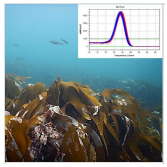

Sep 01, 2021

MarineSediment-BlueC-eDNA

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ABSTRACT

This protocol was established for the Nordic Blue Carbon Project (2017–2020) for tracking the presence of kelp DNA in marine sediments, providing preliminary quantitative information on the original kelp biomass.

Frigstad, H., H. Gundersen, G. S. Andersen, Gunhild Borgersen, K. Ø Kvile, D. Krause-Jensen, C. Boström, T. Bekkby, M. Anglès d'Auriac, A. Ruus, J. Thormar, K. Asdal, and K. Hancke. (2020). Blue Carbon – climate adaptation, CO2 uptake and sequestration of carbon in Nordic blue forests : Results from the Nordic Blue Carbon Project (Nordisk Ministerråd: Copenhagen). TemaNord. http://10.6027/temanord2020-541

Nordic blue forests are coastal vegetated habitats, such as kelp forests, eelgrass meadows and rockweed beds, that are important natural sinks for carbon and thereby climate regulation. They also play an important role in climate adaptation. Simultaneously, blue forests are at high risk from climate change and other human impacts, such as eutrophication and coastal development.
The advent of eDNA methods for tracking animal or vegetal organisms in water or sediment matrices, has enabled not only geographical detection without collection of the organism but also temporal study of their presence in dated sediment cores. The fate of eDNA and its persistence in the environment is intimately related to its biophysical surroundings. eDNA entrapped in sediments may be preserved on long time scales, up to centuries, enabling historical tracking of the presence of a species on a site. The present protocol was developed for tracking eDNA of a predominant Norwegian kelp species, *Laminaria hyperborea*, in dated marine sediment cores. This approach enables confirmation of species specific contribution to carbon sequestration in an effort to evaluate marine sediments operating as carbon sinks.
This work was financed by the Norwegian Environment Agency (Miljødirektoratet) contract #17080044 and published in a NIVA report ISBN 978-82-577-7384-7, Norwegian Environment Agency Report M-2090|2021, Blue Carbon eDNA – A novel eDNA method to trace macroalgae carbon in marine sediments.

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EXTERNAL LINK

https://nordicbluecarbon.no/

PROTOCOL CITATION

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KEYWORDS

eDNA, CO2 sequestration, Blue carbon, Kelp, Blue forests

LICENSE

IMAGE ATTRIBUTION

Eli Rinde, Norwegian Institute for Water research

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48508

MATERIALS TEXT

Gravity corer

KC Denmark **13.540**

The 13.540 gravity corer Ø101,6 mm is designed for taking samples in sandy sediment featuring a detachable core tube with a maximum length of 6 m, (optional). Up to 12 m in soft or muddy sediment. The corer is fully manufactured from AISI 316 stainless steel. Finish: Electro polish.

CFX96 Touch Real-Time PCR qPCR

Bio-Rad **#1855195**

DNeasy PowerSoil Pro Kit (250) **Qiagen Catalog #Cat No./ID: 47014** Step 6

SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix **Bio-rad Laboratories Catalog #1725017** Step 7

Hard-Shell PCR plate thin wall white **BIO-RAD Catalog #HSP9655** Step 7

Optical flat 8-cap strips **BIO-RAD Catalog #TCS0803** Step 7

Core sampling

1 Equipment assembly (Gravity corer)

Manual for (kc-denmark.dk):

1. Add the necessary number of lead weights at the main rack.
2. Mount the supporting frame and secure it by fastening the bolt.
3. Mount the unit with the top lid. Open the fastening device and push the tube with the mechanical stop into the bottom. Lock the handles and secure with a bolt.
4. Attach the carver and fasten with two nails.

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Using the core catcher entails a risk of physically disturb the sediment surface. A top lid is released when the corer hits the sea floor in high speed and close off the top of the corer. Upon retrieval of the corer, a vacuum is then created that retain the sediment core inside the corer during retrieval, given that the corer is handled with caution when exiting the sea water and entering the vessel. This is crucial to avoid the sediment from falling out, especially with coarser sediment types such as sand.

Gravity corer model no. 13.540

2 Core field sampling

2.1 Lower the gravity corer into the sea and towards the sea floor at a controlled speed until it reaches 10m above the sea floor. Let the corer freefall the last 10m, and the corer penetrates the sediments by gravity A top lid is released when the corer hits the sea floor in high speed and close off the top of the corer. Upon retrieval of the corer, a vacuum is then created that retain the sediment core inside the corer during retrieval, given that the corer is handled with caution when exiting the sea water and entering the vessel. This is crucial to avoid the sediment from falling out, especially with coarser sediment types such as sand.

When the core catcher is not used, the corer should be handled with caution when exiting the sea water and entering the vessel to avoid the sediment from falling out.

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Launching and recovering the gravity corer Model no. 13.540

2.2 When the corer enters the vessel: remove the carver (nose piece), seal the bottom of the internal PVC tube with a tight-fitting plastic cap and secure it with waterproof tape. Remove the internal tube carefully from the corer in an upright position in order to keep the sediment-water interface intact. Cap the sediment with sea water from the sampling at the top and seal it with a plastic cap and secure it with waterproof tape. Take care to avoid air being trapped between the sea water and the cap.

Removal of the carver (nose piece) by removing the two nails with a hammer

Removal of the carver (nose piece)

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2.3 In order to reduce the risk of sample contamination, the corer, nose piece and other equipment must be washed between every core cast, either with fresh water (preferrably) or with running sea water from a medium pressure hose on deck, as was done in the current project. The internal PVC tube that collects the core is new for every cast.

3 Equipment required on vessel for core sampling

3.1 Sediment cores were sampled at four sites in Frohavet, Trøndelag (NW coast of Norway, **63-64 °N**). Water depth ranged from 242 to 531 m and the sediment type was silt and very fine sand. The sampled core length ranged from 61 to 121 cm.

3.2 Sediment corer: KC Denmark Gravity Corer. The corer is an open tube fitted with a weight so that gravity can force it sufficiently deep into the sediment in order to retrieve a sediment core. The KC Denmark Gravity Corer recovers an 88.9 mm diameter core of undisturbed sediment. The corer is designed with several detachable core tubes each with a length of 150 cm. The corer is made from stainless steel and consists of a corer body with 6 steering fins and a weight platform, steel corer, internal PVC liners (preferably transparent), lead weights, orange peel closing system ("core catcher") and a carver with cutting edge (nose piece).

Gravity corer

KC Denmark **13.540**

The 13.540 gravity corer Ø101,6 mm is designed for taking samples in sandy sediment featuring a detachable core tube with a maximum length of 6 m, (optional). Up to 12 m in soft or muddy sediment. The corer is fully manufactured from AISI 316 stainless steel. Finish: Electro polish.

Corer body, five lead weights, core catcher ("orange peel closing system") and cutting edge (nose piece) (Photo: NIVA)

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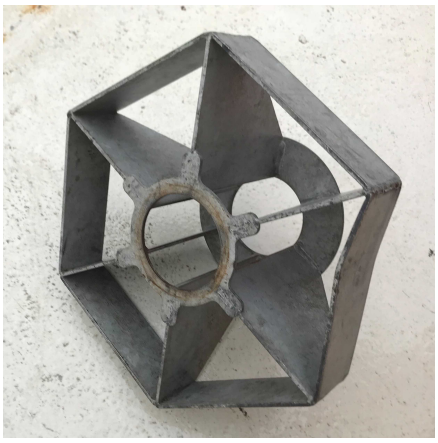

Steering fins (Photo: NIVA).

3.3 Other required equipment include: nails, hammer, cutting tools, ruler, PVC tape/gaff tape, waterproof writing utensils and safety equipment. The vessel must be equipped with a navigation system and being able to position the boat steadily during sampling.

It is necessary with a vessel equipped with hydraulic cranes and winches capable of heavy lifting in order to handle the gravity corer in a safe way. Never walk under the gravity corer, when lifted up by the winch. Make sure the rope or wire used for lifting is strong enough to support the heavy equipment.

4 Core sample transportation and storage

Photograph each core in order to record basic characteristics of sedimentary layers while the sediment is fresh, to be able to identify any major disturbances during transport. Transport and store the cores standing up and maintained in their PVC tubes until opening in the laboratory. Storage until processing: in the dark at **4 °C** .

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Photograph each sediment core

Seal the internal PVC tube with a tight-fitting plastic cap and secure it with waterproof tape.

Vertical storage of sampled cores in their PVC tubes

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5 Core sample aliquoting

5.1 Preparation of whole sediment core for slicing

Open the PVC tube was at both ends and place it on top of a plunger fitting the inner diameter of the tube. Push the tube slowly and carefully downward until all the overlying seawater spills over the top and the sediment surface is even with the top of the tube. Push the tube further down so that a 1 cm thick section of the sediment core can be sliced off with a clean steel plate (slicer). Slice each core into 1 cm thick sections, until the desired depth (50 cm in this project and 12 sections were chosen for further analysis: 0–1, 2–3, 4–5, 6–7, 8–9, 10–11, 13–14, 17–18, 22–23, 28–29, 35–36, 43–44 cm depth layers).

Upper section of the sediment core

Example of an intact sediment core >100 cm long sealed at both ends. Exam the sediment surface and core prior to subsampling to ensure tit is intact.

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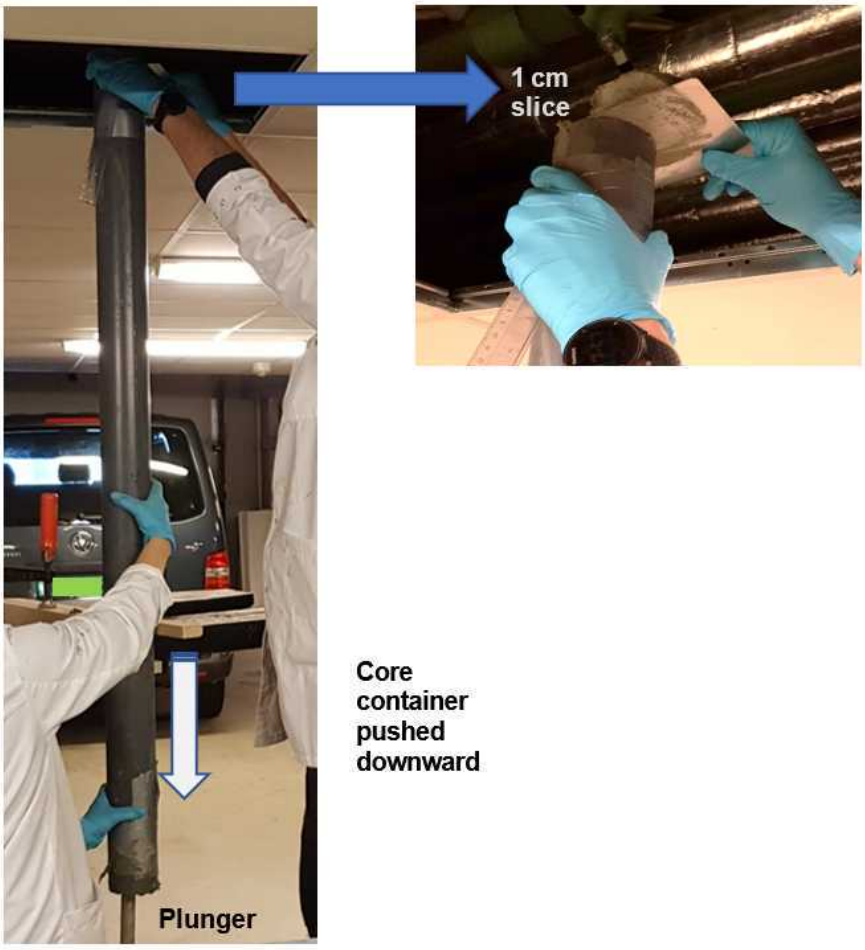

The tube core container is openned at both ends and placed on top of a plunger fitting the inner diameter the tube.

5.2 Individual sediment core slice aliquoting

Portion each 1 cm slice was into equal parts (i.e. aliquote) for different analyses. Rinse the equipment with ☒ Ethanol (95 - 100%), molecular grade **Contributed by users** between each core slice.

Sample the inner part of each slice section in order to further reduce the risk of cross contamination.

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Core 1cm thick slice aliquoted for different analysis. Sample for eDNA analysis is the lower right corner: 2.0 or 1.5mL tubes are completely filled and immediately frozen at -20^{O}C for preservation until DNA extraction is performed, see step 6. Sediment were also analyzed for sediment dating (^{210}Pb), total organic carbon and total carbon content, chlorophyll-a content and 13C and 15N stable isotopes.

eDNA analysis

6 *Laminaria hyperborea* reference sample and core sample DNA extraction

DNA extraction from the core samples as well as from the kelp reference material was performed using

☒ DNeasy PowerSoil Pro Kit

(250) **Qiagen Catalog #Cat No./ID: 47014** with about 250 mg of material (wet weight for the sediment) per sample following the user manual instructions. A final elution volume of 50µL was used. This kit helped mitigate the challenges posed by PCR inhibiting compounds found in environmental samples.

6.1 *L. hyperborea* reference material collection and preservation

Pure reference material was collected from living tangle and sugar kelp biomass near the city of Ålesund at the following geographic positions: **62°42'35.9"N 6°20'33.3"E** on the 12/10/2018. Collected material was preserved dry in silica beads.

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A few g of *L. hyperborea* tissue material preserved in a 15mL Falcon tube filled with Silica gel beads.

7 *Laminaria hyperborea* specific primers & qPCR protocol

The assay was designed to be specific for *L. hyperborea*, with short amplicon, 88bp, to enhance detection sensitivity as DNA fragmentation is expected in sediment eDNA samples. The Bio-Rad SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix kit was chosen for qPCR amplification as it is developed to tolerate inhibitors which are often present in environmental samples and provided best LOD and best specificity when tested in this study. Product specificity of the assay is assessed by using the melt curve analysis to ensure the right melt profile is generated, corresponding to the target amplicon.

A two-step amplification protocol was used with an initial denaturation step at 98 ^{O}C for 2 minutes followed with 45 cycles, 5 seconds 98 ^{O}C denaturation and 20 seconds elongation at 62 ^{O}C. A total mastermix reaction volume of 15 µL was used with 7.5 µL Supermix, with 0.75 µL for each of the two primers (final concentration 0.5 µM), a 1.5 µL sample completed with 4.5 µL sterilized pure water.

Citation: Marc Angles d'Auriac, Kasper Hancke, Hege Gundersen, Helene Frigstad, Gunhild Borgersen (09/01/2021). MarineSediment-BlueC-eDNA. https://dx.doi.org/10.17504/protocols.io.btk4nkyw

A	B	C	D	E	F	G
Target	Species specificity	Primer name	Primers (Forward & Reverse)	Ta. oC	Product (bp)	Reference
cytochrome oxidase subunit-1 (COI)	*Laminaria hyperborea*	L_hyper_coi471F20	CTCCCGGTATGACAATGGAT	62	88	This protocol and associated report
		L_hyper_coi538R21	AAAACAGGAAGCGATAACAGT			

Laminaria hyperborea specific primers

Mastermix:

+ **4.5 µl ddH2O**

+ **0.75 µl Each primer @ [M]10 Micromolar (µM) Primer use solution =**

[M]500 Nanomolar (nM) Final primer concentration

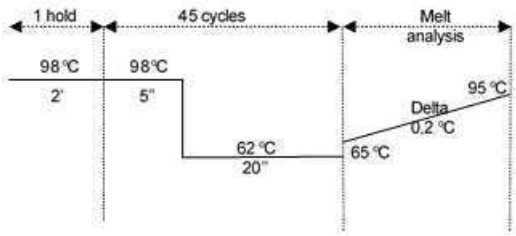
+ **7.5 µl SsoAdvanced MX 2X**

SsoAdvanced™ Universal Inhibitor-Tolerant SYBR® Green Supermix **Bio-rad Laboratories Catalog #1725017**

+ **1.5 µl Sample**

= **15.0 µl Final volume**

CFX96 Touch Real-Time PCR
qPCR
Bio-Rad #1855195



Laminaria hyperborea qPCR cycling conditions using SsoAdvanced Universal Inhibitor-Tolerant SYBR Green Supermix

Usual eDNA precautions were implemented to avoid cross contamination to occur.

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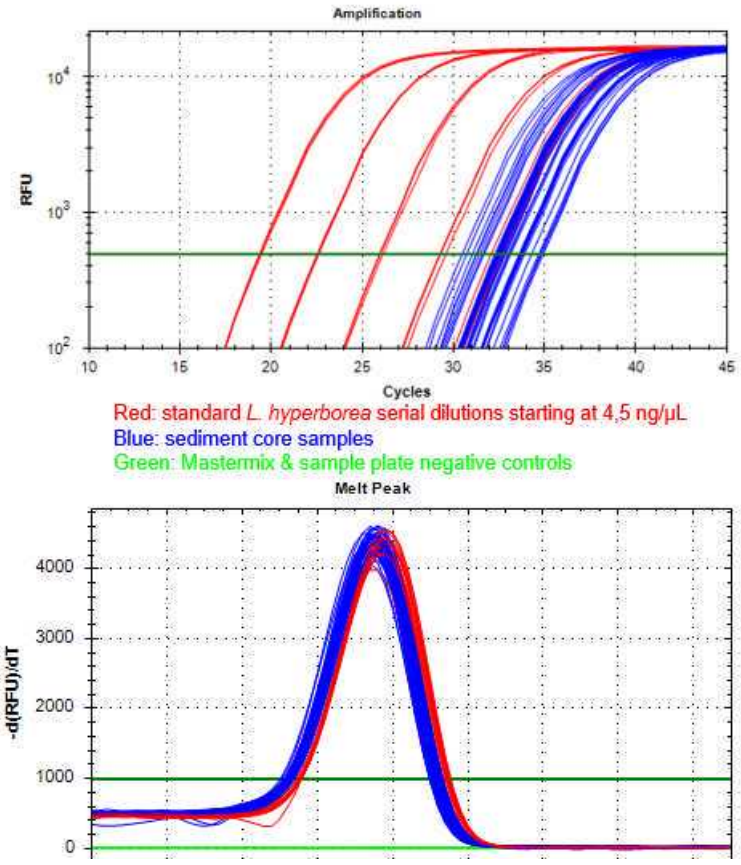
Caren S. Goldberg; Cameron R. Turner; Kristy Deiner; Katy E. Klymus; Philip Francis Thomsen; Melanie A. Murphy; Stephen F. Spear; Anna McKee; Sara J. Oyler-McCance; Robert Scott Cornman; Matthew B. Laramie; Andrew R. Mahon; Richard F. Lance; David S. Pilliod; Katherine M. Strickler; Lisette P. Waits; Alexander K. Fremier; Teruhiko Takahara; Jelger E. Herder & Pierre Taberlet (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods in Ecology and Evolution..
http://10.1111/2041-210X.12595

All laboratory surfaces were wiped with hypochlorite 2% solution to neutralise DNA, followed with a wipe using deionised water to neutralise hypochlorite residues. Cap-strips, rather than films, were used to enable progressive protection of the various plate positions. Reagents and mastermix were prepared and dispatched in the PCR plate in a LAF-bench. Mastermix negative controls, deionized H2O, were dispensed and caped in the LAF-bench. The plate was thereafter transported to the sample laboratory. All field samples were then first dispensed and caped followed by at least 3 sample plate negative controls with deionized water. Finally, the standard samples, also used as positive controls, were dispensed and caped. White PCR plates, rather than see-through PCR plates, were used to provide increased sensitivity. White PCR plates, rather than see-through PCR plates, were used to provide increased sensitivity.

☒ Hard-Shell PCR plate thin wall white **BIO-RAD Catalog #HSP9655**

☒ Optical flat 8-cap strips **BIO-RAD Catalog #TCS0803**



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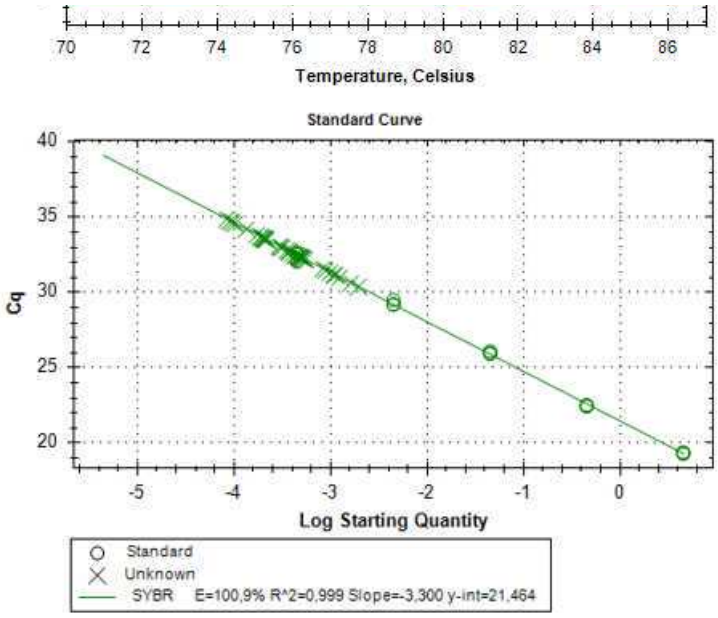

Example of expected results, *L. hyperborea* standard curve (red), eDNA core samples (blue) and negative controls (green).

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