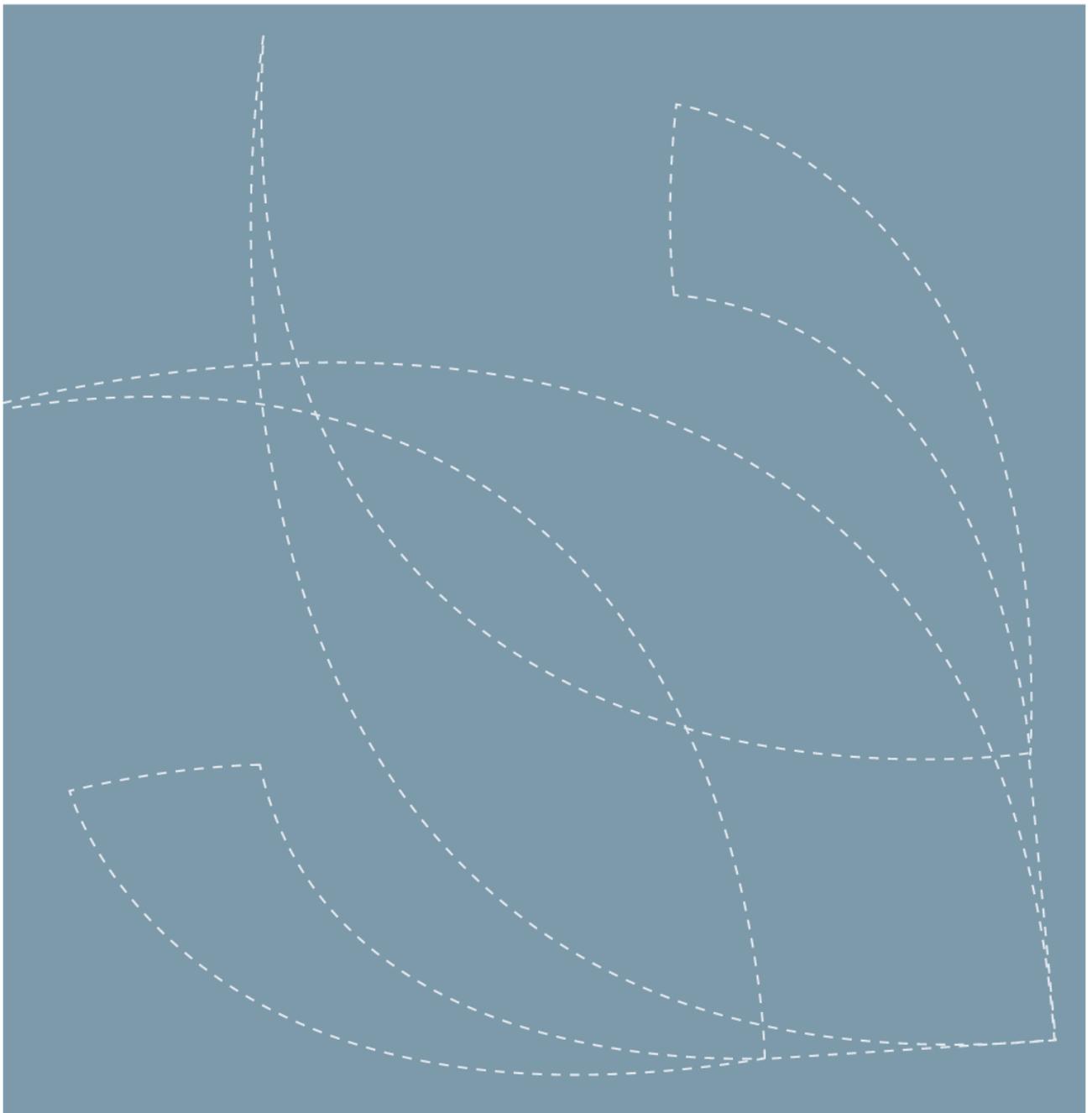


Sea urchin surveying techniques

(Activity A4.1.1 of the NPA URCHIN project)

Philip James, Colin Hannon, Guðrún Þórarinsdóttir, Roderick Sloane and Janet Lohead





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Report

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<p><i>Summary/recommendation:</i> This report introduces the URCHIN project, funded by the Northern Peripheries and Arctic Programme (NPA). It then describes the main techniques used for stock monitoring in sea urchin fisheries around the world, including techniques used in the NPA. Trials in Norway and Iceland run with the aim to develop cheap, effective and reliable methods of stock monitoring suitable for the environmental conditions found in the NPA are described. These include methods for both surveying new fishing areas as well as scientific monitoring. This report describes suitable sea urchin stock assessment techniques for both scenarios and describes the methodology to all participating NPA countries. It also makes recommendations for the most suitable methods for each of the participating NPA countries (Norway, Iceland, Ireland and Greenland).</p>	
<p><i>Summary/recommendation in Norwegian:</i> Denne rapporten omtaler URCHIN prosjektet, finansiert av Northern Peripheres and Arctic Programme (NPA). Rapporten beskriver de viktigste metodene som brukes for bestandsovervåking av kråkebollefiskeriene rundt om i verden, inkludert metoder som brukes i NPA-området. Den beskriver også forsøk i Norge og på Island som er gjennomført med sikte på å utvikle billige, effektive og pålitelige metoder for bestandsovervåking som samtidig er egnet for de miljømessige forholdene som finnes i NPA. Disse omfatter både metoder for oppmåling av nye fiskeområder og metoder for vitenskapelig overvåking. For begge scenariene gis det en beskrivelse av egnede metoder for vurdering av kråkebollebestandene og metodene som benyttes av de deltakende NPA-land. Til slutt presenteres anbefalinger for de mest egnede metodene for hver av de deltakende NPA-landene (Norge, Island, Irland og Grønland).</p>	

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1 Executive summary

In this report, a brief introduction is given to the URCHIN project, funded by the Northern Peripheries and Arctic Programme (NPA).

We then describes the main techniques used for stock monitoring in sea urchin fisheries around the world, including techniques used in the NPA. These include methods for both surveying new fishing areas as well as scientific monitoring. These include the use of SCUBA divers to undertake transect and/or quadrat studies of urchin size and frequency. Included in the report is a full description of the methodology used by Fisheries and Oceans Canada in their SCUBA population surveys in Western Canada. A description of dredge surveys and fixed camera frame surveys are in the following section.

Trials run as part of the NPA URCHIN project and with the aim to develop cheap, effective and reliable methods of stock monitoring suitable for the environmental conditions found in the NPA are described in this report. These include Aerial, Dredge, ROV, Fixed Camera and Trapping surveys. The various techniques are used for either, surveying new fishing areas, or for scientific monitoring surveys and the advantages and disadvantages of each technique are discussed.

Finally, we makes recommendations for the most suitable methods for each of the participating NPA countries (Norway, Iceland, Ireland and Greenland).

2 Introduction

2.1 URCHIN project (Utilisation of the Arctic Sea Urchin Resource)

The URCHIN project aims to utilise the sea urchin resource present in the northern arctic regions. The challenges of fishing, sustainable and responsible harvesting of stocks, legislation and supply chains for sea urchin products from isolated and environmentally harsh and challenging areas in the Northern and Arctic region will be addressed and overcome through innovation and national and transnational technology transfer.

Currently there are small scale (<150 tonne p.a.) intermittent fisheries for sea urchins in the NPA. This is despite there being enormous sea urchin resources present in the area. A number of challenges have prevented the expansion of sea urchin fisheries in the NPA. These include environmental challenges to fishing, inadequate and inappropriate legislation and fisheries management and lack of technology and knowledge regarding sea ranching and roe enhancement of poor quality urchins. Research to overcome these challenges has been disparate and there has been no previous transfer of knowledge between the NPA partner countries.

This project aims to gather the existing expertise from Norway, Iceland, Ireland and Greenland, together with knowledge from Canada to optimise the fishing of high value sea urchins in Northern and Arctic areas.

2.2 Scope of this report

As part of the larger Project aims this report, (Activity A4.1.1 of the URCHIN project) aims to provide details of suitable sea urchin stock assessment techniques for surveying new fishing areas as well as for scientific monitoring in the challenging environment of the NPA.

3 Stock monitoring techniques used in sea urchin fisheries

3.1 Introduction

Stock monitoring of sea urchin populations can be used for two main objectives:

1. Surveying for new fishing sites.
2. Scientific monitoring of sea urchin populations within a given area to monitor population levels and changes in these levels over time.

There is little information in the literature regarding surveying for new fishing areas with most commercial fishing companies having their own tried and tested methods.

Traditionally, population monitoring can be achieved by collating catch and catch per unit effort (CPUE) data from a sea urchin fishery. This is used to effectively monitor and set quotas in a number of countries around the world (e.g. Canada and New Zealand) (Miller and Abraham, 2011) that have long running, established and well-managed sea urchin fisheries. In the NPA countries participating in the URCHIN project, only Iceland and Ireland have catch data from sea urchin fisheries. However, in Ireland the current catches are only a fraction of what they were during the peak of the fishery (see Internal Nofima Report: Report 15/2016, Published March 2016, Sea Urchin Fishing techniques Report) and fisheries catch data is no longer sufficient to use for population monitoring. It is assumed that most of the coastal population of sea urchins in Ireland were effectively removed during the period. In Iceland, the current catch comes entirely from one fisher harvesting in one relatively small area (see Internal Nofima Report: Report 15/2016, Published March 2016, Sea Urchin Fishing techniques Report). Although catch data can be used for monitoring the population over time in this particular area, it delivers little information on the state of the sea urchin population around the entire coast of Iceland.

This report provides techniques for enabling fishers and researchers in the NPA to be able to carry out surveys of sea urchin populations (for finding new fishing areas or as part of a scientific study) as and when required in the harsh environmental conditions experienced in the area. The report does not describe methods of fisheries stock assessment from catch data, nor how to approach stock monitoring using the data provided by surveys. It does introduce this topic based on the experience of the Fisheries and Oceans Canada Department. This is a complex area of research, normally undertaken by Fisheries Research Institutes in the country where the surveys take place, and as part of Fisheries Management Policies.

3.2 Traditional large scale survey techniques

Techniques used for broad scale assessment over large areas (for the purposes of this report a small area is considered to be greater than 1km²) include the following:

3.2.1 SCUBA surveys

This is the traditional method of collecting information on sea urchin populations in a number of countries around the world. There are multiple examples of environmental studies that have used these techniques to monitor sea urchin populations, including ecological studies of multiple species

(e.g. the interaction between sea urchins and macro-algae) as well as looking directly at numbers (density) and size of sea urchin populations. The techniques normally involve the use of SCUBA divers undertaking transect dives where random quadrats are laid down and the number and size of urchins within the quadrat are measured (Figure 1). Alternatively the divers lay down a transect line and count the density of urchins occurring in a set space either side of the transect line. Both techniques require teams of qualified and preferably experienced divers that are familiar with scientific diving. As has been mentioned a number of times the use of divers in the NPA area is both expensive and logistically difficult at different times of the year due to environmental conditions. Subsequently, there have been limited dive surveys undertaken in Norway, Ireland and Iceland and none in Greenland.

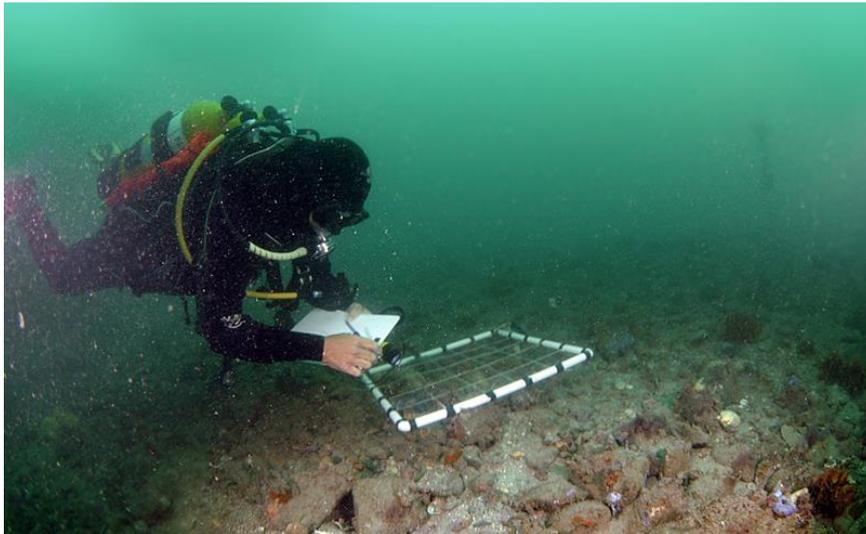


Figure 1 A typical diver quadrat used to sample bottom invertebrates such as sea urchins.

3.2.2 Green Sea Urchin stock monitoring methods in the Pacific Region of Canada

The following is a brief description of the SCUBA survey techniques employed to monitor sea urchin populations in the Pacific region of Canada (written and provided by Janet Lohead, Fisheries and Oceans Canada).

Green Sea Urchin stocks in British Columbia, Canada, are monitored using industry-funded SCUBA dive surveys at index sites. The index sites are chosen through consultation with the commercial fishing industry, which identified fishing ‘hot spots’. The index sites also include control sites in areas closed to the commercial fishery. The objectives of the surveys are to study the natural fluctuations, as well as the effects of commercial fishing, on Green Sea Urchin densities and size.

At each index site, the same 10 – 20 transects are surveyed either annually or biennially. The transects run perpendicular to the shoreline and/or depth contours, from deep to shallow, from 10 m below Chart Datum to 0 m below Chart Datum. Each transect line varies in length, depending on the slope of the bottom. Tide tables are used to establish tide height, stage and range, before each dive to calculate the starting and approximate finishing depth. This ensures that the same area of the seafloor is surveyed at each sampling visit.

The divers count and measure urchins occurring within a one square meter quadrat that is flipped over the substrate for the length of each transect, following either a lead-line or compass bearing. The

surveying scheme depends on average transect length. For transects with average lengths of 0 – 20 metres, the surveying scheme is MEASURE – SKIP – COUNT – SKIP (M S C S). For transects with average lengths of 21 – 100 metres, the surveying scheme is MEASURE – SKIP – COUNT – SKIP – COUNT – SKIP (M S C S C S). For transects with average lengths over 100 metres, the surveying scheme is MEASURE – SKIP – COUNT – SKIP – COUNT – SKIP – COUNT – SKIP – COUNT – SKIP (M S C S C S C S C S). In a MEASURE quadrat, the divers record quadrat number, depth, time, substrate and habitat (algae), and measure every green urchin's test diameter. In a SKIP quadrat, divers do nothing and they skip this quadrat. In a COUNT quadrat, the divers record quadrat number, depth, time, substrate and habitat (algae), and count all green urchins. Substrate is recorded as the three most prominent substrate types, in order of highest to lowest prominence. Percent cover for each substrate type is also recorded. Species and percent cover of algae are recorded under the following categories: canopy (over 2m), understory (20 cm to 2m), turf (5 – 20 cm), encrusting and drift.

A computer program, called “The Green Urchin Analysis Program (GUAP)” was developed by Fisheries and Oceans Canada in 2013 to estimate mean density of Green Sea Urchins from the survey data. Details of the program and its calculations are described in Lochead *et al.* (2015). The analysis is based on the assumption of a continuous distribution of urchins between surveyed quadrats along the transect lines. The program conducts interpolation to fill in data gaps resulting from quadrat subsampling and implements an allometric relationship to convert test diameters to weight (for years when supporting length-weight data were not collected, i.e., 2012 onwards).

Within a transect, GUAP uses linear interpolation to estimate the number of urchins in the uncounted quadrats (skip quadrats) based on the number of urchins in the two closest counted and/or measured quadrats. Then, for each measured quadrat, the probability of an urchin being legal-size (≥ 55 mm) is generated from the observed size-frequencies and according to the beta distribution and. The probability that an unmeasured urchin is either legal (≥ 55 mm) or sublegal (< 55 mm) is called a size-class probability. Linear interpolation is used again to generate size-class probabilities for quadrats without measured urchins (count quadrats and skip quadrats) based on the size-class probabilities from the two closest measured quadrats. The two closest measured quadrats refer to the measured quadrat that precedes and follows the skip or count quadrat in question. If the skip or count quadrat is towards the end of a transect and there is no following measured quadrat, then size-class probability is derived through extrapolation from the preceding measured quadrat only.

For the calculation of biomass densities (g/m^2), the sampling unit is a transect. GUAP incorporates variability among transects through bootstrapping. Transects are re-sampled with replacement and each time a transect is re-sampled, the size-class probabilities of the unmeasured urchins are regenerated as described above. For each re-sample, the ratio-estimator is used to calculate the density for legal and sublegal size-classes. The re-sampling process is repeated and 1000 estimates of density are generated. The frequency of these estimates is used as a proxy for the distribution of density by size-class. The calculation of confidence bounds is further refined using Bias-corrected accelerated percentile intervals (Efron and Tibshirani 1993).

The biomass densities and standard errors from the survey data, along with CPUE from the commercial fishery, are as used as inputs to a Bayesian biomass dynamic model which produces maximum sustainable yield (MSY) probability distributions for each region (DFO 2014, DFO 2016). The MSY probability distributions form the basis of the science advice that is provided to Fisheries Managers, who chose the level of risk and the quotas associated with them.

In addition to the above, Appendix 1 contain protocols from Fisheries and Oceans Canada for measuring benthic marine sponges and 2) analysing still images from fixed camera surveys.

3.2.3 Dredge survey (New fishing areas and Scientific)

Dredge surveys are also relatively uncommon but have been trialed in Iceland as part of the URCHIN Project. The results of these trials are given in Section 4 below.

3.2.4 Drop camera on a fixed frame

This is a technique that has been used for a number of benthic deep water species (eg *Nephrops* Spp.) but it has also recently been used in preliminary studies for benthic invertebrate species such as sea urchins in Canada (Janet Lohead, Fisheries and Oceans Canada, *pers com.*). The trials using a drop camera and fixed frame to measure sea urchin abundance undertaken in Norway as part of the NPA URCHIN Project are discussed further in Section 4.

Appendix 2 contain protocols from Fisheries and Oceans Canada for analyzing still images from fixed camera surveys.

3.3 Traditional small scale survey techniques

Techniques used for assessment of urchin stocks in relatively small areas (for the purposes of this report a small area is considered to be less than 1km²) include the following.

3.3.1 SCUBA survey

The techniques here are the same as used for large-scale surveys in 3.2.1., however, there will be less transects, or quadrats since the area is much smaller.

3.3.2 Snorkel and surface periscope survey

This is a relatively common method used by sea urchin fishers when surveying a new area for fishing. It consists of swimming over areas, or passing over them with a small boat and an underwater viewing periscope, or bathyscope, to look for new sea urchin populations but is not used for scientific monitoring as there is no way to accurately count or estimate urchin size from this technique. However, with sufficient experience it can be a very useful way of assessing urchin presence/absence and abundance on a small scale (R. Sloan, Arctic Caviar AS, *Pers comm.*).

4 Novel survey techniques being used and developed in the NPA

4.1 Aerial survey large scale (>1km²) (Used to establish new fishing areas)

This relatively new method of surveying is not described in the literature but it has been trailed in Norway as part of the URCHIN Project. The trials have involved the use of a fixed wing aircraft (a helicopter would also be ideal for this type of survey) and were conducted in collaboration between Nofima and Arctic Caviar AS. The following is a brief description of the aerial survey employed to search for new fishing areas in the Vestfjord area in north Norway (provided by Roderick Sloane, Arctic Caviar AS).

4.1.1 Aerial survey report for new fishing areas

The aim of the survey was to use light aircraft to map kelp beds - the theory being that big kelp beds equates to no sea urchins, whilst barren rocks surrounded by kelp beds mean it is likely that there will be sea urchins in this area.

Tuesday 23rd Feb 2016

Flight information:

Weather conditions - fairly good visibility with occasional cloud cover and snow flurries.

Sea conditions – 10m/s wind from the North.

A light fixed wing aircraft was used and the flight lasted for 3 hours. Taking off from Bodø we travelled across Vestfjord and then began mapping the northern side of the fjord. The return journey was spent looking at the south side of the fjord (see map in Figure 2). The whole route was tracked using a handheld GPS, which was used to mark waypoints of particular interest. Additionally, cameras were used to capture aerial images of all potential sea urchin sites.

Beneficial aspects of survey technique:

- 1) A large area was covered in the allotted time.
- 2) Huge economic saving, primarily in the time required to survey using traditional methods.
- 3) Birds eye view offered unique insight into surrounding area (e.g. are there any farms or industries nearby, are there mountains that will block sun for macro-algae growth).
- 4) Excellent view of the geological formation of the rocks and the type of rock present in each reef system.

Negative aspects of survey technique:

- 1) This technique is obviously extremely weather dependent.
- 2) You need an airport close to the survey sites.
- 3) Need a skilled pilot (can't do it yourself).
- 4) Can't stop when you want to / conduct counts and a more detailed site investigation. A helicopter would possibly be a better option than a fixed-wing aircraft.

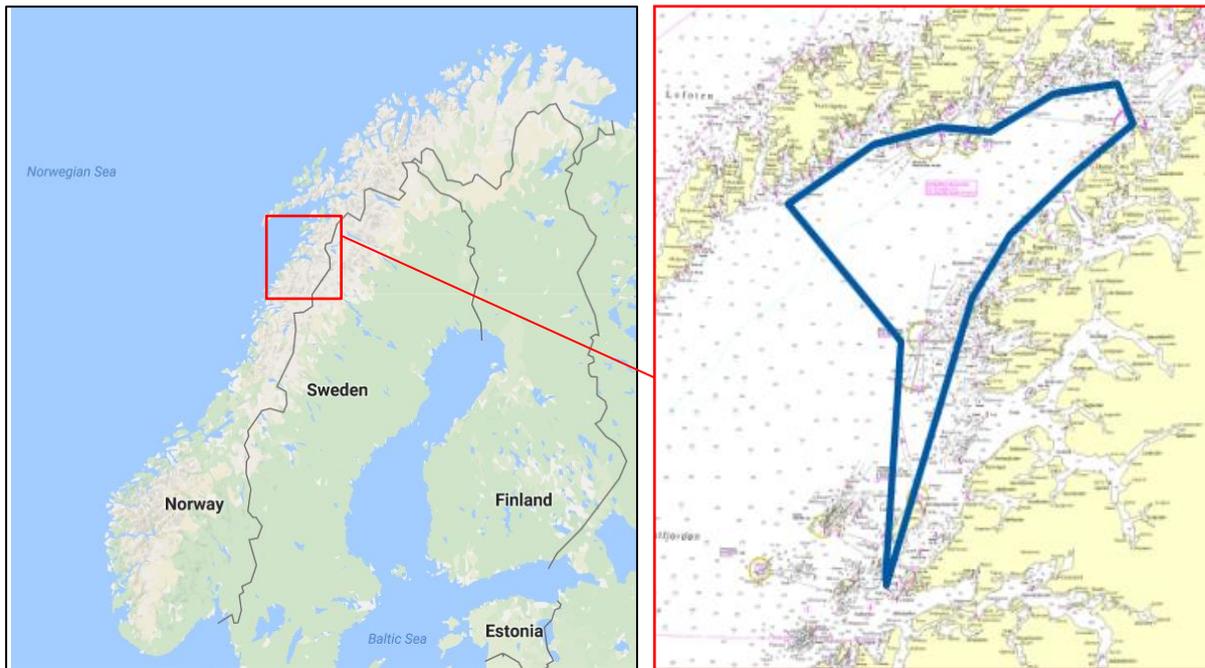


Figure 2 The location of the survey site in Norway (left) and the route taken (solid blue line) in the Vestfjord area (right) during the aerial survey.

General comments:

- From the air it was possible to view extensive kelp beds at certain sites that we could immediately write off as having no potential with regards to future surveying and/or fishing.
- Couldn't actually see any sea urchins from above, yet provided invaluable knowledge of potential sites, and the sheer amount of area covered would take weeks using the traditional survey method utilizing a boat. With this relatively short survey (3 hours) it was possible to identify a handful of key sites that require closer inspection with a boat.

The next phase is to get a diver in the water and have a look at the sites identified as having the most potential. This will give us a deeper insight as only a certain amount of the reef can be viewed from the boat, where as a diver can swim around the entire section.

Thursday 10th Mar 2016

With good boating conditions available, we headed to North Lofoten to implement one of the traditional survey methods used by Arctic Caviar AS, using an underwater viewing periscope. We focused on one of the target areas and slowly made our way south using the on-board GPS of the boat to follow the route the aircraft had taken and locate shallow reefs, again using 'Way Points' to mark locations that could hold potential for the future.

After spending 7 hours at sea, several prospective sites had been examined. Some of these contained sufficient quantities of harvestable, commercial size sea urchins. Other areas contained large populations of small non-commercial size sea urchins. Due to weather conditions certain exposed south facing reefs could not be examined. These areas are known to have the best commercial fishing zones from past survey and fishing experience.

Conclusion:

The initial survey with the aircraft gave a good, broad overview that significantly reduced the amount of time (and cost) involved in identifying sites with commercial potential for harvesting sea urchins. However, this technique still requires the boat and a diver in the water to quantify definitive results.



Figure 3 From left to right: Roderick Sloane (Arctic Caviar AS) planning with pilot; the fixed-wing plane used in the survey; aerial shots of the reefs and surrounding reef structure.

4.2 Aerial survey small scale (<1km²) (Used to establish new fishing areas)

Following on from the large scale aerial survey Nofima has investigated the use of small, relatively cheap drones to survey smaller, more specific areas. This method of surveying has not been previously described in any literature. The following is a short description on the use of drones as a survey tool.

4.2.1 Drone aerial survey in Tromsø, Norway

A preliminary study in Tromsø investigated whether or not the information gathered from drone flights is useful for finding new fishing sites and areas. A drone (Phantom 3 Advanced model, 1280g, operates in 0 - 40°, maximum flight time 23 minutes, approximate cost 11,000NOK) (Figure 4) was used to make both shore based and boat based flights to survey a relatively small area (approximately 0.5km²).

In the first trial the drone was launched from shore and an aerial video survey of approximately 500m of coastline was made. For the second trial the drone was launched from a 5m boat and an area that had been previously surveyed using fixed frame camera and divers was surveyed from the air (Figure 4).

The drone flights produced video of excellent quality that clarified the reef structure and provided excellent information for locating possible fishing locations within the area. The second day of testing which was conducted from a relatively small boat proved that the drone is easily launched and retrieved from a small moving platform and that it was a very effective technique for fast aerial surveys of a relatively small area. This technique could be used by fishers to rapidly identify parts of extended reef systems that are most likely to produce sea urchins. This would eliminate some of the need for extensive in water surveying and enable the fisher to go straight to the sites with the greatest potential.

During the preliminary test the drone was flown at increasing heights and the resulting images were compared. It appeared that the higher the drone flew (maximum height tested was 70m) the better the reef structure was visible with lower heights not adding any clarity, or detail to the image. The relative visibility will likely be affected by the clarity of the water and at the time of the trial the summer algal blooms had already appeared in Tromsø. If the trial was repeated at times of high visibility and sunlight (spring) the results would be better.

The relatively cheap cost of modern drones and the ease that they can be deployed and flown means this is a very realistic tool for sea urchin fishers in remote areas where rapid surveying of a reef system is required. It is reliant on relatively calm weather so that visibility is good and so that the drone is not damaged or lost.



Figure 4 The aerial surveys using the drone model 'the Phantom 3 drone' (top) and aerial shots taken from the drone at a height of approximately 40m showing reef structure along the shoreline (bottom).

4.3 Dredge survey (Used to establish new fishing areas and possibly in scientific surveys)

4.3.1 Large area survey

Surveys were conducted to assess the usable sea urchin biomass in southern Breidafjörður (south 65°10N and East 22°40W) (see Figure 5A) at depths of 8-60m from 13th - 18th of September 2015 and 11th-13th of April 2016. The surveys were conducted by a commercial sea urchin fishing vessel (Fjóla SH 7) (Figure 5B) using a commercial dredge measuring 250 cm in width and with 150 cm long bag (Figure 5C). The mesh size of the catch-bag on the dredge was 100mm. In order to determine the densities/abundances of urchins, each catch was weighed and the distance covered by the dredge was calculated. The total catch weight was divided by the size of the area covered in each tow to give biomass in kg/m². Biomass estimates for any given area were calculated from the mean biomass in that area multiplied by the total size of the area.

The density (ind./m²) was calculated by dividing the mean wet weight of the individuals in an area into the abundance (kg/m²) of the area.

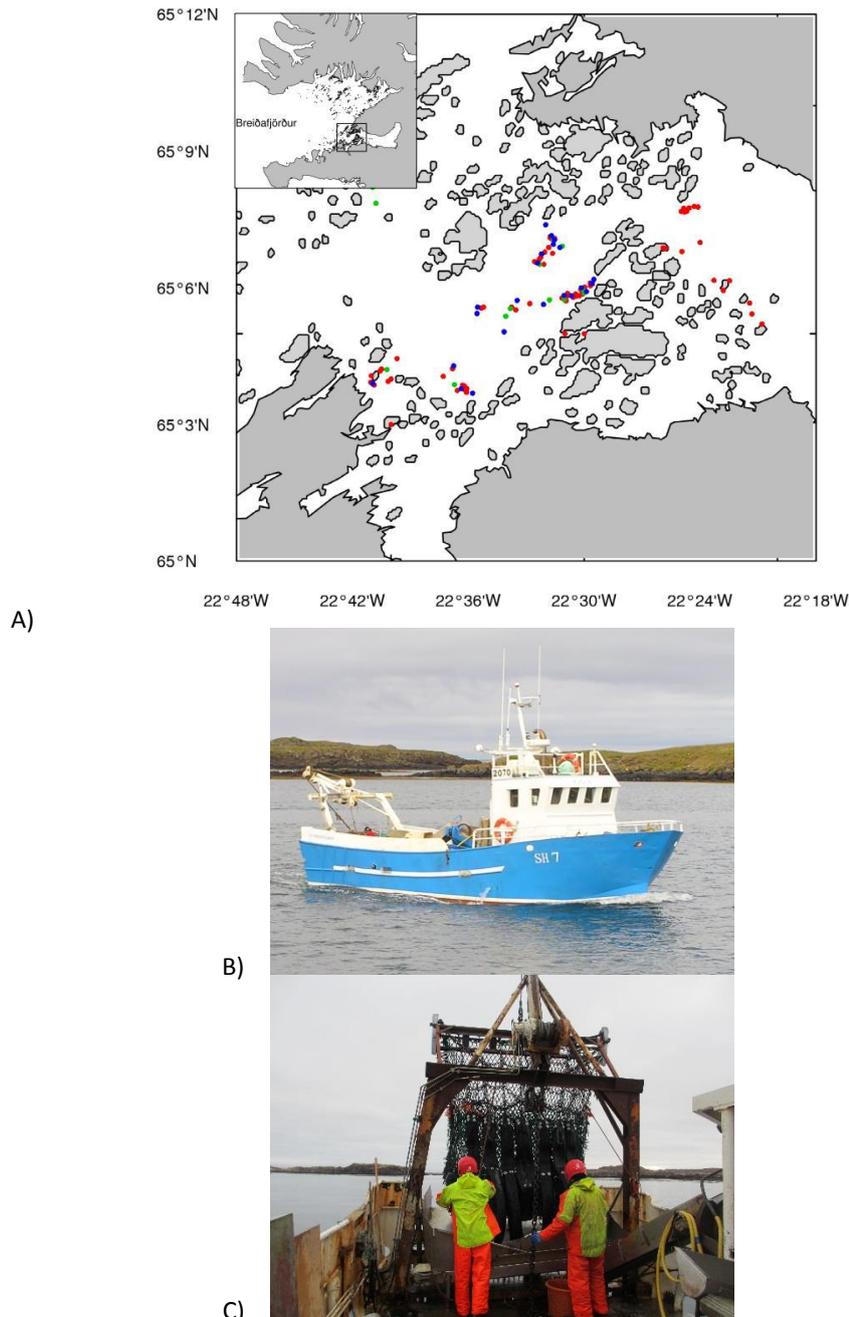


Figure 5 A) A map of the fishing areas investigated in Breidafjörður, Iceland. The red and the green dots denote the stations surveyed in September 2015 and April 2016 respectively, the blue are photo stations from September 2015, B) The fishing vessel used in the survey, C) The sea urchin dredge used in the trials.

During the surveys, sea urchins were found in all areas and at all stations sampled (91) (Figure 5A), regardless of depth (8-60 m) but in varying densities. The whole area investigated measured 9.7 km² and contained seven smaller harvesting areas. Five areas with urchin roe of good quality were observed, these were relatively small (0.3-1.4 km²). The estimated mean abundance varying between 0.17 - 0.52 kg/m² and the density from 2.0 - 8.5 ind./m² which correlated with depth and bottom topography.

The standing stock for all areas combined was 3,395 tonnes. The average efficiency (23%) of the dredge was used when assessing the stock size. The two biggest areas observed, 2.7 and 3.4 km² contained urchins of poor quality (<5 % gonad index) but in considerable abundance.

The distribution of the green sea urchin in Breidifjörður is very patchy with the results showing seven small fishing areas, ranging in size from 0.3 - 3.4 km², at 8-60m depth. Most of the tows (88 %) were made between 8-35 m depth. The green sea urchin is most common in the shallow subtidal zone at depths (less than 50m) but they have also been observed at depths as deep as 300m (Jensen 1974). The density generally decreases with depth to about 20-30m which in many areas corresponds to the deepest distribution of kelp (Himmelman 1986). In the present study no bottom samples were taken but it was obvious that the substrata differed significantly between areas. The most common bottom type was sand and gravel although rocky substrata was also observed. The green sea urchin generally occurs on rocky substrata but is also found on sand and gravel bottoms (Filbee-Dexter and Scheibling 2012). Upper depth limits vary with season and wave action that can dislodge the urchins or limit their ability to graze on macroalgae. On a sedimentary seafloor the urchins rely on drift algae and are more sparsely distributed (Filbee-Dexter and Scheibling 2012).

The bottom type in Area I and II was gravel at mean depth of 35m and the biomass or density at both areas was rather low (0.08kg/m² or 1ind/m²). Kelp was observed at the site not kelp beds as these occur at a maximum depth of 30m depth (Himmelman 1986). The maximum biomass observed was in area III (0.12 kg/m² or 1.6 ind/m²) at lower depth (10m) where there was a gravel seafloor and kelp beds. In area IV the depth was 10m, the density was similar as in the previously mentioned site but here the urchins were of poor quality and small size. Area VI had the greatest depth (50m) and a muddy bottom with the lowest biomass (0.04kg m⁻², 0.5ind. m⁻²). The biggest urchins with the largest roe (gonad index) were observed at area VII at 20 m depth, with a gravel and rocky seafloor and kelp was also observed at the site.

4.3.2 Small area survey

A preliminary small-scale trial was run by Nofima to test the efficacy of a small dredge (see Figure 6 for details of dredge design) at predicting sea urchin biomass in a small survey area. The chosen area had been surveyed previously and had a flat area of seafloor (approximately 500m x 50m) at depths of 2-7m. Three dredge transects were made in the area and the results are compared with the results of two previous dive surveys conducted in the same area and at the same depths (Table 1).

The results show that the estimates for the abundance of urchins was much smaller from the dredge results than from the dive quadrat results. This indicates that the dredge used in the trial is not effectively collecting all the urchins in the sampling area (the area it covers during a single tow). Not surprisingly, the average size in the dredge is slightly higher than in the dive survey and this is likely due to the dredge not picking up very small urchins. If a dredge is to be used for biomass or size estimates of sea urchin populations it would need to be more efficient than the one used in this trial and the efficacy of the dredge would need to be calibrated against dive surveys in an area to be sure the results are accurate. In conclusion, the authors believe there is limited capacity to use mini-dredges as a survey tool but if a more efficient dredge can be used the efficiency of this technique would improve.



Figure 6 The mini-dredge used in the Nofima trial, left and the dredge being pulled onboard (right).

Table 1 The comparative results of three transects using the mini dredge and two previous dive surveys measuring the abundance and size of urchins present in the survey area.

Dredge	Depth (m)	Dredge			Dive survey			
		Urchins in dredge	Length of tow (m)	Square meters covered	Average count / m ²	Average size TD (mm)	Average count / m ²	Average size TD (mm)
1	5	829	64.5	34.8	23.8	25.1	27.3	24.2
2	3	298	66.9	36.1	8.2	21.3	38.2	23.4
3	7.5	249	67.0	36.1	6.9	30.5		
Average	5.2	459	66.1	35.6	12.8	25.6	32.8	23.8

4.4 ROV large and small scale (Used to establish new fishing areas and possibly in scientific surveys)

This is also a relatively new method of surveying has not been described in the literature but preliminary trials have been undertaken in Norway both prior to and as part of the URCHIN Project.

4.4.1 Remote Operated Vehicles, ROV (large and small scale)

Nofima in Norway have previously undertaken a trial using a larger, modified ROV (Figure 7) to fish for sea urchins (see Nofima internal report number 2012, Published March 2012. Test of ROV-based harvesting methods for sea urchins and scallops Part one: Preliminary report on Sea Urchin collection trial). The trial focused on the collection of urchins but one of the clear outcomes was that the effectiveness of the large ROV was reliant on the abundance of sea urchins and the type of bottom substrate. The first 2.5 days of the ROV fishing trial were spent searching for suitable fishing areas and the large ROV was not an effective means of rapidly surveying new areas. It was difficult to launch and retrieve and required the boat to be anchored at all times. It also required a relatively large boat to be launched from and required a powerful and consistent power source. A smaller ROV that was easier to launch and that could be operated from a smaller boat would have been ideal for the start of this trial to rapidly survey large areas and identify those that were suitable for fishing using the larger ROV.

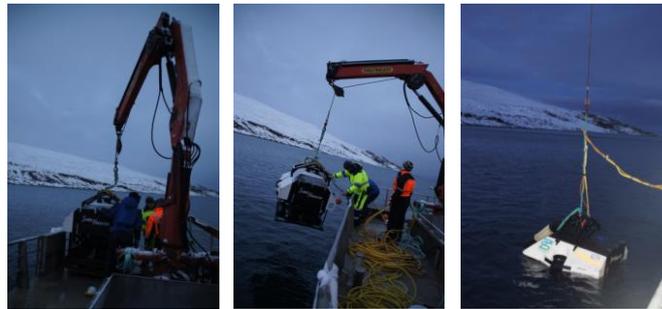


Figure 7 The modified ROV used to fish for sea urchins in a previous Nofima trial and the launching and retrieving process.

Nofima have since tested two types of mini ROV for rapid surveying of underwater sea urchin populations and seafloor habitats, urchin presence/absence and as an initial survey of urchin abundance. This technique is particularly useful when divers are not available, when conditions may not suit divers, when urchin populations are deeper than safe dive limits and when rapid, repetitive surveys are required. The mini ROV comes in a range of models varying in size, ability and price. Two examples are: 1) The battery operated Deep Trekker (Figure 8a) with a total battery life of approximately 3hrs and a 70m umbilical line. The purchase costs of this model is approximately 75,000NOK. Alternatively, the slightly larger, more powerful model SeaBotix LBV300-6 (Figure 8b) is easier to manoeuvre and can handle higher currents and has a 300m umbilical line. It requires a transportable generator but these are relatively light and inexpensive and can be operated from a small boat. The purchase costs of type of mini ROV is approximately 250,000NOK.



Figure 8 The mini ROVs tested by Nofima in Norway for rapid initial site surveying for urchin abundance; Left) the deep trekker, Right) the SeaBotix LBV300-6 mini ROV.

The min ROV's would be very effective for rapid surveying of new fishing areas but would have very limited use as a means of scientific surveying for the following reasons:

- They are very difficult to operate in high current areas, which is where urchins are most likely to be found (Janet Lothead, Fisheries and Oceans Canada, *Pers Comm.*).
- It is very difficult to estimate the field of view, which you need in order to calculate area swept. It is most difficult to estimate field of view on complex substrates, and easier on flat, uniform substrates (Janet Lothead, Fisheries and Oceans Canada, *Pers Comm.*).
- The post-processing of ROV video is very time consuming. For Rockfish video review, for example, which included reviewing the video for all fish observations as well as habitat and field of view, 5 hours of processing were required for every 1 hour of video, but depending on the fish abundance, it could be 4:1 (Janet Lothead, Fisheries and Oceans Canada, *Pers Comm.*).

4.5 Fixed camera quadrats using a drop frame

4.5.1 Methodology for urchin stock assessment with drop-cams used by Nofima

Equipment required:

- A frame constructed from 8mm stainless steel bar with a bottom area of 1m² square and 1m height (Figure 9).
- A GoPro camera, mounted in the top of the frame, faced down so that it could film the entire 1m² frame below.
- A rope marked every half meter was attached to the top of the frame.
- A handheld GPS-unit was set to store positions in its track log every 5 seconds.
- An accurate clock for the boat skipper.
- The camera clock and the skipper's clock was synchronised to match the GPS-clock.



Figure 9 The steel frame used for fixed frame camera surveys in Norway.

Method:

The GoPro camera's time-lapse function was selected so that the camera would take one picture every set period until it was shut down or ran out of power. For these trials we set it to take one picture every 5 seconds. The setup was operated by two people, one driving the boat and taking notes, the other manually lowering the frame to the sea-floor for each quadrat sample. Typically, the boat was

driven close to the shore and the frame dropped at 1 meter depth. The person lowering the frame would transfer the actual depth of the frame to the driver taking notes. The frame was left stationary on the bottom for at least 5 seconds to make sure the camera took at least one image. The driver would take a note of the depth and the exact time (HH:MM:SS). The boat would be driven slowly backwards at a 90 degree angle to the shore line and the camera dropped again at different depths until a straight transect from 1 to 10 meters was obtained (approximately 6 transects on each site to be evaluated).

Post processing of data and counting urchins

The images were imported to a computer and batch rename them to a new filename based on their timestamp: DDMMYY_HHMMSS.jpg using Adobe Bridge. The list of image files was then checked and all images that were not taken when the pyramid was stationary at the sea floor were deleted. The remaining images would be renamed again as such: TransX_Ym_DDMMYYYY_HHMMSS.jpg based on the boat skipper's notes and the image files timestamps. The GPS log was then edited the same way, deleting all entries that didn't match an image within 5-10 seconds.

Each image was then opened in an image editing program (Adobe Photoshop) and a grid overlay with 10 centimeters was applied for easier counting (Figure 10). We would count all visible urchins manually by clicking them with the paint tool, doing one box of the grid at the time.

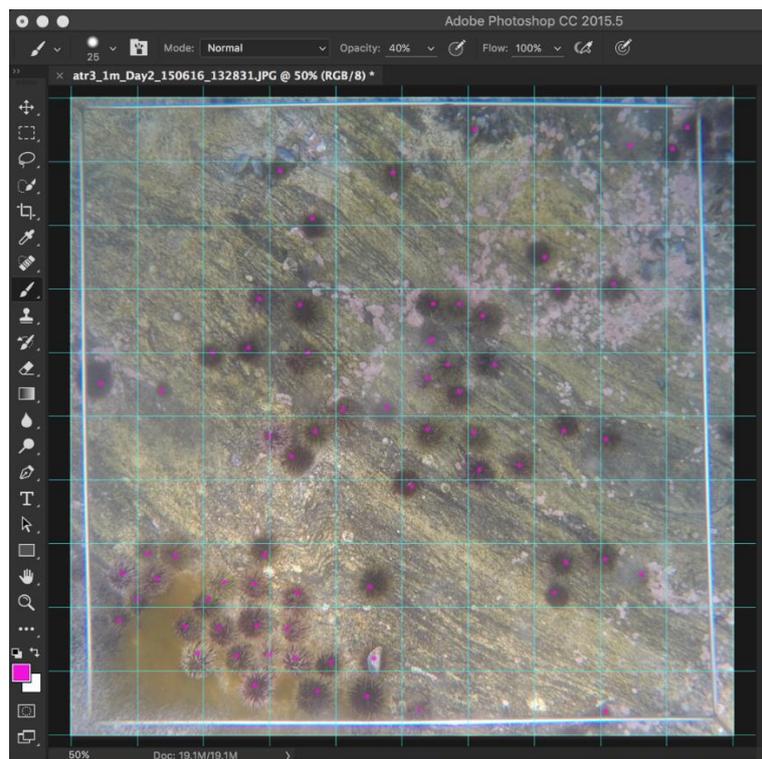


Figure 10 The image processing used on the images after the fixed frame camera survey.

Janet Lohead (Fisheries and Oceans Canada) reported that Fisheries and Oceans Canada have developed publically available software for imaging analysis. There is a very simple analysis program, called 'AVLOG', for green urchin drop camera photos. There is a more complicated program called 'Video Miner', which is for multiple species and habitat variables. Finally, there is a third called 'ImageJ'

which can record measurements and well as counts and would be very useful for urchin assessments (see the Fisheries and Oceans Canada website for further details: <http://www.dfo-mpo.gc.ca>). Galway Marine Institute of Technology also use a program called 'Pro plus' which can record measurements and counts and would also be very useful for urchin image analysis. This program also allows images that have been squeezed due to the curvature of the lens to be squared for easier viewing.

4.5.2 Comparative study between fixed frame camera and dive survey (Norwegian trials)

A survey conducted in Tromsø, Norway tested the efficacy of fixed camera quadrats in comparison with the more traditional technique of using divers to count and measure urchins.

A series of three transects were made in an area that has been extensively surveyed (including mini dredge and trap surveying). It has a rocky shoreline down to approximately 1-2m depth and then a flat shelving sea-floor which consists of shell and gravel. Three fixed frame camera quadrats were measured from each transect as described in the methodology in section 4.5.1. After the photo was taken and before the frame was shifted, all the urchins in the quadrat were collected by a diver and transferred to the boat for counting and measuring. A comparison of the counts was then made between the two techniques.

The results showed that on average the urchin counts taken using the fixed frame camera technique were 83% lower than taken from the same quadrat by diver collection. However, there was a high percentage of very small urchins in the quadrats (average test diameter 23.4mm and only 5 % of the catch were greater than 40mm test diameter) and these urchins would have been difficult to see in the images taken using the fixed frame camera. The authors intend to run a more comprehensive test of the efficacy of this technique to quantify the presence of market size urchins, and to test the efficacy on different substrate types. They believe that if the seafloor is relatively flat and the urchins present are primarily adult urchins the counts would be extremely accurate.

4.5.3 Comparative study between fixed frame camera and dredge survey (Icelandic trials)

As part of the URCHIN project, surveys using bottom photographs were completed between November 2015 and April 2016. These included photographing, counting and identifying all species present in the photos. The number of sea urchins/m² of bottom was estimated and compared to abundance estimated from a survey where swept area method (dredging) was used to assess the abundance (Figure 11).

The efficiency of the dredge ranged from 10 to 33% depending on bottom type and currents. When fishing on hard seafloor, and when there were strong currents present the dredge efficiency decreased considerably.



Figure 11 Bottom photographs from different types of seabed in Breidifjörður. To assess the efficiency of the sea urchin dredge, number of sea urchins observed from the photos was compared to number sea urchin fished at the same site.

4.6 Trapping

4.6.1 Surveying for fishing suitable sites using trapping

Trapping techniques for harvesting sea urchins have been developed in Norway over the past 2-3 years. The Nofima Internal Report (Report 38 B/2014, 2014, Alternative low cost methods of fishing sea urchins) gives a detailed account of the optimal type of traps, the baits and the soak times from these trials. As part of the URCHIN Project a further trial was undertaken using the traps described in the above report (Figure 12) to test for variation in catch rates and urchin size at different sites using the same trapping protocols. Traps were set at six sites (Figure 13a and 13b) with varying environmental conditions.

Site 1: Fokevik

The traps were set near the boulder/sand-line. Approximately 5m depth. Gradually sloping downwards with boulders to 5m and beyond flat sand.

Site 2: Småseines

The traps were set around the 5m transition. Large boulders in depths less than 5m, smaller rock and coarse sand at depths deeper than 5m.

Site 3: Sperkelnes

5 meters or deeper, rock with some sand in between.

Site 4: Myra

Traps set between 2 and 8m. Hard rock with kelp in the shallow area and, in deeper area smaller rock with sand in between (No pictures due to very heavy current).

Site 5: tunnel

Traps set on both solid rock and a little bit deeper where it goes from solid rock to small boulders with sand and coral in between.

Site 6: Nordhellenes

Traps set between 3 and 8m. Solid rock with patches of sand and boulders.



Figure 12 The round collapsible traps designed and tested by Nofima in Norway; sitting on the bottom (left) and being pulled into the boat (right).

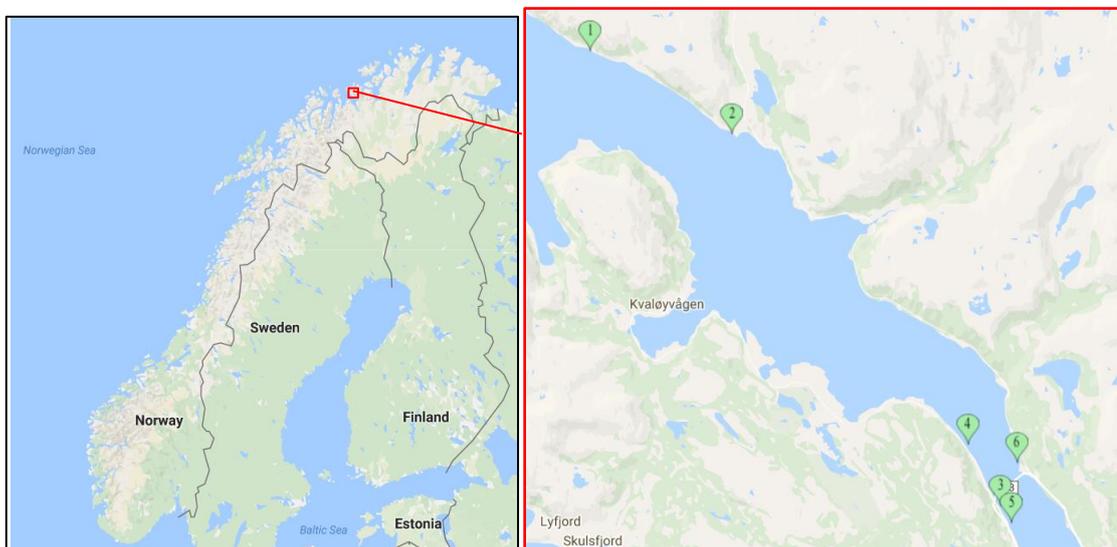


Figure 13 a) The location of the six sites in Kvalsund, Tromsø, used in the trapping experiment.

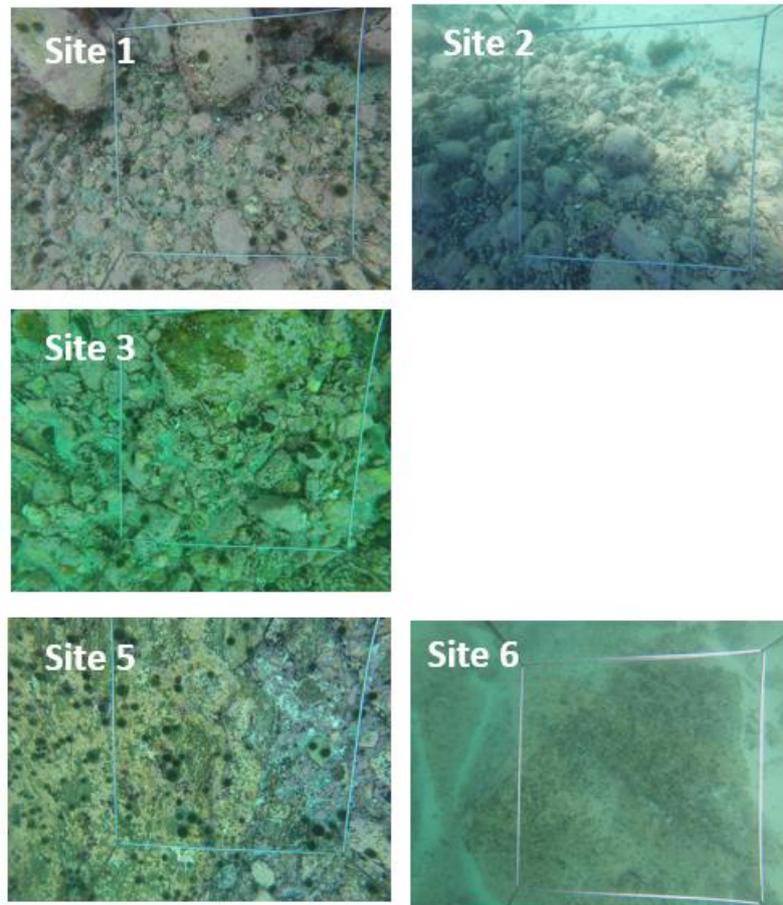


Figure 13b The bottom substrate of the six sites in Kvalsund, Tromsø, used in the trapping experiment (Note site 4 is missing due to extremely high currents at the time of sampling).

The results from the survey are listed in Table 2 and clearly show that trapping could be a viable option for distinguishing sites that have sufficient urchin abundance and urchins of a suitable size for fishing from those that do not. Following on from these trials the technique was also tested on a semi commercial scale (see following section 4.6.2.).

Table 2 The number of traps, average soak time, total catch, average test diameter and total number of urchins greater than 40mm test diameter collected from the 6 sites.

Site	1	2	3	4	5	6
Number of traps	16	14	16	15	16	16
Average of Soak time (days)	8.8	8.4	8.8	8.9	8.8	8.8
Total catch (#)	455	503	648	1973	1015	960
Average diameter (mm)	28.0	29.6	32.9	26.6	33.1	32.6
Total # >40mm	59	126	187	173	358	248

4.6.2 Commercial trapping trial to survey for urchin abundance and size

In addition to the test survey in 4.6.1. a series preliminary commercial trapping trials were used as a means of surveying for new urchin fishing sites in Lyngen, north of Tromsø in Norway. The trials utilised the round collapsible traps developed by Nofima (see Figure 12) and were run in conjunction with the mussel farmer, Arne Samuelsen from Lynsskjellan AS, situated at Rotsund, Lyngen. Each trial involved

setting a minimum of three lines of between 4-10 traps (baited with a variety of algae and fish baits) in areas around Uløya (See Figure 14). The traps were set for periods between 6-9 days and the catch rates are shown in Table 3.

The efficacy of the traps and baits used in the trials has been proven in previous studies (See Internal Nofima Report: Report 38 B/2014, 2014, Alternative low cost methods of fishing sea urchins) and so the catch rates were considered a suitable indication of urchin presence/absence of urchins as well as the urchin size in the area. The results clearly show that trapping is an effective method of distinguishing between suitable sites for urchin fishing. It gives both an indication of the density of urchins present as well as their size. The traps can be set easily and can cover a wide area. Nofima would recommend this is a suitable method of surveying new areas for sea urchin fishing, particularly where diving is difficult, or expensive.

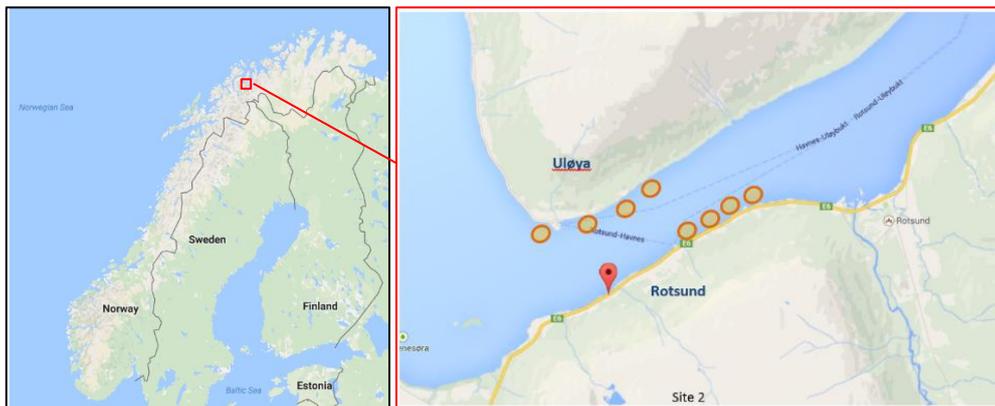


Figure 14 The areas where the trap lines were set around Uløya in Lyngen.

Table 3 The number of traps, trap lines, dates traps went into and out of the water and the catch numbers for the commercial trapping trials conducted in either the Uløya or Rotsund area in Lyngen.

Trap No.	Set 1 (Uløya)			Set 2 (Uløya)			Set 3 (Uløya) (Uløya) (Uløya) (Uløya) (Rotsund) (Rotsund)						Set 4 (Rotsund) (Rotsund) (Rotsund) (Rotsund)			
	Trap line 1	Trap line 2	Trap line 3	Trap line 1	Trap line 2	Trap line 3	Trap line 1	Trap line 2	Trap line 3	Trap line 4	Trap line 5	Trap line 6	Trap line 1	Trap line 2	Trap line 3	Trap line 4
Number of traps	10	10	10	10	10	10	5	5	5	5	5	5	6	4	9	9
Date In	22 Apr	22 Apr	22 Apr	29 Apr	29 Apr	29 Apr	6 May	6 May	6 May	6 May	6 May	6 May	18 May	18 May	18 May	18 May
Date Out	29 Apr	29 Apr	29 Apr	6 May	6 May	6 May	15 May	15 May	15 May	15 May	15 May	15 May	26 May	26 May	26 May	26 May
Number of days	7	7	7	7	7	7	9	9	9	6	6	6	8	8	8	8
Catch urchins < 40mm TD	0	0	46	0	0	10	6	4	6	14	79	13	105	113	44	69
Catch urchins > 40mm TD	0	0	80	0	0	16	5	5	6	15	73	6	63	82	35	50

5 Summary and Recommendations

5.1 Summary

This report describes a number of different techniques for surveying new fishing areas and/or surveying for scientific monitoring of urchin populations. The best method for individual companies, Research Institutes in different countries will depend on a number of factors. The following is a list of recommendations for participating countries in the NPA URCHIN project. The relative advantages/disadvantages of each technique are summarised here.

- SCUBA

Advantages: This is the most commonly used technique and provides very accurate measures of urchin size and numbers. This method also has protocols established for analyzing the resulting data and calculating population biomass in any given area.

Disadvantages: In a number of countries, particularly in the NPA diving as an expensive and unrealistic method of surveying. In addition to the high cost the environmental conditions in the NPA are often not suited to dive operations. SCUBA diving is restricted to relatively shallow depths unless specialised divers are utilized.

- Dredge

Advantage: This a good method of surveying large areas quickly. Particularly if there are already dredge operations in place in an area, using a dredge design that is known to be effective.

Disadvantage: A number of countries within the NPA do not allow dredges to be used for environmental reasons. It is essential that the efficiency of the dredge be calibrated (validated against a known survey technique) prior to using any data from dredge catch surveys. The small-scale dredge used in Norway was not effective at catching the small urchins present at the site.

- Aerial survey

Advantage: This is a very fast and effective means of covering very large areas for a general overview of reef structure and potential new fishing sites. Small drones appear to provide excellent images of smaller areas.

Disadvantage: This technique is very weather dependent and does not give any indication of urchin presence/absence or abundance.

- ROV survey

Advantage: The ROV surveys provide a good, rapid in-water method of surveying new areas for presence/absence of urchins without the need to use divers. Mini ROV's can be used at depths not suitable for divers and can make repeated short exploratory surveys that a diver would not be able to do.

Disadvantage: It is difficult to accurately map where you have surveyed underwater without having expensive sonar equipment. Strong currents also make this method impractical in some areas. Large ROV's (larger than a mini ROV) are too cumbersome for this task.

- Trap survey

Advantage: Traps are cheap and easy to set for fishers that are already established. They give a good reflection of the presence/absence and available biomass and size of urchins in an area.

Disadvantage: The catch rates in traps can be influenced by factors other than urchin presence/absence and so the surveys should be treated with caution.

- Fixed frame camera survey

Advantage: This is a very cheap alternative to dive surveys and give reasonably good catch (and possibly size) estimates depending on the type of substrate and the size of the urchins.

Disadvantage: It can be difficult to see small urchins and urchins in very complex substrate (e.g. boulders).

5.2 Recommendations

The following is a suggested list of possible survey techniques for each of the participating NPA countries. The techniques in bold are the most likely candidates listed.

5.2.1 Norway

Surveying for new fishing areas: **SCUBA, Aerial, ROV, Trap**, Fixed frame camera.

Scientific surveying: **SCUBA, Trap, Fixed frame camera.**

5.2.2 Iceland

Surveying for new fishing areas: SCUBA, **Dredge**, Aerial, ROV, Trap, **Fixed frame camera.**

Scientific surveying: **SCUBA, Dredge, Trap, Fixed frame camera.**

5.2.3 Ireland

Surveying for new fishing areas: **SCUBA**, Aerial, ROV, Trap, Fixed frame camera.

Scientific surveying: **SCUBA, Trap, Fixed frame camera.**

5.2.4 Greenland

Surveying for new fishing areas: SCUBA, **Dredge**, Aerial, ROV, Trap, **Fixed frame camera.**

Scientific surveying: **SCUBA, Dredge, Trap, Fixed frame camera.**

6 References

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Appendix 1

Sea Urchin surveying techniques

(Activity A4.1.1 of the NPA URCHIN project)

Activity 4.1	<i>Activity title</i> Measuring sea urchin stock biomass	<i>Start month</i> 04.2015	<i>End month</i> 07.2016
<i>Description:</i> Develop a cheap, effective and reliable method of stock assessment suitable for the environmental conditions found in the NPA			<i>Deliverables:</i> Report on a suitable sea urchin stock assessment technique
4.1.1	<i>Deliverable</i> Methodology for stock assessment	<i>Target value</i> Supply methodology to all participating NPA countries	<i>Delivery month</i> 06.2015

Followed by the following Activity:

(NOTE: the mini ROV trials will be replaced by drop camera trials following the results in the trials in Activity 4.1)

Activity 4.3	<i>Activity title</i> Implement biomass assessment technique	<i>Start month</i> 09.2015	<i>End month</i> 09.2017
<i>Description:</i> Trials will be conducted using dredging and a mini ROV techniques developed in Activity 4.1 to assess urchin stock size in 3 NPA countries. Greenland: July - August 2015: mini-ROV biomass estimation in key areas of Greenland Iceland: September-October 2015: mini-ROV biomass estimation in key areas of Iceland and stock assessment by a dredge in 2 different areas in September 2015 and September 2016. Norway: September-October 2015: mini-ROV and dredge biomass estimation in 2 sample areas between September 2015 and September 2017.			<i>Deliverables:</i> Comparing techniques and assess stock size in a minimum of two areas in three NPA countries
4.3.1	<i>Deliverable</i> Biomass assessment trial	<i>Target value</i> Completed in two areas from three NPA countries	<i>Delivery month</i> 09.2017

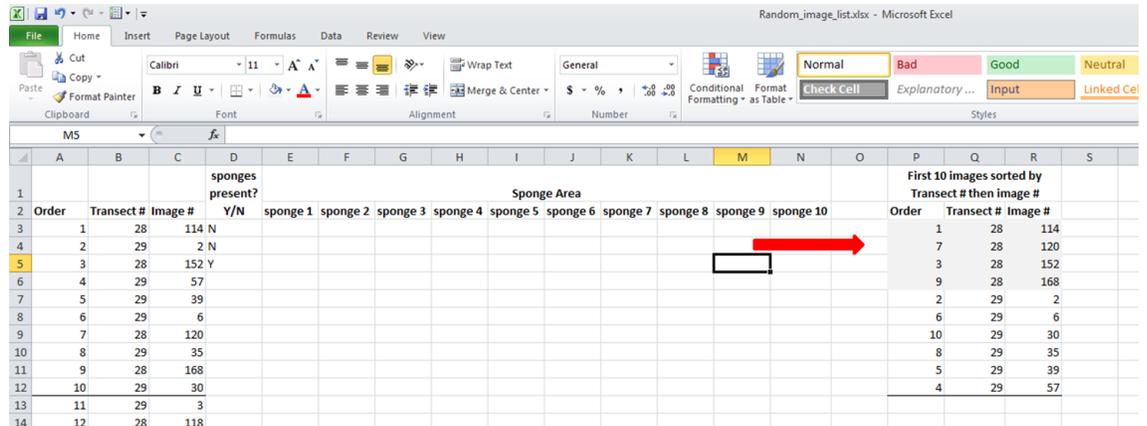
Appendix 2: Measuring Sponge Protocol

Explanation of task

We need to get an idea of sponge size on each of the glass sponge reefs in the Strait of Georgia. To do this, we have randomly selected 10 images taken on the reefs during our survey in 2013. You will measure the size of all of the sponges in those 10 images. If an image does not have any sponges you will include an 11th image, if two images don't have sponges you will review a 12th image, etc until you have measured sponges from 10 images. Some images will have more than 1 sponge; you will measure all sponges in the image (up to 10). There were multiple transects per reef, and the images selected span those transects, this is intentional. You can measure the first 10 images for a reef in any order you like, however, once you move on to extra images (i.e. the 11th, 12th, etc) you must do them in the order provided. Below are step by step instructions.

Measuring sponge protocol

1. Open Image J
2. Open the excel file named "Random_image_list.xlsx"
3. Start with Reef 1
 - a. The first ten images are sorted by transect number and then image number on the left of each sheet (Nanaimo and Parksville only have one transect so this was not done for them).



Order	Transect #	Image #	Y/N	sponge 1	sponge 2	sponge 3	sponge 4	sponge 5	sponge 6	sponge 7	sponge 8	sponge 9	sponge 10	Order	Transect #	Image #
1		28	N											1	28	114
2	2	29	N											7	28	120
3	1	28	N											3	28	152
4	2	29	N											9	28	168
5	3	28	Y											2	29	2
6	4	29	Y											6	29	6
7	5	29	Y											10	29	30
8	6	29	Y											8	29	35
9	7	28	Y											5	29	39
10	8	29	Y											4	29	57
11	9	28	Y													
12	10	29	Y													
13	11	29	Y													
14	12	28	Y													

- i. The images you received from Anya are organized in folders that correspond to the transect number. For example the folder Dive 1 corresponds to Transect 1.
 - ii. The image number is found at the end of each image file name for example 120513_155212_1.jpg is image number 1
4. Open the first image with Image J (File → Open or Control O)

5. Record if sponges are present in the Excel file.

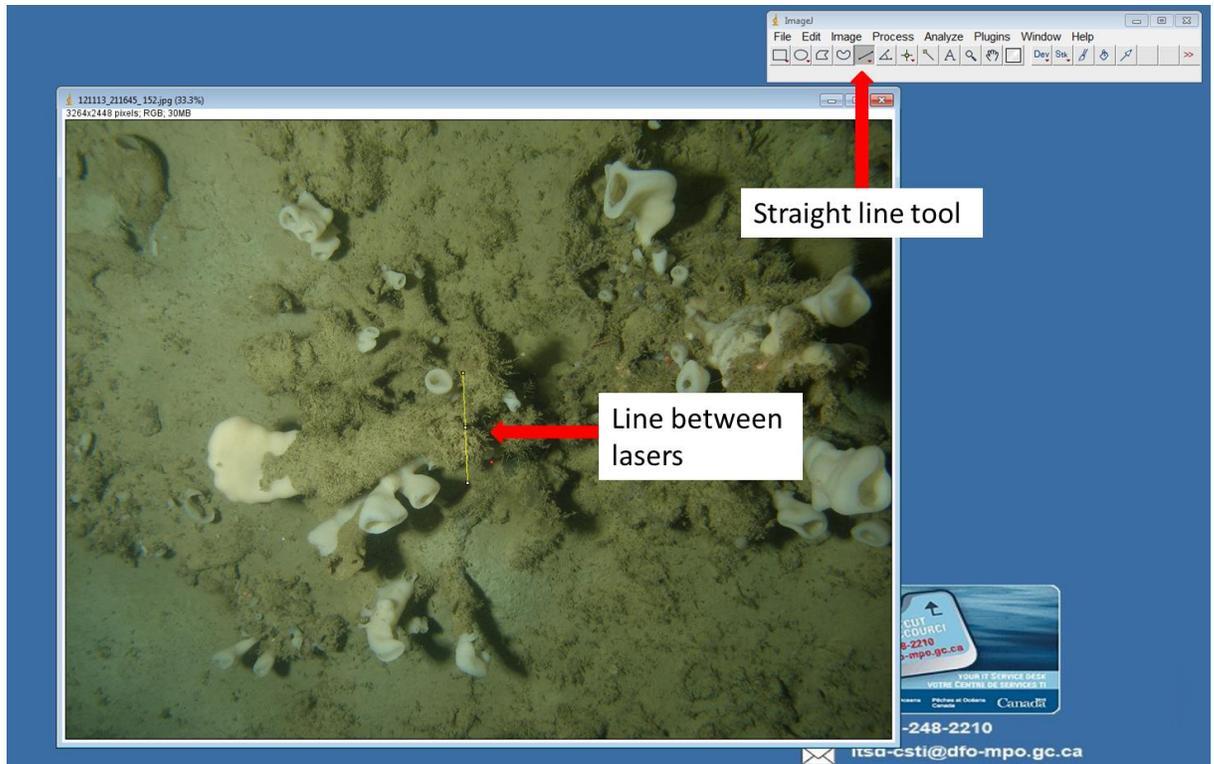
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1				sponges present?															
2	Order	Transect #	Image #	Y/N	sponge 1	sponge 2	sponge 3	sponge 4	sponge 5	sponge 6	sponge 7	sponge 8	sponge 9	sponge 10		Order	Transect #	Image #	
3	1	28	114	N												1	28	114	
4	2	29	2	N												7	28	120	
5	3	28	152	Y												3	28	152	
6	4	29	57													9	28	168	
7	5	29	39													2	29	2	
8	6	29	6													6	29	6	
9	7	28	120													10	29	30	
10	8	29	35													8	29	35	
11	9	28	168													5	29	39	
12	10	29	30													4	29	57	
13	11	29	3																

6. If sponges are not present move on to the next image.

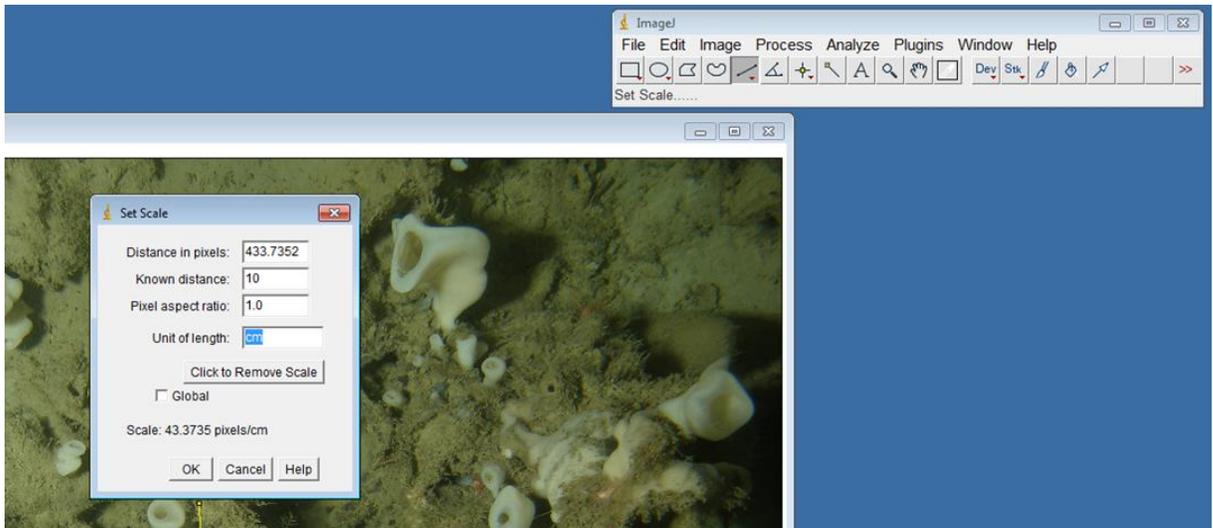
7. If sponges are present select the straight line tool.

i. Draw a line between laser points. There are three laser points. You want to use the two that are in a vertical line. If they are all lined up, use the inner two.

1. NOTE: there are images where the laser points are difficult to see, if you cannot easily see them zoom in and search the center of the image. If you still cannot find the laser points, make a note in the sponges present field and do not analyze the image further.

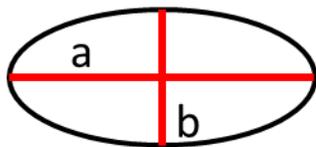
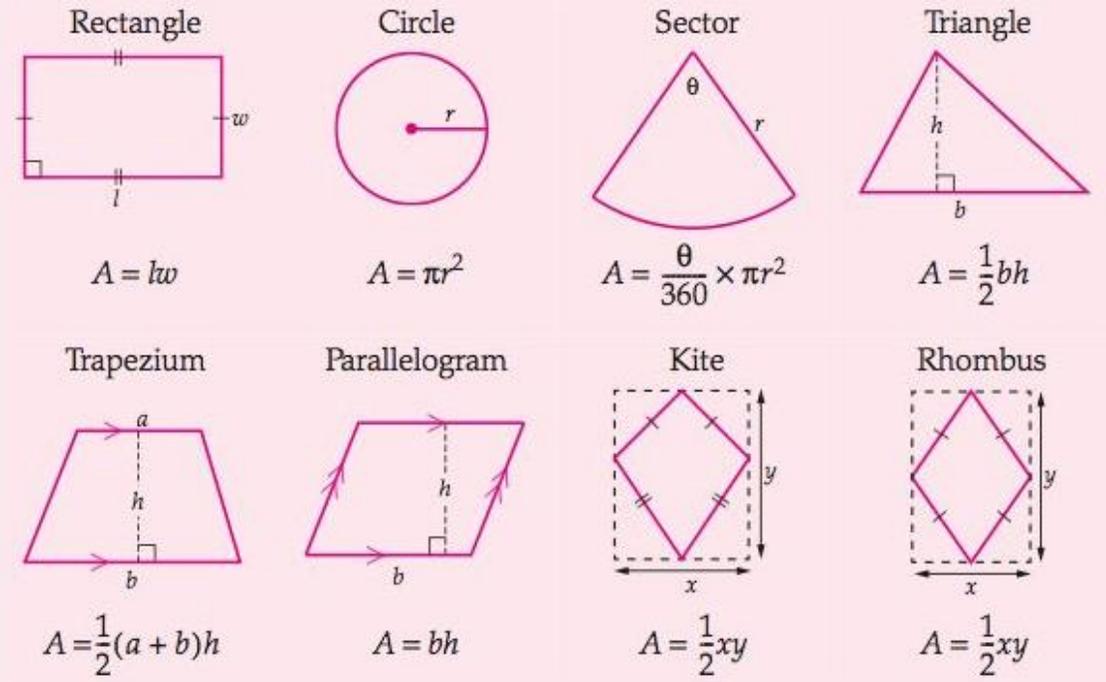


8. Set the scale for the image.
 - a. Go to Analyze → Set scale
 - b. In the “Known distance” field put 10
 - c. Change the “Unit of length” to cm
 - d. Press ok



9. Begin measuring sponges.
 - a. If a sponge is partly in the image DO NOT measure it.
 - b. For each sponge you will decide which geometric shape it most resembles (i.e a circle, square, triangle, etc). Below is a list of shapes and the measurements you should take for each one and the formula to calculate area. Remember, no shape is going to be perfect, do the best you can. Also, this list is not exhaustive.

Area formulas for common shapes

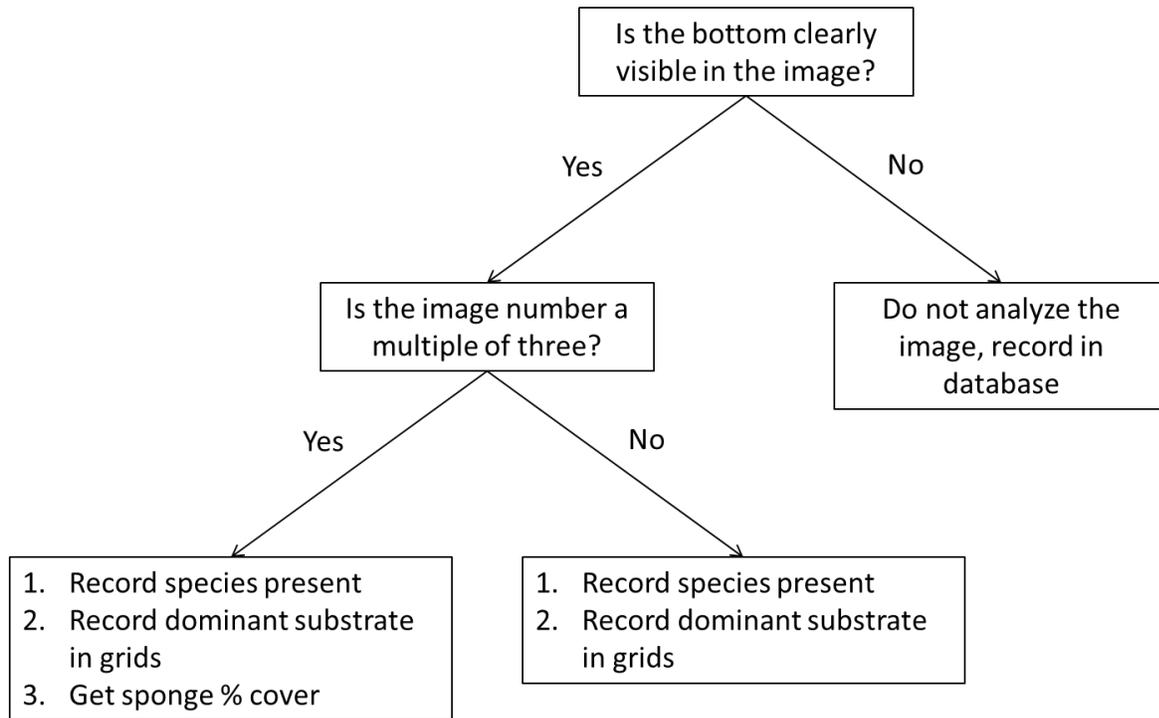


Oval/ Ellipse $A = \pi \left(\frac{1}{2}\right) b * \left(\frac{1}{2}\right) a$

- c. Record the area for each sponge in the image up to 10 sponges. If there are more than 10 sponges in the image do your best to capture the size range present in the image. In other words, measure the smallest and largest sponges then randomly select the other 8 sponges you will measure, being on the lookout for an unconscious bias towards the smaller or larger sponges.
10. Repeat until you have measured sponges in 10 images for Reef 1. The maximum number of sponges you will measure for each reef is 100, the minimum is 10.
11. Move on to Reef 2 and repeat steps 1-10 until all Reefs have had sponges in 10 images measured.

Appendix 3: Protocol for analysing still images

Protocol for analysing still images



1. Record species present in every image
 - a. Record "species" present in each image, their quantity, and your confidence in your ID in the still images database on the hard drive you received. You do not have to get to species, assign organisms to the lowest taxonomic level you feel comfortable. There are three Photo ID guides in the ID_Guides folder on the hard drive you received to aid with identification. If you need help, make a note of the image number and the approximate location of the organism and we can discuss the ID at a later time.
 - b. If there are reef-building sponges in the image
 - i. They should be entered in the database as Hexactinosida.
 - ii. Record if you see any physical signs of damage for any sponge in the image. In the SpongeDamage column.
 - iii. Do not attempt to count individuals, instead we will record the number of osculum visible in the image. For smaller sponges this may be done by easily counting. If the sponge is large enough that you feel you will lose count, then follow these instructions:
 1. If the toolbox is not on your screen press control B or go to Windows→toolbox.
 2. If the tool options dock is not on your screen go to Windows→ Dockable dialogues →tool options
 3. Place a black circle/oval over each osculum by using the ellipse select tool then filling in the selection using the bucket fill tool (shift B, Make sure to select "fill whole selection" in the "Affected Area" section of the

tool options.). The placement of these does not need to be exact. It is important to ensure none of the circles touch.

- a. NOTE: it may be easier to add all the circles at once, then fill them in. To do this, once you have placed the first circle using the ellipse select tool hold down the shift key while placing the remaining circles. Once you are done you can switch to the bucket fill and it will fill all the circles at once.
 4. Select all the circles using the select by color tool (shift O).
 5. Press control I to invert the selection.
 6. Using the bucket fill tool color the background white.
 7. Export the image with the original file name_osculum.
 8. Open the image in ImageJ
 9. Go to Go to Image→Adjust→Color Threshold
 - a. This should automatically select all the dots in the image. If it does not, Use the Wand (tracing) tool to select one filled shape. Press sample in the color threshold dialogue window. If all shapes do not automatically turn red adjust the hue, saturation, and brightness until all shapes red.
 10. Go to Analyze→Analyze Particles
 11. Press ok
 12. The count that shows in the result window is the number of osculum.
Record this number in the database.
2. Record dominant substrate within a 10 cm grid
- a. Create a new .csv file for every image,
 - i. Use the following naming convention YXXTAAIZZZ where XX= the last two digits of the year, AA is the two digit form of the transect number (i.e. transect 2 would be recorded as 02), and ZZZ is the image number. The image number is the very end of the image file name. For example, for image 120513_155212_1.jpg collected in 2013 on transect 1 the file name would be Y13T01I001.csv.
 - b. Open image in image J
 - i. Select the straight line tool.
 - ii. Draw a line between laser points. There are three laser points. You want to use the two that are in a vertical line. If they are all lined up, use the inner two.
 1. NOTE: there are images where the laser points are difficult to see, if you cannot easily see them zoom in and search the center of the image. If you still cannot find the laser points, make a note in the database on the Images_not_processed table and do not analyze the image further for 10 cm grids but DO analyze the image for species present, percent cover (if applicable), and osculum counts (again, if applicable).
 - iii. Press control M or go to Analyze →Measure.
 - iv. The length field in the results box is the number of pixels. Retain this number for use in configuring the grid (see c.ii below)
 - c. Open image in GIMP

- i. Click on view →show grid
- ii. Click on image →configure grid
 1. Set width and height to be the number of pixels between the lasers determined in step b.iv above.
- iii. Record the dominant substrate ($\geq 50\%$ of cell) in each grid cell in the appropriate Excel workbook and worksheet using the classifications in Table 1. The substrate observation from the top left grid cell in the image should be entered in cell A1 of the Excel workbook. The remaining observations from the top row should be entered in row A of the Excel workbook in sequential order, row two from the image should be entered in row B of the workbook, continuing until all rows and column of the image have been entered in the workbook.
- iv. If the substrate in a cell is exactly split between two or more substrates record each in the appropriate place, separating each ID with a period (e.g. a cell with both wood and bedrock, smooth would be 0.1).
- v. Keep image open in GIMP if recording sponge % cover, otherwise move on to next image

Table 1. Possible substrate classifications		
Substrate Id	Substrate Type	Substrate Description
0	Wood	Wood, Bark, or Wood Debris
1	Bedrock, smooth	Bedrock, smooth without crevices
2	Bedrock with crevices	Bedrock with crevices
3	Boulders	Boulders, bigger than a basketball
4	Cobble	Cobble, between 3 inches and basketball size
5	Gravel	Gravel, between 3/4 inch and 3 inch
6	Pea Gravel	Pea Gravel, between 1/8 inch and 3/4 inch
7	Sand	Sand
8	Shell	Shell
9	Mud	Mud
10	Crushed Shell	Crushed Shell (new code 2006)
11	Whole Shell	Whole Shell (new code 2006)
12	Live Reef Sponge	Heterochone calyx and Aphrocallistes vastus
13	Dead Sponge	Dead reef building sponges
14	Boat sponges	Rhabdocalypus spp.
15	Other sponges	All other sponges
16	Man-made object	Marine debris
17	Non-sponge sessile biota	Corals, anemones, etc
18	Mobile biota	Crabs, sea stars, fish, etc

3. Get sponge % cover
 - a. Working in GIMP
 - i. If the toolbox is not on your screen press control B or go to Windows→toolbox.

- ii. If the tool options dock is not on your screen go to Windows→ Dockable dialogues →tool options
- iii. Outline any sponge (live or dead) either using the foreground select tool (instructions [here](#)) or the free select tool.
- iv. Fill in outline using the colors found in Table 2 using the bucket fill option (Shift B).
 - 1. Make sure to select “fill whole selection” in the “Affected Area” section of the tool options.
 - 2. Change the color to fill by clicking on the color swath at the bottom of the tool box.
- v. Once done outlining all sponges, select all filled shapes using the select by color tool (Shift O). Select additional colors by holding the shift button.

Sponge Type	Color
Reef building sponges (Heterochone calyx and Aphrocallistes vastus)- live	Black
Reef building sponges (Heterochone calyx and Aphrocallistes vastus)- dead	Blue
Rhabdocalyptus spp.	White
Other sponges	Orange

- vi. Once all filled shapes are selected press control I to select the background. Fill the background any grey.
- vii. Export file as a .jpg
- b. Open exported file in image J
 - i. Go to Image→Adjust→Color Threshold
 - ii. Unclick the dark background box at the bottom of the dialogue window that opens.
 - iii. Use the Wand (tracing) tool to select one filled shape.
 - iv. Press sample in the color threshold dialogue window
 - 1. If all shapes of that color do not automatically turn red adjust the hue, saturation, and brightness until all shapes of that color are red.
 - v. Press sample in the color threshold dialogue window
 - vi. Press control M or go to Analyze →Measure.
 - vii. The area in the Results window should be recorded in the appropriate column on the Percent Cover table in the database.
 - viii. Repeat until all sponge types present in the image are measured.

