ESTIMATING CONNECTIVITY IN CORAL REEF FISH POPULATIONS: A TOOL FOR UNDERSTANDING STABILITY AND RESILIENCE OF MARINE ECOSYSTEMS

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GENERAL ABSTRACT

The extent of larval dispersal holds a crucial role for both the dynamics and evolution of spatially structured marine populations, determines the scale at which species interact with their environment, respond to perturbations and evolve. Designing effective conservation strategies such as marine reserve networks will depend to some extent in our comprehension of how marine populations are interconnected. However, measuring exchange among populations given the small size of larvae of most marine species and the high dispersal potential in the ocean matrix, remains challenging because of the difficulty of tracking individual larvae in the ocean. As a consequence, there is still little knowledge about the patterns of demographic connectivity in marine environments and the main factors that influence the shape of dispersal. The aim of this dissertation is to provide new leads on the scale of marine larval dispersal in a coastal environment and the role of the parental environment as driver of the variation in magnitude of local population replenishment. A metapopulation consisting of discrete adjacent subpopulations of the anemonefish *Amphiprion polymnus* along 30 km of coastline in Bootless Bay (Papua New Guinea) was used as a model system. Highly variable microsatellite loci as genetic markers and parentage analysis were used to trace back the parental origins of juvenile fish. This method allowed for the estimation of self recruitment and population connectivity within the metapopulation. This dissertation starts by exploring some methodological issues related to the use of parentage analysis in natural populations and estimates the minimal sampling effort required (in terms of number of genetic markers) to attain high parentage assignment accuracy (~94%) in this specific metapopulation. Second, larval retention within and exchange among the subpopulations previously mentioned were quantified over two consecutive years. The results of this chapter suggest that in this coastal metapopulation, self recruitment at small spatial scale (individual reefs) is low (~8% in average), highly variable among sites, but rather constant between years. At the metapopulation level, connectivity and not self recruitment seems to be the dominant pattern. Third, by using individual multilocus profiles as natural tags, parentage analysis and field observations, it is shown for the first time in a natural marine population, that larger females in the local population contribute more than twice to local replenishment than smaller females. In addition, results from this chapter revealed that habitat degradation can have negative consequences on the reproductive output for this species. Finally, I used empirical data from previous chapters to estimate population's

demographic rates (survival, fecundity and size-category transition frequency) and integrated this in a simple matrix model. In this way I was able to show that metapopulation self-persistence is only achieved in this system when considering at least 50 km of coastline. In general, the results of this work reinforce the notion that in scenarios where spacing between suitable habitat patches is small, populations operate as open systems even at relatively large spatial scales. Comparison with previous studies suggests that dispersal of coral reef larvae might be more influenced by geographic settings than species specific life history traits. Finally, in a conservation perspective, these results indicate that protecting large healthy females will be crucial to population replenishment, argue for the implementation of management strategies that will restore and protect size/age structure of marine populations and highlight that, in relatively continuous habitats, a small Marine Protected Area (MPA) could not be considered as self-sustaining if populations outside the MPA are depleted.

RESUME GENERAL

L'étendue de la dispersion larvaire tient un rôle crucial dans la dynamique et l'évolution de la structure spatiale des populations marines. Elle détermine l'échelle à laquelle les espèces interagissent avec leur environnement, répondent aux perturbations et évoluent. La conception des stratégies de conservation efficaces telles que les réseaux de réserves marines dépend, dans une certaine mesure, de notre compréhension de la manière dont les populations marines sont reliées entre elles. Toutefois, étant donné la petite taille des larves de la plupart des espèces marines et de leur grande capacité de dispersion dans l'océan, mesurer l'échange entre différentes populations reste laborieux en raison de la difficulté à suivre les larves dans l'océan. En conséquence, le niveau de connaissances actuelles sur l'échelle de la connectivité démographique dans les environnements marins reste réduit, et l'identité des principaux facteurs responsables de la variabilité de la dispersion larvaire reste méconnue. L'objectif de cette thèse est de fournir de nouvelles informations de l'échelle de la dispersion larvaire dans un milieu côtier, et d'évaluer le rôle de l'environnement parental en tant que facteur de variation de recrutement de larves au niveau local. Le modèle d'étude choisi est une métapopulation côtière du poisson clown *Amphiprion polymnus* qui s'étend sur environ 30 km du littoral en Papouasie Nouvelle Guinée. Des analyses de parenté ont été utilisées pour retrouver les lieux de naissance des juvéniles de cette espèce, à l'aide des marqueurs génétiques hypervariables de type microsatellite. Cette méthode a permis ensuite d'estimer les taux d'autorecrutement et de connectivité entre sous-populations au sein de la méta-population. Le première chapitre de ce travail a étudié certains problèmes méthodologiques liés à l'utilisation des analyses de parenté dans des populations naturelles, et a estimé l'effort d'échantillonnage minimal nécessaire (en termes de nombre de marqueurs génétiques) dans cette métapopulation en particulier, afin d'atteindre une précision des assignations parent-fils élevée (~ 94%). Dans un deuxième chapitre, l'autorecrutement et la connectivité entre sous-populations mentionnées précédemment ont été quantifiés sur deux années consécutives. Les résultats de ce chapitre indiquent que dans cette métapopulation côtière, l'autorecrutement à l'intérieur des sous-populations, est faible (~ 8% en moyenne) mais peut être très variable entre elles. Cependant, ces proportions semblent plutôt constantes dans le temps. Au niveau de la métapopulation, et à différence des études précédentes, la connectivité semble être le modèle dominant et non l'autorecrutement. Le troisième chapitre à permis de montrer pour la première fois dans une population marine, que les femelles plus grandes à l'intérieur de la

metapopulation contribuent plus de deux fois au repeuplement local que les petites femelles. De même, cette approche à permit de montrer que la dégradation de l'habitat peut avoir des conséquences négatives sur la reproduction de cette espèce. Enfin, dans le dernier chapitre, des données empiriques, obtenus au cours des chapitres précédents, ont été utilisées pour estimer des paramètres démographiques de la population (survie, fécondité et fréquence de transition entre les différentes catégories de taille). En suite, ces paramètres ont été intégrés dans un modèle matriciel simple. Dans la suite, l'échelle spatiale à laquelle cette metapopulation devient équilibrée a été estimée à un minimum de 50 km de ligne de côte. De manière générale, les résultats de ce travail ont permis de renforcer la notion que dans des contextes géographiques où l'espacement entre populations est réduit, celles-ci fonctionnent comme des systèmes dynamiques ouverts, même à une échelle spatiale plutôt élevée. De même, la comparaison de ces résultats avec des études antérieures suggère que la dispersion larvaire chez les poissons de récifs coralliens pourrait être plus influencée par le contexte géographique que par la spécificité de certains traits d'histoire de vie. Enfin, dans une perspective de conservation, les résultats de cette thèse indiquent que protéger les femelles de grande taille est crucial pour le repeuplement des populations marines et sont un argument en faveur d'une mise en œuvre de stratégies de gestion qui permettront de restaurer et de protéger la structure de taille/âge des populations marines. Cette approche a également mis en évidence que dans un habitat relativement continu, une AMP de petite taille ne pourrait pas être considéré comme autonome si les populations à l'extérieur de l'aire protégé sont épuisées.

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CHAPTER 1

GENERAL INTRODUCTION

Perhaps one of the most studied yet least understood concepts in ecology and evolution is *movement*, whether it involves the relocation of individuals, propagules or genes. Movement includes all activities where relocation takes place, from daily patterns to ontogenetic life transitions and from single individuals to whole populations (Clobert et al. 2001, Dingle and Drake 2007). Among all the possible forms of movement, dispersal, which includes movement from a natal place to a breeding place, or between two breeding places (Clobert et al. 2001), is the most critical. Dispersal can be a key factor limiting the local distribution and abundance of species (Krebs 1978), patterns of gene flow across space and species biogeographic range (Ronce 2007). On evolutionary time scales, dispersal can determine patterns of genetic structure, diversity and change, within and among populations (e.g. Rocha et al. 2005, Waples and Gaggiotti 2006, Hemmer-Hansen et al. 2007, Zamudio and Wieczorek 2007, White et al. 2010a). For instance, dispersal can help mitigate the effect of drift in small populations, decrease mutation load, and thereby reduce the risk of extinction (Tallmond et al. 2004). On ecological time scales, dispersal plays a crucial role in the dynamics of spatially structured populations (Hanski 1994, Hanski 1998, Hill et al. 2002, Hastings and Botsford 2006). It determines the geographic scale at which species interact with their environment, respond to perturbations and evolve in an ever changing world (Kinlan and Gaines 2003). Despite its importance, the magnitude and scale of dispersal can be the most difficult biological parameters to estimate.

Within the geographic range of most species, individuals are usually distributed to varying degrees into geographically discrete populations (Hanski 1998, Thomas and Kunin 1999, Kritzer and Sale 2004). Discrete populations can result from spatial heterogeneity in the distribution of suitable habitat or a variety of natural or human disturbances that lead habitat fragmentation (Fahrig 2003). The extent to which these populations are linked by dispersal (either larvae, juveniles or adults) is termed connectivity (Palumbi 2003). The behaviour of spatially structured populations can be viewed as a continuum from high connectivity between patches to low connectivity with few dispersal events (Thomas and Kunin 1999). The degree of connectivity can be influenced by life history characteristics such as larval durations or adult migratory ability, physical factors affecting dispersal distance and direction, and the spatial configuration of habitat patches (e.g. Mora and Sale 2002, Ayre et al. 2009, Hellberg 2009, Shima et al. 2010, White et al. 2010a, White et al. 2010b). Both the position of population systems along this connectivity continuum and the dispersal ability of an organism are major determinants of the dynamics of local populations and the geographic range of a species (Bowler and Benton 2005). However, in general, of all the demographic information

required to model spatially discrete populations, patterns of connectivity are the least understood.

Connectivity can have different meanings and implications depending on the scale considered and how it is measured. From an evolutionary perspective, connectivity can be defined as the degree to which gene flow affects evolutionary processes within populations (genetic connectivity) (Waples and Gaggiotti 2006). From an ecological perspective, demographically connected populations are those in which population specific vital rates (survival and birth rates) and population growth rates are affected by dispersal (Lowe and Allendorf 2010). Demographic connectivity has been acknowledged as a vital parameter for understanding the dynamics of populations, how they respond to natural and/or human disturbances, and how to design conservation strategies such as spatial closures or networks of reserves (Roberts 1997, With et al. 1997, Schiegg et al. 2006, Hannah et al. 2007, Munday et al. 2009).

Patterns of dispersal and connectivity are likely to be particularly important in the ecology and evolution of marine species. Populations of the majority of marine organisms can be associated with spatially discrete habitats such as coral reefs, rocky reefs, seagrass and even deep-sea hydrothermal vents (Rocha et al. 2005, Carreras-Carbonell et al. 2007, Cowen et al. 2007, Underwood et al. 2007, Ayre et al. 2009, Hellberg 2009, Puebla et al. 2009, Salas et al. 2010, Vrijenhoek 2010). The majority of marine organisms have complex life cycles including dispersive larval stages. Patterns of connectivity can be strongly influenced by hydrodynamic regimes (White et al. 2010a), larval behaviour (Gerlach et al. 2007), adult movement (Neville et al. 2006) and the distribution of habitat patches (Pinsky et al. 2010). The degree to which larval dispersal connects discrete populations will determine how populations are naturally regulated and how they respond to natural or human disturbances, such as exploitation or habitat loss (Mora and Sale 2002, Kritzer and Sale 2004, Jones et al. 2007, McCook et al. 2009). Our understanding of the scale of larval dispersal and factors that explain natural variations of population replenishment is still very limited. Such information is needed to build accurate population models that can be integrated in to conservation policies to develop more effective conservation strategies such as marine reserve networks (Roberts 1997, Sale et al. 2005a, Mora et al. 2006, Jones et al. 2009b, Munday et al. 2009).

Obtaining direct quantitative measures of the number of individuals moving between patches or populations is logistically challenging in marine systems, particularly for organisms that have a tiny dispersive larval stage that is followed by a relatively sedentary adult stage. Measuring exchange among populations, given the small size of larvae and the

high dispersal potential in the ocean matrix, remains one of the highest challenges for marine ecologists (Mora et al. 2003, Sale et al. 2005a). As a consequence, most of the studies that have addressed connectivity in marine environments overcome this difficulty by using indirect approaches such as classic population genetics, larval behaviour ecology or biophysical models (Swearer et al. 2002, Cowen et al. 2007, Jones et al. 2009b). Classical population genetics is perhaps the most widely used method to make inference about population connectivity in marine environments. This approach remains effective to quantify gene flow at evolutionary time scales (Waples and Gaggiotti 2006), identify major biogeographic barriers (Planes 2002) and evolutionary significant units as well as cryptic species and areas of endemism (Rocha et al. 2007). On the other hand, at the demographic scale it provides little information on the magnitude of exchange among populations because levels of gene flow that are required to impact vital rates are too high to leave detectable genetic signals (differentiation) to accurately infer dispersal rates (Waples and Gaggiotti 2006, Lowe and Allendorf 2010).

Biophysical models that integrate information on hydrodynamic processes, demography and larval behaviour have been extensively developed in the last decade. These models have provided sophisticated predictions of the potential magnitude and direction of larval dispersal (Cowen et al. 2000, Cowen et al. 2006, Treml et al. 2008). At the same time, larval behaviour studies have revealed that marine larvae have highly developed sensory organs, respond to different chemical, sound and visual cues (Lecchini et al. 2005, Simpson et al. 2005, Dixson et al. 2008), and also have well developed orientation and swimming abilities (Leis and Carson-Ewart 2003, Leis 2007). But most important, biophysical models have shown that when passive particles are enabled of movement following some simplified behaviour rules, there are significant changes in the shape of dispersal kernels (Cowen et al. 2000). These advances have highlighted the importance of obtaining reliable direct estimates of demographic connectivity to understand the significance of larval behaviour in dispersal and to test how accurate are existing models for describing real dispersal kernels and how they can be improved. However, only a few empirical studies have managed to obtain direct estimates of self recruitment and none so far has managed to obtain the description of a full dispersal kernel (Jones et al. 2009b).

Among marine ecosystems, coral reefs are perhaps among the most threatened in the planet. Overexploitation of natural resources, pollution and climate change menace these fragile environments (Hoegh-Guldberg 1999, Hughes et al. 2003, Jones et al. 2004, Hughes et al. 2005, Hoegh-Guldberg et al. 2007). Connectivity could be compromised by increased

habitat fragmentation as a consequence of human disturbances and climate change (Munday et al. 2009). Hence, understanding the extent to which coral reef populations are connected in the present and the factors affecting the magnitude and shape of larval dispersal will be essential for implementing effective management strategies, predicting future scenarios and adjusting strategies according to them.

New techniques have emerged such as otoliths microchemistry, otoliths tagging and parentage analysis allowing for empirical quantitative estimation of demographic connectivity (Jones et al. 1999, Swearer et al. 1999, Jones et al. 2005, Almany et al. 2007, Planes et al. 2009, Saenz-Agudelo et al. 2009). Among these techniques parentage analysis is perhaps one of the most promising ones. This approach allows for resolution of dispersal trajectories at the individual level (Jones et al. 2005) and provides valuable genetic data that can be used to answer other relevant ecological questions (Buston et al. 2007). However, parentage analysis relies on theoretical assumptions that are sometimes difficult to meet in natural populations (Marshall et al. 1998, Neff et al. 2000, Jones and Ardren 2003). The effects of violating some of this assumptions and the accuracy of this approach for estimating marine larval connectivity has been seldom been quantified. Testing the accuracy of this approach in different theoretical scenarios will be fundamental to validate and extend its use in this discipline.

So far, empirical estimates of larval connectivity obtained with these methods have been mostly restricted to single geographic locations and estimates of self recruitment (Jones et al. 1999, Swearer et al. 1999, Jones et al. 2005, Almany et al. 2007, Planes et al. 2009, Saenz-Agudelo et al. 2009). These studies have shown that self recruitment can be more important that previously thought, but none so far has managed to obtain direct estimates of exchange among discrete populations and more studies on different species and locations are needed before any general conclusions can be made (Jones et al. 2009b).

Besides the call for empirical descriptions of connectivity, there is also need to identify the main factors that influence the shape of dispersal and the magnitude of replenishment of marine populations (Mora and Sale 2002, Sale et al. 2005a). In benthic species with a larval phase, the number of possible factors that will influence the reproductive output of adults, the quality and survival of early life history phases (eggs and larvae), and eventually lead to dramatic fluctuations in recruitment, is more than vast. However, there is growing evidence that suggests that both the number of larvae produced and their quality (e.g. survival, swimming performance, size, etc) are highly affected by the combination of parental genotype, phenotype and environment, defined as parental effects (reviewed by Green 2008).

Among parental effects, particular attention has been paid to female size and age. Besides a positive relationship between female size/age and fecundity, this trait has been shown to be positively correlated to larval size and quality (Berkeley et al. 2004a). It is widely assumed that parental effects should be maintained through the entire larval phase and influence population replenishment, but this has not yet been verified in wild populations. We are still far from understanding the complexity of factors affecting larval fitness and we still ignore to what extent maternal size effects could be compensated by complex interactions.

The aim of this dissertation was to provide the first estimates of demographic connectivity through larval dispersal for a coastal metapopulation of a coral reef fish species. It describes fine-scale patterns of self-recruitment and connectivity, and explores the role of parental environment in local population replenishment using a combination of genetic data, parentage analysis and capture-mark-recapture methods. It is written as a series of chapters, which consist of stand-alone, though conceptually interconnected papers. This study provides new insights about the magnitude of larval retention and exchange in natural coral reef fish populations, its patterns of spatial and temporal variation and the nature of some of the sources of their variation via parental effects.

Field studies in this dissertation focused on the panda clownfish (*Amphiprion polymnus*), a relatively common coral reef fish distributed across the Western Tropical Pacific that lives in close association with sea anemones. *A. polymnus* is a good model species for *in-situ* demographic studies. The particular life style of its benthic adult phase translates in a very low mobility which in turn makes the observation and manipulation of many individuals relatively simple. *A. polymnus* lays demersal eggs, a relatively common strategy among coral reef fish, and the pelagic larval duration (~12 days) of its larvae is relatively short compared to most marine fish. Given these characteristics, estimates of connectivity for this species can, to some extent, serve as a baseline for other marine organisms.

I start in **chapter 2** by testing the performance of parentage analysis and genetic assignment tests to estimate demographic connectivity under different gene flow scenarios. I used a combination of real and simulated data sets to test these novel genetic methods, evaluate how some deviations from theoretical assumptions influenced individual assignments and estimated error rates linked to statistical tests under different sampling conditions in order to obtain the optimal sampling effort for this study.

In **chapter 3**, I used genetic data collected over two consecutive years (2008 and 2009) in a coastal metapopulation system of *A. polymnus* in Papua New Guinea and estimated self recruitment and connectivity among sub populations and described both their spatial and

temporal variation in this system. The choice of this geographic context (coastal environment) was new compared to previous studies using similar methods (Jones et al. 2005, Planes et al. 2009) and allowed to explore the importance of the geographic spacing to larval exchange.

In **chapter 4**, I explored the link between parental phenotype parental environment and recruitment in this system. I was particularly interested in investigating whether variations in some of these parental characteristics (whether it was phenotype or environment), that had already been shown to influence larval quality under controlled conditions (reviewed by Green 2008), explained differences in the contribution of individual adults to the replenishment of the local population. In addition, during one of the field surveys, a bleaching event affected a third of the anemones been studied. This created an excellent opportunity to explore the effects of habitat degradation on *A. polymnus* dynamics, focusing on reproduction and recruitment.

Chapter 5 was the compilation of most of the results from previous chapters in an attempt to answer one simple but extremely important question of population dynamics: at what spatial scale does a population (or metapopulation) achieves self persistence and how much self recruitment does this represents? I used genetic data gathered over the two years to follow the fate of all fish present in the first year that survived to be counted one year later and estimate specific vital rates (survival, class category transition, fecundity and migration). I integrated this information in a simple deterministic model to explore the conditions (in terms of spatial scale) for which demographic stability could be achieved and how changes in the observed connectivity patterns affected local population's intrinsic growth rates.

Finally, I completed this dissertation with an integrated conclusion of all the major findings in **chapter 6**.

CHAPTER 2

TESTING NOVEL GENETIC TOOLS

This chapter is divided in two sections each of which deals with a different methodological aspect of using genetic tools to estimate marine connectivity. The first section uses empirical data to evaluate the accuracy of parentage analysis and assignment tests both under a situation of high and low gene flow among populations. The second section uses simulated genetic data to explore the effects of different levels of incomplete sampling both in terms of individuals and genetic markers. Results from this section were used to estimate the number of genetic markers that were required to achieve low assignment errors and were used in all posterior analyses.

2.1. EMPIRICAL EVALUATION OF ASSIGNMENT TESTS AND PARENTAGE ANALYSIS UNDER DIFFERENT GENE FLOW SCENARIOS

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ABSTRACT

The application of spatially explicit models of population dynamics to fisheries management and the design marine reserves network systems has been limited due to a lack of empirical estimates of larval dispersal. While new genetic methods provide direct estimates of self-recruitment and connectivity, their accuracy in predicting these parameters at spatial and temporal scales relevant to management is uncertain, particularly when model assumptions are violated. Here we compared assignment tests and parentage analysis for examining larval retention and connectivity under two different gene flow scenarios using panda clownfish (*Amphiprion polymnus*) in Papua New Guinea. A metapopulation of panda clownfish in Bootless Bay with little or no genetic differentiation among 5 spatially discrete locations separated by 2-6km provided the high gene flow scenario. The low gene flow scenario compared the Bootless Bay metapopulation with a genetically distinct population (Fst = 0.1) located at Schumann Island, New Britain, 1,500km to the north-east. We used assignment tests and parentage analysis based on microsatellite DNA data to identify natal origins of 177 juveniles in Bootless Bay and 73 juveniles at Schumann Island. At low rates of gene flow, assignment tests correctly classified juveniles to their source population. On the other hand, parentage analysis led to an overestimate of self-recruitment within the two populations due to the significant deviation from panmixia when both populations were pooled. At high gene flow (within Bootless Bay) assignment tests underestimated self-recruitment and connectivity among subpopulations, and grossly overestimated self-recruitment within the overall metapopulation. However, the assignment tests did identify immigrants from distant (genetically distinct) populations. Parentage analysis clearly provided the most accurate estimates of connectivity in situations of high gene flow. Still, because sampling a consistent fraction of potential parents is limited in marine environments, further research is needed to better evaluate parentage analysis full potential and limitations.

INTRODUCTION

Marine coastal habitats are often discontinuous and species distributions can be fragmented into spatially discrete populations. The dynamics of these populations can potentially be influenced by *self-recruitment* or local retention of juveniles within populations, and by *connectivity*, the degree to which these populations are linked by dispersal (Warner and Cowen 2002, Sale et al. 2005a). Levels of self-recruitment within and connectivity among populations on ecological time-scales are key factors affecting the persistence of marine metapopulations and their resilience to local disturbance (Armsworth 2002, James et al. 2002, Hastings and Botsford 2006). Optimal design of spatially explicit management strategies for marine species, including marine protected areas (MPAs), is also contingent on the extent of population connectivity (Lockwood et al. 2002, Hastings and Botsford 2003, Sale et al. 2005a). In benthic-oriented marine species which are often relatively sedentary as adults, population connectivity largely occurs during a larval phase that extends from reproduction to the completion of the settlement process (Cowen et al. 2007). While an increasing number of methods for estimating population exchange on ecological time-scales are available, the accuracy of the different methods and the degree of concordance among them are seldom known.

Population genetics is the most widely used approach for making inferences about dispersal and connectivity in marine organisms (Planes 2002, Van Oppen and Gates 2006, Hellberg 2007). Estimates of connectivity based on gene flow have also being used to inform the design of marine protected area networks (e.g. Palumbi 2003). However, while clearly a suitable tool for measuring gene flow on evolutionary time-scales, population genetics cannot always distinguish between contemporary and historical gene flow. Standard estimates of migration among populations are increasingly inaccurate at scales where there may be limited population differentiation (Hedgecock et al. 2007). Estimates of dispersal also rely heavily on theoretical models of population structure, such as Wright's island model, which are based on many assumptions that may often be violated in natural populations (Hedgecock et al. 2007). Given that successful management may be reliant on good estimates of population exchange between local populations and successive generations, the accuracy of different approaches needs to be evaluated.

The recent proliferation of molecular and statistical tools has led to the application of genetic tools to provide direct estimates of connectivity in marine populations (Manel et al. 2003). These genetic approaches focus on the assignment of individuals to populations of

origin (assignment methods) (Carreras-Carbonell et al. 2007, Underwood et al. 2007) or to specific parents (parentage analysis) (Gerber et al. 2000, Rodzen et al. 2004, Jones et al. 2005, Castro et al. 2006) Direct estimates of retention and connectivity using assignment tests or parentage analysis can be applied using hypervariable molecular markers such as microsatellites. In assignment methods, an individual is assigned to the most likely source population, based on the expected frequency of its multilocus genotype in various putative sources. The typical assumptions of this approach are that all potential source populations are defined in advance, sampled randomly and do not depart from Hardy-Weinberg or linkage equilibrium. Newer statistical approaches that use maximum likelihood and Bayesian methods involve fewer assumptions and provide higher assignment accuracy (Manel et al. 2005). While it has also been suggested that these approaches are more effective when migration is low ($Nm < 5$) (Waples and Gaggiotti 2006) and consequently genetic structure is high (Underwood et al. 2007), the accuracy of assignment techniques at identifying natal origins at small spatial scales has rarely been examined.

In parentage analysis, individuals are assigned to one single parent or parent pair usually using a likelihood-based approach to select the most likely parent from a pool of potential parents (Jones and Ardren 2003). The main constrain of this approach is that parental allocation success declines dramatically as the proportion of sampled candidate parents drop (Marshall et al. 1998). However, methods have recently been developed that allow to deal with incomplete sampling (Gerber et al. 2003, Duchesne et al. 2005). In addition, parentage analysis assumes that there is random mating in the population. This assumption of panmixia is often violated in wild populations at larger spatial scales, but to our knowledge no empirical studies have tested for the consequences of this violation when parentage models are used to study natural populations.

Coral reef environments are extremely patchy and resident populations of reef fishes can be spatially segregated at small spatial scales, from kilometres to 10's of kilometres (Hellberg 2007). Although fishes have pelagic larval durations that may last weeks to months, recent empirical evidence suggests a high degree of local retention of larvae (Jones et al. 1999, Swearer et al. 1999, Paris and Cowen 2004, Jones et al. 2005, Almany et al. 2007). Standard population genetic techniques vary in their ability to estimate self-recruitment and connectivity at these small spatial scales (Planes 2002), and the application of assignment tests and parentage analysis has been limited (Baums et al. 2005, Gerlach et al. 2007, Underwood et al. 2007). Jones *et al*. (2005) directly estimated levels of self-recruitment in a clownfish by combining parentage analysis and chemical tagging and found similar results

with the two methods. More interestingly, they highlighted that parentage analysis can provide high resolution connectivity information and direct estimates of dispersal distances at the individual level. However, while promising, the effects of violations in model assumptions require further investigation.

The aim of this study was to evaluate and compare estimates of self-recruitment and connectivity from assignment tests and parentage analysis under two different scenarios of gene flow. First, we considered a high gene flow scenario using genetic data from five spatially discrete subpopulations of the panda clownfish *Amphiprion polymnus* in Bootless Bay, Papua New Guinea. Then, we considered a low gene flow scenario by adding a data set from a genetically distinct population (Schumann Island) located more than 1,500 km away in the Bismark Sea (Jones *et al.* 2005). As dispersal between the two locations is extremely unlikely, pooling the two locations provided a means to evaluate the effect of violating the assumption of a panmitic population when classifying parent-offspring relationships.

METHODS

Study species and location

The panda clownfish (*Amphiprion polymnus*) is a Southeast Asian endemic fish that lives in close association with discrete aggregations of two species of anemones (*Stichodactyla hadonni* and *Heteractis crispa*) that occupy sandy habitats associated with coral reefs (Fautin & Allen 1992). Each anemone is usually occupied by one breeding pair and up to eight smaller subadults and juveniles. The female (the largest individual) lays demersal eggs on the upper surface of shells or dead coral next to the anemone. The embryos develop over a period of 6-7days before hatching (Fautin and Allen 1992) and late stage larvae settle into anemones after a pelagic larval phase lasting 9-12 days (Thresher et al. 1989).

We used genetic data from two separate population systems in Papua New Guinea. The first system was located at Bootless Bay, nearby Port Moresby (Figure 1) and consisted of a metapopulation of five discrete subpopulations with no individuals found in adjacent sand or coral habitats. Each population was confined to a discrete ~1ha patch of shallow sand and sea grass separated from the other subpopulations by 2 to 6 km. At each site an exhaustive search for all anemones colonised by *A. polymnus* was made. A total of 85 anemones and 281 adult and subadult *A. polymnus* were distributed among the five subpopulations (Figure 1). The second system was located at Schumann Island (Kimbe Bay, New Britain) over 1500 km to the north east of Bootless Bay. Genetic data from Schumann

Island published by Jones *et al.* (2005) was used to compare the utility of assignment tests and parentage analysis to correctly assign juveniles to geographically distant populations. The Schumann Island population consisted of 40 anemones and 85 adult *A. polymnus* confined to a 1km^2 sand flat adjacent to the island.

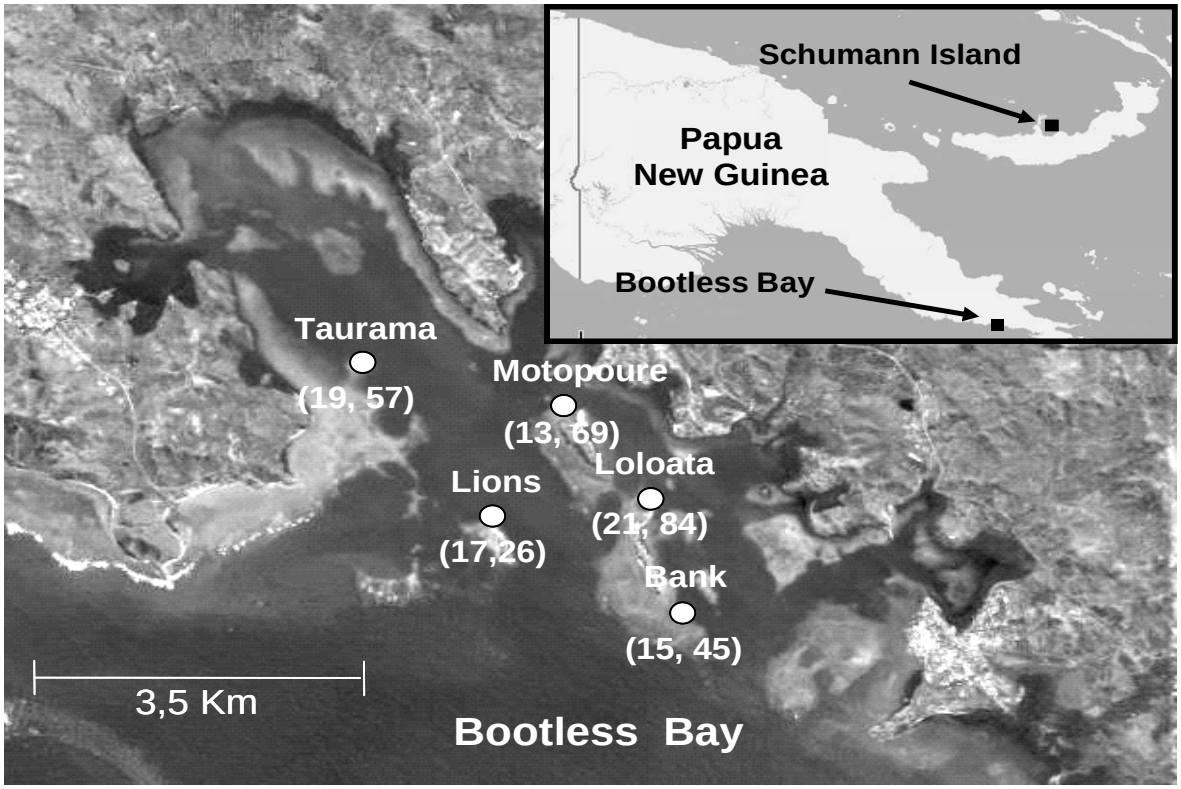

Figure 1 Satellite image showing the sites of five subpopulations of *Amphiprion polymnus* within Bootless Bay. The number of anemones and *A.polymnus* (adult and subadult) at each site are indicated in brackets. Inset: Location of Bootless Bay and Schumann Island study locations in Papua New Guinea.

Sampling and genotyping

A total of 458 individuals (281 adults and subadults and 177 juveniles), representing between approximately 85 and 95% of each of the subpopulations, were sampled after extensive searches at each of the five sites. All resident fish (adult and sub-adult individuals) from the five sites were sampled in December 2005. Each individual was captured on SCUBA using hand nets, fin clipped underwater on site, and then released on the same anemone as captured. All juveniles present at each anemone were captured in December 2005, and at three additional times (January, April, and June 2006). All samples were preserved in 95% ethanol and returned to the laboratory for subsequent genetic analyses. The genetic data set for Schumann Island comprised 158 individuals (85 adults and 73 juveniles).

Adults were fin-clipped in June 2003, and all juveniles settling over a three-months period between August-October 2003 were sampled (see Jones *et al*. 2005 for details).

Details of genotyping procedure are described in (Quenouille et al. 2004). After DNA extraction, 3 multiplex polymerase chain reactions (PCRs) were performed per individual, using fluorescently-labelled primers to process 11 microsatellite loci containing a mixture of dimer and tetramer repeats. PCR products were processed on a Beckman Coulter sequencer CEQ 8000 Genetic Analysis System and the resulting electropherograms were scored manually. Uncertainties were resolved by reamplification and comparison. Alleles were scored as PCR product size in base pairs. None of the 637 individuals screened shared the same diploid genotype. Allelic frequencies, allelic patterns and expected heterozyosities under Hardy Weinberg equilibrium were calculated in GENALEX version 6 (Peakall and Smouse 2006). Tests for Hardy-Weinberg and linkage disequilibrium were conducted using GENEPOP 3.4. (Raymond and Rousset 1995) and significance levels were adjusted with sequential Bonferroni corrections for multiple tests with $p< 0.05$.

A table describing the number of samples, number of alleles, observed and expected heterozygosity for each adult and juvenile group of the ten other loci are shown in the supplementary data (Table 5). While heterozygote deficits were present in at least one site at 3 of the 10 remaining loci, consistent heterozygote deficits were detected across all sites only for one locus (loc 2). This deficit suggested the presence of null alleles and consequently this locus was also excluded from all subsequent analysis. All 9 remaining loci were considered statistically independent since no linkage disequilibrium between loci pairs was observed after Bonferroni correction. One locus was excluded because of difficulties during genotyping.

Population structure

We used *F* statistics via analysis of molecular variance (AMOVA) to measure the proportion of total genetic variation that is geographically structured within Bootless Bay, and between Bootless Bay and Schumann Island. This analysis was performed in GENALEX version 6 (Peakall and Smouse 2006) and partitioned the amount of genetic variation between regions (Bootless Bay and Schumann Island), among sites and within sites with respect to different alleles (*F*st). For this analysis only the genotypes of adult and subadult individuals were used (juveniles were excluded). Tests for statistical significance for all estimates were based on 10^4 random permutations, and significance levels were adjusted with sequential Bonferroni correction for multiple tests. In order to facilitate comparison with other studies,

standardized pairwise *F*st values were estimated using the AMOVA framework as described in Meirmans (2006). Lastly, to visualize these genetic relationships among sites, a genetic distance matrix derived from the pairwise *F*st estimates was used to construct a principal coordinates analysis (PCA) graph in GENALEX.

Assignment tests

Assignment of juveniles was carried out using GENECLASS2 (Piry et al. 2004) under the Bayesian assignment method of Rannala & Mountain (1997). This method performs better in assigning/excluding individuals to their correct population of origin than other likelihood-based and distance-based methods (Cornuet *et al*. (1999). Adult and subadult genotypes of each of the five sites from Bootless Bay and Schumann Island site were used as reference populations. Juveniles from all sites were then either assigned or excluded from each of the populations. We used the Monte Carlo re-sampling algorithm (n = 10,000) of Paetkau *et al.* (2004) to generate statistical thresholds to decide if juveniles could be assigned or excluded. Juveniles were considered immigrants when the probability of been assigned to any population was lower than 0.05 (type I error). When a juvenile showed probabilities of assignment greater than 0.05 to only one population it was assigned to that population. Finally, when a juvenile was assigned to more than one population (with p> 0.5) it was left unassigned.

Parentage analysis

Parentage analysis was performed using FAMOZ (Gerber et al. 2003). The program is based on the calculation of LOD (Log of the odds ratio) scores for parentage relationships and the construction of statistical tests for parentage assignment. These tests are based on simulations that generate offspring from genotyped parents (Ho: the most likely parent is the true parent) or from allele frequencies in the population (Hi: the most likely parent is not the true parent). FAMOZ allows for the introduction of an error rate in the LOD score calculation that takes genotyping errors and null alleles into account (Gerber et al. 2000). It has been shown that introduction of this error, even if it underestimated the true error rate, can reduce type I and type II errors related to the parentage tests (Gerber et al. 2000, Morrissey and Wilson 2005). We evaluated four different error rates to choose the best compromise between introduced error and type I and type II statistical errors. An error rate of 10^{-3} yielded the lowest statistical type I and type II errors using the Bootless Bay dataset (Table 1) and was used for all parentage analysis. Tests evaluations were done using the software option

"parentage test simulation". Thirty test simulations were made for each error rate in order to evaluate mean type I and type II statistical errors.

Table 1. Effects of variation of LOD score introduced error on parentage assignments for the Bootless Bay population. Four different error frequencies were evaluated. For each frequency, the number of assignments in relation with the number of mismatches per assignment is presented as well as the estimation of type I and II statistical errors based on 30 simulations of the parentage.

Introduced error	Simulations Error estimation (%)	
	Type I	Type II
0.01	38±4.8	0.1±0.3
0.001	5.8±1.2	8.1±1.3
0.0001	1.9±0.5	8.8±1.3
0	1.4±0.3	9.3±1.8

To test the effect of violating the assumption of a single panmitic population, parentage analysis was done as follows: First, Bootless Bay and Schumann Island were analysed separately. Second, both datasets (Bootless Bay and Schumann Island) were pooled together. For each analysis, allelic frequencies were estimated from the corresponding adult and subadult genotypes and these estimations were assumed to be close to the real population allele frequencies (Gerber et al. 2003). For each analysis, simulations of sets of 10^4 new recruits were made under the two possible hypotheses and subsequent statistical tests were constructed to decide whether a given parent would be selected as the true parent or true parent pair. The distribution of the simulated LOD scores under the two hypotheses was plotted and the intersection between them was used as the threshold decision value (individuals with LOD scores above the threshold value were accepted as true parents).

Finally, because the presence of full sib or half sib relationships can significantly bias parentage analysis (Marshall et al. 1998, Jones and Ardren 2003), all subadults less than 50mm standard length were excluded from the analysis. While size at the beginning of sexual maturity is not known for *A. polymnus*, individuals of a con-generic species (*Amphiprion clarkii*) under 50mm are sexually immature (Hattori and Yanagisawa 1991), and therefore sub-adults of this size are more likely to be either full or half sibs of juveniles than to be parents.

Effect of number and level of polymorphism of loci used

To explore the sensitivity of each method to the number of loci used, we repeated the analyses excluding the two and four least polymorphic loci and the two and four most polymorphic loci from the data set. Then we compared the percentage of assigned, unassigned and excluded juveniles at each case for assignment tests. In the same way, we compared the statistical error (type I and type II) in parentage analysis by simulating parentage tests when two or four loci were excluded.

RESULTS

Population structure

The AMOVA partitioned 9% (*F*rt= 0.095) of the genetic variation between Bootless Bay and Schumann Island which was significantly different from zero (p< 0.001). Genetic variation among sites within regions was 1% (*F*rs= 0.011) of the total variance and it was also significantly different from zero (p< 0.001). For the low gene flow scenario, pairwise *F*st comparisons showed significant differences for Schumann Island with all the Bootless Bay sites (*F*st values ranging from 0.092 to 0.111 - Table 2). For the high gene flow scenario within Bootless Bay, the Taurama site showed small but significant differentiation from the other four sites (Lions, Loloata, Bank and Motupore) with *F*st values ranging from 0.016 to 0.026. We found no significant genetic differentiation among individuals at Lions, Loloata, Bank and Motupore.

Table 2 A) Pairwise *F*st estimates between sites for *A. polymnus* at Bootless Bay and Schumann Island. Estimates in bold indicate significance based on 10^4 permutations after sequential Bonferroni corrections ($p < 0.05$ for all significant comparisons). **B)** Standardized pairwise *F*st values estimated using the AMOVA framework (Meirmans 2006).

A	Bootless Bay				
	Bank	Lions	Loloata	Motupore	Taurama
Bank	-				
Lions	0.007	-			
Loloata	0.006	0.005	-		
Motupore	0.007	0.000	0.003	-	
Taurama	**0.026**	**0.017**	**0.021**	**0.016**	-
Schumann	**0.111**	**0.099**	**0.104**	**0.101**	**0.092**

B	Bootless Bay				
	Bank	Lions	Loloata	Motupore	Taurama
Bank	-				
Lions	0,029	-			
Loloata	0,022	0,020	-		
Motupore	0,029	0,000	0,013	-	
Taurama	0,109	0,078	0,091	0,067	-
Schumann	0,498	0,483	0,495	0,462	0,459

The *F*st PCA plot (Figure 2) showed a close relationship among Bootless Bay sites, with Taurama been slightly separated. Schumann Island was clearly genetically distinct from all Bootless Bay sites, reflecting its geographic separation.

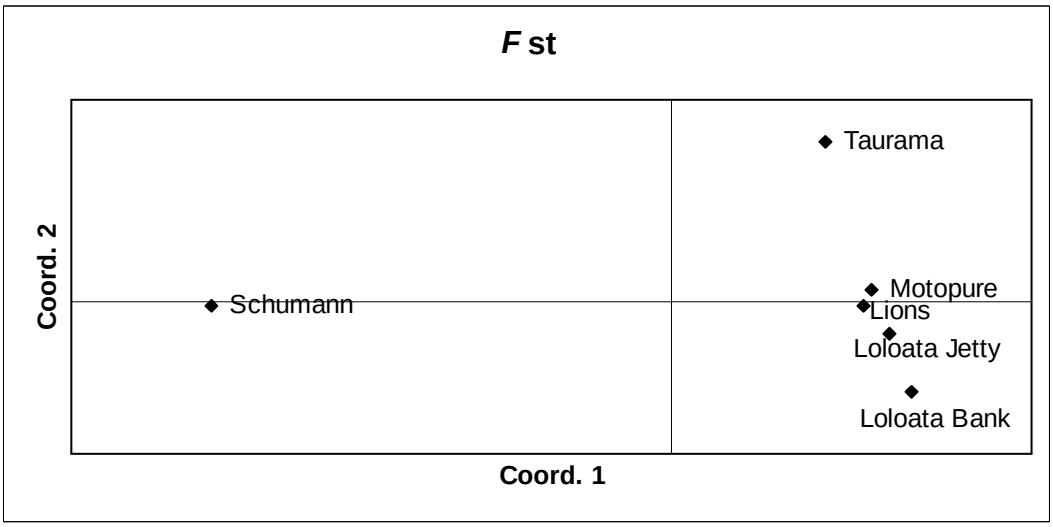

Figure 2 Plots of principal coordinate analysis calculated in GENALEX from standardized distance matrix of pairwise *F*st estimates between sites: the first two axes explain 99% of variation.

Assignment tests

Low gene flow: The assignment method was able to exclude all juveniles sampled in Bootless Bay as being immigrants from Schumann Island with a probability ≥ 95% (Table 3). Likewise, all juveniles from Schumann Island except one were excluded from being immigrants from Bootless Bay (p ≥ 95%). However, the one juvenile from Schumann Island was incorrectly assigned to Bootless Bay (Loloata site) with low probability (p = 0.08).

High gene flow: Within Bootless Bay, 13 juveniles (7%) had a probability greater than 0.05 of belonging to only one of the five sites and were assigned to that site. A further 146 individuals (82%) had a probability greater than 0.05 of belonging to more than one of the five sites within the bay (but were excluded from Schumann Island) and were assigned to the Bootless Bay metapopulation as a whole. In addition, 15 juveniles (8.5%) had a probability lower than 0.05 of belonging to any site and were designated as being immigrants. Finally, 4 juveniles had a probability greater than 0.05 of belonging to either Schumann Island or Bootless Bay and were left unassigned. Within Schumann Island, 60 juveniles (70%) were assigned as having originated from the Schumann Island population, while 24 (28.2%) were excluded from both Bootless Bay and Schumann Island populations and designated as

immigrants. One individual was assigned to both Schumann Island and Bootless Bay and was left unassigned.

Table 3 Results of assignment analysis with GENECLASS2. Juveniles were assigned to one of the six possible sites (sample size in brackets) if the likelihood of their genotype occurring in that site was greater than 0.05, when compared to a distribution of 10^4 simulated genotypes from that site. Juveniles that had a likelihood superior than 0.05 were considered to have being originated from one of the sampled sites. Probability of belonging to the assigned population is given in brackets. If an individual's likelihood was greater than 0.05 for more than one of Bootless Bay sites it was assigned to Bootless Bay as a single unit (all five sites). If the likelihood was greater than 0.05 for both Bootless Bay and Schumann Island it was left unassigned. Juveniles with a likelihood less than 0.05 in all sampled sites were assumed to be immigrants.

Sampling site	Assigned population							Immig	Unassig
	Ba	Li	Lo	Mo	Ta	Bootless	Sch		
Ba(28)	0	1 (0,16)	1 (0,06)	1 (0,16)	1 (0,08)	24	0	1	1
Li(16)	0	1 (0,06)	0	0	0	13	0	2	0
Lo(45)	0	0	0	2 (0.10) (0.12)	0	40	0	3	0
Mo(59)	0	1 (0,15)	0	2 (0.14) (0.31)	2 (0.24) (0.42)	47	0	4	3
Ta(28)	0	0	0	0	1 (0,08)	22	0	5	0
Sch(73)	0	0	1 (0,08)	0	0	0	51	21	1

Parentage analysis

Low gene flow: Parentage analysis was not robust to the deviation in panmixia introduced by pooling samples from Bootless Bay and Schumann Island (Figure 3). For the pooled data set, 39 out of 44 (88.6%) juveniles assigned to parents in Bootless Bay (B) were reassigned there. Five individuals previously assigned to Bootless Bay were excluded, while 10 new juveniles from one of the five sites within Bootless Bay were assigned to Schumann Island. Also, one individual from Schumann Island was assigned to a parent from Bootless Bay (Figure 3A). When the Schumann Island data were tested separately (S), 23 out of 75 juveniles (31.5%) were assigned to one of the 85 sampled parents. When these data were pooled with the Bootless Bay data (B+S), only 15 juveniles assigned when the Schumann Island data were run independently were again assigned a parent from Schumann Island. Additionally, 31 individuals were assigned within Schumann in this test and two juveniles from Schumann Island were assigned to parents in Bootless Bay (Figure 3B).

High gene flow: We examined the possible effect of the slight genetic differentiation among sites in Bootless Bay on the outcome of parentage analysis by analysing the data set with and without the site (Taurama) that was genetically distinct from the other four sites. When parentage analysis was conducted using the 4 sites within Bootless Bay excluding Taurama (B), 26 out of 149 juveniles were assigned to genotyped parents from these sites. When individuals (adults and juveniles) from Taurama were included in the analysis (B+T),

24 of the assigned juveniles from the previous analysis (92%) were reassigned to the same parents. The LOD scores (3.22 and 3.27) of the two juveniles that were not assigned to parents in the pooled analysis were, however, close to the threshold decision value (3.2). An additional 20 juveniles were assigned to parents from Taurama when this site was included.

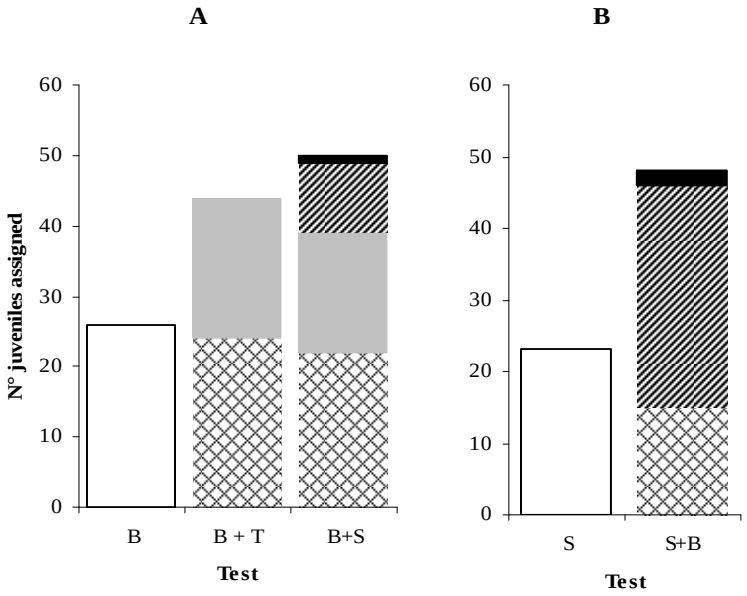

Figure 3. Parentage analysis results using FAMOZ software. **A)** Number of juveniles assigned in Bootless Bay when the test was done using genotypes from Bootless Bay excluding Taurama (B), Bootless Bay all sites (B+T) and Bootless Bay and Schumann Island (B+T+S). Columns show assignments divided in to five categories: (i) Juveniles assigned when test was done excluding Taurama (B) (white fill). (ii) Juveniles reassigned within the four previous sites when Taurama was included (B+T) (squared fill). (iii) New juveniles assigned to/from Taurama (gray fill). (iv) New assignments within the four sites dataset that were not reassigned when Taurama was included in the test (dashed fill). (v) Juveniles from Schumann Island assigned to Bootless Bay (black fill). **B)** Juveniles assigned when the test was done using genotypes from Schumann Island alone (S), and Schumann Island and Bootless Bay (S+B). (i) Juveniles assigned when test was done only with S (white fill). (ii) Juveniles reassigned within S when Bootless Bay was included (gray fill). (iv) New assignments within Schumann that were not assigned in test S (dashed fill). (v) Juveniles from Bootless Bay assigned to Schumann Island (black fill).

Number of loci and degree of polymorphism

We tested the effect of reducing the number and quality of loci for the assignment test under the low gene flow scenario, and for parentage analysis under the high gene flow scenario. The performance of both methods under the opposite scenario was already unsatisfactory and therefore we did not consider the alternative scenarios further. From the 9 loci from our data set, we chose the four loci with the lowest number of alleles as low polymorphic loci (loci: 65, 120, 61 and 55. Increasing number of alleles respectively). Likewise, the four loci with the highest number of alleles were selected as the high

polymorphic loci (loci: 10TCTA, 79, 3GATA and 44. Decreasing number of alleles respectively) (see supplementary data Table 1 for details on number of alleles per loci)
Removing two and four low polymorphic loci had relatively little impact on results from the assignment test compared to results when high polymorphic loci were excluded (Figure 4A). The proportion of juveniles assigned to the population where they were sampled dropped by 6.4% when excluding two low polymorphic loci and by 6.8% when excluding 4 low polymorphic loci. When excluding 2 and 4 high polymorphic loci, juveniles assigned to the population where they were sampled dropped by 13.6% and by 40% respectively. The proportion of juveniles left unassigned (with a probability of assignment > 0.05 to both populations) changed little when 2 or 4 low polymorphic loci were excluded compared to when all loci were used. On the other hand, as many as 42.8% of the juveniles was unassigned when the 4 high polymorphic loci were excluded. The percentage of juveniles excluded from both populations (with a probability of assignment <0.05 to both populations) did not change dramatically in any of the four cases.

In parentage analysis, the effect of excluding high and low polymorphic loci was similar as in assignment tests (Figure 4B). Excluding 2 low polymorphic loci had no significant effect on error rates. Excluding 4 low polymorphic loci had an increase in error rate similar to when 2 high polymorphic loci were excluded. Finally, excluding 4 high polymorphic loci resulted in dramatic increase of type I error (~ 57% of wrong assigned parents). For both cases, excluding two high polymorphic loci had an effect similar as when excluding 4 low polymorphic loci.

DISCUSSION

We have demonstrated that the ability of different genetic techniques to identify natal origins of juvenile coral reef fish depends critically upon the levels of genetic structure within and among focal populations. Standard measures of population differentiation revealed the two distinct gene flow scenarios tested in this study. On one hand, gene flow was extremely limited (*F*st ~0.1) between Schumann Island and Bootless Bay populations of *A. polymnus*. The result was not surprising that the populations were located in different ocean basins separated by over 1,500km and that the pelagic larval duration of this species is 9-12 days (Thresher et al. 1989). Similar *F*st values have also been described for different populations of the same genus *(Amphiprion melanopus*) with similar geographic separation (Doherty et al.

1995). While there may be some gene flow on evolutionary time-scales, this is likely to be of little relevance to local population replenishment or management. On the other hand**,** within the Bootless Bay we found little evidence for genetic structure among subpopulations at 5 sites 2-6 km apart. However, one of the sites (Taurama) was significantly different from the four other sites in the bay even though *F*st values were small. This was an unexpected result, given its proximity to the other populations, although it is perhaps the most isolated of the five subpopulations (Figure 1). Another possible explanation is that the small genetic difference could be due to stochastic variability in reproductive success of past recruitment episodes (Orsini et al. 2008) rather than reproductive isolation of Taumara from the other Bootless Bay sites. Further information is needed to distinguish between these hypotheses.

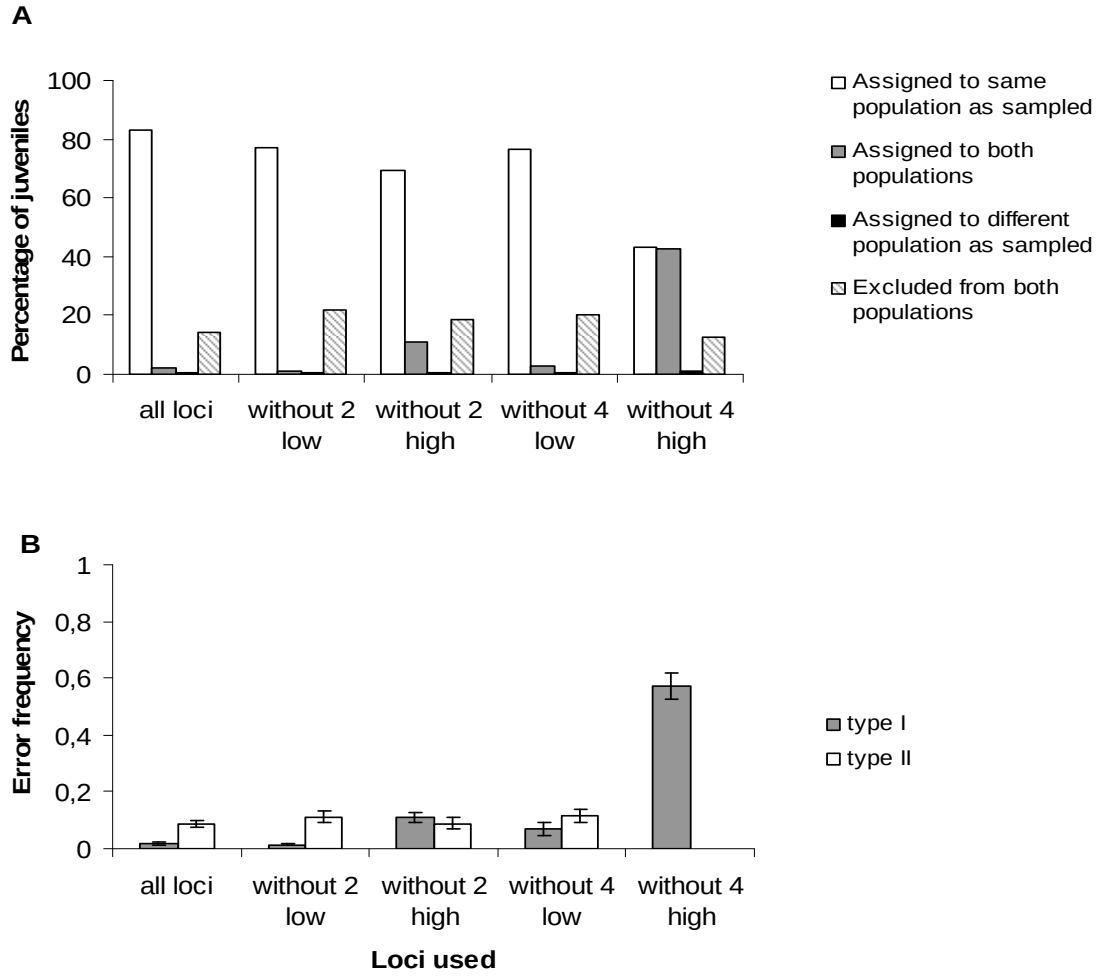

Figure 4. **A)** Assignment test results under the low gene flow scenario (Bootless Bay vs. Schumann Island) using different sets. **B)** Parentage analysis error rate estimates under the high gene flow scenario using different sets of loci (estimated by test simulations in Famoz for each case). The lines on each bar represent the standard deviation after 30 test simulation replicates. The different sets of loci used were: 2 low = two lowest polymorphic loci (loci 120 and 65). 4 low = four lowest polymorphic loci (loci 120, 65, 55 and 61). 2 high = two highest polymorphic loci (loci 10TCTA and 79). 4 high = four highest polymorphic loci (10TCTA, 79, 3GATA and 44).

Low gene flow scenario

We expected the assignment test to perform well when classifying individuals from well differentiated populations given previous results from simulated data (Cornuet et al. 1999, Waples and Gaggiotti 2006) and from empirical studies on populations with strong genetic differentiation (Underwood et al. 2007). Our assignment tests between Bootless Bay and Schumann island populations also support this conclusion. Out of 244 juveniles classified, almost all were assigned to the regional population where they were collected. Only one juvenile from Schumann Island was assigned to the Bootless Bay population, although the assignment probability of this juvenile was fairly low (0.08) and close to the decision threshold (0.05). Given the distance between the populations, we consider this individual to be wrongly assigned by the test.

Alternatively, parentage analysis was not robust to the deviation in panmixia that results from assigning parentage across two differentiated populations. When pooling both populations together, the proportion of parents assigned by both tests (each population separately and both populations pooled) was relatively low (89.6% for Bootless Bay and only 65.2% for Schumann Island). Also, a considerable number of juveniles that were not assigned when the test was done within each population were assigned when the two populations were pooled (20% at Bootless Bay and 67% at Schumann Island). These new assignments were mostly to parents in the same population as the juveniles were collected in, although 3 juveniles were also assigned to parents in the other population. Given that levels of self-recruitment at Schumann have independently been confirmed in larval tagging studies (Jones *et al.* 2005), these additional parent-offspring relationships are most likely errors.

Parentage analysis assumes that all offspring and parents in the data set belong to the same population and LOD scores are estimated using this population's allele frequencies (Gerber et al. 2000). Our results show clearly that significant changes in allele frequencies have major effects on parentage assignments. It is noteworthy that in our study, changes in allele frequencies on parentage assignments increased the number of wrong assignments in higher proportions than they excluded correct assignments (considering that almost all assignments obtained under each separate test were correct). These results suggest that parentage analysis is not appropriate for low gene flow scenarios, where analytical methods such as assignment tests appear to have greater utility.

High gene flow scenario

The degree of population differentiation in Bootless Bay was clearly insufficient for assignment tests to discriminate among subpopulations. The tests failed to assign most of the juveniles to any one of the five subpopulations. Only a small number of individuals were assigned to only one of the sites (13 juveniles) compared to the number of juveniles assigned to at least two sites within the bay (146). Overall, the numbers of recruits assigned to the Bootless Bay metapopulation and to Schumann Island were gross overestimates relative to the parentage analysis. In Bootless Bay, given that Taurama had a small but significant genetic signal, we expected that assignments probabilities from or to this site would be greater than to the other sites. However, we found no difference between the assignment probabilities from juveniles assigned to Taurama and juveniles assigned to the other four sites. Also, from the 13 juveniles assigned to only one site, only three were assigned to the same site as the juveniles assigned by parentage analysis at this level.

The level of accuracy of assignment tests in our study may be lower than those recorded in the literature. For example, using simulated data Cornuet *et al.* (1999) showed that *F*st values as low as 0.01 could yield to ~ 40% accurate assignment with this method. Carreras-Carbonell *et al*. (2007) using microsatellite data on *Tripterygion delaisi* in the NW Mediterranean Sea had a similar problem when attempting to assign individuals to populations that were not genetically different, leaving ~30% of fish with unknown origins unassigned.

Parentage analysis can be considered as the method of choice for estimating retention and connectivity in small, spatially discrete, but genetically similar populations. Unlike assignment tests, they produce high-resolution patterns of self-recruitment and dispersal, and estimates of self-recruitment that have been independently tested in *A. polymnus* using larval marking (Jones *et al.* 2005). Although local genetic heterogeneity was a potential problem, the slight modification of allele frequencies caused by including and excluding the most genetically distinct site at Bootless Bay (Taurama) had little effect on our estimates of parentage within the four other subpopulations. Results for two juveniles apparently produced by parents from Bootless Bay when the analysis was done without Taurama were reversed when Taurama was included. These individuals had LOD scores close to the threshold value and therefore the probability that the identified parent is the right parent is just slightly superior to that of identifying a wrong parent from the population by chance. There were also missing alleles in the genotypes of these juveniles and we therefore suspect that changes in allele frequencies when including Taurama in the analysis had no significant consequences.

This is encouraging because evidence of genetic structure at fine-spatial scale is more common than previously thought in natural populations (Fredsted et al. 2005, Neville et al. 2006, Zamudio and Wieczorek 2007).

The overall levels of self-recruitment and immigration for the two populations as estimated by assignment tests and parentage analysis were very different (Table 4). Assignment tests found 159 juveniles (90.3%) were returning to Bootless Bay populations, and in Schumann Island the self-recruitment estimate was 70%. Parentage analysis, on the other hand, generated self-recruitment estimates of 25% and 31.5% in Bootless Bay and Schuman Island populations, respectively. We believe that estimations of recruitment at this scale based on assignment tests should be treated with caution. When estimating recruitment in marine environments at an ecological level with genetic tools, we assume that the genetic population is larger and extends further than the demographic population under study.

Dispersal can maintain genetic homogeneity over relatively large distances (Fauvelot and Planes 2002) and assignment tests may classify juveniles from the larger genetic population to the local population of interest. The discrepancy between self-recruitment estimates from assignment tests and parentage analysis for Bootless Bay and Schumann Island suggests that close to 65% and 40% respectively of juveniles assigned by GENECLASS2 originated from nearby, genetically similar populations. It is also possible that our parentage analysis has underestimated self-recruitment, because other members of the local populations have yet to be discovered. Other studies of clownfish have shown estimates of self-recruitment as high as 60% (Almany et al. 2007).

Table 4. Comparison of the different estimates obtained with each of the two methods under the high gene flow scenario.

Method	Within sites Self-recruitment	Local connectivity	Overall self recruitment	Immigrants/ unassigned
Bootless Bay				
Assignment	2.2%	5.1%	90.3%	8.5% / 2.2%
Parentage	10.0%	15.0%	25.0%	75.0%
Schumann				
Assignment			69.9%	28.8% / 1.4%
Parentage			31.5%	68.5%

The assignment method performed by GENECLASS2 has the advantage that it takes in to account the possibility of not having sampled all potential populations (Piry et al. 2004). Using this procedure, we found that 8.5% of the new recruits sampled in Bootless Bay and

28.2% of new recruits from Schumann Island came from distinct genetic populations that we failed to characterize. Even if the origin of these juveniles cannot be established, the fact that they were excluded from all sampled populations means that their population of origin is likely to be distant and genetically distinct from the other incorrectly classified individuals, which are likely to have dispersed from nearby populations. The genetically distinct individuals could have travelled long distances before settling on the anemones and therefore correspond to the tail of the distribution of recruitment versus geographic distance. As this information cannot be obtained using parentage analysis, assignment tests may represent a useful technique for defining the tail end of the dispersal kernel. The complementary use of the two techniques may be the best way to define the dispersal kernel as a whole.

Number and polymorphism of loci used

Simulation studies have shown that for a given level of differentiation adding loci usually improves the ability to assign individuals correctly among populations (Cornuet et al. 1999, Waples and Gaggiotti 2006). We found that the quality of loci had a more significant effect than simply the number of loci used. Simulation results have shown that low polymorphic loci produced less accurate assignments than high polymorphic ones (Waples and Gaggiotti 2006), and our results confirmed this situation. This is not surprising since high levels of polymorphism are related to high mutation rates. As gene flow increases, highly polymorphic loci are more informative because new alleles are constantly being generated within subpopulations and shorter times of isolation are needed to detect small population differentiation. At the same time, in parentage analysis, exclusion probabilities are strongly conditioned by the genotypes of the reported relatives, by the frequency of alleles and by the number of loci (Jamieson and Taylor 1997). These exclusion probabilities increase with the number of loci used and their level of polymorphism. In our study, parentage error rates increased more when high polymorphic loci were excluded than they did when low polymorphic ones were excluded, demonstrating again that quality of the loci used is more important than quantity.

Conclusions

While assignment tests perform well at spatial scales over which populations show large genetic differentiation, parentage analysis appears to be a better choice for estimating dispersal at smaller scales among genetically similar populations. Using genetic methods such as assignment tests when trying to measure connectivity at ecologically relevant scales

where migration is high enough to maintain genetic homogeneity remains challenging because these methods still have relatively little power under this circumstances. Parentage analysis on the other hand performs well in conditions of high gene flow. However, incomplete sampling of potential parents can be a major drawback. New likelihood approaches such as the one used in this study need further evaluation to assess this problem. Both techniques appear to lead to overestimates of self-recruitment when applied at scales over which assumptions of the approaches are violated. As parentage analysis appears to be robust to small deviations from panmixia, there may be some intermediate level of differentiation at which both techniques provide useful results. Parentage becomes increasingly difficult to apply as the scale of the study and size of the population increases because the accuracy of assignments relies heavily on the fraction of potential parents sampled. However, more research is needed to explore these new likelihood based parentage methods to quantify their performance under different parental sampling scenarios. Ultimately, a combination of both parentage and assignment tests may be the best way to fully describe dispersal kernels and estimate the scale of demographically important connectivity in marine populations.

Supplementary data:

Table 5. Summary of genetic variation for ten microsatellite loci at five sampling sites in Bootless bay (BA: Loloata Bank, LI: Lion Island, LO: Loloata Jetty, MO: Motopoure Island, Ta: Taurama). **N**, number of analysed individuals; **Na**, number of alleles; **Ho** and **He**, observed and expected heterozygosity respectively; ***F*is**, inbreeding coefficient, significant values ($p<0.05$ after Bonferroni corrections) are in **Bold** capitals.

Pop		**Locus**									
		2A	**10TCTA**	**3GATA**	**44**	**61**	**120**	**65**	**55**	**79**	**45**
BA	**N**	34	34	34	34	34	34	34	34	33	34
	Na	3	14	17	5	7	7	3	5	16	7
	Ho	0.029	0.824	0.882	0.500	0.235	0.765	0.941	0.912	0.697	0.853
	He	0.512	0.792	0.917	0.566	0.649	0.765	0.524	0.658	0.770	0.745
	***F*is**	**0.943**	-0.039	0.038	0.117	**0.638**	0.001	-0.795	-0.386	0.095	-0.145
LI	**N**	27	27	27	27	27	27	27	27	27	27
	Na	4	10	15	7	7	5	4	7	19	8
	Ho	0.037	0.852	0.852	0.556	0.444	0.741	0.667	0.852	0.926	0.926
	He	0.477	0.816	0.912	0.650	0.599	0.715	0.529	0.711	0.903	0.667
	***F*is**	**0.922**	-0.045	0.065	0.146	0.258	-0.035	-0.259	-0.199	-0.025	-0.389
LO	**N**	84	83	83	83	83	84	84	82	82	84
	Na	4	18	22	7	7	7	5	9	20	13
	Ho	0.107	0.831	0.952	0.530	0.337	0.560	0.857	0.939	0.695	0.738
	He	0.318	0.857	0.942	0.605	0.721	0.715	0.598	0.709	0.855	0.754
	***F*is**	**0.663**	0.030	-0.011	**0.124**	**0.532**	0.218	-0.434	-0.324	0.187	0.021
MO	**N**	69	69	69	69	69	69	69	68	69	68
	Na	4	21	21	11	12	7	3	7	21	11
	Ho	0.072	0.855	0.855	0.623	0.391	0.594	0.725	0.956	0.884	0.882
	He	0.395	0.869	0.942	0.673	0.798	0.655	0.510	0.693	0.871	0.697
	***F*is**	**0.816**	0.016	0.092	0.073	**0.509**	0.093	-0.420	-0.380	-0.016	-0.266
TA	**N**	57	57	57	57	57	57	57	57	57	57
	Na	4	18	19	13	12	5	3	7	21	9
	Ho	0.035	0.842	0.965	0.526	0.571	0.737	0.842	0.912	0.842	0.877
	He	0.164	0.852	0.925	0.789	0.724	0.674	0.625	0.723	0.883	0.730
	***F*is**	**0.787**	0.011	-0.043	**0.333**	0.211	-0.093	-0.348	-0.263	0.047	-0.201
Total	**Na**	9	29	23	20	15	13	12	17	29	18

2.2 SAMPLING EFFORT IN PARENTAGE ASSIGNMENTS: HOW MANY LOCI AND WHAT PROPORTION OF CANDIDATE PARENTS ARE NEEDED?

INTRODUCTION

Parentage analysis is a precise form of assignment tests which involves identifying the parents of specific individuals (Manel et al. 2005). Parentage play a central role in the study of diverse ecological and evolutionary topics (reviewed in Jones et al. 2009a) and can be particularly useful for elucidating mating patterns, estimating reproductive success (Rodriguez-Munoz et al. 2010, Serbezov et al. 2010) and larval dispersal in systems with high levels of gene flow (Jones et al. 2005, Christie 2009, Planes et al. 2009, Saenz-Agudelo et al. 2009). Perhaps the first applications of parentage analysis to estimate dispersal were performed in plant biology to obtain estimates of pollen immigration in wild populations (Ellstrand and Marshall 1985). Since then application of these approach to address questions of dispersal has spread to a wide variety of organisms including rodents (Waser et al. 2006, Nutt 2008), insects (Tentelier et al. 2008) and coral reef fish (Jones et al. 2005, Planes et al. 2009, Saenz-Agudelo et al. 2009).

In the last decades a wide range of methods have been developed to infer different kin relationships, the most common ones being parent-offspring, full-sibs and half-sibs. These methods have been classified into six categories: Exclusion, categorical allocation, fractional allocation, full probability parentage analysis, parental reconstruction and sib-ship reconstruction (Manel et al. 2005). Details of each particular approach, their main advantages, disadvantages and associated software packages have been extensively reviewed in recent articles (Jones and Ardren 2003, Jones et al. 2009a) and therefore will not be covered here. Categorical allocation, also known as parentage assignment, is the most commonly used approach to determine patterns of dispersal in natural populations and therefore will be the focus of this section. The principle of categorical allocations consists of using exclusion probabilities in a first step to eliminate any candidate parent who fails to share at least one or more alleles with a given offspring (to account for genotyping error). Then, if complete exclusion fails (when there are more than one candidate parents whose genotypes matches with a given offspring), a likelihood ratio is calculated based on alleles frequencies in the population and the offspring in question is assigned to the candidate parent with the highest likelihood of being the true parent (Jones et al. 2009a). Finally, the significance of this

likelihood ratio can be evaluated statistically, and again different approaches exist to do this (Marshall et al. 1998, Gerber et al. 2003, Anderson and Garza 2006).

One of the advantages of this approach in the context of marine larval connectivity is that it can be applied in situations where neither of the parents is known *a priori* (Marshall et al. 1998), which is often the case in marine species with dispersive larvae where offspring seldom receive paternal care. This approach has previously allowed for direct quantitative estimation of dispersal where potential parents were sampled exhaustively in a particular location and dispersive offspring were tracked back to their place of birth (Planes et al. 2009). However, parentage analysis can have some limitations among which, genotyping errors and the presence of null alleles are where most attention has been addressed. Null alleles and genotyping errors concern all kinds of parentage analysis and are independent of the biological system studied, their implications have been reviewed elsewhere (Marshall et al. 1998, Jones and Ardren 2003, Morrissey and Wilson 2005, Kalinowski et al. 2007) and will not be discussed here. In the context of larval dispersal, two other methodological drawbacks have restricted the use of this approach until now and have obtained less attention from the scientific community. First, parentage assignment assumes that a large fraction (if not all) of the genotypes of all candidate parents in a population is known (Manel et al. 2005). In marine organisms with a larval pelagic phase, population sizes are often large or unknown and exhaustive sampling of potential parents, even in small restricted areas, is unrealistic.

The main aim of this section was to test the accuracy of assignments using the FAMOZ platform and estimate error rates under different scenarios of proportion of parents sampled and number of genetic markers (microsatellites) used and identify at which point increasing the number of genetic markers can compensate for small sample sizes. The accuracy of parentage assignments can decrease drastically when the proportion of candidate parents diminishes, because the probability of assigning false parents when the true parent was not sampled (type II error) increases (Marshall et al. 1998). In addition, some algorithms, like the one implemented in CERVUS also requires that this proportion is known a *priori* (Kalinowski et al. 2007), which is often difficult to estimate in marine populations of organisms with larval dispersal. The methods implemented in the program FAMOZ (Gerber et al. 2003) do not require this *a priori* information and it was the main reason for which it was chosen. However, the relationship between the proportion of sampled candidate parents and statistical errors in this approach has seldom been addressed. Here I used simulated data to explore the relationship between number of genetic markers, the accuracy of assignments

and the proportion of the parental population sampled in two large (n = 500 and n = 1000) simulated populations.

METHODS

Two genotypic data sets were generated using EASYPOP (Balloux 2001). Both data sets were based on a finite island model with 5 subpopulations, each of constant size and equal sex ratio. In the first data set each subpopulation size consisted of 100 reproductive individuals leading to an overall population size of 500 individuals. In the second 200 reproductive individuals per subpopulation were considered which yielded an overall population of 1000 individuals. In both runs at each generation, random mating was simulated to produce a diploid genotype for 20 independent loci for each individual who then had the probability of 0.15 to migrate to another subpopulation. This migration rate was chosen to reflect a scenario of high gene flow (demographic connectivity) among subpopulations, equivalent to 15 and 30 migrants per generation respectively. All loci had the same mutation dynamics which occurred according to the K-allele model (each mutation equally likely to occur at any of K possible sites). Mutation rate (μ) and number of allelic states were considerer to represent highly polymorphic markers like microsatellites ($\mu=1x10^{-4}$, 20 possible allelic states). This mutation rate and number of allelic states are within the ranges published in eukaryotic genomes (Buschiazzo and Gemmell 2006). To attain an approximate mutation-drift equilibrium the simulation was run for 5000 generations (Waples and Gaggiotti 2006). Two genetic data sets were obtained (run1: n_{adults} = 500 and run2: n_{adults} = 1000) and two offspring datasets were created from them using P-LOCI (Matson et al. 2008) as following. In run 1, within each subpopulation, individuals were paired randomly and for each adult pair 4 offspring were generated following mendelian segregation. In run2 the same number of offspring was generated but only half of the adults within each subpopulation where paired and allow to reproduce (to reduce computer analysis time). In both cases the total number of simulated offspring was 1000 (200 per subpopulation).

Parentage assignments were performed using different number of loci and different proportions of sampled parents with the program FAMOZ (Gerber et al. 2003). I tested parentage analysis with 5, 10, 15 and 20 loci and with 20, 40, 60, 80 and 100 % of sampled parents. For each scenario, LOD (log of the odds ratio) scores for parentage relationships were calculated. Then, to decide whether a parent-offspring pair was accepted as true or not, two sets of 10000 offspring were simulated either from genotyped parents or from allele

frequencies. LOD scores of the most likely parents and simulated offspring sets were plotted and the intersection between the distributions of the two sets was taken as the threshold value (parent-offspring pairs with LOD scores higher than threshold were accepted as true pairs). More details on parentage assignment procedures with FAMOZ are available in the section 2.1. Since each true parent-offspring pair was known in advance I was able to estimate type I errors (reject a true parent-offspring pair) and type II errors (accept false parent-offspring pairs that share alleles across all loci by chance knowing that the true parent was not sampled) for each scenario.

RESULTS

For the small data set (n_{adults} = 500) the number of alleles per locus varied from 7 to 14 with a mean value of 10.7 alleles per locus. For the larger data set (n_{adults} = 1000) the number of alleles per loci varied from 10 to18 and the mean value was 14.6 alleles per locus. As expected, both type I and II errors decreased as the analysis were performed with increasing number of loci (Figure 1). Error type I (reject the true parent knowing that it was among the sampled potential parents) was extremely high for all runs with 5 loci, but it was less than 5% for all runs involving 15 and 20 loci. Error type II (assign a parent knowing that the true parent was not sampled) was over 10% for all runs with 5 and 10 loci (with the exception of n = 1000, 80% sampled parents where it was 8.3%). When performing assignments with 15 and 20 loci, type II error differed strongly between the two population sizes. Higher values were obtained for n = 500 with maxima at 20% sampled parents (15 and 6.5% for 15 and 20 loci respectively). For n = 1000, error type II errors were always below 5% with a maximum of 4.2% for 15 loci and 1.7% for 20 loci. In general, when using 20 loci, both errors I and II were low regardless of the proportion of sampled parents and the size of the population.

DISCUSSION

These results show that when dealing with a small fraction of sampled candidate parents, regardless of the number of correct assignments, the total number of observed assigned parent-offspring pairs will be an overestimation of the number of real pairs (due to type II error: assign false parents to offspring whose real parents were not sampled). However the magnitude of this overestimation will vary according to the number of markers involved. Regardless of the size of the parental population, the error linked to sampling a small fraction

of candidate parents can be compensated by increasing the number of loci used. In terms of estimating self recruitment, the overestimation linked to incomplete sampling can be lowered down to reasonable levels using 15 to 20 loci (with only 20% sampled parents, less than 20 and 10% of wrong assignments are achieved with 15 and 20 loci respectively).

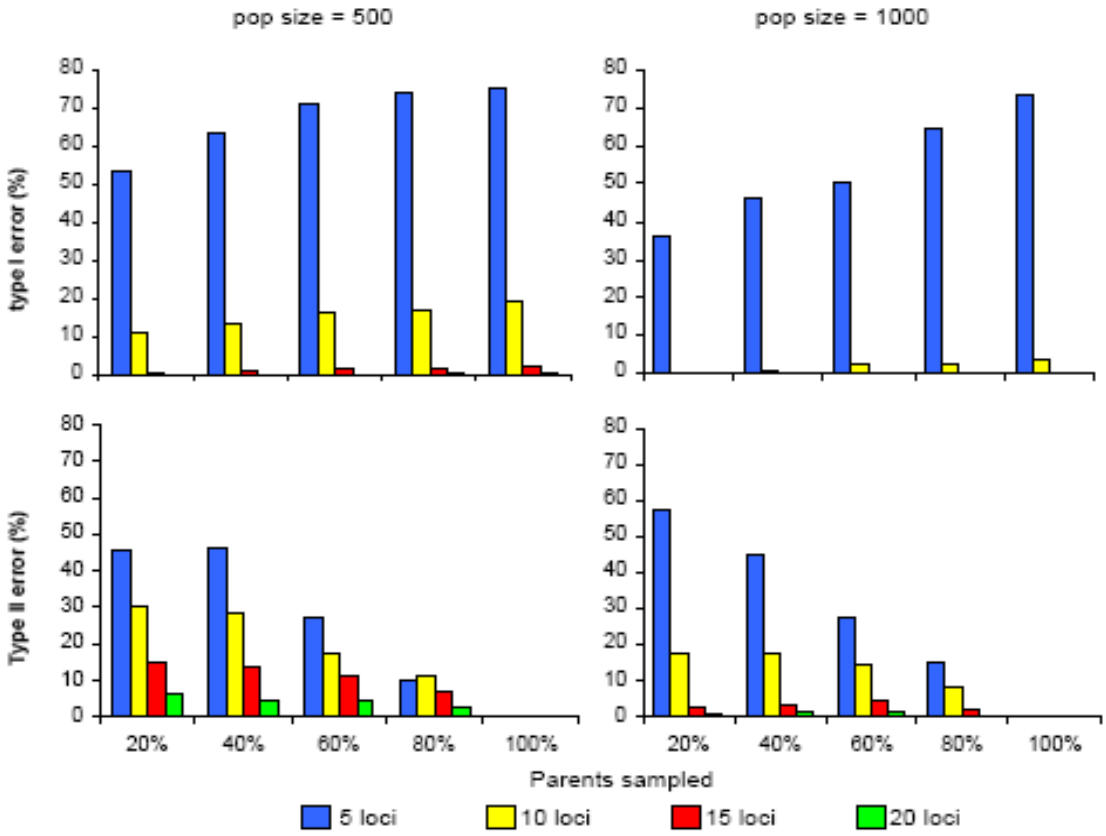

Figure 1 Relationship between statistical errors, number of loci and proportion of candidate parents sampled in parentage analysis for two simulated populations of different sizes (n=500 and n=1000) using the program Famoz

In the present simulations, because mutation rates and population sizes were held constant and selection was ignored, the simulation of a large population (n = 1000) had higher probabilities of creating more neutral genetic variation than the small one (n = 500) and was reflected in the differences in the genetic diversity (number of alleles per locus) between both data sets. In natural populations, despite the fact that population size is never constant, and that genetic drift and selection might be important in shaping genetic diversity, empirical evidence has shown that a positive relationship between genetic variation and population size exists (Frankham 1996, Leimu et al. 2006, White and Searle 2007). In the context of using parentage assignments in natural populations, this positive relationship means that there can be a trade off between population size, sampling effort and accuracy of tests. These results show that in a small population with relatively low genetic variation, more genetic markers

are needed to achieve the same statistical power in parentage assignment tests than in a larger population where genetic variation is higher. On the other hand, in practical terms, the same sampling effort would yield a higher percentage of candidate parents in a small population than in a large one and could compensate to some extent for the lower genetic variation. In the context of marine larval dispersal, it seems that performing preliminary pilot studies to evaluate the number and degree of variation of genetic markers available and the proportion of sampled parents with a given sampling effort will help make decisions in terms of the best strategy (in terms of sampling effort both of individuals and genetic markers) that will yield the best statistical power and cost/benefit ratio.

In cases where the number of genetic markers available is limited, have low variability, and there are no possibilities of increasing their numbers, other methods such as full probability parentage analysis that include non genetic information in their algorithms might provide an alternative option (if this non genetic information is available) (Hadfield et al. 2006). In this kind of approach spatial or behaviour information can be included in the model and compensate the accuracy of the assignments if the genetic data is weak (e.g. change the assumption that all candidate parents are equally likely to be the true parents by taking in to account relevant ecological information). Yet, obtaining large numbers of highly variable genetic markers is no longer unaffordable or extremely time consuming given the recent advances in molecular technology and the application of genetic data-only parentage methods can provide a valuable tool to study dispersal in non-model species. Finally, parentage analysis can be a powerful tool to uncover patterns of dispersal in scenarios with high migration where indirect methods fail (Waples and Gaggiotti 2006). The direct dispersal information gained from this approach could be an important complement of population genetic models (Manel et al. 2003, Luikart et al. 2010) and coupled with ecological or remote-sensing data and biophysical models will definitely help to better understand marine larval dispersal (Christie 2009).

Based on the results from this chapter indicating that approximately 25% of the parents contributing to total recruitment could be sampled (section 1) with a reasonable sampling effort and based on the simulation results presented in this section, I decided to increase the initial number of microsatellite markers (9). After testing several potential loci, nine additional microsatellites were obtained, yielding a total of 18 loci that were used in all the following analyses.

CHAPTER 3

DEMOGRAPHIC CONNECTIVITY: SPATIAL AND TEMPORAL VARIABILITY

This chapter is built upon the results of chapter 2 and is also divided in two sections. Some of the same locations and ideas presented in section 1 of chapter 2 are also shared here. However, while chapter 2 focused on methodological issues, in this chapter these are left behind and all the importance is given to larval connectivity *per-se.* Also the spatial scale of the study was more than doubled. The first section of this chapter deals with spatial connectivity patterns from individual reefs to the metapopulation as a whole. The second section compares focuses on temporal variation by comparing estimates of local connectivity measured on the same locations over two consecutive years.

3.1 SPATIAL PATTERNS OF CONNECTIVITY AND SELF RECRUITMENT IN A COASTAL REEF FISH METAPOPULATION

Publication: Saenz-Agudelo, P., G. P. Jones, S. R. Thorrold, and S. Planes. Connectivity dominates larval replenishment in a coastal reef fish metapopulation. *Proceedings of the Royal Society B.* **In Press.**

ABSTRACT

Direct estimates of larval retention and connectivity are essential to understand the structure and dynamics of marine metapopulations, and optimize the size and spacing of reserves within networks of marine protected areas (MPAs). For coral reef fishes, while there are some empirical estimates of self-recruitment at isolated populations, exchange among sub-populations has been rarely quantified. Here microsatellite DNA markers and a likelihood-based parentage analysis were used to assess the relative magnitude of self-recruitment and exchange among 8 geographically distinct sub-populations of the panda clownfish *Amphiprion polymnus* along 30 km of coastline near Port Moresby, Papua New Guinea. In addition, I used an assignment/exclusion test to identify immigrants arriving from genetically distinct sources. Overall, 82% of the juveniles were immigrants while 18% were progeny of parents genotyped in our focal metapopulation. Of the immigrants, only 6% were likely to be genetically distinct from the focal metapopulation, suggesting most of the connectivity is among sub-populations from a rather homogeneous genetic pool. Of the 18% that were progeny of known adults, two thirds dispersed among the 8 sub-populations and only one third settled back into natal sub-populations. Comparison of our data with previous studies suggested that variation in dispersal distances is likely to be influenced by the geographic setting and spacing of sub-populations.

INTRODUCTION

Most populations of marine organisms are likely to function as metapopulations where numerous sub-populations are connected to varying degrees by larval dispersal (Kritzer and Sale 2004, Sale et al. 2005a, Figueira and Crowder 2006). Estimates of the magnitude of retention within and connectivity among sub-populations is essential to understand natural metapopulation dynamics (e.g. Levin 1974, Armsworth 2002, Hixon et al. 2002) and model human impacts on marine ecosystems (Hughes et al. 2005). In addition, the efficacy of management strategies, such as no-take marine reserve networks, depends on how individual reserve populations function and how they are connected to the metapopulation at larger scale (Botsford et al. 2009, Jones et al. 2009b). How reserves function depends on the degree to which reserves are self-sustaining, are connected to reefs open to fishing and are connected to other reserves in the network (Sale et al. 2005a, Mora et al. 2006, Jones et al. 2009b). These functions cannot be confirmed without quantifying patterns of retention within and connectivity among reef populations. While the nature of demographic connectivity among reef populations is beginning to be described (reviewed by Botsford et al. 2009), the factors that shape variation remain poorly understood.

The metapopulation concept is particularly applicable to coral reef organisms with pelagic larvae, as adult populations are usually restricted to discrete patches of reef habitat (Kritzer and Sale 2004, Jones et al. 2009b). Recent empirical studies have revealed that local replenishment of coral reef fishes is significantly higher than previously envisaged (Jones et al. 1999, Swearer et al. 1999, Jones et al. 2005, Almany et al. 2007, Planes et al. 2009). However, in all these studies a significant proportion of the newly settled juveniles originated from locations beyond the spatial extent of focal populations. Coupled biophysical models have suggested that ecologically relevant larval dispersal in reef fishes occurs over scales of 10 to 100 kilometers in the Caribbean Sea (Cowen et al. 2000, Cowen et al. 2006) and along the Great Barrier Reef (James et al. 2002). These modeling studies have also predicted that levels of self-recruitment may be highly variable among reefs. Testing these model predictions requires estimates of retention within and connectivity among sub-populations on a larger scale than has previously been available.

Empirical connectivity studies have suggested that variations in dispersal distance among species are more likely to be influenced by geographic isolation and spacing of reefs than individual species characteristics (Jones et al. 2009b). Modeling studies have provided some support for this idea, with lower simulated self-recruitment of reef fish species along an

extensive system of barrier reefs (James et al. 2002) than on more isolated oceanic reefs in the Caribbean (Cowen et al. 2006). However, field data on population connectivity remains insufficient to test the accuracy of these simulated dispersal outcomes. The empirical studies conducted to date, using otolith chemistry (Swearer et al. 1999), mass marking of larvae (Jones et al. 1999, Jones et al. 2005, Almany et al. 2007), and DNA parentage analysis (Jones et al. 2005, Planes et al. 2009), have primarily been limited to estimating levels of self-recruitment within populations. While one study has documented dispersal from a small island to distant reefs (Planes et al. 2009), we have no direct quantitative estimates of connectivity in situations where sub-populations are distributed among several sites with suitable habitats.

The aim of this study was to apply parentage analysis and assignment tests based on hyper-variable microsatellite DNA markers to investigate self-recruitment and connectivity among subpopulations using as model the panda anemonefish (*Amphiprion polymnus*) in Bootless Bay, Papua New Guinea. The approach was based on the identification of offspring produced by genotyped parents. Natal origins of recently settled recruits can then be determined providing the location of the parents are known or can be assumed at the time of conception. Parentage analysis based on microsatellite markers has been validated in two species of anemonefishes, *Amphiprion polymnus* (Jones et al. 2005) and *Amphiprion percula* (Planes et al. 2009), by comparing the results with those obtained by simultaneous use of chemical tagging techniques on the same individuals. These data represent the first direct estimates of self-recruitment and connectivity among geographically isolated subpopulations of a coral reef fish.

METHODS

Study species and location

The panda clownfish (*Amphiprion polymnus*) is a southeast Asian endemic that lives in close association with discrete aggregations of two species of anemone (*Stichodactyla hadonni* and *Heteractis crispa*) occurring in sandy habitats associated with coral reefs (Fautin and Allen 1992). Each anemone is usually occupied by one breeding pair and up to eight smaller non-breeders and juveniles. The female (the largest individual) lays demersal eggs on the upper surface of shells or dead coral next to the anemone. Embryos develop over a period of 6-7days before hatching (Fautin and Allen 1992) and post-larvae settle into anemones after a pelagic larval phase lasting 9-12 days (Thresher et al. 1989).

The study location encompassed Bootless Bay and an area of coast adjacent to Port Moresby, Papua New Guinea. This area supported a metapopulation of 8 spatially discrete subpopulations (termed *sites* to avoid confusion with other subpopulation definitions) (Figure 1). Distances among sites varied from 1 to 30 km. With the exception of Fisherman Island (FI) anemones within each site were confined to a ~1ha patch of shallow sand and seagrass. At each site (except for FI), an exhaustive search for all anemones colonised by *A. polymnus* was performed prior to tissue collections. The population of Fishermen Island (FI) was spread over a larger area and it was estimated that near 50% of this population was sampled. In total, 215 anemones hosting *A. polymnus* were found among the 8 sites (Figure 1).

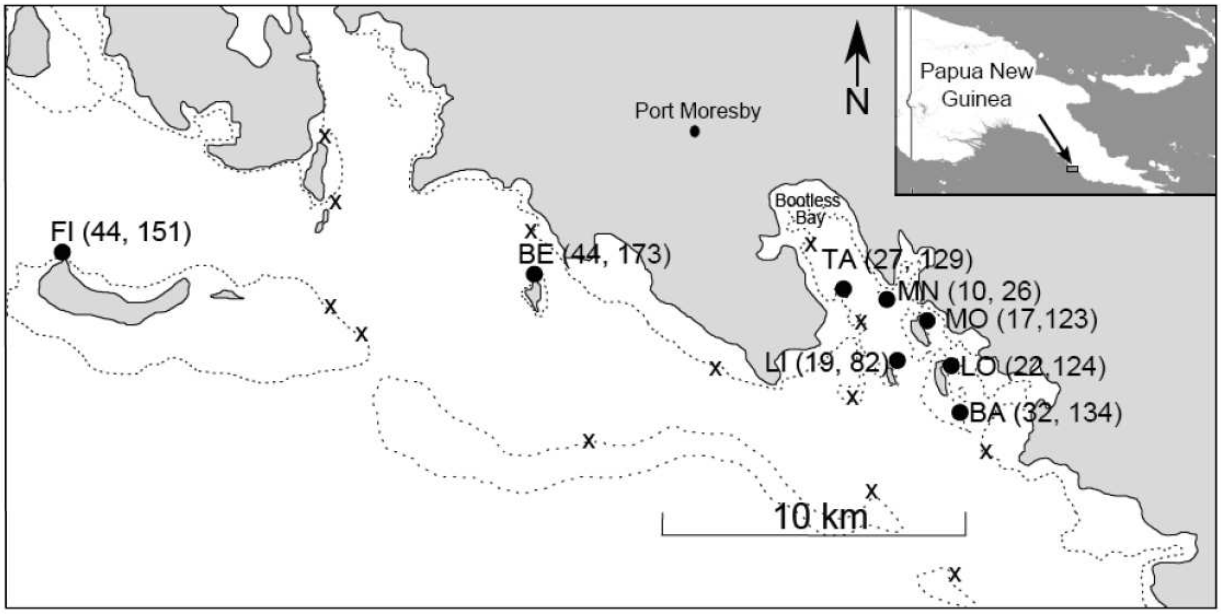

Figure 1 Map showing sites of the 8 sites of anemone aggregations hosting *Amphiprion polymnus* in Bootless Bay area (white filled circles). Crosses (x) indicate sites outside Bootless Bay with potential suitable habitat that were explored but no *A. polymnus* were found. The number of anemones and sampled *A. polymnus* at each site are indicated in brackets. Inset: Location of Bootless Bay in Papua New Guinea. Site abbreviations are as follows: Manubada Island (BE), Lion Island (LI), Taurama (TA), Motupore North Patch reef (MN), Motupore Island (MO), Loloata Island (LO), Loloata South Bank (BA) and Fishermen Island (FI).

Sampling and genotyping

A total of 942 individuals were sampled among the 8 sites between January and April 2008. Each fish was captured by SCUBA using hand nets, measured (total length TL), fin clipped underwater *in situ*, and then released back onto the same anemone. Fish that were too small to be fin clipped (less than 30mm) were collected. In addition, all juveniles settling on each anemone over the sampling period were captured using hand nets. Finally, at the end of the experiment 15-30 fertilized eggs were collected (randomly within the clutch) from 5 egg clutches, each from a different anemone. All samples were preserved in 95% ethanol and

returned to the laboratory for subsequent genotyping. For all analyses fish were divided into 3 categories according to their size. The first category 'breeders' consisted of the female and male (the two biggest individuals) of each anemone. The remaining fish were then divided into 2 arbitrary categories: 'non-breeders' (>50mm) and 'juveniles' (< 50mm).

Details of the 18 microsatellite loci and genotyping procedure are described in Quenouille et al. (2004) and Beldade et al. (2009b). After DNA extraction, 3 multiplex polymerase chain reactions (PCRs) were performed per individual, using fluorescently-labelled primers to process 18 microsatellite loci containing a mixture of dimer and tetramer repeats. PCR products were processed on a Beckman Coulter sequencer CEQ 8000 Genetic Analysis System and the resulting electropherograms were scored manually. Uncertainties were reconciled by re-amplification and comparison. Alleles were scored as PCR product size in base pairs. Allelic frequency and expected heterozygosity under Hardy Weinberg equilibrium were calculated for each locus in GENALEX version 6 (Peakall and Smouse 2006). Tests for Hardy-Weinberg and linkage disequilibrium were conducted using GENEPOP 3.4. (Raymond and Rousset 1995) and significance levels were adjusted with sequential Bonferroni corrections for multiple tests with $P< 0.05$. All 18 loci satisfied Hardy-Weinberg and linkage disequilibrium assumptions.

Population structure

Genetic variability within and among sites and between resident breeders, non-breeders and juveniles was estimated using *F* statistics via analysis of molecular variance (AMOVA) in Arlequin v 3.11 (Excoffier and Lischer 2010). Tests for statistical significance for all estimates were based on 10^4 random permutations, and significance levels were adjusted with a sequential Bonferroni correction for multiple tests.

Parentage analysis

Parentage analysis was performed using FAMOZ (Gerber et al. 2003). The algorithm in this package calculates Log of the odds ratio (LOD) scores for parent-offspring relationships and constructs statistical tests for parentage assignment. Tests are based on simulations that generate offspring from genotyped parents (H_0: the most likely parent is the true parent) or from allele frequencies in the population (H_1: the most likely parent is not the true parent). For each analysis, allelic frequencies were estimated from the 942 genotyped individuals and these estimations were assumed to match the true allele frequencies in the population (Gerber et al. 2003). Then, simulations of sets of 10^4 juveniles were carried out under the two possible

hypotheses (H_0 and H_1 above) and subsequent statistical tests were constructed to decide whether a given parent would be selected as the true parent or true parent pair. The distribution of the simulated LOD scores under the two hypotheses was plotted and the intersection between these distributions was designated as the threshold decision value (individuals with LOD scores above the threshold value were accepted as true parents). FAMOZ also allows for the introduction of an error rate in the LOD score calculation that takes into account genotyping errors and null alleles (Gerber et al. 2000). Introduction of this error, even if it underestimates the real error rate, can reduce type I and II errors related to the parentage tests (Gerber et al. 2000, Morrissey and Wilson 2005). I evaluated four different error rates and chose the best compromise between introduced error and type I and II statistical errors. An error rate of 10^{-3} yielded the lowest statistical type I and II errors (0.10% ± 0.04 and 4.2% ± 0.4 respectively) and was used for all further parentage analyses. Tests evaluations were done using the software option "parentage test simulation". I performed 30 test simulations for each introduced error rate to estimate mean type I and II statistical errors.

All loci showed Mendelian segregation after comparing 36 successfully genotyped eggs of 5 different clutches (from each sampled egg clutch, 8 eggs were randomly sub-sampled and screened for 18 loci) with the respective genotyped parents. None of the 942 screened individuals shared the same diploid genotype. Anemonefish are considered monogamous with only the two biggest fish (breeders) been reproductively active in the fish colony (Fautin and Allen 1992). However, this data set was used to test whether some non-breeder fish were contributing to offspring production in this population. In this preliminary test, all parentage assignments consisted of breeders. None of the sub-adults (non-breeders) was associated with a breeder of the same anemone as the most likely parent pair of any of the juveniles in the sample. However, a few non-breeders were assigned as single parents to juveniles. Given the nature of these assignments I considered them to be more likely full sib or half sib rather than parent/offspring relationships. The presence of full sib or half sib relationships can lead to false positive parent-offspring assignments and significantly bias parentage analysis (Marshall et al. 1998, Jones and Ardren 2003). Therefore to eliminate this source of error a second and final parentage analysis was performed using only breeders as potential parents.

Assignment Test

I used Geneclass2 (Piry et al. 2004) to assign or exclude juveniles from the Bootless Bay population (AMOVA analysis revealed no significant genetic differences between sites,

therefore all sites were considered as one single genetic pool, see results for details). This approach does not assume that the true candidate population has been sampled and can be advantageous in situations where it is not possible to sample all potential populations (Cornuet et al. 1999). Genotypes of all breeders and non-breeders (n = 451) were used as the reference population (assuming a single population, see *population genetic structure* below for details). The likelihood that a new recruit came from the Bootless Bay population was computed with the partially Bayesian criterion of Rannala and Mountain (1997). Then, this likelihood ratio was compared to a distribution of 10^4 genotypes simulated ratios from the reference population with a Monte Carlo algorithm (Paetkau et al. 2004). A new recruit was determined to have originated from a different population when the probability of exclusion from Bootless Bay was > 95% ($P<0.05$).

RESULTS

Population genetic structure

There was no significant genetic differentiation among the 8 sub-populations. The global *Fst* was low (*Fst* = 0.0011) and not significantly different from zero. Pairwise F_{ST} values among all samples were low (<0.0106) and only one out of 120 pair-wise comparisons was significantly greater than 0 after Bonferroni corrections (see annexe, Table 2). It was concluded that the 8 sites were one single genetic pool for all following analyses.

Evaluation of parentage assignment

Parentage analysis assigned 100 juveniles, from a total of 491 that were genotyped, to a sampled parent or parent pair from one of the eight sites. Almost half (45%) of these recruits were assigned independently to both the male and female in the same anemone, while the remaining recruits (55%) were assigned to a single parent. I excluded from further analysis all juveniles assigned to only one parent that presented two or more confirmed mismatches between their genotypes and that of the assigned parent (11 juveniles). The remaining 89 recruits were accepted as being true offspring of the parents to which they were assigned. No juveniles were assigned to two parents from different anemones. Overall, missing values accounted for 1.5 % of the genetic data.

Self-recruitment and connectivity

Local recruitment (n = 89) accounted for 18.2 % of total recruitment (n = 491) to the focal population (Table1, Figure 2). Of these local recruits, 35 (7.1%) individuals settled into

anemones at the same site as their parents (self recruits) while 54 (11.1%) settled in a site other than their natal anemone site (local connectivity). At the site level self-recruitment averaged 7.5% across all sites, but with variability among sites, ranging from 0% at LI site to 27% (16 of 59 individuals) at TA. The number of juveniles that settled in a given site but came from a different site than that of their natal anemone (local connectivity) averaged 12.3% and varied among sites from 5.7% (4 of 70 individuals) in site BA to 20% (2 of 10 individuals) in site MN (Table 1, Figure 2).

Table 1 *Amphiprion polymnus* connectivity matrix among 8 sub-populations in and nearby Bootless Bay, calculated by identifying the natal origins of juveniles using parentage analysis. Numbers in brackets on the Source sites names correspond to the number of breeders that were sampled at each site. The numbers on brackets on the sink sites correspond to the number of juveniles sampled at each site. LD indicates the number of juveniles sampled at each site that had an exclusion probability >0.95 to belong to the genetic pool of Bootless bay and classified as long distance immigrants. In the last two columns, %SR corresponds to the percentage of self-recruitment and %LC to the percentage of local connectivity.

		Source site										
		BA (57)	LO (37)	MO (29)	TA (48)	LI (31)	MN (13)	BE (57)	FI (62)	LD	% SR	%LC
Sink site	BA (70)	4	--	1	1	--	1	1	--	10	5.7	5.7
	LO (69)	3	3	2	1	1	2	1	1	2	4.3	15.9
	MO (70)	1	3	1	3	2	2	--	1	1	1.4	17.1
	TA (59)	--	--	1	16	1	1	--	1	3	27.1	6.8
	LI (42)	1	1	--	3	--	--	1	1	3	0	16.7
	MN (10)	1	--	--	--	--	1	1	--	--	10.0	20.0
	BE (102)	3	1	1	1	1	1	7	1	8	6.8	8.8
	FI (68)	--	--	2	2	--	--	1	3	4	4.4	7.3
	Total (490)	13	8	8	27	5	8	12	8			
	Average										7.5	12.3

Larval dispersal was examined as a function of linear distance among sites for those individuals identified by DNA parentage analysis as being offspring of breeders from the focal metapopulation (Figure 3). Linear distances among sites were grouped in classes (classes' sizes of 2 km each), with self-recruitment considered a separate class.

Approximately 68% of locally-spawned recruits (~12.4% of all juveniles) settled within 3 km of their natal site and 75% of these recruits (~13.5% of all juveniles) settled within 7 km of their natal site. The last 25% of the juveniles identified by the parentage analysis (4.7% of all juveniles) dispersed between 7 and 28 km away from their site of origin. The multimodal dispersal distribution of juveniles was similar to the frequency of linear distances among the 8 sites (Figure 3). Nonetheless, the frequency distributions of juveniles dispersal distances and site distances were significantly different (chi-square = 20.04, df = 9,

P< 0.05). I found that higher numbers of larvae recruited back to their natal sites, with concomitantly lower numbers of larvae dispersing longer distances than predicted based on the distributions of distances among sites.

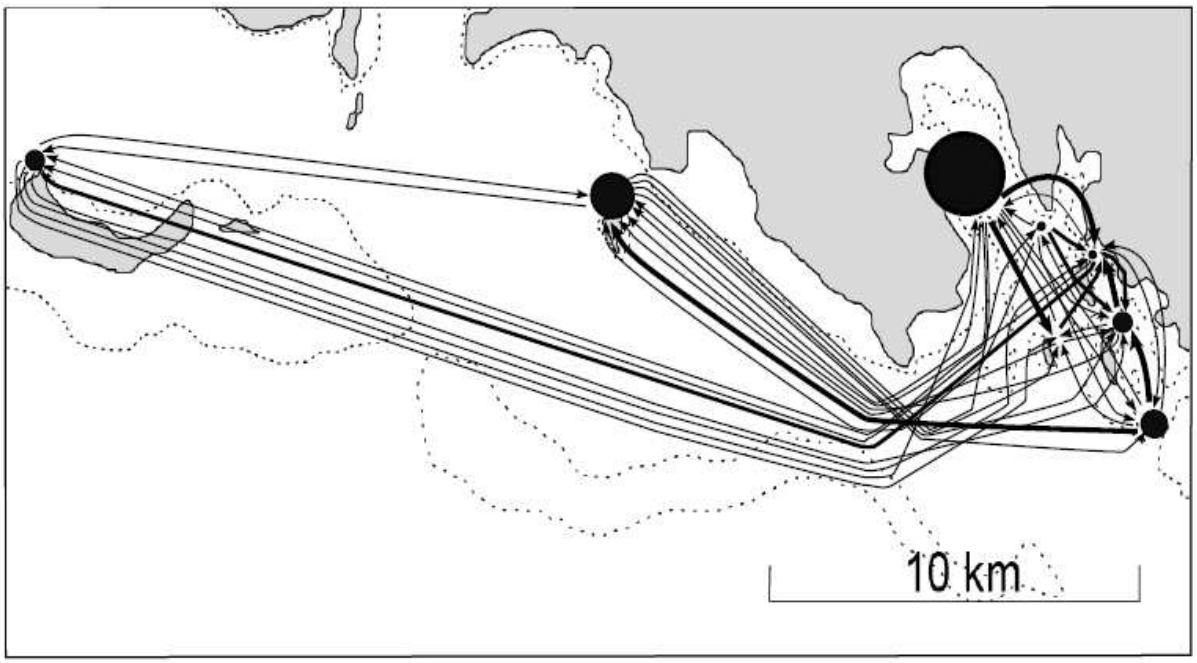

Figure 2 Zoomed map of Bootless Bay area showing each one of the 89 individual trajectories (arrows) of *A. polymnus* juveniles that were assigned by parentage analysis. Self recruitment is represented by black circles. Thickness of arrows and diameter of circles are proportional to the number of juveniles with similar trajectories. For more details about individual trajectories see table 1.

Assignment tests revealed that 31 of 491 juveniles had a probability <0.05 of being from the same genetic pool as the focal metapopulation. These individuals likely came from one or more genetically distinct populations and accounted for 6.3% of total recruitment. Altogether, parentage analysis and assignment tests accounted for 24.5% of sampled juveniles. The remaining recruits ~75 % were sourced from a similar gene pool to that of the focal metapopulation but we can infer little more about the origin and dispersal distances of these individuals.

DISCUSSION

This study provides the first direct estimates of self-recruitment and demographic connectivity among multiple subpopulations in a coastal coral reef metapopulation. Our results indicated that larval retention within the metapopulation was dominated by local exchange among sites, rather than self-recruitment at the site level. At the other extreme, a

small number of individuals came from one or more genetically distinct populations, presumably well beyond the geographic boundaries of our study. The majority of the recruits were genetically indistinguishable from the focal metapopulation, but did not match any of the breeders that we genotyped. Because the sampling within the focal metapopulation was fairly complete, we hypothesize that most of these juveniles represent dispersal from other non-sampled sites along the adjacent coastline.

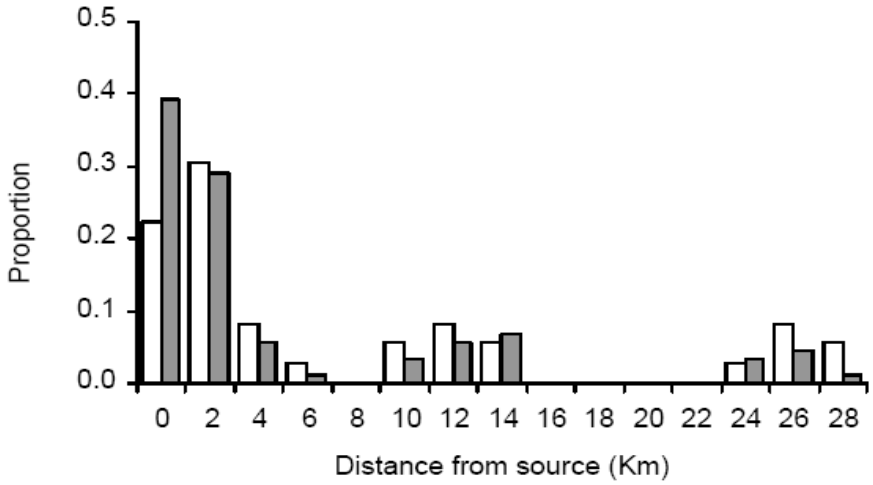

Figure 3 Distribution of the frequency of distances among sites (white bars) and frequency of newly settled juveniles (solid bars) according to the estimated dispersal distance obtained from parentage analysis. Labels on the x axis correspond to the mean value of the distance classes. Note that the zero (0) distance class represents juveniles that settled in the same site as their parents (self recruits)

Compared to our previous study in this location (Saenz-Agudelo et al. 2009), by doubling the number of microsatellite markers used, we reduced the statistical errors linked to likelihood based parentage assignments to less than 5% (both type I and II errors based on simulated data). In addition, we were able to increase substantially the spatial scale and provide for the first time direct estimates of larval exchange among subpopulations spaced up to ~28 km from each other. At this geographic scale, levels of self-recruitment were highly variable among sites, but sites with higher numbers of breeders tended to have more self-recruits than sites with fewer breeders (Table 1). The exception was site TA, which had by far the highest level of self-recruitment despite not representing the largest breeding population. Site TA was located in a relatively protected location close to the head of the bay, while all the other sites with larger breeding populations were outside the bay (BE and FI) or in more exposed locations (BA). Interestingly, in terms of proportions, the site with the second highest self-recruitment rate was MN, a site with a small breeding population also sheltered within the head of the bay. Larvae spawned at these sheltered sites (TA and MN) would therefore

likely be less susceptible to advection by alongshore current flows than larvae from more exposed locations outside Bootless Bay. In addition, the proportion of larvae locally spawned that recruited to their natal sites was over-represented compared to the proportion expected based on the distribution of distances among sites. However, almost half of these self-recruiters were from site TA, indicating that shorter dispersal distances may be a feature of the most protected sites in coastal embayments. Overall, the frequency distribution of known dispersal trajectories appears to be largely explained by the geographic spacing, location and size of the subpopulations. Certainly, the different modes in this distribution coincide with the frequency of spacing between sites.

The high variation in levels of self-recruitment among sites, and the relationship between self-recruitment and population size is consistent with the model of James et al (2002) for the Great Barrier Reef whereby large reefs contributed more than smaller ones to the local larval pool. Our mean estimate of self-recruitment per site (7.5%) is similar to mean simulated values among 321 relatively continuous reefs along the Great Barrier Reef. In their simulations, James and co-workers estimated that virtual larvae returning to their natal reef comprised less than 10% of the settling cohort for most of the reefs. While local retention of larvae may be an advantage in environments were habitat is limited or separated by great distances (Jones et al. 2009b), this advantage may not be extended to situations where habitats are more continuously distributed as in Bootless Bay. Particular sites, with high replenishment rates, such as TA site in this study, could play a crucial role in sustaining the stock in the entire metapopulation (Armsworth 2002, Lipcius et al. 2008).

The coastal geographic setting may be critical in explaining the low self-recruitment pattern of our focal clownfish metapopulation. In the present study, levels of self-recruitment at both 'site' (ranged from 0 to 27%, average 7.5%) and 'metapopulation' level (18%) were relatively low compared with published values for *A. polymnus* and other clownfish species (*A. percula*) at more isolated locations in Kimbe Bay (Papua New Guinea) (Jones et al. 2005, Almany et al. 2007, Planes et al. 2009). These values also correspond to the lowest empirical estimate of self-recruitment measured so far among coral reef fishes (reviewed in Jones et al. 2009b). However, our estimate of self recruitment at the metapopulation level for 2008 (18%) is close to that of our previous estimate of 25% obtained at a smaller spatial scale in Bootless Bay (excluding MN, BE and FI) sampled in 2005-2006 (Saenz-Agudelo et al. 2009), suggesting that these results are not atypical of this region and that the geographic settings do have an important role in determining the observed dispersal pattern.

In contrast of low self recruitment estimates in Bootless Bay for *A. polymnus*, Almany and colleagues (Almany et al. 2007) reported consistent high self recruitment rates in Kimbe Island for two species with contrasting life-history characteristics (*Amphiprion percula*: benthic eggs and ~11days of Pelagic Larval Duration (PLD) and *Chaetodon vagabundus*: pelagic eggs and ~38 days of PLD). Both *Amphiprion* species have similar life-history characteristics and differences between studies in Bootless Bay and Kimbe Island suggest that, at ecological time scales, dispersal kernels may be more influenced by the relative isolation or geographic setting of the focal populations than species specific life-history characteristics (Pinsky et al. 2010). Still, this trend clearly needs to be tested in more species and locations before any conclusion can be made. Besides, other studies based on geochemical signatures in otoliths suggest that this is not a general rule. Patterson et al. (2005) showed that *Pomacentrus coelestis* on Lizard Island exhibited 75% self-recruitment even though it has many other reefs relatively close by, while Patterson and Swearer (2007) showed that *Coris picta* exhibited 26-65% self-recruitment on isolated Lord Howe Island. However, comparisons made between studies that use different approaches to estimate self-recruitment should be made cautiously until these can be cross-validated (Jones et al. 2009b).

Parental analysis suggested that most sites received a higher proportion of recruitment from larvae spawned at different sites within the metapopulation than from self-recruitment. This high connectivity among sites was likely underestimated, in particular that between the inside and outside of Bootless Bay, as it was not possible for us to exhaustively search all potential areas outside of the Bay. This lack of sampling presumably explains a significant proportion of the ~300 juveniles that settled in our study area and were left unassigned either by parentage analysis or assignment tests. It seems that a much larger sampling effort along the coast line will be necessary to find the origin of those juveniles.

Assignment tests detected that a non negligible percentage (6.3%) of the juveniles sampled in this location were genetically distinct from the focal metapopulation. We hypothesize that these recruits were long distance immigrants, but unfortunately, even if this was confirmed, we could not estimate how far these juveniles had travelled. This would require much more extensive sampling of genetic signatures at greater distances to the east and west of Bootless Bay. If indeed these genetically distinct recruits are long distance immigrants they may play an important role in buffering extinction risk in this metapopulation (Hill et al. 2002). However, the fact that these individuals apparently belonged to a different genetic pool suggests that either we have fortuitously captured a very rare dispersal event, or that the juveniles that we collected would not have successfully

reproduced if we had not captured them. This is because a constant exchange of this magnitude with successful reproduction of these individuals should lead to homogenization of these genetic pools (Waples and Gaggiotti 2006). The question that remains is how variable this contribution is over time and whether or not these individuals are capable of successfully integrating into their new population.

In conclusion, given the relatively low observed self-recruitment rates, a high proportion of connectivity among sites, and the relatively high proportion of long distance dispersal, it appears that connectivity and not self-recruitment dominates larval replenishment in this focal clownfish metapopulation. We found that 18% of juveniles in Bootless Bay settled between 0 and 28 km from their place of origin while over 80% were likely to have dispersed from populations beyond our studied sites. These results have significant implications for the design of MPA network in this area as they indicate that a single MPA inside Bootless Bay may not be sufficient to maintain the metapopulation if unprotected sources were to collapse. In addition, while there is consistent evidence that life-history characteristics of individual species can play an important role in terms of dispersal at evolutionary (genetic) time-scales (Rocha et al. 2002, Beldade et al. 2009a, Weersing and Toonen 2009, Reece et al. 2010), the suggestion that the spatial distribution of suitable habitats may have more impact on levels of demographic connectivity than life history characteristics of individual species clearly deserves more attention in future studies. If this happens to be true, it will have encouraging implications for the use of MPAs to offer protection to coral reef fish assemblages (McCook et al. 2009). Testing this hypothesis at more locations, and on more species, remains a top priority for conservation biologists working in coral reef ecosystems.

Annex: Table 2. Pair-wise *Fst* values for all sample sites (below diagonal) and corresponding P-values after 10 000 randomizations (above diagonal). Sample sizes are indicated in parenthesis in the second row. Significant tests after Bonferroni correction are indicated in bold. Last letter for each site code: A (adults, includes breeders and non-breeders), J (juveniles). Negative values are reported as 0.

	BA-A (64)	BE-A (70)	FI-A (82)	LI-A (41)	LO-A (55)	MN-A (16)	MO-A (52)	TA-A (70)	BA-J (71)	BE-J (102)	FI-J (67)	LI-J (42)	LO-J (69)	MN-J (10)	MO-J (70)	TA-J (59)
BA-A		0.4548	0.2604	0.2006	0.3537	0.5217	0.6650	0.0038	0.5935	0.2968	0.0810	0.8141	0.4439	0.6193	0.3929	0.1349
BE-A	0.0002		0.5771	0.2368	0.5639	0.1381	0.1897	0.0058	0.4220	0.3685	0.6801	0.2763	0.6484	0.5027	0.6199	0.0219
FI-A	0.0007	0.0000		0.0205	0.0250	0.2403	0.0852	0.0103	0.1829	0.4708	0.3394	0.2248	0.6086	0.6462	0.2676	0.3222
LI-A	0.0015	0.0012	0.0039		0.4552	0.1843	0.3113	0.0148	0.2901	0.2426	0.4239	0.2498	0.3044	0.8245	0.5022	0.0253
LO-A	0.0006	0.0000	0.0031	0.0001		0.1420	0.6922	0.0021	0.4001	0.4022	0.4523	0.4790	0.4939	0.8332	0.7254	0.0113
MN-A	0.0000	0.0040	0.0022	0.0035	0.0040		0.1044	0.0105	0.1159	0.2659	0.0357	0.0773	0.3942	0.2193	0.0739	0.0336
MO-A	0.0000	0.0013	0.0021	0.0008	0.0000	0.0047		0.0146	0.7691	0.2491	0.2301	0.4645	0.6256	0.8966	0.6831	0.0748
TA-A	0.0045	0.0042	0.0032	0.0047	0.0051	0.0106	0.0039		0.0328	0.0013	**0.0003**	0.0162	0.0146	0.7208	0.0135	0.4091
BA-J	0.0000	0.0001	0.0009	0.0009	0.0002	0.0042	0.0000	0.0027		0.4716	0.3095	0.3768	0.4257	0.8191	0.4195	0.2367
BE-J	0.0005	0.0003	0.0000	0.0010	0.0003	0.0017	0.0007	0.0042	0.0000		0.6703	0.1019	0.4423	0.6284	0.2295	0.0673
FI-J	0.0020	0.0000	0.0004	0.0002	0.0000	0.0066	0.0010	**0.0066**	0.0005	0.0000		0.5201	0.2952	0.8131	0.2105	0.0082
LI-J	0.0000	0.0010	0.0013	0.0013	0.0000	0.0058	0.0000	0.0045	0.0003	0.0020	0.0000		0.3457	0.7712	0.4928	0.0416
LO-J	0.0001	0.0000	0.0000	0.0008	0.0000	0.0005	0.0000	0.0036	0.0001	0.0001	0.0006	0.0005		0.6405	0.6888	0.1089
MN-J	0.0000	0.0000	0.0000	0.0000	0.0000	0.0059	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.8408	0.5655
MO-J	0.0002	0.0000	0.0006	0.0000	0.0000	0.0050	0.0000	0.0032	0.0001	0.0008	0.0009	0.0000	0.0000	0.0000		0.0339
TA-J	0.0017	0.0034	0.0005	0.0040	0.0041	0.0071	0.0025	0.0002	0.0009	0.0020	0.0039	0.0036	0.0019	0.0000	0.0027	

3.2. TEMPORAL VARIATION OF LARVAL CONNECTIVITY IN A CLOWN FISH METAPOPULATION

Publication: Saenz-Agudelo, P., G. P. Jones, S. R. Thorrold, and S. Planes. Temporal stability of larval connectivity in a clownfish metapopulation. **in preparation**

ABSTRACT

Connectivity, the demographic linking of local populations through the dispersal of individuals is one of the fundamental factors determining species distribution. For species with dispersing larvae, empirical estimations of connectivity are challenging and so far there are no empirical studies that have measured how these levels of retention within and exchange among populations vary through time. In an effort to better understand the magnitude of temporal variation of larval dispersal among discrete populations, parentage analysis were used to elucidate the origin of settled juveniles of a coral reef fish (*Amphiprion polymnus*) metapopulation in Papua New Guinea over two consecutive years. Dispersal patterns were estimated by tracing the parental origin of juveniles that were sampled among 9 discrete populations and measured temporal and spatial variation of self recruitment and the contribution in recruitment of each population. I found that both temporal and spatial variations were higher at small scale (between seasons and between populations) than at larger scale (between years and metapopulation). However, temporal variation within sites at the seasonal and inter-annual level in both the proportion of self recruitment and the individual contribution to the metapopulation were lower than spatial variation within the same period of time (season or year). Retention at the metapopulation level (all populations grouped together) was also rather consistent between years (18.2 and 25.4% in 2008 and 2009 respectively). In practical terms, our results suggest that empirical estimates of self recruitment based on parentage analysis might present an advantage over other marking methods because the temporal frame of measurement of this method might better reflect long term dynamics of the population involved. Finally these results also show that highest temporal variation observed at the individual population level is consistent with a local perturbation that degraded the habitat and affected fecundity.

INTRODUCTION

Connectivity refers to the demographic linking of local populations through the dispersal of individuals among them (Sale et al. 2005a). This measure has long been considered as a fundamental factor determining species distribution and population dynamics (e.g. MacArthur and Wilson 1967, Levin 1974, Doak et al. 1992, Taylor et al. 1993, Lindenmayer and Possingham 1996, Schumaker 1996, With et al. 1997, Hanski 1998, Moilanen and Nieminen 2002). Yet, empirical estimation of retention and exchange levels in natural populations is often challenging. Metapopulation studies usually overcome this difficulty by using simplified connectivity measures that are based on landscape structure where the effect of migration is scaled as a function of patch area and patch distance (Moilanen and Nieminen 2002). This simplification assumes a large number of patches in the metapopulation and minimal variation in migration among patches between years (Hanski 1994, Hanski et al. 2000). However, the use of these simplified measures becomes difficult in cases where dispersal is shaped by complex biological and physical interactions.

Marine environments are one such case in which dispersal is highly complex. The vast majority of marine invertebrates and fishes have a planktonic larval stage that is responsible for most of the exchange of individuals among geographically separated populations (Palumbi 2003, Cowen et al. 2007). In addition, the dispersal matrix is far from homogeneous due to currents and oceanographic features (like eddies and fronts) that will influence dispersal patterns (Cowen et al. 2000, Cowen et al. 2006, Treml et al. 2008, White et al. 2010a). Studies of larval behaviour have shown that marine larvae have extremely well developed sensorial capacities, can detect a wide variety of sensory cues and have remarkable swimming capacities (Kingsford et al. 2002, Fisher 2005, Lecchini et al. 2005, Simpson et al. 2005, Leis et al. 2006, Gerlach et al. 2007, Paris et al. 2007, Dixson et al. 2008). The combination of all these factors, coupled with biological mechanisms that will influence larval survival and recruitment success, determine the distribution of settling cohorts (James et al. 2002, Leis 2007) and their variation in time.

Demographic connectivity is a key parameter in the modelling and assessment of population persistence, the design of marine protected areas and fisheries management (Palumbi 2003, 2004b, Sale et al. 2005a, Cowen et al. 2007, Almany et al. 2009, Botsford et al. 2009). Understanding the variability or predictability of population replenishment at the mesoscale (10 to 100 km) is essential because this spatial scale is most relevant to conservation and management decisions (Hamilton et al. 2006). Variation in the magnitude of

replenishment has been widely studied in coral reef fish popupulations (e.g.Doherty and Williams 1988, Wilson and Meekan 2001, Doherty 2002, Sale et al. 2005b, Hamilton et al. 2006). These studies have suggested that while there is considerable temporal variation in the intensity of replenishment at the mesoscale, in some cases, oceanographic and biological factors may intersect to produce predictable, consistent spatial patterns (Hamilton et al. 2006). However, little is known about the magnitude of variation in the proportion of replenishment that is explained either by larval retention within a population, or larval exchanged among populations.

Given the complexity of the dispersal matrix and size of marine larvae, the majority of estimates of dispersal distances are indirect and derived from inferences using biophysical models (Cowen et al. 2000, James et al. 2002, Paris and Cowen 2004, Cowen et al. 2006, Paris et al. 2007, Treml et al. 2008) or population genetics (e.g. Hellberg et al. 2002, Planes 2002, Palumbi 2003, Underwood et al. 2007, Puebla et al. 2009, Salas et al. 2010). Biophysical models that describe larval dispersal based only on environmental factors (excluding larval behaviour) predict high levels of spatial and temporal variation in connectivity and retention among individual reefs (James et al. 2002). Models that incorporate simplified larval behaviour predict higher retention rates than expected based on ocean currents alone (e.g. Cowen et al. 2000, Cowen et al. 2006, Paris et al. 2007). Estimations of the magnitude of larval connectivity derived from direct observations are logistically challenging but are required to validate indirect estimations and model predictions (Botsford et al. 2009, Jones et al. 2009b). Due to logistical constraints, most empirical studies have focused on estimating self recruitment in one population at one single time measure (Jones et al. 1999, Swearer et al. 1999, Thorrold et al. 2001, Jones et al. 2005, Patterson et al. 2005, Almany et al. 2007, Patterson and Swearer 2007, Hamilton et al. 2008, Planes et al. 2009, Saenz-Agudelo et al. 2009). These studies have provided valuable information on how patterns of self recruitment vary among locations and species and suggest that the geography might be more important than species traits (such as pelagic larval duration) (Jones et al. 2009b). However, we are still far from understanding how variable these patterns are over time. Empirical estimation of temporal variability in larval dispersal has not yet been achieved for any species.

Parentage analysis can be particularly useful for detecting ecological dispersal patterns in systems with high levels of gene flow (Christie 2009). This approach involves using molecular data (usually highly variable molecular markers) to calculate the probability that individuals within a population have a given relationship (parent-offspring) given their

respective multilocus genotypes and assuming Mendelian inheritance. This approach has already been successfully used in the estimation of larval connectivity and self recruitment in fishes at the mesoscale level (Jones et al. 2005, Planes et al. 2009, Saenz-Agudelo et al. 2009, Christie et al. 2010). Here this approach was used to evaluate temporal variation of larval retention within and exchange among 9 discrete anemone aggregations hosting the anemonefish *Amphiprion polymnus* near Port Moresby, Papua New Guinea. Larval retention and exchange among anemone aggregations was measured over 2 consecutive years (2008 and 2009) and temporal variation was compared at different time (season and year) and spatial (individual sites and metapopulation) scales.

METHODS

Study species and site

The panda clownfish (*Amphiprion polymnus*) is a Southeast Asian endemic fish that lives in close association with discrete aggregations of two species of anemones (*Stichodactyla hadonni* and *Heteractis crispa*) occurring in sandy habitats associated with coral reefs (Fautin & Allen 1992). Each anemone is usually occupied by one breeding pair and up to eight smaller non-breeders and juveniles. The female (the largest individual) lays demersal eggs on the upper surface of shells or dead coral next to the anemone. The embryos develop over a period of 6-7 days before hatching (Fautin and Allen 1992) and post-larvae settle into anemones after a pelagic larval phase lasting 9-12 days (Thresher et al. 1989).

The study location consisted of 9 spatially discrete anemone aggregations, termed *sites* to avoid confusion with other sub-population definitions, in Bootless Bay, Papua New Guinea (Figure 1). For practical purposes, the group of 9 sites in this study is referred to as a metapopulation. Anemones within each site were confined to a ~1ha patch of shallow sand and sea grass, with distances among sites varying from 1 to 30 km. Each year in all populations, an exhaustive search for all anemones colonised by *A. polymnus* was performed for tissue collections, with the exception of Fishermen Island (FI). Given the size of Fishermen Island (FI) and logistic constraints, only small proportions of the protected side of the island were randomly explored both years, and therefore it is likely that only a fraction of the total fish population was sampled at this site. However, given the number of anemones sampled at this site (44 and 41 in 2008 and 2009 respectively) compared to the size of the other anemone aggregations (mean number of anemones: 25), the sample of FI could be considered representative of the population. In total, 215 anemones hosting *A. polymnus* were

found among the 8 sites (Figure 1). A ninth site (SE) constituting 8 anemones was only found during prospection of potential sites in 2009 and therefore no data is available for this site in 2008.

Sampling and genotyping

A total of 942 individuals were sampled among the 8 sites between January and April 2008 and 927 were sampled among 9 sites between the same periods in 2009 (Figure 1). Each fish was captured by SCUBA using hand nets, measured (total length TL), fin clipped underwater *in situ*, and then released back onto the same anemone. Fish that were too small to be fin clipped (less than 30mm) were collected.

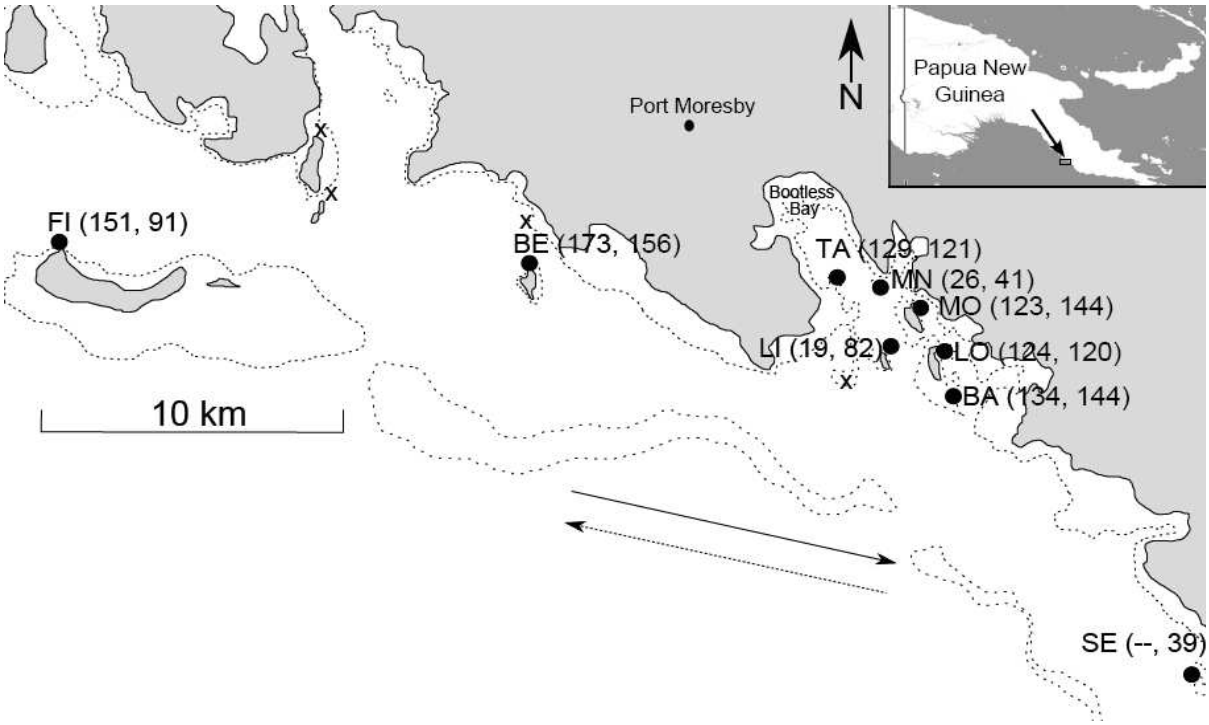

Figure 1 Map showing sites of the 9 anemone aggregations hosting *Amphiprion polymns* in Bootless Bay area (black filled circles) and prevailing surface currents during the summer monsoon (November-March) (solid arrow) and during winter (April-October) (dashed arrow). Numbers of fish sampled each year are indicated in brackets (2008, 2009). Crosses indicate sites outside Bootless Bay with potential suitable habitat for *A. polymnus* host anemones that were explored but no anemones were found. Dashed lines indicate shallow reef limits. Inset: Location of Bootless Bay in Papua New Guinea. Site abbreviations are as follows: Fishermen Island (FI), Manubada Island (BE), Lion Island (LI), Taurama (TA), Motupore north patch reef (MN), Motupore Island (MO), Loloata Island (LO), Loloata South Bank (BA), South East patch reef (SE).

For all analyses fish were divided in to 3 categories according to their size. The first category 'breeders' consisted of the female and male (the two biggest individuals) of each anemone. The remaining fish were then divided in to 'non-breeders' (>50mm) and 'juveniles' (< 50mm). These values were chosen following those of *A. clarkii*, a congeneric species with similar size, where 50mm corresponds to the lower limit of sexually mature individuals

(Hattori and Yanagisawa 1991). It also coincided approximately with maximum size of 1 year old fish (see temporal patterns below).

Details of the 18 microsatellite loci and genotyping procedure are described in Quenouille et al (2004) and Beldade et al (2009b). After DNA extraction, 3 multiplex polymerase chain reactions (PCRs) were performed per individual, using fluorescently-labelled primers to process 18 microsatellite loci containing a mixture of dimer and tetramer repeats. PCR products were processed on a Beckman Coulter sequencer CEQ 8000 Genetic Analysis System and the resulting electropherograms were scored manually. Uncertainties were cleared by reamplification and comparison. Alleles were scored as PCR product size in base pairs. Allelic frequencies and expected heterozyosities under Hardy Weinberg equilibrium were calculated in GENALEX version 6 (Peakall and Smouse 2006). Tests for Hardy-Weinberg and linkage disequilibrium were conducted using GENEPOP 3.4 (Raymond and Rousset 1995) and significance levels were adjusted with sequential Bonferroni corrections for multiple tests with $P< 0.05$. All 18 loci satisfied Hardy-Weinberg and linkage disequilibrium assumptions.

Parentage analysis

I used the FAMOZ platform (Gerber et al. 2003) to assign juveniles (TL< 50mm) back to sampled adults in the metapopulation. This procedure combines exclusion probabilities and maximum likelihood ratios to select the most likely parent for each offspring based on population allele frequencies, genotype matching among parent/offspring pairs and LOD scores distribution of true parent offspring pairs and false pairs (share one allele per locus by chance), allowing for the inclusion of genotype scoring errors (Gerber et al. 2000). Details of parentage analysis procedure can be found in Saenz-Agudelo et al. (2009).

Analysis of temporal patterns

Our objective was to estimate and compare temporal variation of self recruitment and local connectivity at different scales, both in time and space. I began by comparing variation at the smallest scale (seasonal variation and among sites), and increased the level of analysis up to inter-annual variation in self recruitment at the metapopulation level (all sites confounded). I also evaluated differences between years in the shape of the distribution of juveniles according to their dispersal distance from their natal origin based on results of parentage analysis.

Prevailing winds in this region are north-west in the summer (November to March) and south-east trade winds during the winter season (April to October) (Dennis et al. 2001). Since surface currents in the coral sea flow principally in the direction of the winds (Wyrtki 1960) (Figure 1), I investigated if there was a seasonal pattern in *A. polymnus* larval transport. If the influence of larval behaviour and swimming speed was less important than predominant currents, then one should be able to detect directionality in the seasonal larval connectivity patterns reflecting dominant current flows. Under this assumption, during summer, the proportion of larvae transported from South-East to North–West should be greater than in the opposite direction, and opposed patterns should be observed during winter. However, because sampling was only every year the settlement season for all sampled juveniles had to be inferred. To do this, I used a combined approach of otolith reading and multilocus genotype based individual identification to approximately determine the size range, at the moment of sampling, of individuals that settled during the dry or the wet season each year (Figure 2).

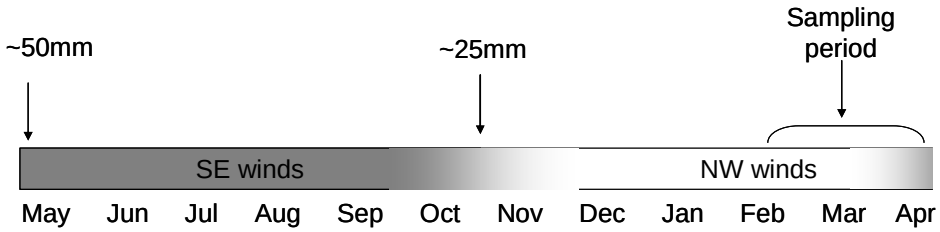

Figure 2 Illustration of the approximated settlement periods in one year (first two arrows from left to right) for *A. polymnus* juveniles of size at capture (TL) 50 mm and 25 mm estimated from otoliths and genetic fingerprints.

Lapilli otoliths were dissected from a subsample of 245 fish collected in the field (up to 30mm). Age of each fish was determined by counting the number of daily increments from the nucleus along the longest axis of the otolith. Unfortunately, daily age determination was not always possible, mostly for larger fish (>26mm) and clear readings were obtained for 145 of these fish (ranging from 7 to 26 mm in total length). The maximum age at size 25mm TL observed from these otoliths readings was ~102 days. Fish of ~100 days old sampled in February should have recruited around the last week of October from the previous year (which coincides roughly with the end of winter season). Therefore the size of 25mm was used as a proxy for the limit between winter and summer (end October beginning November). Fish of 25mm TL or smaller were assumed to have settled in the summer season and fish larger than 26 mm TL were assumed to have settled before this point in time.

To identify the upper size limit of juveniles corresponding to the end of the previous summer (figure 2), I compared individual microsatellite multilocus genotypes from all juveniles, fin clipped in both years, using the Genalex 6 package (Peakall and Smouse 2006).

The combined probability of identity and identity between sibs (Waits et al. 2001) for all loci (18) given the sample size and allele frequencies were small ($3.26x10^{-18}$ and $6.19x10^{-7}$ respectively). Therefore when two multilocus genotypes from different years matched perfectly it was assumed that they corresponded to samples from the same individual. The possibility of genotyping errors was also included by allowing up to 2 mismatches (in 18 loci) in a first run of the pairwise comparisons. Genotypes that matched at all but 1 or 2 loci were re-scored and accepted if discrepancies were subsequently confirmed to be genotyping errors. Of the 80 juveniles between 30 and 50mm captured in 2008 and recaptured in 2009, only 10 (14%) were less than 50mm in 2009 from which only 4 were locally spawned. The rest either died before the 2009 census or were larger than 50mm. Therefore the same arbitrary 50 mm top limit for parentage analysis was also a relatively good indicator of fish younger than 1 year. Finally, I assumed that there was no selective post settlement mortality that would change self recruitment or connectivity proportions between young (<25mm) and old (>26mm) juveniles. Consequences of this assumption are mentioned in the discussion.

RESULTS

Seasonal patterns

No evidence of directionality of larval transport was observed. Larvae in both size categories dispersed both North-West and South-East directions in similar proportions as suggested by the results of the connectivity matrices for <25mm juveniles (summer recruits) and >26 mm (winter recruits) for each year (Table 1.) (See also Table 2 for a summary of each matrix).

Self recruitment (proportion of total recruitment in site A that arrived from site A) was highly variable among sites and seasons, ranging from no self recruitment in various sites to 42%. The highest temporal variation in self recruitment was observed in Taurama. Here no self recruitment varied from 23 to 42% in the first three seasons but dropped to zero in the last summer season (<25mm in 2009) (Figure 3.A). The contribution of each site to the metapopulation (proportion of recruits that were born in a site and settled within the study area) was lower both n magnitude and variation than self recruitment both among sites and seasons. The site with the highest contribution to the metapopulation was Taurama with a contribution of 9% to total recruitment in winter (>25mm<50mm) 2009 (figure 3.B).

Table 1 Representation of the seasonal connectivity matrices for both inferred seasons: winter (26mm<Juv50mm) (matrices A and C) and summer (Juv<25mm) (matrices B and D) in 2008 and 2009. Columns and lines are labelled with the abbreviation of each site and are ordered according to their geographic location (longitude) from East (SE) to West (FI). Each element a_{ij} of each matrix corresponds to the number of juveniles produced on site *i* that settled on site *j*. For each matrix numbers in bold across the diagonal correspond to self recruits, numbers above the diagonal correspond to transport from West to East and numbers below the diagonal correspond to transport from East to West

Source

Sink	SE	BA	LO	MO	LI	MN	TA	BE	FI		SE	BA	LO	MO	LI	MN	TA	BE	FI	
SE																				
BA		**2**	-	-	-	-	-	-	-			**2**	-	1	-	1	-	1	-	
LO		1	**3**	-	1	-	1	1	-			2	-	2	-	2	-	-	1	
MO		-	1	**1**	-	1	2	-	1			1	2	-	2	1	1	-	-	2008
LI		1	1	-	-	-	2	-	1			-	-	-	-	-	1	1	-	
MN		1	-	-	-	**1**	-	1	-			-	-	-	-	-	-	-	-	
TA		-	-	1	-	1	**10**	-	1			-	-	-	1	-	**6**	-	-	
BE		-	-	-	-	-	1	**4**	-			3	1	1	1	1	-	**3**	1	
FI		-	-	1	-	-	2	1	**3**			-	-	1	-	-	-	-	-	
					A										**B**					
SE	**1**	-	-	-	1	-	-	-	-		-	-	-	-	-	-	-	-	-	
BA	--	**7**	-	1	2		2	3	1		-	**2**		-	-	-	-	-	-	
LO	--	3	**1**	2	1	2	2	-	1		-	3	**1**	1	-	-	-	-	-	
MO	1	5	1	**2**	2	2	6	2	2		-	1	-	-	-	3	1	1	1	
LI	--	2	-	-	**2**	-	1	1	-		-	-	1	-	-	-	-	-	-	2009
MN	--	-	-	-	1	-	1	2	-		-	-	-	-	-	-	-	-	-	
TA	--	2	-	-	3	1	**19**	1	1		-	-	-	-	-	-	-	-	-	
BE	--	2	-	-	3	-	1	**5**	-		-	-	-	-	-	-	1	**4**	-	
FI	1	-	-	-	-	1	1	-	**2**		-	-	1	-	-	-	-	-	**1**	
					C										**D**					
	26mm< juv <50mm										**Juv < 25 mm**									

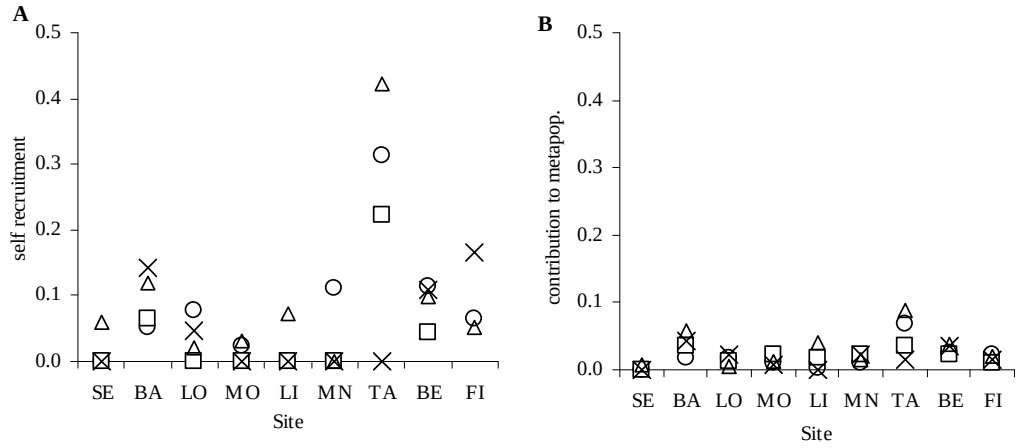

Figure 3 Distribution of relative frequencies of **A)** self recruitment and **B)** contribution of the site to metapopulation among the 9 anemone aggregations hosting *A. polymnus* in Bootless Bay for each size category: 2008 >26mm (circles) <25mm (squares) and in 2009 >26mm (triangles) and <25mm (crosses).

For both self recruitment and contribution of each site to the metapopulation, temporal variation (within sites) was smaller than spatial variation (across sites) within each season. This pattern was more evident for self recruitment than for the contribution of individual sites to the metapopulation. With the exception of Taurama, standard deviations of the mean self recruitment across sites within seasons (spatial variation) were higher than standard deviations of the mean self recruitment within sites across seasons (temporal variation), (figure 4.A).

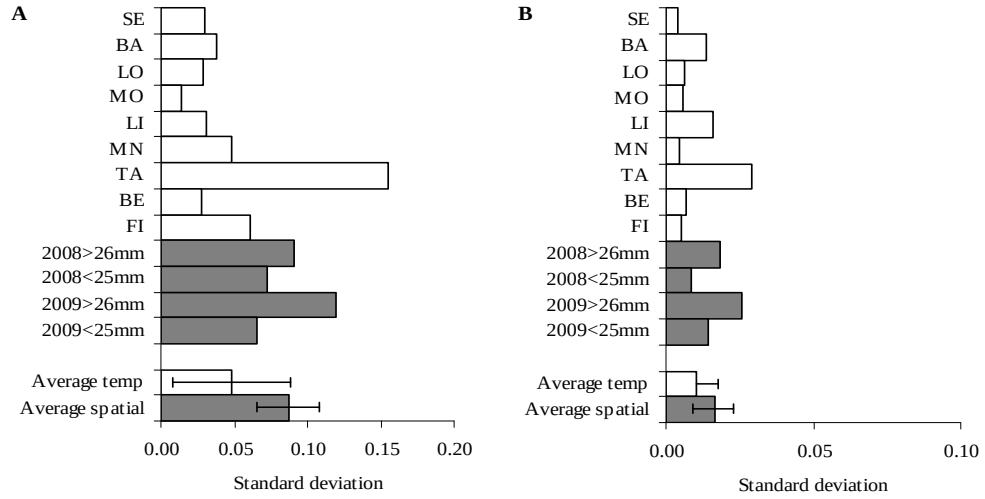

Figure 4 Distribution of the standard variation of the temporal mean (within sites mean across the 4 seasons) (white bars) and spatial mean (across sites within seasons) (grey bars) for **(A)** self recruitment and (B) contribution to metapopulation.

In terms of the contribution of individual sites to the metapopulation, differences between spatial and temporal variation were less evident. However, the average spatial standard deviation (across sites within seasons) was slightly higher than average temporal standard deviation (within sites across seasons) (Figure 4.B).

At the metapopulation spatial scale, the proportion of self recruitment (averaged over all sites) was relatively low in absolute terms in all seasons, ranging from 4.1 to 10.2% (Table 2). This value was also very similar among the same season of different years (4.1 and 5.8% in summer, and 9.4 and 10.2% in winter, 2008 and 2009 respectively). Average local connectivity among sites was higher than self recruitment in all seasons. Average connectivity was very similar for both seasons in 2008 and summer 2009 (~10%), but was almost twice as much in winter 2009 (18.9%). The proportion of self recruitment at the metapopulation level (locally produced juveniles that settled within one of the 9 sites study zone) varied between 16% and 29%.

Table 2 Number of *A. polymnus* juveniles that dispersed South-West and North-East, mean self recruitment and local connectivity per site for Bootless Bay area for each juvenile size category as a proxy for recruitment in different seasons. Last column corresponds to the proportion of juveniles of each size category assigned by parentage analysis, all sites confounded (metapopulation), corresponding to the overall self recruitment for each season.

Year	Size category (Season)	Total juveniles	Assigned by parentage	Juveniles dispersing SW / NE	% Self recruitment mean ± SD	% local connectivity mean ± SD	Overall self recruitment
2008	>25mm<50mm (winter)	264	48	12 / 12	9.4 ± 9.6	11.3 ± 7.7	18.5%
	<25mm (summer)	226	40	15 / 14	4.1 ± 7.7	10.9 ± 8.8	17.6%
2009	>25mm<50mm (winter)	369	106	39 / 28	10.2 ± 13.5	18.2 ± 7.7	29.6%
	<25mm (summer)	138	22	7 / 7	5.8 ± 7.1	8.9 ± 9.9	16.4%

Inter annual patterns

Temporal variability of self recruitment within sites was much lower than spatial variability among sites. Among sites, Taurama (TA) exhibited the highest self recruitment rate in both years, more than twice that at any other site (27 and 35% in 2008 and 2009 respectively). In the remaining sites, self recruitment was lower, varying from 12% to 0% between sites, but also relatively constant between years (Table 3, Figure 5.A).

Table 3 *A. polymnus* connectivity matrix among 9 sites in and near Bootless Bay over two consecutive years (2008 and 2009), calculated by identifying the natal origins of juveniles using parentage analysis. Bold values over the diagonal represent self recruits. Last two rows correspond to the sum of assigned juveniles and total of juveniles sampled at each sink site. For each year, last row corresponds to the number of juveniles produced at each source site that settled within the metapopulation boundaries.

2008	Source										
Sink	BA	LO	MO	TA	LI	MN	BE	CO	SE	Total assigned	Total juveniles
BA	**4**	0	1	1	0	1	1	0	--	8	71
LO	3	**3**	2	1	1	2	1	1	--	14	69
MO	1	3	**1**	3	2	2	0	1	--	13	70
TA	0	0	1	**16**	1	1	0	1	--	20	59
LI	1	1	0	3	**0**	0	1	1	--	7	42
MN	1	0	0	0	0	**1**	1	0	--	3	10
BE	3	1	1	1	1	1	**7**	1	--	16	102
CO	0	0	2	2	0	0	1	**3**	--	8	68
SE	--	--	--	--	--	--	--	--	--	--	--
Total from source	13	8	8	27	5	8	12	8	--	89	491

2009	Source										
Sink	BA	LO	MO	TA	LI	MN	BE	CO	SE	Total assigned	Total juveniles
BA	**9**	0	1	2	2	0	3	1	0	18	73
LO	6	**2**	3	2	1	2	0	1	0	17	68
MO	6	1	**2**	7	2	5	3	3	1	30	95
TA	2	0	0	**19**	3	1	1	1	0	27	57
LI	2	1	0	1	**2**	0	1	0	0	7	40
MN	0	0	0	1	1	**0**	2	0	0	4	20
BE	2	0	0	2	3	0	**9**	0	0	16	89
CO	0	1	0	1	0	1	0	**3**	1	7	44
SE	0	0	0	0	1	0	0	0	**1**	2	21
Total from source	27	5	6	35	15	9	19	9	3	128	507

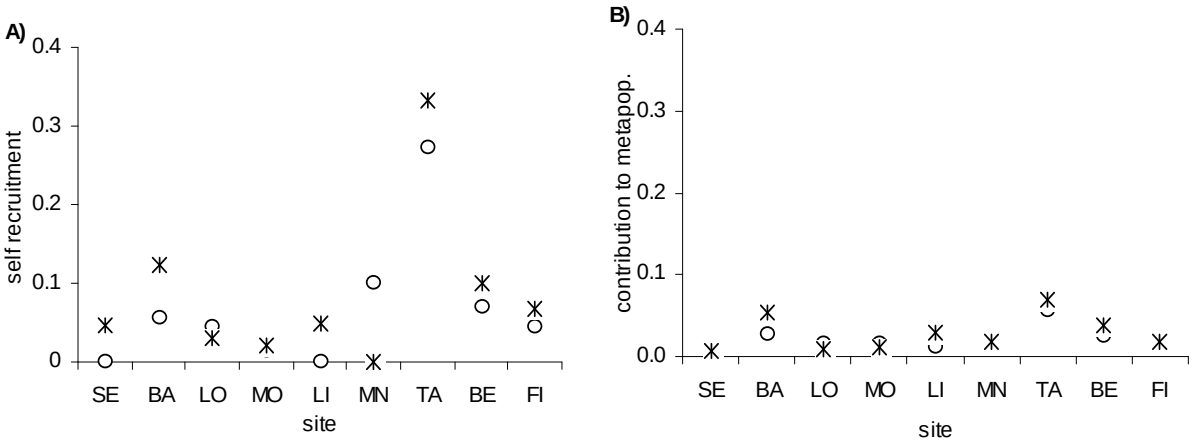

Figure 5 Distribution of relative frequencies of **A)** self recruitment and **B)** contribution to the metapopulation among the 9 anemone aggregations hosting *A. polymnus* in Bootless Bay in 2008 (round symbols) and 2009 (star symbols).

The annual recruitment contribution of each site to the metapopulation was low in general for all sites and both years (maximum contribution was 8% for Taurama in 2009) (Figure 5.B). Average self recruitment across all sites was similar between years (7.5 % and 8.6% for 2008 and 2009 respectively). As for seasonal variation, inter-annual variation was lower than spatial variation among sites both for self recruitment and contribution to metapopulation of each site. Overall self recruitment (comprising all sites) was relatively constant between years with 89 / 491 (18.2%) and 128 / 507 (25.2%) juveniles born and settled in one of the sampled sites in 2008 and 2009 respectively (Table 3).

The distribution of the proportion of juveniles according to the dispersal distances in 2008 was very similar to that in 2009 ($X^2_{,9df} = 3.66$, $P=0.938$). In both years, locally spawned larvae tended to stay relatively close to the sites where they were spawned, with more than 65% of juveniles recruiting within 3 km of their natal anemone in both 2008 and 2009. However not a single larvae returned to the same anemone where it was spawned. Figure 6 shows that self recruitment had a tendency to be overrepresented in both years compared to random expectations based on the distribution of distances among suitable habitat (sites) ($X^2_{,9df}$ 2008 = 20.5, P = 0.015; $X^2_{,9df}$ 2009= 34.6, P< 0.001). In both years, this overrepresentation was the result of consistent high self recruitment in Taurama (see table 3).

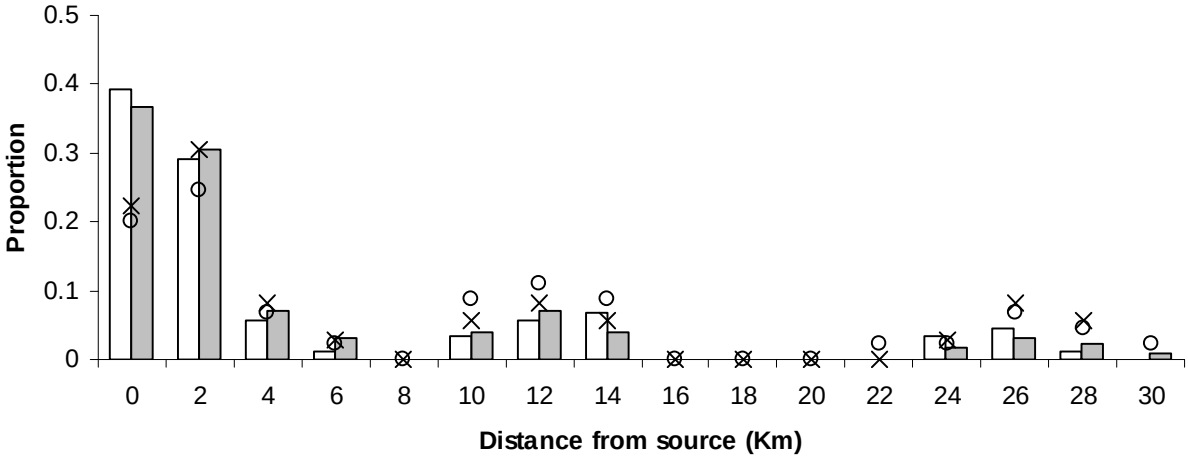

Figure 6 Distribution of the frequency of assigned juveniles according to the estimated dispersal distance obtained from parentage analysis in 2008 (white bars) and in 2009 (grey bars). The distribution of distances among suitable habitat (sites) in 2008 (x symbols) and 2009 (round symbols) are also shown. Labels on the x axis correspond to the central value for each distance class. Note that zero (0) distance class represents juveniles that settled in the same site as their parents (self recruits) and that differences in distribution of suitable habitat between 2008 and 2009 correspond to the incorporation of site SE in 2009.

DISCUSSION

Here self recruitment and exchange among anemone aggregations were estimated over two consecutive years (2008 and 2009) and temporal and spatial variation of larval exchange at different scales were evaluated. These results suggest that at this scale, there is no obvious directional pattern of larval dispersal in accordance to dominant current patterns in the region. That levels of temporal variation in self recruitment, even at small scales (seasons), tend to be of lesser magnitude than spatial variation among individual reefs (sites). They also show that temporal patterns become less variable as both the spatial and temporal scales at which they are estimated is increased. To our knowledge, this is the first time that an analysis of temporal variation is included in an empirical study of marine larval connectivity.

There was no evidence of obvious seasonal directionality in exchange patterns among sites. This could be the combination of bias linked to our estimation of seasonal wind shift, estimation of settlement date, within seasonal variability of current direction and active larval behaviour. Nevertheless, despite all the possible sources of error, there is substantial evidence showing that coral reef fish larvae have extraordinary well developed sensory organs, are extremely sensitive to several different sensory cues (Lecchini et al. 2005, Simpson et al. 2005, Gerlach et al. 2007, Dixson et al. 2008, Simpson et al. 2008), have remarkable swimming capabilities (Irisson et al. 2004, Leis et al. 2006, Leis 2007, Paris et al. 2007) and can swim in precise directions (Leis and Carson-Ewart 2003, Huebert and Sponaugle 2009,

Irisson et al. 2009). Therefore, it seems that the most parsimonious explanation for this lack of directionality between seasons might be linked to active behaviour and directional swimming in directions other than residual current flow. Perhaps residual currents do play a role, but interactions with larval behaviour and the rather small scale of the study simply do not allow us to distinguish between them. Coupling a biophysical model to these empirical observations would definitely help understanding these observations.

I analysed seasonal patterns in self recruitment assuming non selective post-settlement mortality of locally spawned larvae. Selective post-settlement mortality either for or against locally spawned larvae would change self recruitment rates measured among different size classes. For example, newly settled larvae that settled in an anemone containing an unrelated dominant fish may be more (or less) likely to be evicted than settling larvae more or less related. In the case of selection in favour of unrelated individuals, the proportion of self recruitment in small size classes would be larger than the one measured for larger size classes because unrelated newly settled larvae are less likely to be evicted. This could be expected if this behaviour had an evolutionary advantage (e.g. to avoid inbreeding depression) (Buston et al. 2007). However, I believe that such selection in this particular system, if it exists, should be very weak to be detected. First, the weakness of this selection is supported by the work of Buston and collaborators that showed that at least for one anemonefish species (*A. percula*), fish groups are not composed of close relatives. Second, given the low proportion of self recruitment observed in this metapopulation, the probability that fish larvae settle in an anemone with a close relative is extremely small. Therefore if this selection operates in this system, it might be in too few events to actually influence self recruitment proportions.

Self recruitment proportions were highly variable at the smallest scale (between sites and between seasons). The most obvious example of high temporal variation at this level was at Taurama with contrasting patterns of self recruitment ranging from 0 to 42% (0 and 19 self recruits respectively). However, what is interesting here is that temporal variation at this small scale is on average lower than spatial variation (with the exception of Taurama). Proportions of self recruitment and connectivity among sites remained constant in time despite the big differences in the total number of recruits observed between some seasons (369 in 2009 vs 128 between seasons). At a large spatial and temporal scale (metapopulation and year) variation in self recruitment was small with only 7 % difference in self recruitment between both years. Both deterministic and stochastic processes influence levels of population replenishment. Stochasticity can be more important at small scales and therefore, the magnitude of variation is expected to diminish with increasing scale of measurement. This

pattern has been observed in recruitment measurement of other coral reef fish (Hamilton et al. 2006), and the same rules seem to apply here for the proportions of larvae exchanged among or retained within spatial units.

In practical terms, our results suggest that empirical estimates of self recruitment will be highly variable if the temporal scale at which they are measured is small, but becomes more constant as the time frame involved becomes larger. In this sense, a single time assessment of larval dispersal patterns at a small temporal scale (weeks or months) might not be very informative for conservation managers because large fluctuations at this scale of time might not reflect long term dynamics of the population involved. Parentage analysis might present an advantage over other marking methods (Jones et al. 1999, Thorrold et al. 2006, Almany et al. 2007) because the temporal frame of measurement can be more easily adjusted as natural DNA marks only disappear with the death of the individual.

These estimations might provide appropriate guidelines for conservation strategies, and could provide valuable data to validate biophysical models. In the long term, once these models will be validated (and proved sufficiently accurate), they will be able to provide data on dispersal kernels over much larger spatial and temporal scales, for which empirical estimation using available methods such as parentage analysis still remains logistically overwhelming (James et al. 2002, Cowen et al. 2006).

Finally, the low temporal variation found at both seasonal and inter-annual scales might be the consequence of long-term demographic stability in this population system. In this anemone fish metapopulation available habitat seems to be near its maximum carrying capacity (empty anemones were extremely rare during surveys). Under habitat near its maximum carrying capacity, the number of breeders within each site should remain rather constant in time because breeders that die are immediately replaced by non breeders that are queuing for the breeding position (Buston 2004b). As a consequence, self recruitment and the relative contribution of each site to the metapopulation are expected to remain constant in time. In turn, local perturbations may differentially affect individual populations. In such a case, the proportions of self recruitment and individual contributions of each site to the metapopulation should also be affected. Putting our results in this context, with the exception of last season (summer 2009), they suggest that this metapopulation is in a demographic state of equilibrium as all proportions were rather similar among the first three seasons. However, extending this temporal study will be necessary to verify the veracity of this apparent temporal stability.

In terms of the last season, the most representative change was the drastic drop in self recruitment and contribution to the metapopulation by Taurama. This change could be explained by a bleaching event that began in November 2008 that affected all anemones shallower than 7m deep in the entire metapopulation. Anemone bleaching and associated habitat degradation had a significant negative impact on fish fecundity (see chapter 4, section 1). Anemone depth distribution was highly different among sites (see chapter 4 section 1 for details) and Taurama was among the most heavily affected by bleaching which is likely to explain this drastic change at this particular site. In addition, total recruitment in this last season was around 50% less than in all 3 other seasons. This reduction translates into higher stochastic variation and therefore higher temporal variation in relative contributions. The interesting question that arises is to know if after this bleaching event, whether the system will return to its original state and how long it will take. This will depend on how this perturbation affected the structure of individual populations. Only longer time series data will provide the answers and help to better understand long term marine population dynamics. Long term studies are particularly warranted given the increasing frequency and magnitude of perturbations linked to climate change (Munday et al. 2009).

CHAPTER 4

EFFECTS OF PARENTAL ENVIRONMENT ON POPULATION DYNAMICS

This chapter is also structured as two sections. The first one explores how habitat degradations via anemone bleaching affected local dynamics in this metapopulation. The second section investigates if parental effects and their relationship with reproductive success. It focuses particularly in the well known relationship between maternal size, fecundity and larval quality, and tests if this relationship is maintained through the entire pelagic phase and results in higher contribution to local replenishment by larger females.

4.1 SHORT TERM RESPONSE TO HABITAT DEGRADATION

Publication: Saenz-Agudelo, P., G. P. Jones, S. R. Thorrold, and S. Planes. Detrimental effects of host anemone bleaching on anemonefish populations. *Coral Reefs* **30**: 497-506.

ABSTRACT

Coral bleaching and related reef degradation have caused significant declines in the abundance of reef-associated fishes. Most attention on the effects of bleaching has focused on corals, but bleaching is also prevalent in other cnidarians, including sea anemones. The consequences of anemone bleaching are unknown and the demographic effects of bleaching on associated fish recruitment, survival and reproduction are poorly understood. I examined the effect of habitat degradation including host anemone bleaching on fish abundance, egg production and recruitment of the panda anemonefish (*Amphiprion polymnus*) near Port Moresby, Papua New Guinea. Following a high temperature anomaly in shallow waters of the region, most shallow anemones to a depth of 6m (approximately 35% of all the anemones in this area) were severely bleached. Anemone mortality was low but bleached anemones underwent a ~34% reduction in body size. Total numbers of *A. polymnus* were not affected by bleaching and reduction of shelter area. While egg production of females living in bleached anemones was reduced by ~38% in 2009 compared to 2008, egg production of females on unbleached anemones did not differ significantly between years. Total recruitment in 2009 was much lower than in 2008. However, no evidence of recruiting larvae avoiding bleached anemones at settlement was found suggesting that other factors or different chemical cues were more important in determining recruitment than habitat quality. These results provide the first field evidence of detrimental effects of climate-induced bleaching and habitat degradation on reproduction and recruitment of anemonefish.

INTRODUCTION

Severe bleaching events and associated coral mortality are contributing to a world wide decline in the health of coral reefs (Glynn 1993, Pandolfi et al. 2003). Increasing ocean temperatures, directly attributed to climate change, are resulting in increasingly frequent and severe bleaching episodes (Hoegh-Guldberg 1999, Hughes et al. 2003). There is also increasing evidence that coral reef degradation can cause significant declines in the abundance of other reef organisms such as coral reef fishes (Kaufman 1983, Kokita and Nakazono 2001, Adjeroud et al. 2002, Jones et al. 2004, Feary et al. 2007). Bleaching not only affects corals but also a range of other cnidarians including sea anemones, anthipatharians and corallimorpharians, and other taxa containing symbiotic algae such as some sponges and bivalves (McClanahan et al. 2009). As for corals, these habitat-forming organisms can host numerous fishes and invertebrates that depend on them for food or shelter. However, the influence of bleaching of organisms other than corals on reef-associated organisms has received little attention.

While dramatic effects of coral bleaching on reef fish communities have been well-described (Lindahl et al. 2001, Booth and Bereta 2002, Jones et al. 2004, Bellwood et al. 2006, Garpe et al. 2006, Emslie et al. 2008, Pratchett et al. 2008) the demographic processes responsible for population declines and recoveries are poorly understood. Recent studies have shown that bleaching can alter the feeding behavior of fish species that feed directly on corals (Cole et al. 2009) and have a negative effect on their physiological condition (Pratchett et al. 2004), growth (Feary et al. 2007) and mortality (Kokita and Nakazono 2001, Coker et al. 2009). Other studies have shown that reef fishes may be resilient to bleaching, but not actual mortality of host corals (Bonin et al. 2009). While changes in physiological condition may affect egg production of breeders and larval quality (Donelson et al. 2008), there is still no direct evidence that habitat degradation (such as bleaching) has a negative impact on reproductive success. In turn, a negative impact on reproduction could have significant consequences for the population dynamics and resilience of affected fish species (Munday et al. 2009). In general, the demographic consequences and long-term implications of bleaching are unclear, and this is particularly true for anemones and associated organisms.

Anemonefishes (Pomacentridae) live in an obligate association with certain sea anemones in the Indo-Pacific that provide the fish with oviposition sites and protection from predators (Allen 1972). Recent evidence has suggested that host anemones and anemonefishes may be in decline, as a result of destruction of coral habitat due to bleaching and collection

for the aquarium trade (Jones et al. 2008). However, to date no studies have examined the demographic consequences of host anemone bleaching on anemonefishes.

The aim of this study was to examine the immediate effects of anemone bleaching and habitat degradation on the abundance, egg production and larval settlement of the panda anemonefish, *Amphiprion polymnus* (Linnaeus 1758). Endemic to Southeast Asia, *A. polymnus* lives in close association with discrete aggregations of two species of anemones (*Stichodactyla hadonni* and *Heteractis crispa*) that occupy sandy habitats associated with coral reefs. Each anemone is usually occupied by one breeding pair and up to eight smaller sub-adults and juveniles. The female (the largest individual) lays demersal eggs on the upper surface of shells or dead coral next to the anemone. The embryos develop over a period of 6-7 days before hatching (Fautin and Allen 1992) and late stage larvae settle into anemones after a pelagic larval phase lasting 9-12 days (Thresher et al. 1989). Recent studies have indicated a significant proportion of larvae recruiting into adult populations are locally spawned (Jones et al. 2005, Saenz-Agudelo et al. 2009), suggesting that any local effect of bleaching on reproduction or embryo survival could also impact local recruitment.

Our study capitalized on a dramatic bleaching event that occurred in Bootless Bay (PNG) mid-way through a project in which abundance, egg production and recruitment of *A. polymnus* were being monitored. Weekly surveys over 2 months were carried out during 2008, prior to bleaching, and in 2009 when approximately one third of the anemones were bleached. This provided the opportunity to quantify the magnitude of demographic changes in bleached and unbleached anemones.

MATERIALS AND METHODS

Study site and species

This study was conducted at Bootless Bay (09°31'S, 147°17'E) near Port Moresby, Papua New Guinea. It focused on 6 discrete aggregations of the anemones *Stichodactyla hadonni* and *Heteractis crispa* confined to discrete ~1ha patches of shallow sand and sea grass (Figure 1a). These anemones were occupied with adult pairs of *Amphiprion polymnus* that laid eggs on coral fragments and other hard substrata (Figure 1b) or artificial tiles (Figure 1c). The six sites were monitored for two months (February-April) in 2008 and again over the same two months during a bleaching event in 2009, when ~ 35% of the anemones were severely bleached (Figure 1d). Finally, all sites were visited again in February 2010 where only presence and condition of each anemone was recorded.

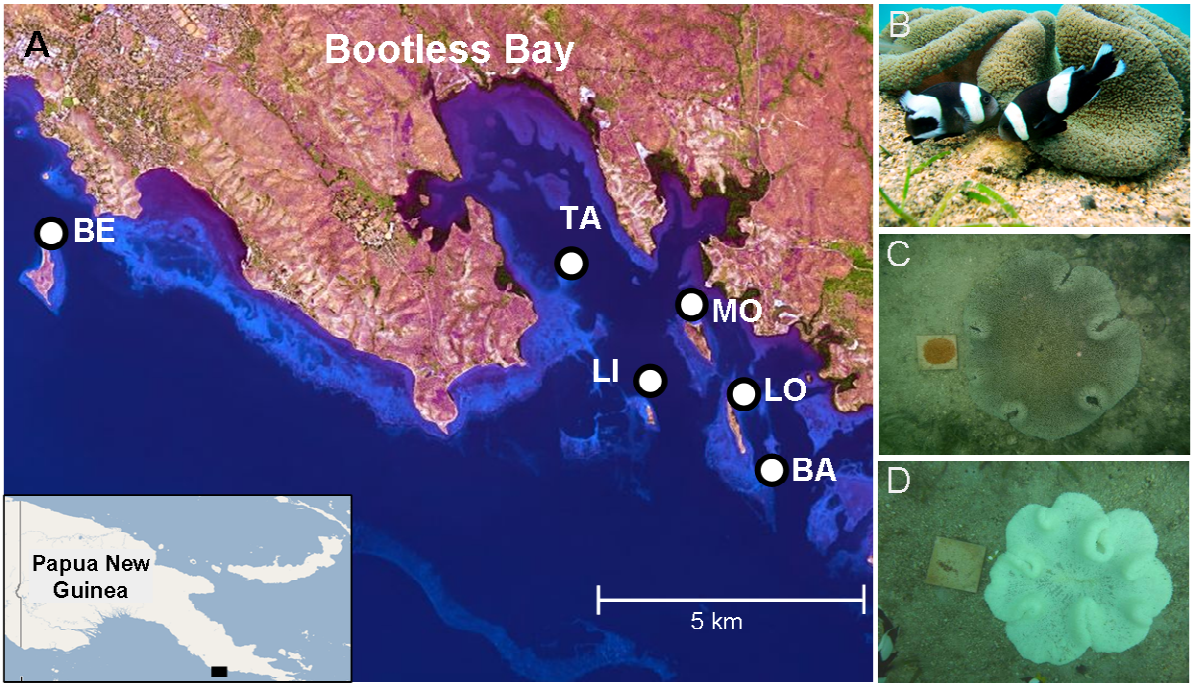

Figure 1. A) Satellite image showing the 6 studied anemone aggregations hosting *Amphiprion polymnus* in Bootless Bay area (white filled circles). Image courtesy of Phill Shearman, University of Papua New Guinea. Inset: Location of Bootless Bay in Papua New Guinea. **B)** Adult pair of *A. polymnus* and their host anemone *Stichodactyla hadonni*, Bootless Bay. **C)** and **D)** same anemone (*Stichodactyla hadonni*) in site TA, Bootless Bay: unbleached in 2008 (C) and bleached in 2009 (D). In both images at the left side of the anemone is one of the tiles used to measure egg production. Tile in C presents an egg clutch (orange color) of *A. polymnus* laid one day before the photo was taken.

Temperature data

Four Odyssey (© Dataflow Systems) submersible temperature data loggers were deployed in February 2008 at 2 sites (LI and MO) at two different depths (shallow ~2m and deep ~10m) to measure in situ water temperature. Temperature readings were recorded every 30 minutes from 1 February 2008 to 22 February 2009.

Field surveys and data collection

In January 2008 all anemones colonized by *A. polymnus* were individually tagged using small underwater buoys and plastic tags attached to metal pins that were buried in the sand next to each anemone. A total of 155 anemones were marked and followed for 2 months each year. In each survey I estimated each anemone's surface area, counted their number of resident *A. polymnus*, estimated the egg production per breeding fish couple and surveyed for the arrival of new recruits. Surface area (cm^{-2}) was estimated by photographing each anemone using an underwater camera and the image processing software ImageJ (Abramoff et al. 2004). Because anemone size can vary slightly from day to day (Buston 2003a) each anemone was photographed 2 times over each survey and the mean area was calculated.

Prior to each survey, all fish at each anemone were captured on SCUBA using hand nets, measured underwater to 1 mm using calipers (total length: TL), fin clipped and then released back on the same anemone. I collected all juveniles that were too small to be fin clipped (less than 30mm). Resident anemonefish may prevent recruitment of new individuals at high densities (Buston 2003b). Therefore removal of the small individuals also homogenized conditions for recruitment among all anemones. For the duration of each survey, all new recruits settling on each anemone over the sampling period were captured using hand nets. Samples were preserved in 95% ethanol and returned to the laboratory for subsequent genetic analyses. For each fish colony, individuals were separated into 4 arbitrary size/maturity categories: (1) the 2 largest individuals measuring at least 50mm TL were considered the breeding pair (Moyer and Steene 1979). (2) The remaining individuals at each anemone larger than 30mm (TL) were considered sub-adults (3) Individuals of 30 mm TL or smaller already present at the beginning of each survey were considered as juveniles. (4) All individuals that settled in anemones within the 8 weeks of surveys were considered new recruits.

Each year after the general fish census and tissue sampling, each anemone was surveyed twice a week over two months (February-April) to record the presence of egg clutches and new recruits. To estimate the egg production for each breeding pair, a 49 cm^2 (7 x7 cm) ceramic tile was placed next to each anemone 2 weeks before the beginning of each survey to standardize access to spawning surfaces among anemones (Fig. 1b and c). All females were using the tiles to lay eggs after two weeks in both years. Egg clutches were then photographed using an underwater digital camera and clutch area (cm^2) was estimated using ImageJ software. I used the cumulative area of egg clutches for each female during the 8 week sampling period as a measure of individual egg production. During both surveys reproductively active *A. polymnus* were only observed in *Stichodactyla hadonni,* with the exception of 1 *Heteractis crispa* where one small clutch was observed in 2008 (Saenz-Agudelo, pers. obs.). As a result, from the 155 marked anemones, the 45 *Heteractis crispa* anemones were excluded from the analysis. From the remaining 111 *Stychodactilya hadonni* anemones, 101 had complete observations in both surveys and were included in statistical analyses of anemone size and *A. polymnus* densities (Table 1). For comparisons of egg production, I selected for analysis only those anemones where the same female *A. polymnus* was observed in both years (see results: *egg production*) to eliminate female identity as an additional source of variation in egg production between years. These females were identified by comparing individual microsatellite multilocus genotypes (see parentage analysis below

for details of genetic markers) from all fish fin clipped in both years using the Genalex 6 package (Peakall and Smouse 2006). Combined probability of identity and identity between sibs (Waits et al. 2001) for all loci (18) given the sample size and allele frequencies were small ($3.26x10^{-18}$ and $6.19x10^{-7}$ respectively). Therefore when two multilocus genotypes from different years matched perfectly it was assumed that they corresponded to samples from the same individual. I also included the possibility of genotyping errors by allowing up to 2 mismatches (in 18 loci) in a first run of the pairwise comparisons, genotypes that matched at all but 1 or 2 loci were re-scored and accepted as well if discrepancies were indeed genotyping errors.

Table 1. Distribution of anemones and *A. polymnus* egg clutches and new recruits among the six study sites in Bootless Bay, Papua New Guinea. **D:** Depth range of anemones per site. **TN:** Total number of anemones per site. **S.h.**: Subset of *Stychodactyla hadonni* with complete observations in both years and included in statistical analyses. **S.h.R**: Subset of anemones where the female surveyed in 2008 survived to 2009, had at least one clutch of *A. polymnus* in one of both surveys and were included in egg production comparisons. **(B):** number of bleached anemones in each category. Finally, the number of *A. polymnus* egg clutches observed (from **S.h** anemones, n = 101) and number of new recruits that arrived at each site during the surveys (observations from **TN** anemones, n = 155).

Site	**Anemones**				***A. polymnus***			
					N° egg clutches		**New recruits**	
	D(m)	**TN (B)**	**S.h. (B)**	**S.h.R (B)**	**2008**	**2009**	**2008**	**2009**
BE	8-15	44 (0)	22 (0)	6 (0)	64	56	36	28
LI	4-18	17 (3)	10 (1)	5 (0)	14	17	12	4
BA	4-12	32 (9)	21 (5)	7 (4)	40	39	17	2
LO	3-10	21 (10)	16 (10)	7 (3)	39	21	11	2
MO	3-11	14 (10)	9 (8)	7 (5)	20	16	9	6
TA	3-7	27 (23)	23 (20)	17 (15)	55	60	10	2
Total		**155 (55)**	**101 (44)**	**49 (27)**	**232**	**209**	**95**	**44**

Statistical analyses

I investigated the effects of bleaching (as an indicator of habitat degradation) on A) anemone size, B) number of *A. polymnus* per anemone and C) egg production using simple non-parametric tests implemented in R (R Development Core Team 2007) as most of the response variables measured here were highly variable and did not follow normality assumptions or homogeneity of variances even after different data transformations. To control for the lack of independence between repeated measures on the same anemone, for each response variable I calculated the within anemone inter-annual variation (ΔX), measured as the difference between 2009 and 2008 per anemone ($X_{2009} - X_{2008}$) where X is the response variable of interest (A, B or C). Under ideal conditions nesting the treatment "bleached/unbleached" within site and depth would have allowed us distinguish between variation linked to spatial heterogeneity and the effects of bleaching and habitat degradation.

However, as bleaching affected only anemones in shallow waters and anemone's depth distribution range differed strongly among sites (as did the proportion of bleached anemones, see figure 2 for details) this approach was not feasible. Therefore, I compared ΔX across sites for both bleached (shallow) and unbleached (deep) anemones independently to test for spatial heterogeneity. Because ΔX was not significantly different for both bleached and unbleached anemones among sites (see results), the effects of bleaching were tested by comparing ΔX among bleached and unbleached anemones (Ho: $\Sigma(\Delta X)_{bleached} = \Sigma(\Delta X)_{unbleached}$), combined across sites, using Wilcoxon rank sum test. Finally, I used Wilcoxon signed rank tests to evaluate if there were consistent differences, averaged across all anemones, between years (Ho: $\Sigma\Delta X = 0$). For each test, the alternative hypothesis with the smallest associated p-value is shown in the results.

Parentage analysis

Parentage analysis was used to investigate whether there was a difference in the proportion of new recruits that were locally spawned (bleached/unbleached) before and after bleaching in Bootless Bay. A total of 546 individuals in 2008 (451 adults and sub-adults, and 95 new recruits) and 464 individuals in 2009 (420 adults and sub-adults and 44 new recruits) were screened for 18 microsatellite DNA loci (Quenouille et al. 2004, Beldade et al. 2009b) that satisfied Hardy-Weinberg equilibrium assumptions. I used the FAMOZ platform (Gerber et al. 2003) to assign new recruits back to sampled adults among the 6 sites described previously. Details of parentage analysis procedure can be found in Saenz-Agudelo et al. (2009).

RESULTS

Temperature and effect on anemones

Bleaching affected all shallow anemones down to 4 m deep (n=27, all sites combined), 20 anemones (85 %) between 5-6 m deep and 9 anemones (50%) between 6-7 m. All anemones below 7 meters did not undergo bleaching (Figure 2). This bleaching pattern corresponded with a spike in the water temperature in shallow water, with temperatures exceeding 32°C from the last week of November to mid-December 2008. Water temperatures measured at 10m depth never went over 30.7°C (Figure 3).

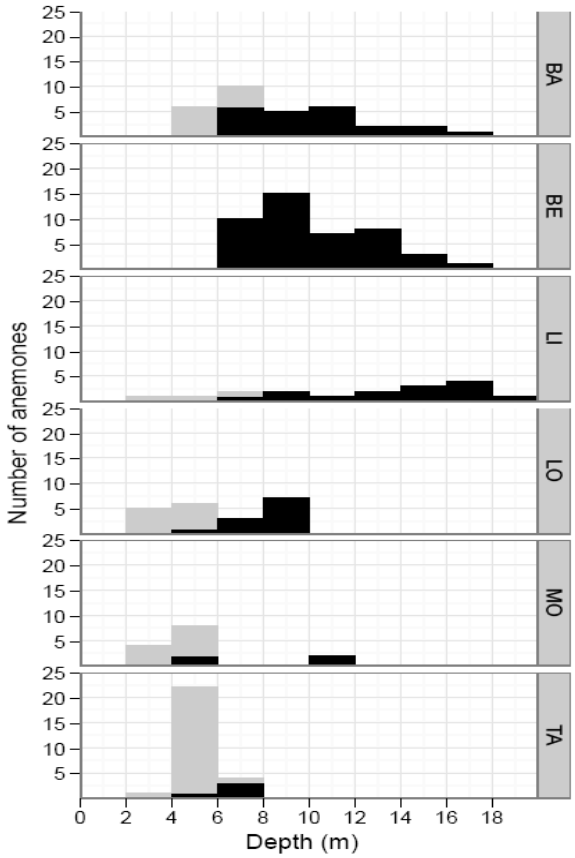

Figure 2. Distribution of all anemones (*Stychodactyla hadonni* and *Heteractis crispa*) by depth (m) among the six sites of this study in Bootless Bay. Grey bars indicate all shallow anemones that were bleached in 2009 and black bars represent deeper anemones that remained unbleached.

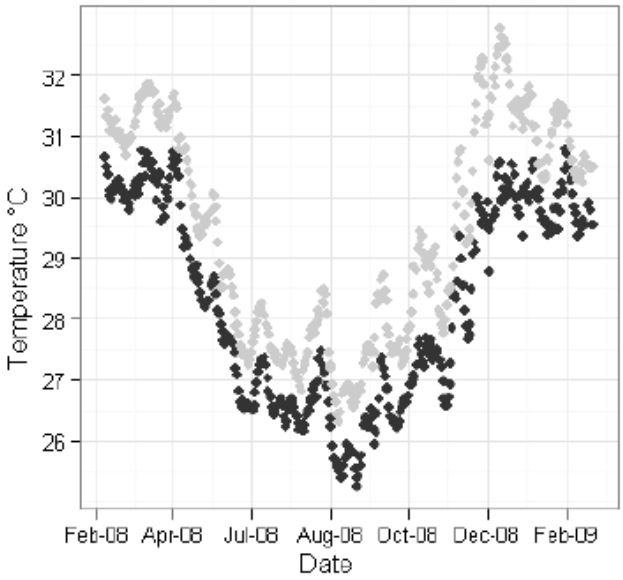

Figure 3 In situ mean daily water temperature profiles from 2 February 2008 to 22 February 2009 at two sites (LI and MO) at 2m (grey symbols) and 10m (solid symbols).

Inter-annual differences among sites for both bleached (shallow) and unbleached (deep) anemones for all variables were not statistically significant (Table 2) and therefore all sites were combined in further analyses to test for effects of bleaching.

Table 2 Summary of Kruskal-Wallis tests performed to evaluate spatial heterogeneity among sites for all variables measured. Tests were performed independently on shallow anemones (**bleached**) and deep anemones (**unbleached**). Sites BE and LI were not included in the "Bleached" test as both sites had less than 2 bleached anemones.

Variable	Test	K-W chi^2	d.f.	P
A) Δ anemone size	bleached	6.46	3	0.092
	unbleached	4.86	5	0.433
B) Δ Fish density	bleached	2.68	3	0.442
	unbleached	7.62	5	0.178
C) Δ egg prod/female	bleached	3.38	3	0.335
	unbleached	9.68	5	0.084

Anemone size was affected by bleaching (Wilcoxon rank sum test, Hi: $\Sigma(\Delta size)_{bleached} < \Sigma(\Delta size)_{unbleached}$, W = 2145.5, P< 0.001). On average, bleached anemones underwent a reduction in size of ~34%, while unbleached anemones apparently grew between 2008 and 2009 (Figure 4.1.4). The difference in anemone size, averaged across all anemones, was not significant between years (Wilcoxon signed rank test, Hi: $\Sigma(\Delta size) < 0$, V= 2241.5, P = 0.129). Despite the significant size reduction, only 3 of the bleached anemones disappeared during the 2009 survey and some of the remaining ones began to show signs of pigmentation recovery at the end of this survey. In February 2010, all remaining anemones were still present and appeared fully recovered.

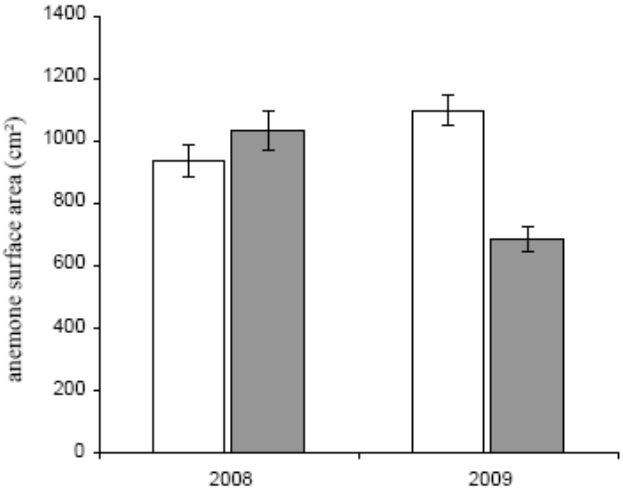

Figure 4 Mean surface area (± SE) of bleached (solid bars) and unbleached (open bars) *S. hadonni* anemones, before (2008) and after (2009) the bleaching event.

Resident fish density

Results of the Wilcoxon rank sum test for all fish categories combined indicated that bleaching had no significant effect on the mean number of resident fish per anemone (res) (Hi: $\Sigma(\Delta res)_{bleached} > \Sigma(\Delta res)_{unbleached}$, W = 1364, P = 0.206). However, the mean number of resident fish per anemone was significantly different between years (Wilcoxon signed rank test, Hi: $\Sigma(\Delta res) > 0$, V= 1314.5, P< 0.001), with more residents per anemone in 2009 (mean ± SD: 4.7 ± 2.7) than in 2008 (mean ± SD 3.7 ± 1.4). To investigate if a particular fish category (breeders, sub-adults or juveniles) was responsible for this change I performed similar tests on each fish category individually. No significant effect of bleaching was observed for any of the fish categories analyzed separately. Of all categories, only sub-adults showed a significant difference between years (Table 3). The number of sub adult fish per anemone in 2009 (mean ± SD: 2.6 ± 1.7) was higher than in 2008 (mean ± SD: 1.9 ± 1.3).

Table 3 Summary of Wilcoxon signed rank tests performed to evaluate differences between years (2008 and 2009) in densities of individual categories of *A. polymnus*. For each test the null hypothesis was no difference between years ($\Sigma \Delta$ number of fish = 0). The alternative hypothesis (A. H.) with the smallest p-value is shown.

Test	A. H.	V-value	P
Breeders (B)	$\Sigma(\Delta B) < 0$	25.0	0.136
Sub-adults (SA)	$\Sigma(\Delta SA) > 0$	2255.0	<0.001
Juveniles (J)	$\Sigma(\Delta J) < 0$	992.5	0.070

Egg production

The comparison of multilocus genotypes from individuals sampled in both years revealed that 54 females were recaptured in 2009 that had been fin clipped in the previous year (n=93). From these, 49 ($n_{unbleached}$: 22, $n_{bleached}$: 27) had complete observations of egg production and were used for subsequent analyses. Egg production, was significantly lower in 2009 than in 2008 (Wilcoxon signed rank test, Hi: $\Sigma(\Delta egg) < 0$, V= 304, P < 0.002). There was a significant differential decline in egg production (eggs) of females present on bleached anemones compared to those on unbleached ones (Wilcoxon rank sum test, Ho: $\Sigma(\Delta eggs)_{bleached} = \Sigma(\Delta eggs)_{unbleached}$, W = 454, P = 0.001). Mean egg production of females on bleached anemones declined by 39.3% from 2008 to 2009 but was not significantly different on females present on unbleached anemones between years (Wilcoxon signed rank test, Hi: $\Sigma(\Delta egg)_{unbleached} < 0$, V= 121, P = 0.582) (Figure 5). Total egg production at the population level (measured as the sum of all egg clutches produced by all females of all sites monitored during each survey) was lower in 2008 than in 2009 for both bleached and unbleached anemones (Table 4). However, this reduction was greater for bleached anemones (~38%) than

for unbleached ones (~20%). In 2008 the relative contribution to total egg production of females in bleached and unbleached anemones was 45% and 55% respectively. In 2009 these percentages changed to 38% and 62% for females in bleached and unbleached anemones respectively.

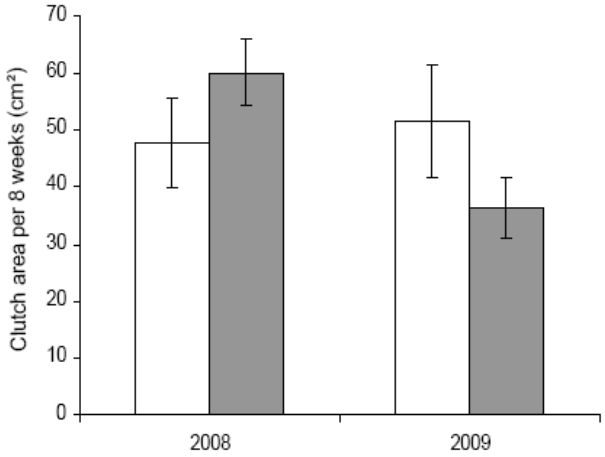

Figure 5 Egg production per female *A. polymnus* during 8 weeks of survey (measured as the sum of the areas of the egg clutches laid in this period), before (2008) and after (2009) the bleaching event. Bars represent mean (± SE) egg production per female that were laid next to bleached (grey bars, n = 27) and unbleached (white bars,n = 22) *S. hadonni* anemones.

Table 4 Distribution of eggs (total clutch area) and new recruits of *A. polymnus* between bleached and unbleached anemones, before (2008) and after (2009) the bleaching event. Egg production is expressed as the sum of areas of clutches laid in either bleached or unbleached anemones. Total new recruits (TR) were classified according to the type of anemone were they settled. Self recruits (SR) were classified first according to the type of anemone were they were born (Born) and then according to the type of anemone were they settled (Set).

	2008				**2009**			
Type of anemone	Eggs (cm²)	TR	SR		Eggs (cm²)	TR	SR	
			Born	(Set)			Born	(Set)
Bleached	2078	30	8	(5)	1292	10	0	(1)
Unbleached	2518	65	7	(10)	1993	34	4	(3)
Total	**4596**	**95**	**15**		**3285**	**44**	**4**	

Recruitment

Bleaching had no significant effect on the number of new recruits (NRs) per anemone (Wilcoxon rank sum test, Hi: $\Sigma(\Delta NRs)_{bleached} < \Sigma(\Delta NRs)_{unbleached}$, W = 1780.5, P = 0.489). However, the mean number of new recruits per anemone was significantly lower in 2009 than in 2008 (Wilcoxon signed rank test, Hi: $\Sigma(\Delta NRs) < 0$, V = 498, P< 0.001). Total recruitment was ~54% less in 2009 than in 2008 (44 and 95 new recruits respectively). Before the bleaching event (2008) ~32% of new recruits (30 of 95) settled in anemones that would suffer bleaching in 2009 and the rest (65 of 95) settled in anemones that did not suffer bleaching.

After the bleaching event (2009) ~23% of new recruits (10 new recruits) settled in bleached anemones and the remaining ~77% settled in unbleached anemones (34 new recruits) (Table 4). The proportion of new recruits settling in bleached anemones was not significantly different between years (chi-square = 0.75, 1df, P = 0.19) and neither were each of these two proportions (2008 and 2009) significantly different from random expectations based on the proportion of anemones that bleached in the population (2008: chi-square= 0.245, 1 df, P = 0.31, 2009: chi-square = 1.99, 1 df, P = 0.08).

Parentage analysis revealed that 15 out of the 95 new recruits (~16%) that settled within the survey period were born in 1 of the 6 sites of this study in 2008, while only 4 out of 44 (~9%) were assigned to local parents in 2009. This difference in proportions of self recruitment between years was not significant (chi-square = 0.643, 1df, P = 0.22). From the 15 new recruits assigned in 2008 (before the bleaching event), 8 individuals were born in anemones from the 'bleached' group and 7 in the unbleached group. After the bleaching event (2009), all 4 new recruits assigned by parentage analysis were born in unbleached anemones. The difference in proportions of those self recruits born in bleached anemones before and after bleaching was not significant (chi-square = 1.82, 1 df, P = 0.09). In 2008, a third (5 individuals) settled in anemones from the 'bleached' group, and in 2009, 1 new recruit of the 4 assigned settled in a bleached anemone.

The difference in proportions of these new recruits settling in bleached anemones before and after bleaching was not significant (chi-square = 0.0, 1 df , P = 1), and neither of them (before and after) was significantly different from the proportion of bleached anemones in the population as what would be expected by chance (chi-square = 0.0, 1 df, P = 0.5 for both years).

DISCUSSION

Our results show that habitat degradation appears to have detrimental effects on both host anemones and anemonefishes, one of the most recognizable symbiotic relationships on coral reefs. Manifested by bleaching and a concomitant decline in anemone size, habitat degradation was associated with a reduction in egg production of *A. polymnus* but did not appear to affect directly the densities of resident fish. These short-term demographic responses, if extended over a long period, could have a negative impact on population

trajectories for this species, particularly if bleaching progressed to significant rates of anemone mortality.

Bleaching causes the anemones to lose their algal symbionts after which they may shrink and eventually die due to the loss of nutrition derived from photosynthesis (Jones et al. 2008). However, there is little available information on the severity of bleaching and related mortality in anemones. Among hard corals bleaching severity and mortality can be highly variable among individuals, species, depths and geographic locations (e.g. Marshall and Baird 2000, Loya et al. 2001, Donner et al. 2007), and the factors responsible for this variation are still poorly understood. In our study, approximately 5 months after the beginning of the bleaching event (April 2009), the proportion of bleached anemones that had died was relatively low (only 3 out of 55). By the end of the study, some of the remaining bleached anemones started to show signs of pigmentation recovery. One year later (2010) the bleached anemones again appeared fully pigmented. Even though mortality was low, bleached anemones decreased in size by an average of ~ 34%. Anemones can be remarkably plastic in their appearance and the observed reduction in size might be in part due to a lack of full expansion as a defense reaction to harsh environmental conditions. On the other hand, bleached and recovering anemones presumably rely on carbon fixed from sources other than autotrophic symbionts, including stored energy reserves and heterotrophy, to meet their daily metabolic energy requirements. Species of hard corals that can obtain most of their daily carbon requirements by heterotrophy are less dependent on energy reserves during bleaching events, can recover their original mass faster, and are more resilient to bleaching events compared to species that cannot efficiently replace their original autotrophic carbon source via heterotrophy (Grottoli et al. 2006). For the bleached anemones in our study, the observed reduction in size probably resulted from the combination of lack of full expansion and the use of energy reserves as a consequence of reduced autotrophic carbon that was presumably obtained from the symbiotic algae before bleaching occurred. Our observations suggest that while this anemone population was tolerant to short term bleaching events, the reduced size of the bleached anemones represented a significant decrease of shelter surface for the anemonefish.

Anemonefish have a strong size hierarchy and rank associated mortality (Buston 2003b). Experimental manipulations have shown that post-settlement mortality and eviction rates are positively correlated with anemone saturation (Buston 2003a). I anticipated a reduction in the number of low rank residents (sub-adults and juveniles) in bleached anemones given that the degree of saturation of bleached anemones increased with their

reduction in size and that fish might be more conspicuous against a bleached background leading to greater harassment from predators. However, no significant differences of *A. polymnus* densities (for all size categories) were found between bleached and unbleached anemones. More sub-adults were observed in 2009 than in 2008, but with no difference between bleached and unbleached anemones. Our results suggest that post-settlement mortality was not severely affected by habitat degradation. A recent study showed that post-settlement mortality rates of *Pomacentrus amboinensis* juveniles settling in bleached corals may be higher than of those settling in live coral (McCormick 2009). *A. polymnus* might be more resistant to habitat degradation than at least some other coral reef fish.

Females on bleached anemones showed a large reduction in egg production compared to those on unbleached anemones. I remain unsure of the mechanisms leading to a reduction in egg production on bleached anemones. However, it seems likely that in this case the observed decline in habitat quality as a function of bleaching and diminution of anemone size reduced access to resources that led in turn to a decline in female condition. Negative effects on body condition and related changes in feeding behavior have been attributed to bleaching only in corallivorous fishes (Pratchett et al. 2004, Cole et al. 2009). Other studies have shown that changes in female condition affect egg quantity and quality (Donelson et al. 2008), and therefore quantity and quality of larvae (Gagliano and McCormick 2007, Donelson et al. 2008). Larval quality may also be strongly influenced by parental behavior during nest-tending in anemonefishes (Green and McCormick 2005b), and males may have a particularly strong contribution (Green and McCormick 2005). Although *A. polymnus* does not feed on its host anemones, bleaching and anemone size reduction may still be additional sources of stress. Besides a reduction of shelter area fish might be more conspicuous against a bleached background leading to greater harassment from other species. Thus harassment may, in turn, affect body condition or/and behavior of resident anemonefish with negative consequences on egg production and larval quality.

At the population level, egg production was lower in 2009 than in 2008, but this reduction was more pronounced in bleached anemones than in unbleached anemones. Parentage analyses revealed that none of the 4 locally spawned recruits in 2009 came from adult pairs breeding on bleached anemones despite ~38% of the total egg production occurring on these anemones. Unfortunately it is difficult to draw any conclusions based on such a low number of new recruits and low statistical power (with 38% of egg production occurring in bleached anemones, the probability that none of the 4 recruits came from bleached anemones just by chance is $(1\text{-}0.38)^4 = 0.14$). However, the tendency of these data

differs with those from 2008 where half of the new recruits that were locally spawned originated from breeding pairs of the same group of anemones that subsequently bleached the following year. If habitat degradation occurs at a large spatial scale and both egg quantity and larval quality are affected, it is certainly possible that recruitment could be dramatically lowered (as observed in this study, where total recruitment was 54% less in 2009 than in 2008) and this may in turn explain why there were no self recruits from bleached anemones in 2009. Again, given our data this remains only a hypothesis but it clearly deserves to be tested more rigorously.

Finally, our data suggest that *A. polymnus* larvae do not avoid bleached anemones when they are ready to settle. These analyses failed to detect any difference between the numbers of new recruits settling in bleached or unbleached anemones. In addition, the proportions of new recruits that settled in bleached anemones did not differ significantly from random expectations based on the proportion of bleached anemones in the population. Reef fish are clearly capable of responding to a wide variety of sensory cues such as chemical signals from anemones (Elliott et al. 1995, Arvedlund and Nielsen 1996, Arvedlund et al. 1999) and conspecifics (Sweatman 1988, Booth 1992, Lecchini et al. 2005), vegetation (Dixson et al. 2008) and sound (Simpson et al. 2005, Simpson et al. 2008). Laboratory tests have shown that anemonefish larvae placed in water choice chambers prefer water from healthy anemones when the other option is water from a bleached anemone, but they prefer a bleached anemone to a control using water without an anemone (DL Dixson, pers. comm.). Although clearly capable of distinguishing between bleached and unbleached anemones in controlled environments, the presence of conspecific odour might be a more dominant settlement cue than anemone odour and may explain why larvae do not avoid bleached anemones in the field. In addition, the degree of anemone saturation may be a critical factor in determining whether or not larvae can successfully join a social group (Buston 2003a). Alternatively, settlers may do best to remain in the first anemone they encounter (Elliott et al. 1995, Buston 2003a, Buston et al. 2007), even if it is bleached, than reject it and try to find a healthy one.

Coral bleaching is only one of several consequences of a changing global climate on coral reefs. Available evidence suggests that spatial scales of population connectivity may be reduced in the future due to effects of climate change on adult reproduction, larval dispersal and habitat fragmentation (Munday et al. 2009). In addition, climate change may reduce the ability of local populations to sustain themselves through self-recruitment (Jones et al. 2009b). However, we are still a long way from understanding the significance of these effects

for the sustainability of reef populations. Our study shows that there are negative short-term effects of habitat degradation on the quantity of egg produced by anemonefish, and possibly the quality of larvae as well, which can by consequence decrease the number of settling larvae. However, our results show that densities of *A. polymnus,* even of small juveniles do not seem to be affected by habitat degradation. Anemonefish appear more resilient to bleaching than at least some other reef fish studied to date (e.g. Kokita and Nakazono 2001). More importantly, because adult anemonefish apparently have a long life span (Buston and García 2007), partial bleaching episodes such as this one may have a relatively small effect on the long term dynamics of this population. Studies of the longer-term implications are warranted to better assess the resilience of these fish to increasing climate induced habitat degradation.

4.2 PARENTAL ENVIRONMENT, REPRODUCTIVE SUCCESS AND LOCAL POPULATION REPLENISHMENT

Publication: Saenz-Agudelo, P., G. P. Jones, S. R. Thorrold, and S. Planes. Size matters: Bigger females produce more successful offspring than smaller ones in a natural anemonefish population. *In preparation*.

ABSTRACT

It has been hypothesized that parental quality can play a major role in explaining variation of replenishment of marine populations. However, although small-scale experiments have documented links between maternal quality and larval size, or between juvenile quality and subsequent survival, the link between maternal quality and offspring success has not yet been demonstrated. In the past, the difficulties associated with following single larvae through the pelagic cycle to recruitment have precluded such an analysis. Here I used field surveys combined with DNA profiling of all members of a wild population of the panda anemonefish (*Amphiprion polymnus*) over two consecutive years to estimate reproductive success of all females that persisted from one year to the next. I examined whether the number of eggs and locally successful recruits produced per female was associated to parental phenotype (female or male size) and environmental characteristics (depth and fish colony size). Both the the number of eggs produced and the number of juveniles that successfully settled within the study boundaries were positively related to female size, but not other measured parental traits (fish density, male size or depth). Although most of the recruitment in this species comes from external sources, larger females in the local population contribute more than twice as much as smaller ones to local replenishment. These findings suggest that protecting large healthy females will be crucial to population replenishment and argue for the implementation of management strategies that will restore and protect size/age structure of marine populations.

INTRODUCTION

The question of what influences the replenishment of marine populations is fundamental to our understanding of their dynamics (Doherty and Williams 1988, Doherty 2002), fisheries management (Birkeland and Dayton 2005) and conservation (Jones et al. 2009b). Many marine species are relatively sedentary as adults, but have a life cycle that includes a pelagic dispersive larval phase that can last from a few days to several weeks (Sale 1980, Thresher et al. 1989, Leis et al. 2006). The time, place, number and quality of larvae that enter the water column are major factors affecting the initial strength of the juvenile year-class (Begg and Marteinsdottir 2000, Vallin and Nissling 2000). Subsequent interactions with the food resources and predators will affect their survival, condition and growth rates during the larval phase, and in turn, may determine the numbers and quality of juveniles recruiting into adult habitat (Jones 1991, Mora and Sale 2002). Elucidating the key factors in the complex series of events affecting population replenishment is critical in order to understand the dynamics of marine populations and how best to implement effective management and conservation strategies (Sale et al. 2005a, Mora et al. 2006, Jones et al. 2009b).

Parental effects potentially play an important role in the early stages of larval development and survival, and may also have carry-over effects that influence performance later in life (Green and McCormick 2005, Gagliano and McCormick 2007, Green 2008). Parental effects represent the effects of the interaction between parental genotype, phenotype and parental environment on offspring characteristics and performance (Beckerman et al. 2006). There are many lines of evidence to suggest parental genotypes and environment can have a major influence on larval quality, which in turn may influence survival (Green 2008). One of the current hypotheses in the literature on parental effects in marine organisms is that because larger females produce more and better quality eggs, which results in more and larger larvae with subsequent higher chances of survival (Berkeley et al. 2004a), they make a greater contribution to population replenishment than smaller females (Roberts and Polunin 1991, Russ 2002, Berkeley et al. 2004a, Berkeley et al. 2004b, Palumbi 2004a, Birkeland and Dayton 2005, Green 2008). It is critical to test this hypothesis, given that fishing pressure invariably results in a differential impact on larger females.

There are three transition stages through a generalised life-cycle of a fish (mother-egg, egg-larvae, larvae-juvenile). For maternal effects to have an impact on juvenile recruitment, they have to carry-over through all 3 stages. Evidence that supports the theory of bigger/older females have higher contributions to population replenishment comes from studies that have a

focus on the different stage-transitions independently. At the early stage, there is support for bigger females producing bigger larvae with higher probabilities of survival than smaller larvae from smaller females (Berkeley et al. 2004a, Venturelli et al. 2010). There is also evidence for a range of species that larger larvae have lower pre and post-settlement mortality (Bergenius et al. 2002, Vigliola and Meekan 2002, Macpherson and Raventos 2005, Raventos and Macpherson 2005, Meekan et al. 2006, Gagliano et al. 2007, Vigliola et al. 2007). Only two studies have shown evidence of the positive relationship between variations in female size and the number of recruits, both for commercial fish species in temperate waters (Gadidae) (Vallin and Nissling 2000, Wright and Gibb 2005). However, the relationship between variation in individual parental quality and the relative contribution to the next generation has never been established in the field.

The presence of a pelagic larval phase, coupled with small size and often cryptic appearance of the larvae, makes it difficult to close the link between the quality of individual parents and their reproductive success at recruitment. However, recent studies that have applied parentage analysis to coral reef fish populations have shown that offspring from local parents can be identified (Jones et al 2005, Planes et al 2009, Saenz Agudelo et al 2009). Here a combination of genetic identification methods, parentage analysis and field observations on egg production were used to assess the relative contribution of different females to local recruitment natural population of the anemone fish *Amphiprion polymnus* in Bootless Bay, Papua New Guinea. I followed the egg production over 2 months and the maturation, and survival of individual female fish over one year and documented their progeny that successfully recruited back to the population after a larval pelagic phase lasting ~12 days. This novel approach allowed us to verify if the number of successful recruits settling within the study's boundaries was associated with particular parental phenotypes (size) and environmental characteristics (depth and fish colony size). I evaluated if egg production, often correlated to female size, was affected by environmental characteristics. Y also investigated if differences in fecundity among females of different sizes could explain most of the variation in recruitment success (number of successful juveniles recruiting within the study area) among females of different sizes. Finally, I tested if female size was related to the average larval dispersal distance.

METHODS

Study population and observational methods

This study was conducted at Bootless Bay (09°31'S, 147°17'E) near Port Moresby, Papua New Guinea. It focused on 7 discrete aggregations of the anemones *Stichodactyla hadonni* and *Heteractis crispa* confined to discrete ~1ha patches of shallow sand and sea grass (termed *sites* from now on) (Figure 1). In January 2008 all anemones colonized by *A. polymnus* (155) were individually tagged using small underwater buoys and plastic tags attached to metal pins that were buried in the sand next to each anemone. Anemone depth was measured to the nearest 0.1m using an underwater computer. All fish at each anemone were counted, captured on SCUBA using hand nets, measured underwater to 1 mm using calipers (total length: TL), fin clipped and then released back on the same anemone. We collected all juveniles that were too small to be fin clipped (less than 30mm). Resident anemonefish may prevent recruitment of new individuals at high densities (Buston 2003b). Therefore removal of the small individuals also homogenized conditions for recruitment among all anemones. Samples were preserved in 95% ethanol and returned to the laboratory for subsequent genetic analyses. The same procedure was repeated one year later in February 2009.

Genetic data and parentage analysis

For all analyses, I defined 3 arbitrary size/maturity categories as follows: (1) Breeders: the 2 largest individuals of anemones with at least 2 individuals measuring at least 50mm TL (Moyer and Steene 1979). (2) Non-breeders: individuals smaller than the breeders that were already present in the first survey. (3) Juveniles: all remaining individuals that settled between both surveys (see determinants of the number of offspring below for details).

A total of 942 individuals in 2008 and 927 individuals in 2009 were screened for 18 microsatellite DNA loci (Quenouille et al. 2004, Beldade et al. 2009b) that satisfied Hardy-Weinberg equilibrium and linkage disequilibrium assumptions. I used the package Genalex v6 (Peakall and Smouse 2006) to compare each of the individual genotypes from fish clipped in 2008 and those clipped in 2009, to identify all pairs of individuals between years that had the same multilocus genotype. Given the number of loci, sample size and allele frequencies, the probability that two individuals drawn at random from a population will have the same genotype at the18 loci (probability of identity) was small (3.26×10^{-18}), and even the more conservative estimate of probability of identity between sibs proposed by Waits et al. (2001) was small (6.19×10^{-7}). Therefore, if two finclips from different years (2008 and 2009) had the same genotype, they were considered as been from the same individual. To account for

possible genotyping errors, mismatches up to 2 loci between genotypes were allowed in pairwise genotype comparisons. Probability of identity and probability of identity between sibs were still small when removing the two loci with the highest exclusion probabilities ($9.10x10^{-13}$ and $2.20x10^{-5}$ respectively) and pairs of individuals that fell in this category were considered if other available data such as size, site and anemone were they were captured corresponded to expectations between years (same size or increment, same anemone or movement to an anemone from the same site).

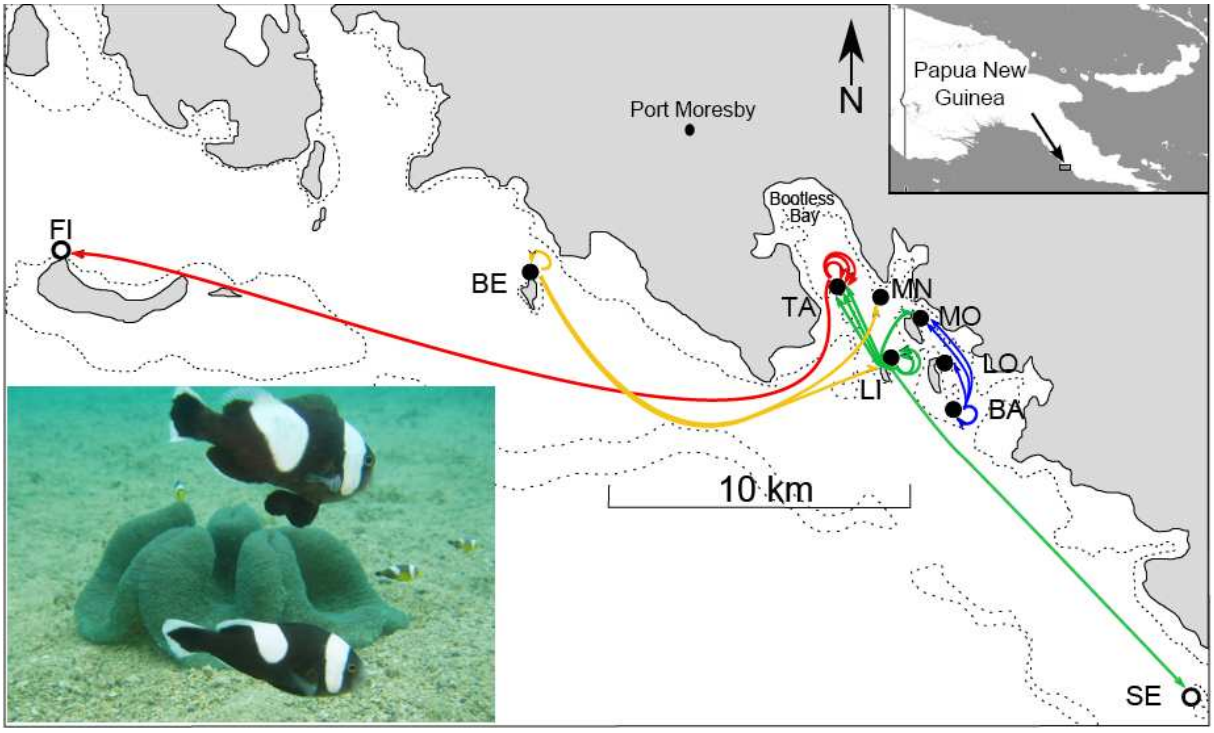

Figure 1 Map of the study area showing 9 sites of anemone aggregations hosting *Amphiprion polymns*. Dotted lines correspond to the limit of shallow reefs. Solid circles correspond to the 7 sites where complete surveys were carried out in both years. Open circles correspond to 2 sites that were partially sampled and for which females were not included in the analyses. Arrows represent individual trajectories of fish larvae produced by 4 different females (each colour represents one female) that were chosen to illustrate how different juveniles from the same mother recruited both within and outside their natal site (only a few trajectories were plotted because the full set would have saturated the image making it unreadable). Site abbreviations are as follows: Manubada Island (BE), Lion Island (LI), Taurama (TA), Motupore north patch reef (MN), Motupore Island (MO), Loloata Island (LO), Loloata South Bank (BA), Fishermen Island (FI), South East Bank (SE). Inset: Location of Bootless Bay in Papua New Guinea. Image: *Amphiprion polymnus* colony in a *Stichodactyla hadonni* anemone, courtesy of S. Planes.

I used the FAMOZ platform (Gerber et al. 2003) to assign juveniles that settled between April 2008 and April 2009 to sampled potential breeders among the 7 sites (this included all potential parents sampled in 2008 plus all new parents sampled in 2009 that replaced fish that died between both surveys). Details of parentage analysis procedure can be found in Saenz-Agudelo et al.(2009). I used an introduced error rate (to account for

genotyping errors) of 0.0001 to simulate the distribution of LOD scores for true and false parents. Based on these simulations, LOD score threshold values to accept parents as been true were set to 4.5 (individual parent) and to 11 (parent couples). Under these conditions 30 assignment tests were ran using simulated data to estimate type I error (probability of excluding true parent while this individual was among the candidate parents) and II error (probability of assigning a wrong parent when the true parent was not sampled), which were acceptably low (~1 and 5% respectively). Because the presence of full/half-sibs is a potential source of type II error, as a conservative measure, I removed manually all single-parent assignments with 2 mismatches between parental-offspring genotypes from the resulting parent-offspring assignments on the real data set (after testing for genotyping errors in the original dataset).

Determinants of egg production

In 2008, after the general fish census and tissue sampling, each anemone was surveyed twice a week over two months (February-April) to record the presence of egg clutches and new recruits. To estimate egg production for each breeding pair, a 49 cm^2 (7 x7 cm) ceramic tile was placed next to each anemone 2 weeks before the beginning of each survey to standardize access to spawning surfaces among anemones. Two weeks was enough time for almost all reproductively active females to start using the tiles to lay eggs. Egg clutches were then photographed using an underwater digital camera and clutch area (cm^2) was estimated using ImageJ software (Abramoff et al. 2004). The mean density of eggs per clutch was estimated from counts along two transects across the clutch (transects were 5mm wide, perpendicular to one another and their length was equal to the diameter of each clutch). In total, 79 breeding pairs had complete observations (all clutches laid in 8 weeks were on tiles and photographed) and were used for analysis. Clutch area and egg density were used to estimate the number of eggs produced per clutch. I used the sum of the number of eggs produced by each female during the 8 weeks of survey as a measure of individual fecundity. Time from laying of eggs to hatching of larvae for this species is ~ 5-6 days in captivity (Rattanayuvakorn et al. 2005). I was able to photograph 95 clutches (40.2%) from 58 breeding pairs in two occasions (with an interval between both photos was 3 or 4 days). Egg loss was estimated as the difference in number of eggs for each clutch between the two observations (number of eggs that disappeared in 3 days). I evaluated if egg production, egg density (as a proxy for egg size) and egg loss (to test if egg production differ from the actual

number of released larvae in a predictable way) were associated with particular parental phenotypes (size) and environmental characteristics (depth and fish colony size).

Determinants of the number of offspring produced in one year

Breeding in *A. polymnus* in this location is likely to occur all year around, as is the case for other anemonefish species in tropical waters of Papua New Guinea (Buston 2004a). To study the relationships between the number of successful offspring produced in one year and parental characteristics (phenotype and environment), the main challenge was in identifying all females that reproduced and survived for the period from 2008 to 2009, and the juveniles that they produced and successfully settled in that time. In both cases multilocus genotype comparisons were used as described previously.

In 2008 I identified 97 anemones that hosted at least a pair of *A. polymnus* and where both fish were large enough to be considered potential active breeders. The minimal size of potential active breeders was chosen to be the size of the smallest female and male couple that was observed laying at least one egg clutch over the entire study period (68mm and 63 mm respectively). A total of 58 females observed in 2008 that had this characteristics were still alive in 2009 and therefore were used in *posteriori* analyses. Among these females, 35 had the same mate in both surveys and the remaining 23 had had their original mate replaced by a large non-breeder from the same anemone or an anemone nearby. Although male replacement could be a source of variation of the number of juveniles produced, I did not take it in to account for two reasons: Firstly, the effect of female size from male replacement could not be separated because females that did not lose their male mate were significantly larger than females that did so (mean ± SE: female $TL_{\text{no replacement}}$ = 110.1 ± 1.7 mm; Female $TL_{\text{male replaced}}$ =103.7 ± 2.7 mm. One-way ANOVA, P = 0.002). Secondly, it has been suggested that in anemonefish where large non-breeders move between anemones (which was the case here), the time for male replacement tends towards zero (Buston 2003a). Therefore, it is likely that male replacement has negligible impact on the number of larvae produced in one year, assuming that only one male replacement occurred per female.

Our procedure to identify juveniles that settled between both surveys was similar as for identifying surviving females previously described. Juveniles that settled between years corresponded to those juveniles sampled in 2009 whose multilocus genotype did not match any of the genotypes from the 2008 database.

Parentage analysis (described previously) was used to identify all of the juveniles produced between both surveys by each of the 58 females mentioned previously

Data analysis

All statistical analyses were performed using R version 2.10.0 (R Development Core Team 2007). I constructed three independent Linear Models (LM) to evaluate the relationship between parental and environmental characteristics and each of the three egg variables (egg production, mean egg density and egg loss) respectively. Fecundity and egg loss values were square-root transformed to fit normality assumptions. Each full model included (1) female TL, (2) difference in size between female and male (Δ_{F-M}), (3) anemone's depth and (4) non-breeders' total length (NBTL) as a measure of fish colony density. I used Δ_{F-M} as a function of male's size because male size is strongly correlated to female size (R^2= 0.77, P<0.001). Model simplification was from the full model using analysis of deviance procedure (Crawley 2007).

General linear models were used to test for correlations between the number of successful recruits produced per female and parental variables. The number of offspring was not normally distributed and could not be transformed; hence a GLM with quasi Poisson errors (to account for over-dispersion) was used. Full model included the same explanatory variables as for egg production. Model simplification was performed using Analysis of Deviance procedure as well.

I evaluated if female size was related to larval dispersal distance using a Mixed Effects Linear Model. This approach enabled us to account for the lack of independence between observations from the same female (many females produced more than one offspring) by including female's identification number as a random effect. For each juvenile (assigned by parentage analysis) the shortest over-water distance between the parental site (hatching site) and the site where the juvenile was sampled (recruitment site) was estimated. Dispersal distance was used as categorical response variable to account for the presence of many zeros (self recruits) in the data set. Dispersal categories were: < 1km (self recruitment), < 5km and > 5km. In addition, to increase our sample size, besides the juveniles produced by 58 females that survived from 2008 to 2009, I included in this analysis all females (and their respective offspring) that acquired this position after the 2008 survey.

Finally, I explored if differences in fecundity among female of different sizes was enough to explain differences in reproductive success (number of juveniles) among females of different sizes. To do this, females present in both surveys (58 in total) were separated into arbitrary size classes of 5mm intervals. Then, I calculated the relative contribution to egg production of each size category (proportion of eggs produced by all the females in one size category divided by the sum of eggs and juveniles produced by all the 58 females

respectively) and based on these proportions and the total number of observed recruits produced by all these females, I estimated the number of offspring produced by each category expected assuming that only egg quantity was responsible for the number of successful juveniles. Using a chi-square test I compared the expected and observed distributions of successful offspring produced by each size class. If significant differences among distributions were found, then the positive relationship between size and egg production would be insufficient to explain the observed positive relationship between female size and the number of successful recruits. To follow chi-square test expectations, all females smaller than 95mm and all females larger than 111mm where grouped respectively in one category, so expected counts within these two classes were > 5.

RESULTS

Determinants of egg production

The linear model on the correlates of fecundity reduced to significant effect of female size only (Analysis of deviance: Female TL, $F_{1, 78} = 22.29$, P= <0.001). The number of eggs produced over 2 months increased with female TL (Figure 2). None of the explanatory variables measured (female TL, male TL, depth and non-breeders total length) had an effect on either egg density or egg loss. Both linear models were reduced to the null model without significant changes in the analysis of deviance (Table 1).

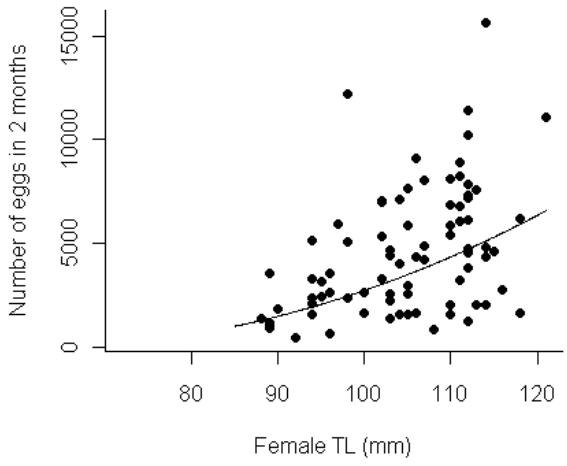

Figure 2 Female size versus fecundity (Sum of the number of eggs laid in 2 months) measured in 2008. Resulting regression line from the reduced Linear Model is shown (sqrt(y) = 1.25x – 66.81. R^2= 0.21, P<0.001, n = 79). Note that the smallest female that was observed laying eggs was 68mm (TL) but was part of females with incomplete observations. Only values for females with complete observations were plotted.

Table 1 Determinants of fecundity in *A. polymnus*. Results of stepwise model reduction using the analysis of variance procedure. Explanatory variables (rows) were removed one at a time, starting from the bottom of the table to the top if P > 0.05.

	Number of eggs		Egg density (eggs cm^{-2})		N eggs lost	
Effect	$F_{(1,77\ df)}$	P-value	$F_{(1,77\ df)}$	P-value	$F_{(1,52\ df)}$	P-value
Female TL	21.72	<0.001	3.02	0.089	1.08	0.303
Depth	0.12	0.728	0.25	0.613	3.73	0.058
NBTL	1.01	0.316	0.18	0.670	1.03	0.314
$\Delta_{F\text{-}M}$ TL	0.14	0.700	0.06	0.802	0.28	0.596

Number and location of juvenile offspring from natal population

Based on individual genotype comparisons 554 juveniles that settled among the 7 sites within the metapopulation between April 2008 and April 2009 were identified. From these (554), 83 were assigned to 58 females older than 1 year and 14 were assigned to individuals that became females in the interval between both surveys (younger than one year). Successful juveniles assigned to local females that settled within the study boundaries settled both within and outside their natal site (some examples are shown in figure 1). Overall, from the 97 locally spawned juveniles ~53% settled within their site of origin, ~26% settled in a different site less than 5 Km away from their site of origin and the remaining 21 settled in sites between 5 and 25 Km away from their natal site (note that maximal distance between two most distant sites was 28 Km).

There was no significant difference in size between groups of females that produced self recruits, larvae that dispersed less than 5km and larvae that dispersed more than 5 Km. (mixed effects linear model: larval dispersal group: $F_{2,43} = 0.0409$, $P = 0.667$). Fecundity was neither directly related to the mean dispersal distance of all larvae produced by each female. (LM, $R^2 = -0.031$, P= 0.906, n=33).

Determinants of number of juvenile offspring produced per female

The General Linear Model on the number of offspring produced per female reduced to a significant effect of female size only. Similar results were obtained for both initial (2008) and final (2009) female total length (TL) (2008). (Female TL_{2008}:. Analysis of deviance: Female TL, $F_{1, 56} = 6.93$, P= 0.0109. The dispersion parameter was taken to be 1.429; Female TL_{2009}:. Analysis of deviance: Female TL, $F_{1, 56} = 6.58$, P= 0.013. The dispersion parameter was taken to be 1.408). Increasing female TL increased the number of successful juveniles that recruited into the population (Figure 3).

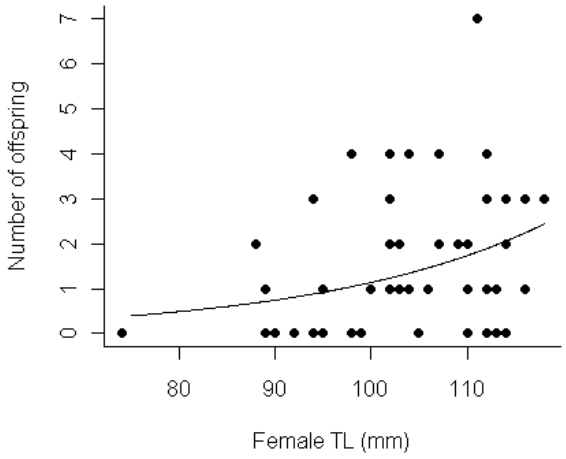

Figure 3 Initial female sizes (2008) versus the number of juveniles that successfully recruited in the population, produced between April 2008 and April 2009. Resulting regression line from the reduced General Linear Model is shown (log(y) = 0.04x – 4.10. n= 58).

There was no significant difference between the distribution of the relative contribution to total fecundity and the distribution of the relative contribution to local replenishment (number of juveniles) among different female size classes (Chi-square = 2.77, 4 df, p = 0.593) (Figure 4). Hence, the great contribution of larger females to local recruitment can be explained by their greater fecundity.

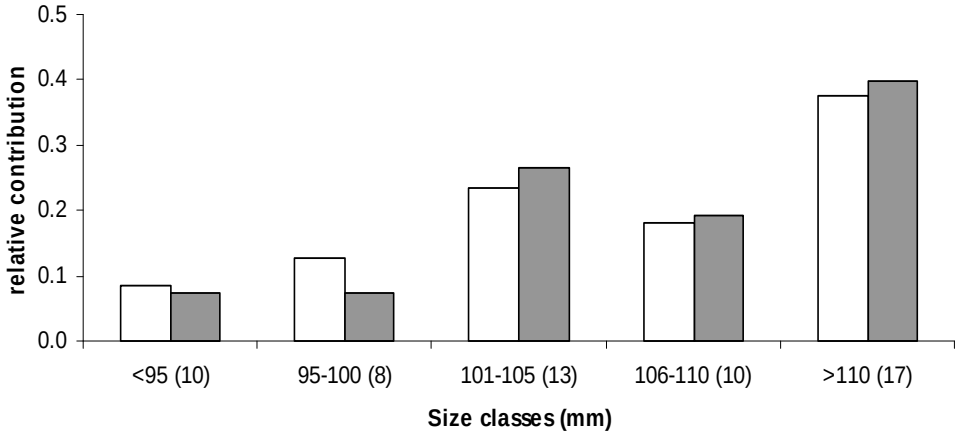

Figure 4 Relative contribution of different size classes to total egg production (white bars) and local replenishment (number of juveniles) (grey bars).

DISCUSSION

Our field application of parentage analysis shows for the first time that individual variation in reproductive success (measured as the actual number of juveniles successfully recruited to the population) can be measured in a natural population of a marine organism with a dispersive larval stage. In doing so, I tested the common assumption that larger females have a higher reproductive success than small females. I confirm, at least at the scale of a small metapopulation, that number of successful recruits produced by individual females is directly related to their size, with larger females accounting for over 3 times the contribution of small females to the recruitment explained by the local population. The magnitude of the difference appears to be largely explained by the difference in egg production, rather than other aspects of egg quality or the local environment.

The assumption that bigger/older females should make a greater contribution to population replenishment than small females is today largely taken for granted (Berkeley et al. 2004b, Kritzer and Sale 2004, Palumbi 2004a, Lucero 2008). This idea is supported by both the common positive relationship between fecundity and female size, and by laboratory experiments that have shown that larval growth and survival can be related to female size or condition (Berkeley et al. 2004a, McCormick 2006). Here I did not find evidence supporting the idea of a significant effect of maternal size on larval fitness, or a synergistic interaction between fecundity and larval fitness. In fact, the differences in the proportion of successful juveniles among female size classes were surprisingly well correlated with the proportion of eggs produced by the corresponding female size class. Despite the fact that female size only explained a low fraction of variation in egg production, the generally higher egg production by large females best explains their higher recorded offspring recruitment. That is, the more eggs produced, the greater the chances of some successfully surviving to recruitment.

These analyses failed to associate most of the variation in egg production and number of successful juveniles per female that was not explained by female size. Fecundity can present high phenotypic plasticity in anemonefish (Green and McCormick 2005) and is in conformity with high levels of unexplained variation observed in this study. In addition, a lack of association between fecundity and fish density is consistent with other studies in other species (Buston 2004a, McCormick 2006). However, fish density and other factors such as body condition or stress may affect larval quality (McCormick 2006, Donelson et al. 2008). The fact that the analyses failed to associate most of the variation of number of successful juveniles to other factors different from female size does not rule out the possibility that larval

quality is also important. This lack of association only suggests that maternal size effects on larval quality might be cancelled by other factors such as stress. As a result, larval quality associated with female size might be less important than fecundity in terms of contribution to local replenishment. However, the high number of uncontrolled and unmeasured possible sources of variation is one of the main drawbacks of studies in the wild, as opposed to laboratory studies where one or few parameters can be tightly controlled. Laboratory situations might in turn remove important sources of selection that can be important in the natural environment. Combining both laboratory and field studies is necessary to quantify the relative importance of larval quality and fecundity to marine population replenishment.

The fact that female size is positively linked with the number of successful recruits in this small reef fish has important implications in terms of conservation and management strategies. First, the effects of female age/size on marine population's dynamics does not seem to be limited to large and long lived exploited species only. Large females of this small coral reef fish species with high population turnover rates can contribute more than twice as much to local replenishment than smaller females. It could be expected that in long lived species, this effect would be more pronounced as bigger females would have higher contributions for longer periods of time. Second, this metapopulation is characterised by rather low self recruitment (see chapter 3), and even under these conditions a significant effect of female size in the contribution to local replenishment can be detected. Besides, there does not seem to be a significant effect of female size with dispersal distance. As a result, there is no reason to think why the female effect on local replenishment would not also be maintained in terms of export outside the metapopulation. However, the degree to which these results can be extrapolated to other scales, species and places requires further investigation. The fact that maternal size effects can be maintained through the three stages of the life-cycle of at least one marine fish warrants further testing of this phenomenon. If it does turn out to be of general importance, there will be strong support for management strategies that not only regulate total mortality, but also for no-take reserves that can help restore age/size structure (Kritzer and Sale 2004, Palumbi 2004a, Sale et al. 2005a, Venturelli et al. 2009).

In conclusion, our study shows that assessing the reproductive success or fitness of individuals in wild marine fish populations is no longer an intractable problem. It can be achieved by combining parentage assignments and long-term monitoring of reproductive status and turnover in the adult population. Although applied here to a clownfish with a relatively short pelagic larval duration, recent data suggests that species with longer larval durations may also exhibit significant levels of local retention (Almany et al. 2007, Jones et

al. 2009b). The combination of these methods is becoming a powerful approach to addressing questions once thought impossible to answer, such as measuring the adaptive significance of different life history and mating strategies (Rodriguez-Munoz et al. 2010, Serbezov et al. 2010). While we still far from fully understand the complexity that drives the variation in marine population replenishment, maternal size is clearly an important piece of the puzzle.

CHAPTER 5

POPULATION SELF-PERSISTANCE: A MATTER OF SPATIAL SCALE

This last data chapter intends to do a compilation of most of the data obtained in previous chapters articulated as a simple demographic model. The idea behind this was to try to answer, in a deterministic way, a simple but important question: Is the amount of self recruitment observed either within local populations or at the metapopulation level enough to assure self persistence? If this is not the case, then what is the spatial scale at which the necessary conditions for demographic stability are met? It is worth mentioning here that it is beyond the scope of this chapter to forecast the future of this metapopulation. The aim was rather to link two "snapshots" of the movie to better understand the action taking place between them.

Publication: Saenz-Agudelo, P., G. P. Jones, S. R. Thorrold, and S. Planes. A model of population persistence in a coastal coral reef metapopulation: importance of spatial scale and connectivity. **In preparation**.

ABSTRACT

An important goal in population biology is to identify the conditions under which populations can persist. Marine populations are often divided into numerous groups of spatially discrete sub-populations that are connected by different levels of dispersal, making their dynamics complex. Models of the behaviour of such populations often lack empirical estimates of critical population parameters such as the magnitude and direction of dispersal, which limits confidence in their predictions. Here I developed and tested a simple matrix-based model of a coastal clownfish metapopulation in Papua New Guinea using novel genetic approaches to estimate population parameters. A DNA fingerprinting approach was used to identify and monitor individuals over two consecutive years to measure survival and size transition rates of resident fish. This information was coupled with estimates of self recruitment and connectivity among subpopulations computed from parentage analysis of all juveniles recruiting over the 2-year period. The model was used to explore conditions of demographic stability by calculating population growth rates at 2 spatial scales: for each subpopulation separately and for the combined metapopulation. Given the observed self recruitment levels and survival rates, the model indicated that all individual subpopulations would have negative growth rates if they relied only on self recruitment over multiple generations. Positive population growth was only achieved by including larval input from outside the boundary of the studied metapopulation. Increasing survival rates of resident fish diminished the geographic scale at which population persistence was achieved. The model highlights the demographic significance of connectivity in this system and suggests that small marine protected areas will not contribute to population persistence, unless they are a part of a larger network of reserves.

INTRODUCTION

Most species are distributed across space in discontinuous populations that are associated with discrete patches of suitable habitat, with varying levels of exchange of individuals among them. Understanding the conditions required for persistence of single populations and groups of populations (metapopulations) is one of the main issues of population biology (Crouse et al. 1987, Hanski 1994, Caswell 2001, Hastings and Botsford 2006, Botsford et al. 2009, White et al. 2010b). Particular attention has been focused in understanding the role of connectivity, the level of exchange among discrete populations, as this is predicted to be a major driver of the dynamics of natural populations and a key factor in population stability and resilience (With et al. 1997, Kritzer and Sale 2004, Figueira and Crowder 2006, Lipcius et al. 2008). Early metapopulation models addressed population persistence by describing how the fraction of occupied habitat patches depends on patch area and isolation (Hanski 1994). This approach assumes that metapopulations are composed of many discrete patches and that local colonisation and extinction events are common and detectable over short periods of time. Under this scenario, the probability of the colonisation of an empty patch can be affected by many factors that are difficult to model and simplified connectivity measures based on patch size and spacing are used to simulate population dynamics (Moilanen and Nieminen 2002). However, one of the limitations of this approach is that colonisation/extinction dynamics may be rare or might not occur at a temporal scale that can be easily measured.

A second approach to determine population persistence involves estimating the growth rate of a population based on demographic parameters such as survival probabilities and fecundity of individuals that are grouped in age or size categories (Caswell 2001). In the simplest form, these population matrix models are based on a single closed population and demographic equilibrium (persistence) is reached when the number of produced offspring that are born is sufficient to offset the number of deaths (White et al. 2010b). This approach can be also used to describe metapopulation systems where exchange among populations exists and can be estimated. In this case, individuals are grouped in sub- or local populations and persistence in the metapopulation can occur as long as at least one of the local populations is self persistent (Armsworth 2002), or if in the absence of a single self persistent population, the total amount of replacement through all possible exchange paths is greater than a certain threshold (Hastings and Botsford 2006). A reliability of matrix population models depends upon the quality of field data on population parameters and establishing the links between

life history and population characteristics (Wisdom et al. 2000). However, the need to link field data and matrix models also restricts their application, as obtaining accurate estimates of the vital demographic rates required for the correct interpretation of model output (Caswell 2001), can be challenging (especially for organisms with complex life cycles).

Most marine species fall in the "challenging" category for modelling because they have complex life cycles that include a dispersive pelagic larval phase. In addition, a variety of human impacts over the last century, including climate change, habitat deterioration and overfishing have drastically declined many populations and fisheries around the planet (Jackson et al. 2001, Hughes et al. 2003, Hoegh-Guldberg et al. 2007, Mora et al. 2009, Worm et al. 2009). To combat these threats, no-take marine protected areas (MPAs) have been widely advocated and implemented as a tool to protect marine biodiversity and restore overfished populations (Roberts 1997). The core of this protection strategy is based on the principle of population self-persistence (Botsford et al. 2009, White et al. 2010b). Marine reserves are assumed to work not only by maintaining sufficient local retention of larvae to assure the maintenance of protected populations within individual MPAs, but also by having sufficient connectivity among locations within an MPA network to rescue populations that suffer a local disturbance (Palumbi 2004b, Sale et al. 2005a, Mora et al. 2006, Almany et al. 2009). Population modeling has revealed how important the width of dispersal kernel can be to population dynamics and how size and spacing of protected areas can affect persistence of exploited populations (Botsford et al. 2001, Lockwood et al. 2002, Hastings and Botsford 2003, Hastings and Botsford 2006, Kaplan et al. 2006, Botsford et al. 2009, Kaplan et al. 2009, White et al. 2010b). However, the lack of empirical data on connectivity has constrained population modelling to a theoretical framework and has hindered their application to specific species and locations.

Empirical data on marine population connectivity is rapidly emerging as a result of new techniques such as chemical analysis of calcified structures, chemical tagging of otoliths and genetic parentage analysis (reviewed in Jones et al. 2009b). These empirical approaches have altered our perceptions of the scale of the dynamics of marine populations, with more local self recruitment than previously thought. However, while they have provided valuable information about the degree of isolation of a marine population (Jones et al. 1999, Swearer et al. 1999, Jones et al. 2005, Patterson and Swearer 2007, Planes et al. 2009, Saenz-Agudelo et al. 2009, Christie et al. 2010), this information alone does not answer the question of whether a population relies on natal larval recruitment for persistence (Botsford et al. 2009, reviewed in Jones et al. 2009b). Inferences about population persistence in these systems can only be

made if these empirical values of self-recruitment and connectivity can be integrated in time with the other vital demographic parameters for the population of interest.

Over the last few decades, a variety of extraordinary genetic tools have been developed that are now being widely applied in different fields of ecology (Jones and Ardren 2003). One of the main advantages of genetic data is that, since DNA samples can be now obtained from small portions of tissue without detrimental effects on individuals, the same genetic information used to reconstruct parent-offspring relationships and estimate self recruitment or connectivity (Jones et al. 2005, Planes et al. 2009, Saenz-Agudelo et al. 2009), can also be used as individual natural tags and provide mark-recapture information (PalsbØli 1999, Waits et al. 2001, Morrissey and Ferguson 2009). That is, DNA-based individual recognition can be used to estimate demographic parameters such as mortality and stage transition rates.

Over the last two years I have gathered genetic data of the anemonefish *Amphiprion polymnus* in a metapopulation system consisting of discrete geographic populations located in Bootless Bay, Papua New Guinea. Population surveys and genotyping (by fin clipping individuals and realising them back) of all individuals within ~ 12 km of coastline has been performed to estimate larval retention and exchange among these populations via parentage analysis (Saenz-Agudelo et al. in preparation). Here, I used individual multilocus genotype comparisons between two genetic data sets of the entire metapopulation (one from each year of survey) to follow the fate of all fish fin clipped in the first year that survived to the next one. In this way survival rates for different size stages were calculated. I coupled these values with connectivity estimates and integrated this information in a simple metapopulation stage-based matrix model (Caswell 2001) to estimate population growth rates at different spatial scales. Given the observed larval dispersal patterns the following questions were evaluated: (1) Can any of the subpopulations persist with inputs only from local self-recruitment? (2) If none of the populations are self-persistent, can persistence of the known metapopulation be achieved on the basis of connectivity among local populations? (3) Or does the metapopulation rely on external larval supply for persistence.

METHODS

Study species, location and demographic parameter estimation

The panda clownfish (*Amphiprion polymnus*) is a Southeast Asian endemic fish that lives in close association with discrete aggregations of two species of anemones

(*Stichodactyla hadonni* and *Heteractis crispa*) that are restricted to sandy habitats associated with coral reefs (Fautin and Allen 1992). Each anemone is usually occupied by one breeding pair and up to eight smaller non-breeders and juveniles. The female (the largest individual) lays demersal eggs on the upper surface of shells or dead coral next to the anemone. The embryos develop over a period of 6-7days before hatching (Fautin and Allen 1992) and post-larvae settle into anemones after a pelagic larval phase lasting 9-12 days (Thresher et al. 1989). *Amphiprion polymnus* is a protandrous hermaphrodite (Moyer and Nakazono 1978). As in all other anemonefishes, when the female of a group dies, the male changes sex and assumes the position vacated by the female, and the largest non breeder from the anemone inherits the position vacated by the sex-changing male (Buston 2004b). These characteristics make these fish excellent candidates to use in stage structured population models.

I used genetic data collected over 2 consecutive years (2008 and 2009) in a metapopulation system consisting of 7 spatially discrete populations in Bootless Bay, Papua New Guinea (Figure 1).

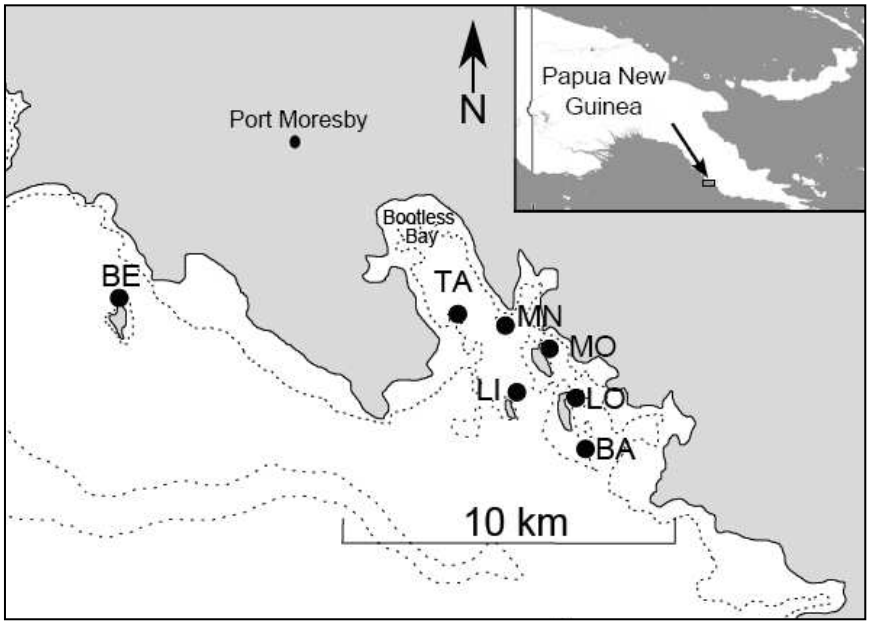

Figure 1 Map showing sites of the 7 anemone aggregations hosting *Amphiprion polymns* in Bootless Bay area (black filled circles) from which demographic parameters were estimated for the model. Inset: Location of Bootless Bay in Papua New Guinea. Site abbreviations are as follows: Manubada Island (BE), Lion Island (LI), Taurama (TA), Motupore north patch reef (MN), Motupore Island (MO), Loloata Island (LO), Loloata South Bank (BA).

Each year, all fish from each anemone were captured, measured to the nearest mm (using callipers) and a small tail clip was taken and stored in 95% ethanol for genetic analysis. A total of 18 microsatellite markers were screened as described in (Quenouille et al. 2004, Beldade et al. 2009b). The package Genalex v6 (Peakall and Smouse 2006) was used to

compare each of the individual genotypes from fish clipped in 2008 and those clipped in 2009. In this way were able to identify all pairs of samples that had the same multilocus genotype. Given the number of loci, sample size and allele frequencies, the probability that two individuals drawn at random from a population will have the same genotype at multiple loci (probability of identity) was really small ($3.26x10^{-18}$), and even the more conservative estimate of probability of identity between sibs proposed by Waits et al. (2001) was small ($6.19x10^{-7}$). Therefore, I assumed that two samples from different years (2008 and 2009) with the same genotype corresponded to the same individual fin clipped in both years. To account for possible genotyping errors, mismatches in up to 2 loci between genotypes were considered in pairwise genotype comparisons. With 16 loci, the probability of identity and probability of identity between sibs remained still small ($9.10x10^{-13}$ and $2.20x10^{-5}$ respectively) and pairs of individuals that fell in this category were considered if after rescoring their genotypes these differences were rectified.

Each individual fish within a group (anemone) was classified in one of three size classes: The biggest fish in was identified as the female, the second largest individual was recognised as the male, and all remaining individuals were classified as juveniles. A life table was constructed where individuals clipped in 2008 that were not recaptured in 2009 were assumed to have died. Individuals that survived were then classified according to whether they had remained in the same size class or they had grown in to the next one. With this data I calculated survival and transition rates for each size category. Finally, I used the data of connectivity and self-recruitment estimated using parentage analysis (Saenz-Agudelo et al *in preparation,* see chapter 3) to calculate fecundity (number of larvae produced per female that settled in one of the seven populations and survived to be counted as a juvenile in one year interval). Self-recruitment is defined as the number of juveniles recruiting to a population that are offspring of parents within the defined population.

Model description

The dynamics of the metapopulation were modelled by using a stage-classified matrix metapopulation model similar to Caswell (2001). For simplicity, I chose a stage classified model where the life cycle was divided into three stages: Juveniles (stage 1), males (stage 2) and females (stage 3). The projection interval of our model was one year because this was the interval between field observations. The number of stages could have been larger to better describe the structure of the groups in this species (composed of up to 8 individuals). However, the number of stages were limited by the time step (interval between observations),

because more stages implied violating the assumption of matrix models of stepwise single stage transitions per time step.

The modelled system was composed of seven populations with local dynamics and larval exchange among them and an additional constant larval input to each population accounting for an external unknown source. The overall dynamics of the metapopulation system with external input is described by

$$\mathbf{n}_{(t+1)} = \mathbf{A}\,\mathbf{n}_{(t)} + \mathbf{m} \qquad \textbf{(eq. 1)}$$

Where $\mathbf{n}_{(t)}$ is a vector composed of subvectors $\mathbf{n}_{x(t)}$ giving the number of individuals at each stage within each population *x* at time (t) (in this case 3 stages and 7 populations, 21 lines). **A** is the population projection matrix describing the metapopulation dynamics:

$$\mathbf{A} = \begin{pmatrix} \mathbf{A}_1 & \mathbf{M}_{2\to1} & \mathbf{M}_{3\to1} & \mathbf{M}_{4\to1} & \mathbf{M}_{5\to1} & \mathbf{M}_{6\to1} & \mathbf{M}_{7\to1} \\ \mathbf{M}_{1\to2} & \mathbf{A}_2 & \mathbf{M}_{3\to2} & \mathbf{M}_{4\to2} & \mathbf{M}_{5\to2} & \mathbf{M}_{6\to2} & \mathbf{M}_{7\to2} \\ \mathbf{M}_{1\to3} & \mathbf{M}_{2\to3} & \mathbf{A}_3 & \mathbf{M}_{4\to3} & \mathbf{M}_{5\to3} & \mathbf{M}_{6\to3} & \mathbf{M}_{7\to3} \\ \mathbf{M}_{1\to4} & \mathbf{M}_{2\to4} & \mathbf{M}_{3\to4} & \mathbf{A}_4 & \mathbf{M}_{5\to4} & \mathbf{M}_{6\to4} & \mathbf{M}_{7\to4} \\ \mathbf{M}_{1\to5} & \mathbf{M}_{2\to5} & \mathbf{M}_{3\to5} & \mathbf{M}_{4\to5} & \mathbf{A}_5 & \mathbf{M}_{6\to5} & \mathbf{M}_{7\to5} \\ \mathbf{M}_{1\to6} & \mathbf{M}_{2\to6} & \mathbf{M}_{3\to6} & \mathbf{M}_{4\to6} & \mathbf{M}_{5\to6} & \mathbf{A}_6 & \mathbf{M}_{7\to6} \\ \mathbf{M}_{1\to7} & \mathbf{M}_{2\to7} & \mathbf{M}_{3\to7} & \mathbf{M}_{4\to7} & \mathbf{M}_{5\to7} & \mathbf{M}_{6\to7} & \mathbf{A}_7 \end{pmatrix}$$

Within this matrix seven sub matrices $\mathbf{A}_\mathbf{x}$ in the diagonal describe the transitions and reproduction of individuals that stay within population x (local dynamics) and the remaining sub matrices $\mathbf{M}_{\mathbf{j}\to\mathbf{i}}$ describe the movement of juveniles from one population (j) to a different one (i).

Each transition matrix $\mathbf{A}_\mathbf{x}$ takes the form:

$$\mathbf{Ax} = \begin{pmatrix} P_{x1} & 0 & F_{x3} \\ G_{x1} & P_{x2} & 0 \\ 0 & G_{x2} & P_{x3} \end{pmatrix}$$

Where individuals in population *x* in stage *i* surviving one year can either remain in the same stage with a probability P_{xi} or they can advance to the stage $i+1$ with a probability of G_{xi}. Fertility F_{x3} in this matrix is represented by self recruitment and defined as the number of larvae produced per female in population **x** that successfully recruited to the same population of origin and survive to be counted in stage 1 (Only individuals in stage 3 produce larvae). Among the seven $\mathbf{A}_\mathbf{x}$ matrices of the model, only fecundity values differ between them. Transition (*G*) and survival (*P*) probabilities were assumed to be equal among populations. Each sub matrix describing the movement of juveniles between populations takes the form

$$\mathbf{M}_{x \to y} \begin{pmatrix} \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{F_{xy}} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} \end{pmatrix}$$

Where F_{xy} is defined as the number of larvae produced per female in population *x* that successfully settled in population *y* and it was calculated from values of connectivity in the empirical data. Finally, the last term in equation 1 **(m)** is a vector describing a constant input of immigrant juveniles to each population coming from an unknown source. Numeric values for this vector were estimated from empirical data and corresponded to the number of juveniles fin clipped in 2009 that did not had a matching genotype in 2008 and were not assigned by parentage analysis.

The population dynamic state (increase, decrease or stable) was evaluated in three different spatial scenarios: 1) for each individual local population with only self recruitment as larval input; (2) for the metapopulation comprising all local populations with self recruitment and larval exchange among them but no external larval supply; 3) same metapopulation but with an external constant larval input which corresponded to the number of juveniles that settled at each site between both surveys that were not assigned to any of the sampled parents by parentage analysis.

In the first scenario each individual population projection takes the form $\mathbf{n}_{\mathbf{x}(t+1)} = \mathbf{A}_{\mathbf{x}} \mathbf{n}_{\mathbf{x}(t)}$. Post multiplying matrix $\mathbf{A}_{\mathbf{x}}$ by the population vector $\mathbf{n}_{\mathbf{x}}$ is used to forecast future population states. The dominant eigenvalue λ_{1x} of the matrix **Ax** describes the long term behaviour of the population **x** assuming a constant environment. The dominant eigenvalue of each stage-class matrix $\mathbf{A}_{\mathbf{x}}$ is equal to e^r, where *r* is the intrinsic rate of change of the population in the equation

$$N_t = N_o e^{rt} \quad \textbf{(eq. 2)}$$

Thus when $\lambda_1 = 1$, $e^r = 1$ and the intrinsic rate of change of the population $r = 0$ and the population is stable (Crouse et al. 1987). If $\lambda_{1x} > 1$ then the population is in exponential growth, and if $\lambda_{1x} < 1$ the population is in exponential decay.

In a similar way the metapopulation projection (second spatial scenario) takes the form $\mathbf{n}_{(t+1)} = \mathbf{A}\ \mathbf{n}_{(t)}$ and the dominant eigenvalue λ_1 describes the long term behaviour of the metapopulation. In both scenarios (1 and 2) the analytical calculation of the dominant eigenvalue allowed to evaluate if the observed larval input at each spatial level was enough for the population (or metapopulation) persistence ($\lambda_1 \geq 1$).

Because the third scenario does not have an analytical solution for λ_1 (because of the migration term), I used a simple routine in R (R Development Core Team 2007) to estimate the population vector $\mathbf{n}_{(t+1)}$ from equation (1) over 40 consecutive years. I considered this period long enough to obtain an approximation of the stable population projection. From equation (2) by plotting the natural logarithm of vector $\mathbf{n}_{(t+1)}$ over time, one can obtain a graphic estimation of *r*. The same R routine was used for scenarios 1 and 2 to obtain graphs for comparison purposes.

It should be noted that the model used here did not attempt to forecast the fate of the metapopulation in the future. Rather, the aim was to examine the dynamics of the population under present conditions with certain assumptions (Caswell 2001). In this case they included constant environment, constant self recruitment and connectivity proportions in time and density-independent vital rates. The first two assumptions are discussed in chapter 3 section 2. Density-independent vital rates are a reasonable assumption because I was only interested in the direction of the population growth rates (positive or negative) and not in its shape (linear or non linear).

Model sensitivity to changes in resident survival rates

One of the advantages of constructing population matrix models is that the sensitivity of the population growth rate to variations in fecundity, growth, or survival rates can be simulated by varying these parameters in the model and then calculating λ_1 for each scenario (Crouse et al. 1987). A simple analytical elasticity analysis (a proportional measure of sensitivity, see Caswell 2001) of the transition matrix A suggested that λ_1 was in general more sensitive to changes in survival rates (especially males and females) than in fecundities (data not shown). Assuming that connectivity patterns depend mostly on the spatial configuration among populations and not in the biological features of the species (Jones et al. 2009b) then it becomes interesting to leave connectivity values fixed in the model and to test how different survival rates will change λ_1 in this particular connectivity scenario. The variation in survival probabilities was evaluated by using published survival and transition probabilities of *Amphiprion percula* (Buston and García 2007). This species has higher survival probabilities than the ones I estimated in this study for *A. polymnus*, and was an interesting real data set to evaluate in our "fixed" connectivity context. In their study survival and transition probabilities for this species were estimated in 6 stages corresponding to size ranks. The survival probabilities of the 4 lower stages corresponding to ranks (6 to 3) were averaged and

used as an estimate of *Pi* in stage 1. Finally, unmodified survival rates for ranks 2 and 1 (males and females respectively) were used as estimates for stages 2 and 3.

RESULTS

Of the 450 resident individuals that were fin clipped in 2008, 273 were still present in 2009 providing estimates of survival and transition probabilities (Table 1). Average survival probabilities were 0.513, 0.77 and 0.628 for juveniles, males and females respectively. These values were then used in the transition matrices **Ax**. The mean number of juveniles that self recruited or were exchanged per year per site (averaged over two years, estimated using parentage analysis) ranged from 0 to 18 (Table 2). From 301 observed juveniles, 39 recruited in their natal populations (self-recruits), 55 recruited in one of the other local populations (local connectivity) and the remaining 207 were immigrants from outside the boundaries of the study. These values were divided by the number of females observed in 2008 in each population to obtain individual fecundity values that were included in the resulting stage class metapopulation matrix **A (**Table 4, appendix).

Table 1 A. Transition matrix showing the probability of surviving and remaining in the initial stage (Pi) and the probability of surviving and advancing in stage (Gi) of *Amphiprion polymnus* anemonefish in Bootless Bay, Papua New Guinea. Numbers in brackets correspond to the number of fish in each stage that were fin clipped in 2008. **B**. Transition matrix using data published in (Buston and García 2007).

A		Initial stage		
Transition probabilities		1 (n = 226)	2 (n = 111)	3 (n = 113)
Final stage	1	0.3186	0	0
	2	0.1947	0.5225	0
	3	0	0.2478	0.6283
Probability of surviving		0.5133	0.7703	0.6283

B		Initial stage		
Transition probabilities		1	2	3
Final stage	1	0.5540	0	0
	2	0.0589	0.8837	0
	3	0	0.0581	0.9310
Probability of surviving		0.6129	0.9418	0.9310

In terms of percentage of recruitment, scenario 1 (local dynamics: self recruitment within populations only) meant that only ~13% of the observed recruitment was considered as source of population replenishment. None of the seven populations had a positive growth rate with the observed self-recruitment rates (Table 3). The highest values of λ_1 (0.8447) and *r* (-0.1688) were to Taurama, the population with highest self recruitment.

Table 2 Estimates of the number of self recruits (bold cases) within and local connectivity among populations per year for *Amphiprion polymnus* based on parentage analyses performed over two consecutive years (2008 and 2009). Last column shows the number of immigrants (not assigned to any of the local populations) that arrived between 2008 and 2009 to each population.

	Source							
Sink	BA n=23	LO n=16	MO n=12	LI n=11	MN N=6	TA n=23	BE n=22	immigrants
BA	**7**	0	1	2	1	0.5	2	47
LO	5	**3**	2.5	2	1	2	0.5	21
MO	4	2	**1.5**	5	2	3.5	1.5	32
LI	2	1	0	**1**	0	2	1	19
MN	1	0	0	1	**0.5**	1	1.5	12
TA	1	0	0.5	2	1	**18**	0.5	32
BE	3	1	0.5	2	0.5	2	**8**	41
Total self recruitment (diagonal)							**39**	
Total local connectivity							55	
Total number of immigrants								207

In scenario 2, exchange of larvae among the seven populations was allowed, but no larvae coming from outside. In terms of recruitment, this meant more than doubling the number of juveniles explained by adults in each sub population, with an ~ 30% self-recruitment. The dominant eigenvalue λ_1 of the metapopulation matrix **A** was $\lambda_{1=}$ 0.8567 and the intrinsic growth rate r = -0.1547. Compared to the previous scenario (self recruitment only), adding local connectivity only had a small positive effect on the overall population growth rate. That is, despite the local connectivity, all else being equal, the populations would still undergo a decline (Figure 2.A).

In scenario 3, the input of larvae from an unknown source at a constant rate per year was allowed. In terms of recruitment, this encompassed 100% of observed recruitment. In this case the intrinsic rate of change was estimated by calculating the slope of the corresponding line of figure 2.A based on equation (2). For this regression I used data beginning at the 15th time step because at this point the system had reached a stable form. Intrinsic rate of change when immigration was allowed was slightly positive (r = 0.0002, R^2= 0.97) and from equation (2) an estimation of the dominant eigenvalue was also estimated ($\lambda_1 = e^r$ = 1.0002).

The same procedure was repeated after increasing survival rates by 10, 17 and 31 % for juveniles, males and females respectively (Table 1B). With these new survival rates, in scenario 1 (allowing only for self-recruitment as replenishment source) individual population λ_1 increased to values between 0.9433 and 0.9883, and respective r from -0.0584 to -0.0118 (Table.3). When connectivity between populations was introduced, the dominant eigenvalue was even closer to 1 (λ_1 = 0.9924) and the intrinsic rate of change was closer to 0 (r = -0.0076) almost reaching demographic equilibrium (Figure 2.B). Finally, when immigrants

where included the population intrinsic rate of change became positive $r = 0.0245$ ($R^2 = 0.99$) and from equation (2) $\lambda_1 = e^r = 1.0248$.

Table 3. Variation of the number of self recruits per female per year among the seven populations of *A. polymnus*. For each population the number of self recruits is also expressed as the percentage of self recruitment estimated from parentage analysis for each population. Resulting population growth rate (λ_1) and intrinsic rate of change (r) are shown for each of the two survival scenarios.

			Low survival (table 1A)		High survival (table 1B)	
Population	Number of self recruits*female^{-1}*year^{-1}	Mean self recruitment (2008-2009)	λ_1	r	λ_1	r
1 (BA)	0.28	9%	0.7585	-0.2765	0.9615	-0.0393
2 (LO)	0.16	4%	0.7161	-0.3339	0.9510	-0.0502
3 (MO)	0.13	2%	0.7123	-0.3393	0.9479	-0.0535
4 (LI)	0.09	3%	0.7123	-0.3393	0.9442	-0.0574
5 (MN)	0.08	5%	0.6902	-0.3707	0.9433	-0.0584
6 (TA)	0.76	30%	0.8447	-0.1688	0.9883	-0.0118
7 (BE)	0.36	8%	0.7771	-0.2522	0.9671	-0.0334

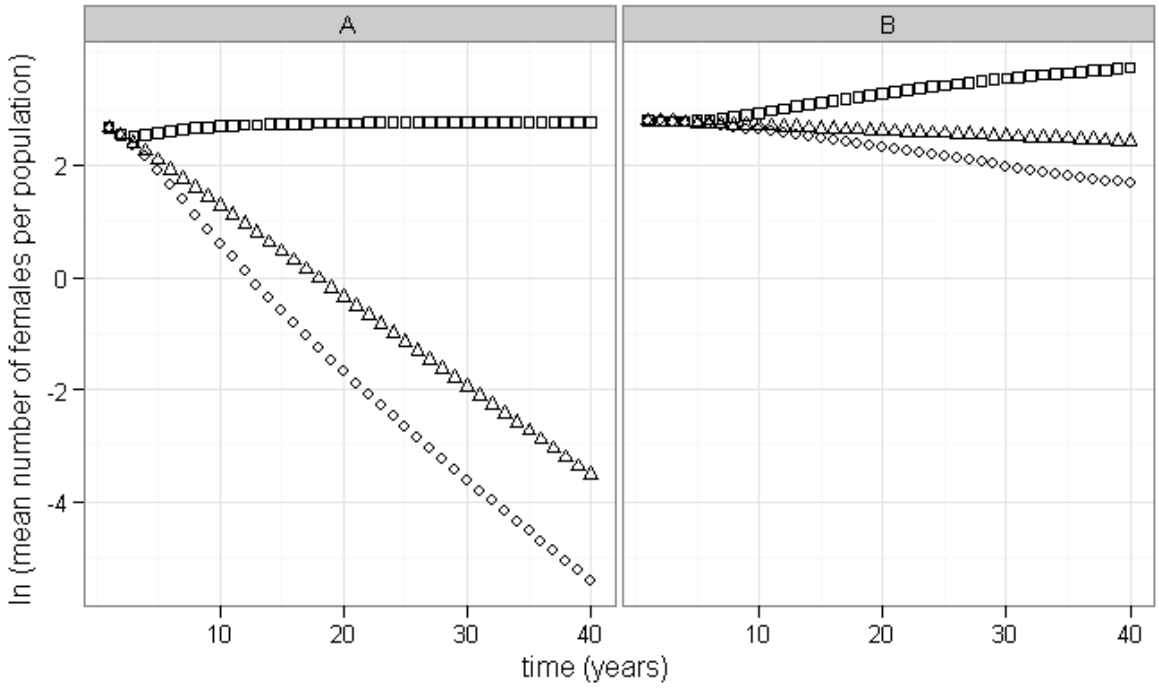

Figure 2. Graphic results of the matrix metapopulation model describing the mean number of female *A. polymnus* per population (average of the 7 populations) over 40 time steps (years). **A)** Low survival scenario using data from table 1A**. B)** High survival scenario using data from table 1B. At each scenario simulation of local dynamics (only self recruitment allowed) are represented by diamonds; metapopulation dynamics (local exchange among populations allowed but no external input) are represented by triangles; Metapopulation dynamics with external larval input is represented by squares. Note that the values of number of females per population are $\log_e$ transformed. In this way from equation (2) the slope of the resulting lines can be taken as an approximation of the intrinsic rate of change (r) of the metapopulation.

DISCUSSION

Overall, the results of this model suggest that for *A. polymnus* at the local population scale, even populations with relatively high self recruitment (~30%) are not self-sustainable. At the metapopulation level, when local connectivity was also included (corresponding to ~33% of total recruitment), there was a positive effect on the overall growth rate. However, this extra larval input was not sufficient to change the negative growth rate of the metapopulation. In other words, when allowing in the model for larval exchange among the seven populations, conditions for persistence were not met either. Stability was achieved when the remaining recruits (immigrants) were included in the model. This study is unique that it integrates DNA-based empirical estimates of population parameters with a model of a known metapopulation of a coral reef fish species. In doing so, it provides evidence of the relative importance of different larval sources in explaining population persistence in this space-limited species.

Before discussing the implications of this modelling approach, it is worth mentioning that the survival rates for *A. polymnus* estimated in this study are considerably lower than those reported for its congener species *A. percula* (Buston and García 2007), used here as an alternative scenario. Even if at first sight both species do not seem to be that different in their ecology to explain such differences, many factors can be at the origin of this variation, including the difference in the geographic location and methods used to estimate survival between studies. Yet, it is worth mentioning that one likely contribution to these differences in mortality might the difference in behaviour between the two species. Large *Amphiprion* species usually venture further away from their host anemones than smaller species (Hattori 1995). In *A. polymnus* adult fish can be seen sometimes a few meters away from the anemone when they are feeding and breeding couples with nests can be aggressive and swim away from their anemone to chase away larger organisms that pass by (including scuba divers) to protect their eggs (personal observations). *A. percula* on the other hand is never more than ~1 meter away from their host anemone and might be less vulnerable to predation that its bigger congener *A. polymnus.* A comparative study using the same techniques in the same location would be interesting to validate this idea.

The spatial scale at which demographic stability was achieved by larval exchange was smaller under scenarios of higher survival rates (slow turn over rates) than for scenarios with lower survival rates. What is interesting about this approach is that it allows to quantitatively estimating how much changes in survival affect the scale at which self-persistence is achieve.

If it is assumed that connectivity patterns are mostly determined by geographic features, and that habitat beyond our study's limit has the same characteristics as it has within (in terms of size and distance among patches),then local connectivity will increase linearly as the area considered increases and self recruitment will remain constant. In numbers this means that the contribution to total recruitment in 10 km of coast-line of the populations within that area will be ~30 % (this includes ~13% of self recruitment and 17% of local connectivity). If this area is doubled then the proportion explained by self recruitment will be the same (~13%) but the proportion explained by local connectivity will increase to 34%. Using this logic, the area needed to reach demographic self persistence can be estimated by adding spatial units and calculating the overall population's growth rate (λ_1). In this case, self-persistence is achieved only when considering between 5 and 6 spatial units (~55km) for a species with low survival rates and between 1 and 2 (15 km) for a species with high survival rates (Figure 3). From a conservation perspective, this means that assessing protection baseline (e.g. size of an MPA) based on a species with rather high turnover rates (high mortality) and easy to study in the field such as *A. polymnus*, should also effectively assure conservation of larger species that are also rather sedentary as adults. However, verifying the relative importance of the geographic context and individual species characteristics is needed before any extrapolation of these particular results can be considered.

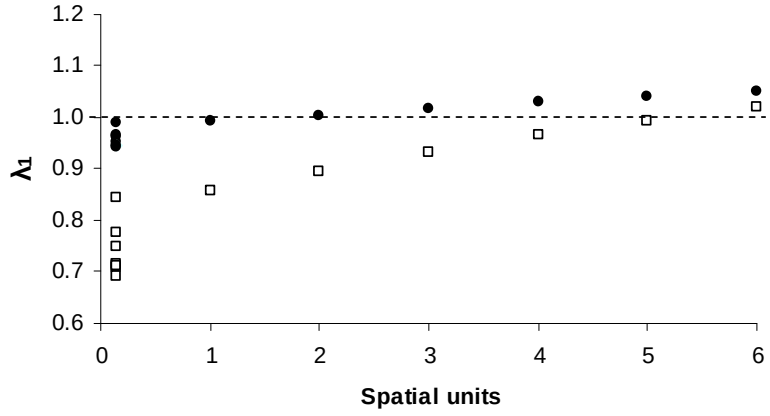

Figure 3 Relationship between population growth rate and the number of spatial units (1 unit ~ 10 Km) for low (open squares) and high (filled circles) survival rates. The broken horizontal line indicates a stable demographic rate. Values of growth rates for each of the individual seven populations assuming only self recruitment are shown near the origin of the x axis.

Another way of interpreting model results is by assuming that the two different survivorship scenarios correspond to two protection levels of this species. On one hand, low survivorship can be interpreted as a baseline, a scenario with no protection measures, or were protection is partial (e.g. focused on protection of habitat for juveniles only and where fishing

of large individuals is allowed to some extent). For example, there is evidence that fish abundance and size of heavily targeted fish in partially open marine reserves does not increase compared to open fishing grounds (Denny and Babcock 2004). In fact some studies have shown that fishing pressure on large fish categories of targeted species may not diminish in partially protected areas (Lester and Halpern 2008). As a consequence, mortality of these targeted fish species remains high (Coleman et al. 2004). In the second scenario, increasing survival rates, especially of large size stages, can be considered as a strategy focused on protecting large individuals (e.g. no take reserve). Under the first strategy (low survival rates) model results suggest that a large area of reserve (around 5 times larger than our study area) would be needed to achieve population persistence. In the second scenario (high survival rates), by increasing survival, especially of breeding fish, population persistence is achieved at a smaller spatial scale (10 to 15 km). In terms of conservation strategies for heavily fished species exogenous larval input in to a reserve will be minimal and the population within the reserve will have to be self-sustaining. What is striking of these results is that they show that the heavier the fishing pressure will be, the larger the area that needs to be protected will be to avoid the population from collapsing. .

From an empirical perspective we are still far from a complete understanding of demographic connectivity, as a full description of a dispersal kernel has not yet been achieved (Botsford et al. 2009). There are still large gaps in the knowledge of the extent of ecologically relevant levels of larval connectivity and the factors that might influence variation across locations and species (Jones et al. 2009b). The present results suggest that stage-based survival and population turn over rates are critical parameters that must be assessed to model population persistence in marine populations and design marine protected areas. This study has shown that individual genetic based methods provide a whole range of tools to obtain estimates of these parameters in marine populations. The model presented here remains relatively simple, but provides useful predictions about the scale that management decisions would need to be applied to successfully protect this species. The same kind of empirical data obtained with these methods could be also used in more complete and complex modelling approaches that take into account several other biologically important factors to forecast future scenarios in more realistic ways (Akçakaya 2000, Hastings and Botsford 2006, Kaplan et al. 2006, Kaplan et al. 2009, White et al. 2010b). In the looming threat of climate change, modelling approaches will play a crucial role in the design and success of our actions to preserve the oceans.

Table 4. Stage-class metapopulation matrix for *A. polymnus*. Values of fecundity were estimated from data on table 2 and *Gi* and *Pi* numeric values are shown in table 5.1.

	BA1	BA2	BA3	LO1	LO2	LO3	MO1	MO2	MO3	LI1	LI2	LI3	MN1	MN2	MN3	TA1	TA2	TA3	BE1	BE2	BE3
BA1	P_1	0	0.280	0	0	0	0	0	0.083	0	0	0.091	0	0	0.083	0	0	0.065	0	0	0.091
BA2	G_1	P_2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BA3	0	G_2	P_3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LO1	0	0	0.188	P_1	0	0.140	0	0	0.208	0	0	0.091	0	0	0.333	0	0	0.065	0	0	0.023
LO2	0	0	0	G_1	P_2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LO3	0	0	0	0	G_2	P_3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MO1	0	0	0.146	0	0	0.111	P_1	0	0.130	0	0	0.182	0	0	0.583	0	0	0.217	0	0	0.068
MO2	0	0	0	0	0	0	G_1	P_2	0	0	0	0	0	0	0	0	0	0	0	0	0
MO3	0	0	0	0	0	0	0	G_2	P_3	0	0	0	0	0	0	0	0	0	0	0	0
LI1	0	0	0.063	0	0	0.056	0	0	0	P_1	0	0.090	0	0	0	0	0	0.087	0	0	0.045
LI2	0	0	0	0	0	0	0	0	0	G_1	P_2	0	0	0	0	0	0	0	0	0	0
LI3	0	0	0	0	0	0	0	0	0	0	G_2	P_3	0	0	0	0	0	0	0	0	0
MN1	0	0	0.021	0	0	0	0	0	0	0	0	0.045	P_1	0	0.080	0	0	0.022	0	0	0.068
MN2	0	0	0	0	0	0	0	0	0	0	0	0	G_1	P_2	0	0	0	0	0	0	0
MN3	0	0	0	0	0	0	0	0	0	0	0	0	0	G_2	P_3	0	0	0	0	0	0
TA1	0	0	0.042	0	0	0	0	0	0.042	0	0	0.182	0	0	0.167	P_1	0	0.760	0	0	0.023
TA2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	G_1	P_2	0	0	0	0
TA3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	G_2	P_3	0	0	0
BE1	0	0	0.104	0	0	0.028	0	0	0.042	0	0	0.182	0	0	0.083	0	0	0.065	P_1	0	0.360
BE2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	G_1	P_2	0
BE3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	G_2	P_3

CHAPTER 6

GENERAL CONCLUSIONS

Marine larval dispersal remains difficult to study because dispersing propagules are minute, have complex behaviour and move in vast spaces that are also complex. Yet, the development of new techniques and models is definitely starting to bring this ambitious quest to a whole new level. Dispersal plays a major role at different scales and levels of organisation, from determining the extent of a species' range of expansion and the interconnection of populations within communities, to the fate of single individuals that venture far from their natal origins. Such a broad potential role makes it critical to continue and increase the efforts to study this process, especially in marine ecosystems where the human impact and climate change are affecting their equilibrium. This thesis represents a new contribution towards a better understanding of demographic connectivity in coral reef fish. It provides evidence of the importance of the geographic setting in the shape of larval dispersal and the size structure within focal populations, but also brings new questions and challenges about the functioning of marine populations that will be interesting to develop in the future.

Demographic connectivity and genetic tools

The main result of chapter 2 proposes that using parentage analyses to obtain direct estimates of larval retention in marine natural populations can be extended to estimate larval exchange among several discrete populations. Parentage analysis using *A. polymnus* data sets performed well in conditions of high gene flow. Small departures from panmixia did not seem to have a significant impact on parent offspring assignments. Besides, it is shown that incomplete sampling, considered as one of the primary limitations to the application of likelihood based parentage analysis to natural populations (Marshall et al. 1998, Neff et al. 2000, Jones et al. 2009a), can be compensated by increasing the number of genetic markers. With the recent advances in molecular technology, obtaining large numbers of highly variable genetic markers for non model species is no longer highly time consuming or unaffordable. Parentage analyses and population inference methods (such as assignment tests) are complementary and the usefulness of each one depends upon specific contexts. Since in most marine organisms with a dispersive larval phase a rather homogenous genetic pool can be found over relatively long distances (hundreds of km), extending parentage analysis to broader spatial scales than the one of this study is not unfeasible. Additionally, individual genetic tags generated from these procedures can be integrated in capture-recapture studies replacing artificial tags, which may have adverse effects on individuals (specially small ones)(e.g. Malone et al. 1999), to obtain estimates of demographic parameters in marine populations (chapters 4 and 5).

One of the challenges that lie ahead for the application of these methods to estimate connectivity in more mobile species (compared to anemonefish) will be to accurately estimate the size of local adult populations. Such is the case in some commercially targeted fish species for which traditional visual surveys and artificial tagging can lead to levels of uncertainty that in the context of population size estimation are acceptable (e.g. Zeller and Russ 2000), but when combined with connectivity estimates can lead to higher levels of uncertainty (Jones et al. 1999, Almany et al. 2007). Natural DNA tags might provide an interesting alternative to artificial tags that will definitely be worth testing in census population size estimation (Luikart et al. 2010).

Some patterns of demographic connectivity through larval dispersal

Results of chapter 3 indicated that in terms of self recruitment, this metapopulation system is characterised by high spatial heterogeneity among individual sites. Yet on average, these values are low compared to previous reports in other localities (Jones et al. 1999, Jones et al. 2005, Almany et al. 2007, Carreras-Carbonell et al. 2007, Planes et al. 2009). Chapter 5 suggests that even at the scale of ~10 km the system operates as an open population, relying on external sources of larvae to achieve positive population's growth rates. The idea that marine populations function as open systems is not new (e.g. Doherty and Fowler 1994), but these findings provide a quantitative description of exchange among geographically distinct populations that had never been achieved before. Besides, while the current conception of marine population dynamics stresses the highly variable and unpredictable nature of the magnitude of replenishment (Doherty and Williams 1988, Dixon et al. 1999, Sale et al. 2005b), results of chapter 3 suggest that relative levels of retention within and exchange among sites seem somewhat constant in time. Temporal stability might be a combination of consistent patterns in marine current regimes in this region (Wyrtki 1960) and demographic stability of these particular populations. In turn, demographic stability in populations of clownfish might be a combination of their particular life stile and the fact that in this region habitat seems to be close to its maximum carrying capacity (most anemones are occupied). However, whether the observed stability is consistent or was just a coincidence will need longer time series of observations.

Similar constant temporal patterns have been reported in a different location with a different time sampling interval (Almany et al. 2007, Planes et al. 2009), supporting the idea that at least the shape and direction of larval dispersal kernels might be less stochastic than previously thought. In contrast, the difference in patterns of levels of self recruitment in this

study (~18-25%) with those reported by Almany et al. and Planes et al. (40-60%) in Kimbe bay, support the idea that spatial distribution of suitable habitats may be more relevant in shaping connectivity among adjacent populations than life history characteristics of individual species (Almany et al. 2007, Ayre et al. 2009, Jones et al. 2009b). Testing this idea will require more independent studies on different locations and species. If this happens to be true, it implies that conservation strategies adapted to a particular location will be effective for a wide range of species.

Chapter 4 was an ambitious attempt to measure parental effects on local population replenishment. The abundance of factors (and complex interactions among them) that can potentially influence survival and fitness of marine pelagic larvae, from the moment they are conceived until recruitment in an adult population, is to a certain extent overwhelming. This complexity was perhaps responsible for most of the unexplained variation in fecundity and reproductive success in the results of this chapter. Yet, despite these high levels of variation, I was able to demonstrate a link between habitat degradation and a diminution in reproductive output of this fish. Also I showed that individual reproductive success (in terms of contribution to local population replenishment) is linked to maternal size. This last finding is the first field evidence for the hypothesis that bigger females have higher contributions to the replenishment of marine populations. These results provide solid evidence supporting the idea that marine population's dynamics behave in ways that are consistent with effects of maternal age/size and that maternal effects do not seem to be limited to large and long lived exploited species only. In contrast, our data does not seem to support the idea that the higher contribution to replenishment by larger females is linked to differences in larval quality that are related to female size. Although more studies are needed to back up our findings, this result highlights the importance of testing hypothesis from laboratory studies in the field. It is clear that bigger females produce better quality larvae under controlled conditions (Berkeley et al. 2004a), and that these "high quality" larvae also do better when reared under homogenous environments (Donelson et al. 2008). But the mechanisms by which different larval traits might be selected in a natural heterogeneous environment remains to be elucidated, as well as the importance of parental effects on larval quality in wild populations.

Connectivity, population stability and future perspectives

The existence of patterns such as temporal stability and geographic determination of population connectivity will have important implications in the way marine resources and conservation strategies will be directed. However, from an empirical perspective we are still

far from understanding demographic connectivity. A full description of a dispersal kernel has not been achieved so far (Botsford et al. 2009), and there are still large gaps in terms of the diversity of available empirical data across locations and species (Jones et al. 2009b). There is still a great effort to be made before any of these proposed patterns can be accepted. Parentage analysis has been proved to be a reliable approach in this field. Given the recent advances in molecular technology, the extension of this type of studies to other marine species in different geographic context is promising. However, extensive field sampling in marine organisms remains logistically challenging and is perhaps the major limit of expanding this approach to larger geographic scales. Coupling this approach with indirect genetic methods such as isolation by distance (Pinsky et al. 2010) and biophysical models (Maurice et al. 2002, Cowen et al. 2006, Treml et al. 2008) will provide a more complete perspective on demographic as well as evolutionary connectivity.

At the same time, recent advances in the study of larval behaviour have shown that larvae are far from being passive particles. Coupling empirical studies like this one, with biophysical modelling at small spatial scales will allow a better understanding of the role of active behaviour in larval dispersal. Likewise, demographic data derived from genetic studies as in this dissertation can also be used to bridge the gap in population biology between theoretical and empirical studies as illustrated in chapter 5 by a simple demographic model. In turn, these individual based demographic models can be integrated as modules in more complex connectivity model systems (CMS) (Sale et al. 2010) that will be soon available on-line to the general public. CMS incorporate species specific biological and site-specific (real-time) physical data to provide output that is specific to particular organisms and regions. Well validated biophysical models (by comparing with empirical connectivity data) that integrate species specific biological data will become powerful tools to generate hypothesis and reveal the importance of particular processes in specific scenarios providing accurate and valuable information to conservation managers (e.g. optimisation of MPA networks) (Hastings and Botsford 2006, Kaplan et al. 2006, Kaplan et al. 2009, Sale et al. 2010, White et al. 2010b).

Concluding remarks

Many marine systems are under high threats that include overexploitation, climate change, pollution and habitat degradation. Habitat loss, fragmentation and distance among suitable habitats is predicted to increase in the next few years, which in turn is likely to reduce dispersal potential of many species and increase the chances of local extinctions by small-scale disturbances (Munday et al. 2009). We are beginning to understand the scale in which

marine populations are connected by larval dispersal and some general patterns that might explain differences in the shape of dispersal kernels (such as configuration of the geographic context) seem to be arising. However more detailed information over a wider range of geographic locations and species remains a top priority for conservation biologists working in marine connectivity. Some of the results presented here provide new field evidence of how individual contribution of adults to the replenishment of local populations depends on their size. These results also provide solid support for a change in management strategies in favour of those that not only regulate total mortality but also aim to protect and restore age/size structure such as no-take marine reserves (Kritzer and Sale 2004, Palumbi 2004a, Sale et al. 2005a, Venturelli et al. 2009). However, this dissertation is just the beginning. More empirical studies are needed to test the hypotheses proposed here and build a solid scientific background that can be integrated in conservation strategies. Only by combining efforts between conservationists and scientists we will hopefully avoid the collapse of marine ecosystems and warrant their sustainable use.

REFERENCES

Abramoff, M. D., P. J. Magelhaes, and S. J. Ram. 2004. Image processing with ImageJ. Biophotonics International **11**:36-42.

Adjeroud, M., D. Augustin, R. Galzin, and B. Salvat. 2002. Natural disturbances and interannual variability of coral reef communities on the outer slope of Tiahura (Moorea, French Polynesia): 1991 to 1997. Marine Ecology Progress Series **237**:121-131.

Akçakaya, H. R. 2000. Population viability analysis with demographically and spatially structured models. Ecological Bulletins **48**:23-38.

Allen, G. R. 1972. Anemonefishes: their classification and biology. 2nd edition. TFH Publications, Neptune city N.J.

Almany, G., S. Connolly, D. Heath, J. Hogan, G. Jones, L. McCook, M. Mills, R. Pressey, and D. Williamson. 2009. Connectivity, biodiversity conservation and the design of marine reserve networks for coral reefs. Coral Reefs **28**:339-351.

Almany, G. R., M. L. Berumen, S. R. Thorrold, S. Planes, and G. P. Jones. 2007. Local replenishment of coral reef fish populations in a marine reserve. Science **316**:742-744.

Anderson, E. C., and J. C. Garza. 2006. The power of single-nucleotide polymorphisms for large-scale parentage inference. Genetics **172**:2567-2582.

Armsworth, P. R. 2002. Recruitment limitation, population regulation, and larval connectivity in reef fish metapopulations. Ecology **83**:1092-1104.

Arvedlund, M., M. I. McCormick, D. G. Fautin, and M. Bildsoe. 1999. Host recognition and possible imprinting in the anemonefish *Amphiprion melanopus* (Pisces: Pomacentridae). Marine Ecology Progress Series **188**:207-218.

Arvedlund, M., and L. E. Nielsen. 1996. Do the anemonefish *Amphiprion ocellaris* (Pisces: Pomacentridae) imprint themselves to their host sea anemone *Heteractis magnifica* (Anthozoa: Actinidae)? Ethology **102**:197-211.

Ayre, D. J., T. E. Minchinton, and C. Perrin. 2009. Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? Molecular Ecology **18**:1887-1903.

Balloux, F. 2001. EASYPOP (version1.7): a computer program for population genetics simulations. Journal of Heredity **92**:301-302.

Baums, I. B., M. W. Miller, and M. E. Hellberg. 2005. Regionally isolated populations of an imperiled Caribbean coral, *Acropora palmata*. Molecular Ecology **14**:1377-1390.

Beckerman, A. P., T. G. Benton, C. T. Lapsley, and N. Koesters. 2006. How effective are maternal effects at having effects? Proceedings of the Royal Society B: Biological Sciences **273**:485-493.

Begg, G. A., and G. Marteinsdottir. 2000. Spawning origins of pelagic juvenile cod *Gadus morhua* inferred from spatially explicit age distributions: potential influences on year-class strength and recruitment. Marine Ecology Progress Series **202**.

Beldade, R., J. Heiser, D. Robertson, J. Gasparini, S. Floeter, and G. Bernardi. 2009a. Historical biogeography and speciation in the Creole wrasses (Labridae, *Clepticus*). Marine Biology **156**:679-687.

Beldade, R., S. Holbrook, R. Schmitt, S. Planes, and G. Bernardi. 2009b. Isolation and characterization of eight polymorphic microsatellite markers from the orange-fin anemonefish, *Amphiprion chrysopterus*. Conservation Genetics Resources **1**:333-335.

Bellwood, D. R., A. S. Hoey, J. L. Ackerman, and M. Depczynski. 2006. Coral bleaching, reef fish community phase shifts and the resilience of coral reefs. Global Change Biology **12**:1587-1594.

Bergenius, M. A. J., M. G. Meekan, D. R. Robertson, and M. I. McCormick. 2002. Larval growth predicts the recruitment success of a coral reef fish. Oecologia **131**:521-525.
Berkeley, S. A., C. Chapman, and S. M. Sogard. 2004a. Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. Ecology **85**:1258-1264.
Berkeley, S. A., M. A. Hixon, R. J. Larson, and M. S. Love. 2004b. Fisheries sustainability via protection of age structure and spatial distribution of fish populations. Fisheries **29**:23-32.
Birkeland, C., and P. K. Dayton. 2005. The importance in fishery management of leaving the big ones. Trends in Ecology & Evolution **20**:356-358.
Bonin, M. C., P. L. Munday, M. I. McCormick, M. Srinivasan, and G. P. Jones. 2009. Coral-dwelling fishes resistant to bleaching but not to mortality of host corals. Marine Ecology-Progress Series **394**:215-222.
Booth, D. J. 1992. Larval settlement patterns and preferences by domino damselfish, *Dascyllus albisella* Gill. Journal of Experimental Marine Biology and Ecology **155**:85-104.
Booth, D. J., and G. A. Bereta. 2002. Changes in a fish assemblage after a coral bleaching event. Marine Ecology Progress Series **245**:205-212.
Botsford, L., A. Hastings, and S. Gaines. 2001. Dependence of sustainability on the configuration of marine reserves and larval dispersal distance. Ecology Letters **4**:144-150.
Botsford, L., J. White, M. Coffroth, C. Paris, S. Planes, T. Shearer, S. Thorrold, and G. Jones. 2009. Connectivity and resilience of coral reef metapopulations in marine protected areas: matching empirical efforts to predictive needs. Coral Reefs **28**:327-337.
Bowler, D. E., and T. G. Benton. 2005. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. Biological Reviews **80**:205-225.
Buschiazzo, E., and N. J. Gemmell. 2006. The rise, fall and renaissance of microsatellites in eukariotic genomes. BioEssays **28**:1040-1050.
Buston, P. 2004a. Does the presence of non-breeders enhance the fitness of breeders? An experimental analysis in the clown anemonefish Amphiprion percula. Behavioral Ecology and Sociobiology **57**:23-31.
Buston, P. M. 2003a. Forcible eviction and prevention of recruitment in the clown anemonefish. Behavioral Ecology **14**:576-582.
Buston, P. M. 2003b. Mortality is associated with social rank in the clown anemonefish (*Amphiprion percula*). Marine Biology **143**:811-815.
Buston, P. M. 2004b. Territory inheritance in clownfish. Proceedings of the Royal Society of London. Series B: Biological Sciences **271**:S252-S254.
Buston, P. M., S. M. Bogdanowicz, A. Wong, and R. G. Harrison. 2007. Are clownfish groups composed of close relatives? An analysis of microsatellite DNA variation in *Amphiprion percula*. Molecular Ecology **16**:3671-3678.
Buston, P. M., and M. B. García. 2007. An extraordinary life span estimate for the clown anemonefish *Amphiprion percula*. Journal of Fish Biology **70**:1710-1719.
Carreras-Carbonell, J., E. Macpherson, and M. Pascual. 2007. High self-recruitment levels in a Mediterranean littoral fish population revealed by microsatellite markers. Marine Biology **151**:719-727.
Castro, J., A. Pino, M. Hermida, C. Bouza, A. Riaza, I. Ferreiro, L. Sanchez, and P. Martinez. 2006. A microsatellite marker tool for parentage analysis in Senegal sole (*Solea senegalensis*): Genotyping errors, null alleles and conformance to theoretical assumptions. Aquaculture **261**:1194-1203.

Caswell, H. 2001. Matrix population models: construction, analysis, and interpretation. 2nd edition. Sinauer Associates, Sunderland, Massachusetts.
Christie, M. R. 2009. Parentage in natural populations: novel methods to detect parent-offspring pairs in large data sets. Molecular Ecology Resources **10**:115-128.
Christie, M. R., D. W. Johnson, C. D. Stallings, and M. A. Hixon. 2010. Self-recruitment and sweepstakes reproduction amid extensive gene flow in a coral-reef fish. Molecular Ecology **19**:1042-1057.
Clobert, J., E. Danchin, A. A. Dhondt, and J. D. Nichols. 2001. Dispersal. Oxford University Press, Oxford, UK.
Coker, D. J., M. S. Pratchett, and P. L. Munday. 2009. Coral bleaching and habitat degradation increase susceptibility to predation for coral-dwelling fishes. Behavioral Ecology **20**:1204-1210.
Cole, A. J., M. S. Pratchett, and G. P. Jones. 2009. Effects of coral bleaching on the feeding response of two species of coral-feeding fish. Journal of Experimental Marine Biology and Ecology **373**:11-15.
Coleman, F. C., W. F. Figueira, J. S. Ueland, and L. B. Crowder. 2004. The Impact of United States Recreational Fisheries on Marine Fish Populations. Science **305**:1958-1960.
Cornuet, J.-M., S. Piry, G. Luikart, A. Estoup, and M. Solignac. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics **153**:1989-2000.
Cowen, R. K., G. Gawarkiewicz, J. Pineda, S. R. Thorrold, and F. Werner. 2007. Population connectivity in marine systems: An overview. Oceanography **20**:14-21.
Cowen, R. K., K. M. M. Lwiza, S. Sponaugle, C. B. Paris, and D. B. Olson. 2000. Connectivity of marine populations: Open or Closed? Science **287**:857-859.
Cowen, R. K., C. B. Paris, and A. Srinivasan. 2006. Scaling of connectivity in marine populations. Science **311**:522-527.
Crawley, M. J. 2007. The R Book. Wiley & Sons Ltd, Chichester, UK.
Crouse, D. T., L. B. Crowder, and H. Caswell. 1987. A Stage-Based Population Model for Loggerhead Sea Turtles and Implications for Conservation. Ecology **68**:1412-1423.
Dennis, D. M., C. R. Pitcher, and T. D. Skewes. 2001. Distribution and transport pathways of *Panulirus ornatus* (Fabricius, 1776) and *Panulirus spp*. larvae in the Coral Sea, Australia. Marine and Freshwater Research **52**:1175-1185.
Denny, C. M., and R. C. Babcock. 2004. Do partial marine reserves protect reef fish assemblages? Biological Conservation **116**:119-129.
Dingle, H., and V. A. Drake. 2007. What Is Migration? BioScience **57**:113-121.
Dixon, P. A., M. J. Milicich, and G. Sugihara. 1999. Episodic Fluctuations in Larval Supply. Science **283**:1528-1530.
Dixson, D. L., G. P. Jones, P. L. Munday, S. Planes, M. S. Pratchett, M. Srinivasan, C. Syms, and S. R. Thorrold. 2008. Coral reef fish smell leaves to find island homes. Proceedings of the Royal Society B: Biological Sciences **275**:2831-2839.
Doak, D. F., P. C. Marino, and P. M. Kareiva. 1992. Spatial scale mediates the influence of habitat fragmentation on dispersal success: Implications for conservation. Theoretical Population Biology **41**:315-336.
Doherty, P. 2002. Variable replenishment and the dynamics of reef populations. Pages 327-355 *in* P. Sale, editor. Coral reef fishes: dynamics and diversity in a complex ecosystem. Academic Press, London.
Doherty, P., and T. Fowler. 1994. An Empirical Test of Recruitment Limitation in a Coral Reef Fish. Science **263**:935-939.
Doherty, P., and D. M. Williams. 1988. The replenishment of coral reef fish populations. Oceanography and marine biology **26**:487-551.

Doherty, P. J., S. Planes, and P. Mather. 1995. Gene flow and larval duration in seven species of fish from the great barrier reef. Ecology **76**:2373-2391.
Donelson, J. M., M. I. McCormick, and P. L. Munday. 2008. Parental condition affects early life-history of a coral reef fish. Journal of Experimental Marine Biology and Ecology **360**:109-116.
Donner, S. D., T. R. Knutson, and M. Oppenheimer. 2007. Model-based assessment of the role of human-induced climate change in the 2005 Caribbean coral bleaching event. Proceedings of the National Academy of Sciences **104**:5483-5488.
Duchesne, P., T. Castric, and L. Bernatchez. 2005. PASOS (parental allocation of singles in open systems): a computer program for individual parental allocation with missing parents. Molecular Ecology Notes **5**:701-704.
Elliott, J. K., J. M. Elliott, and R. N. Mariscal. 1995. Host selection, location, and association behaviors of anemonefishes in field settlement experiments. Marine Biology **122**:377-389.
Ellstrand, N. C., and D. L. Marshall. 1985. Interpopulation gene flow by pollen in wild radish, *Raphanus sativus*. American Naturalist **126**:606-616.
Emslie, M. J., A. J. Cheal, H. Sweatman, and S. Delean. 2008. Recovery from disturbance of coral and reef fish communities on the Great Barrier Reef, Australia. Marine Ecology-Progress Series **371**:177-190.
Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources **10**:564-567.
Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology Evolution and Systematics **34**:487-515.
Fautin, D. C., and G. R. Allen. 1992. Field guide to anemonefishes and their host sea anemones. Western Australia Museum, Perth.
Fauvelot, C., and S. Planes. 2002. Understanding origins of present-day genetic structure in marine fish: biologically or historically driven patterns? Marine Biology **141**:773-788.
Feary, D. A., G. R. Almany, G. P. Jones, and M. I. McCormick. 2007. Coral degradation and structure of tropical reef fish communities. Marine Ecology Progress Series **333**:243-248.
Figueira, W., and L. Crowder. 2006. Defining patch contribution in source-sink metapopulations: the importance of including dispersal and its relevance to marine systems. Population Ecology **48**:215-224.
Fisher, R. 2005. Swimming speeds of larval coral reef fishes: impacts on self-recruitment and dispersal. Marine Ecology Progress Series **285**:223-232.
Frankham, R. 1996. Relationship of Genetic Variation to Population Size in Wildlife. Conservation Biology **10**:1500-1508.
Fredsted, T., C. Pertoldi, M. H. Schierup, and P. M. Kappeler. 2005. Microsatellite analyses reveal fine-scale genetic structure in grey mouse lemurs (*Microcebus murinus*). Molecular Ecology **14**:2363-2372.
Gagliano, M., and M. I. McCormick. 2007. Maternal condition influences phenotypic selection on offspring. Journal of Animal Ecology **76**:174-182.
Gagliano, M., M. I. McCormick, and M. G. Meekan. 2007. Survival against the odds: ontogenetic changes in selective pressure mediate growth-mortality trade-offs in a marine fish. Proceedings of the Royal Society B: Biological Sciences **274**:1575-1582.
Garpe, K. C., S. A. S. Yahya, U. Lindahl, and M. C. Ohman. 2006. Long-term effects of the 1998 coral bleaching event on reef fish assemblages. Marine Ecology-Progress Series **315**:237-247.

Gerber, S., P. Chabrier, and A. Kremer. 2003. FAMOZ: a software for parentage analysis using dominant, codominant and uniparentally inherited markers. Molecular Ecology Notes **3**:479-481.
Gerber, S., S. Mariette, R. Streiff, C. Bodenes, and A. Kremer. 2000. Comparison of microsatellites and amplified fragment length polymorphism markers for parentage analysis. Molecular Ecology **9**:1037-1048.
Gerlach, G., J. Atema, M. J. Kingsford, K. P. Black, and V. Miller-Sims. 2007. Smelling home can prevent dispersal of reef fish larvae. Proceedings of the National Academy of Sciences **104**:858-863.
Glynn, P. W. 1993. Coral reef bleaching: ecological perspectives. Coral Reefs **12**:1-17.
Green, B. S. 2008. Chapter 1 Maternal Effects in Fish Populations. Pages 1-105 *in* D. W. Sims, editor. Advances in Marine Biology. Academic Press.
Green, B. S., and M. I. McCormick. 2005. Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish. Marine Ecology Progress Series **289**:263-272.
Grottoli, A. G., L. J. Rodrigues, and J. E. Palardy. 2006. Heterotrophic plasticity and resilience in bleached corals. Nature **440**:1186-1189.
Hadfield, J. D., D. S. Richardson, and T. Burke. 2006. Towards unbiased parentage assignment: combining genetic, behavioural and spatial data in a Bayesian framework. Molecular Ecology **15**:3715-3730.
Hamilton, S. L., J. Regetz, and R. R. Warner. 2008. Postsettlement survival linked to larval life in a marine fish. Proceedings of the National Academy of Sciences **105**:1561-1566.
Hamilton, S. L., J. W. White, J. E. Caselle, S. E. Swearer, and R. R. Warner. 2006. Consistent long-term spatial gradients in replenishment for an island population of a coral reef fish. Marine Ecology Progress Series **306**:247-256.
Hannah, L., G. Midgley, S. Andelman, M. Araujo, G. Hughes, E. Martinez-Meyer, R. Pearson, and P. Williams. 2007. Protected area needs in a changing climate. Frontiers in Ecology and the Environment **5**:131-138.
Hanski, I. 1994. A practical model of metapopulation dynamics. Journal of Animal Ecology **63**:151-162.
Hanski, I. 1998. Metapopulation dynamics. Nature **396**:41-49.
Hanski, I., J. Alho, and A. Moilanen. 2000. Estimating the Parameters of Survival and Migration of Individuals in Metapopulations. Ecology **81**:239-251.
Hastings, A., and L. W. Botsford. 2003. Comparing designs of marine reserves for fisheries and for biodiversity. Ecological Applications **13**:S65-70.
Hastings, A., and L. W. Botsford. 2006. Persistence of spatial populations depends on returning home. Proceedings of the National Academy of Sciences **103**:6067-6072.
Hattori, A. 1995. Coexistence of two anemonefishes, *Amphiprion clarkii* and *A. perideraion*, which utilize the same host sea anemone. Environmental Biology of Fishes **42**:345-353.
Hattori, A., and Y. Yanagisawa. 1991. Life-history pathways in relation to gonadal sex differentiation in the anemonefish, *Amphiprion clarkii*, in temperate waters of Japan. Environmental Biology of Fishes **31**:139-155.
Hedgecock, D., P. H. Barber, and S. Edmands. 2007. Genetic approaches to measuring connectivity. Oceanography **20**:70-79.
Hellberg, M. 2007. Footprints on water: the genetic wake of dispersal among reefs. Coral Reefs **26**:463-473.
Hellberg, M. E. 2009. Gene Flow and Population Isolation among Populations of Marine Animals. Annual Review of Ecology, Evolution, and Systematics **40**.

Hellberg, M. E., R. S. Burton, J. E. Neigel, and S. R. Palumbi. 2002. Genetic assessment of connectivity among marine populations. Bulletin of Marine Science **70**:273-290.

Hemmer-Hansen, J., E. E. Nielsen, P. Gronkjaer, and V. Loeschcke. 2007. Evolutionary mechanisms shaping the genetic population structure of marine fishes; lessons from the European flounder (Platichthys flesus L.). Molecular Ecology **16**:3104-3118.

Hill, M. F., A. Hastings, and L. W. Botsford. 2002. The effects of small dispersal rates on extinction times in structured metapopulation models. The American Naturalist **160**:389-402.

Hixon, M. A., S. W. Pacala, and S. A. Sandin. 2002. Population regulation: historical context and contemporary challenges of open vs. closed systems. Ecology **83**:1490-1508.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. Marine and Freshwater Research **50**:839-866.

Hoegh-Guldberg, O., P. J. Mumby, A. J. Hooten, R. S. Steneck, P. Greenfield, E. Gomez, C. D. Harvell, P. F. Sale, A. J. Edwards, K. Caldeira, N. Knowlton, C. M. Eakin, R. Iglesias-Prieto, N. Muthiga, R. H. Bradbury, A. Dubi, and M. E. Hatziolos. 2007. Coral reefs under rapid climate change and ocean acidification. Science **318**:1737-1742.

Huebert, K. B., and S. Sponaugle. 2009. Observed and simulated swimming trajectories of late-stage coral reef fish larvae off the Florida Keys. Aquatic Biology **7**:207-216.

Hughes, T. P., A. H. Baird, D. R. Bellwood, M. Card, S. R. Connolly, C. Folke, R. Grosberg, O. Hoegh-Guldberg, J. B. C. Jackson, J. Kleypas, J. M. Lough, P. Marshall, M. Nystrom, S. R. Palumbi, J. M. Pandolfi, B. Rosen, and J. Roughgarden. 2003. Climate change, human impacts, and the resilience of coral reefs. Science **301**:929-933.

Hughes, T. P., D. R. Bellwood, C. Folke, R. S. Steneck, and J. Wilson. 2005. New paradigms for supporting the resilience of marine ecosystems. Trends in Ecology & Evolution **20**:380-386.

Irisson, J.-O., C. Guigand, and C. Paris. 2009. Detection and quantification of marine larvae orientation in the pelagic environment. Limnology and Oceanography: Methods **7**:664-672.

Irisson, J.-O., A. LeVan, M. D. Lara, and S. Planes. 2004. Strategies and trajectories of coral reef fish larvae optimizing self-recruitment. Journal of Theoretical Biology **227**:205-218.

Jackson, J. B. C., M. X. Kirby, W. H. Berger, K. A. Bjorndal, L. W. Botsford, B. J. Bourque, R. H. Bradbury, R. Cooke, J. Erlandson, J. A. Estes, T. P. Hughes, S. Kidwell, C. B. Lange, H. S. Lenihan, J. M. Pandolfi, C. H. Peterson, R. S. Steneck, M. J. Tegner, and R. R. Warner. 2001. Historical Overfishing and the Recent Collapse of Coastal Ecosystems. Science **293**:629-637.

James, M. K., P. R. Armsworth, L. B. Mason, and L. Bode. 2002. The structure of reef fish metapopulations: modelling larval dispersal and retention patterns. Proceedings of the Royal Society B: Biological Sciences **269**:2079-2086.

Jamieson, A., and S. S. Taylor. 1997. Comparisons of three probability formulae for parentage exclusion. Animal Genetics **28**:397-400.

Jones, A. G., and W. R. Ardren. 2003. Methods of parentage analysis in natural populations. Molecular Ecology **12**:2511-2523.

Jones, A. G., C. M. Small, K. A. Paczolt, and N. L. Ratterman. 2009a. A practical guide to methods of parentage analysis. Molecular Ecology Resources **10**:6-30.

Jones, A. M., S. Gardner, and W. Sinclair. 2008. Losing 'Nemo': bleaching and collection appear to reduce inshore populations of anemonefishes. Journal of Fish Biology **73**:753-761.

Jones, G., G. Almany, G. Russ, P. Sale, R. Steneck, M. van Oppen, and B. Willis. 2009b. Larval retention and connectivity among populations of corals and reef fishes: history, advances and challenges. Coral Reefs **28**:307-325.
Jones, G. P. 1991. Postrecruitment processes in the ecology of coral reef fish populations: a multifactorial perspective. Pages 294-328 *in* P. Sale, editor. The ecology of fishes on coral reefs. Academic Press, San Diego CA.
Jones, G. P., M. I. McCormick, M. Srinivasan, and J. V. Eagle. 2004. Coral decline threatens fish biodiversity in marine reserves. Proceedings of the National Academy of Sciences **101**:8251-8253.
Jones, G. P., M. J. Milicich, M. J. Emslie, and C. Lunow. 1999. Self-recruitment in a coral reef fish population. Nature **402**:802-804.
Jones, G. P., S. Planes, and S. R. Thorrold. 2005. Coral reef fish larvae settle close to home. Current Biology **15**:1314-1318.
Jones, G. P., M. Srinivasan, and G. R. Almany. 2007. Population connectivity and conservation of marine biodiversity. Oceanography **20**:100-111.
Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology **16**:1099-1106.
Kaplan, D. M., L. W. Botsford, and S. Jorgensen. 2006. Dispersal per recruit: an efficient method for assessing susteinability in marine reserve networks. Ecological Applications **16**:2248-2263.
Kaplan, D. M., L. W. Botsford, M. R. O'Farrell, S. D. Gaines, and S. Jorgensen. 2009. Model-based assessment of persistence in proposed marine protected area designs. Ecological Applications **19**:433-448.
Kaufman, L. S. 1983. Effects of Hurricane Allen on reef fish assemblages near Discovery Bay, Jamaica. Coral Reefs **2**:43-47.
Kingsford, M., J. Leis, A. Shanks, K. C. Lindeman, S. G. Morgan, and J. Pineda. 2002. Sensory environments, larval abilities and local self-recruitment. Bulletin of Marine Science **70**:Suppl : 309-340.
Kinlan, B. P., and S. D. Gaines. 2003. Propagule dispersal in marine and terrestrial environments: a community perspective. Ecology **84**:2007-2020.
Kokita, T., and A. Nakazono. 2001. Rapid response of an obligately corallivorous filefish *Oxymonacanthus longirostris* (Monacanthidae) to a mass coral bleaching event. Coral Reefs **20**:155-158.
Kritzer, J. P., and P. F. Sale. 2004. Metapopulation ecology in the sea: from Levins' model to marine ecology and fisheries science. Fish and Fisheries **5**:131-140.
Lecchini, D., J. Shima, B. Banaigs, and R. Galzin. 2005. Larval sensory abilities and mechanisms of habitat selection of a coral reef fish during settlement. Oecologia **143**:326-334.
Leimu, R., P. Mutikainen, J. Koricheva, and M. Fischer. 2006. How general are positive relationships between plant population size, fitness and genetic variation? Journal of Ecology **94**:942-952.
Leis, J. 2007. Behaviour as input for modelling dispersal of fish larvae: behaviour, biogeography, hydrodynamics, ontogeny, physiology and phylogeny meet hydrography. Marine Ecology Progress Series **347**:185-193.
Leis, J. M., and B. M. Carson-Ewart. 2003. Orientation of pelagic larvae of coral-reef fishes in the ocean. Marine Ecology Progress Series **252**:239-253.
Leis, J. M., A. J. Southward, and D. W. Sims. 2006. Are larvae of demersal fishes plankton or nekton?. Advances in Marine Biology **51**:57-141.

Lester, S. E., and B. S. Halpern. 2008. Biological responses in marine no-take reserves versus partially protected areas. Marine Ecology-Progress Series **367**:49-56.
Levin, S. A. 1974. Dispersion and population interactions. The American Naturalist **108**:207-228.
Lindahl, U., M. C. Öhman, and C. K. Schelten. 2001. The 1997/1998 Mass Mortality of Corals: Effects on Fish Communities on a Tanzanian Coral Reef. Marine Pollution Bulletin **42**:127-131.
Lindenmayer, D. B., and H. P. Possingham. 1996. Modelling the inter-relationship between habitat patchiness, dispersal capability and metapopulation persistance of the endangered species, Leadbeater's possum, in South-eastern Australia. Landscape Ecology **11**:79-105.
Lipcius, R. N., D. B. Eggleston, S. J. Schreiber, R. D. Seitz, J. Shen, M. Sisson, W. T. Stockhausen, and H. V. Wang. 2008. Importance of Metapopulation Connectivity to Restocking and Restoration of Marine Species. Reviews in Fisheries Science **16**:101 - 110.
Lockwood, D. R., A. Hastings, and L. W. Botsford. 2002. The effects of dispersal patterns on marine reserves: does the tail wag the dog? Theoretical Population Biology **61**:297-309.
Lowe, W. H., and F. W. Allendorf. 2010. What can genetics tell us about population connectivity? Molecular Ecology **19**:3038-3051.
Loya, Y., K. Sakai, K. Yamazato, Y. Nakano, H. Sambali, and R. Van Woesik. 2001. Coral bleaching: the winners and the losers. Ecology Letters **4**:122-131.
Lucero, Y. 2008. Maternal Effects and Time to Recovery. Bulletin of Marine Science **83**:217-234.
Luikart, G., N. Ryman, D. A. Tallmon, M. K. Schwartz, and F. W. Allendorf. 2010. Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. Conservation Genetics **11**:355-373.
MacArthur, R. H., and E. O. Wilson. 1967. The theory of island biogeography. Princeton, New Jersey.
Macpherson, E., and N. Raventos. 2005. Settlement patterns and post-settlement survival in two Mediterranean littoral fishes: influences of early-life traits and environmental variables. Marine Biology **148**:167-177.
Malone, J. C., G. E. Forrester, and M. A. Steele. 1999. Effects of subcutaneous microtags on the growth, survival, and vulnerability to predation of small reef fishes. Journal of Experimental Marine Biology and Ecology **237**:243-253.
Manel, S., O. E. Gaggiotti, and R. S. Waples. 2005. Assignment methods: matching biological questions with appropriate techniques. Trends in Ecology & Evolution **20**:136-142.
Manel, S., M. K. Schwartz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology & Evolution **18**:189-197.
Marshall, P. A., and A. H. Baird. 2000. Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs **19**:155-163.
Marshall, T. C., J. Slate, L. E. B. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology **7**:639-655.
Matson, S. E., M. D. Camara, W. Eichert, and M. A. Banks. 2008. p-loci: a computer program for choosing the most efficient set of loci for parentage assignment. Molecular Ecology Resources **8**:765-768.

Maurice, K. J., R. A. Paul, B. M. Luciano, and B. Lance. 2002. The structure of reef fish metapopulations: modelling larval dispersal and retention patterns. Proceedings of the Royal Society : Biological Sciences **269**:2079-2086.
McClanahan, T. R., E. Weil, J. Cortes, A. H. Baird, and M. Ateweberhan. 2009. Consequences of coral bleaching for sessile reef organisms. Pages 121-138 *in* M. van Oppen and J. Lough, editors. Ecological studies. Springer-Verlag Berlin.
McCook, L., G. Almany, M. Berumen, J. Day, A. Green, G. Jones, J. Leis, S. Planes, G. Russ, P. Sale, and S. Thorrold. 2009. Management under uncertainty: guide-lines for incorporating connectivity into the protection of coral reefs. Coral Reefs **28**:353-366.
McCormick, M. I. 2006. Mothers matter: crowding leads to stressed mothers and smaller offspring in marine fish. Ecology **87**:1104-1109.
McCormick, M. I. 2009. Behaviourally mediated phenotypic selection in a disturbed coral reef environment. Plos One **4**:e7096.
Meekan, M. G., L. Vigliola, A. Hansen, P. J. Doherty, A. Halford, and J. H. Carleton. 2006. Bigger is better: size-selective mortality throughout the life history of a fast-growing clupeid, Spratelloides gracilis. Marine Ecology Progress Series **317**:237-244.
Meirmans, P. G. 2006. Using the AMOVA framework to estimate a standardized genetic differentiation measure. Evolution **60**:2399-2402.
Moilanen, A., and M. Nieminen. 2002. Simple connectivity measures in spatial ecology. Ecology **83**:1131-1145.
Mora, C., S. Andrefouet, M. J. Costello, C. Kranenburg, A. Rollo, J. Veron, K. J. Gaston, and R. A. Myers. 2006. ECOLOGY: enhanced: coral reefs and the global network of marine protected areas. Science **312**:1750-1751.
Mora, C., P. M. Chittaro, P. F. Sale, J. P. Kritzer, and S. A. Ludsin. 2003. Patterns and processes in reef fish diversity. Nature **421**:933-936.
Mora, C., R. A. Myers, M. Coll, S. Libralato, T. J. Pitcher, R. U. Sumaila, D. Zeller, R. Watson, K. J. Gaston, and B. Worm. 2009. Management Effectiveness of the World's Marine Fisheries. PLoS Biol **7**:e1000131.
Mora, C., and P. F. Sale. 2002. Are populations of coral reef fish open or closed? Trends in Ecology & Evolution **17**:422-428.
Morrissey, M. B., and M. M. Ferguson. 2009. Marker-assisted determination of the relationship between body size and reproductive success and consequences for evaluation of adaptive life histories. Molecular Ecology **18**:4330-4340.
Morrissey, M. B., and A. J. Wilson. 2005. The potential costs of accounting for genotypic errors in molecular parentage analyses. Molecular Ecology **14**:4111-4121.
Moyer, J. T., and A. Nakazono. 1978. Protandrous hermaphroditism in six species of the anemonefish genus *Amphiprion* in Japan. Japanese journal of Ichthyology **25**:101-106.
Moyer, J. T., and R. C. Steene. 1979. Nesting behavior of the anemonefish Amphiprion polymnus. Japanese journal of Ichthyology **26**:209-214.
Munday, P., J. Leis, J. Lough, C. Paris, M. Kingsford, M. Berumen, and J. Lambrechts. 2009. Climate change and coral reef connectivity. Coral Reefs **28**:379-395.
Neff, B. D., J. Repka, and M. R. Gross. 2000. Statistical confidence in parentage analysis with incomplete sampling: how many loci and offspring are needed? Molecular Ecology **9**:529-539.
Neville, H. M., D. J. Isaak, J. B. Dunham, R. F. Thurow, and B. E. Rieman. 2006. Fine-scale natal homing and localized movement as shaped by sex and spawning habitat in Chinook salmon: insights from spatial autocorrelation analysis of individual genotypes. Molecular Ecology **15**:4589-4602.

Nutt, K. J. 2008. A comparison of techniques for assesing dispersal behaviour in gundins: revealing dispersal patterns in the absence of observed dispersal behaviour. Molecular Ecology **17**:3541-3556.

Orsini, L., J. Corander, A. Alasentie, and I. Hanski. 2008. Genetic spatial structure in a butterfly metapopulation correlates better with past than present demographic structure. Molecular Ecology **17**:2629-2642.

Paetkau, D., R. Slade, M. Burden, and A. Estoup. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Molecular Ecology **13**:55-65.

Palsbøli, P. J. 1999. Genetic tagging: contemporary molecular ecology. Biological Journal of the Linnean Society **68**:3-22.

Palumbi, S. R. 2003. Population genetics, demographic connectivity, and the design of marine reserves. Ecological Applications **13**:146-158.

Palumbi, S. R. 2004a. Fisheries science: Why mothers matter. Nature **430**:621-622.

Palumbi, S. R. 2004b. Marine reserves and ocean neighborhoods: The spatial scale of marine populations and their management. Annual review of environment and resources **29**:31-68.

Pandolfi, J. M., R. H. Bradbury, E. Sala, T. P. Hughes, K. A. Bjorndal, R. G. Cooke, D. McArdle, L. McClenachan, M. J. H. Newman, G. Paredes, R. R. Warner, and J. B. C. Jackson. 2003. Global trajectories of the long-term decline of coral reef ecosystems. Science **301**:955-958.

Paris, C. B., L. M. Chérubin, and R. K. Cowen. 2007. Surfing, Spinning, or diving from reef to reef: effects on population connectivity. Marine Ecology Progress Series **347**:285-300.

Paris, C. B., and R. K. Cowen. 2004. Direct evidence of a biophysical retention mechanism for coral reef fish larvae. Limnology and Oceanography **49**:1964-1979.

Patterson, H. M., M. J. Kingsford, and M. T. McCulloch. 2005. Resolution of the early life history of a reef fish using otolith chemistry. Coral Reefs **24**:222-229.

Patterson, H. M., and S. E. Swearer. 2007. Long-distance dispersal and local retention of larvae as mechanisms of recruitment in an island population of a coral reef fish. Austral Ecology **32**:122-130.

Peakall, R. O. D., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes **6**:288-295.

Pinsky, M. L., H. R. J. Montes, and S. R. Palumbi. 2010. Using isolation by distance and effective density to estimate dispersal scales in anemonefish. Evolution **64**:2688-2700.

Piry, S., A. Alapetite, J. M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup. 2004. GENECLASS2: A Software for genetic assignment and first-generation migrant detection. Journal of Heredity **95**:536-539.

Planes, S. 2002. Biogeography and larval dispersal inferred from population genetic analysis. Pages 201-220 *in* P. Sale, editor. Coral Reef Fishes. Dynamics and Diversity in a Complex Ecosystem. Academic Press, San Diego.

Planes, S., G. P. Jones, and S. R. Thorrold. 2009. Larval dispersal connects fish populations in a network of marine protected areas. Proceedings of the National Academy of Sciences **106**:5693-5697.

Pratchett, M. S., P. L. Munday, S. K. Wilson, N. A. J. Graham, J. E. Cinner, D. R. Bellwood, G. P. Jones, N. V. C. Polunin, and T. R. McClanahan. 2008. Effects of climate-induced coral bleaching on coral-reef fishes - Ecological and economic consequences. Oceanography and Marine Biology: an Annual Review **46**:251-296.

Pratchett, M. S., S. K. Wilson, M. L. Berumen, and M. I. McCormick. 2004. Sublethal effects of coral bleaching on an obligate coral feeding butterflyfish. Coral Reefs **23**:352-356.
Puebla, O., E. Bermingham, and F. Guichard. 2009. Estimating dispersal from genetic isolation by distance in a coral reef fish (Hypoplectrus puella). Ecology **90**:3087-3098.
Quenouille, B., Y. Bouchenak-Khelladi, C. Hervet, and S. Planes. 2004. Eleven microsatellite loci for the saddleback clownfish *Amphiprion polymnus*. Molecular Ecology Notes **4**:291-293.
R Development Core Team. 2007. R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna.
Rannala, B., and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences **94**:9197-9201.
Rattanayuvakorn, S., P. Mungkornkarn, A. Thongpan, and K. Chatchavalvanich. 2005. Embryonic development of Saddleback anemonefish, *Amphiprion polymnus*, Linnaeus (1758). Kasetsart Journal (Natural sciences) **39**:455-463.
Raventos, N., and E. Macpherson. 2005. Effect of pelagic larval growth and size-at-hatching on post-settlement survivorship in two temperate labrid fish of the genus Symphodus. Marine Ecology Progress Series **285**:205-211.
Raymond, M., and F. Rousset. 1995. GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity **86**:248-249.
Reece, J. S., B. W. Bowen, K. Joshi, V. Goz, and A. Larson. 2010. Phylogeography of two moray eels indicates high dispersal throughout the Indo-Pacific. Journal of Heredity **101**:391-402.
Roberts, C. M. 1997. Connectivity and Management of Caribbean Coral Reefs. Science **278**:1454-1457.
Roberts, C. M., and N. V. C. Polunin. 1991. Are marine reserves effective in management of reef fisheries? Reviews in Fish Biology and Fisheries **1**:65-91.
Rocha, L. A., A. L. Bass, D. R. Robertson, and B. W. Bowen. 2002. Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei: Acanthuridae). Molecular Ecology **11**:243-251.
Rocha, L. A., M. T. Craig, and B. W. Bowen. 2007. Phylogeography and the conservation of coral reef fishes. Coral Reefs **26**:501-512.
Rocha, L. A., D. R. Robertson, J. Roman, and B. W. Bowen. 2005. Ecological speciation in tropical reef fishes. Proceedings of the Royal Society : Biological Sciences **272**:573-579.
Rodriguez-Munoz, R., A. Bretman, J. Slate, C. A. Walling, and T. Tregenza. 2010. Natural and Sexual Selection in a Wild Insect Population. Science **328**:1269-1272.
Rodzen, J. A., T. R. Famula, and B. May. 2004. Estimation of parentage and relatedness in the polyploid white sturgeon (*Acipenser transmontanus*) using a dominant marker approach for duplicated microsatellite loci. Aquaculture **232**:165-182.
Ronce, O. 2007. How does it feel to be like a rolling stone? ten questions about dispersal evolution. Annual Review of Ecology, Evolution, and Systematics **38**:231-253.
Russ, G. 2002. Yet another review of marine reserves as reef fishery management tools. Pages 421-443 *in* P. Sale, editor. Coral reef fishes: dynamics and diversity in a complex ecoystem. Academic Press, San Diego.
Saenz-Agudelo, P., G. P. Jones, S. R. Thorrold, and S. Planes. 2009. Estimating connectivity in marine populations: an empirical evaluation of assignment tests and parentage analysis under different gene flow scenarios. Molecular Ecology **18**:1765-1776.
Salas, E., H. Molina-Ureña, R. Walter, and D. Heath. 2010. Local and regional genetic connectivity in a Caribbean coral reef fish. Marine Biology **157**:437-445.

Sale, P. 1980. The ecology of fishes on coral reefs. Oceanography and marine biology annual reviews **18**:367-421.

Sale, P., H. Van Lavleren, M. C. Ablan Lagman, J. Atema, C. Butler, C. Fauvelot, J. Hogan, G. Jones, K. C. Lindeman, C. Paris, R. Steneck, and H. L. Stewart. 2010. Preserving reef connectivity: a handbook for Marine Protected Area Managers. Connectivity working group, Coral Reef Targeted Research & Capacity Building for Management Program, UNU-INWEH.

Sale, P. F., R. K. Cowen, B. S. Danilowicz, G. P. Jones, J. P. Kritzer, K. C. Lindeman, S. Planes, N. V. C. Polunin, G. R. Russ, Y. J. Sadovy, and R. S. Steneck. 2005a. Critical science gaps impede use of no-take fishery reserves. Trends in Ecology & Evolution **20**:74-80.

Sale, P. F., B. S. Danilowicz, P. J. Doherty, and D. M. Williams. 2005b. The relation of microhabitat to variation in recruitment of young-of-year coral reef fishes. Bulletin of Marine Science **76**:123-142.

Schiegg, K., S. J. Daniels, J. R. Walters, J. A. Priddy, and G. Pasinelli. 2006. Inbreeding in red-cockaded woodpeckers: Effects of natal dispersal distance and territory location. Biological Conservation **131**:544-552.

Schumaker, N. H. 1996. Using landscape indices to predict habitat connectivity. Ecology **77**:1210-1225.

Serbezov, D., L. Bernatchez, E. M. Olsen, and L. A. Vollestad. 2010. Mating patterns and determinants of individual reproductive success in brown trout (*Salmo trutta*) revealed by parentage analysis of an entire stream living population. Molecular Ecology **19**:3193-3205.

Shima, J. S., E. G. Noonburg, and N. E. Phillips. 2010. Life history and matrix heterogeneity interact to shape metapopulation connectivity in spatially structured environments. Ecology **91**:1215-1224.

Simpson, S. D., M. Meekan, J. Montgomery, R. McCauley, and A. Jeffs. 2005. Homeward sound. Science **308**:221-.

Simpson, S. D., M. G. Meekan, A. Jeffs, J. C. Montgomery, and R. D. McCauley. 2008. Settlement-stage coral reef fish prefer the higher-frequency invertebrate-generated audible component of reef noise. Animal Behaviour **75**:1861-1868.

Swearer, S. E., J. E. Caselle, D. W. Lea, and R. R. Warner. 1999. Larval retention and recruitment in an island population of a coral-reef fish. Nature **402**:799-802.

Swearer, S. E., J. S. Shima, M. E. Hellberg, S. R. Thorrold, G. P. Jones, D. R. Robertson, S. G. Morgan, K. A. Selkoe, G. M. Ruiz, and R. R. Warner. 2002. Evidence of self-recruitment in demersal marine populations. Bulletin of Marine Science **70**:251-271.

Sweatman, H. P. A. 1988. Field evidence that settling coral reef fish larvae detect resident fishes using dissolved chemical cues. Journal of Experimental Marine Biology and Ecology **124**:163-174.

Tallmond, D. A., G. Luikart, and R. S. Waples. 2004. The alluring simplicity and complex reality of genetic rescue. Trends in Ecology & Evolution **19**:489-496.

Taylor, P. D., L. Fahrig, K. Henein, and G. Merriam. 1993. Connectivity is a vital element of landscape structure. Oikos **68**:571-573.

Tentelier, C., T. Guillemaud, S. Ferry, and X. Fauvergue. 2008. Microsatellite-based parentage analysis reveals non-ideal free distribution in a parasitoid population. Molecular Ecology **17**:2300-2309.

Thomas, C. D., and W. E. Kunin. 1999. The spatial structure of populations. Journal of Animal Ecology **68**:647-657.

Thorrold, S. R., G. P. Jones, S. Planes, and J. A. Hare. 2006. Transgenerational marking of embryonic otoliths in marine fishes using barium stable isotopes. Canadian Journal of Fisheries and Aquatic Sciences **63**:1193-1197.

Thorrold, S. R., C. Latkoczy, P. K. Swart, and C. M. Jones. 2001. Natal homing in a marine fish metapopulation. Science **291**:297-299.

Thresher, R. E., P. L. Colin, and L. Bell. 1989. Planktonic duration, distribution and population structure of Western and Central Pacific Damselfishes (Pomacentridae). Copeia **1989**:420-434.

Treml, E., P. Halpin, D. Urban, and L. Pratson. 2008. Modeling population connectivity by ocean currents, a graph-theoretic approach for marine conservation. Landscape Ecology **23**:19-36.

Underwood, J. N., L. D. Smith, M. J. H. Van Oppen, and J. P. Gilmour. 2007. Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. Molecular Ecology **16**:771-784.

Vallin, L., and A. Nissling. 2000. Maternal effects on egg size and egg buoyancy of Baltic cod, Gadus morhua: Implications for stock structure effects on recruitment. Fisheries Research **49**:21-37.

Van Oppen, M. J. H., and R. D. Gates. 2006. Conservation genetics and the resilience of reef-building corals. Molecular Ecology **15**:3863-3883.

Venturelli, P. A., C. A. Murphy, B. J. Shuter, T. A. Johnston, P. J. van Coeverden de Groot, P. T. Boag, J. M. Casselman, R. Montgomerie, M. D. Wiegand, and W. C. Leggett. 2010. Maternal influences on population dynamics: evidence from an exploited freshwater fish. Ecology **91**:2003-2012.

Venturelli, P. A., B. J. Shuter, and C. A. Murphy. 2009. Evidence for harvest-induced maternal influences on the reproductive rates of fish populations. Proceedings of the Royal Society B: Biological Sciences **276**:919-924.

Vigliola, L., P. J. Doherty, M. G. Meekan, D. M. Drown, M. E. Jones, and P. H. Barber. 2007. Genetic identity determines risk of post-settlement mortality of a marine fish. Ecology **88**:1263-1277.

Vigliola, L., and M. Meekan. 2002. Size at hatching and planktonic growth determine post-settlement survivorship of a coral reef fish. Oecologia **131**:89-93.

Vrijenhoek, R. C. 2010. Genetic diversity and connectivity of deep-sea hydrothermal vent metapopulations. Molecular Ecology **19**:4391-4411.

Waits, L. P., G. Luikart, and P. Taberlet. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology **10**:249-256.

Waples, R. S., and O. Gaggiotti. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Molecular Ecology **15**:1419-1439.

Warner, R. R., and R. K. Cowen. 2002. Local retention of production in marine populations: evidence, mechanisms and consequences. Bulletin of Marine Science **70S**:245-249.

Waser, P. M., J. D. Busch, C. R. McCormick, and J. A. DeWoody. 2006. Parentage analysis detects cryptic precapture dispersal in a philopatric rodent. Molecular Ecology **15**:1929-1937.

Weersing, K., and R. J. Toonen. 2009. Population genetics, larval dispersal, and connectivity in marine systems. Marine Ecology Progress Series **393**:1-12.

White, C., K. A. Selkoe, J. Watson, D. A. Siegel, D. C. Zacherl, and R. J. Toonen. 2010a. Ocean currents help explain population genetic structure. Proceedings of the Royal Society B: Biological Sciences **277**:1685-1694.

White, J. W., L. W. Botsford, A. Hastings, and J. L. Largier. 2010b. Population persistence in marine reserve networks: incorporating spatial heterogeneities in larval dispersal. Marine Ecology-Progress Series **398**:49-67.
White, T. A., and J. B. Searle. 2007. Genetic diversity and population size: island populations of the common shrew, Sorex araneus. Molecular Ecology **16**:2005-2016.
Wilson, D. T., and M. G. Meekan. 2001. Environmental influences on patterns of larval replenishment in coral reef fishes. Marine Ecology Progress Series **222**:197-208.
Wisdom, M. J., L. S. Mills, and D. F. Doak. 2000. Life stage simulation analysis: estimating vital-rate effects on population growth for conservation. Ecology **81**:628-641.
With, K. A., R. R. Gardner, and M. G. Turner. 1997. Landscape connectivity and population distributions in heterogeneous environments. Oikos **78**:151-169.
Worm, B., R. Hilborn, J. K. Baum, T. A. Branch, J. S. Collie, C. Costello, M. J. Fogarty, E. A. Fulton, J. A. Hutchings, S. Jennings, O. P. Jensen, H. K. Lotze, P. M. Mace, T. R. McClanahan, C. Minto, S. R. Palumbi, A. M. Parma, D. Ricard, A. A. Rosenberg, R. Watson, and D. Zeller. 2009. Rebuilding Global Fisheries. Science **325**:578-585.
Wright, P. J., and F. M. Gibb. 2005. Selection for birth date in North Sea haddock and its relation to maternal age. Journal of Animal Ecology **74**:303-312.
Wyrtki, K. 1960. The surface circulation in the Tasman and Coral seas. CSIRO Division of fisheries and oceanography technical paper **No 8**:44 pp.
Zamudio, K. R., and A. M. Wieczorek. 2007. Fine-scale spatial genetic structure and dispersal among spotted salamander (*Ambystoma maculatum*) breeding populations. Molecular Ecology **16**:257-274.
Zeller, D. C., and G. R. Russ. 2000. Population estimates and size structure of *Plectropomus leopardus* (Pisces : Serranidae) in relation to no-fishing zones: mark-release-resighting and underwater visual census. Marine and Freshwater Research **51**:221-228.