

HOW DOES DISPERSAL AFFECT CHAOS: VISCOUS POPULATIONS AND FAMILIES IN A COMMON  
CARIBBEAN GOBY (*CORYPHOPTERUS PERSONATUS*)

A Thesis

by

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## ABSTRACT

### HOW DOES DISPERSAL AFFECT CHAOS: VISCOUS POPULATIONS AND FAMILIES IN A COMMON CARIBBEAN GOBY (*CORYPHOPTERUS PERSONATUS*)

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Dispersal is a fundamental aspect of metapopulation dynamics. It determines gene flow between populations, genetic structure, population demographics, population stability, population persistence, local adaptation, and divergence. A deep understanding of dispersal processes is one of the key aspects to understanding how a population will respond to environmental variability. Generally in species with bipartite life histories, one phase of the life history is dispersive while the other is sedentary. Marine populations are often homogenous over large spatial scales, with the maximum range of dispersal correlated with the duration of the larval phase. However, there are many biotic processes which can reduce the general dispersal range. These processes can lead to the phenomenon of chaotic genetic patchiness, common among marine species. Chaotic genetic patchiness (CGP) is observed in populations with non-equilibrium or “chaotic” pattern of differentiation among locations in space and/or time. We show that despite having a larval stage, and a wide distribution across Caribbean coral reefs, *Coryphopterus personatus* form groups of related individuals at small spatial scales (<10 metres). These spatially clustered groups of related individuals, families, lead to a pattern of chaotic genetic patchiness. The presence of spatially clustered family groups is a finding that is rarely demonstrated, but could be more common than previously thought. To explain the

observation of relatives inhabiting the same reef we seek to understand the dispersal mechanisms responsible for this pattern.

Despite the benefits of dispersal, it can be costly. It incurs opportunity costs and risk costs, and is often energetically expensive. Because of this, many species have evolved bet-hedging strategies which allow them to take advantage of the benefits with some dispersive individuals, while having other individuals avoid the costs of dispersal by not dispersing. Non-dispersing individuals have to balance the negative effects of kin competition with the benefits gained through inclusive fitness. One way to do that is to have short range dispersal which could potentially disperse individuals outside the scale of kin competition while staying within range of inclusive fitness benefits. We find that within a reef, particular families are found in most of the shoals, and consist of multiple cohorts. This finding supports the hypothesis that there is some degree of short range dispersal away from the shoal from which individuals were spawned, which could act as a bet-hedging dispersal polymorphism. While kin-aggregations are common in terrestrial species there are few examples of consistent family group formation in marine species with a dispersive larval phase. We conclude that *C. personatus* disperse in a similar manner as r-selected terrestrial species which have viscous populations and benefit from inclusive fitness.

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I wouldn't have been able to do any of the field collections without the help of both Alan and our field assistant Emily Anderson, who both quickly became experts in collection *Coryphopterus personatus* while I was busy measuring the positions of all the shoals we were collecting from.

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# **1 CHAPTER 1: KIN-AGGREGATIONS EXPLAIN CHAOTIC GENETIC PATCHINESS, A COMMONLY OBSERVED GENETIC PATTERN, IN A MARINE FISH**

## **1.1 ABSTRACT**

The phenomenon of chaotic genetic patchiness is a pattern commonly seen in marine organisms, particularly those with demersal adults and pelagic larvae. This pattern has been attributed to a number of different biological mechanisms including selection, gene flow and genetic drift. Rarely has chaotic genetic patchiness been attributed to kin relatedness among individuals because such a condition is thought to be unlikely in adult populations of species with highly dispersive larvae. Here we investigate the biological underpinnings of this pattern in a species of marine goby *Coryphopterus personatus*. We find that populations of this species show tell-tale signs of chaotic genetic patchiness including; small but significant differences in genetic structure over large distances, a non-equilibrium or “chaotic” pattern of differentiation among locations in space; and within locus, within population deviations from Hardy-Weinberg equilibrium. We show that despite having a larval stage, and a wide distribution across Caribbean coral reefs, this species forms groups of highly related individuals at small spatial scales (<10 metres). These spatially clustered family groups lead to the pattern of chaotic genetic patchiness, a finding that is rarely demonstrated, but could be more common than previously thought.

## 1.2 INTRODUCTION

Marine organisms with dispersive pelagic larvae are characterized by low levels of genetic differentiation among populations over large areas due to high gene flow resulting in genetic homogenization. However, many marine species exhibit slight, yet significant, levels of genetic heterogeneity over various spatial scales (Selkoe *et al.* 2010; Broquet *et al.* 2013) and molecular markers which deviate from the expectations of Hardy-Weinberg equilibrium (HWE) within some samples. This complex pattern, termed chaotic genetic patchiness (CGP), is often described as ecologically uninformative and attributed to non-equilibrium conditions caused by multiple proposed mechanisms, including selection, drift and population mixing (Larson & Julian 1999). However, by seeking to understand the biological mechanisms underlying these patterns we can deepen our understanding of the ecology and evolution of complex marine populations.

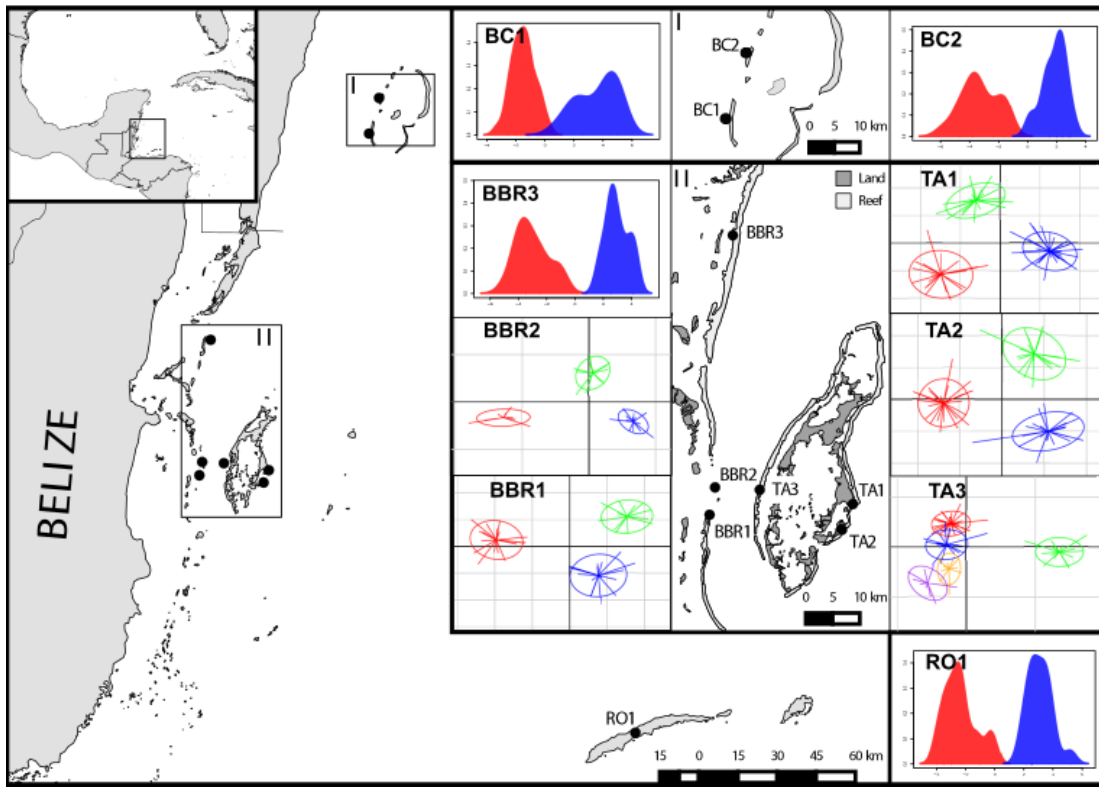
There are several mechanisms that could create CGP across populations of marine organisms (Larson & Julian 1999) including; local habitat differences (Selkoe *et al.* 2010), temporal variability in ocean currents affecting dispersal (Selkoe *et al.* 2006), selection at settlement (Johnson & Black 1984), differential reproductive success (Hedgecock 1994), genetic drift within isolated populations (Broquet *et al.* 2013), and the formation of kin-aggregations (Broquet *et al.* 2013). In marine populations, kin-aggregations are often overlooked as a mechanism to explain CGP. The probability of sampling relatives is expected to be low for species with highly dispersive pelagic larvae (Kinlan & Gaines 2003). However, recent studies show that larvae can remain with kin-aggregations in the plankton (Selkoe *et al.* 2006; Buston *et al.* 2009). Kin-aggregations likely form during the larval stage through aggregations of locally-spawned eggs and larvae by ocean currents and patchiness of food resources (Shapiro 1983). Various life-history traits, including demersal spawning and shoaling might cause kin-aggregation formation in fishes (Shapiro 1983).

Here we investigate the population genetic structure of a shoaling marine goby.

*Coryphopterus personatus*, is found above coral reef habitats throughout the Caribbean, yet little information exists about its life history; particularly, larval duration, dispersal, and population structure. We seek to use observed patterns of genetic structure to test the kin-aggregation hypothesis of CGP in a species of coral reef fish and discuss the consequences of this mechanism for the ecology of this and similar species. We then propose competing alternative hypotheses for the mechanism leading to the formation of kin-aggregations.

### 1.3 METHODS

Fish were collected during summer 2002 from nine sites in the Mesoamerican barrier reef system (Figure 1.1). Within each site, multiple shoals of *C. personatus* were collected by divers from a 1-ha area on the fore-reef and pooled for storage in 95% ethanol. Genomic DNA was extracted from pectoral fin tissue of each individual (Elphinstone *et al.* 2003) and amplified at ten microsatellite loci (Table 3.1; Hepburn *et al.* 2005; Hogan *et al.* 2010). The sizes of DNA fragments were determined using a LiCor 4300 DNA Analyzer with GeneImagIR 4.05 software (Scanalytics, Inc).



**Figure 1.1: Map of Sites with DAPC clustering analysis**

The Mesoamerican Barrier Reef study sites: BC – Banco Chinchorro, Mexico; BBR – Belizean barrier reef, Belize; TA – Turneffe Atoll, Belize; RO – Roatán, Honduras. Insets show scatter plots (and density plots in the case of two clusters) of clusters from DAPC analysis within sampling locations. Ellipses represent inertia ellipses representing 67% of the variance.

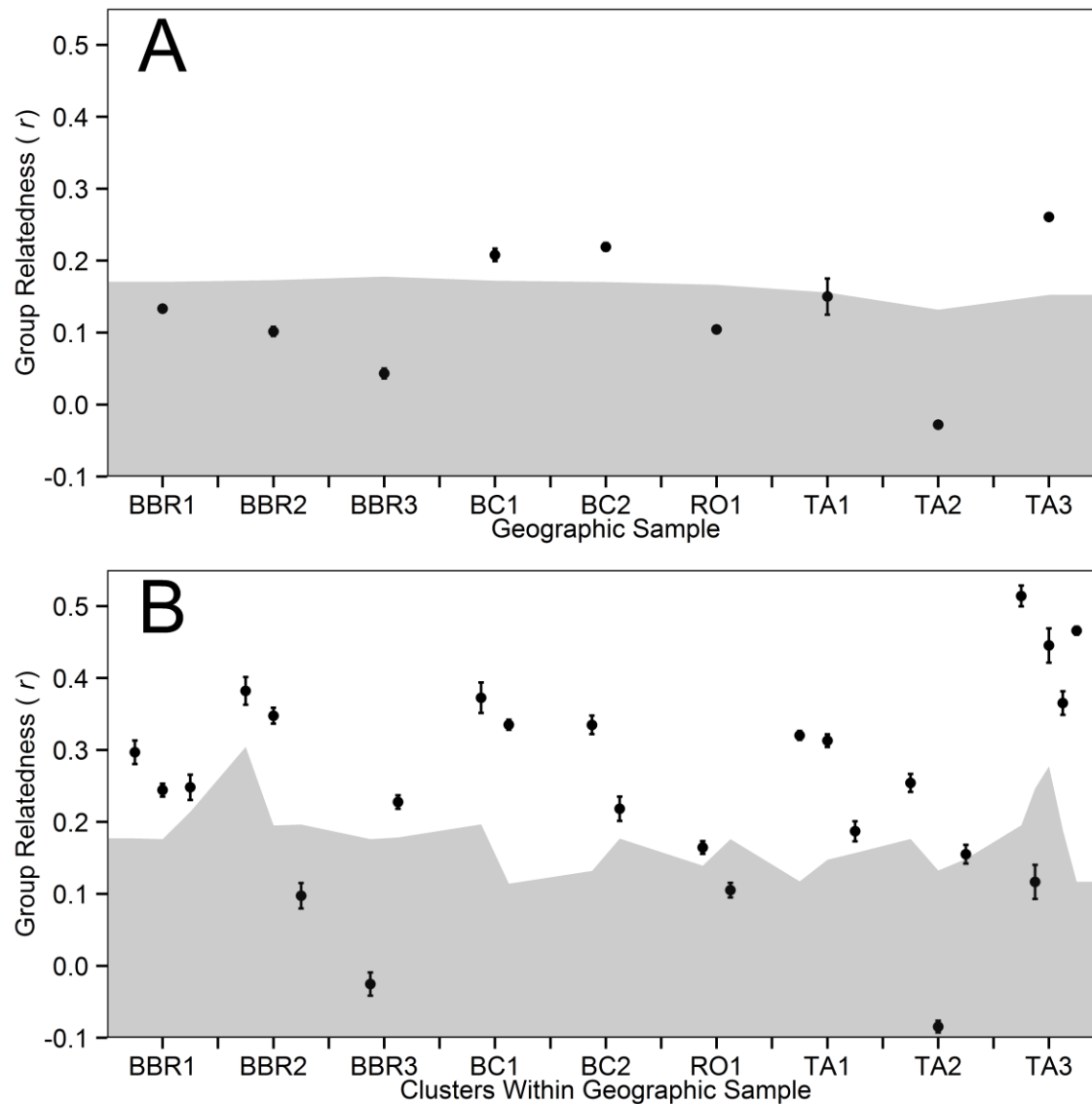
Exact tests for goodness of fit to the expectations of HWE were performed in ADEGENET (Jombart 2008). Monte Carlo tests for homozygote excess were performed using the U-score implemented in HWXTEST (Engels 2014). The proportion of different alleles (PD) and pairwise  $F_{ST}$  between sites were calculated across all loci using ADEGENET (Jombart 2008). A Mantel test was performed in ADE4 to test for isolation-by-distance among sites using PD and  $F_{ST}$  (Dray et al. 2007). Inbreeding coefficients were calculated for each site using GSTUDIO (Dyer 2014). Significance was assessed by permuting genotypes among samples 10,000 times, using sequential Bonferroni to correct for multiple testing.

The coefficient of group relatedness ( $r$ ) was calculated for each geographic sample in R v3.1.1 (Queller & Goodnight 1989). Significance of  $r$  was tested using a one-tail test, where genotypes were permuted among samples 10,000 times. The presence of related groups within geographic samples was tested using a Discriminant Analysis of Principal Components (DAPC) to identify genetic clusters of individuals using ADEGENET (Jombart 2008; Jombart *et al.* 2010). All possible clustering solutions were compared using the k-means clustering algorithm. The coefficient of group relatedness was then recalculated for each cluster within sites generated with the DAPC.

#### 1.4 RESULTS

There were deviations from the expectations of HWE in 48% of site by locus comparisons (Figure 3.1). Homozygote excess was observed in 53% of comparisons (Figure 3.2). Significant differences in  $P_D$  and  $F_{ST}$  were seen in 69% and 97% of the pairwise site comparisons respectively (Table 3.2) and Mantel tests were not significant, indicating no pattern of isolation by distance ( $P_D$ ,  $r=0.22$ ,  $P=0.19$ ;  $F_{ST}$ ,  $r=0.31$ ,  $P=0.14$ ). All loci showed evidence for high levels of inbreeding in a minimum of two sites with an average of  $6.5 \pm 2.6$  (22-100%) significantly inbred sites per locus (Table 3.3).

Within site group relatedness was 103-148% higher than expected from random expectation in sites BC5, BC6 and TA6 ( $p < 0.05$ , Figure 1.2A). Within-site clustering analyses found 2 to 5 highly related groups of individuals (8-36 individuals per cluster) within each of the nine geographic samples (Figure 1.1). Group relatedness measured within these clusters was found to be 128-941% higher than expected in 76% of all clusters ( $p < 0.05$ , Figure 1.2B).



**Figure 1.2: Sample (A) and Cluster (B) group relatedness**

Group relatedness ( $r$ ) values by geographic sample (A) and cluster (B) with error bars calculated through jackknife resampling. The shaded region indicates the area within the 95% confidence intervals calculated using a permutation test with 10,000 iterations.

## 1.5 DISCUSSION

Clusters of highly related individuals were found within geographic samples of *Coryphopterus personatus* explaining apparent CGP. Some geographic samples as a whole showed substantially higher than expected levels of relatedness. However, we found that within

each sample there were between 2 and 5 highly related clusters of individuals. This is unexpected for a species with pelagic larvae where the prevailing paradigm suggests that larvae should be widely dispersed and thoroughly mixed via physical oceanographic processes and populations should be connected over large distances.

We propose two mechanisms driving the formation of related groups in this species. First, larvae could remain together in the plankton and settle onto a reef together. There are many potential selective benefits to larvae remaining in a group throughout the pelagic larval phase including predator avoidance (Hewitt 1981) and maintenance of position within a food patch (Hunter 1980). The presence of kin-aggregations may simply reflect the fact that group formation occurs in the egg phase. Consistent with this mechanism, related individuals have been found within a single larval cohort in several other species (Selkoe *et al.* 2006; Buston *et al.* 2009). Given this mechanism we expect to find individuals of the same cohort within an aggregation to be related to each other, but not related to individuals from other cohorts within the same sample site.

Another mechanism that could explain kin-groups on a coral reef is a lack of larval dispersal. It is possible that larvae do not enter the pelagic environment, remaining on their natal reef. Other marine species that do not have a pelagic larval stage are characterized by low levels of gene flow, frequent population bottlenecks and strong phylogeographic breaks (Bernardi & Vagelli 2004). If *C. personatus* has lost their pelagic life stage, we expect a similar genetic pattern, additionally; we would expect to see highly related individuals across cohorts within a single sample site. Natal recruitment increases the chance of settling in a suitable habitat (Ronce 2007) and given that *C. personatus* needs to feed 2 days post-hatching (Thresher 1984), it is

plausible that rather than entering the plankton where food is sparse and patchy (Medvinsky *et al.* 2001) larvae could remain on the reef where food is more abundant (Hamner *et al.* 1988).

The formation of kin-aggregations is a potentially important evolutionary process due to the likelihood of very little gene flow on extremely small spatial scales and increased rates of inbreeding, potentially leading to greater vulnerability to extirpation (Frankham 1995). The benefits of kin-aggregations (protection from predation, kin-selection, etc. (Hamilton 1964)) may outweigh the costs of inbreeding. The pattern revealed here demonstrates CGP caused by an evolutionarily important biological phenomenon indicating the necessity of exploring the mechanisms of CGP in greater detail.

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## 2 CHAPTER 2: YOU DON'T HAVE TO GO FAR BUT YOU CAN'T STAY HERE: VISCOUS POPULATIONS AND KIN-AGGREGATIONS AS A BET-HEDGING DISPERSAL STRATEGY

### 2.1 ABSTRACT

Dispersal is one of the most fundamental aspects of metapopulation dynamics. It determines gene flow between populations, genetic structure, population demographics, population stability, population persistence, local adaptation, and divergence. Despite these benefits and others, dispersal can be costly. It incurs opportunity costs and risk costs, and is often energetically expensive. Because of this many species have evolved bet-hedging strategies, which allow them to take advantage of the benefits with some dispersive individuals, while having other individuals avoid the costs of dispersal by not dispersing. Non-dispersing individuals have to balance the negative effects of kin competition with the benefits gained through inclusive fitness. One way to do that is to have short range dispersal which could potentially disperse individuals outside the scale of kin completion while staying within range of inclusive fitness benefits. Here we demonstrate a dispersal mechanism in *Coryphopterus personatus*, a common Caribbean goby, consistent with the hypothesis of short range dispersal as a method of balancing kin competition with inclusive fitness. We find nine family groups in a sample of 384 individuals within a single reef (~300 m<sup>2</sup>). Additionally, these families are composed of related individuals across cohorts. This finding is in contrast with the expected pattern of long range dispersal through which relatives would be unlikely to occur in the same location across generations. Finally, the family groups are spread out among shoals found on the reef, indicating some short range dispersal away from the shoal from which individuals were spawned. This pattern is similar to dispersal in r-selected terrestrial populations which benefit from inclusive fitness.

## 2.2 INTRODUCTION

Understanding the patterns and processes of dispersal, “the tendency for organisms to reproduce away from their parents” (Duputié & Massol 2013), is integral to understanding the metapopulation dynamics, which impact all aspects of the evolution and ecology of a species (Hanski & Gilpin 1991; Harrison & Hastings 1996). Dispersal patterns directly impact resistance to perturbation, local adaptation, gene flow and divergence in a population (Cowen *et al.* 2007; Cowen & Sponaugle 2009). By understanding the dispersal characteristics of a population, it becomes possible to effectively manage populations and understand the risk those populations face when confronted with environmental change (Almany *et al.* 2009).

Dispersal is often associated with significant costs to both the individual dispersing and the individual’s parents (for review see Bonte *et al.* 2012). Individuals often face an opportunity cost to dispersing as they may not be able to grow or reproduce while dispersing (Part 1991; Hinsley 2000). Additionally, dispersal is often associated with high levels of mortality, either during the process of dispersing, or by failing to find suitable habitat after dispersing (Greig 1993; Leggett & Deblois 1994; Houde 1997; Cheptou *et al.* 2008).

While costly, dispersal also carries a number of benefits to individuals and populations. These benefits range from population level benefits of increased gene flow (Kokko & López-Sepulcre 2006; Ronce 2007) to individual benefits such as taking advantage of open habitat patches and avoiding inbreeding or kin competition (Moore & Ali 1984; Kokko & López-Sepulcre 2006; Ronce 2007). One consequence of the balancing of costs and benefits of dispersal has been the evolution of various bet-hedging strategies where some offspring disperse (higher risk, higher reward) while others either don’t disperse at all, or do so on a reduced scale (low risk, lower reward) (Toonen & Pawlik 1994, 2001; Nathan & Muller-Landau 2000; Rousset & Gandon 2002; Nanninga & Berumen 2014).

One of the consequences of having a reduced dispersal is living near relatives. Living with relatives introduces the possibility of inclusive fitness benefits, increased fitness associated with reproduction of a relative, mediated through cooperative reproduction and altruism (Hamilton 1964a; b; Griffin & West 2002). Additionally, in situations where inbreeding depression is low inbreeding can become beneficial in the context of inclusive fitness (Puurtilinen 2011; Szulkin *et al.* 2013). However in populations with no dispersal the fitness advantage gained through inclusive fitness can be directly countered by the increased costs of kin competition (Taylor 1992; Wilson *et al.* 1992; West *et al.* 2002). This has led to a number of theoretical and empirical studies investigating dispersal patterns that mitigate the costs of kin competition while maintaining the benefits of inclusive fitness (e.g., budding dispersal, Gardner & West 2006; sex-biased dispersal, Gros *et al.* 2008; limited dispersal, Platt & Bever 2009). However, most of this research hasn't been performed in a marine environment as it's generally assumed marine species with bipartite life histories and potentially dispersive larvae don't form family groups.

Most coastal marine species possess a pelagic larval phase which promotes dispersal among populations and has led to the paradigm that family groups are rarely formed. The duration of the larval period is generally thought to be coupled with the dispersal distance, with longer durations leading to greater dispersal potential (Levin 2006; Bradbury *et al.* 2008). However, many biotic and physical processes can break the link between larval duration and dispersal distance (Sponaugle *et al.* 2002; for review see Cowen & Sponaugle 2009). The mode of reproduction is one biological factor which greatly impacts the potential for dispersal. Species that lay benthic eggs exhibit much higher levels of genetic differentiation than those that broadcast spawn, indicating lower levels of dispersal and connectivity (Riginos *et al.* 2014). Additionally, the developmental competence of the larvae can influence dispersal distance, affecting larval

swimming performance (Stobutzki & Bellwood 1997), sensory abilities (Kingsford *et al.* 2002) and of the timing of vertical migrations (Munk *et al.* 1989). Highly competent larvae can control their position in the water column and orient towards food sources and suitable habitat and possibly prevent advection by currents (Kingsford *et al.* 2002).

Larval competence and reproductive mode have the potential to lead to dramatically reduced effective dispersal ranges that would not be inferred based upon the larval duration alone. For example, larvae of the vagabond butterflyfish (*Chaetodon vagabundus*) have a larval duration of over one month but are just as likely to recruit to their natal reef (60% self-recruitment) as the orange clownfish (*Amphiprion percula*) which has a larval duration of less than two weeks (Almany *et al.* 2007). The spiny damselfish, *Acanthochromis polyacanthus*, and the Banggai cardinalfish, *Pterapogon kauderni*, have lost the pelagic larval phase entirely, instead the larvae complete their development with the adult populations on the reef without dispersing (Planes & Doherty 1997; Bernardi & Vagelli 2004). These species often show evidence of reduced gene flow and strong genetic structuring among populations (Miller-Sims *et al.* 2008).

Spawning behaviour and larval behaviour can also influence the composition of dispersing individuals. Budding dispersal is a phenomenon in which larvae spawned by the same parents are released at the same place and time into the water column and disperse together in a coherent group (Selkoe *et al.* 2006). Often times these coherent larval cohorts settle together, forming related groups of individuals in the populations they recruit to (Planes & Doherty 1997 – *Naso unicornis*; Buston *et al.* 2009 – *Dascyllus aruanus*; Iacchei *et al.* 2013 – *Panulirus interruptus*). *Dascyllus aruanus* recruit together into an existing shoal as a related unit from which they leave and redistribute throughout the reef, potentially to reduce mating competition with relatives, or minimize inbreeding depression (Buston *et al.* 2009).

Gobies (Gobiidae) are the second most specious family of fishes in the world (Nelson 2006), and many of the marine gobies show evidence of reduced dispersal distances. For example, dwarf gobies of the genus *Eviota* are known to have high levels of endemism and restricted species ranges despite the presence of suitable habitat on nearby islands within the potential dispersal range based solely on the 24 – 26 day larval duration (Depczynski & Bellwood 2006; Tornabene *et al.* 2015). Gobies of the genus *Elacatinus* also show regional endemism, strong population structuring and restricted dispersal in the Caribbean (Taylor & Hellberg 2003, 2006). The Caribbean sponge-dwelling goby, *Elacatinus lori*, has a 21 day larval stage (Rickborn & Buston 2015) but almost all larvae disperse less than 5km from the natal reef, and many return to the natal reef (D'Aloia *et al.* 2013). Populations with limited dispersal, like *Elacatinus lori*, are often termed “viscous” populations that are characterized by increased genetic relatedness of neighbours (Hamilton 1964a; b; Taylor 1992; Griffin & West 2002; West *et al.* 2002; D'Aloia *et al.* 2013). The masked goby, *Coryphopterus personatus*, a group-living Caribbean goby with benthic eggs and a larval duration of 26 days (Hogan unpubl. data), appear to show evidence of restricted dispersal, where multiple family groups of related individuals are found to make up local populations within small geographic areas (<100 m<sup>2</sup>; Chapter 1). It is not known whether the formation of family groups in *C. personatus* is a result of budding dispersal, restricted larval dispersal (viscous populations) or even the loss of the pelagic larval phase altogether.

**Table 2.1: Hypotheses**

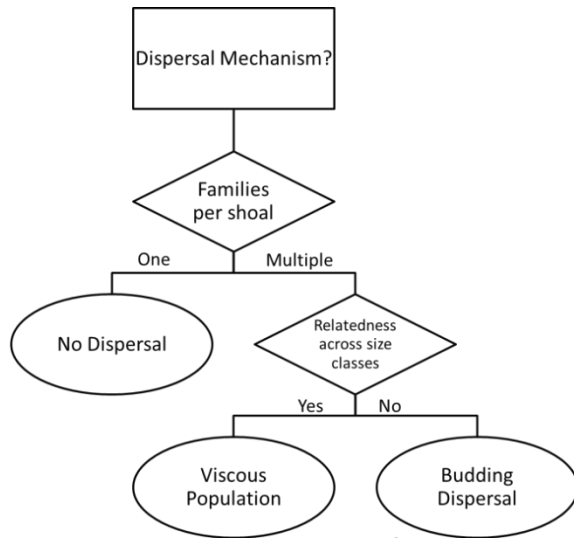
Three competing hypotheses with combination of results supporting each hypothesis

Hypothesis	Families per School	Isolation by Distance	Pairwise Relatedness
No Dispersal	One	Yes	Across and within generations
Budding Dispersal	Many	No	Within generation
Viscous Population	Many	Yes	Across and within generations



Here we seek to infer the mechanism leading to the formation of family groups at spatial scales of cm-m in populations of *C. personatus*. We seek to test three mutually exclusive hypotheses relating to the dispersal of larvae and redistribution of adults on reefs which leads to the presence of family groups (summarized in Table 2.1 and Figure 2.1). Our first hypothesis for the mechanism of family group formation is that *C. personatus* do not disperse (like *A. polyacanthus*), and instead form shoals composed of kin-aggregations. The “no dispersal hypothesis” is supported if shoals are composed entirely of single, multi-generational families. Along with that expectation we would also expect to see related individuals across the body size spectrum as old and young individuals in a shoal will be related. Lastly we would expect to see a negative correlation between pairwise relatedness and geographic distance between individuals (Table 2.1, Figure 2.1).

Our second hypothesis is that *C. personatus* disperse through budding dispersal, where larvae disperse moderate to long distances but stay together throughout the planktonic phase in related groups (Suarez *et al.* 2001; Buston *et al.* 2009; Kümmerli *et al.* 2009). The “budding dispersal hypothesis” would be supported by shoals composed of multiple families and finding related individuals within cohorts (size classes) but unrelated individuals across cohorts (i.e., a negative correlation between pairwise relatedness and difference in size between individuals). We also would not expect any correlation between geographic distance and pairwise relatedness because the recruit dispersal is likely to occur at spatial scales larger than the size of the sampled site (Table 2.1, Figure 2.1).



**Figure 2.1: Hypothesis Tree**

Schematic indicating tests required to differentiate dispersal hypotheses.

Our final alternative hypothesis is that *C. personatus* exhibits viscous populations where dispersal from the natal location is relatively slow and over short distances (Hamilton 1964a; b; Taylor 1992). The “viscous population hypothesis” would be supported by the presence of multigenerational families in the study area but shoals that are composed of multiple families. Additionally, we expect to find related individuals across multiple size cohorts, however, we do expect to see a correlation between pairwise relatedness and geographic distance as the number of dispersers will decline with distance from the source (D’Aloia *et al.* 2013; Table 2.1, Figure 2.1).

## 2.3 METHODS

Thirteen shoals composed of 10 to 67 individuals of *C. personatus* ( $29 \pm 15$  fish per shoal) were collected by divers on SCUBA using hand nets during August 2014 from Turneffe Atoll, Belize and stored in 70% ethanol. Distances between shoals were measured using a compass and transect tape. Upon return to Texas A&M University – Corpus Christi, samples were transferred to 95% ethanol for storage. Genomic DNA was extracted from each individual using E.Z.N.A. ®

DNA extraction kit (Omega Bio-tek). Seven microsatellite loci were amplified (Table 2.2; Hepburn *et al.* 2005; Hogan *et al.* 2010) using the following PCR conditions: 10 ng of template DNA, 1 X PCR buffer, locus specific concentrations (Table 2.2) of fluorescent labelled forward primer and an unlabelled reverse primer, 50  $\mu$ M of each dNTP and 0.5 U Taq polymerase; double stranded DNA was denatured at 94 °C for 3 min, followed by a locus specific number of cycles (Table 2.2) of denature for 15 sec at 94 °C, annealing for 45 sec at a locus specific temperature (Table 2.2), and extension for 30 sec at 72 °C, followed by 5 min extension at 72 °C, and 12 °C hold. The sizes of DNA fragments were determined using an ABI 3730xl Genetic Analyzer and scored with GeneMarker 2.6.4 software (Softgenetics).

**Table 2.2: PCR Conditions**

PCR Conditions for each locus with observed and expected heterozygosities

Locus	MgCl <sub>2</sub> (mM)	Primer ( $\mu$ M)	Anneal (°C)	Cycles	Alleles	Observed Ho	Expected Ho
COPE5	3.0	200	62-54	50	78	0.7282	0.9715
COPE9	3.0	200	62-54	50	12	0.5415	0.7286
CPER26	3.0	500	62-54	50	12	0.5175	0.6571
CPER92	4.5	100	58-52	40	12	0.3070	0.5087
CPER99	4.5	200	68-64	40	5	0.1936	0.3113
CPER119	4.5	100	58-52	40	22	0.4667	0.9094
CER188	4.5	100	58-52	40	15	0.4533	0.4850

Exact tests for goodness of fit to the expectations of Hardy-Weinberg Equilibrium (HWE) were performed in ADEGENET (Jombart 2008). Monte Carlo tests for homozygote excess were performed using the U-score implemented in HWXTEST (Engels 2014). Significance was assessed by permuting genotypes among samples 10,000 times, using sequential Bonferroni to correct for multiple testing.

To test for the presence of genetically similar clusters of individuals (hereafter referred to as families) independent of shoals, we performed a Discriminant Analysis of Principal Components (DAPC) using ADEGENET (Jombart 2008; Jombart *et al.* 2010). The number of families present was determined based on the number of clusters leading to the smallest Bayesian information criterion.

The pairwise relatedness ( $r$ ) was calculated between all pairs of individuals using the triadic likelihood estimator, which has been shown to be the least biased relatedness estimator in many circumstances, using the package RELATED (Wang 2007; Pew *et al.* 2014). The arithmetic mean value for the pairwise relatedness ( $\bar{r}$ ) was determined for each shoal and family. The statistical significance of mean pairwise relatedness was calculated using a one-tail permutation test with individuals permuted among shoals or families 1,000 times.

To determine if pairwise relatedness among individuals was correlated with (1) geographic distance, (2) cohort (inferred from body length), (3) cluster assignment (0 = same cluster, 1 = different clusters), and (4) shoal (0 = same shoal, 1 = different shoal), we performed a multiple regressions on distance matrices analysis (MRDM) using ECODIST (Goslee & Urban 2007; Lichstein 2007; Paquette & Lapointe 2009). Correlations were calculated based on the Spearman rank correlation coefficient to account for nonlinearity with significance assessed using a permutation test with 10,000 permutations.

## 2.4 RESULTS

Measurements of deviations from HWE found many deviations due to homozygote excess. All shoals were found to deviate significantly from HWE in 41% of loci ( $\pm 13\%$ ); all shoals deviated from HWE in at least 2 loci. On average, loci deviated from HWE across 41% ( $\pm 34\%$ )

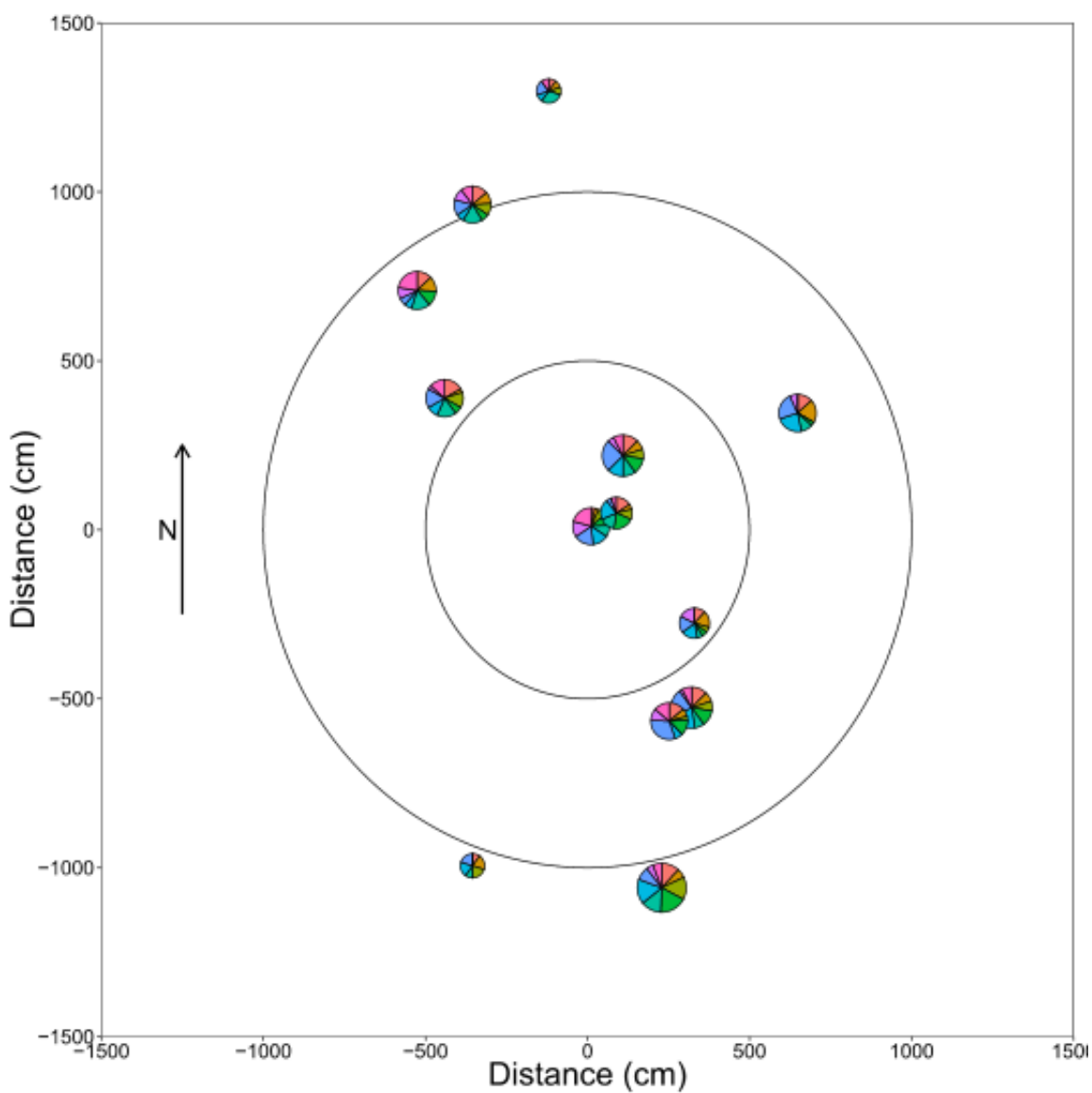
of shoals (Table 2.3); each locus deviated from HWE in at least 1 shoal. An excess of homozygotes explained the deviations from HWE in 63% of comparisons (Table 2.3).

We found nine family groups on the study reef with nearly all families represented in each shoal (Figure 2.2). The mean pairwise relatedness of shoals was not significantly different than randomly generated shoals of equal size ( $\bar{r}=0.089 \pm 0.010$ , all  $p \geq 0.13$ , Figure 2.3A). However the mean pairwise relatedness of families was significantly greater than a random cluster of equal size ( $\bar{r}=0.27 \pm 0.08$ , all  $p \leq 0.003$ , Figure 2.3B).

**Table 2.3: Observed heterozygosity values**

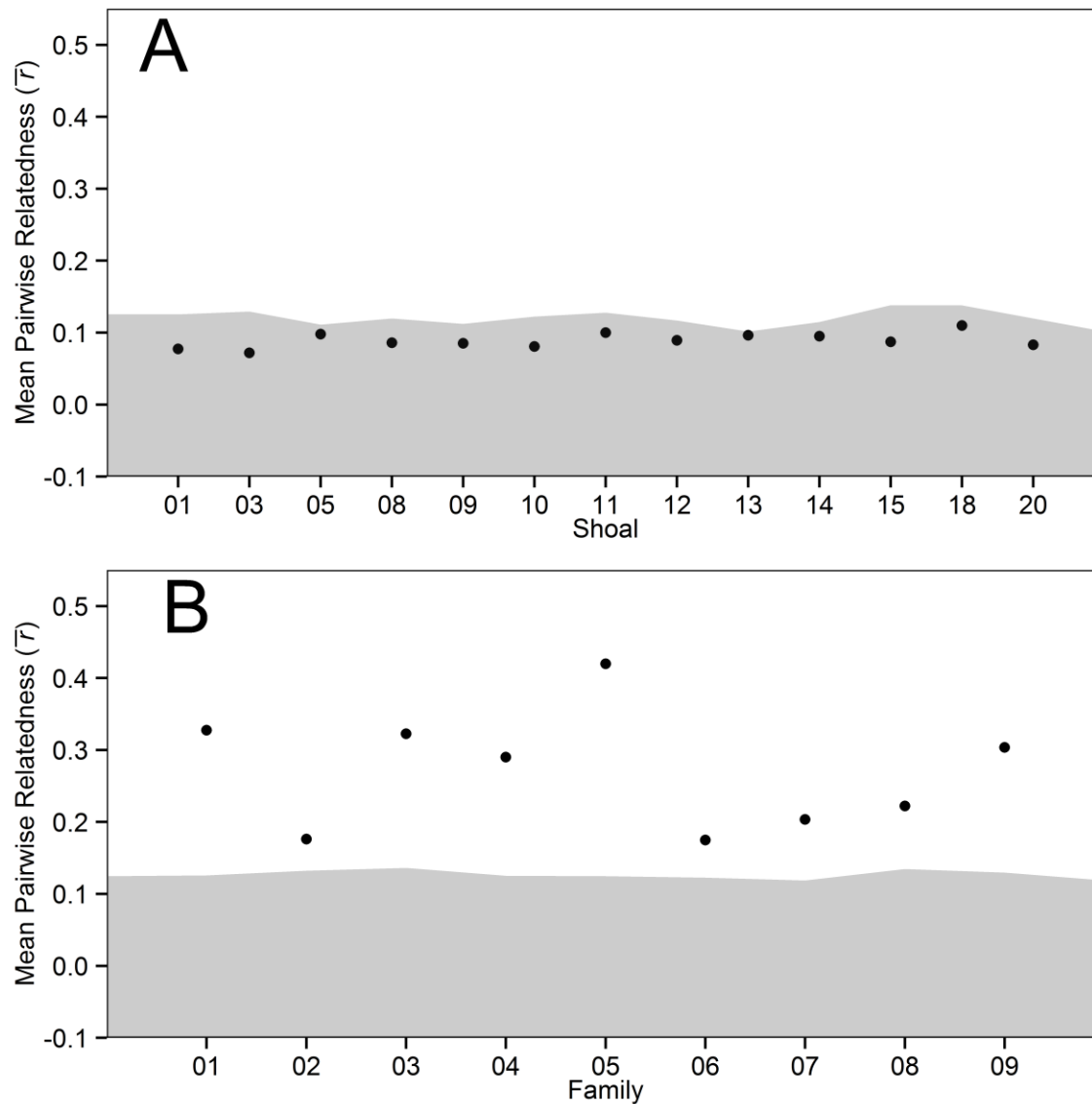
Observed heterozygosity values. Underlined values indicate a significant homozygote excess. Bold values indicate significant deviations from Hardy-Weinberg Equilibrium.

	COPE5	COPE9	CPER26	CPER92	CPER99	CPER119	CPER188
SHOAL-01	<b><u>0.7241</u></b>	0.5517	0.5517	<u>0.1786</u>	0.1724	<b><u>0.5000</u></b>	0.5714
SHOAL-03	<b><u>0.7368</u></b>	<b><u>0.5000</u></b>	0.6316	0.3158	<b><u>0.2105</u></b>	<b><u>0.5556</u></b>	0.4118
SHOAL-05	<b><u>0.8750</u></b>	0.6000	<u>0.4054</u>	<b><u>0.2750</u></b>	0.3250	<u>0.6316</u>	0.4595
SHOAL-14	<b><u>0.7241</u></b>	<b><u>0.3200</u></b>	0.5000	<b><u>0.3333</u></b>	0.2333	<b><u>0.5000</u></b>	0.6000
SHOAL-08	<b><u>0.6452</u></b>	<u>0.5862</u>	0.5807	0.4074	0.2258	<b><u>0.4138</u></b>	<b><u>0.4194</u></b>
SHOAL-10	<b><u>0.8667</u></b>	<u>0.5333</u>	0.5667	0.3462	0.1786	<b><u>0.4231</u></b>	0.6667
SHOAL-09	<b><u>0.7692</u></b>	<u>0.5000</u>	0.4500	<b><u>0.2051</u></b>	0.1316	<b><u>0.5526</u></b>	0.4000
SHOAL-12	<b><u>0.5172</u></b>	<b><u>0.3793</u></b>	0.5172	0.3448	0.1379	<u>0.5517</u>	0.4138
SHOAL-11	<b><u>0.5294</u></b>	0.7059	0.5882	0.5000	0.1177	<b><u>0.4706</u></b>	0.4118
SHOAL-15	<b><u>0.7000</u></b>	0.7000	0.7000	0.2222	<b><u>0.1000</u></b>	<b><u>0.3000</u></b>	0.4000
SHOAL-18	<b><u>0.5000</u></b>	<b><u>0.5714</u></b>	0.5000	<b><u>0.2222</u></b>	0.5000	<u>0.3000</u>	0.3000
SHOAL-20	<b><u>0.7586</u></b>	0.4800	<b><u>0.5385</u></b>	<u>0.3600</u>	<b><u>0.2414</u></b>	<b><u>0.3200</u></b>	<b><u>0.3103</u></b>
SHOAL-13	<b><u>0.7612</u></b>	0.6491	<u>0.4762</u>	0.3115	<b><u>0.1194</u></b>	<b><u>0.3710</u></b>	0.4179



**Figure 2.2: Site map with Family composition of Shoals**

Diagram of shoal position within sampling location. Pie charts represent the proportion of each family assigned to each shoal. Size of the pie chart represents the relative number of *C. personatus* collected from the shoal.

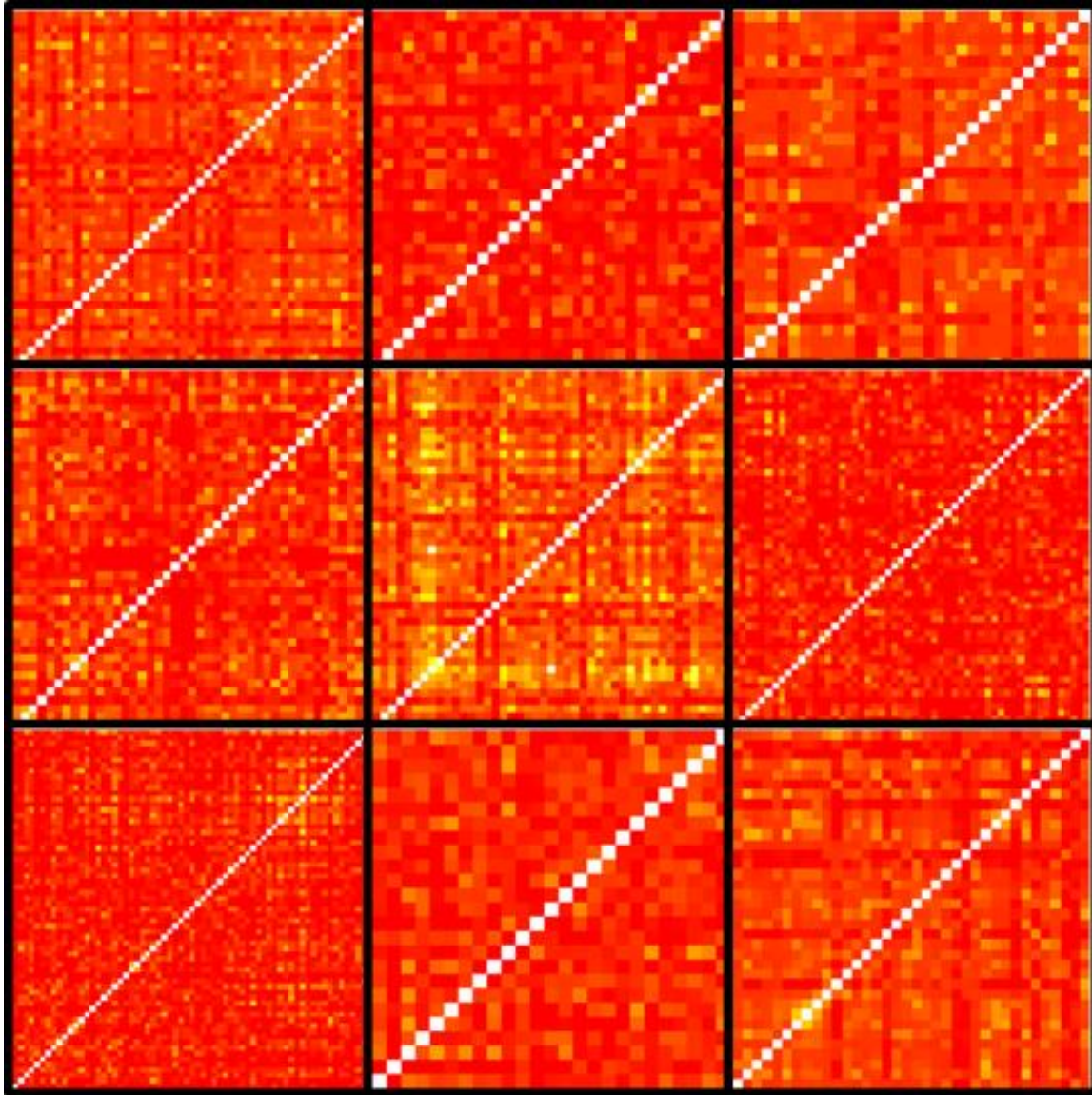


**Figure 2.3: Shoal (A) and Family (B) mean pairwise relatedness**

Mean pairwise relatedness ( $\bar{r}$ ) values by shoal collected from (A) and family assigned to using DAPC (B). The shaded region indicates the area within the 95% confidence intervals calculated using a permutation test with 1,000 iterations.

The MRDM model was able to explain 10.2% of the variation in the pairwise relatedness matrix ( $p \leq 0.0001$ ). Specifically, patterns of relatedness were not correlated with the difference in total length between individuals or the shoal that individuals were collected from (coefficient = 0.0051,  $p = 0.34$  and coefficient = 0.003,  $p = 0.75$  respectively, Figure 2.4). However, pairwise relatedness was significantly, negatively correlated with geographic distance between

individuals and family assignment (coefficient = -0.012,  $p = 0.038$  and coefficient = -0.050,  $p \leq 0.0001$  respectively).



**Figure 2.4: Heatmaps of family pairwise relatedness**

Heatmap of pairwise relatedness of individuals within each family. Individuals are ordered by size such that comparisons of individuals of similar size are found along the diagonal and comparisons of individuals of different sizes are opposite the diagonal. Darker colours indicate lower pairwise relatedness; lighter colours indicate higher pairwise relatedness.



## 2.5 Discussion

We found that within a small area of reef ( $\sim 300 \text{ m}^2$ ) shoals of *C. personatus* were composed of nine families. Our results show that the further apart pairs of individuals were geographically within the reef, the lower their estimated pairwise relatedness. However, we found no relationship between the difference in size of the individuals and their pairwise relatedness, indicating families are made up of multiple cohorts. These results support the viscous population hypothesis (Figure 2.1); *C. personatus* either disperse in short “hops” as larvae, or redistribute within the local geographical area as juveniles/adults after direct development.

The phenomenon of direct development in coral reef fishes is relatively rare, and the few species that do exhibit this phenotype have specific adaptations in the larval phase to allow for this process to occur (Kavanagh 2000). For example *Acanthochromis polyacanthus* larvae undergo flexion prior to emerging from the egg and emerge with fully formed caudal fins (Kavanagh 2000). Additionally, hatching occurs during the day, after which there is an extended period of biparental care (Kavanagh 2000). *Coryphopterus personatus* on the other hand emerges from the egg relatively competent (Hogan unpubl. data) but there is no research indicating that flexion occurs prior to hatching or that have fully formed caudal fins upon hatching (Thresher 1984; Kramer & Patzner 2008). Additionally, other *Coryphopterus sp.* hatch at night and there is no evidence to support the hypothesis that there is any period of parental care (Thresher 1984; Baldwin & Smith 2003; Kramer & Patzner 2008). It should be noted, that this conclusion is based on evidence from congeners and not *C. personatus* specifically, so it is possible this species exhibits direct development but that it has not been confirmed. However, larvae which were putatively identified as *C. personatus* have been collected from the ichthyoplankton from plankton nets moored near reef structures (Baldwin & Smith 2003). Given these larval characteristics it is unlikely that *C. personatus* is a direct developing species, instead this

indicates that it is likely that the larvae emerge and undergo some dispersal albeit over a small average distance with high levels of self-recruitment, similar to *Elacatinus lori* (D'Aloia *et al.* 2013). This short-range dispersal could act to lower the risk associated with dispersal, a bet-hedging strategy which additionally takes advantage of the benefits of living in close association with one's relatives, while minimizing the associated costs, of kin competition and inbreeding depression (Platt & Bever 2009; Duputié & Massol 2013).

Viscous populations exist in an evolutionary balance between the benefits of inclusive fitness (i.e. fitness benefits gained by the reproduction of a relative, mediated through diffusion of costs or added benefits to reproduction, for reviews see Griffin & West 2002; Hatchwell 2009; Bourke 2014) and the costs of reproductive competition between relatives (Hamilton 1964a; b; Taylor 1992; Griffin & West 2002; West *et al.* 2002). In completely viscous populations the effect of reproductive competition between relatives negates the benefit gained through inclusive fitness (Taylor 1992; Johnstone 2008; Cornwallis *et al.* 2009). However, the negative effect of competition with relatives and inbreeding depression can be mitigated if these processes function on a different spatial scale than the benefits of inclusive fitness (Rousset & Gandon 2002; Platt & Bever 2009; Duputié & Massol 2013). If kin competition is a more localized process than reproduction then this form of limited dispersal could lead to the dispersal of individuals beyond the range of kin competition while staying within the range at which inclusive fitness benefits are possible (Platt & Bever 2009). Anemonefish have been found to exhibit high levels of self-recruitment, however recruiting fish avoid settling on the anemone inhabited by their parents (Jones *et al.* 2005; Madduppa *et al.* 2014). Additionally individuals inhabiting the same anemone have been found to be more closely related than those on neighbouring anemones (Madduppa *et al.* 2014). This pattern of self-recruitment with avoidance of parents and maintenance of kin-

aggregations could be another example of the range of dispersal being restricted to maintain the benefits of inclusive fitness while avoiding the costs of direct competition with relatives and inbreeding depression.

While minimizing larval dispersal could be part of a bet-hedging strategy (Philippi & Seger 1989; McPeck & Holt 1992; Ronce 2007) some individuals may disperse a much further distance. Given that *C. personatus* shows a lack of strong genetic differentiation across large spatial scales (Chapter I) and doesn't show evidence of regional endemism throughout the Caribbean (Thacker & Cole 2002; Baldwin *et al.* 2009) there must be some level of dispersal and gene flow across larger spatial scales than observed in this study (Wright 1943, 1949, 1965). The probability density function of dispersal distances (a.k.a., dispersal kernels) for most species includes a long tail of low probability, long-distance dispersal. It is these rare long-distance events that could be the method through which individuals are spread beyond the range of this study (Jones *et al.* 1999; Hogan *et al.* 2012; D'Aloia *et al.* 2013). Given that reproduction is an energetically expensive process (Roff 1983; Jonsson *et al.* 1991; Lambert & Dutil 2000; Palstra & Thillart 2010) and that the pelagic larval phase is characterized by high levels of mortality (e.g., 20 – 80% per day; Houde 1997) and that dispersal success is negatively correlated with dispersal distance (Buston *et al.* 2012) there is a strong selective pressure to minimize the time spent in the pelagic environment (Pechenik 1999; Rousset & Gandon 2002; Bonte *et al.* 2012). However, there is also selective pressure for dispersal (competition avoidance, van Valen 1971; inbreeding avoidance, Moore & Ali 1984; take advantage of vacant patches, Hill *et al.* 1996). These competing selective pressures can lead to the scenario of bet-hedging (see also: “drift-retention dichotomy”, Hannah *et al.* 2000; “dispersal plasticity”, Clobert *et al.* 2001; “dispersal

polymorphism”, Nanninga & Berumen 2014) where there is variation in dispersal distance of offspring among individuals and within clutches (for review see Ronce 2007).

The finding that viscous populations tend to exist in more closely related groups is fairly common in terrestrial species (Hamilton 1964a; b; Lehmann 2007; Cornwallis *et al.* 2009; Banks *et al.* 2011; Bourke 2014). However in marine organisms with pelagic larval phases the phenomenon of family groups is only rarely observed. In terrestrial species where viscous populations are typically observed there are two general strategies of dispersal to reduce kin competition and inbreeding depression. The first of these strategies is generally used by K-selected species such as elephants, *Loxodonta africana*, and yellow baboons, *Papio cynocephalus cynocephalus* (Pianka 1970). This strategy involves the dispersal of a single sex away from a core group of same sex relatives (“sex-biased dispersal”; Douglas-Hamilton 1973; Isbell & van Vuren 1996; Nyakaana & Arctander 1999; Isbell 2004; theoretical basis, Gros *et al.* 2008). Sex-biased dispersal aids in inbreeding avoidance by removing one sex of related individuals from the group to be replaced by dispersal of the same sex from an unrelated group (Gros *et al.* 2008). The sex which doesn’t disperse still gains inclusive fitness benefits from being in a group with relatives of the same sex, through cooperative breeding and altruism (Wild & Taylor 2004). The second strategy of dispersal, generally utilized by r-selected species (Pianka 1970), such as social and sub-social spiders, *Stegodyphus sp.*, involves dispersal away from the natal site by a group of related, newly hatched juveniles (“budding dispersal”; Johannesen & Lubin 2001; theoretical basis, Gardner & West 2006). By dispersing together local competition is reduced through the utilization of open habitat patches while maintaining elevated kinship levels to gain inclusive fitness benefits from cooperative breeding and altruistic behaviour (Gardner & West 2006). As an r-selected species (Pianka 1970) *C. personatus* likely fits the

model of terrestrial r-selected species and more research is needed to determine if the paradigm for terrestrial species dispersal in viscous populations holds true for marine species. Additionally this bet-hedging strategy is potentially beneficial to many other ecologically similar marine species (other crypto-benthic fishes and ephemeral species). While this is one of a very few studies describing the mechanism of dispersal leading to kin-aggregation in marine species it's possible that it is a more widespread phenomenon than previously thought.

## 2.6 ACKNOWLEDGEMENTS

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### 3 Supplementary Materials

#### 3.1 GENETIC ANALYSIS

Genomic DNA was extracted from pectoral fin tissue of each individual following the silica-based 96-well plate protocol of Elphinstone *et al.* (2003). Ten microsatellite loci were chosen from Hepburn *et al.* (2005) and Hogan *et al.* (2010). PCR amplification was then performed in 12.5  $\mu$ L reactions comprised of: approximately 100ng template DNA, 200  $\mu$ M of forward dye-label primer and reverse primer, 200  $\mu$ M of each dNTPs, 0.1 U Taq polymerase (0.025 U for CPER 184, Invitrogen, Burlington, Canada), 1x PCR buffer (provided by the manufacturer) and locus specific concentrations of  $MgCl_2$  and bovine serum albumin. PCR conditions were 94°C for 2 minutes, followed locus specific numbers of cycles of 94°C for 15 s, locus specific annealing temperatures for durations, 72°C for 30 s, with a final extension of 72°C for 90 s (Supplementary Table 1). The size of the PCR products was determined using a LiCor 4300 DNA Analyzer with GeneImagIR 4.05 software (Scanalytics, Inc).

#### 3.2 RELATEDNESS ANALYSIS

To calculate group relatedness we used Equation 3.1 from Queller and Goodnight calculated using code written by the authors in R v3.1.1 (Queller & Goodnight 1989; R Core Team 2014). In this equation  $r$  is the calculated group relatedness value where  $p$  is the allele frequency of allele ( $a$ ) in loci ( $k$ ) in individual ( $j$ ) in group ( $i$ ) and  $m$  is used to index allele frequencies.

**Equation 3.1**

$$r = \frac{\sum_i \sum_j \sum_k \sum_a (p_{i(-j)m} - \bar{p}_{(-i)m})}{\sum_i \sum_j \sum_k \sum_a (p_{ijm} - \bar{p}_{(-i)m})}$$



### 3.3 SUPPLEMENTARY TABLES

**Table 3.1: PCR Conditions**

†: indicates touchdown PCR reaction used; Ta (°C): annealing temperature; Ta (s): annealing time

Locus	Ta (°C)	Ta (s)	MgCl <sub>2</sub> (mM)	BSA (mM)
Cope9 (GACA) <sub>n</sub>	56	15	1.5	0
Cope12 (GT) <sub>n</sub> GG(GT) <sub>n</sub>	56	15	2.5	0
Cper26 (CA) <sub>8</sub>	46	45	2.5	2
Cper92 (TG) <sub>9</sub>	49	15	2.5	0
Cper99 (CA) <sub>7</sub>	46	45	2.5	2
Cper103 (TG) <sub>7</sub>	46	45	2.5	2
Cper119 (TG) <sub>15</sub>	53	15	2.8	0
Cper163 (TG) <sub>12</sub>	65-57	15	2.5	2
Cper184 (TC) <sub>19</sub>	65-57	15	1.25	2
Cper188 (CA) <sub>9</sub>	53	15	2.5	0

**Table 3.2: Pairwise P<sub>D</sub> and F<sub>ST</sub>**

Pairwise P<sub>D</sub> (above diagonal) and F<sub>ST</sub> (below diagonal) with bolded values indicating significant values

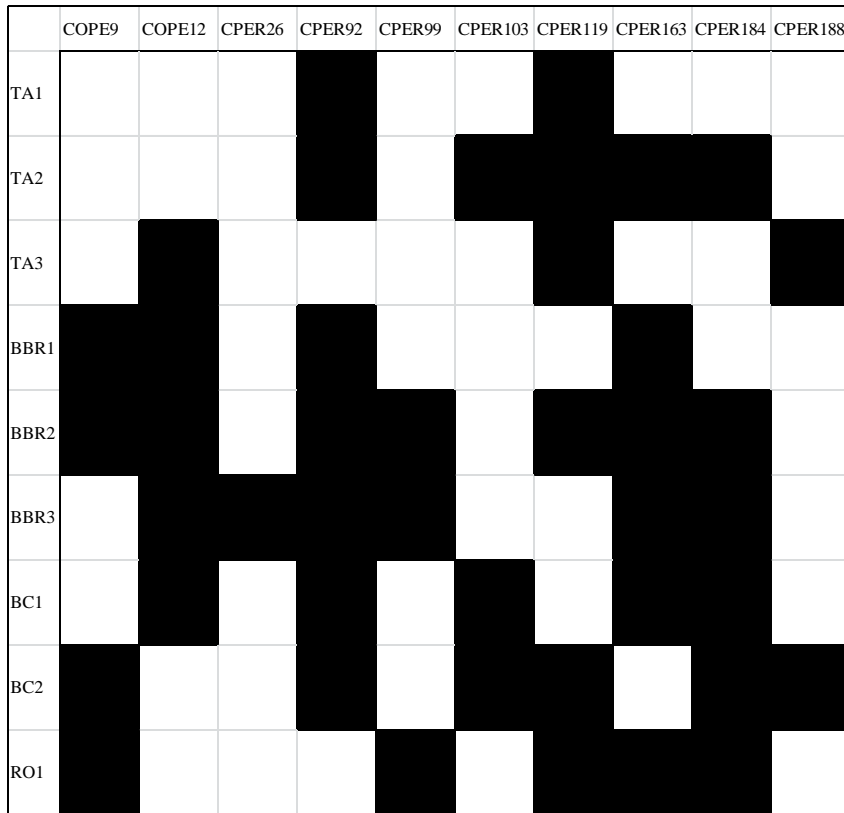
	TA3	TA4	TA6	BBR1	BBR2	BBR3	BC5	BC6	R01
TA3	-	<b>0.31553</b>	0.22794	0.22787	<b>0.2937</b>	<b>0.25648</b>	0.19477	0.24357	<b>0.25998</b>
TA4	<b>0.03702</b>	-	<b>0.29249</b>	<b>0.27325</b>	<b>0.27652</b>	<b>0.32097</b>	<b>0.31922</b>	<b>0.34702</b>	<b>0.32006</b>
TA6	<b>0.02054</b>	<b>0.02624</b>	-	0.22504	<b>0.30487</b>	<b>0.3077</b>	0.21805	0.2428	<b>0.2684</b>
BBR1	<b>0.01588</b>	<b>0.0203</b>	<b>0.01944</b>	-	0.22531	0.24501	0.24724	<b>0.27859</b>	<b>0.26452</b>
BBR2	<b>0.03201</b>	<b>0.01969</b>	<b>0.03385</b>	<b>0.01626</b>	-	<b>0.25638</b>	<b>0.31986</b>	<b>0.33256</b>	<b>0.32675</b>
BBR3	<b>0.02011</b>	<b>0.02222</b>	<b>0.02785</b>	<b>0.01648</b>	<b>0.02149</b>	-	<b>0.30859</b>	<b>0.33874</b>	<b>0.31817</b>
BC5	0.01117	<b>0.03054</b>	<b>0.01623</b>	<b>0.02051</b>	<b>0.04224</b>	<b>0.03005</b>	-	0.24391	<b>0.27915</b>
BC6	<b>0.03652</b>	<b>0.04211</b>	<b>0.03215</b>	<b>0.03913</b>	<b>0.05825</b>	<b>0.04919</b>	<b>0.03143</b>	-	<b>0.28206</b>
R01	<b>0.02828</b>	<b>0.02573</b>	<b>0.02533</b>	<b>0.02012</b>	<b>0.0371</b>	<b>0.02537</b>	<b>0.02689</b>	<b>0.03829</b>	-

**Table 3.3: Inbreeding Coefficient by Locus**

Inbreeding coefficients for each geographic sample by locus. Bold numbers indicate significant results.

	Cope9	Cope12	Cper26	Cper92	Cper99	Cper103	Cper119	Cper163	Cper184	Cper188
TA1	<b>0.2916</b>	0.0901	<b>0.1724</b>	<b>0.4960</b>	<b>0.3579</b>	<b>0.1678</b>	<b>0.2358</b>	<b>0.4596</b>	0.0708	<b>0.1689</b>
TA2	<b>0.3283</b>	0.0045	0.0649	<b>0.5393</b>	<b>0.4440</b>	<b>0.3429</b>	<b>0.4320</b>	<b>0.6568</b>	-0.0368	<b>0.4176</b>
TA3	<b>0.2190</b>	<b>0.4441</b>	0.0459	<b>0.4255</b>	<b>0.4402</b>	-0.1265	<b>0.2523</b>	<b>0.6059</b>	-0.1195	<b>0.4297</b>
BBR1	<b>0.4905</b>	<b>0.7214</b>	0.0977	<b>0.6299</b>	0.0066	0.1188	<b>0.1422</b>	<b>0.8421</b>	<b>0.1339</b>	<b>0.2861</b>
BBR2	<b>0.3297</b>	<b>0.6702</b>	0.0092	<b>0.7067</b>	<b>0.6741</b>	-0.0301	<b>0.4259</b>	<b>0.8763</b>	0.0102	<b>0.5516</b>
BBR3	<b>0.4413</b>	<b>0.4050</b>	<b>0.2849</b>	<b>0.4351</b>	<b>0.5711</b>	0.0645	<b>0.3443</b>	<b>0.8560</b>	<b>0.2072</b>	<b>0.4545</b>
BC1	<b>0.3378</b>	<b>0.2287</b>	-0.2177	<b>0.6089</b>	<b>0.4582</b>	-0.5422	<b>0.3025</b>	<b>0.8224</b>	<b>0.3413</b>	<b>0.4178</b>
BC2	<b>0.3763</b>	0.1994	<b>0.2099</b>	<b>0.3951</b>	0.1825	-0.5595	<b>0.2319</b>	<b>0.5897</b>	0.0880	<b>0.3915</b>
RO1	<b>0.4329</b>	<b>0.3105</b>	0.1417	0.1323	<b>0.4463</b>	-0.0627	<b>0.1479</b>	<b>0.6942</b>	<b>0.1000</b>	-0.0660

### 3.4 SUPPLEMENTARY FIGURES

**Figure 3.1: Deviations from Hardy-Weinberg Equilibrium**

Deviations from Hardy-Weinberg Equilibrium shown at each geographic sample (rows) for each locus (columns). Significant deviations at a particular sample by locus comparison indicated with a black box.

	COPE9	COPE12	CPER26	CPER92	CPER99	CPER103	CPER119	CPER163	CPER184	CPER188
TA1										
TA2										
TA3										
BBR1										
BBR2										
BBR3										
BC1										
BC2										
RO1										

**Figure 3.2: Homozygote Excess**

Homozygote Excess shown at each geographic sample (rows) for each locus (columns). Significant deviations at a particular sample by locus comparison indicated with a black box.

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