CoralFISH protocols for standardised fish census sampling strategies and methodologies

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BACKGROUND

This manual details the methods of the EU-project CoralFISH. It outlines experimental design, sampling procedures for acoustic surveys, long-line-fishing, ROV video footages, sample processing onboard and in the laboratory and the recording of data.

To date much of the deep sea research has focused on mapping the occurrence of WCWs and qualitative descriptions of the habitats. This manual will give guidelines for quantitative estimation of fish and zooplankton abundance and their relationships to CWC habitats and similar non-coral covered topographically complex features. The ultimate goal is to quantify the relationship between fish abundance and the different types of cold water coral ecosystem found from the northern to the southern part of Europe and to make generalizations based on what has been found in the different regions. These types of generalizations describing cold water coral ecosystem functioning will serve important input to the development of management tools serving the whole European region. A manual describing the protocols of the different methods will also be useful in helping in transferring knowledge from partners with high experience in using specific equipment to partners with less experience.

DEEP WATER FISH OCCURRENCE AND THE IMPACT OF FISHERIES

It is often claimed the cold water corals are important habitats for fish. Studies have found that they host both a higher diversity and density of fish than the surrounding sea bed (Husebø et al., 2002; Costello et al., 2005). Most fish recorded around cold water coral reefs are commercially important species that are widespread in the Northeastern Atlantic and not peculiar to coral reefs. It is so far uncertain if the high densities of the fishes observed aggregating in CWC habitats indicate that the corals are important habitats in terms of population processes or not. The importance of shallow water coral reefs for a number of associated fish species has been thoroughly documented. The reason for believing that also cold water coral habitats could be important is that *Lophelia pertusa* forms a major three dimensional structure in deep water where little other cover/shelter for fish is available. CWC reefs could serve as important feeding place, predator refuge, breeding and nursery grounds for fish.

Studies performed in CoralFISH WP 3 will give new insights into which factors are important in controlling fish distribution pattern and habitat selection. The results will also answer the question of whether or not the CWC habitats are important for fish at the population level. The objectives of WP3 are to:

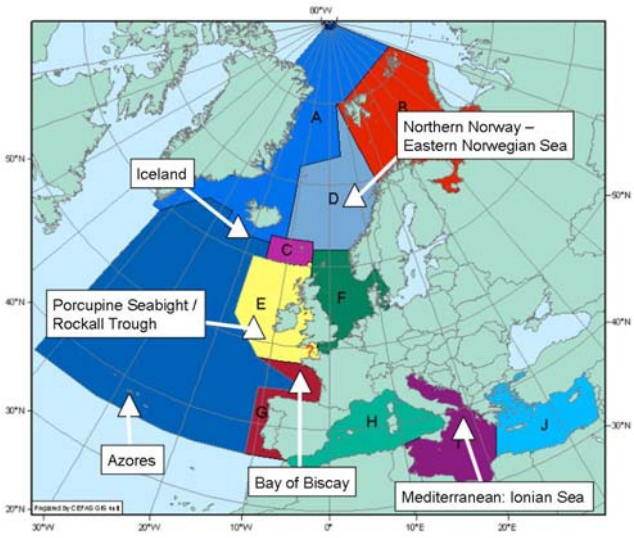
- Compare fish distribution associated with coral habitats, with the distribution associated with other benthic habitats, topographical features and position in the water column
- Assess fish density in relation to habitat characteristics (size of reef, % cover of coral) and in relation to other topographical features on the sea bed
- Assess level of fishery impacts on coral habitats from in situ observations (ROV)
- Assess distribution of fish and corals in relation to the abundance of zooplankton
- Relate zooplankton distribution to topographical bottom features such as coral mounds, ridges and banks
- Assess fish density close to benthic habitats and position in the water column related to diurnal variations

- Make an assessment of the proportion of a regional fish stock that utilize the reef habitat

This will be done by carrying out acoustic assessments of fish and zooplankton distributions, long-line experimental fishing and video inspection of fish in coral and non coral covered topographically complex habitats. In addition, the level of fishing impact from the video footage will be examined. In the appendix section a number of sampling protocols (schemes) have been included. Participants can also choose to use own standardized forms. When this is done make sure that they include all the information that is requested in the CoralFISH forms.

SITES

The CoralFISH study areas have been carefully selected to represent several major eco-regions (biogeographical provinces). They are widely dispersed around Europe, include some ultra-peripheral areas, and can be expected to yield a broad picture of the ways in which fish interact with corals in European waters.



Norway. *Lophelia pertusa* forms thousands of large and well developed CWC reefs in the mid Norwegain shelf (200 to 600 m). Fisheries include all year around or seasonal long-lining and gillnetting targeting redfish (*Sebastes* spp.), tusk (*Brosme brosme*) and ling (*Molva molva*). Some trawling activity might also occur.

Iceland. CWC (predominantly *Lophelia pertusa*) mare mainly confined to the Reykjanes Ridge and near the shelf break off the South Iceland coast, mostly within 500 to 600 m depth.

Porcupine Seabight/Rockall Trough. This area comprises mounds of varying sizes from large mounds up to 380 m high and several km's in length. Depth ranges from 600 to 1000 m. Coral occurrence varies between areas, from dense coverage on summits, open coverage on summits to coverage on flanks only. Fisheries in the region target blue ling (*Molva dypterygia*), angler fish, deep-water red crab (*Chaceon affinis*), orange roughy (*Hoplostethus atlanticus*) and hake (*Merluccius capensis*).

The Azores. Approximately 110 coral species have been identified from the area. Knowledge on the distribution is limited but in general they are confined from the flanks of the islands down to 600 to 1200 m. Fisheries in mainly semi industrial.

Ionian Sea. CWC (including *Lophelia* sp. and *Madrepora* sp.) occur in mounds of the northern Ionian Sea at 600 to 900 m depth. Soft corals occur in the eastern Ionian Sea at 450 to 1050 m depth. The northern Ionian Sea in exploited by large and small trawlers targeting red shrimps (*Aristeus antennatus* and *Aristeomorpha foliacea*), while the eastern part of the Ionian Sea is almost unexploited.

STUDY DESIGN

It is difficult to separate the role of habitat structure on ecological communities from other influences in the marine environment (McCoy and Bell, 1991). The study of CWC reefs and their role as habitat for fish and benthic assemblages cannot generally apply manipulative and intrusive experimental designs. Because of their protective status, studies of coral reefs often depend on observations and so-called mensurative experiments (Hurlbert, 1984). A mensurative experiment does not involve the manipulation of the system being studied, but rather involve the collection of information about the system in its natural state, with all its inherent variability. However, carefully planned observational studies can be applied to test a priori hypotheses (Underwood et al. 2000).

One goal of this project is to better understand and explain natural ecological processes associated with CWC, and the resulting patterns of distribution, abundance, diversity and interactions of species with focus on fish. A simple working hypothesis is that the abundance of selected fish species, and the diversity of fish, is higher in coral reef habitats than elsewhere in similar habitats at similar depths but without coral reefs. When developing study designs to test this hypothesis we should attempt to carefully specify experimental units (geographic plots) that represent coral reef communities in a larger region, and also define associated control areas. The inclusion of control areas is an important part of the observational experiments. The pairing of coral reef study areas with control areas will over time provide stronger documentation of the importance of coral reefs for fish communities. This is especially true if the objective is to document the role of cold water coral reefs more generally.

It is recommended that multiple pairs of coral reef and control plots be surveyed in each experiment, and that standardized sampling with same intensity be conducted in each experimental and control area. Representative and standardized sampling of fish from reef and control areas would allow the measure of differences in relative abundances of species, or in the diversity of the assemblage, for coral reefs versus control areas. Fish assemblages in marine habitats are generally patchy and highly variable through space and time (Underwood, 1996). Assuring that the replicate samples or measurements are dispersed in space (or time) is the most critical aspect of the design of a mensurative coral reef experiment.

Within each broader study area, it is recommended that experimental units (coral reef plots) and appropriate control areas be defined, and then attempt to collect observations from several pairs of coral reef and control areas. This study design will include some desired features of replicated treatment-control experiments approximately, although true replications cannot be achieved without any manipulation of the coral reefs. The sampling of fish by trawl-,

acoustic- , long-line, or video surveys should be standardized within each broader study area. It is strongly recommended that the sampling locations (for trawls and long-lines) or transects (acoustic and video surveys) within each experimental and control unit be selected representatively in time and space. This can be achieved by allocating stations or transects either randomly or systematically (ideally with a random start) within each reef and control area, and by sampling during day and night for a period of time. Acoustic and video sampling is typically conducted along transects. Transects can be allocated systematically in space with equal spacing in each reef and control area, but with a random starting position (see Gilbert 1987, p. 22) for the first transect to eliminate selection bias. When feasible, transect sampling should be in the direction with the highest gradient in habitat conditions, for example across and not within depth contours, and not within depth gradient to increase the precision in fish abundance and diversity indices. Long-line sets also represents a form of transect sampling, and may also be allocated systematically in a similar manner. This allocation of transects would more closely represent the spatial variation in the study area as opposed to transects in a direction with least variable habitat. Sampling with trawls or grabs can either be allocated systematically by placing stations along a grid with random starting position, or randomly. It is recommended that the number of samples in each reef and control area be balanced, with equal number of samples in each. Also, sampling during day and night must be alternated in a manner that cover reef and control areas similarly in time and space. It clearly would be inappropriate to sample reefs during daytime only, and control areas during night.

The representative sampling in space and time should yield unbiased estimates of the global average mean abundance and of diversity. Since reef and control areas are paired, with observations from multiple gears in area over time, the differences in diversity and mean abundance for each gear type could be tested using the method proposed by Schenker and Gentleman (2001). The mean abundance for the reef and control areas would be significantly different if the confidence interval for their difference does not include 0. It should be noted that the use of ANOVA to evaluate differences between reef ("treatment") and control areas would not generally be applicable for this coral reef studies because we do not have true random replication of reef and control areas (Scheiner and Gurewitch 2001).

ACOUSTIC ASSESSMENT OF FISH AND ZOOPLANKTON DISTRIBUTIONS

INTRODUCTION

Description of work

The intention of the acoustic assessments is to collect data on fish and zooplankton distribution and abundance, on and off reefs, in areas where highly detailed information on seabed topography and coral distributions exists, using either ship-borne echo-sounders or with echo-sounders fixed on towed platforms. Fish echoes will be ground truthed by targeted trawling. Data from optical plankton counters should be used where possible in combination with fluorescence meters and ground truthed with net sampling of plankton. Jointly, this would provide information on small-, medium-, and large-scale fish and plankton distribution in relation to the presence of coral reefs and similar non-coral covered topographic features.

Acoustical background information

Acoustical technologies are effective for the sampling of both fish and zooplankton in the water column. The technique has the advantage that large areas can be covered quickly, the areal coverage is continuous and the data collected is of a high resolution. Limitations of the technique are mainly that near-boundary areas (i.e. the surface and sea-bed) cannot be satisfactory sampled and obtaining biological information about the fish (e.g. species, age and maturity) is difficult. Multi-frequency data can be used to discriminate between zooplankton and different types of fish species, however, the great water depth at the 6 survey locations means the usage of higher frequencies to detect fish and zooplankton will be limited. Abundance estimations of fish will be mainly based on data collected at 38 kHz. At shallower depths 120 kHz will be used to target zooplankton more specifically. To obtain an optimal assessment of fish and zooplankton distribution the acoustic surveys will have to be supported by biological sampling (ground truthing). Also prior experience (knowledge about size, depth and habitat preference) will be vital in the interpretation of echo-signal. Acoustic abundance estimation of some species of fish could be greatly enhanced using deep-towed acoustic systems (Dalen et al., 2033). The usage of towed acoustic platforms would also be advantageous for zooplankton abundance estimations. Another option is to use optical plankton counters (OPC) or laser OPCs.

Special considerations

Acoustic assessment of fish and zooplankton distributions will be carried out at Iceland, in Norway, at Rockall/Porcupine, at the Azores and in the Ionian Sea. Seven vessels will participate in the acoustic surveys, the Icelandic RV *Arni Friðriksson* and RV *Bjarni Sæmundsson,* the Italian RV *Universitatis,* the Greek RV *Philia*, the Portuguese RV *Arquipélago*, the Irish RV *Celtic Explorer* and the Norwegian RV *Håkon Mosby.* All the vessels have different acoustic instrumentation (se overview in Table 1, Appendix 4). To obtain high quality, comparable data from all the different regions protocols describing survey and sampling strategies for the acoustic assessment of fish and zooplankton distributions were

developed. Due to the fact that depth varies greatly in the different study locations (i.e. from 200 to 1200 m) and that the coral habitats are shaped differently some factors cannot be standardized. In those instances general guidelines and suggestions for adaptations to the different regions are included. There will be no spatial overlap in working area of the different vessels so no guideline for the inter-calibration of echo-sounders mounted on different vessels has been included.

METHODS

Survey design

The study design used at any of the five locations will be decided upon by the research institution carrying out the survey, however, some general guidelines are included. Most partners will probably adopt a stratified design, i.e. spreading the samples evenly in space to minimize overlap in information and maximize knowledge, however, a random start for the first track in the survey is recommended. Parallel acoustic transects should be run perpendicular to bathymetry with the subsequent transects positioned at a standard spacing from the first both in coral and in non-coral areas. Time spent on and off coral habitats should be equal. Transect length and intervals between will be dependent on time available for the survey. Sampling intensity in any given area should be high enough to allow an abundance assessment with acceptable precision. If the fish or plankton in the area migrate vertically or horizontally in a diurnal cycle the acoustic transects should be run so that fauna is detected consistently throughout the track time. Survey speed should be around 10 knots. In rough seas survey speed may need to be reduced in order to maintain a high quality of the data.

PROTOCOL A1 – Calibration and system performance

Physical calibration of acoustic instruments

Calibration of the echo-sounders is essential to ensure accurate quantitative surveys. The calibration characterizes system parameters relative to expected standard values. It is conducted to ensure that the transducers are operating properly, to ensure that system is operating stably over time and to allow comparisons between different eco-sounders. All frequencies should be calibrated prior to embarking on the survey (vessels from Ireland and Norway operate on 18, 38, 120 and 200 kHz, research vessels from Iceland operate on 18, 38, and 120 kHz, the research vessel from Italy operates on 38 and 200 kHz and the research vessels from the Azores and Greece operates on 38 and 120 kHz). Calibration should ideally also be carried out at the end of the survey to make sure that no significant changes have occurred during the survey.

The calibration of transducers should be conducted in as near the range of environmental conditions as are expected during the ensuing survey (temperature, salinity). Water depth at the calibration site must be sufficient to exceed near-field limitations and system limitations for the different sounder frequencies. During calibration the vessel should be anchored in a location that is calm and sheltered and in an area with no or few fish. Keel-mounted transducers should be calibrated with the keel extended to the depth where it is most likely to be used. The method of calibration employs a standard target whose acoustic scattering properties are known following the procedure described in Foot et al. (1987), i.e. suspending

the target below the transducer, into the sound beam, using lines. Research vessels participating in the CoralFISH surveys are all but one equipped with echo-sounders from Simrad, either EK60 or EK500 (EA400) (see Appendix 4, Table 1). RV *Philia* is equipped with a surface side towed echosounder from Bisonics. The calibration procedures will follow the one recommended in the manufacturer manual (se e.g. http://www.simrad.no/www/01/NOKBG0397.nsf/AllWeb/F2AB311B3F6E6B15C1257106003E0806/$file/164692ca_er60_reference_manual_english.pdf?OpenElement for the SIMRAD ER60 operator manual). Prior to each calibration ambient temperature and salinity conditions shall be measured. The speed of sound will be calculated based on ambient water temperature, salinity and depth using the measures from the depth strata in with the calibration target is located.

Errors detected during the calibration are indicative of the overall systems performance. During the calibration in the 38 kHz system the tolerance error should be ± 0.2 dB for on-axis target strength measurements (Foote, 1983; Simmonds and MacLennan, 2005). If larger than this the source of error should be found and corrected. Only small deviations (<0.3 dB) in parameter setting should be expected from time to time unless parts have been replaced or other modifications to the equipment has been made.

System performance

In addition to the automatic monitoring it is necessary to check the echo-sounder and transducer performance regularly to ensure that high quality data is collected throughout the survey. Inspection of logging process and data flow at fixed time intervals includes a visual control of the echograms and target strength pop-up windows according to the manual of the software used. The clock of the work station shall be checked so that it is synchronized with the GPS time. The logged distance sailed in the post processing software should be checked against the ships log. After each CTD station a mean value for sound velocity shall be calculated and checked against the sound velocity settings for the echo-sounder. If deviation is more that ±10 m/s the new value should be entered. The routine inspection also concerns the internal electronics and processors, transducer and cables. Everything that could influence on the end product must be well documented.

Measurements of test values and passive noise values should be documented following the procedure recommended in manufacturers manual. This will be done outside of the data collection since echo transmissions need to be turned off.

Documentation and archiving

It is vital that all calibration data and supporting information is properly documented and stored. A calibration report scheme can be found in Appendix 1, Table 1.

PROTOCOL A2 – Collection of acoustic data

Instrument settings

There are a number of instrument settings that cannot be changed later during analysis. These are listed below together with suggested values. The acoustic frequency (f) is defined by the

echo-sounder. Two-way integrated beam pattern and beam width is defined by the transducer specifications. G_0 (S_v gain) is obtained from the calibration of the echo-sounder. The sound speed (c) changes with changing salinity, temperature and depth. A CTD probe should be employed in the survey area at the beginning of the survey to collect the required data for the calculation of sound speed in water and sound absorption. The same data can be used throughout the whole survey if oceanographic conditions do not change.

Pulse duration. Pulse duration of 1 ms is sufficient for the pulse envelope to stabilize in 18 kHz echosounder systems with common band widths. Shorter pulse durations will be sufficient if 18 kHz data are not used (Korneliussen et al., 2008). Due to the fact that all frequencies should operate with the same pulse duration we recommend that pulse duration is set to 1 ms.

Ping rate. The ping rate should be set to the fastest rate that will not cause shadow bottoms in the data. Minimum ping rate is estimated by: $i = 3 * 2 * BD/c$, where BD is the expected maximum water depth.

Collection threshold. In Simrad system the collection of raw data does not require the application of a threshold. For Biosonics systems a threshold and a threshold model must be selected. To ensure that all data are recorded data should be collected at the lowest possible threshold, i.e. – 120 dB or less (Korneliussen et al., 2008). In post-processing a threshold can be set to exclude unwanted echoes.

Band width: Wide bandwidths, i.e. 10% of centre frequency, are recommended for the Simrad EK500 for frequencies below 70 kHz. For frequencies above 120 kHz a narrow band width is recommended (i.e. 1% of centre frequency). For the EK60 the bandwidths are calculated by the system.

Power settings: Most theory within fisheries acoustics is based on linear-wave equations, e.g. the time varied gain equations. The power output from a transducer should therefore be selected at a level where the non-linearly generated sound is negligible compared to the linearly generated sound. To achieve this in a Simrad ES38D transducer 15 kW m^{-2} output power is sufficient. With a 60% electro-acoustic efficiency input power would be 25 kW m^{-2}. This is achieved by setting maximum input power on the echosounder to 2500 W. Using higher frequency a lower input power is sufficient to avoid generation of non-line effects. Maximum input power at 120 kHz would be 250 W (Korneliussen et al., 2008).

Additional proposals for the collection of high quality multi frequency acoustic data include: 1) synchronize the transmission from the different transducers, 2) ensure that each ping of each frequency is time-stamped at a resolution of at least 0.01 s 3) operate all frequencies with the same pulse duration, ping rate and digitized sample length (Korneliussen et al., 2008).

Data management

Different software are used by the different partners, i.e. BI500, Echo View, MOVIES, Biosonics and LSSS (Appendix 4, Table 1). These store the data in different formats. This can cause problems when exporting data to other programs. All partners should therefore also store the raw data that is being collected. In EK60 raw data can be collected and saved (*.raw) by setting the echo-sounder to record unprocessed transducer signals (procedure described in

ER60 operator manual). EK500 cannot store raw data. Partners using EK500 could store data also on a separate computer with BI500 (software available for free from SIMRAD). From BI500 data can be exported to all other programs.

GPS. GPS data should be sent to the echo-sounder and stored with the acoustic data string.

Protocol A3 – Post-processing

Data division

The acoustic method of fish abundance estimations will be based on the technique of echo-integration, i.e. the integration of the echo-intensity. Density data will be presented as nautical area scattering coefficient S_A (m^2/nmi^2). EDSU (elementary distance sampling unit), i.e. the length of cruise track along which the acoustic measurements are averaged to give one sample, should be small enough to capture the spatial distribution of fish in relation to coral habitat but not so small that the correlation between pairs of successive samples becomes too large. The best length for the EDSU may be known from previous surveys in the same area. For example in the Træna coral field (where individual coral reefs are about 40 ·150 m) survey EDSU will be set to 0.05 nm. The collection of data should be organized with intervals of time, so that the number of pings in each EDSU is constant. We suggest that depth bins are set to 30-m, referenced to the surface. In the survey special emphasis should be put on examining lowermost 10 m. Here 1 m thick depth channels should be used referenced to the bottom.

Bottom detection. Simrad echosounders include a bottom backstep, used to detect and define the bottom. The size of the backstep will vary depending on weather conditions and between sites. The bottom detection algorithms can fail in topographically complex habitats, as in the CoralFISH study areas and the accuracy of the sounder detected bottom must be verified and corrected manually during post-processing. Biosonics units do not have bottom detection during data collection.

Classification of backscatter - Target identification

The echograms, with their corresponding S_A values, will be scrutinized using BI500, Echoview, MOVIES, Biosonics or LSSS (see Appendix 4, Table 1). The partitioning of data will be either subjective, based on experience of the scientist viewing the echograms, or the echograms will be interpreted automatically in the categorization systems of the software used. Experienced human operators exceed the score of correct identification of any automatic system and are vital for the achievement of good abundance estimations.

During scrutinization contributions from sea-bed, false echoes and noise will be deleted. Echo integration of fish close to the bottom must stop where the spherical wave front reaches the bottom. If not, echoes from fish close to the bottom may merge with the much stronger echoes from the bottom itself. To avoid interference from the bottom echo the integration of fish echo will have to end before the acoustically registered bottom (often 0.5 to 2 m above the acoustically registered bottom). The height of the bottom dead zone is determined by the depth or the beam width, the integrator back step from the bottom echo and the pulse length (Ona and Mitson, 1996). The bottom dead zone will increase if the sea-bed is sloping and in

topographically complex habitats. Threshold S_v should be set to find an optimal balance between eliminating unwanted echoes and preserving the target species S_v. Operator together with the scientist in chief can vary the threshold dependent on how clean the registrations are and how dense e.g. plankton layers are.

Marks in the echogram can be caused by individual fish, single species in schools or layers, mixture of species in aggregations, by zooplankton or mixtures of fish and plankton. The corrected values for integrated echo intensity will be allocated to species subjectively according to the trace pattern of the echograms (target strength, behavior, diurnal migration, habitat preference). The relative frequency response, r(f), will be a useful tool when scrutinizing the data (Korneliussen and Ona 2002). Automatic classification of targets can be done by using r(f) and by comparing differences in mean volume backscattering strength or dB differencing (Jech and Michaels, 2006). Allocation of biological data to the acoustic numbers and abundance estimations of a mixture of species aggregations can be done through automated procedures associating acoustic images and trawl hauls (se e.g. Petitgas et al., 2003) or by partitioning into species level according to the composition of the catch of targeted trawling (Nakken and Dommasnes, 1975). If species cannot be identified larger classification groups can be used, e.g. pelagic fish, meso-pelagic fish and zooplankton. The recorded area echo abundance, i.e. the nautical area backscattering coefficient, will be distribution among the target species of interest and larger classification groups chosen for each study area.

Abundance estimations

Calculations of abundance will be based on the acoustic registrations of the Nautical Area Scattering Coefficient, S_A, mainly at 38 kHz. Conversion of the area echo abundance to numerical fish quantities can be done using the adopted mean target strength ‹TS› to length L relationships for the species identified. For single species the mean backscatter cross section can be determined through in situ measurements of target strength mean during the survey, through the size distribution of the insonified fish and a function which describes the length dependence of the target strength or through more complex scattering models. TS might be known from previous work in the region.

The number of fish (N) in a particular area will be calculated using standard equations (Foote et al., 1987; Toresen et al., 1998):

$$N = \langle S_A \rangle A(4\pi\langle\sigma_{bs}\rangle)^{-1} \text{ or } N = \langle S_A \rangle A(4\pi 10^{\sigma/10})$$

Where $\langle S_A \rangle$ is the mean NASC within the area allocated to the species of interest, A is the size of the area (nmi^2) and $\langle\sigma_{bs}\rangle$ is the mean backscattering cross section of the fish species as estimated from the target strength equation. L will be set to the mean length of the species in the area (as obtained from the biological sampling).

There are numerous other techniques that can be used to estimate numerical fish quantities from area echo abundance (see e.g. Roa-Ureta and Nikilitschek, 2007). They all rely on accurate TS measurements.

Bottom dead zone. The main drawback of the standard acoustic surveys when estimating abundance of demersal and semi demersal fish is the bottom dead zone. Corrections for

unsampled fish in the dead-zone and in the zone above the bottom within the backstep interval must be made in post-processing. This will generate a more representative abundance estimate for demersal fishes. This is commonly done by assuming that the abundance of fish in the acoustic dead zone is not significantly different from the abundance just above the acoustic dead zone. In CoralFISH ROV video footage can be used to check for abundance differences in the zone just above the bottom dead zone and in the dead zone. We also strongly recommend the usage to deep towed high frequency acoustic bodies to improve the acoustic abundance estimation of demersal fish especially in localities with a bottom depth exceeding 500m.

Detection probability

Target far away from the transducer have a lower probability of being detected, i.e. as range increases the signal to noise ratio decreases and the echo-amplitude from the target drop below noise level. This effect is particularly important when the surveying deep habitat and demersal fish species and species without swim-bladder or other gas bearing organs. Detection probability of small targets at great depth at very low signal to noise ratio can be increased by using long pulses in combination with a small band width and low threshold, i.e. < - 80 dB. Noise can be generated both by unsynchronized instruments and by e.g. the vessel itself, other vessels, wind and sea. It is recommended that other acoustic instruments (e.g. fish sonars or echo-sounders operated from the bridge deck) are switched off during the acoustic data collection. (LSSS will remove most of the noise automatically). When working with hull mounted transducers on research or fishing vessels it is particularly difficult to obtain spatially comparable data (Korneliussen et al., 2008). In those instances measurements of vessel noise should be done regularly.

Some species are known to disperse in the water column during darkness and concentrate in the daytime (Hjellvik et al. 2003). The detection probability for those species could be enhanced by sampling during daytime. Species that are known to migrate towards the surface would be more easily detected during night. Timing of the survey will also affect how the fish populations intermingle with in DSL.

Data management and archiving

Raw data files and post processing system files should be logged, written to an external hard-drive and viewed live in the software system installed on the research vessel. An upper limit of the file size of 100 MB is recommended to facilitate file handling and data transfer. At the end of each transects the raw data files should be exported to a second external hard-drive. Raw data, post processing system files and judged data should be burned to a DVD at the end of each day.

Protocol A4 – Targeted trawling

Trawling

Trawls in acoustic surveys are targeted on schools or layers detected on the echo-sounder charts. This sampling is primarily done to validate species composition of the acoustic backscatter and to obtain mean length distribution for target strength estimation. The tows are

of relatively short duration and do not stretch across different habitat types. Aimed trawling requires effective net mensuration instrumentation including door or wing sensors, third-wire sensors, depth sensors and head and foot-rope sensors. Trawl mensuration is also vital to prevent any contact between the gear and the coral. Data from trawling and information on gear used shall be registered (Appendix 1, Table 2). After the net haul the vessel will return directly by the shortest route to the acoustic line and continue the acoustic transect.

Biological sampling

All fish (or a representative sub-selection in the case of large catches) shall be identified and length determined. Length frequency distribution charts for the most abundant species will be produced and mean length should be estimated. Otoliths should be taken from maximum 30 specimens of each species from each haul. On these same specimens weight, sex and (if possible) stage of maturity should be determined. If stomach has not been everted during hauling the stomach and gut should be collected and stored for later analysis in laboratory. The biological data should be recorded on standard observer forms (Appendix 1, Table 3). Stomachs should be but in plastic bags, labeled with station, event and fish number and stored frozen. For most demersal fish species otholits are put directly into paper bags or envelopes, labeled with station, serial and fish number.

If any of the following fish species are caught in the trawl, *Beryx splendens, B. decadactylus*, *Pagelus bogaravoe*, *Heliolenus dactylopterus dactylopterus*, *Trachyscorpia cristulata echinata*, *Hoplostethus atlanticus*, *Chimrea monstrosa*, *Lophius piscatorius*, *Phycis blennoides* or *Molva dipterygia*, samples should be taken for DNA analysis according to the protocol "Sampling corals and associated species in CoralFISH" (http://www.eu-fp7-coralfish.net/).

Data management and archiving

Backup procedures should be put in place to secure the electronic files of the sampling metadata, fish biological data and the position logging files.

Protocol A5 –zooplankton distribution and density estimates

Optical plankton counters (OPC) and laser OPCs

At 200-300 m it is possible to detect larger zooplankton using hull and keel mounted echo-sounders (i.e. 120 kHz and 200 kHz together with noise reduction algorithms) but not beyond that depth. For detection of smaller zooplankton and in deeper areas it is necessary to use towed gear. Optical plankton counters (OPC) and laser OPCs can be towed down to 3000 m and should be used if other deep towed acoustic systems are not used.

Net sampling

Targeted sampling. Directed or targeted net sampling should be carried out to validate and identify acoustic targets detected on the echogram. Different types of plankton tow nets will be used onboard the different research vessels. We recommend that nets should all be

equipped with a flow-meter to estimate the filtered water volume and real time depth recorder should be attached to the gear. A net haul data entry table can be found in appendix 1, Table 4.

After the net haul the vessel will return directly by the shortest route to the acoustic line and continue the acoustic transect.

Abundance estimations. Zooplankton net sampling will also be used to estimate the density or biomass of zooplankton and to relate zooplankton distribution to topographic bottom features such as coral mounds, ridges and banks. The distribution of fish and corals in relation to the zooplankton abundance will be assessed. The horizontal zooplankton sampling could be carried out either along the long-line fishing transects, along the ROV video transects or new zooplankton transects could be selected (randomly) within each study area. As different gears will be used no standardization of the net sampling (duration of haul, speed etc.) will be done. However the net haul data entry table should be filled in carefully. We recommend that the deepest horizontal zooplankton sample should be taken not more than 20 m above the sea-bed. Preferably two different gears should be used, one targeting meso-zooplankton (such as copepods) and one targeting macro-zooplankton (such as krill).

Processing of zooplankton samples

The total volume of the net catch should split into two equal parts. One part will be preserved in 10% buffered formalin solution and will be used for species identification and enumeration. The other half will be size fractionated using sieves of three mesh sizes (the smallest size should match the net mesh size) and used for dry weight measurements on board the vessel. This will give a fast indication on the amount and types of zooplankton (small copepods vs. krill and small fish) at the investigated localities.

These data together with information on fishing depth, start and stop position of sampling and filtered water volume will be used to estimate zooplankton densities at the studied sites. In areas where detailed information on coral distribution is found the zooplankton distribution can be related to topographic bottom features such as coral mounds, ridges and banks. During sample processing special attention should be given to early life stages of fish species. All relevant data should be entered in Table 5, Appendix 1.

Oceanographic measurements

It is recommended that salinity, temperature, fluorescence and turbidity should be measured at each zooplankton collection station.

ASSESSMENT OF FISH DISTRIBUTIONS BY LONG-LINE FISHING

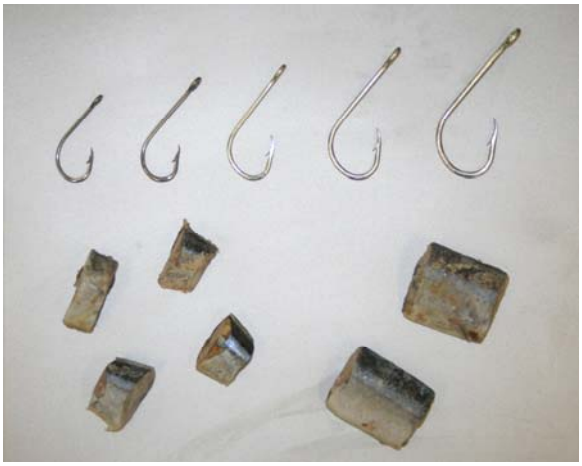
INTRODUCTION

Description of work

Research vessels or hired commercial fishing vessels will be used to carry out long-line fishing on coral ground and in adjacent non-coral areas to assess the distribution of fish in relation to these habitat features. This will ground-truth the results from the video and acoustic surveys. Samples will provide information on catch composition (number and species), sex, age and size of fish, condition factor, stomach and gut content, on and off-reef.

Background information on long-line fishing

Long-line is a passive gear that is well suited to catch fish in fragile and structurally complex habitats. The fishing technique has the advantage that large areas can be covered relatively quicky and that the data collected is of a high resolution when is comes to biological information of the species caught. The long-line fishing can provide information about fish distribution in relation to habitat features with a spatial resolution of about 1 km, i.e. Løkkeborg (1998) showed that cod can be attracted by the smell of bait at 700 m away from the source. Limitations are mainly the selectivity of the gear, i.e. some species are attracted by the bait while others are not and hook size, bait size and bait type will affect what type and size of the fish caught (se chapter below). If long-lines are repeatedly set in the same area, the first line is most likely to catch higher proportion of elasmobranchs compared to subsequent lines, where the catch would be mostly fish. Some long-liners in Iceland set out the first long-line in an area to "get rid of the sharks", and then set the long-line repeatedly on similar location to catch fish. For some species lines set at night produce higher catch rates than those set during the day (Løkkeborg and Pina, 1997), i.e. dusk has been recorded as a period of high feeding activity (Fernö et al., 1986, Løkkeborg et al., 1989). It is therefore important to plan the experimental fishing carefully and to use the same study design at all investigated sites.



Selectvity of the gear

The long-line is both species and size selective. The gear specific factors that add to the selevctivity and that can be controlled are hook size, bait type and size of the bait.

By using large hook smaller fish might be undersampled. It is for example known that smaller hooks catch more haddock than large hooks. Cod and haddock are caught mainly on EZ 10. Also redfish, that has relatively small mouth relative to body size, is more easily caught on EZ10/11 hooks compared to EZ12/13 hooks. EZ14/15 hooks will catch larger fish, such as halibut and elasmobranchs. One idea to increase the size range of species caught is to alternate between small (EZ10 or 11) and large (EZ12 or 13) hooks on the line. By adopting this approach we should be able to catch most of the small and big fish (i.e. small and large mouthed fish) in the study areas. This appoach would also guards against a potential bias in size selectivity, e.g. if there are mainly small species inside the coral grounds and larger ones outside or vice versa. Bait type can be highly influential in species (possibly size) selection. The baits we would use would be saury/mackerel, herring and squid (see Table 2, Appendix 4). These bait types are very different and would allow us to capture a wide range of fish species. One option would be to bait these individually on each hook size, which would result in 9 bait and hook size combinations. A more feasible aproach would be to mix all the baits together and add them to the hooks randomly. Selection in the long-line fisheries varies very much with the bait size, and possibly it is more size (species) selective than hook size. As an example, the size captured of cod on long-line can vary with the bait size. To sue two bait sizes, e.g. 10 g and 20/25 g, would prevent this.

A more simple approach would be to not try to catch all the availabel fish species and sizes in a given area but to focus of the commersialy valuable species targeted by the long-line fishermen, by carrying out experimental long-line fishing using the standard gear of the regional fishermen. This also tells us whether the local fishermen catch more fish in coral than in non-coral habitats.

Special considerations

The CoralFISH study areas are located to Iceland, Norway, Rockall/Porcupine, the Bay of Biscay, the Azores and the Ionian Sea. Six vessels will participate in the experimental long-line fishing. To assure that high quality data is being collected and that the data is collected in a comparable manner between all participating institutions protocols describing survey and sampling strategies for the assessment of fish distributions have been developed. Due to the fact that that the depth varies greatly in the different study locations (i.e. from 200 to 1200 m), that different species of fish are found and that the coral habitats differ in the degree of patchiness and spatial extent the long-liners operating in the 6 study areas use somewhat different gear setup (hook sizes, length between hooks, bait types, bait size, snood length etc.) (Table 2, Appendix 4). We will assume that the gears and methods have been devoloped by the fishermen to most efficiently catch the commersially valuable fish in the area. This protocol will focus on describing standardizations that are vital for allowing us to statistially test the hypothesis more fish are found on or close to coral grounds than on non-coral grounds. All other factors will be left in the way that they are being used or adapted to a specific habitat. For example, the lenght of the lines set will be matched with the coral structures at the site.

Examples describing the experimental long- line fishing to be carried out in Norway, Iceland and Italy/Greece can be found in Appendix 5.

Protocol L1 – Experimental setup

Experimental design

In all six regions multiple coral and non-coral experimental plots/sites should be investigated. Due to the fact that fish can be attracted over distances of close to 1 km we recommend that control sites are placed at least 2 km away from coral sites or even further off if possible. Coral and non-coral plots/sites should range the same bottom depths, should have similar bottom topography and have similar hydrographic conditions.

In all plots/at all sites multiple long-line transects should be set. Transects should be set perpendicularly to the greatest environmental gradient, i.e. most often depth. If lines are set parallel to the current going down current a high precision in the positioning of the line can be achieved, while it can be hard to set the line with strong side currents. Careful consideration should therefore be but into choosing appropriate sites for the long-line transects. To avoid that one line steals fish from another line we recommend that the replicate transects within one experimental block or at one experimental site are placed at least 900 m apart.

The length of the transect should be adapted to the specific coral habitats. All coral and non-coral replicate transects should be of equal length. The number of hooks set in each study block/site should be equal. This can be standardized within each region but not between. Table 2 in Appendix 4 shows that the distance between hooks in the Atlantic long-line fishing fleet is about 1.5 m compared to 5-10 m in the Mediterranean.

Set time of the line should be at least 4 h.

At what time period during the day the line is set will affect to catch efficiency of some species. If all plots are sampled once a day, which plots that should be sampled first should be selected randomly. The next day the order should be altered. Time period of sampling will be of less importance at high latitudes during the summer.

Hook type can influence the catch efficiency. It seems to be that EZ hooks hold better the fish after capture compared to the J hooks. The gear used should be similar in all sites. It is important to use thick long-line (e.g 12 mm) in coral areas, as these are very rough grounds and can basically tear the thinner long-lines.

Environment

Factors that are hard to control are current strength and direction. These are factors that are important in determining the direction and the distance that fish can detect the odour of the bait. One approach to control for this would be to measure the current strength and direction with current meter, as this will determine the spread of the odour of the bait. Similarly, the tidal cycle needs to be taken into acount as the current strength should be lowest between tides.

Protocol L2 – Sampling procedure

Setting the lines

Every time a long-line it set for the sampling of fish, the station information sheet (Appendix 2, Table 1) must be filled in. Some of the fields will be constant throughout the survey (e.g. country and ship). This table also includes detailed information about the fishing, positioning of the gear, duration of the fishing operation, the weather conditions and sample condition.

Collecting catch

When the catch is brought on deck catch should be registered per hook. Empty hooks with bait, empty hook without bait, missing hooks, missing snoods, by-catch such as *Lophelia* sp., soft corals and sponges should also be registered. Fish and by-catch should be collected in baskets á every 250 m of line for later processing.

Abiotic factors

In order to explain spatial and temporal patterns in fish distribution, as many abiotic parameters as possible should be measured. Depth should be recorded as actual depth. We would try to add a DST tags on the line, which would allow us to record automatically the depth of the line and temperature. This allows assessing the composition/abundance of fish relative to temperature and depth at capture. This would be useful if the line is set down a slope and so enabling evaluation on e.g. species composition relative to the depth.

Protocol L2- Catch Processing

Samples from the long-line fishing will provide information on catch composition (number and species), sex, age and size of fish, condition factor, stomach and gut content. This protocol will describe the processing of fish and by-catch in the experimental long-line fishery. Procedures that are described are, field sampling of fish and corals, analysis of fish stomachs, forms and the filling in of forms and sampling for genetic analysis.

Field sampling

All fish shall be identified, length determined and analyzed for age, weight, sex and where applicable, maturity. Stomachs should be collected whenever possible. Stomach and intestine should be cut loose at anus and gullet (throat), placed in plastic bags and frozen immediately. Stomach analyses record the filling, degree of digestion, contents of the stomach, weight, species and number of prey. The biological data should be recorded on standard observer forms (Appendix 1, Table 4).

Due to the large variety of species found in the 6 different regions species specific sampling instructions for the determination of age, maturity and length are not included in this protocol but will have to follow the ones developed by the participating research institutions.

The identity of a sample of otoliths and scales should be station number, serial number, fish number, species, date and vessel. If stomach and gut is collected, i.e. if stomach is not everted, the plastic bags should be labeled similarly. For most demersal fish species otholits are put directly into paper bags or envelopes and labeled. Scales must be frozen immediately to prevent them from drying up. Scales should be selected from the same body part in all fish.

Sampling for genetic analysis

If 25 to 40 individuals from one point or two points close by are caught of any of the following fish species are caught, *Beryx splendens, B. decadactylus*, *Pagelus bogaravoe*, *Heliolenus dactylopterus dactylopterus*, *Trachyscorpia cristulata echinata*, *Hoplostethus atlanticus*, *Chimrea monstrosa*, *Lophius piscatorius*, *Phycis blennoides* or *Molva dipterygia*, samples should be taken for DNA analysis according to the protocol "Sampling corals and associated species in CoralFISH" (http://www.eu-fp7-coralfish.net/). Genetic samples of corals will also be collected according to the same standards. It is important that the samples material is as fresh as possible. Preferably samples should be collected in a cool room or with the fish on ice.

By-catch of corals

While the effect of a single long-line is minor compared to the trawl, the long-term impact of passive gears can be significant. The experimental long-lining will allow us to assess the impact of fishing on coral by registration of coral by-catch. The by-catch of coral can then be compared with the estimated density of coral in the exact same transect, estimated from either acoustics (Svellingen et al. in prep.) or from ROV video recordings. If we are to conclude anything about the commercial fishing and the potential effects of it, it is vital that the experimental fishing in the area is carried out using the same gear and setup as the commercial fishing. By-catch of coral will be recorded á every 250 m of line by entering species, biomass or volume (when possible) on the standard form (Table 2, Appendix 2).

Stomach content analysis

Prey will be identified to lowest practicable level. If not possible prey should be split into groups and then weighed. A detailed observation of digestive stage should be included (Table SC). Five different stages will be included and the degree of digestion will be filled in the form according to the category: 1 = prey undigested, 2 = digestion started, prey easily identifiable, 3 = Advanced digestion, species or groups may be identified, 4 = digestion almost complete, remnants of main prey groups can be identified, 5 = complete digestion, prey cannot be identified or counted. If weight or volume has not been registered this line is left blank. Number of prey should be denoted and the total weight of stomach content and weight of prey/prey groups when possible. Looking at the zooplankton samples from the different areas will help in the identification of prey.

Data management

Data from the fish and zooplankton catch processing and haul operations should be recorded to a PC during the survey and the files should be backed up to an external hard drive or DVD once every 24 hours. Backups of the position logging files and the oceanographic data should also be taken.

UNDERWATER VIDEO ASSESSMENT OF FISH AND COLD WATER CORALS

INTRODUCTION

This document provides operating guidelines and protocols for underwater video assessments carried out within the CoralFISH project. It covers towed transects of pre-determined lines using video platforms and piloted transects along a pre-determined course using ROVs. The guidelines will ensure that the video material is collected in a safe and comparable manner by all participating institutions and by personal working on different shifts on the same research vessels. The protocols will assure that all relevant metadata is being collected.

The aim of the underwater video assessments is to generate fully quantitative datasets describing species composition and the number of fish in cold water coral habitats and in similar non-coral habitats. Underwater video sampling will be used to obtain images of the fishes, to compliment the recordings of the acoustic assessment close to the bottom where trawling is impossible and to provide data on fish abundance in the near-bottom acoustic dead zone. In addition, association between specific habitats and fishes will be obtained on a much smaller scale that what will be obtained in the line-fishing or the acoustic surveys covering the same area.

Due to the variety of systems used in the project (Appendix 4, Table 3) no detailed information on how to operate the gear or run the instrumentation is included.

The text is based on the following documents:

Water quality – Visual seabed surveys using remotely operated and towed observation gear from collection of environmental data. Norwegian Standard. NS 9435:2009.
MESH (2005). Rewiev of standards and protocols for seabed habitat mapping. 192 pp. (http://www.searchmesh.net/)
Reed, H.L. (ed) 2009. Guidelines for the study of the epibenthos of subtidal environments. ICES Techniques in Marine Environmental Science. No. 42.

METHODS

Study design

The number of replicate transects or independent sections of video that are processed should be as large as practicable as this will increase the accuracy of the calculated abundance estimations of the sample populations. We suggest that least five replicate transects are taken from each coral and control ground. To save time, one long transect could be run instead of running several small transects. This long transects could later be subdivided. Care needs to be taken if sub-sections of video tows are to be used as separate samples (avoid pseudoreplication).

Protocol U1 - Calibration and system performance

Set-up and testing of equipment

Make sure that the set-up for the video recording is correct by testing it before deployment. If more than one video-recording method are to be used in a linear sequence (e.g. digital tape, DVD) they must be connected in the right sequence, i.e. with the slowest device last in the sequence. This is because some device only record part of the full signal leading to a loss in the quality of the recorded image. Multiple recording sequences should be data file, DV tape, VHS tape and DVD.

Synchronization of clocks

Clocks in the computers and video recorders must to be synchronized with the GPS time. The clocks should be checked every few days. The synchronization will assure a proper georeferencing of the data.

Size calibration/scaling

For the interpretation of images and subsequent analysis it is important that the scale of the image can be determined. Multiple laser point projected at fixed distance apart onto the sea-bed can be used as a reference scale against which to compare the images. Laser scaling device is the optimal scaling device because the field view of the camera alters when the height above the sea bed changes. If laser scaling devices are not available a scale bar can be fixed as close as possible to the base of the video frame and used to determine size. Recording from an altimeter positioned on the gear can also be used to calculate the area viewed.

Standard operating procedures

Camera field view and lighting should be standardized and recorded. If the camera can be tilted when deployed a set position should be used during survey and this position should be kept when running the transects. Preferably the camera should be pointed slightly forward to prolong the time period each organism will be in the camera field view and aid identification.

If the survey is being performed in an area that has been mapped with multi-beam acoustic the generated x, y and z data should be imported into the navigational system of the vessel to facilitate correct navigation and accurate sample collection. If live positional data of the ROV or the towed video platform is feed into e.g. ArcVeiw or Fledermaus, with topographic maps the camera could also be raised on the approach of oncoming obstructions. A forward looking mounted camera with wide-angel view will also facilitate maneuvering the gear in topographically complex terrain. A video monitor placed on the ships bridge can also be useful.

Georeferencing of images

It is critical that position data is continuously recorded. This information will be used to standardize the video images and make abundance estimations based on area covered (i.e. on a large scale).

When USBL (Ultra Short Base Line) or SSBL (Super Short Base Line) is being used a calibration report involving the USBL system, satellite navigational system and the gyro- and navigational software used should be shown. The standard deviation error for the recorded GPS positions can be calculated by allowing the ROV or towed video platform to rest on the sea-bed for half a minute to collect series of positional data from the same point. Accuracy of the positional data should be <5% of the water depth at the site.

If the video platform does not have a USBL device the actual position of the camera can be calculated by using Pythagoras' Theorem and the distance between the GPS antenna and the place of the ship where the platform was launched. When displayed in a GIS the video track could later be calibrated against known sea-bed features seen in the video record.

Preferably datum and projection used should be standardized (WGS-84 and UTM are recommended).

Protocol U2 - Conducting a video dive – Acquisition of sample

Pre deployment

Enter the relevant metadata onto the video overlay system. Alternately, copy stations data (cruise name and number, station number, survey and gear) onto whiteboard or paper and record video shots of this to mark the beginning of the record for that dive. If a DVD recorder is being used, use the pause function to halt recording.

Test that cameras, lights and lasers are working. Lights and lasers should be switched of during deployment. Set camera to manual focus. Autofocus mode should not be used because particles in the water column could cause focusing error.

Deployment

The vessel should come to a stand-still before deployment. Deployment will be done either at the stern or over the side of the vessel. Once the ROV or video platform is submerged by 1-2 m, the lights, lasers and observations cameras can be switched on.

The transect

When approaching the sea-bed turn on all cameras. Once on the sea-bed allow the ROV/video platform to rest for a while. If desirable allow a couple of minutes to study area in detail. Set the focus of the camera by zooming in as far as possible and adjusting focus. Zoom out until wanted field view is achieved and start. At the starting point of the transect a position fix should be taken and the GPS logging device and the video recording should be started simultaneously. Time, position, water depth and vessel speed should be denoted. At the end of the transect a new position fix should be taken and the GPS logging device and the video recording should be stopped simultaneously. Time, position, water depth and vessel speed should be denoted (Appendix 3, Table 1).

To enable identification of fauna <10 cm cameras should be run less than 3 m from the bottom. The camera height above the bottom should be measured with an acoustic altimeter or mathematically when laser points are used for scaling. Height above the bottom should not vary more than from 1-3 meters. To keep the towed video platform at the same height above the sea-bed during the duration of the tow the length of the cable has to be adjusted continuously. It is recommended that the person operating the winch can see the real-time images from the camera.

Speed should be kept between 0.5 and 1 knots.

During dives even moderately strong currents can have considerable pulling effect on the cable making the ROV difficult to pilot. In areas with strong tides the ROV dives should be performed at slack tide.

Recovery

Before the ROV or video platform reaches the surface the lights and lasers should be switched off. The vessel should come to a complete halt. All power to the ROV or video platform should be switched off before lifting it out of the water. Once on deck the gear should be inspected for any damage on gear, underwater housings and loose connections of cables etc..

Protocol U3 - Data collection, storage and quality control

Logging of data

Complete all sections in the sampling station form for which information is known before deployment of the gear. This involves survey name, date, station number, serial number and weather conditions etc. During the observational phase (i.e. when the transect is run) notes should be made on the field record sheet on the nature of the sea bed, presence of Lophelia sp. and other habitat forming species (such as gorgonians and sponges) and the fishes observed. Changes in the water depth along the transect should be denoted as should the changes made to the length of the cable when towing gears. Notes should be made on the clarity of the image and the field view.

To obtain fast information on the total abundance of fish and the abundance of specific fish species in coral and non-coral habitats Table 2, Appendix 3 could be used. This table should be filled in beforehand with the fish species one expects to find in the regional study area and with some rows left blank. Species that are difficult to identify could be placed in a family or denoted as others. All observed fish are simply placed with species identity in one of the three categories "live", "dead" or "mixed" Lophelia or denoted as "other habitat". For the categories "live", "dead" and "mixed" (live mixed with dead Lophelia) the presence of sponges or gorgonians should be denoted with a g for gorgonians or s for sponge each time they occur.

Data management and archiving

Preferably two video-recording devices should be used. That with the highest quality should be regarded as the master copy and that with the lower quality should be regarded as the back-up. The labels and contents of all recording media should be logged. Tapes and DVDs should be stored in such a manner that degradation of their quality is limited. A back-up should be taken of the media with the highest resolution image from each transect (store on external hard-drive).

Quality check

After each transect check that the field record sheet and station data has been properly completed. The metadata gathered should then be transferred to a database.

Video techniques deliver direct visual information so there will be no need to process the video image to correct errors. However, sometimes pictures are blurry and the view will be reduced. To fast speed will also affect the clarity of the pictures. For towed videos swell will affect quality of image. Images of low quality should be discarded and not used in quantitative analysis.

If a position logging device has been used check the file to see that the position data has been properly logged. This can be done e.g. by crosschecking the ROV/video platform's positional data with the ships positional data by plotting them both in a GIS. Errors could involve the whole track or individual points and should be corrected. When the errors involve individual points wrong values could be replaced with an average estimated from the two positions registered before and two registered after the wrong point. Irrelevant positional data (e.g. data from before transect start) should be deleted from the log file. If whole tracks are wrong the position of the camera has to be calculated by using Pythagoras' Theorem and the distance between the GPS antenna and the place of the ship where the platform was launched.

Protocol U4 - Data processing

The analysis and interpretation concern both the biological and physical structures observed in the image. It relies on the experience of the observer and can be highly subjective. It can be carried out by one experienced marine biologist but it is recommended that some quality assurance tests are carried out letting a second experienced marine biologist reanalyze parts of selected video transect.

Species identification and data tabulation

All fish observed along the transect should be tabulated and identified to lowest practicable taxonomic level. Care should be taken so that the same individual fish are not counted twice. Large sessile epifauna such as gorgonians and sponges and other large physical structures (such as stones) should be tabulated. Coral (i.e. Lophelia) cover estimations should be made. Habitat types that are to be identified in the survey could be determined beforehand if the survey area is well known. Density of Lophelia reef structures along each transect should also be made one a larger spatial scale based on multi-beam images.

Trawl and line catches could be used to verify the identification of difficult species, i.e. after inspecting features that are not visible on video.

Fish will always be registered in combination with a habitat type. In addition, the behavior of the fish should be denoted; swimming, hovering, on/behind habitat forming species or within habitat forming species. Swimming should only be denoted if it is a part of the natural behavior of the fish and not when it is escaping from the ROV/video platform. If gravid female are observed this should be denotes as should any observations of feeding. This data will be used to correlate the occurrence of selected species with small scale habitat features.

Fishing activity can be seen either by marks on the substrate, damaged epifauna or by lost gear. Note that marks on the substrate and damaged fauna can be caused also by other activity. Lost fishing gear such as wire, nets, ropes/line and floats are more certain confirmations of fishing activity. Personnel with experience of fishing can also determine what type of fishing (trawling, line fishing or gillnetting) the fishing gear stems from.

Table 3 results will be used to compare abundances between different transects and between coral and non coral areas.

Statistical analysis

Suggested statistical analyses include testing for correlations between the occurrence of different fish species and habitat forming species and bottom substrate on a smaller spatial scale and an analysis of variance between plots/subareas. Multivariate analysis can be performed as well as geostatistical analyses. A detailed protocol for the analysis of ROV data is being developed and will be available soon after this summer's cruises. This protocol will also describe database development and the linking of species abundance with positional data.

REFERENCES

Gilbert, R.O. 1987. Statistical Methods for Environmental Pollution Monitoring. Van Nostrand reinhold, New York.

Hurlbert, S.H., 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54,187–211.

McCoy, E.D, S.S. Bell, 1991. Habitat structure: the evolution and diversification of a complex topic. In: Bell, S. S., McCoy, E. D., Mushinsky, H. R. (eds.) Habitat structure -the physical arrangement of objects in space. Chapman &
Hall, London, p. 3-27.

Underwood, A.J., 1996b. Spatial patterns of variance in densities of intertidal populations. In: Floyd, R.B., Sheppard, A.W., De Bario, P.J. (Eds.), Frontiers of Population Ecology. CSIRO Publishing, Melbourne, pp. 369–389.

Scheiner, S.M. and J. Gurewitch (editors). 2001. Design and Analysis of Ecological Experiments. 2nd Edition. Oxford University Press.

Schenker, N. and J.F. Gentleman 2001.On Judging the Significance of Differences by Examining the Overlap Between Confidence Intervals. American Statistician 55(3): 182-186

Underwood A.J., M.G. Chapman, S.D. Connell. 2000. Observations in ecology: you can't make progress on processes without understanding the patterns. Journal of Experimental Marine Biology and Ecology 250: 97–115

Appendix 1

Table 1. Survey settings and calibration report for the CoralFISH acoustic survey

Ship:		Date:	
Echosounder:		Localization:	
Sphere:		TS_{sphere}:	
Bottom depth:			
Calibration version:			
Reference target			
TS:		Min. distance:	
TS deviation:		Max. distance:	
Transducer:			
Frequency:		Beamtype:	
Gain:		Two way beam angle:	
Athw. angle sens.:		Along. angle sens.:	
Athw. beam angle:		Along. beam angle:	
Athw. offset angle:		Along. offset angle:	
SaCorrection;		Depth:	
Transciever:			
Pulse duration:		Sample interval:	
Power:		Bandwidth:	
Sounder type:			
TS detection			
Min. value:		Min. spacing:	
Max. beam comp.:		Min. ecolength:	
Max. phase dev.:		Max. ecolength:	
Environment			
Absorption coefficient:		Sound velocity:	
Beam Model results			
Transducer gain:		SaCorrection:	
Athw. Beam angle:		Along. beam angle:	
Athw. Offset angle:		Along. offset angle:	
Data deviation from beam model			
RMS:			
Max.:	No.:	Athw.:	Along.:
Min.:	No.:	Athw.:	Along.:
Data deviation from polynomial model			
RMS:			
Max.:	No.:	Athw.:	Along.:
Min.:	No.:	Athw.:	Along.:
Remarks:			
Wind speed:		**Wind direction:**	
Raw data file:			
File name:			
Created by:			

Table 2. Fishing station form for CoralFISH targeted trawling

	Country	
	Vessel name	
	Cruise number	
	Date	
	Station number	
	Serial number	
	Station type	Acoustic target identification
Gear information	Gear type	
	Net type	
	Mesh size	
	Cod end	
Position start *	Latitude	
	Longitude	
Position stop *	Latitude	
	Longitude	
	Bottom depth	
	Start time *	
	Stop time *	
	Distance trawled	
	Tow duration	
	Speed of trawl	
	Max. fishing depth	
	Min. fishing depth	
	Trawling direction (^{o})	
	Height of opening ±SD	
	Door spread ±SD	
	Wire length	
Weather condition	Wind speed (knots)	
	Wind direction (^{o})	
Sample	Sample size	
	Condition of trawl	
	Quality of catch	
	other comments	

Position, start and stop time should be when trawl arrives and leaves fish depth

Table 3. Specimens form for the CoralFISH targeted trawling

Vessel:	Date:	Station number:	Serial no/sub-section:
Gear:	Type activity:	Species:	

Fish no.	Length	Weight	Sex	Stage	Age	Stomach content	Digestive state	

Table 4. Zooplankton net haul data entry sheet.

	Year	
	Country	
	Vessel name	
	Cruise	
	Date	
	Location	
	Station number	
	Serial number	
	Station type	
Gear information	Gear type	
	Opening width	
	Net type	
	Mesh size	
	Cod end	
Position	Latitude	
	Longitude	
	Bottom depth	
	Start time, net in water (GMT)	
	End time, net out of water	
	Haul duration	
	Flowmeter start	
	Flowmeter stop	
	Flowmeter distance	
	Filtered water volume (m3)	
	Max. fishing depth	
	Min. fishing depth	
Weather	Wind speed (knots)	
	Wind direction (^{o})	
Sample	Sample size (ml)	
Comments		

Table 5. Net sample data entry form

Station number	**Serial number/ sub-section**	**Gear**	**Dominant species/group**	**Biomass (g DW) in sample**			**Filtered volume (m^3)**	**Biomass (g DW m^{-3})**		
				Small size fraction	Medium size fraction	Large size fraction		Small size fraction	Medium size fraction	Large size fraction

Appendix 2

Table 1. Fishing station form for CoralFISH experimental long-line fishing

	Country	
	Vessel name	
	Cruise number	
	Date	
	Station number	
	Serial number	
	Station type	
Gear information	Length of line	
	Number of hooks	
	Hook type	
	Bait type	
	Bait size	
Position	Latitude	
	Longitude	
	Bottom depth	
	Set time , start	
	Set time, stop	
	Pick up time , start	
	Pick up time, stop	
	Max. fishing depth	
	Min. fishing depth	
Weather condition	Wind speed (knots)	
	Wind direction (^{o})	
Sample	Condition of line	
	Quality of catch	
	other comments	

Table 2. Coral and sponge by-catch in line fishing

Vessel:	Date:	Serial no/sub-section:
Gear:	Station number:	

Species/group	Weight	Volume	Name of photo file

Appendix 3

Table 1. Station information

Vessel name		Gear type/name	
Cruise number		Camera field view	
Survey name		Lighting	
Project	CoralFISH	Position logging device	
Date		Kartdatum	
Station number		**Weather condition:**	
Serial number		Wind speed (knots)	
Station type	Video transect	Wind direction (°)	

Transect data

Transect start	Latitude	
	Longitude	
	Time	
	Bottom depth	
Transect stop	Latitude	
	Longitude	
	Time	
	Bottom depth	
	Tow distance	
	Tow duration	
	Speed of tow	
	Max. depth	
	Min. depth	
	Tow direction (°)	
	Wire length	

Filed observations:

Data storage

File name	
DV-tape label	
DVD label	
Harddrive	

Completed by:

Table 2 Field observation sheet recording the abundance of the fish species observed in a few selected habitat types. The table should be filled with species manes of the fish species that are expected to occur in the region in advance of the survey. Their presence in a specific habitat should marked (counted) by making a vertical line each time an individual of a specific species occurs in the habitat. See the example below.

Project	CoralFISH
Date	
Station number	
Serial number	
Station type	Video transect

	Habitat type				
Fish species	**Live L**	**Dead L**	**Mix L**	**other habitats**	**Comments**
Ling	III ggs	IIII	II g	IIII	
Tusk	II	III ss	II sg	II	

For the categories Live, dead and mixed (live mixed with dead) Lophelia the presence of sponges or gorgonians should be denoted with a g (gorgonians) or s (sponge).

Appendix 4

Table 1 Response from partners which have returned the questionnaire for the acoustics

	Vessel	Echosounder	Frequencies	Transducer	Calibration	Software	Experience	Contact person
MRI-Island	RV *Arni Friðriksson*	EK60	18, 38 and 120 kHz	on drop keel		Bi500 or Echoview	Quantitative assesment of pelagic fish	Páll Reynisson pal@hafro.is
MRI-Island	RV *Bjarni Sæmundsson* 1970 Length: 56 Beam: 10.6	EK60	18, 38 and 120 kHz	on hull	using copper spheres	Bi 500 Echoview	Quantitative assessment of pelagic fish	Páll Reynisson pal@hafro.is
CoNISMa-Italy	*Universitatis* Length: 44.8 Beam: 9	EA400 EK60	27 and 200 kHz 38 kHz	on hull		Kongsberg-Simrad EA400 EK60	Qualitative assessment of seabed, fish and plankton	Alessandra Savini Alessandra.savini@unimib.it
IMAR-Azores	RV *Arquipélago* Length 25.4 m Propulsion: 370 kW Cruising speed 9.5 knots	EK500	38 and 120 kHz (split beam)	on hull	Factory manual Foote et al. 1981	MOVIES + v4.4	Quantitative assessment of pelagic fish	Alexandre Morais alexandre.mo.morais@azores.gov.pt Telmo Morato telmo@uac.pt
Ireland	RV *Celtic Explorer* Length: 65.5 Beam: 15 Cruising speed 10 knots	EK60 (single beam) ES 38B (split beam)	18, 38, 120 and 200 kHz	on drop keel	as per manual (metal spheres of different sizes)	ER60 v2.2.0 Ech View/Eco Log (biomass calculation)	Quantitative and qualitative assessment of pelagic fish	Ciaran O'Donnell ciaran.odonnell@marine.ie
IMR-Norway	RV *Håkon Mosby* Length: 47.2 Beam: 10.3	EK60	18, 38, 120 and 200 kHz	on drop keel	Foote et al. 1987	LSSS	Qualitative and quantitative assessments of pelagic fish	Odd Aksel Bergstad oddaksel@imr.no Egil Ona
Greece	RV Philia Length: 26 m	Biosonics DT-X Split beam	38 and 120 kHz	Surface side towed	Standard calibration sphere at start of each trip	Biosonics visual acquisition and analyzer	Quantitative assessments of pelagic fish	Chris Smith csmith@her.hcmr.gr

Table 2 Response from partners which have returned the questionnaire for the longlining

	Iceland	Norway	North Ionian Sea fisheries (Italian coast)	Greece Hake deep-water long-line fishery: **HDW** Wreckfish deep-water long-line fishery: **WLF** Blackspot red seabream deep-water line fishery*1: **BSF**	Ireland	Azores
What is a typical total length of one set of bottom long-line, in (km?) and number of hooks?	Mainly two types of long-line boats; 1) hand baited the line, typically with ≈30 tub of line; each line with 520 hooks, the length of the line ≈625 m. Hand baiters are setting ~ 15600 hooks and 18.7 km in length. 2) auto-liners, more variation. Generally, auto-liner sets ≈50 km of line with max 42,000 hooks.	Number of hooks and length of the line varies from 5000 - 40 000 hooks and 10 - 50 km. An autoliner will have 30 000-40 000 hooks and the line will be 40 to 50 km. A coastal longliner will have 12 000 hooks and the line will be about 23 km in length.	4,5 km and 900 hooks	**HDW:**Total length: 3-20km, usually10km. Number of hooks: 600-2000, usually 1000 **WLF:** Total length: 0.8-4 km, usually2.5 km. Number of hooks: 600-2000, usually 500 **BSF:**Length of the line: usually 200-500 m (depending on the depth), number of hooks: 20-100	1.7 km	??
What is the diameter of the long-line rope? (Often ranging from 4 mm (5/32") to 12 mm (1/2").	Common line would be 9 mm.	9 mm line or 7-5 mm	Generally 8 mm	**HDW:**No rope. Nylon main line is used No200 (2 mm) **WLF:** No rope. Nylon main line is used, No200 (2-3mm diameter) **BSF:**No rope. Nylon main line is used No100-120 (1.1-1.2 mm)	11.5-12 mm	10mm
What type of long-line (mainline) is being used? (monofilament? other?).	Multifilament usually coloured with tare.	Multifilament.	Monofilament	**HDW:** Monofilament **WLF:** Monofilament **BSF:** Monofilament	Nylon	Polyfilament nylon
What is the spacing between the hooks? Spacing between the hooks often ranges from 90 cm (36") to 150 cm (60”).	Ranges from 1meter to 1,4 where 1,2 is most common.	Distance between hooks, 1.25 and 1.35 m for the coastal longliners and 1.85 m for the autoliners.	Generally 5-6	**HDW**:5-10 m, usually 6 or 10 m **WLF:** 5-10 m **BSF:** 10-20 cm	1.4m	25-30 hooks by 45m
What is the most common type of hook used? (types include J-hook, circle hook, EZ-hook)	EZ-hook	EZ-hooks or wide gap depending on whether it’s an autoliner or not.	J-hook	**HDW:** Most common J-hook **WLF:** Most common J-hook **BSF:**Most common J-hook	J hook	J-hook
What is the size of the hook normally used? As an example, size of EZ hooks can range from 11/0 to 15/0.	11/0, 12/0 and 13/0 where 12/0 is most common.	11/0	7/0 (Mediterranean species are smaller than Atlantic ones)	**HDW:** No 10-5, usually No6 **WLF:** No 6 **BSF:**No10-11	13/0	9/0 with 12 mm gap
Are swivels used, and if so, what is the most common type? Many	Most liners use swivels and most of	Yes various kinds	YES	**HDW:** Yes, usually something like the last (bottom) photo (with two fixed stoppers and a swivel between them that can move	Yes/standard	yes

types exist.	them various type of metal swivels (increase sinking speed)			around the main line as well as around itself). **WLF:** Yes, usually something like the last (bottom) photo (with two fixed stoppers and a swivel between them that can move around the main line as well as around itself). **BSF:**Yes		
How long and what type of snoods (gangions) is used? Can be braided or twisted and the length , generally ranging from 35 cm (14") to 60 cm (24")	Most common is 40.5 cm but some use 45.7 cm or even a bit longer.	The length is about 50-60 cm	3.3 m long	**HDW:** Length: 2-3 m. Diameter 1.2 mm. Simple. **WLF:** Length 2-4 m. Diameter 1.2 mm; some times twisted. **BSF:**Length: 10-15 mm. Diameter 0.5-0.6 mm; some times twisted.	40cm	Hook to line – monofilament Nylon 130 mm, length 180 cm
What is the most common bait used	Targeting cod; herring, squid is popular but lately Saury from imported from Asia are widely used. For haddock; sand launce, squid or mackerel are used. Saury is baited as well.	A mixture of squid and mackerel or squid and herring	*Sardina pilchardus*	**HDW:** Sardine (most common), scomber, gilt sardine **WLF:** Scomber, Sardine, gilt sardine, jack mackerel, squid **BSF:**Scomber, sardine, gilt sardine, jack mackerel	Longliners targeting Ling, Molva molva, redfish, halibut Bait: Mainly Squid but small quantities of shark (as bycatch) Longl. targeting shark, bait: Mackerel and Saury-	Mackerel
What is the average weight of bait used per single hook?	10g bait would be small and 30g would be large, around 20g is most likely near the average bait size.	25-30 g	20-30 g	**HDW:** 20-40 g **WLF:** Around 40gr **BSF:**20 gr	Width of the bait is 33-35 cm, weight depends on the bait.	
Are floats and/or weights attached and if so, how are these arranged	Most common: each end of the line is attached to an anchor, sometimes a small weight is attached on the line when the line	Only anchors at the ends, or like fig. 4.30a	No floats are used	**HDW:** Floats with reflectors are used. They are attached to a big cement weight by means of a rope. Usually, there are four floats along the main line. The first and last floats are also connected with a line (of length equal to the bottom depth) at the end of which a 2 kg weight is attached. The main line with the snoods is also connected with this line.	Use 5 floats every 36 cm.	

	is set in U-turn.			**WLF:** Same as for HDW **BSF:**There is one float attached to the main line, but sometimes fishermen do not shoot it to the sea. They keep it on the boat and the gear fishes suspended from the boat.		
What is the approximate depth of the long-lines are set?	Nearby 100meters.	ca 200 m	Between 150 and 500 m.	**HDW:** 350-800 m, usually 400-700 m. **WLF:** 350-800 m, usually 400-700 m. **BSF:**200-700 m, usually 250-500 m.	500-1000	
What is the general set-time (i.e. the time the long-line is on the seabed)	1 to 6 hours	Minimum 4 hours	4 longlines are generally set in 3 hours and the time the longline is on the seabed is around 6 hours.	**HDW:** 1-7 hours, usually 3-5 hours. **WLF:** 2-3 hours **BSF**:0.5-1 hour, usually 0.5 hour.	4 hrs	

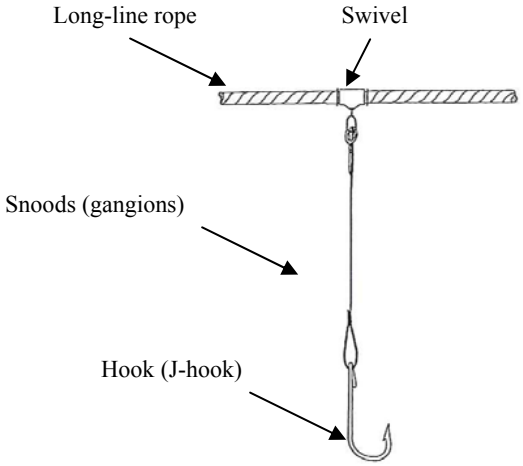
*1Greece:

<u>Blackspot red seabream deep-water line fishery</u>

Gear: A single vertical line at the end of which a weight of about ½ kg is attached by means of a swivel. Another sw[illegible] swivels, many snoods (about 20 in number) are attached at a distance of 10-20 cm between them. The length of eac[illegible] each snood

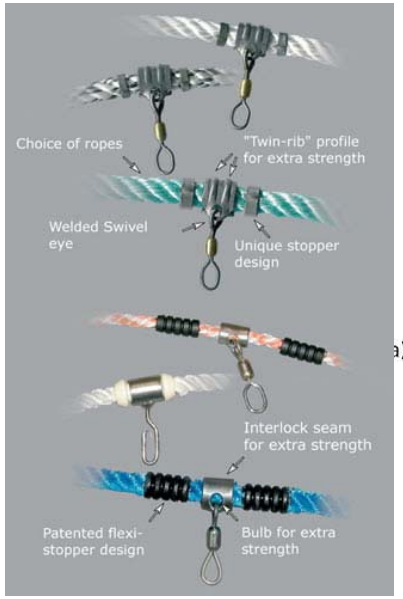
The figures below could be useful for clarification of the long-line gear

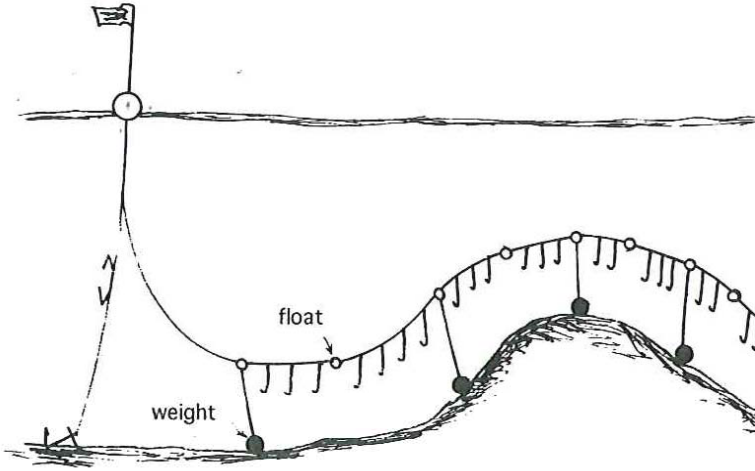
Becket arrangement. loop of lighter line is through the lay of the main line, with snood looped in.



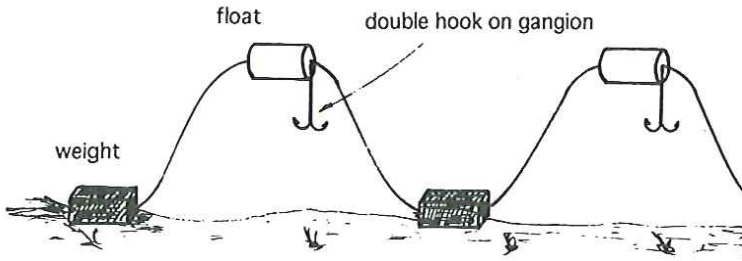
Swivel clip arangment. The gangions and hooks are allowed to rotate about the mainline, and are effective in preventing snood entanglement.

Types of swivels





(a)



(b)

Table 3 from partners which have returned the questionnaire for the ROV

CoralFISH partner number	2	3	5	6
Institute	IMR - Institute of Marine Research, Norway	HAFRO-MRI - Marine Research Institute, Iceland	IMAR-Azores - Instituto dos Mar - Centro dos Acores, Portugal	HCMR - Hellenic Centre for Marine Research, Greece
person in charge	Pål B. Mortensen; Tina Kutti	Stefán Áki Ragnarsson	Fernando Tempera; Gui Menezes	Chris Smith
Email	paal.buhl.mortensen@imr.no; tina.kutti@imr.no		tempera@uac.pt; gui@uac.pt	csmith@her.hcmr.gr
vehicle type	(1) ROV Aglantha and (2) Campod (Tethered passive video platform)	ROV Sub-Atlantic, Aberdeen, Scotland	ROV -SeaBotix LBV300S-6	DSSI Max Rover Mk II - electrical ROV system
min/max speed	optimal between 0-0.7 knots	0-2 knots	0-4 knots (at surface) with 3 knots max operating current intensity	0-1.5 knots
control of speed, altitude, heading	(1) with trusters, (2) with cable length	yes, 6 propeller, auto depth function	speed control and constant depth control	no speed control, auto altitude and heading
max depth [m]	(1) 500-1000 m, (2) 2000m	550 (600) m	200 (300) m	2000
data accumulation (CTD, oxygen etc.)	(1) temperature, (2) CTD and current		temperature umprint of footage; no log file; optionally external time/depth/temperature data logger	-
number of cameras	(1) 3, (2) 2	3	2	5
camera type 1		1 colour camera (OE 14-366-1047 with zoom)	1 colour camera (570 line/02 Lux super HAD color)	2 fixed angle, fixed focus, wide angle DSSI cameras, focal length 10 cm to infinity (no independant controls)
camera type 2		1 black and white camera (EO 15-100-1028)	1 black and white camera (430 LINE/0.03 LUX); manual focus control (90 mm to infinity)	1 wide angle and macrozoom DSSI camera, zoom and focus capablilites, min. focus 30-10 cm
camera type 3		1 still camera (OE 14-208-0201 with OE 11-242-0171 flash)		2 full HDTV camcorder (with zoom focus, WB, aperture, colour temperature etc.); good for min. 5h
orientation of camera(s)	variable	ROV with 1 tilt unit; cameras front-looking; tilt range 180°; pan by turning ROV (360°)	down-, front- and upward-looking; changable; tilt range is of 270° per camera (180° from internal chassis rotation plus 90° from camera lens tilting); no panning possible without moving the ROV itself	on forward-looking pan/tilt camera type 2+3; type 2 camera repositionable on ROV frame
storage of movie material	mini DV/HD-DVC Pro (files)	DVCAM tapes as backup (Sony Digital videocassette recorder DSR-25) and on AVI format by Adobe Premiere Pro	mini-DV tapes; images from only one camera can be recorded/viewed at time	type 1+2 on DVD and hard disk; type 3 on internal hard disk; possibilities for mini-DV, S-VHS, VHS, Betacam, U-matic HB
size calibration	2 laser points	No	No; vertical size estimations from depth gauge variation while moving the ROV vertically	Tritech VMS camera for video measurement; HD camcorder with 4 lasers

time code on movie material	yes	Yes	yes; mini-DV recorder time and the time imprinted on footage by ROV control box is calibrated by the GPS time at the start of each survey day	S-VHS and mini-DV; pilot console with video overlay (date and time) optionally recordable
number of lights	(1) 4, (2) 2	4	1	2 HID lights looking obliquely forward (150nW each), 4 quartz (halogen) lights on the pan and tilt (250 W each)
min/max light intensity	2x400 W	1 HID light; 3 x 250 W underwater lights (plus digital still flash); positionable as required; variable intensity; HID is an on/off light	Luxeon 6 LED array (480 Lumen) mounted internally on the camera chassis; light intensity manually controlled; automatic luminosity and white balance adjustment of the camera	max 300 W plus 1000 W quartz; not dimmable; video recording with HID lights
positioning system	variable between vessels	SIMRAD HPR 400P; SSBL (super short baseline system)	none on ROV; GPS position of vessel logged during each ROV dive	Trackpoint USBL positioning system linked to ship navigational unit for geographic positioning
acuracy of positioning system		approx. 2% of slant length (distance from transducer to transponder). Transducer: SIMRAD PMT 301. Transponder: SIMRAD MST 219/N	ROV dive location known with an uncertainty of 50-100 m	Trackpoint positioning is variable deeper than 500 m; necessary for good positioning to stop the ROV and make sure that ship is not manoeuvring: acuracy +/- 10 m (DVL system in future)
post-processing software available	Video Navigator (developed by IMR)	VARS (video annotation reference system from Monteray Bay Institute); Adobe Premiere for editing	ArcView 9.3; Adelie	VMS camera software for measurements on grabbed images; ArcInfo

Appendix 5

Longline experimental fishing assessment of coral versus non-coral habitat fish distribution – Study area: Ionian Sea (Mediterranean Sea) - (HCMR, partner 6 – CoNISMa, partner 7)

Two longline surveys will be carried out in the NW Ionian (Italian area) and two in the Eastern Ionian (Greek area) during 2010: the first in spring (May?) and the second in late summer (September?). Longline surveys will be carried out by Italian vessel and fishermen in the NW Ionian and by Greek vessel and fishermen in the Eastern Ionian. Commercial vessels will be hired for these surveys.
Methods and gears will be standardized in the study areas as follow.

Methods

Four sampling areas will be considered to study the coral habitat effects on fish distribution:

1) SML coral area in the NW Ionian Sea where fishing does not occur (or only occasionally) (SML-IN);
2) off SML coral area in the NW Ionian Sea with high fishing pressure and without corals (SML-OFF);
3) Greek area in the Eastern Ionian Sea with medium fishing pressure and with corals (to be confirmed during the Aegeo exploration in 2009) (GRE-IN).
4) Greek area in the Eastern Ionian Sea without corals and with no or very low fishing (to be confirmed during the Aegeo exploration in 2009) (GRE-OFF).

Since both fish abundance and sizes can be related to the presence of coral habitat and the absence of fishing, the fourth area could be considered as a control area. In fact, in the first and third area there could be the combined positive effect of coral presence and virtual fishing absence (or low-medium fishing pressure) while in the second one there could be the combined negative effects of coral absence and high fishing pressure presence.

A random-stratified design will be adopted during each survey considering two depth strata: 200-500 and 500-800 m.

	SML-IN	SML-OFF	GRE-IN	GRE-OFF
200-500 m	6 hauls	6 hauls	6 hauls	6 hauls
500-800 m	6 hauls	6 hauls	6 hauls	6 hauls

The number of hauls is only indicative since, on the basis of the available budget for these surveys, it can change in relation to the surface area of each depth stratum and the fishing operation duration (this latter related to the length of longline used and the set-time adopted). Regarding the surface area, the total number of longline hauls will be allocated proportionally to the surface area of each stratum. In relation to the length of longline and set-time (the time the longline is on the seabed), it is better to obtain a greater number of replications in each area and depth stratum. This can be obtained reducing both the length of longline and the set-time.

Gears

The characteristics of the longline to be used by both teams during the surveys in the Ionian Sea could be as follows:

- monofilament;
- length: 4500-5000 m;
- number of hooks: 700-1000 (according to the length and spacing adopted);
- spacing between the hooks: 5-7 m;
- type of hook: J-hook, 6-7/0 in size (this size must be strictly the same);
- length of snoods: 2-3 m;
- bait: sardine;
- weight of bait: 20-40 g;
- depth: 200-500 and 500-800 m;
- set-time: 4-6 hours.

This last point will be dependent on: 1) how many lines are shot, as fishermen tend to pick up the first line shortly after they have shot the last line; 2) the distance between replicates within the depth stratum; 3) the distance between replicates in the different depth strata surveyed in the same day.

However, both methods and gears will be better defined after the Aegeo exploration to be carried out in 2009 by HMCR and further discussion with fishermen.

Experimental long-line fishing on and around the Træna coral reef field - (IMR, partner 2)

Study area

The fishing experiment will be carried out in June 2009 in the 10 * 22 km large coral reef field in the Træna deep, off northern Norway (Figure 1). Multi-beam bathymetric maps (from 2003 and 2005) indicate that there are approximately 1500 reefs in this area. Depth in the coral reef area ranges between 250 and 410 m.

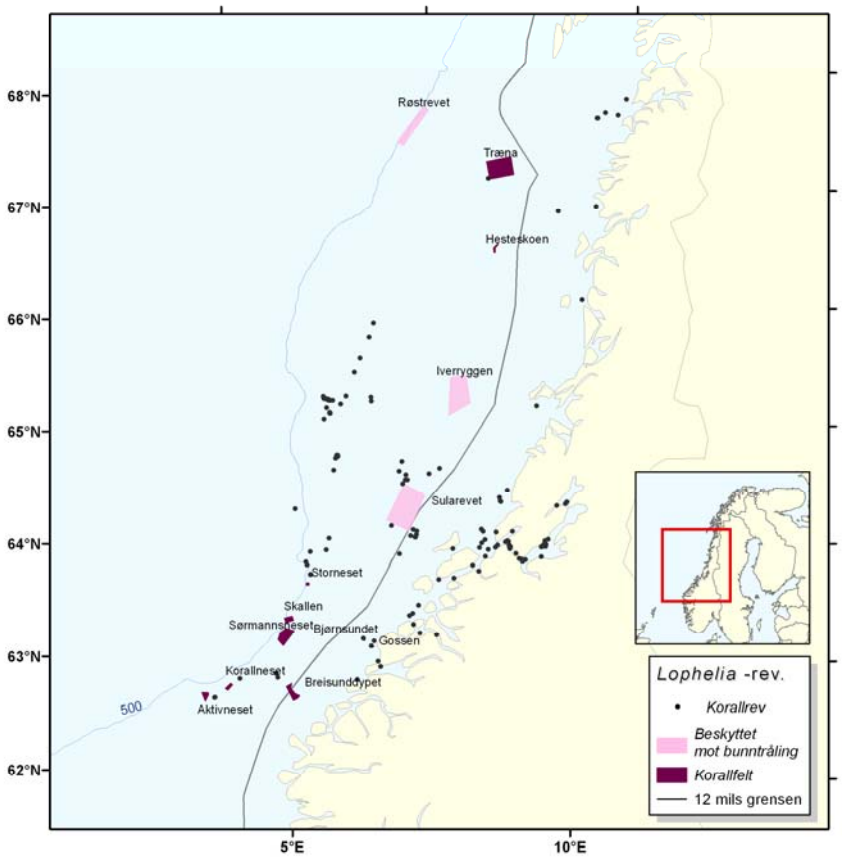


Figure 1. Location of Træna coral reef field.

Fisheries. This coral field is not protected as a MPA. However, the Norwegian salt-water fisheries act prohibits all intentional and negligent destruction of coral reefs and requires precaution when fishing in the vicinity of known coral fields. VMS data from 2004 and 2005 shows that there is no trawling activity in this coral reef area (Figure 2). Long-liners frequently visit the area.

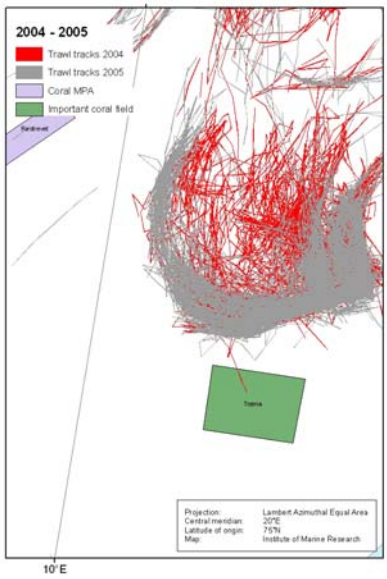


Figure 2. Trawl tracks in 2004 and 2005 at the Røst Bank. The Træna coral field, about 22 km^2, is located just south of the major trawl field (from Fosså and Skaar, 2008). Green box is the coral field.

Reef topography. Each individual reef is about 150 m long, 40 m wide and around 10 m high. The shape and the orientation of the reefs seem to be dictated by local current regimes. Only the up-current part is composed of live *Lophelia* sp. (Figure 3). In the zones with dead/dying *Lophelia* sp. soft corals such *as Primnoa* sp. and *Paragorgea* are abundant.

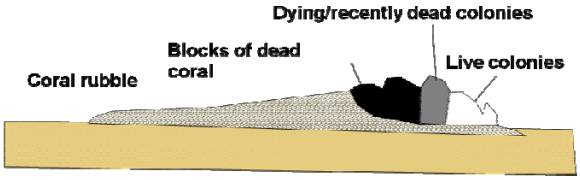


Figure 3. The topography of individual coral reef in the Træna deep.

Hydrography. Bottom currents have been measured at three sites in the Træna deep. Current strenght varied betwen 4 and 20 cm s^{-1} (Figure 5) with peaks observed about 2 times a day. Current direction seemed to the dictated by the topography of the deep basin (Figure 4).

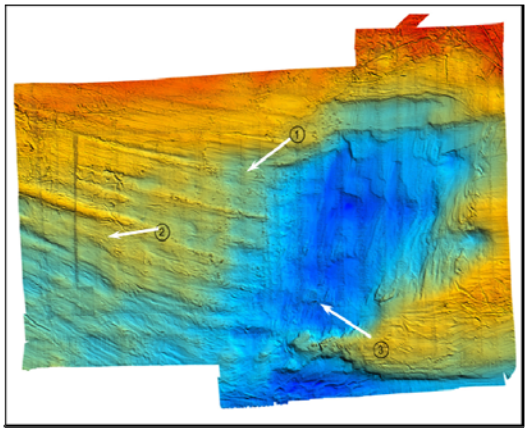


Figure 4. White arrows show the main current direction measured in Træna coral reef field in June 2005.

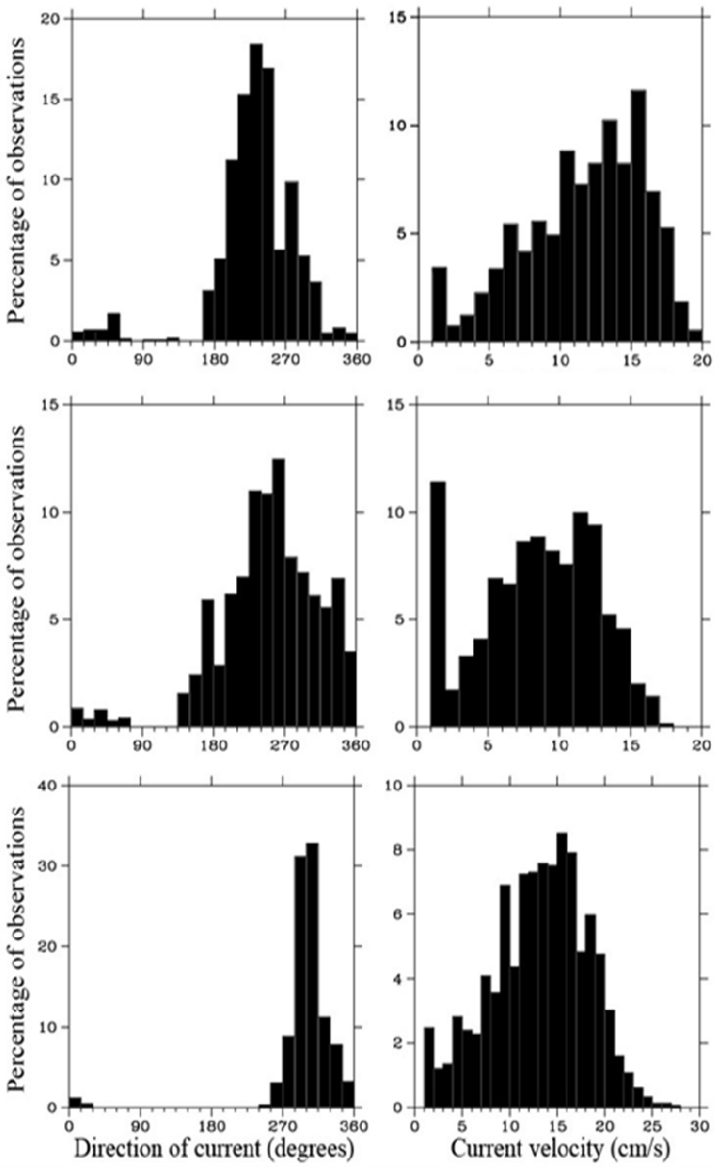


Figure 5. Direction of currents and the current strength measured in Træna coral reef field in June 2006.

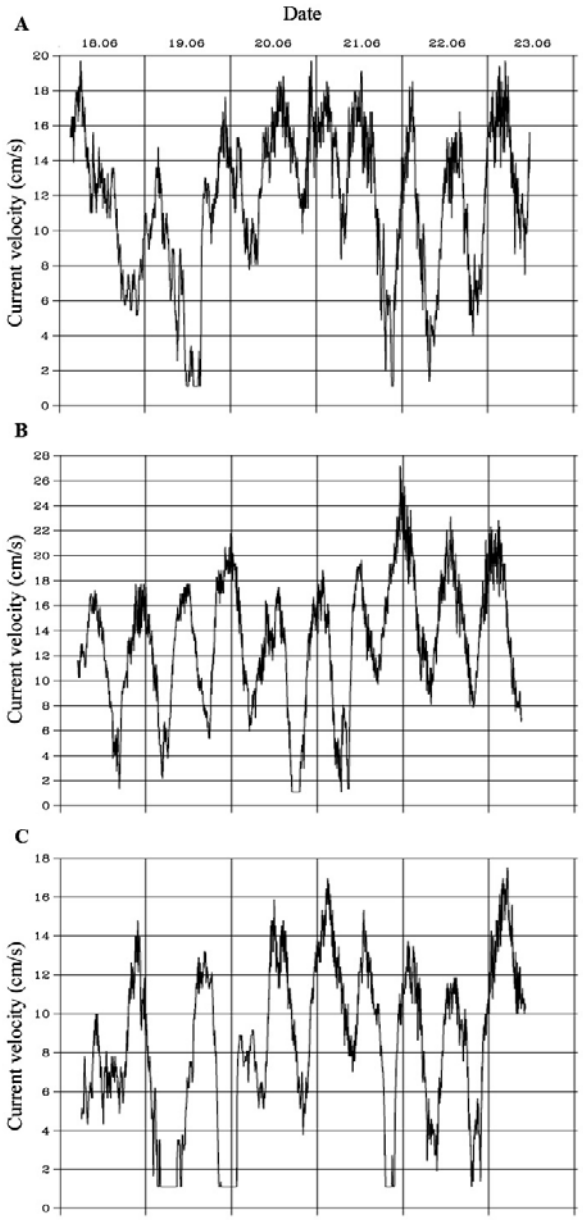


Figure 6. Current cyclicity observed at three locations within the Træna coral reef field in June 2005.

Experimental design

Within the Træna reef field 4 experimental coral (Lophelia) blocks will be examined. Two of the blocks will have a high density of Lophelia reefs (HL) and two blocks will have a low density of Lophelia reefs (LL). Two blocks without corals (NL) will be used as controls to the coral blocks. These will be located either within the 10*22 km large Træna coral reef field or outside the reef field but within the same region with the same depth and topography. Of the 10 experimental blocks shown in figure 7, 6 will be selected for the experimental fishing. The selection of blocks will be done in cooperation with the commercial long-liner that is going to perform the fishing.

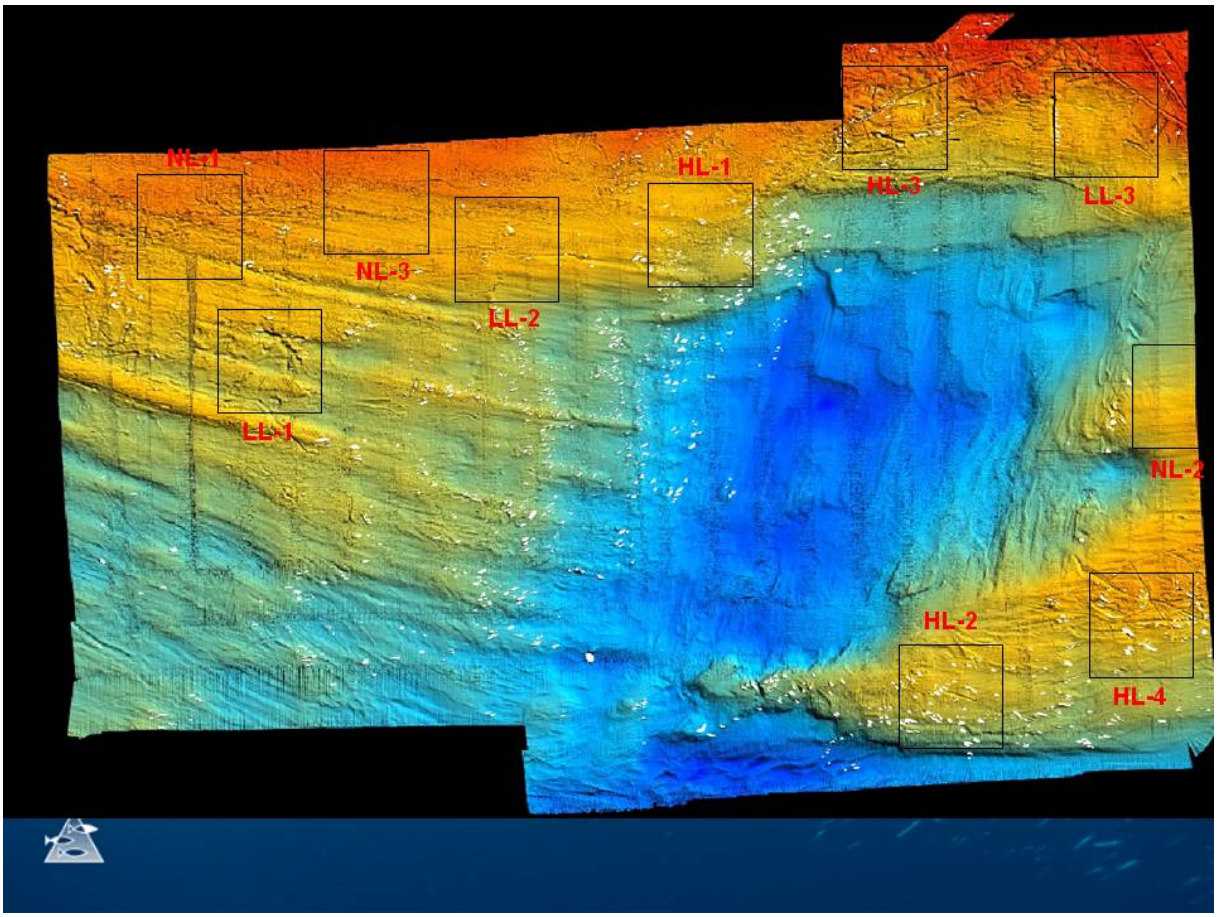


Figure 7. Possible locations of the experimental plots.

The experimental blocks are 2 * 2 km. These experimental blocks will be used in both the acoustic survey, the ROV video footage transects and during the long-line fishing. The block will be divided into 20 * 20 sub-blocks (100 * 100 m) (Figure 8). Starting positions for line transects will be picked randomly from these sub-blocks.

Box	Number of reefs
NL-1	0
LL-1	20 (small)
HL-1	53
NL-2	0?
LL-2	12
HL-2	48
NL-3	0
LL-3	12
HL-3	40
HL-4	47

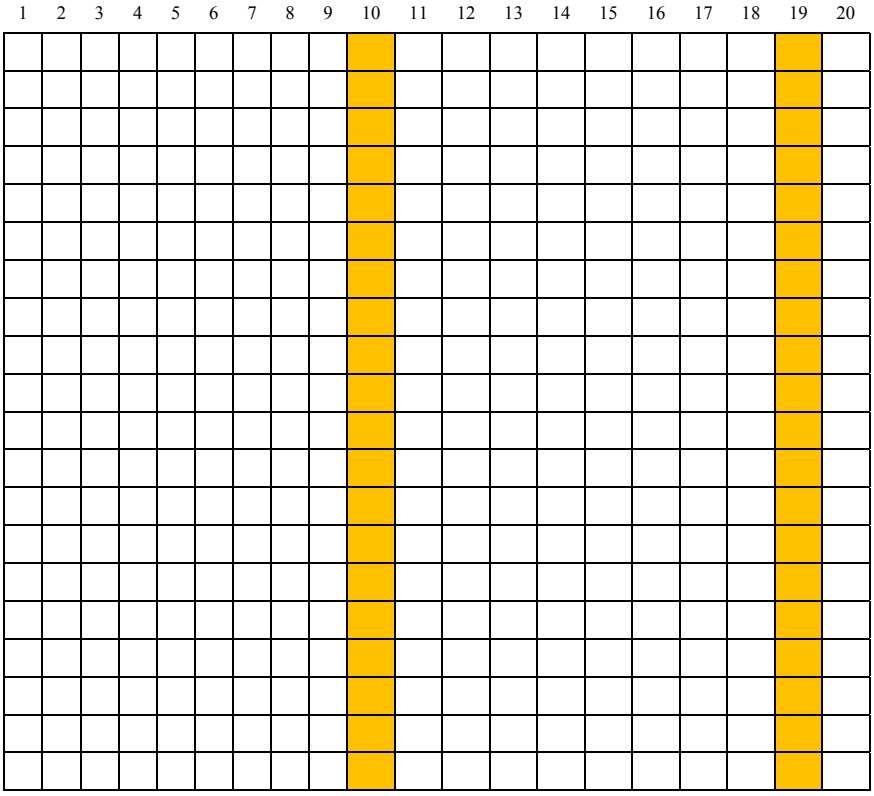


Figure 8. Block structure of the experimental units used during the survey. Orange line showing the positioning of the long-line fleet.

For the experimental long-line fishing two fleets of long-line will be set across each block. Setting direction will be parallel with the main current direction at the site. The first line will be placed in the up current side of the block at with a random start from 1 to 10. The second line will be set 900 m away from the first one, to avoid that one line "steals" fish from the other. Fishing will be carried out two times in each block. The second time with "new" randomly selected positions.

Table1. Number of lines that will be set during the experimental fishing.

	High density Lophelia (HL)	**Low density Lophelia (LL)**	**Control – No Lophelia (NL)**
1	2*2 fleets	2*2 fleets	2*2 fleets
2	2*2 fleets	2*2 fleets	2*2 fleets

Each fleet will be 2 km long and contain approximately 1200 hooks. During the cruise 4 fleets of long-line will be set in each block. In total, 24 fleets of long-line will be set, with 1200 hooks per fleet.

Coordinates for the experimental plots

NL-1: 10.826 66.982; 10.873 66.982; 10.873 66.964; 10.826 66.964
LL-1: 10.861 66.958; 10.905 66. 958; 10.905 66.939; 10.861 66.939
HL-1: 10.060 66.978; 11.105 66.978; 11.105 66.959; 10.060 66.959

NL-2: 11.280 66.945; 11.325 66.945; 11.325 66.926; 11.280 66.926
LL-2: 10.970 66.976; 11.016 66.976; 11.016 66.958; 10.970 66.958;
HL-2: 11.166 66.893; 11.211 66.893; 11.211 66.874; 11.166 66.874

NL-3: 10.912 66.985; 10.959 66.985; 10.959 66.967; 10.912 66.967
LL-3: 11.250 66.996; 11.294 66.996; 11.294 66.977; 11.250 66.977
HL-3: 11.152 66.998; 11.198 66.998; 11.196 66.979; 11.150 66.980

HL-4: 11.256 66.905; 11.304 66.905; 11.304 66.886; 11.256 66.886

Time considerations
Setting a line takes about 1.5 hours (depending on depth and length of line). Retrieving can take place with a speed of approximately 2000 hooks per hour. Lines should be in water for at least 4 hours. Our strategy will be to set 4 lines (e.g. in block HL-1 and NL-1). This will take about 6 hours. Then we will start to haul the first line set and move onwards. Retrieving all lines will take about 6 hours. If we work only daytime we can assume that we can set and haul about 4 fleets per day. If we work both day and night we would be able to set and haul 8 fleets. Setting and retrieving all 24 fleets of long-line would take 6 days or 3 if everything goes fast.

There will be light 24h a day so we will not take into consideration the time period of the day when the lines are set or hauled.

Gear
The experiment will be carried out using a commercial long-liner operating with bottom long-lines in the area, with their own gear.

Catch processing
Each long-line will be divided into 8 units by marking the line every 250 m. Catch will be collected in separate baskets for each of these 250 m subsections (one for fish and one for invertebrates such as corals and sponges). Catch will be registered per sub-section of the line. One man will be responsible for marking the baskets and making sure that the fish, corals and sponges are gathered in the right basket. Hooks that are retrieved empty, with bait or without bait, missing hooks and missing snodds should be counted. Loss of bait when the line is set will not be registered.

Fish. After the fish from one line has been brought onboard all fish should be identified, weighed and length determined. Individuals should also be analyzed for age, sex and where applicable, maturity. Sampling will follow the Manual for sampling of fish and crustaceans (Mjanger et al., 2006). Stomachs should be collected whenever possible. Stomach and intestine should be cut loose at anus and gullet (throat), placed in labeled plastic bags and frozen immediately. Stomach analyses will be done later in laboratory.

Two muscle tissue samples of about 5 g, free of skin and bones, should be collected from ten individuals of each species per experimental plot. After finishing the experimental fishing 2 *10 * 6 tissue samples should have been collected at least for the three fishes tusk, ling and redfish. Muscle pieces have to be collected from a place that does not degrade the quality of the catch, i.e. somewhere around the head area. Make sure to collect from roughly the same

place for all individuals. The samples should be rinsed in distilled water, put into labeled epindorf tubes or plastic bags and stored frozen. These samples will be used for fatty acid and stable isotope analysis and will tell us something about food preference of the fish.

By-catch. Coral and sponges will be identified (to lowest practicable taxonomic level), photographed, with label, and weighed individually. The label will have to include experimental plot, transect number and sub-section. If time allows it samples of sponges and corals should be collected for genetic analysis, se below. Thereafter the catch should be discarded. For the by-catch no tissue samples will be collected for stable isotope and fatty acid composition.

Some of the corals and sponges might become untangled when brought on board (i.e. get lost over the block when line is retrieved). Therefore one man should stand and view the line as it comes in and register by-catch either by taking still photos or video. This lost by-catch should also be registered per 250 m sub-section with either species name or a reference to the file name of still photograph or video file.

Genetic analysis
This will be done to gather information of evolution, ecology and connectivity among coral reefs.

For the DNA analysis samples need to be fixed as soon as possible after collection to avoid DNA degradation or contamination from other samples collected or with human DNA before being fixed in ethanol. Preferably samples should be collected in a cool room or with the fish on ice. Do not use your hands to collect coral branches for DNA preservation. Even if you wear gloves, use forceps and a scalpel to collect the branches for DNA preservation. Using a clean kimwipe, blot excess water from the tissue just before adding it to preservative. Clean (with ethanol) or change the forceps and scalpel after every collection.

Samples should be stored in 70-95% ethanol in eppindorf tubes, i.e. the EtOH volume should exceed 5 to 10 times the volume of tissue that is fixed. Samples should be stored at room temperature, avoid sun exposition or temperatures>30ºC. For each sample record line number, subsection, species name, colony, number of samples and tube number.

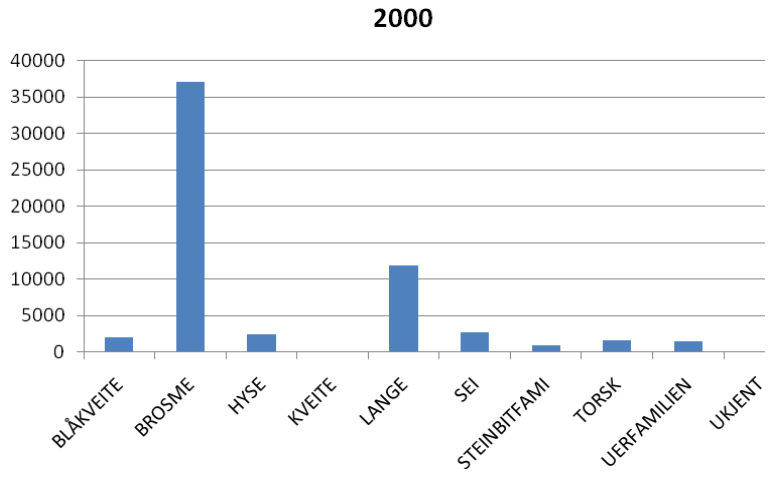
INVERTEBRATES. Any number of the octocoral species caught during the fishing should be collected for identification and phylogentic analysis. The same goes for any sponges.

Stony corals *Lophelia pertusa* and *Madrepora occulata* should be sampled for population genetics. Ideally 30 to 50 individual should be collected per site, i.e. for the whole Træna area. Probably the by-catch of stony corals in the line-fishery will be lower than this so additional sampling of these corals has to be done from *H. Mosby*.

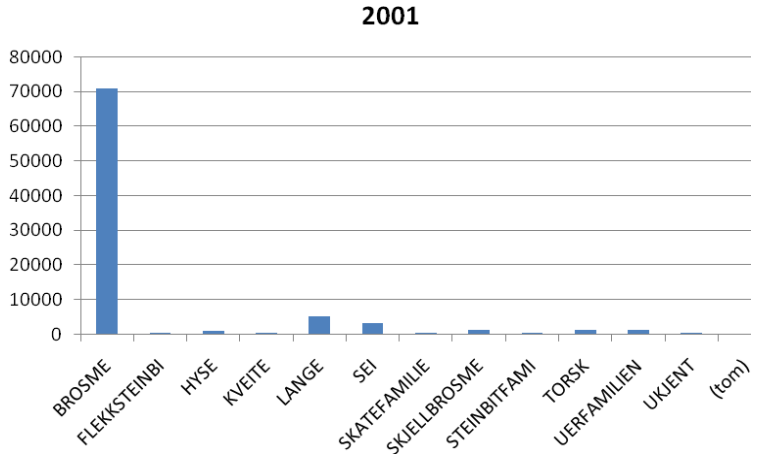
Main considerations
Average catch per long line in Aktivneset and Sørmannsneset (Husebø et al. 2002) was 5.7 redfish per line in coral habitats and 0.8 individuals per line in non-coral habitats. These lines contained approximately 125 hooks. With similar catch rates we would catch 60 redfish, 60 tusk and 10 ling.

Fisheries logbook data

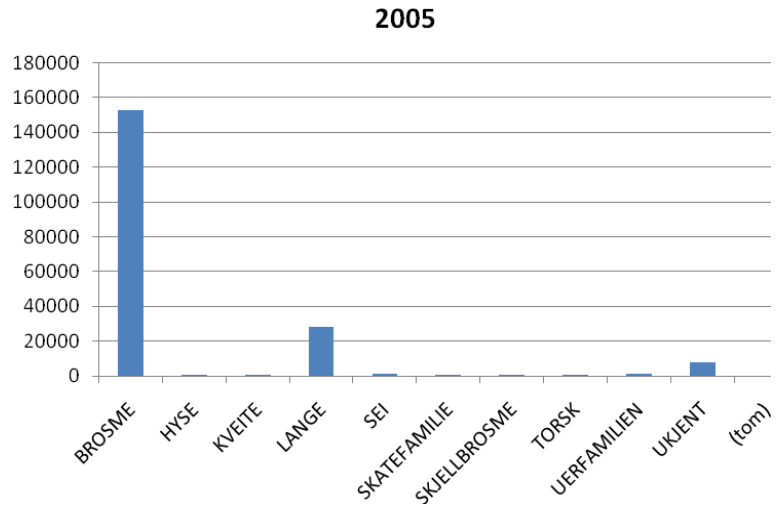
The following graphs show the registered catch from autoliners operating in ICES rectangle 6 locations 29 and 30.

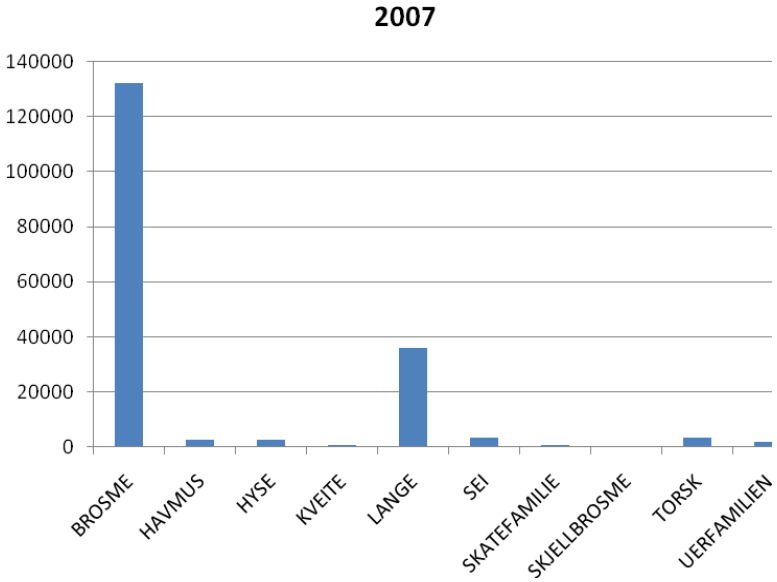


Blåkveite= greenland halibut, Brosme = tusk, Hyse = haddock, Kveite = halibut, Lange = ling, Sei = saith, Steinbitfam = wolffishes, Torsk = cod, Uer fam = redfish family, Ukjent = unknown.



Flekksteinbit = spotted wolffish, Skate = skate family, Skjellbrosme = greater forkbeard



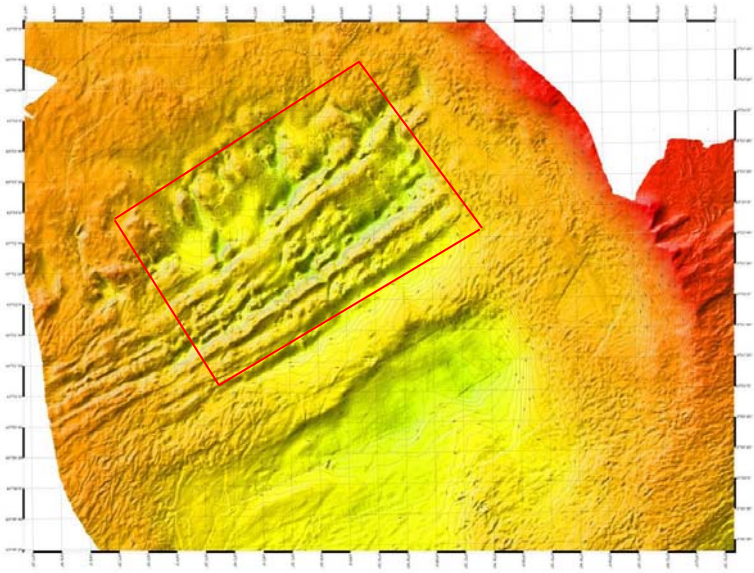


Havmus = rabbit fish

Experimental long-line fishing on and around the possible coral area in Lónsdýpi - (MRI, partner 3)

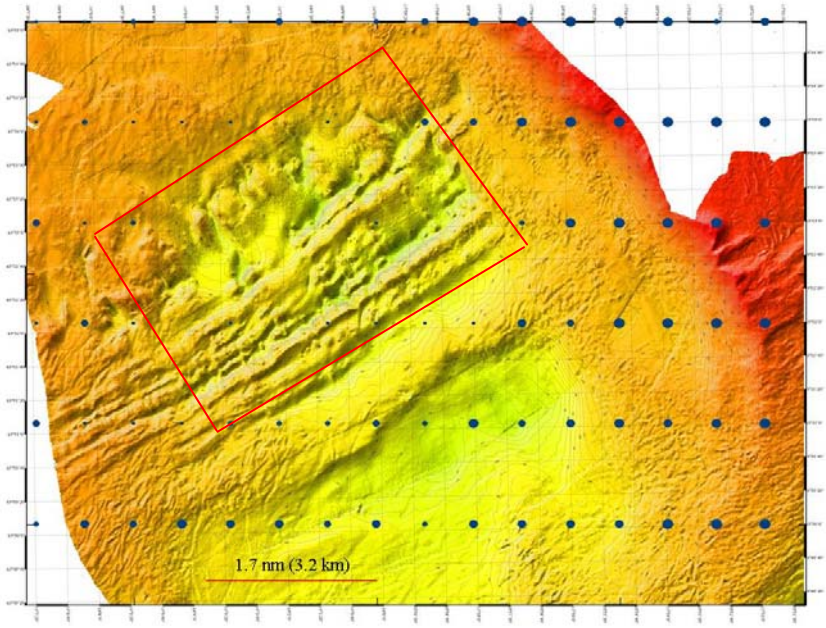
Study area
Lónsdýpi has not yet been surveyed and for that reason it is not certain how extensive coral is to be found there. However anecdotal evidence (e.g. from long-liners) suggests that pristine coral grounds are to be found there.

It is suspected that corals are to be found in the center of the Lónsdýpi but this area remains to be surveyed. The area consists of two parallell ridges of approx 7 km * 2 km in width in the southern part and shorter ridge in the northern part. The depth ranges from 250-300 m.



Multibeam map of the Lónsdýpi area. The red box delimits the area where coral is to be found

Fisheries. There is very limited otter-trawling fishery within the likely coral distribution area. Otter-trawlers avoid trawling in this area due to rough topography (e.g. corals). There is however some long-line fishery within the area , but most of it takes place outside the study area.



Fishing effort (number of tows) for the period 2000-2005 on a spatial resolution of 1' latitude and 1' longitude, based on log-book data.

Reef topography. The area remains to be surveyed and for that reason there is no data available.

Hydrography.

There is very limited data on hydrography within the area.

Experimental design

We will follow the Norwegian initiative as much as it is possible. The study area in the Træna reef field is different from Lónsdýpi that the latter is likely to be much smaller. This means that we will have both fewer longlines and similarly, to ensure sufficient distance between individual longlines, these will be set at different times. We will use short long-lines of between 1500-2000 hooks (2-3 tubs), which corresponds to 1800-2400 m (assuming 1.2 m between hooks). We assume to set 24 long-lines in total, 12 in coral and 12 off coral. After surveying the area in June, we will try to locate high, low and no density areas.

Time considerations

Setting on 7-9 nm speed a 2km long line takes about 30 mins (depending on depth and the sinking speed). Retrieving the line is about ≈2000 hooks per hour. Lines should be in water for at least 4 hours (length of duration depends on the fish species being targeted) and we assume that we can set 4 long-lines a day (i.e. 32 in total). However, on the 8 days we have available we assume that we lose 2 days due to unexpected problems (broken lines, weather). i.e. we would have in total of 24 long lines (12 in corals and 12 off coral). Retrieving a 2-3 km line would take about 1 hour. However, if there is large catch and there are problems retrieving it, this could take much longer time. We will be working during light day hours (8am – 20 pm). We will both try to set the long-line during the afternoon and the morning and compare the differences.

Gear
The experiment will be carried out using a rented research vessel. We will use the long-line available here at MRI or rent it. Longlines should be 12mm or thicker, to reduce the probability that it will be damaged if it gets hooked by a coral. Concerning hook size, we will probably use EZ11, as this hook size collects both large and small fish species. We have not decided on bait size. For bait type we will probably use Saury/mackerel, or herring or squid (or some combination of these).

Catch processing
Each long-line will be divided into 8 units by marking the line every 250 m. Catch will be collected in separate baskets for each of these 250 m subsections (one for fish and one for invertebrates such as corals and sponges). Catch will be registered per sub-section of the line. One man will be responsible for marking the baskets and making sure that the fish, corals and sponges are gathered in the right basket. Hooks that are retrieved empty, with bait or without bait, missing hooks and missing snoods should be counted. Loss of bait when the line is set will not be registered.

Fish. After the fish from one line has been brought onboard all fish should be identified, weighed and length determined. If the catch is large, subsample will be taken and the program Seascale used to estimate number of fish to obtain realistic length distributions. . Individuals should also be analyzed for age, sex and where applicable, maturity. Sampling will follow the Manual for sampling of fish at MRI. Stomachs will be collected when possible. Stomach and intestine should be cut loose at anus and gullet (throat), placed in labeled plastic bags and frozen immediately. Stomach analyses will be done later in laboratory.

By-catch. Coral and sponges will be identified (to lowest practicable taxonomic level), photographed, with label, and weighed individually. The label will have to include experimental plot, transect number and sub-section. If time allows it samples of sponges and corals should be collected for genetic analysis, se below. Thereafter the catch should be discarded. For the by-catch no tissue samples will be collected for stable isotope and fatty acid composition.

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Genetic analysis
Same as for Norway

INVERTEBRATES. Any number of the octocoral species caught during the fishing should be collected for identification and phylogentic analysis. The same goes for any sponges.

Stony corals *Lophelia pertusa* and *Madrepora occulata* should be sampled for population genetics. Ideally 30 to 50 individual should be collected per site, i.e. for the whole Træna area. Probably the by-catch of stony corals in the line-fishery will be lower than this so additional sampling of these corals has to be done from *H. Mosby*.