1. Background

The HERMIONE project is designed to make a major advance in our knowledge of deep-sea ecosystems, with strong connections between deep-sea science and user needs. A wide range of ecosystems including cold-water corals, canyons, cold and hot seeps, seamounts, open slopes and deep basins are studied in study sites stretching from the Arctic down to the Mediterranean Sea.

Cold-water coral ecosystems in this region are mainly created by the reef-building coral *Lophelia pertusa*, hence a key ecosystem engineer by its proper definition. Various threats to *Lophelia* ecosystems have attracted attention over the past decade, such as deep-sea trawling, oil- and gas exploration, acidification and climate change. Quantitative assessment of the destruction from bottom-trawling has made us realize that at the rate we are destroying these ecosystems they will be completely erased long before we have had time to describe the diversity they contain, much less understand the mechanisms governing how they work and function. This has raised international concern and political will to protect and conserve these biologically rich ecosystems before it is too late, but information that allows conservation measures to be based on biologically and ecologically relevant information is largely missing. One of the information needs sought after for *Lophelia* is information regarding population connectivity on small and continental scales. One of problems for the research field that deals with deep-sea science is the availability of the ecosystems. All studies are utterly dependent on technical instruments such as landers, time-lapse cameras, and remotely operated vehicles (ROV). ROVs allow non-invasive sampling of genetic samples that with molecular tools can be used to extract information about population connectivity and to gain novel insight in population demographics.
2. Materials and methods

Study sites and sampling

Genetic samples of *Lophelia pertusa* have been collected at the Atlantic Coral Ecosystem Study (ACES) cruises during the years 1999-2003, HERMES (2005-2009), and HERMIONE cruises (2009-present). 638 genetic *Lophelia* samples from thirty-three locations in the Barents Sea, NE Atlantic Ocean, North Sea and the Mediterranean Sea (Table 1) have been collected. All samples were preserved in pure ethanol (95 - 96%) prior to genetic analysis. 225 samples from the North-East Skagerrak were collected during HERMES and HERMIONE for the purpose of a small-scale population genetic study. At an even finer scale, the intra-reef scale, particularly intensive sampling was performed at the Tisler reef (the largest reef in the NE Skagerrak), where 130 samples were collected. The majority of the samples were collected using a remotely operated vehicle (ROV). Several advantages are offered by the use of ROVs for sampling. Of particular importance is that the damage is kept to a minimum and that precisely geo-referenced samples can be obtained.

Genotyping

Genomic DNA was extracted from coral polyp tissue with the Viogene Blood & Tissue Genomic DNA Extraction Miniprep System, following the manufacturer’s protocol. All samples were genotyped using nine microsatellite loci (Lpe) developed for *Lophelia* (Morrison et al. 2008) and the samples from NE Skagerrak were additionally genotyped with three dinucleotide loci (Lp loci) developed by LeGoff & Rogers (2002). Forward primers were 5’-labelled with fluorescent dyes (WellRED oligos, Proligo). The Lp loci were amplified following the procedure described in LeGoff & Rogers (2002), whereas the Lpe loci were amplified in 10 µL reactions using polymerase chain reaction (PCR) containing 2-60 ng/µL of template DNA, 0.05 U recombinant TaKaRa Taq™, 0.125 µM of forward and reverse primer, 10X buffer (Mg²⁺ free, pH 8.3, 1.5mM MgCl₂, and 0.2 mM of each dNTP. PCR amplification for Lpe loci were performed under the following conditions: initial denaturation at 94°C (2 min), followed by 30 cycles 94 °C (30 s), 58 °C (40 s) and 72 °C (30 s), with a final extension at 72 °C (10 min). Three labelled primer pairs were poolplexed and sized on a CEQ 8000 Genetic Analysis System with two positive controls each run.

Clone discrimination and clonal diversity

Genotyping samples with highly polymorphic microsatellite markers allowed assignment to multilocus genotypes (MLGs) and multilocus lineages (MLLs) for genets represented by slightly distinct MLGs (Arnaud-Haond et al. 2007). GENCLONE v. 2.0 (Arnaud-Haond & Belkhir 2007) was used to calculate genotypic richness and the probability that any of the observed putative clonal genotypes was the result of sexual reproduction. Genotypic richness is the most important indicator of the relative importance of sexual versus asexual
reproduction for population dynamics, and is calculated as the ratio of number of genotypes relative to the number of total samples taken from a population.

Summary statistics
We calculated allele frequencies, observed heterozygosity ($H_O$), expected heterozygosity ($H_E$), inbreeding ($F_{IS}$), Hardy-Weinberg equilibrium (HWE), using GENEPOP 4.0.6 (Rousset 2008). Levels of differentiation between sites were described by the $F_{ST}$ estimator ($\theta$) (Weir & Cockerham 1984), and the null hypothesis of no differentiation was tested using Fisher’s exact test. $F_{ST}$ values were corrected for the presence of null alleles using the program FREENA (Chapuis & Estoup 2007). Allelic richness using the rarefaction method were calculated using FSTAT v.2.9.3.2 (Goudet 2001). Genetic diversity can be defined at several levels due to the mixed mode of reproduction in corals. Genetic diversity sensu stricto (or gene diversity) refers to the amount of variation at the level of individual genes in a population. In contrast to genetic diversity, genotypic richness or diversity is defined as the number of unique multilocus genotypes in a population (see above). For the purpose of this study, genetic diversity is defined and quantified by heterozygosity and allelic richness.

Cluster analysis
We used STRUCTURE version 2.2 (Pritchard et al. 2000) to identify the number of genetic clusters in NE Skagerrak. Traditional estimators of population structure rely on a priori designation of populations, which consequently decreases the informative level if the pre-defined number of populations is incorrect (Pearse & Crandall 2004). The algorithm implemented in STRUCTURE assigns individuals to populations by minimising genotypic disequilibrium under the assumption of HWE and LE in ($K$) number of populations that best fit the data. Mean and variance of log likelihoods of the number of clusters for $K = 1$ to $K = 6$ (the number of sampling locations plus one) were inferred from multilocus genotypes by running STRUCTURE five times under the admixture model with correlated allele frequencies (burn-in = 20 000 steps each with $10^5$ post burn-in MCMC iterations). We calculated the ad hoc statistics $\Delta K$ following the recommendations from Evanno et al. (2005) showing that the second order rate of change of $\ln[Pr(X | k)]$ with respect to $k$ is better at correctly identifying the uppermost hierarchical level of structure at the true value of $K$.

Exclusion/assignment analysis
Recruitment and migration was assessed using genetic assignment performed with GENECLASS 2 (Piry et al. 2004), which uses an individually-based classification method. Groups are defined a priori and individuals are assigned to known sources using the bayesian allele frequency estimation method (Rannala & Mountain, 1997) with the leave-one-out procedure.

Network analysis of spatial genetic variation across the European continental margin
Population graphs are a novel analysis method that allows the genetic structure of populations to be visualized within a graph-theoretic framework. Population relationships are shown in terms of a graphical topology, hence
not quantified in terms of averaging statistics, and therefore utilizing the high-
dimensional genetic covariance relationships among all populations
simultaneously, rather than in a pairwise fashion. Network theory is therefore
an especially powerful tool for addressing issues regarding the genetic
connectivity within a phylogeographical context. Connected populations are
illustrated by the presence of an edge (line) connecting the two different
nodes (populations) in the population graph. The edges are determined by the
genetic covariance of the connected populations. If the populations are
genetically independent, conditional on the remaining data in the model, then
the two populations will not be connected. If they are conditionally dependent,
there will be an edge connecting the nodes. Building and visualization of the
graphs were done in the software suit GeneticStudio (Dyer 2009). The graphs
were created by GENO (using R) and the topologies were visualized by
GRAPH. The program EDENetworks (Kivelä et al. unpublished) was also
used to construct graphs at genet level.

**Age of genets**

Minimum age of three genets at the Tisler reef was estimated by calculating
the time that would be required to grow linearly to the measured area covered
by clones. Linear growth rates for corals in the area have previously been
estimated to 5-7 mm yr\(^{-1}\). For three genets (later referred to as the orange,
blue, and red genet) a sufficiently large number of ramets were found for
calculating the age. Only areas where those genets appeared to be
continuously distributed were used, disregarding the ramets of the same
genotype further afield due to the unknown genetic belonging of the ramets in
between. FLEDERMAUS software was used to calculate the area covered by
the clones. In this way, the calculated area based on distances is seen from
straight above the reef looking down, thus equalling only horizontal growth.
Since corals in large grow vertically, ages were calculated assuming an
average coral colony height of 60 cm and a circular shape of the clone. By
measuring a large number of coral colonies an average of 19-degree angle for
vertical growth was used to calculate genet age.

GENECLONE (Arnaud-Haond & Belkhir 2007) was used to calculate the
expected number of MLGs to be found at the Tisler reef for a given number of
samples. 87 of the 130 Tisler samples were used in the analysis. The
program EstimateS (Colwell 2000) was subsequently used to calculate the
total number of genetic individuals expected at the Tisler reef (i.e. to calculate
when the curve reaches the asymptote). The non-parametric richness
estimators ICE and Chao2 were used (50 randomisations for each sample).
3. Results

Genetic and genotypic diversity

Over-all loci values of $H_e$ ranged between 0.46 and 0.85, $H_o$ from 0.57 to 0.89, and $F_{IS}$ from -0.92 to 0.25 (Table 1). The $F_{IS}$ measures inbreeding of individuals that is due to local non-random fusion of gamets within sub-populations. At panmixia, when gamets fuse randomly, $F_{IS}$ equals zero ($H_E = H_O$). Hence, negative values of $F_{IS}$ correspond to an excess of heterozygots and positive values to a deficit. Nearly all localities show a significant deviation from random mating compared to Hardy-Weinberg assumptions due to heterozygote deficit.

Lopphavet (8.89) exhibited the highest allelic richness in contrast to the Darwin Mounds that had the lowest allelic richness (6.93) (Table 1). Genotypic richness ranged from 0.18 at the Säcken and Osterfjord populations to 1.00 in Stjärnsund, Rockall Bank, Cap de Creus Canyon, and Nord Leksa in the Trondheim fjord (Table 1). The average genotypic richness was 0.60, which indicates the importance of asexual reproduction in population dynamics and demography. There was a clear trend showing that the value of genotypic diversity was decreasing in relation to the number of collected samples (Fig 1). This also emphasizes the importance of quite large sample sizes to be able to estimate the genotypic diversity at a reef locality.

Fig 1. The correlation between number of ramets and genotypic richness (R). The green circles represent pooled samples from (left to right) the Mediterranean Sea, Trondheim fjord, and NE Skagerrak, from left to right, respectively.
Table 1. Sample localities, number of ramets, number of genets, genotypic richness (R), allelic richness (mean number of alleles/loci) (A), expected heterozygosity, observed heterozygosity, the inbreeding coefficient $F_{IS}$. * Denotes significant values at $P < 0.05$

<table>
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<th>Location</th>
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<th>Genets</th>
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**Platforms and low sample localities**

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Continental-scale population-genetic structure

The population subdivision structure based on Wright’s fixation index ($F_{ST}$) reveals a somewhat disorganized genetic structure across the European continental margin (Table 2). Two reef localities, Darwin Mounds and La Galicia, stand out by being genetically differentiated compared to nearly all other populations. Only the pairwise comparison between La Galicia and the Sula reef is above the statistical significance threshold. The Trondheim fjord population is genetically differentiated to most of the southerly-located reef localities around the British Isles but not the coral localities located on the west coast of Norway. The NE Skagerrak is genetically differentiated to all but three other populations, the Korallen reef in the north, the Sula reef, and the Mingulay reef complex.

Table 2: Matrix of pairwise estimates of the $F_{ST}$ estimator ($\theta$) below the diagonal and corresponding significance value above the diagonal. $F_{ST}$ values are corrected for null alleles. Numbers in bold type indicate significantly different from zero at the 0.05 level after Bonferroni corrections. For name abbreviations of the populations see Table 1.
Examination of the topology of the networks based on individuals (Fig. 2) and populations (Fig. 3) reveal distinct topological features. The graph based on populations show a highly connected network whereas the genet network reveals substantial sub-clustering and genets from the same sampling locality can be positioned at very different locations in the network. These networks and topological differences can be used to infer the importance of populations and genets in maintaining the integrity of the system.

Fig. 2 The network of Lophelia pertusa based on unique genetic individuals. The network was created in EDENetworks.

Fig. 3 Network of Lophelia pertusa based on reef localities. The network was created in EDENetworks.

Networks are efficient to deduce important nodes for relaying information through the network by examining the topology of the network. Edges in the network that can be removed without significantly reducing the fit of the network model to the population genetic data can be pruned off to identify the most important nodes. Edges represent significant genetic similarity between pairs of populations once the genetic similarity to other populations has been removed; hence only the vital edges for describing the population genetic covariance structure remains. The four coral populations west of the British Isles are suggested as important nodes for the Norwegian populations in the
north (Fig. 4). All Norwegian populations appear to be dependent on several populations in the south and have a low interconnectivity.

Shifting the focus to the Mediterranean Sea, keeping all four localities separate, and continental shelf reef localities reveal a high interconnectivity within the Mediterranean (Fig. 5). The numerous edges connecting the Mediterranean populations to several different coral localities in the Atlantic suggest that the lower Atlantic populations in turn might be dependent on the gene flow out of the Mediterranean Sea.
**Fine-scale population genetic structure**

The genetic composition of each of the reef localities in NE Skagerrak is visualised in Figure 6, based on STRUCTURE assuming 2-6 genetic clusters. The coinciding plateau of the log probability value and the optimum value for $\Delta K$ at $K=2$ (fig 7) suggests two distinct genetic clusters, where the Säcken reef alone constitutes one cluster. This was also supported by pairwise $F_{ST}$ values, where all pairwise comparisons including the Säcken population showed high and significant values (data not shown).

![Fig. 6 Bar plots for Bayesian STRUCTURE analysis of cold-water coral Lophelia populations using combined data from 13 microsatellite loci. Results are shown for five levels of K (2-6). True K = 2 ($\Delta K=83.3$). Each individual is represented by a vertical line, partitioned into K coloured segments that represent the individual’s estimated membership fractions. Black lines separate individuals from different sampling sites, which are labelled below the Figure. Posterior probabilities are visualised by DISTRUCT.](image-url)
Fig. 7 Values of $L(K)$ ± SD and $\Delta K$, calculated as is Evanno et al. (2005) and based on the log likelihood of the data given by STRUCTURE for each number of cluster assumed ($K$).

All the samples collected at the Säcken locality were assigned back to the sample location by the assignment test in GeneClass2. The majority of individuals sampled at East Søstrene (55%) and Tisler (46%) were assigned back to their sample location and the bulk of the remaining individuals from these locations were assigned to one another (36% of the samples from East Søstrene were assigned to Tisler and 38% of the samples from Tisler were assigned to East Søstrene). No individuals from Fjellknausene were assigned to the sample location; all individuals originate from East Søstrenes (57%) and Tisler (43%). At West Søstrene 50% were assigned to Fjellknausene and the remaining genets from Tisler and East Søstrene in equal proportions (Figure 8). The gene flow among subpopulations in NE Skagerrak is illustrated in figure 9.

Fig 8. Individuals based self-assignment test using the leave-one-out procedure on five Lophelia populations in NE Skagerrak. The vertical bars (y-axis) represent percentage of individuals assigned back to sample location (x-axis). The colours represent assigned locations, orange = West Søstrene, green = East Søstrene, blue = Fjellknausene, yellow = Tisler, red = Säcken.
Intra-reef genetic structure

The 87 ramets analysed from the Tisler reef were distributed among 35 genetic individuals (Fig 10). The expected total number of genotypes at the Tisler reef range from 76 (ICE) to 90 (Chao2) with lower bound 52 and upper bound 219, based on the non-parametric estimators respectively.

Fig 10. Boxplot showing the genotypic richness of genetic samples from the Tisler reef. Central line shows average number of genotypes identified in the sample using X number of sampled ramets. The edges of the box indicate minimum and maximum number of genotypes.
Exhaustive sampling coupled with multi-locus genotyping allowed visualization of the clonal architecture and the spatial distribution of genetic individuals over the Tisler reef (Fig 11). The longest distance between two ramets belonging to the same genet was 253 meters (black dots). The most frequently sampled genet was sampled 14 times (blue dots), the second and third most frequently observed genets were sampled 11 and 7 times, respectively (red and orange dots). The orange clone covered an area of 299 m², which transforms into an estimated age ranging from 4,408 - 6,172 years. Corresponding values for the blue and red clones are 164 and 130 m², with estimated ages in the ranges of 3,251 - 4,569 years and 2,906 - 4,068 years, respectively.

Fig 11. Multibeam map over the Tisler reef facing north-westward. Coloured dots represent spatial distribution of genets. Each colour represents one unique genotype (genet) while white dots indicates multilocus genotypes found only once. The insert shows a zoomed image of the central part of the reef.
Exploring the network shape of ramets from the Tisler reef (Fig. 12) reveals the existence of sub-structures even within the reef.

Fig. 12 Network depicts inter-individual genetic distances between ramets from the Tisler reef using non-shared alleles distance. The network was created in EDENetworks.
4. Discussion and conclusions

This is the first empirical genetical characterisation at the intra reef-scale of a *Lophelia pertusa* cold-water coral reef. The intensive sampling at the Tisler reef provided us with the opportunity of a unique insight of genotypic diversity and spatial distribution of genets at a cold-water coral reef with high resolution.

The rate of discovery of unique genetic individuals is greatest initially and is thereafter gradually decreasing. This can clearly be seen in both Figures 1 and 10. At the Tisler reef, 87 samples were used in the analysis and those were distributed among 35 genetic individuals. That information was used to calculate the expected total number of genets at the Tisler reef, and the result was that less than 100 genetic individuals are expected to build up the Tisler reef.

The spatial distribution of three genets was used to estimate the age of those individuals. A linear growth rate of 5-7 mm yr$^{-1}$ had previously been measured on individuals from the area. Even though clone size is not an optimal method for estimating clone age (Ally et al. 2008), the spatial distribution of genotypes and slow growth rates indicate that clones are of substantial age. It should also be kept in mind that the calculated age in this study is in the lower part of the possible scale, since the age was calculated based only on the size of the ramets that had been sampled and the genetic affinities of the coral colonies outside the area of those included in this study are unknown. Hence, the actual size of the clones may well be considerably larger than what has been measured in this study.

Knowledge of longevity and the number of individuals at a reef gives an important hint about population functioning and dynamics. It also helps to clarify the framework within which interpretation of the genetic structure should be made.

We found significant fine-scale population-genetic structure at the scale of the NE Skagerrak, where two distinct genetic clusters were identified. The Tisler reef and the three reef localities in the outer Oslofjord constitute one genetically diverse and coherent metapopulation, whereas the Säcken reef is genetically isolated and genetically depauperated. Such fine-scale geographical variation emphasizes that connectivity patterns are not solely a species-specific trait but also a reflection of local environmental conditions and stochastic oceanographic processes.

Identification of weak and strong links between populations in a meta-population system is a major challenge of population ecology, and crucial for effective management. To better understand the connectivity, interpretation of the population-genetic structure must be made in the context of relevant life-history characteristics and historical events. This study has shown that clonality is considerably more important than previously anticipated for ecosystem development and maintenance of *Lophelia* cold-water coral reefs. This has a major impact on the evolutionary rate of the species and the
genetic structure is completely permeated and governed by the asexually reproductive mode. Other life-history traits that must be taken into consideration when interpreting the genetic patterns are, (1) small population sizes, (2) low genotypic evenness, (3) extremely long life span of genetic individuals and (4) generation overlap.

Natural populations are structured in space and time, and the contemporary genetic structure is the synergistic result from the joint action of mutation, migration, selection, and drift. The complex life-history traits of *Lophelia* results in reef localities consisting of widely variable assemblages of genotypes, affecting the population-genetic signature in the population as a whole. The power to correctly explain the genetic structure is not only dependent on the number of samples and the genetic markers, but is also dependent on the theoretical models and methodological procedures used to analyse the results. Interpretation of the genetic structure, based on traditional population genetic tools, is very difficult because it is based on averaging summary statistics. The use of novel methods, based on network theory, and comparisons of networks based on both genets and populations provides additional information about population connectivity and phylogeography for the first time.

Evolutionary processes that act to shape the genetic structure operate within a biological and historical context. *Lophelia* has existed for millions of years and during this time six extensive glaciations have occurred and covered large parts of North America, the British Islands, Scandinavia, northern Germany, and the Baltic states with ice. The latest glaciation started approximately 75000 years ago and ended 10000 years ago with the start of the Holocene. During the latest glacial maximum, about 20000 years ago, the ice was up to 3000 meters thick in some areas and covered major parts of today's landmass and oceans. The Scandinavian continental margin and the British Islands, except the south-western parts, were completely covered by the ice. The network analysis reveals that the Norwegian populations originate from reef localities surrounding the British Isles (Fig. 4), which are areas known to have been acting as a refuge during the last glacial maximum. This result is also consistent with the fact that reefs have existed on the mid-Norwegian shelf for approximately 8600 years (Hovland & Mortensen 1999). Based on the low interconnectivity among the Norwegian populations it is also likely that those populations are still dependent on the British populations for larval replenishment, if reefs are damaged or lost in the area. This is also the first study to characterise the genetic structure of Mediterranean *Lophelia* individuals. The numerous edges connecting the Mediterranean and La Galicia populations with the Atlantic populations suggest that the Mediterranean is an important source and reservoir of genetic diversity. The hypothesis that the coral banks in the Porcupine Seabight has a Mediterranean origin has previously been put out by De Mol et al. (2005), who found that the depth range of the coral banks coincides with the Mediterranean Outflow Water.

The network topology on genets and populations are fundamentally different. The network based on populations show the characteristic topology of an exponential network, whereas the network based on individuals is a typical
scale-free network. These two connectivity distribution models exhibit very different properties for resistance and tolerance to disturbance. Scale-free networks are more robust due to their redundant wiring between the components inside the functional network. The inhomogenous connectivity distribution in the scale-free network means that removal of a random node with much higher probability is a node with small connectivity (Albert et al. 2000). However, this robustness comes at the expense of a major weakness. If one of the most highly connected nodes is lost, the integrity of the entire network might be compromised. Translated into Lophelia population connectivity and potential resilience capacity to perturbations, this means that the loss of one or a few important genets might reduce the capacity to transport information (in this case, gene flow) across the network more that an entire reef if it does not hold any individuals that are highly connected. The fact that loss of a few genets might cause the collapse of the entire network, which consequently would break down into small isolated fragments, implies that conservation decisions should preferably be based on information from the level of genetic individuals. This is not realistic, but it emphasizes the importance of protection of cold-water coral reefs in general. Even small components in a large system can be critically important for the overall function.
5. References


Goudet (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3).


HERMIONE
Month 6 scientific progress report

Each HERMIONE partner is required to complete a progress report detailing scientific activities and advances during the period 1 April - 30 September 2009. Please use this document to outline your activities during this period and return by email to Vikki Gunn by 21 September 2009 (vkg@noc.soton.ac.uk).

Partner organisation name: UGOT
Partner number: 22
Lead scientist: Tomas Lundälv

1. Scientific progress over the past 6 months (April – Sept 09)

Please give a brief (3 pages max.) account of your institute's progress and contribution to HERMIONE during the last 6 months. Please divide your report according to work package and include details of any deviation from the workplan or delays incurred in your work.

WP4: Over the period, seven cruises (59 shiphours) related to HERMIONE were undertaken with R/V Lophelia. The objectives of the cruises ranged from exploring new areas for coral occurrence, re-examining fishery-destroyed coral habitats in the Kosterfjord for possible recruitment, launch and recovery of long-term recording instruments, documentation of coral recovery transect at the Tisler reef and sampling of corals for genetics and experiments on coral recovery and physiology. The planned cruise schedule for the summer of 2009 had to be reduced due to a major breakdown in the ROV-system, causing a halt to operations over a 2-month period due to slow delivery of spare parts.

Two members of the UGOT-team (T. Lundalv, M. Dahl) took part in Cruise 391 with R/V Poseidon in September 2009 (organised by IFM-GEOMAR) along the Norwegian Margin, from the northernmost known reefs to the Oslofjord entrance. Objectives for the UGOT-team was to collect samples for genetics and to obtain measurements of hydrodynamics in relation to coral status. Most of the planned work was dependent on a large number of scheduled dives with the research submarine JAGO. Due to unfavourable weather conditions, the number of JAGO dives that could be carried out was unfortunately reduced from over 20 planned dives at 5 locations to only 4 dives on 2 locations. Still, a substantial (c. 25) number of genetic samples were obtained, and collection of data on hydrodynamics in relation to coral status was initiated. The known northern limit for occurrence of *Lophelia pertusa* could be extended by c. 800 m.

The documentation of the coral recovery transect at Tisler reef in August 2009 has not yet been fully analysed, but showed an additional mortality in the sponge *Geodia baretti* in the transect (c. 118 m depth) of about 10% over the last year, likely in response to a period of high temperatures recorded in late autumn of 2008. In total, the population of *Geodia baretti* in the recovery transect has suffered a mortality of c. 60% over the period 2007 – 2009, likely in response to two periods of elevated temperatures in the late autumns of 2006 and 2008 respectively. Mortality rates of
over 90% were observed in populations at more shallow depths (c. 70 – 95 m). A manuscript describing these observations is in preparation.

The analysis of small-scale genetic connectivity within- and between coral populations in the Kosterfjord-Hvaler area has continued, and a manuscript on the findings is in the final stages of preparation.

An event of successful spawning in *Lophelia pertusa* in February 2009 has been followed up by obtaining high-quality SEM photographs of different larval stages and by studies of genetic relations between different batches of larvae and parent colonies. A publication is in preparation.

A pilot experiment on the effects of electrodereposition on growth and survival of *Lophelia pertusa* has been successfully carried out. The experiment demonstrated slightly increased rates of growth and budding when corals were exposed to low levels of current density (0.06 A m\(^{-2}\)), while higher current densities gave no or negative effects. It is concluded that electrodereposition with low current densities could offer a way of enhancing coral recovery rates. A manuscript on the findings is in preparation.

**WP6:** Postdoc Andrea Morf has started collection of relevant data for socioeconomic studies related to the establishment of two Marine National Parks in the Koster/Hvaler areas (both parks were inaugurated on September 9, 2009).

**WP8:** Contribution of a large number of photographs, video clips and interviews for books, newspaper articles, TV-programs and information material.

2. **Scientific objectives for the next 6 months (Sept 09 – March 10)**

   *Again, please separate by WP where possible.*

   **WP4:** (1) Finalisation of results from the studies on coral genetics. (2) Further documentation and analysis of development within coral recovery site at Tisler reef. Publication of results on mortality in populations of *Geodia baretti*. (3) Publication of results on spawning in *Lophelia pertusa*. (4) Documentation of new possible coral sites, weather permitting. (5) Possibly establishment of a seafloor observatory connected to internet at Tisler reef, depending on granting of necessary permits, and delivery of hardware from ESONET.

   **WP5:** If weather permits, ROV-documentation of pockmarks in Bratten area, open Skagerrak

   **WP6:** Further collation of background material for socioeconomic studies related to the two recently established Marine National Parks in Koster and Hvaler.

3. **Publications, presentations and conferences**
Please list below any publications or presentations related to HERMIONE that your team has produced in the last 6 months. You should include details of any conferences or workshops attended as part of HERMIONE research.


Presentations at GEOHAB meeting in Trondheim, Norway, May 3 – 7, 2009:

**Genoveva Gonzalez-Mirelis**: Georeferencing video images – issues and approaches.

**Tomas Lundälv and Vikram Unnithan**: Video-mosaicing techniques

**Tomas Lundälv, Jan Helge Fosså, Pål Buhl Mortensen, Lisbeth Jonsson, and Vikram Unnithan**: Development in a trawl-damaged coral habitat (Tisler reef, NE Skagerrak) during four years of trawl protection

4. Media contact/public outreach

Please list below any HERMIONE-related media coverage you have been involved with (including date and details of newspaper, radio station, etc.)

Contribution with a large number of photographs, interview and information for a book entitled “Kosterhavet”, about the new Marine National Park “Kosterhavet”, inaugurated in September 2009

Magazine VI (9), September 2009: Contribution of photographs and information for 2-page article.

Contribution with photographs and information to a special issue of the Norwegian magazine “Fjord og Friluft”, September 2009.


Contribution with interview and photographs for an article in the regional newspaper Bohuslänningen, September 1, 2009.
Contribution with interview and photographs for an article about marine biodiversity in the large Swedish newspaper “GoteborgsPosten”, September 4, 2009.

Contribution with video footage and interview to the Swedish TV-channel 4, in a program about the new Marin National Park Kosterhavet, September 2009.

Contribution of a large number of photographs for various brochures, posters, picture shows etc. that were released in relation to the creation of two new Marine National Parks on the Swedish and Norwegian sides of the border between Norway and Sweden in NE Skagerrak

5. Staff working on the project

Please list below the people working on the HERMIONE project at your institute. You should indicate whether they are permanent or temporary members of staff, or post-doc researchers. For gender monitoring purposes (a FP7 requirement), please indicate male/female.

<table>
<thead>
<tr>
<th>Staff name</th>
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<th>Perm/Temp</th>
<th>Male/Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomas Lundalv</td>
<td>Researcher</td>
<td>Perm</td>
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<tr>
<td>Lisbeth Jonsson</td>
<td>Postdoc</td>
<td>Temp</td>
<td>Female</td>
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<tr>
<td>Carl André</td>
<td>Assoc. Professor</td>
<td>Perm</td>
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<tr>
<td>Lars Hagstrom/Annnci Niklasson</td>
<td>Administrative staff</td>
<td>Perm</td>
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<tr>
<td>Mats Lindegarth</td>
<td>Assoc. Professor</td>
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<tr>
<td>Ann Larsson</td>
<td>Postdoc</td>
<td>Temp</td>
<td>Female</td>
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</tbody>
</table>

6. Students working on the project

Please list below any students working on HERMIONE at your institute (regardless of whether they are funded by the project or not). Please include a brief description of their work (PhD title, etc) and at what level they are working (PhD, MSc, undergraduate project, etc).

<table>
<thead>
<tr>
<th>Student name</th>
<th>Level (PhD, MSc etc)</th>
<th>Supervisor</th>
<th>Research topic</th>
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<tbody>
<tr>
<td>Mikael Dahl</td>
<td>PhD-student</td>
<td>C. André/T. Lundalv</td>
<td>Coral genetics</td>
</tr>
<tr>
<td>Genoveva Gonzales Mirelis</td>
<td>PhD-student</td>
<td>M.Lindegarth/T.Lundalv</td>
<td>Predictive habitat mapping</td>
</tr>
<tr>
<td>Susanna Stromberg</td>
<td>Msc-student</td>
<td>T. Lundalv</td>
<td>Coral biodiversity/recovery</td>
</tr>
</tbody>
</table>

7. Person-effort per workpackage

Please enter the person-month effort contributed by your organisation to each WP in the table below for the period 1 April – 30 September 2009. Please note that under FP7 rules, you are obliged to keep records to support these figures (e.g., timesheets).

<table>
<thead>
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<th>WP1</th>
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HERMIONE
Month 12 scientific progress report

Each HERMIONE partner is required to complete a progress report detailing scientific activities and advances during the period 1 October 2009 - 31 March 2010. Please use this document to outline your activities during this period and return by email to Abigail Pattenden by 19 March 2010 (adcp@noc.soton.ac.uk).

Partner organisation name: UGOT (University of Gothenburg)
Partner number: 22
Lead scientist: Tomas Lundälv

1. Scientific progress over the past 6 months (Oct 09 – March 10)

Please give a brief (3 pages max.) account of your institute's progress and contribution to HERMIONE during the last 6 months. Please divide your report according to work package and include details of any deviation from the workplan or delays incurred in your work.

WP4: Over the period, two cruises (18 shiphours) related to HERMIONE were undertaken with R/V Lophelia. The primary objective for these cruises was ROV-aided recovery and redeployment of long-term recording bottom-deployed instruments at the Tisler reef in collaboration with NUIGALWAY partners. Further cruises were planned during the period, but had to be postponed due to extremely cold weather and heavy ice conditions, preventing R/V Lophelia from leaving harbour between late December 2009 – late March 2010.

Intense preparations for the establishment of a cabled and internet-connected Seafloor Observatory at the Tisler Reef, in collaboration with partners from Jacobs University, Bremen, took place during the period. This involved production of detailed drawings and maps of the planned installation, negotiations with and applications to relevant Norwegian authorities (County Governor of Østfold, Fisheries Directorate and Hvaler municipality) concerning necessary permits. All the permits needed for the installation were obtained by mid-March 2010, and it is now anticipated that the Observatory can be operational within the first half of 2010.

Over the period, much effort was put into the preparation of various publications and presentations related to the data collected within the HERMES-, CORAMM- and early HERMIONE projects (see Publications below). One article was published, one submitted and eight articles are in various stages of preparation. Analysis of material related to observed high mortality in the poriferan Geodia baretti, coinciding with periods of unusually high temperatures, has continued. An example from the regularly monitored “coral-recovery” transect at Tisler reef, covering the latest observed period of high temperatures in the autumn of 2008, is shown in Fig. 1. Video mosaics from part of this transect dominated by poriferans, obtained in July 2008 and August 2009 demonstrated an approximate 10% mortality in the sponge population covered by the mosaics over the period. This mortality is in addition to the approximate 50% mortality observed after the first instance of high temperatures (autumn 2006). Considerably higher mortality rates (up to approximately 90% of the already in 2007 highly reduced populations) in Geodia baretti was observed in other localities and in lesser depths.
Over the period, funding has been obtained for the acquisition of a new ROV and for refurbishing of R/V Lophelia, to optimise her capabilities for handling of ROV:s and other instrumentation, as well as improving her speed capability. Consequently, much effort has gone into the planning of these acquisitions, which should substantially enhance our capabilities for field work related to HERMIONE.

**WP6:** Postdoc Andrea Morf has continued collection of relevant data for socioeconomic studies related to the recently established marine national parks in Koster and Hvaler, as well as the potential marine SAC Bratten in the open Skagerrak:

a) Case studies Koster/Hvaler/Bratten (AM's actual assignment: deliverable 6.8)
   - Status of cases: National parks in Koster and Hvaler area under implementation and possible to study. Planning in Bratten area not started yet, preliminary contacts between authorities and stakeholders. Lobby work ongoing from NGOs and researchers to protect the area. NB: Open how much of a case Bratten will be until data-collection has to be concluded (latest in Jan 2011).
   - Status of work: Field work in Koster/Hvaler area under way spring 2010. Analysis of data summer 2010. Keeping in contact with authorities on development of Bratten as a case study.

b) Related work on science-policy interface and maritime spatial planning and ecosystem based management (related to synthesis work within WP 6)
   - 6. October 2009, Göteborg: Meeting Sweden/Norway/Denmark preparing an application within INTERREG Kattegatt-Skagerrak programme on maritime planning and management of biodiversity and climatic change around the Kattegatt/Skagerrak
- CERF conference Portland, Oregon, USA 8 1.-6. Nov: Presentation of Koster case from an ecosystem approach perspective
- 9. December 2009, Göteborg: Meeting Sweden/Norway/Denmark preparing an application within INTERREG Kattegatt-Skagerrak programme on maritime planning and management of biodiversity and climatic change around the Kattegatt/Skagerrak (asked to sit in reference group for the project)
- 12. March 2010, Copenhagen: Participating as Swedish expert in the 1st meeting of a complementary expert group on maritime spatial planning within the Nordic Council's Aquatic Ecosystem Group (AEG)
- July 2009 - Jan 2012: Participation in INTERREG Baltic Sea programme BaltSeaPlan on maritime spatial planning for the Baltic Sea area, presently working within BaltSeaPlan's WP 3 with analysis of national maritime policies relevant for maritime spatial planning (national reports March 2010).

WP8: Contribution of a large number of photographs, video clips and interviews for books, newspaper articles, TV-programs and information material.

2. Scientific objectives for the next 6 months (April – Sept 10)

Again, please separate by WP where possible.

WP4: (1) Installation of internet-connected Seafloor Observatory at Tisler reef, in collaboration with partners from Jacobs University. (2) Documentation of Tisler coral recovery transect. Further development of techniques for obtaining high-quality video-mosaics. (3) Joint intense effort with partners from NUIGALWAY, ULIV and NIOZ in a field campaign at Tisler reef to address some of the missing aspects to previous measurements, particularly those regarding dissolved carbon, as well as quantifying the total alkalinity state of the reef waters. It is intended to correlate biogeochemical variability with hydrographic & water quality conditions, zooplankton abundance & variability (incl diurnal migrations) and general reef activity (via imaging) at the reef. Data will be utilized in a food web model for the reef. (4) Continuation of experiments with re-colonisation of trawl-damaged coral habitats. (5) continued work to finalise the many manuscripts in preparation.

WP5: Cruise to the Bratten area for ROV-documentation of chemosynthetic habitats.

WP6: Field work in Koster/Hvaler area under way spring 2010. Analysis of data summer 2010. Keeping in contact with authorities on development of Bratten as a case to study.

WP8: We have been asked to edit videos illustrating the marine national parks on both the Swedish and Norwegian sides of the border. Establishment of the internet-connected seafloor observatory at Tisler reef should offer a unique possibility for public outreach to a broad audience.

3. Publications, presentations and conferences

Please list below any publications or presentations related to HERMIONE that your team has produced in the last 6 months. You should include details of any conferences or workshops attended as part of HERMIONE research.


Martin White, Damien Guihen, George A. Wolff, Kostas Kiriakoulakis, Marc Lavaleye, Gerhard Duineveld and Tomas Lundälv, in prep. Are cold-water coral ecosystems hotspots for carbon cycling?

Damien Guihen, Martin White, Tomas Lundälv, in prep. Flow dynamics and turbulence generation at a cold-water coral reef.

Tomas Lundälv, Damien Guihen, Martin White, in prep. Massive mortality in populations of the poriferan *Geodia baretti* in NE Skagerrak: An effect of recurring temperature chocks?


4. Media contact/public outreach

*Please list below any HERMIONE-related media coverage you have been involved with (including date and details of newspaper, radio station, etc.)*

Contribution with image material and interview to the Norwegian TV and radio Broadcasting Corporation NRK, February 2010. [http://www.nrk.no/nyheter/distrikt/ostfold/1.6983290](http://www.nrk.no/nyheter/distrikt/ostfold/1.6983290)

Contribution with image material and facts to information folders published by the Norwegian Directorate for Nature Management in February 2010 ([http://www.dirnat.no/content.ap?thisId=1500](http://www.dirnat.no/content.ap?thisId=1500))

Contribution with a large number of photographs to a book about National Parks in Sweden, issued by the Swedish Environmental Protection Agency in March 2010.

5. Staff working on the project

*Please list below the people working on the HERMIONE project at your institute. You should indicate whether they are permanent or temporary members of staff, or post-doc researchers. For gender monitoring purposes (a FP7 requirement), please indicate male/female.*

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<td>Andrea Morf</td>
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<td>Lars Hagstrom/Annzi Niklasson</td>
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Please list below any students working on HERMIONE at your institute (regardless of whether they are funded by the project or not). Please include a brief description of their work (PhD title, etc) and at what level they are working (PhD, MSc, undergraduate project, etc).

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<thead>
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<td>M. Lindegarth/T. Lundalv</td>
<td>Predictive habitat mapping</td>
</tr>
<tr>
<td>Susanna Stromberg</td>
<td>MSc-student</td>
<td>T. Lundalv</td>
<td>Coral biodiversity/recovery</td>
</tr>
</tbody>
</table>

7. Person-effort per workpackage

Please enter the person-month effort contributed by your organisation to each WP in the table below for the period 1 October 2009 – 31 March 2010. Please note that under FP7 rules, you are obliged to keep records to support these figures (e.g., timesheets).

<table>
<thead>
<tr>
<th>WP1</th>
<th>WP2</th>
<th>WP3</th>
<th>WP4</th>
<th>WP5</th>
<th>WP6</th>
<th>WP7</th>
<th>WP8</th>
<th>WP9</th>
<th>WP10</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td>2.5</td>
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<td></td>
<td>3.0</td>
</tr>
<tr>
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<td>1</td>
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<td></td>
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<td></td>
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<td>0.5</td>
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<td>9.5</td>
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</tbody>
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