Development of sustainable fisheries management and monitoring for sensitive soft-bottom habitats and species in the Kattegat

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Colophon

Preface

The overall aim of the project was to improve the knowledge base for development of fisheries management and measures for protection of sensitive habitats and species in relation to the bottom trawling of the Norway lobster (*Nephrops norvegicus*) and mixed fish species in the Kattegat. This project focused on sensitive benthic faunal habitats and species included in the Marine Strategy Framework Directive (MSFD), Natura 2000 and the OSPAR list of threatened and/or declining species, especially sea pens (*Pennatula phosphorea* and *Virgularia mirabilis*), sponges (Porifera), northern horse mussel (*Modiolus modiolus*), ocean quahog (*Arctica islandica*) and the tubiculous crustaceans of the *Haploops* genus.

In this project, we investigated selected areas of the Kattegat based on the local knowledge of fishers and the findings contained in studies undertaken by the scientific community. The projects aimed to: i), locate potential areas with sensitive habitats and species; ii), mapping of the spatial distribution of bottom trawling intensity; iii), comparatively investigate the occurrence, density and biodiversity of sensitive habitats and species in un-trawled areas and across a gradient of fishing intensity using four different sampling methods; iv), test the effectiveness of two non-invasive survey gears (UWTV, BRUV) for use in future monitoring of sensitive habitats and species; v), develop biodiversity indicators for the monitoring of sessile and mobile fauna and the effects of new management measures, such as areas of seabed closures, under different environmental conditions and vi), disseminate the project results to the wider public by a virtual table, and to the scientific community *via* publications, and to prove advice for development of management measures related to areal closures of fishery to protect sensitive habitats and species. With regard to iii) above, this comparative study was undertaken using side scan sonar and a light video sledge operating from a fishing vessel, and to conduct a research survey using a quantitative video sledge (UWTV), baited stereo-cameras (BRUV), the KASU survey trawl (TV3) and a quantitative sediment sampler (Van Veen $0.1m^2$) operated from the *R/V Havfisken*.

The expected outcome of the project was thus to provide new: i), knowledge of potential areas in the Kattegat with sensitive habitats which may need protection; ii), biological insights into the correlations between benthic habitats, bottom trawling and the distribution of sensitive habitats and iii), knowledge of the function of sensitive habitats with regards to the ecological status of biodiversity and the ecosystem. The project results thus improve the scientific knowledge base for advice related to the implementation of the MSFD and Natura 2000 protection of sensitive habitats and species and further development of sustainable, ecosystem based fisheries management.

The final draft version of the project report was submitted in March 2019. The final report presented herein benefited from further data analyses and new information on macrofaunal indicators of fisheries impacts on seabed habitats, which are presented as an addition to Chapter 6 together with the two resulting international peer-reviewed papers.

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Grete E. Dinesen

Contents

Summary

The Kattegat is home to an intensive bottom trawl fishery for Norway lobsters (*Nephrops norvegicus*), and although the fishery on the target species is sustainable, intensive bottom trawling is known to significantly impact sensitive seabed habitats and species. In this project, we investigated how bottom trawling for *N. norvegicus* affects the seabed fauna and the demersal fish assemblages in the Kattegat by sampling selected stations with bottom grabs and trawls, by recording the fauna on the seabed with a towed video camera, and by counting and identifying the species attracted to baited stereo-video cameras. The sampling stations were primarily located in the deeper, soft sediment areas where *N. norvegicus* is abundant, but samples were also collected in shallower areas where sensitive species had been identified in previous investigations. Prior to sampling, local fishers were interviewed to identify potential areas of *N. norvegicus* habitats that were '*de facto*' closed to fishing due to the presence of obstacles on the sea bed, such as reefs and boulders. In some of these areas, side scan sonar was used to map the seabed. Results from previous investigations and maps of fishing intensity were used to select the sampling locations, in order to ensure that a bottom fishing pressure gradient ranging from zero to high intensity was represented in the sampling design.

Four different sampling devices were deployed to compare their individual suitability to record different elements of the seabed and demersal fauna. The sediment grab sampler mostly contained invertebrate species that dwell in the sediment (infauna) and some of those which reside on the surface of the seabed (epifauna). The towed video camera primarily recorded the larger (megafauna) epifaunal species. These included epifaunal invertebrate species known to be sensitive to bottom trawling, such as sea pens (*Virgularia mirabilis*, *Pennatula phosphorea*), northern horse mussel (*Modiolus modiolus*), tube building crustaceans (*Haploops* spp.), and larger sea anemones (*Pachycerianthus multiplicatus*, *Bolecera tuediae*). The baited stereo-video cameras recorded scavenging invertebrates and fish species that were attracted to the bait. Most noteworthy was the large numbers of hagfish (*Myxine glutinosa*) recorded at all the deeper (>50 depth) stations. This species was rarely caught by the grab sampler and the bottom trawl deployed in the project. The bottom trawl caught a variety of other fish species as well as some of the larger, mobile, epifaunal, invertebrate species, which were not recorded by any of the other sampling devices. Given the differences in the species recorded at each station by the four sampling methods we conclude that adequate monitoring of the ecological impacts of bottom trawling requires the application of a number of different sampling devices to fully record the changes in the fauna affected by bottom trawling, such as shifts in density and species composition of benthic macrofauna, megafauna and demersal fish assemblages. It is therefore also necessary to apply different sampling devices to investigate the mitigating effects of management measures, such as areal closures.

During the field survey we collected a total of 84 sediment grab samples across the Kattegat. After collection, each sample was passed through two different sieve sizes, to separate the macrofaunal community into small and large individuals. This size-separation was carried out because we expected large-bodied faunal benthos to be particularly sensitive to trawling disturbance and, therefore, have the potential to be effective indicators of trawling impacts on the seabed habitats. The macrofaunal data were analysed with fishing intensity data and six

additional environmental variables known to have a strong influence on benthic communities. The results describe the distribution of benthic communities across the study area, the distribution of pre-selected (*a-priori*) sensitive species, the effects of trawling pressure and other drivers on the composition of species and traits within the community, and the response of several ecological indicators and benthic life history traits to trawling. The pre-selected sensitive species were found to be relatively uncommon across the fishing grounds. Of these, the sea pen *Virgularia mirabilis* and sea anemones were only recorded at stations with low to intermediate trawling intensity, while *Pennatula phosphorea*, *Modiolus modiolus* and *Haploops* spp. were only recorded at a single station each. On the other hand, other species known to be sensitive to bottom trawling such as the bivalve *Arctica islandica* and several species of tube-building worms were more widely distributed. Analysis of the data showed a number of key life history traits were associated with high sensitivity to trawling. These included biological traits such as immobility (sessile life-habits), suspension feeding, and deposition of bioturbated material at the sediment surface. Further to this, large fauna with a lifespan of >10 years and a burrowing to depth of 6-10cm in the sediment were also significantly impacted by trawling. The sensitivity to trawling of common, large-bodied infauna and epifauna, such as bivalves and tube-building polychaetes, was strongly reflected in the performance of the ecological indicators. These results show that trawling remains a significant driver of macrofaunal density of individuals and biomass, even when differences in the local physical and hydrodynamic conditions are accounted for.

Stakeholder meetings were held in order to engage the fishers in the project and in discussions about how trawling impacts on sensitive species and habitats can be managed. The results of the project were visualized by an interactive model of the Kattegat seabed, showing where sensitive species and habitats were found, and where the *Nephrops* fishery takes place. We conclude that areas closed to trawling primarily should be established where sensitive species and communities occur. Closing areas that are already heavily trawled cannot guarantee that sensitive species and communities will re-establish themselves in these areas, and may furthermore lead to fishing effort being diverted from these areas to others where the sensitive species are currently found.

1. Introduction

In this project we have focused on habitats and species in the Kattegat that are listed as threatened and/or declining within the area covered by the 1992 OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic (which unified and up-dated the 1972 Oslo and 1974 Paris Conventions, www.ospar.org/news/increased-protection-for-endangered-marine-life-of-the-north-east-atlantic). The listed habitats and species included in this project were: i), sea pens and burrowing megafauna communities; ii), deep-sea sponge aggregations; iii), *Modiolus modiolus* (northern horse mussel) beds; iv), *Arctica islandica* (ocean quahog) and v), *Haploops* spp. (tube-building amphipods) (OSPAR Commission, 2008; 2010; ICES, 2018). *Nephrops norvegicus* (Norway lobster) lives as burrowing megafauna, and often co-occurs with the habitats and species listed above.

Nephrops norvegicus is the target primary species of the fishery within the Danish Economic Exclusive Zone (EEZ) in the Kattegat area at depths exceeding 15 meters. The habitats and species focussed upon in this project were also included as potentially sensitive to mobile demersal fisheries with bottom contacting gears in the first MSC certification of the fishery for *Nephrops norvegicus* (hereafter denoted the *Nephrops* fishery) in the Kattegat and Skagerrak (MSC in 2014). The *Nephrops* fishery was MSC certified in 2014. Assessments of the environmental sustainability of the fishery regarding co-occurring sensitive species of sponges, sea pens, bivalves and tube-building crustaceans have, however, not been undertaken. The *Nephrops* fishery in the Kattegat and Skagerrak is considered to be biologically and economically sustainable with respect to its impact on the target species and the value of the landings (Frandsen, 2015). In the Kattegat and Skagerrak area, a recent study based on at-sea-sampling data in fished areas showed that time of day, season, depth, temperature, year, trawl type and location all significantly affected the catch rates of *Nephrops* (Feekings *et al.*, 2015). In the Danish EEZ in the Kattegat and Skagerrak, *N. norvegicus* is primarily caught by bottom trawls as the target species of the most important Danish fishery in the area, with annual landings in 2016-2018 ranging between 3.5-5.2 tones (in fresh weight) with values of between 228-265 mill DKK (~€29-35 mill) (table 1.1). In the Swedish EEZ of the Kattegat and Skagerrak, *Nephrops* fisheries by Swedish fishers are conducted using stationary gears, mainly creels (Frandsen, 2015).

Table 1.1. Annual *Nephrops norvegicus* landings by the Danish fishing fleet in the Danish Economic Exclusive Zone for the period 2016-2018
(from: www.fd-ststweb.fd.dk/landingsrapport/rapport/main.html)

	2016		2017		2018	
	Fresh weight (T)	Value (DKK)	Fresh weight (T)	Value (DKK)	Fresh weight (T)	Value (DKK)
Kattegat	1,181	83,572,633	1,585	97,385,787	2,197	119,193,557
Skagerrak	2,258	154,618,450	2,115	120,842,520	2,934	144,745,265
Total	3,439	238,191,083	3,700	218,228,307	5,131	263,938,822

The spatial distribution of the Danish *Nephrops* fishery is fairly stable between years with the highest landings (and trawling intensities) occurring in the northern Kattegat (in the Vinga trench east of the island of Læsø) and on the Danish shelf and slope of the Skagerrak (as an example see the landings from 2016 in figure 1.1 below).
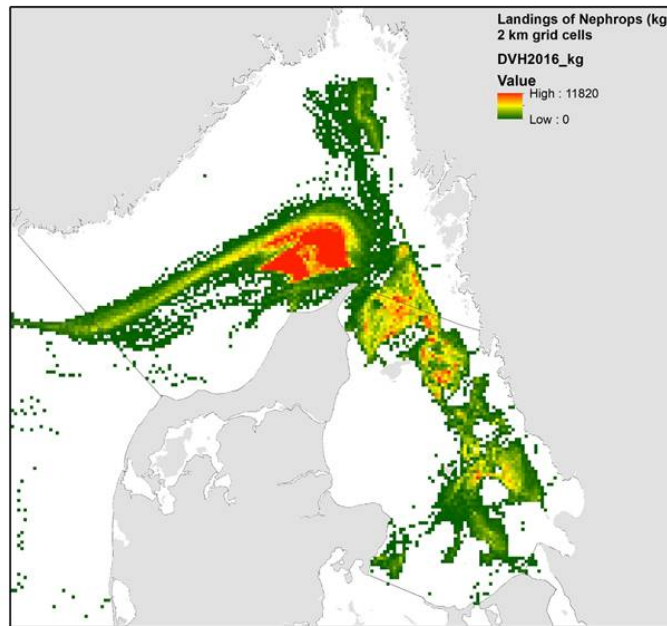
Figure 1.1. Distribution and density of landings (kg) of the Danish *Nephrops* fishery in the Danish Economic Exclusive Zone (EEZ) of the Kattegat and Skagerrak. The landings are based on data from the Vessel Monitoring System (VMS) and logbook information from vessels of and exceeding 12 meters of length.

Nephrops norvegicus (Linnaeus 1758) (Danish: Jomfruhummer) lives in the seafloor where it excavates complex burrows in soft sediments. Individual burrows may reach depths of between 2-5m in the sediment and have multiple openings at the seabed surface. More than 50 individuals may inhabit a single burrow complex. The species is thus one of the most important benthic bioturbators within its geographic range on the continental shelves and slopes of the Northeast Atlantic. The habitat suitability for *N. norvegicus* has been modelled using MAXENT based on presence-only data from the OBIS database and environmental data from the Hexacoral database (see details in Johnson *et al.*, 2013). The density of *N. norvegicus* was explained largely by the combined silt and clay content of the sediment. West of Ireland the density of *N. norvegicus* burrows were highest in sediments composed of 40-80% silt and clay, although actual density varied between sites of similar silt and clay composition (~0.4-1.25 burrows m^{-1}) (Campbell *et al.*, 2009b). More sandy sediments are less cohesive and appear unsuitable for burrow building. Thus, the distribution of the EUNIS level 3 habitats, Sublittoral mud (A5.3.) and Deep-sea mud (A6.4.) may provide an indication of the distribution of potential *Nephrops* habitats in the Kattegat and Skagerrak (figure 1.2).

Predicted EUNIS level 3 habitat type

EUNIS code, EUNIS name

- A3.1, Atlantic and Mediterranean high energy infralittoral rock
- A3.2, Atlantic and Mediterranean moderate energy infralittoral rock
- A3.3, Atlantic and Mediterranean low energy infralittoral rock
- A3.5, Baltic moderately exposed infralittoral rock
- A4.1, Atlantic and Mediterranean high energy circalittoral rock
- A4.2, Atlantic and Mediterranean moderate energy circalittoral rock
- A4.3, Atlantic and Mediterranean low energy circalittoral rock
- A4.5, Baltic moderately exposed circalittoral rock
- A4.6, Baltic sheltered circalittoral rock
- A4.7, Features of circalittoral rock
- A5.1, Sublittoral coarse sediment
- A5.2, Sublittoral sand
- A5.3, Sublittoral mud
- A5.4, Sublittoral mixed sediments
- A5.5, Sublittoral macrophyte-dominated sediment
- A5.6, Sublittoral biogenic reefs
- A6.1, Deep-sea rock and artificial hard substrata
- A6.2, Deep-sea mixed substrata
- A6.3, Deep-sea sand
- A6.4, Deep-sea muddy sand
- A6.5, Deep-sea mud
- NA, Data not available

Figure 1.2. Predicted distribution of the EUNIS level 3 habitats in the Kattegat and Skagerrak.

However, as pointed out by the potential habitat model by Johnson *et al.* (2013), other environmental parameters and biological processes are also essential in determining the distribution of *N. norvegicus*. These include high salinity levels (salinity minimum ~31.8) and oxygen concentrations (minimu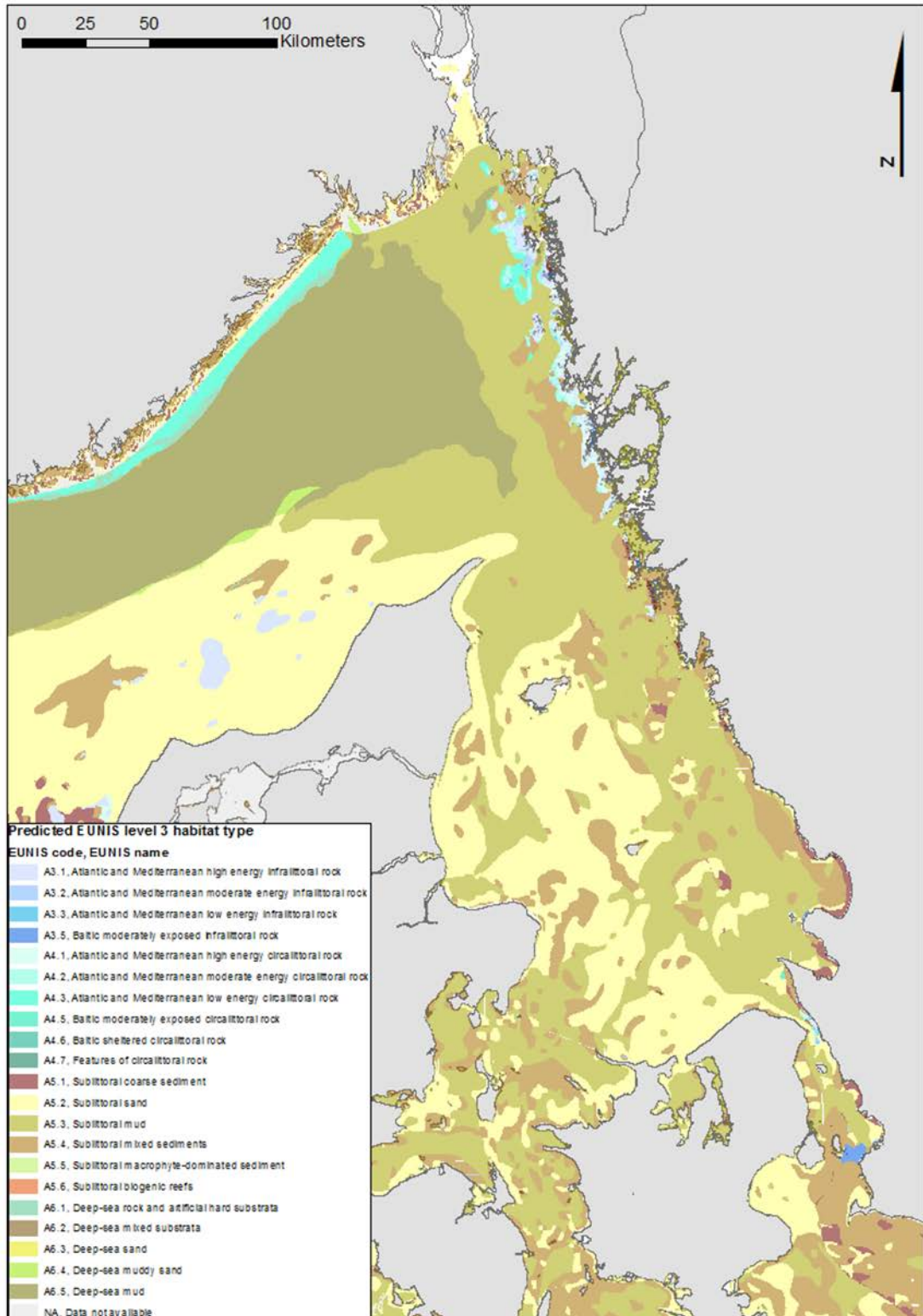m ~5.9mg $O_2 L^{-1}$) (Johnson *et al.*, 2013). Moreover, mean annual bottom temperature, mean depth, and mean annual surface chlorophyll may delineate essential habitat boundaries in the northeast Atlantic (Johnson *et al.*, 2013).

Nephrops norvegicus feeds on a variety of benthic fauna, including foraminifera, molluscs, polychaetes, crustaceans and echinoderms, as well as fish, and may also scavenge carrion or suspension-feed on plankton (Johnson *et al.*, 2013). The main predator of *N. norvegicus* throughout most of its distribution is thought to be Atlantic cod, *Gadus morhua* (Linnaeus, 1758) (Johnson *et al.*, 2013). Hagfish, *Myxine glutinosa* (Linnaeus, 1758), has been observed to attack living *N. norvegicus* and, in deeper, muddy, areas with high hagfish abundances the species may also be an important predator of *N. norvegicus*. The population structure and ecological importance of hagfish are little known however.

Whereas *N. norvegicus* forages on the seafloor, resting time is spent in its burrow. The burrowing character of the species is a challenge when estimating population size and structure. Population analyses today are based on standardised Under Water Video Transect (UWTV) assessments of burrow-complexes visible on the sediment surface (ICES, 2009). Moreover, stock assessments are conducted primarily in the fished areas, which are probably the most productive ones with medium to high densities of individuals. Thus, population structure in non-fished areas are little known, as are the habitat preferences throughout its wider distribution (reviewed by Johnson *et al.*, 2013), including the Kattegat and Skagerrak.

The first review of the reproductive biology of *N. norvegicus* by Farmer (1974) has been updated more recently by Powell and Eiriksson (2013). *Nephrops norvegicus* is dioecious. Following maturity female growth rates decrease in comparison with the same-sized males, which results in proportionally fewer females in the size groups larger than those at the onset of fecundity of ~2.5cm carapace length (Powell and Eiriksson, 2013). Females reproduce annually (or biennially in the northern part of its geographic distribution) and carry out partial brood protection. After spawning of the fertilised eggs, the female carries the developing embryos attached to her abdominal segments where they are held by the pleopods. Females stay in their burrows to a larger extent than males, providing additional protection to the embryos. Embryo development lasts for 6-10 months and is sustained by yolk reserves. After hatching the larva is called a zoea. The reproductive cycle has been correlated with depth and geographic distribution accounting for climate and weather related parameters such as temperature and photoperiod (Powell and Eiriksson, 2013). The *N. norvegicus* larval stages (zoea I-III and mysis I) are pelagic for up to 50 days and may experience horizontal advection of up to ~50km day^{-1} in the Skagerrak-Kattegat area. They can therefore move over considerable distances before they settle on the seafloor as post-larvae (mysis II or PL1 stage) (Powell and Eiriksson, 2013).

Recruitment patterns of *N. norvegicus* in the Kattegat are less well known, however. Here larvae are restricted to the mainly inflowing bottom layer of high saline (and lower temperature) water below the permanent pycnocline which typically is found at ~15-25m depth. This may explain why *N. norvegicus* typically occurs in deeper waters exceeding 25m in the Kattegat, with

a few exceptions where high saline waters enter shallower, muddy, trench systems, such as the Læsø trench (where *N. norvegicus* occurs from as shallow as 16m and downwards). In the Kattegat, therefore, the stock of likely recruits is mainly obtained from drifted larvae produced by populations in the northern North Sea and Norwegian trench-Skagerrak area (up to ~1000km away), although part of the population may be of local origin. In areas, where deeper water masses are retained locally, such as in the Skagerrak (and the Irish Sea, see Johnson *et al.*, 2013), *N. norvegicus* stock recruitment relies more heavily on locally reared offspring.

2. Sensitive habitats and species in the Kattegat

Information on the potentially sensitive habitats and species and their distribution in the Danish EEZ of the Kattegat was retrieved from historical sources (e.g. Petersen, 1913; 1918) and more recent monitoring surveys (NOVANA 2005-2013) and supplemented with single-point samples, including the OCEANA localities in the Kattegat sampled in 2011 and 2012, and the stations sampled in the proposed MSFD area in 2015 (Naturstyrelsen, 2016).

2.1 Sea pens and burrowing megafauna

This community comprises sea pens and large solitary sea anemones (Anthozoa) as well as several burrow-building crustaceans, such as *Nephrops norvegicus* and *Callianassa tyrrhena* (Petagna, 1792). The sea pens especially are negatively affected by bottom trawling (for *N. norvegicus*) and have thus been listed by OSPAR as threatened and/or declining species (OSPAR Commission, 2008; 2010).

Sea pens are colonial marine cnidarians belonging to the order Pennatulacea. All sea pens live in soft sediments in a mucous-lined burrow into which they can withdraw either in part or completely. The reason for this ability is not understood (Hoare and Wilson, 1977; Langron *et al.*, 1990; Ambroso *et al.*, 2013; Chimioeni *et al.*, 2018). In the cases of *Virgularia mirabilis* and *Pennatula phosphorea* an anthropogenic effect may be that the fast-retreating *V. mirabilis* is less affected by trawling with bottom contacting gears than the slower *P. phosphorea*. Another poorly understood ability in many, if not all, sea pens is their bioluminescence, which may be a predator avoidance mechanism.

2.1.1 Pennatula phosphorea

Pennatula phosphorea Linnaeus, 1758, (English: phosphorescent or red sea pen. Danish: Almindelig eller Rød søfjer) (figure 2.1). The colonies comprise a thick fleshy axis carrying two opposite lateral rows of large leaf-like branches, each composed of ~20 or more partly fused polyps. The siphonozoids (reduced individual water-pumping polyps) are arranged in a long conspicuous field along the rear of the axis. The colour both in life and as preserved specimens is a deep red with white tipped polyps. Numerous sclerites are present in the surface, especially of the polyps; they are red and give the colony its conspicuous colour. In Danish waters the colony can be up to ~20cm long (Carlgren, 1945; Manuel, 1981).

Distribution: Kattegat, north into Skagerrak, south into the Sound. It is especially common in the northern part of the Kattegat.

Habitat: Seabed of sand and muddy sand to mud, generally at depths exceeding 15m. The species occurs in high densities in some areas of the northern Kattegat experiencing low trawling intensity or no fishing for the past 10 years ('*de facto*' closed areas).

Biology: Free-living, digging a mucous-lined vertical burrow into which the whole colony slowly withdraws (3-6 minutes) with polyps retracted when continually disturbed (Chimienti *et al.*, 2013, *P. rubra*). When the colony is stretched out, the lower part is buried in the burrow, the upper part protruding into the near-bottom water. The outstretched polyps catch small planktonic

organisms. All polyps of a colony are of the same sex, and the gametes are shed into the water column (Edwards and Moore, 2008; Eckelbarger *et al.*, 1998, *P. aculeate*). The zygotes form small ciliated larvae, which after some days of free-swimming life allowing dispersal by bottom currents over distances at the km-scale, settle on the bottom and develop into the axial polyps of new colonies. With physical disturbance, a colony shows bioluminescence (Nicol, 1958).

Figure 2.1. Dense aggregation of *Pennatula phosphorea*. Image from a UWTV sledge transect (start position: 57° 22.886N, 11° 26.073E). The distance between the green laser lines at the bottom of the image is 80cm.

2.1.2 Virgularia mirabilis
Virgularia mirabilis (Mueller, 1776) (English: slender sea pen. Danish: Søstrå) (figure 2.2). The colony is slender with a 2-4mm thick axial component carrying two opposing lateral rows of short branches, each one composed of up to 16 partly fused polyps. The siphonozoids (reduced water-pumping polyps) are arranged in transverse rows on the axis, but are not conspicuous. Colour in life varies from creamy white to yellowish orange. There are no sclerites (skeletal needles). In Danish waters, the colonies can be up to ~50cm long (Carlgren, 1945; Manuel, 1981).

Distribution: Kattegat north into Skagerrak, south into the Sound and the belts. It is especially common in the southern part of the Kattegat (Carlgren, 1945).

Habitat: Soft seabed with muddy sand to soft mud, generally at depths exceeding 10m (Manuel, 1981).

Biology. Free-living, digging a mucus-lined vertical burrow into which the whole colony retracts fast with polyps extended when disturbed by a pressure wave (Ambroso *et al.*, 2013). Normal position is with the lower part of the colony in the upper end of the burrow, and the upper part protruding upright into the near-bottom water. The outstretched polyps catch both small planktonic organisms and suspended particles (Hoare and Wilson, 1977). All polyps of a colony are of the same sex. Reproduction is supposed to take place annually, with the gametes shed into the water and the zygotes forming a small ciliated lecithotrophic larva (planula larva). This

stage is free-swimming for several days allowing dispersal by near-bottom currents over distances at the km-scale. The larval stage settles on the bottom and develops (by metamorphosis) into the axial polyp of a new colony. The colonies show bioluminescence in response to physical disturbance. *Virgularia mirabilis* seems not to be especially sensitive to bottom trawling (Tuck *et al.*, 1998).

Figure 2.2. Several adult *Pennatula phosphorea* with a single *Virgularia mirabilis* colony to the right. Image from the UWTV sledge transect (start position: 57° 22.886N, 11° 26.073E). Distance between laser lines at the bottom of the image is 80cm.

2.1.3 Pachycerianthus multiplicatus

Pachycerianthus multiplicatus Carlgren, 1912 (English: fireworks anemone. Danish: Tyk cylinderrose) (figure 2.3). Large, stout body with two rings of tentacles on the oral disc. The colour of the body is yellowish- to reddish-brown and the tentacles are light grey - the marginal ones with brown bands and often white tips. The ~180 marginal tentacles are long, while the numerous central tentacles are short and cover the mouth and most of the oral disc. In Danish waters up to ~20cm high and ~3cm in diameter, broadest just under the oral disc, and somewhat tapering towards the lower end (Carlgren, 1945; Manuel, 1981).

Distribution: In the Kattegat, *P. multiplicatus* is known only from the deep eastern part south to the Sound (Carlgren, 1950; Jägerskiöld, 1971).

Habitat: Mud or muddy sand, generally at depths greater than 10m (Manuel, 1981).

Biology: *P. multiplicatus* is free-living, digging a vertical, up to 1m deep mucous-lined, thick tube, into which it can withdraw completely on disturbance. It is carnivorous, catching smaller planktonic organisms. Details of the reproduction are poorly known. The species is hermaphroditic, presumably with a life span of several years. At spawning the male gametes are shed first, stimulating the release of the eggs (Thorson, 1950). The larva has not been formally described (Manuel, 1981), but it is suggested that it is short-lived, stays near the bottom and has limited dispersal potential (Carlgren, 1950; Molodtsova, 2004).

Figure 2.3. *Pachyserianthus multiplicatus* **in the upper right side of the photo. Image from the UWTV sledge.**

2.1.4 Bolocera tuediae

Bolocera tuediae (Johnston, 1832) (English: deeplet sea anemone. Danish: Brændende søanemone) (figure 2.4). The cylindrical, soft, smooth body is up to 20cm high, but rather variable in height, sometimes even kept so short that it is hidden by the up to ca. 200 tentacles, which are thick, up to 15cm long and easily detached. The body colour is light to darker brown-reddish, that of the tentacles often darker. Detached tentacles remain alive for several days, but are not able to regenerate into new sea anemones (Carlgren, 1945; Manuel, 1981).

Distribution: In the Kattegat, *B. tuediae* has been widely recorded from the northern and eastern parts, south to the Sound (Carlgren, 1950; Jägerskiöld 1971). It is the largest actinian species in Danish waters.

Habitat: Typically on hard substrata such as shells and small stones on soft bottoms, generally at depths greater than 15m (Manuel, 1981).

Biology: Sessile, epifaunal. Although a hard-bottom species, it is also widely found on the soft seabed. Here, the planula larva has settled upon a smaller, hard-faced substratum (e.g., a shell), which is subsequently overgrown. It is carnivorous, with rather powerful cnidocytes. The species is oviparous (Carlgren, 1945), but further reproductive details are not known.
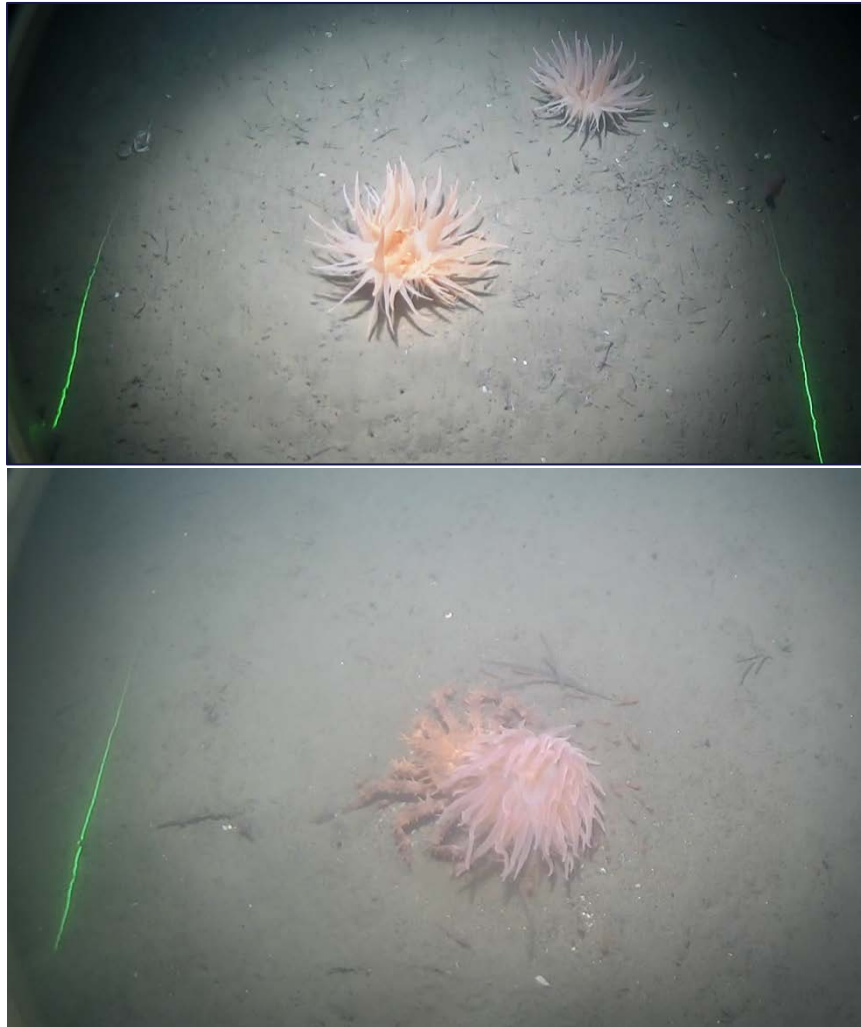
Figure 2.4. Top image: Two individuals of *Bolocera tuediae*, from the soft seabed in the northern Kattegat. Bottom image: a Norway king crab, *Lithodes maja* (Linnaeus, 1758), is using *B. tuediae* for protection.

2.2 Sponges (Porifera)

Deep-sea sponge aggregations do not occur in the Kattegat within the Danish EEZ. There are, however, other sponges (Porifera) in the area, which may be impacted by bottom trawling.

Suberites ficus (Johnston, 1842) (English: fig sponge; Danish: Figensvamp). The species name should be considered tentative, as it is used for sponges of different morphologies and growth forms distributed over a large part of the North Atlantic and beyond. Also there is a wealth of literature expressing different opinions on names, generic placements and species delimitations (see for example Van Soest, 2000).

Distribution: Found all over the Kattegat into the Sound, the Belts and the Western Baltic (Arndt, 1935; Alander, 1942).

Habitat: Sandy to muddy seabed at depths from a few metres to more than 100m. The sponges occurring in the Kattegat are massive, smooth, grey in colour and with a maximum dimension of ~5cm. It occurs in two growth forms. One is massively encrusting gastropod shells inhabited by a pagurid, often growing larger than the shells and forming at the same time a larger space for the pagurid. It occurs in both shallow and rather deep waters, but generally between 20 and 50m depth. The other growth form is stalked, more or less fig-shaped growing on the end of a common tusk shell, *Antalis entalis* (Linnaeus, 1758); it is generally found somewhat deeper, at 40-100m depth (Arndt, 1935; Alander, 1942).

Biology: Although in reality a hard bottom species, *S. ficus* is common on a variety of soft seabed types because of its use of mollusc shells as a substratum. The reproduction and dispersal means are poorly known. It is probably oviparous, with a ciliated larva swimming free for only a short time. It also has the potential for asexual reproduction through a kind of budding.

2.3 Northern horse mussel

Modiolus modiolus (Linnaeus, 1758) (English: northern horse mussel. Danish: Hestemusling) belongs to the bivalve family, Mytilidae, but differs in most biological respects from its smaller, fast-growing, intertidal, relative, the blue mussel, *Mytilus edulis* Linnaeus, 1758. *Modiolus modiolus* is a large, slow-growing, subtidal mussel, the individuals of which may reach a shell length of 23cm and an age of 45 years or more (see review by Dinesen and Morton, 2014).

Distribution: Horse mussels occur from the infralittoral down to ~200m depth in northern boreal waters of the Atlantic Ocean and adjacent seas. Horse mussel beds in deeper, higher salinity waters are known to harbour a highly diverse associated infauna and epifauna. In Danish waters this habitat has been recorded from the North Sea and Skagerrak, Kattegat, the Sound (Øresund) and Great Belt, and the western Baltic Sea. Horse mussels sometimes occur in shallow inshore waters, such as the Limfjord, but here patches are relatively small and of low diversity compared to the off-shore beds in deeper waters (i.e. at depth greater than 20m).

Habitat: The species is habitat-forming (known as the *Modiolus* habitat or community), and may cover 1-10s of km² of the seabed. It is highly patchy in distribution, with some of the known local populations seeming to have diminished in the past decades. It is not known whether this is due to increasing seawater temperatures, oxygen depletion, direct physical disturbance or smothering by suspended sediments from fishing and shipping activities (e.g. bottom trawling, catamaran ferries), or cumulative effects thereof.

Biology: The species is dioecious and reaches maturity at between 4-6 years of age. The larvae are planktotrophic for 1-2 months and thus have the potential to spread quite far distances. Competent larvae settle preferentially in response to chemical cues from adult individuals which would favour recruitment in existing populations (Dinesen and Morton, 2014). The species is highly sensitive to fishery activities, primarily from dredging for co-occurring scallops (Cook *et al.*, 2013). Various efforts to re-establish horse mussel beds in areas where they have become depleted, such as in the Irish Sea, have so far proven unsuccessful (OSPAR Commission, 2008). This has been linked to the pattern of recruitment where the planktotrophic larva settles from the water column in response to pheromone cues from established adults residing on the seabed (Dinesen and Morton, 2014).

2.4 Ocean Quahog

Arctica islandica (English: ocean quahog. Danish: Molboøster) (figure 2.5) belongs to the bivalve order Venerida. The sturdy shells of this species may reach a length of 12.5cm. It is considered to be one of few solitary marine species that can reach an individual age exceeding 400 years. The species can change to anaerobic respiration and thus withstand longer periods (of 20 days or more) of oxygen depletion. It is considered a common prey of Atlantic cod (see details in Morton, 2011).

Distribution: The species occurs in the Skagerrak, Kattegat and in the northern part of the Sound and Belt seas, at depths greater than ~15m.

Habitat: The species lives in soft seabed habitats.

Biology: The species is considered sensitive to bottom trawling (OSPAR Commission, 2008). In this study, the species was retrieved from the sediment by the Van Veen grab (figure 2.6.).

Figure 2.5. Top: *Artica islandica*, image from the UWTV sledge recordings. Bottom: The sieves used to fractionate the benthic macrofauna from the Van Veen grab. Several *Arctica islandica* are seen in the sieve to the left (4mm mesh size). In the sieve to the right (1mm mesh size) are seen a few larger polychaetes and numerous small individuals of different invertebrates groups.

Figure 2.6. The Van Veen grab (seabed area: 0.1m^2) deployed in this project to sample sediment and macrobenthos.

2.5 Tube-building Haploops

Haploops tubicola Liljeborg, 1856 (Danish: Almindelig haploops) and *Haploops tenuis* Kanneworff, 1966 (Danish: Fin haploops) (figure 2.7). The amphipod, *H. tubicola* is up to 10mm long, transparent, with a violet intestine and red eyes. *Haploops tenuis* is slightly smaller, up to 8mm long, pale brown, and the tube entrance is set in the top of the straight cut tube. These two species are known to occur together in Danish waters (in Øresund, see Kanneworff, 1966). They are tube-building and can occur in great numbers in patches of different extent. There are several other species of the genus which form similar *Haploops* communities in other geographical areas. The *Haploops* communities have recently been provisionally added to the OSPAR list of threatened and/or declining species and habitats, although information about their biology and the effects of population-induced changes upon them are unknown (ICES, 2018).

Distribution: The two species in Danish waters are considered northern boreal in their distribution. A century ago, high numbers of *Haploops* tubes formed dense mats covering 1-10s of km^2 of seabed, creating a *Haploops* community in some parts of the Kattegat (Stephensen, 1928) as well as in the Skagerrak and North Sea. It also occurred in the northern parts of the Belts and the Sound (Stephensen, 1928) at densities of between 1500-4000 individuals m^{-2} (Petersen, 1913). Since the 1930s, the *Haploops* community has either diminished in extent, or disappeared from certain areas, although today's distribution in Danish waters is unknown.

Habitat: Muddy seabed, from 20m and deeper (Stephensen, 1928).

Biology: *Haploops tubicola* builds a smooth tube of mucus and fine mud particles. The tube wall is ~1mm thick, up to 6cm long and ~1cm wide. Most of the tube is buried vertically in the seabed, with about 1cm projecting into the water column. The amphipod is positioned in the opening of the tube and catches detritus particles and small planktonic creatures with its extended, branched, antennae. On disturbance, it withdraws into the lower part of the tube. It is a short-lived species, with a longevity of between 24-36 months. The sexes are separate. The

fertilized eggs develop directly in the brood pouch of the female. Each female may brood up to 40-50 eggs in a lifetime. The combination of a short life span, sessile dwelling, tube-building and a low dispersal capacity of the offspring is likely to account for the low recoverability of this species in areas from which it has disappeared. Existing *Haploops* communities, however, may expand rapidly in areas where they already occur, such as was seen by the Lusitanian species, *Haploops nirae*, in the Bay of Biscay (Rigolet *at al.*, 2012). Recent studies of this species, however, estimated the *Haploops* community productivity to be P = ~13gDW m^{-2} y^{-1} and, thus, a third of the productivity of a neighbouring *Amphiura* community (P= ~31gDW m^{-2} y^{-1}) (Rigolet *at al.*, 2012). In a study of the biodiversity, however, 33% of the associated fauna was found to be unique to this *Haploops* community (i.e. 33% of the species were not found in neighbouring communities) (Rigolet *et al.*, 2014). If this is similar to other *Haploops* species, therefore, the presence of a *Haploops* community may reduce the overall productivity of the area it occurs in while, at the same time, increasing the biodiversity of a wider geographic area (Rigolet *et al.*, 2014).

Figure 2.7. Seabed with dense aggregations of the tube-building *Haploops* spp. from a '*de facto*' closed area in the Kattegat. Image from a UWTV sledge transect (start position: 57° 03.997N, 11° 32.395E).

3. Mapping of areas closed to fishery with bottom towed gears

3.1 Areas closed to fishery with bottom contacting gears

The Sound (Øresund) between Denmark and Sweden was first closed to fisheries with bottom towed gears as a bilateral Royal Convention nearly 100 years ago to (Ministry of Foreign Affairs of Denmark, 1932; 1933; 1990). Dredging for seabed raw materials is, however, still occurring in some parts of the Sound.

North of the Sound, bottom trawling is prohibited within 3nm of the Danish coast and 4nm of the Swedish coast. Only the central part, known as 'Kilen' is, thus, open to bottom trawling. Since 2009, the Kilen area has been open to trawling from between 1 April – 31 December. This seasonal closure is part of more recent fisheries regulations implemented to protect local cod stocks. This also includes two areas in the south-eastern Kattegat that are seasonally and permanently closed to bottom trawling, respectively (Ministry of Foreign Affairs of Denmark, 2009; 2010).

The geographic boundaries of the Natura 2000 areas and the suggested Marine Strategy Framework Directive (MSFD) areas in the Danish part of the Kattegat were collated into a GIS database (figure 3.1). Restrictions on bottom trawling have been implemented in the Natura 2000 areas, with designated protection of boulder reefs and reefs related to leaking gases. In these, bottom trawling is prohibited closer than 240m from the structures (http://mscfiskere.fiskeriforening.dk/beskyttedelukkede-omraader/). Other restrictions on fisheries with bottom contacting gears in certain areas have been proposed in relation to the MSFD. Six MSFD areas have been identified in the Kattegat, but have not yet been implemented.

Further, as part of the MSC certification of the *Nephrops norvegica* fishery in the Kattegat-Skagerrak area, selected positions were closed to bottom trawling as part of a voluntary agreement between DFPO and WWF (http://mscfiskere.fiskeriforening.dk/beskyttedelukkede-omraader/). Among others, this includes selected sites with the known occurrences of sensitive habitats listed under the OSPAR Convention (OSPAR Commission, 2008; 2010).

Danish & Swedish SAR 2013-2016

Value

High : 88,0186

Low : 0

◼ Proposed MSFD areas
◼ Natura2000 areas
— EEZ
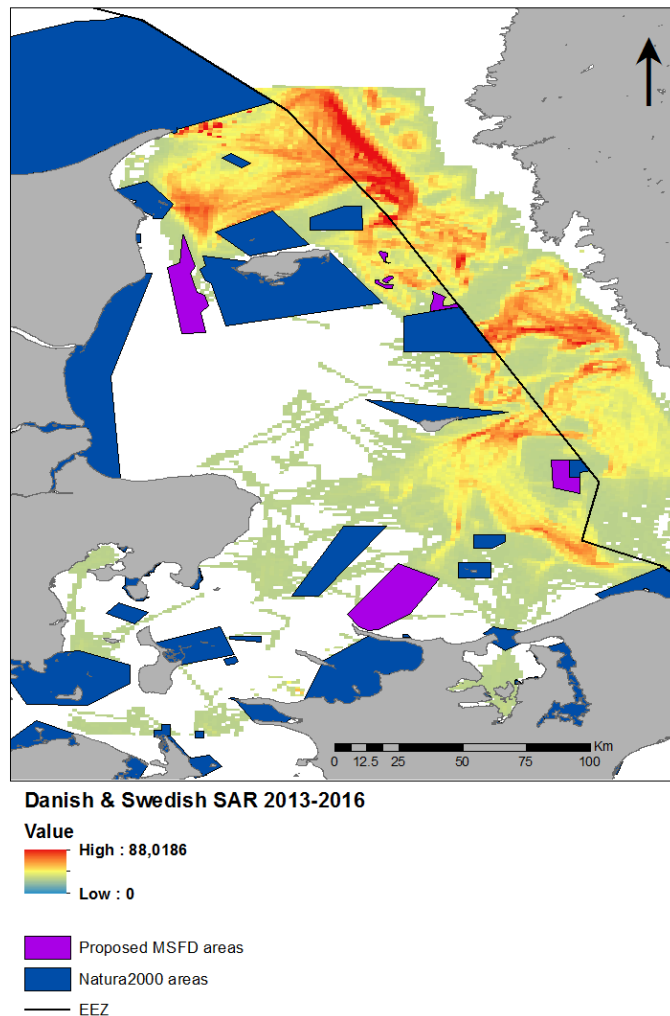
Figure 3.1. Map of the Kattegat area, with the Danish Natura 2000 sites (blue) and proposed MSFD (Marine Strategy Framework Directive) sites (purple) superimposed on the footprint of fisheries with bottom contacting gears modelled as SAR (Swept Area Ratio) based on VMS (Vessel Monitoring System) and logbook information cumulative for the period 2013-2016 (grid cells with low SAR values are green, medium SAR are yellow and high SAR are read). The black line shows the EEZ (Exclusive Economic Zone) of Denmark (to the west) and Sweden (to the east).

3.2 Acoustic and visual mapping of fishers appointed de facto closed areas

3.2.1 Fishers map of 'de facto' closed areas in the Northern Kattegat

Based on information from interviews with local *Nephrops norvegicus* fishers from the island of Læsø conducted by DTU Aqua in June 2016 potential areas of 'de facto' closed areas in the Vinga trench east of Læsø were identified. Furthermore, the Læsø-based fisher, Willy Christensen, provided a consensus map of changes in the Kattegat fishery, as experienced by local fishers in the area (figure 3.2).

Figure 3.2. Consensus map of '*de facto'* closed areas (marked with red) in the Kattegat as perceived by local fishers (drawn by the fisher, Willy Christensen, June 2016).

3.2.2 Field survey with a local fisher

DFPO identified a fisher (Captain Thomas Christensen) from Østerby Havn, Læsø, to collaborate in the project. DTU Aqua subsequently conducted a 3-day field survey in June 2016 with the captain and crew on board the commercial vessel, *FN226 Andrea Klitbo*. The side scan sonar (SSS) and an underwater video sledge were employed at five selected sites to test for seabed sediment structures, *N. norvegicus* burrows and fishing gear footprints (e.g. marks from otter trawl doors, ground gears). It is however, uncertain for how long individual trawl tracks remain visible on the sea floor, especially in the deep aphotic soft sediment habitat. *Nephrops norvegicus* burrows in soft sediment were only detected at two sites. These two localities were included as stations in the research survey conducted in September – October 2016.

An Edgetech 4125 SAR 600/1600 kHz side scan sonar connected to an AirMar PB150 Weather Station for positioning was used for mapping the seabed in the five selected areas in the northern Kattegat. We used the 600 kHz frequency on the side scan sonar, towed it at a depth of approximately 10 to 15m above the seafloor and at a speed of around 3 knots, covering an area of between 100 and 150 m of the seabed on each side. As a rule of thumb, we aimed for the towing depth above the seafloor to be ~1/10 of the area covered.

Mosaics of the side scan recordings were created after the end of the survey, using the post processing software SonarWiz 5 from Chesapeake Technology Inc. Here, the transects were mosaicked, layback corrected and exported to GeoTiff files and KMZ files for visualization in

ArcGis and Google Earth (figure 3.3) with examples of the images recorded by the side scan sonar (figure 3.4-3.6) and the video drop camera (figure 3.7).

Figure 3.3. An overview of the first test area and the four survey areas in the Vinga trench system stretching from north to south east off Læsø island.
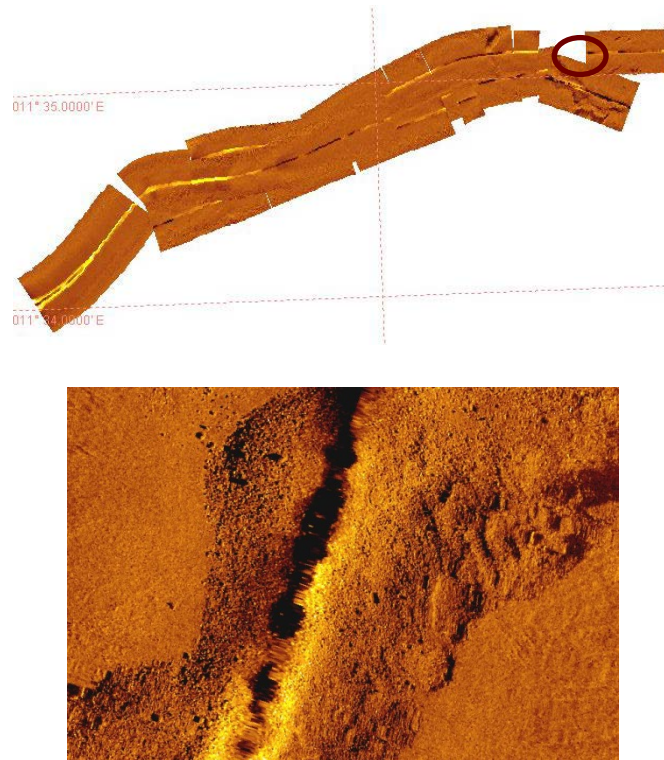
Figure 3.4. Top, an overview of survey area #2. Bottom, a close-up of an area with a steep slope, piles of gravel and a few larger hard structures (boulders and similar).
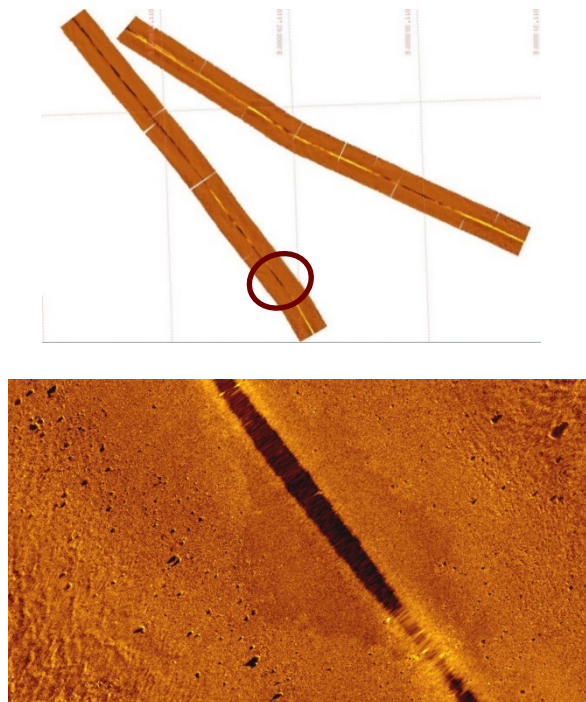
Figure 3.5. Top, overview of survey area #3. Bottom, a close-up of a flat area from survey area #3. It seems a softer sediment (darker brown) just below the sonar (around the black line) with a harder sediment surrounding it with spread out tall hard structures, possibly of leaking gas structures.
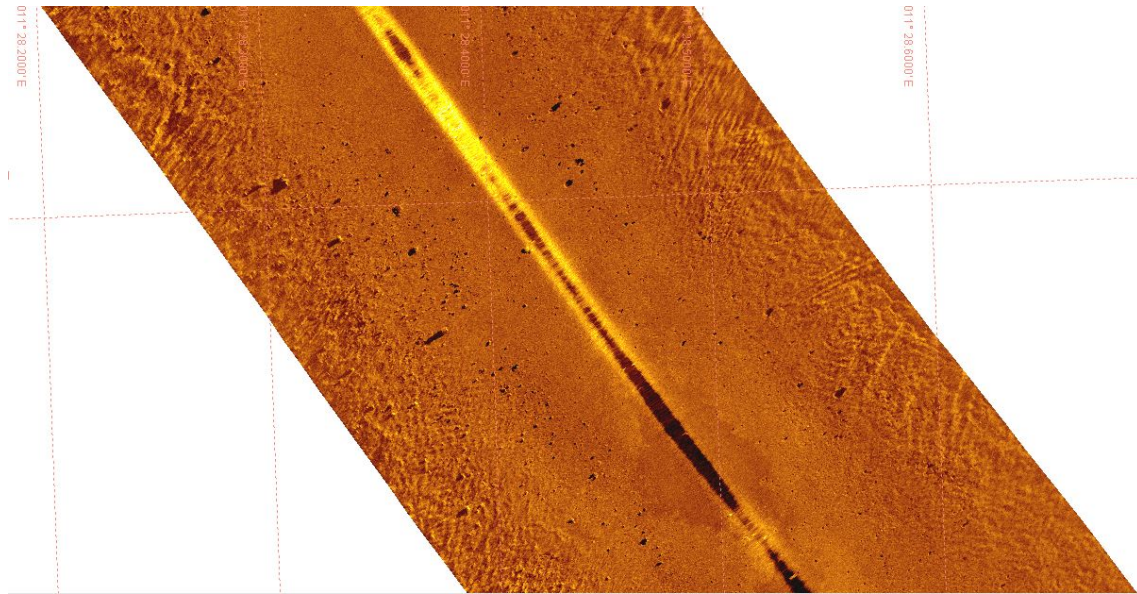
Figure 3.6. A close up of potential leaking gas structures from survey area #3.

Figure 3.7. Image from the video drop camera used to verify seabed structures, primarily burrows of _Nephrops norvegicus_.

3.2.3 SODENA data

SODENA data still in use are owned by the individual fishers and the data are confidential and thus not available as open access information (figure 3.8). In this project, we instead tested if the SODENA data can be used to geographically identify un-trawled polygons that can then be used to validate and/or adjust new VMS and AIS based models of fishing intensity. This 'translation' appeared promising at a relatively high resolution, and such a data 'translation' will be continued based on availability with the aim to cover the Kattegat area for use in future assessments of trawling impacts on marine benthic habitats and species.
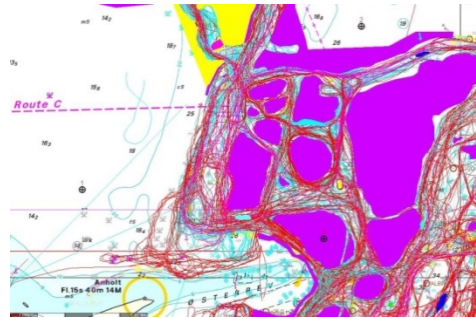
Figure 3.8. Example of high resolution SODENA information of trawl tracks in the Kattegat from anonymous commercial, bottom trawling vessels.

3.2.4 Research survey with R/V *Havfisken*

The 14-day research survey with the *R/V Havfisken* was conducted in September-October 2016 by DTU Aqua with deployment of four sampling gears: i), Baited Remote Underwater stereo Video cameras (BRUV); ii), the Kattegat Survey (KASU) fisheries monitoring trawl (TV3); iii), the Underwater TeleVision (UWTV) sledge developed and used in the monitoring survey for stock assessment of *N. norvegicus* in the Kattegat and Skagerrak and iv), a sediment sampler of a size that is suitable for the sampling of small and larger macrofauna (Van Veen grab, seabed surface samples: 0.1m^2). The geographic position of the individual sampling sites and the VMS based fishing intensity (swept-area ratio, SAR modelled as the cumulative intensity over the 36 months prior to sampling) at each site is shown in figure 3.9.
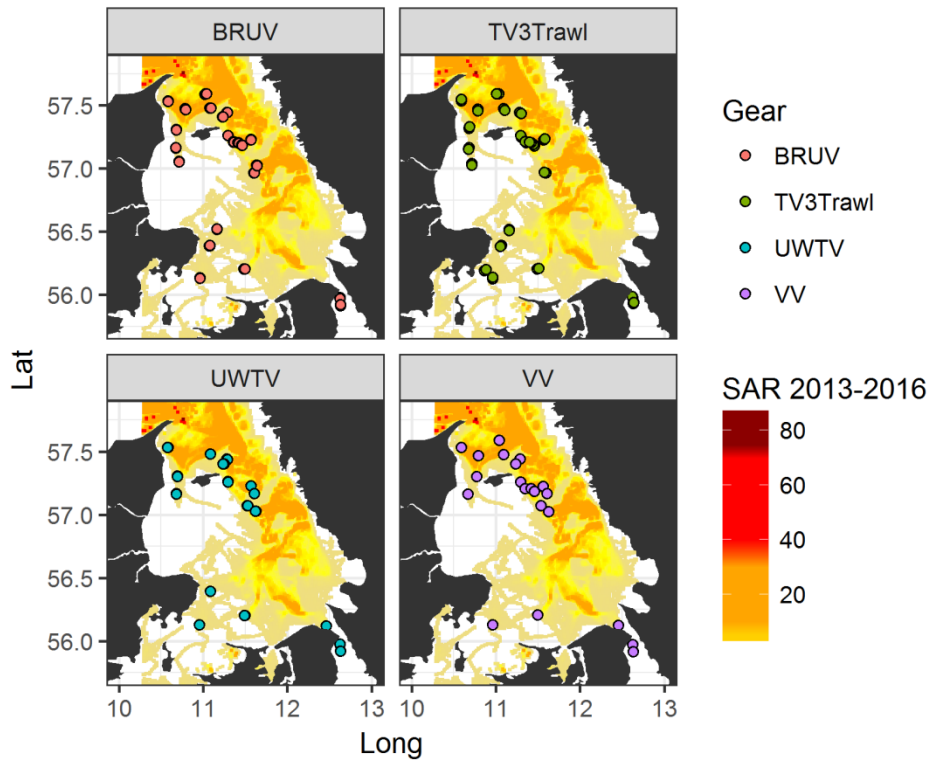
Figure 3.9. Sampling sites investigated from the *R/V Havfisken* during September-October 2016 by DTU Aqua with deployment of four sampling gears and the VMS based fishing intensity (swept-area ratio, SAR, modelled as the cumulative intensity over the 36 months prior to sampling) (see text for details).

4. Quantification of bottom trawling intensity

4.1 Data and modelling methods of fishing pressure (fishing intensity)

Since 2006, all fishing vessels ≥15m operating in European Union waters have been required to carry a VMS receiver on board, and since 2012 vessels ≥12m have also been included in this requirement. In the Kattegat, vessels of length exceeding 15m constitute the great majority of bottom trawlers (Danish AgriFish Agency, 2016). VMS receivers collect and send data regarding vessel location, heading, and speed, with a polling frequency of once every hour (in Danish and Swedish waters). For the analyses conducted in this project, raw VMS data from all Danish and Swedish vessels above 15m for the period 2005 to 2016 were cleaned and filtered to retain only trawling activity (speed ranging between 2 and 4 knots, with a minimum distance of 3km from port). These raw data points were then used to reconstruct vessel trawl tracks using cubic Hermite spline interpolation (Hintzen *et al.*, 2010). The trawl tracks were coupled with information on vessel size and gear type (from EU logbooks) and with modelled gear dimensions to estimate gear-width (Eigaard *et al.*, 2016). The tracks were then aggregated to calculate the area of seabed 'swept' during each logbook trip. Modelling of trawling effort and estimation of swept area was based on the approach in Eigaard *et al.* (2017) using the VMStools package (Hintzen *et al.*, 2012). These individual trip-based swept area estimates were then further aggregated using two approaches, and serving three purposes:

i), To assess the historic fishing pressure in the Kattegat and identify a suite of soft-bottom sampling stations representing approximately the full gradient of fishing pressure exerted during the period from 2005-2016, annual maps of gridded Swept Area Ratios (SARs) were produced. The gridded annual SARs were defined as the ratio of the area swept (cumulative over 12 months) to the area of the grid cell, which was set at a size of 1 × 1 minutes of longitude and latitude (~1.9km^2 at 56° north). At this spatial scale, bottom trawling tends to be randomly distributed within years but also tends to be uniformly spread on longer timescales (Amoroso *et al.*, 2018; Ellis *et al.*, 2014). This mechanism does, however, to some extent, depend on the specific fishery and underlying bathymetry (Eigaard *et al.*, 2017). Because vessels between 12 and 15 meters of length were only included in the later years of the time-series (from 2012 and onwards), and because these smaller vessels contribute marginally to the total bottom trawling pressure in the Kattegat, only the vessel group of ≥15m length was included.

ii), For each of the 21 fishing pressure gradient stations identified from the gridded SAR maps, point-based estimates of circular SARs were calculated at a three year scale back in time from the point of the actual benthic sampling (during September - October 2016). The circular SARs were defined as the ratio of the area swept (summed over 3 years [the 36 months] prior to sampling) to the size of the circular area surrounding each benthic station within a 1km radius. The locations of the 21 stations were overlain with a gridded SAR map of fishing pressure from September 2013 to September 2016 (figure 4.1A) and with a map of EUNIS level3 habitat types (figure 4.1B) to illustrate the methodological approach taken for the gradient analysis.

iii), For a time series analysis of soft bottom trawling impacts in the Kattegat (based on an existing benthic fauna data set from 22 fixed stations sampled annually in April or May in the years 2005–2008, 2010, 2011 and 2013 as part of the national NOVANA monitoring programme (figure 4.2, from Gislason *et al.*, 2017), point-based estimates of circular SARs with a radius of 2km were calculated at an annual scale. To take into account spring-recruitment of benthos the

SARs were cumulated over the period from May in the preceding year to April in the year where the bottom samples had been collected.
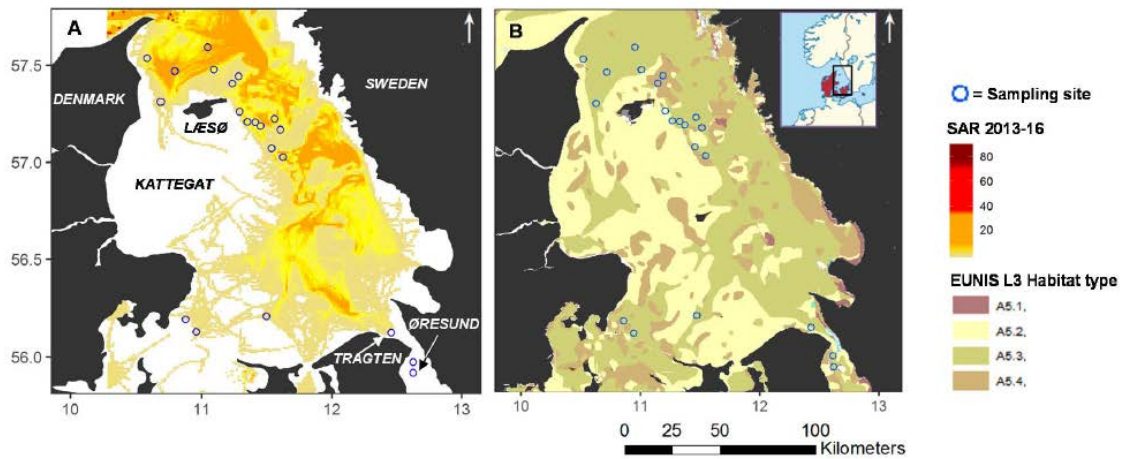
Figure 4.1. Map of sampling sites in respect to (A) total gridded Kattegat fishing pressure from September 2013 to September 2016 (B) EUNIS level 3 habitat types. Swept Area Ratio (SAR) values are average values for all Danish and Swedish bottom contacting gears between September 2013 and September 2016 for vessels of at least 15 meters length. Habitat types included A5.1 = sublittoral coarse sediment, A5.2 = sublittoral sand, A5.3 = sublittoral mud, A5.4 = sublittoral mixed sediment. The locations of sampling sites found within areas closed to trawling (Tragten and Øresund, or the Sound) are highlighted in part (A).
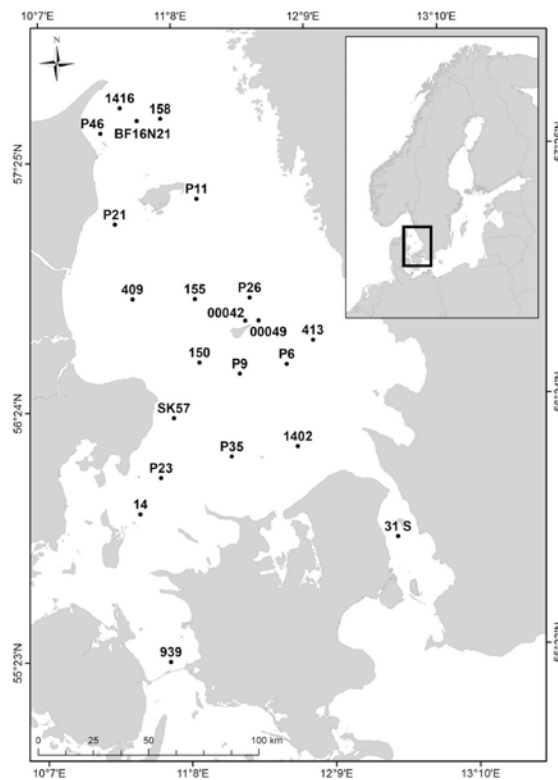
Figure 4.2. Map of NOVANA sampling stations (from Gislason *et al.*, 2017).

4.2 Application in the project

In preparation of the design of the research cruise with *R/V Havfisken* in September- October 2016, we applied the above methodology to calculate the SAR for the period covering the last 10 years (2005-2015) to identify the areas of long-term trawling grounds in the Kattegat survey area. Subsequently, we modelled the SAR for the period October 2013 to August 2016 for the Kattegat survey area. Stations were selected across a gradient of fishing pressure (SAR) and included true zero stations (i.e. '*de facto*' closed areas), see results in Chapters 5 and 6.

In the first investigation of fisheries effects on benthic macrofauna in the Kattegat applying high resolution VMS and logbook information, we used a radius of 2000m and one year (12 months) of fishing prior to the sampling of benthic fauna. The benthic macrofauna data were retrieved from the national monitoring programme NOVANA (data available in the ODA database at http://dce.au.dk/overvaagning/databaser/oda/) for the period from 2005-2013. These data comprise benthic macro fauna density (of individuals and biomass) in 5(10) replicate HAPS corers (surface area of $0.0143m^2$) collected annually in either late April or early May. The overall results showed that the number of individuals of macrofauna correlated negatively with fishing pressure. The biodiversity indicator, DKI (the Danish Quality Index), proved inadequate for assessment of fisheries effects of benthic macrofauna. Detailed results and conclusions are presented and discussed in Gislason *et al.* (2017).

In the investigation of fisheries effects on benthic megafauna and fish, fishing pressure was estimated for the gear types deployed, including the TV3 trawl (standard trawl used in the Danish fisheries monitoring programme in the Kattegat, KASU), the BRUV (3 baited stereo video cameras) and the UWTV (Under Water TeleVision) sledge. Different radii were used for different gear types, but all were based on the cumulative SAR for the three years prior to sampling (see results in Chapter 5). In the investigation of fisheries effects on benthic macrofauna sampled by the Van Veen grab sampler (surface area of $0.1m^2$), we also tested fishing pressure patterns of different radii and numbers of cumulative years of fishing (see results in Chapter 6).

5. Sampling and analysing species composition

One of the aims of the project was to estimate fish and macrofaunal diversity and density using different sampling devices in order to test their efficiency and suitability for future ecosystem based monitoring of environmental variation and anthropogenic pressures such as bottom trawling.

To asses this, during the *R/V Havfisken* research survey a number of different gears were used: i), A 0.1 m² Van Veen grab was used for sampling of macrofaunal; ii), a baited stereo video-camera (Baited Remote Underwater Video, BRUV) was used to attract and identify mobile scavenging fish and epifauna; iii), a towed sledge on which a video camera was mounted (Under Water TeleVision, UWTV) was used to identify large attached and mobile epifauna and iv), a TV3 trawl was used to sample fish and megafauna. Each of the gears differed with regard to the efficiency by which they recorded different species and functional groups, and it was therefore considered an advantage to compare and combine the observations.

5.1 Sampling devices and procedures for fish and megabenthos

Information on fish and megabenthos was sampled remotely using baited stereo cameras (BRUV) and an underwater video sledge (UWTV), while live material was sampled using a standard survey trawl (TV3).

5.1.1 BRUV - Baited Remote Underwater Video

A stereo camera rig (BRUV) consists of a SeaGIS stainless steel stereo camera frame supplied with two camera housings, two GoPro cameras, two 100 W lamps, and a pole with a bait bag (figure 5.1). Three 5kg weights were attached to the bottom of the frame to ensure its correct positioning on the seabed. The two cameras recorded the species approaching the bait from different angles allowing the combined images to be used to estimate the distance to each individual and, thus, its size.

To ensure accurate estimates of the size of the fish in front of the cameras each BRUV was calibrated before the survey using a standard SeaGis calibration cube. Three stereo BRUVs were applied at each station to provide an estimate of within-station variance. At each station the three stereo BRUVs were placed in a line on the seabed with ~200m between them and left for one hour before retrieval. Before deployment the bait bag was filled with ~0.5kg of herring cut into ~4cm slices. The herring were thawed overnight before being placed in the bag. After retrieval, the cameras were removed from their housings and the video recordings were transferred to portable hard drives, and the batteries of the cameras and the lamps were recharged. The video-recordings were subsequently analysed at DTU Aqua using the software supplied by SeaGis (EventMeasure). For each species, the maximum number of individuals visible in the left camera during the deployment period was recorded and used as a measure of the relative densities of the species.

Figure 5.1. Top: SeaGis Stereo BRUV turned 120 degrees counter clockwise on the afterdeck of *R/V Havfisken* before the research survey. Note the two white camera housings, the two black lamps between the camera houses, and the weights attached to the steel frame. A bait bag made of white netting (in the upper left hand corner of the photograph), and filled with pieces of herring, is mounted on the pole in front of the cameras. During the survey the dark grey plastic pole was removed and the bait bag was fastened to the tip of the stainless rod extending from the frame. This was done to allow visual identification of fish in the turbid waters encountered at some of the deeper stations. The BRUV was left recording on the seabed for one hour after which it was retrieved by means of the blue rope to which a red buoy was attached (seen in the lower right half of the photo). Note that the bait pole and the BRUV both are marked with a single stripe of blue tape, indicating that this is BRUV and pole no. 1. Bottom: The BRUV and pole no. 2 was marked with two stripes.

5.1.1.1 Results

The BRUV was easy to deploy and retrieve, but the unloading of the camera's memory cards and the recharging of the batteries on-board *R/V Havfisken* were cumbersome and time consuming.

A total of 13 species of macro benthos and 14 species of fish were recorded by the left camera at the 22 stations where the BRUV was deployed. The species composition changed considerably with depth and/or salinity, with whiting and hagfish being at the deeper high salinity stations while the shallower lower salinity stations were characterized by dab, greater weaver, swimming crabs, hermit crabs and whelk (figures 5.2 and 5.3).
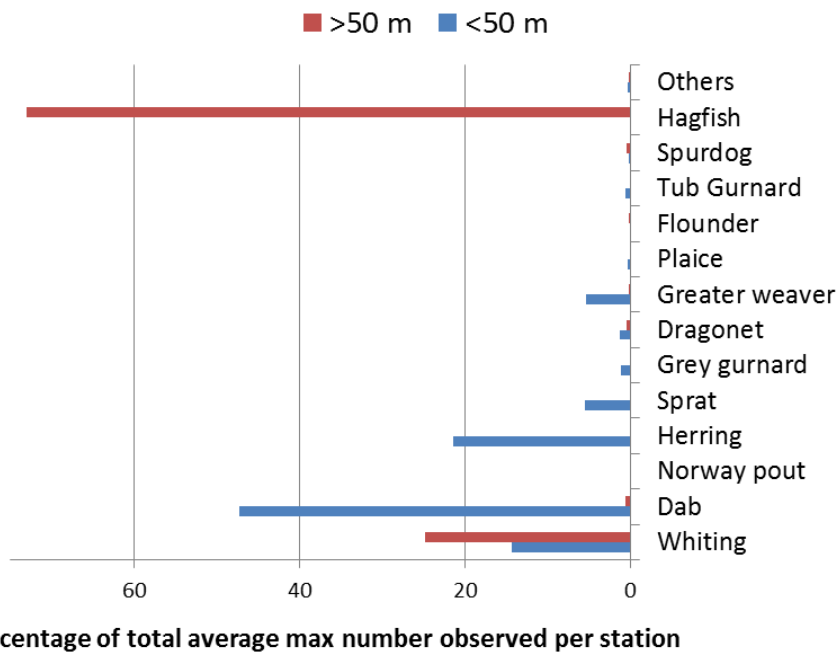
Figure 5.2. Percentage species composition (relative maximal number of individuals) of each fish species observed in the BRUV video recordings, at a depth above (blue) and below (red) 50m.
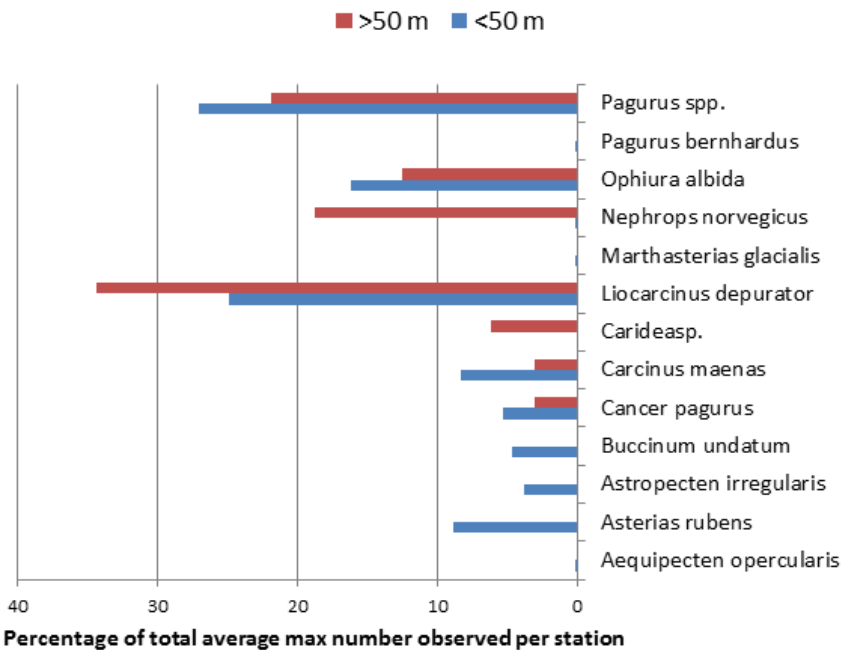
Figure 5.3. Percentage species composition (relative maximal number of individuals) of each invertebrate species observed in the BRUV video recordings, at a depth above (blue) and below (red) 50m.

A multivariate analysis (DistLM routine in Primer-e Permanova+ v.7, see details in the materials and methods in Chapter 6) showed significant effects of depth (p<0.001), which explained the largest proportion of the variation explained by the model of fish and megafauna species composition in the BRUVs (dbRDA1: 25.1% of total variation) (figure 5.4). Fishing intensity (VMS based swept-area ratio, SAR, accumulated for the 36 months prior to sampling) explained a smaller but significant (p<0.001) proportion of the variation explained by the model (dbRDA2: 3.8% of total variation).

Figure 5.4. Plot of distance based redundancy analysis (dbRDA) displaying fitted values of models of the taxonomic composition of fish and megabenthos recorded in the BRUVs. Vectors of significant predictors are overlaid onto each plot. Sites are categorised according to trawling intensity group 0-3.

5.1.2 UWTV

The UWTV is regularly deployed in the *Nephrops* monitoring programme where it is used to identify and count their burrow-complexes on the seabed. It consists of a fixed video camera mounted on a towed sledge supplied with artificial light and lasers. A cable connects the video camera to a monitor on-board the vessel. On the seabed, the video covers an 80cm wide track demarcated by two clearly visible laser beams. Compared to the grab and BRUV samples, the sledge covers a large area per unit time. Larger mobile species that do not flee and sedentary ones either on or protruding from the seabed can be observed and counted on the video. Species identification may, however, be difficult particularly in turbid waters and rough weather where movements of the ship sometimes course the sledge to lift from the seabed. During the survey the UWTV sledge was towed at ~1 knot for 10 minutes at each station. Video sequences were later analysed in the laboratory and where the visible individuals were, as far as possible, identified to species level and enumerated. Only few data were available from the deeper stations due to windy weather and rough seas.

5.1.2.1 Results

Only six species of fish were observed in the UWTV video. These were hagfish, sole, dab, tub gurnard, European eel, and greater weaver. In addition, several flatfish that could not be identified to either species or family were present (figure 5.5). All species occurred in low numbers. Thirty-two invertebrate species or species groups were identified from the 17 stations where the UWTV was deployed. From a few of the stations, the slender sea pen, *Vigularia mirabilis*, and the brittle stars, *Ophiura* spp., were identified in larger numbers (figure 5.6).

Percentage of total number of individuals
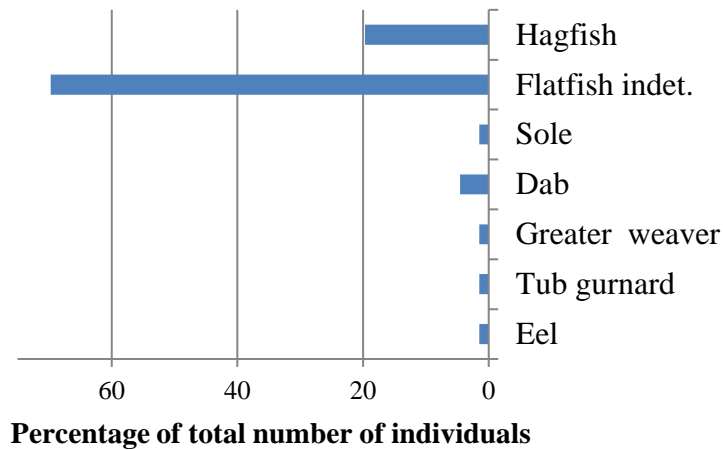
Figure 5.5. Percentage fish species composition (relative number of individuals) identified in the UWTV recordings.

A multivariate analysis showed significant effects of depth (p<0.01) which explained the largest proportion of the variation explained by the model of fish and megafauna species composition in the UWTV transects (dbRDA1: 14.3% of total variation) (figure 5.7). Fishing intensity (VMS based swept-area ratio, SAR, accumulated for the 36 months prior to sampling) explained a smaller and non-significant (p<0.07) proportion of the variation explained by the model (dbRDA2: 5.1% of total variation). Inclusion of more sampling stations is expected to resolve the proportion of fish and megafauna community composition explained by the UWTV data in relation to fishing intensity. Furthermore, increasing the number of sampling stations and replicates across all levels from un-trawled and low to high fishing intensities will provide information for assessment of sensitivity and recoverability of the habitat forming epifaunal species, such as sea pens, *Haploops* and horse mussel beds.

Figure 5.6. Percentage of the invertebrate species (relative number of individuals) recorded from the UWTV recordings.
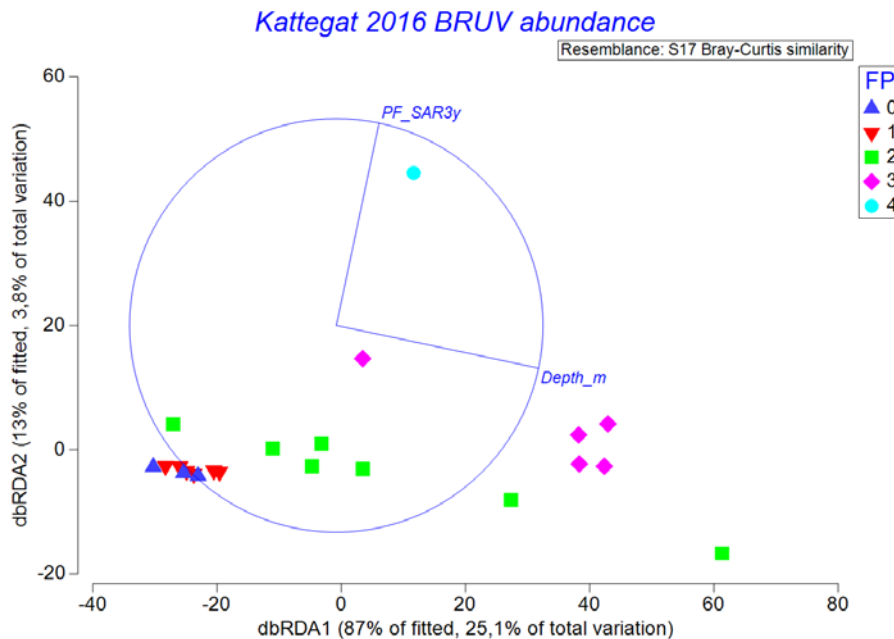

Figure 5.7. Plot of distance based redundancy analysis (dbRDA) displaying fitted values of models of the taxonomic composition of fish and megabenthos recorded in the UWTV transects. Vectors of significant predictors are overlaid on each plot. Sites are categorised according to trawling intensity group 0-3.

5.1.3 TV3

The TV3 trawl is the standard trawl used by DTU Aqua on the fish monitoring surveys in the Kattegat and Skagerrak. Trawling speed on *R/V Havfisken* is 3 knots and the wingspread of the TV3 is approximately 15m, depending on the length of the sweeps. One haul of 30 minutes duration was made on each of the 21 stations that were trawled. The haul was placed ~200m away from and in parallel with the line BRUVs if these were used on the station. On board *R/V Havfisken* the catch was sorted into species and weighed, and for each species the number of individuals (or a subset thereof) was recorded. Fish and invertebrates were treated separately. Due to time constraints parts of the invertebrate catch were frozen and analysed in the laboratory at DTU Aqua.

5.1.3.1 Results

Whiting and dab where the most frequently caught species at the <50m shallow sampling stations. At the deeper stations >50m, a total of 29 fish species were recorded with whiting and Norway pout dominating the catch (figure 5.8). A total of 36 fish species was recorded from the hauls made in the shallower (<50m) stations of the Kattegat. Note, however, that species richness most likely depends on the number of individuals, and thus stations, sampled, and only five hauls were made at depths >50m. The benthic bycatch was dominated by swimming crabs at both depths. Large numbers of the gastropod *Philine adspersa* and the sponge *Suberites ficus* were caught on a few stations in shallow waters (figure 5.9).
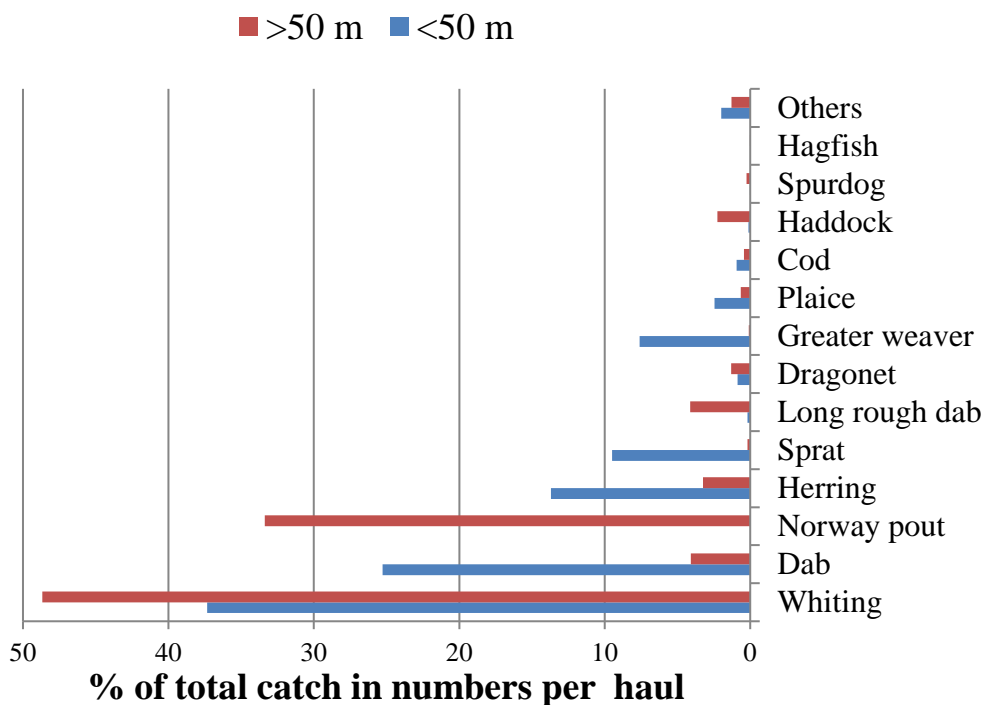
Figure 5.8. Percentage catch composition of the fish caught by the TV3 trawl above and below depths of 50m.
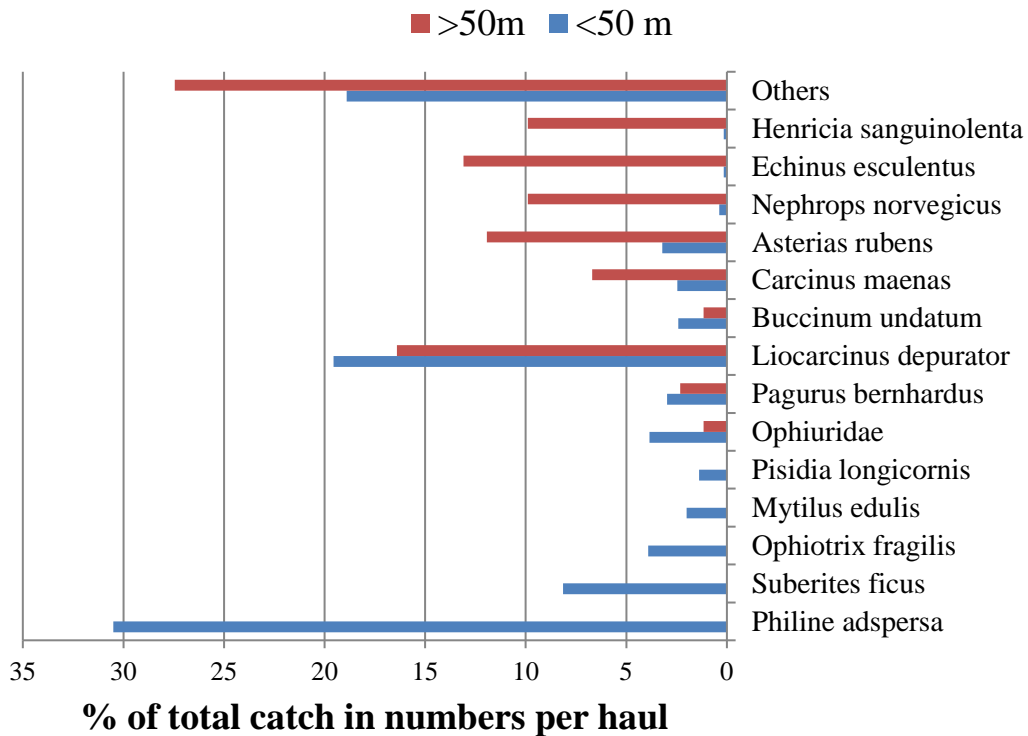
Figure 5.9. Percentage species composition of the benthos caught by the TV3 trawl above and below depths of 50m.

A multivariate analysis showed significant effects of depth (p<0.001) which explained the largest proportion of the variation explained by the model of fish and megafauna species composition in the UWTV transects (dbRDA1: 24.7% of total variation) (figure 5.10). Fishing intensity (VMS based swept-area ratio, SAR, accumulated for the 36 months prior to sampling) explained a smaller but significant (p<0.007) proportion of the variation explained by the model (dbRDA2: 3.8% of total variation). Inclusion of more sampling stations is expected to resolve the proportion of fish and megafauna community composition explained by the UWTV data in relation to fishing intensity. Furthermore, increasing the number of sampling stations and replicates across all levels from un-trawled and low to high fishing intensities will provide information for assessment of sensitivity and recoverability of the habitat forming epifaunal species, such as sea pens, *Haploops* and horse mussel.
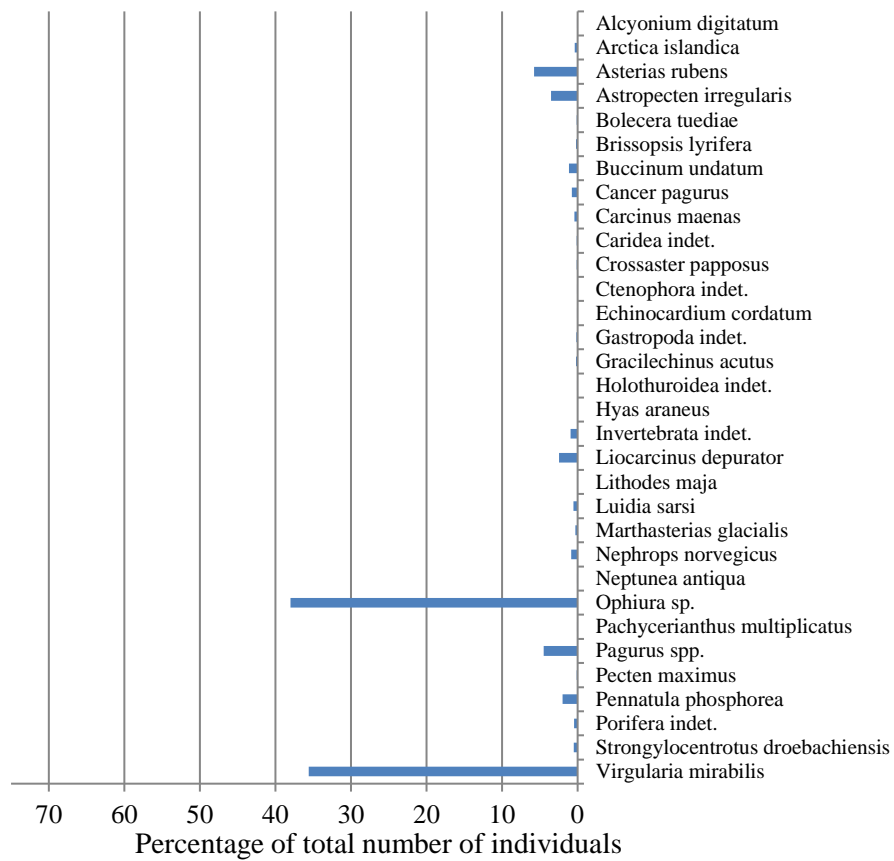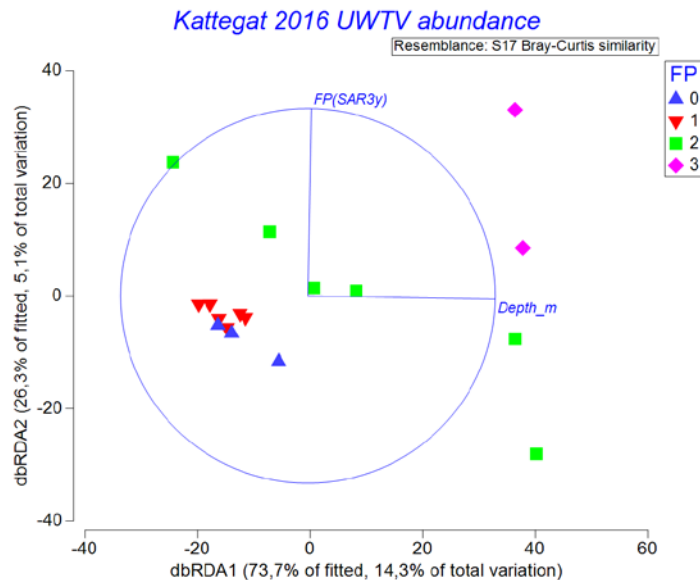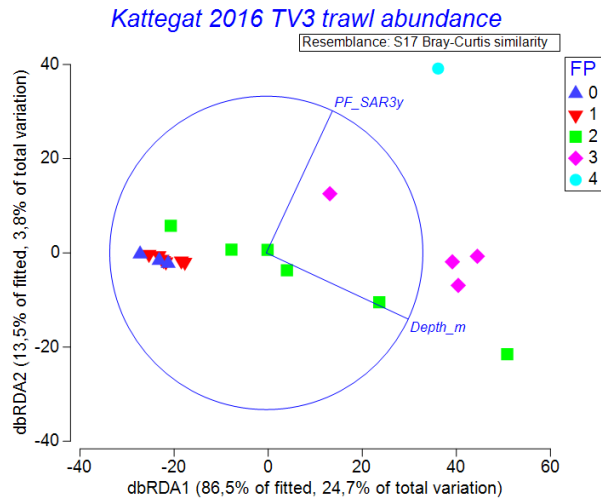
Figure 5.10. Plot of distance based redundancy analysis (dbRDA) displaying fitted values of models of the taxonomic composition of fish and megabenthos recorded from the TV3 trawl hauls. Vectors of significant predictors are overlaid onto each plot. Sites are categorised according to trawling intensity group 0-4.

5.2 Relative efficiency of each sampling method

5.2.1 BRUV

The species attracted to the BRUV bait bag differed from those caught in the TV3 trawl at the same stations. First of all only one hagfish was observed in the trawl catch at one of the deeper stations below 50m, while a total of at least 329 hagfish (based on the maximum numbers recorded from each station) were observed on the BRUV videos from the same stations. In general, however, the BRUV observed ~100 times fewer individuals than the trawl (figure 5.11). Only 14 out of the 45 species of fish and 13 out of the 54 invertebrate species recorded in the deeper trawl hauls, respectively, were identified on the BRUV video suggesting it only reflects a certain proportion of the overall fauna.

A simple regression was used to compare the number of individuals caught by the BRUV and in the TV3 trawl. Although a positive relationship could be identified between the number of whiting and dab observed in the BRUV and in the TV3 catch, there was no direct linear relationship between the two measures of relative density (figures 5.12, 5.13 and 5.14).

BRUV/TV3-trawl
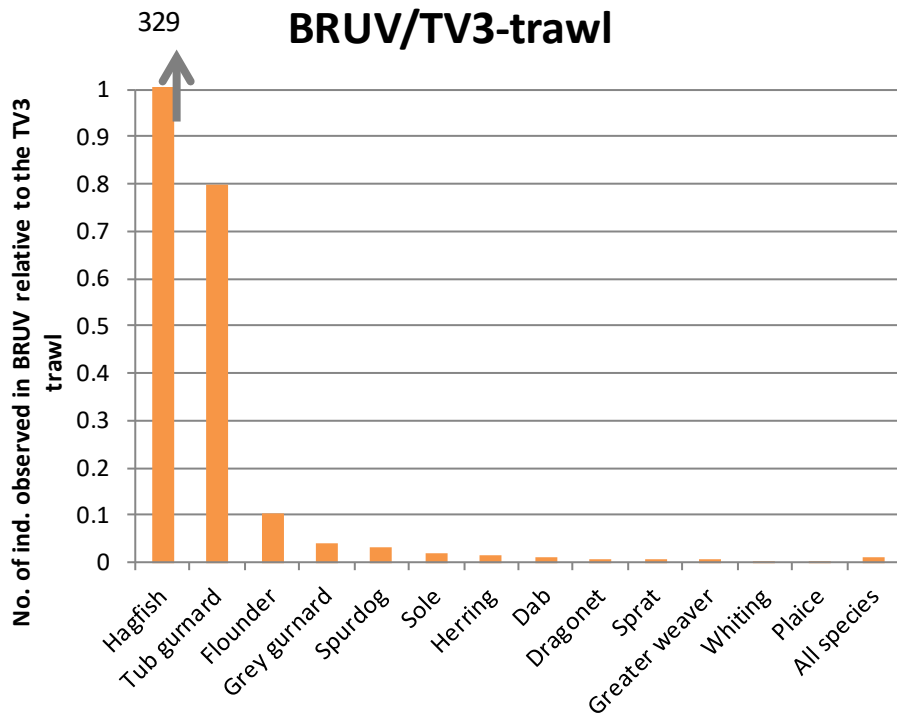
Figure 5.11. Relative total numbers of individuals (maximum number) observed in the three BRUVs compared with the total TV3 catch. Note that 329 hagfish were observed in the BRUV, but only one was caught in the TV3. Note also that the total numbers of individual fish caught in the TV3 trawl is ~100 times larger than the maximum numbers of individuals observed in the three BRUVs at the stations where both gears had been deployed.

Whiting

$y = 0.0018x + 6.5544$
$R^2 = 0.15$

Dab

$y = 0.0253x - 8.9579$
$R^2 = 0.66$
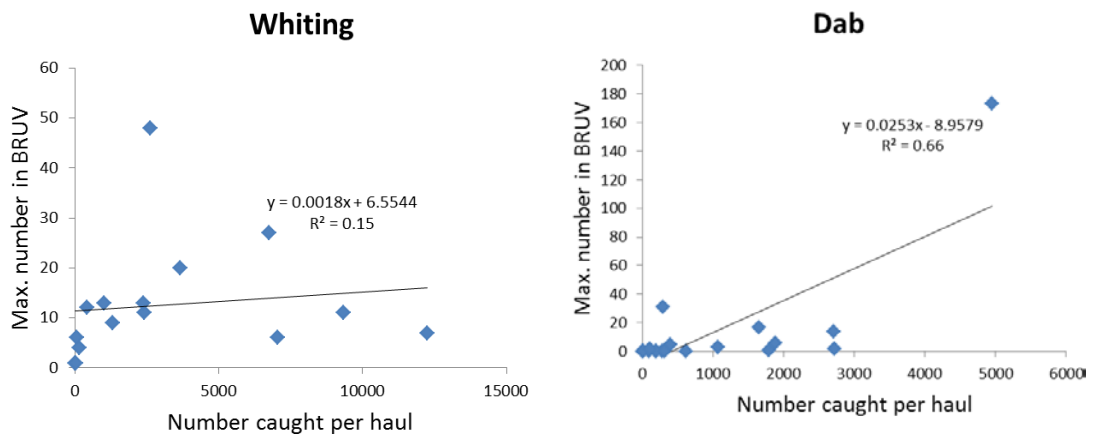
Figure 5.12. Total number of whiting and dab observed in the three BRUVs (maximum number) at each station, compared with the TV3 catch per haul of the two species at the same station.

All fish species

$y = 0.0047x + 8.8421$
$R^2 = 0.4001$

All invertebrate species

$y = 0.0715x + 33.716$
$R^2 = 0.3431$
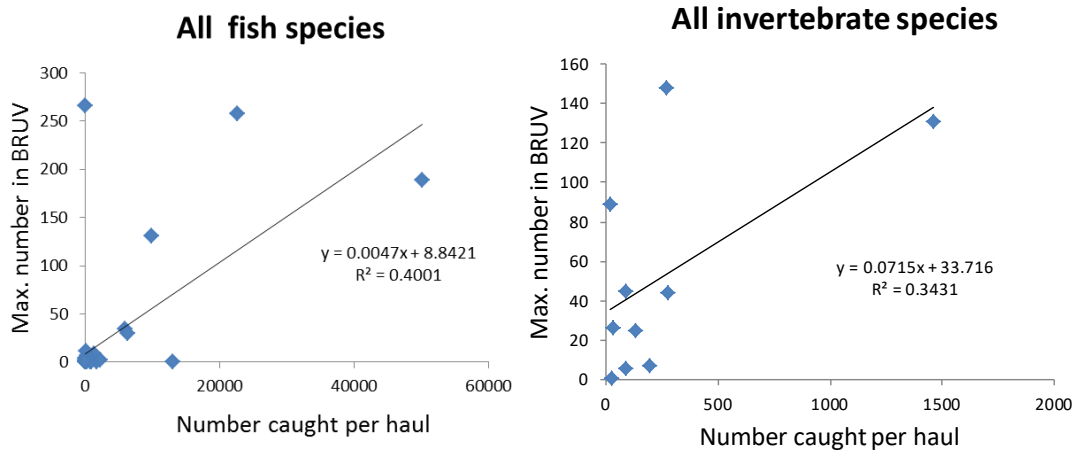
Figure 5.13. Total numbers of individuals observed in the three BRUVs (maximum number) at each station, compared with the TV3 catch per haul of these species at the same station.

Asterias rubens

$y = 0.9658x + 6.8248$
$R^2 = 0.1054$

Pagurus sp.

$y = 1.1163x + 5.8776$
$R^2 = 0.7224$

Astropecten irregularis

$y = 2.0611x + 5.577$
$R^2 = 0.7377$

Ophiura sp.

$y = 0.0526x + 0.456$
$R^2 = 0.7938$
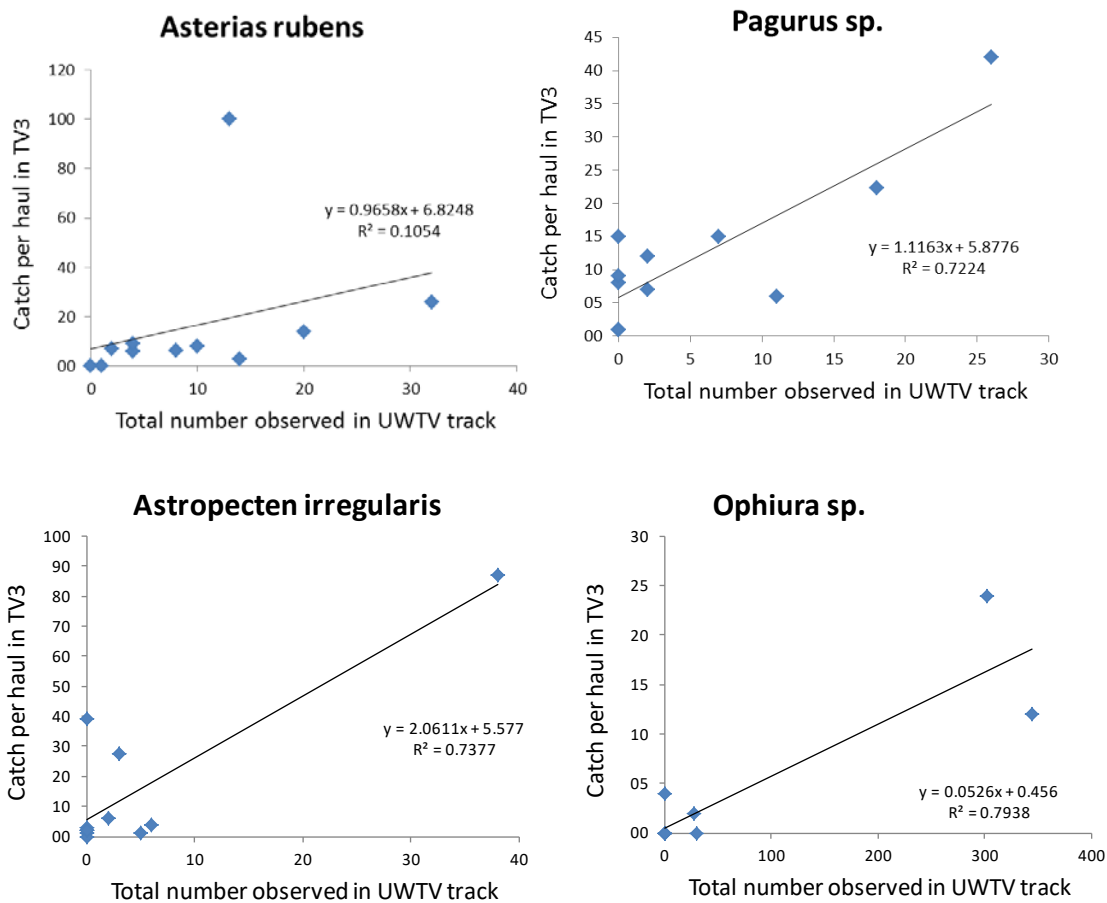
Figure 5.14. Total numbers of individuals of *A. irregularis* and *Ophiura* spp. observed in the UWTV track at each station, compared with the numbers recorded in theTV3 catch per haul at the same station.

5.2.2 UWTV

The efficiency of the UWTV can be assessed by comparing its catch to that of the TV3 trawl at the 11 stations where both devices were used.

On average 171 benthic invertebrate and 6 fish individuals were recorded from the UWTV videos per station, while the TV3 on average caught 212 benthic invertebrate and 3487 fish individuals per haul. While the TV3 caught 37 fish and 38 benthic invertebrate species at the 11 stations, the UWTV recorded 7 fish and 28 benthic invertebrate species (figure 5.15).

Plotting the number of the four most abundant species in the UWTV and TV3 samples against each other reveals some correspondence between high density stations in both gears. This was most pronounced for hermit crabs, *Pagurus* spp., where a simple linear regression explains 72% of the variation. For the starfish, *A. irregularis* and the brittle stars *Ophiura* spp. the proportion of variation explained was also high, but driven by one or two high points.

5.3 Discussion

The four different sampling devices employed in this project differed widely in how efficiently they caught and recorded the different species and functional groups.

The BRUV is a non-invasive gear where scavengers who find fish bait attractive will be recorded on video. The BRUV, however, only provides semi-quantitative density estimates, as it may be difficult to obtain relative density estimates without information about the area from which animals may become aware of and attracted to the smell of the bait under different current speeds and turbulence conditions. Without knowing how the behaviour of the animals is affected by other stimuli, such as the artificial light employed, the time of day, their feeding status, the presence of potential predators etc., it is difficult to know how well the BRUV reflects the relative density of each of the species. Clearly, some species do not seem to be attracted to the bait. However, for typical scavengers, such as hagfish that otherwise appears to escape or burrow, the BRUV is able to verify their presence, and may provide a potential way of evaluating changes in densities. Only one hagfish was collected in the entire trawl catch and 13 individuals were seen swimming over the bottom in the UWTV transects. However, at the deeper stations the BRUV recorded a total of 329 individuals. We had not expected to see that many hagfish feeding on the bait. Hagfish are likely to be attracted to direct casualties and discards from the *Nephrops* fishery and may have experienced population increases in fished areas where these are readily available. Little is known about the ecology and potential interactions with other species on the deeper muddy grounds of the Kattegat.
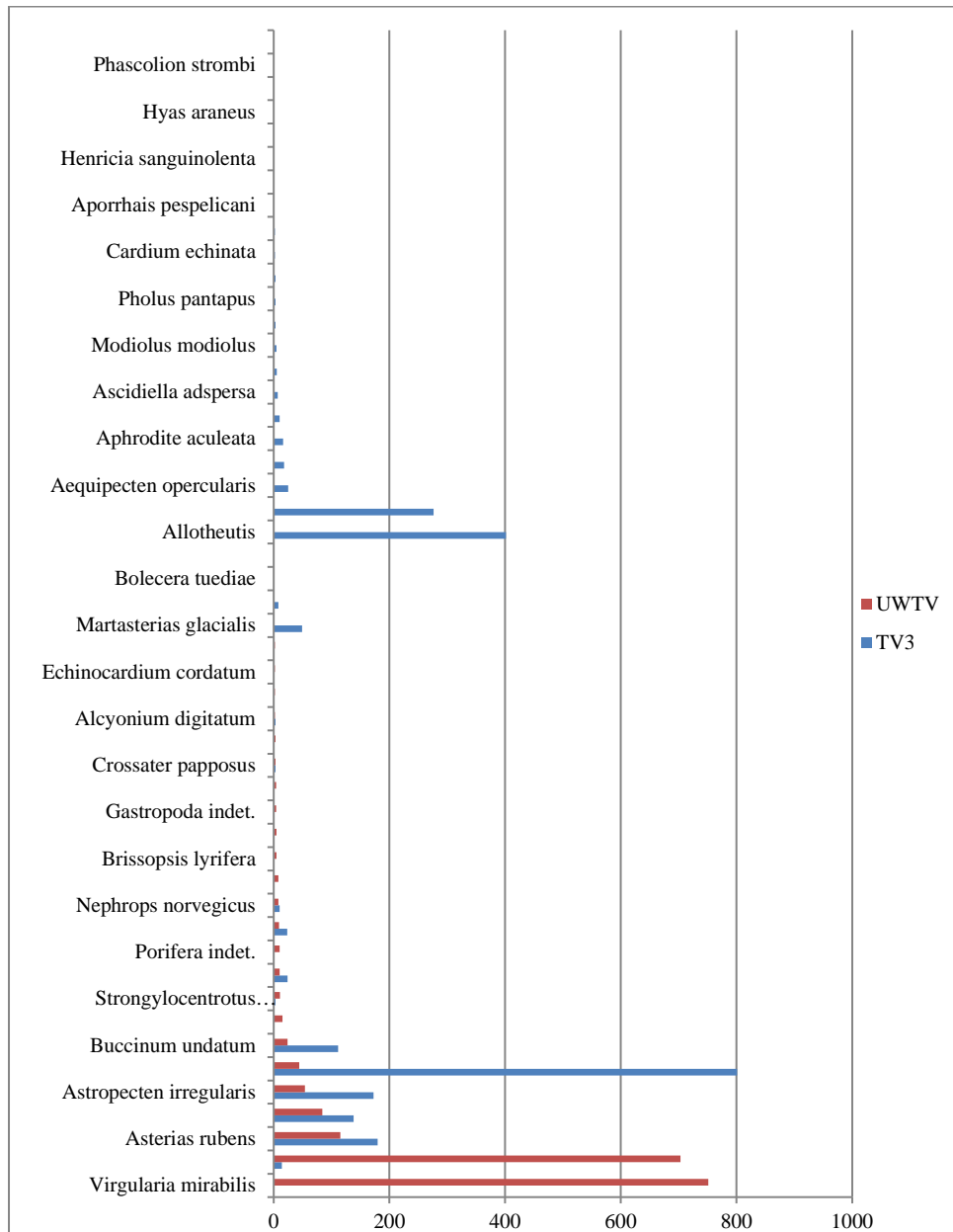
Figure 5.15. Comparison of the total catch of the UWTV (no. per 10 minutes transect) and the TV3 trawl (no. per 30 minutes haul) at the 11 stations were both gears were operated.

There were more than two orders of magnitude in difference between the number of fish observed by the three BRUVs and those caught in the TV3 trawl. The TV3 trawl is a traditional fish trawl with a bycatch of benthos. The sweeps and bridles of the trawl herd fish into the mouth of the trawl where they try to keep their position relative to the gear until they get exhausted and fall back to either pass through the meshes or be retained in the cod-end. The relative efficiency of the trawl depends on the vertical position of the individuals in the water column and the seabed, the size and swimming speed of the individual and the escape behaviour of the species including its reaction to the cloud of suspended sediment generated by the trawl doors that may provide additional herding of some species. How selective the trawl is

with respect to megafauna (mostly epifauna) is unknown, but it seems likely that swimming crabs and other large mobile benthic animals may have a higher probability of passing over the ground-rope than smaller and flexible attached epibenthos such as the slender sea pen and the phosphorescent sea pen, and various tube building animals (including both the amphipods, *Haploops* spp., and several larger polychaetes), the kinds of animals that are generally believed to be most sensitive to bottom trawling.

The UWTV is able to provide recordings of all kinds of discernible megafauna on the seabed and supplement the trawl fish recordings. Species identification may be difficult in case of smaller species and/or individuals, and may generate differences in efficiency across species, and there is no tissue to provide genetic material to aid the identification. Like the BRUV, the UWTV is, however, a non-invasive survey device, and it appears to be considerably more efficient than the TV3 trawl when it comes to registering individuals attached to the seabed, such as larger sea anemones (*Pachycerianthus multiplicatus*, *Bolecera tuidae*), sea pens (*Pennatula phosphorea*, *Virgularia mirabilis*) and tube building amphipods (*Haploops* spp.) and polychaetes (*Sabella pavonina* Savigny, 1822), but much less efficient when it comes to fish, where most of the observed individuals were flatfish that tend to remain motionless until an approaching threat becomes imminent. Larger, mobile invertebrate predators and scavengers, such as gastropods, decapods and starfish, were regularly recorded from both the BRUV and UWTV videos, and in the TV3 trawl hauls.

Table 5.1. Employment and sampling characteristics of the four different gears used in the project.

Gear	Duration of employment (minutes)	Approximate area covered (m^2 per deployment)	No of fish registered per deployment	No of invertebrates registered per deployment	Animals rarely recorded by any of the other gears	Time spend on working up samples (hours, approx.)	Area worked up per unit of time (m^{2}h^{-1})
4 x 0.1m^2 Van Veen	30	0.4	0	1145	Small infauna	30	0.013
3 BRUV	60	4	48	75	Highly mobile scavengers	10	0.4
UWTV	10	250	5.5	269	Large erect attached megabenthos	6	41
TV3 trawl	30	40000	5452	156	Less abundant fish species	8	5000

Infauna can only be recorded quantitatively by the use of grab and corer sediment samplers. But samples are time consuming to analyse in the laboratory and each sample only covers a small area of the seabed. A bottom grab may therefore be likely to miss some of the larger epifaunal animals (megabenthos), such as the reef-forming *Modiolus modiolus*, tube-building crustaceans, *Haploops* spp., the sea pens, *Pennatula phosphorea* and *Virgularia mirabilis,* and larger sea anemones, such as *Bolecera tuidae*, and starfish *Luidia sarsi*, some of which have been reported to be moderately to highly sensitive to bottom trawling. The UWTV dredge is able to cover large attached epifaunal species (megabenthos) not recorded regularly in any of the other sampling devices. It is furthermore able to provide visual information about bottom topography and trawl marks, and provide much information about the animals on the surface of the sediment. The BRUV is able to attract scavenging species, such as the burrowing hagfish

(*Myxine glutinosa*) not otherwise caught regularly by any of the other gears. It also attracts a number of mobile scavenging invertebrates and may thus be used to monitor the effects of fishery discards on the local seabed fauna. Finally, the TV3 trawl provided much information about fish faunal diversity as well as semi-quantitative information about some of the larger invertebrates such as *Modiolus modiolus*, hermit crabs, *Pagurus* spp., the common whelk *Buccinum undatum*, and different crab species, such as *Liocarcinus depurator*, *Carcinus maenas* and *Cancer pagurus*. However, although small individuals of sea pens and sea anemones were sometimes observed in the grab samples, they were rarely recorded from the TV3 trawl.

Overall, we conclude that adequate monitoring of seabed communities and their reaction to bottom trawling will require a multitude of sampling devices to fully cover the potential changes in the local benthic invertebrate, scavenger and fish communities, and that synoptic sampling is particularly important for investigating indirect effects among the different species and functional groups. Moreover, we conclude that at least four different gears can provide supplementary information about the effects of bottom trawling on the biodiversity of fish and invertebrates in the Kattegat and that each of these gears have their advantages and drawbacks (table 5.1).

6. Suitability of benthic macrofaunal indicators in fishery impact assessments

6.1 Introduction

Benthic macrofaunal indicators are commonly used in environmental assessments to describe the ecological state and detect changes in seabed ecosystems. Typically, variations in species richness and relative abundances of either individuals or groups of species are used as indicators. Densities of individuals and species are often relatively easy to measure and calculate and, thus, allow complex ecological information to be presented in a simple manner. However, for species to be useful as indicators, they need to be both widely occurring and constrained by environmental conditions and anthropogenic disturbances such that they are sensitive to changes therein (Zettler *et al.,* 2013). Species, however, can be inadequate as indicators if their abundances and geographical extent are low and are, thereby, unable to convey reliable information regarding environmental and anthropogenic driven changes (Reiss *et al.*, 2009). Furthermore, single species are often less suitable to accurately describe changes in the macrofaunal assemblages of specific seabed habitats and in the wider ecosystem.

Assessing the state and changes in density of individuals, species richness and composition of wider macrofaunal assemblages is a widely applied alternative to single species indicators. Density, species richness and composition refer to the taxonomic relationships and the spatial pattern of occurrence of organisms that comprise either an assemblage or a community (Rice *et al.*, 2012). A more recent assessment approach further includes biological traits or phenotypic characteristics (i.e. morphological, physiological and behavioural features) of an organism, which can be used to predict the vulnerability of communities to disturbance (Mouillot *et al.*, 2013). When combined, such information can be used as ecological indicators to more effectively detect and link changes in ecological conditions that occur as a result of environmental and anthropogenic pressures (e.g. Pearson and Rosenberg, 1978) and, thus, form the basis for impacts assessments on seabed habitats.

Ecosystem based assessments of seabed habitat status are required to guide management advice for human activities under the European Union's Marine Strategy Framework Directive (MSFD, Descriptor "Biodiversity "and Descriptor 6 "Seafloor Integrity"), the Habitat Directive (HD), and the Water Framework Directive (WFD). Ecological indicators are often applied to assess human impacts on species, habitat and environmental state as they are easily calculated, monitored, and interpreted. Indicators allow for the characteristics of the system to be translated into easily interpretable values, such as total biomass. In order to be effective, however, the indicators should be sensitive to changes in ecological state and capture and convey information on the ecosystem and not be overly affected by short-term variations or auxiliary drivers. Measuring all aspects of ecosystem state is neither practical nor possible, and well-designed macrofaunal indicators of anthropogenic disturbance, such as bottom trawling, are therefore required to support management (Rice *et al.*, 2012; Van Hoey *et al.*, 2010).

Large benthos are comparatively sensitive to bottom trawling, as demonstrated by a number of experimental and model based studies (Duplisea *et al.*, 2002; Jennings *et al.*, 2001; McConnaughey *et al.*, 2005; Queirós *et al.*, 2006; Reiss *et al.*, 2009). The sensitivity of seabed

habitats to physical disturbance by bottom trawling can, thus, be assessed using the body-size composition of a benthic fauna (Rice *et al.*, 2012) and the overall mortality, as larger individuals will have to live long enough to reach a large size within a given habitat and area (Rice *et al.*, 2012). The sensitivity of the larger macrofauna to bottom trawling can be partly explained by the observed relationship between body size and several key life history characteristics (Begon *et al.*, 2006). As a result, considerable potential exists to develop size-based macrofaunal indicators and improve their reliability and sensitivity to detect trawling impacts on seabed habitats. The majority of research dedicated to the development of ecological indicators based on biological traits has focused on how benthic macrofaunal assemblages respond to gradients of organic enrichment and derived oxygen depletion (Borja *et al.*, 2009). This is partly to be expected given the relatively good understanding and maturity of research within this field. Despite this, trait-based methods are now being increasingly used to assess trawling impacts on seabed habitats. The concept of trawling-specific trait-based indicators was first proposed by Bremner *et al.* (2003), and subsequent to this, several studies have used trait composition to examine the effects of bottom trawling (Bolam *et al.*, 2017, 2014; Tillin *et al.*, 2006; Van Denderen *et al.*, 2015). However, it remains that few studies have examined the use of macrofaunal trait-based univariate indicators, and their potential to monitor and manage trawling impacts on seabed habitats. A number of additional factors can affect relationships between macrofaunal composition and bottom trawling. These includes the type of habitat (Kaiser *et al.*, 2006) and trawl gear used (Eigaard *et al.*, 2016; Hiddink *et al.*, 2017), the effects of anthropogenic and natural disturbance (Diesing *et al.*, 2013; Van Denderen *et al.*, 2015) and the potential adaptations of macrofaunal communities to disturbance (Kaiser *et al.*, 2000; Reiss *et al.*, 2009). These various factors can obscure the detection of measurable bottom trawling effects, and the challenge remains to identify indicators that most effectively can 'disentangle' trawling effects from other human pressures and natural processes.

The main objective of this macrofaunal study was therefore to explore the response of benthic macrofaunal communities to bottom trawling across a fishing pressure gradient and relative to the underlying environmental conditions. We do this by examining the response of: i), the entire community (species and trait composition; ii), widely used benthic indicators (taxonomic and trait-based); iii), 'sensitive species' either identified a-priori or determined from the above analyses and iv), novel trait-based indicators. In the case of parts i), ii) and iv), we further examined the responses as a function of individual body-size by splitting the community into small and large size fractions. To facilitate this, we collected 84 grab samples of the seabed from 21 locations in the Danish Exclusive Economic Zone (EEZ) (figure 6.1). Sampling locations were selected over a wide gradient of trawling intensity, ranging from 'true' zero (determined by long-standing closed areas) to regionally high trawling intensities. To undertake the comparative analysis across size fractions, we used sieves to split the benthic community at each location into three size categories: a ≥4mm fraction and 1-4mm fraction, and the pooled full community. Chapter 6 herein summarizes the results of McLaverty *et al.* (2020), (attached as Appendix A.3) and McLaverty *et al* (in prep.), (attached as Appendix A.4).

6.2 Materials and methods

6.2.1 Sampling design
The sampling design was stratified using key environmental criteria across comparable seabed habitat within the Danish Exclusive Economic Zone (EEZ). These criteria included: i), a

minimum depth of 15m; ii), mud (EUNIS A5.3) and mixed sediments (EUNIS A5.4) habitats (figure 6.1b) and iii), within a gradient of bottom trawling intensity over the period 2013-2016 (figure 6.1a). Two sampling sites were located in the Sound (i.e. Øresund) between Denmark and Sweden (figure 6.1) which has been subject to a trawl ban since 1932, and a single sampling site was located in the 'Tragten' area, in the southern Kattegat (figure 6.1) closed to trawl fisheries since 2009 (Angangtyr and Holm-Hansen, 2018). The comparability of the sampling sites were also statistically analysed (see Appendix A.3 for details).

6.2.2 Data collection

Sampling was conducted at 21 sites (figure 6.1) between 22 September and 6 October 2016. At each site, five sediment samples were collected with a $0.1m^2$ Van-Veen grab. Four samples were acquired for faunal analysis, and one for particle size analysis (PSA) and organic content estimation *via* loss-on-ignition (LOI%). To divide the samples into size categories, the samples were processed over two sieve mesh sizes; 1mm and 4mm. A 1mm mesh size is widely used as benthic macrofauna are generally defined as metazoans retained by a 1mm sieve, and a 4mm threshold is used to distinguish larger macrofauna (also known as megafauna). Samples were fixed in 4% borax-buffered formaldehyde solution in ambient seawater. In the laboratory, samples were rinsed, sorted, and all animal material identified to species level, where possible. These three size categories resulted in three datasets carried forward for analysis; the small (1-4mm) fraction, the large (≥4mm) fraction and the pooled full community fraction.

6.2.3 Biological trait classification

A set of 10 trait categories (developed from Bolam *et al.*, 2017) were used to describe the trait composition of the benthic community. For a full description of the methodology and the list of categories and trait modes, see McLaverty *et al.* (2020, and *in prep*). (Appendix A.3 and A.4 herein). The trait categories included in the analysis comprised - size range; longevity/maximum age; larval development type; morphology; egg development type; living habitat; sediment position (depth in sediment); feeding mode; mobility; bioturbation mode.

6.2.4 Estimating trawling pressure

VMS and logbook data were cleaned and filtered to retain only 'trawling activity' (defined as speed ranging between 2-4 knots, with a minimum distance of 3km from port). These raw data points were then used to reconstruct vessel trawl tracks using cubic Hermite spline interpolation (Hintzen *et al.*, 2012). The tracks were coupled with information on vessel size and gear dimensions (Eigaard *et al.*, 2016) to estimate gear-width, and aggregated to calculate the area of seabed 'swept' during each logbook trip. Trawling effort and swept areas were estimated using the VMStools package (Hintzen *et al.*, 2012). The total swept areas (for all Danish and Swedish ≥12m vessels and trips) were aggregated within a 1km radius around each benthic sampling site. Fishing pressure, in the form of bottom trawling intensity, is described as swept-area ratio (SAR) values, defined as the accumulated swept area within a circle divided by the size of the circle. SAR can therefore be interpreted as the number of times the seabed within the circle is trawled within the given time period. To account for inter-annual variation in trawling activity at infrequently fished sampling sites, and due to the focus of the analysis on larger, longer-lived, macrofauna, SAR estimates were based on three years of fishing effort. Fishing pressure was back-calculated from the day prior to sampling for each replicate sample (September-October 2013 to September-October 2016).
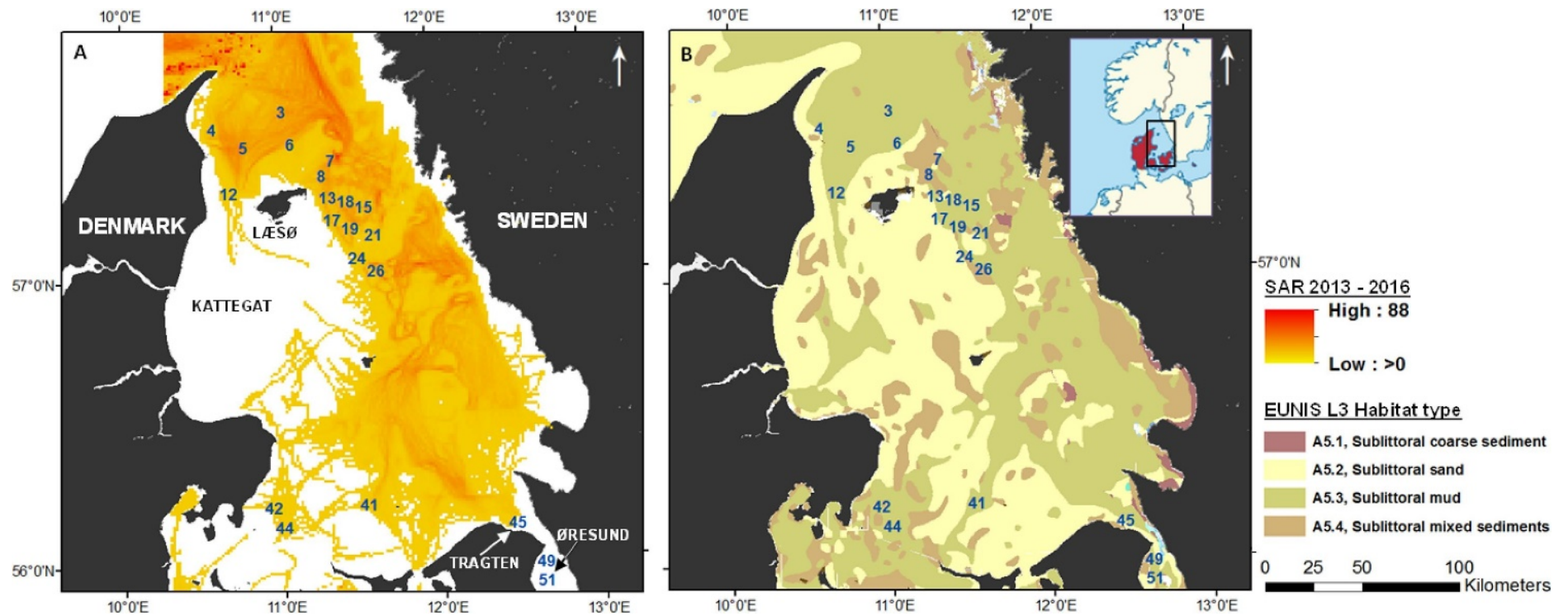
Figure 6.1. Map of the 21 Van Veen grab sampling sites with respect to (a) fishing effort and (b) EUNIS Level 3 habitat types. Swept Area Ratio (SAR) values are average values for Danish and Swedish bottom contacting gears between September 2013 and September 2016. The locations of sampling sites found within areas closed to trawling (Tragten and Øresund) are highlighted in part (a).

6.2.5 Environmental predictors
The structure and composition of benthic communities are known to be closely linked to ambient environmental conditions. To account for key environmental drivers and ascertain potentially confounding effects, we included six environmental parameters in addition to trawling intensity. These included depth (*in situ*), LOI%, and mud content (derived from sediment samples), average bottom current velocity (m/s), bottom temperature (°C), and minimum bottom salinity (PSU) (all modelled using the Baltic Sea Ice-Ocean Model (BSIOM) (Lehmann *et al.*, 2014). Detailed information on the model can be found in McLaverty *et al.* (2020) (Appendix A.3).

6.2.6 Ecological indicators
Ecological indicators were the focus of McLaverty *et al.* (2020, see Appendix A.3) and are outlined in Section 6.3.5. The indicators were based on benthic macrofaunal samples and chosen to reflect the ecological, taxonomic and functional characteristics of the community. Taxonomic indicators (density, species density, Shannon diversity and biomass) were calculated using PRIMER v.7 (Clarke and Gorley, 2015) and functional indicators (functional diversity, functional richness, functional evenness, and functional dispersion) were calculated using the R package 'FD' (Laliberte, Legendre and Shipley, 2015). Appendix A.3 provides a description of the indicators and their interpretation. The functional indicators were weighed using density data, as this is more commonly recorded in benthic impact studies (Hiddink *et al.*, 2017). The results of functional indicators based on biomass data are available in McLaverty *et al.* (2020, Supplement table S2).

6.2.7 *A-priori* selected sensitive species
Several benthic species and taxonomic groups were identified prior to the analysis as possible indicators of sensitive habitats in the Kattegat. These included sponges (Porifera), larger sea anemones (Hexacorallia), the sea pens *Pennatula phosphorea* and *Virgularia mirabilis*, the northern horse mussels *Modiolus modiolus*, ocean quahog *Arctica islandica*, and the tube-building amphipods *Haploops tubicola* and *H. tenuis*.

6.2.8 Statistical approach
The following provides a summary of statistical analyses and methods used in the study. Detailed descriptions of the statistical approach can be found in Appendix A.3 and A.4.

6.2.9 Community composition
Relationships between community (taxonomic and trait) composition, trawling, and other environmental drivers were quantified using PRIMER v.7 with PERMANOVA+ (Anderson *et al.*, 2008). Distance-based linear models (DistLM) were used to combine data relating to benthic fauna, and abiotic information, and calculate the relationship between the multivariate data cloud and each predictor variables. This approach allowed for the determination of the main drivers of community composition and the main species and traits that accounted for these relationships. Relationships between community composition and abiotic variable were calculated using distance-based redundancy analysis (dbRDA) (Anderson *et al.*, 2008). In each case, resemblance matrices of faunal data were based on Bray-Curtis similarity and matrices of environmental data were based on Euclidean distance. The final models are presented visually using dbRDA plots. Each point in the plots represents the taxonomic/trait composition at each site. The sites were grouped into categories based on trawling effort to aid visual presentation. Sites located in the closed areas were categorised as 'un-trawled', and the remaining sites were

grouped in the following categories in the plots; low (0.1 – 1.5 SAR), medium (1.5 – 5.5 SAR), high (5.5 – 9.7 SAR), and very high (9.7 – 24.1 SAR).

6.2.10 Identification of sensitive species and traits

The DistLM routine also provides the facility as an exploratory hypothesis generating tool (Anderson *et al.*, 2008) and allows for the calculation of relationships between individual species/traits. To examine the most sensitive species and traits in each dataset we examined the correlation coefficients (Pearson's R) between each species/trait and primary axis of the redundancy plot, which also correlated significantly with trawling intensity. The larger the correlation coefficient, the larger is the contribution of that trait to the dbRDA ordination. Using this method, we identified 10 species and traits that demonstrated the strongest (negative and positive) correlations with the RDA axes and which, thus, best represented trawling. Relationships were determined based on Pearson R^2 values and critical values. Analysis of sensitive species and traits is provided in Section 6.3.7. Development of trait-based indicators is provided in Section 6.3.8. (see details in Appendix A.4).

6.2.11 Analysis of benthic indicators

The response of benthic indicators to trawling and other environmental drivers were analysed using generalised linear mixed effects models (GLMMs). With the aim to examine their ability to detect bottom trawling impacts, we calculated four taxonomic-based indicators including density of individuals (N) and biomass (B), species density (S), and Shannon diversity (H') and four functional-based indicators including functional diversity ($RaoQ$), functional richness (F_{ric}), functional evenness (F_{eve}) and functional dispersion (F_{dis}). As each replicate sample represented an observation, we analysed the data using mixed effects models. This modelling approach is particularly suitable to quantify potential correlations in repeated measure designs (i.e. replicate samples nested within sites) (Bolker *et al.*, 2009). The sampling site was therefore included as a random effect term to account for non-independence of samples, while all other predictors were included as fixed effect terms. Indicators were modelled using either a negative binomial, Poisson, Tweedie, or Gaussian distribution depending on the data type (see Appendix A.3 for details).

6.3 Results

6.3.1 Distribution of trawling effort

Trawling intensity at the sampling sites ranged from 0 to 43.4 over the three year period (equivalent to ~14.5 yr^{-1}). The higher levels of trawling effort were concentrated mainly in the northern parts of the Kattegat (east of the towns of Skagen and Frederikshavn), and in the deeper areas of the Vinga trench, at site 3 (SAR 33.57), site 5 (SAR 43.4), site 17 (SAR 35.02) and site 26 (SAR 16.39) (see figure 6.1 and 6.3c for the geographic locations of sampling sites). The lowest SAR values were recorded at sites 45, 49, and 50 (all closed areas – SAR 0), site 12 (SAR 0.42), and site 44 (SAR 3.28). Trawling effort was highly habitat specific, and concentrated in areas of sublittoral mud (EUNIS A5.3) and sublittoral mixed sediments (EUNIS A5.4) (figure 6.1b). Trawling pressure estimates for each site can be found in the supplementary material of McLaverty *et al.* (2020, see Appendix A.3).

The 21 Van Veen grab sampling sites from the Kattegat area were distributed across a depth gradient. Gradients from low to higher values of bottom trawling intensity (SAR) and sediment

mud content (%) were recorded from both shallow (<50m) and deep water (>50m) sites, as well as for minimum bottom salinity in shallow water (<50m), while salinity in the deep water (>50m) sites were always high (figure 6.2). Although Pearson correlation coefficients (r) were <0.7 for all pairs of predictor variables, the correlation between depth and salinity was relatively high (r=0.66). Depth as a variable thus inherently also explains, to some extent, data variance linked to salinity, especially.
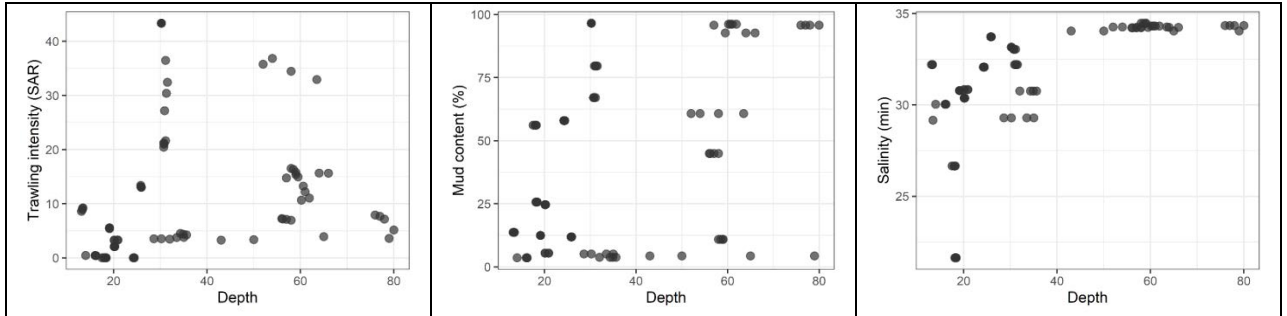
Figure 6.2. Pair plots of depth in metres correlated with bottom trawling intensity expressed in swept area ratio (r=0.27), sediment mud content in percentage terms (r=0.48) and minimum bottom salinity (r=0.66) for the 21 Van Veen grab sampling sites. Pearson correlation coefficients are provided in brackets.

6.3.2 Macrofaunal density and species density

A total of 285 taxa and 30,783 individuals were identified and enumerated. Boxplots describing density and species density across sampling sites are presented in figure 6.3. Greater density and species density were generally recorded from sites associated with lower fishing effort i.e. area adjacent to the Vinga trench system (Site 12), to the north east of Læsø (Sites 7 and 8) and in the closed areas (Sites 45, 49, 51) (figure 6.3). Lower density and species density values were associated with areas of high fishing pressure to the north of Læsø (Sites 3, 5, and 6) and those in the Vinga trench with the combination of deep water and high trawling effort (sites 15, 17, 18, and 19) (figure 6.3). The clear exception to this was site 26, which exhibited particularly high species density, but also had one of the highest SAR values recorded (SAR 16.39).
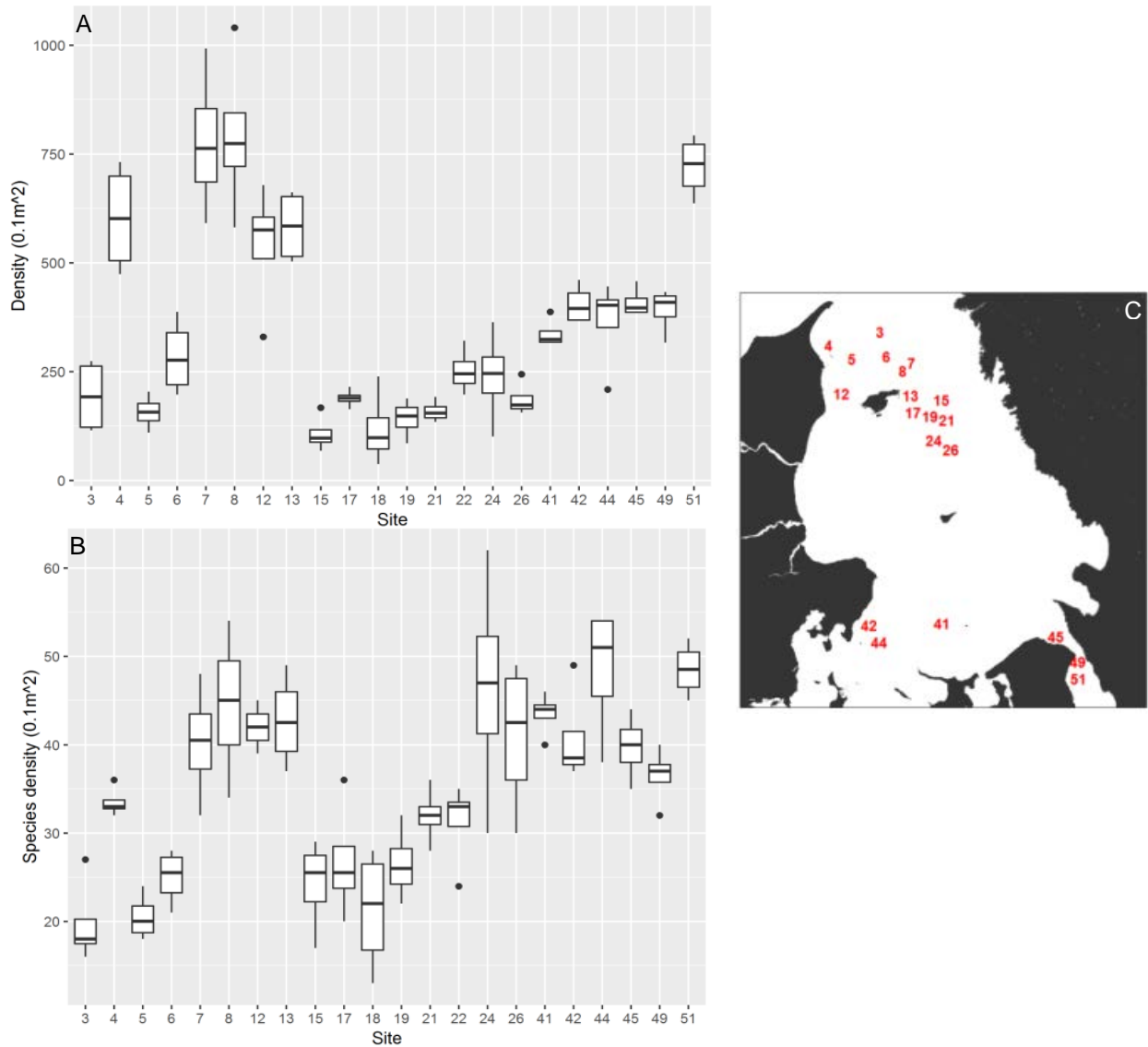
Figure 6.3. Box plots of (A) density and (B) species density across sampling sites. Locations of sampling sites are shown in (C).

6.3.3 Effect of bottom trawling intensity and environmental variables on macrofaunal composition

The redundancy analysis (dbRDA) plot of taxonomic composition is shown in figure 6.4A. The diagram shows that species composition was relatively similar in areas which were subject to similar levels of bottom trawling intensity. Sites associated with 'un-trawled' and 'low' trawling intensity grouped together and was ordinated towards the bottom and bottom-left of the plot. Sites with higher trawling intensities, i.e. the 'medium', 'high' and 'very high' categories, graduated towards the top and top-right of the dbRDA plot. In particular, the communities in the 'un-trawled' (closed areas) and some of the 'low' trawl sites were more distinct from the other categories. This would indicate that macrofaunal species composition in closed areas differs from that of more regularly trawled areas.

The redundancy analysis (dbRDA) plot of trait composition is shown in figure 6.4B. Similarly, the trait composition of the community observed in 'un-trawled' and 'low' trawling sites are ordinated towards the bottom-left of the plot, graduating towards 'very high' in the top-right. However, the gradient is not as clear as shown by the taxonomic data, and there is a greater degree of overlap between the 'un-trawled' and 'low' categories. This would suggest that although the trait composition at un-trawled sites are distinct from more highly trawled conditions, there is a larger degree of similarity in the trait composition of 'un-trawled', 'low', and 'medium' trawling intensity sites.
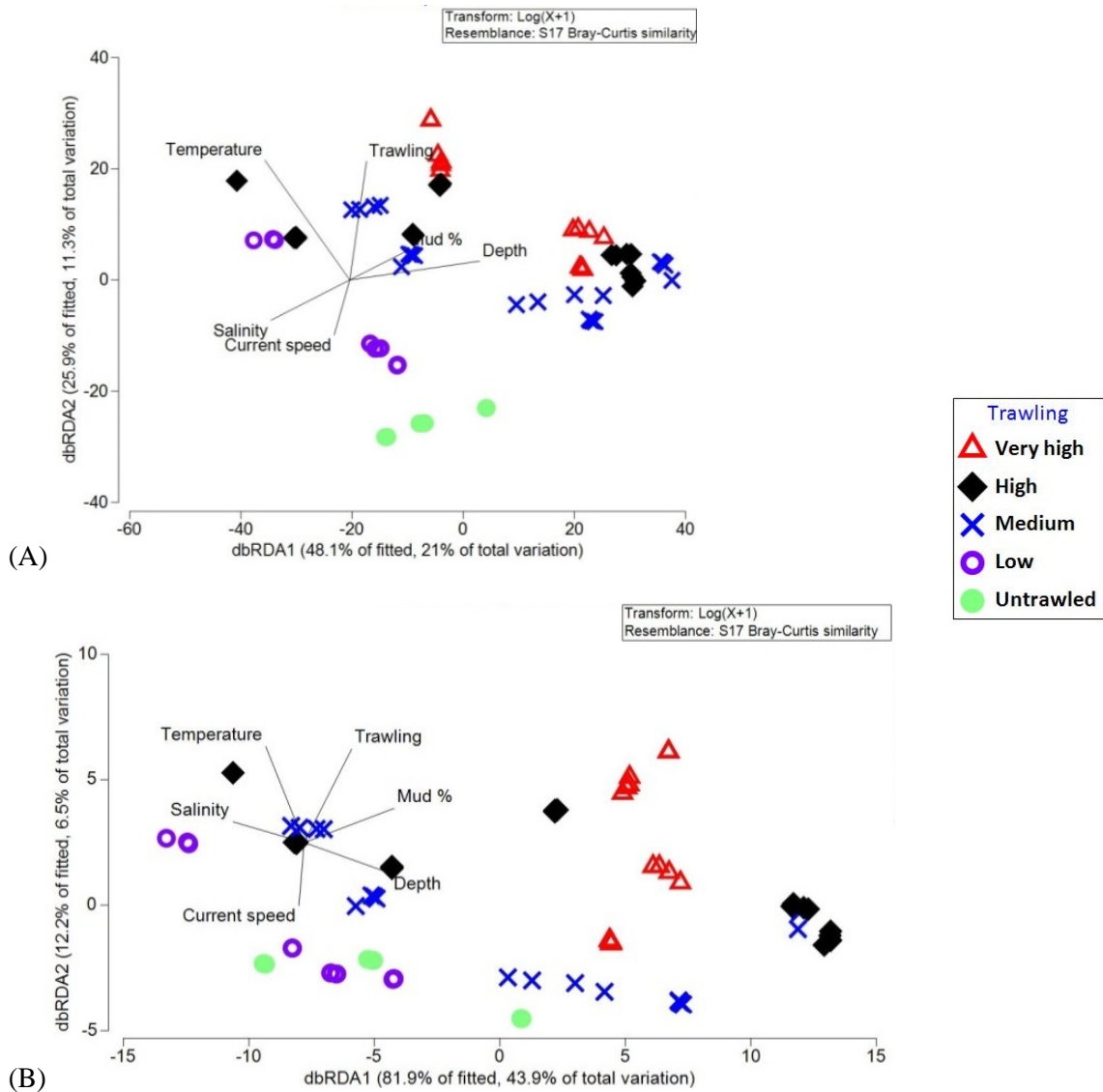
Figure 6.4. Plots of distance based redundancy analysis (dbRDA) displaying fitted values of models of (A) taxonomic composition, and (B) trait composition. Vectors of significant predictors are overlaid on each plot. Sites are categorised according to trawling intensity.

6.3.4 Effect of bottom trawling intensity on macrofaunal composition within size categories

To compare the effect of trawling intensity on different species' size categories, we quantified trawling disturbance to taxonomic and trait composition in the 1-4mm, ≥4mm fractions and the pooled full community separately. Figure 6.5 describes the relative importance of each of the predictors in determining macrofaunal composition. With regard to species composition (figure 6.5a), the model based on the full community explained a total 43.6% of variability in the community, with all six predictors significant. Depth and temperature were particularly important and accounted for 19.1% (p=0.001) and 10% (p=0.001) of variance, respectively. When added to the model, trawling accounted for an additional 3.3% (p=0.001) of variance. When compared to the model based on small fauna (1-4mm fraction), roughly similar proportions of variance were allocated between the variables, although the variance explained by trawling was marginally less. The model for large fauna (≥4mm fraction) also had a high explanatory power (43.5% of total variation). In this case, mud content was excluded from the final model and depth was again the most important predictor (22.9% of variance). The addition of trawling to the model accounted for 4.8% (p=0.001) of variance, which was comparatively higher than the full and 1-4mm communities.

Figure 6.5b provides a comparison of trait variance explained by each predictor. Over 53% of variance was explained by the full community model, with all six predictors significant. Depth was a particularly important predictor explaining 33% (p=0.001) of variance, along with mud content (10.8% p=0.001). Trawling accounted for 4.2% (p=0.001) of variance in the full community. Similar proportions are observed in the 1-4mm model, although variance explained by trawling is particularly low (1.5%, p= 0.048). The ≥4mm model has a lower explanatory power (38.4% of total variation) than the other size categories, and salinity is excluded from the final model. However, the addition of trawling to the model accounted for 10.2% (p=0.001) of explained variability.

Bottom trawling thus had an overall significant effect on benthic macrofauna at the community level; and the effect was relatively greater on the larger bodied species and their co-occuring biological traits. The relatively small influence of salinity across the models can be explained by salinity and depth showing a relatively high degree of correlation. This means the potential influence of salinity is at least partly captured by the depth predictor.
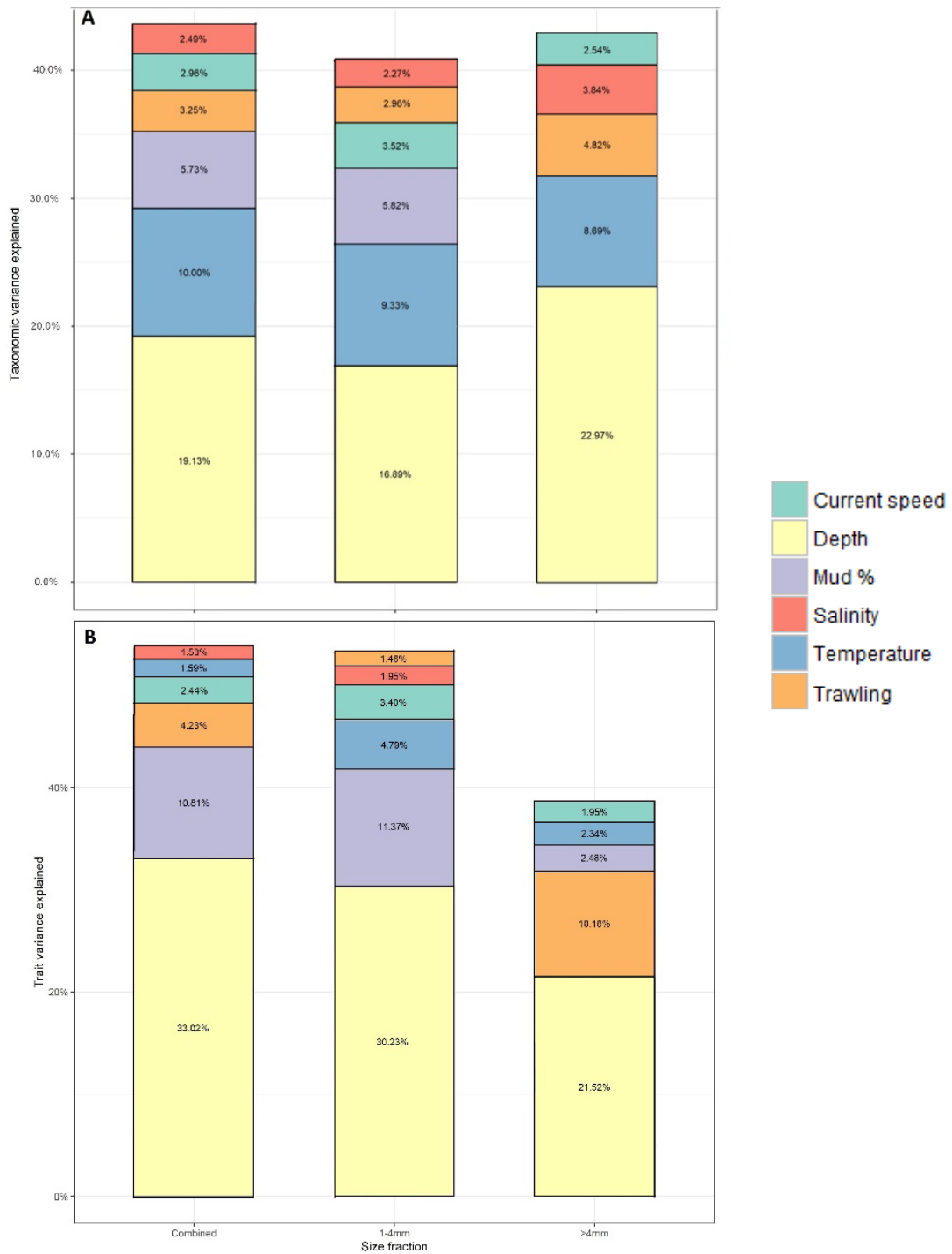
Figure 6.5. Proportions of variance explained by significant predictors in models of (A) taxonomic composition and (B) trait composition. A and B present the results of models based on the full (pooled) community (left column), 1-4mm fraction (middle column), and >4mm fraction (right column). The percetentages provided are calculated from a conditional test, such that variance allocated to each predictor takes into account the effect of the other predictors in the final model.

6.3.5 Macrofaunal indicators

The effect of bottom trawling and other environmental drivers on benthic indicators was similarly examined across size fractions. Indicators based on the full community demonstrated a varied performance to detect trawling disturbance. Of the eight indicators examined, four exhibited a significant negative response to trawling. These included density (N), biomass, functional richness (F_{ric}), and functional dispersion (F_{dis}) (table 6.1). Conversely, there was no observed effect of trawling on species density (S), Shannon diversity (H'), and functional diversity (RaoQ) (figure 6.6). In the small fraction, none of the taxonomic based indicators (N, S, H', biomass) responded to trawling, whereas two of the functional indicators (F_{ric} and F_{dis}) demonstrated significant negative relationships with trawling. Both these indicators were distinctive as they demonstrated significant negative relationships with trawling intensity across all size categories. In the large fraction, each of the eight indicators examined declined significantly with trawling. Further, trawling was the sole explanatory variable in the most parsimonious models of S, H', F_{ric}, F_{eve}, and F_{dis}, which indicated a reduced influence of other environmental variables in the large fraction. Comparison of model parameter estimates across size fractions further indicated that the effect of bottom trawling was greater per unit N, biomass, RaoQ and F_{dis}, in the large fraction (see details in Appendix A.3)

Table 6.1. Summary output for generalised linear mixed models of macrofaunal indicators. The values shown are parameter estimates, and associated standard error (shown in brackets). The significance level is denoted by asterisks (* = P < 0.05; ** =P < 0.01; *** = P < 0.001). Conditional R² values describe the proportion of variance explained by both the fixed and random terms. Significant negative and positive relationships are shown in orange and green, respectively.

	Indicator	Intercept	Current	Depth	Mud%	Temperature	Trawling	Salinity	Density†	Conditional R²
Full	N	7.16(0.19)	-0.206 (0.065)**	-0.017 (0.003)***	-0.007 (0.001)***		-0.013 (0.005)*		-	0.79
	S	4.63 (0.29)			-0.005 (0.001)***	-0.139 (0.037)***			0.25 (0.004)***	0.75
	H'	3.56 (0.58)	0.066 (0.027)*			-0.08 (0.031)*			-	0.33
	Biomass	4.19 (1.20)		-0.032 (0.006)***		-0.368 (0.147)*	-0.017 (0.009)*		-	0.35
	RaoQ	0.64(0.07)	0.020 (0.009)*			-0.025 (0.010)*			-	0.52
	Fric	0.47(0.01)			-0.001 (<0.001)**		-0.004 (0.001)**		-	0.47
	Feve	0.31(0.07)						0.010 (0.002)***	-	0.51
	Fdis	5.86(0.16)	0.167 (0.076)*				-0.018 (0.005)**		-	0.48
Small fraction	N	6.92(0.22)	-0.240 (0.077)**	-0.017 (0.003)***	-0.011 (<0.002)***				-	0.82
	S	2.67 (0.46)	0.101 (0.031)***	0.004 (0.001)*	-0.005 (0.001)***	-0.138 (0.03)***			0.005 (0.002)***	0.73
	H'	2.07(0.15)	0.243 (0.066)**			0.250 (0.075)			-	0.76
	Biomass	-0.81(0.14)		-0.018 (0.003)***	-0.004 (0.002)*				-	0.38
	RaoQ	0.779(0.08)	0.022 (0.009)**			-0.042 (0.011)***			-	0.58
	Fric	0.38 (0.02)		-0.001 (>0.001)*			-0.003 (0.001)**			0.38
	Feve	0.26(0.06)						0.011 (0.002)**	-	0.48
	Fdis	7.63(0.78)				-0.202 (0.103)*	-0.017 (0.006)*			0.46
Large fraction	N	7.07(0.73)		-0.018 (0.003)***		-0.246 (0.089)**	-0.025 (0.005)***		-	0.53
	S	0.66(0.28)					-0.016 (0.004)*		0.423 (0.06)***	0.75
	H'	1.55(0.08)					-0.019 (0.005)***		-	0.43
	Biomass	0.95(0.32)		-0.027 (0.008)***			-0.027 (0.012)*		-	0.31
	RaoQ	0.43(0.02)		-0.001 (<0.0001)*			-0.004 (0.001)***		-	0.50
	Fric	0.49 (0.03)					-0.007 (0.001)***		-	0.47
	Feve	0.67(0.01)					-0.004 (0.001)***		-	0.21
	Fdis	7.77(0.55)		-0.048 (0.013)***			-0.042 (0.021)*		-	0.65

N = density, S = species density, H' = Shannon diversity, RaoQ = functional diversity, Fric = Functional richness, Feve = Functional evenness, Fdis = Functional dispersion
Model families: N = Negative binomial. S = Poisson. H/Biomass/RaoQ/Fric/Feve/Fdis = Gaussian. Models of H were fitted using a log link.
† = density (N) included as a predictor for models of species density (S), only

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Figure 6.6. Relationships between trawling intensity and macrofaunal indicators derived from the full community. Regression lines represent the predicted values from GLMMs presented in Table 7.1. Greyed area represents 95% confidence intervals. The raw observations are overlaid as data points. N = density, S = species richness, H = Shannon diversity, Feve = functional evenness, Fric = functional richness, Fdis = functional dispersion, RaoQ = functional diversity.

6.3.6 *A-priori* selected sensitive species and traits

Figure 6.7 shows the location and relative abundance of five *a-priori* identified sensitive species, namely, large sea anemone species (including *Hormathia digitata, Peachia boeckii, Peachia cylindrical,* and *Actiniaria* spp.), *Arctica islandica*, *Haploops tubicola and H. tenuis, Pennatula phosphorea,* and *Virgularia mirabilis*. These species were selected based on their expected sensitivities to chronic bottom trawling disturbance. Large sea anemones were recorded at sites 4 (SAR 7.01), 12 (SAR 0.42), 22 (SAR 2.11) and 24 (SAR 3.31), which together represent sites categorised by intermediate levels of bottom trawling intensity. *Arctica islandica* was recorded from sites 7 (SAR 1.54), 41 (SAR 0.64), 42 (SAR 0.12), 44 (SAR 0.47), 45 (SAR 0.00), 49 (SAR 0.00) and 51 (SAR 0.00), all of which are low in trawling intensities. *Haploops tubicola* and *Pennatula phosphorea* were recorded at site 24 (SAR 3.31) and site 17 (SAR 9.73), respectively. *Virgularia mirabilis* was recorded from sites 15 (SAR 5.92), 42 (SAR 0.12), 44

(SAR 0.47), 45 (SAR 0.00), 51 (SAR 0.00), representing both low and intermediate trawling intensities.
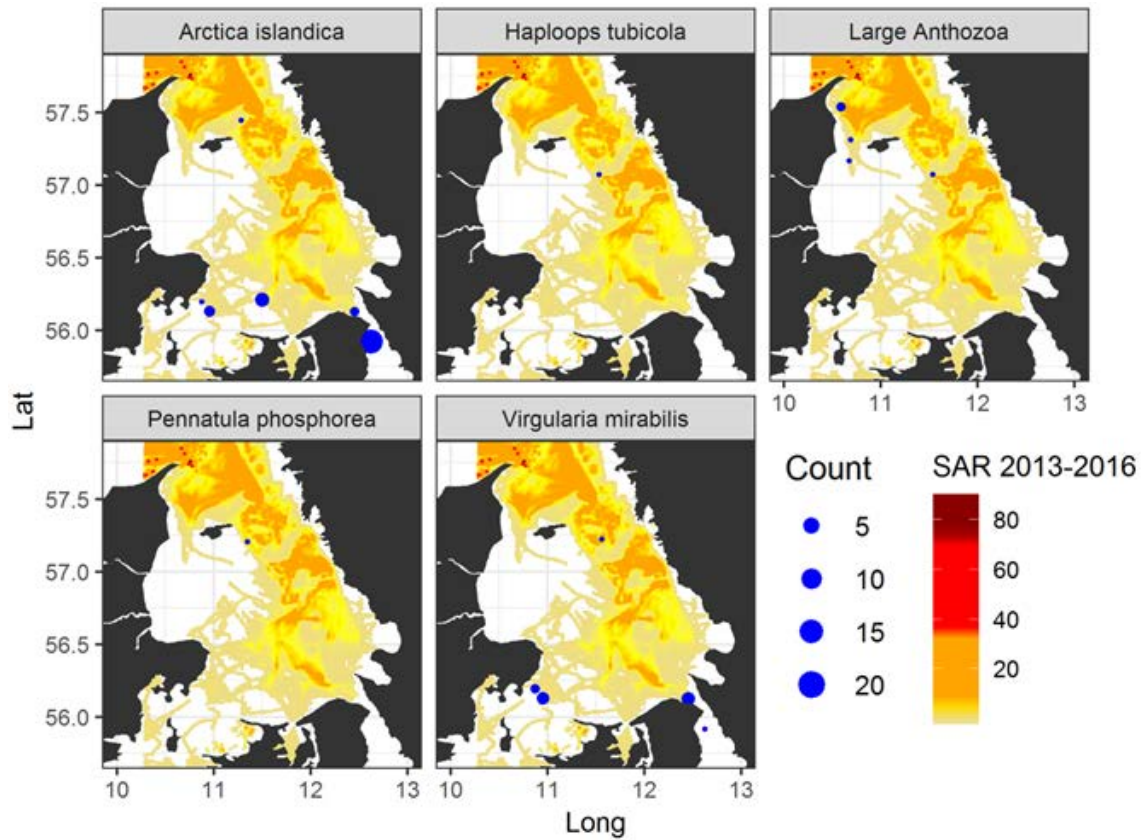
Figure 6.7. Distribution and abundace of *a-priori* determined sensitive species recorded in the Van Veen grab.

6.3.7 Sensitive species and traits

To determine the species and traits most sensitive to trawling, correlation coefficients were calculated between individual species/traits and the most relevant dbRDA axes (figure 6.4 - dbRDA axes were chosen based on their significant correlation with trawling). The species which exhibited the highest negative correlation values (table 6.2) with dbRDA2 was dominated by a group of tubicolous polychaetes. This group included mainly maldanid (*Maldane sarsi, Praxillella praetermissa, Rhodine gracilior*), and terebellid (*Anobothrus gracilis, Pectinaria auricoma, Terebellides stroemii)* polychaetes. Two species of bivalve, *Abra nitida* and *Thyasira flexuosa*, also had high correlation values. The species which showed the strongest positive relationships with trawling mainly comprised a group of gastropod molluscs (*Euspira nitida* and *Hyala vitrea)* and mysid shrimps (*Meterythrops robustus and Acanthomysis longicornis*), with a single species each of polychaete, echinoderm, and sipunculid.

The results of the macrofaunal trait sensitivity analyses form the basis for McLaverty *et al.* (*in prep.*) (included herein as Appendix A.4). Using the same approach for species outlined above, the trait modality which exhibited the greatest negative relationship with trawling was the

suspension feeding modality (suspension) within the feeding mode category (table 6.2). Other important trait modalities included having a soft external morphology (soft), depositing material at the surface during bioturbation (surface deposit), and having a long lifespan of >10 years (l>10y). A small number of traits exhibited a positive response with trawling, such as maximum longevity <1 year (l<1y) although these relationships were not significant.

Table 6.2. Linear relationships between individual species (a) and traits (b) with the relevant axes of distance based redundancy analysis (taxonomic components = dbRDA2, trait components = dbRDA1) (figure 6.4). The 10 species and traits with the highest significant correlation values are presented.

(A) Taxonomic components			
Negative correlation		**Positive correlation**	
Species (major taxonomic group)	**Pearson R^2**	**Species (major taxonomic group)**	**Pearson R^2**
Anobothrus gracilis (Polychaeta)	-0.77	*Hyala vitrea* (Gastropoda)	0.63
Prionospio fallax (Polychaeta)	-0.72	*Euspira nitida* (Gastropoda)	0.49
Rhodine gracilior (Polychaeta)	-0.66	*Cylichna cylindracea* (Gastropoda)	0.39
Terebellides stroemii (Polychaeta)	-0.64	*Diastylis lucifera* (Crustacae)	0.28
Maldane sarsi (Polychaeta)	-0.62	*Amphiura chiajei* (Echinodermata)	0.27
Praxillella praetermissa (Polychaeta)	-0.61	*Golfingia spp. (Sipunculidea)*	0.26
Thyasira flexuosa (Bivalvia)	-0.57	*Meterythrops robustus* (Crustacae)	0.25
Chaetoderma nitidulum (Mollusca)	-0.55	*Sorgenfreispira brachystoma* (Gastropoda)	0.23
Pectinaria auricoma (Polychaeta)	-0.50	*Notomastus latericeus* (Polychaeta)	0.22
Abra nitida (Bivalvia)	-0.50	*Acanthomysis longicornis* (Crustacae)	0.21

(B) Trait components	
Negative correlation	
Trait modality (trait category)	**Pearson R^2**
Suspension (feeding mode)	-0.55
Soft (morphology)	-0.54
Surface deposition (bioturbation)	-0.51
>10 years (longevity)	-0.51
Sessile (mobility)	-0.51
Pelagic eggs (egg development)	-0.5
Planktotrophic (larval development)	-0.5
Exoskeleton (morphology)	-0.49
101 - 200mm (size range)	-0.49
Burrow-dwelling (sediment stability)	-0.48

6.3.8 Traits as indicators of trawling disturbance

In the analyses, 10 traits were examined in terms of their sensitivity to trawling and other environmental drivers (Appendix A.3). Similar to the analysis of community composition and indicators outlined above, this was also carried out across size fractions. The 10 traits examined

included 'exoskeleton' (morphology), 'pelagic eggs' (egg development), 'planktotrophic' (larval development), 'sessile' (morphology), 'surface deposition' (bioturbation), 'suspension' (feeding mode), 'tube-dwelling' (living habit), '101-200mm' (size range), '6-10cm deep' (sediment position), and '>10 years' (longevity). Of these, five traits, namely 'planktotrophic', 'sessile', 'surface deposition', 'suspension', 'tube-dwelling', exhibited a significant relationship with trawling (table 6.3; figure 6.8). Further, seven of the traits examined exhibited a significant relationship with trawling in the large fraction. This would suggest that the traits of large benthic fauna are particularly sensitive to bottom trawling. In contrast, the traits of small-bodied benthos were not sensitive to trawling, with the sole exception being 'sessile'.
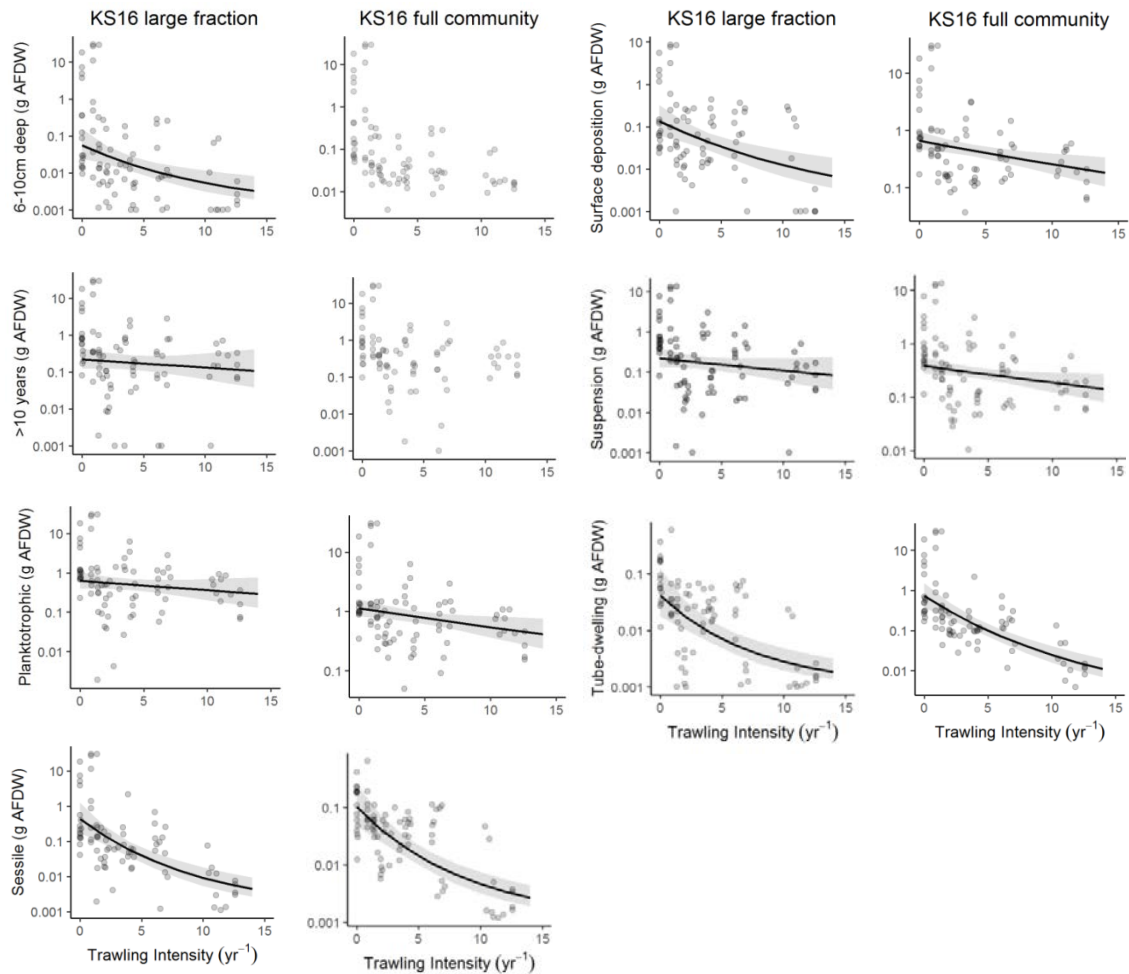
Figure 6.8. Relationships between trawling intensity and sensitive benthic traits. The results are presented comparatively for the large fraction and full community. Regression lines denote a significant relationship, and represent the predicted values from GLMMs presented in table 7.3. Shaded areas represent 95% confidence intervals. The raw observations are overlaid as data points. To aid visual interpretation, trait values are shown on a log scale.

Table 6.3. Summarised results of GLMMs for sensitive traits. Significant relationships with trawling denoted by an 'x'. Significant relationships with other environmental variables are shown in the 'other' column, and denoted by C = bottom current speed, D = depth, M = mud content, T = bottom temperature and S = bottom salinity. Model coefficients of determination (R^2) provided as conditional R^2 values (both marginal and random effects combined). Significant negative (-) and positive (+)relationships are shown in orange in green, respectively

Trait	KS16 (2016)								
	Full community			Small fraction			Large fraction		
	Trawling	Other	R^2	Trawling	Other	R^2	Trawling	Other	R^2
Exoskeleton		D-, T-	0.61		T+	0.52		C+, D-, T-	0.57
Pelagic eggs		C+, D-, T-	0.62		S-, T+	0.40		D-, T-	0.58
Planktotrophic	x-	D-	0.61		S-, T+	0.42	x-	D-	0.58
Sessile	x-	D-	0.82	x-	C-	0.74	x-	D-	0.83
Surface deposition	x-	D-	0.69		S-, T+	0.44	x-	D-, T-	0.67
Suspension	x-	D-	0.72		S-, T+	0.48	x-	D-	0.69
Tube-dwelling	x-	T-	0.87		M-, T-	0.81	x-	T-	0.80
101-200mm		D-, T-	0.70		S-, T+	0.48		D-, T-	0.68
6-10cm deep		D-, T-	0.81		M-, S-	0.67	x-	D-, T-	0.83
>10 years		D-, T-	0.69		D-	0.67	x-	D-	0.70

6.4 Discussion

This study represents the first examination of bottom trawling impacts on benthic trait composition in the Kattegat and reveals that the signal of trawling disturbance is stronger for the trait composition of large fauna compared to the small ones. The use of a size fractioning and trait-based approach significantly improved the quantification and detection of macrofaunal responses to trawling intensity across all metrics. The observed changes in the macrofaunal composition were only partly reflected by the indicators based on the full community (i.e. the small and large sieve fractions combined) where significant declines were observed for density and functional diversity. This indicated that full community indicators may not be adequate for describing trawling impacts on benthic communities. Using large fauna to calculate indicators consistently resulted in significant negative correlations with trawling intensity. Further, the use of large fauna indicators resulted in the reduced influence of environmental variables which can mask trawling effects.

6.4.1 Macrofaunal composition and distribution

The study showed benthic macrofaunal composition is impacted significantly by bottom trawling in the most intensively fished areas of the Kattegat. These areas were typically associated with lower macrofaunal density of individuals and biomass as well as lowered functional, trait-based, richness in comparison to the low or no-fished areas studied herein. Several important environmental drivers were included in the analyses, and this demonstrated sediment type and depth (and depth-derived variables) as important structuring variables across the study area. Furthermore, their inclusion in the analysis also showed that trawling remains a significant pressure shaping macrofaunal composition even when differences in physical and hydrodynamic conditions across sites are accounted for.

The highest fishing intensities were generally recorded in the northern Kattegat (sites 3, 5, and 6) in the deeper sites of the Vinga trench. These areas were associated with the lowest macrofaunal density, biomass and species richness. In the case of the deepest site (site 18), trawling intensity was relatively high. In this study, most of the areas deeper than 70m had experienced relatively high trawling intensities. The consequence of this is that it is difficult to characterise accurately the true effects of trawling at these deeper areas due to lack of comparable un-fished (i. e. reference) habitats. Conversely, the highest diversity areas examined in this study were identified in the proximity of Læsø (to the north-east and east), and in some of the deep areas (~60m) of the Kattegat trench system (site 24 and site 26).

The results demonstrated that highly trawled communities across the study areas were associated with significant reductions in large-bodied macrofauna and their associated traits. These larger-sized members of the community play an important role in several benthic processes. These individuals are particularly important for nutrient cycling (Solan *et al.*, 2004), and significant drivers of local sedimentary characteristics, biogeochemical fluxes, community dominance structures (Thrush *et al.,* 2006), and ecosystem function (Norkko *et al.*, 2013). The majority of this understanding comes from theoretical and empirical studies, however, and there is currently a lack of research which describes the significance of these losses to the functionality of benthic communities across fishing grounds.

6.4.2 Effect of trawling intensity on species composition

Recent attempts to quantify trawling effects to taxonomic composition in the Kattegat have produced differing outcomes. When analysing Danish monitoring data gathered between 2005 and 2009, Pommer *et al.* (2016) were unable to detect significant differences in macrofaunal community composition over a gradient of trawling disturbance. This lack of measurable response was assumed to be due to an absence of suitable reference conditions, and a high correlation between trawling and depth which made it difficult to disentangle trawl effects from environmental gradients (Pommer *et al.*, 2016). Conversely, Sköld *et al.* (2018) demonstrated small but significant shifts in both density and biomass based taxonomic composition, using Swedish monitoring data from 2009 to 2014. The results of Sköld *et al.* (2018) were determined using comparable multivariate models to ours, where we also revealed a significant effect of trawling. This, coupled with the similar levels of community variance accounted for by trawling in their study, may indicate that the effects of trawling are relatively similar in the Swedish and Danish sectors of the Kattegat.

In our study, changes in community composition included reductions in the relative occurrence of attached, filter-feeding, long-lived species, and sessile organisms. These results are similar to those from comparable muddy grounds in the North Sea (Tillin *et al.*, 2006). The effect on sessile species was particularly notable in the case of tubicolous polychaetes (maldanids and terebellids), which were highly abundant in un-trawled and lightly trawled areas, and reduced with increasing trawling intensity. These polychaete species can form dense aggregations of tubes protruding from the seabed, which provide important habitat structure in soft sediment communities, and play a role in the cycling of seawater and nutrients at the sediment water interface. The loss of functionally important traits and species , which may reduce the diversity of the taxonomic and trait composition, is thought to not only affect overall community function, but also lead to reduced community stability and resilience of communities (Bremner *et al.*, 2003; De Juan *et al.*, 2007; Frid *et al.*, 2005). This has not yet, however, been tested at the scale of fishing grounds.

6.4.3 Macrofaunal indicators

The observed changes in macrofaunal community composition were partly reflected by the indicators based on the full community, where density (N), biomass, functional richness (Fric), and functional dispersion (Fdis) declined significantly. The effect of trawling on density and biomass had been demonstrated in several studies (Gislason *et al.,* 2017; Hiddink *et al.,* 2006). This study represents the first examination of trait-based indicators in the Kattegat. In particular, functional richness (Fric) and functional dispersion (Fdis) were particularly affected to trawling, even across size fractions. Fric is largely influenced by either the loss or addition of unique traits, while Fdis provides a measure of the distinctiveness of traits within the community. The relative sensitivity of these indicators compared to taxonomic diversity indicators (e.g. species richness) would suggest that trawling may have a greater effect on the functional characteristics of the community. If that is the case, then communities in heavily trawled areas may become functionally impoverished, whereas a base level of species diversity may be comparatively unaffected. This would have implications for the functionality of benthic communities across fishing grounds and suggests that monitoring of functional indicators should be undertaken in conjunction with taxonomic approaches. Alternatively, the lack of response observed in the taxonomic diversity indicators may indicate that full community indicators are not adequate for describing trawling impacts on benthic macrofaunal communities. This is because measures of

diversity (e.g. species richness) can be highly sensitive to factors such as size of sampling area, sampling intensity and the taxonomic properties of the species in question. Furthermore, small species are relatively less affected by trawling, and the high abundances of these small-bodied fauna can therefore essentially mask the measurable effects of trawling at the full community level. The size-separation of the community, however, showed that all taxonomic and functional characteristics of large benthos were impacted significantly by trawling, and represents an alternative method by which to measure and monitor impacts of bottom trawling intensity on seabed habitats.

6.4.4 *A-priori* selected sensitive species

Among the *a-priori* identified sensitive species, *Haploops tubicola* and *Pennatula phosphorea* were only recorded with the Van Veen grab from a single site each. Both of these species are assumed to be highly sensitive to trawling disturbance. The suitability of these species as indicators of trawling effects in this study, however, is limited by low spatial distribution and abundances. In particular, *Haploops* spp. has seen significant declines in the Kattegat area since the beginning of the last century, where high densities patches of these tube building amphipods occurred (Petersen, 1918). The reductions in spatial extent and abundance are thought to be linked to demersal trawling (Göransson, 1999; Josefson *et al.*, 2018; Sköld *et al.*, 2018), as the species is now only found sporadically except in areas closed to trawling in the Kattegat and the Sound (Naturstyrelsen, 2016). The single record from the Van Veen grab samples of *P. phosphorea* was from a highly trawled site (site 17) (figure 6.9). This record was of a juvenile individual, and indicates that recruitment of these sensitive species also takes place in the highly trawled areas, but that growth through to the adult stage may be compromised by regular and high fishing pressure. This and other megabenthic epifauna species, such as larger sea anemones, *V. mirabilis*, *M. modiolus* and *Haploops* spp., are monitored more effectively by the use of remote sensing systems, such as the UWTV sledge. The greater occurrence of *A. islandica* in the Van Veen grab samples, and at the low and unfished sites, combined with their generally wider distribution across the study site, would indicate that this species may be a suitable indicator of trawling impacts. In particular, we observed that *A. islandica* contributed significantly to the biomass in unfished and lightly fished sites. This large, periodically deep living, and particularly long lived (>100-400 years) (Abele *et al.,* 2008; Morton, 2011) species, can contribute to nearly half of total benthic community production, and can be an important prey item for commercially important species such as cod (Brey *et al.*, 1990). *Arctica islandica* is also reported to be highly sensitive to chronic bottom trawling disturbance (Rumohr and Krost, 1991). Generally, sites in the southern Kattegat and closed areas had the highest abundances of this *a-priori* identified species.

Figure 6.9. Specimen of *Pennatula phosphorea* recorded at site 17 in the Van Veen grab.

6.4.5 Sensitive species and traits

Results of the multivariate analysis identified a number of species and traits with high negative and positive correlation values with trawling. The negative correlations observed for various tube-building maldanid polychaetes (e.g. *Rhodine gracilior* and *Maldane sarsi*)*,* terebellid polychaetes (e.g. *Anobothrus gracilis*), and horseshoe worms (*Phoronis muelleri*) can be expected due to their fragility, and their requirement for stable substrata in which to build their tubes (Colson and Hughes, 2004). A number of these polychaete species share a common set off traits which were also highlighted in the analysis. These species shared traits relating to morphology ('soft')*,* their living habit the sediment surface ('tube-dwelling'), and their limited mobility ('sessile'). Aggregations of tubicolous polychaetes have been shown to actively support increased benthic diversity and to benefit other species by improving sediment stability (Friedrichs *et al.,* 2000). Further, high densities of tubes have been shown to improve conditions for larval settlement (Bolam and Fernandes, 2002), and promote increased food supply for associated benthic species (Holte, 2001). Although trawling is known to reduce the abundance of polychaetes (Kenchington *et al.*, 2006) and phoronids (Hinz *et al.*, 2009), tube-building has not previously been commonly identified as a potential indicator of trawling disturbance. Tube-dwelling species typically live either at or near the sediment surface and require stable substrata to construct their tubes. Further, several tube-building polychaetes and amphipods species brood their offspring within the tubes and, thus, can be slow to recolonise areas after disturbance events (Bolam and Fernandes, 2002).

The high negative correlation values for traits such as sessile, suspension feeding and tube-building may point to potentially sensitive species that share this trait in-combination. These traits together may therefore predispose species to experience high rates of direct and in-direct mortality. In addition to the tube-building polychaetes described above, this specific trait combination is also shared by *Haploops* spp. *Haploops* spp. are recognised for their functional

importance (Rigolet *et al.*, 2014), and were once the dominant component of the macrofauna in the Kattegat at the beginning of the 1990s. If this was the case, however, this trait combination could also explain the observed decline in distribution and abundance of the species. Further, due to the highly patchy distribution *Haploops* spp. is not suitable as a generic indicator of trawling disturbance to soft seabed habitats in the Kattegat.

High negative correlation values were recorded for two bivalve species, *Thyasira flexuosa* and *Abra nitida*. These species also share sensitive traits such as shallow sediment position (Infauna: 0-5cm)*,* relatively long life-spans (longevity: 3-10y), and a subsurface feeding mode (Subsurface deposit). Further, both species have a distinctly thin and brittle shell structures, and have previously been identified as sensitive to trawling in comparable studies (Ball *et al.*, 2000; Hinz *et al.*, 2009; Reiss *et al.*, 2009).

Species which demonstrated a positive relationship with trawling included the brittle stars *Amphiura chiajei* and *Amphiura filiformis*. In a similar study undertaken in the Swedish EEZ of the Kattegat (Sköld *et al.*, 2018), *A. chaijei* was also observed at higher densities in highly trawled areas. This may not be expected based on their biological traits, given that brittle stars are both relatively large and long-lived (longevity: >10 years). This observation was, however, explained by the authors to be a result of reduced predation pressure from the lower abundances of demersal fish species in highly trawled areas. While this indeed may the case, the behaviour and life history of the species may also explain the lack if trawling effect. This is as *Amphiura* spp. can reside in excavated chambers within the sediment down to a depth of 6.8 cm (O'Reilly *et al.*, 2006). Further, *Amphiura* spp. are also able to alternate between suspension and deposit feeding depending on food availability (Sköld and Gunnarsson, 1996) and have the ability to regenerate lost arms successfully, a capability that has been observed in areas of high trawling intensities (Sköld and Rosenberg, 1996). Accordingly, species such as *Amphiura* spp. may be inherently robust to trawling impacts in muddy grounds. This is an important observation as many studies have not observed positive relationships between macrofauna and fishing intensities. Such results provide a better understanding of how bottom trawling alters seabed habitats.

6.4.6 Size dependency

The results of our study demonstrate a greater effect of trawling on the ≥4mm fraction (large fauna) compared to the full community and small fauna. Furthermore, the traits of large fauna were particularly sensitive to trawling disturbance. This differences across size fractions can be explained by the size selective sensitivity of large fauna to trawling in community size spectra (Duplisea *et al.*, 2002; Queirós *et al.*, 2006). Using body size provides a proxy for a large amount of embedded ecological information, as it is related to a several life history traits strongly linked to community structure and sensitivity (Woodward *et al.*, 2005). The sensitivity of certain larger faunal species is therefore likely due the combination of specific life history traits, such as a low reproductive capacity and population growth rate and the greater longevity of individuals. Separating the community into small and large size fractions looks to have effectively captured this sensitivity and shows that using a numerically reduced dataset composed of large fauna (22% of total abundance) has a stronger relationship with trawling intensity and, therefore, improves the sensitivity of the analyses.

These results also highlight the potential for benthic macrofaunal indicators of bottom trawling disturbance based on the biological traits of the community and provide insights into trawling impacts on benthic ecosystem functioning. Traits of large benthic species such as 'sediment position: 6-10 deep', 'longevity: >10 year' were distinct in their sensitivity and rare at heavily trawled sites. These traits were chiefly accounted for by bivalve species (e.g. *A. islandica*, *Thracia* spp.), as well as large sea urchins (*E. cordatum*), and sea pens (*V. mirabilis*). Marine bivalves are functionally important members of the benthic macrofaunal communities, but can experience relatively high rates of trawling induced mortality. Large bivalves contributed significantly to total benthic community production, and are an important prey item for commercially important species such as cod. Further, large benthic fauna enhance the exchange the water and oxygen between the sediment and water column (Forster and Zettler, 2004), and irrigate deeper sediments with water and oxygen (Osinga *et al.*, 1995). Other sensitive traits identified in this study included 'bioturbation: surface deposition', 'feeding mode: suspension', and 'living habitat: tube-dwelling'. The large benthos which exhibit these traits perform important functional roles such as benthic-pelagic coupling and the provision of benthic habitat (Bolam *et al.*, 2002; Gili and Coma, 1998). Although generally less abundant, the loss of large individuals from benthic faunal communities may precede the loss of ecosystem function (Solan *et al.*, 2004). Accordingly, the size-dependant effect of bottom trawling on benthic faunal communities has the potential to degrade ecosystem function in heavily fished areas.

In terms of seabed habitats and benthic community indicators, total density, biomass, functional richness (F_{ric}) and functional dispersion (F_{dis}) (full community) exhibited significant negative relationships with trawling, while the four other indicators tested showed no such response (table 6.1). Moreover, none of the 1-4mm indicators responded to trawling, but did respond to various environmental predictors such as current speed, mud content, and temperature. The mixed response of full community indicators, and lack of response in the 1-4mm fraction, highlights the underlying issue of using full community indicators to detect trawling impacts. In our dataset, the small fraction was the numerically dominant (78%) part of the community, but is mainly composed of macrofauna with typically *r*-selected life history traits, such as higher population growth rate, early reproductive onset, and wide-spread recruitment. The resilience of this group can therefore mask responses of the more sensitive components of the community, such as the larger fauna. When we tested indicators based on the ≥4mm fraction, all eight community indicators demonstrated a significant negative relationship with trawling. The strong response of the large fauna indicators, coupled with the reduced effect of other environmental predictors, may indicate that by the time individuals reach an asymptotic size, ambient environmental conditions will have already selected for certain species or traits. Large fauna may therefore be less susceptible to variation caused by natural processes and local recruitment success, which are known to complicate the outcome of trawling impact assessments, and may be better suited to track trawling impacts over time and space. From a practical perspective, therefore, large fauna are considerably less abundant than small fauna, meaning that fewer individuals and species are required for analysis and monitoring. This is a particularly noteworthy output of this study, as it may demonstrate the suitability of larger fauna to be used in ecological monitoring of fishery impacts.

7. Interactive Ocean Floor Model

The results of the project were used to develop an interactive ocean floor model (figure 7.1) that depicts the areas where sensitive habitats and species occur, and where trawling for *Nephrops norvegica* lobster occurs in the Kattegat. The ocean floor model was developed by DFPO in collaboration with DTU Aqua and WWF. The ocean floor model can be used for public communication, now and in the future. It was first presented at the People's Festival of Nature 2019, where both DTU Aqua and DFPO had stands.
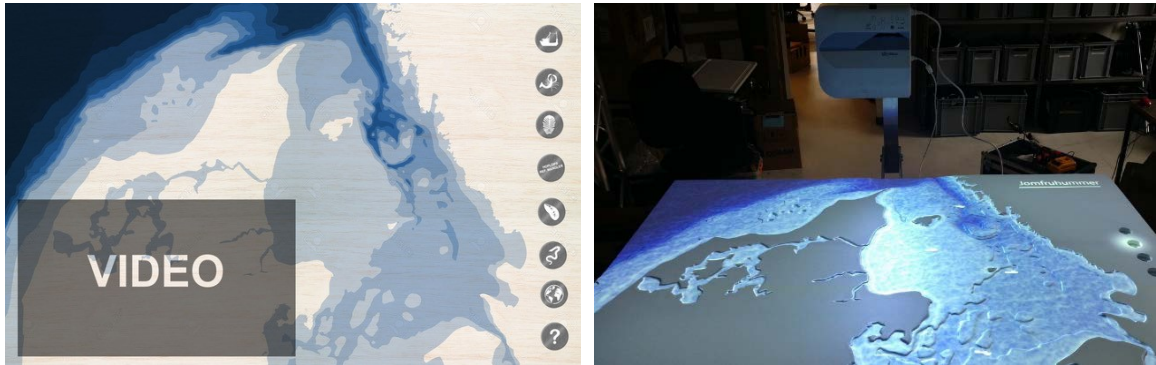
Figure 7.1. Left: Original layout of the model. Right: The actual interactive ocean floor model.

8. Stakeholder engagements

Stakeholder engagement was the specific focus of both the stakeholder forum and fisheries-scientist workshop. Both events were organised by DFPO in collaboration with DTU Aqua.

The stakeholder forum took place on the 21st of November 2018 at Axelborg in Copenhagen. The forum was attended by DFPO board members, DTU Aqua, WWF and the Ministry of Environment and Food of Denmark. Regretfully, the Danish Fisheries Agency was not able to attend but it was agreed to set up a meeting at the end of the project to communicate results directly to this party. The forum was divided into two parts; DTU Aqua presented the results of the project and, thereafter, discussions were encouraged with the focus on two main questions:

i), What needs to be considered during the designating of areas closed to fishery activities and the designing of the management plans?
ii), How to ensure that the overall aims of individual management measures (e.g. fishing closures) are assessed and the expected targets are reached

The workshop for fishers and scientists was conducted on the 13th December 2018 at Strandby Badehotel in Strandby (Appendix A.2). The workshop was attended by DFPO, DTU Aqua and *Nephrops* fishers trawling on fishing grounds in the Kattegat and Skagerrak. WWF was initially going to attend, but unfortunately needed to prioritise other work. DTU Aqua communicated the results of the project, and further engaged the workshop participants in a discussion on how the *Nephrops* fishery could be managed sustainably with respect to both the fishing industry and the environmental protection of sensitive habitats and species in the Kattegat.

During the project period regulations on fisheries had already been implemented in appointed Natura 2000 areas in the Kattegat (http://mscfiskere.fiskeriforening.dk/wp-content/uploads/sites/6/2018/04/Natura-2000-DK.pdf). This workshop gave the fishers the ability to discuss both implemented management measures and their effectiveness in light of the results of this project. Discussions from the workshop were communicated to other stakeholders, and to the wider public through an article in *FiskeriTidende* (Appendix A.2).

9. Conclusions relevant for sustainable management of fisheries effects

The project provides new results on how the diversity of benthic habitats and associated invertebrates and fish species in the Kattegat are affected by fisheries with bottom contacting gears. The results further improve the knowledge base required to further develop and improve ecosystem based management of demersal fisheries and for implementing the habitat and species protection required by Natura 2000 and the MSFD.

The project results show that improving the spatial and temporal resolution of the fishing footprint (SAR, Swept-Area Ratio day^{-1}), based on the Vessel Monitoring System (VMS, since 2005) data and logbook information, can help improve the accuracy of estimates of fishing pressure from towed bottom contacting gears and enhance the possibilities for assessing their effects on benthic macrofauna, habitats, and species.

Modelling results based on existing data from the Danish environmental monitoring programme, NOVANA, showed a significant negative response of the individual density (N) of benthic macrofauna to increasing SAR (sum of 1 year prior to benthic sampling) in the Kattegat (Gislason *et al.*, 2017). The results, however, also revealed that the Danish Quality Index (DKI) masks an inherently high correlation between the density of individuals (N) and the density of species (S) and thus cannot properly relate index variations to those of environmental conditions and anthropogenic pressures. A build-in salinity calibration further masks effects of individual drivers and their interactions with index variations. The DKI cannot separate the effect of fishing from the effect of other drivers and is, therefore, inadequate as an indicator of fisheries impacts on seabed habitats and species in the Kattegat. Also, due to the small sediment sampling size of the HAPS corer used in the NOVANA programme (seabed area covered per sample: 0.0143m^2), larger benthic faunal species risk being under-sampled. Among the megafauna and habitat forming species included by the OSPAR list of threatened and declining species, those sensitive to bottom trawling, such as sea pens, large sea anemones, sponges and horse mussels, are rarely (if ever) caught by the HAPS corer. In areas of high densities, tubicolous crustaceans are adequately caught by the HAPS corer. This gear is, however, less suitable for mapping of the spatial extent of seabed habitats.

In this project, we deployed a larger benthic sampler (Van Veen grab seabed area: 0.1m^2) and fractionized the macrofauna into large and small individuals (by sieving using 4mm and 1mm mesh sizes). The analysis revealed that benthic indicators calculated from the full community showed a varied ability to detect trawling disturbance, i.e. only four of the eight full community indicators exhibited a significant negative correlation with trawling intensity (i.e. density, biomass, functional richness and functional dispersion). When the same indicators were calculated using only the large-bodied macrofaunal benthos, however, then all eight of the indicators examined exhibited a significant negative response to trawling. This result demonstrates the sensitivity of large benthos to bottom trawling, and further demonstrates a method by which benthic indicators can be refined and improved in future seabed monitoring. Developing this theme, the project also investigated the sensitivity of forty-eight benthic life history traits, and identified seven that show a particularly strong response to bottom trawling.

These include the traits '6-10 deep' (sediment position), 'surface deposition' (bioturbation), '>10 year' (longevity), 'planktotrophic' (larval development), 'sessile' (mobility), 'suspension' (feeding mode), and 'tube-dwelling' (living habit). Given the importance of many of these traits in processes such as benthic-pelagic coupling and nutrient cycling, it is likely that the loss or decline of these traits will have considerable implications for the functioning of benthic ecosystems across fishing grounds. Of these, the sensitivity of traits such as '6-10 deep' and 'l>10 year' were observed only in the large-bodied macrofaunal dataset. This would suggest that these functionally important benthic traits may be overlooked when analyses are based on the full community.

Based on the above results we expect some of the areas with no or little fishing pressure to harbour a higher biodiversity and density of sensitive species compared to nearby seabed habitats under comparable environmental conditions (e.g. salinity, temperature, current regimes, oxygen levels) which have experienced medium to high fishing pressure. The use of high resolution data of the spatial and temporal distribution of fishing pressures can, therefore, be used in combination with detailed habitat (substratum) maps to identify geographic areas with no or low fishing pressure within the fishing grounds. Should these areas harbour dense adult populations of sensitive species and function as larval source areas which spread to adjacent more frequently fished areas, then they could be considered as candidates for future sensitive species MPAs. Where available, SODENA data and derived polygons of un-trawled areas could be used to further optimize delineation of new potential areas to be closed to fishing and other human activities to meet the ecological targets of the Natura 2000 and MSFD implementations.

The project results also confirm the OSPAR assessments that the sea pens *Pennatula phosphorea* and *Virgularia mirabilis*, as well as some bivalves and tubicolous crustaceans and polychaetes are sensitive to bottom trawling. Our results improve the knowledgebase for the development of robust methods of assessment of Good Environmental Status (GES) as required for the implementation of Natura 2000 regulations and for fulfilling the future requirements of the MSFD implementation. Although the Van Veen grab adequately samples juvenile sea pens (*V. mirabilis*, *P. phosphorea*), the highly patchy distribution and the large size of adult sea pens, as well as horse mussels and tubicolous amphipods (*Haploops* spp.), requires coverage of a larger sampling area to generate significant results. These organisms are better surveyed using the UWTV (Under Water TeleVision) sledge developed for monitoring and assessment of the *Nephrops norvegica* stocks for fisheries management in the Kattegat and Skagerrak. The video from the UWTV sledge can also be used to estimate the density of larger epibenthic macrofauna, including the species and faunal groups found on the OSPAR list of threatened and declining species in the area. The monitoring programme for *Nephrops norvegica* stock assessment presently only covers areas of medium to high bottom trawling intensity (SAR) with low (or zero) densities of the sensitive habitats and species. To assess the ecological status of sensitive habitats and species in the Kattegat and Skagerrak, therefore, the *Nephrops norvegica* monitoring programme should be extended to also cover soft sediment areas with no and low bottom trawling intensities. This would be a cost-effective method to acquire the information and data necessary for assessing the overall impact of the fishery on sensitive benthic megafaunal and macrofaunal habitats and species in the area and for evaluating whether the establishment of the proposed MSFD areas and other spatial closures to bottom trawling achieve the intended Good Ecological Status.

The TV3 trawl is the standard gear used in the Danish fish survey of the Kattegat (KASU). This gear proved to be the most effective for the monitoring of fish diversity among the four sampling gears employed in the project. The baited stereo cameras (BRUV) recorded some of the most abundant fish species, but many of the rarer fish species encountered in the TV3 trawl were not recorded by the BRUV. Interestingly, the BRUV proved highly effective for recording and quantifying the presence of both invertebrate and fish scavengers. Most remarkable was the efficiency in which the gear recorded hagfish, *Myxine glutinosa*. This species is rarely caught in bottom trawls, including the TV3, where only one individual was caught during the survey. The hagfish, together with whiting, was, however, shown to be highly abundant in the BRUV recordings in deeper waters (exceeding 50m) in the Kattegat. In more shallow waters (15-40m), no hagfish were identified, while larger decapods, dab and whiting dominated among the highly mobile scavengers, and common whelk and starfish approached the bait along the bottom at a slower pace. The BRUV would, therefore, be the most effective gear for assessing relative scavenger abundance and causal relations to the amount of discards produced by the fishery.

We conclude that adequate monitoring of seabed habitats and species, and their responses to bottom trawling, will require several sampling devices to fully describe the changes in the local benthic ecosystem resulting from bottom trawling. These changes include shifts in large (mega) and smaller (macro) invertebrate infauna and epifauna assemblages, scavenger abundance and fish community composition. Accordingly, sampling programmes designed to monitor and detect these changes are required in order to quantify the direct and indirect effects on the different species and functional groups characteristic for the seabed habitats in the Kattegat.

Knowing the distribution of sensitive species and habitats is a pre-requisite for establishing areal closures (MPAs) to protect such features from bottom trawling and/or other anthropogenic disturbances as required by the MSFD. Selecting such areas can, however, be undertaken in several ways. From a biological point of view it seems most relevant to focus on closing areas where the sensitive species and/or habitats are present. In this project we have identified evidence that habitats where trawling intensity is low tend to have higher benthic species diversity, higher densities of individuals, and higher biomass of benthos per unit area than similar habitats subject to high trawling intensity. Furthermore, the sensitive species defined by OSPAR were either absent or rare in areas of high trawling intensity, and were mostly present where trawling intensity was either low or zero. Closing areas with high trawling intensity will therefore be less likely to increase the overall protection of sensitive species and habitats. Although trawling may contribute significantly to the absence of sensitive species from intensively trawled areas, other environmental factors such as salinity, currents, predators and food availability may either limit or hinder the re-establishment of viable populations of the sensitive species in these areas. Further, bottom trawling activities may have altered the surface sediment composition whereby the seabed is no longer a suitable habitat for some of the sensitive species. There is, thus, no guarantee that a sensitive species can invade an area where it presently does not occur even though it is closed to bottom trawling. Closing an area with high fishing intensities will, moreover, re-allocate the current fishing effort to surrounding areas. In the worst case scenario this will displace fishing effort to areas where sensitive habitats and species are present. This points to the importance of focusing area–based protection measures of sensitive species and habitats in '*de facto*' closed areas or areas of low trawling intensity, so that they are effectively protected from bottom trawling in the future.

Acknowledgements

References

Abele, D., Strahl, J., Brey, T., Philipp, E.E.R. 2008. Imperceptible senescence: Ageing in the ocean quahog Arctica islandica. Free Radic. Res. 42, 474–480. https://doi.org/10.1080/10715760802108849

Alander, H. 1942. Sponges from the Swedish west coast and adjacent waters. Göteborg, H. Struwes Boktryck, 1-95 pp.

Ambroso, S., Dominguez-Carrió, C., Grinyó, J., López-González, P,J., Gili, J.-M.,Purroy, A., Requena, S. & Madurell, T. *et al.* 2013 In situ observations on withdrawal behaviour of the sea pen *Virgularia mirabilis*. Mar Biodiversity 43: 257-258.

Amoroso, R. O., Pitcher, C. R., Rijnsdorp, A. D., McConnaughey, R. A., Parma, A. M., Suuronen, P., ... and Jennings, S. 2018. Bottom trawl fishing footprints on the world's continental shelves. Proceedings of the National Academy of Sciences of the United States of America, 115(43), E10275-E10282 .DOI: 10.1073/pnas.1802379115

Anderson, M.J., Gorleyt, R.N., Clarket, K.R., 2008. PERMANOVA + for PRIMER : t. Guid. to Softw. Stat. Methods 1, 1-186. https://doi.org/10.1016/j.isatra.2014.07.008

Arndt, W. 1935. Porifera. – Die Tierwelt der Nord- und Ostsee III a, 1-140.

Ball, B. 2000. Long- and short-term consequences of a Nephrops trawl fishery on the benthos and environment of the Irish Sea. ICES J. Mar. Sci. 57, 1315–1320. https://doi.org/10.1006/jmsc.2000.0924

Bolam, S.G., Fernandes, T.F. 2002. Dense aggregations of tube-building polychaetes: response to small-scale disturbances. J. Exp. Mar. Bio. Ecol. 269, 197–222. https://doi.org/10.1016/S0022-0981(02)00003-5

Bolam, S.G., Garcia, C., Eggleton, J., Kenny, A.J., Buhl-Mortensen, L., Gonzalez-Mirelis, G., van Kooten, T., Dinesen, G., Hansen, J., Hiddink, J.G., Sciberras, M., Smith, C., Papadopoulou, N., Gumus, A., Van Hoey, G., Eigaard, O.R., Bastardie, F., Rijnsdorp, A.D. 2017. Differences in biological traits composition of benthic assemblages between unimpacted habitats. Mar. Environ. Res. 126, 1–13. https://doi.org/10.1016/j.marenvres.2017.01.004

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.-S.S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24, 127–135. https://doi.org/10.1016/j.tree.2008.10.008

Borja, A., Ranasinghe, A., Weisberg, S.B. 2009. Assessing ecological integrity in marine waters, using multiple indices and ecosystem components: Challenges for the future. Mar. Pollut. Bull. 59, 1–4. https://doi.org/10.1016/j.marpolbul.2008.11.006

Bremner, J., Frid, C.L.J., Rogers, S.I. 2003. Assessing Marine Ecosystem Health: The Long-Term Effects of Fishing on Functional Biodiversity in North Sea Benthos. Aquat. Ecosyst. Health Manag. 6, 131–137. https://doi.org/10.1080/14634980301470

Brey, T., Arntz, W.E., Pauly, D., Rumohr, H. 1990. *Arctica (Cyprina) islandica* in Kiel Bay (Western Baltic): growth, production and ecological significance. J. Exp. Mar. Bio. Ecol. 136, 217–235. https://doi.org/10.1016/0022-0981(90)90162-6

Campbell, N., Dobby, H., Baily, N. 2009a. Investigating and mitigating uncertainties in the assessment of Scottish *Nephrops norvegicus* populations using simulated underwater television data. ICES Journal of Marine sciences 66, 646-655.

Campbell, N., Allan, L., Weetman, A., Dobby, H. 2009b. Investigating the link between *Nephrops norvegicus* burrow density and sediment composition in Scottish waters. ICES Journal of Marine Science 66, 2052-2059.

Carlgren, O. 1945. Polypdyr III. Koraldyr. – Danmarks Fauna Bd. 51. G.E.C. Gad, København, 1-167.

Chimienti, G., Angeletti, L, Mastrototaro, F. 2018. Withdrawal behaviour of the red sea pen *Pennatula rubra* (Cnidaria: Pennatulacea). The European Zoological Journal 85, 64–70.

Colson, I., Hughes, R.N. 2004. Rapid recovery of genetic diversity of dogwhelk (*Nucella lapillus* L.) populations after local extinction and recolonization contradicts predictions from life-history characteristics. Mol. Ecol. 13, 2223–2233. https://doi.org/10.1111/j.1365-294X.2004.02245.x

Cook, R., Farina-Franco, J.M., Gell, F.R., Holt, R.H.F., Lindenbaum, C. *et al.* 2013. The substantial first impact of bottom fishing on rare biodiversity hotspots: A dilemma for evidence-based conservation. PLOS ONE 8(8): e69904, 1-10.

Danish AgriFish Agency. 2016. Yearbook of Fishery Statistics 2015. Danish AgriFish Agency. Ministry of Environment and Food, December 2016, 1-278.

de Juan, D., Thrush, S., Demestre, M. 2007. Functional changes as indicators of trawling disturbance on a benthic community located in a fishing ground (NW Mediterranean Sea). Mar. Ecol. Prog. Ser. 334, 117–129. https://doi.org/10.3354/meps334117

Diesing, M., Stephens, D., Aldridge, J. 2013. A proposed method for assessing the extent of the seabed significantly affected by demersal fishing in the Greater North Sea. ICES J. Mar. Sci. 70, 1085–1096. https://doi.org/10.1093/icesjms/fst066

Dinesen, G.E., Morton, B. 2014. Review of the functional morphology, biology and perturbation impacts on the boreal, habitat-forming horse mussel *Modiolus modiolus* (Bivalvia: Mytilidae: Modiolinae). Marine Biology Research 10(9), 845-870. https://doi.org/10.1080/17451000.2013.866250

Duplisea, D.E., Jennings, S., Warr, K.J., Dinmore, T.A., 2002. A size-based model of the impacts of bottom trawling on benthic community structure. Can. J. Fish. Aquat. Sci. 59, 1785–1795. https://doi.org/10.1139/f02-148

Eckelbarger, K.J., Tyler, P.A., Langton, R.W.1998. Gonadal morphology and gametogenesis in the sea pen *Pennatula aculeata* (Anthozoa: Pennatulacea) from the Gulf of Maine. Marine Biology 132, 677–690.

Edwards, D.C.B., Moore, C.G. 2008. Reproduction in the sea pen *Pennatula phosphorea* (Anthozoa: Pennatulacea) from the west coast of Scotland. Marine Biology 155, 303–314.

Eigaard, O.R., Bastardie, F., Breen, M., Dinesen, G.E., Hintzen, N.T., Laffargue, P., Polet, H. 2016. Estimating seabed pressure from demersal trawls, seines, and dredges based on gear design and dimensions. ICES J. Mar. Sci.: Journal du Conseil 73 (1), i27–i43. https://doi.org/10.1093/icesjms/fsv099

Eigaard, O.R., Bastardie, F., Hintzen, N.T., Buhl-Mortensen, L., Buhl-Mortensen, P., Catarino, R., Dinesen, G.E., Rijnsdorp, A.D. 2017. The footprint of bottom trawling in European waters: distribution, intensity, and seabed integrity. ICES J. Mar. Sci.: Journal du Conseil 74 (3), 847–865.

Ellis, N., Pantus, F., and Pitcher, C.R. 2014. Scaling up experimental trawl impact results to fishery management scales — a modelling approach for a "hot time". Canadian Journal of Fisheries and Aquatic Sciences, 71, 733–746.

Feekings, J., Christensen, A., Jonsson, P., Frandsen, R., Ulmestrand, M., Munch-Petersen, S., Andersen, B. 2015. The use of at-sea-sampling data to dissociate environmental variability in *Nephrops norwegicus* (*Nephrops norwegicus*) catches to improve resource exploitation efficiency within the Skagerrak/Kattegat trawl fishery. Fisheries Oceanography 24(4), 383-392.

Frandsen, R. 2015. Sustainable development of the *Nephrops* fishery in the Kattegat-Skagerrak region. DTU Aqua report 298-2015, 1-23, + appendices.

Frid, C., Paramor, O., Scott, C. 2005. Ecosystem-based fisheries management: progress in the NE Atlantic. Mar. Policy 29, 461–469. https://doi.org/10.1016/j.marpol.2004.07.004

Friedrichs, M., Graf, G., Springer, B. 2000. Skimming flow induced over a simulated polychaete tube lawn at low population densities. Mar. Ecol. Prog. Ser. 192, 219–228. https://doi.org/10.3354/meps192219

Gislason, H., Bastardie, F., Dinesen, G.E., Egekvist, J. and Eigaard, O.R. 2017. Lost in translation? Multi-metric macrobenthos indicators and bottom trawling. Ecological Indicators, 82, 260-270. https://doi.org/10.1016/j.ecolind.2017.07.004

Hiddink, J.G.G., Jennings, S., Kaiser, M.J., Queirós, A.M., Duplisea, D.E., Piet, G.J. 2006. Cumulative impacts of seabed trawl disturbance on benthic biomass, production, and species richness in different habitats. Can. J. Fish. Aquat. Sci. 63, 721–736. https://doi.org/10.1139/f05-266

Hiddink, J.G., Jennings, S., Sciberras, M., Szostek, C.L., Hughes, K.M., Ellis, N., Rijnsdorp, A.D., McConnaughey, R.A., Mazor, T., Hilborn, R., Collie, J.S., Pitcher, C.R., Amoroso, R.O., Parma, A.M., Suuronen, P., Kaiser, M.J. 2017. Global analysis of depletion and recovery of seabed biota after bottom trawling disturbance. Proc. Natl. Acad. Sci. 114, 8301–8306. https://doi.org/10.1073/pnas.1618858114

Hintzen, N.T., Piet, G.J., Brunel, T. 2010. Improved estimation of trawling tracks using cubic Hermite spline interpolation of position registration data. Fish. Res. 101(1), 108–115.

Hintzen, N.T., Bastardie, F., Beare, D., Piet, G.J., Ulrich, C., Deporte, N., Egekvist, J., *et al.* 2012. VMStools: open-source software for the processing, analysis and visualisation of fisheries logbook and VMS data. Fish. Res. 115–116, 31–43.

Hiddink, J.G.G., Jennings, S., Kaiser, M.J., Queirós, A.M., Duplisea, D.E., Piet, G.J. 2006. Cumulative impacts of seabed trawl disturbance on benthic biomass, production, and species richness in different habitats. Can. J. Fish. Aquat. Sci. 63, 721–736. https://doi.org/10.1139/f05-266

Hiddink, J.G., Jennings, S., Sciberras, M., Szostek, C.L., Hughes, K.M., Ellis, N., Rijnsdorp, A.D., McConnaughey, R.A., Mazor, T., Hilborn, R., Collie, J.S., Pitcher, C.R., Amoroso, R.O., Parma, A.M., Suuronen, P., Kaiser, M.J. 2017. Global analysis of depletion and recovery of seabed biota after bottom trawling disturbance. Proc. Natl. Acad. Sci. 114, 8301–8306. https://doi.org/10.1073/pnas.1618858114

Hinz, H., Prieto, V., Kaiser, M.J. 2009. Trawl disturbance on benthic communities: chronic effects and experimental predictions. Ecol. Appl. 19, 761–773. https://doi.org/10.1890/08-0351.1

Hoare, R. and Wilson, E.H. 1977. Observations on the behaviour and distribution of *Virgularia mirabilis* O.F. Müller (Coelenterata: Pennatulacea) in Holyhead harbour. In Proceedings of the Eleventh European Symposium on Marine Biology, University College, Galway, 5-11 October 1976. Biology of Benthic Organisms, (ed. B.F. Keegan, P.O. Ceidigh & P.J.S. Boaden. Oxford: Pergamon Press. Oxford: Pergamon Press, 329-337.

Holte, B. 2001. Possible ecological effects from maldanid (annelida, polychaeta) "super-dominance" in a small North Norwegian sill system. Ophelia 55, 69–75. https://doi.org/10.1080/00785236.2001.10409474

ICES. 2009. Report of the Benchmark Workshop on *Nephrops* assessment (WKNEPH). ICES CM:2009/ACOM:33.

ICES. 2018. OSPAR request on review of the OSPAR case report for the addition of *Haploops* communities to the OSPAR list of threatened and/or declining species and habitats. ICES Special Request Advice, North Atlantic Ecoregion, sr.2018.33, 1-19. https://doi.org/10.17895/ices.pub.4654

Jennings, S., Dinmore, T.A., Duplisea, D.E., Warr, K.J., Lancaster, J.E. 2001. Trawling disturbance can modify benthic production processes. J. Anim. Ecol. 70, 459–475. https://doi.org/10.1046/j.1365-2656.2001.00504.x

Johnson, M.P., Lordan, C. and Power, A.M. 2013. Habitat and Ecology of *Nephrops norvegicus*. Advances in Marine Biology 64, 27-63.

Josefson, A.B., Loo, L.-O., Blomqvist, M., Rolandsson, J. 2018. Substantial changes in the depth distributions of benthic invertebrates in the eastern Kattegat since the 1880s. Ecol. Evol. 8, 9426–9438. https://doi.org/10.1002/ece3.4395

Jägerskiöld, L.A. 1971. A survey of the marine benthonic macro-fauna along the Swedish Wesrcoast 1921-1938. Acta Regiae Societatis Scientiarum et Litterarum Goteburgensis. Zoologica 6, 1-146.

Kaiser, M., Clarke, K., Hinz, H., Austen, M., Somerfield, P., Karakassis, I. 2006. Global analysis of response and recovery of benthic biota to fishing. Mar. Ecol. Prog. Ser. 311, 1–14. https://doi.org/10.3354/meps311001

Kaiser, M.J., Ramsay, K., Richardson, C.A., Spence, F.E., Brand, A.R. 2000. Chronic fishing disturbance has changed shelf sea benthic community structure. J. Anim. Ecol. 69, 494–503. https://doi.org/10.1046/j.1365-2656.2000.00412.x

Kanneworff, E. 1966. On some amphipod species of the genus *Haploops*, with species reference to *H. tubicola* liljeborg and *H. tenuis* sp. nov. from the Øresund. Ophelia 3(1), 183-207.

Kenchington, E.L.R., Gilkinson, K.D., MacIsaac, K.G., Bourbonnais-Boyce, C., Kenchington, T.J., Smith, S.J., Gordon, D.C. 2006. Effects of experimental otter trawling on benthic assemblages on Western Bank, northwest Atlantic Ocean. J. Sea Res. 56, 249–270. https://doi.org/10.1016/j.seares.2006.03.010

Langton, R.W., Langton, E.W., Theroux, R.B., Uzmann, J.R. 1990. Distribution, behaviour and abundance of sea pens, Pennatula aculeata, in the Gulf of Maine. Marine Biology 107, 463-469.

Lehmann, A., Hinrichsen, H.-H., Getzlaff, K., Myrberg, K. 2014. Quantifying the heterogeneity of hypoxic and anoxic areas in the Baltic Sea by a simplified coupled hydrodynamic-oxygen consumption model approach. J. Mar. Syst. 134, 20–28. https://doi.org/10.1016/j.jmarsys.2014.02.012

Manuel, R.L. 1981. *British Anthozoa.* London: Academic Press. Synopses of the British Fauna 18.

McConnaughey, R. A., Syrjala, S. E., Dew, C. B. 2005. Effects of chronic bottom trawling on the size structure of soft-bottom ben- thic invertebrates. In P. W. Barnes, & J. P. Thomas (Eds.), Benthic habitats and the effects of fishing. Bethesda, Maryland: American Fisheries Society, 425–437.

McLaverty, C., Eigaard, O.R., Gislason, H., Bastardie, F., Brooks, M.E., Jonsson, P., Lehmann, A., Dinesen, G.E., 2020. Using large benthic macrofauna to refine and improve ecological indicators of bottom trawling disturbance. Ecological Indicators 110, 105811. https://doi.org/10.1016/j.ecolind.2019.105811

Ministry of Foreign Affairs of Denmark. 1933a. Bekendtgørelse om en under 31. December 1932 mellem Danmark og Sverige angaaende fiskeriforholdende i de til Danmark og Sverige grænsende farvande. BEK no. 228 of 21/06/1933, Ministry of Foreign Affairs of Denmark, J.nr. 63.D.31, 1-5. https://www.retsinformation.dk/pdfPrint.aspx?id=163290

Ministry of Foreign Affairs of Denmark, Royal Regulation 1933b. Anordning om frednings- og ordensbestemmelser af fiskeriet i det til kongerigerne Danmark og Sverige grænsende farvande. AND no. 230 of 29/06/1933, 1-2. https://www.retsinformation.dk/Forms/R0710.aspx?id=79587

Ministry of Foreign Affairs of Denmark. 1990. Bekendtgørelse om vedtægt for fiskeriet i farvandet omkring København og Nordlige Øresund. BEK no. 639 of 17/09/1990, 1-2.

Ministry of Foreign Affairs of Denmark. 2009. Bekendtgørelse om forbud mod visse former for fiskeri i nærmere afgrænsede områder i Kattegat og nordlige del af Øresund. BEK no. 182 of 13/03/2009, Ministry of Food, Agriculture and Fishery, Fisheries Agency, J.nr. 2009-01657, 1-8. https://www.retsinformation.dk/pdfPrint.aspx?id=123851

Ministry of Foreign Affairs of Denmark. 2010. Bekendtgørelse om forbud mod visse former for fiskeri i nærmere afgrænsede områder i Kattegat og nordlige del af Øresund. BEK no. 391 of 16/04/2010, Ministry of Food, Agriculture and Fishery, Fisheries Agency, J.nr. 2010-00997, 1-9. https://www.retsinformation.dk/pdfPrint.aspx?id=131444

Mouillot, D., Graham, N.A.J., Villéger, S., Mason, N.W.H., Bellwood, D.R. 2013. A functional approach reveals community responses to disturbances. Trends Ecol. Evol. 28, 167–177. https://doi.org/10.1016/j.tree.2012.10.004

Molodtsova, T.N. 2004. On the taxonomy and presumable evolutionary pathways of planktonic larvae of Ceriantharia (Anthozoa, Cnidaria). Hydrobiologia, 530/531, 261-266.

Morton, B. 2011. The biology and functional morphology of *Arctica islandica* (Bivalvia: Arcticidae) - A gerontophilic living fossil. Marine Biology Research 7(6), 540-553.

Naturstyrelsen. 2016. Kortlægning af blødbundsområder i Kattegat. Miljø- og Fødevareministeriet report available at https://mst.dk/media/121035/kortlaegning-af-bloedbundsomraader-i-kattegat.pdf

Nicol, J.A. 1958: Observations on the luminescence of *Pennatula phosphorea*, with a note on the luminescence of *Virgularia mirabilis.* Journal of the Marine Biological Association of the United Kingdom, 37, 551-563.

O'Reilly, R., Kennedy, R., Patterson, A. 2006. Destruction of conspecific bioturbation structures by Amphiura filiformis (Ophiuroida): evidence from luminophore tracers and in situ time-lapse sediment-profile imagery. Mar. Ecol. Prog. Ser. 315, 99–111. https://doi.org/10.3354/meps315099

OSPAR Commission. 2008. OSPAR list of threatened and/or declining species and habitats. Reference no. 2008-6, 1-5.

OSPAR Commission. 2010. Background document for sea pen and burrowing megafauna communities. Biodiversity Series 2010, 481, 1-26.

Pearson, T., Rosenberg, R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology: An Annual Review 16, 229–311.

Petersen, C.G.J. 1913. Om havbundens dyresamfund og disses betydning for den marine zoogeografi. Havets Bonitering II. Beretning fra den danske biologiske station, 21. Köbenhavn 1913.

Petersen, C.G.J. 1918. The sea bottom and its production of fish food. A survey of the work done in connection with the valuation of the Danish waters from 1883-1917. Rep Danish Biol Station 25, 1–62.

Pommer, C., Olesen, M., Hansen, J. 2016. Impact and distribution of bottom trawl fishing on mud-bottom communities in the Kattegat. Mar. Ecol. Prog. Ser. 548, 47–60. https://doi.org/10.3354/meps11649

Powell, A. and Eiriksson, S.P. 2013. Reproduction: Life cycle, larvae and larviculture. Advances in Marine Biology 64, 201-245.

Queirós, A.M., Hiddink, J.G., Kaiser, M.J., Hinz, H. 2006. Effects of chronic bottom trawling disturbance on benthic biomass, production and size spectra in different habitats. J. Exp. Mar. Bio. Ecol. 335, 91–103. https://doi.org/10.1016/j.jembe.2006.03.001

Reiss, H., Greenstreet, S., Sieben, K., Ehrich, S., Piet, G., Quirijns, F., Robinson, L., Wolff, W., Kröncke, I. 2009. Effects of fishing disturbance on benthic communities and secondary production within an intensively fished area. Mar. Ecol. Prog. Ser. 394, 201–213. https://doi.org/10.3354/meps08243

Reiss, J., Bridle, J.R., Montoya, J.M., Woodward, G. 2009. Emerging horizons in biodiversity and ecosystem functioning research. Trends Ecol. Evol. 24, 505–514. https://doi.org/10.1016/j.tree.2009.03.018

Rice, J., Arvanitidis, C., Borja, A., Frid, C., Hiddink, J.G., Krause, J., Lorance, P., Ragnarsson, S.Á., Sköld, M., Trabucco, B., Enserink, L., Norkko, A. 2012. Indicators for sea-floor Integrity under the European Marine Strategy Framework Directive. Ecol. Indic. 12, 174–184. https://doi.org/10.1016/j.ecolind.2011.03.021

Rigolet, C., Dubois, S.F., Droual, G., Caisey, X. and Thiebaut, E. 2012. Life history and secondary production of the amphipod *Haploops nirae* (Kaim-Malka, 1976) in the Bay of Concarneau (South Brittany). Estuarine, Coastal and Shelf Science 113, 259-271.

Rigolet, C., Dubois, S.F. and Thiebaut, E. 2014. Benthic control freaks: Effects of the tubicolous amphipod *Haploops nirae* on the specific diversity and functional structure of benthic communities. Journal of Sea Research 85, 413-427. https://doi.org/10.1016/j.seares.2013.07.013

Rumohr, H., Krost, P. 1991. Experimental evidence of damage to benthos by bottom trawling with special reference to *Arctica islandica*. Meeresforschung/Rep Mar. Res. 3, 340–345.

Sköld, M., Gunnarsson, J. 1996. Somatic and germinal growth of the infaunal brittle stars Amphiura filiformis and A. chiajei in response to organic enrichment. Mar. Ecol. Prog. Ser. 142, 203–214. https://doi.org/10.3354/meps142203

Sköld, M., Göransson, P., Jonsson, P., Bastardie, F., Blomqvist, M., Agrenius, S., Hiddink, J., Nilsson, H., Bartolino, V. 2018. Effects of chronic bottom trawling on soft-seafloor macrofauna in the Kattegat. Mar. Ecol. Prog. Ser. 586, 41–55. https://doi.org/10.3354/meps12434

Sköld, M., Rosenberg, R. 1996. Arm regeneration frequency in eight species of ophiuroidea (Echinodermata) from European sea areas. J. Sea Res. 35, 353–362. https://doi.org/10.1016/S1385-1101(96)90762-5

Solan, M. 2004. Extinction and ecosystem function in the marine benthos. Science 80, 306, 1177–1180. https://doi.org/10.1126/science.1103960

Stephensen, K. 1928. Storkrebs II. Tanglopper. – Damarks Fauna 32. G.E.C. Gads Forlag. København, 1-399.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. Biological Reviews, 25, 1-45.

Tillin, H., Hiddink, J., Jennings, S., Kaiser, M. 2006. Chronic bottom trawling alters the functional composition of benthic invertebrate communities on a sea-basin scale. Mar. Ecol. Prog. Ser. 318, 31–45. https://doi.org/10.3354/meps318031

Tuck, I.D., Hall, S.J., Robertson, M.R., Armstrong, E. and Basford, D.J. 1998. Effects of physical trawling disturbance in a previously unfished sheltered Scottish sea loch. Marine Ecology Progress Series, 162, 227-242.

van Denderen, P., Bolam, S., Hiddink, J., Jennings, S., Kenny, A., Rijnsdorp, A., van Kooten, T. 2015. Similar effects of bottom trawling and natural disturbance on composition and function of benthic communities across habitats. Mar. Ecol. Prog. Ser. 541, 31–43. https://doi.org/10.3354/meps11550

Van Hoey, G., Borja, A., Birchenough, S., Buhl-Mortensen, L., Degraer, S., Fleischer, D., Kerckhof, F., Magni, P., Muxika, I., Reiss, H., Schröder, A., Zettler, M.L. 2010. The use of benthic indicators in Europe: From the Water Framework Directive to the Marine Strategy Framework Directive. Mar. Pollut. Bull. 60, 2187–2196. https://doi.org/10.1016/j.marpolbul.2010.09.015

Van Soest, R.W.M. 2000. Family Suberitidae Schmidt, 1870. Pp. 227-244 in: Systema Porifera Vol 1. A guide to classification of sponges. KluwerAcademic/Plenum Publishers. New York, 1-1101.

Woodward, G., Ebenman, B., Emmerson, M., Montoya, J.M., Olesen, J.M., Valido, A., Warren, P.H. 2005. Body size in ecological networks. Trends Ecol. Evol. https://doi.org/10.1016/j.tree.2005.04.005

Zettler, M.L., Proffitt, C.E., Darr, A., Degraer, S., Devriese, L., Greathead, C., Kotta, J., Magni, P., Martin, G., Reiss, H., Speybroeck, J., Tagliapietra, D., Van Hoey, G., Ysebaert, T. 2013. On the Myths of Indicator Species: Issues and Further Consideration in the Use of Static Concepts for Ecological Applications. PLoS One 8, e78219. https://doi.org/10.1371/journal.pone.0078219

Appendices

A.1 Project organisation

This project, "Development of sustainable fisheries management and monitoring of sensitive soft-bottom habitats and species in the Kattegat" (in Da.: "Udvikling af bæredygtig forvaltning af følsomme habitater og arter i Kattegat"), was conducted as a collaboration between the National Institute of Aquatic Resources (DTU Aqua), the National History Museum of Denmark (SNM), the Danish Fisheries Production Organisation (DFPO) and the World Wide Fund for Nature in Denmark (WWF DK).

DTU Aqua was responsible for leading and conducting of the scientific tasks in the project (WP1-4), as well as of the scientific dissemination of WP5, D5.1 and WP5, D5.4). Participants: Henrik Gislason (lead), Grete E. Dinesen (co-lead), Ciaran McLaverty, Ole R. Eigaard and Eva Maria Petersen.

SNM contributed to the scientific tasks, and especially to Chapter 2 of this report. Participants: Ole S. Tendal, †Passed away 7th April 2020.

DFPO was responsible for the administrative lead of the project (WP5, D5.5) and for several tasks, including the engagement of fishers to participate in the acoustic seabed mapping (WP2, D2.1), conducting fisheries interviews, acoustic cruise planning and retrieving of SODENA data (WP1, D1.2), conducting a fisheries-scientist workshop (WP5, D5.2) and stakeholder forum (WP5, D5.3), as well as for the development of an interactive ocean floor model for public dissemination (WP5, D5.1). Participants: Jonathan B. Jacobsen (initial lead), Sofie Smedegaard Mathiesen (lead) and Henrik Lund.

WWF Denmark contributed to discussions during the stakeholder forum (WP5, D5.3) and participated in the development of the interactive ocean floor model for public dissemination (WP5, D5.1). Participants: Iben Wiene Rathje, Peter Blanner and Thomas Kirk Sørensen.

The project comprised five work packages (WPs). The individual deliverables (Ds) and were to find the results are listed below.

WP1. Mapping of sensitive habitats and species and fishery with bottom towed gears

D1.1: Analysis of the spatial distribution of fishing intensity and identification of non-fished areas.
Completed, see results in Chapter 4.

D1.2: Fisheries interviews and input data from SODENA
Completed, see results in Chapter 3.

D1.3: Existing data of sensitive species collated
Completed, see results in Chapter 2.

D1.4: KASU survey data of relevance identified and prepared for use
Completed.

D1.5: Data collated in a GIS database
Completed. The GIS information was used to plan the 14-days field survey in September-October 2016, and further used for extracting of environmental variables used in the faunal analyses; see results in Chapter 5 and 6.

D1.6. Fisher-scientist workshop and acoustic cruise planning
Completed, see Chapter 3 and 7.

D1.7. New data collated in a GIS database
Completed.

WP2. Acoustic and visual mapping of sensitive habitats and species
Completed in part, see below and results in Chapter 3 and 7.

D2.1: Selection of fishers and 4-5 fishing vessels to take part in the acoustic field survey
Completed, however, it was only possible to engage one commercial fishing vessel in this project.

D2.2: 15 days of acoustic field surveys with commercial vessels
The acoustic field survey included recording of acoustic information of the seafloor using side scan sonar (SSS) and under water video. Due to the availability of one fishing vessel (from Østerby Havn, Læsø), the acoustic field survey was downscaled to 3 days in the Vinga trench in June 2016. This area is topographically the most complex area in the Kattegat, which encompass both the highest fisheries intensity and non-fished areas and several of the suggested MSFD areas. Instead, more stations and samples were included in the research survey conducted by DTU Aqua in September – October 2016.

D2.3: SSS and under water video screening of physical and biological structures
Conducted at five selected sites in the northern Kattegat, see Chapter 3.

D2.4: SSS and video data work up and analyses for AP4
Completed, see methods and results Chapter 3.

D2.5: Protocol for acoustic methods for identification of physical and biological structures
Completed, see results in Chapter 3.

WP3. Quantitative biodiversity survey of sensitive habitats in closed and fished areas

D3.1: Meetings to plan the research survey design.
Completed.

D3.2: 14 days research survey of the quantitative biodiversity
Conducted with the *R/V Havfisken* from 23 September to 6 October 2016.

D3.3: Data laboratory workup, calibration and preparation for analyses in WP4.
Completed, see methods in Chapter 5 and 6.

D3.4: Photo documentation selected and prepared for use in WP5.
Completed, see results in Chapter 2, 5 and 7.

WP4. Sustainable management of sensitive habitats and species

D4.1: Review of sensitive habitats and species in the Kattegat.
Completed, see Chapter 2.

D4.2: Review of the use of fishery closed areas as management measure to protect marine sensitive habitats and species.
Completed, see conclusion in Chapter 9.

D4.3: Analyse of the distribution of sensitive habitats and species based on data collated in WP1-3.
Completed, see results in Chapter 5 and 6.

D4.4: Test of UWTV and BRUV as environmental sensitive and cost-effective monitoring methods
Completed, see results in Chapter 5.

D4.5: Modelling of fauna across a fisheries gradient including un-trawled ('*de facto*' closed) areas
Completed, see results in Chapter 5 and 6.

WP5 Communication and administration

D5.1: Development of interactive ocean floor model
Completed. The results of the project were used to develop an interactive ocean floor model that depicts the areas where sensitive habitats and species are found, and where trawling after *Nephrops norvegicus* occurs in the Kattegat.

D5.2: Fisheries-scientist workshop
Completed. A workshop for fishers and scientists took place on the 13th December 2018 at Strandby Badehotel in Strandby (see Appendix A.2).

D5.3: Stakeholder forum
Completed. The stakeholder forum took place on the 21st of November 2018 at Axelborg in Copenhagen (see Appendix A.2).

D5.4: Communication about the activities and the results
Completed. Communication about the project activities and results was done in multiple ways at stakeholder workshops, Danish and international conferences, university courses, scientific journals and in public papers as well as videos (see details in Appendix A.2).

D5.5: Administration
Completed.

A.2 List of project deliberations

All public project deliberations listed below as document A-K were attached as appendices to of the final project report submitted on the 29th March 2019.

A.2.1 Peer reviewed paper and submitted manuscript
A. Available online at DOI:10.1016/j.ecolind.2017.07.004:
Gislason, H., Bastardie, F., Dinesen, G.E., Egekvist, J. and Eigaard, O.R. 2017. Lost in translation? Multi-metric macrobenthos indicators and bottom trawling. Ecological Indicators, 82, 260-270.

B.
McLaverty, C., Eigaard, O.R., Gislason, H., Bastardie, F., Brooks, M.E., Jonsson, P., Lehmann, A. and Dinesen, G.E. 2019. A size-based approach to assessing the response of benthic communities to bottom trawling. Manuscript submitted to Ecological Indicators, February 2019. See Appendix A.3 herein for the pre-published version.

A.2.2 Conference contributions, abstracts of oral presentations and posters
C. Abstract of the oral presentation is available online:
Gislason, H., Bastardie, F., Dinesen, G.E., Egekvist, J., and Eigaard, O.R. 2017. Hvordan påvirker bundtrawlfiskeriet Kattegats bundfauna? En analyse af ændringer i densitet og artsrigdom og en diskussion af potentielle indikatorer. Abstract of oral presentation at the 19th Danish Marine Science Meeting, Helsingør, 25.-27. January 2017.

D. The poster abstract is available online (in Danish). The associated two minutes underwater video is available upon request to DTU Aqua:
Dinesen, G.E., Gislason, H., McLaverty, C., Bastardie, F., Veicherts, NM. And Feekings, J. 2017. Scavenging strategies of hagfish in the Kattegat. Poster presentation at the 19th Danish Marine Science Meeting, Helsingør, 25.-27. January 2017.

E.
Gislason, H. Bastardie, F., Dinesen, G., Egekvist, J. and Eigaard, O.R. 2018. Macrobenthos biodiversity indicators – density and species density revisited. Oral presentation at The World Conference of Marine Biodiversity (WCMD), Montreal, 13-16 May 2018.

F.
McLaverty, C., Gislason, H., Eigaard, O.R., Lehmann, A. and Dinesen, G.E. 2018. Effect of trawling on taxonomic and functional diversity in benthic communities: Does size matter? Oral presentation at The World Conference of Marine Biodiversity (WCMD), Montreal, 13-16 May 2018.

G. Available online at doi.org/10.7287/peerj.preprints.26739v1:
Dinesen, G.E., Gislason, H., McLaverty, C., Bastardie, F. and Eigaard, O.R. 2018. Bottom trawling effects on marine macrobenthos: Changes in ecological functioning interpreted by a biological multiple traits approach. Oral presentation at The World Conference of Marine Biodiversity (WCMD), Montreal, 13-16 May 2018.

A.2.3 Stakeholder meetings
H. Invitation to:

Stakeholder forum: Stakeholder forum – udvikling af bæredygtig forvaltning af følsomme habitater og arter i Kattegat. København, 21 November 2018 kl. 9:00am-12:00am.

I. Invitation to:

Fishers and scientists workshop: Workshop – udvikling af bæredygtig forvaltning af følsomme habitater og arter i Kattegat. Strandby, 13 December 2018, 9:30am-12:30am.

A.2.4 Public papers
J.

Jensen, L.D. 2018. Følsomme arter og lukkede områder under luppen. Fiskeritidende, Saturday 1 December 2018, p. 13.

K.

Bundtrawl har ikke stor påvirkning på havbundens dyreliv. Fiskeritidende, Saturday 22 December 2018, p. 10-11.

A.2.5 Teaching
The project results were presented in part at the postgraduate course *Biological Oceanography* (Technical University of Denmark, Lyngby) for ~25 students in March 2017 and March 2018, and at the postgraduate course *Marine Organism* (University of Copenhagen, Helsingør) for ~20 in August 2017 and August 2018. PowerPoint presentations in pdf format are available upon request to DTU Aqua, contact: gdi@aqua.dtu.dk).

A.3 Pre-printed version of McLaverty *et al.*, 2020

Using large benthic macrofauna to refine and improve ecological indicators of bottom trawling disturbance

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Keywords

Keywords: benthic community; benthic indicators; ecosystem-based approach; fisheries management; functional traits; seafloor disturbance; trawling impacts.

Abstract

Bottom trawling alters the abundance, diversity, size-composition, and function of benthic communities. However, the ability to detect these impacts over large spatial scales can be obscured by various complicating factors, such as community adaptation to disturbance and co-varying environmental conditions. An ecosystem-based approach to fisheries management therefore requires ecological indicators which can 'disentangle' trawling effects from other natural and human drivers, and respond effectively to shifts in ecological quality. We collected benthic macrofaunal samples at 21 sites across a Norway lobster *Nephrops norvegicus* fishing ground in the Kattegat, and separated the benthic community into small (1-4mm) and large (>4mm) size fractions. Four taxonomic indicators (total density, species density, Shannon diversity, and biomass) and four functional indicators (functional diversity, functional richness, functional evenness, and functional dispersion) were calculated based on each size fraction, and the two fractions combined (pooled community). Here, we compare the ability of these indicators to detect trawling impacts across size categories. We show that indicators derived from large macrofauna were highly effective in this regard, and were less influenced by other environmental drivers, such as depth, sediment grain size, bottom current velocity, salinity, and temperature. This suggests that the taxonomic and functional characteristics of benthic communities display a size-dependent sensitivity to trawling disturbance, and therefore community metrics based on large benthic macrofauna may provide useful indicators. By contrast, indicators derived from the small fraction performed poorly, and those based on the pooled community demonstrated a varied ability to detect trawling. Small macrofauna are typically characterised by high density, diversity, and population growth rates, and their relative resilience to trawling may mask the response of the more sensitive

macrofauna. This highlights an underlying issue with calculating indicators based on the whole benthic community. The approach outline here is easily applied, improves indicator performance, and has the potential to reduce laboratory workloads due to the fewer taxa and individuals required for analyses.

1. Introduction

In recent years, the mapping and quantification of commercial trawling effort has greatly improved. This has come about due to the increased availability of spatially resolved information on fishing vessel activities, and the development of open source methods to estimate fishing effort (Bastardie et al., 2010; Hintzen et al., 2012). As a result, there is an improved understanding of the distribution of commercial trawling effort (Amoroso et al., 2018; Eigaard et al., 2017; Puig et al., 2012), and how gradients of trawling intensity alter the structure and ecological functioning of benthic macrofaunal communities (Bolam et al., 2017; Hiddink et al., 2017; Hinz et al., 2009; Tillin et al., 2006). Growing societal concern regarding fisheries effects has prompted the adoption of an 'Ecosystem Approach to Fisheries Management' (EAFM) (FAO, 2003) in the European Union. This has seen increased scientific interest and resources dedicated to the monitoring and assessment of 'Sea-floor Integrity' (Anon., 2010) and 'Good Environmental Status' of seafloor ecosystems, under the European Union Marine Strategy Framework Directive (MSFD) (Anon., 2008). By definition, an EAFM requires assessments of human impacts at the level of species, communities, and ecosystems to guide management advice. Ecological indicators are often a favoured instrument in this regard as they are easily calculated, monitored, and interpreted. However, in order to be effective, indicators should be sensitive to changes in ecological state, capture and convey information on the ecosystem, and not be overly affected by short-term variation or auxiliary drivers. Measuring all aspects of ecosystem state is neither practical nor possible, and well-designed indicators of bottom trawling impacts are therefore required to support management (Rice et al., 2012; Van Hoey et al., 2010).

As knowledge of the benthic effects of trawling has advanced, various co-varying and interacting factors have also been revealed. These can include the sensitivity of specific seabed habitats (Kaiser et al., 2006), the impact of different gear types (Eigaard et al., 2016; Hiddink et al., 2017), the magnitude of background human and natural disturbance (Diesing et al., 2013; Van Denderen et al., 2015), and gradual adaptations of macrofaunal communities to disturbance (Kaiser et al., 2000; Reiss et al., 2009). Alone, or in combination, such factors can obscure the measurable effects of trawling in benthic communities, and may explain why indicators sometimes display varied performance (Atkinson et al., 2011; Kaiser et al., 1998; Mangano et al., 2014). On the other hand, experimental and model based studies have revealed that large macrofauna are disproportionately sensitive to chronic trawling disturbance (Duplisea et al., 2002; Jennings et al., 2001; McConnaughey et al., 2005; Queirós et al., 2006). This vulnerability is linked to a relationship between body size and several key life history traits (Begon et al., 2006), whereby larger macrofauna tend to grow and reach maturity at a slower rate, have comparatively lower mortality and population growth rates, and are therefore more vulnerable to trawling induced mortality. Although small benthic fauna are also vulnerable trawling

disturbance (Hinz et al., 2008), they often display relatively high growth and turnover rates, resulting in shorter recovery times.

While body size and sensitivity may vary across taxa, sensitivity may also change within the lifespan of an individual. During development from offspring to adult, sensitivity to physical disturbance can vary across size classes, trophic levels, and functional roles (Brose et al., 2016). For this reason, indicators based on e.g. the fraction of individuals above a certain minimum body size have been suggested and applied to monitor fish communities (Greenstreet et al., 2011; Shin et al., 2005), yet no such size-based indicators exist for benthic macrofauna. Recent studies by Hiddink et al., (2018) and Rijnsdorp et al., (2018) have shown that the relative abundances of long-lived macrofauna can indicate the sensitivity of benthic communities to trawling. This approach combines information on age composition of the community with fishing pressure data, and allows for the assessment of risks and impacts of trawling to taxa with defined longevity or recovery times (Hiddink et al., 2018). While this approach has shown to be effective, the longevity of macrofauna can be difficult to measure, may be be uncertain, and may be lacking for many species in less studied areas. It would therefore be advantageous if macrofaunal indicators of trawling impacts could be devised based on the size composition of the benthic organisms. Such indicators could be easily and accurately quantified, would not require knowledge of life history traits, would have the potential to be applied across habitat type and geographic region, and could potentially reduce sample processing times.

The main objective of this study is to explore if the performance of several taxonomic and functional indicators of trawling disturbance would improve if they were based on large macrofauna, rather than the whole community. To test this, data were collected in a fishing ground with a long history of bottom trawling. Sampling sites were selected to represent a wide spatial gradient of trawling intensity, ranging from zero (determined by long-standing closed areas) to regionally high trawling intensities. During sampling, sieves were used to separate the benthic community into two size fractions. Eight macrofaunal indicators were then calculated within each size fraction (small and large), as well as the fractions combined (pooled community). We investigate and compare the ability of these indicators to detect bottom trawling impacts across size categories using generalised linear mixed models (GLMMs).

2. Materials and Methods

2.1. Study area

The Kattegat is a shallow transitional sea separating the marine North Sea and the brackish Baltic Sea (Figure 1). Its seabed is composed of sandy sediments in the west, and of mud and mixed sediments in the north, east, and deeper areas (Figure 1b). Physical conditions on the seabed are characterised by gradients in salinity, temperature, current velocity, and by a two-layer stratified flow pattern. An inflow of saline water from the North Sea occurs in the deeper areas, which is offset by an outflow of brackish water from the Baltic Sea at the surface. The two water masses are separated by a pycnocline which sits between 15m (westwards) and 25m (eastwards) (Al-Hamdani et al., 2007). Commercial fishing for cod *Gadus morhua* (Linnaeus, 1758) and plaice *Pleuronectes platessa* (Linnaeus, 1758) has taken place in the Kattegat for over 100 years (Petersen, 1918). In recent years, high fishing effort has led to significant declines in the cod population and associated quota (Svedäng and Bardon, 2003; ICES, 2018). Conversely, there has been a considerable rise in landings of Norway lobster *Nephrops norvegicus* (Linnaeus, 1758). Although this may occur as a result of population increase (ICES, 2018b), or community shifts associated with reduced predation pressure (Howarth et al., 2014), time series data of commercial CPUE (catch per unit effort) suggest that increased fishing effort is the likely explanation (Eggert and Ulmestrand, 1999). *Nephrops,* now the primary target species in the Kattegat, is caught almost exclusively by Danish and Swedish vessels using demersal otter trawls (Hornborg et al., 2017). As *Nephrops* reside in shallow burrows, the fishery is characterised by relatively low catchability and high trawling intensity. As a result, the Kattegat is an area of relatively high trawling intensity at European (Eigaard et al., 2017) and global scale (Amoroso et al., 2018). Based on trawling effort in 2017, we estimate that ~53% of all sublittoral mud habitat in the Kattegat (A5.3 - EUNIS level 3) (Figure 1b) was impacted by mobile bottom-contacting fishing gear.

Figure 1: Map of sampling sites in respect to (a) trawling effort and (b) EUNIS Level 3 habitat types. Trawling intensity is described as the swept Area Ratio (SAR), and represents the cumulative trawling effort over 3 years (September 2013 to September 2016) for all Danish and Swedish vessels >12m, using bottom contacting gears.
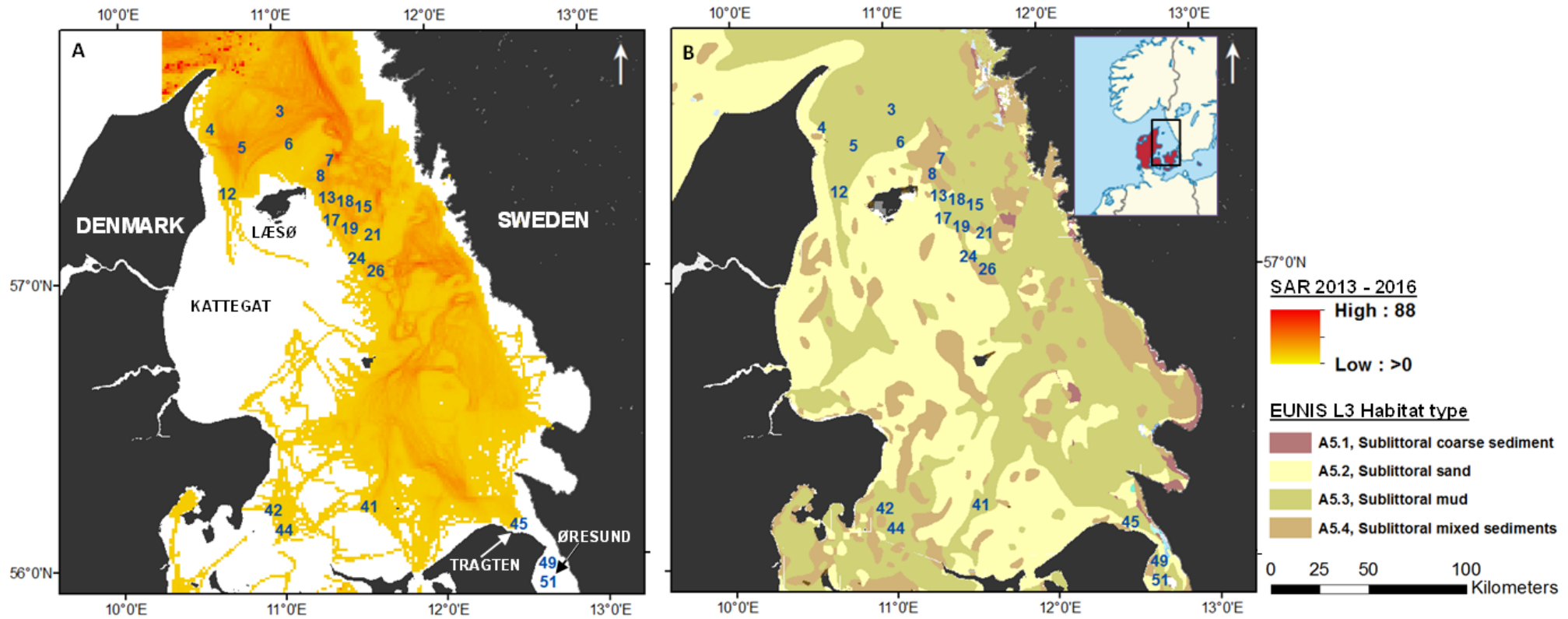
2.2.   Sampling design

Sampling sites were selected within areas characterised by the sediment type and depth suitable for *Nephrops*. The locations of sites were restricted to Danish waters and were chosen based on, i) a minimum depth of 15m, ii) mud (EUNIS A5.3) and mixed sand/mud (EUNIS A5.4) habitat (Figure 1b), iii) within a gradient of trawling intensity between 2013 -2016 (see section 2.7 - *Estimating trawling pressure*) (Figure 1a). Furthermore, some sites were determined by side scan sonar and consultation with local fishermen. As depth has been shown to be a potentially confounding factor in similar studies (Pommer et al., 2016), we selected areas of high and low trawling intensity in both shallow and deeper areas. The sampling design also included three sites located in areas subject to long-standing trawling closures; two in the Øresund, and the a third in nearshore areas of the 'Tragten' (Figure 1a). These areas have been subject to a trawl ban since 1932 under a Danish-Swedish Royal Convention (Ministry of Foreign Affairs of Denmark, 1933a; 1933b, 2009). Comparability of abiotic habitat characteristics were verified post-sampling by means of statistical analysis (detailed in the section *2.8 Environmental drivers*).

2.3.   Data collection and sample processing

Macrofauna communities were sampled at 21 sites (Figure 1) between 22 September and 6 October 2016. Five sediment samples were taken at each site using a 0.1m^2 Van-Veen grab. Four samples were processed for faunal analysis, and a fifth was sub-sampled for particle size analysis (PSA) and organic content. The faunal samples were sieved consecutively over 4mm and 1mm mesh sizes to separate the community into two size fractions. A 1mm mesh size is widely used as benthic macrofauna are generally defined as metazoans retained by a 1mm sieve, and a 4mmthreshold is used to distinguish larger macrofauna (also known as megafauna). The sieved samples were then fixed in 4% borax-buffered formaldehyde in ambient seawater. In the laboratory, all animal material were identified to the lowest taxonomic level possible, and biomass estimates per species were determined as ash-free dry weight. All tubicolous polychaetes were removed from their tubes prior to weighing. Taxa were validated and standardised in accordance with World Register of Marine Species (WoRMS Editorial Board, 2018). The size-separation the community provided three datasets carried forward for analysis; the 1-4mm (small) fraction, the ≥4mm (large) fraction, and the two fractions combined (pooled community). Samples acquired for PSA and LOI% were first frozen on board at - 18°C. PSA was undertaken via mass loss following wet sieving (Danish Standard (DS) 405.9), and resulted in the estimation of mud% (<63µm), sand% (0.063–2mm), and gravel% (≥2mm). Organic content was estimated via loss-on-ignition (LOI%), and calculated by mass loss of 4mg of homogenised sediment placed in a muffle furnace at 560°C for 4 hours, or until a constant weight was achieved (DS 204).

2.4.   Biological trait classification

An overview of the biological traits and associated categories (modalities) used to describe the functional characterises of the community is presented in Table 1. Some 10 traits and 48 descriptive modalities were developed by Bolam et al.(2017), based on their potential sensitivity to trawling. As marine taxa often exhibit more than one modality within a trait (e.g. multiple feeding modes), we followed a 'fuzzy coding' approach (Chevene et al., 1994). If a species exhibited full affinity to a single modality with in a trait (e.g. age), a score of 3 was assigned. Where species exhibited more than one modality, they were assigned a score relative to its importance (e.g. a predator which occasionally scavenges was coded as Predator = 2, Scavenger = 1). After coding, the scores for each modality were standardised to 1 within each trait type. The coded trait scores (taxa-by-trait matrix) were then combined with abundance data (abundance-by-site), to generate a trait-by-site matrix.

Table 1: Biological traits and associated modalities used in the study

Trait	Modalities
Size range	<10mm | 11-20mm | 21-100mm | 101-200mm | 201-500mm | >500mm
Longevity	<1 year | 1-<3 years | 3-10 years | >10 years
Larval development	Planktotrophic | Lecithotrophic | Direct
Morphology	Soft | Crustose | Cushion | Stalked | Tunic | Exoskeleton (chitin/calcium carbonate)
Egg development	Asexual/Budding | Sexual: shed eggs pelagic | Sexual: shed eggs benthic | Sexual: brood eggs
Living habit	Tube-dwelling | Burrow-dwelling | Free-living | Crevice/hole/under stone | Attached to substratum | Epi/endozoic/phytic
Sediment position	Surface | Infauna: 0-5cm | Infauna: 6-10cm | Infauna: >10cm
Feeding mode	Suspension | Scavenger/Opportunist | Surface Deposit | Subsurface deposit | Predator | Parasite
Mobility	mob Sessile | Burrower | Swim | Crawl/creep/climb
Bioturbators	Diffusive mixing | Surface deposition | Downwards conveyer | Upward Conveyor | None

2.5.   Ecological indicators

Ecological indicators were based on the benthic macrofauna, and chosen to reflect the ecological, taxonomic, and functional characteristics of the community. Taxonomic indicators (density, species density, Shannon diversity and biomass) were calculated using PRIMER v.7 (Clarke and Gorley, 2015) and functional indicators (functional diversity, functional richness, functional evenness, and functional dispersion) were calculated using the R package 'FD' (Laliberte, Legendre & Shipley, 2015). Table 2 provides a description of the indicators and their interpretation. The functional indicators were weighed using density data, as this data is more

commonly recorded in benthic impact studies (Hiddink et al., 2017). For completeness, we also provide the results of functional indicators based on biomass data (Supplement – Table S2).

Table 2: Macrofaunal indicators used in the study

Indicator	Label	Description	Range of values	Reference
Density	N	Total number of individuals per sample.	0 - ∞	
Species density	S	Total number of species per sample.	0 - ∞	
Shannon diversity	H'	A measure of taxonomic diversity, which accounts for S and N, and the structure (dominance) of a community. Calculated using Log_e.	0 - ∞	(Shannon, 1948)
Log biomass	Biomass	Total ash free dry weight biomass per sample. Log_e transformed.	0 - ∞	
Functional diversity	RaoQ	A measure of trait diversity, which accounts for trait richness and relative abundance. Rao's quadratic entropy measures the trait dissimilarity of two random individuals (akin to Simpson's diversity).	0-1	(Botta-Dukát, 2005; Lepš et al., 2006)
Functional richness	Fric	A measure of the richness of traits expressed in the community, measured by the total volume of multivariate trait space occupied by the community. Calculated using minimum convex hull.	0-1	(Villéger et al., 2008)
Functional evenness	Feve	A measure of trait dominance, defined by the evenness in distribution of trait abundance in multivariate trait space. Calculated using minimum convex hull.	0-1	(Villéger et al., 2008)
Functional dispersion	Fdis	A measure of the average distance of species to the centre of multivariate trait space.	0 - ∞	(Laliberté and Legendre, 2010)

2.6. Potential recruitment effect on indicators

The recruitment of benthic macrofauna typically takes place between August and November in the Kattegat. As sampling was conducted in mid-September/early October, there is the risk that newly settled recruits may be recorded in the data. These individuals may consequently have had little or no exposure to trawling, and could potentially bias results. However, recruits would need to settle (or begin ontogenetic development) and reach ≥1mm in size by the end of September for this to occur. We therefore considered the potential contribution of new recruits to our measures of density (N). Of the most abundant taxa (Table 3), early juveniles of *Phoronis* sp., *Kurtiella bidentata*, *Nucula nitidosa*, *Thyasira flexuosa* and *Scalibregma inflatum*, were considered too small or soft bodied to be retained on a 1mm sieve at the time of sampling. Moreover,

early juveniles of these taxa were not observed during the processing of samples. Conversely, *Amphiura filiformis* and *Amphiura chiajei* are relatively large macrofaunal taxa and have the potential for rapid post-settlement growth rates (Josefson and Jensen, 1992). Recently settled (<1 year) individuals were also observed in the small fraction during sample processing. We thus estimated the potential growth rate of newly settled *Amphiura* spp., in accordance with Sköld et al. (2001), to determine if our results could be affected by an influx of *Amphiura* spp. prior to sampling. The details of these estimates are outlined in the Supplement – Text S1. Accordingly, we took the conservative measure of also removing 50% of all *Amphiura* spp. from the dataset, and re-analysing pooled community N and small fraction N without these individuals. The results are provided in section 3.4.1.

2.7. Estimating trawling pressure

Since 2012, all fishing vessels ≥12m operating in European Union waters have been required to carry a vessel monitoring system (VMS) receiver on board. VMS receivers collect and send data regarding the vessel location, heading, and speed, with an hourly polling frequency (in Danish and Swedish waters). Raw VMS data were cleaned and filtered to retain only 'trawling activity' (defined as speed ranging between 2 and 4 knots, with a minimum distance of 3km from port). These raw data points were then used to reconstruct vessel trawl tracks using cubic Hermite spline interpolation (Hintzen et al., 2012). The tracks were coupled with information on vessel size (from EU logbooks) and gear dimensions (Eigaard et al., 2016) to estimate gear-width, and aggregated to calculate the area of seabed 'swept' during each logbook trip. Trawling effort and swept areas were estimated using the VMStools package (Hintzen et al., 2012). The total swept areas (for all Danish and Swedish ≥12m vessels and trips) were aggregated within a 1km radius around each benthic sampling site. This spatial scale represents a trade-off between precision limitations in i) VMS-positioning, ii) gear position in relation to vessel position, and iii) interpolation of trawl tracks. With the choice of a 1 km radius, trawling would tend to be randomly distributed within years and uniformly spread on longer timescales (Amoroso et al., 2018; Ellis et al., 2014), and the potential error associated with the spline interpolations is likely to be limited (Lambert et al., 2012). Trawling intensity is described as swept-area ratio (SAR) values, defined as the accumulated swept area within a circle divided by the size of the circle. SAR can therefore be interpreted as the number of times the seabed within the circle is trawled within the given time period. To account for inter-annual variation in trawling activity at infrequently fished sampling sites, and due to the focus of the analysis on larger, longer-lived, macrofauna, SAR estimates were based on three years of fishing effort. Trawling intensity was back-calculated from the day prior to sampling for each replicate sample (September/October 2013 to September/October 2016).

2.8.   Environmental drivers

The structure and composition of benthic communities are known to be closely linked to ambient environmental conditions. To account for key natural drivers, and ascertain potentially confounding effects, we included six environmental parameters in addition to trawling intensity. Depth (*in situ*), LOI%, and mud% (described above) were collected in the field. Hydrodynamic data in the form of bottom current velocity (m/s), bottom temperature (°C), and minimum bottom salinity (PSU), were extracted from the high-resolution Kiel Baltic Sea Ice-Ocean Model (BSIOM) (Lehmann et al., 2014) (further information can be found in the Supplement – Text S2). Hydrodynamic data were back-calculated at a monthly scale and averaged over the year prior to sampling (September 2015 - September 2016). The same environmental data values were applied to each replicate with a site. To investigate comparability of environmental conditions across sampling sites, an analysis of similarity was undertaken by multivariate clustering analysis, using the package PRIMER v.7 (Clarke and Gorley, 2015). Clustering was based on normalised Euclidean distances and significance was determined using the SIMPROF routine, which tests for statistically significant station clusters. The analysis resulted in one single cluster of similar habitat characteristics (Supplement – Figure S1). Tests for collinearity between predictors are discussed below.

2.9.   Statistical approach

We used generalised linear mixed models (GLMMs) to test the ability of macrofaunal indicators to detect trawling impacts across size fractions. As each replicate sample represented an observation, we analysed the data using mixed effects models. This modelling approach is particularly suitable to quantify potential correlations in repeated measure designs (i.e. replicate samples nested within sites) (Bolker et al., 2009). 'Sampling site' was therefore included as a random effect term to account for non-independence of samples, while all other predictors were included as fixed effect terms. The exception to this was for models which contained species density (S) as a response variable. In such cases density (N) was included an additional predictor, as per Gislason et al., (2017) and Sköld et al., (2018). This step was taken to account for changes in the detection rate new species as abundance increases (Gotelli and Colwell, 2001), and therefore improve our ability to detect the effects of other predictors, aside from N. Indicators were modelled using either a negative binomial, Poisson, or Gaussian distribution depending on the data type (count/discrete or continuous). The response variable Biomass was log transformed. To avoid issues related to multicollinearity of predictor variables, pair-wise correlations between the predictor variables were determined. Predictors with correlation coefficients >0.7 were excluded from the analysis (Dormann et al, 2013). As a result, LOI% was removed due to a high correlation with mud% (R = 0.95, p = <0.001). Pairwise correlation coefficients were visualised using the corrplot package (Wei and Simko, 2016) and are presented in the Supplement –

Figure S2. Variance inflation factor (VIF) values were checked in a model of the remaining predictor variables (Fox and Weisberg, 2011). VIF values for all predictor variables were <2. GLMMs were fit using the glmmTMB package (Brooks et al., 2017). To determine the best fit model for each indicator, we used the MuMin package (Barton, 2013) to calculate all possible combinations of the predictor variables. The routine returns all sub-models in ascending order based on small-sample size corrected Akaike information criterion (AICc) value. The most parsimonious models were selected based on the lowest AICc (Burnham and Anderson, 2004). To deal with potential difficulties associated with model validation of mixed models, we applied a quantitative (simulation) based approach for determining model diagnostics using the DHARMa package (Hartig, 2016). Marginal effects of GLMMs were plotted using the sjPlot package (Lüdecke, 2016). All analyses were conducted using R version 3.5.0 (R Development Core Team, 2018).

3. Results

3.1. Dataset description

The study identified 30,783 individuals across 285 different taxa. Of these, 276 (97%) were identified to species level, 7 (2%) to genus, 1 (<1%) to class, and 1 (<1%) to phylum. Out of the total number of taxa recorded, 91% were represented in the small fraction (268 taxa), 52% represented in the large fraction (147 taxa), and 26% were unique to the large fraction (74 taxa). The dominant taxa in the pooled community were *Amphiura filiformis* (Class Ophiuroidea), *Phoronis* sp. (Phylum Phoronida), *Kurtiella bidentata*, *Nucula nitidosa*, *Thyasira flexuosa*, *Eunucula tenuis*, *Abra nitida* (Class Bivalvia), *Scalibregma inflatum*, *Diplocirrus glaucus*, *Spiophanes kroyeri*, *Rhodine gracilior*, *Prionospio fallax*, *Scoloplos armiger*, *Pholoe inornata* (Class Polychaeta) , and *Hyala vitrea* (Class Gastropoda) (Table 3). A similar group of dominant taxa, and relative contributions to total density, were observed in the small fraction, indicating the importance of this fraction to the overall community. The relative contribution of dominant taxa in the large fraction was greater than in the other size categories, with the top 15 taxa contributing 89% of total density (Table 3). The dominant taxa in the large fraction also included *Amphiura chiajei* and *Ophiura* spp. (Class Ophiuroidea), *Maldane sarsi*, *Terebellides stroemii*, *Praxillella praetermissa* (Class Polychaeta), *Turritella communis* (Class Gastropoda), and *Echinocardium cordatum* (Class Echinoidea).

3.2. Trawling effort

Over the three year period, fishing intensity (SAR) ranged from 0 to 43.4 (equivalent to ~14.5 yr^{-1}). Sites 3, 5 and 6 (northern Kattegat), and Sites 17 and 19 (south east Læsø) were subject to the highest fishing intensities. Untrawled conditions were observed in the closed areas (Sites 45, 49, 51), and low SAR values

were observed at various locations across the study area, particularly at Sites 8, 12, 24, and 41. Figure 2 illustrates the relative abundance of the dominant taxa as a function of trawling intensity. The polychaetes *Anobothrus gracilis*, *P. fallax*, *S. inflatum,* and the unrelated horseshoe worm *Phoronis* sp., were observed in relatively high densities at the untrawled and lightly trawled sites (left of the plot), while being either absent or observed at low densities in the highly trawled sites (right of the plot). This same pattern was also observed for the bivalve species *T. flexuosa* and *N. nitidosa*. Conversely, *A. filiformis* and *K. bidentata* were recorded at high densities across the range of trawling intensity, while numbers of *D. glaucus* were also relatively consistent. The tubicolous polychaete *S. kroyeri* were observed to peak in numbers at intermediate levels of trawling, while *H. vitrea* occurred in relatively high densities at the most heavily trawled sites. Summary information of site-by-site SAR values and macrofaunal counts are provided in the Supplement – Table S1.

Table 3: Dominant macrofauna (based on density) of the pooled community, small fraction, and large fractions

Pooled community		Small fraction (1-4mm)		Large fraction (>4mm)	
Taxa	Density (cumulative contribution %)	Taxa	Density (cumulative contribution %)	Taxa	Density (cumulative contribution %)
Amphiura filiformis	8153 (26%)	*Amphiura filiformis*	5358 (22%)	*Amphiura filiformis*	2795 (41%)
Phoronis sp.	3942 (38%)	*Kurtiella bidentata*	3729 (37%)	*Phoronis* sp.	1498 (62%)
Kurtiella bidentata	3729 (50%)	*Phoronis* sp.	2444 (46%)	*Spiophanes kroyeri*	364 (68%)
Nucula nitidosa	1370 (54%)	*Nucula nitidosa*	1192 (51%)	*Rhodine gracilior*	343 (72%)
Thyasira flexuosa	1110 (58%)	*Scalibregma inflatum*	1062 (55%)	*Amphiura chiajei*	202 (75%)
Scalibregma inflatum	1068 (61%)	*Thyasira flexuosa*	1015 (59%)	*Nucula nitidosa*	178 (78%)
Hyala vitrea	749 (63%)	*Hyala vitrea*	749 (62%)	*Turritella communis*	124 (80%)
Diplocirrus glaucus	732 (66%)	*Diplocirrus glaucus*	725 (65%)	*Ophiura sp.*	113 (81%)
Spiophanes kroyeri	611 (68%)	*Ennucula tenuis*	540 (68%)	*Maldane sarsi*	100 (83%)
Ennucula tenuis	550 (69%)	*Pholoe inornata*	497 (70%)	*Thyasira flexuosa*	95 (84%)
Rhodine gracilior	527 (71%)	*Prionospio fallax*	493 (72%)	*Anobothrus gracilis*	87 (86%)
Prionospio fallax	504 (72%)	*Scoloplos armiger*	489 (73%)	*Terebellides stroemii*	56 (86%)
Scoloplos armiger	503 (74%)	*Abra nitida*	362 (75%)	*Abra nitida*	54 (87%)
Pholoe inornata	499 (76%)	*Anobothrus gracilis*	303 (76%)	*Praxillella praetermissa*	52 (88%)
Abra nitida	416 (77%)	*Cylichna cylindracea*	303 (77%)	*Echinocardium cordatum*	51 (89%)
Pooled community total	**30,783**	**Small fraction total**	**23,996**	**Large fraction total**	**6,787**
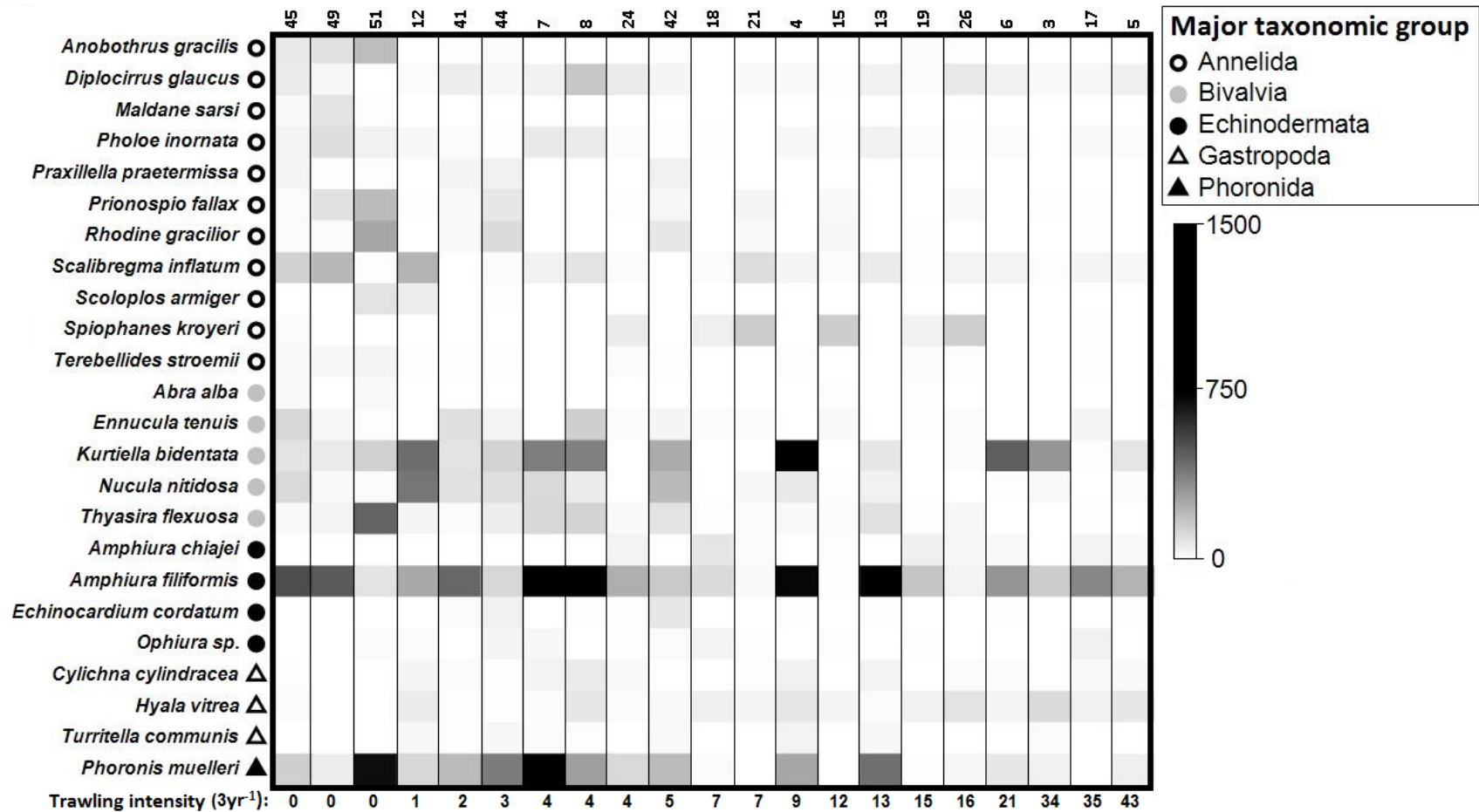
Figure 2: Relative density of dominant fauna across the gradient of trawling intensity, illustrated as a shade plot. Dark shading indicates high density, light shading indicates low density, and values represent the total density of macrofauna per site (or per 0.4 m². SAR values are based on three years of trawling effort (3 yr⁻¹), and increase from left to right (bottom of the plot). Sampling site numbers are provided at top of the plot.

3.3. Response of macrofaunal indicators to trawling

Macrofaunal indicators based on the pooled community demonstrated a varied performance, with four of the eight indicators exhibiting a significant negative response to trawling. These included density (N), biomass, functional richness (Fric), and functional dispersion (Fdis) (Table 4). Although species density (S), Shannon diversity (H'), functional diversity (RaoQ) also showed a tendency to decline over the gradient of trawling intensity (Figure 3), these relationships were not statistically significant. On the contrary, functional evenness (Feve) slightly increased over the trawling gradient, although again this trend was not significant. In the small fraction, none of the taxonomic based indicators (N, S, H', biomass) responded to trawling, whereas two of the functional indicators (Fric and Fdis) demonstrated significant negative relationships with trawling. These indicators were distinctive, in that they demonstrated significant negative relationships with trawling intensity across all size categories. In the large fraction, each of the eight indicators examined declined significantly with trawling. Moreover, trawling was the sole explanatory variable in the most parsimonious models of S, H', Fric, Feve, and Fdisp. Comparison of model parameter estimates across size fractions indicates that there was also a larger effect of trawling per unit N, biomass, RaoQ and Fdisp, in the large fraction. Results for the functional indicators calculated using biomass data are presented in Table S2, and in general, these indicators performed similarly to their density-based counterparts. The main discrepancies being that Fdis (pooled community) showed no relationship with trawling, and RaoQ (small fraction) responded to negatively to trawling.

Table 4: Summary output for generalised linear mixed models of macrofaunal indicators. The values shown are parameter estimates, and associated standard error (shown in brackets). The significance level is denoted by asterisks (* = P < 0.05; ** =P < 0.01; *** = P < 0.001). Conditional R^2 values describe the proportion of variance explained by both the fixed and random terms.

	Indicator	Intercept	Current	Depth	Mud%	Temperature	Trawling	Salinity	Density†	Conditional R^2
Pooled	N	7.16(0.19)	-0.206 (0.065)**	-0.017 (0.003)***	-0.007 (0.001)***		-0.013 (0.005)*		-	0.79
	S	4.63 (0.29)			-0.005 (0.001)***	-0.139 (0.037)***			0.25 (0.004)***	0.75
	H'	3.56 (0.58)	0.066 (0.027)*			-0.08 (0.031)*			-	0.33
	Biomass	4.19 (1.20)		-0.032 (0.006)***		-0.368 (0.147)*	-0.017 (0.009)*		-	0.35
	RaoQ	0.64(0.07)	0.020 (0.009)*			-0.025 (0.010)*			-	0.52
	Fric	0.47(0.01)			-0.001 (<0.001)**		-0.004 (0.001)**		-	0.47
	Feve	0.31(0.07)						0.010 (0.002)***	-	0.51
	Fdis	5.86(0.16)	0.167 (0.076)*				-0.018 (0.005)**		-	0.48
Small fraction	N	6.92(0.22)	-0.240 (0.077)**	-0.017 (0.003)***	-0.011 (<0.002)***				-	0.82
	S	2.67 (0.46)	0.101 (0.031)***	0.004 (0.001)*	-0.005 (0.001)***	-0.138 (0.03)***			0.005 (0.002)***	0.73
	H'	2.07(0.15)	0.243 (0.066)**			0.250 (0.075)			-	0.76
	Biomass	-0.81(0.14)		-0.018 (0.003)***	-0.004 (0.002)*				-	0.38
	RaoQ	0.779(0.08)	0.022 (0.009)**			-0.042 (0.011)***			-	0.58
	Fric	0.38 (0.02)		-0.001 (>0.001)*			-0.003 (0.001)**		-	0.38
	Feve	0.26(0.06)						0.011 (0.002)**	-	0.48
	Fdis	7.63(0.78)				-0.202 (0.103)*	-0.017 (0.006)*		-	0.46
Large fraction	N	7.07(0.73)		-0.018 (0.003)***		-0.246 (0.089)**	-0.025 (0.005)***		-	0.53
	S	0.66(0.28)					-0.016 (0.004)*		0.423 (0.06)***	0.75
	H'	1.55(0.08)					-0.019 (0.005)***		-	0.43
	Biomass	0.95(0.32)		-0.027 (0.008)***			-0.027 (0.012)*		-	0.31
	RaoQ	0.43(0.02)		-0.001 (<0.0001)*			-0.004 (0.001)***		-	0.50
	Fric	0.49 (0.03)					-0.007 (0.001)***		-	0.47
	Feve	0.67(0.01)					-0.004 (0.001)***		-	0.21
	Fdis	7.77(0.55)		-0.048 (0.013)***			-0.042 (0.021)*		-	0.65

N = density, S = species density, H' = Shannon diversity, RaoQ = functional diversity, Fric = Functional richness, Feve = Functional evenness, Fdis = Functional dispersion

Model families: N = Negative binomial. S = Poisson. H/Biomass/RaoQ/Fric/Feve/Fdis = Gaussian. Models of H were fitted using a log link.

† = density (N) included as a predictor for models of species density (S), only

Pooled community   Small fraction   Large fraction

Trawling Intensity (3 yr^{-1})   Trawling Intensity (3 yr^{-1})   Trawling Intensity (3 yr^{-1})
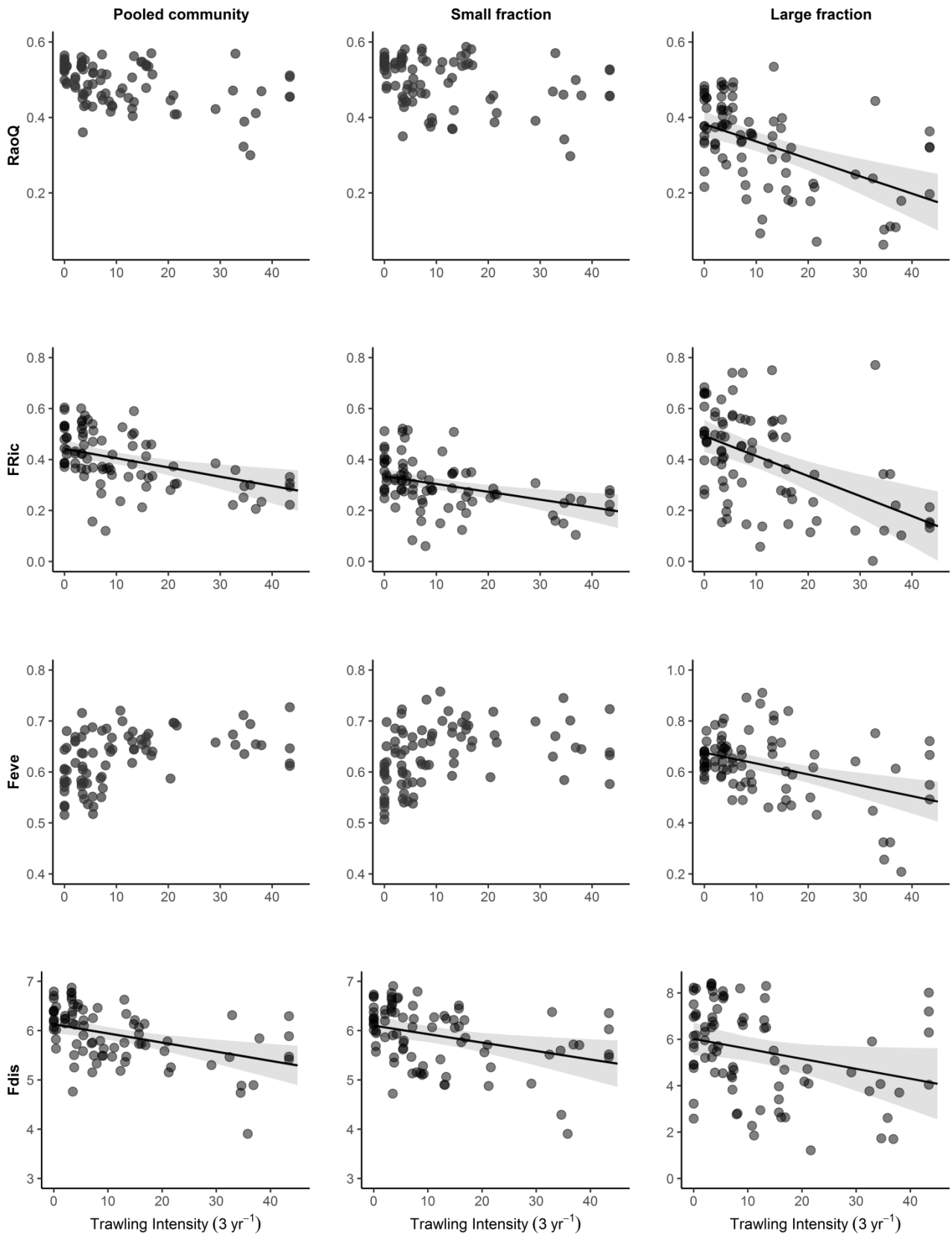
Continued…

Figure 3: Relationships between trawling intensity and macrofaunal indicators derived from the pooled, small, and large fractions, respectively. Regression lines represent the predicted values from GLMMs

presented in Table 4. Greyed area represent 95% confidence intervals. The raw observations are overlaid as data points. Note: Y-axis scale differs in large fraction plots, with the exception of biomass, RaoQ, and Fric.

3.4.   Response to environmental drivers

Overall, the indicators based on the pooled community and small fraction were strongly influenced by environmental drivers. A number of significant relationships were observed between indicators and current speed (mainly positive relationships) and temperature (mainly negative relationships) in both these categories, while depth was more prominent in the small fraction. Salinity was observed to be a poor predictor overall (pooled, small and large fractions), with Feve being the only indicator to respond to salinity. The overall influence of environmental drivers on large fraction indicators was considerably less. The large fraction indicators of N, biomass, RaoQ, and Fdis responded significantly to depth, while N also responded to temperature.

3.4.1.   Potential effect of recruits on abundance

Based on estimates provided by Sköld et al. (2001), the post-metamorphic settlers of *Amphiura* spp. would be expected to be ~0.4 - 0.5mm in diameter. In the event of an early settlement from August onwards, and a daily increase in disk diameter of 0.42% and arm length of 1.76%, individual *Amphiura* spp. would thus need a minimum of 75 days to reach ≥1mm in diameter. As data collection was completed ~65 days after the beginning of August, it was deemed unlikely that many recruits would have reached ≥1mm by the time of sampling. Furthermore, empirical evidence of Sköld et al 2001 also show that the vast majority of *Amphiura* spp. sampled in early October in the Kattegat were <1mm in length. Nevertheless, the precautionary step to exclude 50% of *Amphiura* spp. individuals from pooled community N and small fraction N did not change the overall outcome. , The model for N minus 50% *Amphiura* spp. included trawling (β = -0.017, se = 0.004, p = <0.001), Mud% (β = -0.006, se = 0.001, p = <0.001), depth (β = -0.02, se = 0.001, p = <0.001), and current (β =-0.117, se = 0.05, p = 0.002). Likewise, the model for small fraction N minus 50% *Amphiura* spp. included Mud% (β = 0.01, se = 0.002, p = <0.001), depth (β = 0.017, se = 0.003 p = <0.001), and current (β =-0.188, se = 0.07, p = 0.009). The conditional R^2 for these models were 0.77 and 0.81, respectively. Although spring sampling would have been optimal (logistical constraints did not allow for this), the retrospective steps taken to validate our results demonstrate that recruitment effects are unlikely to have affected our results. Furthermore, we provide the results of biomass and biomass based indicators, which are less likely to be affected by recent recruitment events.

4. Discussion

In this analysis we describe and compare the ability of eight ecological indicators to detect trawling impacts across macrofaunal size categories. Our results suggest that benthos display a size-dependent sensitivity to trawling, whereby large fauna (≥4mm) and their associated traits are most affected. Indicators derived from large fauna were able to capture this sensitivity, effectively detect trawling disturbance, while being less affected by other environmental drivers. Studies which apply macrofaunal indicators to assess human and natural pressures in the marine environment are typically undertaken using indicators based on all members of the benthic community (equivalent of the pooled community in this study). This approach may therefore not be the most suitable for the assessment of trawling impacts to benthic communities. In the following sections, we discuss the performance of indicators, as well as the ecological and management implications of these findings.

4.1. Response of pooled community indicators to trawling

The community-level indicators of density (N), biomass, functional richness (Fric), and functional dispersion (Fdis) declined significantly with increasing trawling disturbance in our study. Under chronic trawling conditions, rates for the depletion of biota (density and biomass combined) have been estimated at ~6% per pass of an otter trawl (Hiddink et al., 2017). As the most heavily fished sites in this study were associated with SAR values of up to 43.4 over a three year period (or ~14.5 yr^{-1}), N and biomass would be expected to considerably decline in heavily trawled areas. In general, declines in these indicators will occur when the fishery exerts mortality rates higher than what can be replaced by the wider population, either through recruitment or potentially immigration. Although this rate may vary between location, the efficacy of N as indicator of trawling impacts in gradient studies has been demonstrated in a number of regions, including in the Kattegat (Gislason et al., 2017), Irish Sea (Hinz et al., 2009), Mediterranean (Mangano et al., 2014), and New Zealand (Thrush et al., 1998). Equally, biomass has been shown to be an effective indicator of trawling disturbance. Repeated and intense trawling will typically result in shifts from communities dominated by high biomass taxa, to those dominated by highly abundant small macrofauna (Hiddink et al., 2006; Jennings et al., 2002; Kaiser et al., 2000). Furthermore, total biomass has been shown to persistently decline in highly trawled areas, even when other indicators (such as N) have become unresponsive (Reiss et al., 2009). Despite this, the performance of N and biomass can also vary across studies. A good example of this is provided by Sköld et al., (2018). This study was undertaken in nearby *Nephrops* grounds, and across a similar gradient of trawling intensity (up to 15.8 yr^{-1}), yet did not detect significant shifts in N or biomass. A possible explanation is that the models used in their study were characterised by high uncertainty at the heavily fished sites (due to relatively few observations), which may have affected overall trends. Furthermore, samples were collected

from long-standing monitoring sites, while our data are based on a 'snapshot' sampling event. While our approach has the disadvantage of lacking information on inter-annual trends, it does allow for the sampling design to be tailored to the observed range of recent trawling effort, while accounting for effort across other environmental factors, such as depth. Aside from these differences, Sköld et al., (2018) also observed that the density of *Amphiura chiajei*, a particularly dominant taxa in their dataset, showed a positive relationship with trawling. This may demonstrate that when a community is dominated by 'robust' taxa, their response has the potential to mask that of the remaining community. By contrast, abundances of *Amphiura* spp. (mostly *A. filiformis*, but also *A. chiajei*) were relatively stable over the trawling gradient in our study (Figure 2), while taxa which increased over the trawling gradient (e.g. *Hyala vitrea*) contributed less to total abundance. Moreover, taxa such as *H. vitrea* were completely absent from the large fraction, and may partly explain the clearer trends observed in this size category (discussed in section 4.2). Observations from *Nephrops* grounds in the Irish Sea have conversely shown *A. filiformis* to be highly sensitive to trawling (Queirós et al., 2006; Hinz et al., 2009). While the exact reasons for these discrepancies are unclear, it is possible that local sedimentary and hydrodynamic conditions may affect the relationship between some taxa and trawling.

Although species density and diversity (S and H') showed a negative relationship with trawling in our study, these relationships were not significant. This lack of response is in agreement with other trawling gradient studies (Ball et al., 2000; Currie et al., 2011; Reiss et al., 2009), and may add weight to general concerns about their suitability in ecological monitoring. Measures of species richness can be highly sensitive to factors such as the size of sampling area, sampling intensity, and taxonomic properties of the species in question (Fleishman et al., 2006). A further complicating factor is that species density is often positively correlated with density (Gislason et al., 2017; Gotelli and Colwell, 2001). To account for observed variation in N between our sampling stations, we included N as a predictor for models of S. Although this step allows for a more accurate assessment of the role of other predictors, this did not result in the detection of trawling. Nonetheless, this relationship between N and S is theoretically relevant for all indicators derived from estimates of species richness, such as diversity measures (e.g. Shannon-diversity, functional diversity), and for multi-metric indicators commonly used in national benthic monitoring programs. Currently, most diversity indicators are applied without accounting for this relationship, or by e.g. using rarefied species richness. For this reason we did not attempt to standardize our diversity indicators with respect to N, but followed common practice, and future work is needed to quantify the effect of this relationship and its relevance for indicator use.

Our study also tested the performance of several functional indicators, which provide alternative and complimentary information to taxonomic measures. Trawling has been shown to negatively affect trait composition, and characteristics such as the size, age, nutrient cycling, and trophic profile of benthic communities (Bolam et al., 2017; Bremner et al., 2003; Tillin et al., 2006). Nonetheless, comparatively few studies have formally tested the ability of functional indicators to reflect these changes. In contrast to measures of species density and diversity, functional richness (Fric) and functional dispersion (Fdis) demonstrated significant negative relationships with trawling, doing so across all size categories. Fric is largely influenced by the loss or addition of unique traits, while Fdis provides a measure of the distinctiveness of traits within the community. The results thus suggest that trawling may have a greater effect on trait richness/diversity than on taxonomic counterparts. If that were the case, then communities in heavily trawled areas may become functionally impoverished, while a base level of species diversity may be comparatively unaffected. This would have implications for the functionality of benthic communities across fishing grounds, and suggests that monitoring of functional indicators should be undertaken in conjunction with taxonomic approaches.

4.2. Response of large and small fraction indicators to trawling

It has been suggested that the body-size composition of a community can provide a proxy for a large degree of embedded ecological information, such as ecological quality and sensitivity to disturbance (Woodward et al., 2005). This is supported by our results, where each of the indicators derived from the large size fraction displayed significant negative relationships with trawling. Although previous studies have investigated trawling impacts to large epifauna (Hinz et al., 2009), and the production rates of large macrofauna (≥4mm) (Reiss et al., 2009), none have explicitly tested community indicators based on body-size. Accordingly, the results of this study are not readily comparable to others, although the processes which underpin the sensitivity of large fauna are relatively well described. While, some large-bodied taxa are comparatively unaffected by trawling, due to e.g. burrowing depth, high mobility, or robust physical structure, populations of larger fauna are unable to withstand the high rates of mortality imposed by chronic trawling (Duplisea et al., 2002). Consequently, trawling leads to the disproportionate loss of large individuals, and an associated steepening in the slope of the size spectrum (Jennings et al., 2001; Queirós et al., 2006). The effects of trawling on large fauna is also thought to be greater than that of other influential environmental factors, such as depth and sediment characteristics (Duplisea et al., 2002), and may explain the reduced influence of natural drivers to indicators based on this size fraction. By the time an individual is near its asymptotic size, ambient environmental conditions will have selected for certain species or traits. Large fauna may therefore be less susceptible to variation caused by natural processes and local recruitment success, which are known

to complicate the outcome of trawling impact assessments, and may be better suited to track trawling impacts over time and space. Furthermore, large fauna are considerably less abundant than small fauna, meaning that fewer individuals and species are required for analysis and monitoring. Overall, indicators derived from the small fraction were less responsive to trawling, and demonstrated several significant relationships with environmental drivers such as depth, current speed, mud content, and temperature. Small macrofauna typically exhibit higher growth rates, earlier reproductive onset, wide-spread recruitment, and elevated abundances of small fauna have been shown to be effective indicators of other environmental pressures, such as nutrient enrichment (Pearson and Rosenberg, 1978). Nevertheless, trawling is thought to reduce community abundance and biomass across a range of benthic size classes (Hinz et al., 2008), and may explain why proliferations of opportunistic species have not been observed in highly trawled area (Hinz et al., 2009; Jennings et al., 2001). While indicators based on small fauna were relatively unresponsive in our study, individual taxa (and traits) within this fraction may be potentially affected by trawling. A more detailed size-based investigation of species and trait-level responses would therefore help to clarify potential relationships. Such results may also be relevant for less productive areas, where comparatively lower abundances and biomass of large fauna may reduce the effectiveness of large community indicators. Regardless, the varying responses observed between the two size fractions highlight an underlying issue with using pooled community indicators to detect trawling impacts. This is as species, taxonomic groups, and traits may display contradictory responses to trawling and to other environmental drivers.

4.3.   Ecological implications

The ecosystem effects of trawling can range from changes in the functioning of marine sediments (Sciberras et al., 2016), to bottom-up interactions with higher trophic levels (Eddy et al., 2017; Hiddink et al., 2011; Hinz et al., 2017). The results of this study provide additional evidence that chronic trawling has a strong negative effect on the abundance (i.e. density and biomass) (see Hiddink et al., 2017; Sciberras et al., 2018) and functional capacity of benthic macrofaunal communities (see Bremner et al., 2003; de Juan et al., 2007; Tillin et al., 2006), while demonstrating the value of closed areas to scientific studies of demersal fishing. In particular, we show that highly trawled communities are associated with significant reductions in large macrofauna, and their associated traits. Large macrofauna play a particularly important role in several benthic processes, and it is thought that their importance in nutrient cycling is such that losses cannot be replicated, or offset, by smaller species (Solan et al., 2004). Manipulative experiments have shown that the removal of large individuals from intertidal communities have significant negative effects on local sedimentary characteristics, biogeochemical fluxes, and community dominance structures (Thrush et al., 2006). In addition, variation in intraspecific size has been recognised as a stronger predictor of ecosystem

function than that of density or species density (Norkko et al., 2013). Theoretical and empirical evidence would therefore suggest that the loss of large fauna may have significant consequences for the functionality of benthic communities. The role and decline of large fauna from wider ecological systems has received increased attention in recent years (see Malhi et al., 2016), yet the significance of this for benthic systems across fishing grounds is poorly understood.

4.4. Conclusions and applications

Using a whole community approach, four of the eight macrofaunal indicators examined were unable to detect trawling impacts. By contrast, all indicators based on large macrofauna responded consistently and effectively to trawling intensity. These results were demonstrated using data from a one-off sampling event, and in an area where fisheries may have altered the benthic community over time (Josefson et al., 2018). Although this approach cannot be retrospectively applied to existing data, our findings may have implications for future ecological monitoring of bottom trawling activities. By size-separating the benthic community, improved indicator performance was achieved from a numerically reduced subset, comprising some 22% of all individuals and 52% of taxa. The laboratory based processing of macrofaunal samples is a labour intensive and costly task. Considering this, we estimate that the time taken to screen the whole community (i.e. pick all biota from the sieved residuum), and taxonomically identify and determine biomass just for the large fraction, would have reduced the total laboratory processing time by approximately 75%. This time difference is mainly due to the fewer individuals and species requiring identification. The approach outlined in this study is easily employed, does not require prior ecological knowledge of the system, and future work should seek to test its application across different habitat types and geographic regions. We suggest that this, along with other promising methodologies (Hiddink et al., 2018; Rijnsdorp et al., 2018), have the potential to provide a suite of improved tools to better detect, assess, and monitor bottom trawling impacts on benthic communities.

Supplementary information

[See supplement]

References

Al-Hamdani, Z.K., Reker, J., Leth, J.O., Reijonen, A., Kotilainen, A.T., Dinesen, G.E., 2007. Development of marine landscape maps for the Baltic Sea and the Kattegat using geophysical and hydrographical parameters. Geol. Surv. Denmark Greenl. Bull. 13, 61–64.

Amoroso, R.O., Pitcher, C.R., Rijnsdorp, A.D., McConnaughey, R.A., Parma, A.M., Suuronen, P., Eigaard, O.R., Bastardie, F., Hintzen, N.T., Althaus, F., Baird, S.J., Black, J., Buhl-Mortensen, L., Campbell, A.B., Catarino, R., Collie, J., Cowan, J.H., Durholtz, D., Engstrom, N., Fairweather, T.P., Fock, H.O., Ford, R., Gálvez, P.A., Gerritsen, H., Góngora, M.E., González, J.A., Hiddink, J.G., Hughes, K.M., Intelmann, S.S., Jenkins, C., Jonsson, P., Kainge, P., Kangas, M., Kathena, J.N., Kavadas, S., Leslie, R.W., Lewis, S.G., Lundy, M., Makin, D., Martin, J., Mazor, T., Gonzalez-Mirelis, G., Newman, S.J., Papadopoulou, N., Posen, P.E., Rochester, W., Russo, T., Sala, A., Semmens, J.M., Silva, C., Tsolos, A., Vanelslander, B., Wakefield, C.B., Wood, B.A., Hilborn, R., Kaiser, M.J., Jennings, S., 2018. Bottom trawl fishing footprints on the world's continental shelves. Proc. Natl. Acad. Sci. 201802379. https://doi.org/10.1073/pnas.1802379115

Anon. 2008. Directive 2008/56/EC of the European Parliament and of the Council of 17 June2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive)

Anon. 2010. Commission Decision of 1 September 2010 on criteria and methodological standards on good environmental status of marine waters 2010/477/EU

Atkinson, L.J., Field, J.G., Hutchings, L., 2011. Effects of demersal trawling along the west coast of southern Africa: Multivariate analysis of benthic assemblages. Mar. Ecol. Prog. Ser. https://doi.org/10.3354/meps08956

Ball, B.J., Fox, G., Munday, B.W., 2000. Long- and short-term consequences of a Nephrops trawl fishery on the benthos and environment of the Irish Sea. ICES J. Mar. Sci. 57, 1315–1320. https://doi.org/10.1006/jmsc.2000.0924

Barton, K. (2013) MuMIn: Multi-Model Inference. R Package Version 1.40.4 http://CRAN.R-project.org/package=MuMIn

Bastardie, F., Nielsen, J.R., Ulrich, C., Egekvist, J., Degel, H., 2010. Detailed mapping of fishing effort and landings by coupling fishing logbooks with satellite-recorded vessel geo-location. Fish. Res. https://doi.org/10.1016/j.fishres.2010.06.016

Begon, M., Harper, J.L., Townsend, C.R., 2006. Ecology: From Individuals to Ecosystems, fourth ed. Blackwell Science, Oxford.

Bolam, S.G., Garcia, C., Eggleton, J., Kenny, A.J., Buhl-Mortensen, L., Gonzalez-Mirelis, G., van Kooten, T., Dinesen, G., Hansen, J., Hiddink, J.G., Sciberras, M., Smith, C., Papadopoulou, N., Gumus, A., Van Hoey, G., Eigaard, O.R., Bastardie, F., Rijnsdorp, A.D., 2017. Differences in biological traits composition of benthic assemblages between unimpacted habitats. Mar. Environ. Res. 126, 1–13. https://doi.org/10.1016/j.marenvres.2017.01.004

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.S.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. https://doi.org/10.1016/j.tree.2008.10.008

Botta-Dukát, Z., 2005. Rao's quadratic entropy as a measure of functional diversity based on multiple traits. J. Veg. Sci. 16, 533–540. https://doi.org/10.1111/j.1654-1103.2005.tb02393.x

Bremner, J., Frid, C.L.J., Rogers, S.I., 2003. Assessing Marine Ecosystem Health: The long-term effects of fishing on functional biodiversity in North Sea benthos, in: Aquatic Ecosystem Health and Management. https://doi.org/10.1080/14634980301470

Brose, U., Blanchard, J.L., Eklöf, A., Galiana, N., Hartvig, M., R. Hirt, M., Kalinkat, G., Nordström, M.C., O'Gorman, E.J., Rall, B.C., Schneider, F.D., Thébault, E., Jacob, U., 2016. Predicting the consequences of species loss using size-structured biodiversity approaches. Biol. Rev. 49, 684–697. https://doi.org/10.1111/brv.12250

Burnham, K.P., Anderson, D.R., 2004. Multimodel Inference. Sociol. Methods Res. 33, 261–304. https://doi.org/10.1177/0049124104268644

Chevene, F., Doleadec, S., Chessel, D., 1994. A fuzzy coding approach for the analysis of long-term ecological data. Freshw. Biol. 31, 295–309. https://doi.org/10.1111/j.1365-2427.1994.tb01742.x

Clarke KR, Gorley RN (2015) Primer v7:user manual/tutorial. PRIMER-E Ltd, Plymouth. www.primer-e.com/primer 7_download.htm

Currie, D.R., Dixon, C.D., Roberts, S.D., Hooper, G.E., Sorokin, S.J., Ward, T.M., 2011. Relative importance of environmental gradients and historical trawling effort in determining the composition and distribution of benthic macro-biota in a large inverse estuary. Fish. Res. https://doi.org/10.1016/j.fishres.2010.11.003

de Juan, S., Thrush, S.F., Demestre, M., 2007. Functional changes as indicators of trawling disturbance on a benthic community located in a fishing ground (NW Mediterranean Sea). Mar. Ecol. Prog. Ser. 334, 117–129. https://doi.org/10.3354/meps334117

Diesing, M., Stephens, D., Aldridge, J., 2013. A proposed method for assessing the extent of the seabed significantly affected by demersal fishing in the Greater North Sea. ICES J. Mar. Sci. 70, 1085–1096. https://doi.org/10.1093/icesjms/fst066

Dormann, C.F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J.R.G., Gruber, B., Lafourcade, B., Leitão, P.J., Münkemüller, T., McClean, C., Osborne, P.E., Reineking, B., Schröder, B., Skidmore, A.K., Zurell,

D., Lautenbach, S., 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography (Cop.). 36, 27–46. https://doi.org/10.1111/j.1600-0587.2012.07348.x

Duplisea, D.E., Jennings, S., Warr, K.J., Dinmore, T.A., 2002. A size-based model of the impacts of bottom trawling on benthic community structure. Can. J. Fish. Aquat. Sci. 59, 1785–1795. https://doi.org/10.1139/f02-148

Eddy, T.D., Lotze, H.K., Fulton, E.A., Coll, M., Ainsworth, C.H., de Araújo, J.N., Bulman, C.M., Bundy, A., Christensen, V., Field, J.C., Gribble, N.A., Hasan, M., Mackinson, S., Townsend, H., 2017. Ecosystem effects of invertebrate fisheries. Fish Fish. 18, 40–53. https://doi.org/10.1111/faf.12165

Eigaard, O.R., Bastardie, F., Breen, M., Dinesen, G.E., Hintzen, N.T., Laffargue, P., Mortensen, L.O., Nielsen, J.R., Nilsson, H.C., O'Neill, F.G., Polet, H., Reid, D.G., Sala, A., Sköld, M., Smith, C., Sørensen, T.K., Tully, O., Zengin, M., Rijnsdorp, A.D., 2016. Estimating seabed pressure from demersal trawls, seines, and dredges based on gear design and dimensions. ICES J. Mar. Sci. 73, i27–i43. https://doi.org/10.1093/icesjms/fsv099

Eigaard, O.R., Bastardie, F., Hintzen, N.T., Buhl-Mortensen, L., Buhl-Mortensen, P., Catarino, R., Dinesen, G.E., Egekvist, J., Fock, H.O., Geitner, K., Gerritsen, H.D., González, M.M., Jonsson, P., Kavadas, S., Laffargue, P., Lundy, M., Gonzalez-Mirelis, G., Nielsen, J.R., Papadopoulou, N., Posen, P.E., Pulcinella, J., Russo, T., Sala, A., Silva, C., Smith, C.J., Vanelslander, B., Rijnsdorp, A.D., 2017. The footprint of bottom trawling in European waters: distribution, intensity, and seabed integrity. ICES J. Mar. Sci. J. du Cons. 74, fsw194. https://doi.org/10.1093/icesjms/fsw194

Ellis, N., Pantus, F., Pitcher, C.R., II, T.Q., 2014. Scaling up experimental trawl impact results to fishery management scales — a modelling approach for a "hot time." Can. J. Fish. Aquat. Sci. 71, 733–746. https://doi.org/10.1139/cjfas-2013-0426

FAO (2003) Fisheries management 2: The ecosystem approach to fisheries. FAO Technical Guidelines for Responsible Fisheries (FAO, Rome), 4(Suppl 2):112.

Fleishman, E., Noss, R.F., Noon, B.R., 2006. Utility and limitations of species richness metrics for conservation planning. Ecol. Indic. https://doi.org/10.1016/j.ecolind.2005.07.005

Fox, J., Weisberg, S., 2011. An R Companion to Applied Regression, second edition [WWW Document]. Sage, Thousand Oaks CA.

Frid, C., Paramor, O., Scott, C., 2005. Ecosystem-based fisheries management: Progress in the NE Atlantic. Mar. Policy. https://doi.org/10.1016/j.marpol.2004.07.004

Gilkinson, K., Paulin, M., Hurley, S., Schwinghamer, P., 1998. Impacts of trawl door scouring on infaunal bivalves: Results of a physical trawl door model/dense sand interaction. J. Exp. Mar. Bio. Ecol. https://doi.org/10.1016/S0022-0981(97)00207-4

Gislason, H., Bastardie, F., Dinesen, G.E., Egekvist, J., Eigaard, O.R., 2017. Lost in translation? Multi-metric macrobenthos indicators and bottom trawling. Ecol. Indic. 82, 260–270. https://doi.org/10.1016/j.ecolind.2017.07.004

Gotelli, N.J., Colwell, R.K., 2001. Quantifying biodiversity: Procedures and pitfalls in the measurement and comparison of species richness. Ecol. Lett. https://doi.org/10.1046/j.1461-0248.2001.00230.x

Greenstreet, S.P.R., Rogers, S.I., Rice, J.C., Piet, G.J., Guirey, E.J., Fraser, H.M., Fryer, R.J., 2011. Development of the EcoQO for the North Sea fish community. ICES J. Mar. Sci. 68, 1–11. https://doi.org/10.1093/icesjms/fsq156

Hartig, F. (2016). DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models . DHARMa version 0.2.0. URL https://cran.r-project.org/web/packages/DHARMa/index.html.

Hiddink, J.G., Jennings, S., Kaiser, M.J., Queirós, A.M., Duplisea, D.E., Piet, G.J., 2006. Cumulative impacts of seabed trawl disturbance on benthic biomass, production, and species richness in different habitats. Can. J. Fish. Aquat. Sci. 63, 721–736. https://doi.org/10.1139/f05-266

Hiddink, J.G., Jennings, S., Sciberras, M., Bolam, S.G., Cambiè, G., McConnaughey, R.A., Mazor, T., Hilborn, R., Collie, J.S., Pitcher, R., Parma, A.M., Suuronen, P., Kaiser, M.J., Rijnsdorp, A.D., 2018. Assessing bottom-trawling impacts based on the longevity of benthic invertebrates. J. Appl. Ecol. https://doi.org/10.1111/1365-2664.13278

Hiddink, J.G., Jennings, S., Sciberras, M., Szostek, C.L., Hughes, K.M., Ellis, N., Rijnsdorp, A.D., McConnaughey, R.A., Mazor, T., Hilborn, R., Collie, J.S., Pitcher, C.R., Amoroso, R.O., Parma, A.M., Suuronen, P., Kaiser, M.J., 2017. Global analysis of depletion and recovery of seabed biota after bottom trawling disturbance. Proc. Natl. Acad. Sci. 114, 8301–8306. https://doi.org/10.1073/pnas.1618858114

Hiddink, J.G., Johnson, A.F., Kingham, R., Hinz, H., 2011. Could our fisheries be more productive? Indirect negative effects of bottom trawl fisheries on fish condition. J. Appl. Ecol. https://doi.org/10.1111/j.1365-2664.2011.02036.x

Hintzen, N.T., Bastardie, F., Beare, D., Piet, G.J., Ulrich, C., Deporte, N., Egekvist, J., Degel, H., 2012. VMStools: Open-source software for the processing, analysis and visualisation of fisheries logbook and VMS data. Fish. Res. 115–116, 31–43. https://doi.org/10.1016/j.fishres.2011.11.007

Hinz, H., Moranta, J., Balestrini, S., Sciberras, M., Pantin, J.R., Monnington, J., Zalewski, A., Kaiser, M.J., Sköld, M., Jonsson, P., Bastardie, F., Hiddink, J.G., 2017. Stable isotopes reveal the effect of trawl fisheries on the diet of commercially exploited species. Sci. Rep. 7, 6334. https://doi.org/10.1038/s41598-017-06379-6

Hinz, H., Prieto, V., Kaiser, M.J., 2009. Trawl disturbance on benthic communities: Chronic effects and experimental predictions. Ecol. Appl. 19, 761–773. https://doi.org/10.1890/08-0351.1

Hinz, H., Hiddink, J.G., Forde, J., Kaiser, M.J., 2008. Large-scale responses of nematode communities to chronic otter-trawl disturbance. Can. J. Fish. Aquat. Sci. 65, 723–32.

Howarth, L.M., Roberts, C.M., Thurstan, R.H., Stewart, B.D., 2014. The unintended consequences of simplifying the sea: Making the case for complexity. Fish Fish. 15, 690–711. https://doi.org/10.1111/faf.12041

ICES, 2018. ICES Advice on fishing opportunities, catch, and effort, Greater North Sea Ecoregion. Cod (Gadus morhua) in Subdivision 21 (Kattegat). Published 29 June 2018

Jennings, S., Dinmore, T.A., Duplisea, D.E., Warr, K.J., Lancaster, J.E., 2001. Trawling disturbance can modify benthic production processes. J. Anim. Ecol. 70, 459–475. https://doi.org/10.1046/j.1365-2656.2001.00504.x

Jennings, S., Nicholson, M.D., Dinmore, T.A., Lancaster, J.E., 2002. Effects of chronic trawling disturbance on the production of infaunal communities. Mar. Ecol. Prog. Ser. 243, 251–260. https://doi.org/10.3354/meps243251

Josefson, A.B., Jensen, J.N., 1992. Growth patterns of Amphiura filiformis support the hypothesis of organic enrichment in the Skagerrak-Kattegat area. Mar. Biol. 112, 615–624. https://doi.org/10.1007/BF00346179

Josefson, A.B., Loo, L.O., Blomqvist, M., Rolandsson, J., 2018. Substantial changes in the depth distributions of benthic invertebrates in the eastern Kattegat since the 1880s. Ecol. Evol. https://doi.org/10.1002/ece3.4395

Kaiser, M.J., Clarke, K.R., Hinz, H., Austen, M.C.V., Somerfield, P.J., Karakassis, I., 2006. Global analysis of response and recovery of benthic biota to fishing. Mar. Ecol. Prog. Ser. 311, 1–14. https://doi.org/10.3354/meps311001

Kaiser, M.J., Edwards, D.B., Armstrong, P.J., Radford, K., Lough, N.E.L., Flatt, R.P., Jones, H.D., 1998. Changes in megafaunal benthic communities in different habitats after trawling disturbance. ICES J. Mar. Sci. 55, 353–361. https://doi.org/10.1006/jmsc.1997.0322

Kaiser, M.J., Ramsay, K., Richardson, C.A., Spence, F.E., Brand, A.R., 2000. Chronic fishing disturbance has changed shelf sea benthic community structure. J. Anim. Ecol. https://doi.org/10.1046/j.1365-2656.2000.00412.x

Laliberté, E., Legendre, P., & Shipley, B. (2015). FD: measuring functional diversity from multiple traits, and other tools for functional ecology. R Package, Version 1.0-12

Laliberté, E., Legendre, P., 2010. A distance-based framework for measuring functional diversity from multiple traits. Ecology 91, 299–305. https://doi.org/10.1890/08-2244.1

Lambert, G.I., Jennings, S., Hiddink, J.G., Hintzen, N.T., Hinz, H., Kaiser, M.J., Murray, L.G., 2012. Implications of using alternative methods of vessel monitoring system (VMS) data analysis to describe fishing activities and impacts. ICES J. Mar. Sci. 69, 682–693. https://doi.org/10.1093/icesjms/fss018

Lehmann, A., Hinrichsen, H.H., Getzlaff, K., Myrberg, K., 2014. Quantifying the heterogeneity of hypoxic and anoxic areas in the Baltic Sea by a simplified coupled hydrodynamic-oxygen consumption model approach. J. Mar. Syst. https://doi.org/10.1016/j.jmarsys.2014.02.012

Lepš, J., De Bello, F., Lavorel, S., Berman, S., 2006. Quantifying and interpreting functional diversity of natural communities: practical considerations matter. Preslia 78, 481–501. https://doi.org/halsde-00293183

Lüdecke, D., 2016 sjPlot: Data Visualization for Statistics in Social Science. 2019; Available online: http://cran.r-project.org/package=sjPlot

Malhi, Y., Doughty, C.E., Galetti, M., Smith, F.A., Svenning, J.-C., Terborgh, J.W., 2016. Megafauna and ecosystem function from the Pleistocene to the Anthropocene. Proc. Natl. Acad. Sci. 113, 838–846. https://doi.org/10.1073/pnas.1502540113

Mangano, M.C., Kaiser, M.J., Porporato, E.M.D., Lambert, G.I., Rinelli, P., Spanò, N., 2014. Infaunal community responses to a gradient of trawling disturbance and a long-term Fishery Exclusion Zone in the Southern Tyrrhenian Sea. Cont. Shelf Res. 76, 25–35. https://doi.org/10.1016/j.csr.2013.12.014

McConnaughey, R. a, Syrjala, S.E., Dew, C.B., 2005. Effects of chronic bottom trawling on the size structure of soft-bottom benthic invertebrates. Benthic habitats Eff. fishing. Am. Fish. Soc. Symp. 41 425–437.

Ministry of Foreign Affairs of Denmark, 1933b. Anordning om frednings- og ordensbestemmelser af fiskeriet i det til kongerigerne Danmark og Sverige grænsende farvande. Royal Regulation, AND no. 230 of 29/06/1933, 2 pp. DOI: https://www.retsinformation.dk/Forms/R0710.aspx?id=79587

Ministry of Foreign Affairs of Denmark. 1933a. Bekendtgørelse om en under 31. December 1932 mellem Danmark og Sverige angaaende fiskeriforholdende i de til Danmark og Sverige grænsende farvande. BEK no. 228 of 21/06/1933, Ministry of Foreign Affairs of Denmark, J.nr. 63.D.31, pp 1-5. DOI: https://www.retsinformation.dk/pdfPrint.aspx?id=163290

Ministry of Foreign Affairs of Denmark. 2009. Bekendtgørelse om forbud mod visse former for fiskeri i nærmere afgrænsede områder i Kattegat og nordlige del af Øresund. BEK no. 182 of 13/03/2009, Ministry of Food, Agriculture and Fishery, Fisheries Agency, J.nr. 2009-01657, pp 1-8. DOI: https://www.retsinformation.dk/pdfPrint.aspx?id=123851

Norkko, A., Villnäs, A., Norkko, J., Valanko, S., Pilditch, C., 2013. Size matters: Implications of the loss of large individuals for ecosystem function. Sci. Rep. 3, 2646. https://doi.org/10.1038/srep02646

Pearson, T.H., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. Ann. Rev. 16, 229–311

Petersen CGJ (1918) The sea bottom and its production of fish food. A survey of the work done in connection with the valuation of the Danish waters from 1883-1917. Rep Danish Biol Station 25: 1–62

Pommer, C.D., Olesen, M., Jhansen, Ø.L.S., 2016. Impact and distribution of bottom trawl fishing on mud-bottom communities in the Kattegat. Mar. Ecol. Prog. Ser. 548, 47–60. https://doi.org/10.3354/meps11649

Puig, P., Canals, M., Company, J.B., Martín, J., Amblas, D., Lastras, G., Palanques, A., Calafat, A.M., 2012. Ploughing the deep sea floor. Nature. https://doi.org/10.1038/nature11410

Queirós, A.M., Hiddink, J.G., Kaiser, M.J., Hinz, H., 2006. Effects of chronic bottom trawling disturbance on benthic biomass, production and size spectra in different habitats. J. Exp. Mar. Bio. Ecol. 335, 91–103. https://doi.org/10.1016/j.jembe.2006.03.001

R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria URL. https://www.R-project. org/.

Reiss, H., Greenstreet, S.P.R., Sieben, K., Ehrich, S., Piet, G.J., Quirijns, F., Robinson, L., Wolff, W.J., Kröncke, I., 2009. Effects of fishing disturbance on benthic communities and secondary production within an intensively fished area. Mar. Ecol. Prog. Ser. 394, 201–213. https://doi.org/10.3354/meps08243

Rice, J., Arvanitidis, C., Borja, A., Frid, C., Hiddink, J.G., Krause, J., Lorance, P., Ragnarsson, S.Á., Sköld, M., Trabucco, B., Enserink, L., Norkko, A., 2012. Indicators for sea-floor integrity under the european marine strategy framework directive. Ecol. Indic. 12, 174–184. https://doi.org/10.1016/j.ecolind.2011.03.021

Rijnsdorp, A.D., Bolam, S.G., Garcia, C., Hiddink, J.G., Hintzen, N.T., van Denderen, P.D., van Kooten, T., 2018. Estimating sensitivity of seabed habitats to disturbance by bottom trawling based on the longevity of benthic fauna. Ecol. Appl. 28, 1302–1312. https://doi.org/10.1002/eap.1731

Sciberras, M., Hiddink, J.G., Jennings, S., Szostek, C.L., Hughes, K.M., Kneafsey, B., Clarke, L.J., Ellis, N., Rijnsdorp, A.D., McConnaughey, R.A., Hilborn, R., Collie, J.S., Pitcher, C.R., Amoroso, R.O., Parma, A.M., Suuronen, P., Kaiser, M.J., 2018. Response of benthic fauna to experimental bottom fishing: A global meta-analysis. Fish Fish. 19, 698–715. https://doi.org/10.1111/faf.12283

Sciberras, M., Parker, R., Powell, C., Robertson, C., Kröger, S., Bolam, S., Geert Hiddink, J., 2016. Impacts of bottom fishing on the sediment infaunal community and biogeochemistry of cohesive and non-cohesive sediments. Limnol. Oceanogr. 61, 2076–2089. https://doi.org/10.1002/lno.10354

Shannon, C.E., 1948. The mathematical theory of communication. Bell Syst. Tech. J. https://doi.org/10.2307/3611062

Shin, Y.J., Rochet, M.J., Jennings, S., Field, J.G., Gislason, H., 2005. Using size-based indicators to evaluate the ecosystem effects of fishing. ICES J. Mar. Sci. 62, 384–396. https://doi.org/10.1016/j.icesjms.2005.01.004

Sköld, M., Göransson, P., Jonsson, P., Bastardie, F., Blomqvist, M., Agrenius, S., Hiddink, J.G., Nilsson, H.C., Bartolino, V., 2018. Effects of chronic bottom trawling on soft-seafloor macrofauna in the Kattegat. Mar. Ecol. Prog. Ser. 586, 41–55. https://doi.org/10.3354/meps12434

Sköld, M., Josefson, A., Loo, L., 2001. Sigmoidal growth in the brittle star Amphiura filiformis (Echinodermata: Ophiuroidea). Mar. Biol. 139, 519–526. https://doi.org/10.1007/s002270100600

Solan, M., Cardinale, B.J., Downing, A.L., Engelhardt, K.A.M., Ruesink, J.L., Srivastava, D.S., 2004. Extinction and ecosystem function in the marine benthos. Science (80-.). https://doi.org/10.1126/science.1103960

Svedäng, H., Bardon, G., 2003. Spatial and temporal aspects of the decline in cod (Gadus morhua L.) abundance in the Kattegat and eastern Skagerrak. ICES J. Mar. Sci. 60, 32–37. https://doi.org/10.1006/jmsc.2002.1330

Thrush, A.S.F., Hewitt, J.E., Cummings, V.J., Dayton, P.K., Cryer, M., Turner, S.J., Funnell, G.A., Budd, R.G., Milburn, C.J., Wilkinson, M.R., 2008. Disturbance of the Marine Benthic Habitat by Commercial Fishing : Impacts at the Scale of the Fishery Published by : Ecological Society of America Stable URL : http://www.jstor.org/stable/2641273 8, 866–879. https://doi.org/10.1890/1051-0761(1998)008[0866:DOTMBH]2.0.CO;2

Thrush, S.F., Hewitt, J.E., Gibbs, M., Lundquist, C., Norkko, A., 2006. Functional role of large organisms in intertidal communities: Community effects and ecosystem function. Ecosystems 9, 1029–1040. https://doi.org/10.1007/s10021-005-0068-8

Tillin, H.M., Hiddink, J.G., Jennings, S., Kaiser, M.J., 2006. Chronic bottom trawling alters the functional composition of benthic invertebrate communities on a sea-basin scale. Mar. Ecol. Prog. Ser. 318, 31–45. https://doi.org/10.3354/meps318031

Van Denderen, P.D., Bolam, S.G., Hiddink, J.G., Jennings, S., Kenny, A., Rijnsdorp, A.D., Van Kooten, T., 2015. Similar effects of bottom trawling and natural disturbance on composition and function of benthic communities across habitats. Mar. Ecol. Prog. Ser. 541, 31–43. https://doi.org/10.3354/meps11550

Van Hoey, G., Borja, A., Birchenough, S., Buhl-Mortensen, L., Degraer, S., Fleischer, D., Kerckhof, F., Magni, P., Muxika, I., Reiss, H., Schröder, A., Zettler, M.L., 2010. The use of benthic indicators in Europe: From the water framework directive to the marine strategy framework directive. Mar. Pollut. Bull. 60, 2187–2196. https://doi.org/10.1016/j.marpolbul.2010.09.015

Villéger, S., Mason, N.W.H., Mouillot, D., 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. Ecology.

Wei T, Simko V (2016) Package 'corrplot'. R package version 0.77. https://cran.r-project.org/web/packages/corrplot/.

Woodward, G., Ebenman, B., Emmerson, M., Montoya, J.M., Olesen, J.M., Valido, A., Warren, P.H., 2005. Body size in ecological networks. Trends Ecol. Evol. https://doi.org/10.1016/j.tree.2005.04.005

WoRMS Editorial Board (2018). World Register of Marine Species. Available from http://www.marinespecies.org at VLIZ. Accessed 05/06/2018. doi:10.14284/170

Supplementary info- "Using large benthic macrofauna to refine and improve ecological indicators of bottom trawling disturbance"

Text S1 - Post-metamorphic growth of *Amphiura sp.*

Since the recruits of most species recorded herein settle during autumn (from September to November), sampling in mid-September to early October could have resulted in an overrepresentation of newly settled individuals in the small fraction (and therefore also the full community). Thus recruits could result in low response/lack of response to trawling in the small fraction and full community. Of the six most abundant species (>1000 individuals in the total dataset) in the small fraction, and in the full community, notable numbers of juveniles were not encountered for Phoronis muelleri, Kutiella bidentata, Nucula nitidosa, Thyasira flexuosa and Scalibregma inflatum. This is probably due to the early juveniles being too small and/or soft bodied to be retained on a 1mm sieve. However, few juvenile individuals of *Amphiura spp.* were encountered in the samples. the absence of significant numbers of (<1 year) juveniles is in line with earlier findings in the Kattegat area, where total diameter of Amphiura individuals in October is typically <1mm (Skold et al 2001) (see below). Furthermore, daily mortality of post-metamorphic juveniles are considered high (estimated 160 individuals day^{-1} in Sköld *et al.*, 2001) and annual recruitment into adult populations appears low (Muus, 1981; Sköld *et al.*, 2001). Recruitment of Amphiura takes place between August and November (peaking in October). Typically, the diameter of post-metamorphic settlers is ~0.46mm (disc diameter ~0.3mm and 2x arm length ~0.08mm in Muus, 1981 and Sköld *et al.*, 2001). Using the estimated growth models of Sköld *et al.*(2001), and a daily increase in disk diameter of 0.42% and arm length of 1.76%, even early August recruits are unlikely to have reached a total size of 1mm prior to the sampling time (considering that sampling began ~50 days after August 01, and ended ~65 days after). Furthermore, empirical evidence from Sköld et al 2001 (frequency distributions of the mean arm length and disk diameter) show that the vast majority of individuals sampled in early October were <1mm in length. To be allow for a precautionary approach, we excluded 50% off all Amphiura sp from the dataset and analysed N (small fraction). Model output is provided in section 3.3.3.

Calculation of post-metamorphic growth of *Amphiura filiformis* from Sköld et al 2001:

	Average size at settlement (μm)	Daily growth (in % day^{-1})			
Disc diameter:	300 μm	0.42%			
Arm length:	80 μm	1.76%			
Growth equation: Size x (1+ (%/100))^n where **size** is the length at Day 0, **%** is the daily growth in percentages, and **n** is the number of days of growth					
	Day 0	**Day 30**	**Day 60**	**Day 75**	**Day 90**
Disc diameter	300	300 x (1+ (0.42/100))^{30}=340	300 x (1+ (0.42/100))^{60}=386	411	300 x (1+ (0.42/100))^{90}=438
Arm length (two arms)	80 (160)	80 x (1+ (1.76/100))^{30}=135 (270)	80 x (1+ (1.76/100))^{60}=228 (456)	296 (592)	80 x (1+ (1.76/100))^{90}=385 (770)

Total diameter, μm (1 disc + 2 arms):	460	510	842	1003	1208

References

Sköld, M., Josefson, A., Loo, L., 2001. Sigmoidal growth in the brittle star Amphiura filiformis (Echinodermata: Ophiuroidea). Mar. Biol. 139, 519–526. https://doi.org/10.1007/s002270100600

Text S2 - Kiel Baltic Sea Ice-Ocean Model

We used hydrological data from the high-resolution hydrodynamic Kiel Baltic Sea Ice-Ocean Model (BSIOM, Lehmann and Hinrichsen, 2000); Lehmann et al. 2002) which covers the period 1979-2016. The horizontal resolution of the coupled sea-ice ocean model is at present 2.5 km, and in the vertical 60 levels are specified, meaning that the upper 100 m are resolved into levels of 3 m thickness. The model domain comprises the Baltic Sea, Kattegat and Skagerrak. The oxygen conditions in the entire Baltic Sea are described by an oxygen consumption sub-model coupled to BSIOM (Lehmann et al. , 2014). At the western boundary, a simplified North Sea is connected to the model domain to represent characteristic North Sea water masses in terms of characteristic temperature and salinity profiles resulting from different forcing conditions. The model is forced by low frequency sea level variations in the North Sea/Skagerrak calculated from the BSI (Baltic Sea Index, Lehmann et al., 2002; Novotny et al., 2006). The current model setup is forced by ERA-Interim reanalysis data (Dee et al. 2011) which provide surface air pressure, 2 m air temperature, 2 m dew point temperature, cloudiness at 6-hourly, and total precipitation at 12-hourly resolution. The atmospheric forcing have a horizontal resolution of 0.5x0.5 degrees, and the 6- and 12-hourly base data are linearly interpolated onto 3-hourly resolution to force the ocean model. Wind speed and direction at 10 m height are calculated from geostrophic winds according to the gradient in surface air pressure, where the impact of differences in surface roughness of coastal and open sea areas on wind speed and direction are accounted for (Bumke et al. 1998). Surface heat and momentum fluxes (from wind stress, short wave radiation and sensible and latent heat) are calculated according to Rudolph and Lehmann (2006). River runoff into the Baltic Sea is prescribed from a runoff dataset for the period 1979—2016 corrected using annual accumulated river runoff for extension into the year 2016 (Kronsell and Andersson 2012, see e.g. Lehmann et al. 2014). Modelled data extracted and used in the analysis included average bottom current velocity (m/s), bottom temperature (°C), and bottom salinity (PSU) values.

References

Bumke, K., Karger, U., Hasse, L. Niekamp, K., 1998. Evaporation over the Baltic Sea as an example of a semi-enclosed sea. Contr. Atmos. Phys., 71(2), 249-261

Dee DP, et al. 2011. The ERA-interim reanalysis: configurationand performance of the data assimilation system. Quart.. J. Roy. Meteor. Soc. 137, 1972 – 1990.

Kronsell, J., Andersson, P., 2012. Total regional runoff to the Baltic Sea. HELCOM Indicator Fact Sheets 2011. Online. (http://www.helcom.fi/environment2/ifs).

Lehmann, A., Krauß, W., Hinrichsen, H.-H., 2002. Effects of remote and local atmospheric forcing on circulation and upwelling in the Baltic Sea. Tellus, 54A: 299–316.

Lehmann, A., Hinrichsen, H.-H., Getzlaff, K., Myrberg, K., 2014. Quantifying the heterogeneity of hypoxic and anoxic areas in the Baltic Sea by a simplified coupled hydrodynamic-oxygen consumption model approach. Journal of Marine Systems, 134:20-28.

Novotny, K., Liebsch, G., Lehmann, A., Dietrich, R., 2006. Variability of sea surface heights in the Baltic Sea: An intercomparison of observations and model simulations, Marine Geodesy, 29, 113-134.

Rudolph, C., Lehmann, A., 2006. A model-measurements comparison of atmospheric forcing and surface fluxes of the Baltic Sea. Oceanologia, 48(3), 333-380.

Figure S1: (a) nMDS plot of environmental characteristics of sampling sites (b) Cluster analysis of environmental characteristics of sampling sites. Parts (a) and (b) are based on Euclidean distance.
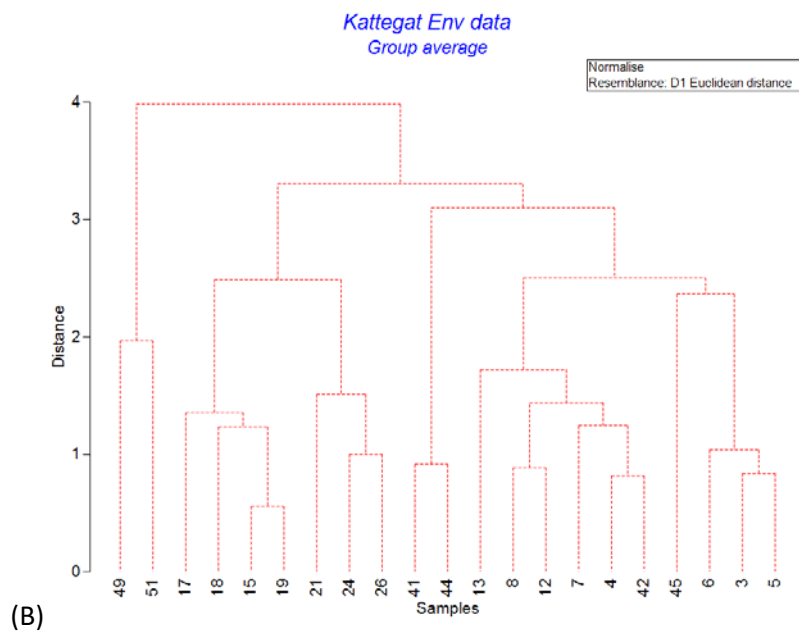

(A)

(B)
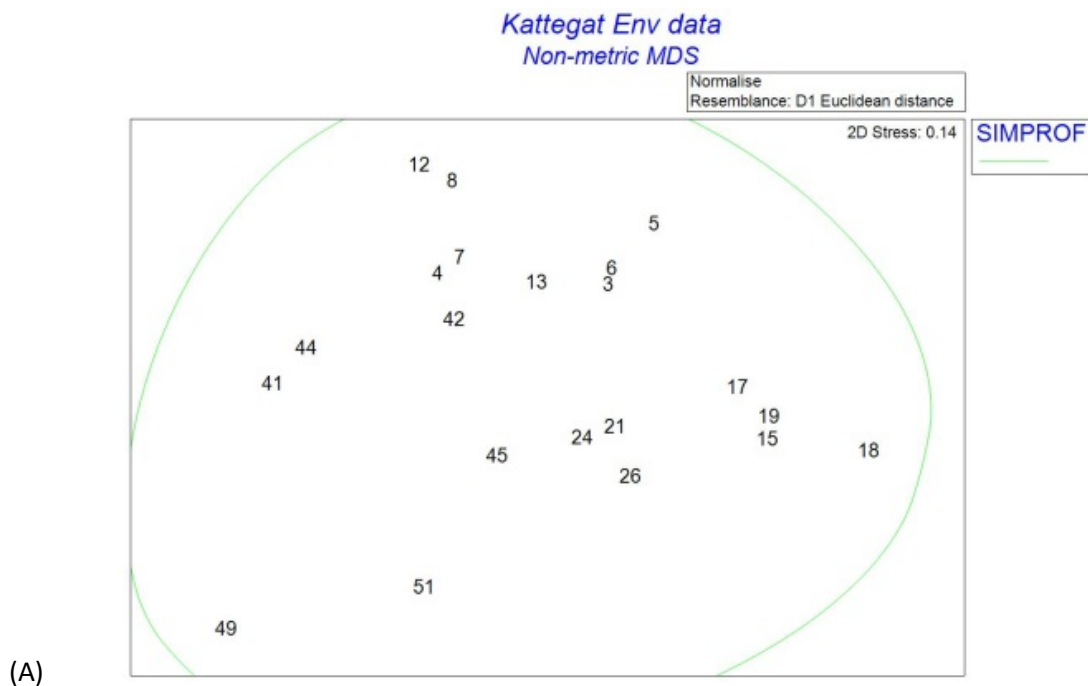
Figure S2: Pairs plot of predictor variables (fishing intensity and environmental variables) and associated Pearson correlation coefficients. LOI = loss on ignition. SAR = swept area ratio. Correlations of >0.7 were excluded from the analysis, which resulted in Loss-on-ignition (LOI) being removed.
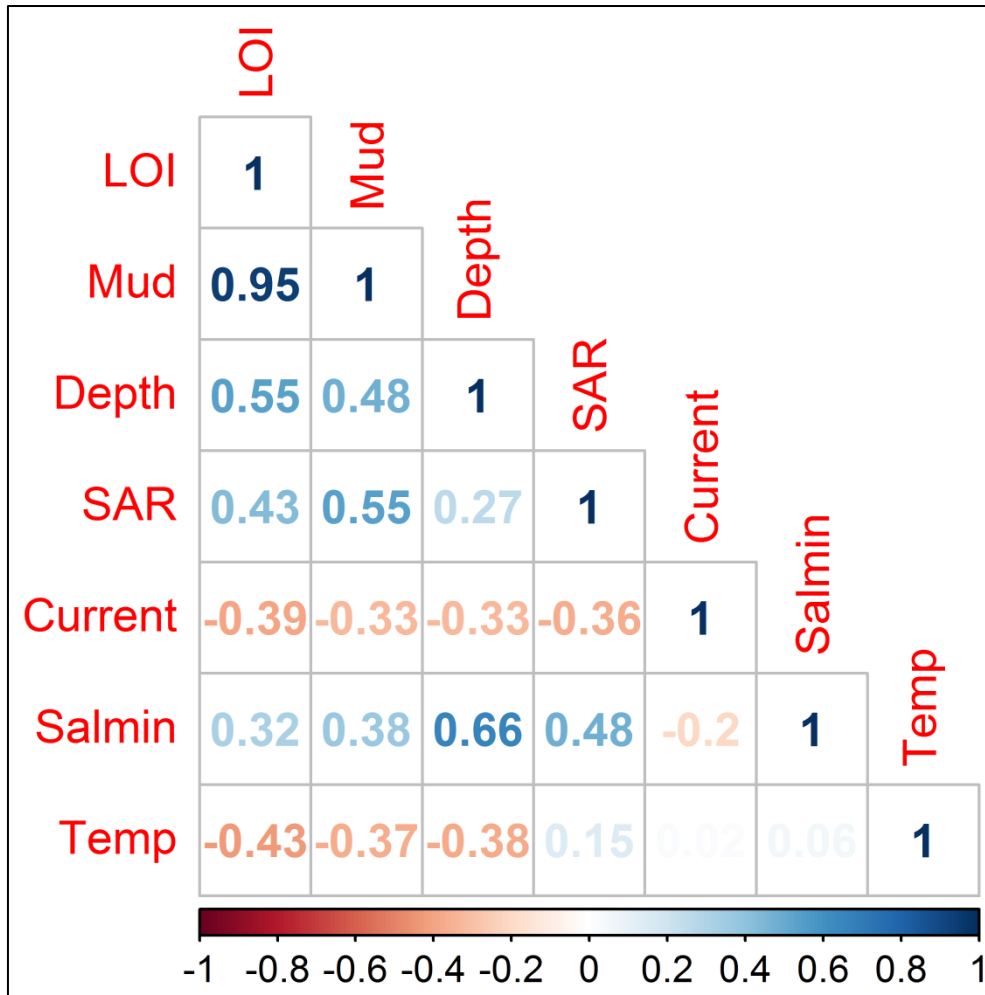
Table S1: Site-by-site summary information for macrofaunal density, species density, trawling intensity, and environmental variables. Density and species density are totals over four replicates. As SAR values were calculated and analysed for each replicate sample, the values presented here are averaged over four replicates (standard deviation shown in brackets). Data for environmental variables were calculated per sampling site.

Sampling site	Density			Species density			Trawling Intensity	Current speed	Depth	Mud content	Min Salinity	Temperature
	1-4mm	>4mm	Full community	1-4mm	>4mm	Full community	SAR (3 yr^{-1})	cm/s	m	% <63μm	PSU	Celsius
3	629	144	773	36	7	39	33.53 (3.7)	1.66	34.43	79.59	32.20	8.12
4	1850	558	2408	52	22	59	8.95 (0.2)	1.69	16.25	13.67	29.16	8.98
5	552	76	628	36	10	38	43.4 (0)	1.36	32.90	96.54	33.15	8.54
6	947	189	1136	40	10	44	21.06 (0.4)	0.84	33.18	67.08	33.04	8.17
7	2889	219	3108	73	18	81	4.14 (0.4)	1.86	38.65	3.78	30.76	8.54
8	2882	286	3168	75	33	89	3.64 (0.1)	1.02	33.00	5.10	29.28	8.85
12	1847	311	2158	62	31	73	0.42 (0)	0.98	18.58	3.57	30.03	8.99
13	1872	461	2333	73	25	81	13.15 (0.2)	0.73	28.03	11.89	33.72	7.82
15	318	111	429	47	11	50	11.91 (1.1)	1.45	63.68	96.16	34.31	6.92
17	558	197	755	47	18	52	35.02 (1.6)	0.42	60.90	60.77	34.26	6.95
18	258	214	472	42	17	46	7.2 (1.2)	0.41	81.08	95.76	34.34	6.80
19	403	166	569	51	14	53	15.3 (0.5)	0.95	64.90	92.67	34.24	7.02
21	480	155	635	53	16	57	7.11 (0.1)	2.54	59.93	44.93	34.22	7.33
24	709	246	955	83	38	101	3.54 (0.3)	1.93	59.30	4.32	34.04	7.50
26	624	122	746	73	14	80	16.39 (0.5)	1.35	62.08	10.90	34.47	6.89
41	752	599	1351	74	36	82	2.08 (0)	4.11	22.45	24.67	30.37	7.54
42	1242	375	1617	61	31	69	5.48 (0.1)	1.46	19.88	12.46	30.78	7.77
44	975	483	1458	78	33	89	3.28 (0.1)	3.56	23.13	5.48	30.83	7.89
45	1100	535	1635	53	33	62	0 (0)	2.35	26.78	57.94	32.07	6.77
49	1018	548	1566	52	27	63	0 (0)	1.76	19.25	25.68	21.63	6.41
51	2091	792	2883	67	39	75	0 (0)	1.10	20.93	56.17	26.66	6.25

Table S2: Output of generalised linear mixed models for macrofaunal indicators. Parameter estimates are presented and standard error is shown in brackets. Significance level is denoted by asterisks (* = P < 0.05; ** =P < 0.01; *** = P < 0.001).

Macrofaunal indicator	Intercept	Current	Depth	Mud%	Temperature	Trawling	Salinity	Density†	Marginal R²	Conditional R²
Pooled community										
RaoQ	0.23 (0.02)		-0.001 (<0.001)*	-0.001 (<0.001)*					0.09	0.18
Fric	0.47(0.01)			-0.001 (<0.001)*		-0.003(0.001)**			0.36	0.47
Feve	0.20(0.09)						0.006(0.003)*		0.08	0.24
Fdis	4.31(0.40)		0.022(0.010)*	-0.013(0.006)*					0.09	0.13
Small (1-4mm) fraction										
RaoQ	0.43(0.06)				-0.014(0.008)	-0.001 (<0.001)***			0.17	0.17
Fric	0.38(0.02)		-0.001 (<0.001)*			-0.003(0.001)**			0.26	0.38
Feve	0.21(0.08)						0.007(0.002)**		0.11	0.11
Fdis	5.76(0.13)					-0.025(0.008)**			0.10	0.11
Large (≥4mm) fraction										
RaoQ	0.28(0.02)	-0.024) 0.011)*	-0.001 (<0.001)*			-0.002(0.001)***			0.28	0.28
Fric	0.49(0.03)					-0.007(0.001)***			0.26	0.46
Feve	0.46(0.03)					-0.005(0.001)**			0.11	0.11
Fdis	4.37(0.30)					-0.053(0.018)**			0.12	0.21

N = density, S = species density, H' = Shannon diversity, RaoQ = functional diversity, Fric = Functional richness, Feve = Functional evenness, Fdis = Functional dispersion

Model families: N = Negative binomial. S = Poisson. Biomass/RaoQ/Fric/Feve/Fdis = Gaussian

† = density (N) included as a predictor for models of species density (S), only

A.4 Pre-submitted version of McLaverty *et al.*, *in prep.*

Benthic life history traits as ecological indicators of bottom trawling

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Abstract

An ecosystem approach to fisheries management aims to strike a balance between the exploitation of marine resources, and inevitable impacts to benthic biodiversity and ecosystem function. In this regard, trait-based approaches represent a promising method to understand the impacts of trawling on benthic ecosystem function, and as a basis to develop indicators which can be used to quantify benthic impacts. In this study, we describe the sensitivity of benthic communities to trawling using community-weighted mean (CWM) traits, which have the potential to act as univariate indicators of trawling disturbance. Using benthic data collected from commercial fishing grounds in the Kattegat, we demonstrate that the traits of large benthic macrofauna are particularly sensitive to trawling impacts. In addition, benthic fauna which are sessile, suspension feeding, surface depositing, tube-dwelling and demonstrating long lifespans, were also particularly sensitive in this regard. Given the importance of many of these traits in processes such as benthic-pelagic coupling, it is therefore likely that the loss or decline of these traits may have implications for the functioning of benthic ecosystems across fishing grounds. Additionally, we validated our observations using an independent benthic monitoring dataset. Our results suggest that chronic bottom trawling has a strong negative effect the traits of benthic macrofauna, and that data collected from a carefully designed one-off sampling event can provide results which are representative of long-term datasets.

1. Introduction

Ensuring a balance between fisheries exploitation, biodiversity, and ecosystem function represents a basic tenet of an Ecosystem Approach to Fisheries Management (EAFM) (FAO, 2003; Pikitch et al., 2004). Research of the ecological effects of bottom trawling have thus far mainly focussed on the links between fishing pressure and the structure, diversity, and composition of benthic species (Kaiser et al. 2006, Hiddink et al. 2017, Sciberras et al. 2018). Such taxonomic (i.e. species-based) approaches, however, face several limitations regarding their ability to effectively monitor biodiversity (Reiss et al. 2009), while being unable to convey information regarding ecosystem processes or describe mechanisms of sensitivity (Bremner et al. 2006). As a result, biological trait approaches are being increasingly used in community ecology to characterise community composition (McGill et al. 2006). Biological traits describe the phenotypic (i.e. morphological, physiological, behavioural) characteristics of an organism (Violle et al. 2014), and as a result can be used to predict the vulnerability of communities to disturbance (Mouillot et al., 2013; Beauchard et al., 2017).

The implementation of major European marine policy directives in recent years, such as the European Union Water Framework Directive (Anon. 2000) and Marine Strategy Framework Directive (Anon. 2008), have also led to an increased focus on the monitoring of human impacts to seabed ecosystems. These Directives have generally favoured the use of ecological indicators to monitor such pressures. Ecological indicators provide the facility to combine numerous environmental factors in a single, easily measurable, and calculable value. Furthermore, they allow for often complex information to be presented in a simple manner to a range of stakeholders (Jorgensen et al. 2013). Nonetheless, for indicators to be useful in a fisheries management context, they must be able to both accurately describe the ecological state of seabed biota, and be responsive to changes in state which occur as a result of fishing. This is important as the ecological status of the seabed varies naturally across fishing grounds, meaning that effective ecological indicators must be resilient to the influence of other natural or manmade environmental drivers, or by spatial or temporal variation in seabed conditions (Rice et al. 2012, Hiddink et al. 2020).

Considering the relative advantages of trait-based approaches, and the legislative ambition to integrate benthic indicators in national monitoring programmes (Van Hoey et al. 2010), significant scope exists to further develop trait-based trawling indicators. While most research to date has focussed on trawling impacts on community trait composition (Bremner et al. 2003, Tillin et al. 2006, Bolam et al. 2014, 2017, Van Denderen et al. 2015), comparatively few studies have examined the if trait-based indicators can be used to monitor and manage trawling. Notable exceptions include the development of a trawling disturbance indicator based on defined functional groups (De Juan & Demestre 2012), and using the longevity

characteristics of the community to describe benthic sensitivity to trawling (Rijnsdorp et al. 2018, Hiddink et al. 2019). The value of these trait-based approaches is that the sensitivity of benthos to trawling is intrinsically linked to an individual's biological attributes or traits. The main features which define an individual's sensitivity is typically composed of a balance between its tolerance to disturbance (i.e. rate of mortality from a given pressure) and its recoverability (recolonization following disturbance) (Hiscock & Tyler-Walters 2006). As a result, the sensitivity of an individual, or indeed a community, can be underpinned by the relative abundance of several key traits. These may include feeding mode (i.e. suspension-feeder vs. scavenger), living habit (free-living vs. tube-dwelling), sediment position (surface vs. deep), egg development (parental brooding vs. pelagic dispersal), as well as general biological traits such as body-size, longevity, and morphology (Bremner et al. 2003, Tillin et al. 2006, Bolam et al. 2017).

A further advantage of a trait-based approach to indicator development is that indicators can be calculated to reflect the most sensitive characteristics of the community. Bottom trawling results in uneven mortality to benthic fauna, meaning that some highly sensitive species will experience high mortality rates while others may increases in abundance in response to trawling (Sciberras et al. 2018). This can lead to the situation where indicators calculated using all members of the benthic community can exhibit a varied ability to detect trawling disturbance (McLaverty et al. 2020). Furthermore, benthic communities are often predominantly composed of highly abundant small-bodied individuals, which are relatively less sensitive to bottom trawling and may be more closely linked to variations in other environmental drivers (Duplisea et al. 2002). The sensitivity of benthic indicators to trawling disturbance can be improved by using applying a body-size threshold to the community, thereby separating the larger individuals and species, and using these in the calculation of indicators (McLaverty et al. 2020). While this has demonstrated a clear size-dependent sensitivity of the community to trawling, it is not currently known if individual benthic traits are similarly sensitivity to trawling, or whether body-size is simply the dominant trait determining sensitivity.

The main aims of this study were to (i) examine which traits are the most effective indicators of bottom trawling, (ii) if this performance of indicators varied as a function of body-size, and (iii) discuss the implications of our findings for benthic ecosystem function. To do this, benthic samples were collected along a wide gradient of trawling intensity from a heavily fished commercial fishing ground in the Kattegat. The benthic community was separated into small (1 – 4mm) and large (≥4mm) fractions to investigate the potential size-sensitivity of traits and relative indicator performance. Finally, we supplemented our analysis with data from a long-running monitoring dataset from the same area. This allowed us to determine if our results were representative of long-term bottom trawling impacts, which may not be potentially captured by a one-off sampling event.

2. Materials and methods

2.1. Data sources

Data analysed in this study comprised two separate benthic surveys undertaken in Danish waters between 2005 and 2016. The Kattegat 2016 (KS16) survey was undertaken as a one-off or 'snapshot' survey in September 2016, designed to assess the impact of *Nephrops norvegicus* fisheries in the Kattegat. Across 21 sampling sites, each benthic sample was size-separated into large (>4mm) and small (1 – 4mm) size fractions. This resulted in three separate datasets; the small fraction, the large fraction, and the two fractions combined (full community). The study also included a comparison of results with a data from a long-term benthic data. These data were extracted from the Danish National Monitoring programme (NOVANA), which undertakes benthic sampling to monitor the effects of anthropogenic pressures, such as nutrient enrichment, in Danish waters (Svendsen et al., 2005). The NOVANA dataset comprised 22 sampling (fixed) sites across the Kattegat, sampled in the years 2005, 2006, 2007, 2008, 2010, 2011 and 2013. Accordingly, four datasets were used in the analysis; three from the KS16 survey, and one combining the various NOVANA surveys.

2.2. Study area

Both the KS16 and NOVANA surveys took place in the Kattegat within the Danish Exclusive Economic Zone (EEZ) (Figure 1a). The area represents an important fishing ground for Danish and Swedish vessels targeting mainly Norway lobster *Nephrops norvegicus* (Linnaeus, 1758). The western areas of the Kattegat are shallow and sandy, while the northern and eastern areas are generally deeper, and composed of a mosaic of muddy canyons and mounts of mixed sediments. *Nephrops* habitats generally occur below 20m depth where muddy sediments dominate. The highest trawling intensities typically occur in the northern areas, around the Kattegat trench system in the east, and sporadic areas in the south (Figure 1b).
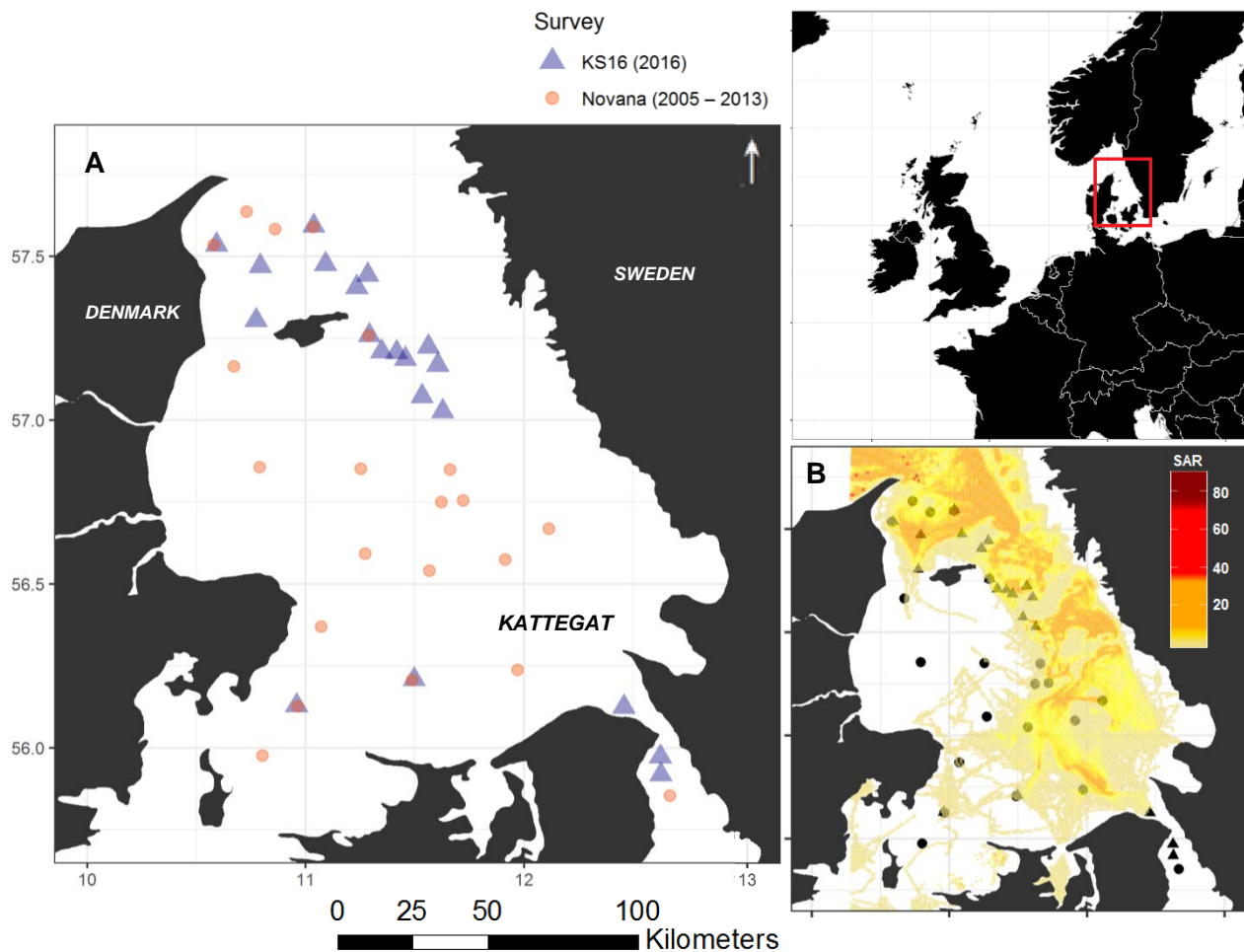
Figure 1. (a) Location of sampling sites for KS16 and NOVANA surveys (b) location of sampling sites in respect to typical trawling effort in the Kattegat (based on fishing effort between 2013 and 2016).

2.3. Data collection and sample processing

KS16 survey (2016): The KS16 sampling sites were selected based on criteria such as sediment type (mud and mixed sand/mud), depth (≥15m), and a gradient of trawling intensity based on 2013-2016 data (Figure 1b). At each site, five sediment samples were acquired using a 0.1m² Van Veen grab. One sample was used to determine the sediment grain size, and four samples were processed for benthic fauna. Overall, 84 faunal samples were collected and processed. Each of these samples were sieved consecutively over 4mm and 1mm mesh sizes to separate the community into large and small size fractions. The sieved samples were then fixed in 4% borax-buffered formaldehyde in ambient seawater. In the laboratory, all animal material was identified

to the lowest taxonomic level possible, and biomass estimates per species were determined as ash-free dry weight.

NOVANA survey (2005 – 2013): At each of the fixed NOVANA sampling sites, four or five replicate sediment samples are collected using a 0.0143m^2 Haps corer for faunal analysis. Combining data for all years between 2005 and 2013 resulted in a total of 827 samples. Each sample was rinsed over a 1mm sieve, and persevered in 96% ethanol solution. In the laboratory, all animal material was sorted and identified to the lowest taxonomic level possible. Biomass estimates per species were determined as wet weight.

2.4. Estimating trawling pressure

Trawling intensity was calculated for the KS16 and NOVANA surveys as a swept area ratio (SAR). The SAR values are calculated within a circular 'impact area' placed around each benthic sampling site. Within these impact areas, cumulative trawling effort is calculated within the circle, and then divided by the size of the circle to estimate the SAR. The SAR values can thus be interpreted as the number of times the seabed within the circle is trawled in a given time period. The impact area was delineated by a 2km radius. This was selected based on the findings of Lambert et al. (2012), who found a 2km x 2km resolution to be relatively consistent when determining relationships between fishing intensity and community biomass (Lambert et al. 2012). The cumulative trawled area was estimated by combining Vessel Monitoring System (VMS) data with information regarding vessel and gear specifications (provided by EU logbooks). VMS receivers collect and send data regarding the vessel location, heading, and speed at an hourly polling frequency (for Danish and Swedish vessels). The raw VMS data were then processed to preserve only points which represented trawling activity, defined as sailing speeds between 2 and 4 knots, and with a minimum distance of 3km from port. To reconstruct trawling tracks, the raw data points were interpolated using a cubic Hermite spline method (Hintzen et al. 2012). These tracks are then combined with logbook data on vessel size and gear dimensions (Eigaard et al. 2016), which are used to estimate and calculate the area of seabed 'swept' during each logbook trip. All processing of VMS data and estimation of SAR were done using the VMStools package (Hintzen et al. 2012). In order to ensure comparability between the KS16 and NOVANA data, data for fishing vessels ≥15m were used in the study. While VMS has been mandatory all vessels ≥12m operating in European waters since 2012, VMS was only mandatory on vessels ≥15m between 2005 and 2012, meaning that data are not available for vessel between 12-15m for much of the NOVANA samples.

2.5. Environmental drivers

Aside from trawling intensity, data for five environmental drivers were included in the analysis. These included mud content (%), depth (m), bottom current speed (cm^{-s}), bottom water salinity, and bottom

temperature (°C). Data were collected in the field, while mud content was determined from sediment samples collected at each sampling site. Particle size analysis (PSA) was undertaken via mass loss following wet sieving (Danish Standard (DS) 405.9), and resulted in the estimation of mud% (<63μm), sand% (0.063–2mm), and gravel% (≥2mm). Modelled hydrodynamic data for bottom current speed, bottom salinity, and bottom temperature were extracted from the high-resolution Baltic Sea Ice-Ocean Model (BSIOM) (Lehmann et al., 2014). These data were back-calculated based on a 2 x 2km grid cells at a monthly scale, and averaged over the 12 months prior to sampling. Due to the resolution of the environmental data, the same values were applied to all replicates at each site.

2.6. Calculation of trait composition and indicators

The trait information for each species were described using 10 trait categories and 48 descriptive modalities (traits) (Table 1). The majority of trait information was extracted from an existing trait database (Bolam et al., 2017), with 10 genera coded based on available literature, and the BIOTIC database (www.marlin.ac.uk/biotic). The trait composition in each dataset was calculated based on 368 (NOVANA), 285 (KS16 full community), 144 (KS16 large fraction) and 259 (KS16 small fraction) taxa. As benthic species often exhibit multiple traits per category, the traits were described using a 'fuzzy coding' approach (Chevenet et al. 1994). This allows several traits to be expressed within a category, and indicates the relative importance of each trait. When taxa showed full affinity to a trait (e.g. in the case of maximum body size) a score of 3 was assigned. Conversely, if taxa exhibited multiple traits per category, a score relative to its importance would be assigned (e.g. the brittlestar *Amphiura filiformis* is a deposit feeder and facultative suspension feeder, this scored as suspension = 2 and surface deposit = 2). After all traits were scored, the values were standardised to 1 within each category. Trait scores where weighted by biomass data, and trait-by-site matrices generated. To generate a matrix, the trait scores were averaged across all species present in a sample and then multiplied by the relative biomass of those species, resulting in a single community-weighted mean (CWM) value for each trait per sample. Biomass was chosen to weight the trait values as it is considered to better reflect an organism role in ecosystem functioning (Bolam & Eggleton 2014). CWM values were calculated using absolute values, rather than relative (Beauchard et al. 2017), in order to preserve spatial and temporal contrasts in trait biomass across the study area.

Table 1. Biological trait categories and traits used in the study.

Category	Trait
Feeding mode	Suspension Scavenger/Opportunist Surface Deposit Subsurface deposit

	Predator
	Parasite
Larval Development	Planktotrophic
	Lecithotrophic
	Direct
Egg development	Asexual/Budding
	Sexual: eggs pelagic
	Sexual: eggs benthic
	Sexual: brood eggs
Mobility	Sessile
	Burrower
	Swim
	Crawl/creep/climb
Size range	<10mm
	11-20mm
	21-100mm
	101-200mm
	201-500mm
	>500mm
Longevity	<1 year
	1-3 years
	3-10 years
	>10 years
Morphology	Soft
	Crustose
	Cushion
	Stalked
	Tunic
	Exoskeleton (chitin/calcium carbonate)
Living habit	Tube-dwelling
	Burrow-dwelling
	Free-living
	Crevice-dwelling
	Attached to substratum
	Epi/endozoic/phytic
Sediment position	Surface
	0-5cm deep
	6-10cm deep
	>10cm deep
Bioturbation	Diffusive mixing
	Surface deposition
	Downwards conveyer
	Upward Conveyor
	None

2.7. Statistical approach

Multivariate analysis – determining sensitive traits

Analyses of trait composition were carried out using distance-based linear models (DistLMs). DistLMs fit a relationship between community data and predictors (in our case environmental data) using a distance-

based redundancy analysis (dbRDA) approach (Legendre & Andersson 1999). Using this approach, constrained ordinations are undertaken using non-Euclidean distance measures. As absolute values were used, we log transformed (log(x+1)) the trait scores prior to analysis, and constructed resemblance matrices based on Bray-Curtis similarity. Step-wise sequential tests and the small-sample size corrected Akaike information criterion (AICc) were used for the selection of predictors in the DistLM, and p-values were obtained based on 999 permutations. Following this, the dbRDAs visualise the fitted values from the DistLM model. In each of the plots, vector overlays visualise the multiple partial correlations for the significant predictors. We used these multiple partial correlations to compare the importance of the trawling predictor in each of the datasets. The most sensitive traits in each dataset were determined via correlation coefficients (Pearson's R) between each trait and primary axis of the redundancy plot, which also correlated significantly with trawling intensity. The larger the correlation coefficient, the larger the contribution of that trait to the dbRDA ordination. This approach has been suggested as a useful method to generate exploratory hypotheses from multivariate data (Anderson et al., 2008), and has been used examine the sensitivity of individual species to trawling disturbance (Sköld et al. 2018). Given that each of the four datasets contain 48 traits, we limited our analysis to the ten traits with the largest correlation coefficients in each dataset. All analyses were carried out using PRIMER v.7 with PERMANOVA+ (Anderson et al. 2008).

Univariate analysis - Sensitivity of CWM traits to trawling

The response of CWM traits to trawling were analysed using generalised linear mixed effects models (GLMMs), an approach suitable for the quantification of potential correlations in repeated measure designs (Bolker et al. 2009). Given the CWM traits were weighted with biomass data, the trait data were non-negative, continuous, containing zeros, and right skewed. Log-transformations are commonly applied to such data structures in ecological studies. However, this has the potential to distort the underlying meaning of the response variable and even produce spurious results (Feng et al. 2014). We therefore applied an alternative modelling approach, and used a Tweedie distribution to model the CWM traits. This distribution is particularly suited to this data as it can handle continuous data that is greater than or equal to zero. The Tweedie distribution and its extensions have been shown to effectively fit fish biomass data (Foster & Bravington 2013). We fitted the GLMMs and Tweedie distribution with a log-link and using the glmmTMB package (Brooks et al., 2017). The final model was determined via backwards model selection and likelihood ratio tests. Model diagnostics were checked using the DHARMa package (Hartig, 2016), a simulation based approach designed for the interpretation of linear mixed models. To avoid issues related to multi-collinearity of predictor variables, all pair-wise correlations were checked, and all demonstrated correlation values of

<0.7 (Dormann et al., 2013). Pairwise correlation coefficients are presented in the Supplement – Table S1. All analyses were conducted using R version 3.5.0 (R Development Core Team, 2019).

3. Results

3.1. Comparison of surveys data

Although the KS16 survey involved less sampling effort (in terms of the number of samples and surveys), the area of seabed sampled across the two surveys was roughly similar (Table 2). This was due the assize of the respective 0.1 m^2 Van Veen grab sampler and 0.0143m^2 Haps corer used in KS16 and NOVANA, respectively. The size fractionation of the KS16 data revealed that ~90% of the biomass was attributable to the large fraction. The large fraction, however, contained considerably fewer species than the full community, resulting in comparatively less time required taken to process the samples. The means and ranges of trawling intensity were relatively comparable across sampling sites in both surveys, indicating that the trawling gradient sampled in 2016 was largely representative of longer-term patterns in trawling activity.

Table 2. Summary of the sampling effort, benthic community parameters, and fishing intensity recorded in the KS16 (2016) and NOVANA surveys (2005-2013).

Survey	KS16 (2016)			NOVANA (2005 – 2013)
dataset	Large fraction	Small fraction	Full community	Full community
Size fraction	≥4 mm	1 – 4 mm	≥1 mm	≥1 mm
Number of surveys	1			7
Number of samples	84			827
Area sampled	8.4 m^2			11.8 m^2
CWM traits represented	46	48	48	48
Total biomass	199.04 g*	21.46 g*	220.50 g*	4,814.13 g†
Total number of taxa	144	259	285	368
Range of trawling intensity (SAR)	0 - 12.6 yr^{-1}			0 - 14.8 yr^{-1}
Mean trawling intensity (SAR)	3.8 yr^{-1}			3.4 yr^{-1}
* Ash free dry weight biomass † Wet weight (blotted) biomass				

3.2. Determining sensitive traits

The primary axis of the redundancy analyses (dbRDA1) accounts for the majority of variance in the multivariate trait ordination, and in each dataset, dbRDA1 was significantly correlated with trawling. DbRDA1 was therefore used to calculate relationships with the CWM traits in each of the datasets (Table 3a). Traits such as 'suspension', 'sessile', 'burrow-dwelling', '0-5cm deep' and '6-10cm deep' exhibited large negative correlation coefficients in both the KS16 and NOVANA surveys. Across size fractions, traits of large benthos (KS16 large fraction) showed a high degree of similarity with traits identified at the community level (KS16

full community). Exceptions to this were '6-10cm deep', 'tube-dwelling and 'soft'. On the other hand, the traits identified from the small benthos (KS16 small fraction), e.g. 'subsurface deposit', 'lecithotrophic', 'predators', '0-5cm deep', and 'diffusive mixing', were comparatively different from the other size fractions. On the other hand, a few traits ('free-living' and 'crevice dwelling') exhibited positive relationships with dbRDA1. The redundancy analysis also revealed that trawling had relatively greater influence on the KS16 large fraction dataset than the other datasets. This was demonstrated by the marginally larger multiple partial correlation coefficients, which describe the effect of a predictor on the ordination conditional of the effects of all predictors (Table 3b). Based on this, the most sensitive traits identified in the large fraction chosen as a basis for univariate modelling. These traits included 'suspension', '>10 years', 'surface deposition', '101-201mm', 'sexual: eggs pelagic', 'planktotrophic', 'exoskeleton', 'tube-dwelling', 'sessile', and '6-10cm deep'.

Table 3 (a) Correlation coefficients describing the relationship between CWM traits and dbRDA1. The bdRDA1 accounted for the majority of trait variation, and was significantly correlated with trawling. The ten strongest correlations are presented for each dataset. Plus/minus symbol indicates if relationship is positive/negative. (b) Multiple partial correlations between trawling and dbRDA1.

		KS16 large	KS16 small	KS16 Full community	NOVANA
(a)					
Category	Trait				
Bioturbation	Surface deposition	-0.49		-0.51	
	Non-bioturbator				-0.21
	Diffusive mixing		-0.43		
Egg development	Pelagic eggs	-0.48	-0.52	-0.5	
Feeding	Predator		-0.45		
	Subsurface deposit		-0.43		
	Suspension	-0.52		-0.55	-0.20
Larval development	Lecithotrophic		-0.46		
	Planktotrophic	-0.48		-0.5	
Living habit	Crevice-dwelling				+0.42
	Free-living				+0.21
	Tube-dwelling	-0.47			
Longevity	1 – 3 years				-0.20
	>10 years	-0.50		-0.51	
Mobility	Sessile	-0.47	-0.45	-0.51	-0.24
Morphology	Exoskeleton	-0.47		-0.49	
	Soft		-0.51	-0.54	

Sediment position	0-5cm deep		-0.47		-0.23
	6-10cm deep	-0.45	-0.53		-0.23
Sediment stability	Burrow-dwelling		-0.41	-0.48	-0.26
Size range	21 - 100mm				-0.22
	101 - 200mm	-0.49		-0.49	
(b)					
	Predictor				
	Trawling	0.45	0.30	0.41	0.13

3.3. Sensitivity of CWM traits to trawling

Of the ten traits examined from the KS16 large fraction, seven exhibited a significant negative relationship with trawling i.e. 'suspension', 'planktotrophic', 'sessile', 'surface deposition', '>10 years', '6-10cm deep' and 'tube-dwelling' (Table 4, Figure 3 & 4). The sensitivity of these traits to trawling were mostly reflected at the full community level, in both the KS16 full community and NOVANA data. The exception to this were the traits '>10 years' and '6-10cm deep' in the KS16 full community, and 'suspension' in the NOVANA data. This high degree of congruence would indicate that traits identified from the large fraction were highly important in driving the sensitivity of benthos across the entire community, and that these results were reflective of long-term trawling effects. Across each of the datasets, depth, temperature, and current speed were key drivers of trait biomass. Model outputs from the GLMMs are provided in Table S3 in the Supplement.

Table 4. Summarised results of GLMMs for CWM traits. Significant relationships with trawling denoted by an 'x'. Significant relationships with other environmental variables are shown in the 'other' column, and denoted by C = bottom current speed, D = depth, M = mud content, T = bottom temperature, S = bottom salinity. Direction of the relationship is shown as positive (+) or negative (-). Model coefficients of determination (R^2) provided as conditional R^2 values (both marginal and random effects combined).

Trait	KS16 (2016)									NOVANA (2005 – 2013)		
	Large fraction			Small fraction			Full community			Full community		
	Trawling	Other	R^2	Trawling	Other	R^2	Trawling	Other	R^2	Trawling	Other	R^2
Exoskeleton		C+, D-, T-	0.57		T+	0.52		D-, T-	0.61		C+, S+	0.29
Pelagic eggs		D-, T-	0.58		S-, T+	0.40		C+, D-, T-	0.62		S+, T+	0.42
Planktotrophic	x-	D-	0.58		S-, T+	0.42	x-	D-	0.61	x-	C+, S+	0.41
Sessile	x-	D-	0.83	x-	C-	0.74	x-	D-	0.82	x-	C+, M+, S+, T-	0.90
Surface deposition	x-	D-, T-	0.67		S-, T+	0.44	x-	D-	0.69	x-	C+, M-, S+	0.43
Suspension	x-	D-	0.69		S-, T+	0.48	x-	D-	0.72		C+, M-	0.43
Tube-dwelling	x-	T-	0.80		M-, T-	0.81	x-	T-	0.87	x-	C+, M-, T-	0.82
101-200mm		D-, T-	0.68		S-, T+	0.48		D-, T-	0.70		C+, M+	0.52
6-10cm deep	x-	D-, T-	0.83		M-, S-	0.67		D-, T-	0.81	x-	C+, D-, M-, S+	0.67
>10 years	x-	D-	0.70		D-	0.67		D-, T-	0.69	x-	C+, D+, T+	0.41
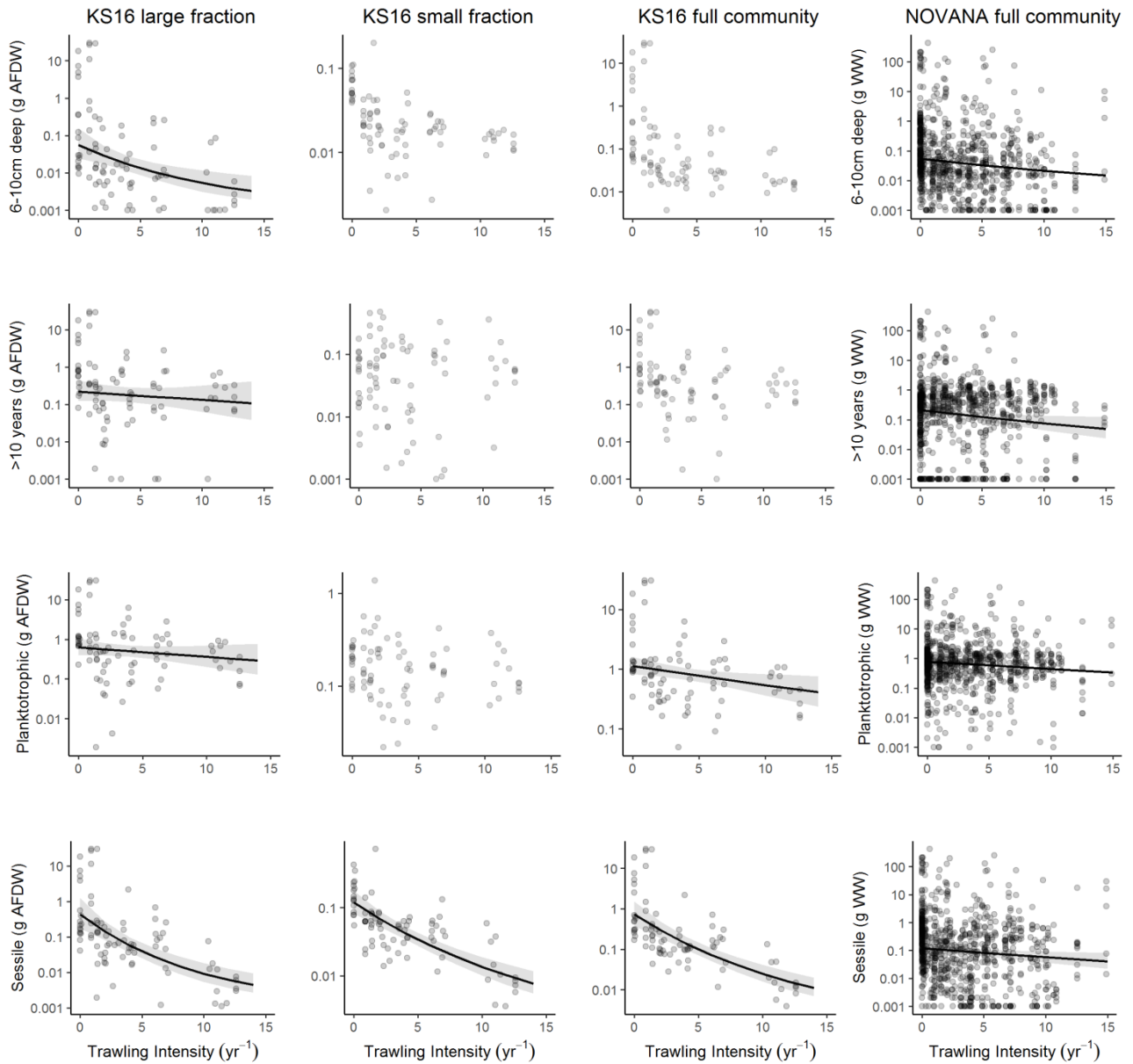
Figure 2. Relationships between trawling intensity and the CWM traits 6-10cm deep, >10 years, planktotrophic, and sessile. The results are presented comparatively for the KS16 and NOVANA surveys. Regression lines denote a significant relationship, and represent the predicted values from GLMMs presented in Table 4. Shaded areas represent 95% confidence intervals. The raw observations are overlaid as data points. To aid visual interpretation, trait values are shown on a log scale.

Figure 3. Relationships between trawling intensity and the CWM traits surface deposition, suspension, and tube-dwelling. The results are presented comparatively for the KS16 and NOVANA surveys. Regression lines denote a significant relationship, and represent the predicted values from GLMMs presented in Table 4. Shaded areas represent 95% confidence intervals. The raw observations are overlaid as data points. To aid visual interpretation, trait values are shown on a log scale.
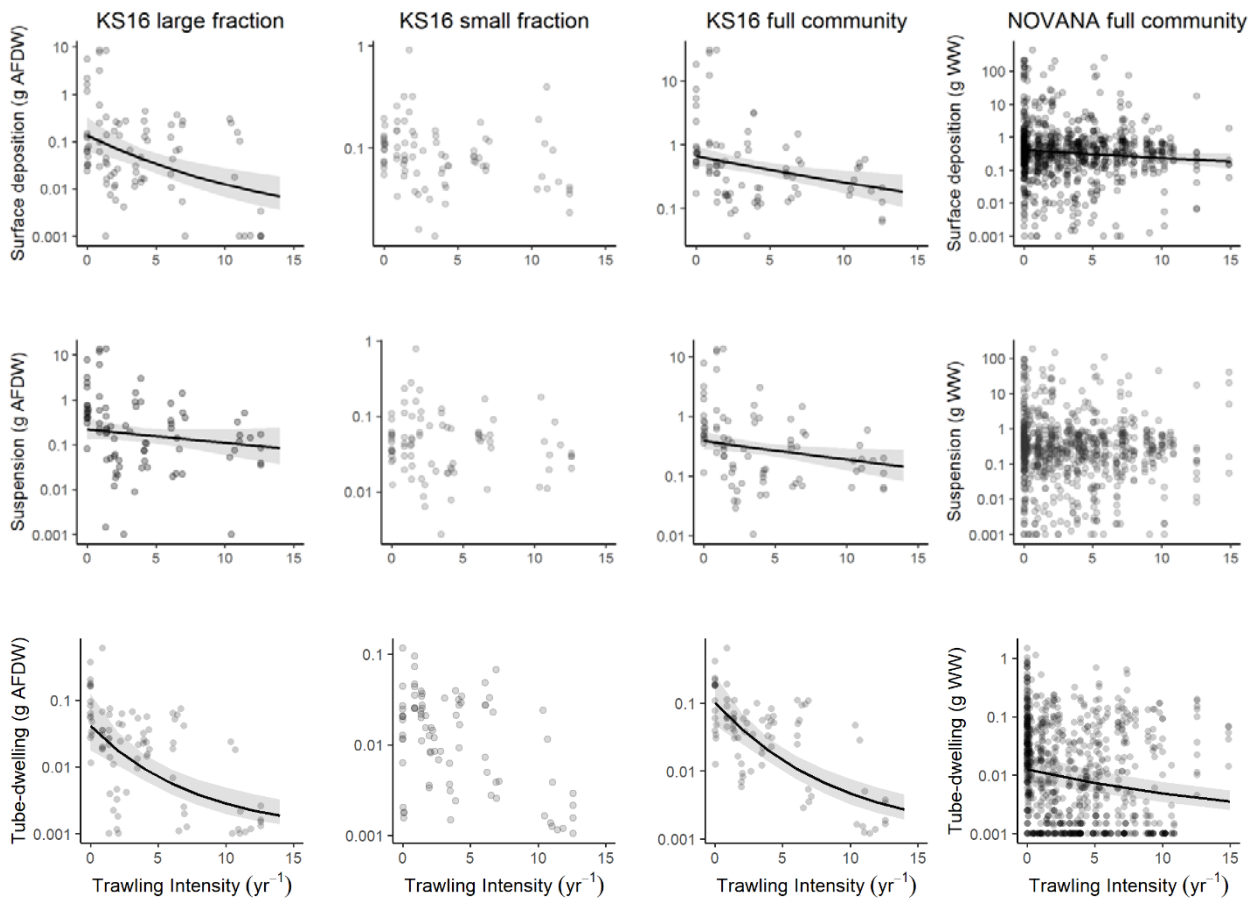
4. Discussion

An ecosystem approach to fisheries management (EAFM) aims to sustain ecosystem function where commercial fisheries take place (Garcia et al., 2003). In this regard, assessments of ecosystem functionality based on biological traits can provide a valuable alternative to direct measurements of ecological function, such as nutrient flux experiments, which are methodologically challenging to implement in the field. We have investigated the sensitivity of forty-eight benthic life history traits as part of this study, and identified seven that show a particularly strong response to bottom trawling. These were identified from a dataset comprising the larger members of the community (≥4mm in size), and included the traits '6-10 deep' (sediment position), 'surface deposition' (bioturbation), '>10 year' (longevity), 'planktotrophic' (larval development), 'sessile' (mobility), 'suspension' (feeding mode), and 'tube-dwelling' (living habit). Using a full community approach, as is the standard approach for benthic community analysis, five of the seven traits responded significantly to trawling. These exceptions to this were '6-10 deep' and '>10 year'. This would suggest that these the deep-living and long-lived benthos are highly sensitive to trawling, and yet their sensitivity to trawling may be overlooked when analyses are based on the full community. The majority of these traits also showed a significant response to trawling in an independent spatio-temporal dataset spanning seven sampling events (NOVANA surveys). This means that a targeted one-off (or snapshot) sampling programme has the ability to describe longer-term trends caused by chronic trawling, particularly when the focus is on the response of large-bodied benthic fauna. This result, and indeed the examination of trait sensitivity as a function of body-size, both represent novel aspects of our study.

4.1. The loss of large benthic fauna to ecosystem function

Large benthic fauna are known to be disproportionately sensitive to bottom trawling (Kaiser et al. 2000, Pitcher 2000, Queirós et al. 2006). The implications of these losses to the functionality of fishing grounds are, however, poorly understood. Given that body-size is an important ecological driver of ecosystem function (Woodward et al. 2005, Reiss et al. 2009), it could be argued that greater attention to size in functional assessment is required as part of an EAFM. Generally, large benthos account for a large degree of bioturbation and sediment-reworking (Sandnes et al. 2000), they improve nutrient fluxes and benthic production (Lohrer et al. 2004), and offer substantial sources of labile carbon available for higher trophic levels (Ellingsen et al. 2007, Hiddink et al. 2016). Specifically, our results showed that large benthic traits such as '6-10 deep', '>10 year' were distinct in their sensitivity and were rare at heavily trawled sites. These traits were chiefly accounted by bivalve species (e.g. *Arctica islandica*, *Thracia* sp., *Mya arenaria*), as well as large sea urchins (*Echinocardium cordatum*), and sea pens (*Virgularia mirabilis*). Marine bivalves are functionally important member of the benthos, yet can experience relatively high rates of trawling induced mortality

(Bergman & Van Santbrink 2000). In particular, we observed that *Arctica islandica* contributed significantly to the biomass in unfished and lightly fished sites. This large, periodically deep living, and particularly long lived species (>100 years) (Abele et al. 2008), can contribute to nearly half of total benthic community production, and are an important prey item for commercially important species such as cod (Brey et al. 1990). *A. islandica* are also highly sensitive to chronic trawling disturbance (Rumohr & Krost 1991). Similarly large, deep-living, and functionally important species such as *M. arenaria, E. cordatum,* and *V. mirabilis* were reduced in biomass at heavily fished sites, owing to their sensitivity to physical disturbance (Beukema 1995, Bergman & Van Santbrink 2000, Sköld et al. 2018). These species exchange water and oxygen between the sediment and water column (Forster & Zettler 2004), and irrigate deeper sediments with water and oxygen (Osinga et al. 1995). Other sensitive traits identified in this study included 'surface deposition' (bioturbation), 'suspension' (feeding mode), and 'tube-dwelling' (living habit). These traits perform are important functional roles such as benthic-pelagic coupling and the provision of benthic habitat (Gili & Coma 1998, Bolam et al. 2002). Although generally less abundant, the loss of large individuals from benthic communities may precede the loss of ecosystem function (Solan et al. 2004). Accordingly, the size-dependant effect of trawling on benthos has the potential to denude ecosystem function in heavily trawled areas. While the results of laboratory based experiments would support this idea (Norkko et al. 2013), there is currently a lack of empirical evidence from commercial fishing grounds to support this.

4.2. Large fauna as functional indicators

Aside from a strong response to trawling, the use of indicators based on large benthic fauna can have several advantages. Large individuals are relatively less abundant and less specious, meaning that indicators are relatively easy to measure and calculate, as well as being cheaper and faster to process compared to using the full community (McLaverty et al. 2020). Furthermore, if biomass estimates are available, our results would suggest that a large proportion of biomass will be represented by the large animals. This would mean that there may not be a need to process and analyse the entire community in order to calculate more useful indicators. A further advantage of this approach is that indicators of bottom trawling need to be robust to the effects of other environmental pressures in order to be effective. Indeed our results would suggest that large fraction indicators were less influenced by variables such as mud content, salinity and temperature, compared to the small fraction. This may mean that indicators calculated using large benthic fauna would have a greater ability to respond directly to variations in trawling intensity. This aspect may be particularly relevant in areas influenced by multiple environmental drivers, such as the Kattegat, where the effectiveness of benthic indicators can be inhibited by auxiliary drivers (Gislason et al. 2017).

The similarity in indicator performance between a snapshot survey and a multi-year monitoring data also suggests that large fauna indicators provide a better representation of chronic impacts. Relationships between ecological units and their environment are most often investigated using snapshot surveys (Fisher et al. 2010), commonly as a result of limitations to resources and funding. While snapshot surveys are often more practical, there remains the issue that ecological communities may vary from year-to-year, particularly in dynamic systems. Our results suggest large fauna indicators provide a better reflection of long-term trends than full community indicators. This is likely due to the biomass of large benthic fauna being less affected by inter-annual variation in e.g. spawning success, and the likelihood that past trawling events at a given location will have a greater effect on the longer-lived fauna.

The use of larger sieve sizes to undertake ecological assessments has support from other fields of research. Comparisons of sieve sizes use to undertake ecological status assessments under the Water Framework Directive have shown that the choice of 1 mm, 2 mm, and 4 mm sieve do not overly affect the outcome of the assessment (Pinna et al., 2014, 2013). Given that the larger sieve sizes are associated with significantly reduced sampling and processing times, their use may be justified in certain circumstances. It should also be noted that large fauna indicators cannot replicate the resolution of data provided by small sieve sizes or indeed long-term datasets, and their use should be linked to specific hypothesis or research goals. However, a potential limitation of this method could be the loss of a high degree of ecological information when large sieve sizes are used. This is as relatively fewer species are represented in the data, and comparatively less information of community composition can be gathered (Pinto et al., 2009).

4.3. Other sensitive traits

'Sessile' was an unusual trait in our analysis in that it was highly responsive to trawling regardless of size fraction and across surveys. Although several studies have demonstrated that sessile benthos are highly sensitive to trawling (Kaiser et al., 2018; M. J. Kaiser et al., 2000; Sciberras et al., 2018), this would suggest that small sessile fauna are equally vulnerable to trawling impacts. At the full community level, suspension feeders were also significantly impacted by trawling. Benthos which share this trait are typically sessile, possess fragile feeding appendages, and may be vulnerable to sediment resuspension due to clogging of their feeding apparatus (Jennings & Kaiser 1998). Suspension feeders are functionally important member of the benthos due to their role in benthic-pelagic coupling in sedimentary environments (Griffiths et al. 2017), cycling nutrients via the capture and transfer of organic material into the sediment matrix (Rosenberg 2001, Lohrer et al. 2004). Unfished and lightly fished sites in our study were characterised by relatively high biomass tube-building maldanid polychaetes (e.g. *Rhodine gracilior and Maldane sarsi*), terebellid polychaetes (e.g. *Anobothrus gracilis*), and horseshoe worms (*Phoronis* sp.). Aggregations of tubicolous worm have also been

shown to actively support increased benthic diversity (Bolam, 1999), and benefit other species by improving sediment stability (Noji & Noji 1991, Friedrichs et al. 2000). Furthermore, high densities of tubes have been shown to improve conditions for larval settlement (Bolam & Fernandes 2002), and promote increased food supply for associated benthic species (Holte 2001). Although trawling is known to reduce the abundance of polychaetes (Kenchington et al. 2006) and phoroinid worms (Hinz et al. 2009), tube-building has not previously been commonly identified a potential indicator of trawling disturbance. Tube-dwellers typically live at the sediment surface, require stable substrate to construct tubes, and many species spawn within tubes and may be slow to recolonise areas after disturbance events (Bolam & Fernandes 2002).

Our study provides an overview of individual benthic traits which exhibit a high degree of sensitivity to trawling. Although not formally examined, it would appear that certain trait combinations could play a more important role than individual traits in governing the sensitivity of benthos to trawling, and thus have important implications in the development of trait-based indicators going forward. For example, the combination of sessile, suspension feeding, and tube-building traits often occur together in a number of trawling sensitive species. Species which share this combination of traits, or similar combinations, may therefore be likely to experience high rates of direct and in-direct mortality. This specific trait combination is also shared by *Haploops* sp., a tube building amphipod, which has experienced large-scale declines across the Kattegat. *Haploops* sp. are recognised for their functional importance (Rigolet et al. 2014), and were once the dominant component of the macrofauna in the Kattegat at the beginning of the 1990s (Petersen, 1913). It has been previously suggested that the large-scale loss of these species from the Kattegat may be as a direct result of increased bottom trawling in the region (Göransson, 1999, Josefson et al., 2018; Sköld *et al.,* 2018). Indeed, despite its historical abundance, we recorded *Haploops* sp. at only a handful of sites in either survey. This would suggest that while *Haploops* sp. can no longer act as an indicator of trawling disturbance in the Kattegat, its trait combinations may have potential to predict the vulnerability of other key species in the future.

4.4. Conclusions

Trait-based approaches provide a promising method to understand the effect of chronic trawling disturbance on benthic macrofaunal communities. Although the Kattegat is one of the most heavily trawled areas in the world (Amoroso et al. 2018) there are few studies which have used biological traits to interpret trawling impacts in the area. In this study we have demonstrated the sensitivity of several benthic traits as indicators of trawling disturbance, and outlined some of the mechanisms which may underpin their sensitivity. Given the importance of many of these traits in processes such as benthic-pelagic coupling and nutrient cycling, it is likely that the loss or decline of these traits will have considerable implications for the functioning of

benthic ecosystems across fishing grounds. In particular, the traits of large benthic macrofauna were particularly sensitive to bottom trawling. This was established by size separating the benthic community into small and large size categories. This approach has the dual advantage of being able to improve detection rates and reduce sample processing time, as only a subset of the full community is required for analysis. Furthermore, the validation of our observations using long-running monitoring dataset suggests that a carefully designed one-off sampling event can provide results which are representative of the information contained in long-term datasets. Finally, the monitoring of ecological systems is often constrained by high ecosystem complexity, and there is a need develop ecological indicators which effectively distil this information into metrics of ecological impact and state which are easy to use, interpret and communicate. The results of this study may therefore help develop fishery-specific indicators, using traits that are common to benthic communities across geographic area, habitat, and survey type.

Acknowledgements

Supplementary information

See Supplement below.

References

Abele D, Strahl J, Brey T, Philipp EER (2008) Imperceptible senescence: Ageing in the ocean quahog Arctica islandica. Free Radic Res 42:474–480.

Amoroso RO, Pitcher CR, Rijnsdorp AD, McConnaughey RA, Parma AM, Suuronen P, Eigaard OR, Bastardie F, Hintzen NT, Althaus F, Baird SJ, Black J, Buhl-Mortensen L, Campbell AB, Catarino R, Collie J, Cowan JH, Durholtz D, Engstrom N, Fairweather TP, Fock HO, Ford R, Gálvez PA, Gerritsen H, Góngora ME, González JA, Hiddink JG, Hughes KM, Intelmann SS, Jenkins C, Jonsson P, Kainge P, Kangas M, Kathena JN, Kavadas S, Leslie RW, Lewis SG, Lundy M, Makin D, Martin J, Mazor T, Gonzalez-Mirelis G, Newman SJ, Papadopoulou N, Posen PE, Rochester W, Russo T, Sala A, Semmens JM, Silva C, Tsolos A, Vanelslander B, Wakefield CB, Wood BA, Hilborn R, Kaiser MJ, Jennings S (2018) Bottom trawl fishing footprints on the world's continental shelves. Proc Natl Acad Sci 115:E10275–E10282.

Anderson MJ, Gorleyt RN, Clarket KR (2008) PERMANOVA + for PRIMER : t. Guid to Softw Stat Methods 1:186

Anon (2000) Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy (Water Framework Directive).

Anon (2008) Directive 2008/56/EC of the European Parliament and of the Council establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive).

Ball BJ, Fox G, Munday BW (2000) Long- and short-term consequences of a Nephrops trawl fishery on the benthos and environment of the Irish Sea. ICES J Mar Sci 57:1315–1320

Beauchard O, Veríssimo H, Queirós AM, Herman PMJ (2017) The use of multiple biological traits in marine community ecology and its potential in ecological indicator development. Ecol Indic 76:81–96

Bergman MJN, Santbrink JW Van (2000) Mortality in megafaunal benthic populations caused by trawl fisheries on the Dutch continental shelf in the North Sea in 1994. In: ICES Journal of Marine Science.p 1321–1331

Beukema JJ (1995) Long-term effects of mechanical harvesting of lugworms Arenicola marina on the zoobenthic community of a tidal flat in the Wadden Sea. Netherlands J Sea Res 33:219–227.

Bolam SG, Coggan RC, Eggleton J, Diesing M, Stephens D (2014) Sensitivity of macrobenthic secondary production to trawling in the English sector of the Greater North Sea: A biological trait approach. J Sea Res 85:162–177.

Bolam SG, Eggleton JD (2014) Macrofaunal production and biological traits: Spatial relationships along the UK continental shelf. J Sea Res 88:47–58

Bolam SG, Fernandes TF (2002) Dense aggregations of tube-building polychaetes: Response to small-scale disturbances. J Exp Mar Bio Ecol 269:197–222

Bolam SG, Fernandes TF, Huxham M (2002) Diversity, biomass, and ecosystem processes in the marine benthos. Ecol Monogr 72:599–615.

Bolam SG, Garcia C, Eggleton J, Kenny AJ, Buhl-Mortensen L, Gonzalez-Mirelis G, van Kooten T, Dinesen G, Hansen J, Hiddink JG, Sciberras M, Smith C, Papadopoulou N, Gumus A, Van Hoey G, Eigaard OR, Bastardie F, Rijnsdorp AD (2017) Differences in biological traits composition of benthic assemblages between unimpacted habitats. Mar Environ Res 126:1–13.

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JSS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24:127–135

Bremner J, Frid CLJ, Rogers SI (2003) Assessing Marine Ecosystem Health: The long-term effects of fishing on functional biodiversity in North Sea benthos. In: Aquatic Ecosystem Health and Management.

Bremner J, Frid CLJ, Rogers SI (2005) Biological traits of the North Sea benthos: does fishing affect benthic ecosystem function? Am Fish Soc Symp 41:477–489

Bremner J, Rogers SI, Frid CLJ (2006) Methods for describing ecological functioning of marine benthic assemblages using biological traits analysis (BTA). Ecol Indic 6:609–622

Brey T, Arntz WE, Pauly D, Rumohr H (1990) Arctica (Cyprina) islandica in Kiel Bay (Western Baltic): growth, production and ecological significance. J Exp Mar Bio Ecol 136:217–235.

Chevenet F, Doleadec S, Chessel D (1994) A fuzzy coding approach for the analysis of long-term ecological data. Freshw Biol 31:295–309

Clarke KR, Gorley RN (2015) Primer v7:user manual/tutorial. PRIMER-E Ltd, Plymouth. www.primer-e.com/primer 7 _ download.htm

De Juan S, Demestre M (2012) A Trawl Disturbance Indicator to quantify large scale fishing impact on benthic ecosystems. Ecol Indic 18:183–190.

Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G, Marquéz JRG, Gruber B, Lafourcade B, Leitão PJ, Münkemüller T, McClean C, Osborne PE, Reineking B, Schröder B, Skidmore AK, Zurell D, Lautenbach S (2013) Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography (Cop) 36:27–46.

Duplisea DE, Jennings S, Warr KJ, Dinmore TA (2002) A size-based model of the impacts of bottom trawling on benthic community structure. Can J Fish Aquat Sci 59:1785–1795.

Eigaard OR, Bastardie F, Breen M, Dinesen GE, Hintzen NT, Laffargue P, Mortensen LO, Nielsen JR, Nilsson HC, O'Neill FG, Polet H, Reid DG, Sala A, Sköld M, Smith C, Sørensen TK, Tully O, Zengin M, Rijnsdorp AD (2016) Estimating seabed pressure from demersal trawls, seines, and dredges based on gear design and dimensions. ICES J Mar Sci 73:i27–i43.

Ellingsen KE, Hewitt JE, Thrush SF (2007) Rare species, habitat diversity and functional redundancy in marine benthos. J Sea Res 58:291–301.

FAO (2003) Fisheries management 2: The ecosystem approach to fisheries. FAO Technical Guidelines for Responsible Fisheries (FAO, Rome), 4(Suppl 2):112.

Feng C, Wang H, Lu N, Chen T, He H, Lu Y, Tu XM (2014) Log-transformation and its implications for data analysis. Shanghai Arch Psychiatry

Fisher JAD, Frank KT, Leggett WC (2010) Dynamic macroecology on ecological time-scales. Glob Ecol Biogeogr.

Forster S, Zettler ML (2004) The capacity of the filter-feeding bivalve Mya arenaria L. to affect water transport in sandy beds. Mar Biol.

Foster SD, Bravington M V. (2013) A Poisson–Gamma model for analysis of ecological non-negative continuous data. Environ Ecol Stat 20:533–552

Friedrichs M, Graf G, Springer B (2000) Skimming flow induced over a simulated polychaete tube lawn at low population densities. Mar Ecol Prog Ser 192:219–228

Garcia, S. M., Zerbi, A., Aliaume, C., Do Chi, T., and Lasserre, G. (2003) The ecosystem approach to fisheries. Issues, terminology, principles, institutional foundations, implementation and outlook. FAO Fisheries Technical Paper, 443. 71 pp.

Gili JM, Coma R (1998) Benthic suspension feeders: Their paramount role in littoral marine food webs. Trends Ecol Evol 13:316–321.

Gislason H, Bastardie F, Dinesen GE, Egekvist J, Eigaard OR (2017) Lost in translation? Multi-metric macrobenthos indicators and bottom trawling. Ecol Indic 82:260–270.

Griffiths JR, Kadin M, Nascimento FJA, Tamelander T, Törnroos A, Bonaglia S, Bonsdorff E, Brüchert V, Gårdmark A, Järnström M, Kotta J, Lindegren M, Nordström MC, Norkko A, Olsson J, Weigel B, Žydelis R, Blenckner T, Niiranen S, Winder M (2017) The importance of benthic–pelagic coupling for marine ecosystem functioning in a changing world. Glob Chang Biol

Hiddink JG, Jennings S, Sciberras M, Bolam SG, Cambiè G, McConnaughey RA, Mazor T, Hilborn R, Collie JS, Pitcher R, Parma AM, Suuronen P, Kaiser MJ, Rijnsdorp AD (2018) Assessing bottom-trawling impacts based on the longevity of benthic invertebrates (V Trenkel, Ed.). J Appl Ecol

Hiddink JG, Jennings S, Sciberras M, Szostek CL, Hughes KM, Ellis N, Rijnsdorp AD, McConnaughey RA, Mazor T, Hilborn R, Collie JS, Pitcher CR, Amoroso RO, Parma AM, Suuronen P, Kaiser MJ (2017) Global analysis of depletion and recovery of seabed biota after bottom trawling disturbance. Proc Natl Acad Sci 114:8301–8306

Hiddink JG, Kaiser MJ, Sciberras M, McConnaughey RA, Mazor T, Hilborn R, Collie JS, Pitcher R, Parma AM, Suuronen P, Rijnsdorp AD, Jennings S (2020) Selection of indicators for assessing and managing the impacts of bottom trawling on seabed habitats. J Appl Ecol 57:0–3.

Hiddink JG, Moranta J, Balestrini S, Sciberras M, Cendrier M, Bowyer R, Kaiser MJ, Sköld M, Jonsson P, Bastardie F, Hinz H (2016) Bottom trawling affects fish condition through changes in the ratio of prey availability to density of competitors. J Appl Ecol 53:1500–1510

Hintzen NT, Bastardie F, Beare D, Piet GJ, Ulrich C, Deporte N, Egekvist J, Degel H (2012) VMStools: Open-source software for the processing, analysis and visualisation of fisheries logbook and VMS data. Fish Res 115–116:31–43.

Hinz H, Prieto V, Kaiser MJ (2009) Trawl disturbance on benthic communities: Chronic effects and experimental predictions. Ecol Appl 19:761–773.

Hiscock K, Tyler-Walters H (2006) Assessing the Sensitivity of Seabed Species and Biotopes – The Marine Life Information Network (MarLIN). Hydrobiologia 555:309–320.

Holte B (2001) Possible ecological effects from maldanid (annelida, polychaeta) "super-dominance" in a small North Norwegian sill system. Ophelia 55:69–75.

Jennings S, Kaiser MJ (1998) The effects of fishing on marine ecosystems. Adv Mar Biol:201–212.

Jorgensen SE, Burkhard B, Müller F (2013) Twenty volumes of ecological indicators-An accounting short review. Ecol Indic 28:4–9.

Josefson AB, Loo LO, Blomqvist M, Rolandsson J (2018) Substantial changes in the depth distributions of benthic invertebrates in the eastern Kattegat since the 1880s. Ecol Evol:1–13.

Kaiser M, Clarke K, Hinz H, Austen M, Somerfield P, Karakassis I (2006) Global analysis of response and recovery of benthic biota to fishing. Mar Ecol Prog Ser 311:1–14.

Kaiser MJ, Hormbrey S, Booth JR, Hinz H, Hiddink JG (2018) Recovery linked to life history of sessile epifauna following exclusion of towed mobile fishing gear. J Appl Ecol 55:1060–1070

Kaiser MJ, Ramsay K, Richardson CA, Spence FE, Brand AR (2000) Chronic fishing disturbance has changed shelf sea benthic community structure. J Anim Ecol 69:494–503

Kaiser MJ, Spence FE, Hart PJB (2000) Fishing-gear restrictions and conservation of benthic habitat complexity. Conserv Biol.

Kenchington ELR, Gilkinson KD, MacIsaac KG, Bourbonnais-Boyce C, Kenchington TJ, Smith SJ, Gordon DC (2006) Effects of experimental otter trawling on benthic assemblages on Western Bank, northwest Atlantic Ocean. J Sea Res 56:249–270

Lambert GI, Jennings S, Hiddink JG, Hintzen NT, Hinz H, Kaiser MJ, Murray LG (2012) Implications of using alternative methods of vessel monitoring system (VMS) data analysis to describe fishing activities and impacts. ICES J Mar Sci 69:682–693.

Legendre P, Andersson MJ (1999) Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. Ecol Monogr 69:1–24.

Lohrer AM, Thrush SF, Gibbs MM (2004) Bioturbators enhance ecosystem function through complex biogeochemical interactions. Nature 431:1092–1095.

McGill BJ, Enquist BJ, Weiher E, Westoby M (2006) Rebuilding community ecology from functional traits. Trends Ecol Evol 21:178–185.

McLaverty C, Eigaard OR, Gislason H, Bastardie F, Brooks ME, Jonsson P, Lehmann A, Dinesen GE (2020) Using large benthic macrofauna to refine and improve ecological indicators of bottom trawling disturbance. Ecol Indic 110:105811.

Mouillot D, Graham NAJ, Villéger S, Mason NWH, Bellwood DR (2013) A functional approach reveals community responses to disturbances. Trends Ecol Evol 28:167–177.

Noji CI-M, Noji TT (1991) Tube lawns of spionid polychaetes and their significance for recolonization of disturbed benthic substrates. Meeresforsch 33:235–246.

Norkko A, Villnäs A, Norkko J, Valanko S, Pilditch C (2013) Size matters: Implications of the loss of large individuals for ecosystem function. Sci Rep 3:2646.

Osinga R, Lewis WE, Wopereis JLM, Vriezen C, van Duyl FC (1995) Effects of the sea urchin Echinocardium cordatum on oxygen uptake and sulfate reduction in experimental benthic systems under increasing organic loading. Ophelia.

Petersen CGJ (1918) The sea bottom and its production of fish food. A survey of the work done in connection with the valuation of the Danish waters from 1883-1917. Rep Danish Biol Station 25: 1–62

Pikitch, E., Santora, C., Babcock, E., Bakun, A., Bonfil, R., Conover, D.,Dayton, P., Doukakis, P., Fluharty, D.,Heneman, B., Houde, E., Link, J.,Livingston, P., Mangel, M., McAllister, M., Pope, J. & Sainsbury, K. (2004) Ecosystem-based fishery management. Science, 305, 346 –347.

Pitcher C (2000) Implications of the effects of trawling on sessile megazoobenthos on a tropical shelf in northeastern Australia. ICES J Mar Sci 57:1359–1368.

Queirós AM, Hiddink JG, Kaiser MJ, Hinz H (2006) Effects of chronic bottom trawling disturbance on benthic biomass, production and size spectra in different habitats. J Exp Mar Bio Ecol 335:91–103.

Reiss J, Bridle JR, Montoya JM, Woodward G (2009) Emerging horizons in biodiversity and ecosystem functioning research. Trends Ecol Evol 24:505–514.

Rice J, Arvanitidis C, Borja A, Frid C, Hiddink JG, Krause J, Lorance P, Ragnarsson SÁ, Sköld M, Trabucco B, Enserink L, Norkko A (2012) Indicators for Sea-floor Integrity under the European Marine Strategy Framework Directive. Ecol Indic 12:174–184.

Rigolet C, Dubois SF, Thiébaut E (2014) Benthic control freaks: Effects of the tubiculous amphipod Haploops nirae on the specific diversity and functional structure of benthic communities. J Sea Res 85:413–427

Rijnsdorp AD, Bolam SG, Garcia C, Hiddink JG, Hintzen NT, Denderen PD van, Kooten T van (2018) Estimating sensitivity of seabed habitats to disturbance by bottom trawling based on the longevity of benthic fauna. Ecol Appl 28:1302–1312

Rosenberg R (1995) Benthic marine fauna structured by hydrodynamic processes and food availability. Netherlands J Sea Res 34:303–317

Rosenberg R (2001) Marine benthic faunal successional stages and related sedimentary activity. Sci Mar 65:107–119

Rumohr H, Krost P (1991) Experimental evidence of damage to benthos by bottom trawling with special reference to Arctica islandica . Meeresforschung/Rep Mar Res 33:340–345.

Sandnes J, Forbes T, Hansen R, Sandnes B, Rygg B (2000) Bioturbation and irrigation in natural sediments, described by animal-community parameters. Mar Ecol Prog Ser 197:169–179.

Sciberras M, Hiddink JG, Jennings S, Szostek CL, Hughes KM, Kneafsey B, Clarke LJ, Ellis N, Rijnsdorp AD, McConnaughey RA, Hilborn R, Collie JS, Pitcher CR, Amoroso RO, Parma AM, Suuronen P, Kaiser MJ (2018) Response of benthic fauna to experimental bottom fishing: A global meta-analysis. Fish Fish 19:698–715

Sköld M, Göransson P, Jonsson P, Bastardie F, Blomqvist M, Agrenius S, Hiddink JG, Nilsson HC, Bartolino V (2018) Effects of chronic bottom trawling on soft-seafloor macrofauna in the Kattegat. Mar Ecol Prog Ser 586:41–55.

Solan M, Cardinale BJ, Downing AL, Engelhardt KAM, Ruesink JL, Srivastava DS (2004) Extinction and ecosystem function in the marine benthos. Science (80-) 306:1177–1180.

Svendsen, L.M. & Norup, B. (eds.) 2005: NOVANA. Nationwide Monitoring and Assessment Programme for the Aquatic and Terrestrial Environments. Programme Description – Part 1. National Environmental Research Institute, Denmark. 53 pp. – NERI Technical Report No. 532

Tillin HM, Hiddink JG, Jennings S, Kaiser MJ (2006) Chronic bottom trawling alters the functional composition of benthic invertebrate communities on a sea-basin scale. Mar Ecol Prog Ser 318:31–45.

Van Denderen PD, Bolam SG, Hiddink JG, Jennings S, Kenny A, Rijnsdorp AD, Van Kooten T (2015) Similar effects of bottom trawling and natural disturbance on composition and function of benthic communities across habitats. Mar Ecol Prog Ser 541:31–43.

Van Hoey G, Borja A, Birchenough S, Buhl-Mortensen L, Degraer S, Fleischer D, Kerckhof F, Magni P, Muxika I, Reiss H, Schröder A, Zettler ML (2010) The use of benthic indicators in Europe: From the water framework directive to the marine strategy framework directive. Mar Pollut Bull 60:2187–2196.

Violle C, Reich PB, Pacala SW, Enquist BJ, Kattge J (2014) The emergence and promise of functional biogeography. Proc Natl Acad Sci 111:13690–13696.

Woodward G, Ebenman B, Emmerson M, Montoya JM, Olesen JM, Valido A, Warren PH (2005) Body size in ecological networks. Trends Ecol Evol 20:402–409.

Supplement: "Benthic life history traits as ecological indicators of bottom trawling"

Table S1. Pair-wise correlations between the predictor variables used in the analyses of the KS16 NOVANA benthic fauna data. Values represent Pearson's R values and range between -1 and 1.

KS16	Trawling	Mud	Depth	Current	Salinity
Mud	0.58813				
Depth	0.288834	0.475688			
Current	-0.38924	-0.3251	-0.32779		
Salinity	0.51561	0.378989	0.662901	-0.20062	
Temp	0.159995	-0.35519	-0.37505	0.020433	0.058533

NOVANA	Trawling	Mud	Depth	Current	Salinity
Mud	0.347546				
Depth	0.600782	0.690657			
Current	-0.17703	-0.16523	-0.19854		
Salinity	0.42274	0.349985	0.509452	-0.14439	
Temp	-0.23702	-0.24371	-0.56769	0.020077	-0.03251

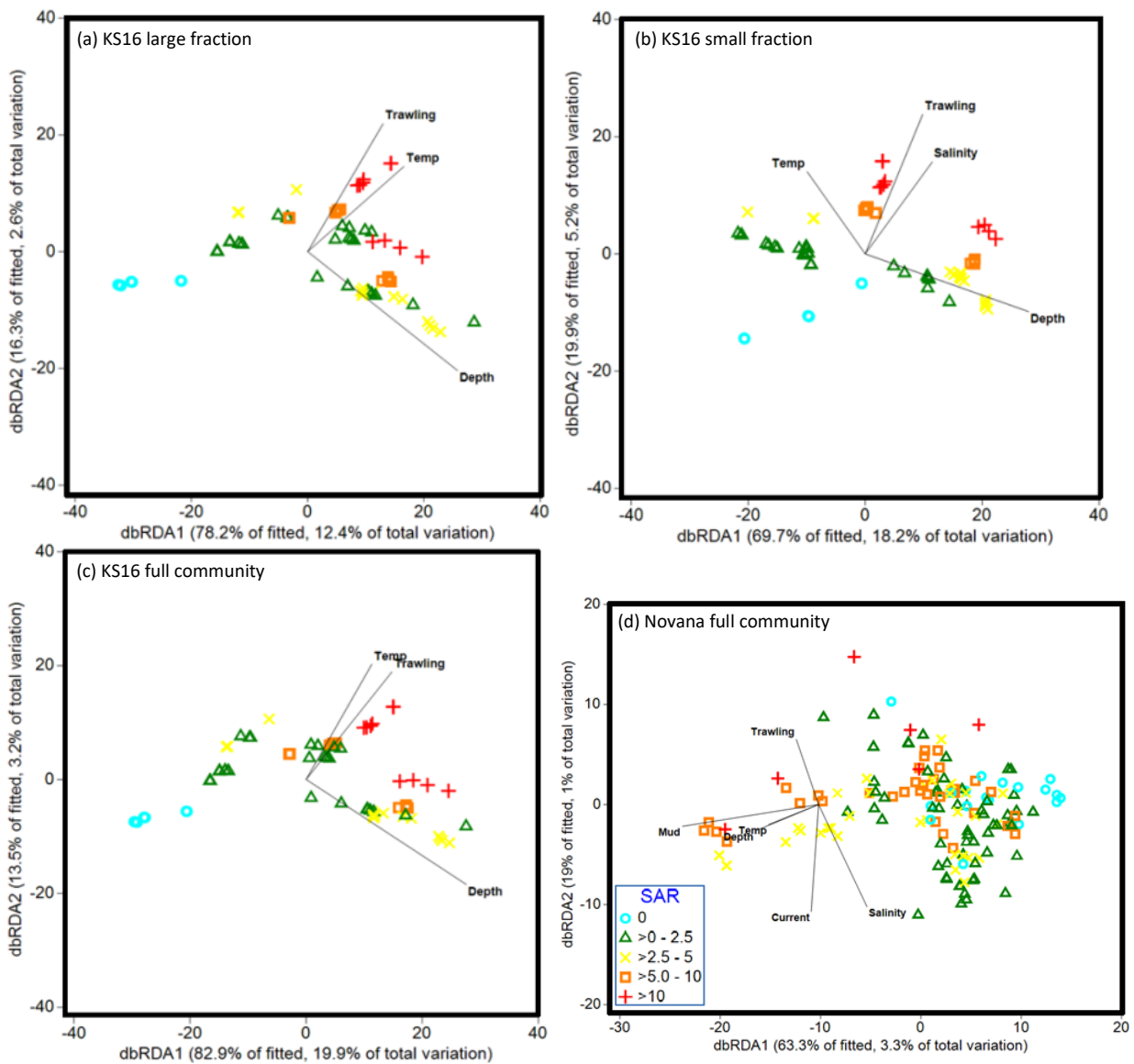**Figure S1. Distance-based redundancy analysis (dbRDA) ordinations of trait composition, in the (a) KS16 large fraction, (b) KS16 small fraction, (c) KS16 full community, and (d) NOVANA datasets. Sampling stations are colour coded by their associated trawling intensity (SAR) category. The length and direction of the vectors indicate the relative effect of each predictor variable on the constrained ordination.**

Table S2. Multiple partial correlations correlation coefficients between predictor variables and dbRDA1 axes . Large fraction: ≥4 mm mesh size; small fraction: 1 – 4 mm mesh size.

	dbRDA1	Pearson's R					
	Variance explained	Current	**Depth**	Mud	Salinity	**Temp**	**Trawling**
KS16 (full community)	19.91%		**0.82**			**0.34**	**0.41**
KS16 (small fraction)	18.22%		**0.83**		0.35	**-0.29**	**0.30**
KS16 (large fraction)	12.38%		**0.75**			**0.49**	**0.45**
Novana	3.29%	n/s	**0.37**	0.81	-0.29	**0.29**	**0.13**
Pearson correlation critical values: KS16 = 0.21 | Novana = 0.07							

Table S2. Multiple partial correlations correlation coefficients between predictor variables and dbRDA1 axes . Large fraction: ≥4 mm mesh size; small fraction: 1 – 4 mm mesh size. Significant relationships with trawling are shown in bold. Only statistically significant relationships are shown.

	Pearson's R						dbRDA1
Survey dataset	Current	Depth	Mud	Salinity	Temp	**Trawling**	% of total variance
KS16 (large fraction)	-	0.75	-	-	0.49	**0.45**	12.4
KS16 (full community)	-	0.82	-	-	0.34	**0.41**	19.9
KS16 (small fraction)	-	0.83	-	0.35	-0.29	**0.30**	18.2
Novana	-	0.37	0.81	-0.29	0.29	**0.13**	3.3

Table S3. Summary output for generalised linear mixed models. The values shown are parameter estimates, and associated standard error (shown in brackets). The significance level is denoted by asterisks (* = P < 0.05; ** =P < 0.01; *** = P < 0.001). Conditional R^2 values describe the proportion of variance explained by both the fixed and random terms.

KS 16 Full community	Intercept	Current	Depth	Mud	Salinity	Temperature	Trawling	R^2
>10	6.99 (2.52)		-0.05 (0.01)***			-0.69 (0.3)*		0.69
101-200mm	7.81 (2.51)		-0.04 (0.01)***			-0.82 (0.30)**		0.70
Exoskeleton (chitin/calcium carbonate)	5.94 (2.04)		-0.03 (<0.01)***			-0.55 (0.24)*		0.58
Infauna: 6-10cm	11.51 (3.23)		-0.08 (0.01)***			-1.33 (0.39)***		0.81
Planktotrophic	1.73 (0.37)		-0.02 (<0.01)**				-0.12 (0.04)**	0.61
Sessile	1.5 (0.51)		-0.03 (0.01)**				-0.35 (0.06)***	0.82
Sexual shed eggs- pelagic	4.68 (1.86)	0.39 (0.17)*	-0.03 (0.01)***			-0.51 (0.21)*		0.62
Surface deposition	1.7 (0.42)		-0.03 (<0.01)***				-0.15 (0.05)**	0.70
Suspension	1.34 (0.36)		-0.04 (<0.01)***				-0.12 (0.04)**	0.72
Tube-dwelling	3.70 (1.59)					-0.79 (0.21)***	-0.27 (0.04)***	0.84

KS16 Large fraction	Intercept	Current	Depth	Mud	Salinity	Temperature	Trawling	R^2
>10	1.49 (0.64)		-0.03 (0.01)**				-0.44 (0.08)*	0.69
101-200mm	9.75 (2.89)	0.89 (0.36)*	-0.05 (0.01)***			-1.08 (0.35)**		0.68
Exoskeleton (chitin/calcium carbonate)	6.24 (2.16)	0.40 (0.19)*	-0.03 (0.01)***			-0.70 (0.25)**		0.57
Infauna: 6-10cm	11.79 (4.01)		-0.08 (0.02)***			-1.30 (0.50)**	-0.31 (0.11)**	0.83
Planktotrophic	1.65 (0.45)		-0.03 (0.01)*				-0.13 (0.05)*	0.58
Sessile	1.49 (0.64)		-0.03 (0.01)*				-0.44 (0.08)***	0.83
Sexual shed eggs- pelagic	9.28 (2.2)		-0.05 (0.01)***			-0.92 (0.27)**		0.58
Surface deposition	7.18 (2.47)		-0.05 (0.01)***			-0.67 (0.30)*	-0.13 (0.05)*	0.67
Suspension	1.38 (0.42)		-0.04 (0.01)***				-0.13 (0.05)*	0.69
Tube-dwelling	4.24 (1.85)					-0.93 (0.24)***	-0.27 (0.05)***	0.80

KS16 Small fraction	Intercept	Current	Depth	Mud	Salinity	Temperature	Trawling	R²
>10	-2.15 (0.46)		-0.02 (0.01)*					0.67
101-200mm	-2.51 (1.69)				-0.07 (0.04)*	0.31 (0.15)*		0.48
Exoskeleton (chitin/calcium carbonate)	-5.74 (1.4)					0.47 (0.18)**		0.52
Infauna: 6-10cm	1.13 (1.01)			-0.01 (<0.01)*	-0.14 (0.03)***			0.57
Planktotrophic	-2.42 (1.19)				-0.06 (0.02)*	0.35 (0.10)**		0.42
Sessile	-1.69 (0.31)	-0.23 (0.13)*					-0.21 (0.03)***	0.74
Sexual shed eggs- pelagic	-1.04 (1.04)				-0.08 (0.02)***	0.26 (0.09)**		0.40
Surface deposition	-2.09 (1.27)				-0.07 (0.03)**	0.29 (0.11)*		0.44
Suspension	-2.91 (1.72)				-0.09 (0.04)*	0.38 (0.16)*		0.45
Tube-dwelling	6.65 (2.08)			-0.02 (<0.01)***		-1.35 (0.26)***		0.81

NOVANA	Intercept	Current	Depth	Mud	Salinity	Temperature	Trawling	R²
>10	-6.07	0.37 (0.07)***	0.05 (0.01)***			0.37 (0.11)**	-0.09 (0.02)**	0.41
101-200mm	-2.54 (0.22)	0.32 (0.06)***		0.01 (<0.01)***				0.52
Exoskeleton (chitin/calcium carbonate)	-2.62 (0.51)	0.23 (0.06)***			0.05 (0.01)***			0.29
Infauna: 6-10cm	-6.81 (0.71)	0.31 (0.07)***	-0.05 (0.01)***	-0.01 (<0.01) ***	0.18 (0.02)***		-0.11 (0.03)***	0.84
Planktotrophic	-2.39 (0.47)	0.21 (0.05)***			0.06 (0.01)***		-0.05 (0.02)*	0.41
Sessile	-0.16 (1.14)	0.35 (0.08)		0.03 (<0.01)***	0.09 (0.02)***	-0.56 (0.11)***	-0.09 (0.03)**	0.91
Sexual shed eggs- pelagic	-2.30 (0.47)				0.04 (0.01)***	0.25 (0.05)**		0.42
Surface deposition	-2.76 (0.51)	0.13 (0.05)*		-0.01 (<0.01)*	0.06 (0.01)***		-0.04 (0.02)*	0.43
Suspension	-1.67 (0.22)	0.27 (0.06)***		-0.01 (<0.01)**				0.43
Tube-dwelling	3.78 (0.78)	0.37 (0.06)***		-0.02 (<0.01)***		-1.09 (0.09)***	-0.12 (0.02)***	0.76