

**Thesis title:**

Testing spatial models of genetic connectivity for European *Raja clavata* and *Sepia officinalis* in response to seascape resistance

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**Date of submission:**

10 October 2014

**Date of corrected submission:**

9 December 2014

## Declaration

This thesis is written in the form of a journal article from of the *Methods in Ecology and Evolution*.

I wish to acknowledge the following assistance in the research detailed in this report:

My supervisor Associate Professor Adam Stow, and PhD candidate Paolo Momigliano who assisted in the study design and editorial recommendations of this thesis. Furthermore PhD candidate Paolo Momigliano assisted in and designed the original ArcGIS friction surfaces for the study area of interest and proofed the final R script forms. I would like to acknowledge like to acknowledge feedback on preliminary forms of the 'Introduction' and 'Methods and Materials' Coordinators for the Master of Research 2014 program by Associate Professor Grant Hose and Associate Professor Andrew Barron.

All other research described in this report is my own original work.



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## **Australian Higher Ed Graduation statement abstract**

Testing influences of habitat shape on movement and genetic connectivity for wild populations has largely been neglected in marine systems. We apply this approach to two demersal species, the common cuttlefish and thornback ray, to test how the relationship between genetic connectivity and movement between locations can inform on how environmental features and natural history influence connectivity. Isolation by ocean distances explained the connectivity of rays while cuttlefish connectivity was influenced by habitat configuration and the least-cost distance over habitat. We provide a simple framework for developing tests of genetic connectivity that reveal the processes underpinning patterns of genetic structure.

## Abstract

Predicting influences of habitat distribution and heterogeneity on species gene flow is now widely acknowledged as a key step in optimising effective marine management practices. Commonly applied models of geographic influence such as isolation by distance (IBD) and isolation by oceanic distances (IBO), though often effective at predicting the genetic connectivity of pelagic species, fail to incorporate potential influences of habitat by single corridor or multiple corridors on the genetic and movement connectivity for non-pelagic and demersal-obligate species, as found on continental shelf systems. Least-cost path (LCP) and isolation by resistance (IBR); a novel approach based on Circuit Theory principles of random walk, counters these limitations by integrating the effects of single and multiple pathways on connectivity by distances taken between locations over habitat shapes of assigned resistances optimised *a priori*. Applications of these models have been largely restricted to terrestrial systems despite the potential for improving predictions of connectivity for marine habitat specialists. Using available genetic, distribution and habitat data from two co-distributed and predominately demersal species *Sepia officinalis* and *Raja clavata*, we were able to identify that IBO explained the connectivity of *R. clavata* while *S. officinalis* connectivity was influenced by IBR and the LCP by habitat configuration. With this study we provide a simple framework for developing tests of genetic connectivity that indicate some of the processes underpinning patterns of genetic structure.

Keywords: fisheries management, geographical information systems, landscape genetics, seascape connectivity

## INTRODUCTION

Genetic and demographic compositions of natural populations are in part the emergent properties of adaption to the spatial and temporal distribution of their realised niches (Baguette et al. 2013; Hutchinson, 1957; Riddle et al. 2008; Wiens & Graham, 2005). The degree to which particular compositions and conditions in the environment facilitate or offer friction to movement has been proposed to determine the connectivity within a species distribution (McRae, 2006; McRae & Beier, 2007). The degree of connectivity influences a range of processes, including the conveyance of evolutionary potential and variance (*i.e.* gene flow), source-sink dynamics of meta-populations, resource migration, emigration to ‘islands’ in matrices of resistance, demographic fluctuations, gamete and larval dispersal, response to climate change expansions and the persistence of biodiversity and their ecological services (Garrido-Garduno et al. 2013). Connectivity described herein refers to gene flow among regional ‘units’ to reflect the indeterminate nature of population limits (Stow & Magnusson, 2012). In the following sections I review the models that are typically employed to predict connectivity (see also Table 1), predicting connectivity in the seascape and objectives of this paper.

### Models predicting connectivity

Identifying environment features that reduce or facilitate connectivity among fragmented and exploited populations is vital for long-term conservation and management (Manel et al. 2003; McRae & Beier, 2007; Shirk et al. 2010). Effective planning and practices for reserve design should aim to preserve broad-scale processes such as gene flow and migration (Manel et al. 2003; McRae & Beier, 2007; Shirk et al. 2010). However such spatially and quality-specific aspects are often studied and applied based on ad-hoc inferences of coincident landscape features and qualities in association to genetic structure (McRae, 2006; McRae & Beier, 2007; Shirk et al. 2010).

Table 1. List of key terminology and geographic models, their descriptions and further explanatory references as applied in this study. Model terminology includes, known assumptions and published examples of each geographic distance model.

Term	Description	Assumptions and/ or limitations	References
Population	For the application of this investigation, is defined as individuals from a spatially specified locality, wherein the true bounds of theoretical populations are not assumed.	The application of this term is limited to estimates of targeted localities wherein effective interbreeding and migration within that locality are assumed to be greater than among localities.	Diniz-Filho et al. 2013
Connectivity	The status of gene flow and dispersive potential between populations.	The resolution and configuration of connectivity is dependent on the complexity of the model applied.	McRae, 2006; McRae et al. 2008.
Isolation by distance (IBD) model	Euclidean spatial predictor which assumes a positive ‘ <i>unbounded</i> ’ and linear relationship between the pairwise locations’ geographic and genetic distance matrices.	Model does not incorporate spatial heterogeneity into pairwise matrices.	Wright, 1942. McRae & Beier, 2007.
Isolation by oceanic distance (IBO) model	Spatial predictor which assumes a positive near-linear relationship between two pairwise location geographic and genetic distance matrices, bounded only by the spatial configuration of hypothesised ‘ <i>potential habitat</i> ’ of uniform resistance and ‘ <i>non-habitat</i> ’ of infinite resistance to gene flow. Also known as two-dimensional isolation by distance.	Model incorporates heterogeneity only by the spatial configuration of two environmental feature classes in the study range. For the purposes of this investigation potential habitat was classes as all continuous marine waters in range and non-habitat as above-sea landforms.	(Two-dimensional terrestrial IBD terrestrial analogue): McRae & Beier, 2007.
Least cost path (LCP) model	Spatial predictor which assumes a positive near-linear relationship between two pairwise cost distances and their genetic distance matrices. This cost distance is determined to be the path which accumulates the lowest cost in terms of distance travelled by a hypothesised ‘preferred habitat’ as opposed to ‘non-preferred habitats between sites. Preferred habitat configurations for the purpose of this investigation assume a uniform resistance, while non-preferred habitats assume a resistance greater than one.	Model calculates a single optimal path between locations. It incorporates heterogeneity by the spatial configuration of three environmental feature classes in the study range. For the purposes of this investigation the hypothesised preferred habitat was designated as the bounds of the continental shelf (<1m to 200m sea floor depth) and non-preferred habitat was the spatial configuration of all water beyond the depth of the continental shelf.	McRae & Beier, 2007.
Isolation by resistance (IBR) model	Spatial predictor which assumes a positive relationship between two pairwise ‘resistance’ distances and their genetic distance matrices. The resistance is determined by the simultaneous calculation of all cost path contributions between the hypothesised preferred habitat and non-habitat features.	Model incorporates the spatial heterogeneity and influence of multiple pathways contributors into the prediction of genetic connectivity, addressing the limitations of IBD and LCP respectively, while hypothetically increasing the resolution of connectivity over IBO	McRae, 2006; McRae & Beier, 2007; McRae et al. 2008.

By in large the most frequently applied spatial model of genetic differentiation in land and seascape systems is isolation-by-distance (IBD) based on geographic distance alone (Manel et al. 2013; McRae & Beier, 2007). A test of IBD is asking whether geographically closer population samples will have less genetic divergence than those further apart, it can only incorporate an assumption of homogeneous connectivity on all planes of movement (McRae, 2006). The use of straight line distances is often nonsensical in marine environments because this distance measure may include the terrestrial environment. Therefore a common model feature in marine population analyses is isolation by oceanic distance, whereby the closest pairwise geographic distances that exclude the terrestrial environment are used.

Another frequently utilised distance model is the least-cost path (LCP) model, which incorporates a single optimal (*i.e.* suitable habitat) path of least distance between two sample locations. Thus the LCP model offers a potentially higher resolution for fragmented populations in complex habitats by incorporating an element *a priori* deductions of specific biology and landscape features (McRae, 2006). That said the LCP model cannot calculate the collective contribution of multiple paths of migration and gene flow between two sites, and has typically relied upon course-distribution data (McRae, 2006; McRae & Beier, 2007).. Offering a limited resolution of biologically relevant, complex connectivity (McRae, 2006; McRae & Beier, 2007).

To address the limitations of IBD and LCP McRae (2006) formulated a new model which replaces pure or optimal geographic distance with a resistance distance format that correlates predetermined spatially explicit variations in landscape features (*i.e.* resistance) with genetic structures. This model, referred to as isolation-by-resistance (IBR), was based on Circuit Theory principles of random walk and cumulative resistances between nodes in an electrical array. In IBR all cumulative resistance pathways (analogous to connectivity or gene flow) are calculated simultaneously between two points of the array, addressing the limitation of LCP. All resistances between the nodes are assigned ranks based on spatially explicit univariate or multivariate features, addressing IBD (McRae, 2006). Comparative model-fit tests via R-squared Mantel and partial r Mantel tests have also demonstrated that IBR can explain significantly more genetic differentiation when influences of IBD and LCP are controlled for (Mantel, 1967; McRae & Beier, 2007). In this theoretical paper McRae (2006) discusses the theoretical premise and applicability for a novel landscape genetics model based on Circuit Theory, 'isolation by resistance' (IBR). The main aim was to explain the mathematical

principles that can be applied to and account for variations in species connectivity, in response to spatially explicit features selected *a priori*. McRae (2006) describes how IBR improves conventional IBD and LCP by incorporating spatial heterogeneity and multiple pathway contributions into the model respectively. This argument is supported by his R-squared Mantel and partial r Mantel analyses of a simulated population, whereby IBR of a variable feature explains significantly more of the observed genetic structuring than either IBD or LCP. McRae's (2006) model has a wide applicability, where a variety of spatially explicit univariate or multivariate landscape resistances can be fed into the model based on an *a priori* evaluation of the target species' connectivity resistances. This paper outlines the mathematical and conceptual framework for employing and contrasting IBR co-distributed genetic and spatial data for species in the seascape. McRae and Beier (2007) demonstrated how IBR can significantly improve the resolution and optimise the inference of population connectivity over IBD and LCP, using previous genetic and landscape data in two previous studies where only IBD was calculated. The authors' aim was to provide a terrestrial proof of concept for the potential improvement IBR can offer based on data from 12 North American populations ( $n = 461$ ) of wolverines (*Gulo gulo*) typed to 12 microsatellite loci (Kyle & Strobeck, 2001) and 8 North American populations ( $n = 287$ ) of big-leaf mahogany (*Swietenia macrophylla*) typed to seven microsatellites (Novick et al. 2003). For both species IBR explained significantly more variation than IBD and moderately more than LCP in R-squared Mantel and partial r Mantel comparisons. This terrestrial proof of concept for IBR is informative in several aspects, namely it demonstrates that even relatively simple models of resistance are a significant improvement over pure distance measures. There have been several subsequent terrestrial studies which employed IBR modelling, however, this approach is yet to be evaluated with marine examples (Hagerty et al. 2011).

#### Predicting connectivity in seascapes

In their follow up review Manel and Holderegger (2013) summarised the methodological advances and limitations over the last decade in the field of landscape genetics (Manel & Holderegger, 2003). Synthesising the current body of understanding, particular attention was given to the need to increase and improve models for marine species. Concerning seascape genetics Manel and Holderegger's (2013) qualitative review revealed that there were substantially less seascape genetics papers in comparison to terrestrial systems. Two key explanations were provided, namely the difficulty in incorporating rapid temporal and spatial shifts in currents and temperatures; and difficulty in obtaining a representative sample at a sufficient spatial scale (also see; Liggins et al. 2013; Riginos & Liggins 2013; White et al. 2010). The majority of the papers discussed within these reviews tested the associations



between pelagic or wide dispersing species with genetic differentiation and conventional distance measures (Liggins et al. 2013; Riginos & Liggins 2013; White et al. 2010). Therefore for the purposes of design simplification for this proof of concept thesis species selection criteria has excluded those with pelagic larval life histories in favour of potential coastal or benthic invertebrate or invertebrate candidates. Furthermore as current can also have a significant effect on the dispersal, species which attach their clutches to substrate were favoured. Cuttlefish (*Sepia* spp.), Octopus (*Octopus* spp.) and Rajiformes (Skates and allies) in particular contain numerous examples coastal-benthic specialisation. With examples such as *Sepia officinalis* (common cuttlefish) and *Raja clavata* (thornback ray) limiting their depth preference from approximately 10 to several tens of meters over distinct substrate (Ellis et al. 2005; Sifner, et al. 2009). The target species were then selected based on the availability of pre-existing adequate genetic and complementary distribution data. The selection of a study with suitable genetic data required further scrutiny to ascertain whether the sample size and spatial scale reflected the likely dispersal ability to ensure that connectivity was evaluated on an ecologically meaningful scale (Anderson et al. 2010).

#### Preliminary methods

Following species selection spatially explicit barriers or facilitators to gene flow were identified for each species. These features corresponding to population occurrence data will serve as either the univariate or multivariate resistance proxies to associate with genetic differentiation. In their paper Shirk et al. (2010) presented a methodology for selecting appropriate resistance surfaces. Starting with expert opinion of species specific resistances (*e.g.* museum curators, specialist academics), they outlined how their parameters can then be systematically adjusted and contrasted to attain optimal resistance specifications. The authors' aim was to develop and test the application of formulae based on an *a priori* selection for resistance. This study supports the inclusion of expert opinion as a starting point for selecting relevant resistances, which can be univariately or multivariately optimised to provide the most meaningful resistances. Another aspect of creating a resistance surface was ranking the degree of resistance in order to create an IBR matrix for spatial modelling purposes. In their paper Spear et al. (2010) present a methodology for translating specified environmental resistances of IBR into relative ranks suitable for the geographic information system (GIS) environment. Spear et al. (2010) demonstrate how resistance values can be assigned to continuous variables and then optimised through computation simulations.

#### Objectives

Despite Circuit Theory's isolation by resistance (IBR) model's potential to measure connectivity as a product of their environment, this model has been applied relatively little in terrestrial systems and never in marine spaces. The central theme of this thesis has focused on developing simple binary seascape resistance models of least cost path and isolation by resistance with which we could contrast with oceanic distance models for selected marine species. I intend this paper to provide proof of concept for whether IBR can significantly improve the resolution of connectivity over previous models in the marine environment (McRae, 2006; Shirk et al. 2010; Spear et al. 2010). To address this knowledge gap, I specifically ask whether models accounting for differential resistance of habitats account for more variation in genetic structure than isolation by oceanic distance. We intend this paper to provide proof of concept for whether IBR can significantly improve the resolution of connectivity over previous models in the marine environment (McRae, 2006; Shirk et al. 2010; Spear et al. 2010). To address this knowledge gap, I specifically ask whether models accounting for differential resistance of habitats account for more variation in genetic structure than isolation by oceanic distance.

## METHODS

### Species selection criteria and application to model build

We selected two species, the common European cuttlefish, *Sepia officinalis* and the thornback ray, *Raja clavata* from the citation indexing service 'Web of Knowledge', from Pasolini et al. (2011) and Pérez-Losada et al. (2007) respectively. These papers were selected on the basis of several criteria. Namely, the availability of pairwise genetic distance values and their corresponding coordinates; measures of non-significant p-values ( $\alpha \geq 0.05$ ) of R-squared Mantel test or low explanations (*i.e.* the bench mark for a low Mantel test being R-squared  $\leq 0.5$ ) of genetic connectivity by isolation-by-distance (IBD), supporting evidence that the candidate species did not exhibit pelagic larval stages and that their range may correspond to the range of the continental shelf configuration. The International Union for Conservation of Nature (IUCN) Chondrichthyan expert group designates non-pelagic species as those which are demersal or benthos obligate or require interaction with the substrate during significant stages of their breeding or feeding cycles. The coordinates of these study sites were used to generate geographic distance matrices under three competing models tested for model fitness of genetic connectivity; the null model isolation-by-oceanic distance (IBO), connectivity by least-cost path (LCP) and isolation-by-resistance (IBR). The explanations of these models and their builds are explained in the third paragraph section below (and Table 1). The data and life history traits

for the criteria listed above and several additional population and physical characteristics for each of the selected studies has been explained in sections below.

### Sample data summaries

#### *Raja clavata* species and data summary

*R. clavata* is an obligatory demersal batoid ray from the order Rajiformes (Reid et al. 2005). Pasolini et al. (2011) study provided two fixation index matrices; pairwise difference matrix  $\Phi_{ST}$  ( $\Phi_{ST}$ ) and multilocus estimated  $F_{ST}$  matrix (see Pasolini et al. 2011: Supplementary Material S4) generated approximately 421bp of mitochondrial DNA control region (mtDNA CR) for 42 sequences at five locations and 80-500bp of amplified fragment length polymorphisms (AFLPs) at six (see Fig.1 for map of sites, Supplementary material: S3 for coordinated used). The data applied to this investigation contained six and five sites respectively for a total of 181 individuals from coastal European waters. The location of one site (the east Mediterranean) could not be resolved to an explicit coordination and was excluded in Pasolini et al. (2011) final Mantel analyses. While their Mantel tests were significant their R-squared were low in explanatory power (for AFLP: coastal distance vs linearised,  $F_{ST}$  = Mantel R-squared 0.28,  $p$  0.012; and for mtDNA not significant,  $\alpha \geq 0.05$ ). *R. clavata* are typically found at depths between 10-60m (preferred depth 60m), however maximums of 300m have been recorded (Ellis, 2005). Females and males migrate to the shallows yearly after sexual maturity to spawn up to 140 egg cases. Females typically move into shallower water than males for protracted periods (between February to September, with peaks recorded between May and June) to attach their eggs to the substrate. These points qualify their agreed non-pelagic status. Their estimated distribution ranges overlap the extent of the continental shelf between the NE Atlantic, Mediterranean Sea, and western Africa to the tip of southern Africa (Fowler et al. 2005; Smale & Cowley 1992; Rousset, 1990; Stehmann, 1995; Walker, 1998). Although *R. clavata* specifically targeted trawl net fishing is listed low by the IUCN *R. clavata* remains vulnerable to this technique and frequently forms a dominant proportion of the by-catch for fishery and research vessels in North Sea and heavily fished regions (Fowler et al. 2005). The IUCN red list has categorised their population status as near threatened and in decline (IUCN, 2014). This species has a longevity of approximately 12 years for males and 15 years for females. On average females grow to dimensions of 60-85cm total length (TL) and 45-54cm disc width (DW), while 60-77cm (TL) for males and 38-48cm (DW). This species is slow to mature with females reaching sexual maturity at around seven years and males eight.

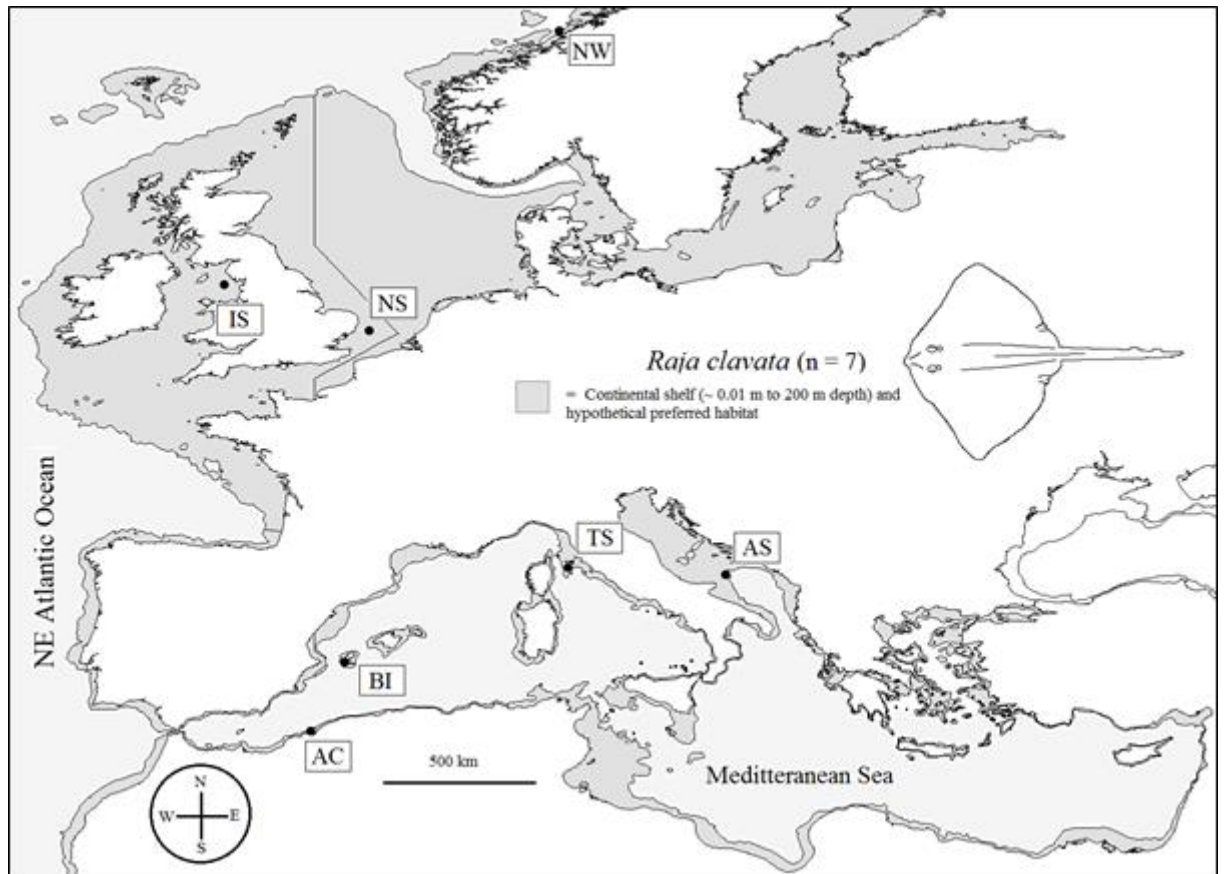


Fig 1. Map of the North-eastern Atlantic Ocean and Mediterranean Sea showing the seven locations (black points) where *R. clavata* was sampled by Pasolini et al. (2011). The dark grey shading represents the approximate bounds of the hypothetical preferred habitat; the maximum continental shelf (approx. <0.01 m to 200 m maximum depth). Map bathymetry was sourced from GEBCO (2003) as one minute grid data and visualised using ArcGIS 10.2.2 (ESRI, 2014).

#### *Sepia officinalis* species and data summary

*S. officinalis* from the order Sepiida is one of the largest and most studied of the cuttlefish. Pérez-Losada et al. (2007) study provided a pairwise ( $\Phi_{ST}$ ) difference matrix (see Pérez-Losada et al. 2007: Supplementary Material S1) generated from 659bp of mtDNA cytochrome oxidase (COI) gene (259 sequences) from 25 locations in European and northern West African coastal waters (see Fig. 2 for map of sites, Supplementary material: S4 for coordinated used) for 259 individuals. Their Mantel tests were significant however this was across all sites, after they had established that there were four well defined clades and may be confounded by non-random mating sub-populations or closely related species (for mtDNA COI: IBD vs linearised,  $\Phi_{ST}$  = Mantel  $r = 0.3$ ,  $p < 0.001$ ). *S. officinalis* are typically found at depths between 100-200m, with 100m as the preferred depth, but come to shallower waters near the coast to breed (April to July) and migrate to deeper waters in winter months. Females can attach between 150 to 4000 eggs to the substrate, or anchored plant mater in their only breeding cycle. Additionally this

species performs nocturnal vertical migrations and they are dependent on the demersal submerges into the substrate for preservation and ambush foraging on a regular basis, qualifying their generally agreed non-pelagic status. Their estimated ranges overlap the extent of the continental shelf and beyond between the North Sea in the North eastern Atlantic Ocean, Mediterranean to north western African coast but is notably absent in the Baltic Sea (Reid et al. 2005). The IUCN red list has categorised their population status as of least concern with unknown population trends, though presumed to be resilient to harvesting (IUCN, 2014). This species is short lived with a longevity of approximately two years, as is typical with cuttlefish species. *S. officinalis* is also one of the largest cuttlefish species, growing up to 2kg and 300-480mm in length (Reid et al. 2005).

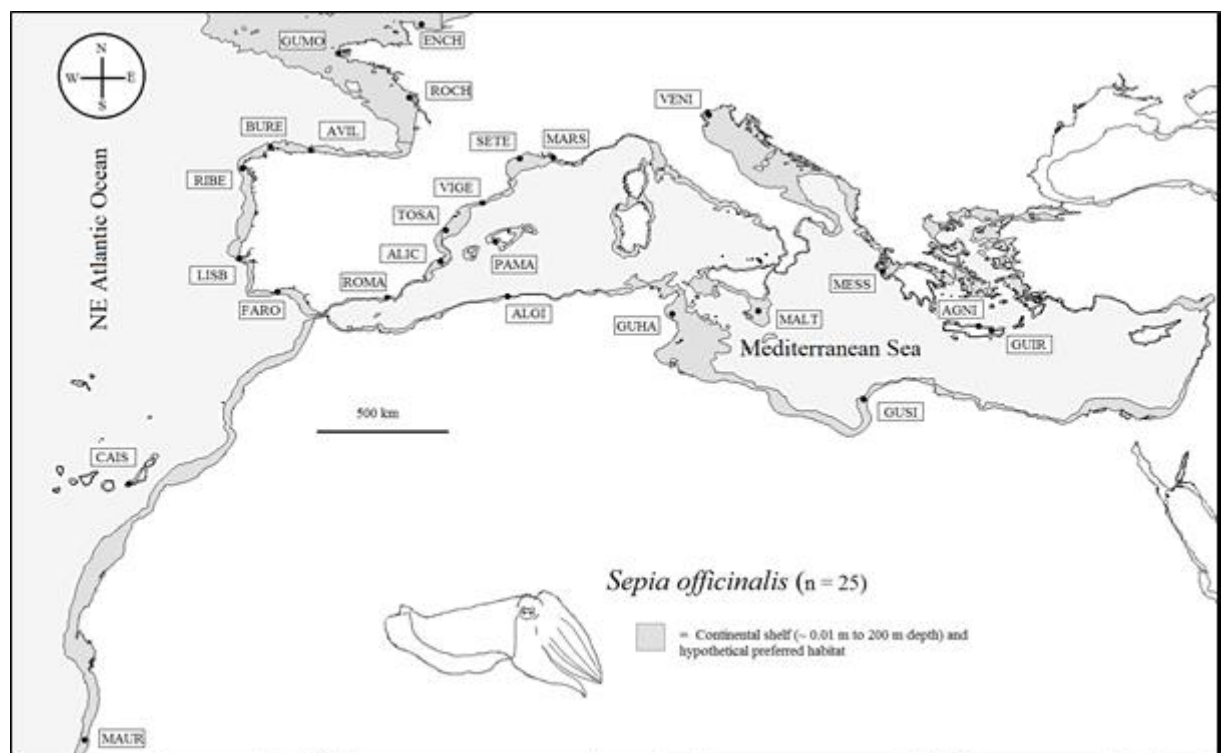


Fig 2. Map of the North-eastern Atlantic Ocean and Mediterranean Sea showing the 25 locations (black points) where *S. officinalis* was sampled. The darker grey shading represents the approximate bounds of the hypothetical preferred habitat; the maximum continental shelf (approx. <0.01 m to 200 m maximum depth). Map bathymetry was sourced from GEBCO (2003) as one minute grid data and visualised using ArcGIS 10.2.2 (ESRI, 2014).

#### Model build with ArcGIS

Bathymetric data between 14.60° and 55.68° of 1 arc minute resolution was imported from the ‘General Bathymetric Chart of the Oceans’ repository (GEBCO, 2014) capturing the coordinates for each study. This bathymetry provided the two environmental polygon bounds of differential permeability for visualisation and analysis in ArcGIS 10.2.2. For repeatable and

wide applicability of resistance analysis, two habitat classes, preferred habitat in the form of the extent of the continental shelf (bodies of water above 1-200m sea floor depth) and the non-preferred habitat (bodies of water above 201-10000m sea floor depth) covering the range extent for the continental shelf and beyond as two-dimensional polygon features. The preferred habitat for range for the study species, was given the feature of a resistance of 1 (*i.e.* least possible resistance) to its assigned polygon. This permeability value was kept constant relative to the non-habitat off the continental shelf (201m-10000m) whose permeability of assigned polygons in turn was iteratively lowered (*i.e.* higher resistance) to generate comparative distance matrices. These iterations generated distance matrices were optimised based on cross checks with Mantel R-squared in the Isolation by Distance web service, where the closet ‘true ideal’ resistance was ascribed to the non-habitat resistance that corresponded with the asymptote (based on trend plots of non-habitat-resistance setting and R-squared score) for the three model geographic distances IBO, LCP and IBR (described in section 2.4 and table 1 in further detail) to genetic distances provided. Initially low relative resistances to the continental shelf were applied (*e.g.* 5, 10, 20, 50, 75, 100) up to 1000, 2000 and 10000, until the real resistance was approached.

#### Seascape model description and implementation

Three models were applied in turn to the three genetic distance matrices (for *R. clavata* mtDNA CR and AFLPs and *S. officinalis* mtDNA COI) until the highest Mantel R-squared was approached, before declining. These models were IBO, LCP and IBR. IBO (*i.e.* oceanic distance) model predicts a positive correlation between the closest linear distance which avoids landforms and genetic distance, ignoring habitat influences. IBO represents a two-dimensional lattice bound only by the landmass configuration (the two polygon sets outer boundary limits). The distance matrices were generated by applying the uniform (1 to 1 = continental shelf to off continental shelf) resistance to the friction surface for the LCP analysis from the ArcGIS extension program Landscape Genetic v 1.2.3 (Etherington, 2011). LCP model predicts a positive linear relationship between the lowest cost-weighted distance and genetic distance between sites. LCP was thus the shortest, a single optimal path calculation optimised through iteration. The LCP generations were applied through the same package and process with the difference of a 1 to higher resistance value, iterating to the true resistance or highest explanatory R-squared. The IBR model predicts a positive linear relationship between (theoretical true) resistance distance and genetic distance, enforced upon the populations as a direct consequence of structuring by habitat configuration agency. IBR distances were generated using the Circuitscape for ArcGIS extension (v BETA) using raster inputs of node to node (here pixel

resolution by 1 arc minute) resistance as the friction surface configuration generated by the polygon value assignment process described in the previous section (McRae et al. 2008).

#### Phylogenetic analysis for *Sepia officinalis*

Pérez-Losada et al. (2007) investigation indicated that their *S. officinalis* sequence data appeared to be well coalesced into four clades. To confirm and visualise these results we performed a reconstruction of phylogenetic relationship between the *S. officinalis* samples. Where sites in clades are well coalesced and have adequate individual representation (*i.e.* eight or more for robustness) we would generate seascape models of their distance for Mantel R-squared and partial r Mantel tests (to control for either the genetic or geographic distance factor) based on molecular data retrieved under the accession no. EF416306 –E416564 GenBank, for *S. officinalis* 259 mtDNA COI sequences. These were aligned by the ClustalW function and a phylogeny estimated using the Maximum Likelihood (ML) in MEGA with at a tree fit iteration of 1000BS permutations as per Pérez-Losada et al. (2007; Tamura et al. 2013). The best phylogenetic model based on the lowest AICc was determined to be the General Time Reversible (GTR + I +  $\Gamma$ ) model with invariable sites and gamma distribution as per (Tamura, 1992). This model had a log-likelihood of -618.514, I= 22.37 and  $\Gamma$  shape = 4.78. For initial trees heuristic searches were obtained by Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach. The process involved 73 nucleotide sequences and a total of 65 positions in the final sequence (Nei & Kumar, 2000; Tamura et al. 2013).

#### Best model fit selection

For all three models we compared model predictions to genetic distances measured between the sampled populations using Mantel tests of significance (1967) and major axis regressions (Jensen et al. 2005, 2014; RStudio, 2014). The highest significant ( $\alpha = 0.05$ ) values by each seascape and genetic data model were tested by partial r to identify best fit using IBD web service with one genetic distance matrix, by one geographic distance matrix and the null IBO, indicator matrix (Jensen et al. 2005, RStudio, 2014). This analysis provides a method to observe the Mantel score outputs whilst controlling for the variation and potentially confounding effects of the null IBO model.

## RESULTS

#### Phylogenetic inference for *Sepia officinalis* and by clade analysis

Molecular data was retrieved from GenBank under the accession no. EF416306 – E416564. As found in Pérez-Losada et al. (2007) there were no indels with 659bp available following alignment. These sequences were resolved into 73 distinctive haplotypes which fell into four clades (see Supplementary material: S1). Given strong genetic partitioning and the non-significant Mantel R-squared and p values ( $\alpha > 0.05$ ) when LCP and IBR were across all sites (Supplementary material: S2), within clade Mantel analyses were required. The clades 1 and 4 (whose individuals fell in to six and 12 sites respectively) had a sufficient number of samples for analysis. For sites within each of these two clades pairwise  $\Phi_{ST}$  and conventional  $F_{ST}$  distance matrices were generated using the population genetics program Arelquin v.3.5. Using major axis regressions LCP and IBR models were run against clade 1 and clade 4 variations of these genetic metrics (including their linearised form). Clade 1 was best explained by  $\Phi_{ST}$  metrics while clade 4 was best explained by convention were best explained by  $F_{ST}$  distance, in their non-linearised form.

#### Best model fit selection

##### *Raja clavata* AFLPs

We applied R-squared (simple) Mantel and partial r Mantel tests to the AFLPs data for *R. clavata*. Partial r Mantel analysis showed that the best fitted-model was the simplest model for IBO (R-squared = 0.902,  $p = 0.007$ ), where controlling for IBO did not result in significant partial r Mantel scores (IBO; see Table 4). Observations of R-squared Mantel outputs appear to corroborate these statistic outputs with IBO and least cost path having also most the same explanatory power whilst the IBR optimised estimate is much lower and marginally significant (Fig. 4). Major axis regressions meet assumptions of linearity and spread (Fig 5.).

##### *Raja clavata* mtDNA CR

We applied R-squared Mantel and partial r Mantel tests to the mtDNA CR data for *R. clavata*. Partial r Mantel analysis showed that the best fitted-model was the simplest model for IBR (R-squared = 0.677  $p = 0.023$ ), where controlling for IBO did not result in significant partial r Mantel scores (IBO; see Table 4) Mantel outputs appear to corroborate these statistic outputs with IBO and least cost path having also most the same explanatory power, whilst the IBR optimised (Fig 4.) estimate is much lower and marginally significant. Major axis regressions meet assumptions of linearity and spread (Fig. 5).

Table 4. Summary of R-squared Mantel and partial r Mantel results of interest for thornback ray (*R. clavata*) AFLP and mtDNA sample analyses. Scores were generated in IBD web service, following 10000 permutations (Goslee & Urban, 2007; IBDWD, 2014). Partial r Mantel tested



the correlation that was not explained by the variable being control for, denoted by ('|'). Genetic distance metrics were extracted from pairwise-distance  $F_{ST}$  (y variable) calculated by Pasolini et al. (2011). Resistance distances are the resistances multiplied by the resistance on the continental shelf (least). With uniform being equal resistance (and maximum permeability between hypothetical habitat and no-habitat) and higher resistances being the differential resistance of the continental shelf.

Geographic distance metric (x)	AFLPs		mtDNA CR	
	R-squared	p-value	R-squared	p-value
Isolation by Oceanic Distance (IBO)	0.902	0.007	0.431	0.094
Isolation by Resistance (IBR by uniform)	-	-	0.64	0.023
Isolation by Resistance (IBR by 5)	0.371	0.05	-	-
Least cost distance (LCP by 5)	0.903	0.009	0.453	0.076
	partial r	p-value	partial r	p-value
IBR by uniform   IBO	-	-	0.677	0.068
IBR by 5   IBO	0.198	0.222	-	-
LCP by 5   IBO	0.216	0.292	0.246	0.304

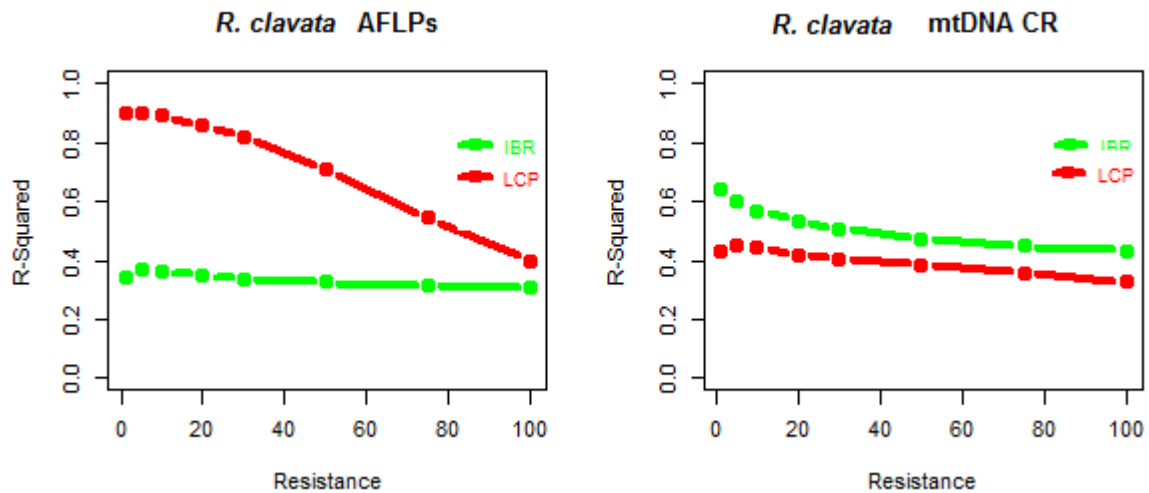


Fig. 4. Line plot of the R-squared values simulated from the LCP and IBR models based on the assignment of resistance iterations based on the mtDNA control region data for *R. clavata*. The assigned resistance for the AFLPs IBR model resistance 5 off the shelf (non-habitat) and for the LCP model resistance of 5. The mtDNA CR IBR model of uniform resistance off the shelf (non-habitat) and for the LCP model resistance of 5 off the shelf.

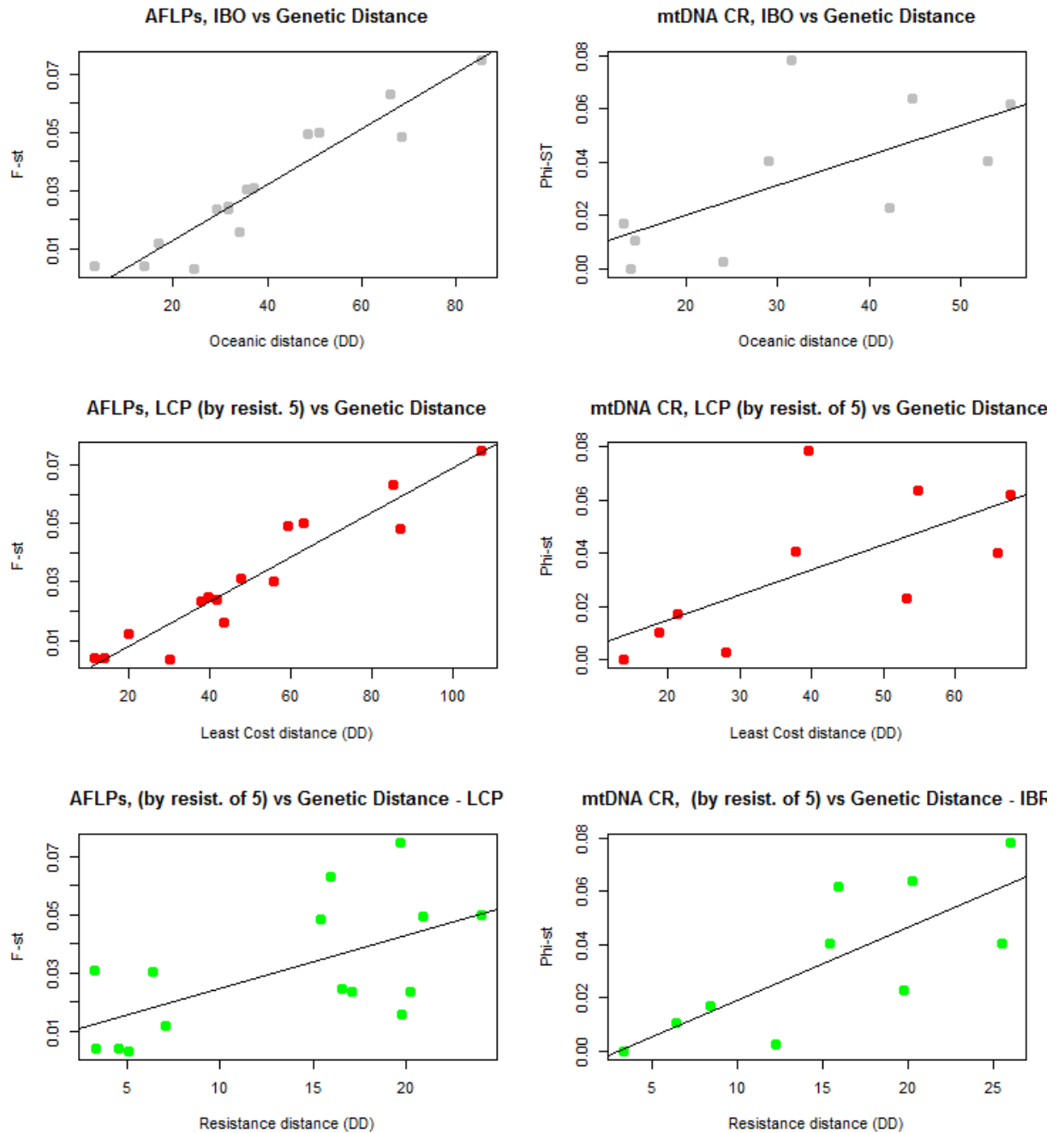


Fig. 5. Major axis regression plots showing the three optimised models for IBO, and the best-fit resistances (resist.) in comparison the continental shelf resistance of  $1\Omega$ , for LCP and IBR based on the AFLPs and mtDNA CR data for *R. clavata*.

#### *Sepia officinalis* mtDNA COI Clade 1

We applied R-squared Mantel and partial r Mantel tests to the mtDNA COI clade 1 *S. officinalis*. Considering partial r Mantel analysis the best fitted-models appear to be both the IBR model by 10000 ( $r = 0.766$ ,  $p = 0.032$ ) and LCP by 50 times the continental shelf resistance ( $r = 0.764$ ,  $p = 0.026$ ) when IBO is controlled for (see Table 5). However partial r Mantel outputs controlling for LCP against IBR and counter-wise did not show significant partial r Mantel

scores, indicating a possible interaction of the two models. Observations of R-squared Mantel outputs appear to corroborate these statistic outputs with optimised IBR and LCP having also most the same explanatory power (Fig. 6) estimate is much lower and marginally significant. Major axis regressions reveal though that while balanced the points are not evenly spread and may not meet assumptions (Fig 8.).

#### *Sepia officinalis* mtDNA COI Clade 4

We applied R-squared Mantel and partial r Mantel tests to the mtDNA COI clade 4 *S. officinalis*. Considering R-squared and partial r Mantel analyses, we found that there were no significant models of best-fit (Table 5). Observations of R-squared Mantel iteration outputs appear to corroborate these statistic outputs with weak R-squared plots with little variation between the highest score and lowest (Fig. 7). Major axis regressions however do not appear to meet assumptions of linearity and spread (Fig 9.).

Table 5. Summary of R-squared Mantel and partial r Mantel results of interest for the common cuttlefish (*S. officinalis*) clade 1 and 4 mtDNA data analyses. Scores were generated in IBD web service, following 10000 permutations (Goslee & Urban, 2007; RStudio, 2014). Partial r Mantel tested the correlation that was not explained by the variable being control for, denoted by ('|'). Genetic distance metrics were extracted from pairwise-distance  $F_{ST}$  (y variable) calculated by Pérez-Losada et al. (2007). Resistance distances are the resistances multiplied by the resistance on the continental shelf.

Geographic distance metric (x)	Clade 1		Clade 4	
	R-squared	p-value	R-squared	p-value
Isolation by oceanic distance (IBO)	0.147	0.163	0.0463	0.163
Isolation by resistance (IBR by 500)	-	-	0.117	0.096
Isolation by resistance (IBR by 10000)	0.592	0.002	-	-
Least cost path (LCP by 50)	0.506	0.042	0.0538	0.178
	partial r	p-value	partial r	p-value
IBR by 500   IBO	-	-	0.336	0.096
IBR by 10000   IBO	0.766	0.032	-	-
LCP by 50   IBO	0.764	0.026	0.125	0.108
IBR by 10000   LCP by 50	0.492	0.129	-	-

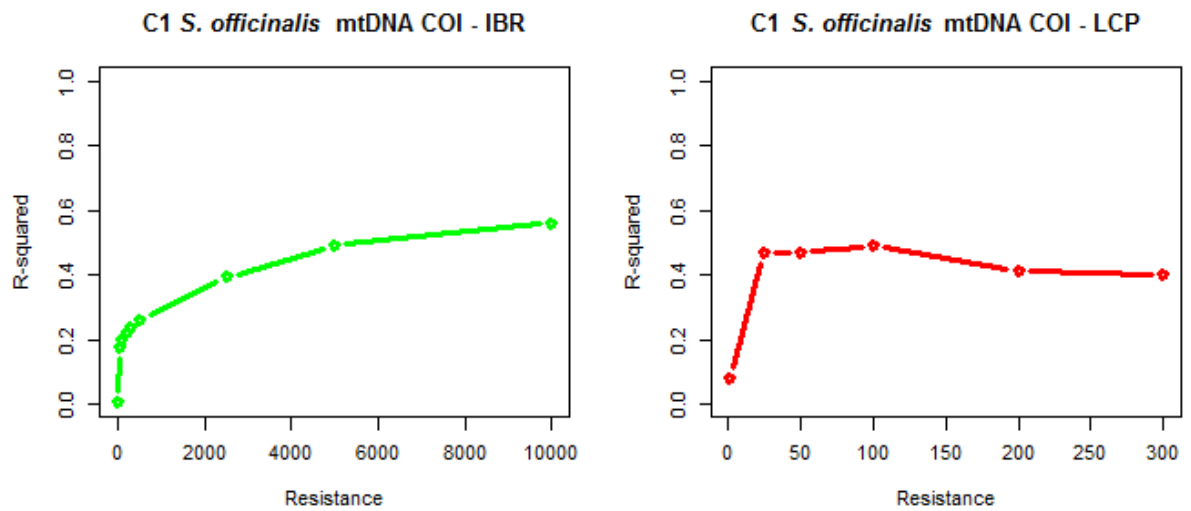


Fig. 6. Line plot of the R-squared values simulated from the IBR and LCP models based on the assignment of resistance iterations based on the clade 1 (C1) mtDNA COI region data for *S. officinalis*. The assigned resistance for the IBR model of 10000 resistance off the continental shelf (non-habitat) and for the LCP model resistance of 50 off the shelf.

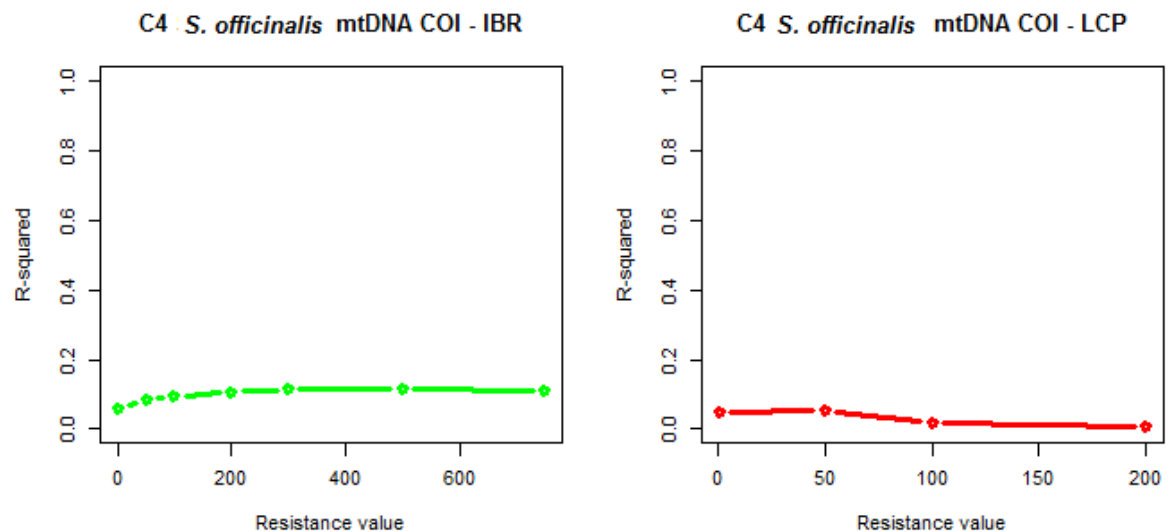


Fig. 7. Line plot of the R-squared values simulated from the IBR and LCP models based on the assignment of resistance iterations based on the clade 4 (C4) mtDNA COI region data for *S. officinalis*. The assigned resistance for the IBR model of 500 resistance off the shelf (non-habitat) and for the LCP model of 50 resistance.

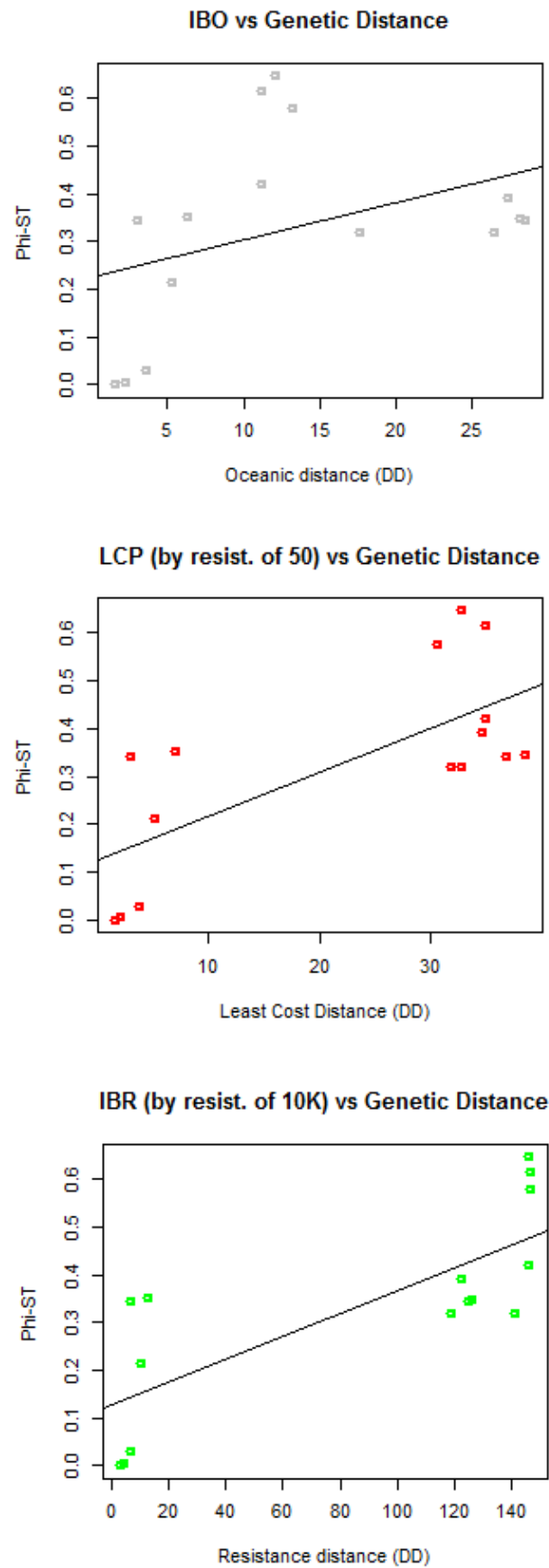


Fig 8. Major axis regression plots showing the three optimised models for IBO, and the best-fit resistances (resist.) in comparison the continental shelf resistance of  $1\Omega$ , for LCP and IBR based on the clade 1 (C1) mtDNA COI data for *S. officinalis*.

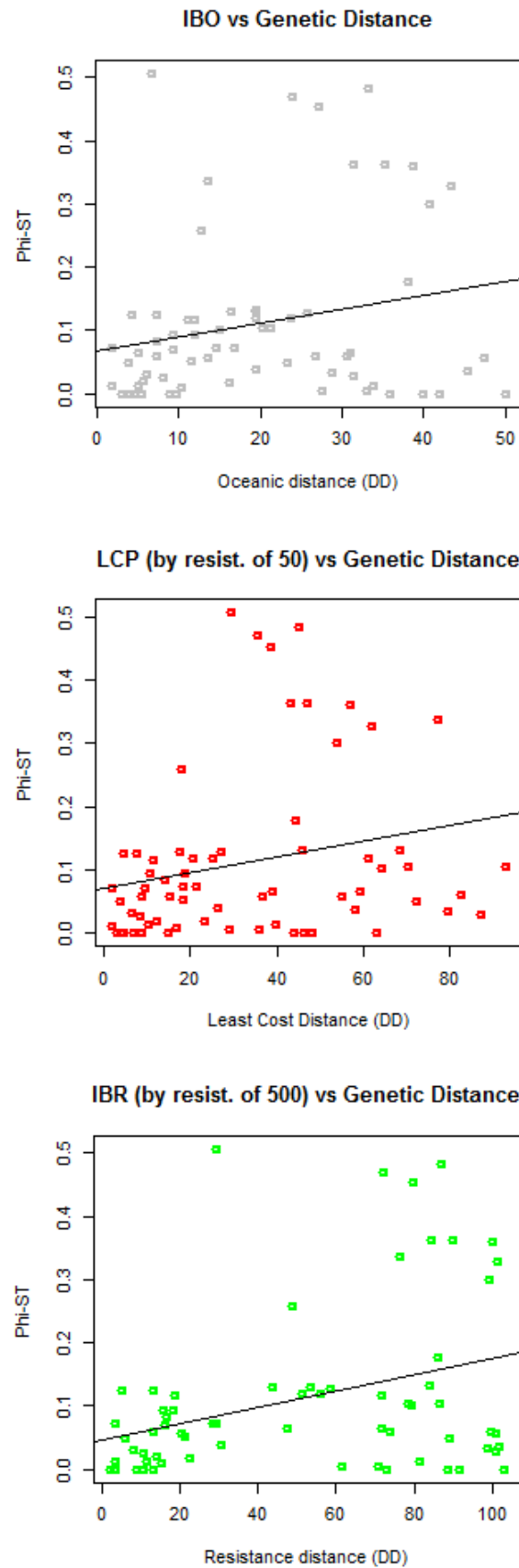


Fig 9. Major axis regression plots showing the three optimised models for IBO, and the best-fit resistances (resist.) in comparison the continental shelf resistance of  $1\Omega$ , for LCP and IBR based on the clade 4 (C4) mtDNA COI data for *S. officinalis*.

## DISCUSSION

### *Raja clavata*

The analysis of the role that depth has on genetic connectivity resulted in three key outcomes which have relevance for future analysis of connectivity in the marine environment. With the *R. clavata* AFLP and mtDNA datasets we failed to reject the null hypothesis of isolation by oceanic distance. Partial  $r$  Mantel correlation estimates, which controlled for potentially confounding variation in the null oceanic distance model were not significant. This finding suggests that connectivity between these sites was significantly structured by geographic distance with little influence from the presence of preferred habitat. Alternatively the coordinate distances of the data source sites might be far enough apart that more complex and subtle resolution was masked by the effect of geographic distance. There is support for this interpretation as this species requires shallow substrate to spawn and form shelter from strong currents and predators for developing vulnerable juveniles (Ellis, 2005; Fowler et al. 2005). Given the next best models for genetic connectivity were least cost path and isolation by resistance, it would be of interest to examine these same distance models with higher numbers of sites and smaller scale resolutions. During the selection phase of this preliminary study the availability of pairwise genetic metrics was not published for several larger and more spatially sampled studies. Future analyses for demersal-obligate and non-pelagic species genetic data could be improved and made more efficient from the wider adoption of metadata submission to repositories such as dryad. This said the genetic structuring by ocean distance remains robust for this sample study.

### *Sepia officinalis*

The 25 sampled sites for *S. officinalis* were partitioned into four well supported clades, indicating that a collective sample wide analysis would not be appropriate. Two clades, assigned identification numbers 1 and 4 were the only clades which contained sites with adequate numbers of sampled individuals. As such we performed R-squared Mantel and partial  $r$  Mantel tests of the three seascape models separately for each clade. First to begin with the analysis for clade 1 which contained six sites, we found that oceanic distance was significantly outperformed by both the isolation by resistance model and least cost path. However when both least cost path and isolation by resistance variations were each in turn controlled for, there was no measurable significant difference to determine the best fitting model. This finding suggests that the path selection of *S. officinalis* for this region might be an interaction between deliberate path and random walk around the putative barriers. Regardless, there appears to be good support

that the more complex seascape models better describe genetic connectivity. Next for clade 4 represented by individuals from 12 sites, there was a lack of any statistically significant models and observations of major axis regression graphs showed distinctive clustering away from the line of best fit, up in the Maximum likelihood for phylogeny within this clade for the model selected. Additionally this finding may indicate non-random mating between sites.

### Model Simulations

For accurate connectivity models sample sets should be large and replicated enough to measure seascape agencies for genetic structure. In the future we would like to include alternative candidate seascape variables for non-pelagic and obligate-demersal species such as the influence of vertical depth variation and the distribution of preferred substrate. For both of these species a fine substrate is required for foraging. With *S. officinalis* being reported as routinely burying itself in the substrate, potentially to reduce predator detection and improve their success at ambush by camouflage (Allen et al. 2010; Barratt & Allcock, 2012, Reid et al. 2005). Behavioural ecology studies have shown that this species favours a finer grain of substrate, potentially to reduce damage to their soft bodies and reduce friction for increased ambush speed. Additionally *R. clavata* hunts via the electro-magnetic filter pits, known collectively as the Ampullae of Lorenzi, for hidden invertebrates and fish, which requires access to demersal plains lacking in protruding bed rock systems and coarse substrate (Ellis, 2005; Fowler et al. 2005).

### CONCLUSIONS

Despite the lack of conclusive improvement by isolation by resistance by habitat, these tests ignore the configuration and quality of heterogenetic features. Additionally, modelling by parameterised seascape and landscape variables of importance to the species are likely to offer more theoretically justifiable inferences (Hagerty et al. 2011; McRae, 2006; McRae & Beier, 2007). Furthermore, a higher sampling density may be necessary to take advantage of these resistance models. Consequently, I think it is well worth pursuing more sophisticated ‘seascape’ modelling approaches to value add to genetic data. These approaches offer great potential to identify important habitat corridors in both the marine and terrestrial environment and provide the basis for conservation strategies.



## ACKNOWLEDGEMENTS

I would like to thank first my Master of Research supervisor Associate Professor Adam Stow for whom this thesis would not have been possible. His guidance and years of expertise in the field of conservation genetics has been a humbling and deeply appreciated experience. Next I would like to extend my deep gratitude to PhD student and candidate Paolo Momigliano, a bright and growing star in the on the plane of Conservation genetics in Australia. Your innate understanding and guidance in all concepts and programs concerned with genetic connectivity was a cornerstone to my research development and thesis structure. I would like to thank I would also like to thank the extended Conservation Genetics group of late 2013-2014, for your hospitality and guidance into the world of academia. Next I would like to thank equally associate Professor Andrew Barron and Associate Professor Grant Hose for orchestrating the Master of Research Program in its first run at Macquarie University, this has been a hugely exciting process and your guidance and workshops helped myself and fellow 'MRes' students to feel assured in our purpose and place within our faculties. Next I would like to thank my cohort of MRes students finishing in 2014, I have known many of you as an undergraduate and grown to know more of each of you in the delightfully concentrated classes of MRes 700-800. Next I would like to thank my family and friends. To my parents going to university would not have been possible without your unconditional love and support.

## REFERENCES

- Allen, J. J., Mäthger, L. M., Barbosa, A., Buresch, K. C., Sogin, E., Schwartz, J., Chubb, C., and R. T. Hanlon. 2010. Cuttlefish dynamic camouflage: responses to substrate choice and integration of multiple visual cues. *Proceedings of the Royal Society B-Biological Sciences* **277**(1684): 1031-1039.
- Anderson, C. D., Anderson, C. D., Epperson, B. K., Fortin, M-J., Holderegger, R., James, P. M. A., Rosenberg, M. S., Scribner, K. T., and S Spear. 2010. Considering spatial and temporal scale in landscape-genetic studies of gene flow. *Molecular Ecology* **19**(17): 3565-3575.
- Baguette, M., Blanchet, S., Legrand, D., Stevens, V. M., and C. Turlure. 2013. Individual dispersal, landscape connectivity and ecological networks. *Biological Reviews* **88**(2): 310-326.

- Barratt, I. & L. Allcock. 2012. *Sepia officinalis*. The IUCN Red List of Threatened Species. Version 2014.2. Retrieved 6 June 2014. Available from <http://www.iucnredlist.org/details/162664/0>
- Ellis, J. 2005. *Raja clavata*. The IUCN Red List of Threatened Species. Version 2014.2. Retrieved 6 June 2014. Available from <http://www.iucnredlist.org/details/39399/0>
- ESRI, 2014. ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute.
- Etherington, T.R. 2011. Python based GIS tools for landscape genetics: visualising genetic relatedness and measuring landscape connectivity. *Methods in Ecology and Evolution*, **2**(1): 52-55.
- Fowler, S.L., Cavanagh, R.D., Camhi, M., Burgess, G.H., Cailliet, G.M., Fordham, S.V., Simpfendorfer, C.A. and Musick, J.A. (comps and eds). 2005. *Sharks, Rays and Chimaeras: The Status of the Chondrichthyan Fishes. Status Survey*. pp. x + 461. IUCN/SSC Shark Specialist Group, IUCN, Gland, Switzerland and Cambridge, UK.
- Garrido-Garduno, T. and E. Vazquez-Dominguez. 2013. Genetic, spatial and connectivity methods of analysis in landscape genetics. *Revista Mexicana De Biodiversidad* **84**(3): 1031-1054.
- GEBCO, 2003. The GEBCO Digital Atlas published by the British Oceanographic Data Centre on behalf of IOC and IHO, 2003)
- Goslee, S.C. and D. L. Urban. 2007. The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software* **22**(7):1-19.
- Hagerty, B. E., Nussear, K. E., Esque, T. C., and C. R. Tracy. 2011. Making molehills out of mountains: landscape genetics of the Mojave Desert tortoise. *Landscape Ecology* **26**(2): 267-280.
- Hutchinson, G. E. 1957. *A Treatise on Limnology*. New York, NY: Wiley. p. 1015.
- Holden, M.J. 1975. The fecundity of *Raja clavata* in British waters. *Journal du Conseil International de l'Exploration de la Mer* **36**: 110-118.
- IUCN 2014. The IUCN Red List of Threatened Species. Version 2014.2. Retrieved 24 July 2014. Available from <http://www.iucnredlist.org>
- Jensen, J.L., Bohonak, A.J., and Kelley, S.T. 2005. Isolation by distance, web service. BMC Genetics 6: 13. v.3.23 <http://ibdws.sdsu.edu/>

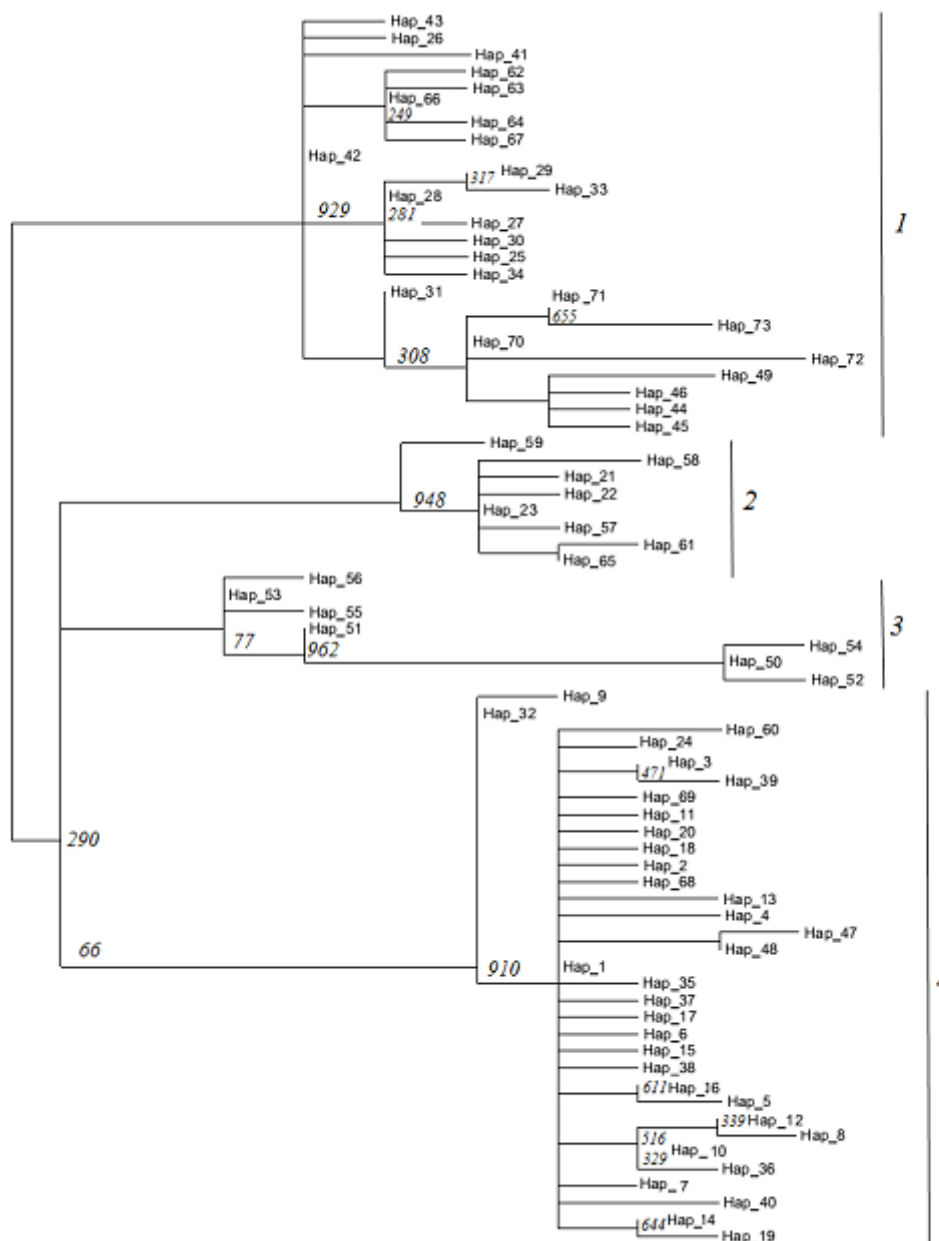
- Kyle, J.C., and C. Strobeck. 2001. *Molecular Ecology* **10**, 337–347.
- Liggins L., Trem, E. A., and C. Riginos. 2013. Taking the Plunge: An Introduction to Undertaking Seascape Genetic Studies and Using Biophysical Models. *Geography Compass* **7**(3): 173–196,
- Lowe, W. H., and F. W. Allendorf. 2010. What can genetics tell us about population connectivity? *Molecular Ecology* **19**, 3038–3051.
- Manel, S., and R. Holderegger. 2013. Ten years of landscape genetics. *Trends in Ecology & Evolution* **28**(10): 614-621.
- Manel S., Schwartz M. K., Luikart G., and P. Taberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution* **18**(4): 189-197.
- Mantel, N. 1967. *Cancer Research* **27**: 209 –220.
- McRae, B. H. 2006. Isolation by resistance. *Evolution* **60**(8):1551-1561.
- McRae, B. H., and P. Beier. 2007. Circuit theory predicts gene flow in plant and animal populations. *PNAS* **104**(50): 19885-19890.
- McRae, B.H., and V. B. Shah. 2009. Circuitscape user's guide. ONLINE. The University of California, Santa Barbara. Available from <http://www.circuitscape.org>
- Nei M. and S. Kumar. 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.
- Novick, R.R., Dick, C.W., Lemes, M.R., Navarro, C., Caccone, A., and E. Bermingham . 2003. *Molecular Ecology* **12**: 2885–2893.
- Pérez-Losada, M., Nolte, M. J., Crandall, K. A., and P. W. Shaw. 2007. Testing hypotheses of population structuring in the Northeast Atlantic Ocean and Mediterranean Sea using the common cuttlefish *Sepia officinalis*. *Molecular Ecology* **16**: 2667–2679.
- Pasolini, P, Ragazzini, C., Zaccaro, Z., Cariani, A., Ferrara, G., Gonzalez, E. G., Landi, M., Milano, I., Stagioni, M., Guarniero, I., and F. Tinti. 2011. Quaternary geographical sibling speciation and population structuring in the Eastern Atlantic skates (suborder Rajoidea) *Raja clavata* and *R. straeleni*. *Marine Biology* **158**: 2173–2186
- Reid, A., Jereb, P. and C. F. E. Roper. 2005. Family Sepiidae. In: P. Jereb and C.F.E. Roper (eds), *Cephalopods of the World. An Annotated and Illustrated Catalogue of Cephalopod*

- Species Known to Date. Volume 1.* Chambered Nautiluses and Sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae), pp. 54-152. FAO, Rome.
- Riddle, B. R., Dawson, M.N, Hadly, E. A., Hafner, D. J., Hickerson, M. J., Mantooth, S. J., and D. A. Yoder. 2008. The role of molecular genetics in sculpting the future of integrative biogeography. *Progress in Physical Geography* **32**(2): 173-202.
- Riginos C., and L. Liggins. 2013. Seascape Genetics: Populations, Individuals, and Genes Marooned and Adrift. *Geography Compass* **7**(3): 197–216.
- Rousset, J. 1990. Catches and geographical distribution of selachians on the western coast of Brittany. *Journal of the Marine Biological Association of the United Kingdom* **70**: 255-260.
- RStudio, 2014. RStudio: Integrated development environment for R (Version 0.96.122) [Computer software]. Boston, MA. Retrieved January 20, 2014. Available from <http://www.rstudio.org/>
- Royer, J., Pierce, G.J., Foucher, E., and J. P. Robin. 2006. The English Channel stock of *Sepia officinalis*: Modelling variability in abundance and impact of the fishery. *Fisheries Research* **78**: 96-106.
- Shirk, A. J., Allin, D. O. W., Cushman, S. A., Rice, C. G., and K. I. Warheit. 2010. Inferring landscape effects on gene flow: a new model selection framework. *Molecular Ecology* **19**: 3603–3619.
- Sifner, S. K., Vrgoč, N., Dadić, V., Isajlović, I., Peharda, M., and C. Piccinetti. 2009. Long-term changes in distribution and demographic composition of thornback ray, *Raja clavata*, in the northern and central Adriatic Sea. *Journal of Applied Ichthyology* **25**: 40-46.
- Smale, M. J. and P.D. Cowley. 1992. The feeding ecology of skates (Batoidea: Rajidae) off the Cape south coast, South Africa. *South African Journal of Marine Science* **12**: 823-834.
- Spear, S. F., Balkenhol, N., Fortin, M-J. F., McRae, B. H., and K. Scribner. 2010. Use of resistance surfaces for landscape genetic studies: considerations for parameterization and analysis. *Molecular Ecology* **19**: 3576–3591
- Stehmann, M. 1995. A record of *Raja clavata*, the eastern Atlantic thornback skate, from the southern Madagascar Ridge at Walters Shoal (Elasmobranchii, Rajidae). *Journal of Ichthyology* **35**(5): 63-74.

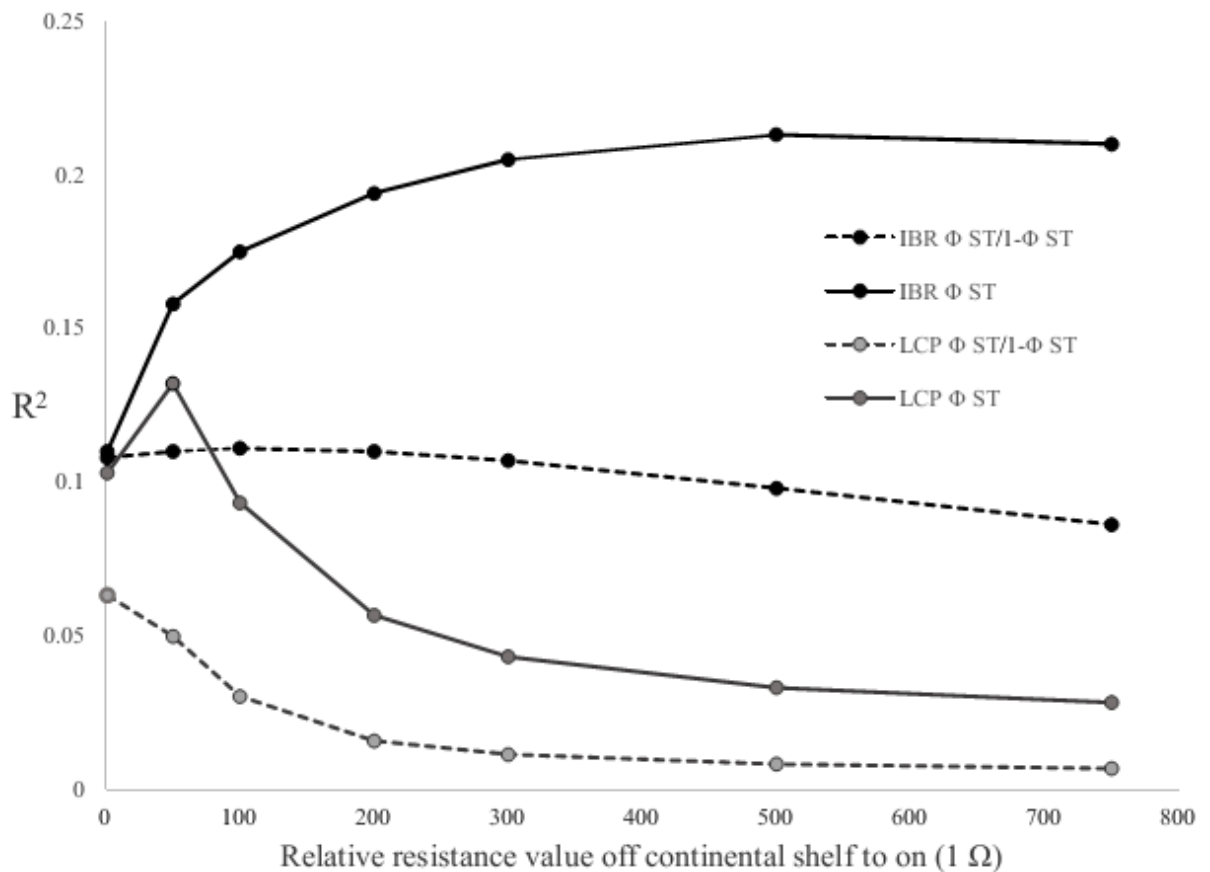
- Stehmann, M. and D. L. Buerkel. 1984. Rajidae. In: P.J.P. Whitehead, M-L., Bauchot, J-C., Nielsen, H. J., and E. Tortonese (eds), *Fishes of the northeastern Atlantic and Mediterranean*, UNESCO, Paris, France.
- Stow, A. J. and W. E. Magnusson 2012. Genetically defining populations is of limited use for evaluating and managing human impacts on gene flow. *Wildlife Research* **39**(4): 290-294.
- Tamura, K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution* **9**:678-687.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., and S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725-2729.
- Walker, P.A. 1998. *Fleeting Images: Dynamics of North Sea Ray Populations*. Ph.D. Thesis, University of Amsterdam.
- Walker, P.A., Howlett, G. and R. Millner 1997. Distribution, movement and stock structure of three ray species in the North Sea and eastern English Channel. *ICES Journal of Marine Science* **54**: 797-808.
- Wheeler, A. 1969. *The Fishes of the British Isles and North-West Europe*. Macmillan, London, UK.
- White, C., Selkoe, K. A., Watson, J., Siegel, D. A., Zacherl, D. C., and R. J. Toonen. 2010. Ocean currents help explain population genetic structure. *Proceedings of the Royal Society - Biological Sciences* **277**(1688): 1685-1694.
- Wiens, J. J. and C. H. Graham. 2005. Niche conservatism: Integrating evolution, ecology, and conservation biology. *Annual Review of Ecology, Evolution, and Systematics* **36**: 213-245.

## SUPPLEMENTARY MATERIAL

S1. Evolutionary tree reconstruction by Maximum Likelihood. Molecular phylogenetic tree for *S. officinalis* samples constructed by the Maximum Likelihood (ML) method (log likelihood - 624.0) in MEGA6 by BS1000 permutations as done Pérez-Losada et al. 2007 (Tamura et al. 2013). Evolutionary history was constructed on the lowest AIC scored model for ML, the General Time Reversible (GTR + I +  $\Gamma$ ) model with invariable sites and gamma distribution (Tamura, 1992). The process involved 73 nucleotide sequences and a total of 659 positions in the final sequence (Nei and Kumar, 2000; Tamura et al. 2013). The percentage of trees in which the associated taxa clustered along the tree is shown above the branches. For initial trees heuristic searches were obtained by Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach. The tree is drawn to scale.



S2. Initial scatterplot for all 25 *S. officinalis* sites seascape model parameterisations represented by the Mantel Test R-squared scores for genetic distance (seascape model trend lines (pairwise genetic distance vs differential geographic distance ) as a function of relative resistance cost iterations off-continental shelf sea body to on continental shelf (on being a uniform value of  $\Omega = 1$ ). We found relatively low explanations of variation in genetic for all models, which along with observations of phylogenetic reconstruction (see S1) prompted investigations to resolve whether disjunct population clades could be confounding outputs. Peaks represent the nearest ‘true’ resistance presented by the hypothetical barrier beyond the continental slope.



S3. Location data summary of *R. clavata* including corresponding location codes, location names,  $N$  = individuals processed by site, and coordinates by degrees decimal (by World Geodetic System 1984 hereafter WSG 84) sourced by Pasolini et al. (2011). mtDNA CR were sampled from all sites with a total sample number of  $N = 181$ .

<b>Code</b>	<b>Name</b>	<b><math>N</math></b>	<b>Longitude (DD)</b>	<b>Latitude (DD)</b>
NW	Norwegian Sea	-	9.9000	64.2176
NS	North Sea	38	2.2365	52.1048
IS	Irish Sea	22	-3.6149	53.9756
BI	Balearic Islands	-	1.2300	38.7390
TS	Tyrrhenian Sea	52	10.2570	42.5480
AC	Algerian Coast	27	-0.1070	35.9290
AS	Adriatic Sea	42	16.600	42.2692



S4. Location data summary of *S. officinalis* including corresponding location codes, location names, N = individuals processed by site, and coordinates by degrees decimal (by WSG 1984) sourced by Pérez-Losada et al. (2007).

<b>Code</b>	<b>Site name</b>	<b>N</b>	<b>Longitude (DD)</b>	<b>Latitude (DD)</b>
RIBE	Ribeira	14	-9.2900	42.8000
BURE	Burela	10	-7.9900	43.8100
PAMA	Palma de Mallorca	10	2.5475	39.3736
TOSA	Torre la Sal	10	0.2252	39.9097
VIGE	Vilanova i la Geltrú	11	1.9475	41.2000
SETE	Sete	10	3.6975	43.2500
AVIL	Avilés	12	-6.0892	43.6455
ROCH	La Rochelle	11	-1.5000	46.1200
GUMO	Gulf of Morbihan	10	-4.8000	48.1954
ENCH	English Channel	10	-0.9169	49.5500
MARS	Marseille	10	5.2700	43.2900
VENI	Venice	10	12.5514	45.2800
FARO	Faro	10	-7.6575	37.0113
LISB	Lisboa	11	-9.4600	38.6000
ALGI	Algiers	4	3.1256	36.7900
ALIC	Alicante	10	-0.0220	38.4321
ROMA	Roquetas del Mar	10	-2.5214	36.7500
GUHA	Gulf of Hammamet	10	10.8167	35.9939
MALT	Malta	10	14.8410	36.1237
GUSI	Gulf of Sidra	16	19.7919	32.0000
MESS	Mesolongion	10	20.6900	37.9962
GUIR	Gulf of Iraklion	10	25.7712	35.1800
AGNI	Agios Nikolaos	10	25.1669	35.4000
CAIS	Canary Islands	10	-14.6200	28.0211
MAUR	Mauritania-Senegal border	10	-16.7000	16.0597