

# Occurrence of pearls in mussels (*Mytilus* spp.) from the Norwegian coast.



# REPORT

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<p>Summary</p> <p>Mussels (<i>Mytilus</i> spp.) from the Norwegian coast produce pearls, up to as many &gt;360 pearls per individual, and it seems to be a southern-northern gradient with more pearls in mussels from the south than from the north. Out of the 280 mussels studied, nearly 2000 pearls were found, and the mussels producing pearls were all &gt;4 cm. Size and condition index did correlate with the pearl frequency, while microplastics did not. Mussel health is important to study as they are important actors in the coastal ecosystem, as bioindicators for environmental monitoring, as a food source as well as the recent reports on changes in their distribution across the Norwegian coast.</p>
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This report is quality assured in accordance with NIVA's quality system and approved by:

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# Table of contents

<b>1</b>	<b>Introduction .....</b>	<b>9</b>
1.1	Scope of the report .....	9
1.2	Pearl formation in molluscs with focus on mussels and oysters .....	9
1.3	Microplastics and pearls in <i>Mytilus</i> spp. from the Norwegian coast.....	10
<b>2</b>	<b>Methods.....</b>	<b>11</b>
2.1	Sampling.....	11
2.2	Sample processing and analysis.....	11
2.3	Statistical analysis .....	11
<b>3</b>	<b>Results and Discussion.....</b>	<b>12</b>
3.1	Pearl occurrence in <i>Mytilus</i> spp. from the Norwegian coast.....	12
3.2	Size, condition index and microplastics as possible parameters correlating with pearl formation in <i>Mytilus</i> spp. ....	17
3.3	Possible implications of pearl production in <i>Mytilus</i> spp. ....	20
<b>4</b>	<b>References .....</b>	<b>22</b>

## Summary

Pearls, composed mainly of calcium carbonate ( $\text{CaCO}_3$ ), are formed by mollusc species that contains an inner layer of nacre, also termed *mother-of-pearl*. Pearls formation is thought to be a defence system against irritants, such as parasites and/or particles, with pearls being secreted from the nacre to encapsulate the irritant. The pearl oyster pearl industry has used this to their advantage to create artificial pearls, while pearls in wild mussels (*Mytilus* spp.) are much less studied. Mussels, like other molluscs, are thought to produce pearls to neutralise possible threats, but which threats and pearl inducing stimuli that are most important, is not well understood. Extensive pearl formation is likely to be a metabolic cost for the mussel individuals, and it has been hypothesised that it can impair the mussel's energy reserves. Parasite infection from trematodes have been identified as a pearl inducing stimuli in mussels, but other causes such as particulates (e.g. sand) may also represent additional stimulants. With growing concern surrounding microplastics in the marine environment as an additional stressor to marine biota together with e.g. ocean acidification, environmental pollutants and increasing sea temperatures, understanding how these factors impact marine organisms should be prioritized. Mussels along the Norwegian coast are monitored annually for the impact of inorganic and organic contaminants. Microplastics are not currently included in this monitoring program for the time being, but some preliminary studies have been conducted on the occurrence of microplastics in Norwegian mussels (Lusher et al. 2017; Bråte et al. 2018). To date these preliminary investigations have found microplastics in individuals from almost all sites around the coast. It was also observed that pearls were frequently found alongside microplastic particles. This report aims to investigate pearl abundance in mussels from the Norwegian coast, and to see whether size, condition of the mussels or microplastic content are correlating with pearl occurrence. No other explanatory variables have been considered, such as as water parameter (i.e. pH, temperature, turbidity), persistent organic pollutants (POPs), proximity to city, and substrate etc.

A total of 280 mussels were studied for pearl occurrence, with 20 individuals from each site. Pearls were found in Norwegian mussels from 10 out of 14 sites, with most pearls being found in mussels from the inner Oslofjord. No pearls were found in mussel populations from Færder, Bodø, Tromsø and Skallneset. A total of 1937 pearls were found in 36% of the mussels analyzed with an average number of 7 ( $\pm$  31) pearls per individual. The number of pearls per individual varied between location from 0 pearls to a maximum of 362 pearls. A retrospective statistic power analysis found that based on the differences in the pearl mean between the 14 groups and the total variance (SD), the samples size was sufficient (power >0.8) to find any significant differences for pearl occurrence.

Pearls varied in size (0.07 to 2.50 mm), colour (white to blue) and shape (spherical, irregular, droplet, fused). It is likely that this diversity is related to a similar diversity in nuclei. Location and mussel size did influence the presence of pearls, whilst microplastic occurrence did not. A south-north gradient appears to exist in this survey; mussels from southern populations contained more pearls than northern populations, with exception to mussels from Færder that did not contain any pearls. It is currently unknown what is causing such a possible trend and should be further assessed, but difference in degree of parasite infection and water parameters (such as particle exposure) might explain this trend. Pearls were only found in mussels > 4 cm, and with exception to mussels from Tromsø (n=20), pearls were found in at least one individuals from all other locations where mussels were larger than 4 cm.

Microplastic occurrence in mussels does not appear to influence pearl production in Norwegian mussels. No significant correlation between number of microplastics detected in mussels and

number of pearls detected in mussels was identified. However, any pearl and microplastic relationship should be further investigated since it is unknown if pearls can embed microplastics.

In general, pearl formation in mussels should be further investigated, to decide whether pearl occurrence is a useful environmental parameter in assessing the degree of anthropogenic impact to mussels, given the ecological importance of mussels in the coastal zone, the recent reports on their change in abundance, their suitability as a sentinel species for monitoring as well as their importance as a food resource.

# Sammendrag

Tittel: Tilstedeværelsen av perler i blåskjell (*Mytilus* spp.) fra Norskekysten

År: 2018

Forfatter(e): Inger Lise Nerland Bråte, Nina Buenaventura, Amy Lusher

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Alle bløtdyr som har en perlemorhinne, også kalt nacre, kan i teorien produsere perler. Disse perlene består i hovedsak av kalsiumkarbonat/kalk ( $\text{CaCO}_3$ ). Perledannelse er trolig en forsvarsmekanisme mot irriterende substanser, som for eksempel parasitter og/eller partikkeleksponering, hvor objektet antakelig blir innkapslet i perlen. Perleindustrien har brukt denne kunnskapen til deres fordel for å produsere kunstige perler i østers, mens mye mindre kunnskap eksisterer angående perledannelse i blåskjell (*Mytilus* spp.). Blåskjell, som andre bløtdyr, antas å produsere perler for å nøytralisere mulige trusler, men nøyaktig hvilke trusler og perleinduserende stimuli som er viktigst, er fortsatt usikkert. Dersom et blåskjell produserer store mengder perler innebærer dette trolig en metabolsk kostnad for blåskjellet, og det har blitt framsatt hypoteser om at det kan påvirke energireservene deres.

Parasittinfeksjon fra trematoder har blitt identifisert som en perleinduserende stimulus i blåskjell, men andre årsaker som partikkeleksponering (for eksempel sand) kan muligens også representere ytterligere årsaker. Det er en økende bekymring for effekter av mikroplast på havmiljøet sammen med andre stressfaktorene som marin biota utsettes for, som havforsuring, miljøgifter og økende havtemperaturer, og kunnskap om hvordan dette påvirker dyrelivet bør prioriteres. Årlig overvåkes blåskjell langs norskekysten for å studere påvirkningen av uorganiske og organiske miljøgifter. Mikroplast er foreløpig ikke inkludert i dette overvåkingsprogrammet, men det finnes innledende studier på forekomsten av mikroplast i norske blåskjell (Lusher et al. 2017; Bråte et al. 2018). Mikroplast ble funnet i skjell fra nesten alle stasjonene som ble studert langs kysten. I forbindelse med disse studiene ble perledannelse ofte observert sammen med mikroplastpartiklene. Formålet med denne rapporten er å beskrive forekomsten av perler i blåskjell fra norskekysten, og undersøke om blåskjellets størrelse (og dermed alder), kondisjonen eller mengden mikroplast korrelerer med perleforekomsten. Begrensede faktorer er at ingen andre forklarende variabler har blitt vurdert, som for eksempel ulike vannparameter (f.eks. pH, temperatur, turbiditet), miljøgifter, grad av urbanisering eller substrat de vokser på.

Totalt ble 280 blåskjell undersøkt for tilstedeværelse av perler fordelt på 20 individer per stasjon. Perler ble identifisert i blåskjell fra 10 av 14 stasjoner fra norskekysten, hvor flest perler ble funnet i blåskjellpopulasjoner fra Indre Oslofjord. Ingen perler ble funnet i skjell fra Færder, Bodø, Tromsø og Skallneset. I alt ble det identifisert 1937 perler, og disse perlene ble funnet i 36% av de analyserte individene. Dette resulterte i et gjennomsnittlig antall perler på 7 ( $\pm 31$ ) per blåskjell. Antallet perler varierte fra 0 til maksimalt 362 perler per individ. En retrospektiv statistisk «power» analyse ble utført basert på forskjellene mellom perle-gjennomsnittet funnet i de 14 ulike gruppene sammen med den totale variansen (SD), og antallet blåskjell undersøkt (20 per stasjon) var nok for å finne eventuelle signifikante forskjeller med høy nok sikkerhet (power >0.8).

Perlene varierte i størrelse (0,07 til 2,50 mm), farge (hvit til blå) og form (sfærisk, uregelmessig, dråpeformede, og sammenvokste perler). Sannsynligvis representerer dette mangfoldet et lignende mangfold når det kommer til nukleusen, årsaken, av perlene. Basert på denne studien ser det ut som blåskjellets plassering (stasjon) og størrelse påvirket tilstedeværelsen av perler, mens mengden

mikroplast derimot ikke påvirket mengden perler. Det så også ut til å være en sør-nord-gradient; blåskjell fra sørlige populasjoner inneholdt flere perler enn nordlige populasjoner, med unntak av blåskjell fra Færder hvor ingen av blåskjellene inneholdt perler. Grunnen til denne mulige trenden er uklar og mer forskning trengs, men forskjeller i grad av parasittinfeksjon og vannparametere (som temperatur eller partikkeleksponering) kan forklare denne trenden. Perler ble bare funnet i skjell > 4 cm, og med unntak av skjell fra Tromsø (n = 20) ble perler funnet i minst et individ fra alle andre steder hvor muslinger var større enn 4 cm.

Det ble ikke funnet noen signifikant korrelasjon mellom mengden perler og mikroplast i norske blåskjell. Det bør midlertidig undersøkes nærmere om det er noen interaksjon mellom perler og mikroplast da det er ukjent om perler har evnen til å kapsle inn mikroplast.

Perledannelse i blåskjell bør undersøkes videre for å avgjøre om perledannelse kan være en nyttig miljøparameter for å forstå den totale antropogene påvirkningen blåskjellet utsettes for, gitt den økologiske betydning av blåskjell i kystnære strøk, den sentrale rollen den har som bioindikator i miljøovervåking og som matressurs, og de siste rapportene om endringer i utbredelsen av blåskjell.



# 1 Introduction

## 1.1 Scope of the report

Lusher et al. 2017 revealed the frequent presence of sometimes numerous and small pearls in Norwegian mussels (*Mytilus* spp.) while assessing microplastic occurrence in mussels from the Norwegian environment. Subsequently, the Norwegian Environment Agency (Miljødirektoratet) tasked NIVA to study pearl occurrence in more detail by investigating whether there was any correlation between the number of pearls and the number of microplastics identified in mussels. Site, size of mussels and microplastic occurrence is assessed as possible explanatory factors, though no other explanatory variables have been considered, such as water parameter (i.e. pH, temperature, turbidity), persistent organic pollutants (POPs), proximity to city, substrate etc. Initially, a short literature review on pearl formation in mussels and oysters is presented.

## 1.2 Pearl formation in molluscs with focus on mussels and oysters

Pearls, composed of calcium carbonate ( $\text{CaCO}_3$ ), are formed in the mantle of molluscs. Most research on pearl formation has been generated in relation to the pearl industry and focuses on pearl oysters (family Pteriidae). Pearls are also formed in many other mollusc species although research is scarce. It is considered that any mollusc with an inner shell-lining of “*mother-of-pearl*”, or nacre, can produce pearls under certain circumstances (Farn 1986). Pearl formation in mussels (*Mytilus* spp.) has received little attention and most of the research dates to the turn of the nineteenth century (reviewed in Fernandes & Seed 1983).

The nacre is an organic-inorganic composite layer which is formed during a biomineralization process that leads to a highly organized internal structure, giving nacre very robust mechanical properties and characteristic shine (Nudelman et al. 2006; Rousseau et al. 2009). Nacre is composed of multiple aragonitic sheets (a form of calcium carbonate) with a polygonal shape, with each “tablet” being around 5–15  $\mu\text{m}$  in diameter (Watabe 1965). These aragonitic units are piled on top of each other horizontally to form a lamella which is around 0.5  $\mu\text{m}$  thick (Grigoire 1957; Nudelman et al. 2006). In between these layers of mineralized aragonite, are extremely thin sheets of organic materials termed conchiolin that primarily consist of a protein complex (Bowen & Tang 1996). Overall, nacre structural organisation can be described to resemble a brick wall (Grigoire 1957). This structure has inspired material scientists to create new types of resilient concrete by mimicking nacre, since it can lead to a 300 to 500 times more tensile strain capacity than normal concrete<sup>1</sup>.

Pearls are secreted within the nacre (Fernandes and Seed 1983) and they consist of 90%  $\text{CaCO}_3$ , while the remaining parts are water and conchiolins (Farn 1986). The composition of nacre and pearls are well-studied and well-understood, yet the exact biological processes and pearl inducing stimuli leading to pearl formation are much less understood (Fernandes and Seed 1983).

To produce artificial oysters in pearls, a small slit is made in the pearl oyster’s gonad before a piece of mantle tissue from a donor oyster is implanted into the recipient oyster together with a small pre-produced bead (termed pearl nucleus or mother-of-pearl). Usually, pearl nucleus is made perfectly spherical (and at best case pure white) and produced from the shell of a freshwater mussel (family Quadrulidae) (Farn 1986). If the pearl induction was successful, oysters will then start to heal the

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<sup>1</sup> <https://www.iem.com/product-development/news/21006734/bendable-concrete-inspired-by-seashells>.

wound and form a pearl sac (epithelium tissue) around the pearl nucleus before nacre is deposited in layers (Sato et al. 2013). Pearl sac formation starts with the pearl nuclei being encapsulated by hemocytes; both granular and agranular hemocytes. Granular hemocytes phagocytose tissue debris, and agranular hemocytes cause epithelium tissue formation (Suzuki & Funakoshi 1992). Oysters are then left to grow their pearls for sometimes as much as six years after a successful implantation before pearls are harvested.

Mussels, like other molluscs, are thought to produce pearls to neutralise possible threats, but there does not appear to be a single pearl inducing stimuli for *Mytilus* (Farn 1986). Originally, it was thought that pearls were only encapsulating inorganic foreign objects such as sand particles (reviewed by Farn 1986 and Wilcox 2013). Later research has suggested that pearl formation may be partially or wholly due to infection by a parasitic trematode, suggesting that pearl formation may be considered a morbid condition (Lutz 1978, 2009; Farn 1986 Wilcox 2013). The trematode is thought to be a gymnophallid larvae of i.e. genus *Gymnophallus* (Lutz 2009, Wilcox 2013). The trematodes life history is interesting, with the first larval stage living in a small mollusc (i.e. the grooved carpet shell, *Tapes decussatus*, or the common cockle, *Cerastoderma edule*), and possibly a second larval stage in a larger molluscs (often *Mytilus edulis*) while the mature form can be found in the common scoter (*Melanitta nigra*) or the common eider (*Somateria mollissima*) (Farn 1986 and Wilcox 2013).

The presence of pearls in wild mussels might therefore be due to trematode infections, but other pearl inducing stimuli such as pollutants and particles cannot be ruled out. With the growing concern surrounding the presence of microplastics in the marine environment and potential effects on aquatic biota, it is relevant to investigate the possible impact of microplastics on pearl formation in mussels.

### **1.3 Microplastics and pearls in *Mytilus* spp. from the Norwegian coast**

NIVA studied microplastic occurrence in mussels sampled from the Norwegian coast on behalf of The Norwegian Environment Agency (Lusher et al. 2017), and this study was later expanded to cover a total of 15 sites spanning from the Oslofjord to the Barents Sea (Bråte et al. 2018). Microplastics were found in mussels from 14 out of the 15 sites. Three sites were significantly different (both semi-quantitative and qualitative), with no microplastics detected in mussels from Ørland, while Skallneset in Northern Norway and Akerhuskaia within the Oslofjord had elevated microplastic levels and a wider range of polymer types were identified. Through this study it was observed that many mussels contained numerous pearls, including small pearls down to ~ 50 µm. Due to the digestion of soft tissue with 10% potassium hydroxide (KOH), all pearls associated to mussel tissue could be identified. A similar method with KOH has previously been used to study pearl formation in mussels at a lower concentration (5% solution; Fernandes & Seed 1983). However, Fernandes and Seed (1983) used boiling KOH while the current study incubated samples with KOH at 60°C. Despite these differences, KOH degradation of soft tissue seems to be an appropriate method to study both microplastic and pearl occurrence simultaneously. Many of the pearls required a microscope to identify them, as did microplastic particles. Another aspect of pearl and microplastics occurrence, is that the very small pearls may be mistaken for small plastic beads if researchers are not aware that mussels (and other molluscs) can produce these spherical pearls that resembles plastic beads.

## 2 Methods

### 2.1 Sampling

Data from a total of 280 individuals were analysed from 14 sites (20 individuals from each site) around the Norwegian coast, spanning from the Oslofjord to Skallneset in north to study pearl and microplastic data. All of the mussels were gathered in 2017 as a part of Norway's contribution to the OSPAR CEMP monitoring program. Sampling procedures and details of microplastics in mussels are presented in Green et al (2018), and are not included in this report. Therefore, the microplastic data (except from correlation study) will not explicitly be presented in this report.

### 2.2 Sample processing and analysis

Shell length of mussels was set by determining the maximum anterior-posterior axis to the nearest 0.1 mm with calipers. Each mussel was weight (wet weight, w.w.) with two decimal places, without removing pearls. The individual pearls were not weighted. The length condition index (BCI) was calculated as following based on Kagley et al. (2003):  $BCI (ln) = \text{tissue w.w. (gram)}/\text{shell length (mm)} \times 100$ .

To detect microplastic and pearls in mussels, pre-treatment of mussel soft tissue was conducted, modified from Dehaut et al. (2016). In short, each individual mussel was added to a 10% solution of KOH and incubated for 24 hours at 60°C and 120 rpm. The solution was then filtered onto GF/F filter papers and visually assessed for microplastics, followed by a spectroscopy method, micro Fourier Transform Interferometer ( $\mu$ FT-IR), to confirm polymeric particle composition. Detection limits for both pearls and microplastics was  $>70 \mu\text{m}$ . For in-depth information of the analysis see Lusher et al. 2017 and Bråte et al. 2018. Each pearl was recorded per individual and size measurements were performed on sub-samples of the detected pearls (249 pearls measured; from 13 individuals at three sites) by measuring their longest dimension using a stereomicroscope couple with camera (Infinity 1-3C camera and INFINITY ANALYZE and CAPTURE software). Any fused pearls were counted as a single pearl.

### 2.3 Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, Inc., USA) and XLSTAT 2018.1 statistical software. Normal distribution and homogenous variance were tested (D'Agostino & Pearson omnibus normality test). Non-parametric tests were conducted because data did not conform to normal distribution after log-transformation. A Kruskal-Wallis test was performed to test for site effects, followed by multiple pairwise comparisons using Dunn's procedure (post-hoc test). Spearman's rank order test was performed to investigate correlations between pearl occurrence with size, condition index and microplastic abundance. A retrospective power analysis was also performed to investigate the number of mussels that were required to detect any differences in pearl formation by using the G\* Power tool<sup>2</sup>. A F-test was performed, with a one-way ANOVA and 14 groups adding the individual mean and the overall variation (SD = 30,64). The effect size (f) was calculated to 0.542, the alpha value was set to 0.05 (p-value) and the power to 0.80.

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<sup>2</sup> <http://gpower.hhu.de/>

## 3 Results and Discussion

### 3.1 Pearl occurrence in *Mytilus* spp. from the Norwegian coast

This study found that Norwegian mussels are producing pearls (Table 1). A total of 1937 pearls were found in 36% (101) of 280 individual mussels analyzed. Mussels from 10 of the 14 sites contained pearls, with an overall average of 7 ( $\pm$  31) pearls ind<sup>-1</sup>. There was a large variance between number of pearls produced per individuals ranging from 0 pearls ind<sup>-1</sup> to a maximum of 362 pearls ind<sup>-1</sup>. At least 60% of individuals from the Oslofjord contained pearls. No pearls were found in mussels from Færder, Bodø, Tromsø or Skallneset.

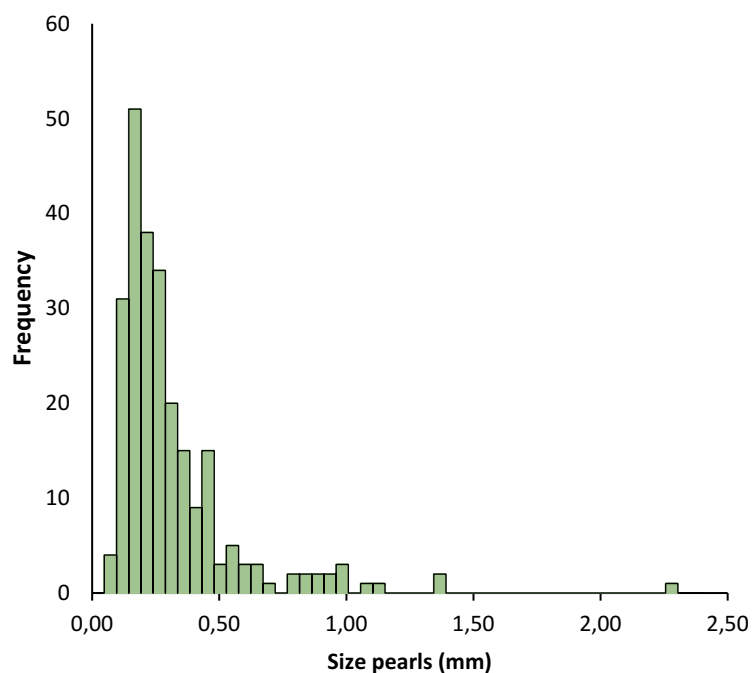
Based on a sub-sample of 249 pearls, the size of the pearls ranged from 0.07 mm to 2.50 mm with an average size of 0.31 ( $\pm$ 0.25) mm (Figure 1). Pearl occurrence in wild and commercial mussels might, due to the pearls overall small size, be a more frequent event than previously considered, as their often-small size prevent them from being detected without using microscope and dissolving the soft tissue. Based on the power analysis, it was calculated that the samples size needed to detect any significant differences regarding pearl occurrence between mussels populations was a total of 84 mussels (for 14 sites), which gave the analyses a power of 0.88. More than 80% power is often considered sufficient to detect any differences.

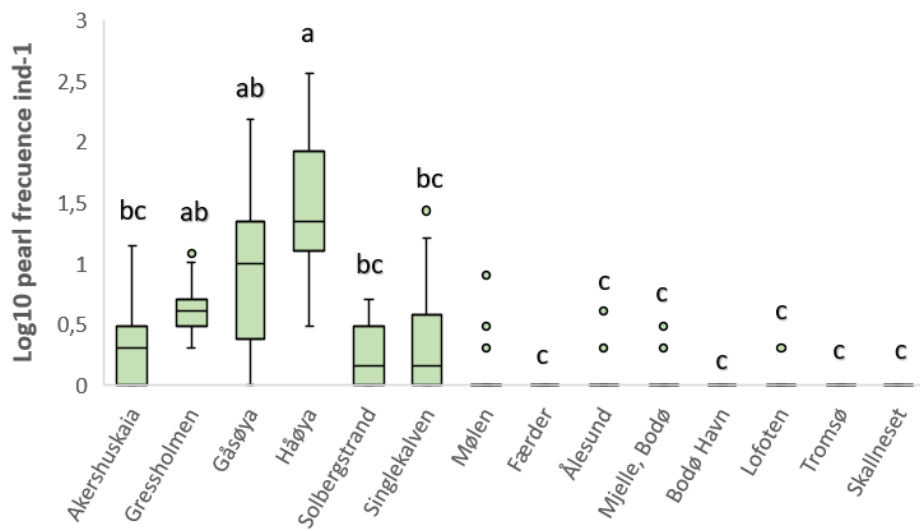
Based on our study, location and mussel size did influence the presence of pearls, whilst microplastic occurrence did not. The highest abundance of pearls was found in mussels from Håøya located in the Inner Oslofjord and the levels found was significant different (Kruskal-Wallis;  $p < 0.0001$ ) from most of the other sites except from Gåsøya and Gressholmen (Table 1; **Figure 2**). Gåsøya and Gressholmen had the second and third highest levels, respectively, and were significantly different from most sites except from Håøya, Akershuskaia, Solbergstrand and Singlekalven. A south-north gradient appears to exist in this survey; where mussels from southern populations produce more pearls than northern populations, with exception to mussels from Færder. Reasons for this eventual trend are currently unknown and should be further assessed. We hypothesise that differences in trematode infections or water parameters, such as temperature or particulate matter, along the gradient may explain the variance. Since parasite infections are thought to be a main driver for pearl production in mussels, it can also be hypothesised that there is a higher level of trematode infected mussel in the southern part of Norway. Therefore, trematode infections in these populations, especially within Oslofjord, should be studied.

Pearls varied in shape from spherical to highly irregular with uneven surface, droplet formed pearls and fused pearls, including a helix shaped pearl and a flat pearl were found (Figure 3; Figure 4). Pearls had diverse colors from bright white and off-white to blue. The abundance of irregular shapes and variation in colors is in accordance with a previous study in North Wales that found a large diversity in the shapes and the colours of pearls, including fused pearls (Fernandes & Seed 1983). These fused pearls may be pearls that are under production, rather than several pearls that are fused post-production. Research from pearl oyster farming shows that it is important to introduce perfectly spherical and perfectly white beads to avoid any deformation or decoloring of the produced pearl. Therefore, it is likely that the nuclei of the pearls, of unknown origin, were diverse in shape and colour. Particularly, the helix formed pearl is of special interest, and similar pearls have not, to the authors knowledge, been reported previously.

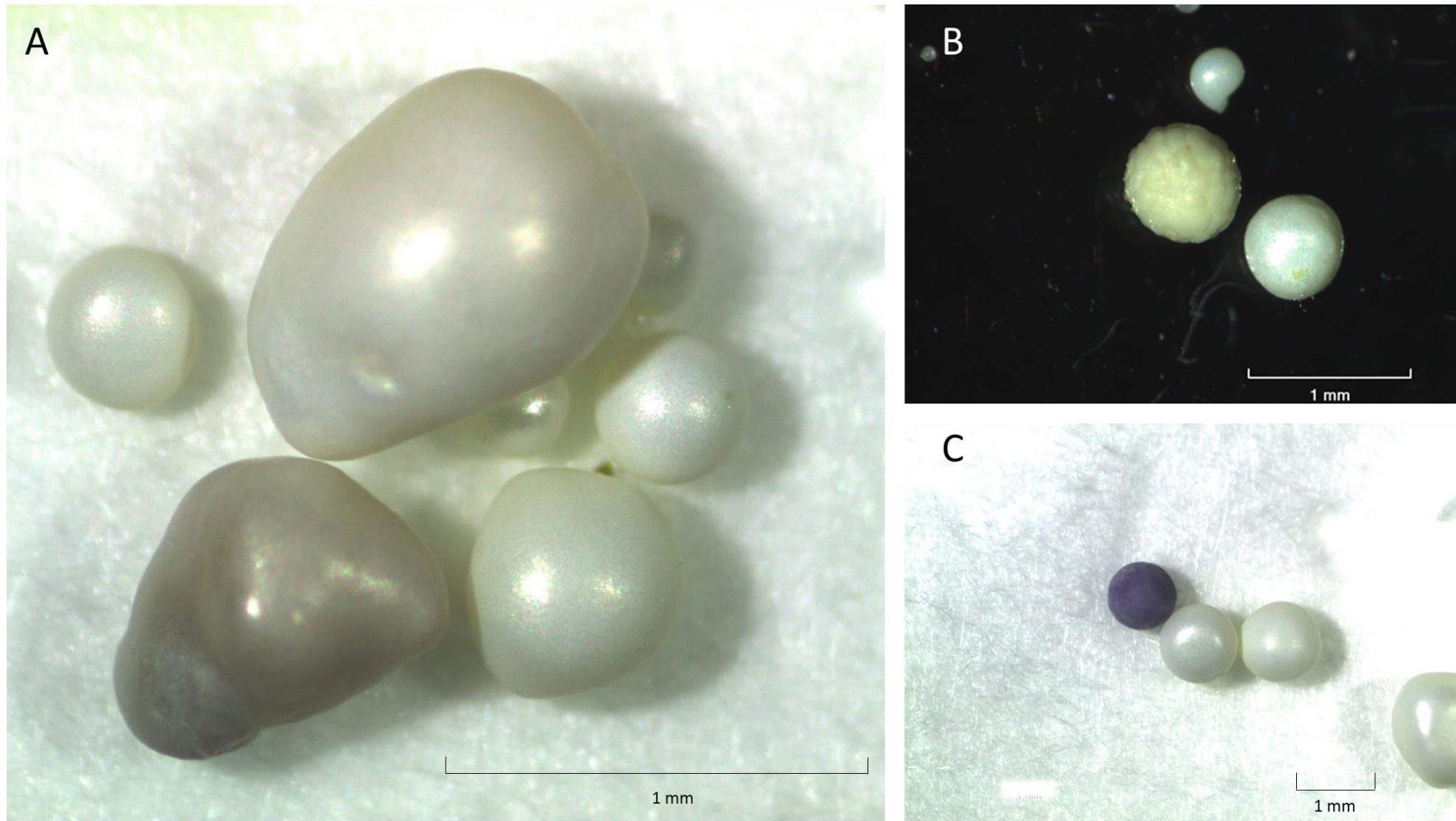
**Table 1:** Pearl occurrence in *Mytilus* spp. at different sites along the Norwegian coast. \* per 20 mussel individuals.

Site	Total no. of pearls *	Average no. of pearls ind <sup>-1</sup>	SD ( $\pm$ )	Max	Min	% of ind. w/pearls
Akershuskaia	36	2	3	13	0	60
Gressholmen	72	4	3	11	1	100
Gåsøya	493	25	39	152	0	80
Håøya	1228	61	91	362	2	100
Solbergstrand	19	1	1	4	0	50
Singlekalven	64	3	6	26	0	50
Mølen	11	1	2	7	0	20
Færder	0	0	0	0	0	0
Ålesund	7	0	1	3	0	15
Mjelle, Bodø	6	0	1	2	0	20
Bodø Havn	0	0	0	0	0	0
Lofoten	1	0	0	1	0	5
Tromsø	0	0	0	0	0	0
Skallneset	0	0	0	0	0	0
<b>Total</b>	<b>1937</b>	<b>7</b>	<b>31</b>	<b>362</b>	<b>0</b>	<b>36</b>

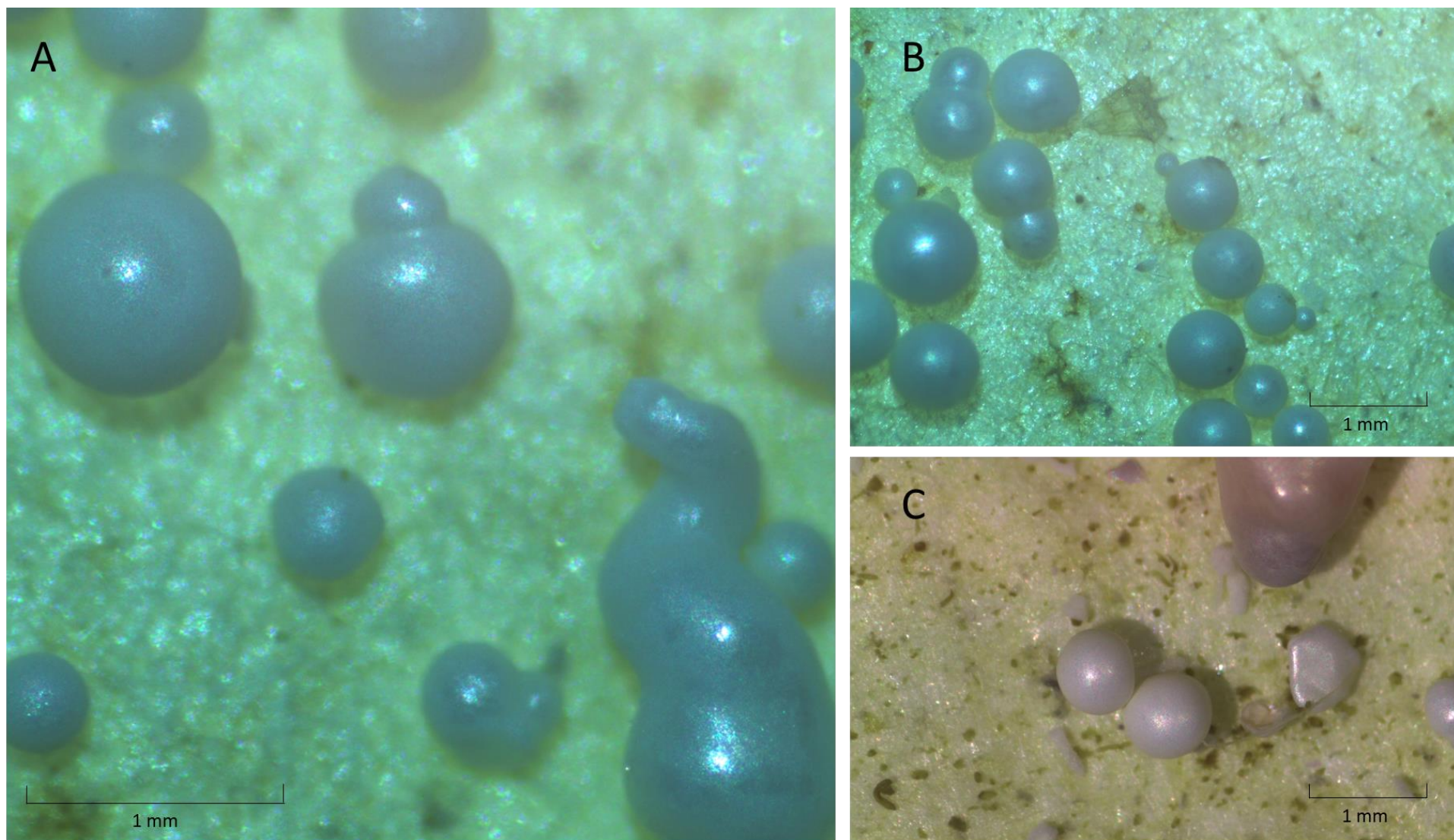
**Figure 1:** Size distribution of a sub-sample of pearls in *Mytilus* spp. isolated from mussels from sites along the Norwegian coast (n=249 pearls from 13 individuals located across three sites).



**Figure 2:** Box and whisker plots of log transformed data of pearl frequency per mussel per site (20 individuals), Sites that were significant different from each other (Dunn's test;  $p < 0.05$ ) are marked with letters a, b and c indicating which groups they belong to.



**Figure 3:** Examples of pearl found in Norwegian *Mytilus* spp. **A:** several different shapes **B:** different surfaces **C:** different colors.



**Figure 4:** Examples of pearls found in Norwegian *Mytilus* spp. **A:** fused and helix formed pearls **B:** fused pearls **C:** a flat pearl.



### 3.2 Size, condition index and microplastics as possible parameters correlating with pearl formation in *Mytilus* spp.

Mussel length (cm), likely corresponding to age, seems to be an important aspect of pearl occurrence. A positive correlation was found between mussel length and number of pearls (Figure 5A; Spearman  $r = 0.686$ ;  $p < 0.0001$ ). Mussels in this study ranged from 2.14 to 8.91 cm in size, and no pearls were identified in mussels smaller than 4 cm (Figure 5B). Therefore, older mussels seem more likely to contain pearls than younger mussels. In this study, no pearls were found in mussels <4 cm which is in accordance with previous research where almost no pearls were found in mussels between 3-4 cm from North Wales (Fernandes & Seed 1983). Increased pearl incidence amongst larger mussels may reflect their increased relative area of secretory mantle epithelium with increased size, but also prolonged exposure to pearl inducing stimuli (Fernandes & Seed 1983). Mussel farming industry has experienced that older mussels starts to produce pearls and have advised harvesting individuals before they reach three years to prevent pearl occurrence (Morse & Rice 2010).

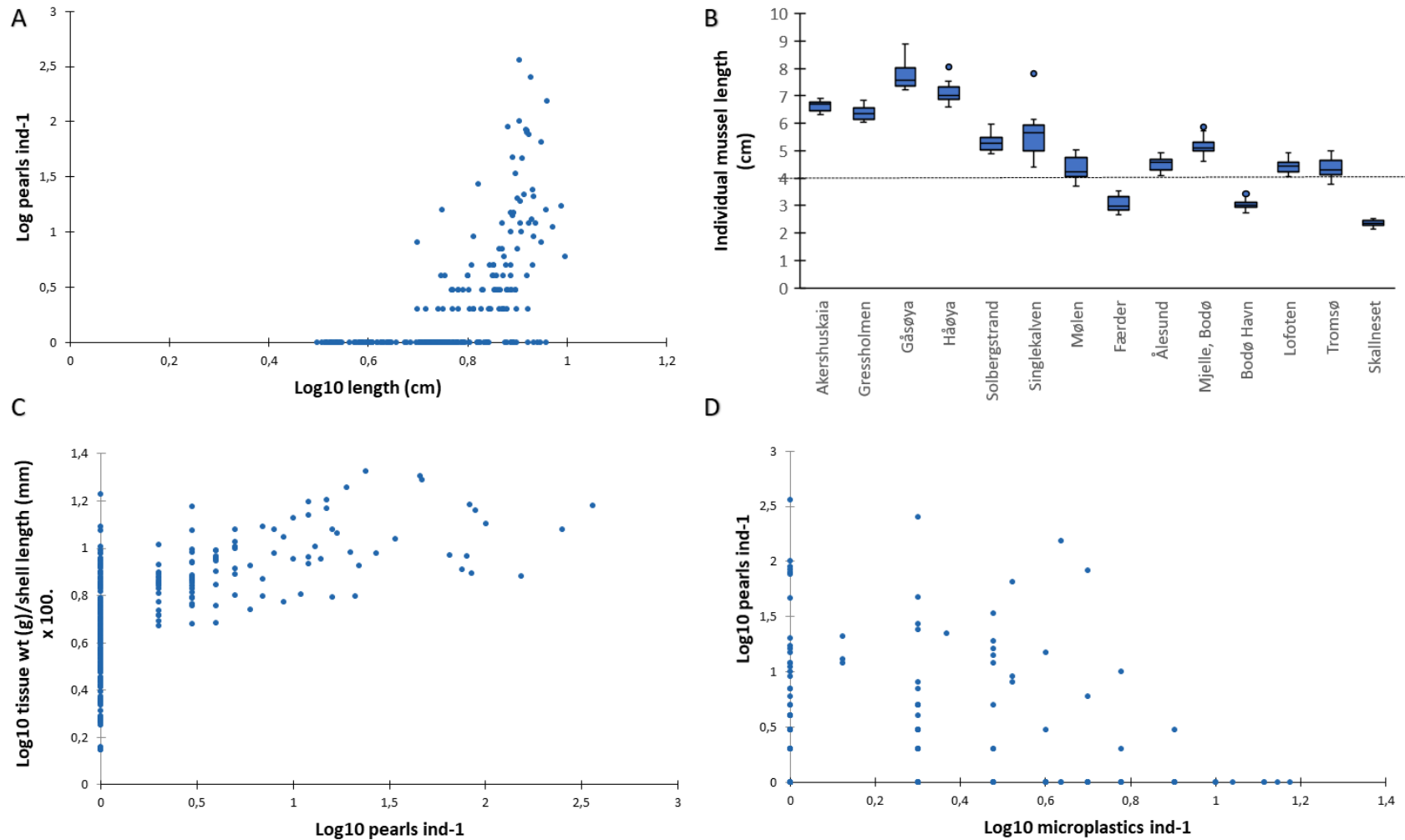
If a mussel produces a lot of pearls, it is likely that this could be highly costly for the mussel. Seed (1991) did suggested that high levels of pearls could represent a significant metabolic cost for the mussel individual (Seed 1991). Due to this theory, the mussels condition was assessed (condition index) and sequentially a correlation test was performed between the condition index and pearl frequencies. Surprisingly, a positive correlation was found between the number of pearls and the condition index (Figure 5C; Spearman  $r = 0.434$ ;  $p < 0.0001$ ). However, results may be an artefact since pearl weight was not excluded from mussel weight. A study from North Wales found that pearls accounted for 12.8% of mussel total body weight (dry weight) (Seed 1991), and numerous pearls (or large pearls) could therefore strongly impact mussel soft tissue weight. In this current study, the same correlation test was also performed by including only mussels <4 cm and this results still shows a positive correlation between condition index and pearl frequencies (Spearman  $r = 0.611$ ;  $p < 0.0001$ ).

The maximum amount of pearls previously reported in an individual *Mytilus edulis* was 264 pearls (Fernandes & Seed 1983), whilst the maximum number found in this study was 362 pearls. The mussel from North Wales was 6.2 cm long, while the Norwegian mussel was 7.0 cm long. However, since length (and probably the age), of mussel positively correlates with pearl formation, same-sized mussels should be assessed to investigate spatial differences. Same sized mussels from different coastal locations in the north-eastern United States, had varied pearl production, and this lead to some areas being considered better for mussel farming than others (Scattergood & Taylor 1949).

Nevertheless, size/age cannot alone explain pearl formation in Norwegian mussels. Multiple other factors may be involved in inducing pearl formation, and our results indicated a site-specific variation. With the exception of one individual from Lofoten which contained a single pearl, mussels from Tromsø and Lofoten, which were all above >4 cm, did not contain any pearls. Whether this is by chance, or whether this reflects variation in specific environmental factors and stimuli is unknown. Site-specific differences for mussel populations >4 cm should be studied to investigate underlying mechanisms leading to pearl production or lack of pearl production.

It has been hypothesized that the presence of microplastics might encourage pearl formation (Lusher et al., 2017). However, based on this study, microplastic occurrence does not appear to significantly influence pearl production in Norwegian mussels. No significant correlation between number of microplastics and number of pearls was identified (Figure 5D; Spearman  $r = -0.101$ ;  $p < 0.091$ ). Again, the same correlation was performed for mussels only  $\geq 4$  cm, and there was still no significant

correlation (Spearman  $r = -0,011$ ;  $p = 0.873$ ) Hypothetically, if mussels do encapsulate microplastics in pearls, previous microplastic data from locations such as the Oslofjord would be an underestimation of the microplastic load in these mussels. Further, a positive correlation between microplastics and pearls may not be possible to find if microplastics are embedded in the pearls. Ideally, pearls should be investigated for microplastic content enabling an assessment into whether these parameters are related. Mussels from Skallneset, where high concentrations of microplastics in mussels have been detected (Bråte et al., 2018) did not contain any pearls, demonstrating that the link between microplastics and pearls is not clear. However, it should be noted that the mussels from Skallneset were significantly smaller than other sites, and it cannot be ruled out that pearls could be produced by these mussels if they were larger than 4 cm.



**Figure 5:** **A:** Scatter plot of logarithmic pearl data and length of mussels merged from all sites. **B:** Box and whisker plots of the length of the mussels from the different sites. The dotted line represents 4 cm mussels, with no pearls being detected in mussels below this size **C:** Scatter plot of logarithmic condition index and pearl data merged from all sites **D:** Scatter plot of logarithmic pearl and microplastic data merged from all sites.

### 3.3 Possible implications of pearl production in *Mytilus* spp.

Understanding the cause of pearl formation in mussels is an important aspect of mussel ecology, both as indicator organisms for monitoring of coastal pollution as well as a commercially important species. Based on this limited study, increased knowledge on pearl occurrence in Norwegian mussels have been established. Although it has demonstrated a need for further studies on stressors that might act as pearl inducing stimuli and how this may impact the condition of mussels.

Mussel populations are disappearing from locations around the coast of Norway, and the same phenomenon has been reported from other European countries including France, the Netherlands and Scotland (Mortensen & Strohmeier 2018). Mortensen and Strohmeier (2018) highlights that mussels are disappearing from some localities while others are found in new locations such as Svalbard (since 2004). There are many unanswered questions regarding this phenomenon, primarily due to lack of available data. However, Mortensen and Strohmeier (2018) highlights potential reasons for these changes; 1) *Changes in climate/environment* leading to altered food supplies for the larvae reducing larvae survival, or lowering of pH (ocean acidification) that can impair shell formation (and thereby possibly also pearl formation; Li et al. 2016); 2) *Changes in prey* due to changes in water bodies; 3) *Increased competition* due to invasive species such as from Pacific Oysters (*Crassostrea gigas*) or local species such as barnacles; 4) *Changes in predation* of either the larvae and/or adult stage; 5) *Changes in genetic structure of the populations* due to “genetic drift” that might change their adaption 6) *Diseases* caused by bacteria or parasite infections such *Marteilia pararefringens*. 7) *Pollution (including plastics)* that might represent an additional stressor (Mortensen & Strohmeier 2018). Additionally, it has also been raised concerns about periodically deficiency of thiamine (vitamin B1) in several classes of animals, including mussels from the Baltic Sea, and possible associating negative health effects such as impaired reproduction in mussels (Balk et al. 2016).

Pearl formation is important to understand in the context of environmental monitoring, since the presence of pearls could possibly affect weight of the mussel and therefore also the reported load of organic and inorganic contaminants per weight unit measured in individual mussels. For example, the total concentration of PCB in mussel tissue is given as  $\Sigma\text{PCB ng g}^{-1}$  wet weight (or dry weight). Furthermore, extensive pearl formation might affect the mussels health which again might impair biomarker responses if pearl occurrence in itself is a symptom of a stressed mussel. Another aspect of pearl formation in mussels in relation to environmental monitoring, is the possible concept of using pearl formation as a health parameter, since pearl formation might be a morbid condition and could give valuable insight into overall mussel health.

Additionally, mussel farming is important for the Norwegian economy and mussels are important as a human food source worldwide. Lutz (1980) have reviewed the implications of pearl formation in farmed mussels. For instance, pearls can lead to marketing problems, at least if the pearls exceed 1 mm (Lutz 1978).

With continued growing concern surrounding the stressor affecting marine coastal ecosystems such as ocean acidification, pollutants and microplastics in the marine environment, understanding how this impact marine organisms should be at the forefront of research. Most available pearl occurrence data has either originated from commercial pearl oysters or predates to the turn of the nineteenth century, this report is therefore a much-needed contribution to empirical data. However, more research is required since the exact biological processes and environmental pearl inducing stimuli leading to pearl formation is not well-understood. *Mytilus* spp. are often considered as an optimal sentinel species for monitoring of coastal environmental pollutants, as recently reviewed by Beyer et

al. (2017). Mussels have been used to monitor organic and inorganic hazardous contaminants through the joint Oslo Paris Convention for the Protection of the Marine Environment of the North East Atlantic (OSPAR) monitoring since 1981 (Green et al. 2017). Lately, they are also proposed as a possible sentinel species for microplastic pollution by ICES (OSPAR 2015; Li et al. 2019) and recent empirical data have supported their usefulness to monitor small microplastics semi-quantitatively and qualitatively by finding spatial trends along the Norwegian coast (Lusher et al. 2017; Bråte et al. 2018). As pearls in wild Norwegian mussels might be partly due to trematode infections, and as there seems to be a south-north gradient when it comes to pearl occurrence, the degree of parasite infections in mussels from the southernmost and northernmost sites should be assessed and compared, as well as their associated host species. Additional parameters should also be assessed regarding pearl formation such as the levels of persistent organic pollutants (POPs) and water parameters such as as turbidity. Pearls nuclei should also be investigated to find out what is embedded, and to see if particles such as microplastics might be encapsulated. Furthermore, pearl mass should be measured to give more exact quantities of pearls.

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