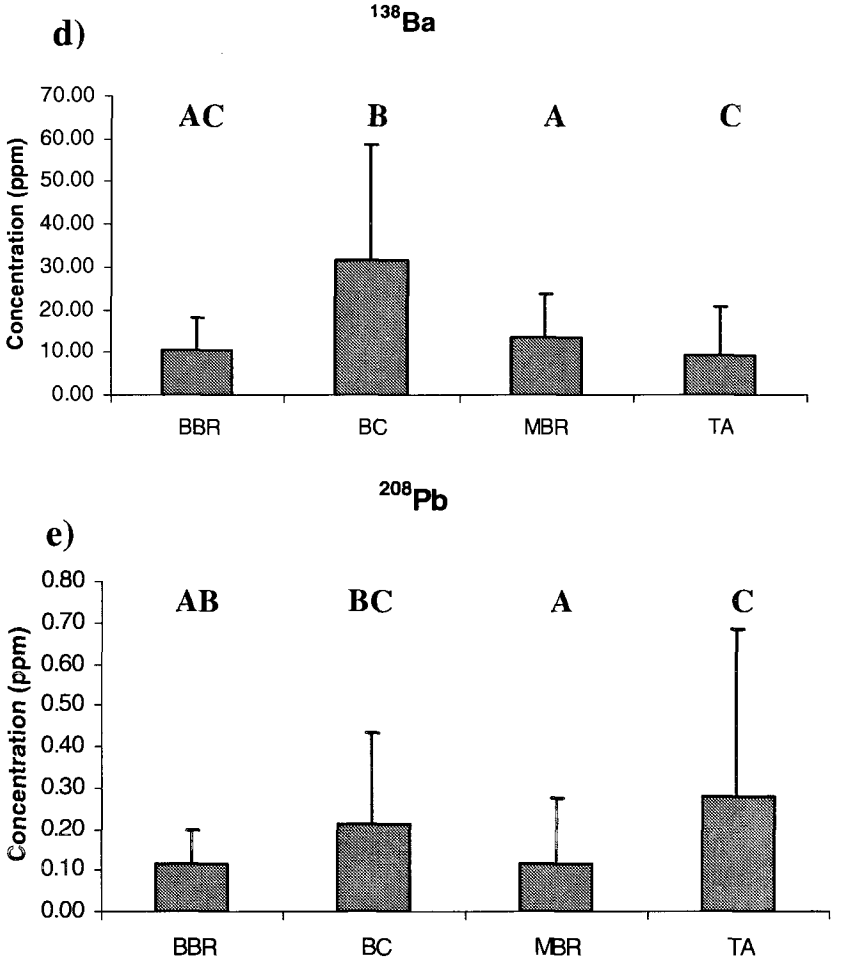


Figure 4.2: Continued from previous page.



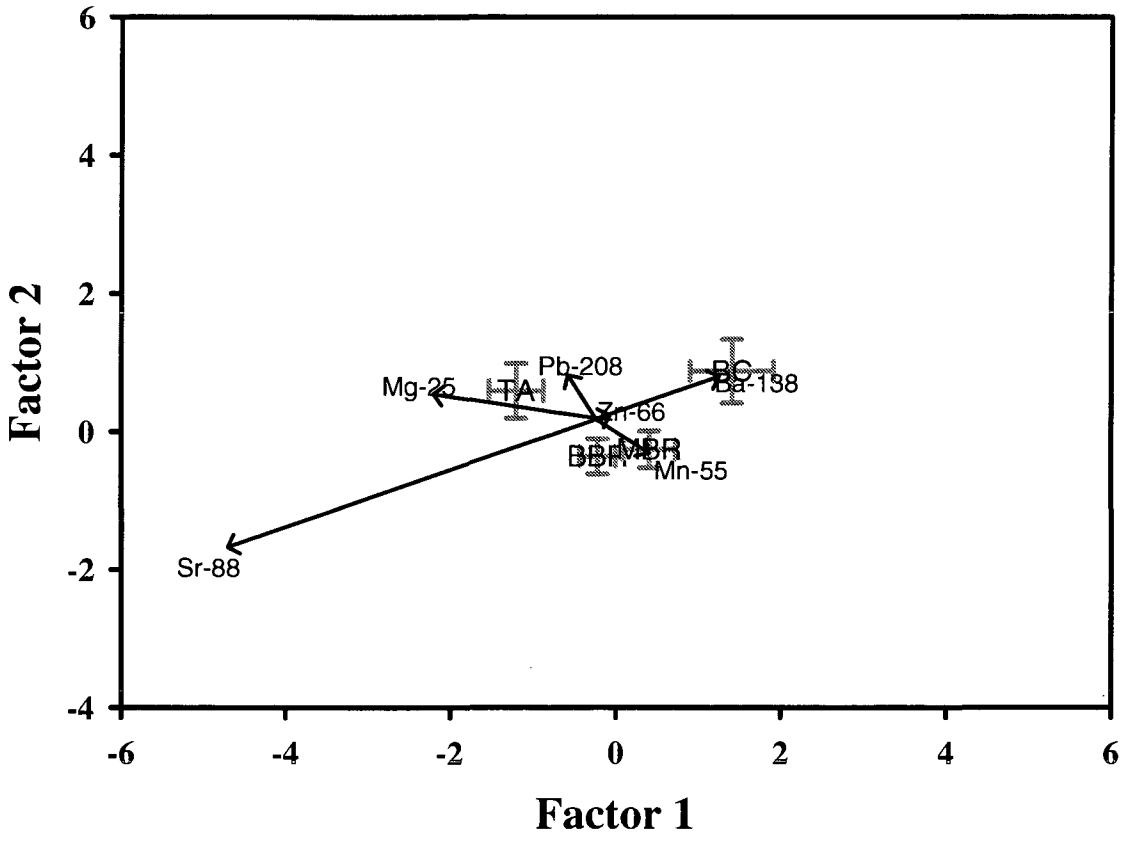


Figure 4.3: Mean canonical factor scores (± 95% confidence limits) describing the relationships between the four regions sampled in SP2 in terms of their multi-elemental otolith edge chemical signatures. Region codes are the same as Table 4.1.

Figure 4.4: Mean concentrations of Mn, ^{86}Sr, ^{88}Sr, ^{137}Ba, ^{138}Ba and Pb from the otoliths of fishes from the sites in the mixed stock dataset (sampled during SP2). Error bars are standard deviations.

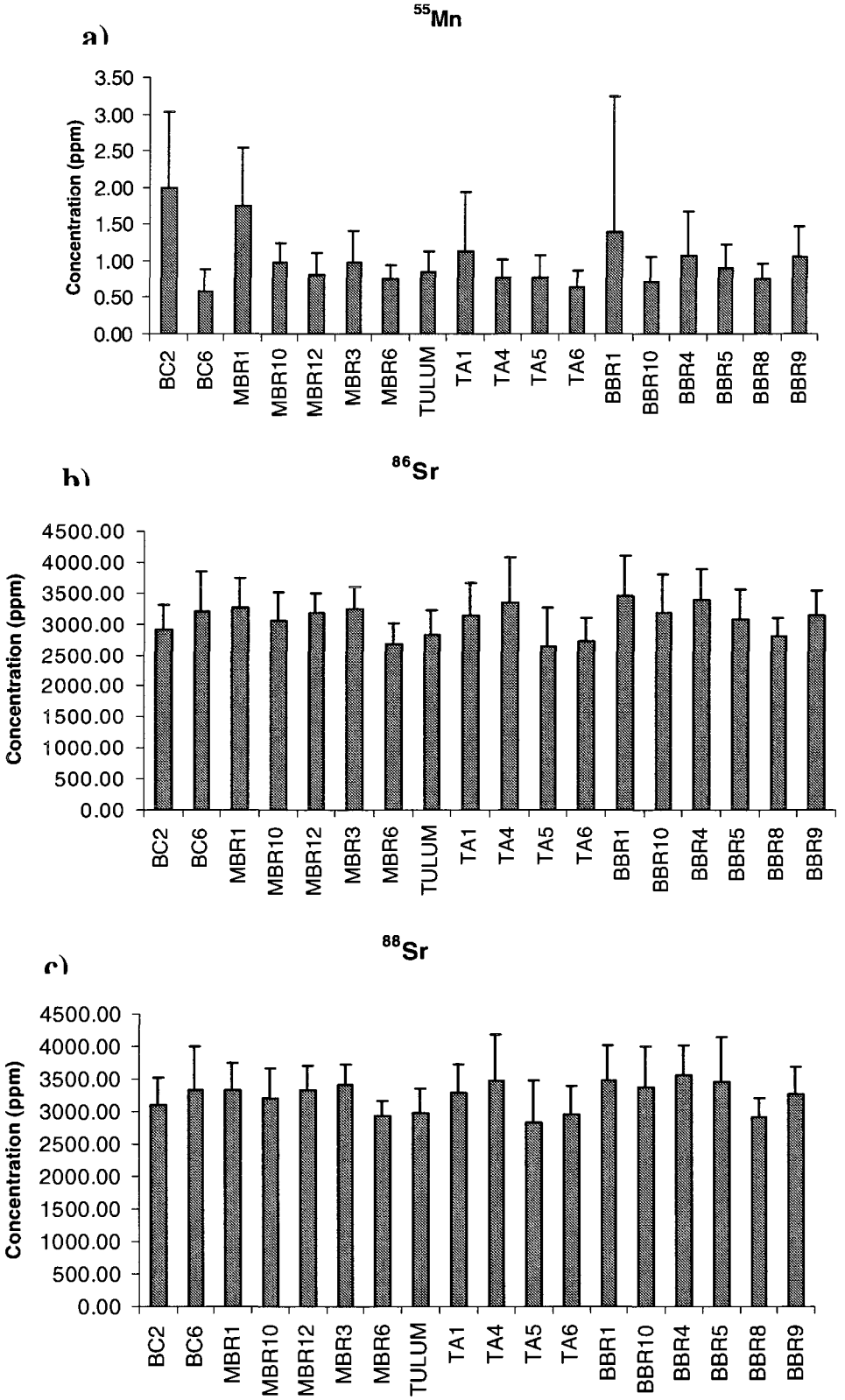
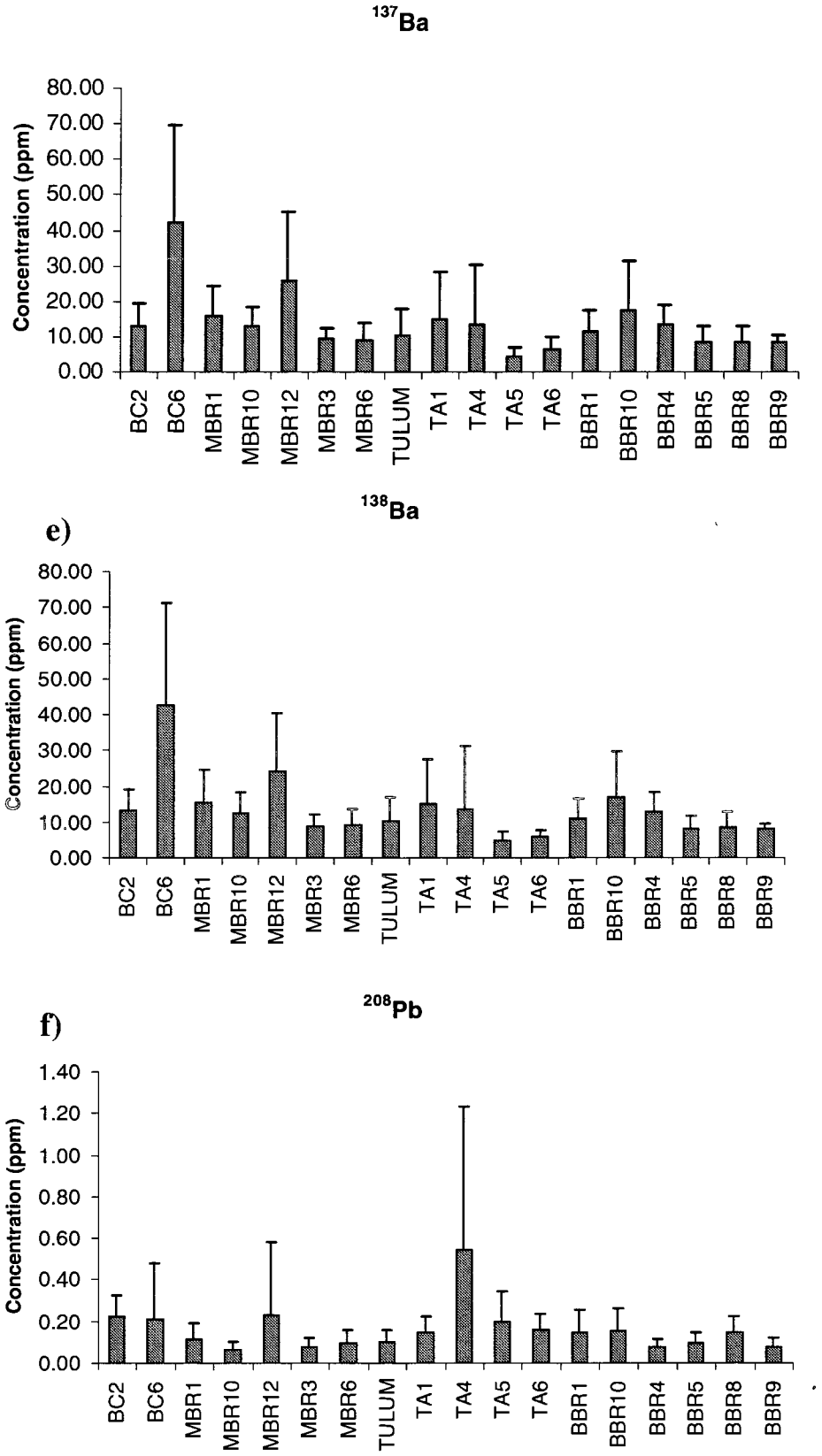


Figure 4.4: Continued from previous page.



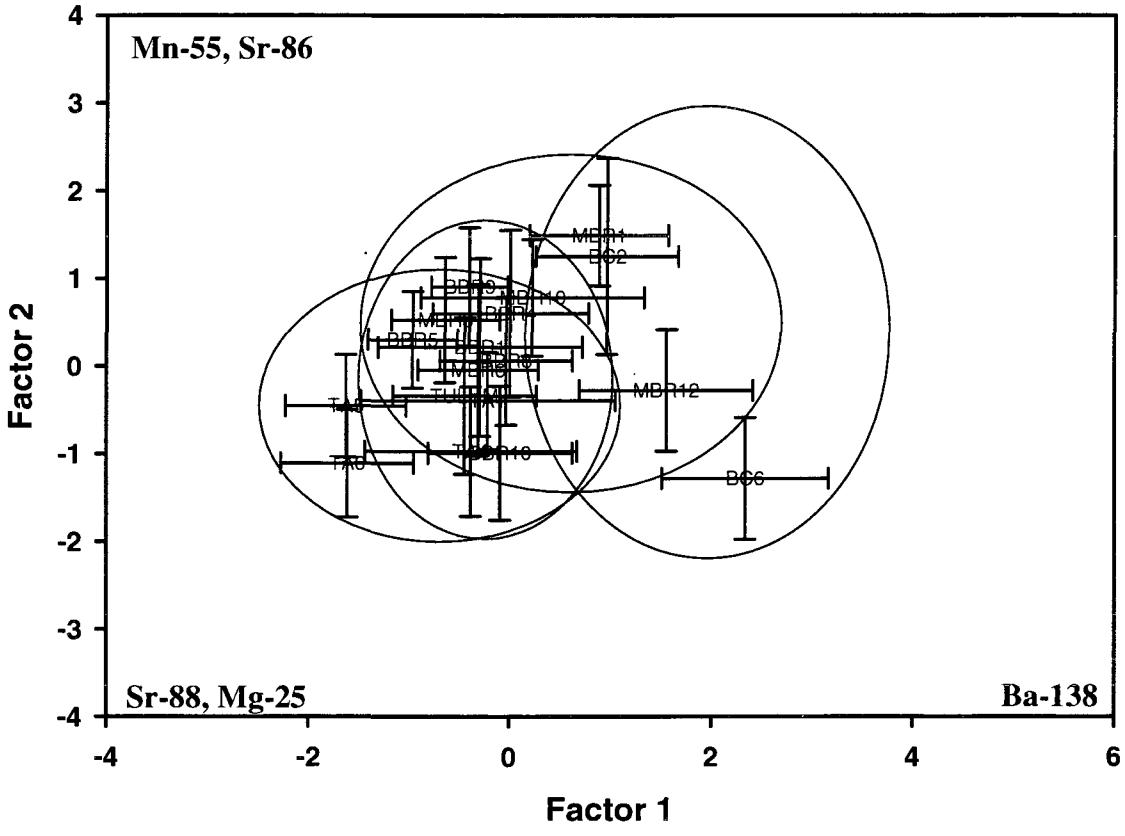
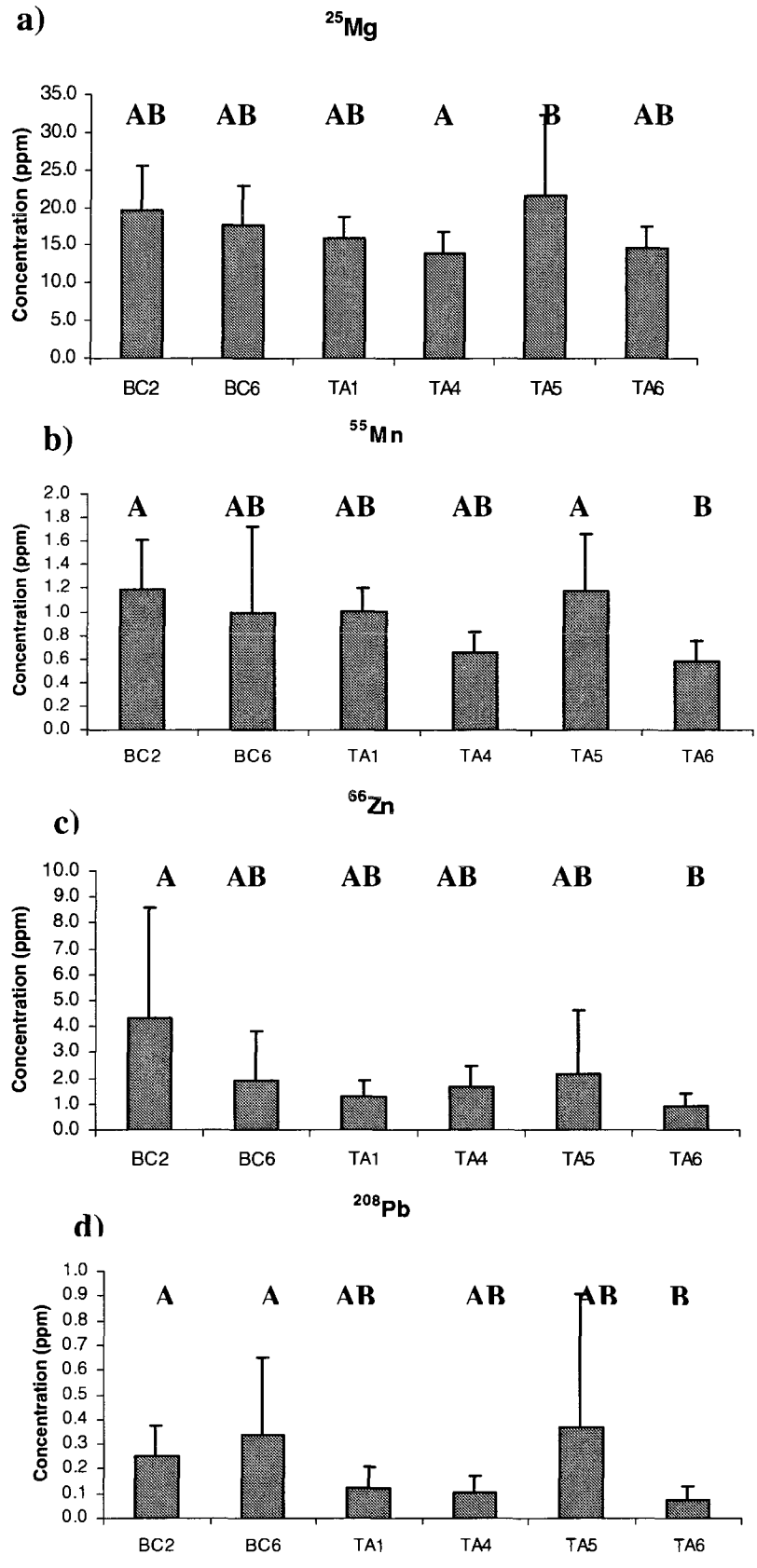


Figure 4.5: Mean canonical factor scores (± 95% confidence limits) describing the relationships between the 18 sites sampled in SP2 (mixed stock populations) in terms of their multi-elemental otolith edge chemical signatures. Site codes are the same as Table 4.1. Ellipses are placed around sites within the same region but do not reflect 95% confidence ellipses.

Figure 4.6: Mean concentrations of Mg, Mn, Zn and Pb from the otoliths of fishes from the baseline populations. Error bars are standard deviations. Letters correspond to groupings; sites that are not significantly different share letters.



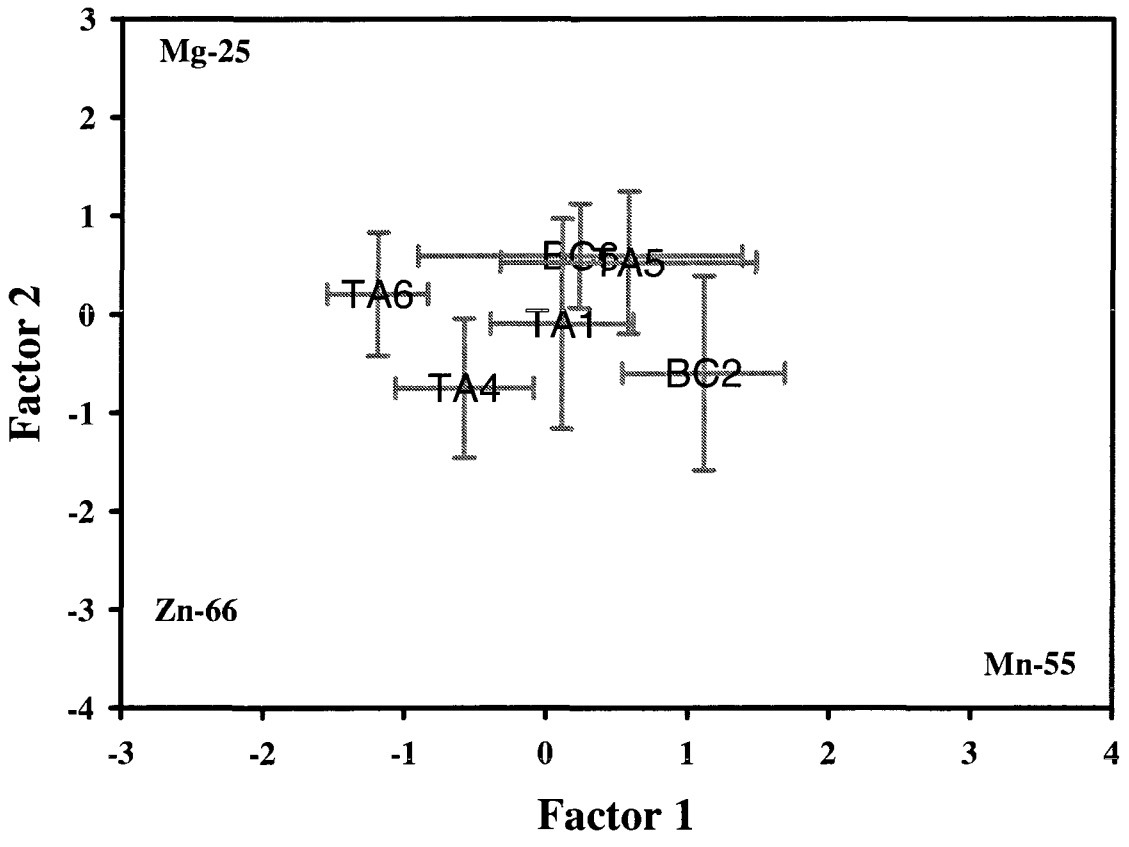


Figure 4.7: Mean canonical factor scores (± 95% confidence limits) describing the relationships between the six sites sampled in SP1 (baseline populations) in terms of their multi-elemental otolith edge chemical signatures. Site codes are the same as Table 4.1.

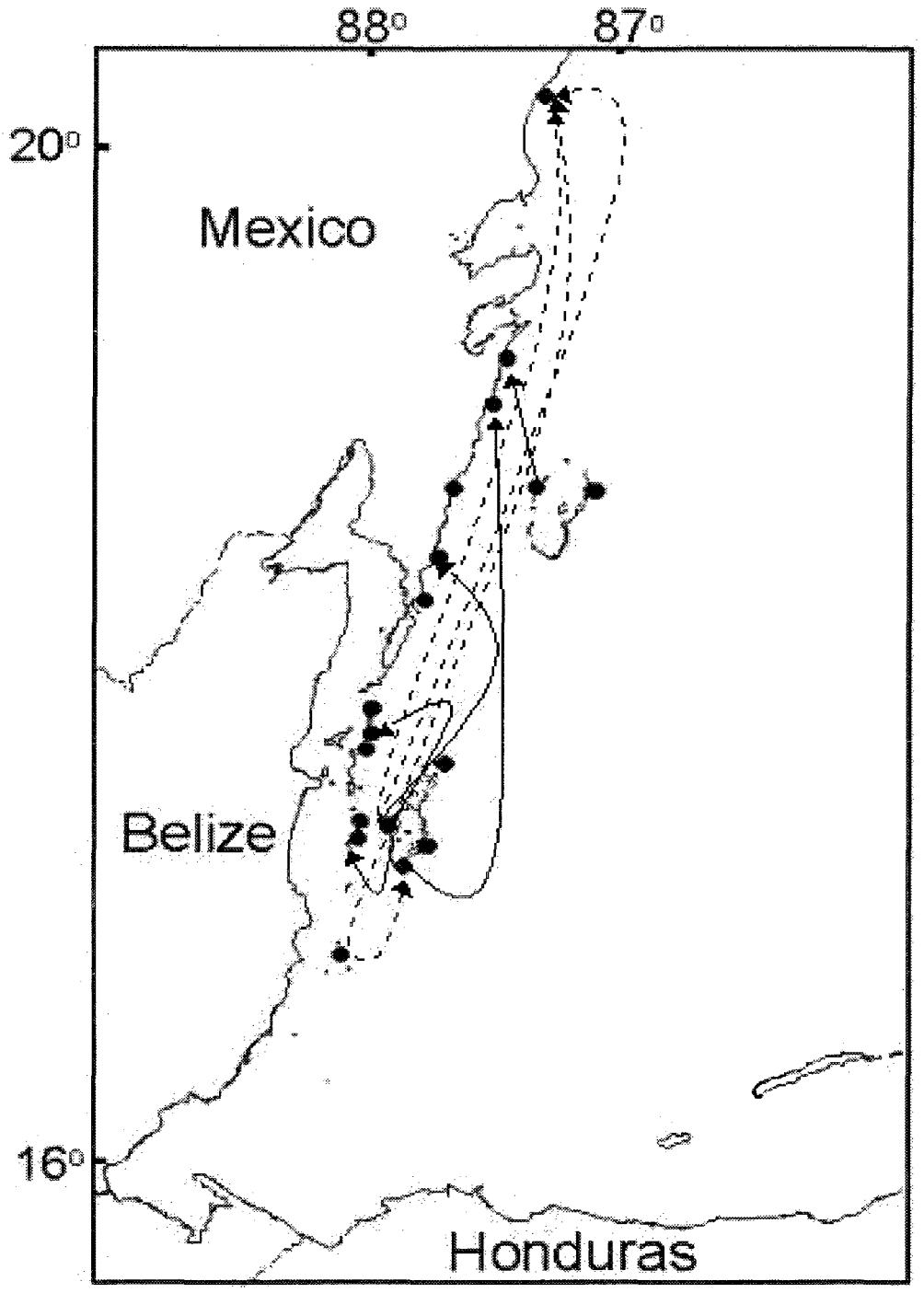


Figure 4.8: Map of the Mesoamerican Barrier Reef System showing the Patterns of larval dispersal among sites. Only those individuals from the mixed stock are included for which the discriminant function analysis predicted site membership with > 90% posterior probability. Dotted lines are only for clarity of visual interpretation.

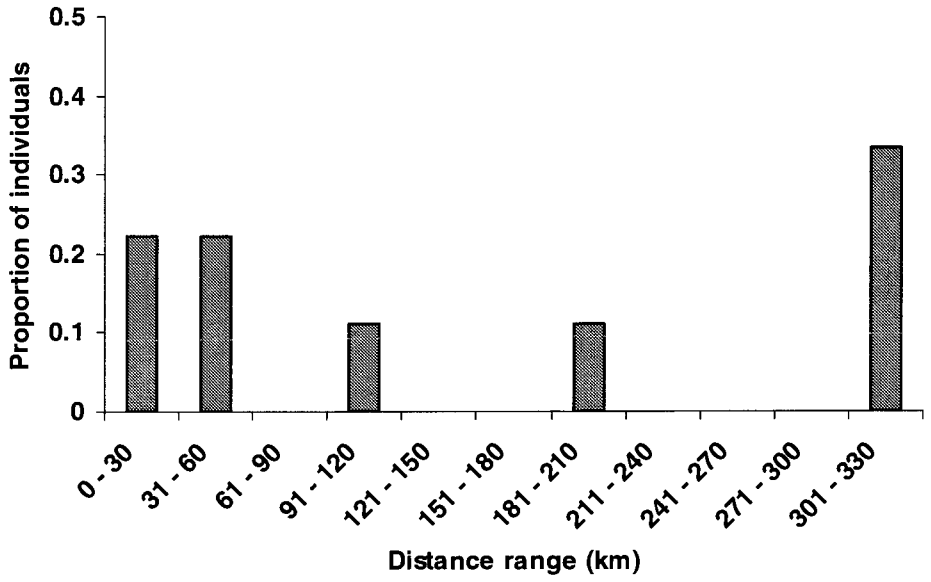


Figure 4.9: Histogram showing the frequency distribution of estimated dispersal distances of individuals. Only those individuals from the mixed stock, that the discriminant function analysis predicted site membership with > 90% posterior probability, are included (n = 9).

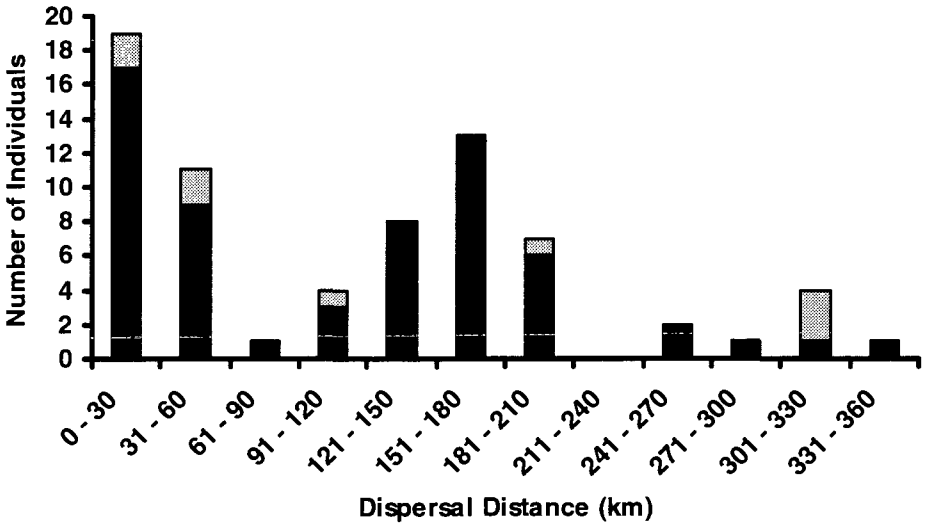


Figure 4.10: Histogram showing the frequency distribution of estimated dispersal distances of individuals within the Mesoamerican barrier reef system. Dispersal estimates are from both this study (grey bars) and from Mora, 2004 (black bars). From this study, only those individuals from the mixed stock, that the discriminant function analysis predicted site membership with > 90% posterior probability, are included. This figure is modified from figure 3.4 in Mora (2004).

Chapter 5: Critical Swimming Abilities of Late-stage Coral Reef Fish Larvae From The Caribbean: A methodological and intra-specific comparison.

A modified version of this chapter is published in the Bulletin of Marine Science with the following authors:

J.D. Hogan, R. Fisher and C. Nolan.

INTRODUCTION

Much is known of the swimming capabilities of coral reef fish larvae. Experiments have shown that late-stage larvae can sustain swimming at ambient current speeds for hundreds of hours (Stobutzki and Bellwood, 1997). They can also demonstrate short-term sustained swimming speeds of up to 50 body lengths (bl) per second (Bellwood and Fisher, 2001). Larval swimming speeds increase throughout ontogeny (Fisher and Bellwood, 2000) with a marked increase in undisturbed swimming speeds around the time of settlement (Fisher and Bellwood, 2003). *In situ*, larvae have been shown to swim approximately 50% of the speed as determined in swimming chamber experiments (Leis and Fisher, in press), this is also the same speed that larvae are capable of sustaining for periods of at least 24 hours (Fisher and Bellwood 2002, Fisher and Wilson, 2004). This speed also corresponds to the speed at which larvae will swim at when given the behavioural freedom to choose their swimming speed (Hogan and Mora, 2005). This wealth of knowledge has been used to surmise not only that reef fish larvae may be able to influence their settlement and dispersal, but to what extent this may be possible. While this has yet to be definitively determined, swimming abilities have been recognised as an important component of dispersal. In fact, many more recent dispersal models have included a fish swimming component (Wolanski et al., 1997; Armsworth, 2000, Paris et al., 2005).

One common technique used to measure fish swimming is the U_{crit} method (See Kolok 1999). This is because U_{crit} is an easy means of measuring swimming performance and involves swimming fish at incrementally increasing speeds until

exhaustion. This technique is favourable because the experiments are of short duration but they give a measure of maximum aerobic swimming speed that can be sustained for short periods (Plaut, 2001). Because U_{crit} measures aerobic sustained swimming performance it can be translated into terms of effective swimming abilities relatively easily. There are strong positive correlations between U_{crit} and *In situ* swimming speeds (Leis and Fisher, in press), as well as strong correlations with speeds sustainable for 24-hour periods. (Fisher and Wilson, 2004). This method is also useful because the experiments are simple and can be replicated easily allowing for a comparison of swimming abilities from a breadth of taxonomic groups and geographic regions. While the U_{crit} method does provide a comparative measure of swimming ability, the length of time these speeds can be maintained is unknown and may be sensitive to the speed and time increments used by the observer (Kolok, 1999; Plaut, 2001), thereby potentially confounding comparisons between studies.

In this paper I present a list of the U_{crit} swimming speeds from settlement-stage individuals for species of tropical reef fish of the Caribbean Sea. I investigate the validity of comparing U_{crit} estimates as measured by different observers by investigating the potential confounding effects of methodology on U_{crit} by comparing fish captured using different sampling methods, those fish swum using long (15 min.) and short (two min.) time intervals, and also fish swum in schools or singly within the chamber. I make intra-specific comparisons of the swimming speeds between individuals caught in the coastal waters of Belize, Central America and those from a previous study (Fisher et al., 2005) caught at the Turks and Caicos Islands.

MATERIALS AND METHODS

Data from the Turks and Caicos Islands were obtained from specimens collected using light traps. All fish were swum the same day as collection using a three-channel swimming flume, for more details see Fisher et al. (2005). Those experiments conducted in Belize were done so using settlement-stage fishes from a range of species occurring at Calabash Caye, Turneffe Islands Atoll, Belize (17° 16.414' N, 87° 48.674' W), during the summer months (May through September) of 2003, 2004 and 2005. Specimens were collected using a variety of techniques including crest nets, channel nets, light traps and night-light lift nets, although most specimens were collected using light traps and crest nets. Some specimens of *A. saxatilis* were collected with hand nets from a fish attracting device which was deployed over a seagrass bed from a dock. All specimens of *C. parrae* were collected with handnets from deep fore-reefs. Although they had already settled to the fore-reef, these individuals had yet to undergo complete metamorphosis and were included in the list of species regardless.

After capture, individuals were held in fresh seawater, with an aeration stone, in 24 L buckets to reduce stress prior to swimming trials. All individuals were swum within 24 hours of capture, most of which were swum within 6 hours of collection from nets or traps. Settlement-stage individuals were swum in either a single-lane swimming chamber or a three-lane swimming chamber similar in design to that used by Stobutzki and Bellwood (1997), both were constructed from Plexiglass™ (internal dimensions of swimming area: 185mm X 50mm X 50mm). A removable lid, sealed with an O-ring was used to introduce fish to, and remove them from both the chambers. One section of flow straighteners, 45-mm long, was placed just after the inflow in order to reduce turbulence

within the chamber. Fish were forced to swim within the swimming area by two 4.0mm mesh metal retaining fences, which were covered with a finer mesh when required for very small larvae.

U_{crit} was measured by incrementally increasing water velocity until the individual could no longer maintain position in front of the metal retaining fence for the full interval. Water velocity was increased by a rate of three body lengths per second for each increment, following the methods of Bellwood and Fisher (2001). U_{crit} swimming speed was calculated following Brett (1964): $U_{crit} = U + (t/t_i \times U_i)$, where U is the penultimate speed, U_i is the velocity increment, t is the time swum in the final velocity increment and t_i is the time interval for each increment. Four swimming protocols were used which varied either in the length of the time interval or the number of individuals in a single lane of the chamber, they were: (1) single individuals for 2 min intervals; (2) single individuals for 15 min intervals; (3) two individuals for 2 min intervals; (4) three individuals for 2 minute intervals. One-way ANOVAs were used to test for differences in swimming speed across all species as well as across species within individual families.

Total lengths (TL) were measured either pre-trial using calipers (2003 and 2004), or post-trial from photographic analysis (2005). Photographs were taken using a Minolta XG9 SLR camera with a 50mm lens and scanned from film into digital format. "Image Tool" software was used for image analysis. Specimens were preserved in 95% ethanol and each individual was identified to the lowest taxonomic level possible by either keying out preserved individuals, identifying individuals at the time of capture based on distinct colourations or by rearing individuals in aquaria until their juvenile colourations revealed their identity.

When comparing U_{crit} estimates between studies there are some potential sources of error that must be accounted for, such as differences in fish collection methods, and differences in U_{crit} estimation methods (i.e. length of time intervals, number of fish per lane in the swimming channel). To test for any bias in U_{crit} estimates between fish captured from different sampling devices I compared the U_{crit} values of individuals of one species, *C. capistratus*, caught in Belize during the 2005 field season using the two most commonly used sampling devices (light traps and crest nets). A paired t-test was used to test for differences in the U_{crit} estimates of fishes caught using the different techniques.

One-way analysis of variance was used to test for differences in U_{crit} between fish swum singly in the swimming chamber and those swum in schools, using the unknown Gerreid sp1. This species was chosen for this analysis because fishes of this species tended to school in the wild and were repeatedly caught in light traps and crest nets numbering in large numbers. I tested the effects for three experimental treatments, one fish per lane, two fishes per lane or three fishes per lane.

Another source of error in U_{crit} estimates could arise from differences in the length of the time interval between each incremental increase in current speed, because this will dramatically increase the length of time the fish is forced to swim. Fish swum for five intervals at an interval length of two minutes would have swum for only 10 minutes, whereas a fish swum for the same number of intervals at an interval length of 15 minutes would swim for 75 minutes. The length of time spent swimming could potentially affect the estimate of swimming speed if U_{crit} estimates are sensitive to energetic resource exhaustion. I experimentally examined the effect on U_{crit} estimates of swimming fish at interval lengths of two minutes and 15 minutes, using six common

species in Belize including, *S. partitus*, *S. diencaeus*, unknown Gerreid sp1, *C. capistratus*, *A. puncticulatus* and *A. quadrisquamatus*. A nested ANOVA was used to test the effects of varying the time interval in U_{crit} experiments.

We compared the swimming speed estimates of fish caught in two locations within the Caribbean Sea, Belize (BLZ) and the Turks and Caicos Islands (TCI). This comparison was possible for only six species from three families for which there were at least two individuals swum from each location (Table 5.3). U_{crit} was regressed against total length and the residual U_{crit} was taken for further analysis. Nested ANOVAs were used to test for significant differences between species for each location (TCI and BLZ) in both total length and residual U_{crit} measures. Sequential Bonferroni post hoc tests were used to determine specifically which species showed differences between locations in both total length and residual U_{crit}.

RESULTS

We measured the U_{crit} swimming speeds for 383 individuals from 46 species and 22 families (Table 5.1). There were significant differences in the mean U_{crit} among the species examined ($F_{37,344} = 15.43$, $p < 0.001$). The fastest swimming species was the Holocentrid, *S. coruscum* which had a mean U_{crit} of 72.07 $cm \cdot s^{-1}$ (Table 5.1). The slowest swimming species was the Ogcocephalid species, *O. nasutus*, individuals of which swam, on average, only 0.29 $cm \cdot s^{-1}$ (Table 5.1). The coefficient of variation (CV) at the individual level was calculated for all species with greater than one replicate. The average variation in U_{crit} at the individual level was 27% of the mean. On average, total length only explained 14% of the variation in swimming ability within species (Table

5.2), and varied only 17% of the mean species size. There was a significant positive correlation between total length and U_{crit} for individuals of two species, *A. puncticulatus* and *L. mahogoni* (Figure 5.1).

The level of variation across species (within families) was 29% of the mean (for n = 11 families). Analysis of variance showed that U_{crit} varied significantly among species in the family Monacanthidae ($F_{2,10} = 14.58$, $p < 0.01$), *S. setifer* being the fastest swimming species and the two *Monacanthus* species swimming nearly half as fast (Table 5.1). There were no significant differences found for all other families with more than two species for which greater than three individuals were measured, including Apogonidae, Lutjanidae, Pomcentridae and Tetradontidae. Variation across families was even higher than within families (CV = 63%), reflecting the large range in U_{crit} speeds at the family level. Thirty-six percent of the variation in U_{crit} among families was explained by body length ($P = 0.005$; Figure 5.2). The family with the fastest swimming species was the Holocentridae, followed by the Acanthuridae (Table 5.1). The slowest swimming family was the Ogcocephalidae. The Sygnathidae were the next slowest swimming family despite the fact that they were one of the largest families in terms of total length (Table 5.1; Figure 5.2).

Methodological Effects on U_{crit} Estimates

We tested for any bias in U_{crit} estimates associated with method of specimen collection. I found no significant differences in the swimming speeds of fishes caught using light-traps or crest-nets ($t = 0.73$; d.f. = 24; $p = 0.47$), there was also no difference in the size of fishes caught by the different devices ($t = -0.42$; d.f. = 24; $p = 0.68$). I also

tested for any possible effects of schooling (i.e. putting more than one fish per lane in the swimming chamber) on my estimates of U_{crit}. There was no significant difference in the U_{crit} estimates between fish swum singly or in a school of either two or three fishes ($F_{2,39} = 2.19$; $p = 0.13$). There was also no effect of varying the length of the time interval (t_i) in terms of swimming speed between fish swum at two minute intervals and those swum at 15 minute intervals for all six species (Nested ANOVA: $F_{6,178} = 2.00$; $p = 0.06$).

Regional Comparison of U_{crit} Estimates

There were significant differences in the total length between fishes from TCI and those from Belize ($F_{6,106} = 5.15$; $p < 0.001$). Two species (*A. bahianus* and *S. diencaeus*) showed significantly different total lengths between locations, in both cases the fish from TCI were larger than those from Belize (Figure 5.3a). Regression analysis was used to obtain residual U_{crit} values removing the effect of length. There were significant differences in the residual swimming speeds between fish from TCI and those from Belize ($F_{6,106} = 11.35$; $p < 0.001$). Post hoc tests revealed that there were significant differences between the two locations for five out of six species (Figure 5.3b). In all cases, both U_{crit} and residual U_{crit} were higher for fish from the Turks and Caicos Islands (Figure 5.3b).

DISCUSSION

The late-stage larvae of Caribbean reef fishes show the same exceptional swimming abilities as those from the Pacific (Fisher et al. 2005), with some species critical speeds reaching as fast as 72cm·s^{-1}. Indeed there is quite a large range of

swimming abilities across families and species, emphasising that swimming abilities may play a widely varying role in the ecology of the settlement stages of different taxa. I report here the slowest U_{crit} value for any species so far reported, the Ogcocephalid *O. nasutus* (mean U_{crit} 0.29 $cm{\cdot}s^{-1}$). It has been suggested that late-stage larvae can sustain swimming speeds roughly equivalent to 50% of their U_{crit} for 24 hours, which can be considered a measure of sustained swimming speed (Fisher and Bellwood, 2002; Fisher and Wilson, 2004). In terms of what has been considered an effective swimming speed, 71% of species reported here can sustain swimming speeds greater than the average minimum current speed around Turneffe Atoll (7.7$cm{\cdot}s^{-1}$) but only two percent can sustain speeds greater than the average mean current speed (28.4 $cm{\cdot}s^{-1}$) of the same area (current speed data from Tang et al., in review).

The mean variation in U_{crit} within species was 27% and only a small percentage of the variation was explained by length (Table 5.2). This is likely because the within species variation in length was very small (17%). Variation in U_{crit} within families was similar to the within species variation (29%). This value is higher than CV values previously reported at this taxonomic level on the Great Barrier Reef (CV = 14%, Fisher et al., 2005), and may reflect a greater degree of within family variation in size and/or body morphology in the present study. The variation across families was very high, 63% of the mean, much higher than previously reported values (Fisher et al. 2005). In addition, body length explained 36% of the variation in swimming speeds at the family level. The relationship between swimming ability and length has been shown over and over again in the reef fish literature (Leis and Carson-Ewart, 1997; Stobutzki and Bellwood, 1997; Fisher and Wilson, 2004, Fisher et al., 2005), as well as the non-reef

literature (Bainbridge, 1960) and length often explains only a modest portion of the variation in swimming abilities, other morphometric measures (or a combination thereof) are proving to be better predictors of swimming ability than simply length alone (Fisher et al. 2005).

Methodological Effects on U_{crit} Estimates

There are numerous studies that have used U_{crit} to examine the swimming abilities of fishes in relation to environmental factors (Brett and Glass, 1973; Green and Fisher, 2004), to pollutants (Kovacs and Leduc, 1982; Kumaragaru and Beamish, 1983; Cripe et al, 1984), to growth rate (Kolok and Oris, 1995). Although many of these studies will cite Brett (1964) as the source of the methodology, most have modified the original technique in some way. As a result, there exists no standard protocol in the literature for conducting U_{crit} experiments (Kolok, 1999) where such aspects as velocity increment size, interval length and the number of fish per channel will vary between studies. Furthermore, the sampling technique used to collect fish samples is not standardised among studies, this too could bias U_{crit} estimates if certain sampling methods are selecting for fish of a certain condition or developmental age. Light-traps could select for fishes in good condition since the fish are required to actively move toward the trap to be captured. Alternatively, crest-nets could bias U_{crit} by selecting for fishes that are later in development, possibly even those that have already settled.

These variations in methodology could be important sources of error potentially biasing comparative studies of U_{crit}, specifically intraspecific comparisons (Kolok, 1999). Here, I sought to compare the U_{crit} of individuals which varied in the methodology used

to measure them in terms of the sampling technique used to collect the specimens (light-traps versus crest-nest), in the number of fish swum per channel (between one and three fishes per channel), and the length of the time intervals (t_i, 2 min versus 15 min).

We found that there was no difference in the Ucrit estimates for individuals of the butterflyfish *C. capistratus* that were collected by either light-traps or crest-nets despite the potential for differential selection by the different techniques. I also found no difference in the size of *C. capistratus* collected by the different devices suggesting that, at least for this species, individuals caught by the two methods are at the same developmental stage. One noticeable difference between the two techniques was the species composition of the catches. Crest-nets collected a greater breadth of taxa than did light traps, this may be because light traps require active behaviour on the part of larvae, whereas crest-nets sample passively. Schooling is a well known phenomenon for many fishes and possible benefits include increased energy savings due to drafting and increased swimming performance caused by turbulent waves from schooling partners (Liao et al. 2003). As such, it seems possible that varying the number of fishes in the swimming channels may influence estimates of U_{crit} swimming speed. Despite these potential benefits I found no significant effect of schooling on the U_{crit} estimates of individuals of one Gerreid species, regardless of whether there was one, two or three fishes in the channel. This is especially remarkable given that this Gerreid species is found naturally to occur in schools. The fact that U_{crit} appears insensitive to possible advantages of schooling may be due to the fact that U_{crit} measures maximum speed, and that fishes do not reach metabolic exhaustion (Plaut, 2001). Schooling may, however, be important for energy savings during long term, sustained swimming experiments.

Aside from being robust to both the capture device used to obtain specimens, as well as, to schooling behaviour, U_{crit} estimates also appear to be relatively robust to the length of the time increments used during the experiments. I found no significant effect of varying the length of the time interval on U_{crit} estimates between 2 minute and 15 minute intervals. While there was no difference in the estimates based on time intervals of two and 15 minutes, it has been suggested that time intervals of greater than 30 minutes may lead to a decrease in U_{crit} estimates, subjecting fishes to trials that are more like sustained performances than U_{crit} performances (Kolok, 1999). The results I present here suggest that U_{crit} estimates are fairly insensitive to changes in time increment length, and that experiments of varying length produce similar estimates of maximum speed.

Regional Comparison of U_{crit} Estimates

Residual U_{crit} swimming speeds differed significantly between TCI and Belize for all but one species (*A. coeruleus*, Figure 5.3b). These differences in swimming ability are not due to differences in sizes of the fish between the two locations, with total length differing significantly between locations for only two of the six species (*A. bahianus* and *O. chrysurus*, Figure 5.3a), and total length explained less than three percent of the variation in the U_{crit} of those five species that showed regional differences in U_{crit} (Table 5.2). It is possible that the differences in intra-specific U_{crit} estimates can be explained by geographic distance/isolation between TCI and Belize. The Turks and Caicos Islands is a tropical Atlantic Ocean nation of eight major islands located southeast of the Bahamas, north of Dominican Republic and northeast of Cuba. Belize is a western Caribbean nation located immediately south of Mexico, in Central America. It is possible that

populations of fishes from these two locations are isolated and that region-specific selection and/or environmental conditions could explain differences in swimming performance.

However, the U_{crit} estimates from the two locations may be temporally confounded, because experiments in Belize were conducted primarily in the summer of 2005 (albeit some were done in summers 2003 and 2004), whereas the experiments in TCI were done during the winter of 2003. It is possible that conditions that affect swimming performance (i.e. food availability, Alsop and Wood, 1997; Fisher and Bellwood, 2001) could differ significantly between years and/or times of year, so it cannot be said definitively whether spatial or temporal factors are responsible for the observed differences in U_{crit} between conspecifics from these two locations. Regardless, this study represents the first temporal/spatial comparison of U_{crit} swimming performance and highlights that the swimming abilities of fishes can vary for species both spatially and/or temporally. It appears that spatial-temporal differences in swimming performance must be considered when comparing or combining work done in different places, during different years, or at different times of the year. It would be beneficial for future studies to further investigate the temporal and spatial variability of intra-specific U_{crit} estimates, focusing in on smaller geographic distances, and focusing on seasonal differences (winter vs. summer months). Interestingly, the differences observed here between the two locations appear to be consistent among all species examined, with speeds consistently faster in the Turks and Caicos Islands than in Belize. The spatial and/or temporal variability in swimming performance of fishes may have important consequences for spatial-temporal variability in the ecology of settlement-stage coral reef fishes.

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Table 5.1. U_{crit} for late-stage larvae of 47 species from 22 families of fishes caught from Calabash Caye, Turneffe Atoll, Belize.

Family/Genus/Species	N	U_{crit} ($cm \cdot s^{-1}$)	TL (mm)
Acanthuridae	**16**	**38.09 ± 6.66**	**32.56 ± 7.95**
Acanthurus bahianus (Castelnau, 1855)	11	36.3 ± 5.76	31.27 ± 9.42
Acanthurus chirurgus (Bloch, 1787)	1	42.00	33.98
Acanthurus coeruleus (Bloch & Schneider, 1801)	4	42.03 ± 8.57	35.77 ± 0.54
Antennariidae	**3**	**6.23 ± 6.37**	**10.40 ±2.95**
Unknown sp1	1	1.90	7.20
Unknown sp2	2	8.39 ± 7.28	12 ± 1.40
Apogonidae	**58**	**19.94 ± 5.26**	**17.31 ± 4.28**
Apogon maculatus (Poey, 1860)	11	19.85 ± 3.93	17.59 ± 3.25
Apogon planifrons (Longley & Hildebrand, 1940)	4	20.34 ± 4.45	19.16 ± 3.99
Apogon quadrisquamatus (Longley, 1934)	24	20.96 ± 6.45	20.29 ± 3.03
Astrapogon puncticulatus (Poey, 1867)	19	18.54 ± 4.83	13.33 ± 3.81
Carangidae	**7**	**20.61 ± 5.51**	**11.93 ± 4.26**
Unknown sp1	2	26.23 + 5.29	17.86 ± 1.62
Unknown sp2	5	18.33 ± 4.16	9.55 ± 1.37
Chaetodontidae	**37**	**32.31 ± 6.27**	**17.96 ± 3.48**
Chaetodon capistratus (Linnaeus, 1758)	36	31.67 ± 5.01	17.88 ± 3.52
Chaetodon striatus (Linnaeus, 1758)	1	55.22	20.00
Diodontidae	**1**	**6.67**	**11.93**
Chilomycterus antennatus (Cuvier, 1816)	1	6.67	11.93
Gerreidae	**32**	**28.72 ± 6.67**	**12.04 ± 3.02**
Unknown sp1	32	28.72 ± 6.67	12.04 ± 3.02
Haemulidae	**2**	**33.84 ± 9.48**	**16.15 ± 0.09**
Haemulon flavolineatum (Desmarest, 1823)	2	33.84 ± 9.48	16.15 ± 0.09
Holocentridae	**3**	**72.07 ± 16.05**	**36.19 ± 8.75**
Sargocentron coruscum (Poey, 1860)	3	72.07 ± 16.05	36.19 ± 8.75
Labridae	**12**	**26.97 ± 10.60**	**13.56 ± 4.14**
Clepticus parrae (Bloch & Schneider, 1801)	6	27.85 ± 1.78	16.88 ± 1.32
Doratonotus megalepis (Günther, 1862)	3	36.14 ± 13.50	8.16 ± 0.28
Xyrichtys spA	1	25.07	17.00
Unknown sp1	2	11.53 ± 4.63	10.00 ± 0.00
Lutjanidae	**29**	**32.37 ± 4.44**	**21.77 ± 4.26**

Lutjanus apodus (Walbaum, 1792)	16	32.22 ± 3.23	20.28 ± 3.10
Lutjanus mahogoni (Cuvier, 1828)	7	35.02 ± 6.18	25.99 ± 5.25
Ocyurus chrysurus (Bloch, 1791)	6	29.66 ± 3.72	20.59 ± 2.16
Monacanthidae	**13**	**22.88 ± 10.86**	**14.80 ± 7.79**
Monacanthus ciliatus (Mitchill, 1818)	5	15.14 ± 3.36	10.52 ± 1.89
Monacanthus tuckeri (Bean, 1906)	4	19.30 ± 8.67	19.02 ± 9.47
Stephanolepis setifer (Bennett, 1831)	4	36.14 ± 5.50	15.94 ± 9.59
Ogcocephalidae	**3**	**0.29 ± 0.13**	**6.37 ± 0.55**
Ogcocephalus nasutus (Cuvier, 1829)	3	0.29 ± 0.13	6.37 ± 0.55
Ostraciidae	**13**	**14.03 ± 2.85**	**8.06 ± 0.82**
Lactophrys bicaudalis (Linnaeus, 1758)	12	14.07 ± 2.98	8.14 ± 0.81
Lactophrys triqueter (Linnaeus, 1758)	1	13.52	7.21
Pomacanthidae	**2**	**29.30 ± 11.62**	**23.36 ± 2.6**
Holocanthus ciliaris (Linnaeus, 1758)	2	29.30 ± 11.62	23.36 ± 2.6
Pomacentridae	**105**	**34.18 ± 10.79**	**14.28 ± 2.46**
Abudefduf saxatilis (Linnaeus, 1758)	22	30.86 ± 13.32	15.02 ± 2.22
Microspathodon chrysurus (Cuvier, 1830)	1	31.62	16.80
Stegastes diencaeus (Jordan & Rutter, 1897)	39	37.49 ± 12.12	12.01 ± 0.99
Stegastes dorsopunicans (Poey, 1868)	1	31.52	14.97
Stegastes leucostictus (Müller & Troschel, 1848)	2	31.53 ± 0.01	14.21*
Stegastes partitus (Poey, 1868)	40	33.03 ± 7.22	16.06 ± 1.96
Scaridae	**1**	**6.00**	**9.00**
Unknown sp1	1	6.00	9.00
Serranidae	**10**	**24.15 ± 16.43**	**11.36 ± 2.26**
Epinephelus mystacinus (Poey, 1852)	4	13.61 ± 3.74	10.15 ± 1.26
Epinephelus spA	4	40.77 ± 13.42	13.75 ± 0.5
Unknown sp1	2	12.00 ± 2.12	9.00 ± 0.76
Sphyrinidae	**11**	**18.38 ± 3.12**	**18.55 ± 1.99**
Sphyraena barracuda (Walbaum, 1792)	11	18.38 ± 3.12	18.55 ± 1.99
Sygnathidae	**7**	**4.03 ± 2.78**	**30.42 ± 9.94**
Cosmocampus elucens (Poey, 1868)	7	4.03 ± 2.78	30.42 ± 9.94
Tetradontidae	**13**	**19.22 ± 4.82**	**16.14 ± 5.91**
Canthigaster rostrata (Bloch, 1786)	4	20.00 ± 5.18	18.36 ± 0.86
Sphoeroides testudineus (Linnaeus, 1758)	4	20.38 ± 2.53	21.26 ± 5.50
Sphoeroides spA	5	17.65 ± 6.29	10.26 ± 2.72

Table 5.2. Summary statistics for regressions carried out between U_{crit} and total length analysed at the individual level for 14 species.

Species	N	R^2	Slope	P-value
A. bahianus	11	0.01	-0.41	0.74
A. maculatus	11	0.05	0.26	0.51
A. quadrisquamatus	24	0.13	0.8	0.81
A. puncticulatus	**19**	**0.66**	**1.04**	**0.001**
C. capistratus	36	0.01	-0.12	0.66
C. parrae	6	0.00	0.04	0.95
L. apodus	16	0.09	0.33	0.26
L. mahogani	**7**	**0.67**	**0.96**	**0.02**
O. chrysurus	6	0.01	0.20	0.82
L. bicaudalis	12	0.18	1.55	0.19
A. saxatilis	22	0.06	1.47	0.30
S. diencaeus	39	0.02	-1.9	0.42
S. partitus	40	0.01	0.75	0.70
Gerriidae sp1	32	0.11	0.48	0.14

Table 5.3. Summary of sample sizes for cross-regional, intra-specific comparison of U_{crit} estimates.

Family/Species	Location	N
Acanthuridae		
A. bahianus	BLZ	10
	TCI	20
A. coeruleus	BLZ	4
	TCI	8
Lutjanidae		
L. apodus	BLZ	14
	TCI	2
O. chrysurus	BLZ	6
	TCI	2
Pomacentridae		
S. partitus	BLZ	20
	TCI	12
S. diencaeus	BLZ	14
	TCI	6

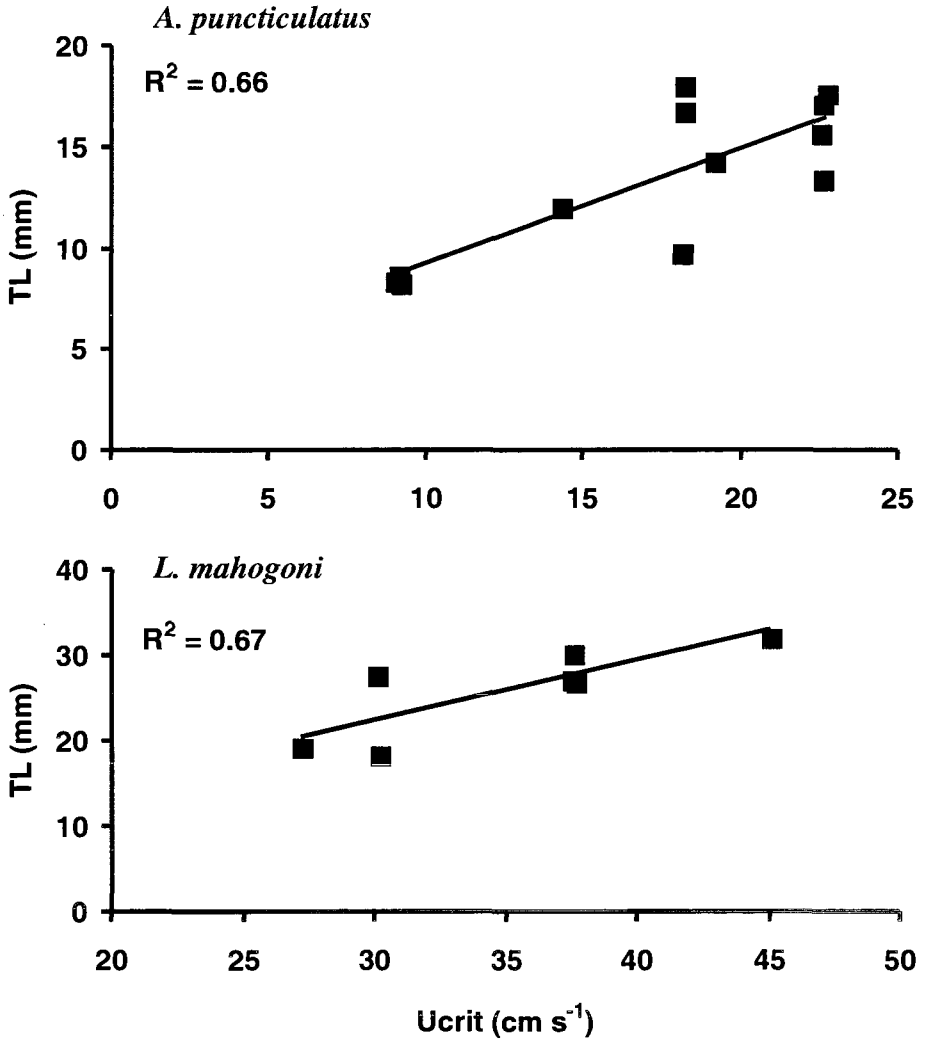
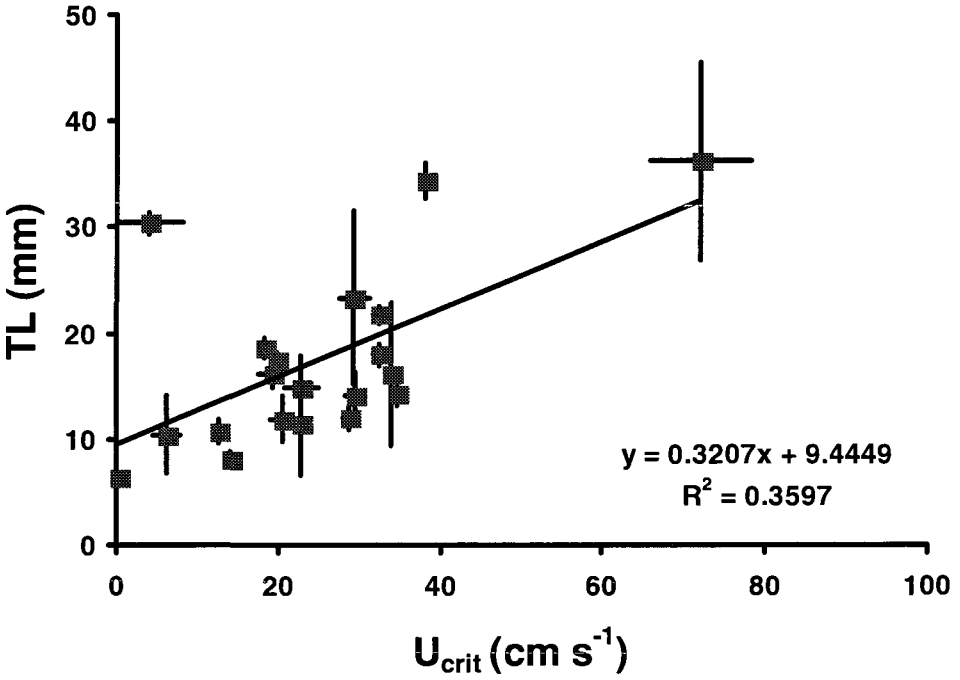


Figure 5.1: The relationship between U_{crit} and total length for individuals of two species, *A. puncticulatus* and *L. mahogoni*.

Figure 5.2: The relationship between mean U_{crit} and mean total length of the family for



those families with greater than one individual replicate. Error bars represent standard errors.

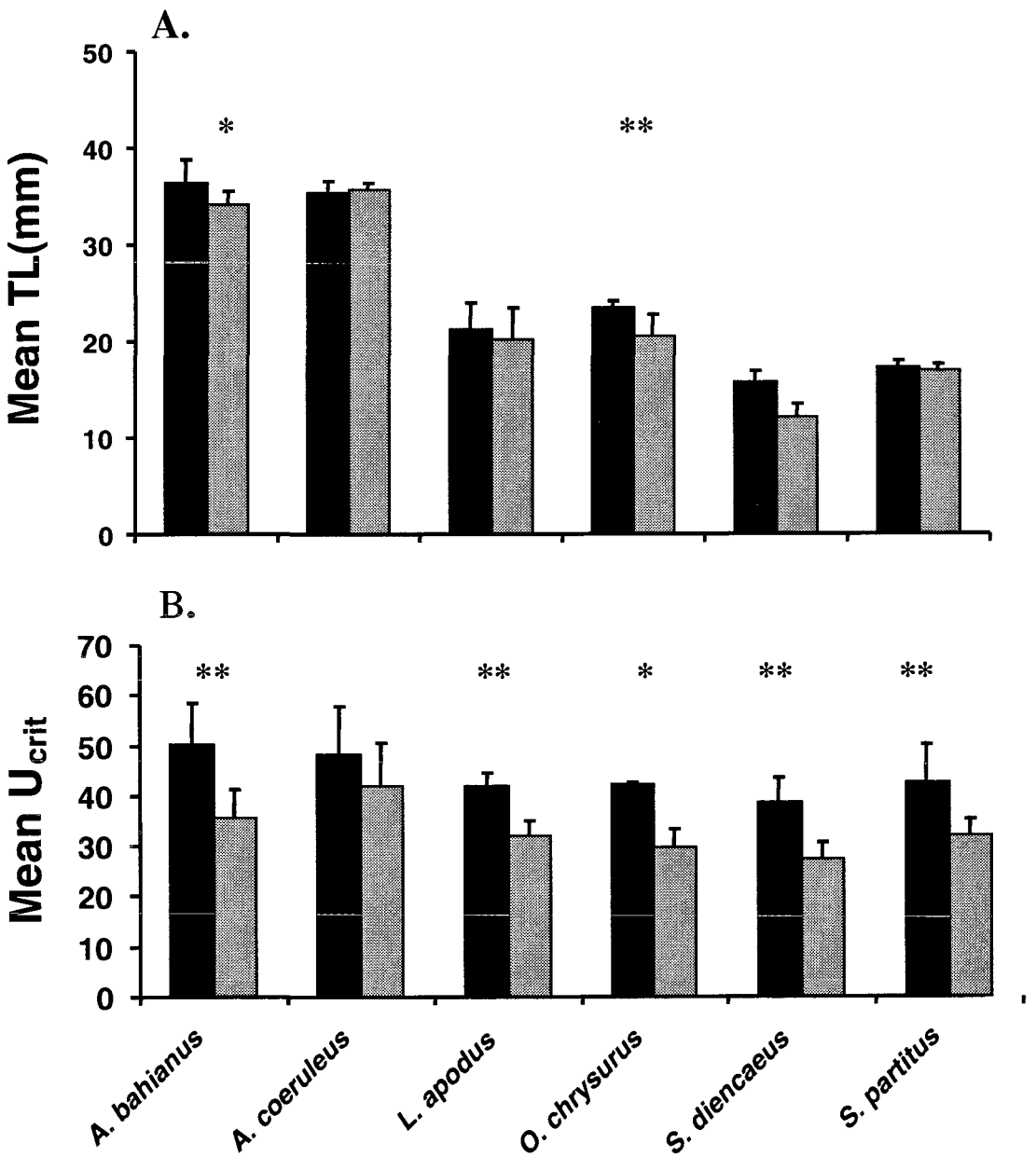


Figure 5.3: The mean total lengths (a) and mean raw U_{crit} values (b) for six species from both the Turks and Caicos Islands (black bars) and Belize (grey bars). Asterisks indicate significant differences between the two locations as determined by nested ANOVAs, * = $p < 0.05$ and ** = $p < 0.01$. In Figure 5.3b, Nested ANOVA was preformed using residual U_{crit} values. All error bars represent standard deviations.

Chapter 6: Experimental analysis of the contribution of swimming and drifting to the displacement of reef fish larvae

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J.D. Hogan and C. Mora.

INTRODUCTION

Dispersal is a key process in several aspects of the biology and conservation of marine species, such as reef fishes and other benthic organisms with pelagic larvae (Roberts 1997, Shulman 1998, Underwood & Keough 2001, Mora & Sale 2002, Planes 2002, Mora et al. 2003). However, studying dispersal in reef fishes has been challenging mainly because of technical difficulties associated with tracking small and behaviourally active propagules in vast expanses of water and the limited precision of alternative indirect approaches for tackling this process (reviewed by Leis 2002, Mora & Sale 2002).

Traditionally, there was a prevailing assumption among coral reef fish ecologists that larval dispersal was passively mediated by hydrodynamic processes (Sale 1970, Leis & Miller 1976, Roberts 1997). However, the recent recognition of behavioural and physiological capabilities of reef fish larvae has led to the belief that larvae are active in the pelagic environment and possibly capable of directing their own dispersal (reviewed in Kingsford et al. 2002, Leis 2002). Specifically, recent evidence shows that reef fish larvae are quite capable swimmers in terms of both speed and endurance (Stobutzki & Bellwood 1994, Leis & Carson-Ewart 1997, Stobutzki & Bellwood 1997, Stobutzki 1998, Bellwood & Fisher 2001) and can detect aural (Tolimieri et al. 2000, Leis et al. 2002) and olfactory (Atema et al. 2002) cues, both of which could be used for orientation. The realisation that larvae can propel themselves and possibly orient themselves in the pelagic environment has led to the idea that the larvae of reef fishes can navigate. Such an idea has profoundly affected our assessment of how reef fish populations are regulated (the open-closed population paradigm, see Warner & Cowen 2002, and associated papers,

Leis 2002, Mora & Sale 2002). The possibility of larval navigation has led to a reassessment of how hydrodynamic processes affect larval dispersal (cf. Roberts 1998, Sale & Cowen 1998, Bellwood et al. 1998), how dispersal should be mathematically modeled (Wolanski et al. 1997, Armsworth et al. 2001) and how marine reserves should be designed (Stobutzki 2000). Strong genetic differentiation found among populations has been attributed to navigational capabilities of larvae allowing them to return to their natal reefs (Taylor & Hellberg 2003). However, the extent of navigational capabilities and their use for dispersal purposes is poorly understood, and a subject of some interest in marine ecology (e.g. Roberts 1998, Colin 2003, Warner & Palumbi 2003), which highlights the need for detailed studies about larval navigation in reef fishes.

Effective larval navigation requires several physiological and behavioural attributes. Physiologically it requires the ability to detect and swim towards a reef while behaviourally it requires the larvae to "decide" to actively move to that reef (Mora & Sale 2002). Current knowledge suggests that larvae can detect reefs and have the swimming capabilities to reach them (see above), however, little is known about the behavioural component to navigation (but see Fisher & Bellwood 2003). The behavioural decision of reef fish larvae to swim is critically important because this is what determines the importance of the swimming ability itself (i.e. whether it is used for dispersal or not). At present, experimental trials intended to determine the swimming capabilities of reef fish larvae have been particularly important in putting an upper bound to the possible swimming performance of reef fish larvae. However, the behavioural link to this performance (or in my words the behavioural "decision" of larvae to use these capabilities) is missing because in these experiments larvae have been forced to swim (by

using small chambers with retaining fences; e.g. Stobutzki & Bellwood 1994, Stobutzki & Bellwood 1997, Stobutzki 1998) and because species that refuse to swim in these chambers have often been excluded from analyses (Leis and Stobutzki 1999). Some important attempts have been made to follow larval and post larval reef fishes in the field but the conclusions drawn from these studies are limited by the short duration of such observations (5-10 min) and by the fact that behaviours might be affected by the presence of divers (Leis & Carson-Ewart 1997, Leis & Stobutzki 1999, Hindell et al. 2003). This paper is intended to experimentally assess this behavioural component of swimming to larval displacement.

Larval displacement in the pelagic environment results from the interaction between hydrodynamics and the "navigational skills" of a larva. In the absence of active movement, hydrodynamics can contribute to displacement in the form of drifting. Drifting can be a component of larval displacement if water currents exceed the swimming capabilities of larvae and/or if larvae "decide" not to swim. This paper is intended to experimentally assess the contribution of swimming and drifting to the displacement of reef fish larvae. Here I used late-stage larvae of the Sergeant major, *Abudefduf saxatilis,* as my focal species. I first quantified the displacement of larvae in an extended flow chamber that reduced space constraints and gave larvae the behavioural freedom of movement (i.e. swim forward, backward, hold position or not swim at all). The contribution of swimming and drifting to this displacement was quantified by comparing the displacement observed in the larvae with that expected from passive particles. Similarities in displacement between the larvae and that expected from passive particles will indicate the existence of drifting while differences will indicate the

contribution of swimming. To assess the causes of drifting, I compared the swimming speeds observed in this extended chamber with the speeds achieved in a traditional chamber, which forces larvae to swim due to space limitations and a retaining fence. I expect the latter chamber to indicate the full extent of the swimming capabilities of the larvae while the former to indicate voluntary swimming speeds. Thus the comparison of swimming speeds between these chambers will indicate the extent to which larvae are voluntarily using their swimming capabilities, and to what extent larval displacement is influenced by the current. There have been many studies focusing on the physiology of swimming in fishes (reviewed by Webb 1993). The energetics of swimming in temperate fishes is well understood, and how this pertains to migrations has been investigated (see Dodson 1997). However, the capabilities of temperate fishes are not necessarily the same as those of coral reef fishes (Leis & Stobutzki 1999). Besides phylogenetic differences between the two groups, most coral reef fishes have fully developed fins at a smaller size than temperate taxa (Leis & Carson-Ewart 1997). Studies of swimming, and energetics of movement in tropical reef fishes are fewer and knowledge in this field is less comprehensive (Leis and McCormick 2002).

MATERIALS AND METHODS

Species analyzed and collection of specimens

I focus this study on the late-stage larvae of *Abudefduf saxatilis* (Pomacentridae). This species was chosen because it was the most abundant larval fish species at the study site. This species is commonly found throughout the Caribbean inhabiting shallow fore and back reefs (Humann & Deloach 2002). As larvae, *A. saxatilis* spends on average 18.2 days (± 1.1 SD) in the plankton (Wellington & Victor 1989). In this study, larvae were defined following Leis and Stobutzki (1999) as any reef fish that was still in the pelagic phase of life. Larval fish were collected and tested at the Institute for Marine Studies Research Station at Calabash Caye, in Turneffe Islands Atoll, Belize (17° 16.4'N, 87° 48.7'W). Late-stage larvae of *A. saxatilis* were collected from Fish Attracting Devices (FADs) using hand nets. A FAD consists of a black plastic hose joined together to form a cylindrical frame, which is covered in a black plastic mesh (4 cm mesh size). The FADs were placed at the end of the dock of the research station and were quickly colonized by late-stage larvae of this species. I used fish of the smallest size class present in the collections, and chose individuals within a similar range of body sizes (mean ± 1SD total length = 1.58 ± 0.24 cm) to avoid individuals that delayed their settlement, or had settled some time ago, and to reduce variations in swimming performance related to development. All specimens were collected in the morning and were held in fresh seawater with an aeration stone in a 24 L black plastic bucket for at least 4 hours before

trials. This allowed us to test fish right after collection and reduce stress associated with captivity (Pankhurst & Sharples 1992).

Laboratory Water System

Water to feed the two different chambers was provided through a unique water delivery system. Water was pumped directly from the ocean (via a 0.55 kw waterfall pump, Cal Pump model PW5000) through PVC pipe (diameter = 3.3 cm) to a head of 3.8 m. At the head, a U-joint returned water to the ocean. This allowed a constant water pressure at the base of the pipe where the chambers were joined to the system with a T-joint.

Contribution of swimming and drifting to larval displacement

The displacement of larvae was quantified in an extended swimming chamber, which reduces space constraints and gives larvae the freedom of swimming (i.e. swim forward, backward, hold position or not swim at all). This 280 cm long chamber was constructed from white plastic eaves trough (see Figure 6.1 for more details). Two identical sections of flow straighteners (each 10 cm long) were placed directly in front of the water inflow in order to reduce the turbulence of the flow throughout the chamber. The top of the inflow/flow-straightener section of the chamber was covered with clear plastic (further reducing the turbulence of the water entering the swimming area) and sealed with silicone adhesive. The swimming area of the chamber was left uncovered. Although I did not calculate the boundary layers of the extended chamber I observed that fish tended to

swim in the middle of the chamber, and did not seem able to take advantage of the boundary layers as they commonly drifted with the currents (see Figure 6.3c). I conducted some gross profiling of water flow within the chamber using passive neutral particles and found no eddy formation along the swimming area. Different flow rates within the chamber were attained by the use of a multi-turn gate valve at the inflow and a plastic gate at the outflow of the chamber (Figure 6.1). Both the valve and the gate were adjusted accordingly to attain the desired current speeds. Water speed was calculated by dividing the volume of water flowing over the outflow gate in a unit time by the cross-sectional area of the chamber. A 60 cm high wooden observation blind covered in black plastic sheet was placed over top of the chamber forming walls on all sides (Figure 6.1) to minimise observer disturbance. The observation blind reduced external visual cues that could be used for orientation. A tape measure was placed along the outer edge of the wall to allow observers to measure larval position along the length of the chamber.

Once the chamber was set, individual larvae were introduced into a mesh holding pen located in the middle of the swimming area (Figure 6.1) and allowed to acclimatise for 5 minutes at a current speed of 5 $cm{\cdot}s^{-1}$. Acclimatisation at 5 $cm{\cdot}s^{-1}$ was used to reduce the stress of the following exercise trial and to reduce the effects of disorientation associated with a sudden exposure to a current. The speed of the water was then adjusted accordingly to one of five experimental speeds: 5, 10, 20, 30 and 40 $cm{\cdot}s^{-1}$. These speeds were chosen to simulate the range of current regimes that could be experienced by a larva in the pelagic environment. Once the acclimatisation period was over, the holding pen was removed and the start and end positions of the larva were recorded at time intervals corresponding to the amount of time it would take a passive, neutral particle to travel 100

cm at each current speed (i.e. every 20 sec at 5 $cm{\cdot}s^{-1}$; 10 sec at 10 $cm{\cdot}s^{-1}$; 5 sec at 20 $cm{\cdot}s^{-1}$; 3.3 sec at 30 $cm{\cdot}s^{-1}$; 2.5 sec at 40 $cm{\cdot}s^{-1}$). A trial ended after either the larva swam to the front of the chamber (0 cm), or to the rear of the chamber (280 cm), or after seven time intervals had elapsed.

For each time interval in a trial, the difference between the start and end position of the larva in the chamber was taken as its displacement. The net displacement of that larva at that particular current speed was the average displacement for all time intervals of the trial. In comparison to the passive particle, a larval net displacement of 100 cm is equivalent to total passive drift. Negative values of net displacement indicate that swimming was forward into the current and that the larva swam faster than the water current (Figure 6.2). Positive values between 0 and 100 indicate that the larva faced the current but was pushed backwards by it (Figure 6.2). Positive values larger than 100 indicate that the larva swam in the same direction as the current (Figure 6.2).

The specific contributions of swimming (active movement) and drifting to the net displacement were quantified independently for each larva. I considered active movement as the amount of net displacement not due to the movement induced by the current (Figure 6.2). This was calculated as:

Active movement = $|$*Net displacement of the larva - Displacement of the passive particle*$|$

Drift was considered as the contribution of water current to the net displacement of the larva (Figure 6.2) and was quantified as:

Drift = Displacement of the passive particle - Active movement

Drift was quantified only in larvae with positive values of net displacement (i.e. that ended downstream of the releasing point; Figure 6.2). Any larva with negative net displacement (i.e. that ended upstream of the releasing point) did not experience any drift. In these cases drift values were recorded as zero. In any case where the net displacement of the larva exceeded 100 cm, a value of 100 cm of drift was assigned since in those cases drifting would have contributed maximally to larval displacement. In almost all cases, larval displacement in this chamber was less than 100 cm (see Figure 6.3a) therefore, values of drifting commonly varied between zero (i.e. swimming speed was stronger than or equal to the current speed) and 100 cm (not swimming at all). I used one-way ANOVAs to test for variations in net displacement, active movement and drifting among current speeds.

Causes of drifting

Larval displacement due to the effect of the current (i.e. drifting) can result from the water flow exceeding the swimming capabilities of the larvae and/or if larvae decide not to swim despite being capable of doing so. To assess these possibilities I compared the swimming speeds observed in the extended chamber (speeds voluntarily selected) with the set of speeds achievable for this species when forced to swim. Larval speeds in the extended chamber were calculated by dividing the amount of active movement in a trial by the length of that trial (Figure 6.2). Here I quantified achievable swimming speeds as U_{crit} using a smaller chamber similar to that originally designed by Stobutzki & Bellwood (1994).

The single lane flow chamber has a swimming area with internal dimensions of 18.5 cm in length and 5.0 cm for both width and height. The fish are restricted to the swimming area by the use of metal retaining fences on the inflow and outflow ends. Water flow into the chamber was regulated with a multi-turn gate valve and water velocity was controlled with a second multi-turn gate valve exiting the chamber. The second gate valve was calibrated, based on the number of turns, to provide different current speeds within the chamber. Current speeds were calculated by dividing the volume of water that passed through the chamber in a unit time by the cross-sectional area of the swimming area. The maximum water velocity achieved by this system was 60 $cm{\cdot}s^{-1}$. In the smaller chamber used to measure U_{crit}, the size of the boundary layer was calculated to be 4mm in size along all sides at a current speed of 32 $cm{\cdot}s^{-1}$. This value was less than 10% of the total volume of the swimming area and was very small compared to the size of the fish used.

Individual fish were introduced into the chamber at a current speed of 2 $cm{\cdot}s^{-1}$ and allowed to acclimate for 10 min. Current speed was then increased incrementally by three body lengths per second (where body length is the total length of the individual), at intervals of two minutes, until the larva could no longer maintain position in front of the metal retaining fence. U_{crit} swimming speed was calculated following Brett (1964) where:

$$U_{crit} = U + (t/t_i \, X \, U_i)$$

U is the penultimate speed, U_i is the velocity increment, t is the time swum in the final velocity increment and t_i is the time interval for each increment.

RESULTS

All the behavioural responses measured in the extended chamber showed strong trends with current speed (Figure 6.3a-c and Figure 6.4) and showed significant differences among current speeds (Displacement: $F = 9.79$, $p < 0.00004$; Active swimming $F = 9.79$, $p < 0.00004$; Passive movement: $F = 9.13$, $p < 0.00007$; Swimming speed: $F = 9.72$, $p < 0.00004$; n = 34 larvae for all tests). Overall, larval displacement was positive and increased with current speeds (Fig 3a), meaning that fish ended downstream of their starting position. Only at a current speed of 5 $cm \cdot s^{-1}$ did larvae tend to overcome the current, and finish upstream of their starting position. The contribution of active movement to net displacement tended to decrease with increasing current speed (Figure 6.3b) while the contribution of drifting increased exponentially with current speed (Figure 6.3c). During the behavioural trials, larvae were observed swimming in the centre of the chamber. General swimming direction was more variable at the slowest current speed ($5 cm \cdot s^{-1}$) but in general larvae faced the current, did not exhibit erratic swimming bursts at any current speed, and most drifted backwards with the current particularly as current speeds increased (Figure 6.3c).

Within the extended chamber there was a four-fold variation in larval speeds among currents. Larval swimming speed tended to increase proportionally at each successive current speed until ~ 20 $cm \cdot s^{-1}$ at which point swimming speeds tended to remain constant despite any further increases in current speed (Figure 6.4). In the extended chamber, the minimum and maximum mean swimming speeds were 5.22 $cm \cdot s^{-1}$ and 22.5 $cm \cdot s^{-1}$ respectively, while fish in the smaller chamber achieved faster swimming

speeds (mean U_{crit} = 31.8 $cm \cdot s^{-1}$, SD = ±11.0 $cm \cdot s^{-1}$, n = 7, maximum = 59.5 $cm \cdot s^{-1}$) (Figure 6.4). No individual larva in the extended chamber achieved a swimming speed greater than the mean U_{crit} value for this species.

DISCUSSION

Experimental studies have been critical in advancing our understanding of ecological processes that would otherwise be impossible to assess under complex natural conditions. However, in extrapolating experimental data to natural conditions it is also important to be aware of potential biases. In this study, I was conscious of two possible limitations that could have affected the results. I observed that almost all larvae faced the oncoming current and that their swimming speed mimicked slower current speeds. This indicates larval awareness of the hydrodynamic environment they are in. This was very likely achieved through the use of visual, tactile or mechanosensory references within the chamber that inform larvae of their displacement by the water flow. Such references are likely absent in the open water (unless larvae have capabilities permitting long-distance detection of reefs; note that the longest distance larvae have been shown able to orient towards reefs is 1 km; Leis et al 2002). The fact that larvae drifted in the chamber despite the presence of such visual references suggests that the estimates of drifting are conservative and that drifting may be more extensive in natural conditions where visual and/or other orientation cues are likely to be absent. The second limitation to address is the possibility of non-laminar water flow in the chamber. Turbulence is an important limitation influencing the results of all experimental studies on swimming ability and

behaviour. Although this bias is difficult to control, I attempted to reduce the turbulence of the flow by adding two sections of flow straighteners. In spite of this potential bias, I observed some larvae, when trials were over, holding position at the outflow gate of the chamber. This suggests that larvae were capable of swimming faster than the current despite any effect of turbulence and the fact that they did not do so in experimental trials suggests that they voluntarily drifted during those trials.

The relative contribution of drifting and swimming to the net displacement of larvae in the plankton has been a topic of debate in the recent reef fish literature (cf. Roberts 1998 vs. Sale & Cowen 1998, Bellwood et al. 1998 and Taylor & Hellberg 2003 vs. Colin 2003) but it certainly remains as one of the current challenges to reef fish ecologists. My experimental approach showed that net downstream displacement of late-stage larvae of *Abudefduf saxatilis* tended to increase as the current speed in the chamber increased (Figure 6.3a). Since active movement tended to decrease with current speed (Figure 6.3b) and larval swimming stayed relatively constant beyond moderate current speeds (Figure 6.4), the increase in net displacement with current speeds was better explained by drifting (Figure 6.3c). However, drifting itself can be caused by (1) current speeds exceeding the capabilities of the larvae and/or (2) the larva exercising behavioural control over its swimming speed. Here I found that in the extended chamber, larvae swum at speeds between one and six times slower than those they achieved in the smaller swimming chamber. This suggests that when the behavioural choice is given to larvae they do not use the entire potential of their swimming capabilities. This result certainly raises caution for the use of published swimming capabilities of reef fish larvae to model the patterns of larval dispersal. Interestingly enough, drifting increased rapidly with

incremental increases in current speed (Fig 3c). This is very likely due to the combination of behavioural drifting, observed at almost all speeds, and the effect of high-speed currents that exceeding larval capabilities (Figure 6.4). Although it is difficult to determine whether behavioural choices in the laboratory will reflect those made in the field, the decision to drift rather than resist the current is a feasible option particularly in terms of energy saving.

Energy resources allocated to swimming will have major impacts on metabolic resource bases, net energy gain from foraging, and reserves required for migrations (Webb 1993). In reef fish, energy reserves are necessary for successful settlement (McCormick 1998) and post-recruitment survival (Bergenius et al. 2002). It is also know that sustained swimming has significant effects on lipid and carbohydrate concentrations in the body (Stobutzki 1997), and that reef fish get exhausted faster as current speeds increase (up to 50 times faster with only a four fold increase in water current; Fisher & Bellwood 2002). Therefore, there are demonstrated benefits of conserving energy during the pelagic stage, which might be achieved by drifting. Although, this balance between energy and swimming can be further complicated by the availability of food (Fisher & Bellwood 2001) the estimates of drifting are reliable because I tested larvae soon after their collection. This reduced the likelihood of starvation effects on the results.

Gross cost of transport (GCOT) is the measure of the overall impact that swimming speed has on energy costs. GCOT is defined as the energy required to move a unit mass through a unit distance (Webb 1993). The GCOT – swimming speed relationship is U-shaped (Webb 1993), the result is that optimal swimming performance (i.e. the most energy efficient swimming speed) will fall somewhere below a fish's

maximum swimming velocity. For instance, Bernatchez and Dodson (1987) showed that the energetic cost of migration increased as fish deviated from their optimal displacement rate. In *Abudefduf saxatilis*, I observe that the maximum swimming speed in the extended chamber is ~ 70% of its mean U_{crit}, and ~ 40% of the maximum U_{crit} value. The U-shaped relationship between GCOT and swimming speed implies that as current speed increases, it becomes no longer economical to attempt to overcome that current. This further supports the idea that, under natural conditions, larval fish may choose to swim well below the swimming capabilities previously reported for reef fishes.

Although I describe drifting only for the late-stage larvae of a single coral reef fish species, I expect this behaviour to vary both among taxa and ontogenetically within species. Indeed, drifting has been previously observed in pelagic larvae. Bishai (1960), observed larvae maintaining position in the current at slow current speeds, however at higher speeds, the larvae drifted backward while still swimming against the current. Similarly, Hindell et al. (2003) doing *in situ* observations on pelagic post-larval fishes of temperate species found the movement patterns of these fishes were related to the speed and direction of currents in ocean waters greater than 7 m depth. Based on variations in the cost of swimming among species of different body size one also could expect inter-specific variations in the degrees of drifting. Among species, the optimum swimming speed tends to decrease with increasing size of the species (Webb 1993), due to increased energetic cost of swimming with increasing body size. This suggests that under similar current regimes, larger species might be expected to drift to a greater degree than smaller species. Within species, swimming abilities tend to be poor early in ontogeny and increase exponentially with age (Fisher & Bellwood 2000). Furthermore, newly hatched

larvae experience low to intermediate Reynolds numbers (Bellwood & Fisher 2001) which are characterised by viscous forces, creating drag (McHenry et al. 2003). This will increase the amount of energy required for active movement in young larvae and probably will affect their "decision" to swim. Since swimming abilities tend to be poor early in ontogeny it is likely that the late-stage larvae I used give a conservative estimate of the degree to which fish choose to drift, and that early larval stages are more likely to choose this option.

Here I have shown the importance of drifting on the net displacement of larvae in experimental conditions. In natural conditions drift can reduce energy expenses, which can increase the probability of successful completion of the larval period and survival after settlement. Furthermore, this study suggests that published data on swimming capabilities of reef fish larvae cannot be easily used to assess the extent to which these fish actively affect their dispersal. Finally, it is important to consider that the strong swimming abilities with which reef fish larvae are equipped may have evolved for purposes other than dispersal, such as improving prey capture or predator avoidance and/or for the location of suitable habitat at settlement by late-stage larvae. Further behavioural studies are required if we are to fully understand how larvae behave in open water and how this affects their dispersal.

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Figure 6.1: Schematic representations of the lateral, over-head and cross-sectional views of the extended swimming chamber. Diagram not to scale. See text for description.

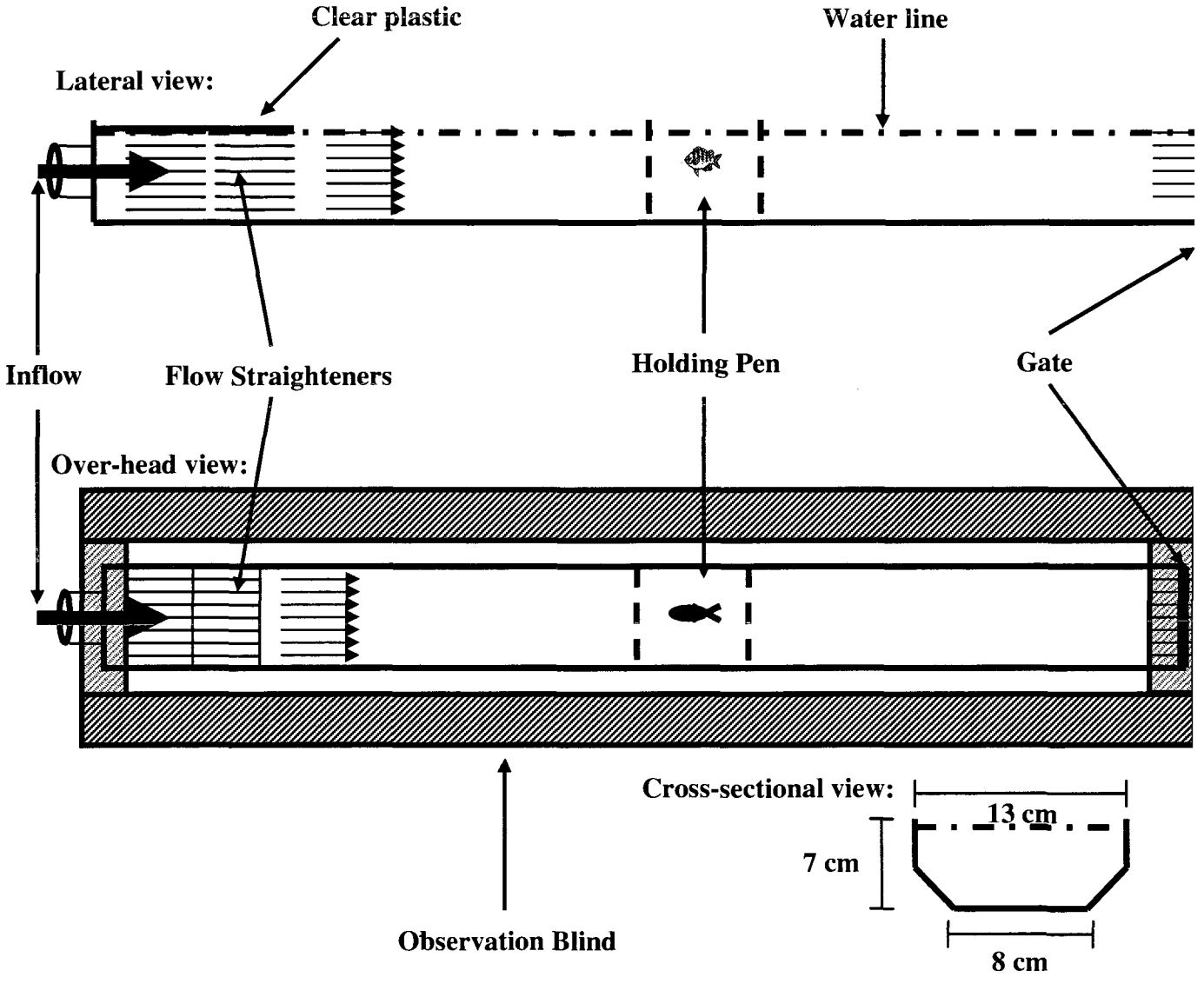
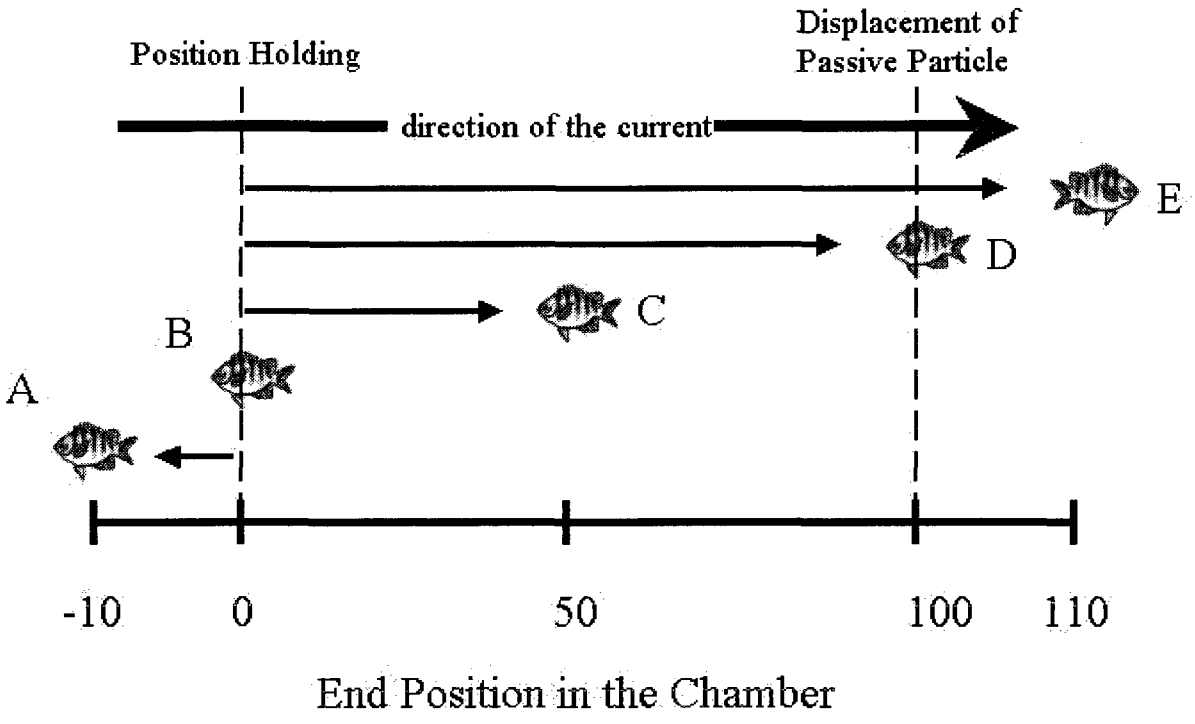


Figure 6.2: Possible behavioural responses of fish larvae in the swimming chamber and the respective values corresponding to each of the four variables measured in this study. The end position of each larva was recorded after a time interval equal to the time required for a passive particle to travel 100 cm at that current speed. As a case example, larva "A" ended 10 cm ahead of the starting position. Its displacement was -10 (negative sign indicates that it swum forward and faster than current). The active swimming value of this larva was the 100 cm it swam to overcome the current plus the 10 cm it displaced forward thus totaling 110. Although water current reduced the absolute displacement of the larva from its starting position, in this case it did not contribute directly to the displacement. Therefore, drifting (or the contribution of water current to displacement) was zero. Swimming speed for this larva was calculated by dividing the distance it traveled actively by the length of the time trial. Similar examples are given for when larvae ended behind the releasing point (larvae B to E).

Figure 6.2: See page above for figure caption.



	A	B	C	D	E
Displacement	-10	0	50	100	110
Active Movement	110	100	50	0	10
Passive Movement	0	0	50	100	100
Swimming Speed	110 / t	100 / t	50 / t	0 / t	10 / t

Figure 6.3: Variations in larval displacement at different current speeds (a) and how that displacement is accounted for by active swimming (b) and drifting (c). In the plot (a), the dashed line represents the displacement of a passive particle for the duration of one time interval. Numbers in brackets indicate sample size. All values expressed as mean (± 1SD). Trend lines were fit to mean values.

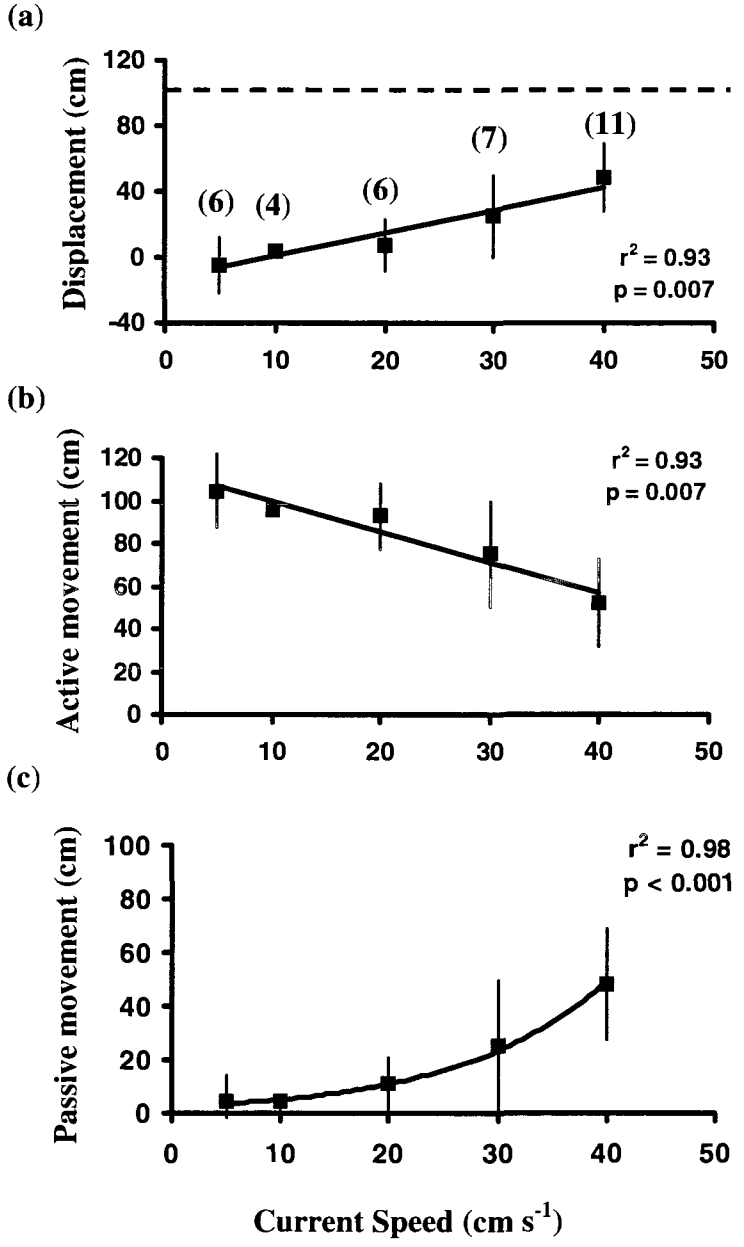
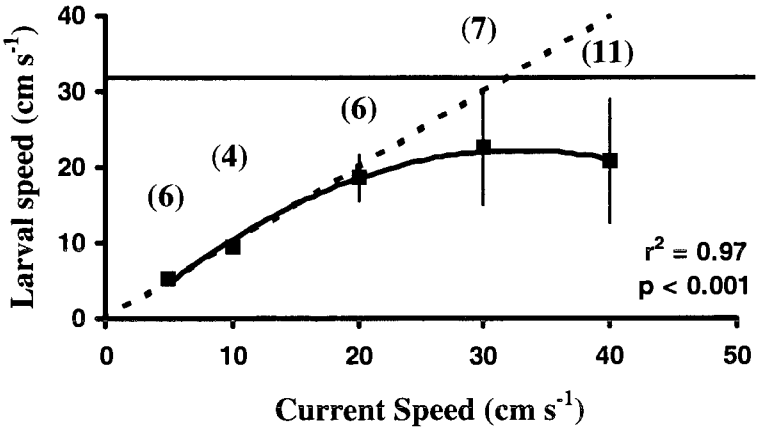


Figure 6.4: The relationship between current speed and voluntary swimming speed. The solid horizontal line represents the mean U_{crit} for *Abudefduf saxatilis*, and the dashed line represents the one to one ratio. All values expressed as mean (± 1SD). The trend line was fit to mean values. Sample sizes in brackets.



Chapter 7: General Discussion

In this thesis I have endeavoured to gain insight into the life history of larval reef fishes. I have used a variety of techniques to investigate the biology and ecology of these animals as it pertains to their dispersal and recruitment to reef populations. The results of my studies have shown that reef fish larvae are active animals capable of affecting their dispersal trajectories (see Chapters 5 and 6). I have shown, for one damselfish species *Stegastes partitus*, that long distance dispersal of larvae is possible, however, the majority of dispersal likely occurs at the meso-scale level (Chapter 4). While dispersing, larval fishes possibly stay in discrete patches, and these larvae in patches can synchronously replenish reefs spaced tens of kilometers apart (Chapters 2 and 3). I found that the spatial scale of coherence in recruitment varies by taxa, but coherent replenishment tends to occur on a meso-scale (Chapter 2). Understanding the biology of larval reef fishes and defining the scales of dispersal and variation in recruitment will allow future studies to investigate the processes that affect the replenishment of populations on reefs.

Refining our understanding about larval behaviour and swimming performance

It was initially assumed that reef fish larvae were no more than plankton floating along with ocean currents (Leis and McCormick, 2002). The earliest studies of the behaviour of temperate fish larvae showed that these animals were relatively poor swimmers (Blaxter, 1986; Miller et al., 1988) and reef fish larvae were assumed to be similar in capacity. However, starting in the mid-90s studies, conducted in the Pacific, on the behaviour of reef fish larvae showed that these animals were very competent swimmers (Stobutzki and Bellwood, 1994) and it was recognized that larval swimming

could potentially affect the ecology of these species up until the point of replenishment (Stobutzki and Bellwood, 1997; Wolanski et al., 1997; Armsworth et al., 2001; Leis, 2002). Data in chapter 5 show that Caribbean reef fishes have similarly strong swimming abilities compared with their Pacific cousins. In chapter 6, I attempt to refine estimates of swimming performance to give a more realistic idea of the speeds that larvae will likely maintain in the wild. Other recent studies have also helped refine these swimming performance estimates (Fisher and Bellwood, 2003; Leis and Fisher, 2006) and it is now thought that larvae will be capable of sustaining swimming for extended periods at speeds that are approximately 50 – 70% of those measured in experiments (Leis and Fisher, 2006; Chapter 6 of this volume). I also demonstrate for the first time regional variation in swimming performance of the same species (Chapter 5). There appears to be some spatial and/or temporal variation in swimming performance that could potentially influence the spatial and temporal variation in replenishment of reef fish populations. Future studies could focus on this spatio-temporal variation to better define the scales at which it occurs and relate it back to variation in dispersal or recruitment.

The scale of larval dispersal and its consequences for population replenishment

In order to investigate the factors that affect variation in replenishment of reef fish populations, we must define the scales of larval dispersal. Estimates of the scales of dispersal in reef fishes range from 10s to 100s of kilometers (Palumbi, 2003; Planes 2002; Cowen, 2002). Data from chapter 4 suggest there is a range of dispersal distances that are likely. I found that some larvae of the damselfish *Stegastes partitus* dispersed over 300 km from their natal reef in the span of a single larval period. However, during

the same period of time, approximately 45% of larvae for which dispersal distances were estimable dispersed 60 km or less from their natal sites. The results of this study were corroborated by two other studies conducted on the same system with the same species (Mora, 2004; Chittaro, 2005). The fact that dispersal is variable is not surprising considering the number of factors that can potentially affect the outcome of dispersal, including variable pelagic resources, hydrodynamic features and larval behaviours. While I show only spatial variability in dispersal, it is likely that there is temporal variability as well. This idea is corroborated by Chittaro (2005), where he showed that connections between sites at Turneffe Atoll were variable across months and years. The studies by Mora and Chittaro as well as the results from chapter 4 here show that a moderate proportion of larvae disperse distances 60 km or less. This is most interesting because this suggests that processes that act on this scale (the meso-scale) that affect the success of larvae are likely the most important factors for determining variability in replenishment of populations and this is the scale that is most relevant to metapopulation ecology and ultimately fisheries management.

Recruitment coherence, larval patches and connectivity among reefs

Much is already known about recruitment of larval reef fishes. At the scale of a single reef it can be highly variable through time, and at any given time recruitment to two reefs, even closely spaced together, can be highly variable (Doherty and Williams, 1988). One important observation is the coherence between sites in the timing and relative magnitude of replenishment. Recruitment coherence can lend insight into the dynamics of larval fish assemblages, and can be used to infer the scales of connections

between reefs and the scales at which pelagic processes will act to cause variation in replenishment (Victor, 1984; Pitcher, 1988; Myers, 1997; chapter 2 of this volume). In chapter 2, I determine the spatial scale of recruitment coherence for a variety of common reef fish species from the Caribbean. I show that for the most part the spatial scale of recruitment coherence occurs between sites spaced between 10 and 50 km apart. This suggests that processes that act on a meso-scale are most likely to cause variation in recruitment, like certain hydrodynamic features such as eddies, environmental factors like storms etc.

The scale of recruitment coherence might indicate the scale at which sites are linked by the supply of larvae from source populations. In chapter 3, I show that site-pairs coherent in recruitment received larvae that had experienced more similar pelagic environments (at least in the latter portion of the larval stage). This result suggests that patches of larvae arriving to reefs may remain coherent for some time during the development of the larvae. This again supports the idea that factors acting on a scale relevant to the size of larval patches (meso-scale) should be the most important factors for causing variation in replenishment. From these data it was not possible to tell whether fishes recruiting in coherent pulses originated from the same source populations. The next evolution of this research should attempt to use environmental signals as recorded chemically by otoliths to back track to the source of these larvae and determine whether the larval patches remain coherent from spawning until settlement.

General Conclusions

The goal of this thesis was to use a variety of studies to gain insight into the poorly understood larval life stage of reef fishes. One unifying theme from the results of this work is that there is a lot of variability in replenishment of reef fish populations. That variability can arise from factors that affect the pelagic larvae. In this thesis I elucidate patterns from the variability in an attempt to more accurately focus research into the factors that cause the variability. I have used recruitment surveys and otolith microchemistry to better understand the spatial and temporal scale of recruitment coherence and to infer the distribution of larval fishes prior to settlement. I used otolith chemistry in an attempt to determine the spatial scale of larval dispersal. I also measured swimming performance and behaviour in an attempt to explain some of the variation in dispersal and recruitment. I conclude that future studies on the ecology of larval reef fishes should focus on processes occurring on the meso-scale. It seems likely that this is the scale at which processes will act to cause variation in larval abundance and distribution and possibly variation in the replenishment of reef fish populations.

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Vita Auctoris

Name:	James Derek Hogan
Place of Birth:	Etobicoke, Ontario, Canada
Date of Birth:	17 October 1978
Education:	2002 – 2007 Ph.D. University of Windsor, Canada.
	1997 – 2001 B.Sc. Brock University, Canada.