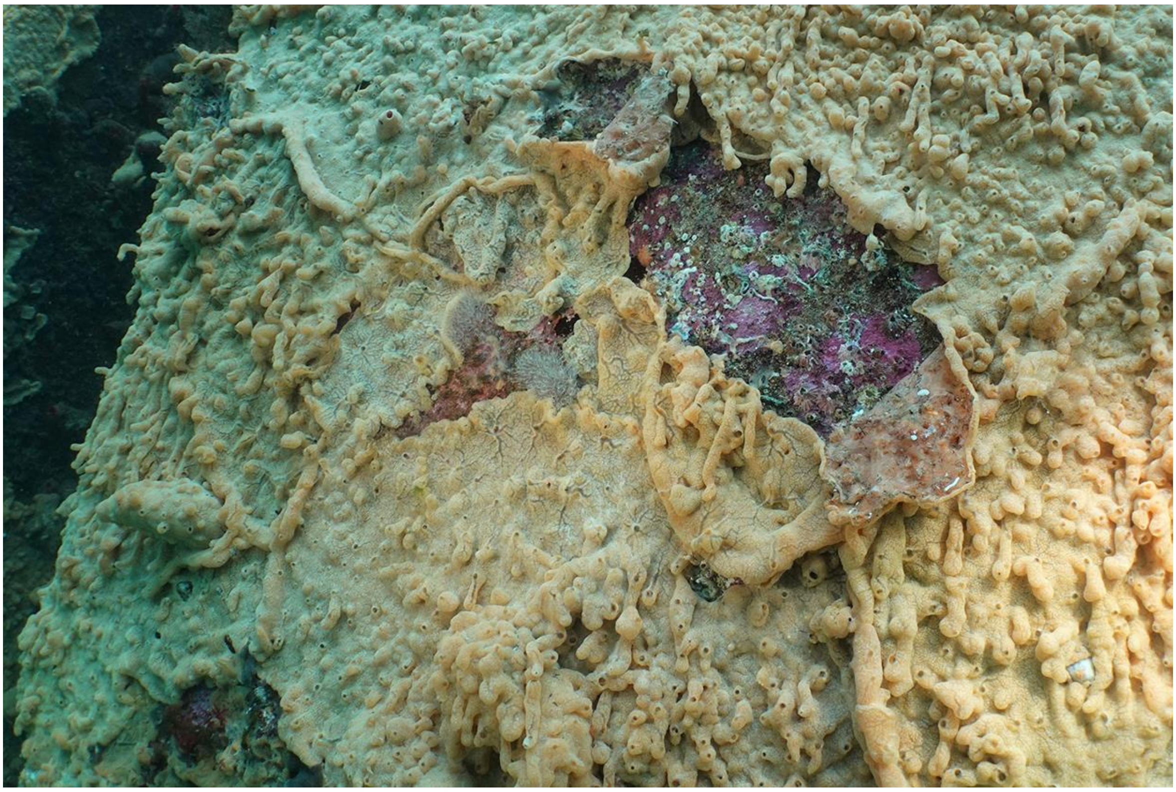

VKM Report 2023: 7

# Assessment of risk and risk-reducing measures related to the introduction and dispersal of the invasive alien carpet tunicate *Didemnum vexillum* in Norway

**Scientific Opinion of the Panel on Biodiversity of the Norwegian Scientific Committee for Food and Environment**

VKM Report 2023: 7
Assessment of risk and risk-reducing measures related to the introduction and dispersal of the invasive alien carpet tunicate *Didemnum vexillum* in Norway

Scientific Opinion of the Panel on Biodiversity of the Norwegian Scientific Committee for Food and Environment
17.02.2023

ISBN: 978-82-8259-416-5
ISSN: 2535-4019
Norwegian Scientific Committee for Food and Environment (VKM)
Postboks 222 Skøyen
0213 Oslo
Norway

Phone: +47 21 62 28 00
Email: vkm@vkm.no

https://vkm.no

Cover photo: Åge Wee

Suggested citation: VKM, Johanna Järnegren, Bjørn Gulliksen, Vivian Husa, Martin Malmstrøm, Eivind Oug, Paul Ragnar Berg, Anders Bryn, Sonya R. Geange, Kjetil Hindar, Lars Robert Hole, Kyrre Kausrud, Lawrence Kirkendall, Anders Nielsen, Brett K. Sandercock, Eva Thorstad, Gaute Velle (2023). Assessment of risk and risk-reducing measures related to the introduction and dispersal of the invasive alien carpet tunicate *Didemnum vexillum* in Norway. Scientific Opinion of the Panel on Biodiversity of the Norwegian Scientific Committee for Food and Environment. VKM Report 2023:7, ISBN: 978-82-8259-416-5 , ISSN: 2535-4019. Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway.

# Assessment of risk and risk-reducing measures related to the introduction and dispersal of the invasive alien carpet tunicate *Didemnum vexillum* in Norway

## Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft the opinion. The project group consisted of one VKM member, one VKM staff and three external experts. Two referees commented on and reviewed the draft opinion. The Committee, by the Panel on Biodiversity, assessed and approved the final opinion.

## Authors of the opinion

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and/or the VKM Panel on … /the VKM Scientific Steering Committee/an interdisciplinary VKM approval group, appointed specifically for the assignment.


**Members of the project group** (in alphabetical order after chair of the project group):

Johanna Järnegren – Chair of the project group. Member of the VKM Panel on Biodiversity. Affiliation: 1) VKM; 2) Norwegian Institute of Nature Research

Bjørn Gulliksen – External expert. Affiliation: Department of Arctic and Marine Biology, UiT, The Arctic University of Norway, Tromsø

Vivian Husa – External expert. Affiliation: Institute of Marine Research

Martin Malmstrøm – Project leader, VKM staff. Affiliation: VKM.

Eivind Oug – External expert. Affiliation: Norwegian Institute for Water Research

**Members of the Panel on Biodiversity that assessed and approved the opinion** (in alphabetical order before chair of the Panel):

Paul Ragnar Berg – Affiliations: 1) VKM; 2) Norwegian Institute for Water Research (NIVA)

Anders Bryn – Affiliations: 1) VKM; 2) Natural History Museum, University of Oslo; 3) NIBIO

Sonya R. Geange – Affiliations: 1) VKM; 2) University of Bergen (UiB)

Kjetil Hindar – Affiliations: 1) VKM; 2) Norwegian Institute for Nature Research (NINA)

Lars Robert Hole – Affiliations: 1) VKM; 2) Norwegian Meteorological Institute (MET)

Johanna Järnegren – Affiliations: 1) VKM; 2) Norwegian Institute of Nature Research (NINA)

Kyrre Kausrud – Affiliations: 1) VKM; 2) Norwegian Veterinary Institute (NVI)

Lawrence Kirkendall – Affiliations: 1) VKM; 2) University of Bergen (UiB)

Anders Nielsen – Affiliations: 1) VKM; 2) Norwegian Institute of Bioeconomy Research (NIBIO)

Brett K. Sandercock – Affiliations: 1) VKM; 2) Norwegian Institute for Nature Research (NINA)

Eva Thorstad - Affiliations: 1) VKM; 2) Norwegian Institute for Nature Research (NINA)

Gaute Velle – Chair of the VKM Panel on Biodiversity. Affiliations: 1) VKM; 2) NORCE Norwegian Research Center; 3) UiB.

## Acknowledgement

VKM would like to thank Anna Dimming (Länsstyrelsen Västra Götaland, Sweden) for valuable information on the findings in Sweden.

VKM would like to thank the referees Oliver Floerl (Cawthron Institute, New Zealand and SINTEF Ocean, Norway) and Adriaan Gittenberger (GiMaRIS, The Netherlands) for their valuable comments through critical review of the draft opinion. VKM emphasises that the referees are not responsible for the content of the final opinion. In accordance with VKM's routines for approval of a risk assessment (VKM, 2018), VKM received their comments before evaluation and approval by VKM Panel on Biodiversity, and before the opinion was finalised for publication. VKM would also like to thank Erling and Rudolf Svendsen for contribution photographs and helpful information on local spreading of *D. vexillum*.

## Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third-party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

# Table of Contents

# Summary

**Key words**: VKM, Norwegian Scientific Committee for Food and Environment, Norwegian Environment Agency, biofouling, negative effects, kelp forests, Zoostera, mearl bed, coral reef, aquaculture, predators, eradication.

**Introduction**

*Didemnum vexillum* is colonial sea squirt, a marine species which originates from the northwest Pacific; it was first recorded in Norway in November 2020. *Didemnum vexillum* is an alien species, meaning that it is a species that has been transferred from its original region to other regions of the world through human activity, and it had not previously been recorded in Norwegian waters. The species is regarded as having great invasive potential and having strong negative ecological effects on biodiversity. It is also considered to pose a risk to marine industries such as shipping and aquaculture, with possible major negative economic impacts.

Alien species can create changes and disrupt ecosystem functions by, for example, displacing native species. There are many examples in marine ecosystems of invasive species that spreads rapidly and becomes the dominating species in areas with suitable living conditions: Japanese wireweed (*Sargassum muticum*) is a well-known example in Norway. Japanese wireweed was introduced to Europe from Japan and has since spread through drifting material or as epigrowth on ships. Spreading of alien species occurs either through the species own reproduction or by moving objects to which the species is attached. Ship traffic is the most important vector for fouling organisms (algae or animal that attaches to a surface that is immersed in seawater) to spread over long distances.

Solitary and colonial sea squirts (phylum Chordata, class *Ascidiacea*) are well represented in Norway. Colonial sea squirts are characterised by the individuals being connected, creating a cover or carpet over the surface. Most alien species do not have a significant impact on existing ecosystems. But *D. vexillum*, that creates carpet colonies, have great potential to outcompete other sessile species by overgrowing them and thereby reducing their growth and performance. *Didemnum vexillum* can grow rapidly and spread though both sexual and asexual reproduction. Larvae resulting from sexual reproduction cannot disperse over long distances; larvae develop during the summer months and must settle within a few days. Asexual reproduction occurs all year via fragmentation of a colony. Even very small pieces that are broken off from the main colony can create new colonies, increasing the species' dispersal potential. Additionally, *D. vexillum* has few natural predators that could reduce the size of new and growing colonies.

**Methods**

VKM has based its evaluations of *D. vexillum* on a synthesis of relevant scientific articles and reports, and expert opinions. VKM also reached out to relevant experts globally that

contributed their experience to the assessments. The report includes supplementary information on morphology, systematics and ecology of sea squirts, but with an emphasis on *D. vexillum*.

**Results**

The first record of *D. vexillum* in Norway was from Engøysundet in Stavanger in November 2020. The species has now been found at five separate areas of western Norway, with dispersal leading to local spread. In September 2022, *D. vexillum* was discovered at the Koster Islands off the west coast of Sweden, close to the Norwegian border, which increases the risk of spreading to the Oslo fjord and southern Norway.

VKM considers it likely that *D. vexillum* arrived at the Norwegian coast as epigrowth on a cargo ship or other vessel. Whether there was a single introduction followed by additional spreading or several separate introductions is not known, but the affected locations are either busy ports or storage areas for petroleum installations. Small marinas nearby are also affected and can play a central role in spreading *D. vexillum* to surrounding areas. Spreading of *D. vexillum* over longer distances (>100 km) is assessed by VKM to primarily occur through larger vessels or transport of large installations, such as oil rigs. Humans thereby facilitate rapid spread over long distances.

Sexual reproduction and spreading by larval dispersal will likely have a northernmost limit close to Tromsø. The sea temperatures further north are too cold to allow larval development. VKM highlights that the largest risk for spreading is through epigrowth on vessels and subsequent fragmentation. Fragments of *D. vexillum* can grow into colonies at low temperatures and consequently have the potential to colonize the entire Norwegian coast, Bear Island and Svalbard.

VKM assesses *D. vexillum* as presenting a high risk for a number of different ecosystems in Norway: hard bottom communities, shallow cold-water coral reefs, kelp forests, maerl beds of coralline algae, eelgrass meadows, and gravel and sand bottoms containing European oysters or horse mussels. For kelp forests, VKM assesses *D. vexillum* as posing a moderate risk. However, for the kelp forests north of Trondheimsfjord, which are under strong pressure from grazing sea urchins, there is a high risk for negative effects. The main effects on kelp forest are through reduced nutrient availability, growth and reproduction. Moreover, there are effects on the associated communities within the kelp forest through overgrowth and competition for space with other species associated with kelp. *Didemnum vexillum* also has the potential to transmit diseases to different types of bivalves.

VKM has also assessed the risk for negative effects of *D. vexillum* on aquaculture in coastal Norway. For fish aquaculture, VKM notes that a need for removal of growth on installations and nets will lead to increased production costs. The increase in costs would primarily be due to cleaning of nets, shells or other structures in a way that would prevent further spread of the *D. vexillum,* but cleaning nets would also prevent reduced production due to overgrowth of aquaculture organisms e.g., mussels and oysters.

Possible measures to eliminate and prevent further spreading of *D. vexillum* from current populations are suggested at different spatial levels and time frames. Short-term measures include treatment of small marinas, private boats and equipment. More long-term measures for discovering and eliminating *D. vexillum* include implementing "clean hull"-certification for international vessels, and inspection and cleaning of vessels and installations for transit within Norway. Monitoring of populations is important both as a short- and long-term measure. Measures to remove *D. vexillum* from all habitats must be conducted with methods that will prevent spreading of fragments.

Measures carried out to prevent new introductions of *D. vexillum* to Norway, and spreading within Norwegian waters, will also increase the possibility of preventing other invasive species that can cause biofouling from entering Norway.

**Conclusions**

VKM considers it highly unlikely that *D. vexillum* can be eradicated from Norwegian waters. However, VKM assess it possible to slow down negative effects of the species on important ecosystems and habitats if measures are implemented quickly and efficiently. Slowing the spread would give time to put long-term measures in place. Measures such as monitoring and limiting spread through private boats and equipment are considered to be particularly important in the early stages of the establishment of the species. In the long term, efforts must include international collaboration to stop new introductions and long distance spreading through commercial vessels. Measures implemented against *D. vexillum* will also have effects on other alien species not yet arrived in Norway.

# Sammendrag på norsk

**Innledning**

*Didemnum vexillum* er en kolonidannende sjøpung som har sin opprinnelse i vestlige Stillehavet (Japan), og som ble registrert i Norge for første gang i november 2020. *D. vexillum* er en introdusert fremmed art i Norge, det vil si en art som er overført fra sitt naturlige utbredelsesområde med menneskelig aktivitet. Arten anses å ha stort invasjonspotensiale og vil kunne ha stor negativ økologisk påvirkning på stedegent biologisk mangfold. Den anses også å utgjøre en stor risiko for ulike former av marin kommersiell og industriell aktivitet.

Fremmede arter kan skape forandringer og problemer i økosystemer, bl.a. ved å fortrenge stedegne arter. I marine økosystemer finnes mange eksempler på fremmede arter som har spredd seg raskt og utviklet dominerende bestander der miljøforholdene er gunstige. I Norge er japansk drivtang (*Sargassum muticum*) et velkjent eksempel. Drivtangen kom til Europa fra Japan og har siden spredd seg o med drivende planterester eller som påvekst på fartøyer. Fremmede arter kan spres både ved artenes egne spredningsstadier og ved forflytning av objekter som arten er etablert på. For begroingsorganismer på fast underlag er spredning med båttrafikk en viktig kilde til spredning over større avstander.

Det finnes mange arter i gruppen sjøpunger (*fylum Chordata*, klasse *Ascidiacea*) i Norge, både solitære og kolonidannende. De kolonidannende er karakterisert ved at individene kan danne teppeformede kolonier på underlaget. De fleste fremmede arter skaper ikke nødvendigvis problemer og store forandringer i eksisterende økosystemer. Ettersom *D. vexillum* danner teppeformete kolonier, har arten imidlertid et stort potensial til å konkurrere ut andre arter ved å gro over disse og derved redusere deres livsfunksjoner og vekstmuligheter. Arten kan vokse meget raskt og kan dessuten spres både ved kjønnet og ukjønnet formering. Ved ukjønnet formering kan selv små fragmenter som brytes av moderkolonien danne nye kolonier på nye steder. Det gjør at arten er vanskelig å bekjempe fysisk, fordi små løsrevne fragmenter kan danne nye kolonier og derved intensivere begroingsproblemet. I tillegg har *D. vexillum* få naturlige predatorer som kan redusere størrelsen av nye bestander og bestander i vekst.

**Metode**

VKM har primært basert sine vurderinger om *D. vexillum* på informasjon som er tilgjengelig i internasjonale vitenskapelige artikler og rapporter, og ekspertenes forståelse av hvor relevante internasjonale funn er for norske forhold. I tillegg har det vært direkte kontakt med relevante forskere i utlandet som har bidratt med sin ekspertise. Rapporten omfatter også supplerende og relevant informasjon om morfologi, systematikk og økologi hos sjøpunger, med mest vekt på *D. vexillum*.

## Resultater

Den første registreringen av *D. vexillum* i Norge var i Engøysundet i Stavanger i november 2020. Arten er nå registrert på fem forskjellige steder på Vestlandet, og det er også påvist lokal spredning nær disse lokalitetene. I september 2022 ble det også gjort funn av *D. vexillum* ved Kosterøyene på Sveriges vestkyst nær grensen mot Norge. Dette funnet øker risikoen for spredning til bl.a. Oslofjorden og sørlandskysten.

VKM anser det som sannsynlig at *D. vexillum* har kommet til norskekysten som påvekst på skroget av lasteskip eller andre fartøy. Det er uvisst om det er snakk om én enkelt introduksjon og videre spredning fra denne, eller om det har vært flere introduksjoner, men de berørte områdene er enten travle handelshavner og/eller opplagssteder for petroleumsinstallasjoner. Småbåthavner i nærheten av disse er også berørt og det er frykt for at de kan spille en sentral rolle i videre spredning til nærliggende områder.

VKM vurderer at spredning over lengre distanser (>100km) primært vil kunne finne sted med større skip eller flytting av større installasjoner, som oljerigger. Sistnevnte type spredning betyr at arten kan spres raskt over store avstander. Det er grunn til å presisere at den største risikoen for spredning er som påvekst på fartøyer og påfølgende fragmentering (ukjønnet formering).

Kjønnet formering og spredning ved larver vil sannsynligvis ha en nordlig grense nær Tromsø. Lenger nord er sjøtemperaturen for lav til utvikling av larver. Fragmenter av *D. vexillum* kan vokse til kolonier ved lavere temperaturer, noe som medfører at arten har potensiale til å kunne kolonisere hele norskekysten, Bjørnøya og Svalbard.

VKM har vurdert at *D. vexillum* utgjør en høy risiko for en rekke ulike marine økosystemer i Norge. Det omfatter bl.a. økosystem på hardbunns-habitater, grunne kaldtvannskorallrev, ruglbunn, ålegressamfunn og grus- og sandbunn med forekomster av østers og o-skjell. For tareskogen konkluderer VKM med at *D. vexillum* på generelt grunnlag utgjør en moderat risiko. For tareskogene nord for Trondheimsfjorden, som allerede er under hardt press fra beiting av kråkeboller, er det imidlertid høy risiko for negativ påvirkning. I hovedsak dreier det seg om effekter på taren i form av redusert stoffomsetning, vekst og reproduksjon, men også for biologisk mangfold i tareskogen på grunn av overgroing og konkurranse om plass med andre organismer knyttet til taren. *D. vexillum* har også potensiale til å overføre sykdommer til ulike typer skjell.

VKM har også vurdert risiko for at *D. vexillum* har negativ innvirkning på ulike typer av akvakultur i Norge. VKM konkluderer med at fjerning av begroing på installasjoner og nøter vil kunne føre til økte produksjonskostnader for fiskeoppdrett. Kostnadsøkningen kommer særlig som følge av at nøtene må renses på en forsvarlig måte, det vil si uten at det frigjøres fragmenter av koloniene. For oppdrett av tare og skjell vil påvirkningen i hovedsak dreie seg om redusert produksjon på grunn av begroing på organismene.

VKM har gjennomgått mulige tiltak for å bekjempe og forhindre at *D. vexillum* spres videre fra allerede etablerte kolonier, og sett på positive og negative sider ved disse. f Kortsiktige tiltak inkluderer behandling av småbåthavner, private båter og utstyr. Mer langsiktige tiltak for å registrere og bekjempe *D. vexillum* inkluderer implementering av «rent skrog»-sertifisering av internasjonale skip, og inspeksjon og rensing av skip og installasjoner i transitt innad i Norge. Overvåking av forekomster er viktig både som kortsiktig og langsiktig tiltak. Tiltak som gjennomføres for å fjerne *D. vexillum* fra alle habitater må gjøres på en måte som forhindrer spredning av fragmenter.

Tiltak som gjennomføres for å hindre ny innførsel av *D. vexillum* til Norge og spredning i norske farvann, vil også kunne føre til at andre skadelige begroingsorganismer ikke etablerer seg i Norge.

**Konklusjoner**

VKM anser det som svært usannsynlig at *D. vexillum* kan utryddes fra norske farvann. Imidlertid vurderer VKM at det er mulig å bremse artens skadevirkninger på viktige økosystemer og habitater dersom det raskt og målrettet iverksettes tiltak. Ved å bremse spredning, vil det være mulig å iverksette mer tidkrevendetiltak. Overvåkning for å oppdage nye områder tidig, samt og begrensning av videre spredning med fritidsbåter og ulike typer marint utstyr, som bøyer og ruser, anses som særdeles viktige tiltak i artens etableringsfase i Norge. På lengre sikt vil det være nødvendig med internasjonalt samarbeid for å hindre nye introduksjoner og langdistansespredning med kommersielle fartøy. Tiltak som settes inn mot *D. vexillum* også vil kunne ha effekt på andre fremmede arter, som ennå ikke er kommet til Norge.

# Glossary

**Alien species:** (= non-indigenous species, =non-native species) A species, subspecies or lower taxon, introduced outside its natural past or present distribution; includes any part, gametes, seeds, eggs, or propagules of such species that might survive and subsequently reproduce (Convention of Biological Diversity – CBD; Norwegian Ministry of the Environment-Strategy on Invasive Alien Species 2007)

**Door knocker:** An alien species that is not yet established in the country or territory of concern. In Norway, it is further considered that the species would be able to establish within 50 years

**Introduced species:** Any species that has spread by a human-mediated vector intentionally or accidentally) to habitats outside its native range (International Council for the Exploration of the Sea – ICES)

**Invasive species:** A term that has been and still is used in somewhat different meanings, varying from invading species (species extending their distribution into new environments/habitats) to introduced species with harmful effects. In this report, the term refers to "A taxon whose introduction and/or spread threatens biological diversity" (Convention of Biological Diversity- Global Register of Introduced and Invasive Species)

**Invasive alien species:** An alien species whose introduction and/or spread threaten biological diversity (Convention of Biological Diversity - CBD)

**Secondary introduction:** A secondary introduction takes place as the result of an intentional or unintentional introduction into a new area, when the species disperses from that point of entry into areas it could not have reached without the initial (primary) human-mediated introduction (Norwegian Ministry of Environment- Strategy on Invasive Alien Species 2007)

**Vector:** Any living or non-living carrier that transports living organisms intentionally or unintentionally (ICES)

# Background as provided by the Norwegian Environment Agency

The Norwegian Environment Agency refers to the agreement between the Norwegian Environment Agency and the Norwegian Scientific Committee for Food and Environment (VKM) on 31 January 2019, as well as an authorization for assignments to VKM relating to risk assessment in 2021. VKM hereby agrees to carry out a risk assessment regarding establishment of the invasive species *Didemnum vexillum* along the Norwegian coast, as well as listing possible measures to prevent new establishments and further dispersion, and if possible, measures of eradication

**Introduction**

*Didemnum vexillum*, a colonial sea squirt, has been discovered at several locations on the west coast of Norway. The species was first observed in Norway on November 2nd, 2020, in Engøysundet in Stavanger by the diver Erling Svensen. It has so far been found in Engøysundet in Stavanger, at several locations in Karmøy and Haugesund, and at Askøy north of Bergen. An updated overview of confirmed findings can be seen in the Norwegian Species Map Service. *Didemnum vexillum* is an invasive alien species that naturally lives in the Pacific Ocean. The Norwegian Biodiversity Information Centre considers the species to be of very high risk with great invasion potential and high ecological effect on biodiversity. It is also considered to pose a potential risk to marine industry, with possible major negative impact.

The Institute of Marine Research, which has done some mapping of *D. vexillum* in Norway, believes it is likely that it was introduced by biofouling and international ship traffic. The species has dispersed to large parts of the world and is known in Europe from, among others, the Netherlands, France, Ireland, and the United Kingdom.

After the species was discovered in Engøysundet in Stavanger, collaboration was established between the Institute of Marine Research (IMR), the County Governor in Rogaland, Stavanger Museum, Stavanger Harbour, and Stavanger Municipality. The recommendation from the researchers was initially to map and monitor the development of the species, as well as have an overview of boat traffic in the area. In December 2020, IMR launched a monitoring and mapping project of *D. vexillum*, in collaboration with Stavanger Museum and Stavanger Diving Club, to monitor the development of the colonies in Engøysundet. Through grants from the Norwegian Environment Agency to the County Governor, Stavanger Municipality was able to initiate a mapping of 70 locations in Rogaland in the spring / summer of 2021, carried out by IMR. No new colonies of *D. vexillum* were detected. During the autumn of 2021, however, several new findings of the species has been registered in Rogaland and Vestland counties.

Internationally, attempts have been made to remove *D. vexillum* from areas it has invaded, but we do not know of any successful attempts without the species returning immediately. Measures to limit dispersion can, however, limit the growth where the species is already established and avoid introduction to new places along the coast. On its own, dispersion of *D. vexillum* to new areas takes a long time, but with the help of vectors such as ships, barges, leisure boats, fishing gear or other, parts of the colonies that grow on these can drip down to new locations and thus disperse to new places quickly.

The Norwegian Environment Agency has so far given priority to obtaining information about the situation and guidance to the authorities and persons responsible for activities in the relevant areas. Activities that can lead to faster dispersion of *D. vexillum* will be affected by the general precautionary provision in section 18 of the Regulations on Alien species.

**Assignment for VKM**

The Norwegian Environment Agency needs more knowledge about *Didemnum vexillum* in Norway as a basis for prioritizing measures to reduce possible consequences. Good understanding of the species dispersion biology, as well as an assessment of the risk and impact of the establishment, is important to be able to prioritize efficient measures. In the Action Plan on the control of invasive alien species, it is specified that "comprehensive analyses in line with the assessment instructions should be used as a basis for prioritizing which measures are to be implemented". The knowledge base and the assessment of measures from VKM will be used by the Norwegian Environment Agency to make a professional assessment of which measures are correct to implement.

**Description**

According to the risk assessment by The Norwegian Biodiversity Information Centre for *Didemnum vexillum* in 2018, the species is considered to pose great ecological impact. The species can overgrow and outcompete other species where it establishes. On gravel and stone beds, it forms a mat-shaped cover that changes the living conditions for small species that depend on protection and access to natural substrate. Strong overgrowth has been reported in shellfish farms in New Zealand. In Engøysundet in Stavanger, where the species was first discovered in Norway, it has been reported that the species now covers more than 50 % of the seabed in parts of the area. The species has been discovered in several places in Vestland and Rogaland County, and based on what is known about dispersion, it is likely that similar development and spread will occur as in Engøysundet. Both dispersal from established colonies and new imports are major threats along the coast. In addition to the ecological effects, it is expected that the species can have major economic consequences for the aquaculture industry as well as negatively affecting fishing industry, ship traffic and general activity along the coast in infected areas.

**Objectives**

Objectives for the assignment will be reviewed in collaboration with VKM after start-up. The Norwegian Environment Agency's goal is to reduce the consequences for biological diversity, and any other negative consequences the species' establishment and dispersion may have.

**Legal background**

The results from the report will be used in work with regulatory development.

**Relevant articles**

Artskart: Vis utvalg i kart | Artskart 2 (artsdatabanken.no) Fremmedartslista 2018: https://artsdatabanken.no/fremmedarter/2018/N/2199 Miljødirektoratets temaside om havnespy: Havnespy (japansk sjøpung) - Miljødirektoratet (miljodirektoratet.no)

**Conditions**

The risk assessment report must be written in English with a Norwegian summary. The report is published in dialogue with the Norwegian Environment Agency. We also refer to the collaboration agreement between the Norwegian Environment Agency and VKM.

**Deadline for submission**

The first part of the assignment will be delivered on 20 April 2022.

An evaluation of the assignment description can be done in dialogue between VKM and the Norwegian Environment Agency after the first part of the assignment has been delivered. The deadline for delivery of the assignment part two in the autumn of 2022 will be decided after part one is handed over.

# Terms of reference as provided by the Norwegian Environment Agency

**Assignment**

The Norwegian Environment Agency ask VKM for a risk assessment of the alien species *Didemnum vexillum* which is now established in Western Norway. The assignment's delivery is divided into two. In the first part, relevant immediate short-term measures (1-3 years) will be listed and assessed. In the second part, a comprehensive report shall be submitted in response to the assignment. The assignment includes two different time periods: Short term (1-3 years) and long term (3-20 years). This includes:

- Background assessment
- Knowledge of the species dispersion biology and experiences from 4 international environments where this is established.
- Description of the species' total distribution area, distribution in Norway and any areas the species does not have the potential to establish in (based on the Norwegian Biodiversity Information Centre risk assessment in the alien species list of 2018).
- Assess important dispersion paths and vectors, as well as the expected timeline for dispersion along the Norwegian coast and to Svalbard.
- Describe the null alternative (Expected development without new measures and instruments).
- Assess the potential consequences of *Didemnum vexillum* for biological diversity, industries and other activity along the coast, and the probability that these will occur. All activities the species may have consequences for must be identified. The assessment shall include a description of the uncertainty in the estimates.
- Describe possible measures that can limit the spread of established colonies, combat established colonies and prevent new colonies from establishing.
- Identify current risk-reducing measures.
- Suggest any action plan towards goal achievement.
- Give a description of the positive and negative consequences of the relevant measures, compared with the null alternative. Including assessment of uncertainty compared to risk described under the null alternative.

# 1 Introduction

## 1.1 The carpet tunicate *Didemnum vexillum*

The carpet tunicate *Didemnum vexillum* Kott, 2002 (Norw. *havnespy*, *japansk sjøpung*) is an invasive alien species of marine animal that is native to Japan but presently spreading to major temperate cold-water seas around the world. In invaded areas, the species has the capacity to outcompete native species, deteriorate environmental integrity, and cause significant economic harm to the marine aquaculture industry (McKenzie et al. 2017). The species is a biofouling organism that can spread over long distances by shipping or other human-mediated transport activities. *Didemnum vexillum* was first reported from Norwegian waters in autumn 2020. In the latest Norwegian risk assessment of alien species (2018), it was included as a door knocker and classified to the highest risk category 'severe impact' due to its anticipated environmental impact and a high potential for establishment and spread (NBIC 2018)[1].

## 1.2 Biofouling

Biological fouling or biofouling is a term used to refer to the settlement and development of aquatic organisms primarily on man-made structures such as ship hulls, docks and piers, water inlets, underwater pipelines, and aquaculture farm infrastructure. However, the term is also used to describe unwanted epigrowth on natural surfaces (Bannister et al. 2019). In this assessment, we use biofouling also to describe growth on natural substrates and other organisms.

The International Maritime Organization (IMO) defines biofouling with a focus on anthropogenic activities: "accumulation of aquatic organisms on surfaces and structures immersed in or exposed to the aquatic environment" (IMO 2011). Biofouling organisms comprise all types of micro-organisms, plants, algae, and invertebrates that can attach to surfaces. Biofouling also includes boring organisms that are capable of making holes in wood, shells, rock and other substrates. The boring organisms causing damage to shellfish aquaculture include clionid sponges, molluscs, and worms (Bannister et al. 2019). Other economically important borers include molluscs and crustaceans that bore into wood piles supporting piers and other wooden support structures in marine environments (Lopez-Anido et al. 2004). In this report, we focus mainly on *D. vexillum* but also briefly discuss other macro-organisms growing on the surface of marine structures to describe the problem.

[1] https://www.artsdatabanken.no/taxon/Didemnum%20vexillum/126850

### 1.2.1 Important biofouling organisms world-wide

More than 4000 species have been identified in fouling communities on ships. The most common groups of fouling macrobiota are barnacles, tubeworms, bryozoans, mussels, ascidians, and algae (Davidson et al. 2009, Bressy and Lejars 2014). The extent of biofouling on a man-made structure is dependent on time deployed in the sea, movement and drag actions on the structure, substrate surface characteristics and antifouling measures, such as antifouling coating and cleaning of the surface. On a clean surface, a biofilm starts to develop after a short time in the sea, soon followed by settlement of fast-growing opportunistic species, such as barnacle larvae and, if surface is exposed to light, green algae. When the structure is immersed in seawater for a longer period, a more advanced biofouling community starts to develop that can harbour crustaceans, corals, polychaetes, sponges, hydroids, cnidarians, and even small fishes in between thick layers of barnacles and mussels.

### 1.2.2 Introduction of alien biofouling organisms

Transport of alien species with ships from one biogeographic region to another in ballast water, with ballast weight (stones) or as biofouling on vessel hulls has been recognized since preindustrial times and has led to the global spread of a wide variety of biofouling organisms. Opening of canals through continents, such as the Panama and Suez canals, has led to greater spreading of alien organisms among ocean basins. Shipping activity has increased worldwide in the past 50 years (Brooks and Faust 2018). With the implementation of the international Convention for the Control and Management of Ships' Ballast water and Sediments by IMO, which requires treatment of the water before it is released in a new harbour, the problem with alien marine species in ballast water has diminished, but still is an issue as a domestic pathway. Similar regulations for biofouling action have been suggested by the International Maritime Organization (IMO 2011b). Compliance is voluntary but there is ongoing work to legislate international regulations. The biofouling guidelines focus on keeping the hull of the vessel clean and protected with anti-fouling coats at all times. The global ban of TBT (Tributyltin) in 2008 in anti-fouling paint, which led worldwide to the masculinisation of females of certain marine snails ('imposex' disorder), significantly decreased the effect of the paint. Over time, more efficient and environmentally friendly treatments have been developed. Vessels can be cleaned by dry docking, by divers or remotely operated underwater vehicles (ROVs) designed for cleaning, some with suction systems that allow collection of biological waste. Such ROVs are currently most efficient in an early stage of the fouling when a thin biofilm occurs and are less efficient when the vessel is heavily fouled. Normally, the smooth portion of the hull is easy to clean while difficult areas around the propellers, and sea chests, moon pools and other recesses in the ship's hull are often left unattended.

Alien biofouling organisms can also be spread as 'hitch-hikers' with transport of commercial species from one region to another for aquaculture. In Europe, several alien biofouling organisms are suspected of having been introduced with importation of Pacific oysters for commercial cultures that starting in the 1960s.

Biofouling on vessels leads to increased frictional drag on the hull which requires greater energy use during transit and most shipping companies clean their vessels regularly to avoid this. However, niche areas like propellers and ports in the hull of the vessels on the vessels are mostly left unattended. For larger types of marine infrastructure, such as oil rigs and barges, cleaning can be difficult and costly. There is also growing evidence that biofouling organisms on vessels can carry pathogens from one area to another that may have detrimental effects on wild and farmed mollusc species (Georgiades et al. 2021).

Biofouling can also be a problem for marine constructions, such as the submerged parts of wind turbines. In the fish farming industry, biofouling on net pens is a huge problem. Biofouling organisms thrive on the nets as they have surplus nutrients and small particles of organic waste, however they prevent the flow of water through the nets. The solution is to treat the nets with antifouling compounds, mainly copper-based, or to clean the nets regularly during the summer- and autumn seasons. Due to accumulation of copper in the sediments around fish farms, many farmers have started to use untreated nets with procedures for frequent cleaning.

### 1.2.3 Spread of alien marine species to the Norwegian coast and within Norway

#### *1.2.3.1 Vectors*

After the legislation of the International Convention for the Control and Management of Ships' Ballast Water and Sediments in 2017, biofouling on vessels is considered to be the most important vector for spread of alien species into the Norwegian coast (Husa et al. 2022). Import and release of alien species into the environment is strongly regulated in Norway, but other paths of introduction and vectors, such as the aquarium trade, import of shellfish for consumption, transport of live cleaner fish for salmon lice treatment, transport with fishing gear, and transport with floating debris can still play a role in their spread. The areas of Norway with the highest risk for introduction of fouling organisms are the Oslo fjord area and the southwest coast (Rogaland and Vestland Counties), as harbours in these areas receive most vessels directly from foreign ports (Husa et al. 2022). Further dispersal of established alien marine species into new areas within Norway likewise happens with the same vectors. However, transport of propagules (larvae, spores, fragments) can also happen with domestic ballast water as there are no regulations on intake and release within Norwegian areas. Larvae and fragments can also be transported together with farmed fish in well-boats with tanks for storage and transport of live fish. Biofouling organisms, such as *D. vexillum,* can also be transported from one area in Norway to another by transport of any object that has been in the sea at an infected area for a while. Floating fenders and jetties, barges used for various purposes, and equipment for the aquaculture industry are frequently transported among Norwegian ports.

#### *1.2.3.2 Secondary introductions and self-dispersal*

Several alien marine species in Norwegian waters are present due to so-called secondary introductions – alien species that have been introduced to neighbouring countries and then subsequently spread to Norway, either by human-mediated dispersal or by self-dispersal. Unaided self-dispersal can occur in species where adults or propagules are able to swim, crawl or follow the currents over long distances from neighbouring countries where the species are established. Species with short pelagic or non-swimming larval stages, as in most ascidians, normally do not spread far from the source population and hence largely depend on man-made vectors or natural floating material to disperse over longer distances. When growing on seaweed or seagrass, long-range drifting can occur if the plants detach. Similarly, fragments or detached specimens of colonial species may drift over longer distances, depending on speed-rate of sinking and currents, but this has not been studied.

## 1.3 Tunicates: subphylum Tunicata – class Ascidiacea

The authors have decided to include some general information about the subphylum Tunicata and especially the class Ascidiacea and selected species within the class in this report. The main reason is that the class includes species in addition to *Didemnum vexillum* that already are causing fouling problems or may cause problems in the future in Norwegian waters. The selection of species is primarily limited to species occurring in Norwegian waters. The diverse ascidian class has around 3000 species world-wide (Shenkar et al. 2023) and is not only regarded as a class creating fouling problems. Several species are collected as a food source, and some species are even cultured for food and feed (Lambert 2005).

### 1.3.1 Natural history

In scientific classification, the tunicates (Tunicata or Urochordata) are classified as 1 of 3 subphyla within the animal phylum Chordata, which otherwise includes the cephalochordates and vertebrates. Tunicates lack a backbone but possess a notochord at some time in the life cycle, which is a stiff rod-shaped structure on the dorsal side of the body. All tunicates are marine. Most species belong to the class Ascidiacea, usually called sea squirts, which are benthic as adults and usually attached to a hard surface, but some species live in mud or sand. The larvae are free-swimming and of quite different shape than the adult, looking like a microscopic tadpole. The tail of the larva is supported by a notochord, but the notochord degenerates and is lost when the larva settles on the bottom and develops into the sessile adult form.

All ascidians are water filtering organisms. The adult body is typically more or less barrel-shaped and has openings for water intake (oral siphon) and water outlet (atrial siphon or cloaca), usually situated terminally (Figure 1.3.1-1). The body is covered by an external tunic or test consisting primarily of polysaccharides quite similar in chemical structure to that of cellulose (tunicin). Internally the body consists of an anterior part with a large water filtering net (branchial sac) and a posterior part with stomach, intestine, and gonads. The oral siphon leads into the branchial sac which is surrounded by an atrial chamber leading to the atrial siphon. Details of the branchial sac, shape of stomach, location and shape of gonads and coiling of intestine are of taxonomic importance and usually need to be examined for morphological identification of the species. The arrangement and number of slits (stigmata) in the branchial sac is of particular importance.

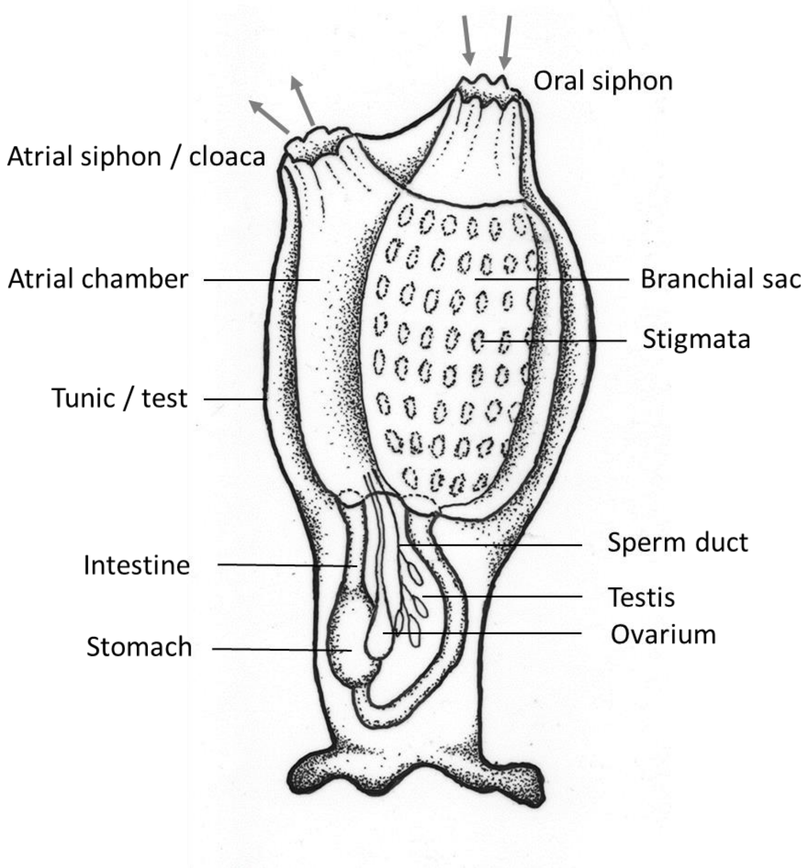


**Figure 1.3.1-1:** General structure of ascidians. Redrawn from Lützen (1967) by Eivind Oug.

Ascidian species are either solitary or colonial. Colonial species attain different appearances compared to solitary species and can look like undefined masses of jelly, spherical or oval balls, or depressed carpets depending on the shape of the substrate. Colonies often cover more than one square meter. The individual specimens (zooids) are embedded in a common tunic of the colony. Each zooid has an oral siphon for water intake, and the atria of several zooids are connected and lead into common and usually enlarged openings for water outlet (cloaca). In some species, the zooids are arranged in regular star-shaped or longitudinal patterns and can easily be discerned, whereas in other species they are more scattered and totally covered by the tunic.

Larvae of ascidians are planktonic, but the duration of the free-swimming stage can be very short. In some species, it is not uncommon that the larva uses the mother individual as substrate. Two or three generations of the same solitary species attached to each other are therefore not uncommon. Such aggregates of solitary ascidians are not regarded as colonial ascidians.

## 1.3.2 Solitary ascidians

Solitary species can reach a size of several centimetres and usually look like a vase or a bag with oral and atrial siphons that are usually located terminally. A well-known example in Norwegian waters is the common, cosmopolitan, and often very abundant *Ciona intestinalis* (L., 1767), a fouling species of ship hulls and other man-made structures in the marine environment. *Ciona intestinalis* can reach a length of 30 cm, but lengths between 10 and 20 cm are most common. It is usually greenish or whitish and has strong longitudinal muscle bands that are visible through the tunic and serve to recognize the species (Van Name 1945). The species can actively pump water into the branchial sac through the oral siphon, whereas water pumping from muscular activity is otherwise not common among ascidians. In localities with a surplus of organic particles in the water, *C. intestinalis* can grow fast and attain high densities in a short time. *Ciona intestinalis* is native to European waters but has been introduced to other areas of the world where it is considered a pest organism.

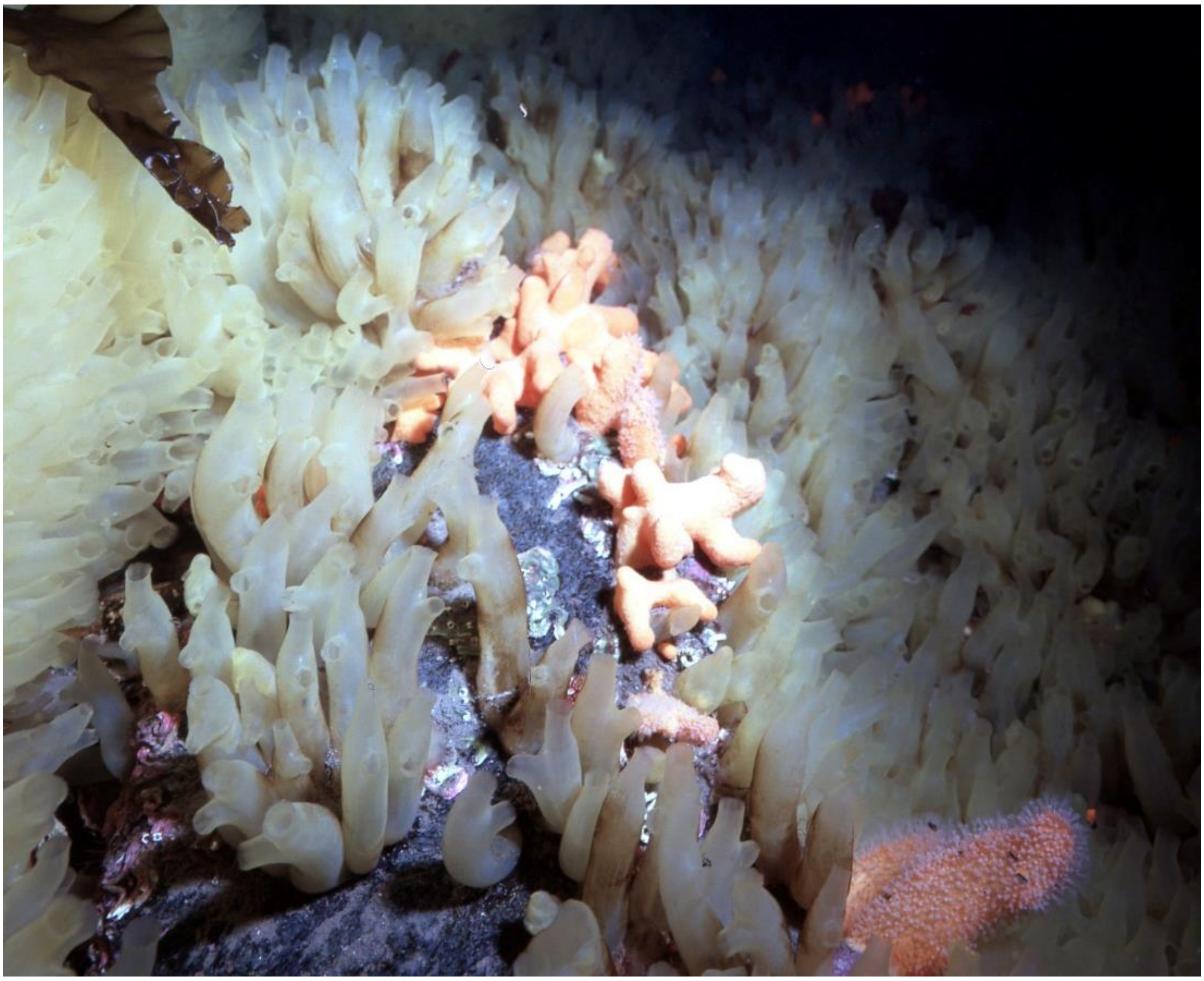

**Figure 1.3.2-1:** The solitary vase tunicate *Ciona intestinalis* can occur in large aggregations. (Photo: Bjørn Gulliksen).

Other solitary species may be more rounded in form and can be transparent or opaque, and several are brilliantly coloured with red, yellow or brown hues. Some species can be overgrown by small algae, hydroids, and bryozoans. Several species form aggregations where individuals of different taxa are densely packed together.

## 1.3.3 Colonial ascidians

In colonial ascidians, the individual animals are generally very small, but the colonies may reach sizes of several square meters. The colony grows by budding of new individuals from existing specimens, allowing rapid colony expansion.

### *1.3.3.1 Family Didemnidae*

The species *Didemnum vexillum* is taxonomically placed in the family Didemnidae, which is here treated in some detail providing a background for further assessments of *D. vexillum*. Didemnidae comprises about ten genera and, according to the Ascidiacea World Database (http://www.marinespecies.org/ascidiacea/), 237 species are recognized as belonging to the genus *Didemnum.*

All species of Didemnidae are colonial, usually flat and incrusting, sometimes thick and massive and usually attached to the substrate by a broad base (van Name 1945). The zooids are generally much smaller than in any other group, very numerous and are scattered around in a common tunic (Figure 1.3.3.1-1). The specimens are located separately in the tunic but have common canals for outflowing water (cloaca). The tunic (test) commonly contains minute calcareous objects (spicules) that protect the colonies from grazing.

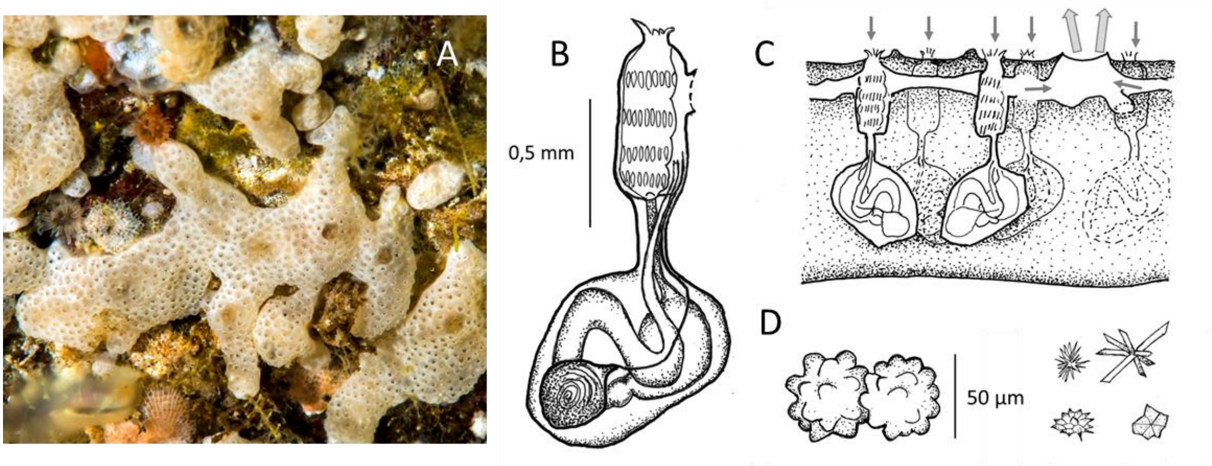


**Figure 1.3.3.1-1:** Characteristics of family Didemnidae. Left: Fotograph showing the shape of encrusting colony (*Didemnum helgolandicum*), the surface dots are water intake openings of individual specimens (Photo by Erling Svensen). Right: B) A single individual (zooid) of *D. helgolandicum*. C) Section through tunic showing location of zooids and common water outflow canals. D) Spicules (microscopic) from different species. B, C, D. Redrawn from Millar (1966) and Lützen (1967) by Eivind Oug.

The identification of species is difficult, partly because many species are morphologically similar and easily confused and partly because morphological characters may be difficult to observe. For instance, already van Name (1945) referred to problems by noting that colonies were too opaque to study in a living state, and that zooids, when preserved, usually attained a violently contracted condition that made zooid internal organs difficult to observe.

### 1.3.4 Ascidians in Norway

Presently, 97 species of ascidians are known from the Norwegian coast, the Svalbard archipelago (including Bear Island), Jan Mayen, and neighbouring oceans (Artsdatabanken 2022). Most species were treated by Millar (1966) in a reference work of Scandinavian ascidians that includes distribution maps, notes on substrate preferences, and keys for identification based on morphology and anatomy. Most species live in shallow waters or at moderate depths (< 200 – 300 m), but a few species have been recorded in deep waters. Millar (1966) reported 85 species in Norway, of which 26 species are colonial. The present number of species is mainly due to new records and introductions of alien species since the 1960s.

Millar (1966) reported five genera in the family Didemnidae from Norwegian waters (*Trididemnum, Leptoclinides, Didemnum, Diplosoma,* and *Lissoclinum*). He separated the genera by the number of rows of branchial stigmata (three or four), how the lower part of the sperm duct is coiled, morphology of the atrial opening, and morphology of the calcareous spicules in the tunic. The genus *Didemnum* is characterised by having four rows of stigmata, a coiled sperm duct and an atrial opening without a siphon (Millar 1966). The genus currently comprises seven species in Norwegian waters (Artsdatabanken 2022). Two of the species were reported by Millar (1966) (*Didemnum albidum* Verril, 1871 and *Didemnum helgolandicum* Michaelsen, 1921) and two species have been described later (*Didemnum romssae* Marks, 1996 and *Didemnum vexillum* Kott, 2002). The three additional species have few records in Norwegian waters and may be synonyms or specimens that were identified incorrectly.

Another group of colonial ascidians in Norway is the carpet-forming botrylloids in the family Styelidae. In this group, the zooids are easily recognizable and arranged in distinct patterns. The genus *Botryllus,* with the species *B. schlosseri,* forms fleshy and encrusting colonies often several centimetres across but usually not more than 1–2 cm thick. The zooids are grouped in a stellate or oval system around a cloacal opening. The genus *Botrylloides* is similar in appearance to *Botryllus* but the zooids are arranged in long rows or chains. Millar (1966) reported three species from Norwegian waters: *Botryllus schlosseri* (Pallas, 1766), *Botrylloides leachii* (Savigny, 1816) (Figure 1.3.4-1, left and right respectively), and *Botrylloides aureus* Sars, 1851.

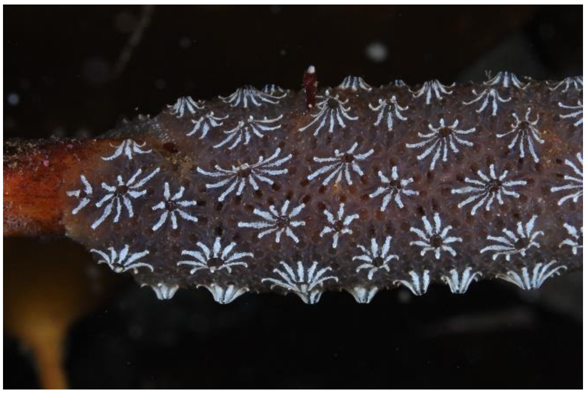


**Figure 1.3.4-1:** Left: *Botryllus schlosseri* (Photo: Erling Svensen). Right: *Botrylloides* sp. (Photo: Bjørn Gulliksen).

## 1.3.5 Alien ascidians in Norway

In addition to *Didemnum vexillum*, three other alien ascidians have been recorded from Norway—the solitary species *Corella eumyota* and *Styela clava*, and the colonial species *Botrylloides violaceus*. There has also been much discussion about whether a fourth species, *Molgula manhattensis*, is native or an alien species (see below).

*Corella eumyota* is a recent invader that was recorded at Egersund, southwest Norway, in January 2022. In the latest Norwegian risk assessment of alien species (2018), the species was included as a door knocker and classified in the next highest risk category 'high impact' due to its anticipated environmental impact and a high potential for establishment and spread (NBIC 2018). The species is native to the waters around Antarctica and the southern hemisphere and was first reported in Europe (Bretagne, France) in 2002. It is a gelatinous sea squirt with a relatively thin and transparent tunic and may reach sizes up to 8–10 cm. The larvae have a short pelagic life-stage and settle within a few minutes after they are released. Individuals are therefore often found living close together forming dense clumps. The species is epifaunal and can be found attached to a variety of hard substrates. It may become a nuisance species in aquaculture by growing on nets and lines and by reducing the growth of cultured bivalves via competition for food.

*Styela clava* was recorded for the first time in Norway in the 1990s (NBIC 2022)[2] and is now a common species in the south-western part of Norway (Figure 1.3.5-1). It is native to the north-western waters of the Pacific Ocean and was probably brought to Europe as epifauna on fouled warships returning with international forces from the Korean war in 1952 (Minchin & Duggins 1988). It has a tough, leatherlike and usually brownish tunic with lighter warts. Larger specimens (up to 20 cm in length) have a stalk that raises the body with the oral siphon from the substrate and gives the species an advantage compared to many other filter-feeding species. It could pose a problem in mussel and oyster farming by outcompeting

[2] https://www.artsdatabanken.no/taxon/Styela%20clava/120283

the cultured species for space and food. It is also a fouling organism, which may attach to man-made structures such as ship-hulls, propellers, rudders, and intakes and outlets of submarine pipelines, and installations of aquaculture farms.

The colonial carpet species *Botrylloides violaceus* is native to the Northwest Pacific from northern Japan to southern Korea. It spread to the north American Pacific coast in the 1960s and was first recorded on the coast of the eastern US in the late 1970s where it now is an abundant component of fouling communities. The transport of the species from the west coast of the US to the east coast was done by a scientist who released the species in the Eel Pond in Woods Hole in 1973 to have a supply of experimental animals (Carlton 1989). *Botrylloides violaceus* may encrust bottom areas in colonies exceeding one square meter in size and varies in colour, ranging from purple, red, yellow, and brown (Snowden 2008). The thickness usually varies between 2 and 4 mm. The tunic is soft, and the zooids are organized in ladder-like chains. The zooids have 10 to 11 rows of stigmata and 9 to 12 stomach folds. The zooids are hermaphroditic, and eggs may be self-fertilized or fertilized by sperm from neighbouring colonies. The larva is up to 3 mm in length and released fully developed from a common cloacal opening. The pelagic stage usually has a short duration of 4 to 10 hours (Saito et al. 1981).

The first specimens of *B. violaceus* in the Northeast Atlantic were collected from the lagoon of Venice, Italy in 1993 (Zaniolo et al. 1998), and the species has since been recorded from several other European localities: Netherlands (2000), British waters (2005), and three locations in Spain (2009). The exact time of arrival to Norway is difficult to assess but it seems to have arrived in the early twenty-first century based on available underwater photographs of botrylloids from the Norwegian coast. The species is not included in the reference work by Millar (1966) treating Scandinavian ascidians, but it is now accepted that this species is relatively common in Norway. The species has a morphology quite similar to *Botrylloides leachi*, and it is difficult to separate the two species based on external examination.

In 2017, *B. violaceus* was recorded by DNA metabarcoding of sediments from Svalbard (van den Heuvel-Greve 2021), and a thorough study of occurrence of botrylloids from the Norwegian coast would probably reveal that the species is quite common.

A curiosity is that Milne-Edwards (1841) presented a species called *Botrylloides violaceus* from the English Channel, but the drawing is not detailed enough to establish whether the specimen was the same species we now accept as *Botrylloides violaceus* (Figure 1.3.5-1).

There are several reports indicating that *B. violaceus* is frequently recorded on submerged man-made structures, such as pier pilings, aquaculture structures, and boat hulls, and it is also reported that the colonies frequently displace other fouling organisms (Carman et al. 2010, 2016, Davidson et al. 2009; Simkanin et al. 2012, 2013). This colonial ascidian is therefore assumed to potentially have distinct impact on shipping, aquaculture and fishery of benthic species, such as clams and oysters.

**Figure 1.3.5-1:** Left: *Botrylloides violaceus*. Right: *Styela clava*. Photos: Erling Svensen

*Molgula manhattensis* is a solitary ascidian, normally globular with a diameter of 20 to 25 mm, but specimens measuring up to 35 mm in diameter have been recorded. The atrial siphon may attain half the body diameter in length with a square aperture, while the oral siphon is shorter, stouter and has a six-lobed aperture. The siphons are fairly close together. The test is grey or pale green and may have hair-like processes, which often catch particles, such as sand grains, mud, and shell-fragments that may disguise the morphology of the species but is also a characteristic in other species in the genus. *Molgula manhattensis* may live in highly polluted water, and it is also one of the few ascidians that will live in water of somewhat lower salinity (van Name 1945).

There are currently 12 species of *Molgula* along the Norwegian coast (NBIC 2022)[3], and conclusive identification to species level cannot be done without dissection and examination of the anatomy. Species of *Molgula* have gonads on each side, and *M. manhattensis* is characterized with six folds on each side of the branchial sac (Millar 1966). The other species in this genus in Norway have either five or seven folds. A difference in the orientation of the long axis of the gonads on the left and right side can also be used to verify the identification of *M. manhattensis*, and this difference can sometimes be observed through the test of 'clean' animals', according to van Name (1945).

The species produces many tiny eggs that develop into small tadpole larvae whose development takes place outside the body. It is rare to find any larvae in the parent individual. The planktonic stage of the larvae lasts from 1 to 10 hours (van Name 1945).

*Molgula manhattensis* is one of the most common, abundant, and conspicuous solitary ascidians along the US Atlantic coast (van Name 1945). However, whether populations of the east Atlantic coast of Europe are indigenous or not is unclear (Millar 1966, Lützen 1967). Haydar et al. (2011) sequenced the mitochondrial gene cytochromeoxidase I (COI) and concluded that '*Molgula manhattensis* is native in north-east America. However, whether it was introduced or is native to Europe remains equivocal'. The species was recorded at Jan

---

[3] https://www.artsdatabanken.no/taxon/Molgula/107800

Mayen in 1978 (Gulliksen et al. 1980). In 2017, the species was also recorded by DNA-metabarcoding of sediments from Svalbard (van den Heuvel-Greve 2021).

*Molgula manhattensis* primarily inhabits shallow water (ca. <30 m depth), often growing in abundance on pier pilings, bottoms of anchored boats and floats, and other substrates where it frequently forms large groups or masses, or on eel grass, but it can also be found buried in soft sediments (Van Name 1945). This solitary ascidian is assumed to have some impact as a fouling species in shipping and aquaculture but has not been assessed to have any negative impact on biodiversity in Norwegian waters and The Norwegian Biodiversity Information Centre (2022) has categorized it as having "low impact" in Norway[4].

## 1.4 Biology of *Didemnum vexillum*

### 1.4.1 Taxonomy and identification

#### *1.4.1.1 Morphological identification*

*Didemnum vexillum* was first described in New Zealand by Patricia Kott in 2002. The species was at that time considered to be a native but little-known species in New Zealand (Kott 2002). It has later been confirmed that the species was introduced, and at that time had also been introduced to Europe, USA, and Canada. In these areas, it was confused with native species or simply referred to as an unknown species of *Didemnum* (*Didemnum* sp. A) (Lambert 2009, McKenzie et al. 2017). The long-lasting confusion reflected the challenging identification of species of *Didemnum* due to the morphological similarity of several species from different parts of the world (Lambert 2009, McKenzie et al. 2017). With the advance of molecular tools in species taxonomy and systematics, identification has become more certain (Stefaniak et al. 2009, Graham 2015). Both mitochondrial genes and nuclear genes have been successfully sequenced from colonies around the world and have verified that one single species is involved. The analyses have also provided evidence that the most likely area of origin was Japan (Lambert 2009, Stefaniak et al. 2009).

In the original description, Kott (2002) described several characteristics of the colonies of *D. vexillum*, including extensive growth, the tendency to overgrow other organisms (sea weeds and worm tubes), the formation of flexible irregular frond-shaped cylindrical or branched outgrowths projecting from the surface, and the arrangement of zooids along common deep cloacal canals that extend the full depth of the zooids (Figure 1.4.1.1-1). On vertical substrates or on overhangs, the outgrowths develop into long fleshy lobes (tendrils). The species name *vexillum* (Latin for flag or banner) refers to the waving of the tendrils in currents. The colonies usually attain sizes of 10–50 cm in diameter or more, and adjoining colonies can cover several square meters of bottom substrate. Colonies can also have a

[4] https://www.artsdatabanken.no/fremmedartslista2018

varying degree of distinct surface pattern of veins from cloacal canals that are visible in the tunic, depending on the colony size.

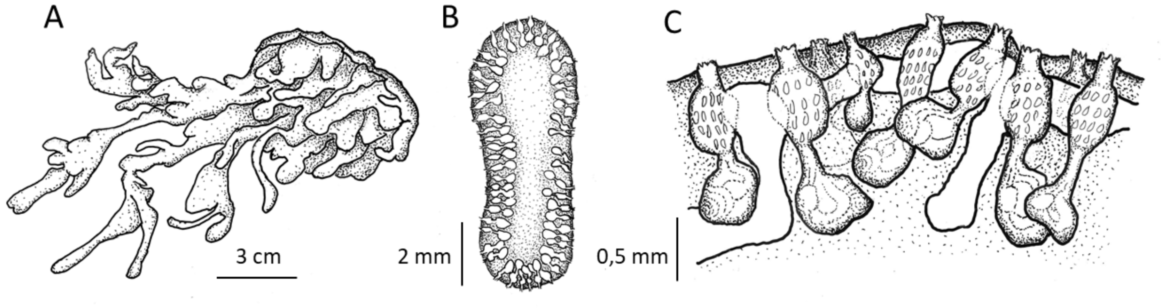


**Figure 1.4.1.1-1:** *Didemnum vexillum*. A: part of colony with well-developed tendrils. B: cross-section of colony lobe showing layer of zooids at surface. C. vertical section of tunic showing zooids and cloacal canals. Modified from Kott (2002) by Eivind Oug.

The colonies recorded as *D. vexillum* from Norway agree with the morphological and ecological descriptions given by Kott (2002), Stefaniak et al. (2009), and Lambert (2009). The initial identifications were based on external features, in particular the growth form, development of large and small lobes, and vein patterns on the colony surfaces, characteristics that are rare in other Norwegian species of *Didemnum.* Lambert (2009) presented underwater pictures of *D. vexillum* colonies that look similar to colonies recorded in Norway. In addition, underwater pictures of Norwegian colonies have been verified by international specialists (among them Gretchen Lambert) to be *D. vexillum.* Characteristics of Norwegian colonies are illustrated in Figure 1.4.1.1-2. However, it should be borne in mind that other species of *Didemnum* are present in Norway, and the potential chance of misidentification is great for non-experts.

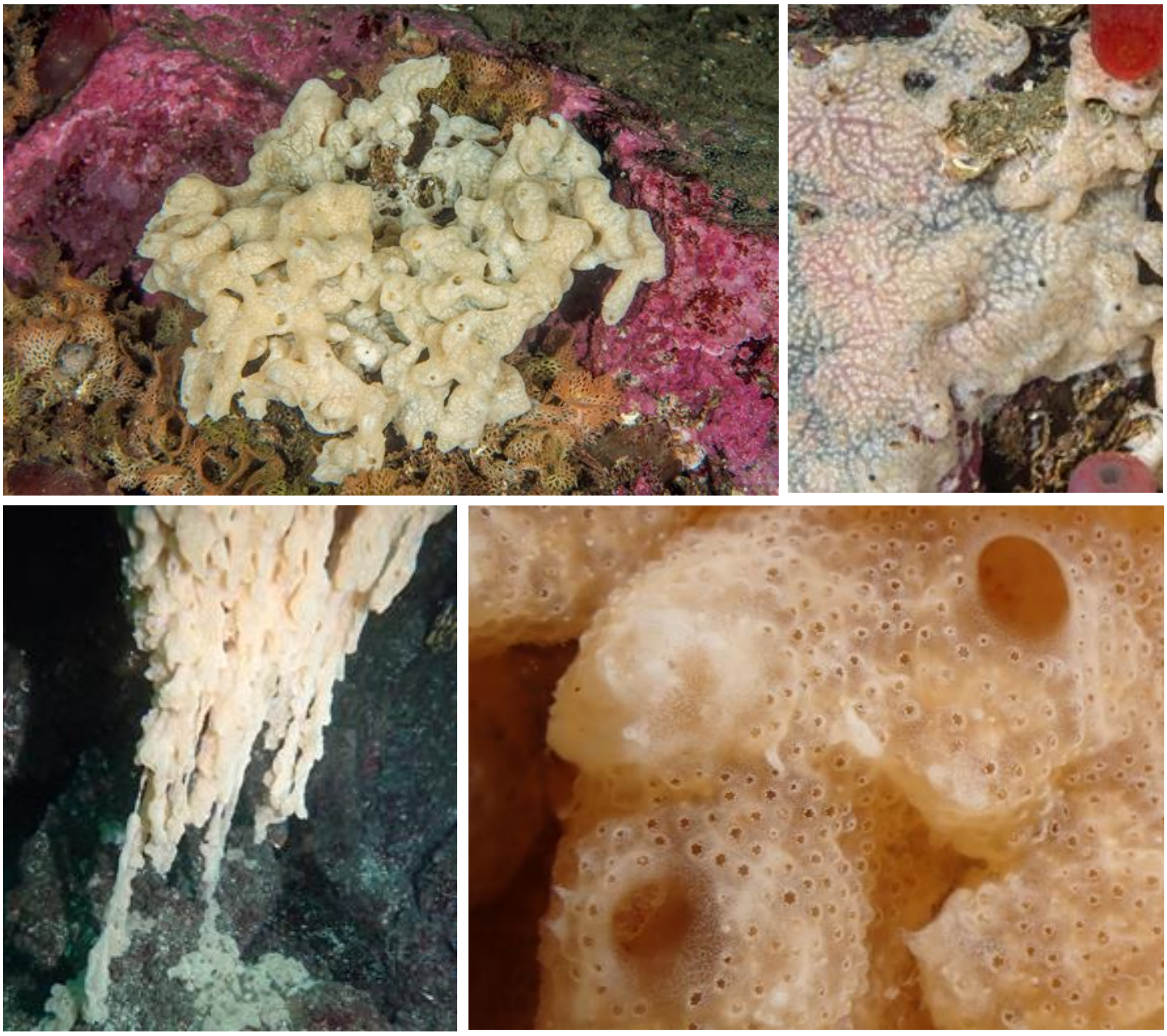

**Figure 1.4.1.1-2:** Colonies of *Didemnum vexillum* from South-Western Norway. Upper left: Colony overgrowing encrusting alga (red) and bryozoa (brown). Upper right: colony with well-developed surface pattern of veins. Lower left: colony from overhang with long tendrils. Lower right: close-up of colony showing two exhalant siphons (cloaca) for water outflow and numerous openings of oral siphons of individuals for water intake. Photo: upper row Erling Svensen, lower row Anne Mari With Ottesen.

The addition of a new congener has now led to increased difficulty in correct identification of *D. vexillum* based solely on external morphological characters. Turon et al. (2020) described a new species, *Didemnum pseudovexillum* that coexists with *D. vexillum*, and there is virtually no external difference between the two species, but examination of the spicules and larvae morphology will show the difference (Turon et al. 2020). See also Figure 1.4.1.1-3 for species that are often misidentified as *D. vexillum*.

**Figure 1.4.1.1-3:** Other marine organisms can potentially be mistaken for *D. vexillum* by inexperienced observers. Upper left: The whitish layers are sponges (Photo: Bjørn Gulliksen). Lower left: the ascidian *Diplosoma listerianum* (Photo: Erling Svensen). Upper right: mat of whitish bacteria on the bottom below seawater cages with cultured salmon (Photo: Bjørn Gulliksen). Lower right: Breadcrumb sponge *Halichondria panicea* (Photo: Erling Svensen).

#### *1.4.1.2 Genetic identification*

In its native area Japan, the species is differentiated into two main genetic clades, clade A and clade B, but only clade A has spread worldwide (Stefaniak et al. 2012, Casso et al 2019). The genome of *D. vexillum* has also recently been described (Parra-Rincón et al. 2021). The first records of *D. vexillum* in Norwegian and Swedish waters have been confirmed by genetic methods. To be able to quickly detect and monitor *D. vexillum* over large areas, analysis of environmental DNA (eDNA) has been demonstrated to be a useful tool (Matejusova et al. 2021, Fossøy et al. 2022, Gargan et al. 2022). A species-specific genetic marker for *D. vexillum* has been developed, allowing detection of the species through analysing water-samples using qPCR (quantitative Polymerase Chain Reaction) (Gargan et al.

2022). However, e-DNA methods in the marine environment have some uncertainties depending on current speed and direction, varying amount of emitted DNA and degradation rates of DNA from different species and taxonomic groups, which might make it difficult to decide on the distance from a positive water sample to the organism and also to be sure that a negative sample means that the species is not present in the area.

### 1.4.2 Habitat and environmental tolerance

*Didemnum vexillum* colonises both natural and artificial hard substrates. Benthic hard bottoms, kelp forests, and pebble areas are attractive sites of attachment, as well as vessel hulls, harbour facilities, undersides of wharves, and fishing and aquaculture equipment. Although sheltered areas, where wave action and sedimentation are minimized, appear to be preferred (Bullard et al. 2007, McKenzie et al. 2017), the species is fully capable of colonising off-shore areas with higher water movement, such as Georges Bank in the northwest Atlantic where 230 $km^2$ of pebble-gravel habitat has been colonized (Bullard et al. 2007, Valentine et al 2007). Shifting sandy substrates appear not to be colonized (Valentine et al. 2007).

*Didemnum vexillum* is found in temperate waters in both coastal and offshore environments, from lowest intertidal to at least 81 m depth (Bullard et al. 2007). It prefers temperatures between 14 and 20°C but tolerates from -2 to >24°C, with daily fluctuations of up to 11°C (Valentine et al. 2009, Bullard et al. 2007). *Didemnum vexillum* has its highest growth rates in marine environments with a salinity >26 psu (Bullard and Whitlatch 2009). Experiments from the Irish sea, which has a salinity of 34 psu, shows that survival was reduced by 20 % when salinity was permanently reduced to 27 psu and by 40 % at a salinity of 20 psu. *D. vexillum* showed no mortality during short term exposure to 10 psu (2h every second day for seven days) (Gröner et al. 2011). The species is found in the Dutch Wadden Sea (Netherlands) at salinities between 21.8 and 25.6 psu (Gittenberger et al. 2019). It does not tolerate desiccation very well and exposure to air for more than 3 h per day over a month is lethal, although time required will vary with location and seasonal climate (Valentin et al. 2007a, 2007b).

### 1.4.3 Feeding

Like other ascidian species, *D. vexillum* is an active filter feeder, where adults primarily feed on phytoplankton but also on particulate organic matter, diatoms, and suspended bacteria; food items are usually between 0.5-2 µm, although the ascidian can ingest larger particles (Lambert 2005, Daniel and Therriault 2007). Ascidians are capable of filtering large amounts of water, but the filtering capacity of colonial ascidians is difficult to determine and is not known (Daniel and Therriault 2007). As a filter feeding organism, *D. vexillum* is likely vulnerable to smothering by fine sediment that blocks siphons and causes cessation of water pumping (Valentine et al. 2007), but this has so far not been validated (Fletcher et al. 2018). At the location Skipavika in Norway, discharge of finely ground stone in the vicinity of colonies of *D. vexillum* does not appear to affect them (Vivian Husa, IMR, pers. obs.). Larvae rely on a yolk sac and do not actively feed.

## 1.4.4 Reproduction and growth

### *1.4.4.1 Asexual reproduction*

Growth through asexual reproduction takes place in two ways. Colonies can grow by budding, where each zooid buds off a genetically identical zooid called a blastozooid and through this expands the colony size from the original settled larva (McKenzie et al 2017). Growth can also occur through fragmentation, when a piece of the colony breaks off, floats away to settle in a suitable habitat and continue to grow. New colonies established by fragmentation will be genetically identical to the mother colony. Even small fragments can establish new colonies (Morris and Carman 2012). Fragmented pieces can also contain eggs and larvae that can be released for up to three weeks, further increasing the spreading potential (Morris and Carman 2012).

*Didemnum vexillum* can form chimeric clones (Rinkevich and Fiddler 2014, Weinberg et al 2019) which likely are stable over time (Casso et al. 2019). Chimerism is a union of different colonies to produce a genetically composite entity, an individual colony combined of multiple genotypes. Chimeras may last for the lifetime of the colony or only for a few days. Chimeric colonies have greater genetic variability and possibly display a wider range of physiological tolerances, enhanced growth rates, reproduction, survivorship and competition, but also significant disadvantages such as developmental instability and somatic and germ cell parasitism (Stoner et al. 1999, Rinkevich 2011).

The ability to form large chimeric colonies may partly explain the extreme invasive success of *D. vexillum* (Smith et al. 2012, Rinkevich and Fiddler 2014, Stefaniak 2017, Casso et al. 2019b). *Didemnum vexillum* experienced a significant reduction in genetic diversity when colonizing New Zealand, likely due to a founder effect (Smith et al. 2012). Other introduced populations in Europe, East North America and West North America also display reduced diversity when compared to Japan (Smith et al. 2012). However, reduced genetic diversity in the non-native areas are correlated with high rates of chimerism and colony fusion. Fusion occurs in 80 % of the colonies in *D. vexillum* in the non-native range in New Zealand versus 27 % of the colonies in its native range (Smith et al. 2012). Densely settled larvae may use fusion to rapidly increase colony size (Stefaniak 2017).

### *1.4.4.2 Sexual reproduction*

*Didemnum vexillum* is a hermaphrodite and also ovoviviparous. Individuals possess both male and female reproductive organs and spawning takes place through broadcast spawning where only sperm is released ('spermcast' mating). The sperm enters a zooid in a different colony and eggs are fertilized. Fertilized eggs remain within the tunic of the colony until they are ready to hatch, each zooid containing 1-20 eggs (McKenzie et al. 2017). The brooding can take several weeks after which 1.4 mm long larvae are released as free-swimming tadpoles (Lambert 2009). Temperature plays an important role in both spawning and release of larvae, but the thresholds are variable both regionally and globally. The overall pattern shows that an increase in temperature is required for the onset of the spawning but not for cessation (Valentine et al. 2009, Fletcher et al. 2013). It appears that the first release of larvae is not linked to a specific temperature but is dependent on the local temperature trends. The larvae are released at the end of the developmental period as the water temperature increases (Valentine et al. 2009). The first occurrences have generally been observed above 13°C and up to 23°C, while recruitment ceases in the range of 9–11°C (Valentine et al. 2009, Fletcher et al. 2013).

The tadpole larvae are lecithotrophic and depend on their yolk sac for a short pelagic phase and are able to settle after only a few minutes. Most settle within 24 h (>50 %) (Fletcher and Forrest 2011), but there are still viable larvae up to 36 h, but none after 48 h (Fletcher et al. 2012). Once larvae have settled, they undergo metamorphosis and begin feeding. Metamorphosis involves a series of steps that transform the motile, non-feeding larvae into a sessile, feeding individual. Three days after larval release, 73 % had successfully settled and after three weeks the majority had undergone asexual budding and formed small colonies (Fletcher et al. 2012). Field trials studying dispersal have shown the ability to spread at least 250 m from the source colony and models predict a maximum dispersal of more than 1 km is possible under favourable conditions (Fletcher et al. 2012).

It has been suggested that colonies that undergo regression where they are reduced in size during winter months and experience water temperatures below 4°C require longer time to regenerate, spawn, brood and release larvae than colonies that experience higher winter temperatures and less regression annually (Valentine et al. 2009, Fletcher et al. 2013). Colonies that are not reproducing due to low water temperatures during winter, but are not regressed, have late larval stages present in the tissue, suggesting that the species has the potential to reproduce year around (Fletcher et al. 2013). Environmental variation is reflected in the duration of the reproductive season, which can vary from 3.5–5 months in areas with large variability in temperature (Valentine et al. 2009), whereas areas with less annual variation, and therefore less regulation of productive output, have an extended reproductive season of nine months (Fletcher et al. 2013).

#### *1.4.4.3 Growth and reproduction*

As a successful invasive colonial ascidian, *D. vexillum* has the ability for rapid growth and expansion through both sexual and asexual reproduction. It may overgrow sessile plants and animals, quickly overtaking any suitable substrate, both man-made structures and natural marine habitats. Under optimal conditions, growth can increase colony size 6- to 11-fold in 15 days (Valentine 2007b).

Colonial ascidians generally grow in their first year, followed by breeding and then death in subsequent years. Age determination is difficult due to the periodic regeneration and reduction that the colonies undergo. Colonies typically live from one to three years (Daniel and Therriault 2007). *Didemnum vexillum* shows a high plasticity in growth, tuned to thermal conditions. It regresses and shrinks during winter in colder environments, whereas it regresses during warm summer months in warmer environments. Minimum temperature, maximum temperature and temperature variability all influence reproduction and growth rates. Minimum temperatures below zero cause the colonies to regress, but not all die (Valentine 2007b). In tidal pools in New England where temperature may vary between –1°C in winter to +24°C during summer months with large daily fluctuations, *D. vexillum* has a seasonal cycle of growth with small patches of colonies becoming more visible during spring, growing rapidly during the warm summer months and declining during the lower water temperatures in the autumn and winter (Valentine 2007b). Maximum temperatures above 20°C also cause the colonies to degenerate (Gittenberg 2010, Ordóñez et al. 2015). In the Dutch Wadden Sea, colonies started declining and rates of growth decreased at temperatures above 20°C (Gittenberg 2010). In the Mediterranean, with water temperatures ranging between 8 and 28°C, colonies start growing in autumn (November) and grow through the winter, reaching maximum size in spring (May) when they start to regress at 25°C during the warm summer months and become almost absent in September (Ordóñez et al. 2015). At deeper sites with less variation in temperature, such as the Georges Bank fishing grounds (4–15°C, NW Atlantic), *D. vexillum* does not show this annual variation in growth. In areas with higher variability in temperature some colonies would begin to degenerate at a certain temperature, while the colonies at Georges Bank showed no signs of degeneration at the same temperature. A high variability in temperature during the warm season appears to inhibit the reproduction process and therefore successful colonisation (Valentine 2007).

Temperature is a significant factor in reproduction and growth. Other important environmental factors include food availability, salinity (Bullard and Whitlatch 2007), substrate availability (Fletcher et al. 2018) and health status of potential substrate (Roth et al. 2018).

## 1.5 Distribution and spread of *Didemnum vexillum*

### 1.5.1 Native distribution

It is widely assumed that *D. vexillum* is native to Japan (Lambert, 2009), and both mitochondrial and nuclear DNA analyses support this (Stefaniak et al. 2012, Casso et al. 2019). The species was first formally identified in 2009 from a sample taken in Mutusu Bay in northern Japan in 1926 (Lambert 2009) that had previously been described as *D. pardum* (Nishikawa 1990). There were no reports of this species outside Japan prior to the 1970s. In its native area, *D. vexillum* is differentiated into two main genetic clades, clade A and clade B, of which only clade A has spread worldwide (Stefaniak et al. 2012, Casso et al 2019b).

### 1.5.2 Invasive range outside Norway

It appears that *D. vexillum* first spread to the north-eastern North America before it reached New Zealand. It later spread to the west coast of the United States and later to Europe (Casso et al 2019). In Europe, by 2017 the species had been found in the Netherlands (1991), France (2002), Ireland (2005), United Kingdom (2008), Italy (2010), and Spain (Mediterranean 2012) (Griffith et al 2009, McKenzie et al. 2017). Subsequently, the species was recorded for the first time in Sweden at the Koster Islands close to the Norwegian border in September 2022. According to occurrence data from the Global Biodiversity Information Facility (GBIF- https://www.gbif.org/species/7707408), the invasive distribution, as of 15 September 2022 now also includes Nakhodka region in the southeast part of Russia. The known locations are presented in Figure 1.5.2-1.

**Figure 1.5.4.1-1:** World map showing known locations of *Didemnum vexillum* recorded in GBIF.

## 1.5.3 Presence in Norway as of winter 2022/23

*Didemnum vexillum* was first reported in Norway in November 2020 on a steep wall on the north side of Engøysundet north of Stavanger by underwater photographer Erling Svensen. By closer examination of the area the following summer, it was clear that the species was locally abundant on rocks, stones and gravel almost covering the entire seafloor in an area of approximately 9 $km^2$ on the south side of the sound. The epicentre seemed to be two moored prams where commonly smaller vessels could be moored and used as storage for Yokohama fenders. Subsequent mapping showed that patches of colonies were found on nearby shores and islands, in addition to one site close to Stavanger harbour. In August 2021, reports on a unidentified ascidian was coming in from divers in Haugesund 60 km north of the first observation site and confirmed to be *D. vexillum*. The area has not been mapped systematically but reports from divers suggest a colonized area of 6 $km^2$ in the central harbour areas of city, with satellite populations on the west coast of Karmøy and 5-6 km north of Haugesund city. In this area, the species is growing on step walls below piers, on gravel and stones and also growing on kelp and other seaweeds. In September 2021, *D. vexillum* was observed on an oil rig at a shipyard at Askøy north of Bergen. By examination of the surrounding area, it was clear that the species was established here, growing on seafloor walls and stones and it had also spread to the kelp forest on small islands nearby. An e-DNA sampling campaign from Rogaland and Vestland county were initiated that winter (Fossøy et al. 2022) and showed clear detections from several sites in Vestland county, and a visual inspection of these sites was performed. Most of the sites examined showed no signs of *D. vexillum*, but the species was found in Skipavika in Gulen approximately 70 km north of Bergen in February 2022. The area is a lay-up port where oil rigs and other vessels can be moored for longer periods. In this area, the species has colonized the steep walls, where few other species are present due to sedimentation from industry on land down to 30 meters depth but was most abundant between 10 and 15 meters. Under the piers, where there is more shadow, the species coverage is almost complete up to the intertidal zone. In December 2021, *D. vexillum* was also recorded from a site in Egersund, 90 km south of Stavanger. The species is here spreading fast in natural habitats, including kelp forest and hard-rock communities and is patchily present in an area of approximately 0,7 km$^2$, occurring mostly below 25 meters depth due to a brackish layer in the surface water (Erling Svensen, pers. comm.). The disjunct spatial distribution in coastal Norway (Figure 1.5.3-1.) suggests that dispersal is aided by human vectors. The almost simultaneous records in five separated areas suggests that either the species has been present at the Norwegian coast for several years before it was discovered, or alternatively, that multiple introduction events have occurred.


**Figure 1.5.3-1:** The five areas in Norway and Koster Islands in Sweden known to be colonised by *D. vexillum* as of January 2023 (Distribution map from GBIF).

### 1.5.4 Pathways for spreading

A thorough description of pathways for spreading of alien marine species is presented in section 1.2.3.

**Figure 1.5.4-1:** *Didemnum* spp*.* can be spread to new localities with motile and crawling organisms to new localities. The *Didemnum* sp. illustrated here is attached to the carapace of a crab (*Hyas* sp.). For orientation: the eye of the crab can be observed in the center of the picture. Photo: Erling Svensen.

There are no specific pathways given for spreading of *D. vexillum*, but commercial vessels that have been stationary for a long time prior to moving, thus allowing time for the ascidian to colonize and grow, is presumably the main contributors to spreading between Norwegian sites. As *Didemnum* species can grow on other mobile organisms (Figure 1.5.4-1), they are able to spread shorter distances within an area. Also, fragments of larger colonies are often detached from the rest in a typical droplet form (Figure 1.5.4-2) and the fragments can be transported by currents or by other means to new locations.

**Figure 1.5.4-2:** A fragment in the process of being released from a larger colony of *Didemnum vexillum*. Such fragments can spread the organism to new localities. Egersund December 2022. Photo: Erling Svensen.

## 1.6 Possible impacts of *Didemnum vexillum* in Norway

### 1.6.1 On biodiversity

*Didemnum vexillum* is regarded as an ecosystem engineer (Wallentinus & Nyberg 2007), although there are relatively few studies that have quantified impacts on native biodiversity (but see Gittenberger 2007 and Gittenberger et al 2019). One explanation is that, although it has been present in New Zealand for about 20 years, the species is newly introduced to several parts of the world. Another is the difficulties of studying such impacts. One could design a study to observe changes before and after colonisation, but the uncertainty of whether or when the species will establish in a study area of interest is a major obstacle (Epstein and Smale 2017). Another approach is to study changes in communities that have previously been studied (baseline), but the results could be confounded with recent temporal changes in environmental factors, such as ocean warming or changes in salinity, nutrient concentrations or organic loading (Husa et al. 2008).

Although there are few specific studies on the impact of *D. vexillum* worldwide, there are some reports of the observed changes in biodiversity in the areas where the tunicate has established. One such report comes from Georges Bank, outside the east coast of North America, where bottom surfaces were investigated. Lengyel et al. (2009) reported that *D.*

*vexillum* covered up to 70 % of the gravel seafloor and that this had led to a significant change in the epibenthic community, such as an increase of polychaetes. Kaplan et al. (2018) also identified *D. vexillum* as a probable key driver of biodiversity decline. They found an increase in polychaetes and mobile predators like crabs and sea stars, in areas where *D. vexillum* had established, and that these had replaced sessile organisms in the habitat. Similar changes to the ecosystem composition were reported in a study from Long Island Sound (Mercer et al. 2009). These observations led to the general assumption among researchers in Norway that one potential effect, following establishment and spread of *D. vexillum*, would be changes in the composition of the benthic micro-, and macro-fauna. Whether a reduction in total biomass is to be expected is not clear, but the biodiversity is likely to be reduced.

Aside from various effects on bottom surface habitats, it is likely that *D. vexillum* will have a negative impact on key species of marine plants, like seaweed and seagrass. In Norway, the brown algae *Laminaria hyperborea* (common names: tangle or stortare) and the seagrass *Zostera marina* (common name: ålegras) are especially important species as they form the basis for important habitats like kelp forests and seagrass meadows. Several observations have been made of *D. vexillum* growing on *Z. marina* or similar species (Carman and Grunden 2010, Long and Grosholz 2015). *D. vexillum* has not been reported to grow on kelp in other regions but overgrowth has been documented in Norway (Vivian Husa IMR, pers. obs.), as depicted in Figure 1.6.1-1. It might be expected that *D. vexillum* could negatively impact these marine plants in Norway. Both eelgrass communities and kelp forests are important habitat for recruitment of numerous species of marine invertebrates and vertebrates, and habitat loss would certainly have a negative impact on biodiversity if these habitats became infested with *D. vexillum*.

*Didemnum vexillum* also overgrows and competes with shellfish, such as mussels, scallops and oysters (Figure 1.6.1-2), negatively affecting these bivalves through reducing both food availability and gas exchange (Morris et al. 2009, Auker 2010, Dijkstra and Nolan 2011, Switzer et al. 2011). The effects of overgrowth are also likely to be seen in Norway, where the environmental conditions allow the coexistence of these species. Last, but not least, *D. vexillum* could also impact other shallow nature types, like maerl communities (calcified red algae), high diversity filter feeding communities on hard bottom in strong tidal currents, and shallow sea pen and coral communities.

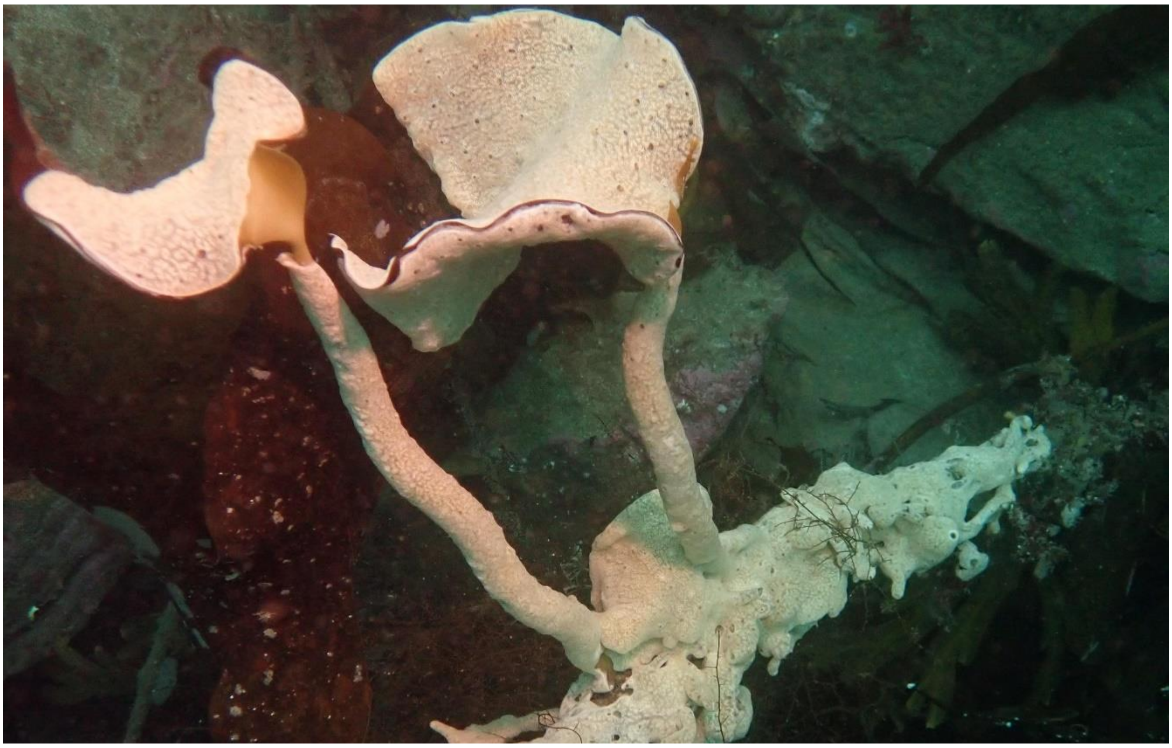

**Figure 1.6.1-1:** *Didemnum vexillum* covering young *Laminaria hyperborea* plants in Haugesund. Photo: Torbjørn Brekke.

**Figure 1.6.1-2:** *Didemnum vexillum* growing on a Pacific oyster at Koster Islands (Kosteröarna), Sweden (Photo: Örjan Karlsson).

### 1.6.2 On aquaculture

Establishment of invasive ascidians on shellfish cultures is a worldwide problem and causes reduced spat collection and stock growth and increased expenses for removal of biofouling. A study of the effect of *D. vexillum* on cultures of green-lipped mussels (*Perna canaliculus*) in New Zealand showed that the density of small mussels was significantly reduced when overgrown by colonies, while growth and performance were less affected (Fletcher et al. 2013); the establishment of the species on these shellfish cultures has led to a significant loss of income for the farmers.

In addition to causing problems for net pens in fish farms and baskets for oysters, *Didemnum vexillum* could also cause problems for the organisms produced in aquaculture facilities. In the same way that the species could impact kelp in the natural environment, it could establish in kelp farms and reduce growth and quality of the kelp. In Norway, the main culture of seaweeds in long line culture (where the kelp is cultured on long lines) of the sugar kelp *Saccharina latissima* (common name: sukkertare). In Sweden, *D. vexillum* is now abundant at an oyster culture facility, where it is causing problems on baskets and oysters, causing soft spots in the shell where the colonies have been situated (Andrea Ljung, Havs och Vattenmyndigheten, Sverige, pers. comm.).

Biofouling on net pens on fin fish farms is a major problem mainly during summer due to reduced water flow and poor oxygen conditions for the farmed organisms (Bloecher et al. 2013, Bannister et al. 2019). Establishment of *Didemnum vexillum* on net pens may increase this problem.

## 1.7 Predation on Ascidians

Studies of prey-predator relationships are traditionally based on examination of the stomach and gut contents of the predator. Prey lacking slowly- or non-digestible parts are usually more difficult to detect, record and identify compared to prey with hard parts, such as bones, shells, or spicules. Most Ascidiacea lack hard parts, and this is one of the reasons ascidians generally are regarded as having few predators (Millar 1971, Lützen 1967, Goodbody & Gibson 1974). In addition, high vanadium concentrations (Swinehart et al. 1974, Danskin 1978) and/or bladder cells containing sulphuric acid (Carlisle 1968, Swinehart et al. 1974) may also act as defences against predation. Stoecker (1980) do claim that the lack of predation by generalists on many common benthic ascidians is due to chemical defences.

However, the increased numbers of underwater *in situ* observations by SCUBA-divers suggest that ascidians play a more important role as food resource in benthic food chains than earlier believed. Predators are most frequently found in such taxa as turbellarians (Ching 1977, Jennings 1957, Lambert 1968), prosobranch snails (Behrens 1981, Diehl 1956, Gulliksen 1975, Laxton 1971) and echinoderms (Ferguson 1969, Gulliksen & Skjæveland 1973, Keough & Butler 1979), but opisthobranch snails (Birkeland 1974), fish (Hobson 1974, Denstadli 1972, Lande 1973, 1976) and reptiles (sea turtles) (Ferreira 1968) may also occasionally eat ascidians. Some of the prosobranch lamellarid snails are also well camouflaged and observed only by careful examination of the ascidian.

Certain genera of prosobranch snails (for example *Trivia*, *Velutina*, *Lamellaria*) are specialized predators preferring ascidians as food. *Velutina velutina* (common name: sjøpungsnegl) probably engulf too few ascidians to reduce the size of carpet ascidians significantly. Lamellarid snails are relatively small, often resembling their colonial tunicate prey, so do not need to move much when attached to prey because the feeding rate appear to be equalized by the growth of the carpet ascidian (Gulliksen, unpublished data). Figures 1.7-1 and 1.7-2 show various predators of ascidians.

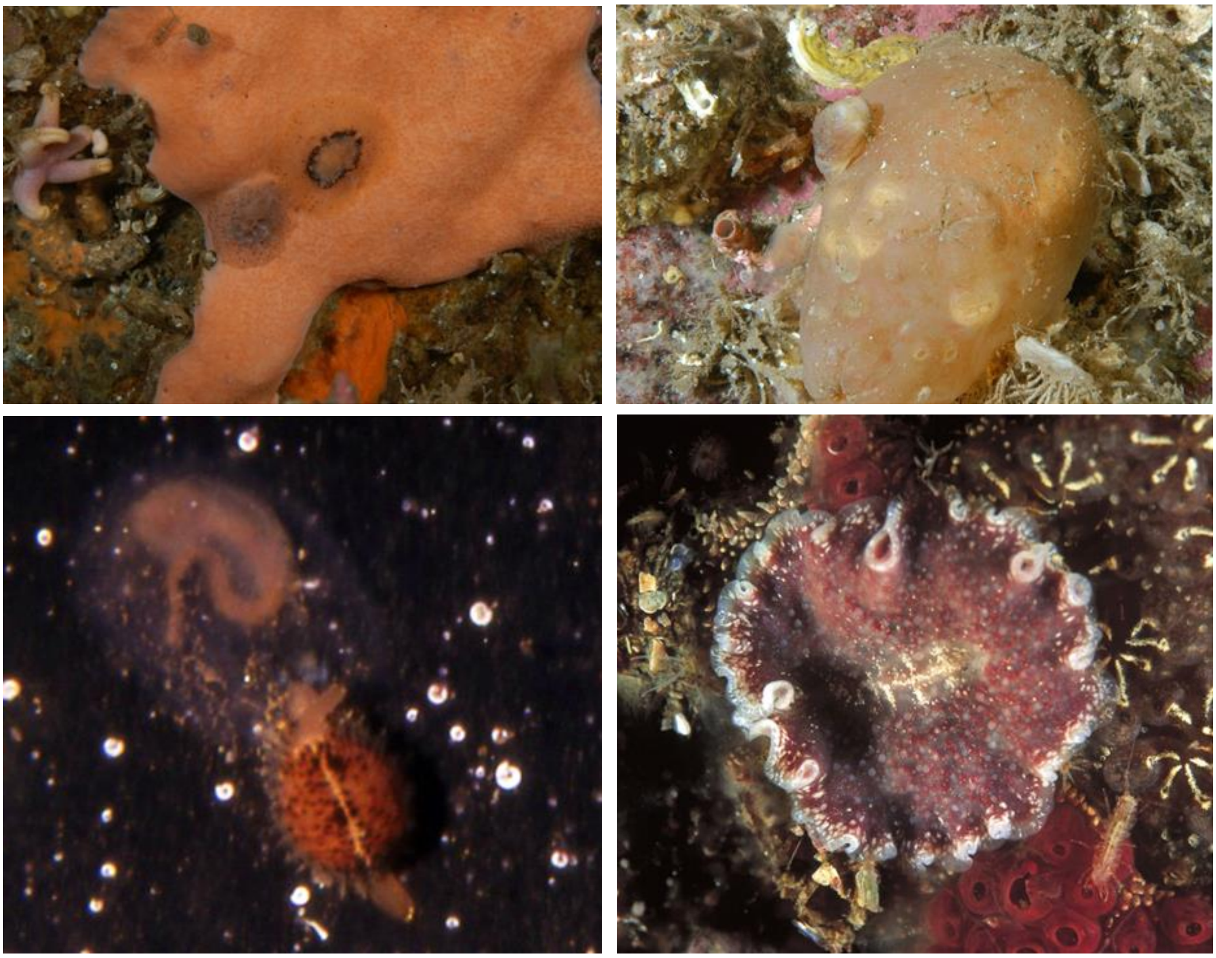

**Figure 1.7-1:** Upper left: Two camouflaged lamellarids feeding on a *Didemnum colony*. Upper right: *Velutina velutina* on a solitary ascidian in the genus *Ascidia*. Note the yellow patches which are eggs the snail has laid in the ascidian. Lower left: The snail *Trivia arctica* feeding on the ascidian *Ascidia conchilega* (Photos: Bjørn Gulliksen). Lower right: A flatworm feeding on botrylloids (Photo: Erling Svensen).

**Figure 1.7-2:** Close-up photograph of three specimens of the lamellarian snail *Lamellaria perspicua* feeding on colonies *of Didemnum vexillum.* December 2022 near Egersund. Photo: Erling Svensen.

It is possible, but not very realistic, that dense populations of echinoderms, like the sea urchin *Strongylocentrotus* spp. and the sea-star *Asterias rubens* (common name: vanlig korstroll) could remove carpets of colonial ascidians. There are, for example, evidence of dense populations of *Asterias rubens* eradicating dense populations of the Iceland scallop *Chlamys islandica* (common name: haneskjell) in Norwegian waters (Brun 1968), and sea-stars are voracious on many benthic organisms including ascidians. However, it is a rare incident with such high densities of sea star as shown in figure 1.7-3.

Several sea urchins *Echinus esculentus* (common name: svabergsjøpiggsvin) were observed possibly feeding on a steep rock in Haugesund in September 2022 (Figure 1.7-4). One aspect of this is that the feeding behaviour of most sea urchins is that fragments of the ascidian colony removed by the teeth of the sea urchin will not be absorbed but spread in the water masses and function as a nucleus for production and vegetative growth of new colonies (Figure 1.7-5).

**Figure 1.7-3:** A dense population of the starfish *Asterias rubens* eradicating a dense population of the Icelandic scallop *Chlamys islandica* (Brun 1968). (Photo: Einar Brun).

**Figure 1.7-4:** *Echinus esculentus* on *Didemnum vexillum* colonies in Haugesund (Photo: Åge Wee).

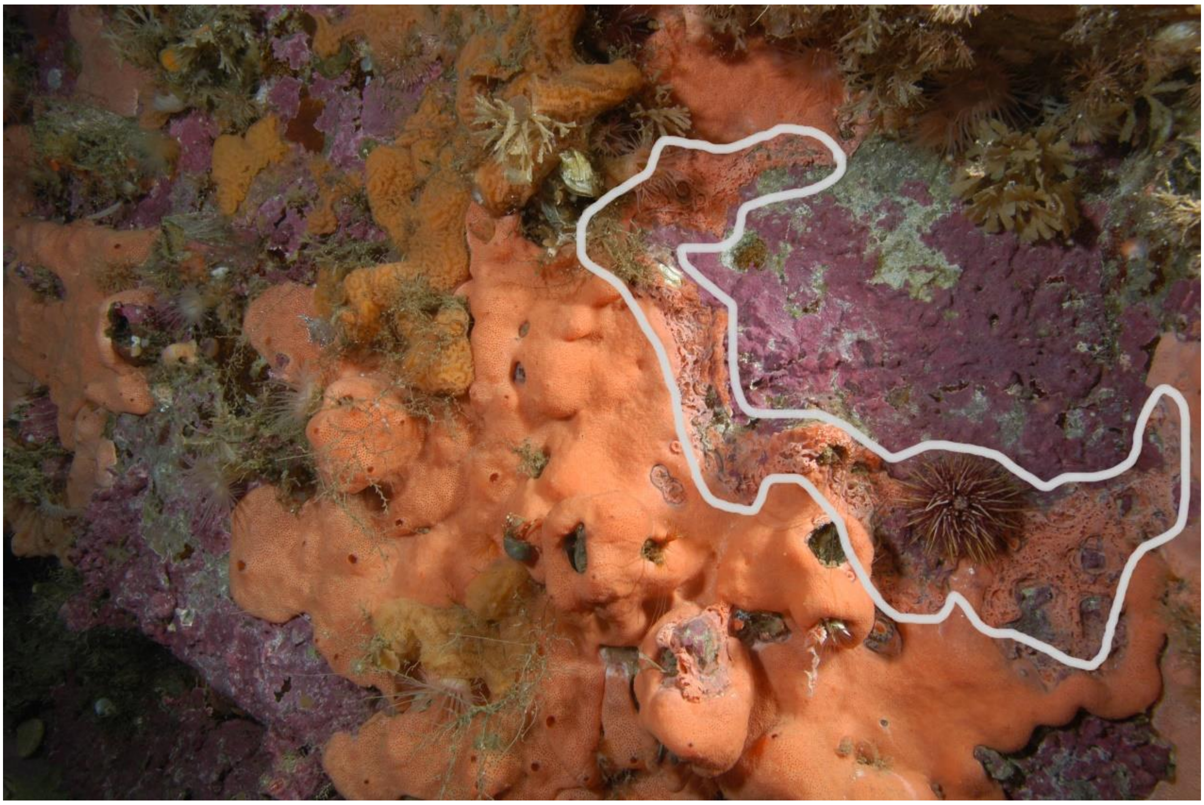

**Figure 1.7-5:** A colony of *Didemnum* sp. A sea urchin (*Strongylocentrotus droebachiensis*) is feeding on the colony and areas where the sea urchin has fed on the didemnid is marked with the white line (Photo: Bjørn Gulliksen).

Laboratory experiments have indicated that *Cancer* crabs (e.g., *Cancer pagurus,* common name: taskekrabbe) can prey on solitary ascidians such as *Ascidiella aspersa*, *Ciona intestinalis* and *Styela clava*, but not on colonial ascidians such as *D. vexillum* (Dijkstra & Harris 2007).

### 1.7.1 Predation on *Didemnum vexillum*

The scarce records of specific predation on the relatively newly described *D. vexillum* Kott, 2002 do probably not differ from the general picture regarding predation on ascidians. The records are in general based on observations of predators feeding on its prey and not on the occurrence of spicules in the stomach of the predator even though *D. vexillum* contain spicules. Gastropods (Gittenberger 2007, Kleeman 2009, Carman et al. 2009, Lambert 2009) and echinoderms (Bullard et al. 2007, Lambert 2009) are reported as predators on *D. vexillum.*

The occurrence of *D. vexillum* in Norway and increased amount of information and focus have resulted in several records of predators on the species. Erling Svensen (pers. comm.) brought to the surface a submarine rock with a colony of *D. vexillum* (ca 5 – 6 $dm^2$) near Egersund and the colony hosted 24 individuals of lamellarid snails (probably two species). Rudolf Svensen (Stavanger Museum, pers. comm.) has also observed lamellarian predators on the colonies of *Didemnum vexillum* near Stavanger, but the predation rate is low compared to the growth of the ascidian. Echinoderms (sea stars and the urchin *Gracilechinus acutus*) have also been recorded as predators. Sea stars apparently feeding on *D. vexillum* are shown in figure 1.7.1-1.

**Figure 1.7.1-1**: Sea stars on *Didemnum vexillum*, possibly feeding. Foto: Torbjørn Brekke.

## 1.8 Implications of climate change

As a successful invasive species with high adaptability and tolerance for changes in the physical environment, the effects of climate change will likely be positive for *D. vexillum* in Norwegian waters. It is already theoretically possible for the species to colonize the entire Norwegian coast and Svalbard, including Bear Island by means of fragmentation or boat traffic. Based on the water temperature we expect that the northernmost limit for larval spreading is currently approximately in the Tromsø area. The border will likely not move much further north within a 30-year perspective, even with predicted increase in temperatures, but the likelihood of successful spawning will increase (Chapter 3 and Figs 3.1-3 and 3.1-4).

# 2 Methodology and Data

## 2.1 Risk assessment

### 2.1.1 General

The International Union for Conservation of Nature (IUCN) has recently developed general guidelines for classification of the impact of alien species on the environment. The system - 'Environmental Impact Classification for Alien Taxa' (EICAT) - is not a method for risk assessments *per se*, but rather a method for classifying alien taxa into impact categories according to the magnitude of the detrimental impacts to the environment. The system is similar to the IUCN system for red listing of threatened species and habitats and is mainly established for informing scientists, environmental managers and relevant stakeholders of the impacts of alien taxa and provide a basis for the evaluation of management policies and actions. Alien species are classified into five categories from negligible impacts (Minimal Concern) to small effects (Minimal) and further to Moderate, Major and Massive impacts. Impacts that fall within the categories Moderate, Major or Massive are termed 'harmful' (IUCN 2020).

To describe how an organism poses a threat to biodiversity (and aquaculture), VKM utilises the EICAT system to describe the key mechanisms that determine how alien invasive species affect biodiversity in Norway. The twelve potential mechanisms identified by IUCN include:

1) Competition
2) Predation
3) Hybridization
4) Transmission of disease to native species
5) Parasitism
6) Poisoning / toxicity
7) Biofouling or other direct physical disturbance
8) Grazing / herbivory / browsing
9) Chemical impact on ecosystems
10) Physical impact on ecosystems
11) Structural impact on ecosystems
12) Indirect impact through interactions with other species

Of these mechanisms, the project group identifies factors which are relevant for each organism and assesses the risk the alien invasive species pose through each of them for the specific hazards, following the steps outlined below.

### 2.1.2 Assessment of specific hazards

For the questions outlined in the Terms of Reference (ToR), hazards were identified and assessed independently. VKM assesses each potential hazard in four standardized steps: hazard identification, hazard characterization, likelihood, and risk characterization. These steps are judged by the project group experts. Table 2.1.1 describes the ratings for the level of confidence the project group has given the assessments.

**Table 2.1-1:** Ratings used for describing the level of confidence.

| Rating | Descriptors |
| --- | --- |
| **Low** | There is limited information on the specific subject, in particular from comparable environmental settings. Subjective expert judgements may be introduced without supporting evidence.<br><br>Little peer reviewed literature available and there are limited empirical and quantitative data to support the assessment. |
| **Medium** | Relevant information on the specific subject is available, but only limited information from comparable environmental settings. Some subjective expert judgements are introduced.<br><br>Both grey literature and peer reviewed literature are used and there are some empirical and quantitative data to support the assessment. |
| **High** | There is extensive information on the specific subject, also from comparable environmental settings. Little or no subjective expert judgements is introduced.<br><br>Primarily peer reviewed literature is used and there are empirical and quantitative data to support the assessment. |

Under "**Hazard identification**" we describe the specific hazard and why this hazard is considered in the current assessment. Examples include specific species relevant for import, predation on or competition with native species, and a hitchhiking disease-causing organism. The known effects of the hazard are presented and referenced with examples of the known impacts from other countries.

Under "**Hazard characterization**" the specific potential effects of the hazard in question are described under Norwegian conditions. Examples include which areas or habitats that a species can thrive in, which species the invading species would compete with or prey upon, and which species can be infected by pathogens from the hitchhiking organism. The potential magnitude of the specific hazard is then characterized from "Minimal" to "Massive" as described in Table 2.1-2.

**Table 2.1-2:** Ratings for the magnitude of impact on biodiversity in Norway.

| Rating | Descriptors |
|---|---|
| **Minimal** | Potential impact is limited to occasional deaths of individuals. No expected effects on the local-, regional-, or national population size. |
| **Minor** | Potential impact includes limited reductions in local abundance of one or a few species and these effects are temporary and spatially limited. No expected effects on the regional-, or national population size. |
| **Moderate** | Potential impact can result in moderately reduced abundance of one or more species, with potential implications on population viability on a regional level. |
| **Major** | Impacts may cause severe reductions in the abundance of one or more species, including potential extinction of local and regional populations. Consequences may also affect ecosystem functions and services. The consequences are regarded to be reversible should the alien species be eradicated. |
| **Massive** | Impacts may cause detrimental reductions in the abundance of more than one species, including extinction of local populations and potentially threaten the extinction of the national population. Consequences is likely to affect ecosystem functions and services and are regarded to likely be irreversible. |

**Table 2.1-2b:** Ratings of the potential magnitude of impact on aquaculture facilities in Norway. Numbers are (loosely) based on expert assumptions of the need for extra cleaning (*i.e.*, including suction and filtration of wastewater).

| Rating | Descriptors |
|---|---|
| **Minimal** | Potential impact is limited to a 0-5% increase in maintenance costs due to increased surveillance (all types of aquacultures). |
| **Minor** | Potential impact includes a 5–10% increase in net or line cleaning costs (fish and shellfish)<br><br>or<br><br>Impact may cause a 0–5% decrease in production output (seaweed/kelp) |
| **Moderate** | Potential impact includes a 10–20% increase in net or line cleaning costs (fish and shellfish)<br><br>or<br><br>Impact may cause a 5–20% decrease in production output (seaweed/kelp) |
| **Major** | Potential impact includes a 20–50% increase in net or line cleaning costs (fish and shellfish)<br><br>or<br><br>Impact may cause a 20–50% decrease in production output (seaweed/kelp) |
| **Massive** | Potential impact includes increase in net or line cleaning costs of more than 50% (fish and shellfish)<br><br>or<br><br>Impact may cause a 50–100% decrease in production output (seaweed/kelp). |

Under “**Likelihood**” we assess how likely it is that the characterized hazard occurs. Likelihood intervals range from “Very unlikely” to “Very likely”, as described in Table 2.1-3.

**Table 2.1-3:** Ratings for the likelihood of the specific impacts in the assessment.

| Rating | Descriptors |
|---|---|
| **Very unlikely** | Negative consequences would be expected to occur with a likelihood of 0–5% |
| **Unlikely** | Negative consequences would be expected to occur with a likelihood of 5–10% |
| **Moderately likely** | Negative consequences would be expected to occur with a likelihood of 10–50% |
| **Likely** | Negative consequences would be expected to occur with a likelihood of 50–75% |
| **Very likely** | Negative consequences would be expected to occur with a likelihood of 75–100% |

Finally, under “**Risk characterization**”, the risk biodiversity or aquaculture in Norway, posed by the specific hazard, is characterized as either “Low”, “Medium” or “High”, based on the magnitude of potential impact of that hazard and the overall likelihood of this occurring. This characterization follows the matrix presented in **Figure 2.1.3-1.**

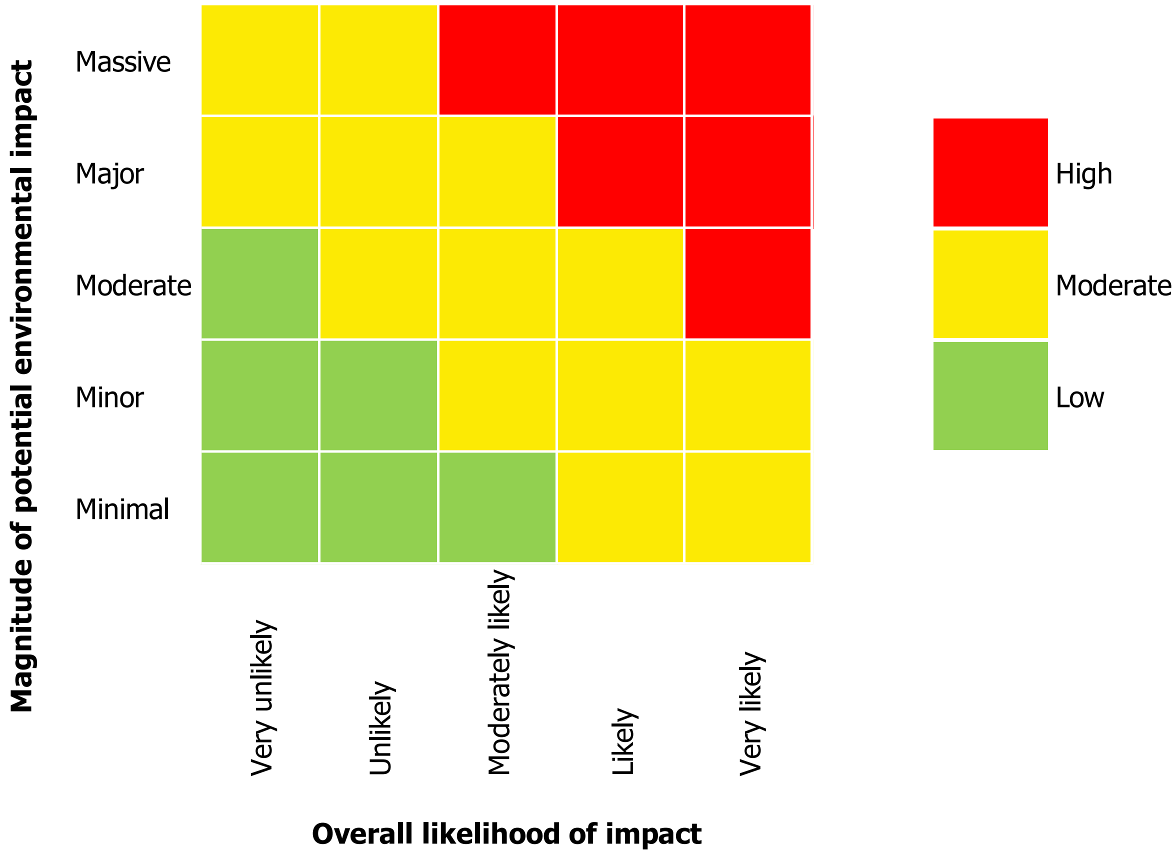


**Figure 2.1.3-1:** The conclusion of the risk assessments (Low, Moderate, or High) is based on the overall likelihood of the impact and the magnitude of the potential consequences of that impact on Norwegian biodiversity.

## 2.2 Data and information gathering

Site records of *D. vexillum* in Norway were retrieved from the Norwegian Biodiversity Information Centre. In addition, the Institute of Marine Research and NINA have been surveying and testing various areas to detect occurrence of the species in new locations.

The Institute of Marine Research has, in collaboration with other institutes and parties, conducted various tests on eradication of *D. vexillum* during summer and autumn of 2022.

Data on ocean temperatures, used to predict suitable habitats for survival and sexual reproduction, were provided from the Norwegian Meteorological Institute in Bergen.

Additional information on effects of measures and various aspects of *D. vexillum* biology has been obtained through personal communication with the renowned underwater photographer Erling Svensen.

## 2.3 Literature search

We have used ISI Web of Science core collection as primary source of scientific information. In addition, we performed general searches in Google Scholar. The searches included species name (or synonyms, or common name) and specific terms, such as "invasive", "introduced", "disease", "parasite", "virus", "bacteria", "eradication", "measures", "spreading" in combination with both common and scientific names of the species. In addition, terms for habitats, such as "gravel", "sand", "seagrass", "coralline algae", "kelp", and vectors ("ballast water", "ship hulls", etc) were used.

We conducted general Google searches using some of the same terms as mentioned above. These searches sometimes revealed webpages or grey literature with relevant information, such as other risk assessments conducted for this species in other countries. Finally, the participating experts used their extensive databases of relevant scientific literature.

# 3 Risk assessment

## 3.1 Assessment of suitable habitat in Norway

Spreading of *D. vexillum* within Norway by means of sexual reproduction and larvae is limited by temperature and space. Release of larvae generally requires temperatures of at least 13°C but there is not a specific temperature when this happens, as it is dependent on minimum temperature during winter or the level of colony regression during this time (See sections 1.4.2 and 1.4.4). Higher winter temperatures are favourable for colonies already containing late-stage larvae ready for spawning that require shorter periods of warmer water before spawning starts. Most tadpole larvae settle within 250 m from the maternal colony under normal conditions, but modelling suggests that they could spread over 1 km under favourable conditions depending on direction and strength of water currents. Settlement patterns regulate the distance and speed with which *D. vexillum* may spread through sexual reproduction on a regional scale, but the species can readily spread locally from established populations. Considering maximum surface temperatures along the Norwegian coast (Figure 3.1-1), the current northernmost limit for sexual reproduction is approximately in the Tromsø area. That winter temperatures rarely drop below 2°C (Figure 3.1-2) and often are higher makes it likely that short periods of warm water could initiate spawning. With climate change and increasing temperatures in the ocean, this border will likely not move much further north within a 30-year perspective, but the likelihood of successful spawning will increase (Figures 3.1-3 and 3.1-4).

The largest risk for spreading is connected to the ability of *D. vexillum* to spread through fragmentation. Fragments of a colony can settle and grow: asexual growth is not limited by temperatures within Norwegian waters (tolerance limits between -2 to +24°C). Thus, it is possible for *D. vexillum* to colonize the entire Norwegian coast and Svalbard, including Bear Island. The potential range will not change within a 30-year climate change perspective.

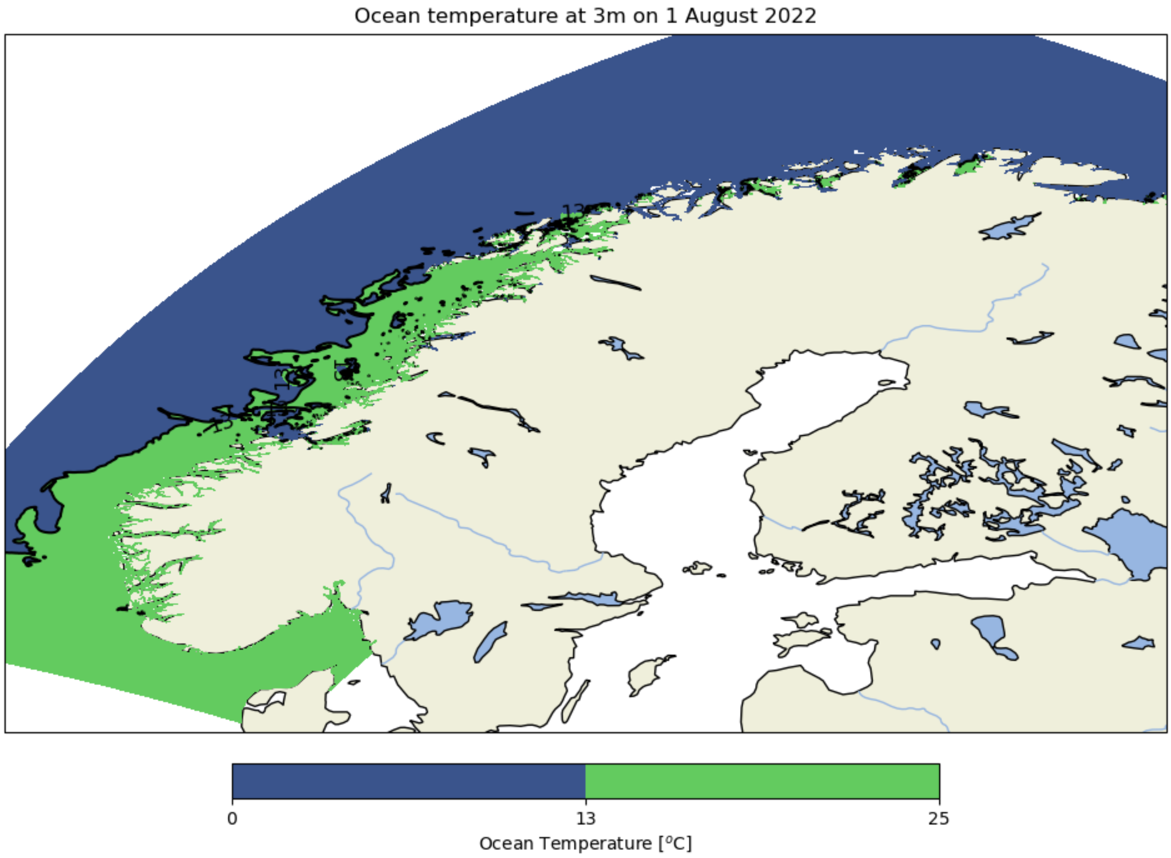


**Figure 3.1-1** Ocean temperatures in Norwegian waters in the warmest month of the year (August) at 3m depth. Green area indicates temperatures above 13 °C, the minimum temperature necessary for sexual reproduction of *Didemnum vexillum*. This area indicates where *D. vexillum* potentially can spread by means of larvae. Data source: NorKyst800 (Albretsen et al. 2011, Myksvoll et al 2018).

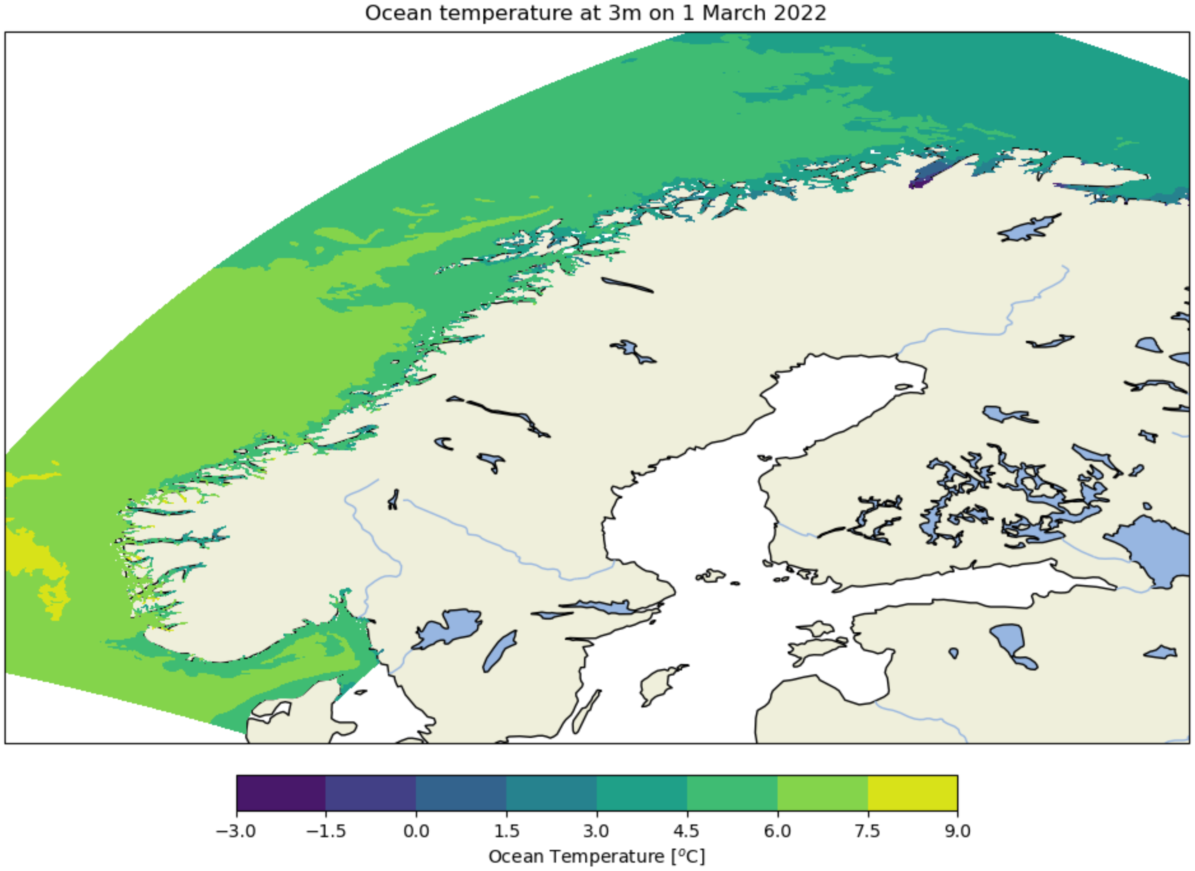


**Figure 3.1-2** Ocean temperatures in Norwegian waters during the coldest month of the year (March) at 3m depth. The range maps show that winter temperatures will likely not be low enough to cause severe regression of the colonies of *Didemnum vexillum* during cold periods. The pattern indicates that a shorter time of warm temperatures (<13 °C) in the spring or summer is needed to initiate spawning, thereby increasing the potential success of sexual reproduction. Data source: NorKyst800 (Albretsen et al. 2011, Myksvoll et al. 2018).

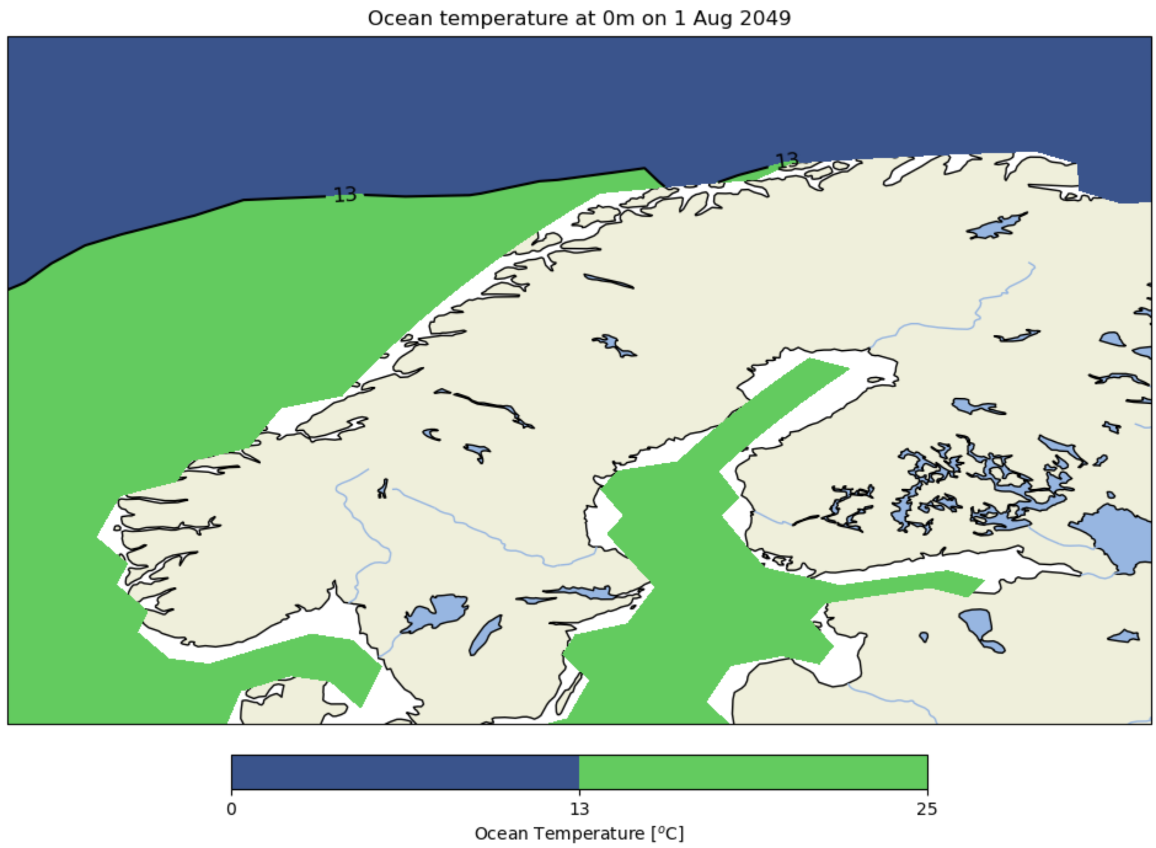


**Figure 3.1-3** Modelled ocean surface (0m) temperatures in Norwegian waters in year 2049 (commonly used reference period for climate change, 30 years). Green shows the area with temperatures above 13 °C, where *Didemnum vexillum* has the potential to spread through sexual reproduction. The northern distribution has not increased significantly compared to 2022 (Fig. 3.1-1) but the potential for successful spawning has likely increased. Data from HadGEM3/CMIP6 climate model, RCP4.5 (Roberts 2017).

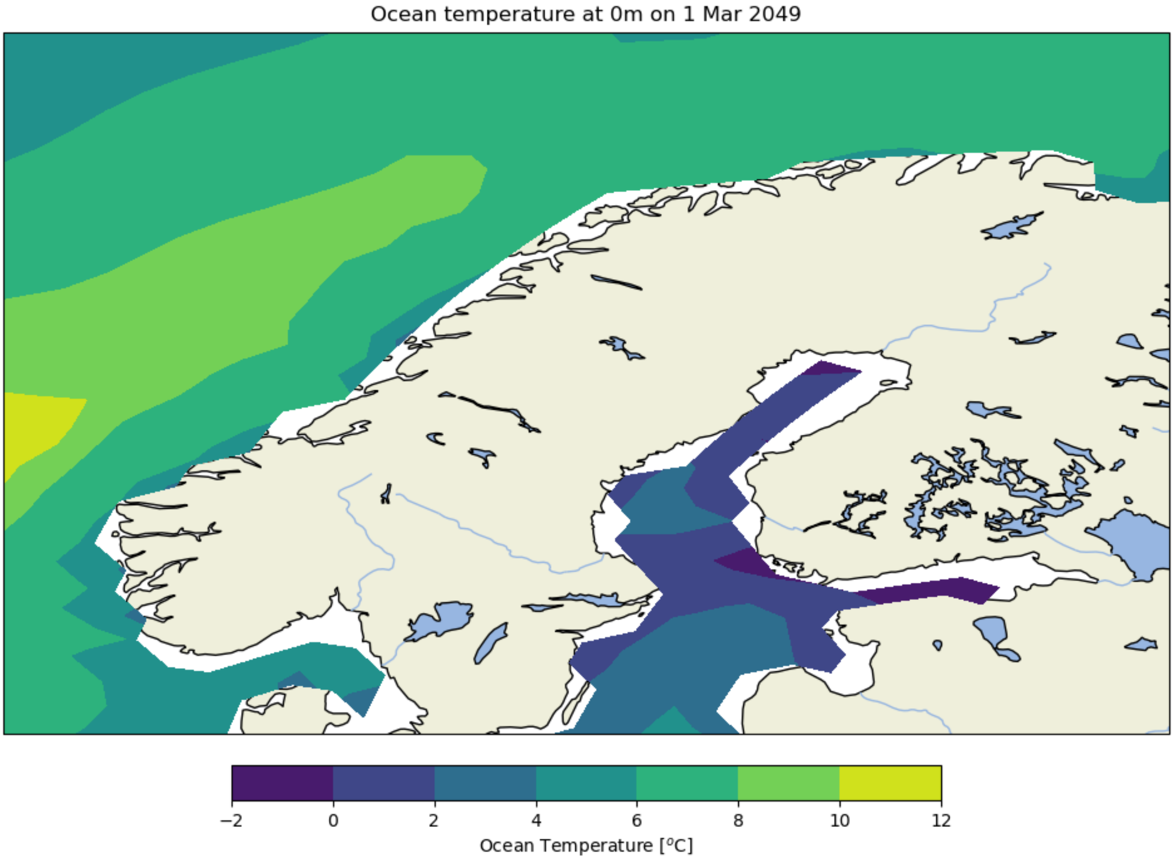


**Figure 3.1-4** Modelled ocean surface (0m) temperatures in Norwegian waters in year 2049 during the coldest month of the year (March). Winter temperature has increased compared to 2022 (Fig. 3.1-2) likely increasing the potential success of sexual reproduction of *Didemnum vexillum*. Data from HadGEM3 climate model, RCP4.5 (Roberts 2017).

## 3.2 Assessment of potential mechanisms for impact

*Didemnum vexillum* can represent a hazard to biodiversity and aquaculture through multiple mechanisms. The IUCN has defined 12 different mechanisms through which an alien taxon could have impact (section 2.1.1). The mechanisms are based on factors proposed by Nentwig et al. (2010), Kumschick et al. (2012) and Blackburn et al. (2014) and are aligned with mechanisms identified in the IUCN Global Invasive Species Database[5]. Of these, the project group finds that *D. vexillum* could has a negative impact through four different mechanisms: competition (for food and space), biofouling (overgrowing other organisms), transmission of diseases, and structural impacts on marine ecosystems. The four

[5] http://www.iucngisd.org/gisd/

mechanisms are discussed in detail where applicable for each of the specific hazards in section 3.4 (impact on wild organisms and biodiversity) and 3.5 (impact on aquaculture).

## 3.3 Risk assessments of potential impacts on biodiversity in Norwegian waters

To assess the impact *D. vexillum* could have on biodiversity in Norwegian waters, the project group identified different habitats that could be affected by this species. Some important habitats and key species, such as beds of dwarf eelgrass (*Zostera noltii*) were not included in the assessments because this species is primarily found in the tidal zone where *D. vexillum* does not thrive. Some brackish water habitats were excluded from further investigation due to the low tolerance of *D. vexillum* for low salinity. The assessments concerning likelihood of the events described in this chapter were done with a 3-year perspective under the assumption that no measures, or inadequate measures, are taken to eradicate and counteract further spread.

The occurrence of *Didemnum vexillum* is primarily dependent upon the composition and structure of the substrate. Colonies primarily are established where there are firm, stable surfaces, such as larger rocks or rock walls, as well as solid man-made structures, such as pier pilings, ship's hulls and aquaculture rigs. The tunicate can also form dense mats on gravel surfaces.

### 3.3.1 Potential impact on macroalgal communities

#### *3.3.1.1 Hazard identification*

All settlement of epiphytes on photosynthesizing organisms, such as seaweeds and marine angiosperms, will shade the sunlight and reduce photosynthetic rates and will hinder uptake of nutrients and gas exchange in the cells, reducing growth and energy storage of the host organisms (Levin et al. 2002, Andersen et al. 2011). Both native and alien species can have a negative effect through the mechanism of biofouling.

Settlement of dense colonies of *D. vexillum* in kelp forests could have a structural impact on this ecosystem. Dense establishment of a sheet-forming species like *D. vexillum* could change the faunal community in the different sections of the macroalgae including the hapterons attached to the substrate, the stipe (or stalk) and the lamina (or blades) of the kelp plant itself in a detrimental way (Levin et al. 2002, Andersen et al. 2011, Krumhansl et al. 2011).

Kelp forests provide the basis for the highly diverse communities of marine organisms (Christie et al. 2003). These unique habitats consist of highly diverse fauna and flora communities and act as shelter, nursery ground and food supply for a wide range of species, including crustaceans and fish of commercial interest. However, outside Norway, *D. vexillum* is primarily observed in disturbed areas or on man-made structures, and no report have been made on establishment in kelp forests. Therefore, little is known of the exact impact of the mechanism of biofouling on kelp forests worldwide.

### *3.3.1.2 Hazard characterization*

In Norway, the most common epigrowth on kelp lamina are usually filamentous seaweeds and bryozoans such as *Electra pilosa* and *Membranipora membranacea* (Haugland et al. 2021), but both colonial and solitary ascidians have also been observed on kelp. Bryozoan biofouling can lead to weakening, breakage, and detachment of the lamina (Krumhansl et al. 2011).

*Laminaria hyperborea* is the most important kelp species in Norway, forming large forests subject to harvesting on the western coasts. This species of macroalgae has the main growth period in winter and spring when the new blade is produced. On *L. hyperborea* it has been observed that *D. vexillum* colonizes the hapterons (attachment structure), the stipes (stalk) and the lamina (blade) (Vivian Husa IMR, pers. obs., Figure 3.3.1.2-1). The old blade will fall off during early summer and give rise to a new blade from the junction between the blade and the stipes. *Didemnum vexillum* colonies will continue to grow from stipes and onto the new blade reaching a diameter of approximately 10-15 cm on the blade in August (Vivian Husa IMR, pers. obs.).

**Figure 3.3.1.2-1:** Left: *Didemnum vexillum* covering the lamina. Right: *D. vexillum* covering hapterons (attachment structure, holdfast) and stipes (stalk) of *Laminaria hyperborea* in Haugesund. (Photo: Vivian Husa)

In Norway *D. vexillum* is observed to be growing on both sides of the lamina in a thick sheet, but also covering the natural inhabitants on the stipes and in the hapterons of the kelp (Figure 3.3.1.2-1). The colonial ascidians weigh substantially more than the thin sheets of bryozoans and could lead to an earlier detachment of the old blade in spring, depriving the new blade for stored products for growth.

**Figure 3.3.1.2-2:** *Didemnum vexillum* attached to macroalgae near Egersund in December 2022. Photo: Erling Svensen.

The *L. hyperborea* forest provides habitat and food supply for numerous marine species, which also potentially will be affected as overgrowth of the stipe community is observed. Dense settlement of ascidian colonies on hard substrate in the kelp forest might also prevent the settlement of new kelp recruits.

In summary, *D. vexillum* might negatively affect both the growth rate, recruitment and survivorship of kelp plants, in addition to reducing the biodiversity in kelp communities. As kelp plants are crucial for sustaining kelp forest communities, VKM assesses the potential impact of *D. vexillum* on biodiversity in Norway through biofouling of wild *L. hyperborea* kelp forests as **"Moderate"** in Norway. However, for kelp forests in northern Norway (Norwegian Sea and the Barents Sea), where these habitats are affected by grazing by sea urchins and are already listed as threatened (according to the Norwegian Biodiversity Information Centre 2021 Red List), the impact is expected to be **"Major"**. Although *D. vexillum* is observed colonizing kelp plants in Norway, there are no published research on this, and these assessments are therefore made with **"Low"** confidence.

#### ***3.3.1.3 Likelihood***

*D. vexillum* is found in several areas in Norway already, and further spread is likely. As it has been shown to grow on kelp in Norway, the likelihood of having a negative impact on biodiversity is therefore primarily driven by where the species can survive (see section 3.1). VKM assesses that the likelihood of *D. vexillum* having a negative impact on biodiversity, through biofouling *L. hyperborea* kelp forests, in Norway is **"Likely"** for all areas with suitable habitat. Due to the likely spreading of *D. vexillum* as biofouling on vessels, VKM assesses it as likely that *D. vexillum* can spread long distances up to Svalbard just as fast as shorter distances, such as Trondheimsfjord. These assessments are made with **"Medium"** confidence.

#### 3.3.1.4 Risk characterization

Biofouling of *D. vexillum* on kelp forests is assessed as having potentially "Moderate" and "Minor" effects on biodiversity in southern- and northern Norway, respectively. VKM assesses that it is "Moderately likely" and "Unlikely" that these effects will occur for the two areas, respectively. In sum, VKM finds that *D. vexillum*, through biofouling on *L. hyperborea* kelp forests, poses a **"Moderate" risk** to biodiversity in Norwegian waters in general, but a **"High" risk** to habitats further north that are already threatened. This assessment is made with **"Medium"** to **"Low"** confidence.

### 3.3.2 Potential impact on *Zostera marina* communities

#### 3.3.2.1 Hazard identification

Biofouling of seagrass communities will, as with kelp communities, shade sunlight and reduce photosynthetic rates of the host organisms, as well as hindering uptake of nutrients and gas exchange, thus reducing growth and energy storage (Levin et al. 2002, Andersen et al. 2011, Long and Grosholz 2015). Seagrasses are angiosperm organisms with developed root systems that are found on subtidal and some lower intertidal soft-bottom substrates in fully marine areas. In Norway, the eelgrass *Zostera marina* is the most important species that forms seagrass meadows. Well-developed and dense *Zostera* patches create three-dimensional meadows that are habitats for a number of associated species (Fredriksen et al. 2005, Boström et al. 2014) and function as nursery grounds and hiding places for fish spat and crustaceans. Eelgrass meadows also bind sediments and increase the resistance of soft-bottom environments to erosion from currents and waves. The eelgrass *Zostera marina* has a wide distribution in the northern hemisphere from warm-temperate to subarctic regions. In Europe the eelgrass is common in Scandinavian, British and French Atlantic waters. Eelgrass habitats in the Skagerrak/Kattegat area have in recent years undergone large-scale losses most probably related to habitat destruction, eutrophication and overfishing of cod and other top predators, the latter leading to decreased grazing and increased epiphyte loads (Boström et al. 2014).

Several ascidians, both solitary and colonial species, have been reported to attach to eelgrass. The first reported occurrence of *Didemnum vexillum* from eelgrass (*Zostera marina*) was made from the east coast of USA (New England) where it was recorded together with several other alien invasive ascidians (Carman and Grunden 2010). Later, *D. vexillum* was reported from similar habitats in California (Long and Grosholz 2015). The species attaches to the stalk and blade of eelgrass plants, usually growing up from the base but may also spread out on the sediment close to the stalk (Long and Grosholz 2015). Fragments of *D. vexillum* and larvae attach readily to eelgrass plants even at fairly low temperatures (6–10 °C), which in turn could contribute to long distance spread by rafting on detached eelgrass blades and plant debris (Carman et al. 2014).

Long and Grosholz (2015) demonstrated that overgrowth of *D. vexillum* reduced plant growth and production, presumably by blocking light and gas fluxes. In the field, above-ground biomass production was reduced about 30% when plant shoots were covered up to about 20% of plant lengths. With regard to associated fauna, eelgrass with overgrowth showed an unexpected higher mean invertebrate density than uncovered eelgrass. However, only two taxa of small benthic species contributed to the change, and several were unchanged. For the increasing taxa, *D. vexillum* may have provided shelter from predation. Larger taxa and more mobile fauna were not well estimated by the methods used so that general ecosystem effects were essentially not assessed (Long and Grosholz 2015).

In a study of a related species, *Didemnum perlucidum,* generally causing similar problems as *D. vexillum* in Australian waters, measurably negative effects were recorded for overgrown seagrasses (including *Zostera*) and resident fauna in an Australian estuary (Simpson et al. 2016). A dominant mud snail playing a key ecological role in the estuary was significantly reduced leading to potentially severe effects for ecosystem functioning. It was also indicated that the acidic tunic of didemnids deters fish predators and snails, and also covers the decomposing seagrass leaves.

#### ***3.3.2.2 Hazard characterization***

In Norway, eelgrass meadows are best developed in sheltered inshore areas in southern and middle Norway. In northern Norway (Troms and Finnmark county) eelgrass is more scattered and often found in the lower intertidal zone. The depth distribution is down to about 8 m. Eelgrass habitats are generally threatened by several human activities. Eelgrass is often found in sheltered bays and inlets that are preferred places for leisure craft harbours. Vessel traffic, shading from docked vessels, and oil spills can destroy eelgrass. In many areas, especially in south-eastern Norway, sheltered localities are subject to eutrophication from nutrient runoff from urbanised areas and areas with intensive agriculture. A consequence of eutrophication is that eelgrass may be overgrown by short-lived epiphytic algae that shade surfaces and reduce photosynthetic activity. Physically, the habitat can be damaged from dredging and construction work for moorings, docks, cables and underwater pipelines. Norwegian environmental authorities are generally restrictive in allowing new activities in areas with well-developed eelgrass meadows to protect these sensitive habitats as much as possible. In the inner Oslofjord, trials have been carried out to experimentally restore damaged eelgrass meadows.

It must be assumed that the reported effects of *Didemnum vexillum* on *Zostera* will be rather similar in Norwegian waters. *Zostera* plants can be seriously affected by restricting growth and production of new leaves, resulting in a lower canopy density of the meadows. The associated fauna will most probably be reduced, especially mobile and swimming species using the canopy for shelter and foraging, though little mobile species on stems and shoots may increase. The value as nursery grounds for fish spat could be lost. VKM hence assess that the potential impact of *D. vexillum*, through biofouling, on *Zostera* habitat is **"Major"** in Norway. This assessment is made with "**Low**" confidence.

#### 3.3.2.3 Likelihood

At present, no eelgrass habitats in Norway appear to be infected by *D. vexillum*. The rather few reports of invasion of seagrass habitats elsewhere could imply that seagrasses are not very susceptible to colonisation by *D. vexillum*. Carman and Grunden (2010) observed that *D. vexillum* was found on *Zostera* in the vicinity of infested docks and artificial structures and indicated that the colonisation of eelgrass might be due to sparsity of preferred substrates in the invaded area. Long and Grosholz (2015) observed in mesocosm experiments that *D. vexillum* grew better on hard substrates than on *Zostera*. Carman and Grunden (2010), however, commented that their observations were the first record of what is probably occurring in many locations. There is not much information on local spread from infested artificial structures into natural habitats. It may be worth mentioning that for the related *D. perlucidum*, Simpson et al. (2016) noted a consistently higher prevalence on seagrasses in areas with boat moorings, jetties and yacht clubs. In particular, the first colonies at the start of the growing season were recorded around these structures, suggesting that they facilitated the recruitment into seagrass beds.

The presence of *D. vexillum* at Koster close to the Norwegian border increases the possibility of invasion also in southeastern Norwegian waters. There is a high traffic of leisure crafts in Skagerrak between Norway and western Sweden that could potentially transmit *D. vexillum*. In addition, the prevalent surface currents flow northwards from Sweden to Norway suggesting a risk of raft transfer with detached seaweeds or driftwood and other flotsam. VKM assesses the likelihood of invasion and negative impact on *Zostera* habitats and associated biodiversity from *D. vexillum* in Norway to be **"Moderately likely",** except for areas in close vicinity to typical 'hot spots' for *D. vexillum* (e.g., harbours and docks with high vessel traffic) where effects would be **"Likely"** due to local spread. These assessments are made with **"Medium"** confidence.

#### 3.3.2.4 Risk characterization

Based on the magnitude of potential impact and the likelihood of occurrence VKM finds that *D. vexillum* in general poses a **"Moderate" risk** to eelgrass habitats in Norwegian waters including both plants and associated biodiversity, but a **"High" risk** to habitats in the vicinity of infested harbours, docks and other human-made environments with artificial substrates. This assessment is made with **"Medium"** confidence.

### 3.3.3 Potential impact on maerl beds

#### *3.3.3.1 Hazard identification*

Maerl beds are built up by calcareous red algae that form loose aggregations in soft bottom environments, particularly in areas with strong currents. The beds often have a lower layer of several meters of dead coralline sand and an upper layer of living individuals, which forms a 3-dimensional habitat for many species. Species-forming maerl is slow-growing (1–2 mm a year) and maerl beds are therefore difficult to restore when destroyed (Blake & Maggs 2003). The habitat is vulnerable to shading, competition for food and space, biofouling and impact from invasive alien species (see Figure 3.1-1a, c, d).

Development of dense mats of colonial ascidians on maerl beds can reduce the light available for photosynthesis and hinder gas exchange in the cells, which again will reduce the growth rates of the maerl beds. As maerl species are calcareous algae with $CaCO_3$ in the cells, they are vulnerable to acidification (Cornwall et al. 2022). *Didemnum vexillum* has a low pH on their body surface which may conflict with calcification rates and thereby growth in the algae when colonizing the surfaces.

Maerl-forming species usually get rid of most of their epiphytes by rolling and turnover at the sediment surface caused by waves and currents. The rapid growth of *D. vexillum* could hinder this natural way to get rid of epiphytes once a dense and firm mat is established during calm weather conditions in summer.

Encrusting species can have large effects on biodiversity in sedimentary habitats with gravel, pebbles or other firm objects that the species may attach to. At Georges Bank and Long Island Sound on the east coast of North America, *D. vexillum* forms extensive mats on the surface of gravel substrates. The mats essentially 'glue' gravel and pebbles together, thereby transforming the habitats from basically three-dimensional systems into more two-dimensional systems (Mercer et al. 2009). Presumably the mats act as barriers for exchange of oxygen and nutrients between the sediment column and the water above. Studies of biodiversity in the invaded areas showed that epibenthic species were significantly reduced whereas some species living inside the mats, contrary to expectation, became more abundant, probably because the mats could provide protection against predators (Mercer et al. 2009, Lengyel et al. 2009, Kaplan et al. 2018). In case of colonisation of maerl beds by *D. vexillum,* similar effects would be expected but effects could as well be stronger because the maerl itself will be affected. Reduced growth or die-off of maerl combined with reduced water exchange below *Didemnum* mats may lead to hypoxic conditions in the sediments that in turn will have critical consequences for the fauna.

#### *3.3.3.2 Hazard characterization*

Norway has extensive maerl beds (calcareous red algae) in the northern part of the country, which are considered to be an important habitat for other marine species by providing a 3-dimensional habitat. The maerl beds in Norway are not mapped, but Trøndelag, Nordland,

and Troms & Finnmark Counties are the areas where larger maerl beds are more frequent; in the southern part of the country, only small areas have been reported. Norway probably has the largest and most pristine maerl beds in Europe.

Dense establishment of *D. vexillum* on maerl beds will have negative effects through different mechanisms. Effects include shading and reduces photosynthesis and growth in the maerl forming species, competition for food and space for associated fauna, biofouling and both structural and chemical impacts that alter these communities.

In sum, VKM determines that the joint effects of these mechanisms can have **"Major"** impact on maerl bed communities, should *D. vexillum* establish and form dense beds. This assessment is made with "**Medium"** confidence.

#### 3.3.3.3 Likelihood

*Didemnum vexillum* is found in several areas in Norway already, and further spread is likely. In the Haugesund area, the species is reported with a few colonies outside Karmøy. which is some kilometres away from a small maerl bed. However, there are no records of *D. vexillum* in this habitat yet. As the further spread in the northernmost counties depends on where it can survive and reproduce, the likelihood of colonization and impact in northern maerl beds is uncertain. VKM assess that the likelihood of *D. vexillum* will have negative impact in maerl bed communities in Norway to be "**Likely"** in suitable habitats (see section 3.1) in Norwegian waters. These assessments are made with "**Low"** confidence.

#### 3.3.3.4 Risk characterization

Biofouling of *D. vexillum* on maerl communities is assessed as having a potentially **"**Major effect" on biodiversity in Norwegian waters. VKM assesses that it is "Likely" that these effects will occur. In sum, VKM finds that *D. vexillum* poses a **"High" risk** to biodiversity in maerl communities in Norwegian waters. This assessment is made with **"Low"** confidence.

### 3.3.4 Potential impact on soft bottom communities

#### 3.3.4.1 Hazard identification

In the broad sense, soft bottom includes all sediments from very fine mud to gravel and cobbles, in practise all bottoms where the bottom material can be moved by currents and waves. Typically, soft bottom habitats harbour organisms living either within the sediment or on the sediment surface. The living conditions and species communities differ substantially from fine-grained mud in calm and sheltered waters to sand, gravel and pebbles in wave- and currents-swept areas. Many species perform activities that either move sediments or bind sediments and thus contribute to shape the environmental conditions.

Whereas *D. vexillum* is a typical encrusting species mainly found on firm and stable substrates, it is also able to colonise habitats with sand, gravel and pebbles. The most well-documented case is the colonisation of large areas (> 230 $km^2$) of sand/gravel regions at

50–80 m depth in Georges Bank on the east coast of North America (Valentine et al. 2009, Lengyel et al. 2009, Kaplan et al. 2018). Colonisation of pebble and cobble habitats is also reported from Long Island Sound, USA (Mercer et al. 2009). In these areas, *D. vexillum* forms extensive mats essentially gluing gravel and pebbles together. The mats could alter the flux of materials between the water column and the sediment by creating a barrier to water flow at the sediment surface (Mercer et al. 2009). On Georges Bank, the effects on the resident fauna have been strong. Before and after studies of benthic fauna in invaded areas (Lengyel et al. 2009) and comparative studies of epifauna in invaded and uninvaded areas (Kaplan et al. 2018) showed that most resident species were negatively affected. Only a few motile bristle worms and large predatory species such as crabs and sea stars increased in population size. In addition to the direct overgrowth of resident species (hydroids, barnacles, bryozoans), the acidic nature of the tunic may repel larvae of benthic species from settling and thereby inhibit recruitment to the resident species of the marine communities. On Georges Bank, impacts on recruitment are of particular concern for recruitment of scallops (*Pecten magellanicus*) that are a commercially harvested species (Kaplan et al. 2018). It has also been indicated that the environmental changes reduce the value of the habitat as feeding grounds for juvenile Atlantic cod and haddock (Lengyel et al. 2009).

The increased abundances of motile bristle worms suggest that *D. vexillum* may create a favourable habitat for some species. It is possible that the dense tunic mat provides protection from predators, such as bottom feeding fish (Lengyel et al. 2009). Crabs and sea stars may easily traverse areas with *D. vexillum* and prey on damaged or immobilised animals or take advantage of discards from the fishing activities in areas open to bottom fishing (Kaplan et al. 2018).

In Long Island Sound, the seabed fauna appeared to respond differently and to some extent opposite to the fauna at Georges Bank. Mercer et al. (2009) found that total abundance and species richness of benthic fauna were either not different or significantly higher in samples from *Didemnum* mats compared to samples collected just outside of the mats. Their results were contrary to expected, but they suggested that the *Didemnum* mats might provide protection from epibenthic predators and thus could explain the higher abundances. Yet, Kaplan et al. (2017) pointed out that the different results might as well depend on the size and types of organisms studied. Whereas small-bodied organisms were in focus in Long Island Sound, larger organisms were studied at Georges Bank. The contrasting results hence suggest that the effects of the *Didemnum* may differ for different components of the fauna, and probably then most clearly expressed between epifaunal species living on the sediment surface and species living in the sediment layers.

Muddy sediments are generally not colonised by *D. vexillum*. It is assumed that establishment will not be possible in sediments that lack firm objects to attach and where small sediment particles could clog the water filtering system (Valentine et al. 2009). It has been observed, however, that colonies established on patches of firm substrates or epibenthic organisms may spread out on nearby soft sediments. In seagrass beds, for instance, *D. vexillum* may spread out from plant stems onto the bottom and may stabilise sediment (Long and Grosholz 2015). Gittenberger (2007) observed colonies of *D. vexillum* that expanded on sand after overgrowth of solitary ascidians.

#### *3.3.4.2 Hazard characterization*

In Norway, muddy and sandy sediments cover extensive areas of the seabed from the intertidal to the largest depths in fjords and offshore. Coarser sediments dominated by gravel or pebbles are largely present at more shallow depths in current and wave-swept areas. The distribution of sediment types is roughly known for Norwegian coastal areas from early sediment mapping for public navigational charts. At present, there are ongoing mapping activities producing updated and detailed sediment charts for offshore and coastal areas through the Mareano program and related activities (www.ngu.no > maringeologi). In the coastal areas, parts of the Oslofjord including Færder national park, the fjords at Stavanger, the region from Gulen to Sunnmøre on the west coast, scattered fjords in Trøndelag and Nordland and some larger fjord systems in Troms and Finnmark have been mapped. In addition, several municipalities have gathered local information of seabed sediments for planning and management of coastal activities.

The coarse sediment types from gravel to pebbles with more or less admixture of sand and mud are considered to be the soft bottoms that could be successfully colonised by *D. vexillum*. The hazard characterisation is therefore restricted to these sediment types. Very little is known about the benthic fauna in Norwegian coarse sediment habitats, but it may be assumed that a variety of epifaunal organisms (sponges, hydroids, tube-building bristle worms, bivalves, bryozoans, echinoderms, ascidians, as well as some macroalgae) will be found unless the bottom is very unstable from currents or wave action. In some cases, species of particular concern are found in dense populations and then considered as habitats in itself, for instance the European oyster (*Ostrea edulis*, common name: østers) and the horse mussel (*Modiolus modiolus*, common name: o-skjell) that are considered as threatened habitats by OSPAR. Both species may be seriously affected by overgrowth of *D. vexillum,* as has recently been reported for the Pacific oyster in Swedish waters (see Figure 1.6.1-2 above).

Based on the reports from Georges Bank and Long Island Sound, it may be assumed that several benthic species will be negatively affected by *D. vexillum,* whereas some species may be little affected, and some species essentially favoured. VKM assesses that *D. vexillum* generally may have "**Moderate"** impact on coarse sediment bottoms but may have **"Major"** impact on habitats dominated by European oyster or horse mussel. These assessments are made with **"Medium"** confidence.

#### *3.3.4.3 Likelihood*

In Norway, *D. vexillum* has been recorded from gravel or sand-mixed gravel sediments in the harbour area of Stavanger. It is therefore to be expected that *D. vexillum* will colonise similar substrates elsewhere in Norway when it becomes established in near habitats, for instance on hard bottom substrates or artificial structures. Small, detached fragments from colonies transported by currents will likely settle on muddy, sandy or gravel substrates where current-transported items are deposited. VKM assess that colonisation of coarse sediment habitats in Norway, including oyster and horse mussel habitats, is **"Likely"**. This assessment is made with **"Medium"** confidence.

#### *3.3.4.4 Risk characterization*

Based on the magnitude of potential impact and the likelihood of occurrence VKM finds that *D. vexillum* in general poses a **"Moderate" risk** to coarse sediment habitats in Norwegian waters, except for habitats dominated by European oyster or horse mussels where there is a **"High risk"**. These assessments are made with **"Medium"** confidence.

Soft fine-grained sediments dominated by clay, silt or fine sand have not been assessed. Such habitats are very unlikely to be colonised, possibly with exception for small patches adjacent to hard substrates or organisms overgrown by *D. vexillum*.

### 3.3.5 Potential impact of *D. vexillum* on benthic communities on hard bottom surfaces

#### *3.3.5.1 Hazard identification*

The main hazard of *D. vexillum* on hardground is probably the active overgrowth of other surface-dwelling organisms that are attached to the substrate. Vulnerable species are found within several phyla, such as sponges, cnidarians (corals, gorgonians, hydroids), polychaete worms, barnacles and ascidians. Species within such groups could contain valuable chemicals and are as such valuable in addition to their specific role in benthic ecosystems ('keystone' organisms or species with less importance). Some species may have defensive mechanisms protecting them for overgrowth such as some species of sea anemones and the ascidian genus *Halocynthia,* but these mechanisms are relatively rare. Many overgrown organisms can therefore suffer from the overgrowth by *D. vexillum*, and the overgrowth may therefore cause a hazard for the community composition (Gittenberger 2007).

#### *3.3.5.2 Hazard characterization*

A stable substrate, such as found on large rocks and rock walls will, under favourable conditions concerning water current and food supply, host marine benthic communities with both high biomass and high species diversity. Examples of such biotopes can be found in tidal currents such as Saltstraumen in Nordland County and Rystraumen in Troms, in sounds such as Skarnsundet in Trøndelag and steep rocky walls in many Norwegian fjords. Overgrowth by *D. vexillum* may in such habitats have profound negative effects for the biodiversity.

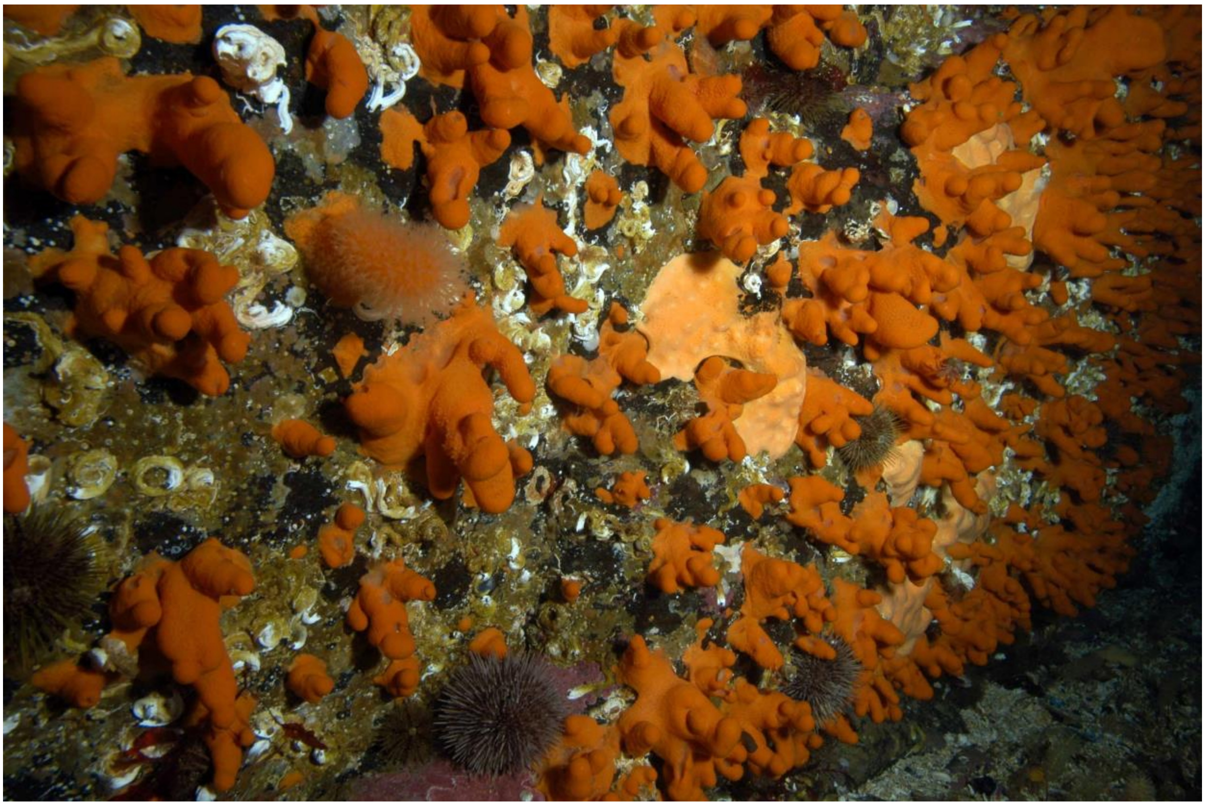

**Figure 3.3.5.2-1:** Vertical rocky wall in Rystraumen dominated by soft coral 'Dead man's finger' (*Alcyonium digitatum*). Lighter patches with didemnids (NB. Not *Didemnum vexillum*) occur between coral colonies. Photo: Bjørn Gulliksen.

Within the context of global climate change, an important process is the sequestration of dissolved inorganic carbon dioxide from the atmosphere and surface waters for periods of interest to global warming. In the marine environment, sequestration and storage of carbon dioxide takes place in calcareous biogenic structures such as coral reefs, polychaete reefs, bryozoan colonies and the shells of molluscs. Overgrowth of such structures by *D. vexillum* may reduce the ability of such structures to store inorganic carbon structure.

Some benthic organisms with hard and calcareous exoskeletons, which are common on hard surfaces, build a secondary substrate and increase substrate complexity with crevices and cavities (Dean 1981; Senn and Glasstetter 1989; Sebens 1991). Such biogenic structures do usually increase the species diversity in a locality. Examples from Norwegian waters are especially found within cold-water corals and polychaetes.

Norway has the highest occurrence of documented cold-water coral reefs in the world (Järnegren and Kutti 2014). The major reef building species is the stony coral *Lophelia pertusa* (syn. *Desmophyllum pertusum*) that constructs some of the most complex three-dimensional structures in the deep sea (Figure 3.3.5.2-2). The structures are made of calcium carbonate and are considered biodiversity hot spots, housing an associated fauna of more than 1300 species in the NW Atlantic (Roberts et. al 2006). Normally occurring between 200 and 400 m depth along the continental shelf, cold-water corals are commonly found at shallower depths in the fjords and up to 39 m (Tauterryggen, Trøndelag). A

Lophelia-reef consists of up to 70% of dead coral structure (Vad et. al 2017) while the living coral constitutes the outer 10–30 cm of the structure. The majority of the associated organisms dwell in the dead coral skeleton, making it as important as the living corals. Other important species of cold-water corals include the gorgonians *Paragorgia arborea*, *Primnoa resedaeformis* and *Paramuricea placomus*, as constructors of coral gardens which also function as an important habitat for other species (Figure 3.3.5.2-3). These species are found at depths similar to *L. pertusa*, and also occur shallower in the fjords where they can be found from 20 m and below.

The fjords in Norway harbour the shallowest locations of cold-water corals in the world, starting at 39 m depth which puts them at risk for *D. vexillum* that has been found down to 81 m. The hard structure of the calcium carbonate reefs would likely provide a suitable surface for *D. vexillum* to settle and grow on. Studies on tropical corals have shown that a *Didemnum* sp. rapidly overgrew a reef area consisting of both live and dead coral, with the ascidian covering the coral colonies completely when affected (Roth et al. 2018). However, the tropical coral reefs differ much from the cold-water corals in Norway. A perhaps more comparable habitat is the before-mentioned Georges Bank in NW Atlantic, which harbours 230 km$^2$ of thriving *D. vexillum* populations, having tidal currents coming up in speeds of 1–2 knots (50–100 m/s). These currents correspond to the currents over shallow cold-water coral reefs that are also tidal driven and nutrient rich, giving the organisms time to feed in the calmer periods when the tide is changing.

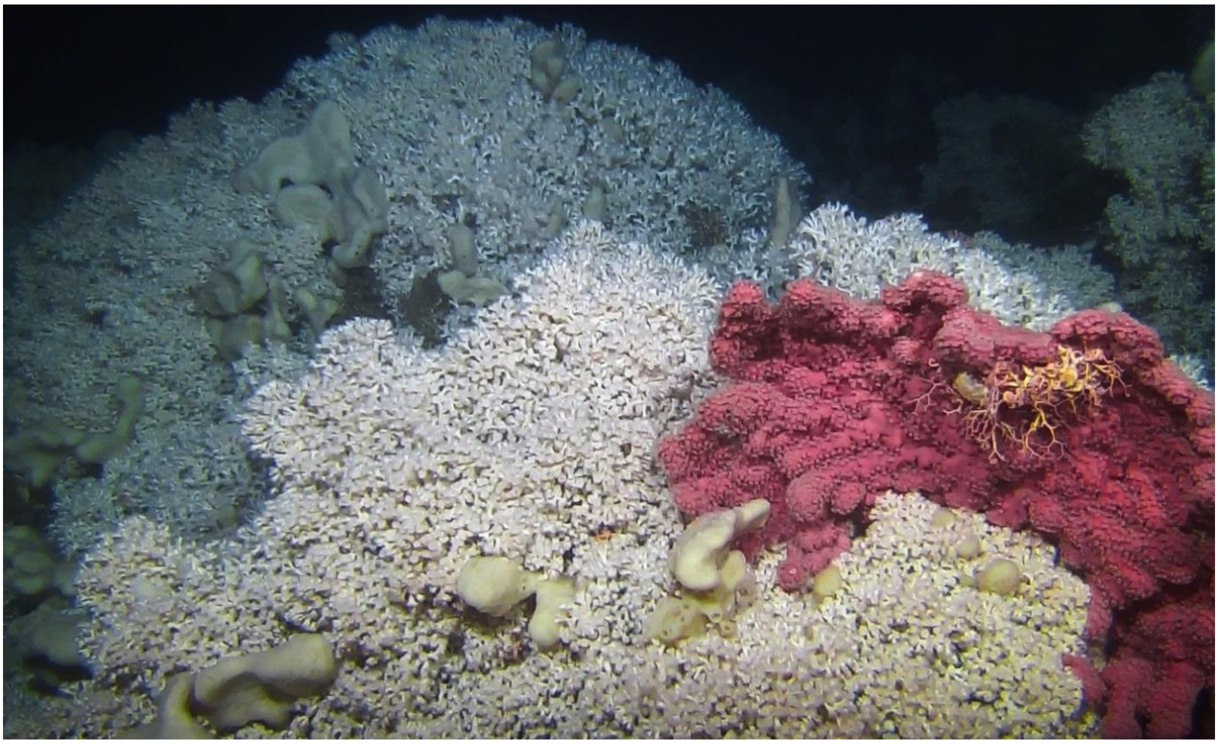

**Figure 3.3.5.2-2:** *Lophelia pertusa* from Trondheimsfjord (Photo: Johanna Järnegren)

This type of habitat appears, as seen on Georges Bank, to suit *D. vexillum* well. As with maerl-beds, the acidic tunic will likely also affect the carbonate structure negatively. In the event of *D. vexillum* gaining foothold on a coral reef or coral garden, it is unlikely that the coral, or its associated fauna, could survive being covered for a prolonged period of time.

**Figure 3.3.5.2-3:** Coral gardens in Trondheimsfjorden. *Paramuricea placomus* and *Paragorgia arborea.* (Photo: Johanna Järnegren)

Serpulid polychaetes cement their tubes to firm substrates and occur throughout the world, often aggregating in unstable environments. The polychaete genus *Filograna* is a Norwegian example of a polychaete forming small calcareous reefs. For example, more than 100 species comprising only 160 g of biomass were found within only a 4.4 l volume of serpulid polychaete aggregations in the tidal current of Rystraumen in Troms (Haanes & Gulliksen 2011). Rystraumen is now (as of 2020) a protected area in Norway, mainly due to the high diversity of the benthic communities occurring in this tidal current.

**Figure 3.3.5.2-4:** Calcareous reefs of *Filograna implexa* from the protected tidal stream Rystraumen in northern Norway. Photo: Bjørn Gulliksen.

Arthropods, such as shrimps, prawns, lobsters and crabs have hard exoskeletons to which *D. vexillum* can attach, but growth of such organisms is accomplished by periodic moulting and shedding of the exoskeleton. The hazard of *D. vexillum* is therefore probably only temporary concerning such organisms, though a shed exoskeleton with *D. vexillum* on it could function as a source for further spreading the ascidian.

The phylum Mollusca includes solitary species, such as snails and bivalves with hard and calcareous shells, and these shells functions as hard substrates and are thus attractive for settlement of *D. vexillum*. See section 3.3.6 for assessment of the impact on bivalves.

VKM assess that the negative impact, through biofouling (overgrowth) and competition for space, on biodiversity from *D. vexillum* establishment would be as follows on the different types of hardbottom habitats in Norwegian waters:

A. Hard surfaces in general – **"Major"** (**"Medium"** confidence)

B. Specific habitats with high nutrition flow and high biodiversity – **"Major"** (**"Medium"** confidence)
C. Shallow cold-water coral reefs – **"Major"** (**"Medium"** confidence) bordering to "Massive" as these effects are likely to be irreversible due to the slow growth rate of cold-water corals.

#### ***3.3.5.3 Likelihood***

The most likely effect of *D. vexillum* on hardground is connected to competition for space with other species and overgrowth of other species. VKM assess that spreading of *D. vexillum* to hardbottom habitats in Norway is **"Likely"** (with **"Medium"** confidence). In regard to the specific areas, with high biomass and high biodiversity, mentioned above, VKM assess that the spread to, and thus impact on, these habitats is also **"Likely"** with **"Medium"** confidence. Finally, regarding shallow cold-water coral reefs, VKM assess that the negative impacts described above is **"Likely"** to occur if measures are not taken to reduce the spread of *D. vexillum*. This assessment is made with **"Medium"** confidence.

#### ***3.3.5.4 Risk characterization***

Overgrowth by *D. vexillum* will reduce the supply of food and oxygen, and eventually kill the overgrown organism, and result in a lower species diversity. The effect of reduced species diversity will be increased if biogenic structures containing many other species are overgrown.

VKM thus assess that the negative impact on biodiversity from *D. vexillum* establishment represent the following risk to the different types of hardbottom habitats in Norway:

A. Hardbottom surfaces in general – **"High"**, bordering to **"Moderate"**, with **"Medium"** confidence.
B. Specific habitats with high nutrition flow and high biodiversity – **"High"**, bordering to **"Moderate"**, with **"Medium"** confidence.
C. Shallow old-water coral reefs – **"High"**, bordering to **"Moderate"**, with **"Medium"** confidence.

### 3.3.6 Potential impact on wild bivalves

The animal class bivalves (Bivalvia) belong to the phylum Mollusca. Molluscs are a species-rich phylum with around 100 000 existing known species (Moen & Svensen 2020) and contain well-known groups such as snails (class Gastropoda), clams, scallops, oysters and mussels (class Bivalvia), and squids, octopus and cuttlefish (class Cephalopoda). NBIC (2022) reports 851 species of molluscs in Norway.

Bivalves occur in a wide variety of habitats and substrates. Several bivalve species live embedded in the substrate with just their siphons sticking out above the sediment surface, and some of these species have an ecological role as food for organisms, such as fish, birds and marine mammals. Many bivalves embedded in the substrate are also edible for humans such as razor shells (fam. Solenidae) and cockles (fam. Cardiidae), which have commercial value in many countries although not in Norway.

Scallops and oysters are examples of bivalves living as epifauna on the bottom but not strongly attached to the substrate. Scallops are quite motile; they can swim short distances and thus escape from predators.

#### *3.3.6.1 Hazard identification*

Epigrowth, which is quite common on the calcareous shells of scallops, may have both positive and negative effects. A positive effect is that the epigrowth may protect the shells from predation. A negative effect is that the epigrowth can reduce uptake of nutrients and gas exchange and ultimately reduce the growth of the host organisms (Morris et al. 2009, Auker 2010). Another hazard concerning biofouling is related to the swimming ability of scallops. However, relatively little research has been carried out regarding identification of *D. vexillum* hazards for wild populations of bivalves compared to hazards for species in aquaculture (See section 3.4.2 for assessment of risk posed to bivalve aquaculture).

Experiments have been performed to examine the effects on the swimming behavior of the sea scallop *Placopecten magellanicus,* which is indigenous to the northwest Atlantic from the waters of Newfoundland and Labrador to Cape Hatteras (Dijkstra & Nolan 2011). Dijkstra and Nolan (2011) found that scallops covered by *D. vexillum* became exhausted more quickly and were not able to swim as far in either horizontally or vertically as the control sea scallops without *D. vexillum* encrustation. They conclude that the expansion of *D. vexillum* into sea scallop habitat may increase the vulnerability of sea scallops to predation and limit their ability to access food rich habitats. Also, *D. vexillum* may affect recruitment of bay scallops (*Argopecten irradians*), as scallop larvae have been observed to avoid settlement of *D. vexillum* colonies (Morris et al 2009).

Interaction of *D. vexillum* with the Atlantic Sea scallop *P. magellanicus* is described from Georges Bank, a shallow and highly productive submarine plateau off the west coast of New England, USA (Kaplan et al. 2017). Georges Bank supports a number of valuable commercial fisheries (Butman & Beardsly, 1987). The study of the relationship between the invasive tunicate (initially observed on Georges Bank in 1998) and the scallop took place in the northeast portion of the bank with both sand and gravel substrates and high densities of sea scallops and *D. vexillum* in some locations. Their results suggest that *D. vexillum* competes for habitat with *P. magellanicus*, and that there is a negative relationship the two species on commercially important fishing grounds. Scallop spat cannot settle on *D. vexillum*, likely as a result of its acidic tunic (Morris et al. 2009). Overgrowing of gravel by the tunicate do also cement grains making it more difficult for scallops to burrow into the substrate (Mercer et al. 2009).

Auker et al. (2014) report epigrowth of *D. vexillum* on the blue mussel (*Mytilus edulis*, common name: blåskjell) in the Gulf of Maine. The authors reported that epigrowth decreases mussel growth and reproduction, while also decreasing predation on the mussel by a common predator, the green crab *Carcinus maenas* (common name: strandkrabbe).

In the Netherlands, *D. vexillum* is observed to be spreading in European oyster and blue mussel banks in the Wadden sea (Gittenberger et al. 2019). *D. vexillum* have shown significant negative impacts on mussels, scallops and oysters by overgrowing them via biofouling and thus reducing available food and hindering gas exchange and recruitment (Morris et al. 2009, Auker 2010, Switzer et al. 2011, Dijkstra and Nolan 2011).

Some ascidians have been shown to transmit diseases to bivalves. Introduced species that have not lost their associated pathogens or parasites can potentially transmit novel parasites to native hosts (Dunn and Hatcher 2015, Costello et al. 2021). Further, introduced invasive species may promote transmission of pathogens by native species and act as reservoirs and facilitate replication in areas where disease occurs (Costello et al. 2021). Introduced ascidians have been shown to transmit viruses, bacteria and haplosporidian protists, such as *Bonamia ostreae, Minchinia* to oysters and cockles that in turn can cause mass mortalities of bivalves. Costello et al. (2021) showed from Ireland that *D. vexillum* was infected by the bacterium *Vibrio aestuarianus* in the vicinity of infected oysters at oyster aquaculture sites. It appeared that *D. vexillum* had to be in contact with oysters to maintain the disease, but it could function as a reservoir for reinfection or contribute to circulate *Vibrio* among other species, thus heightening disease transmission. It should also be considered that the combined effect of the physical impact from overgrowth and the maintenance of potentially lethal pathogens increases the threat that *D. vexillum* represents for susceptible species of bivalves (Costello et al. 2021).

#### *3.3.6.2 Hazard characterization*

An important hazard in Norway concerns biofouling of *D. vexillum* on wild bivalves with economic importance. The hazard is related to epigrowth on the king scallop (great scallop) (*Pecten maximus* common name: kamskjell) and the Icelandic scallop *C. islandica* (Figure 3.3.6.2-1).

*P. maximus* usually occurs on coarse sandy bottom from 15–35 m depth (Moen & Svensen 2020) all along the Norwegian coast but is more abundant south of the Lofoten islands than further north. It may attain a diameter of up to 210 mm and the annual catches in Norway the last ten years have been between 500 and 1000 tonnes per year.

*Chlamys islandica* has a diameter up to 110 mm in diameter and is thus somewhat smaller than the king scallop, but most often recorded in higher densities in so called 'scallop banks'. The distribution is more northern, and it is not very abundant south of the Lofoten islands. The highest abundances are usually between 20 and 40 m depth. The yearly catch has been low during the last years.

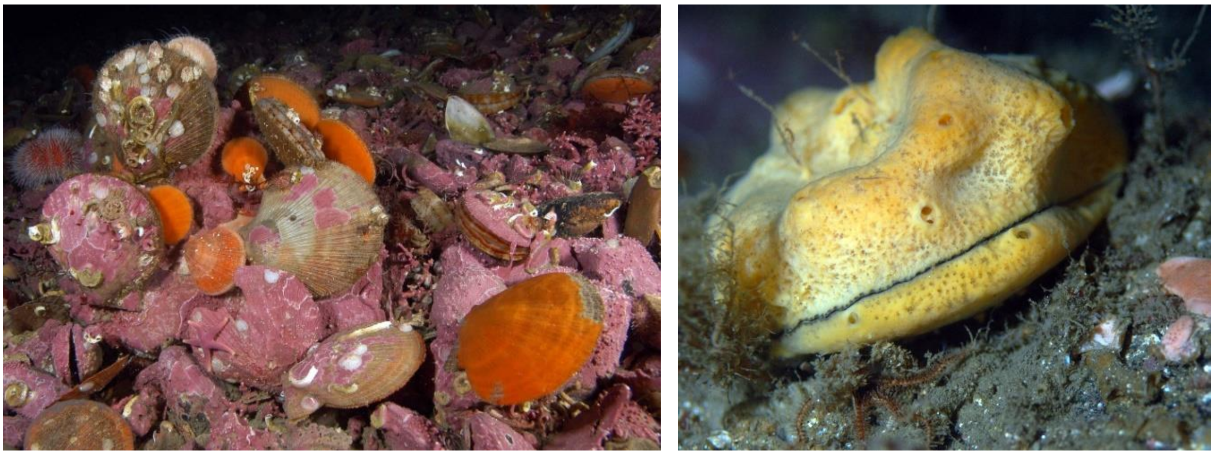

**Figure 3.3.6.2-1:** *Chlamys islandica* on gravel bottom near Berg, Balsfjord, in northern Norway. The picture to the right shows a scallop overgrown by sponge (Porifera). Photos: Bjørn Gulliksen

Biofouling in the form of epigrowth is also expected to have a negative impact on other, non-economically important, species. The only native oyster in Norway is the European flat oyster (*O. edulis*). The species is recorded all along the Norwegian coast from the tidal zone to a few meters depth but needs a water temperature above 16–18 °C to become ripe, and it is therefore not very abundant north of Trondheimsfjorden. It is most common in localities that have high summer temperature such as in semi-landlocked basins (polls). It is valued as a delicacy and is collected from the wild, but not in large enough quantities to be regarded as having economic importance.

**Figure 3.3.6.2-2:** Left: Saltstrømmen in Nordland County. Mussels attached to the rocky wall between sea anemones. Right: A horse mussel overgrown by a didemnid (not *D. vexillum)* Photos: Bjørn Gulliksen.

The Pacific cupped oyster *Magallana gigas* (syn. *Crassostrea gigas,* common name: Stillehavsøsters) was introduced intentionally to European waters and introduced to the Nordic countries in 1977—1979. In 2007, it was recorded in the wild in great numbers south-eastern Norway and has since spread further west and north along the Norwegian coast, at least to Vestland county north of Bergen. There are ongoing projects in Norway collecting this species for sale, but not yet with great economic success.

The blue mussel (*M. edulis*) and the horse mussel (*M. modiolus*) in the family Mytilidae attach to the substrate with strong proteinaceous threads called byssus threads and can also be overgrown by didemnids (Figure 3.3.6.2-2)

The potential hazard and effects of *D. vexillum* will vary among the different species of bivalves in Norwegian waters. The most vulnerable species will probably be scallops, especially *P. maximus* because it has a more southern distribution overlapping with the present distribution of *D. vexillum*. The other bivalve of some economic importance, the Icelandic scallop *C. islandica,* has its main distribution north of the northern recorded distributional limit in Norway, but that may change depending on the expansion northwards of *D. vexillum*. VKM assess that the potential impacts on wild bivalves, through biofouling (overgrowth) and to some extent competition for food, are "**Moderate"** for the two species that are harvested as food: *P. maximus* and *C. islandica.* Similarly, the potential impact on the wild populations of flat oyster (*O. edulis*), the Pacific oyster (*M. gigas*), the blue mussel (*M. edulis*) and the horse mussel (*M. modiolus*) are also regarded as "**Moderate"**. These assessments are made with "**Medium"** confidence.

#### *3.3.6.3 Likelihood*

VKM assess that the likelihood of *D. vexillum* will have a negative impact on biodiversity, through biofouling wild bivalves in Norway to be **"Moderately likely"** for the scallop *C. islandica* and the oysters *O. edulis* and *C. gigas*, blue mussel (*M. edulis*), the horse mussel (*M. modiolus*), and *P. maximus.* These assessments are made with "**Low"** confidence.

#### *3.3.6.4 Risk characterization*

VKM assesses that *D. vexillum,* through biofouling, poses a **Moderate risk** to all of the bivalve species investigated. These assessments are made with **"Low"** confidence

## 3.4 Assessment of potential impacts on aquaculture in Norway

Biofouling organisms are a common nuisance for all aquaculture facilities. Bryozoans, seaweeds, cnidarians, and solitary ascidians are routinely removed from net pens, baskets and other structures, requiring manual labour that increases production costs. Culture organisms, such as oysters, mussels and kelp can also be fouled in the same way as the aquaculture structures and reduce growth and quality of the product. However, as these organisms mostly are native species, they can be removed from the pens *in situ* without further actions taken. In the case of *D. vexillum*, the same treatment would result in fragmentation and further spread of the organism and a different approach will need to be implemented, further increasing the production costs and potentially lowering the output production of the facility.

The project group finds that *D. vexillum* can negatively affect aquaculture facilities and various cultured species, through biofouling on equipment, through both increased production cost (due to increased need for surveillance and net cleaning) and reduced production output (increased mortality, lower production rate etc.). See Table 2.1-2b for definitions of impact categories on net cleaning costs.

### 3.4.1 Potential impact on fish aquaculture

#### *3.4.1.1 Hazard identification*

There have been no international studies of impact of *D. vexillum* in areas where there currently is fish farming in open net pens. The mechanisms behind such impact could be clogging of nets, hindering efficient oxygen supply to the fish in the pens. Examples of such organisms are found in groups as hydrozoans and bryozoans. Common species found on net pens include blue mussels, the alien Japanese skeleton shrimp (*Caprella mutica*) and native copepod *Jassa falcata*, flowerhead polyp (*Ectopleura larynx)*, sugar kelp (*Saccharina latissima*) and other seaweeds, bryozoans, and ascidians like *Ciona intestinalis* and the alien *Styela clava* (Bloecher et al. 2013, Håvardsson 2013).

#### *3.4.1.2 Hazard characterization*

In Norway, there is an annual production of 1.6 million tonnes of salmonid fish in open net pens (Directorate of Fisheries 2021). Biofouling of nets is already a major issue, and the use of copper-treated nets has led to accumulation of copper in the sediment in many places. Subsequently, many farmers have stopped used antifouling treatment on their nets and rely instead on frequent flushing of nets. It is a common practice to flush the nets with different types of underwater devices whether the nets are treated or not. To date there has been no observations of *D. vexillum* on net pens in Norwegian fish farms, but some farms are close to areas that are affected and establishment on these farms are to be expected. Flushing of nets with *D. vexillum* will inevitably break colonies into small fragments which will follow the currents and establish on new sites. A study on the effect of antifouling treatment (copper) against *D. vexillum s*hows that this treatment is efficiently hindering settlement of *D. vexillum* on the nets (figure 3.4.1.2-1).

**Figure 3.4.1.2-1:**. Net pens treated with copper (to the left) and with no treatment (to the right) after 5 months in the sea in the vicinity of *Didemnum vexillum* colonies (Photo: Vivian Husa).

This may lead to more use of antifouling treatments, followed by increased environmental impact from fish farming. The establishment of fast-growing biofouling species such as *D. vexillum* will evidentially increase the industry's cost to deal with fouling on nets, as it is likely that the management authorities will demand cleaning of the nets with technology that assures secure collection of the material.

VKM assesses that the potential impact of *D. vexillum* on fish aquaculture facilities in Norway through biofouling of pens is **"Major"** in all Norwegian waters where there is fish aquaculture. These assessments are made with **low** confidence.

#### *3.4.1.3 Likelihood*

VKM assesses that the likelihood that *D. vexillum* will have a negative impact on fish aquaculture facilities, through biofouling, in Norway to be **"Likely"** in Norwegian waters. These assessments are made with **"Low"** confidence. The likelihood will be dependent on the efficiency of the treatment on the nets.

#### *3.4.1.4 Risk characterization*

The risk posed to fish aquaculture in Norway is assessed to be **"Major"**, bordering to **"Moderate"**. As there is high uncertainty concerning how costly it will be to rinse the nets in such a way that it does not induce further spread of the organism, this assessment is made with **"Low"** confidence.

### 3.4.2 Potential impact on shellfish aquaculture

Fouling by *D. vexillum* and other ascidians has caused major problem for shellfish farmers in areas where the species is introduced (Sinner & Coutts 2003, Auker 2010). In Norway, shellfish production is relatively low compared to finfish production and is consists mainly of blue mussels and European oysters. Small initiatives to culture European lobsters in semi-locked areas also exist. There is currently no commercial production of scallops in Norway.

#### *3.4.2.1 Hazard identification*

Bivalves are filter feeders consuming both phytoplankton and zooplankton in the water masses. They are relatively passive filter feeders, and shell growth is therefore enhanced by water currents. The impact of *D. vexillum* is mainly two-fold: first, competition for food as the ascidian also is a filter-feeder; second, epigrowth of the ascidians may reduce or clog the shell openings or structures for holding bivalves, such as baskets for oyster culture.

The main impact of *D. vexillum* epigrowth on oysters is probably decreased growth rates. On the west coast of Canada, oysters (*M. gigas*) fouled by *D. vexillum* were shown to have a lower condition index than oysters that underwent chemical or mechanical treatment to reduce fouling (Switzer et al. 2011). Negative impacts can also be the result of decreased water flow to shellfish, limiting access and creating competition for food resources (McKenzie et al. 2017). When farmed in cages, epigrowth of *D. vexillum* may reduce water flow through the cages and thus reduce living conditions for the oysters. Oyster farmers in Sweden report that soft spots in the shell of the oyster appear where *D. vexillum* colonies have been situated (Anna Dimming, Länsstyrelsen Västra Götaland, pers. com). This phenomenon may be caused by low pH in the *Didemnum* colonies.

Auker (2010, 2019) studied the effects of *D. vexillum* on biology and ecology of blue mussels *M. edulis* in the Gulf of Maine. She reported that *D. vexillum* had been documented as a pest in the area since 2000 and that there had been an increasing frequency in important fishing and aquaculture locations. Growth of *M. edulis* may decrease when overgrown by *D. vexillum* because the ascidian often smothers the mussel (Bullard et al. 2007), and mussels with complete overgrowth have high mortality (Auker 2010). Overgrowth may also affect reproduction (Bullard et al. 2007, Dijkstra et al. 2007). Ascidians that grow on mussel cultures can also increase the weight of biota on the mussel lines causing mussels to fall from the lines (Gittenberg 2009, Kott 2002). However, Auker (2010) also suggests a positive effect on *M. edulis* in natural populations by providing an anti-predator defence against crabs (*Carcinus maenas*) and sea stars (*Asterias rubens*), two species also quite abundant on the

Norwegian coast, but concludes that *D. vexillum* may have a profound negative effect on mussel populations in areas with abundant ascidian growth. Blue mussels can tolerate wide fluctuations in salinity, desiccation, temperature, and oxygen tension. The ability of *M. edulis* to live in estuarine and brackish waters may protect the species from epigrowth of *D. vexillum* under such conditions. In the Netherlands where there is a developed blue mussel industry, it seems that usually young mussels stay clean the first two years, probably due to antifouling mechanisms, and epigrowth of *Didemnum vexillum* and other organisms is mainly a problem in older mussels with less commercial value than young mussels (Arjan Gittenberger, GIMARIS, pers. comm.).

#### 3.4.2.2 *Hazard characterization*

The shellfish industry in Norway is based on two species of bivalves, the blue mussel (*M. edulis*) and the European flat oyster (*O. edulis*). Currently, 121 culture facilities are listed at the Directorate of Fisheries, but it is likely that many of these are not active (Directorate of Fisheries 2021).

The number of people involved in the commercial production of molluscs, crustaceans (mainly lobster) and echinoderms (sea urchins) in Norway are reported to be 114 persons, which is a relatively low number compared to the 7,070 persons involved in the production of salmon and trout in the country in 2021 (Directorate of Fisheries 2021).

Farming of blue mussel (*M. edulis*) in Norway started with a few attempts in early 1970s, but the volume of production remained relatively low for about 30 years. In 2000, the production was only about 850 tons, increasing to nearly 5000 tons in 2005. The importance of the species as a commercial product can be measured by sales, and in the period 2010–2021, the production was around 2000–2500 tons annually (Directorate of Fisheries 2021). Most blue mussels are farmed on ropes hanging from the surface from floating objects like buoys (longlines). Pelagic spat of blue mussels is usually intercepted by the ropes, and the mussels grow attached to the ropes.

Other species of shellfish of economic importance in Norway include scallops and oysters. Oysters (*O. edulis*) are farmed, and the scallops (*P. maximus*) are mainly collected in nature. The net sale in Norway was respectively 15 tons for oysters and 13 tons of scallops (Directorate of Fisheries 2021).

The oyster *O. edulis* occurs naturally along the Norwegian coast north to the county of Trøndelag. The best quality of the oyster is obtained when the oyster is produced with relatively few specimens in open boxes hanging from the surface in localities with sufficient water exchange and oxygenated water. Concerning temperature, the European oyster is near its northern distributional limit in Norway, and best results for farming are therefore often found in localities with high temperatures, such as polls.

The scallop *P. maximus* occurs all along the Norwegian coast from about 5 m depth down to about 100 m depth. The highest number of registrations along the coast are recorded in the counties Trøndelag and Nordland where *D. vexillum* not yet has been recorded.

The impact of *D. vexillum* on the scallop *P. maximus* is connected to its habitat and behaviour. It is not attached to its bottom habitat (usually, nearly horizontal sandy bottoms) and is able to swim ('jump') for short distances. Dijkstra & Nolan (2011) have reported that overgrowth by *D. vexillum* can decrease swimming ability in sea scallops (*Plagopecten magellanicus*), which may limit their ability to escape predation and access food-rich habitats, which may ultimately affect growth and survival. Also, *D. vexillum* may affect recruitment of bay scallops (*Argopecten irradians*), as scallop larvae have been observed to avoid settlement on *D. vexillum* colonies (Morris et al. 2009).

It is expected that the hazards for the shellfish industry in Norway will be highest for the blue mussel *M. edulis*.

VKM assesses that the potential impact of *D. vexillum* on shellfish aquaculture facilities in Norway through biofouling is:

1. **"Minor"** for blue mussel (*M. edulis*) production in Norwegian waters.
2. **"Minor"** for European flat oyster (*O. edulis*) production in Norwegian waters.

These assessments are made with **"Medium"** confidence.

#### *3.4.2.3 Likelihood*

VKM assesses that the likelihood that *D. vexillum* will have a negative impact on shellfish aquaculture facilities through biofouling in Norway is:

3. **"Likely** for blue mussel (*M. edulis*) production in Norwegian waters.
4. **"Likely"** for European flat oyster (*O. edulis*) production in Norwegian waters.

These assessments are made with **"Medium"** confidence.

#### *3.4.2.4 Risk characterization*

Although the expected impact on the two species of bivalves assessed here is characterized as **"Minor"**, the likelihood of this happening is high. Therefore, the risk posed to shellfish aquaculture in Norway is assessed to be **"Moderate"**. This assessment is made with **"Medium"** confidence.

### 3.4.3 Potential impact on seaweed aquaculture

#### *3.4.3.1 Hazard identification*

One of the major problems in the world's culturing of seaweeds is fouling by other organisms. Fouling on seaweeds decreases photosynthesis and growth of the seaweed and also increases the chances for the fouled specimen to be detached from the aquaculture structure. Heavy biofouling will also degrade the final product as it would need to be cleaned before being used in any way.

#### *3.4.3.2 Hazard characterization*

The production of cultured seaweeds in Norway is currently low but increasing. The annual production is around 200–300 tonnes a year, mainly of sugar kelp (*S. latissima*) and Dabber locks (*Alaria esculenta*). There are currently 210 permits listed in the Directorate of Fisheries, but it is unclear how many are active. Most of the seaweed farms are situated in Vestland and Nordland counties and employed 69 persons in 2021.

Seedlings are usually set out on longline cultures in autumn (Sept–Nov) and harvested before the fouling problem becomes too big in spring or early summer. In this way the production period becomes very short and ends before the main growth season of the seaweeds. As *Didemnum vexillum* is a fast colonizer and is observed in a dense layer on both sides of the blades, this species may have severe impact on the growth and quality of cultured kelp. If *D. vexillum* larvae settles on the kelp during autumn the colonies will continue to grow during winter and be extensive by harvesting in spring. If kelp seedlings are set out late in the autumn, when there is low risk for *D. vexillum* larvae in the water due to low temperatures and harvested before the species starts to reproduce in early summer the risk for settlement on kelp is lower. An examination of Dutch kelp farms (*S. latissima)* at harvesting time in spring/early summer showed no sign of *D. vexillum* on the kelp plants (Arjan Gittenberger, GIMARIS, pers.com). However, effects of *D. vexillum* could be different for other seaweed species which is dependent on summer growth.

VKM assesses that the potential impact of *D. vexillum* on seaweed aquaculture facilities in Norway through biofouling is **"Moderate"**. This assessment is made with **"Medium"** confidence.

#### *3.4.3.3 Likelihood*

Due to the potential spread of *D. vexillum*, VKM assesses that the likelihood of *D. vexillum* having a negative impact on seaweed aquaculture facilities in Norway, through biofouling, to be **"Likely"** if harvesting in late summer. However, this likelihood can be reduced by harvesting earlier in the year, but with lower yield. This assessment is made with **"Medium"** confidence.

#### *3.4.3.4 Risk characterization*

The risk posed by *D. vexillum* to seaweed aquaculture in Norway is assessed to be **"Moderate"**, bordering to **"High"**. This assessment is made with **"Medium"** confidence.

## 3.5 Summarized risks to biodiversity and aquaculture without any measures

### 3.5.1 Risk to biodiversity

In terms of the risk posed to biodiversity in Norway, VKM has found that *D. vexillum* poses a potentially **High risk** to a variety of different habitats. Sensitive habitats include:

- *Laminaria hyperborea* kelp forest in Northern Norway
- Eelgrass meadows in areas with reduced environmental status
- Mearl beds of coralline algae
- Hardbottom habitats (Hardbottom surfaces in general, specific habitats with high nutrition flow and high biodiversity and coral reefs in suitable environment
- Soft bottom habitats consisting of sand, gravel and pebbles dominated by European oyster or horse mussels

Further, VKM assess that *D. vexillum* pose a potentially **Moderate risk** to kelp forests in Southern Norway and eelgrass meadows in non-disturbed areas in addition to six species for wild shellfish and coarse sediment soft bottom habitats in general.

### 3.5.2 Risk to aquaculture

In regard to posing a threat to aquaculture, VKM has assessed that *D. vexillum* poses a **High risk** to fish aquaculture, and a **Moderate risk** to shellfish and seaweed aquaculture.

The main mechanisms that VKM have identified for *D. vexillum* posing a threat are biofouling and competition for space by overgrowing other organisms. These mechanisms are relevant for all habitats and also the three different types of aquaculture facilities. In addition, VKM finds that *D. vexillum* can have a negative impact through structural changes of the mearl bed ecosystem, and potentially through transmission of disease-causing agents to shellfish, including viruses, bacteria and haplosporidians.

# 4 Aims

Chapter 3 describes the risk *D. vexillum* currently poses to biodiversity and aquaculture in Norway and presents the possible impacts this organism will have if no actions are taken to reduce the risk (the "null alternative"). As *D. vexillum* is classified as an invasive alien species, it is unwanted in Norwegian waters, and the overall aim is to reduce its presence and potential impact as much as possible. Here, we describe more detailed aims and in short what measures that need implementation in order to achieve these aims. The risk reducing measures are described in more detail in chapter 5. The aims are divided into two timeframes; the near future of 0–3 years and for a longer time perspective over 3–20 years, depending on how long time it takes to possibly achieve them.

## 4.1 Monitoring

In order to combat *D. vexillum* it is crucial to have information on where it is and whether it spreads to new areas. Thus, establishing some sort of monitoring program would be the primary aim. *Didemnum vexillum* may spread naturally from established populations in native habitats, either by larval drift or by detached plants from colonized kelp forests and seagrass beds in addition to human-mediated spread from harbours and small marinas. To prevent establishment in new areas, early detection of spreading products or incipient colonies in new areas is crucial. Areas that may be of particular focus include:

- Marine protected areas, such as the marine national parks of Hvaler and Færder
- Commercial harbours with high vessel traffic
- Popular guest harbours for leisure crafts
- Areas with aquaculture facilities for fish and shellfish production

## 4.2 Short-term aims

### 4.2.1 Prevent further spread from small marinas

First and foremost, on a short time scale, it is desirable to prevent further spread from *D. vexillum*-infested marinas harbouring privately owned boats. Aims include preventing spread from:

A. The infrastructure of the marinas including stationary and floating structures
B. The privately owned boats
C. Privately owned equipment, including fishing equipment, such as nets and traps.

### 4.2.2 Local eradication

Eradication of this invasive alien species would be a both a short-term and long-term aim. Eradication is perhaps feasible in some areas, where the infestation of *D. vexillum* is not yet extensive, or in areas where new established colonies would be discovered.

## 4.3 Long-term aims

We here describe long-term aims, as these are assessed to take some time to achieve. However, if possible, measures should be taken to achieve these aims as soon as possible.

### 4.3.1 Prevent new introductions of *D. vexillum* to Norwegian waters

To reduce the rapid spread and potentially negative impacts of *D. vexillum,* it is an important aim to prevent further introductions of this biofouling species. To achieve this aim, measures would need to be put in place to ensure regular cleaning and hull maintenance of large vessels entering Norwegian waters from potentially infected areas, such as:

A. Commercial cargo vessels
B. Commercial tourist vessels
C. Tankers
D. Special crafts
E. Private recreational vessels

### 4.3.2 Prevent further spread through commercial vessels

Although *D. vexillum* is likely to be dispersed smaller distances with privately owned boats, larger vessels are the more likely vectors for long distance dispersal within Norway. An important aim is to reduce biofouling on vessels traveling in Norwegian waters, both between harbours and between offshore sites and the coast. These include:

A. Cargo vessels
B. Cruise ships
C. Tankers
D. Special crafts

# 5 Risk reducing measures

The desired outcomes described in chapter 4 are based on the risks to biodiversity and aquaculture in Norway that are described in chapter 3. Here in chapter 5, we describe measures that could be taken to achieve the aims and how effective they would be. We also identify, to the best of our knowledge, any relevant parties that would be affected if the measure is put into effect or not. Finally, we discuss any potential negative effects on biodiversity that each measure could have and list any actions that require investments that we have identified.

## 5.1 Monitoring

Both in Scotland and in Norway, monitoring methods based on environmental (eDNA) extracted from water samples and analysed for mtDNA markers have been used with success to detect *Didemnum vexillum* (Matejusova et al. 2021, Fossøy et al. 2022). Uncertainties remain about how many water samples one needs to take in one area to be sure to obtain detections of *D. vexillum* if it is present but preliminary studies show promising results. Negative results do not guarantee that *D. vexillum* is not established on a nearby site if it is not detected during sampling, but positive detections should lead to more detailed visual monitoring of the area. Ensuring that a sufficient number of samples are taken in each area and that enough seawater is filtered from each sample point will minimize the risk of not detecting the species.  A study performed at the west coast of Norway in late 2021, reported detections in areas where the species had not been reported yet, followed up by confirmation with the help of a small ROV (Fossøy et al. 2022). Additional eDNA samples were collected from 55 sites along the Norwegian coast from the Oslofjord to Trondheim in summer 2022, but all results are not ready yet. Results from the Oslofjord area were negative (Frode Fossøy, NINA, pers. comm.). Water samples for eDNA methods are fairly easy to collect by a portable water pump, and one can cover large areas in few days and are such a cost-efficient method that will give a good indication on where to start monitoring with visual methods. A variable volume and filter types have been used in the different studies. Fossøy et al. 2022 recommend that a minimum of 5-10 litres of water is filtered. Such e-DNA surveys should always be followed by visual inspection of the area to confirm the presence of Didemnum vexillum.

Classic monitoring includes the so-called "rapid assessment techniques" where one or several target species are searched for by trained specialists in areas that are particularly likely to be invaded. Assessments can be carried out by direct visual inspection or using Remote Operated Vehicles. Sampling or visual inspection are generally carried out according to standardised methods (protocols).

In the Netherlands, they have installed settlement plates in harbours as a test. The plates are regularly inspected by personnel in harbours, which are reporting with photos. Sentinel monitoring with settlement plates seem to be a successful and cost-efficient method, which can also be used on aquaculture sites.

For the Norwegian coast we find that the optimal surveillance scheme would consist of a combination of e-DNA techniques and visual monitoring by video rigg or small UW drones. Increased information on the species to boat owner, diver clubs, diver- and other inspection companies and the general public may harvest additional information on spread of the species.

### 5.1.1 Early detection

Early detection methods can also be carried out more informally by personnel at aquaculture installations, oil installations, and small boat harbours, recreational amateurs and divers. However, reports have to be examined and confirmed by specialists given the difficulties of accurate species identification. Surveillance for alien species can also be included as parts of national biodiversity monitoring programs, but such programs are generally less efficient for early detection by following strict and long-term designs for sampling and monitoring sites.

#### *5.1.1.1 Positive effects*

Early detection is crucial for controlling the invasion of *D. vexillum*. Present methods for killing and new methods under development may be able to eradicate incipient populations (see sections 5.2.2-5.2.5), whereas eradication may be impossible for established and large populations. In addition, early detection is important for early implementation of measures against further spread and their possible success (See Sambrook et al 2014).

#### *5.1.1.2 Relevant parties that are affected by the measure*

Various governmental institutions could be affected by this measure as it requires additional funding.

#### *5.1.1.3 Negative effects*

Monitoring aim to increase the knowledge about presence of alien species and the potential negative effects of those species. VKM therefore assesses that there would not be negative consequences for biodiversity related to this measure.

#### *5.1.1.4 Potential costs or investments needed to perform the measure*

Monitoring measures require manual labor and time, both to perform RAS (Rapid Assessment Survey) and sampling, and to analyze the collected samples. For many of the tasks, like identification and verification, experts are needed.

## 5.2 Short-term measures

### 5.2.1 Treatment of marinas and private boats to prevent further spread

It is desirable to prevent further spread of *D. vexillum* from marinas that are already infested (section 4.1.1). Possible mitigation includes a suite of different minor measures, with variation in what actions could be taken, which can be determined based on what is most feasible at each location. In short, the aim is to ensure that all boats (and other equipment) that are harboured in areas where *D. vexillum* has been identified, or close to such areas, are free from biofouling organisms, and free of *D. vexillum* in particular.

**Figure 5.2.1-1:** *Didemnum vexillum* growing under floating dock. Photo: Anne-Mari With Ottesen

To prevent further spread, measures should be taken to kill or safely remove *D. vexillum* from all parts of a marina, including underneath floating ocks and other structures (See Figure 5.2.1-1). Ideally, control measures should be conducted during the colder periods of the year to prevent fragmentations and potential spread. However, for practical reasons, combining the measures with normal spring maintenance is perhaps more feasible. Importantly, special care should be taken to treat equipment deeper in the water column (below the upper brackish layer).

Killing or eradication of *D. vexillum* can be accomplished through five methods: desiccation, suffocation, osmotic shock, chemical treatment, or mechanical removal.

- Desiccation of *D. vexillum* can be achieved through storing the boat or equipment under dry conditions for up to one week.

- Suffocation can be achieved through covering the submerged structures in wrap-on PVC plastic sheets for at least one week. Coverage will result in lethal anoxic conditions underneath the plastic sheet (see Figures 3 and 7 in Pannell and Coutts 2007).
- Osmotic shock can be achieved through submerging the equipment in a container of freshwater for at least 6 h. For boats or other floating structures, this can be done by pulling a large tarpaulin underneath, removing as much seawater as possible and replace it with freshwater (see Figure 9 in Pannell and Coutts, 2007). If there is a river outlet or estuary in the vicinity, boats could also be moored there for this treatment to be effective.
- Chemical killing can be achieved by mixing acetic acid in saltwater to obtain a >5% solution. The treatment can be used for treating boats on the water in the same manner as freshwater (using tarpaulins).
- Mechanical removal of *D. vexillum* should be carried out in such a way that no fragments of the organism are released back into the ocean. Cleaning can be achieved by constructing a sand box underneath the area where boats are cleaned, so that any water is filtered thoroughly before returning to the ocean. Water can be collected and sterilized with UV-light and both 300µm and 100µm mechanical filters. Use of freshwater for cleaning is also highly recommended.

#### *5.2.1.1 Positive effects*

If the above measures are carried out it will have a very positive effect in terms of containing the infestation and thus prevent nearby areas of being affected.

However, unless also the ocean bottom is cleaned or treated (see section 5.4) so that the harbour is free of *D. vexillum*, control measures need to be repeated once a year.

#### *5.2.1.2 Relevant parties that are affected by the measure*

Owners of recreational boats and equipment will be affected in the sense that there would be additional maintenance costs, and possibly a short time-window where the boat cannot be used.

Owners and employees at marinas would be affected through potentially new need for storage of boats, or equipment and infrastructure needed to safely clean boats and other equipment.

Commercial companies working with marine pollution would be affected positively as there could be a greater demand for their services.

Divers would be affected through loss of biodiversity in areas with high density of *D. vexillum*, as this reduces the value of the ecosystem service that they enjoy. However, they could also benefit from doing work at marinas, aiding the work of eradicating *D. vexillum*.

Aquaculture facilities, including farmers of fish, shellfish and seaweed will be negatively affected in different ways, either through increased maintenance costs or reduced harvests, if *D. vexillum* should spread to their facilities (see section 5.2 for details).

Governmental institutions are affected in various ways.

The public in general is also affected by *D. vexillum* as there will be a degradation of the natural resources and ecosystem services (*i.e.,* all use of coastal areas) in affected areas.

#### *5.2.1.3 Negative effects*

VKM assesses that the measures described here will have minimal negative effects on biodiversity. Some organisms other than the target species will die as a result of the treatments, but incidental mortality represents negligible parts of the total populations. Marinas are highly urbanized areas with low biodiversity. Therefore, we expect that there would not be red-listed species in the treated areas, however, this needs to be confirmed before measures are taken.

#### *5.2.1.4 Potential costs / investments needed to perform the measure*

Many marinas already have some sort of infrastructure in place for hoisting, cleaning and storage of small to medium sized boats. Also, winter storage, and thus also cleaning of boat hulls, is relatively common, so in many marinas there will only be minor adjustments to already existing procedures. Therefore, additional costs or investments needed to perform these measures should be limited. However, some modifications or acquisitions are likely to be made. These include:

- Establishing a storage area that can hold a number of boats (approximately 25% of the marina’s capacity) on land at one time.
- Establishing a suitable area for secure cleaning of boat hulls, as described above.
- Ensuring freshwater capacity for cleaning.
- Acquisition of acetic acid.

Additionally, there will be costs associated with manual labour in regard to removal and disposal of *D. vexillum* infested substrates and treatment of submerged structures and larger boats and floating structures that are too big to be brough on land (dry docked).

### 5.2.2 Local eradication through physical removal

Physical removal of *D. vexillum* from the substrate can be performed by manually scraping the colonies off the substrate. Removal should be done in combination with some sort of suction to ensure that small fragments are not lost and dispersed, thus function as nodules for new colonies. Also remaining fragments in cracks and crevices will also serve as a reservoir. Physical removal is thus best performed from manmade structures with smooth surfaces and in combination with equipment (suction) that can ensure that no fragments are lost.

#### *5.2.2.1 Positive effects*

If removal is conducted correctly with suction and filtering, the measure will reduce the *D. vexillum* colonies and thus reduce the potential impact. However, caution is needed, and the positive effects might be limited to manmade structures.

#### *5.2.2.2 Relevant parties that are affected by the measure*

Infected harbours and constructions in seawater influenced by biofouling will be affected by the measure.

#### *5.2.2.3 Negative effects*

If removal is not conducted in a safe manner with suction and filtration, the measure could result in increased spread of *D. vexillum*.

#### *5.2.2.4 Potential costs or investments needed to perform the measure*

Cost for an operation in the field will depend upon size and depth of the area that needs to be treated. Physical conditions such as water current, substrate angle, and substrate characteristics can also influence operational costs.

### 5.2.3 Local eradication through lime treatment

Hydrated lime (Ca $(OH)_2$) is a recommended treatment to clean cultured mussels of *D. vexillum* epigrowth (Switzer et al. 2011, Rolheiser et al. 2012). Lime particles have been used to diminish the populations of urchins grazing the kelp forest in northern Norway (Strand et al. 2020). Preliminary results from lab studies on the effect of lime on *D. vexillum* show that coarse and fine lime particles in treatments with a duration of 12 hours cause respectively 95 to 100 % mortality of the colonies. Treatment with hydrated lime for one hour also has an effect on survival of *D. vexillum* (85% mortality), but not as strong as the particles (Legrand & Husa in prep).

#### *5.2.3.1 Positive effects*

Lime particles can be used in nature in local areas to diminish the abundance of *D. vexillum*. Treatment of heavily infected harbours may minimize the risk of spreading of the species to new areas. Such treatment will also be beneficial to the harbours in order not to lose customers due to occurrence of *D. vexillum* in the area and reduce the cost for removal of *Didemnum* from vessels in the area. Hydrated lime treatment of cultivated mussels and oysters may also be a solution for the shellfish industry.

#### *5.2.3.2 Relevant parties that are affected by the measure*

Infected harbours, shellfish farmers and the shipping industry will be positively affected by the measure.

#### *5.2.3.3 Negative effects*

Lime treatment could also introduce a high risk for negative impact on non-target species and thus reduce the biodiversity of a locality. However, some of the habitats where *D. vexillum* is found in Norway tend to be disrupted by human activities and low biodiversity, so the negative effects on these localities would be small, however, a mapping of biodiversity and vulnerable species should be performed before measures are taken in an area. Should *D. vexillum* spread to other, more pristine habitats, additional assessments of each case need to be conducted in order to reduce the possible negative impacts on local biodiversity.

#### *5.2.3.4 Potential costs / investments needed to perform the measure*

Cost for an operation in the field will depend upon size and depth of the area that needs to be treated. Physical conditions, such as water current, substrate angle, and substrate characteristics, will also influence operational costs. Lime is a relatively cheap chemical, and the distribution of particles can be done with fairly cost-efficient methods.

### 5.2.4 Local eradication through ozone treatment

Ultrafine bubbles of ozone are currently used in the aquaculture industry to eliminate bacteria and viruses on vessels that goes from farm to farm. There are two facilities in Norway using this technology in closed systems where the vessels can be treated, and all microorganisms on the hull dies after a 1–3-hour treatment (Stian Teige, DEFLOAT pers.comm). Trials with ozone treatment of *D. vexillum* for two hours in recommended doses did not have any effect on *D. vexillum*. All treated colonies and controls were still alive after two weeks of recovery in the sea (Legrand & Husa in prep).

#### *5.2.4.1 Positive effects*

There are no positive effects on *D. vexillum* identified for this treatment.

#### *5.2.4.2 Relevant parties that are affected by the measure*

Not applicable.

#### *5.2.4.3 Negative effects*

Such treatment administrated in nature could possibly have negative effect on non-target species. Absence of red-listed species needs to be confirmed before measures are taken.

#### *5.2.4.4 Potential costs / investments needed to perform the measure*

Equipment for ozone-gas distribution is expensive and requires additional infrastructure.

### 5.2.5 Local eradication through use of predators

Biological control of invasive species has been mostly used in agriculture areas, but a few attempts have been made in the marine environment with varying success (reviewed in Secord 2003). Biological control can have several forms, like introduction of another natural enemy from the target species natural range or enhancement of natural local enemies.

As reported in section 1.7, several species and animal groups have been recorded as predators on both solitary and colonial ascidians but with most published observations related to solitary ascidians as prey. The most frequently recorded predators are found among lamellarid prosobranch snails, crabs, sea urchins, sea stars and fish.

Laboratory experiments have shown that *Cancer* crabs can prey on solitary ascidians, such as *Ascidiella aspersa*, *Ciona intestinalis*, and *Styela clava*, but not on colonial ascidians such as *D. vexillum.* Kaplan et al. (2018) have recorded a positive correlation of occurrence between *D. vexillum* and predators, such as crabs (*Cancer* sp.) and sea stars (*Asterias* sp.).

From Borgenfjorden, a side-arm of Trondheimsfjorden, cod (*Gadus morhua*), plaice (*Pleuronectes platessa*), common dab (*Limanda limanda*) and the sea star *Asterias rubens* have been recorded as preying on the solitary ascidian *Ciona instestinalis* (Denstadli 1972, Gulliksen & Skjæveland 1973, Lande 1973, 1976). However, observations indicated that heavy predation by fish and sea stars had no obvious influence on the total population of *C. intestinalis* in the fjord.

Predators on colonial ascidians are primarily echinoderms and prosobranchs in the family Velutinidae. Examples of predators are the Arctic cowrie *Trivia arctica*, the velvet shell *Velutina velutina* and lamellarid snails. such as *Lamellaria perspicua,* all of which are relatively common along the Norwegian coast. Lamellarid prosobranchs are however, quite small (usually < 2 cm), and it has been observed that their food-uptake are more than balanced by the rapid growth of the prey, even in cases where lamellarid prosobranchs were recorded in relatively high numbers on *D. vexillum* (Gittenberger 2007).

Several reports conclude that biological projects testing susceptibility of *D. vexillum* or other ascidians to various predators, such as urchins, sea stars, and gastropods, are unlikely to limit biofouling (Carman et al. 2009, Epaulbaum et al. 2009, Switzer et al. 2011, McKenzie et al 2017).

*In situ* experiments/projects using predators to reduce the occurrence of *D. vexillum* would need permission from national authorities.

#### *5.2.5.1 Positive effects*

The positive effect of using predators such as crabs, echinoderms and lamellarid prosobranchs is that no foreign elements (chemicals) are introduced into the environment.

Concerning the lamellarids, they seem to be quite specialized ascidian predators, and predation would probably not change the general biodiversity of the habitat significantly.

Echinoderms, especially the sea stars of current interest, are omnivorous predators. Introduction of echinoderms and crabs would probably change and reduce the general biodiversity of the habitat and not have the same positive effect on ascidian grazing as the above mentioned lammelarids.

#### *5.2.5.2 Relevant parties that are affected by the measure*

Relevant parties will depend upon choice of selected scenarios. Three scenarios using predators to eradicate *D. vexillum* are selected based on their strategy concerning food uptake, either introduction of (1) gastropods (snails), (2) sea urchins and crabs feeding either by claws or stout, calcareous jaws, or (3) sea stars. The most voracious and common sea stars in Norwegian waters, for example *Asterias rubens,* digest their prey externally by everting the stomach around its prey.

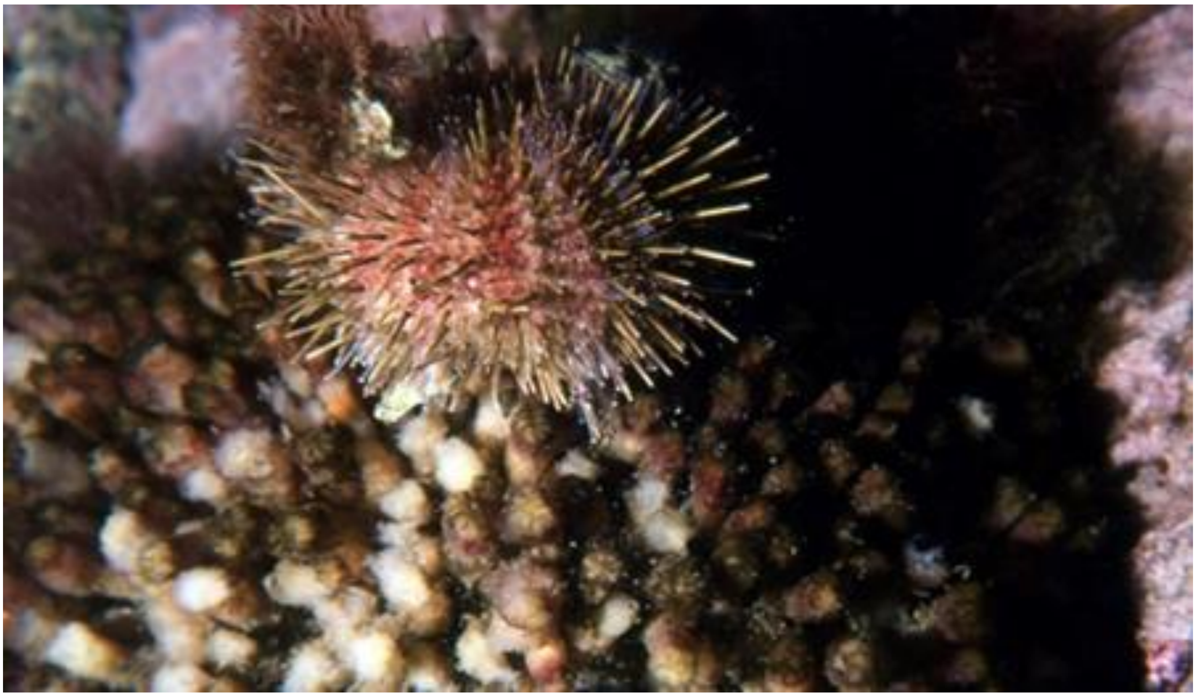

**Figure 5.4.4.2-1:** A sea urchin *Strongylocentrotus* sp. nipping on a colonial ascidian (*Synoicum turgens*). The feeding technique may cause spread of fragments and could therefore induce further spread of a carpet ascidian, such as *Didemnum vexillum*. Photo: Bjørn Gulliksen

In the first scenario, gastropod specimens can primarily be collected by scuba divers. Collection of specialized prosobranchs directly from the sea bottom from genera, such as *Velutina*, *Trivia*, and *Lamellaria,* would be quite labour intensive. It would require numerous dives in the field in localities where these genera occur and involve SCUBA -divers with biological competence and experience, among others because these species are quite cryptic and not easy to find unless you are trained. Information about production of relevant gastropods in aquaculture is not available.

In the second and third scenarios, collection of crabs, sea stars and sea urchins can also be collected by scuba divers, but collection can probably also be done by scraping the sea bottom using different types of dredges. The personnel do not necessarily need to have the same biological competence as these species are easy to identify.

#### *5.2.5.3 Negative effects*

Biological control, in contrary to chemical treatment, is consider having irreversible ecological effects when species or pathogens are introduced to an area. Such species may have a strong impact on non-target organisms if they are not host/prey specific (Secord 2003). For example, an enhancement of urchin population to diminish the abundance of *D. vexillum* in kelp forest, may lead to heavy grazing of the kelp plants and associated flora as this preferable diet to urchins. Large sea urchin populations tend to be present in the area for a long time when first established, which is the case for northern Norway where they have caused barren grounds in for the last 60 years.

Studies of use of different species of sea slugs for grazing of the introduced green algae *Caulerpa taxifolia* in the Mediterranean Sea showed that one of the species contributed to further dispersal of the algae by fragmentation of the plant while eating (Zuljevic 2001). Such mechanisms may also be the case during consumption of *D. vexillum*.

Using predators to reduce the presence of *D. vexillum* will have different potential negative effects depending on whether sea urchins, crabs or sea stars would be used. Sea urchin and crabs can lead to spread of fragments and could therefore induce further spread of the carpet ascidian. Additional negative effect is that all groups (sea urchins and crabs, and sea stars) could reduce the total biodiversity within a habitat.

Least negative effects are assumed to be connected to the use of prosobranchs within the family Velutinidae as predators. The snails are very selective concerning food and do probably select ascidians before other types of prey. Species within genera as *Velutina*, *Trivia* and *Lamellaria* will probably only attack and eat ascidians, but not solely *D. vexillum* and thus they could reduce the diversity of Ascidiacea as a group.

Collection of predators will also affect the local area from where they are harvested, causing disturbance in the habitat balance.

#### *5.2.5.4 Potential costs / investments needed to perform the measure*

Potential costs would be connected to collection of the predators at different localities, transport to localities where *D. vexillum* occur and release of the predators close to or on *D. vexillum.* Sampling and release of prosobranch predators requires scuba divers, surface personnel and most often ships if the divers cannot operate from land. Collection of predators within echinoderms can be done either by scuba-divers or by dredging the sea bottom at selected localities where the species of interest are known to occur. Dredging will have lower costs related to personnel because fewer people are needed and those involved do not need to have the same biological competence as when using scuba-divers. However, large ships are costly, and collection using scuba-divers could reduce this cost as they can either dive from land or use smaller and cheaper boats.

## 5.3 Long-term measures

### 5.3.1 "Clean hull" certification for entry to Norwegian harbours to prevent new introductions

After the implementation of the international ballast water convention, which ensures cleaning of ballast water before releases into coastal waters in Norway, biofouling on vessels is now regarded as posing the largest risk for introduction of alien marine species. The International Maritime Organization (IMO) adopted the guidelines for the control and management of ships biofouling to minimize the transfer of invasive aquatic species in 2011. A similar protocol has later been published for leisure crafts (https://www.imo.org/en/OurWork/Environment/Pages/Biofouling.aspx). The guidelines are currently voluntary to follow and ongoing work in IMO continues to evaluate and revise the guidelines. Some countries and states have taken steps towards making similar guidelines mandatory, like New Zealand, Australia, Canada and California, USA. In order to prevent new entries of *Didemnum vexillum* and other alien marine species into Norwegian waters, the IMO guidelines could be mandatory for ships entering Norway while waiting for international agreement. Documentation on proper maintenance of the hull, like regularly cleaning and protection coating, could also be rewarded in the harbours in form of reduced harbour fees. To prevent spread to new areas within Norway with commercial vessels, we recommend that vessels that have been docked in areas with *D. vexillum* for a longer period of time (weeks to months, depending on time of year) be inspected in a proper way before being allowed to be moved to another area such that any *D. vexillum* can be removed and collected. The risk of settlement of *Didemnum* larvae on vessels are temperature dependent, in winter at the Norwegian coast there will be a low risk for settlement since the species is not reproducing sexually at this time of year. In summer, when there will be plenty of larvae in the water which will settle within approximately 24 hours, the risk will be much higher even if the vessel only stays for a few days.

#### *5.3.1.1 Positive effects*

The measures to prevent biofouling would, to a large degree, prevent new entries and rapid spread of *D. vexillum* in Norway. They would also contribute to preventing entry of other alien species.

#### *5.3.1.2 Relevant parties that are affected by the measure*

Various parties related to the shipping industry and oil industry would be affected. New measures to prevent biofouling might be costly to implement but could have economic benefits if they reduce hull drag and operating costs for commercial vessels.

#### *5.3.1.3 Negative effects*

To make the IMO guidelines for biofouling mandatory for all ships entering Norwegian waters would require large operations in form of inspection and documentation of species growing on the ship hulls and cleaning hulls which that are not up to standards before entering. Currently, there are no such systems in place in our harbours and the capacity for hull cleaning facilities is low. Several systems to maintain the biofouling at an early stage (biofilm) are under development and more will come if there is an international agreement to make these guidelines mandatory. A system that economically favours vessels with a good cleaning and protection history in Norwegian harbours will not have any negative effect for any parties.

#### *5.3.1.4 Potential costs or investments needed to perform the measure*

The cost of cleaning hulls will depend on the size of the vessel, the degree of fouling and if the material should be collected or not. An operation to clean an oil rig (during docking) with collection of all material with filtering of the wastewater will cost between 5–7 million NOK (Thor Østervold, EcoSubsea pers. comm).

### 5.3.2 Inspection and cleaning of commercial vessel hulls to prevent further spread within Norway

Information on vessel movements would aid management practices, as shown in Husa et. al (2022). Inspections in harbours with known locations of *D. vexillum* and high vessel traffic should be more frequent than other areas. Prolonged durations of vessels in port can result in increased biofouling. It is not feasible to inspect all ships, but by identifying and targeting ships that have longer residency time in harbours where *D. vexillum* is known to occur, there is potential to monitor ships with higher risk. Similar to entry of ships into Norwegian waters (section 5.2), documentation of proper maintenance of the hull (biofouling management book) could be rewarded with reduced harbour fees within Norway.

A national monitoring program to detect *D. vexillum* would be a necessary complement to these actions (see Section 5.1.2).

Cleaning of ballast water is not currently required within Norwegian waters. Ballast water is not considered a main pathway of spreading for *D. vexillum,* but it may be possible for fragments to survive transport in ballast water within the country. The same requirements as in the international ballast water convention could be applied to vessels leaving areas with known occurrences of *D. vexillum*, particularly during summer when growth rates of *D. vexillum* are at its highest.

#### *5.3.2.1 Positive effects*

Measurements will prevent or delay spreading of *D. vexillum* within Norwegian waters. It will also prevent spreading of other alien species. A clean hull improves fuel economy, reduces the environmental footprint, and improves overall vessel performance.

#### *5.3.2.2 Relevant parties that are affected by the measure*

Shipping industry, energy sector (oil and gas, offshore wind power), aquaculture and commercial fisheries.

#### *5.3.2.3 Negative effects*

VKM assess that implementation of a system to prevent transport of biofouling organisms in general, and potentially alien invasive species would not have any negative effects on biodiversity in Norway.

#### *5.3.2.4 Potential costs / investments needed to perform the measure*

The cost of cleaning hulls would depend on the size of the vessel, the time the ship is not operational, the degree of fouling and if the material on the should be collected or not. An operation to clean an oil rig with collection of all material with filtering of the wastewater would cost 5–7 million NOK.

# 6 Uncertainties

## 6.1 Taxonomic uncertainty

The numerous species within the genus *Didemnum* Savigny, 1816 can be difficult to identify by morphological traits. Van Name (1945) writes (page 80): '… that instead of *Didemnum* being one of the largest genera of ascidians the species are in reality rather few', but a revision reducing the number of species has not taken place since 1945. On the contrary, the number of described species has increased, exemplified with the relative recent descriptions of congeneric species such as *D. vexillum* Kott, 2002, *Didemnum romssae* Marks, 1994 and *Didemnum pseudovexillum* Turon & Viard, 2020. In the published description of *D. pseudovexillum*, Turon et al. (2020) indicate that *D. pseudovexillum* can only be distinguished from *D. vexillum* based on their morphology/anatomy (spicules and larvae) but not easily based on their appearance in the field. A combination of genomic analysis, morphological traits, and mitochondrial sequences of the cytochrome oxidase I gene supported the description of the new species (Turon et al. 2020). However, sampling of material for genomic analysis can be challenging in some didemnids due to the high density of calcareous spicules making the upper surface of some species very hard. Also, the low pH of the tunic (<3.0) may degrade the genomic DNA in field samples (Parra-Rincon et al. 2021).

Marks (1994) used larval morphology and spicule shape to separate three native species of *Didemnum* occurring in Norwegian waters (*D. albidum, D. polare*, *D. romssae*). An uncertainty in a possible program to reduce or eradicate *D. vexillum* from Norwegian waters is therefore dependent upon correct identification of the species in cases where identification is only based on morphological features. This is important as eradication efforts for *D. vexillum* could have negative implications for nontarget native species in the same genus.

## 6.2 Uncertainty regarding the species distribution in Norwegian waters

So far, the species *D. vexillum* has been documented in five separate locations in Norway. Environmental DNA testing of several other locations is still being conducted, so we do not know the full extent of the invasion and current distribution along the Norwegian coastline. Furthermore, there is a high uncertainty regarding the presence of this species on offshore localities, like windfarms and oil- and gas production platforms, which could function as steppingstones for new introductions to the coast.

## 6.3 Uncertainty regarding spread

We have identified large commercial vessels as the primary agents that are likely responsible for introduction and spread, but it remains highly uncertain how rapidly the organism can establish on these vessels. Whether a few hours in an infected marina is sufficient for potential recruitment, or if this takes one or more weeks is still unknown. Spread will vary according to of time of year, water temperature, level of hull fouling. Although not observed in Norway yet, VKM also strongly believes that recreational boats can function as vectors for spreading, and the same uncertainty thus applies to settlement on these.

## 6.4 Uncertainty regarding ecological impact

Uncertainties concerning *D. vexillum* also include a lack of basic biological knowledge of the populations occurring in Norway. Most of our knowledge of the species is based on field studies in other countries, and we do not know how relevant these results are related to Norwegian conditions. For example, *D. vexillum* was first detected in New Zealand in 2001, caused a lot of concern over run-away spread and impact for several years (Coutts & Forrest 2007). However, the species did not in the end attain the level of abundance and associated impacts that were initially anticipated. It is therefore high uncertainty regarding the long-term impacts of this species in Norway.

## 6.5 Uncertainty regarding effects of climate change

An uncertainty is also related to the effect of climate change on reproduction, growth, and geographical spread in Norwegian waters. including Svalbard and Jan Mayen. A comprehensive study of the biology of didemnids is therefore necessary before an extensive program to reduce or eradicate the invasive *D. vexillum* from Norwegian waters. It is expected that the increase in water temperature could increase the impact of *D. vexillum* in Norwegian waters as it will have longer time to grow with increased temperature.

# 7 Conclusions (with answers to the terms of reference)

The colonial ascidian *Didemnum vexillum* is native to Japan in the southwest Pacific Ocean and is regarded as globally invasive. The colonies create carpets that overgrow other organisms thus reducing the general biodiversity of a location, and foul man-made structures such as boats, oilrigs, and aquaculture installations. Overgrowth of oysters, mussels and scallops creates problems for shellfish aquaculture. The occurrence of *D. vexillum* as a rapidly growing and efficiently spreading species has caused ecological problems and increased economic costs in several countries where it has become established as an alien species.

*** Knowledge of the species dispersion biology and experiences from 4 international environments where this is established.**

Dispersion biology in *D. vexillum* is similar in all four international environments but local and regional differences in temperature is the most important controlling factor. *Didemnum vexillum* can disperse both sexually and asexually. Spreading through sexual reproduction is limited by a short larval stage (<48 h) and dispersal over short distances (< 1000 m) and mainly affects spreading of the local populations. Asexual reproduction takes place through fragmentation of the colony where a fragment can survive a prolonged period of time in the water mass (3 weeks) before settling and continuing growth. The ability of *D. vexillum* to reproduce through fragmentation causes the largest risk for spreading.

See section 1.4.4 for details.

*** Description of the species' total distribution area, distribution in Norway and any areas the species does not have the potential to establish in (based on the Norwegian Biodiversity Information Centre risk assessment in the alien species list of 2018).**

*Didemnum vexillum* is currently widely distributed along the coasts of northeastern and northwestern North America, and along the coasts of several European countries. The species is also locally abundant in northern New Zealand and at some locations in Australia and the southeast of Russia. See subchapter 1.5.2 for a complete list of countries and map of known locations.

In Norway, presence of *D. vexillum* has so far been documented at five separate locations (see map in subchapter 1.5.3). The species is strictly marine and is unlikely to establish in areas with low salinity, such as estuaries, or in the littoral zone due to air exposure.

Fragments of *D. vexillum* has the capacity to grow into colonies at low temperatures and consequently have the potential to colonize the entire Norwegian coastline, Bear Island and

Svalbard. Sexual reproduction and larval spreading will likely have their northernmost boundary close to Tromsø. The sea temperature further north is too low to allow larval development.

*** Assess important dispersion paths and vectors, as well as the expected timeline for dispersion along the Norwegian coast and to Svalbard.**

*D. vexillum* can be dispersed by commercial shipping or transport of larger marine infrastructure (long distance dispersal) or by private boats or gear (shorter distances). See section 1.5.4 for details. Husa et al. (2022) assessed the Oslofjord area and the west coast to be of highest risk for introductions of alien marine species into Norway by vessel.

The project group has concluded that the organism is likely to spread to all parts of Norwegian waters with commercial shipping. The time frame for spreading depends on what measures are taken to prevent new introductions and further spread from the existing colonies in Norway.

*** Describe the null alternative (Expected development without new measures and instruments).**

Without any measures taken to reduce the presence and spread of *D. vexillum*, VKM concludes that it is likely that the organism can spread to the entire coastline of the country, including Svalbard and Bear Island, within 10 years. This conclusion is based on the ability of *D. vexillum* to spread through fragmentation not being limited by temperatures within Norwegian waters.

*** Assess the potential consequences of *Didemnum vexillum* for biological diversity, industries and other activity along the coast, and the probability that these will occur. All activities the species may have consequences for must be identified. The assessment shall include a description of the uncertainty in the estimates.**

Based on the "Likely" spread of the organism, without measures taken, VKM has assessed the potential risk that *D. vexillum* presents to biodiversity in Norway is assessed in section 3.3. These are summarized in section 3.5.1 and illustrated in Figure 7-1.

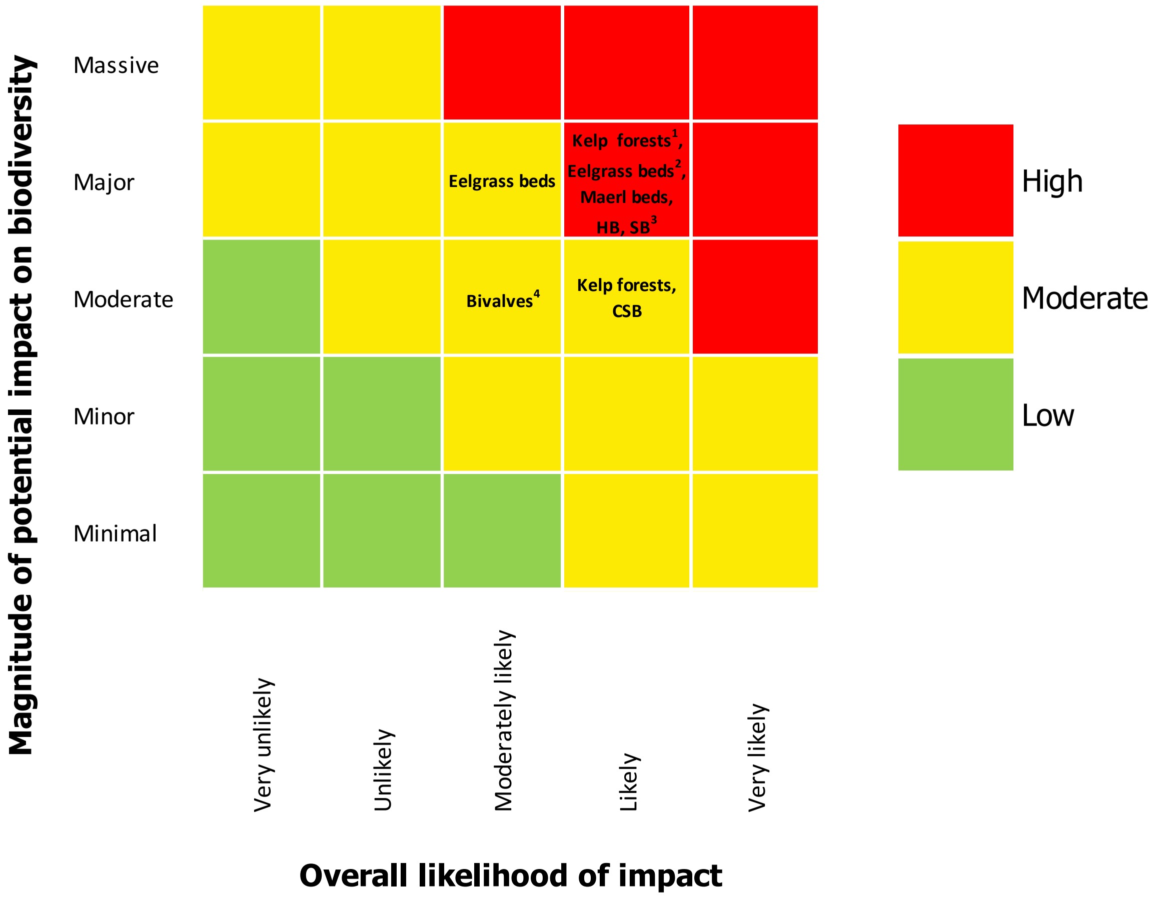


**Figure 7-1:** Summarized risk of negative impact on various habitats and natural populations of shellfish species in Norway. HB = Hard bottom habitats, SB = Soft bottom habitats, CSB = Coarse sediment bottom habitats. [1] Kelp forests in Northern Norway that are affected by grazing by sea urchins and are already listed as threatened. [2] Eelgrass habitats in the vicinity of infested harbours, docks and other human-made environments with artificial substrates. [3] Soft bottom habitats consisting of sand, gravel and pebbles dominated by European oyster or horse mussels. [4] *C. islandica*, *O. edulis*, *C. gigas*, *M. edulis*, *M. modiolus* and *P. maximus.*

The risk posed to various types of aquacultures is assessed in subsection 3.4, summarized in subsection 3.5.2 and illustrated in Figure 7-2.

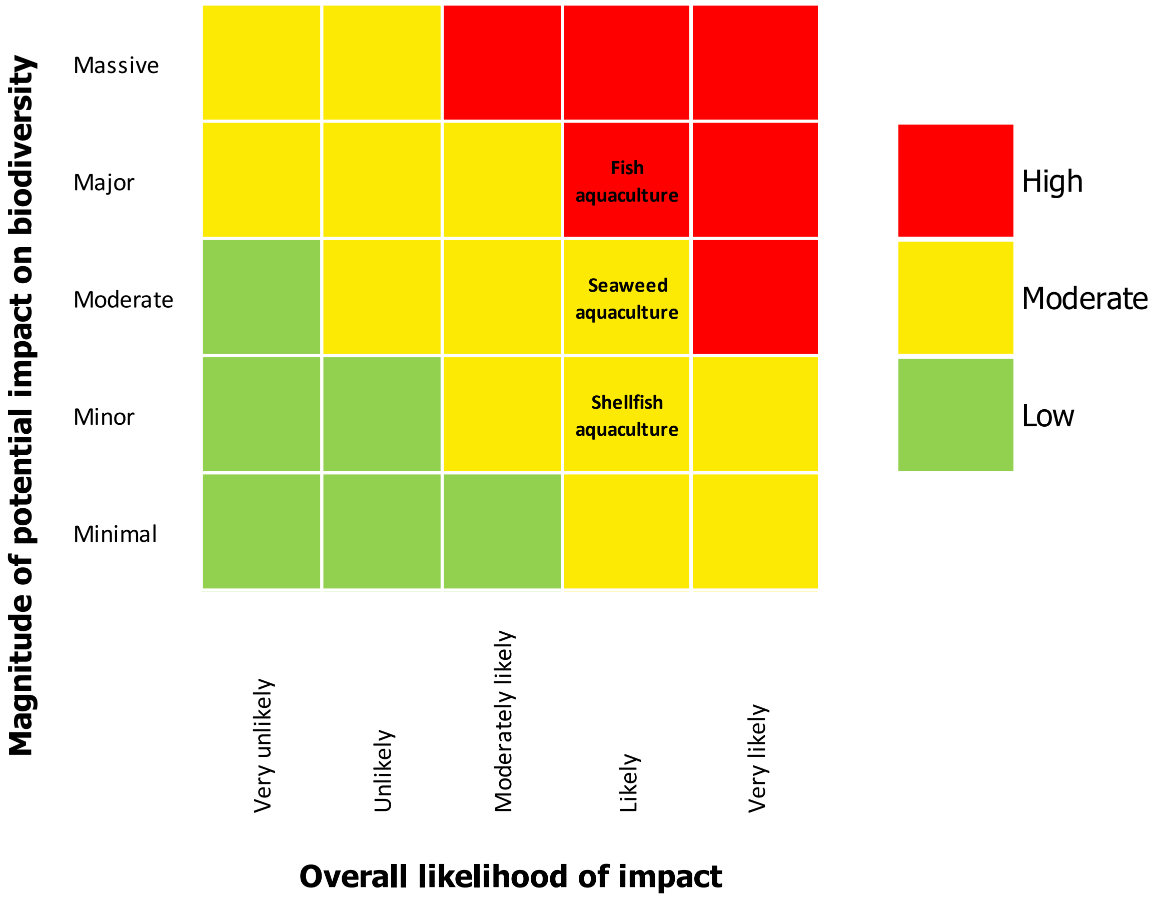


**Figure 7-2:** Summarized risk of negative impact on different types of aquacultures in Norway

*** Describe possible measures that can limit the spread of established colonies, combat established colonies, and prevent new colonies from establishing.**

Short-term measures include treatments of small marinas, private boats and equipment to remove colonies and educational campaigns. More long-term measures for finding and eliminating *D. vexillum* include implementation of "clean hull" certification for international vessels, and inspection and cleaning of vessels and installations before transit within Norway. Monitoring of populations is important both as a short- and long-term measure. Measures to remove *D. vexillum* must be conducted with methods that prevent spreading of fragments that have the potential to establish new colonies. Processes for long-term measures also needs to commence as soon as possible as some of them will require international collaboration which takes time. Measures are presented in detail in chapter 5.

*** Identify current risk-reducing measures**

Risk-reducing measures would in this aspect strongly mirror the possible measures described above. Short-term measure, that could be implemented within the first couple of years, include establishment of a monitoring program for early detection of new introductions and spreading, thorough cleaning of recreational boats and marinas, and possible eradication of smaller colonies outside the urbanized marinas. Measures that need more time to implement include establishment of international collaboration for "clen hull" certification of large vessels and a systematic approach to prevent further spread of *D. vexillum* as biofouling on various vessels within Norway. These measures are described in full in chapter 5.

*** Suggest any action plan towards goal achievement.**

As it is outside the mandate of VKM to determine management goals, VKM has instead listed desirable outcomes for the various measures that can be taken and listed these as short-term (0–3 years), and long-term (3–20 years) aims. These are listed in chapter 4.

*** Give a description of the positive and negative consequences of the relevant measures, compared with the null alternative. Including assessment of uncertainty compared to risk described under the null alternative.**

Positive consequences of the measures include containment of the infestation and thus prevent spread to nearby areas while early detection is crucial for controlling the invasion. The methods suggested, including cleaning of recreational boats and marinas and killing the alien species with lime, could possibly eradicate small incipient populations, whereas eradication may be impossible for established and large populations. These measures would, to a large degree, prevent new entries and rapid spread of *D. vexillum* in Norway. They would also contribute to preventing entry of other alien species.

VKM assess that the measures described will have minimal negative effects on biodiversity. Some organisms other than the target species will die as a result of the treatments, but these represent negligible parts of the total populations, and we expect that there would not be red-listed species in the treated urbanized habitats, however, this needs to be verified.

It will require large operations to make the IMO guidelines for biofouling mandatory for all ships entering Norwegian waters. Currently there are no such systems in place in Norwegian harbours and the capacity for hull cleaning facilities is low.

Compared to the null alternative, that no measures are taken, VKM assesses that the positive consequences of the suggested measures are clearly large. If no measures are implemented, VKM assess that it is likely that the organism will continue to spread and can reach any part of Norwegian waters within 5-10 years. Implementing measures to reduce new introductions and further spread from established colonies will not only have positive effects in regard to *D. vexillum*, but also for other alien species.

The positive effects or the measures, in regard to the described aims, are described in a subsection for each of the measures described in chapter 5. Likewise, any potential negative effects on biodiversity (given implementation of the measure) are presented in a separate subsection. The uncertainty is discussed under each measure. Finally, VKM emphasize the need for individual assessments of each site before measures that can have negative effects on biodiversity are put into action.

# 8 Data gaps

The availability of data on the environmental effects of invasive ascidians is generally low, especially for Norwegian coastal ecosystems. In addition to the uncertainties listed in chapter 6, we specifically find that there is a lack of data on:

- Growth rate of both larvae and sessile adults of *D. vexillum* at different temperatures
- Survival rate of *D. vexillum* at different salinities and pH.
- Maximum depth distribution of *D. vexillum*
- Presence on offshore oil riggs and other installations.
- Effects of limestone treatment under different conditions on the mortality of *D. vexillum.*
- Potential negative effects (*i.e.,* allowing spread) of physical removal of *D. vexillum*, if not conducted in a correct manner (including suction).
- Effect of various native predators on *D. vexillum* under Norwegian conditions.
- Presence and spread of *D. vexillum* via fragments and larvae in ballast water in vessels operating within Norway.

# References

Albretsen, J., Sperrevik, A.K., Staalstrøm, A., Sandvik, A.D., Vikebø, F., Asplin, L., 2011. NorKyst-800 Rapport nr. 1 : Brukermanual og tekniske beskrivelser. NorKyst-800 Report No. 1 : User Manual and technical descriptions. Data at: https://thredds.met.no/thredds/dodsC/fou-hi/norkyst800m-1h/

Andersen, G.S., Steen, H., Christie, H., Fredriksen, S., and Moy, F. E. (2011). Seasonal patterns of sporophyte growth, fertility, fouling, and mortality of *Saccharina latissima* in Skagerrak, Norway: implications for forest recovery. Journal of Marine Biology 2011: art690375. https://doi.org/10.1155/2011/690375

Auker, L. A. (2010) The effects of *Didemnum vexillum* overgrowth on Mytilus edulis biology and ecology. PhD Thesis, University of New Hampshire, Durham, New Hampshire, USA. 152 pp.

Auker, L. A, Majkut, A. L. and Harris, L. G. (2014) Exploring biotic impacts from *Carcinus maenas* predation and *Didemnum vexillum* epibiosis on *Mytilus edulis* in the Gulf of Maine. Northeastern Naturalist 21: 479–494, https://doi.org/10.1656/045.021.0314.

Auker, L. A. (2019) A decade of invasion: changes in the distribution of *Didemnum vexillum* Kott, 2002 in Narragansett Bay, Rhode Island, USA, between 2005 and 2015. BioInvasions Records 8: 230-241.

Bannister, J., Sievers, M., Bush. F. and Bloecher, N. (2019) Biofouling in marine aquaculture: a review of recent research and developments. Biofouling 35: 631-648.

Behrens, D. W. (1981) The Lamellariidae of the Northeastern Pacific. The Veliger 22:323-339.

Birkeland, C. E. (1974) Interactions between a sea pen and seven of its predators. Ecological Monographs 44: 211-232.

Blackburn, T. M., Essl, F., Evans, T., Hulme, P. E., Jeschke, J. M., Kühn, I. et al. (2014) A unified classification of alien species based on the magnitude of their environmental impacts. PLoS Biol 12: e1001850. https://doi.org/10.1371/journal.pbio.1001850

Blake, C. and Maggs, C. A. (2003). Comparative growth rates and internal banding periodicity of maerl species (Corallinales, Rhodophyta) from northern Europe. Phycologia, 42(6): 606-612.

Bloecher N, Olsen Y, Guenther J. 2013. Variability of biofouling communities on fish cage nets: A 1-year field study at a Norwegian salmon farm. Aquaculture 416-417: 302-309.

Boström, C., Baden, S., et al. (2014) Distribution, structure and function of Nordic eelgrass (*Zostera marina*) ecosystems: implications for coastal management and conservation. Aquatic Conserv. Mar. Freshw. Ecosyst. 24: 410-434.

Bressy, C. and Lejars, M. (2014). Marine Fouling: An overwiev. Journal of Ocean Technology 9: 19-28.

Brooks, M. R., and Faust, P. (2018). 50 years of review of maritime transport, 1968-2018: Reflecting on the past, exploring the future (No. UNCTAD/DTL/2018/1).

Brun, E. (1968) Extreme population density of the starfish *Asterias rubens* L. On a bed of Icelandic scallop, *Chlamys islandica* (O.F.Müller). Astarte 1(32): 1-4.

Bullard, S. G., Lambert, G., Carman, M. R., Byrnes., J., Whitlatch, R. B. et. al. (2007) The colonial ascidian Didemnum sp. A: Current distribution, basic biology, and potential threat to marine communities of the northeast and west coasts of North America. Journal of Experimental Marine Biology and Ecology 342: 99–108, https://doi.org/10.1016/j.jembe.2006.10.020.

Bullard, S. G. and Whitlatch, R. B. (2009) In situ growth of the colonial ascidian *Didemnum vexillum* under different environmental conditions. Aquatic Invasions 4: 275–278, https://doi.org/ 10.3391/ai.2009.4.1.27.

Butman, B., and Beardsley, R. C. (1987) Physical oceanography. In The Georges Bank, First edition, pp. 88–98. Ed. by R. H. Backus. The MIT Press, Cambridge, MA.

Carlisle, D. B. (1968) Vanadium and other metals in ascidians. Proceedings of the Royal Society, B. 18: 276-279.
Carlton, J. T. (1989) Man's role in changing the face of the ocean: biological invasions and implications for conservation of near-shore environments, Conservation Biology 3: 265-273.
Carman, M. R., Allen, H. M., and Tyrrell, M. C. (2009) Limited value of the common periwinkle snail *Littorina littorea* as a biological control for the invasive tunicate *Didemnum vexillum*. Aquatic Invasions, 4: 291–294.
Carman, M. R., Morris, J. A., Karney, R. C., Grunden, D. W. (2010) An initial assessment of native and invasive tunicates in shellfish aquaculture of the North American east coast. Journal of Applied Ichthyology 26 (Suppl. 2): 8-11
Carman, M. R. and Grunden, D. W. (2010b) First occurrence of the invasive tunicate *Didemnum vexillum* in eelgrass habitat. Aquatic Invasions 5: 23-29.
Carman, M. R., Grunden, D. W. and Ewart, D. (2014) Coldwater reattachment of colonial *Didemnum vexillum* fragments to natural (eelgrass) and artificial (plastic) substrates in New England. Aquatic Invasions 9: 105-110.
Carman, M. R., Colarusso, P. D., Nelson, E. P., Grunden, D. W., Wong, M. C., McKenzie, C. et. al. (2016). Distribution and diversity of tunicates utilizing eelgrass as substrate in the western North Atlantic between 39 and 47 north latitude (New Jersey to Newfoundland). Management of Biological Invasions, 7: 51-57.
Casso, M., Tagliapietra, D., Turon, X. and Pascual, M. (2019). High fusibility and chimera prevalence in an invasive colonial ascidian.Scientific Reports 9:15673 https://doi.org/10.1038/s41598-019-51950-y.
Casso, M., Turon, X. and Pascual, M. (2019b) Single zooids, multiple loci: independent colonisations revealed by population genomics of a global invader. Biol Invasions 21: 3575-3592. https://doi.org/10.1007/s10530-019-02069-8
Ching, H. L. (1977) Redescription of *Eurylepta leoparda* Freeman, 1933 (Turbellaria: Polycladida), a predator of the ascidian Corella willmeriana, 1898. Can. J. Zool. 56:1372-1376.
Christie, H., Jørgensen, N. M., Norderhaug, K. M., Waage-Nielsen, E. (2003) Species distribution and habitat exploitation of fauna associated with kelp (*Laminaria hyperborea*) along the Norwegian coast. J. Mar. Biol. Ass. U.K. 83: 687-699.
Cornwall, C. E., Comeau, S., DeCarlo, T. M., Larcombe, E., Moore, B., Giltrow, K. et al. (2022) A coralline alga gains tolerance to ocean acidification over multiple generations of exposure. Nature Climate Change 10: 143–146. www.nature.com/natureclimatechange
Costello, K. E., Lynch, S. A., McAllen, R., O'Riordan, R. M., Culloty, S. C. (2021) The role of invasive tunicates as reservoirs of molluscan pathogens. Biological Invasions 23: 641-655.
Daley, B. A. and Scavia, D. (2008) An integrated assessment of the continued spread and potential impacts of the colonial ascidian, *Didemnum* sp. A, in U.S. waters. http:// coastalscience.noaa.gov/publications/nccos78.pdf. Accessed 13 May 2015
Daniel, K. S. and Therriault, T. W. (2007) Biological synopsis of the invasive tunicate *Didemnum* sp. Can Manuscr Rep Fish Aquat Sci 2788. Fisheries and Oceans Canada, Ottawa.
Danskin, G. P. (1978) Accumulation of heavy metals by some solitary tunicates. Canadian Journal of Zoology 56: 547-551.
Davidson, I. C., Brown, C. W., Sytsma, M. D. and Ruiz, G. M. (2009) The role of containerships as transfer mechanisms of marine biofouling species. Biofouling 25: 645-655.
Dean, T. (1981) Structural aspects of sessile invertebrates as organizing forces in an estuarine fouling community. J Exp Mar Biol Ecol 53: 163–180.
Denstadli, S. O. (1972) Forplantning, vekst og ernæring hos torsk (*Gadus morhus* L.) I Borgenfjorden, Nord-Trøndelag. 111 pp.
Diehl, M. (1956) Die raubschnecke *Velutina velutina* als feind und bruteinmieter der ascidia *Styela coriacea*. Kieler Meeresforschungen 12: 180-185.

Dijkstra, J. A., Sherman, H. and Harris, L. G. (2007) The role of colonial ascidians in altering biodiversity in marine fouling communities. Journal of Experimental Marine Biology and Ecology 342: 167-171.

Dijkstra, J. A. and Nolan, R. (2011) Potential of the invasive colonial ascidian, *Didemnum vexillum*, to limit escape response of the sea scallop, *Placopecten magellanicus*. Aquatic Invasions 6: 451–456. doi: 10.3391/ai.2011.6.4.10.

Directorate of Fisheries (2021) Rapport: Nøkkeltall fra norsk havbruksnæring 2020. 28 pp http://www.fiskeridir.no

Dunn, A. M. and Hatcher, M. J. (2015) Parasites and biological invasions: parallels, interactions, and control. Trends in Parasitology 31: 189-199.

Ekdale, A. A., Bromley, R. G. and Pemberton, S. G. (1984) Ichnology: Trace Fossils in Sedimentology and Stratigraphy: SEPM, Short Course 15.

Epelbaum, A., Pearce, C. M., Barker, D. J. et al (2009) Susceptibility of non-indigenous ascidian species in British Columbia (Canada) to invertebrate predation. Mar Biol 156:1311–1320. doi:10.1007/s00227-009-1172-7

Epstein, G., Smale, D. A. (2017) *Undaria pinnatifida*: A case study to highligth challenges in marine invasion ecology and management. Ecol. Evol. 7: 8624-8642.

Ferguson, J. C. (1969) Feeding activity in *Echinaster* and its induction by dissolved elements Biol. Bull. 136: 374-384.

Ferreira, M. M. (1968) Sobre a alimentacao da aruana, *Chelonia mydas* L., ao longo da costa do estado do ceara. Arq. Est. Biol. Mar. Univ. Fed. Ceara 8: 83-86.

Fletcher, L. M. and Forrest, B. M. (2011) Induced spawning and culture techniques for the invasive ascidian *Didemnum vexillum* (Kott, 2002). Aquatic Invasions 6: 457–464. doi: 10.3391/ai.2011.6.4.11

Fletcher, L. M., Forrest, B. M. and Bell, J. J. (2012) Natural dispersal mechanisms and dispersal potential of the invasive ascidian *Didemnum vexillum*. Online version. Biol Invasions (2013) 15: 627-643.

Fletcher, L. M., Forrest, B. M., Atalah, J. and Bell J. J. (2013) Reproductive seasonality of the invasive ascidian *Didemnum vexillum* in New Zealand and implications for shellfish aquaculture. Aquaculture Environment Interactions 3: 197–211. doi: 10.3354/aei00063.

Fletcher, L. M., Atalah, J. and Forrest, B. M. (2018) Effect of substrate deployment timing and reproductive strategy on patterns in invasiveness of the colonial ascidian *Didemnum vexillum*. Mar Environ Res 141: 109-118. doi: 10.1016/j.marenvres.2018.08.006.

Fredriksen, S., Christie, H. and Sæthre B. A. (2005) Species richness in macroalgae and macrofauna assemblages on *Fucus serratus* L. (Phaeophyceae) and *Zostera marina* L. Angiospermae) in Skagerrak, Norway. Marine Biology Research 1: 2-19. DOI: 10.1080/17451000510018953

Fossøy, F., Sivertsgård, R., Ambjørndalen, V.M., Brandsegg, H., Andersskog, I.P.Ø., Husa, V. & Forsgren, E. 2022. Kartlegging av den fremmede marine arten havnespy *Didemnum vexillum* ved hjelp av miljø-DNA. En rask respons undersøkelse. NINA Rapport 2092. Norsk institutt for naturforskning.

Garcia, E. G. (2006). The fishery for Iceland scallop (*Chlamys islandica*) in the Northeast Atlantic. Adv Mar Biol. 51: 1-55.

Gargan, L.M., Brooks, P.R., Vye, S.R. et al. (2022) The use of environmental DNA metabarcoding and quantitative PCR for molecular detection of marine invasive non-native species associated with artificial structures. Biol Invasions 24: 635–648. https://doi.org/10.1007/s10530-021-02672-8

Georgiades, E., Scianni, C., Davidson, I., Tamburri, M. N., First, M. R., Ruiz, G. et al. (2021). The role of vessel biofouling in the translocation of marine pathogens: Management considerations and challenges. Frontiers in Marine Science 8: 660125

Gittenberger, A. (2007) Recent population expansions of non-native ascidians in The Netherlands. Journal of Experimental Marine Biology and Ecology 342: 122-126.

Gittenberger, A. (2009). Invasive tunicates on Zeeland and Prince Edward Island mussels, and management practices in The Netherlands. Aquatic Invasions, 4: 279-281

Gittenberger, A. (2010). Risk analysis of the colonial sea-squirt *Didemnum vexillum* Kott, 2002 in the Dutch Wadden Sea, a UNESCO World Heritage Site. GiMaRIS report 2010.08: 32 pp. Issued by the Dutch Ministry of Agriculture. Nature & Food Quality.

Gittenberger, A., and Moons, J. J. S. (2011). Settlement and possible competition for space between the invasive violet tunicate *Botrylloides violaceus* and the native star tunicate *Botryllus schlosseri* in The Netherlands. Aquatic Invasions, 6: 435-440.

Goodbody, I. and Gibson, J. (1974 )The biology of *Ascidia nigra* (Savigny). V. Survival in populations settled at different times of the year. Biol. Bull. 146:217 – 237.

Graham, J., Collins, C., Lacaze, J. P., Brown, L., and McCollin, T. (2015) Molecular identification of *Didemnum vexillum* Kott, 1982 from sites around the UK coastline. BioInvasions Record, 4(3).

Griffith, K., Mowat, S., Holt, R. H., Ramsay, K., Bishop, J. D., Lambert, G., and Jenkins, S. R. (2009) First records in Great Britain of the invasive colonial ascidian *Didemnum vexillum* Kott, 2002. Aquatic Invasions, 4(4), 581-590.

Gröner F, Lenz M, Wahl M, Jenkins SR (2011) Stress resistance in two colonial ascidians from the Irish Sea: The recent invader Didemnum vexillum is more tolerant to low salinity than the cosmopolitan *Diplosoma listerianum*. Journal of Experimental Marine Biology and Ecology 409: 48–52. http://dx.doi.org/10.1016/j.jembe.2011.08.002

Gulliksen, B. (1975) The prosobranch *Trivia arctica* as predator on the solitary ascidian *Ascdiia conchilega.* Proc. Malac. Lond. 41:377-378.

Gulliksen, B. and Skjæveland, S. H. (1973) The sea-star, *Asterias rubens* L., as predator on the ascidian Ciona intestinalis (L.) in Borgenfjorden, North-Trøndelag, Norway. Sarsia 52:15-20.

Gulliksen, B., Haug, T. and Sandnes, O.H. (1980) Benthic macrofauna on new and old lava grounds at Jan Mayen. Sarsia 65:137-148.

Haanes, H. and Gulliksen, B. (2011) A high local species richness and biodiversity within high-latitude calcareous aggregates of tuve-building polychaetes. Biodivers. Conserv. 20: 793-806

Haugland, B. T., Armitage, C. S, Kutti, T., Husa, V., Skogen, M. D., Bekkby, T. et al. (2021) Large-scale salmon farming in Norway impacts the epiphytic community of *Laminaria hyperborea*. Aquacult Environ Interact 13:81-100.

Haydar, D., Hoarau, G., Olsen, L., Stam, W. T. and Wolff, W. J. (2011) Introduced or glacial relict? Phylogeography of the cryptogenic tunicate *Molgula manhattensis* (Ascidiacea, Pleurogona). Diversity and Distributions 17: 68-80.

Husa, V., Agnalt, A-L., Berntsen, H., Falkenhaug, T., Fossøy, F., et. al. (2022) Alien marine species in Norway. Mapping monitoring and assessment of vectors for introduction. Rapport fra havforskningen 2022-08.

Husa, V., Sjøtun, K., Brattenborg, N. and Lein, T. E. (2008) Changes of macroalgal diversity in sublittoral sites in southwest Norway: Impact of an introduced species or higher temperature? Marine Biology Research 4: 414-428.

Hobson, E. S. (1974) Feeding relationships of teleostean fishes on coral reefs in Kona, Hawaii. Fishery Bulletin 72: 915-1031.

Håvardsson, B. (2013). Den lille groeboken. Steen-Hansen.

IOM (2011). Annex 26: Resolution MEPC.207(62) adopted on July 15th 2011. Guidelines for the control and management of ships' biofouling to minimize the transfer of invasive aquatic species. The Marine Environment Protection Committee. Available at: https://wwwcdn.imo.org/localresources/en/KnowledgeCentre/IndexofIMOResolutions/MEPCDocuments/MEPC.207(62).pdf

IOM (2011b). Annex 2: Resolution MEPC.196(62) adopted on July 15th 2011. Guidelines for the development of ship recycling plan. The Marine Environment Protection Committee. Available at: https://wwwcdn.imo.org/localresources/en/OurWork/Environment/Documents/RESOLUTION%20MEPC.196(62).pdf

IUCN (2020). IUCN EICAT Categories and Criteria. The Environmental Impact Classification for Alien Taxa. First edition. Gland, Switzerland and Cambridge, UK. 22 pp.

Järnegren, J. and Kutti, T. (2014) *Lophelia pertusa* in Norwegian waters. What have we learned since 2008? NINA rapport 1028: 1-40.

Jennings, J. B. (1957) Studies on feeding, digestion, and food storage in free-living flatworms. (Platyhelminthes: *Turbellaria*). Biol. Bull. mar. biol. Lab., Woods Hole 112:63-80.

Kaplan, K. A., Hart, D. R., Hopkins, K., Gallager, S., York, A., Taylor, R. and Sullivan, P. J. (2017) Evaluating the interaction of the invasive tunicate *Didemnum vexillum* with the Atlantic sea scallop *Placopecten magellanicus* on open and closed fishing grounds of Georges Bank. ICES Journal of Marine Science (2017), 74: 2470–2479. doi:10.1093/icesjms/fsx076

Kaplan, K. A., Hart, D. R., Hopkins, K., Gallager, S., York, A., Taylor, R. and Sullivan, P. J. (2018) Invasive tunicate restructures invertebrate community on fishing grounds and a large protected area on Georges Bank. Biological Invasions 20: 87-103.

Keough, M. J. and Butler, A. J. (1979) The role of asteroid predators in organization of a sessile community on pier piling. Marine Biology 51:167-177.

Kleeman, S. N. (2009) *Didemnum vexillum* – Feasibility of Eradication and/or Control. CCW Science report Report No: 875, 53 pp, CCW, Bangor.

Kott, P. (2002) A complex didemnid ascidian from Whangamata, New Zealand. Journal of the Marine Biological Association of the UK. 82: 625-628.

Krumhansl, K. A., Lee, M. and Scheibling, R. E. (2011) Grazing damage and encrustation by an invasive bryozoan reduce the ability of kelps to withstand breakage by waves. Journal of Experimental Marine Biology and Ecology. 407 : 12-18.

Kumschick, S., Bacher, S., Dawson, W., Heikkilä, J., Sendek, A., Pluess, T., Robinson, T.B. & Kuhn, I. (2012) A conceptual framework for prioritization of invasive alien species for management according to their impact. NeoBiota 15: 69–100.

Lambert, G. (1968) The general ecology and growth of a solitary ascidian, *Corella willmeriana*. Biol. Bull. mar. biol. Lab., Woods Hole 135:296-307.

Lambert, G. (2005) Ecology and natural history of the protochordates. Canadian Journal of Zoology 83: 34–50, https://doi.org/10.1139/z04-156

Lande, R. (1973) Food and feeding habits of plaice (*Pleuronectes platessa* L.) in Borgenfjorden, North-Trøndelag, Norway. Norwegian Journal of Zoology. 21:91-100.

Lande, R. (1976) Food and feeding habits of dab (*Limanda limanda* (L.)) in Borgenfjorden, North-Trøndelag, Norway. Norw. J. Zool. 24:225-230.

Laxton, J. H. (1971) Feeding in some Australasian Cymatiidae (Gastropoda: Prosbranchia). Zool. J. Linn. Soc. 50: 1-9.

Legrand, E. and Husa, V. (in prep). Testing of eradication methods for *Didemnum vexillum*.

Lengyel, N. L., Collie, J. S. and Valentine, P. C. (2009) The invasive colonial ascidian *Didemnum vexillum* on George's bank- Ecological effects and genetic identification. Aquatic Invasions 1: 143-152.

Levin, P. S., Coyer, J. A., Petrik, R. and Good, T. P. (2002) Community-wide effects of nonindigenous species on temperate rocky reefs. Ecology 83: 3182-3193.

Locke, A., and Carman, M. (2009). Adventures of a sea squirt sleuth: unraveling the identity of Didemnum vexillum, a global ascidian invader. *Aquatic Invasions*, *4*(1), 5-28.

Long, H. A. and Grosholz, E. D. (2015) Overgrowth of eelgrass by the invasive colonial tunicate *Didemnum vexillum*: consequences for tunicate and eelgrass growth and epifauna abundance. Journal of Experimental Marine Biology and Ecology 473: 188-194.

Lopez-Anido R., Michael A.P., Goodell B., Sandford. T.C. (2004) Assessment of wood pile deterioration due to marine organisms. Journal of Waterway, Port, Coastal, and Ocean Engineering 130:70-76. DOI: 10.1061/(asce)0733-950x(2004)130:2(70).

Lützen, J. G. (1967) Sækdyr. Danmarks Fauna. Bd. 75. 267 pp.

Marks, J. A. (1994) Three sibling species of didemnid ascidians from northern Norway: *Didemnum albidum* (Verrill, 1871), *Didemnum polare* (Hartmeyer, 1903), and *Didemnum romssae* sp.nov. Can. J. Zool. 74: 357-379.

Matejusova I, Graham J, Bland F, Lacaze J-P, Herman G, Brown L, Dalgarno E, Bishop JD, Kakkonen JE, Smith KF and Douglas A (2021) Environmental DNA based surveillance for the highly invasive carpet cea squirt *Didemnum vexillum*: A targeted single-species approach. Front. Mar. Sci. 8:728456. doi: 10.3389/fmars.2021.728456

McKenzie, C. H., Reid, V., Lambert, G., Matheson, K., Minchin, D., Pederson, J.,Brown, L. et al. (2017) Alien species alert: *Didemnum vexillum* Kott, 2002: Invasion, impact, and control. ICES Cooperative Research Report No. 335. 33 pp. http://doi.org/10.17895/ices.pub.2138.

Mercer, J.M., Whitlatch, R. B. and Osman, R. W. (2009) Potential effects of the invasive colonial ascidian (*Didemnum vexillum* Kott, 2002) on pebble-cobble bottom habitats in Long Island Sound, USA. Aquatic Invasions 4: 133–142, https://doi.org/10.3391/ai.2009.4.1.14

Millar, R.H. (1966) Marine invertebrates of Scandinavia. No. 1. Tunicata, Ascidiacea. Universitetsforlaget, Oslo. 123 pp.

Millar, R. H. (1971) The biology of Ascidians. In “Advances in Marine Biology” (FS Russel & CM Yonge, eds.). Academic Press, London. 9:1-100.

Milne-Edwards, M. (1841) Observations sue les Ascidies composées des Cotes de la Manche.

Minchin, D. and Duggan, C. B. (1988) The distribution of the exotic ascidian, *Styela clava* Herdman, in Cork Harbour. Irish Naturalists’ Journal 22: 388-393

Moen, F. E. and Svensen, E. (2020) Dyreliv i Havet. Norsk Marin Fauna. 7. ed. KOLOFON Forlag AS 800 pp.

Morris, J. A., Carman, M. R., Hoagland, K. E., Green-Beach, E. R. M. and Karney, R. C. (2009) Impact of the invasive colonial tunicate *Didemnum vexillum* on the recruitment of the bay scallop (*Argopecten irradians irradians*) and implications for recruitment of the sea scallop (*Placopecten magellanicus*) on Georges Bank. Aquatic Invasions, 4: 207–211.

Morris, J. A., and Carman, M. R. (2012) Fragment reattachment, reproductive status, and health indicators of the invasive colonial tunicate *Didemnum vexillum* with implications for dispersal. Biological Invasions 14: 2133–2140.

Myksvoll, M.S., Sandvik, A.D., Albretsen, J., Asplin, L., Johnsen, I.A., Karlsen, Ø., Kristensen, N.M., Melsom, A., Skardhamar, J., Ådlandsvik, B., 2018. Evaluation of a national operational salmon lice monitoring system—From physics to fish. PLOS ONE 13: e0201338. https://doi.org/10.1371/journal.pone.0201338

NBIC - Norwegian Biodiversity Information Centre (2018) The Alien Species List of Norway – ecological risk assessment 2018. https://www.biodiversity.no/alien-species-2018

NBIC - Norwegian Biodiversity Information Centre (2022) https://www.biodiversity.no/

Nentwig, W., Kuehnel, E. and Bacher, S. (2010) A generic impact scoring system applied to alien mammals in Europe. Conservation Biology, 24: 302-311. https://doi. org/10.1111/j.1523-1739.2009.01289.x

Nishikawa, T. (1990) The ascidians of the Japan Sea. I. Publications of the Seto Marine Biological Laboratory, 34(4–6): 73–148.

Ordóñez, V., Pascual, M., Fernandez-Tejedor, M., Pineda, M.C., Tagliapietra, D., and Turon, X. (2015) Ongoing expansion of the worldwide invader *Didemnum vexillum* (Ascidiacea) in the Mediterranean Sea: High Plasticity of its biological cycle promotes establishment in warm waters. Biological Invasions, 17: 2075–2085. doi: 10.1007/s10530-015-0861-z.

Pannell, A., and Coutts, A. D. (2007) Treatment methods used to manage *Didemnum vexillum* in New Zealand. New Zealand Marine Farming Association.

Parra-Rincón, E., Velandia-Huerto, C. A., Gittenberger, A., Fallmann, J., Gatter, T., Brown, F. D., ... & Bermúdez-Santana, C. I. (2021). The genome of the "Sea Vomit" *Didemnum vexillum*. Life 11: 1377.

Rinkevich, B. (2011) Quo vadis chimerism? Chimerism 2, 1–5.

Rinkevich, B. and Fidler, A. E. (2014) Initiating laboratory culturing of the invasive ascidian *Didemnum vexillum*. Management of Biological Invasions 5:55-62.

Roberts, M (2017). *MOHC HadGEM3-GC31-LL model output prepared for CMIP6 HighResMIP*. Version 20221001.Earth System Grid Federation. https://doi.org/10.22033/ESGF/CMIP6.1901 . Data at: https://cds.climate.copernicus.eu/

Rolheiser, K. C., Dunham, A., Switzer, S. E., Pearce, C. M., and Therriault, T. W. (2012) Assessment of chemical treatments for controlling *Didemnum vexillum*, other biofouling, and predatory sea stars in Pacific oyster aquaculture. Aquaculture, 364: 53–60

Roth, S. K., Powell, A., Smith, D. J., Roth, F. and Schierwater, B. (2018) The highly competitive ascidian *Didemnum* sp. threatens coral reef communities in the Wakatobi Marine National Park, Southeast Sulawesi, Indonesia. Reg Stud Mar Sci 24: 48-54.

Saito, Y., H. Mukai, and Watanabe, H. (1981) Studies on Japanese compound styelid ascidians II. A new species of the genus *Botrylloides* and redescription of *B. violaceus* Oka. Publ. Seto Mar. Biol. Lab. 26: 357-368.

Sambrook, K., Holt, R. H., Sharp, R., Griffith, K., Roche, R. C., Newstead, R. G., and Jenkins, S. R. (2014) Capacity, capability and cross-border challenges associated with marine eradication programmes in Europe: the attempted eradication of an invasive non-native ascidian, *Didemnum vexillum* in Wales, United Kingdom. Marine Policy, 48, 51-58.

Sebens, K. P. (1991) Habitat structure and community dynamics in marine benthic systems. In: Mushinsky HR (ed) Habitat structure: the physical arrangement of objects in space. Chapman and Hall, London Sebens.

Secord D. 2003. Biological control of marine invasive species: cautionary tales and land-based lessons. Biological Invasions 5: 117-131

Senn, D. G. and Glasstetter, M. (1989) On the occurrence of barnacle-reefs around Cocos-Island, Costa Rica. Senck Marit 20:241–249.

Shenkar, N.; Gittenberger, A.; Lambert, G.; Rius, M.; Moreira da Rocha, R.; Swalla, B.J.; Turon, X. 2023. Ascidiacea World Database. Accessed at https://www.marinespecies.org/ascidiacea on 2023-01-19. doi:10.14284/353

Simkanin, C., Davidson, I. C., Dower, J. F., Jamieson, G. and Therriault, T. W. (2012) Anthropogenic structures and the infiltration of natural benthos by invasive ascidians, Marine Ecology 33: 499-511.

Simkanin, C., Dower, J. F., Filip, N., Davidson, I. C., Dower, J. F., Jamieson, G. and Therriault, T. W. (2013) Biotic resistance to the infiltration of natural benthic habitats: Examining the role of predation in the distribution of the invasive ascidian *Botrylloides violaceus*, Journal of Experimental Marine Biology and Ecology 439: 76-83.

Simpson, T. S., Wernberg, T. and McDonald, J .I. (2016) Distribution and localised effects of the invasive ascidian *Didemnum perlucidum* (Monniot 1983) in an urban estuary. Plos One 11: e0154201.

Sinner, J. and Coutts, A. D. M. (2003) Benefit-cost analysis of management options for *Didemnum vexillum* in Shakespeare Bay. Cawthron Report, 924. 24 pp.

Smith, K. F., Stefaniak, L., Saito, Y., Gemmil,l C. E. C., Cary, S. C. and Fidler, A. E. (2012) Increased inter-colony fusion rates are associated with reduced COI haplotype diversity in an invasive colonial ascidian *Didemnum vexillum*. PLoS ONE 7: e30473. http://dx.doi.org/10.1371/journal.pone.0030473

Snowden, E. 2008. *Botrylloides violaceus* A colonial sea squirt. In Tyler-Walters H. and Hiscock K. (eds) *Marine Life Information Network: Biology and Sensitivity Key Information Reviews*, [on-line]. Plymouth: Marine Biological Association of the United Kingdom. [cited 01-02-2023]. Available from: https://www.marlin.ac.uk/species/detail/2186.

Stefaniak, L., Lambert, G., Gittenberger, A., Zhang, H., Lin, S. and Whitlatch, R. B. (2009) Genetic conspecificity of the worldwide populations of *Didemnum vexillum* Kott, 2002. Aquatic Invasions 4: 29-44.

Stefaniak, L., Zhang, H., Gittenberger, A., Smith K., Holsinger, K., Lin, S. and Whitlatch, R. (2012) Determining the native region of the putatively invasive ascidian *Didemnum vexillum* Kott, 2002. J Exp Mar Biol Ecol 422−423: 64−71

Stoecker, D. (1980) Chemical defenses of ascidians against predators. Ecology 61(6):1327-1334.

Stoner, D. S., Rinkevich, B. and Weissman, I. L. (1999) Heritable germ and somatic cell lineage competitions in chimeric colonial protochordates. Proc. Natl. Acad. Sci. USA 96, 9148–9153.

Strand, H. K., Christie, H., With Fagerli, C., Mengede, M. and Moy, F. (2020) Optimizing the use of quicklime (CaO) for sea urchin management — a lab and field study. Ecological Engineering: X (2020), doi: https://doi.org/10.1016/j.ecoena.2020.100018

Swinehart, J. H., Biggs, W. R., Halko, D. J.and Schroeder, N. C. (1974) The vanadium and selected metal contents of some ascidians. Biol. Bull. 146:302 – 312.

Switzer, S. E., Therriault, T. W., Dunham, A., and Pearce, C. M. (2011) Assessing potential control options for the invasive tunicate *Didemnum vexillum* in shellfish aquaculture. Aquaculture, 318: 145–153.

Turon, X., Casso, M., Pascual, M. and Viard, F. (2020) Looks can be deceiving: *Didemnum pseudovexillum* sp. Nov. (ascidiacea9 in European habours. Marine Duiversity. 50:48.

Vad J., Orejas C., Moreno-Navas J., Findlay H. S. and Roberts J. M. (2017) Assessing the living and dead proportions of cold-water coral colonies: Implications for deep-water marine protected area monitoring in a changing ocean. PeerJ 5: e3705. doi: 10.7717/peerj.3705.

Valentine, P. C., Carman, M. R., Blackwood, D. S. and Heffron, E. J. (2007a) Ecological observations on the colonial ascidian *Didemnum* sp. in a New England tide pool habitat. Journal of Experimental Marine Biology and Ecology 342: 109–121. https://doi.org/10.1016/j.jembe.2006. 10.021

Valentine, P. C., Collie, J. S., Reid, R. N., Asch, R. G., Guida, V. G. and Blackwood, D. S. (2007b) The occurrence of the colonial ascidian *Didemnum* sp. on Georges Bank gravel habitat – ecological observations and potential effects on groundfish and scallop fisheries. Journal of Experimental Marine Biology and Ecology 342: 179–181. https://doi.org/10.1016/j.jembe.2006. 10.038.

Valentine, P.C., Carman, M. R., Dijkstra, J. A. and Blackwood, D. S. (2009) Larval recruitment of the invasive colonial ascidian *Didemnum vexillum*, seasonal temperatures in New England coastal and offshore waters, and implications for spread of the species. Aquatic Invasions 4: 153-168.

van den Heuvel-Greve, M., van den Brink, A., Glorius, ST., Arjen de Groot, G., Laros, I., Renaud PE., Pettersen, R., Weslawski, JM., Kuklinski, P., Murk, Aj. (2021) Early detection of marine non-indigenous species on Svalbard by DNA metabarcoding of sediment. Polar Biology https://doi.org./10.1007/s00300-021-02822-7

Van Name, W. G. (1945) The North and South American ascidians. Bull. Am. Mus. Nat. Hist. 84:1-476.

VKM (2018). Rutine for godkjenning av risikovurderinger. https://bit.ly/3HxXHA9

VKM (2019). Kriterier for forfatterskap og faglig ansvar i VKMs uttalelser. https://bit.ly/3jeDbex
Wallentinus, I. and Nyberg, C. D. (2007) Introduced marine organisms as habitat modifiers. Marine Pollution Bulletin 7-9: 323-332.
Weinberg, R. B., Clancy, D. L., & Cohen, C. S. (2019). Genotypic variability following fusion in the invasive colonial tunicate *Didemnum vexillum*. Invertebrate Biology, 138(3), e12263
Zaniolo, G, Manni, L., Brunetti, R. and Burighel, P. (1998) Brood pouch differentiation in *Botrylloides violaceus*, a viviparous ascidian (Tunicata). Invert. Reprod. Devel. 33: 11-24.
Zuljevic A, Thibaut T, Elloukal H and Meinesz A (2001) Sea slug disperses the invasive Caulerpa taxifolia. Journal of the Marine Biological Association of the United Kingdom 81: 343–344