**Final project report**
**Reporting template**

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| Project acronym | | MARFOR |
| Project title | | **Functional Variability and Dynamics of Responses of Marine Forests to Global Change** |
| Project coordinator | Person (Title, Full Name) | Prof. Ester Serrao |
| | Entity (Company/organization) | CCMAR |
| Project period (Start date – End date) | | 1-1-2017 to 30-11-2020 |
| Project website, if applicable | | http://maree.ccmar.ualg.pt/marfor/ and https://www.marineforests.com |

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| Indicate a contact person for the project, if different from the author of the report | the same |
| Title, first name, surname | |
| Telephone | |
| E-mail | |

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| List of partners involved in the project (company/organization and principal investigator). Please use partner numbers to specify the tasks, work packages and inputs of each partner in sections 4.3, 5 and 6.2 to 6.4. | **Funded:**<br>Partner 1: CCMAR-Univ. Algarve, E. Serrao<br>Partner 2: AWI (Alfred-Wegner Inst.), Klaus Valentin<br>Partner 3: AMU (Aix-Marseille Univ.), Thierry Thibaut<br>Partner 4: CNRS-Roscoff, Myriam Valero<br>Partner 5: IMAR-Univ. Azores, Pedro Afonso<br>Partner 6: UC (Univ. Cologne), Gernot Glockner<br>Partner 7: UGOT (Univ. Goteborg), Kerstin Johannesson<br>Partner 8: UM (Univ. Malaga), Antonio Flores-Moya<br><br>**Self-financed:**<br>Partner A: AU (Aarhus Univ.), Dorte Krause-Jensen<br>Partner B: UniBo (Univ. Bologna), Laura Airoldi |

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## 1. Short description for publicity

Marine forests of large brown algae are ecosystem-structuring habitats upon which many ecosystem functions depend. The MARFOR project discovered the geographical variation in biodiversity components that affect fitness and consequently the fate and future trajectories of these keystone ecosystems along the coastlines of Europe. The project revealed the functional and genetic biodiversity and connectivity of populations that build the most important marine forest ecosystems of Europe. A very intensive field monitoring, experimental testing effort and genotyping and sequencing effort was developed to understand population genetic diversity for the marine forests of Europe. This was an unprecedented mega collaborative effort that benefited greatly from the very good scale of integration both across geographical regions and across multi-disciplinary approaches. Across all studied species there were common patterns of genomic, ecological and evolutionary responses of species that create marine forest ecosystems, although the affinities and geographies of such patterns were contingent on the specific genetic lineages and regions.The consequences of the ongoing and predicted spatial shifts in biodiversity for the future of European marine forest ecosystems resulted in a vast array of implications and recommendations for practical applications of biodiversity for blue growth and for coastal green infrastructures and management measures.

Image: A diver conducting a demographic survey at the southermost marine forest of *Laminaria ochroleuca* along its continuous range in continental Europe.  Cape Espichel, Marine Protected Area of the Arrabida Natural Park, Portugal. Photo credit: Emanuel Gonçalves.

Image: Edge of a deep marine forest of *Laminaria ochroleuca* in the Azores islands, which was discovered in this project to be very genetically distinct from any other known populations of the same species. Photo credits: GUE/IMAR/DOP-UAz, 2014.

## 2. Summary

Marine forests of large brown algae are ecosystem-structuring habitats upon which many ecosystem functions depend. A very intensive field monitoring, experimental testing effort and genotyping and sequencing effort was developed to understand population genetic diversity for the marine forests of Europe. The MARFOR project discovered the geographical variation in biodiversity components that affect fitness and consequently the fate and future trajectories of these keystone ecosystems along the coastlines of Europe. The project revealed the functional and genetic biodiversity and connectivity of populations that build the most important marine forest ecosystems of Europe. Along a large number of surveys and experiments that resulted in many research puplications, the project unraveled intra-specific genetic and demographic structure and functional traits between and within populations of marine forests of large brown algae across distinct European regions and habitats.

This was an unprecedented mega collaborative effort that benefited greatly from the very good scale of integration both across geographical regions and across multi-disciplinary approaches. Across all studied species there were common patterns of genomic, ecological and evolutionary responses of species that create marine forest ecosystems, although the affinities and geographies of such patterns were contingent on the specific genetic lineages and regions. The consequences of the ongoing and predicted spatial shifts in biodiversity for the future of European marine forest ecosystems resulted in a vast array of implications and recommendations for practical applications of biodiversity for blue growth and for coastal green infrastructures and management measures.

## 3. Objectives of the research

Specific objectives - for marine forests of large brown algae along European shores:

Objective 1 • Discovery of the variability in selective (i.e., adaptive) and non-selective **genetic/genomic diversity** and **differentiation** across European coastlines.

Objective 2 • Discovery of functional (**ecological, ecophysiological**) differences between populations and species.

Objective 3 • Prediction of **species/genetic group trajectories** under possible **future climate scenarios** and their implications for genetic and functional trait conservation.

Objective 4 • Identification of **critical features** revealed by the previous objectives for management of blue-green growth and infrastructures **(e.g., aquaculture and ecosystem restoration)**.

## 4. Project activities and achievements

### *4.1. General description of activities over the duration of the project*

This project produced great scientific outcomes (see below lists of publications and description of results) with major consequences for the capacity of European institutions to manage, conserve and value their marine forests. The scales of the integrated approaches taken by geographically well-distributed partners across all major dominant large brown algae of Europe were unprecedented, and the results and outputs demonstrated in this report so far are only the tip of the iceberg, as all the initiative and collaborative links will continue to extend and develop long after the end of the project. The impact of this project is not completed, but rather it is expected to become even higher after the end of the project as many activities are still in preparation or will be continuing, including also those that became delayed or postponed by the pandemic situation, and will resume even after the funding of the project is completed, given the long-lasting partnerships that were built.

The activities within each of all the objectives were integrated within the MARFOR consortium, as the same species and populations were compared for all traits including genomic, ecological, and ecophysiological (objectives 1 and 2) and finally used for future predictions and implications (objectives 3 and 4).

The main focus was on the dominant European kelp species, which are mainly *Laminaria digitata, Saccharina latissima, L. hyperborea* and *L. ochroleuca*. These were mainly the focus of studies by core central partners CCMAR, AWI ans CNRS, in several cases with the other partners also including AU. These joined efforts to infer variability in selective (i.e., adaptive) and non-selective genetic/genomic diversity and differentiation across European coastlines for all of these species (objective 1).

Other species of kelp were also studied, particularly the Mediterranean *L. rodriguezii* by this consortium led by AMU and UniBo, and the Arctic *L. solidungula* was mainly the focus of AWI and UC and AU. The partners located at sites with peripheral populations focused especially on applying the questions at the scale of their geographical region, such that the genus *Fucus* and other fucoids was the main study subject for UGOT focused on Baltic Sea algae, and for UMA on their unique populations of this genus. It was also a topic of study by IMAR in the Azores archipelago, but IMAR concentrated further on Azorean populations of kelp, *Laminaria ochroleuca*.

All partners but UC conducted sampling activities for inferences about the functional (ecological, ecophysiological) differences between populations and species (Objective 2). This was focused on the same species and populations that were studied in objective 1. Partner CCMAR led the integration of the data collected by all partners into modeling efforts for the development of predictions of future trajectories for genetic lineages (objective 3). These efforts were and will continue to be used for helping in management decisions concerning various types of implications for the human society (objective 4). This is particularly including aquaculture by leading partner CNRS. The selection of populations for restoration of kelp forests in MPAs in SW Europe was guided by the research results here achieved. One major impact of great significance was the contribution to integrating these marine forests of kelp in the OSPAR convention, with major contributions by partners IMAR, CNRS, AWI and Roscoff. The identification of critical features revealed by the previous objectives for management of blue-green growth and infrastructures (including aquaculture and ecosystem restoration) is being included in a major joint publication for stakeholders developed by this consortium with a large number of collaborators from institutions across Europe.

The project further produced many results and conclusions that are specific for each species and genetic lineage and biogeographic region. The space provided here is insufficient to present them, but they are detailed in the publications with the respective figures and maps.

The main conclusions of the project can be summarized as the discovery, description and mapping of genetic traits and subspecies diversity in what was previously managed and applied as homogeneous marine biodiversity units. The implications for stakeholders of this novel understanding, for example to transform into management measures, was demonstrated to not be a direct straightforward process because our modeling predictions of future trajectories have revealed that the marine biodiversity that we discovered and characterized today is part of a changing trajectory. It is of utmost importance to understand these future trends and account for them in stakeholders activities. This, although we discovered metapopulation structures in every marine forest species analysed across their entire geographic distribution ranges, our results also highlight that stakeholders must be aware that the scenario of European marine forests is rapidly changing, and adaptive measures are only possible if taken within the appropriate time ranges.

### *4.2. Table of deliverables*

| **Deliverable Name** | | | **Lead partner (country and designation)** | **Date of delivery (mm/yyyy)** | | **Comments** |
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| | | | | **Initially planned** | **Delivered** | |
| ***Work Package*** | ***Deliverable or Milestone*** | ***Full Name*** | | | | |
| WP1 | D1.1.1. | Report on collection of dry samples, DNA and genotypes from different populations and species | France, CNRS. | 31 Dec 2018 | Completed April 2020 | **CNRS, CCMAR, AMU, UGOT:** Collections of > 3500 dry samples for kelp species (*Lo, Ld, Lh, Sl* and *Lr*) and many species of *Cystoseira sensu lato* (including *Cystoseira, Carpodesmia, Treptacantha*) were completed. All of these samples were genotyped (see details Tables 4, 5 and 6), including genotyping of temporal data set, and the genotyping with the newly developed microsatellites and NGS markers.<br>**IMAR and AMU** collected 58 samples of *L. ochroleuca* from 3 locations in the Azores to integrate with the genetic studies. Many dry samples were also collected for other taxa like *Fucus guiryi* around several Azorean islands. These included also hundreds of samples of *Cystoseira sensu lato* from 9 locations in the Azores.<br>**UM** collected 200 samples of *Fucus guiryi* from populations in the Strait of Gibraltar (n=50, 4 sites) and 30 samples of *Carpodesmia tamariscifolia* from one population in the Alboran sea. Material was sent to **CCMAR** for genetic analyses. |

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| WP1 | D1.1.2 | Report on gametophyte culture collection from different populations and species | France, CNRS. | 31 Dec 2018 | completed September 2020 | **AWI, CNRS, CCMAR:** Isolation of diverse strains of kelp populations completed. These and old strains already available in AWI, CNRS and CCMAR provide a first stock for a European conservation center to preserve the genetic diversity of European kelps, especially those which are under threat of disappearance.<br>AWI: *L. rodriguezii*: 7 clonal male and female strains. *L. digitata*: 6 locations, 7 male and female clonal strains per population, in addition per strain 5 sister clones isolated.<br>CCMAR: *L. ochroleuca*: 1 location; at least one male and one female gametophytes were isolated from 6 sporophytes<br>*L. pallida*: 2 locations; at least one male and/or one female gametophytes were isolated from 6 sporophytes per population<br>**IMAR**: 8 dives ranging in depth between 37 and 51 m were made to try and collect samples of living reproductive tissue of *Laminaria ochroleuca* for the experiments requiring gametophyte production in cultures. These attempts were made between June and September on kelp beds located off 3 islands in central and eastern Azores. No reproductive tissue was found suggesting that reproduction concentrates in a period where ocean storms make sampling generally complex to accomplish. |
| WP1 | D.1.2.2 | Standard protocols for teaching kelp biology in school to raise awareness | Germany, AWI | 31 Aug 2019 | completed July 2020 | **AWI**: Pupils of higher school classes developed a first concept for a teaching unit ‘Kelp forests under climate change’ which was delivered to the AWI |

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| | | of this important European ecosystem | | | | HighSea program (https://www.awi.de/en/work-study/out-of-school/as-highsea-scholar.html). |
| WP2 | D2.3.2 | A comprehensive dataset about the distribution of marine forests made available on the World Wide Web. | Portugal, CCMAR | 31 Oct 2019 | completed September 2020 | **CCMAR:** The dataset was compiled. The website is under development but is already functioning and freely available to the public: www.marineforests.com<br>**IMAR**: A compilation of all existing kelp occurrences identified in the Azores has been published with the respective metadata on EUROBIS/EMODNET Biology (DOI: 10.14284/423; Tempera et al. 2020) |
| WP1 | D1.2.3 | Identification of suitable cultivars able to withstand altered environmental conditions for kelp aquaculture | Germany, AWI | 31 Dec 2019 | completed September 2020 | **AWI**: Clonal isolates collected along the distribution gradient of *L. digitata* all characterised with respect to their temperature performance (see report above). Resilience of cultivars could be enhanced in future with adaptive breeding methods, generating hybrids between distant isolates. |
| WP1 | D1.3.1 | Report on comprehensive dataset on macroalgal transcriptomes related to temperature and nutrient gradients for future assessment of the status of macroalgal forests | Germany, UC | 31 Dec 2019 | *L. digitata* transcriptome : completed November 2020;<br>*L. ochroleuca* transcriptome: in progress;<br>*Saccharina latissima* transcriptome: 2019 | **AWI, UC and CCMAR:** Nutrient x temperature experiments have only been performed on *L. ochroleuca* under the lead of CCMAR. AWI focused on transcriptomic analyses of genetic crosses and selfings in *L. digitata*.<br>**UGOT** contributed to generating macroalgae transcriptomes for hyposalinity treatments for *Fucus vesiculosus* in the Baltic Sea.<br>**CCMAR, AWI, UC** have generated transcriptomes related to thermal experiments and evolutionary studies. |

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| | | | | | | **IMAR** collected 28 samples of *Laminaria ochroleuca* from 2 different locations in Eastern Azores for transcriptomic purposes. |
| WP1 | D1.3.2. | Report on prediction of tolerable and severe stress levels, e.g, limiting temperatures, at the physiological, genome and transcriptome level | Germany, UC | 31 Dec 2019 | *L. digitata*: completed 2020; S. latissima: completed 2020; in preparation for publication; CCMAR in coop with AWI/UC: L. ochroleuca / L. hyperbora | **AWI/UC in coop with UBremen**: Experiments with *Sacharina latissima* and *Laminaria digitata* have been performed involving heat and/or salinity stress and published in a series of manuscripts and student theses (Li et al 2019, 2020, Monteiro et al 2019, 2020a, b, Liesner et al 2020, Gauci 2020, Schimpf 2021, Diehl et al. in progress).<br>**CCMAR**: Thermal responses were compared between populations from different parts of the ranges of *Laminaria hyperborea* and *Laminaria ochroleuca* , revealing locally adapted populations, and showing a genetic basis that is kept even in the next generation in common garden.<br>**UM** conducted a study comprising a twenty-six-year time series about the intermittent occurrence of Fucus guiryi thalli at Punta Calaburras (Málaga, Spain). A teleconnection between atmospheric oscillations (NAO) and seasonal survival of this population, its easternmost distribution limit in the Mediterranean, was demonstrated (Melero et al., 2017). This population is currently extinct, no inividuals were found since 2018 and no recovery has been detected. |
| WP1 | D1.3.3. | Report detailing three publications planned in task 1.3. | Germany, UC | 31 Dec 2019 | completed 2020 | **AWI, UC and CCMAR**, in cooperation with **UBremen** and **Bodö:** In task 1.3 seven publications have been achieved (Li et al. 2019, 2020, Monteiro et |

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| | | | | | | al. 2019, 2020; Liesner et al, in prep; Pearson et al. 2019; Rana et al. 2019 |
| WP1 | D1.4.1 | Report about transcriptomic responses to thermal changes and differential exhibition of valuable kelp compounds across species and lineages under thermal change conditions | Germany, UC | 31 Dec 2019 | completed | **AWI/UC**: Sampling and extraction of biochemical compounds has been mostly achieved in two species (*L. digitata, S. latissima*) |
| WP2 | D2.1.1 | Report describing detailed genetic structures of target species and highlighting populations of high priority for conservation | Sweden, UGot | completed September 2020 | completed September 2020 | **UGOT:** Three manuscripts are being prepared which report on detailed genetic structures at the range margin of Fucus vesiculosus in the Baltic Sea. One describing the expansion and formation of clones, one describing the detailed systematics of the Baltic Sea Fucus with suggestions of isolated and unique populations, and a third manuscript compare genetic biodiversity of populations inside and outside MPAs. |
| WP2 | D2.2.1. | Report detailing manuscript comparing realised and potential estimates of connectivity of marine forest along european coast | France, AMU | 31 Dec 2019 | completed | **CCMAR, CNRS, UniB:** This task has been already addressed for four species (Assis et al., 2018, J Biogeography; Buonomo et al, Mol Ecol, 2017; Neiva et al., 2018; Neiva et al., 2020; Guzinski et al., 2020).<br>**AMU** in collaboration with **CNRS:** This task is almost achieved. A paper is under writing on connectivity in *Carpodesmia zosteroide*s (Reynes et al. in prepartion for Evolutionary Application). Concerning *L. rodriguezii* Reynes et al is under-review in Mol. Ecol. |

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| WP2 | D2.2.2 | Report about repartition of genetic diversity and connectivity network for management and conservation purposes | France, AMU | 31 Dec 2019 | Completed | Genotyping and sampling is completed, this task will be completed in January 2021 for *L rodrigueszii* and *C. zosteroides*. The PhD defense of L. Reynes is scheduled for March 2021 (postponed because of the sanitary crisis).<br>**CNRS + CCMAR**: One published manuscript highlighting the role of ocean currents in mediating regionl gene-flow and the location of genetic breaks (Nicastro et al. 2020). Two manuscripts are in preparation at the scale of the Britany coast for *L.digitata*: Fouqueau et al a and b, in prep;)<br>The first one about modelling the environmental and physical drivers of connectivity at this scale and the second one about the spatio-temporal variation of genetic diversity in marginal compared to core populations.<br>**CNRS**: a theoretical model was developed to study the selective forces acting on reproductive mode evolution in the central and marginal parts of the distribution range of a species. The models shows that asexuality is often favored in marginal populations, as it can preserve adaptation to extreme conditions. Increasing maladaptation at range limits leads to stronger selection for asexuality (Fouqueau et al. submitted to Evolution). |

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| WP2 | D2.3.1 | Report about the conservation status of NE Atlantic marine forests inferred from Citizen Science | Portugal, CCMAR | 31 Dec 2019 | completed . | **CCMAR:** The citizen science platform is launched and collecting data. |
| WP2 | D2.4.1 | Report on species distribution modelling considering population connectivity and realized and fundamental niches submitted | Portugal, IMAR | 31 Dec 2019 | completed | **CCMAR**: distribution models published in Assis et al. (2017, 2018) for all major kelp species of Europe<br>**IMAR**: new *L. ochroleuca* presence-absence compilation used to investigate the environmental controls regulating the distribution of the species in the Azores archipelago and map its ecological niche around 3 islands and one euphotic seamount summit for which high-resolution covariates were available. (report by Schmiing et al. 2020)<br>**UM:** recorded vertical range limits and thermal profile of the intertidal fucoid *F. guiryi* during the summer period. A maximum temperature of 41.5 ºC was registered during the study period. |
| WP2 | D2.4.2. | Web-GIS integrating large-scale information about the distribution of NE Atlantic marine forests (online) | Portugal, IMAR | 31 Dec 2019 | completed . | **CCMAR:** The platform marineforests.com is being upgraded to perform this function of producing maps online. |
| WP3 | D3.1.1. | Report on spatial units of connectivity, their functional traits/diversity and predictions for future scenarios. | Portugal, CCMAR | 31 Dec 2019 | completed | **CCMAR:** The data were analysed with all the partners that provided samples and data. |

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| WP3 | D3.1.2. | White paper assisting stakeholders on implementing climate policies. | Portugal, CCMAR | 31 Dec 2019 | completed | **All partners:** a first draft was completed, the information was used to contribute to OSPAR |
| WP3 | D3.1.3. | Layman online booklets, containing information about european marine forests for stakeholders | Portugal, CCMAR | 31 Dec 2019 | completed | **All partners:** This report was replaced by various contributions on this topic to the OSPAR proposal to include kelp forests |
| WP3 | D3.2.1. | Report on implications of the results for conservation, habitat restoration, and policy. | Italy, UniBo | 31 Dec 2019 | completed | **All partners:** This report was replaced by various contributions on this topic to the OSPAR proposal to include kelp forests |
| WP3 | D3.2.2 | White paper including guidelines for conservation of marine forests and restoration techniques. | Italy, UniBo | 31 Dec 2019 | completed | **All partners:** This report was replaced by various contributions on this topic to the OSPAR proposal to include kelp forests |
| WP3 | D3.3.1. | Report detailing manuscript on implications for the seaweed aquaculture industry | France, CNRS | 31 Dec 2019 | completed Nov 2020 | **CNRS:** The analysis of genetic structure of *S. latissima* populations helped to identify the risk of cross breeding (due to existing gene flow) between wild and farmed strains. The minimum geographical distance required to avoid gene flow between unrelated populations was estimated of about 100km (Ruggeri et al., in prep) |
| WP3 | D3.3.2. | White paper for seaweed blue economy | France, CNRS | 31 Dec 2019 | Completed | **CNRS:** This deliverable was considered the “seaweed manifesto” where such questions about seaweed economy are addressed |

4.3. Table of Milestones

| **Milestone Name** | | | **Lead partner (country and designation)** | **Date of delivery (mm/yyyy)** | | **Comments** |
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| | | | | **Initially planned** | **Delivered** | |
| ***Work Package*** | ***Milestone*** | ***Full Name*** | | | | |
| WP2 | M2.2.1 | Development of genetic markers | France, AMU | 30 Sep 2017 | completed September 2020. | **UGOT:** RADSeq was established for *Fucus* species. UGOT generated ca. 20,000 SNP markers for the Baltic-Skagerrak geographic range.<br>**CCMAR:** developed microsatellite markers for *Cystoseira* species<br>**CCMAR+CNRS:** cross calibrated markers for species of *Laminaria*.<br>**CNRS+CCMAR+AMU:** development of 20 new microsatellites for L.digitata and cross amplification in *L. hyperborea, L. ochroleuca, L. rodriguezii* and *L. pallida*; development of a PCR amplification methods to distinguish *Laminaria* species from *Hedophylum nigripes*<br>**AMU:** microsatellite markers have been tested for *Cystoseira zosteroides* and *Laminaria rodriguezii*. AMU in collaboration with CNRS : development of ddRAD-seq derived SNPs in *C. zosteroides* and *L. digitata.*<br>**CNRS:** development of ddRAD-seq derived SNPs for S. latissima |
| WP2 | M2.4.1 | Collation of relevant mesoscale oceanographic | Portugal, IMAR | 31 Oct 2017 | Was completed on time. | **CCMAR and UGhent:** work was completed and has been made available in Bio-Oracle2: Assis et al. (2017) |

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| | | variables for the NE Atlantic | | | | |
| WP2 | M2.4.2. | Collation of georeferenced kelp occurrences for correlative niche modeling | Portugal, IMAR | 31 Dec 2017 | Was completed on time. | **CCMAR, CNRS, IMAR:** It was completed and has been made available in publications: Assis et al. (2017, 2018); Tempera et al (2020) |
| WP3 | M3.1.1 | Local meeting engaging stakeholders to collaborate in the process of providing guidelines assisting marine managment and climate policy | Portugal, CCMAR | 31 Dec 2017 | Was completed on time. | **All partners:** See list of meetings above: A meeting was organized at the Station Biologique de Roscoff including scientists and management and conservation officers. A paper with essential information for stakeholders was outlined and is undergoing. |
| WP3 | M3.3.1. | Local meeting engaging stakeholders (industry) to collaborate in the procces of providing guidelines assisting the seaweed blue economy | France, CNRS | 31 Dec 2017 | Was completed on time. | **All partners:** See list of many meetings with aquaculture industry stakeholders above. This milestone was achieved by the participation of MARFOR to several events and workshops involving aquaculture stakeholders from European countries and some international ones too |
| WP1 | M1.1.1. | Spatial and temporal sampling of different populations and species | France, CNRS | 30 Jun 2018 | Completed, even more than planned . | **CNRS, AWI, CCMAR:** Spatial and temporal sampling along the distribution gradient was conducted for four kelp species: *S. latissima, L. digitata, L. hyperborea* and *L. ochroleuca*. Sampling for demography,(see table XX) physiology, biochemistry and genetic temporal sampling was achieved |

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|  |  |  |  |  |  | **CCMAR, AU, IMEDEA, CIIMAR, IMAR:** Sampling of many kelp and fucoid populations spatially and temporally. See tables for more detail.<br>**AMU, UniBo, UT:** For the Mediterranean species please see tables for more detail.<br>**UM:** Sampling of fucoid populations spatially and temporally from the Strait of Gibraltar and Alboran sea.<br>Wide variations in demography and reproductive ecology variables among populations of *F. guiryi*, due to microclimate and local effects (thermal regime, exposure, standing biomass). Demographic parameters from these populations follow the latitudinal comparison by range index made by Zardi et al 2015.<br><br>Populations with low cover and density tend to present small and non-fertile individuals.<br><br>Extinct populations were detected at Punta de Calaburras (Málaga) and several locations in Ceuta. |
| WP3 | M3.1.2. | Collation of relevant mesoscale oceanographic variables from Atmospheric-Ocean General Circulation Models under different Representative Concentration Pathways | Portugal, CCMAR | 30 Jun 2018 | Completed on time. | **CCMAR, IMAR** and **UGhent:** work was completed and has been made available in: Bio-Oracle2: Assis et al. (2017a) and used in several publications (e.g., Assis et al. 2017b, 2018; Buonomo et al. 2017) |

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| WP3 | M3.2.1. | Selection of potential donor populations for restoration | Italy, UniBo | 30 Jun 2018 | Completed | **UGOT** identified donor populations for restoration of *Fucus* populations in the Baltic Sea.<br>**UniBo, AU, IMEDEA** and **CCMAR** identified appropriate donor populations for some species of *Cystoseira*.<br>**CIIMAR, CCMAR** and **UT** for *Fucus* in the Mediterranean.<br>**CCMAR, AU** and **CNRS** for some kelp species |
| WP1 | M1.1.2. | Sampling of sporophytes in order to obtain gametophyte for crosses between different populations in the different study species | France, CNRS | 31 Aug 2018 | completed | **CNRS:** Sampling and crosses are already done for L. *digitata and S latissima. Two paprs are in preparations*<br>**AWI** and **CCMAR** completed crosses among *L. digitata* and between *L. digitata* and S-Atlantic *L. pallida* (Liesner 2020, Liesner et al. in prep, Martins et al. 2019). |
| WP1 | M1.3.3. | Significantly regulated transcripts identified and correlated to treatments and origin of strains | Germany, UC | 31 Aug 2018 | Completed | **AWI**: Numerous genes significantly induced by temperature and salinity stress have been identified in two *S. latissima* strains from contrasting environments (Spitsbergen / Brittany) (see Li et al. / Monteiro et al.). More studies dealing with *L. digitata* are finished but not yet sequenced or analysed.<br>**CCMAR** with **AWI, CNRS, IMAR, AU, IMEDEA, UT, CIIMAR:** analyses of transcriptomes of several kelp and fucoid species ongoing. |
| WP2 | M2.2.2. | Biophysical modeling | France, AMU | 31 Aug 2018 | Completed for some species. Still, extra species | **CCMAR** and **UniBo:**<br>Completed this work for a kelp and a fucoid (*Cystoseira*) species: |

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|  |  |  |  |  | are planned to be added to the original ones. | Assis (2017b) ; Buonomo et al. (2017)<br>**AMU** the work is almost completed a fucoid has been published Thibaut et al., (2017); on *C. zosteroides* the ms is underwriting. |
| WP3 | M3.3.2. | Demonstration of the importance of selecting life stages with specific natural traits in regions of seaweed blue economy | France, CNRS | 30 Set 2018 | Completed. | **ALL:** This question was addressed in the stakeholder meeting in Roscoff and in the white paper in progress |
| WP1 | M1.2.2 | Temperature gradient experiments on gametophyte reproduction including sampling for gene expression | Germany, AWI | 31 Oct 2018 | Completed in 2020 | **AWI / CCMAR** in coop with **CNRS, Bodö, UBremen:** Focus on *Laminaria digitata*: Achieved with a diversity of strains from contrasting environments along full distribution gradient resulting in several publications and student theses. Martins et al. 2017, 2020, Liesner et al. 2020, Gauci 2020, Schimpf 2021; Pearson et al. 2019, Monteiro et al. 2019)<br>**UM:** Focused only on intertidal fucoids. Thermal experiments on early-life stages reported on M 1.3.1. |
| WP2 | M2.4.3 | Collation of ecophysiological response/tolerance limits from the literature and previous project tasks for mechanistic niche modeling | Portugal, IMAR | 31 Oct 2018 | Completed. | **AWI, CCMAR, NIBIO, UGhent:** This collation has been completed for the white paper and is under preparation for publication. |

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| WP1 | M1.2.1. | Temperature gradient experiments on sporophyte reproduction of kelps including sampling for gene expression | Germany, AWI | 30 Nov 2018 | cancelled | **AWI:** This milestone was not achievable as induction of fertility failed. As the experiments are long-lasting and include massive field sampling they were cancelled. |
| WP2 | M2.1.1 | Comprehensive sampling of populations along the European coast | Sweden, UGot | 31 Dec 2018 | Completed for the target species. | **UGOT** has comprehensive sampling along the Baltic-Skagerrak coast (44 localities, including Sweden, Finland, Estonia, Russia, Latvia, Germany and Denmark, and additional samples from Norway, Wales, France and Spain. **CCMAR, AWI, CNRS, AMU, AU, UGhent, UniBo, UT, MBA, NUIG, UR, FRI,** all have been contributing for the sampling of Atlantic and Mediterranean species, even more than originally planned.<br>**CNRS/CCMAR/AMU** The same for the five study kelp species (Ld, Lh, Lo and Lr) |
| WP2 | M2.2.3 | Population genotyping with microsatellites and NGS | France, AMU | 31 Dec 2018 | completed | AMU, CNRS; CCMAR: This milestone is completed for all microsatellite genotyping (see Table 4) and SNPs |
| WP2 | M2.3.1. | Engage coastal users and small local business | Portugal, CCMAR | 31 Dec 2018 | Completed, | **CCMAR:** Several workshops and meetings took place with stakeholders linked to seaweed uses. Dissemination over social media promoted wide participation of citizens. |
| WP2 | M2.4.4. | Fitting of final models, production of maps and comparisons | Portugal, IMAR | 31 Dec 2018 | Completed, | **CCMAR** and **IMAR, AU, IMEDEA, UM, IBERS, CNRS, UniBo:** models, maps and comparisons across many species of European marine forests. See papers: Assis et al. (2018); Bermejo et al. (2018); Buonomo et al. (2018). |

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| WP3 | M3.2.2 | Experimental assays of population recovery | Italy, UniBo | 31 Dec 2018 | Completed,. | **UniBo, UT, CCMAR, CIIMAR:** Kelp and fucoid restoration experiments were conducted in the Atlantic and Mediterranean.<br>**UM:** Optimization of protocols for zygote extraction and culture of *Fucus guiryi* and *Carpodesmia tamariscifolia* from the Strait of Gibraltar and Alboran Sea, respectively, for future restoration purposes. |
| WP1 | M1.3.1. | Key temperature conditions identified for species under consideration and for different isolates along the planned geographical gradient in short and long-term experiments and through physiological measurement | Germany, UC | 28 Feb 2019 | Completed | **AWI, CCMAR; CNRS AMU:** Key temperature responses identified among populations and key species collated along distributional gradients.<br>L. digitata: Liesner et al. 2020 a, b, Gauci 2020, Schimpf 2021, Martins et al. 2017, 2020).<br>Crosses between *L. digitata* and *L. pallida:* Martins et al. 2019<br>*S. latissima:* Diehl et al, in prep.<br>*L. ochroleuca:*<br>Barreto et al. in prep<br>*L. hyperborea:*<br>Barreto et al.in prep.<br>*L. rodriguezii*: new data were generated for the first time - one location: Pageault 2020; Pageault et al, in prep; Martins et al. in prep<br>**UM:** Conducted several experiments with adults and early-life stages of *Fucus guiryi* from Tarifa and |

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| | | | | | | *Carpodesmia tamariscifolia*, measuring growth, survival, developmental stages and photosynthetic responses.<br>**Thermal limits of early-life stages of *Fucus guiryi* shifted seasonally.** Recruits from early summer had lower thermal tolerance and narrower thermal limits than winter ones. Mean optimal temperature for recruits was between 15-23ºC. **Extreme temperatures at the onset of summer would hamper recruit survival for this species.** A manuscript is under review (Sánchez de Pedro et al).<br>· **A simulated marine heat-wave experiment** was performed in July 2019 with different life-stages of *F. guiryi*. Emersion promotes thermal resilience in adults of *F. guiryi*, withstanding short exposures up to 36-39ºC, but recruitment ceased after the simulated heat-wave. Samples for gene expression of HSP proteins were taken but pending final analyses in 2021. Photosynthetic responses are still under analysis.<br>· A comparative study along three populations of *C. tamariscifolia* from a natural thermal gradient in the Alboran sea was conducted. Strong influence of local conditions on responses to thermal resilience was detected. Thermal resilience increased spatially towards the most Mediterranean population. Results reported in a conference paper (Campos-Cáliz et al 2019). |
| WP1 | M1.2.3. | Biochemical characterization of kelps from different populations | Germany, AWI | 31 Mar 2019 | completed | AWI, in coop with UBremen/ URostock:<br>*L. digitata*: mannitol, pigments, CN (Liesner et al. 2020; Gauci 2020) |

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| | | / species and after experimental treatments | | | | *S. latissima*: mannitol, phlorotannin, pigments, CN (Diehl et al. in prep)<br>*L. solidungula*:<br>mannitol, CN, pigments (Diehl et al. 2020)<br>**UM**: Measurements of total carbon and nitrogen in embryos of *Fucus guiryi* from Tarifa. Seasonal differences detected and reported in manuscript under review (Sánchez de Pedro et al) |
| WP1 | M1.3.2. | Transcriptomes assembled | Germany, UC | 30 Apr 2019 | 31 Dec 2019 | **AWI, UC, CCMAR:** Transcriptomes were assembled for all the main kelp species and *Fucus* species of Europe |
| WP1 | M1.1.3. | Sporophyte and gametophyte life span estimates | France, CNRS | 30 Jun 2019 | Completed, | **CNRS/AWI:**<br>life span of gametophytes in the laboratory is probably endless (>40 years reported without loss of fertilty). We were not able to design and perform in situ experiments but there is published evidence for perennuality in situ as well.<br>**CNRS/ AWI / CMMAR:** demographic datasets all over Europe have been gathered for 4 kelp species. *L. digitata*: a clear gradient from the Arctic to the southern distribution egde is visible: Long lived plants in the Arctic (4-7 years), short lived plants in the south (1-3 y). This will only be possible for sporophytes, based on demography in *L. digitata* and *L. hyperborea*. The longevity of gametophytes appears to last much longer than the duration of this project, at least under certain environmental conditions. |

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| WP2 | M2.2.4. | Statistical analyses and integration of genetic and modelling results | France, AMU | 30 Jun 2019 | Completed, | **AMU:** Matrix of connectivity is under comparison with genetic connectivity on *C. zosteroides, L. rodriguezii.* **CCMAR, UGOT, CNRS, UT, UniBo,** et al. have done this for several species. |
| WP3 | M3.1.3. | Fitting of final models for 2050 and 2100, integrating seascape genetics Identification of regions of sink, source and corridors for range shifts. | Portugal, CCMAR | 30 Jun 2019 | Completed, | **CCMAR, SAMS, IMAR, UGhent, MBA, UP:** Tools for this were developed, when all data were available. |
| WP3 | M3.3.3. | Assessment of the risks of gene flow between cultivated and natural populations | France, CNRS | 30 Jun 2019 | Completed, | **CNRS:** This milestone was completed in connection with the GENIALG project. The risk of cross breeding (due to existing gene flow) between wild and farmed strains was estimated by two approaches. A set of 30 microsatellite markers have been applied to perform an assignment test (Geneclass2; Piry et al., 2004) that was used (1) to estimate the likelihood for an individual to be assigned to its locality of origin on a collection of 20 localities and distributed across the NE Atantic and (2) by comparing three farms/wild populations. The results suggest that local gene flow among geographically close locations can naturally happen for distances up to ~100 km |
| WP2 | M2.1.2. | Successful application of genomic marker approach, and | Sweden, UGoat | 31 Aug 2019 | Completed, | **UGOT** has DNA extracted, RADSeq libraries prepared and sequenced samples from: |

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| | | establishment of RAD libraries. | | | | 46 localities Baltic-Skagerrak, and we have DNA extracted, RADSeq libraries prepared from additional 4 localities.<br>**CNRS / AMU**<br>ddRAD seq SNPs were developed and analysed in four species *L. digitata, S latissima, L. rodriguezii* and C. zosteroides . We sequenced the genome of C. zosteroides. The RAD data of *Laminaria* were analysed thanks to the genome provided by the Phaeoexplorer project (M. Cock) |
| WP3 | M3.1.4. | Local meetings with stakeholders to collaboratively deliver guidelines assisting marine management and climate policy | Portugal, CCMAR | 31 Aug 2019 | Completed, | ALL: Many meetings with stakeholders have been taking place with this goal, and more will continue. UGOT has in collaboration with the Swedish Agency for Water Management (SWAM) launched the first pilot program for monitoring of genetic biodiversity in which *Fucus vesiculosus* is one of ten target species. This program will hopefully be permanent from 2022 and include sampling of *Fucus vesiculosus* around the complete Swedish coast (approx 20 sites) each 3rd year to follow genetic changes that are expected in parallel with temperature increase of the Baltic Sea. The program will also lay the foundation for science-based management of *Fucus* and the other target species in the area. |
| WP3 | M3.3.4. | Local meeting with stakeholders (industry) to collaboratively deliver guidelines assisting the seaweed blue economy | France, CNRS | 31 Aug 2019 | Completed | Workshop<br>Roscoff<br><br>Forum IDEALG 27 November 2019 in Roscoff. |

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| | | | | | | This meeting was open to local stakelhoders and the discussion was centered on the challenges of algal resources in Brittany and in Europe |
| WP3 | M3.4. | Marine Forest conference | Portugal, CCMAR | 31 Dec 2019 | Cancelled due to the pandemic. Expected to take place when the pandemic is over. | **ALL:** This will be planned when real meetings in person will be possible again after the pandemic. Sponsors are being sought. |
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*4.3. Scientific outcomes*

This is an incomplete description because there isn't enough space to describe so many outcomes. We therefore report the readers to the pdfs of the papers to see the figures and tables with results.

The activities of all the objectives were developed in a very integrated mode within the MARFOR consortium, as the same species and populations were compared for all traits including genomic, ecological, and ecophysiological (objetives 1 and 2) and finally used for future predictions and implications (objectives 3 and 4). The main focus was the dominant European kelp species, which are mainly *Laminaria digitata, Saccharina latissima, L. hyperborea* and *L. ochroleuca*, but many other species were studied, includin the Mediterranean kelp *Laminaria rodriguezii* and fucoid species from the Arctic to Mediterranean.

## Cold-adapted kelp: *Laminaria digitata*:

Partner AWI together with CNRS analysed the genetic diversity and its functional response towards summer heat wave scenarios of one major coastal bioengineering and economically important brown kelp species, *Laminaria digitata*. We sampled six populations along the species' whole Northeastern Atlantic distribution gradient ranging from the southernmost distributional limit in Quiberon (Brittany, France), over Roscoff (Brittany, France) and Helgoland (North Sea, Germany) to Bodo, Trömso (Norway mainland) and one of its northernmost Arctic sites in Spitsbergen (Norway). The environment of all populations was very different with respect to the amplitude of annual mean monthly sea-surface water temperatures, ranging between 0 and 7°C (Spitsbergen) to 5-17°C (Brittany). We wanted to provide an understanding for the thermal plasticity of a coastal foundation species across its latitudinal distribution gradient and correlate it with genetic features and phylogeography. It was hypothesized that populations from Arctic and cold-temperate locations are less heat resilient than populations from warm distributional edges. Results from the heat stress experiment together with other published evidence suggest that the **upper temperature limit of *L. digitata* is nearly identical across its distribution range**, but **subtle differences in growth and stress responses were revealed for three populations from the species' ecological range margins**. In *L. digitata* from the northernmost population (Spitsbergen, Norway), showed a slightly higher sensitivity towards heat stress. Parallel microsatellite genotyping revealed all sampled populations to be genetically distinct, with a **strong hierarchical genetic differentiation between southern and northern clades**. **Genetic diversity** was lowest in the isolated population of the North Sea island of Helgoland and **highest in Roscoff in the English Channel**. All together, these results support the hypothesis of moderate local differentiation across *L. digitata's* European distribution, whereas effects are likely too weak to ameliorate the species' capacity to withstand ocean warming and marine heatwaves at the southern range edge (see Liesner et al. 2020, Ecology and Evolution).

As it became evident that despite presence of ecotypic differentiation in the kelp *L. digitata* (Liesner et al. 2020, EcolEvol), the overall differences between populations were subtle, partner AWI further quantified the degree of phenotypic plasticity within single kelp populations. Phenotypic plasticity (genotype by environment interaction) is an especially important ecological means for sessile organisms, such as kelps, to cope with environmental variation. But knowledge about plasticity in kelps and also seaweeds as a whole is poor. We focused on one population (Helgoland), trying to evaluate for the first time in kelps, the plasticity of reaction norms and its dependence on the thermal history the species may encounter during ontogeny. This was achieved by following the reaction norms of juvenile kelps from five distinct genetic lines of *L. digitata* in a full factorial approach raised under a set of different thermal histories. We hypothesized that thermal plasticity increases trait performance of juvenile sporophytes in experimental temperatures that match the temperature experienced during ontogeny (gametogenesis, recruitment), and that plasticity differs among genetic lines (genetic variation for plasticity). Two major results became evident that both provide evidence that only cold temperatures during early ontogeny may (1) promote growth in adolescent sporophytes and (2) induce a high variation for plasticity in reaction norms towards cold and high temperatures. This was in contrast to expectations and provides evidence that **the cold-temperate to Arctic kelp species, *L. digitata* is impaired by warm temperature during gametogenesis and recruitment in growth and plasticity and that resilience in nature may be dependent on cold temperatures** (Liesner et al. 2020, Frontiers).

The **importance of cold temperatures for the development of *L.digitata*** was further supported in the frame of a master thesis (Gauci 2020) (involvement of partner AWI, AMU, CCMAR). A cross-generational beneficial effect of cold temperatures became evident. When sporophytes of *L. digitata* were recruited at 10°C from parental gametophytes which had been kept at either 5 or 15°C for 3 years, ontogenetic development was faster in material from cold parents. In addition, during further development, the sporophytes which developed from cold parental gametophytes showed a higher resilience towards sub-lethal cold (0°C) and high (20°C) temperatures than respective counterparts from warm parental gametophytes (Gauci et al, in preparation)

The differentiation of immediate ecophysiological response patterns of *L. digitata* populations along temperature gradients was investigated in a BSc thesis (Schimpf 2021, in preparation) to substantiate the data provided by Liesner et al. (2020, EcolEvol) and to detect possible local adaptation by partner AWI, CCMAR, CNRS, partially in cooperation with external partner Bodö. We hypothesized that local adaption is rather visible at low temperatures as these are experienced in situ by all populations, though with a different degree, while lethal temperatures are seldom encountered in the field over vast areas and thus may not have provided an environment for selection. The ontogeny of gametophytes (gametogenesis, recruitment, survival) from different populations collected along the south-north distribution gradient was thus compared under high to lethal warm temperatures (20,21,22,23,24, 25°C) and at the lower to optimum temperature range (0,3,6,9,12, 15°C). It became evident that as detected in Lienser et al. (2020) for sporophytes , the upper survival temperature of gametophytes from all populations was uniform - surviving long-term 23°C (14 days) without damage, but 24 and 25°C for up to 8 days with differential rates. However, **sporophyte recruitment along the temperature gradient differed significantly showing a clear north-south gradient with southern populations producing more sporophytes at higher temperatures**. Similarly at the lower temperature range there was a **clear differentiation between populations with respect to speed of fertility and survival partial in accordance with the south-north distribution gradient and thereby supporting that local adaptation** has taken place in this kelp species.

The subtle but nevertheless clear differentiation between the northern population from Spitsbergen and the southern Helgoland population was further substantiated by mechanistic common-garden experiments (AWI, CCMAR) contrasting the capacity of *L. digitata* gametophytes to withstand short-term heat-wave scenarios combined with high or cool recovery temperatures. **The Arctic population was better adapted to low recovery temperatures than the southern population** (Martins et al. 2020).

Preliminary population genomic analyses of the Atlantic species *L. digitata*, by CNRS and AMU, showed lower levels of genetic structure than in *L. rodriguezii*. We observed variable levels of population genomic diversity in *L. digitata*, with the population of Helgoland showing lower diversity and higher genetic drift than other populations. We will then use the developed SNPs to test for genetic association with environment in *L. digitata*: this will be compared with neutral differentiation (Leisner et al., 2020) and with observed ecophysiological response patterns of *Laminaria digitata* populations along temperature gradients (Reynes et al., in prep).

**Thermal tolerance crosses: cold adapted *Laminaria digitata x warm-adapted Laminaria pallida***

In two hybridisation studies between genetically and functionally different strains of *L. digitata* and between *L. digitata* and the genetically different southern Atlantic species *L. pallida* partners AWI, UC and CCMAR investigated the inheritance of thermal traits in juvenile offspring sporophytes. *L. pallida* was used instead of *L. ochroleuca* to represent a warm-adapted species because they are sister taxa genetically similar but only this way the hybrid crosses were not incompatible (Bartsch pers. obs). It became evident that either mid-parent or better-parent heterosis was expressed in hybrid sporophytes compared to parental selfing sporophytes with respect to their survival and growth capacity at sublethal to lethal warm temperature limits (Martins et al. 2019; Liesner 2020, Liesner et al, in prep.). The effect was mild for within species crosses and no clear difference in physiology at upper temperature limits was observed for crosses as compared to Helgoland selfings. However, hybrids needed to express less extra genes at lower expression levels to survive at upper temperature limit than selfings (Liesner 2020), **indicating that future conservation of kelp forest species could be facilitated via adaptive and selective breeding programs under the premise that clonal gametophytes of a range of populations will be kept in conservation centers and an ethical discussion about advantages and disadvantages of 'genetic contamination' versus loss *of kelp forests* is initiated and results implemented into European law.** The clear better-parent heterosis exhibited by interspecific F1 hybrids of *L. digitata x L. pallida* (Martins et al 2019) was the subject of a further study using allele-specific expression to identify genes, pathways and regulatory mechanisms of dominance contributing to increased hybrid thermal tolerance (Pearson et al in prep.). The results indicate that expression conservation is widespread (broadly indicating hybrid genome stability), but that expression level dominance of the more thermally tolerant genome (*L. pallida*) is dynamic, and results suggest a dramatic (4-5 fold) increase in the number of *L. digitata* genes regulated by the *L. pallida* genome under thermal stress. **These analyses may provide a useful tool for dissecting the mechanisms of heterosis, and have implications for strain selection and breeding in a commercial context**.

**Temporal changes in genetic parameters** were examined by Partner 4 (CNRS) in order to get information about population demography. Genetic differentiation between 10 years-sampling intervals in *L digitata* was significant for all isolated/marginal populations while allelic frequencies did not change with time in the well-connected/core populations. This result suggests that the **demographies of isolated/marginal populations were not stable in time** (Fouqueau et al. in prep). In this context, a theoretical model was developed to study the selective forces acting on reproductive mode evolution in the central and marginal parts of the distribution range of a species. The model shows that asexuality is often favored in marginal populations, as it can preserve adaptation to extreme conditions. Increasing maladaptation at range limits leads to stronger selection for asexuality (Fouqueau et al. submitted to Evolution).

## Arctic kelp: *Alaria esculenta*

Partners CCMAR and AWI also compared the performance and reproductive success of early life stages of the kelp species *A. esculenta* and *L. digitata* under **simulated Arctic** environmental conditions. The microscopic gametophytes from both species were able to **endure long periods of darkness in the winter,** however **only *L. digitata* was able to resume reproduction and sporophyte recruitment** after recovery in simulated spring conditions. Therefore, *L. digitata* may rely on banks of microscopic gametophytes persisting during winter months for the development of a new generation of sporophyte recruits later in spring and summer. During summer conditions, the gametophytes of *L. digitata* require irradiance protection by parental kelp canopy for their survival, reproduction and recruitment, while *A. esculenta* gametophytes showed no dependence on natural shading to become reproductive. **This study revealed differences in the survival, recovery and reproductive output of microscopic gametophytes of these two sympatric kelp species to different Arctic season conditions** (Silva et al. in preparation). In addition, the effect of light conditions (daylength and light intensity) on the gametogenesis and the recruitment success of the Arctic *Alaria esculenta* was also investigated. Although continuous daylight, characteristic of Arctic summers, enhanced gametogenesis and increased gametophyte vegetative growth, the recruitment of sporophytes was higher under long days of 16:8h daylength. Previous exposure to different daylengths during early life stages influences the sporophyte growth under different temperatures; overall the sporophytes previously developed under continuous daylight grew less compared to the sporophytes developed under shorter daylengths. However, no major differences were observed regarding the thermal tolerance limits between the sporophytes from different daylengths. This study showed that the **synchronization of fertilization in *A. esculenta* seems to be disrupted in continuous light leading to hampered recruitment** (Martins et al. in preparation).

## Arctic kelp: *Laminaria solidungula*

UC and AWI investigated for the first time the deep water Arctic endemic kelp *L. solidungula* with respect to possible genomic adaptations to its cold and low-light habitat and we looked into the molecular phylogeny of the species (Rana et al (2019). Draft genome sequencing was performed and besides genomic contigs also the complete plastid genome was assembled unambiguously. The *L. solidungula* plastid genome is colinear with all other known brown algal plastid genomes, i.e. all genes in such plastomes genes are at the same relative locations and in the same orientations. This finding allowed to easily reconstruct whole plastid genome alignments resulting in a robust **phylogenetic placement of *L. solidungula* within the brown algae, directly related to *L. digitata*.** Moreover, colinearity of plastomes allowed to identify genes absent or present in any species in the alignment. Whereas all other brown algae, including distantly related species such as *Fucus vesiculosus* and *Ectocarpus siliculosus*, contain an identical set of genes, one gene is missing in *L. solidungula*. The missing gene, ycf37, is of unknown function but it seems essential as it is present in many plastid genomes and in cyanobacteria. Functional analysis of a knockout mutant of ycf37 in *Synechococcus* revealed its involvement in building of photosystem I, where it seems to be required under high light conditions. It is possible that this protein is dispensable under the relatively lower light conditions in higher latitudes where *L. solidungula* exclusively thrives.

MARFOR supported a study of UBremen on the impact of **temperature and lowered salinity** of the endemic Arctic species *Laminaria solidungula*. Both drivers had significant and interacting impacts, either in an additive or antagonistic way, dependent on the respective response variable. The maximum quantum yield of photosystem II (Fv/Fm) significantly declined with temperature increase and low salinity. Even though the absolute pigment content was not affected, the deepoxydation state of the xanthophyll cycle increased with intensified stress. Higher temperatures affected the C:N ratio significantly, mainly due to reduced nitrogen uptake, while reduced salinity supported the nitrogen uptake, resulting in an attenuation of the effect. While the concentration of the reserve sugar mannitol decreased at low salinities it was not affected at control salinities between 0 and 10 °C but significantly decreased at 15 °C, a sub-lethal temperature for the species. Conclusively, it was revealed that **the interaction of salinity decrease and temperature increase will be especially detrimental to this Arctic kelp species** (Diehl et al. 2020).

Through cooperation of UBremen, AWI and UC we also studied the adaptive capacity of kelps at the transcriptomic level in different life stages (gametophytes versus sporophytes), from different habitats (Spitsbergen versus Roscoff), and for different stressors (temperature, salinity). Results were published in a series of 4 papers (Monteiro et al. 2019a, b, Li et al. 2020a, b).

### Northern-central kelp: *Saccharina latissima*

There was a great focus on the very economically important European kelp species, *Saccharina latissima*. The physiological responses of *S. latissima* from Spitsbergen and Roscoff did not differ a lot in reaction to enhanced temperature or reduced salinity yet the trancriptomes could clearly be distinguished for both treatments and origin in a principal component analysis. On the molecular level, Roscoff *S. latissima* was more tolerant to higher temperatures, whereas the Spitsbergen isolate was better adapted to low salinity. The basis of this analysis was the number and nature of regulated genes and not their physiological responses. We conclude that (1) **local adaptation exists in kelps at the level of gene expression** and (2) **that transcriptomic studies allow to determine the nature and quality of local adaptation more precisely than physiological parameters**.
In another experiment we compared the response of male versus female gametophytes to temperature treatments on the level of growth, photosynthesis and gene expression in *S. latissima*. Again the **physiology between sexes did not differ whereas a clear separation was observed in gene expression patterns for females versus males at all temperatures tested**. As the gametophytes of both sexes stemmed from one sporophyte we conclude that **differences in transcriptomic responses could be related to the sex-linked loci** rather than genetic variability among gametophytes.
For Arctic *S. latissima* we compared a combination of temperature and low salinity treatments to assess the possible threat of global change, expressed by enhanced temperatures and reduced salinity through glacier melting, for Arctic kelps. We found that **expected warming in Arctic waters lies well within the tolerance of *S. latissima* whereas reduced salinity poses a threat**. This was true at all levels from physiology to transcriptomic responses but most pronounced at the level of gene expression.

Partners CCMAR and AWI investigated transcriptional changes during the transition from vegetative growth to reproduction in male and female (i.e., dioicous) haploid gametophytes of *S. latissima* (Pearson et al. 2019). In haplodiplontic species, sexual reproduction occurs uncoupled from meiosis.

Transcriptional responses to gametogenic conditions yielded results describing both core sex-independent genes and pathways (e.g., ribosome biogenesis, transcription and translation) as well as the sex-biased activation of specific male (e.g., mitotic proliferation) and female (e.g., ROS signalling) pathways for sperm and egg production. **The results add significantly to genome-level understanding of gametogenesis in this ecologically and economically important lineage**.

AWI and UC in cooperation with UBremen, URostock and Bodö investigated the resilience of *S. latissima* populations collected along the latitudinal gradients under heat wave scenarios and in interaction with lowered salinity. The latter aspect is especially relevant at Arctic sites where glacier melting and increased freshwater run-off may lead to a decrease in coastal salinity, especially during summer time. A high degree of plasticity was observed between local populations indicating slight local adaptation, especially with respect to **salinity tolerance which was better in Arctic material** (Monteiro et al. 2019, 2020). Increase of **local summer temperatures up to 6 °C for a week was not harmful** at all for northern populations (Spitsbergen, Tromsö, Bodö) but rather ameliorated their response while two populations from the southern margin (Helgoland, Locmariaquer) suffered at the rise by 6°C treatment but did not die (Diehl et al., in prep). In contrast to the study with *L. digitata*, precise survival limits of local gametophytes and sporophytes under identical conditions have not been investigated and thus the general survival potential of this kelp species at warm temperature limits is not known. **Biochemical parameters and growth activity were highly plastic in *S. latissima* irrespective of the latitudinal gradient of sampling suggesting a great variety of genotypes and the need to support this unknown source of genotypes for mariculture or conservation management**.

Partner CNRS conducted an analysis to test for statistical correlation between genetic marker allele frequencies and three environmental proxies (sea surface temperature, salinity, and water turbidity) in the kelp *S. latissima,* at the Pan-European scale (from Northern Iberia to Spizbergen). **Seven SNPs and 12 microsatellite alleles were found to be significantly associated with at least one of the three environmental variables** (Guzinski et a., 2020). We speculate on the putative adaptive functions of the genes associated with the outlier markers and the importance of these markers for successful conservation and aquaculture strategies for *S. latissima* in this age of rapid global change.

The question of the variation of the compatibility of crossbreeding between individuals according to their similarity and of the selective value of their descendants was addressed in *L. digitata* and *S. latissima* by the CNRS partner. For both species we found **a significant effect of inbreeding depression but the effect was population dependant and increasing with the age of the offspring**). **We were not able to detect a general effect of outbreeding depression or heterosis, but again results were population dependant**. This knowledge has important consequences on the management of natural resources and on the development of aquaculture (a paper is in prep and should be published in 2021).

## Southern kelp: *Laminaria ochroleuca* and Central kelp: *Laminaria hyperborea*:

CCMAR in cooperation with AWI and CNRS investigated the ecophysiological responses to simulated marine heatwaves in central and edge populations of *Laminaria hyperborea* (cold temperate) and *Laminaria ochroleuca* (warm temperate) along their respective distributions (PhD thesis of Barreto

2022, in preparation). The general hypotheses tested were that population differentiation in thermal resilience correlates with environmental variation along a latitudinal thermal gradient, and that the capacity for local adaptation may interact with variation in genetic diversity/marginality. **Common garden experiments with *L. hyperborea* adults showed a surprising population differentiation with no resilience of the Southern edge population to the highest temperature** tested (23ºC) with 100% mortality during recovery in both nutrient enriched and unenriched treatments. Similar experiments with ***L. ochroleuca* adults showed that nutrient depletion had a higher effect than the temperature gradient.** All populations were able to recover at 25ºC with nutrients, but lower photosynthetic performance ($rETR_{max}$ and $F_v/F_m$) was observed in unenriched conditions.
**To remove possible environmental carry-over effects, *L. ochroleuca* F1 juveniles were raised** from isolated gametophyte cultures. Here, population differentiation was found despite the absence of habitat differences, **revealing lower photosynthetic performance at 25ºC for the northern population** under both nutrient enriched and unenriched treatments. A similar approach is planned to compare *L. hyperborea* F1 sporophytes at the population level, while transcriptome profiling (RNA-seq) experiments to identify gene expression variation in *L. ochroleuca* F1 sporophyte populations are underway.

**CCMAR in collaboration with AWI** performed experiments with F1 sporophytes of 5 Atlantic *Laminaria* species (*L. solidungula, L. digitata, L. hyperborea, L. ochroleuca, L. pallida*) derived from gametophytes (AWI culture collection). The aim was a comparative analysis of species-specific thermal responses, using common protocols and exposures between 5-22ºC for 7 days. Growth and physiological parameters together with RNA-seq data for transcriptional profiles were obtained (Martins et al., in prep.).

**All the above mentioned studies clearly support the existence of local ecotypes along latitudinal gradients. Differences however mostly appear at sub-lethal temperatures while there are indications that lethal temperatures are quite fixed within species.**

### Deep-water kelp of the Mediterranean: *Laminaria rodriguezii*

The MARFOR consortium facilitated cooperation between partner AWI, AMU, UC and CCMAR that resulted in the participation to a **deep-water expedition** organized by AMU in summer 2019 in the Mediterranean Sea. The aim was to sample and further investigate the genomic diversity and the ecophysiology of the unique deep-water kelp species *Laminaria rodriguezii*. No ecophysiological information was available beforehand. AWI managed to isolate gametophyte cultures for the first time. AWI and CCMAR both performed first experiments on growth, survival and photosynthetic responses of *L. rodriguezii* (MSc thesis Pageault 2020, Martins et al, unpublished). It became evident that this species presents **warm-temperate characteristics with a growth optimum between 15 and 20°C and sporophyte survival capacities ranging between 22 and 24°C,** depending on the development stage, with juveniles being more susceptible than adults. The obtained results also showed that ***L. rodriguezii* is well adapted to the low irradiances encountered at its deep collection site (approx 60 m) and probably is restricted from shallow sites by a combination of high water temperatures and high irradiances**.

Partners CNRS and AMU observed an important and significant genetic structure in the deep Mediterranean species *L. rodriguezii*. The use of ddRAD-Seq allowed us to show signals **of clonality**

**(heterozygote excesses, linkage disequilibrium) at different levels depending on the population of *L. rodriguezii***. We were also able to analyse the impact of partial clonality among loci in the genome (Reyneset al., under revision).

**AMU** assessed the demographic structure of *L. rodriguezii*. The population structure is still under study and shows a great ease of cuttings by short runners, which corroborates the results of population genetics which show a **strong clonality** within the populations of Magaud Bank and no inter-population exchange. Questions remain on the lifespan of this species, observations on the number of blades produced per year do not correspond to the size of the individuals nor to the age assumed by dendrochronology. This supports the need to put in place **strong conservation measures** for each population discovered.

Through a MARFOR cooperation of AWI, UC and AMU we attempted to identify genomic adaptations shared by the deep water species *L. solidungula* from the Arctic and *L. rodriguezii* from the Mediterranean Sea, performed draft genome sequencing, and assembled complete plastid and mitochondrial genomes, respectively, from *L. rodriguezii*. Again, the organellar genomes are colinear with other brown algal organelle genomes yet, opposed to *L. solidungula*, *L. rodriguezii* still contains the ycf37 gene in its plastid genome. However, when comparing brown algal mitochondrial genomes we detected an introduction of a foreign gene into the mtGenome of *L. rodriguezii*. This gene has no homologue in any database and is thus of unkown function. However, parts of the new gene we also found in the mitochondrial genome of a distantly related brown alga, *Desmarestia viridis*. The overlapping part of these genes are well conserved indicating that this is a functional gene. Introduction of genes into organellar genomes are very rare and sometimes facilitated by so-called mito viruses. We therefore assume that a yet unknown mito virus has independently infected *D. viridis* and *L. rodriguezii*, respectively, in the past and left its traces in their mitochondrial genomes. The level of conversation suggests a possible function of the introduced sequences (Rana et al. under revision).

## Kelp and Fucoids:

CCMAR used ecological niche modelling to predict genetic diversity hotspots for eight species of large brown algae with different thermal tolerances (Arctic to warm temperate), estimated as regions of persistence throughout the Last Glacial Maximum (20,000 YBP), the warmer Mid-Holocene (6,000 YBP), and the present (Assis et al., 2018).

A fine-tuned global distribution dataset of marine forests (Assis et al. 2020) of large brown algae was compiled, curated, and made available with its publication.

The MARFOR consortium facilitated cooperation between partners CNRS, AWI, IMAR, AMU and CCMAR to increase the coverage of the sampling over the range distribution of several European kelp species (*L. digitata, L. hyperborea, L. ochroleuca, L. rodriguezii, S. latissima*) in order to test the prediction of these models concerning hotspot of genetic diversity and persistence of refugia. The MARFOR consortium also facilitated the use and **development** of new markers across the different laboratories increasing the power of estimation of genetic diversity. CNRS and CCMAR partners worked on the calibration of microsatellites between labs in order to combine their data sets. Twenty new microsatellite were developed in *L. digitata* by CNRS and their cross amplification was tested on 4 sister species (*L. hyperborea, L ochroleuca, L. rodriguezii* and *L. pallida* ) on sampled that were provided by CCMAR and AMU. Mauger et al, in prep). Next Generation Sequencing markers were also developed in collaboration between CNRS, AMU and CCMAR for 3 kelps species (Guzinski et al., 2020; Reynes et al, submitted to Mol Ecol) and in *Carpodesmia zosteroides* (Reynes et al., in prep).

The MARFOR consortium described the pattern of genetic diversity and structure of most of the kelps encountered along the Nort Eastern Atlantic and the Western Mediterranean coasts (in the cold-artic water species: ***S. latissima***: Neiva et al., 2018; Guzinski et al., 2020 and in ***L. digitata***: Liesner et al. 2020; Neiva et al., 2020 and paper in prep. ; in the temperate-cold water species: ***L. hyperborea***: Schoenrock et al, 2020 and Thomas et al. in prep; and in the warm-temperate/deep water species ***L. ochroleuca***: Assis et al. 2018 and ***L. rodriguezii***: Reynes et al. (under revison). The increased effort of sampling that was possible in the Marfor project allowed to better identify all the LGM refugia for these species with overlapping range distribution but different temperature affinities. The importance of the Artic colonization and secondary contact from southern peri-glacial refugia acting as reservoirs of genetic diversity was emphasized for the two cold-arctic kelp species while for the two warm-water species, hot spots of genetic diversity are observed in deep water populations. In ***Saccharina latissima***, three divergent, parapatric and to a large degree reproductively isolated phylogroups were identified in the Arctic/Atlantic, that are reminiscent of incipient species. They illustrate well the effects of past climates and vicariance as drivers of diversification and highlight the need to take into account cryptic intra-specific diversity in e.g. kelp farming, to avoid unintended genetic contamination of regional gene-pools or conversely to actively select for locally adapted populations. Additional work is required to determined how functional traits vary in these biogeographically and genetic distinct pools of *Saccharina*. In ***Laminaria digitata***, evidence for distinct trans-Atlantic pools exist, but genetic data cannot exclude concusively post-glacial colonization of the American side, where some degree of niche unfilling is apparent when compared to Europe where the species reaches the high-Arctic archipelago of Svalbard. Globally, These genetic data confirmed the niche modelling predictions made by Assis et al. (2018). Overall, **all kelp forests exhibited strong levels of genetic structure and the effect of habitat continuity to maintain genetic connectivity was confirmed.** The island of Helgoland was characterised by an extremely low within-population genetic diversity for the two kelp species found in this area. It suggests that this site experienced elevated levels of genetic drift that can be explained by its geographic isolation ("island" of rocky substrate surrounded by several hundreds of kilometers of sandy beaches) and their low effective population size. One can wonder about the maintenance of kelp forest in this site in the near future.

### Arctic fucoid: *Fucus distichus*

Additonal studies dealing with fucoid forests have been completed. *F. distichus*, one of the most polymorphic, plastic and widespread amphiboreal seaweed of the Northern hemisphere was shown to have distinct Pacific vs Atlantic gene pools, with most diversity unexpectedely concentrated in the Pacific. This patterns probably reflects a later colonization of the Atlantic, but modelling data also shows that climatic refugia are vast in the Pacific but negligeable in the Atlantic – in this latter the species is only present today in areas heavily glaciated during the last glacial (Neiva et al., in prep). Its not completely clear if its absence along temperate Atlantic latitudes reflects intra-specific trans-Arctic niche differences or competitive exclusion by related fucoids, but both are poorly understood and largely overlooked factors potentially constraining marine species distributions. Another CCMAR/ SBR collaboration revealed that modern oceanographic patterns (i.e. ocean currents), in addition to historical factors, explain modern population structure of an estuarine fucoid (Nicastro et al. 2020). Other collaborative efforts with independent partners clarified several aspects regarding the systematics, ecology and regional biogeography of *Cystoseira* spp. sensu lato (Mulas et al., 2020, Medrano et al. 2020, Sogadurska et al, in press.).

Two partners (CCMAR and CNRS) of the Marfor consortium were invited to participate at a review paper about the Phylogeny and Evolution of the Brown Algae (Bringloe et al. 2020) to synthesize these principal results describing the phylogenetic processes occurring in the kelp forests of the North western Atlantic.

**AMU** set up different restoration experiments. In the Mediterranean, large scale experiments on ***Carpodesmia amentacea*** have been set up on ports (Marseille, Antibes) in order to restore lost habitat during port extension. Preliminary results gave a survival success decreasing with time but reproduction occurs and new recruits have been observed and they became fertile.
A similar experiment has been performed on *C. mediterranea* (Agde) where the species had completely disappeared from the 80's. Results are similar than observed in *C. amentacea*. The critical features to recreate an ecosystem service with sea-surface dwelling species is the management of herbivorous fish predation and the slope of the substrate.
These results are encouraging toward a large-scale restoration of sea-surface marine forests but useless for subtidal species.

**Predicting the future species diversity of marine forests of large brown algae in Europe**

Table 4.3.1. Number of species per family included in species distribution modelling to predict the distribution of large brown algae.

| Family | Number of species |
|---|---|
| Agaraceae | 1 |
| Alariaceae | 5 |
| Chordaceae | 2 |
| Fucaceae | 21 |
| Halosiphonaceae | 1 |
| Himanthaliaceae | 1 |
| Laminariaceae | 12 |
| Phyllariaceae | 4 |
| Sargassaceae | 74 |
| Tilopteridaceae | 2 |

The marine forests dataset retrieved 390987 occurrence records for species of large brown algae in European Waters (Table 1; Figure 1). These were used in stacked species distribution modelling to predict current and future diversity patterns (species richness).

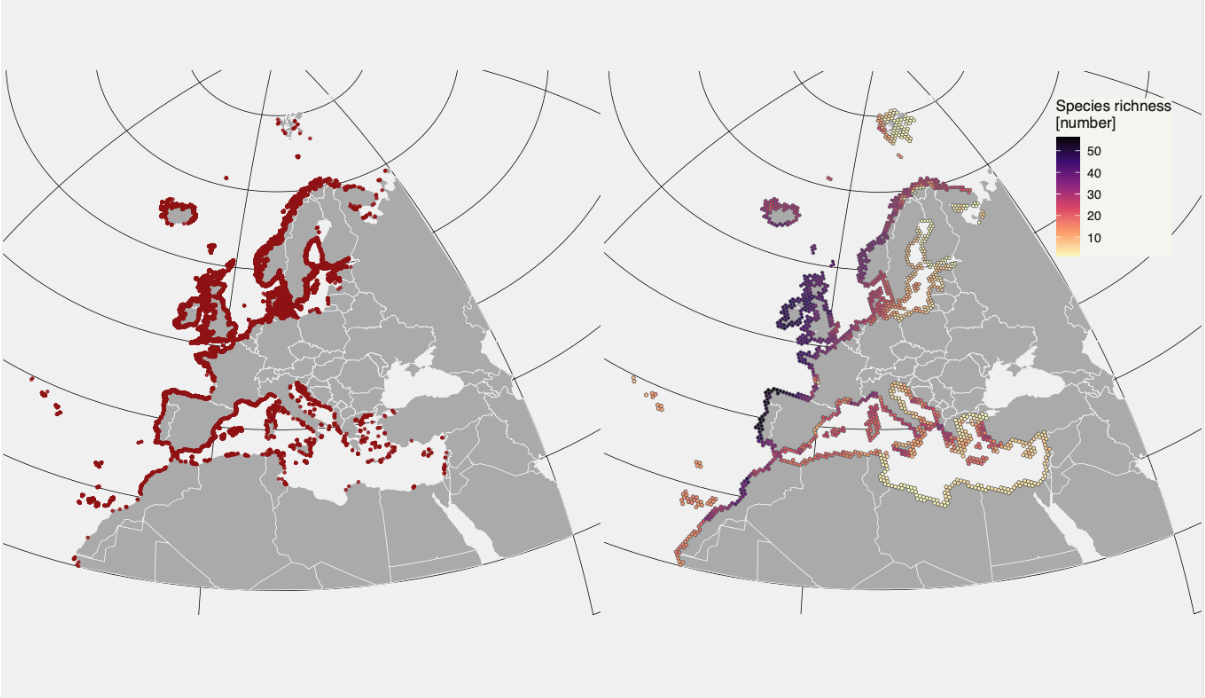

Figure 4.3.1. (left) Records of occurrence use in stacked species distribution modelling to infer the distribution of large brown algae. (right) Current predicted distribution of the European diversity (species richness) of large brown algae.

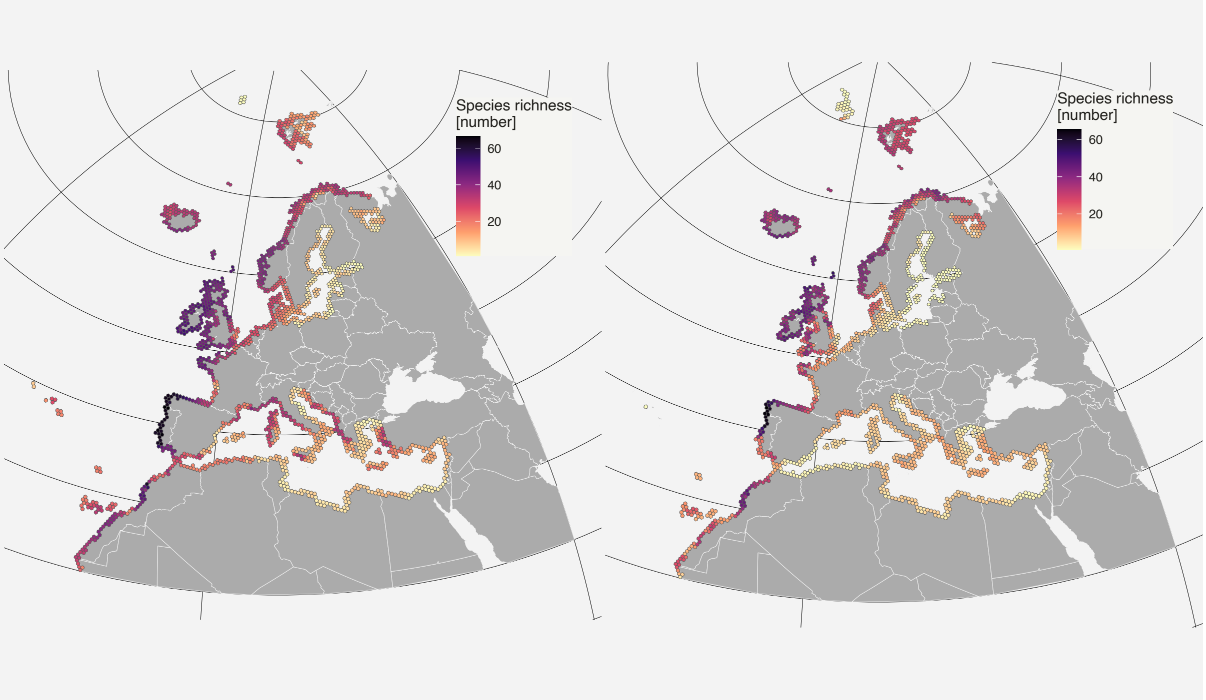

Figure 4.3.2. Future potential distribution of the European diversity (species richness) of large brown algae inferred with species distribution modelling under the (left) RCP26 and (right) RCP85 scenarios.

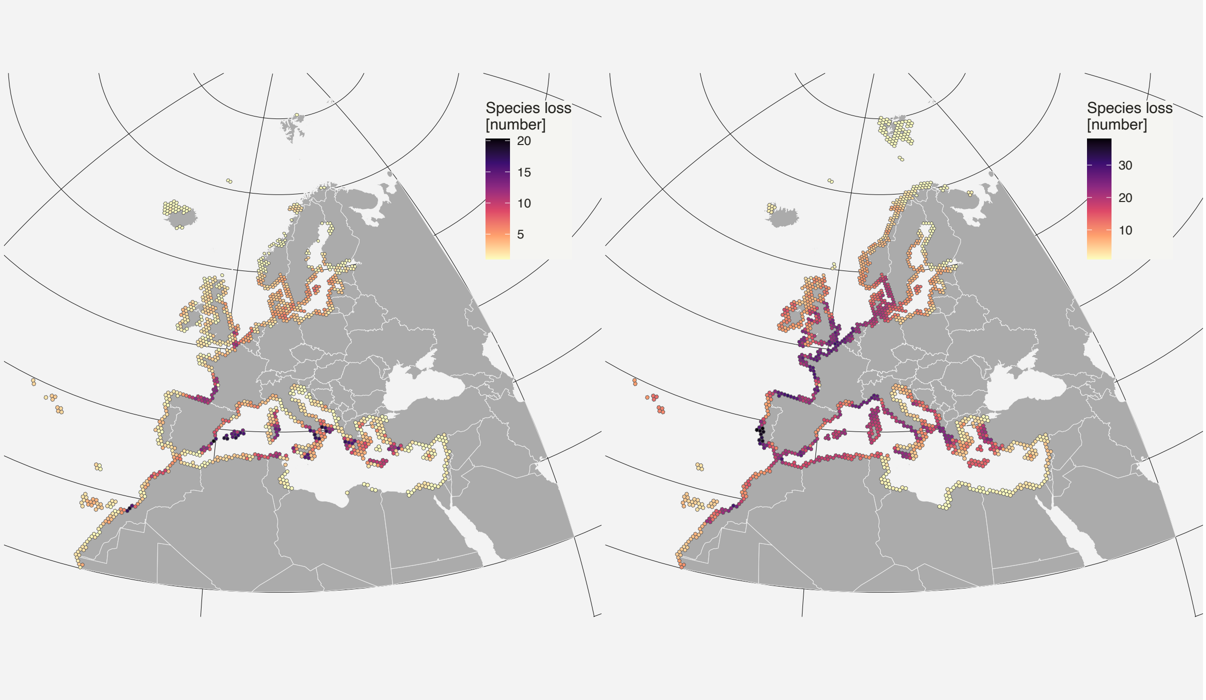

Figure 4.3.3. Number of species of large brown algae potentially loss under the (left) RCP26 and (right) RCP85 future scenarios.

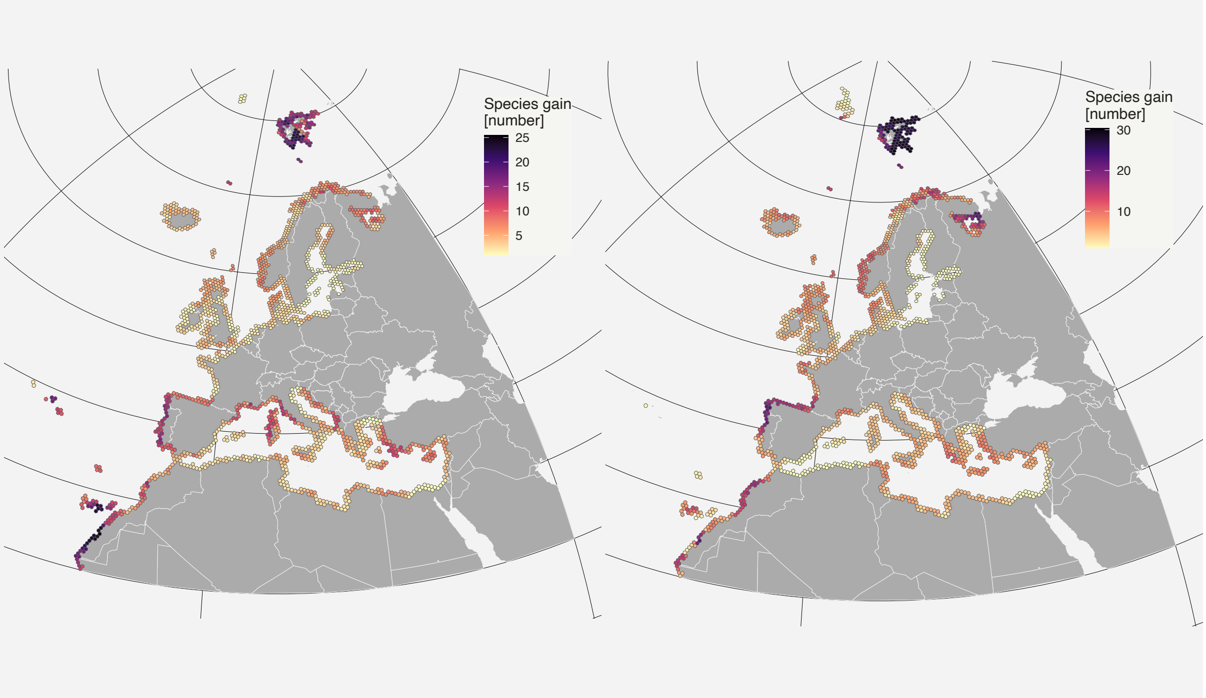

Figure 4.3.4. Number of species of large brown algae potentially gained under the (left) RCP26 and (right) RCP85 future scenarios.

## Predicting the future species diversity of kelp forests in Europe

Table 4.3.2. Number of species per family included in species distribution modelling.

| Family | Number of species |
|---|---|
| Alariaceae | 5 |
| Laminariaceae | 12 |
| Phyllariaceae | 4 |
| Tilopteridaceae | 2 |

The marine forests dataset retrieved 105019 occurrence records for kelp forest species (families Alariaceae, Laminariaceae, Phyllariaceae and Tilopteridaceae) in European Waters (Table 1; Figure 1). These were used in stacked species distribution modelling to predict current and future diversity patterns (species richness).

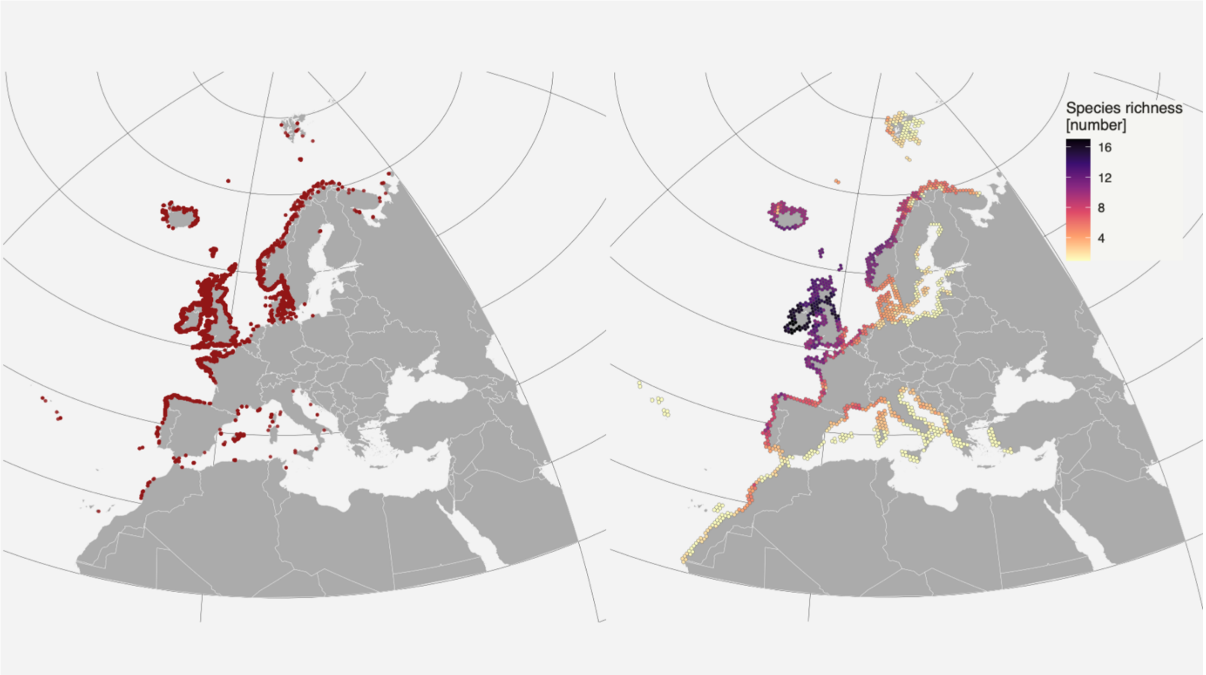

Figure 5. (left) Records of occurrence use in species distribution modelling to predict the distribution of kelp forests' diversity with stacked species distribution modelling. (right) Current predicted distribution of the European diversity (species richness) of kelp forests.

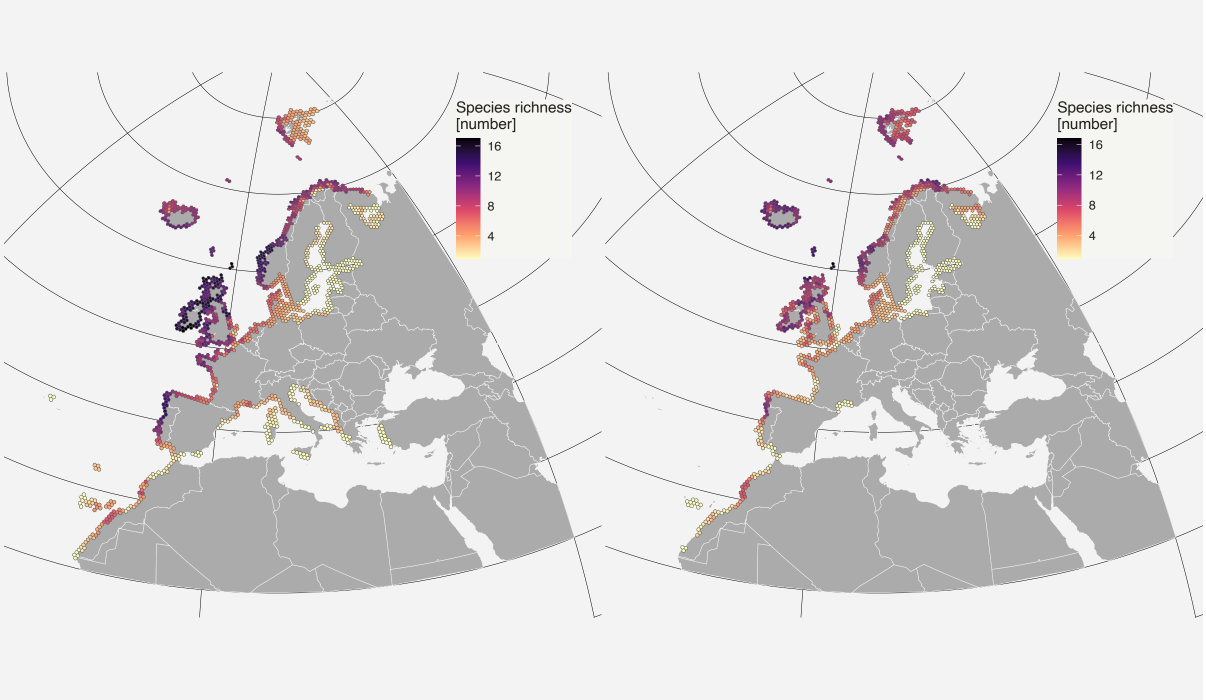

Figure 6. Future distribution of the European diversity (species richness) of kelp forests' diversity under the (left) RCP26 and (right) RCP85 scenarios.

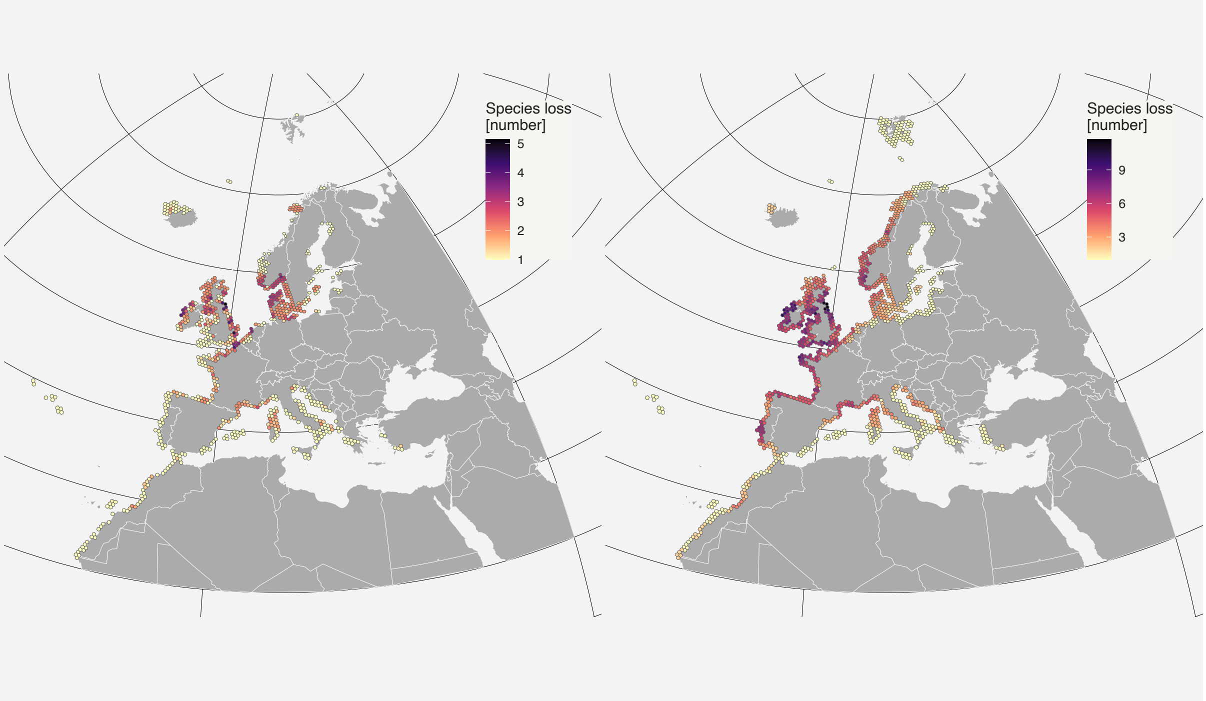

Figure 7. Number of species of kelp forests potentially loss under the (left) RCP26 and (right) RCP85 future scenarios.

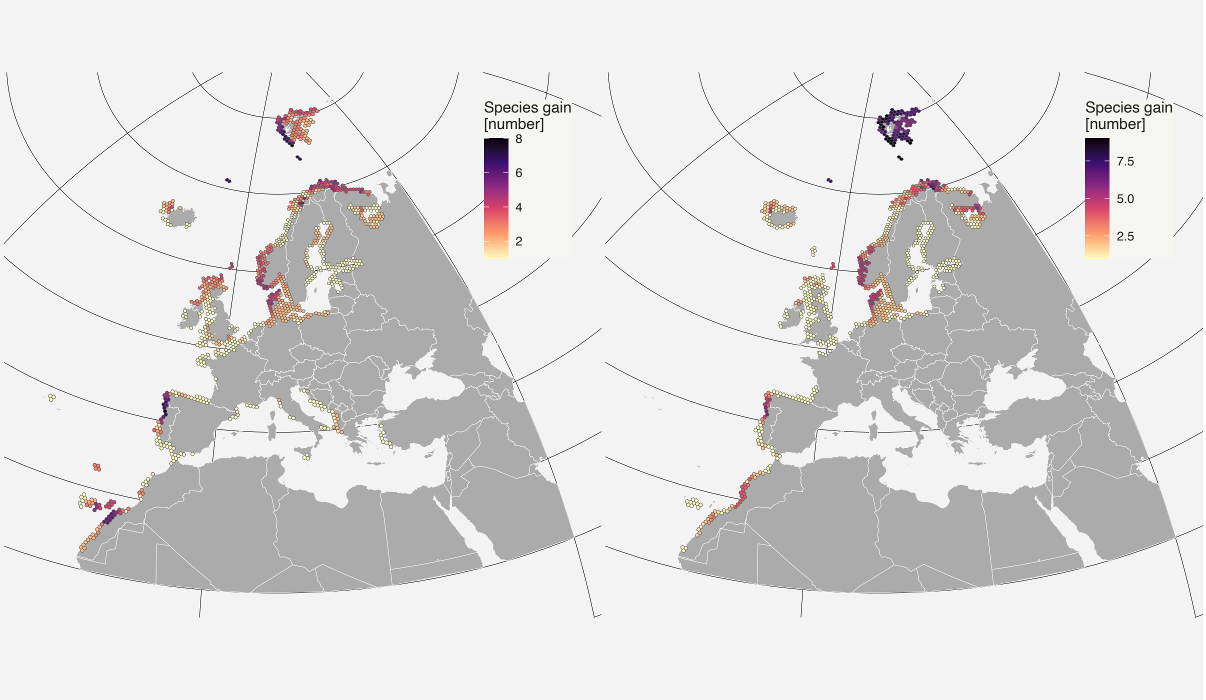

Figure 8. Number of species of kelp forests potentially gained under the (left) RCP26 and (right) RCP85 future scenarios.

See more examples of results in Annex 4.

### *4.4. List of project meetings*

| Date | Place | Participating partners | Meeting title and object |
|---|---|---|---|
| Jan 2017 | Faro, Portugal | CCMAR, AWI, CNRS, IMAR, UC, UGOT (video), UM, UniBo, IBERS, UT, FRI, IMEDEA (video), CIIMAR, UB, UGhent, JRC (video) | Discussion and planning of all tasks |
| Feb 2017 | Roscoff, France | CCMAR, CNRS, UniBo, IMAR, AMU, UT, NUIG | Workshop with management stakeholders, on marine conservation and management. Twenty-three participants joined the meeting from various institutions, organizations and countries. Sponsored jointly by Euromarine. |
| April 2017 | AWI; Bremerhaven | AWI, UC, Uni Bremen (self-funded), CCMAR, univ Hamburg | Networking of students and PIs within WP1; planning, student talks |
| April 2017 | Brussels, Belgium | CCMAR, CNRS | BiodivERsA meeting (with aquaculture stakeholders) |

| | | | |
|---|---|---|---|
| May 2017 | Oban, Scotland | CCMAR, CNRS, SAMS | Dissemination of the project MARFOR to the community interested in macroalgal diseases (linked to a course) |
| June 2017 | AWI; Bremerhaven | AWI, UC, Uni Bremen (self-funded), univ Hamburg | Networking of students and PIs within WP1; planning, student talks |
| Jun 2017 | Porto, Portugal | CCMAR; CIIMAR, CNRS | Dissemination of the project MARFOR to the Euromarine community (linked to Euromarine meeting) |
| Jul 2017 | Nantes, France | CCMAR, NIBIO, AU | Presentation of the project to seaweed aquaculture stakeholders and discussion of possible synergies as a special session during a Congress of the international society for applied phycology, (sponsored jointly by euromarine-MARFOR and COST-Phycomorph) |
| Nov 2017 | Den Hague, the Netherlands | CCMAR | Presentation of the project to seaweed aquaculture stakeholders and discussion of possible synergies (linked to seagriculture meeting) |
| Jan 2018 | AWI; Bremerhaven | AWI, UC, Uni Bremen (self-funded), CCMAR, univ Hamburg | Networking of students and PIs within WP1; planning, student talks |
| Jan 2018 | Southend, UK | CCMAR, IBERS, MBA, SAMS | Presentation and discussion of research from the project (linked to British Phycological Society Meeting) |
| Jan 2018 | Faro, Portugal | CCMAR, CNRS, AWI | Follow-up and joint planning of the ecophysiological work on kelp species |
| April 2018 | Brussels, Belgium | CCMAR, UniBo | Dissemination of the project at the workshop “Ocean Frontiers for Sustainable development”. |
| Sep 2018 | Hobart, Tasmania | CCMAR; CIIMAR, AWI, UP | Contribution of project MARFOR to implementation of “Macroalgal Cover and Composition” as an Essential Ocean Variable (GOOS). |
| Sep 2018-10-01 | Roscoff, France | CCMAR, CIIMAR | Participation in the APPRISE (**A**nticipating **P**otential **P**athways and **R**outes for **I**nnovation towards desirable **S**ocio-**E**conomic impacts) workshop to build a competitive, sustainable and responsible European macroalgae industry. |

| | | | |
|---|---|---|---|
| Oct 2018 | Uni Bremen, Bremen | AWI, UC, Uni Bremen (self-funded), univ Hamburg | Networking of students and PIs within WP1; student talks |
| November 2019 | AWI, Bremerhaven | AWI, CCMAR, CNRS | 4-days workshop on demographic kelp data collected along the European coastlines within the frame of MARFOR and beyond that |
| March 2020 | virtual meeting (due to Corona) | AWI, CCMSR, CNRS | 1-day workshop, continuation of demographic data analysis |
| several dates | Cologne | AWI, UC | partners met to develop bioinformtics analyses |
| Jan 2018-Dec 2020 | Virtual | UGOT, SWAM (authority) | 8 planning meetings to launch the first genetic monitoring program in Europe, which started as a pilot program 2020 and will (according to plan) be implemented as a permanent program from 2022 |

### *4.5 Follow up activities and plans for further exploitation of the results*

## 5. Stakeholder engagement in the project

### *5.1 Before the project's start*

During project planning we consulted our national conservation institutes. In Portugal the marine protected area of Arrabida was involved in the project planning.

### *5.2 During the project*

A long list of Stakeholders have been involved in the project by being informed about our actions and discussing their implications with us. These include:
Finn L. Aachmann : NTNU - Norwegian University of Science and Technology, Norway
Helena Abreu : ALGAPlus, Portugal
Urd Bak : Ocean Rainforest, Faroe Islands
André Berthou : Talibreizh et Syndicat des Récoltants Professionnels d'Algues de Rive de Bretagne, France
Calanques National Park, Marseille (France)
Olavur Gregersen : Ocean Rainforest, Faroe Islands
Damien Guiffant : Vidon, Parc Scientifique Blue Valley, Roscoff, France
Franck Hennequart : ALGAIA, France
Philippe Kerrison : Scottish Association for Marine Science (SAMS), UK
Aurélie Loaec : Cargill Starches, Sweeteners & Texturizers, France
Maria Matard : Olmix Group, France
Angela Mead : BIOME Algae Ltd.
Pi Nyvall-Collén : Olmix Group, France
Ronan Pierre : Centre d'Etude et de Valorisation des Algues, (CEVA), France

Birgit Quack : GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel, Germany
Monique Ras : Blue Science Consulting and Management, Saint Pol de Leon, France
Céline Monique Odile Rebours : Moreforsk, Norway
Douglas Robinson : Laboratory for Interdisciplinary studies of Science Innovation and Society (LISIS),
Jean-Marc Salou : EuroFin Consulting, France
Rachel Sellin : Pole Mer Bretagne
Marta Sofia Marques Rodrigues Da Silva : University of Bergen, Norway
Melanthia Stavroulaki : Hellenic Centre for Marine Research (HCMR), Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Greece
Ronan Sulpice : National University of Ireland, Galway, Ireland
Florian Weinberger : GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel, Germany
Cat Wilding : Marine Biological Association of the UK
And also (listing the institutions rather than the personal names): Oceana, Unesco-OBIS, GBIF, European Phycological Societies (federation), Pew Foundation, Oceano Foundation, Seaforester, Hortimare, C-weed, National Park of Mer d'Iroise, Natural History Museum of Paris, Arrabida Marine Park, EMEPC, Kosterhavet National Park, Azores Regional Government, Federation of the European Phycological Societies, Submaris, French National Agency of Protected Marine Areas, Subnauta underwater tourism, UN intergovernmental panel on climate, State agency for agriculture, environment and rural affairs of Germany, IUCN, Natura 2000 Biodiversity network France, Underwater Federations of France and Portugal, Global Ocean Observatory.

2. Provision of data by stakeholders; use of field/experiments allowed by stakeholders

Many stakeholders have provided data, in addition to individual citizens. The major ones were the Reef Life Survey, OBIS, GBIG, Macroalgal Herbarium Portal. Support of field research on Helgoland via State Agency for Rural Sites and Environment, Flintbek, Germany (LLUR_SH). Support for field sampling in the study of gene flows between farms and wild populations in S. latissima.

3. Involvement of stakeholders considered as research objects (e.g. Participatory meetings used to assess biodiversity and service values by them)

See list of meetings with stakeholders above in the list of meetings. Many were devoted to aquaculture stakeholders, and a major one took place with management stakeholders, resulting in preparation of a paper (ongoing).

4. Other meetings and activities (to be specified)

UGOT has several times met with the Swedish Agency for Marine and Water Management (SWAM) in Sweden for consultation on how to implement knowledge on genetic diversity into management. A first pilot project was initiated during 2018-2019 on genetic monitoring of Fucus vesiculosus/F. radicans in the Baltic Sea funded by SWAM. From 2020 a full genetic monitoring program targeting Fucus and 9 additional aquatic species are launched. Several participants from CCMAR and CNRS have met with marine park authorities to provide advice on management of exploitation, conservation and restoration practices of kelp and fucoid forests.

The Directorate for Maritime Affairs of the Azores Regional Government was informed and acknowledged the new results regarding the genetics of the Azores kelp population contained in Assis et al. (2018).

CNRS and CCMAR had a local meeting engaging stakeholders (industry) to collaborate in the process of providing guidelines assisting the seaweed blue economy. MARFOR participated in the APPRISE (Anticipating Potential Pathways and Routes for Innovation towards desirable Socio-Economic impacts (focus on macro algae)) workshop that brought together stakeholders from all parts of the macro algae innovation ecosystem (researchers, algae producers and harvesters, processing firms, user firms and social scientists) to provide the right mix of expertise to develop a robust analysis of the challenges ahead.

### *5.3 Foreseen after the project's end*

We are waiting for the end of the pandemic-linked restrictions to organize a major meeting.

## 6. Dissemination of results

### *6.1 List of scientific publications*

These are listed in the tables 1-3.

### *6.2. Dissemination of results to scientists and scientific organisations (1-page max)*

These are listed in the tables 1-3. Furthermore, the impact on training of a young generation of scientists is noteworthy.

### *6.3 List of dissemination activities with stakeholders*

Following this Biodiversa project, we answered to the last Biodiversa call on the "Conservation and restoration of degraded ecosystems and their biodiversity, including a focus on aquatic systems", with a consortium including CCMAR, AMU and CNRS, among other partners. This project is under evaluation.

Following the research developed in Marfor in complement with the other project IDEALG and GENIALG, since September 2020, the Station Biologique de Roscoff is the headquarter of the seaweed manifesto (http://www.seaweedmanifesto.com/) initiated by the Lloyd's Registrer Foundation and supported by the Sustainable Ocean Business Action Platform of the United Nations Global Compact. This manifesto aims to define a vision for the seaweed industry: an upscaled, responsible and restorative seaweed industry, playing a globally significant role in food security, climate change mitigation, and support to the marine ecosystem, as well as contributing to job-creation and poverty alleviation. In that context, all partners of Marfor should represent a major force to participate to this project for their expertise in conservation and restauration actions.

### *6.4 Dissemination of results to stakeholders (1-page max)*

*Dissemination of results to stakeholders (except general public)*

*Information / technology transfer*
In addition to the many meetings listed above (see table of meetings), an extra one connected to the MARFOR project but funded separately, KJ has been the main organiser of a 2-day joint workshop for managers and researchers on how genetic biodiversity should be used in management of the Baltic Sea ecosystem. This meeting gathered 60 participants, representing managers, government authorities, NGOs and scientists.

Contributed information towards the proposal to include kelp forests in OSPAR, participated in meetings with the national representatives. The seaweed aquaculture community was involved in joint planning on relevant issues. The project information was used to define donor populations for marine habitat restoration in Marine Protected Areas along the entire portuguese coastlune. Marine conservation authorities were consulted via 4 meetings, to define monitoring protocols for permanent monitoring.

*Outreach to the general public*

Some of our outreach has been conducted by social media, website and the marineforests.com initiative. Participants disseminated the project in several events, including open science day and European researchers' night at the various countries. During the European Researchers Night in 2017 CCMAR (E Serrao) organized a marine forests event at the Natural History Museum in London (with local scientist Juliet Brodie).

During European Researchers Night in 2019 UMA organized the practical workshop "Seaweeds, marine forests and global change" including the design of two outreach posters and scientific experiences guide (in Spanish) (LINK: shorturl.at/nEFLS).

The new results from Assis et al. (2018) regarding the Azores kelp population were disseminated via the IntraDOP webpage (LINK: http://www.horta.uac.pt/intradop/index.php/pt/86-notas/3372-os-acores-como-refugio-de-laminaria-ochroleuca).

The results of Neiva et al. (2020) about the location of refugia in the kelp L. digitata were dissiminated Svia TV News the 28th Jul 2020 https://news.stv.tv/highlands-islands/ice-age-kelp-found-by-scientists-off-coast-of-scotland?top

During the "Fête de la science" in Station Biologique de Roscoff, CNRS organised an exhibition stand for the general public, on the domestication of seaweed, September 27, 2020

*Education projects*

The school actions developed so far have involved dissemination of the participation of children in reporting marine forests by taking photos and adding them to marineforests.com under the organization of the school teachers.

At AWI a cooperation between pupils from the AWI school project Highsea and scientists took place developing a first manual for teachers on the topic ‘Kelp forest and climate change’.

For the International Day of Women and Girls in Science (February 11th) , UMA organised a practical workshop for high school students about MARFOR in 2019 and about ocean warming and marine heat waves in 2020.

## 7 Global Impact assessment indicators

### *7.1 Impact statement*

Project MARFOR is having major impacts on:

- global outreach to the international scientific community of the research results related to marine forests of large brown algae of Europe, a scientific field that has been traditionally centered in California and Australia. This is achieved via publications and talks, including invited ones.

- impact on marine conservation and management of key ecosystems that support biodiversity, as the project contributed with much information towards the proposal to include kelp forests in OSPAR

- impact on the seaweed aquaculture community (built from dialog and direct invitation to talk and collaborate on relevant issues)

- impact on blue-green infrastructures as the project information was used to define donor populations for marine habitat restoration.

- impact on defining monitoring protocols for permanent monitoring.

- Partners are planning a European Center for Conservation (genetic resources, with a focus on seaweeds, kelps)

.

### *7.2 Synthetic figures for the project publications (including interactions with stakeholders)*

**51** published papers, plus **30** in preparation (listed in the publication list template, but not taken into account in the following table). **14** published papers with impact factor **>4**

**Analysis of the *project* publications:**

| Journal | Number of papers | Impact Factor |
|---|---|---|
| Global Change Biology | 1 | 8.997 (2017) |
| Critical Reviews in Plant Sciences | 1 | 6.235 (2019) |
| Molecular Ecology | 1 | 6.131 (2017) |
| Global Ecology and Biogeography | 1 | 5.958 (2017/2018) |
| Scientific Data | 1 | 5.541 (2019) |
| Diversity and Distributions | 1 | 4.830 (2011) |

| | | |
|---|---|---|
| Journal of Biogeography | 1 | 4.154 (2017/2018) |
| Physiologia Plantarum | 1 | 4.148 (2019) |
| BMC Genomics | 1 | 4.093 (2019) |
| Scientific Reports | 5 | 4.011 (2018/2019) |
| Marine Pollution Bulletin | 1 | 3.782 (2018/2019) |
| Genes | 1 | 3.759 (2019) |
| Evolution | 1 | 3.698 |
| Frontiers in Marine Sciemce | 7 | 3.661(2019) |
| BMC Plant Biology | 1 | 3.497 (2019/20) |
| Mar Env Research | 1 | 3.159 (2017/2018) |
| BMC Evol Biol | 1 | 3.027 (2017) |
| BMC Ecology | 2 | 2.922 (2018) |
| ICES JMS | 1 | 2.760 (2016) |
| European J Phycology | 3 | 2.756 (2019) |
| PloS ONE | 3 | 2.740 (2019/2020) |
| Ecology and Evolution | 2 | 2.540 (2019) |
| J Evolutionary Biology | 1 | 2.538 (2017) |
| J Applied Phycology | 1 | 2.401 (2017/2018) |
| MEPS | 1 | 2.326 (2019/20 |
| Peer J | 1 | 2.12(2017) |
| Mediterranean Marine Science | 1 | 2.071 (2018/2019) |
| Polar Biology | 1 | 2.0 (2019) |
| Cryptogamie Algologie | 3 | 1.791 (2019) |
| Aquatic Botany | 1 | 1.787 (2017) |
| Phycological Research | 1 | 1.147 (2019) |
| Botanica Marina | 1 | 0.989 (2017/2018) |
| Aquatic Living Resources | 1 | 0.525 (2017/2018) |

**International dimension and multi-partnership for publications**

| | published | **Number of publications** |
|---|---|---|
| **Multi-partner publications** | **Peer-reviewed journals** | 27 + 21 in prep |
| | **Books or chapters in books** | 0 |
| | **Communications (conferences)** | 23 |
| **Single-partner publications** | **Peer-reviewed journals** | 27 + 9 in prep |
| | **Books or chapters in books** | 6 |
| | **Communications (conferences)** | 25 |
| **Outreach initiatives including** | **Popularization articles** | 4 |
| | **Popularization conferences** | 12 |

| | | |
|---|---|---|
| **interactions with stakeholders** | **Others** | 19 |

*7.3. Other scientific outputs*

| | **Number, years and comments (Actual or likely outputs)** |
|---|---|
| **International patents obtained** | 0 |
| **International patents pending** | 0 |
| **National patents obtained** | 0 |
| **National patents pending** | 0 |
| **Operating licences (obtained / transferred)** | sampling licenses were obtained from the respective national authorities in each country |
| **Software and any other prototype** | developed Bio-ORACLE v2.0: marine data layers for bioclimatic modelling. DOI: 10.1111/geb.12693 |
| **Company creations or spin-offs** | 0 |
| **New collaborative projects** | applications pending |
| **Scientific symposiums** | cancelled due to covid |
| **Others (please specify)** | many graduate student theses |

### *7.4. Assessment and follow-up of personnel recruited on fixed-term contracts (excluding interns)*

| Identification | | | Before recruitment for the project | | | Recruitment for the project | | | | After the project | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Surname and first name | Sex M/F | E-mail address | Last diploma obtained at time of recruitment | Country of studies | Prior professional experience, including post-docs (years) | Partner who hired the person (Organisation and Country) | Position in the project (1) | Duration of missions (months) (2) | End date of mission on project | Professional future (3) | Type of (4) |
| BLANFUNE Aurélie | F | aurelie.blanfune-thibaut@mio.osupytheas.fr | PHD | France | Research assistant (6 years) | AMU (France) | Post-doc | 18 | July 2019 | still working on the project | Lecturer- |
| SÁNCHEZ DE PEDRO CRESPO, Raquel | F | rsdpc@uma.es | PHD | Spain | Research assistant (6 years) | UMA | Post-doc | 22.5 | November 2019 | Fixed-term contract | Teaching research |
| FERNÁNDEZ FERNÁNDEZ, Andrea Nieves. | F | an.fernandez@uma.es | MSC | Spain | - | UMA | Technician | 18 | July 2019 | Fixed-term contract | Other pri |
| LIESNER, Daniel | G | daniel.liesner@awi.de | MSC | Germany | BSC and MSC student | AWI (Germany) | PhD | 39 | 30 August 2020 | Postdoc at MPI Tübingen | Research |
| RANA, Shivani | G | srana1@uni-koeln.de | MSC | Germany | BSC and MSC student | UC (Germany) | PhD | 39 | ?? | unknown | unknow |
| Louise Fouqueau | F | louise.fouqueau@sb-roscoff.fr | MSC | France | - | CNRS (France) | PHD | 36 | 30 November 2020 | still working on the project | PHD complem fellowsh |

### *7.5. Data Management and timeline for open access*

All the occurrence records for European shorelines (and for the entire world) were published and availability is described here:

Assis, J., Fragkopoulou, E., Neiva, J., Abecasis, D., Faugeron, S., Serrão, E.A., 2020. A fine-tuned global distribution dataset of marine forests. Sci. Data 7. https://doi.org/https://doi.org/10.1038/s41597-020-0459-x

All papers include datasets published in the respective databases, listed in each paper. Genbank is the database used for all DNA datasets.

The genetic and genomic data developed during this project are ready for further analyzes (planned) on adaptation to local environments. These data will also be useful for comparative meta-analyzes of the genetic diversity and genetic structure of marine forests

**Index of annexes attached:**

Annex 1
Table 1: List of peer-reviewed publications accepted, in press or published
Table 2: List of peer-reviewed publication in review or in preparation at time of reporting
Table 3: Other publications

Annex 2
File with presentation of examples of the results