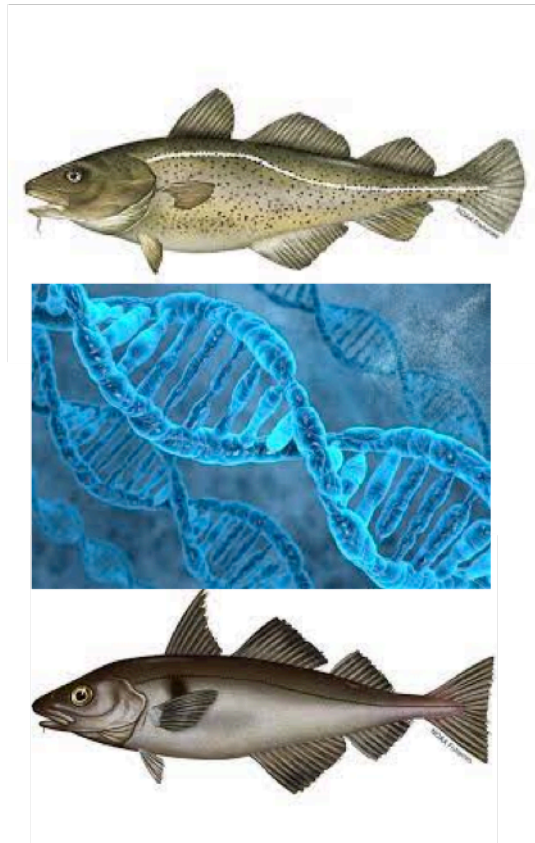


BANK-e-DNA: Assessing the recovery of Cod, Haddock and Saithe on the Faroe Bank using environmental DNA

Havstovan Project: 8195
Fiskivinnuroyndir 18/00652



HAVSTOVAN
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1. Summary

The overall conclusion from Bank-e-DNA is that environmental DNA in water samples can be used to elucidate the biomass of different fish stocks on the Faroe Bank.

The main results of Bank-e-DNA are:

1. It was possible to detect DNA fragments of both Haddock (Hysa, *Melanogrammus aeglefinus*) and Atlantic cod (Toskur, *Gadus Morhua*) in bottom water samples from the Faroe Bank.
2. The detection rate and the concentration of DNA in water samples were higher for haddock than Atlantic cod, consistent with data from the trawl survey showing higher haddock biomass.
3. There were strong seasonal differences, with both detection rates and concentrations higher during the spring Surveys (March) compared to the autumn surveys (September). It is likely that this is due to spawning activity in spring which results in both physical aggregations of fish and release of sexual gamete cells, both of which serve to augment DNA concentrations in the surrounding water.

2. Introduction

The Faroe Bank (FB) is a bathymetric feature SW of the Faroe Islands with a minimum depth of 100m. It is home to what is believed to be a uniquely self-sustained and local cod stock characterised by its own population dynamics, lack of interaction with surrounding cod stocks and individual growth rates that are extremely rapid and amongst the highest of any cod in the North Atlantic. As such it is a potentially valuable commodity for the Faroese fishing economy. The Faroe Bank also hosts other commercially important demersal fish species, including haddock and saithe. Faroe Bank cod represents a distinct biological management unit with respect to stock assessment due to its characteristic stock dynamics. In contrast the haddock

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and saithe found on the Faroe Bank are believed to be mix more extensively with non-bank populations such that they are not regarded as a distinct biological management unit. This has important implications for managing the bank as a mixed demersal fishery if different commercial stocks show variable rate of decline and recovery.

Ongoing demersal survey work on the Faroe bank have shown a serious decline in cod stocks, which appeared to crash around 2005/2006, leading to the closure of the bank to major fishing gears in January 2009. Similarly, the catch per unit effort for both haddock and saithe exhibited a serious decline during the same period (Figure 1). Despite the decision to close the bank to commercial fishing activity, the populations of cod, haddock and saithe have remained very low in the period 2009-2015. However, in the last three years (2015-2018), haddock populations appear to be exhibiting signs of a remarkable recovery, with catch per unit efforts increasing to 1200 kg / hr in 2018, matching the peak observed almost 15 years earlier. During the last year saithe populations also appear to have increased above background levels, although it is too early to diagnose if this is the start of a recovery pattern or an anomalous year. In contrast, populations of cod do not appear to be showing these signs of recovery.

Over the last few years it has recently emerged that environmental DNA may serve as a complimentary tool to understand patterns in the spatial and temporal distribution of fish biomass. It is increasingly recognised by ICES, the EU, and several national monitoring programmes as an important method for studying mixed fisheries and their corresponding management policies.

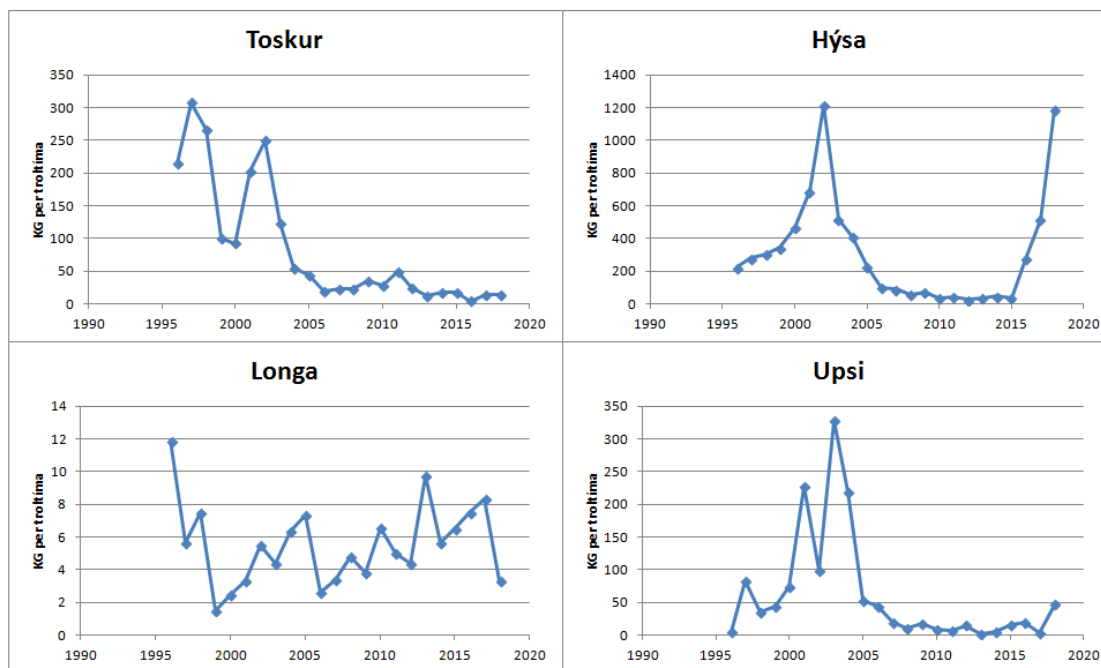


Figure 1: Temporal variability in cod, haddock and saithe populations from the summer ground survey conducted on the Faroe Bank. The catch per unit effort indices that show a significant decline in cod, haddock and saithe around 2005-2006 that have subsequently remained low for the best part of a decade. In 2015 the haddock population shows significant recovery, saithe appears to have started a recovery whilst cod stocks remain low. *Data courtesy of Petur Steingrund and Luis Cruz (Havstovan).*

Just like criminals that leave behind their DNA at the scene of a crime, fish also leave traces of their DNA in the ocean. Fish constantly exude DNA into the environment through a number of pathways that includes sloughed epithelial cells, intestinal cells, faeces and urine production (Thomson and Willerslev, 2015). This so-called environmental DNA has the potential to record patterns on the size and timing of commercial stocks passing through specific fishing grounds.

In a previously funded project by Fiskivinnuoyndir (Toska-u-DNA / Code-DNA mal: 16/00642-2) we have pioneered the approach to combine environmental DNA seawater sampling with quantitative PCR to map the spatial distribution of Atlantic cod biomass around the Faroe Islands (Salter et al. 2019). The detection of cod DNA fragments in bottom water samples shows similar spatial patterns to

detection in standard trawl surveys (Figure 2a and 2b) and the concentration of cod DNA fragments is highest in the spawning areas where highest cod biomass was recorded from trawl data (Figure 2c and 2d).

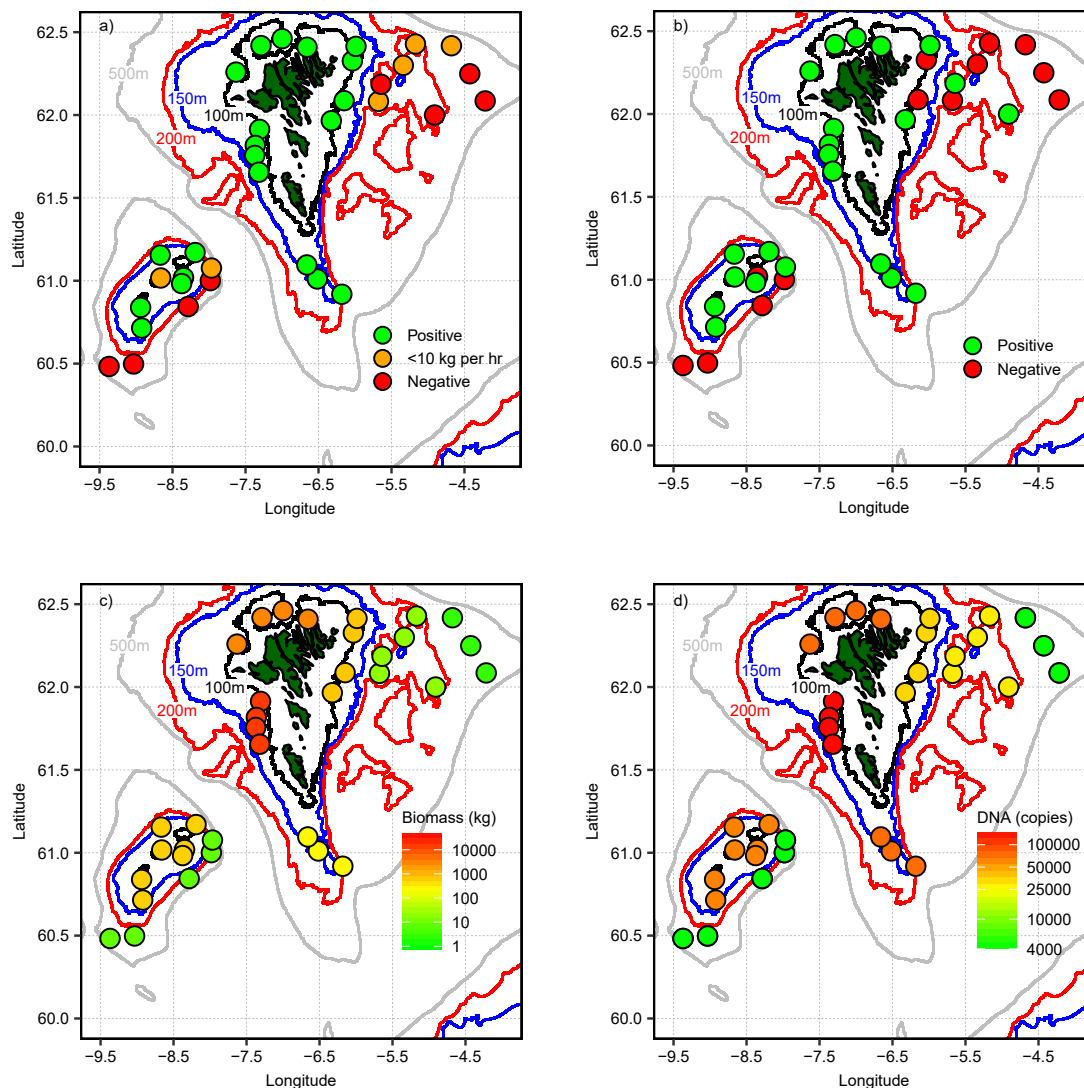


Figure 2 Regional detection rates and quantities from demersal trawl and eDNA survey. Panel (a) shows the detection of Atlantic cod from demersal trawl survey. Green and red filled circles denote positive and negative detection, respectively. Orange filled circles show positive detection at a biomass of $<10 \text{ kg h}^{-1}$. Panel (b) shows positive and negative detections of Atlantic cod from environmental DNA samples. Green and red filled circles show positive and negative detections, respectively. Panel (c) shows region quantities of Atlantic cod from demersal trawl survey. Values represent the sum of biomass within each region; data is expressed logarithmically. Panel (d) shows region quantities of cod eDNA copies.

The integration of this data demonstrates that there is a statistically positive relationship between the concentration of environmental DNA of Atlantic cod in seawater and catch per unit effort as determined from standardized demersal trawl surveys. These results indicate that environmental DNA can map the spatial distribution of Atlantic cod around the Faroe Islands across several orders of magnitude. Substituting space for time therefore suggests that it is theoretically possible to follow temporal fluctuations in the biomass of a commercially relevant demersal fish target from analysing residual DNA fragments in seawater. However, it remains unclear whether these preliminary findings can be applied to different species and to what degree seasonal variation plays a role. It is well known that the degradation of environmental DNA in seawater can exhibit strong temporal variability depending on environmental factors (Salter, 2018).

3. Objectives

The overall objective of Bank-e-DNA is to apply the proven eDNA approach to address the quantification of haddock and saithe biomass on the Faroe Bank, in addition to the work recently carried out for cod. Given the variable patterns of recovery displayed by these three commercially important stocks on the Faroe Bank, the project aims to address the hypothesis that eDNA concentrations in the water display a temporal variability that matches the patterns in biomass obtained from trawl surveys. If it can be proven that the eDNA approach can successfully document patterns in stock recovery and decline in mixed demersal fisheries it will be an important step forward. Given the observed differences in the patterns of haddock, saithe and cod recovery on the Faroe bank in 2018, the timing to carry out such a project is critical.

In order to meet the overall project objective, three specific project aims have been identified:

1. Apply a qPCR-eDNA assay for haddock and saithe
2. Compare the relationships between eDNA and biomass for cod, haddock and saithe on the Faroe Bank

3. Establish whether eDNA concentrations in the water change over time as a function of stock recovery.

4. Approach

As explained above the original objective of Bank-e-DNA was to examine the ability of environmental DNA to describe variations in the biomass of cod, haddock and Saithe. Unfortunately the project was not granted full funding. The original application requested an amount of 540355 DKK over a 2-year period (2019 and 2020), but was granted 447500 DKK. The reduction of 95855 DKK precluded the possibility of performing the study for cod, haddock and saithe and so it was conducted for cod and haddock only.

The project combined environmental DNA samples collected on the Faroe Bank as part of a previously funded project (toska-u-DNA) that was carried out with Magnus Heinason in 21st-28th March 2018 (Tur 1810) and 5th-11th September 2018 (1840) with new samples collected explicitly as part of bank-e-DNA. These samples were collected during two demersal trawl surveys carried out by Magnus Heinason in 2019: Tur 1908 and Tur 1938.

(i) Tur 1908

Tur 1908 was the spring demersal trawl survey on the Faroe Bank, which took place between 20th March – 27th March 2019 on board Magnus Heinason. The weather was problematic during the trip and 15 of the 29 planned trawl stations were successfully carried out. They were conducted mainly in the shallow area (< 200m), which is the basis of the environmental DNA comparison. As part of the survey, 16 CTD stations were carried out immediately prior to the trawl stations to collect bottom water samples for environmental DNA. The CTD stations were carried out immediately prior to the trawl commencing to minimise contamination from disturbed sediments and large quantities of fish present on the trawl deck during sampling. Upon recovery, water samples were taken from the Niskin bottles on the CTD, filtered onto 0.2 um Sterivex filters and stored frozen for DNA extraction at iNOVA.

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(ii) *Tur 1938*

Tur 1908 was the autumn demersal trawl survey on the Faroe Bank, which took place between 4th September – 11th September 2019 on board Magnus Heinason. The weather was very good during the trip all 29 of the planned trawl stations were successfully carried out. As part of the survey, 27 CTD stations were carried out, 19 of which were used for Bank-e-DNA. Immediately prior to the trawl stations to collect bottom water samples for environmental DNA. The CTD stations were carried out immediately prior to the trawl commencing to minimise contamination from disturbed sediments and large quantities of fish present on the trawl deck during sampling. Upon recovery, three replicate water samples were taken from the Niskin bottles on the CTD, filtered onto 0.2 um Sterivex filters and stored frozen for DNA extraction at iNOVA.

Table 1 CTD stations for bank-e-DNA samples from Tur 1908. MQ Blank corresponds to blank water samples that were taken on board to check for contamination of the work area and filtration equipment.

Dato	Støð nr	Botndypi (m)	Prøva dypi (m)	DNA prøva nr
21/03/19	19080001	148	142.1	F1-F3
21/03/19	19080003	96	83	F4-F5
21/03/19	19080006	100	87.7	F7-F9
21/03/19	19080009	108	100.6	F10-F12
25/03/19	19080012	110	101.1	F14-F16
25/03/19	19080014	102	93.7	F17-F19
25/03/19	19080016	105	97	F20-F22
25/03/19	19080018	140	127	F23-F25
25/03/19	19080020	125	119	F26-F28
26/03/19	19080022	140	130	F31-F33
26/03/19	19080025	208	196	F34-F36
26/03/19	19080027	194	182	F37-F39
26/03/19	19080030	118	108	F40-F42
25/03/19	MQ Blank	na	na	F13
25/03/19	MQ Blank	na	na	F29
26/03/19	MQ Blank	na	na	F30
26/03/19	MQ Blank	na	na	F43

Table 2 CTD stations for bank-e-DNA samples from Tur 1938. MQ Blank corresponds to blank water samples that were taken on board to check for contamination of the work area and filtration equipment.

Dato	Støð nr	Botndypi (m)	Prøva dypi (m)	DNA prøva nr
05/09/19	1938-0001	114	105.2	H1-H3
05/09/19	1938-0003	95	86.8	H4-H6
05/09/19	1938-0006	109	102	H7-H9
06/09/19	1938-0015	130	122	H11-H13
06/09/19	1938-0017	95	86	H14-H16
06/09/19	1938-0019	104	97.3	H17-H19
06/09/19	1938-0021	100	91	H20-H22
07/09/19	1938_0026	135	129	H24-H26
08/09/19	1938-0034	246	237	H27-H29
08/09/19	1938-0036	134	127	H30-H32
08/09/19	1938-0038	124	118	H33-H35
08/09/19	1938-0040	104	95	H37-H39
08/09/19	1938-0042	141	133	H41-H43
08/09/19	1938-0044	132	125.1	H44-H46
09/09/19	1938-0045	109	106	H48-H50
09/09/19	1938_0048	211	207	H51-H53
09/09/19	1938-0050	180	177	H54-H56
09/09/19	1938-0052	194	191	H57-H59
09/09/19	1938-0055	138	133	H62-H64
05/09/19	MQ Blank	na	na	H10
06/09/19	MQ Blank	na	na	H23
08/09/19	MQ Blank	na	na	H40
08/09/19	MQ Blank	na	na	H47
09/09/19	MQ Blank	na	na	H60

Upon return to the laboratory the DNA water samples were extracted in sterile laboratory conditions according to the protocols developed during Toska-u-DNA. Subsequently the samples were analysed by quantitative polymerase chain reaction (qPCR) targeting both Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) and checking for inhibition of samples using an internal DNA marker and primer. The sampling, extraction and analysis protocols used are those that were developed for Atlantic cod in a previous project (Toska-u-DNA 16/00642) were

applied for haddock as part of the current project bank-e-DNA). Full details of the methods can be found in the publication Salter et al. 2019.

5. Results

The trawl survey indicated that the biomass of cod (Toskur) on the Faroe Bank during spring was low (Figure 3a). Compared to the peak of 1400 kg hr⁻¹ in 2004, the Catch Per Unit Effort (CPUE) was only 35 kg hr⁻¹ (29 stations) in 2018 and 97 kg hr⁻¹ (15 stations) in 2019. This continues the trend of chronically low biomass since the crash in 2006. Similarly, during the autumn survey (Figure 3b) cod biomass was very low in 2018 (15 kg hr⁻¹, 29 stations). However, in 2019 there was an approximate six-fold increase to 80 kg hr⁻¹, 29 stations).

In contrast, the trawl survey indicated that the biomass of haddock (hysa) on the Faroe Bank during spring continued to increase (Figure 3c). In 2018, CPUE was 565 kg hr⁻¹, increasing further to 1204 kg hr⁻¹ in 2019. Similarly in the autumn survey, hysa CPUE reached approximately 1200 kg hr⁻¹ in both 2018 and 2019 (Figure 3d), which is among the highest biomass recorded on the Faroe Bank (2002), since the continued standardized survey began in 1997. All data are summarised in Table 1.

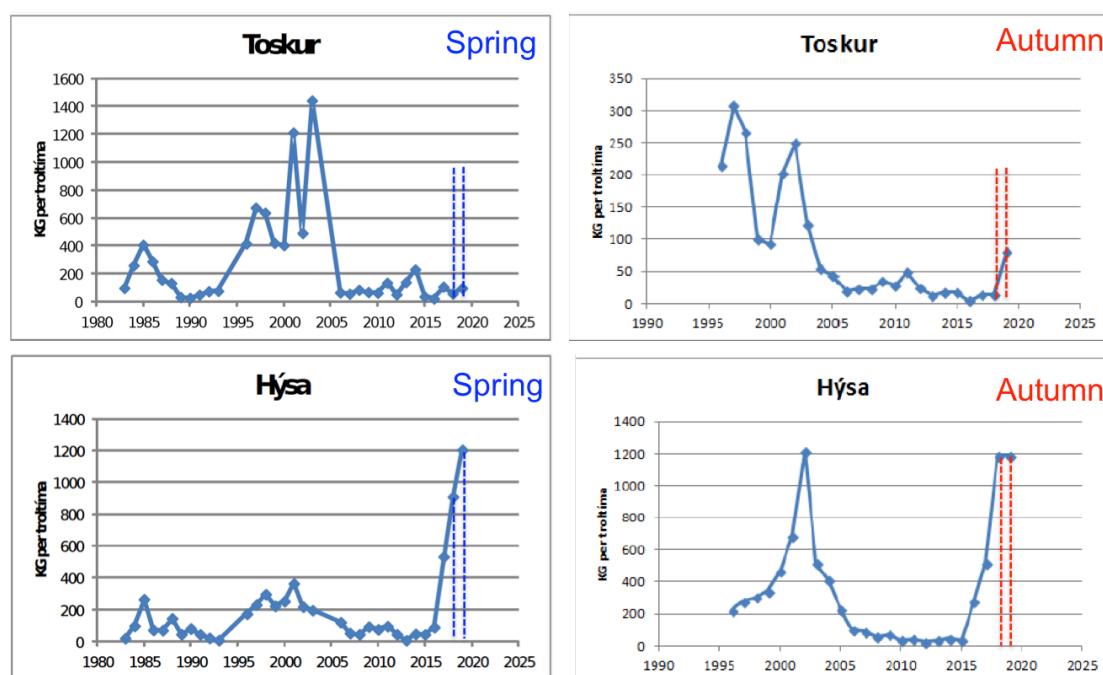


Figure 3 Time-series of Catch per unit effort (CPUE) in kg hr⁻¹ on the Faroe Bank from demersal trawl survey in spring and autumn for Atlantic cod (Toskur) and haddock (Hysa).

Table 1 Summary of biomass of Atlantic cod (Toskur) and haddock (Hysa) caught during the demersal trawl survey on the Faroe Bank during spring and summer/autumn periods. The total catch (kg) is divided by the number of trawl stations (1 hr each) to derive the CPUE in kg hr⁻¹.

Year	Season	Fish	Nr Trólstød	Total Catch (kg)	CPUE (kg hr ⁻¹)
2018	Spring	Toskur	29	1039	36
2018	Spring	Hysa	29	16381	565
2019	Spring	Toskur	15	1466	98
2019	Spring	Hysa	15	18064	1204
2018	Autumn	Toskur	29	431	15
2018	Autumn	Hysa	29	34541	1205
2019	Autumn	Toskur	29	2347	80
2019	Autumn	Hysa	29	34518	1190

The environmental DNA results are summarised in Table 2, separated into sub-tables (a-d) for each trip. Target DNA from both atlantic cod and haddock was successfully amplified from water samples from all trips, and there was significant spatial, seasonal and interannual variability

In spring 2018 (Tur 1810), the proportion of replicates in which cod DNA was positively detected ranged from 0-1. Integrating all the samples together the proportion of all samples that amplified was 0.54. The average concentration of cod eDNA in samples was 30 copies / μ L, ranging from 0-145 copies / μ L.

In summer 2018 (Tur 1840), the proportion of replicates in which cod DNA was positively detected ranged from 0-0.5. Integrating all the samples together the proportion of all samples that amplified was 0.08. The average concentration of cod eDNA in samples was 7 copies / μ L, ranging from 0-100 copies / μ L.

Table 2 Environmental DNA results for spring 2018 (2a), summer 2018 (2b), autumn 2018 (2c) and autumn 2019 (2d). Positive column is the proportion of replicates at each station that tested positive for the presence of target (cod or haddock) DNA in bottom water samples. Total is the proportion of all replicates across the survey that tested positive. [DNA] is the concentration of target (copies / μL) at each station. Total [DNA] is the summed concentration of target copies (copies / μL) and average is the total divided by the number of stations occupied.

Table 2a

1810 spring 2018	Toskur (cod)		Hysa (haddock)	
Station	Positive	[DNA]	Positive	[DNA]
18100001	1.00	47	0.75	49
18100005	0.75	45	1.00	90
18100008	1.00	145	0.75	31
18100013	0.50	20	0.75	24
18100016	0.50	10	1.00	34
18100018	0.50	25	1.00	218
18100021	0.75	53	1.00	135
18100027	0.25	4	0.75	20
18100032	0.25	13	0.75	33
18100034	0.00	0	0.75	26
18100036	0.50	12	0.75	30
18100041	0.25	0	0.00	0
18100043	0.50	14	1.00	57
18100046	0.75	32	0.50	5
Total	0.54	418	0.77	753
Average		29.8		54

In spring 2019 (Tur 1908), the proportion of replicates in which cod DNA was positively detected ranged from 0-1. Integrating all the samples together the proportion of all samples that amplified was 0.56. The average concentration of cod eDNA in samples was 125 copies / μL , ranging from 0-581 copies / μL .

In summer 2019 (Tur 1938), the proportion of replicates in which cod DNA was positively detected ranged from 0-0.5. Integrating all the samples together the proportion of all samples that amplified was 0.13. The average concentration of cod eDNA in samples was 16 copies / μL , ranging from 0-241 copies / μL .

Table 2b

1840 autumn 2018	Toskur (cod)		Hysa (haddock)	
Station	Positive	[DNA]	Positive	[DNA]
18400001	0.125	3	0.50	23
18400003	0.01	0	0.25	5
18400007	0.01	0	0.63	21
18400009	0.01	0	0.50	16
18400011	0.01	0	1.00	103
18400015	0.5	100	1.00	228
18400016	0.01	0	0.88	29
18400018	0.01	0	0.88	38
18400020	0.5	18	1.00	513
18400025	0.01	0	0.75	180
18400027	0.01	0	1.00	191
18400035	0.01	0	1.00	38
18400037	0.01	0	1.00	55
18400039	0.25	2	0.75	40
18400043	0.01	0	0.88	61
18400044	0.01	0	1.00	85
18400046	0.01	0	1.00	358
18400048	0.01	0	1.00	12
18400050	0.01	0	1.00	5064
Total	0.08	124	0.84	7059
Average		7		372

In spring 2018 (Tur 1810), the proportion of replicates in which haddock DNA was positively detected ranged from 0-1. Integrating all the samples together the proportion of all samples that amplified was 0.77. The average concentration of haddock eDNA in samples was 54 copies / μL , ranging from 0-218 copies / μL .

In summer 2018 (Tur 1840), the proportion of replicates in which haddock DNA was positively detected ranged from 0.25-1. Integrating all the samples together the proportion of all samples that amplified was 0.84. The average concentration of haddock eDNA in samples was 372 copies / μL , ranging from 5-5064 copies / μL .

Table 2c

1908 spring 2018	Toskur		Hysa	
Station	Positive	[DNA]	Positive	[DNA]
1908_0001	0.01	0	0.63	27
1908_0003	0.01	0	0.88	23
1908_0006	0.375	7	1.00	200
1908_0009	0.625	23	1.00	90
1908_0012	0.5	16	1.00	72
1908_0014	0.875	47	1.00	52
1908_0016	0.75	42	1.00	199
1908_0018	0.125	1	1.00	153
1908_0020	0.5	94	1.00	2146
1908_0022	1	135	1.00	201
1908_0025	1	421	1.00	551
1908_0027	0.5	581	1.00	698
1908_0030	1	258	1.00	334
Total	0.56	1625	0.96	4746
Average		125		365

In spring 2019 (Tur 1908), the proportion of replicates in which haddock DNA was positively detected ranged from 0.63-1. Integrating all the samples together the proportion of all samples that amplified was 0.96. The average concentration of haddock eDNA in samples was 365 copies / μL , ranging from 23-2146 copies / μL .

In summer 2019 (Tur 1938), the proportion of replicates in which haddock DNA was positively detected ranged from 0.88-1. Integrating all the samples together the proportion of all samples that amplified was 0.97. The average concentration of haddock eDNA in samples was 75 copies / μL , ranging from 10-582 copies / μL .

Table 2d

1938 autumn 2019	Toskur		Hysa	
Station	Positive	[DNA]	Positive	[DNA]
1938-0001	0.125	4	0.88	21
1938-0003	0.01	0	1.00	15
1938-0006	0.01	0	0.88	14
1938-0015	0.25	11	1.00	13
1938-0017	0.01	0	1.00	22
1938-0019	0.01	0	1.00	15
1938-0021	0.01	0	1.00	21
1938_0026	0.5	241	0.88	64
1938_0034	0.01	0	1.00	10
1938_0036	0.01	0	0.88	15
1938_0038	0.01	0	1.00	25
1938_0040	0.01	0	1.00	43
1938_0042	0.01	0	1.00	278
1938_0044	0.75	27	1.00	582
1938_0045	0.5	12	1.00	22
1938_0050	0.01	0	1.00	71
1938_0052	0.01	0	1.00	67
1938_0055	0.01	0	1.00	46
Total	0.13	296	0.97	1342
Average		16		75

The proportion of samples that analysed positively for haddock was consistently higher (0.77-0.96) than that of Atlantic cod (0.08-0.56), and similar between spring (0.77-0.96) and summer (0.84-0.96). In contrast, the proportion of samples that analysed positively for Atlantic cod was considerably larger in spring (0.54-0.56) compared to summer (0.08-0.13) (**Figure 4**). There was a positive relationship between the average concentration of target DNA in water samples and Catch Per unit Effort as recorded in the trawls (**Figure 5**).

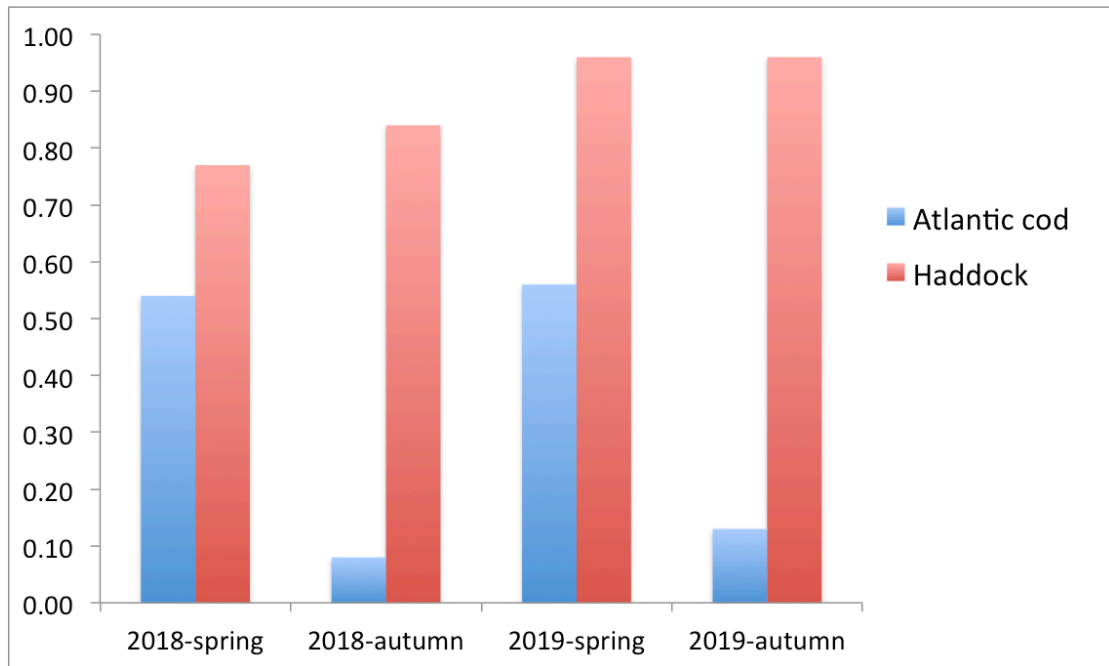


Figure 4 Proportion of replicates that target DNA was detected for Atlantic cod and haddock across different seasons and years.

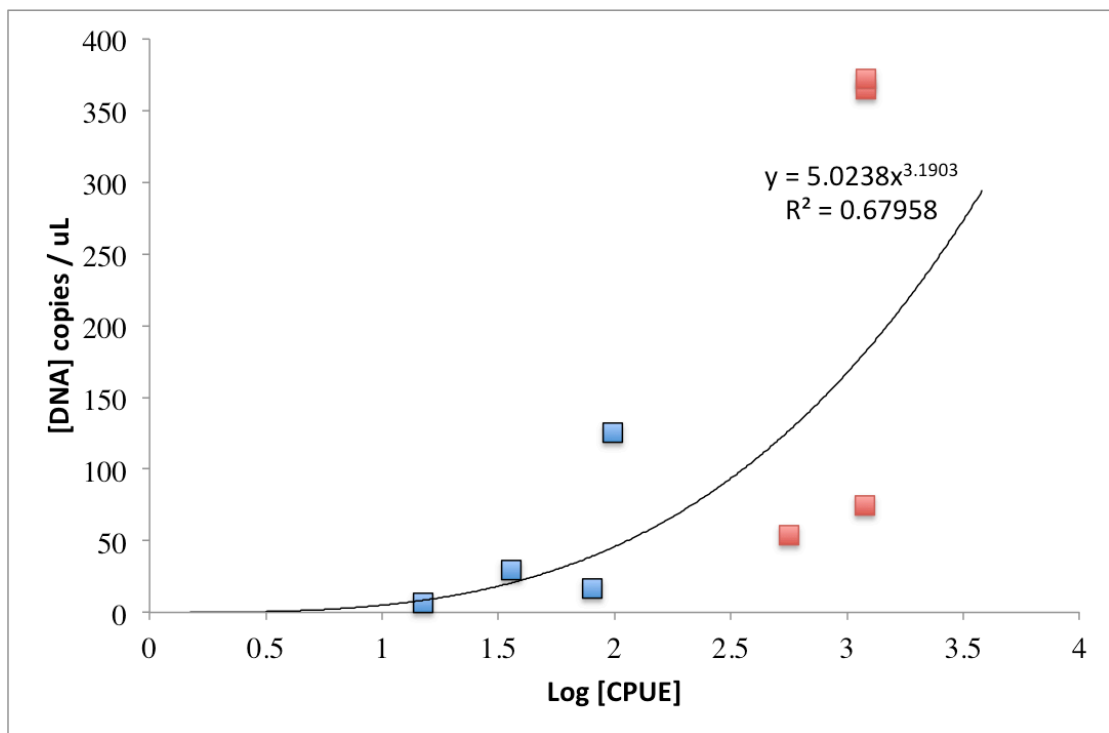


Figure 5 Synthesis of results. The relationship between the catch per unit effort of Atlantic cod (blue) and haddock (red) and the concentration DNA copies in seawater. Catch per unit effort (kg hr⁻¹) is the log transformed.

6. Discussion

The project Bank-e-DNA has been successfully executed. The work plan has been executed as described in the original application and the objectives have been addressed. The key difference between the project plan and the project results is the absence of data for Saithe. As explained above, Saithe as a target species was removed from the project plan due to bank-e-DNA only receiving partial funding.

Objective 1: Apply a qPCR-eDNA assay for haddock and saithe

During the project we successfully adapted the methodologies developed during cod-e-DNA and applied it to a new target species; haddock. We tested this methodology and archived samples from 2018 and newly collected samples from 2019 and could demonstrate the successful amplification of haddock environmental DNA from oceanic samples.

Objective 2: Compare the relationships between eDNA and biomass for cod, haddock and saithe on the Faroe Bank

During the project we planned and implemented the sampling of environmental DNA for two demersal survey cruises that took place in March 2019 (Tur 1908) and Septemer (2019). The project lead planned, organised and participated in the survey cruises ensuring that the maximum number of water samples could be collected to compare with trawl data. The participation of the project lead also assured that careful and clean procedures were followed to ensure the samples were of the highest quality possible. Upon return to the laboratory the samples were extracted for DNA and analysed by qPCR facilitating a detailed comparison of environmental DNA concentrations and biomass from trawl surveys.

Objective 3: Establish whether eDNA concentrations in the water change over time as a function of stock recovery

The overall findings from bank-e-DNA support the conceptual approach that as fish biomass increases in a survey area, there is a corresponding increase in both the proportion of positive samples and concentration of DNA fragments in seawater. In the present project this is best demonstrated by the comparison of eDNA and biomass

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relationships between cod and haddock, which have showed variable recovery patterns during the project period.

Haddock biomass is now considerably higher on the Faroe Bank than Atlantic cod, and this corresponds to a higher proportion of positive samples and higher average concentrations of haddock DNA in bottom water samples. These data provide further support to the idea developed in a previous project (Salter et al. 2019) that statistically significant and quantitative relationships exist between oceanic DNA concentrations and the size of a demersal fish stock.

An interesting observation from bank-e-DNA was the seasonal differences in DNA concentrations between Atlantic cod and haddock. Cod eDNA was typically present at higher concentrations in spring, despite relatively similar biomass metrics between spring and summer. Our interpretation is that this related to physical aggregations and release of gametes into the water that characterise spawning activity. This appears to elevate eDNA concentrations in Spring despite relatively low biomass. In contrast, summer biomass is similar but the fish are distributed over a wider area and unlikely to be spawning. This raises some interesting issues regarding using eDNA as a survey tool when comparing between different times of year. It also indicates that eDNA could itself be applied as a spawning index if molecular markers that distinguish between gametes and somatic cells were applied.

In contrast haddock eDNA concentrations were found to be high in both spring and autumn, although there was some variability between the study years 2018 and 2019 that remains unexplained. These data would suggest that when biomass of a fish stock increases, processes other than spawning activity become important for generating eDNA particles in surrounding water samples. It is possible that size structure of the fish stock is relevant and allometric scaling may further define these relationships. In order to assess this properly it will be necessary to follow the relationships between stock fluctuations and eDNA concentrations over a longer time period, whilst factoring in other features of the stock such as age distribution, size structure and spawning activities. Future projects should place emphasis on clarifying these issues. Environmental DNA remains a promising and exciting approach to

follow the dynamics of commercially important fish stocks in Faroese waters (Ray et al. 2020).

7. References

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