ATLAS Deliverable 4.4

Reproduction, dispersal and genetic connectivity in benthos and fishes

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Author:	Marina Carreiro-Silva, Alan Fox, Jens Carlsson, Sophie Arnaud-Haound (see next page for full list of authors)

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Author list: Marina Carreiro-Silva[1], Alan Fox[2], Jens Carlsson[3], Jeanette EL Carlsson[3], Covadonga Orejas[4], Christopher Nicolai Roterman[5] Maria Rakka[1], Joana Boavida[6], José González- Irusta[7], Telmo Morato[1], Meri Bilan[1], Juancho Movilla[8], António Godinho[1], Sophie Arnaud-Haound[6]

Contributors: All partners of DL 3.3 who provided data on species presence records, Ricardo Aguillar[9], Marzia Bo[10] Jordi Grinyo[11]

1- IMAR-University of Azores, Portugal carreirosilvamarina@gmail.com

2- Scottish Association for Marine Science (SAMS), UK

3- Area52 Research Group, School of Biology and Environmental Science/Earth Institute, University College Dublin, Belfield, Dublin 4, Ireland

4- Instituto Español de Oceanografía, Centro Oceanográfico de Baleares, Palma, Spain

5- University of Oxford, Oxford, UK

6- Ifremer, UMR 248 MARBEC (Marine Biodiversity, Exploitation and Conservation) Sète - France

7- Instituto Español de Oceanografia, Centro Oceanográfico de Santander, Spain

8- Instituto Español de Oceanografía, Estación de investigación Jaume Ferre, Centro Oceanográfico de Baleares, Menorca, Spain

9 - -Oceana Europe, Madrid, Spain

10 - Università degli Studi di Genova, Dipartimento di Scienze della Terra, dell'Ambiente e della Vita, Genova,Italy

11 - Institut de Ciències del Mar (CSIC), Barcelona, Spain

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1 Executive summary

In the marine realm, the management of fishery resources, the control and prevention of invasive species and the conservation plans for threatened species or vulnerable ecosystems (some already suffering well characterized declines such as coral reefs –Aichi target 10-, seagrass, and mangroves) requires the knowledge of interconnection and interdependency of stocks, populations and communities constituting these ecosystems. A particular challenge in marine systems is that most marine organisms exhibit a complex life cycle with two phases, including an adult stage with limited or no movements and a larval dispersive stage. In addition, many fish species can have dispersive phases during the larval, juvenile and adult phases. The direct observation and study of migratory movements is almost impossible in the oceans due to i) the reduced potential to fully access the marine environment, ii) the often minute size of the dispersing stages, iii) the often extremely large population sizes *sensu stricto*, and iv) the generally substantial migration distances, at adult or larval stages, with no strict *a priori* relationship with life history traits suspected to influence dispersal potential (Riginos et al., 2011). All those technical challenges to direct observation are of course exacerbated in the deep-sea, making models of predictive connectivity and indirect inferences a strong need. Molecular data interpreted in the theoretical framework of population genetics (Hellberg et al., 2002), ideally integrated with modelling approaches, have thus a central role to play in the study of marine connectivity.

Both predictive modelling and indirect inferences based on population genomics (and in some cases on biochemical analysis of calcified structures such as otoliths or shells) need to be fed by a good knowledge of the biology and ecology of species being studied. More precisely, Lagrangian modelling of particles requires input from a diversity of fields to deliver the most accurate predictions. Mostly three fields of research are at stake that are all represented in ATLAS consortium (Figure 1). First, **oceanographic data** are needed to establish the way currents can act as conveying belt for the dispersal of particles or in some cases adult stages. Second, **habitat mapping and modelling** to feed the model with knowledge of the geographic locations where dispersing life stages can be emitted from and those they can viably settle because many marine, and in particular deep-sea species have a spatially fragmented distribution. Third, **reproductive biology** information including the nature, duration and behaviour of the dispersing life stage because most deep-sea species' dispersal relies on the pelagic larval stages (Cowen and Sponaugle 2009, Hilário et al. 2015), is required to accurately choose the oceanographic data in terms of season when dispersal can take place and define a confidence interval for its duration, chose the

depth of currents to be considered at different time steps, and account for the ability to mitigate or enhance their influence through active dispersal.

Genomic data need to be interpreted with a good knowledge of historical habitat modifications (at evolutionary time scales) to understand the signature left on the genomic composition of species by past patterns of past connectivity and indirectly reconstruct their modification, as well as that of species distribution range under the effect of past environmental changes. These can be done through the analysis of sequence divergence, allelic frequency and modelling. Multi-locus genotype based analysis can then be used to infer more contemporary patterns of connectivity that would correspond to a similar time scale as the predictions issued from Lagrangian modelling. In all cases, a good knowledge of the reproductive biology of species (particularly their ability to reproduce and persist through sexual reproduction, but also to self-fertilize) is also required to accurately transform observations of the geographic distribution of genetic polymorphism into inferences as to the present or past patterns of connectivity.

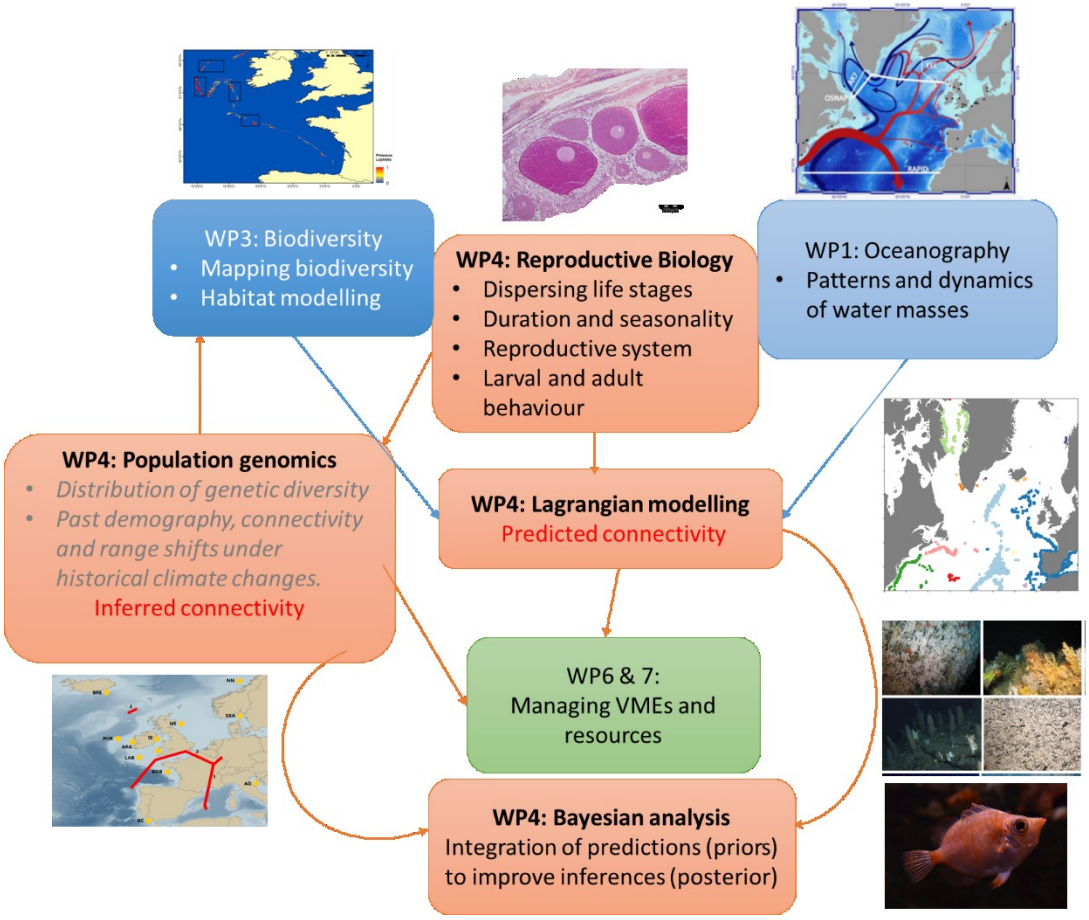


Figure 1: Scheme summarizing the Atlas Work Packages providing the information needed to predict and infer connectivity through Lagrangian modelling and population genomics. Components of WP4 included in this report are shown in orange boxes.

However, deep-sea species are harder to manipulate than their coastal counterparts, making it extremely difficult to observe or keep them in aquaria facilities in order to obtain the relevant information required for sound modelling predictions. During the ATLAS project, we successfully induced spawning and reared larvae of the octocoral *Viminella flagellum* under aquaria conditions (see Table 1). This was the first time that the larvae biology of a deep-sea octocoral has been studied. In addition, we have characterized the reproductive biology and gametogenic cycle of four CWC species that form important Vulnerable Marine Ecosystems (VMEs) in the Mediterranean and the Azores (section 3).

Studies on coral reproduction revealed that all species studied were gonochoric (separate sexes), broadcast spawners (release of gametes into the water column) with continuous gametogenic cycles. The dendrophyllids *Dendrophyllia cornigera* and *Dendrophyllia ramea* from the Mediterranean come from relatively shallow depth ca. 50-100 m depth and their spawning seems to be coupled with seawater temperature. In contrast, spawning in the octocorals *Dentomuricea meteor* and *V. flagellum* collected from 200-500m deep the Azores seem to follow seasons with high primary productivity, in spring and autumn. Assexual reproduction was also studied for the octocorals *Acanthogorgia armata* and *Acanella arbuscula* based on aquaria observations. These octocorals displayed the ability of polyp bailout, with polyp dissociation from the mother colony resulting in free negatively buoyant polyps without any calcareous material. Polyp bailout is described as a stress response and an expression of reverse development, i.e. the ability of adult forms to develop into earlier developmental stages, which have higher probabilities of dispersal. It therefore may represent an important mechanism of dispersal under unfavourable conditions caused by increasing anthropogenic activities and climate change.

Larvae biology of the octocoral *V. flagellum* was studied under aquaria conditions, and the data produced used for the connectivity modelling studies in section 4. This is the first study on the larvae biology of a deep-sea octocoral, with results indicating a lower pelagic larvae duration (PDL) than the reef-bulding species *Lophelia pertusa* (12 days in *V. flagellum* compare with 3-5 weeks in *L. pertusa*).This influences the dispersal ability of this species, which seems to be much shorter that *L. pertusa* (see modelling results).

Table 1: Availability of data for the species targeted in WP4. Bold crosses indicate data collected during ATLAS, unbold crosses indicate data avaialable in the literature. Species for which there was enough data for the modelling connectivity studies are highlighted in blue.

Species	*Habitat Modelling (at the N Atlantic basin scale)*	*Population genetics*	*Larval Behaviour* ***PLD***	*Larval behaviour* ***Vertical distribution***	*Reproduction*
Lophelia pertusa	X	**X**	X	X	X
Dendrophyllia cornigera	-	-	-	-	**X**
Dendrophyllia ramea	-	-	-	-	**X**
Dentomuricea cf. meteor	-	-	-	-	**X**
Viminella flagellum	**X**	-	**X**	**X**	**X**
Nephrops norvegicus	-	**X**	X	X	X
Cidaris cidaris	-	**X**	X	X	X
Capros aper	-	**X**	X	X	X
Trachurus trachurus	-	**X**	X	X	X
Helicolenus dactylopterus	**X**	X	(inferred)	X	X

On the basis of this knowledge, and the one produced in WP3 for habitat mapping and modelling (for species for which enough data were available, see Table 1) and WP1 for water masses movements (Figure 1), the Lagrangian modelling could be adapted to fit several species studied in ATLAS, for which a benthic adult stage rendered coherent the hypothesis of a mainly larval driven dispersal. This included 2 invertebrates (the reef-building coral *L. pertusa* and the octocoral *V. flagellum*) and one fish species (*Helicolenus dactylopterus*) (section 5). Modelling of dispersal of the echinoid *Cidaris cidaris* was attempted but proved impossible due to the limited presence records of this species on public databases that could be used in habitat distribution models; likely erroneous records of presence derived from video and photographic data owing to morphological similarity with other taxa, and poor constraint in the population genetics of *C. cidaris* populations in the North Atlantic and Mediterranean (see section 6.3). In order to account for uncertainties for many other species for which the reproductive biology could not be studied and information is lacking, nine 'generic' models were produced at the scale of the North Atlantic. Those include lower, average and higher bounds for larval duration and movement in the water column. The aim of this exercise was to produce connectivity matrices that could be i) compared to inferences based on population genetics data in order to select the prediction that would best match the inference of connectivity and ii) be available for future studies as knowledge of the reproductive biology of species will inform the choice of one of the nine available scenarios that would best fit

the species being studied (available at Zenodo). These generic connectivity matrices clearly show the increased dispersal potential of increasing PLDs and drifting closer to the surface. They also highlight the regions of stronger currents as important sources of larvae, particularly currents along the continental slopes.

Individual species models for the corals *L. pertusa*, *V. flagellum* and the fish *H. dactylopterus* demonstrate how hydrodynamically-based modelled connectivity can be combined with species-specific habitat suitability models to suggest connectivity by species. Model outputs show how this predicted connectivity varies considerably with the assumptions made about larval behaviour. One common feature was that populations along the eastern boundary of the North Atlantic may be generally more strongly connected. However, as currents are generally weak here (compared to those on the western and northern boundaries of the basin) this conclusion is probably dependent on the existence of a near-continuous band of suitable habitat from the mouth of the Mediterranean northwards.

Connectivity models for *L. pertusa* under future climate scenarios suggest present day regions of high connectivity - important sources and sinks of larvae – will have much reduced connectivity, with the best connectivity future sites found northwards along the coast of Greenland and Canada. In addition, in the south areas, the reduced population in the Azores, previously supplying larvae to the US and European coasts via intermediate seamounts, becomes connected only along the mid-Atlantic ridge, with some larvae still coming in from the US coast. Predictions of future distribution and connectivity for *H. dactylopterus* show expansion in the suitable habitat range to the north and west particularly into the Labrador Sea. However, predictions show that with the exception of the Azores and seamounts in the Mid-Atlantic Ridge, *H. dactylopterus* may become a single strongly connected component under future conditions, suggesting that *H. dactylopterus* population may become more robust and resilient under changed future conditions.

Finally, both sampling and genomic resources could be gathered to deliver inferences of connectivity at different time scales for two invertebrate VME indicator species (*L. pertusa* and *Madrepora oculata*), and their commensal polychaete *Eunice norvegica*, the associated invertebrate species to VMEs (*Cidaris cidaris*) and three species exploited by fisheries, i.e. two fish species (*Capros aper* and *Trachurus trachurus*) and one crustacean (*Nephrops norvegicus*) (section 6).

Results from genetic analyses and modelling simulations are mostly concordant for , indicating that *L. pertusa* forms a large panmictic genetic cluster (ie, a group with random mating) along most of the NE Atlantic European margins (excluding the Mediterranean Sea). The large individual

aggregations associated to a long larval dispersal time mediated by ocean currents may lead to high gene flow among the distant NE Atlantic cold-water coral reefs. Cases of inconsistent findings between observed genetic data and dispersal simulations, such as the location of hypothetical past climatic refugia that may have acted as post-glacial colonization sources, highlighted that other processes not yet captured may be operating (eg, the degree to which larval dispersal is driving genetic patterns across the seascape, habitat quality, variation in reproduction, population density and local selection), as well as differences between the location of genetic samples and modelled spatial extent. Despite local discrepancies, biophysical models have helped understand the complex process of gene flow and can inform future work.

With the echinoid *Cidaris cidaris*, the population genetics results are tentative as the high-resolution genomic single nucleotide polymorphism (SNP) dataset is still undergoing quality control and analysis. The preliminary examination of COI mitochondrial gene fragments indicates no clear barriers to connectivity between North Atlantic and Western Mediterranean specimens, or between specimens collected between 200 m and 1200 m depth; consistent with a theorised highly dispersive planktotrophic larval phase in the *Cidaris* genus, whereby larvae feed near the surface and are transported by faster surface currents. The higher resolution genomic SNP dataset may yet reveal subtle patterns of geographic constraints on long-distance gene flow, or regionally determined variability in selective pressures, however.

The genetic studies of *Caprus aper* clearly demonstrated that the species does not constitute a single panmictic population across the sampled range. Samples from the Mediterranean Sea showed the largest genetic differentiation. Similarly, studies on *Nephrops norvegicus* indicated that the Mediterranean samples from the Adriatic Sea are significantly differentiated from the Atlantic samples analysed. Furthermore, both species showed further diferentiation, although weaker, among the Atlantic samples. The studies of *Trachururs trachurus* are ongoing (samples from the Mediterranenan Sea are yet to be analysed) but the preliminary results indicate that the species is represented by multiple populations. In summary, there is clear population structure within all three species exploited by fisheries and this information could be used by managers to improve the management of these marine resources.

Among all ten species for which advances could be made either in terms of knowledge of the reproductive biology, dispersal modelling or population genomics, a full set of information could be gathered for the emblematic reef-building *L. pertusa*, and the consortium is also trying to finalize the genomic data production for the exploited fish *H. dactylopterus*.

Perpectives beyond this report include the finalization of data production and analysis, and the test of a new method to go beyond the usual "side by side" comparison of predicted (lagrangian modelling) versus realized (inferences from population genomics) dispersal by integrating the Lagrangian matrices of connectivity as priors of a Bayesian analysis (Gaggiotti, 2017) of the genetic dataset characterizing the metapopulation system being studied. Such integrative framework has long been expected by the scientific community addressing connectivity through the use of a diversity of tools and theoretical framework as the ones detailed in this report. The recent development of this new analytical tool will allow testing this approach on the scleractinian coral *L. pertusa*. All results obtained here will be used to deliver information useful for the conservation and management of VMEs and fisheries resources (WP 6 and 7), an advance that will be formalized in the next report of WP4, DL4.5 "Integrated management considering connectivity patterns".

2 General Introduction

Connectivity is as a key component to any ecologically coherent management plan. While our understanding of coastal marine ecosystem connectivity is advancing (Cowen et al 2006), knowledge of deep-water ecosystem connectivity is poor, particularly at the basin scale due to the natural challenges posed by collecting information at large water depths (McClain and Hardy et al 2010).

Because of these inherent technical challenges of working in the deep-sea, the ATLAS project used a combination of predictive modelling and indirect inferences on population genomics to improve our understanding of connectivity patterns in the deep ocean. This included collecting data on key species life history traits (including reproductive and larvae biology) and genomic data using genes as markers of the individual movements and population connections. Data on life history traits was used in combination with oceanographic data from WP1 and species distribution models in WP3 to produce connectivity modelling maps for key deep-sea species. The approaches were chosen to understand and how connectivity between populations of key species depend on ocean and climate dynamics at two different, but equally relevant, timescales: (1) a phylogeographic reconstruction of historic demography, connectivity and range shifts under the influence of climate changes, and (2) a combined seascape genomics–life history approach to understand the factors influencing present day genetic diversity and migration paths. This approach identified the source areas and ocean transport pathways most critical to supplying ecosystems and marine Genetic resources (source-sink dynamics), and important zones of contact (admixture or

hybridization) for gene pools found in both the Atlantic and Mediterranean. Indirect approaches such as population genetics, using genes as markers of the individual movements and population connections, allowed predictions of connectivity such as those derived from WP1 to be tested.

Specific objectives of this DL were to:

1) Improve our knowledge of life history traits, including reproduction and larvae biology and behaviour of key deep-sea species, particularly CWCs for which information is very limited;
2) Compare genomic connectivity patterns using species that have differing life history traits with the VIKING20 model (WP1) to understand how biological life history traits (based on objective 1 and on the literature) mediate the outcomes of physical transport mechanisms; including the identification of source and stepping-stone areas that maintain meta-populations of VME indicator taxa at present and under future scenarios of climate change;
3) Use population genetics tools to deliver inferences of connectivity at different time scales for VME indicator species and species exploited by fisheries;
4) Genetically delimit different fish stocks. Reconstruct historic and present day population genetics through Bayesian inferences based on genome scan data to seek evidence for recent genetic bottlenecks related to exploitation.

These key objectives were addressed in the different sections 3-6 of this report. Results obtained here will be used to deliver information useful for the conservation and management of VMEs and fisheries resources (WP 6 and 7), an advance that will be formalized in the next report of WP4, DL4.5 “Integrated management considering connectivity patterns”.

3 Reproduction and larvae biology of deep-sea corals (IMAR-UAZ, IEO)

3.1 Overview of reproductive biology of cold-water corals

Despite the fundamental importance of cold-water corals (CWCs) as habitat formers and ecosystem engineers, our understanding on their reproductive biology and ecology is still scarse. The reproductive biology of CWCs has been studied only for the past two decades, with the first studies conducted in the North West Atlantic on *Oculina varicosa* (Brooke, 2002) and in the North

East Atlantic on *Fungiacyathus marenzelleri* Waller et al. (2002). Current available information is mostly limited to basic reproductive characteristics such as sexuality and reproductive mode, with only a few studies on reproductive seasonality and gametogenesis, and available for less than 20 species mostly in the North East and South Atlantic and Antarctic waters (see Tables 2, 3). This is related to difficulties in sampling in the deep-sea across all seasons due to high costs and complicated logistics, and difficulties with the analysis of samples. In CWCs there is often no sexual dimorphism, therefore, gametes can only be observed after fixation and decalcification and through histological processing (Brooke, 2002; Waller, 2003).

Overall CWCs exhibit sexual and asexual reproductive strategies. Among the sexual features, most CWCs are gonochoric, meaning that sex is allocated in different colonies, however several cases of hermaphroditism have been also documented for some solitary cup corals (e.g. three *Caryophyllia* species studied by Waller et al. 2003, 2005) and the octocoral *Drifa* sp. (Sun et al., 2010).

Although sexual reproduction is vital to preserve the genetic variability of species, CWCs have developed asexual reproductive strategies which allow them to colonise larger areas and disperse, overcoming potential unfavourable environmental conditions for sexual reproduction. These asexual strategies have been described and documented mostly in tropical corals and comprise: (1) fragmentation (Highsmith, 1982), also documented in the CWC *L. pertusa* (Dahl et al., 2012), (2) polyp bail-out (Rosen and Taylor, 1969; Sammarco 1982) observed in some cold water octocorals (see Rakka et al. 2019 and section 3.2.3), but not in scleractinians until now, (3) parthenogenesis (Stoddart, 1983; Ayre and Resing, 1986; Yeoh and Dai, 2010) unknown in CWCs, and (4) production of genetically identical larvae due to cleavage (Heyward and Negri, 2012), also an aspect still not investigated in CWCs.

The knowledge on the reproductive basic characteristics of CWCs as well as on their gametogenic cycle is has mostly restricted to a few species in the North East and South Atlantic, and some in Antarctic waters (Tables 2 and 3). There are several reasons for the scarcity of studies on CWCs reproduction, including, as previously mentioned, the difficulties to sample in the deep-sea across all seasons, and also that the histological study is time-consuming process. The histological investigations performed up to date, revealed that in scleractinian corals sperm develop in spermacysts or spermatic cysts, which are held together by a mesogleal envelope within the mesenteries; oocytes are often found in groups also included in the mesenteries. All gametes develop in the lamellae of the mesenteries, eventually migrating into the mesoglea (Brooke, 2002; Waller et al., 2002; Waller, 2005; Waller and Feehan, 2013).

Table 2: Reproductive characteristics of the cold-water coral scleractinians investigated up to date.

Species	Average oocyte size (µ)	Nr Oocyte/Polyp	Incubation mode (reproductive strategy)	Geographical location /Depth (m)	References
Lophelia pertusa	140[#]/60[$]	3146[#]	♀♂, annual broadcaster spawner	Porcupine Seabight[#]/900 Trondheim Fjord[$]/147	Waller (2003)[#], Brooke & Järnegren (2013)[$], Pires et al. (2014)
Enallopsammia rostrate	400	144	♀♂, broadcast spawner	Chatham Rise/800-1000	Burguess & Babcok (2005), Waller (2005), Pires et al. (2014)
Madrepora oculata	350[#]	10[#]	♀♂, broadcaster spawner	Porcupine Seabight[#]/900 Chatham Rise/800-1000	Waller (2002), Waller & Tyler (2005), Pires et al. (2014)
Oculina varicose	100	550	♀♂, broadcast spawner, 22 d. planula	Florida/3-100	Brooke (2002), Brooke & Young (2003), Brooke & Young (2005)
Goniocorella dumosa	135	480	♀♂ sup., broadcast spawner	Chatham Rise/800-1000	Waller (2005), Burguess & Babcok (2005)
Solenosmilia variabilis	165	290	♀♂, broadcast spawner	Chatham Rise/800-1000	Burguess & Babcok (2005), Waller (2005), Pires et al. (2014)
Caryophyllia ambrosia	630	2900	♀ broadcast spawner	Porcupine Seabight/1100-3000	Waller et al. (2003,3005)
Caryophyllia sequenzae	450	940	♀ broadcast spawner	Porcupine Seabight/960-1900	Waller et al. (2003,3005)
Caryophyllia cornuformis	340	-	♀ broadcast spawner	Porcupine Seabight/435-2000	Waller et al. (2003,3005)
Dendrophyllia cornigera	150	1044 (July)	♀♂, broadcaster spawner	Off Galicia (NE Atlantic)/ ~100	Orejas et al. In prep
Dendrophyllia ramea	--	--	♀♂, broadcaster spawner	Off Granada (SW Mediterranean)/~50	Antón et al. In prep.
Fungiacyathus marenzelleri	750	1290^-2900	♀♂, broadcast spawner	California^ and Rockall Through (N Atlantic), 2000	Waller et al. (2002), Flint et al. (2007)^, Waller & Feehan (2013)
Flabellum alabastrum	1010	550	♀♂, broadcast spawner	Porcupine Seabight/1800-2250	Waller and Tyler (2011)
Flabellum angulare	814	2800	♀♂, broadcast spawner	Porcupine Seabight/1647-2875	Mercier et al. (2011), Waller and Tyler (2011)
Flabellum thouarsi	4800	2412	♀♂, brooder	W Antarctic Peninsula /500	Waller et al. (2008)
Flabellum curvatum	5120	1618	♀♂, brooder	W Antarctic Peninsula /500	Waller et al. (2008)
Flabellum impensum	5167	1270	♀♂, brooder	W Antarctic Peninsula /500	Waller et al. (2008)

Table 3: Reproductive features of cold-water octocoralls investigated up to date. IB: Internal Brooder; BS: Broadcast Spawner, G: Gonochoric; H: Hermaphrodite, F: Only female colonies encountered. Features annotated with asterisk are results reported as indications, requiring further studies

Order	Species	Reproductive mode	Sexuality	Reproductive Timing	Sex Ratio	Polyp Fecundity[1]	Oocyte diameter[1]	Spawning Seasonality	Source
Alcyonacea	*Anthomastus grandiflorus*	IB	G	Annual	1:4		1100	Seasonal	Mercier & Hamel, 2011
	Anthomastus ritteri	IB	G	Continuous		5.4±2.7	376±178.8	Continuous	Cordes *et al.*,2001
	Drifa glomerata	IB	G	Continuous				Continuous	Sun *et al.*, 2010
	Duva florida	IB							Sun *et al.*, 2011
	Gersemia fruticosa	IB							Sun *et al.*, 2011
	Keratoisis ornata	BS		Annual	F	10-60	700	Annual	Mercier & Hamel, 2011
	Primnoa resedoeformis	BS		Continuous/Overlapping	F	84.3±3.1	1000	Repeated*	Mercier & Hamel, 2011
	Drifa sp.		H						Sun *et al.*, 2009
Gorgonacea	*Acanella arbuscula*	BS	G	Seasonal/Overlapping	1:1	18.8±16.2	136.1±125.1		Beazley *and Kenchington.*, 2012
	Corallium lauuense	BS*	G	Seasonal/Quasicontinuous		5.3	660	Seasonal	Waller & Baco, 2007
	Corallium secundum	BS*	G	Seasonal/Quasicontinuous			600	Seasonal	Waller & Baco, 2007
	Gerardia sp.	BS	G	Seasonal			300	Seasonal	Waller & Baco, 2007
Pennatulacea	*Anthoptilum grandiflorum*	BS	G	Annual	1:1	13±1.8	1100	Seasonal	Baillon *et al.*, 2013
	Anthoptilum murrayi	BS	G	Continuous/Overlapping	1:1	90	1200	Continuous	Pires *et al*, 2009
	Funiculina quadrangularis	BS	G	Continuous/Quasicontinuous	1:1		800	Annual	Edwards & Moore, 2009
	Kophobelemnon stelliferum	BS	G	Continuous			800	Continuous*	Rice *et al.*, 1992
	Pennatula aculeata	BS	G	Continuous				Continuous*	Eckelbarger *et al.*, 1998
	Umbellula durissima	BS	G						Tyler *et al.*, 1995
	Umbellula lindahli	BS	G	Continuous			800	Continuous*	Tyler *et al.*, 1995
	Umbellula monocephalus	BS	G						Tyler *et al.*, 1995
	Umbellula thomsonii	BS	G						Tyler *et al.*, 1995

Possible reproductive modes include broadcast spawning, the release of gametes in the water column where fertilization occurs externally, and brooding which involves fertilization and embryogenesis on or within the colony (Kahng et al., 2011). The reef-forming colonial species studied so far are gonochoristic broadcast spawners: *Oculina varicosa* (Brooke and Young, 2003), *Goniocorella dumosa, Solenosmilia variabilis* and *Enallopsammia rostrata* (Burgess and Babcock, 2005*), L. pertusa* and *Madrepora oculata* (Waller, 2005; Waller and Tyler, 2005; Pires et al., 2014), also the currently ongoing studies within ATLAS reveal *D. cornigera* and *D. ramea* as gonochoristic and broadcaster spawners as no larvae has been identified through the currently conducted histological studies (see section 3.2.1); conversely the solitary CWC scleractinians, display different reproductive strategies, including hermaphroditism, gonochorism, brooding, and broadcast spawning. The *Flabellum* species (*Flabellum thouarsii, F. curvatum and F. impensum*) studied by Waller et al. (2008), *Flabellum angulare* investigated by Mercier et al. (2011a) and *Flabellum alabastrum* and *F. angulare* also studied by Waller and Tyler (2011) were all gonochoric, some of them were broadscast spawners and others brooders; whereas the three *Caryophyllia* species (*Caryophyllia ambrosia, C. cornuformis, and C. sequenzae*) studied by Waller et al. (2005) were hermaphrodites, and *Fungiacyathus* sp. (Waller et al., 2002; Flint et al., 2007; Waller and Feehan, 2013) was revealed as a gonochoric and most probably broadcaster spawner (Table 2).

Within the octocorals, species from the Order Pennatulacea reported so far, are broadcast spawners (Brooke & Young, 2003; Waller et al., 2002; Waller & Tyler, 2005; Watling et al., 2011). However, deep octocoral species within the order Alcyonacea exhibit great flexibility regarding reproductive mode with both broadcast spawners and internal brooders being reported, even within the same genera (Watling et al., 2011) (see Table 3).

Reproductive seasonality is highly variable among species, with some exhibiting clear annual cycles, such as *Oculina varicosa* (Brooke & Young, 2003) and *L. pertusa* (Waller et al., 2002; Waller and Tyler, 2005; Brooke and Järnegren, 2013; Pires et al., 2014). Such clear cycles are scarcer in octocoral species as most of them exhibit continuous gametogenesis with multiple oocyte cohorts within one polyp and periodic or seasonal spawning events (Watling et al., 2011) in both shallow and deep ecosystems. However, continuous gametogenesis has been also reported in deep-sea scleractinians, such as *M. oculata* (Waller and Tyler, 2005). Although there are indications of the existence of environmental cues that can affect reproductive processes and timing such as seasonal carbon input, temperature and lunar cycles (Orejas, 2001; Mercier et al., 2011b) the extent to which reproductive seasonality and synchronicity are affected by environmental factors or internal phylogenetic constrains is yet to be determined. However, it is likely that phytodetritus input from surface waters (Billett et al., 1983; Tyler et al., 1992; Rice and Lambshead, 1994) may

act as a more important control of reproduction (Waller and Tyler, 2005; Mercier et al., 2011a; Brooke and Järnegren, 2013) than other environmental factors, as CWCs thrive in dark environments below the thermocline.

3.2 ATLAS Case studies

3.2.1 ATLAS Case study 1: Scleractinian corals *Dendrophyllia cornigera* and *Dendrophylia ramea*

The aim of this study was to investigate the reproductive features as well as gametogenic cycle of two species from the genus *Dendrophyllia*. *Dendrophyllia cornigera* is a CWC species that seems to be restricted to particular geographical areas (Gori et al. ,2014 and references therein), it occurs forming dense mono-aggregations in some areas (Gori et al., 2014 and references therein), and present a singular physiological performance compared to other CWCs. It has been documented that the species can maintain its physiological functions between 12 and 16 ºC, allowing the species to be the most abundant CWC species in deep-sea ecosystems where temperatures are too warm for other CWCs (e.g., Canary Islands). These special characteristics of the species, as well as its critical status in some locations (the species has been included two years ago in the Barcelona Convention treaty), makes it especially interesting to study the reproduction of this coral, as one of the main and basic biological functions of any living animal. This information is fundamental to better understand the population dynamics, and species functioning and performance. Regarding *D. ramea*, this dendrophyllidae has been considered a species shallower distributed compared to *D. cornigera*, however recent findings reveal the occurrence of this species at higher depths (ca. 150 m) in the Levantine Mediterranean. There is still scarce information on many aspects on the biology and ecology of this species with the exception of two recent publications (Orejas et al., 2019; Reynaud & Ferrier Pagès, 2019). The species has been also included in the Barcelona Convention treaty. Having the opportunity to get access to samples from the two species offer the opportunity to increase the still scarce knowledge, limited to a couple of species, on the reproduction of two more colonial CWC species.

Materials & Methods

Specimens from *D. cornigera* (Fig. 2a) have been collected in la Costa da Morte (off Galicia, Spain) in May, August and October in 2016 and 2017 at ca. 100 m depth and in May, June, October, November 2017 and February and July 2018 from *D. ramea* (Fig. 2b) off Granada. Samples have

been preserved in buffered 10% formaldehyde. In the lab, three colonies per month and three polyps per colony have been decalcified in a solution of 10% formic acid until the skeletons were completely dissolved. Samples have been histologically processed following standard procedures, consisting of dehydration in subsequent immersions in different solutions: alcolin 60', ethanol 70% 120', ethanol 75% 120', ethanol 80% 60', ethanol 95% 60', ethanol 100% 3 x 60', Microclearing X0026® 2 x 90', DParafine Diawax 56 – 58ºC ® 180', DParafine Diawax 56 – 58ºC ® 120'). Subsequently, samples were embedded in paraffin blocks, sectioned in longitudinal section (4 microns) using a Microm HM330 microtome and stained using a standard Hematoxylin-Eosin protocol. Histological slides were observed under a dissecting microscope to verify the presence and size of gametes.

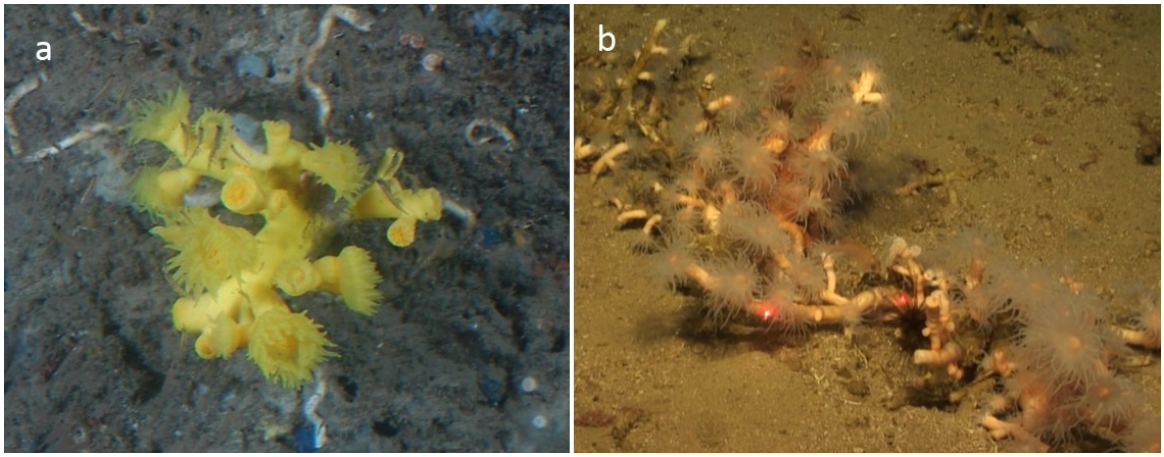


Figure 2: a) Dendrophyllia cornigera *(Cap de Creus canyo, Gulf of Lions, NW Mediterranean, ©ICM-CSIC & IFM-GEOMAR), b)* Dendrophyllia ramea *(off Cyprus, Levantine Mediterranean, © CYCLAMEN project).*

Results and discussion

Dendrophyllia cornigera ongoing study (preliminary data are presented in Figure 4) reveal the species as gonochoric. No hermaphrodite specimens have been detected in the analysed colonies. Sex ratio female: male was biased to the males (1:1.63). The histological study (Figure 3) of the gametogenic cycle reveal two oocyte size classes that could correspond to two cohorts; the larger gametes correspond to late vitellogenic oocytes whereas the smaller ones respond to early vitelogenic developmental stage (Figure 5). The corals proceed from a relatively shallow area (around 100 meters depth) and the gametogenic cycle seem to be coupled with the water temperature (Orejas et al. in prep.). Regarding other aspects that could influence CWCs reproduction, Waller and Tyler (2005) observed for *L. pertusa* that under trawling stress there

were nosexually mature colonies, while they were all in a reproductive state in a preserved area. Therefore, it seems that at least some anthropogenic effects can influence the reproductive biology of CWCs species.

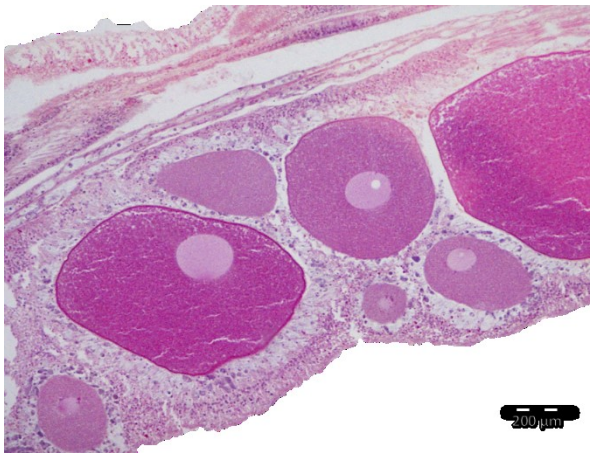


Figure 3: Oocytes of Dendrophyllia cornigera *inside a mesentery. Different developmental stages (early and late vitellogenic) of oocytes can be observed in the histological section.*

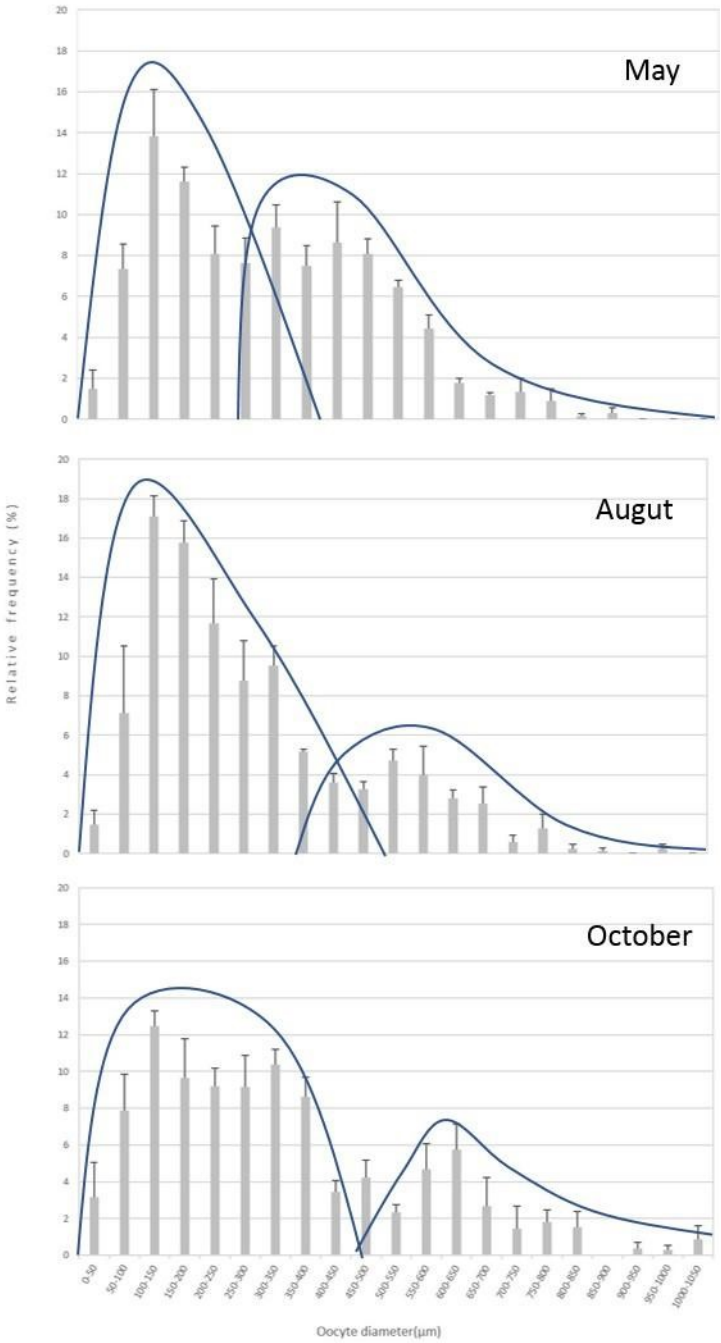


Figure 4: Female gametogenic cycle of Dendrophyllia cornigera *off Galicia. The blue lines indicated the two possible cohorts (oocyte size classes) identified.*

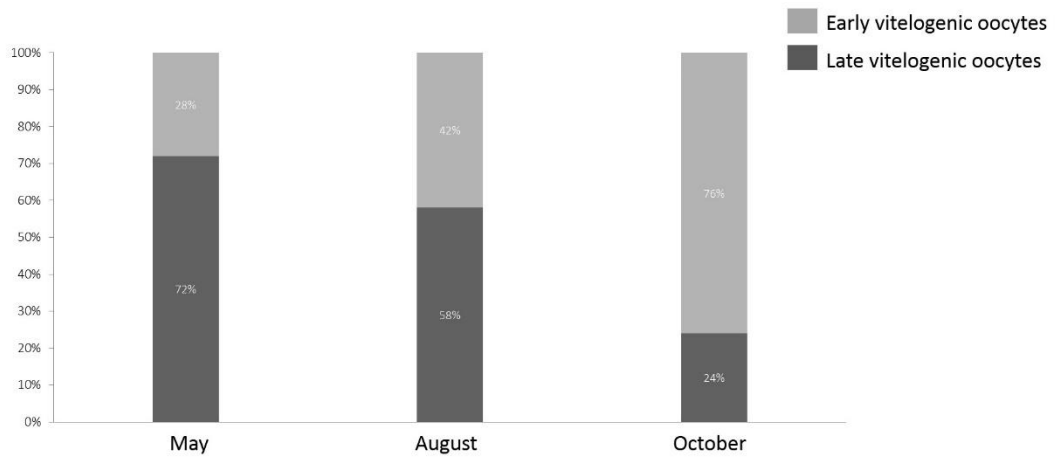


Figure 5: Female gametogenic cycle of Dendrophyllia cornigera *off Galicia. Percentage of early and late vitelogenic oocytes in May, August and October*

Dendrophyllia ramea ongoing histological study revealed the species as gonochoric with a 1:1 sex ratio (Figure 6). Preliminary data on gametogenesis (May, July and October samples have been processed) suggest two differentiated oocyte size classes, similarly to *D. cornigera* (see Figure 7). The spawning seems to take place at some point between July and August as the largest oocyte size classes are not present after October. The analyses of the polyps collected in winter will contribute to reveal if these pattern of two oocyte classes observed in spring and summer persist.

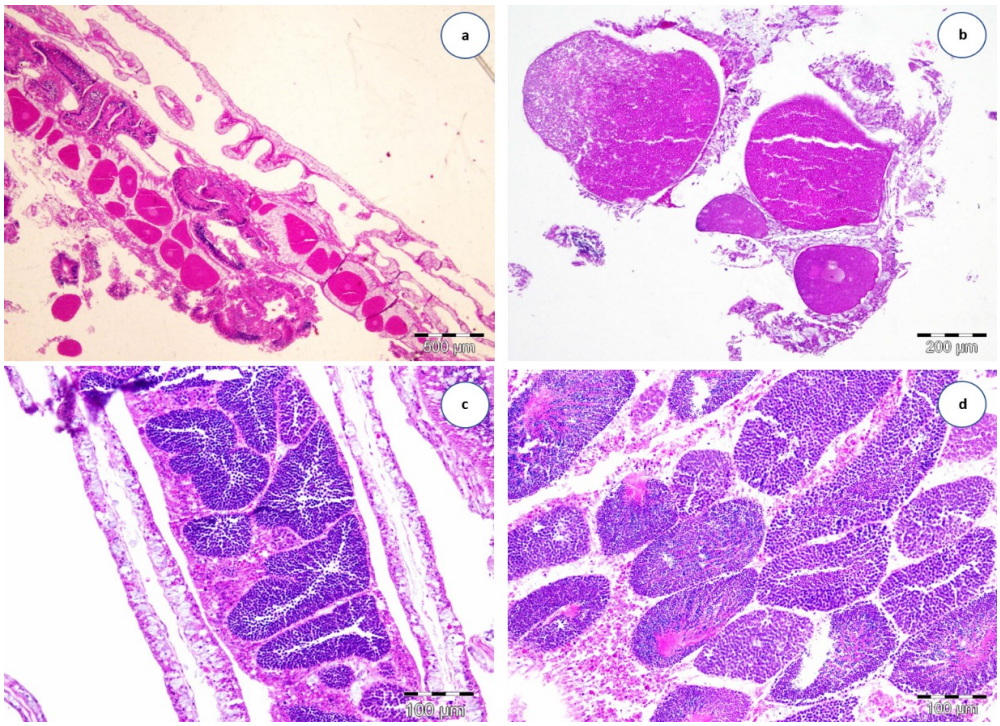


Figure 6: Histological slides of the reproductive tissue of Dendrophyllia ramea. Panel "a" display a mesentery with the oocytes and panel "b" a detail of three mature oocytes; panel "c" show the spermatic cysts packed in a mesentery and panel "d" a detail of mature spermatic cysts.

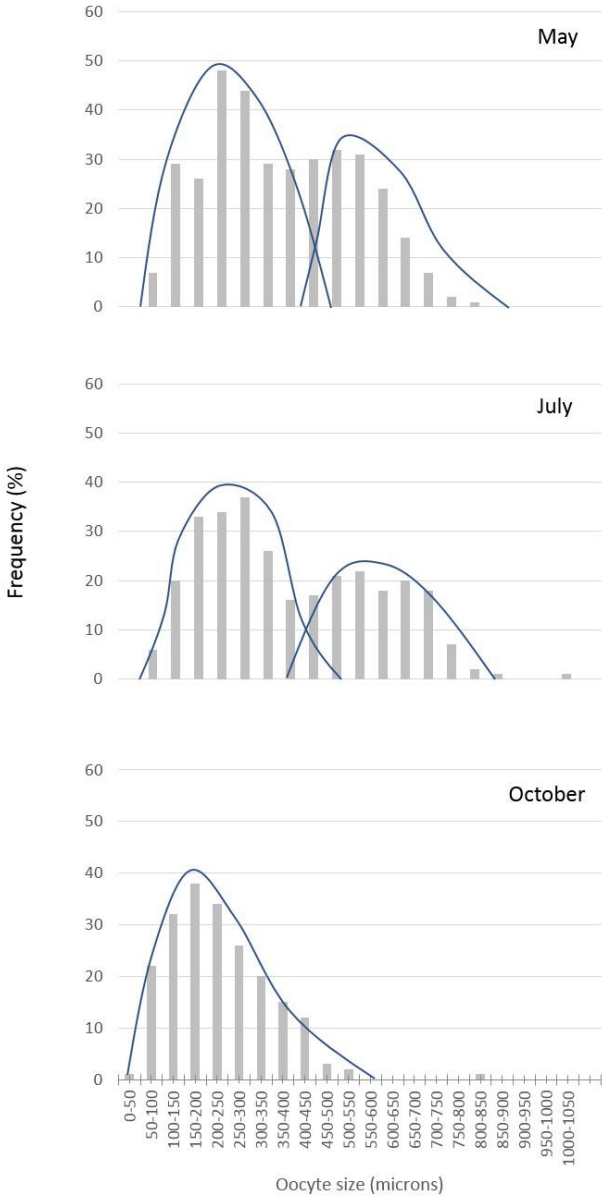


Figure 7: Female gametogenic cycle of Dendrophyllia ramea *off Granada. The blue lines indicated the two possible cohorts (oocyte size classes) identified. Note that gamete spawning take place between July and October.*

3.2.2 ATLAS case study 2: Octocorals *Dentomuricea meteor* and *Viminella flagellum* (IMAR-UAz)

Cold- water octocorals form dense aggregations known as "coral gardens" which are classified as Vulnerable Marine Ecosystems (VME) due to their high vulnerability and great importance as habitat-forming communities. The octocorals *D. meteor* and *V. flagellum* are common cold- water coral species in the Azores Archipelago, where they form dense coral gardens between 200 and

600 meters of depth, often co-existing in the same coral garden (Figure 8). The aim of the study was to (1) describe their basic reproductive biology i.e. sexuality and reproductive mode (2) investigate their gametogenic cycle and (3) provide insights to the reproductive seasonality and timing.

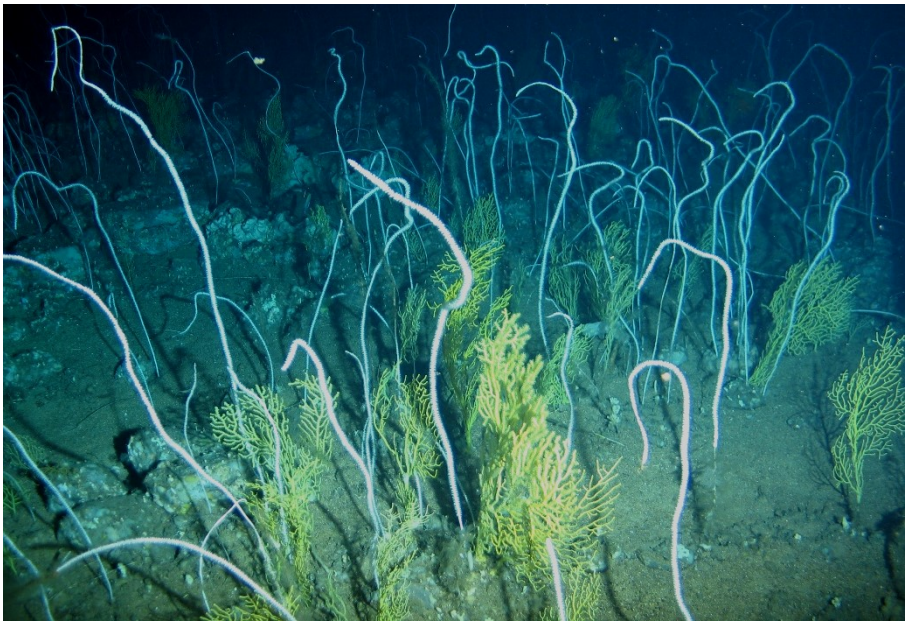


Figure 8: Mixed coral garden of the two target octocoral species: fan shaped Dentomuricea aff. meteor and whip-like Viminella flagellum*. ©Greenpeace*

Materials & Methods

Specimens of *D. meteor* and *V. flagellum* were obtained during 2008-2011, either during scientific expeditions or as by-catch from commercial deep-sea bottom longline fisheries. Specimens were collected at depths between 200-500 meters while sampling sites covered a wide area in the Azores Archipelago. Branchlets of 5-10 cm were cut from each specimen and preserved in 10% seawater formalin. Five polyps were dissected from each specimen and decalcified with 10% formic acid until no traces of sclerites were seen in the tissue. A standard histological procedure was used, consisting of dehydration in subsequent immersions in ethanol solutions (70%, 80% for 30 minutes, 90%, 95% for 15 minutes and 100% for 60 minutes), followed by clearing in xylene for 20 minutes and infiltration in paraffin (Merck Histosec, 56-58^{o}C) at 60 °C for one hour. Dehydration and clearing were performed under a vacuum hood. Subsequently, samples were embedded in paraffin blocks, sectioned in longitudinal section using a Leica 2035 microtome and stained using a standard Hematoxylin-Eosin protocol. Histological slides were observed under a dissecting microscope to verify the presence and size of gametes.

Results & Discussion

Both *D. meteor* and *V. flagellum* were found to be gonochoric at the polyp and colony level and no sexual dimorphism was observed. Sex ratios were female skewed for both species with female to male ratio being 1.86:1 for *D. meteor* and 3.2:1 for *V. flagellum.* Most octocoral species in shallow and deep environments exhibit a 1:1 sex ratio, however female skewed ratios are the most common form of sex disequilibrium with no male skewed ratios being reported so far (Beazley and Kenchington, 2012). Such skewed sex ratios may indicate a variety of processes, such as the existence of differential survival rates for the two sexes or the existence of inbreeding. However, estimates of sex ratios might be influenced by the lower probabilities of encountering fertile males, due to their commonly shorter gametogenic cycles which can interfere with sex determination (Khang et al., 2011).

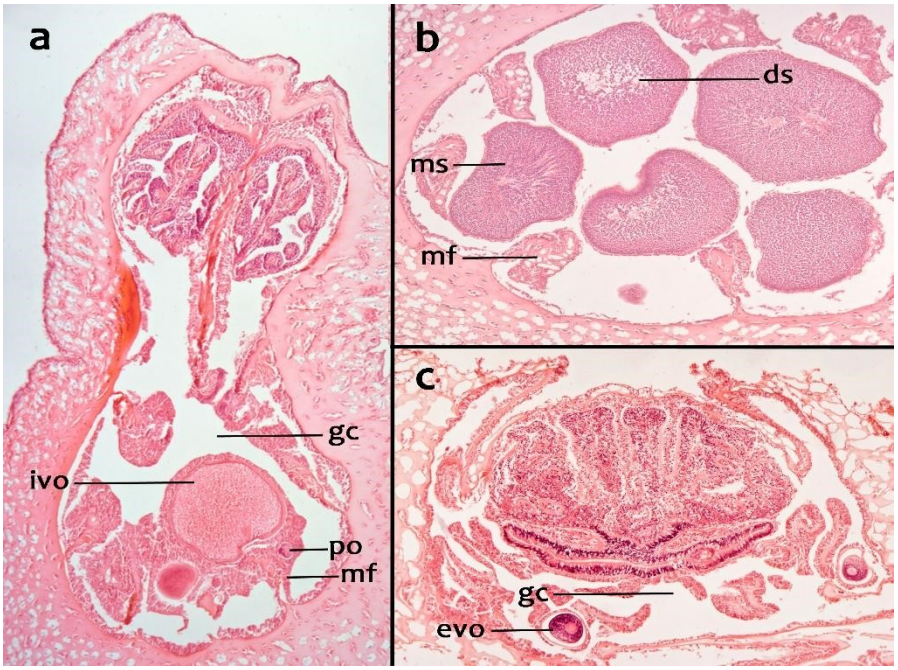


Figure 9: Histological sections of female polyp (a) and male polyp (b) of Viminella flagellum *and female polyp of* Dentomuricea meteor *(c). gc: gastrovascular cavity ; po: previtellogenic oocyte; mf: mesenterial filament; ivo: vitellogeic oocyte; ms: mature spermatocyst; ds: developing spermatocyst; evo: early vitellogenic oocyte.*

Gametes of the two species were encountered either attached to mesenterial walls or free at the base of the gastrovascular cavity (Figure 9). Four oocyte stages were identified: pre-mature (stage 1); pre-vitellogenic (stage 2), early vitellogenic (stage 3) and vitellogenic (stage 4). Oocyte size increased gradually with stage (Figure 10) for both species. Oogenesis of *D. meteor* and *V. flagellum* exhibited stages similar to the ones reported for most octocoral species (Watling et al., 2011; Quintanilla et al., 2013; Beazley and Kenchington, 2012). Three stages of spermatogenesis

were distinguished, depending on the amount of spermatocytes, spermatids (early stages of sperm) and spermatozoa (mature stages of sperm) encountered within the spermatocysts: early (stage 1), developing and mature spermatocysts (stage 3). Because of the low number of male colonies encountered in the present study for both species, further studies on the spermatogenesis are essential.

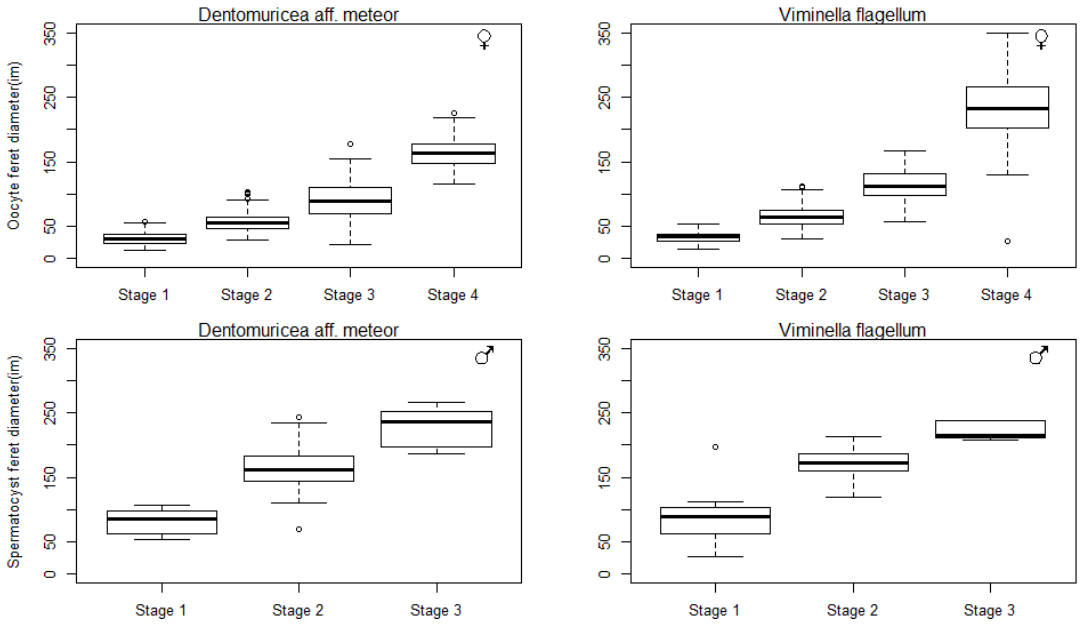


Figure 10: Size of oocyte and spermatocysts of the gorognians Dentomuricea meteor *and* Viminella flagellum *in each developmental stage.*

Specimens collected in 2010 and 2011 were used to study reproductive timing and seasonality as they were the most complete years considering collection of specimens in different months. Gamete presence was observed during the whole year, indicating continuous reproduction. Oocyte size distributions were unimodal or bimodal depending on the existence of mature oocytes (Figure 11), and revealed the existence of two cohorts: one consisting of immature oocytes (stages 1-3) and another consisting of vitellogenic oocytes (stage 4). The difference between the two cohorts is evident due to the large increase in oocyge size during the vitellogenic stage (Figure 10). Colonies of *D .meteor* collected at the same month appeared to be mostly synchronized (Figure 11).

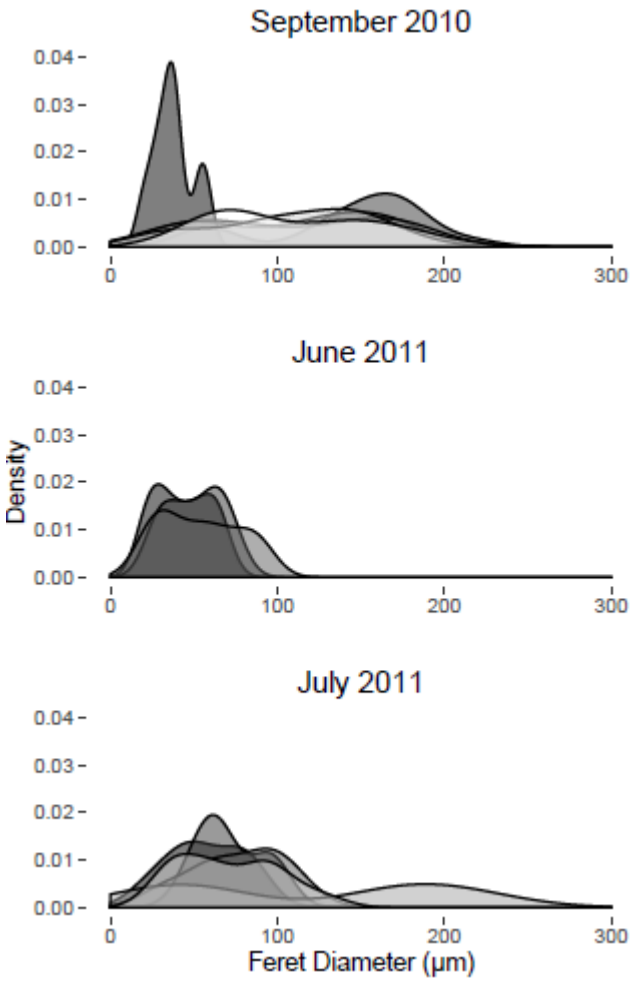


Figure 11: Oocyte size distributions of female colonies of Dentomuricea meteor *collected during the same sampling month.*

Collectively, these results indicate the existence of continuous gametogenesis and reproductive effort with repetitive spawning. This was confirmed by the size of the latest cohort encountered in each specimen, which displayed fluctuations throughout the study period with seasonal peaks (Figure 12). Reproductive peaks appeared in similar periods in the two years: in late spring for *V.flagellum* and in spring and autumn for *D. meteor,* highlighting the fact that although gametogenesis is continuous, spawning might be seasonal. This is the case for other plexaurid species such as *Paramuricea clavata* (Coma et al., 1995) and *Anthoplexaura dimorpha* (Seo et al., 2015) for which annual reproductive cycles have been reported, however inspection of their oocyte size distributions can reveal continuous gametogenesis and overlapping oogenic cycles with annual peaks. Due to the large oocyte size of many octocorals, it is quite common for such species to exhibit long gametogenic periods that can last more than a year (Orejas et al., 2007; Waller et al., 2014). Nevertheless, *D. meteor* and *V. flagellum* displayed relatively short oogenic cycles (4-6 months and 8-9 months respectively) which are also reported for many plexaurid and

ellisellid species (Coma et al., 1995; Gori et al., 2007; Chang, 2007). Likewise, it is likely that the longer reproductive season of *V. flagellum*, comparatively to the one of *D. meteor*, is owed to its relatively larger oocytes.

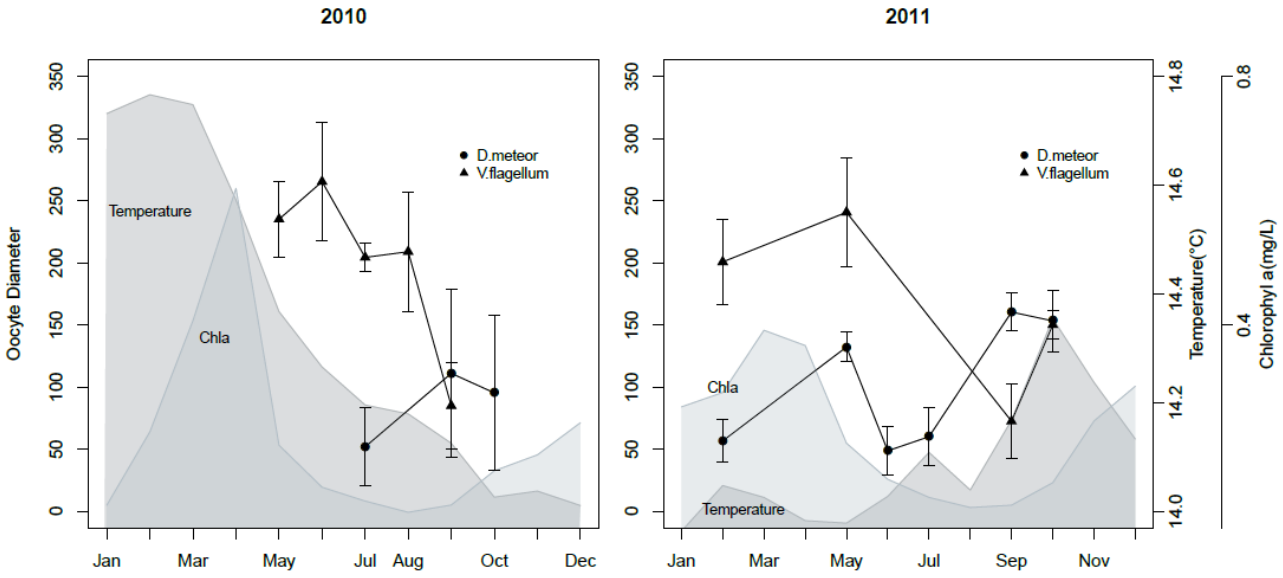


Figure 12: Oocyte diameter of the latest cohort encountered in specimens of the species Dentomuricea meteor *and* Viminella flagellum *during sampling months in 2010 and 2011, along with temperature and chlorophyll a levels during the two years.*

Temperature, lunar cycles, tidal patterns and productivity seem to be important factors that affect reproductive seasonality in both shallow and deep-sea octocorals (Watling et al., 2011). While some of these factors may act directly as cues that synchronize reproductive processes such as vitellogenesis or spawning, others may indirectly affect reproduction by influencing energy availability, metabolism or other internal processes essential for reproduction (Tyler et al., 1995). In the case of the two studied species, peaks in oogenesis seem to follow seasons with high primary productivity, in spring and autumn. A number of deep-sea species display similar behaviour, with potential spawning linked to influx of phytodetritus or zooplankton increase coming from seasonal phytoplankton blooms, e.g. the sea pen *Anthoptilum grandiflorum* (Ballion et al., 2013), the alcyonaceans *Gersemia fruticosa* (Sun et al., 2011) and *Anthomastus grandiflorus* (Mercier and Hammel, 2011) display similar seasonalities. It is possible that in these periods available food sources and thus energy provide the essential energy for the completion or onset of reproductive processes (Gooday, 2002), however further insights to the mechanisms of metabolism, energy allocation and reproduction are essential to explain the existence of such seasonal patterns.

3.2.3 ATLAS case study 3: Polyp-bail out in the octocorals *Acanthogorgia armata* and *Acanella arbuscula*

Life histories of clonal benthic animals are inherently complicated compared to those of aclonal organisms due to their higher plasticity (Jackson and Coates, 1986). Cnidarian species are characterized by high structural simplicity which allows them to employ a high diversity of life history strategies including various forms of asexual reproduction and mechanisms to withstand or escape unfavourable conditions (Piraino et al., 2004). The objective of this study was to describe two distinct cases of a stress/escape behaviour displayed by two octocoral species, *A. armata* (Verrill 1878) and *A. arbuscula* (Johnson 1862). Both species are common encounters in deep-sea habitats in the Archipelago of the Azores (Figure 13) with *A. armata* being mostly encountered between 200-800 m of depth and *A. arbuscula* between 800-3000m (Braga-Henriques, 2013).

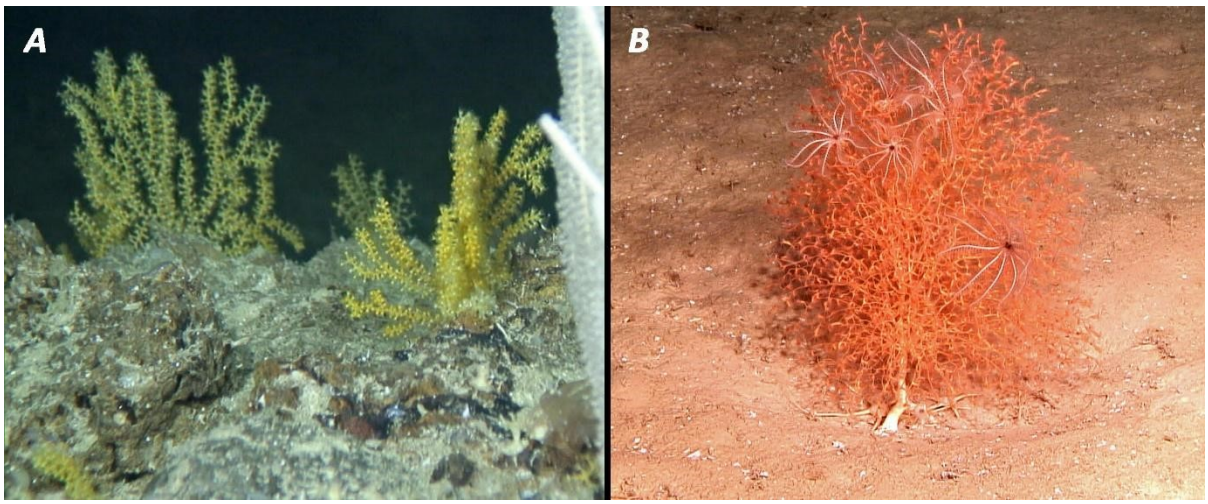


Figure 13: Octocoral species Acanthogorgia armata *(A) and* Acanella arbuscula *(B) ©MEDWAVES*

Materials & Methods

Three colonies of *A. armata* were collected from the ‘‘Coral Garden Hill’’ in the Pico-Faial channel in Azores (38°29.374’N 028°37.262’W) at 350 m deep with the manned submersible Lula (Rebikoff-Niggeler Foundation) in September 2009. Corals were transferred to the IMAR-Uaz facilities (DeepSeaLab) in coolers. The aquaria were equipped with a continuous flow-through open system supplied continuously with seawater treated by sandfilters and UV-light (Vecton 600, TMCTM) before distributed to aquaria. Aquaria were kept in a thermostatic room in darkness, keeping temperature at 14 ± 0.9 C. Specimens were maintained in a 35-L aquarium with seawater

renewal of approximately 12 L per hour, mixed with submersible pumps and were fed daily with frozen zooplankton and a liquid mixture of marine particles between 10 and 450 ml (Marine Active Supplement, Bentos Nutrition Maim, Vic, Spain). During the 5-month maintenance period, coral colour and polyp expansion were closely monitored, while measurements of temperature and oxygen were performed daily.

Four colonies of *A. arbuscula* were collected from the Formigas Bank off the Azores (37°12.140'N 024°826.717'W) at depths between 1094 and 1127 m, using the Remotely Operated Vehicle (ROV) Liropus 2000 (IEO), during the MEDWAVES cruise on-board the Research Vessel (RV) Sarmiento de Gamboa in October 2016. After emersion, which lasted approximately 40 min, colonies were transferred to a 20-L tank where they were fixed on plastic bases with epoxy. The tank was kept inside a dark, thermoregulated room onboard keeping temperature at 12.0 ± 0.5°C. Water renewal was made daily with water collected from 1000 m with a CTD rosette and kept refrigerated at 4°C. Corals were fed every other day with the Marine Active Supplement described above. Maintenance of all specimens lasted for 4–5 d after collection. During this period, no measurements of aquaria parameters were possible; however, observations on the colour, polyp expansion and tissue state of coral specimens were made daily.

Results & Discussion

Colonies of *A. armata* did not show signs of stress during the first 2 months of maintenance (September, October) (Figure 14a, b), however by November 2009, the colour of the colonies changed from vibrant yellow to dark brown and polyps retracted (Figure 14c, d). This event coincided with a short malfunction of the refrigerating system which caused an increase in water temperature from 14°C to 16.5°C and which was accompanied by a drop in oxygen saturation from an average of 85% to 70%. Thereafter, sporadic coenosarc withdrawal (Figure. 14c), sclerite release and massive polyp detachment were observed. Coenosarc withdrawal spread progressively, and disassociated polyps started accumulating at the bottom of the aquarium, solitary or in small aggregates. By January 2010, most of the skeleton was exposed and only small traces of coenosarc were left on the colony. Detached polyps were negatively buoyant and contained sclerites, with exposed aboral sides, i.e. open and free of sclerites (Figure 14f).

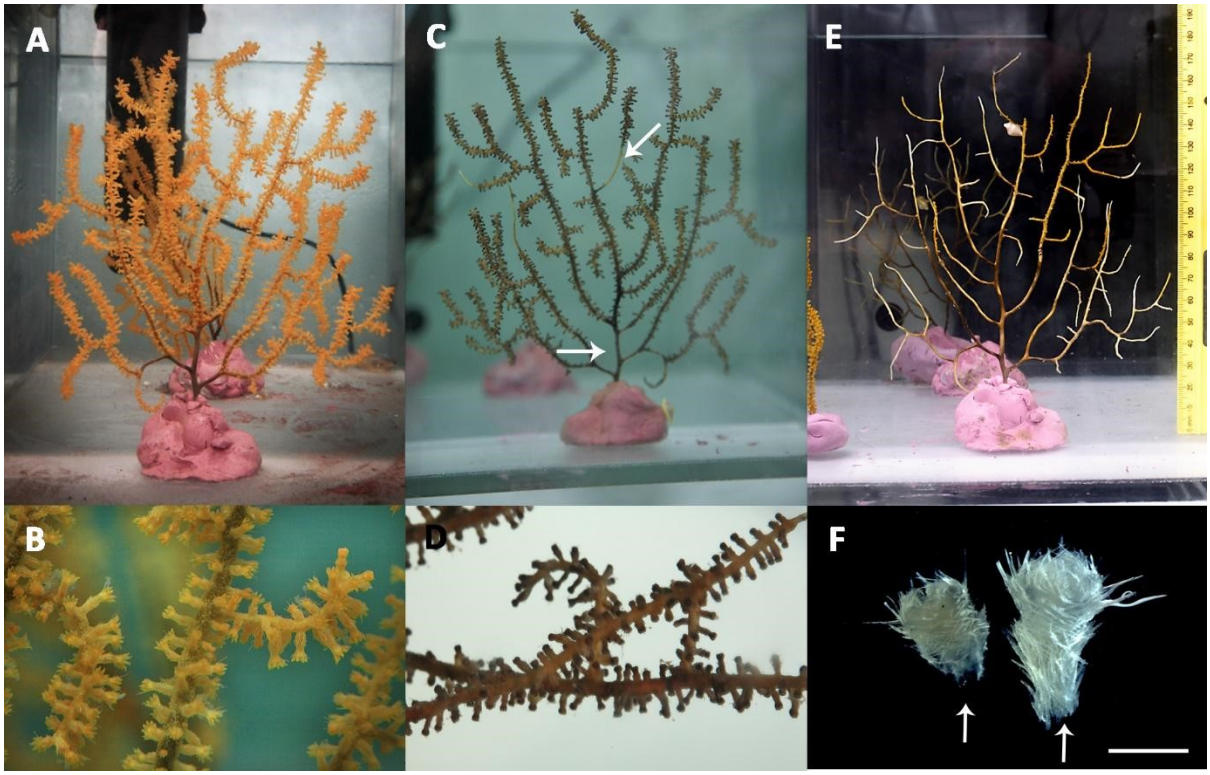


Figure 14: Full frame and close-up perspective of Acanthogorgia armata *colonies during maintenance period: a, b colonies during the first 15 d, with vibrant yellow colour and extended polyps; c, d colonies after 3 months, coloured dark brown with retracted polyps. Arrows indicate sporadic withdrawal of tissue; e colony after 5 months in the aquarium, with no visible tissue; f close-up image of detached polyps with exposed aboral sides (arrows). Scale bar: 1 mm. (Rakka et al., 2019)*

Immediately after collection, colonies of *A. arbuscula* released large quantities of mucus which persisted during the whole maintenance period (Figure 15). Approximately twelve hours after transfer to the aquaria, polyps started detaching from the mother colonies and accumulated at the bottom of the tank (Figure 15a). Polyps seemed to be released gradually from the rest of the skeleton after slow detachment of tissue and sclerites from the lateral polyp side (Figure 15b). Detachment continued until day 4 after which colonies had lost most of their tissue and polyps. Despite continuous detachment of polyps, remaining polyps on the mother colony remained open until the end of day 3, after which all polyps retracted. Tissue loss and polyp detachment also led to the release of oocytes which are usually found at the polyp base (Beazley and Kenchington 2012). Oocytes were neutrally buoyant and were floating in the water column or attached to mucus. Oocytes had a diameter between 350–470 µm and were therefore mature (Beazley and Kenchington 2012), however oocyte release seemed an inevitable result of polyp detachment rather than spawning. Free polyps during the first 48 h of polyp detachment were mostly intact with exposed aboral sides and were covered with sclerites (Fig. 15c). However, most polyps

collected on the third day were smaller and more spherical with a closed aboral side, discrete oral side and only a few incorporated sclerites, resembling sclerite-free spheres (Fig. 15d).

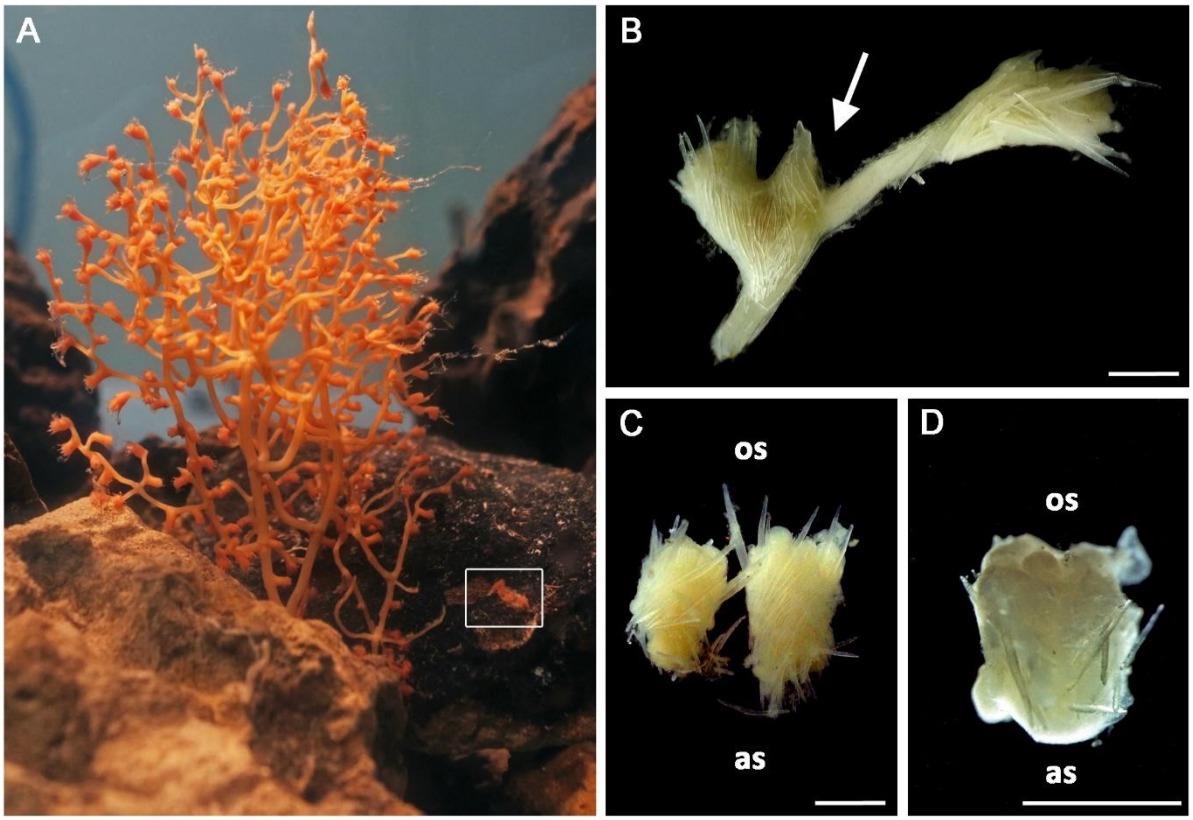


Figure 15: Polyp bailout in specimens of Acanella arbuscula*: a live colony with mucus nets and expelled polyps accumulating on adjacent rocks (white rectangle) on the second day of maintenance, b polyp in the procedure of detachment, with detached tissue and sclerites on the lateral side (arrow), c expelled polyps covered with sclerites, open oral sides and exposed aboral sides, collected during the first 2 d of maintenance d sphere-like form of an expelled polyp with a few sclerites attached, collected on the third day. os oral side, as aboral side. Scale bars: 1 mm (Rakka et al., 2019)*

The reactions of both octocoral species included tissue contraction, sclerite loss and massive polyp detachment. Moreover, sclerite-free spheres found in *A. arbuscula* suggest the existence of a transformational process from intact free polyps to sclerite-free spheres. These observations are consistent with the description of polyp bailout in scleractinian corals (first described by Goreau and Goreau 1959; termed by Sammarco 1982), including partial mortality of the coenosarc and polyp dissociation resulting in free, usually negatively buoyant polyps without any calcareous material. Polyp bailout is a stress response and an expression of reverse development, the ability of adult forms to develop into earlier developmental stages (Richmond 1985; Jackson and Coates 1986) which have higher probabilities of dispersal. It has been associated with a variety of inducing factors such as fluctuations in temperature (Kruzic, 2007), salinity (Shapiro et al., 2016), pH (Kvitt et al. 2015) and accumulation of byproducts in closed aquaria systems

(Serrano et al., 2017a). In the case of *A.armata* it is most likely that rapid temperature changes and oxygen decrease led to the stress response. As for *A. arbuscula*, a combination of factors may have led to the polyp bailout response, including rapid temperature and pressure changes during the long ascent of the ROV and maintenance in closed system which might have prevented oxygen circulation and clearance of metabolic products such as carbon dioxide, ammonia and nitrite (e.g. Parent and Morin 2000; Crab et al. 2007).

To our knowledge, this is the first record of a polyp bailout response in cold-water octocoral species. Polyp bailout has been widely described in scleractinian including both tropical (Capel et al. 2014; Shapiro et al. 2016) and temperate species (Kruzic 2007; Serrano et al. 2017a). It highlights the capacity of coral species to alternate between soft bodied and calcifying forms and showcase their high developmental plasticity. This plasticity has been suggested as a possible developmental pathway that allowed scleractinians to persist throughout periods of extremely unfavourable conditions during their evolutionary history (the ‘‘naked coral hypothesis’’, Medina et al. 2006; Kvitt et al. 2015). It is therefore an important life history trait that can be very important in possible unfavourable conditions caused by increasing anthropogenic activities and climate change.

3.3 Overview of larvae biology and behaviour (IMAR-UAz)

Dispersal and connectivity have been recognized as fundamental characteristics of marine ecosystems, defining species survival and distribution and constituting essential knowledge for effective marine conservation (Cowen et al., 2006). For marine benthic species, dispersal is mainly performed during the early life history stages, namely the larva stage. While increasing studies focus on hydrodynamic controls of dispersal, knowledge on larval biology is scarce for most marine species creating a gap in our understanding and providing poor support to attempts of dispersal modelling (Cowen and Sponaugle, 2009; Hilário et al., 2015).

Dispersal can be affected by a variety of larval characteristics such as size, developmental rate, buoyancy, swimming behaviour, vertical migrations, pelagic larval duration (PLD), attachment behaviour and physiological tolerance (Hilário et al., 2015; Treml et al., 2015). Furthermore, successful dispersal and recruitment is bound by ecological processes such as predation, competition and mortality (Lacharité and Metaxas, 2013; Treml et al., 2015). To date, such information is extremely scarce for most deep marine species including CWC, despite their recognized importance as habitat formers and ecosystem engineers in the deep sea. Furthermore, recent studies highlight the existence of great variability in larval traits among species.

The larvae stage in CWCs has only been studied in six species due to the difficulty of collecting larvae at several hundreds or thousands of meters depth, as well as to induce spawning in aquaria conditions (but see examples in Orejas et al. 2019). So far, knowledge on larvae traits of cold water scleractinian species include the common reef building corals *Oculina varicosa* (Brooke and Young, 2002) and *L. pertusa* (Larsson et al., 2014; Stromberg & Larsson, 2017) and the solitary coral *Flagellum angulare* (Mercier et al., 2011a). In the case of *O. varicosa*, embryos reached the planula stage (size: 120μm) within 9 hours at 25°C. Ciliated planulae displayed upward swimming even in the dark, and bottom probing behaviour was observed after 1-2 weeks (Brooke and Young, 2002). Embryos of *L. pertusa* reached the planula stage approximately within 7-9 days after fertilization, and were able to swim by day 5, using cilia in water of 7-8°C (Larsson et al., 2014). Under higher temperature (11-12°C), development was faster, with swimming blastula appearing by day 3 (Stromberg and Larsson, 2017). Planula (size: 120–270 μm) appeared to be able to feed on a variety of small food particles by day 20 when their oral pore developed in a protractible mouth (Larsson et al., 2014). Moreover, larvae seemed to increase their swimming speed under food presence (Stromberg and Larsson, 2017). During the first two weeks planula were swimming upwards and could swim through salinity gradients without lowering their swimming speed (Stromberg and Larsson, 2017). Bottom probing behaviour became common after 4-5 weeks and most planulae survived between 8 weeks (Larsson et al., 2014) to a year (Stromberg and Larsson, 2017). Although settlement was not observed, indications of the onset of metamorphosis were reported (Stromberg and Larsson, 2017). Lastly, larvae of *F. angulare* reached the planula stage (2-3mm long) within 24h and survived for a few weeks in aquaria without settling (Mercier et al., 2011b).

Larvae of the soft corals *Gersemia fruticosa, Drifa glomerata*, *Duva florida* (Sun et al., 2010; 2011) and *Anthomastus ritteri* (Cordes et al., 2001) have also been acquired in laboratory conditions, displaying very different larval traits from the aforementioned scleractinian species. The four octocoral species are internal brooders, with fertilization occurring within the polyps and embryos being released after reaching the planula stage. Planulae of the species *G. fruticosa* were rod shaped with length between 1.5-2.5 mm. They could control their buoyancy and displayed settlement after on average 11.4±1.7 days. In total 48.2% of the observed planulae settled and metamorphosis coincided with settlement. However, in a small percentage of larvae, metamorphosis occurred before settlement with some larvae reaching the polyp stage without having settled permanently (Sun et al., 2011). Planulae of *D. florida* had similar size (1-2.5 mm) however their buoyancy was negative and displayed crawling behaviour (Sun et al., 2011).

Similarly, *A ritteri* released planulas 3 mm long that displayed initial settlement within 2-3 days and metamorphosis within 4 days (Cordes et al., 2001).

In previous studies Rinkevich and Loya (1979) first stipulated that all CWC larvae are lecithotrophic; this is in agreement with the observations of large mature oocytes in *M. oculata*, *S. variabilis* and *E. rostrata* (Table 2). However, Larsson et al. (2014) observed that *L. pertusa* larvae were rather planktotrophic as previously suggested by Waller and Tyler (2005). Before settlement, larvae have a planktonic life, and their swimming behaviour is similar to that of other scleractinian larvae. Most larvae swim in a rotary clockwise or counter-clockwise mode around the oral-aboral axis, and many of them are characterised by positive phototactic and/or negative geotactic responses (Fadlallah 1983). Therefore, in *O. varicosa*, early stage larvae swim towards the surface responding to geotactic signals, whereas later stage larvae display negative phototactic behaviour (Brooke and Young 2005). In L. pertusa, larvae show strongly negatively geotactic signal during the 1st week following fertilisation (Larsson et al. 2014), reaching the bottom 3 weeks after fertilisation. Such behaviour avoids the risk of being eaten by benthic predators (Brooke and Young 2003), and/or is a way to reach the photic layer with a higher quality and quantity of food.

3.3.1 ATLAS case study: Octocoral *Viminella flagellum*

The aim of this study was to provide insights into the larvae biology of the habitat forming cold-water octocoral *V. flagellum*. The data produced in this study was used to model larval dispersal in the North Atlantic and Mediterranean (see sections 4.4.2).

Materials & Methods

Successful spawning and fertilization of the species *V. flagellum* occurred on 18 August 2017 during maintenance of the species in the aquaria facilities of IMAR-UAz (DeepSeaLab). A total of 20 fertilized oocytes were collected and transferred to a 250 beaker placed within a water bath keeping temperature stable at 14±0.6 °C. Embryos and larvae were observed every 12h and six samples were collected during the first 48h and fixed in formalin in order to describe larval development. During observations, information on the developmental stage, number of larvae and position in the water column were noted. Data on horizontal swimming speed were collected on day 10. Larvae were transferred in a 1cm (diameter) labyrinth, one at a time, and 1 min videos were recorded with a calibrated dissecting microscope (DinoLite AM7013MT). Swimming speed was estimated in segments where larvae displayed straight movement, by using the software Image J. During larvae maintenance, water was changed weekly and small quantities of food were given weekly, consisted by a mixture of freshly harvested zooplankton and phytoplankton. Upon

observing downward swimming behaviour, a piece of basalt was offered to the larvae to observe potential attachment.

Results

Spawning occurred on August 2017 in one aquaria holding several fragments of *V. flagellum.* Embryos were encountered already on the 8-cell stage, with average diameter 1216.1±21.3 μm and had neutral buoyancy, floating in the mid-water. Three hours later most embryos were already in the 32-cell stage and reached the blastula stage within 24 hours from the first encounter and the planula stage (Figure 16) within 72 hours. During that period all embryos had neutral buoyancy.

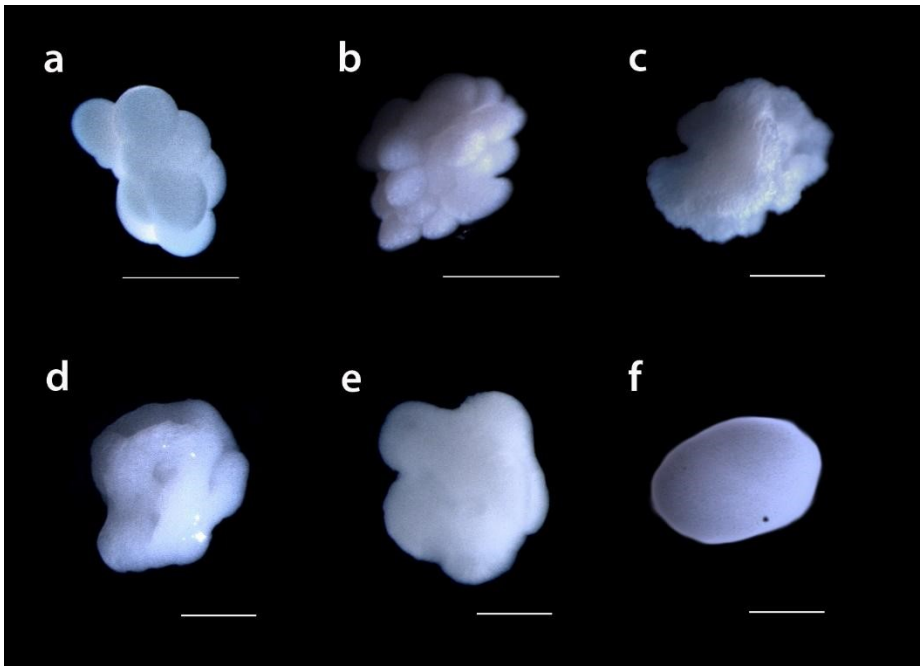


Figure 16: Embryo development of the species Viminella flagellum. *(a) 8 cell embryo; (b) morula; (c) 1 day blastula; (d) 2 days gastrula; (e) 3 days gastrula; (f) early planula. Scale bar: 500 μm*

Early stage planulae were mainly spherical having a diameter of 1522.6±19.3 μm. They could alternate between spherical and elongated shape, which has been observed before in other deep sea octocorals (e.g. *Drifa glomerata*, Sun et al., 2010) and scleractinians (*L. pertusa*, Larsson et al., 2014). Planulae after day 8 were mostly elongated and their length could reach 4-8 mm which is much larger than the size reported for *L. pertusa,* but is similar to sizes reported for other octocoral species (Cordes et al., 2001; Sun et al., 2011). On the fifth day 12 larvae were encountered on the surface and displayed upward swimming behaviour even if moved lower in the water column. Bottom probing was first observed on day 7 and by day 12 all larvae had returned to the bottom (Figure 17), resulting to an average pelagic larval duration (PDL) of 9.5

days, which is quite short compared to *L. pertusa* which displayed bottom probing behaviour only after 3-5 weeks (Larsson et al., 2014).

After reaching the bottom most larvae were moving horizontally on the provided substrate and attached temporarily in crevices. Swimming was performed by using cilia and was circular with a clockwise direction. Swimming velocity was on average 5.07±1.64 mm/sec. Although this swimming speed estimate is much higher than the one reported for *L. pertusa* (0.51±0.09 mm/sec at 11°C, Larsson et al., 2014), and *O. varicosa* (0.66 mm/sec at 35C, Brooke and Young, 2003) this difference might be attributed to the different larval sizes as the planula of *V. flagellum* is much larger.

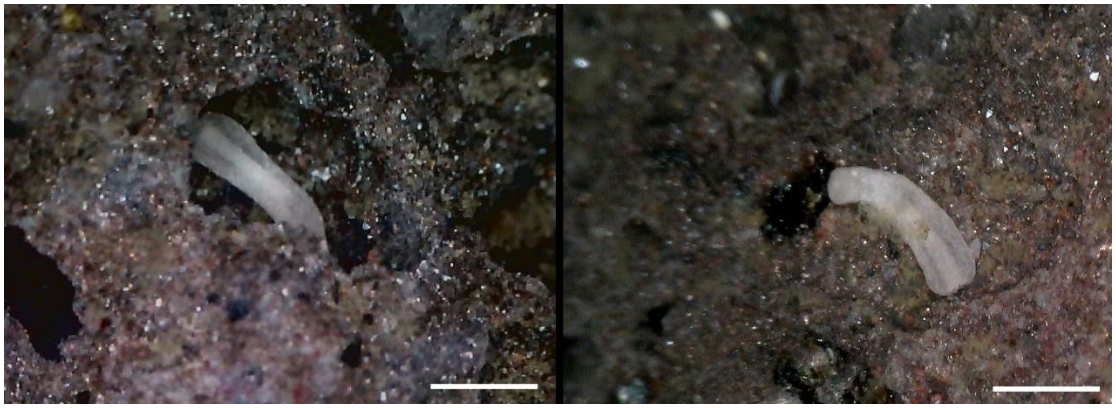


Figure 17: Bottom probing behaviour displayed by larvae of the gorgonian Viminella flagellum. *Scale 7 mm*

Survival on the first 12 days was 40%, but declined with time reaching 5% within three months, with only one larva surviving. This larva survived for a total of 210 days. During that time it performed very limited movements between crevices and attached temporarily (Figure 17). No permanent attachment was observed. Although the present results provide basic information on the early life history stages of the studied species, including important variables such as larval size, position in the water column and bottom probing behaviour, the sample size of obtained larvae was extremely small. Further studies are needed to provide further insights to the larval developmental stages, vertical swimming speed, feeding and attachment behaviour.

4 Modelling dispersal and connectivity in the deep-sea (UEDIN, IMAR-UAz, IFREMER)

4.1 Introduction

Many ocean bottom dwelling species release their larvae into the water column so that the larvae can both be recruited into the local population and spread further field to support remote populations and colonize new sites. A better understanding of larval pathways and downstream colonization is an important factor determining population connectivity, informing studies of natural and man-made networks, feeding into the design of Marine Protected Area networks and ultimately impacting how the marine environment is used. During their transit, larvae exhibit a range of different behaviours for maximizing their immediate survival (finding food and avoiding predation) and their long-term survival (finding a suitable spot to settle). Key strategies are pelagic duration and control of the vertical position in the water column.

Connectivity of marine ecosystems is fundamental to survival, growth, spread, recovery from damage and adaptation to changing conditions, on ecological and evolutionary timescales (James et al. 2002; Cowen and Sponaugle 2009; Burgess et al. 2014). Empirical evidence shows the benefits of connectivity information to conservation management (Planes et al. 2009; Olds et al. 2012). Knowledge of the characteristics of marine connectivity is rapidly expanding, with recent studies using seascape genetics approaches combining particle tracking in high resolution ocean models with state-of-the art genetic techniques (Foster et al. 2012; Teske et al. 2016; Truelove et al. 2017). Many populations in the deep sea are spatially fragmented and vulnerable to damage from increasing exploitation of the deep sea; understanding the connectivity between the subpopulations is critical for spatial management (see Cabral et al. 2016, for a review). However, knowledge of deep-sea connectivity remains limited – from both the physical and biological perspective. Direct evidence of deep-sea population connectivity, through tagging and tracking, remains almost unknown, but indirect estimates of connectivity can be constructed by using genetic methods, elemental fingerprinting or particle modelling.

A common indirect method of estimating connectivity is through tracking of virtual adults, juveniles or, more usually, larvae, within a hydrodynamic model – so called 'Lagrangian', individual-based models (IBMs) or particle tracking modelling. To date, in the deep sea, this approach is limited to relatively few studies (e.g. Lavelle et al. 2010; McGillicuddy et al. 2010; Yearsley and Sigwart 2011; Young et al. 2012; Fox et al. 2016; Breusing et al. 2016). Such modelled connectivity and dispersal estimates are affected by life history traits – timing of spawning, larval behaviour, and effective pelagic larval duration (PLD). Knowledge of these biological parameters,

and estimates of their relative importance to dispersal and connectivity in the deep sea is sparse. Bradbury et al. (2008) review estimates of marine dispersal, for fish species estimates are primarily based on otolith microstructure and show a correlation between habitat depth and PLD – fishes living at deeper depths have generally longer PLD. For non-fish species, Bradbury et al. (2008) estimated PLD from biogeography and population separation distances.

Hilário et al. (2015) provide a thorough, updated review of the challenges of estimating dispersal distances in the deep sea. They found estimates of PLD in the literature for fewer than 100 species living below 200m, over 80% of these estimates are for species on sedimentary slopes, predominantly echinoderms. PLDs from a few days to over a year were found. Direct observations of larval position in the water column has also been used to infer PLD and spawning times and to ground-truth larval dispersal models (Mullineaux et al. 2005; Arellano et al. 2014), predominantly for species of mollusc (with larger, more substantial larvae) living around vents and seeps.

Dispersal depth is also thought to be a critical parameter for deep-sea connectivity, since current speeds, directions and turbulence can vary significantly with depth. For example, Lagrangian modelling demonstrates that larvae of the deep-sea mussel *Bathymodiolus childressi* drifting in the upper water layers of the Gulf of Mexico can potentially seed most known seep metapopulations on the Atlantic continental margin, whereas larvae drifting demersally cannot (McVeigh et al. 2017). In the northeast Atlantic, Fox et al. (2016) showed how *L. pertusa* larvae following vertical swimming behaviour traits predicted from observations in the laboratory (Larsson et al. 2014; Strömberg and Larsson 2017) may disperse much more widely than passive larvae.

The occurrence of seep species of molluscs, crustaceans, and other taxonomic groups across the Atlantic Ocean suggest broad connectivity (e.g. Cordes et al. 2007), while recent examination of deep-sea sponges in the NE Atlantic (Van Soest and De Voogd 2015) finds communities at Mingulay were faunistically closer to the geographically distant shelf reefs at Skagerrak than to the geographically closer bathyal reefs of the Porcupine–Rockall area. The Atlantic meridional overturning circulation (AMOC) can achieve large-scale larval dispersal across ecological timescales as observed in the cold-water-coral *L. pertusa* (Henry et al. 2014). Dispersal modelling, coupled with pelagic larval duration estimates and population genetics, has also recently (Young et al. 2012; Breusing et al. 2016) been used to predict the existence of further vent ecosystems between neighbouring known sites on the mid-Atlantic ridge.

Hilário et al. (2015) identify the physical component of biophysical connectivity models as one of the gaps in estimating deep sea connectivity. While these models are far from perfect, the errors

are better defined than those associated with the larval biology, with widespread verification against, and assimilation of, *in-situ* and remotely sensed data covering many variables at all depths. These models are described in detail in the physical oceanography and modelling literature, output from many is available online. Of more concern is the risk that ecologists use such models as a 'black box', choosing a model which appears to work but whose inner workings are unknown, potentially resulting in misuse and misunderstanding of the models capabilities (Ross et al. 2016). The current generation of global- and basin-scale models run at horizontal resolutions of 3–4 km, modelling mesoscale eddies, and, when used for particle tracking, are able to reproduce the coherent structures, patch stretching and straining, attractors and barriers described by Harrison et al. (2012). Of particular concern in the deep-sea is the difficulty in modelling the small-scale turbulence, mixing and cross-slope exchanges associated with the steep topography (upper continental slope, seamounts and ocean ridges) which forms typical adult habitat and which may be fundamental to early-life larval dispersal and later settling.

For deep sea species observational evidence of the characteristics of the larval behaviour strategies are severely lacking, while the little evidence available suggests these strategies to be important for larval dispersal and population connectivity. Particle tracking modelling can perhaps be most useful for predicting which larval behaviour traits have the strongest influence on larval dispersal, and thereby helping to guide future observational work.

Working with the ATLAS Case Study regions in the North Atlantic, in ATLAS Deliverable 1.6 we tried to answer two questions to help identify whether larval behaviours are impacting their long-term spreading: (1) Is there any evidence that any of these behaviours, either alone or combined, cause greater spreading and, (2) do any of these behaviours cause larvae to follow particular pathways so they settle in specific locations? To answer these questions we used particle tracking with ARIANE within the VIKING20 model of the North Atlantic. The details of the methods used are in Deliverable 1.6. Here we reproduce the summary of the findings of D1.6:

- Larval behaviours are predicted to impact their long-term spreading, with wider spreading being everywhere associated with more time spent higher in the water column.
- The strength of this enhanced dispersal varies regionally (from strong to very strong).
- Dispersal pathways are predicted to be affected by larval behaviour in ways which could influence the distribution of species.

- For deep-sea populations, the uncertainty in modelled dispersal and connectivity associated with vertical larval positioning in the water column is potentially an order of magnitude larger than that associated with pelagic larval duration or model hydrodynamics.

- The knowledge gaps in larval behaviour which contribute most to the uncertainty concern settling – the age at which larvae start to sink, and the sinking rate.

- In the absence of detailed knowledge of larval development, time-series observations of larval position in the water column could be used to constrain models, hugely reducing uncertainty in predictions.

- Under the most dispersive behaviour modelled, populations throughout the North Atlantic would be connected. Seamount populations may be crucial stepping stones in this wider connectivity.

- In the more dispersive scenarios two large-scale closed connectivity loops were identified, one anticlockwise around the North Atlantic basin with west-east return via the Azores, the second smaller loop following the sub-polar gyre.

- Even for the least dispersive behaviour modelled, populations along the continental slope may be connected anticlockwise around the North Atlantic, depending on the detailed habitat distribution.

- These conclusions are based on a large, systematic Lagrangian modelling experiment, tracking about 10 million virtual particles over 50 years in contrasting dynamical regimes.

The aim of the current work was to use current knowledge of basin-scale population distribution and reproductive and larval life history traits to focus connectivity modelling on various key deep-sea species and to extend the modelling work of Deliverable 1.6 from Case Study regions to the full North Atlantic basin. However the results of D1.6 showing very strong dependence of dispersal on larval vertical distribution and life span, coupled with almost total lack of knowledge of these for any deep sea species, suggested a change of strategy which came out of an ATLAS workshop in February 2019. So here we first model basin-scale connectivity using passive particles released at a range of depths with a range of lifespans. We then tailor these connectivity matrices to individual species using the closest approximate behaviour and results from habitat distribution models. Models of current and future habitat distribution were used to try to assess the effects of changing conditions on population connectivity.

4.2 Methods

Particle tracking modelling Particles tracks are simulated with a modified version of the ARIANE software (Döös 1995; Blanke et al. 1999) coupled to the velocity, potential temperature, T, and salinity, S fields in the VIKING20 configuration (Böning et al. 2016) of the NEMO ocean model (Madec 2008). In the VIKING20 hindcast run, forced by the CORE2 dataset (Large and Yeager 2009), a 0.05° resolution grid spanning the North Atlantic was two-way nested (Debreu et al. 2008) within a 0.25° resolution global ocean. VIKING20 output from 1958 to 2009, at a temporal resolution of average fields over every 5 days, is used here.

Particles were released quarterly over 50 years at three positions in the water column: near-surface (model level 2, at 9 m), near-bed (the mid-point of the deepest level locally), and 1000 m (wherever depth > 1000 m). These were chosen to reproduce surface-drifting, near-bed passive drifting and drifting mid-column but below the seasonal thermocline. We did not attempt here to reproduce early stage vertical movement or settling behaviour as these are generally unknown. Each quarter in each year at each of the three (or two in shallow water) depths, a particle was released in the model from a random selection of the horizontal gridsquares. The release squares were chosen at lower density in model regions with depths over 3000 m, because these abyssal depths are not the focus of ATLAS. Releasing particles from 67% of gridsquares (33% in depths over 3000 m) resulted in 10 million particles released each year. Each particle was tracked for 6 months.

The resulting 500 million tracks were then used to assess connectivity. We have assessed connectivity at a reduced resolution of 10x10 gridsquares – an approximate resolution of 0.5°. This loses some of the fine detail but individual connections are based on larger numbers of tracks giving greater confidence and the problem becomes computationally tractable. For each track a connection was recorded between the source gridsquare and all other squares along the track from the start to the chosen PLD. With little knowledge of ages of competence we have assumed particles can settle at any age from zero to the PLD. This method gives a connectivity matrix containing relative measures of connection strength between any two cells based on the fraction of tracks starting in one which pass through the other. It is not an absolute measure of connection strength or probability as there are many unknowns – local population density, fecundity, habitat availability, settling likelihood, mortality with age, and others. As most unknowns would act to reduce the likelihood of any connection, the results presented here probably represent an upper bound to the range of dispersal.

Theoretical work of Hastings and Botsford (2006), Kininmonth (2014), Fox et al. (2019) predict that for population persistence it is important that networks of sub-populations are well connected – with either strong larval retention within a site or multigenerational pathway back to the original source. We can examine connectedness using similar ideas from network theory of strongly-connected subnetworks. In a strongly connected subnetwork, any sub-population is connected to all others (in multiple steps). For each species considered we break the overall network down into the list of strongly-connected sub-networks. The larger, and fewer, these subnetworks, the better connected and more resilient the network should be.

To produce connectivity matrices for individual species we need to try to reduce some of the unknowns mentioned above. Where we have some information on PLD and drifting depth we can use these to select from the above generic connectivities – for example *L. pertusa* larvae are predicted to drift in the surface waters for up to 60-days.

Selected species From a list of 17 potential species discussed with a group of experts on modelling, larvae biology and population genetics during the ATLAS "Deep sea particle dispersal and connectivity" workshop that took place on 21-22 January 2019 at University of Edinburgh (see minutes of the workshop in ATLAS partners area), the reef-building species *L. pertusa*, the octocoral *V. flagellum*, the fish *H. dactylopterus* and the sea-urchin *Cidaris cidaris* were selected for the modelling exercises (see Table 1). Species used in the models were selected based on the availability of data on reproduction, larvae characteristics and habitat suitability models. Modelling of dispersal of *C. cidaris* was not possible due to difficulties of modelling its distribution based on the limited presence records of this species on public databases and poor constraint in the population genetics of *C. cidaris* populations in the North Atlantic and Mediterranean (see section 6.3.2).

Species occurrence data sources Georeferenced presence-only records were obtained for all species from institutional databases of partners as well as from public databases such as the Ocean Biogeographic Information System portal (OBIS), the NOAA Deep Sea Coral Data Portal , and the ICES Vulnerable Marine Ecosystems (VMEs) data portal (see DL 3.3 for details). In order to reduce potential errors in the spatial position of the records, the depth associated to the OBIS and NOAA's presence records were compared with the depth value extracted from the depth raster layer. Data points with no depth information or with differences in depth values greater than 30% and more than 50 m in absolute depth were excluded. In the case of the ICES VMEs database, those records with a position accuracy lower than 5000 m were excluded. Data directly provide from the partners were considered accurate.

Habitat suitability modelling Habitat suitability modelling was used to extrapolate from observed species presence to basin-scale habitat maps. We used an ensemble modelling approach to predict the habitat suitability under present-day (1951-2000) conditions and forecasted changes under future (2081-2100) projected climate conditions. We employed three widely used modelling methods (González-Irusta et al., 2015) capable of dealing with presence-only data using pseudo-absences: the maximum entropy model (Maxent, Phillips et al., 2006; 2017), Generalized Additive Models (GAMs, Hastie and Tibshirani, 1990) and the Random Forest machine learning algorithms (Breiman, 2001). Detailed description of the models used in this study is included in DL3.3 Chapter 3. A set of terrain and environmental variables were used in this study as candidate predictors of present-day and future conditions distribution. All predictor variables were rescaled to a final grid cell resolution of approximately 3x3 km comprising about 3.8 million cells. Terrain variable depth was extracted from a bathymetry grid built from two data sources: the EMODnet Digital Bathymetry portal (EMODnet, 2016) and the General Bathymetric Chart of the Oceans (GEBCO 2014; Weatherall et al., 2015). The original resolution from EMODnet (0.002°) was up-scaled to the GEBCO resolution (0.008°) using a bilinear interpolation. The bathymetry layers were merged by using EMODnet data when available and completing it with GEBCO 2014, and rescaled to the final resolution of 3x3 km using bilinear interpolation. The importance of each predictor variable to the ensemble habitat suitability model predictions was estimated as the average of the variable importance in the individual models weighted by the models evaluation metrics. Environmental variables of present-day conditions, including particulate organic carbon (POC) flux at 100-m depth (epc100, mg C·m-2·d-1), dissolved oxygen concentration at seafloor (mL·L-1), pH at seafloor, and potential temperature at seafloor (°C) were downloaded from the Earth System Grid Federation (ESGF) Peer-to-Peer (P2P) enterprise system5. See details in DL3.3 Chapter 3.

The habitat suitability models produce maps of suitability index (HSI) in the range [0,1]. We use this index (above a threshold of 0.1) as a proxy for both the number of larvae released from a site (combined population density and fecundity) and the probability of larvae settling. So for each grid-square we multiply all outward connection strengths by the local HSI to scale for population density and fecundity, we then multiply all inward connection strengths by the HSI to scale for settling probabilities. The major remaining unknown which we do not address here is age-related mortality.

4.3 Modelling exercise for generic connectivity matrices

The major outputs of this work are large 2-d connectivity matrices, listing directional connection strengths between all pairs of grid-squares. These matrices contain large amounts of information

and are extremely difficult to visualise, so here we give a few selected results. The full matrices will be made available via Zenodo.

Figure 18 shows maps of unweighted out-degree for 12 generic connectivity matrices: surface, mid-water column and near-bed releases; each with PLD of 20, 60, 120 and 180 days (3 release depths x 4 PLDs = 12 cases). Unweighted out-degree – counts of the number of sites linked to from each source – is a good guide to which areas might be important sources for wider larval distribution and colonisation of new areas. These plots clearly show the increased dispersal potential of increasing PLDs and drifting closer to the surface. They also highlight the regions of stronger currents as important sources of larvae, particularly currents along the continental slopes. The western subtropical basin and the Gulf Stream do not show up as major sources because, while currents are strong, suitable downstream habitat (depths 200-2000 m) is lacking. Maps of in-degree, the number of particles arriving in a site (not shown) display similar characteristics.

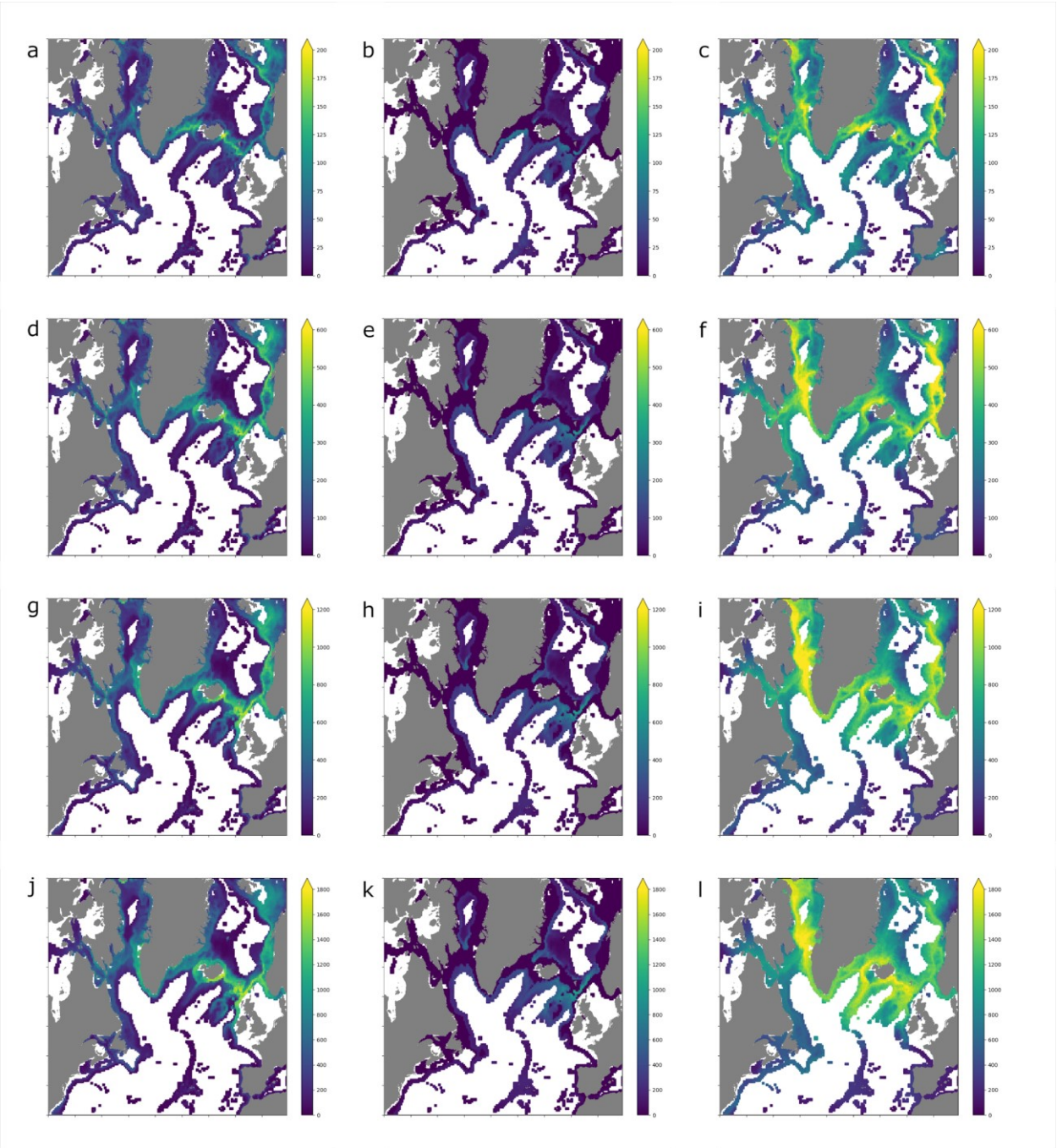


Figure 18: Modelled 50-year connectivity network out-degree (the number of other grid-squares linked to). Only connections to and from grid-squares with depth 200-2000 m are included. a-c: 20 day PLD (Pelagic Larval Duration); d-f: 60-day PLD; g-i: 120-day PLD; j-l: 180-day PLD. Notice the change in colour ranges with increasing PLD, network out-degree increases approximately linearly with PLD. Left column (a,d,g,j): bed releases; centre column (b,e,h,k): releases at 1000 m; right column (c,f,l,l): surface releases. Particles released at the surface disperse more widely than those released at depth for any given PLD.

4.4 Modelling exercises for selected species

4.4.1 ATLAS Case Study 1: Scleractinian CWC *Lophelia pertusa*

Connectivity matrices for *L. pertusa* were obtained from the general connectivity matrix for 60-day PLD and surface drifting as this most closely represents the known characteristics of *L. pertusa* larval behaviour (Larsson et al. 2014). These basin-wide connectivity values were then multiplied by the habitat suitability index (HSI) for both larval outflow and inflow as described above. Locations with values of HSI less than 0.1 were considered unsuitable. Figure 19 shows maps of present day HSI, predicted connectivity in-degree and out-degree. Connectivity maps are dominated by HSI rather than the underlying connectivity (compare with figure 18f, 60-day surface connectivity). There are subtle differences in major source and sink regions (Figures 19c,d), particularly around the Greenland-Shetland Ridge, but as a first approximation more important sources and sinks coincide with the major determining factor being predicted habitat quality.

In Figure 20 we explore possible recolonization routes for northern populations from southern refugia. The smallest number of steps (c.f. generations) to get from the source region to any other region is calculated for three possible source regions: Azores, near Gibraltar, and US Mid-Atlantic coast. All three represent regions of potential *L. pertusa* refugia in the last ice-age when more northern populations were lost (Boavida et al 2019a and references therein, section 6.3.1). Recolonisation from sources near the Mediterranean appears slowest of these three, with no direct route to the Azores and only gradual spreading northwards along the shelf slope. Ultimately everywhere is reached, with the Azores and the western Atlantic reached via routes around Rockall bank and the Mid-Atlantic ridge and then colonised from the north. Conversely, larvae from the Azores are predicted to reach the Iberian and US coasts directly (or with single stepping-stones), and spread north along the mid-Atlantic ridge before crossing to the Rockall Bank, and spreading north and east. From US coastal refugia, particles are predicted to be able to reach the mid-Atlantic ridge and the Azores, travelling in the strong eastward currents, from there colonisation of NE Atlantic is relatively rapid, with the last area reached being the Bay of Biscay. These results might suggest colonisation of NE Atlantic by *L. pertusa* after the last ice-age is more likely to have been from refugia in the west Atlantic or the Azores than the Mediterranean.

These results are highly dependent on the larval characteristics simulated in the model. Slightly longer PLD, or deeper drifting depths, could complete the link from Mediterranean outflow to the Azores, altering the predicted spreading rates. The connectivity matrices presented here are based on the full 50 years of modelled connectivity, so recorded links might only appear once in

the 50 years or be present every year. We have not attempted to calculate the most likely number of generations for *L. pertusa* to spread northwards, it could be via more, shorter, steps with stronger links. The results here represent maximum spreading rates, and routes which are not observed here – such as Mediterranean-Azores – were not recorded at any time in the 50 years. Searching for strongly-connected subnetworks we find the calculated connectivity predicts the whole basin to form a single strongly-connected network. In theory this implies that, given enough time, the whole network could be regenerated from a remnant population in any single grid-square. This may not represent the true picture as the habitat suitability modelling seems to overpredict habitat suitability in some areas when compared with local models (see details in DL3.3 Chapter 3). However, these differences are mainly located in some specific areas and only relevant at lower scales so its effect on the connectivity at basin scale should be minor.

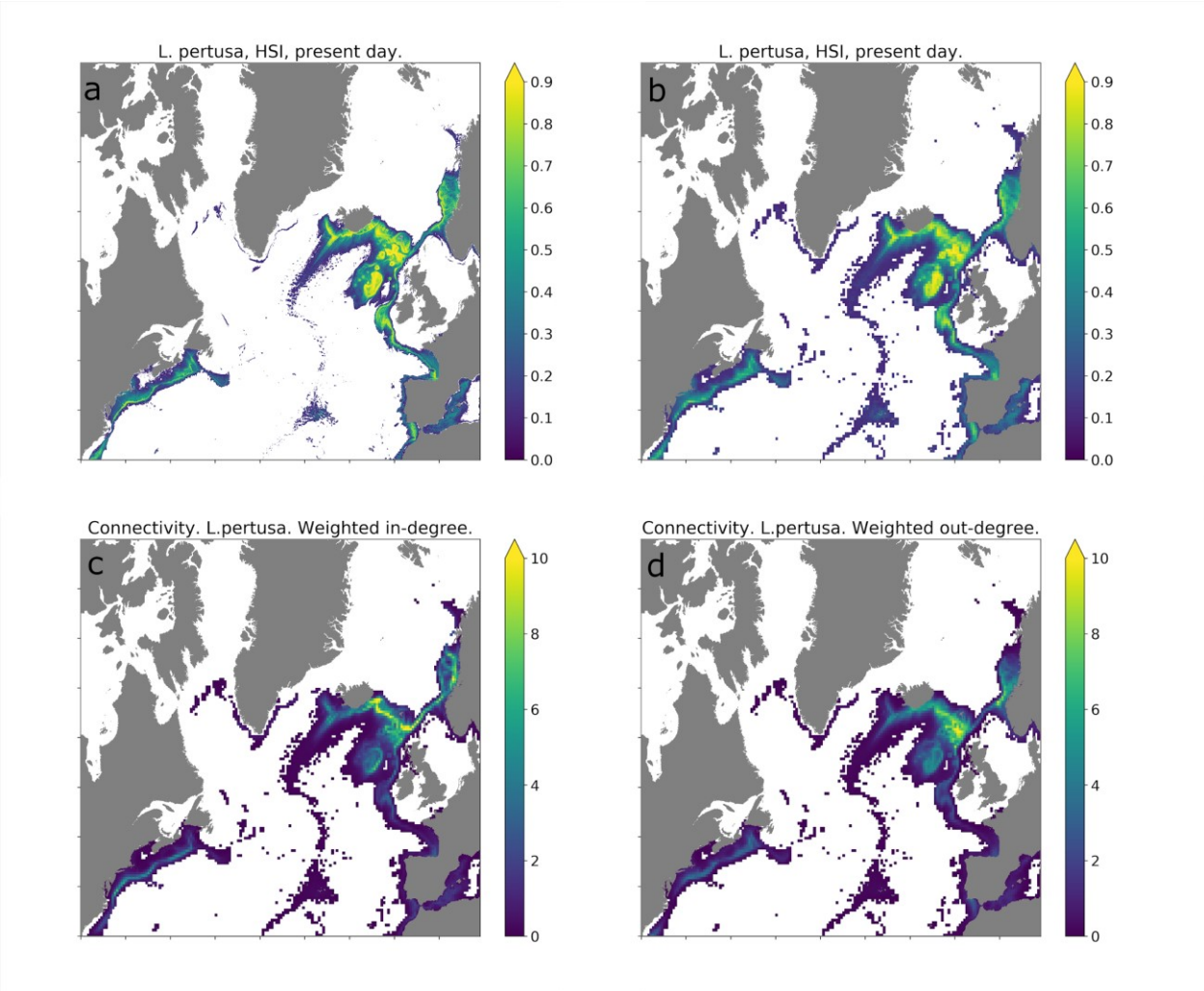


Figure 19: Lophelia pertusa *connectivity. a) Present day predicted habitat suitability index on the model grid. b) low resolution HSI used in connectivity calculations. c) Weighted in-degree, this is a measure of the relative numbers of larvae arriving and settling in each square d) Weighted out-degree, this is a measure of the number of larvae leaving each gridsquare which subsequently settle in suitable habitat elsewhere. Units on c) and d) are arbitrary.*

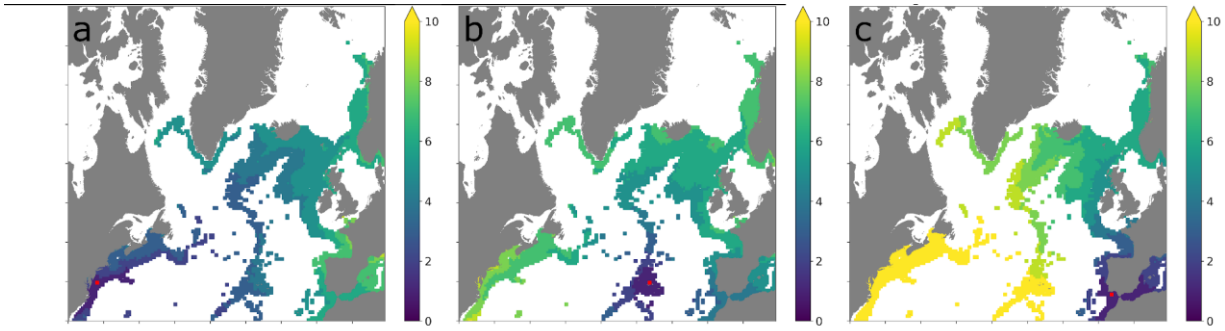


Figure 20: A measure of the smallest number of steps (generations) to link from the source gridsquare (marked in red) to all other gridsquares. This gives an idea of possible colonisation routes. Note we see no direct routes from the Mediterranean outflow to the Azores (middle panel).

4.4.2 Case study 2: Octocoral *Viminella flagellum*

Information on both the distribution and larval behaviour for the octocoral *V. flagellum* is much sparser than for *L. pertusa* so the results presented here have correspondingly lower confidence associated. Laboratory observations of *V. flagellum* larvae suggest they return to the bed after only 12 days (see section 3.3.1). Once at the seabed larval further drift can be considered insignificant. During the 12 days however, rapid vertical movement was observed. With this minimal information we decided to model *V. flagellum* dispersal and connectivity using a 20-day PLD and drifting at all levels in the water column described in the Methods section, above (surface, near-bed and mid-depth). Figure 21 shows the HSI and network in- and out-degree for *V. flagellum.* Note first that both HSI and network degree values are significantly lower than for *L. pertusa.* This reflects the lower confidence in the distribution, calculated from fewer observations, and the weaker predicted connectivity associated with shorter PLD. *Viminella flagellum* HSI fields appear to have more small-scale structure than those for *L. pertusa* above. This could indicate a shorter dispersal distances, but more likely is a function of the sparsity of data used in the HSI calculation. Once again network connectivity, as depicted by in- and out-degree, strongly reflects the HSI structure. But with reduced larval dispersal there is low connectivity predicted for remote seamounts and the mid-Atlantic ridge compared to the higher predicted connectivity for more continuous habitat in shelf slope regions. It should be noted that HSM for *V. flagellum* predicts a suitable habitat in the Northeast Atlantic and US coast but the present know distribution of *V. flagellum* includes only the Mediterranean and Eastern Atlantic (Azores Islands, Cape Verde Islands, Canaries Islands, Madeira, Josephine Bank, Great Meteor Bank and Moroccan coast). These modelling results mean that there is suitable habitat for *V. flagellum* in these areas but the species is not present there, potentially because of the short PDL for this species.

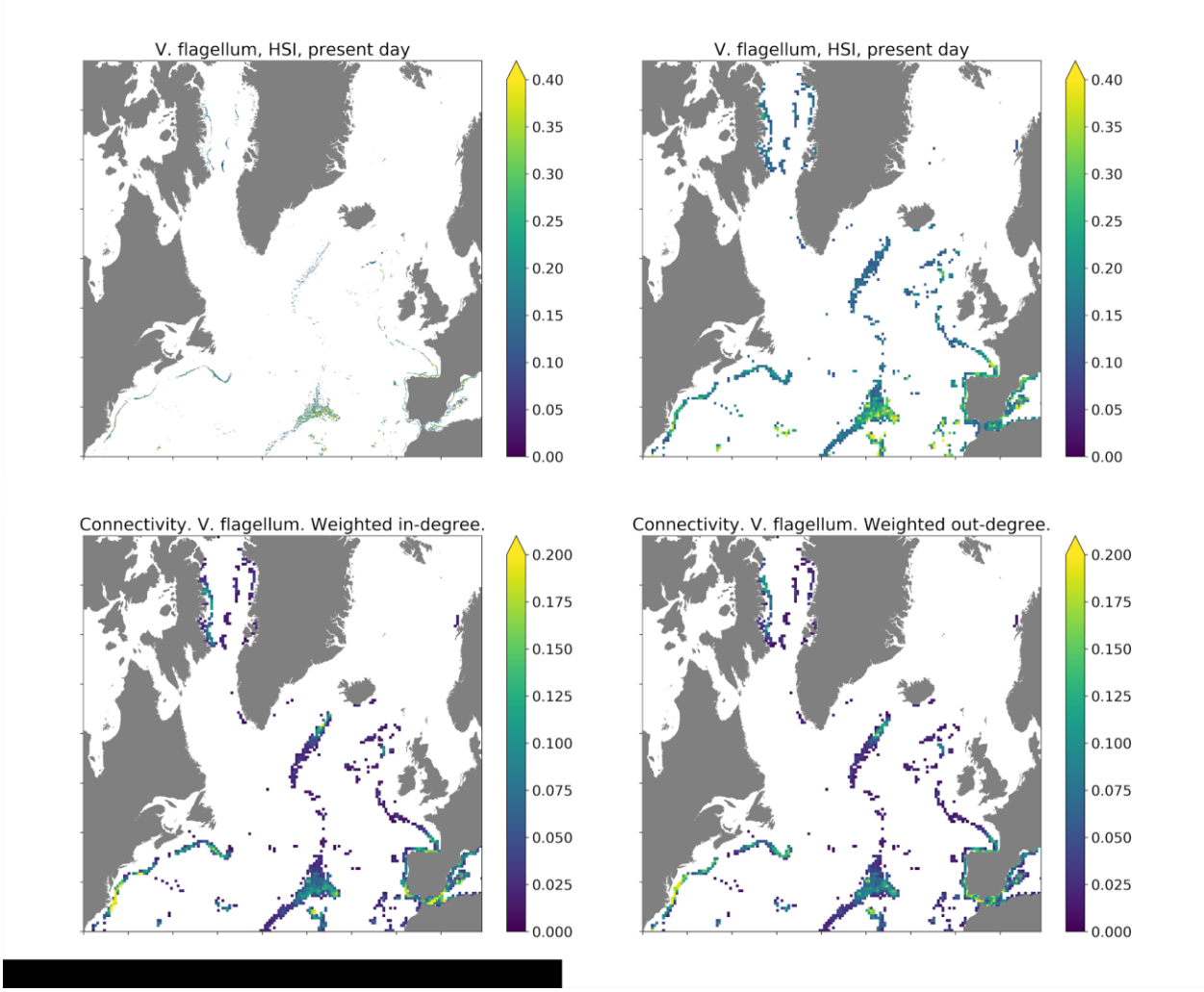


Figure 21: Viminella flagellum *connectivity. a) Present day predicted habitat suitability index on the model grid. b) low resolution HSI used in connectivity calculations. c) Weighted in-degree, this is a measure of the relative numbers of larvae arriving and settling in each square d) Weighted out-degree, this is a measure of the number of larvae leaving each gridsquare which subsequently settle in suitable habitat elsewhere. Units on c) and d) are arbitrary.*

In Figure 22 we again explore the network connectivity by testing re-colonisation scenarios from refugia near the Mediterranean, Azores and US coast. The contrast with *L. pertusa* is stark, with much slower possible spreading and no single refugia site source able to fully recolonise the basin. This is supported by identification of strongly-connected sub-networks, Figure 23. With larvae with relatively short lifespans, the predicted strongly connected components are typically separated by regions of abyssal depths. On the US shelf slope, separate components exist without large physical separation. It is important to note that belonging to the same strongly connected component does not necessarily imply little genetic differentiation, although as mentioned earlier, *V. flagellum* is not recorded at present in this area.

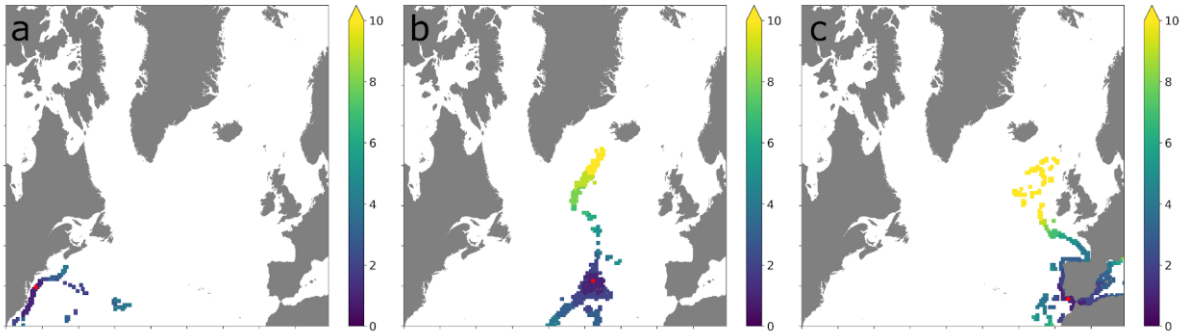


Figure 22: The smallest number of steps (generations) to link from the source gridsquare (marked in red) to other occupied gridsquares. This gives an idea of possible colonisation routes. Note we see no overlap in the regions which can be reached from each source, showing they each belong to separate subnetworks.

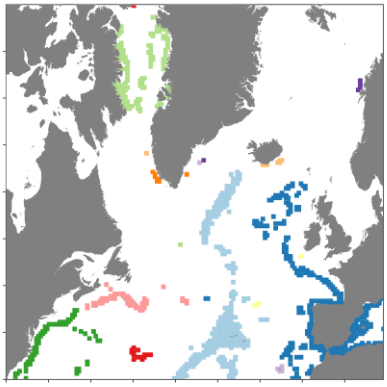


Figure 23: Strongly-connected components of the predicted network of V. flagellum habitat. Different colours represent different components. Separate components can be linked BUT ONLY IN ONE DIRECTION.

4.4.3 Case study 3: Fish *Helicolenus dactylopterus*

Using particle tracking methods to predict the connectivity of fish habitat is fraught with difficulties, adult fish are often highly mobile and fish larvae and juveniles may have greater swimming abilities than the simple coral larvae modelled above. From the range of fish species of interest in ATLAS (see appendix 1), we have chosen to model connectivity of *H. dactylopterus,* primarily because of the highly sedentary adult phase (Aboim et al 2005), although the larval biology is very poorly known. Estimates of the position of larvae in the water column range around the upper 100 m or so, so are best modelled here with surface particle releases. *H. dactylopterus* larvae have been observed in the water column in various places and seasons (Sabatés 2004; Sequeira et al 2012), the shortest period over which larvae were observed to be present is about 2 months so we can take this as an upper bound on our PLD estimate.

To investigate the effect of this possibly constituting an overestimate of PLD we also calculate connectivities with a shorter 20-day PLD (Figures 24-26). The predicted distribution of *H. dactylopterus* (Figure 24a,b) is generally shallower than *L. pertusa* or *V. flagellum,* with suitable habitat predicted on shelf, particularly in the North Sea. *H. dactylopterus* displays a smaller suitable habitat than *L. pertusa* limited to areas south of the Flemish Cap, south of Iceland, the Azores, and around the British Isles. Reducing the modelled PLD from 60 to 20 days significantly reduces the modelled connection strengths (Figure 24, c-f) although the spatial patterns of major sources and sinks remains similar, with highest connectivity predicted in narrow bands close to the shelf break on the US and UK/Irish coasts. This peak connectivity is predicted in similar regions, but at shallower depths (< 300 m) than for *L. pertusa.*

Figure 25 again shows potential multi-generational connection routes northwards from the US coast, the Azores and near Gibraltar. For 60-day PLD *H. dactylopterus* larvae could again reach all predicted habitat areas, as for *L. pertusa* with a similar predicted PLD. However, the predicted shortest routes are different, primarily due to the sparse predicted extent of suitable habitat along the mid-Atlantic Ridge eliminating northward connections (though southward connections are retained). With the shorter estimated PLD the model predicts that the Azores population would be isolated, and no routes connecting west and east N Atlantic populations. Analysis of the strongly-connected components confirms this picture, almost the whole N Atlantic is predicted to form a single strongly-connected component if a 60-day PLD is assumed, while 20-day PLD results in more fragmentation with large connected components on the western and eastern boundaries of the basin but small, potentially isolated, populations on seamounts and the mid-Atlantic ridge (Figure 26).

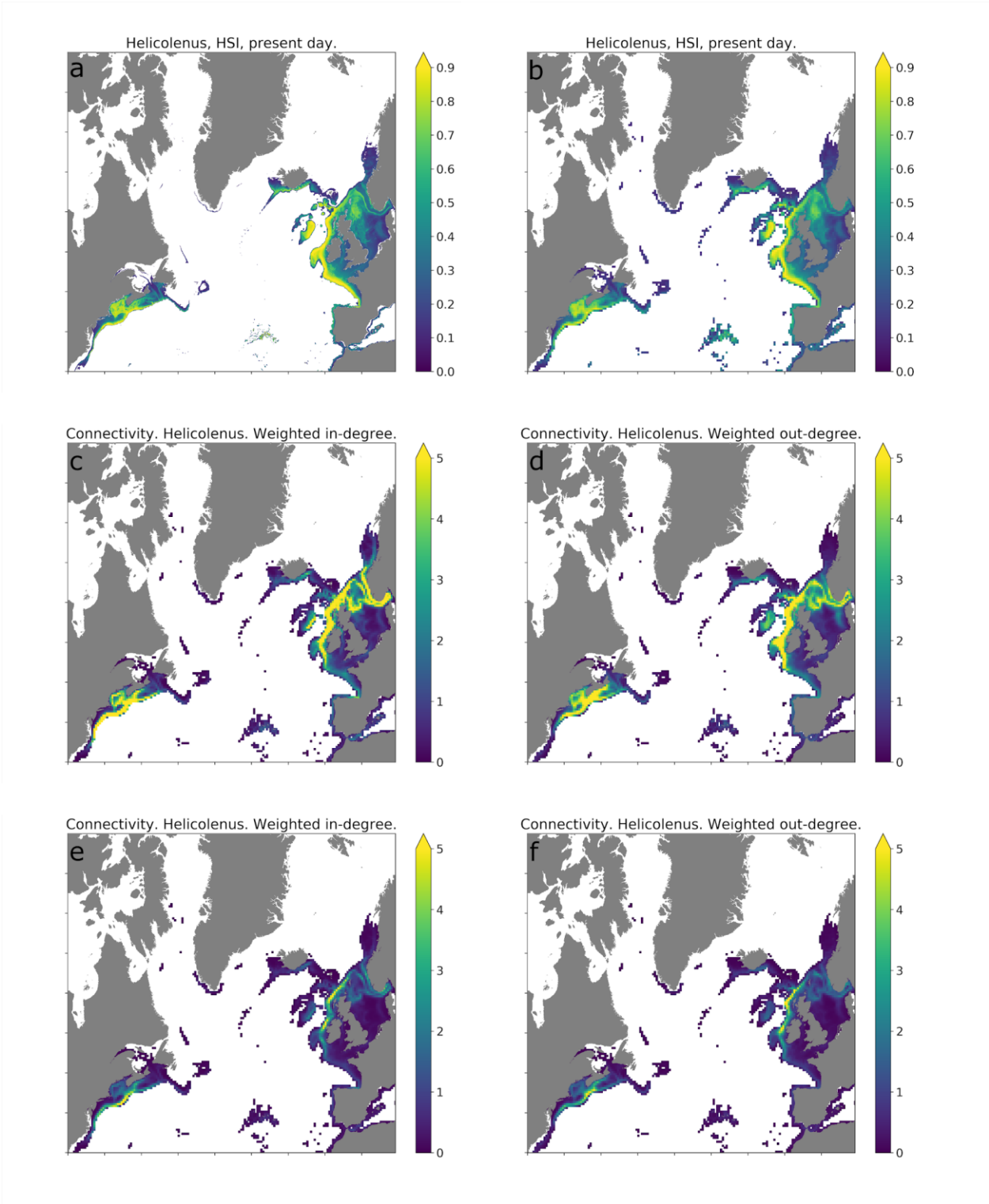


Figure 24: Helicolenus dactylopterus *modelled connectivity. a) Present day predicted habitat suitability index on the model grid. b) low resolution HSI used in connectivity calculations. c) Weighted in-degree for* ***60-day PLD****, d) Weighted out-degree for* ***60-day PLD****, e) Weighted in-degree for shorter* ***20-day PLD****, d) Weighted out-degree for shorter* ***60-day PLD****. Units on c) and d) are arbitrary.*

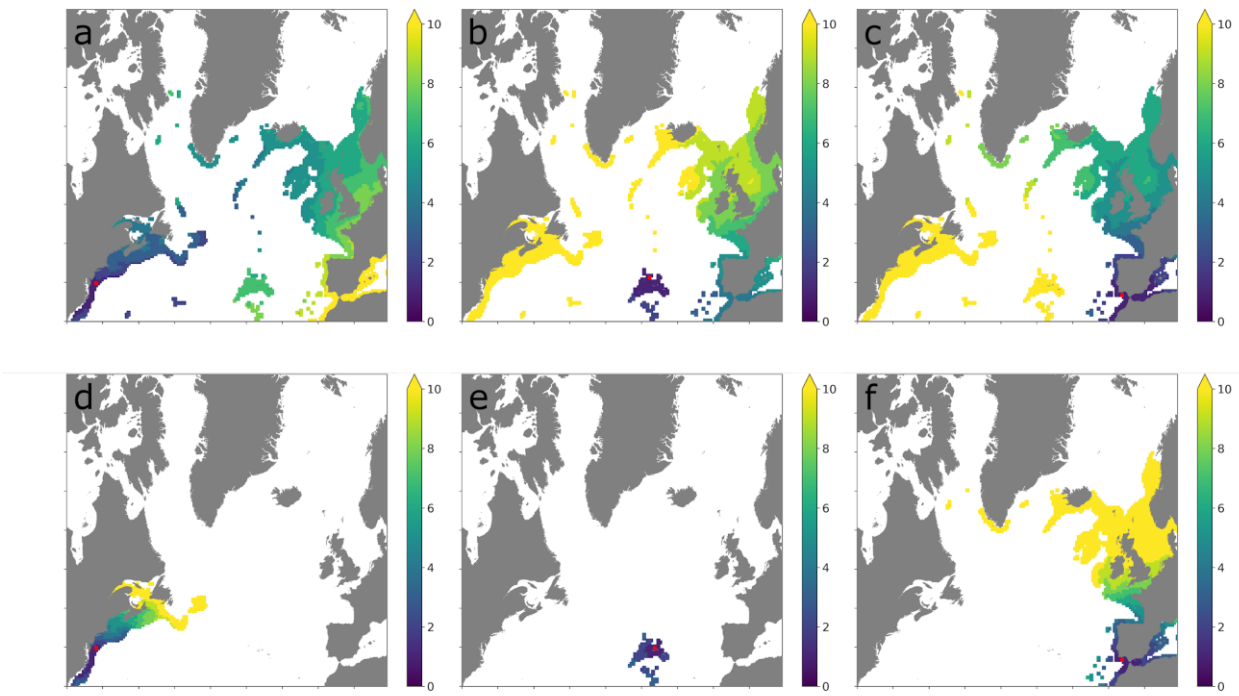


Figure 25: Helicolenus dactylopterus. *The smallest number of steps (generations) to link from the source gridsquare (marked in red) to other occupied gridsquares. This gives an idea of possible colonisation routes. Panels a – c, assuming a 60-day PLD. Panels d – f, assuming a 20-day PLD.*

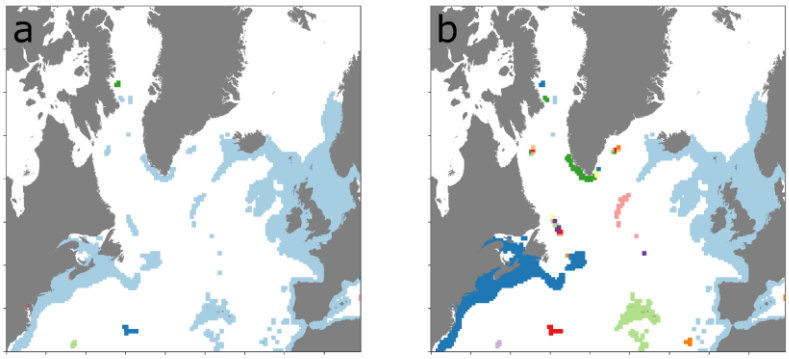


Figure 26: Helicolenus dactylopterus. *The strongly-connected components for, (a) 60-day PLD and (b) 20-day PLD. The contrast in predicted connectivity due to unknown of larval behaviour can be clearly seen.*

4.5 Summary of individual species experiments

We have only been able to show a small subset of the possible large array of connectivity metrics here. The raw matrices are very difficult to visualise. This data will be made available on Zenodo for further analysis. The results shown demonstrate how hydrodynamically-based modelled connectivity can be combined with species-specific habitat suitability models to suggest

connectivity by species. We show how this predicted connectivity varies considerably with the assumptions we have to make about larval behaviour. One common feature which seems to be emerging is that populations along the eastern boundary of the North Atlantic may be generally strongly connected. However, as currents are generally weak here (compared to those on the western and northern boundaries of the basin) this conclusion is probably dependent on the existence of a near-continuous band of suitable habitat from the mouth of the Mediterranean northwards. The basin-scale HSMs though are only predicting areas where is possible to find *H. dactylopterus*. Spawning areas could be more limited in space and therefore not using substrate information, or information on fishing effort, which may break up this continuous band of predicted suitable habitat and could be not directly applicable to larval dispersal. It is very difficult to ascribe levels of uncertainty to these results. Connectivity predictions for *H. dactylopterus* showed the variability which could be associated with a factor of three in the PLD (20 to 60 days). For most species, including those modelled here, it would be optimistic to claim that we know the PLD to within a factor of three. The vertical behaviour is even less well understood. We can possibly conclude that for widespread species, with larvae drifting higher in the water column, a 60-day PLD is sufficient to keep all populations connected (over multiple steps) under current flow conditions and would potentially allow recolonisation of the whole basin from single refugia anywhere in the basin. But for species with shorter PLD – less than about three weeks with drifting throughout the water column – connectivity appears much weaker with potentially isolated populations.

5 Predicting connectivity under present and future scenarios (UEDIN, IMAR-UAZ, IFREMER)

5.1 Connectivity models under future scenarios were built for two broadly distributed species in the North Atlantic, the reef-building *L. pertusa* and the fish *Helicolenus dactylopterus*. Models were not built for the octocoral *V. flagellum* because of the restricted geographical distribution and limited number of presence records in public databases (i.e. there was a bias towards presence records in the Azores and the Mediterranean) which made projections for future scenarios not reliable. The reef building coral *Lophelia pertusa*

Habitat suitability modelling has been performed for *L. pertusa* (DL 3.3, Chapter 4) under projected future conditions of increasing temperature, decreased pH, aragonite saturation state

and changes in near-particulate organic carbon (see methods description, Figure 27 a,b). This predicts reducing habitat suitability in most areas along with an extension of the range into the Labrador Sea. We have calculated the network connectivity (Figure 27 c,d) from these future habitat suitability maps using results from particle dispersal experiments using current (1959-2008) model flows. While ocean currents will probably also change under future conditions, the magnitude and direction of those changes is highly uncertain and dispersal modelling has shown that such effects are small compared to the uncertainty in our connectivity models (ATLAS deliverable 1.6). Under these changed conditions the suggest present day regions of high connectivity - important sources and sinks of larvae – have much reduced connectivity, with the best connectivity future sites now found along the coast of Greenland and Canada. However these regions are dominated by connectivity in the anticlockwise direction.

While the idea of southern refugia is probably not relevant for immediate future scenarios, the possible colonization routes from the south area is interesting (Figure 28). These show that the reduced population in the Azores, previously supplying larvae to the US and European coasts via intermediate seamounts, is now connected only along the mid-Atlantic ridge, with some larvae still coming in from the US coast. Location of strongly-connected sub-networks shows that even though connectivity is weaker, the whole basin may still constitute a single strongly-connected network (with the exception of 10 out of 4530 gridcells which may be isolated).

For *L. pertusa,* these results suggest that the dominant factors in future distribution are likely to be the changes in local conditions, particularly temperature and acidity. With relatively long PLD and corresponding wide dispersal, *L. pertusa* larvae are likely to continue to reach most of the N. Atlantic basin.

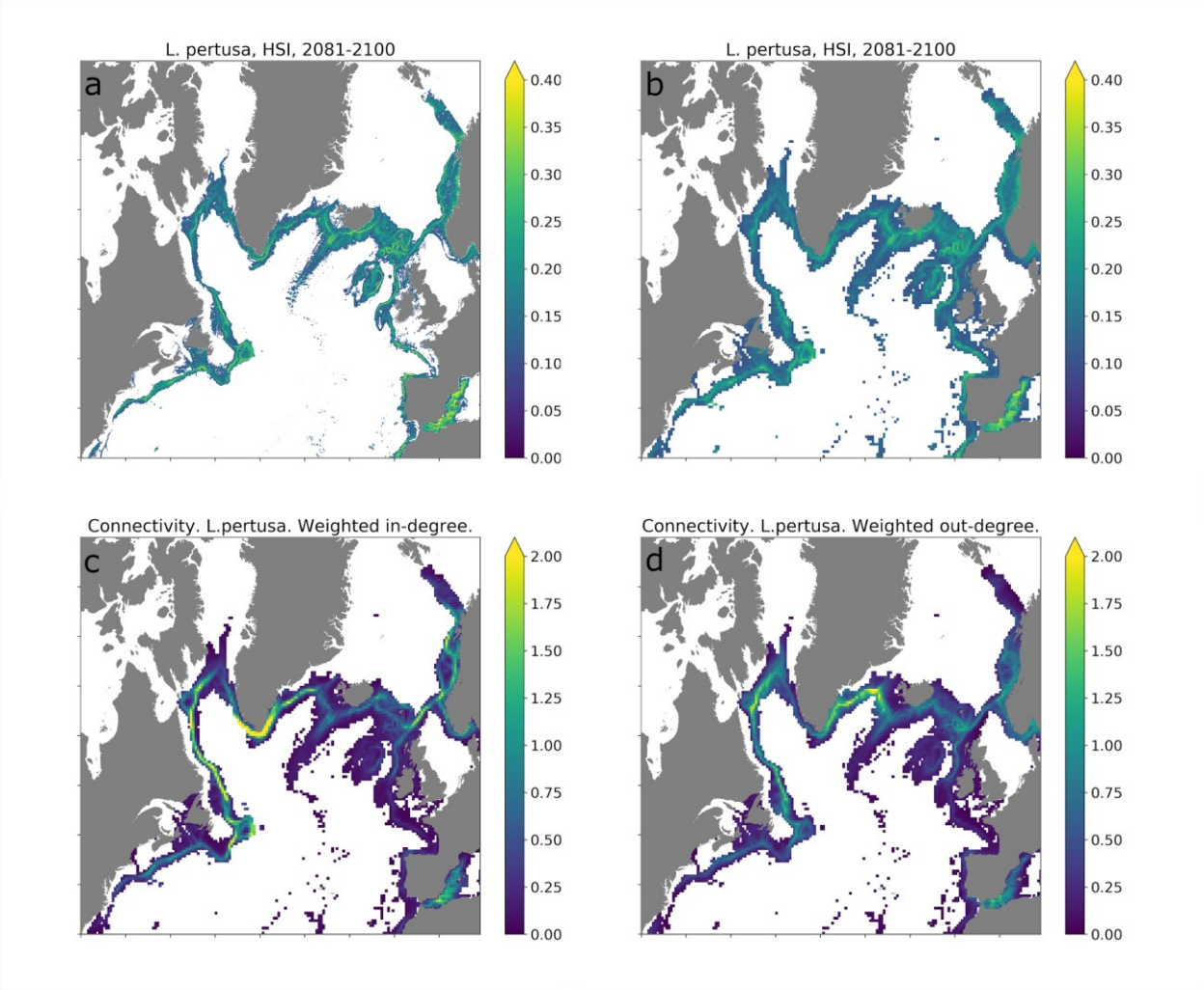


Figure 27: Projected future (2081-2100) Lophelia pertusa *connectivity. a) Present day predicted habitat suitability index on the model grid. b) low resolution HSI used in connectivity calculations. c) Weighted in-degree, this is a measure of the relative numbers of larvae arriving and settling in each square d) Weighted out-degree, this is a measure of the number of larvae leaving each gridsquare which subsequently settle in suitable habitat elsewhere. Units on c) and d) are arbitrary. Compared with Figure 2 notice the lower habitat suitability indices and connectivity values. Also notice the major shift northwards and westwards in habitat suitability distribution.*

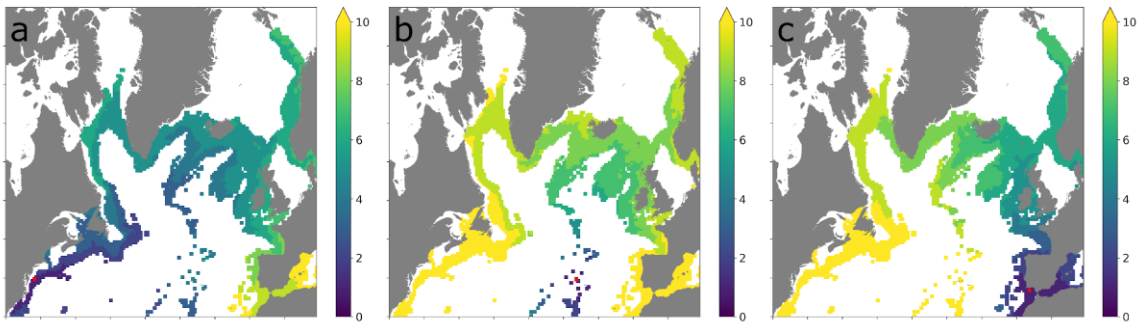


Figure 28: The smallest number of steps (generations) to link from the source gridsquare (marked in red) to other occupied gridsquares. This gives an idea of possible colonisation routes. Compare with Figure 3 for current connectivity. The predicted lower habitat suitability on the south, coupled with corresponding reduction in connectivity increases the number of generations theoretically required to recolonise from the south.

5.2 Deep-sea fish *Helicolenus dactylopterus*

Predictions of future distribution and connectivity for *H. dactylopterus* provide an interesting contrast to those for *L. pertusa.* Again the suitable habitat range is predicted to expand to the north and west -- particularly into the Labrador Sea (Figure 29a,b), but for *H. dactylopterus* there is less reduction in suitability of habitat in the rest of the basin – some reduction of habitat suitability is seen along the US Atlantic coast. Comparing resulting connectivities (Figure 30c-f against Figure 31c-f) shows some corresponding predicted reduction in connectivity along the US Atlantic coast, but also increased connectivity in the North Sea and along the coast of Norway together with expansion into the Labrador Sea. Looking at the spreading pathways (Figures 31 vs. Figure 26), the expansion of suitable habitat northwards and westwards makes little difference to the pathways and shortest connections for the 60-day PLD larvae. These larvae disperse widely enough under present conditions to cross the gaps between suitable habitat. But for shorter PLD (20-days), the additional suitable habitat regions predicted for 2081-2100 open up pathways in both directions between the west and east of the N Atlantic basin. If 20-day larval PLD correctly represents *H. dactylopterus,* the Azores are predicted to be isolated for in both present and future conditions. Decomposing the population into the strongly connected components highlights these changes. The future conditions make little difference to the structure of the network for the test case with longer PLD, whereas for shorter PLD the main part of the network – excluding the Azores and a few Mid-Atlantic Ridge and Seamount sites – becomes a single strongly connected component under future conditions. This suggests that *H. dactylopterus* population may become more robust and resilient under changed future conditions.

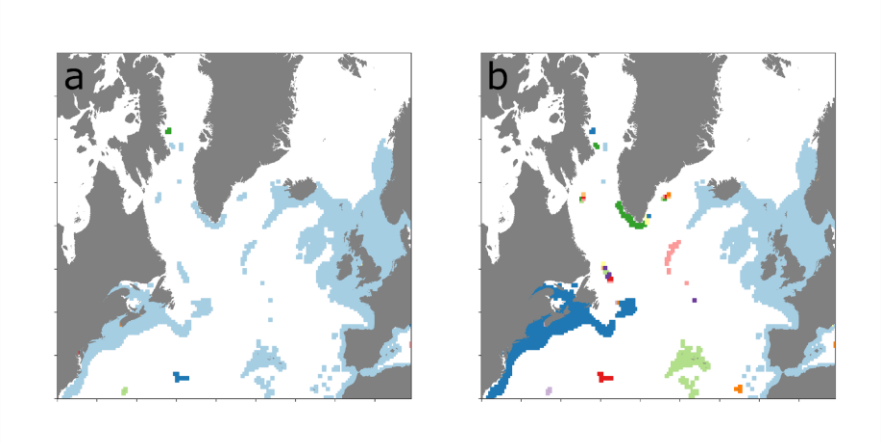


Figure 29: Helicolenus dactylopterus. *The strongly-connected components for, (a) 60-day PLD and (b) 20-day PLD. The contrast in predicted connectivity due to unknown of larval behaviour can be clearly seen.*

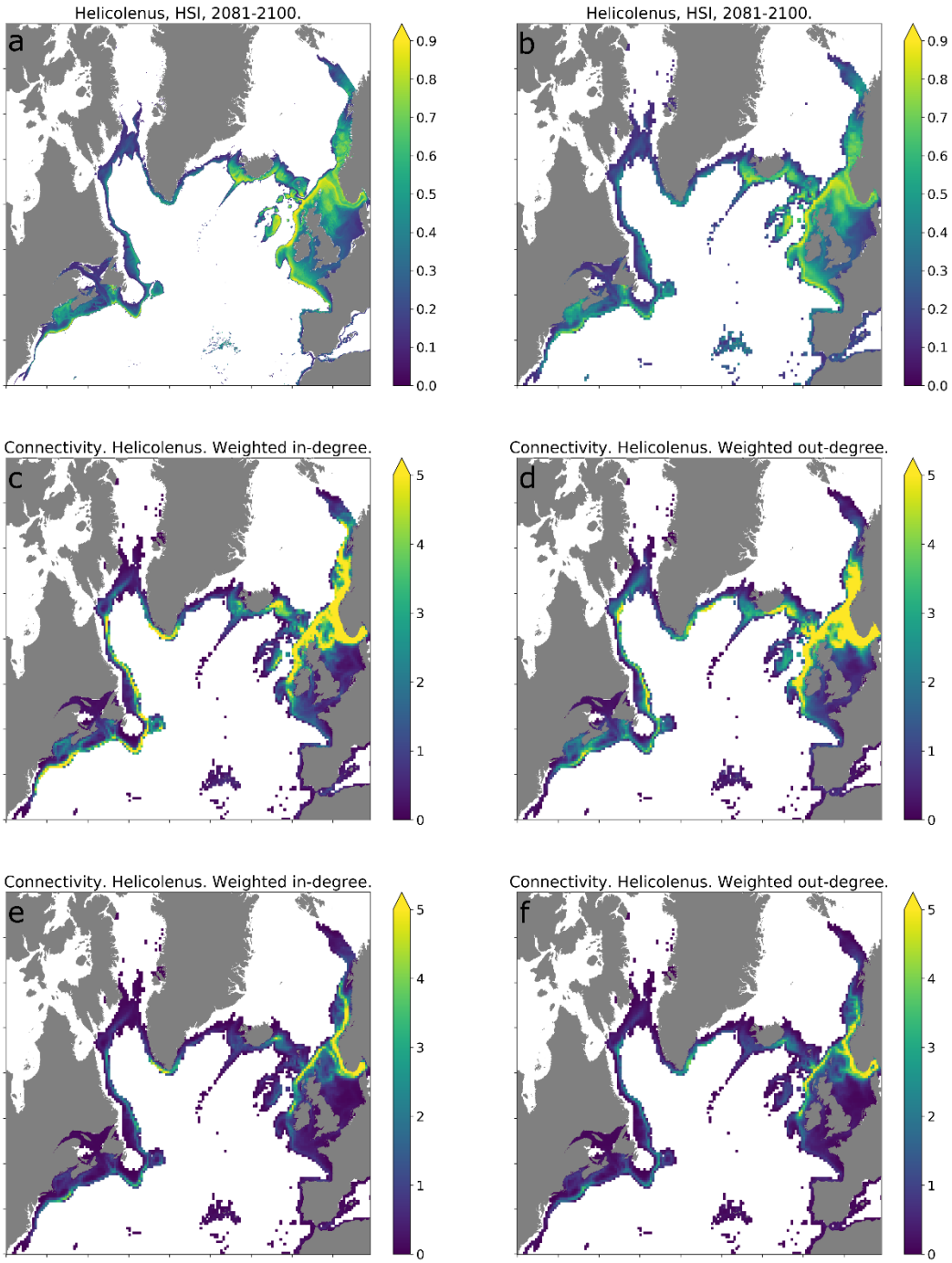


Figure 30: Helicolenus dactylopterus *modelled connectivity under predicted future conditions (2081-2100). a) Present day predicted habitat suitability index on the model grid. b) low resolution HSI used in connectivity calculations. c) Weighted in-degree for* ***60-day PLD****, d) Weighted out-degree for* ***60-day PLD****, e) Weighted in-degree for shorter* ***20-day PLD****, d) Weighted out-degree for shorter* ***60-day PLD****. Units on c) and d) are arbitrary.*

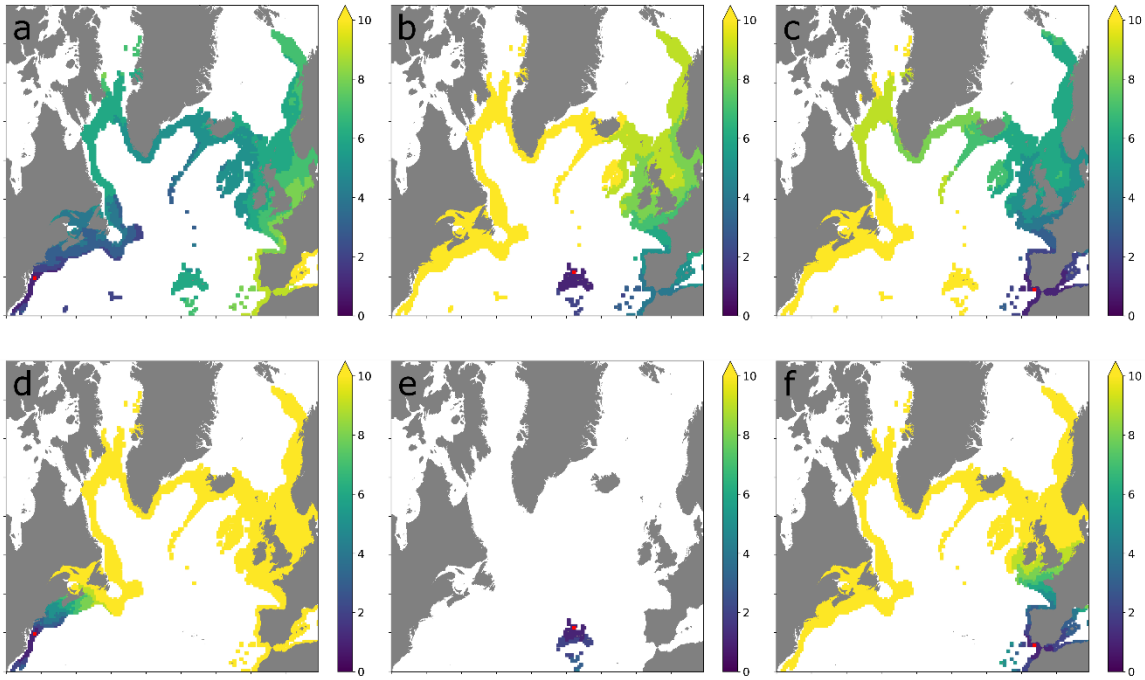


Figure 31: Helicolenus dactylopterus *connectivity under future conditions (2081-2100). The smallest number of steps (generations) to link from the source gridsquare (marked in red) to other occupied gridsquares. This gives an idea of possible colonisation routes. Panels a – c, assuming a 60-day PLD. Panels d – f, assuming a 20-day PLD. Colour scale has saturated but we keep the scale for comparison with other figures. In d and f links between US coast and Gibraltar take about 25 steps (generations).*

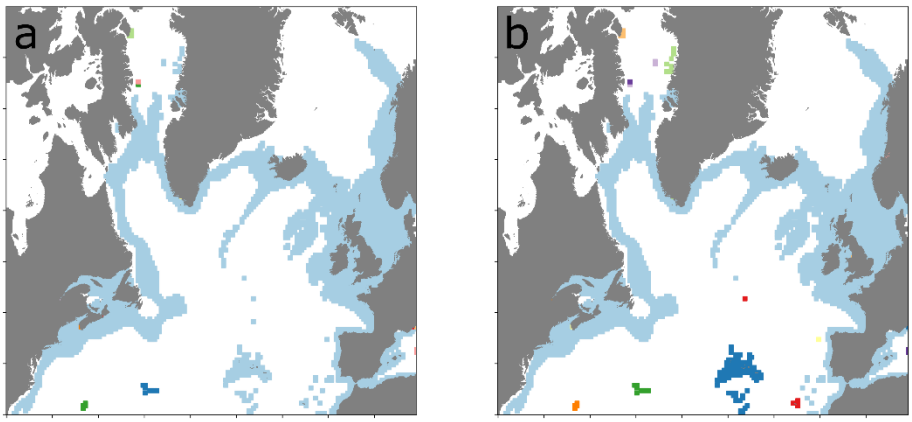


Figure 32: Helicolenus dactylopterus *under predicted future conditions (2081-2100). The strongly-connected components for, (a) 60-day PLD and (b) 20-day PLD. Comparison with figure 9 shows how the increased predicted geographical range of H. dactylopterus increases connectivity around the basin margins.*

5.3 Summary

These predictions of population connectivity under future conditions contain many sources of uncertainty – the habitat distribution and its use as a proxy for fecundity and settlement success,

PLD, larval behaviour – which we have not tried to quantify. Also we have not considered the effects of changes in ocean currents – the magnitude and direction of those changes is highly uncertain and dispersal modelling has shown that such effects are small compared to the uncertainty in our connectivity models (ATLAS deliverable 1.6) – or of unknown environmental influences on PLD. However, the results shown here demonstrate several interesting features. Firstly, that there are species for which future conditions probably imply reduced connectivity (and corresponding reduction in resilience) and others for which connectivity (and resilience) may increase. Secondly, looking at the results based on two separate, but equally valid, assumptions of PLD for *H. dactylopterus,* the changes in network connectivity over the next 80 years are within the range of current uncertainty. Finally, there are cases where changing conditions make little difference to connectivity strength (beyond movement of range) and others where tipping points may be crossed (e.g. in the predicted introduction of basin-scale connectivity for *H. dactylopterus* under the short PLD assumption). These results highlight the importance of focussing on improving knowledge of current (and past) connectivity in order to make robust predictions.

6 Genetic connectivity in deep-sea species (IFREMER, UCD, UOX)

6.1 Defining genetic connectivity

Most species show fragmented range distribution, with groups of individuals forming populations more or less connected among them by migratory flows. The distribution, importance and direction of these flows, a set of parameters described as 'connectivity', plays a major role in population and community dynamics both at the long and short term, by determining the demographic, ecological and evolutionary inter-dependency of populations and consequently the evolutionary trajectories and fate of species. Migratory movements allow the persistence of populations of limited size, temporarily demographically impacted (rescue effect, or source-sink systems), or genetically impoverished (limiting drift), and thus strongly influence their capacity to cope with spatially and temporally fluctuating environmental conditions. On the other hand, connectivity directly driven by human activities (i.e. biological introductions) is impacting the structure and functioning of natural communities, and promotes secondary contacts between previously allopatric lineages. From a population genetics point of view, migration is often the homogenizing antagonistic evolutionary force that opposes mutation, random drift and disruptive

selection in native ranges. While fragmentation is an important concern in conservation genetics of continental species (Lindenmayer and Fischer,2006), the problem has been neglected for too long in the marine realm, let alone in the deep-sea. Connectivity is thus a fundamental process, extremely hard to observe directly in the marine realm in particular, that needs to be understood and accounted for to improve conservation and move towards sustainable environmental services.

6.2 Using genetic methods to assess connectivity

Genetic methods can be used to infer "realized" connectivity. Depending on the markers and analysis used, both past and present day patterns of connectivity can be inferred (Gaggiotti, 2017). In ATLAS, the analysis of sequence divergence has been used to check the taxonomic status of species through barcode analysis (for example it was discovered that the polychaete *Eunice norvegica* associated to scleractinian likely encompasses two species, one of them undescribed; Boavida et al., in prep.), and reconstruct past phylogeography (Boavida et al, 2019; Gallagher et al., in prep.). More recent but still past connectivity assessments were performed on several invertebrates and fish species based on the analysis of allelic frequency either using microsatellites or taking advantage or recently produced genome scan data based on Next Generation Sequencing (most data obtained but almost all still being anayzed, see here below). These last, newly produced high density data will allow improving the assessment of present day connectivity based on Multi Locus Genotype analysis.

6.3 ATLAS case studies on population genetics

In this section, we present five case studies on population genetics of invertebrate species and fish, including the VME indicator reef-building coral species *Lophelia pertusa* and *Madepora oculata*, together with their commensal polychaete *Eunice norvegica* and an often associated invertebrate species to VMEs, the echinoid *Cidaris cidaris*. Studies were also conducted with 3 species exploited by fisheries, i.e. the fish species *Capros aper* and *Trachurus trachurus* and one crustacean (*N. norvegicus*).

6.3.1 ATLAS case study 1: Reef building cold-water coral species *Lophelia pertusa*, *Madrepora oculata* and their commensal polychaete *Eunice norvegica*

Introduction

For this DL we consider three basin-scale sampled species, pertinent to the North Atlantic region: the reef-building CWC *L. pertusa* and *M. oculata*, and their commensal polychaete *Eunice norvegica* (Figure 33), which has been shown to enhance reef calcification (Mueller et al., 2013).

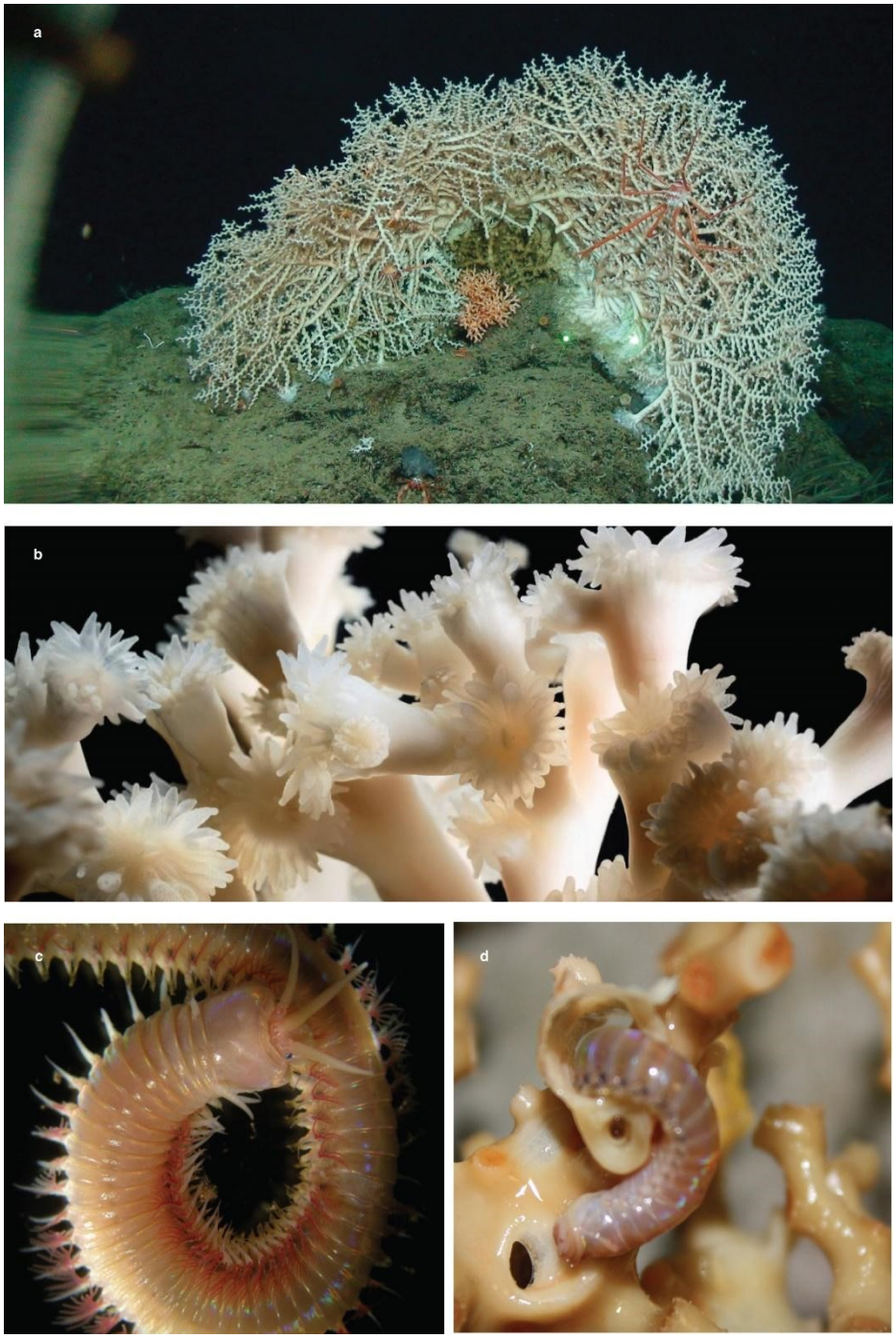


Figure 33: Reef building cold-water coral species and their commensal polychaete. a) Madrepora oculata *is one of the deepest reef building stony corals in the world, known to occur as deep as 2020 meters. Colonies are distinguished by the zig-zag appearance of their branches. This colony is home to several squat lobsters at 750 meters depth in Roatan, Honduras. The green laser points in the lower center are 10 cm apart. b) A living colony of* Lophelia pertusa. *c) Eunice norvegica photographed in the laboratory. d) Live* Eunice norvegica *moving between sections of a calcified tube in a L. pertusa colony, after collection by ROV. Copyright: a and d) NOAA; b) anonymous from the internet; c) Dr. S. Strömberg.*

Analyses of the spatial distribution of genetic diversity can be used to detect connectivity pathways across reefs and to define key areas for the conservation of biodiversity. Studying the

phylogeography (ie, the past biogeography) of CWC offers the chance to understand the responses of CWC to climatic changes that occurred in the recent past (thousands of years), which in turn allows making inferences and creating testable hypotheses about on-going and future climatic changes that may affect CWC.

The biogeographic connectivity between Mediterranean and NE Atlantic CWC during and after Pleistocene (c. 2.7 Ma to 12 ka) glaciations can partly be inferred from palaeo-records. While CWC have maintained a continuous presence in the Mediterranean Sea since at least the early Pleistocene (Vertino et al., 2014), in the NE Atlantic CWC presence may have been more affected by changes in climate, with a consequent local demise during the Last Glacial Maximum (LGM; Frank et al., 2011). Notably, the last cold oscillation, during the Younger Dryas (12.9–11.7 ka), represented a favourable period for CWC reef growth in the western Mediterranean Sea, as well as in adjacent Atlantic regions in the Gulf of Cadiz and African margins (McCulloch et al., 2010; Schröder- Ritzrau et al., 2005; Taviani et al., 2011). In contrast, growth episodes of CWC in the NE Atlantic have been restricted to warm climate stages and coral fossils are absent from strata corresponding to glacial episodes (Frank et al., 2009). Radiometric dating shows that NE Atlantic CWC are younger than those from the Mediterranean Sea, with ages estimated to be post-LGM (under 12,000 years; Freiwald and Roberts, 2005; Schröder-Ritzrau et al., 2005). Given the persistent occurrence of CWC, it has been argued that the Mediterranean basin may have acted as a CWC glacial refugium during range contractions in the North Atlantic Ocean during Pleistocene glaciations (De Mol et al., 2002; Henry et al., 2014). This putative refugium may have constituted the source for the Atlantic northward recolonization of CWC at the end of the LGM by larvae, which were transported with intense flows of Mediterranean Outflow Water (MOW) beginning in 50 ka (Stumpf et al., 2010; Voelker et al., 2006).

The CWC reef framework depends on the balance between growth and bio-erosion processes, of which an important mechanism is the association of coral species with eunicid polychaete worms. Among the most ubiquitous eunicid species in the North Atlantic is *Eunice norvegica* (Fig. 1a), found in high numbers in association with *L. pertusa* and *M. oculata* (12-17 inds m-2; (Buhl-Mortensen et al. 2006; Mortensen et al. 2001). *Eunice norvegica* reinforces the coral framework by thickening its branches and connecting them. The polychaete develops a tube through the coral, initially in a parchment-like material that is then calcified by the coral, strengthening it (Roberts 2005). Corals with *E. norvegica* can display up to four times more calcification than those without it (in aquaria; Mueller et al. 2013). The coral host acts as a shelter and possibly as a nutrition vector to *E. norvegica*, while remaining unaffected (eg, Mueller et al. 2013). Furthermore, the polychaete keeps the coral hosts clean by removing sediment from the surface

of coral polyps, decreasing the risk of infection and colonisation by sessile invertebrates (Mortensen and Fossa 2006).

Recent studies have pointed to physiological differences between *L. pertusa* and *M. oculata*, particularly in terms of optimal growth temperatures (Lartaud et al., 2014, 2017). *Lophelia pertusa* presents potential for high larval dispersal (Strömberg and Larsson, 2017), high fecundity (Waller and Tyler, 2005) and variable microbiome compositions (Meistertzheim et al., 2016), while the reproductive strategy of *M. oculata* is different, with much lower fecundity (Waller and Tyler, 2005) and a resilient microbiome (Meistertzheim et al., 2016; and unknown larval characteristics). The biology of *E. norvegica*, including mating system, dispersal or other major life-history traits, and genetic connectivity remain largely unknown (Boavida et al. in press; Sylvestre, 2017). Previous CWC studies have shown reduced genetic connectivity at an inter-basin scale for *L. pertusa*, along with moderate to high gene flow within regions (Dahl et al., 2012; Flot, et al., 2013; Morrison et al., 2011). Nevertheless, the extent of CWC connectivity along the European margins and the role that extant populations had in the past, such as during past climatic changes, remained to be tested until ATLAS' sampling and research efforts (Boavida et al., 2019b; Orejas et al., 2018).

We have been investigating the population genetic diversity and structure of the CWC *L. pertusa* and *M. oculata* and the coral-commensal polychaete *E. norvegica* along the deep margins of the NE Atlantic using three types of molecular markers, nuclear and mitochondrial data (microsatellites and gene sequences) and restriction-associated DNA high-throughput DNA high-throughput sequencing (RAD-seq) on thousands of samples. As this report is being produced, two new RAD-seq libraries for *L. pertusa*, one for *M. oculata* and one for *E. norvegica* have been received and are being processed with bioinformatic pipelines (*L. pertusa* 64 samples; *M. oculata* 95 samples; *E. norvegica* 80 samples). Some of the markers were specifically developed for these studies (all RAD-seq *de novo* loci and *E. norvegica* microsatellites). This work is partially published (Boavida et al., 2019a; Boavida et al. in press; Sylvestre, 2017) and partially being prepared for submission to international peer-reviewed journals.

Materials & Methods

Sampling Samples were collected, during several cruises along European margins (BobEco, IceCTD, Corsaro, ALTRO, MAGIC, MARCOS, MedCor, MS35, Belgica/BobGeo, M70-1, B6-2009, Arqdaço-27-P07, Medwaves), from 10 locations, corresponding to different reefs, canyons,

seamounts and decommissioned oil platforms in the Northeast Atlantic Ocean (North Sea, South Iceland, Southeast Rockall Bank off Ireland, Bay of Biscay, the Mid Atlantic archipelago of Azores and the Ormonde seamount on the Gorringe ridge) and the Mediterranean Sea (Alboran Sea, Bay of Lion, Malta and, Italy), using remotely operated vehicles and grabs. The North Sea oil platforms were sampled haphazardly during platform disassembly. Otherwise, sampling in the Atlantic was done using 200x100 m^2 quadrats within which random sampling of corals and associated polychaetes occurred (see Becheler et al., 2015 and Boavida et al., 2019a for details), whereas in the Mediterranean sampling was opportunistic. Once on board, live specimens were either extracted directly or stored in absolute alcohol. DNA extraction was performed using the CTAB method (Doyle and Doyle, 1988), except when stated otherwise, and frozen at −20 or −80°C prior to DNA extraction. *Lophelia pertusa* DNA was extracted aboard using the Fast DNA®SPIN for soil kit, according to the manufacturer's protocol (MP Biomedicals, France). Sampling permits for the Bay of Biscay, Rockall bank and Iceland were obtained for the entire cruise by the fleet manager (IFREMER; No 347/11; NV/ USEN/No 3278/2012); in the Mediterranean Sea, sampling was conducted in compliance with all relevant regulations for national and international waters; the Azores samples were collected as fishing by-catch without the need for permits.

The nuclear internal transcribed spacer (ITS) ribosomal sequence was amplified using primers developed by Diekmann, Bak, Stam, and Olsen (2001). ITS sequences (262 samples of *L. pertusa* 1,130 base pairs, bp, 200 samples of *M. oculata* 1,124 bp) were proofread and aligned using Geneious 6.1 (Kearse et al., 2012). Nine *L. pertusa* (Morrison et al. 2011; Becheler et al., 2017) and six *M. oculata* microsatellite markers were amplified following Becheler et al. (2017). Products were scored using Geneious 5.6.4. One *L. pertusa* locus was discarded due to the high frequency of null alleles (>30%). Clones were removed from each dataset (ITS and microsatellites).

Mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified for *E. norvegica* samples using the universal primers LCO1490 and HCO2198 described by Folmer et al. (1994). PCR amplifications were carried out in 50 µL volumes containing 50 ng DNA, 1× reaction buffer (GoTaq, Promega), 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 0.4 U Taq DNA polymerase (GoTaq, Promega, Madison, WI, USA) and 0.6 µM of each primer. The PCR amplification was conducted in a Perkin-Elmer Gene-Amp System 7200 (Waltham, MA, USA) with the following program: 2 min. at 95 ºC; 35 cycles composed of 1 min denaturation at 95 ºC, 1 min at 52 ºC and 1.5 min elongation at 72 ºC, followed by a final elongation step of 7 min at 72 ºC. PCR products were purified and sequenced at GATC Biotech AG (Germany). Sequences were proofread and aligned using Geneious. Eunice spp. COI sequences were retrieved from Genbank and aligned using Geneious.

To develop specific microsatellite markers for *Eunice norvegica*, whole genomic DNA was isolated using the CTAB method (Doyle and Doyle, 1988) from three individuals of E. norvegica from the Mediterranean Sea. To isolate the microsatellite sequences, a combination of an SSR-enrichment protocol with 454 pyrosequencing was performed (Ecogenics GmbH, Zürich, Switzerland). One CT/GT enriched library was generated, the insert size of the libraries was 500-800 bp and the average read length from the 454 sequencer was 137 bp. A total of 29,684 sequences were obtained, of these 1,064 had microsatellite repeats. A total of 122 primer pairs were designed using primer3 core (Untergrasser et al. 2012), from which 48 primer pairs were delivered, after an amplification test on three individuals. We tested all primers for polymorphism using seven individuals. An M13-tail (TGTAAAACGACGGCCAGT) was added at the 5' end of all forward primers to enable fluorescent-dye labelling (Schuelke 2000). Of all the primer pairs tested, we were able to optimize 6 polymorphic markers. Amplification reactions for the six microsatellite markers we developed for *E. norvegica* were prepared in a 10 µL volume containing 10 ng of genomic DNA, 1x Qiagen HotStart Taq buffer, 200 µM of dNTP's, 0.3 µM of each primer and 0.5 U of HotStart Taq polymerase (Qiagen). PCR amplifications were conducted in a Perkin-Elmer Gene-Amp System 7200 (Waltham, MA, USA) with the following program: 15 min at 95° C; 10 cycles composed of 30 s of denaturation at 95º C, 1 min. touchdown 55ºC to 50ºC, 20 s elongation at 72° C; 25 cycles composed of 30s at 95ºC, 1 min at 50ºC and 20 s at 72ºC, followed by 8 cycles composed of 30s at 95ºC, 45s at 53ºC and 20s at 72ºC; and a final 30 min elongation step at 72º C. Fragments were separated on an ABI 3130 XL automatic sequencer (Applied Biosystems, Foster City, CA, USA) with the internal size standard Rox 350. Alleles were scored using Peak Scanner version 1.0 (Applied Biosystems).

Restriction associated DNA library preparation and sequencing Between one and three and three and five coral polyps were dissected, respectively, from 70 *L. pertusa* and 111 *M. oculata* samples for genomic DNA (gDNA) extraction (NucleoSpin® Plant II Macherey Nagel, following the manufacturer's instructions). On a first stage, two small sample sets for each species (12 *L. pertusa* and 16 *M. oculata*) were selected. gDNA was quantified using a Qubit fluorometer (Broad Range Assay kit) and gDNA quality was assessed by running 100 ng of DNA for each sample on a 1% agarose gel. Concentration of the highest quality DNA samples was normalized to 20 ng ul^{-1} and sent to Floragenex Inc. (Eugene, OR, USA) for RAD-seq library preparation with the 6-cutter *PstI* enzyme. Sequencing was done on two lanes of an Illumina HiSeq2500, producing single-end 100 bp reads for each of the two coral species. On a second stage, ninety-five *M. oculata* samples were later sent to Floragenex Inc. for library preparation and sequencing in the same way.

Fifty-eight *L. pertusa* samples were used to construct two independent RAD libraries (Restriction site Associated DNA genotyping) based on the protocol of Etter et al (2011), with 32 samples in each library. gDNA quality was assessed by running 100 ng of DNA for each sample on a 1% agarose gel. Individual gDNA samples were distributed between the two libraries, so that all populations were represented in each library. Library 1 included the samples with the highest gDNA quality and library 2 included DNA of lower quality (genomic DNA was more or less degraded. In each library two samples, one from the Atlantic and one from the Mediterranean, were included twice in the library to assess intra-library variability. Two individual coral samples with high quality gDNA were included in both libraries, to allow library comparison. In library 1, gDNA concentration was standardised for all samples at 30 ng μl^{-1}. In library 2, which presented a more variable quality of genomic DNA, samples with higher DNA quality were standardised to 25 ng μl^{-1}, and samples with lower DNA quality were standardised to 30 ng μl^{-1}. Libraries were prepared with P2 adapters compatible with paired-end sequencing. After each reaction, libraries were purified using SpriSelect Magnetic beads (Agentcourt, Beckmann). Due to the small genome size of corals, whole genomic DNA was first digested with *PstI*-HF (New England Biolabs). Within each library, individuals were labelled with unique 5 or 6 bp barcodes following an Illumina sequencing adapter. Variable barcode length is known to improve clustering quality. After tagging, samples were pooled in equal volumes, producing an equimolar sample solution for library 1 (high quality gDNA). For library 2, we chose to slightly over-represent samples with lower quality gDNA. DNA was shredded using a Covaris S220 ultrasonicator. Fragments between 200 and 800 bp were selected with SpriSelect Magnetic beads (Agentcourt, Beckmann) with a first beads/DNA ratio of 0.45 and then a ratio of 0.95. The last steps of library construction were done following exactly the protocol of Etter et al (2011). Library quantification and quality assessment was done on a High Sensitivity D5000 ScreenTape (Agilent). Libraries were sequenced twice on an Illumina HiSeq4000 (Genoscope, France) producing paired-end sequences of 150 bp (including barcode). Sequence data for *L. pertusa* was recently received and is being analysed.

DNA from *E. norvegica* samples used for restriction associated DNA sequencing (RAD-seq) were extracted with the Nucleospin Tissue kit (Macherey-Nagel) following the manufacturer's instructions. DNA was quantified with a Qubit 2.0 fluorometer (Broad Range Assay kit). Quality was assessed by running 100 ng of DNA per sample on a 1% agarose gel, and concentrations were subsequently normalized to 20 ng ul^{-1} for library preparation. One RAD-seq library was built from 32 *E. norvegica* samples following the protocol of Baird (2008), with the high fidelity 8-cutter restriction enzyme *sbfI*. We used a sequencing adapter containing a primer and an individual-specific barcode (5-6 bp, minimum 2 bp distance). The library was sequenced in two lanes on an

Illumina HiSeq 2500 flow cell (Illumina Inc., San Diego, CA, USA; Macrogen Inc., South Korea) producing single-end sequences of 201 bp (including barcode). Eighty additional *E. norvegica* samples were sequenced in one Illumina HiSeq lane at Floragenex Inc.; this library was recently received (end of July) and is waiting further processing.

Quality control of all RAD-seq datasets was performed using FastQC (Babraham Bioinformatics). The two smaller RAD-seq coral datasets and that of *E. norvegica* were analysed with Stacks 1.6 (Rochette and Catchen, 2017), whereas the latest datasets for all three species are being analysed with ipyrad (Eaton, 2014; Eaton and Overcast, 2016).

Data analyses Statistical power was assessed for the two coral species and for the commensal polychaete with POWSIM (Ryman and Palm, 2006) to test if the eight and six microsatellite loci used have enough resolving power to test Fst values significantly different from zero. For the whole range of Fst values estimated for the three datasets, power was 100%. Inference of spatial population structure with Bayesian clustering on microsatellite data was performed with the R package tess3r (Caye, Jay, Michel, and François, 2018), using location priors. Statistical parsimony networks were estimated on TCS 1.2.21 (Clement et al., 2000) and visualised with TCSbu (Múrias dos Santos et al., 2015) with nuclear ITS sequences for *M. oculata* (1,124 base pairs) and *L. pertusa* (1,130 bp), and mitochondrial COI sequences for *E. norvegica* (499 bp). We visualized the distribution of genomic diversity with a Principal Component Analysis (PCA) using the adegenet and ggplo2 R packages (Jombart, 2008; Wickham, 2016). For more details, see Boavida et al (2019a,b).

Results & Discussion

Results obtained by different genetic markers (*M. oculata* - nuclear internal transcribed spacer (ITS) ribossomal sequences, six microsatellites (270 samples) and two libraries of RAD-seq loci (107 samples); *L. pertusa* - nuclear ITS sequences, eight microsatellites (260 samples) and one library of RAD-seq loci (12 samples); *E. norvegica* - mitochondrial cytochrome oxidase I (COI) sequences, six microsatellites (270 samples) and one library of RAD-seq loci (32 samples)) are concordant and show that most variation is driven by geography or geography-associated factors. For all three species, the strongest genetic structure found at the NE Atlantic scale, estimated with Bayesian clustering (Figure 34) and principal component analyses (Figure 35), is found t between the Atlantic Ocean and the Mediterranean Sea samples. Nevertheless, *M. oculata*'s

Mediterranean genetic group, which encompasses the eastern and western Mediterranean Sea basins, extends into the Bay of Cádiz in the adjacent NE Atlantic Ocean (Figures 34c, 35a and 36a), although with some structure between the Mediterranean and the Bay of Cádiz samples (Figure 36a). *Lophelia pertusa* and the commensal polychaete *E. norvegica* exhibit a much lower degree of genetic connectivity within the Mediterranean Sea. *Lophelia pertusa* is strongly structured between the eastern and western Mediterranean basins, and *E. norvegica* is structured even within the Ionian Sea in the eastern Mediterranean, between Santa Maria di Leuca reef *Atlantis*, located closer to the Adriatic Sea, and the Malta Plateau (Figure34c).

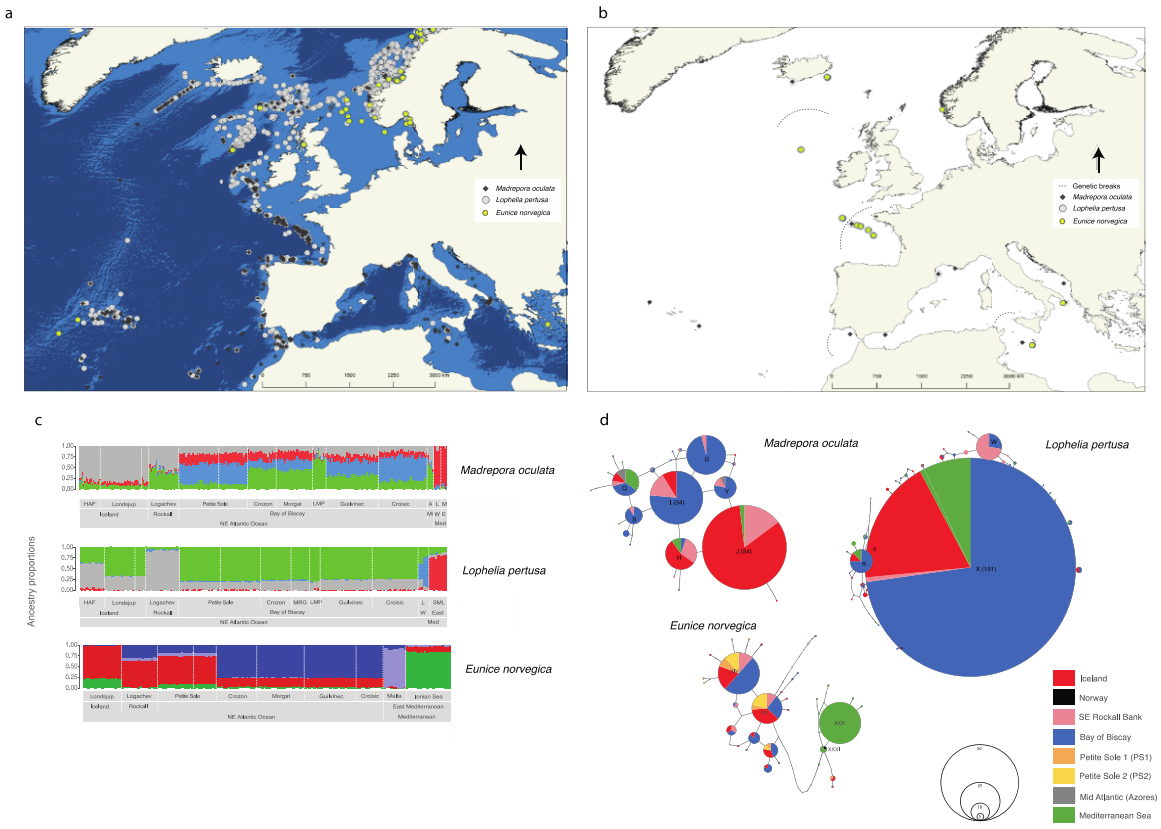


Figure 34: a) Contemporary distribution of the cold-water corals Madrepora oculata (black diamonds), Lophelia pertusa (grey circles) and their commensal polychaete Eunice norvegica (yellow circles) in the NE Atlantic (data from 2017 UNEP database; http://data.unep-wcmc.org and 2019 GBIF https://doi.org/10.15468/dl.1rk01w); b) Sampling sites; c) Bayesian clustering showing the ancestry proportion of each species along the NE Atlantic Ocean, for K=4 genetic groups estimated by cross-validation with tess3r package based on 6-8 microsatellite loci; each vertical bar corresponds to one individual's genetic ancestry; four colour-coded genetic ancestries are shown; d) Statistical parsimony networks estimated on TCS with internal transcribed spacer sequences for M. oculata *(1,124 base pairs) and* L. pertusa *(1,130 bp), and mitochondrial cytochrome oxydase I sequences for E. norvegica (499 575 bp). Abbreviations as follow: HAF - Hafadsjup, LMP - Lampaul canyon, L - Lacaze-Duthiers canyon (Gulf of Lion), W / E - Western / Eastern Mediterranean Sea, A - Azores, M - Montenegro (Adriatic Sea), Mi - Mid Atlantic, SML - Santa Maria di Leuca reef Atlantis (Ionian Sea). *Mediterranean sea - all Mediterranean samples were pooled together in M. oculata and L. pertusa network analyses. Maps created on QGIS with Mollweide's equal area projection. c) and d) adapted from Boavida et al. (2019a).*

Within the NE Atlantic (excluding the Mediterranean Sea), some differences in the genetic structure of the three species are evident. *Lophelia pertusa* presents the lowest differentiation. *Lophelia pertusa* NE Atlantic populations are composed by one homogeneous genetic group

(green genetic ancestry in the Bayesian clustering plot, Fig. 1c; dominant, ie largest, ITS haplotype, Figure 34d), excluding only South East (SE) Rockall Bank samples. In the PCAs that included the RAD-seq datasets, *L. pertusa* samples from the Mid-Atlantic (Azores archipelago) separated from all other *L. pertusa* samples along PC1 (Figure 2b), which explained 12.8% of the variation, while the Mediterranean Sea and adjacent Bay of Cádiz samples formed a distinct cluster on PC2, explaining 11.4% of variation. Mediterranean Sea samples also formed well-separated clusters for *M. oculata* (PC1 28.9%) and *E. norvegica* (PC1 15.7%). However, while all NE Atlantic *L. pertusa* samples (excluding Mid-Atlantic and Mediterranean samples) formed a gradient along PC2 (from Iceland to Bay of Biscay), *M. oculata* samples formed three distinct clusters on PC2, which explained 8.8% of variation, one containing most Bay of Biscay samples, a second containing all samples from Iceland, and a third containing all Mid-Atlantic samples and a few from the Bay of Biscay and one from SE Rockall Bank. The coral-commensal polychaete, *E. norvegica*, also presents three well-separated clusters on PC2, explaining 8.4% of variation, a heterogeneous group containing all samples from Iceland, most from the Bay of Biscay (Petite Sole, Guilvinec and Croisic in Figure 35c) and a few from SE Rockall Bank (Logachev on Figure 35c), one cluster formed by two SE Rockall Bank samples that separated out from all other, and another cluster containing two northern Bay of Biscay samples, from the Petite Sole canyon. In PC1, these two *E. norvegica* clusters (SE Rockall Bank and Petite Sole) segregate from all other NE Atlantic samples, in a similar way as found for microsatellite Bayesian clustering (Figure 34c), where SE Rockall Bank and Petite Sole samples form a mostly homogeneous cluster, albeit with some Bay of Biscay and Mediterranean ancestry.

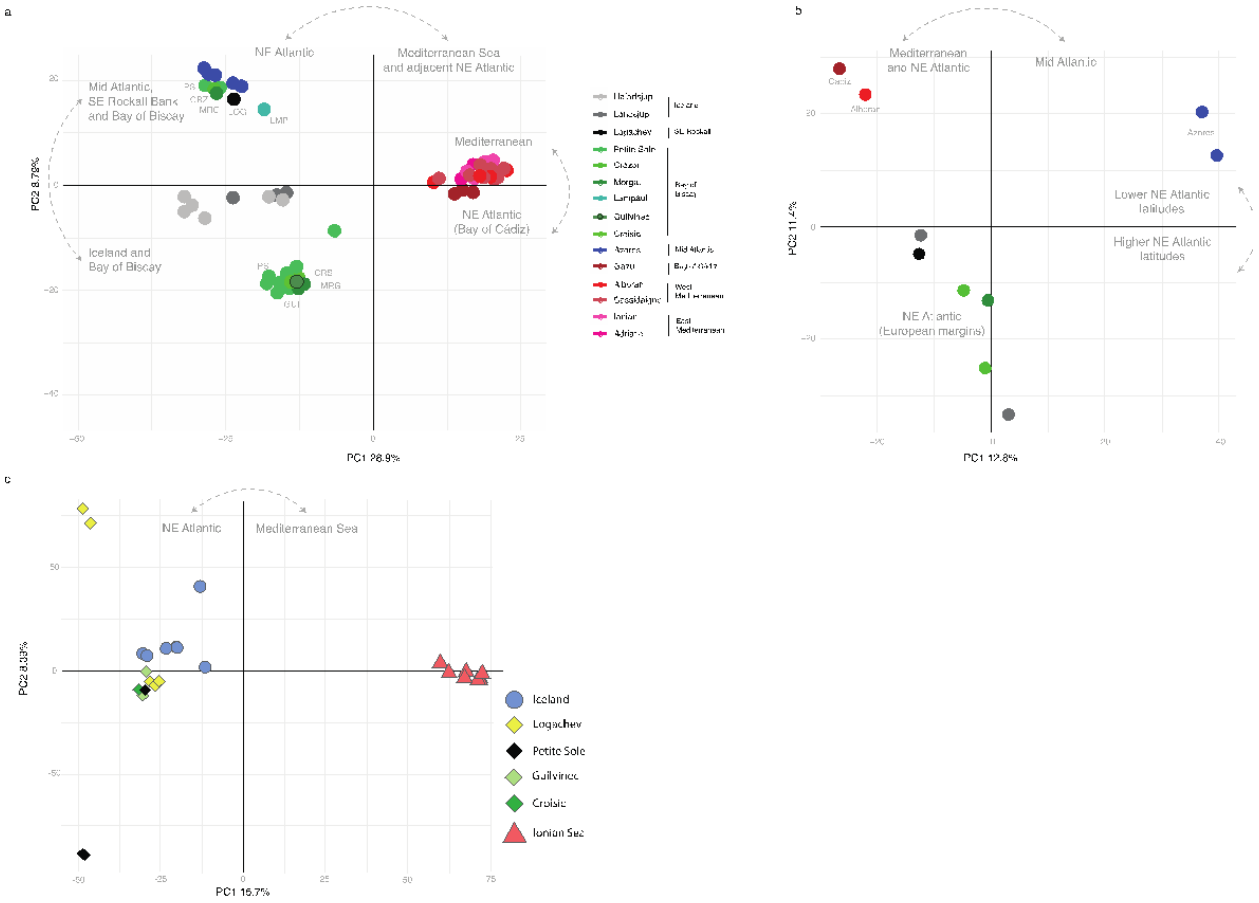


Figure 35: Genetic affinities defined by Principal component analysis (PCA) based on high-throughput sequences of North East Atlantic cold-water corals and their commensal polychaetes. Most variation is driven by geography. a) Madrepora oculata *genetic groups based on 95 samples with 12,896 single nucleotide variants (SNV). b)* Lophelia pertusa *based on 12 samples with 32,554 SNV. c) Eunice norvegica based on 32 samples with 3,300 SNV. All data obtained with restriction associated DNA sequencing (RAD-seq), a) and b) with the enzyme PstI and c) with SbfI. Abbreviations as follows: LOG - Logachev mounds, PS - Petite Sole canyon, CRZ - Crozon canyon, MRG - Morgat-Douarnez canyon, LMP - Lampaul canyon, GUI - Guilvinec canyon, CRS - Croisic canyon.*

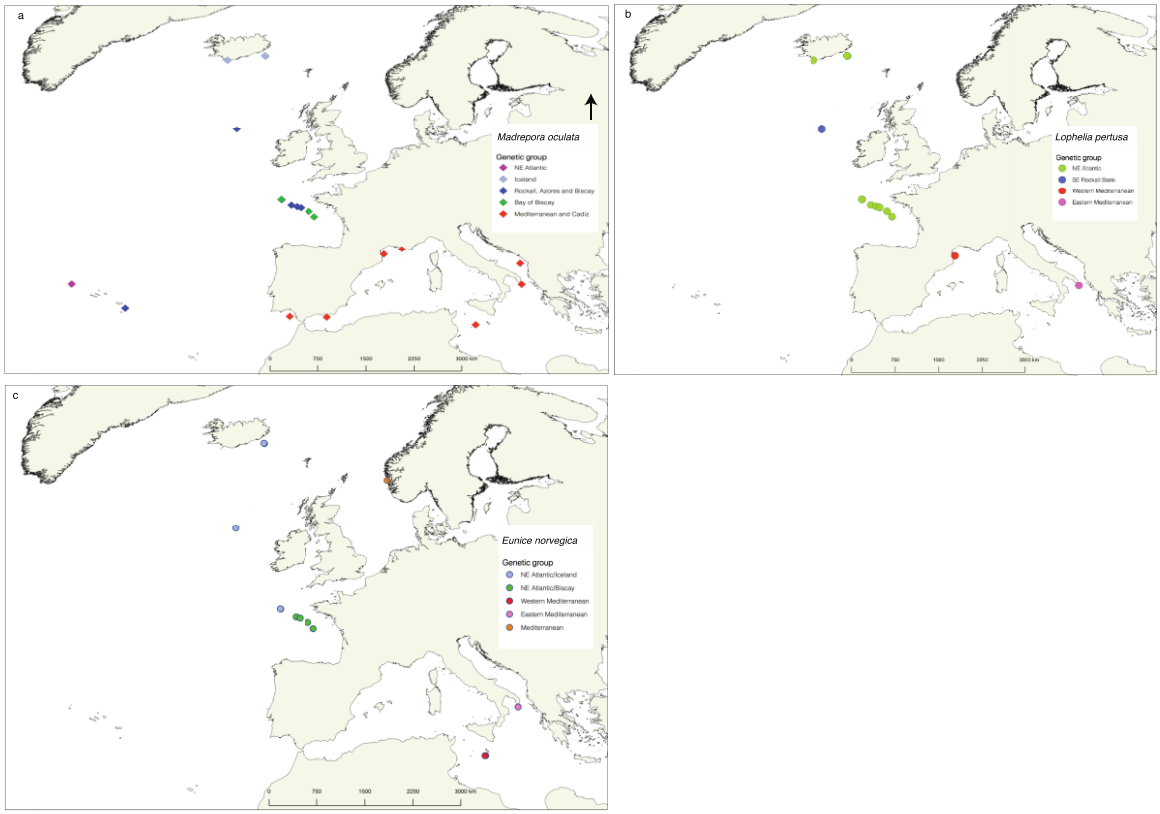


Figure 36: Geographic distribution of North East Atlantic Ocean genetic groups for selected cold-water corals and commensal polychaetes in the deep-sea, based on 6-8 microsatellite loci, nuclear and mitochondrial gene sequences and 3,000 to 12,000 RAD-seq loci. Maps created on QGIS with Mollweide's equal area projection.

While the moderate to high gene flow of *L. pertusa* homogenized nearly the whole Atlantic genetic pool producing a panmictic population, *M. oculata* seems to have progressed slower than *L. pertusa*, particularly in high latitudes and might have been additionally recolonized from other areas. *Madrepora oculata*'s more differentiated populations are perhaps relicts of previous vicariance during glacial periods, a hypothesis that is being tested by ATLAS team. According to the present estimations, *L. pertusa* expanded swiftly along the NE Atlantic at a rate of 0.7–2 km per year (considering 4,000 km of expansion in the past 10,000–30,000 years; Boavida et al., 2019) during postglacial range expansions.

Long-distance dispersal and gene flow, which would erase patterns of population structure, may be unlikely in *M. oculata*. This may be the case if this species of CWC presents different reproduction and dispersal modes compared to *L. pertusa*, for example, a shorter pelagic larval duration in *M oculata* than in *L. pertusa*, or brooding. This hypothesized poor dispersal ability of *M. oculata* may be associated with the complex habitat geomorphology (canyons, seamounts) along the NE Atlantic, along with the large depth gradient (hundreds to thousands of metres) and

may contribute to fragmentation, reduce gene flow and maintain or reinforce large-scale patterns of the NE Atlantic population structure in *M. oculata*.

The mutualistic coral-commensal polychaete, *E. norvegica*, is more similarly structured to *L. pertusa* (Figures 34c and d, 36b and c), with a panmictic cluster along most of the NE Atlantic European margins (excluding the Mediterranean Sea). Although the reproductive biology of this elusive species is unknown, a literature revision revealed that all other studied eunicid worms present evidence of broadcast spawning with swarming of individuals (eg., Gambi and Cigliano, 2006; Fage and Legendre, 1927) and many present evidence of a pelagic larva (Schulze, 2006, Zanol et al., 2010, Hofman, 1975). Such large individual aggregations with concomitant reproduction and assumed dispersal mediated by ocean currents, may lead to high gene flow among distant cold-water coral reefs. Interestingly, *E. norvegica* samples collected recently by ATLAS partners in decommissioned oilrigs from the North Sea belong to the mitochondrial COI "Mediterranean" lineage (Figure 34d), along with a few other NE Atlantic samples (Iceland, Bay of Biscay and Gorringe seamounts). The RAD-seq samples that are segregated from all others in both PCA axes, SE Rockall Bank and Petite Sole canyon, also hint into a more complex population history, which is currently under study.

The contrasting patterns of genetic diversity observed here strongly support differing present-day dispersals of *L. pertusa* and *M. oculata* and that past environmental changes had different influences on these CWC species. The wide geographic sampling scheme of ATLAS work allowed identifying the major axes of structure and on-going work will allow inferring the underlying processes. Our study provides an important warning for managers that even taxa with seemingly similar ecological roles, geographic distributions and tolerances may differ in their response to global change. Therefore, multispecies models are required to ensure conservation measures, such as truly representative and connected networks of marine protected areas.

6.3.2 ATLAS case study 2: Echinoid *Cidaris cidaris*

Introduction

Cidaris cidaris is a species of cidaroid urchin (Echinodermata: Echinoidea) – typically referred to as 'pencil urchins' – which may be found in the eastern Atlantic from St. Helena in the South Atlantic to the Norwegian Sea, as well as in the Mediterranean (Figure 37). This wide geographical distribution appears to be matched by a wide depth range of ~50-2000 m (Emlet 1995; OBIS 2019). The recorded distribution of *C. cidaris* is largely based on photographic and video survey data,

raising the possibility that the true distribution may be more limited, with some specimens attributed to *C. cidaris* belonging to superficially similar cidaroid pencil urchins in other genera, such as *Histocidaris purpurata*, or *Stereocidaris ingolfiana*. Nevertheless, *C. cidaris* is likely to be one of the most common urchins found to inhabit the upper bathyal slope in the northeast Atlantic and Mediterranean. Information on the precise habitat preferences of *C. cidaris* is limited. Surveys of seamounts northwest of the UK and also west of Spain indicate that *Cidaris* urchins are found both on hard and soft substrates, on cold water coral and sponge grounds and also on sandy, sedimented settings (Howell et al. 2010; Serrano et al. 2017b). Focusing on UK seamounts, Howell et al. (2010) and Davies et al. (2015) found them to be commonly around *Lophelia* reefs and sponge grounds, but also often numerically dominant in mixed sandy and cobble settings as well. Serrano et al. (2017c) reported *Cidaris* urchins to be dominant at 1000-1200 m depth in a sandy setting along with *Acanella* bamboo corals at the Galicia Bank Seamount west of Spain. Analyses of the gut contents of *C. cidaris*, reveals a broad diet of hard corals (*Lophelia* and *Madrepora*), hydroids, sponges, crinoids, sediment and foraminifera, making these urchins a likely important structuring force in cold-water coral habitats (Stevenson and Rocha, 2013).

Despite the common occurrence of these urchins, there is relatively little information regarding their mode of dispersal and general life history characteristics. Emlet (1995) determined from direct observation, as well as morphological examination of *C. cidaris* eggs that they produce planktotrophic (~110 µm diameter echinopluteus) larvae. This seems to be the case for other *Cidaris* urchins found in the Atlantic Ocean. Working on *Cidaris blakei*, a congeneric species commonly found around the Bahamas and the eastern Caribbean, Bennett et al. (2012) estimated a planktonic larval duration (PLD) of ~120 days; with larvae developing optimally at temperatures around 11-12°C and being unable to develop at temperatures above ~15 ˚C. If *C. cidaris* larvae develop in a similar manner to *C. blakei* and in similar thermal conditions, then given that in the northern Atlantic, surface temperatures ≤15 ˚C are common throughout much of the year, it seems likely that *C. cidaris* larvae may be capable of drifting on surface currents for at least 3 months, giving them a substantial dispersal capability.

The primary aim of this study is to apply RADseq (Restriction Site Associated DNA sequencing) technology to generate a reduced representation genome-wide multi-locus dataset of bi-allelic SNPs (Single Nucleotide Polymorphisms) in order to investigate patterns of fine-scale population genetic connectivity in *C. cidaris* in the Northeast Atlantic, with a view to focusing on patterns of connectivity with depth. A secondary aim is to pool specimens from various museum and institution collections to characterise the phylogeography of Atlantic *Cidaris* urchins, using mitochondrial DNA sequence data.

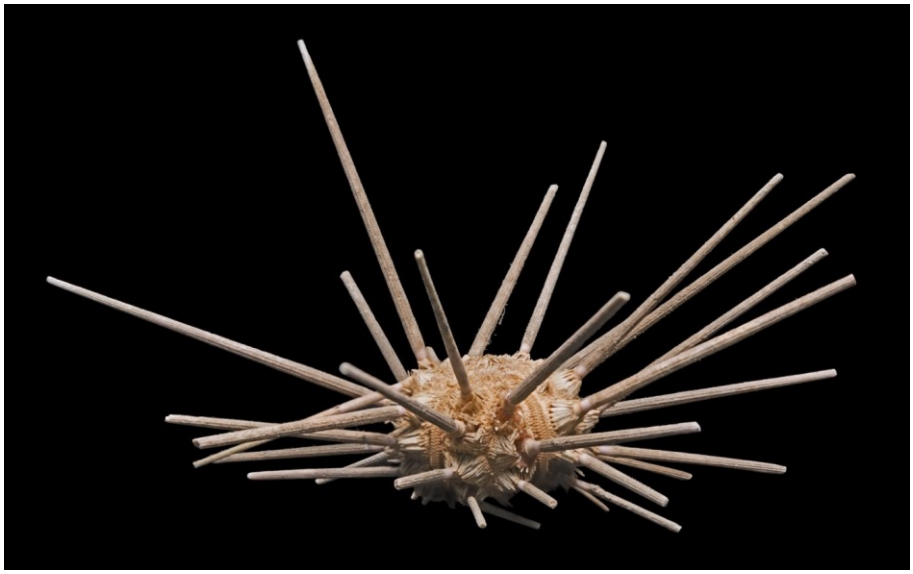


Figure 37: Photograph of Cidaris cidaris *by Didier Descouens, CC 3.0, https://commons.wikimedia.org/w/index.php?curid=30709860*

Methods

The core component of the dataset comprises specimens collected aboard the research vessel *RRS James Cook* during the DeepLinks expedition JC136 in 2016 from seamounts roughly 200 nautical miles northwest of Scotland at depths ranging from around 100-1200 metres. Additional specimens were also collected from the western Mediterranean during the MEDWAVES expedition aboard the research vessel *Sarmiento de Gamboa* and others from the SeaRover survey off the west of Ireland aboard the *ILV Granuaile* (Figure 38). More specimens in the *Cidaris* genus (including *C. abyssicola* and *C. rugosa* from the W. Atlantic, as well as *C. cidaris*) were acquired from the Smithsonian Institute in the USA, from the Senckenburg Society for the Study of Nature.

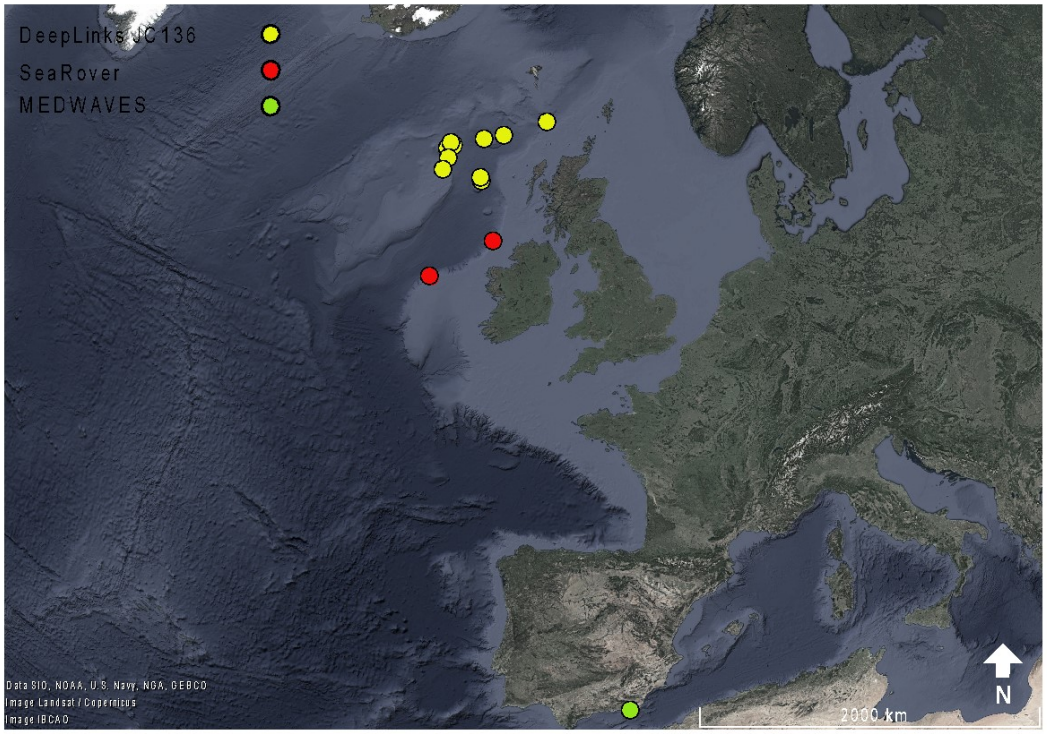


Figure 38: Map of sample sites for C. cidaris urchins collected in the NE Atlantic and the Mediterranean. In the case of freshly collected specimens, tissue was excised from within the urchin tests (preferably the gonad tissue) and immediately preserved in 95% ethanol, before being chilled in refrigerators at ~4 ˚C, or in freezers at -20 ˚C. Tissue from museum collections was selected based on being less than 20 years old and having only ever been preserved in 95% ethanol. DNA was extracted with the DNeasy Tissue Extraction Kit (Qiagen, Crawley, West Sussex, United Kingdom) as directed by the manufacturer with an additional step of adding 5 µl of RNase A (10 mg/µl) with incubation for 10 minutes at 36 ˚C after the proteinase K digestion phase. DNA was examined for quality by examination on gel electrophoresis plates and DNA concentrations were measured using Qubit Fluorometric Quantification with the brand range assay.

PCR cycling reactions were conducted on all DNA extract that yielded detectable DNA in order to confirm morphological identification and for phylogeographic analyses. A ~650 bp fragment of COI was amplified for all urchins using the forward and reverse primers COI e5 (5'–GCY TGA GCW GGC ATG GTA GG–3') and COI er (5'–GCT CGT GTR TCT ACR TCC AT–3') respectively, from Jeffery et al. (2003). A ~600 bp fragment of 16S was amplified from all *Cidaris* urchins using forward and reverse primers 16SarL (5'–CGC CTG TTT ACC AAA AAC AT–3') and 16S ech 3 (5'–TCG TAG ATA GAA ACT GAC CTG–3') from Stockley et al. (2005). Reactions were performed in 30 µl volumes, containing 2 µl of each primer (forward and reverse at 4 pmol/µl), 15 µl of Qiagen HotStarTaq Plus Master Mix, 5 µl of DNA template and 6 µl of double-distilled water. The PCR cycling protocols for both gene fragments were the same: an initial denaturation at 95 ˚C for 5 minutes, followed by 40 cycles of 94 ˚C for 45 seconds, the annealing step at 52 ˚C for 90 seconds, an extension at 72 ˚C for 1 minute, and a final extension of 5 minutes at 72 ˚C. PCR clean up, sequencing reactions and clean up, as well as final Sanger sequencing were undertaken by the Zoology Department sequencing facility at the University of Oxford. Forward and reverse sequences were assembled using Geneious 6.1.8.

For SNP generation using RADseq, a randomised subset of *Cidaris* urchin DNA extract (normalised to 20 ng/µl) was sent to Floragenex Inc. in Portland, Oregon, USA. Only DNA that met the desired standard of sufficient quality and concentration (at least 20 ng/µl of high genomic weight DNA) was selected. No more than 10 specimens per sample station were chosen, with the subset being selected using random number sequences generated by Random.org. Two plates were sent to Floragenex in December 2018, of which 145 were *Cidaris* spp., with the remaining being *Stereocidaris* aff. *ingolfiana*. 13 of the 145 *Cidaris* specimens were duplicates to assess the consistency of the RADseq. At Floragenex, a high-density scan using the restriction enzyme PSTI was performed across four lanes of Illumina HiSeq.

Results & Discussion

DNA was extracted from a total of 526 urchins, of which 318 were *C. cidaris* according to DNA barcoding with COI and 16S. A further 35 were assigned to *C. rugosa* and 36 to *C. abyssicola*, giving a total for *Cidaris* of 389, with the remaining being either *Stereocidaris* aff. *ingolfiana* (85), *Histocidaris purpurata* (3) or *Goniocidaris* sp. (3). The remainder (46) either failed to produce detectable DNA or generate sequence data. As of late August 2019, this sequence data is still being quality controlled, before undergoing phylogeographic analyses. However, a very preliminary examination of a subset of the *C. cidaris* COI data collected from the JC136 and SeaRover expeditions does not at this point indicate any geographic population structure, based on a median-joining haplotype network (Figure 39), although population genetics statistics have yet to be applied to the data.

This lack of apparent *C. cidaris* structure over ~1000 km from the continental slope on the west of Ireland to seamounts off the northwest of Scotland is consistent with panmixia across the region as a consequence of long-range dispersal of planktotrophic larvae combined with large populations. Alternatively, it could reflect a more recent range and population expansion, with insufficient time passing for genetic divergence to have occurred. Further examination of the quality-controlled mitochondrial data and incorporating data from the Mediterranean may yet reveal a population structure at a larger geographic scale and/or a strong signal of recent demographic expansion. The multilocus SNP data generated by RADseq should also provide greater resolution; showing any hidden patterns of structure, both geographically and with depth. The raw data (~200 GB of single-end reads) was received from Floragenex in May 2019 and is currently undergoing preliminary quality control in the Stacks 2.41 pipeline. The aim is to complete the phylogeographic analyses and submit for publication by the end of 2019, with the fine-scale RADseq SNP study being submitted for publication in early-to mid 2020.

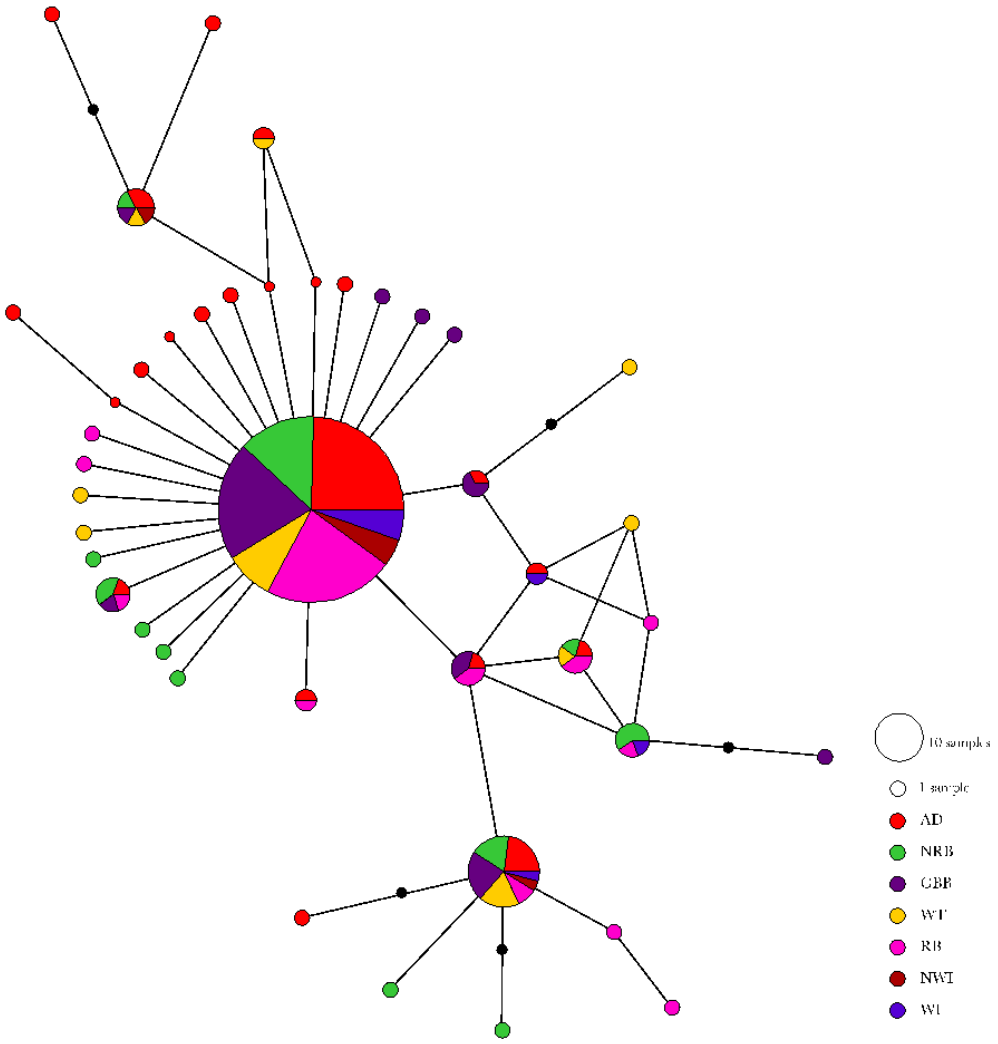


Figure 39: Median-joining haplotype network of Cidaris cidaris *urchins collected from the continental slope and seamounts off the W and NW coasts of Ireland and the UK. AD = Anton Dorhn seamount, NRB = North Rockall Bank, GBB = George Bligh Bank, WT = Wyville-Thomson Ridge, RB = Rockall Bank, NWI = N*

6.3.3 ATLAS case study 3: The Dublin Bay prawn *Nephrops norvegicus*

Introduction

Nephrops norvegicus (Figure 40) is a benthic dwelling crustacean, which is distributed through the north-east Atlantic from the coast of North Africa, around Iceland reaching northern Norway and Skagerrak and, in the Mediterranean, Adriatic and Aegean Seas (Maltagliati et al., 1998; Johnson et al., 2013).

Figure 40: The benthic dwelling crustacean Nephrops norvegicus *(source: https://upload.wikimedia.org/wikipedia/commons/thumb/c/cb/Nephrops_norvegicus_1.jpg/1280px-Nephrops_norvegicus_1.jpg).*

Adult *N. norvegicus* live approximately five to ten years in burrows on patches of soft muddy sediment on the seafloor at ~4 to ~800m depth (Johnson et al., 2013). Adults do not migrate or leave their mud patches at any point with dispersal occurring in the larval stage of the life cycle (Aguzzi and Sardà, 2008). Larval pelagic planktonic movement may last up to fifty days (Hill, 1990; Johnson et al., 2013; O'Sullivan et al., 2014).

In the North Atlantic *N. norvegicus* is currently managed under several authorities. The European Union (EU) manages the fisheries collectively under the Total Allowable Catch (TAC) scheme with the Common Fisheries Policy (CFP; Casey et al., 2016). As non-EU members, Norway, Iceland and the Faroe Islands manage their fisheries independently. The International Council for the Exploration of the Seas (ICES) is the scientific authority on the eastern side of the North Atlantic and divides this area into ICES rectangles or geographic zones used to report on and manage *N. norvegicus*. However, due to the limited dispersal capabilities of the species, these ICES areas usually encompass more than one *N. norvegicus* ground or unit. Assessment and management are therefore based on a smaller scale of stock unit known as functional units (FU). There are over thirty of these FUs in the North Atlantic and thirty geographical sub areas (GSA) in the Mediterranean (Casey et al., 2016).

Stock assessment of *N. norvegicus* via commercial fishing methods can be difficult as individuals are more commonly caught in commercial gear during periods of emergence from the burrow

which may depend upon a number of variables including: time of day, size, sex, reproductive stage, mating, hunger state, food presence, and territorialism (Morello et al., 2007; Aguzzi and Sardà, 2008; Sbragaglia et al., 2015). Fisheries-independent data are therefore important for assessing and managing this species. Burrow counts via underwater television surveys are undertaken annually in many areas to estimate stock size and exploitation status (ICES, 2014; Lordan et al., 2015). The data gathered from these surveys are utilised for subsequent annual catch advice. For many marine species, the existing management definition of populations or stocks does not necessarily apply directly to the actual biological unit (Carvalho and Hauser, 1994). Matching individual species' biological processes and subsequent fisheries management action is therefore important to ensure ongoing sustainability.

The current study aims to identify mitochondrial (mtDNA) and nuclear DNA (nDNA) genetic markers to assess the population structure of *N. norvegicus* throughout the species' range using Sanger sequencing and by adopting a High Throughput Sequencing (HTS) based Genotyping By Sequencing (GBS) approach by:(i) identify suitable primer regions in the mtDNA D-loop region; (ii) develop a de novo panel of nDNA microsatellite markers; (iii) deploy both mtDNA and nDNA on samples from the species' range to assess population genetic structure to assist management of the species. Part of the results based on mitochondrial DNA has been published in Gallagher et al (2018).

Materials & Methods

Nephrops norvegicus samples were obtained across the geographic distribution from commercial fishing or research vessels between 2013-2017 (Figure 1 and 2). First and second pereiopods were removed from each individual before being stored in 80% EtOH. Whole samples that were collected were stored at -20 °C before tail tissue was removed and stored in 80% EtOH. Individuals sampled were grouped in two size distributions (16-37mm and 37-79mm carapace length) to minimise the risk of including only one generation in the study. Including only one generation could potentially cause family effects or reflect non-temporally stable data and could bias the conclusions (Haynes et al., 2016). Total genomic DNA was extracted using a modified chloroform/isoamyl alcohol protocol (Petit et al., 1999). Extracted DNA was quantified on a NanoDrop® ND-1000 spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA). All samples were standardised to 40ng/µl.

Primers (NN3DF 5'-ACA GCG TTA AGA YAC CAT AG-3' and NnDR 5'-GCT CTC ATA AAC GGG GTA TGA-3') were designed initially using Primer-3 as implemented in Geneious® 7 (https://www.geneious.com; Kearse et al., 2012) and the D-loop *N. norvegicus* mtDNAl genome (GenBank Accession: LN681403.1). The resulting amplicons were relatively larger (~880bp) than had been designed for (~600bp), and were Sanger sequenced to discover a ~280bp fragment of the D-loop area missing from within the GenBank data (Figure 2.1). Subsequently, new primers JG2 F 5'-CTA CAG ATT TCG TCT ATC AAC-3' and NnD R 5'-GCT CTC ATA AAC GGG GTA TGA-3' were designed on these returning sequences to incorporate the newly discovered ~280bp for a ~680bp amplicon. Primer sequences' specificity was confirmed using BLASTn (Basic Logical Alignment Search Tool; Zhang et al., 2003). Optimal annealing temperature was determined using a gradient PCR.

A reduced representation library based on two individual *N. norvegicus* was created for shotgun sequencing in an effort to detect nDNA variation (microsatellites) as per Carlsson et al. (2013). Resulting sequences in the form of raw FASTQ sequence data were downloaded from Illumina BaseSpace. Quality control was performed using FastQC (Babraham Bioinformatics). Nextera library kit adaptors were removed from the sequences using Trim-Galore (Babraham Bioinformatics). Paired-end sequences that were overlapping were aligned and assembled in PANDAseq (Masella et al., 2012). Microsatellite-containing sequences were identified and extracted using QDD_pipe 1: Microsatellite detection in QDD-VM version 3.1. (Meglécz et al., 2010). These sequences were then assembled using MIRA 4.0 and converted to .ACE file format using MIRA convert (Chevreux et al., 1999). Assembled reads were imported into Geneious® 7 (https://www.geneious.com; Kearse et al., 2012) and analysed visually to identify polymorphic microsatellite loci with a suitable flanking region for primer design. To check that the same microsatellites were not present in multiple sequences, consensus sequences were extracted and assembled de novo using Geneious® 7 under default settings. Resulting assemblies were screened for redundancy by ensuring that each microsatellite was represented only once. Forward and reverse primers were designed on individual contigs containing variable loci using Primer-3 as implemented in Geneious® 7. Primers were designed to be ~20bp in length, have a Tm of 60°C and a product size range of 80-280bp. Primers were screened for redundancy by mapping them back to the de novo assembled sequences. Those that bound to more than one assembly were altered or removed. Universal tails CAG (5'-CAG TCG GGC GTC ATC A-3') or M13-R (5'-GGA AAC AGC TAT GAC CAT-3') were included on the 5' end to allow for future incorporation of barcodes onto resulting amplicons as described in Vartia et al., (2016). Primers with tails were subsequently tested for the formation of hairpins, primer dimer and heterodimer using IDT OligoAnalyzer 3.1

(https://eu.idtdna.com/calc/analyzer). Additionally, available *N. norvegicus* sequences were downloaded from GenBank (Accession numbers: AF221987.1, AF221989.1, AF221991.1, GU559883.1, GU559884.1, GU559887.1, GU559888.1, GU559890.1) and primers were designed on these. Primers were then ordered with universal tails attached according to the protocol of Vartia et al. (2016) to allow for future combinatorial barcode incorporation.

The developed primers (both mt and nDNA) were deployed on samples from throughout *N. norvegicus*' distribution (Figure 41 and 42). Samples for mtDNA were subjected to commercial Sanger sequencing (Macrogen) and GBS was performed using an Ion S5XL HTS amplicon sequencing (NGI, Uppsala, Sweden) and sequences were analysed as per Farrell et al. (2016).

Results & Discussion

The analyses based n mtDNA clearly indicated genetic separation of samples from the Mediterranean from Atlantic samples (Figure 1). Of the the 85 de novo developed microsatellite markers, 25 produced amplicons that could be genotyped using the approach developed in Vartia et al. (2016). Of these 15 were retained for further analyses (markers showing deviations from Hardy-Weinberg expectations were discarded). The resulting genotypes were used to assess population structure and demographics in *N. norvegicus*. The results indicated, based Fst, structure, and barrier analyses (Figure 42, 43a,b and 44), that *N. norvegicus* could be divided into at least four to five populations throughout the sampled geographical range (Figure 43). Further, Fst analyses indicated genetic differentiation increased with geographic distance both when the Mediterranean sample were included and excluded (Figure 43a and b). Analyses for rapid declines of effective population sizes that could have been caused by overfishing were assessed using the Bottleneck software (version 1.2.02 - 16.II.1999, Piry et al., 1999) assuming a TMP models (80% SMM and 20% IAM) did not detect any indication of recent bottlenecks in any sample.

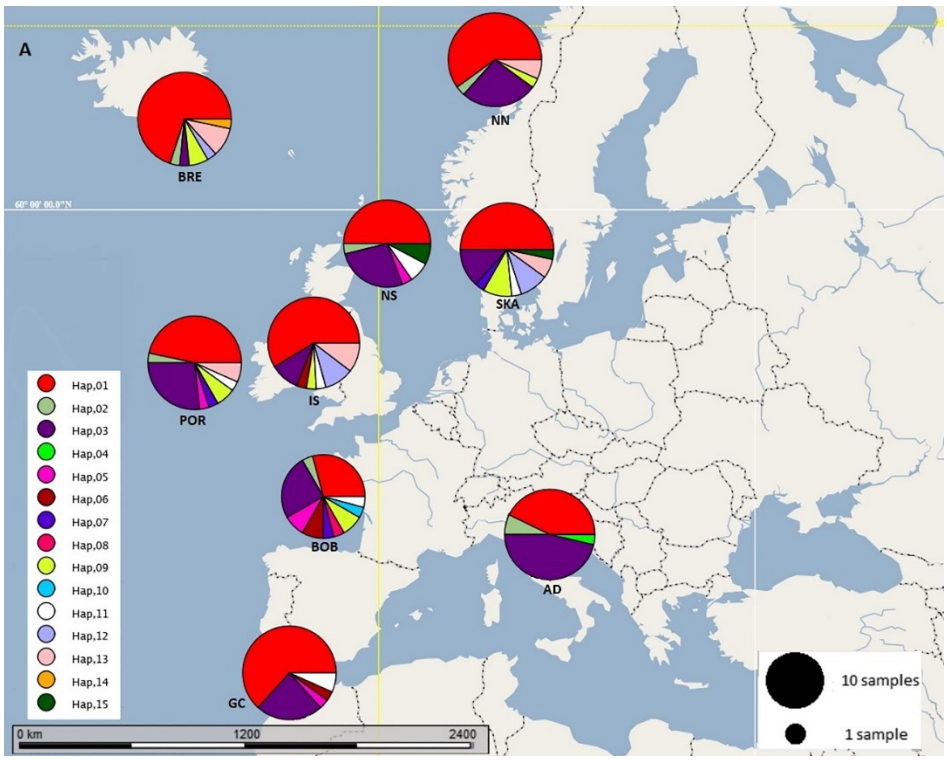


Figure 41: Map of the geographic distribution and frequency of mtDNA haplotype groups in nine sample sites of Nephrops norvegicus. *AD: Adriatic, BOB: Bay of Biscay, BRE: Breiðamerkurdjúp, GC: Gulf of Cadiz, IS: Irish Sea, NN: Northern Norway, NS: North Sea, POR: Porcupine, SKA: Skagerrak haplotypes. Sample sizes Adriatic 28, Bay of Biscay 24, Breiðamerkurdjúp 30, Gulf of Cadiz 30, Irish Sea 29, Northern Norway 30, North Sea 26, Porcupine 30, Skagerrak 30.*

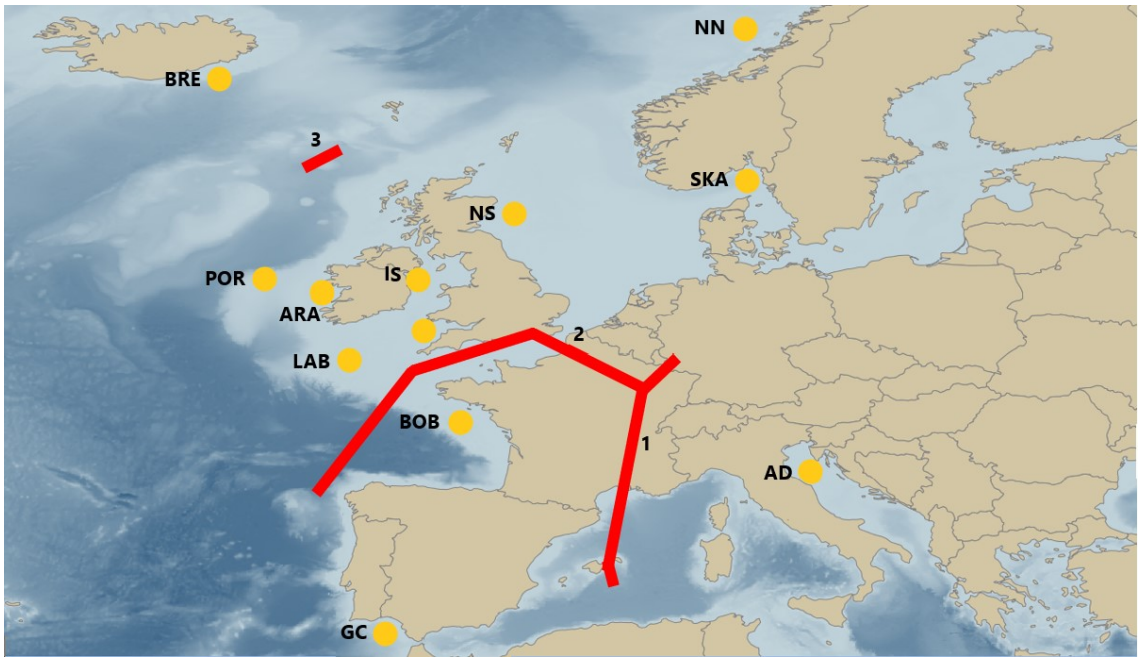
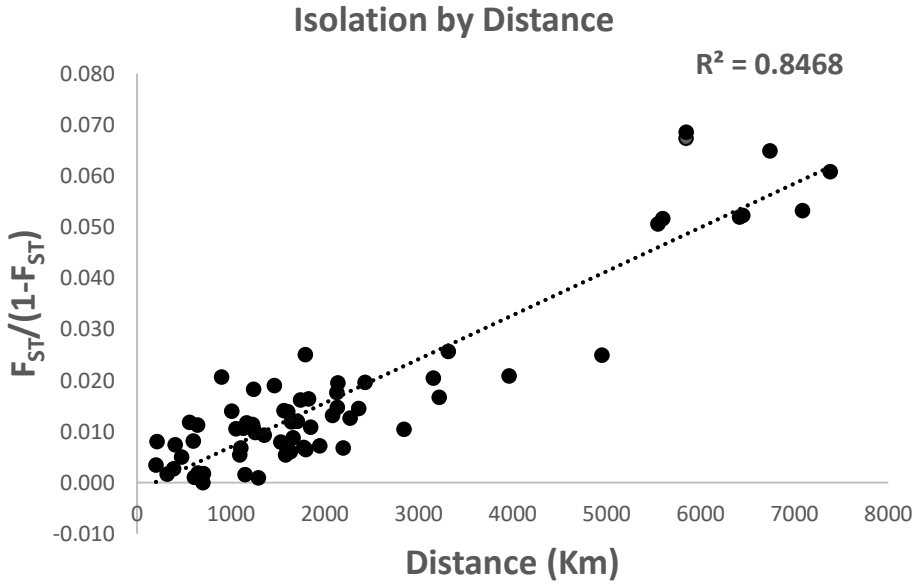


Figure 42: Potential genetic barriers to gene flow detected using microsatellites for Nephrops norvegicus *detected with a bootstrap support over 70. Sample sizes: AD 86, ARA 95, BOB 95, BRE 96, GC 87, IS 87, LAB 95, NN 95, NS 91, POR 96, SKA 88, SMA 96.*

The results revealed new evidence for the population structure and demographics of *N. norvegicus*. Structure analyses suggested that *N. norvegicus* group into four main clusters; 1) Adriatic, 2) Bay of Biscay, Gulf of Cadiz, 3) Back of Aran, Breiðamerkurdjúp, Irish Sea, Labadie, Porcupine, Smalls and 4) Northern Norway, North Sea, Skagerrak. However, it is evident from closer inspection for the Structure output (Figure 44) that the Gulf of Cadiz and Bay of Biscay samples do not show the same genetic signature and that those two samples could represent genetically differentiated populations.



A

B

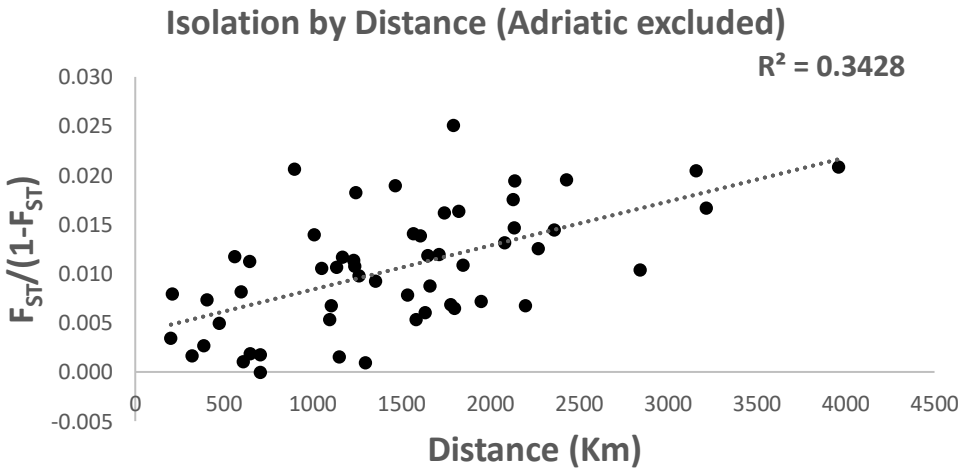


Figure 43: Scatter plots of genetic and geographical distances in Nephrops norvegicus *sample sites for a) all samples P <0.001, b) Adriatic sample removed P <0.001.*

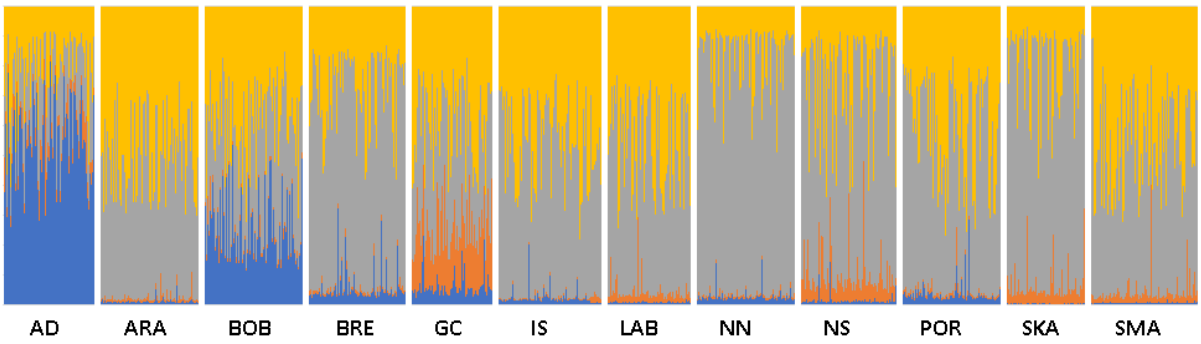


Figure 44: Cluster assignment of Nephrops norvegicus *from structure analysis of the 15 loci dataset across 12 sample sites where K=4.*

The study clearly demonstrated the efficiency of NGS based GBS for de novo marker discovery, genotyping and subsequent population genetic analyses. Further it demonstrated that *N. norvegicus* does not comprise a single panmictic population throughout the species' distribution, rather, at least 4 to 5 populations could be identified as per Structure and Fst analyses (c.f. Figure 42 and 44) both in mtDNA and nDNA. In a fisheries context, these results do not correspond to current delineations for fisheries surveys and management. (c.f. Fig. 1.1 in Bell et al. 2018) As the four/five population clusters cover multiple functional units, the connection between these sites needs to be taken into account. For example, the sites covered in this study around the Irish coast group into one cluster but currently cover five functional units. Grouping into one cluster, as opposed to five, means a sudden change in one site may have a knock-on effect on the others within the same cluster and they are not independent as previously suspected. These results suggest that fisheries management could benefit from incorporating molecular studies into survey and management practices for a more cohesive determination of biological and management units.

This study provides the first significant evidence for population structure in *N. norvegicus*. The results show support for *N. norvegicus* being divided into four/five main clusters with a clear separation between east-Mediterranean and Atlantic populations (supported both by mt and nDNA). Within the Atlantic there is evidence for further separation between the south (Bay of Biscay, Gulf of Cadiz) and north, and further distinction within the northern samples between east and west. Recent expansion or recovery from a population bottleneck is possible. Sampling these

sites again, or including markers under selection for local environmental variables in future studies, may also be useful for further explanation. The outcomes achieved in this study provide novel and important knowledge and tools for not only the conservation of *N. norvegicus*.

6.3.4 Case Study 4: The boardfish *Capros aper*

Introduction

The boarfish (*Capros aper*) (Fig 45) is a small marine pelagic shoaling species distributed in Atlantic shelf waters from Norway to Senegal, including the Mediterranean (Blanchard and Vandermeirsch 2005).

Figure 45: The boardfish Caprus aper *(Source: https://commons.wikimedia.org/wiki/File:Boar_Fish.jpg)*

Boarfish in the northeast Atlantic area are a long-lived species that reach a maximum age of more than 30 years with a length at maturity of 9.7 cm total length at age of 3.4 years (Farrell et al. 2012; Hüssy et al. 2012). In the northeast Atlantic, boarfish have historically been considered a low abundance species that show periodical fluctuations in abundance with increases in the Bay of Biscay, the Galician continental shelf and the Celtic Sea between the 1980s and 2000 (Farina et al. 1997; Pinnegar et al. 2002; Blanchard and Vandermeirsch 2005). The increases in abundance have been tentatively attributed to enhanced adult growth and recruitment as a result of climate-related changes in environmental conditions (Blanchard and Vandermeirsch 2005; Coad et al.

2014). As a consequence of the increased abundances a pelagic trawl fishery developed in shelf waters southwest of Ireland (ICES 2014). Analyses of survey data from bottom trawl landings indicated discontinuity in the distribution of boarfish between the northern Spanish Shelf and Portuguese waters (ICES 2014). In addition, recent reports (Farrell et al. 2012; Sequeira et al. 2015) indicate differences in reproductive characteristics of boarfish both in the northeast Atlantic region and in Portuguese waters. For fisheries management and assessment purposes, one single northeast Atlantic 'stock' is considered to exist north of Portuguese waters (ICES Subareas 6, 7 and 8) and the current management strategy only considers the 'stock' in this area. It is not known if this 'stock' comprises a single or multiple genetically differentiated populations and the delineation of the 'stock' boundaries remain uncertain. Further, the connectivity among units of this 'stock' with boarfish to the south, in the Mediterranean Sea and isolated insular boarfish elements remains unknown. Similarly, it is not understood if the recent increases in abundance of boarfish [Farina et al. 1997; Pinnegar et al. 2002; Blanchard and Vandermeirsch 2005) are caused by population expansion within the northeast Atlantic or an immigration of boarfish from southern or oceanic regions. To enable accurate assessment and sustainable management of boarfish the issues about population structure must be resolved.

The current study aims to resolve these issues using Next Generation Sequencing (NGS) for marker discovery using a modified protocol based on Carlsson *et al*. (2013) and an improved version of Genotyping By Sequencing (GBS) of microsatellites building on the protocol from Vartia *et al*. (2016) to: (i) develop a *de novo* panel of microsatellite markers for boarfish; (ii) assess the genetic population structure of boarfish across the species' range; and (iii) investigate if purported recent increases in abundance in the northeast Atlantic area are the results of an immigration of boarfish from other regions or caused by a population expansion. This study has been published as: Farrell ED, Carlsson JEL, Carlsson J. 2016. Next Gen Pop Gen: implementing a high-throughput approach to population genetics in boarfish (Capros aper). R. Soc. open sci. 3: 160651. (http://dx.doi.org/10.1098/rsos.160651)

Materials & Methods

Samples of boarfish were collected from the catches of fisheries surveys and commercial fishing throughout the species' range. Tissue samples were collected and total genomic DNA was extracted using a chloroform/isoamyl alcohol protocol. Two individuals from the northern and southern extremes of the distribution, respectively, were selected as template for microsatellite discovery using shot gun sequencing on an Illumina MiSeq Platform (Illumina Inc.) with a 500-

cycle MiSeq Reagent Kit V2 to yield 250 bp paired-end (PE) reads. Resulting sequence reads were interrogated for the presence of microsatellites using methods in Vartia et al. (2016). Primers for microsatellites were designed in accordance with Vartia et al. (2016). This includes incorporating M13 or CAG tail-sequences on the 5' end of forward and reverse primers. These tails were used to bind individual barcodes facilitating combinatorial barcoding to amplicons to enable identification of sequences to individual. A second MiSeq (250PE) run was performed on a single library containing 972 barcoded individuals at 85 microsatellite loci from 20 sample locations (Fig. 1).

Results and Discussion

Of the 85 de novo developed microsatellite markers, 42 produced amplicons that could be genotyped using the approach developed in Vartia et al. (2016). The resulting genotypes were used to assess population structure and demographics in boarfish (cf. Farrell et al. 2016). These results indicated, based Fst, structure, divMigrate and barrier analyses, that boarfish could be divided into at least seven populations throughout the sampled geographical range (Figure 46, 47 and 48).

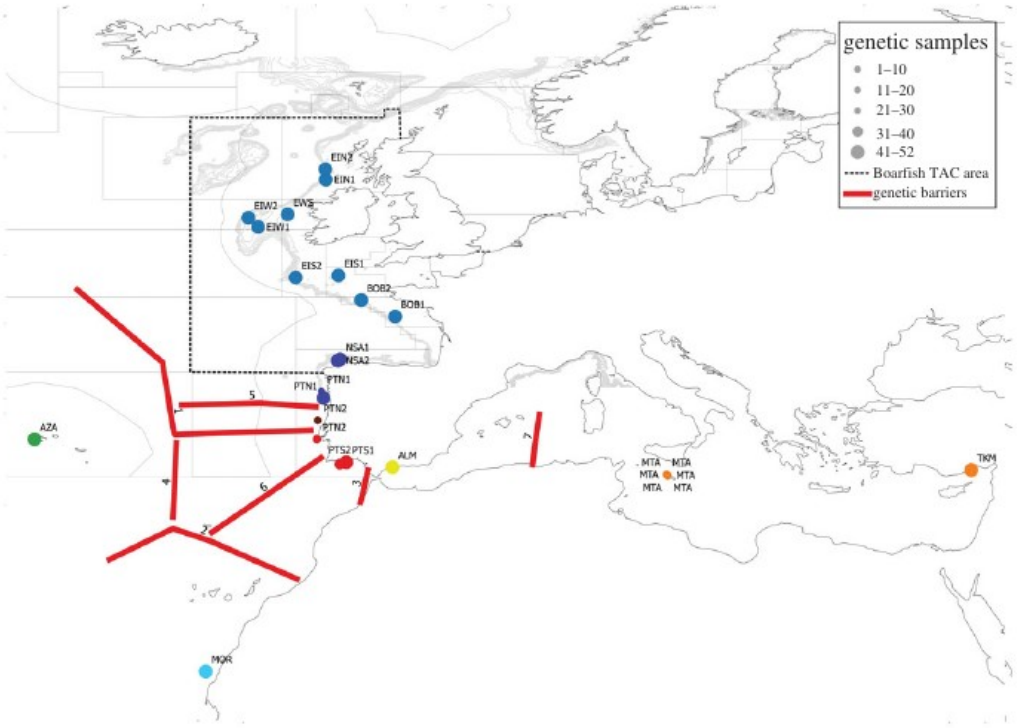


Figure 46: (from Farrell et al. 2016). Map of sampled locations. Location colour is in accordance with Structure analysis cluster. Red lines indicate genetic barriers and the dotted line indicates current management areas for boarfish.

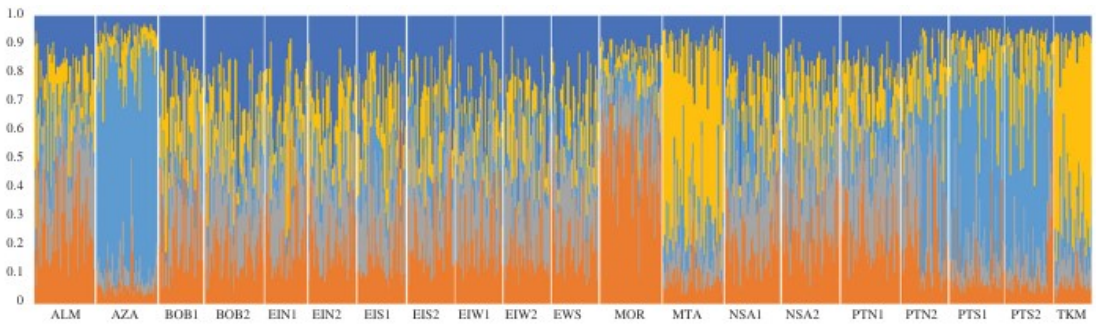


Figure 47: (from Farrell et al. 2016). Structure analysis indicating the genetic affinity of individual boarfish at K 5.

Further, the analyses indicated that the increased abundances in the norther distribution is better explained by a regional demographic expansion with the regions rather than immigration from southern region. Analyses for rapid declines of effective populatin sizes that could have been caused by overfishing were assessed using the Bottleneck software (version 1.2.02 - 16.II.1999,

Piry et al., 1999) assuming a TMP models (80% SMM and 20% IAM) and did not detect any indication of recent bottlenecks in any sample.

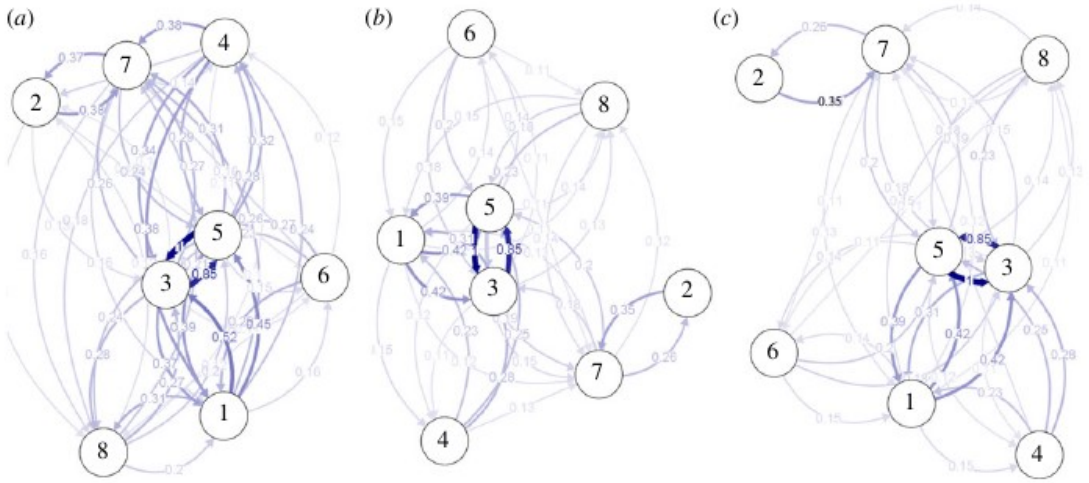


Figure 48: (from Farrell et al. 2016). Directional relative gene flow among boarfish samples throughout the species range using population genetic structure indicators (a) Jost's D (b) GST (c) Nm. The sample clusters are indicated by number: 1, ALM; 2, AZA; 3, NEA; 4, MOR; 5, NSA-PTN1; 6, PTN2A; 7, PTN2B-PTS; 8, MED.

The study clearly demonstrated the efficiency of NGS based GBS for *de novo* marker discovery, genotyping and subsequent population genetic analyses. Further it demonstrated that boarfish doe not comprise a single panmictic population throughout the species' distribution, rather, at least seven populations could be identified. There was no evidence of immigration to the northern distribution from southern areas and hence it is evident that the recent increase in abundance observed in the northern distribution is likely caused by population expansion occurring in situ in the northern distribution. The current study represents the first extensive population genetic analyses of boarfish which also required development of genomic resources for the species. Taking into account the observed population structure is essential for the development of sustainable and adequate management plans for boarfish.

6.3.5 Case study 5: The Horse Mackerel *Trachurus trachurus*

Introduction

The horse mackerel, *Trachurus trachurus* (Linnaeus, 1758) (Figure 49) is distributed throughout the East Atlantic from Norway in the north to West Africa in the south and the Mediterranean Sea in the east.

Figure 49: The horse mackerel Trachurus trachurus

It is a pelagic shoaling species often targeted by commercial fisheries on the continental shelf and is a widely distributed species in shelf waters. Horse mackerel in the northeast Atlantic region is managed as three stocks; the Western, the North Sea and the Southern. The accuracy of alignment of these stocks with biological populations is uncertain. In an effort to resolve these uncertainties we undertook in collaboration with industry and the ATLAS project the largest and most comprehensive population genetic study undertaken on Atlantic horse mackerel.

Materials & Methods

Atlantic samples were provided by industry and sampling was conducted over three consecutive years and three spawning seasons and covered a large area of the distribution of the species including the Western, North Sea and Southern stock areas and also West African waters. Further samples from the Mediterranean (c. 100 individuals), provided by ATLAS partners, are currently being analysed and hence the results presented here are preliminary. Only the mitochondrial COI sequences from the Mediterranean samples are available. In total 33 samples, comprising 2,295 individual fish were collected between 2015 to 2017 across the study area. Total genomic DNA was successfully extracted from 2208 of these specimens. To ensure that individual samples were

all Trachurus trachurus, the COI section of the mitochondrial genome was sequenced using approaches developed by Farrell et al. (unpublished).

Results & Discussion

It was clear that the samples from West African waters contained a high level of species misidentification while only one individual was misidentified in samples from the Mediterranean. Individuals in spawning condition were analysed with a panel 37 microsatellite markers (Farrell et al. unpublished) and population genetic statistical analyses indicated that horse mackerel in the northeast Atlantic region does not represent a single biological unit. On the highest level there are mixed species catches in African waters and to a smaller extent in the Mediterranean. Further there is a clear separation of the southern North Sea from other regions and further, less pronounced, structure along the northeast Atlantic continental shelf.

7 Future perspectives: Combining genetic and modelled connectivity

The study of connectivity is traditionally made separately in each discipline (e.g. population genetics, modelling), and a conceptual integrative framework is needed. One main innovation is to depart from usual separate analysis and comparative interpretation of data to move toward a unified framework of analysis and interpretation of information from different sources, disciplines and methods. Thus far, the framework of seascape genomics has moved toward such a goal, allowing the integration of genomics and environmental data, yet with many gaps and pitfalls still to be overcome (Riginos et al., 2016; Selkoe et al., 2016). We aim to go a step further, using layers of data issued from predictive habitat and dispersal modelling (Galindo et al., 2006) in a Bayesian framework to inform the analysis of population genomics data modelling (Galindo et al., 2006) in a Bayesian framework to inform the analysis of population genomics data (Gaggiotti et al., 2017). This will be performed in particular on *Lophelia pertusa* for which we have a connectivity matrix based on a well-informed Lagrangian modelling (both in terms of habitat mapping and reproductive knowledge), and a RAD-seq dataset allowing to analyse the distribution of Multi Locus Genotypes to infer present day connectivity.

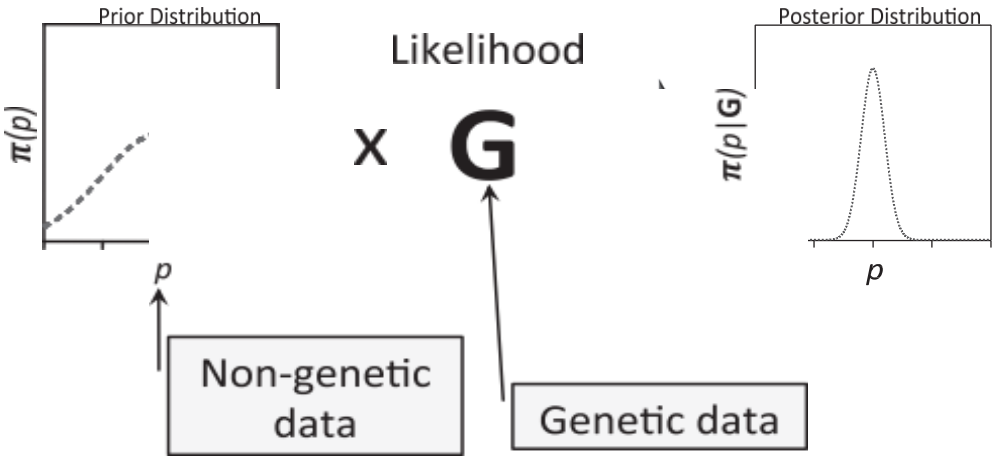


Figure 50. Schematic representation of the Bayesian approach as implemented in e.g., Gaggiotti et al. (2002, 2004). In this example, there is only one parameter to estimate, which could be the allele frequency, p, at a particular locus. The prior distribution, π(p), is used to introduce the non-genetic data and the likelihood function allows the incorporation of genetic data, G, with which we estimate the posterior distribution of p, π(p|G).

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Authors (Partner)				
Responsible Authors	**Name**	Marina Carreiro Silva	**Email**	carreirosilvamarina@gmail.com

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