



HAL
open science

Evaluation of conservation efficiency for gorgonian species at a regional scale based on an existing Marine Protected Area network and modeling scenarios accounting for hydrodynamical connectivity

Mariana Padrón

► To cite this version:

Mariana Padrón. Evaluation of conservation efficiency for gorgonian species at a regional scale based on an existing Marine Protected Area network and modeling scenarios accounting for hydrodynamical connectivity. Oceanography. Université Pierre et Marie Curie - Paris VI; Università degli studi (Bologne, Italie). Dipartimento di scienze della terra e geologico-ambientali, 2015. English. NNT : 2015PA066652 . tel-01488971

HAL Id: tel-01488971

<https://theses.hal.science/tel-01488971v1>

Submitted on 14 Mar 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Université Pierre et Marie Curie

Università di Bologna

Ecole doctorale des Sciences de l'Environnement d'Île de France

Laboratoire d'Ecogéochimie des Environnements Benthiques

Evaluation of conservation efficiency for gorgonian species at a regional scale based on an existing Marine Protected Area network and modeling scenarios accounting for hydrodynamical connectivity

Par Mariana Padrón

Thèse pour l'obtention du grade de docteur en océanographie biologique

Dirigée par:

Katell GUIZIEN, CNRS, UMR 8222 UPMC LECOB

Marco ABBIATI, UB, Dipartimento di Scienze Biologiche, Geologiche ed Ambientali (BiGeA).

Présenté et soutenue publiquement le 24 Novembre 2015

Devant un jury composé de (par ordre alphabétique):

Marco ABBIATI

Professeur, UB

Co-directeur

Didier AURELLE

Maître de conférences, HDR

Rapporteur

Lorenzo BRAMANTI

Chercheur contractuel

Responsable scientifique

Federica COSTANTINI

Maître de conférences, UB

Examinatrice

Katell GUIZIEN

Chargé de recherches, CNRS

Directrice

Claire PARIS

Professeur, University of Miami

Rapporteur

Eric THIEBAUT

Professeur, UPMC

Président

WHEN I heard the learn'd astronomer,
When the proofs, the figures, were ranged in columns before me,
When I was shown the charts and the diagrams, to ad, divide,
and measure them,
When I, sitting, heard the astronomer, where he lectured
with much applause in the lecture room,
How soon, unaccountable, I became tired and sick,
Till rising and gliding out, I wander'd off by myself,
In the mystical moist night-air, and from time to time,
Look'd up in perfect silence at the stars.

Walt Whitman
Leaves of Grass

Acknowledgements

Et voilà, c'est fini!

It is never easy to organize your thoughts when it comes to acknowledging the people that has, in any way, helped you or accompanied you in the process of achieving a goal in life. There are so many faces in the recollection of memories...so many people to thank.

I guess I will start by commemorating the past. After all, I wouldn't be here writing these pages if it wasn't for all of the choices I made and, of course, the big contribution of serendipity that lead me to this point in my life. I thank you all, the good and the not so good moments and all of the people that, most likely without knowing it, have helped me gain experience both personally and professionally.

To my sweet and wonderful Matt, thank you so much for following me in this journey! I'm so grateful for all the joy, the laughs and unconditional support. All this work and stress were only bearable thanks to your amazing patience and love.

A mis papas, que aún desde la distancia consiguen envolverme de magia y hacerme sentir segura y confiada como cuando era niña. Gracias por ese apoyo inquebrantable ante mi decision de salir a aventurar por el mundo, y por alimentar mi argentinito en los momentos de dudas.

To my supervisors, I honestly couldn't have asked for better guidance and support. You have showed me a great deal of determination and strength. I feel very fortunate for having the opportunity to have worked and learned from you. Thank you for taking me in, I will consider you life-long colleagues and friends. To the French team (Katell and Lorenzo), thanks for the freedom to develop my own ideas, and the bossing around when I needed some pressure. That balance kept me motivated, and it is a rare quality to find in an advisor. I particularly appreciate our long discussions about science and life. Your enthusiasm for what you do is truly inspiring. To the Italian team (Marco and Federica), I couldn't be more grateful for the support and trust that you put in me. You made me feel so welcome in your lab! Thanks for the guidance and the lab meetings that allow me to interact and learn more about what you do.

To my friends from Banyuls, Italy, MARES, Venezuela and San Francisco, enjoying work would have been much more difficult if you didn't take the time to have a pause café, a drink, send a message or a Skype call with me. Thank you! You are way too many to make a list, and I don't trust my memory with such a task anyway so, you know who you are! I'm sure I have let you know by now. Thanks for listening when I needed to vent, for telling me to shut up when I complained for no reason, for the laughs, the tears, the swimming, the dancing... And thank you Banyuls for the summer!!!



Abstract

Connectivity is expected to strongly influence the dynamics and persistence of marine populations. Studying the development and maintenance of connectivity patterns among marine populations is, thus, essential for spatial planning and the proper design and management of Marine Protected Areas (MPAs). However, understanding the complex processes driving marine population connectivity requires the use of tools that integrate bio-physical models with information regarding the demographic and genetic linkages resulting from the larval exchange among populations. Therefore, the aim of this PhD dissertation is to evaluate the patterns of genetic connectivity among gorgonian populations at a regional scale, and disentangle the processes that shape the observed connectivity by using model simulations accounting for hydrological, demographic and genetic connectivity. The first chapter presents a spatially explicit metapopulation model that, using stochastic connectivity matrices, assesses the effect of demography on allele frequencies in a marine metapopulation of sessile benthic species. The model is then used to evaluate the effect of demographic traits and connectivity structure on the genetic diversity of a marine metapopulation. The second chapter examines the patterns of genetic connectivity of two common and widely distributed gorgonian species at a regional scale: *Paramuricea clavata* in the Ligurian Sea, and *Eunicella singularis* in the Gulf of Lions. Both species exhibit strong patterns of genetic structure at a regional scale, although the dispersal capacity of each species does not seem limited (>100 m). The third chapter discerns among the potential processes shaping the realized connectivity of *E. singularis* in the Gulf of Lions by applying the model presented in Chapter 1, and comparing the modeled patterns of genetic structure to the results obtained from empirical genetic data in Chapter 2. Modeled and empirical results show similar patterns of genetic structure among populations of *E. singularis* in the region. Genetic and demographic differentiation among populations is demonstrated to result from the spatial structure of dispersal alone. The ability to evaluate the expected development of genetic structure among populations under different demographic and hydrological scenarios using the seascape model presented in Chapter 1 provides a useful tool with relevance for marine spatial planning and the persistence of marine populations.

Keywords: connectivity, marine metapopulation, population genetics, genetic structure, spatially-explicit model, seascape genetics, gorgonians, demography, larval dispersal, Gulf of Lions, Ligurian Sea, *Paramuricea clavata*, *Eunicella singularis*, Marine Protected Areas, marine spatial planning.





Résumé

La connectivité est supposée influencer fortement la dynamique et la pérennité des populations d'organismes marins. Ainsi, étudier l'évolution et le maintien des patrons de connectivité au sein des populations marines semble essentiel pour la planification spatiale, la création et la gestion des Aires Marines Protégées (AMP). Cependant, comprendre la complexité des processus régissant la connectivité entre les populations marines nécessite l'utilisation d'outils, qui associent des modèles biophysiques et des informations caractérisant les relations entre démographie et génétique, résultant des échanges larvaires entre les populations. L'objectif de cette thèse de doctorat est donc, d'évaluer les patrons de connectivité génétique au sein des populations de gorgones à une échelle régionale et d'explorer les processus induisant la connectivité observée, grâce à des simulations de modèles prenant en compte les connectivités hydrologique, démographique et génétique. Le premier chapitre présente un modèle de métapopulation spatialement explicite qui, grâce à l'utilisation de matrices de connectivité stochastique, permet d'évaluer l'effet de la démographie sur la fréquence des allèles dans une métapopulation d'espèces marines benthiques sessiles. Le modèle est alors utilisé pour déterminer l'effet des traits démographiques et de la structure de connectivité sur la diversité génétique de la métapopulation. Le deuxième chapitre présente une analyse des patrons de connectivité génétique de deux espèces communes et largement répandues de gorgones, à une échelle régionale : *Paramuricea clavata* en mer de Ligurie et *Eunicella singularis* dans le Golfe du Lion. Les deux espèces présentent de forts patrons de structure génétique à l'échelle régionale, bien que leur capacité de dispersion ne semble pas limitante (>100m). Le troisième chapitre vise à tester si la dispersion larvaire seule permet d'expliquer la connectivité réalisée d'*E. singularis* dans le Golfe du Lion, en appliquant le modèle présenté dans le Chapitre 1, et en comparant les patrons de structure génétique modélisés, aux résultats obtenus dans le Chapitre 2, par les méthodes empiriques d'analyse de données génétiques. Les patrons de structure génétique modélisés en ne prenant en compte que la structure spatiale de dispersion sont similaires à ceux observés entre les populations d'*E. singularis* dans cette région, suggérant le rôle prépondérant de la connectivité hydrologique dans la distribution régionale de l'espèce, tant au niveau démographique que génétique. La capacité d'évaluer le développement des structures génétiques entre populations, sous différents scénarios démographiques et hydrologiques, avec le modèle de paysage sous-marin présenté dans le Chapitre 1 s'avère donc un outil efficace pour la planification spatiale et la pérennité des populations marine.



Mots clés : connectivité, métapopulation marine, génétique des populations, structure génétique, modèle spatialement explicite, génétique des fonds marins, gorgones, démographie, dispersion larvaire, Golfe du Lion, Mer de Ligure, *Paramuricea clavata*, *Eucnicella singularis*, Aires Marines Protégées, planification spatiale marine.



Abstract

La connettività sembra influenzare fortemente la dinamica e la persistenza delle popolazioni marine. Comprendere l'evoluzione e il mantenimento dei pattern di connettività tra le popolazioni è perciò essenziale per la pianificazione spaziale marittima e per la creazione e la gestione delle Aree Marine Protette (AMPs). Ciononostante, analizzare la complessità dei processi che guidano la connettività delle popolazioni marine richiede l'utilizzo di strumenti che integrino modelli bio-fisici con informazioni derivanti dalle relazioni tra demografia e genetica che derivano dagli scambi larvali tra le popolazioni. Lo scopo di questa tesi di dottorato è quindi quella di valutare i pattern di connettività genetica tra popolazioni di gorgonie ad una scala spaziale regionale, e di comprendere i processi che determinano la connettività osservata attraverso simulazioni di modelli che tengano conto dei pattern idrodinamici, demografici e genetici. Il Primo Capitolo presenta un modello spazialmente esplicito di metapopolazione, che, attraverso l'utilizzo di matrici stocastiche di connettività, permetta di comprendere gli effetti della demografia sulle frequenze alleliche di una metapopolazione di specie marine bentoniche sessili. Il modello è stato poi utilizzato per valutare gli effetti delle caratteristiche demografiche e della connettività sulla diversità genetica di una metapopolazione marina. Il Secondo Capitolo esamina i pattern di connettività genetica su scala regionale di due specie di gorgonie ampiamente distribuite lungo le coste del Mar Mediterraneo: *Paramuricea clavata* nel Mar Ligure e *Eunicella singularis* nel Golfo del Leone. Entrambe le specie mostrano forti pattern di strutturazione genetica su scala regionale anche se la capacità dispersiva di tali specie non sembra essere particolarmente ridotta (>100 m). Il Terzo Capitolo cerca di comprendere quali sono i processi che determinano l'effettiva connettività tra le popolazioni di *E. singularis* del Golfo del Leone attraverso l'utilizzo del modello presentato nel Primo Capitolo e confrontando tali risultati con quelli ottenuti nel Capitolo 2 attraverso l'utilizzo di dati genetici empirici. I pattern di struttura genetica modellizzati prendendo in considerazione solo la struttura spaziale di dispersione sono simili a quelli osservati empiricamente. Questo suggerisce che la connettività idrodinamica debba svolgere un ruolo importante nella distribuzione regionale della specie così come nella sua struttura genetica e demografica. La capacità di valutare l'evoluzione della struttura genetica tra le popolazioni sotto differenti scenari demografici e idrodinamici attraverso l'utilizzo del modello presentato nel Capitolo Primo rappresenta un ottimo strumento per la pianificazione spaziale marittima e per la persistenza delle popolazioni marine.

Parole chiave: connettività, metapopolazione marina. Genetica di popolazione, struttura genetica, modello spazialmente esplicito, genetica del paesaggio marino, gorgonie, demografia. Dispersione larvale, Golfo del Leone, Mar Ligure, *Paramuricea clavata*, *Eunicella singularis*, Aree Marine Protette, pianificazione spaziale marittima.



Table of Contents

List of Figures	xvii
List of Tables	xix
Introduction	1
Larval dispersal and marine connectivity	4
Methods to estimate marine connectivity	8
References	14
Chapter 1: Modelling the effect of demographic traits and connectivity on the genetic structuration of marine metapopulations of sedentary benthic invertebrates	19
1.1 Abstract	21
1.2 Introduction	22
1.3 Methods	24
1.3.1 A seascape genetic model for benthic invertebrates with a pelagic larval phase	24
1.3.2 Test cases	28
1.3.3 Metrics and statistics	31
1.4 Results	32
1.4.1 Do differences in demographic parameters affect genetic diversity in closed populations?	32
1.4.2 Do different patterns of connectivity affect the maintenance of genetic diversity in a metapopulation	36
1.4.3 How does local population stability affect heterozygosity in a metapopulation?	38
1.5 Discussion	40
1.6 Acknowledgements	45
1.7 References	46
1.8 Supplementary material	51
Chapter 2: Genetic connectivity of gorgonian species at a regional scale	55
Introduction	57
2.1 Connectivity helps marine coastal populations recover from climate-induced threats	67

2.1.1	Abstract	69
2.1.2	Introduction	70
2.1.3	Material and Methods	73
	2.1.3.1 Sampling	73
	2.1.3.2 Microsatellite genotyping	74
	2.1.3.3 Genetic diversity	76
	2.1.3.4 Population genetic structure	76
	2.1.3.5 Migration and genetic drift	78
2.1.4	Results	79
	2.1.4.1 Genetic diversity	79
	2.1.4.2 Population genetic structure	81
	2.1.4.3 Migration and genetic drift	83
2.1.5	Discussion	85
	2.1.5.1 Do disturbed populations show a signal of reduced genetic diversity?	86
	2.1.5.2 Did connectivity play a role in the recovery of the affected populations?	87
	2.1.5.3 Implications of connectivity in a climate change scenario	88
2.1.6	Acknowledgements	89
2.1.7	References	89
2.2	Genetic connectivity of the white gorgonian, <i>Eunicella</i> <i>singularis</i>, in the Gulf of Lions	99
2.2.1	Abstract	101
2.2.2	Introduction	102
2.2.3	Material and Methods	104
	2.2.3.1 Study area and regional hydrodynamics	104
	2.2.3.2 Sampling	105
	2.2.3.3 DNA extraction and microsatellite genotyping	108
	2.2.3.4 Genetic diversity	108
	2.2.3.5 Genetic structure	108
	2.2.3.6 Migration patterns	110
2.2.4	Results	111
	2.2.4.1 Genetic diversity	111
	2.2.4.2 Genetic structure	111
	2.2.4.3 Migration patterns	115
2.2.5	Discussion	116

2.2.6	Acknowledgements	120
2.2.7	References	121
2.2.8	Supplementary Material	129
Chapter 3: Testing processes of genetic structuration for <i>Eunicella</i>		
<i>singularis</i> in the Gulf of Lions		
3.1	Abstract	135
3.2	Introduction	137
3.3	Methods	140
3.3.1	Hydrological connectivity	141
3.3.2	Demographic connectivity	143
3.3.3	Seascape genetics model	145
3.3.4	Realized connectivity: genetic population structure	146
3.4	Results	147
3.4.1	Hydrological connectivity vs. realized connectivity	147
3.4.2	Demographic connectivity vs. realized connectivity	152
3.4.3	Modeled genetic connectivity vs. realized connectivity	153
3.5	Discussion	155
3.6	Acknowledgements	158
3.7	References	159
3.8	Supplementary material	163
Conclusion and perspectives		
	References	165
		172

List of Figures

Introduction

1	Schematic representation of the metapopulation concept	4
2	Schematic representation of the successive processes along a benthic species life cycle driving realized connectivity	5
3	Representation of a connectivity matrix	11

Chapter 1

1.1	Schematic representation of seascape genetic model	25
1.2	Effect of different life expectancies on the loss of genetic diversity for an isolated population at initial saturating capacity	33
1.3	Effect of population growth rate over allele fixation time (T_f) for three different initial conditions of population abundance	35
1.4	Effect of different initial population abundances on the loss of genetic diversity	36
1.5	Spatial pattern of genetic diversity caused by distinct connectivity structures	37
1.6	Relationship between local retention (LR) and the number of population turnovers to start losing genetic diversity (T_d/L_e), in a metapopulation over time	38
1.7	Effect of fluctuations in local population stability on heterozygosity over time	39

Chapter 2

2.1	Two of the most common gorgonian species in the Mediterranean Sea .	58
2.1.1	Map of the seven sampling locations of <i>Paramuricea clavata</i> in the Ligurian Sea	74
2.1.2	Population genetic structure of <i>Paramuricea clavata</i> in the Ligurian Sea, as revealed by the software Structure	81
2.1.3	Autocorrelogram based on the kinship coefficient of Loiselle, estimated for multilocus genotypes within 13 spatial distance classes	83
2.1.4	Pattern of contemporary migration of <i>Paramuricea clavata</i> in the Ligurian Sea	85
2.2.1	Regional hydrodynamics and sampling locations of the populations of <i>Eunicella singularis</i> in the NW Mediterranean	106
2.2.2	Population structure of <i>E. singularis</i> in the Gulf of Lions and Spanish Catalan Coast	113
2.2.3	Plots of principal coordinate analysis from genetic distance matrix estimated with pairwise F_{ST} estimates of <i>E. singularis</i> sites for each genetic	

cluster	114
2.2.4 Migration patterns of <i>Eunicella singularis</i> in the NW Mediterranean . .	115
2.2.5 Autocorrelogram based on the kinship coefficient of Loiselle	116
Chapter 3	
3.1 Conceptual model of the seascape genetic approach	141
3.2 Geographic distribution of eight sites considered for the release of larvae in Lagrangian model of larval dispersal in the Gulf of Lions . .	142
3.3 Schematic representation of use of connectivity matrices to calculate hydrological distance among populations of <i>E. singularis</i> in the Gulf of Lions	143
3.4 Connectivity and hydrological distance matrices resulting from a biophysical model of larval dispersal of <i>E. singularis</i> in the Gulf of Lions	149
3.5 Population structure of <i>E. singularis</i> in the Gulf of Lions, as revealed by Structure software	150
3.6 Graphic representation of migration patterns and migration rates among sampled populations of <i>E. singularis</i> in the Gulf of Lions . .	151
3.7 Analysis of Isolation By Distance (IBD) for both geographical and hydrological distances of samples of <i>E. singularis</i> in the Gulf of Lions	151
3.8 Fluctuations in population density of <i>E. singularis</i> in the Gulf of Lions over time, as resulted from metapopulation dynamics modelling. . .	152
3.9 Heterozygosity values among populations of <i>E. singularis</i> in the Gulf of Lions	153
3.10 Principal Coordinate Analysis (PCA) of genetic distance among sites of <i>E. singularis</i> in the Gulf of Lions, as resulted from model simulations	154

List of Tables

Chapter 1

1.1	Settings of six simulation groups	30
-----	---	----

Chapter 2

2.1.1	Sampling locations of <i>Paramuricea clavata</i> in the Ligurian Sea	75
2.1.2	Parameters of genetic diversity for 12 sites of <i>P. clavata</i> at six micro-satellite loci	80
2.1.3	Pairwise F_{ST} values among sites in the Ligurian Sea	82
2.2.1	Description of sampling locations of <i>Eunicella singularis</i> in the NW Mediterranean	107
2.2.2	Parameters of genetic diversity of <i>E. singularis</i> at eight microsatellite loci in 30 sites along the Gulf of Lions and Spanish Catalan coast	112

Introduction





The world's oceans are increasingly threatened by multiple anthropogenic stressors. A large range of land and ocean-based activities, coupled with human population expansion and migration towards the coast, are rapidly causing the modification and/or degradation of the ocean's functions and biodiversity (Lubchenko 2003). Coral bleaching, fisheries collapse, changes in species composition and mass mortality events are a few among the severe consequences of the progressive alteration of the marine ecosystems around the world (Halpern *et al.*, 2008).

Within this context, the need of ensuring the persistence of marine species' populations becomes evident, as so does the realization that the resilience of these ecosystems will partly depend on our ability to establish appropriate management measures for conservation. As a consequence, growing concern regarding the number and efficiency of Marine Protected Areas (MPAs) has been evidenced in the scientific literature (Agardy 1994; Babcock *et al.*, 2010; Edgar *et al.*, 2014; Lagabrielle *et al.*, 2014). Furthermore, the Convention on Biological Diversity (CBD) has set a target of protecting 10% of the coastal and marine areas by 2020, which has led to a rapid increase in the creation of MPAs worldwide.

Our ability to effectively protect marine biodiversity, however, depends as well on our understanding of the species' ecology, dynamics, and its interactions with the environment. In the marine environment, species habitats are usually discontinuous either for natural reasons (e.g. coral reef patches, seagrass beds) or due to climatic or anthropogenic disturbances (e.g. hurricanes, coastal development). This discontinuity or fragmentation of habitat leads to the geographical separation of marine populations. The maintenance of the spatial distribution of these discrete populations suggest that they must be more or less connected by the exchange of individuals among them, forming what is commonly known as a metapopulation (Hanski 1999) (Figure 1).

The degree of connectivity, as well as the temporal and spatial scales of the connections among populations are, thus, key to the functioning of metapopulations. Theoretical studies suggest that population connectivity plays a fundamental role in





local and metapopulation dynamics, community dynamics and structure, genetic diversity, and the resilience of populations to human exploitation (Hastings and Harrison, 1994; Botsford *et al.*, 2009). Previous studies have demonstrated how connectivity may enhance the persistence of a metapopulation after disturbances, by enhancing their recovery and/or recolonization (Hastings and Botsford 2006; Guizien *et al.*, 2014). Therefore, understanding the degree and patterns of connectivity among populations is also central to the success of conservation management strategies, like the design of MPAs (Sale *et al.*, 2006).

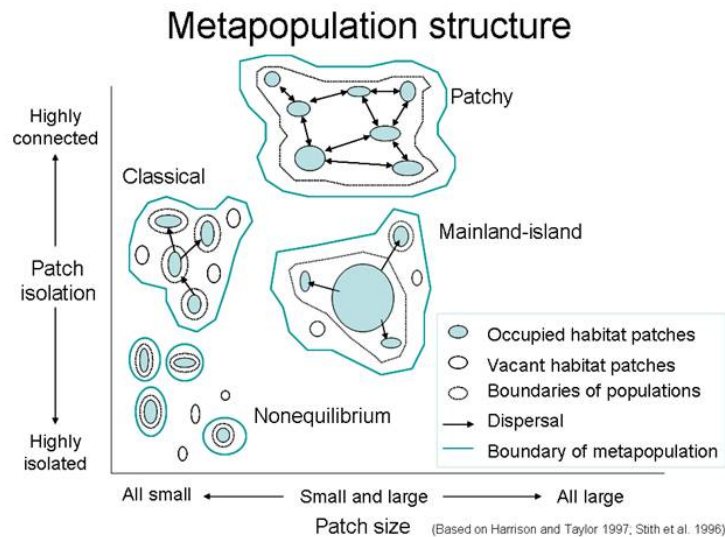


Figure 1.- Schematic representation of the metapopulation concept.

Larval dispersal and marine connectivity

The exchange of individuals among populations can occur over different times during a species life cycle: dispersal of larvae, and movement of juveniles and/or adults. However, for benthic sessile species, dispersal can only take place during their larval phase. Therefore, larval dispersal defines the spatial scale of connectivity for these species (Cowen and Sponaugle 2009).

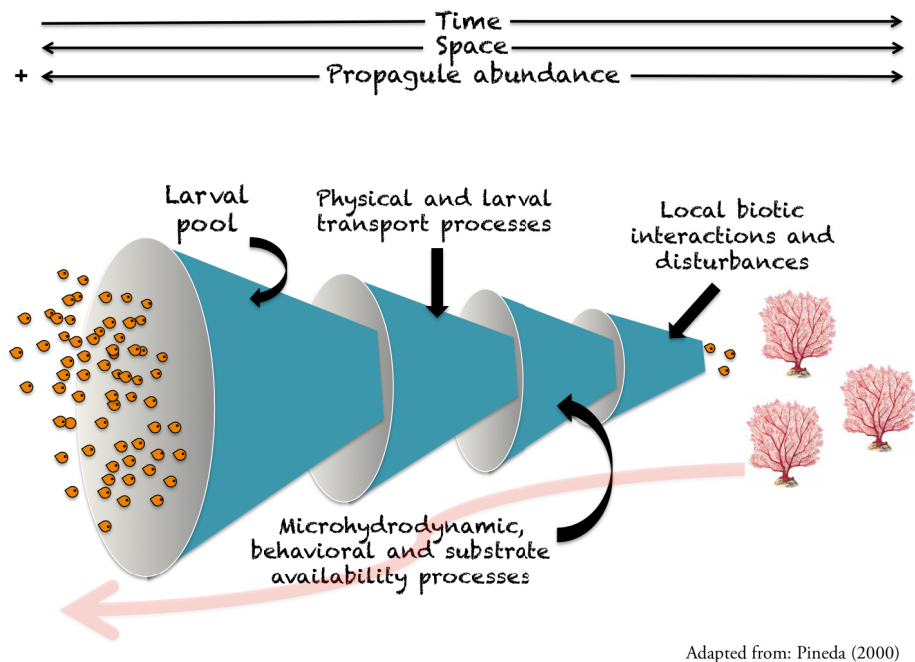
Several biophysical processes influence larval dispersal. Hydrodynamical features and environmental forcing at the location and timing of spawning will determine the





duration and direction of transport of larvae (Pineda 2000). Larval Pelagic Duration (PLD) and larval behavior may also alter the vertical distribution of the larvae in the water column, exposing it to different hydrodynamic conditions and potentially modifying their dispersal patterns (Butler *et al.*, 2011). Moreover, larval mortality can dilute the larval pool and limit connectivity (Paris *et al.*, 2007).

Connectivity patterns resulting from larval dispersal will also be affected by several processes acting at different temporal and spatial scales during the different stages of dispersion, from spawning to reproduction of the settled larvae (Pineda 2007) (Figure 2).



Adapted from: Pineda (2000)

Figure 2.- Schematic representation of the successive processes along a benthic species life cycle driving realized connectivity.

It is important to highlight that, after transport, not all of the arriving larvae will be able to settle. As highlighted by Pineda (2000), settlement rate is not a function of the number of larvae arriving. The availability of suitable substrate can also limit the exchange of individuals among populations. Also, post-settlement mortality will affect the effective connection among populations, as the larvae that successfully settle will





still face local biotic interactions and potential disturbances before it reaches sexual maturity and is able to reproduce (Chesson 1998). Furthermore, adult demography can vary reproductive output over time and space and thus control the number of larvae available to disperse.

Taking into account the variability in the spatial and temporal scales at which all of these processes act on population connectivity, it is then important to distinguish between the different types of connectivity that can be found according to the different processes that drive them. An understanding of larval dispersal can only determine population connectivity until the moment of larval settlement, which is known as *larval connectivity* or *potential connectivity* (Pringle 2003). Knowledge of larval connectivity may be useful for some objectives related, for example, to the persistence of a metapopulation. However, it only provides partial information regarding the exchange of individuals that will eventually reproduce (Pineda *et al.*, 2007).

For connectivity to enhance metapopulation persistence, the sum of births and immigration in a population must be equal or exceed the sum of deaths and emigration (Pineda *et al.*, 2007). When the contribution from the exchange of individuals among populations is large enough to influence the demographic parameters of a population, it is referred as *demographic connectivity* (Sale *et al.*, 2010). Within an ecological temporal scale, persistence is, therefore, only possible if the level of demographic connectivity among populations allows the replacement of an adult individual that is able to reproduce, even if its population of origin is not self-persistent (Burgess *et al.*, 2014).

In an evolutionary scale, on the other hand, when we refer to the exchange of genes among populations over several generations, we talked about *genetic connectivity*. The patterns of genetic connectivity in marine species will determine the level of genetic differentiation among populations, which could influence local adaptation and eventually speciation (Banks *et al.*, 2013). However, the level of exchange of individuals necessary to maintain genetic connectivity and even genetic homogeneity among populations, unlike demographic connectivity, are much lower (Greenbaum *et al.*, 2014).





Although all of aforementioned processes (biophysical and demographic) play a fundamental role in driving the connectivity patterns, their relative importance to connectivity and metapopulation dynamics can vary depending on the system and species-specific properties. For a long time, the relative homogeneity of the marine environment, coupled with a perceived lack of conspicuous barriers lead to the expectation that marine populations were highly connected (Caley *et al.*, 1996). The characteristic of these “open” populations would, however, depend on the school of thought. For some researchers, they would be characterized by high recruitment of larvae coming from external sources, although at a level that would still allow populations to fluctuate below saturating capacity. While for others, density-dependent processes acting after settlement would control the excess of incoming larvae (Hixon *et al.*, 2002). However, a growing body of evidence has revealed that patterns of dispersal can limit demographic exchange among populations and that, in fact, marine populations rarely constitute fully open or closed systems (Sponaugle *et al.*, 2002). In 2000, Cowen *et al.*, using numerical simulations of larval dispersal, showed that larval retention at a small scale was possible. An assertion that was later confirmed by field studies showing high levels of self-recruitment in coral reef systems (Almany *et al.*, 2007; Planes *et al.*, 2009; Gilmour *et al.*, 2013).

As an attempt to discern the processes behind these observations, several studies evaluated the fit of Larval Pelagic Duration (PLD) as a proxy to explain the dispersal potential and population connectivity of marine species. However, the results have been contradictory. Shanks *et al.* (2003) and Siegel *et al.* (2003) found a strong correlation between PLD and inferred dispersal distance. Conversely, other studies demonstrated strong population subdivision in species with long PLD (Thomas *et al.*, 2013; Damerau *et al.*, 2014). Whether limited dispersal capacity, regional hydrodynamics, or perhaps local demography, are responsible for the high levels of larval retention and/or self-recruitment observed in many marine species it is still unclear, and remains a scientific challenge. Quantifying the spatial scale of connectivity and identifying the factors that





regulate it has now become crucial for the establishment of appropriate management measures for the conservation of marine populations (Kritzer and Sale 2004; Pineda *et al.*, 2007; Jones *et al.*, 2009).

Methods to estimate marine connectivity

Our ability to measure connectivity by tracking larvae and following their movements between two populations is very limited. The classical techniques of physical tagging, although the most accurate, are just not feasible when dealing with such a large amount of very small size individuals (Thorrold *et al.*, 2002). Nonetheless, this type of direct tracking could not provide relevant information at an evolutionary time scale (Kool *et al.*, 2013). In order to overcome these limitations, other methods for estimating connectivity have been developed, which can be grouped into two main categories: empirical and modelling.

Within the empirical category of methods to assess connectivity among marine populations, chemical and genetic tools have been commonly used in a wide variety of fish and invertebrate species (Hedgecock *et al.*, 2007; Campana 1999). The chemical methods are based on the property of certain calcified tissues (e.g. shells and otoliths) to permanently incorporate chemical elements from the environment during their development. Notoriously among the techniques of chemical tagging, the method developed by Thorrold *et al.* (2006), allows the transgenerational transference of the chemical tag (a stable isotope of ¹³⁷Barium) from the mothers to their offsprings by an injection in the abdominal cavity of the individual. However, the use of this method is limited to species with calcified tissue, therefore, its application has been mostly limited to the analysis of otoliths in fish species.

Genetic tools, on the other hand, have been applied for the evaluation of population connectivity for a wide variety of marine taxa. Genetic inferences of gene flow among populations contribute to our understanding of the evolutionary consequences of dispersal. Furthermore, population genetics provides a mean to evaluate population connectivity integrated over many generations, which would not be





observable with other approaches (Lowe and Allendorf 2010). The methods used in population genetics allow both direct and indirect estimates of connectivity by assessing changes in the frequency of alleles (defined as alternative forms of a gene), and the assignment of individuals to their population of origin, respectively (Hedgcock *et al.*, 2007).

Changes in allele frequency are due to synergistic action of four main evolutionary forces: the mutation of genes, the stochastic fluctuations in finite populations called random genetic drift, the gene flow that results from the migration of individuals among populations, and selection. Over long periods of time, random genetic drift and selection can cause population differentiation, while migration acts to homogenize genetic diversity among populations (Roughgarden 1979). This effect of migration on allele frequencies is the basis of the usefulness of population genetics in inferring connectivity. However, it is important to highlight that the indirect methods estimate gene flow from the level and the pattern of genetic divergence among populations, under the assumption that the populations are at drift-gene flow equilibrium (Slatkin 1987). This assumption, of a balance between the influence of genetic drift driving the fixation on an allele in all populations, and the opposite effect of gene flow bringing new alleles to the populations, if not met, will generally lead to the overestimation of the current amount of gene flow (Lowe and Allendorf 2010).

In fact, most indirect methods used for inferring connectivity rely on theoretical models of population structure, like the Wright' Island Model (Wright 1931), an unrealistic model that assumes equal gene flow among populations of the same size. Therefore, it is important to understand the limitations of these indirect methods when inferring connectivity patterns. These methods can estimate connectivity over evolutionary scales, but are incapable of estimating contemporary exchange among populations (Hedgcock *et al.*, 2007). The most common metrics of genetic differentiation obtained from these methods are F_{ST} (a standardize variance of allele frequencies among populations), and a variety of conceptually similar metrics like G_{ST} , Φ_{ST} , AMOVA, etc.).





More recently, in order to overcome some of the shortcomings from unrealistic model assumptions and take advantage of variable molecular markers, methods that allow the direct estimation of gene flow by assigning individuals to their populations of parents of origin have been developed, and are considered to be analogous to non-genetic approaches of estimating migration among populations (Lowe and Allendorf 2010). Based on the expected frequency of its multilocus genotype, the probability that an individual originated from a given population or set of parents can be calculated (Manel *et al.*, 2005). Maximum Likelihood and Bayesian approaches, assuming only Hardy-Weinberg equilibrium and Linkage disequilibrium and, therefore, providing higher accuracy estimates, can also calculate inferred rates of migration among populations (Wilson and Rannala 2003). Nonetheless, assignment tests are sensitive to sample size and do not work well at high levels of gene flow, when population differentiation becomes difficult (Hedgcock *et al.*, 2007).

Although empirical methods of assessing population connectivity can provide estimates of the spatial and temporal variability of the observed connectivity patterns, these approaches are costly and labor intensive, which limits their wide application over several scales. Moreover, the fact that the observed patterns of genetic connectivity are integrated over multiple generations brings additional challenges when attempting to discern the processes that influenced them over time. In that regard, the use of numerical simulations of larval dispersal and connectivity enhances the testing of hypothesis over multiple and large temporal and spatial scales (Kool *et al.*, 2013).

As any other field, modelling tools are not free of challenges and, as a consequence, the models used to assess marine connectivity have evolved and greatly improved over time. Currently, biophysical models allow to quantify connectivity by simulating larval dispersal at different temporal and spatial scales. The incorporation of 3-dimensional coastal circulation models and spatially-explicit Individual Based Models (IBMs) are used to project individual (Lagrangian) larval trajectories under realistic conditions while, in some cases, taking into account some biological traits of the larvae





as well (Guizien *et al.*, 2006; Werner *et al.*, 2007). Several modelling approaches exist to estimate connectivity from larval dispersal trajectories (Kool *et al.*, 2013). However, the most commonly used in the literature is the study of connectivity matrices (Figure 3).

The connectivity matrix resulting from a biophysical model can provide essential information for understanding persistence in a marine metapopulation. With origin locations along the columns and destination locations along the rows, each one of the cells represents the probability that an individual produced at a given origin location either settles in the same location or disperses to settle in a given destination location at any given time (Burgess *et al.*, 2014). However, the use and interpretation of data resulting from biophysical models depends on the spatial and temporal resolution of the model, as well as the biological characteristics attributed to the larvae (Paris *et al.*, 2007).

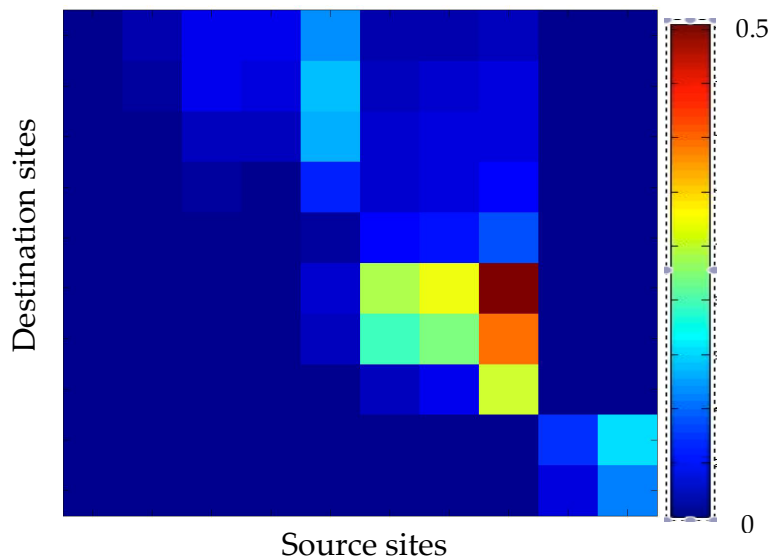


Figure 3.- Representation of a connectivity matrix

With so many different methods (empirical and modelling) available to estimate the different types of connectivity (larval, demographic and genetic) over a species life cycle, a true understanding of how spatial management can maintain the persistence of populations calls for a need to integrate multiple approaches. Furthermore, the





combination and comparison of connectivity patterns resulting from the use of different tools could allow to disentangle the relative role of the seascape processes shaping the observed connectivity patterns among marine populations.

The integration of ecological and genetic metapopulation models has been shown to be an important tool for understanding the complexities of population connectivity (Levin 2006; Epperson *et al.*, 2010). Previous studies have attempted to link biophysical dispersal models and population genetic structure (Galindo *et al.*, 2006; Foster *et al.*, 2012). However, these models do not seem to explain the observed connectivity patterns in sessile marine species at small spatial scales, a mismatch that could be explained by the lack of integration of the demographic dynamics of their populations, and/or the interaction between larval dispersal and population genetics.

Within this context, the objective of my research thesis is to discern the seascape processes responsible for driving and maintaining genetic connectivity in sessile invertebrate species at a regional scale, by confronting the observed realized connectivity to a combined approach of modelling methods that integrate connectivity over several stages of a species life cycle.

In **Chapter 1**, I present the development of a coupled seascape genetics model that allows to project the evolution of allele frequencies in a metapopulation over time, taking into account demographic traits and connectivity variability. With the application of this model I examine how: 1) demographic traits influence the development of genetic structure in closed populations over time, 2) different patterns of connectivity affect the loss or maintenance of genetic diversity at a regional scale in a metapopulation, 3) fluctuations in local population density help maintain genetic diversity over time and 4) local population demographic stability impacts genetic diversity to provide relevant information for conservation decisions in the Gulf of Lions, France.

In **Chapter 2**, I used a combination of direct and indirect genetic methods to evaluate the connectivity patterns of two gorgonian species in two regional scales. I analyse the genetic population structure of the red gorgonian (*Paramuricea clavata*) in the





Ligurian Sea, and the white gorgonian (*Eunicella singularis*) in the Gulf of Lions. Given the history of mass mortality events recorded for *P. clavata* in the Ligurian Sea, I attempt to answer: 1) whether disturbed populations show a signal of reduced genetic diversity, and 2) if connectivity may have played a role in the recovery of the populations. In the case of *E. singularis* in the Gulf of Lions, I aim to answer the following questions: 1) does *E. singularis* have dispersal capacity?, 2) is there genetic differentiation between the populations inside and outside the Gulf of Lions?, and 3) can the pattern of genetic population structure be explained by the regional hydrodynamics?.

In **Chapter 3**, I attempt to identify the underlying processes driving the observed patterns of genetic connectivity for *E. singularis* in the Gulf of Lions by comparing the observations to the modelled patterns of connectivity resulting from the coupled seascape genetic model presented in Chapter 1. The main question rose for this analysis was: can larval dispersal alone drive the patterns of genetic connectivity of *E. singularis* in the Gulf of Lions?.





References

- Agardy, M. 1994. Advances in marine conservation: the role of marine protected areas. *Trends in Ecology & Evolution*, 9: 267–270.
- Almany, G., Berumen, M., Thorrold, S., Planes, S., and Jones, G. 2007. Local replenishment of coral reef fish populations in a marine reserve. *Science*, 316: 742–744.
- Babcock, R., Shears, N., Alcala, A., Barrett, N., Edgar, G., Lafferty, K., McClanahan, T., *et al.* 2010. Decadal trends in marine reserves reveal differential rates of change in direct and indirect effects. *Proceedings of the National Academy of Sciences*, 107: 18256–18261.
- Banks, S., Cary, G., Smith, A., Davies, I., Driscoll, D., Gill, A., Lindenmayer, D., *et al.* 2013. How does ecological disturbance influence genetic diversity? *Trends in Ecology & Evolution*, 28: 670–679.
- Botsford, L., White, J., Coffroth, M., Paris, C., Planes, S., Shearer, T., Thorrold, S., *et al.* 2009. Connectivity and resilience of coral reef metapopulations in marine protected areas: matching empirical efforts to predictive needs. *Coral Reefs*, 28: 327–337.
- Burgess, S., Nickols, K., Griesemer, C., Barnett, L., Dedrick, A., Satterthwaite, E., Yamane, L., *et al.* 2014. Beyond connectivity: how empirical methods can quantify population persistence to improve marine protected-area design. *Ecological Applications*, 24: 257–270.
- Butler, M. J., Paris, C., Goldstien, J., Matsuda, H., and Cowen, R. 2011. Behavior constrains the dispersal of long-lived spiny lobster larvae. *Marine Ecology Progress Series*, 422: 223–237.
- Caley, M., Carr, M., Hixon, M., Hughes, T., Jones, G., and Menge, B. 1996. Recruitment and the local dynamics of open marine populations. *Annual Review of Ecology and Systematics*, 27: 477–500.
- Campana, S. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series*, 188: 263–297.





- Chesson, P. 1998. Recruitment limitation: a theoretical perspective. *Australian Journal of Ecology*, 23: 234–240.
- Cowen, R. 2000. Connectivity of marine populations: open or closed? *Science*, 287: 857–859.
- Cowen, R., and Sponaugle, S. 2009. Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, 1: 443–466.
- Damerau, M., Matschiner, M., Salzburger, W., and Hanel, R. 2013. Population divergences despite long pelagic larval stages: lessons from crocodile icefishes (Channichthyidae). *Molecular Ecology*, 23: 284–299.
- Edgar, G., Stuart-Smith, R., Willis, T., Kininmonth, S., Baker, S., Banks, S., Barrett, N., *et al.* 2014. Global conservation outcomes depend on marine protected areas with five key features. *Nature*, 506: 216–220.
- Epperson, B., McRae, B., Scribner, K., Cushman, S., Rosenberg, M., Fortin, M., James, P., *et al.* 2010. Utility of computer simulations in landscape genetics. *Molecular Ecology*, 19: 3549–3564.
- Foster, N., Paris, C., Kool, J., Baums, I., Stevens, J., Sanchez, J., Bastidas, C., *et al.* 2012. Connectivity of Caribbean coral populations: complementary insights from empirical and modelled gene flow. *Molecular Ecology*, 21: 1143–1157.
- Galindo, H., Olson, D., and Palumbi, S. 2006. Seascape genetics: A coupled oceanographic-genetic model predicts population structure of Caribbean corals. *Current Biology*, 16: 1622–1626.
- Gilmour, J., Smith, L., Heyward, A. J., Baird, A. H., and Pratchett, M. S. 2013. Recovery of an isolated coral reef system following severe disturbance. *Science*, 340: 69–71.
- Greenbaum, G., Templeton, A., Zarmi, Y., and Bar-David, S. 2014. Allelic richness following population founding events – A stochastic modeling framework incorporating gene flow and genetic drift. *PLoS ONE*, 9: e115203.
- Guizien, K., Belharet, M., Moritz, C., and Guarini, J. M. 2014. Vulnerability of marine benthic metapopulations: implications of spatially structured connectivity for





conservation practice in the Gulf of Lions (NW Mediterranean Sea). *Diversity and Distributions*, 1–11.

Guizien, K., Brochier, T., Duchêne, J. C., Koh, B. S., and Marsaleix, P. 2006. Dispersal of *Owenia fusiformis* larvae by wind-driven currents: turbulence, swimming behaviour and mortality in a three-dimensional stochastic model. *Marine Ecology Progress Series*, 311: 47–66.

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., Bruno, J. F., *et al.* 2008. A global map of human impact on marine ecosystems. *Science*, 319: 948–952.

Hanski, I. 1999. Habitat connectivity, habitat continuity, and metapopulations in dynamics landscapes. *Oikos*, 87: 209–219.

Harrison, S., and Taylor, A. 1997. Empirical evidence for metapopulation dynamics: a critical review. In: *Metapopulation Dynamics: ecology, genetics and evolution* (Eds. Hanski, I and Gilpin, A.) Academic Press, San Diego, CA. 27-42.

Hastings, A., and Botsford, L. 2006. Persistence of spatial populations depends on returning home. *Proceedings of the National Academy of Sciences*, 103: 6067–6072.

Hastings, A., and Harrison, S. 1994. Metapopulation dynamics and genetics. *Annual Review of Ecology, Evolution, and Systematics*, 25: 167–188.

Hedgecock, D., Barber, P., and Edmands, S. 2007. Genetic approaches to measuring connectivity. *Oceanologica Acta*, 20: 1–10.

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

Kool, J., Moilanen, A., and Treml, E. 2013. Population connectivity: recent advances and new perspectives. *Landscape Ecology*, 28: 165–185.

Kritzer, J., and Sale, P. 2004. Metapopulation ecology in the sea: from Levin's model to marine ecology and fisheries science. *Fish and Fisheries*, 5: 131–140.

Lagman, M., Crochelet, E., Andrello, M., Schill, S., Arnaud-Haond, S., Alloncle, N., and Ponge, B. 2014. Connecting MPAs - eight challenges for science and management.





- Aquatic Conservation: Marine and Freshwater Ecosystems, 24: 94–110.
- Levin, L. 2006. Recent progress in understanding larval dispersal: new directions and digressions. *Integrative and Comparative Biology*, 46: 282–297.
- Lowe, W., and Allendorf, F. 2010. What can genetics tell us about population connectivity? *Molecular Ecology*, 19: 3038–3051.
- Lubchenco, J., Palumbi, S., Gaines, S., and Andelman, S. 2003. Plugging a hole in the ocean: the emerging science of marine reserves. *Ecological Applications*, 13: S3–S7.
- Manel, S., Gaggiotti, O., and Waples, R. 2005. Assignment methods: matching biological questions with appropriate techniques. *Trends in Ecology & Evolution*, 20: 136–142.
- Paris, C., Cherubin, L., and Cowen, R. 2007. Surfing, spinning, or diving from reef to reef: effects on population connectivity. *Marine Ecology Progress Series*, 347: 285–300.
- Pineda, J. 2000. Linking larval settlement to larval transport: assumptions, potentials and pitfalls. *Oceanography of the Eastern Pacific*, I: 84–105.
- Pineda, J., Hare, J., and Sponaugle, S. 2007. Larval transport and dispersal in the coastal ocean and consequences for population connectivity. *Oceanologica Acta*, 20: 22–39.
- Planes, S., Jones, G., and Thorrold, S. 2009. Larval dispersal connects fish populations in a network of marine protected areas. *Proceedings of the National Academy of Sciences*, 106: 5693–5697.
- Pringle, C. 2003. What is hydrologic connectivity and why is it ecologically important? *Hydrological Processes*, 17: 2685–2689.
- Roughgarden, J. 1979. *Theory of population genetics and evolutionary ecology: an introduction*. Macmillan, New York. 634 pp.
- Sale, P., Hanski, I., and Kritzer, J. 2006. Chapter 1 - The merging of metapopulation theory and marine ecology: establishing the historical context. In: *Marine Metapopulations* (Ed. Sale, P.) Academic Press, Burlington. 3–28.
- Sale, P., Van Lavieren, H., Lagman, M., Atema, J., Butler, M., Fauvelot, C., Hogan, J., *et al.* 2010. Preserving reef connectivity: A handbook for marine protected area





managers. Connectivity Working Group, Coral reef targeted research and capacity building for management program, ONU-INWEH. 80 pp.

- Shanks, A., Grantham, B., and Carr, M. 2003. Propagule dispersal distance and the size and spacing of marine reserves. *Ecological Applications*, 13: 159–169.
- Siegel, D., Kinlan, B., Gaylord, B., and Gaines, S. 2003. Lagrangian descriptions of marine larval dispersion. *Marine Ecology Progress Series*, 260: 83–96.
- Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science*, 236: 787–792.
- Sponaugle, S., Cowen, R., Shanks, A., Morgan, S., Leis, J., Pineda, J., Boehlert, G., *et al.* 2002. Predicting self-recruitment in marine populations: biophysical correlates and mechanisms. *Bulletin of Marine Science*, 70: 341–375.
- Thomas, L., and Bell, J. J. 2013. Testing the consistency of connectivity patterns for a widely dispersing marine species. *Heredity*, 111: 345–354.
- Thorrold, S., Jones, G., Hellberg, M., Burton, R., Swearer, S., Neigel, J., Morgan, S., *et al.* 2002. Quantifying larval retention and connectivity in marine populations with artificial and natural markers. *Bulletin of Marine Science*, 70: 291–308.
- Thorrold, S., Jones, G., Planes, S., and Hare, J. 2006. Transgenerational marking of embryonic otoliths in marine fishes using barium stable isotopes. *Canadian Journal of Fisheries and Aquatic Sciences*, 63: 1193–1197.
- Werner, F., Cowen, R., and Paris, C. 2007. Coupled biological and physical models. *Oceanologica Acta*, 20: 1–16.
- Wilson, G., and Rannala, B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, 163: 1177–1191.
- Wright, S. 1931. Evolution in Mendelian populations. *Genetics*, 16: 97–159.



CHAPTER 1

Modelling the effect of demographic traits and connectivity
on the genetic structuration of marine metapopulations of
sedentary benthic invertebrates





Mariana PADRON^{1*} and Katell GUIZIEN¹

¹ Sorbonne Universités, UPMC Univ Paris 06, CNRS, Laboratoire d'Ecogéochimie des Environnements Benthiques (LECOB), Observatoire Océanologique, 66650, Banyuls sur Mer, France.

N.B. This manuscript is now published in the ICES Journal of Marine Science.

1.1 Abstract

The genetic structure of populations is commonly used to infer connectivity among distant populations. Accounting for connectivity is essential in marine spatial planning and the proper design and management of Marine Protected Areas (MPAs), given that their effectiveness depends on the patterns of dispersal and colonisation between protected and non-protected areas. Here, we present a spatially explicit coupled metapopulation gene flow model that simulates the effect of demographic fluctuations on the allele frequencies of a set of populations. We show that in closed populations, regardless of population growth rate, the maintenance of genetic diversity at the initial saturating population density increases with species life expectancy as a result of density-dependent recruitment control. Correlatively, at low initial population density, the time at which a population begins to lose its genetic diversity is driven by recruitment success – the larger the recruitment success, the quicker the genetic drift. Different spatial structures of connectivity established for soft bottom benthic invertebrates in the Gulf of Lions (NW Mediterranean, France) lead to very different results in the spatial patterns of genetic structuration of the metapopulation, with quick genetic drift in sites where the local retention rate was larger than 2%. The effect of recruitment failure and the loss of key source populations on heterozygosity confirm that transient demographic fluctuations have a major effect on the maintenance of genetic diversity in a metapopulation. The loss of a population with high betweenness values could be crucial in genetic differentiation and metapopulation subdivision, even more than the loss of source sites identified as key contributors for metapopulation persistence. The model provides a tool for evaluating different scenarios of conservation





spatial planning and the need for finding a compromise between species demographic persistence requirements and the conservation of genetic diversity, pleading for a MPAs network as opposed to isolated MPAs.

1.2 Introduction

Connectivity strongly impacts the dynamics and persistence of marine populations because it influences key processes, from short-term demography to long-term evolution, including resistance to disturbances and environmental changes. Therefore, understanding the development and maintenance of connectivity patterns is essential in marine spatial planning and the proper design and management of Marine Protected Areas (MPAs) (Palumbi 2004; Botsford *et al.*, 2009). However, the quantitative study of connectivity and the identification of connectivity bottlenecks that could compromise the ability for populations to recover after disturbances are current scientific challenges (Pineda 2000; Burgess *et al.*, 2014).

For sedentary marine benthic species, understanding connectivity translates into understanding larval dispersal, given that it is during the pelagic larval stage that the exchange of individuals and genes among populations takes place (Pineda *et al.*, 2007; Cowen and Sponaugle 2009). Hence, to analyse the patterns of connectivity among populations and study, in more detail, the mechanisms that shape and maintain connectivity patterns through time, the dispersal of organisms should be taken into account (Hastings and Botsford 2006; Aiken and Navarrete 2011).

Different approaches have been undertaken to try to quantify dispersal and connectivity in marine ecosystems. Population genetics has been one of the most frequently used tools to provide direct and indirect measures of connectivity in several marine species (Hellberg *et al.*, 2002; Saenz-Agudelo *et al.*, 2009; Lowe and Allendorf 2010). The dispersal potential of the larvae produced by sedentary benthic species has usually correlated with gene flow and was even used as a predictor of population genetic structure (Siegel *et al.*, 2003).

Using estimates of Pelagic Larval Duration (PLD) as a proxy to measure realised





dispersal distance, several studies showed that the correlation between PLD and genetic Isolation by Distance (IBD) was consistent with the expectation that migration was the key factor driving the genetic structuration of marine populations (Hellberg 1996; Gilg and Hilbish 2003). However, recent data set reviews (Riginos *et al.*, 2011; Selkoe and Toonen 2011; Faurby and Barber 2012) have now shown that the relationship between PLD and genetic structuration might be weaker than previously thought.

The influence of several processes (e.g. larval behaviour, availability of suitable habitat, and biotic interactions) can cause the patterns of population connectivity to be different from what would be expected from flow integration along PLD alone (Pineda 2000; Guizien *et al.*, 2006). Among these processes, settlement or recruitment regulation of dispersal alters gene flow (Pineda *et al.*, 2007), in addition to the local demography. This temporal and spatial variability in the strength of gene and demographic flow due to bottlenecks during larval settlement and recruitment implies that there is a set of interacting processes operating across various scales, which introduces complexity into the system. Therefore, inferring connectivity by only one disciplinary approach (either genetic or bio-physic) does not provide the necessary information to properly describe population persistence in a network of MPAs (Hastings and Harrison 1994; Marko and Hart 2011).

It is therefore necessary, in order to efficiently inform conservation management decisions, to use tools that integrate bio-physical models with information regarding local demographic traits driving genetic linkages that result from larval exchange among populations (Werner *et al.*, 2007; Cowen and Sponaugle 2009). However, detecting the genetic signatures caused by the transient dynamics of demographic changes remains a challenge (Alcala *et al.*, 2013).

Although genetic models incorporating larval dispersal and demographic parameters have recently been developed to quantify connectivity among marine populations (Galindo *et al.*, 2006; Kool *et al.*, 2009; Munroe *et al.*, 2012), they have some limitations when attempting to understand connectivity patterns among non-





equilibrium populations. The model Galindo *et al.* (2006) developed, for example, does not take into account demographic variability, as population sizes were kept constant over time. The approach Kool (2009) developed, although allowing changes in population size, does not account for overlapping generations but, more importantly, being an Individual-Based Model (IBM), population sizes were limited to 10 individuals. Recently, Munroe *et al.* (2012), presented a modelling case study for Eastern oysters in Delaware Bay (USA) in which they relaxed population size limitations and assigned each population its own demographic dynamics.

In an attempt to generalise Munroe *et al.*'s (2012) approach, we present a spatially explicit coupled demographic gene flow model that assesses the effect of demography variability on allele frequencies in a marine metapopulation of sedentary benthic species with realistic population densities. The seascape model was used to examine how: 1) demographic traits influence the development of genetic structure in closed populations over time, 2) different patterns of connectivity affect the loss or maintenance of genetic diversity in a metapopulation, 3) fluctuations in local population density help maintain genetic diversity over time and 4) local population stability impacts genetic diversity to provide relevant information for conservation decisions in the Gulf of Lions, France.

1.3 Methods

1.3.1 *A seascape genetic model for benthic invertebrates with a pelagic larval phase*

To simulate the transient demographic effect on local allele frequencies arising from environmental variability modulating connectivity through time, a seascape genetic model was developed. The seascape model relies on the forcing of a spatially explicit dynamical model of allele probability by a spatially explicit metapopulation model (Guizien *et al.*, 2014, adapted from Hastings and Botsford 2006) (Fig. 1.1).



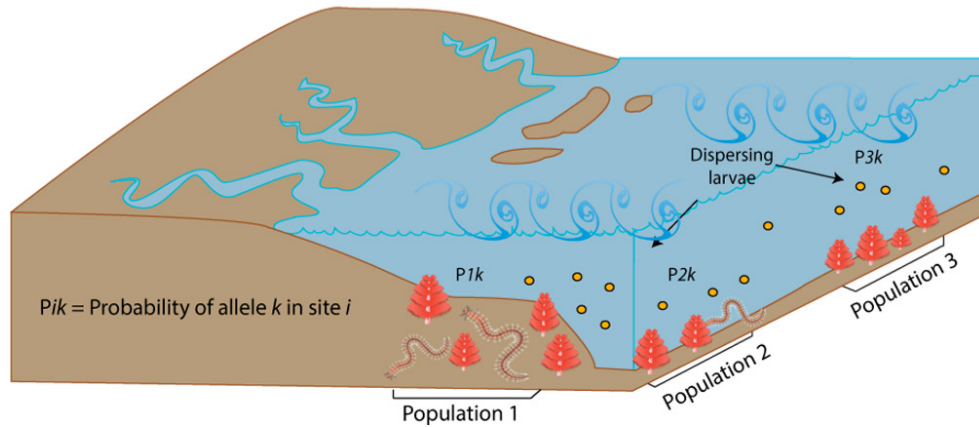


Figure 1.1.- Schematic representation of seascape genetic model. The frequency of each allele k is calculated at any given time in every site i , as a result of mixing between the surviving individuals with allele k in site i and to the input of new recruits with allele k , coming from any other site.

The combination of these models allows us to project the development of a population genetic structure through time, resulting from demography dynamics driven by local survival and larval transfer on a regional scale.

The spatially explicit metapopulation model simulates the population density $N_i(t)$ at each site i at any time t resulting from the balance of adult survival and input of new recruits resulting from reproduction of adults present in the previous time step. Therefore, N_i represents the adult census size per unit surface, and it is equal to the effective number of breeders per unit surface for the next reproductive event (as defined by Waples *et al.*, 2013). It is important to note that such formulation implicitly hypothesized that age at first reproduction and the time lapse between reproductive events are both equal to the model time step, ensuring that effective number of breeders does not vary with population age structure. Larval transfer rate among sites is estimated from a set of realistic connectivity matrices obtained using the larval dispersal model (Guizien *et al.*, 2012), and recruitment success (inter-specific competition or predation) is limited by saturating population density (intra-specific competition) (Eq. 1 and 2) (Guizien *et al.*, 2014).





$$N_i(t + 1) = \min(G(t)N_i(t), N_{max}) \quad (1)$$

$N_i(t + 1)$ = Vector of spatial density of individuals at each site at time $t+1$ [individual per m²]

$N_i(t)$ = Vector of spatial density of individuals at each site at time t [individual per m²]

N_{max} = Saturating population density based on space limitation [individual per m²]

with the transfer matrix $G(t)$ defined by:

$$G_{ji}(t) = a_i C_{ji}(t) b_j + s_{jj} \delta_{ji} \quad (2)$$

a_i = Propagule production rate at site i [larvae per adult]

$C_{ji}(t)$ = Propagule transfer rate from site i to site j multiplied by the ratio of surface of site i to surface of site j [no units]

b_j = Recruitment success at site j [adults per larva]

s_{jj} = Adult survivorship rate at site j [no units]

δ_{ji} = Kronecker symbol (1 when $i=j$, 0 otherwise)

Maximum population growth rate (P_i) of a closed population in site i is then defined by:

$$P_i = a_i b_i + s_{ii} \quad (3)$$

The time it takes for a population at any initial density N_o to reach its saturating density N_{max} , from any given initial density was estimated as:

$$T = \frac{\ln\left(\frac{N_{max}}{N_o}\right)}{\ln(P_i)} \quad (4)$$





In the seascape genetics model, the frequency of each allele k is calculated at any given time in every site i , as a result of mixing between the surviving individuals with allele k in site i and the input of new recruits with allele k , coming from any other site (Eq. 5). Given the large number of larvae produced by each adult and the high recruitment success (i.e. larval and juveniles survival) values assigned (10%) compared to estimated regional retention rates (3%), genetic drift due to larval and juveniles mortality is neglected and, thus, a proportional and uniform loss was applied to the larval pool with allele k produced at each site embedded in the recruitment success parameter. The effect of genetic drift is therefore simulated by the random sampling of the alleles lost during two different periods in the life cycle: larval dispersal and settlement. Simplifying assumptions were that: 1) mating takes place randomly every year among diploid organisms, 2) allele frequencies are identical among sexes, 3) generations are discrete but overlapping, 4) populations are not age-structured, 5) given the relatively short period of time considered in the simulations (100 years), mutation is assumed to be negligible compared to genetic drift and migration, and thus set to zero and, 6) selection can be neglected.

During larval dispersal, the pool of larvae with allele k produced by each site, remaining in the region (accounting for regional retention rate), is randomly sampled before being re-distributed among the destination sites according to the connectivity matrix (Eq. 6 and 7). The number of larvae with allele k that will survive dispersal is computed by sorting out the rank of the allele carried by the surviving larvae. During settlement, saturating density limits the number of arriving larvae that can actually settle at each site, therefore, a random subset of alleles carried by the pool of larvae arriving at each site actually settle.

$$p_{ik(t+1)} = \frac{(s_{ii}N_{i(t)}p_{ik(t)} + b_j \sum_j \frac{c_{ij} s_{ij} n'_{jk}}{\sum_l c_{lj} s_{lj}})^i}{N_{i(t+1)}} \quad (5)$$





where n'_{jk} is a random number between 0 and n_{jk} , with the constraint that $\sum_k n'_{jk} = n'_j$

$$n'_j{}^i = A_j a_j \left(\sum_l C_{lj} \frac{S_l}{S_j} \right) \quad (6)$$

$$n_{jk} = p_{jk} A_j a_j \quad (7)$$

$p_{ik(t+1)}$ = Frequency of allele k at site i at time $t+1$ [no units]

$p_{ik(t)}$ = Frequency of allele k at site i at time t [no units]

$p_{jk}(t)$ = Frequency of allele k at site j at time t [no units]

S_i = Surface area of site i [m²]

A_j = Density of individuals in site j [individual per m²]

$n'_{jk}{}^i$ = Number of larvae produced per unit surface in site j having allele k that remain in the region and will survive until sexual maturity in site i

$n'_j{}^i$ = Number of larvae produced per unit surface in site j that remain in the region and survive until sexual maturity in site i

n_{jk} = Number of larvae with allele k produced in site j per unit surface

1.3.2 Test cases

The simulations were designed to evaluate the changes in allele frequencies due to variations in species demographic parameters and connectivity structure. Although this modelling approach applies to any sedentary species with a reproductive dispersive stage, to test the sensitivity of the model, we focused on marine benthic species with four different life expectancies (2, 5, 10 and 20 years) that reproduce each year. The values of recruitment success were varied in order to test the effect of maximum population growth in the maintenance of genetic diversity over time. Recruitment success was also the same in all populations (10%), although it was limited by saturating spatial density, which was set to $N_{max}=6250$ individuals/m² per population.





Six groups of simulations were performed (Table 1.1). All simulations were run for 100 iterations, one iteration being the time lapse between two reproductive events. The initial genetic structure was described by ten different alleles present simultaneously in every site. In order to examine the variability due to specific initial conditions of genetic structure, simulations were repeated at least 100 times with different and random initial conditions of allele frequencies each time.

The first group consisted of simulations of a closed population (no larval inputs) in which life expectancy was raised from two to 20 years. Groups 2, 3A, 3B, 4A and 4B, consisted of simulations of a metapopulation of a species with a life expectancy of 10 years, distributed among 32 sites along the Gulf of Lions (northwestern Mediterranean) (Figure S1), in which spatial structure was driven by connectivity only. These groups of simulations correspond to the test case of dominating species of the soft-bottom benthic communities in the Gulf of Lions (polychaetes). The continuous sandy-bottom habitat of these polychaetes, which is delimited by the 10 and the 30 meters isobaths, was described by sites of approximately the same width (~ 20 km) contiguously distributed along the coast. Based on these criteria, the areas of the 32 sites ranged from 6.2 to 121 km². Survival and fecundity rates were the same among populations within the metapopulation and were kept constant over time in all of the simulations.

Larval transfer between the 32 sites was computed from Lagrangian larval dispersal simulations based on regional hydrodynamic simulations performed with the coastal circulation model SYMPHONIE (Marsaleix *et al.*, 2008). Larval dispersal was simulated by releasing larvae at the average site depth (20 m). Connectivity matrices containing larval transfer probabilities were produced for 20 sets of 10-day long spawning periods in 2004 and 2006 with an average PLD of four weeks (Guizien *et al.*, 2014). A total of 20 different connectivity matrices were used for this analysis.



Table 1.1.- Settings of six simulation groups. Connectivity, when present, was set either deterministically (D) using the same matrix every year (either one of the 20 variants) or stochastically (S) picking a different matrix among the 20 variants every year. Disturbance scenarios were only applied to the groups with stochastic connectivity.

	Group 1	Group 2	Group 3A	Group 3B	Group 4A	Group 4B
Connectivity	NO	D	S	S	S	S
Initial population density (Individuals/m²)	50, 1000, Nmax	50	50	50	50	50
Fecundity	1000	2000	2000	2000	2000	2000
Life expectancy	2,5,10 and 20 years	5 years	10 years	10 years	10 years	10 years
Number of simulations	100	100	500	500	500	500
Disturbance	NO	NO	NO	Recruitment failure	Habitat loss (source sites)	Habitat loss (betweenness)



Simulations of Group 2 were performed to evaluate the effect of the distinct connectivity structure on heterozygosity. In this case, connectivity was set deterministically using the same connectivity matrix every year (either one of the 20 variants) and, thus, simulations were run 100 times.

Groups 3A, 3B, 4A and 4B were performed in order to test the effect of recruitment (3A and 3B) and habitat loss (4A and 4B) on metapopulation size and the maintenance of heterozygosity within a metapopulation over time. Metapopulation size was represented as average regional coverage, defined as the ratio between regional average population density and saturating density. Connectivity was set stochastically using a different connectivity matrix among the 20 variants every year and, thus, simulations were run 500 times to reach the convergence of the mean when combining stochasticity in connectivity and initial condition. Group 3A simulated gene flow among all populations, and recruitment success was constant every year. Group 3B explored a scenario where recruitment failure (no recruitment) occurred every three years. Group 4A simulated the loss of four populations (sites 17, 18, 19 and 20). These populations were identified as being essential for the regional persistence of species at minimum recruitment success in metapopulation modelling, comparing the vulnerability to habitat loss of four ports in the Gulf of Lions (Guizien *et al.*, 2014). Nonetheless, it is important to mention that for the present simulations, the values of minimum recruitment success and fecundity were set to ensure long-term persistence of the metapopulation after removing those four sites, unlike in Guizien *et al.* (2014). Group 4B evaluated a scenario where only one population (site 21) was lost from the metapopulation. This site was characterised by consistently showing the highest values of betweenness in the metapopulation when applying graph theory analysis to connectivity matrices (Costa, *pers. comm.*).

1.3.3 Metrics and statistics

Heterozygosity and allelic richness are two measures of genetic diversity commonly used in the population genetics literature, and in the present study both were





used. Heterozygosity (He_i), was estimated taking into account all individuals at each site i over time (Eq. 8).

$$He_i(t) = 1 - \sum_{k=1}^n (p_{ik}(t))^2 \quad (8)$$

where $p_{ik}(t)$ = frequency of the k th allele of n alleles in site i at time t [no units].

However, heterozygosity and allelic richness describe the state of genetic diversity of a population at a particular time and not its dynamics. In order to measure the speed at which genetic diversity varied as a function of demographic parameters (regulating genetic drift) and connectivity structure, new metrics describing the initial and average slope of the evolution of genetic diversity over time are introduced: 1) Allele Drifting Time (T_d), which is the moment at which allelic diversity starts decreasing, meaning the time when the first allele is lost from the population or the metapopulation. 2) Allele Fixation Time (T_f), which is the time lapse between the first loss of an allele (T_d) and the fixation of any allele (Figure S2).

These metrics were defined on allelic richness rather than heterozygosity to avoid ambiguity in defining genetic diversity reference points and given the known sensitivity of allelic richness to the loss of rare alleles (Allendorf 1986).

1.4 Results

1.4.1 Do differences in demographic parameters affect genetic diversity in closed populations?

Figures 1.2A and 1.2B display the loss of genetic diversity in closed populations at saturating initial density as a function of the ratio between mortality (being complementary to unity of survival) and recruitment success. This ratio, having the dimensions of larvae/adult/year, depicts the origin of recruitment regulation (*sensu* by Hixon *et al.*, 2002) and informs on the relative contribution of intrinsic vs. extrinsic drivers: high values indicate recruitment regulation due to low recruitment success





driven by inter-specific competition or predation, while low ratio values indicate recruitment regulation due to space limitation at a high population density (intra-specific competition). It should be stressed here that, in closed populations, low recruitment success depicts not only limitation in larval and juveniles survival but it also includes limitation in the proportion of settlers by local retention rate. Therefore, the acceleration of the loss of genetic diversity (measured as Td) shown when recruitment success increases in these simulations, is also expected when local retention rate increases.

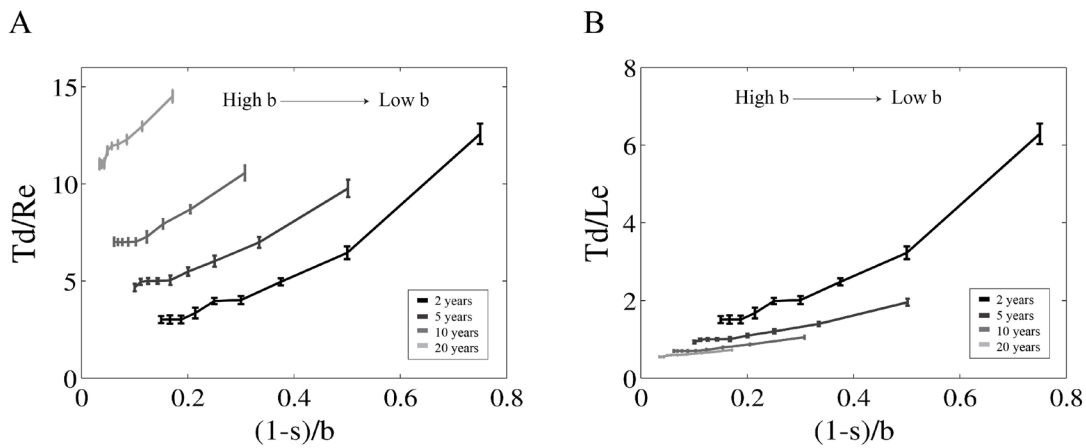


Figure 1.2.- Effect of different life expectancies on the loss of genetic diversity, measure as allele drifting time (Td), for an isolated population at initial saturating capacity (6250 individuals/m²). Adimensionalizing allele drifting time by the number of reproductive events (Td/Re) (A) represents the differences in the number of generations it takes for a species with different life expectancies to start losing its genetic diversity as a function of the ratio between mortality and recruitment success. In contrast, adimensionalizing allele drifting time by the life expectancy (Td/Le) of a species (B) provides a different vision corresponding to population turnover.

Fig. 1.2A shows that the number of reproductive events after which the population begins to lose its genetic diversity (Td/Re) increases with the ratio between mortality and recruitment success. This demonstrates a reduction of genetic drift when recruitment success decreases for any given life expectancy. However, genetic drift is





inhibited at high levels of recruitment success, as depicted by low and almost constant T_d/Re for the lowest values of the ratio between mortality and recruitment success. Such minimum T_d/Re is of the order of magnitude of the number of reproductive events in a life expectancy. For a recruitment frequency of 0.18, short-lived species (two years) could start losing their genetic diversity after three years, while it would take around 14 years for a long-lived species (20 years). This reflects the effect of genetic drift inhibition by the reduction of reproductive events due to the lack of space caused by the presence of adults as life expectancy increases.

Similarly, the maintenance of genetic diversity, measured by the number of reproductive events before allele fixation (T_f/Re), in closed populations at saturating initial density increases with life expectancy, regardless of whether the population growth rate is low (<2.5) or high (>2.5) (Fig. 1.3). This confirms that genetic drift is limited by population inertia due to recruitment inhibition as a result of space limitation, as neither fecundity nor recruitment success affects the relationship.

However, when dealing with overlapping generations, it must be taken into account that the reduction of genetic drift by space limitation during the first population turnover decreases with species longevity. This is exhibited by the different hierarchies between life expectancy curves when representing the time at which the population starts to lose its genetic diversity in terms of population turnover (adimensionalising T_d by the life expectancy of the species) (Fig. 1.2B). Species with short life expectancies begin to experience a reduction of their genetic diversity after a higher number of population turnovers than do long-lived species, as fewer reproductive events occur during a short life expectancy compared to during a long one. This result confirms that genetic drift can be controlled by effective reproduction (which may be limited by long life expectancy or recruitment success intensity) and its frequency along the species lifespan in a context of finite population size.



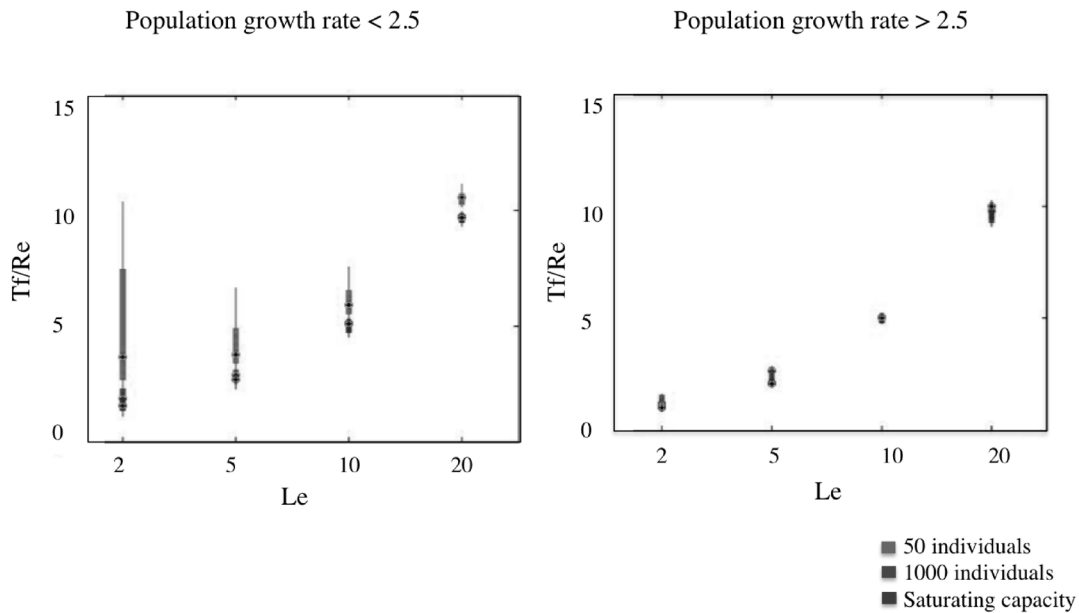


Figure 1.3.- Effect of population growth rate over allele fixation time (T_f) for three different initial conditions of population abundance (50, 1000 and 6250 individuals per m^2) as a function of species life expectancy (L_e) in a closed population.

Different initial population densities also affect the time at which a population starts losing genetic diversity (Fig. 1.4A) – for any value of the ratio between mortality and recruitment success, the lower the initial density of the population, the slower the loss of genetic diversity. This result points out that genetic drift is slower when considerable space is available for recruitment compared to at saturating density where space availability is regulated by mortality.

Quantifying space availability by the time it will take a population to reach saturating density (6250 individuals/ m^2) shows that it would take twice as long for a population with an initial density of 50 individuals/ m^2 compared to a population with an initial density of 1000 individuals/ m^2 (Fig. 1.4B). Allele drifting time increases linearly with the time necessary to reach saturating density.



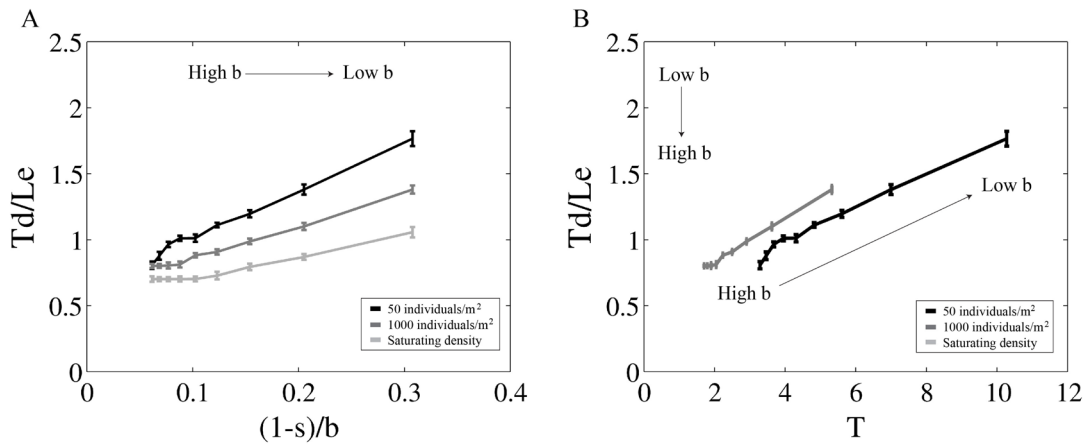


Figure 1.4.- Effect of different initial population abundances (50, 1000 and 6250 individuals per m^2) on the loss of genetic diversity, measured as allele drifting time (Td), as a function of A) the ratio between mortality and recruitment success ($(1-s)/b$), and B) the time to reach saturating capacity (T). Td is adimensionalized by the life expectancy (Td/Le) of a species that lives 10 years, thus representing the number of population turnovers to start losing genetic diversity in an isolated population.

The population growth rate triggers the effect of initial population density when it comes to the maintenance of genetic diversity, measured as Tf (Fig. 1.3). While in fast growing populations, the maintenance of genetic diversity is not sensitive to initial density, in slow growing populations, the maintenance of genetic diversity lasts longer and is more variable when the initial population size is small (50 individuals/ m^2) compared to when it is at saturating density. Such sensitivity to initial population size is amplified at small life expectancies.

Overall, in closed populations, genetic drift is the quickest when the population is at saturating density with high recruitment success, slowing down at low recruitment success or when population size is lower than saturating density. This result demonstrates the importance of transient demography in regulating the speed of genetic drift.

1.4.2 Do different patterns of connectivity affect the maintenance of genetic diversity in a metapopulation?





Simulated heterozygosity patterns among populations forming a metapopulation varied according to each connectivity matrix. The two most contrasting spatial patterns with low heterozygosity values either in the west (A) or in the east (B) of the Gulf of Lions are shown in Fig. 1.5. A plausible explanation is the different spatial patterns of local retention (defined as the proportion of larvae produced locally that remain in the same spatial unit) vs. import (estimated as the proportion of all larvae produced among all sites that settled within the focal site). For connectivity matrix 1, the ratio between local retention and import was higher in the west than in the east (0.074 and 0.027, respectively). On the contrary, for connectivity matrix 2 the values of the same ratio were higher in the east compared to the west (0.069 and 0.0085, respectively). However, simulations demonstrated that the relationship between T_d and import do not exhibit any trend with no linear correlation ($R^2 = 0.011$; $P < 0.01$) (Figure S3).

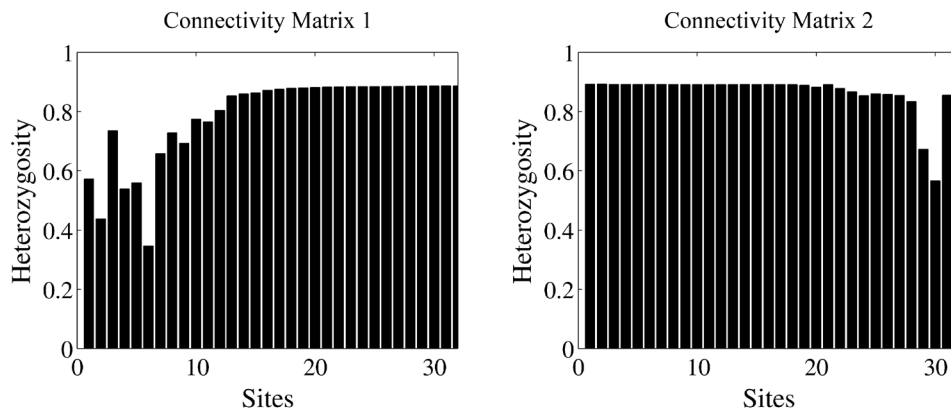


Figure 1.5.- Spatial pattern of genetic diversity caused by distinct connectivity structures. Variability of heterozygosity for 32 sites forming a metapopulation in the case of a species with a life expectancy of five years.

Similarly, T_d was not linearly correlated to local retention rate values obtained from the analysis of the 20 connectivity matrices ($R^2 = 0.031$; $P > 0.05$). However, T_d was delimited by a piecewise function enabling the discrimination between two groups (Fig. 1.6) – populations with low levels of local retention ($< 2\%$) that show high variability in the time they begin to lose their genetic diversity (T_d/Le ranging from 0.2 to 50) and





populations with high local retention ($> 2\%$) where genetic drift is much quicker and less variable (Td/Le ranging from 0.2 to 12). This indicates that the speed of genetic drift is regulated locally only at a high local retention rate (larger than 2%) while, for local retention rates lower than 2%, the strength of genetic drift depends on the regional structure of connectivity. According to the direction of the transfer and the genetic characterisation of the contributing populations, genetic drift will then be accelerated or slowed down in the focal population.

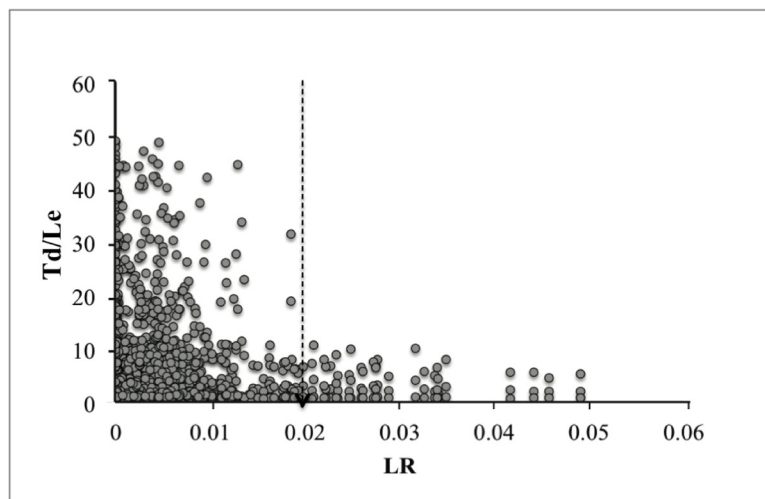


Figure 1.6.- Relationship between local retention (LR) (defined as the proportion of larvae produced in one population that remain in the same population) and the number of population turnovers to start losing genetic diversity (Td/Le), in a metapopulation over time. Values correspond to 20 connectivity matrices, and species with three different life expectancies (2, 5 and 20 years). Dashed arrow indicates the threshold at which the effect of LR on Td/Le begins to decrease.

1.4.3 How does local population stability affect heterozygosity in a metapopulation?

When looking into the variation of the average heterozygosity in the metapopulation through time for a species with a life expectancy of 10 years, it becomes clear that changes in population density have a major effect on the loss of genetic diversity in a metapopulation (Fig. 1.7). The rate at which a metapopulation reaches a





stable regional coverage influences its possibility to maintain genetic diversity over time, even in the presence of migration.

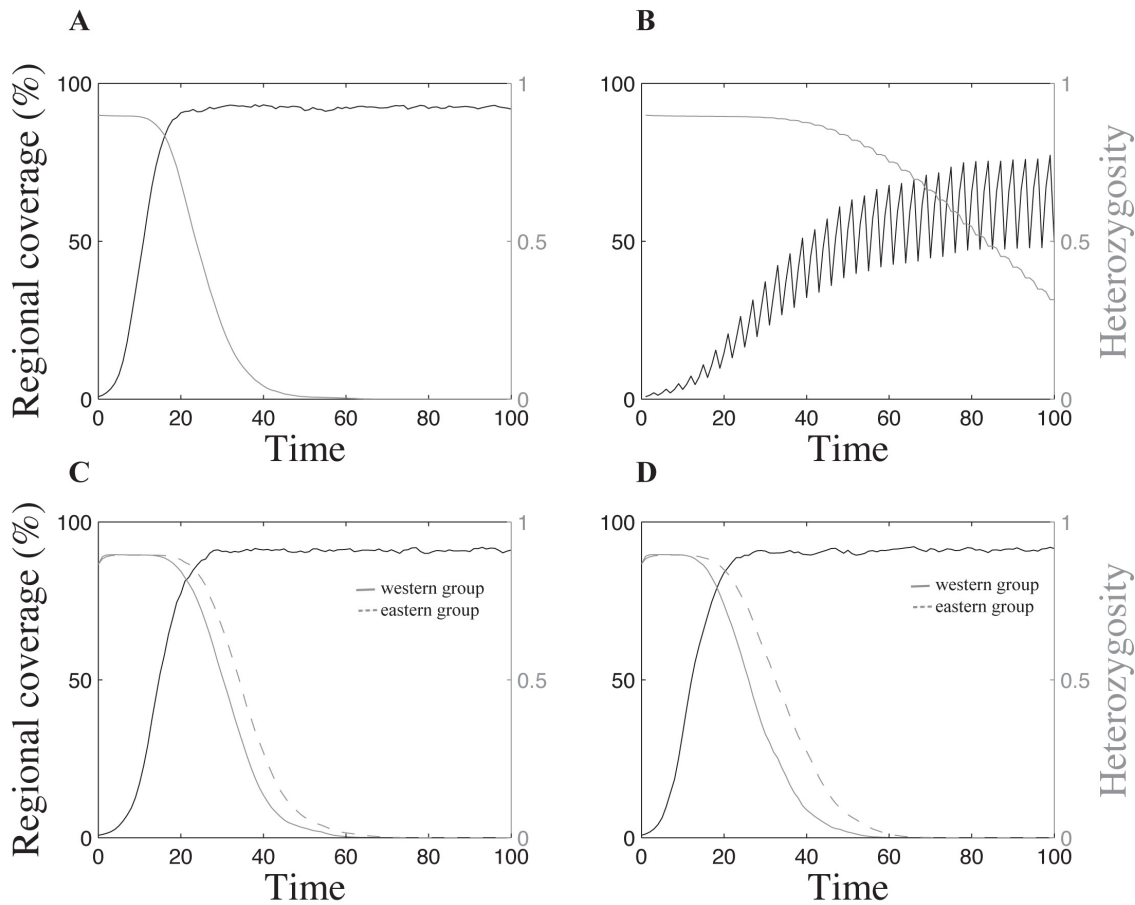


Figure 1.7.- Effect of fluctuations in local population stability on heterozygosity over time. Scenarios: A) Simulates gene flow among all of the populations forming a metapopulation with constant recruitment success every year. B) Simulates recruitment failure (no recruitment) every three years C) Simulates the loss of four source sites (sites 17, 18, 19 and 20) in the metapopulation, while keeping recruitment success constant every year. D) Simulates the loss of one key population (site 21) for maintaining genetic connectivity in the metapopulation.

Overall, we observe that as soon as regional coverage reaches 80%, heterozygosity values decrease rapidly and genetic diversity is lost. Scenario A shows how, with constant recruitment success, the effect of migration among populations





homogenises allele frequencies over time and leads to the fixation of the same allele in all populations over a relatively short period of time. Most interestingly, in Scenario B, we find that high variability in metapopulation regional coverage due to recurrent failures in recruitment actually helps maintain genetic diversity over time. Stochastic connectivity leading to demographical fluctuations in populations enables the maintenance of genetic diversity.

On the other hand, Scenario C exemplifies how, in the Gulf of Lions, removing populations (sites 17–20) responsible for bringing demographic persistence to the metapopulation at minimum recruitment success divides it into an eastern and western group, causing a slightly faster decrease of genetic diversity in the latter group than in the former. However, Scenario D shows how the loss of only one site, identified as essential for maintaining the integrity of the connectivity graph, indeed disrupts the genetic connectivity of the metapopulation by augmenting the differentiation between the eastern and western groups in terms of the loss of heterozygosity, with drifting starting earlier in the eastern part. In Scenarios C and B, recruitment success was set in order to ensure that the loss of sites within the metapopulation would still allow for species persistence. It should be noticed that in both scenarios, a similar constant metapopulation size was maintained, even if not at saturating density. The effect of migration in this case will also lead to the loss of genetic diversity in the metapopulation over time, although at a slower rate.

1.5 Discussion

The present study highlights that genetic drift increases considerably with settlement limitations by lack of space when the effective number of breeders approaches saturating capacity, causing a strong reduction of effective reproduction.

Early authors had extensively shown how changes in genetic diversity are related to effective population size (Wright 1931; Kimura 1955). Nonetheless, it has been





demonstrated theoretically that some life cycle processes, like overlapping generations (Jorde and Ryman 1995), and demographic parameters can also have an effect on the amount of temporal allele frequency change and should be taken into account (Ryman 1997). Our results highlight the role of life cycle processes, other than age at first reproduction (Lee *et al.*, 2011), in reducing effective reproduction in populations with large number of breeders and increasing the effect of genetic drift on a short evolutionary time scale. In closed populations with saturating density, actual recruitment regulates the speed of genetic drift, demonstrating the relevance of transient demography in shaping the genetic structuration of marine species. In open populations, genetic drift is slowed down as a result of larval exchange. The effect of recruitment failure and the loss of key source populations on heterozygosity confirm that transient demographic fluctuations, when remaining above the risk of regional extirpation and actually avoiding reaching saturating density, help maintain genetic diversity over time.

Recently, several meta-analyses and empirical studies evaluating the relationship between PLD and population genetic differentiation across different marine species indicated a weak correlation between the two (Selkoe and Toonen 2011; Faurby and Barber 2012). In some cases, the high uncertainty in the estimates of larval dispersal and measures of population differentiation were suggested as the main causes for the unexpected results, while other studies suggested that differential mortalities and levels of abundance among populations were more relevant (Munroe *et al.*, 2012). The results from our simulations support these findings, while further demonstrating that considering other demographic parameters (such as life expectancy and population growth rate) and the inclusion of transient dynamics caused by recruitment failure, would help explain better the variability of genetic connectivity in a metapopulation.

Dawson *et al.* (2014), found that differences in fecundity, population size and PLD could account for the differences in population genetic structure between eight co-distributed rocky intertidal invertebrate species in the eastern North Pacific. They found that population size was positively related to population genetic structure and they





attributed the variability among species to differences in recruitment and demography. Furthermore, they highlighted that the differences in migration potential found between theoretical and empirical data could be attributed to the role of genetic drift and natural selection. Here, we corroborate their hypotheses by demonstrating how the strength of genetic drift is influenced by recruitment success, local retention and fluctuations in population density due to habitat fragmentation and recruitment.

The present study exhibits an acceleration of genetic drift when the number of reproductive adults increases in a population. Although counterintuitive, such acceleration depicts the strong decrease in effective reproduction when recruitment is reduced by lack of space. However, relating genetic drift acceleration explicitly to a strong reduction in effective adult population size was not possible in the present case given that the filter controlling effective reproduction applies to the fate of the larvae and not on the adults contributing to reproduction. Yet, we demonstrate that a long life expectancy and low recruitment success regulate the temporal variation of allele frequencies and reduce the loss of genetic diversity over time. This provides an alternative explanation to empirical evidence of strong genetic structure in long-lived species, when recruitment success is likely limited by space availability (Costantini *et al.*, 2007).

Population regulation occurs when at least one demographic rate is density-dependent (Hixon *et al.*, 2002). Clear evidence of genetic drift acceleration due to recruitment regulation is observed in our simulations for closed populations at the saturating initial density. Furthermore, the evaluation of the ratio between mortality and recruitment success allowed for disentangling the relative contribution of intrinsic (intra-specific competition) vs. extrinsic drivers (inter-specific competition or predation).

In open systems, it was already suggested that the amount of local retention could have a strong influence on the relative importance of demographic vs. connectivity parameters in the dynamics of metapopulations (Figueira 2009). Our results confirm those findings and indicate that local retention rate values larger than 2%





diminish the influence of the connectivity structure over genetic drift, as genetic drift accelerates in those populations close to saturating density. This is particularly relevant for the spatial planning of MPAs. Whether an MPA is created for the conservation of biodiversity or for fishery management, its main purpose is to maintain the persistence of its populations. In the case of marine metapopulations, local retention is considered the currency of persistence: the higher the local retention, the closer the population is to its saturating capacity (Burgess *et al.*, 2014). Nonetheless, it is important to note that, as Figueira (2009) suggested, under the same flow structure, differences in PLD among species could cause distinct patterns of local retention for different species in the same place, making biodiversity conservation efforts even more difficult. In any case, the loss of genetic variability through genetic drift at a high local retention rate can diminish future adaptability to a changing environment, which might be detrimental to local conservation efforts, strongly advocating for a network of MPAs formed by sites with lower local retention rate.

The maintenance of genetic diversity in the metapopulation over at least 100 years in the case of regular recruitment failure, creating regional demographic fluctuations, was not unexpected. These results, incorporating realistic spatially explicit connectivity matrices, extend previous theoretical studies that found that demographic instability and fluctuations in migration produce major changes in the evolution of genetic variation and population differentiation (Whitlock 1992). Given that disturbances can cause variations in key demographic and biological processes, disturbance history may be one of the major drivers shaping the patterns of genetic diversity in many natural populations (Banks *et al.*, 2013). The pattern of genetic diversity maintenance over time observed in our simulations has been reported in an empirical study on natural populations of frogs, where no losses of genetic diversity after a disturbance were found when survival was high or when recovering populations recruited many individuals from multiple sources (Spear *et al.*, 2012). In addition, Larson *et al.* (1984) suggested, based on a survey of 22 species of salamanders, that historical





influences are much more important in describing genetic variation patterns than is recent migration. Exhibiting that recruitment limitation by lack of space can be a strong filter to gene flow, even when larval dispersal connects populations, the present study provides an explanation for the discrepancy between modelled and observed connectivity between coral populations in the Caribbean (Foster *et al.*, 2012).

Although demographic disturbances leading to population bottlenecks have also been shown to induce strong genetic drift, in the present study, demographic disturbances around saturating capacity can reversely reduce the effect of genetic drift. The present study, thus, highlights that demographic disturbances can have contrasting effects on genetic drift whether they affect populations with small or large size compared to their saturating capacity: 1) decelerating genetic drift when population census size is close to saturating capacity (release of recruitment limitation), or 2) accelerating genetic drift when population census size is low compared to saturating capacity (reduction of effective number of breeders).

Previous studies in the Gulf of Lions identified the spatial structuration of population vulnerability to habitat loss driven by hydrodynamical connectivity. Four sites essential for species regional persistence at minimum recruitment success were identified from metapopulation modelling around the port of Sète (sites 17–20, Guizien *et al.*, 2014). However, analysis of the same connectivity graph used in the metapopulation model depicted those four sites as high bridging centrality, spanning across the two subclusters of the region. Another site (site 21) was instead identified for maintaining network integrity, as depicted by the highest betweenness (Costa, *et al.*, *pers. comm*). Here, we show that both the loss of four essential populations and the loss of the highest betweenness site divide the metapopulation into two genetically distinct subpopulations (eastern and western). Nevertheless, the loss of only site 21 leads to a higher level of genetic differentiation in the metapopulation. This result suggests that the highest betweenness of hydrodynamical connectivity graph could be interpreted for relaying gene flow and maintaining genetic connectivity, similar to how Rozenfeld *et al.*





(2008) interpreted the highest betweenness of genetic distance. This example encourages further investigation of the potential of graph analysis of hydrodynamical connectivity matrices to identify sites important for conservation of genetic diversity.

By pinpointing the differential effect of demographic parameters and flow connectivity on population persistence and genetic diversity the present study opens a question for management: in order to protect species persistence and genetic diversity, should we be seeking a compromise between preserving populations that are low in terms of diversity but are also demographically stable vs. highly diverse but unstable populations? The present model, by combining demography and gene flow in realistic conditions allows to answer this question by 1) disentangling the relative contribution of local demography and environmental connectivity in shaping seascape genetics, and 2) performing *in silico* evaluations of different scenarios for marine spatial planning.

1.6 Acknowledgements

This work was (co-)funded through a MARES Grant. MARES is a Joint Doctorate programme selected under Erasmus Mundus coordinated by Ghent University (FPA 2011-0016). Check www.mares-eu.org for extra information. This work was also partly funded by the French National Program LITEAU IV of the Ministère de l'Écologie et de l'Environnement Durable under project RocConnect—Connectivité des habitats rocheux fragmentés du Golfe du Lion (PI, K. Guizien, Project Number 12-MUTS-LITEAU-1-CDS-013). We would like to thank Proof-reading-service.com for English edition and corrections of the manuscript, and Dr. Lorenzo Bramanti for an early critical review of the manuscript.





1.7 References

- Aiken, C., and Navarrete, S. 2011. Environmental fluctuations and asymmetrical - dispersal: Generalized stability theory for studying metapopulation persistence and marine protected areas. *Marine Ecology Progress Series*, 428: 77–88.
- Alcala, N., Streit, D., Goudet, J., and Vuilleumier, S. 2013. Peak and persistent excess of genetic diversity following an abrupt migration increase. *Genetics*, 193: 953–971.
- Banks, S. C., Cary, G. J., Smith, A. L., Davies, I. D., Driscoll, D. A., Gill, A. M., Lindenmayer, D. B., *et al.* 2013. How does ecological disturbance influence genetic diversity? *Trends in Ecology & Evolution*, 28: 670–679.
- Botsford, L., White, J., Coffroth, M., Paris, C., Planes, S., Shearer, T., Thorrold, S., *et al.* 2009. Connectivity and resilience of coral reef metapopulations in marine protected areas: Matching empirical efforts to predictive needs. *Coral Reefs*, 28: 327–337.
- Burgess, S., Nickols, K., Griesemer, C., Barnett, L., Dedrick, A., Satterthwaite, E., Yamane, L., *et al.* 2014. Beyond connectivity: How empirical methods can quantify population persistence to improve marine protected-area design. *Ecological Applications*, 24: 257–270.
- Butler, M.J., Paris, C.B., Goldstein, J.S., Matsuda, H., Cowen, R.K. 2011. Behaviour constrains the dispersal of long-lived spiny lobster larvae. *Marine Ecology Progress Series*. 422: 223-237.
- Costantini, F., Fauvelot, C., and Abbiati, M. 2007. Genetic structuring of the temperate gorgonian coral (*Corallium rubrum*) across the western Mediterranean Sea revealed by microsatellites and nuclear sequences. *Molecular Ecology*, 16: 5168–5182.
- Cowen, R., and Sponaugle, S. 2009. Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, 1: 443–466.
- Dawson, M., Hays, C., Grosberg, R., and Raimondi, P. 2014. Dispersal potential and population genetic structure in the marine intertidal of the eastern North Pacific. *Ecological Monographs*, 84: 435–456.





- Faurby, S., and Barber, P. 2012. Theoretical limits to the correlation between pelagic larval duration and population genetic structure. *Molecular Ecology*, 21: 3419–3432.
- Figueira, W. 2009. Connectivity or demography: Defining sources and sinks in coral reef fish metapopulations. *Ecological Modelling*, 220: 1126–1137.
- Foster, N.L., Paris, C.B., Kool, J.T., Baums, I.B., Stevens, J.R., Sanchez, J.A., Bastidas, C., et al. 2012. Connectivity of Caribbean coral populations: complementary insights from empirical and modelled gene flow. *Molecular Ecology*. 21(5): 1143-1157.
- Galindo, H., Olson, D., and Palumbi, S. 2006. Seascape genetics: A coupled oceanographic-genetic model predicts population structure of Caribbean corals. *Current Biology*, 1622–1626.
- Gilg, M., and Hilbish, T. 2003. The geography of marine larval dispersal-coupling genetics with fine-scale physical oceanography. *Ecology*, 1–11.
- Guizien, K., Belharet, M., Marsaleix, P., and Guarini, J. M. 2012. Using larval dispersal simulations for marine protected area design: Application to the Gulf of Lions (northwest Mediterranean). *Limnology and Oceanography*, 57: 1099–1112.
- Guizien, K., Belharet, M., Moritz, C., and Guarini, J. M. 2014. Vulnerability of marine benthic metapopulations: Implications of spatially structured connectivity for conservation practice in the Gulf of Lions (NW Mediterranean Sea). *Diversity and Distributions*, 1–11.
- Guizien, K., Brochier, T., Duchêne, J. C., Koh, B. S., and Marsaleix, P. 2006. Dispersal of *Owenia fusiformis* larvae by wind-driven currents: Turbulence, swimming behaviour and mortality in a three-dimensional stochastic model. *Marine Ecology Progress Series*, 311: 47–66.
- Hastings, A., and Botsford, L. 2006. Persistence of spatial populations depends on returning home. *Proceedings of the National Academy of Sciences*, 103: 6067–6072.
- Hastings, A., and Harrison, S. 1994. Metapopulation dynamics and genetics. *Annual Review of Ecology, Evolution, and Systematics*, 25: 167–188.





- Hellberg, M. 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. *Evolution*, 50: 1167–1175.
- Hellberg, M., Burton, R., Neigel, J., and Palumbi, S. 2002. Genetic assessment of connectivity among marine populations. *Bulletin of Marine Science*, 70: 273–290.
- Hixon, M., Pacala, S., and Sandin, S. 2002. Population regulation: Historical context and contemporary challenges of open vs. closed systems. *Ecology*, 83: 1490–1508.
- Jorde, P., and Ryman, N. 1995. Temporal allele frequency change and estimation of effective size in populations with overlapping generations. *Genetics*, 139: 1077–1090.
- Kimura, M. 1955. Random genetic drift in multi-allelic locus. *Evolution*, 9: 419–435.
- Kool, J. 2009. An object-oriented, individual-based approach for simulating the dynamics of genes in subdivided populations. *Ecological Informatics*, 4: 136–146.
- Larson, A., Wake, D., and Yanev, K. 1984. Measuring gene flow among populations having high levels of genetic fragmentation. *Genetics*, 106: 293–308.
- Lee, A., Engen, S., and Saether, B. 2011. The influence of persistent individual differences and age at maturity on effective population size. *Proceedings of the Royal Society B: Biological Sciences*, 278: 3303–3312.
- Lowe, W., and Allendorf, F. 2010. What can genetics tell us about population connectivity? *Molecular Ecology*, 19: 3038–3051.
- Marko, P., and Hart, M. 2011. The complex analytical landscape of gene flow inference. *Trends in Ecology & Evolution*, 26: 448–456.
- Marsaleix, P., Auclair, F., Floor, J., Herrmann, M., Estournel, C., Pairaud, I., and Ulses, C. 2008. Energy conservation issues in sigma-coordinate free-surface ocean models. *Ocean Modelling*, 20: 61–89.
- Munroe, D., Klinck, J., Hofman, E., and Powell, E. 2012. The role of larval dispersal in metapopulation gene flow: Local population dynamics matter. *Journal of Marine Research*, 1–2441–4677.





- Palumbi, S. 2004. Marine reserves and ocean neighborhoods: The spatial scale of marine populations and their management. *Annual Review of Environment and Resources*, 29: 31–68.
- Pineda, J. 2000. Linking larval settlement to larval transport: assumptions, potentials and pitfalls. *Oceanography of the Eastern Pacific*, 84–105.
- Pineda, J., Hare, J., and Sponaugle, S. 2007. Larval transport and dispersal in the coastal ocean and consequences for population connectivity. *Oceanography*, 20: 22–39.
- Riginos, C., Douglas, K., Jin, Y., Shanahan, D., and Treml, E. 2011. Effects of geography and life history traits on genetic differentiation in benthic marine fishes. *Ecography*, 34: 566–575.
- Rozenfeld, A., Arnaud-Haond, S., Hernández-García, E., Eguíluz, V., Serrão, E., and Duarte, C. 2008. Network analysis identifies weak and strong links in a metapopulation system. *Proceedings of the National Academy of Sciences*, 105: 18824–18829.
- Ryman, N. 1997. Minimizing adverse effects of fish culture: Understanding the genetics of populations with overlapping generations. *ICES Journal of Marine Science*, 54: 1149–1159.
- Saenz-Agudelo, P., Jones, G., Thorrold, S., and Planes, S. 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.
- Selkoe, K., and Toonen, R. 2011. Marine connectivity: A new look at pelagic larval duration and genetic metrics of dispersal. *Marine Ecology Progress Series*, 436: 291–305.
- Siegel, D., Kinlan, B., Gaylord, B., and Gaines, S. 2003. Lagrangian descriptions of marine larval dispersion. *Marine Ecology Progress Series*, 260: 83–96.
- Spear, S., Crisafulli, C., and Storfer, A. 2012. Genetic structure among coastal tailed frog populations at Mount St. Helens is moderated by post-disturbance management. *Ecological Applications*, 22: 856–869.





Waples, R., Luikart, G., Faulkner, J., and Tallmon, D. 2013. Simple life-history traits explain key effective population size ratios across diverse taxa. *Proceedings of the Royal Society B: Biological Sciences*, 280: 20131339.

Werner, F., Cowen, R., and Paris, C. 2007. Coupled biological and physical models. *Oceanography*, 1–16.

Whitlock, M. 1992. Temporal fluctuations in demographic parameters and the genetic variance among populations. *Evolution*, 46: 608–615.

Wright, S. 1931. Evolution in Mendelian populations. *Genetics*, 16: 97–159.





1.8 Supplementary material

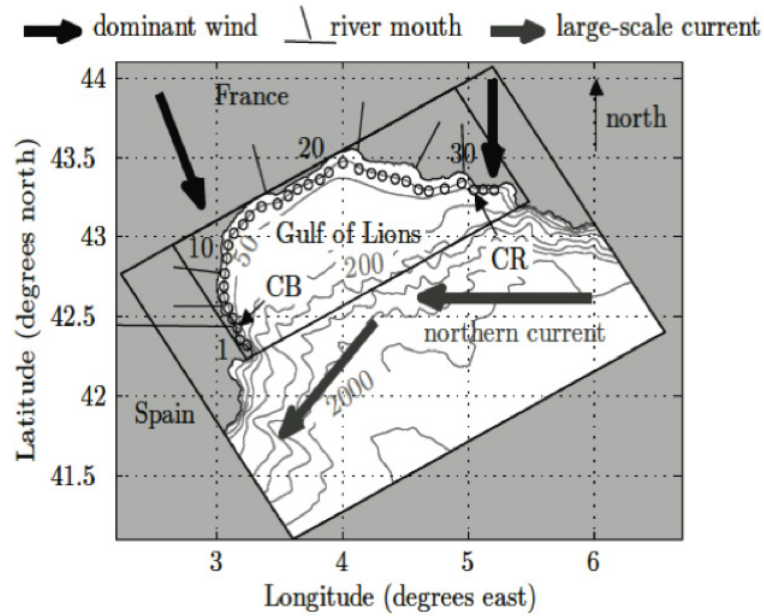


Figure S1.- Gulf of Lions (NW Mediterranean Sea) bathymetric map with depth contours (20, 50, 100, 500 m and spacing is 500 m below 500 m deep). Nearshore soft bottom habitat (depth between 10 and 30 m) was gridded into 32 sites, shown by circles and numbers from west to east. For dispersal simulations larvae was released from the centre of each circle. Solid boxes represent the extension of the two nested computational domains (1500 m and 750 m resolution grids). (from Guizien *et al.*, 2012).

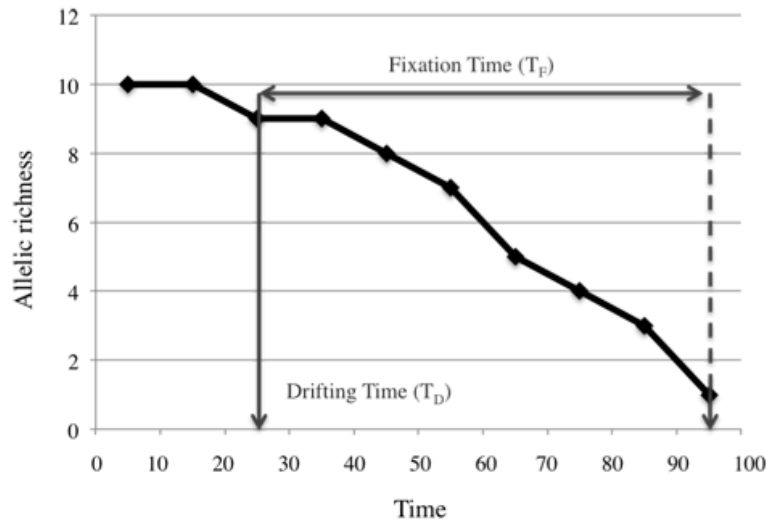


Figure S2.- Schematic definition of Allele Drifting Time (T_D) and Allele Fixation Time (T_F). Allele Drifting Time is the moment at which allelic richness decreases for the first time. Allele Fixation Time is the time lapse between the first loss of an allele (T_D) and the fixation of any allele.

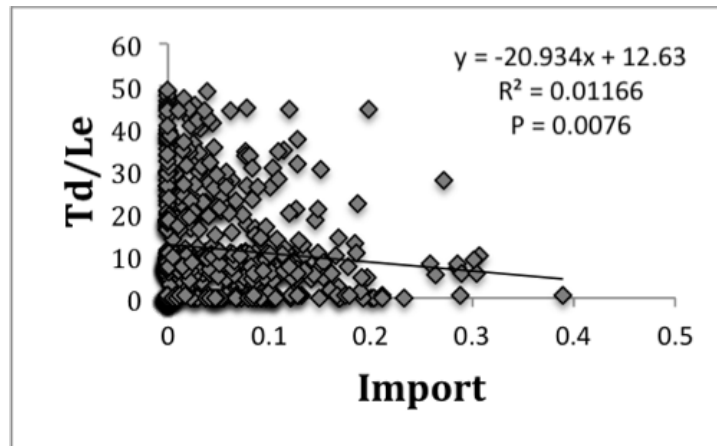


Figure S3.- Relationship between Td/Le and import (estimated as the proportion of all larvae produced among all sites that settled within the focal site) in a metapopulation.

CHAPTER 2

Genetic connectivity of gorgonian species at a regional scale





Introduction

Multiple ecological and anthropogenic stressors are increasingly affecting a growing number of marine species around the world, causing diseases and mass mortalities particularly in tropical coral reefs (Hughes *et al.*, 2003; Hoegh-Guldberg and Bruno 2010). In the Mediterranean Sea, coralligenous concretions are the unique calcareous formations of biogenic origin that allow the formation of reefs and are considered to be one of the main biodiversity hotspots in the region (Ballesteros 2006). These coralligenous outcrops have been identified as Mediterranean ‘priority habitats’ by the European Union Habitats Directive (92/43/CE). Severe cases of mass mortality events of several sessile benthic organisms inhabiting these coralligenous communities, in particular gorgonian species, have been reported in the coasts of the Ligurian Sea and Gulf of Lions (NW Mediterranean Sea) (Cerrano *et al.*, 2000; Perez *et al.*, 2000; Bramanti *et al.*, 2005; Cupido *et al.*, 2008; Coma *et al.*, 2006; Garrabou *et al.*, 2009; Santangelo *et al.*, 2015).

Gorgonians are of great interest for conservation. As engineering species, they play a fundamental structural and functional role in benthic ecosystems, which makes them key to the persistence and stability of hundreds of other species in the Mediterranean Sea (Gili and Coma 1998; Ballesteros 2006). For this reason, the future of benthic coralligenous communities depends on the ability of gorgonians to respond to increasing climate change and human disturbance (Ponti *et al.*, 2014).

Out of approximately 20 different gorgonian species inhabiting the Mediterranean Sea (Carpine and Grasshoff 1975), the red gorgonian *Paramuricea clavata* (Riso, 1826) and the white gorgonian *Eunicella singularis* (Esper 1791) are the most frequent and conspicuous species in the rocky sublittoral zone (Weinberg 1978; Gili and Ros 1985; Harmelin 1995; Ballesteros 2006) (Figure 2.1). While *P. clavata* is mostly found in sciaphilic rocky walls, *E. singularis* is a zooxanthellate species found in abundance on horizontal or subhorizontal rocky bottoms (Weinberg and Weinberg 1979). In the NW Mediterranean, particularly around the Gulf of Lions and Cap Creus, *E. singularis* is





commonly found in relatively higher densities than *P. clavata* (Gori *et al.*, 2011). Conversely, in the Ligurian Sea *P. clavata* is generally found in higher densities than *E. singularis* (Cocito 2002).

Regarding their reproductive ecology, these two species share some common traits: gonochoric (separate sex) colonies that reproduce sexually every year, with a long duration of oogenesis as compared to spermatogenesis, and releasing their offspring into the water column during summer (Coma *et al.*, 1995; Ribes *et al.*, 2007). Nonetheless, there are fundamental differences among *P. clavata* and *E. singularis*. *E. singularis* is an internal brooder species, in which embryogenesis takes place inside the female polyps and the mature planulae larvae is released from the polyp mouth (Weinberg and Weinberg 1979).

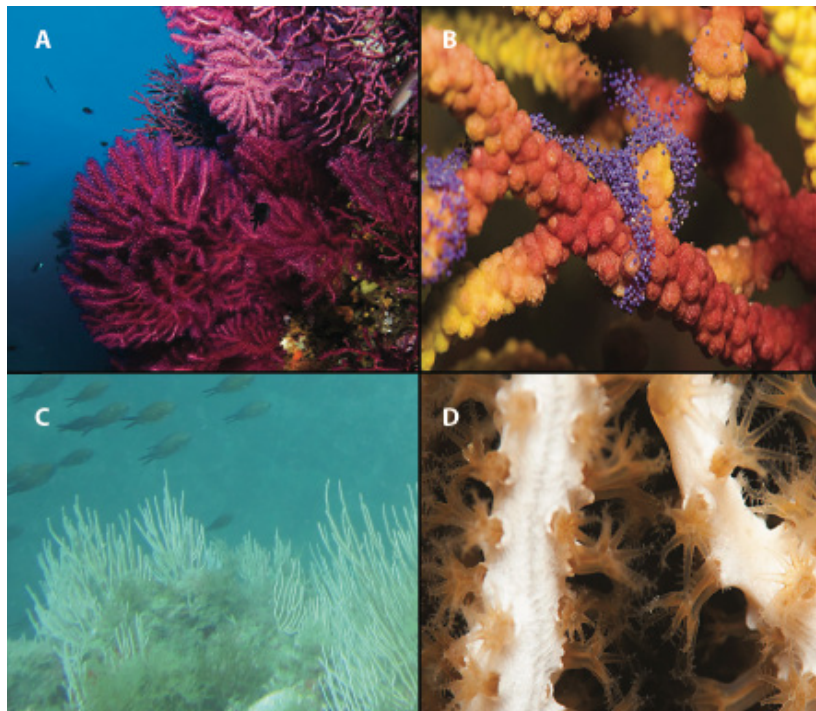


Figure 2.1.- Two of the most common gorgonian species in the Mediterranean Sea. A) Population of the red gorgonian *Paramuricea clavata*; B) Branch of *P. clavata* with cloud of eggs; C) Population of *Eunicella singularis*; D) Branch fragment with polyps of *E. singularis*. Photos: Regàs, J (A, B and D); Padrón, M (C).





Conversely, *P. clavata* is a surface brooder and embryogenesis occurs on the surface of the female colonies (Coma *et al.*, 1995). It has been reported that its larvae presents a short swimming period (Coma *et al.*, 1995) and low energetic content (Viladrich 2015), suggesting that the species may have limited dispersal capacity and, thus, settle close to the maternal colonies. A short dispersal range of the larvae and settlement near parental colonies has also been observed in *E. singularis* (Théodor 1967). However, the presence of symbiotic algae in the larvae (Weinberg and Weinberg 1979), and the high energetic content found in it (Viladrich 2015), has led to believe that the larvae of this species may have high dispersal capacity. Nonetheless, a true understanding on the dispersal capacity of these species is still limited by the lack of precise knowledge regarding their Pelagic Larval Duration (PLD) and competency period.

Many studies have focused their interest on evaluating the causes and consequences of the mortality events that affected both *E. singularis* and *P. clavata* populations in the NW Mediterranean. Multiple comparisons of the demographic structure of these populations before and after the climate-related disturbances have demonstrated their ability to recover after relatively short periods of time (Perez *et al.*, 2000; Coma *et al.*, 2006; Santangelo *et al.*, 2007; Cupido *et al.*, 2009; Santangelo *et al.*, 2015). Nonetheless, population recovery has been usually attributed to the species' life history traits and/or local population dynamics (e. g. high reproductive output and high recruitment rates) (Cerrano *et al.*, 2005; Santangelo *et al.*, 2015), disregarding the potential connectivity among populations at small regional scales arising from larval dispersal.

Under these circumstances, the use of genetic tools to evaluate the patterns of connectivity among populations seems an appropriate approach for assessing the realized dispersal of *E. singularis* and *P. clavata* (Hellberg 2002; Lowe and Allendorf 2010). However, because connectivity for marine species is the product of a series of filters driving population structure, starting by the production and dispersal of the larvae, followed by their settlement and survival, analysis of population genetic





structure can only be seen as proxy for characterizing connectivity (Jones *et al.*, 2009; Treml *et al.*, 2015). Our ability to discern patterns will also be affected by the spatial and temporal scales of the analysis.

Previous studies have evaluated the genetic structure among populations of *E. singularis* and *P. clavata* at different spatial scales in the NW Mediterranean (Calderón *et al.*, 2006; Cataneo 2011; Mokhtar-Jamaï *et al.*, 2011; Mokhtar-Jamaï *et al.*, 2013; Arizmendi-Mejía *et al.*, 2015). Using mitochondrial DNA (COI), Calderón *et al.* (2006) showed no genetic differentiation among sampled populations of *E. singularis*, not even allowing its distinction from a closely related species, *E. cavolinii*. On the other hand, using five polymorphic microsatellite loci, the work of Cataneo (2011) showed somewhat contradictory results in the analysis of the genetic structuration of *E. singularis* within the Mediterranean basin: although a weak pattern of IBD was found at the regional scale, populations separated by 100s of kilometers (France and Algeria) were grouped into the same genetic cluster (Cataneo 2011). Conversely, assessments of genetic population structure of *P. clavata*, using microsatellite loci, have evidenced strong genetic structure among populations separated by less than 30 meters (Mokhtar-Jamaï *et al.*, 2011; Arizmendi-Mejía *et al.*, 2015).

Nonetheless, the spatial scale of these studies has been either too small (5 m²) or too large (Mediterranean basin) to provide a clear understanding of how connectivity may enhance population resilience at a metapopulation scale. Indeed, at the large scale, their findings of strong genetic differentiation among populations are not very surprising, given that each one of the genetic clusters identified correspond to different hydrodynamic provinces, which, as defined by Rossi *et al.* (2014), are areas that are well connected internally but with limited exchange of larvae among them. Therefore, to evaluate what could be the processes involved in shaping connectivity within a hydrodynamic province, and the importance of distant connectivity in population functioning of these two abundant and widely distributed gorgonian species, the objective of this chapter was to evaluate the genetic structuration of *P. clavata* and *E.*





singularis at a regional scale, relying on a common approach and sampling design considering spatial scales from meters to kilometers. This chapter is thus structured in two sections to explicitly evaluate: 1) the role of genetic connectivity on the recovery of disturbed *P. clavata* populations in the Ligurian Sea; and 2) Genetic structure of *E. singularis* in the Gulf of Lions and Spanish Catalan Coast and its relationship with the local hydrodynamics.

References

- Arizmendi-Mejía, R., Linares, C., Garrabou, J., Antunes, A., Ballesteros, E., Cebrian, E., Diaz, D., *et al.* 2015. Combining genetic and demographic data for the conservation of a Mediterranean marine habitat-forming Species. PLoS ONE: 1–19.
- Ballesteros, E. 2006. Mediterranean coralligenous assemblages: a synthesis of present knowledge. *Oceanography and Marine Biology*, 44: 1–74.
- Bramanti, L., Magagnini, G., De Maio, L., and Santangelo, G. 2005. Recruitment, early survival and growth of the Mediterranean red coral *Corallium rubrum* (L 1758), a 4-year study. *Journal of Experimental Marine Biology and Ecology*, 314: 69–78.
- Calderón, I., Garrabou, J., and Aurelle, D. 2006. Evaluation of the utility of COI and ITS markers as tools for population genetic studies of temperate gorgonians. *Journal of Experimental Marine Biology and Ecology*, 336: 184–197.
- Carpine, C., and Grasshoff, M. 1975. Les gorgonaires de la Méditerranée. *Bulletin de l'Institut Océanographique Monaco*, 71: 1–140.
- Cataneo, J. 2011. Adaptation d'*Eunicella singularis* en milieu perturbé: symbiose et structuration génétique. PhD Thesis, Université de Nice-Sophia Antipolis. 220 pp
- Cerrano, C., Arillo, A., Azzini, F., Calcinai, B., Castellano, L., Muti, C., Valisano, L., *et al.* 2005. Gorgonian population recovery after a mass mortality event. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 15: 147–157.
- Cerrano, C., Bavestrello, G., Bianchi, C., Cattaneo-Vietti, R., Bava, S., Morganti, C., Morri, C., *et al.* 2000. A catastrophic mass-mortality episode of gorgonians and other





- organisms in the Ligurian Sea (North-western Mediterranean), summer 1999. Ecology Letters: 1–10.
- Cocito, S., Bedulli, D., and Sgorbini, S. 2002. Distribution patterns of the sublittoral epibenthic assemblages on a rocky shoal in the Ligurian Sea (NW Mediterranean). *Scientia Marina*, 66: 175–181.
- Coma, R., Linares, C., Ribes, M., Diaz, D., Garrabou, J., and Ballesteros, E. 2006. Consequences of a mass mortality in populations of *Eunicella singularis* (Cnidaria: Octocorallia) in Menorca (NW Mediterranean). *Marine Ecology Progress Series*, 327: 51–60.
- Coma, R., Ribes, M., Zabala, M., and Gili, J. M. 1995. Reproduction and cycle of gonadal development in the Mediterranean gorgonian *Paramuricea clavata*. *Marine Ecology Progress Series*, 117: 173–183.
- Cupido, R., Cocito, S., Barsanti, M., Sgorbini, S., Peirano, A., and Santangelo, G. 2009. Unexpected long-term population dynamics in a canopy-forming gorgonian coral following mass mortality. *Marine Ecology Progress Series*, 394: 195–200.
- Cupido, R., Cocito, S., Sgorbini, S., Bordone, A., and Santangelo, G. 2008. Response of a gorgonian (*Paramuricea clavata*) population to mortality events: recovery or loss? *Aquatic Conservation: Marine and Freshwater Ecosystems*, 18: 984–992.
- Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonné, P., Cigliano, M., Diaz, D., *et al.* 2009. Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. *Global Change Biology*, 15: 1090–1103.
- Gili, J. M., and Coma, R. 1998. Benthic suspension feeders: their paramount role in littoral marine food webs. *Trends in Ecology & Evolution*, 13: 316–321.
- Gili, J. M., and Ros, J. 1985. Study and cartography of the benthic communities of Medes Islands (NE Spain). *PSZNI Mar. Ecol.*, 6: 219–238.
- Gori, A., Rossi, S., Berganzo, E., Pretus, J., Dale, M., and Gili, J. M. 2011. Spatial distribution patterns of the gorgonians *Eunicella singularis*, *Paramuricea clavata*, and *Leptogorgia sarmentosa* (Cape of Creus, Northwestern Mediterranean Sea). *Marine*





Biology, 158: 143–158.

Gori, A., Linares, C., Rossi, S., Coma, R., and Gili, J. M. 2007. Spatial variability in reproductive cycle of the gorgonians *Paramuricea clavata* and *Eunicella singularis* (Anthozoa, Octocorallia) in the Western Mediterranean Sea. *Marine Biology*, 151: 1571–1584.

Harmelin, J. 1995. Gorgones. *Océanorama*, 24: 3–9.

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

Hoegh-Guldberg, O., and Bruno, J. 2010. The impact of climate change on the world's marine ecosystems. *Science*, 328: 1523–1528..

Hughes, T., Baird, A., Bellwood, D., Card, M., Connolly, S., Folke, C., Grosberg, R., *et al.* 2003. Climate change, human impacts, and the resilience of coral reefs. *Science*, 301: 929–933.

Jones, G., Almany, G., Russ, G., Sale, P., Steneck, R., van Oppen, M., and Willis, B. 2009. Larval retention and connectivity among populations of corals and reef fishes: history, advances and challenges. *Coral Reefs*, 28: 307–325.

Linares, C., Coma, R., Garrabou, J., Diaz, D., and Zabala, M. 2008. Size distribution, density and disturbance in two Mediterranean gorgonians: *Paramuricea clavata* and *Eunicella singularis*. *Journal of Applied Ecology*, 45: 688–699.

Lowe, W., and Allendorf, F. 2010. What can genetics tell us about population connectivity? *Molecular Ecology*, 19: 3038–3051.

Mokhtar-Jamäi, K., Coma, R., Wang, J., Zuberer, F., Féral, J. P., and Aurelle, D. 2013. Role of evolutionary and ecological factors in the reproductive success and the spatial genetic structure of the temperate gorgonian *Paramuricea clavata*. *Ecology and Evolution*, 3: 1765–1779.

Mokhtar-Jamäi, K., Pascual, M., Ledoux, J. B., Coma, R., Féral, J. P., Garrabou, J., and Aurelle, D. 2011. From global to local genetic structuring in the red gorgonian *Paramuricea clavata*: the interplay between oceanographic conditions and limited





- larval dispersal. *Molecular Ecology*, 20: 3291–3305.
- Perez, T., Garrabou, J., Sartoretto, S., Harmelin, J. G., Francour, P., and Vacelet, J. 2000. Mortalité massive d'invertébrés marins: un événement sans précédent en Méditerranée nord-occidentale. *C. R. Acad. Sci. Paris, Sciences de la vie*, 323: 853–865.
- Ponti, M., Perlini, R., Ventra, V., Grech, D., Abbiati, M., and Cerrano, C. 2014. Ecological shifts in Mediterranean coralligenous assemblages related to gorgonian forest loss. *PLoS ONE*, 9: e102782.
- Ribes, M., Coma, R., Rossi, S., and Micheli, M. 2007. Cycle of gonadal development in *Eunicella singularis* (Cnidaria: Octocorallia): trends in sexual reproduction in gorgonians. *Invertebrate Biology*, 126: 307–317.
- Rossi, V., Ser-Giacomi, E., López, C., and Hernandez-García, E. 2014. Hydrodynamic provinces and oceanic connectivity from a transport network help designing marine reserves. *Geophysical Research Letter*, 41: 2883–2891.
- Santangelo, G., Bramanti, L., and Iannelli, M. 2007. Population dynamics and conservation biology of the over-exploited Mediterranean red coral. *Journal of Theoretical Biology*, 244: 416–423.
- Santangelo, G., Cupido, R., Cocito, S., Bramanti, L., Priori, C., Erra, F., and Iannelli, M. 2015. Effects of increased mortality on gorgonian corals (Cnidaria, Octocorallia): different demographic features may lead affected populations to unexpected recovery and new equilibrium points. *Hydrobiologia*: 1–17.
- Théodor, J. 1967. Contribution à l'étude des gorgones. VII. Ecologie et comportement de la planula. *Vie Milleu*, 18: 291–301.
- Treml, E., Ford, J., Black, K., and Swearer, S. 2015. Identifying the key biophysical drivers, connectivity outcomes, and metapopulation consequences of larval dispersal in the sea. *Movement Ecology*, 3: doi: 10.1186/s40462-015-0045-6.
- Viladrich, N. 2015. Study of environmental and biological factors that affect larval survival in sessile coastal marine organisms. PhD Thesis, Universitat Autònoma de





Barcelona.

Weinberg, S. 1978. Mediterranean octocorallian communities and the abiotic environment. *Marine Biology*, 49: 41–57.

Weinberg, S., and Weinberg, F. 1979. The life cycle of a gorgonian: *Eunicella singularis*. *Bijdragen tot de Dierkunde*, 48: 1–14.



CHAPTER 2.1

Connectivity helps marine coastal populations recover
from climate-induced mass mortality events



Mariana PADRÓN^{1*}, Federica COSTANTINI², Lorenzo BRAMANTI¹, Katell GUIZIEN¹ and Marco ABBIATI^{2,3}

¹ Sorbonne Universités, UPMC Univ Paris 06, CNRS, Laboratoire d'Ecogéochimie des Environnements Benthiques (LECOB), Observatoire Océanologique, 66650, Banyuls sur Mer, France.

² Dipartimento di Scienze Biologiche, Geologiche ed Ambientali (BiGeA) & Centro Interdipartimentale di Ricerca per le Scienze Ambientali (CIRSA), University of Bologna, ULR CoNISMa, Via S. Alberto 163, I-48123 Ravenna, Italy

³ ISMAR, Consiglio Nazionale delle Ricerche - Istituto di Scienze Marine, Bologna, Italy

N.B. This manuscript has been prepared for submission to the journal of Global Change Biology.

2.1.1 Abstract

Climate-induced threats are increasingly affecting marine populations worldwide. For benthic sessile species depending on a larval stage to disperse, the connection among populations may be key to ensure their persistence. Several mass mortality events have been recorded to affect multiple benthic species in the NW Mediterranean, particularly gorgonian species. Although previous studies have evaluated the demographic effect of these climate-related disturbances, the ability of population connectivity within the seascape to mitigate the loss of genetic diversity of benthic species has not been investigated empirically in the Mediterranean Sea. In this study, we examine the genetic diversity and population genetic structure of *P. clavata* among affected and non-affected populations in the Ligurian Sea using six polymorphic microsatellite loci, as a proxy to understand the patterns of realized connectivity in the region and their potential role in the recovery of its populations. Strong genetic differentiation was found between sites for *P. clavata* in the Ligurian Sea. We did not detect evidence of severe bottlenecks in all affected populations. The analysis of migration over several generations revealed that all populations are well connected in both directions. Patterns of surface currents in the





region seem to explain the spatial heterogeneity in the levels of gene flow. Although no temporal comparisons can be made given the lack of data on the genetic structure before the mass mortality events of *P. clavata* in the Ligurian Sea, our results suggest that the contribution of immigration could have helped the fast recovery of the genetic diversity and density of recruits in the most affected populations.

2.1.2 Introduction

Marine biodiversity at a global scale is under increasing pressure from environmental change caused by human activities, including climate change (Hoegh-Guldberg and Bruno 2010). Trends in rising sea temperatures, decreasing ocean pH and sea level rise, along with climate perturbations increasing the frequency and magnitude of extreme events (storms and heat waves) have already threatened several marine species and communities (Doney *et al.*, 2012). However, the threshold of populations and communities' resilience to climatic disturbances is still an open question in contemporary ecology and biodiversity conservation (Wernberg *et al.*, 2014). Among the threats affecting community structure and population persistence, mass mortality events and coral cover decline related to warming ocean temperatures are threatening the integrity of reef habitats and their associated high biodiversity (Pandolfi *et al.*, 2011; Selig *et al.*, 2012; Johns *et al.*, 2014; Riegl and Purkis 2015).

Severe and sudden reductions in population size (demographic bottleneck) are expected to lead to a loss of genetic diversity, particularly allelic diversity, causing a genetic bottleneck (Lacy 1987). Furthermore, populations that have suffered a genetic bottleneck and which remain of small size during a long period of time could lose additional genetic variation as a consequence of genetic drift, and eventually be affected by inbreeding (Nei *et al.*, 1975). However, the loss of genetic diversity caused by a population collapse can rapidly be offset or reversed by immigration (Crow and Kimura 1970; Keller *et al.*, 2001). Therefore, connectivity among populations could be an important asset for the mitigation of the loss of genetic diversity.





In marine benthic populations, connectivity between distant populations ensured by larval dispersal should allow the recovery of populations after disturbances and is expected to be a key aspect of their resilience (Almany *et al.*, 2009). Nonetheless, the efficacy of connectivity in the recovery after disturbance is controversial: while biophysical modeling approaches forecast wide potential connectivity through larval dispersal (Shanks *et al.*, 2003; Lipcius *et al.*, 2008), realized connectivity assessed by genetics is often more restricted in stable populations, as expected from the filtering due to settlement and recruitment limitations (Pineda 2007). Yet, within the framework of the interaction between climate change and connectivity, most attention has been put to investigating how potential connectivity may be affected by climate change (Munday *et al.*, 2009; Lett *et al.*, 2010), while there is almost none empirical evidence on how realized connectivity may influence the response of marine species and confer resilience to disturbances caused by climate change (Vollmer and Palumbi 2007; Underwood *et al.*, 2007; Gilmour *et al.*, 2013; Caplins *et al.*, 2014; Magris *et al.*, 2014).

Determining the genetic diversity and genetic structure of marine populations can provide an understanding of the natural genetic variability of the populations, as well as their ability to withstand perturbations, their potential resistance to local extirpation and their natural rate of recovery (van Oppen and Gates 2006; Kovach *et al.*, 2015). In particular, the use of tests that allow the assignment of individuals to their population of origin can provide more detailed estimates of gene flow among populations, enabling the inference of contemporary migration patterns (Waser and Strobeck 1998).

In temperate waters, several mass mortality events coinciding with anomalous temperature increases have also affected benthic sessile species along the rocky shores of the north-western Mediterranean during the last two decades (Cupido *et al.*, 2008). Among the sessile invertebrates species concerned, gorgonian corals' populations particularly suffered the most damaging effects, resulting in the loss of more than 50% in density and biomass of different species (Cerrano *et al.*, 2000; Bramanti *et al.*, 2005;





Cupido *et al.*, 2008; Coma *et al.*, 2009; Garrabou *et al.*, 2009; Santangelo *et al.*, 2015).

Gorgonian corals are long-lived, slow-growing, habitat-forming species considered to be one of the main components of the Mediterranean coralligenous assemblages (Ballesteros 2006). They are of great interest for conservation given their role in the persistence and stability of several associated species. At the same time, their slow dynamics make them particularly vulnerable to disturbances (Linares *et al.*, 2008). Therefore, the future of the communities of Mediterranean coralligenous assemblages depends on the capability of gorgonian species to respond to increasing climate change and human disturbances (Ponti *et al.*, 2014).

Among the gorgonian species affected by sea temperature anomalies, the red gorgonian *Paramuricea clavata* (Risso 1826) suffered the most drastic reductions in density and biomass over a broad spatial scale. The impact of mass mortality events on the demographic structure and dynamics of this species has been assessed in detail, providing valuable information and tools to understand the species' ability to respond to perturbations in the face of climate change (Santangelo *et al.*, 2007; Cupido *et al.*, 2009; Santangelo *et al.*, 2015). However, the role of connectivity on the observed recovery of the affected populations has been ignored, attributing the increase of recruits after adult mortality events to local replenishment by the high reproductive output of surviving colonies (Santangelo *et al.*, 2015). An assumption motivated by the low dispersal potential attributed to *P. clavata* based on high genetic differentiation at small spatial scales (less than 30 meters) observed in non-impacted populations (Mokthar-Jamaï *et al.*, 2011; Arizmendi-Mejía *et al.*, 2015).

In this study, we examine the genetic diversity, genetic structure and contemporary migration patterns of *P. clavata* among populations affected and non-affected by mass mortality events related to heat waves in the Ligurian Sea. In particular, we attempt to answer the questions: 1) do disturbed populations show a signal of reduced genetic diversity? and 2) did connectivity play a role in the recovery of the affected populations?





Furthermore, this study represents a regional baseline for future comparative studies of genetic variation of the species in the face of increasing environmental change and potential disturbances induced by climate variability.

2.1.3 Material and Methods

2.1.3.1 Sampling

Colonies of the red gorgonian *P. clavata* were sampled by SCUBA diving at seven different locations (depth ranging from 25 to 40 meters) along the Ligurian Sea during May of 2014 (Figure 2.1.1), following a hierarchical sampling design. Colonies height varied from 30 to 60 cm, with most of them measuring between 35-45 cm, thus being older than 15 years (Cupido *et al.*, 2012). Small (< 3 cm) branch fragments from 26 to 37 colonies were collected in two different sites (separated by a distance of 30 m - 1 km) within each location, except in Punta Mesco and Cerboli Island. In Punta Mesco and Cerboli Island, colonies were sampled only at one site due to restricted sampling permission (Table 2.1.1). All samples were preserved in 95% ethanol and stored at 4 °C.

In the Ligurian Sea, mass mortality events due to heat waves were reported in 1999 and 2003. The sampling design included two populations of *P. clavata* in which those events have been investigated: Portofino (POF1 and POF2) (Cerrano *et al.*, 2005;) and Portovenere (POV1 and POV2) (Cupido *et al.*, 2008; Santangelo *et al.*, 2015). A reduction of 78.3% in density was recorded in Portovenere, while up to 50% of the colony density was lost in Portofino. After the mass mortality events in Portovenere, recruitment increased five-fold with respect to pre-mortality values, with recruits becoming the dominant class (Santangelo *et al.*, 2015). In Portofino, Cerrano *et al.* (2005) observed a two-fold increase in recruitment inducing significant population recovery after three years, even reaching higher densities than before the mortality event. Although mass mortality events of *P. clavata* were not reported in other locations within the Ligurian Sea, other sessile invertebrates were affected by the increases in water temperature at same time in the area of Bergeggi (Parravicini *et al.*, 2010).



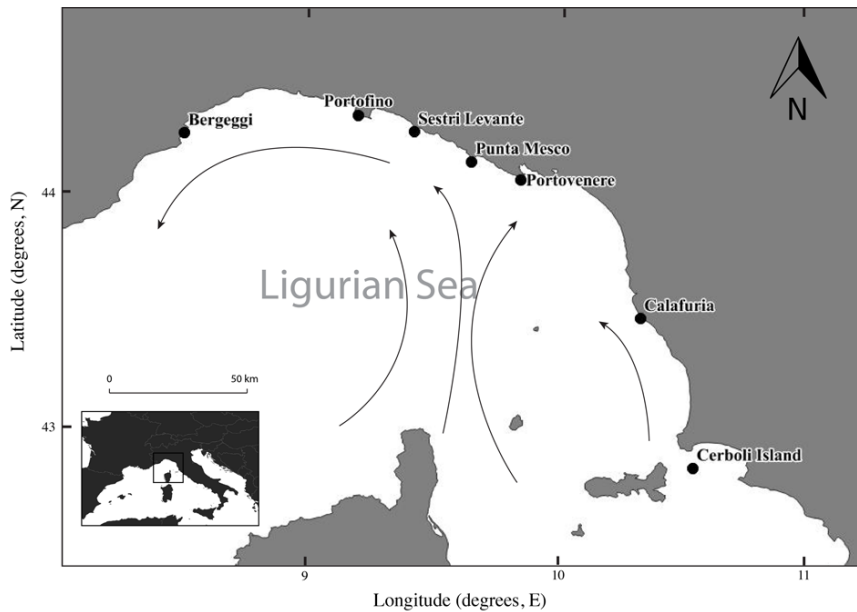


Figure 2.1.1.- Map of the seven sampling locations of *Paramuricea clavata* in the Ligurian Sea. The dominant sea surface currents according to the Istituto Idrografico della Marina (1982) for the month of June (beginning of spawning season for *P. clavata* in the Mediterranean Sea (Linares *et al.* 2008) are indicated by arrows.

2.1.3.2 *Microsatellite genotyping*

Total genomic DNA was extracted from a total of 372 colonies using the DNA extraction kit NucleoSpin Tissue by Macherey-Nagel. All individuals were genotyped at six polymorphic microsatellite loci developed for *P. clavata*, as used in Mokhtar-Jamäi *et al.*, (2011): Parcla 09, Parcla 10, Parcla 12, Parcla 14, Parcla 17 (Molecular Ecology Resources Primer Development Consortium *et al.*, 2010) and Par_d (Agell *et al.*, 2009). Genotyping was carried out on an ABI 3130 Genetic Analyzer using an internal size standard (GeneScan 600 LIZ; Applied Biosystems), and allele scoring was performed using Peak Scanner Software version 1.0 (Applied Biosystems).



Table 2.1.1.- Sampling locations of *P. clavata* in the Ligurian Sea. Rocky bottom patch size was measured as the maximum continuous length of rocky bottom found along the coast. Reports of mass mortality events were obtained from Cupido *et al.*, (2008) and Santangelo *et al.*, (2015).

Location	Site	Latitude	Longitude	Depth (m)	Samples	Rocky bottom patch size (Km)	Mass mortality reported
Bergeggi	BER1	44.2416	8.4461	37	32	2	No
	BER2	44.2416	8.4461	38	30		
Portofino	POF1	44.2980	9.2188	36	31	13	Yes
	POF2	44.2980	9.2188	40	31		
Sestri Levante	SES1	44.2482	9.3963	30	32	3	No
	SES2	44.2482	9.3963	30	32		
Punta Mesco	PME	44.1247	9.6322	29	26	5	No
Portovenere	POV1	44.0227	9.8511	25	30	6	Yes
	POV2	44.0238	9.8505	25	32		
Calafuria	CAL1	43.4700	10.3338	35	32	12	No
	CAL2	43.4700	10.3338	25	29		
Cerboli Island	CER	42.8555	10.5469	30	37	2	No



2.1.3.3 Genetic diversity

Observed and Nei's (1987) unbiased expected heterozygosity (H_o and H_e , respectively) were estimated using Genetix v.4.05 (Belkhir *et al.*, 2004). Allelic richness (N_a) was estimated using FSTAT v.2.9.3.2 (Goudet 2001), while rarefied allelic (A_r) and private allelic richness (A_p) were calculated with a rarefaction procedure using the HP-Rare software (Kalinowski 2005). Linkage disequilibrium was tested for all loci using Genepop v.4.2 (Rousset 2008) as implemented for online use (<http://genepop.curtin.edu.au>). The test for Hardy-Weinberg equilibrium was computed for each locus using the null hypothesis of no heterozygote deficiency, and the level of significance was calculated with a Markov Chain algorithm with parameters set as default (100 batches and 1000 iterations per batch). Single and multilocus F_{IS} were estimated using Weir and Cockerham's (1984) fixation index using Genepop. The presence of null alleles was estimated using the Expectation Maximization (EM) algorithm of Dempster *et al.*, (1977) in Freena software (Chapuis and Estoup 2007).

Due to the mass mortality events that affected *P. clavata* populations in the region during the past decade (Santangelo *et al.*, 2015), evidence of recent changes in effective population size was tested using Bottleneck v.1.2.02 following the Step-wise Mutation Model (SMM) (Piry *et al.*, 1999).

2.1.3.4 Population genetic structure

Population genetic structure was estimated using the F_{ST} estimates of Weir and Cockerham (1984) with Genepop, and following the Excluding Null Allele (ENA) method in Freena. The significance of pairwise genetic differentiation among samples was computed with an exact test using the default parameters in Genepop (100 batches and 1000 iterations per batch).

For linkage disequilibrium and F_{ST} estimates, FDR corrections (Benjamini and Hochberg 1995) were applied in order to adjust significance levels for false discovery rates.





Isolation by Distance (IBD) was tested at a regional scale through the correlation between pairwise $F_{ST}/(1-F_{ST})$ values and the geographical distance between populations with a Mantel test (Mantel 1967) using the Isolde program implemented in Genepop. Geographical distances were measured in Google Earth by using set points (determined as the center of the area of distribution where the samples were collected) and measuring both straight-line and along the coast distances. Significance level of correlation between genetic differentiation and geographical distance among samples was computed using 10,000 permutations.

To estimate the extent of gene diffusion, multilocus genotypes were autocorrelated within spatial distance classes using the kinship coefficient of Loiselle as implemented in Spagedi v.1.4 revealing the distance at which the random effects of genetic drift, not gene flow, are the primary determinants of genetic composition (Hardy and Vekemans 2002). Significant positive coefficients suggest that colonies within a specific distance class are more genetically related than colonies randomly taken from any distance class. The first x-intercept of the autocorrelogram, which corresponds to the average distance at which the genetic similarity between any two quadrats drops below 0.0, is termed the 'genetic patch size' (Sokal and Wartenberg 1983). Spatial distance intervals for kinship comparisons were constructed in such a way that the number of pairwise comparisons within each distance interval was approximately constant (between 3000 and 8000 pairs among distance classes). A total of 13 distance classes were considered: class 1: 0 – 1 km; class 2: 2 – 23 km; class 3: 23 – 28 km; class 4: 28 – 40 km; class 5: 40 – 75 km; class 6: 75 – 89 km; class 7: 89 – 104 km; class 8: 104 – 125 km; class 9: 125 – 153 km; class 10: 153 – 177 km; class 11: 177 – 229 km; class 12: 229 – 251 km; class 13: 251 – 283 km. Significance of the observed coefficients relative to the expected was tested for each class using 10,000 random permutations of locations.

To evaluate the number of clusters (K) in the dataset without prior information regarding the geographic location of the samples, a Bayesian method implemented in Structure v.2.3.4 (Pritchard *et al.*, 2000; Falush *et al.*, 2003, 2007) was used. Structure was





run under the admixture model, choosing the assumption of correlated allele frequencies and the option of recessive alleles to cope with null alleles, as suggested by Falush (2007). Mean and variance of log likelihoods were estimated for the number of clusters, $K=1$ to $K=12$. Ten runs were performed for each K with 500,000 iterations and a burn-in period of 50,000. In order to identify the number of clusters that best fit the data, the resulting output files were then analyzed using the Evanno method in Structure Harvester v.0.6.94, as implemented for online use (<http://taylor0.biology.ucla.edu/structureHarvester/>) (Earl and vonHoldt 2012). For the chosen K , the results were averaged using Clumpp v.1.1.2 (Jakobsson and Rosenberg, 2007), and the graphical representation was performed in Distruct v.1.1 (Rosenberg 2004).

2.1.3.5 Migration and genetic drift

Recent average migration rates and first generation migrants were estimated as proxies of contemporary connectivity among populations. The amount and direction of gene flow over the last several generations among locations was estimated by performing a maximum-likelihood-based analysis. Migrate-n v.3.6 was used to estimate Θ and M based on microsatellite data (Beerli and Felsenstein 2001). Theta is defined as $4N_e\mu$ for a diploid system with nuclear microsatellite loci, where N_e is the effective population size and μ is the mutation rate per generation and site. M is the mutation scaled migration rate, defined as m/μ , representing the relative contribution of immigration and mutation to the variability brought into the population, whereas m is the fraction of new immigrants found in the population per generation. On the other hand, first generation migrants were detected with a threshold of 95% confidence using the Bayesian computation of Rannala and Mountain (1997) ($n=10000$; $\alpha = 0.05$) in GeneClass v.2.0 (Piry *et al.*, 2004).

The level of genetic drift was estimated using the standard linkage disequilibrium method (Hill 1981) with Waples' (2006) correction to compute a contemporary value of the effective population size (N_e). The computations were





performed with NeEstimator v. 2.01 (Do *et al.*, 2014) under the random mating model, excluding rare alleles with frequencies of less than 0.02 and using the jackknife option to estimate confidence intervals.

2.1.4 Results

2.1.4.1 Genetic diversity

All of the microsatellite loci were polymorphic with POF1 showing the highest number of alleles (11), while BER1 presented the lowest (7). Mean allelic richness (N_a), rarefied allelic richness (A_r) and private allelic richness (A_p) among populations were 8.98 ± 1.25 , 5.96 ± 0.52 and 0.40 ± 0.15 , respectively. Rarefied allelic richness ranged from 4.96 for BER1 to 6.55 for POV2. The private allelic richness varied from 0.08 for SES2 to 0.58 for POV1. Observed (H_o) and unbiased expected heterozygosity (H_e) ranged from 0.49 for CER to 0.71 for SES2 with a mean value of 0.59 ± 0.06 , and from 0.67 for BER1 to 0.76 for POF1, with a mean value of 0.70 ± 0.03 , respectively (Table 2.1.2). No significant linkage disequilibrium was found among loci ($P > 0.05$ after FDR correction).

Estimates of null allele frequencies varied between 0 for Parcla09, Parcla10, Parcla12, Parcla14 and Par_d to 0.26 for Parcla12.

Multilocus estimates of F_{is} ranged between 0.04 (BER1) to 0.33 (CAL1 and CER). All the values were significantly different from zero ($P < 0.001$) with the exception of BER1, meaning that this population does not show significant levels of heterozygosity deficiency. All loci showed significant deviations from Hardy-Weinberg expected genotype frequencies.

Recent changes in effective population size were evidenced in BER2 and POV1 due to an excess of the heterozygosity expected from the observed number of alleles, given the sample size under the assumption of mutation-drift equilibrium for the Step-wise Mutation Model (SMM) ($P = 0.008$ and $P = 0.015$, respectively). Despite the fact that the sites in Portofino (POF1 and POF2) were affected by mass mortality events, no recent changes in effective populations size were observed ($P > 0.05$).



Table 2.1.2.- Parameters of genetic diversity for the 12 sites of *P. clavata* at six microsatellite loci

Site	H _o	H _E	Na	Ar	Ap	F _{IS}	Ne	95% CI
BER1	0.65	0.67	6.83	4.96	0.13	0.04	51	24
BER2	0.63	0.73	7.00	5.42	0.2	0.17	180	30
POF1	0.61	0.66	9.67	5.24	0.55	0.22	4707	52
POF2	0.64	0.76	10.67	6.29	0.5	0.21	342	36
SES1	0.59	0.68	8.83	6.01	0.34	0.14	156	50
SES2	0.71	0.70	8.83	6.34	0.3	0.14	66	30
PME	0.56	0.70	9.83	6.41	0.51	0.20	197	55
POV1	0.55	0.69	9.00	6.32	0.42	0.21	36	16
POV2	0.58	0.69	8.17	6.55	0.28	0.10	66	26
CAL1	0.52	0.74	10.00	6.25	0.55	0.33	176	54
CAL2	0.54	0.68	8.33	5.54	0.42	0.27	Inf	195
CER	0.49	0.71	10.55	6.19	0.62	0.33	131	27
Mean value	0.59 ± 0.06	0.70 ± 0.03	8.98 ± 1.25	5.96 ± 0.52	0.40 ± 0.15	0.20 ± 0.08	509 ± 1325	

H_o, observed heterozygosity; H_E, unbiased expected heterozygosity; Na, Number of alleles; Ar, rarefied allelic richness; Ap, private allelic richness; F_{IS}, Weir and Cockerham's (1984) estimate of Wright's (1951) fixation index with significant values in bold (0.05 threshold after FDR correction); Ne, Effective population size; 95% CI, 95% Confidence interval of Ne.



2.1.4.2 Population genetic structure

Global multilocus F_{ST} indicated strong genetic differentiation between sites for *P. clavata* in the Ligurian Sea ($F_{ST} = 0.154$; $P = 0.0001$). Pairwise F_{ST} values ranged from 0 to 0.181, and all but three pairwise comparisons (BER1 vs. BER2, SES1 vs. SES2 and PME vs. POV1) were significant after FDR correction (Table 2.1.3). No significant differences were found between pairwise F_{ST} and pairwise F_{ST} corrected for null alleles (t-test, $P = 0.163$), which suggests that the effect of null alleles is negligible. A significant but weak pattern of Isolation by Distance (IBD) was detected for both measures of distance: straight-line ($R^2 = 0.22$; $P < 0.01$) and along the coast ($R^2 = 0.17$; $P < 0.001$).

Six genetic clusters were identified by the Bayesian clustering analysis ($K = 6$, $\Delta K = 12.26$) (Fig. 2.1.2): 1) BER1 and BER2 composed the BER cluster; 2) POF1 and POF2 formed the POF cluster; 3) SES1 and SES2 composed the SES cluster; 4) PME, POV1 and POV2 were grouped in the PMP cluster; 5) CAL1 and CAL2 formed the CAL cluster; and 6) CER was designed as the CER cluster.

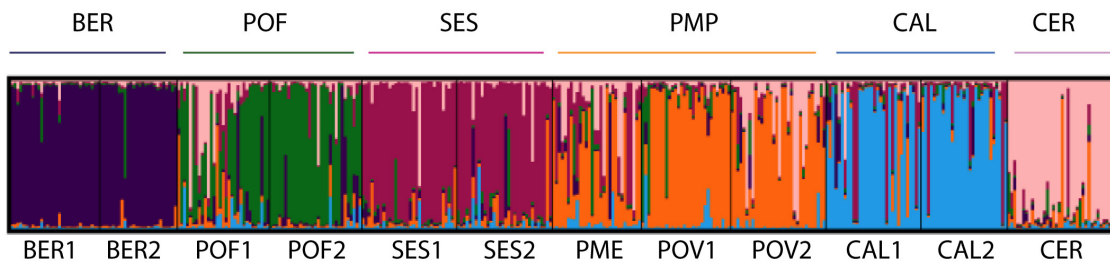


Figure 2.1.2.- Population genetic structure of *Paramuricea clavata* in the Ligurian Sea, as revealed by the software Structure. Each individual is represented by a vertical line, where the different colors indicate the individual proportion of membership to each cluster ($K = 6$). The color purple represents the cluster of Bergeggi (BER), the color green indicates the cluster of Portofino (POF), the color fuchsia represents the cluster of Sestri Levante (SES), the color yellow indicates the cluster of Punta Mesco and Portovenere (PMP), the color blue indicate the cluster of Calafuria (CAL) and the color pink the cluster of Cerboli (CER).

Table 2.1.3.- Pairwise F_{ST} values among sites in the Ligurian Sea. Significant values, after FDR correction, are highlighted in bold ($P < 0.05$).

	BER1	BER2	POF1	POF2	SES1	SES2	PME	POV1	POV2	CAL1	CAL2	CER
BER1	0											
BER2	0.004	0										
POF1	0.116	0.116	0									
POF2	0.132	0.144	0.066	0								
SES1	0.127	0.131	0.089	0.125	0							
SES2	0.099	0.096	0.069	0.108	0.011	0						
PME	0.158	0.149	0.097	0.143	0.083	0.066	0					
POV1	0.141	0.139	0.095	0.136	0.088	0.092	0.061	0				
POV2	0.125	0.116	0.094	0.122	0.086	0.074	0.037	0.029	0			
CAL1	0.189	0.181	0.139	0.177	0.111	0.081	0.143	0.177	0.164	0		
CAL2	0.112	0.117	0.089	0.139	0.075	0.052	0.103	0.121	0.112	0.032	0	
CER	0.143	0.145	0.074	0.138	0.091	0.079	0.071	0.062	0.094	0.154	0.102	0



The kinship coefficient of Loiselle was positive and significant for the 0-23 km ($P < 0.001$), 23 – 28 km ($P < 0.001$) and 28 – 41 km ($P < 0.001$) distance classes (Fig. 2.1.3). The genetic patch size was estimated to be 40 km.

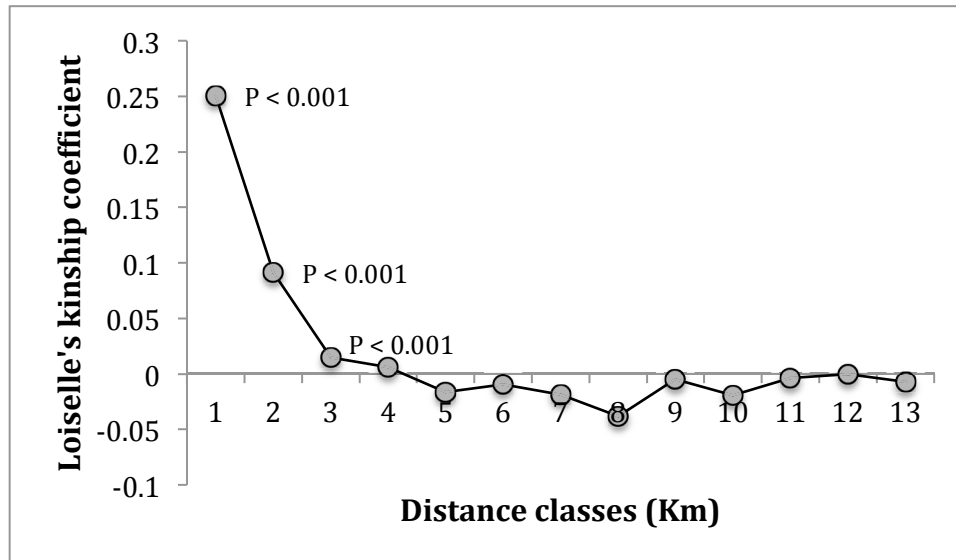


Figure 2.1.3.- Autocorrelogram based on the kinship coefficient of Loiselle, estimated for multilocus genotypes within 13 spatial distance classes. Spatial distance classes were designated in such a way that the number of pairwise comparisons within each distance interval is approximately constant. Class 1: 0 – 1 km; class 2: 2 – 23 km; class 3: 23 – 28 km; class 4: 28 – 40 km; class 5: 40 – 75 km; class 6: 75 – 89 km; class 7: 89 – 104 km; class 8: 104 – 125 km; class 9: 125 – 153 km; class 10: 153 – 177 km; class 11: 177 – 229 km; class 12: 229 – 251 km; class 13: 251 – 283 km.

2.1.4.3 Migration and genetic drift

Maximum likelihood-based analysis using Migrate-n revealed that values of $M (= m/\mu; \text{migration/mutation})$ were >1 in all populations, meaning that the effect of migration (m) is larger than the effect of mutation (μ). Overall, migration was found to be bi-directional among closely distributed sites (less than 1 km), and unidirectional (westward) among distant sites. Migration rate decreased as distance between sites increased, finding still evidence of migration among the most distant locations (> 250 km). The cluster that received most immigrants over multiple generations was PMP





(mean $M = 18.21 \pm 5.53$), which comprises one of the populations affected by mass mortality due to heat waves in the region (Portovenere). The cluster POF, corresponding to the other affected population (Portofino) showed a mean value of $M = 10.50 \pm 7.92$. The cluster of CER was identified as the one receiving the lowest amount of immigrants (mean $M = 6.14 \pm 3.02$). In terms of contribution of migrants, the cluster of PMP showed the highest value (mean $M = 18.02 \pm 8.16$), and the cluster of CAL the lowest (mean $M = 6.15 \pm 3.02$). The effective number of migrants per generation $Nem (= \Theta M/4)$ was > 0.22 in all sites.

The analysis of first generation migrants revealed that the clusters of populations affected by mass mortality events were the largest contributor of migrants (PMP = 40.62% and POF = 28.12%). On the other hand, the clusters of BER and CAL were identified as the smallest contributors of immigrants with 3.12% in both cases (Fig. 2.1.4). In terms of reception of immigrants, the clusters CAL and PMP received the highest proportion of migrants (15.9% and 14.81%, respectively), with the latter receiving immigrants for almost all other clusters (except BER). On the contrary, the cluster BER received the lowest amount of immigrants (3.33%), all coming from the neighboring cluster POF.

The harmonic mean estimates of N_e ranged from 36.2 for POV1 to 4707.4 for POF2, with a negative value obtained for CAL2 (Table 2.1.2), indicating that the effect of drift would be different for each population (Waples and Do 2010). There is no indication that disturbed populations exhibited the lowest values of N_e .



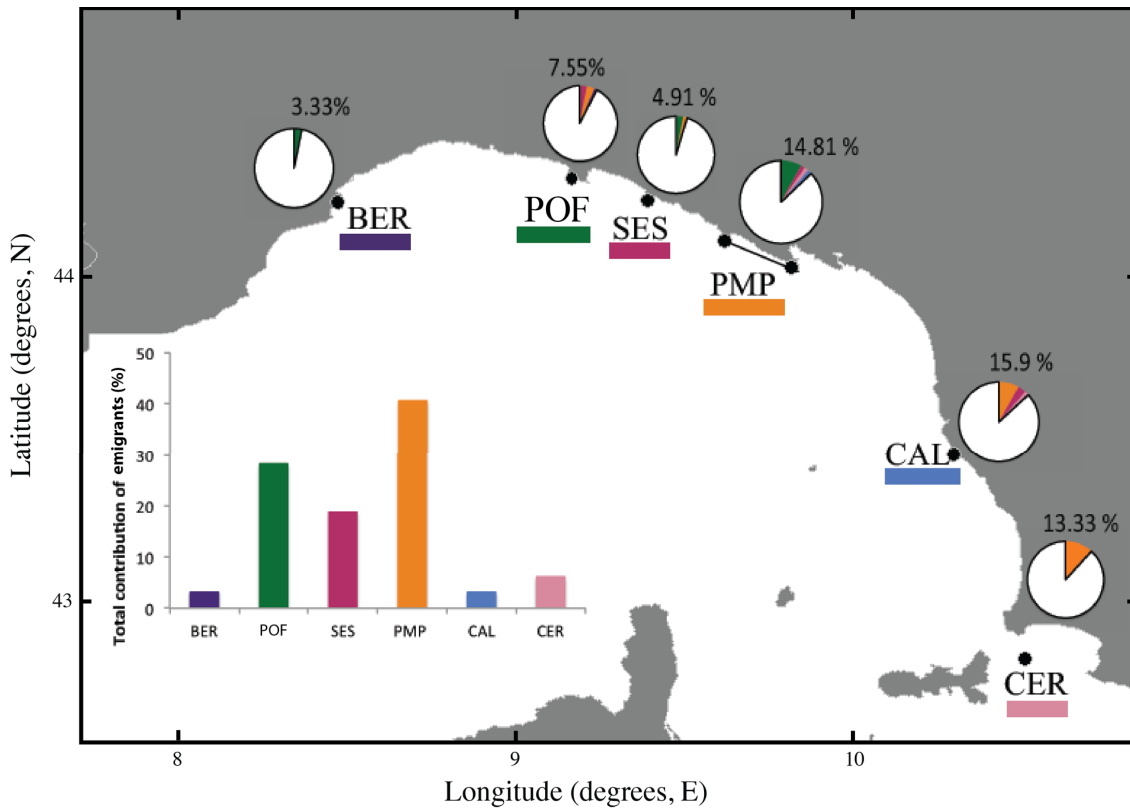


Figure 2.1.4.- Pattern of contemporary migration of *Paramuricea clavata* in the Ligurian Sea. The pie graphs represent the total proportion of immigration in each cluster. The white portion of the pie indicates the proportion of individuals identified as non-immigrants, while the colors represent the proportion of individuals identified as immigrants coming from different locations. The color purple represents the cluster of Bergeggi (BER), the color green indicates the cluster of Portofino (POF), the color fuchsia represents the cluster of Sestri Levante (SES), the color yellow indicates the cluster of Punta Mesco and Portovenere (PMP), the color blue indicates the cluster of Calafuria (CAL) and the color pink the cluster of Cerboli (CER). The bar plot indicates the total contribution of immigrants (%) coming from each cluster.

2.1.5 Discussion

We present genetic evidence that supports the role of connectivity in the recovery of marine populations affected by climate-induced disturbances. Although no





temporal comparisons can be made given the lack of data on the genetic structure of *P. clavata* before the mass mortality events in the Ligurian Sea, our results suggest that the contribution of immigration and gene flow within a network of connected populations helped the fast recovery of the affected populations by increasing the density of recruits and maintaining their genetic diversity (see Santangelo *et al.*, 2015).

2.1.5.1 *Do disturbed populations show a signal of reduced genetic diversity?*

Among the affected populations, evidence of a genetic bottleneck was only observed in Portovenere. No sign of a reduction in effective population size was found in Portofino. In fact, the largest value of N_e was estimated for that population. However, as reported by Waples and England (2011), it is likely that the estimated N_e values are upwardly biased given the high migration rates ($m > 0.2$) found among populations. Values of observed and expected heterozygosity (H_o and H_E , respectively), allelic richness (N_a) and effective population size (N_e) were smaller in Portovenere than in Portofino. However, given that the population of Portofino significantly recovered after three years, showing higher densities than before the mortality event (Cerrano *et al.*, 2005), it is not surprising to find a faster recovery of genetic diversity in this population.

Several authors had previously shown that bottleneck tests can fail to detect well-known population collapses in several species of conservation concern due to a combination of factors like the timing and duration of the bottleneck, immigration and the amount of pre-bottleneck genetic diversity (Spong and Hellborg 2002; Aguilar *et al.*, 2008; Henry *et al.*, 2009). Also, model simulations have demonstrated that when settlement is limited by space availability, population disturbances can lead to opposite effects of drift on genetic diversity depending on the conditions of the population prior to the disturbance: accelerating genetic drift when population census size is high with recruitment limitation, or decelerating genetic drift when population census size is low with no recruitment limitation (Padrón and Guizien, 2015).

Evidence of a population bottleneck was also found in the cluster of Bergeggi. Although, to our knowledge, no reports of mass mortality events exist for *P. clavata* in





that area, we believe that the observed signal may be also due to the heat waves that affected other sessile invertebrate species in the area (Parravicini *et al.*, 2010). In addition, the cluster of Bergeggi is characterized by the lowest contribution and reception of migrants from other populations, thus, with the lowest values of allelic richness, the retention of a genetic bottleneck signature in this site and its high genetic differentiation is not unexpected.

2.1.5.2 *Did connectivity play a role in the recovery of the affected populations?*

The analysis of migration over several generations revealed that all neighbouring populations are connected in both directions. In all populations, the effective number of migrants per generation suggests that, even though migration brings additional genetic variability into populations, local populations will continue diverging due to the combined effects of mutation and genetic drift, with the population of Bergeggi being the most noted case.

As observed by Arizmendi-Mejía *et al.*, (2015) when analyzing the genetic structure of *P. clavata* in the Balearic Islands (Spain), contemporary migration seems to play a major role in shaping the genetic structure of *P. clavata* populations in the Ligurian Sea. However, the percentage of individuals identified as migrants in our analysis was larger than previously reported and not limited to neighboring clusters, indicating that effective connectivity, even among distant clusters, is not negligible. The two populations affected by mass mortality events (Portofino and Portovenere) were found to be the most important contributors of migrants in the region, with Portovenere also receiving most of the migrants coming from all of the other populations. A possible explanation for the larger contribution of migration observed in this study maybe due to a combination of the increased reproductive output of the surviving colonies (Santangelo *et al.*, 2015), along with the sudden availability of suitable habitat after the mass mortality events (e.g. Bramanti *et al.*, 2015).

According to our results, spatial heterogeneity in the levels of gene flow coincide with the predominant pattern of surface currents recorded in the region during the





spawning period of *P. clavata*, which is characterized primarily by westward surface currents, and eastward currents around the areas of Portovenere and Calafuria creating a small gyre (Istituto Idrografico della Marina 1982; Cupido *et al.*, 2012). As an example, we found that the population of Bergeggi receives five times less migrants than the Calafuria population and, in general, it seems that the populations on the eastern part of the Ligurian Sea tend to receive more migrants than the populations on the western part of the region.

The genetic patch size for *P. clavata* was estimated to be 40 km in the Ligurian Sea. A size much larger than the continuous extent of suitable habitat (rocky bottom) around the seven locations sampled (see Table 2.1.1). The extension of the genetic patch size found in our study, compared to previous findings in other regions (Mokhtar-Jamaï *et al.*, 2011), suggests that dispersal limitation for *P. clavata* depends on local hydrology, demography and ecology rather than being related only to the life history trait of the species.

2.1.5.3 Implications of connectivity in a climate change scenario

This study represents a case in which, a species that has previously shown high genetic structuration and very limited dispersal, seems to exhibit higher connectivity after disturbances induced by climate change. This is particularly relevant now that the paradigm shift from “open” to “closed” marine populations has been nourished recently by growing evidence on the ability of populations to recover in isolation, due to high values of local retention (Gilmour *et al.*, 2013; Beger *et al.*, 2014). Nonetheless, model simulations have shown that high rates of local retention can weaken connectivity among populations (Figuereido *et al.*, 2014) and reduce their genetic diversity (Padrón and Guizien 2015).

Seascape genetic inference can be strongly influenced by the recent demographic history of populations. Without knowledge of the recent demographic collapse of some of the studied populations, the same results could have been ascribed to high isolation among populations, disregarding the effect of the seascape on the observed pattern of





spatial genetic structure. Therefore, our study highlights the importance of integrating demographic and genetic data in order to accurately assess the impact of climate-induced disturbances. Particularly with the application of population genetic tools that allow the estimation of contemporary migration in order to evaluate different scales of connectivity, and its role on populations' resilience.

According to climate change projections, extreme events will increase in frequency in the future (Meehl *et al.*, 2000), causing more frequent and severe demographic fluctuations, especially in small and isolated habitat patches. According to our results, this could drive the loss of genetic variability in sessile marine populations, with selection most likely acting within a context of increasing influence of genetic drift. This study increases our understating on the scale of connectivity of *P. clavata* in the Ligurian Sea, and helps identify key populations in need of protection. Our results provide empirical evidence on the role of connectivity in maintaining genetic diversity in unstable populations affected by climate change, and should be taken into account in effective marine spatial planning.

2.1.6 Acknowledgments

This work was (co-)funded through a MARES Grant. MARES is a Joint Doctorate programme selected under Erasmus Mundus coordinated by Ghent University (FPA 2011-0016). Check www.mares-eu.org for extra information. This work was also partly funded by the French National Program LITEAU IV of the Ministère de l'Écologie et de l'Environnement Durable under project RocConnect—Connectivité des habitats rocheux fragmentés du Golfe du Lion (PI, K. Guizien, Project Number 12-MUTS-LITEAU-1-CDS-013).

2.1.7 References

Agell, G., Rius, M., and Pascual, M. 2009. Isolation and characterization of eight polymorphic microsatellite loci for the Mediterranean gorgonian *Paramuricea clavata*.





Conservation Genetics, 10: 2025–2027.

- Aguilar, A., Jessup, D., Estes, J., and Garza, J. 2008. The distribution of nuclear genetic variation and historical demography of sea otters. *Animal Conservation*, 11: 35–45.
- Almany, G., Connolly, S., Heath, D., Hogan, J., Jones, G., McCook, L., Mills, M., *et al.* 2009. Connectivity, biodiversity conservation and the design of marine reserve networks for coral reefs. *Coral Reefs*, 28: 339–351.
- Arizmendi-Mejía, R., Linares, C., Garrabou, J., Antunes, A., Ballesteros, E., Cebrian, E., Díaz, D., *et al.* 2015. Combining genetic and demographic data for the conservation of a Mediterranean marine habitat-forming Species. *PLoS ONE*: 1–19.
- Aurelle, D., Ledoux, J., Rocher, C., Borsa, P., Chenuil, A., and Féral, J. 2011. Phylogeography of the red coral (*Corallium rubrum*): inferences on the evolutionary history of a temperate gorgonian. *Genetics*: 1–17.
- Beerli, P., and Felsenstein, J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences*, 98: 4563–4568.
- Beger, M., Selkoe, K., Treml, E., Barber, P., Heyden, von der, S., Crandall, E., Toonen, R., *et al.* 2014. Evolving coral reef conservation with genetic information. *Bulletin of Marine Science*, 90: 159–185.
- Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., and Bonhomme, F. 2004. GENETIX 4.05, Logiciel sous Windows™ pour la génétique des populations. Laboratoire, Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier, France.
- Benjamini, Y., and Hochberg, Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol*, 57: 289–300.
- Bramanti, L., Iannelli, M., Fan, T., and Edmunds, P. 2015. Using demographic models to project the effects of climate change on scleractinian corals: *Pocillopora damicornis* as a case study. *Coral Reefs*: 505–515.





- Bramanti, L., Magagnini, G., De Maio, L., and Santangelo, G. 2005. Recruitment, early survival and growth of the Mediterranean red coral *Corallium rubrum* (L 1758), a 4-year study. *Journal of Experimental Marine Biology and Ecology*, 314: 69–78.
- Caplins, S. A., Gilbert, K. J., Ciotir, C., Roland, J., Matter, S. F., and Keyghobadi, N. 2014. Landscape structure and the genetic effects of a population collapse. *Proceedings of the Royal Society B: Biological Sciences*, 281: 20141798.
- Cerrano, C., Arillo, A., Azzini, F., Calcinai, B., Castellano, L., Muti, C., Valisano, L., *et al.* 2005. Gorgonian population recovery after a mass mortality event. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 15: 147–157.
- Cerrano, C., Bavestrello, G., Bianchi, C., Cattaneo-vietti, R., Bava, S., Morganti, C., Morri, C., *et al.* 2000. A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-western Mediterranean), summer 1999. *Ecology Letters*: 1–10.
- Chapuis, M., and Estoup, A. 2007. Microsatellite null alleles and the estimation of population differentiation. *Molecular Biology and Evolution*, 24: 612–631.
- Coma, R., Ribes, M., Serrano, E., Jiménez, E., Salat, J., and Pascual, J. 2009. Global warming-enhanced stratification and mass mortality events in the Mediterranean. *Proceedings of the National Academy of Sciences*, 106: 6176–6181.
- Crow, J., and Kimura, M. 1970. *An introduction to population genetics theory*. Harper and Row.
- Cupido, R., Cocito, S., Manno, V., Ferrando, S., Peirano, A., Iannelli, M., Bramanti, L., *et al.* 2012. Sexual structure of a highly reproductive, recovering gorgonian population: quantifying reproductive output. *Marine Ecology Progress Series*, 469: 25–36.
- Cupido, R., Cocito, S., Barsanti, M., Sgorbini, S., Peirano, A., and Santangelo, G. 2009. Unexpected long-term population dynamics in a canopy-forming gorgonian coral following mass mortality. *Marine Ecology Progress Series*, 394: 195–200.
- Cupido, R., Cocito, S., Sgorbini, S., Bordone, A., and Santangelo, G. 2008. Response of a gorgonian (*Paramuricea clavata*) population to mortality events: recovery or loss?





Aquatic Conservation: Marine and Freshwater Ecosystems, 18: 984–992.

- Dempster, A., Laird, N., and Rubin, D. 1977. Maximum likelihood from incomplete data via EM algorithm. *J R Stat Soc Series B Stat Methodol*, 39: 1–38.
- Do, C., Waples, R., Peel, D., Macbeth, G., Tillett, B., and Ovenden, J. 2014. NeEstimatorv2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Molecular Ecology Resources*, 14: 209–214.
- Doney, S., Ruckelshaus, M., Emmett Duffy, J., Barry, J., Chan, F., English, C., Galindo, H., *et al.* 2012. Climate change impacts on marine ecosystems. *Annual Review of Marine Science*, 4: 11–37.
- Earl, D., and vonHoldt, B. 2011. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4: 359–361.
- Falush, D., Stephens, M., and Pritchard, J. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, 164: 1567–1587.
- Falush, D., Stephens, M., and Pritchard, J. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*, 7: 574–578.
- Figueiredo, J., Baird, A., Harii, S., and Connolly, S. 2014. Increased local retention of reef coral larvae as a result of ocean warming. *Nature Climate Change*, 4: 498–502.
- Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonné, P., Cigliano, M., Díaz, D., *et al.* 2009. Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. *Global Change Biology*, 15: 1090–1103.
- Gilmour, J., Smith, L., Heyward, A., Baird, A., and Pratchett, M. 2013. Recovery of an isolated coral reef system following severe disturbance. *Science*, 340: 69–71.
- Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available: <http://www.unil.ch/izea/software/fstat.html>.





- Haig, S. 1998. Molecular contributions to conservation. *Ecology*, 79: 413–425.
- Hardy, O., and Vekemans, X. 1999. Isolation by distance in a continuous population: reconciliation between spatial autocorrelation analysis and population genetics models. *Heredity*, 83: 145–154.
- Henry, P., Miquelle, D., Sugimoto, T., McCullough, D., Caccione, A., and Russello, M. 2009. *In situ* population structure and *ex situ* representation of the endangered Amur tiger. *Molecular Ecology*, 18: 3173–3184.
- Hill, W. 1981. Estimation of effective population size from data on linkage disequilibrium. *Genetical Research*, 38: 209–216.
- Hoegh-Guldberg, O., and Bruno, J. 2010. The impact of climate change on the world's marine ecosystems. *Science*, 328: 1523–1528.
- Hughes, T., Baird, A., Bellwood, D., Card, M., Connolly, S., Folke, C., Grosberg, R., *et al.* 2003. Climate Change, Human Impacts, and the Resilience of Coral Reefs. *Science*, 301: 929–933.
- Hughes, T., Graham, N., Jackson, J., Mumby, P., and Steneck, R. 2010. Rising to the challenge of sustaining coral reef resilience. *Trends in Ecology & Evolution*, 25: 633–642.
- Hutchinson, D., and Templeton, A. 1999. Correlation of pairwise genetic and geographic distance measures: Inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, 53: 1898–1914.
- Istituto Idrografico della Marina. 1982. Atlante delle correnti superficiali dei mari italiani: 1–45.
- Jakobsson, M., and Rosenberg, N. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, 23: 1801–1806.
- Johns, K., Osborne, K., and Logan, M. 2014. Contrasting rates of coral recovery and reassembly in coral communities on the Great Barrier Reef. *Coral Reefs*, 33: 553–563.
- Kalinowski, S. 2005. HP-RARE 1.0: a computer program for performing rarefaction on





measures of allelic richness. *Molecular Ecology Notes*, 5: 4015–4026.

Keller, L. F., Jeffery, K. J., Arcese, P., Beaumont, M. A., Hochachka, W. M., Smith, J. N. M., and Bruford, M. W. 2001. Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers. *Proceedings of the Royal Society B: Biological Sciences*, 268: 1387–1394.

Kovach, R., Muhlfeld, C., Wade, A., Hand, B., Whited, D., DeHaan, P., Al-Chokhachy, R., *et al.* 2015. Genetic diversity is related to climatic variation and vulnerability in threatened bull trout. *Global Change Biology*, 21: 2510–2524.

Lacy, R. 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection and population subdivision. *Conservation Biology*, 1: 143–158.

Ledoux, J., Garrabou, J., Bianchimani, O., Drap, P., Féral, J., and Aurelle, D. 2010. Fine-scale genetic structure and inferences on population biology in the threatened Mediterranean red coral, *Corallium rubrum*. *Molecular Ecology*, 19: 4204–4216.

Lett, C., Ayata, S., Huret, M., and Irisson, J. 2010. Biophysical modelling to investigate the effects of climate change on population dispersal and connectivity. *Progress in Oceanography*, 87: 106–113.

Linares, C., Coma, R., Garrabou, J., Díaz, D., and Zabala, M. 2008. Size distribution, density and disturbance in two Mediterranean gorgonians: *Paramuricea clavata* and *Eunicella singularis*. *Journal of Applied Ecology*, 45: 688–699.

Lipcius, R., Eggleston, D., Schreiber, S., Seitz, R., Shen, J., Sisson, M., Stockhausen, W., *et al.* 2008. Importance of metapopulation connectivity to restocking and restoration of marine species. *Reviews in Fisheries Science*, 16: 101–110.

Loiselle, B., Sork, V., Nason, J., and Graham, C. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany*, 82: 1420–1425. 6 pp.

Magris, R., Pressey, R., Weeks, R., and Ban, N. 2014. Integrating connectivity and climate change into marine conservation planning. *Biological Conservation*, 170: 207–221.





- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research*, 27: 209–220.
- Meehl, G., Zwiers, F., Evans, J., Knutson, T., Mearns, L., and Whetton, P. 2000. Trends in extreme weather and climate events: Issues related to modeling extremes in projections of future climate change. *Bulletin of the American Meteorological Society*, 8: 427–436.
- Mokhtar-Jamaï, K., Coma, R., Wang, J., Zuberer, F., Féral, J. P., and Aurelle, D. 2013. Role of evolutionary and ecological factors in the reproductive success and the spatial genetic structure of the temperate gorgonian *Paramuricea clavata*. *Ecology and Evolution*, 3: 1765–1779.
- Mokhtar-Jamaï, K., Pascual, M., Ledoux, J. B., Coma, R., Féral, J. P., Garrabou, J., and Aurelle, D. 2011. From global to local genetic structuring in the red gorgonian *Paramuricea clavata*: the interplay between oceanographic conditions and limited larval dispersal. *Molecular Ecology*, 20: 3291–3305.
- Molecular Ecology Resources Primer Development Consortium, Mokhtar-Jamaï, K., Bottin, L., Chaix, A., Aurelle, D., and Forcioli, D. 2010. Characterization of microsatellite loci for the Mediterranean red gorgonian, *Paramuricea clavata*. *Molecular Ecology Resources*: 1–8.
- Munday, P., Leis, J., Lough, J., Paris, C., Kingsford, M., Berumen, M., and Lambrechts, J. 2009. Climate change and coral reef connectivity. *Coral Reefs*, 28: 379–395.
- Nei, M. 1987. *Molecular evolutionary genetics*. Columbia University Press, New York.
- Nei, M., Maruyama, T., and Chakraborty, R. 1975. The bottleneck effect and genetic variability in populations. *Evolution*, 29: 1–10.
- Padrón, M., and Guizien, K. 2015. Modelling the effect of demographic traits and connectivity on the genetic structuration of marine metapopulations of sedentary benthic invertebrates. *ICES Journal of Marine Science*. doi: 10.1093/icesjms/fsv158.
- Palumbi, S. 2003. Population genetics, demographic connectivity and the design of marine reserves. *Ecological Applications*, 13: 5146–5158.





- Pandolfi, J., Connolly, S., Marshall, D., and Cohen, A. 2011. Projecting coral reef futures under global warming and ocean acidification. *Science*, 333: 418–422.
- Parravicini, V., Guidetti, P., Morri, C., Montefalcone, M., Donato, M., and Bianchi, C. 2010. Consequences of sea water temperature anomalies on a Mediterranean submarine cave ecosystem. *Estuarine, Coastal and Shelf Science*, 86: 276–282.
- Peery, M., Kirby, R., Reid, B., Stoelting, R., Doucet-B eer, E., Robinson, S., V asquez-Carrillo, C., *et al.* 2012. Reliability of genetic bottleneck tests for detecting recent population declines. *Molecular Ecology*, 21: 3403–3418.
- Pineda, J., Hare, J., and Sponaugle, S. 2007. Larval transport and dispersal in the coastal ocean and consequences for population connectivity. *Oceanologica Acta*, 20: 22–39.
- Piry, S., Alapetite, A., Cornuet, J., Paetkau, D., Baudouin, L., and Estoup, A. 2004. GENECLASS2: A Software for Genetic Assignment and First-Generation Migrant Detection. *Journal of Heredity*, 95: 536–539.
- Piry, S., Luikart, G., and Cornuet, J. 1999. BOTTLENECK: a program for detecting recent effective population size reductions from allele data frequencies. *Journal of Heredity*, 90: 502–503.
- Ponti, M., Perlini, R., Ventra, V., Grech, D., Abbiati, M., and Cerrano, C. 2014. Ecological shifts in Mediterranean coralligenous assemblages related to gorgonian forest loss. *PLoS ONE*, 9: e102782.
- Pritchard, J., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155: 945–959.
- Rannala, B., and Mountain, J. 1997. Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences*, 94: 9197–9201.
- Riegl, B., and Purkis, S. 2015. Coral population dynamics across consecutive mass mortality events. *Global Change Biology*, doi: 10.1111/gcb.13014.
- Rosenberg, N. 2004. DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, 4: 137–138..
- Rousset, F. (n.d.). GENEPOP'007: a complete re-implementation of the GENEPOP





- software for Windows and Linux. *Molecular Ecology Resources*, 8: 103–106.
- Sale, P., Cowen, R., Danilowicz, B., Jones, G., Kritzer, J., Lindeman, K., Planes, S., *et al.* 2005. Critical science gaps impede use of no-take fishery reserves. *Trends in Ecology & Evolution*, 20: 74–80.
- Santangelo, G., Bramanti, L., and Iannelli, M. 2007. Population dynamics and conservation biology of the over-exploited Mediterranean red coral. *Journal of Theoretical Biology*, 244: 416–423.
- Santangelo, G., Cupido, R., Cocito, S., Bramanti, L., Priori, C., Erra, F., and Iannelli, M. 2015. Effects of increased mortality on gorgonian corals (Cnidaria, Octocorallia): different demographic features may lead affected populations to unexpected recovery and new equilibrium points. *Hydrobiologia*: 1–17.
- Selig, E., Casey, K., and Bruno, J. 2012. Temperature-driven coral decline: the role of marine protected areas. *Global Change Biology*, 18: 1561–1570.
- Shanks, A., Grantham, B., and Carr, M. 2003. Propagule dispersal distance and the size and spacing of marine reserves. *Ecological Applications*, 13: 159–169.
- Sokal, R., and Wartenberg, D. 1983. A test of spatial autocorrelation analysis using an isolation-by-distance model. *Genetics*, 105: 219–237.
- Spong, G., and Hellborg, L. 2002. A near-extinction event in lynx: do microsatellite data tell the tale? *Conservation Ecology*, 6: 15.
- Underwood, J., Smith, L., van Oppen, M., and Gilmour, J. 2006. Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. *Molecular Ecology*, 16: 771–784.
- van Oppen, M., and Gates, R. 2006. Conservation genetics and the resilience of reef-building corals. *Molecular Ecology*, 15: 3863–3883.
- Vollmer, S., and Palumbi, S. 2006. Restricted gene flow in the Caribbean staghorn coral *Acropora cervicornis*: Implications for the recovery of endangered reefs. *Journal of Heredity*, 98: 40–50.
- Waples, R. 2006. A bias correction for estimates of effective population size based on





linkage disequilibrium at unlinked gene loci. *Conservation Genetics*, 7: 167–184.

Waples, R., and Do, C. 2010. Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evolutionary Applications*, 3: 244–262.

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

Waser, P., and Strobeck, C. 1998. Genetic assignment tests as indices of interpopulation dispersal. *Trends in Ecology & Evolution*, 13: 43–44.

Weir, B., and Cockerham, C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution*, 38: 1358–1370.

Wernberg, T., Russell, B., Thomsen, M., and Connell, S. 2014. Marine biodiversity and climate change. *In* *Global Environmental Change*, pp. 181–187. Ed. by B. Freedman. Springer Netherlands.



CHAPTER 2.2

Genetic connectivity of the white gorgonian,
Eunicella singularis, in the NW Mediterranean



Mariana PADRÓN^{1*}, Federica COSTANTINI², Sandra BAKSAY¹ and Katell
GUIZIEN¹

¹ Sorbonne Universités, UPMC Univ Paris 06, CNRS, Laboratoire d'Ecogéochimie des Environnements Benthiques (LECOB), Observatoire Océanologique, 66650, Banyuls sur Mer, France.

² Dipartimento di Scienze Biologiche, Geologiche ed Ambientali (BiGeA) & Centro Interdipartimentale di Ricerca per le Scienze Ambientali (CIRSA), University of Bologna, ULR CoNISMa, Via S. Alberto 163, I-48123 Ravenna, Italy.

N.B. This manuscript has been prepared for submission to the journal of Molecular Ecology.

2.2.1 Abstract

Understanding the development and maintenance of connectivity patterns is essential in marine spatial planning and the proper design of Marine Protected Areas (MPAs). However, the mapping of past and future connectivity is a current scientific challenge. Population genetics has been one of the most frequently used tools to provide direct and indirect measures of connectivity given that, for benthic sedentary species, it has been assumed that the dispersal potential of the larvae was correlated with gene flow. Here we present an analysis of the genetic population structure and regional migration patterns for *Eunicella singularis* in the Gulf of Lions and Spanish Catalan Coast using eight polymorphic microsatellite loci. Our results show that *E. singularis* is able to disperse over 200 km. Nonetheless, high levels of genetic population structure were observed at various spatial scales: a strong separation was observed between populations of the Gulf of Lions and the Spanish Catalan Coast, in agreement with the hydrodynamical isolation of the Gulf of Lions by the Northern Current. Moreover, within the Gulf of Lions, genetic structuration of *E. singularis* populations could be explained by flow structuration.





2.2.2 Introduction

For marine sessile benthic organisms with a pelagic larval phase, dispersal is crucial for the maintenance of demographic and genetic connection among populations. Long dispersal facilitates gene flow among distant populations, contributing to outcrossing and reducing genetic differentiation among populations. Conversely, limited dispersal increases the probability of inbreeding and leads to high genetic differentiation among populations (Hellberg *et al.*, 2002). Furthermore, the scale and patterns of connectivity will determine the ability of these sessile species to withstand severe disturbances (Cowen and Sponaugle 2007). The resilience of some populations may depend on the supply of larvae from distant and/or undisturbed populations. In some other cases, local production may be the only source of recruitment and could lead to slow and limited recovery (Almany *et al.*, 2009).

Given the difficulties in assessing patterns of dispersal through direct empirical data, genetic methods are frequently used to estimate population connectivity, by quantifying gene flow and barriers to gene flow (Lowe and Allendorf 2010). The existence of tests that allow the assignment of individuals to their populations of origin through the use of highly variable DNA markers (e.g. microsatellites) also enables the inference of current migration patterns and can reveal additional information about the effect of seascape processes on the reproductive success of immigrants (Hedgecock *et al.*, 2007; Underwood *et al.*, 2007; Baguette *et al.*, 2012).

Ecosystem engineers, such as gorgonian corals, play a fundamental ecological role in maintaining the diversity, biomass and structural complexity of hard-bottom communities in the Mediterranean Sea (Weinberg 1978; Ballesteros 2006). Several anthropogenic and climate-related disturbances, such as destructive fishing practices (Bavestrello *et al.*, 1997) and seawater temperature increases have severely affected gorgonian species causing multiple mass mortality events along the northwestern Mediterranean (Perez *et al.*, 2000; Cerrano *et al.*, 2005; Coma *et al.*, 2006, 2009). From these gorgonian species, the white gorgonian *Eunicella singularis*, is the most abundant and





widely distributed in the north western Mediterranean (Weinberg 1978; Linares *et al.*, 2008; Gori *et al.*, 2011).

E. singularis is an internal brooder species that reproduces annually, which means that every year its larvae are produced sexually through internal fertilization of the eggs, brooded within the polyp, and then released into the water column during the summer months of June and July (Weinberg and Weinberg 1979; Ribes *et al.*, 2007). The larvae of *E. singularis* exhibit high activity frequency and energetic content (Villadrich 2015). Furthermore, the presence of symbiotic algae may supply them extra energy (Weinberg and Weinberg 1979), suggesting that the species may have high dispersal capacity.

Being such an abundant and widely distributed species, identifying the factors that drive the patterns of population structure is not straightforward. The variability in spatial distribution and density of *E. singularis* populations across the NW Mediterranean are believed to be related to the requirement for hard bottom substrates (Gori *et al.*, 2011), and other factors (e.g. predation, interspecific competition, disturbances) rather than intraspecific competition (Linares *et al.*, 2008). Although Cataneo (2011) studied the population genetic structure of *E. singularis* in the Mediterranean basin, discerning the pattern of realized connectivity among populations was not possible, probably due to the limited number of microsatellite loci analyzed. Therefore, the processes shaping the population density and spatial distribution of the species at a regional scale have not yet been revealed.

The Gulf of Lions (NW Mediterranean Sea) is one of the most productive areas of the Mediterranean. Characterized by high concentration of nutrients, low temperature and low salinity due the influence of the Rhône river (the largest river of the basin), the waters of the Gulf of Lions are confined over the shelf by a density front, which is in dynamic equilibrium with Liguro-Provençal current (UNEP-MAP-RAC/SPA 2013). This area has been identified as an isolated hydrodynamic province in larval dispersal studies (Guizien *et al.*, 2012, 2014; Rossi *et al.*, 2014), and in biogeochemistry studies





(Nieblas *et al.*, 2014), suggesting it would be the scale of closure of metapopulation for benthic sessile invertebrates. Although an area of interest for studies of genetic structuration on commercial fish species (Lenfant and Planes 1996; García de León *et al.*, 1997; Morat *et al.*, 2014), no analysis of population genetic structure has been carried out for other widely distributed sessile invertebrate species in the region.

Therefore, by assessing the genetic population structure and regional migration patterns using eight polymorphic microsatellite loci for *E. singularis* in the Gulf of Lions and along the Spanish Catalan Coast, this work represents the first analysis of genetic connectivity for a non-commercial species in the region, and aims to answer the following questions: 1) does *E. singularis* have dispersal capacity? 2) does genetic differentiation enables to identify the Gulf of Lions as the closure scale of a metapopulation?, and 3) can the pattern of genetic population structure be explained by the regional hydrodynamics?.

2.2.3 Material and Methods

2.2.3.1 Study area and regional hydrodynamics

The Gulf of Lions (hereafter GoL) is a large, microtidal continental shelf of 100 km radius located in northwestern Mediterranean Sea (Figure 2.2.1). It is mostly covered by soft sandy sediment down to 30 m depth. However, fragmented patches of hard bottom substrate can be found in the eastern (Marseille), central (Leucate and Agde), and western (Banyuls and Cap de Creus) parts of the Gulf (Guizien *et al.*, 2012). Circulation in the GoL is driven by three main forcings: 1) Strong continental winds (the Mistral and the Tramontane) that frequently generate upwellings and downwellings along the coast, as well as transient currents and eddies 2) the Northern Current (NC), which usually flows along the shelf break of the GoL from the Ligurian Sea to the Catalan Sea; 3) several rivers, particularly the Rhône, that provide freshwater and important quantities of nutrients and organic matter (Millot 1990; Hu *et al.*, 2009; Campbell *et al.*, 2013).





A general westward circulation characterizes the GoL, with the NC acting as a barrier limiting the transport of particles and larvae across the shelf break. In fact, numerical simulations have shown that the only successful way for anchovy larvae to escape the GoL is in front of Cap de Creus (Nicolle *et al.*, 2009). During summer (spawning period of *E. singularis*), both simulated and empirical data have shown the presence of a persistent anticyclonic circulation in the southwestern part of the GoL. During the LAgrangian Transport EXperiment (LATEX), this eddy was observed between mid-July to mid-August, at which point it finally approaches the Northern Current and interacts with it (Hu *et al.*, 2009). Further south, along the Catalan shelf, intense and persistent eddies have also been registered in summer. These so called Catalan eddies are believed to originate in the GoL, in the lee of Cap de Creus (Rubio *et al.*, 2009; Garreau *et al.*, 2011). It has been hypothesized that the continuity between the LATEX and Catalan eddies, along with the presence of a northward coastal current off the Cap de Creus could transport passive larvae from the Catalan Sea towards the Gulf of Lions (Garreau *et al.*, 2011).

2.2.3.2 Sampling

Small apical fragments (<3 cm) of 13-36 colonies of *E. singularis* were randomly collected by scuba divers in 30 different sites along the NW Mediterranean, from Marseille (France) to Blanes (Spain) (Figure 2.2.1; Table 2.2.1). The sites were distributed from less than 1 km to over 300 km apart. A total of seven locations were visited (Côte Bleue, Plateau des Aresquiers, Cap d'Agde, Côte Vermeille, Cap de Creus, Montgri and Costa Brava) and the number of sites sampled in each location varied according to the density of *E. singularis* and the size of the patch with suitable habitat (rocky bottom).

In France, four locations were visited (Côte Bleue, Plateau des Aresquiers, Cap d'Agde, Côte Vermeille). In Côte Bleue, the location with the lowest density of colonies, three sites were sampled; four sites in Plateau des Aresquiers and Cap d'Agde, while six sites were sampled in the Côte Vermeille due to the extensive coverage of rocky bottom in the area. In Spain three locations were visited (Cap de Creus, Montgri and Costa Brava). In





Cap de Creus, the location with the highest frequency of occurrence and the highest densities of *E. singularis* (Gori *et al.*, 2011) eight sites were sampled; while, given the lower occurrence of *E. singularis* in the Costa Brava (Linares *et al.*, 2008), only two sites were sampled. All of the samples were preserved in 95% ethanol and kept at -20 °C until DNA extraction.

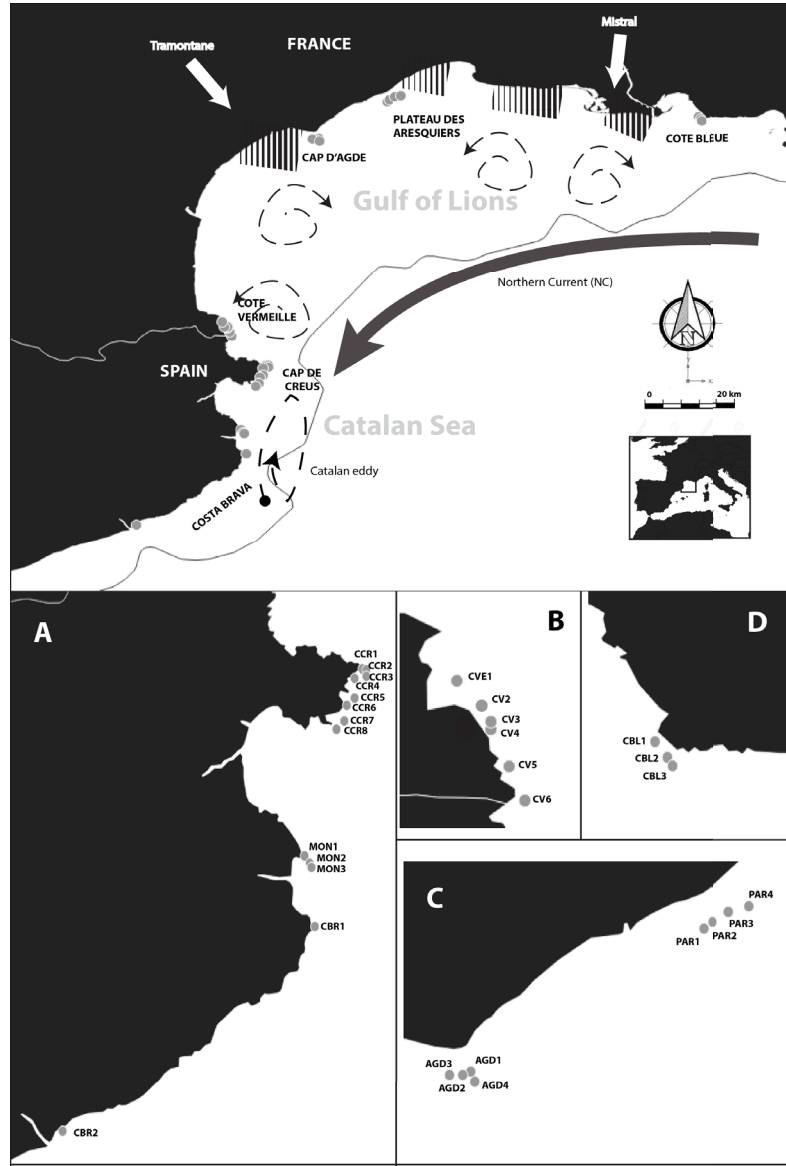


Figure 2.2.1.- Regional hydrodynamics and sampling locations of the populations of *Eunicella singularis* in the NW Mediterranean (grey dots).



Table 2.2.1.- Description of sampling locations of *Eunicella singularis* in the NW Mediterranean.

Country	Location name	Site	Latitude	Longitude	Depth (m)	Sample size
France	Côte Bleue	CBL1	43.3443	5.0098	23	17
		CBL2	43.3335	5.0197	16	22
		CBL3	43.3280	5.0232	21	13
	Plateau des Aresquiers	PAR1	43.4060	3.7865	15	36
		PAR2	43.4132	3.7964	10	30
		PAR3	43.4240	3.8162	10	27
		PAR4	43.4301	3.8392	11	28
	Cap d'Agde	AGD1	43.2560	3.5146	19	28
		AGD2	43.2525	3.5048	17	26
		AGD3	43.2525	3.4899	15	26
		AGD4	43.2452	3.5197	27	21
	Côte Vermeille	CVE1	42.4425	3.1741	14	25
		CVE2	42.4589	3.1663	22	29
		CVE3	42.4768	3.1572	17	33
		CVE4	42.4804	3.1572	21	25
		CVE5	42.0480	3.1451	12	14
CVE6		42.5003	3.1398	24	13	
Spain	Cap de Creus	CCR1	42.3247	3.3111	18-21	30
		CCR2	42.3230	3.3186	18	30
		CCR3	42.3144	3.3186	10-20	30
		CCR4	42.3119	3.2977	17-20	30
		CCR5	42.2841	3.2977	15-17	30
		CCR6	42.2733	3.2844	15-17	30
		CCR7	42.2511	3.2808	17	25
		CCR8	42.2391	3.2675	20	30
	Montgri	MON1	42.0613	3.2125	16-19	25
		MON2	42.0513	3.2213	18-20	30
MON3		42.0452	3.2241	16-19	30	
Costa Brava	CBR1	42.9613	3.2308	12-15	30	
	CBR2	41.6727	2.8027	15-17	30	





2.2.3.3 DNA extraction and microsatellite genotyping

Total genomic DNA was extracted from 793 colonies of *E. singularis* using the Promega's DNA extraction kit Wizard. All individuals were genotyped at eight polymorphic microsatellite loci specifically developed for *E. singularis*: C21, C30 and C40 (Molecular Ecology Resources Primer Development Consortium 2010) and *E. verrucosa*: Ever003, Ever004, Ever009, Ever013 and Ever014 (Holland *et al.*, 2013). All the loci were amplified using the PCR protocol described in Holland *et al.*, (2013). PCR products were analyzed on an ABI 3130 Genetic Analyzer using an internal size standard (GeneScan 500 LIZ; Applied Biosystems). The scoring of alleles was carried out with GeneMapper v.3.5 software (Applied Biosystems).

2.2.3.4 Genetic diversity

We analyzed the genetic diversity of *E. singularis* samples by estimating observed heterozygosity (H_o) and Nei's (1973) unbiased expected heterozygosity (H_e) with Genetix v.4.05 (Belkhir *et al.*, 2004).

Allelic richness (N_a) was estimated using Genodive v.2.0b.27 (Meirmans and van Tienderen 2004), while rarefied allelic (A_r) and private allelic richness (A_p) were calculated with a rarefaction procedure using the HP-Rare software (Kalinowski 2005) with the minimum number of genes set to 20. In order to investigate levels of inbreeding in each site, single and multilocus F_{is} were estimated using Weir and Cockerman's (1984) fixation index using Genepop.

2.2.3.5 Genetic structure

Population structure was calculated using different approaches: measure of genetic differentiation (F_{ST}), which estimates levels of genetic differentiation among populations based on the variance of allele frequencies; a Bayesian clustering method, which by sorting individuals into Hardy–Weinberg/linkage equilibrium populations, creates clusters of individuals that share similar patterns of variation; and a hierarchical analysis of molecular variance (AMOVA), which allows to estimate the partition of molecular variance (F-statistics) into its components, among groups of populations,



among populations within groups and within populations.

Global and pairwise population genetic differentiation was measured with Weir and Cockerman's θ estimator of F_{ST} in Genepop, and its significance was computed with the exact test of genic differentiation using the default parameters. Pairwise F_{ST} estimates were also calculated following the excluding null allele (ENA) method in Freena (Chapuis and Estoup 2007). In order to visualize the genetic relationships among sites, a genetic distance matrix derived from the pairwise F_{ST} was used to construct a graph of principal coordinates analysis (PCA) in Genalex.

The spatial genetic structure was analyzed by testing the presence of a pattern of Isolation By Distance (IBD). A linear regression between pairwise $F_{ST} / (1-F_{ST})$ values and the linear geographical distances between samples was performed, and the significance of the regression was tested with a Mantel test ($n=10,000$) (Mantel 1967) using the Isolde program implemented in Genepop. For all analyses, significance levels were corrected using a false discovery rate (FDR) correction for multiple tests (Benjamini & Hochberg 1995) when necessary.

Unlike Cataneo (2011), the Bayesian clustering method implemented in Structure v.2.3.4 (Pritchard *et al.*, 2000; Falush *et al.*, 2003, 2007) was used to evaluate the number of clusters (K) in the dataset without prior information regarding the geographic location of the samples. As suggested by Falush (2007) when dealing with non-discrete populations, we selected the admixture model and chose the assumption of correlated allele frequencies and the option of recessive alleles to cope with null alleles. Mean and variance of log likelihoods were estimated for the number of clusters, $K=1$ to $K=30$. Ten runs were performed for each K with 1,000,000 iterations and a burn-in period of 100,000. In order to identify the number of clusters that best fit the data, the resulting output files were then analyzed using the Evanno method in Structure Harvester v.0.6.94, as implemented for online use (<http://taylor0.biology.ucla.edu/structureHarvester/>) (Earl and vonHoldt 2012). The clustering observed for $K=2$ were then retained to subdivide the samples in two groups





and evaluate additional sub-structuring within each cluster. Clumpp v.1.1.2 (Jakobsson and Rosenberg, 2007) was used to average the results for the chosen K, and the graphical representation was performed in Distruct v.1.1 (Rosenberg 2004).

A hierarchical analysis of molecular variance (AMOVA) (1,000 permutations) was performed with Arlequin v.3.5 (Excoffier *et al.*, 2005) using the clusters (K=2) and subclusters (K=3) defined by Structure.

2.2.3.6 Migration patterns

Contemporary connectivity among populations was analyzed by estimating first generation migrants and average migration rates over the last several generations. The amount and direction of gene flow over the last several generations among sites was estimated by performing a Bayesian analysis using BayesAss 1.3 (Wilson and Rannala 2003). First generation migrants were detected using the Bayesian computation of Rannala and Mountain (1997) ($n=10,000$; $\alpha = 0.01$) in GeneClass v.2.0 (Piry *et al.*, 2004).

In order to complement the information on contemporary connectivity and estimate the extent of gene diffusion, multilocus genotypes were autocorrelated with spatial distance classes using the kinship coefficient of Loiselle, as implemented in Spagedi v.1.4 (Hardy and Vekemans 2002). The kinship coefficient of Loiselle (Loiselle *et al.*, 1995) provides a relative index of relatedness between each pair of individuals. Significant positive coefficients suggest that colonies within a specific distance class are more genetically related than colonies randomly taken from any distance class. Spatial distance intervals for kinship comparisons were constructed in such a way that the number of pairwise comparisons within each distance interval was approximately constant (around 30000 pairs among distance classes). A total of 11 distance classes were considered: class 1: < 1 km; class 2: 1 – 5 km; class 3: 6 – 22 km; class 4: 23 – 30 km; class 5: 31 – 48 km; class 6: 49 – 90 km; class 7: 91 – 105 km; class 8: 106 – 121 km; class 9: 122 – 135 km; class 10: 136 – 178 km; class 11: 179 – 260 km. Significance of the observed coefficients relative to the expected was tested for each class using 10,000 random permutations of locations.





2.2.4 Results

2.2.4.1 Genetic diversity

Observed heterozygosity (H_o) varied from 0.46 to 0.67 with a mean value of 0.53 ± 0.05 . Expected heterozygosity (H_e) ranged from 0.47 to 0.64 and presented a mean value of 0.56 ± 0.03 . The highest number of alleles (N_a) was found in Montgri (MON3) (6.00), while Côte Bleue (CBL2) exhibited the lowest value (3.25). Rarefied allelic richness (A_r) and private allelic richness (A_p) among populations were 2.82 ± 0.21 and 0.06 ± 0.05 , respectively. Rarefied allelic richness ranged from 2.35 for Cap d'Agde (AGD2) to 3.74 for Montgri (MON1). The private allelic richness varied from 0.01 for Cap d'Agde (AGD3) and Cap de Creus (CCR6) to 0.20 for Montgri (MON1). No significant differences were found in the values of number of alleles, rarefied allelic richness and private allelic richness among the clusters identified by Structure (Kruskal-Wallis, P -values > 0.05). Multilocus F_{IS} values ranged from -0.136 for Plateau des Aresquiers (PAR3) to 0.251 for Cap de Creus (CCR2). Significant differences in F_{IS} were found among clusters (Kruskal-Wallis, P -value < 0.001) (Table 2.2.2).

2.2.4.2 Genetic structure

Overall F_{ST} value was high (0.185) and statistically significant (P -value < 0.001). Pairwise F_{ST} values (Table S1) ranged from -0.004 for PAR1 vs. PAR2 to 0.305 for MON2 vs. CBL2. No significant differences were found between pairwise F_{ST} and pairwise F_{ST} corrected for null alleles (t-test, $P > 0.05$). Almost all pairwise differentiation tests were significant (after FDR correction). The non-significant comparisons included some sites separated by 10 km or less (PAR1 vs. PAR2, PAR1 vs. PAR4, PAR2 vs. PAR3, AGD2 vs. AGD3, CVE4 vs. CVE6, CCR6 vs. CCR2 and CCR8 vs. CCR2), while others involved one site in Côte Vermeille separated by over 100 km (CVE5 vs. PAR1, CVE5 vs. PAR3, CVE5 vs. PAR4 and CVE5 vs. AGD1).





Table 2.2.2.- Parameters of genetic diversity of *E. singularis* at eight microsatellite loci in 30 sites along the Gulf of Lions and Spanish Catalan Coast.

Site	H _O	H _E	Na	Ar	Ap	F _{IS}
CBL1	0.47	0.51	4.12	2.73	0.06	-0.040
CBL2	0.46	0.47	3.25	2.59	0.02	0.096
CBL3	0.55	0.55	3.50	2.73	0.05	-0.030
PAR1	0.51	0.53	3.75	2.79	0.06	0.055
PAR2	0.47	0.54	3.87	2.82	0.08	-0.091
PAR3	0.59	0.59	4.75	2.93	0.02	-0.136
PAR4	0.54	0.53	4.00	2.67	0.03	0.090
AGD1	0.63	0.59	4.75	2.61	0.06	0.074
AGD2	0.52	0.56	4.00	2.35	0.02	0.092
AGD3	0.65	0.59	4.37	2.56	0.01	-0.006
AGD4	0.60	0.58	3.50	2.65	0.02	0.022
CVE1	0.51	0.54	4.12	2.69	0.04	0.198
CVE2	0.58	0.55	3.50	3.07	0.16	0.073
CVE3	0.50	0.53	5.25	2.68	0.02	-0.032
CVE4	0.57	0.53	4.50	2.94	0.11	-0.060
CVE5	0.67	0.59	4.50	2.83	0.04	0.063
CVE6	0.48	0.52	4.37	3.05	0.08	-0.017
CCR1	0.50	0.55	4.50	2.65	0.08	0.132
CCR2	0.47	0.58	4.25	2.98	0.05	0.251
CCR3	0.54	0.58	4.37	2.91	0.06	0.106
CCR4	0.49	0.58	4.00	2.83	0.02	0.169
CCR5	0.52	0.61	5.37	3.19	0.07	0.214
CCR6	0.53	0.58	4.00	2.88	0.01	0.134
CCR7	0.51	0.60	5.50	3.07	0.18	0.244
CCR8	0.49	0.58	4.87	2.91	0.04	0.163
MON1	0.60	0.64	5.50	3.24	0.20	0.187
MON2	0.51	0.55	3.62	2.66	0.04	0.070
MON3	0.60	0.63	6.00	3.18	0.15	0.086
CBR1	0.55	0.58	4.12	2.83	0.03	0.100
CBR2	0.48	0.55	3.62	2.67	0.02	0.127
Mean	0.53 ±	0.56 ±	4.32 ±	2.82 ±	0.06 ±	0.078 ±
value	0.05	0.03	0.68	0.21	0.05	0.098

H_O, observed heterozygosity; H_E, unbiased expected heterozygosity; Na, Number of alleles; Ar, rarefied allelic richness; Ap, private allelic richness; F_{IS}, Weir and Cockerham's (1984) estimate of Wright's (1951) fixation index with significant values in bold (0.05 threshold after FDR correction).





The Bayesian clustering analysis identified two genetic clusters ($K = 2$) (Figure 2.2.2A): one cluster grouping all of the sites within the Gulf of Lions (17 sites), and another cluster composed of all the sites sampled along the Spanish Catalan Coast (13 sites). The results of the AMOVA confirmed the clustering structure ($P < 0.001$) (Table S2). Analysis of sub-structuring within each cluster, on the other hand, showed contrasting results. The cluster of Gulf of Lions exhibited significant levels of genetic structuration ($P < 0.001$), identifying three genetic sub-clusters ($K=3$) corresponding to the geographic origin of the samples (Figure 2.2.2B; Table S3). Conversely, although the results from Structure indicated genetic subdivision within the cluster of the Spanish Catalan Coast as well ($K=3$), the pattern of structuration was not statistically significant ($P > 0.05$) (Figure 2.2.2C; Table S4).

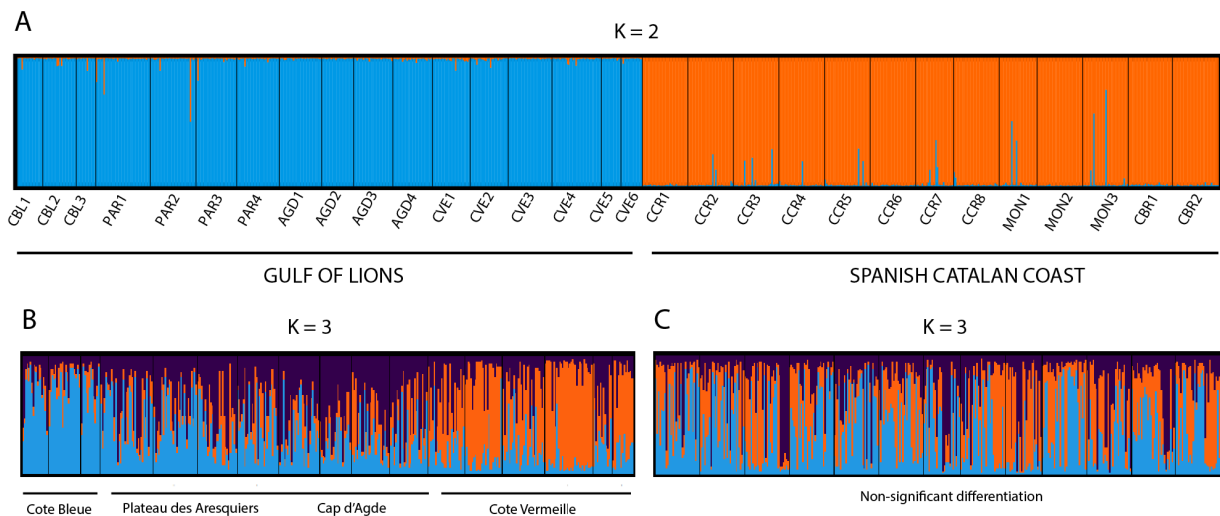


Figure 2.2.2.- Population structure of *E. singularis* in the Gulf of Lions and Spanish Catalan Coast, as revealed by Structure software. Each individual is represented by a vertical color line, with each color representing the proportion of membership to each cluster. Black lines represent the division among sampled populations. A) Analysis of all samples from the NW Mediterranean ($K = 2$). B) Substructure analysis of the cluster of France ($K = 3$). C) Substructure analysis of the cluster of Spain ($K = 3$).

The PCA analysis illustrated the close relationship among sites within each location for the Cluster of France, and the overall distinctiveness of each site in the



cluster of Spain (Figure 2.2.3). A positive and significant correlation between $F_{ST} / (1 - F_{ST})$ and linear geographic distances was found for the GoL and Spanish Catalan Coast ($R^2 = 0.19$; P -value < 0.01 ; Figure S1). A pattern of IBD was also observed within each genetic cluster, although a stronger relationship was found for the cluster of the GoL, relative to the cluster of the Spanish Catalan Coast ($R^2 = 0.39$, $R^2 = 0.20$, respectively; P -value < 0.01 in both cases; Figure S1). However, the slope of the linear regression suggests that the pattern of IBD is weak.

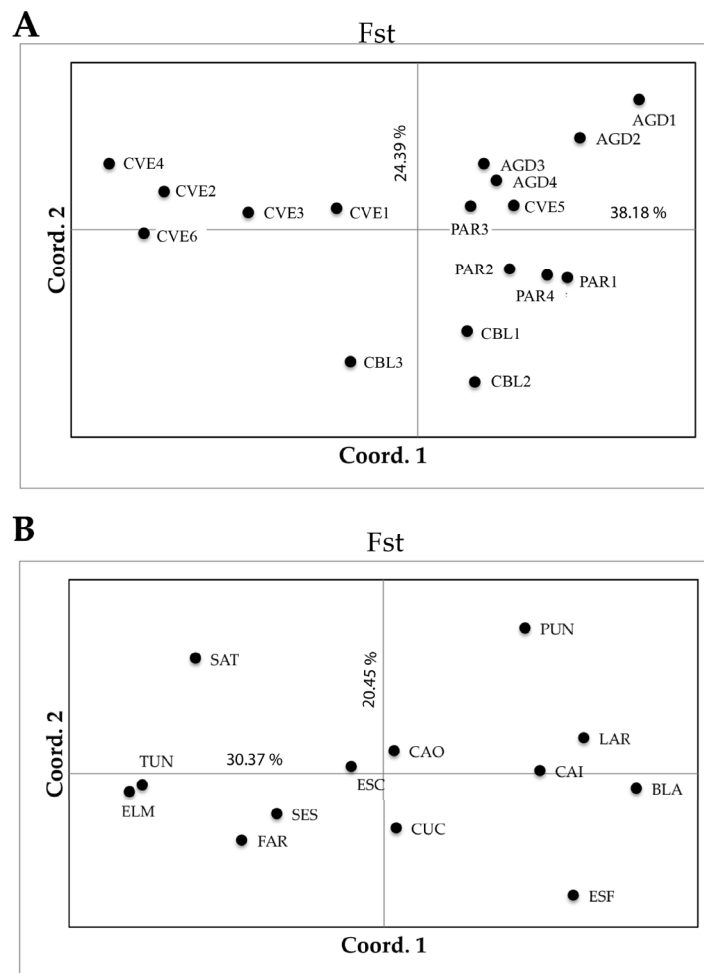


Figure 2.2.3.- Plots of principal coordinate analysis from genetic distance matrix estimated with pairwise F_{ST} estimates of *E. singularis* sites for each genetic cluster identified by Structure. A) Cluster of Gulf of Lions. First two axes explain 60.41% of variation. B) Cluster of Spanish Catalan Coast. First two axes explain 50.82% of variation.





2.2.4.3 Migration patterns

The analysis of first generation migrants identified 22 colonies (2.77% of all individuals; Figure S2) as originating from a site different to the one they were collected at. This result revealed a higher intra-cluster migration than inter-cluster migration between the two genetic clusters (Gulf of Lions and Spanish Catalan Coast), and showed that inter-cluster migration is uni-directional and only takes place in a westward fashion (from France to Spain), although over very long distances. Two colonies in Montgri (MON3) were identified as immigrants coming from Côte Vermeille (CVE4) (>50 km) and Côte Bleue (CBL2) (>200 km) (Figure 2.2.4). Within each cluster, on the other hand, migration can be bi-directional and also over long distances (> 150 km). In the cluster of France, for example, first generation migrants found in two different sites of Côte Vermeille (CVE1 and CVE2) were identified as coming from Côte Bleue (CBL1 and CBL2). While in the cluster of the Spanish Catalan Coast, one first generation migrant found in the northernmost population of Cap de Creus (CCR1) was identified as originating from the southernmost population of Costa Brava (CBR2).

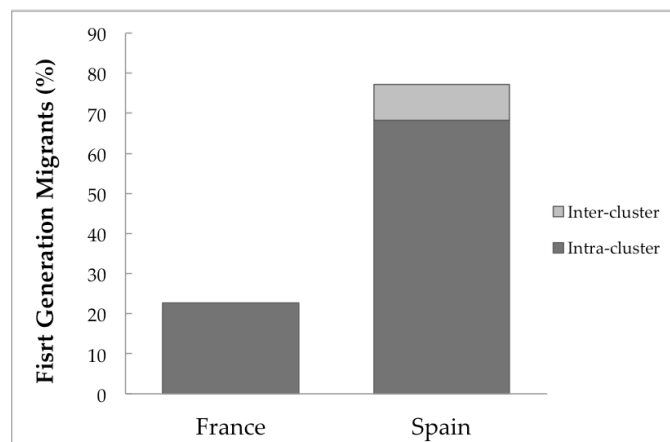


Figure 2.2.4.- Migration patterns of *Eunicella singularis* in the NW Mediterranean. Each bar represents the percentage of First Generation Migrants to each cluster (Gulf of Lions and Spanish Catalan Coast). The dark gray corresponds to the percentage of recent migrants coming from populations within the same cluster (intra-cluster immigration), and the light gray indicates the percentage of recent migrants coming from another cluster (inter-cluster immigration), as detected by Geneclass 2.0.



The kinship coefficient of Loiselle was positive and significant for the first three distance classes: < 1 km ($P < 0.001$), 1 - 5 km ($P < 0.001$) and 2 - 22 km ($P < 0.001$) distance classes (Figure 5). The genetic patch size, based on the first x-intercept of the autocorrelogram, was estimated to be 30 km.

Recent migration rates among *E. singularis* populations were mainly uni-directional in the NW Mediterranean, with some cases of bi-directional migration at low rates. Highest values of migration rate were found between Cap d'Agde and Plateau des Aresquiers within the Gulf of Lions, and between Cap de Creus and Costa Brava along the Spanish Catalan Coast (Table S5).

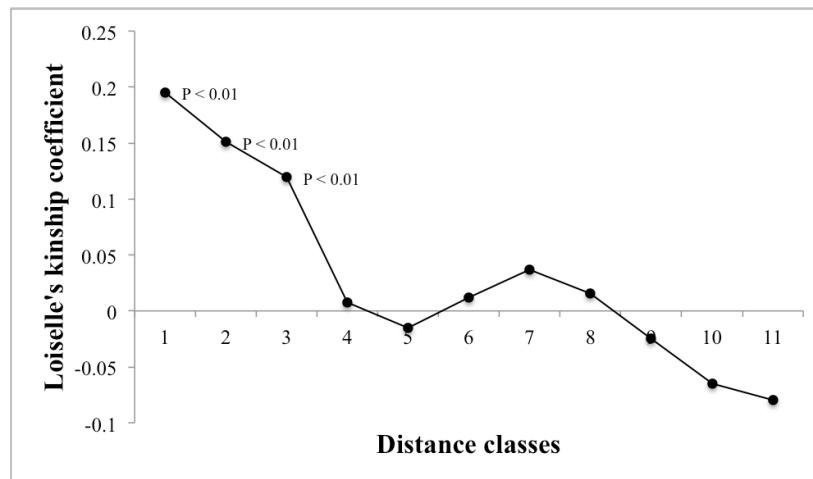


Figure 5.- Autocorrelogram based on the kinship coefficient of Loiselle, estimated for multilocus genotypes within 11 distance classes. Class 1: < 1 km; class 2: 1 - 5 km; class 3: 6 - 22 km; class 4: 23 - 30 km; class 5: 31 - 48 km; class 6: 49 - 90 km; class 7: 91 - 105 km; class 8: 106 - 121 km; class 9: 122 - 135 km; class 10: 136 - 178 km; class 11: 179 - 260 km.

2.2.5 Discussion

The present study supports the expectation of dispersal capacity for *E. singularis*, commonly within 30 km of their natal colony, as demonstrated by spatial autocorrelation analysis, and supplemented by successful migrants over very long distances (> 200 km).





The dispersal distance found for *E. singularis* in this study is much larger than those reported for other common gorgonian species, like the red coral (*Corallium rubrum*) and the red gorgonian (*Paramuricea clavata*) (Costantini *et al.*, 2007; Mokhtar-Jamaï *et al.*, 2013; Ledoux *et al.*, 2010). The variability of inferred dispersal distances based on genetic studies of gorgonian species suggests that regional hydrodynamics may play an important role in filtering the dispersive potential of a species, causing the size of genetic patches to be related to the sites rather than the species. Nonetheless, it must be taken into account that gene flow between distant localities does not necessarily take place over one generation. It may also occur over multiple generations following a stepping-stone model, as it has been observed in other invertebrate species (Kimura and Weiss 1964; Crandall *et al.*, 2012). And even if long distance migration did take place over one generation, as evidenced by the first generation migrant identified in Montgri (MON3) as coming from Côte Bleue (CBL2), it does not mean that it is a recurrent event.

It is well known that only a few successful larvae may be enough to provide sufficient genetic connectivity across broad spatial scales over evolutionary time (Hedgecock *et al.*, 2007). Successful dispersal of *E. singularis* over such long distances in the NW Mediterranean could actually be a rare event happening only once during the lifetime of the species.

Strong genetic differentiation was found between the Gulf of Lions and Spanish Catalan Coast genetic clusters. Unlike the few studies of population genetic structure in the region, which found no genetic differentiation among populations of commercial fishes in the Gulf of Lions and the Catalan Sea (Lenfant and Planes, 1996; García de León *et al.*, 1997; Morat *et al.*, 2014), this study evidences a clear discontinuity of gene flow between populations north and south of Cap de Creus, separated by only ~20 km. The influence of the NC may be a good explanation for the genetic discontinuity among populations along the French and Spanish coasts.

This study confirms the presence of a biophysical barrier that seems to interrupt gene flow for sessile benthic invertebrates across the north and the south of Cap de





Creus, at least when larval dispersal takes place during summer, as shown by Mokhtar-Jamaï *et al.* (2011). Guizien *et al.* (2014) identified the Gulf of Lions as the scale of closure for metapopulation simulations of soft-bottom species based on arguments regarding hydrodynamical isolation by the NC and habitat spatial distribution. The present study suggests that metapopulation modelling for *E. singularis* could also be performed within the Gulf of Lions, although hard bottom substrate extends south of Cap de Creus.

Similarly, patterns of population genetic connectivity within each cluster may be well explained by the local hydrodynamics. The observed pattern of genetic population structure of *E. singularis* in the NW Mediterranean has a weak relationship with geographic distance. Overall, our results are evidence of the strong influence of regional hydrodynamics on the dispersal, successful recruitment and genetic connectivity of *E. singularis*. In the GoL, the potential interaction of the LATEX and Catalan eddies, along with the general circulation patterns may explain the observed structuration. The patterns of genetic connectivity of *E. singularis* presented here confirm the findings of Guizien *et al.*, (2014), in which numerical simulations of a metapopulation model for benthic invertebrate species in the GoL showed that hydrological connectivity could spread individuals over the entire region. They identified areas of the western tip of the GoL as sink sites given that low retention rates did not allow for local persistence, while the center of the Gulf was characterized by areas that enhance metapopulation resistance by creating loops of persistence (Hastings and Botsford 2006). Moreover, low levels of larval retention, which would limit outcrossing among populations, may explain the high genetic differentiation of Côte Vermeille, relative to other locations in the GoL.

A strong pattern of genetic structure subdividing the cluster of France into three subgroups: Côte Bleue, Cap d'Agde and Côte Vermeille, evidence the possibility of finding genetic structuration even in the presence of the migration. Within the cluster of France, samples from Côte Vermeille seem to be more genetically distinct when compared to all other sites. The only exception was the site CVE5, which did not show significant differences when compared to most of the sites in Plateau des Aresquiers



(except PAR2) and one site in Cap d'Agde (AGD1), indicating that the LATEX eddy may indeed facilitate the transport of larvae between Plateau des Aresquiers and Côte Vermeille. Furthermore, the presence of first generation migrants found in two sites of Côte Vermeille that were identified as originating from Côte Bleue, in addition to the lack of immigrants found in Côte Bleue, evidences the influence of the general westward circulation in the GoL. Bi-directionality in the direction of immigration was only observed between the sites of Plateau des Aresquiers and Côte Vermeille.

Within the cluster of the Spanish Catalan Coast, all sites were found to be significantly different from each other, although no spatial pattern of structure was observed. First generation migrants were found both upstream and downstream from their natal colony, demonstrating that the local pattern of present-day migration in this cluster is bi-directional. A pattern that also reflects the local hydrodynamics and suggest that the Catalan eddy influences the transport of larvae of *E. singularis*, retaining most of the larvae in that region and limiting its transport north of Cap de Creus.

Even though this study allows making inferences on the seascape processes driving the genetic structuration of *E. singularis* in the NW Mediterranean, which seems to be primarily the regional hydrodynamics, we cannot discard the influence of local demography, nor disentangle the role and relative contribution of hydrology and demography in the shaping of the observed patterns of genetic connectivity. Differences in patterns of genetic structure and IBD observed for *E. singularis* among different spatial scales may also influenced by ecological and environmental factors over gene flow. Processes disrupting dispersal or selecting on post-settlement survival are well recognized as potential drivers of genetic differentiation among populations, which becomes particularly relevant when dealing with in non-equilibrium populations (Sexton *et al.*, 2013). Observed patterns of genetic population structure could be driven by local recruitment regulation, temporal variability of dispersal patterns or equilibrium between local recruitment and immigration. Their influence on genetic structure has been inferred from previous empirical studies on species with larval dispersal (White *et*





al., 2010; Nanninga *et al.*, 2014). Numerical simulations have demonstrated that high local retention can lead to high genetic drift, despite the presence of immigration, as a result of quick convergence to saturating density (Padrón and Guizien 2015).

Although several gorgonian species exhibit low and episodic recruitment (Lasker 1990; Coma *et al.*, 2004; Linares *et al.*, 2008), Lasker and Porto-Hannes (2015) found no difference in the genetic population structure of recruits and adults of the gorgonian *Antillogorgia elisabethae*, suggesting that limited variation in recruitment patterns could explain the adult population structure of a gorgonian species in the Caribbean Sea. In the NW Mediterranean, small colonies of *E. singularis* have been usually found in high proportions relative to adult colonies, which has led to indications that the species is characterized by high recruitment rates (Linares *et al.*, 2008; Gori *et al.*, 2011). Nonetheless, temporal variability has also been reported with an alternative dominance of recruits vs. adult colonies (Linares *et al.*, 2008). Furthermore, low recruitment rates have been found as a result of mortality events (Coma *et al.*, 2006).

It would be therefore necessary to combine approaches that integrate biophysical, demographic and genetic data to truly understand the seascape dynamics in the region and its influence on the evolutionary history of *E. singularis*. Nonetheless, the present results have important implications for the recovery and management of *E. singularis* in the face of increasing climate change by identifying the GoL as the appropriate scale of closure for the metapopulation of *E. singularis*. We provide an important baseline for the understanding of genetic connectivity among populations of one of the most common gorgonian species in the northwestern Mediterranean Sea.

2.2.6 Acknowledgements

This work was (co-)funded through a MARES Grant. MARES is a Joint Doctorate programme selected under Erasmus Mundus coordinated by Ghent University (FPA 2011-0016). Check www.mares-eu.org for extra information. This work was also partly funded by the French National Program LITEAU IV of the Ministère de l'Écologie et de l'Environnement Durable





under project RocConnect—Connectivité des habitats rocheux fragmentés du Golfe du Lion (PI, K. Guizien, Project Number 12-MUTS-LITEAU-1-CDS-013. The authors particularly thank the scientific managers of the Gulf of Lions MPA : S. Blouet, E. Charbonnel, B. Ferrari and J. Payrot for valuable interactions in the study design. The authors gratefully acknowledge the helpful assistance during sampling of R. Bricout, F. Cornette, S. Fanfard, B. Hesse, C. Labrune, L. Lescure, J.-C. Roca, P. Romans, and staff of the Réserve Naturelle Marine de Cerbère-Banyuls, Aire Marine Protégée Agatoise, Parc Naturel Marin du Golfe du Lion and Parc Marin de la Côte Bleue. We would also like to thank the contribution of samples from Spain to Andrea Gori, Josep Maria Gili and Sergio Rossi.

2.2.7 References

- Almany, G., Connolly, S., Heath, D., Hogan, J., Jones, G., McCook, L., Mills, M., *et al.* 2009. Connectivity, biodiversity conservation and the design of marine reserve networks for coral reefs. *Coral Reefs*, 28: 339–351.
- Baguette, M., Blanchet, S., Legrand, D., Stevens, V., and Turlure, C. 2012. Individual dispersal, landscape connectivity and ecological networks. *Biological Reviews*, 88: 310-326.
- Ballesteros, E. 2006. Mediterranean coralligenous assemblages: a synthesis of present knowledge. *Oceanography and Marine Biology*, 44: 1–74.
- Bavestrello, G., Cerrano, C., Zanzi, D., and Cattaneo-Vietti, R. 1997. Damage by fishing activities to the gorgonian coral *Paramuricea clavata* in the Ligurian Sea. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 7: 253–262.
- Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., and Bonhomme, F. 2004. GENETIX 4.05, Logiciel sous Windows™ pour la génétique des populations. Laboratoire, Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier, France.
- Benjamini, Y., and Hochberg, Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol*, 57: 289–300.





- Campbell, R., Diaz, F., Hu, Z., Doglioli, A., Petrenko, A., and Dekeyser, I. 2013. Nutrients and plankton spatial distributions induced by coastal eddy in the Gulf of Lion. Insights from a numerical model. *Progress in Oceanography*, 109: 47–69.
- Cataneo, J. 2011. Adaptation d'*Eunicella singularis* en milieu perturbé: symbiose et structuration genetique. PhD Thesis, Université de Nice-Sophia Antipolis. 220 pp.
- Cerrano, C., Arillo, A., Azzini, F., Calcinai, B., Castellano, L., Muti, C., Valisano, L., *et al.* 2005. Gorgonian population recovery after a mass mortality event. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 15: 147–157.
- Chapuis, M., and Estoup, A. 2007. Microsatellite null alleles and the estimation of population differentiation. *Molecular Biology and Evolution*, 24: 612–631.
- Coma, R., Ribes, M., Serrano, E., Jiménez, E., Salat, J., and Pascual, J. 2009. Global warming-enhanced stratification and mass mortality events in the Mediterranean. *Proceedings of the National Academy of Sciences*, 106: 6176–6181.
- Coma, R., Linares, C., Ribes, M., Diaz, D., Garrabou, J., and Ballesteros, E. 2006. Consequences of a mass mortality in populations of *Eunicella singularis* (Cnidaria: Octocorallia) in Menorca (NW Mediterranean). *Marine Ecology Progress Series*, 327: 51–60.
- Consortium of Molecular Ecology Resources Primer Development. 2010. Permanent genetic resources added to molecular ecology resources database 1 August 2009 - 30 September 2009. *Molecular Ecology Resources*, 10: 232–236.
- Costantini, F., Fauvelot, C., and Abbiati, M. 2007. Genetic structuring of the temperate gorgonian coral (*Corallium rubrum*) across the western Mediterranean Sea revealed by microsatellites and nuclear sequences. *Molecular Ecology*, 16: 5168–5182.
- Cowen, R., and Sponaugle, S. 2009. Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, 1: 443–466.
- Crandall, E., Tremblay, E., and Barber, P. 2012. Coalescent and biophysical models of stepping-stone gene flow in neritid snails. *Molecular Ecology*, 21: 5579–5598.
- Earl, D., and vonHoldt, B. 2011. STRUCTURE HARVESTER: a website and program for



- visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4: 359–361.
- Excoffier, L., and Lischer, H. 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.
- Falush, D., Stephens, M., and Pritchard, J. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, 164: 1567–1587.
- Falush, D., Stephens, M., and Pritchard, J. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*, 7: 574–578.
- García de León, F., Chikhi, L., and Bonhomme, F. 1997. Microsatellite polymorphism and population subdivision in natural populations of European sea bass *Dicentrarchus labrax* (Linnaeus, 1758). *Molecular Ecology*, 6: 51–62.
- Garreau, P., Garnier, V., and Schaeffer, A. 2011. Eddy resolving modelling of the Gulf of Lions and Catalan Sea. *Ocean Dynamics*, 61: 991–1003.
- Gori, A., Viladrich, N., Gili, J. M., Kotta, M., Cucio, C., Magni, L., Bramanti, L., *et al.* 2012. Reproductive cycle and trophic ecology in deep versus shallow populations of the Mediterranean gorgonian *Eunicella singularis* (Cap de Creus, northwestern Mediterranean Sea). *Coral Reefs*, 31: 823–837.
- Gori, A., Rossi, S., Berganzo, E., Pretus, J., Dale, M., and Gili, J. M. 2011. Spatial distribution patterns of the gorgonians *Eunicella singularis*, *Paramuricea clavata*, and *Leptogorgia sarmentosa* (Cape of Creus, Northwestern Mediterranean Sea). *Marine Biology*, 158: 143–158.
- Guizien, K., Belharet, M., Marsaleix, P., and Guarini, J. M. 2012. Using larval dispersal simulations for marine protected area design: Application to the Gulf of Lions (northwest Mediterranean). *Limnology and Oceanography*, 57: 1099–1112.
- Guizien, K., Belharet, M., Moritz, C., and Guarini, J. M. 2014. Vulnerability of marine



benthic metapopulations: implications of spatially structured connectivity for conservation practice in the Gulf of Lions (NW Mediterranean Sea). *Diversity and Distributions*, 1–11.

Hardy, O., and Vekemans, X. 1999. Isolation by distance in a continuous population: reconciliation between spatial autocorrelation analysis and population genetics models. *Heredity*, 83: 145–154.

Hastings, A., and Botsford, L. 2006. Persistence of spatial populations depends on returning home. *Proceedings of the National Academy of Sciences*, 103: 6067–6072.

Hedgecock, D., Barber, P., and Edmands, S. 2007. Genetic approaches to measuring connectivity. *Oceanologica Acta*, 20: 1–10.

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

Holland, L., Dawson, D., Horsburgh, G., Krupa, A., and Stevens, J. 2013. Isolation and characterization of fourteen microsatellite loci from the endangered octocoral *Eunicella verrucosa* (Pallas 1766). *Conservation Genetics Resources*, 5: 825–829.

Hu, Z. Y., Doglioli, A. M., Petrenko, A. A., Marsaleix, P., and Dekeyser, I. 2009. Numerical simulations of eddies in the Gulf of Lion. *Ocean Modelling*, 28: 203–208.

Jakobsson, M., and Rosenberg, N. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, 23: 1801–1806.

Johnson, M. S., and Black, R. 1984. The Wahlund effect and the geographical scale of variation in the intertidal limpet *Siphonaria* sp. *Marine Biology*, 79: 295–302.

Kalinowski, S. 2005. HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Molecular Ecology Notes*, 5: 4015–4026.

Kimura, M., and Weiss, G. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, 49: 561–576.

Lasker, H. 1990. Clonal propagation and population dynamics of a gorgonian coral. *Ecology*, 71: 1578–1589.





- Lasker, H., and Porto-Hannes, I. 2015. Population structure among octocoral adults and recruits identifies scale dependent patterns of population isolation in The Bahamas. *PeerJ*, 3: e1019.
- Ledoux, J., Garrabou, J., Bianchimani, O., Drap, P., Féral, J., and Aurelle, D. 2010. Fine-scale genetic structure and inferences on population biology in the threatened Mediterranean red coral, *Corallium rubrum*. *Molecular Ecology*, 19: 4204–4216.
- Lenfant, P., and Planes, S. 1996. Genetic differentiation of white sea bream within the Lion's Gulf and the Ligurian Sea (Mediterranean Sea). *Journal of Fish Biology*, 49: 613–621.
- Linares, C., Coma, R., Garrabou, J., Díaz, D., and Zabala, M. 2008. Size distribution, density and disturbance in two Mediterranean gorgonians: *Paramuricea clavata* and *Eunicella singularis*. *Journal of Applied Ecology*, 45: 688–699.
- Loiselle, B., Sork, V., Nason, J., and Graham, C. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany*, 82: 1420–1425.
- Lowe, W., and Allendorf, F. 2010. What can genetics tell us about population connectivity? *Molecular Ecology*, 19: 3038–3051.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research*, 27: 209–220.
- Meirmans, P., and van Tienderen, P. 2004. Genotype and Genodive: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Resources*, 4: 792–794.
- Millot, C. 1990. The Gulf of Lions' hydrodynamics. *Continental shelf research*, 10: 885–894.
- Mokhtar-Jamaï, K., Coma, R., Wang, J., Zuberer, F., Féral, J. P., and Aurelle, D. 2013. Role of evolutionary and ecological factors in the reproductive success and the spatial genetic structure of the temperate gorgonian *Paramuricea clavata*. *Ecology and Evolution*, 3: 1765–1779.





- Mokhtar-Jamaï, K., Pascual, M., Ledoux, J. B., Coma, R., Féral, J. P., Garrabou, J., and Aurelle, D. 2011. From global to local genetic structuring in the red gorgonian *Paramuricea clavata*: the interplay between oceanographic conditions and limited larval dispersal. *Molecular Ecology*, 20: 3291–3305.
- Morat, F., Letourneur, Y., Dierking, J., Pécheyran, C., Bareille, G., Blamart, D., and Harmelin-Vivien, M. 2014. The great melting pot. common sole population connectivity assessed by otolith and water fingerprints. *PLoS ONE*, 9: e86585.
- Nanninga, G., Saenz-Agudelo, P., Manica, A., and Berumen, M. L. 2014. Environmental gradients predict the genetic population structure of a coral reef fish in the Red Sea. *Molecular Ecology*, 23: 591–602.
- Nicolle, A., Garreau, P., and Liorzou, B. 2009. Modelling for anchovy recruitment studies in the Gulf of Lions (Western Mediterranean Sea). *Ocean Dynamics*, 59: 953–968.
- Nieblas, A., Drushka, K., Reygondeau, G., Rossi, V., Demarcq, H., Dubroca, L., and Bonhommeau, S. 2014. Defining Mediterranean and Black Sea biogeochemical subprovinces and synthetic ocean indicators using mesoscale oceanographic features. *PLoS ONE*, 9: e111251.
- Padrón, M., and Guizien, K. (2015). Modelling the effect of demographic traits and connectivity on the genetic structuration of marine metapopulations of sedentary benthic invertebrates. *ICES Journal of Marine Science (in press)*.
- Perez, T., Garrabou, J., Sartoretto, S., Harmelin, J. G., Francour, P., and Vacelet, J. 2000. Mortalité massive d'invertébrés marins: un événement sans précédent en Méditerranée nord-occidentale. *C. R. Acad. Sci. Paris, Sciences de la vie*, 323: 853–865.
- Piry, S., Alapetite, A., Cornuet, J., Paetkau, D., Baudouin, L., and Estoup, A. 2004. GENECLASS2: A Software for genetic assignment and first-generation migrant detection. *Journal of Heredity*, 95: 536–539.
- Pritchard, J., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155: 945–959.





- Rannala, B., and Mountain, J. 1997. Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences*, 94: 9197–9201.
- Ribes, M., Coma, R., Rossi, S., and Micheli, M. 2007. Cycle of gonadal development in *Eunicella singularis* (Cnidaria: Octocorallia): trends in sexual reproduction in gorgonians. *Invertebrate Biology*, 126: 307–317.
- Rosenberg, N. 2004. DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, 4: 137–138.
- Rossi, V., Ser-Giacomi, E., López, C., and Hernandez-García, E. 2014. Hydrodynamic provinces and oceanic connectivity from a transport network help designing marine reserves. *Geophysical Research Letter*, 41: 2883–2891.
- Rubio, A., Barnier, B., Jordà, G., Espino, M., and Marsaleix, P. 2009. Origin and dynamics of mesoscale eddies in the Catalan Sea (NW Mediterranean): Insight from a numerical model study. *Journal of Geophysical Research*, 114: C06009.
- Sexton, J., Hangartner, S., and Hoffmann, A. A. 2013. Genetic isolation by environment or distance: which pattern of gene flow is most common? *Evolution*, 68: 1–15.
- Underwood, J., Smith, L., van Oppen, M., and Gilmour, J. 2006. Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. *Molecular Ecology*, 16: 771–784.
- UNEP-MAP-RAC/SPA. 2013. Description of the ecology of the Gulf of Lions shelf and slope area and identification of the areas that may deserve to be protected. Sardà, J.M.G and Dominguez-Carrió, C. Ed. RAC/SPA, Tunis. 69 pp.
- Viladrich, N. (2015). Study of environmental and biological factors that affect larval survival in sessile coastal marine organisms. PhD Thesis. Universitat Autònoma de Barcelona.
- Weinberg, S. 1978. Mediterranean octocorallian communities and the abiotic environment. *Marine Biology*, 49: 41–57.
- Weinberg, S., and Weinberg, F. 1979. The life cycle of a gorgonian: *Eunicella singularis*. *Bijdragen tot de Dierkunde*, 48: 1–14.





Weir, B., and Cockerham, C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution*, 38: 1358–1370.

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

Wilson, G., and Rannala, B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, 163: 1177–1191.





2.2.8 Supplementary Material

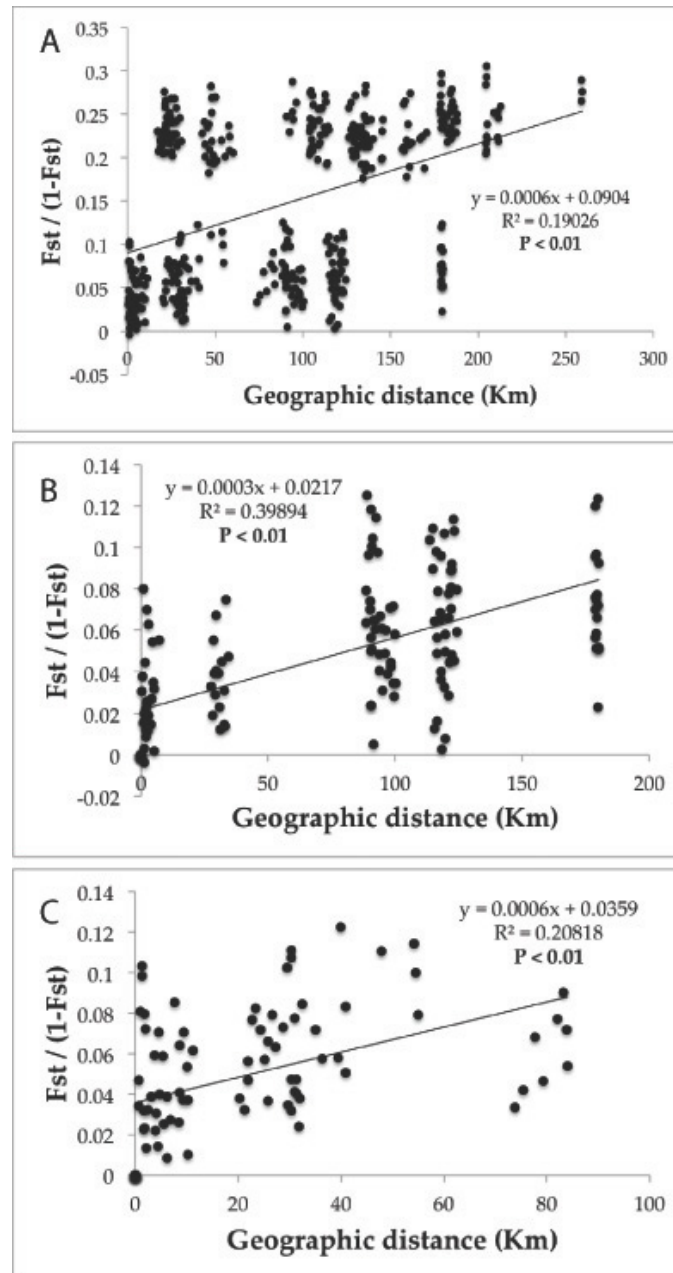


Figure S1.- Isolation by Distance (IBD) for *Eunicella singularis* in the Gulf of Lions. Correlation between genetic distances ($F_{st} / (1-F_{st})$) and geographic distances (Km). A) All samples, B) Samples from the cluster of France, C) Samples from the cluster of Spain.



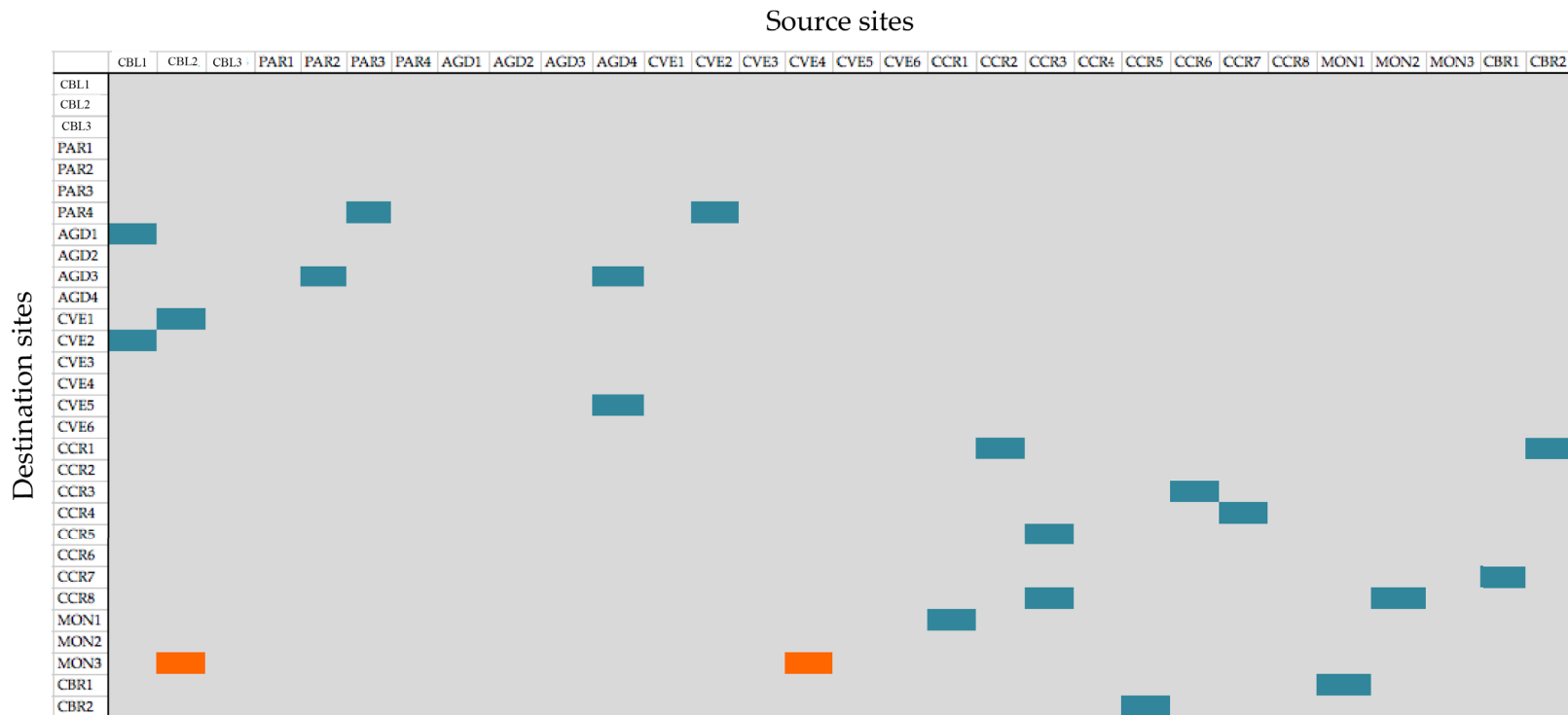


Figure S2.- Matrix of first generation migrants. Sites on the top row represent the sites where the migrant was originated, while sites on the left column indicate those where the migrants were found. The cells in color blue indicate intra-cluster migration, and cells highlighted in orange represent inter-cluster migration.

Table S1.- Pairwise F_{ST} values among sites of *E. singularis* in the Gulf of Lions. Significant values, after FDR correction, are highlighted in bold (P <0.05).

	CBL1	CBL2	CBL3	PAR1	PAR2	PAR3	PAR4	AGD1	AGD2	AGD3	AGD4	CVE1	CVE2	CVE3	CVE4	CVE5	CVE6	CCR1	CCR2	CCR3	CCR4	CCR5	CCR6	CCR7	CCR8	MON1	MON2	MON3	CBR1	CBR2		
BL1	0																															
BL2	0.021	0																														
BL3	0.026	0.038	0																													
AR1	0.035	0.058	0.035	0																												
AR2	0.042	0.072	0.028	0.004	0																											
AR3	0.039	0.071	0.045	0.014	0.009	0																										
AR4	0.031	0.049	0.060	0.002	0.015	0.015	0																									
GD1	0.045	0.081	0.092	0.033	0.039	0.023	0.031	0																								
GD2	0.089	0.114	0.108	0.055	0.067	0.045	0.075	0.016	0																							
GD3	0.046	0.080	0.060	0.041	0.040	0.014	0.047	0.012	0.003	0																						
GD4	0.066	0.089	0.070	0.019	0.029	0.012	0.014	0.016	0.045	0.025	0																					
VE1	0.052	0.093	0.051	0.040	0.033	0.029	0.048	0.061	0.067	0.040	0.049	0																				
VE2	0.077	0.124	0.066	0.079	0.068	0.050	0.078	0.098	0.114	0.065	0.061	0.022	0																			
VE3	0.051	0.097	0.059	0.064	0.049	0.036	0.058	0.065	0.101	0.050	0.057	0.027	0.025	0																		
VE4	0.096	0.120	0.076	0.109	0.098	0.065	0.107	0.105	0.118	0.070	0.074	0.054	0.016	0.030	0																	
VE5	0.023	0.072	0.072	0.012	0.016	0.003	0.008	0.005	0.051	0.024	0.024	0.035	0.063	0.020	0.080	0																
VE6	0.070	0.075	0.057	0.104	0.090	0.056	0.096	0.096	0.125	0.064	0.079	0.055	0.032	0.019	0.011	0.070	0															
CR1	0.286	0.297	0.271	0.258	0.264	0.237	0.271	0.277	0.275	0.244	0.231	0.230	0.213	0.276	0.228	0.267	0.252	0														
CR2	0.252	0.261	0.255	0.223	0.233	0.201	0.230	0.236	0.236	0.208	0.203	0.229	0.224	0.263	0.236	0.221	0.244	0.034	0													
CR3	0.242	0.253	0.234	0.223	0.227	0.204	0.235	0.261	0.272	0.221	0.211	0.220	0.205	0.246	0.223	0.225	0.222	0.103	0.081	0												
CR4	0.240	0.249	0.233	0.209	0.218	0.202	0.224	0.243	0.237	0.213	0.208	0.207	0.214	0.259	0.238	0.221	0.248	0.023	0.013	0.080	0											
CR5	0.228	0.248	0.218	0.211	0.215	0.192	0.218	0.243	0.257	0.215	0.197	0.221	0.206	0.246	0.225	0.207	0.227	0.070	0.040	0.059	0.039	0										
CR6	0.259	0.274	0.253	0.227	0.235	0.212	0.242	0.257	0.258	0.226	0.217	0.233	0.228	0.268	0.241	0.227	0.257	0.039	0.009	0.059	0.014	0.023	0									
CR7	0.243	0.259	0.227	0.228	0.227	0.213	0.244	0.265	0.273	0.228	0.235	0.223	0.224	0.268	0.241	0.227	0.246	0.064	0.041	0.085	0.027	0.022	0.032	0								
CR8	0.242	0.250	0.229	0.215	0.216	0.188	0.222	0.233	0.236	0.194	0.192	0.216	0.202	0.242	0.214	0.217	0.219	0.037	0.010	0.071	0.026	0.025	0.030	0.032	0							
ON1	0.209	0.217	0.205	0.210	0.212	0.178	0.215	0.215	0.223	0.176	0.193	0.228	0.216	0.239	0.209	0.195	0.193	0.107	0.047	0.103	0.073	0.066	0.072	0.056	0.038	0						
ON2	0.293	0.305	0.284	0.261	0.266	0.239	0.274	0.283	0.275	0.247	0.249	0.241	0.235	0.282	0.252	0.269	0.270	0.040	0.047	0.111	0.034	0.079	0.057	0.077	0.032	0.099	0					
ON3	0.222	0.238	0.224	0.209	0.220	0.189	0.217	0.223	0.218	0.192	0.185	0.201	0.183	0.219	0.194	0.203	0.197	0.038	0.024	0.078	0.032	0.063	0.037	0.082	0.047	0.072	0.047	0				
BR1	0.246	0.253	0.224	0.221	0.224	0.188	0.229	0.244	0.231	0.199	0.199	0.220	0.202	0.237	0.208	0.224	0.206	0.083	0.050	0.122	0.058	0.058	0.072	0.084	0.041	0.061	0.053	0.037	0			
BR2	0.276	0.289	0.266	0.252	0.251	0.219	0.259	0.265	0.279	0.235	0.222	0.248	0.230	0.287	0.244	0.253	0.264	0.072	0.054	0.090	0.077	0.046	0.068	0.042	0.033	0.079	0.100	0.114	0.111	0		



Table S2.- AMOVA results for all samples of *E. singularis* in the NW Mediterranean

Source of variation	d.f.	Percentage of variance	P
Among groups	1	18.17	0.0000
Among populations within groups	28	4.38	0.0000
Among individuals within populations	763	6.63	0.0000
Within individuals	793	70.81	0.0000

Table S3.- AMOVA results for samples of *E. singularis* corresponding to the cluster of France.

Source of variation	d.f.	Percentage of variance	P
Among groups	2	3.90	0.0000
Among populations within groups	14	2.76	0.0049
Among individuals within populations	396	1.67	0.0000
Within individuals	413	91.66	0.0000

Table S4.- AMOVA results for samples of *E. singularis* corresponding to the cluster of Spain.

Source of variation	d.f.	Percentage of variance	P
Among groups	2	0.21	0.3294
Among populations within groups	10	5.37	0.0000
Among individuals within populations	367	14.39	0.0000
Within individuals	380	80.02	0.0000

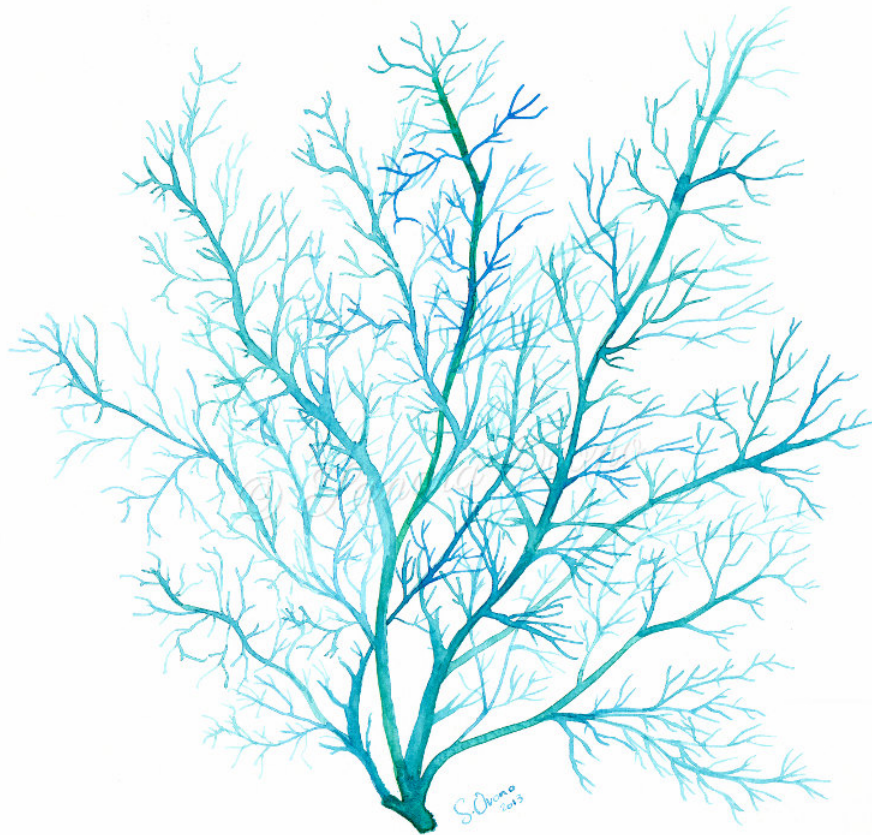


Table S5.- Migration rates among *E. singularis* populations in the NW Mediterranean, as resulted from the Bayesian inference in BayesAss (Wilson and Rannala 2003).

	Côte Bleue	Plateau des Aresquiers	Cap d'Agde	Côte Vermeille	Cap de Creus	Montgri	Costa Brava
Côte Bleue	0.672	0.002	0.296	0.017	0.002	0.004	0.004
Plateau des Aresquiers	0.001	0.662	0.292	0.03	0.001	0.002	0.002
Cap d'Agde	0.005	0.317	0.669	0.002	0.001	0.001	0.001
Côte Vermeille	0.018	0.306	0.001	0.669	0.002	0.001	0.001
Cap de Creus	0	0	0.003	0.004	0.668	0.19	0.302
Montgri	0.007	0.008	0.001	0.001	0.308	0.666	0.001
Costa Brava	0.004	0.005	0.002	0.002	0.311	0.002	0.672

CHAPTER 3

Testing processes of genetic structuration for *Eunicella singularis* in the Gulf of Lions





Mariana PADRÓN^{1*}, Lorenzo BRAMANTI¹, and Katell GUIZIEN¹

¹ Sorbonne Universités, UPMC Univ Paris 06, CNRS, Laboratoire d'Ecogéochimie des Environnements Benthiques (LECOB), Observatoire Océanologique, 66650, Banyuls sur Mer, France.

N.B. This manuscript is in the early stages of preparation.

3.1 Abstract

Understanding the complex processes driving marine population connectivity is a challenge that requires the use of tools that integrate bio-physical models with information regarding the demographic and genetic linkages resulting from the larval exchange among populations. To identify the most relevant processes driving demographic and genetic differentiation among populations of the white gorgonian (*Eunicella singularis*) in the Gulf of Lions, genetic structure patterns were simulated at the metapopulation scale, and compared to empirical analysis of genetic population structure using eight polymorphic microsatellite loci. The comparison between the modeled pattern of genetic structure of *E. singularis* with and without connectivity and the observations, suggests that migration structure in the Gulf of Lions induces both demographic and genetic differentiation among populations. However, some discrepancies persist.

3.2 Introduction

The declining health of marine ecosystems around the world has led to an increasing concern about the organization of uses of the marine space in order to balance human development with the protection of marine ecosystems, which is commonly known as marine spatial planning (Douvere 2008). However, given the complexity in the heterogeneity of marine populations and ecosystems, marine spatial planners cannot assume that populations will recover once the stressors are reduced. Hence, there is a





need to understand the processes that drive and maintain the spatial distribution of marine biodiversity (Crowder and Norse 2008).

Connectivity, understood as the exchange of individuals between populations (Cowen *et al.*, 2000), is a key characteristic affecting the dynamics, demography and genetics of marine metapopulations (Hanski 1999; Kritzer and Sale 2004). The effectiveness of Marine Protected Areas (MPAs) is thought to be dependent on the connectivity that exists among populations (Almany *et al.*, 2009; Gaines *et al.*, 2010). The demographic effect of the movement of individuals from one population to another is related to the potential rescue and re-colonization of disturbed populations, while the exchange and mixing of genes among populations enhances genetic diversity within populations and reduces the erosion of genetic variability (Baguette *et al.*, 2013).

For sessile benthic species with a pelagic larval phase, connections between populations are possible through larval dispersal only, which is also the time at which gene flow takes place. Therefore, connectivity among MPAs depends on the patterns and strength of larval dispersal. However, connectivity is not only a function of larval dispersal, but also of post-larval survival, which implies multiple spatial and temporal scales that are variable between and within species and locations (Pineda *et al.*, 2007).

Given the difficulties in conducting field studies of larval stages and their movements, due to the small size and tracking difficulty of larvae, inferences about levels of larval connectivity have commonly resulted from the use of two different approaches: biophysical modelling and population genetics (Paris *et al.*, 2007; Werner *et al.*, 2007). Modelling tools allow Lagrangian particle tracking driven by hydrological models to simulate the potential patterns and levels of dispersal among populations based in oceanographic and climatic data (Cowen *et al.*, 2006). The method using forecast of ocean currents in different climate change scenarios also has the potential to forecast larval connectivity in the future (Werner *et al.*, 2007). This approach allows us to make inferences on demographic connectivity, which has been defined as the degree to which population growth and vital rates are affected by dispersal (Lowe and Allendorf 2010).





The genetics approach, on the other hand, allows inferring aspects of realized connectivity integrated over many generations from empirical genetic data. The degree to which gene flow affects evolutionary processes within populations is known as genetic connectivity (Lowe and Allendorf 2010). Nonetheless, both approaches present limitations: neither larval post-settlement nor survival, which can significantly affect demographic connectivity, are considered explicitly in larval dispersal modelling (Armsworth 2002). Similarly, genetic methods are not capable to discern between dispersal and settlement bottlenecks in realized connectivity, and have no predictability potential in a changing ocean (Lowe and Allendorf 2010). Furthermore, population genetic models assume a mutation-drift equilibrium that is rarely reached in marine species, and thus, gene flow patterns can be strongly influenced by past population expansions and contractions (Benzie 1999). Therefore, none of the approaches on itself can provide all the information necessary to understand population connectivity and properly describe population persistence in a network of MPAs (Hastings and Harrison 1994; Marko and Hart 2011).

Despite the unsuitability of larval dispersal modelling and population genetic approaches to fully determine marine connectivity on their own, their complementary nature calls for an integration. A combination of ecological, demographic and genetic metapopulation models can provide an important tool for understanding the complexity of population connectivity (Levin 2006; Epperson *et al.*, 2010). Clarification of the influence that environmental and demographic factors have on connectivity and the patterns of genetic structuring are essential for the design of MPAs.

Recently, models that couple biophysical data with demography and population genetics have been developed (Galindo *et al.*, 2006; Kool *et al.*, 2009; Munroe *et al.*, 2012; Padrón and Guizien *in press*). Using these spatially-explicit models, it is possible to project the development of population genetic structure through time, resulting from demographic dynamics driven by local survival and larval transfer on a regional scale. However, these tools have rarely been applied in order to disentangle the seascape





processes driving the observed patterns of genetic structure in marine metapopulations of benthic sessile species (but see Foster *et al.*, 2012). Furthermore, reconciling the factors that are believed to be responsible for generating modeled genetic structure with the observed genetic patterns will lead to a better understanding of what the important processes actually are, and what would be the expected consequences if they were to change.

Gorgonian corals are an ideal study case for applying this approach. They have a sessile adult stage and a larval pelagic phase that allows the use of modelling tools to simulate their dispersal trajectories, without the confounding effect of adult migration. They are long-lived and slow-growing organisms, which facilitate the sampling of the same cohorts over different years. Their spatial distribution in discrete habitats allows the assessment of connectivity among separated sites. In this chapter, I test the effect of larval dispersal on the realized connectivity of the white gorgonian, *Eunicella singularis*, in the Gulf of Lions (northwestern Mediterranean Sea), as observed from empirical genetic data (Chapter 2). By combining realistic connectivity matrices resulting from a Lagrangian model of larval dispersal (Guizien *et al.*, 2012), and a theoretical seascape demo-genetic model that projects the development of genetic structure for benthic sessile marine invertebrates (Padrón and Guizien 2015; Chapter 1), I evaluate the expected development of genetic structure among populations, and compare it to the observed patterns of genetic structure of *E. singularis* in the region. In particular, I ask the question: can larval dispersal alone explain driving the patterns of genetic connectivity observed in *E. singularis* within the Gulf of Lions?

3.3 Methods

In order understand how populations of *E. singularis* are connected in the Gulf of Lions, I used a seascape genetic approach that combines the results obtained from three different levels of integration along a species life cycle of assessing connectivity (Figure 3.1). Firstly, larval connectivity was examined by using connectivity matrices that result





from a biophysical model, which incorporates a coastal circulation model and a Lagrangian model of larval dispersal. Then, demographic connectivity among populations was studied by using a spatially-explicit metapopulation dynamics model parameterized for the species. Finally, the genetic connectivity of *E. singularis* populations was assessed by analyzing empirical data and comparing the results to those obtained by the modelling approach.

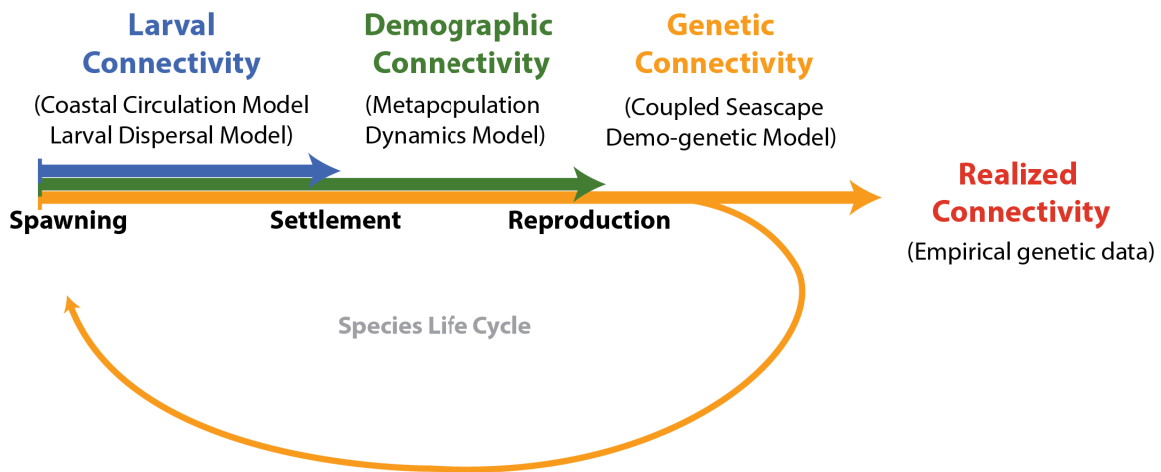


Figure 3.1.- Conceptual model of the seascape genetic approach.

3.3.1 Larval connectivity

Lagrangian larval dispersal simulations based on regional hydrodynamic simulations performed with the coastal circulation model SYMPHONIE, with a horizontal resolution of 750 meters (Marsaleix *et al.*, 2008). The fragmented rocky-bottom habitat of the white gorgonian in the Gulf of Lions, which is delimited by the 10 and the 30 meters isobaths, was described by sites of approximately the same width (~ 20 km) patchily distributed along the coast (Figure 3.2). Based on these criteria, larval transfer between eight sites was computed.

Larval dispersal was simulated by releasing larvae at the average site depth (20 m). Connectivity matrices containing larval transfer probabilities were produced for 20





sets of 10-day long spawning periods in spring 2004 and 2006 (Guizien *et al.*, 2014). Given the uncertainty on the Pelagic Larval Duration (PLD) of *E. singularis*, simulations were run for three different PLDs (two, three and four weeks). A total of 20 different connectivity matrices for each PLD were used for this analysis. In each connectivity matrix, columns represent source populations, rows represent destination populations, and cell contents describe the probability with which a given larvae migrates from a source population to a destination. Elements along the diagonal of the matrix correspond to levels of self- recruitment within a population.

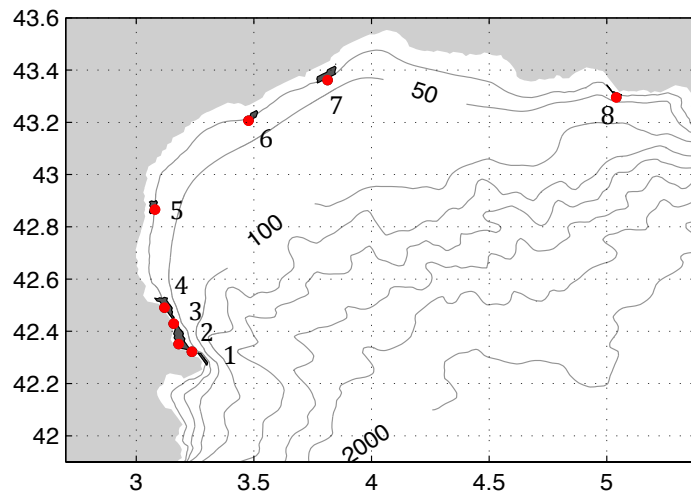


Figure 3.2.- Geographic distribution of eight sites considered for the release of larvae in Lagrangian model of larval dispersal in the Gulf of Lions.

To estimate the hydrological distance among populations using the realistic connectivity matrices from the rocky sites in the Gulf of Lions, I calculated a weighted average of 20 replicate connectivity matrices for two different years (2004 and 2006), for the three different PLDs (2, 3 and 4 weeks duration). In order to reverse the hierarchy of transfer probabilities among sites into distance, while maintaining linearity, the conversion of probability values into a measure comparable to distance of the mean connectivity matrices for each PLD, was done using the equation developed by Costa *et al.*, (in prep) (Figure 3.3):



$$d_{ij} = \ln\left(\frac{1}{\alpha_{ij}}\right)$$

where,

α_{ij} = connectivity probability given by the connectivity matrices used in the metapopulation model.

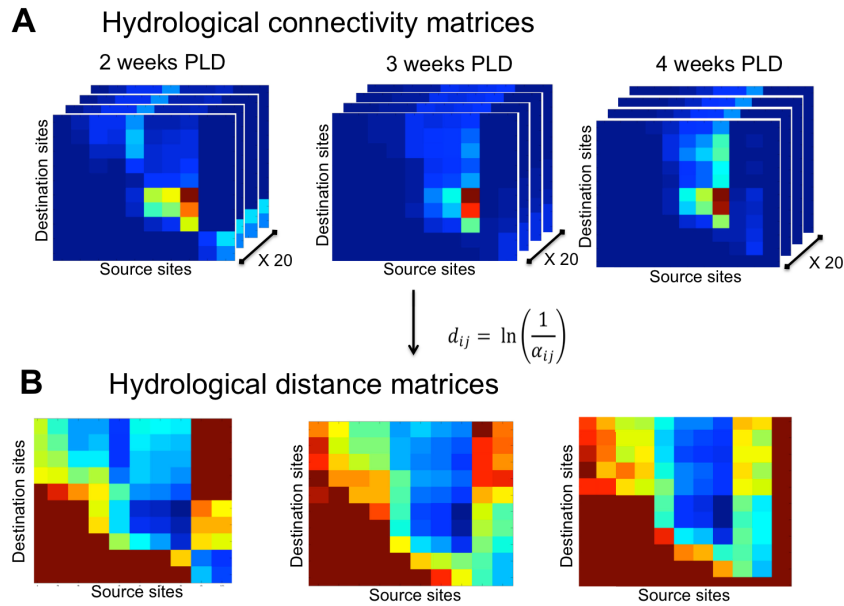


Figure 3.3.- Schematic representation of use of connectivity matrices to calculate hydrological distance among populations of *E. singularis* in the Gulf of Lions. A) Hydrological connectivity matrices, where color represents probability of larval retention from one site to another varying from low (dark blue) to high (red). B) Hydrological distance matrices, where dark blue represents short distances and red large distances between populations.

3.3.2 Demographic connectivity

A spatially-explicit metapopulation dynamics model that incorporates realistic connectivity matrices, and where recruitment success is limited by saturating population density was used to evaluate the fate of *E. singularis* in the Gulf of Lions (Eq. 1 and 2) (Guizien *et al.*, 2014).

$$N_i(t + 1) = \min(G(t)N_i(t), N_{max}) \quad (1)$$





$N_i(t + 1)$ = Vector of spatial density of individuals at each site at time $t+1$
[individual per m²]

$N_i(t)$ = Vector of spatial density of individuals at each site at time t [individual per m²]

N_{max} = Saturating population density based on space limitation [individual per m²]

with the transfer matrix $G(t)$ defined by:

$$G_{ji}(t) = a_i c_{ji}(t) b_j + s_{jj} \delta_{ji} \quad (2)$$

a_i = Propagule production rate at site i [larvae per adult]

$c_{ji}(t)$ = Propagule transfer rate from site i to site j multiplied by the ratio of surface of site i to surface of site j [no units]

b_j = Recruitment success at site j [adults per larva]

s_{jj} = Adult survivorship rate at site j [no units]

δ_{ji} = Kronecker symbol (1 when $i=j$, 0 otherwise)

Based on values obtained from previous demographic studies of *E. singularis*, the life expectancy of the species was set to 18 years. Considering a mean fecundity value of 27.8 larvae per cm, with an average colony branch length of 50 cm, total fecundity was set to 1400 propagules per individual. Saturating density was set to 40 individuals per m².

In order to test the effect of larval dispersal (larval connectivity) on demographic connectivity, all demographic parameters in each site were the same and two groups of simulations were run: 1) One group that simulated a metapopulation, where the connectivity matrices resulting from the Lagrangian model of larval dispersal were used





to run the model for 100 years (sorting out randomly one of the 20 connectivity variants), and evaluate the fate of the metapopulation due to larval connectivity; 2) A second group simulating isolated populations, with the same procedure except that no connectivity (all diagonal terms) was set to zero. In this way the fate of the metapopulation could be explained by the demographic traits of the species and local retention alone.

Population abundance in each site was then compared among group of simulations.

3.3.3 Seascape genetics model

The seascape genetics model (Chapter 1, Padrón and Guizien 2015), simulates the frequency of each allele k at any given time in every site i , as a result of mixing between the surviving individuals with allele k in site i and the input of new recruits with allele k , coming from any other site. All simulations were run for 100 iterations, one iteration being the time lapse between two reproductive events. The initial condition of genetic structure was defined by ten different alleles present simultaneously in every site. Simulations were repeated at least 200 times with different and random initial conditions of allele frequencies each time in order to evaluate output variability due to specific initial conditions.

As one of the most common measures of genetic diversity, Heterozygosity (He_i), was estimated taking into account all individuals at each site i over time following the equation:

$$He_i(t) = 1 - \sum_{k=1}^n (p_{ik}(t))^2$$

where $p_{ik}(t)$ = frequency of the k th allele of n alleles in site i at time t [no units].

The values of heterozygosity were then compared to the ones obtained from the analysis of microsatellite data. Nei's standard genetic distance among sites was also calculated and used to construct a graph of principal coordinates analysis (PCA) in Genalex, also to be compared with the results obtained by the empirical analysis of





genetic data.

3.3.4 *Realized connectivity: genetic population structure*

The patterns of genetic population structure of *E. singularis* in the Gulf of Lions were studied using eight polymorphic microsatellite loci (see Chapter 2.2 for detailed description of methods). For this particular analysis, a subset of the dataset presented in Chapter 2.2 was used, as the closure boundary of the *E. singularis* metapopulation was identified within the Gulf of Lions. A total of 413 colonies from 17 sites distributed along four different locations in the Gulf of Lions were examined (Côte Bleue, Plateau des Aresquiers, Cap d'Agde and Côte Vermeille).

Different analysis were carried out to compare the results obtained from model simulations of hydrological, demographic and genetic connectivity to the observed pattern of genetic structure of *E. singularis* in the Gulf of Lions, respectively:

1) *Larval connectivity vs. realized connectivity*: To test the influence of larval connectivity on the spatial genetic structuration of *E. singularis* in the region I assessed the presence of a pattern of Isolation By Distance (IBD). A linear regression between pairwise F_{ST} / ($1 - F_{ST}$) values and both the linear geographical and hydrological distances between populations was performed, and the significance of the regression was tested with a Mantel test ($n=10,000$) (Mantel 1967) using the Isolde program implemented in Genepop. Geographical distances were measured between all sampled locations using Google Earth. Hydrological distances were calculated from the larval connectivity matrices, as it was previously described.

The resulting patterns of migration among populations were compared to the simulations on larval connectivity. Additionally, the results from the Bayesian clustering method implemented in Structure v.2.3.4 (Pritchard *et al.*, 2000; Falush *et al.*, 2003, 2007) (see Methods Chapter 2.2) were used to evaluate if the observed patterns of population structure corresponded to the patterns of larval exchange resulting from the connectivity matrices.

2) *Demographic connectivity vs. realized connectivity*: First generation migrants of *E.*





singularis were estimated as proxies of contemporary connectivity among populations in the Gulf of Lions (see Methods Chapter 2.2). Effective population size (N_e) was calculated using the NeEstimator software (Do *et al.*, 2014) and compared to the modeled values of density in each population.

3) *Modeled genetic connectivity vs. realized connectivity*: Modeled and observed levels of genetic diversity of *E. singularis* in the different populations along the Gulf of Lions were estimated and compared based on heterozygosity (H_e) metrics. It is important to mention that, due to the spatial resolution of the connectivity matrices used to run the seascape genetics model, heterozygosity values could not be calculated for all three sites in each location. Instead, values were obtained for only one site in Cap d'Agde, Plateau des Aresquiers and Côte Bleue. Furthermore, in order to visualize the genetic relationships among sites, a genetic distance matrix derived from Nei's standard genetic distance measures, for both modeled and empirical genetic data, was used to construct a graph of principal coordinates analysis (PCA) in Genalex.

3.4 Results

3.4.1 Larval connectivity vs. realized connectivity

The analysis of larval connectivity among populations of *E. singularis* in the Gulf of Lions, showed that local larval retention can vary according to the different PLDs (two, three and four weeks). For a PLD of two weeks, the mean probability of local retention ranged from $0.02 \pm 0.09\%$ in Côte Vermeille to $1.03 \pm 1.2\%$ in Plateau des Aresquiers. For a PLD of three weeks, mean values ranged from 0 to $0.05 \pm 0.04\%$ also for Côte Vermeille and Plateau des Aresquiers, respectively. While the mean values of local retention when considering a PLD of four weeks varied from 0.01 ± 0.01 in Côte Vermeille, to $0.56 \pm 0.13\%$ in Cap d'Agde. Overall, the highest probability of larval retention was found in the sites along the central part of the Gulf, between Cap d'Agde and Plateau des Aresquiers. On the two extremes of the diagonal of the connectivity





matrices (Côte Bleue and Côte Vermeille), the second highest values of larval retention were observed in Côte Bleue, while the sites of Côte Vermeille showed the minimum values of local retention, regardless of PLD (Figure 3.4).

The observed patterns of hydrological distance obtained from the connectivity matrices indicate that the shortest hydrological distances between sites are also found along the central region of the Gulf of Lions, between Plateau des Aresquiers and Cap d'Agde (Figure 3.4). Matrices of hydrological distances are not symmetric: hydrological distances are lower from east to west, than from west to east. The hydrological distance between Côte Bleue and all of the other sites in the Gulf of Lions is shorter than the distance between Côte Vermeille and any other site, whatever the PLD values. Furthermore, it seems that the hydrological distance between Côte Bleue and Côte Vermeille is also shorter than the distance from Côte Bleue to any other site within the Gulf, even to the closest site in terms of geographical distance (Plateau des Aresquiers).

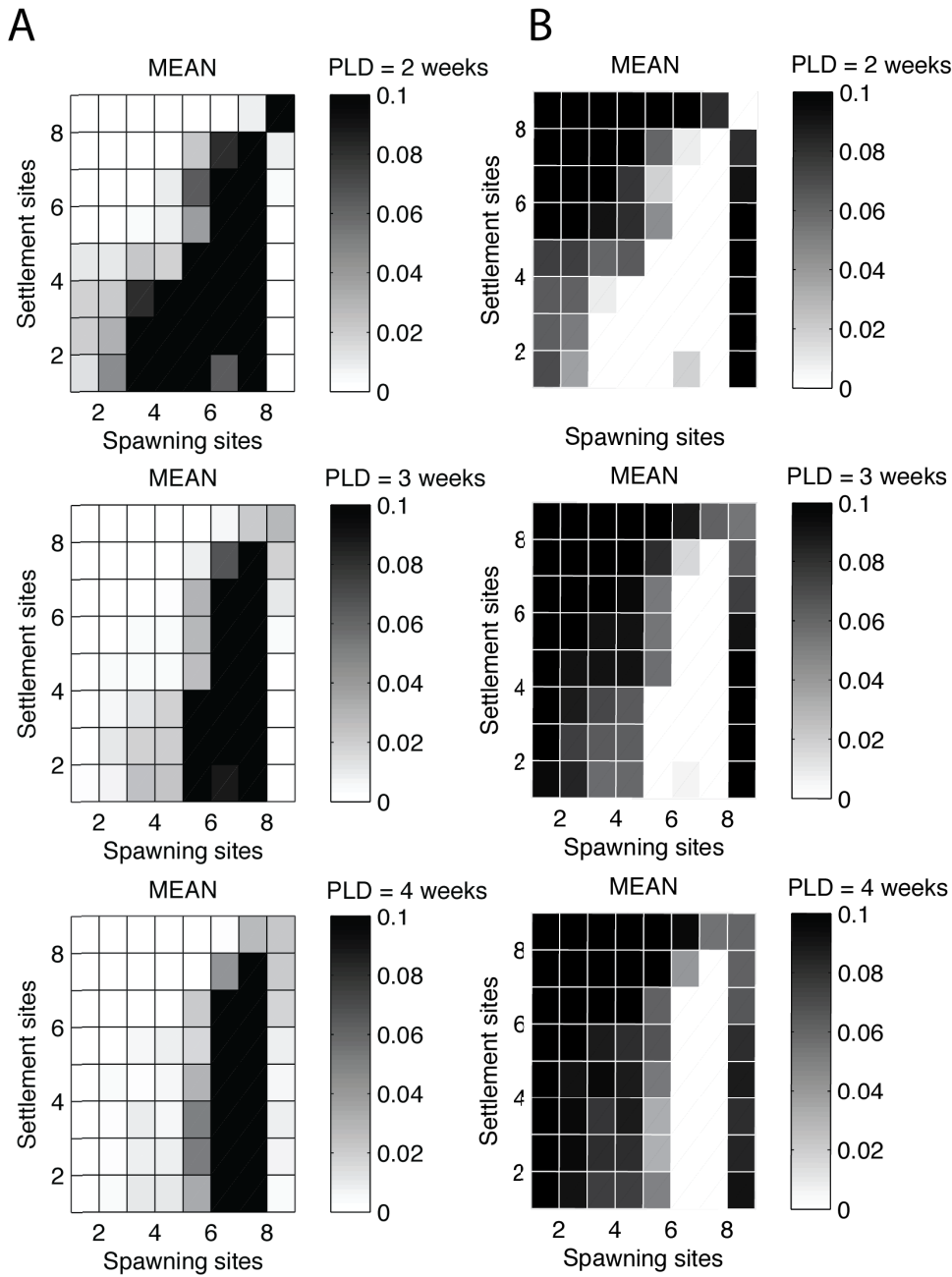


Figure 3.4.- Connectivity and hydrological distance matrices resulting from a biophysical model of larval dispersal of *E. singularis* in the Gulf of Lions, considering 11 sites distributed from east to west of the Gulf (Côte Bleue, CBL; Plateau des Aresquiers, PAR; Cap d’Agde, AGD; Côte Vermeille (CVE). A) Transition probability matrices. Each color cell represents the probability (in percentage) of larval retention from one site to another. B) Hydrological distance matrices. Each color cell represents the hydrological distance (inverse of larval connectivity) from one site to another.





Regarding the genetic population structure of *E. singularis* in the Gulf of Lions, the Bayesian clustering analysis identified three genetic clusters ($K = 3$) which, although not completely isolated, allows the differentiation between Côte Bleue, Côte Vermeille and the grouping of Plateau des Aresquiers and Cap d'Agde (Figure 3.5).

$K = 3$

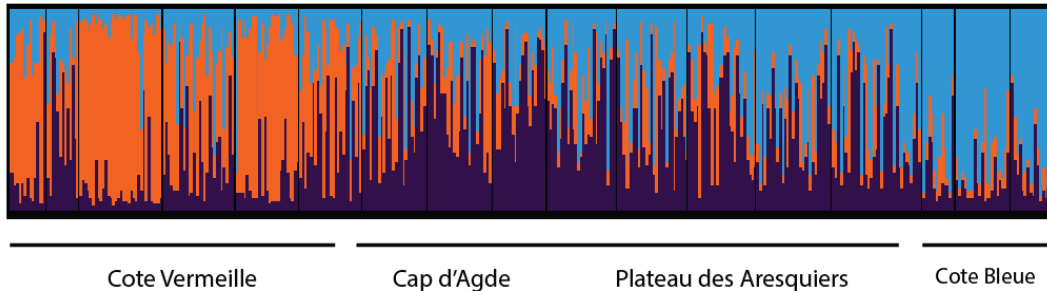


Figure 3.5.- Population structure of *E. singularis* in the Gulf of Lions, as revealed by Structure software. Each individual is represented by a vertical color line, with each color representing the proportion of membership to each cluster ($K = 3$). Black lines represent the division among sampled populations.

The analysis of contemporary connectivity resulted in the identification of five recent migrants, which corresponds to 1.84% of the total number of samples analyzed. The origin of all the first generation migrants was identified: two immigrant colonies were found in Cap d'Agde, one originating from Côte Bleue and the other being native of Plateau des Aresquiers. The remaining three immigrants were found in Côte Vermeille, each one coming from a different population: Cap d'Agde, Plateau des Aresquiers and Côte Bleue. On the other hand, migration rates obtained from the Bayesian analysis revealed a bi-directional pattern of migration among all populations, except Côte Bleue, which did not show evidence of receiving migration from any other population within the Gulf of Lions. Also, although bi-directional migration was observed, migration rate values were generally higher from east to west (Figure 3.6).

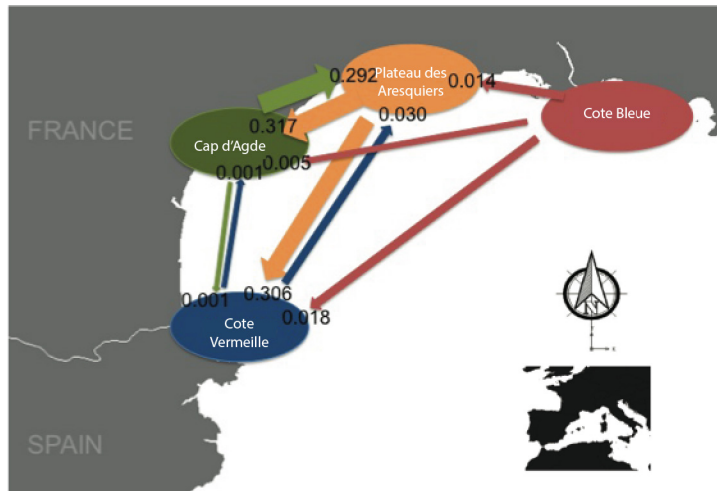


Figure 3.6.- Graphic representation of migration patterns and migration rates among sampled populations of *E. singularis* in the Gulf of Lions.

A positive and significant correlation between $F_{ST} / (1-F_{ST})$ and linear geographic distances was found ($R^2 = 0.43$; P -value < 0.01). The correlation increased when $F_{ST} / (1-F_{ST})$ was related to hydrological distances, for all the PLDs considered (Figure 3.7).

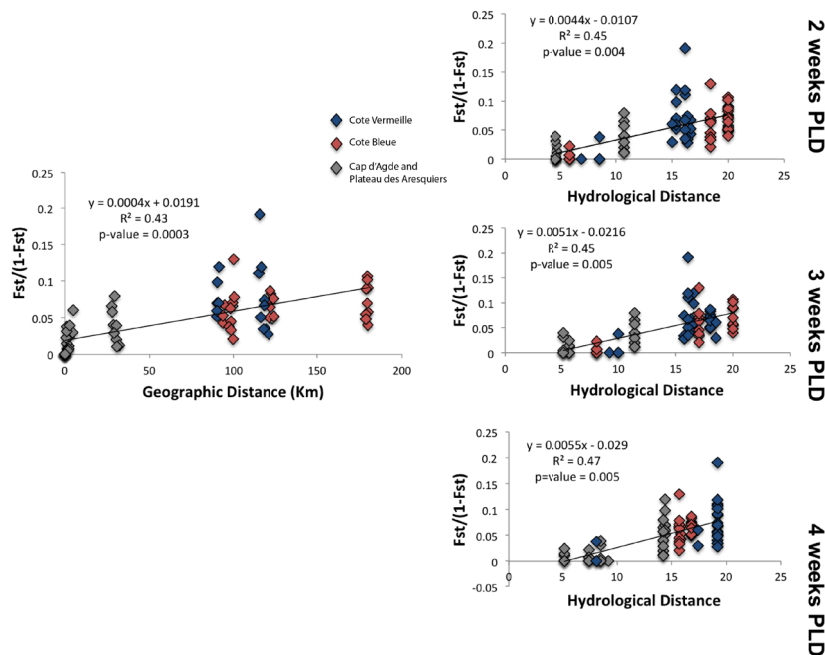


Figure 3.7.- Analysis of Isolation By Distance (IBD) for both geographical and hydrological distances of samples of *E. singularis* in the Gulf of Lions.





3.4.2 Demographic connectivity vs. realized connectivity

The analysis of the metapopulation dynamics of *E. singularis* with and without connectivity scenario, showed how population density fluctuates and is maintained over time in all sites of the Gulf of Lions (Figure 3.8).

In the absence of connectivity (isolated populations), the populations of Plateau des Aresquiers and Cap d'Agde displayed population density close to saturation, while the sites in Côte Vermeille, Leucate and Côte Bleue exhibited local extirpation. When connectivity was incorporated, the populations from Plateau des Aresquiers, Leucate, Cap d'Agde and Côte Vermeille exhibited similar values of population density, while the lowest values were found in Côte Bleue

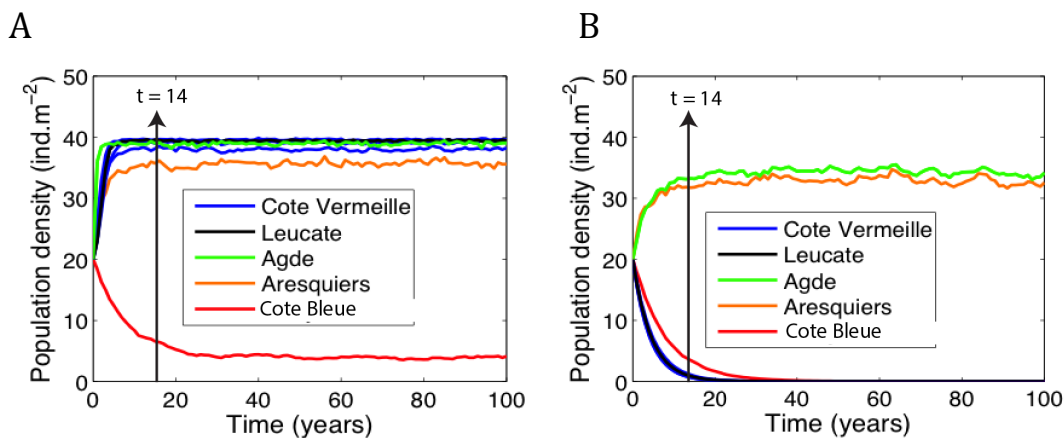


Figure 3.8.- Fluctuations in population density of *E. singularis* in the Gulf of Lions over time, as resulted from metapopulation dynamics modelling. A) Scenario considering connectivity among all populations. B) Scenario with connectivity, where all populations are isolated. Black arrow highlights the status of the metapopulation after 14 years.

Values of effective population size (N_e) were higher in Côte Vermeille (305) and Côte Bleue (204), relative to Plateau des Aresquiers and Cap d'Agde (142 and 100, respectively). In all case the effective population size, although not represented in terms of density, were lower than the estimated number of individual colonies of *E. singularis* in each population.





3.4.3 Modeled genetic connectivity vs. realized connectivity

Observed heterozygosity (H_o) varied from 0.53 to 0.59 with a mean value of 0.54 ± 0.05 . Modeled values of heterozygosity, after one population turnover (14 years), were very different between the two different connectivity scenarios (metapopulation vs. isolated populations). In the case of isolated populations, heterozygosity values remained high in all populations, and the variability around each value was relatively small among simulations (except in the cases of Cap d'Agde and Plateau des Aresquiers). Conversely, for the metapopulation scenario, modeled values of heterozygosity were similar to the ones obtained by the analysis of microsatellite data for *E. singularis*, except for Côte Bleue (Figure 3.9). However, variability around the mean was much higher for all populations in this latter case except, again, in the case of the Côte Bleue.

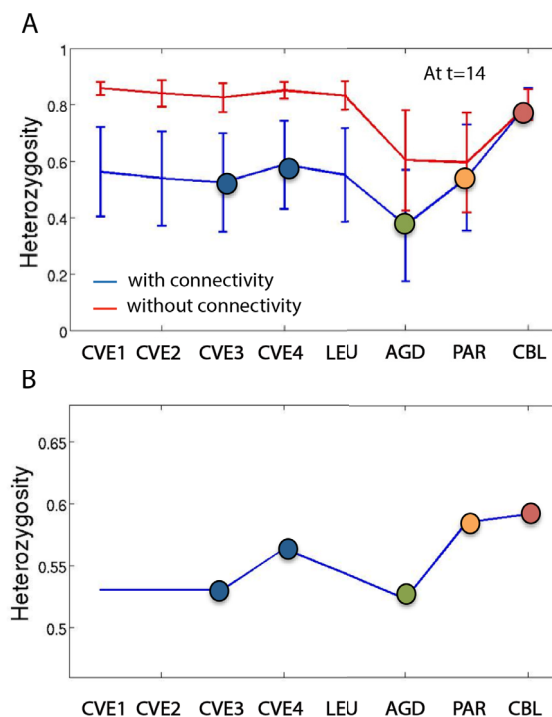


Figure 3.9.- Heterozygosity values among populations of *E. singularis* in the Gulf of Lions. A) Modeled results for two connectivity scenarios. B) Observed results from the empirical analysis of microsatellite data. Heterozygosity values correspond to results obtained a time $t=14$ years.





The PCA analysis of empirical genetic data revealed grouping among sites within each location, reflecting the genetic differentiation of three major clusters: eastern (Côte Bleue), central (Cap d'Agde and Plateau des Aresquiers) and western (Côte Vermeille) areas of the Gulf of Lions (Figure 3.10A). The sum of the two principal coordinates explained 83.81% (observations) and 99.42% (model) of the variability. The results from the PCA analysis from the modeled data also showed genetic differentiation among populations, which partially match the pattern obtained from the empirical genetic data (Figure 3.10B).

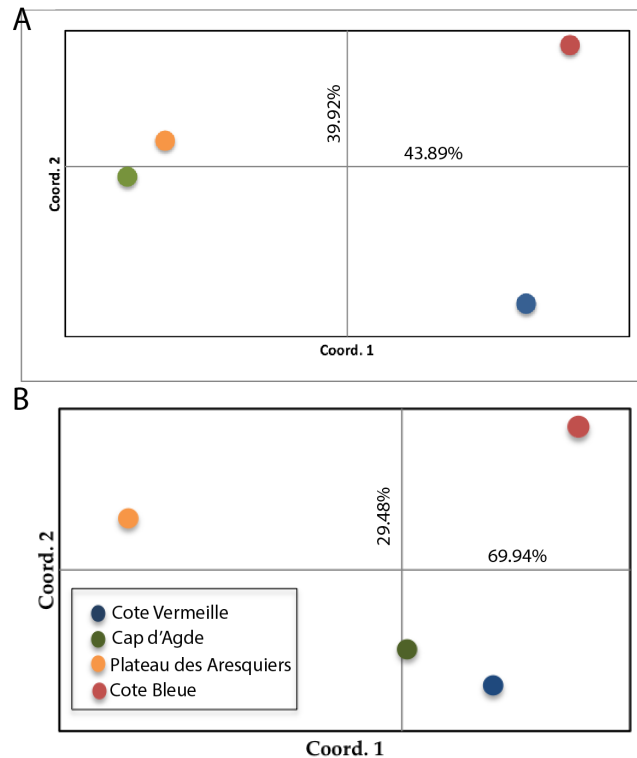


Figure 3.10- Principal Coordinate Analysis (PCA) of genetic distance among sites of *E. singularis* in the Gulf of Lions, as resulted from model simulations at time $t=14$ years. A) Results from empirical genetic data, B) Results from model simulations.



3.5 Discussion

The use of a combined modelling-observation approach to evaluate the genetic structuration of *E. singularis* in the Gulf of Lions, enable to test the relative importance of the processes driving demographic and genetic patterns of *E. singularis* in the region. Larval connectivity alone was necessary and sufficient to reproduce the demographic hierarchy in the Gulf of Lions, with comparable density from Plateau des Aresquiers to Côte Vermeille, and lower density in Côte Bleue (Guizien and Bramanti *pers. comm*). Without connectivity, populations at the extremities of the Gulf of Lions would have been exterminated, which is not the case. Contemporary migration patterns inferred from the population genetic structure of *E. singularis* agree very well with larval connectivity hindcast in recent years, although based on spring ocean circulation. Thus highlighting potential connectivity of other benthic sessile invertebrate species with high dispersal capabilities in the region.

Recent migration observations suggest that connectivity between the central and western populations (Plateau des Aresquiers, Cap d'Agde and Côte Vermeille) to Côte Bleue is low. However, on a longer term, the inference of migration rates suggest bi-directional migration between Côte Vermeille and the populations in the central part of the Gulf (Plateau des Aresquiers, Cap d'Agde), which were not present in simulated hydrological matrices. Yet, such reversal flow may happen in the Gulf of Lions depending on the wind curls in the region (Tramontane and Mistral) (Estournel *et al.*, 2003), or when anticyclonic eddies form in stratified conditions (Hu *et al.*, 2009). This latter possibility should be further explored by simulating how connectivity evolved considering a spawning time in late spring-early summer, as observed off the Catalan coast (Gori *et al.*, 2007).

The estimation of effective population sizes (N_e), which determines the rate at which the change of the genetic composition of population caused will take place due to genetic drift, was lower than the census population size in the Gulf of Lions. However,





these results are no unexpected, violations of the assumptions of Hardy-Weinberg equilibrium, like inbreeding, overlapping generations, fluctuating population density and spatial structure are expected to reduced N_e and thus, induce a stronger effect of genetic drift (Charlesworth 2009). More interestingly is the finding that the relative values of N_e among *E. singularis* populations in the Gulf of Lions is in accordance with theoretical expectations (Charlesworth 2009; Padrón and Guizien 2015): lowest values of N_e found in populations characterized by highest levels of local retention (Cap d'Agde and Plateau des Aresquiers), and higher N_e estimates for populations with low values of local retention.

The results of this study have relevant implications for regional conservation and the spatial management of marine biodiversity at a regional scale. Both modeled and empirical genetic data indicates the presence of three genetically distinct clusters of *E. singularis* characterizing the eastern, central and western parts of the Gulf of Lions. Furthermore, the contemporary patterns of migration, with the support of the simulations of demographic dynamics, allow the identification of populations that are key to the persistence of the metapopulation: 1) being sustained mainly by self-recruitment and able to persist without connectivity, Cap d'Agde and Plateau des Aresquiers act as source populations for the region; 2) with the highest level of genetic diversity and high contribution of immigrants to all the sites in the Gulf of Lions the population of *E. singularis* in Côte Bleue seems to act as a source of gene flow in the metapopulation; 3) exhibiting local extirpation without connectivity and the lowest levels of contribution of immigrants, the population of *E. singularis* in Côte Vermeille is a sink in the dynamics of the metapopulation.

Presently, three MPAs have been designed in the extreme tips of the Gulf of Lions: Parc Marin de la Côte Bleue, the Réserve Naturelle Marine de Cérberes-Banyuls (both targeted for the protection of hard-bottom species mainly) and, more recently, the Parc Naturel Marin de Golfe du Lion. The patterns of connectivity of *E. singularis* presented here suggest a low value of conservation for the Réserve Naturelle Marine de





Cérbere-Banyuls, while providing evidence of the importance of the Parc Marin de la Côte Bleue in protecting a source of genetic diversity.

Guizien *et al.*, (2014), based on numerical simulations of larval dispersal of polychaete species in the Gulf of Lions, had already identified the western and central areas of the Gulf as sink and source regions based on their values of retention rate, respectively. The findings of this thesis confirm their suggestions, while further demonstrating the genetic consequences of this pattern of connectivity in the metapopulation of a fragmented distributed gorgonian species in the region. Furthermore, it becomes evident that it is possible to observe genetic differentiation among populations even in the presence of migration. Overall, the modeled patterns of genetic structure are consistent with the field-based observations of genetic connectivity for *E. singularis* in the Gulf of Lions.

It is well known that larval dispersal does not necessarily equals to gene flow, given that migrants after successful settlement may fail to reproduce (Pineda *et al.*, 2007; Hedgecock *et al.*, 2007). A clear understanding of population connectivity should consider not only larval dispersal, but also include factors that affect post-recruitment survival (Munroe *et al.*, 2012). Numerical simulations have already shown how recruitment limitation enhances the strength of genetic drift when population size reaches saturating density (Padrón and Guizien 2015). This effect, for example, could explain the low values of genetic diversity observed in Cap d'Agde and Plateau des Aresquiers, which approach saturating density. Reversely, population density far from saturation seems to preserve a higher genetic diversity in Côte Bleue. Here, by incorporating aspects of density dependence, size structure and small-scale factors affecting dispersal and recruitment success (Armsworth 2002), I present a seascape genetics approach that provides a more realistic understating of the seascape processes driving connectivity and allows to disentangle their relative influence in driving patterns of realized connectivity.

It is important to mention that the model provides explanations rather than





predictions. One of the main objectives of the seascape genetics model used is to test whether a given set of parameters is consistent with the observed results, which, in the case of a positive confirmation, would indicate that the assumptions that were taken regarding the system are correct. Here, the assumption was that larval connectivity driven by the local hydrodynamics and habitat location and size was the only force shaping the genetic structuration of populations of *E. singularis* in the Gulf of Lions. Although in this case the potential connectivity of the species matched its realized connectivity, the results could be different for other sessile invertebrate species in the region, or even for the same species in a different geographical area. Furthermore, it must be noted that the temporal and spatial scales of the biophysical model used to simulate the potential trajectories of larval dispersal in the region influence the projected connectivity patterns. Increasing the horizontal spatial resolution of the hydrodynamical model, for example, extending spawning period to late spring or incorporating larval behavior could improve the description of hydrodynamic features that potentially affect larval transport in the western part of the Gulf of Lions (Guizien *et al.*, 2006).

Nonetheless, the use of seascape genetic approaches like the one presented here, which projections can be validated by field observations, provides a useful and effectively tool for understanding the processes that produce and maintain connectivity among marine populations.

3.6 Acknowledgements

This work was (co-)funded through a MARES Grant. MARES is a Joint Doctorate programme selected under Erasmus Mundus coordinated by Ghent University (FPA 2011-0016). Check www.mares-eu.org for extra information. This work was also partly funded by the French National Program LITEAU IV of the Ministère de l'Écologie et de l'Environnement Durable under project RocConnect—Connectivité des habitats rocheux fragmentés du Golfe du Lion (PI, K. Guizien, Project Number 12-MUTS-LITEAU-1-CDS-013). The authors particularly thank the scientific managers of the Gulf of Lions MPA : S. Blouet, E. Charbonnel, B. Ferrari and J. Payrot





for valuable interactions in the study design. The authors gratefully acknowledge the helpful assistance during sampling of R. Bricout, F. Cornette, S. Fanfard, B. Hesse, C. Labrune, L. Lescure, J.-C. Roca, P. Romans, and staff of the Réserve Naturelle Marine de Cerbère-Banyuls, Aire Marine Protégée Agatoise, Parc Naturel Marin du Golfe du Lion and Parc Marin de la Côte Bleue.

3.7 References

- Almany, G., Connolly, S., Heath, D., Hogan, J., Jones, G., McCook, L., Mills, M., *et al.* 2009. Connectivity, biodiversity conservation and the design of marine reserve networks for coral reefs. *Coral Reefs*, 28: 339–351.
- Armsworth, P. 2002. Recruitment limitation, population regulation, and larval connectivity in reef fish metapopulations. *Ecology*, 83: 1092–1104.
- Baguette, M., Blanchet, S., Legrand, D., Stevens, V., and Turlure, C. 2013. Individual dispersal, landscape connectivity and ecological networks. *Biological Reviews*, 88: 310–326.
- Benzie, J. 1999. Genetic structure of coral reef organisms: ghosts of dispersal past. *Integrative and Comparative Biology*, 39: 131–145.
- Charlesworth, B. 2009. Fundamental concepts in genetics: Effective population size and patterns of molecular evolution and variation. *Nature Reviews Genetics*, 10: 195–205.
- Costa, A., Doglioli, A., Guizien, K., and Petrenko, A. (in prep). Interpretation of graph theory metrics for species persistence in a metapopulation. The Gulf of Lion study case.
- Cowen, R. K., Lwiza, K., Sponaugle, S., Paris, C., and Olson, D. 2000. Connectivity of marine populations: open or closed? *Science*, 287: 857–859.
- Cowen, R. K., Paris, C., and Srinivasan, A. 2006. Scaling of Connectivity in Marine Populations. *Science*, 311: 522–527.
- Cowen, R., and Sponaugle, S. 2009. Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, 1: 443–466.





- Crowder, L., and Norse, E. 2008. Essential ecological insights for marine ecosystem-based management and marine spatial planning. *Marine Policy*, 32: 772–778.
- Do, C., Waples, R., Peel, D., Macbeth, G., Tillett, B., and Ovenden, J. 2014. NeEstimatorv2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Molecular Ecology Resources*, 14: 209–214.
- Douve, F. 2008. The importance of marine spatial planning in advancing ecosystem-based sea use management. *Marine Policy*, 32: 762–771.
- Epperson, B., McRae, B., Scribner, K., Cushman, S., Rosenberg, M., Fortin, M., James, P., *et al.* 2010. Utility of computer simulations in landscape genetics. *Molecular Ecology*, 19: 3549–3564.
- Estournel, C. 2003. Observation and modeling of the winter coastal oceanic circulation in the Gulf of Lion under wind conditions influenced by the continental orography (FETCH experiment). *Journal of Geophysical Research*, 108: 8059.
- Falush, D., Stephens, M., and Pritchard, J. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, 164: 1567–1587.
- Falush, D., Stephens, M., and Pritchard, J. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*, 7: 574–578. 5 pp.
- Foster, N., Paris, C., Kool, J., Baums, I., Stevens, J., Sanchez, J., Bastidas, C., *et al.* 2012. Connectivity of Caribbean coral populations: complementary insights from empirical and modelled gene flow. *Molecular Ecology*, 21: 1143–1157.
- Gaines, S. D., White, C., Carr, M. H., and Palumbi, S. R. 2010. Designing marine reserve networks for both conservation and fisheries management. *Proceedings of the National Academy of Sciences*, 107: 18286–18293.
- Galindo, H., Olson, D., and Palumbi, S. 2006. Seascape genetics: A coupled oceanographic-genetic model predicts population structure of Caribbean corals.





Current Biology: 1622–1626.

- Gori, A., Linares, C., Rossi, S., Coma, R., and Gili, J. M. 2007. Spatial variability in reproductive cycle of the gorgonians *Paramuricea clavata* and *Eunicella singularis* (Anthozoa, Octocorallia) in the Western Mediterranean Sea. *Marine Biology*, 151: 1571–1584.
- Guizien, K., Belharet, M., Moritz, C., and Guarini, J. M. 2014. Vulnerability of marine benthic metapopulations: implications of spatially structured connectivity for conservation practice in the Gulf of Lions (NW Mediterranean Sea). *Diversity and Distributions*: 1–11.
- Guizien, K., Belharet, M., Marsaleix, P., and Guarini, J. M. 2012. Using larval dispersal simulations for marine protected area design: Application to the Gulf of Lions (northwest Mediterranean). *Limnology and Oceanography*, 57: 1099–1112.
- Guizien, K., Brochier, T., Duchêne, J. C., Koh, B. S., and Marsaleix, P. 2006. Dispersal of *Owenia fusiformis* larvae by wind-driven currents: turbulence, swimming behaviour and mortality in a three-dimensional stochastic model. *Marine Ecology Progress Series*, 311: 47–66.
- Hanski, I. 1999. Habitat connectivity, habitat continuity, and metapopulations in dynamics landscapes. *Oikos*, 87: 209–219.
- Hastings, A., and Harrison, S. 1994. Metapopulation dynamics and genetics. *Annual Review of Ecology, Evolution, and Systematics*, 25: 167–188.
- Hedgecock, D., Barber, P., and Edmands, S. 2007. Genetic approaches to measuring connectivity. *Oceanologica Acta*, 20: 1–10.
- Hu, Z. Y., Doglioli, A. M., Petrenko, A. A., Marsaleix, P., and Dekeyser, I. 2009. Numerical simulations of eddies in the Gulf of Lion. *Ocean Modelling*, 28: 203–208.
- Kool, J. 2009. An object-oriented, individual-based approach for simulating the dynamics of genes in subdivided populations. *Ecological Informatics*, 4: 136–146.
- Kritzer, J., and Sale, P. 2004. Metapopulation ecology in the sea: from Levin's model to marine ecology and fisheries science. *Fish and Fisheries*, 5: 131–140.





- Levin, L. 2006. Recent progress in understanding larval dispersal: new directions and digressions. *Integrative and Comparative Biology*, 46: 282–297.
- Lowe, W., and Allendorf, F. 2010. What can genetics tell us about population connectivity? *Molecular Ecology*, 19: 3038–3051.
- Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research*, 27: 209–220.
- Marko, P., and Hart, M. 2011. The complex analytical landscape of gene flow inference. *Trends in Ecology & Evolution*, 26: 448–456.
- Marsaleix, P., Auclair, F., Floor, J., Herrmann, M., Estournel, C., Pairaud, I., and Ulses, C. 2008. Energy conservation issues in sigma-coordinate free-surface ocean models. *Ocean Modelling*, 20: 61–89.
- Millot, C. 1990. The Gulf of Lions' hydrodynamics. *Continental shelf research*, 10: 885–894.
- Munroe, D., Klinck, J., Hofman, E., and Powell, E. 2012. The role of larval dispersal in metapopulation gene flow: Local population dynamics matter. *Journal of Marine Research*: 1–2441–4677.
- Padrón, M., and Guizien, K. 2015. Modelling the effect of demographic traits and connectivity on the genetic structuration of marine metapopulations of sedentary benthic invertebrates. *ICES Journal of Marine Science*, doi: 10.1093/icesjms/fsv158.
- Paris, C., Cherubin, L., and Cowen, R. 2007. Surfing, spinning, or diving from reef to reef: effects on population connectivity. *Marine Ecology Progress Series*, 347: 285–300.
- Pineda, J., Hare, J., and Sponaugle, S. 2007. Larval transport and dispersal in the coastal ocean and consequences for population connectivity. *Oceanologica Acta*, 20: 22–39.
- Pritchard, J., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155: 945–959.
- Werner, F., Cowen, R., and Paris, C. 2007. Coupled biological and physical models. *Oceanologica Acta*: 1–16.





3.8 Supplementary material

Table S1.- AMOVA Results

Source of variation	d.f.	Percentage of variance	P
Among populations	3	4.21	0.040
Among individuals within populations	268	0.25	0.000
Within individuals	272	96.04	0.552



Conclusion and perspectives





Within a context of growing concern regarding the conservation of marine biodiversity and the maintenance of population persistence in the face of increasing human and natural disturbances to the marine ecosystems (Lubchenko 2003), the aim of this thesis was to improve our understanding on the patterns of connectivity among populations of sessile invertebrate species and the processes driving their spatial structure at a regional scale. Connectivity, understood as a fundamental factor in the dynamics and genetic structure of metapopulations, affects the ability of marine populations to mitigate ecological perturbations (Hastings and Botsford 2006). However, with the variety and variability of the processes shaping the patterns of connectivity over a species life cycle being integrated over time, how can we identify the underlying processes that create and maintain the patterns of realized connectivity, observed from empirical genetic data?

This question is the yarn that threads the connection among the different chapters of the present work. Although different methods that allow estimating different types of connectivity (e.g. hydrological, demographic and genetic) have been developed by several researchers in the past (Kool *et al.*, 2013), the challenge remained in the integration of the results obtained from different approaches (Cowen and Sponaugle 2009; Burgess *et al.*, 2014). Chapter 1 examined how demographic traits and dispersal structure affect the genetic diversity and patterns of genetic differentiation among populations of benthic sessile invertebrate species. Presenting a novel 'seascape' genetics model that can be used as a tool for disentangling the relative contribution of seascape processes (e.g. hydrology and demography) on the genetic differentiation among populations, it is shown how pre- and settlement limitations have a strong influence on the genetic structure. Furthermore, this chapter exhibits how local disturbances may affect genetic diversity over a relatively short period of time, highlighting the role of transient demography in the genetic structuration of marine populations.

In Chapter 2, an assessment of the patterns of genetic connectivity of two common and widely distributed gorgonian species is presented as case studies for





evaluating the potential processes driving population genetic structure at a regional scale. The results from this chapter highlight the role of connectivity in the potential recovery of populations after ecological disturbances. For *Paramuricea clavata* in the Ligurian Sea, frequent migration was found within 40 km. *Eunicella singularis* in the NW Mediterranean Sea exhibited strong spatial patterns of genetic structure at a regional scale, allowing the identification of the limit of closure of a metapopulation formed by fragmented rocky habitats within the Gulf of Lions. Levels of migration demonstrated the dispersal capabilities of both species over long geographic distances (>100 km). However, the inference of population connectivity from genetic data alone cannot reveal the underlying processes that create and maintain the patterns of genetic structure (Lowe and Allendorf 2010).

It is in Chapter 3 where an attempt to discern the seascape processes responsible for the observed pattern of genetic connectivity of a benthic sessile species is presented. Given the lack of a high-resolution hydrodynamical model for the Ligurian Sea, the assessment was only possible for the case of *E. singularis* in the Gulf of Lions. To test whether larval dispersal alone could lead to the realized connectivity of this species, a comparison of the modeled vs. empirical patterns of population genetic structure was carried out. The results from this chapter put in evidence the possibility of observing high levels of genetic differentiation among populations even in the presence of migration in the Gulf of Lions. Moreover, it is shown that both genetic and demographic differentiation among populations of *E. singularis* can be explained by the regional spatial structure of dispersal alone.

The integrative and quantitative approach employed in this thesis, involving regional hydrodynamics, larval dispersal, demography and population genetics goes significantly beyond the qualitative measures typically used to evaluate the effect of some aspects of life history of marine species on genetic structuring, such as PLD (Selkoe and Toonen 2011). The lack of a consistent fit with expectations (the longer the PLD the weaker the genetic differentiation among populations) is a proof of the complexity of





factors influencing settlement and the overestimation of genetic metrics used as proxies to infer genetic connectivity, such as F_{st} .

Within the context of metapopulations, it is also important to consider that one of the characteristics that differentiate a metapopulation from a set of discrete populations with high levels of connectivity is the asynchrony in the dynamics of each one of the subpopulations that constitute it (Sale *et al.*, 2006). A property that must be evaluated in order to understand how its temporal variability could influence management and have potential implications for conservation. Asymmetrical dispersal within a metapopulation may result in source-sink population dynamics that are particularly relevant in the design of MPAs (Crowder *et al.*, 2000).

The model presented in Chapter 1, as has been demonstrated in Chapter 3, provides a tool for quantitatively assessing the genetic response of a metapopulation due to changes in some of the seascape processes that are assumed to influence the system, which is particularly relevant in marine spatial planning. The results from this type of sensitivity analysis can help identify key populations for conservation. In the case of *P. clavata* in the Ligurian Sea, the populations of Punta Mesco and Portovenere seem to act as a source of migrants potentially contributing to the genetic diversity of the metapopulation. For *E. singularis* in the Ligurian Sea, on the other hand, from the viewpoint of demographic connectivity, the populations of Cap d'Agde and Plateau des Aresquiers are identified as a source of larvae enhancing the persistence of the metapopulation. While from the genetics point of view, the population of Carry-le-Rouet seems to be the source of genetic diversity. Interestingly, the sink dynamics of the Côte Vermeille in the Gulf of Lions is congruent in both demographic and genetic approaches. Nonetheless, there are many ways in which further research could improve the sensibility of the model and, thus, our capacity to discern the relative contribution of the processes shaping the patterns of genetic structure at smaller spatial scales.

From the modeling perspective, higher spatial and temporal resolution data could enhance our understanding on the fine-scale hydrodynamic features that affect





near shore processes, and influence the estimated levels of local retention in the populations of interest. Some of the discrepancies found between the modeled and observed patterns of genetic differentiation among populations may, in fact, be due to this issue. The spatial resolution of the model used in this work may not be high enough to accurately estimate the levels of local retention in areas with high geographic heterogeneity. For example, levels of local retention in Côte Vermeille may be underestimated, given that high levels of genetic differentiation (as observed from the analysis of empirical genetic data in Chapter 2.2) are usually expected from sites with higher levels of local retention. In addition, the lack of knowledge about the behavior of the larvae as well as the PLD of the species (*E. singularis*) constrained a more realistic simulation of the larval trajectories in the region.

In terms of demography, one important limitation of the model is that, although taking into account overlapping generations, it does not incorporate distinct age/size classes. Temporal variability of settlement in gorgonian species has been previously reported (Linares *et al.*, 2008; Gori *et al.*, 2011), which could potentially lead to different patterns of connectivity among cohorts. Sensitivity analysis carried out by Bramanti *et al.* (2015) showed that variation of demographic parameters have different effects on the fate of a metapopulation depending on the size classes affected. Furthermore, although much difficult to estimate, field measurements of population size, survival and fecundity within subpopulations could also improve the accuracy of the model outputs.

The potential temporal variability of connectivity patterns among cohorts could be examined by other empirical estimates of genetic data. Although genetic assignment tests have been proven very useful to evaluate contemporary migration among populations (Lowe and Allendorf 2010), parentage analysis provides a direct measure of pedigree. The advantage of this method is that allows identifying the parents of the recruits collected in the population, permitting to determine the relationship between dispersal and the demographic structure of the population. However, to establish these relationships it is necessary to collect an exhaustive number of samples, representative





of the studied population. An interesting alternative approach would be to evaluate the patterns of genetic connectivity for each age/size class in order to identify if the relative contribution of the processes shaping connectivity changes over time and/or during the lifetime of the species (Goffredo and Lasker 2006). However, the complex growth trajectories of gorgonian species can mask the relationship between size and age (Lasker and Porto-Hannes (2015) assessed and compared the genetic structure of recruits and adults over several years for the gorgonian *Antillogorgia elisabethae* in the Caribbean, however, they did not find significant differences among them.

The selection of an appropriate metric to infer genetic connectivity among populations must be highlighted as well. The most common measures of population differentiation (e.g. F_{st} and G_{st}) could be misleading due to the effect of variance in effective population size and the non-equilibrium conditions of most natural populations (Waples and Gaggiotti 2006). Furthermore, analysis of population genetic structure and the identification of genetically distinct clusters, does not allow inferring the degree of 'isolation' or 'connectedness' of marine populations. Populations identified as being genetically distinct, like the case of Côte Vermeille from Chapter 2.2, may, in fact, not be able to persist over time without connectivity, as observed in Chapter 3. The use of direct methods, like assignment tests and parentage analysis, should then be the priority in studies attempting to evaluate genetic connectivity among marine populations.

Overall, it becomes clear that, given the complexity of the processes that drive and maintain marine population connectivity over time, there is no reason to believe that two similar patterns of connectivity will be the result of the exact same processes. Disentangling their relative contribution to the realized connectivity of benthic sessile species should be approached with careful regards of the species' life history and the region it inhabits. In this study, genetic and demographic differentiation of *E. singularis* in the Gulf of Lions seemed to be explained by larval dispersal alone. However, a





different scenario may be found for the same species in another region, or for a different species in the same region.

Nonetheless, the sensitivity of genetic structure to the patterns of hydrological connectivity has important implications for genetic connectivity in a changing world. Climate change is expected to influence PLD, local retention and thus, connectivity patterns among marine populations in the Mediterranean (Andrello *et al.*, 2014). Predictions on the potential effect are, however, complicated by the synergetic (additive or compensating) effects of increasing seawater temperature on environmental (current velocity and direction) and biological (survival, reproductive output, etc.) factors. An increase of the hydrological distance among populations may reduced gene flow and, thus, augments their genetic differentiation. Conversely, increased dispersal may enhance gene flow among populations and lead to a reduction of population differentiation and potential for local adaptation.

To reach a more realistic integration of connectivity on conservation planning within a context of changing marine ecosystems, it would be then necessary to integrate through modelling the potential effect of multiple demographic traits and spatial structure of dispersal for several marine sessile species and how they will be influenced by human activities and climate change at a regional scale. A challenging task that calls for a collaborative multi-disciplinary approach that could greatly influence marine spatial planning for the persistence of marine populations over time.

References

- Andrello, M., Mouillot, D., Somot, S., Thuiller, W., and Manel, S. 2014. Additive effects of climate change on connectivity between marine protected areas and larval supply to fished areas. *Diversity and Distributions*: 1–12.
- Bramanti, L., Iannelli, M., Fan, T. Y., and Edmunds, P. J. 2015. Using demographic models to project the effects of climate change on scleractinian corals: *Pocillopora damicornis* as a case study. *Coral Reefs*: 1–11.





- Burgess, S., Nickols, K., Griesemer, C., Barnett, L., Dedrick, A., Satterthwaite, E., Yamane, L., *et al.* 2014. Beyond connectivity: how empirical methods can quantify population persistence to improve marine protected-area design. *Ecological Applications*, 24: 257–270.
- Cowen, R., and Sponaugle, S. 2009. Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, 1: 443–466.
- Crowder, L., Lyman, S., Figueira, W., and Priddy, J. 2000. Source-sink population dynamics and the problem of siting marine reserves. *Bulletin of Marine Science*, 66: 799–820.
- Goffredo, S., and Lasker, H. 2006. Modular growth of a gorgonian coral can generate predictable patterns of colony growth. *Journal of Experimental Marine Biology and Ecology*, 336: 221–229.
- Gori, A., Rossi, S., Berganzo, E., Pretus, J., Dale, M., and Gili, J. M. 2011. Spatial distribution patterns of the gorgonians *Eunicella singularis*, *Paramuricea clavata*, and *Leptogorgia sarmentosa* (Cape of Creus, Northwestern Mediterranean Sea). *Marine Biology*, 158: 143–158.
- Hastings, A., and Botsford, L. 2006. Persistence of spatial populations depends on returning home. *Proceedings of the National Academy of Sciences*, 103: 6067–6072.
- Kool, J., Moilanen, A., and Treml, E. 2013. Population connectivity: recent advances and new perspectives. *Landscape Ecology*, 28: 165–185.
- Lasker, H., and Porto-Hannes, I. 2015. Population structure among octocoral adults and recruits identifies scale dependent patterns of population isolation in The Bahamas. *PeerJ*, 3: e1019–DOI 10.7717–peerj.1019–. 1066.
- Linares, C., Coma, R., Garrabou, J., Diaz, D., and Zabala, M. 2008. Size distribution, density and disturbance in two Mediterranean gorgonians: *Paramuricea clavata* and *Eunicella singularis*. *Journal of Applied Ecology*, 45: 688–699.
- Lowe, W., and Allendorf, F. 2010. What can genetics tell us about population connectivity? *Molecular Ecology*, 19: 3038–3051.





Lubchenco, J., Palumbi, S., Gaines, S., and Andelman, S. 2003. Plugging a hole in the ocean: the emerging science of marine reserves. *Ecological Applications*, 13: S3–S7.

Sale, P., Hanski, I., and Kritzer, J. 2006. Chapter 1 - The merging of metapopulation theory and marine ecology: establishing the historical context. Academic Press, Burlington. 26 pp.

Selkoe, K., and Toonen, R. 2011. Marine connectivity: a new look at pelagic larval duration and genetic metrics of dispersal. *Marine Ecology Progress Series*, 436: 291–305.

Waples, R., and Gaggiotti, O. 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: 1519-1439.

