UNIVERSITY OF CALIFORNIA, SAN DIEGO

Rocky Reef Fish Connectivity: Patterns, Processes, and Population Dynamics

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

in

Oceanography

by

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The Dissertation of Geoffrey Scott Cook is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

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DEDICATION

To Mom and Dad. Thanks for everything.

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ACKNOWLEDGMENTS

I recall visiting various schools prior to commencing my PhD, and at one of them I interviewed with a former student of Lisa. During our discussion I mentioned that I was contemplating working with Lisa at SIO. At hearing this he described her as the best mentor he had ever had or known, suggesting further that if I happened to get into Scripps, I should go. He did not steer me wrong. Lisa is an amazing mentor, and in large part this dissertation was made possible by her support and encouragement. She has incredible insight, unparalleled depth of knowledge, and an uncanny ability to not lose focus of the big picture. For advising and mentoring me, and providing me with a scientific home over the past few years I am forever grateful.

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ABSTRACT OF THE DISSERTATION

Rocky Reef Fish Connectivity: Patterns, Processes, and Population Dynamics

by

Geoffrey Scott Cook

Doctor of Philosophy in Oceanography

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Professor Lisa A. Levin, Chair

The extent to which marine populations are connected by dispersing larvae and the ramifications of this connectivity for population dynamics was investigated for the temperate damselfish, *Hypsypops rubicundus*, in San Diego County, USA. Surveys identified six source populations for this species: Carlsbad, Cardiff, Torrey Pines, La Jolla, Mission Point, and Zuniga Point. Three of these reefs are within or adjacent to existing marine protected areas. Trace elemental fingerprinting was used to quantify the connectivity of populations in 2008-2009. High-resolution sampling over a protracted spawning season revealed that elemental fingerprints of reefs earlier in the spawning season became indistinguishable from other reefs later in the spawning

season, resulting in inaccurate assignment of natal origin of post-dispersal fish. When natal origins of fish were assessed using appropriately binned data, one reef, La Jolla, emerged as the predominant source population, supplying itself and three other reefs with recruits. The northernmost reef, Cardiff was a "pure" sink, in that it unilaterally imported fish. Dispersal trajectories predominantly were in a northerly direction, but sporadic southerly dispersal was documented, corresponding to empirically measured current reversals. On intra-annual time scales this network of reefs resembles a source-sink metapopulation, but over annual time scales it functions as an open metapopulation with a well-mixed larval pool. To assess the demographic significance of observed connectivity patterns, empirically parameterized, stage-based matrix models were coupled with connectivity matrices. Elasticity analyses suggest inter-reef connectivity acts primarily to regulate which vital rates are demographically most significant; at low levels of connectivity adult survivorship has the greatest influence on population growth rate; at high levels of connectivity, juvenile growth is most influential. Quantitative metrics of sources and sinks were developed and node deletion experiments conducted to better characterize reef connectivity within the metapopulation. La Jolla was identified as the most valuable reef within the metapopulation in terms of connectivity; it may regulate how populations of fish at other reefs persist over time, and as such should be a conservation priority. New knowledge of the magnitude, directionality and variability of connectivity, and its roles in regulating local and metapopulation dynamics will aid local marine conservation efforts.

CHAPTER I.

INTRODUCTION

Over the past decade there has been a growing interest in the subject of marine population connectivity (Cowen and Sponaugle 2009). Connectivity broadly-defined is the movement of individuals among discrete patches of suitable habitat (Kritzer and Sale 2004). This movement among habitat patches is tied inherently to the functioning of ecosystems (Lubchenco et al. 2003); and for those interested in the conservation of marine biodiversity its purported effects can be viewed as both positive and negative. On one hand, high levels of connectivity can act negatively by enabling the spread of invasive species, threatening the persistence of endemic populations (Levin 2006 and references therein); on the other hand, high levels of connectivity among populations of a threatened species can, in theory, facilitate the recovery and restoration of a population, increasing its resilience to predicted changes in climate and its probability of persistence over time (Hastings and Botsford 2006, Botsford et al. 2009).

Marine protected areas (MPAs) are an emerging tool for protecting biodiversity, sustaining productivity, and allowing for continued extractive uses of the marine environment (Dayton et al. 2000, Russ and Zeller 2003). Over the past decade, MPAs have gained rhetorical acceptance in fishery management plans as a means of mitigating uncertainty and bet-hedging against management failure while promoting marine habitat protection (Pauly et al. 2002, Russ 2002). The design of MPAs rests on two critical principles: 1) spillover (export of biomass via juvenile/adult emigration)

from within a reserve into adjacent waters; and 2) population connectivity on a regional scale via pelagic egg/larval dispersal. Many coastal marine species have a complex life history, with individuals being relatively sedentary during juvenile and adult life stages while eggs and/or larval life stages disperse broadly through the water column. Although there is evidence that MPAs provide spillover effects (see Palumbi 2002, Russ 2002); the degree to which population connectivity occurs is debatable (Russ 2002, Sale et al. 2005).

Historically it was believed that early life stages could disperse passively for up to 1000s of kms, resulting in open population dynamics (Caley et al. 1996, Shanks et al. 2003 and references therein). However, in recent years there has been growing evidence that larvae do not disperse as widely as first imagined (reviewed in Levin 2006, Cowen et al. 2007, Cowen and Sponaugle 2009), and some larvae are able to settle back to the same benthic environment in which they were spawned, a process deemed self-recruitment (Jones et al. 1999, Jones et al. 2005). Generalizations regarding the extent to which these connectivity patterns impact population dynamics along open coastlines and non-coral reefs is largely unknown, as there have been few empirical estimates of self-recruitment and the magnitude of larval exchange between local patches has been rarely quantified.

Due to a lack of connectivity-model validation and conflicting results from field trials using various stocks, much uncertainty remains. Many regard larval dispersal in marine ecosystems to be one of the "major unsolved problems of biological oceanography" (Palumbi 1999), and it is seen as "one of the crucial gaps in

scientific knowledge" facing marine ecologists, resource managers, and policy makers (Sale et al. 2005). Accurate and robust estimates of well-defined larval dispersal and well-defined local retention are essential prerequisites to resolving marine recruitment patterns and to design effective marine protected areas (Thorrold et al. 2002, Botsford 2005, Botsford et al. 2009, Cowen and Sponaugle 2009). In addressing this challenge, the unifying goal of this project was to increase our understanding of the larval connectivity of nearshore rocky-reef fishes inhabiting a network of MPAs located along the San Diego County coastline, and to develop methods for exploring the implications of observed connectivity patterns for population dynamics, ultimately bettering our ability to preserve and protect marine biodiversity. Using garibaldi (Appendix 1.1) as a model system this study addresses these shortcomings by: (1) gathering empirical connectivity data on sources and sinks of larval fish settling on San Diego County rocky reefs (Chapter 2), (2) examining the spatial and temporal variability in otolith trace elemental fingerprints to assess the applicability of this method for quantifying connectivity within the study region (Chapter 3), (3) using these elemental fingerprints to re-construct larval dispersal trajectories and from these create connectivity matrices describing the connectedness of garibaldi populations inhabiting the study region (Chapter 4), (4) developing the use of matrix population and metapopulation (see Appendix 1.2 below) models to better assess the demographic consequences of the documented connectivity patterns (Chapter 5), and from these data (5) adapting metrics for the analysis of quantitative food webs, and applies these in conjunction with node deletion experiments to critically examine the

connectivity "value" of individual reefs to the marine protected area network currently located along the open coastline of San Diego County.

San Diego County Marine Protected Areas

This study examines the extent of connectivity among garibaldi populations inhabiting six nearshore rocky reefs spanning 60 km of the San Diego County coastline (Figure 1.1); three of the study reefs are or are adjacent to existing MPAs: the Cardiff-San Elijo SMCA, the La Jolla SMCA, and the Mia J. Tegner SMCA The Cardiff-San Elijo SMCA is situated at the base of sandstone cliffs, was established in 1989, and spans an area of approximately 2.39 km^2. It ranges between 0 to 18m water depth, has high- and low-relief reefs spanning from the intertidal to the subtidal zone (Becker et al. 2005), and has substantial *Macrocystis* (i.e. giant kelp) beds approximately 300 m offshore (CDFG 2004). The La Jolla SMCA was established in 1971 and protects approximately 2.16 km^2 of marine habitat (Parnell et al. 2005). It is composed of rocky reefs and outcrops, surfgrass, and approximately 0.10 km^2 of the 8.25 km^2 of the La Jolla kelp forest (CDFG 2004, Parnell et al. 2005). The Mia J. Tegner SMCA was established in 1978, has an approximate area of 0.02 km^2, and while being small in area, is adjacent to the largest kelp forest in the Southern California Bight (North et al. 1993, CDFG 2004). The habitat here is mainly rocky reef with surfgrass, *Egregia*, understory patches of smaller kelps (e.g. *Pterogophora and Eisenia*), articulated coralline algae turf, and extensive (approximately 10 km^2) *Macrocystis* forests offshore (Dayton and Tegner 1984, Dayton et al. 1992, CDFG 2004). There is currently an effort to re-zone the protected areas within this region as

mandated by the California Marine Life Protection Act. At this point there have not been any official changes in the location, protective measures, or names to existing reserves or the overall reserve network design. However in the preferred alternative plan that is currently under review, two of the MPAs within the regions would be re-named and their borders slightly altered (see Chapter 2 below).

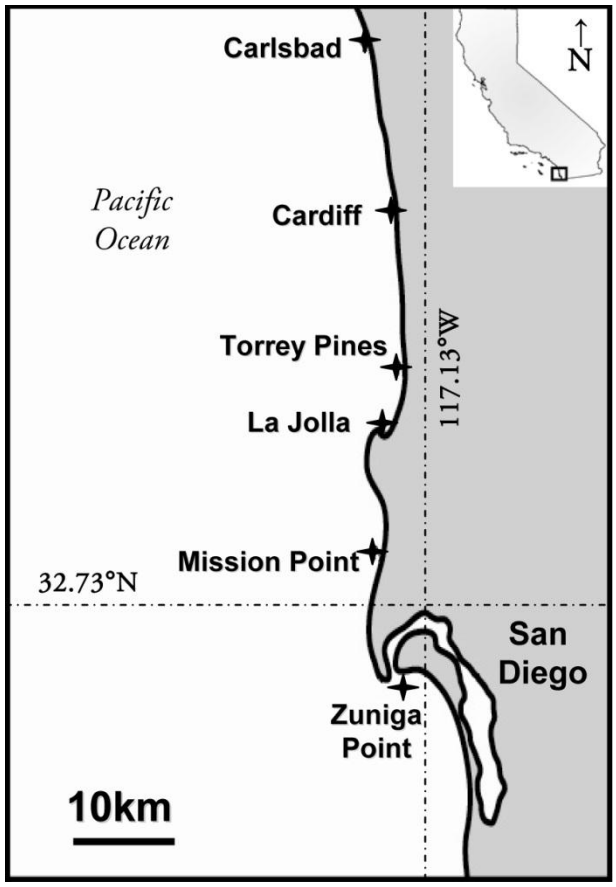

Figure 1.1 Map of study reefs spanning San Diego County coastline. Cardiff and La Jolla are MPAs, while Zuniga Point is adjacent to an existing MPA. Figure taken from Cook 2011 (see Chapter 3).

Otolith Trace Elemental Chemistry

Trace elemental fingerprinting was used to quantify the connectivity of garibaldi populations inhabiting reefs within the study region. This method relies on the fact that fishes and other organisms contain calcareous structures that incorporate chemical properties of their surrounding water masses, creating spatially-explicit identifiable signatures (Thorrold et al. 2002, Walther and Thorrold 2006, see Thorrold et al. 2007 for a recent review). Within the inner ear of teleosts lie three calcareous structures, the sagittal, lapillal, and asterisci otoliths. Sagittal and lapillal otoliths are formed primarily of aragonite, while asterisci otoliths are composed of vaterite (Campana 1999).

A useful trait of otoliths is that they grow (i.e. accrete new calcium carbonate) on a daily basis (Pannella 1971), and as this occurs, trace elements of ambient waters are incorporated into the calcareous matrix of the otolith (e.g. strontium ions can substitute for calcium ions within the calcium carbonate crystal lattice of the otolith) in proportion to the dissolved concentrations of these elements in the surrounding environment (Campana 1999, Thorrold and Hare 2002, Elsdon and Gillanders 2003, Walther and Thorrold 2006). Therefore as the otolith grows it creates a time-specific chemical record of the water masses it has inhabited. A laser ablation – inductively coupled plasma mass spectrometer (LA-ICPMS) can then be used to measure the ratio of trace elements in the otolith to the amount of calcium (i.e. the material ablated; e.g. Sr/Ca, Warner et al. 2005) allowing for spatial inferences of natal origin when

comparing otoliths of settlers of unknown origin against known otolith-derived reference trace element fingerprints.

Thesis Overview

To address the role of connectivity in population dynamics it was first necessary to choose a species that well-represents the various families of fish inhabiting nearshore rocky-reefs within the study region. To avoid the 'shifting baselines' syndrome (Pauly 1995), and enable an assessment of connectivity patterns in a "pristine" setting I selected garibaldi, a species that has been protected for approximately 30 years (Appendix 1.1, Chapter 2). Hundreds of exploratory dives were completed to identify the largest populations of garibaldi in San Diego County (Chapter 2). After identifying the possible source populations, it was necessary to test the spatial and temporal stability of otolith trace elemental fingerprints to validate the utility of this method for quantifying population connectivity via larval dispersal (Chapter 3). Using high resolution sampling over the three month garibaldi spawning season I re-created larval dispersal trajectories, and from these data developed estimates of larval dispersal distance, directionality, and magnitude (Chapter 4). From these connectivity data I quantified local retention, and regional settlement within the MPA network located along the open coastline of San Diego County (Chapter 4). Coupling these connectivity data with stage-based matrix population models I tested the relative contribution of vital rates and inter-reef connectivity on reef-level and metapopulation growth rates (Chapter 5). Using simulation modeling I explored the role of connectivity in metapopulation dynamics, providing insights into the possible

roles of connectivity in managing natural resources (Chapter 5). Building upon these data generated in earlier chapters I applied qualitative and quantitative food-web metrics to realized connectivity data and using node deletion experiments created a method of quantifying the value of individual reefs to the six reef metapopulation (Chapter 6).

This thesis provides one of the few empirical data sets describing larval dispersal and metapopulation connectivity among a network of MPAs, specifically among those MPAs located along the coastline of San Diego County. Coupling realized connectivity data with concurrently collected demographic data provides a manner of better understanding the role of connectivity in population dynamics. The methods and metrics developed for quantifying connectivity and the connectivity value of individual reefs within a reserve network can hopefully be used by scientists, resource managers, and policy makers in a manner that will increase the sustainability of marine fisheries while simultaneously protecting marine biodiversity.

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Appendix 1.1 Supplementary information on Model Species, *Hypsypops rubicundus*

Model Species-Garibaldi

The patchy distribution of subtidal rocky reef habitat spanning San Diego County creates a spatially-defined metapopulation of garibaldi. Garibaldi (*Hypsypops rubicundus*), a member of the damselfish family (Pomacentridae), are a representative rocky reef species within this region (Pondella et al. 2005, Allen and Pondella 2006). Adult garibaldi have a conspicuous bright orange color, a unique characteristic of the rocky reef fish fauna of southern California. They inhabit rocky regions from the shallow subtidal to a depth of 30m (Limbaugh 1964). Both males and females are territorial as adults, occupying a region of ~10m^2 which typically includes a region for forage, shelter, and for adult males, a benthic nest of turf-forming red algae (i.e. >98% *Tiffaniella snyderae*, *Murrayellopsis dawsonii*, and *Pterosiphonia dendroidea*; Limbaugh 1964, Clarke 1970, Foster 1972).

Between May and October female garibaldi spawn tens of thousands (Mean = 129,000 per annum) of elliptical (1mm x 2mm) eggs in male-tended benthic nests; onset of spawning occurs shortly after water temperatures breach 15°C (Clarke 1970). Numerous females will spawn eggs within a single nest, and the embryos within the brood will develop on the nest for approximately 12 days (depending on temperature; Sikkel 1988). After the benthic developmental period, larvae hatch out during the two hours following dusk over a period of several days and spend approximately 20 days dispersing in the pelagic before settling to the shallow (i.e. 5-15 m) rocky subtidal (Clarke 1970, Sikkel 1989, Alcalay and Sikkel 1994). Settlement is followed by a

period of two years as a juvenile and three additional years as a subadult before reaching sexual maturity at approximately 5-6 years of age; estimates of longevity are 12 to 13 years (Clarke 1970; Figure 1.2). In conjunction with their spawning mode, spawning period, and pelagic larval duration, there are numerous life-history traits that make garibaldi an excellent model species for fishes inhabiting rocky reefs of southern California; see Table 1.1).

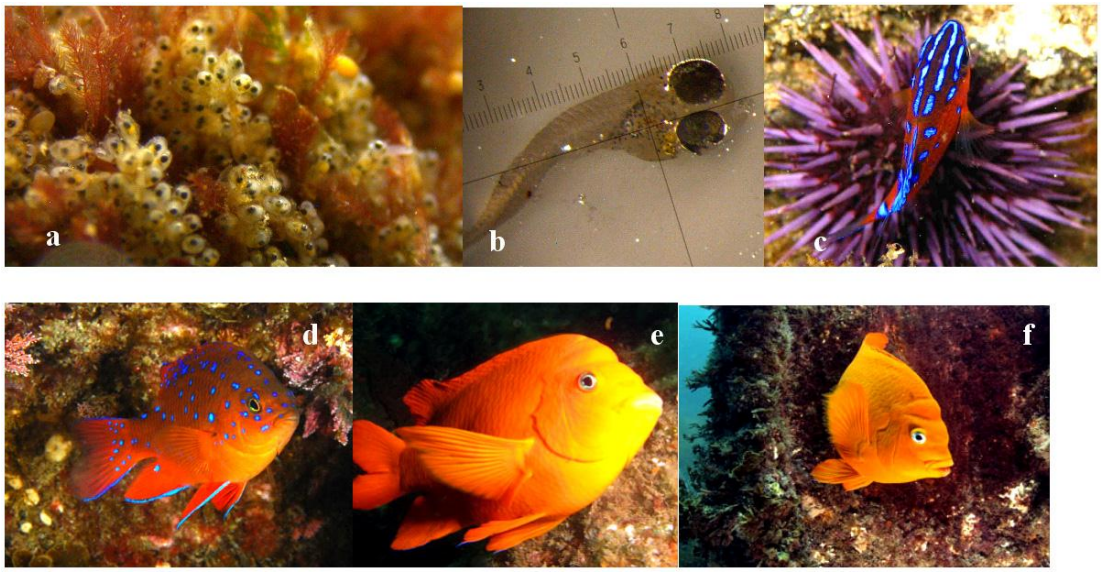

Figure 1.2 Garibaldi life stages: a) late stage embryos b) 1 day post-hatch (~3.5mm total length) c) young-of-the-year settler (~15mm total length) d) juvenile stage (~90mm total length) e) sub adult ~180mm total length f) adult stage (~280mm total length)

Garibaldi inhabit rocky habitat patches separated by a sand-dominated matrix, presenting an ideal model system to study population connectivity via larval dispersal. Along the San Diego County coastline five nearshore marine protected areas have

been established, one of which, the La Jolla State Marine Conservation Area (SMCA), is a no-take' marine reserve. The other four reserves, the Mia J Tegner SMCA, the San Diego-Scripps SMCA, and the adjoining Encinitas and Cardiff-San Elijo SMCAs range from Point Loma in the south to Encinitas in the North, and allow limited recreational and commercial take (see Figure 1.1, Map of Study Area). Notably garibaldi are protected from both recreational and commercial extraction throughout California. However, these reserves, like others found in Californian waters, "were established on a piecemeal basis" (CMLPA 2007) and their ability to meet mandated resource protection needs, including the connectivity (via larval dispersal) of the species found within their borders, is unknown.

Table 1.1 Garibaldi life history traits as compared with average values of other nearshore* fishes (values are given as mean ± sd; adapted from Shanks and Eckert 2005)

	Garibaldi	Nearshore Fishes (N = 89 spp.)		
		Demersal Spawner	Live Birth	Pelagic Spawner
Spawning Mode	Demersal Eggs	51% of spp. Demersal Eggs	26% of spp. Live Birth	23% spawn pelagic eggs
Spawning -Settlement Period	May 01 – Sept 30	Mar 16 – Sept 01		Mar 14 – Sept 21
Pelagic Larval Duration (days)	21	41.9 ± 3.8	n/a	47.4 ± 8.1
# broods/yr.	1	1.8 ± 0.3		45.2 ± 21.3
Max. Fecundity	129000	59698 ± 32945		976,400 ± 444,519
Max. Age (yrs)	13	12.1 ± 1.95		20.6 ± 5.2

**Nearshore species are defined as those inhabiting estuaries, the intertidal zone, and waters ≤ 30 m depth whose range is found primarily between 30° N and 47° N.*

Appendix 1.2 Background Information on Marine Metapopulations

Metapopulations

Many marine species form metapopulations (Kritzer and Sale 2006). A metapopulation is a population composed of a number of subpopulations inhabiting suitable habitat patches embedded within an uninhabitable matrix (e.g. patches of rocky reef separated by sand bottom), whose dynamics are connected via individuals dispersing between discrete habitat patches. As originally defined by Levins (1969, 1970), the dynamics of a metapopulation are described by the balance between colonization and extinction rates. As models have changed and grown more explicit to better address management needs of marine species, so too has the definition of a metapopulation evolved (Kritzer and Sale 2004).

In this study overall metapopulation dynamics are assumed to be governed by four basic rates: fertility, survival, immigration, and emigration of individuals dispersing between the various subpopulations comprising the metapopulation. If these four rates in concert result in a metapopulation λ greater than or equal to one, the population as a whole will persist over time, whereas if λ is consistently less than one, the metapopulation will with high probability go extinct. It is perhaps due to these apparently simple dynamics that the concepts inherent in metapopulation theory are invoked by policy makers and resource managers involved in the siting of marine reserves (i.e. a no-take marine protected area) and marine reserve networks (i.e. those joined by inter-reserve dispersal events). Their appeal is in large part due to a promise

of both protecting marine biodiversity while simultaneously protecting populations of fishes from fisheries-related overexploitation (Cook and Heinen 2005).

Connectivity within a metapopulation framework is cited frequently as a consideration in planning marine reserves and marine reserve networks. Yet despite its apparent importance, there have been few if any marine reserves or marine reserve networks sited with an explicit incorporation of empirically-derived estimates of population connectivity (but see Planes et al. 2009). This in large part stems from the difficulty in tracking small (i.e. mm-scale) dispersing larvae over an extended (i.e. weeks) pelagic larval period, coupled with complexities arising from myriad patterns of dispersal across taxa.

CHAPTER II.

INFLUENCE OF PROTECTED STATUS AND HABITAT STRUCTURE ON THE DISTRIBUTION AND ABUNDANCE OF A TEMPERATE ROCKY REEF DAMSELFISH, *HYPSYPOPS RUBICUNDUS*

Abstract

One measure used to prevent species overexploitation is the designation of protected status. However it is challenging to assess to success of this form of protection without adequate long-term data. The garibaldi, *Hypsypops rubicundus*, is an iconic damselfish with protected status in the State of California. The primary goals of this study were 1) to explore the influence of protected status on garibaldi abundance and 2) to evaluate whether the territorial behavior of *H. rubicundus* in conjunction with habitat complexity results in higher conspecific survivorship and relative condition for post-settlement stage young-of-the-year (YOY). Quantitative data on the status, distribution, and abundance of *H. rubicundus* and their spawning sites were collected from April 2002 – June 2010. We compared *H. rubicundus* abundance data from 1965 (prior to protected status) with data collected after fully protected status was designated (1995); in this period of time adult densities were found to have more than doubled (from 0.07 m^{-2} to 0.2 m^{-2}) to levels previously undocumented. However, the causative role of fully protected status in the observed population increases in adult density remains uncertain. Neither post-settlement survival nor condition factor of YOY were significantly different among study sites, although YOY condition factor in 2009 was significantly higher than in 2008 ($F_{1,87}$=

37.7, $p < 0.001$). Young-of-the-year survivorship in areas with high adult densities but lacking habitat complexity was lower than at sites having high adult density and habitat complexity (0% vs. 32%), suggesting YOY survivorship varies with habitat complexity. YOY survivorship is more dependent on the presence of small-scale physical refugia created by high habitat complexity than the presence of biotic refugia created by the territorial behavior of conspecific adults.

INTRODUCTION

Since Hjort (1914) first postulated on the role of physical and biological factors leading to fluctuations in year-class success in fish populations, researchers have attempted to identify what mechanisms most influence recruitment. For many fishes exhibiting a bipartite life cycle, with relatively sedentary adults and a possibly wide-ranging larval dispersal phase, recruitment success is highly variable, and regulated by numerous factors during early life stages (i.e., stages spanning from post-hatch to breeding-age). Two of the foremost causes of mortality during early life stages are predation (Houde 1987; Bailey and Houde 1989; Houde 2008) and competition (Hixon 2006). One form of competition that has received much attention, particularly in coral reef fishes is territoriality (Ehrlich 1975; Doherty 1983; Myrberg and Thresher 1984; Doherty and Williams 1988). While an extensive body of work exists on density dependence and territoriality in coral reef fishes (see review by Hixon and Jones 2005), comparatively little is known of the role territoriality plays in population regulation of temperate demersal fishes (but see Hixon 2006).

Territorial behavior has been described as a regulatory mechanism involving intraspecific interference competition, where individuals of a single species compete for a share of a limited resource (Clarke 1971). For territorial reef fishes individuals establish a given territory that includes shelter and feeding area(s) and defend this home area against conspecifics. This agonistic behavior is thought to regulate populations by constraining the maximum number of individuals found within a given region based on suitable substrate and the number of territories that can fit within this area. Under this scenario territorial behavior results in intraspecific competition, the premise upon which maximum sustainable yield (MSY) was built. This concept (of MSY) was used to guide fisheries management in a "sustainable" manner for the better part of the 20th century (*cf.* Sette 1943 as described by Hixon 2006).

A variety of techniques have been implemented by resource managers to protect fisheries from overexploitation. These methods include spatial zoning (e.g., marine protected areas), implementing catch quotas, and infrequently by granting some form of protected status to a species (Jennings et al. 2001). Few protected species exhibit territoriality, and to our knowledge there has not been an assessment of the efficacy of granting protected status to a territorial nearshore marine species. In this study we attempt to address this question in temperate Californian waters, where few species are protected by law. However one species that does allow for pre- vs. post-protection comparisons of abundance is the California State marine fish, garibaldi (*Hypsypops rubicundus*).

Garibaldi Life History

Three researchers have completed work on various components of garibaldi life history at various stages of protected status. Limbaugh (1964) was the first to collect broad-scale presence-absence and life history data on garibaldi (*Hypsypops rubicundus*) prior to protected status. Clarke (1970, 1971) built upon this work by providing detailed descriptions of territorial behavior and abundance of garibaldi after partial-protection was conferred, and just prior to official "no-take' status was conferred. Sikkel (e.g., 1995) provided in-depth data describing garibaldi reproductive life history and spawning behaviors. In this study we build upon this foundation by extending our understanding of how factors, such as protected status, influence the distribution and abundance of garibaldi. This analysis has allowed us to test how post-settlement survivorship of YOY is influenced by both physical environment and territorial behavior of adult garibaldi. The following is a brief review of garibaldi life history, including novel observations made as part of this study.

The garibaldi is one of two members of the damselfish family (Pomacentridae) in southern California. Garibaldi inhabit rocky regions from the shallow subtidal to a depth of 30 m (Limbaugh 1964) and are a representative rocky reef species within this temperate region (Pondella et al. 2005; Allen and Pondella 2006). Adult garibaldi are the second largest of all damselfish, reaching a maximum total length of ca. 35 cm (Allen 1991). Adults have a conspicuous bright orange color. Anecdotally the common name, garibaldi, is attributed to Giuseppe Garibaldi, the Italian revolutionary known for wearing a bright red shirt. Both males and females are territorial as adults, occupying a region between ~ 3 and 10 m^2 that typically includes a region for forage,

shelter, and for adult males, a benthic nest (Limbaugh 1964; Clarke 1970; Foster 1972; Cook unpubl. data). Between May and October female garibaldi spawn tens of thousands (mean = 129,000 per annum) of elliptical (1 mm x 2 mm) eggs that stick to male-tended red algal nests; onset of spawning occurs shortly after water temperatures consistently remain above 15°C (Limbaugh 1964; Clarke 1970).

Numerous females will spawn eggs within a single nest, and the embryos within the brood develop on the nest for ~12 to 23 days (depending on temperature; Sikkel 1988). After the benthic developmental period, larvae hatch out during the two hours following dusk over a period of several days and spend approximately 21 days in the pelagic dispersing before settling to shallow (i.e., <15 m) subtidal rocky reefs (Clarke 1970; Sikkel 1989; Alcalay and Sikkel 1994; Cook unpubl. data). Larvae are active swimmers following hatch (Cook unpubl. data), but their position in the water column for the duration of the 21-day pelagic larval period remains unknown. Qualitative laboratory experiments conducted by the first author revealed a shift in larval swimming behavior over 18 days following hatch. The first day was spent near the surface of a 120-liter drum (approximately 75 cm deep), followed by reversing periods spent at depth and at the surface. After settlement individuals spend ~ three years as juveniles and two additional years as sub adults before reaching sexual maturity at approximately 5 - 6 years of age. Within the study region estimates of longevity are 12 to 13 years (Clarke 1970; Clarke 1971).

Juvenile and sub adult life-stages have bright iridescent-blue markings that slowly fade over the first five years of life, eventually taking on the characteristic

bright orange coloration of an adult by the time an individual reaches sexual maturity (see Kritzler 1950 for an in-depth description of developmental changes in coloration and Limbaugh 1964 for figures). Sub adults (individuals retaining iridescent-blue coloration) are generally tolerated within the established territories of adults until they develop a solid orange adult coloration, although agonistic behavior has been observed between YOY from different settlement cohorts (i.e., a ~25 mm TL YOY was seen chasing a ~15 mm TL YOY from a "preferred" shelter site; Cook pers. obs.)

Here, we explore the influence of protected status on the abundance of garibaldi off San Diego County, provide updated information on its distribution and habitat preferences, and test the effects of a biotic interaction on survivorship of post-settlement stage YOY. To address the first component of this study it was necessary to determine when protected status was officially conferred to garibaldi. This was unclear from the literature and so we conducted a review of the literature on the history of garibaldi protection, consulted experts in the field, and consolidated historical abundance data. We hypothesized that protected status would result in an increase in garibaldi abundance over time. In addition we examine whether a putative form of intraspecific facilitation results in reduced mortality and increased condition factor (see below) for YOY garibaldi. We propose that the territorial behavior of adult garibaldi in combination with substrate complexity should act to lower conspecific YOY mortality, by creating *de facto* refugia from predation.

METHODS

Distribution and abundance data were collected using SCUBA at numerous rocky reefs spanning the San Diego County coastline (Fig. 2.1). Possible survey sites were identified through reading the published literature and by speaking with key scientific personnel, active scientific divers and recreational divers. Garibaldi abundance was assessed utilizing band transects (30 x 4 m) oriented along the major and minor axes of reef habitat, subsequently these data were used to calculate garibaldi density. These surveys (n = 587) occurred between March 2002 and May 2008. From these exploratory surveys of three broad geographic regions (North County, La Jolla, and Point Loma; Fig. 2.1), six major populations of garibaldi were identified along the San Diego County coastline. The primary study sites were chosen based on density of individuals (i.e., the largest populations within a contiguous region) and proximity (i.e., primary sites were to be separated by $\geq$ 5 km of uninhabited substrate, specifically soft-bottom habitat).

Abundance and size-class surveys of garibaldi were conducted at the six primary study sites by dive teams in fixed band transects (30 x 4 m). Individual fish were enumerated by size class using the total length classes of 0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm, 20-25 cm, >25 cm. Mean values of abundance and density were calculated from two replicate surveys conducted during each visit to individual study sites. When benthic nests were present, they were enumerated, and notes were made on whether the nest was empty, had "fresh" eggs (i.e., early-stage embryos yellow in color), or "mature" eggs (i.e., late-stage embryos grey in color) as defined by Sikkel (1988, 1989).

Settlement cohorts were surveyed from June 2008 through June 2010 to estimate annual mortality of YOY (i.e., over two spawning-settlement seasons). Annual mortality (z) was calculated as:

(1) $$z = 1 - (n_{(t+1)} / n_{(t)})$$

where $n_{(t)}$ is the maximum number of YOY settlers at time t and $n_{(t+1)}$ is the number of individuals in the same settlement cohort enumerated at the next time step (i.e., z is a measure of the YOY that settled and survived to grow into the next larger size-class). Sites were visited bi-weekly during the spawning and settlement season. Size-class surveys were conducted monthly, but were occasionally omitted during winter months due to poor diving conditions (swell or visibility). Surveys were conducted every 2-6 weeks between spawning/settlement seasons.

To test for a putative link between study site and physical condition of settlement-stage fish, collections of YOY were made after the 2008 and 2009 spawning/settlement periods. Mass (g) and Standard Length (mm) data were collected from unpreserved post-mortem individuals on the day of capture. Mass was obtained from individuals following pat-drying to remove moisture from their exterior. A relative condition factor was calculated using a length/weight relationship of individual fish; broadly speaking a "heavier" individual is considered to be in better physical condition than a "lighter" individual of the same size (see Nash et al. 2006 for a brief review of condition factors in fisheries science). In this study relative condition factor (CF) was calculated as:

(2) $$CF = \text{Mass (g)} / \text{Standard Length}^3 \text{ (mm)} * 100{,}000$$

Individuals with CF >1 standard deviation above the mean CF were considered fish in optimal condition, while those <1 standard deviation below the mean were considered to be in poor condition. The length-weight data used to calculate CF were combined with data describing fishes spanning larger size classes to create a general relationship of length and weight for garibaldi (Fig. 2.2).

As the size of rocks and boulders comprising the rocky substrate decreases smaller shelter holes are created (i.e., increasing habitat complexity). These holes may act as YOY refugia from larger predators (e.g., kelp bass, *Paralabrax clathratus*; Cook pers. obs.). Early attempts to assess reef rugosity using standard methods failed to capture smaller-scale habitat complexity. Therefore to quantify habitat complexity, individual components of the rocky substrate were measured every 3 m along multiple 30 m transect surveys at each of the six primary study sites (n = 60). In this process divers extended a transect line along the major axis of each reef, and measurements were made of haphazardly selected rocks/boulders (i.e., those falling under each 3 m mark of the primary transect line) along 3 major axes (length, width, and height) to generate a rough volumetric estimate of rocky substrate at each site. To achieve normality and homogeneity of variance data were $\log_e$ transformed prior to statistical analyses. Habitat data in combination with those describing adult conspecific density (see above) were used in a stepwise multiple regression to assess the putative relationship among adult density, habitat complexity (i.e., mean volume of rocks/boulders comprising hard substrate), and YOY survivorship at the six primary study sites.

Description of Primary Study Sites

The northernmost study site, **Carlsbad rocky reef** (Fig. 2.1) runs roughly perpendicular to shore, is approximately 30-50 m wide and ~2 km long. It is comprised of small and mid-sized rocky boulders and cobble, and rises from the sandy sea floor to within 5m of the surface at the highest regions of the reef. There is limited giant kelp (*Macrocystis pyrifera)*; the feather boa kelp (*Egregia menziesii*) is abundant in nearshore subtidal regions and the gorgonian *Muricea californica* becomes increasingly abundant with distance offshore (~300 m). The **Cardiff-San Elijo State Marine Conservation Area** (SMCA, Fig. 2.1) is situated at the base of sandstone cliffs, was established in 1989, and spans an area of approximately 2.39 km^2. It ranges between 0 and 18m water depth, has high- and low-relief reefs spanning from the intertidal to the subtidal zone (Becker et al. 2005), and has substantial *M. pyrifera* (i.e., giant kelp) beds approximately 300 m offshore (CDFG 2004).

The **Torrey Pines Artificial Reef** was established in 1975 by the California Department of Fish and Game (CDFG). It is ~13 m deep and spans ~100 m in an alongshore direction and ~30 m cross-shore. *Macrocystis pyrifera* is absent year-round at this site, but large boulders (3,000 tons of quarry rock taken from San Clemente Island), are covered with gorgonians and other encrusting organisms. The **La Jolla SMCA** was established in 1971 and protects approximately 2.16 km^2 of marine habitat (Parnell et al. 2005). It is composed of rocky reefs and outcrops, surfgrass, and a limited amount of the kelp *M. pyrifera* (approximately 0.10 km^2 of the 8.25 km^2 comprising the adjacent La Jolla kelp forest (CDFG 2004; Parnell et al.

2005)). As part of the California Marine Life Protection Act a new suite of marine protected areas have been mandated for the study region, and within the agreed upon "integrated preferred alternative" plan, the La Jolla SMCA has been slated for a formal name/designation change to the Matlahuayl State Marine Reserve (SMR; Fig. 2.1). Therefore the La Jolla SMCA shall hereafter be referred to as Matlahuayl, a Kumeyaay word meaning "place of caves", and La Jolla will refer to the broader geographic region surrounding La Jolla.

The two southernmost sites, **Mission Point** and **Zuniga Point** are artificial sites made of rip-rap composed of large to mid-sized quarry boulders (0.2 – 4.7 m^3). Mission Point spans ~3.5 km, lining the channel entrance to Mission Bay. Habitat suitable for garibaldi is located from ~1.5 m water depth to ~8 m water depth. There is limited growth of *M. pyrifera*, and the gorgonian *M. californica* is present at depths > 4 m. Zuniga Point forms the eastern boundary of the San Diego Bay inlet. It is an approximately 1.8-km long submerged jetty composed of rip-rap (i.e., mean (± SE) rocky substrate volume = 4.7 ± 0.7 m^3) stretching in a primarily north-south direction. Rocky substrate ranges from 3 m to 10 m water depth. *Macrocystis* is absent from this site, but like Torrey Pines, large boulders are covered with *Muricea* and encrusting organisms.

Data describing the natural history and the status, distribution, and abundance of garibaldi were collected from the primary literature. To clarify specific points, informal interviews were conducted with senior resource management personnel,

emeritus professors, dive safety officers, and collection managers. Archival material housed in the Scripps Institution of Oceanography Library was also utilized.

RESULTS

Clarification of the Protected Status of Garibaldi

Despite garibaldi's current status as a fully protected (i.e., no-take) species in California, and the fact that they are the official California state marine fish, the history of their protected status has not been well-documented. The scientific literature indicates that there was little to no legal protection afforded to garibaldi in the early parts of the 20th century. For example, Jordan and Evermann (1898) in their species description of garibaldi state it "is of some value as food" and in studies by Sumner and Fox (1935) on the ability of various marine taxa to synthesize carotenoids (i.e., carotene and xanthophyll), their experimental design involved the authors feeding various species of fish "the chopped flesh and skin of the garibaldi (*Hypsypops rubicunda* (Girard))." In a follow-up study, Fox (1936) again describes feeding test subjects the "chopped flesh of the brilliant orange fish, garibaldi."

Prior to the 1960s, garibaldi were taken frequently as part of the recreational fishery by both spear and hook-and-line; spear-fishing comprised the largest component of the recreational catch (James Stewart, Dive Safety Officer Emeritus, Scripps Institution of Oceanography, pers. comm.). However, around the late-1960s and early-1970s there were informal discussions about making garibaldi the official

state marine fish of California, and a moratorium was put in place on the recreational capture of garibaldi. This moratorium was never removed (James Stewart pers. comm.). Between 1950 (when a study by Kritzler et al. makes no mention of their protected status) and a posthumous note by Conrad Limbaugh in Pacific Science in 1964 there was a legal change in the protected status of garibaldi. Limbaugh (1964) describes the natural history of garibaldi and blacksmith (*Chromis punctipinnis*), two damselfishes found in Californian waters, stating, "spearfishermen represent a potential predator of considerable importance, but garibaldis are now protected by law from this danger." He continues further writing that garibaldi are partially protected by law (i.e., from recreational fishing), but that "they are still taken in quantity for aquarium use…by collectors having commercial licenses." This same sentiment is repeated in numerous publications over the next two decades.

Eschmeyer et al. (1983) state that "it is illegal to spear or retain this species (if caught, release alive)" in California. However, it is difficult to ascertain whether or not this modification to protection of garibaldi was legal or one that was informally adopted among the fishermen of California. There is anecdotal evidence that the California Department of Fish and Game suggested full protection for garibaldi in the early 1970s, but apparently no legal motions were passed to further protect the species (James Stewart pers. comm.). In 1993 a motion was passed in the California State legislature to protect garibaldi from a burgeoning demand in the aquarium trade. This motion stated that garibaldi could only be taken by those with a valid commercial (i.e.,

aquarium) collector's permit, and that collections were restricted to a portion of the year when garibaldi were not breeding (November 01 – January 31).

With rumors growing that the status of garibaldi was in jeopardy due to over-harvesting by commercial collectors at some locations, a motion was put forward in 1994 by Assembly member Bill Morrow as part of California State Assembly Bill 77, to temporarily impose a moratorium on all take of garibaldi. Within this bill Mr. Morrow added section 425.6 to Chapter 2 of Division 2, within Title 1 of the California Government Code to declare that, "The garibaldi (*Hypsypops rubicundus*) is the official state marine fish." This bill was first heard on the floor of the Assembly on December 22, 1994, and after some delays relating to other components of Bill 77, it was signed into law by Governor Wilson on October 16, 1995 making garibaldi the official state marine fish of California. With this proclamation, official 'no-take' status was conferred to garibaldi.

Garibaldi Distribution and Abundance

A total of 542 surveys were conducted between 2002 and 2007. Based on these survey data, it was apparent garibaldi preferentially inhabit shallow-water regions of relatively high-relief (i.e., > 1m) rocky substrate (Parnell unpubl. data.). From 2007 until 2010 an additional 105 surveys were conducted at the six primary study sites. From these survey data densities of adult garibaldi (i.e., those larger than 20 cm TL) at the six primary sites were estimated to range from 0.089 - 0.35 m^{-2} (Table 2.1). These are the highest recorded densities of garibaldi to date. The mean densities of garibaldi within the broader geographic regions surveyed between 2002

and 2007 ranged from 0.001 - 0.01 individuals m^{-2}. Densities of garibaldi at sites considered to be optimal habitat (i.e., where rocky substrate had vertical relief ≥ 1m) ranged from 0.0038 – 0.35 individuals m^{-2}.

The maximum number of YOY settlers arriving at a given site ranged from 0 to 25 per 30-m belt transect. Two of the six primary study sites, Zuniga Point and Torrey Pines, did not receive any YOY settlers in 2008. Cardiff Reef and Mission Point did not receive YOY settlers in 2009. In surveys conducted in the La Jolla region during 1965, Clarke (1970) calculated the mean (± 1 SE) density of garibaldi was 0.072 (0.01) m^{-2}. This value is similar to densities found in optimal garibaldi habitat within the broad geographic regions surveyed (i.e., North County, La Jolla, and Point Loma) as part of this study but is significantly less than the density of garibaldi at the primary La Jolla study site (Matlahuayl) between 2008 and 2010 (Fig. 2.3; ANOVA $F_{9,188} = 156.5$, $p < 0.0001$, Post-hoc Tukey HSD $p < 0.001$).

The density of nests at the six primary study sites ranged from 0.03 m^{-2} at Cardiff Reef to as high as 0.15 m^{-2} at Torrey Pines Reef. Clarke (1970) had previously determined the mean (± 1 SE) number of eggs spawned by a female was 129,000. The mean density of eggs within a given nest was 1.4 (0.05) mm^{-2}. Prior to receiving protected status Clarke (1970) determined the mean density of garibaldi nests within the La Jolla region was 0.04 (0.009) m^{-2}. This value was not significantly different from the density of nests found in La Jolla in this study. For the region examined in this study, egg developmental period was approximately 12 days (Cook pers. obs.).

Young-of-the-Year Annual Mortality and Condition Factor

The maximum density of YOY settling to a given site in 2008 was 0.2 m^{-2} (i.e., 25 YOY over a 30 m transect), while the maximum density observed in 2009 was an order of magnitude less (0.02 m^{-2}, only 2 YOY observed over a 30-m long belt transect). Mean (± 1 SE) density of YOY in 2008 was 0.07 m^{-2} (0.04) while mean density in 2009 was 0.01 m^{-2} (0.002). Annual YOY mortality estimates for 2008 and 2009 ranged from 0.50 - 1.0, while the mean annual mortality rate was 0.8 (0.09; Table 2.1). Highest annual YOY mortality (100%) was documented at Mission Point in 2008 and Torrey Pines and Zuniga Point in 2009. Lowest annual YOY mortality rates (50%) were seen at Cardiff in 2008 and Carlsbad and La Jolla in 2009 (Table 2.1).

Site-specific condition factor (CF) values for YOY ranged from 3.7 to 6.7. Across 2008 and 2009 the mean (± 1 SE) CF was 4.64 (0.05). When YOY condition factor data were pooled across years, there were no significant differences among study sites (One-way ANOVA $F_{4,84} = 2.1$, $p = 0.09$). When CF was pooled among sites for a given year, the condition of YOY in 2009 was significantly greater than in 2008 (Fig. 2.4; One-way ANOVA $F_{1,87} = 37.7$, $p < 0.0001$).

Habitat Complexity

Rocky substrate at Zuniga Point and Torrey Pines was significantly greater in size than rocky substrate at Mission Point, Cardiff, and Carlsbad (Fig. 2.5; One-way ANOVA $F_{5,54} = 21.0$, $p < 0.0001$, Post Hoc Tukey HSD $p < 0.05$). The size of rocky substrate at Matlahuayl was significantly less than the size of rocky substrate at Zuniga Point, but was significantly greater than rocky substrate at Mission Point, Cardiff, and

Carlsbad (Fig. 2.5; One-way ANOVA $F_{5,54} = 21.0$, $p < 0.0001$, Post Hoc Tukey HSD $p < 0.05$).

Interaction of Adult Density, Habitat Complexity, and YOY Survivorship

The relationship among adult density, rocky habitat complexity, and YOY survivorship was not significant (stepwise multiple regression model $F_{2,3} = 1.48$, $p = 0.36$). While this relationship is not statistically significant, the best-fitting equation relating these variables was, YOY survivorship = 1.32 – 0.24 (Adult Density) – 0.083 (ln (Rocky Substrate Volume)); $R^2 = 0.497$, $p = 0.21$). The vast majority of the variation in this relationship was conferred by the volume of rocky substrate ($R^2 = 0.487$), with the remainder of the variability explained by the model coming from adult density ($\Delta R^2 = 0.011$). Retrospective power analyses suggest (if additional data retained similar statistical properties) an alpha < 0.05 would be achieved through 7 additional substrate surveys and ~300 additional adult density surveys. Pairwise correlations further support the stronger (relative to adult density) relationship between habitat complexity and YOY survivorship. Young-of-the-year survivorship was not significantly correlated with adult density ($r = -0.29$, $p = 0.58$), but it was negatively correlated with substrate volume (i.e. sites with higher habitat complexity (due to smaller substrate volume) had higher YOY survivorship; $r = -0.70$, $p = 0.12$). This relationship, again while not quite statistically significant, is ecologically relevant and suggests a positive relationship between habitat complexity and YOY survivorship, warranting further study.

DISCUSSION

Garibaldi Protection

Earlier work by Limbaugh (1964) and Clarke (1970) laid much of the groundwork for our understanding of garibaldi natural history. Limbaugh (1964) produced the first presence-absence dataset describing the distribution and abundance of garibaldi in the San Diego region, and Clarke (1970) built upon this work by conducting high-resolution sampling and surveys within a subset of the regions visited by Limbaugh. Building upon this body of knowledge, we have been able to test the consequences of granting protected status to a previously fished species fifteen years after its official designation.

The results of this study suggest that within the region studied by Clarke (1970; i.e., the western edge of the Matlahuayl SMR), there were significantly lower densities of adults at the onset of partial protection (i.e., in 1965 just after the spearfishing moratorium was put in place) than were found in recent surveys ($F_{1,28} = 35.4$, $p < 0.0001$). Adult densities observed in this study suggest that the individual size of a given territory at Matlahuayl reefs is approximately 5 m^2, half the area of an individual territory when Clarke (1970) conducted surveys in 1965. This suggests that there has been a doubling of the number of territories within this same region over the past forty-five years. Survey data are unavailable to test if this increase in adult density occurred since partial protection was mandated in the 1960s or since complete protection came into force fifteen years ago in 1995. However partial protection from

spearfishing may have been one factor contributing to the observed increase in adult density.

Garibaldi Habitat

An increase in the availability of suitable habitat is another factor that would allow for increases in adult density. There are several non-biological factors which could influence the availability of adult garibaldi habitat. On a seasonal (i.e., intra-annual) time scale, the movement of sand and other sediment is sufficiently dynamic within the study region to both expose new suitable rocky substrate that could act as shelter or areas for grazing, and conversely, may re-cover these newly exposed sites within a period of weeks to months (Clarke 1970; Cook pers. obs.). In addition to the presence of a shelter hole and grazing area, the density of adults (particularly breeding males) may be influenced by the availability of a nesting sites (Clarke 1970). The nests of garibaldi are composed primarily (> 98%) of the turf-forming algae *Tiffaniella snyderae*, *Murrayellopsis dawsonii*, *Pterosiphonia dendroidea*, and *Ophidocladus californicus* (Limbaugh 1964; Foster 1972). It can take from two to three years for a new nest site to develop a thick enough algal mat that females will deposit eggs on it (Clarke 1970). Therefore on ecological time scales the optimal number and size of territories for garibaldi in any one area should tend to be stable over time as successful nesting sites and their associated territories are occupied by other individuals (Clarke 1970; Sikkel 1988; Cook pers. obs.).

Survivorship of YOY appears to vary as a function of the rocky substrate complexity. As sandstone and mudstone are weathered over time through mechanical

forces, such as wave action, and/or biological forces, such as boring organisms (e.g., piddock clams - Pholads), habitat complexity increases. This increase in habitat complexity provides relatively small physical refugia for YOY garibaldi. These refugia are large enough for YOY garibaldi to enter, but small enough to prevent larger predators such as kelp bass (*Paralabrax clathratus*) or California moray (*Gymnothorax mordax*) from pursuing them (Cook pers. obs.).

At larger space and time (i.e., evolutionary) scales, active subduction of an oceanic plate under the area currently occupied by San Diego produced the penisular mountains 150-80 million years ago; this resulted in very little deposition of sedimentary rocks (Abbott 1999). From 80 million years ago until the present, the broad-scale geology of the region has been dominated by transform faulting, three of which run through the Matlahuayl region: the Rose Canyon Fault, the Mt. Soledad Fault, and the Country Club Fault. This shift from a subduction to transform-fault dominated region has allowed for the accumulation of relatively large volumes of sediment, and over geologic time scales the formation of sedimentary rocks (primarily sandstone and mudstone) in the Matlahuayl region (Abbott 1999). It is these relatively-soft sedimentary rocks, and the mechanical and biological erosion of this rocky substrate, that ultimately creates relatively complex rocky habitat suitable for garibaldi.

Garibaldi Survivorship and Biological Interactions

Biological refugia created by intra-specific facilitation (i.e., refugia created by the agonistic behavior of territorial conspecific adults toward fishes entering their

territory) have not been documented to our knowledge. The putative intraspecific facilitation hypothesized to increase YOY garibaldi survivorship was not statistically supported by the data in this study. However, this does not rule out this form of intra-specific interaction. In the same geographic region as this study, Tegner and Dayton (1981) reported on a similar intraspecific facilitation. Young-of-the-year red sea urchins (*Strongylocentrotus franciscanus,* > 40 mm) find refuge from predators under the long spine canopy of adult conspecifics, and YOY garibaldi have been observed seeking shelter from predators in the spine canopy of sea urchins (*Strongylocentrotus spp.*; Limbaugh 1964).

Previous studies of reef fishes have shown a wide-range of density-dependent interactions with varying results. Many studies have documented a negative relationship between the density of juveniles and the density of conspecific adults. Forrester (1995) showed that for the bridled goby (*Coryphopterus glaucofraenum*), a common demersal fish inhabiting Caribbean coral reefs, the density of juveniles was negatively related to adult density. Forrester (1995) suggested adults were regulating the density of new recruits in one of two manners: reduced larval settlement, which Forrester (1999) subsequently disproved, or via a reduction in post-settlement survivorship. In a similar manner, Webster (2004) showed juvenile mortality of fairy basslet (*Gramma loreto*), a planktivorous coral reef fish, increased as a function of density of conspecific adults. In Webster (2004) the increase in juvenile mortality was not attributed to direct agonistic behavior of the adults, but rather to interference competition. Adults were able to outcompete juveniles during feeding, resulting in

increased predation risk to juveniles as they fed in sub-prime feeding locations (Webster 2004).

Historically density-dependent regulation of populations has been couched in terms of negative interactions; competition and predation within- and among species decreases population growth as the density of individuals increases. Hixon and Jones (2005) showed that for many demersal fishes, predation is a key driver of density dependence, but they suggest that the importance of competition in density dependence is underestimated due to the short temporal scale of many studies. Competition is the ultimate cause of density dependence, while predation is merely a proximal agent resulting in mortality. Samhouri et al. (2009) in a recent test of inter-cohort competition and density- dependent mortality in *Gnatholepis thompsoni* found a negative relationship between adult density and conspecific juvenile survivorship. However, there was not a consistent effect of refuge availability on juvenile survivorship. In the present study system, and perhaps due to ontogenetic-associated changes in feeding preferences, YOY garibaldi do not directly compete with adults. Young-of-the-year garibaldi feed on planktonic and semi-planktonic crustaceans whereas adults feed primarily on sponges and bryozoans (Clarke 1970). Adult garibaldi tolerate the presence of conspecifics within their territories until individuals reach ~15-20 cm in length (Limbaugh 1964; Clarke 1970; Cook pers. obs.); this length typically coincides with the body size of individuals that have lost almost all juvenile coloration (Clarke 1970). While predation pressure was not directly addressed in this study, previous experimental work with damselfish has shown the effect of predators

is dependent on habitat complexity; similar to the findings of this study, juvenile survivorship is higher when substrate is more complex and provides numerous prey refuges (Beukers and Jones 1997; Hixon and Jones 2005).

In recent years there has been an increased appreciation for the role of positive interactions (e.g., facilitation) in population regulation (see Hixon and Webster 2002 and references therein); this finding is often described as inverse density dependence. Data describing the putative relationship between territorial behavior of adult garibaldi and survivorship of conspecific YOY were equivocal in this study, but positive impacts of territorial behavior have been documented in studies of tropical damselfish previously. In a community-level example, Hixon and Brostoff (1983) found the territorial behavior of the yelloweye damselfish (*Stegastes fasciolatus*) increased the overall diversity of algae present within territories of individuals (i.e., other predators of the algae were chased from the territories by yelloweye damsels, resulting in lower overall predation on the algae). Booth (1992) showed the settlement of larval *Dascyllus albisella* was positively correlated with the density of conspecific juveniles, and Jones (1988b) studying the influence of habitat complexity and competition on juvenile survivorship found slight positive effects among density of juveniles and survivorship. In their study of the blue chromis, *Chromis cyanea,* Anderson et al. (2007) experimentally tested the relationships among density of resident conspecifics, settlement, and recruitment; settlement occurred in locations where conspecifics were present, and this relationship was inversely density dependent. It is evident from the literature that the mechanisms influencing YOY survivorship are complex with few

viable generalizations; further study is required to better our understanding of these processes.

By consolidating data from the primary and grey literature to clarify the protected status of garibaldi in the State of California, we allow for a comparison to be made between study sites surveyed prior to and after protected status was conferred to this species. There has been an increase in the density of garibaldi within the study region since protective measures were put in place informally over 40 years ago and formally 15 years ago; we suggest that this increase in density may be attributable to these protective measures. This suggests that command and control (see Vig and Kraft 2003 and references therein) resource management techniques, such as the implementation of protected areas or setting catch quotas, may be successful in protecting stocks. However, consideration must be given to the asymmetries present in these situations. There are direct and immediate influences on consumptive users in the near-term, while benefits derived from the protection of a species are more diffuse and long-term in nature (Cook and Heinen 2005). Therefore, efforts should be made to utilize other management techniques in concert with strict protective measures, if the ultimate goals of moving fisheries toward sustainability and protecting marine biodiversity are to be achieved successfully.

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Table 2.1 Adult density, YOY density and mortality of garibaldi at six primary study sites and within the three broad regions surveyed, North County, La Jolla, and Point Loma (Max. YOY density calculated using the maximum number of YOY recruits observed on a transect after a settlement pulse). Annual YOY mortality was calculated as the proportion of YOY that settled and survived to grow into the next largest size class (i.e., from 0-5 cm TL to 5-10 cm TL). na indicates 'not applicable' (i.e., no YOY were present).

Site	Number of Surveys	Adult Density ind. m^{-2} (± 1 SE)	Tukey HSD ($p < 0.001$)[a]	2008 Max. YOY Density (ind m^{-2})	2009 Max. YOY Density (ind m^{-2})	2008 YOY Annual Mortality	2009 YOY Annual Mortality
Carlsbad	15	0.346 (0.007)	A	0.05	0.02	0.67	0.50
Cardiff	16	0.089 (0.007)	E	0.02	0.008	0.50	1.00
Torrey Pines	21	0.335 (0.006)	A	na	0.008	-	1.00
Matlahuayl	23	0.212 (0.006)	C	0.21	0.02	0.92	0.50
Mission Point	16	0.155 (0.007)	D	0.008	na	1.00	-
Zuniga Point	14	0.276 (0.007)	B	na	0.008	-	1.00
North County[b]	70	0.013 (0.003)	F	na	na	-	-
La Jolla [b]	264	0.004 (0.002)	F	na	na	-	-
Point Loma [b]	208	0.0008 (0.002)	F	na	na	-	-

a Sites not connected by similar letters are significantly different (One-way ANOVA $F_{8,638} = 936.9$, $p < 0.00001$; Tukey HSD $p < 0.001$)

b Surveys in broad geographic regions (North County, La Jolla, Point Loma) were conducted between March and June prior to settlement of YOY.

Figure Legends

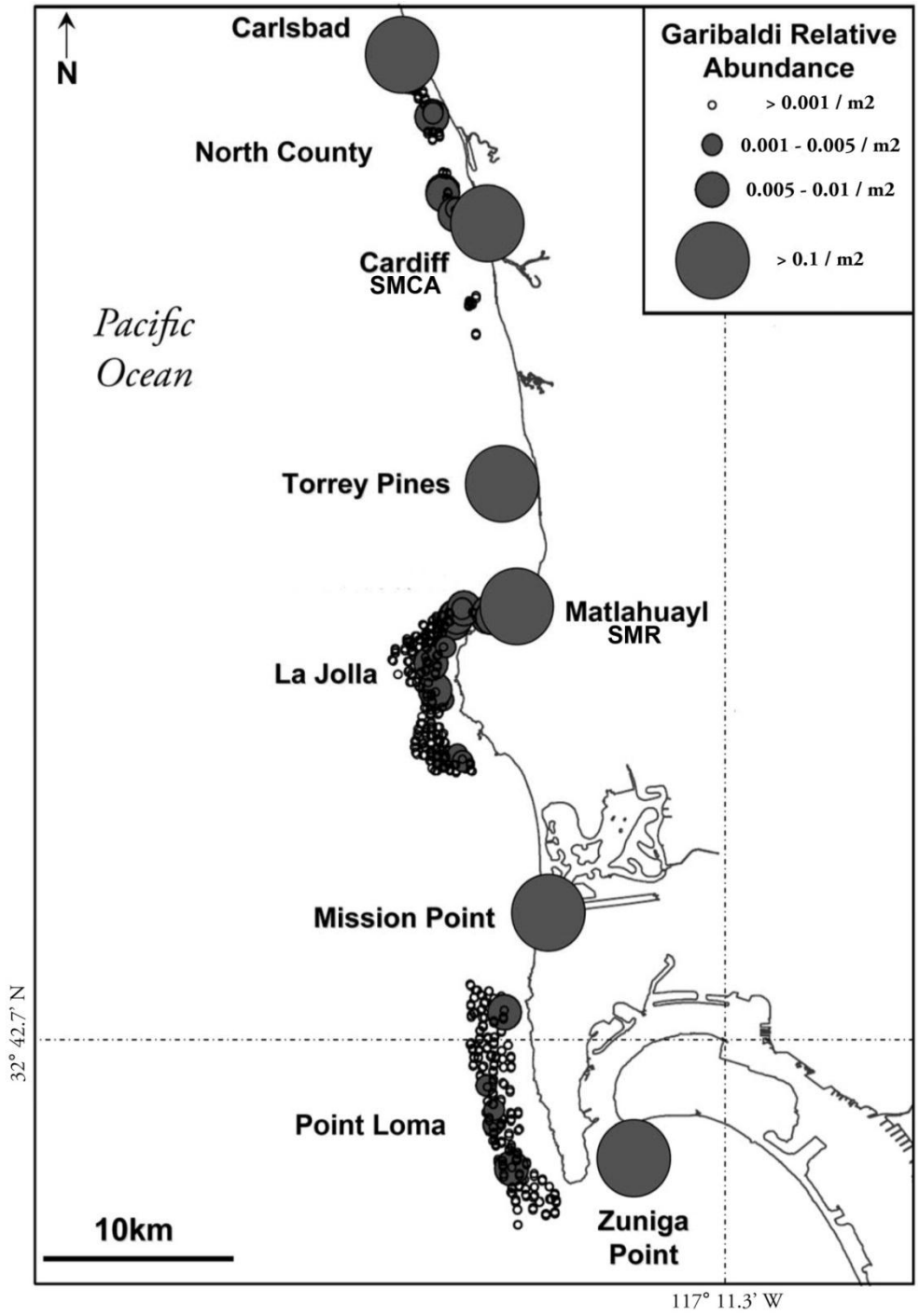
Figure 2.1 Map of study area with garibaldi density. Relative abundance of garibaldi at individual survey locations is indicated by size of circle.

Figure 2.2 Garibaldi weight-length relationship based on collection of individuals in 2008 and 2009 (n = 102). Solid triangles indicate individual fish.

Figure 2.3 Density of garibaldi at locations with rocky substrate vertical relief ≥ 1 m (i.e., optimal garibaldi habitat). Box plots indicate median, 75^{th} and 25^{th} percentiles; whiskers indicate 95^{th} and 5^{th} percentiles. Smaller lines within box plots indicate mean (± 1 SE). Locations not connected by common letters are significantly different (One-way ANOVA $F_{9,188} = 156.5$, $p < 0.0001$; Tukey HSD $p < 0.001$).

Figure 2.4 Relative condition factor (see text for description) of young-of-the-year fish in 2008 and 2009. Box plots indicate median, 75^{th} and 25^{th} percentiles; whiskers indicate 95^{th} and 5^{th} percentiles. Horizontal lines bisecting box plots indicate value of mean condition factor for each year (One-way ANOVA $F_{1,87} = 37.7$, $p < 0.0001$).

Figure 2.5 Comparison of habitat complexity, as estimated by volume of rocky substrate, across study sites. Box plots indicate median, 75^{th} and 25^{th} percentiles; whiskers indicate 95^{th} and 5^{th} percentiles. Horizontal lines bisecting box plots indicate mean value for each location. Locations not connected by common letters are significantly different (One-way ANOVA $F_{5,54} = 21.0$, $p < 0.0001$, Post Hoc Tukey HSD $p < 0.05$).



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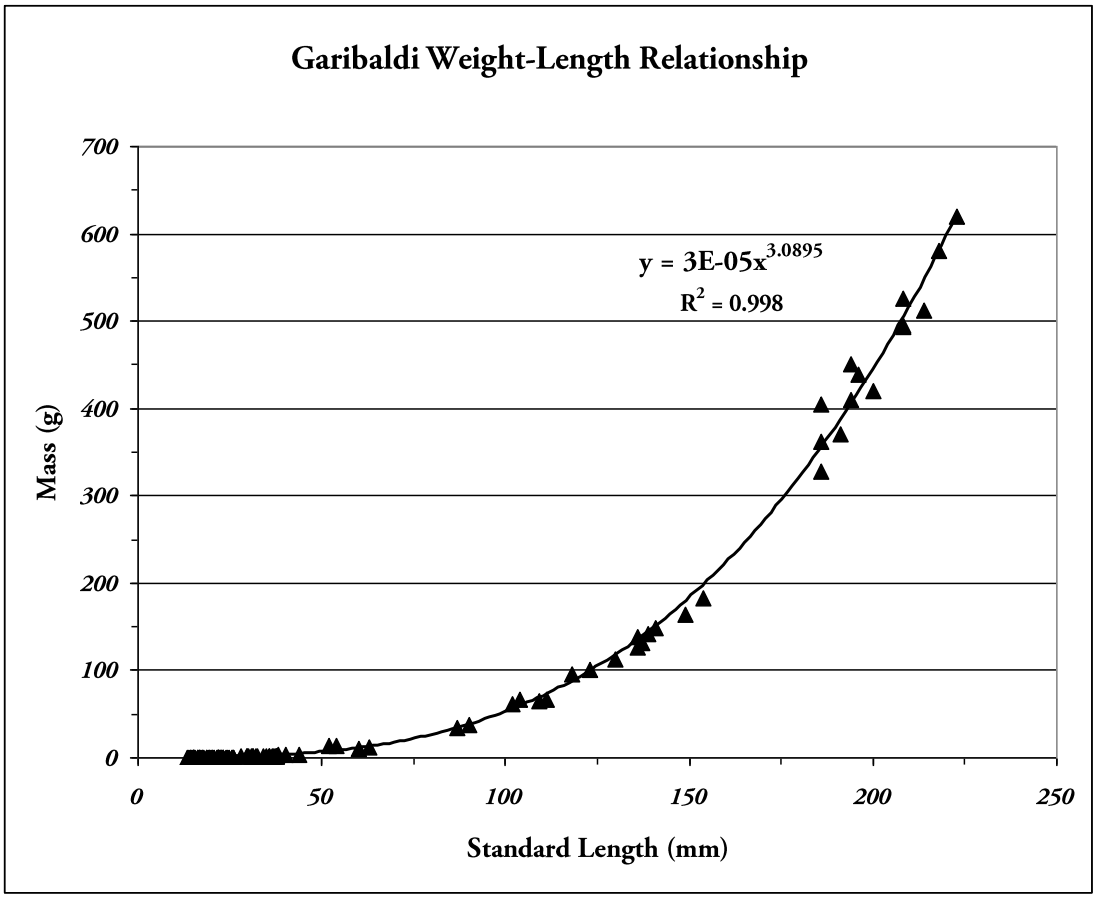
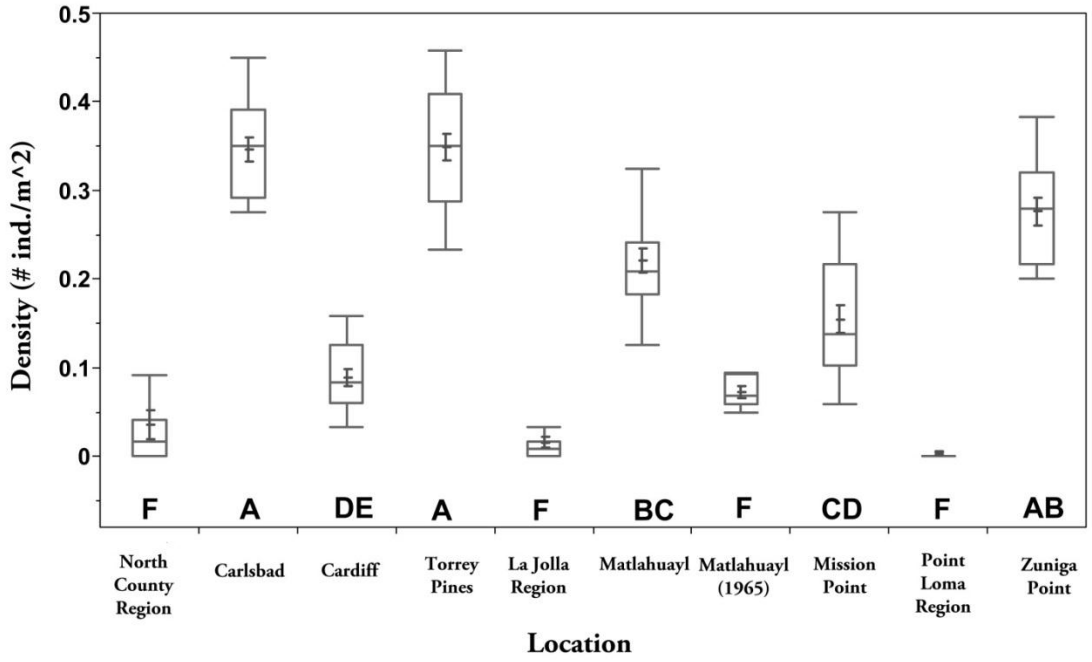


Figure 2.2 Garibaldi weight-length relationship based on collection of individuals in 2008 and 2009 (n = 102). Solid triangles indicate individual fish.



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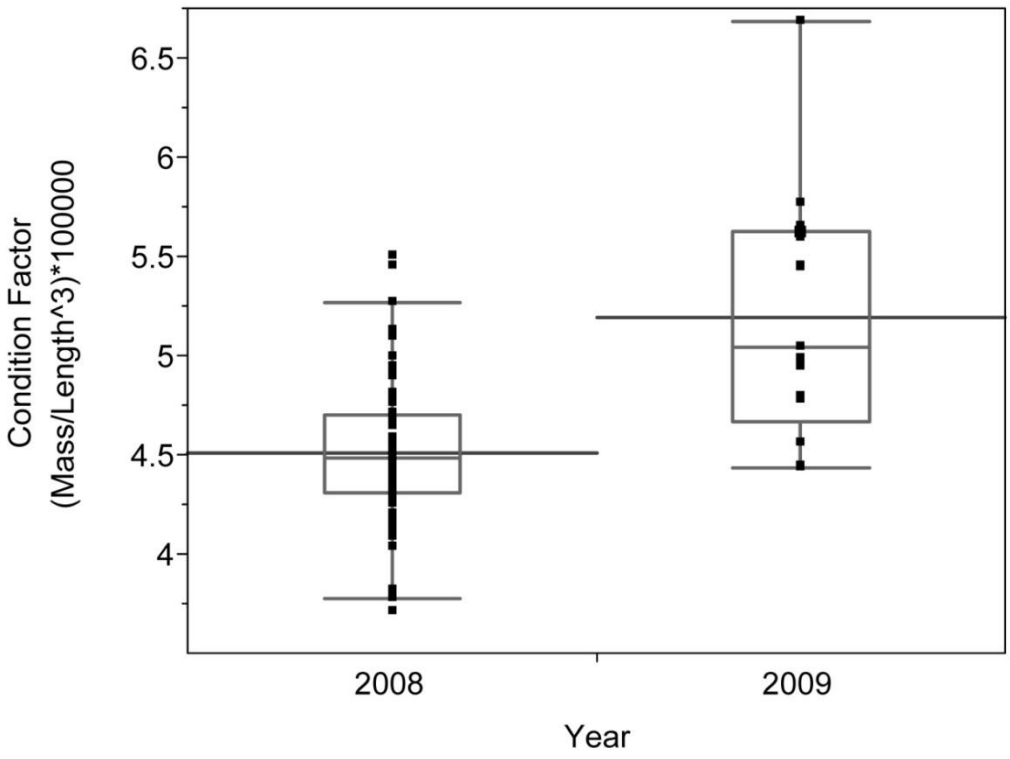
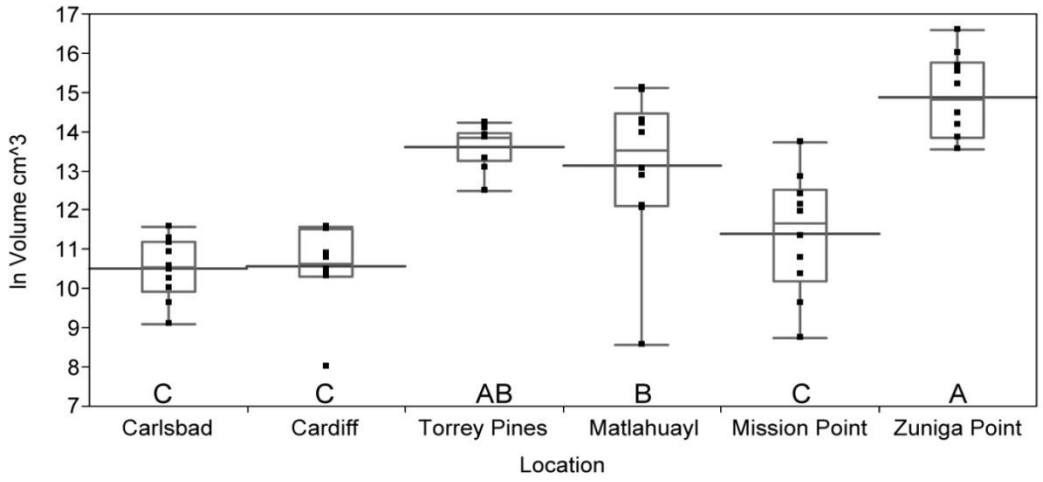


Figure 2.4 Relative condition factor (see text for description) of young-of-the-year fish in 2008 and 2009. Box plots indicate median, 75th and 25th percentiles; whiskers indicate 95th and 5th percentiles. Horizontal lines bisecting box plots indicate value of mean condition factor for each year (One-way ANOVA $F_{1,87} = 37.7$, $p < 0.0001$).



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CHAPTER III.

CHANGES IN OTOLITH MICROCHEMISTRY OVER A PROTRACTED SPAWNING SEASON INFLUENCE ASSIGNMENT OF NATAL ORIGIN

Abstract

A complete understanding of the role dispersal plays in marine population dynamics remains elusive. One method used to quantify movement among populations is otolith microchemistry. A challenge with this method is gaining an understanding of not only the spatial variability, but also the temporal variability of microchemistry that occurs over the course of a protracted spawning season. In this study high-resolution sampling was used to explore the spatial and temporal variability of elemental fingerprints of *Hypsypops rubicundus* at six rocky reefs spanning the southern California coastline. Embryonic fish were sampled from benthic nests during the entire 2008 spawning season (June 03 – September 02, 2008). Otolith microchemistry of 1101 larval fish and 72 juveniles was analyzed using a laser ablation inductively coupled plasma mass spectrometer. Within-reef elemental fingerprints were more similar than among-reef elemental fingerprints. The primary elements enabling discrimination among reefs, and their relative contribution (%) to the unique elemental fingerprint of each study reef were U (47%), Pb (31%), Ba (14%), Mg (9%), and Sr (1%). Over the course of the spawning season the two elements with the most consistent shifts in elemental chemistry from the onset to cessation of the spawning season were Ba (decreased across all study sites) and U (increased across all study sites). More than a third of the time (34%) the elemental

fingerprint of a given reef was indistinguishable from the elemental fingerprint of a different reef later in the spawning season. When natal origins of juveniles are classified using microchemical data spanning a protracted spawning season, the number of source locations and number of individuals predicted to have originated from individual source populations within the study region are underestimated. Researchers must utilize natural history information together with otolith microchemistry data to ensure natal origin of juveniles is assigned using data that account for both spatial and temporal changes in reef-specific elemental fingerprints.

Introduction

In light of pressures currently facing the marine environment, resource managers and policy makers are in need of methods that simultaneously protect marine biodiversity and move fisheries toward sustainability. One of the tools frequently used to achieve these management goals is the creation of marine protected areas (MPA). However to create successful MPAs, and MPA networks (i.e. MPAs connected via migration and/or larval dispersal), resource managers need empirical data describing the connectivity of populations on management-relevant time scales (i.e. 1-5 years). Over larger spatial and temporal scales, advances in molecular methods and large-scale physical oceanographic models have shown promise (see Cowen et al. 2007 and references therein), but one of the most promising techniques for quantifying the connectedness of fish populations on ecological time scales is otolith microchemistry (Thorrold et al. 2007).

Use of otolith microchemistry to quantify population connectivity is a two-stage process. First, it is necessary to ensure there are spatial differences in the reference "elemental fingerprints" imparted to otoliths developing within larval fish at the study sites of interest; for a recent review, see Thorrold et al. (2007). Second, to examine connectivity, it is necessary to capture individual fish after the pelagic dispersal phase and compare their natal chemical signatures with the reference chemical fingerprints. The natal origin of a post-dispersal fish can be inferred by using algorithms to compare otolith core signatures (i.e. the natal portion) of juveniles with the chemical reference data generated from larval fish otoliths collected at known locations earlier in the spawning season.

Early chemical fingerprinting research focused on connectivity in discrete locations and time periods (e.g. Swearer et al. 1999). However recognition of variability inherent in microchemistry led to multi-year studies searching for stability between years. At this point many studies have examined the inter-annual stability of otolith microchemistry in diverse geographic regions (e.g. Channel Islands (Warner et al. 2005, Standish et al. 2008), Gulf of Mexico (Patterson et al. 2008), Hawaiian Islands (Ruttenberg et al. 2008), southern California (Fodrie and Levin 2008), United States; Galapagos Islands, Ecuador (Ruttenberg and Warner 2006); Victoria, Australia (Barbee and Swearer 2007)). These studies have met with varied success in finding inter-annual stability in chemical fingerprints in island settings and along open coastlines. Some show that otolith microchemistry can be used to assess population connectivity over 10s of kilometers within a given year (Warner et al. 2005).

However, as one moves to larger spatial scales (i.e. 100s of kilometers), variation in geographic locale and natural history of the study species may influence the success of this method. On this larger spatial scale otolith microchemistry is most successful with diadromous species, where freshwater imparts an identifiable chemical signature to otoliths (e.g. Barbee and Swearer 2007, Clarke et al. 2009, Walther and Thorrold 2009). Successful application of this method in other systems has been challenging because of a lack of consistency in chemical fingerprints resulting in lower classification success and reduced confidence in inferred patterns of population connectivity (e.g. with a Hawaiian Island reef fish, *Abudefduf sordidus*; Ruttenberg et al. 2008). To infer patterns of connectivity successfully, these and other studies have suggested a need to match the life history and scale of larval dispersal distance with the spatial and temporal scale of variability in otolith microchemistry (Ruttenberg and Warner 2006, Ruttenberg et al. 2008, Walther and Thorrold 2009).

Elemental fingerprinting applications must contend not only with the spatial variability in otolith chemistry, but also the natural variability in chemical signatures over time. Understanding how otolith microchemistry temporally varies is critical for accurate interpretation of otolith-derived population connectivity estimates. Temporal variation in water chemistry can occur on decadal, interannual, or shorter time scales. An ability to recognize changes in the elemental fingerprint of a given reef over the course of a protracted spawning season has not been examined in detail. Commonly young-of-the-year (YOY) fish are collected at a given location, and an otolith-derived chemical reference map generated from larval fish collected during one segment of a

protracted spawning season is used to infer YOY natal origin. Usually the assumption is made that elemental signatures imparted to larval otoliths do not change over the course of the spawning season. If such change occurs, however, the chemical reference map used may incorrectly classify a recruit as originating at one location, when in reality it was spawned at a different location. To infer patterns of population connectivity using elemental fingerprinting one must ensure dispersing fish are classified against the appropriate chemical reference map (Walther and Thorrold 2009).

There is a paucity of data available to address the uncertainty of connectivity estimates associated with intra-seasonal variability of otolith microchemistry (i.e. chemical fingerprint data spanning a protracted spawning season, but see Hamer et al. 2003 or Becker et al. 2005 for an invertebrate example). Hamer et al. (2003) found little intra-annual variability in otolith microchemistry over a two month period while Becker et al. (2005) found relatively high stability in weekly-scale chemical fingerprints derived from *Mytilus* mussel shells collected over a four week period from 26 January – 21 February 2002. Many demersal species exhibiting a bipartite life cycle spawn over an extended period of time (i.e. several months), with lengthy planktonic durations. Here I ask whether intra-seasonal temporal changes in elemental chemistry may confound the interpretation of natal origin in an eastern Pacific temperate damselfish.

This study, spanning the entirety of a 12-week spawning season, enables the first in-depth examination of intra-annual temporal stability of otolith microchemical

signatures. It highlights the value of high-resolution temporal sampling to infer spatial patterns of connectivity among populations inhabiting discontinuous rocky reefs by examining otolith microchemistry across a protracted spawning season. The inherent variability in chemical signatures of larval fish otoliths is quantified over the three-month spawning season of garibaldi (*Hypsypops rubicundus*), a rocky reef damselfish inhabiting waters of the Southern California Bight, USA. Otolith microchemistry data are assessed on bi-weekly time scales over three spatial scales: (1) within nest (<1 m), (2) within reef (among nests separated by meters to 10s of meters), and (3) among reefs separated by 5 - 65 km. Due to broad environmental changes occurring over the protracted spawning season (e.g. within the study region mean water temperature at 5 m water depth increased from ~18 °C to 21 °C from onset to cessation of spawning), I hypothesized (a) that otolith chemical variability among samples collected from different nests at a single reef will not vary significantly at one point in time, (b) that there will be significant differences in elemental signatures within a study reef over the spawning season, and (c) changes in the otolith chemical signatures, both at the within- and among-reef scales, will increase the complexity and uncertainty inherent in identifying the putative natal origins of post-dispersal phase YOY.

Methods

Garibaldi as a Model Species

Damselfish (Pomacentridae) are widely distributed in shallow tropical, subtropical, and temperate waters (Allen 1991). Garibaldi (*Hypsypops rubicundus*), a pomacentrid of the San Diegan biogeographic province, primarily inhabits moderate relief (i.e. >30 cm) rocky bottom habitat from Bahia Magdalena, Mexico in the south to Point Conception, California, USA in the north (~ 24° N to 34° N; Hubbs 1960, Limbaugh 1964). *Hypsypops rubicundus* is a demersal spawner, a trait present in ~50% of nearshore fish species of this region (Miller and Lea 1972; Sikkel 1988, 1989; Allen 1991; Love 1996). Here nearshore is defined as by Shanks and Eckert (2005) as the estuarine, the intertidal, and waters ≤ 30 m depth for species whose range falls between 30° N and 47° N. In San Diego waters, male *H. rubicundus* tend a filamentous red algae nest between March and September; shortly after water temperatures consistently remain above 16 °C onset of spawning commences (Cook unpubl. data). Females deposit tens of thousands (mean = 129,000) of elliptical eggs that remain affixed to the red algal nest by sticky filamentous tendrils; typically spawning begins in mid-May and ends by early September (Limbaugh 1964, Clarke 1970). The otoliths of embryonic fish develop while attached to the benthic nest for approximately two weeks. During this embryonic-developmental period prior to hatching the otoliths incorporate various trace elements into their calcareous matrix, a process that ultimately enables the identification of a natal elemental fingerprint. Just prior to hatching, the sagittal otoliths of the embryonic fish are ~40 μm in diameter along the major axis and 10 μm "thick" (Cook unpubl. data). After larval fish hatch from their egg capsules, they spend between 18 to 22 days dispersing in the pelagic realm prior to settling on relatively shallow (i.e. ~5-10 m) rocky reefs (Clarke 1970, Moser 1996).

Study Region and Sample Collections

Samples were collected from six major rocky reef spawning sites in San Diego County, USA. These sites span ~65 km of coastline, and in sum comprise over 90% of the *H. rubicundus* population in San Diego County (Figure 3.1, Map of Study Region), a population estimate based on >650 demographic transect surveys conducted between 2002 and 2010 (Cook and Parnell unpubl. data). Benthic nests were located on rocky reef substrate between 3 m and 15 m water depth. The distance between adjacent sites ranged from as little as 5 km (i.e. La Jolla and Torrey Pines) to as much as 15 km (i.e. La Jolla and Mission Point); the nearest suitable habitat/spawning sites outside of the study region are located 35 km to the south of the southernmost study site, near Rosarito, Baja California and 50 km north of the northernmost study site, near Dana Point, California. Median depth-averaged water velocities of < 2 cm sec^{-1} measured in this region (Rasmussen et al. 2009, Rasmussen unpubl. data) suggest the six study reefs span more than twice the distance a passively dispersing larval fish would travel over an 18 – 22 day pelagic larval period.

Trends in otolith microchemistry data across the 2008 spawning season are presented for all six reefs. Of these reefs, Torrey Pines had the longest spawning season in 2008 (i.e. individual study sites had spawning seasons 4 -12 weeks in duration, Table 3.1). Using SCUBA, nests were monitored throughout the 2008 spawning season, and biweekly collections of late-stage embryos (i.e. those just prior to hatch) were made from four subjectively-selected benthic nests on each dive from

June 03 2008 – September 02 2008. Immediately after collection, samples from individual nests were placed on ice, and upon return to the laboratory were placed in acid-washed 50mL centrifuge tubes and frozen until further analysis.

Larval Collections

In total 32 nests were collected at Torrey Pines, of these, 456 larval fish from 23 nests provided a single sagittal otolith of sufficient size to generate usable trace-elemental data (i.e. otoliths were large enough to be individually extracted, cleaned, mounted, and analyzed). In a similar fashion, benthic nest collections were made from June 03, 2008 until August 21, 2008 at the other five study reefs, yielding a combined total of 1101 larval fish otoliths from 77 nests across all six study sites (Table 3.1).

Otolith Extraction, Preparation, and Elemental Analysis

Most larval fish hatched from collected egg capsules between the time of collection on a benthic nest and the collector's return to the water's surface. Samples were frozen at -20 °C prior to processing, and upon thawing single larval fish were placed in an individual drop of MilliQ water (i.e. quartz-distilled water with resistivity >18.1 MΩ) on an acid-washed slide. A single sagittal otolith was extracted using a fine-tipped tungsten probe; subsequently otoliths were transferred among slides using a nasal hair affixed to a wooden dowel (methods developed by Becker et al. (2005) and Motomitsu Takahashi (Seikai National Fisheries Research Institute)). After this

point, any glassware coming into direct contact with extracted otoliths was not acid-washed, but rather rinsed 10X in MilliQ to prevent dissolution caused by acidic residue remaining on acid-washed slides (Koch pers. comm., Cook pers. obs.). These otoliths were subsequently moved from a clean lab area to a Class 100 clean room.

To eliminate contaminants and remove organic tissue, otoliths were transferred from the extraction slide to a drop of 15% H_2O_2 buffered with 0.05 mol L^{-1} NaOH. They were then sonicated for 5 minutes, transferred to a single drop of MilliQ, and sonicated for an additional 5 minutes, then rinsed three more times. After the final MilliQ rinse, otoliths were mounted on double-sided tape affixed to petrographic slides, and stored in a Class 100 laminar flow hood located within a Class 100 clean room.

All otoliths were analyzed using a New Wave UP 213 nm laser ablation unit coupled to a Thermoquest Finnegan Element 2 Inductively Coupled Plasma Mass Spectrometer (at UC Santa Barbara). Three standards were used to ensure proper calibration of the instrument: a solution-based dissolved $CaCO_3$ reference material (OTO), and two solid standards, NIST 612 (a commonly used glass standard) and the newly available USGS MACS3 $CaCO_3$ standard. All reference materials, both solution- and solid-based, were analyzed at the beginning and the end of each run; mounting medium and instrument blanks were run multiple times during a sequence.

Based on preliminary testing, ^{24}Mg, ^{55}Mn, ^{87}Sr, ^{138}Ba, ^{208}Pb, and ^{238}U were selected for analysis in low resolution. Isotopes were included in subsequent analyses if counts were >3 standard deviations of background levels when blanks were run. All

isotope data are given as concentration relative to ^{48}Ca in either mmol mol^{-1} or μmol mol^{-1} (hereafter, "concentration"). For ablations, laser intensity was set at 50% with a 40-μm spot size and a four second dwell time. As larval fish otoliths were relatively small (range = 8.5 – 44.6 microns in diameter) it was necessary to ablate all otolith material during a single ablation, this precluded multiple ablations of the larval fish otoliths and YOY natal core (see below). Estimates of the external precision for standards, given as relative standard deviation (% RSD) for the various isotopes, and detection limits are given in Appendix 3.1.

Young-of-the-Year Collections

In 2008, post-dispersal phase YOY (n = 72) were collected from four of the six study reefs using hand nets. Two reefs, Zuniga Point and Torrey Pines, did not receive any YOY settlers in 2008. The YOY collections occurred from August 26 until November 20, 2008. The sagittal otoliths from these fish were extracted using a ceramic scalpel, and were cleaned, processed, and stored in the same manner as the larval otoliths. However, in the final stage of processing, rather than being mounted on double-sided tape, otoliths were mounted on petrographic slides using cyanoacrylate. Otoliths were polished to within 15 - 30 microns of the core using a lapping wheel with 30 μm and 3 μm diamond polishing film, and prior to analysis cleaned with 1% HNO_3^- and MilliQ water. One sagittal otolith was used for microchemical analysis, while the remaining sagittal otolith was used for aging fish by counting daily growth rings. To identify core signatures of the otoliths, eight

consecutive vertical pits each consuming a “disc” of otolith material approximately 40 microns in diameter and 10 microns “thick”. Ablations over the core region of each otolith were preceded by pre-ablations to remove possible contaminants; the putative core disc was identified by a characteristic Mn spike (Ruttenberg et al. 2005). The microchemical data of the core discs were compared subsequently with the reef-specific chemistry created by the larval fish otoliths to infer the natal origin of the post-dispersal phase YOY fish (see Statistical Analyses below). Independent seawater samples were not collected as part of this study as previous work in the region has shown that the elemental chemistry of aragonitic structures (e.g. larval fish otoliths) becomes integrated over periods of multiple days and does not resemble point samples of water collected during the same period of time (Becker et al. 2005, Warner et al. 2005).

Estimates of YOY age were derived by counting otolith daily growth rings using light transmission microscopy. Rings were counted from the edge of putative core regions (identified by measurements of larval fish otoliths) to an outer edge. However, otoliths proved challenging to unequivocally age because of poor light transmission near otolith primordia. To be conservative, YOY were placed within two-week bins reflecting the putative two-week period when they were developing embryos on benthic nests (Figure 3.2).

Statistical Analyses

Multivariate outliers were identified using jack-knifed mahalanobis distances. Univariate outliers (those falling outside of 95% confidence intervals) were identified by visual inspection, and to be conservative these otoliths were removed from statistical analyses. All elements violated the univariate assumption of normality (as assessed by the Shapiro-Wilk's goodness of fit test, $p < 0.05$). Natural log transformed data improved assumptions of normality but qualitatively were similar to untransformed data in parametric univariate and multivariate analyses. Therefore results and significance levels are presented for untransformed data. One-way analysis of variance (ANOVA) was used to examine univariate temporal differences in elemental chemistry at a given study site (i.e. how a given element changed over the spawning season); ANOVA also was used to test for spatial differences in individual elements (among nests within a reef and among reefs during a given segment of the spawning season). Significant results of the omnibus tests were assessed with a Tukey honestly significant difference (HSD) test. Nested multivariate analysis of variance (MANOVA) was used to assess temporal and spatial differences in multi-elemental fingerprints. Pillai's trace was chosen as the test statistic to assess significant differences of multi-elemental concentrations as it is more robust to violations of multivariate normality (Zar 1999). All ANOVAs and MANOVAs were completed using JMP (Version 6.0.3).

To examine the relative influence of temporal and spatial scales on the trace elemental fingerprints, non-parametric multivariate analysis of similarity (ANOSIM) procedures were conducted with otolith microchemistry from individual reefs binned

across three temporal periods: biweekly, monthly, and over six weeks (i.e. early vs. late spawning season). A post-hoc similarity percentages procedure (SIMPER) was conducted to determine which elements were responsible for driving the chemical variability within and between reefs across all temporal scales (Clarke and Warwick 2001, Clarke and Gorley 2006).

ANOSIM is a permutation procedure applied to the Euclidean similarity matrix using a calculated R statistic, and here tests the null hypothesis that there is no difference in the chemical fingerprints between two reefs (Clarke and Warwick 2001, Clarke and Gorley 2006). R ranges from 0 (i.e. otolith chemistry within and between reefs is the same) to 1 (i.e. chemically all otoliths from one reef are more similar to each other than to otoliths from a different reef). In this procedure 10000 permutations were run. Subsequently the value of R for the empirical microchemistry data is compared with the distribution of R derived from the permutation test to calculate a probability that the observed R statistic is no different than an R achieved by a randomly generated data set. As p values are greatly influenced by sample size, results of the ANOSIM are best interpreted by examining R values. An $R < 0.25$ indicates reefs are chemically indistinguishable from one another, while an $R > 0.5$ implies otoliths from reefs are chemically well separated.

To test if chemical similarity was related to the geographic distance separating reefs or to the length of time between sampling events (i.e. a proxy for when otoliths acquired their natal signatures), the data from the ANOSIM procedures were used to divide the reef-to-reef comparisons into two groups based upon R values. To

determine if reefs with more distinct elemental fingerprints have more geographic distance between them and/or more time between sampling events than those reefs with poor separation, a t-test was conducted between reef pairs with R values greater than 0.5 (n = 78, reefs with higher discriminatory ability) and those with R values less than 0.25 (n = 87, reefs with lower discriminatory ability). All ANOSIM and SIMPER routines were conducted on natural log transformed data to compute the Euclidean distance matrix and subsequent dissimilarity matrix using PRIMER Version 6.1.6 (Clarke 1993, Clarke and Warwick 2001, Clarke and Gorley 2006).

Linear DFA was used to test how temporal binning of microchemistry data (i.e. two, four, six, and 12 weeks) influences the prediction of natal origins of YOY. Quadratic DFAs were also performed on these data for comparative reasons; however, these neither resulted in significant differences from linear DFA (i.e. differences in classification success between the two methods were less than 1%), nor different classification of natal origin of YOY fish. Results are presented only from the linear DFA. In the DFA classification procedure, larval otoliths were used to create a training dataset, and YOY cores were classified as unknowns against this training set to determine their putative natal origin. To compare the DFA jackknife reclassification success to that expected from a randomly generated data set, 1000 runs were made of randomized data sets. All DFAs and randomization procedures were run in Matlab Version 7.4, modified from White and Ruttenberg (2007).

Results

Of the initial 1101 larval fish otoliths, 72 otoliths were removed from subsequent analyses because they fell below detection limits for individual elements (52 for Pb, 6 for U) or because they were multivariate outliers (n = 14), reducing the total sample size of larval otoliths included in analyses to 1029. This removal of outliers decreased the total number of clutches analyzed from 77 to 76 (i.e. one nest from Torrey Pines was not included in analyses). The median number of otoliths per nest providing usable microchemistry data ranged from 10 to 19 (Table 3.1). In addition, manganese yielded measureable concentrations in larval otoliths, but consistently fell below detection limits. Therefore Mn data are presented for comparative purposes, but are excluded from multivariate analyses.

Spatial Variability of Otolith Microchemistry

Within-Reef (10s of meters) Elemental Variability

Significant differences were detected in among-nest, multi-elemental chemical fingerprints (MANOVA $p < 0.001$), but generally there were no significant differences in univariate elemental concentrations among nests collected at a given reef for a given date. At Torrey Pines across the 36 possible one-way ANOVAs (i.e. six elements over six dates) spanning the 2008 spawning season, 26 had no significant differences. Two of the elements, Mg and Mn, were never significantly different among nests. Three of the elements, Sr, Ba, and Pb, had significant among-nest

differences on two dates, and U was significantly different among-nests on four of the six sampling dates. Upon further review with post-hoc Tukey HSD tests ($p < 0.05$) it was apparent that significant results in element-by-element ANOVAs were attributable typically to one nest with a significantly higher or lower concentration of a single element.

Among-Reef (5 - 65 km) Elemental Variability

Study reefs are separated by distances between 5 and 65 km. At this spatial scale when otoliths from individual reefs were grouped across the entire 2008 spawning season, reef-specific elemental fingerprints differed significantly from one another (MANOVA Pillai's Trace = 0.354, $F_{25,5115} = 15.58$, $p < 0.0001$). Zuniga Point and Torrey Pines otoliths had significantly higher levels of Mg compared to Cardiff, La Jolla or Mission Point otoliths (One-way ANOVA $F_{5,1023} = 5.74$, $p < 0.0001$). La Jolla otoliths had significantly higher levels of Mn than those from Carlsbad, Mission Point, or Zuniga Point (One-way ANOVA $F_{5,1023} = 3.74$, $p < 0.002$). Levels of Sr in larval fish otoliths were significantly lower at La Jolla compared to all other sites (One-way ANOVA $F_{5,1023} = 48.44$, $p < 0.0001$); Ba levels were significantly higher at Carlsbad, La Jolla, and Zuniga Point (One-way ANOVA $F_{5,1023} = 5.64$, $p < 0.0001$). Levels of Pb were significantly lower in otoliths at Torrey Pines and La Jolla (One-way ANOVA $F_{5,1023} = 3.12$, $p < 0.008$), and U was significantly lower in otoliths from La Jolla and Mission Point (One-way ANOVA $F_{5,1023} = 23.04$, $p < 0.0001$).

When elemental fingerprints of reefs are compared for only the period of time when all sites had spawning fish (mid-July – mid-August), reef elemental fingerprints

were significantly different (MANOVA Pillai's Trace = 0.503, $F_{25,1835} = 8.22$, $p < 0.0001$). Carlsbad otoliths had significantly higher levels of Mg than did Mission Point otoliths (One-way ANOVA $F_{5,367} = 3.16$, $p = 0.008$), La Jolla otoliths had significantly higher levels of Mn than those from Cardiff (One-way ANOVA $F_{5,367} = 3.05$, $p = 0.01$), Carlsbad and Cardiff otoliths had significantly higher levels of Sr than otoliths at all other reefs (One-way ANOVA $F_{5,367} = 29.87$, $p < 0.0001$), Mission Point and La Jolla otoliths had significantly lower levels of Ba (One-way ANOVA $F_{5,367} = 13.89$, $p < 0.0001$), and Torrey Pines and Zuniga Point otoliths had significantly higher levels of U when compared against all other reefs. Pb levels did not differ significantly among any of the reefs (One-way ANOVA $F_{5,367} = 1.00$, $p = 0.42$; Figure 3.3).

Temporal Variability of Otolith Microchemistry

Within-Reef (10s of meters) Elemental Variability

When individual reefs were examined for temporal changes in elemental fingerprints over the 2008 spawning season, results varied by reef. At Carlsbad, the northernmost site, multi-elemental fingerprints were similar over the one month spawning season in 2008 (MANOVA Pillai's Trace = 0.148, $F_{5,70} = 2.07$, $p = 0.08$).

At Torrey Pines (the reef with the most protracted spawning season in 2008) elemental fingerprints changed significantly from early June to early September (MANOVA Pillai's Trace = 0.882, $F_{30,2145} = 15.32$, $p < 0.0001$; Figure 3.4). Three of

the elements, Mg, Pb, and U, increased in relative concentration over the course of the spawning season, while Mn, Sr, and Ba decreased (all $p < 0.001$). Elemental fingerprints at all other study reefs changed significantly over the course of the 2008 spawning season (MANOVA Pillai's Trace associated p values < 0.0001 for all reefs).

Among-Reef (5 – 65 km) Elemental Variability

When otolith-derived chemical data from individual reefs were binned across two, four, or six weeks, and compared against other reefs, highly significant differences were observed in study reef multi-elemental fingerprints at all temporal scales (MANOVA Pillai's Trace $p < 0.0001$). In a subsequent univariate comparison, relative concentrations of all six elements comprising otoliths collected during the first half (i.e. June 03 2008 – July 15 2008) of the spawning season were significantly different from otoliths collected during the second half of the spawning season (i.e. July 16 – September 02 2008; all one-way ANOVA p values < 0.001, Tukey HSD $p < 0.05$). Similarly, at the shortest temporal scale (i.e. bi-weekly), chemical fingerprints of reefs changed significantly over the course of the 2008 spawning season, in both multi-elemental fingerprints (MANOVA Pillai's Trace = 1.095, $F_{115,5025} = 12.25$, $p < 0.0001$) and individual elements compared among reefs (One-way ANOVA $F_{23,1005}$ ranged from 3.7 to 29.3, all $p < 0.0001$; all Tukey HSD p values < 0.05).

Interaction of Spatial and Temporal Variability of Otolith Microchemistry

Of the 15 reef-to-reef comparisons spanning the entirety of the 2008 spawning season, none had an R statistic greater than 0.16 ($p < 0.001$), suggesting that at this temporal scale (~12 weeks) the reefs were essentially indistinguishable from a microchemical standpoint. As the data were broken down to shorter temporal bins, the R values increased. The highest separation among reefs occurred when data were compared in two-week bins indicating that the greatest chemical separation among study reefs occurred at this shortest temporal scale (ANOSIM $R = 0.8$, $p < 0.0001$; Bonferroni corrected value of alpha < 0.05).

When chemical fingerprints of individual reefs were assessed at this shortest temporal scale, there were 300 reef-to-reef comparisons possible (including combinations when the elemental fingerprint of one reef was compared against itself at a later point in the spawning season). When only distinct reefs are compared there are 255 reef-to-reef comparisons. In 87 of these (34% of the comparisons), one reef was chemically indistinguishable from a different reef at a later segment of the spawning season (ANOSIM $R < 0.25$, $p < 0.0001$; Bonferroni corrected value of alpha < 0.05 for all reef-to-reef comparisons). Among these 87 comparisons, the primary elements driving the chemical differences between reefs (and relative contribution of these elements to chemical differences) were Pb (46%), U (26%), Ba (17%), and Mg (10%). There were 78 instances where reef pairs were well separated by otolith microchemistry (ANOSIM $R > 0.5$, $p < 0.0001$, Bonferroni corrected value of alpha < 0.05); all other reef pairs had intermediate ANOSIM R values. The primary difference between reefs that chemically were well separated and those that were not well

separated, was in U, which accounted for the greatest chemical variability (66%), while Pb, Ba, and Mg contributed 22%, 7% and 5% respectively. In all comparisons, Sr typically accounted for less than 1% of the chemical differences between reefs.

To assess the relative importance of temporal and spatial scale on the ability to discriminate between two different reefs, the distance between reefs and the time between otolith collections was compared for reef pairs with low ($n = 87$, $R < 0.25$) and high ($n = 78$, $R > 0.5$) ANOSIM R values. In these two groups there was no statistical difference in the distance between reefs; on average 20.6 km separated reefs with distinct chemical signatures and 25.4 km separated reefs with similar chemical signatures (One-way ANOVA $F_{1,163} = 3.37$, $p = 0.07$). In a similar comparison, there was a statistically significant difference in the duration of time between sampling, 5.7 weeks for chemically distinct reefs and 3.1 weeks in chemically similar reefs (One-way ANOVA $F_{1,163} = 59.9$, $p < 0.0001$).

Classification of YOY Fish

Discrimination among reefs (using DFA) was most successful (i.e. had highest classification success) when data were analyzed in two week bins (e.g. mean biweekly classification success = 71% vs. 53% for the entire 2008 season). However, all combinations comparing otolith microchemistry among reefs across all four temporal bins (i.e. biweekly to seasonally) resulted in DFA classification success rates

significantly higher than classification success rates that would have been observed given a randomly generated data set ($p < 0.001$).

When elemental signatures from YOY fish were compared against the various temporal bins of larval otolith microchemistry data, different natal origins were predicted. Use of larval reference data for the complete spawning season suggested all but two of the 72 YOY collected in 2008 originated at the reef in La Jolla (n=70, 97%); the remaining two individuals appear to have originated from Mission Point. Predicting natal origin of YOY using the chemical reference map generated from the entire 2008 data set, for example, suggests 7 of 8 individuals that were developing embryos in the first two weeks of August (based on otolith aging) originated from La Jolla reefs. However, based on empirical survey data, there was no spawning occurring in La Jolla at this point in time. As YOY microchemistry was compared with larval reference data from shorter periods of time (i.e. closer to the two week bins in which individuals are actually developing embryos acquiring natal signatures on benthic nests), a third natal source, Torrey Pines, appeared to contribute ~6% of YOY. When natal origins of YOY are classified using larval reference data from discrete two-week bins, La Jolla remains the predominant source population within the study region, but the proportion of YOY initially predicted to be from La Jolla decreased from 97% to 82%, while the proportion of YOY predicted to originate at Mission Point increased to ~13%. Using these 2-week datasets to predict the natal origin of the same eight YOY considered in the example above suggests that the seven individuals initially predicted to originate from La Jolla (using the entire 2008 data set), are now

predicted to originate from Mission Point, a site where empirical data show spawning was still occurring (Figure 3.5).

Discussion

The prevalence of otolith microchemistry as a tool for quantifying larval dispersal distances and population connectivity has grown since its first successful application (Swearer et al. 1999). However, we must strive to increase our understanding of the factors that may influence interpretation of these types of data particularly when the impetus for many of these studies is to quantify the connectedness of spatially discontiguous populations, in attempts to better the effectiveness of spatial management of marine resources. The findings of this study suggest that if resource managers do not have sufficient data to pair accurately the life history of focal species with data describing population connectivity, conservation efforts may be inadvertently misdirected. For example, if data from this study were viewed holistically and the entire 2008 data set were utilized to identify source populations, one of the three source populations, namely Torrey Pines, would not have been identified. In addition, the relative importance of Mission Point, a location that supplied YOY to reefs 15 km to 50 km away, may have been downplayed or even overlooked by stakeholders during the site-selection process.

If otolith microchemistry is to be used as a tool for inferring population connectivity, there is a necessity for ion uptake to be regulated by environmental

rather than physiological factors (for a recent in-depth discussion see Elsdon et al. 2008). Laboratory studies have started to shed light on which ions are regulated primarily by environmental parameters, and as such are the most appropriate for utilizing otolith microchemistry to address questions regarding population connectivity. Bath et al. (2000) show otolith incorporation of Sr and Ba is proportional to concentrations found in ambient waters. In a complementary study examining the relative importance of ambient water chemistry and diet in otolith chemical composition, Walther and Thorrold (2006) show the majority of strontium and barium ions comprising otoliths are regulated by surrounding water chemistry (83% and 98% respectively), further suggesting otolith microchemistry of these ions reflects ambient water chemistry.

In an examination of inter-annual variability of otolith microchemistry, Walther and Thorrold (2009) found that in order to successfully utilize otolith microchemistry to identify natal origins of *Alosa sapidissima* (American shad), one must be aware of how variable microchemistry is on inter-annual time scales, and suggested that if considerable inter-annual variability exists, efforts must be made to accurately match microchemical data from larval fish to the cohort of interest. This study builds upon Walther and Thorrold's work by showing that efforts must also be made to understand the intra-annual variability in otolith microchemistry, particularly for those species with protracted spawning seasons. However, Hamer et al. (2003), in a comparison of two months of *Pagrus auratus* (Sparidae) otolith microchemistry data from southeastern Australia, determined that intra-annual (i.e. monthly-scale)

chemical variability spanning the February-March recruitment season did not influence the classification accuracy of adults to their juvenile origin, and hence temporal mismatches did not influence conclusions regarding connectivity patterns. In Hamer et al. (2003), within-year differences in classification success were driven primarily by barium. The different conclusions being drawn between this and the present study may stem from the environmental history of the study organisms. *Pagrus auratus* spends its early life in estuarine inlets prior to migrating to open coast habitat, while *H. rubicundus* spends its entire life along the open coast. The strength of the barium "signal" incorporated into the otolith during this early juvenile estuarine phase, as evidenced by its preponderance in driving discrimination among inlets being studied by Hamer et al., appears to override the input of other elements comprising the otolith. However, with *H. rubicundus*, and perhaps for other species inhabiting open-coast regions, the relative importance of individual elements enabling discrimination among sites, in part due to a lack of this estuarine period, may mean more high resolution sampling (relative to the species in question) is necessary to adequately resolve connectivity patterns.

While we continue to refine our use of this tool, generalizations regarding connectivity patterns remain few, with many differences among taxa and geographic locale. This study addresses the challenges associated with and the necessity for understanding how microchemical fingerprints of reefs vary over time. For studies to accurately assess patterns of population connectivity and estimate larval dispersal distances efforts must be made to collect and analyze samples spanning the entire

spawning season of the species in question. Trace elemental fingerprints derived from otolith microchemical data reported here change over time scales ranging from weeks to months. These data emphasize challenges faced by scientists and resource managers using otolith microchemistry to quantify connectivity among marine populations inhabiting open coastlines, and show the extent of data collection necessary to capture the intra-annual temporal and spatial variability that occurs in otolith microchemistry. Without this knowledge, estimates of dispersal distance and population connectivity can perhaps be viewed best as first order approximations of reality, and should be interpreted with caution.

Key to successful natal assignment is matching empirical data collection efforts to the natural history of the organism in question. In this study embryonic *H. rubicundus* incorporate their natal signatures over a two week benthic period prior to dispersing in the pelagic realm. For *H. rubicundus,* two weeks turns out to be the most appropriate time scale at which to bin data. However, if the species in question was incorporating its natal signatures over a 30-day or 7-day period, perhaps the most appropriate scale at which to collect samples and bin data would differ.

The results of this study suggest two things: (1) the chemical composition of otoliths collected more closely in space and time tend to be more similar, and (2) the spatial chemical variability, while significant (in the statistical sense of the word), cannot be used to assess connectivity patterns, without an understanding of the temporal changes that occur in otolith microchemistry.

Conclusions

In ecology it has often been said that one must ask questions at the appropriate spatial and temporal scale (e.g. Levin 1992). In this instance, it has been shown that efforts must be made not only to ask questions at the appropriate scale, but to design studies at spatial and temporal scales relevant to the natural history of the organism of interest. To understand the role of dispersal in population dynamics, particularly for species approximately described as metapopulations due to dispersive developmental stages connecting spatially discontiguous habitat patches, this can be a daunting task. If the dispersal stage spans periods of several months, or if the spawning period is greatly protracted, as is the case for numerous marine species, the use of otolith microchemistry to quantify population connectivity may remain a tremendous logistical challenge.

The primary goal of this study was to demonstrate that changes in otolith microchemistry occur over the course of an extended spawning season, and these changes could result in the "confusion" of one reef for another (as the ANOSIM data show more than a third of the time). These temporal changes in reef-specific chemical fingerprints could influence an investigator's ability to accurately assess natal origin, larval dispersal distances, and connectivity patterns. This suggests that regardless of the forces influencing otolith microchemistry over a spawning season, having empirical data describing the biology of the species in question is invaluable and necessary to accurately quantify population connectivity using microchemical methods.

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Table 3.1 Number of nests collected, otoliths generated, mean (± sd) number of otoliths analyzed per nest, range of 2008 spawning season, and number of young-of-the-year (YOY) collected at each reef.

	Study Reef						
	Carlsbad	Cardiff	Torrey Pines	La Jolla	Mission Point	Zuniga Point	Total
Total Nests Collected	10	12	32	18	11	12	95
Nests Analyzed by LA-ICPMS	8	9	23	18	8	11	77
Otoliths Analyzed by LA-ICPMS	80	114	456	212	123	116	1101
Mean Otoliths per Nest Analyzed (± sd)	9.5 ± 2.5	10.8 ± 5.0	19.8 ± 5.9	10.9 ± 4.9	14.8 ± 7.0	9.5 ± 3.0	
First 2008 Collection Date	July 24	July 10	June 03	June 03	July 15	July 22	
Last 2008 Collection Date	Aug 21	Aug 21	Sept 02	July 29	Aug 12	Aug 19	
YOY Collected	18	10	0	42	1	0	72

Figure Legends

Figure 3.1 Map of Study Region, including location of six study reefs where *H. rubicundus* were collected 1) Carlsbad, 2) Cardiff, 3) Torrey Pines, 4) La Jolla, 5) Mission Point, and 6) Zuniga Point. Stars indicate location of study sites. Inset map shows location of study region within California, USA.

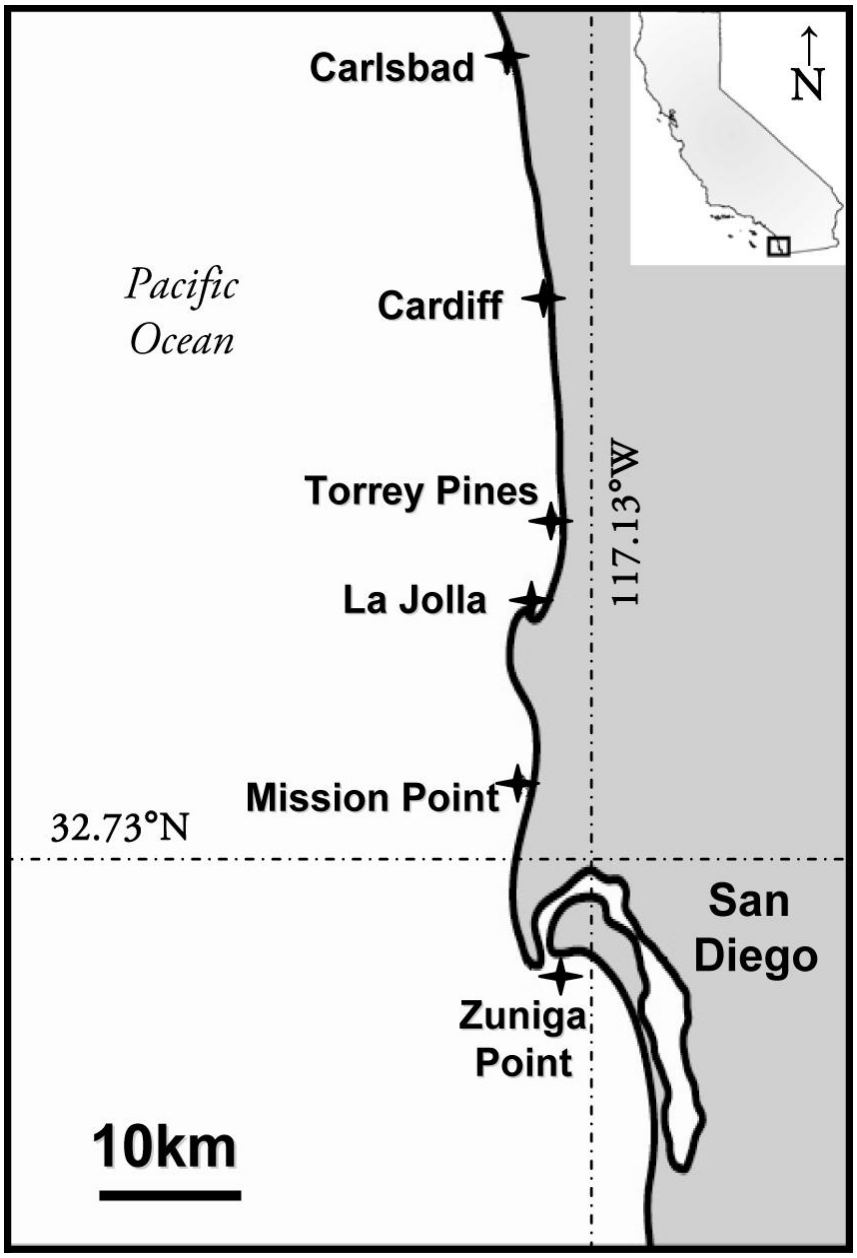
Figure 3.2 Putative benthic nest period of young-of-the-year (YOY) collected in 2008 (n = 72). Each two week period is the time period during which the YOY were acquiring their natal elemental signatures. Cross-hatched portions of the histogram indicate the proportion of YOY predicted to have originated from Torrey Pines (~6%). Grey portions of the histogram indicate the proportion of YOY predicted to have originated from La Jolla (~82%), and black portions of the histogram indicate the proportion of YOY predicted to have originated from Mission Point (~13%).

Figure 3.3 Inter-reef spatial variability of *H. rubicundus* otolith microchemistry during period of concurrent spawning (i.e. mid-July – mid-August 2008). Box plots indicate median, 75^{th} and 25^{th} percentiles; whiskers indicate 95^{th} and 5^{th} percentiles. Nests not connected by similar letters are significantly different (Tukey HSD $p < 0.05$). Continuous horizontal grey lines indicate grand mean. CD = Carlsbad, CF = Cardiff, TP = Torrey Pines, LJ = La Jolla, MP = Mission Point, and ZP = Zuniga Point. Note different scales on Y axes.

Figure 3.4 Temporal variability of *H. rubicundus* otolith microchemistry at Torrey Pines Reef across the 2008 spawning season. Box plots indicate median, 75^{th} and 25^{th}

percentiles; whiskers indicate 95th and 5th percentiles. Two-week bins of time not connected by similar letters are significantly different (Tukey HSD $p < 0.05$). Continuous horizontal grey lines indicate grand mean. Suffixes after month indicate the first 15 days of the month (a) or last fifteen days of the month (b). Note different scales on Y axes.

Figure 3.5 Natal classification of eight young-of-the-year (YOY) fish that were acquiring elemental signatures between August 01 and August 15 2008 using data spanning (A) the entire 2008 spawning season or (B) August 1 – August 15. Numbers indicate the proportion of YOY dispersing between two reefs. Arrows indicate directionality of dispersal, and the thickness of each arrow is proportional to the number of YOY dispersing. Stars indicate sampling sites.



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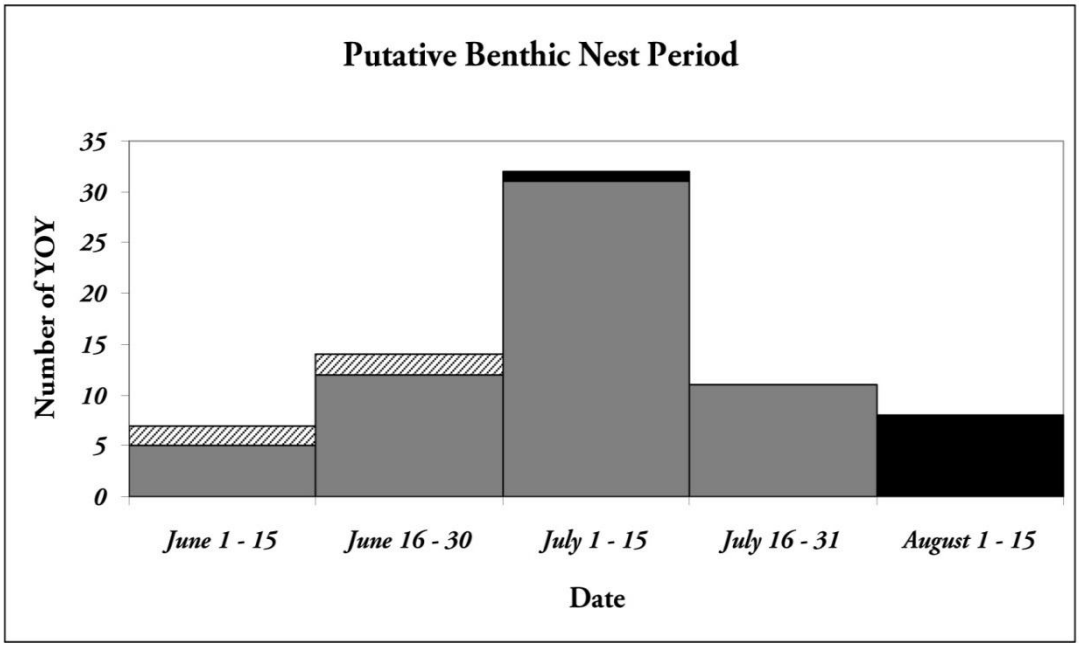

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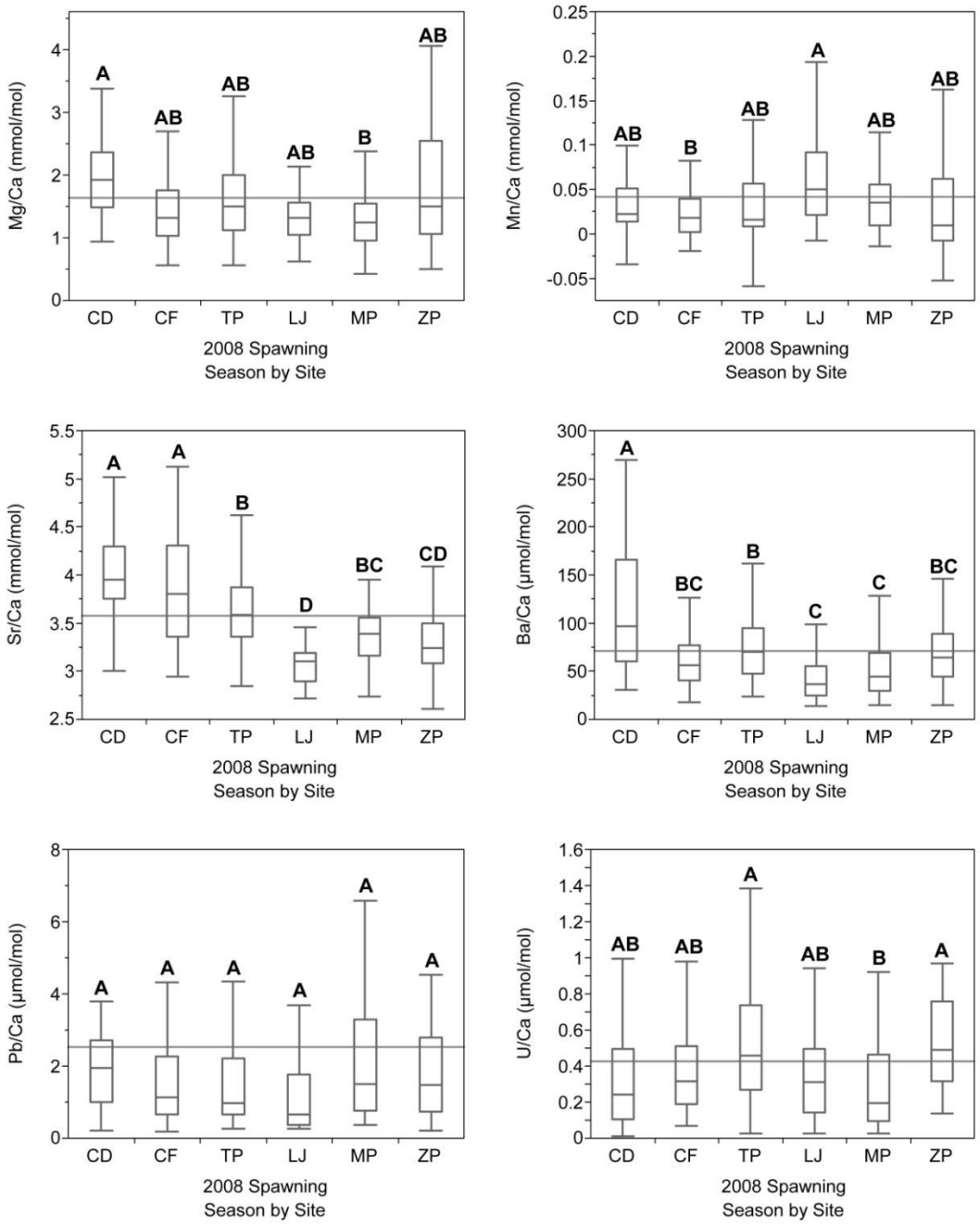

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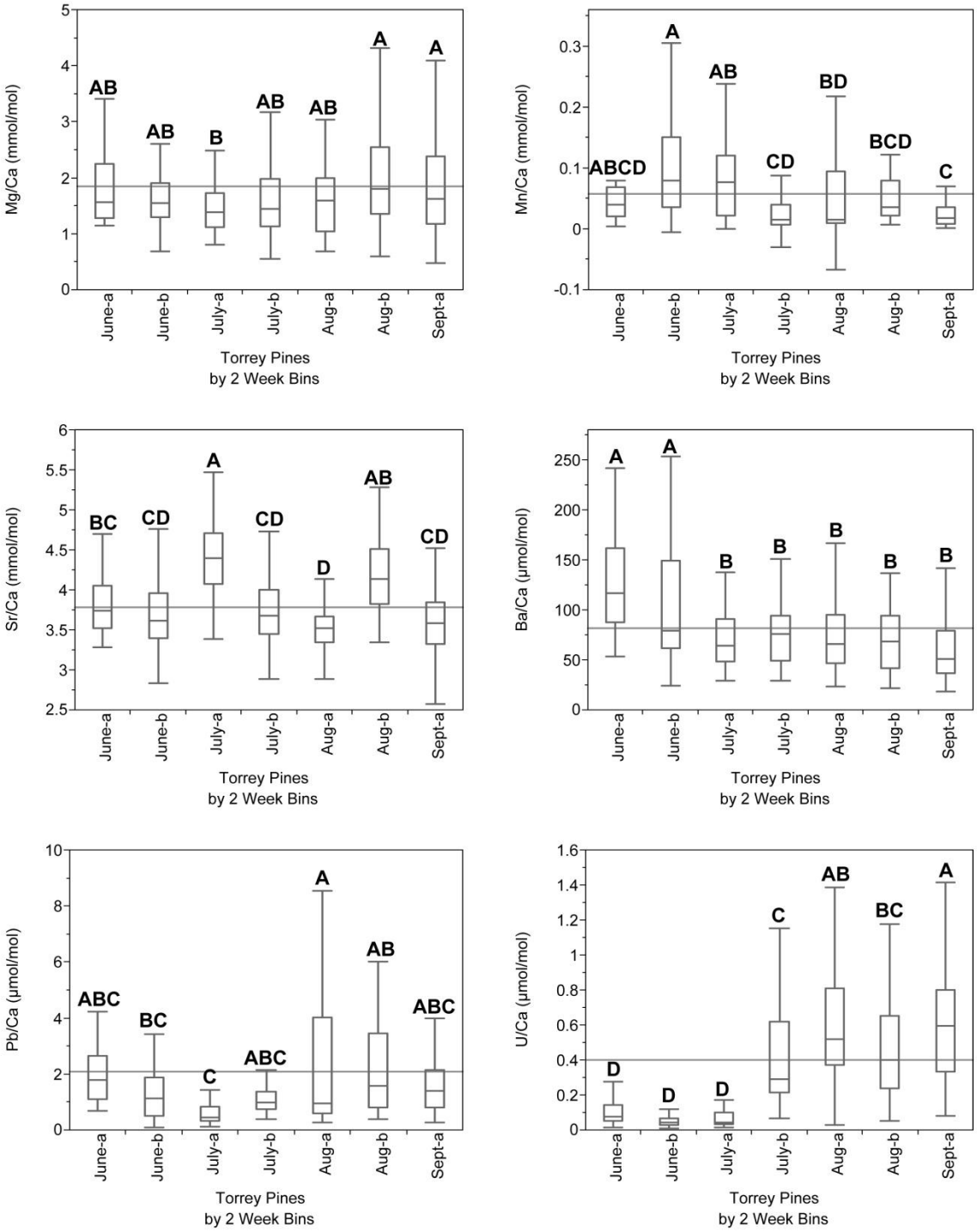

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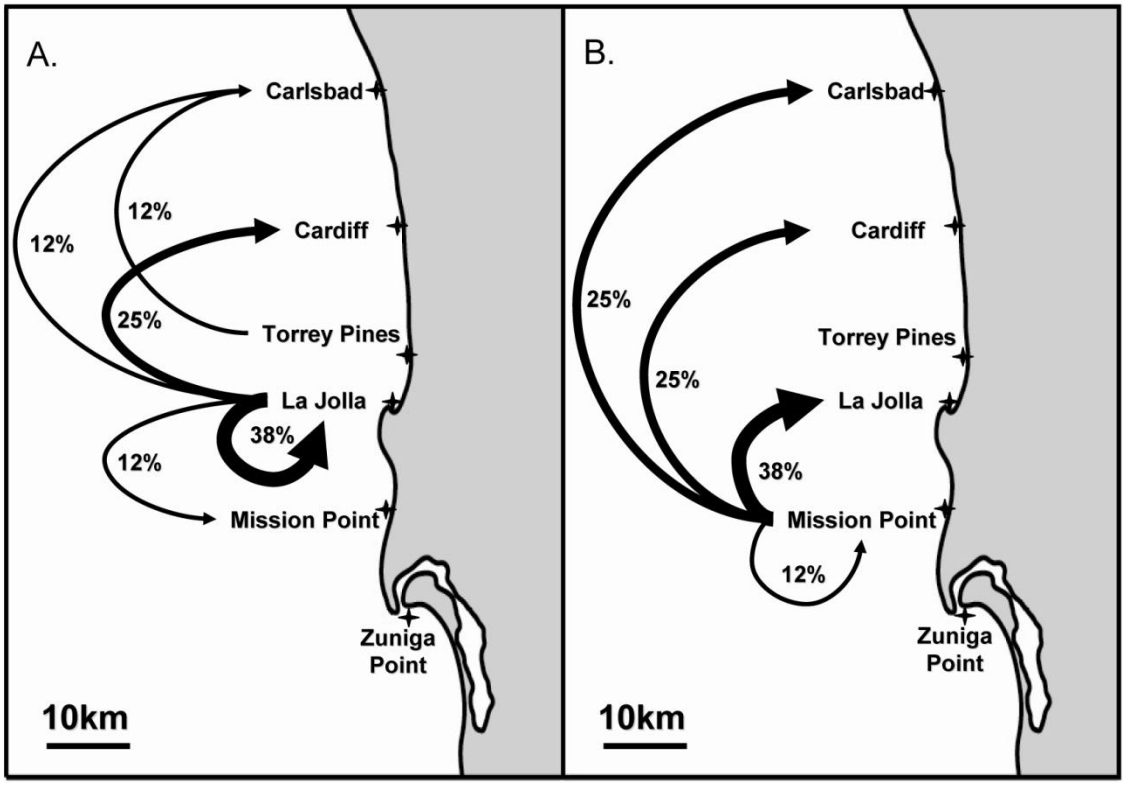

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Appendix 3.1 Estimates of precision (relative standard deviation, % RSD) for LA-ICPMS analyses and limits of detection for elements of interest. RSDs were calculated from more than 6500 means of 6 scans (i.e. number of scans per sample), and are presented for a dissolved $CaCO_3$ reference material (OTO), and two solid reference materials: NIST 612 and USGS MACS3

	%RSD			
Element	**OTO**	**NIST 612**	**MACS 3**	**Detection Limit**
Mg	**1.03**	**9.50**	**7.68**	**0.04 mmol/mol Ca**
Mn	**1.63**	**8.87**	**6.02**	**0.03 mmol/mol Ca**
Sr	**2.18**	**8.23**	**4.74**	**0.04 mmol/mol Ca**
Ba	**3.22**	**10.01**	**8.93**	**0.31 µmol/mol Ca**
Pb	**4.41**	**10.07**	**8.17**	**0.25 µmol/mol Ca**
U	**5.27**	**8.68**	**13.43**	**0.01 µmol/mol Ca**

CHAPTER IV.

HIGH-FREQUENCY SHIFTS IN CONNECTIVITY PATTERNS WITHIN AN OPEN-COAST MARINE PROTECTED AREA NETWORK: IS THE WHOLE GREATER THAN THE SUM OF ITS PARTS?

Abstract

A complete understanding of population connectivity via larval dispersal remains an unresolved challenge to the effective design of marine protected areas. Empirical estimates of larval dispersal trajectories and population connectivity patterns are few, continuing to stymy a more complete understanding of marine population dynamics needed successfully manage marine populations. Here we use high resolution spatial and temporal sampling to quantify connectivity patterns of new recruits of the temperate rocky reef damselfish, *Hypsypops rubicundus,* over three time scales: biweekly, seasonally, and interannually. Empirical data for this species, which serves as a model for nearshore fishes of the eastern temperate Pacific, suggest three MPAs spanning 46 km within a larger network of reefs are connected by larval dispersal, but the magnitude and direction of these connections reverse between years. Counter to existing hypotheses, self-recruitment, where an individual is spawned and subsequently settles to the same location, was documented to be significant at two reefs along the open coastline, one of which is an existing MPA. Within individual years, MPA self-recruitment ranged from 50-84%. Over the 2008 and 2009 spawning seasons, self-recruitment accounted for 45% of all individuals recruiting within the study region, suggesting that populations could persist without larval inputs from

outside the local study region. On biweekly time scales there is high-frequency variability seen in both current data and re-created larval dispersal trajectories. However, this variability is damped by persistent unilateral northerly dispersal when data are viewed across longer time scales. Counter to expectations, the results of the high frequency sampling has implications for evolutionary-scale population dynamics, suggesting a well-mixed larval pool, while low frequency annual-scale connectivity patterns suggest the network of reefs behaves more as a source-sink metapopulation with ecological-scale implications for natural resource management.

INTRODUCTION

Marine larval dispersal has been called one of the "major unsolved problems of biological oceanography", and it is viewed as "one of the crucial gaps in scientific knowledge" facing marine ecologists, resource managers, and policy makers (Palumbi 1999, Sale et al. 2005). Accurate and robust estimates of larval dispersal and local retention are essential prerequisites to resolving marine colonization patterns and designing effective marine protected areas (MPA; Cowen et al. 2007). Many studies predict numerous ecological benefits both within and outside the boundaries of MPAs; within MPAs these include increases in abundance, diversity, and productivity. Hypothesized ecological benefits of MPAs outside of their boundaries are two-fold; MPAs may (1) provide spillover effects enabling adjacent areas to be more productive and (2) augment populations on a regional scale via larval dispersal and/or emigration of adults and juveniles.

Numerous ecological benefits are purported to exist both within and outside the boundaries of MPAs (Palumbi 2002, Lubchenco et al. 2003, Lipcius et al 2008). Within MPAs these include increases in abundance, diversity, and productivity of marine communities; lower levels of mortality within MPAs also decrease the probability of local and regional extinction (Lubchenco et al. 2003, Worm et al. 2006). Ecological benefits of MPAs outside of their boundaries are potentially two-fold. MPAs can provide spillover effects allowing surrounding areas to be more productive and may augment populations on a regional scale via larval transport and/or emigration of adults and juveniles (Sale et al. 2005). Documentation of self-recruitment and MPA connectivity are fundamental to both the successful design of MPAs and advancing our understanding of the benefits MPAs can provide to those tasked with protecting marine biodiversity and moving fisheries closer to sustainability. One of the purported ecosystem services provided by networks of MPAs linked by larval dispersal is the ability of "upstream" populations to re-seed "downstream" regions open to resource extraction, yet despite its importance the evidence for this reseeding remains elusive. Accurate and robust estimates of larval dispersal and self-recruitment are critical prerequisites to designing effective MPAs (Botsford et al. 2009).

Theoretical and simulation modeling suggest the importance of population connectivity and self-recruitment to marine population dynamics and the success of MPA networks (see Botsford et al. 2009 and references therein). However for the field to develop a more mechanistic understanding of connectivity and its role in

population dynamics will require empirical evidence; evidence which until recently has remained elusive for the majority of ecosystems. Elegant studies in insular settings have recently shed some light on the extent of self- recruitment and connectivity within MPAs. Startlingly high self-recruitment has been documented within populations of two coral reef fishes inhabiting a single MPA at Kimbe Island, and subsequent work by this research group provided the first unequivocal documentation of fish dispersing among the locations proposed for a suite of MPAs in Kimbe Bay, Papua New Guinea (Almany et al. 2007, Planes et al. 2009). Levels of self-recruitment within this embayment were estimated to be ~40% and larval dispersal up to 35 km was documented over an approximately 11-day pelagic larval period. Similar studies attempting to quantify self-recruitment and connectivity along open coastlines, including among a suite of MPAs, have met with some success. Mytilid mussels exhibit both self-seeding within and connectivity among broadly defined regions along an open coastline in southern California (Becker et al. 2007). Differing reproductive seasons (spring vs. fall) combine with seasonal reversal of physical transport mechanisms to yield different dispersal trajectories for two co-occurring species (Carson et al. 2010), with major lessons for interpreting connectivity among MPAs sited within this region. Spillover effects of protection from fishing, manifested as decreasing recruitment with distance from MPAs has been documented for mussels along the South African coastline (Pelc et al. 2009). However, and despite valiant efforts to overcome the logistical challenges associated with tracking minute particles moving through a fluid matrix, robust empirical quantification of self-recruitment and connectivity among a suite of MPAs subject to advection along an

open coastline remain largely in the realm of simulation modeling (Cowen et al. 2006, Siegel et al. 2008, Mitarai et al. 2009, Watson et al. 2010).

This study, building upon the methodological foundation laid down by these earlier researchers, is now able to quantify for the first time self-recruitment and connectivity among a suite of MPAs sited along the open coastline of the northeast Pacific Ocean. Using a protected oviparous fish, *Hypsypops rubicundus* as a model for a relatively pristine (i.e. unfished) population, this project captures the emergence of annual patterns of connectivity, and highlights the challenges and conservation ramifications of these for MPA design. More specifically, high-resolution sampling over the course of a three-month spawning season enabled an in-depth exploration of how patterns of marine population connectivity evolve over three distinct time scales: biweekly, seasonally, and inter-annually and systematically documents how source and sink populations emerge and disappear on timescales heretofore undescribed. In turn these data are used to explore how variability in connectivity across temporal scales influences the interpretation of population structure and ramifications of these for the successful management of marine populations.

This study was designed to answer two questions fundamental to the success of MPAs as management tools: (1) are MPAs located along an open coastline in southern California connected by larval dispersal? and (2) does self-recruitment occur along open coastlines to the extent that reef-level populations can persist without larval inputs from other source populations? In addition to answering these fundamental questions, this data set enabled the testing of four closely-related but distinct

hypotheses: (1) the magnitude and directionality of larval dispersal vary spatially and temporally (2) all sites within the MPA network contribute evenly to the larval pool (3) Eulerian predictions of passive larval dispersal are adequate proxies for larval dispersal and population connectivity and (4) metapopulation structure describing this network of reefs. In addressing these questions and hypotheses, data describing the magnitude and variability in larval exchange between MPAs provide empirical validation of theoretical and simulation-based predictions of connectivity, and will ultimately aid in the stewardship of marine resources.

METHODS

Model Species

Many nearshore fishes have a bipartite life cycle in which isolated populations of relatively sedentary adults are connected by a widely dispersing larval stage. From a heuristic standpoint these spatially separated adult populations form a metapopulation (a population of smaller subpopulations linked by individuals dispersing among locations). In this study garibaldi, *Hypsypops rubicundus*, was selected as a model for rocky reef species for three primary reasons: (1) it has life-history traits similar to numerous inshore rocky reef fishes (see Chapter 1), (2) it has been informally and formally protected from commercial or recreational take for over 30 years, enabling it to be viewed as a pristine species (i.e. avoiding the shifting baseline syndrome (see Pauly 1995, Chapter 2)) and (3) it is territorial and has benthic

nests enabling collections of embryonic fish necessary to quantify population connectivity using trace elemental fingerprinting (see below). The natural history of garibaldi has been described in detail in Limbaugh (1964) and Clarke (1970). Briefly, spawning females deposit eggs on male-guarded benthic nests between late May and late August each year. During this three-month spawning season clutches of embryos develop on benthic nests for approximately two weeks (Clarke 1970, Sikkel et al. 1988). After this benthic developmental period, embryonic fish hatch and disperse in the pelagic realm for 18 – 22 days before settling to shallow rocky reef habitat (Clarke 1970, Moser 1996). Individual fish subsequently go through a series of phenotypic changes as they develop (over 5-6 years) into reproductively active adults (Clarke 1970).

Identification of Putative Source Populations

To identify possible source populations of garibaldi, thereby framing the boundaries of the study region, over 600 habitat/population surveys were conducted using SCUBA between 2002 and 2008 (Chapter 2). These data indicated that the population spanned ~60 km of open coastline; the next nearest possible source populations were >50 km to the north of the northernmost study site and >30 km to the south of the southernmost study site (see Chapter 2 for a detailed description of the study region). Within this region, six sites were identified as putative source populations; these six sites support >90% of the population within the study region (Chapter 2). Of these six sites, two are within the boundaries of MPAs, namely

Cardiff and La Jolla, and one, Zuniga Point, is adjacent to a third MPA, the Mia J Tegner State Marine Conservation Area (Figure 4.1, adapted from Chapter 2).

Empirically Quantifying Larval Dispersal and Population Connectivity

Otolith microchemistry was used to infer larval dispersal distances and generate population connectivity estimates. Otoliths are aragonitic structures found in the inner ear of fishes. They serve to keep the fish properly oriented in the water column and aide in hearing. As an embryonic fish grows, so does its otoliths. As the aragonite matrix is precipitated during otolith formation (Pannella 1971), elements from the surrounding water mass become incorporated, creating a unique chemical fingerprint that can be used to distinguish among different geographic regions (Swearer et al. 1999). At the time of hatch these otoliths are approximately 45 microns along the major axis and 10 microns "thick" (Figure 4.2).

The first stage of this process occurs prior to larval dispersal, during the benthic embryonic phase. Study sites were visited biweekly and collections of late-stage (i.e. just prior to hatching) embryonic fish were made. Data describing sample collections in 2008 and 2009 can be found in Table 4.1; otolith microchemistry data can be found in Appendix 4.1. Otoliths were extracted from these larval fish (n = 1784), trace elemental composition was analyzed, and linear discriminant function analysis was then applied to create a chemical reference map of the study region for each two-week period. Late in the three-month spawning season, young-of-the-year fish become visible (i.e. they have taken on their characteristic juvenile coloration; iridescent-blue and orange) on rocky reefs. These early juvenile fish were collected (n

= 89), and similar to the larval fish, their otoliths were extracted. After processing, the microchemistry of the otolith core (i.e. the natal portion ~45 microns in diameter) was analyzed (see below). The natal origin of a post-dispersal fish can be inferred by using algorithms to compare the otolith core chemical signature against the appropriate chemical reference map (i.e. the two-week period of the spawning season when the juvenile was an embryonic fish developing on a benthic nest). These data can be used to identify sources and sinks within the study system as well as to calculate larval dispersal distances, estimate dispersal trajectories, and assuming an 18 - 22 day pelagic larval period, mean dispersal rates. For a detailed description of methods involved in this process please see Chapter 3.

Otolith Processing

Methods for otolith extractions and processing of larval and YOY fish otoliths followed (Cook 2011); a brief description of otolith processing follows. Randomly chosen sagittal otoliths were removed from individual larval fish using fine-tipped tungsten probes. Subsequently a nasal hair fastened to a wooden dowel was used to transfer otoliths through a series of cleaning, sonicating, and rinsing steps using 15% H_2O_2 buffered with 0.05 mol L^{-1} NaOH (to remove organic material) and MilliQ water (i.e. quartz-distilled water with resistivity >18.1 MΩ). After the final rinse with MilliQ water, larval fish otoliths were mounted on double–sided tape affixed to petrographic slides, and stored until chemical analysis (see below).

Left sagittal otoliths from YOY fish were removed using ceramic scalpels and transferred with Teflon-coated forceps. Initial cleaning steps were the same as those

for larval fish otoliths, except that YOY fish otoliths were mounted in cyanoacrylate on petrographic slides and left for a period of 48 hours to enable hardening of the mounting medium. Subsequently, YOY fish otoliths were polished to within 15 microns of the otolith core using 30-μm and 3-μm diamond polishing film. After polishing, otoliths were cleaned with 1% HNO_3^- , and rinsed with MilliQ water. After the final processing steps, YOY and larval fish otoliths were stored within sealed petri dishes and placed under a Class 100 laminar flow hood housed within a Class 100 clean room.

Right sagittal otoliths were used to estimate the period within the spawning season when YOY were developing on benthic nests. After polishing otoliths (as above), light transmission microscopy was used to count daily growth rings. Counts were made outward from the edge of the natal region to an outer edge of the otolith. However the core region of YOY otoliths was frequently difficult to see clearly due to poor light transmission. Therefore YOY were placed conservatively within 14-day bins reflecting their putative two-week benthic embryonic period. The natal chemical signature of the left sagittal otolith removed from the same fish was compared with the chemical reference map from the corresponding period of the spawning season to infer natal origin (see statistical analyses below).

Laser Ablation – Inductively Coupled Plasma Mass Spectrometry

All trace elemental analyses were conducted using a New Wave UP 213 nm laser ablation unit coupled to a Thermoquest Finnegan Element 2 Inductively Coupled Plasma Mass Spectrometer at the UC Santa Barbara MSI Analytical Lab. Field

samples collected in 2008 were analyzed between April 16 - April 30 2009, and field samples collected in 2009 were analyzed from August 23 – September 10 2010.

Analytical methodology followed (Cook 2011). Seven isotopes were selected for analysis: ^{24}Mg, ^{48}Ca, ^{55}Mn, ^{87}Sr, ^{138}Ba, ^{208}Pb, and ^{238}U. Individual isotopes were included in subsequent analyses if their relative concentration was greater than 3X the standard deviation of blanks run during each sequence. Hereafter "concentration" will imply relative concentration of a given isotope to ^{48}Ca; values for ^{24}Mg, ^{55}Mn, and ^{87}Sr are reported in mmol/mol ^{48}Ca and values for ^{138}Ba, ^{208}Pb, and ^{238}U are reported in μmol/mol ^{48}Ca. For ablations of 2008 samples, laser intensity was set at 50% with a 40-μm spot size and a four second dwell time. With 2009 samples, signal stability was highest when laser intensity was reduced to 30%, and dwell time was increased to 5 seconds. Larval fish otoliths ranged in diameter from 8.5 – 46.4 microns. Due to this relatively small size, it was necessary to ablate the entire larval fish otolith and the entire natal core region of YOY otoliths to obtain stable signals and high enough counts to remain above detection limits. Estimates of instrument precision (as % Relative Standard Deviation) and detection limits for 2008 and 2009 samples are provided in Appendix 4.2.

Flow-Based Predictions of Larval Dispersal

Currents were measured intermittently between May 2008 and September 2009, spanning both garibaldi spawning seasons included in the two-year study. Only those data temporally appropriate for the study were included in the analyses. An RDI 660kHz ADCP (Acoustic Doppler Current Profiler) was deployed on the ocean bottom

at a depth of ~32 m between the two southernmost sites, Zuniga Point and Mission Point (approximately 32.7°N, 117.3°W). The ADCP sampled data in one minute ensembles over 2 meter depth bins between May 7, 2008 and May 31, 2008, and in 5 minute ensembles from June through September. Current data were lowpass filtered to remove the tides (<2 days) using a Butterworth filter. Lowpass filtered currents were then plotted in depth/time space with color indicating direction and opacity indicating magnitude. Mean current magnitudes were also plotted by bin for each summer after decimating the one-minute data from the first deployment (see above) to 5 minutes using a Chebyshev Type I filter. Data were analyzed using the digital signal processing toolbox in Matlab.

Statistical Analyses

All descriptive statistics and univariate analyses of dispersal data were conducted using JMP (Version 6.0.3).

Otolith Microchemistry

Statistical analyses of otolith microchemical data follow (Cook 2011), and are described in detail therein. Initially data were assessed for normality and homogeneity of variances, and where warranted, outliers were removed. Multivariate outliers were identified using jack-knifed mahalanobis distances and univariate outliers were identified by visual inspection (i.e. samples falling beyond 95% confidence intervals; Zar 1999). This resulted in 72 (of 1101) larval fish otoliths from 2008 and 50 (of 683) larval fish otoliths from 2009 being removed from statistical analyses. To improve

normality, data were either ln (x) or $\sqrt{(x + 3/8)}$ transformed prior to analyses (Zar 1999).

Due to the length of the benthic developmental period the most appropriate period of time in which to temporally bin data with this study system was approximately 14 days (i.e. the period of time in which the embryonic fish are acquiring their natal signatures; Cook 2011). To assess chemical differences among reefs at this temporal scale, linear discriminant function analysis (DFA) was used. All DFAs were run in a stepwise manner; only elements with an F-to-remove statistic greater than 2.5 ($p < 0.05$) were included in each model. One of the challenges with using DFA to predict natal origin is that individual fish are predicted to originate at one of the "choices" given by the chemical reference map. To address this we conducted extensive surveys prior to the study to ensure all possible source populations were accounted for (see above). In addition, during data exploration a seventh "unknown" location was added as a possible source population to test if YOY would be assigned to a virtual location not included among the six study sites. The prior probability of assignment to this "unknown" location was fixed at ~0.14 (1/7); however no YOY in either 2008 or 2009 were predicted to originate from this "unknown" source population. Therefore DFA results are presented for analyses using only the six primary study sites. For comparative purposes and to assign significance levels to the classification success of the DFA, data were compared against the classification success generated from 1000 randomized data sets (i.e. a null expectation of classification success); all DFAs and randomization procedures

presented were run in Matlab Version 7.4, with code modified from White and Ruttenberg (2007).

RESULTS

Across 2008 and 2009 spawning seasons, the trace elemental chemistry of 1662 larval fish otoliths was used to create biweekly chemical reference maps of the study region (Appendix 4.1). Subsequently the natal origins of 89 post-dispersal young-of-the-year (YOY) fish were determined using the chemical reference maps generated from the trace elemental chemistry of pre-dispersal larval fish otoliths. Mean classification success of larvae of known origin collected biweekly from egg capsules was 71% in 2008 and 66% in 2009; both are significantly greater than classification success of randomly generated data sets ($p < 0.001$).

Larval Dispersal and Natal Origins

In 2008, YOY appeared to originate from one of three natal reefs: Torrey Pines, La Jolla, or Mission Point. Of these locations, the sole no-take MPA, located within the center of the study region (i.e. La Jolla) was the largest source population; 82% of all captured YOY were predicted to have come from La Jolla. Mission Point and Torrey Pines produced 13% and 6% of post-dispersal recruits, respectively (Figure 4.6A). Net dispersal distance of individual larval fish ranged between 0 km and 46 km. When dispersal distances from the three natal origins were aggregated (including individuals predicted to self-recruit to a given reef which were assigned a

dispersal distance of 0), mean (± s.e.m) dispersal distance from June – August 2008 was 12.3 km (± 1.8 km). The mean larval dispersal distances for larvae from Torrey Pines, La Jolla, and Mission Point were 10.5 (± 7.1) km, 10.7 (± 1.8) km, and 23.6 (± 4.7) km, respectively. Larval fish originating at Mission Point dispersed significantly farther than those originating in La Jolla (23.6 km vs. 10.7 km; (One-way ANOVA $F_{2,69} = 3.23$, $p < 0.05$); Tukey HSD $p < 0.05$). Over shorter (biweekly) time-scales, mean dispersal distances differed significantly over the course of the 2008 spawning season; mean dispersal distance for larvae that were developing embryos during August 1 – 15 2008 (24.8 ± 4.9 km) was greater than for those developing during July 16 - 31 2008 (5.8 ± 4.2 km) (One-way ANOVA $F_{4,67} = 2.7$ $p = 0.04$, Tukey HSD $p < 0.05$). A temporal relationship with dispersal distance was not evident when comparing dispersal distances across the entire 2008 spawning season. Excluding self-recruiting individuals, and assuming an 18-22 day pelagic larval period, this suggests mean larval transport rates across 2008 ranged from 0.26 cm s^{-1} to 2.96 cm s^{-1}. When directionality of larval dispersal was assessed, 44% of larval fish dispersed 27.2 km (± 1.53) in a northerly direction, 51% self-recruited, while the reminder (5%) dispersed in a southerly direction (5.0 ± 0 km).

In 2009, four (of six) reefs were determined to serve as natal origins of post-settlement stage fish: Cardiff, La Jolla, Mission Point and Zuniga Point. La Jolla was again the predominant source of post-dispersal fish within the study region, producing 53% of settlers. Mission Point, Cardiff, and Zuniga Point produced 29%, 12%, and 6% of YOY, respectively (Figure 4.7B). From May - August 2009 individual larval

fish dispersed between 0 km and 59 km; mean dispersal distance over all source populations was 27.5 ± 3.7 km. Mean larval dispersal distances from Cardiff, La Jolla, Mission Point, and Zuniga Point were 19.0 (± 10.3) km, 20.8 (± 4.9) km, 36.8 (± 6.5) km, and 59.0 (±14.6) km, respectively. There were no significant differences in larval dispersal distances for larvae originating from different reefs or developing at different times during the study period. Making the same assumptions regarding pelagic larval duration, and excluding individuals that self-recruited, empirical data suggests mean net larval transport rates over the 2009 spawning season ranged between 0.74 cms^{-1} and 3.79 cms^{-1}. When directionality of dispersal trajectories was assessed in 2009, 65% of YOY dispersed in a northward direction (36.6 ± 3.6 km), while approximately 18% self-recruited or dispersed in a southerly direction (21.7 ± 2.7 km).

When comparing larval dispersal distances between years, garibaldi larvae on average dispersed significantly longer distances in 2009 (27.5 km) vs. 2008 (12.3 km; One way ANOVA $F_{1,87} = 13.9$, $p < 0.001$; Figure 4.3). When locations were pooled across years, larval fish from Zuniga Point dispersed significantly greater distances than those originating from La Jolla or Torrey Pines (59.0 km vs. 12.4 km or 10.5 km, respectively; Tukey HSD $p < 0.05$). Including individuals that self-recruited, mean larval dispersal distance across both years of the study was 15.2 km (± 1.7; Figure 4.4).

Flow-Based Results

Subtidal frequency currents deeper than ~5 m were directed mainly alongshore with mean velocities of ~2 cm sec^{-1} and rarely exhibited vertical shear (Figures 4.5A and 4.5B). These currents were unidirectionally northward (most common) or southward at periods of a few days to more than a week. Near-surface currents were strongly affected by winds with mean current magnitudes at least an order of magnitude greater than subsurface currents and were mainly directed shoreward and southward – the predominant direction of the afternoon sea breeze during summer (Figures 4.5A and 4.5B).

Connectivity and Self-recruitment in MPAs

MPA Connectivity

The three sites which encompass or are adjacent to existing MPAs are Cardiff, La Jolla and Zuniga Point. In 2008 two of these three sites, Cardiff and La Jolla, were connected via larval dispersal; Zuniga Point was not a source or destination of any YOY (Figure 4.6A). While sample sizes were relatively low (N=72) we assume that the YOY captured accurately reflect the greater unsampled portion of the population, it can be estimated that 80% of the YOY at Cardiff are supplied by La Jolla, while the remaining 20% are derived from Mission Point. When viewed more broadly, this 80% represents 11% of the YOY dispersing within the greater metapopulation. In 2009, however, this connectivity pattern was reversed; 33% of the YOY settling in La Jolla originated at Cardiff, while none of the YOY settling to Cardiff were supplied by La Jolla. This value (33% of YOY) represents approximately 12% of the metapopulation YOY (Figure 4.6B). When connectivity among existing MPAs was

assessed by summing the 2008 and 2009 dispersal trajectories, data suggest ~7% of all YOY within the study region dispersed northward from La Jolla to Cardiff, and 56% (50/89) of all YOY settling within the study region contributed in some manner to MPA connectivity (i.e. through self-recruitment to an MPA or dispersing between two MPAs; Figure 4.7).

Zuniga Point did not exchange larvae with any of the other study reefs in 2008, but it did act as a source and destination for YOY in the second year of this study. This location supplied ~11% of the YOY to Carlsbad in 2009, a site almost 60 km to the north, and it received a single southward dispersing YOY from one of the other MPA sites (La Jolla), a distance of almost 30 km. During some of the biweekly or annual time periods, Zuniga Point was not connected with other study sites, however high-resolution sampling over the entire 2008 and 2009 spawning seasons showed Zuniga Point acted intermittently as both a source and sink (~1% each) within the greater metapopulation (Figure 4.3 – 2D, 2E).

MPA Self-recruitment

Over the course of the 2008 and 2009 spawning and settlement seasons, self-recruitment occurred at La Jolla and Mission Point; La Jolla was the only MPA within the study region to exhibit self-recruitment. In 2008, 84% (36/43) of the YOY collected in La Jolla appear to be self-recruits (Appendix 4.3). These larvae, represent 50% of all larval fish dispersing within the study region in 2008. In 2009, La Jolla was the only location where self-recruitment occurred (Appendix 4.3). However, the extent of the self-recruitment was lower in 2009 than in 2008. Over shorter time-

scales La Jolla had relatively persistent self-recruitment over eight weeks of the 12-week spawning season in 2008 (Figure 4.3-1B-1E). Self-recruitment was more intermittent in 2009, occurring in late May, and again in early July (Figure 4.3-2A, 2D). In 2009, self-recruitment again supplied a large proportion of YOY to La Jolla (50%), but this value represents only 18% of all YOY collected within the study region in this year. Over both years of the study self-recruitment in MPAs accounted for 44% (39/89) of all YOY settling to rocky reefs (Appendix 4.3).

DISCUSSION

To this point, empirical studies of larval connectivity have provided only snapshots in time (i.e. once or twice a year) (Almany et al. 2007, Becker et al. 2007, Planes et al. 2009, Carson et al. 2010), making extrapolations beyond and interpolations between data sets tenuous. To our knowledge, this is the first study to document the evolution of larval connectivity patterns at high frequencies over multiple years. The high-resolution sampling in this study revealed that patterns of connectivity and larval dispersal, while generally northward when viewed on annual time scales, can in the short-term be highly variable, not only in direction, but also magnitude (e.g. Figure 4.3). Previous studies explicitly assessing the extent of self-recruitment and connectivity in MPAs have shown relatively high levels of self-recruitment and connectivity in island settings (Almany et al. 2007, Planes et al. 2009). However, self-recruitment of fishes in MPAs along open coastlines has been harder to resolve, and until now there has not been an explicit test of self-recruitment

and connectivity via larval dispersal in populations inhabiting MPAs located along an oceanographically dynamic open coastline.

Examination of empirically estimated mean dispersal velocities with concurrently collected ADCP data allowed a post-hoc comparison of empirically derived estimates of larval transport with Eulerian-based predictions for a passively dispersing particle (i.e. a proxy for a dispersing larval fish). These physical data suggest a passively dispersing particle would travel very different distances depending upon where it was located in the water column. If a larval fish spent the entirety of a ~20 day pelagic larval period (Range 18-22 days) passively dispersing in the upper 4m of the water column, ADCP data suggest that in the absence of any swimming behavior it could travel a gross distance > 150 km. However, if a larval fish were to spend those same 20 days below 4 m water depth it would be predicted to passively disperse only 35 km. The range of dispersal velocities generated by empirical data as part of this study suggest larval fish are remaining in mid to lower parts of the water column rather than in surface waters. Experimental studies of swimming behavior of other pomacentrids suggest that late-stage larval fish can, in the absence of feeding, swim constantly against a 13.5 cms^{-1} current for between 25 and 250 hours; over this period of time they would swim an equivalent distance ranging between 12 and 123 km (Stobutzki and Bellwood 1997). Swimming ability also varies with ontogeny, at hatch *Pomacentrus amboinensis* (a tropical damselfish) can sustain swimming at 3.5 cms^{-1} for a period of minutes, but by the end of a 20-day pelagic larval period, can swim at a sustained speed of > 30 cms^{-1} for > 90 hours (Fisher et al. 2000). Recent

efforts to describe larval swimming speed and behavior *in situ* suggest larval fish are cognizant of swimming directionality, and are able to able to maintain swimming speeds of > 13 cms^{-1} (a velocity that matched the mean current speed; Leis et al. 2007). The influence of swimming behavior on dispersal distances and empirically-derived connectivity patterns suggest that the assumption of larval fish passively dispersing in the surface waters, and subsequent predictions of passive dispersal distance are not valid. Microchemically-derived estimates of larval fish dispersal distances are an order of magnitude below the mean current velocities measured in surface waters (>20 cms^{-1}) but fall within the range of velocities measured at water depths below 4 m as part of this study (~2 cms^{-1}). When otolith-derived estimates of connectivity are compared with ADCP data, there appears to be a general concordance in directionality. For example embryonic fish predicted to be developing on benthic nests between July 16-31, 2009 (Figure 4.3-2E), all dispersed in a northerly direction. This two week nesting bin is followed by unilateral southerly dispersal from August 1-15 (Figure 4.3-2F). When ADCP data are viewed over this same period of time (Figure 4.5B) a similar pattern is observed; currents are flowing predominantly in a northerly direction for a period of ~3 weeks, followed by a reversal in current direction occurring at the approximate time the otolith-derived estimates suggest a change in dispersal direction. This suggests that if swimming behavior is known, physical proxies, can with caution, be used to roughly predict larval dispersal directionality, and when viewed at depth (i.e. below wind-forced surface waters) can resolve approximate larval dispersal magnitude.

Qualitative evidence suggests that these larval fish, as with other pomacentrids, are active swimmers upon hatch (Cook pers. obs.). Therefore they may be able to maintain their position in the water column, by either placing themselves in portions of the water column where there are lower current velocities (e.g. near the bottom), moving vertically to take advantage of currents flowing in opposite directions, constantly swimming against currents, or some combination thereof. The breadth of dispersal trajectories and subsequent connectivity patterns exhibited by larval fish in this study suggests that an accurate mechanistic understanding of population connectivity via larval dispersal will require focused studies pairing the collection of appropriate physical oceanographic data concurrently with biological data (e.g. vertical swimming behavior); enabling a more surgical dissection of the processes ultimately responsible for generating larval dispersal trajectories.

In this study three MPAs within a larger system of reefs were connected by larval dispersal over a two-year period. However the direction of this connectivity as well as the proportions of individuals varied between years. By assessing larval dispersal and subsequent population connectivity over various temporal scales, we show the importance of high-resolution sampling to the accurate re-construction of population connectivity patterns. To fully-resolve connectivity patterns will however require protracted time-series (e.g. see Carson et al 2010) and the need to incorporate synoptic sampling into future studies of MPA design, particularly in light of self-recruitment and connectivity being central tenets for the success of MPAs and MPA networks. At the local scale, and counter to existing hypotheses (Cowen et al. 2006),

there appears to be sufficiently high self-recruitment within one of the MPAs located in the study region that, taken in conjunction with the mean life span (~13 years; Clarke 1970), this population may be able to persist without larval inputs from outside the local area. Despite the high current velocities measured within the study region, larval dispersal distances for garibaldi larvae were between 0 km and 59 km over a three-week period, suggesting the importance of larval swimming behavior to realized patterns of self-recruitment and reef connectivity. The variability observed in connectivity patterns has important ramifications for the design and management of MPAs; if connectivity patterns are viewed over short temporal scales in isolation, data would suggest this six-reef metapopulation functions as a source-sink metapopulation. However, when connectivity patterns are viewed on inter-annual time scales, the metapopulation is more aptly viewed as an open population with a well-mixed larval pool. The ability to distinguish between these two metapopulation models enables those involved in the MPA design process to make better-informed decisions. In conclusion, this study provides new insights into the relative importance of various processes influencing connectivity patterns, lends support to the growing body of evidence for limited larval dispersal, and can be used by stake holders to better the design of MPA networks, moving marine fisheries closer to sustainability while simultaneously protecting marine biodiversity for future generations.

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Table 4.1 Number of nests collected, otoliths analyzed, mean (± 1 sd) and number of otoliths analyzed per nest, range of spawning season, and number of young-of-the-year (YOY) collected at each reef.

	Study Reef						
	Carlsbad	Cardiff	Torrey Pines	La Jolla	Mission Point	Zuniga Point	Total
Nests Collected 2008	10	12	32	18	11	12	95
Nests Collected 2009	23	21	26	17	21	26	136
Larval Fish Otoliths Analyzed 2008	80	114	456	212	123	116	1101
Larval Fish Otoliths Analyzed 2009	118	107	132	101	100	125	683
Otoliths per Nest Analyzed 2008	9.5 ± 2.5	10.8 ± 5.0	19.8 ± 5.9	10.9 ± 4.9	14.8 ± 7.0	9.5 ± 3.0	
Otoliths per Nest Analyzed 2009	6.9 ± 4.9	7.1 ± 5.2	10.2 ± 6.0	7.2 ± 6.0	7.7 ± 6.4	6.6 ± 6.9	
First 2008 Collection Date	July 24	July 10	June 03	June 03	July 15	July 22	
Last 2008 Collection Date	Aug 21	Aug 21	Sept 02	July 29	Aug 12	Aug 19	
First 2009 Collection Date	June 11	June 11	May 29	May 29	June 12	June 12	
Last 2009 Collection Date	Aug 26	Aug 11	Aug 26	July 29	Aug 12	Aug 28	
YOY Collected 2008	18	10	0	42	1	0	72
YOY Collected 2009	9	1	0	6	0	1	17

Figure Legends

Figure 4.1 Map of study area with relative abundance of model species. Locations of study sites are given, and sites currently designated as MPAs encircled in red. Zuniga Point is encircled in a dashed red line as is not officially within an MPA but rather is adjacent to the Mia J Tegner MPA. Figure adapted from Chapter 2.

Figure 4.2 Scanning electron micrograph of larval-stage *H. rubicundus* otolith. (magnification 4699X, scale bar = 20 microns).

Figure 4.3 Bi-weekly connectivity patterns in 2008 and 2009. Grey region is land and white region is ocean. Purple arrows indicate northward dispersal, green arrows indicate southward dispersal, and black arrows indicate self-recruitment. Thickness of arrows is proportional to the number of individuals dispersing among reefs. Crosses indicate location of primary study sites.

Figure 4.4 Mean larval dispersal distances (km) of all (n=89) YOY fish collected in 2008 and 2009. Green histograms and negative values indicate southward dispersal. Purple histograms and positive numbers indicate northward dispersal, and the grey histogram depicts the number of YOY that self-recruited (i.e. that were spawned at and settled to the same reef).

Figure 4.5 Figure 4.5A. ADCP data from 2008. Purple colors indicate a northerly direction, green colors indicate a southerly direction, while red indicates onshore flow. Intensity of color represents magnitude of flow. Black regions represent periods of time where ADCPS were not collecting data. Figure 4.5B. ADCP data from 2009.

Purple colors indicate a northerly direction, green colors indicate a southerly direction, while red indicates onshore flow. Intensity of color represents magnitude of flow. Black regions represent periods of time where ADCPS were not collecting data.

Figure 4.6 Annual aggregate connectivity patterns derived from bi-weekly connectivities in 2008 (Figure 4.6A) and 2009 (Figure 4.6B). Purple arrows indicate northward dispersal, green arrows indicate southward dispersal, and black arrows indicate self-recruitment. Thickness of arrows is proportional to the number of individuals dispersing among reefs. Percentages given are the proportion of YOY within the entire study system (e.g. in 2008, 36 of 72 (50%) of YOY collected across all study sites appear to have self-recruited). Crosses indicate location of primary study sites; the hollow star adjacent to Zuniga Point indicates the location of the Mia J Tegner MPA.

Figure 4.7 Interannual connectivity and self-recruitment across the 2008 and 2009 spawning seasons. Values indicate the proportion of all recruits summed across both years traveling among reefs. Arrows are proportional to the number of individuals dispersing between two reefs, and indicate the direction of larval dispersal. Purple arrows indicate northward dispersal, green arrows indicate southward dispersal, and black arrows indicate self-recruitment. Crosses indicate location of primary study sites.

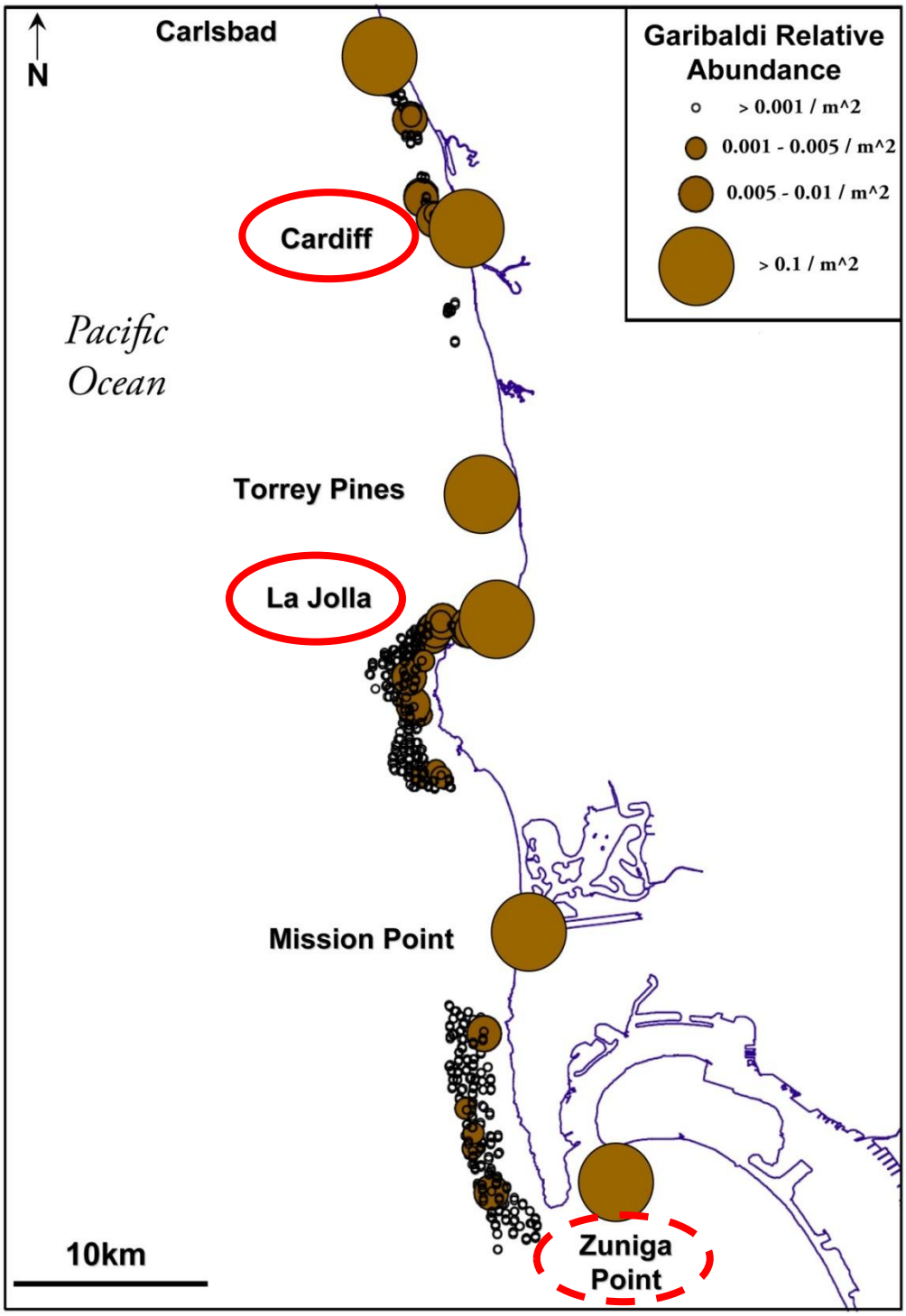

Figure 4.1 Map of study area with relative abundance of model species. Locations of study sites are given, and sites currently designated as MPAs encircled in red. Zuniga Point is encircled in a dashed red line as is not officially within an MPA but rather is adjacent to the Mia J Tegner MPA. Figure adapted from Chapter 2.

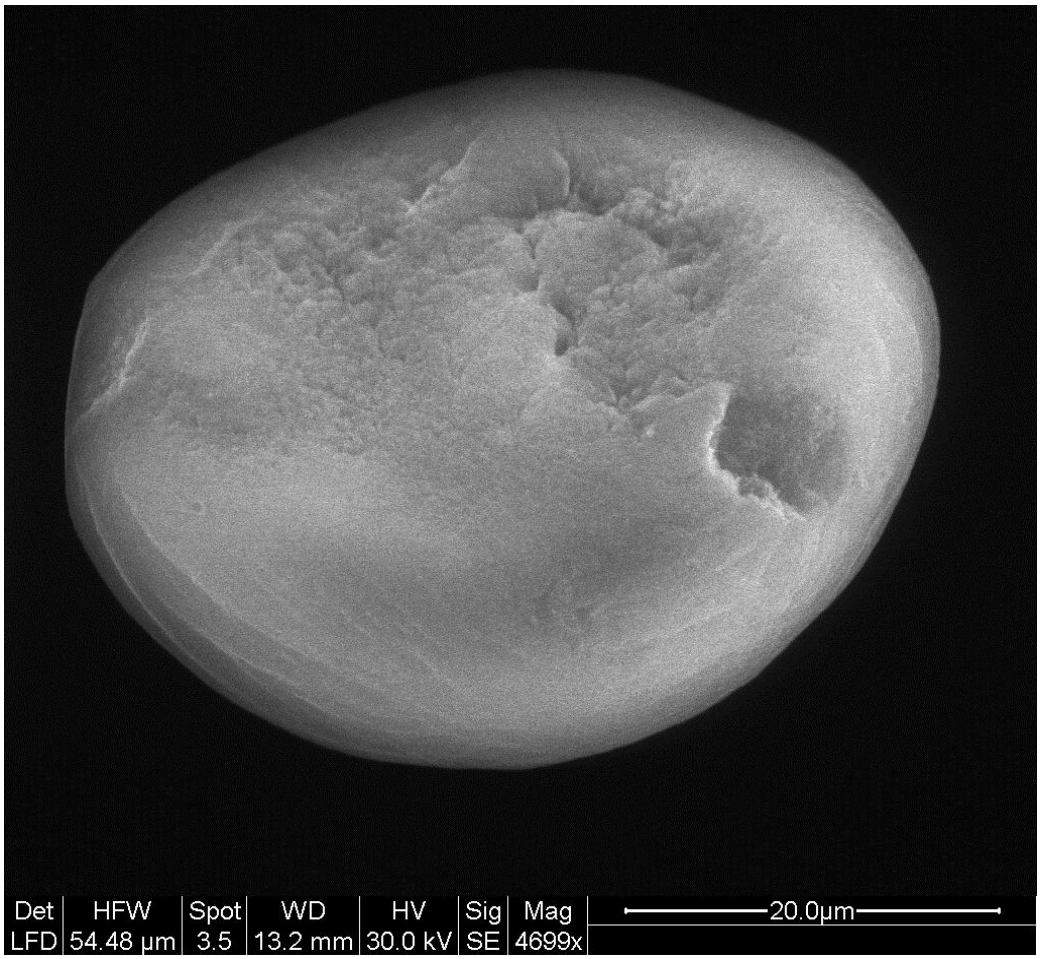

Figure 4.2 Scanning electron micrograph of larval-stage *H. rubicundus* otolith. (magnification 4699X, scale bar = 20 microns).

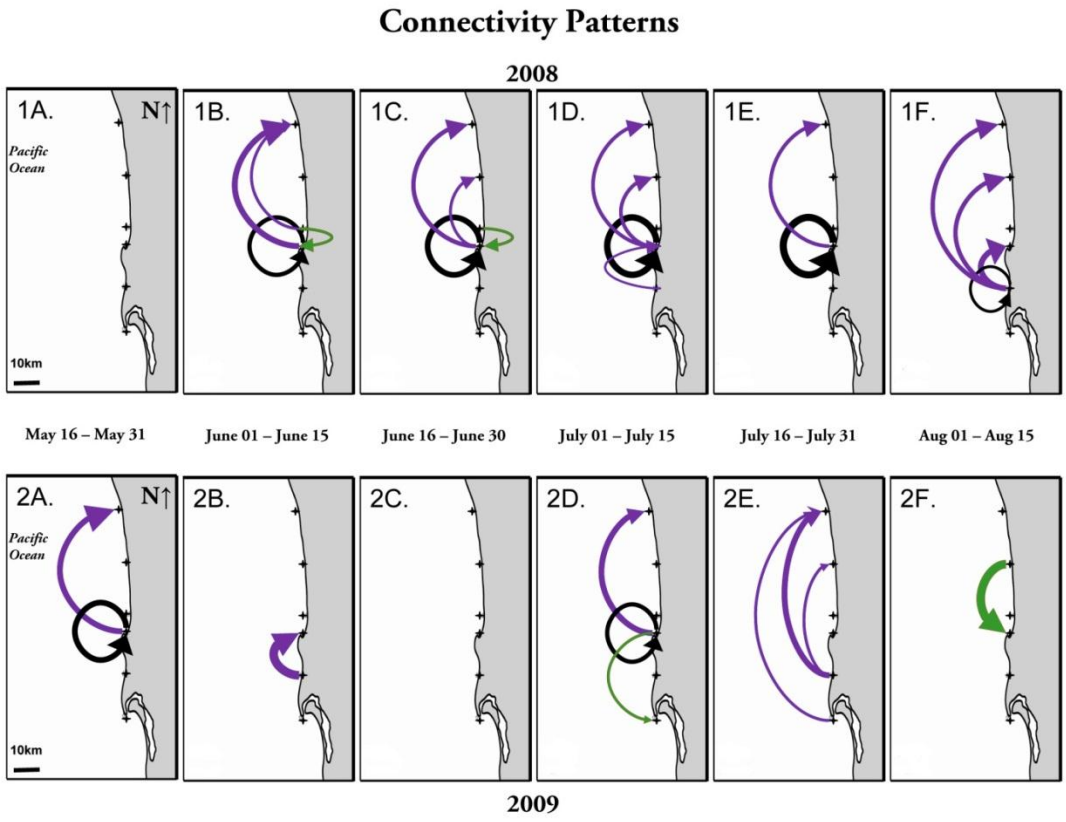

Figure 4.3 Bi-weekly connectivity patterns in 2008 and 2009. Grey region is land and white region is ocean. Purple arrows indicate northward dispersal, green arrows indicate southward dispersal, and black arrows indicate self-recruitment. Thickness of arrows is proportional to the number of individuals dispersing among reefs. Crosses indicate location of primary study sites.

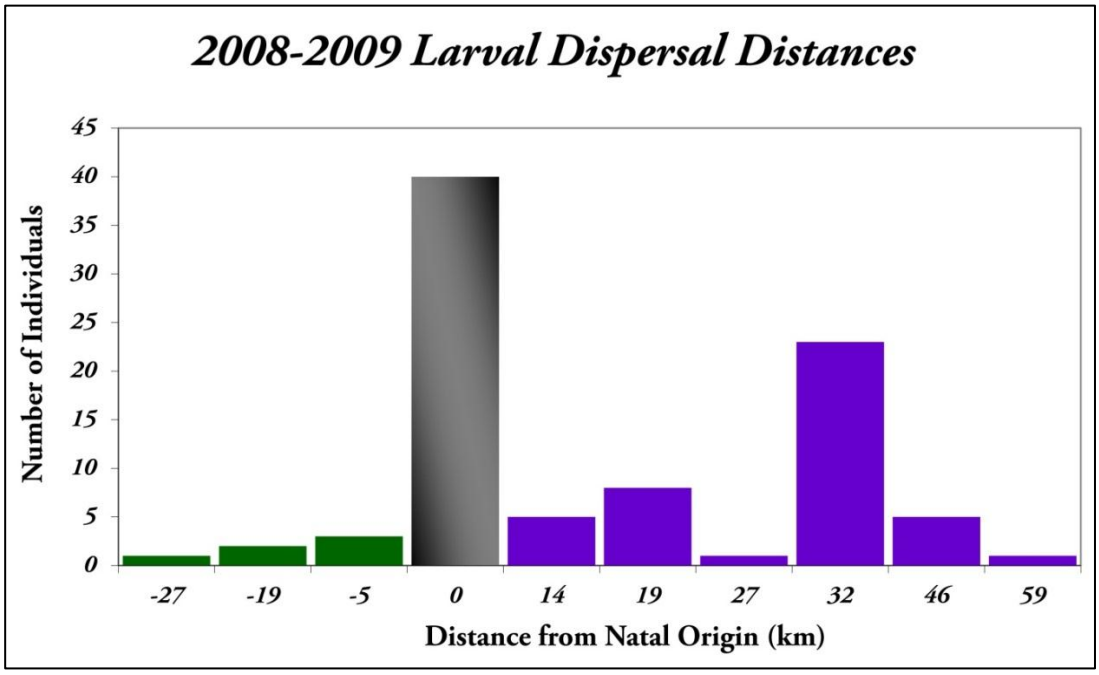

Figure 4.4 Mean larval dispersal distances (km) of all (n=89) YOY fish collected in 2008 and 2009. Green histograms and negative values indicate southward dispersal. Purple histograms and positive numbers indicate northward dispersal, and the grey histogram depicts the number of YOY that self-recruited (i.e. that were spawned at and settled to the same reef).

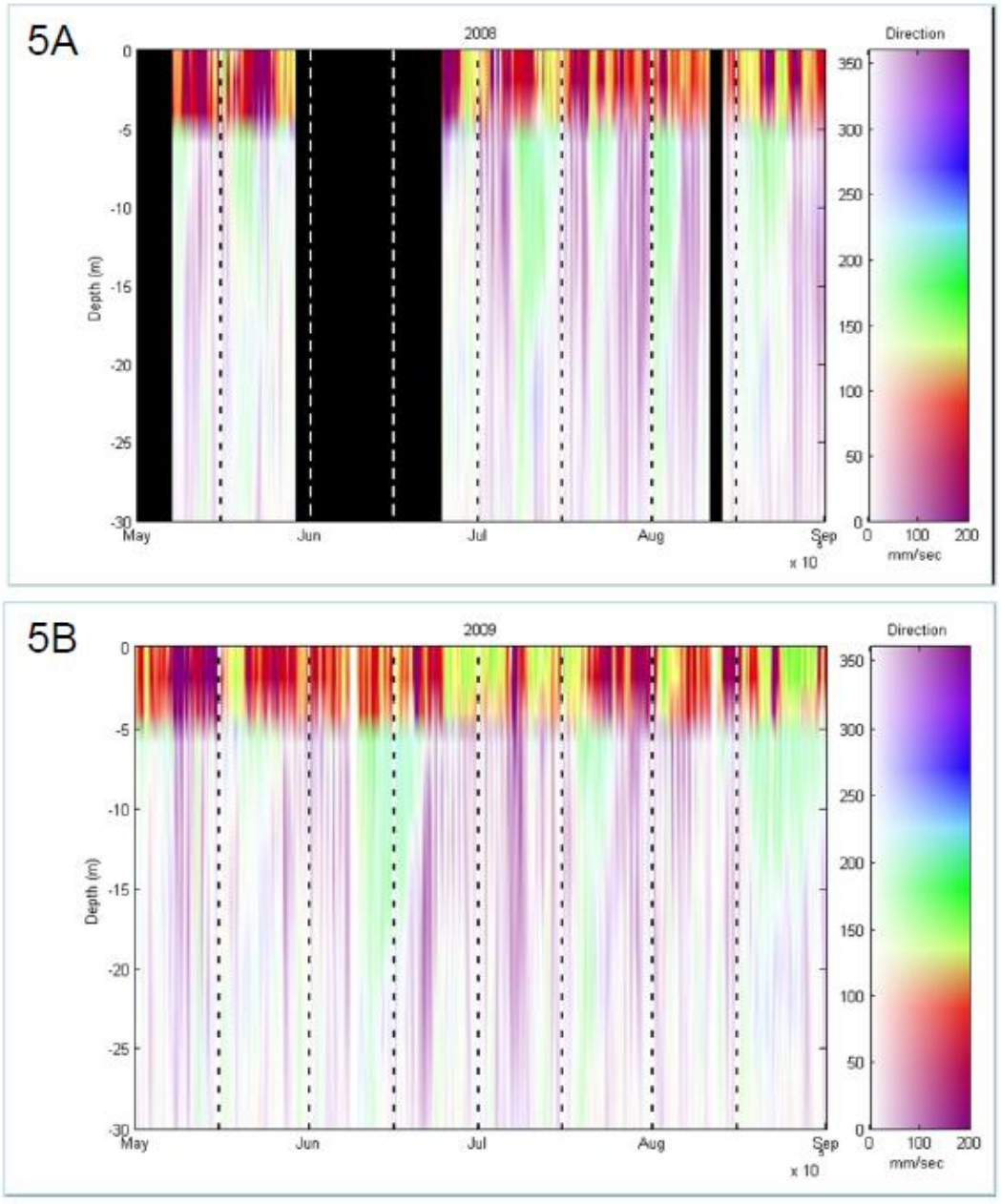

Figure 4.5 Figure 4.5A. ADCP data from 2008. Purple colors indicate a northerly direction, green colors indicate a southerly direction, while red indicates onshore flow. Intensity of color represents magnitude of flow. Black regions represent periods of time where ADCPs were not collecting data. Figure 4.5B. ADCP data from 2009. Purple colors indicate a northerly direction, green colors indicate a southerly direction, while red indicates onshore flow. Intensity of color represents magnitude of flow. Black regions represent periods of time where ADCPs were not collecting data.

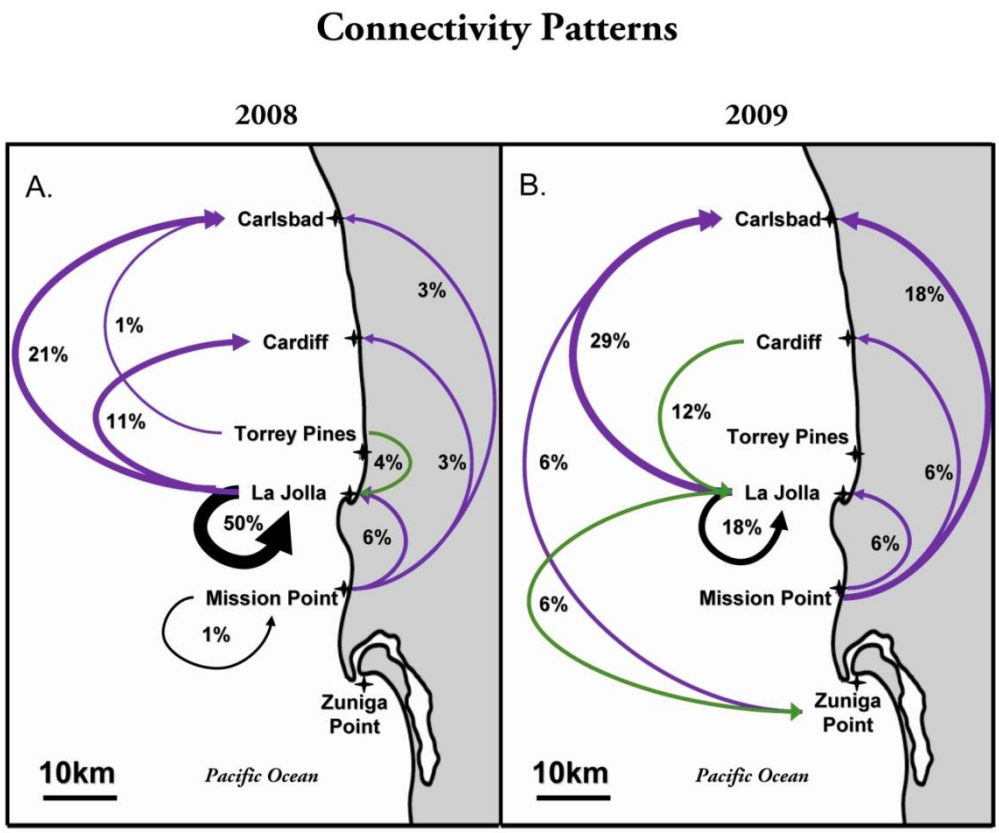

Figure 4.6 Annual aggregate connectivity patterns derived from bi-weekly connectivities in 2008 (Figure 4.6A) and 2009 (Figure 4.6B). Purple arrows indicate northward dispersal, green arrows indicate southward dispersal, and black arrows indicate self-recruitment. Thickness of arrows is proportional to the number of individuals dispersing among reefs. Percentages given are the proportion of YOY within the entire study system (e.g. in 2008, 36 of 72 (50%) of YOY collected across all study sites appear to have self-recruited). Crosses indicate location of primary study sites; the hollow star adjacent to Zuniga Point indicates the location of the Mia J Tegner MPA.

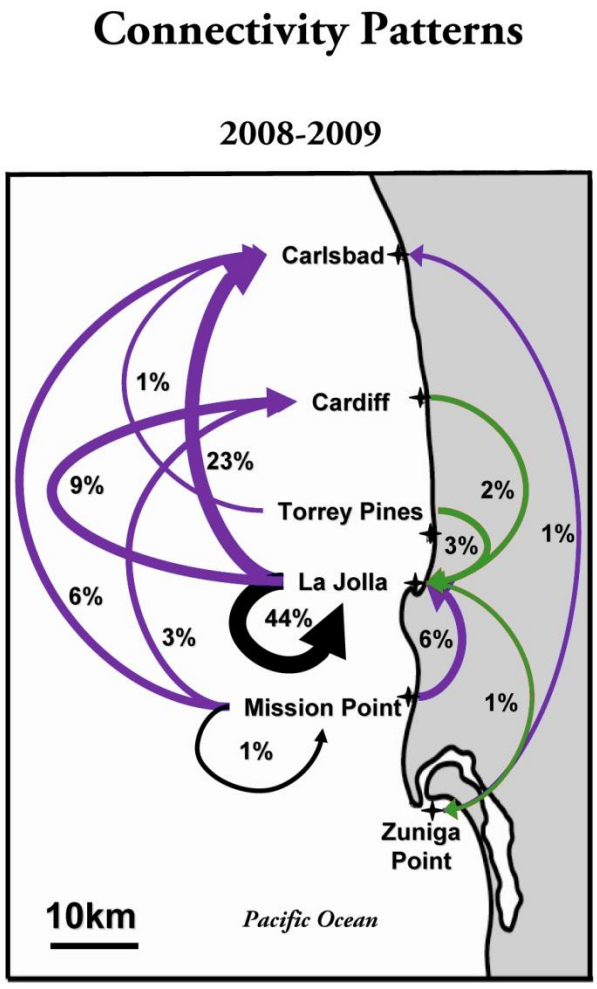

Figure 4.7 Interannual connectivity and self-recruitment across the 2008 and 2009 spawning seasons. Values indicate the proportion of all recruits summed across both years traveling among reefs. Arrows are proportional to the number of individuals dispersing between two reefs, and indicate the direction of larval dispersal. Purple arrows indicate northward dispersal, green arrows indicate southward dispersal, and black arrows indicate self-recruitment. Crosses indicate location of primary study sites.

Appendix 4.1 Mean (± se) elemental "concentrations" of otoliths collected from nests in 2008 and 2009. Values of Mg, Mn, and Sr are given in mmol/mol Ca, and Ba, Pb, and U are in µmol/mol Ca.

		Elemental Concentration											
NEST	Samples	Mg/Ca (mmol/mol)	se	Mn/Ca (mmol/mol)	se	Sr/Ca (mmol/mol)	se	Ba/Ca (µmol/mol)	se	Pb/Ca (µmol/mol)	se	U/Ca (µmol/mol)	se
CD20082	9	2.10	0.13	0.04	0.01	4.16	0.11	157.53	21.33	5.88	2.10	0.50	0.06
CD20083	10	1.90	0.17	0.05	0.02	3.78	0.12	119.03	25.54	2.18	0.34	0.23	0.04
CD20084	9	2.03	0.24	0.03	0.01	4.54	0.17	129.88	20.70	1.32	0.29	0.05	0.02
CD20085	10	1.81	0.24	0.02	0.02	3.66	0.11	66.96	7.37	2.68	1.06	0.66	0.14
CD20086	10	1.56	0.23	0.05	0.03	4.04	0.16	80.17	7.02	5.95	1.85	0.47	0.08
CD20087	4	1.45	0.29	0.12	0.06	3.80	0.16	91.68	43.61	0.91	0.43	0.18	0.06
CD20089	12	1.62	0.21	0.02	0.01	3.85	0.17	99.57	12.95	3.46	1.28	0.29	0.05
CD200810	12	1.40	0.09	0.03	0.01	3.78	0.11	90.87	12.41	2.15	0.34	0.59	0.09
CF20081	13	2.04	0.21	0.28	0.06	3.94	0.11	119.53	18.81	2.03	0.33	0.26	0.09
CF20082	1	1.49	.	0.06	.	3.60	.	93.52	.	1.23	.	0.36	.
CF20083	14	1.17	0.09	0.02	0.01	3.45	0.09	55.20	6.09	1.07	0.11	0.82	0.09

Appendix 4.1 Continued

CF20084	18	1.40	0.17	0.04	0.01	3.57	0.10	54.22	8.05	1.46	0.52	0.31	0.04
CF20085	12	1.18	0.11	0.03	0.01	3.61	0.15	65.00	9.47	1.33	0.25	0.40	0.07
CF20086	10	1.89	0.24	0.01	0.01	4.47	0.09	73.40	11.09	2.05	0.68	0.26	0.04
CF20087	6	2.88	0.77	0.05	0.01	4.07	0.06	82.03	20.73	5.05	2.47	0.33	0.07
CF20088	9	1.59	0.21	0.02	0.02	4.57	0.15	69.93	14.80	5.46	1.45	0.19	0.04
CF200811	14	1.82	0.29	0.04	0.01	3.74	0.11	65.95	8.15	3.53	0.76	0.52	0.07
TP20088	21	1.79	0.14	0.06	0.02	3.86	0.09	139.32	16.03	1.92	0.20	0.10	0.02
TP20089	18	1.84	0.23	0.10	0.02	3.72	0.08	113.56	16.91	3.05	0.38	0.07	0.01
TP200810	17	1.90	0.13	0.14	0.03	4.05	0.10	137.36	14.91	1.11	0.10	0.06	0.01
TP200811	19	1.80	0.19	0.08	0.02	3.51	0.08	136.94	17.48	1.49	0.29	0.08	0.02
TP200812	19	1.30	0.06	0.09	0.02	3.47	0.05	55.57	4.05	1.00	0.55	0.05	0.02
TP200815	21	1.41	0.09	0.09	0.02	4.23	0.13	87.42	10.96	0.80	0.11	0.10	0.03
TP200816	19	1.57	0.14	0.10	0.02	4.51	0.09	64.00	6.63	0.43	0.07	0.07	0.02
TP200817	19	1.73	0.20	0.03	0.01	3.85	0.11	79.46	12.08	1.19	0.19	0.33	0.04
TP200818	19	1.77	0.31	0.03	0.01	3.65	0.10	72.84	5.34	2.30	1.08	0.25	0.04
TP200819	18	1.83	0.25	0.01	0.01	3.69	0.08	93.04	8.81	1.97	1.11	0.75	0.07

Appendix 4.1 Continued

TP200821	10	1.44	0.18	0.04	0.03	3.45	0.07	88.42	12.21	0.95	0.28	0.23	0.06
TP200822	20	1.71	0.14	0.10	0.02	3.63	0.10	69.73	8.46	0.80	0.12	0.69	0.08
TP200823	20	1.59	0.19	0.04	0.01	3.47	0.07	63.81	7.29	1.74	0.42	0.50	0.04
TP200824	19	1.78	0.16	0.04	0.02	3.53	0.08	74.08	7.43	8.89	1.17	0.74	0.05
TP200825	16	2.18	0.27	0.07	0.02	4.53	0.13	66.02	9.94	3.06	0.69	0.23	0.04
TP200826	9	2.43	0.40	0.04	0.01	4.12	0.17	50.43	4.94	3.15	1.08	0.32	0.05
TP200827	18	2.06	0.29	0.06	0.02	3.90	0.08	118.75	14.38	2.35	0.34	0.67	0.07
TP200828	19	2.08	0.23	0.04	0.01	4.10	0.08	76.23	12.34	1.75	0.42	0.50	0.05
TP200829	30	2.58	0.28	0.03	0.00	3.66	0.08	59.47	5.84	2.89	0.46	0.50	0.06
TP200830	25	1.46	0.12	0.03	0.01	3.61	0.08	63.05	6.10	1.59	0.19	0.82	0.07
TP200831	38	2.30	0.26	0.02	0.00	3.64	0.07	53.81	5.28	1.86	0.54	0.63	0.06
TP200832	22	1.59	0.20	0.04	0.01	3.45	0.09	78.20	10.93	1.78	0.35	0.50	0.05
LJ20081	3	2.75	0.54	0.06	0.03	3.63	0.19	236.69	65.12	2.03	0.38	0.02	0.01
LJ20082	6	1.40	0.12	0.01	0.00	3.31	0.10	250.86	45.80	2.04	0.20	0.13	0.03
LJ20083	9	1.97	0.24	0.14	0.03	2.97	0.13	189.94	35.08	2.51	0.60	0.12	0.02

Appendix 4.1 Continued

LJ20084	13	1.81	0.14	0.06	0.02	3.61	0.06	115.02	12.12	2.70	0.56	0.14	0.06
LJ20085	15	2.26	0.33	0.07	0.02	3.20	0.05	121.17	23.47	3.21	0.95	0.06	0.01
LJ20086	5	2.11	0.45	0.04	0.02	3.18	0.13	125.18	29.67	2.12	0.59	0.07	0.02
LJ20087	5	1.98	0.35	0.10	0.03	3.41	0.09	58.84	11.87	1.97	1.16	0.01	0.00
LJ20088	15	1.59	0.14	0.13	0.02	3.26	0.05	54.31	8.22	1.12	0.21	0.04	0.01
LJ20089	10	1.54	0.12	0.04	0.01	3.22	0.14	85.05	12.03	0.65	0.08	0.11	0.06
LJ200810	10	1.75	0.34	0.07	0.02	3.16	0.12	78.70	18.22	2.00	0.78	0.07	0.03
LJ200811	10	1.34	0.16	0.04	0.01	3.31	0.10	106.18	15.22	0.93	0.16	0.06	0.01
LJ200812	10	1.17	0.12	0.09	0.01	3.40	0.11	52.50	9.23	0.40	0.19	0.11	0.04
LJ200813	21	1.35	0.14	0.09	0.02	3.30	0.05	77.53	10.73	2.22	0.97	0.03	0.01
LJ200814	20	1.72	0.18	0.06	0.02	3.30	0.10	75.26	18.15	1.00	0.28	0.18	0.03
LJ200815	10	1.62	0.31	0.07	0.02	3.10	0.06	50.28	9.38	1.16	0.36	0.30	0.07
LJ200816	10	1.48	0.16	0.08	0.02	2.99	0.08	52.13	12.54	0.95	0.20	0.33	0.07
LJ200817	15	1.21	0.07	0.06	0.01	3.09	0.05	37.34	5.91	0.96	0.35	0.27	0.06
LJ200818	10	2.28	0.55	0.05	0.02	3.04	0.06	69.83	18.48	3.52	1.37	0.66	0.13

Appendix 4.1 Continued

MP20081	15	1.41	0.13	0.02	0.00	3.57	0.11	48.48	10.79	1.36	0.32	0.04	0.01
MP20083	3	3.26	1.28	0.05	0.02	4.17	0.50	173.71	35.06	3.48	0.59	0.25	0.16
MP20084	19	1.34	0.09	0.04	0.01	3.83	0.12	112.23	11.50	1.34	0.17	0.09	0.01
MP20085	9	1.47	0.27	0.03	0.02	3.51	0.21	62.80	10.91	4.38	1.70	0.35	0.14
MP20086	9	1.87	0.38	0.04	0.01	3.39	0.24	83.47	19.63	1.36	0.29	0.23	0.07
MP20088	23	1.09	0.09	0.04	0.01	3.53	0.07	58.30	5.38	2.43	0.42	0.32	0.05
MP20089	20	1.32	0.08	0.06	0.01	3.28	0.04	30.60	2.55	3.64	1.24	0.26	0.05
MP200811	20	1.63	0.14	0.07	0.01	3.35	0.08	64.89	7.12	1.43	0.21	0.35	0.05
ZP20081	7	2.89	0.67	0.05	0.03	3.96	0.20	151.90	13.69	1.26	0.06	0.10	0.02
ZP20083	14	1.64	0.25	0.05	0.03	3.65	0.07	108.27	18.57	1.47	0.26	0.14	0.07
ZP20084	15	1.46	0.13	0.05	0.01	4.01	0.08	90.05	12.79	0.84	0.14	0.07	0.02
ZP20085	10	1.46	0.17	0.02	0.01	3.36	0.16	59.47	11.26	3.61	1.23	0.46	0.08
ZP20086	6	2.60	0.42	0.00	0.02	3.34	0.07	133.36	37.09	3.14	0.81	0.67	0.10
ZP20087	9	1.96	0.31	0.06	0.02	3.28	0.10	71.23	7.76	2.31	0.97	0.60	0.08
ZP20088	9	1.39	0.26	0.04	0.02	3.25	0.17	54.26	10.36	1.54	0.75	0.37	0.07

Appendix 4.1 Continued

ZP20089	10	1.74	0.33	0.05	0.02	3.69	0.19	62.67	11.04	2.52	0.80	0.63	0.05
ZP200810	10	2.11	0.26	0.06	0.03	3.78	0.13	116.39	22.53	3.41	1.55	0.67	0.08
ZP200811	10	3.37	0.64	0.04	0.01	3.66	0.11	89.32	23.85	4.55	2.60	0.49	0.05
ZP200812	5	3.48	0.69	0.08	0.04	3.60	0.19	127.27	17.78	4.24	0.89	0.79	0.19
CD20091	14	2.56	0.75	0.13	0.06	4.09	0.29	65.14	21.12	0.93	0.28	0.42	0.09
CD20092	4	1.67	0.55	0.07	0.00	4.15	0.22	56.97	23.17	6.02	3.51	0.90	0.32
CD20093	4	1.20	0.2	-0.01	0.01	3.27	0.13	18.29	2.8	0.47	0.23	0.11	0.01
CD20094	2	1.32	0.23	-0.01	0.01	3.21	0.13	97.50	35.53	2.50	0.85	1.06	0.07
CD20095	11	1.13	0.31	0.04	0.02	3.67	0.14	53.04	5.5	2.34	1.05	0.54	0.14
CD20096	1	1.62	.	0.15	.	3.13	.	53.58	.	3.83	.	1.83	.
CD20097	10	0.86	0.07	0.04	0.01	3.33	0.1	39.15	5.07	0.93	0.14	0.16	0.03
CD20098	3	0.78	0.24	0.15	0.07	3.56	0.28	109.55	80.66	1.37	0.62	0.45	0.11
CD20099	5	4.53	1.89	0.19	0.11	3.40	0.12	188.33	63.46	2.87	1	0.31	0.06
CD200910	2	2.19	0.34	0.06	0.04	3.22	0.36	87.63	24.85	3.57	0.35	0.93	0.14

Appendix 4.1 Continued

CD200911	2	1.23	0.3	0.04	0.02	3.61	0.06	56.93	1.26	2.13	0.54	0.83	0.2
CD200912	11	1.55	0.19	0.19	0.06	3.62	0.1	121.39	22.2	7.74	2.56	0.37	0.06
CD200914	1	2.25	.	0.04	.	3.37	.	269.54	.	2.13	.	0.39	.
CD200915	14	2.48	1.64	0.08	0.05	3.03	0.06	78.39	37.94	2.85	1.07	0.21	0.08
CD200917	4	3.30	1.25	0.05	0.02	3.06	0.02	65.81	32.4	2.00	1.09	0.42	0.25
CD200920	11	4.86	1.51	0.14	0.08	2.89	0.29	108.96	18.79	5.44	1.79	0.82	0.17
CD200922	5	1.13	0.14	0.02	0.00	3.23	0.14	34.77	10.55	0.39	0.17	0.09	0.05
CD200923	14	1.88	0.38	0.06	0.02	3.28	0.13	89.67	30.36	1.76	0.5	0.19	0.05
CF20091	5	0.64	0.14	0.04	0.02	4.04	0.1	51.80	15.26	1.22	0.22	1.37	0.2
CF20092	11	0.78	0.09	0.05	0.02	4.49	0.22	57.02	6.9	2.56	0.66	0.58	0.11
CF20093	10	1.57	0.33	0.06	0.02	3.70	0.13	54.39	10.58	1.50	0.44	0.71	0.16
CF20094	3	5.60	4.46	0.69	0.49	4.19	0.48	661.22	633.69	6.28	4.93	1.29	0.15
CF20095	5	10.26	3.28	0.12	0.03	3.86	0.3	266.51	101.74	3.44	1.21	1.23	0.19
CF20096	1	0.80	.	0.15	.	4.18	.	20.34	.	0.91	.	0.61	.

Appendix 4.1 Continued

CF20097	12	1.43	0.35	0.09	0.02	4.06	0.16	50.11	20.1	8.49	5.08	0.34	0.08
CF20098	7	1.68	0.5	0.09	0.03	4.35	0.37	77.54	27.33	2.11	0.8	0.35	0.06
CF200911	16	1.83	0.35	0.23	0.06	4.49	0.09	58.67	12.01	7.41	3.47	0.50	0.15
CF200915	5	3.13	0.71	0.31	0.23	3.07	0.1	46.50	13.6	3.12	0.54	0.71	0.15
CF200916	9	0.98	0.21	0.03	0.11	3.63	0.15	20.58	1.44	0.77	0.15	0.24	0.04
CF200917	4	3.93	1.26	0.35	0.12	3.50	0.5	132.25	68.87	45.31	40.05	0.79	0.27
CF200918	1	0.98	.	0.05	.	4.22	.	80.83	.	1.51	.	1.12	.
CF200920	17	1.04	0.07	0.12	0.03	3.47	0.07	29.84	6.66	1.64	0.63	0.22	0.04
CF200921	1	3.07	.	1.23	.	3.22	.	102.84	.	11.00	.	3.16	.
TP20091	7	5.90	1.65	0.34	0.19	3.76	0.13	199.69	43.86	2.29	0.55	0.47	0.11
TP20092	5	1.88	0.37	0.17	0.11	3.39	0.06	64.82	6.9	1.43	0.33	0.66	0.15
TP20093	8	2.92	0.65	0.06	0.02	3.88	0.24	157.25	75.49	2.45	0.81	0.35	0.1
TP20094	8	4.98	1.04	0.11	0.05	4.04	0.21	197.05	104.47	9.40	2.95	0.72	0.08
TP20095	4	1.88	0.36	-0.01	0.02	3.16	0.04	39.69	17.58	2.32	0.72	0.58	0.11

Appendix 4.1 Continued

TP20096	2	1.66	0.33	0.09	0.05	3.33	0.21	25.31	5.26	5.07	1	0.25	0.14
TP20097	12	0.96	0.1	0.04	0.02	3.22	0.11	30.46	3.73	1.87	0.93	0.26	0.12
TP200912	14	1.69	0.31	0.08	0.04	3.48	0.1	69.77	11.74	2.76	1.35	0.32	0.06
TP200914	18	1.77	0.23	0.04	0.01	4.00	0.09	79.14	7.07	3.15	0.8	0.13	0.02
TP200917	15	0.63	0.05	0.10	0.03	3.22	0.05	61.39	4.85	1.07	0.3	0.22	0.03
TP200918	3	1.58	0.53	0.16	0.05	3.11	0.2	68.14	17.13	14.75	6.18	0.49	0.13
TP200924	18	0.63	0.05	0.07	0.03	4.23	0.17	96.37	12.61	1.91	0.26	0.40	0.1
TP200925	18	1.58	0.25	0.08	0.02	3.40	0.08	65.06	13.59	3.60	1.57	0.12	0.03
LJ20091	4	4.20	1.74	0.07	0.02	3.36	0.46	102.81	29.2	7.57	2.92	1.61	0.55
LJ20092	8	1.23	0.28	0.05	0.01	3.28	0.06	34.80	5.81	0.92	0.21	0.61	0.09
LJ20094	14	5.77	1.63	0.16	0.08	3.61	0.18	121.79	44.88	4.67	1.27	1.00	0.23
LJ20095	3	1.46	0.75	0.06	0.02	2.89	0.15	51.53	17.31	6.72	2.44	1.64	0.33
LJ20096	11	1.50	0.23	0.02	0.01	3.45	0.14	55.74	25.91	2.07	1.05	0.28	0.12
LJ20097	1	1.33	.	0.04	.	3.21	.	7.66	.	0.23	.	0.11	.

Appendix 4.1 Continued

LJ20098	3	1.01	0.15	0.05	0.01	3.08	0.05	48.54	25.48	1.55	0.22	1.63	0.31
LJ20099	3	1.25	0.31	0.06	0.02	3.19	0.04	65.34	42.15	0.78	0.54	0.17	0.03
LJ200910	12	0.80	0.13	0.03	0.01	2.95	0.06	51.79	13.91	1.03	0.35	0.27	0.05
LJ200912	4	1.52	0.47	0.05	0.02	3.02	0.05	73.55	56.87	0.85	0.39	0.18	0.04
LJ200913	2	1.34	0.14	0.03	0.00	2.90	0.09	26.20	19.42	3.38	3.05	1.16	1.15
LJ200914	2	1.21	0.06	0.10	0.00	3.07	0.15	6.94	2.14	0.13	0.02	0.09	0.07
LJ200916	14	1.65	0.31	0.02	0.01	3.13	0.11	57.22	5.31	2.14	0.38	0.61	0.16
LJ200917	20	1.87	0.53	0.05	0.05	2.86	0.19	79.04	18.39	3.02	0.4	0.59	0.11
MP20092	15	2.49	0.83	0.12	0.04	3.86	0.15	70.05	21.47	3.30	0.79	0.79	0.18
MP20094	4	2.29	1.55	0.15	0.11	3.72	0.5	95.63	62.74	5.96	4.71	1.32	0.41
MP20095	4	0.62	0.11	0.06	0.03	3.39	0.09	24.88	1.88	0.67	0.14	1.10	0.25
MP20096	3	0.89	0.27	0.14	0.12	3.51	0.03	45.26	3.87	2.84	1.71	1.56	0.38
MP20097	8	2.00	1.21	0.14	0.07	3.42	0.13	58.24	27.28	1.84	0.45	0.83	0.04
MP20098	1	1.30	.	0.06	.	2.62	.	17.47	.	4.03	.	1.14	.

Appendix 4.1 Continued

MP200910	2	1.74	0.97	0.07	0.07	3.13	0.39	81.75	37.95	1.97	1.02	0.67	0.46
MP200911	15	1.11	0.22	0.04	0.01	3.67	0.08	37.67	10.81	3.42	2.55	0.21	0.05
MP200913	2	1.93	1.02	0.02	0.00	3.20	0.29	35.77	0.64	3.30	1.25	1.77	0.69
MP200914	1	3.27	.	0.04	.	3.66	.	41.59	.	7.44	.	0.21	.
MP200915	16	3.56	0.78	0.04	0.01	3.73	0.18	59.53	6.09	2.10	0.39	0.29	0.04
MP200919	17	1.17	0.24	0.07	0.03	2.80	0.07	28.00	3.57	1.83	0.33	0.60	0.13
MP200921	12	4.33	1.29	0.07	0.12	3.52	0.16	164.09	47.53	2.33	0.4	0.79	0.12
ZP20092	7	1.23	0.4	0.05	0.03	3.76	0.15	58.10	5.39	2.38	0.54	0.72	0.11
ZP20093	7	1.05	0.19	0.31	0.11	4.13	0.11	55.63	11.25	3.53	0.56	1.85	0.26
ZP20094	3	0.95	0.3	0.03	0.01	4.10	0.14	96.72	21.53	3.32	1.34	2.10	0.42
ZP20095	2	0.96	0.03	0.07	0.01	3.22	0.19	89.72	68.92	4.35	1.54	1.13	0.02
ZP20097	16	1.28	0.24	0.07	0.02	3.72	0.1	39.78	3.89	2.02	0.81	0.75	0.23
ZP20099	3	0.76	0.3	0.06	0.02	3.92	0.23	24.93	9.77	1.95	1.28	0.45	0.17
ZP200910	13	0.89	0.05	0.11	0.04	4.08	0.1	28.68	3.52	0.74	0.2	0.21	0.04

Appendix 4.1 Continued

ZP200911	1	1.02	.	0.42	.	4.39	.	22.90	.	1.06	.	0.61	.
ZP200912	2	1.77	1.17	0.34	0.28	4.52	0.36	100.35	75.06	6.89	4.75	0.59	0.19
ZP200913	15	1.76	0.33	0.06	0.03	3.90	0.09	95.89	28.5	4.17	0.82	0.71	0.18
ZP200915	3	1.86	0.61	0.03	0.01	3.91	0.28	41.52	3.11	1.93	0.62	1.42	0.06
ZP200917	23	1.66	0.29	0.07	0.2	3.46	0.13	75.49	26.74	10.47	6.77	0.24	0.03
ZP200918	1	0.93	.	0.01	.	3.09	.	26.16	.	2.70	.	2.20	.
ZP200919	1	0.37	.	0.07	.	3.51	.	16.96	.	0.57	.	0.74	.
ZP200922	3	1.43	0.61	0.22	0.2	3.74	0.25	57.36	14.46	4.41	0.39	1.37	0.78
ZP200924	3	15.86	14.56	0.10	0.07	1.97	0.99	39.75	18.67	1176.21	1174.35	0.69	0.33
ZP200926	1	0.49	.	0.01	.	3.42	.	35.64	.	1.13	.	0.18	.
ZP200927	3	1.78	0.83	0.20	0.09	3.56	0.15	54.39	15.38	3.10	2.18	0.51	0.24
ZP200928	18	1.68	0.37	0.08	0.02	3.80	0.13	91.42	45	4.61	1.55	0.19	0.04

Appendix 4.2 Estimates of precision (relative standard deviation, % RSD) for LA-ICPMS analyses and limits of detection for elements of interest. RSDs were calculated from more than 6500 means of 6 (2008 data) or 12 (2009 data) scans (i.e. number of scans per sample), and are presented for a dissolved CaCO3 reference material (OTO), and two solid reference materials: NIST 612 and USGS MACS3.

2008 % RSD and Detection Limits

Element	OTO	NIST 612	MACS 3	Detection Limit	Units
Mg	1.03%	9.50%	7.68%	0.04	mmol/mol Ca
Mn	1.63%	8.87%	6.02%	0.03	mmol/mol Ca
Sr	2.18%	8.23%	4.74%	0.04	mmol/mol Ca
Ba	3.22%	10.01%	8.93%	0.31	µmol/mol Ca
Pb	4.41%	10.07%	8.17%	0.25	µmol/mol Ca
U	5.27%	8.68%	13.43%	0.01	µmol/mol Ca

2009 % RSD and Detection Limits

Element	OTO	NIST 612	MACS 3	Detection Limit	Units
Mg	1.90%	3.84%	3.79%	0.06	mmol/mol Ca
Mn	1.95%	2.75%	6.83%	0.02	mmol/mol Ca
Sr	1.77%	3.18%	7.29%	0.13	mmol/mol Ca
Ba	1.77%	3.05%	14.43%	0.49	µmol/mol Ca
Pb	2.14%	3.27%	12.49%	0.36	µmol/mol Ca
U	3.67%	2.48%	17.89%	0.04	µmol/mol Ca

Appendix 4.3 Connectivity matrices for 2008, 2009, and 2008-2009. Values represent numbers of YOY fish. Rows are predicted natal reefs, columns are reefs where post-dispersal YOY were captured.

2008 Population Connectivity Matrix

	Carlsbad	Cardiff	Torrey Pines	La Jolla	Mission Point	Zuniga Point
Carlsbad	0	0	0	0	0	0
Cardiff	0	0	0	0	0	0
Torrey Pines	1	0	0	3	0	0
La Jolla	15	8	0	36	0	0
Mission Point	2	2	0	4	1	0
Zuniga Point	0	0	0	0	0	0

2009 Population Connectivity Matrix

	Carlsbad	Cardiff	Torrey Pines	La Jolla	Mission Point	Zuniga Point
Carlsbad	0	0	0	0	0	0
Cardiff	0	0	0	2	0	0
Torrey Pines	0	0	0	0	0	0
La Jolla	5	0	0	3	0	1
Mission Point	3	1	0	1	0	0
Zuniga Point	1	0	0	0	0	0

Interannual (2008-2009) Population Connectivity Matrix

	Carlsbad	Cardiff	Torrey Pines	La Jolla	Mission Point	Zuniga Point
Carlsbad	0	0	0	0	0	0
Cardiff	0	0	0	2	0	0
Torrey Pines	1	0	0	3	0	0
La Jolla	20	8	0	39	0	1
Mission Point	5	3	0	5	1	0
Zuniga Point	1	0	0	0	0	0

CHAPTER V.

DEMOGRAPHIC CONNECTIVITY DECONSTRUCTED: AN EXPLORATION OF THE DEMOGRAPHIC CONSEQUENCES OF LARVAL DISPERSAL

Abstract

Population connectivity is a key element in the maintenance of species that live in fragmented habitats. These species frequently function as metapopulations, where the movement of individuals among local populations links the dynamics of local habitat patches across larger spatial scales, thus connectivity figures prominently in their management. The logistical challenges associated with tracking dispersing individuals have slowed the generation of empirical data needed to test metapopulation theory and its application in the management of marine populations Here we use realized larval connectivity data for a temperate rocky reef damselfish, *Hypsypops rubicundus*, to explore the influence of connectivity on population and metapopulation growth rates. By linking stage-based matrix population models populated with local demographic data to connectivity matrices and the use of simulation modeling we were able to assess the importance of population connectivity to the functioning of marine metapopulations. Elasticity analyses suggest that at the reef and metapopulation level, adult survivorship has the greatest overall contribution to the persistence of marine populations at observed levels of connectivity. However, as the magnitude of connectivity increases within a metapopulation of six rocky reefs, there is a shift in the vital rates that have the greatest influence on local and metapopulation growth rates. The probability of successfully surviving and growing

from the first-to-second and second-to-third year of life replace adult survivorship as the primary contributors to growth rate. In metapopulations with growth rates (λ) below unity, equivalent incremental increases in patch connectivity have a larger proportional influence on metapopulation growth rate than in populations where λ is above unity. These findings suggest that while connectivity is not the greatest contributor to local or metapopulation growth rate, it does act to regulate the relative importance of other vital rates. For the successful management of populations in decline or facing extinction, efforts should be made to not only increase the survivorship of adults, but where possible to optimize levels of connectivity within the metapopulation to increase its probability of persistence in perpetuity.

Introduction

There has been a growing interest in the role of connectivity in the persistence of populations (Hastings and Botsford 2006 and references therein). In terrestrial systems this interest has been driven in large part by the recognition that human activities are fragmenting patches of natural habitat, an action that ultimately threatens terrestrial biodiversity (Chapin et al. 2000). To mitigate these threats, theoretical and empirical efforts have been made to understand the dynamics of populations living in fragmented populations, and to better comprehend how we can maximize the persistence of populations living in these altered systems. In contrast, many organisms living in marine systems inhabit patches of habitat that are naturally

fragmented. One of the theoretical frameworks frequently used to examine the dynamics of organisms inhabiting patches of habitat surrounded by an inhospitable matrix, whether they are anthropogenically or naturally formed, is metapopulation theory.

A metapopulation simply defined is a population comprised of smaller discrete local populations connected by dispersing individuals (Hanski 1998). This framework was initially developed by Levins (1969) as a way to examine how various spatial configurations of agricultural fields could be used to slow the spread of agricultural pests. It described equilibrium dynamics where the presence or absence of a pest within a given patch of habitat could be determined by the balance between the rate of immigration among patches and the rate of extinction within patches. If the rate of colonization was greater than the rate of extinction, the pest would persist within the metapopulation and, conversely, if the rate of extinction was greater than the rate of colonization, the pest would eventually go extinct. The broader conservation implications of this study resonated with researchers, and over the ensuing years it became widely adopted within the field of terrestrial ecology (Hanski and Simberloff 1997).

While the utility of this concept has been relatively well tested and developed in the field of terrestrial ecology, its development in the marine environment has been slower (Kritzer and Sale 2006). In part this is attributable to the logistical challenges associated with tracking the dispersal of individuals among spatially separated patches of habitat. Making this challenge greater still is the fact that many marine species

have a bipartite life cycle, where relatively sedentary adults inhabit patches of habitat that are connected by a widely dispersing mm-scale larval stage that can last from days to months (Cowen and Sponaugle 2009). For much of the past century the common belief was that marine larvae dispersed 100s to 1000s of km, as propagules drifted passively along with the ocean currents (Caley et al. 1996).

However, emerging methodologies developed at the end of the 20th century have started to shed some light into the black box of marine larval dispersal, and mounting empirical evidence suggests the magnitude of larval dispersal distance is not as great as once believed (Swearer et al. 1999, Jones et al. 1999, Thorrold et al. 2002, Jones et al. 2005, Almany et al. 2007, Becker et al. 2007, Planes et al. 2009, Carson et al. 2010, Chapter 4-this dissertation). These studies have brought to light the gaps in our fundamental understanding of how connectivity influences marine population dynamics, and the extent to which connectivity influences populations on local and metapopulation scales. Due to the paucity of empirical data describing the connectedness of populations, the examination of the interaction between local and metapopulation dynamics in the marine environment has remained principally in the realm of theory and simulations (e.g. Gerber et al. 2005, Bode et al. 2006, Figueira and Crowder 2006). The next step forward in this field will be to build upon the emerging empirical estimates of larval dispersal and population connectivity in an attempt to quantify the demographic consequences of connectivity for marine metapopulations (Pineda et al. 2007).

Here we couple spatially explicit demographic data with complementary empirical estimates of population connectivity generated as part of a broader study examining the connectedness of a temperate damselfish, *Hypsypops rubicundus* inhabiting a network of coastal rocky reefs in the northeast Pacific Ocean (Chapters 2 and 4 - this dissertation). The objective of this paper is to integrate these unique connectivity data with demographic data formulated as stage-based matrix models (sensu Caswell 2001) to explore the influence of connectivity on local and metapopulation dynamics. We combine stage-based matrix models, elasticity analyses, and simulation modeling to address three fundamental questions regarding the demographic consequences of connectivity: (1) What is the influence of realized inter-reef connectivity on local and metapopulation growth rates? (2) What is the relative importance of connectivity and other vital rates to population growth rates? and (3) Under what conditions does connectivity result in higher population growth rates? In answering these questions we improve our theoretical understanding of marine population dynamics and our ability to manage these natural systems in a manner that can maximize the persistence of these populations over time.

Methods

Study Region and Model Species

The study region encompasses nearshore (i.e. < 30 m water depth) rocky reefs found in the coastal zone of the extreme southwestern United States. Over 600

transect surveys were conducted using SCUBA between 2002 and 2008 to identify possible source locations within this region. From these survey data, a metapopulation of six rocky reefs spanning 60km was identified (Figure 5.1, taken from Cook 2011). Chapter 2 of this dissertation provides a detailed description of survey methodology, survey results, and study region. Between June 2008 and June 2010 these six reefs were visited biweekly throughout the spawning season of *Hypsypops rubicundus* (see below), and every four to six weeks between spawning seasons. Chapter 4 gives a detailed description of field methods. The model species for this study was *H. rubicundus*, a temperate damselfish (see Limbaugh 1964, Clarke 1970 for detailed life history information on this species). Briefly, this species inhabits nearshore rocky reef territories of ~5-10 m^2 that include a shelter, forage area, and for males, a benthic nest. Over a protracted spawning season (typically spanning June – August) males tend benthic red algal nests. During this three-month period females deposit tens of thousands of eggs on the male-guarded benthic nests; after fertilization embryos develop for approximately two weeks prior to hatching. After hatching the larval fish disperse in the water column for a period of approximately 20 days before settling to nearshore rocky reefs. Individuals remain sexually immature for a period of approximately 5 years before recruiting into the adult population. For a detailed description of the model species within the study region please see Clarke 1970 and Chapter 2 - this dissertation).

Empirically Estimating Metapopulation Connectivity

Empirical connectivity data for *H. rubicundus* are given in Chapter 4. This paper assigns the natal origin of post-dispersal settlement-stage fish during 2008 and 2009 over a 60 km stretch of coastline in San Diego County, USA (Appendix 5.1).

Demographic Matrix Model

A deterministic 4-stage population matrix model (sensu Caswell 2001) was constructed to assess the population growth rate of individual reefs. The four stages in this model were: young-of-the-year (YOY), juveniles, subadults, and adults. Due to the length of each of the model stages (≥1 yr), data describing the two-week egg stage and ~3-week larval stage were omitted from the demographic model (Davis and Levin 2002). The YOY stage spanned from settlement until transition into the next size-class (~ 1 year; see below). The juvenile stage spanned approximately 1 year, the subadult stage 3 years, and the adult stage 8 years. Therefore the time step of the model was set to one year.

The stage-based matrix model can be used to assess the growth of a given population by:

$$n_{(t+1)} = A_i n_{(t)} \quad (1)$$

where A_i is a 4x4 matrix describing the stage-specific probability of growth (G), survivorship (P), and fecundity (F) at reef *i* (Figure 5.2):

$$A_i = \begin{bmatrix} 0 & 0 & F_{SA} & F_A \\ G_{YJ} & 0 & 0 & 0 \\ 0 & G_{JSA} & P_{SA} & 0 \\ 0 & 0 & G_{SAA} & P_A \end{bmatrix}$$

and n*(t)* is a vector describing the abundance of individuals in each stage.

Estimating Vital Rates

From June 2008 – June 2010 monthly demographic transect surveys were conducted at each of the six study reefs. During these surveys a pair of SCUBA divers moved along a fixed 30-m long, 4-m wide transect line enumerating fish in 5-cm bins (e.g. 0-5 cm, 5-10 cm, 10-15, cm etc.). The mean of these count data were used to calculate size-specific annual estimates of density, growth, and mortality at each of the study reefs (e.g. the mean of adults surveyed in 2008 was compared against the mean of all adults surveyed in 2009 to estimate annual mortality; see below). These estimates of the vital rates were in turn used to populate a single, stage-based matrix model for each reef, representative of the vital rates from 2008-2009. The 0 to 5-cm size class was comprised entirely of young-of-the-year (YOY) fish and the 5 to 10-cm size class was comprised of second-year juvenile fish. Individual variability in growth resulted in overlapping year-classes in the 10-20 cm size class; these individuals were binned together and deemed subadults. The smallest fish observed spawning between 2007 and 2010 was ~20 cm in total length, so individuals greater than 20 cm were deemed adults for the purpose of this study; these size-class bins agree with earlier work by Clarke (1970).

From these empirical data the probability of growth for a given stage (G*i*) was calculated following Caswell (2001):

$$G_i = \sigma_i \gamma_i \tag{2}$$

where γ_i is the reciprocal of the stage duration (Ripley and Caswell 2008), and σ_i is the stage-specific probability of survival. It was assumed that adults could not grow out of the adult stage, and so G_A was set to zero. Stage-specific survival probability was estimated as:

$$\sigma_i = e^{-z_x} \tag{3}$$

where z_x is the annual mortality rate in stage x (following Caswell 2001, Houde 2002, and Ripley and Caswell 2008). The probability of remaining in a given size-class was calculated as:

$$P_i = \sigma_i (1 - \gamma_i) \tag{4}$$

where σ_i and γ_i are defined as above. For the YOY and juvenile stages, the probability of stasis, P_i , was zero as these two stages were 1 year in duration.

As it was impossible to track the fate of tens of thousands of larval fish, fecundity of adult females was calculated as per Ripley and Caswell (2008). Specifically, fecundity was estimated as:

$$F_i = \frac{YOY / m^2}{adults / m^2} \tag{5}$$

and for our model we assume a post-breeding census. Therefore the values of F_i are dependent on the probability of surviving the previous year and successfully breeding. As a result of this, there is a small contribution to F from the proportion of subadults that successfully grow into the adult size-class in a given year.

Once parameters were estimated for each of the six study reefs, the dominant eigenvalue (λ) of the reef-specific projection matrices, A_i , was calculated to estimate the population growth rate of each reef. Subsequently we assumed that the population at each reef was in approximate equilibrium (a necessary component for the use of deterministic matrix models; Caswell 2001) with the understanding that the empirical estimates of the vital rates were applicable only for the years 2008-2009. Standardized reef-specific projection matrices (by iterating values of F_i) so that $\lambda = 1$. When λ was standardized to 1, the reefs that had the lowest observed densities of YOY fish (i.e. Torrey Pines, Mission Point, and Zuniga Point) had to have their values of Fs multiplied by the largest factors to achieve a $\lambda = 1.0000$. This was a function of these parameters (F_i) having relatively low elasticities (Table 5.1B), so large changes in these parameters were necessary to alter λ as was the case in Ripley and Caswell (2008). The lowest iterated value of F was 0.99 at Torrey Pines (Appendix 5.3), this value was similar to maximum values measured in the study region (0.74); to achieve $\lambda = 1.0000$, the greatest increase in F was necessary at Torrey Pines, an increase of almost 500X empirical estimates (Appendices 2 and 3). These standardized matrices were then used to assess the elasticities of the individual elements in the reef-specific projection matrices to compare the relative importance of reef-specific vital rates to λ.

Elasticities measure the proportional sensitivity of λ to proportional perturbations in the individual matrix elements a_{ij} (Caswell 2001). Elasticity was calculated as:

$$E_{ij} = \frac{a_{ij}}{\lambda} \times S_{ij} \quad (6)$$

where S_{ij} is the sensitivity matrix. S_{ij} was calculated as:

$$S_{ij} = \frac{v_i w_j}{< w, v >} \quad (7)$$

where v is the left eigenvector of A_i, w is the right eigenvector of A_i, $<w,v>$ is the scalar product of the right and left eigenvectors, v_i is the ith element of v, and w_j is the jth element of w.

To explore the influence of connectivity on population growth rate (λ) at the reef-level, empirical estimates of connectivity were used to determine the proportion of settlers originating from each of the study reefs. This enabled us to vary the estimates of fecundity at the reef-level such that we could calculate values of λ for each of the study reefs with and without connectivity among reefs (i.e. assuming that the proportion of YOY empirically predicted to originate from each of the reefs was representative of the greater population, we could reduce the value of F for each reef by the proportion of YOY predicted to originate at other reefs in the metapopulation and recalculate λ). This gave a proportional change in λ due to connectivity. Retrospectively, we created an additional metric to assess the relative importance of connectivity to the local reef, eC. This connectivity-elasticity index was calculated as:

$$eC = \frac{eF_{SA_i} + eF_{A_i}}{eP_{Ai}} \quad (8)$$

where eF_{SAi} and eF_{Ai} are the elasticity values of the fecundity terms in the reef-level population projection matrices attributed to connectivity (see below) and eP_{Ai} the elasticity values for the probability of remaining in the adult size class for reef *i*. This index was first calculated for each reef with "full" connectivity (i.e. including YOY from all reefs in the calculation of *F*), and then re-calculated when only self-recruitment was included in the values of *F*. The change in the two ratios was then multiplied by 100 to provide a percent change describing the decrease in λ due to removal of inter-reef connectivity, in effect placing a relative value on the "importance" of connectivity to the individual reefs. In addition the elasticities of the various vital rates were ranked to show the importance of connectivity terms relative to other vital rates for each of the study reefs.

Metapopulation Mode with Connectivity

To assess the role of connectivity at the metapopulation scale we coupled the 2008-2009 stage-based matrix models for each of the six reefs with connectivity matrices generated in Chapter 4 (this dissertation) following the methods of Gerber et al. (2005). In this study the connectivity matrices describe the proportion of all metapopulation YOY dispersing among the six study reefs across the two-year study period (i.e. connectivity was summed across the 2008 and 2009 spawning seasons). A model two-reef life-cycle diagram can be viewed in Figure 5.3; however initial model runs were performed with a 6 reef configuration.

Initially our intent was to examine the relative contribution of inter-reef connectivity to metapopulation growth rate by assessing the elasticities of metapopulation-level vital rates and connectivity parameters as described for the reef-specific matrix models above. However, empirical connectivity data for *H. rubicundus* in Southern California resulted in full six-reef metapopulation model being a reducible matrix. One of the challenges inherent in calculating the elasticities of reducible matrices is that the metapopulation λ will take on the λ of the reef (i.e. the diagonal submatrix) with the highest lambda; with the empirical data in hand this results in adult survivorship at Torrey Pines (the reef with the highest population growth rate ($\lambda = 0.98$)) dominating the elasticities. When all submatrix λs are equivalent and set to 1, the imbalanced empirical connectivity patterns describing the dispersal among reefs results in the two reefs in the metapopulation with two-way flow (i.e. Cardiff and La Jolla) dominating the elasticities (Figure 5.4). As reducible matrices are not amenable to elasticity analyses, this result precluded an analysis similar to the reef-level matrix models (see Caswell 2001 and Stott et al. 2010 for a description of the challenges associated with calculating and interpreting the eigenvalues and eigenvectors of reducible matrices as they pertain to demographic and metapopulation matrix models). Therefore to circumvent this challenge, metapopulation simulation experiments were conducted to explore the relative influence of connectivity within irreducible connectivity-demography matrix models.

In these simulation experiments the submatrices describing dynamics on the reef-level were varied so that λ was equivalent in all reefs (i.e. submatrices running

along the diagonal), and connectivity was assessed when submatrix λs were less than, equal to, and greater than 1. These three scenarios are representative of a metapopulation in decline, at equilibrium, and growing, respectively. Over these three metapopulation fitness scenarios, the connectivity parameters were varied over several orders of magnitude (i.e. inter-reef connectivity values ranged from 0.0 – 1100) to examine the behavior of the elasticities attributed to connectivity parameters. In all three reef-level fitness scenarios the resultant values of metapopulation lambda were then tested to examine the relationship between connectivity and metapopulation fitness, as well as to compare the relative changes in metapopulation growth rate with varying levels of population connectivity. All statistical and matrix analyses were conducted in JMP (Version 6.0.3) and MATLAB (v. 7.4), respectively.

Results

Metapopulation-level connectivity matrices and reef-level connectivity rankings across 2008-2009 can be found in Appendix 5.1 (adapted from Chapter 4 - this dissertation). The reef with the highest connectivity was La Jolla (1.315), followed by Carlsbad (0.303), Mission Point (0.169), and Cardiff (0.146). The two reefs with the lowest connectivity values were Torrey Pines (0.045) and Zuniga Point (0.022; Chapter 4 - this dissertation)

Reef-Level Vital Rates

The pooled 2008-2009 estimates of reef-specific vital rates and the subsequently calculated population growth rates (λ =dominant eigenvalues) suggest that none of the study reefs were at equilibrium (Table 5.1A, Appendix 5.2). The empirical values of λ for individual reefs ranged from 0.70 to 0.98, with a mean (± 1 se) across all six reefs of 0.89 (0.04). La Jolla, the site with the highest connectivity value had the second lowest empirically-estimated population growth rate (0.83), while the two reefs with the lowest connectivity values (Torrey Pines and Zuniga Point) had the highest reef-level population growth rates, 0.98 and 0.97, respectively. The changes in the values of λ when population growth rate for reefs was recalculated in the absence of inter-reef connectivity (i.e. when values of F were decreased by the proportion of YOY predicted to have originated at a different reef) were relatively minor; for three of the study reefs λ was equal to P_A, while population growth rates at the remaining three reefs decreased between 0.22% and 1.43% (Table 5.1A).

When λ was standardized to 1.0000 and the influence of connectivity re-calculated, there was a significantly larger decrease in λ as compared to the empirical ($\lambda \neq 1$) estimates of λ (One-way ANOVA $F_{1,10} = 5.1$, $p < 0.05$; Table 5.1B; Appendix 5.3). When λ was initially set to 1.00 across all six reefs, the removal of inter-reef connectivity caused a change in decrease in λ of -10.1 % (4.3); the range of decrease in λ was -2.3 % to -30.7% (Table 5.1B).

When the elasticities of reef-level demographic matrix models with standardized values of λ were assessed to rank the relative contribution of individual vital rates to population growth rate, P_A always had the largest elasticity. The relative

importance of G_{YJ}, G_{JSA}, and the summed values of F_{SA} and F_A (as a proxy for inter-reef connectivity, C), was approximately equal, while either growth from the subadult to adult stage (G_{SAA}) or the probability of remaining in the subadult stage (P_{SA}) had the lowest elasticities (Table 5.2). When the elasticity-connectivity index was assessed with and without inter-reef connectivity, the mean decrease in eC was -16.0% (0.09) suggesting that at the reef-level the relative importance of connectivity to adult survivorship increased as connectivity among reefs decreased (Table 5.1B).

Metapopulation Simulation Experiments

When all six reef-level population growth rates were equivalent there was a strong positive correlation between the magnitude of the connectivity-related parameters (connectivity submatrix elements $a_{13} + a_{14} = C$) and λ; this correlation was relatively invariant if λ was less than, equal to, or greater than unity ($r = 0.92$ for all three scenarios, respective p values were 0.0002, 0.03, and 0.001 respectively; Table 5.3). When lambdas of submatrices were not equivalent, as was mentioned earlier, the metapopulation takes on the λ of the reef with the highest λ. In the case where there was zero connectivity among reefs but all reefs had equivalent λs, the result was in essence six populations existing in isolation, and the λ of the metapopulation was equivalent to the individual population growth rates of each of the reefs (Figure 5.4B).

As with the reef-level elasticities, at low levels of connectivity, probability of adult survivorship (P_A) had the largest elasticities in the metapopulation models. However, across all three scenarios (population growth rate less than, equal to, or greater than 1) as connectivity was increased among the reefs in the metapopulation

the importance of adult survivorship (P_A) decreased relative to the other parameters in the metapopulation model; as connectivity levels increased to higher levels of connectivity, the probability of growing from a YOY to a juvenile and growing from a juvenile to a subadult had the highest elasticities (Figures 5.4C and 5.4D). As this occurred the relative importance of connectivity increased, but never surpassed these two transition probabilities, suggesting that the magnitude of connectivity influences which vital rates are most important to reef-level and metapopulation-level growth rates. When the relative change in population growth rate with increasing connectivity was compared across the three scenarios, no significant differences were detected (Table 5.3; One way ANOVA $F = 0.17$, $p = 0.85$). However, there was a noticeable difference among the relative impact of increasing connectivity when comparing the three population growth groups at lower levels of connectivity. For example, when metapopulation connectivity was increased from 0.1 (a value falling among the empirical estimates of connectivity) to 1 (a value slightly greater than those observed) there was a 15% increase in metapopulation growth rate (λ increased from 0.83 to 0.95; Table 5.3). However, when this same increase in connectivity was made in the $\lambda = 1$ scenario, metapopulation growth rate increased only 2.3% when connectivity was increased from 0.1 to 1, suggesting that the influence of increasing connectivity may be more evident when populations are in decline (i.e. $\lambda < 1$).

Discussion

There have been numerous studies both theoretical and empirical examining the role of dispersal and connectivity in terrestrial settings (e.g. Boudjemadi et al. 1999, Miller and Tenhumberg 2010, Morales et al. 2010). However, studies assessing the demographic consequences of connectivity in the marine environment have been less in number and are primarily theoretical or modeling studies (e.g. Gerber et al. 2005, Hastings and Botsford 2006, Mantzouni et al. 2007, Figueira 2009). In an explicit test of the role of dispersal in the design of marine reserves, Gerber et al. (2005) found the probability of adult survival was closely tied to population growth rate; within a hypothetical marine reserve adult survivorship and population growth rate were higher than in non-reserve locations. Hastings and Botsford (2006) used a single-species matrix model to assess the factors influencing the persistence of populations inhabiting discrete habitat patches. By assessing how population persistence varied with varying larval production they were able to show that no single habitat patch was sufficient to enable population persistence; for a population to persist over time it must receive and contribute larvae to other populations, and it must over longer time scales have some level of self-recruitment (Hastings and Botsford 2006).

Empirical tests of the influence of larval connectivity within in a marine metapopulation have been rare, in large part due to the logistical challenges associated with tracking dispersing larvae (Cowen and Sponaugle 2009). Carson et al. (in review) assessed the influence of connectivity to population growth rate within a two-region metapopulation of two congeneric mytilid mussels. One of their primary

findings was that the dispersal of larval mussels influenced where and when given vital rates were of primary importance, in a similar fashion to the findings in this study. However, and counter to one of the primary findings of Carson et al. for mussels, increases in connectivity of *H. rubicundus* always resulted in an increase in metapopulation growth rate, regardless of whether the populations were decreasing, at equilibrium, or increasing. This difference may be due in part to seasonal differences in which the studies took place (Spring and Fall (Carson et al.) vs. Summer (this study)), as well as the life histories of the focal species. In mytilid mussels, juvenile mortality and growth have the highest elasticities, suggesting these parameters were most important to population fitness (Carson et al. in review). While at levels of connectivity documented in Chapter 4 (this dissertation), adult survivorship was most important for *H. rubicundus*. This pattern is in accordance with the findings of Ripley and Caswell (2008), who demonstrated that for bivalve species with shorter lifespans, growth parameters tended to be most important to overall population fitness, while for longer-lived bivalves survivorship is most important.

Empirical data for *H. rubicundus* suggest that similar to the findings of Ripley and Caswell (2008), at the reef-level, the probability of adult survivorship (P_A) was the largest contributor to population fitness. Considering the variability and episodic nature of recruitment seen in many marine systems (Dixon et al.1999), this perhaps is not a surprising result. In a review of studies using matrix population models to assess population growth rates, Pfister (1998) showed that the most variable life-history parameters tend to have the smallest contribution to population fitness, and

suggested that evolutionary forces act to minimize the influence of highly variable life- history stages such as fecundity. Stemming from this, it can be better understood why adult survivorship is necessary for the population to persist over time. As garibaldi are a relatively long-lived species (i.e. lifespan > 12 years), the annual contribution of surviving adults to reef-level fitness, can primarily be viewed as a buffer to negative population growth between bouts of successful recruitment. As suggested by Ripley and Caswell (2008) the longer the reproductive lifespan of an organism, the less relative importance is given to each spawning bout. Over the course of an individual's lifetime, it has to merely survive long enough to allow itself to be replaced by one of its offspring successfully recruiting into the reproductive population.

During 2008-2009 none of the reefs in this study region had a population growth rate greater than 1, suggesting that each of the reefs in isolation were not in steady-state equilibrium; this suggests that the six reefs are functioning as a metapopulation. As this species has been protected from exploitation for several decades (Chapter 2 – this dissertation), we hypothesized that all of the reefs would be near their carrying capacity, but examination of the reef-level population growth rates suggest that only two of the reefs (Torrey Pines and Zuniga Point) are near equilibrium. Empirical connectivity and demographic survey data show that during the study period these two reefs have the lowest connectivities (i.e. received the lowest number of YOY each year) and during this same period of time these were the only reefs to not receive YOY settlers every year (Chapter 2 – this dissertation).

Conversely, the reef with the highest documented levels of connectivity (La Jolla) had the second lowest population growth rate, suggesting that exporting YOY to other reefs may on some level have a detrimental influence on reef-level population growth rate.

When reef population growth rates were standardized to equilibrium and the relative importance of connectivity was assessed, all reefs experienced an increase in population growth rate when connectivity was included, although the extent of influence was variable among reefs. The reef with the largest increase in population growth rate due to connectivity was Cardiff, the reef with the lowest empirically estimated population growth rate ($\lambda = 0.70$) while the reefs that were closest to equilibrium (Torrey Pines and Zuniga Point) were least affected by a loss of inter-reef connectivity. The decreases seen in the elasticity-connectivity index support this finding and suggest that for each of the reefs, as the level of connectivity is increased, population fitness becomes less reliant on adult survivorship, and conversely, as connectivity decreases population fitness is more closely tied to adult survivorship.

There were three patterns that arose during the metapopulation simulation modeling. As with the reef-level analyses (see above), as the magnitude of connectivity increases, the relative importance of adult survivorship to metapopulation fitness decreases, and the relative importance of the probability of growing from smaller to larger size classes increases in relative importance. According to the findings of Ripley and Caswell (2008) this pattern would suggest a move from broadcast spawning to brooding. As garibaldi are benthic spawners (i.e. females

spawn eggs onto benthic nests where embryos develop for ~2 weeks prior to dispersing for approximately three weeks), they fall roughly midway along the broadcast and brooding continuum.

Secondly, as metapopulation connectivity was varied from low to high levels, the relative contribution of connectivity to metapopulation fitness climbed above some of the within-reef vital rates including adult survivorship, but never surpassed all vital rates. What it did do was act to regulate what vital rates were most important to metapopulation fitness. At lower levels of connectivity, adult survivorship was the largest contributor to population fitness, while at higher levels of connectivity, growth from the YOY stage into the juvenile stage, and from the juvenile stage to the subadult stage were the largest contributors to metapopulation fitness. This result may be due in part to increases in connectivity causing a concomitant increase in the rate at which individuals move through the metapopulation. As was mentioned earlier, adults are more important to population growth rate when successful recruitment into the adult population is episodic, but as connectivity increases (i.e. in essence increasing the number of successful offspring being produced each unit of time), the relative importance of adult survivorship decreases, as the population receives a constant influx of new recruits that can replace adults leaving the system through mortality.

A final pattern that emerged from the metapopulation simulation modeling was the apparent larger influence of connectivity to metapopulation population growth rate when population growth rates at the reef-level were below 1 (suggesting they were in decline). While there were no significant differences in the relative changes seen in

metapopulation growth rate when viewed across all four orders of magnitude, at the lower levels of connectivity, which happen to coincide with the empirical connectivity data generated in Chapter 4, there was a consistently larger influence of increasing connectivity in metapopulations when reef-level population growth rates were decreasing (i.e. $\lambda < 1$). From a management perspective, this suggests that in situations where local populations comprising a metapopulation are in decline, management actions that are able to increase the connectivity within the broader metapopulation may be more successful at increasing the overall metapopulation growth rate relative to actions aimed at increasing metapopulation growth rates in systems where populations are at equilibrium (i.e. $\lambda = 1$). As this finding may have relevance for those involved in the conservation and management of natural resources, this intriguing result warrants further study.

In conclusion, the results of this study suggest connectivity plays an active role in regulating which vital rates are the greatest contributors to population and metapopulation growth rates. At low levels of connectivity, adult survivorship is the greatest contributor to population growth rate. The development of coupled connectivity-demography models should increase our understanding of the demographic consequences connectivity. This in turn, will enable resource managers to more successfully protect marine species living within metapopulations, possibly moving fisheries closer to sustainability while simultaneously protecting marine biodiversity.

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Table 5.1 Table 5.1A. Change in empirically constructed reef-level projection matrices with and without metapopulation connectivity. Full connectivity implies all recruits at a reef were self-recruits, ignoring empirical predictions of natal origin, whereas metapopulation-level lambda accounts for natal origin.

Reef	2008-2009 Full Connectivity Lambda	2008-2009 Metapopulation-Level Connectivity Lambda	Lambda Metapopulation-Level % Δ
Carlsbad	0.93	0.92	-0.86
Cardiff	0.69	0.69	-0.22
Torrey Pines	0.98	0.98	0.00
La Jolla	0.83	0.82	-1.43
Mission Point	0.91	0.91	0.00
Zuniga Point	0.97	0.97	0.00

Table 1B. Change in standardized (λ=1) reef-level projection matrices with and without metapopulation connectivity, and resultant change in elasticity-connectivity index (eC)

Reef	2008-2009 Full Connectivity Lambda	2008-2009 Metapopulation-Level Connectivity Lambda	Lambda Metapopulation-Level % change	Elasticity-Connectivity Index (eC) % Δ
Carlsbad	1.00	0.92	-8.30	-9.82
Cardiff	1.00	0.69	-30.70	-57.92
Torrey Pines	1.00	0.98	-2.30	-2.41
La Jolla	1.00	0.93	-7.50	-12.03
Mission Point	1.00	0.91	-8.90	-10.65
Zuniga Point	1.00	0.97	-2.80	-2.97

Table 5.2 Ranking of vital rates (P and G) and connectivity terms (C) based upon elasticity values (in parentheses). A higher ranking connotes a higher elasticity value (i.e. a greater relative contribution to population growth rate).

	Reef					
	Carlsbad	Cardiff	Torrey Pines	La Jolla	Mission Point	Zuniga Point
Elasticity Ranking						
1	P_A (0.65)	P_A (0.27)	P_A (0.90)	P_A (0.40)	P_A (0.69)	P_A (0.88)
2	P_{SA} (0.10)	C, $G_{Y\text{-}J}$, $G_{J\text{-}SA}$ (0.16)	C, $G_{Y\text{-}J}$, $G_{J\text{-}SA}$ (0.02)	P_{SA} (0.18)	C, $G_{Y\text{-}J}$, $G_{J\text{-}SA}$ (0.07)	C, $G_{Y\text{-}J}$, $G_{J\text{-}SA}$ (0.03)
3	C, $G_{Y\text{-}J}$, $G_{J\text{-}SA}$ (0.064)	P_{SA} (0.13)	$G_{SA\text{-}A}$ (0.02)	C, $G_{Y\text{-}J}$, $G_{J\text{-}SA}$ (0.11)	$G_{SA\text{-}A}$ (0.07)	$G_{SA\text{-}A}$ (0.03)
4	$G_{SA\text{-}A}$ (0.06)	$G_{SA\text{-}A}$ (0.12)	P_{SA} (0.01)	$G_{SA\text{-}A}$ (0.09)	P_{SA} (0.02)	P_{SA} (0.01)

Table 5.3 Metapopulation simulations depict influence of connectivity on metapopulation λ when reef-level submatrices λ are less than, equal to, or greater than 1.

			Reef-Level λ less than 1		Reef-Level λ equal to 1		Reef-Level λ greater than 1	
Connectivity		Connectivity Sum	Metapopulation Lambda	Relative Change in λ (%)	Metapopulation Lambda	Relative Change in λ (%)	Metapopulation Lambda	Relative Change in λ (%)
C_3	C_4							
0.01	0.10	0.11	0.83		1.00		1.02	
0.10	1.00	1.10	0.95	14.92	1.03	2.67	1.06	4.12
1.00	10.00	11.00	1.25	31.07	1.19	15.53	1.28	21.19
10.00	100.00	110.00	1.90	52.00	1.68	41.18	1.90	48.45
100.00	1000.00	1100.00	3.22	69.66	2.77	64.70	3.23	69.36

Figure Legends

Figure 5.1 Map of study area showing location of six primary study reefs. Figure taken with permission from Cook 2011.

Figure 5.2 Four-stage single reef life cycle diagram for garibaldi. G = stage transition probability, P = probability of remaining within same stage-class, and F = fertility.

Figure 5.3 Sample 4 stage-class, 2-reef life cycle diagram for garibaldi, where G = stage transition probability, P = probability of remaining within same stage-class, and F = fertility. Red lines indicate connectivity terms.

Figure 5.4 Figure 4A. Metapopulation elasticity values generated from standardized demographic (i.e. all reef-level and metapopulation $\lambda = 1.00$) coupled with empirical connectivity data. The two clusters of columns represent the various vital rates for Cardiff (blue) and La Jolla (green-yellow). For each reef adult survivorship had the highest elasticity. Figure 4B. Metapopulation elasticity values generated when all reef-level λ are equal in magnitude (i.e. $\lambda = 1.01$), but with no inter-reef connectivity. All reef-level vital rates are equivalent to empirical estimates of P and G from La Jolla, except F_{SA} and F_A have been increased to 1, and 10, respectively. Figure 4C. Metapopulation elasticity values generated when all reef-level λ are equal in magnitude (i.e. $\lambda = 1.01$; reef-level vital rates as defined in Figure 4B), and inter-reef connectivity C_{SA} and C_A are set to 0.1 and 1, respectively ($C_{total} = 1.1$); overall

metapopulation growth rate = 1.06. As with Figures 4A, and 4B probability of adult survivorship at each reef has the highest contribution to metapopulation growth rate.

Figure 4D. Metapopulation elasticity values generated when all reef-level λ are equal in magnitude (i.e. λ = 1.01; reef-level vital rates as defined in Figure 4C), and inter-reef connectivity C_{SA} and C_A are set to 100 and 1000, respectively (C_{total} = 1100); overall metapopulation growth rate = 3.23. Unlike Figures 4A, 4B, and 4C the probability of growth from the YOY to juvenile stage and from juvenile to subadult stage has the highest contribution to metapopulation growth rate, and the total contribution of connectivity to metapopulation growth rate is greater than adult survivorship.

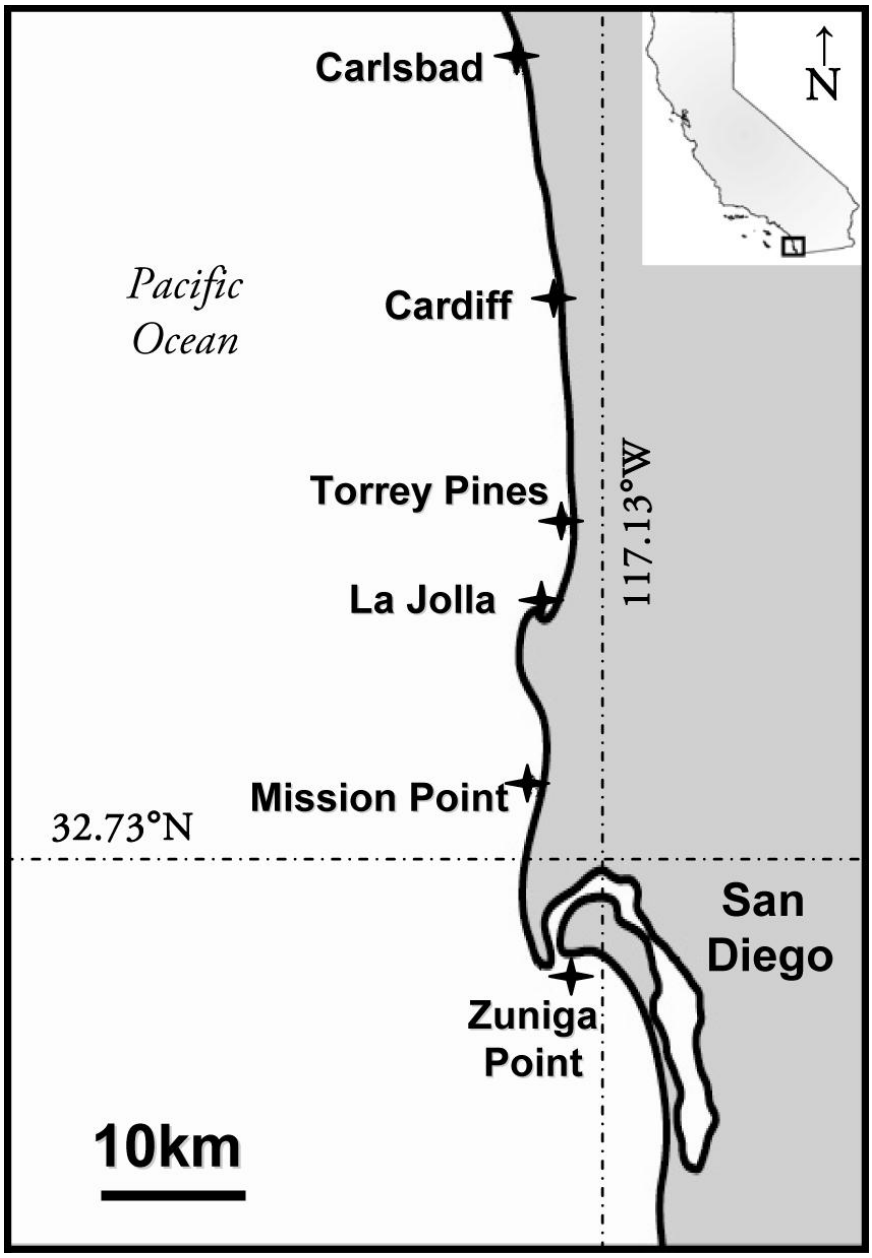
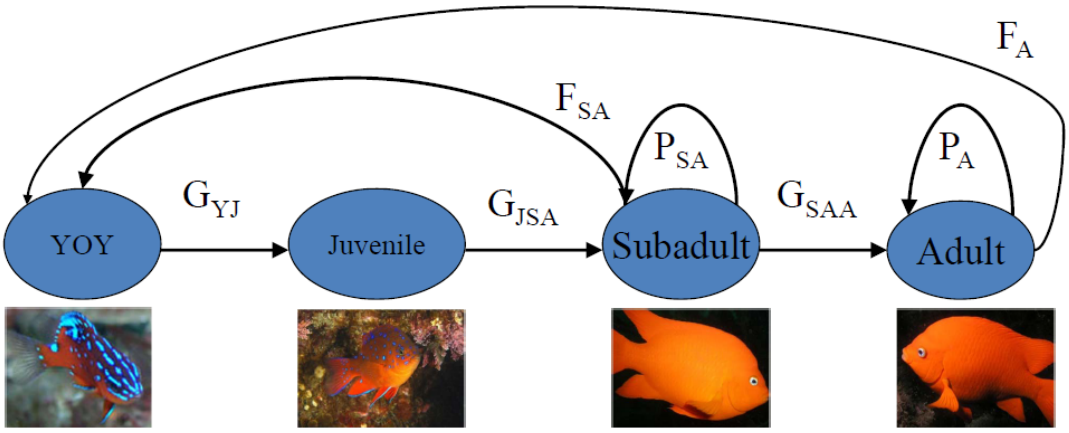


Figure 5.1 Map of study area showing location of six primary study reefs. Figure taken with permission from Cook 2011.



4 Stage Population Projection Matrix			
0	0	F_{SA}	F_A
G_{YJ}	0	0	0
0	G_{JSA}	P_{SA}	0
0	0	G_{SAA}	P_A

Figure 5.2 Four-stage single reef life cycle diagram for garibaldi. *G* = stage transition probability, *P* = probability of remaining within same stage-class, and *F* = fertility.

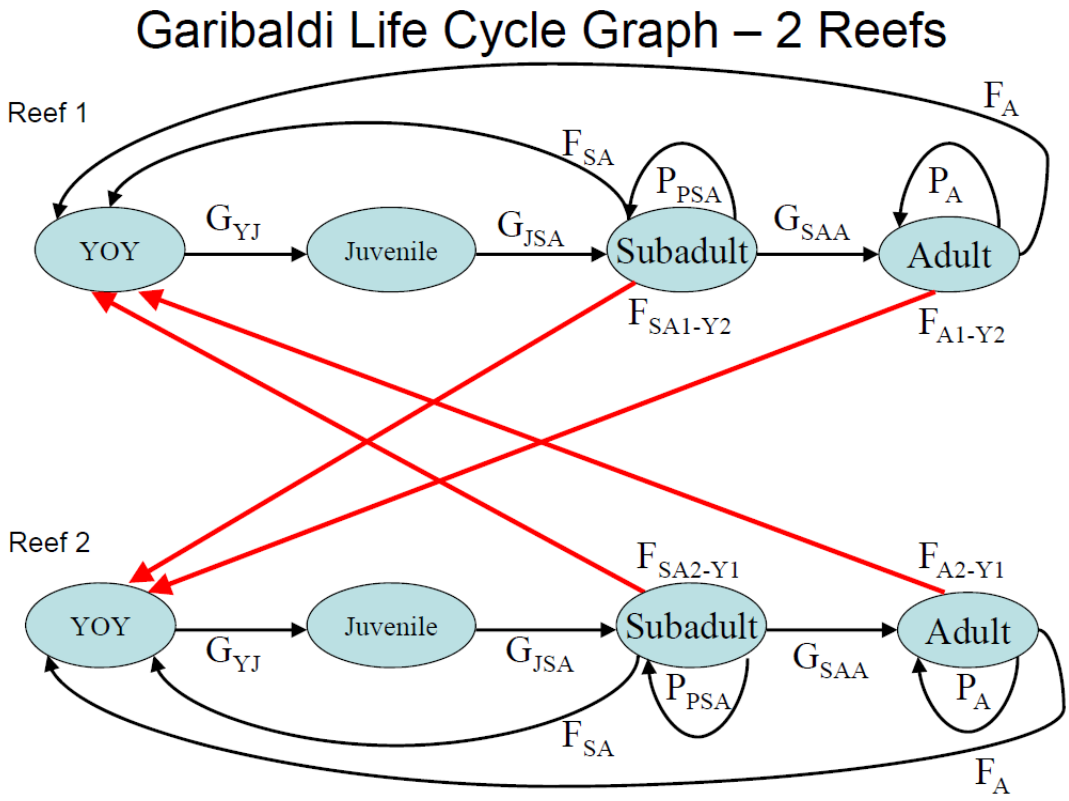


Figure 5.3 Sample 4 stage-class, 2-reef life cycle diagram for garibaldi, where *G* = stage transition probability, *P* = probability of remaining within same stage-class, and *F* = fertility. Red lines indicate connectivity terms.

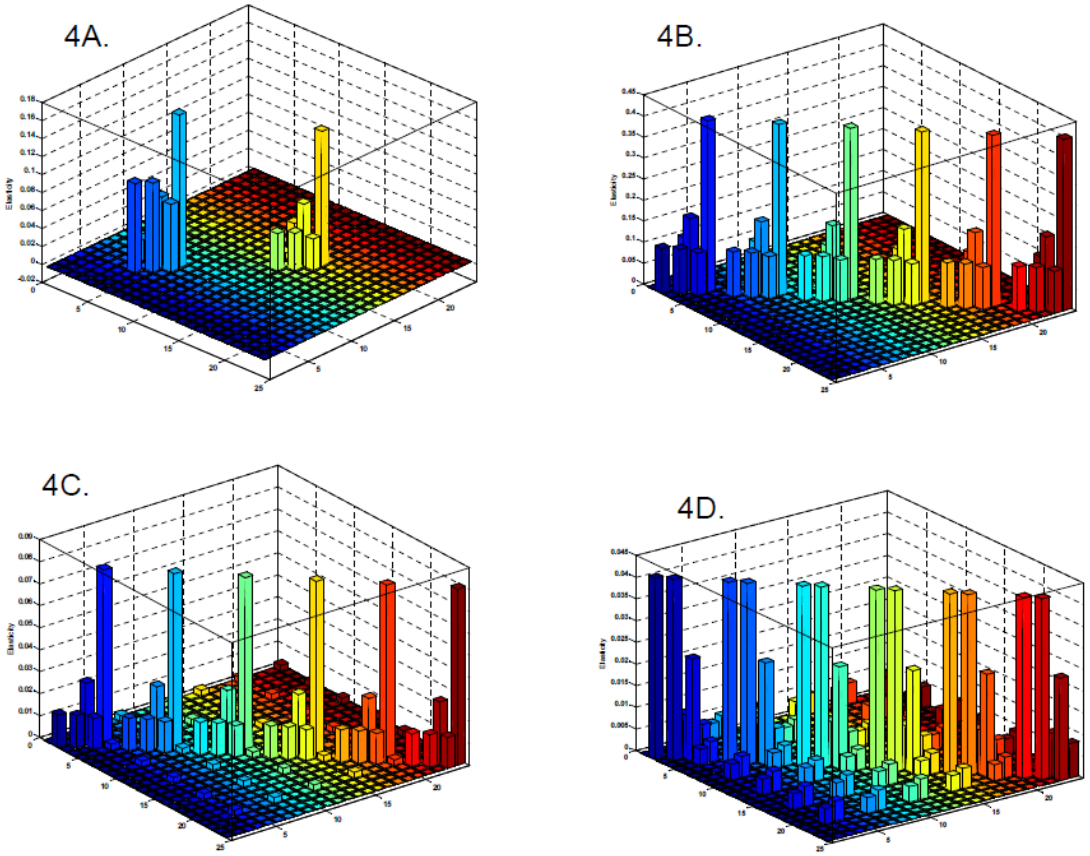


Figure 5.4 Figure 4A. Metapopulation elasticity values generated from standardized demographic (i.e. all reef-level and metapopulation $\lambda = 1.00$) coupled with empirical connectivity data. The two clusters of columns represent the various vital rates for Cardiff (blue) and La Jolla (green-yellow). For each reef adult survivorship had the highest elasticity. Figure 4B. Metapopulation elasticity values generated when all reef-level λ are equal in magnitude (i.e. $\lambda = 1.01$), but with no inter-reef connectivity. All reef-level vital rates are equivalent to empirical estimates of P and G from La Jolla, except F_{SA} and F_A have been increased to 1, and 10, respectively. Figure 4C. Metapopulation elasticity values generated when all reef-level λ are equal in magnitude (i.e. $\lambda = 1.01$; reef-level vital rates as defined in Figure 4B), and inter-reef connectivity C_{SA} and C_A are set to 0.1 and 1, respectively (C_{total} = 1.1); overall metapopulation growth rate = 1.06. As with Figures 4A, and 4B probability of adult survivorship at each reef has the highest contribution to metapopulation growth rate. Figure 4D. Metapopulation elasticity values generated when all reef-level λ are equal in magnitude (i.e. $\lambda = 1.01$; reef-level vital rates as defined in Figure 4C), and inter-reef connectivity C_{SA} and C_A are set to 100 and 1000, respectively (C_{total} = 1100); overall metapopulation growth rate = 3.23. Unlike Figures 4A, 4B, and 4C the probability of growth from the YOY to juvenile stage and from juvenile to subadult stage has the highest contribution to metapopulation growth rate, and the total contribution of connectivity to metapopulation growth rate is greater than adult survivorship.

Appendix 5.1 Connectivity matrices for 2008, 2009, and 2008-2009. Values represent proportion of all YOY fish dispersing among reefs. Rows are predicted natal reefs, columns are reefs where post-dispersal YOY were captured.

Interannual (2008-2009) Population Connectivity Matrix (proportions based on entire metapopulation)

	Settlement Reef						
Natal Reef	Carlsbad	Cardiff	Torrey Pines	La Jolla	Mission Point	Zuniga Point	row sum
Carlsbad	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cardiff	0.000	0.000	0.000	0.022	0.000	0.000	0.022
Torrey Pines	0.011	0.000	0.000	0.034	0.000	0.000	0.045
La Jolla	0.225	0.090	0.000	0.438	0.000	0.011	0.764
Mission Point	0.056	0.034	0.000	0.056	0.011	0.000	0.157
Zuniga Point	0.011	0.000	0.000	0.000	0.000	0.000	0.011
column sum	0.303	0.124	0.000	0.551	0.011	0.011	
Connectivity Total	0.303	0.146	0.045	1.315	0.169	0.022	
Connectivity Rank	2	4	5	1	3	6	

Appendix 5.2 Empirical stage-based demographic matrix models. Values represent individual vital rates. Subdiagonal values are growth transition, diagonal values are stasis probabilities. Values in row 1 are fecundity terms.

Carlsbad Empirical Demography

0.00	0.00	0.02	0.07
0.13	0.00	0.00	0.00
0.00	0.77	0.61	0.00
0.00	0.00	0.30	0.92

Cardiff Empirical Demography

0.00	0.00	0.02	0.09
0.02	0.00	0.00	0.00
0.00	0.44	0.45	0.00
0.00	0.00	0.22	0.70

Torrey Pines Empirical Demography

0.00	0.00	0.003	0.01
0.001	0.00	0.00	0.00
0.00	0.18	0.38	0.00
0.00	0.00	0.19	0.98

La Jolla Empirical Demography

0.00	0.00	0.14	0.43
0.03	0.00	0.00	0.00
0.00	0.87	0.63	0.00
0.00	0.00	0.31	0.81

Mission Point Empirical Demography

0.00	0.00	0.003	0.03
0.01	0.00	0.00	0.00
0.00	0.13	0.25	0.00
0.00	0.00	0.13	0.91

Zuniga Point Empirical Demography

0.00	0.00	0.003	0.02
0.002	0.00	0.00	0.00
0.00	0.14	0.34	0.00
0.00	0.00	0.17	0.97

Appendix 5.3 Empirical stage-based demographic matrix models, lambda set to 1. Subdiagonal values are growth transition, diagonal values are stasis probabilities. Values in row 1 are fecundity terms.

Carlsbad λ=1.0000			
0.00	0.00	0.30	0.99
0.13	0.00	0.00	0.00
0.00	0.77	0.61	0.00
0.00	0.00	0.30	0.92
Cardiff λ=1.0000			
0.00	0.00	14.92	66.40
0.02	0.00	0.00	0.00
0.00	0.44	0.45	0.00
0.00	0.00	0.22	0.70
Torrey Pines λ=1.0000			
0.00	0.00	79.42	413.00
0.001	0.00	0.00	0.00
0.00	0.18	0.38	0.00
0.00	0.00	0.19	0.98
La Jolla λ=1.0000			
0.00	0.00	2.52	8.02
0.03	0.00	0.00	0.00
0.00	0.87	0.63	0.00
0.00	0.00	0.31	0.81
Mission Point λ=1.0000			
0.00	0.00	48.57	387.00
0.01	0.00	0.00	0.00
0.00	0.13	0.25	0.00
0.00	0.00	0.13	0.91
Zuniga Point λ=1.0000			
0.00	0.00	69.94	373.00
0.00	0.00	0.00	0.00
0.00	0.14	0.34	0.00
0.00	0.00	0.17	0.97

CHAPTER VI.

QUANTIFYING CONNECTIVITY IN MARINE METAPOPULATIONS

Abstract

As empirical estimates of population connectivity emerge for marine systems, there is a need to move from qualitative to quantitative metrics of connectivity. This study couples intuitive metrics of connectedness developed by those studying quantitative food webs with the tools emerging from graph theory to create easy-to-comprehend metrics of reef-level and metapopulation-level connectivity. Qualitative and quantitative metrics of population and metapopulation connectivity are developed to identify source and sink populations within the study region, and using node deletion experiments we are able to quantify the connectivity value of individual reefs to a greater metapopulation. When realized connectivity data are compared against the connectivity potential of the system (i.e. potential topological connectance per node and the maximum topological connectance per node), simple ratios describing the extent of connectivity within the metapopulation are created. Quantitative metrics of metapopulation connectivity combined with node deletion experiments more accurately depict the value of individual reefs to the connectedness of the metapopulation than qualitative metrics alone. Using an empirical example of fish population connectivity data using elemental fingerprinting generated for a network of temperate, rocky reef marine reserves, we show how these intuitive metrics are easily comprehensible, require minimal data inputs, and can be used by those involved in the

reserve selection process to make quantitative comparisons of alternative reserve designs.

Introduction

The importance of dispersal to population dynamics has been explicitly acknowledged for over a century (Reid 1899, Hjort 1914, Tansley 1923, Elton 1925, and Nicholson 1933). This long-held interest of ecologists in dispersal, the immigration and emigration of individuals, and their influence on the dynamics of spatially separated populations should not however be a surprise, because as Elton (1927) so eloquently put it, "When we are studying any particular animal or community of animals, we are brought up, sooner or later, against questions connected with dispersal". This same sentiment was presaged by Wood-Jones (1912):

> *Those creatures that are settled and established are the elect, and they are appointed out of a countless host of competitors, all of whom have had equal adventure but have gone under in the struggle, through no fault of their own. They are the actual colonists, the survivors of a vast army of immigrants, every one of which was a potential colonist.*

While the role of dispersal was being studied by ecologists in the early 1900s, it was not, with few exceptions, formalized analytically until almost a half century later (Skellam 1951, 1952).

Research on dispersal continued through the mid-20th century with theoretical advances being made by numerous individuals. MacArthur and Wilson (1963, 1967) invoked the role of dispersal capacity as an implicit mechanism in their theory of island biogeography. Levins (1969, 1970), adapting this framework to apply spatial population dynamics to the control of agricultural pests, showed the balance between extinction rates and colonization rates governed the rise and fall of spatially separated populations. Since the inception of Levins' (1969, 1970) seminal work, the field of metapopulation ecology has flourished, particularly in terrestrial systems as anthropogenically-driven habitat fragmentation was acknowledged to be one of the primary drivers of biodiversity decline.

This concern over habitat-related loss of biodiversity gave rise not only to the field of metapopulation biology, but also to landscape ecology. Landscape ecologists are concerned primarily with describing attributes of the mosaic of habitat patches and the influence of this matrix on dispersing individuals while metapopulation ecologists have focused their efforts on describing dynamics of populations inhabiting discrete patches of suitable habitat connected by migrating individuals (Hanski 1998). While the relationship between landscape and metapopulation ecology has been tempestuous at times, they are in fact very complementary fields of study, and as such advances in the understanding of dispersal and its role in the conservation of biodiversity has been made through both avenues of study (Wiens 1997). These advances have moved the importance of migration and dispersal among patches of habitat comprising metapopulations beyond theoretical prediction, yet debate still remains among the

primacy of the various processes governing population growth and persistence (e.g. Gadgil 1971, Brown and Kodric-Brown 1977, Fahrig and Merriam 1985, Pulliam 1988, Hanski and Gilpin 1997, Mouquet and Loreau 2003, Bowler and Benton 2005, Fagan and Lutscher 2006, Levin 2006, Holland and Hastings 2008, Griffen and Drake 2009, Miller and Tenhumberg 2010).

A growing number of terrestrial, lacustrine, and riverine studies have started to shed light on the frequency and magnitude with which spatially separated populations interact with one another (e.g. Paradis et al. 1998, Ricketts 2001, Kneitel and Miller 2003, Smith and Green 2005, Stevens et al. 2010). As these empirical dispersal data have been generated, the definition of a metapopulation has evolved (Kritzer and Sale 2006). For the sake of this study, a metapopulation shall be defined as a network of reefs each separated by kilometers of an uninhabited sand-bottom matrix, where larval connectivity among reefs is sufficiently high that there is demographic connectivity and low enough that the metapopulation does not behave as one single population (*sensu* Kritzer and Sale 2006). Efforts are being made to characterize the variability inherent in population connectivity patterns, and tools have been developed to facilitate advances in our understanding of connectivity, particularly in the fields of landscape and conservation ecology. One of the approaches adopted for its ease of comprehension and ability to simplify large amounts of connectivity data, has been the field of graph theory (Urban et al. 2009). From a conservation standpoint, this analytical framework has proven useful for examining actual and hypothetical systems of reserves. In large part the growing acceptance for the use of graphs can be

attributed to the ease with which patches of habitat separated by an uninhabitable matrix can (at least visually) be viewed as a set of nodes connected by arcs, describing the magnitude and directionality of individuals dispersing among patches (Urban et al. 2009).

The use of graphs to analyze and assess habitat and population connectivity patterns in terrestrial and riverine systems has proven successful in conservation-oriented studies (Keitt et al. 1997, Bunn et al. 2000, Urban and Keitt 2001, Pascual-Hortal and Saura 2006, Schick and Lindley 2007, Urban et al. 2009, and references therein), but this practice has been adopted more slowly in marine systems (but see Treml et al. 2008). One reason for this lag in the use of graph theory in marine systems may be attributed to the immense logistical challenges associated with estimating population connectivity in marine systems. To track a dispersing seed or flying insect in a terrestrial system is daunting in its own right but for many marine species, movement between populations of relatively sedentary adults occurs via a wide-ranging larval dispersal stage that is essentially invisible as it disperses through a hydrodynamically complex environment for periods of time ranging from days to months. The constant interaction of larval behavior and a shifting mosaic of oceanographic currents ultimately delivers larvae to their settlement destination.

Fortunately methodological and technological advances in tracking larval dispersal have emerged recently in the marine environment and the extent to which spatially separated populations are linked by larval dispersal and the role dispersal plays in local and metapopulation dynamics is moving from theoretical prediction

toward a more mechanistic understanding through empirical validation (see Kritzer and Sale 2006 and references therein). One promising method for quantifying population connectivity is elemental fingerprinting. This method is predicated upon spatial differences in trace elemental chemistry that are great enough to distinguish among reefs of interest, and can only be applied to organisms that are capable of recording these differences during the dispersal stage (see Thorrold et al. 2007). Many marine organisms (e.g. fish, mussels, squid) have calcareous ($CaCO_3$) hard parts that grow incrementally (similar to the growth rings seen in trees), and as these structures grow, they integrate the chemical signatures of their natal location (Thorrold et al. 2007). For fishes, the application of otolith (ear-stone) microchemistry as a tool for estimating population connectivity has been greatly refined since its first successful application (Swearer et al. 1999).

A record of recruit natal origin can be used to generate a connectivity matrix describing the number of individuals moving between reefs per unit time. Subsequently these connectivity matrices can be used to generate data describing mean dispersal distance and rate, dispersal directionality, and proportion of self-recruitment (i.e. individuals being spawned at and settling to the same location). For most studies, this descriptive assessment of connectivity has been the primary goal, and given the logistical challenges of generating these estimates, has been the intellectual stopping point (e.g. Cowen et al. 2006, Almany et al. 2007, Becker et al. 2007, Siegel et al. 2008, Standish et al. 2008, Mitarai et al. 2009, Planes et al. 2009, Carson et al. 2010, Watson et al. 2010, Chapter 4 - this dissertation).

However, if the field is to advance further and our understanding move beyond gross descriptions of connectivity and their implications for reserve design, we must move from qualitative descriptions to quantitative measures of connectivity. In this study we utilize empirically-derived larval connectivity matrices generated in Chapter 4 (this dissertation) for a temperate rocky-reef damselfish, to develop metrics for quantifying connectivity in marine metapopulations. By building upon the groundwork laid by those attempting to quantitatively describe the properties of food-webs through the use of ecosystem flow networks (Ulanowicz and Wolff 1991, Bersier et al. 2002), we develop an inherently intuitive framework for ecologists to quantify and explore the properties of metapopulation connectivity. We compare insights derived from qualitative and quantitative metrics of connectivity. Using empirical connectivity data in conjunction with node deletion experiments (see below), we explore the value of individual reefs to overall metapopulation connectivity. These data and indices subsequently are used to rank and make relative comparisons among reefs thereby creating an elegant decision-making tool for natural resource managers, policymakers, and vested stakeholders involved in the reserve selection process.

Methods

Model Species

The model species for this study was garibaldi, *Hypsypops rubicundus*. Its life history and habitat characteristics within the study region have been described in detail in Limbaugh (1964), Clarke (1970), and Chapter 2 (this dissertation). Garibaldi are benthic spawning temperate damselfish inhabiting nearshore rocky reefs from Point Concepcion, California in the north to Bahia Magdalena, Baja California in the south. In the center of this range, in San Diego County, USA between mid-May and mid-August of each year male garibaldi guard benthic nests on shallow (i.e. primarily < 15 m water depth) rocky reefs. During this period of time females periodically deposit eggs on the nests tended by males. Embryos develop on benthic nests for approximately two weeks, after which they hatch and disperse for 18 – 22 days (Moser 1996, Cook 2011) prior to settlement on nearshore rocky reefs. In conjunction with their spawning mode, spawning period, and pelagic larval duration, there are numerous life-history traits that make garibaldi an excellent model species for fishes inhabiting rocky reefs of southern California (Pondella et al. 2005, Allen and Pondella 2006, please see Chapter 1 – this dissertation for a more detailed description of the model species).

Study Region

To define the boundaries of the study region over 600 population and habitat surveys were conducted using SCUBA between 2002 and 2008 (Figure 6.1, adapted from Chapter 2 – this dissertation). From these survey data, six reefs supporting over

90% of all garibaldi within the ~60 km study region, were identified (a detailed description of the study region is available in Chapter 2 – this dissertation). Three of these six populations are located within or adjacent to existing marine reserves: Cardiff, La Jolla, and Zuniga Point, San Diego County, USA.

Qualitative Metrics of Connectivity

For the purpose of this study aggregate annual and interannual estimates of dispersal and population connectivity will be discussed (for a description of biweekly patterns of connectivity please see Chapter 4 - this dissertation). These microchemistry-derived estimates of annual and interannual connectivity were used to calculate the number of YOY dispersing between the six reefs comprising the study system (see Appendix 6.1 for a detailed description of methods used to empirically estimate population connectivity). Individuals predicted to originate at a given location were enumerated as individuals exported by a reef, and individuals arriving at a reef were enumerated as individuals imported. These same data were used to describe qualitative reef connectance. For this metric the total number of reefs a given location provided YOY settlers to (i.e. acted as a source population for) was tallied, and the number of reefs a given reef received YOY settlers from (i.e. acted as a sink for) was tallied. In graph theory parlance, these values are equivalent to the out-degree and in-degree of the individual reefs, respectively (please see Treml et al. 2008, Urban et al. 2009 for descriptions of graph definitions).

These source-sink data were then utilized to calculate qualitative functional connectivity for each reef within the metapopulation. This metric describes the disequitability between the number of reefs a location receives larval fish from (imports) and the number of reefs it provides larval fish to (exports), and thus determines whether a reef acts primarily as a source or sink population. The unweighted qualitative functional connectivity for reef *k* is calculated as:

$$c_k = \frac{\sum SN_k}{\sum SN_k + \sum SRC_k}$$

where SN_k is the number of locations reef *k* imports settlers from (acts as a sink for) and SRC_k is the number of locations reef *k* exports settlers to (acts as a source for). Reef *k* will be considered a "pure" sink population if $1 - c_k = 0$ (i.e. reef *k* only receives settlers from other reefs but does not contribute settlers to other reefs within the metapopulation). It is a "pure" source population if $1 - c_k = 1$ (i.e. reef *k* only contributes settlers to other reefs, but does not receive settlers from any reefs within the metapopulation), and an intermediate source-sink otherwise (i.e. $0 < c_k < 1$). However c_k fails to account for the magnitude of YOY dispersing among all reefs in the metapopulation. A weighted version of c_k accounts for this by weighting SN_k and SRC_k by the proportion of all YOY flowing to and from reef *k* by the total number of YOY being imported and exported among all reefs within the metapopulation. These proportionally weighted connectivity values can subsequently be utilized to determine which reefs within the reserve network were, using the definitions above, behaving as source and/or sink populations within the greater metapopulation.

To assess the connectivity of the metapopulation as a whole, two widely used metrics for describing the connectivity of systems were used: link density (*LD*) and directed connectance (*DC*). Link density describes the average number of reefs each reef in the metapopulation is connected to. It is calculated as *l/s*, where *l* is the number of links and *s* is the total number of reefs within the study system. Directed connectance is calculated as l/s^2; this value describes the actual number of links in the system (including self-recruitment) divided by the total number of links possible (Bersier et al. 2001).

Quantitative Metrics of Connectivity

To quantitatively determine the functional status of a given reef (i.e. whether it is a source or sink population) we shall utilize the Shannon measure of diversity (aka entropy or uncertainty), *H* (Shannon 1948). This metric was selected for diversity of flows as, relative to other indices (e.g. Simpson's measure of evenness, Simpson 1949); it provides a measure of balance between precision and accuracy (Bersier et al. 2002). Using Shannon's index the diversity of both inflows and outflows will reach a maximal value when all events occur in equal proportion (i.e. the magnitude of larval exchange between all reefs within the metapopulation is equal). Adapting the positional index (*c*), from Ulanowicz and Wolff (1991) and Bersier *et al*. (2002), individual reefs can be deemed sources, sinks, or intermediate source-sinks based on their connectivity with other reefs within a metapopulation. For each focal reef (*S* =

total number of reefs comprising metapopulation) within a metapopulation one can measure the diversity of settlers arriving at a given location (H_I) and the diversity of settlement reefs for larvae originating from a given reef within the metapopulation (H_E). For reef *k*,

$$H_{I,k} = -\sum_{i=1}^{S} \frac{s_{ik}}{s_{.k}} \log_2 \frac{s_{ik}}{s_{.k}}$$ = diversity of inflows (i.e. settlers imported) at reef *k*

$$H_{E,k} = -\sum_{j=1}^{S} \frac{s_{kj}}{s_{k.}} \log_2 \frac{s_{kj}}{s_{k.}}$$ = diversity of outflows (i.e. settlers exported) from reef *k*

The column sum $s_{.k}$ and row sum $s_{k.}$ represent the total number of settlers arriving at and emanating from reef *k*, respectively. If either the row or column sum is undefined (i.e. if a reef did not import or export any settlers in a given period of time), the value of $H_{(I \text{ or } E),k}$ is assumed to be zero. Taking the reciprocal of *H*, 2^H, which conceptually is the number of settlers being dispersed equally among all local populations comprising the metapopulation, allows one to recover the original units (i.e. number of settlers), and allows for a more intuitive understanding of the metric being applied. The reciprocals of $H_{I,k}$ and $H_{E,k}$ are:

$$n_{I,k} = \begin{cases} 2^{H_{I,k}} \\ 0 \quad if\ s_{.k} = 0 \end{cases}$$

$$n_{E,k} = \begin{cases} 2^{H_{E,k}} \\ 0 \quad if\ s_{k.} = 0 \end{cases}$$

These values describe equivalent numbers of source (n_I) and sink (n_E) populations, and are used to calculate the unweighted quantitative functional connectivity (QC_k) of a given reef:

$$QC_k = \frac{n_{I,k}}{n_{I,k} + n_{E,k}}$$

Like the unweighted qualitative functional connectivity (c_k), this metric disregards the magnitude of immigrating ($s_{.k}$) and emigrating ($s_{k.}$) settlers from each reef. Therefore to account for the magnitude of larval settlers coming from source locations and arriving at sink populations within the metapopulation, the proposed weighted quantitative functional connectivity index for reef *k* is:

$$QC_k = \frac{s_{.k} n_{I,k}}{s_{.k} n_{I,k} + s_{k.} n_{E,k}}$$

As with the qualitative metrics of functional connectivity, a reef will be considered a "pure" sink population if 1 - QC_k = 0 (i.e. reef *k* only receives settlers from other reefs but does not contribute settlers to other reefs within the metapopulation), a "pure"

source population if 1 - $QC_k = 1$ (i.e. reef k only contributes settlers to other reefs, but does not receive settlers from any reefs within the metapopulation), and an intermediate source-sink population otherwise (i.e. $0 < QC_k < 1$). In the situation where a given reef was neither a source nor a sink for YOY settlers (e.g. Zuniga Point in 2008 or Torrey Pines in 2009), the diversity and functional connectivity metrics were undefined.

Building upon the diversity of inflows and outflows of settlers among reefs within the metapopulation, the degree of connectivity for the metapopulation will be assessed by determining the entire systems effective connectance per node, m, as defined by Ulanowicz and Wolff (1991). The effective connectance per node is the mean, or effective number of flows, originating at and emanating from a reef within the metapopulation (Bersier et al. 2002). It measures the effective connectance per reef averaged over all reefs within the study system and incorporates the total magnitude of settlers dispersing throughout the metapopulation. Computationally it is defined as:

$$m = 2^{\Phi/2}$$

where

$$\Phi = \sum_{k=1}^{S} \frac{s_{k.}}{s_{..}} H_{E,k} + \sum_{k=1}^{S} \frac{s_{.k}}{s_{..}} H_{I,k}$$

Adapting the description of Bersier et al. (2002) to a connectivity framework, Φ is the sum of the diversity of settlers emigrating from a reef weighted by the total number of

settlers emigrating from all reefs, and the diversity of immigrating settlers to a reef weighted by the total number of immigrating settlers within the metapopulation.

To compare the realized connectivities of the study system with a hypothetical optimally (from a diversity of flow standpoint) connected network of reefs, the topological connectance per node, m^*, was calculated as per Ulanowicz and Wolff (1991). The topological connectance per node, m^*, represents the "connectivity potential" of a system of reserves in that m^* is the potential topological value of connectance for the metapopulation, constrained by the connectivity linkages described by the empirical data (see Figure 6.2). Intuitively the topological connectance per node represents the value that m would take on if all the nonzero values in the connectivity matrix are assumed to be equal in magnitude. Therefore the effective connectance per node (m) will always be smaller (or possibly equal) to the topological connectance per node (m^*; Bersier et al. 2002); flows between reefs in a network are rarely (if ever) uniform (Ulanowicz and Wolff 1991).In the same manner, the maximum topological connectance per node, m^{max} will be calculated for a hypothetical network of six reefs. Qualitatively, m^{max} is the value that m would have if all reefs in the study system were connected by larval dispersal, and the magnitude of larval fish being imported from and exported to each reef was equivalent; it represents the greatest connectivity value that m could achieve in a network of reefs of the same order (i.e. having the same number of reefs). Comparing the empirically-derived value of m with m^* and m^{max} yields an easily understandable ratio describing the relative extent of connectivity within the system of interest.

Subsequently node deletion experiments were run to test the "value" of individual reefs to the connectivity of the metapopulation as a whole (following Urban and Keitt 2001 and Treml et al. 2008). In this process, individual nodes (reefs) were systematically removed from the system and the value of the three metrics describing metapopulation connectivity, namely *LD*, *DC*, and *m* of the reconfigured metapopulation was recalculated. The relative change in these metrics for the altered metapopulations was then assessed to rank the relative importance of individual reefs to the connectivity of the metapopulation. Reefs whose removal results in a greater decrease in these metrics, would from a connectivity standpoint, receive a higher priority ranking for conservation.

Results

Qualitative and quantitative metrics of population connectivity derived from the empirically-derived rocky reef fish connectivity matrices reveal which reefs behaved as sources, sinks, or intermediate source-sinks within the study system (Appendix 6.2, Table 6.1). In each year of the study there was one reef that was not connected to the metapopulation, Zuniga Point in 2008 and Torrey Pines in 2009. However, these reefs were connected in the alternate year of the study.

Source and Sink Populations

In 2008 4 reefs that behaved as source-sinks, two of which, Carlsbad and Cardiff, were "pure" sinks. The in-degree of the sink reefs ranged from 1 – 3, indicating they received YOY from 1-3 reefs. In 2008 3 reefs behaved as source populations, their out-degree ranged from 2-4. Only one of these three reefs, Torrey Pines, was a "pure" source ($C_k = 1$).

In 2009, the number of sinks, and the range of in-degree of individual reefs did not change, however the composition of reefs acting as sinks did. Zuniga Point, which was not connected to the other nodes in this network in 2008, acted as an intermediate source-sink in 2009, while Carlsbad was a "pure" sink population ($C_k = 0$). The number of sources increased to four; Mission Point was only one "pure" source population, producing 29% of the YOY that successfully settled in 2009. When connectivity is considered across both years of the study, only Carlsbad was consistently a "pure" sink population, suggesting that during this period it is a net importer within the study system. Torrey Pines appeared as a "pure" source across both years, although this reef was "unconnected" from the system in 2009. The remaining four reefs within the metapopulation behaved as intermediate source-sink populations ($0 < C_k < 1$) when viewed across 2008-2009; the study reef (i.e. node) with the highest degree (i.e. the sum of qualitative reef connectance values) was La Jolla (6 in 2008 and 2009, 8 across both years of the study; Table 6.1).

Comparing qualitative vs. quantitative descriptors of connectivity

There were no differences in ranked qualitative or quantitative connectivity function when reefs were deemed "pure" sources or sinks (Table 6.1), but for intermediate source-sink locations the weighted metrics had consistently lower values than the unweighted metrics and provide a more accurate description of the extent to which a reef is a source and/or a sink population. The higher values observed in weighted (vs. unweighted) metrics reflect the fact that within individual years most intermediate source-sink populations exported more YOY than they imported (i.e. they were more similar to "pure" source populations than the unweighted metrics would suggest). This was not true for Cardiff in 2008-2009, when ~ 85% of YOY were imported into Cardiff, while only 15% of YOY were exported by this reef. This caused the bi-annual weighted value of C_k to decrease, suggesting that Cardiff was more of a sink than a source population.

At the individual reef level, qualitative and quantitative estimates of functional connectivity (C_k and QC_k) had strong accordance (all pairwise correlations between weighted and unweighted metrics ranged between 0.963 and 0.998, all $p < 0.0001$). At the metapopulation-scale, qualitative connectivity metrics (i.e. *LD* and *DC*) suggest greater levels of connectivity within the metapopulation than quantitative metrics. For example in 2008, when effective connectance per node (m) is compared against potential topological connectance per node (m^*) it appears as though ~76% (2.1/2.75) of the potential connectance is reached. However when the comparable qualitative metrics are used to assess the extent of connectivity, it appears as though both *LD* and *DC* are equivalent to the values measured in an optimally connected metapopulation,

incorrectly suggesting that the empirical system has reached 100% of its connectivity potential.

Metapopulation Connectivity

Qualitative link density (*LD*) of the metapopulation in 2008 was 3.0 meaning that each reef, on average, imported YOY from three reefs and exported YOY to three reefs. This value decreased to 2.7 in 2009, but when data are viewed across both years of the study, this value climbs to 4.0 (2/3 of m^{max}). As can been seen, the values of *DC* are not independent from *LD*, as an alternative method for calculating *DC* is *LD/S*, where S is the total number of reefs comprising the metapopulation; numerically *DC* provides the proportion of m^{max} achieved by the metapopulation. The quantitative metric of metapopulation connectivity, effective connectance per node (*m*), ranged between 2.10 and 2.26 within years, and climbed to 2.32 when summed over both years of the study. These values, incorporating the imbalance of YOY dispersing to and from individual reefs, may provide a more accurate depiction of metapopulation connectivity than *LD* or *DC*. As a proportion of potential connectivity, *m*, m* ranged from 76% in 2008 to 99% of *m** in 2009. When *m* was averaged over 2008-2009, empirical metapopulation connectivity achieved 82% of potential connectivity, and comparing *m* against m^{max} suggests empirical connectivity between 2008 and 2009 achieved between 35% and 39% of maximum connectivity possible for the study system (Figure 6.2 A-C).

Node Deletion Experiments

Systematic deletion of individual reefs within the study system provided insight into the connectivity "value" of individual reefs within the study system (Table 6.2). When reefs are ranked, qualitative metrics of connectivity (i.e. *LD* and *DC*) agree on the relative connectivity "value" of individual reefs within the study system, but when overall % change is used to assess the value of individual reefs to the study system, the ranking shifts slightly enabling a more precise ranking of individual reef value to network connectivity (Table 6.3). In all years, and across all metrics, the most important reef (from a connectivity standpoint) was La Jolla. When this single reef was deleted from the network of reefs there was a mean change in metapopulation *LD* across all years of the study of -50.7 %, a decrease of *DC* by 41.4%, and a decrease in *m* of 32.1%. From a conservation standpoint, La Jolla would be a top priority for ensuring system connectivity.

Averaging among qualitative and quantitative metrics of connectivity, the second and third most influential reefs in maintaining a diversity of YOY flowing among reefs within the metapopulation are Carlsbad and Cardiff. Depending on whether overall rank or overall % Δ is used to determine primacy, these two reefs alternate in importance. The two reefs least important to metapopulation connectivity during the study were Torrey Pines and Zuniga Point. Overall rank provided by the metapopulation connectivity metrics suggest that Torrey Pines and Zuniga Point are of equal importance, but overall % change suggests Zuniga Point should be ranked slightly above Torrey Pines as a conservation priority (Table 6.3).

Discussion

The connectivity metrics presented here were developed to create a quantitative framework for measuring connectivity measured within a system of marine reserves. Until recently empirical estimates of population connectivity in marine systems have been lacking, relegating prediction of population connectivity and generation of hypotheses to simulation modeling of passively dispersing particles and inference from biophysical models (Treml et al 2008, Cowen and Sponaugle 2009). While these studies have provided a framework with which one can generate testable hypotheses of the connectedness of marine populations and the ramifications of these patterns to population structure, empirical data necessary to validate these predictions has not been forthcoming. Recent technological and methodological advances have been made in tracking the dispersal of marine larvae, enabling first order approximations of connectivity in marine metapopulations (e.g. Becker et al. 2007, Planes et al 2009, Carson et al. 2010, Chapter 4 - this dissertation). These empirical data can now be used to begin the process of model validation, ultimately improving our predictive ability and understanding of the role dispersal and connectivity play in maintaining populations.

The terms source and sink are frequently used to describe populations, and this has been used along with demographic data to describe the growth rates of populations as influenced by immigration and emigration of individuals (Pulliam 1988, Crowder et al. 2000). This study used these same terms to describe the ecological role of individual reefs within a well-defined metapopulation by building upon a foundation

created by those quantifying connectedness in weighted food webs (e.g. Bersier et al. 2002, Dunne et al. 2002), by providing simple mathematical definitions to connectivity terms, we can make reef valuation less arbitrary. The tools of graph theory readily define sources and sinks within metapopulations by assessing the degree of each subpopulation, and assessing the imbalance between the number of sources a reef delivers individuals to and the number of sources a reef receives individuals from. The imbalance of these flows, weighted by the magnitude of individuals moving along the arcs connecting each reef in the metapopulation can then be used to place a numerical value on individual reefs, and these can in turn be used to calculate overall metapopulation-level metrics of connectivity.

Based on the empirical data generated using *H. rubicundus* as a model it would appear that certain reefs (e.g. Torrey Pines) act as "pure" source populations, others act as "pure" sink populations (e.g. Carlsbad) and still others have balanced flows of larvae, behaving like intermediate source-sinks (e.g. La Jolla). From a descriptive level, and over this two-year period it would appear that this system of MPAs could be classified as a source-sink metapopulation (*sensu* Harrison 1991). Whether this system actually functions as a source-sink metapopulation or as an open population is probably more a matter of perspective and scale than empirical data would suggest (see Chapter 4 - this dissertation). If this study were continued indefinitely and the data set temporally expanded, one may eventually capture inter-generational (i.e. decadal-scale) connectivity, providing insights into whether or not the system ultimately functions as a source-sink or open population. The magnitude of

connectivity seen within the two years of this study would suggest that all populations sited within the region are connected to some degree, and at a time scale relevant to decisions being made by most resource managers (i.e. 1-5 years). Therefore, the question of what type of metapopulation best describes a network of reefs should not be framed by the data, but rather by the goal of the end-user. Over ecological time-scales this system functions as a source-sink but over decades (i.e. multiple generations) encompassing large-scale sources of climate variability (e.g. El Niño) functions most probably as an open population (Chapter 4 – this dissertation).

The unweighted vs. weighted metrics describing the functional value of an individual reefs in the study system (i.e. "pure" source, "pure" sink or intermediate source-sink) did not differ substantially. The weighted values did however provide a more accurate depiction of the balance of flows among the individual reefs. In addition, the metapopulation-scale metrics of connectivity were weighted by the flow of individuals not just immigrating and emigrating from individual reefs, but by all individuals dispersing throughout the system allowing assessment of reef contribution and source-sink role within the greater metapopulation. Use of the reef-level and metapopulation-level metrics of connectivity in concert generates a more comprehensive understanding of the functional role of individual reefs and the importance of connectivity to the metapopulation as a whole.

The qualitative metrics developed in food web studies (e.g. *LD* and *DC*), describe the basic structural properties of the metapopulation in question, but they do not enable more rigorous assessments to be made regarding the connectedness of

systems, and do not lend themselves well to the evaluation of the functional value of individual reefs. Recent efforts to generate network-based metrics of connectivity in terrestrial systems (e.g. betweenness centrality, Bodin and Saura 2010) are not as applicable to marine systems at the meso-scale (i.e. 10s of km) as pelagic larvae may disperse to any reef within the metapopulation within a single time step, rendering distance-based metrics such as path length (at least on ecological time-scales) irrelevant, at least at the spatial scales (i.e. < 100 km) and the life histories addressed in this study. One of the advantages of the more quantitative metrics defined here is that they can be used to test and evaluate the value of individual reefs to the connectivity of a system as a whole over management-relevant time scales. By viewing these values as proportions of potential connectivity and maximum topological connectance within the study system, they provide insights into the current extent of metapopulation connectedness. These data can then be used to value and prioritize individual reefs for conservation, and in so doing the system can be managed in such a way as to achieve desired connectivity, whether that goal is to minimize connectivity (e.g. to reduce the risk of invasion by invasive species) or to maximize connectivity (e.g. to ensure a well-mixed genetic pool or population replenishment).

In the case of marine protected area (MPA) networks, the aim of reserve creation is typically to increase the probability of regional persistence of populations and ecosystems by having a series of reserves that are linked via dispersal. These conservation goals are, in an ideal network, balanced with those of extractive users by ensuring the reserve network provides some benefit to adjacent regions. In this study

region a suite of MPAs are being created to meet a number of ecological criteria, while balancing the desires of consumptive and non-consumptive stakeholders living and working within the region. Two of the focal study reefs (La Jolla and Cardiff) are currently designated as MPAs, and a third, Zuniga Point is adjacent to a third MPA. Managers could use the quantitative metrics developed here to place a value on individual reefs in an effort to quantitatively compare alternative MPA scenarios. The qualitative metrics of metapopulation connectivity pooled over 2008 and 2009 would suggest efforts should be made to protect Torrey Pines (a "pure" source), and to remove or lessen restrictions in regions such as Carlsbad (a "pure" sink) within the region (Table 6.1). However when effective connectance per node is used in conjunction with *post hoc* node deletion analyses to quantify metapopulation connectivity, it becomes apparent that the reefs most important to maintaining connectivity within the region are La Jolla, Carlsbad, and Mission Point. Conversely, and counter to the result suggested by the qualitative metrics of connectivity, Torrey Pines is one of the reefs least valuable to the maintenance of connectivity within the system, and its loss would result in a minor decrease in overall network connectivity.

While terrestrial studies have made advances in understanding metapopulation dynamics (e.g. Hanski 1998 and references therein), parallel knowledge has remained elusive in marine systems, in large part due to the logistical challenges associated with tracking virtually transparent mm-scale larvae through a constantly shifting fluid environment. By utilizing empirically-derived estimates of annual connectivity patterns of dispersing larvae within a marine metapopulation this study has allowed us

to move from coarse descriptors of connectivity, to more quantitative metrics describing the connectivity of populations. These metrics should provide a framework that can be used to make relative comparisons of the "value" of individual reefs to overall metapopulation connectivity, ultimately bettering our understanding of connectivity and its function in marine metapopulations.

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Table 6.1 Reef-specific and metapopulation level qualitative and quantitative metrics of connectivity.

		Qualitative Connectivity Metrics						Quantitative Connectivity Metrics						
		Number of YOY		Reef Connectance		Qualitative Functional Connectivity		Diversity of		Effective Number		Functional Connectivity		Metapopulation Effective Connectance Per Node
Location	Year	Import	Export	Sink for	Source for	unweighted C_k	weighted C_k	Reefs Importing YOY From	Reefs Exporting YOY To	Importing $n_{I,k}$	Exporting $n_{E,k}$	unweighted QC_k	weighted QC_k	(m, m*, or m^{max})
Carlsbad	2008	18	0	3	0	0.00	0.00	0.80	0.00	1.74	0.00	0.00	0.00	2.10
Cardiff	2008	10	0	2	0	0.00	0.00	0.72	0.00	1.65	0.00	0.00	0.00	
Torrey Pines	2008	0	4	0	2	1.00	1.00	0.00	0.81	0.00	1.75	1.00	1.00	
La Jolla	2008	43	59	3	3	0.50	0.58	0.80	1.33	1.74	2.51	0.59	0.66	
Mission Point	2008	1	9	1	4	0.80	0.97	0.00	1.84	1.00	3.57	1.00	1.00	
Zuniga Point	2008	0	0	0	0	undef.	undef.	undef.	undef.	undef.	undef.	undef.	undef.	
Carlsbad	2009	9	0	3	0	0.00	0.00	1.35	0.00	2.55	0.00	0.00	0.00	2.26
Cardiff	2009	1	2	1	1	0.50	0.67	0.00	0.00	1.00	1.00	0.50	0.67	
Torrey Pines	2009	0	0	0	0	undef.	undef.	undef.	undef.	undef.	undef.	undef.	undef.	
La Jolla	2009	6	9	3	3	0.50	0.60	1.46	1.35	2.75	2.55	0.48	0.58	
Mission Point	2009	0	5	0	3	1.00	1.00	0.00	1.37	0.00	2.59	1.00	1.00	
Zuniga Point	2009	1	1	1	1	0.50	0.50	0.00	0.00	1.00	1.00	0.50	0.50	
Carlsbad	2008-2009	27	0	4	0	0.00	0.00	1.12	0.00	2.18	0.00	0.00	0.00	2.32
Cardiff	2008-2009	11	2	2	1	0.33	0.08	0.85	0.00	1.80	0.00	0.00	0.00	
Torrey Pines	2008-2009	0	4	0	2	1.00	1.00	0.00	0.81	0.00	1.75	1.00	1.00	
La Jolla	2008-2009	49	68	4	4	0.50	0.58	1.03	1.43	2.05	2.70	0.57	0.65	
Mission Point	2008-2009	1	14	1	4	0.80	0.98	0.00	1.81	0.00	3.50	1.00	1.00	
Zuniga Point	2008-2009	1	1	1	1	0.50	0.50	0.00	0.00	1.00	1.00	0.50	0.50	

Table 6.1 Continued

		Qualitative Connectivity Metrics						Quantitative Connectivity Metrics						
		Number of YOY		Reef Connectance		Qualitative Functional Connectivity		Diversity of		Effective Number		Functional Connectivity		Metapopulation Effective Connectance Per Node (m, m*, or m^{max})
Location	Year	Import	Export	Sink for	Source for	unweighted C_k	weighted C_k	Reefs Importing YOY From	Reefs Exporting YOY To	Importing $n_{I,k}$	Exporting $n_{E,k}$	unweighted QC_k	weighted QC_k	
Optimal Topological Connectance Per Node (m*)														
Carlsbad	2008	24	0	3	0	0.00	0.00	1.58	0.00	3.00	0.00	0.00	0.00	2.75
Cardiff	2008	16	0	2	0	0.00	0.00	1.00	0.00	2.00	0.00	0.00	0.00	
Torrey Pines	2008	0	16	0	2	1.00	1.00	0.00	1.00	0.00	2.00	1.00	1.00	
La Jolla	2008	24	24	3	3	0.50	0.50	1.58	1.58	3.00	3.00	0.50	0.50	
Mission Point	2008	8	32	1	4	0.80	0.94	0.00	2.00	0.00	4.00	1.00	1.00	
Zuniga Point	2008	0	0	0	0	undef.	undef.	undef.	undef.	undef.	undef.	undef.	undef.	
Carlsbad	2009	6.38	0	3	0	0.00	0.00	1.58	0.00	3.00	0.00	0.00	0.00	2.28
Cardiff	2009	2.13	0.33	1	1	0.50	0.13	0.00	0.00	0.00	0.00	undef.	undef.	
Torrey Pines	2009	0	0	0	0	undef.	undef.	0.00	0.00	0.00	0.00	undef.	undef.	
La Jolla	2009	6.38	1.67	3	3	0.50	0.21	1.58	1.58	3.00	3.00	0.50	0.50	
Mission Point	2009	0	1.67	0	3	1.00	1.00	0.00	1.58	0.00	3.00	1.00	1.00	
Zuniga Point	2009	2.13	0.33	1	1	0.50	0.13	0.00	0.00	0.00	0.00	undef.	undef.	
Carlsbad	2008-2009	29.67	0	4	0	0.00	0.00	2.00	0.00	4.00	0.00	0.00	0.00	2.83
Cardiff	2008-2009	14.83	7.42	2	1	0.33	0.20	1.00	0.00	2.00	0.00	0.00	0.00	
Torrey Pines	2008-2009	0	14.83	0	2	1.00	1.00	0.00	1.00	0.00	2.00	1.00	1.00	
La Jolla	2008-2009	29.67	29.67	4	4	0.50	0.50	2.00	2.00	4.00	4.00	0.50	0.50	
Mission Point	2008-2009	7.42	29.67	1	4	0.80	0.94	0.00	2.00	0.00	4.00	1.00	1.00	
Zuniga Point	2008-2009	7.42	7.42	1	1	0.50	0.50	0.00	0.00	0.00	1.00	1.00	1.00	
Maximum Topological Connectance Per Node (m^{max})*														
Each reef	2008-2009	14.83	14.83	6	6	0.50	0.50	2.58	2.58	6.00	6.00	0.50	0.50	6.00

** m^{max} is only provided for one sample reef for 2008-2009 as all connectivity metrics (other than the number of YOY imported and exported by a location) are equivalent when examining a hypothetical system with maximum connectivity.*

Table 6.2 Results of node removal analysis on metapopulation-level connectivity metrics. Link Density (*LD*), Directed Connectance (*DC*), and Effective Connectance per Node (*m*).

	Qualitative Connectivity Metrics						Quantitative Connectivity Metric		
	Metapopulation Connectance						Metapopulation Effective Connectance Per Node		
Node Removed	***LD***	*LD* % Δ	Rank % Δ	***DC***	*DC* % Δ	Rank % Δ	***m***	% Δ	Rank % Δ
Actual 2008	3.0	-	-	0.50	-	-	2.1	-	-
Carlsbad	2.4	-20.0	3	0.48	-4.0	3	1.69	-19.5	2
Cardiff	2.8	-6.7	4	0.56	12.0	4	1.81	-13.8	3
Torrey Pines	2.8	-6.7	4	0.56	12.0	4	1.97	-6.2	5
La Jolla	1.6	-46.7	1	0.32	-36.0	1	1.15	-45.2	1
Mission Point	2.0	-33.3	2	0.40	-20.0	2	1.83	-12.9	4
Zuniga Point	3.6	20.0	6	0.72	44.0	6	2.1	0	6
Actual 2009	2.7	-	-	0.44	-	-	2.26	-	-
Carlsbad	2.0	-25.9	2	0.40	-9.1	2	1.83	-19.0	2
Cardiff	2.4	-11.1	4	0.48	9.1	4	2.14	-5.3	5
Torrey Pines	3.2	18.5	6	0.64	45.5	6	2.26	0.0	6
La Jolla	1.2	-55.6	1	0.24	-45.5	1	1.57	-30.5	1
Mission Point	2.0	-25.9	2	0.40	-9.1	2	1.83	-19.0	2
Zuniga Point	2.4	-11.1	4	0.48	9.1	4	2.04	-9.7	4
Actual 2008-2009	4.0	-	-	0.67	-	-	2.3	-	-
Carlsbad	3.2	-20.0	2	0.64	-8.6	2	1.91	-17.0	2
Cardiff	3.6	-10.0	4	0.72	2.9	4	2.00	-13.0	4
Torrey Pines	4.0	0.0	5	0.8	14.3	5	2.19	-4.8	5
La Jolla	2.0	-50.0	1	0.4	-42.9	1	1.83	-20.4	1
Mission Point	3.2	-20.0	2	0.64	-8.6	2	1.99	-13.5	3
Zuniga Point	4.0	0.0	5	0.8	14.3	5	2.25	-2.2	6

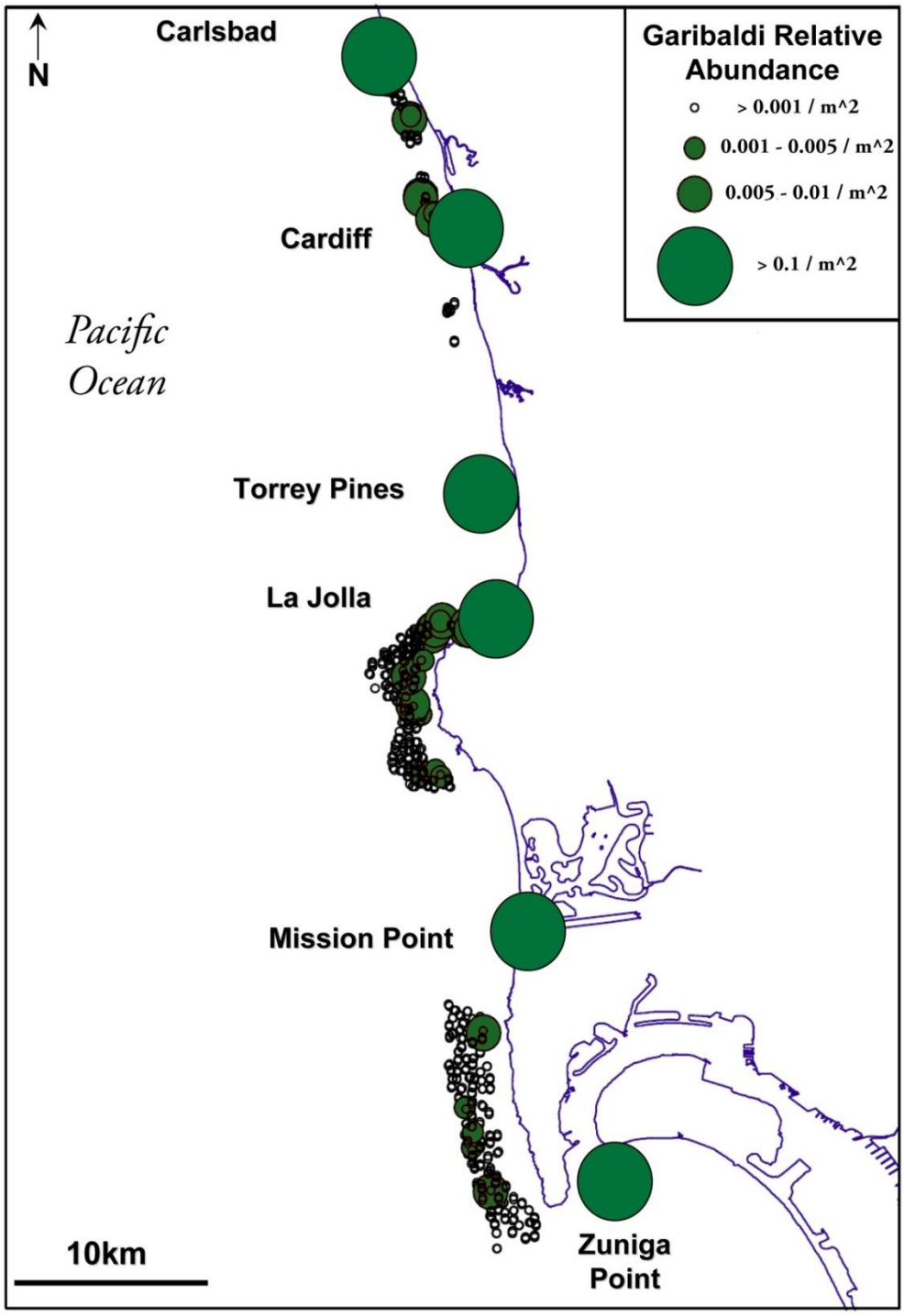
Table 6.3 Relative importance of individual study reefs based on % change in qualitative and quantitative metrics of metapopulation connectivity during node removal analysis. Overall rank represents the mean rank given across all three metapopulation connectivity metrics.

Reef	Mean Rank % Δ (LD)	Mean % Δ	(± 1 se)	Mean Rank % Δ (DC)	Mean % Δ	(± 1 se)	Mean Rank % Δ (*m*)	Mean % Δ	(± 1 se)	Overall Rank
Carlsbad	2.3	-22.0	2.0	2.3	-7.2	1.6	2.0	-18.5	0.8	2.2
Cardiff	4.0	-9.3	1.3	4.0	8.0	2.7	4.0	-10.7	2.7	4.0
Torrey Pines	5.0	4.0	7.5	5.0	23.9	10.8	5.3	-3.7	1.9	5.1
La Jolla	1.0	-50.7	2.6	1.0	-41.4	2.8	1.0	-32.1	7.2	1.0
Mission Point	2.0	-26.4	3.9	2.0	-12.6	3.7	3.0	-15.1	2.0	2.3
Zuniga Point	5.0	3.0	9.1	5.0	22.5	10.9	5.3	-4.0	3.0	5.1

Figure Legends

Figure 6.1 Map of study area with relative abundance of model species. Names of primary study reefs are provided, Cardiff and La Jolla are currently designated MPAs and Zuniga Point is adjacent to the Mia J Tegner MPA. Center of study region is located at approximately 32° 51'59.9" N, 117° 15'30.1" W. Figure adapted from Cook 2011.

Figure 6.2 Figure 2A, Aggregate 2008-2009 metapopulation effective connectance per node (m), Figure 2B, Potential topological connectance per node (m*), and Figure 2C, maximum topological connectance per node (m^{max}). Nodes represent study reefs, and values along arcs represent the proportion of all larval fish dispersing among study reefs comprising the metapopulation.



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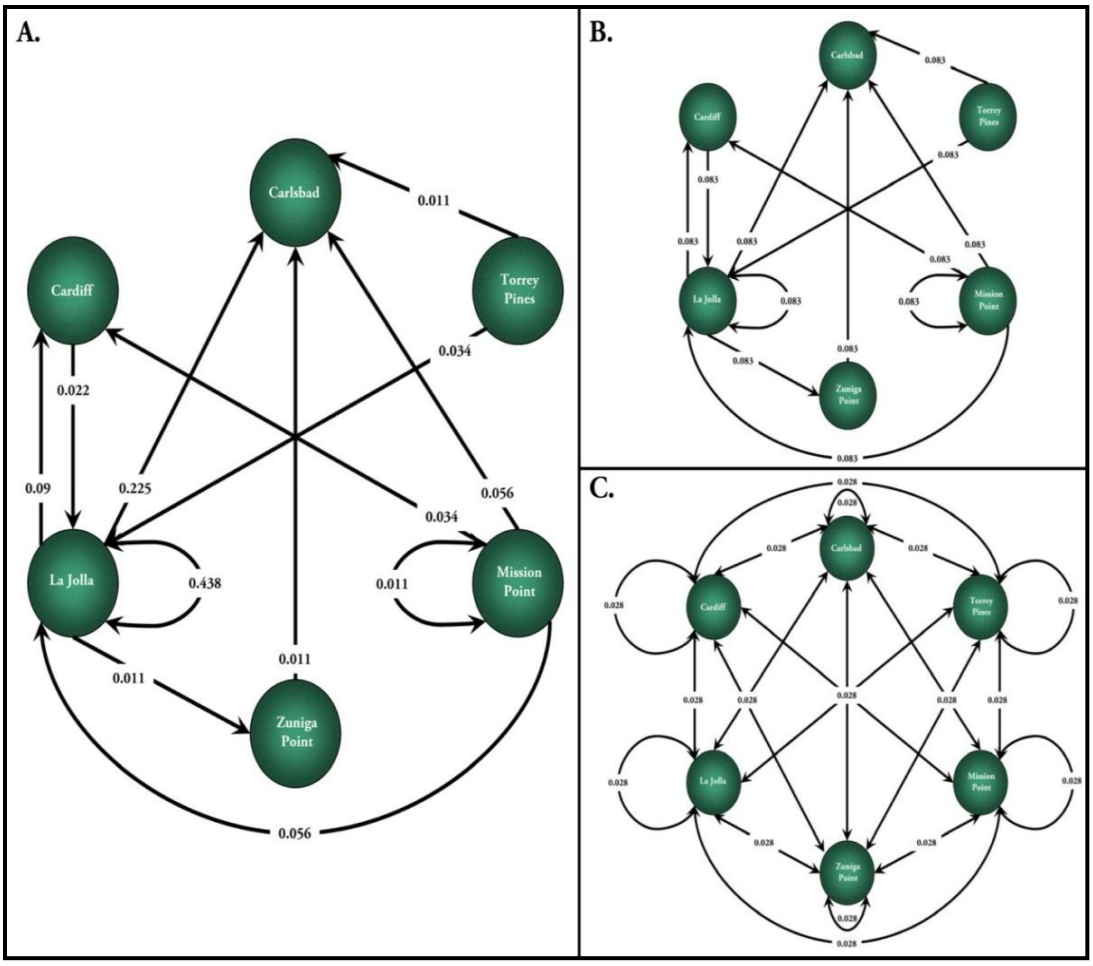

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Appendix 6.1 Description of Methods for Empirically Estimating Population Connectivity

Empirically Estimating Population Connectivity

Trace elemental fingerprinting of otoliths (see above) was used to estimate the extent to which the six populations comprising this metapopulation were connected by larval dispersal. For an in-depth description of otolith processing and analytical methodology see Cook 2011. Briefly, embryonic fish were collected from benthic nests biweekly for the duration of the 2008 and 2009 spawning seasons, typically from early June until mid-August. The otoliths of larval fish were extracted, processed, and analyzed using a LA-ICPMS to create biweekly chemical reference maps of the study region (see below). Toward the end of the spawning season (early-September – early-November) post-dispersal stage young-of-the-year (YOY) fish were collected at reefs. The otoliths of these fish were extracted and using algorithms, their natal origin was predicted (see statistical analyses below). Subsequently, these empirically-derived estimates of dispersal were used to create connectivity matrices describing the number and proportion of individuals dispersing among the six reefs within the study region.

Otolith Microchemistry - Statistical Analyses

Statistical methodology for analysis of otolith trace elemental chemistry followed Cook 2011. Briefly, a suite of six trace elements was selected for analysis using the LA-ICMPS: ^{24}Mg, ^{55}Mn, ^{87}Sr, ^{138}Ba, ^{208}Pb, and ^{238}U. All otoliths were analyzed with a New Wave UP 213 nm laser ablation unit coupled to a Thermoquest Finnegan Element 2 Inductively Coupled Plasma Mass Spectrometer housed at the UC

Santa Barbara MSI Analytical Lab. Otoliths collected in 2008 were analyzed between April 16 and April 30 2009, and samples collected in 2009 were analyzed between August 23 and September 10 2010. Over both years of this study the trace elemental chemistry of 1784 larval fish otoliths and 89 YOY otoliths was analyzed. Of these, 1662 larval fish otoliths and all YOY otoliths were included in subsequent statistical analyses (i.e. 122 larval fish otoliths were removed from analyses because they did not surpass instrument detection limits or were deemed statistical outliers).

Linear discriminant function analysis (DFA) was used to create chemical reference maps of the study region. In this process, the trace elemental "concentration" (actually a relative concentration of a given element to ^{48}Ca) of larval fish otoliths collected at the six primary study reefs was used to create biweekly chemical reference maps of the study region using DFA. The classification success generated using these data was compared against 1000 randomized data sets to generate a null expectation of classification success (as per White and Ruttenberg 2007). Then using the otolith core elemental fingerprints (i.e. the natal portion) of the post-dispersal YOY the natal origin of individuals was predicted (for a detailed description of this process see Cook 2011 and Chapter 4 – this dissertation). All DFAs and randomization procedures were completed in Matlab Version 7.4 adapting code provided by White and Ruttenberg 2007.

Appendix 6.2 Connectivity matrices for H. rubicundus in San Diego County, USA in 2008, 2009, and 2008-2009. Rows are predicted natal origin, and columns are capture locations of post-dispersal YOY. Values indicate the number of YOY.

2008 Population Connectivity Matrix

	Settlement Reefs					
Natal Origin	Carlsbad	Cardiff	Torrey Pines	La Jolla	Mission Point	Zuniga Point
Carlsbad	0	0	0	0	0	0
Cardiff	0	0	0	0	0	0
Torrey Pines	1	0	0	3	0	0
La Jolla	15	8	0	36	0	0
Mission Point	2	2	0	4	1	0
Zuniga Point	0	0	0	0	0	0

2009 Population Connectivity Matrix

	Settlement Reefs					
Natal Origin	Carlsbad	Cardiff	Torrey Pines	La Jolla	Mission Point	Zuniga Point
Carlsbad	0	0	0	0	0	0
Cardiff	0	0	0	2	0	0
Torrey Pines	0	0	0	0	0	0
La Jolla	5	0	0	3	0	1
Mission Point	3	1	0	1	0	0
Zuniga Point	1	0	0	0	0	0

Interrannual (2008-2009) Population Connectivity Matrix

	Settlement Reefs					
Natal Origin	Carlsbad	Cardiff	Torrey Pines	La Jolla	Mission Point	Zuniga Point
Carlsbad	0	0	0	0	0	0
Cardiff	0	0	0	2	0	0
Torrey Pines	1	0	0	3	0	0
La Jolla	20	8	0	39	0	1
Mission Point	5	3	0	5	1	0
Zuniga Point	1	0	0	0	0	0

CHAPTER VII.

CONCLUSIONS

The original intent of this dissertation was to develop and apply empirical methods for quantifying the connectivity (via larval dispersal) of nearshore rocky reef fish populations. Many of the questions laid out at the onset of this project were able to be answered, in one manner or another. But as is often the case, not all of the methods initially proposed for answering these scientific questions were successful. The ontogeny of this dissertation was in many regards sequential, where more advanced steps necessary for advancing our understanding of connectivity had to be preceded first by the careful collection of empirical data describing the natural history of the organism of interest, under the water with a pencil and slate in hand (Chapter 2).

In building this natural history foundation (Chapter 2) I was grateful to stand on the shoulders of those before me who had completed the preliminary descriptions of my chosen model species (Limbaugh 1964; Clarke 1970; Sikkel 1988, 1989). It was their hard work that enabled me to verify and test previously generated hypotheses, to imagine a feasible project at the onset, and attempt to answer the questions they generously left unanswered. Incrementally building upon their bodies of work I was able to increase our understanding of the distribution and abundance of garibaldi, to test hypotheses about the role of territorial adults and habitat complexity, and their synergistic interaction that may possibly influence the survivorship of post-settlement individuals (Chapter 2).

By spending substantial amounts of time underwater I was able to gain a more thorough understanding of my chosen study system, and through repeated observations develop hypotheses of my own. While observing the high mortality of young-of-the-year (YOY) fish I began to contemplate the various factors that may influence their post-settlement survival. Two possible thoughts entered my mind: 1) small scale physical refugia provided shelter from predators, and 2) the territorial behavior of adult conspecifics may create biological refugia from predation. In testing these hypotheses, data suggested small-scale physical refugia increased YOY survivorship; however supporting evidence for the positive influence of territorial behavior was tenuous (Chapter 2). Further experimental studies on the factors influencing YOY survivorship are warranted as they may shed light on methods of altering habitat during restoration efforts to maximize survivorship of post-dispersal individuals.

In addition to testing hypotheses about the various factors influencing YOY survivorship, I was able to assess the role of protected status in population recovery. Over 30 years after the unofficial protection of garibaldi, adult densities have more than doubled (Chapter 2). While the causative role of its designation as a protected species in increasing population density remains uncertain, these optimistic results suggest that traditional management techniques can positively influence the persistence of populations, and should be used in conjunction with methods predicated upon the connectedness of populations (e.g. MPAs).

Previous connectivity studies have been hampered by an inability to identify possible source populations, and as such it was difficult to say with certainty if they had identified all of the possible source populations contributing to their larval pool. However, and thanks to the earlier work of Ed Parnell, between 2002 and 2007 over 500 surveys were conducted within the study region. By coupling these data with the 200 surveys conducted as part of this dissertation, the major garibaldi populations in San Diego County were identified (Chapter 2).

Once the possible source populations were identified, it was necessary to develop a method for quantifying the movement of larval fish dispersing through the study region. To do this again required that I build upon the foundation laid by those before me (e.g. Becker et al. 2005, Fodrie and Levin 2008). The method chosen to track dispersing larvae was trace elemental fingerprinting, but for this method to be used first it had to be tested and proven. Building upon the methods of Becker et al. (2005, 2007) and Fodrie and Levin (2008) I was able to develop a suite of elements that could be used to distinguish among the study reefs spatially, and by sampling across a protracted spawning season extend our knowledge of the intra-annual temporal variability inherent in otolith microchemistry (Chapter 3). Initial analyses suggested that the method could be used to successfully distinguish among study reefs, however, further scrutiny showed a flaw in current methodology. For species that spawn over protracted periods of time, it was shown that the elemental fingerprint generated for a particular reef changes over time, and in each year of the study over a third of the time an elemental fingerprint of one reef was indistinguishable from the

elemental fingerprint of a different reef later in the spawning season (Chapter 3). These changes in elemental chemistry confound the ability to accurately determine the natal origin of individual fish, result in underestimates of the number of source populations, and ultimately call into question predicted patterns of connectivity and the function of individual reefs within the study region (Chapter 3). For the growing number of researchers using trace elemental fingerprinting to assess population connectivity, these challenges can be overcome by coupling a thorough understanding of the natural history of the species in question (e.g. length of spawning season) and high resolution sampling capable of capturing variability in elemental fingerprints.

When properly utilized trace elemental fingerprinting data can be used for quantifying the magnitude of larval dispersal within the study region, and the variability of these dispersal patterns within and among years (Chapter 4). High resolution sampling increased the accuracy of assignment of individual reefs by more than 30% (Chapter 4), increasing the confidence of predictions in natal origin. Three of the MPAs located within the study region are connected by larval dispersal, but this connectivity was only documented when data were viewed over a two year period, suggesting short-term studies may fail to capture the extent of connectivity within a region of interest (Chapter 4). Over the course of this study self-recruitment was documented at two of the study reefs, one of which, La Jolla, is a San Diego County MPA, and over both years of the study self-recruitment accounted for 50-84% of recruits within this MPA Directionality of realized dispersal trajectories while being variable on biweekly scales over the summers of 2008 and 2009, were predominantly

in a northerly direction on annual time-scales; these findings are supported by concurrently collected ADCP data (Chapter 4). This suggests larval inputs from regions to the south of the study region (i.e. Mexico) may influence regional population dynamics over longer time scales, and speak to the value of international collaboration.

These connectivity data in turn were necessary to identify the sources and sinks of larval fish dispersing within the study region (Chapters 5 and 6). When coupled with demographic transect survey data generated as part of the exploratory diving done earlier in this thesis (Chapter 2), I was able to assess the demographic consequences of connectivity to garibaldi population and metapopulation growth (Chapter 5). Simply stated, the purpose of this work was to answer the question, from a demographic (or population growth) standpoint does connectivity matter? One realization coming from this portion of the study was that in order to accurately answer this question more study is needed. Due to the relatively long lifespan of garibaldi, and the time it takes for an individual to become sexually mature (i.e. ~5 years), the demographic consequences of the realized connectivity patterns documented over 2008 and 2009 will not be truly evident for another three to four years. This suggests that if we want to improve our understanding of the demographic consequences of connectivity, long-term monitoring of long-lived species (i.e. relative to the time it takes to reach sexual maturity) will be required. An alternative method of increasing our understanding would be to focus future connectivity-demography

studies on species with relatively short lifespans; this would enable the demographic consequences of connectivity to be measured over the lifetime of most research grants.

When assumptions are made about the realized connectivity patterns documented in this study (i.e. that they represent typical connectivity values), demographic stage-based matric models and subsequent elasticity analyses can be used to assess the relative contribution of connectivity to population and metapopulation growth rates. Briefly, connectivity matters to population growth rate. But the manner in which connectivity matters to metapopulation growth rates is more complex than initially thought. At the onset of this study, I hypothesized that higher levels of connectivity would results in higher population growth rates, and at the reef level this finding appears to be supported (Chapter 5). At each study reef when connectivity was removed, the population growth rate decreased (Chapter 5). However connectivity is never the single greatest factor contributing to local and metapopulation growth rate; at empirical levels of connectivity measured in this study (Chapter 4), the primary contributor to population and metapopulation growth rate is adult survivorship. Connectivity is as important a contributor to growth rate as any of the other vital rates that were measured as part of this study (Chapter 5), but it is never the greatest contributor.

Using simulation modeling to explore the role of connectivity at the metapopulation level did uncover some unexpected results. Again, while connectivity was never the primary contributor to metapopulation growth rate, it was the parameter that regulated what vital rates were most important (Chapter 5). So while connectivity

may not be the who of population and metapopulation dynamics, it is the what, when, where, and why of population and metapopulation dynamics. Connectivity acts to increase local and metapopulation growth rates (Chapter 5), and as connectivity within a system is increased, it shifts the primary drivers of local and metapopulation growth from adult survivorship to the juvenile growth transitions (Chapter 5).

In order to assess the realized connectivity patterns in terms of conservation and MPA design it was necessary to develop a manner of placing a relative connectivity "value" on individual reefs. To enable this metrics for quantitative food webs were adapted and combined with node deletion experiments (Ulanowicz and Wolff 1991, Bersier *et al*. 2002). This novel method placed quantitative values on the qualitative terms source and sink population (Chapter 6). In so doing it was possible to rank (qualitatively and quantitatively) the value of each reef to the greater metapopulation, as well as compare the "quality" of realized connectivity patterns with those of hypothetical patterns of population connectivity (Chapter 6). When these metrics were applied to the empirical data generated in this study, La Jolla was, from a connectivity standpoint, the most valuable reef within the study region, suggesting this reef should be considered a high priority for conservation within the study region (Chapter 6). This tool for assessing individual nodes in networks of MPA enables those involved in the design, analysis, and implementation of marine protected areas networks a method for systematically quantifying the relative value of source and sink populations within larger networks of reserves.

The overall results of this study on the role of connectivity on population and metapopulation dynamics has broad implications for those interested in the conservation of marine biodiversity, the restoration of threatened populations and habitats, and those involved in the design of MPA networks. A promising finding from this study is that for populations in decline (i.e. $\lambda < 1$), management efforts that lead to increases in connectivity (e.g. by increasing larval output from reserves), may result in (relatively speaking) larger increases in population growth rate (Chapter 5). Therefore if managers can couple efforts to increase adult survivorship (i.e. the greatest contributor to population and metapopulation growth rates) with increases in connectivity they can maximize the growth rate of populations of interest. However, if it is easier to manipulate juvenile growth rates (e.g. through enhanced food supply), managers can, if possible, attempt to increase the levels of connectivity within the study system of interest to a point where the primary contributor to population growth is not adult survivorship, but juvenile growth.

As was mentioned earlier in this dissertation (Chapter 1), some of the goals in understanding connectivity are to eradicate invasive species and increase the probability of persistence of threatened species. The findings from this dissertation are able to provide insight and advice about the most appropriate manner to achieve these desired management goals. Utilizing the connectivity metrics and node deletion experiments (Chapter 6) in conjunction with the elasticity analyses, will also provide insights into not only what life stage to focus efforts, but also where to focus management efforts. Given the same scenario as above (i.e. the management goal is to

eradicate an invasive species), you can also determine which of the local populations was most important, from a connectivity standpoint, to the local and metapopulation growth of the invasive species, and focus the limited amount of conservation-related funding available at this location.

By increasing our understanding of connectivity and its role in population growth this study has improved our ability to manage natural resources, whether the ultimate goal of a resource manager is to eradicate an invasive species, or increase the probability of persistence of a species threatened by predicted climate change. This study has provided a framework for estimating the magnitude of connectivity among populations (e.g. using otolith microchemistry or using ADCP data; Chapter 4) and created a tool for resource managers to determine the most effective manner of using connectivity to manage populations (Chapters 5 and 6). Connectivity acts to regulate how important various vital rates are to population and metapopulation growth (Chapter 5). Fortunately for those populations in decline, it appears as though efforts to increase population connectivity (e.g. by implementing catch restrictions to ensure the largest most fecund individuals can successfully reproduce, thereby augmenting larval output) will be one of the most useful methods of increasing population growth rates; hopefully increasing the persistence of populations threatened with extinction. But connectivity must be viewed as only one of the tools of spatial conservation, and should be utilized along with management actions that will address critical facets of an organism's life history (Chapter 5) to ensure the successful protection of marine biodiversity in perpetuity.

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