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THE DISTRIBUTION OF THE MUSSEL *MYTILUS* SPECIES ALONG THE NORWEGIAN COAST

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ABSTRACT Mussels (*Mytilus* spp.) are used frequently in biological effects studies and biomonitoring programs either as transplanted groups or native populations. However, as a result of the similarities in the external morphology of *Mytilus*, which are influenced by environmental factors, visual identification is often never certain. Because differences in contaminant bioaccumulation and biomarker responses may occur among the 3 mussel species *Mytilus edulis*, *Mytilus trossulus*, and *Mytilus galloprovincialis*, bias in biological effects studies and biomonitoring programs is likely. In the current study, mussels were collected from strategic locations along the 25,000-km Norwegian coastline and identified to species by polymerase chain reaction amplification of gill tissue DNA. Specific primers were used to amplify the *Glu* gene (polyphenolic adhesive protein), which has been validated previously to identify the different *Mytilus* species. The amplified products were electrophoresed, showing specific bands for *M. edulis* (180 bp), *M. trossulus* (168 bp), and *M. galloprovincialis* (126 bp), with the presence of 2 bands suggesting *Mytilus* hybrids. The results identified the presence of all 3 *Mytilus* species in a patchy distribution around the Norwegian coast, with the Mediterranean species *M. galloprovincialis* located up to 60°N. The implications of this situation for biomonitoring programs and exposure studies are discussed.

KEY WORDS: mussels, monitoring, *Mytilus edulis*, *Mytilus galloprovincialis*, *Mytilus trossulus*, DNA amplification, hybrids

INTRODUCTION

Mussels, *Mytilus* species, are ideal biomonitoring organisms because they are sessile, can bioaccumulate many environmentally important contaminants, and have a wide range of biological effect end points that can be measured and often quantified. For these reasons, the mussel has been used consistently as a model organism in both coastal and offshore biomonitoring programs worldwide, where they are often collected from locations along the coast and either analyzed immediately or transplanted into exposure studies (e.g., Brooks et al. 2009, Raftopoulou & Dimitriadis 2010, Brooks et al. 2011a, Brooks et al. 2011b, Pereira et al. 2011). Biomarker end points can be used to determine the health of the mussel, which in turn can provide important information on the health status of a particular water body (Brooks et al. 2009, Corsi et al. 2011), or the potential impact of chemical point discharges (Brooks et al. 2011a).

Until relatively recently, the distribution of *Mytilus* on the Norwegian coastline was identified as solely consisting of *Mytilus edulis* (L.), with an *M. edulis*/*Mytilus trossulus* (Lmk.) hybrid zone on the Swedish coastline, and *Mytilus galloprovincialis* (Gould) within the Mediterranean (Gosling 1992). However, recent investigations have discovered a very different and often patchy distribution, with *M. trossulus* found on the west coast of Norway (Ridgway & Naevdal 2004) and even as far north as the Arctic Circle in the Barents and White seas (Kijewski et al. 2011, Väinölä & Strelkov 2011). Although these studies have been relatively extensive geographically, they have focused mainly on the Baltic and Arctic regions, with only a few sampling stations around the Norwegian coastline.

Because environmental factors such as temperature, salinity, and exposure to wave action can have significant effects on the mussel's external morphology, it is not possible to distinguish reliably among *Mytilus* species by mere visual inspection, which can lead to misrepresentation of *Mytilus* in exposure studies. Differences in chemical bioaccumulation and biomarker responses among the *Mytilus* spp. are not fully known, but are thought to occur to a certain extent (Lobel et al. 1990). For example, different assessment criteria values have been suggested for the different *Mytilus* species for certain biological effects measurements, including micronuclei formation and acetylcholine esterase inhibition (ICES/OSPAR SGIMC 2011). If mixed species of *Mytilus* are used unknowingly in exposure studies and biomonitoring programs, it could lead to potential difficulties in the interpretation of chemical and biomarker data, which could impinge on regulatory decisions.

The method used to identify the *Mytilus* species was first described by Inoue et al. (1995), and represents a highly specific and sensitive high-throughput method without the need for DNA sequencing. Although not disclosing the full phylogenetic relationships and the process of *Mytilus* hybridization, because they are linked only to 1 nuclear allele, it has been used as the preferred technique for species identification in several recent studies (Bignell et al. 2008, Brooks et al. 2009).

The aims of the current study were to collect mussels from strategic locations on the Norwegian coastline and to sample biological tissue to determine the correct species. The mussels were sampled from the same locations as those used by the OSPAR Co-ordinated Environmental Monitoring Program (CEMP), which is an annual monitoring program that uses mussels to assist in determining the environmental status of a particular water body or coastline by measuring chemical bioaccumulation.

METHODS

Collection of Mussels

Mussels were collected from 13 locations along the Norwegian coastline as part of the national monitoring program (OSPAR CEMP). The sampling stations were spread out evenly to encompass the entire coastline from Oslo in the South to Finnmark

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in the Arctic Circle. Mussels between 4 cm and 5 cm in length were collected from the shore for species determination. The local habitat at each sampling site is described in Table 1. The entire mussel, including shell, was frozen at –20°C until required for analysis.

DNA Isolation, Amplification, and Gel Electrophoresis

Total DNA was extracted from 20–40 mg gill tissue from frozen mussels using DNAzol reagent (Invitrogen, Madison, WI) following the manufacturer's recommended protocol. Briefly, the tissue was homogenized in 1 mL DNAzol using Precellys 24 bead mill (Bertin, Montigny-le-Bretonneux, France), using ceramic CK14 beads at 5,000*g* for 10 sec. Cell debris was then removed by centrifugation at 10,000*g* for 10 min (4°C) before DNA was precipitated from the supernatant by the addition of 500 µL 100% ethanol. After 2 wash steps with 75% ethanol, the DNA was pelleted by centrifugation at 4,000*g* for 2 min, then air-dried and dissolved in 8mM NaOH. The resulting DNA was quantified and quality controlled on a nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA), and all samples had OD 260/280 > 1.8, indicative of pure DNA. For species identification, polymerase chain reaction (PCR) was used to amplify a specific 180-bp segment for *Mytilus edulis*, a 168-bp segment for *Mytilus Trossulus,* and a 126-bp segment for *Mytilus galloprovincialis* of the *Glu* gene (polyphenolic adhesive protein) as described by Inoue et al. (1995). The 50-µL PCRs contained 10 µL DNA template, 300 µM forward and reverse primers, and VWR 2× Taq mastermix (VWR, Radnor, PA, USA), and were subjected to a 5-min preheating stage at 95°C followed by 35 cycles of 30 sec at 95°C, 30 sec at 55°C, 30 sec at 72°C, and a final extension step of 10 min at 72°C.

One microliter of the PCR product was loaded onto a DNA 1000 chip (Agilent Technologies, Santa Clara, CA) and run in a Bioanalyzer instrument (Agilent Technologies) for visualization of amplicon size.

DNA Sequencing

A subset of the samples were finally sequenced in an ABI3730 automatic sequencer (Applied Biosystems, Foster City, CA), using BigDye chemistry (v3.1). For *Mytilus* hybrids, the different amplicons were first separated on a 2% agarose gel and recovered individually using the Zymoclean gel DNA recovery kit (Zymo Research Corporation, Orange, CA). A 1-µL template was used in a 20-µL reaction together with 0.6 µL Big Dye mix, 3.6 µL 5× sequencing buffer (Applied Biosystems), and 5 µM Me15 or Me16 primer, and was subjected to the following temperature program: 1 min of preheating at 95°C followed by 25 cycles of 10 sec at 96°C, 5 sec at 50°C, and 4 min at 60°C. Sequences were then read in both directions separately before creating the contig sequence, which was subjected to a Basic Local Alignment Search Tool (BLAST) search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) for species verification.

RESULTS

Gel Electrophoresis and Sequence Information

The method of DNA amplification and gel electrophoresis of mussel gill tissue was successful in differentiating between the 3 *Mytilus* species and their respective hybrids. Examples of gel like images displaying amplicon sizes for the *Mytilus* species found on the Norwegian coastline are presented in Figure 1. Single bands were identified at 180 bp for *Mytilus edulis* (columns 2, 3, and 10), 168 bp for *Mytilus trossulus* (columns 4 and 5), and 126 bp for *Mytilus galloprovincialis* (column 8). In addition, hybrids were identified as a double band at 180 bp and 168 bp for *M. edulis M. trossulus* (columns 6 and 7), and at 180 bp and 126 bp for *M. edulis*/*M. galloprovincialis* (column 9). The *M. trossulus*/*M. galloprovincialis* hybrid was not detected in the mussel samples analyzed.

The DNA sequence information obtained from the specific amplified bands corresponded well with the sequence information from the BLAST database. The anticipated *Mytilus edulis* DNA fragment aligned to both gb|AY845258.1 (*M. edulis* clone 21 foot protein 1 (fp-1) mRNA, complete cds) and emb|X54422.1 (*M. edulis* gene for polyphenolic adhesive protein) with 99% identities (179/180) and an E value of 9e-87. For the anticipated *Mytilus trossulus* fragment, the alignment was 99% (119/120), with an E value of 2e-54, to the following nucleotide sequences: gb|DQ640589.1 (*M. trossulus* voucher MtII8 polyphenolic adhesive protein gene, partial cds), gb|DQ640588.1 (*M. trossulus*

TABLE 1.

The coordinates of the sampling locations from where mussels were collected along the Norwegian coastline.

No.	Sample location	Latitude	Longitude	Habitat type
1	Inner Oslo fjord	59.882	10.712	Intertidal zone, rock/gravel shore
2	Outer Oslo fjord	59.488	10.498	Intertidal zone, rocky shore
3	Østfold	59.102	11.045	Intertidal zone, rocky shore
4	Telemark	59.023	9.754	Intertidal zone, rocky shore
5	Agder	58.125	7.989	Intertidal zone, sand and small stones
6	Rogaland	59.326	5.318	Intertidal zone, rocky beach, mussels exposed at low tide
7	Hardanger	60.421	6.405	Intertidal zone, rock and kelp, mussels exposed at low tide
8	Møre og Romsdal	62.81	8.275	Collected from a floating jetty; depth, <1 m; mussels totally submerged
9	Nordland	66.319	14.128	Intertidal zone, rocky shore
10	Nord-Trøndelag	64.967	11.661	Intertidal zone, rocky beach near fish farm
11	Lofoten	68.158	14.653	Intertidal zone, rocky shore
12	Finnmark (Brashavn)	70.104	30.262	Intertidal zone, rocky shore
13	Finnmark (Varangerfjord)	69.899	29.744	Intertidal zone, rocky shore

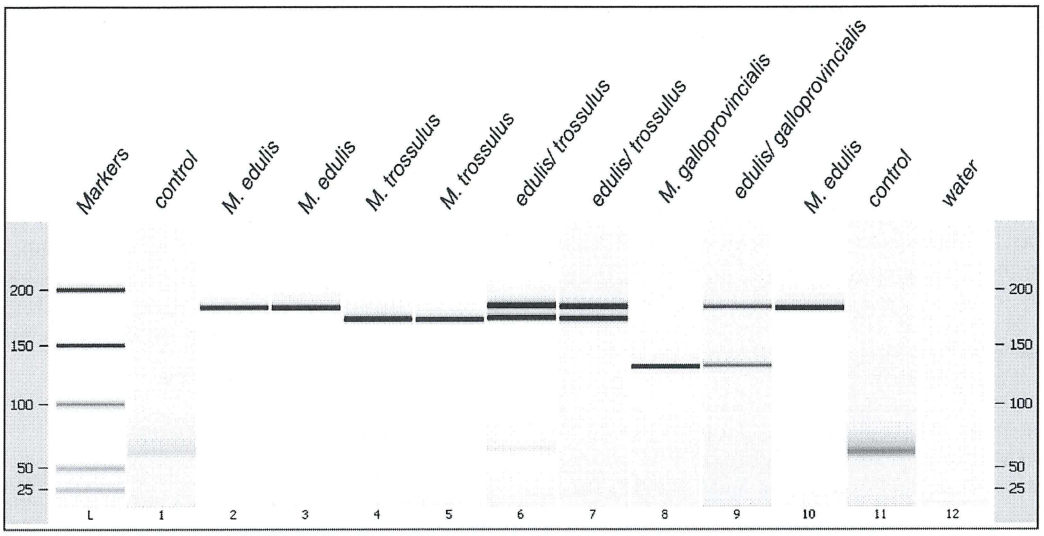

Figure 1. Output of DNA amplification and gel electrophoresis (as a gellike image) showing clear bands for the *Mytilus* species: *Mytilus edulis* (180 bp), *Mytilus trossulus* (168 bp), and *Mytilus galloprovincialis* (126 bp). In addition, double bands denote hybrids at columns 6 and 7 for an *M. edulis*/*M. trossulus* hybrid, and at column 9 for an *M. edulis*/*M. galloprovincialis* hybrid.

voucher MtI3 polyphenolic adhesive protein gene, partial cds), and dbj|D50553.1 (MSLADP *M. trossulus* gene for adhesive protein, partial cds). The anticipated *Mytilus galloprovincialis* amplicon aligned to both gb|AF489933.1 (*Mytilus* sp. JHX-2002 adhesive plaque protein precursor, mRNA) and dbj|D63778.1 (MSLAP *M. galloprovincialis* mRNA for adhesive plaque protein, complete cds) with 100% (126/126) identities and an E value of 1e-58. Sequencing results thereby confirmed the presence of the 3 *Mytilus* species and their hybrids.

The representation of the different *Mytilus* species and their hybrids on the Norwegian coastline is shown graphically in Figure 2. Overall, *Mytilus edulis* was the most abundant, found at all of the 13 stations sampled. The only species type found within the inner and outer Oslofjord as well as farther south in Østfold was *M. edulis*. The mussel *M. edulis* was dominant (90%) in the samples collected from Telemark and Agder, with 1 *M. edulis*/*Mytilus galloprovincialis* hybrid and one *M. edulis*/*Mytilus trossulus* hybrid found in Telemark and Agder, respectively. Farther west to Rogaland revealed 60% *M. edulis*/*M. galloprovincialis*, with 40% *M. edulis*. Although *M. galloprovincialis* was not found at this sampling point, the high proportion of the *M. edulis*/*M. galloprovincialis* hybrid would suggest it was present but not sampled. At sampling stations Hardanger, Møre, and Romsdal, *M. trossulus* was by far the dominant *Mytilus* species sampled. Sample sizes at these 2 stations were considerably higher ($n = 22$ and $n = 83$, respectively) and clearly show *M. trossulus* with a high number of *M. edulis*/*M. trossulus* hybrids and a few *M. edulis*. Three *M. trossulus*/*M. galloprovincialis* hybrids were found at Møre and Romsdal, but were not found at any of the other 12 stations.

Farther north from Nordland, up to Finnmark in the Arctic Circle, *Mytilus edulis* was the dominant *Mytilus* species found, although *Mytilus trossulus* was also found at Nordland and Finnmark (Brashavn) and *Mytilus galloprovincialis* was also found in Nord Trondelag and Finnmark (Varangerfjord). The *M. edulis*/*M. galloprovincialis* hybrid was found at all stations within this northern region, whereas *M. edulis*/*M. trossulus* hybrids were found at all northern stations except for Lofoten.

DISCUSSION

The distribution of *Mytilus* around the Norwegian coastline was found to be particularly patchy, with *Mytilus edulis*, *Mytilus galloprovincialis*, *Mytilus trossulus*, and both hybrids of *M. edulis*/*M. galloprovincialis* as well as *M. trossulus*/*M. galloprovincialis* all found. This situation is very different from that reported previously during the early 1990s, when only *M. edulis* was thought to exist (Gosling 1992), but did support the recently reported distribution of *M. trossulus* on the west coast (Ridgway & Naevdal 2004) and the Barents Sea (Kijewski et al. 2011, Väinölä & Strelkov 2011).

Only the Inner and Outer Oslo fjord as well as locations farther southeast of the Oslo fjord (Østfold) could be considered as being comprised solely of *Mytilus edulis*. Because of the nature of the current study and the inability to sample all mussels in the environment, the study can merely confirm the presence of the different *Mytilus* species and their hybrids. Therefore, the absence of a particular *Mytilus* species and/or hybrid from the different sampling locations does not necessarily mean that it was not present at that location, but rather that it was not sampled, and care should be taken when assuming pure populations based on the current data.

In areas where *Mytilus* thrive, hybrid zones have been reported and studied; these include, for example, *Mytilus edulis* and *Mytilus galloprovincialis* hybrid zones on the Cornwall coast in the southwest of England (Gilg & Hilbish 2003), as well as *M. edulis* and *Mytilus trossulus* hybrid zones on the southeast coast of Sweden (Gosling 1992, Riginos & Cunningham 2005). From the current study, hybrid zones were not defined clearly along the Norwegian coastline. Previously, a hybrid zone at the

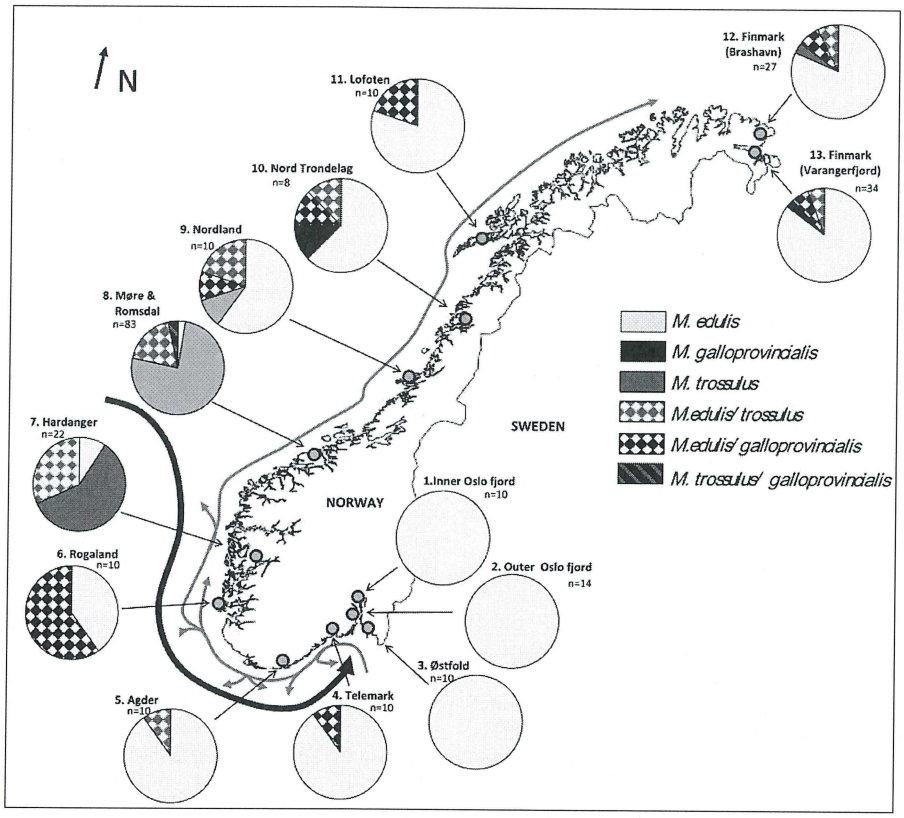

Figure 2. Locations of the sampled mussels around the Norwegian coastline, including the proportion of the different *Mytilus* and hybrids at each location. The number of mussels (*n*) sampled at each location is provided. Bold lines indicate the prevailing water currents around the coastline.

salinity gradient between the North Sea and the Baltic Sea was known to exist where *M. trossulus* gives way to *M. edulis* as the salinity increases (Väinölä & Hvilsom 1991). However, the presence of *M. edulis*/*M. trossulus* hybrids on the Norwegian coastline from Telemark all the way north as far as Finmark suggest that the hybrid zone has become extended from what was originally thought, and supports the data from recent studies (Kijewski et al. 2011, Väinölä & Strelkov 2011).

Pure or hybrid forms of *Mytilus trossulus* were detected at almost all sampling stations, which would suggest that the presence of *M. trossulus* north from the Baltic was possibly a gradual process, with the dispersal of gametes following the direction of the coastal water currents northward. However, it cannot be excluded that *M. trossulus* was there from the beginning, because limited genetic identification of *Mytilus* was available prior to the review by Gosling (1992).

The presence of only *Mytilus edulis* individuals in the inner and outer Oslo fjord as well as farther southeast in Østfold may reflect local current directions preventing the transport of gametes for colonization within these habitats. The mussel *M. edulis* is known to withstand extreme cold—down to temperatures of –20°C in laboratory exposures (Bourget 1983). In recent years populations of *M. edulis* have been found as far North as the island of Svalbard in the Barents Sea, located deep within the Arctic Circle at approximately 75°N (Berge et al. 2005). This extension of the mussels' range was attributed to the unusually high northward mass transport of warm Atlantic water in 2002, resulting in elevated sea-surface temperatures in the North Atlantic and along the west coast of Svalbard (Berge et al. 2005). In contrast, *Mytilus galloprovincialis* is regarded as a warm-water species typically found in the warmer waters of the Mediterranean and Adriatic seas, with its northern most limit on the shores of Britain and Ireland (Gosling 1992). On the west coast of North America, the distribution of *M. galloprovincialis* was found to be restricted to approximately 41°N, equivalent to southern Europe (Suchanek et al. 1997). Therefore, it was surprising to find *M. galloprovincialis* living as far North as Finnmark in the Arctic Circle (approximately 70°N). Temperatures in these waters during the winter can approach 0°C, with air temperatures from –30°C. To our knowledge, 64°N is the most northerly limit at which *M. galloprovincialis* has been found previously to inhabit.

For *Mytilus trossulus*, individuals were found at 1 of the 2 most northerly stations in the Arctic Circle (at 70°N), with the *Mytilus edulis*/*M. trossulus* hybrid also present. On the west coast of North America, *M. trossulus* was found to have a distribution between 43°N and 60°N, and was considered to be far more suited to colder waters than *Mytilus galloprovincialis* (Suchanek et al. 1997). The presence of *M. trossulus* in the Arctic Circle is supported by previous studies (Kijewski et al. 2011,

Väinölä & Strelkov 2011). However, in Europe, *M. trossulus* has become specifically adapted to the low-salinity waters of the Baltic Sea. Its distribution northward from the Baltic has been restricted in part through this adaptation to freshwater and the competition from *M. edulis* (Riginos & Cunningham, 2005). Consequently, the increased distribution of *M. trossulus* on the Norwegian coast may be a result of some form of physiological adaptation to full seawater, enabling individuals to compete better for space on the Norwegian coastline. Alternatively, transport of gametes and/or adults of the *M. trossulus* populations from the Pacific coast of North America may be the source of the new distribution. However, transport of offspring from these individuals seems only possible through ballast water from commercial shipping activities and, although possible, is probably unlikely. Genetic fingerprinting or DNA barcoding of *M. trossulus* on the Norwegian coast with Baltic and North American populations may assist in uncovering the population phylogeny and possible source, although this was outside the scope of the current study.

The farming of mussels around the Norwegian coastline is commonplace, and although the mussels are considered to be mostly (if not entirely) *Mytilus edulis,* no detailed studies have taken place. In 2011, more than 99% of all farmed mussels sold in Norway originated from Nordland and Trøndelag, although prior to this the farms were distributed more evenly around the coast (Directorate of Fisheries 2012). The influence of the farmed mussels on the distribution and apparent patchy distribution of the mussel complex on the Norwegian coast cannot be ignored, although to what extent is not known. The prevailing water currents are likely to transport the released gametes and developing larvae from both natural-occurring and farmed mussels northward along the coast, and thus contribute to the distribution of the mussel complex.

Physiological differences and tolerances between the main mussel species can influence their distribution along the shore and potentially influence habitat preferences (Riginos & Cunningham 2005). Consequently, site selection and position on the shore could potentially influence *Mytilus* selection. From the information acquired from the sample sites, mussels from almost all sites were collected from the intertidal zone, with no obvious difference between positions on the shore. There was only 1 site where mussels were collected from a floating jetty (Møre and Romsdal). These mussels would have experienced 100% submergence, and it was from this site that a high proportion of *Mytilus trossulus* were found. Whether this was the result of the different habitat is uncertain, although a high proportion of *M. trossulus* was also collected from the neighboring site of Hardanger, collected from an intertidal rocky shore.

The patchy distribution of all 3 *Mytilus* species and their hybrids on the Norwegian coastline has potential implications for biomonitoring programs and exposure studies using transplanted wild mussels. The potential differences in the bioaccumulation of contaminants as well as biological responses to stress, either environmental or chemical, among the different *Mytilus* species have the potential to reduce the effectiveness of biomonitoring programs. With respect to contaminant exposure, higher metal concentrations were detected in *Mytilus trossulus* compared with *Mytilus edulis* when collected from the same habitat and in the same size range (Lobel et al. 1990). The differences in the bioaccumulation of metals between these 2 *Mytilus* species was suggested by Lobel et al. (1990) to be the result of the slower growth rate of *M. trossulus* compared with *M. edulis*, rather than any direct differences between the element metabolisms of the species. Hence, *M. trossulus* of the same size as *M. edulis* would, in fact, be older and have a longer exposure history, resulting in higher contaminant concentrations.

Differences in biomarker responses among the different species have, to our knowledge, not been investigated. However, biological effects assessment criteria have been developed in mussels to assess the environmental status of water bodies in marine biomonitoring programs (ICES OSPAR SGIMC 2011). Different background assessment criteria for the 3 *Mytilus* species have been suggested for micronuclei formation, indicating that genotoxic responses differ among the 3 *Mytilus* species. As yet, interspecies differences for other biological effects end points in *Mytilus* have not been reported. However, there are a few cases in which differences in general physiology and behavior of the mussel could affect the overall fitness and biological response. For example, differences in the reproductive strategy of *Mytilus edulis* and *Mytilus galloprovincialis* have been found to occur, with the former spawning earlier and investing more in their replenishment than the latter from similar localities (Hilbish et al. 2002). Such differences are likely to alter the energy budgets between the species and affect general fitness at different times of the year. In addition, *Mytilus* species have been found to be differentially susceptible to parasitism, with the 2 parasites the pea crab *Pinnotheres pisum* and the trematode parasite *Prosorhynchus squamatus* reported to infect *M. edulis* preferentially over *M. galloprovincialis* (Seed 1969, Coustau et al. 1991).

Histological parameters including adipogranular rate and gonadal status were found to be statistically different between *Mytilus edulis*, *Mytilus galloprovincialis*, and their hybrids sampled from the same location in the United Kingdom (Bignell et al. 2008). It was stated that because different *Mytilus* species can display different physiological phenotypes, differences among sites cannot be compared reliably if the species compositions are not known and are not the same.

In conclusion, based on the results of the current study, it is highly recommended that the correct mussel species be known before their use in both future biomonitoring programs and laboratory exposure experiments. This requirement is particularly important when using mussels from the Norwegian coastline, because of their highly variable distribution. The PCR amplification of the *Glu* gene (polyphenolic adhesive protein) represents a basic, yet fast, highly specific and sensitive method of speciation that will help to ensure that species differences are known and do not bias unknowingly the interpretation of biological effects studies. Last, as a result of the vast expanse of coastline around Norway and the limited number of sites and samples acquired in this study, it is recommended that a more comprehensive study be performed in the future.

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