Universidade de Aveiro Departamento de Biologia
Ano 2017

SOFIA ALEXANDRA PINTO RAMALHO

TOLERÂNCIA DE ECOSSISTEMAS BENTÓNICOS DE MAR PROFUNDO A PERTURBAÇÃO INDUZIDA POR PESCA DE ARRASTO

TOLERANCE OF DEEP-SEA BENTHIC ECOSYSTEMS TO TRAWLING DISTURBANCE

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Ciências do Mar, realizada sob a orientação científica da Professora Doutora Maria Marina Ribeiro Pais da Cunha, Professora Auxiliar do Departamento de Biologia da Universidade de Aveiro e co-orientação da Professora Doutora Ann Vanreusel, Professora Catedrática do Departamento de Biologia da Universidade de Ghent, Bélgica e Doutor Nikolaos Lampadariou, Investigador do Hellenic Center for Marine Research, Grécia.

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" … *beneath the waves, there are many dominions yet to be visited, and kingdoms to be discovered; and he who venturously brings up from the abyss enough of their inhabitants to display the physiognomy of the country, will taste that cup of delight, the sweetness of whose draught those only who have made a discovery know. Well do I remember the first day when I saw the dredge hauled up after it had been dragging along the sea-bottom depth of more than one hundred fathoms*".

This description, made by Edward Forbes in The Natural History of the European Seas in 1859, let me think about my own excitement when I first saw the seabed through the ROV video cameras onboard of the RV Belgica, or when each sample come on deck after a long waiting. This and all the other new experiences that came along with my PhD were only possible with the help of my supervisors, to whom I will always be gratefully. I cannot thank you enough for your constant support and teaching that you offered me. Specifically, I would like to express my sincere “Obrigado” to professor Marina Cunha, for her constant availability, encouragement and for her help when I needed to look at my work with a new perspective. Thank you also for all your help with the identifications of macrofauna. I would like also to thank professor Ann Vanreusel, for letting me join the world of the deep-sea research and the opportunities that you have given me over these past years. Thank you for making me feel welcome at the Marine Biology research group at UGent and for your help in many steps of this work. To Dr. Nikolaos Lampadariou, I am also very gratefully for your encouragement and support.

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palavras-chave

comunidades bentónicas, talude continental superior, biodiversidade, funcionamento dos ecossistemas, pesca de arrasto de profundidade, margem Ibérica ocidental

resumo

A pesca de arrasto de fundo é considerada a atividade antropogénica mais difundida e destrutiva que atualmente ameaça os ecossistemas de mar profundo. Geralmente, esta atividade está associada à redução da abundância e biomassa de organismos bentónicos, alterações na estrutura das comunidades e perda de biodiversidade em habitats de substrato rochoso (nomeadamente montes submarinos e recifes de coral de águas profundas), onde taxas de recuperação ocorrem tipicamente num período de várias décadas. No entanto, é nas regiões de fundos sedimentares, nomeadamente no talude continental e canhões submarinos, onde se concentram a maior parte dos pesqueiros e a maior pressão por parte dos arrastões de profundidade. No entanto, os efeitos sobre a biodiversidade e o funcionamento dos ecossistemas são pouco conhecidos, inclusive na margem Ibérica Oeste, uma das regiões Europeias mais impactadas por artes de arrasto de fundo. Como tal, a presente tese teve como objetivo principal investigar os efeitos da perturbação física induzida pelos arrastões de profundidade na composição, diversidade e estrutura trófica das comunidades bentónicas, bem como na manutenção de funções essenciais dos ecossistemas mediadas pela fauna (por exemplo, transformação da matéria orgânica, reciclagem de nutrientes, respiração e produção secundária).

No total, foram selecionadas para o presente estudo três áreas de interesse, representativas de vários graus de perturbação física (não arrastado, e baixa e alta pressão por arrasto) na transição da plataforma para o talude continental da margem Sudoeste Portuguesa. Estas áreas foram estabelecidas a partir de dados de sistemas satélite de monitorização de embarcações (VMS). Foram realizados levantamentos de vídeo através de um veículo de operação remota (ROV) e amostradas sete estações dentro das três áreas de interesse, que permitiram a comparação das comunidades bentónicas, em termos de abundância, biomassa total, composição e diversidade (taxonómica e trófica), incluindo os grupos de meiofauna, macrofauna e mega-epifauna. Além disso, foi também realizado um trabalho experimental de curta duração (5 dias) com sedimentos colhidos em dois locais sujeitos a diferentes níveis de perturbação por arrasto.

Aos sedimentos colhidos foram adicionadas algas marcadas com ^{13}C, de forma a investigar vários *proxys* de funções tipicamente mediadas por comunidades bentónicas, nomeadamente mineralização de carbono por comunidades microbianas, biomassa bacteriana total (através da absorção do ^{13}C e estimada a partir de ácidos gordos derivados de fosfolipídios específicos de bactérias - PLFAs), bioturbação (através da absorção do ^{13}C no sedimento) e bioirrigação (analisada a partir da variação das concentrações de amónia no sedimento). Além disso, taxas de respiração e a respiração total da fauna foram utilizadas como *proxy* para a função metabólica do ecossistema.

No geral, os resultados da presente tese demostraram que a pesca de profundidade por artes de arrasto de fundo resulta na degradação da integridade dos fundos marinhos (por exemplo, áreas perturbadas demonstraram uma topografia aplanada, pouca evidência de bioturbação e marcas de portas e redes de arrasto). As componentes da fauna de maior dimensão (mega-epifauna e macrofauna) apresentaram composições distintas nas áreas investigadas. Em condições de elevada perturbação física, observou-se a diminuição da riqueza taxonómica induzida pela perda de espécies raras e sensíveis à perturbação (por exemplo, organismos filtradores ou suspensívoros). Acresce que, no geral, a baixa dissimilaridade entre as áreas de pesqueiro e áreas adjacentes sujeitas a baixa perturbação, sugere que os efeitos negativos detectados podem estender-se para além das áreas directamente afectadas (por exemplo, resultados indirectos associados a plumas de sedimentos em suspensão). Correlações negativas significativas foram detectadas entre vários índices de diversidade da mega-epifauna e esforço de pesca, bem como com a abundância, riqueza especifica e riqueza de grupos tróficos de macrofauna. No entanto, não foram detectadas correlações entre esforço de pesca e outros índices de diversidade estimados para macrofauna (Shannon-Wiener e a equitabilidade de Pielou), apesar de diferenças na composição das comunidades evidentes através da análise multivariada e na interpretação das comunidades nucleares (compostas por espécies características, dominantes ou frequentes).

resumo (cont.)

Estes resultados sugerem que alguns índices de biodiversidade tipicamente utilizados em estudos de impacto ambiental podem não ser suficientemente sensíveis para identificar alterações das comunidades sob perturbação física. Apesar das alterações na composição da macrofauna, a complexidade trófica foi no geral mantida (presença de todos grupos tróficos em todas as áreas). No entanto, a redundância trófica (número médio de espécies por grupo trófico) diminuiu, pelo que cada função (representada por cada grupo trófico) passou a ser assegurada por um menor número de espécies ou até mesmo uma única espécie. Esta alteração traduz-se numa maior vulnerabilidade a perturbações adicionais e/ou continuadas que induza novas extinções locais de espécies. Os resultados experimentais sugerem que as práticas de pesca de arrasto de profundidade na área de estudo parecem não afectar a biomassa bacteriana, nem a composição e a diversidade de organismos da meiofauna.

A deplecção de várias funções realizadas pelos ecossistemas nas áreas sujeitas a de alta perturbação por pescas de arrasto, inclusive fluxos de energia e matéria nos sedimentos, foram relacionadas com alterações relevantes na composição da macrofauna, bem como alterações no espectro de tamanhos corporais dos organismos (prevalência de espécies de menor tamanho sob condições de perturbação generalizada). Tanto a produção secundária bacteriana, como a bio-irrigação e bioturbação apresentaram uma redução em sedimentos obtidos nas áreas de arrasto intenso. Adicionalmente, foi observada uma correlação positiva entre a respiração total e a riqueza específica da macrofauna, sustentando a nossa hipótese de que funções fundamentais do ecossistema podem sofrer depleções sob condições de perturbação física por arrasto de profundidade.

Em resumo, a presente tese demonstrou que as atividades de arrasto de profundidade têm efeitos prejudiciais nas comunidades bentónicas de habitats sedimentares, em particular na mega-epifauna e macrofauna. Estes efeitos manifestam-se numa redução de funções regulatórias essenciais do ecossistema, normalmente mediadas pela fauna afetada. Estes resultados sugerem que a exploração continuada dos recursos biológicos ao longo da Margem Portuguesa, estão atualmente a pôr em risco os ecossistemas de mar profundo, e em particular as suas comunidades bentónicas.

resumo (cont.)

É importante salientar que os efeitos negativos detectados podem nem sempre ser identificados pelos actuais indicadores utilizados na avaliação dos impactos e programas de monitorização em sistemas marinhos e, portanto, deverão ser acompanhados por outros indicadores da composição das comunidades, condição do ecossistema e vulnerabilidade, de modo a adequadamente determinar o estado ambiental de ecossistemas de mar profundo ao longo das margens Europeias.

keywords

benthos, upper continental slope, biodiversity, ecosystem functioning, bottom-trawling fisheries, West Iberian Margin

abstract

Bottom-trawling fisheries are considered the most pervasive and destructive anthropogenic activity presently threatening deep-sea ecosystems. In general terms, this activity is associated with the reduction of the benthos standing stocks, alterations of the benthic community structure and loss of fauna biodiversity in hard substrate habitats (i.e. seamounts and cold-water coral reefs), where recovery rates are estimated to be within decades. Yet, it is within the soft sediment regions, such as the continental slopes and submarine canyons, where a large amount of the trawling pressure is presently concentrated, and the effects on biodiversity and ecosystem function derived from this practice are barely known. This is particularly applicable for the West Iberian Margin, one of the most disturbed regions by bottom trawlers in Europe. Hence, this research aimed to investigate the effects of the long-term induced physical disturbance by bottom trawlers on the deep-sea soft-sediment benthic assemblages composition, diversity and trophic structure, and how this was translated into the maintenance of essential ecosystem functions (e.g. organic matter transformation and nutrient cycling, secondary production, ecosystem metabolism).

Three main areas were selected based on various degrees of disturbance (no, low, and high trawling pressure) along a continental slope area off the SW Portuguese margin, established from Vessel Monitoring Systems (VMS) data. Remotely Operated Vehicle (ROV) video surveys and a total of seven stations within these three areas were sampled to compare the benthic assemblages, in terms of total standing stocks (abundance and biomass), composition and diversity (both taxonomic and trophic), including meiofauna, macrofauna and mega-epifauna groups. Additionally, an onboard short-term pulse-chase experiment (5 days) was performed on sediment cores obtained from two selected locations, and enriched with ^{13}C labeled algae, to investigate several proxies of ecosystem functions in the sediment typically promoted by the benthic assemblages. These included carbon mineralization and production by bacteria communities and their total biomass (^{13}C uptake estimated through bacteria specific phospholipid-derived fatty acids – PLFAs), bioturbation (^{13}C sediment uptake profile with sediment depth) and bioirrigation (ammonia concentrations in the sediment depth profile).

abstract (cont.)

Additionally, infauna respiration rates and total respiration were used as a proxy for ecosystem metabolic function.

Overall, the main results of the present thesis showed an evident compromise of the seabed integrity at the highly disturbed area (e.g. often flattened topography, low bioturbation evidence, and numerous trawl scars). Furthermore, the larger sized component of the benthic biota (megafauna and macrofauna) showed distinct assemblages between the areas investigated, and a lower morphospecies/species richness under conditions of high trawl disturbance, due to the loss of rare and trawl sensitive groups (e.g. sessile filter feeding fauna). Besides, a lower dissimilarity between assemblages were found in the main fishing ground areas (high trawled) and the adjacent low-disturbance locations, suggesting that the potentially negative effects of trawling are extended beyond the main targeted areas (e.g. by the plumes of re-suspended sediments). Significant negative correlations were generally detected between various mega-epibenthic diversity indices and trawling pressure, as well for macrofauna abundance, species/trophic guild richness. However, diversity indices related with macrofauna community structure (Shannon-wiener and Pielou's evenness) failed to detect the observed differences in community structure observed by the multivariate analysis and the structure of the core assemblages (i.e. characteristic, dominant or frequent taxa). We suggest that such indices may not be sensitive enough to identify changes under conditions of physical disturbance. Besides, even though alterations of macrofauna community composition were not reflected in an impoverished trophic complexity (all feeding guilds present in all areas), as a result of an increase trawling pressure, macrofauna trophic redundancy (average number of species per trophic guild) declined, reflecting a higher vulnerability under conditions of disturbance, as each function (trophic guild) was insured by a low number of species. Contrariwise, trawling practices seemed to have little effect on either bacterial biomass or meiofauna standing stocks and composition.

abstract (cont.)

A depletion of important ecosystem functions, such as energy and matter fluxes in the sediments at the high trawling pressure areas was particularly linked with changes in macrofauna assemblages and size structure, towards a dominance of smaller sized species under conditions of permanent disturbance. Both bacterial production and bioirrigation/ bioturbation (e.g. the higher build-up of ammonia at the sediment deeper layers), was reduced in high trawled sediments. Furthermore, the general decline in macrofauna species richness across the study region was correlated with the depletion of macrofauna total respiration, supporting our hypothesis that the depletions of fundamental regulatory ecosystem functions occur under high trawling disturbance regimes.

In summary, this thesis demonstrated that trawling activities have deleterious effects on soft-sediment benthic assemblages, mainly within mega-epifauna and macrofauna, and are linked with the depletion of essential regulatory ecosystem functions normally mediated by the affected biota. These suggest that the exploitation of the deep-sea natural resources in the SW Portuguese Margin, one of the most disturbed regions by bottom trawlers in Europe, is currently endangering its benthic habitats. Finally, the deleterious effects on the benthic habitats associated with trawling disturbance may not be perceived by the current routinely used monitoring tools for impact assessment and monitoring programmes in marine systems (e.g. univariate indices of diversity) and therefore should be accompanied by other indicators of community composition, ecosystem condition and vulnerability to adequately determine and achieve a Good Environmental Status is deep-sea areas within the European margins.

trefwoorden

benthos, bovenste continentale helling, biodiversiteit, ecosysteemfunctie, bodemvisserij, West-Iberische randen

abstract

Boomkorvisserij wordt beschouwd als één van de meest destructieve antropogene activiteiten die thans diepzee-ecosystemen bedreigt. In het algemeen wordt deze activiteit geassocieerd met een afname in benthische standing stock, veranderingen in de benthische gemeenschapsstructuur en verlies van biodiversiteit op harde substraten (dwz zeebergen en koudwater koraalriffen), waar herstel naar schatting decennia kan duren. Echter vooral op zachte bodems zoals langsheen de continentale hellingen en onderzeese canyons, waar een grote deel van de bodemvisserij momenteel geconcentreerd is, zijn de effecten op biodiversiteit en ecosysteemfuncties nauwelijks bekend. Dit geldt in het bijzonder voor de West-Iberische continentale rand, één van de meest door bodemvisserij verstoorde regio's in Europa. Vandaar dat in dit onderzoek de effecten werden bestudeerd van langdurige fysische verstoring door bodemvisserij op de bodemdieren van zachte sedimenten, meer bepaald de impact op de samenstelling van deze gemeenschappen, als ook op hun biodiversiteit en trofische structuur werd onderzocht, en hoe dit zich vertaald heeft in het behoud van essentiële ecosysteemfuncties (bijv. Afbraak van organisch materiaal, de trofische cyclus, secundaire productie, en ecosysteemmetabolisme).

Drie gebieden werden geselecteerd op basis van verschillende gradaties van verstoring (geen, lage en hoge bodemvisserijdruk) langsheen de zuidwestelijke Portugese rand. De selectie gebeurde op basis van ‘Vessel Monitoring System’ (VMS) data. ROV-video-transecten en in totaal zeven stations verspreid over deze drie gebieden werden bemonsterd om de benthische gemeenschappen te vergelijken, in termen van totale standing stock (densiteiten en biomassa), samenstelling en diversiteit (zowel taxonomisch als trofisch), waarbij zowel meiofauna, macrofauna als mega-epifauna werden bestudeerd. Daarnaast werd een ex situ ‘pulse-chase’-experiment (5 dagen) uitgevoerd op sedimentstalen verzameld in twee geselecteerde locaties.

abstract (cont.)

Deze sedimentstalen werden verrijkt met ^{13}C gemerkte algen, met als doel een aantal proxies voor ecosysteemfuncties in het sediment te onderzoeken, die typisch gerelateerd worden aan de activiteit van bodemdieren. Deze functies omvatten koolstofmineralisatie/-productie door microbiële gemeenschappen en hun totale biomassa (^{13}C opname geschat door bacterie-specifieke fosfolipide-afgeleide vetzuren - PLFA's), bioturbatie (^{13}C sedimentopnameprofiel met sedimentdiepte) en bioirrigatie (ammoniakconcentraties in het sedimentdiepteprofiel). Daarnaast werden infauna respiratie ratio's en totale respiratie bepaald als proxy voor de metabolische functie van het bodemecosysteem.

In het algemeen wijzen de belangrijkste resultaten van dit doctoraatsproefschrift op een duidelijke impact op de integriteit van de zeebodem in het meest verstoorde gebied (bijvoorbeeld afgevlakte topografie, lage bioturbatie, talrijke afdrukken van visserijactiviteiten). Bovendien vertoonde een groot deel van de benthische biota (megafauna en macrofauna) een verschillende samenstelling tussen de onderzochte gebieden, en een lagere morfospecies/soortenrijkdom onder omstandigheden van hoge bodemvisserijverstoring doordat zeldzame en trawlgevoelige groepen afwezig zijn (bv. filtervoedende fauna). Verder is er een kleiner verschil tussen gemeenschappen aanwezig in de belangrijkste visserijgebieden (hoge druk) en de aangrenzende laag verstoorde locaties, wat suggereert dat de potentieel negatieve effecten van bodemvisserij zich ook buiten de doellocatie uitbreiden (bijv. door sedimenten in suspensie). Significante negatieve correlaties werden in het algemeen geobserveerd tussen verschillende mega-epibenthische diversiteitsindices en visserijdruk, als ook voor macrofaunadensiteiten, soorten/trofische diversiteit. Diversiteitsindices voor macrofauna (Shannon-Wiener en Pielou's eveness) geven geen verschillen zoals wel waargenomen voor gemeenschapsstructuur op basis van multivariate analyse en de structuur van de kernsoorten (dat wil zeggen karakteristieke, dominante of frequente taxa).

abstract(cont.)

We besluiten daarom dat dergelijke indices niet gevoelig genoeg zijn om veranderingen te identificeren als gevolg van fysische verstoring. Bovendien, hoewel veranderingen in de macrofauna gemeenschapssamenstelling zich niet weerspiegelen in een verarmde trofische complexiteit, als gevolg van een toenemende visserijdruk, is er een afname in de trofische redundantie (gemiddelde aantal soorten per trofische groep), wat een hogere functionele kwetsbaarheid weerspiegelt onder omstandigheden van verstoring, aangezien elke functie (trofische gilde) verzekerd was door een laag aantal soorten. In tegenstelling lijkt de ‘trawling’ praktijk weinig effect te hebben op bacteriële biomassa of meiofauna standing stocks en samenstelling.

Een afname in belangrijke ecosysteemfuncties, zoals energie- en materiaalfluxen in de sedimenten van de meeste beviste gebieden (hoge druk), ging opmerkelijk gepaard met veranderingen in macrofauna-gemeenschappen en grootteverdeling van de organismen, met een meer uitgesproken dominantie van kleinere soorten onder omstandigheden van permanente verstoring. Zowel bacteriële productie als bioirrigatie/bioturbatie (bijv. De hogere opbouw van ammoniak in de diepere lagen van het sediment) toonden een afname in sterk verstoorde sedimenten. Bovendien was de algemene afname in de rijkdom van de macrofauna-soorten in het studiegebied gecorreleerd met een afname in de totale respiratie van de macrofauna, waardoor de vooropgestelde hypothese dat er een afname plaatsvindt van fundamentele ecosysteemfuncties onder hoge trawlverstoringsregimes niet wordt verworpen.

Samengevat toont dit proefschrift aan dat trawlactiviteiten schadelijke effecten hebben op de bodemdiergemeenschappen van zachte sedimenten, hoofdzakelijk wat betreft de mega-epifauna en macrofauna. Hieraan gekoppeld wordt ook een afname waargenomen in essentiële regulerende ecosysteemfuncties die normaal gesproken worden gemedieerd door de aangetaste biota.

abstract (cont.)

Deze resultaten suggereren dat door de exploitatie van de diepere continentale randen in Europa de aanwezige benthische habitats worden bedreigd. Tenslotte worden de schadelijke effecten op de bodemgebieden als gevolg van boomkorvisserij niet waargenomen door de huidige routinematig gebruikte monitoringinstrumenten voor effect-beoordelings- en bewakingsprogramma's in mariene systemen (bijv. Univariate diversiteitsindices). Daarom wordt het gebruik van andere indicatoren zoals gemeenschapssamenstelling, ecosysteemfuncties en -kwetsbaarheid aanbevolen om de beoogde ‘Good Environmental Status’ van diepzeegebieden langsheen de Europese randen adequaat te bepalen en te bereiken.

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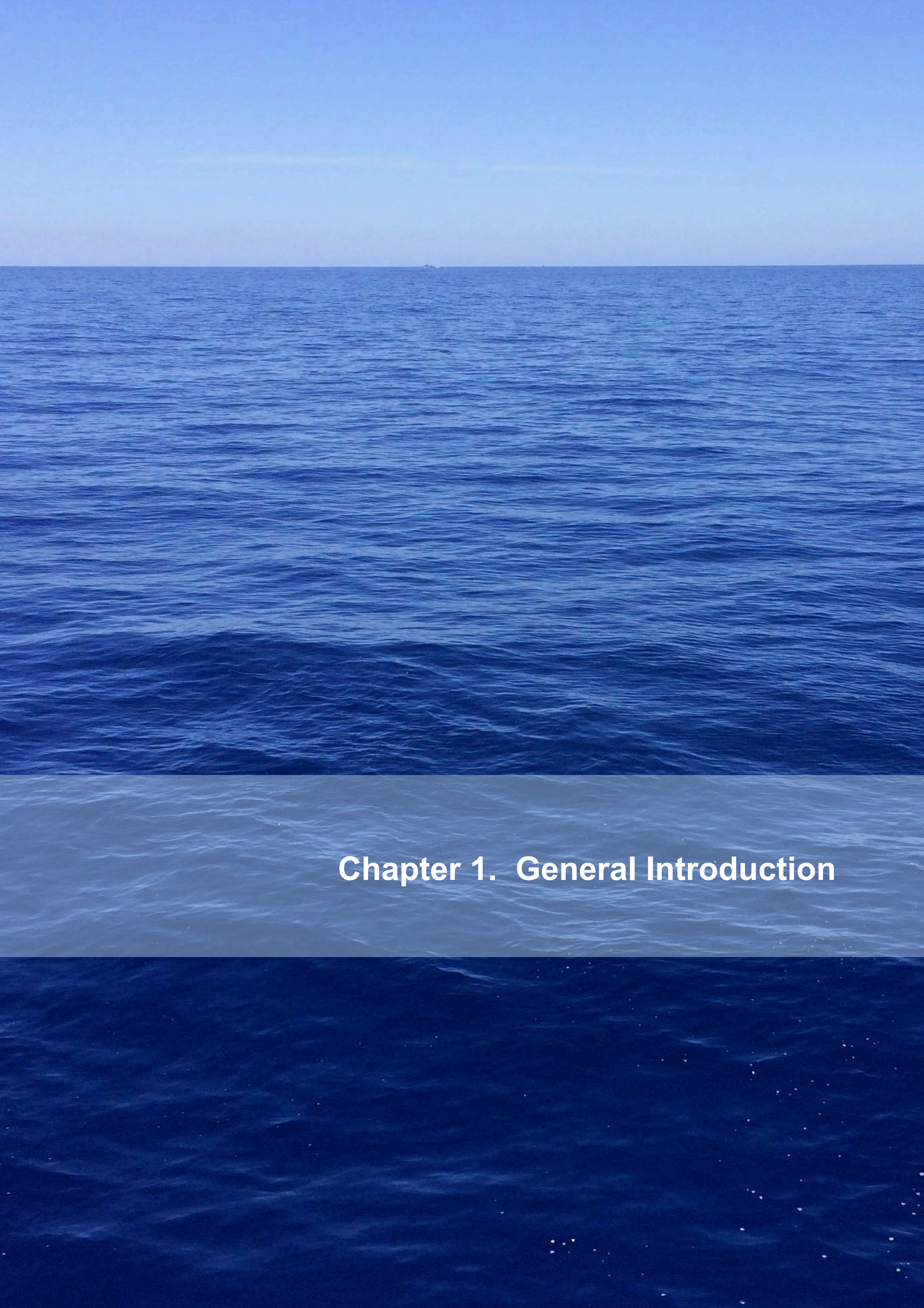
Chapter 1. General Introduction



1.1 General background

Continental margins are the submerged outer edges of continents that occupy approximately 15% of the surface of the planet. Usually, these are divided into three main regions (Fig. 1.1): a shallow extension of the continent designated as continental shelf; a steep transition zone which connects the continental shelf and the deep ocean floor, the continental slope; and the continental rise, which connects the continental slope to the deep abyssal basins. The shelf break marks the abrupt transition from continental shelf to the continental slope, and it is usually considered as the shallowest limit of the deep-sea ecosystems (ca. 200m water depth) (Tyler, 2003).

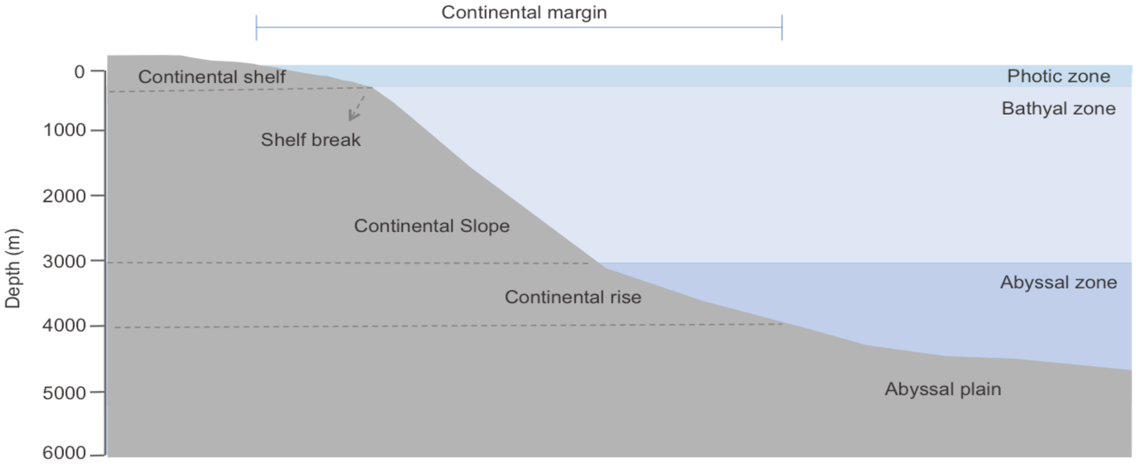


Figure 1.1 Schematic profile of a continental margin and deep-sea system, showing the major physiographic regions. Adapted from Gage and Tyler (1991).

Owing to constraints related with accessibility and exploration costs, our understanding of the continental margins ecosystems is still limited in comparison to other marine environments. Nevertheless, it is generally accepted that these regions accommodate a large amount of essential supporting functions and provisioning, and regulatory ecosystem services (Levin and Sibuet, 2012; Thurber et al., 2014). These include carbon and other nutrients cycling processes (e.g. > 40 % of total carbon transformation/burial occurs within continental margins; Muller-Karger, et al., 2005), and climate regulation (ca. 25% of the annual CO_2 produced by human activities is sequestrated in the deep ocean; Heinze et al., 2015). Moreover, continental margins generate the highest primary and secondary production in marine regions (ca. 80% of total marine animal biomass is concentrated within these regions; Wei et al., 2010) and provide important resources which human populations exploit, namely food and energy including oil, gas and rare minerals (Levin and Sibuet, 2012; Thurber et al., 2014). The high relevance of continental margins in ecosystems functioning and services is due largely to the high spatial and temporal heterogeneity of their habitats and the diversity of associated biota. Severe environmental gradients occurring at continental margins are determined by tectonics, sediment diagenesis, terrestrial inputs and various oceanographic processes, that create a high habitat heterogeneity with distinctive faunal communities, such as the sedimentary continental slopes, submarine canyons, seamounts, cold-water corals reefs, and even chemosynthetic-based habitats such as pockmarks and mud volcanoes (Levin and Dayton, 2009; Levin and Sibuet, 2012).

The steep continental slopes host typically a large component of the biological diversity and their biological assemblages contrast considerably in composition from those observed on the continental shelf regions (Hessler and Sanders, 1967; Levin et al., 2010; Rex and Etter, 2010). The marked depth-related environmental gradients (e.g. oxygen, temperature, salinity, productivity), recurrent and episodic hydrodynamic disturbance events (e.g. currents, internal waves, tidal energy, storm-induced waves conditions, strong near bottom currents, sedimentary transport, landslides) and biological interactions (e.g. predation and competition) are among the main drivers for the complex and high biodiversity of these regions (Carney, 2005; Levin et al., 2001). In general terms, distribution of benthic faunal standing stocks (abundance and biomass) and the diversity of benthic assemblages along the slopes may vary depending on fauna components, environmental characteristics and spatial scales (Levin and Sibuet, 2012). Benthic standing stocks, with the exception of the microbiota, typically decrease severely with increasing water depth and the associated reduction of the food supply and quality derived from the surface (Carney, 2005; Rex et al., 2006; Smith et al., 2008). Diversity (e.g. expected number of species) usually follows a unimodal pattern peaking at mid to lower slope (1000-3000 m) and the main species turnover (β-diversity) is observed at the shelf-slope transition (300-500m) (Rex, 1981; Grassle and Maciolek, 1992; Carney, 2005). However, exceptions occur, for example in oxygen minimum zones (OMZs), where the severe conditions disrupt these characteristic patterns (Levin, 2003; Carney, 2005) or in some canyons where the accumulation of organic matter may lead to high-dominance and low-diversity opportunistic assemblages (Cunha et al. 2011). In addition to the influence of natural factors, biodiversity in deep-sea regions such as continental slopes is also influenced, at least to some extent, by the increasing pressure from anthropogenic activities (Levin and Sibuet, 2012; de Leo et al., 2017).

1.1.1 Anthropogenic disturbance in the deep sea

The most relevant cumulative effects caused by anthropogenic activities are concentrated in both terrestrial and shallow marine ecosystems (< 200 m water depth). Yet, the depletion of both biological and mineral resources in these regions, and the lower availability of safe disposal sites for many types of waste in several regions around the world, have caused an increase of human pressure towards deeper regions, which until recently have been kept off the influence from human disturbance (Ramirez-Llodra et al., 2011). This does not mean that deep-sea regions were necessarily kept in pristine conditions, as the lack of adequate legislation for these unexplored areas has allowed, for instance, the routinely disposal of waste materials (e.g. toxic or radioactive waste, munitions). It was only in 1972, that the London convention prohibited the practice of regular waste disposal in the deep ocean (Ramirez-Llodra et al., 2011).

The existence of current stricter regulations has not been successful in reducing waste accumulation in deep-sea regions. With the increase of worldwide populations inhabiting along coastal regions, unintentional and/or careless disposal of litter is still transported to deep-sea areas. For example, accumulation of litter, predominantly plastic, is regularly found in submarine canyon regions (Pham et al., 2014). Moreover, in recent years the scientific community has also stressed the importance of plastic debris of small size, microplastics, present in high abundances in deep-sea sediments and likely putting at risk many organisms that may feed on these small particles and integrate them up the food webs. Yet, the precise effects of microplastics to the environment and fauna are still largely unknown (Woodall et al., 2014; Courtene-Jones et al., 2017; Katija et al., 2017). In addition to plastic, lost or discarded fishing gear is also frequently reported (Pham et al., 2014; Vieira et al. 2015). Moreover, litter is not the only type of disposed materials that presently reach the deep-sea floor. For example, areas of contaminants' accumulation may occur associated with terrestrial and river runoff waters that are rich in organic pollutants (de Jesus Mendes et al., 2011; Jesus et al., 2013) or from deposition of contaminated sediments and mine tailings (Ramírez-Llodra et al., 2015; Hughes et al., 2015; Mestre et al., 2017). The later have been reported to negatively affect deep-sea communities, particularly those of low mobility, which are unable to escape from contamination zones (e.g. Hughes et al., 2015; Mevenkamp et al., 2017).

While disposal is one of the many ways human populations are currently altering the deep-sea habitats, exploitation of deep-sea resources such as the extraction of oil and gas, and in the near future, the foreseeable regular extraction of important minerals (e.g.

copper, manganese, cobalt) also have drastic effects in the deep-sea habitats (Glover and Smith, 2003; Ramirez-Llodra et al., 2011). Adverse environmental impacts from oil and gas extraction can derive from infrastructure installation to routine activities (e.g. physical disturbance by drilling, release and deposition of organic enriched and contaminated seawater/sediments - drilling muds), and have been detected as far as 5 km away from the drilling sites (Jones et al., 2012; Cordes et al., 2016 and references therein). Furthermore, major environmental disasters related with the release of large amounts of hydrocarbons have occurred during exploitation accidents, such as Deep-water horizon accident in the Gulf of Mexico in 2010, or spills during vessel transportation. These hydrocarbon releases have been associated with contamination and alterations over large extensions of the deep-seabed and that may last for decades (Montagna et al., 2013; Cordes et al., 2016). Moreover, although deep-sea mining activities are still to be proven cost-effective, there is increasing evidence in test zones that the removal of hard substrates such as manganese nodules in the abyssal zones may result in the large decline of associated fauna (Ramirez-Llodra et al., 2011; Vanreusel et al., 2016; De Smet et al., 2017). At the continental margins, mining exploitation will be mainly focused on the extraction cobalt-rich crusts in seamounts, which will also likely present negative effects on the associated fauna, although not thoroughly studied. These regions sustain highly diverse and in many cases endemic fauna (Koslow et al., 2000) that is often structurally distinct from the fauna inhabiting other seamounts without cobalt-rich crusts, highlighting its vulnerability to exploitation (Schlacher et al., 2013).

None of the present exploitation or disposal activities influence as strongly the deep-sea pelagic and benthic regions as the exploitation of biological resources (e.g. fishes, crustaceans and shellfish). Fisheries are the most pervasive and destructive anthropogenic activity currently in practice along the continental margins worldwide, and should be of outmost concern when considering conservation measures in deep-sea regions (Glover and Smith, 2003; Ramirez-Llodra et al., 2011; Clark et al., 2015). Because this is the main focus of the present thesis, a detailed exploration of the deep-sea fisheries and known effects in benthic ecosystems is described in more detailed in sections 1.3 and 1.4.

In the present scenario of accelerated human-induced global change, the vulnerability of margin ecosystems to human disturbances is likely to increase. Hypoxia, low pH and higher temperature conditions, may compromise the resistance and the resilience of biotic assemblages to other types disturbance (both natural and anthropogenic; Fig. 1.2), with major implications to deep-sea biodiversity and their contribution to the functioning of ecosystems and the services they provide (Ramirez-Llodra et al., 2011; Levin and Sibuet, 2012; Jones et al., 2014; Sweetman et al., 2017).

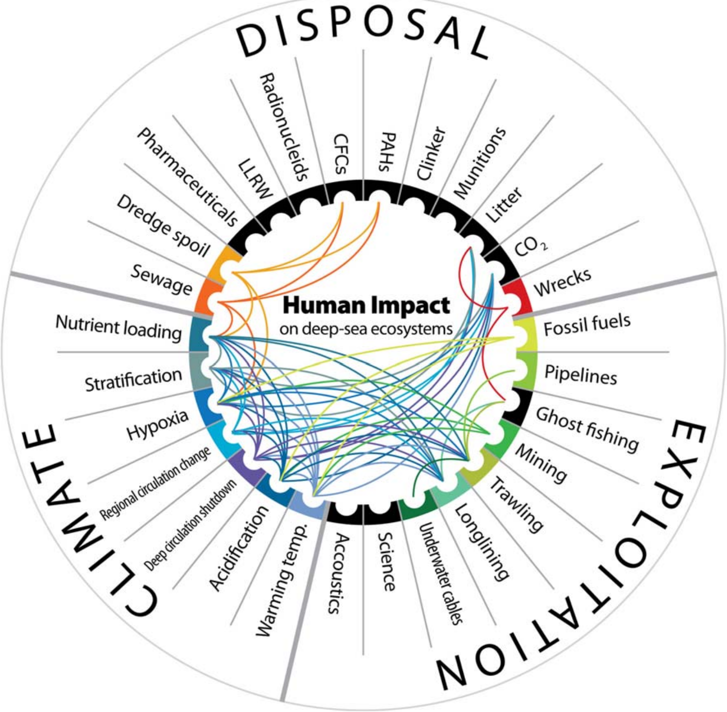


Figure 1.2 Links between different types of anthropogenic activities impacting deep-sea habitats and its faunal assemblages. LLRW: Low-level radioactive waste; CFCs: chlorofluorocarbons; PAHs: polycyclic aromatic hydrocarbons. From Ramírez-Llodra et al. (2011).

As the pressure from anthropogenic activities increases, their cumulative effects and synergies with natural disturbance in deep-sea regions is not likely to slow down, the implementation of mitigation and conservation measures, such as the creation of marine protected areas (MPAs) in strategic regions, will be fundamental to preserve and maintain a sustainable exploitation of deep-sea ecosystems, and restore areas that have been devastated from past activities. For this to happen, regional managers must establish priority areas and determine which aspects of the deep-sea biodiversity are fundamental to protect (e.g. species diversity, inclusive at the genetic level, maintenance of particular habitats and ecosystems, such as the enigmatic ecosystems such as hydrothermal vents fields, or the deep-water coral reefs). Assessing biodiversity, as well as how it changes under conditions of disturbance, is a fundamental step for informed decision-making for the conservation of the deep sea. Noteworthy is that the deep sea accounts for the majority of the marine systems in terms of area but most of its biodiversity remains undetermined and most of the species recovered (ca. 90%) are likely still new to science (Ebbe et al., 2010; Higgs and Attrill, 2015).

1.1.2 Deep-sea fisheries

Fisheries are the most widespread anthropogenic source of physical disturbance in deep-sea environments (Clark et al., 2015). The decline of the shallow water fishing stocks and development of more efficient and powerful types of vessels and gears in the 1960s and 1970s, associated with an increasing demand for marine resources (i.e. fish and shellfish), led fisheries to a generally progress towards deeper fishing grounds (Roberts, 2002; Morato et al., 2006).

Global fisheries are presently concentrated at an average depth range of 500-600 m (Watson and Morato, 2013), but may reach as far as 2000 m water depths in some regions of the globe (Rogers and Gianni, 2010). Furthermore, it is likely that targeted depths will continue to increase. Watson and Morato (2013) estimated an approximate increase of 63 m per decade for the mean fishing depth for global fisheries (Fig. 1.3). The overexploitation of the fish and shellfish stocks together with the unsustainable nature of deep-sea fisheries (in particular bottom trawling; Norse et al., 2012), may lead to even faster rates of increasing the mean fishing depth, in most cases supported by government subsidies (Norse et al., 2012).

One of the main reasons why deep-sea fisheries are fundamentally unsustainable owes to the fact that the few commercially important deep-sea fish species tend to exhibit low productivity (i.e. slow growth, late maturity/reproductive age, long life spans) and thus low resilience to exploitation when compared to fish species living in shallow areas (Roberts, 2002). For example, the Atlantic round-nose grenadier (*Coryphaenoides rupestris*) can live over 70 years, and only matures at 14–16 years old (Bergstad, 1990), while the orange roughy (*Hoplostethus atlanticus*) can reach 150 years in age, and does not mature before it is 20 to 30 years old (Horn et al., 1998)

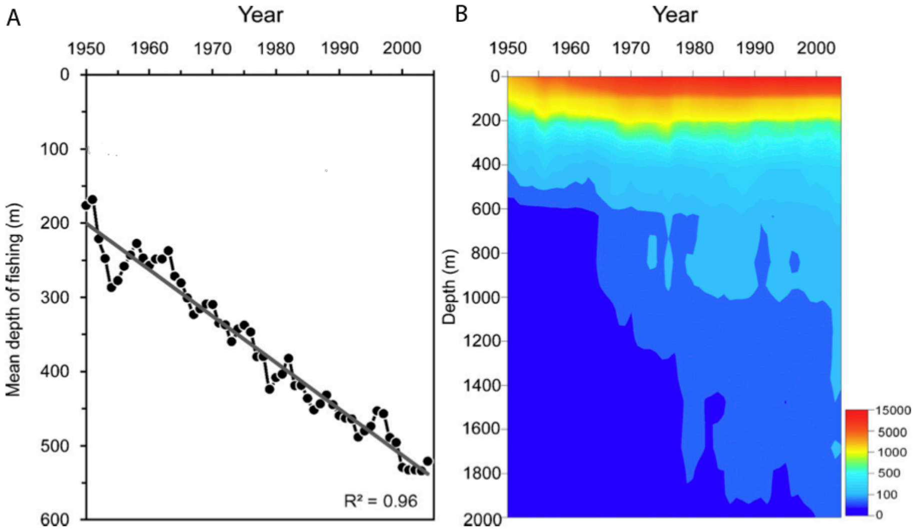


Figure 1.3 Depth of world marine bottom fisheries catches from 1950–2004. (A) Trend line fitted using a linear regression model and taking into account both within- and between-species changes in mean depth; (B) time series of world marine bottom fisheries catches by depth strata. Catch are in million tonnes. From Watson and Morato (2013).

Additionally, the most common deep-water fishing technique, bottom trawling, produces enormous amounts of by-catch (incidental catches that are not commercialised due to various reasons) and indirectly prompts the decline of fishing stocks and other fauna by damaging many deep-sea habitats. Bottom trawlers target regularly many regions of the globe, but are mostly concentrated within sedimentary continental slopes, seamounts and submarine canyon habitats, where the highest levels of fishing stocks are found (Roberts, 2002; Ramirez-Llodra et al. 2011; Clark et al., 2015). Many long-lived corals and sponges commonly reported in high abundances along seamounts, ridges and canyons provide nursery grounds and refuge for the early stages of commercially important species (Jennings and Kaiser, 1998; Costello et al., 2005; Clark et al., 2010). The unselective removal or damage of all benthic groups by trawl gears, including long-lived species such as sponges and corals, may increase predation and mortality of early life stages of commercially important fish species, and in time result in the reduction of valuable deep-sea fish stocks globally (Costello et al., 2005; Clark et al., 2010).

1.1.3 Bottom-trawling fisheries and known impacts in the marine benthic environments

The effects of bottom-trawling fisheries on the deep sea, particularly on benthic environments, are still far from being well understood (Dayton et al., 1995; Gage et al., 2005; Clark et al., 2015). In fact, most of our current knowledge on the effects of trawling on marine benthic ecosystems arises largely from the well-documented shallow water studies (e.g. general reviews and meta-analysis done by Dayton et al., 1995; Thrush and Dayton, 1995; Jennings and Kaiser, 1998; Collie et al., 2000; NRC, 2002; Kaiser et al., 2002), which is complemented by scarcer information obtained from deep-sea studies carried in a variety of habitats and scattered regions across the globe (e.g. Gage et al., 2005; Clark et al., 2015 and references therein; Murillo et al., 2016; Oberle et al., 2016; de Leo et al., 2017).

In general, effects reported from both coastal areas and continental shelf studies demonstrate that the low selectivity of bottom contact fishing practices such as trawling, results in (Fig. 1.4; NRC, 2002): i) high mortality of target species and incidental catches (by-catch); ii) increased food availability from discard practices or in-situ mortality/damage of organisms that makes them susceptible to predation; iii) loss of habitat and/or severe alteration of seabed habitat structure and complexity, caused by re-working of the surface and subsurface of the sediments, induced sediment suspension, as well as removal of erect sessile habitat-forming species.

As a consequence of bottom-trawling disturbance, indirect and interconnected alterations of ecosystem processes and benthic fauna structure are usually described (Fig. 1.4; NRC, 2002), but are dependent on habitat characteristics (Collie et al., 2000; NRC, 2002; Queirós et al., 2006; Sciberras et al., 2016). In general, most studies report marked alterations of the benthic community composition and biodiversity loss, particularly of rare species or sensitive species (Kaiser et al., 2002; NRC, 2002; Hiddink et al., 2006). For example, in regions where trawling is recurrent, long periods of sediment re-suspension induced by the trawl gears may indirectly affect certain faunal groups, namely filter-feeding fauna either by suffocation or by inefficient feeding behaviour (Lindeboom and de Groot, 1998; Pile and Young, 2006; Maldonado et al., 2012; Leys, 2013). Noteworthy, is that trawling disturbance is not always translated in a reduction of diversity, as changes in community structure result from increases or decreases of both species richness and evenness (NRC, 2002). Moreover, in chronically disturbed areas under a permanent altered state, benthic communities may become readapted, by for example, long-term shifts in their size-structure, towards small-bodied species, which have a lower potential of removal or damage than large-sized fauna (Jennings et al., 2001a; Jennings et al., 2002; Duplisea et al., 2002). Still, even small-sized fauna suffers changes in community composition (Schratzberger and Jennings, 2002; Lampadariou et al., 2005), and in some extreme cases, deleterious effects within meiofauna/nematode assemblages standing stocks and diversity were observed (Hinz et al. 2008).

Trawling disturbance can also induce alterations of faunal behaviour and intra/inter-specific relationships (e.g. disruption of predator-prey relationships) and alterations of trophic webs connectance and complexity are frequently reported, even if as short-term effects (Jennings et al., 2001b; Jennings et al., 2002; NRC, 2002). For example, the increased food availability from on-site mortality and/or accumulation of carrion on the seabed from discarding practices attracts high abundances of opportunistic scavengers and predators, which otherwise would be present in relatively low abundances (Smith et al., 2000). In such cases, short-term enhancement of secondary production and nutrient flow in the sediment may occur (Ramsay et al., 1998; Groenewold and Fonds, 2000).

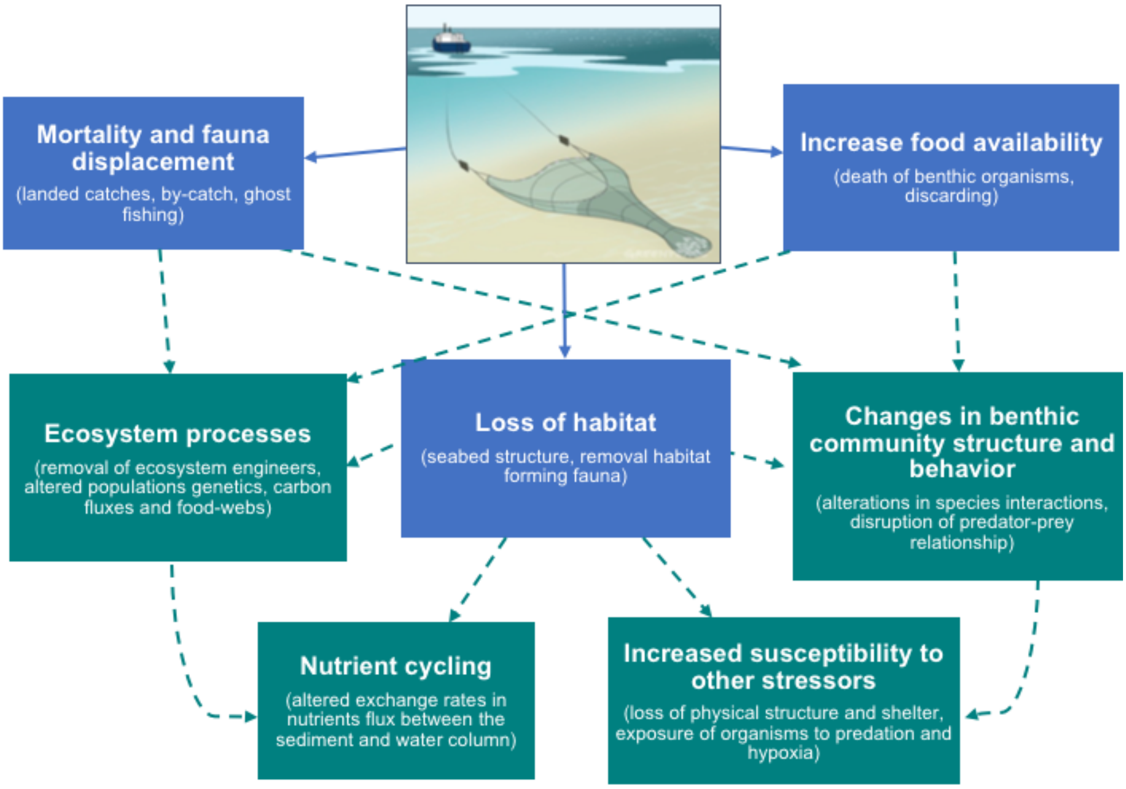


Figure 1.4 Schematic relations demonstrating the main direct (full blue lines) and indirect effects (dashed green line) of bottom-trawling disturbance in marine systems identified by NRC (2002).

Indirect alterations of sediment processes and biogeochemistry are also expected (NRC, 2002; Sciberras et al., 2016). The induced direct alterations of the sediment structure and porosity through trawl gear seabed ploughing and revolving, as well sediment re-suspension, may increase the availability of organic matter and pollutants buried in the deeper layers of the sediment. The synergy of these alterations, with the induced mortality/damage of important ecosystem engineers (20-50%) that stimulate sediment bioturbation and bioirrigation processes may also lead to alterations of the sediment biogeochemistry (Kaiser et al., 2006; Olsgard et al., 2008; Sciberras et al., 2016). Although these effects are still largely unknown even in shallow water regions (NRC, 2002; Kaiser et al., 2002) this is likely to result in changes nutrient cycling processes (e.g. carbon and nitrogen), because of the shortage in the provision of a regular turnover of oxygen and nutrients in the sediment pore water (Widdicombe et al. 2004; Trimmer et al., 2005; Olsgard et al., 2008). Moreover, as trawls plough the seabed a short-term increase in oxygen provision occurs into the deeper sediments layers, compromising both denitrification and anamox processes responsible for the conversion of nitrogen gas released to the overlaying sediment water, and sediments may suffer an increase in carbon mineralization (Duplisea et al., 2002; Trimmer et al., 2005). In addition, high turbidly periods induced by trawlers, may also lead to an increased load of several nutrients to the water column (i.e. ammonia, nitrate, silicate content), where for example denitrification processes are impaired (Pilskaln et al., 1998; Duplisea et al., 2001; Durrieu de Madron et al., 2005),

The conjuncture of the direct and indirect alterations of habitat, sediment processes and associated fauna enhance the vulnerability of these impacted sites to human-induced stressors or natural disturbance, such as strong episodic hydrodynamic conditions, alterations of water temperature and pH associated with climate change, etc. (Kaiser et al., 2002).

1.1.4 Recovery from bottom-trawling disturbance

The spatial and temporal variation associated with bottom-trawling fisheries, suggests that the magnitude of the impacts will not only depended on the frequency and intensity, extension of the area disturbed, and the type and configuration of the gears used (e.g. beam or otter trawl, weight of doors), but also on the complexity of the habitats affected (physical and biological characteristics) and the capacity of the impacted assemblages to resist and/or recover after disturbance (Kaiser et al., 2002; NRC, 2002; Lambert et al., 2014; Hiddink et al., 2017). Nonetheless, it is expected that chronic trawling disturbance of the seabed may significantly exceed the background levels and frequency of many natural disturbance events, both biotic (e.g. predation, competitions) and abiotic (regular, such as daily currents and tides; or episodic, such as winter storms) (Fig. 1.5), although this is not always evident in areas that are naturally under recurrent elevated natural disturbance conditions (van Denderen et al., 2015).

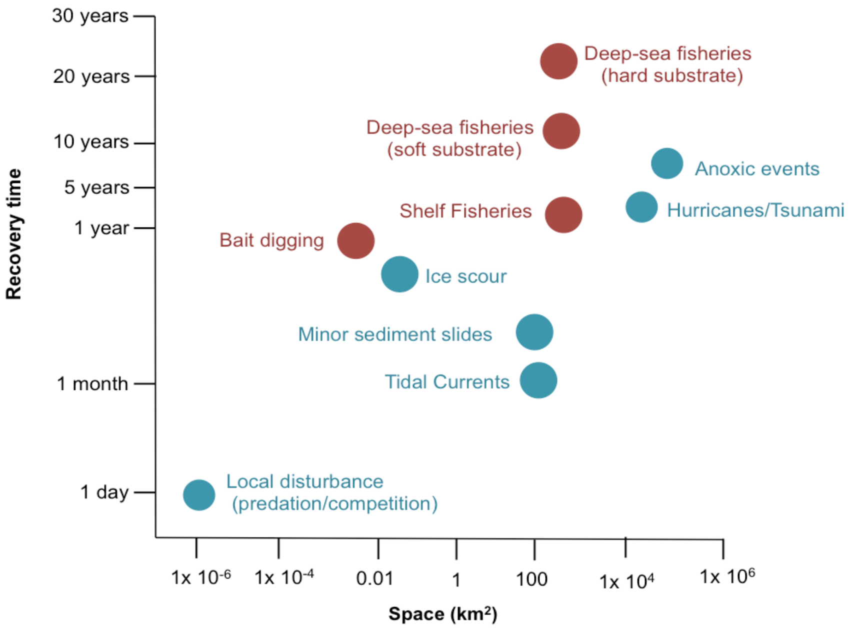


Figure 1.5 General recovery rate at different spatial and temporal scales for both natural and anthropogenic disturbance in marine environments (mostly coastal and shelf regions, unless mentioned otherwise). Adapted from Kaiser et al., 2002 and Yesson et al. (2017).

Intuitively, more complex habitats and those that are naturally not subjected to strong natural disturbance events (e.g. biogenic reefs, muddy sediments) are predictably more susceptible to trawling disturbance; their recovery is expected to be slow (Kaiser et al., 2002; NRC, 2002; Lambert et al., 2014), and developing a capacity to adjust to frequent disturbance would require an evolutionary time-scale (100s-1000s of years). Contrariwise, fauna from shallow continental shelf areas, often of unconsolidated sediments (e.g. sands), and subjected to frequent highly hydrodynamic conditions (e.g. strong currents, tides, wave action), typically exhibit fauna that is more adapted and resilient to periodic disturbance events, and present a faster turnover and recolonization capacity (Jennings & Kaiser 1998; Collie et al., 2000). Collie et al., (2000) indicated a recovery period for coastal sandy habitats from the North Sea of approximately 100 days, which suggests that these habitats could endure 2-3 events of trawling disturbance per year without drastic alterations in their assemblages. Nevertheless, the patchy character of bottom-trawling fisheries makes it unlikely that important fishing grounds will be trawled such few times. Rijnsdorp et al. (1998) reported that some areas in the North Sea can be trawled more than 400 times within a single year, meaning they are in a permanent (daily) state of disturbance. Thus, it is important to consider that the scale and frequency at which fishing disturbance typically occurs could easily exceed the tolerance threshold of the benthic assemblages, as their capacity to recover is dependent on the life history of the organisms in question, often equivalent to 1-5 times the generation time (Collie et al., 2000; Emeis et al., 2001). In this context, it is not surprising that estimates of recovery are highly contradictory, varying from days to decades (Collie et al., 2000; Lambert et al., 2014). In a recent revision, Hiddink et al., (2017) point out that, depending on the type of trawl gears used and their frequency, recovery time for macrofauna assemblages inhabiting sedimentary environments is more likely to be within a scale of years and not days. Moreover, Hiddink et al., 2017 indicated that in soft sediments with a 5 to 50% of removed faunal biomass, showed a post-trawling median recovery time ranging between 1.9 to 6.4 years, depending on the type of trawl gear used.

As mentioned before, faunal vulnerability as well as its recovery capacity to trawling disturbance is chiefly linked to the body-size and other life-history traits. The expected shift in the assemblage size spectrum towards dominance of small, fast-growing fauna under conditions of chronic trawling disturbance (Kaiser et al., 2002; Duplisea et al., 2002; Queirós et al., 2006) may ultimately result in a genetic selection for different faunal behaviour and reproductive traits fitted to tolerate extreme disturbance conditions (e.g. earlier maturity age; Kaiser et al., 2002; Tillin et al., 2006). It is generally accepted that a greater vulnerability is linked to large-sized organisms, particularly sessile or low-mobility fauna inhabiting the seabed surface with slow turnover rates such as mega-epibenthos (organisms recognized in photographs), and in some cases macrofauna (>250 /500μm). Those organisms are more susceptible to removal and/or damage by trawl gears and associated alterations in the environmental setting (e.g. turbidity) (Jennings et al., 2001a; Duplisea et al., 2002; Queirós et al., 2006; Dimitriadis et al., 2014). On the other hand, even though small-sized fauna (microfauna (bacteria and archaea) and meiofauna (> 32μm)) can easily be suspended and even subjected to mortality, total standing stocks are usually not affected by trawling disturbance, or may even increase. Their fast turnover rates, together with the reduction of competition and/or predation by larger organisms, and in some cases the higher organic matter availability reported in some trawled sites are crucial to explain such trends (Jennings et al., 2001a; Duplisea et al., 2002).

1.1.5 Bottom-trawling in the deep sea: constraints and state of knowledge

Similarly to shelf studies, pressure induced by bottom-trawling fisheries depends upon a large variety of factors (i.e. gear used, area disturbed, complexity of the habitats affected (Jennings and Kaiser, 1998; NRC, 2002). Among those factors essential differences arise when comparing shallow and deep-sea trawling practices. For example, trawl gears used in deep-sea regions are typically heavier, and will consequently create a greater and likely more persistent on-site pressure than in shelf regions (Clark et al., 2015).

Yet, the most fundamental difference highlighted by many authors, is the fact that deep-sea communities are rarely exposed to strong disturbance conditions (Gage et al., 2005; Clark et al., 2015). Moreover, typical biological and metabolic rates of deep-sea organisms make them more vulnerable to any type of disturbance, including trawling, than fauna inhabiting costal and continental shelf regions (Clark et al., 2015). It is generally believed that many deep-sea species may present k-selected life history traits (slow growth, late maturity, high longevity, low productivity; Gage and Tyler, 1991) implying a low turnover rate that makes deep-sea fauna less tolerant to the frequent trawling disturbance when compared to shallow water assemblages (Gage and Tyler, 1991; Gage et al., 2005; Clark et al., 2015). These traits together with the variable larvae dispersal capacity, intermittent recruitment and settlement potential (Lacharité and Metaxas, 2013) are indicative of a lower recovery capacity to background conditions. For example, in seamounts and cold-water coral reef habitats, heavy trawl gear has been shown to damage and remove a large amount of the sessile habitat-forming organisms, including slow growing and long-living corals and sponges, and recovery of these sites, if not disturbed again will likely take decades if not centuries (Roberts et al., 2000; Koslow et al., 2001; Fosså et al., 2002; Althaus al., 2009; Clark and Rowden, 2009; Clark et al., 2015; Yesson et al., 2017).

Also, the investigation of trawling impacts in deep-sea regions is hindered by several methodological limitations. The issue of trawling effects in marine systems is usually assessed by means of two main methodologies: the experimental and the comparative approach (Jennings and Kaiser, 1998; Clark and Rowden, 2009). An experimental approach usually implies an assessment of seabed habitat conditions and/or associated assemblage's structure prior and after induced disturbance; recovery time can be assessed by monitoring these areas over a period of time after disturbance (e.g. Kaiser and Spencer, 1996; Schratzberger et al., 2002; de Biasi, 2004; Pitcher et al., 2009). This approach may include laboratory manipulations where fauna is removed simulating the effects of trawl nets in order to examine the alterations in sediment properties and small-sized fauna (e.g. Lohrer et al., 2004; Ingels et al., 2014). Both practices are usually unfeasible in deep-sea regions, either due to the vulnerability status of the few undisturbed regions and/or expensive and logistically difficult monitoring experimental programmes (e.g. high costs and availability of ship time, operability in rough weather conditions). On the other hand, comparative studies, which typically are the approach followed in deep-sea studies, compare undisturbed (control) and disturbed regions (e.g. covering a gradient of fishing intensity), with similar habitat characteristics. This approach is constrained by the still largely incomplete knowledge on deep-sea biodiversity in most areas of the globe, including those that are frequently disturbed and by the difficulty in finding suitable reference sites with environmental conditions (e.g. seabed composition, depth) similar to the disturbed sites (Clark and Rowden, 2009; Clark et al., 2015). This implies that we often lack the background knowledge on the composition of deep-sea assemblages prior to disturbance hindering the full interpretation of comparative results.

Nonetheless, the few deep-sea studies investigating persistent bottom-trawling pressure in benthic habitats, showed long-term alterations of the community composition, large losses in epifaunal diversity and biomass, and significant deleterious effects on important long-lived, habitat-forming organisms (i.e. sponges and corals) and their associated fauna (Koslow et al., 2001; Cryer et al., 2002; Hall-Spencer et al., 2002; Fosså et al., 2002; Clark and O'Driscoll, 2003; Gage et al., 2005; Althaus al., 2009; Clark and Rowden, 2009). Most of these studies were carried out in hard substrate habitats known to be vulnerable, such as cold-water coral areas and seamounts (Clark et al., 2015 and references therein). In soft-sediment habitats, some of the most noticeable effects were reported by Puig et al., (2012) who showed large-scale changes of the seabed topography and sediment dynamics of a submarine canyon subjected to long-term chronic trawling disturbance (e.g. Puig et al., 2012; Martín et al., 2014a). Altered surface and sub-surface sediment properties, namely organic matter concentrations, grain size composition and porosity (Martín et al., 2014b; Wilson et al., 2015; Oberle et al., 2016), as well as increased pollutants' availability was also observed by Oberle et al. (2016). Information on the effects of trawling on soft sediment faunal assemblages are barely known and not consistent. Because many organisms create in large part the structure (e.g. burrows, mounds) of soft-sediment habitats, deleterious effects on fauna are likely to strongly degrade the complexity and integrity of the seabed (Gage et al., 2005). Studies focused on sedimentary slopes and canyons seem to point out for faster recovery capacity of fauna inhabiting these regions when compared to seamounts and cold-water corals regions (Yesson et al., 2016; Almeida et al. 2017). Nevertheless, these assemblages are still subjected to strong alterations, particularly depletion of biomass, changes in community structure and loss of rare species and sensitive faunal traits (e.g. filter feeding organisms, such as sponges), particularly from mega-epifauna, but sometimes also from infaunal assemblages (e.g. Cryer et al., 2002; Atkinson et al., 2011; Buhl-Mortensen et al., 2015; Clark et al., 2015; Murillo et al., 2016; Yesson et al., 2016; de Leo et al., 2016; Almeida et al., 2017). It is important to mention that most of these studies have caveats related to the lack of reference information prior to exploitation or the synergistic effects of strong environmental gradients (e.g. trawling effects vs. oxygen minimum zone, de Leo et al., 2017).

The changes in structure and composition of the assemblages, including the loss of rare taxa or certain faunal traits, observed in sedimentary slopes and submarine canyons associated with trawling disturbance (e.g. Cryer et al., 2002; Gage et al., 2005; Atkinson et al., 2011; Buhl-Mortensen et al., 2015; Murillo et al., 2016; Yesson et al., 2016) may have profound effects on the food web and numerous processes supported by both infauna and epifauna, including nutrient fluxes, bentho-pelagic coupling and trophic interactions (Gage et al., 2005; Clark et al., 2015). In fact, few studies, and much less in deep-sea regions (Leduc et al., 2016), have evaluated how the fisheries' pressure translates into changes in ecosystem functioning (Duplisea et al., 2001; Lohrer et al., 2004; Tillin et al. 2006; Oslgard et al., 2008; Sciberras et al., 2016), herein considered as the "*processes that transform or translocate energy or materials in the ecosystem*" (in the sense of Solan et al., 2012; Strong et al., 2015), and in ecosystem services, herein considered as "*the direct and indirect contributions that ecosystems make to human wellbeing*" (in the sense of de Groot et al., 2010; Böhnke-Henrichs et al. 2013).

1.1.6 Relevance of disturbance to biodiversity and ecosystem functioning

Biological diversity or biodiversity is "*an aggregation of highly inter-connected ecosystem components, encompassing all levels of biological organization from genes, species, populations, communities to ecosystems, with the diversity of each level having* *structural and functional attributes, which can be assessed at various temporal or spatial scales*" (Cochrane et al., 2016). It is generally accepted that high biodiversity, may act as a buffer against environmental fluctuations and temporal variability, but also likely to punctual disturbance events from anthropogenic sources (Yachi and Loreau, 1999; Loreau, 2000; Cochrane et al., 2016). A higher number of species is likely to enhance efficiency in the use of resources and insure stability to ecosystem functions in variable or disturbed environments, while species-poor assemblages are likely less capable to resist and to recover from change (resistance and resilience, respectively; Strong et al., 2015 and references therein).

Long-term effects of biodiversity that contribute to maintain or enhance ecosystem functioning in the face of environmental fluctuations can be considered as "insurance effects" (Yachi and Loreau 1999). In general terms, species richness, through compensatory dynamics of individual species with similar functional roles, ensures the ecosystems against declines in their functions ("the Insurance Hypothesis") and it is a critical feature to the reliability of ecosystems functioning and their long-term capacity to provide goods and services (Naeem and Li, 1997; Naeem, 1998). In high diversity ecosystems, functional traits are likely safeguarded by several species (functional redundancy: number of species within each functional entity), and in such cases the exclusion of redundant species could have little immediate consequence to the functional performance of a disturbed system (Tillin et al., 2006; Loreau, 2008, Tyler-Walters et al., 2009). Yet, long-term loss of species will lead to decreased functional redundancy (e.g. trophic redundancy) and ultimately to the decrease of various ecosystem functions, inclusive within the complexity of food webs (total number of functional/trophic entities and their interactions) (Loreau and Hector, 2001; Hooper et al., 2005).

Noteworthy is that high diversity or functional redundancy, per se, will not ensure the resilience of ecosystems to disturbance (e.g. capacity to recover from the depletion of standing stocks, loss of species or decreased food-web complexity which may be caused by trawling). Ecosystem resilience will vary with the environmental context (e.g., climate, resource availability, and natural disturbance (Ives and Carpenter, 2007) and human pressures that may act cumulatively or synergistically with the natural drivers (Hooper et al., 2005). Ultimately, the replacement of local extinctions in disturbed systems will also depend on the probability of recolonization from adjacent habitats and/or from a regional pool of species (Naeem and Li, 1997).

The increased pressure from a wide range of anthropogenic activities, including fisheries, raises serious concerns regarding the future maintenance of essential supporting functions and provisional, and regulatory ecosystem services (Worm et al., 2006; Danovaro et al., 2008), that may be impaired as the result of alterations and likely loss of biodiversity in the affected areas, including in the deep-sea (Glover and Smith 2003; Loreau, 2008). Thus, the understanding of how biodiversity and ecosystem functioning are related may help predict the effects of future changes (Strong et al., 2015). Biodiversity and ecosystem functioning relationship (BEF) is overall not well understood, but general three main types of BEF relationships have been encountered in the deep-sea: positive linear (Pape et al., 2013, Baldrighi et al., 2017) positive exponential (Danovaro et al., 2008; Narayanaswamy et al., 2013; Baldrighi et al., 2017), and non-existent (Leduc et al., 2013). Positive linear BEF model suggests a proportional increment of functions with addition of species, where each species has a unique role in the ecosystem functions (Cardinale et al., 2011). Positive exponential BEF relationship implies that even minor losses of diversity will result in a marked decline of functions provided, in which case rare species are functionally unique and mutualistic interactions (individual species perform better in mixed communities through facilitation mechanisms – complementarity effects) prevail over competition (selection effects) (Loreau and Hector, 2001; Naeem and Wright, 2003; Loreau et al., 2008). There are several inter-connected ways in which deep-sea high taxonomic biodiversity can influence the ecosystem functioning (Fig. 1.6; Solan et al., 2012; Strong et al., 2015). For example, the larger species (e.g. macrofauna) are known to promote particle sediment mixing (bioturbation) and solute transferring (bio-irrigation) to deeper layers in the sediment providing oxygen and food to organisms in the anoxic layers (Braeckman et al., 2011). The selective removal of a sizeable amount of such species will consequently translate into a decrease in benthic nutrient cycling fluxes (Lorher et al. 2004) and redistribution of food within the sediment. Furthermore, high biodiversity levels can also promote higher rates of detritus processing, digestion and reworking, thus resulting in faster rates of organic matter re-mineralisation, while loss of diversity within the highest trophic groups, such as predator species might have more severe effects on the stability of food webs through top-down control, and thus could lead to changes in secondary production at the intermediate and lower levels of the food chain, thereby modifying carbon cycling (e.g. biomass; Spiers et al., 2016).

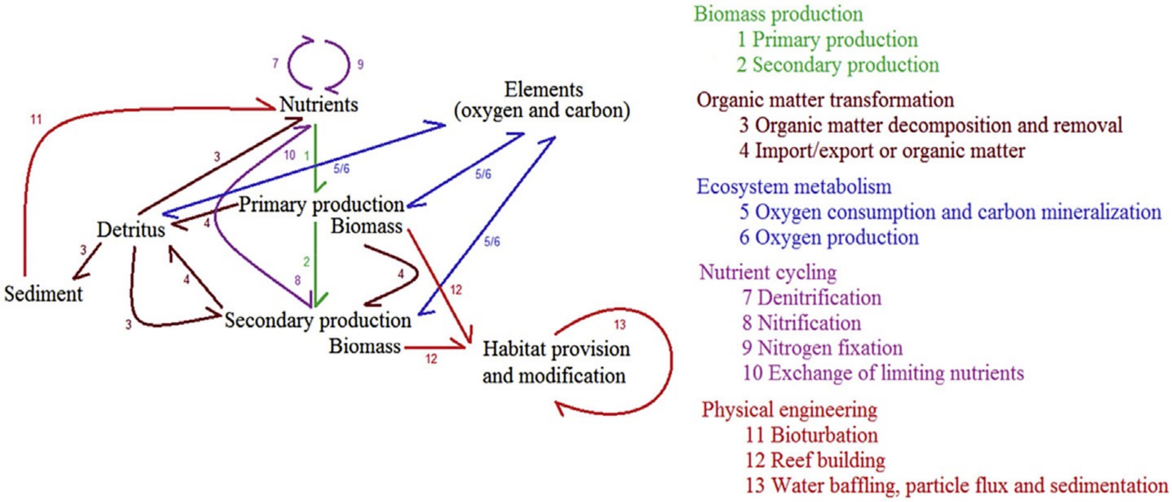


Figure 1.6 Relationships between essential provisioning, supporting and regulatory ecosystem functions. From Strong et al., (2015).

Nonetheless, the absence of diversity-functioning relationship in highly diverse communities found by Leduc et al. (2013) might indicate that effects of biodiversity loss on ecosystem functioning may also be unpredictable (idiosyncratic model) or even non-existent (null model), due to high niche overlap, strong control by environmental factors or extremely variable biotic/abiotic interactions (Cardinale et al., 2011). Hence, alterations of benthic assemblages and loss of biodiversity in the deep sea associated with trawling disturbance may not always necessarily represent a proportionate loss of ecosystem functions. These differences in BEF relationships found may be related to the different spatial scales, taxonomical level (genus vs. species) and group, as well the level of biodiversity (Leduc et al., 2013) considered in these studies. Assessment of BEF relationships but also functional redundancy in deep-sea regions may help predict the ecosystem's efficiency and stability (resistance and resilience; Strong et al., 2015) under (anthropogenic) disturbance conditions within the study region.

1.2 The study region: The West Iberian Margin

1.2.1 General environmental characterisation

The West Iberian Margin (WIM) is characterised by a relatively narrow shelf with a steep and irregular continental slope, incised by several large submarine canyons and deep gullies (Pinheiro et al., 1996). Seabed sediment composition across the continental shelf is diverse, but predominantly composed of by biogenic carbonate sandy bottoms of different grain sizes, although in some areas at the mid-shelf, particularly north of Cabo Raso, large extensions of gravel-dominated sediments may occur (Dias and Nittrouer, 1984, 1987). A significant decrease in grain size occurs towards the outer shelf, from medium to very fine sands (Dias and Nittrouer, 1984; Martíns et al., 2012), and at the upper slope the sediments become predominantly finer, transitioning from fine-sand to muddy-sand and mud, with high content of silt and clay (>10%) and a large contribution of pelagic and hemipelagic organic matter fractions (Martíns et al., 2012). The presence of rocky outcrops is observed along the whole margin.

The diverse geomorphological and sedimentary features of the WIM interact with several oceanographic processes (e.g. current systems and water masses), which determine in large part the spatial (both vertical and horizontal) and temporal variability in the environmental conditions of the water column and seabed, namely in terms of salinity, temperature and oxygen content (Fiúza 1983; Relvas et al. 2007; Kämpf and Chapman, 2016). The West Iberian Margin (WIM) is under the influence of the northern component of the Iberian Upwelling System (Kämpf and Chapman, 2016). By their relevant contribution to total standing stock and primary production, upwelling events have a significant impact on both pelagic and benthic food webs at the WIM (Santos, 2001)

The Iberian Upwelling current system results from the complex and seasonally variable wind-driven conditions, current systems, fronts and the underlying water masses (Kämpf and Chapman, 2016 and references therein). The properties of distinct water masses mainly influence the upper and middle continental slope regions, while they are of less importance to the lower slope and abyssal plain (Kämpf and Chapman, 2016). In detail, during upwelling events the surface waters (ca. the first 100m) are primarily determined by the Eastern North Atlantic Central Water mass conditions (ENACW; surface down to 500-600m). During the spring and summer, the upwelling favourable conditions are driven by intense northerly winds and the surface cold and nutrient rich water currents that direct towards the equator, i.e. offshore Portugal current and the Portugal coastal current (Fig. 1.7A), while Portugal coastal undercurrent influences the slope northwards (Fiúza 1983; Relvas et al., 2007; Kämpf and Chapman, 2016). By contrast, during autumn and winter downwelling-favouring processes occur with changes in the predominant southward winds, that promote a reversal of the surface circulation poleward, i.e. the Iberian Polar current (Fig. 1.7B; Peliz et al., 2005; Relvas et al., 2007; Kämpf and Chapman, 2016). Although, during winter prevailing downwelling conditions typically reduce surface productivity, episodic upwelling events may induce productivity pulses throughout the year (Relvas et al., 2007).

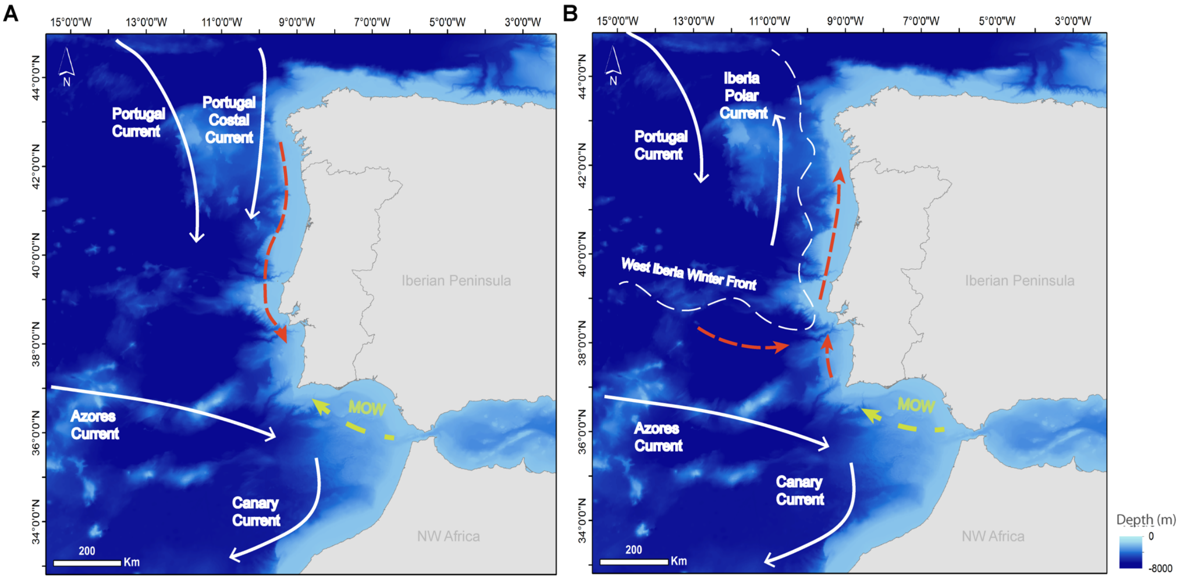


Figure 1.7 Schematic overview of the main surface circulation features (arrows) of the West Iberian Margin during A) Spring - Summer and B) Autumn - Winter. MOW: Mediterranean outflow water. Adapted from Kämpf and Chapman (2016).

Total annual surface productivity estimates for the WIM vary from ca. 360 gC.m^{2}.y^{-1} for the shelf, ca. 270 gC.m^{2}.y^{-1} for the continental slope between depths of 200 to 2000 m and ca. 230 gC.m^{2}.y^{-1} in the open basin bellow 2000 m (Epping et al., 2002). Yet the majority of the yearly productivity is formed during the coastal spring-summer upwelling peaks (Fiúzia, 1983). During these periods, large filaments of phytoplankton blooms are transported several kilometres offshore, often 30–40 km, but can reach as far as 200-300 km transported along shelf areas through complex circulation patterns (Salgueiro et al., 2010; Relvas et al., 2007).

The seasonally varied surface productivity regimes (upwelling/downwelling), are in large part responsible for both a spatial (horizontal and vertical) and temporal patchiness of particulate organic matter (POC) flux from the surface water to the seabed in this region

(Fiúza, 1983; Relvas et al., 2007). They also represent a significant contribution of the primary productivity that supports the benthic and pelagic food webs and the productive fisheries, which characterise the WIM (Santos, 2001; Picado et al., 2014: Kämpf and Chapman, 2016). The provision of organic rich detritus material associated with the high productivity of the WIM (e.g. mostly phytoplankton but also from terrestrial derived materials) into deeper areas, is prompted by various circulation processes and hydrodynamic conditions, either with a periodic (near bottom currents, internal waves and tides) or episodic character (e.g. strong winter storms) (Relvas et al., 2007; Llave et al., 2015).

1.2.2 Importance of deep-sea fisheries at the WIM

The dynamic conditions of the WIM are also under the influence of both physical and chemical disturbance from anthropogenic sources, which are likely influencing the deep seafloor and its associated fauna (e.g. fisheries, litter, pollution; e.g. Morais et al., 2007; Mordecai et al., 2011; de Jesus Mendes et al., 2011). For example, high levels of heavy metals and organic contaminants, as well high density of litter concentrations have been recurrently found in areas close to shore or in submarine canyons connected to a river system, such as the Lisbon and Setubal canyons, in the vicinity of a high population density coastal areas (Mordecai et al., 2011; Neves et al., 2015). The most common litter items observed or collected at the WIM are lost fishing gears, such as lines, nets and cages which is not surprising given the importance of fisheries in the region (Neves et al., 2015; Oliveira et al., 2015; Vieira et al., 2015).

Fishing activities in Portugal are of great importance in the social-economical but also to the cultural context (Hill and Coelho, 2001; Leitão et al., 2014). Even with a reduction of the fleet, number of fishermen and total annual catches since in the 1980's, Portugal is still the European country with the highest fish consumption (ca. 57 kg per capita in 2014; European Commission, 2017), more than twice the European average (25.5 kg per capita; European Commission, 2017). Presently, Portuguese fisheries are comprised of a diversity of *métiers* targeting a large variety of species (including crustaceans, cephalopods and fishes), using numerous gear types and fishing techniques. The activity is mostly artisanal and family-based. Among the various *métiers*, crustacean bottom-trawling fisheries typically represent a very small percentage of the total annual landings with no more than 5% of the total catches. However the high profit associated with the landing of several species of deep-water crustacean targeted by this practice, results in a total contribution of more than 30% of total annual sales values (Campos et al., 2007; Silva et al., 2015). The designation of crustacean bottom trawling, is usually associated with the *métier* that uses an otter trawl and targets several species of deep-water crustacean, such as the Norway lobster (*Nephrops norvegicus*), red and rose shrimps (*Aristeus antennatus* and *Parapenaeus longirostris*, respectively), but also a few demersal fish species such as blue whiting (*Micromesistius poutassou*) and the European hake (*Merluccius merluccius*) (Campos et al., 2007).

Otter trawling is a fishing technique in which a net towed along the seabed behind the fishing vessel, held by two main otter boards or doors and fixed between the wraps and bridles that allow maintaining the wings of the net open at varying distance depending on characteristics of the vessel; a series of buoys attached to the headline maintain the net vertically open (Fig. 1.8) (Jennings et al., 2009). Otter trawls typically produce less by-catch than beam trawls and towed scallop dredges, disturbing an approximately sediment depth of 2.4 cm in average and remove about 6% of benthic fauna with a single tow, by comparison to beam trawl and towed scallop dredges with average sediment penetration of 2.7 and 5.5 cm and 14 and 20% fauna captured, respectively (Hiddink et al., 2017). Noteworthy is that penetration depth of otter trawl gears in deep-sea sediment, particularly when targeting prawns and flatfishes, are likely to be higher than the estimates of Hiddink et al. (2017). In such cases, trawlers are usually required to tow with heavier gears due to the greater depth (Clark et al., 2015), and may also present attached tickler chains to the otter boards in order to dig deeper inside the sediment (Jennings et al., 2009).

The crustacean otter trawling fleet in Portugal is presently comprised of approximately 24-26 relatively small licensed vessels from 20-29 m of overall length (INE, 2015; Silva et al., 2015), which have to comply with several legal restrictions imposed by the Portuguese government[1]. Legal restrictions include an exclusion zone within six nautical miles from the coastline[2] where trawling is banned, a compulsive closure period during the month of January with other possible additional regional restrictions that may be applied according to captures during the year[3], and a minimum gear mesh size (i.e. 55 - 59 mm when targeting crustaceans, or > 70 mm for all organisms) [1]. These measures

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[1] Diário da Répulica, Portaria n.º 1102-E/2000 de 22 de Novembro, Ministério da Agricultura, do Desenvolvimento Rural e das Pescas, Série I-B - nº270 de 20 de Novembro de 2000

[2] Diário da Répulica, Decreto regulamentar nº 43/87 de 17 de Julho, Ministério da Agricultura, Pescas e Alimentação, 1ª Série - nº162 de 17 de Julho de 1987

[3] Diário da Répulica, Portaria n.º 43/2006, de 12 de janeiro, Ministério da Agricultura, do Desenvolvimento Rural e das Pescas, Série I-B-319, 12 de janeiro de 2006

aim to reduce the capture of small size individual and by-catch. Moreover, the Portuguese government has issued a total ban for bottom-trawling activities in the high-sea areas comprising the Azorean EEZ and the claimed extended continental shelf beyond the 200 nautical miles[4], yet these interdictions do not include most of the targeted fishing grounds in the mainland, including the continental slope and submarine canyon areas.

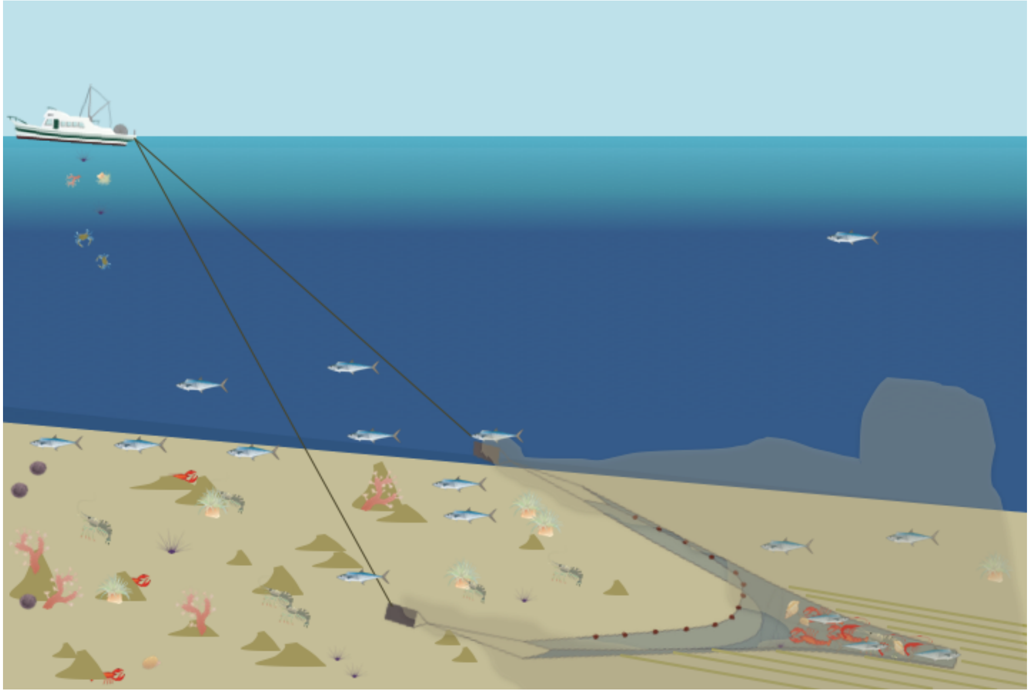

Figure 1.8 Schematic of otter trawler fishing in the SW Portuguese Margin, with associated suspended sediment plumes, alteration of seabed surface topography, and removal of large size fauna.

Despite of the imposed regulations, the low selectivity and typically high by-catch rates (60% of the total catches; Costa and Erzini 2008) of bottom-trawling fisheries has relevant consequences to the depletion of not only commercially important stocks, but also of many non-target species. Another issue of concern to the scientific community is the discard, often onsite, of by-catches and fish below legal minimum landing size; discard ratios vary considerably, with conservative estimates indicating an average of 40%

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[4] Diário da Répulica, Portaria n.º 114/2014, de 28 de Maio, Ministério da Agricultura e do Mar 1ª Série - nº102, 28 de Maio de 2014

discarding, but values of up to 70% of the total biomass reported for crustacean trawlers (Borges et al., 2001; Monteiro et al., 2001; Costa et al., 2008)

Moreover, none of the different fishing activities currently in practice along the Iberian margin affect as strongly the deep-sea seabed habitats, as the bottom-trawling fisheries. Specifically, estimates of seabed integrity indices, although hindered by the limited information on the biology of deep-sea species, are among the lowest in European waters (Eigaard et al., 2016). Seabed integrity indices evaluate the trawling intensity taking into account of the sensitivity of the benthic component to trawling pressure (i.e. taxa biomass proportion within longevity classes). Eigaard et al. (2016) also estimated the average bottom-trawling fisheries footprint per unit of landing (for all types of bottom-contact gears) in approximately 17 $km^{-2}t^{-1}$ in shallow areas down to 200 m, and 12.6 $km^{-2}t^{-1}$ in areas deeper than 200 m in the Iberian margin. Based on Vessel Monitoring System (VMS) satellite data, 90% of the total managed seabed areas in the region is trawled at least once a year. These estimates correspond to 5-15 times the trawling footprint of most of the European regions for the period of 2010 to 2012, and only equivalent to estimates for the Aegean Sea (Eigaard et al., 2016).

Bueno-Pardo et al. (2017) analysed VMS data compiled from Direção-Geral de Recursos Naturais, Segurança e Serviços Marítimos - DGRM (MAMAOT, 2012) for the period of 2012-2014. The authors estimated that total trawling pressure by crustacean bottom trawlers alone in the mainland ranged from 60988 to 69596 $h.y^{-1}$ accounting for more than 100 fishing trips per trawler in a fleet of 24-26 licensed vessels. The same study highlighted the locations of the main fishing grounds, typically found in the outer shelf and upper continental slope and at the flanks of submarine canyon areas in the south and southwest regions (Fig. 1.9 ; Bueno-Pardo et al., 2017). With particular interest to the present research, is the trawling pressure in the SW region, that encompassed depths from 100 to 600 meters (98% of the total trawl pressure; Fig. 1.9), and where the distribution of the effort showed an increase (e.g. more area affected in 2014 than in 2013) despite the relative constancy of the total trawling effort at national level (Bueno-Pardo et al., 2017; Fig. 1.9).

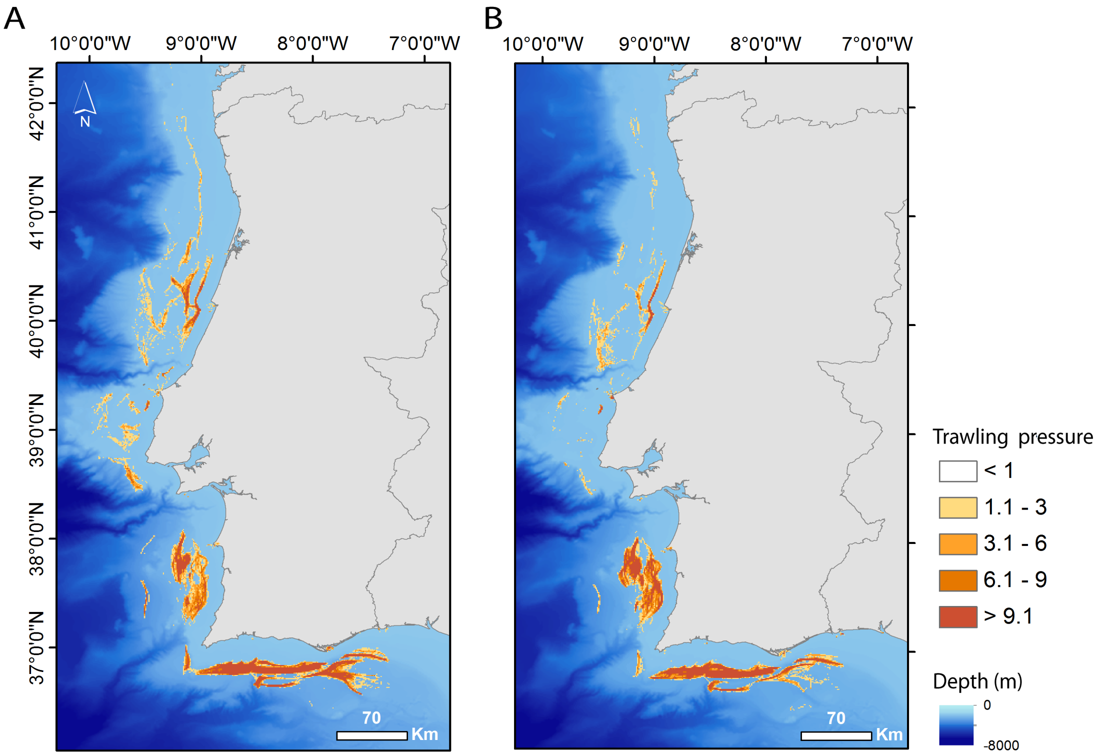

Figure 1.9 Distribution of the annual trawling pressure (h.cell^{-1}.y^{-1}) by the crustacean bottom trawlers in A) 2013 (66766 h.cell^{-1}.y^{-1}) and B) 2014 (63427 h.cell^{-1}.y^{-1}) along the West Iberian Margin. Modified from Bueno-Pardo et al. (2017).

In accordance with several other studies, Bueno-Pardo et al., (2017) identified the habitats targeted by crustacean bottom trawlers as mostly soft sediments with high percentages of silt and clay (>10%), further designated here as deep-sea mud and muddy-sand habitats following the EUNIS habitat classification (Fig 1.10; Davies et al., 2004). This habitat type is mainly overlapping the distribution of several target species. Many burrowing deep-water crustaceans species such as the Norway lobster, are unable to construct and maintain burrows and tunnels systems in unstable sediments such as sandy sediments (Afonso-Dias, 1998). Furthermore, the bathymetric distributions of most of the targeted deep-water crustaceans overlap at depths around 300-500m: the rose shrimp (*Parapenaeus longirostris*) is preferably found at depths between 200-400 m, the red shrimp (*Aristeus antennatus*) in areas between 300-600m and the Norway lobster (*Nephrops norvegicus*) at depths 200-700, with the highest concentrations usually bellow 500 m depth (de Figueiredo & Viriato, 1992; Monteiro et al., 2001; Silva et al., 2015).

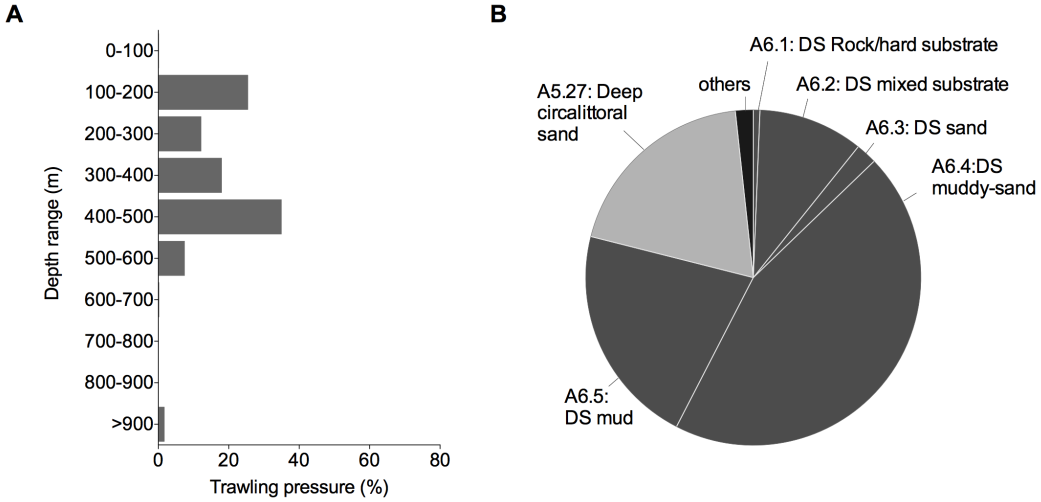

Figure 1.10 Trawling pressure distributions along the depth profile (A) and seabed habitats (B) at the SW Portuguese region between 2012 and 2014. Habitats represented in light colours indicate circalittoral habitats, dark grey deep-sea habitats and in black habitat that are undetermined, following the EUNIS habitat classification (Davies et al., 2004). From Bueno-Pardo et al. (2017).

1.2.3 Bottom-trawling disturbance and benthic biodiversity in Iberian waters

In the context of the European Union's Marine Strategy Framework Directive 2008/56/EC (MSFD; European Commission, 2008), which established a guideline for the state members of the EU aiming to achieve or maintain a Good Environmental Status (GES) in marine environments, the initial assessment made by the Portuguese government - DGRM (MAMAOT, 2012) highlights trawling fisheries as one of the most pervasive activities along the Portuguese margin. Several descriptors of GES are of particular relevance for bottom-trawling fisheries: biodiversity is maintained (descriptor 1); population of commercial fish species is healthy (descriptor 3); elements of food webs ensure long-term abundance and reproduction (descriptor 4) and the seafloor integrity ensures functioning of the ecosystem (descriptor 6) (European Commission, 2008). However, the existing assessments of these descriptors have a low degree of confidence because of the insufficiency of available data to determine the condition of the benthic assemblages in the extensive areas affected by trawling fisheries and by the low availability of adequate no-trawling control areas (MAMAOT, 2012).

In fact, to the present only few studies have dealt with the impacts of physical disturbance on benthic assemblages either by dredging, beam or otter trawling in the

WIM, and most were conducted at depths shallower than 100 m (Chicharo et al. 2002, Gaspar et al. 2003; Falcão et al. 2003), despite the clear importance and unsustainable nature of bottom-trawling fisheries in the deep-sea areas. To my best knowledge, only two published studies have explored a link between deep-water trawling fisheries and the biodiversity and structure of benthic assemblages in the Southern Portuguese region and Portimão submarine canyon, off Portugal. Moreover, both studies focused on large mega-epibenthos assemblages visible in photographs/video captured by Autonomous (AUV) or Remotely operated vehicles (AUV, ROV), in Morais et al. (2007) and Fonseca et al. (2014), respectively. Extensive areas severely impacted by trawling, where seabed morphology was altered by frequent trawl scars were observed in both studies. Also, the authors report a depletion of faunal abundances and diversity in highly disturbed sites, although the link between these alterations and trawling was compromised by the differences in habitat attributes at the different locations investigated. For example, Fonseca, et al. (2014) reported the occurrence of an extensive bed of the crinoid *Leptometra celtica* in an enclave of gravelly sand, while the surrounded muddy sediments, where trawlers usually fish for the Norway lobsters, showed generally lower species richness and a depletion of sensitive groups, such as sponges and crinoids. Similarly, Morais et al., (2007), detected differences in species composition between different habitats associated with distinct trawling disturbance regimes, but trawlers were particularly concentrated in mud and muddy sand habitats.

The Iberian margin may harbor biodiversity hotspots that we might not yet be aware of, and hence, it is crucial to increase the research effort on the ecosystem of this region, and establish reliable baseline knowledge for the prediction and mitigation of expected impacts of trawling practices. We may expect that in a near future currently undisturbed areas might be targeted, considering the global shift of deep-water fisheries towards deeper areas (Watson and Morato, 2013). Moreover, such information may urge the current management actions and future decision-making for a sustainable exploitation of deep-sea fishery resources, while maintaining a good environmental status in the mainland of the Portuguese margin.

1.3 Main objectives and thesis outline

The main objective of this PhD thesis is to gain insight on how chronic bottom-trawling fisheries induced pressure is altering deep-sea benthic assemblages, integrating the different faunal size components (meio-, macro and megafauna) and how this is translated into the maintenance of essential ecosystem functions (e.g. organic matter transformation, nutrient cycling, secondary production, ecosystem metabolism), with a focus on the continental slope areas of the SW Portuguese margin. The primary hypothesis raised by this thesis, is that the long history of bottom-trawling disturbance along the upper continental slope induced significant alterations of the benthic communities' composition and diversity. Secondly, the alterations of benthic assemblages, particularly within infauna (meio- and macrofauna) will be reflected in the depletion of important ecosystem functions (i.e. inefficiency in carbon mineralization, reduced sediment-water nutrient fluxes).

The thesis is structured in three main sections: a general Introduction where a general background is given on the anthropogenic disturbances in the deep sea, particularly focused on deep-sea bottom-trawling fisheries and their known impacts, together with a characterization of the study region (**Chapter 1**), followed by the main results sections (Chapters 2, 3 and 4) and a general discussion (Chapter 5). In specific:

Chapter 2, describes the comparison of mega-epibenthic faunal abundance, composition and diversity along soft-sediment areas subjected to distinct trawling disturbance regimes (no, low, and high trawling pressure) using Remotely Operated Vehicle (ROV) video imagery. In addition to the identification and quantification of the mega-epibenthic assemblages, the video recordings were used to characterise the seabed integrity, including seafloor structure, evidence of faunal activity, and by the presence and conditions of trawl scars. The results were complemented with satellite data information on trawling pressure established from Vessel monitoring systems (VMS) data, and temporal information on surface productivity aiming to relate with the observed changes of the faunal patterns across the different trawl pressure areas. This work was accepted for publication in the research topic "Anthropogenic disturbances in the Deep Sea" in the journal Frontiers in Marine Science as "*Ramalho SP, Lins L, Bueno-Pardo J, Cordova EA, Amisi JM, Lampadariou N, Vanreusel A, Cunha MR (2017) Deep-sea mega-epibenthic assemblages from the SW Portuguese margin (NE Atlantic) subjected to bottom-trawling fisheries*. Front Mar Sci. doi: 10.3389/fmars.2017.00350"

Analogously, in **Chapter 3** these same areas subjected to distinct trawling disturbance regimes (no, low, and high trawling pressure) were investigated but in terms on the macro-infauna standing stocks (abundance and biomass), community structure and taxonomical and trophic diversity. Moreover, a further detail was given on the core assemblages, which included a subset of the whole assemblage composed by the most abundant, frequent and distinctive taxa in each trawl pressure group, so that functional (trophic) complexity but also redundancy was explored in detail. This work was submitted for publication to PLOS One and is currently under review as "*Ramalho SP, Almeida M, Esquete P, Génio L, Ravara A, Rodrigues CF, Lampadariou N, Vanreusel A, Cunha MR. Bottom-trawling fisheries influence on standing stocks, composition, diversity and trophic redundancy of macrofauna assemblages from the West Iberian Margin*".

In **Chapter 4,** the field observations were combined with an isotope pulse-chase enrichment experiment on sediments obtained from two stations of interest under (low and high trawling pressure), which were compared then in terms of meio- and macrofauna (infauna) biodiversity (both taxonomic and trophic), and several ecosystem function proxies, which included: bacterial production, infauna respiration rates and evaluation of biogeochemical function and bioturbation through ^{13}C uptake and pore-water nutrients concentrations along the sediment profiles. The pulse-chase experimental results were then complemented with the full biological dataset available within this thesis, and additional information on meiofaunal diversity collected within the framework of the present project, to investigate the relations between structural and functional diversity and ecosystem functioning (i.e. ecosystem metabolism, inferred from biomass, respiration rates and total respiration). This chapter is under preparation for submission to the journal Deep-Sea Research part I as "Ramalho, SP, Lins L, Soetaert K, Lampadariou N, Cunha MR, Vanreusel A, Pape E, *Altered ecosystem functions under condition of bottom-trawling disturbance: experimental approach and field observations*".

Finally, **Chapter 5** provides an integration of the general observations of this thesis and briefly provides some general guidelines for future monitoring and research at the study region.

Noteworthy is that because the main result Chapters (2, 3 and 4) of this thesis are presented as research articles, either accepted, submitted or in preparation, there is inevitably some degree of overlap among these chapters, particularly within the introduction and methods section.

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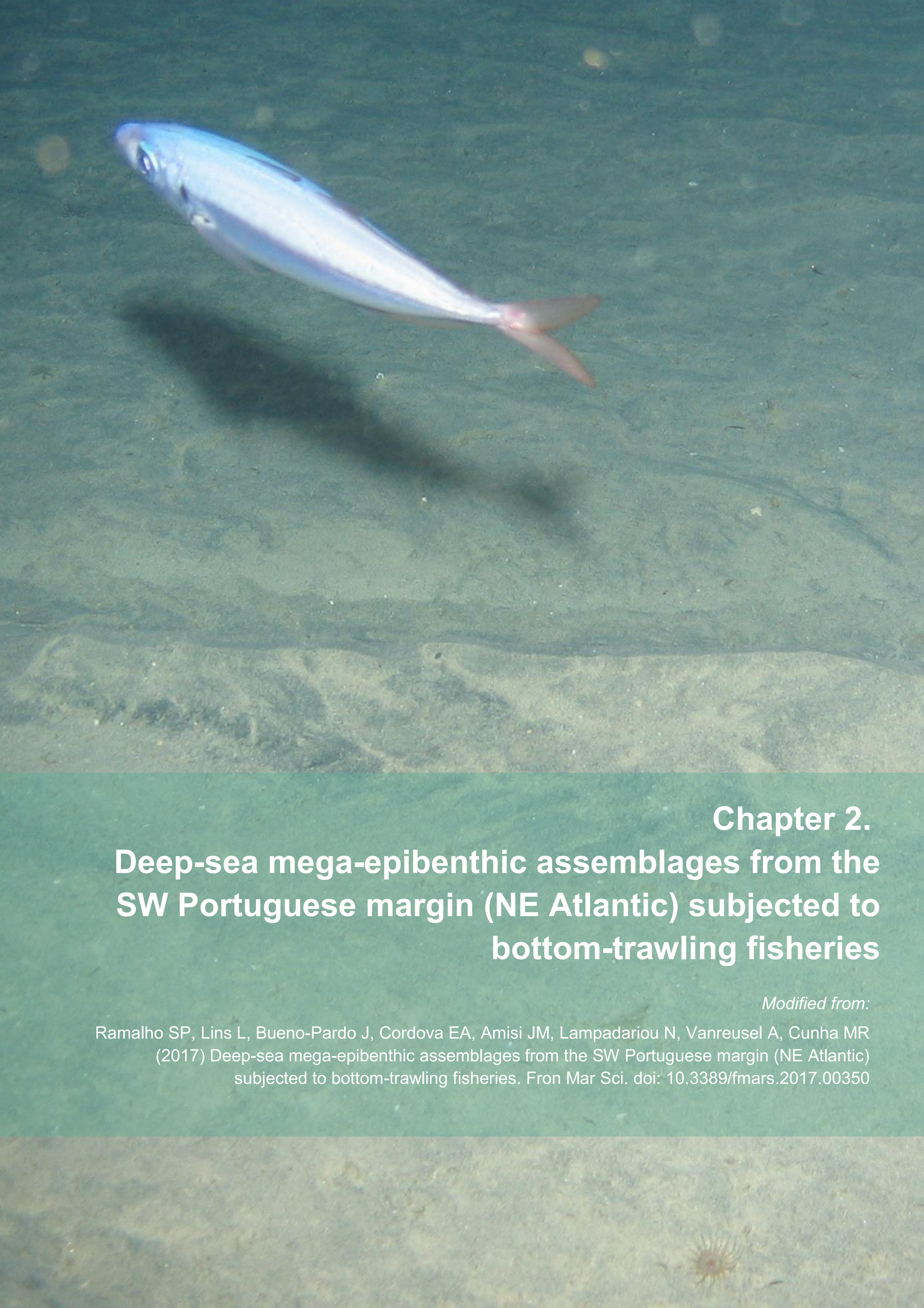

Chapter 2.

Deep-sea mega-epibenthic assemblages from the SW Portuguese margin (NE Atlantic) subjected to bottom-trawling fisheries

Modified from:

Ramalho SP, Lins L, Bueno-Pardo J, Cordova EA, Amisi JM, Lampadariou N, Vanreusel A, Cunha MR (2017) Deep-sea mega-epibenthic assemblages from the SW Portuguese margin (NE Atlantic) subjected to bottom-trawling fisheries. Fron Mar Sci. doi: 10.3389/fmars.2017.00350

Abstract

Bottom-trawling fisheries are a common threat to the health of continental margins worldwide. Together with numerous environmental and biological processes, physical disturbance induced by trawlers can largely shape the benthic habitats and their associated assemblages. At the SW Portuguese Margin, crustacean bottom trawlers have exploited deep-sea habitats for a few decades, but its effects on the benthic biodiversity are practically unknown. During the spring-summer of 2013 and 2014, several Remotely Operated Vehicle (ROV) video transects were used to investigate mega-epibenthic abundance, composition and diversity in soft-sediment areas subjected to varying trawling pressures off Sines and Setúbal (200-800 m). Differences in mega-epibenthic assemblages were linked with environmental changes (depth, grain size, primary productivity) and trawling disturbance. The effect of trawling was assessed between segments with similar habitat characteristics, i.e. muddy-sand bottoms between 300–500 m. Areas subjected to intensive trawling pressure showed a generally flattened seabed, with abundant recent trawl marks (up to 3 scars.100 m^{-1}), indicating that the seabed physical integrity was compromised. Significant negative correlations were detected between various mega-epibenthic diversity indices (S, H' and $ET_{(20)}$) and trawling pressure ($h.cell^{-1}.y^{-1}$). Furthermore, the distinct mega-epibenthic assemblages and absence of several sessile erect morphospecies at both low and highly disturbed locations by trawling off Sines, namely all seapen morphospecies found in non-trawled areas, demonstrates the negative influence of trawling fisheries on the benthic component of the study area. Also, low dissimilarity between assemblages from the main fishing grounds and the adjacent low-disturbance locations, suggests that the potentially negative influence of trawling can extend beyond the targeted areas (e.g. by the plumes of re-suspended sediments). The observed deleterious effects of trawling on mega-epibenthic fauna together with the intensification of trawling pressure in the study area, stress the need for adequate monitoring programs and regulatory measures to halt the long-term loss of biodiversity and allow the sustainability of fisheries at the SW Portuguese Margin.

2.1 Introduction

Continental margins are considered productive and diverse regions in the deep sea (Levin and Dayton, 2009). They encompass several unique habitats, such as submarine canyons, seamounts and even chemosynthesis-based habitats (e.g.

pockmarks and mud volcanoes). Hence, benthic faunal biodiversity at margins is complex as it is shaped by the interaction of numerous environmental and biological processes (e.g., substrate sorting, water-mass properties, productivity regimes, predation, competition), but also to some extent, by the increasing pressure from anthropogenic activities (e.g. fisheries, pollution, mineral and oil extraction; Levin and Dayton, 2009; Levin et al., 2001; Ramírez-Llodra et al., 2011).

Among the several anthropogenic activities occurring in deep waters worldwide, bottom-trawling fisheries are identified as one of the most destructive, affecting primarily the continental shelf and upper slope, seamounts and submarine canyons (Ramírez-Llodra et al., 2011). General impacts caused by trawling practices are relatively well established for the shelf areas, although the magnitude and duration of the effects largely depends on the characteristics of the targeted habitats, gears used, and trawl intensity and frequency (Jennings and Kaiser, 1998; NRC, 2002). Moreover, the low selectivity of trawling practices directly causes a high mortality of both target and non-target species and alterations or destruction of seabed habitats (NRC, 2002). Indirectly, trawling also promotes shifts in benthic community composition and diversity, while trophic webs may also be affected, namely by the increase in carrion available from both on-site mortality and discard practices (NRC, 2002). Also, effects of trawling are highly dependent on the faunal size-groups, as a greater vulnerability is linked with the large-sized fauna (macro and megafauna; Jennings et al., 2001; Duplisea et al., 2002). In this context, megafaunal organisms, defined by Grassle et al. (1975) as animals >1 cm either easily detected in photographs/videos or collected by trawl nets, are particularly sensitive to repeated trawling disturbance. Subsequently, changes in megafauna assemblages can result in depletion of several ecosystem functions, since megafauna is known to promote important benthic-pelagic coupling processes (Soltwedel et al., 2009), and function as “ecosystem engineers”. For example, mega-epibenthic organisms can promote habitat complexity and induce changes in the sediment biogeochemistry via bioturbation, but also by serving as biogenic habitats for smaller fauna (e.g. corals; Buhl-Mortensen et al., 2010).

It is postulated that trawling practices may have stronger effects on the deep-sea mega-epibenthic fauna in comparison with shallower areas (Clark et al., 2015). This arises from the typical characteristics of deep-sea species, particular life-history traits (k-selected; e.g., slow growth, high longevity), metabolic rates (low productivity) and reproductive strategies (e.g. intermittent spawning events), which make them more vulnerable and less resilient to the effects of trawling practices (Thrush and Dayton 2002). Heavier trawl gears and more localized practices can also exert a stronger pressure on deep-sea habitats (Clark et al., 2015). Yet, the effects on deep-sea benthic habitats and mega-epibenthic assemblages are still debated and geographically dependent, since in most cases we lack either background knowledge of the biodiversity on the long-term targeted areas, or an obligatory legislation that requires impact assessment and monitoring programmes at recent fishing grounds (Clark et al., 2015).

The most obvious effects identified so far include the large-scale changes of the seabed topography and sediment dynamics (e.g. fishing grounds at the upper flank of La Fonera canyon, Catalan margin; Puig et al., 2012). With each trawling haul, the seafloor is flattened and large amounts of sediment are re-suspended, often resulting in alterations of both surface and sub-surface sediment properties, namely organic matter concentrations, grain size composition and porosity (Martín et al., 2014; Oberle et al., 2016). These high turbidity periods often extend beyond the fishing grounds, indirectly impacting non-target areas by smothering filter-feeding organisms and increasing mortality rates of their faunal assemblages (Greathead et al., 2007; Leys, 2013; Clark et al., 2015). Effects on mega-epibenthic fauna include the decline of both standing stocks (abundance and biomass) and species richness, and changes in community composition (Clark et al., 2015 and references therein). In addition, the damage of long lived habitat-forming organisms (i.e. sponges and corals) in seamounts areas, have shown a very low recoverability and marked community shifts of their associated fauna (Koslow et al., 2001; Clark and Rowden, 2009; Williams et al., 2010, Yesson et al., 2016). In more extreme cases, alterations of the mega-epibenthic faunal distribution patterns at different spatial scales can also occur (Althaus et al., 2009).

It is important to stress that current knowledge pertains mostly to rather charismatic and vulnerable hard substrate habitats such as cold-water coral areas and seamounts (Clark et al., 2015 and references therein). Less focus has been directed to study the effects of bottom trawling on fauna inhabiting soft sediments from slopes and canyons along continental margins worldwide (e.g. Atkinson et al., 2011; Buhl-Mortensen et al., 2015; Murillo et al., 2016; Yesson et al., 2016). Yet, some indications arise regarding their potential for a faster recovery after disturbance (Yesson et al., 2016). Hence, it is crucial to increase our knowledge related to trawling effects at these areas that naturally contrast from hard-bottom areas, so we can adequately adjust the current management actions to allow for a sustainable exploitation of natural resources, and maintain a good environmental status.

The Iberian Margin has been identified as one of the most disturbed regions by bottom-trawling fisheries in Europe. This activity affects 40 to 90% (depending on the substrate type) of the areas beyond the six nautical miles limit down to ca. 1000 m water depths and is associated with a large footprint per unit of landing with ca. 13-17 km^{-2}t^{-1} depending on the depth range considered (Eigaard et al., 2016; Bueno-Pardo et al., 2017). Moreover, few studies have attempted to understand the trawling impacts on the benthic assemblages and are limited by the absence of adequate control areas (Morais et al., 2007; Fonseca et al., 2014). The present study aims to address this issue by investigating the upper slope mega-epibenthic assemblages in a southwest Iberian margin area subjected to long-term crustacean bottom trawling. Specifically, we hypothesised that i) the spatial and temporal environmental heterogeneity in the study region (i.e. water depth, sediment composition, annual productivity) will affect mega-epibenthic composition and community structure; ii) changes in the mega-epibenthic abundance, diversity, composition and community structure are altered by different degrees of bottom-trawling pressure (including no-, low-and high trawling pressure). These hypotheses will be tested using multivariate analyses.

2.2 Methodology

2.2.1 Study area

The West Iberian Margin (WIM) is characterised by a relatively narrow shelf with a steep and irregular continental slope, incised by several large submarine canyons. It is exposed to complex seasonal hydrodynamic processes, driven by wind forcing, local bathymetry and prominent topographic features, such as the Setúbal canyon (Fiúza, 1983; Relvas et al., 2007). During spring and summer, northerly winds induce relatively weak upwelling regimes, reaching a maximum off cape of Sines (SW Portugal). The inverse tends to occur during winter, with downwelling regimes and strong storm events, driven by south-westerly winds, although pulse episodes can occur at all seasons (Fiúza, 1983; Relvas et al., 2007). The high surface primary production generated during upwelling extends often for ca. 30–40 km offshore, but in some areas phytoplankton bloom filaments can reach as far as 200 km offshore. The relevant contribution of the surface productivity peaks to total standing stock and primary production have a significant impact on the food webs, supporting productive fisheries along the WIM (Picado et al., 2014).

Fishing activities along the WIM comprise various *métiers*, of which deep-water otter trawling, often designated as “crustacean bottom trawling”, as one of the most economically important, accounting for more than 30% of the total landing sale values (Campos et al., 2007). Crustacean trawling fisheries at the WIM are typically restricted to the South and Southwest regions off Portugal, where the most landed and valuable species include several deep-water crustaceans species, such as the Norway lobster (*Nephrops norvegicus*), red and rose shrimps (*Aristeus antennatus* and *Parapenaeus longirostris*, respectively), but also a few demersal fish species such as blue whiting (*Micromesistius poutassou*) and the European hake (*Merluccius merluccius*) (Campos et al., 2007; Bueno-Pardo et al., 2017). In 2014, the total declared landings of these species altogether for the SW Portuguese region were relatively low (ca. 50 t y^{-1}, and about 5% of the total landings). Yet, this region yielded approximately 30% of the total trawling effort in Portugal (Bueno-Pardo et al., 2017). While not all of these species show the same habitat preferences, their distribution often overlaps at the soft sediment areas (mud and muddy-sand) between 200–800 m water depths (Monteiro et al., 2001). Fishing grounds along the Portuguese margin are delimited by legal restrictions defined by the initial official regulation from July in 1987[5], which prohibits trawling within six nautical miles from the coastline.

Based on the vessel monitoring system (VMS) satellite data compiled by DGRM (MAMAOT, 2012), a region of interest in the SW Portuguese margin was delimited at approximately 37º40'–38º20'N; 08º50'–09º20'W, along the upper continental slope (200-800 m water depth) off Sines and in the vicinity of Setúbal canyon (Fig. 2.1 A, Table 1). Here, two main seabed types can be identified considering the habitats scheme of the European Union Nature Information System (EUNIS; Davies et al., 2004) and detailed sediment charts from Instituto Hidrográfico[6,7]: coarser sediments (A6.3: deep-sea sand) at shallower depths (ca. 200–300 m) until the self-break/upper slope transitions areas, while finer sediment types occur at deeper locations (>300 m; A6.4: deep-sea muddy-sand, with variable mud and carbonate contents). Owing to the occurrence of the Norway lobster habitat (fine sediments near the shelf break) and proximity to Sines harbour, this region is heavily targeted by crustacean trawlers. On the other hand, the 6 nm limit creates a

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[5] Diário da Républica, Decreto regulamentar nº 43/87 de 17 de Julho, Ministério da Agricultura, Pescas e Alimentação, 1ª Série - nº162 de 17 de Julho de 1987

[6] Instituto Hidrográfico. (2005a). "Carta dos Sedimentos Superficiais da Plataforma Continental Portuguesa"- Folha 5 - Escala 1:150 000

[7] Instituto Hidrográfico (2005b). Carta dos Sedimentos Superficiais da Plataforma Continental Portuguesa - Folha 6A - Escala 1:150 000

trawling-free area between cape Sines and cape Espichel (Setúbal area), allowing the comparison between heavily fished and non-fished areas at similar depths and sediment types (Fig. 2.1A).

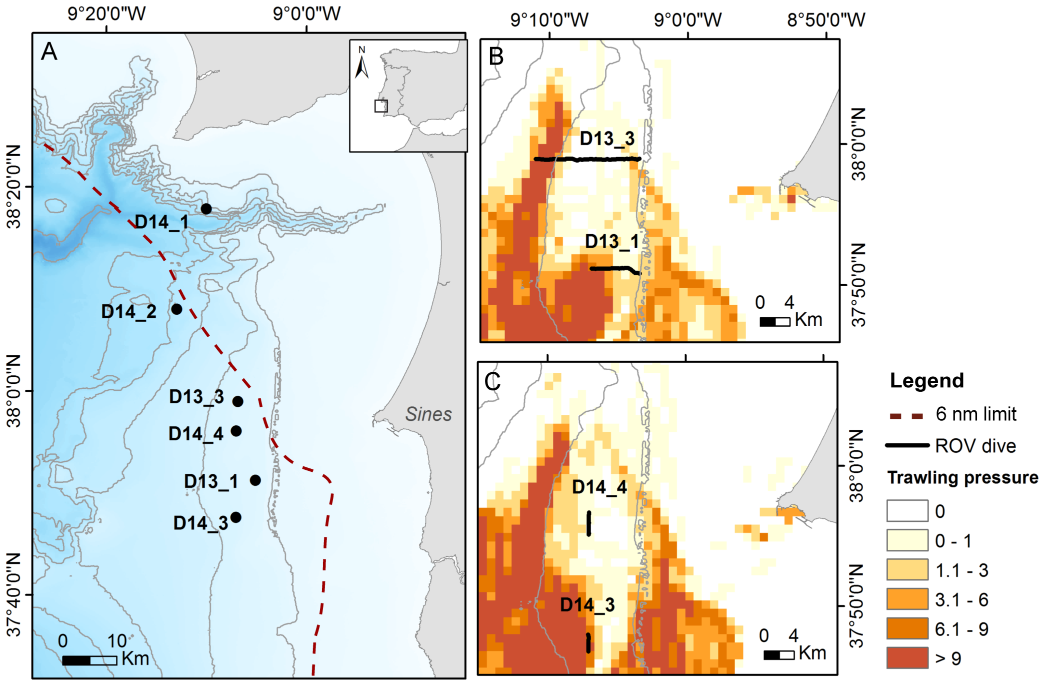

Figure 2.1 (A) Map of the study area indicating the locations of ROV dive transects in relation to the distribution of the crustacean trawlers annual trawling pressure (h.cell^{-1}.y^{-1}) for (B) 2013 and (C) 2014. Setúbal canyon area is not shown (0 h.cell^{-1}.y^{-1}). Red dashed line establishes the legal six nautical miles from the coastline.

Table 2.1 Metadata on ROV dive transects.

Cruise	**Dive Code**	**Date**	**Position coordinates**				**Depth range (m)**	**Total length (m)**	**Segments analysed (%)**	**EUNIS Habitat**	**Trawling pressure**
			Start		**End**						
			Lat (N)	**Long (W)**	**Lat (N)**	**Long (W)**					
RV Belgica 2013/17	D13_1	11/06/13	37.85257	-9.117838	37.84722	-9.049816	208–318	6002	60	A6.3/ A6.4	LT / HT
	D13_3	12/06/13	37.98379	-9.187216	37.98314	-9.05709	228–441	11405	78.9	A6.3/ A6.4	LT / HT
RV Pelagia 64PE387	D14_1	03/05/14	38.292665	-9.169028	38.29948	-9.162458	425–720	951	66.7	A6.4	NT
	D14_2	04/05/14	38.133224	-9.219712	38.133139	-9.21361	740–786	534	80	A6.4	LT
	D14_3	04/05/14	37.772635	-9.117301	37.799775	-9.117666	343–348	3020	43.3	A6.4	HT
	D14_4	05/05/14	37.906349	-9.116855	37.946467	-9.116353	287–309	4400	59.1	A6.4	LT

EUNIS Habitats classification (Davies et al., 2004): A6.3: Deep-sea sand; A6.4: Deep-sea muddy-sand; Trawling pressure includes: NT: no, LT: low and HT: high trawling pressure.

2.2.2 ROV dive surveys and sampling design

A total of six ROV survey transects were performed. The surveys were designed taking into consideration the available information from the VMS satellite data and the known distribution of sediment types. In 2013 (RV Belgica, cruise 2013/17), two transects (6000 m and 11500 m; D13_1 and D13_3, respectively) were outlined perpendicularly to the coastline from the upper continental slope to shallower areas. These covered several types of sediments (sand to muddy sand) and crossed a gradient of trawling pressures, including heavily fished deeper areas and the transition to less or no fished shallower areas (Fig. 2.1B). In 2014 (RV Pelagia, cruise 64PE387), four shorter transects (< 4000 m) were delineated only in areas of similar sediment type (muddy-sand). Two transects running parallel to the coastline focused on trawling target and adjacent non-target areas (D14_3 and D14_4; respectively Fig. 2.1C) in the main fishing ground off Sines. Additionally, two other transects (D14_1 and D14_2, Fig. 2.1A), were initiated near the flanks of the Setúbal canyon, where trawling pressure is null, and in the case of D14_1, it was located within the 6 nm limit. Both dives were not fully completed as planned (longer transects) owing to safety reasons, due to the risk of entanglement in the numerous fishing traps deployed at depths of ca. 450 m.

The video transects were performed using the ROV *Genesi*s, a sub-Atlantic Cherokee-type Remotely Operated Vehicle from VLIZ (Vlaams Instituut voor de Zee). Each video recording was obtained using two forward-looking standard definition black and white (Kongsberg OE15–100a) and colour cameras (Kongsberg OE14–366/367) at a speed of ~0.4 m. s^{-1} and altitude of ~1 m above the seabed. In addition, digital still images were acquired at approximate 30-second intervals using a high definition camera (Canon PowerShot G5). Accurate geo-positioning of both video and stills was obtained though the IXSEA global acoustic positioning system.

2.2.3 Image analysis and faunal characterization

Video recordings were analysed in segments of 100 m (linear distance sampling unit) calculated from the geo-positioning data. At each segment, all specimens visible in the footage were counted and identified to the lowest taxonomic level possible using additional high-resolution stills taken during the dives. Digital identification of some morphospecies was confirmed with specimens collected for macrofauna studies within the same sampling campaigns. In many cases, it was not possible to accurately assign specimens to species level and they were thus grouped into separate morphospecies, based on distinct morphological characteristics. Taxonomic classification followed the World Register of Marine Species database (WoRMS Editorial Board, 2016). Typical pelagic organisms (Ctenophora, Scyphozoa, and pelagic fishes) were also identified but not counted, since these organisms sometimes followed the ROV lights for long distances, not allowing their accurate quantification. Note that demersal fish species were included in our analyses, due to their direct interaction with the seabed. Video observations also included the description of seabed characteristics (e.g. bioturbation evidence, topography, ripple marks, phytodetritus patches) and any evidence of disturbance by trawling operations (trawl scars). Trawl scars were classified into "eroded" - scars where evident bioturbation and/or collapsed tracks; and "recent" - scars that were clearly undisturbed by bottom currents or faunal activity.

Due to technical issues, the reference scale normally provided by the laser points was not available and consequently the field view area was not estimated, which hindered biomass estimates and estimates of abundance per area (thus expressed per 100 m). The segments were performed at a relatively constant camera position and altitude, allowing the comparison among dives in both years. When this was not possible (e.g. no visual contact or varying altitude, high sediment resuspension, strong illumination), segments of "poor image quality" were excluded from the analysis to avoid low confidence level observations, resulting in the analysis of approximately 65% of the video recordings (Table 2.1).

2.2.4 Environmental parameters

Geographical information system software ArcGIS v10.3.1 was used to compile environmental data pertaining to each segment obtained from various sources as mentioned below.

Seabed habitats and bathymetric data were acquired from the European Marine Observation and Data Network portal - EMODnet (European Commission, 2016). Seabed habitats were classified following the EUNIS scheme (Davies et al., 2004) and the refined information from the available seabed sediment charts from Instituto Hidrográfico[8,9]. Deep-

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[8] Instituto Hidrográfico. (2005a). "Carta dos Sedimentos Superficiais da Plataforma Continental Portuguesa" - Folha 5 - Escala 1:150 000

sea sand (A6.3) included MdS1 (medium sand, grain size dominant fraction: 500-250 mm with <10% mud and <30% carbonate content) and FiS1 (fine sand, grain size dominant fraction: 250mm-63µm with <10% mud and <30% carbonate content). Deep-sea muddy-sand (A6.4) included SM2 (sandy-mud with 25–50% mud and 30–50% carbonate content), MS2 (muddy-sand with 10–25% mud and 30–50% carbonate content) and MS1 (muddy-sand with 10–25% mud and <30% carbonate content). Charts referring to the sediment composition were confirmed by several sediment samples collected for macrofauna studies within the same sampling campaigns.

The monthly average surface Net Primary Production (avNPP; g.C.m^{-2}.month^{-1}) values were obtained from the Vertically Generalised Productivity Model (VGPM) available on the Ocean productivity database (Behrenfeld and Falkowski, 1997). The VGPM model uses a standard algorithm calculated based on MODIS aqua satellite data for chlorophyll *a* concentrations, photosynthetically active radiation and sea-surface temperature. Temporal variability of the monthly surface Net Primary Production over one year prior to each sampling campaign was expressed as the seasonal variation index (SVI), calculated from dividing the standard deviation by the monthly average of the NPP (Lutz et al., 2007):

$$SVI = \frac{\sigma(NPP)}{\overline{NPP}}$$

2.2.5 Trawling pressure

Annual trawling pressure estimates (h.cell^{-1}.y^{-1}; where each cell size corresponds to 0.01 x 0.01 decimal degrees) were used as a proxy for the intensity of disturbance caused by crustacean trawlers to the seabed during the two years of this study. Trawling pressure was calculated based on VMS position data of the deep-water otter trawlers in operation along the Portuguese Margin, often designated as “crustacean trawlers”. This data was provided by DGRM and processed according to Bueno-Pardo et al. (2017). Trawling pressure data allowed to classify each segment into one of the following classes: no (NT: 0 h.cell^{-1}.y^{-1}), low (LT: 0.1 – 1.5 h.cell^{-1}.y^{-1}), and high (HT: >1.5 h.cell^{-1}.y^{-1}) trawling pressure. In fact, both NT and LT locations are assumed to be not directly disturbed. However, NT label was attributed to the segments within the 6 nm limit and with null

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[9] Instituto Hidrográfico (2005b). Carta dos Sedimentos Superficiais da Plataforma Continental Portuguesa - Folha 6A - Escala 1:150 000

trawling pressure values, while LT segments were assigned to segments that corresponded to relatively undisturbed areas adjacent to the main fishing ground (HT).

2.2.6 Data analysis

Mega-epibenthic faunal abundances (ind.100m^{-1}: individuals per 100 m of linear distance), composition and diversity were investigated using both uni- and multivariate data analyses performed with the software PRIMER v6 and PERMANOVA+ (Anderson et al., 2008; Clarke and Gorley, 2006). Prior to the exploration of the biological dataset in relation to trawling disturbance, the relationship between the mega-epibenthic assemblages and all acquired environmental variables (depth, sediment type (categorical predictor variable based on mud and carbonate content percentage range), avNPP, SVI, and trawling pressure) was computed by means of the distance-based linear model (DISTLM) analysis. The DISTLM routine was run using the adjusted-R^2 as selection criterion and the stepwise selection procedure on normalised environmental data and the distance-based redundancy analysis (dbRDA) plot was computed to illustrate the DISTLM model (Anderson et al., 2008).

In addition to trawling pressure, a strong relation between the other environmental variables and the biological dataset was observed in the DISTLM analysis. Thus, to further investigate the sole influence of trawling on the mega-epibenthic assemblages, only a subset of the dataset with relatively similar habitat characteristics was analysed: segments characterised by muddy-sand sediments within two narrow bathymetric ranges (either 300–400 m or 400–500 m) for each year. Each bathymetric range was analysed separately, as follows: a 2-factor layout, with “Year” as fixed factor and “Trawling” as a random factor nested in “Year”, was used for the 300–400 m depth range, and a 1-factor layout, with “Trawling” as the fixed factor, was used for the 400–500 m (replicate samples from both years were not available). A Non-metric multidimensional scaling (nMDS) ordination based on the Bray-Curtis similarity matrix after 4th root transformation was performed followed by the permutational multivariate analysis of variance (PERMANOVA) to test for differences in mega-epibenthic assemblages among groups (1-factor and 2-factor nested design for the subset of data). Morphospecies contributions (%) for the observed similarity within and dissimilarity between groups were analysed through the SIMPER analysis.

Species richness (S), Shannon-Wiener diversity (H’), evenness (J) (Pielou, 1966), and Hurlbert’s expected number of taxa ($ET_{(20)}$; Hurlbert, 1971) were used to examine diversity patterns. k-dominance (Lambshead et al., 1983) and Hurlbert's rarefaction curves were plotted to assess for differences in community structure. Lastly, non-parametric Spearman correlations were calculated between trawling pressure and mega-epibenthic faunal abundance, as well as trawling pressure and various diversity values (S, H' and $ET_{(20)}$)), assuming no dependence among variables (Quinn and Keough, 2002). Significant correlation values were adjusted by using the Bonferroni correction (Shaffer, 1995), which was calculated by dividing the significance value of each test by the number of hypothesis tested. Correlation analyses were run using the software GraphPad PRISM v6 (GraphPad Software, www.graphpad.com).

2.3 Results

2.3.1 Environmental variability

2.3.1.1 General seabed characterisation

Overall, the distribution of the different sediment types mapped in the geological charts was confirmed by the video observations. Coarser sediments (medium and fine sands included in A6.3, surveyed in 2013) were concentrated at shallower locations (ca. 200–300 m) along the self-break/upper slope transitions and characterised by a little phytodetritus coverage. In opposition, finer sediments (A6.4 deep-sea muddy-sand) were mostly found at depths greater than 300 m. Most segments surveyed in 2013 presented frequent ripple marks and heterogeneous patches of organic detritus material deposited on the seafloor. In 2014, most segments were deprived of evident phytodetritus coverage across all segments, which contrast with the higher annual average surface net primary production (avNPP; $g.C.m^{-2}.month^{-1}$) and smaller monthly fluctuations (lower SVI values) observed for 2014 (Table 2.2). Segments from the flanks of the Setúbal canyon were characterised by a heterogeneous seabed microtopography, with muddy-sand sediments (A6.4) and little evidence of detrital material.

Table 2.2 Summary of average surface Net Primary Production (avNPP; g.C.m^{-2}.month^{-1}) and seasonal variation index (SVI) values per dive (average of 100m segments).

Dive	**avNPP**	**SVI**
D13_1	345.5±46.25	0.51±0.018
D13_3	339.2±46.14	0.51±0.017
D14_1	438.1±50.30	0.41±0.031
D14_2	356.5±34.40	0.36±0.000
D14_3	410.7±53.54	0.43±0.029
D14_4	410.2±51.35	0.41±0.000

2.3.1.2 Mega-epibenthic assemblages in relation to environmental variables

A total of 27953 individuals were counted and subsequently assigned to 71 different morphospecies, belonging to at least 50 families and eight phyla. Six pelagic species and eight benthic morphospecies present in the reduced visibility segments could not be quantified and therefore were not included in further analyses. The list of all observed taxa is provided in the Annex 1 and Annex 2 of this thesis. Overall, the most abundant phylum was Annelida (66% of the total abundances), however only represented by four morphospecies. Contrastingly, the phyla Cnidaria (13%; 11 morphospecies) and Chordata (11%; 18 morphospecies) showed intermediate abundances but high taxa richness. The remaining phyla were less abundant, but not necessarily less diverse: Echinodermata (4%; 15 morphospecies), Arthropoda (3%; 11 morphospecies), Mollusca (1%; 9 morphospecies), Porifera (2%; 2 morphospecies), and Nemertea (<0.01%; 1 morphospecies).

The mega-epibenthic assemblages showed a large variation within and among dives, where spatial (depth, sediment composition, trawling disturbance) and temporal (years) factors appeared to, at least partially, determine the observed variability (Fig. 2.2 and Fig. 2.3). In detail, shallower areas off Sines (c.a. 200–300 m, only surveyed in 2013) yielded the highest abundances of the study, reaching 531 ind.100m^{-1} at 250 m depth, and the lowest diversity, with $ET_{(20)}$ ranging from 3.0 to 3.4. Here, mega-epibenthic fauna was typified by high numbers of the polychaete *Hyalinoecia tubicola* (83–88% of the total assemblage) regardless of the sediment type (sand or muddy-sand) (Fig. 2.3).

Muddy-sand sediments at the upper slope off Sines (ca. 300–500 m, surveyed both in 2013 and 2014) showed much lower abundances, typically under 150 ind.100m^{-1}, but higher diversity, with $ET_{(20)}$ ranging from 6.2 to 8.5 (Fig. 2.3). Faunal composition gradually changed with increasing water depth. Yet, the assemblages were generally dominated by different morphospecies of tube-dwelling anemones (sub-class Ceriantharia - Spirularia ind.; 19–57%) and hexacorallian anemones (2–52%), namely epibenthic actiniarians (mostly *Actinauge richardi)* and zoantharians (commensal, attached to hermit crabs). Several benthic fish morphospecies (Actinopterii: 6–21%) and few crustaceans morphospecies (Malacostraca: 3–17%) were also well represented. The 2014 surveys were marked by the presence of higher abundances of Crinoidea (10–17%), but also Porifera (21%) and Ophiuroidea (18%) in D14_4.

Muddy-sand sediments at the Setúbal region (450-800 m) showed also low abundances, with 22.5±3.75 and 71.8±11.6 ind.100m^{-1}, but higher evenness leading to ET$_{(20)}$ values of 8.2 and 10.5 for D14_1 and D14_2 respectively (Fig. 2.3). Communities were typically composed by the tube-dwelling anemones from the anthozoan sub-classes Ceriantharia (16–47%) and Octocorallia (15–17%), but also with relevant contributions of various other taxa such as Actinopterii (9–35%), Malacostraca (5–17%) and Polychaeta (2–15%).

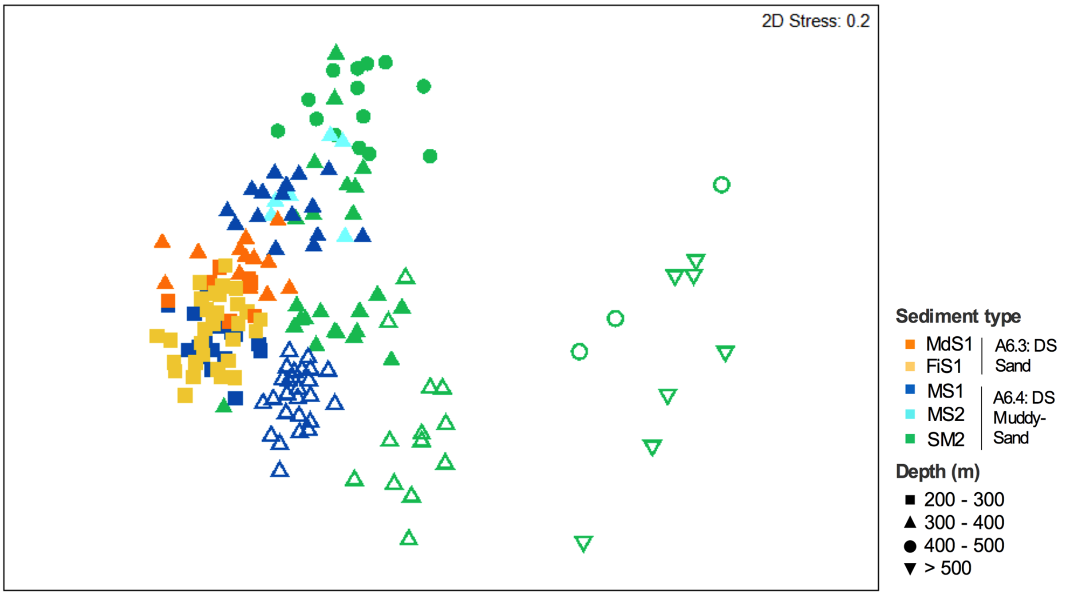

Figure 2.2 nMDS plot for comparison of mega-epibentic assemblages in relation to depth and sediment type (MdS1: medium sand; FiS1: fine sand; MS1: muddy-sand with <30% carbonate content; MS2: muddy-sand with 30–50% carbonate content; SM2: sandy-mud). Closed symbols represent segments from 2013 and open symbols samples from 2014 dives.

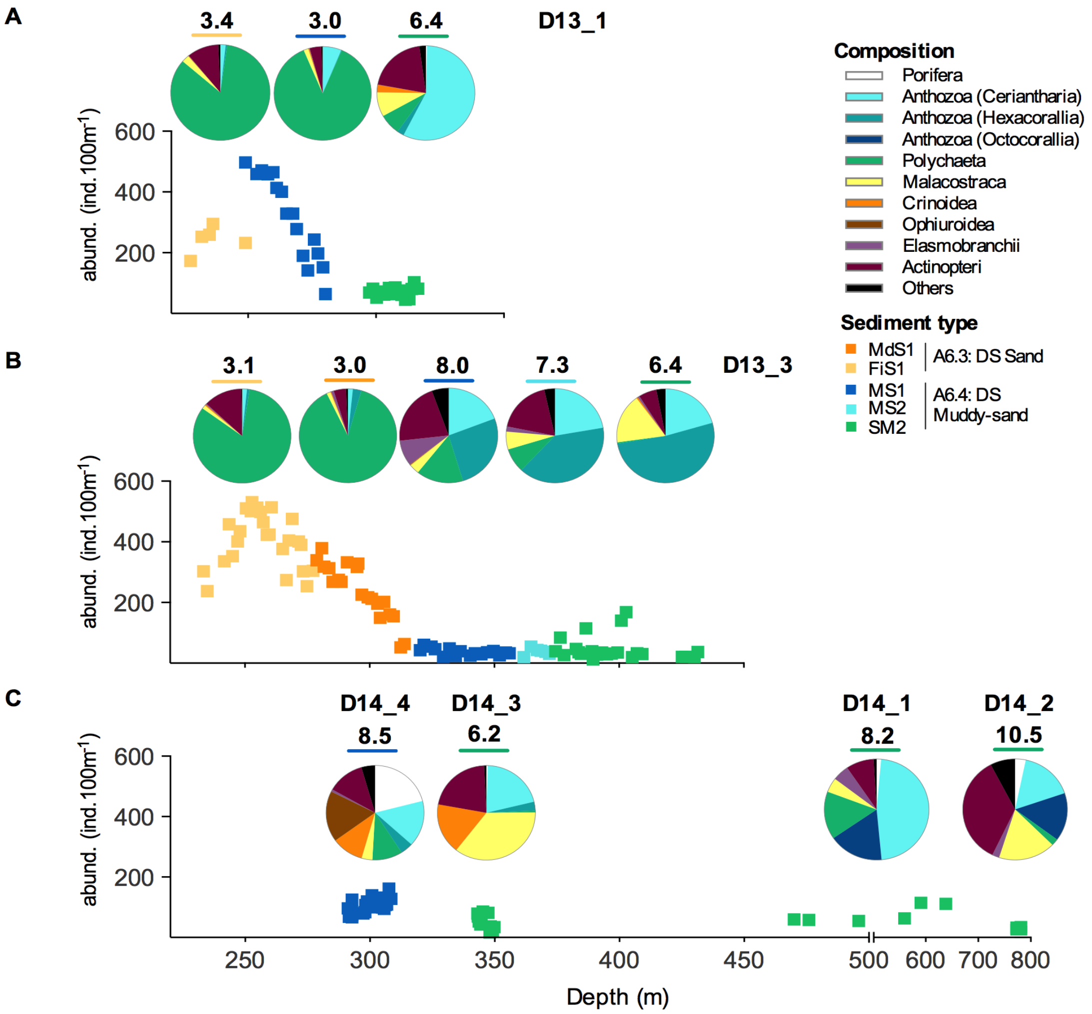

Figure 2.3 Distribution of the mega-epibenthic fauna abundance (ind.100m-1) in relation to depth and sediment type (MdS1: medium sand; FiS1: fine sand; MS1: muddy-sand with <30% carbonate content; MS2: muddy-sand with 30–50% carbonate content; SM2: sandy-mud). The pie charts show the taxonomic composition for each sediment type in the different dives: (A) D13_1; (B) D13_3 and (C) all 2014 dives. Hulbert's expected number of taxa (ET$_{(20)}$) for each assemblage is indicated above the respective pie charts. "Others" represent all the taxa that contribute with <1% to the total abundance.

PERMANOVA main test and pair-wise test results (Table 2.3; Supplementary Table 2.1), confirmed the differences between mega-epibenthic assemblages from different depths ranges ($p<0.01$), sediment types ($p<0.01$) and years ($p<0.001$), as well as between the interaction of depth and sediment type ($p<0.01$). Moreover, year and depth differences were more important for community structure, as indicated by the higher estimated component of variation (Table 2.3). Yet, the significant multivariate dispersion within each factor, tested though the PERMDISP analysis (p<0.01; Supplementary Table 2.2) together with the large amount of the estimated component of variation attributed to the residuals, indicates that a considerable amount of the observed variability in the mega-epibenthic assemblages remained unexplained by these factors alone. Note that "Year" differences may be confounded by differences in the sampling design and types of sediment surveyed in each year. In addition, trawling pressure, mostly concentrated between 400-500 m, was not taken into account in this analysis.

Table 2.3 PERMANOVA main results of the 3-factor crossed design (Year x Depth x Sediment type) based on the mega-epibenthic faunal community composition dataset.

Source of variation	df	SS	MS	Pseudo-F	P	Perm	ECV
Year (Ye)	1	14272	14272	13.295	**0.0001**	9942	1542
Depth (De)	3	44068	14689	13.684	**0.0001**	9817	1564.5
Sediment type (Sed)	4	36942	9235.5	8.603	**0.0001**	9998	370
DexSed	1	4820.6	4820.6	4.491	**0.0002**	9935	875.6
Res	165	177120	1073.5				1073.5
Total	174	330530					

Values in bold represent significant values. No test possible between YexDe; YexSed and YexDexSed. ECV: Estimated component of variation.

The DISTLM model analysis demonstrated that all six individual environmental variables were significantly correlated with the mega-epibenthic community structure (marginal tests; p <0.01; Supplementary Table 2.3). The best explanatory model (adjusted R^2= 0.42852) and sequential tests recognised by order of importance, sediment type (18%), SVI (11%), depth (9%), avNPP (4%) and trawling pressure (TP; 2%), explaining a total of 44.8% of the observed variability (Fig 2.4; Supplementary Table 2.3). Thus, because of the strong separation between the assemblages surveyed in the years 2013 and 2014, but also depth, sediment type and trawling pressure (Fig. 2.3), the putative effect of trawling disturbance on the mega-epibenthic assemblages was further analysed only within segments pertaining muddy-sand sediments at two major depth ranges: 300–400 m and 400–500 m (see section 2.3.2).

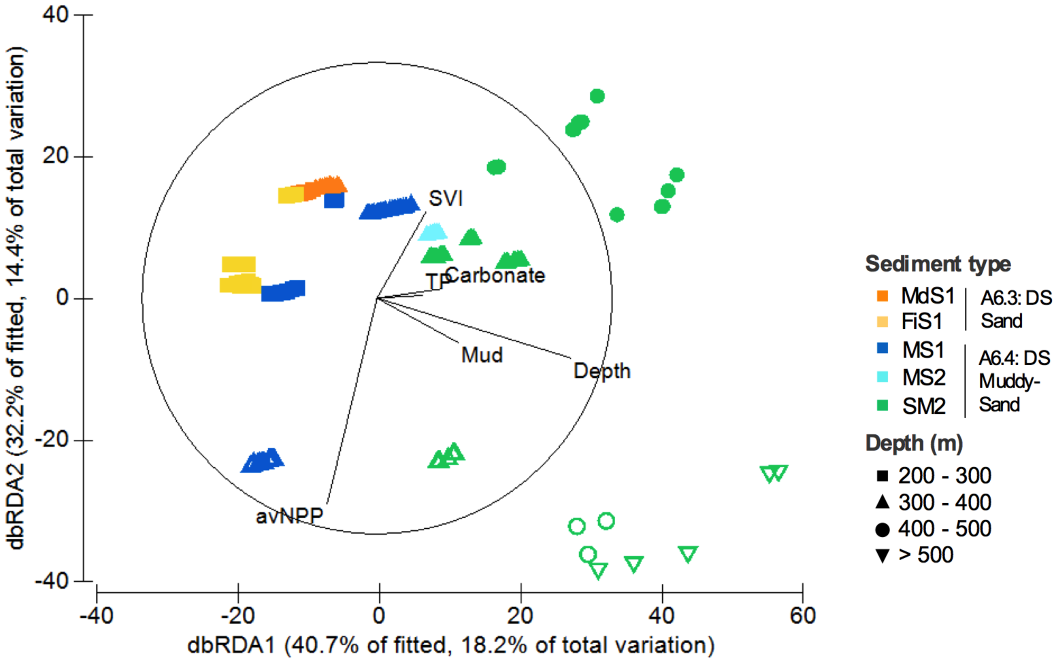

Figure 2.4 Distance-based redundancy (dbRDA) plot illustrating the DISTLM model exploring the relationship between megafauna community composition and environmental variables (vectors). Environmental parameters included in the analysis were: depth (m); sediment type (categorical variable determined by the mud and carbonate content (%, indicated as vectors); average net primary production (avNPP); seasonal variation index (SVI) and annual trawling pressure (TP; h.cell^{-1}.y^{-1}). MdS1: medium sand; FiS1: fine sand; MS1: muddy-sand with <30% carbonate content; MS2: muddy-sand with 30–50% carbonate content; SM2: sandy-mud. Closed symbols: 2013 segments; Open symbols: 2014 segments.

2.3.2 Bottom-trawl fisheries disturbance

2.3.2.1 Evidence of trawling disturbance on the seabed

In total, 149 trawl scars were detected in the present study, mostly associated with the higher trawling pressure areas (HT; 61.1%) and muddy-sand sediments (73.8%).

Undisturbed locations (NT) near the Setúbal canyon flanks were not associated with trawl marks (Table 2.4) and showed an overall heterogeneous microtopography and frequent evidence of faunal activity and bioturbation, numerous tracks and variously sized burrows and mounds, which are often associated with mud-burrowing decapods, such as the Norway lobster, *N. norvegicus* (Fig. 2.5A,B). In contrast, both low (LT) and highly disturbed (HT) segments were characterised by the presence of either discontinuous or continuous ripple marks. Particularly in 2013, comparatively considerable less bioturbation evidence (e.g. fewer and smaller burrows and tracks) (Fig. 2.5C-F) was observed for these areas. LT segments showed consistently low numbers of trawl scars (ca. 0.15 trawl scars/100m). Most scars observed at LT segments in 2013 were classified as “recent”, while scars observed in 2014 were mostly characterized as “eroded” (Table 2.4). The number of scars observed in the trawling target areas (HT) was up to 19 times higher than at the LT areas (Table 2.4). Note that this number may be greatly underestimated owing to the repeated operation of trawlers over the same trajectories.

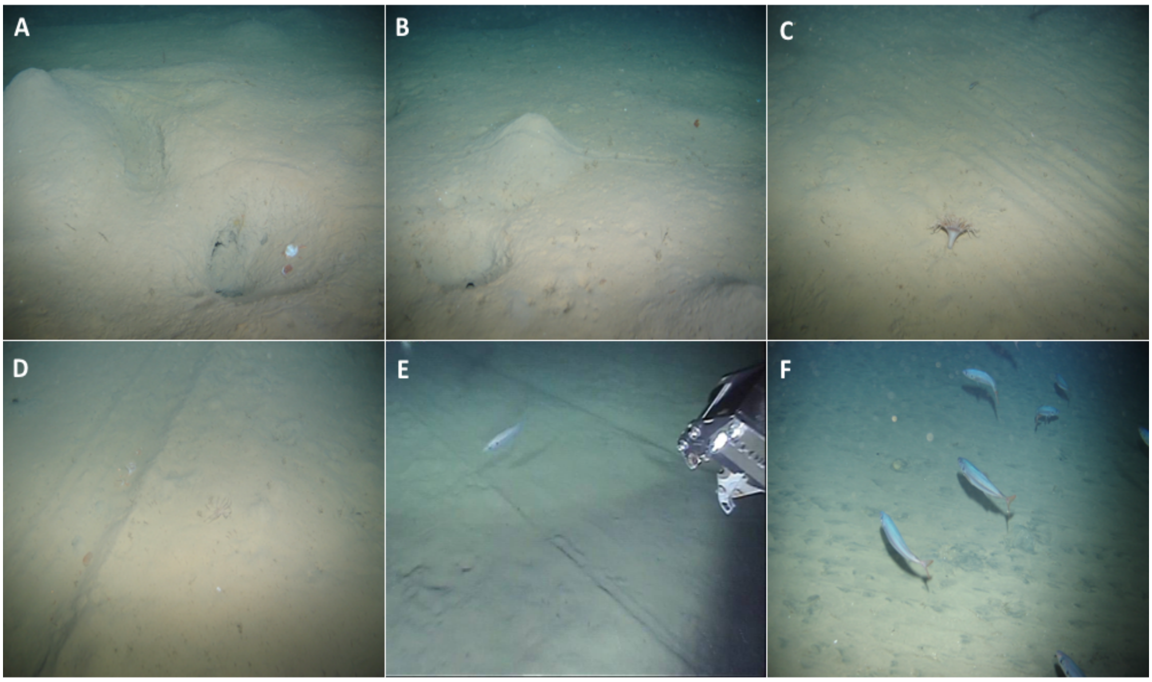

Figure 2.5 Seabed image samples from the study area within muddy-sand sediments (A6.4). (A,B) Undisturbed locations at the Setúbal area (NT) showed heterogeneous topography and large faunal activity (e.g. tracks, burrows and mounds of various sizes). Older evidence of trawling disturbance was demonstrated by (C) chain/net scars and (D) trawl door marks with clear signs of bioturbation activity. Recent passages by trawlers were evidenced by (E) large door marks and F) adjacent locations with flattened seabed surface with no recent faunal activity evidence. Photo credits: VLIZ and UGent.

Table 2.4 Characterization of the trawling scars observed in muddy-sand sediment (A6:4) segments within 300–400 m and 400–500 m depths (selected dataset).

Area	Nº of 100 segments	TP (h.cell^{-1}.y^{-1})	Trawl scars	
			Average ± SE	Eroded scars (%)
300–400 m				
LT (13)	16	0.03±0.027	0.18±0.136	25
LT (14)	26	0.39±0.051	0.15±0.072	100
HT (13)	15	5.55±0.393	0.53±0.192	50
HT (14)	13	8.90±0.191	2.85±0.406	18.9
400–500 m				
NT (14)	3	0.00±0.000	0.00±0.000	0
HT (13)	23	11.24±1.622	2.09±0.492	4.3

TP: trawling pressure. NT: no, LT: low and HT: high trawling pressure.

2.3.2.2 Mega-epibenthic assemblages in relation to trawling disturbance

The nMDS plot (Fig. 2.6) shows a segregation of the mega-epibenthic assemblages according to trawling pressure and years. PERMANOVA results (Table 2.5) confirms significant differences in mega-epibenthic assemblages from different “trawling pressure” groups ($p<0.001$) within the same depth range, independently of the sampling year ($p=0.3181$). Morphospecies contributions for these differences analysed through the SIMPER analysis, showed a maximum dissimilarity of 90.5% between assemblages from NT and HT segments, while dissimilarity between LT and HT segments was 64.3% (Supplementary Table 2.4 and Supplementary Table 2.5). The comparison between NT and LT was not computed due to depth-range differences.

The major contributors to the dissimilarity between NT and HT segments (400-500 m; Table 2.6 and Supplementary Table 2.5) were the dominant morphospecies in these groups: Spirularia ind. 1, *Kophobelemnon* sp., *Galeus melastomus*, and other Pennatulidae at NT segments; anthozoan anemones, such as *Actinauge richardi* and the tube-dwelling Spirularia ind. 2, and high abundances of the motile predator hermit crabs with their commensal anemones (Zoantharia ind.) in HT segments. Differences between LT and HT segments (300–400 m) were largely explained by the presence of Porifera ind. 2 and Ophiuroidea ind. 1, limited to LT segments in 2014, high abundance of the predator shrimp, *Plesionika sp.*, in HT segments, but also by various morphospecies with low individual contributions (e.g. *H. tubicola*, Spirularia ind. 2, *Caryophyllia* sp., small sized Comatulida ind. 1 and Comatulida ind. 2.; Table 2.6 and Supplementary Table 2.4).

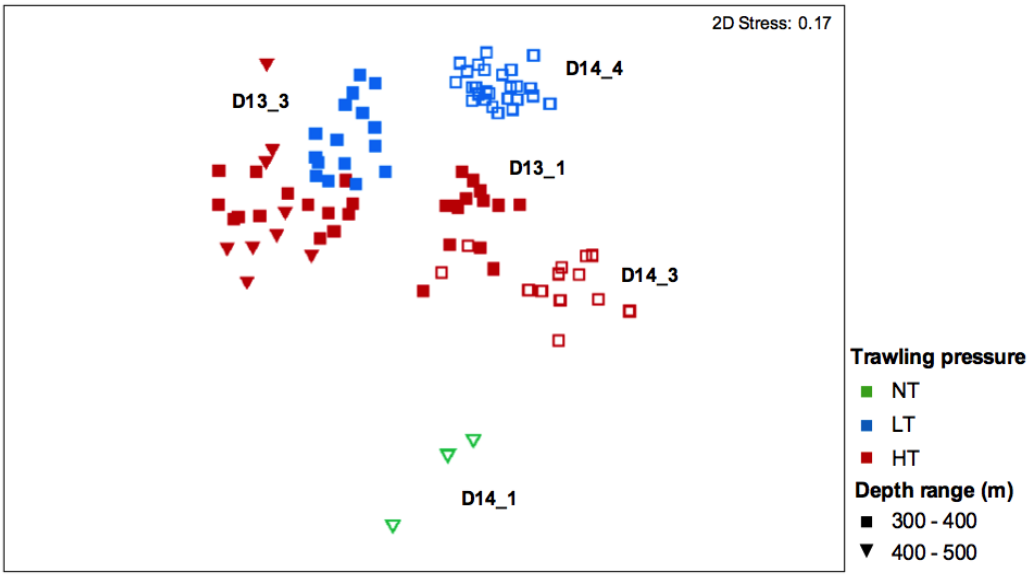

Figure 2.6 nMDS plot for comparison of mega-epibenthic assemblages from muddy-sand sediments segments between 300–400 m and 400–500 m subjected to varying trawling pressure (selected dataset). NT: no, LT: low and HT: high trawling pressure. Closed symbols: 2013 segments; Open symbols: 2014 segments.

Table 2.5 PERMANOVA main results based on the mega-epibenthic faunal community composition dataset of the 2-factor nested design (Year x Trawl (Year)) for muddy-sand sediment between 300–400 m water depths and 1-factor design (Trawl) for depths 400–500 m. For tests with permutations lower than 100, Monte Carlo results were considered; Values in bold represent significant values. ECV: Estimated component of variation.

Source of	**df**	**SS**	**MS**	**Pseudo-**	**P**	**Per**	**P(MC)**	**ECV**
300 – 400 m								
Year	1	31733	31733	1.5406	0.1715	6	0.2502	297.4
Trawl (Year)	2	40863	20431	22.532	**0.0001**	9907	0.0001	1051.3
Res	77	69821	906.77					906.77
Total	80	142420						
400 – 500 m								
Trawl	1	18309	18309	21.365	**0.0007**	2546	0.0001	3288.1
Res	24	20567	856.96					856.96
Total	25	38876						

Table 2.6 Abundance and biodiversity results from muddy-sand sediment areas (300-500 m) subjected to varying trawling disturbance (selected dataset).

Trawling pressure	**n**	**N**	**S**	**ET$_{(20)}$**	**H'**	**J'**	**Dominance**			
							Taxa	**Morphospecies**	**FG**	**%**
300–400 m										
LT (13)	16	42.4±2.99	26	8	2.25	0.69	HEX	*Actinauge richardi*	Pr/Su	20.1
							CER	Spirularia ind. 2	Pr/Su	19.0
							ACT	*Gadiculus argenteus*	Pr	18.5
							POL	*Hyalinoecia tubicola*	Sc/Dt	15.5
							ELA	*Galeus melastomus*	Pr	7.2
LT (14)	26	101.7±4.82	40	8.5	2.38	0.646	POR	Porifera ind. 2	Su	20.9
							OPH	Ophiuroidea ind. 1	Dt	18.5
							CER	Spirularia ind. 2	Pr/Su	15.4
							POL	*Hyalinoecia tubicola*	Sc/Dt	10.6
							ACT	*Gadiculus argenteus*	Pr	9.8
HT (13)	15	71.1±4.16	36	7.4	2.09	0.582	CER	Spirularia ind. 2	Pr/Su	35.0
							DEC	*Plesionika* sp.	Pr/Om	18.1
							ACT	*Gadiculus argenteus*	Pr	16.1
							CRI	Comatulida ind. 1	Su	8.2
							CER	Spirularia ind. 3	Pr/Su	4.5
HT (14)	13	66.2±10.59	21	6.2	1.84	0.603	DEC	*Plesionika* sp.	Pr/Om	34.8
							ACT	*Gadiculus argenteus*	Pr	20.0
							CRI	Comatulida ind. 1	Su	17.2
							CER	Spirularia ind. 3	Pr/Su	11.6
							CER	Spirularia ind. 5	Pr/Su	8.6
400–500 m										
NT (14)	3	53.0±4.82	20	9.1	2.33	0.778	CER	Spirularia ind. 1	Pr/Su	27.7
							OCT	*Kophobelemnon* sp.	Su	23.3
							ELA	*Galeus melastomus*	Pr	12.4
							OCT	Pennatulacea ind.1	Su	11.3
							ACT	*Gadiculus argenteus*	Pr	4.4
HT (13)	23	47.3±1.53	29	6.4	1.88	0.558	HEX	Zoantharia ind.	Pr/Su	39.2
							CER	Spirularia ind. 2	Pr/Su	17.2
							DEC	Paguroidea ind. 1	Pr/Su	16.7
							HEX	*Actinauge richardi*	Pr/Su	12.4
							ACT	*Gadiculus argenteus*	Pr	3.9

NT: no, LT: low and HT: high trawling pressure; n: number of the pooled segments; N: average abundance±SE: standard error; S: morphospecies richness; ET$_{(20)}$: Hulbert's expected number of species per 20 individuals; H': Shannon–Wiener diversity (ln base); J': Pielou's evenness. Taxa include: POR (Porifera), CER (Anthozoa: Ceriantharia - Spirularia), HEX (Anthozoa: Hexacorallia), OCT (Anthozoa: Octocorallia), POL (Polychaeta), DEC (Malacostraca: Decapoda), CRI (Crinoidea), ELA (Elasmobranchii), ACT (Actinopterii). Feeding group (FG) includes: Pr: Predator, Sc: Scavenger, Om: omnivores; Dt: Detritus feeder, Su: Suspension/Filter feeder.

Differences in composition between disturbed and undisturbed areas were supported by the consistently higher diversity and evenness values of the mega-epibenthic assemblages at NT (H'=2.33; J=0.778; $ET_{(20)}$= 9.1; K_1=27.7), and LT (H'=2.25–2.38; J=0.646–0.690; $ET_{(20)}$= 8.0–8.5; K_1=20.1–20.9), when compared to HT (H'=1.84–2.09, J=0.558–0.603, $ET_{(20)}$=6.2–7.4; K_1=34.8–39.2; Table 2.6). This is further confirmed by the lower rarefaction curves and higher dominance curves displayed by the HT assemblages at both depth ranges (Fig. 2.7). All rarefaction curves approximate asymptotic values, apart from the NT segments at the deeper areas (400–500 m, Fig. 2.7D), indicating that the survey was insufficient to fully evaluate the biodiversity at the Setúbal sites.

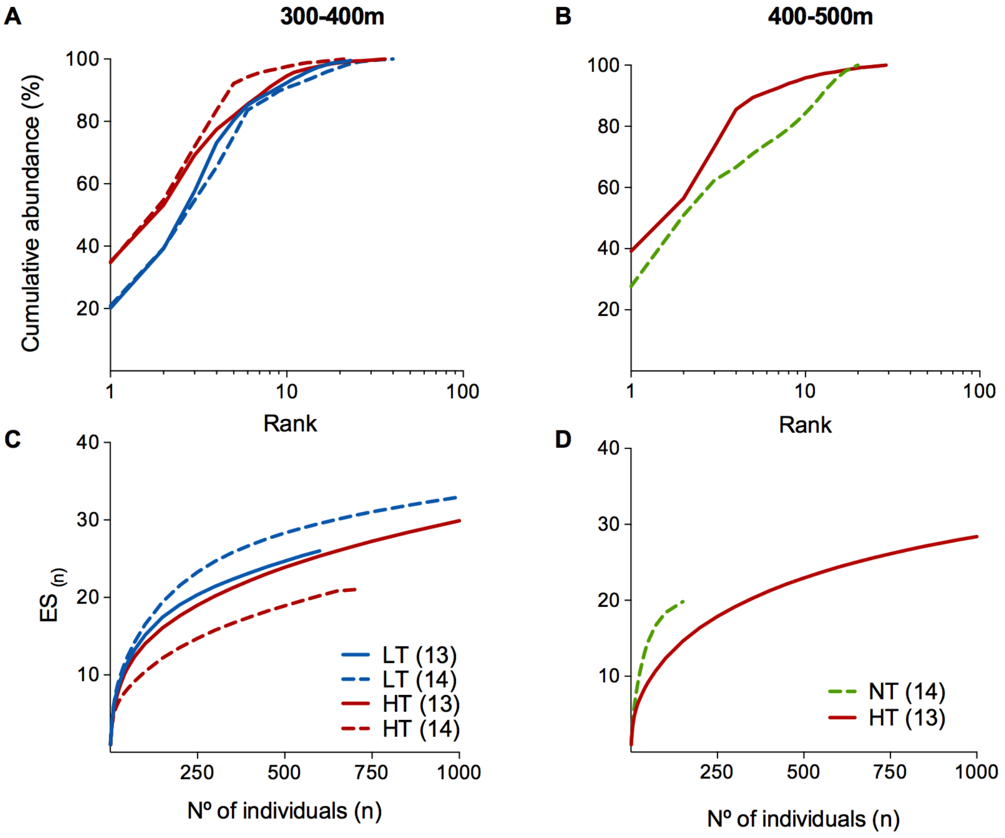

Figure 2.7 Diversity comparison among the different disturbed areas through (A, B) k-dominance curves and (C, D) rarefaction curves (Hurlbert's expected number of species) computed based on the selected dataset for mega-epibenthic assemblages at depth of 300–400 m (Left) and 400–500 m (Right) within muddy-sand sediments. NT: no, LT: low and HT: high trawling pressure.

A significant negative correlation (after Bonferroni correction) was detected between trawling pressure and the estimated diversity indices: species richness (R= -0.5169, p<0.001), Shannon-Wiener diversity (R= -0.6347, p<0.001) and $ET_{(20)}$ (R= -0.6335, p<0.001) (Figure 6B,C,D). Contrastingly, no significant correlation between trawling pressure and mega-epibenthic faunal abundances was observed (Figure 6A). It is noteworthy the record of large aggregations of the hermit crab Paguroidea ind. 1 in two segments under high trawling pressure (19 $h.cell.^{-1}.y^{-1}$). The high abundances of this species largely contributed to the high variability in faunal abundances observed in the HT areas.

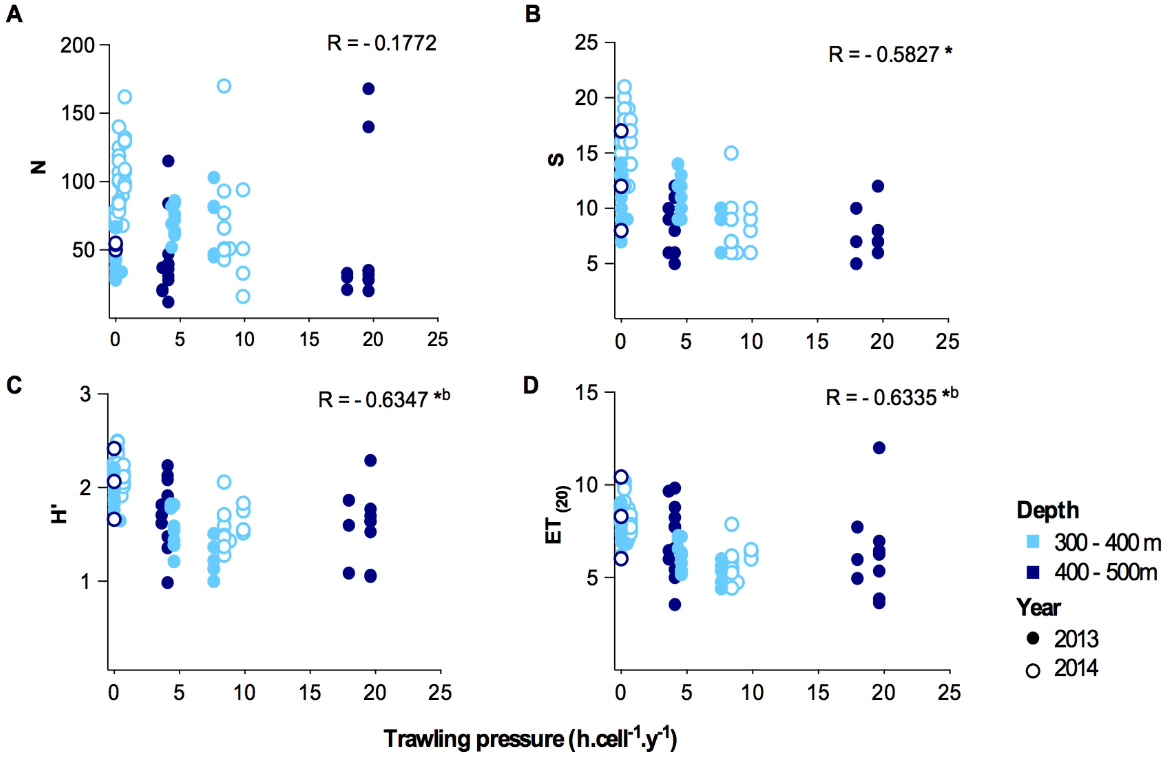

Figure 2.8 Relationship between annual trawling pressure ($h.cell^{-1}.y^{-1}$) and (A) abundance (N; $ind.100m^{-1}$) (B) morphospecies richness (S), (C) Shannon-Wiener diversity index (H') and (D) $ET_{(20)}$ Hulbert's expected number of taxa for 20 individuals per depth range. *indicates significant correlation; ^{b} indicates significant correlations after Bonferroni correction.

2.4 Discussion

The sustainable exploitation and management of deep-sea resources can only be achieved by a good knowledge on the biodiversity and ecosystem functions of the concerned area. This has been proven difficult when, in addition to the environmental and biological processes, anthropogenic activities, particularly fisheries, are also influencing the mega-epibenthic assemblages (Ramírez-Llodra et al., 2011). This work was fundamentally driven by the limited information available on the impacts caused by crustacean bottom-trawling fisheries, which have been active along the Portuguese coast since the late 70's. To our knowledge, only few in-situ observations were performed aiming to describe the mega-epibenthic faunal biodiversity there, and those were mostly concentrated in submarine canyon areas (Pattenden, 2008; Duffy et al., 2012; Fonseca et al., 2014; Gomes-Pereira et al., 2015). Yet, even less attempt has been made to identify the possible impact of fisheries on the benthic habitat and faunal assemblages (Morais et al., 2007; Fonseca et al., 2014).

It is important to refer that some limitations are associated within the present study. Specifically, the low taxonomical resolution associated with identification certain taxa (e.g. Porifera and Anthozoa), may have resulted in the underestimation of the overall biodiversity in study region as we only assigned a separate morphospecies when clear morphological characters were identified. This issue is usually associated with photographic/video surveys, in areas where the understanding of biological biodiversity is still limited and is not associated with additional sampling, however it represents currently the best available tool to accurately quantify mega-epibenthic specimens (Bicknell et al., 2016). Furthermore, imagery surveys are essential to describe both faunal distribution and activity (e.g. bioturbation and feeding behaviour), but also more importantly, to investigate direct evidence of physical disturbance on the seabed (e.g. presence and condition of trawl marks), otherwise impossible or counterproductive when using destructive methods such as trawl samplers (Bicknell et al., 2016). Secondly, because the laser points were not available due to technical issues, we were not able to estimate biomass differences across areas, even though the influence of bottom trawling fisheries on this measure has been frequently reported (NRC, 2002).

2.4.1 Mega-epibenthic assemblages associated with environmental variability

The effects of trawling fisheries on mega-epibenthic assemblages are fundamentally difficult to isolate from the environmental variability. Here, we observed marked differences in faunal assemblages linked with both spatial and temporal variability of the environmental and trawling disturbance conditions experienced along a relatively narrow depth range (c.a. 200–800 m). Depth-related changes in sediment sorting and fishing disturbance conditions (trawling pressure), together with the expected decrease in food supply (not directly investigated here) were accompanied by changes in mega-epibenthic fauna abundance, composition and diversity.

In the area off Sines, the overall higher abundances that characterised the shelf-break assemblages (c.a. 200–300 m), regardless of the sediment type, contrasts with the sharp abundance decline at depths greater than 300 m both at Sines and Setúbal areas. An abrupt decline in the benthic standing stocks (both abundance and biomass) is usually observed with increasing water depth. These declines in standing stocks are generally linked with a major decline of particulate organic matter supply to the seafloor (Rex et al., 2006). Furthermore, the high abundance and low diversity values at shallower depths resulted from the dominance of a single species, the onuphid polychaete *Hyalinoecia tubicola*, present in large aggregations and often feeding on carrion. This opportunistic scavenger has been reported in several regions of the NW Atlantic, including at the Portuguese margin (Fauchald and Jumars, 1979; Ravara and Moreira, 2013). *Hyalinoecia tubicola* displays a wide bathymetric distribution, but is only dominant in relatively shallow and hydrodynamic areas (Grassle et al., 1975), thus it is not surprising that here shallower coarser sediment areas seemed to create a suitable habitat for this polychaete species, otherwise mainly absent at deeper locations. Furthermore, remains of dead crabs and other animals were frequently observed during the surveys off Sines. They probably originated from discarding practices which are common along the Portuguese margin (Monteiro et al., 2001), and may allow the maintenance of the abundant *H. tubicola* populations.

The upper slope segments off Sines (>300 m) were characterised by a shift to finer sediments (but also different trawling regimes). This area showed distinct mega-epibenthic assemblages from the ones observed at the shelf-break, typified by the presence of tube-dwelling anemones and other mud-burrowing fauna (e.g. the Norway lobster). Sediment preferences by both epibenthic and infaunal organisms are often reported in other studies and have been linked to life style and feeding habits (e.g. deposit feeders may select certain grain-size classes; Levin et al., 2001; Murillo et al., 2016). The preference of burrowing organisms for finer sediment types has been related with the higher stability of these sediments. A higher sediment stability allows for example the construction of burrows and tunnels (or even large galleries in the case of the Norway lobster), which otherwise would collapse in unstable sandy sediments (Afonso-Dias, 1997).

Differences in both morphospecies composition and diversity were also largely associated with different geographic locations (Setúbal and Sines areas; >300 m) and distinct long-term trawling disturbance regimes (discussed in more detail in section 4.2 and 4.3). While we recognize the possible influence of canyon conditions (e.g. high energy bottom currents) at the Setúbal region (reference areas - NT), the naturally high dynamic conditions and productivity regimes of the WIM (Lavaleye et al., 2002), may attenuate the normally observed dissimilarities in community composition between canyon and slopes habitats (e.g. Ramírez-Llodra et al., 2010). In contrast with the typical dominance of deposit-feeders in other European regions (e.g. the Celtic Margin), the upper slope assemblages along the WIM tended to exhibit a naturally high proportion of sessile filter-feeders communities, often described as “canyon indicators” (Lavaleye et al., 2002). These “canyon indicators” were represented here by several morphospecies of the sub-class Octocorallia. The presence of current ripple marks parallel to the isobaths lines confirms the high energy hydrodynamic conditions along the self-break and upper slope off Sines.

Besides spatial variability, the mega-epibenthic assemblages also showed differences between years. As stressed before, these temporal changes must be interpreted with caution because of the differences in the alignment of the dives (perpendicular or parallel to the coastline) and of sediment types and depths surveyed in 2013 and 2014. Temporal fluctuations in environmental conditions, namely the lower seasonal fluctuations and higher surface productivity in 2014 may explain the observed increase in dominance of detritivores (e.g. ophiuroids). The influence of other stressors that we were not able to directly investigate here (e.g. water masses properties, bottom currents, etc.), likely also contributed to these interannual differences. It is also important to mention that extreme storms occurred during the winter of 2013-2014[10], and those were

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[10] Instituto Português do Mar e da Atmosfera, 2014. Informação mais Detalhada Sobre a TempestadeStephanie.https://www.ipma.pt/pt/media/noticias/news.detail.jsp?f=/pt/media/noticias/arquivo/2014/tempestade-stephanie.html (accessed 01 November 2016).

not recorded in the winter of 2012-2013. These extreme events resulted in severe beach erosion and transport of large amounts of OM rich sediments from terrestrial origins towards deeper areas (Sanchez-Vidal et al., 2012; Diogo et al., 2014), likely providing additional food sources for detritivores and deposit feeders in the surveyed area.

2.4.2 Crustacean trawling fisheries and seabed physical integrity

The initial characterization made by the Portuguese government - Direcção Geral dos Recursos Naturais, Segurança e Serviços Marítimos (DGRM) (MAMAOT, 2012) in the context of the European Union's Marine Strategy Framework Directive highlights trawling fisheries as one of the most pervasive activities along the Portuguese margin. Furthermore, the Portuguese government has issued a ban for bottom-trawling activities in the high seas areas comprising the Azorean EEZ and the claimed extended continental shelf beyond the 200 nautical miles[11]. However, these interdictions do not include continental slope and submarine canyon areas along the Portuguese mainland, which are the principal target habitats of deep-water crustacean trawlers.

Fishing effort distribution patterns in the mainland differ greatly between northern and southern regions (north and south of Cape Espichel, respectively). These differences are primarily related to the distribution of different target species and their preferred habitats. In the north, the most landed species include several cephalopod and demersal fish species that occur in coarse sediments along the continental shelf; in the south region, the most valuable species include several deep-water crustacean species (e.g. the Norway lobster, red and rose shrimps), which typically occur at muddy and muddy-sand habitats between the shelf break and 700 m water depths (Bueno-Pardo et al., 2017; Campos et al., 2007). Our results show the highest evidence of disturbance (trawl scars) in muddy-sand sediment bottoms (300-500 m depth) and an increase of up to 5 times in the observed number of trawl scars from 2013 to 2014, which are consistent with the fishing effort distribution and the increase in trawling pressure off Sines reported by Bueno-Pardo et al. (2017). This recently observed shift in trawling activity towards the Southwest region, mostly towards deeper locations (Bueno-Pardo et al., 2017), is of particular concern because it is likely to exert an unprecedented pressure on the deep-dwelling benthic assemblages and should be followed by an adequate monitoring programme.

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[11] Diário da Républica, Portaria nº 114/2014 de 28 de Maio, 1ª série nº102 de 28 de Maio de 2014

While the most direct evidence of trawling pressure on benthic habitats are illustrated by the trawl scars, other seabed features could also help to characterise the effect of trawling in this area. Both the direct evidence of trawl fisheries impact (number and condition of the trawl scars), as well as the microtopography and bioturbation evidence (as proxy of the "ecosystem engineers" faunal activity) could help to infer the physical integrity of the seafloor; which is crucial for benthic biodiversity and ecosystem functioning (Rice et al., 2012; Thurber et al., 2014). The studied areas included in this research suggest that seabed integrity was largely compromised at disturbed locations off Sines. In the most severe cases (several HT segments) the seabed showed a completely flat appearance, and overall both HT and LT areas displayed low structural complexity. These observations contrasted with the area off Setúbal, which has never been trawled, and where the presence of a complex microtopography, represented by numerous tracks from crawling fauna, variously sized burrows and mounds was observed. These mentioned seafloor characteristics are indicative of the presence of "ecosystem engineering" fauna, responsible for performing several fundamental functions in the environment, such as promoting sediment carbon cycling, enhancement of water-sediment fluxes, microhabitat provision, and refuge for associate fauna (Thurber et al., 2014).

2.4.3 Mega-epibenthic fauna vulnerability to physical disturbance

Among the most evident impacts associated with the low selectivity of bottom-trawling practices are the direct removal of large biomasses of target species, incidental catches of non-target species (by-catch), and overall increased in-situ mortality of damaged individuals. The indirect effects on the benthic habitats may include compromised seabed integrity (mentioned above), changes in benthic community trophic structure and size spectrum, and decreased mega-epibenthic fauna diversity (Jennings and Kaiser, 1998; NRC, 2002). However, the results of different studies are often inconsistent. For example, Atkinson et al., (2011) reported a decline in both mega-epibenthic faunal abundance and species richness from low to highly disturbed areas (reference conditions not available). In the Barents Sea, Buhl-Mortensen et al., (2015) investigated a wide range of soft and hard-substrate bottoms, and they have observed significant declines in abundance in sand and hard substrates locations, while muddy bottoms showed no distinct patterns regarding changes in abundance. In the south Portuguese margin, Morais et al., (2007) and Fonseca et al., (2014) identified a depletion of mega-epibenthic organisms abundances and diversity in fine sediment locations that suffered intense exploitation by crustacean trawlers with little evidence of recovery, while rocky and coarse sand substrates (avoided by trawlers to not damage the nets), promoted refuge for several sensitive species that included a large crinoid bed of *Leptometra celtica* (Fonseca et al., 2014). Moreover, most studies on soft sediment faunal assemblages impacted by trawling are flawed by the lack of reference pristine areas of the same habitat type.

By comparing mega-epibenthic assemblages subjected to different levels of trawling pressures only in areas with similar sediment types and depth ranges, our study attempts to minimize the effects of other confounding environmental variables. Overall, the mega-epibenthic assemblages under higher levels of trawling pressure showed low diversity (taxa richness and evenness) in agreement with previous reports form the Southern Portugal coast (Morais et al., 2007; Fonseca et al., 2014). Differences in community composition were mostly marked between undisturbed locations (NT) and highly impacted sites (HT). Undisturbed areas were characterised by a more diverse fauna, showing a wider range of feeding modes and life styles. Among the most dominant taxa here were small tube-dwelling Spirularia ind. 1, several filter-feeding seapen species (e.g. *Kophobelemnon* sp., *Pennatula* sp.) anchored to the seabed and small predatory sharks (*Galeus melastomus*). In contrast, the typical dominant fauna of impacted areas included large and robust anemone species (*A. richardi* and tube-dwelling Spirularia ind. 2) and several highly mobile fish species and decapods with an opportunistic feeding behaviour (predatory-scavenging; e.g. the arrow shrimp - *Plesionika* sp. and the hermit crabs - Paguroidea ind. 1). The presence of abundant motile predators or scavengers in HT segments is consistent with previous observations reporting a rapid response after disturbance of such species (e.g. Dannheim et al., 2014; Almeida et al., 2017) but also experimental works performed in the deep sea (Bluhm, 2001, Gerdes et al., 2008). In fact, there is often an increased food availability for these trophic groups in recurrently trawled areas, which results from both the on-site mortality or injured fauna, but also from discarding practices (Ramsay et al., 1996; NRC, 2002; Castro et al., 2005). The low commercial value of many by-catch species (e.g. Henslow's crab) at the WIM often leads to discarding of an average of 40-70% of the fished biomass by crustacean trawlers (Borges et al., 2001; Monteiro et al., 2001).

Differences between LT and HT mega-epibenthic assemblages were less pronounced than between NT and HT. Because LT areas are adjacent to the main fishing grounds (HT areas), they are likely influenced by trawling-induced turbidity. Pervasive high turbidity owing to sediment re-suspension during trawling operations (Puig et al., 2012; Martín et al., 2014) causes smothering of filter feeding fauna and can lead to overall lower abundances (Greathead et al., 2007). Lastly, the lower dissimilarity between HT and LT assemblages off Sines (64%) when compared to NT vs. HT areas (91%), suggests that the long-term influence of physical disturbance led to a significantly altered state of the mega-epibenthic assemblages in areas beyond the ones directly targeted by crustacean trawlers.

2.5 Conclusions

The marked differences in morphospecies community composition and lower diversity in the disturbed locations, as well evidence of deleterious effects in areas beyond the ones directly targeted by crustacean trawlers, are indicative of strong effects of bottom-trawling activities on the mega-epibenthic assemblages off the SW Portuguese margin. Future recovery assessments would require historical analysis on both trawling pressure and community-based information (not currently available to our knowledge). Nevertheless, the observed deleterious effects of trawling on mega-epibenthic fauna, together with the intensification of trawling pressure in the study area stress the need for adequate monitoring programs and regulatory measures to halt the long-term loss of biodiversity and allow the sustainability of fisheries at the SW Portuguese Margin.

Lastly, it is important to point that trawl disturbance evidence on the seabed, assessed through the number and condition of the trawl scars, supports the Vessel Monitoring Systems (VMS) mapping and trawling pressure estimates performed by Bueno-Pardo et al., (2017), for the Portuguese Margin. While this method shows constraints related with data acquisition and background information of benthic habitat biodiversity, VMS data shows great potential for the identification of areas of interest in the deep sea that may need further monitoring.

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PT2020 Partnership Agreement and Compete 2020. S. P. Ramalho work was (co-) funded through a MARES Grant. MARES is a Joint Doctorate programme selected under Erasmus Mundus coordinated by Ghent University (FPA 2011-0016). Check www.mares-eu.org for extra information. L. Lins work was funded by BOF (12/DOS/006) and CAPES (BEX 11595/13-2) grants. JB was funded by the Calouste Gulbenkian Foundation, through the project "The Economic Valuation and Governance of Marine and Coastal Ecosystem Services" (MCES) (BPD/UI88/6454/2014)

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Supplementary Material

Supplementary Table 2.1 PERMANOVA pair-wise test results of the 3-factor crossed design (Year x Depth x Sediment type) based on the megafaunal community composition dataset. Values in bold represent significant values; nt: no test possible. Sediment type: MdS1: medium sand with <10% mud and less 30% carbonate content; FiS1: fine sand with <10% mud and <30% carbonate content; MS1: muddy-sand with 10-25% mud and < 30% carbonate content; MS2: muddy-sand with 10-25% mud and 30-50% carbonate content; SM2: sandy-mud with 25-50% mud and 30-50% carbonate content

Pair-wise Test 1 (Year)	t	P(perm)	unique perms
2013, 2014	6.9271	**0.0001**	9940

Pair-wise Test 2 (Depth)	t	P(perm)	unique perms
Groups			
300-400, 200-300	5.2258	**0.0001**	9939
300-400, 400-500	2.7186	**0.0001**	9934
300-400, >500	4.4922	**0.0001**	9931
200-300, 400-500	5.5002	**0.0001**	9931
200-300, >500	6.0146	**0.0001**	9931
400-500, >500	3.1688	**0.0002**	9740
300-400, 200-300	5.2258	**0.0001**	9939

Pair-wise Test 3 (Sediment type)	t	P(perm)	unique perms
Groups			
SM2, MS1	4.161	**0.0001**	9940
SM2, FIS1	4.6367	**0.0001**	9938
SM2, MS2	1.7059	**0.0179**	9955
SM2, MDS1	3.4702	**0.0001**	9948
MS1, FIS1	3.803	**0.0001**	9942
MS1, MS2	2.5358	**0.0002**	9955
MS1, MDS1	3.1272	**0.0001**	9935
FIS1, MS2	4.6705	**0.0001**	9921
FIS1, MDS1	3.1327	**0.0001**	9932
MS2, MDS1	3.3833	**0.0001**	9729

Supplementary Table 2.2 PERMDISP and pair-wise comparison results of the 3-factor design (Year x Depth x Sediment type) based on the megafaunal community composition dataset. Values in bold represent significant values; MdS1: medium sand with <10% mud and <30% carbonate content; FiS1: fine sand with <10% mud and <30% carbonate content; MS1: muddy-sand with 10-25% mud and < 30% carbonate content; MS2: muddy-sand with 10-25% mud and 30-50% carbonate content; SM2: sandy-mud with 25-50% mud and 30-50% carbonate content.

Test 1 (Year)
Deviations From Centroid

df1	df2	F	P(perm)
1	173	18.772	**0.0003**

Pairwise comparisons

Groups	t	P(perm)
2013, 2014	4.3327	**0.0004**

Test 2 (Depth)
Deviations from centroid

df1	df2	F	P(perm)
3	171	17.522	**0.0001**

Pairwise comparisons

Groups	t	P(perm)
300-400, 200-300	7.3096	**0.0001**
300-400, 400-500	1.0314	0.3914
300-400, >500	0.58155	0.6837
200-300, 400-500	4.9277	**0.0002**
200-300, >500	2.6611	0.0675
400-500, >500	0.74179	0.579

Test 3 (Sediment type)
Deviations from centroid

df1	df2	F	P(perm)
4	170	57.369	**0.0001**

Pairwise comparisons

Groups	t	P(perm)
SM2, MS1	6.9007	**0.0001**
SM2, FiS1	10.408	**0.0001**
SM2, MS2	4.8825	**0.0001**
SM2, MdS1	8.3429	**0.0001**
MS1, FiS1	9.0137	**0.0001**
MS1, MS2	4.6007	**0.0089**
MS1, MdS1	7.2205	**0.0001**
FiS1, MS2	0.3471	0.8216
FiS1, MdS1	0.55818	0.585
MS2, MdS1	0.65571	0.6288

Supplementary Table 2.3 Results of the distance-based linear model (DISTLM) analysis for exploring relationship between megafauna community composition and the investigated environmental variables. Marginal tests: explanation of variation for each variable taken separately. Sequential tests: conditional tests of individual variables in constructing the best model (selection procedure: stepwise; selection criterion: adjusted R²). Environmental variables included: depth (m), sediment type (categorical variable established from the mud and carbonate content as percentage), annual average net primary production (avNPP), seasonal variation index (SVI) and trawling pressure (h.cell^{-1}.y^{-1}). Values in bold represent significant values.

Marginal tests						
Variable/ Group	SS(trace)	Pseudo-F	P	Prop.	res.df	regr.df
Depth	52384	32.582	0.0001		173	2
Sediment type	60891	19.421	0.0001		172	3
Trawling	29902	17.208	0.0001	9.0468	173	2
avNPP	40772	24.343	0.0001		173	2
SVI	42127	25.271	0.0001		173	2

Sequential tests								
Variable/ Group	Adj R^2	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df	regr.df
Sediment type	0.17474	60891	19.421	0.0001	0.18422	0.18422	172	3
SVI	0.28454	37235	27.398	0.0001	0.11265	0.29688	171	4
Depth	0.38231	32932	28.067	0.0001	9.9636E-2	0.39651	170	5
avNPP	0.41548	11821	10.647	0.0001	3.5765E-2	0.43228	169	6
Trawling pressure	0.42852	5269.3	4.8539	0.0003	1.5942E-2	0.44822	168	7

Best solution				
Adj R^2	R^2	RSS	No.Vars	Selections
0.42852	0.44822	1.8238E5	5	All

Supplementary Table 2.4 Abundance and breakdown of percentual contributions from SIMPER analysis for (dis)similarities comparisons between low trawling pressure (LT) and high trawling pressure (HT) segments within 300-400 m water depth range in muddy-sand sediments (A6.4). The taxa listed contribute at least with 2% of the total abundance. Numbers in bold mark the five dominant taxa.AS: average similarity; AD: Average dissimilarity; *: contributions lower than 2%.

	Abundance (ind/100m)				% Contribution		
	LT(13)	LT(14)	HT(13)	HT(14)	LT	HT	LT/HT
Total (ind/100m)	45.2	101.5	62.4	51.1	AS: 63.0	AS: 42.9	AD: 64.3
Porifera							
Porifera ind. 2	-	**21.2**	0.2	0.2	9.4	*	7.4
Cnidaria							
Spirularia ind. 2	**5.0**	**15.6**	**26.7**	0.3	13.4	16.5	4.4
Spirularia ind. 3	-	0.2	-	**5.9**	*	2.7	2.7
Spirularia ind. 5	0.2	-	0.6	**4.4**	*	4.6	2.8
Actinauge richardi	**5.6**	2.3	2.7	1.1	5.8	11.2	3.1
Zoantharia ind.	1.6	-	**9.3**	0.4	*	2.5	2.9
Caryophyllia sp.	0.6	2.0	0.2	0.1	5.3	*	4.1
Annelida							
Hyalinoecia tubicola	**15.6**	**10.8**	1.0	0.3	11.7	*	6.7
Arthropoda							
Plesionika sp.	0.4	0.8	**3.2**	**17.8**	*	15.0	4.8
Paguroidea ind. 1	0.6	-	**4.1**	0.1	*	*	2.2
Munida sp.	0.4	1.1	-	-	2.8	-	3.1
Mollusca							
Colus sp.	0.8	0.3	0.5	0.2	*	*	2.2
Calliostoma granulatum	-	0.7	-	-	*	-	2.2
Galeodea rugosa	0.6	0.4	0.2	-	*	*	2.1
Bivalvia ind.	0.6	0.7	-	-	*	-	2.3
Echinodermata							
Comatulida ind. 1	-	8.5	1.1	**8.8**	7.6	9.3	4.3
Comatulida ind. 2	-	1.9	-	-	4.1	-	4.0
Ophiuroidea ind. 1	0.2	**18.7**	-	-	10.0	-	8.0
Chordata							
Galeus melastomus	**2.6**	0.4	0.1	-	*	18.9	2.6
Gadiculus argenteus	**8.4**	**10.0**	**5.8**	**10.2**	12.7	*	2.8
Merluccius merluccius	-	0.8	1.5	0.7	*	6.4	2.9
Triglidae ind. 2	-	0.8	-	-	2.3	-	2.7
Lepidorhombus boscii	0.6	0.8	0.9	-	2.2	2.4	2.7
% Contribution of selected taxa	96.9	96.7	93.0	98.8	87.2	89.6	83.0

Supplementary Table 2.5 Abundance and breakdown of percentual contributions from SIMPER analysis for (dis)similarities comparisons between no trawling pressure (NT) and high trawling pressure (HT) segments at depths 400-500m in muddy-sand sediments (A6.4). The taxa listed contribute at least with 2% of the total abundance. Numbers in bold mark the five dominant taxa. AS: average similarity; AD: Average dissimilarity; *: contributions lower than 2%.

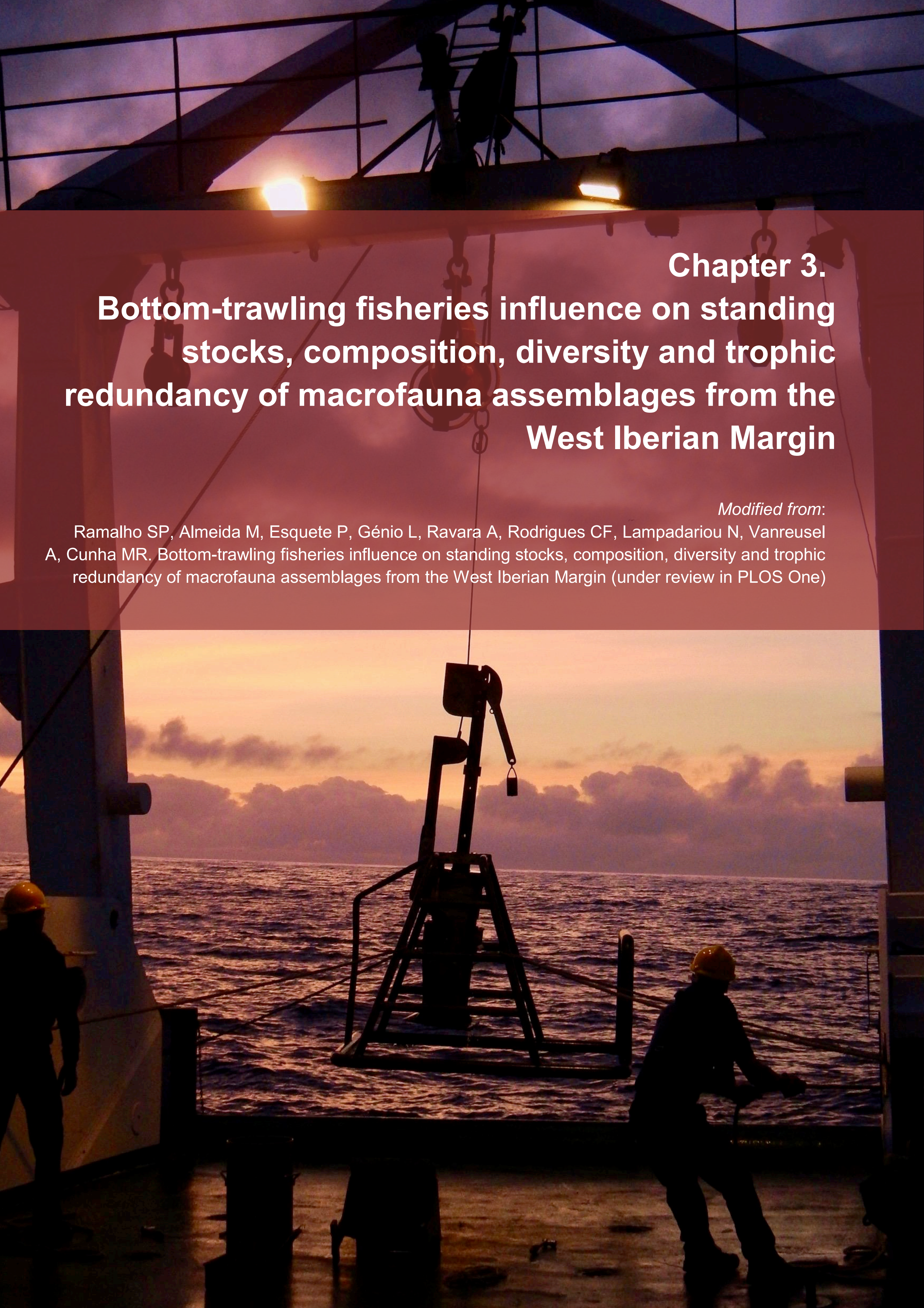
	Abundance (ind/100m)		% Contribution		
	NT(14)	HT(13)	NT(14)	HT(13)	NT (14)/ HT (13)
Total (ind/100m)	52.3	46.9	AS: 59.61	AS: 60.5	AD: 90.5
Cnidaria					
Spirularia ind. 1	**14.7**	-	20.7	-	8.3
Spirularia ind. 2	-	**8.1**	-	23.9	7.1
Spirularia ind. 3	1.3	-	*	*	2
Spirularia ind. 4	1.7	0.8	*	*	2.8
Spirularia ind. 5	1	0.7	*	*	2.4
Actinauge richardi	-	**5.8**	-	19.8	6.2
Zoantharia ind.	-	**18.4**	-	20.6	7.2
Caryophyllia sp.	-	0.1	-	*	
Pennatula sp.	1.3	-	10.9	-	4.5
Kophobelemnon sp.	**12.3**	-	19	-	8
Pennatulacea ind. 1	**2.3**	-	13	-	5.2
Annelida					
Hyalinoecia tubicola	-	0.1	-	*	
Bonellia viridis	1.3	-	3.5	-	3.3
Polychaeta ind.	1.3	-	3.2	-	2.9
Arthropoda					
Aristeus antennatus	1.3	-	3.5	-	3.4
Paguroidea ind. 1	-	**7.8**	-	16.6	5.8
Mollusca					
Colus sp.	0.3	0.7	*	3.0	2.2
Chordata					
Galeus melastomus	**6.0**	0.1	14.5	*	6
Coryphaenoides	0.7	-	4.2	-	3.1
Gadiculus argenteus	**2.3**	**1.8**	3.8	5.6	3
Merluccius merluccius	1.3	0.4	3.8	*	2.8
% Contribution of selected taxa	94.3	95.7	100	89.3	86.3

Chapter 3.

Bottom-trawling fisheries influence on standing stocks, composition, diversity and trophic redundancy of macrofauna assemblages from the West Iberian Margin

Modified from:

Ramalho SP, Almeida M, Esquete P, Génio L, Ravara A, Rodrigues CF, Lampadariou N, Vanreusel A, Cunha MR. Bottom-trawling fisheries influence on standing stocks, composition, diversity and trophic redundancy of macrofauna assemblages from the West Iberian Margin (under review in PLOS One)



Abstract

Bottom-trawling fisheries operating in Portugal (West Iberian Margin) impose one of the largest footprints per unit of biomass landed in European waters at depths greater than 200 m, affecting the seafloor integrity and the associated benthic fauna. To investigate how trawling pressure is affecting the macrofaunal assemblages, we compared the standing stock (abundance and biomass), community structure and taxonomical and trophic diversity in areas subjected to varying trawling pressure at the SW Portuguese upper slope, between 200-600 m. In addition to trawling pressure, several environmental variables, namely depth, grain size and organic matter were correlated with the biological component, which suggest that the longstanding trawling pressure presents cumulative effects to the habitat heterogeneity known to characterise the West Iberian Margin fauna. Furthermore, our results showed a depletion of macro-infaunal abundances (up to 3 times lower) in both low and highly trawled areas. The observed decrease in abundance with increasing trawling pressure was also associated with a loss of species and trophic richness, but univariate diversity indices related with community structure (i.e. Shannon-Wiener index, Pielou's evenness) failed to detect consistent differences across areas. Also observed was a decrease in the number of taxa - trophic guilds combinations of the core assemblage (i.e. characteristic, dominant or frequent taxa) with increasing trawling pressure. We suggest that, in disturbed sediments, the lower functional redundancy resulting from the loss of species within most feeding guilds increases the vulnerability of trophic interactions and therefore the whole assemblage to further increases in natural and anthropogenic disturbance or their synergistic effects.

3.1 Introduction

The West Iberian Margin (WIM) is characterised by a narrow and irregular shelf and steep continental slope incised by several submarine canyons under the influence of the northern component of the Iberian Upwelling system (Pinheiro et al., 1996; Kämpf and Chapman, 2016). It is exposed to high hydrodynamic and productivity regimes, driven by seasonal wind forcing shifts that interact with the local water masses and the complex bathymetry (Fiúza, 1983; Relvas et al., 2007). By their relevant contribution to total standing stocks and primary production, upwelling events have a significant impact on both pelagic and benthic food webs supporting the productive fisheries along the Iberian western coast (Santos, 2001; Picado et al., 2014).

At the WIM, fisheries are characterised by a fleet composed of various small and medium fishing vessels encompassing numerous *métier,* which have great cultural and economic importance (Hill and Coelho, 2001; Leitão et al., 2014). Among these, crustacean bottom-trawling fisheries typically represent a small percentage of the total landings (ca. 5%), but are considered highly profitable reaching approximately 30% of total landing sales values (Campos et al., 2007; Silva et al., 2015). Yet, they are globally recognized as one of the most destructive and unsustainable fishing techniques presently operating worldwide (Jennings and Kaiser, 1998; Pauly et al., 2003; Clark et al., 2015). In Portuguese waters, the main fishing grounds exploited by crustacean bottom trawlers are typically located along the shelf break and upper continental slope and at the flanks of submarine canyon areas in the South and Southwest regions (ca. 85% of the total fishing effort for the period of 2013-2014) (Campos et al., 2007; Bueno-Pardo et al., 2017). Trawling pressure by this *métier* has increased in the past years particularly at the Southwest Portuguese region and is concentrated at depths of ca. 200-600 m (Bueno-Pardo et al., 2017). Moreover, seabed integrity indices estimated for bottom-trawling practices in Portugal (including all types of bottom-contact gears) are among the lowest in European waters, resultant from both the large footprint per unit of landing (ca. 17 $km^{-2}t^{-1}$) and total area trawled annually (93.6%) at depths between 200 and 1000 m (Eigaard et al., 2016), which expresses the enormous pressure imposed by trawling to the benthic habitats.

The increased awareness on the putative impacts of trawling has promoted research, and thus increased knowledge on marine biodiversity and ecosystem functioning, but has been focused mainly on continental shelf areas (Jennings and Kaiser, 1998; Kaiser et al., 2002; NRC, 2002; Lohrer et al., 2004; Tillin et al., 2006). The magnitude of bottom-trawling pressure depends largely on the gear type and the spatial and temporal scales associated with trawling (NRC, 2002; Hiddink et al., 2017). On the other hand, the resistance (capacity to resist change) and resilience (capacity to recover from change) of the ecosystem is largely determined by the life history traits of the inhabiting fauna (e.g. reproductive and dispersal capacity), the characteristics of the targeted habitats (including depth) and their regional setting (biogeography, latitude, connectivity with similar, non-impacted habitats). Known direct effects associated with trawling fisheries include primarily: i) mortality of both target and non-target populations; ii) increased food availability for both predators and scavengers owing to discarding practices and on-site faunal mortality or injury; and iii) alterations or even loss of habitat complexity – e.g. sediment reworking and loss of habitat-forming fauna (NRC, 2002;

Thrush and Dayton, 2002). Indirect effects are derived from the former, and may involve long-term changes on infauna standing stocks, shifts in community composition, and eventually weakening food web stability (NRC, 2002; Thrush and Dayton, 2002: Kaiser et al., 2002). The loss of disturbance-sensitive species, for instance filter-feeding fauna such as sponges, bivalves and polychaetes, is usually observed in highly disturbed areas by trawl fisheries, as these organisms are easily smothered or are unable to efficiently feed during high turbidity periods induced by the re-suspension of sediments during trawl ploughing (Lindeboom and de Groot, 1998; Jennings et al., 2001a; Leys, 2013; Clark et al., 2015). Although rare in marine systems, trophic cascading effects due to loss of species were also reported in areas subjected to high intensity and frequent trawling pressure (Pauly et al., 1998; Pace et al., 1999; Coleman and Williams, 2002).

Loss of species leads to decreased functional redundancy (number of species within each functional entity) and, ultimately, also decreased complexity of food webs (total number of functional entities and their interactions) (Hooper et al., 2005). Species richness has both a buffering effect (reduces temporal variance) and a performing-enhancing effect on ecosystem functions (Yachi and Loreau, 1999). In general terms, species richness, through compensatory dynamics, ensures the ecosystems against declines in their functions (“the Insurance Hypothesis”) and it is a critical feature to the reliability of ecosystems functioning and their long-term capacity to provide goods and services (Naeem and Li, 1997; Naeem, 1998). There is theoretical and accumulating empirical evidence (Liu et al., 2016 and references therein) that this compensatory dynamics may also limit the strength of trophic cascades (designated by Frank et al., 2006 as “Community Regulation Hypothesis”); it increases food web connectance by promoting additional interactions among (e.g. omnivory) and within trophic guilds (e.g. competition, intraguild predation) and diffuses the direct effects of consumption and productivity throughout the trophic spectrum. (Frank et al., 2006). Trophic cascades are generally believed to be less frequent and weaker in functional redundant detritus-based food webs that deviate from a linear food chain (Liu et al., 2016).

High diversity has also been related with greater stability, resistance and resilience of ecosystems (Strong et al., 2015 and references therein). However, high diversity, or even functional redundancy, per se does not ensure resilience, because the replacement of local extinctions in disturbed systems depends on the probability of recolonization from adjacent habitats and/or from a regional pool of species (Naeem and Li, 1997). More importantly, the relationship between diversity and stability is a complex problem that cannot be understood outside the context of the environmental drivers (e.g., climate, resource availability, and natural disturbance (Ives and Carpenter, 2007). Additionally, human activities can modify and act synergistically with all of these drivers (Hooper et al., 2005).

The need to ensure the sustainable functioning of ecosystems is acknowledged by marine policy obligations such as the European Marine Strategy Framework Directive 2008/56/EU (MSFD; European Commission, 2008), but our understanding of the effects of trawling practices on benthic ecosystems in Portugal, is still very limited and predominantly restricted to studies on large-sized mega-epifauna (Morais et al., 2007; Fonseca et al., 2014), or related with coastal bivalve dredging (Chícharo et al., 2002; Gaspar et al., 2003; Falcão et al., 2003). The MSFD definition of Good Environmental Status (GES) includes the requirement that "*the structure, functions and processes of the constituent marine ecosystems allow those ecosystems to function fully and to maintain their resilience to human-induced environmental change*". However, reference data on benthic assemblages prior to fishing exploitation is often scarce, or even inexistent for deeper habitats, and adequate control areas are difficult to find, hindering a rigorous assessment of the environmental status of the impacted ecosystems. Thus, the present study aims to investigate putative changes in macrofauna assemblages resulting from long-term crustacean bottom trawling at the upper slope of the Southwest Iberian margin. Specifically, we assessed the differences in macrofaunal assemblages collected from areas with three levels of trawling pressure (no, low and high) in terms of their standing stocks (abundance and biomass), community structure and structural and trophic diversity and redundancy. The results were interpreted in relation to the environmental setting of the study area.

3.2 Materials and Methods

3.2.1 Study area

The West Iberian margin (WIM) presents complex and diverse geomorphological and hydrographic features (Relvas et al., 2007; Voelker et al., 2009; Maestro et al., 2013). Among the numerous sources of heterogeneity in this region are various topographic features (submarine canyons, rocky outcrops) and sediment types which interact with several oceanographic processes, such as various water masses and fronts determining spatial and temporal variability in salinity, temperature and oxygen content (Relvas et al., 2007). Periodic and episodic natural disturbance events (e.g. strong near-bottom currents, high energy winter storms) promote the erosion of sediments from the shelf and their transport and deposition into deeper areas (Vitorino et al., 2002; Diogo et al., 2014). Seasonally variable surface productivity regimes (upwelling and downwelling) are responsible for the horizontal and vertical patchiness of particulate organic matter (POC) flux to the seabed in this region (Kämpf and Chapman, 2016). Typically, the major peaks in surface primary production occur during spring and summer as a consequence of seasonal upwelling events forced by intense northerly winds. During these periods, large filaments of phytoplankton blooms reach several kilometres offshore (often 30–40 km but as far as 200-300 km) or are transported along shelf areas through complex circulation patterns. During winter, low productivity regimes are derived from downwelling under south-westerly winds and mixing by strong storm events may occasionally take place (Fiúza, 1983; Relvas et al., 2007; Kämpf and Chapman, 2016). However, pulse episodes of reverse winds can occur during all seasons (Kämpf and Chapman, 2016). The WIM is also under the influence of both physical and chemical disturbance from anthropogenic sources (e.g. fisheries, litter, pollution; e.g. Morais et al., 2007; Mordecai et al., 2011; de Jesus Mendes et al., 2011).

Bottom-trawling fishery grounds at the WIM are delimited by legal measures that prohibit trawling practices within six nautical miles from the coastline (Fig. 3.1A; MAMAOT, 2012). This adds to the narrow shelf and steep slope prompting the concentration of bottom-trawling activity at the shelf break and upper slope (200–800 m depth), primarily in the South and Southwest regions off Portugal, within soft sediment areas (mud and muddy-sand), the preferred habitat of several targeted species. This *métier* targets several species of deep-water crustaceans such as the Norway lobster (*Nephrops norvegicus*), red and rose shrimps (*Aristeus antennatus* and *Parapenaeus longirostris*, respectively), but also a few fish species such as the blue whiting (*Micromesistius poutassou*) and the European hake (*Merluccius merluccius*) (Campos et al., 2007; Bueno-Pardo et al., 2017). Lastly, this *métier* is highly unselective, usually resulting in large rates of by-catch and discarding. Conservative estimates reported that 28-40% of the total catches of crustacean trawlers are by-catch, while more severe estimates have reported up to 70% of by-catch (Borges et al., 2001; Monteiro et al., 2001).

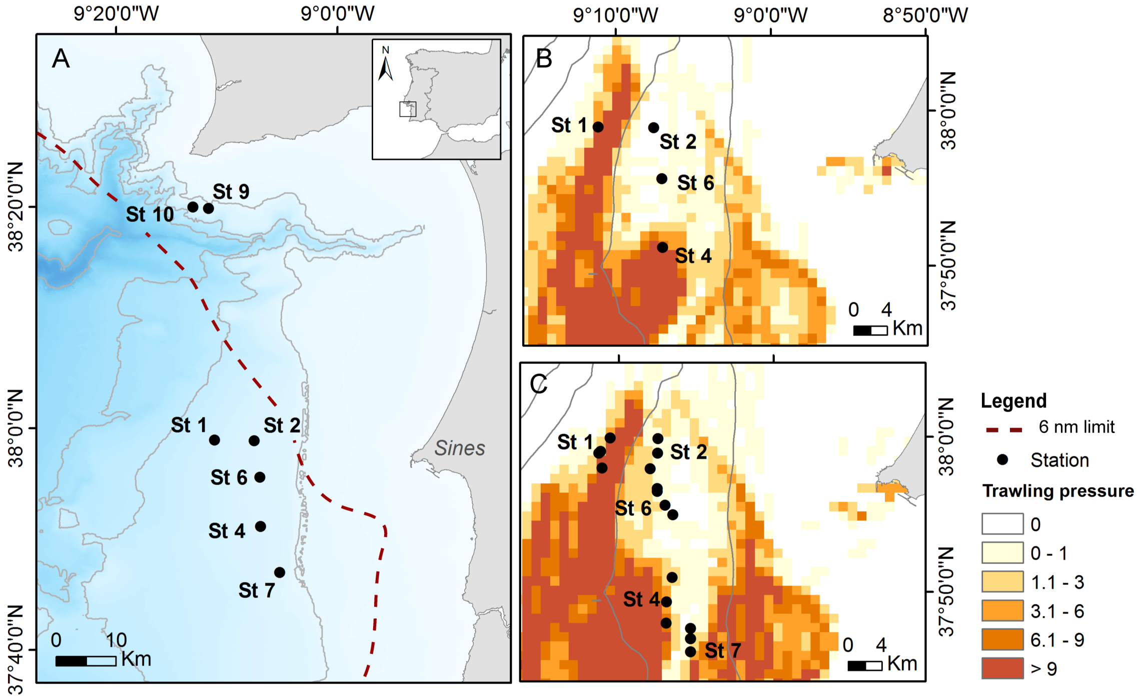


Figure 3.1 A) Map of the study area indicating the sampled stations (3-4 replicates per station) and distribution of the crustacean trawlers annual trawling pressure (h.cell^{-1}.y^{-1}) for (B) 2013 and (C) 2014. Setúbal canyon area (st. 9 and st. 10) is not shown (0 h.cell^{-1}.y^{-1}). Red dashed line establishes the legal six nautical miles from the coastline.

3.2.2 Sample collection and processing

During the RV Belgica cruises B2013/17 (10/06/2013–18/06/2013) and B2014/15 (02/06/2014–10/06/2014) several sites were selected to investigate macrofauna assemblages and sediment properties from areas subjected to different degrees of trawling pressure (TP). TP (h.cell^{-1}.y^{-1}) was estimated according to Bueno-Pardo et al. (2017), for individual cells with an area of 0.01 x 0.01 decimal degrees (ca.1 km^2), based on Vessel monitoring systems (VMS) position data of crustacean bottom trawlers operating at the study area, compiled by Direção Geral de Recursos Marinhos - DGRM (MAMAOT, 2012). In total, seven stations were sampled with replicates (n=3 or n=4) from: no- (st. 9 and st. 10), low- (st. 2 and st. 6) and high- (st. 1, st. 4 and st. 7) trawling pressure locations along the upper continental slope off Sines and near the Setúbal canyon between depths of ca. 200 and 600 m water depth (Fig. 3.1 and Table 3.1). No trawling pressure (NT), low trawling pressure (LT) and high trawling pressure (HT) areas corresponded to 0, 0.1–1.5 and >1.5 h.cell^{-1}.y^{-1}, respectively. Note that NT label was only

Table 3.1 Metadata on sampled stations.

Cruise	Station code	Deployment	Sample code	Area code	Date	Latitude (N)	Longitude (W)	Depth (m)	Sampler	Sampled area (dm²)
B2013/17	1_13	4	1.1_13	HT_13	13/06/13	37°59'006	09°11'107	445	BOX	8.04
	1_13	8	1.2_13	HT_13	13/06/13	37°58'962	09°11'111	445	BOX	8.04
	1_13	9	1.3_13	HT_13	13/06/13	37°58'948	09°11'099	445	BOX	8.04
	2_13	22	2.1_13	LT_13	15/06/13	37°58'888	09°07'528	335	BOX	8.04
	2_13	23	2.2_13	LT_13	15/06/13	37°58'896	09°07'506	335	BOX	8.04
	2_13	24	2.3_13	LT_13	15/06/13	37°58'894	09°07'514	335	BOX	8.04
	6_13	49	6.1_13	LT_13	17/06/13	37°55'598"	09°06'997"	298	MUC	3.14
	6_13	50	6.2_13	LT_13	17/06/13	37°55'598"	09°07'003"	299	MUC	3.14
	6_13	53	6.3_13	LT_13	17/06/13	37°55'601"	09°07'001"	298	MUC	2.36
	6_13	54	6.4_13	LT_13	17/06/13	37°55'602"	09°07'011"	298	MUC	2.36
	6_13	56	6.5_13	LT_13	17/06/13	37°55'621"	09°07'012"	298	MUC	1.57
	4_13	36	4.1_13	HT_13	16/06/13	37°51'168"	09°06'950"	325	MUC	2.36
	4_13	37	4.2_13	HT_13	16/06/13	37°51'168"	09°06'959"	325	MUC	1.57
	4_13	38	4.3_13	HT_13	16/06/13	37°51'169"	09°06'948"	325	MUC	2.36
	4_13	39	4.4_13	HT_13	16/06/13	37°51'166"	09°06'943"	325	MUC	1.57
	4_13	40	4.5_13	HT_13	16/06/13	37°51'166"	09°06'963"	325	MUC	3.14
	4_13	41	4.6_13	HT_13	16/06/13	37°51'172"	09°06'948"	325	MUC	2.36

Cruise	Station code	Deploy-ment	Sample code	Area code	Date	Latitude (N)	Longitude (W)	Depth (m)	Sampler	Sampled area (dm^2)
B2014/15	1_14	70	1.1_14	HT_14	09/06/14	37°59'949	09°10'528	443	BOX	8.04
	1_14	68	1.2_14	HT_14	09/06/14	37°59'065	09°11'143	449	BOX	8.04
	1_14	69	1.2_14	HT_14	09/06/14	37°58'969	09°11'271	451	BOX	8.04
	1_14	67	1.3_14	HT_14	09/06/14	37°58'010	09°11'045	430	BOX	8.04
	2_14	66	2.1_14	LT_14	09/06/14	37°59'902	09°07'454	350	BOX	8.04
	2_14	65	2.3_14	LT_14	09/06/14	37°58'969	09°07'480	336	BOX	8.04
	2_14	64	2.3_14	LT_14	09/06/14	37°57'955	09°07'953	342	BOX	8.04
	4_14	63	4.1_14	HT_14	09/06/14	37°50'952	09°06'523	318	BOX	8.04
	4_14	34	4.2_14	HT_14	04/06/14	37°49'364	09°06'897	330	BOX	8.04
	4_14	33	4.3_14	HT_14	04/06/14	37°47'997	09°06'911	330	BOX	8.04
	6_14	31	6.1_14	LT_14	04/06/14	37°56'498	09°07'486	323	BOX	8.04
	6_14	32	6.2_14	LT_14	04/06/14	37°56'670	09°07'486	325	BOX	8.04
	6_14	30	6.2_14	LT_14	04/06/14	37°55'590	09°06'997	300	BOX	8.04
	6_14	29	6.3_14	LT_14	04/06/14	37°54'977	09°06'494	285	BOX	8.04
	7_14	28	7.1_14	HT_14	04/06/14	37°48'488	09°05'447	299	BOX	8.04
	7_14	25	7.2_14	HT_14	04/06/14	37°47'598	09°05'496	291	BOX	8.04
	7_14	26	7.2_14	HT_14	04/06/14	37°47'584	09°05'493	290	BOX	8.04
	7_14	27	7.3_14	HT_14	04/06/14	37°46'842	09°05'437	295	BOX	8.04
	9_14	73	9.1_14	NT_14	09/06/14	38°20'505	09°12'084	329	BOX	8.04
	9_14	72	9.1_14	NT_14	09/06/14	38°19'872	09°11'645	326	BOX	8.04
	9_14	71	9.2_14	NT_14	09/06/14	38°19'426	09°11'150	340	BOX	8.04
	10_14	76	10.1_14	NT_14	09/06/14	38°20'469	09°13'644	360	BOX	8.04
	10_14	75	10.2_14	NT_14	09/06/14	38°19'998	09°13'063	550	BOX	8.04
	10_14	74	10.3_14	NT_14	09/06/14	38°19'475	09°12'530	407	BOX	8.04

Trawling areas code: NT: no, LT: low and HT: high trawling pressure and sampling year. BOX: boxcorer sampler and MUC: Multiple corer sampler.

assigned to the stations safeguarded by current legal restrictions and where trawling has not occurred for the past decades (i.e., stations in the vicinity of the Setúbal canyon head). In opposition, LT stations correspond to areas that have been undisturbed or only subjected to very few trawl passages in time and space but are adjacent to the main fishing grounds where the highest pressure occurred (HT).

3.2.2.1 Environmental variables

Replicated sediment samples (min. n=3) were collected to characterise the environmental setting. In 2013, these samples were collected using the MUC sampler equipped with four Plexiglas tubes (∅ 10cm), while in 2014 a small sub-sample of sediment was collected from the NIOZ boxcorer used to sample for macrofauna. Samples for grain-size and biogeochemical analyses were stored at -20°C and -80°C, respectively. The grain-size distribution was later determined using a particle size analyser (Malvern Mastersizer 2000) with a particle size range of 0.02–2000 µm and then classified into five categories following the Wenthworth scale (1922): silt+clay, very fine sand, fine sand, medium sand and coarse sand. Total organic carbon and total nitrogen (TOC and TN, respectively, expressed as percentage of sediment dry weight) were measured using a Carlo Erba 25 elemental analyser, after acidification with 1 % HCl to eliminate carbonates present. Chlorophyll a content (Chl-a, expressed as µg per g of sediment dry weight) was determined via reverse-phase HPLC (High-Performance Liquid Chromatography) after extraction (90 % acetone) from lyophilised and homogenised sediment samples using a Gibson fluorescence detector (Wright and Jeffrey, 1997).

3.2.2.2 Fauna

At each station macrofauna samples were collected using a NIOZ box corer (∅ 32 cm). For each core the overlaying water was sieved through a 250 µm mesh in order to retain any swimming specimens, and the fauna at the sediment surface was carefully picked. The sediment was then sub-sampled at three depth layers (0-1; 1-5 and 5-15 cm) and washed through a set of sieves of 1 mm, 500 µm and 250 µm mesh-size. The retained material was immediately fixed with 96% ethanol and stored for further laboratory processing. In addition, due to strong winds and rough sea conditions the box corer (BOX), our preferred gear to collect samples for investigating macrofauna biodiversity, was substituted halfway through the RV Belgica 2013/17 campaign, by the lighter and thus easier to handle, multiple-corer sampler (MUC). Thus, st. 1 and st. 2 were sampled by means of the box-corer sampler, while the multiple-core sampler equipped with four Plexiglas tubes (∅ 10 cm) were used to collect samples from st. 4 and st. 6, where sediment samples from the same deployment were pooled together to increase sampled surface area (Fig. 3.1; Table 3.1). Since a sampler effect was clear during the sample processing, the direct comparison of trawling pressure effects on the macrofauna assemblages collected with the MUC was not further explored for st. 4 and st. 6, although a similar sample processing methodology as in the main dataset was implemented on these samples (see supplementary results and discussion of this chapter).

Back in the laboratory, each sub-sample was sorted to family level under the stereomicroscope. Macrofaunal biomass was weighted for specimens grouped at the family level. In order to keep the physical integrity of the specimens the biomass was determined as wet weight and expressed as mg.10 dm^{-2} (all individuals belonging to the same family in each sub-sample were transferred to previously weighed microtubes containing 96% ethanol that were then weighed again to obtain the wet weight of the lot). Note that both molluscs and echinoderms were weighted with their shell and exoskeleton, respectively. Mean individual biomasses (MIB; expressed in mg) were obtained by dividing the wet weight of each lot by the respective number of individuals. Subsequently, all individuals were counted and identified to the lowest taxonomical level possible. In the cases where a match with a species name was not possible, each taxon was ascribed with a consistent code across all sampled stations. Typical “meiofaunal” taxa, i.e. Nematoda, Copepoda and Ostracoda, were excluded. Macrofaunal densities were expressed as individuals per 10 dm^2 (ind.10 dm^{-2}). Furthermore, each species was assigned to a trophic guild according to its food source (or foraging behaviour), feeding mode and food type/size, following the classification proposed by MacDonald et al. (2010) and other relevant literature available (e.g. Fauchald and Jumars, 1979; Jumars et al., 2015). The following categories were considered for: a) food source: epibenthic (EP), sediment surface (SR), and sediment subsurface (SS); b) feeding mode: omnivorous (Om), deposit feeders (De), detritus feeders (Dt), grazers (Gr), scavengers (Sc), predators (Pr), suspension/filter feeders (Su), mixotrophs (Mx) and suctorial parasites (Sp); and c) food type/size: sediment (sed), particulate organic matter (poc), microfauna (mic), meiofauna (mei), macrofauna (mac), zooplankton (zoo) and fish (fis).

3.2.3 Data analysis

A non-metric multidimensional scaling (nMDS) analysis was carried out, based on the Bray-Curtis similarity matrix estimated after square-root transformation on the macrofaunal abundances. Significant differences among the macrofaunal assemblages were tested by means of a permutational multivariate analysis of variance (PERMANOVA). In the cases where the number of permutations was low (< 100) the Monte Carlo p-values (P_{MC}) were considered instead of the permutation p-value. Because of the unbalanced sampling design between years, i.e. in 2013 (2 stations; 2 areas: LT_13 and HT_13) and in 2014 (7 stations; 3 areas: NT_14, LT_14, HT_14), the PERMANOVA analysis was performed separately for each year. Specifically, the following design was applied: a 1-factor layout with "trawling pressure" (TP) as the fixed factor for the 2013 dataset; and a 2-factor layout for 2014, with TP as fixed factor and "station" (St) as a random factor nested in TP. When significant differences were detected by the PERMANOVA main test, the respective pairwise comparisons were also tested. The homogeneity of the multivariate dispersions were also tested by means of the PERMDISP test. A SIMPER analysis was then performed to determine the species contributions (%) for the observed similarity within groups and dissimilarity between groups. The relation between environmental parameters and macrofaunal assemblages was investigated through a distance-based linear model analysis (DISTLM), computed using the full untransformed normalized environmental dataset. These analyses were performed with the software PRIMER v6 and PERMANOVA+ (Anderson et al. 2008; Clarke and Gorley 2006).

The "core assemblage" composition, i.e. the most prominent species, for each trawling pressure group and year was then established according to the following criteria of dominance, constancy (C) and fidelity (F): i) dominant (top 10 most abundant species), ii) distinctive (exclusive or elective species - F≥ 67% with a constancy≥ 50%) and iii) all other constant species (C≥ 50%). Constancy is herein defined as the frequency of occurrence of each species in a given group of samples (number of samples where the species is present divided by the total number of samples, expressed as a percentage; Dajoz, 1971). Fidelity is herein defined as the degree of association of a species to a given group of samples (number of samples of a given assemblage where the species is present divided by the total number of samples where the species is present; Retièrie, 1979). Trophic redundancy (TR, average number of species per trophic guild), trophic over-redundancy (TOR, percentage of trophic groups represented by a number of species greater than TR) and trophic vulnerability (TV, percentage of trophic guilds represented by a single species) were estimated for each core assemblage (see Mouillot et al., 2014 for details and equations given for the concepts of functional redundancy, functional vulnerability and functional over-redundancy).

Taxonomic and trophic biodiversity patterns were examined using several diversity indices, namely: species richness/trophic guilds richness (S/TG), Shannon-Wiener diversity (H'), evenness (J'; Pielou, 1966) and Hurlbert's expected number of taxa or trophic guilds ($ES_{(n)}/ETG_{(n)}$) for 50 and 100 individuals (Hurlbert, 1971). These biodiversity indices were estimated using the software PRIMER v6 (Clarke and Gorley, 2006). Diversity partitioning was assessed for the number of species, Hurlbert's expected number of species ($ES_{(50)}$) and Shannon–Wiener index, and their equivalents for trophic diversity. The total diversity ($\gamma=\alpha+\beta$) is partitioned into the average diversity within the lowest level of sampling (α) and among sampling levels (β) and therefore β-diversity can be estimated by $\beta= \gamma-\alpha$ (Wagner et al., 2000; Magurran, 2004). To extend the partition across multiple scales (β_1= within stations, β_2= between stations and β_3= between TP groups) the smallest sample unit for level 1 are replicates from each station (α diversity), while for the upper levels sampling units are formed by pooling together the appropriate groups of nested samples. The diversity components are calculated as $\beta_m= \gamma-\alpha_m$ at the highest level and $\beta_i= \gamma-\alpha_{i+1}- \alpha_i$ for each lower level. The additive partition of diversity is $\gamma = \alpha1 + \beta1 + \beta2 + \ldots + \beta m$. The total diversity can therefore be expressed as the percentage contributions of diversity in each hierarchical level (Crist et al., 2003). Partitioning was carried out by weighting each sample according to its respective abundance. Values of α_i were therefore calculated as a weighted average (according to the number of replicates pooled). Diversity partitioning was estimated for each year separately with two β-diversity levels in 2013 and three levels in 2014.

Differences in macrofaunal densities and biomasses among trawling pressure groups were assessed by non-parametric Mann-Whitney U-tests (2013 dataset) and Kruskal-Wallis tests (2014 dataset) using the software GraphPad PRISM v6. Non-parametric Spearman's rank correlations between macrofaunal variables (density, S, TG, taxonomic and trophic H', $ES_{(50)}$, $ETG_{(50)}$) and trawling pressure were computed using the same software. Significant correlation values were adjusted by using the Bonferroni correction (Shaffer, 1995), which was calculated by dividing the significance value of each test by the number of hypothesis tested.

3.3 Results

3.3.1 Environmental characterization

Environmental parameters measured for each station and trawling pressure (TP) group are summarised in Table 3.2. The study region was generally characterised by muddy-sand bottoms (silt+clay > 10 %), with the total organic carbon (TOC) content ranging from 0.28-0.83%. C/N ratio values measured for the whole study region ranged from 5.6 to 10.0, which indicates the predominant algal origin of sedimentary organic matter derived from surface primary productivity. Overall, grain size composition of LT stations showed the highest proportion of coarser sediments (over 60% content in fine, medium and coarse sands; Table 2). The main bottom-trawling fishery grounds (HT) showed a more heterogeneous group of stations with finer grained sediments but with st. 7, closer in composition to LT stations and st. 1 and st. 4 closer to the ones from NT stations (over 50% content in very fine sands and silt+clay; Table 3.2). On the other hand, the sediment biogeochemistry results in NT stations showed higher average contents of chlorophyll a, TN and TOC than HT stations, which also resulted in slightly higher values of C/N ratios. All these environmental variables showed the lowest values at LT stations.

Table 3.2 Summary of the environmental parameters (average ± standard error) investigated, including grain-size composition (%), total organic carbon (TOC,%), total nitrogen (TN, %), carbon/nitrogen (C/N), chlorophyll a content (chl-a; μg.g^{-1}) and trawling pressure (h.cell^{-1}.y^{-1}).

Station code	n	Silt+Clay (%)	Very Fine sand (%)	Fine sand (%)	Medium sand (%)	Coarse sand (%)	TOC (%)	TN (%)	C/N	Chl-a	Trawling pressure
9_14	3	39.07 ± 2.258	21.17 ± 4.984	25.77 ± 1.770	12.44 ± 4.894	1.55 ± 1.398	0.52 ± 0.019	0.059 ± 0.0020	8.8 ± 0.10	0.06 ± 0.013	0.00±0.000
10_14	3	52.66 ± 13.486	21.79 ± 3.625	19.05 ± 7.189	6.08 ± 2.750	0.42 ± 0.122	0.83 ± 0.182	0.085 ± 0.0230	10.0 ± 0.61	0.21 ± 0.171	0.00±0.000
6_14	4	15.34 ± 0.581	14.01 ± 1.046	32.64 ± 0.961	29.41 ± 1.258	8.60 ± 1.148	0.29 ± 0.009	0.043 ± 0.0016	6.8 ± 0.12	0.02 ± 0.003	0.23±0.132
2_13	3	13.39 ± 0.309	12.34 ± 0.311	32.70 ± 0.473	32.70 ± 0.407	8.86 ± 0.397	0.28 ± 0.014	0.049 ± 0.0012	5.6 ± 0.16	0.01 ± 0.012	0.00±0.000
2_14	3	16.63 ± 0.272	12.26 ± 0.742	31.36 ± 1.983	30.42 ± 0.512	9.32 ± 2.114	0.28 ± 0.007	0.042 ± 0.0029	6.9 ± 0.31	0.01 ± 0.014	1.08±0.566
7_14	4	20.55 ± 1.390	12.27 ± 1.641	23.88 ± 1.070	29.72 ± 2.364	13.58 ± 1.581	0.34 ± 0.012	0.050 ± 0.0020	6.8 ± 0.06	0.02 ± 0.003	2.51±0.848
4_14	3	40.66 ± 1.725	26.09 ± 1.743	24.20 ± 1.164	8.83 ± 1.187	0.21 ± 0.200	0.59 ± 0.020	0.081 ± 0.0028	7.3 ± 0.16	0.03 ± 0.003	4.58±1.988
1_13	3	23.96 ± 1.329	24.92 ± 0.485	37.03 ± 0.718	14.01 ± 0.331	0.07 ± 0.030	0.41 ± 0.017	0.053 ± 0.0027	7.6 ± 0.13	-	3.63±0.000
1_14	4	31.69 ± 3.015	23.70 ± 0.593	32.79 ± 2.008	11.58 ± 0.656	0.22 ± 0.141	0.47 ± 0.021	0.055 ± 0.0026	8.6 ± 0.54	0.01 ± 0.005	8.58±3.802
LT_13	3	13.39 ± 0.309	12.34 ± 0.311	32.70 ± 0.473	32.70 ± 0.407	8.86 ± 0.397	0.28 ± 0.014	0.049 ± 0.0012	5.6 ± 0.16	0.01 ± 0.012	0.00±0.000
HT_13	3	23.96 ± 1.329	24.92 ± 0.485	37.03 ± 0.718	14.01 ± 0.331	0.07 ± 0.030	0.41 ± 0.017	0.053 ± 0.0027	7.6 ± 0.13	-	3.63±0.000
NT_14	6	45.87 ± 6.829	21.48 ± 2.760	22.41 ± 3.636	9.26 ± 2.886	0.98 ± 0.677	0.67 ± 0.106	0.072 ± 0.0118	9.4 ± 0.38	0.14 ± 0.084	0.00±0.000
LT_14	7	15.89 ± 0.419	13.26 ± 0.719	32.09 ± 0.945	29.85 ± 0.729	8.91 ± 1.018	0.29 ± 0.006	0.042 ± 0.0014	6.8 ± 0.13	0.02 ± 0.006	0.60±0.284
HT_14	11	30.08 ± 2.796	20.20 ± 2.045	27.21 ± 1.556	17.43 ± 3.081	5.08 ± 2.100	0.45 ± 0.033	0.060 ± 0.0043	7.6 ± 0.31	0.02 ± 0.004	2.84±1.600

Stations are ordered by the increasing average trawling disturbance of the station.

3.3.2 Macrofaunal assemblages

A total of 4695 macrofaunal individuals examined during this study were ascribed to 310 different taxa, of which 77 were singletons (24.8% of the total species richness). The full list of all macrofauna taxa encountered in the present study is provided in the Annex 3. The most abundant phylum was the Annelida (59.9% of the total abundance; 95 species), while Arthropoda was the most species-rich (24.5% of the total abundance; 147 species). Mollusca showed an intermediate relative importance in terms of abundance and number of species (10.1% of total abundance; 48 species). The remaining phyla were less represented both in terms of abundance and number of species, namely: Echinodermata (2.1%; 9 species); Cnidaria (1.0%; 5 species); Sipuncula (2.0%; 1 species); Nemertea (0.3%; 3 species); Platyhelminthes (< 1%; 1 species) and Cephalorhyncha (Class Priapulida; < 1%; 1 species).

3.3.3 Multivariate analyses

The results of the nMDS plotted in Figure 3.2 show a clear segregation of the three TP groups of samples. The statistical significance of the differences in the macrofaunal assemblages from NT, LT and HT groups is supported by the PERMANOVA results for the 2014 dataset (p_{perm}<0.05; Table 3.3) across all levels (pairwise comparisons of the levels NT, LT_14 and HT_14; p<0.05, Supplementary Table 3.1) but not for 2013 (LT_13 vs. HT_13; P_{MC}=0.23; Table 3.3). Furthermore, significant differences between stations within each TP group were also identified for 2014 (station (TP); p_{perm}<0.05; Table 3.3). Although pairwise comparisons between stations (random factor) were not computed, their position in the nMDS plot suggests that the variability and, in some cases, the segregation of stations within the same TP group may be linked with the depth gradient and interannual variability. In fact, even though trawling pressure was overall an important factor in the PERMANOVA (based on ECV value), both PERMDISP analysis (Supplementary Table 3.2) and the high ECV value of the residuals (Table 3.3), indicate that a large proportion of the variability in the assemblages remains unexplained.

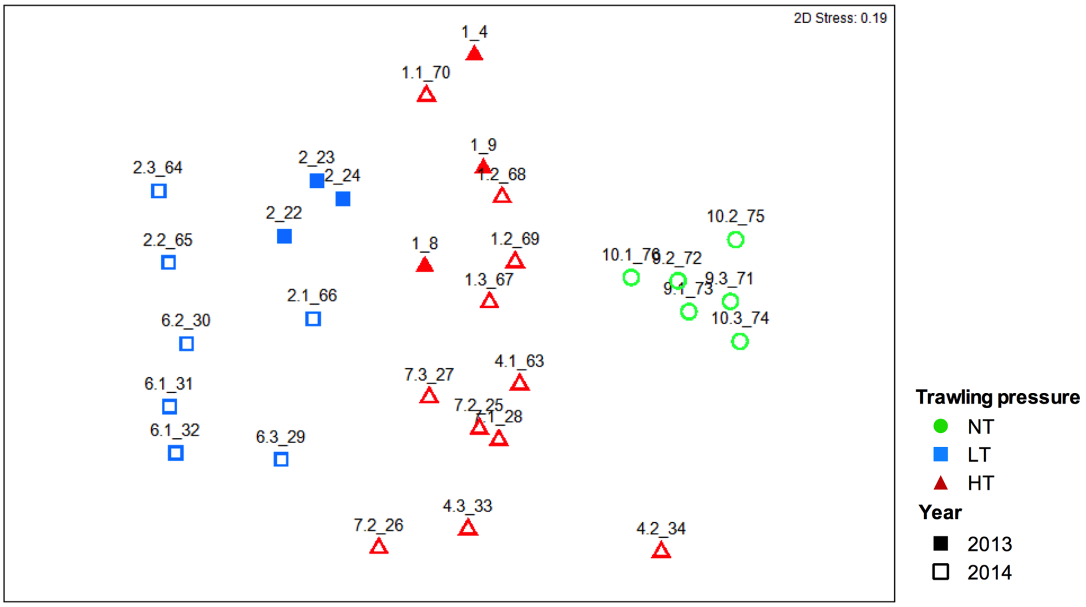


Figure 3.2 nMDS plot for comparison of macrofauna assemblages subjected to varying trawling pressure. NT, LT and HT: no, low and high trawling pressure, respectively. Closed symbols: 2013 samples; open symbols: 2014 samples. Numbers above each symbol correspond to the replicate codes (station and deployment number).

Species contributions to the differences between TP groups were examined through SIMPER analyses (Supplementary Table 3.3 and Supplementary Table 3.4). Pairwise dissimilarities in community composition in 2014 ranged between 62.9 and 72.6% (LT_14 vs. HT_14 and NT_14 and LT_14, respectively). In 2013, the dissimilarity among groups was slightly lower (58.1% for LT_13 vs. HT_13). These values resulted mainly from numerous species with low contributions to the total dissimilarity (e.g. species with individual contributions > 1.5% only accounted for 12.7-15.6% of the total dissimilarity between groups; Supplementary Table 3.3). Such arises from the overall low densities of the species and high evenness of the assemblages. In fact, the highest contributions to the similarity within groups and/or dissimilarity between groups are due to fluctuations in the density of common species, mostly surface deposit feeding polychaetes (e.g. Aricidae, Cirratulidae, Ampharetidae, Spionidae), shared across groups (Supplementary Table 3.3 and Supplementary Table 3.4).

Table 3.3 Results of the PERMANOVA main tests of the: 1-factor design (TP: trawl pressure - Test 1) applied 2013 samples; and 2-factor design (TP: trawl pressure and station (TP) - Test 2) applied to the 2014 dataset. Significant values are in bold; ECV: Estimated component of variation.

Source of variation	df	SS	MS	Pseudo-F	P_{perm}	Perm	P_{MC}	ECV
Test 1 - 2013								
TP	1	2210.1	2210.1	1.5401	0.1049	10	0.2295	258.4
Res	4	5740	1435					1435
Total	5	7950.1						
Test 2 - 2014								
Trawl	2	13224	6612.1	2.9744	**0.0099**	1258	-	569.4
Station (TP)	4	8916.3	2229.1	1.5639	**0.0001**	9741	-	234.7
Res	17	24230	1425.3					1425.3
Total	23	46371						

To further explore the observed variability in the macrofauna assemblages, the measured environmental parameters and biological dataset were modelled through the DISTLM routine (marginal tests) and illustrated in the dbRDA plot (Fig. 3.3). Nine out of the eleven examined environmental variables contributed significantly to the variation in macrofaunal composition (Supplementary Table 3.5). Furthermore, the variables that best contributed to the construction of the fitted model (adjusted R^2= 0.17866), included, by order of importance, silt+clay content (12.3%), water depth (7.0%), C/N ratio (4.8%), trawling pressure (TP; 4.2%), coarse sand (3.5%) and very fine sand contents (3.2%), accounting for 35.0% of the total variability. The dbRDA plot, further confirms the heterogeneity within HT group encompassing stations with more variable grain size composition and a greater depth range. Although the contribution of trawling pressure for the fitted model is low, the interpretation of this result is complex because of the possible interactions with other examined variables (e.g., grain size, TOC).

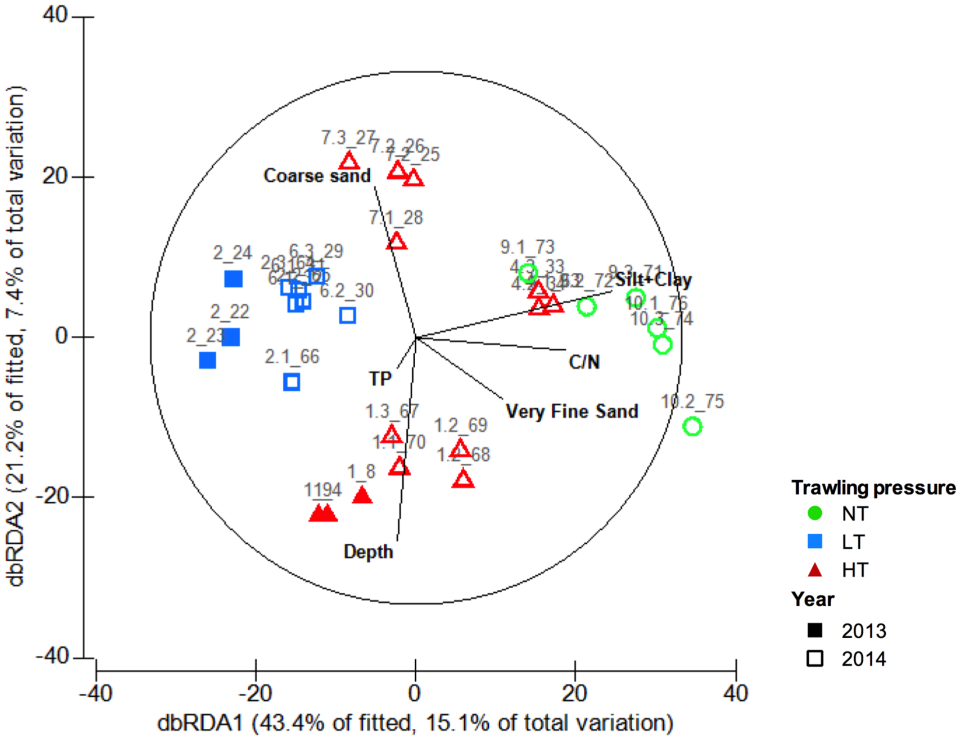


Figure 3.3 Distance-based redundancy (dbRDA) plot illustrating the DISTLM model illustrating the relation between macrofaunal assemblages and the fitted environmental variables (vectors). Environmental parameters included in the analysis were: depth (m), silt+ clay content, very fine sand (%), coarse sand (%), trawling pressure (TP), and Carbon/Nitrogen ratio (C/N). Closed symbols: 2013 samples; Open symbols: 2014 samples. NT, LT and HT: no, low and high trawling pressure, respectively.

3.3.4 Biomass, abundance, and biodiversity

The average macrofaunal biomass (wwt, mg.10dm^{-2}) varied greatly across the stations investigated (395.9–1495.5 mg.10dm^{-2}). Despite the higher average biomass recorded in NT stations (1077.8±458.71 mg.10dm^{-2}), no significant differences were detected between TP groups either in 2013 (U-test=3.0; p=0.700) or 2014 (K=3.485; p=1.146) (Fig. 3.4A,B). Because the mean individual biomass (MIB) of most organisms was much smaller than 1 mg (71.2–85.2%; Fig. 3.4C,D), differences in the total biomasses were determined by the presence of weightier individuals (mostly with MBI >>100 mg). For instance, in st. 10_14 (NT) biomass was mostly accounted for by one anthozoan preying on zooplankton (Spirularia sp1, 1372.2 mg, 38.0% of the total biomass) and five individuals of the suspension feeder *Amphiura borealis* (786.9 mg, 21.8%). Weightier individuals were overall absent from LT areas but were also observed in HT stations (Fig. 3.4C,D): a single specimen (1408.0 mg) of a polychaete belonging to the family Acoetidae, preying on macrofauna, accounted for 64.3% of the total biomass at st. 4_14 and one *Aristeus* sp., a generalist omnivore shrimp (877.5 mg), accounted for 46.0% at st. 7_14.

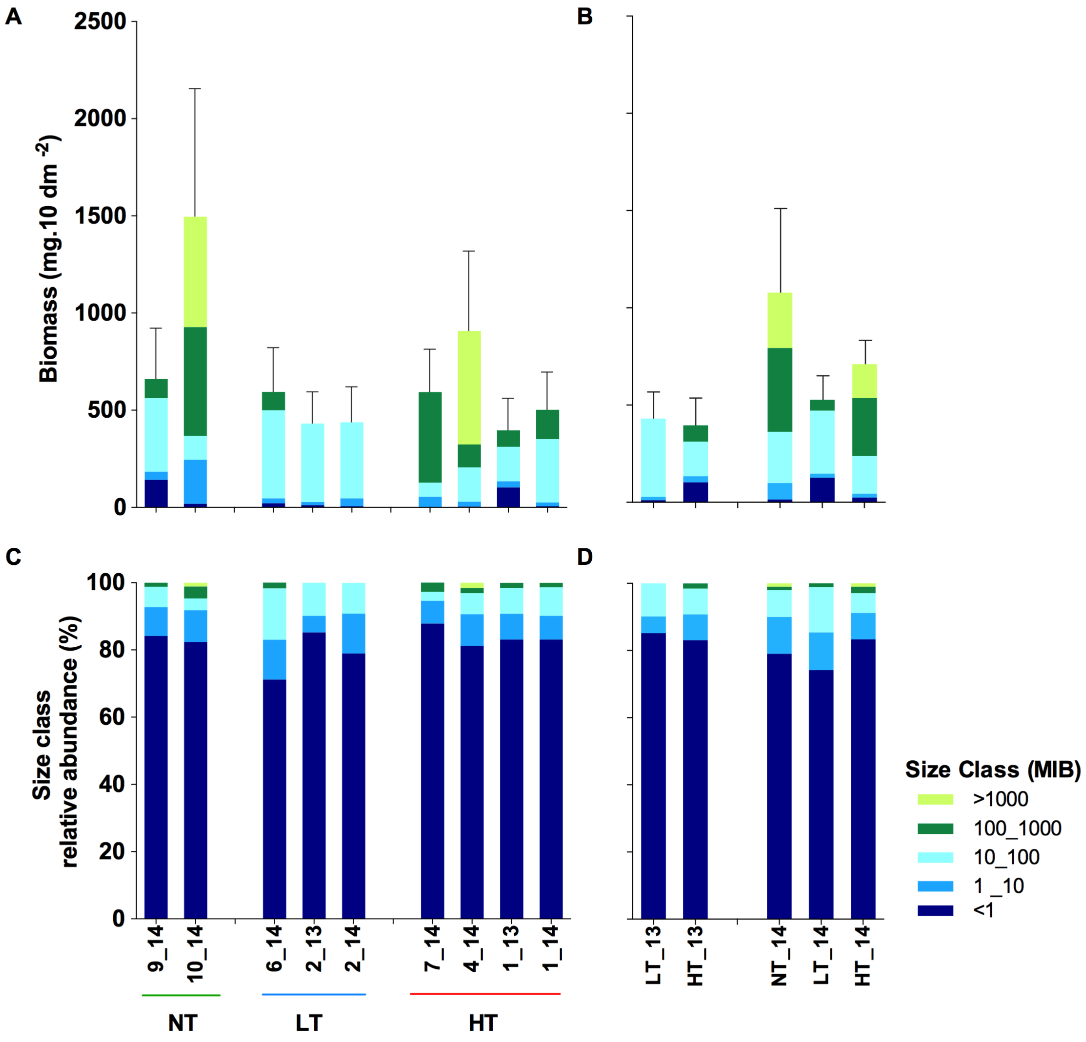


Figure 3.4 Total macrofaunal biomass (average ± standard error) (A) per station and (B) trawling pressure group from each year, and matching results for the relative abundance of the different size classes per (C) station and (D) trawl pressure group. MIB: mean individual biomass (mg); NT, LT and HT: no, low and high trawling pressure, respectively.

The highest macrofaunal densities were consistently observed at NT stations (401.4±41.17 ind.10dm^{-2}; Fig. 3.5; Table 3.4). In fact, densities at NT stations were 1.8 to 3.7 times higher and significantly differed from those in either LT or HT stations in 2014 (K=12.94; $p<0.05$; with $p<0.05$ in Dunn's post hoc test for NT_14 vs. LT_14 and NT_14 vs. HT_14), while LT and HT densities did not significantly differ either in 2014 (Dunn's post hoc test) or in 2013 (U=2.00; p=0.400). The same pattern was observed for the average species richness per sample with significantly higher values in NT stations in 2014 (S_{av}: 74.5±3.9; Table 3.4; K=12.13; $p<0.05$; with $p\leq0.05$ in Dunn's post hoc tests for NT_14 vs. LT_14 and NT_14 vs. HT_14) and no significant differences between LT and HT (U=3.00; p=0.700 in 2013). As for the average number of trophic guilds per sample, the higher value at NT stations (TG_{av}: 16.0±0.45) was only significantly different from HT in 2014 (K=10.36; $p<0.05$; with $p<0.05$ in Dunn's post hoc test for NT_14 vs. HT_14 and no significant differences in 2013: U=0.00; p=0.100). Note that the higher number of pooled species for HT_14 stations shown in Figure 3.5F may be partly explained by the higher number of replicates (11) taken in this TP group. Noteworthy, biodiversity indices across all stations were characterised by a relatively high taxonomic diversity and evenness (S: 88–137; H': 3.88–3.99; J': 0.804–0.876; $ES_{(50)}$: 29.6–32.1; $ES_{(100)}$: 44.3–50.3), as well as trophic diversity and evenness (TG: 15–20; H': 2.00–2.30; J': 0.704–0.797; $ETG_{(50)}$: 10.8–12.6; $ETG_{(100)}$: 12.7–14.8; Table 3.4).

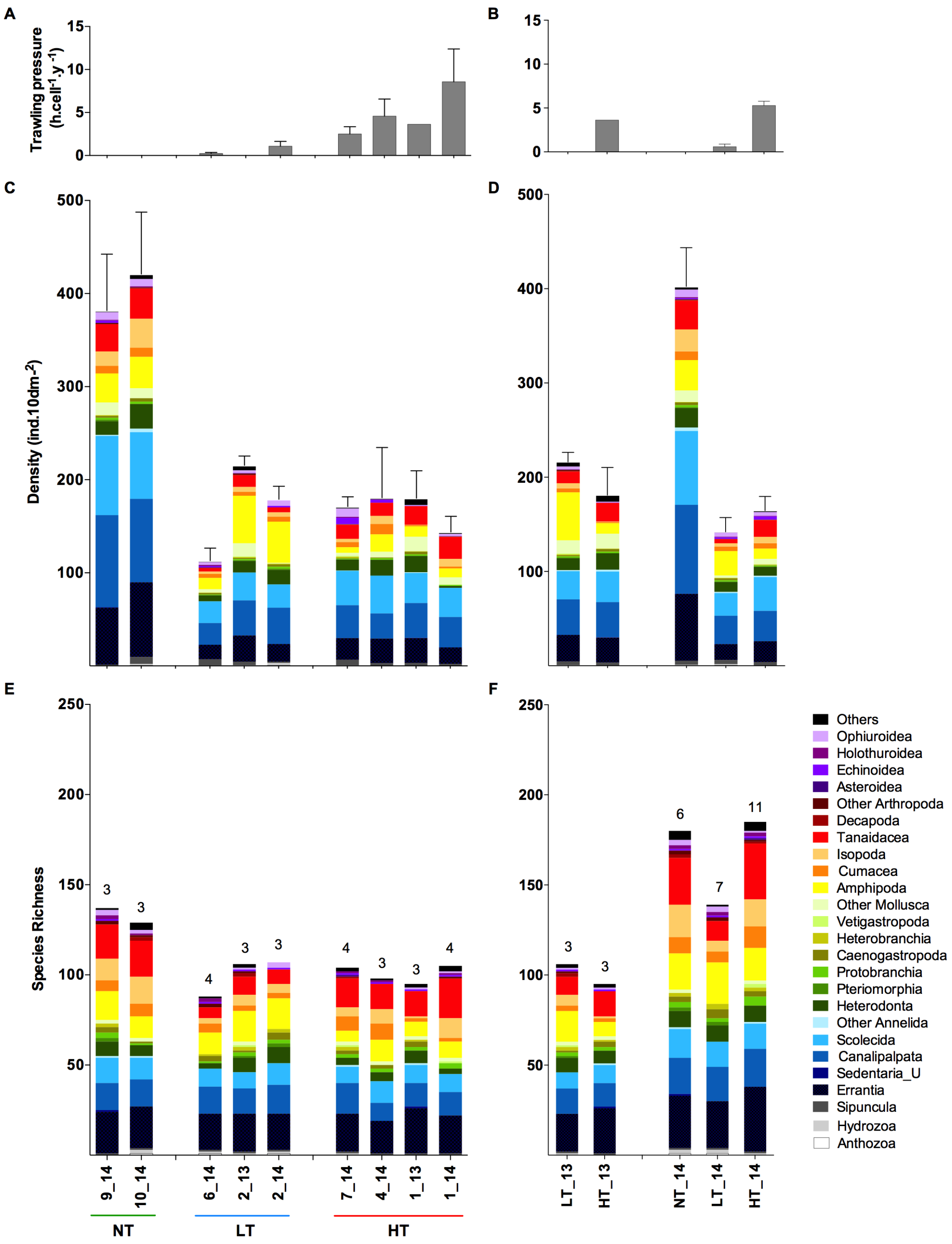


Figure 3.5 Overview of macrofauna density and species richness patterns in relation to trawling pressure. (A) Trawling pressure (TP in h.cell^{-1}.y^{-1}) per station and (B) trawling pressure group in each year, and matching results for to macrofaunal density (C) and (D), respectively) and pooled species richness (E) and (F), respectively). The number of replicates pooled in each case are indicated above the bars. NT, LT and HT: no, low and high trawling pressure, respectively.

Table 3.4 Overview of the macrofaunal density (average±SE), biomass (average±SE), and biodiversity (both taxonomic and trophic) results for each station, trawling pressure areas per year and study region (All).

Station code	n	Area (dm^2)	Density (ind. 10dm^{-2})	Biomass (mg.10 dm^{-2})	Taxonomic diversity							Trophic diversity					
					N	S_{po}	S_{av}	H'	J'	$ES_{(50)}$	$ES_{(100)}$	TG_{po}	TG_{av}	H'	J'	$ETG_{(50)}$	$ETG_{(100)}$
9_14	3	24.1	381.7±60.66	660.4±260.73	867	137	76.0±3.21	3.96	0.804	29.5	45.8	19	16.7±0.33	2.07	0.704	11.6	14.1
10_14	3	24.1	421.1±66.38	1495.5±656.68	943	129	73.0±7.94	3.91	0.804	28.9	44.3	18	15.3±0.67	2.23	0.772	12	13.8
6_14	4	32.2	113.1±13.36	594.4±226.31	327	88	39.3±3.12	3.88	0.867	30.8	47.5	17	13.4±0.29	2.25	0.794	12.6	14.7
2_13	3	24.1	215.5±10.06	430.9±162.81	462	106	56.0±0.00	3.91	0.839	30	46.9	19	15.7±0.33	2.26	0.766	12.3	14.7
2_14	3	24.1	179.0±14.14	437.4±182.52	411	107	56.0±5.51	3.95	0.846	30.5	48.5	17	14.3±0.33	2.16	0.763	12.3	14.8
7_14	4	32.2	171.3±10.48	593.5±219.35	492	104	47.8±1.44	3.89	0.838	29.6	45.3	17	14.0±0.41	2.13	0.753	11	12.9
4_14	3	24.1	180.7±53.89	907.5±410.110	403	98	47.0±11.15	3.99	0.878	31.4	48	18	14.3±0.88	2.3	0.797	12.4	14.6
1_13	3	32.2	180.3±29.43	395.9±165.83	363	95	49.0±4.62	3.99	0.876	32.1	50.3	15	12.3±0.67	2.12	0.783	10.9	12.7
1_14	4	24.1	143.9±16.85	501.9±193.43	427	105	44.0±3.19	3.93	0.844	30.6	48.1	17	12.3±0.48	2	0.707	10.8	13.2
LT_13	3	24.1	215.5±10.06	430.8±162.81	462	106	56.0±0.00	3.91	0.839	30	46.9	19	15.7±0.33	2.26	0.766	12.3	14.7
HT_13	3	32.2	180.3±29.43	395.9±165.83	363	95	49.0±4.26	3.99	0.876	32.1	50.3	15	12.3±0.67	2.12	0.783	10.9	12.7
NT_14	6	48.3	401.4±41.17	1077.8±458.71	1810	180	74.5±3.89	4.07	0.783	30.1	46.8	19	16.0±0.45	2.18	0.741	12	14
LT_14	7	56.3	141.4±15.13	527.1±148.97	738	139	46.4±4.31	4.14	0.838	31.8	50.3	18	13.9±0.26	2.25	0.777	12.6	14.9
HT_14	11	80.4	163.9±15.02	710.3±148.92	1322	185	46.2±2.90	4.23	0.81	31.9	50.3	19	13.5±0.41	2.18	0.741	11.6	13.7
All	30	241.3	283.7±39.97	658.1±116.76	4695	310	53.2±2.61	4.47	0.779	33.4	53.8	20	15.2±0.76	2.24	0.747	12.2	14.3

Area: surface area sampled; N: abundance (total number of specimens); $S_{po:}$ pooled species richness; S_{av} average species richness per sample (average±SE); H': Shannon-Wiener diversity index (ln-based); J': Pielou evenness; $ES_{(50)}$ and $ES_{(100)}$: Hurlbert's expected number of species per 50 and 100 individuals, respectively; TG_{po}: pooled number of trophic guilds; TG_{av}: average number of trophic guilds per sample (average±SE), $ETG_{(50)}$ and $ETG_{(100)}$: Hurlbert's expected number of trophic guilds per 50 and 100 individuals, respectively. Stations are ordered by the increasing average trawling disturbance (TP) of the station.

Biodiversity partitioning of the 2014 assemblages in terms of species richness (Fig. 3.6B) estimates a large component of β-diversity (β-diversity: 78.6% vs α-diversity: 21.4%) with the largest percentage explained by differences between TP groups (β3: 39.6%) and then decreasing towards smaller special scales (β2: 20.1%; β1: 18.9%). This reflects the overall high percentage of singletons and rare (infrequent) species, but also the occurrence of distinctive species in NT and LT stations. In terms of the other indices, $ES_{(50)}$ and H' (Fig. 3.6B), the largest biodiversity component is estimated for α-diversity (>80%) because of the little variation in community structure across all spatial scales (e.g. all assemblages, either at replicate, station or TP level, showed low dominance). Nevertheless, differences between TP groups (β3) always accounted for about one third of the total β-diversity. Similar patterns were observed in 2013 (Fig. 3.6A), but with higher values estimated for α-diversity (53.3, 94.1 and 85.5% for S, $ES_{(50)}$ and H', respectively) which demonstrates the relevance of NT stations (not sampled in 2013) to the overall β-diversity in the region. On the other hand NT stations had much lower contribution in the differences of trophic diversity partition in 2013 and 2014 (Fig. 3.6C,D). The highest contribution was from the α-diversity (TG: 70.4, 70.7%; $ETG_{(50)}$: 86.9, 88.4%; H': 93.9, 94.5%, for 2014 and 2013, respectively) because most trophic guilds were represented at the replicate level. Also the difference in α-diversity contribution for TG was closer to the contributions for $ETG_{(50)}$ and H' because the limited number of trophic guilds (much lower than the possible number of taxa).

A significant negative correlation (Fig. 3.7), after Bonferroni correction, was detected between trawling pressure ($h.cell^{-1}.y^{-1}$) and trophic guild richness (R=-0.6079; p=0.0016); macrofaunal density, species richness, and $ETG_{(50)}$ also showed significant correlations, but only before Bonferroni correction (R=-0.4349; p=0.0337; R=-0.4903; p=0.0150; R=-0.4558, p=0.0252, respectively). Although not statistically significant (mainly because of the high dispersion of values at 0 $h.cell^{-1}.y^{-1}$), negative trends were also observed between trawling pressure and all the other estimated biodiversity indices and total biomass. Note that these values concern only the 2014 samples; the correlations were not estimated for 2013 because of the small number of samples and narrower range of trawling pressure values (Fig. 3.7).

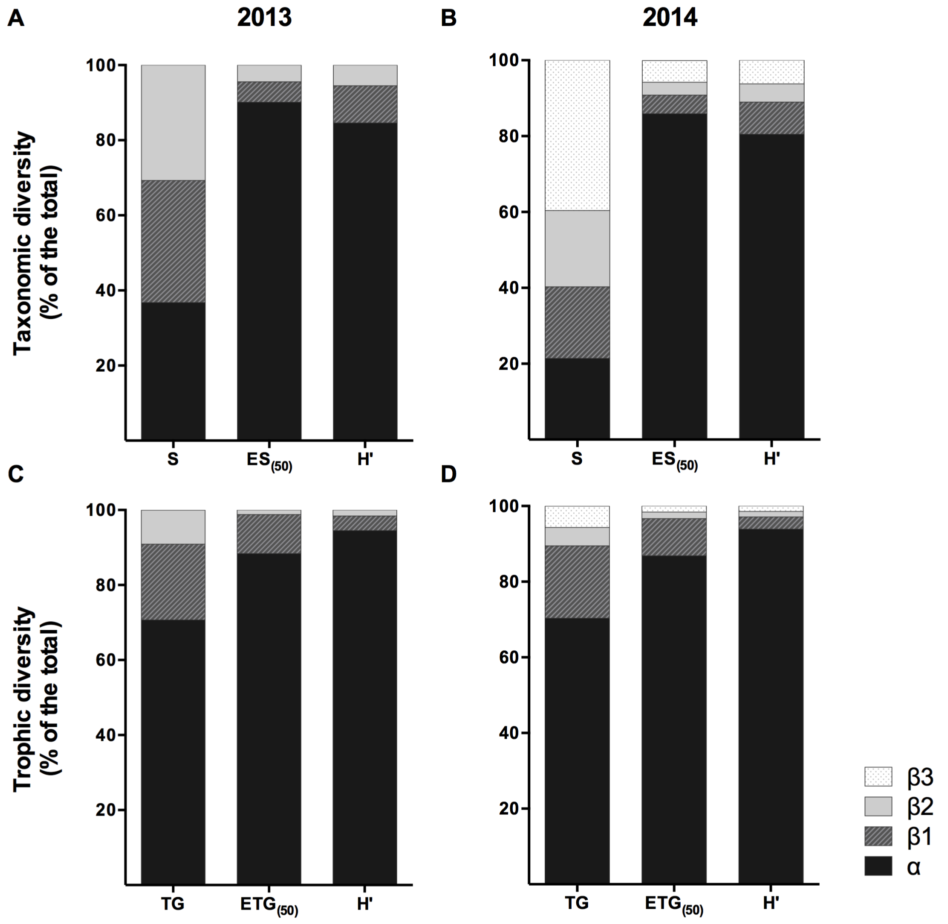


Figure 3.6 Partitioning of the taxonomic and trophic diversity for (A, C) 2013 and (B, D) 2014. S: number of species; H’: Shannon-Wiener diversity (log-based); $ES_{(50)}$: Hurlbert's expected number of species per 50 individuals; TG: number of trophic guilds; $ETG_{(50)}$: Hurlbert´s expected number of trophic guilds per 50 individuals; α: α-diversity of the sampled level - deployments; β1: β-diversity between deployments (within station); β2: β-diversity between the different stations (within areas); β3: β-diversity between areas.

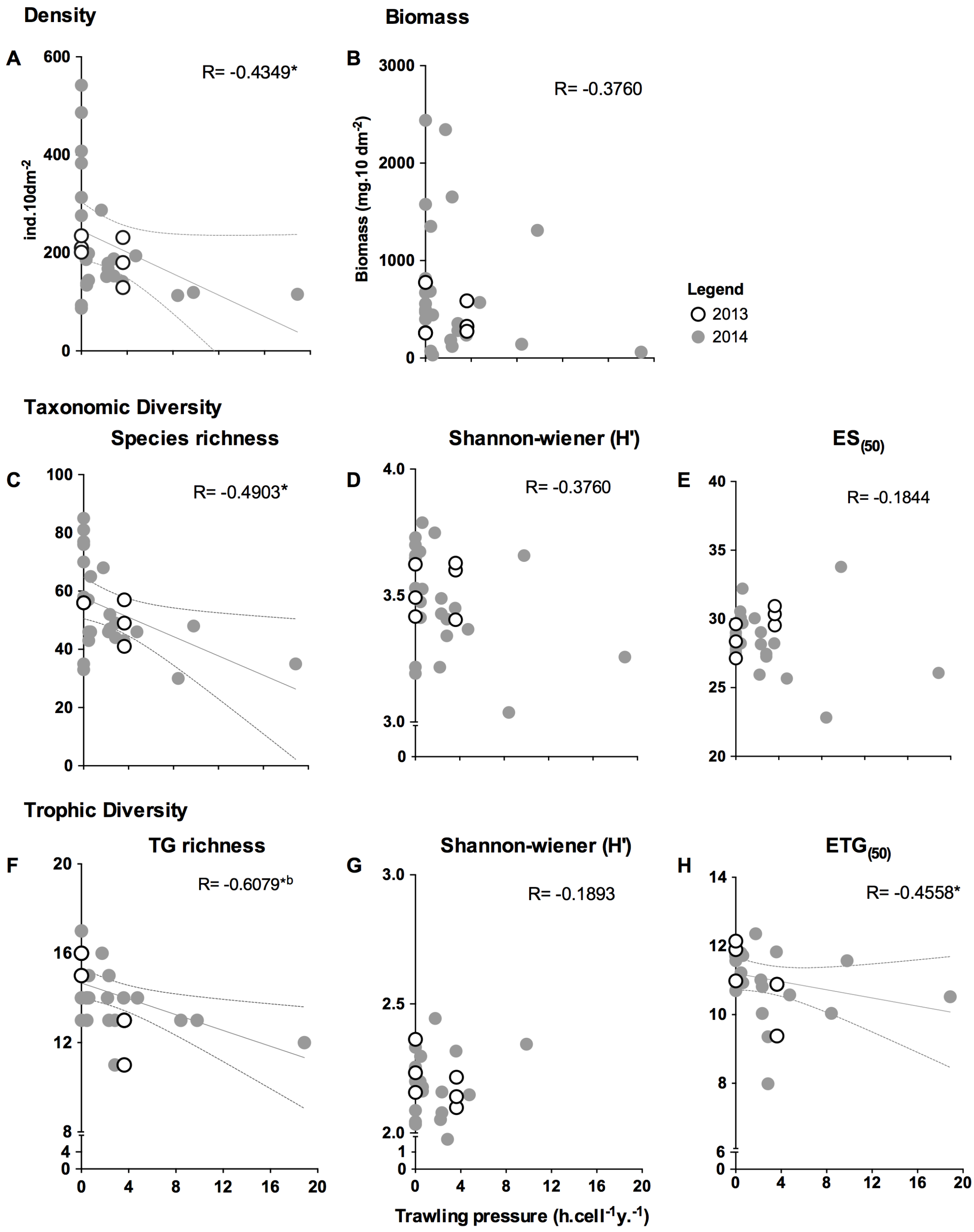


Figure 3.7 Trawling pressure (h.cell^{-1}.y^{-1}) relationship with macrofauna (A) density (ind.10dm^{-2}); (B) biomass; taxonomic diversity indices including: (C) species richness (S), (D) Shannon-Wiener taxonomic diversity (H'), (E) Hulbert's expected number of taxa per 50 individuals; and trophic diversity indices: (F) number of trophic guilds (TG); (G) Shannon-Wiener trophic diversity (H'), (H) Hurlbert´s expected number of trophic guilds per 50 individuals.*Indicates significant correlation for 2014 samples; ^{b}indicates significant correlations after Bonferroni correction.

3.3.5 Core assemblage composition in relation to trawling pressure

The core assemblage (Fig. 3.8) in NT stations was composed by a higher number of taxa (both at species level and major groups), and feeding guilds than the ones from LT and HT stations sampled in the same year (2014). In total, NT core assemblage was represented by 45 different species (13 major taxa and 14 trophic guilds) grouped in 24 different combinations of major taxa and feeding guilds (Fig. 3.8A, Fig. 3.9). These values contrast with the core assemblage of HT_14 stations composed by only 26 species (10 major taxa and 11 trophic guilds) grouped in 16 different combinations (Fig. 3.8C), while LT_14 showed intermediate values (31 species, 11 major taxa, 13 trophic guilds and 21 different combinations; Fig. 3.8B).

Overall, surface and sub-surface deposit feeders (mostly polychaetes) were the most well-represented trophic guilds in all assemblages. Additionally, both NT_14 and LT_14 core assemblages showed distinctive species from a variety of trophic guilds (11 each; Fig. 3.9), but HT_14 showed no distinctive species, and a lower representation of suspension feeders and predators with an absence of microbial grazers. Distinctive species in NT_14 were suspension-feeder bivalves (*Kelliella* sp1, *Abra longicallus, Mendicula ferruginosa*), isopods preying on macrofauna (*Bullowanthura* sp., Anthuridae sp1), omnivore polychaetes (Exogoninae sp4) and oligochaetes (Oligochaeta sp1), detritivore crustaceans (*Carangoliopsis spinulosa*, *Pseudotanais denticulatus*) and deposit feeder polychaetes (Capitellidae sp1). Distinctive species in LT_14 included suspension-feeder bivalves (*Thyasira tortuosa*), crustaceans and polychaetes predators on macrofauna (*Stenothoe* cf. *bosphorana*) and on meiofauna (*Lumbrineris* sp4, *Nannastacus* cf. *unguiculatus*), omnivore polychaetes (*Aponuphis bilineata*) and bivalves (*Yoldiella philippiana*), detritivore crustaceans (*Pedoculina* cf. *garciagomezi*, *Araphura* sp1) and deposit feeder polychaetes (*Aonidella* sp1, *Polycirrus* sp1). In fact, the core assemblage in HT_14 stations is an impoverished subset of the other core assemblages and is formed mostly by generalist feeding guilds (deposit feeders, detritivores and omnivores) and some predator species (Fig. 3.9). Trophic redundancy was higher in NT_14 core assemblage and trophic vulnerability was higher in HT_14 while LT_14 showed the highest trophic over-redundancy (TR: 3.5, 2.4, 2.4 species per trophic guild; TV: 30.8, 38.5, 54.5%; TOR: 30.8, 46.2, 27.3; for NT, LT an HT, respectively).

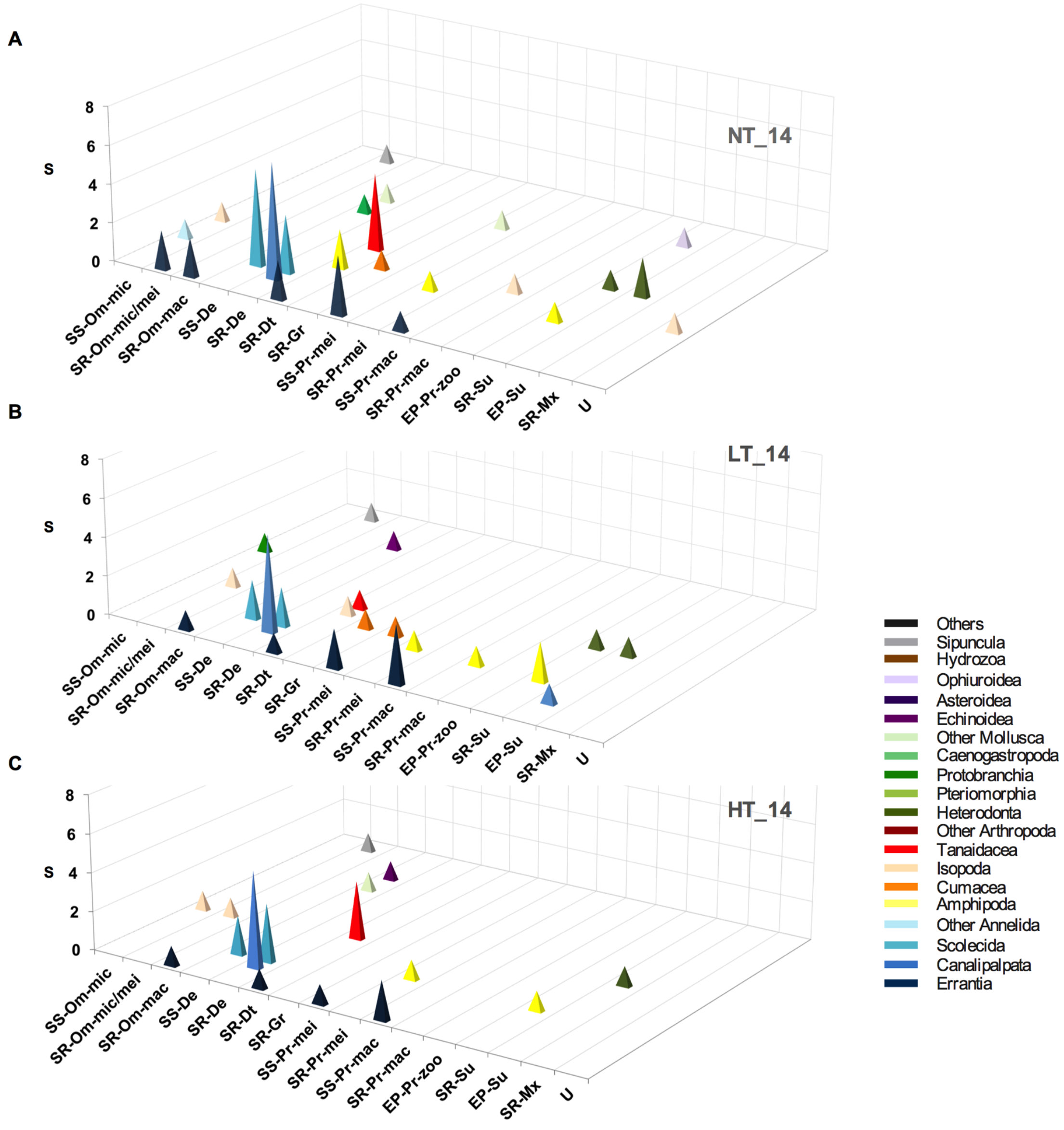


Figure 3.8 Core assemblage illustrated as the number of species grouped in different combinations of major taxa and trophic guilds for each trawling pressure group in 2014: (A) NT, (B) LT_14 and (C) HT_14: no, low and high trawling pressure, respectively. Each cone represents a different combination of major taxa and trophic guild and the height of the cone represents the number of species in each combination. Macrofauna trophic guilds codes were composed of: the food source (epibenthic (EP), seafloor surface (SR) and sediment subsurface (SS)); food type/size (particulate organic matter/microfauna (mic), meiofauna (mei), macrofauna (mac)); and feeding mode (omnivorous (Om), detritus (Dt) and deposit (De) feeders, grazers (Gr), predators (Pr), mixo trophs (Mx), suspension/filter feeders (Su)). U: no information.

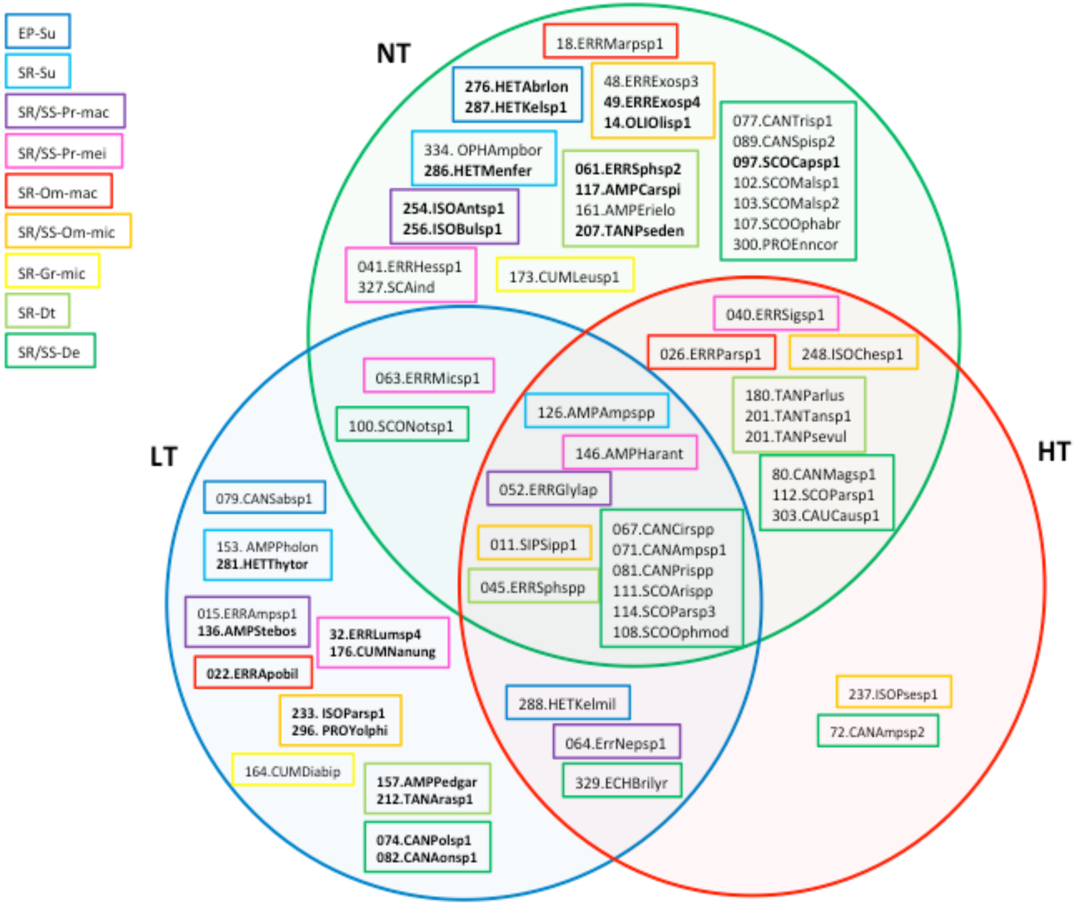


Figure 3.9 Core assemblage's species composition and their corresponding trophic guilds for each trawling pressure group in 2014. NT, LT and HT: no, low and high trawling pressure, respectively.

Macrofauna trophic guilds are composed of a combination of food source (epibenthic (EP), seafloor surface (SR) and sediment subsurface (SS)); food type/size (particulate organic matter/microfauna (mic), meiofauna (mei), macrofauna (mac)); and feeding mode (omnivorous (Om), detritus (Dt) and deposit (De) feeders, grazers (Gr), predators (Pr) and suspension/filter feeders (Su)). Species codes ordered by major taxa/ID number: SIPUNCULA (SIN): Sipuncula sp1 (011); OLIGOCHAETA (OLI): Oligochaeta sp1; POLYCHAETA – ERRANTIA (ERR): Amphinomidae sp1 (015), *Marphysa* sp1 (018), *Aponuphis bilineata* (022), cf. *Paradiopatra* sp1 (026), *Lumbrineris* sp4 (032), Sigalionidae sp1(040), Hesionidae sp1 (041), *Sphaerosyllis* spp. (045), Exogoninae sp3 (048), Exogoninae sp4 (049), *Glycera lapidum* (052), Sphaerodoridae sp2 (061), *Micronephthys* sp1 (063), *Nephtys* sp1 (064); POLYCHAETA – CANALIPALPATA (CAN): Cirratulidae spp. (067), Ampharetidae sp1 (071), Ampharetidae sp2 (072), *Polycirrus* sp1 (074), Trichobranchidae sp1(077), Sabellidae sp1 (079), *Magelona* sp1 (080), *Prionospio* spp. (081), *Aonidella* sp1 (082), *Spiophanes* sp2 (089); POLYCHAETA – SCOLECIDA (SCO): Capitellidae sp1 (097), *Notomastus* sp1 (100), Maldanidae sp1 (102), Maldanidae sp2 (103), *Ophelina abranchiata* (107), *Ophelina modesta* (108), *Aricidea* spp. (111), Paraonidae sp1 (112), Paraonidae sp3 (114); AMPHIPODA (AMP): *Carangoliopsis spinulosa* (117), *Ampelisca* spp. (126), *Stenothoe* cf. *bosforana* (136), *Harpinia antennaria* (146); *Photis longicaudata* (153), *Pedoculina* cf. *garciagomezi* (157), *Eriopisa elongata* (161); CUMACEA (CUM): *Diastyloides* cf. *biplicatus* (164), Leuconidae sp1 (173), *Nannastacus* cf. *unguiculatus* (178), TANAIDACEA (TAN): *Paranarthrura lusitanus* (180), *Tanaopsis* sp1 (201), *Pseudotanais pseudotanais vulsella* (204), *Pseudotanais pseudotanais denticulatus* (207), *Araphura* sp1 (212); ISOPODA (ISO): *Paramunna* sp1 (233), Pseudarachna sp1 (237), *Chelator* sp1 (248), Anthuridae sp1 (254), *Bullowanthura* sp1 (256); BIVALVIA - HETERODONTA (HET): *Abra longicallus* (276), *Thyasira tortuosa* (281), *Mendicula ferruginosa* (286), *Kelliella* sp1 (287), *Kelliella miliaris* (288); BIVALVIA – PROTOBRANCHIA (PRO): *Yoldiella philippiana* (296), *Ennucula corbuloides* (300); CAUDOFOVEATA (CAU): Caudofoveata sp1 (303), SCAPHOPODA (SCA): Scaphopoda indet (327); ECHINOIDEA (ECH): *Brissopsis lyrifera* (329); OPHIUROIDEA (OPH): *Amphiura borealis* (334). Species in bold are distinctive of each trawl pressure group.

The results obtained for the core assemblages in 2013 (Fig. 3.10) showed overall the same patterns (impoverished core assemblage in HT, with higher trophic vulnerability), but are not explored in detail here due to the limited number of replicates and stations (two stations, one LT and one HT, each represented by only three replicates).

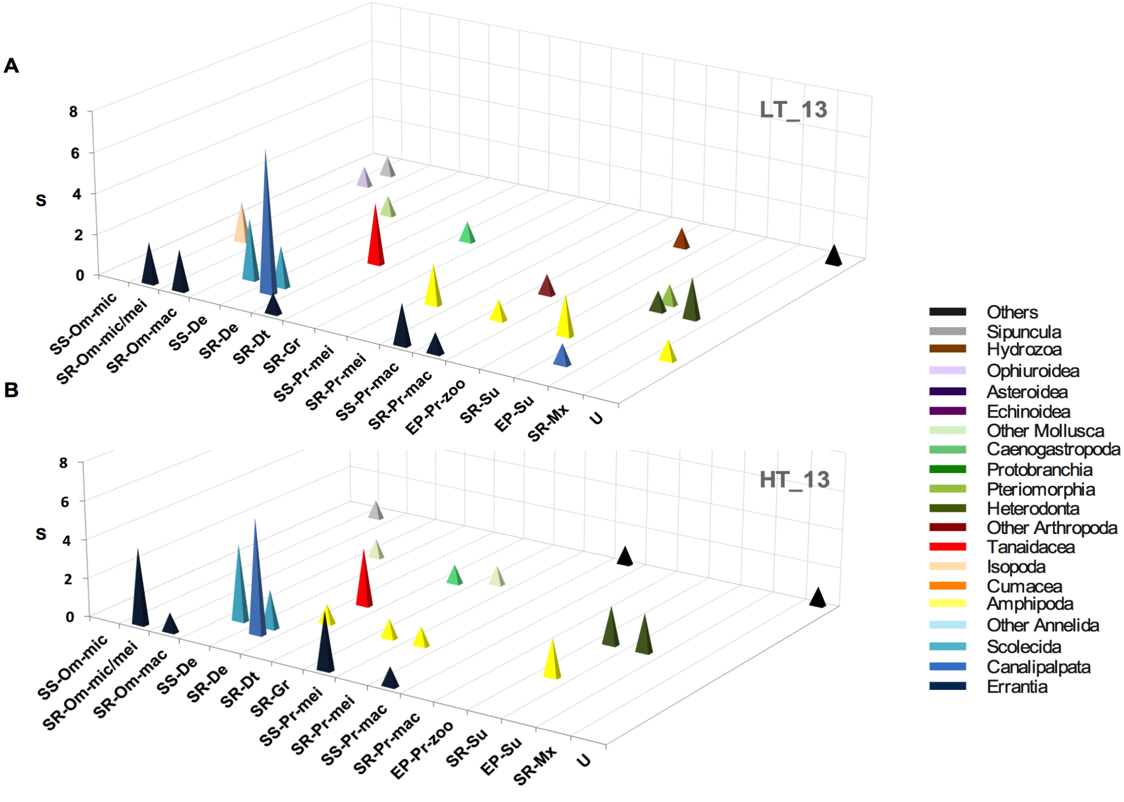


Figure 3.10 Core assemblage illustrated as the number of species grouped in different combinations of major taxa and trophic guilds for each trawling pressure group in 2013. (A) LT_13 and (B) HT_13: low and high trawling pressure, respectively. Macrofauna trophic guilds composed of the food source: epibenthic (EP), seafloor surface (SR) and sediment subsurface (SS); food type/size: particulate organic matter/microfauna (mic), meiofauna (mei), macrofauna (mac); and feeding mode included omnivorous (Om), detritus (Dt) and deposit (De) feeders, grazers (Gr), predators (Pr), mixotrophs (Mx), suspension/filter feeders (Su). U: no information.

3.4 Discussion

The magnitude of the effects imposed by trawling on benthic habitats depends on the interaction of numerous factors, namely frequency and intensity of trawling activities, gears used and characteristics of the target habitats and their faunal assemblages (NRC, 2002; Kaiser et al., 2002; Hiddink et al., 2017). As such, the assessment of trawling effects on the ecosystem requires a regional perspective for understanding the impacts, as well as regionally-adapted monitoring programmes to determine the sustainability of deep-sea fisheries (Eigaard et al., 2016).

The historical importance of bottom-trawling fisheries in Portugal has lead to one of the largest footprint per unit of landing in Europe bellow 200m depth, particularly in the south and southwest Portuguese margin (Eigaard et al., 2016; Bueno-Pardo et al., 2017). While both national and European programmes perform relatively frequent stock assessments of economical valuable species (MAMAOT, 2012), the condition of benthic habitats and their assemblages in the continental Portuguese deep-sea areas remains poorly known (Morais et al., 2007; Fonseca et al., 2014; Chapter 2). Moreover, the existing assessments of Good Environmental Status (GES) have a low degree of confidence and are hindered by the limited availability of adequate control areas and inexistence of pristine habitats (MAMAOT, 2012). Current legislation and imposed regulative measures have been incorporating mostly the increment of the fishing gear selectivity by defining minimum net mesh sizes according to the target species (Campos et al., 2007). Yet, the need to decrease the high existing bottom-trawling fisheries footprint, and determine adequate protected areas that insure overall resilience of the ecosystems and preserve habitats of major biological interest, makes imperative further research on the trawling impacts.

In the Portuguese margin, bottom trawlers typically target several species of deep-water crustaceans (Campos et al., 2007, Bueno-Pardo et al., 2017), thus the main fishing grounds (in the study area at depths between 300-500 m water depths) are overlapping the distribution of species, such as the Norway lobster and rose shrimp. These are typically found in muddy and muddy-sand habitats; since coarser sediments are more unstable and hinder the construction and maintenance of burrows and tunnels by the Norway lobster (Afonso-Dias, 1997). Habitat characteristics also change with increasing depth (e.g. finer sediments with higher organic content at deeper locations). In this context, our results have demonstrated the importance of the environmental setting for the assessment of trawling impacts. The DISTLM results confirmed that the observed variability in macrofauna assemblages was associated with both trawling pressure and a combination of several environmental drivers (depth, sediment grain size, C/N values). Still, a large component of the variability remained unexplained probably due to other natural and anthropogenic drivers not examined in this study. The study area is located between the shelf break and upper slope close to the boundary (ca. 500 m water depth) between the North Atlantic Central Water and the Mediterranean outflow water (Llave et al., 2015) and subjected to temporal variability in the oceanographic regime (e.g. winter storms, seasonal upwelling). The different sources of spatial heterogeneity and temporal variability are typically considered as determinant in shaping the infaunal assemblages (Levin et al., 2001 and references therein).

Furthermore, we may also assume that the long trawling history in the study area may have contributed to changes in the environmental setting. For instance, seabed topography showed clear differences among the study areas (NT, LT, HT), visually confirmed by ROV video observations (Chapter 2). Besides the flattened seabed, observed the ploughing by trawl gears promotes sediment re-suspension and changes in the sediment biogeochemistry (Puig et al., 2012). Examples are trawling induced changes in surface and sub-surface organic matter concentration, grain size composition and porosity reported by Martín et al. (2014) and Oberle et al. (2016) in the Iberian Margin and the Mediterranean Sea. These authors mention that trawling induced changes may act synergistically with natural sources of disturbance stressors.

3.4.1 Influence of trawling disturbance on macrofauna standing stocks and diversity

The present study identified the negative influence of trawling pressure influence on macrofauna density (negative trends on biomass as well), but also the decline of species richness and changes in the community structure shown by the multivariate analysis. The reduction of the epi-benthic and infaunal standing stocks (abundance and biomass) and alterations of the community composition is one of the most frequently reported indirect effects of chronic trawling disturbance in shallow areas (NRC, 2002; Kaiser et al., 2002; Queirós et al., 2006; Hinz et al., 2009), and may derive either from the direct removal or damage of the large-sized organisms or for example from indirect changes in the sediment biogeochemistry processes and in predator-prey relationships (Duplisea et al., 2001; Jennings et al., 2001b). Although less frequent, similar observations were reported from some deep-sea areas (Gage et al., 2005; Clark et al., 2015). For example, in the Mediterranean at similar depth ranges of the present study, Smith et al. (2000) found a significant decrease of the macrofauna abundance and biomass, particularly within the echinoderm and sipunculid species.

Noteworthy is that while we observed a loss of abundance of infaunal macrobenthos, mega-epibenthic abundances did not differ between trawling pressure groups at the study region (Chapter 2), possibly due to the presence of a fauna that includes robust anemone species (Spirularia ind. 5) apparently tolerant to the physical disturbance, and highly mobile species that are able to avoid disturbance and/or recolonise disturbed areas over short-term periods. Infaunal macroinvertebrates present typically lower mobility, and may take longer to recolonise newly disturbed sediments. Furthermore, flattened surface and low evidence of bioturbation by large sized burrowing species in HT areas, contrasted with the more heterogeneous LT and NT areas (Chapter 2). Such differences in sediment properties result in loss of habitat complexity and refugia, but also likely in alterations in the water-sediment exchanges fluxes, namely oxygen and organic matter provision deeper into the sediment (Martín et al., 2014; Oberle et al., 2016), that may all contribute to the decline of infauna standing stocks in disturbed locations (e.g. up to 3 times more individuals in NT areas, compared to LT and HT).

Declines in biomass were less clear, but trawling disturbance appeared to have prompted changes in the macrofauna size structure. The biomasses in HT areas were mostly defined by the accidental occurrences of a few specimens of relatively large-sized, mobile fauna (e.g. Acoetidae, *Aristeus* sp or *Natatolana* sp. 1). Contrarily, the biomasses in NT areas were determined by the presence of common speciesorganisms (with relatively high MBI), including sensitive taxa to trawling, namely by the tube dwelling anemones and several individuals of the brittle stars from the *Amphiura* genera (e.g. Smith et al., 2000, Atkinson et al., 2011, Pommer et al., 2016).

Noteworthy is that despite the differences in the composition of macrofaunal assemblages from areas with different trawling pressure shown by the multivariate analysis, univariate diversity indices that are primarily based on community structure (e.g. Shannon-Wiener diversity and Pielou's evenness failed to detect such differences, as also reported by Atkinson et al. (2011). Benthic diversity in continental slope regions is characterised by high richness and evenness (Grassle and Maciolek, 1992), and under some types of disturbance (e.g. organic pollution, eutrophication) the loss of intolerant or vulnerable species often relieves competition and is accompanied by increased abundance and dominance of opportunistic species that take advantage from the high resource availability. Bottom-trawling disturbance is predominantly physical (reworking and resuspension of sediments) and our results showed that the significant decrease both in number of species and abundance in HT areas was not compensated by increased abundance of more tolerant species. Instead it resulted in impoverished but even assemblages (no compensatory abundance effects by other species) and therefore univariate biodiversity indices (e.g. Shannon-Wiener diversity) that are used frequently as a standard monitoring tool for impact assessment in marine systems may not adequately reflect these important changes in assemblages disturbed by trawling. In the context of the MSFD 2008/56/EU descriptor 1 "*biological diversity is maintained*" (European commission, 2008), these indices may even incorrectly indicate the maintenance of the Good Environmental Status (GES), and should be accompanied by other indicators of community composition, ecosystem condition and functional diversity (Strong et al., 2015).

3.4.2 Influence of trawling disturbance on macrofauna core community and functional diversity

Direct effects of trawling disturbance on the fauna assemblages include high mortality of both target and non-target populations; increased food availability and loss of habitat complexity (NRC, 2002). Indirect effects of trawling disturbance on the benthic component are usually much more difficult to assess, particularly in deep-sea habitats, and include typically changes in the faunal community structure, diversity and distribution (Jennings et al. 2001b; NRC, 2002; Kaiser et al., 2002). These changes may result in alteration of the in biological interactions and trophic composition, inevitably altering the food-web structure and ecosystem functioning (Jennings et al. 2001a,b; NRC, 2002; Kaiser et al., 2002).

In the present study, we observed an overall high macrofauna structural and functional diversity (and evenness), characteristic of the environmentally heterogeneous habitats of the shelf-slope transition region (Grassle and Maciolek, 1992; Levin et al., 2001). The investigation of compositional changes in relation to increasing levels of trawling pressure was focused in the core assemblage – a subset of the whole assemblage composed by the most abundant, frequent and distinctive taxa in each TP group of stations. The less diverse core assemblages in HT areas diverged greatly from the NT areas, likely in response to differing local conditions over long periods (decades). With the absence of distinctive taxa and packing of taxa under generalist trophic guilds (deposit feeders, detritivores and omnivores), HT core assemblage was mostly an impoverished subset of NT and LT core assemblages. Although trophic complexity was maintained in HT areas, the depleted number of taxa across most trophic guilds represents a loss of trophic redundancy, and therefore a higher trophic vulnerability (Naeem, 1998) of these highly disturbed assemblages.

Local extinctions of species do naturally occur as a result of environmental fluctuations (Mouillot et al., 2014) and are usually compensated by increased abundances of sympatric, trophically redundant species and/or by the recolonization from adjacent areas, allowing in time the re-establishment of the ecosystem functions (Naeem and Li, 1997; Naeem, 1998; Liu et al., 2016). The loss of functional redundancy in HT assemblages indicates one or several of the following: i) the time between successive disturbance events prevented the re-establishment of the abundance of depleted populations; ii) the time between successive disturbance events prevented recolonization from adjacent areas; iii) there were no other trophically redundant species available locally; iv) there were no other trophically redundant species available in adjacent areas. When the loss of redundancy and/or weakening of the trophic links occurs in association with a low recolonization rate, the assemblages may either take longer to re-establish, or not recover at all, ultimately leading to trophic cascading and regime shifts (Yachi and Loreau, 1999; Belgrano, 2005; Liu et al., 2016). This shows that the resilience of assemblages affected by trawling depends crucially on the frequency of disturbance and on the existence of regional undisturbed refugia that can replenish depleted populations through recolonization.

In the case of the Portuguese margin an impressive 93.6% of the total area at depths between 200 and 1000 m are trawled annually (Eigaard et al. 2016). Areas adjacent to the fishing grounds (e.g. LT) show affected assemblages and even the few existing refugia are not exempt of anthropogenic pressures (e.g. baited traps for Norway lobster are allowed in the NT area near Setúbal canyon). Also important is the natural variability in the oceanographic regime (e.g. upwelling events; Kämpf and Chapman, 2016), and the putative increased occurrence of climatic episodic events (e.g. winter storms; Vitorino et al., 2002; Diogo et al., 2014). In the present scenario of global change, which may act cumulatively with trawling to increase the frequency of disturbance.

3.5 Conclusions

The present study indicated a depletion of macro-infaunal standing stocks (mainly abundance), as well as taxonomic and trophic richness in areas subjected to both low and high trawling pressure. On the contrary univariate biodiversity indices, routinely used to assess the GES in marine systems, failed to detect important compositional changes in the assemblages.

The core assemblage composition in areas subjected to high trawling pressure was an impoverished subset of the assemblage from undisturbed areas and was typified by generalist trophic guilds (deposit feeders, detritivores and omnivores) common across the studied region. The macrobenthic assemblages in the shelf break and upper slope of the Portuguese margin have likely adapted over time to high intensities and frequencies of natural disturbance and they maintain a relatively high biodiversity and trophic complexity under trawling pressure. However, our results indicate a loss of trophic redundancy, which makes these assemblages more vulnerable to further increases in trawling pressure and their synergistic effects with natural disturbance.

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Supplementary material

Supplementary Table 3.1 PERMANOVA pair-wise comparison results of the macrofauna community composition dataset. Pair-wise test were only applied to the Test 2, factor Trawl pressure: TP, while the factor Station (TP) for the 2014 was not test (random factor). Additionally, because Test 1: 1-factor design for 2013 (TP) only presented two levels (LT/HT) so no pair wise comparison were presented here. Values in bold represent significant values. NT, LT and HT: no, low and high trawling pressure.

Pair-wise test 2 (Trawl pressure**)**	t	P(perm)	unique perms
HT, LT	1.6865	**0.0002**	9829
HT, NT	1.9126	**0.0002**	9606
LT, NT	2.5321	**0.0005**	4637

Supplementary Table 3.2 PERMDISP and correspondent pair-wise comparison results. Test 1: 1-factor design for 2013 (Trawl pressure: TP) and Test 2: 2-factor nested design (TP and Station (TP)) for 2014 macrofauna community composition dataset. No pair-wise comparisons were performed for the Test 3 random factor). Values in bold represent significant values. NT, LT and HT: no, low and high trawling pressure.

Test 1 (Trawl pressure - 2013)

Deviations from centroid

df1	df2	F	P(perm)
1	4	0.79038	0.6001

Pairwise Comparisons

Groups	t	P(perm)
LT_13 vs. HT_13	0.88903	0.6028

Test 2 (Trawl pressure - 2014)

Deviations from Centroid

df1	df2	F	P(perm)
2	21	14.18	**0.0007**

Pairwise comparisons

Groups	t	P(perm)
NT vs. LT_14	1.1686	0.3546
NT vs. HT_14	4.6513	**0.0015**
LT_14 vs. HT_14	5.6294	**0.0005**

Test 2 (Stations (TP) - 2014)

Deviations from Centroid

df1	df2	F	P(perm)
6	17	6.0242	**0.0438**

Supplementary Table 3.3 Abundance and breakdown of percentual contributions from SIMPER dis(similarities) comparisons between low (LT_13) and high trawling pressure (HT_13) areas sampled in 2013, including taxa with a contribution of at least 1.5% of the total abundance. Numbers in bold mark indicate the ten dominant taxa. Macrofauna feeding guilds composed of the food source: epibenthic (EP), seafloor surface (SR) and sediment subsurface (SS); food type/size: particulate organic matter/microfauna (mic), meiofauna (mei), macrofauna (mac); and feeding mode included omnivorous (Om), detritus (Dt) and deposit (De) feeders, grazers (Gr), predators (Pr), suspension/filter feeders (Su). AS: average similarity; AD: Average dissimilarity; *: contributions lower than 1.5%

	Taxa	**Mobility**	**Trophic group**	**Average density (ind.10dm-2)**		**% Contribution**		
				LT_13	**HT_13**	**LT_13**	**HT_13**	**LT_13/HT_13**
				191.48	**150.45**	**AS: 49.4**	**AS: 44.2**	**AD: 58.1**
Scyphozoa	Scyphozoa sp1	S	U	**3.73**	**4.97**	1.7	5.8	*
Sipuncula	Sipuncula sp1	DB	SR-Om-mic	**3.73**	**3.32**	2.5	4.6	*
Annelida								
Errantia	Amphinomidae sp1	MF	SS-Pr-mac	2.49	0.83	2.8	*	*
	cf. *Paradiopatra* sp1	DT	SR-Om-mac	1.66	1.66	*	1.5	*
	Sphaerosyllis spp.	MF	SR-Dt	2.07	0.41	2.8	*	*
	Exogoninae sp1	MF	SR-Om-mic	2.9	2.49	3.1	1.6	*
	Glycera lapidum	MF	SS-Pr-mac	**7.46**	**4.97**	5.3	4.4	*
	Phyllodoce madeirensis	MF	SR-Pr-mac	3.32	0.83	2.8	*	*
Scolecida	*Notomastus* sp1	DF	SS-De	1.66	2.49	*	4.6	*
	Maldanidae sp1	DT	SS-De	1.24	2.49	*	1.6	*
	Ophelina modesta	MF	SS-De	2.07	**5.8**	2.8	5.9	*
	Aricidea spp.	DB	SR-De	**14.51**	**8.29**	7.5	7.7	*
	Paraonidae sp1	DB	SR-De	4.56	**4.97**	3.7	2.4	*
Canalipalpata	Cirratulidae spp.	DF	SR-De	2.9	**5.39**	3.5	4.6	*
	Ampharetidae sp1	DT	SR-De	2.49	**3.32**	*	2.1	*
	Ampharetidae sp2	DT	SR-De	**4.14**	0.83	4.3	*	1.6
	Aonidella sp1	DT	SR-De	**4.14**	0.41	3.5	*	1.6
	cf. *Pseudopolydora* sp1	DT	SR-De	-	**3.73**	-	*	*
	Laonice sp1	DT	SR-De	1.24	0.41	2.5	*	*
	Prionospio spp.	DT	SR-De	**18.24**	**19.07**	8.3	10.8	*
Mollusca								
Caudofoveata	Caudofoveata sp1	MF	SS-De	2.49	**3.32**	2.8	3.6	*
Scaphopoda				0.41	2.07	*	1.7	*
Heterodonta	*Kelliella miliaris*	DF	EP-Su	4.97	**4.14**	1.6	3.6	*
	Kurtiella tumidula	DF	EP-Su	-	**4.56**	-	*	*
	Mendicula ferruginosa	DB	SR-Su	0.41	2.9	*	3.9	*
Arthropoda								
Amphipoda	*Ampelisca* spp.	DT	SR-Su	**12.02**	2.9	7.1	3.9	1.9
	Haploops sp1	DT	SR-Su	-	1.66	-	3.2	*
	Harpinia antennaria	MB	SR-Pr-mei	0.83	1.66	*	3.2	*
	Harpinia zavodniki	MB	SR-Pr-mei	**9.53**	-	5.1	-	3
	Photis longicaudata	DT	SR-Su	**15.34**	-	3.1	-	3.2
	Liljeborgia sp1	DC	U	**4.6**	-	*	-	*
Tanaidacea	*Tanaopsis* sp1	DT	SR-Dt	1.66	0.83	2.5	*	*
Ophiuroidea	*Ophiura (Dictenophiura) carnea*	MF	SR-Om-mic	2.07	-	2.8	-	1.5
% Contribution of selected taxa				**72.5**	**66.9**	**81.7**	**80.8**	**12.7**

Supplementary Table 3.4 Abundance and breakdown of percentual contributions from SIMPER dis-(similarities) comparisons between no- (NT), low- (LT_14) and high trawling pressure (HT_14) areas sampled in 2014, including taxa with a contribution of at least 1.5% of the total abundance. Numbers in bold mark indicate the ten dominant taxa. Macrofauna feeding guilds composed of the food source: epibenthic (EP), seafloor surface (SR) and sediment subsurface (SS); food type/size: particulate organic matter/microfauna (mic), meiofauna (mei), macrofauna (mac); and feeding mode included omnivorous (Om), detritus (Dt) and deposit (De) feeders, grazers (Gr), predators (Pr), suspension/filter feeders (Su). AS: average similarity; AD: Average dissimilarity; *: contributions lower than 1.5%

				Density (ind.10dm-2)			**% Contribution**					
	Taxa	**Mobility**	**Trophic group**	**NT_14**	**LT_14**	**HT_14**	**NT_14**	**LT_14**	**HT_14**	**NT_14/ LT_14**	**NT_14/ HT_14**	**LT_14/ HT_14**
				375.1	**131.1**	**149.4**	**AS:53.1**	**AS:43.3**	**AS:41.0**	**AD: 72.6**	**AD: 62.9**	**AD: 65.5**
Sipuncula	Sipuncula sp1	DB	SR-Om-mic	4.4	**4.3**	**3.7**	*	3.6	4.6	*	*	*
Annelida												
Oligochaeta	Oligochaeta sp1	MF	SS-Om-mic	2.5	-	0.1	*	-	*	*	*	*
Errantia	Amphinomidae sp1	MF	SS-Pr-mac	0.6	1.1	0.2	*	2.1	*	*	*	*
	Aponuphis bilineata	DT	SR-Om-mac	-	1.4	0.1	-	1.9	*	*	*	*
	cf. *Paradiopatra* sp.	DT	SR-Om-mac	**14.3**	0.9	**3.2**	3.6	*	3.7	2	1.5	1.5
	Sphaerosyllis spp.	MF	SR-Dt	**20.9**	0.9	2	4.8	1.8	*	2.4	2.3	*
	Glycera lapidum	MF	SS-Pr-mac	**9.5**	**3**	**6.1**	3.5	4.4	6.6	*	*	*
	Sphaerodoridae sp2	MF	SR-Dt	1.9	-	0.5	1.5	-	2.8	*	*	*
Scolecida	*Notomastus* sp1	DF	SS-De	0.2	**2**	1.2	*	3	*	*	*	*
	Maldanidae sp2	DT	SS-De	4.8	0.5	0.9	2.1	*	*	*	*	*
	Ophelina abranchiata	MF	SS-De	**8.5**	0.2	0.2	*	*	*	*	1.6	*
	Ophelina modesta	MF	SS-De	5.4	**2.7**	**7.2**	2.4	1.5	4.9	*	*	1.9
	Aricidea spp.	DB	SR-De	**10.2**	**12.4**	**12.8**	3.1	11.2	9.5	*	*	*
	Paraonidae sp1	DB	SR-De	**33**	1.6	**5**	6.4	*	3.9	3.1	2.7	1.7
	Paraonidae sp3	DB	SR-De	**7.3**	**2**	**4.1**	3	2.9	2.1	*	*	*
Canalipalpata	Cirratulidae spp.	DF	SR-De	**15.7**	**4.6**	**4.5**	4.7	5.7	5.6	*	*	*
	Ampharetidae sp1	DT	SR-De	**13.3**	**8**	**6.4**	3.6	6.2	5.6	*	*	*
	Ampharetidae sp2	DT	SR-De	0.8	0.9	2.4	*	*	1.7	*	*	*
	Polycirrus sp1	DT	SR-De	0.2	1.8	0.1	*	2.9	*	*	*	*
	Trichobranchidae sp1	DT	SR-De	6	0.4	0.1	1.8	*	*	*	1.5	*
	Magelona sp1	DF	SR-De	**8.1**	0.2	2.8	2.1	*	4	1.5	*	1.6
	Aonidella sp1	DT	SR-De	0.2	**3.2**	0.2	*	5.1	*	*	*	1.6
	Prionospio spp.	DT	SR-De	**41.2**	**5.7**	**10.6**	7.5	4.7	8.1	2.8	2.5	*

				Density (ind.10dm-2)			**% Contribution**					
	Taxa	**Mobility**	**Trophic group**	**NT_14**	**LT_14**	**HT_14**	**NT_14**	**LT_14**	**HT_14**	**NT_14/ LT_14**	**NT_14/ HT_14**	**LT_14/ HT_14**
				375.1	**131.1**	**149.4**	**AS:53.1**	**AS:43.3**	**AS:41.0**	**AD: 72.6**	**AD: 62.9**	**AD: 65.5**
Mollusca												
Caudofoveata	Caudofoveata sp1	MF	SS-De	3.5	-	1.7	1.9	-	*	*	*	*
Caenogastropoda	*Abra longicallus*	DT	SR-De	2.5	-	-	1.9	-	-	*	*	*
Heterodonta	*Kelliella miliaris*	DF	EP-Su	2.5	**5.2**	**6.3**	*	4.9	2.4	*	*	1.8
	Kelliella sp1	DF	EP-Su	**10.2**	-	1.4	*	-	*	1.6	1.7	*
	Thyasira tortuosa	DB	SR-Su	-	**2.8**	0.3	-	*	*	*	*	*
Protobranchia	*Ennucula corbuloides*	MF	SS-De	1.7	0.4	0.6	*	*	*	*	*	*
Arthropoda												
Amphipoda	*Ampelisca* spp.	DT	SR-Su	**6.4**	**6.9**	2.6	2.6	5.6	3	*	*	*
	Harpinia antennaria	MB	SR-Pr-mei	**12**	**2.7**	1.1	3.7	1.9	*	*	1.9	*
	Photis longicaudata	DT	SR-Su	0.2	**7.1**	0.7	*	1.9	*	*	*	1.9
Isopoda	*Paramunna* sp1	MF	SR-Om-mic	-	0.7	0.1	-	*	*	*	*	*
	Chelator sp1	MF	SS-Om-mic	**6.4**	-	0.9	1.9	-	*	*	*	*
Cumacea	*Diastyloides* cf. *biplicatus*	MF	SR-Gr-mic	0.2	**2.1**	0.6	*	2.8	*	*	*	*
	Leuconidae sp1	MF	SR-Gr-mic	5.4	-	1.5	1.9	-	*	*	*	*
Tanaidacea	*Araphura* sp1	DT	SR-Dt	0.8	1.1	0.3	*	*	*	*	*	*
	Paranarthrura lusitanus	DT	SR-Dt	2.1	0.4	1.8	*	*	1.5	*	*	*
	Tanaopsis sp1	DT	SR-Dt	5.8	0.4	**4.2**	2	*	3	*	*	1.6
Echinodermata												
Echinoidea	*Brissopsis lyrifera*	DB	SS-De	1.2	1.6	**3.5**	*	1.7	2.1	*	*	*
% Contribution of selected taxa				**71.9**	**67.8**	**68.4**	**65.8**	**75.7**	**74.9**	**13.4**	**15.6**	**13.5**

Supplementary Table 3.5 Results of the distance-based linear model (DISTLM) analysis for exploring relationships between macrofauna community composition and environmental variables. Marginal tests: explanation of variation for each variable taken separately. Sequential tests: conditional tests of individual variables in constructing the best model (selection procedure: stepwise; selection criterion: adjusted R²). Values in bold represent significant values.

Marginal tests

Environmental variables		SS(trace)	Pseudo-F	P	Prop.
1	Silt+Clay (%)	7137.7	3.9135	**0.0001**	0.1226
2	Very Fine Sand (%)	4741.4	2.4831	**0.001**	0.0815
3	Fine Sand (%)	4878	2.5612	**0.0004**	0.0838
4	Medium Sand (%)	7070.4	3.8715	**0.0001**	0.1215
5	Coarse sand (%)	5443.9	2.889	**0.0004**	0.0935
6	TN (%)	4581.6	2.3923	**0.0004**	0.0787
7	TOC (%)	6375.9	3.4444	**0.0001**	0.1095
8	C/N	6472	3.5029	**0.0001**	0.1112
9	Chl *a*	2336.7	1.1711	0.1598	0.0401
10	Depth (m)	4180.8	2.1668	**0.0031**	0.0718
11	Trawl pressure (TP)	2739.8	1.3831	0.0707	0.0471
	res.df: 173				

Sequential tests

Variable	Adj R^2	SS(trace)	Pseudo-F	P	Prop.	Cumul. (5)	res.df
Silt+Clay (%)	0.091294	7137.7	3.9135	**0.0001**	0.12263	0.12263	28
Depth (m)	0.13254	4059.3	2.3315	**0.0001**	0.069741	0.19237	27
+C/N	0.15287	2801.8	1.6478	**0.0087**	0.048136	0.2405	26
TP	0.16729	2424	1.4503	**0.0339**	0.041645	0.28215	25
Coarse sand (%)	0.1743	2008.9	1.2122	0.1737	0.034513	0.31666	24
Very fine sand (%)	0.17866	1858.4	1.1273	0.2807	0.031927	0.34859	23

Best solution

Adj R^2	R^2	RSS	No.Vars	Selections
0.17866	0.34859	37916	6	1,2,5,8,10,11

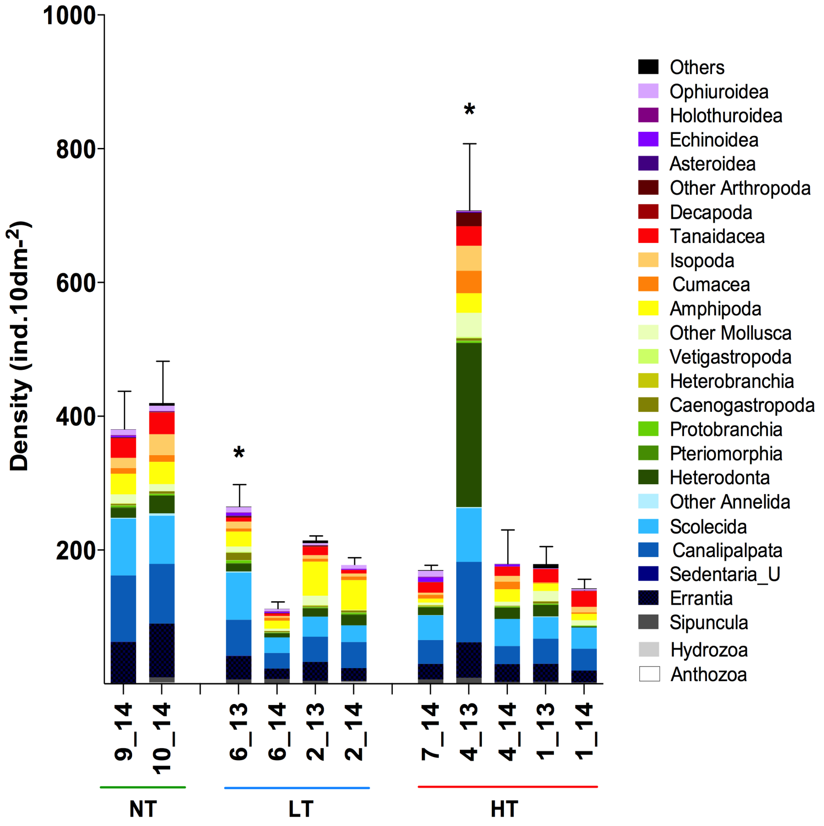
Supplementary results and discussion

The importance of sampling consistency in trawling impact assessment studies

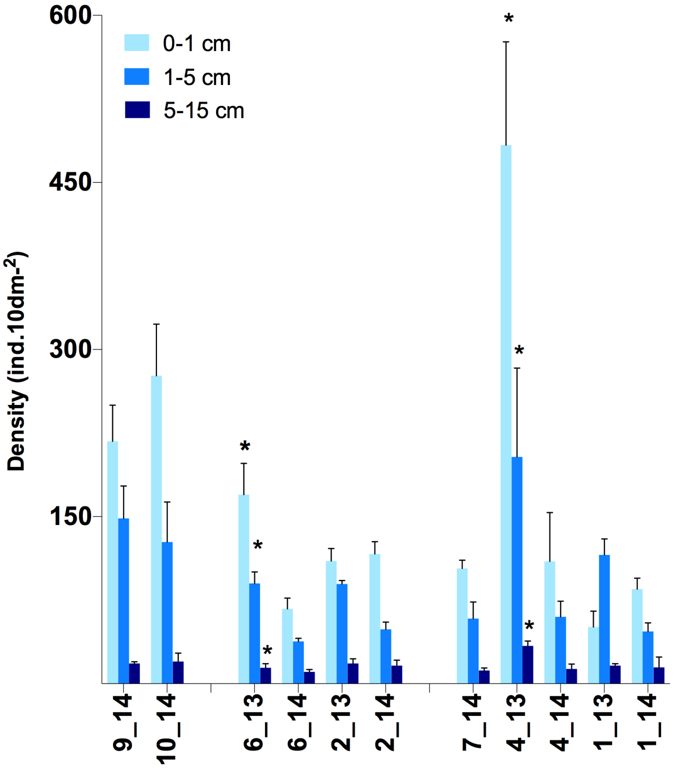
The accurate quantification and representativeness of a certain target marine habitat and benthic fauna is only possible upon a well-defined sampling design (e.g. scale, replication and sample independence, sampling gears used). Yet, challenges associated with deep-sea surveys may supersede ideal sampling conditions (Clark et al. 2016). Examples of common constrains during deep-sea sampling campaigns includes the time vacant for sampling within multidisciplinary teams, gears available on-board, and of major importance for the present study, the weather conditions.

Due to strong winds and rough sea conditions the use the boxcorer (BOX), our preferred gear to collect samples for investigating macrofauna biodiversity, was substituted halfway through the RV Belgica 2013/17 campaign, by the lighter and thus more easily to handle, multiple-corer sampler (MUC). Several studies have highlighted the generally lower quality of the samples collected by means of the box-corer, when compared to those collected with the multiple-corer sampler, when aiming the characterisation of both the sediment surface biogeochemistry and meiofaunal assemblages (Bett et al 1994; Shirayama and Fukushima 1995), although sampler effects are not always evident (Thistle and Sherman 1985; Montagna et al. 2016). The lower quality of samples collected with the boxcorer seem to primarily result from the designated down wash or bow-wave effect, which occurs as the heavy boxcorer enters the seabed often washing away the surface “lighter” materials (Bett et al 1994). Yet, even though the multiple-corer is irrefutably designed to collect undisturbed samples and thus theoretically preserving higher faunal density, the smaller sampled area recovered by comparison to the boxcorer usually produces much lower macrofaunal taxa richness estimates and consequently marked differences in community composition depending on the chosen method (Montagna et al. 2016), observed also in the present study (Supplementary Figure 3.1, Supplementary Figure 3.2; Supplementary Figure 3.3). Specifically, Montagna et al. (2016) found that macrofauna taxa richness in sediment samples collected using the box corer was up to 60% higher, by comparison to the results obtained using the multiple-core sampler at similar sampling locations. Thus, it is not surprising that the even though the bow-wave effect and associated loss of lighter macrofauna organisms may be expected, deep-sea researchers tend to opt by the use of the boxcorer sampler in macrofauna biodiversity studies, and try to minimize bow-wave effects by reducing the penetration velocity of the boxcorer gear as it approximates to the seabed.

Because in the present study, the use of the two different sampling methods was not applied at the same stations, the sampler effect cannot be directly investigated here. Thus, the comparison of macrofaunal assemblages subjected to the different trawl pressure (NT, LT, HT) was only applied to samples obtained by the same sampling method, the boxcorer. The highest abundances of the study were observed in NT areas showed, up to 3 times higher densities than LT and HT locations sampled using the same sampling method. Yet, when comparing these abundances to those collected by mean of the MUC, an opposite trend arises, with densities in disturbed sediments (HT_13; st. 4), with greater of those in LT_13 sediments for the same year (st. 6; Supplementary Figure 3.1). Furthermore, these high abundances at st. 4 reached values of approximately two times higher than those of NT areas, although not directly comparable.



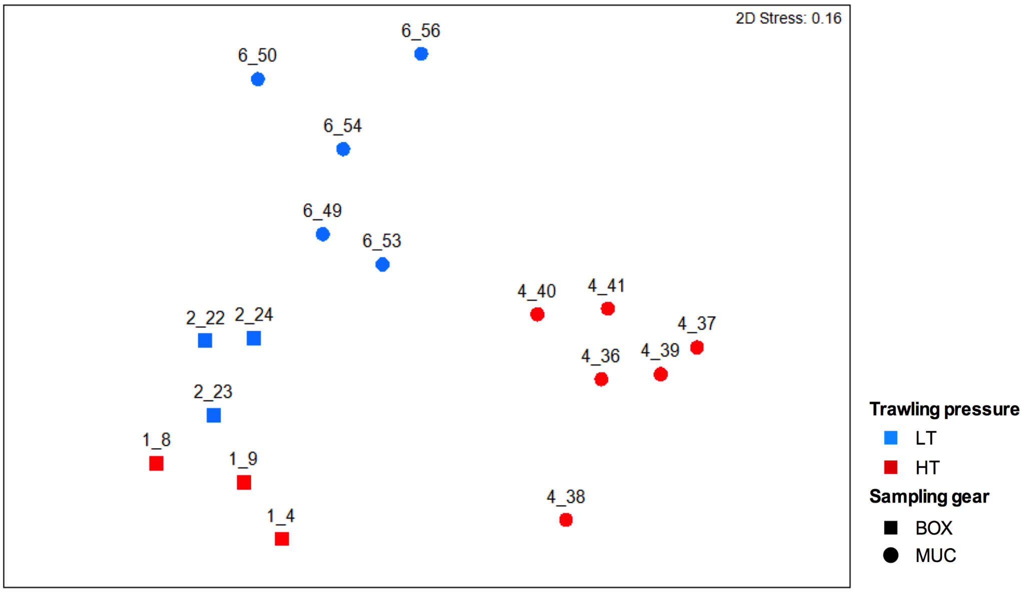
Supplementary Figure 3.1 Average macrofauna density (ind.10dm^{-2}) per station.*indicates samples collected with the multiple-core sampler (MUC). NT: no, LT: low and HT: high trawling pressure.



Supplementary Figure 3.2 Average macrofauna density (ind.10dm^{-2}) vertical distribution in the sediment.* indicates samples collected with the multiple-core sampler (MUC). NT: no, LT: low and HT: high trawling pressure.

The high abundances at st. 4 were predominantly determined by the high abundances of small sized *Kelliella miliaris*, most juveniles, and other small sized molluscs and crustacean taxa (e.g. Cumaceans), that can easily be washed away with the box-corer. Additionally, the larger abundances found on the uppermost sediment layer (0-1) in the sediment samples collected by means of the multiple-core sampler (both LT_13 or HT_13; Supplementary Figure 3.2), support the observation that MUC samples are by comparison less disturbed at the sediment surface, and that we may expect that at least some of the macrofauna component may have been lost during sampling.

Lastly, clear differences in community composition between trawling pressure groups were observed in the nMDS when accounting all samples collected in 2013 (BOX and MUC; Supplementary Figure 3.2). Also, despite no significantly different assemblages were detected by the PERMANOVA test on the two stations sampled with the boxcorer ($p>0.05$; st 1 vs. st 2; Table 3.3), when accounting with samples of the multiple-core samplers these showed highly significant differences (st. $p<0.001$), which themselves differed from those collected by the box corer ($p<0.05$; with significant pair-wise comparisons: st. 4 vs. st. 1; st 4 vs. st. 2; st. 6 vs. st. 1; st. 6 vs. st. 2). These results suggest that also in 2013 macrofaunal assemblages were likely distinct at the different trawling disturbance regimes areas, however to which extent we cannot know based on these current samples. Hence, we highlight the importance of future studies in this study region to maintain a strict consistency in the sampling methodologies and processing, always keeping in mind that both box-corer or multiple-core samplers present distinct limitations regarding the quality of samples when analysing the macrofauna assemblages.



Supplementary Figure 3.3 nMDS plot for comparison of macrofauna assemblages subjected to varying trawling pressure using two types of sediment samplers: box corer (BOX) and multiple-core sampler (MUC) in 2013. LT: low trawling pressure; HT: high trawling pressure. Sample code above each symbol indicate of station and deployment number.

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Chapter 4.

Altered ecosystem functions under conditions of bottom-trawling disturbance: experimental approach and field observations

Ramalho, SP, Lins L, Soetaert K, Lampadariou N, Cunha MR, Vanreusel A, Pape E, Altered ecosystem functions under conditions of bottom-trawling disturbance: experimental approach and field observations (in preparation for submission to Deep-Sea Research part I)

Abstract

Understanding the effects of trawling induced changes on benthic community structure, diversity and ecosystem functioning across the different benthic-size components (micro-, meio- and macrofauna) is imperative to determine the future sustainability of bottom-trawling fisheries in deep-sea regions. In this study, we combined field sampling with an isotope pulse-chase enrichment experiment on sediments obtained from two stations of interest differentiated by distinct trawling pressures (low (LT) and high (HT) trawling pressure) along the West Iberian Margin (WIM), and compared them in terms of meio- and macrofauna (infauna) biodiversity (both taxonomic and trophic) and several ecosystem function proxies. These proxies included: i) ^{13}C uptake by bacterial communities and infauna respiration rates relating to carbon mineralization and secondary production and ii) penetration of ^{13}C in the sediment and pore-water nutrients concentrations profiles in the sediment as a proxy for biogeochemical functioning typically promoted by faunal induced bioturbation and bioirrigation. The pulse-chase experimental results were then complemented with a larger biological dataset obtained in the study area to investigate general structural and functional diversity and ecosystem functioning (total respiration) patterns across the WIM. Our observations indicated that different regimes of trawling negatively influenced macrofaunal size structure. Macrofauna biomass and respiration rates were significantly reduced at high disturbance locations, and they were predominantly composed of deposit/detritus feeding smaller-sized species. The total biomass of small-sized biota, including bacteria and meiofauna, did not show marked differences between stations, although bacterial production appeared to be reduced in HT sediments. These results suggest that trawling activities may affect benthic assemblages, as well as regulatory ecosystem functions, such as sediment biogeochemical fluxes and bacterial secondary production. Also, the general decline in macrofauna species richness across the study region impacted areas was correlated with a depletion of total respiration, suggesting that the long history of trawling disturbance at the WIM is affecting fundamental ecosystem functions. These results can be an alert for the imperceptible impacts of trawling on the benthic ecosystems, overlooked by the current tools used in monitoring programmes.

4.1 Introduction

There is cumulative evidence on the influence of anthropogenic activities on marine biodiversity. This includes the deep sea, where exploitation of marine resources have been frequently associated with the changes in the benthic structure and loss of diversity (Ramirez-Llodra et al., 2011 and references therein). Moreover, biodiversity is potentially linked with ecosystem functions - "the *processes that transform or translocate energy or materials in the ecosystem*" (in the sense of Solan et al., 2004; Strong et al., 2015) and services - "*the direct and indirect contributions that ecosystems make to human wellbeing*" (in the sense of de Groot et al., 2010; Böhnke-Henrichs et al. 2013). Thus, the increased pressure of human-induced disturbance raises serious concerns on deterioration of the ecosystem functioning and its integrity (Worm et al., 2006; Danovaro et al., 2008).

The relationship between biodiversity and ecosystem functioning (BEF) revealed different patterns until the present: positive linear (Pape et al., 2013, Baldrighi et al., 2017), positive exponential (Danovaro et al., 2008; Narayanaswamy et al., 2013; Baldrighi et al., 2017) or non-existent (Leduc et al., 2013). Positive linear BEF models indicate a proportional increment of functions with gain of species, as each species contributes uniquely to ecosystem functions (Cardinale et al., 2011). A positive exponential BEF relationship implies that even minor losses of diversity will result in a marked decline of functions provided, as rare species are functionally unique and mutualistic interactions (complementarity effects) prevail over competition (selection effects; Loreau, 2001; Naeem and Wright, 2003; Loreau, 2008). The absence of a clear trend in BEF relationship as observed by Leduc et al. (2013), on the other hand suggests that the effects of biodiversity loss on ecosystem functioning may also be unpredictable (idiosyncratic model) or even non-existent (null model) in certain habitats. Absence of BEF relationships characterise an ecosystem that is primarily controlled by environmental factors or when there is a high niche overlap so that changes in relative abundance and species richness will not alter ecosystem processes (Hooper et al., 2005; Cardinale et al., 2011). The idiosyncratic model considers that BEF relations are strongly determined by extremely variable biotic/abiotic interactions - here, alterations of the ecosystem functions will be largely dependent on the context of the local extinctions of species, e.g. environmental context (Hooper et al., 2005; Cardinale et al., 2011; Strong et al., 2015). Hence, loss of biodiversity in the deep sea may not always necessarily represent a proportionate loss of ecosystem functions and services. Differences in BEF relationships may also be related to the different spatial scales of the observations, taxonomical resolution (genus vs. species) and faunal compartment, as well as the local biodiversity (Leduc et al., 2013). The assessment of how biodiversity relates to ecosystem functions in deep-sea regions can assist predicting ecosystem's efficiency and its resistance and resilience under (anthropogenic) disturbance conditions (Strong et al., 2015).

Among the most destructive anthropogenic activities affecting the deep sea, bottom-trawling fisheries severely affects organisms dwelling at the seabed and may consequently have an impact on the ecosystem efficiency and stability (Ramírez-Llodra et al., 2011, Clark et al., 2015). High damage and mortality rates of the affected fauna and marked alterations of seabed habitats have been reported in both shelf and deep-sea studies (NRC, 2002; Clark et al., 2015 and references therein). In soft sediments, as trawl nets typically homogenise the sediment surface and, depending on trawling pressure (frequency and intensity), may also modify sediment biogeochemistry and pollutants' availability (Oberle et al., 2016). Sediment removal and remixing by trawl gears, causes high turbidly periods, impoverish the sediment surface organic matter concentrations and increases sediment sorting and porosity, which inevitably weakens water-sediment nutrient fluxes (Martín et al., 2014a,b; Oberle et al., 2016). Moreover, the induced faunal mortality and alteration of habitat can change faunal interactions and benthic community structure, and induce biodiversity loss (NRC, 2002; Clark et al., 2015). The negative effects of trawling on the benthos appear to be size-dependent. Larger-sized faunal compartments, i.e. megafauna (recognized in photographs) and also macrofauna (> 250/500μm), are more susceptible to removal or damage by trawl gears (Jennings et al., 2001a,b, Queirós et al., 2006; Clark et al., 2015). In comparison, small-sized biota, i.e. microbenthos (typically bacteria and archaea, < 32μm) and meiofauna (> 32μm), are temporarily re-suspended, and may not suffer significant alterations in terms of standing stocks in the long term. In some cases, they may even benefit from the lower predation pressure (i.e. by macrofauna), which allows elevated turnover rates of the small-sized biota and an increase in the local benthic secondary productivity (Jennings et al., 2001b; Schratzberger et al., 2002; Schratzberger and Jennings, 2002; Lampadariou et al., 2005). Noteworthy, negative influence of chronic trawling on meiobenthos abundance has been identified by Pusceddu et al. (2014) in the La Fonera submarine canyon (NW Mediterranean Sea), suggesting that the absence or even beneficial effects of physical disturbance by trawling on small fauna observed in shelf areas (Scharatzberger et al., 2002; Schratzberger and Jennings, 2002; Lampadariou et al., 2005), may not necessarly be transposed to highly dynamic deep-sea regions. Moreover, although meiofauna abundances seem to recover fast, most studies also reported changes in the community structure of the nematodes, which are the dominant group within the metazoan meiofauna (Schratzberger et al., 2002; Schratzberger and Jennings, 2002; Lampadariou et al., 2005).

Dependent on their size and traits (e.g. mobility capacity, feeding strategies), benthic organisms may be responsible for supporting various key ecosystem functions. For example, macrofauna organisms are fundamental in sustaining sediment biogeochemistry fluxes and the diversity and efficiency of microbial communities, either through bioturbation (particle mixing) and bio-irrigation (solute transfer and sediment permeability; Aller, 1982; Lohrer et al., 2004; Braeckman et al., 2010), or through biological interactions (e.g. carbon transfer by predation). Note that the role of certain meiofauna groups (i.e. foraminifera, nematodes) on sediment processes via micro-bioturbation can also be of importance, particularly in the absence of diverse macrofaunal assemblages (Rysgaard et al., 2000; Bonaglia et al., 2014). A decline in benthic standing stocks may result in reduced sedimentary oxygen and penetration depth of nutrient concentrations leading to changes in microbial metabolism and affecting microbial-mediated processes such as carbon remineralisation and nutrient cycling (e.g. nitrogen; Aller, 1982; Lohrer et al., 2004; Braeckman et al., 2010).

So far, only few studies have addressed the study of the structure and diversity of benthic communities in parallel with the investigation of ecosystem functions that these communities facilitate in deep-sea areas affected by bottom trawling (Duplisea et al., 2001; Hiddink et al., 2006; Leduc et al., 2016; Sciberras et al., 2016). In this context, the present study examined macro- and meiofaunal diversity and composition in concert with several ecosystem functions at the Western Iberian Margin (WIM), an area subjected to bottom-trawling fisheries for decades. The first part of the study compares an area under low (LT) and a high trawl (HT) pressure in terms of environmental and faunal parameters assessed from field sampling, and ecosystem functions (i.e. bacterial production and biogeochemical functioning typically promoted by faunal induced bioirrigation and bioturbation) by conducting an on-board pulse chase experiment. The second part of the study aims at determining the existence of a putative BEF relationship at the WIM by relating existing biodiversity data to the measured proxies for ecosystem functioning (respiration rates and total respiration).

4.2 Material and Methods

4.2.1 Study area

The West Iberian continental margin (WIM) presents complex and diverse geomorphological features (Relvas et al. 2007, Maestro et al. 2013), such as submarine canyons and rocky outcrops. These features interact with several water masses and fronts, determining the spatial and temporal variability in salinity, temperature and oxygen content (Relvas et al. 2007).

Under the influence of the Iberian upwelling system, the high seasonal primary production along the WIM (associated with upwelling) is determinant to sustain the productive fisheries (Santos, 2001; Picado et al. 2014; Kämpf and Chapman, 2016) that are also one of the most pervasive and economically important anthropogenic activities in the region (Hill and Coelho, 2001). From the various *métiers* operating in Portuguese waters, bottom-trawling fisheries target several species of crustaceans is particularly threatening deep-sea areas. With low selectivity and target areas concentrated in muddy and muddy sand bottoms along the South and Southwest regions off Portugal, crustacean bottom trawling has been in practice for several decades and has high economic relevance. These are among the most disturbed areas in Europe; Eigaard et al., (2016), estimated that, the majority of the areas between 200 and 1000 m water depth in the Portuguese Iberian region (93.6% of the total seabed) are disturbed by trawling annually. These fisheries are also associated with an enormous footprint per unit of landings (ca. 17 $km^{-2}t^{-1}$). Moreover, because of the low selectivity of trawling practices, crustacean trawlers have usually high by-catch and discard rates (c.a. 40 - 70 %; Borges et al. 2001; Monteiro et al. 2001).

4.2.2 Sampling strategy and onboard sample processing

During the RV Belgica cruises B2013/17 (10/06/2013–18/06/2013) and B2014/15 (02/06/2014–10/06/2014) a total of seven distinct stations were sampled along the upper continental slope off Sines and near the Setúbal canyon (ca. 250 - 550m depth) for the analysis of sediment environmental parameters, meiofauna and/or macrofauna assemblages in areas subjected to varying trawling pressure (Fig. 4.1; Supplementary Table 4.1 and Supplementary Table 4.2). Sampling stations were primarily selected based on trawling pressure information obtained from Vessel monitoring systems data compiled by DGRM (MAMAOT 2012). Annual trawling pressure ($h.cell^{-1}.y^{-1}$) estimates for each sampling deployment was then obtained (hours per an area of 0.01 x 0.01 decimal degrees: ca.1 km^2) from the Vessel Monitoring Systems (VMS) position data provided by the Direção Geral de Recursos Marinhos (DGRM) and analysed as in (Bueno-Pardo et al., 2017). This allowed categorising each station into the following disturbance categories: no- (NT; 0 $h.cell^{-1}.y^{-1}$), low- (LT; 0.1–1.5 $h.cell^{-1}.y^{-1}$) or high (st 1; HT; >1.5 $h.cell^{-1}.y^{-1}$) trawling pressure (TP). Note that NT label was only assigned to the stations safeguarded by current legal restrictions and where trawling has not occurred for the past decades (i.e., st. 9 and st. 10 in the vicinity of the Setúbal canyon head). Samples for environmental and meiofauna analysis were collected with a multicorer (MUC, ∅ 10 cm), whereas those for macrofauna analysis were collected with a NIOZ box corer (∅ 32 cm). Meiofauna and environmental samples were sliced every centimetre down to 10 cm depth and preserved in borax-buffered 4% formalin or frozen at -20ºC. Macrofauna samples were initially processed collecting the overlaying water through a sieve of 250 μm mesh, and then the sediment was sub-sampled at three depth layers (0-1; 1-5 and 5-15 cm). Each layer was washed through a set of sieves of 1mm, 500μm and 250 μm mesh-size and fixed with 96% ethanol. Amongst our total of seven stations, we selected two (stations 6 and 7) with a similar environmental setting, but distinct trawling disturbance (LT and HT) where we collected additional MUC cores for onboard pulse-chase experiments (see section 4.2.4).

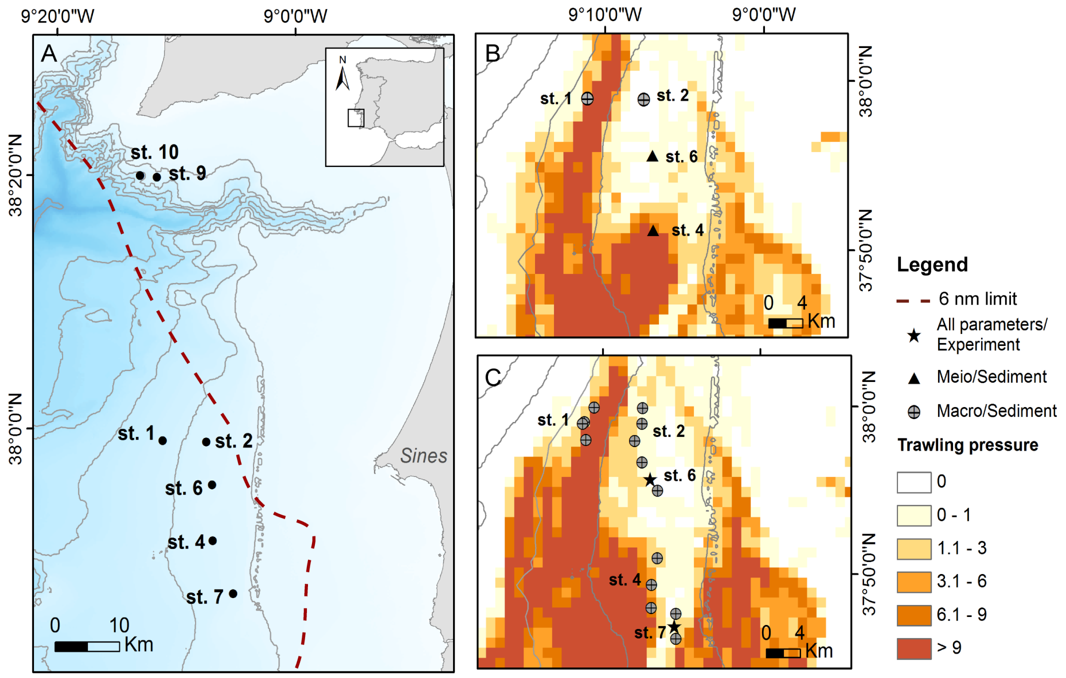

Figure 4.1 (A) Study area with an indication of all sampled stations and their position in relation to trawling pressure (h.cell^{-1}.y^{-1}) for the year of (B) 2013 and (C) 2014, and corresponding analysis: meiofauna, macrofauna and sediment environmental parameters (sediment) or isotope enrichment experiment. The Setúbal canyon area was only sampled for macrofauna and environmental parameters; details are not shown here due to null trawling pressure values (NT; 0 h.cell^{-1}.y^{-1}). Red dashed line establishes the legal six nautical miles from the coastline.

4.2.3 Field sample analyses

4.2.3.1 Environmental parameters

Environmental parameters included sediment grain size, total organic carbon and total nitrogen contents and were obtained from Lins et al. (2017) and Chapter 3 (Supplementary Table 4.1 and Supplementary Table 4.2). Grain-size distribution was determined using a particle size analyser (Malvern Mastersizer 2000) with a particle size range of 0.02–2000 µm and then classified into five categories following the Wenthworth scale (1922): silt+clay (< 63µm), very fine sand (63 - 125µm), fine sand (125 – 250µm), medium sand (250 – 500µm), and coarse sand (500µm – 2mm). Total organic carbon (TOC) and total nitrogen (TN), expressed as percentage of sediment dry weight, were measured using a Carlo Erba 25 elemental analyser, after acidification with 1 % HCl to eliminate carbonates present.

4.2.3.2 Macro- and meiofaunal community analyses

Meiofauna, retained in between 32-μm and 1000-μm mesh sieves, was extracted from the sediment using a density gradient solution in a centrifugation procedure using colloidal silica polymer LUDOX HS-40 (specific gravity 1.19). This dataset was obtained from Lins et al. (2017) and included total abundances of the metazoan meiobenthic organisms classified following Higgins and Thiel, (1988) and Giere, (2009). In addition, a list of the nematode genera abundance was also provided from the sediment surface slice (0-1cm), estimated from a randomly picked subset of 100 to 120 nematodes mounted on permanent slides (or all nematodes when abundances were lower than 120 per sample). The nematodes were identified to genus level using the pictorial keys provided by Platt and Warwick (1983,1988) and Warwick et al. (1998), online identification keys and other relevant literature available on the Nemys Database (Guilini et al., 2016). The full list of all meiofauna taxa encountered is provided in the Annex 4. Each nematode genus was allocated to a matching trophic group, following the Wieser (1953) classification: selective deposit feeders (1A), non-selective deposit feeders (1B), epistratum feeders (2A), and predators/scavengers (2B).

Macrofauna abundance data was obtained from Chapter 3. The full list of all macrofauna taxa encountered is provided in the Annex 3. All individuals sorted were identified to the lowest taxonomical level possible, and in the cases where a match with a species name was not possible; each taxon was ascribed with a consistent code across all sampled stations. Typical “meiofaunal” taxa, i.e. Nematoda, Copepoda and Ostracoda, were excluded from this daraset. Each taxon was assigned to a matching trophic guild according to its food source (or foraging behaviour), feeding mode and food type/size, following the classification proposed by MacDonald et al. (2010) and other relevant literature available (e.g. Fauchald and Jumars 1979; Jumars et al., 2015). The following categories were considered for: a) food source: epibenthic (EP), sediment surface (SR), and sediment subsurface (SS); b) feeding mode: omnivorous (Om), deposit feeders (De), detritus feeders (Dt), grazers (Gr), scavengers (Sc), predators (Pr), suspension/filter feeders (Su), mixotrophs (Mx) and suctorial parasites (Sp); and c) food type/size: sediment (sed), particulate organic matter (poc) microfauna (mic), meiofauna (mei), macrofauna (mac), zooplankton (zoo) and fish (fis).

4.2.3.3 Biomass

Nematode biomass was determined for the subsample of 100-120 individuals per sediment layer. Individual nematode length (excluding tail tips; L (µm)) and maximum body width (W (µm)) was measured under the compound microscope (Olympus BX-50) with Olympus Cell^D software, and body volume estimated by applying Andrassy's formula (wet weight; Andrassy, 1956; Wieser, 1960). A ratio of a 0.124 was assumed to convert nematode wet weight into carbon weight (µgC; Baguley et al., 2004). Individual mean biomass was calculated as the weight of the taxon group divided by the number of individuals counted, while total biomass was calculated as the sum of the products of individual biomass and abundance of each taxon.

Macrofauna biomass data obtained from Chapter 3, as wet weight (mg) grouped by specimens of the same family for sample and sediment layer (0-1; 1-5 and 5-15), was converted into carbon weight (mgC) f following the taxa-specific conversion factors of Rowe (1983). Due to their small values, macrofaunal wet weights were measured by transferring all individuals belonging to the same family in each sub-sample to previously weighed microtubes containing 96% ethanol that were then weighed again to obtain the wet weight of the lot.

Total fauna biomasses were expressed as μgC.10cm^{-2} and mgC.10dm^{-2}, for meiofauna and macrofauna, respectively.

4.2.3.4 Allometric respiration rates

Allometric respiration estimates were calculated for both nematode (meiofauna) and macrofauna assemblages following Mahaut's formula (Mahaut et al., 1995). The mass dependent respiration rate (R, d^{-1}) was calculated as:

$$R = aW^b$$

, where *W* is the mean individual biomass (in mgC), and the constant a=7.4*10-3 and b=-0.24. Total community respiration of both meiofaunal and macrofaunal assemblages was calculated as the product of the mass-dependent respiration rate (*R*) and total biomass (in mgC.m^{-2}), expressed as mgC.m^{-2}.d^{-1}.

4.2.4 Time-series isotope enrichment experiment

4.2.4.1 Experimental set-up

During the B2014/15 cruise, two stations of interest were selected from a similar environmental setting, but distinct trawling disturbance regimes (st 6 (LT) and st 7 (HT) at ca. 300 m water depth; Fig. 4.1). Here, we determined various proxies of ecosystem functioning, i.e. bioturbation, bacterial biomass/production and bio-irrigation at three distinct time points: start of experiment (T0), after 3 (T3) and 5 (T5) days.

In total, 18 MUC cores were collected: nine for each trawl pressure group (HT and LT) accounting for three replicates per each of the three time points (Supplementary Table 2). The cores were initially maintained in the cold room in the dark for 24h at approximated *in situ* water temperature, i.e. 12°C, and constant oxygen flow provided by aquarium pumps. After acclimatization, each core was randomly assigned to a distinct sampling time step (n= 3 for T0, T3 and T5) and, except for the cores assigned as T0 that were used as controls, a suspension of ^{13}C labeled algae (*Skeletonema costatum*) was added homogeneously to the sediment surface of each core with a long pipette (ca. 2.6 mgC per core; 26% of ^{13}C enrichment). *S. costatum* was chosen because it is a common diatom species in phytoplankton assemblages, both in winter and summer periods, along the Iberian Margin (Silva et al., 2009). At each time step, the selected cores from each trawl pressure group were sliced per centimetre down to the bottom of the core, and subsampled for the analysis of: ^{13}C uptake by sedimentary total organic carbon (ca. 2 ml), ^{13}C uptake by bacteria-specific phospholipid-derived fatty acids (PLFAs) (ca. 10 ml), and pore-water ammonium concentrations (remaining sediment). Sub-samples for pore-water nutrient ammonium concentrations were stored at -20ºC, while the remaining sub-samples were stored at -80ºC for further laboratory analysis. Bacterial biomass from the T0 samples was used in conjunction with meiofaunal and macrofaunal biomass to compare infaunal standing stocks between LT and HT.

4.2.4.2 Assessment of biogeochemical functions, bioturbation and bacterial biomass and production

The pore-water dissolved inorganic nitrogen concentrations; in specific ammonium concentrations (expressed as $\mu mol.l^{-1}$), were investigated along the vertical sediment profile (down to 10 cm) as a proxy for biogeochemical functioning typically promoted by faunal bioirrigation and bioturbation. The pore-water was extracted from each sub-sample through Whatman GF/C filters and analysed using a continuous flow analyser the SKALAR SAN.

Bioturbation was inferred from ^{13}C incorporation in the sediment for the duration of the experiment. Each sediment sub-sample was first freeze-dried and grinded. Quantification of organic carbon content and isotopic ratios were then carried using a Thermo Flash EA 1112 element analyser, coupled with a Thermo Delta V Advantage Isotopic mass spectrometer (Thermo Fisher scientific). Due to laboratory and analysis constraints, ^{13}C labelled algae content in the sediment and corresponding total organic carbon and total nitrogen values in the experimental cores were only measured down to 5 cm depth.

Bacteria ^{13}C algae uptake (production) and biomass were derived from the concentrations of bacteria-specific phospholipid-derived fatty acids PLFA's, for the layers 0-1 cm and 4-5 cm as described by van Oevelen et al. (2006). The polar lipid fraction was extracted from the freeze-dried and grinded sediments and derivatized using the mild alkaline methanolysis to yield fatty acid methyl esters (FAMEs), following the Bligh and Dyer method (Bligh and Dyer, 1959; Boschker 2004). ^{13}C concentrations of this component were analysed with a gas chromatography combustion interface isotope-ratio mass spectrometer (GC-c-IRMS). We analysed only 0-1 and 4-5 cm for comparison. The bacteria specific PLFA's used included the i14:0 and ai15:0, present in all of our samples, and accounted roughly with 8% of all bacterial PLFA's (Middelburg et al, 2000) and 5.6% of the total carbon content in bacterial cells (Brinch-Iversen and King, 1990), allowing the estimation of total bacterial biomass.

4.2.5 Data analyses

The environmental and biological data (field samples) collected for the pulse-chase experiment (st. 6 and st. 7, from here on designated as LT and HT stations respectively), were tested for differences by means of non-parametric Mann-Whitney U-tests, after rejection of normality and homogeneity of dispersion (Quinn and Keough, 2002), using the software GraphPad PRISM v6 (GraphPad Software, www.graphpad.com). The environmental parameters tested included: grain-size class group, porosity, TOC and TN (expressed as percentage). Biological parameters from the surface layer (0-1 cm) included total bacteria biomass; meiofauna and macrofauna total abundance, mean individual biomass, total biomass and total respiration. Note that comparisons for the deeper layers were not evaluated here due to the absence of consistent data obtained for all benthic size-groups at the different sediment depth layers. Because nematodes were the dominant fraction of meiofauna, when considering meiofauna diversity and biomass, we are referring only to nematode assemblages. Taxonomic and functional (trophic) biodiversity patterns were also analysed for meiofauna and macrofauna for both stations (HT and LT) using several diversity indices, namely: species or genus richness/trophic guilds richness (S/TG), Shannon-Wiener diversity (H'), evenness (J') (Pielou, 1966) and Hurlbert's expected number of taxa or trophic guilds ($ES_{(n)}/ETG_{(n)}$) for 20 individuals (Hurlbert, 1971). These biodiversity indices were estimated using the software PRIMER v6 (Clarke and Gorley, 2006), and were also tested for differences by means of non-parametric Mann-Whitney U-tests, using GraphPad PRISM v6.

Ecosystem functions investigated during the enrichment experiment included: biogeochemical functioning (ammonium and nitrate concentrations), bioturbation (^{13}C sediment uptake) and bacterial production (^{13}C bacteria uptake). These variables were tested for differences between stations (trawling pressure) over time and accounted for sediment depth dependency, by means of a permutational multivariance analysis of variance (PERMANOVA) using PRIMER v6 and PERMANOVA+ add-on (Clarke and Gorley, 2006; Anderson et al., 2008). These tests were applied on Euclidean distance matrix after normalization of the main datasets. PERMANOVA design followed a 4-factor layout, with "Trawling pressure" as a fixed factor (levels: HT and LT); "Time" as a fixed factor (levels: T0 (only for ammonium/nitrate concentrations), T3, T5); "Sediment depth" as a fixed factor (levels: every centimeter down to 10 cm for biogeochemical functioning established from nutrients concentrations, and 0-1 and 4-5 cm for the other variables), and "replicate core" as a random factor nested in "Trawling pressure x Time". In case a significant effect ($p \leq 0.05$) found for any of the factors investigated in the PERMANOVA main test, pair-wise pseudo-t tests were then carried out.

Lastly, the correlations between structural diversity and ecosystem functions (i.e. respiration rate and total respiration); as well structural and functional diversity (trophic guild richness and predator richness) for the whole meiofauna and macrofauna field dataset, was explored by means of non-parametric Spearman rank correlations using the software GraphPad PRISM v6. Significant correlation values were adjusted using the Bonferroni correction (Shaffer, 1995), by dividing the significance value of each test by the number of hypotheses tested. Biodiversity indices estimated for all seven sampled stations were calculated using the software PRIMER v6 (Clarke and Gorley, 2006).

4.3 Results

4.3.1 Comparison between the LT and HT area

4.3.1.1 Environmental parameters

Generally, similar environmental conditions were observed at the two stations sampled for the pulse-chase experiment (HT and LT) (Table 4.1). Overall sediments were characterised as muddy-sand (silt+clay content >10%) composed of high proportions of both fine and medium sand content (ca. 50-60%), with no significant differences in terms of sediment porosity (U=9; p=0.610) (Table 2). TOC (%) concentrations were also similar in both stations (U=100; p=0.747): 0.422±0.0178 and 0.433±0.0138 at LT and HT respectively. Also, TN (%) concentrations did not significantly differ between stations (U=88; P=0.408).

Table 4.1 Overview of the sediment environmental characteristics at the LT and HT stations (average ± standard error) in the sediment surface (0-1 cm).

Environmental Variables	LT (st. 6)	HT (st. 7)
Silt+Clay (%)	10.5±0.73	15.6±0.61
Very Fine Sand (%)	17.1±0.41	13.7±0.39
Fine Sand (%)	37.8±0.38	25.6±0.26
Medium Sand (%)	28.5±0.59	30.4±0.52
Coase sand (%)	6.1±0.38	14.8±0.58
Porosity	0.51±0.011	0.52±0.022
TOC (%)	0.422±0.0178	0.433±0.0138
TN (%)	0.0490±0.00402	0.0518±0.00190

4.3.1.2 Infaunal standing stocks, diversity and trophic composition

The infauna (including both meiofauna and macrofauna) showed consistently higher abundances in the 0-1 cm layer at HT (st. 7) than LT (st. 6; Fig. 4.2A). Total macrofauna abundances accounted in average 67.2±9.73 and 103.5±14.62 ind.10dm^{-2} at LT and HT stations respectively and differed significantly (U=0; p<0.05). Meiofauna was typified by the dominance of nematodes (68-90%) and total abundances amounted on average from 393.7±34.35 and 490.7±38.28 ind.10cm^{-2} in LT and HT stations respectively, but these differences were not significant (U=1; p= 0.114). Similar patterns were detected at the sediment sub-surface layers for both faunal groups (>1cm depth; data not shown).

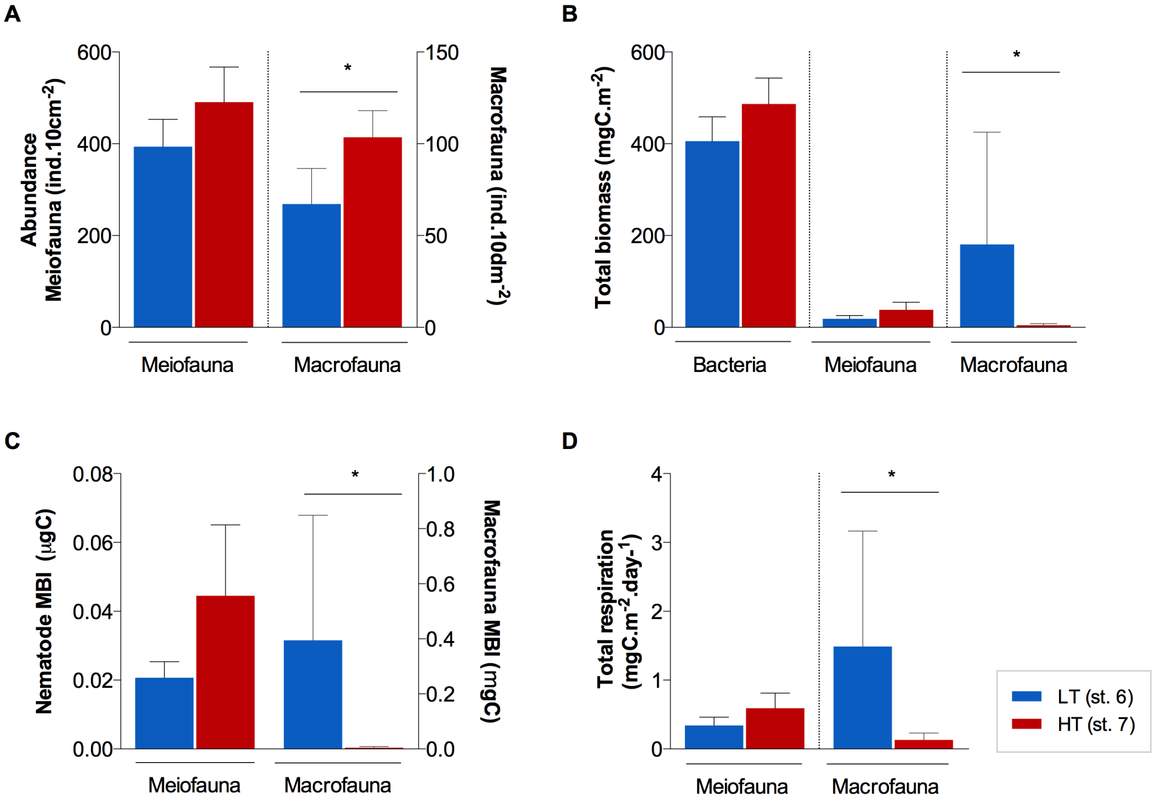

Figure 4.2 Average (± standard error) benthic (A) abundances, (B) biomass, (C) mean individual biomass (MBI) and (D) total respiration per fauna size groups (bacteria, meiofauna and macrofauna) at the surface of the sediments (0-1cm) of station LT and HT. Note that for bacteria, only biomass measurements were available. *Indicates significant differences between treatments ($p \leq 0.05$).

Unlike abundance, benthic biomass, expressed as carbon content, showed contrasting trends between stations, dependent of the size group. Overall, bacteria were the main contributor to the total biomass at both stations (Fig. 4.2B), with a higher average contribution at HT (91%) than at LT (67%), although not significantly different (U=1; p=0.400). Meiofauna was identified as the second most important contributor to the total benthic biomass at the HT, while macrofauna relative contribution prevailed over meiofauna at LT (macrofauna ca. 30% in LT vs. 1% in HT station). Macrofauna biomasses were significantly different between these stations (U=0; p<0.05; Fig. 4.2B), associated with a much higher, yet variable, mean individual weight at the sediment surface (0-1cm) of LT (st 6; 0.34±0.227 mgC; Fig. 4.2C). Noteworthy is that the sub-surface layer of the HT station (1-5 cm) was comprised of weightier individuals resulting in similar core total biomasses at both stations (data not shown; at 1-5 cm 21.6±20.59 and 324.2±133.56 $mgC.m^{-2}$ for LT and HT respectively).

Macrofauna and nematode biodiversity indices did not significantly differ between LT and HT stations ($p>0.05$), with exception of macrofauna $ETG_{(20)}$ ($U=0$; $p<0.05$), as general trends indicated a higher trophic (functional) diversity at LT when compared to HT (Table 4.2). Macrofauna trophic structure was more complex (Fig. 4.3) and diversity was higher in the LT sediments (Table 4.2). This resulted from relatively even contributions of the various trophic groups that comprised the macrofauna assemblages at LT. At LT station, the relative contribution of deposit and detritus feeders (ca. 37%) was highest, followed by predators (23%), suspension feeders (16%), omnivores (8%) and gazers (5%; Fig. 4.3B). The HT station was characterised by a much larger contribution of both surface and subsurface deposit and detritus feeders (56%). Nematode trophic composition at the LT was also composed by lower contributions of Wieser's (1953) equivalent to deposit/detritus feeding guilds (1A+1B; 41%) when compared to HT (53%; Fig. 4.3A).

Table 4.2 Meiofauna (Nematoda) and macrofaunal taxonomic and trophic diversity (average±SE) at LT and HT stations.

	Meiofauna - Nematoda		**Macrofauna**	
	LT (st 6)	**HT (st 7)**	**LT (st 6)**	**HT (st 7)**
Taxonomic Diversity				
S	46.3±1.45	50.3±2.87	25±5.83	33.3±6.90
J'	0.896±0.0109	0.896±0.0124	0.926±0.016	0.901±0.0183
$ES_{(20)}$	14.8±0.33	15.0±0.40	14.2±0.84	14.1±0.79
H'	3.44±0.068	3.51±0.078	2.96±0.219	3.14±0.202
Trophic Diversity				
TG	4.0±0.00	4.5±0.29	12.3±0.5	11.5±2.08
J'	0.892±0.0042	0.855±0.0311	0.884±0.0481	0.816±0.0341
$ETG_{(20)}$	3.90±0.021	3.93±0.047	9.4±0.46	7.5±0.60
H'	1.24±0.006	1.27±0.02	2.21±0.127	1.98±0.116

S: species richness; H': Shannon-Wiener diversity index (ln-based); J': Pielou evenness; $ES_{(20))}$: Hurlbert's expected number of species per 20 individuals; TG: number of trophic guilds; $ETG_{(20)}$: Hurlbert's expected number of trophic groups per 20 individuals.

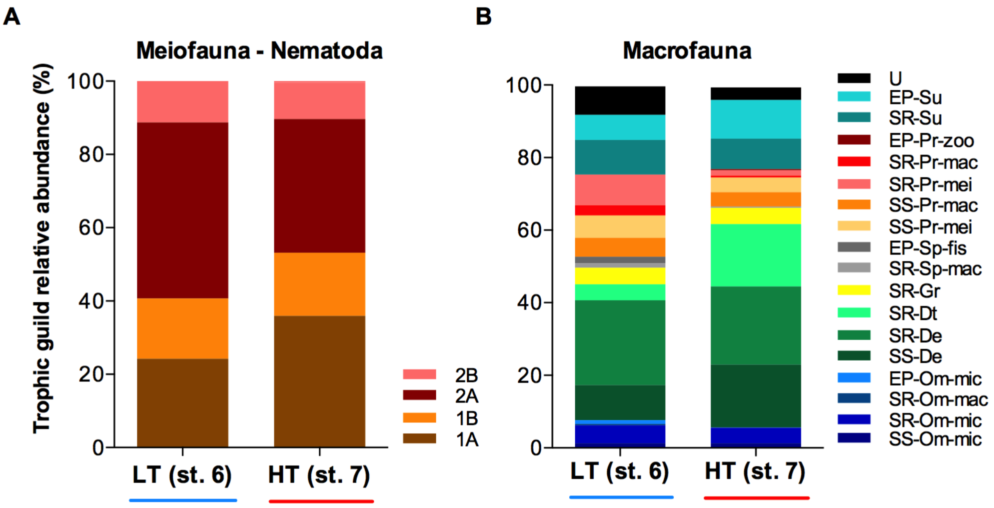

Figure 4.3 (A) Meiofauna (Nematoda) and (B) macrofauna trophic guild relative contribution (%). Nematoda feeding guilds included: selective deposit feeders (1A); non-selective deposit feeders (1B); epigrowth feeders (2A), predators/scavengers (2B) and parasite (P). Macrofauna feeding guilds code was composed of: food source (epibenthic (EP), seafloor surface (SR) and sediment subsurface (SS)); food type/size (particulate organic matter/microfauna (mic), meiofauna (mei), macrofauna (mac)); and feeding mode (omnivorous (Om), detritus (Dt) and deposit (De) feeders, grazers (Gr), predators (Pr), suctorial parasites (Sp), suspension/filter feeders (Su)). U: no information.

4.3.1.3 Ecosystem functions

Total respiration estimates for nematodes varied in average between 0.34±0.069 and 0.59±0.111 mgC.10m^{-2}d^{-1} in LT and HT sediments respectively, not differing significantly. Total respiration estimates for the macrofauna assemblages inhabiting the surface sediment layer showed significantly higher values in LT sediments (1.49±1.676 mgC.m^{-2}d^{-1}) when compared to HT (0.13±0.098 mgC.m^{-2}d^{-1}) (U=0; p<0.05; Fig. 4.2D).

Biogeochemical functioning investigated through pore-water nutrients concentrations, showed a significant effect of the different stations on ammonium concentrations (F= 5.3926; p<0.05), sediment depth (F=27.609, p<0.01) but also replication (F=2.8056; p<0.01) (Supplementary Table 4.3). Concentrations of ammonium were significantly higher in HT at the surface and in the subsurface layers (0-4 cm; Supplementary Table 4.4), followed by marked increased ammonium concentrations at the deeper layers (mostly bellow 4/5 cm; Supplementary Table 4.4), within the first three days of experiment (Fig. 4.4 A,B). Note that no significant differences in pore-water nitrate concentrations across the study period and ammonium profiles after five days were observed between trawling regimes (Fig. 4.4 C,D,E, F).

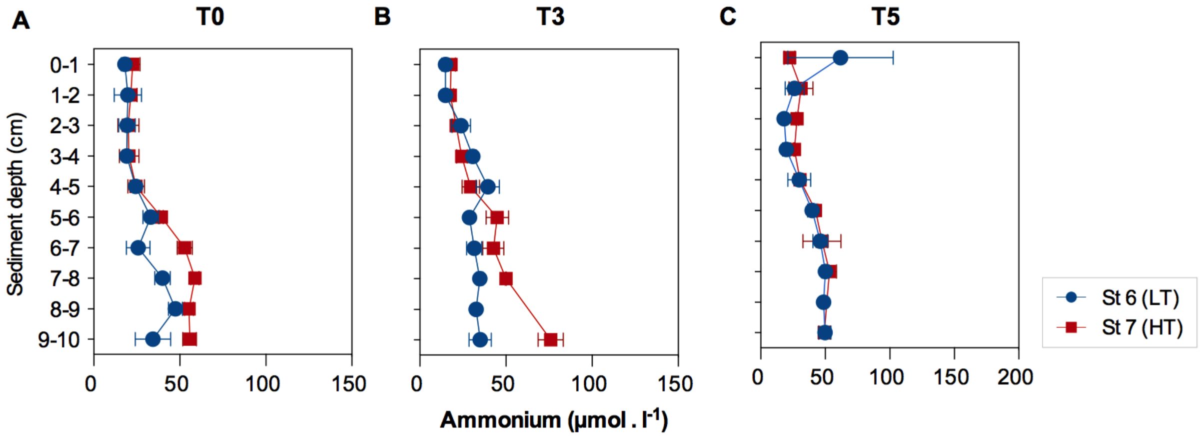

Figure 4.4 Pore-water concentrations of ammonium (average ± standard error) across the core sediment profile in LT and HT trawl pressure groups A) after acclimatization (T0), B) three (T3) and C) five (T5) days.

The ^{13}C labelled algae added was detected within the initial three days of the experiment down to 3-4 cm sediment depth, however only at HT (data not shown). After five days, the ^{13}C labelled algae signal was detected in both LT and HT down to the deepest sediment layer (4-5cm) - supported by significant differences between times (F=5.5494; p= 0.045) and sediment depths (F=68.702; p<0.01), yet no significant differences were detected between LT and HT (F=0.50507; p=0.488) (Fig. 4.5 A; Supplementary Table 4.5).

The average uptake of the ^{13}C labelled algae by bacteria (or bacterial production) showed significant differences between HT and LT (F=12.175; p<0.05), sediment depth (F=11.935; p<0.05) and the interaction of both factors (F=9.7769; p<0.05) (Supplementary Table 4.7). Higher bacteria uptake was consistently observed at the LT station after both three and five days (Fig. 4.5 B). By contrast, bacterial biomasses were consistently higher at both layers in sediments from HT over the course of the experiment, yet not significant (Fig. 4.5 C).

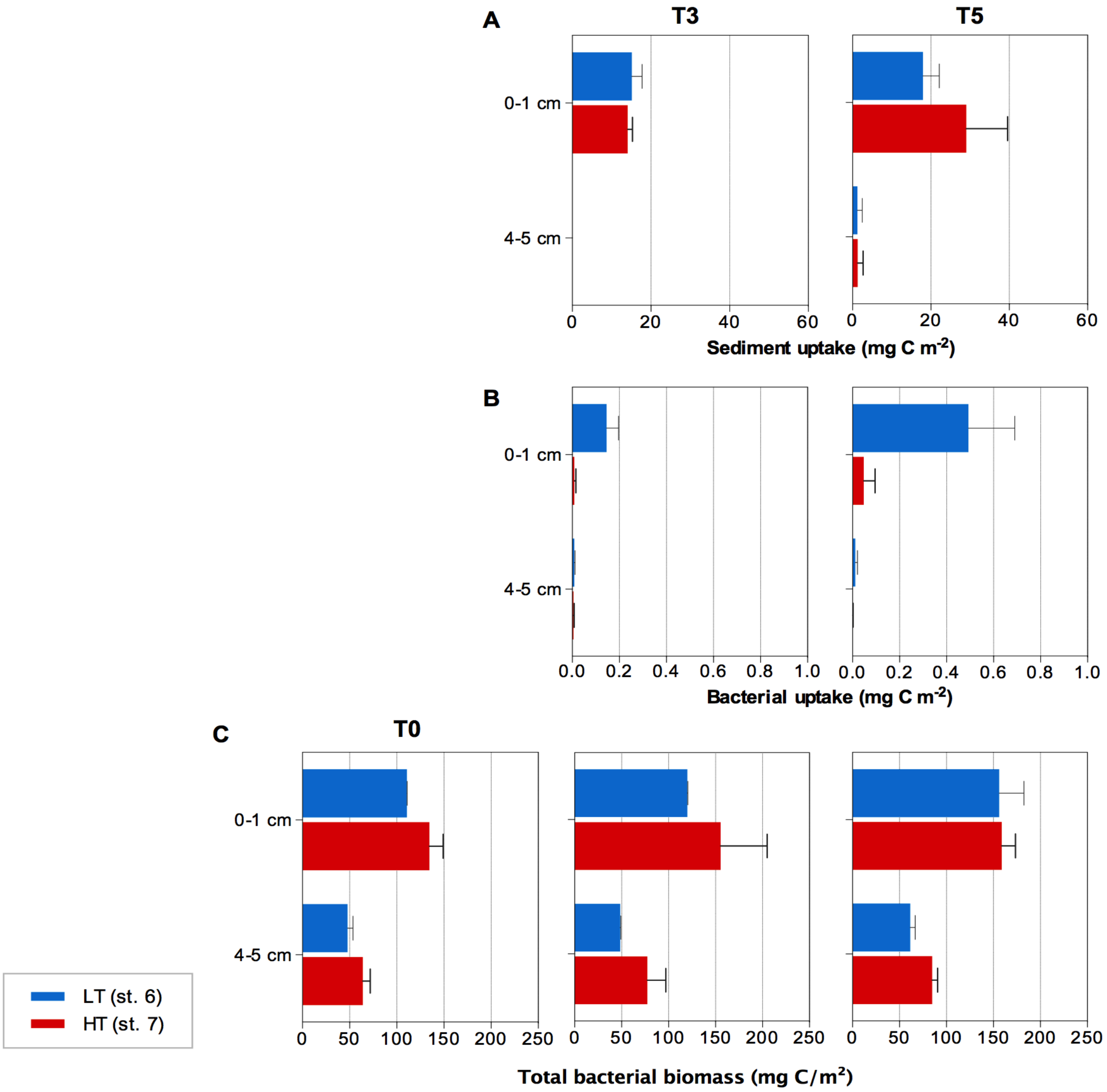

Figure 4.5 ^{13}C algae uptake by the (A) sediment and (B) bacterial communities; and its relationship with (C) total bacteria biomass in LT and HT trawl pressure groups after acclimatization (T0) three (T3) and five (T5) days. Values expressed as average ±standard error.

4.3.2 BEF relationships under different trawling regimes at the WIM

We identified significant negative correlations, after Bonferroni correction, between trawling pressure and macrofauna total respiration (R=-0.5147; $p<0.01$; Fig. 4.6F), and total biomass (R= -0.5156; $p<0.01$; Fig. 4.6 B). Also, a significant correlation was found between trawling pressure and macrofauna respiration rate (R=-0.3818; $p<0.05$) but only before Bonferroni correction; Fig. 4.6D). Note that between trawling pressure and different measures of meiofauna/nematode abundance and diversity no significant correlations were detected (Supplementary Figure 4.1), nor between trawling pressure and respiration rates and total respiration (measures of functioning; Fig. 4.6A, C, E).

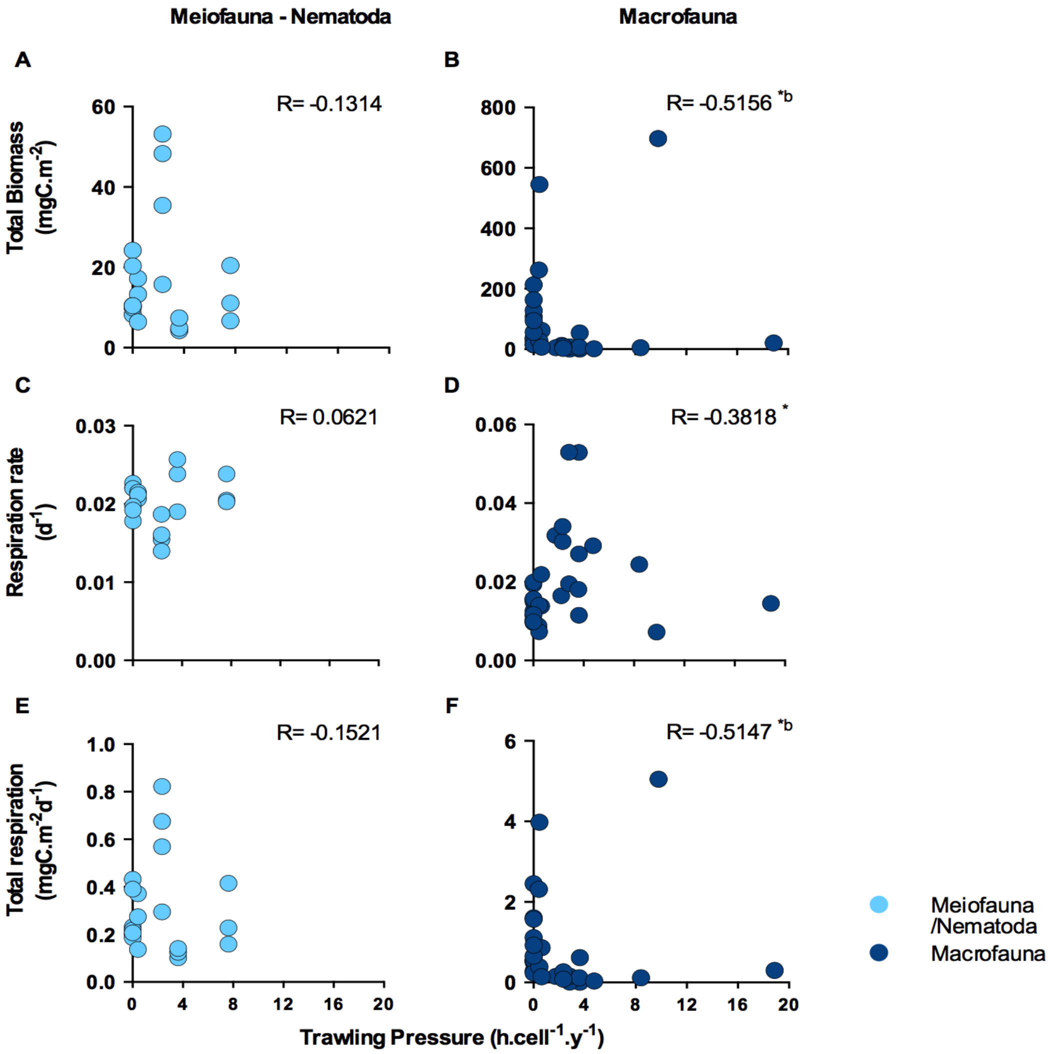

Figure 4.6 Trawling pressure relationship with nematoda and macrofauna: A, B) total biomass; C,D) respiration rate and E, F) total respiration on the sediment surface (0-1 cm). *Indicates significant correlation; ^{b} indicates significance after Bonferroni correction.

Macrofauna BEF relationships investigated through correlations between species richness and ecosystem metabolism proxies (respiration rates and total respiration) were only significant (positive) for total respiration (R= 0.4326; $p<0.05$) (Fig. 4.7C; Table 4.3). Although no clear patterns were perceived when investigating correlations for each trawling pressure group (Table 4.3), total respiration were typically higher in LT and NT stations (Fig. 4.7 C), while respiration rates were higher in HT stations (Fig. 4.6 C and Fig. 7B). Significant correlations were also identified between macrofauna species richness and biomass (R= 0.0298; $p<0.05$; Fig. 4.7 A; Table 4.3). BEF (negative) correlations within meiofauna were identified between nematode genus richness and respiration rates (R=-0.7173, $p<0.01$), however only within HT stations (Supplementary Figure 4.2; Supplementary Table 4.9.

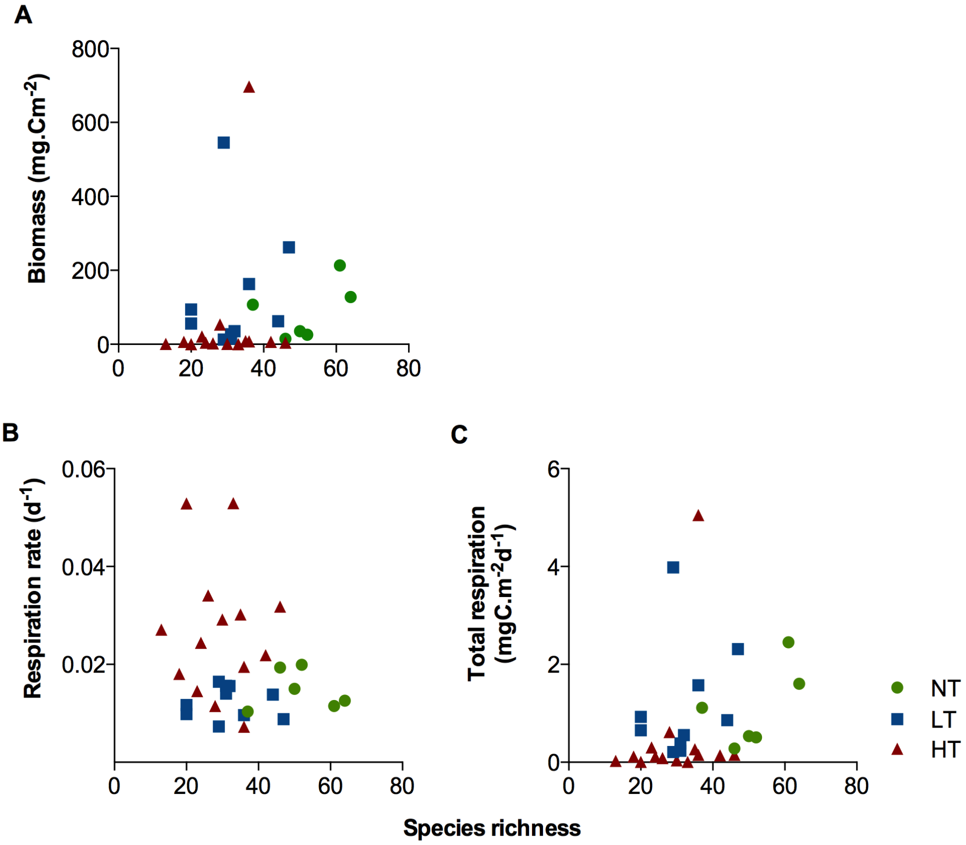

Figure 4.7 Relationship between macrofauna species richness and (A) biomass, (B) respiration rates and (C) total respiration. Spearman-rank correlation and p-values are shown in Table 4.3.

Significant positive relations after Bonferroni corrections were identified between macrofauna species richness and trophic (functional) diversity (R= 0.7540; p<0.01; Fig. 4.8A; Table 4.3). Specifically, predator-feeding guilds were positively linked with species richness, (R=0.7322; p<0.01; Fig. 4.8B), despite the comparable relative contribution of these feeding guilds to the macrofauna trophic structure among all stations (Fig. 4.8C). Note that nematode genus diversity was also related to predator richness (R=0.5231; p<0.05) even though trophic diversity did not vary markedly among groups (LT and HT) (Supplementary Figure 4.3; Supplementary Table 4.9).

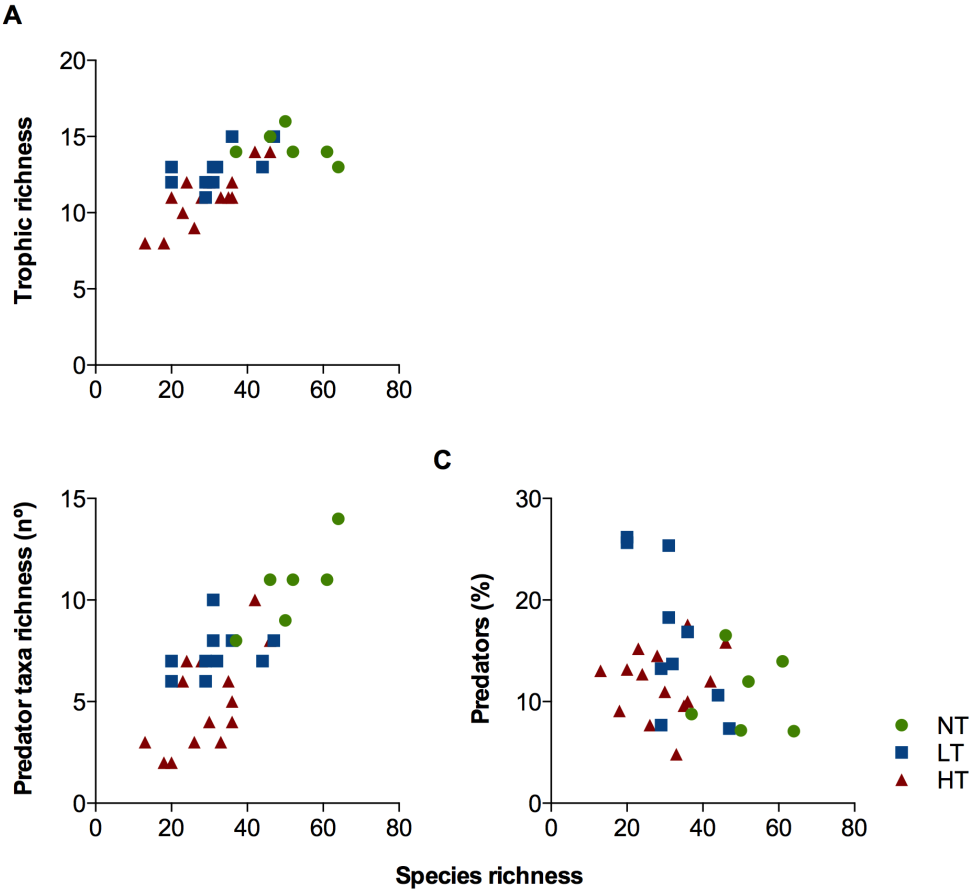

Figure 4.8 Relationship between macrofauna species richness and (A) trophic guild richness, (B) predator richness and (C) predators relative contribution to the trophic structure. Spearman-rank correlation and p-values are shown in Table 4.3.

Table 4.3 Overview of the non-parametric Spearman-rank correlations results for macrofauna species richness and macrofauna biomass, macrofauna associated ecosystem function (respiration) and functional (trophic) diversity.

		Macrofauna species richness	
		Spearman R	**P-value**
Macrofauna Respiration rate	NT	0.08571	0.9194
	LT	-0.1040	0.7496
	HT	-0.0022	0.9911
	ALL	-0.1711	0.3661
Macrofauna Total respiration	NT	0.5429	0.2972
	LT	0.2141	0.5499
	HT	0.4422	0.1143
	ALL	0.4326	**0.0170**
Macrofauna Total biomass	NT	0.5429	0.2972
	LT	0.2141	0.5499
	HT	0.3718	0.1897
	ALL	0.0298	**0.0298**
Macrofauna Trophic diversity	NT	-0.5768	0.1899
	LT	0.6800	**0.0356**
	HT	0.7636	**0.0020**
	ALL	0.7540	**<0.0001**
Macrofauna Predator diversity	NT	0.8197	0.0667
	LT	0.5453	0.1052
	HT	0.6116	**0.0224**
	ALL	0.7322	**< 0.0001**

4.4 Discussion

Bottom trawling activities are associated with the deterioration of the seabed integrity, not only by altering the substrate structure, but also by producing both direct and indirect changes to the benthos composition. Since changes in taxonomic and functional diversity (e.g. Duplisea et al., 2001; NRC, 2002; Lohrer et al., 2004), may alter ecosystem functions in the sediment, our primary goal was to investigate changes in infaunal standing stocks and diversity in areas subjected to different regimes of trawling pressure and relate with several sediment ecosystem functions. To our knowledge, this issue has received little attention in deep-sea regions, particularly those subjected to recurrent anthropogenic disturbance. This issue is of major significance in the context of the studied region, the Western Iberian margin (WIM), where trawling is known to impose an enormous pressure on benthic habitats (Eigaard et al., 2016). In the context of the European Union's Marine Strategy Framework Directive 2008/56/EC (MSFD, European commission, 2008) the existing assessment of Good Environmental Status (GES) have a low degree of confidence, and are hindered by the limited availability of data (MAMAOT, 2012), including for key descriptors, such as descriptor 1 (biodiversity is maintained) and descriptor 6 (seafloor integrity insures functioning of the ecosystems) (European commission, 2008).

4.4.1 General characterisation of the LT and HT areas selected for the pulse-chase experiment

The alteration of the seabed structure (e.g. grain size sorting, porosity) as well as pollutants availability is one of the most significant trawling effects in soft-sediment habitats (Martín et al., 2014a, b; Oberle et al., 2016), including the WIM (Oberle et al., 2016). Even though we cannot exclude the influence of long-term trawling disturbance on the present sediment structure across the study region, the locations where we performed the pulse-chase experiment did not markedly differ either in terms of sediment grain-size or porosity. Because our primary goal was to compare two areas under different trawling regimes for both infauna assemblages and ecosystem functions, for the on-board pulse-chase experiment we deliberately chose two sites subjected to distinct trawling pressure but with relatively similar sediment composition (muddy-sand sediments[12,13]). The need to perform this experiment in sediments with similar characteristics was crucial to exclude the influence of varying environmental conditions (e.g. grain size and food availability), known to structure deep-sea infauna assemblages (Levin et al., 2001), but also sediment biogeochemical processes (e.g. differences in permeability will determine the variable oxygen supply to the sediments) (Glud, 2008). Furthermore, the use of adequate local

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[12] Instituto Hidrográfico. (2005a). Carta dos Sedimentos Superficiais da Plataforma Continental Portuguesa - Folha 5 - Escala 1:150 000

[13] Instituto Hidrográfico (2005b). Carta dos Sedimentos Superficiais da Plataforma Continental Portuguesa - Folha 6A - Escala 1:150 000

trawling pressure information determined from Vessel Monitoring Systems (VMS) mapping, and in-situ video observations from a Remotely Operation Vehicle (ROV), ensured that sediments with different state of disturbance were collected. Trawling intensity in the area was also shown to relate with differences in mega-epibenthic assemblage's biodiversity (Chapter 2).

The video observations obtained ca. one month prior to the sampling of sediments/experiment start, demonstrated that the seabed surface structure between the LT/HT areas was considerably different (Chapter 2). LT sediments generally presented a clearer evidence of bioturbation, and the few trawl scars existent were scattered and mostly eroded. While a direct inspection of the sampled site in the HT region (st. 7) was not possible due to ship time limitations (hence chosen only based on VMS data), the adjacent surveyed area showed numerous trawl scars and generally flattened seabed surface over large extensions. Also, both regions exhibited overall different mega-epifaunal assemblages. Higher diversity at LT location where sediment was collected for the pulse-chase experiment, were mostly determined by a high abundance of a small-sized undetermined species of sponges, Porifera ind. 2, not present in HT sites (Chapter 2). Although, technical constraints (malfunction of the laser pointer scale) did not allow us to estimate total biomass and respiration rates of mega-epibenthic assemblages, the observed differences in certain community groups and compromised seabed integrity suggest a putative deleterious influence of trawling on ecosystem functioning. The absence of abundant suspension and filter-feeding sponges in HT, but present in relatively large abundance at the LT site, may indicate a depletion of ecosystem functions, as sponges are documented to enhance benthic-pelagic coupling processes, through capturing of settling and laterally advecting hemipelagic organic matter and facilitation of microbial nutrient cycling processes, or by direct processing of several dissolved nutrients (Pile and Young, 2006; Maldonado et al., 2012).

Finally, even though we recognized the importance of including an area close to pristine conditions and legally protected (NT) in the experimental set up, due to ship time limitations, this area could not be included. Nonetheless, the NT area was sampled for macrofauna within the framework of this PhD project (Chapter 3), and therefore included when investigating macrofauna biodiversity and ecosystem functions relationships (BEF) (result section 4.3.2 and discussion section 4.4.3).

4.4.2 Alterations of ecosystem functions in association with variations in benthic size structure and faunal traits within the pulse-chase experiment

Ecosystem functions supported by the benthos are associated with several key processes, namely primary and secondary production, ecosystem metabolism, organic matter transformation, nutrient cycling and physical engineering (as defined in Strong et al., 2015). Also, because different faunal groups and ecosystem processes are largely interconnected, changes in fauna assemblages caused by anthropogenic disturbance (i.e. bottom-trawling fisheries), are likely to influence several ecosystem functions (Strong et al., 2015).

In addition to changes in community structure, both shifts in the size-structure and productivity of benthic assemblages under conditions of trawling disturbance have been observed in coastal and shelf regions (Lindeboom and de Groot 1998; Duplisea et al., 2002; Queirós et al., 2006). One of the main reasons such changes are frequently reported in chronically disturbed locations (NRC, 2002) likely relates to the dissimilar capacity of the benthos groups to recolonize and re-establish after one or several persistent disturbance events. This will depend on the assemblage's resistance and resilience traits, turnover rates, faunal interactions (e.g. prey-predator relations, facilitation processes), but also post-disturbance habitat conditions (Clark et al., 2015 and references therein; Yesson et al, 2016). Post-disturbance environmental conditions in soft sediments habitats, will be determined by direct changes in sediment structure (e.g. porosity and permeability) but also by alterations of the biotic and abiotic processes that follow. During remixing, sediment deeper anoxic layers experience an immediate input of organic matter and oxygen, promoting a short-term aerobic remineralisation and reoxidation processes, followed by a release of nutrients locked in the sediment that will temporally increase dissolved nutrients in the water column (Duplisea et al., 2001; Sciberras et al., 2016). However, this short-term increase in bacterial productivity and accelerated carbon and nitrogen cycling processes can result in enhanced high oxygen consumption, and thus followed by hypoxic or anoxic episodes along the whole sediment column (Polymenakou et al. 2005). Reduced conditions can have a strong impact in the infaunal assemblages, including the reduction of macrofauna biomass (Levin 2002), which is one of the main responsible groups for bioturbation and bioirrigation processes (Aller, 1982; Aller, 1994; Lohrer et al., 2004; Braeckman et al., 2010), and thus affecting both bacterial productivity and nutrient cycling processes.

Overall, the present study identified several important differences between LT and HT stations, which are suggestive of altered ecosystem functions under conditions of varying disturbance history. These key differences were perceived not only by changes in macrofaunal abundance, but also by a difference in trophic structure and a shift in the macrofauna community size-structure towards smaller-sized species in the HT area, composed by a large proportion of surface and subsurface deposit and detritus feeders. Moreover, the influence of trawling disturbance on both bacterial production and ecosystem metabolism investigated for each benthic faunal component through total respiration and respiration rates, also suggest that the different conditions are leading to decreased ecosystem metabolic efficiency.

A higher vulnerability of large-sized fauna organisms is associated with both their lower turnover rates and to the ease of direct removal or injury by the trawl gears (Lindeboom and de Groot 1998; Queirós et al., 2006). Additionally, certain faunal groups may suffer indirectly from the changes of environmental conditions and high turbidity periods. For example, the absence of mega-epibenthic sponges in HT sediments was likely the result of both direct removal from trawl nets and long periods of suspended sediments that leads to suffocation or reducing feeding capacity of these organisms (Leys, 2013; Chapter 2). Because sponges are known to promote bentho-pelagic coupling processes and contribute to nutrient cycling (Pile and Young, 2006; Maldonado et al., 2012), their absence in the HT area may be contributing to the detected changes in ecosystem functions, although to which extent we do not know.

On the other hand, the potential of a infaunal assemblages (in the sense of Sloan et al., 2004; Queirós et al., 2013) to influence nutrient fluxes (both carbon and nitrogen), either through bioturbation and bio-irrigation (Aller, 1982; Lohrer et al., 2004; Braeckman et al., 2010; Laverock et al., 2011), will depend not only on abundance, but also largely on the size (individual biomass) and life-history traits (i.e. feeding more, mobility capacity) of the community (Lohrer et al., 2004; Queirós et al., 2013). Thus, the observed reduction of larger macrofauna organisms and shifts in functional (trophic) diversity towards omnivory (detritus and deposit feeding) at HT locations may have triggered the observed changes on bacterial uptake rates (lower ^{13}C uptake in HT) associated carbon transformation processes in the sediments (Aller, 1982; Aller, 1994; Lohrer et al., 2004; Braeckman et al., 2010; Laverock et al., 2011). In addition, these sift in size-structure may also contribute to altered nitrogen cycling processes, highlighted here by the presence of higher ammonium concentrations detected at the deeper layers at T0 and T3 in HT sediments while such trend was not detected in LT samples. This increase in ammonium concentrations can occur under alterations of the denitrifying bacterial communities and/or depletion oxygen concentrations required to convert ammonium into N_2 via anaerobic ammonium oxidation (anammox) (Laverock et al., 2011).

Both the presence of significantly smaller macrofauna groups (lower MBI), and larger nematode genera in HT sediments, are also in support of deprived oxygen provision inside the sediments of highly disturbed areas. A decrease in macrofauna abundances and biomass is often observed in deep-sea regions under low oxygen levels (Levin 2002), while the presence of larger-sized nematodes (observed in HT sediments) has been suggested as an adaption to maximise oxygen absorption under oxygen-deprived conditions (Jensen, 1986). Larger size nematodes also show a higher mobility capacity to escape from unfavourable conditions (Jensen, 1986).

Noteworthy, is that contrary to macrofauna, the absence of a negative significant effect on meiofauna stranding stocks (both abundance and biomass), community composition (Lins et al., 2017) and respiration at both experimental stations but also generally across the whole study region, advocate for an absence of an effect in the measured metrics and seems to contradict the results obtained by Pusceddu et al. (2014) in La Fonera Canyon. Meiofaunal standing stocks are usually linked with food availability and quality in deep-sea sediments (e.g. Ingels et al., 2009; Lins et al., 2017). Thus, it is likely that the contrasting results between these two studies are relate to the fact that Pusceddu et al. (2014) observed a significant reduction in organic matter content at the high trawled areas, not noticed here. Moreover, while meiofauna (e.g Foraminifera, Nematoda) may play an important role in ecosystem processes via micro-bioturbation, in highly diverse systems under the influence of strong faunal interactions with diverse macrofauna assemblages (competition and predation) such as the study area (Chapter 3), the relevance of meiofauna to sediment functioning may be comparatively low (Rysgaard et al., 2000; Bonaglia et al., 2014).

4.4.3 General diversity and ecosystem function trends across the WIM

The observed impairment of various functions (including trophic diversity) in the highly disturbed (HT) sediments during our experiment was not necessarily associated with significant alterations (loss) of taxonomic diversity, which suggests the absence of or an idiosyncratic diversity–ecosystem function relationship as was also observed by Leduc et al. (2013). Yet, under physical disturbance conditions, univariate diversity indices may fail to detect important structural changes under disturbance conditions particularly in highly diverse and dynamic regions such as the West Iberian Margin, otherwise detected by e.g. multivariate analysis (Chapter 3).

General diversity trends in the study region (Chapter 3; Lins et al., 2017) allowed to account for the spatial heterogeneity beyond the two sites investigated during the experiment. It also allowed identifying a general decrease in species richness with increasing trawling pressure for macrofaunal assemblages, but not meiofaunal genus richness (Supplementary Figure 1). It is important to highlight that this may not signify a lack of relationship between trawling pressure and nematode diversity, as the same taxonomic resolution to macrofauna, achieved to species level, was not achievable for nematodes. Moreover, similarly to the two stations where the pulse-chase experiment was conducted, highly disturbed locations showed a decrease in macrofaunal biomass with increasing trawling pressure, which suggests that the shift in the benthos size structure under condition of high disturbance may be constant across the study region. With respect to ecosystem functions we could also estimate meiofauna and macrofauna respiration rates, total respiration and functional (trophic) diversity for the entire region. We identified significant positive relations between macrofauna species richness and total respiration, and with trophic (functional) diversity, where both NT and LT displayed consistently the highest functional diversity, including predator richness. Energy transfer in marine systems (across the food web) is predominantly determined by biotic interactions (e.g. particularly predation, but also competition, facilitation) among the organisms that compose an ecosystem (Strong et al., 2015; Spiers et al., 2016). Capture and conversion of the primary production into secondary production by consumers is a key function undertaken by the benthos (Strong et al., 2015). Thus, the observed alterations of the trophic structure, respiration rates and benthic secondary production (indirectly assessed by biomass), in relation to trawling disturbance, may influence the nutrient and energy fluxes across the food web. Moreover, there is an increased evidence that loss of species at higher trophic levels would have more severe effects on the stability of food webs through top-down control, and thus groups such as predators can have a unique role in carbon and energy cycling (Atwood et al., 2015; Spiers et al., 2016). The decreased predator's abundance and diversity could thus lead to changes in secondary production at the intermediate and lower levels of the food chain, thereby modifying carbon cycling (e.g. biomass; Spiers et al., 2016).

4.5 Conclusions

The present study suggested a negative influence of trawling disturbance on the benthos and related ecosystem functions. The most evident effects were detected for the macrofauna assemblages, which suffered a marked decrease in total abundance and total respiration, and a prevalence of small-sized species under high physical disturbance conditions. In contrast, the biomass of the small-sized biota (meiofauna and bacteria) showed no marked differences between trawling regimes, although bacterial production (^{13}C uptake) was reduced at the highly disturbed site. The difference in macrofauna size structure may relate with t a reduced bioturbation and bioirrigation under disturbance conditions, associated with the observed changes in ecosystem functions, including lower bacterial production (carbon mineralization), as well as effects on nutrient cycling. Although we require further investigation to substantiate the observed function impairment across the study area, as these functions were mostly explored within two stations for all faunal components (bacteria, meiofauna and macrofauna) and lack the comparison with pristine locations; the general decline in macrofauna species richness, functional (trophic) diversity and total respiration, suggests that the long history of trawling disturbance along the Western Iberian margin is affecting the ecosystem's integrity and its capacity to provide fundamental ecosystem functions and services.

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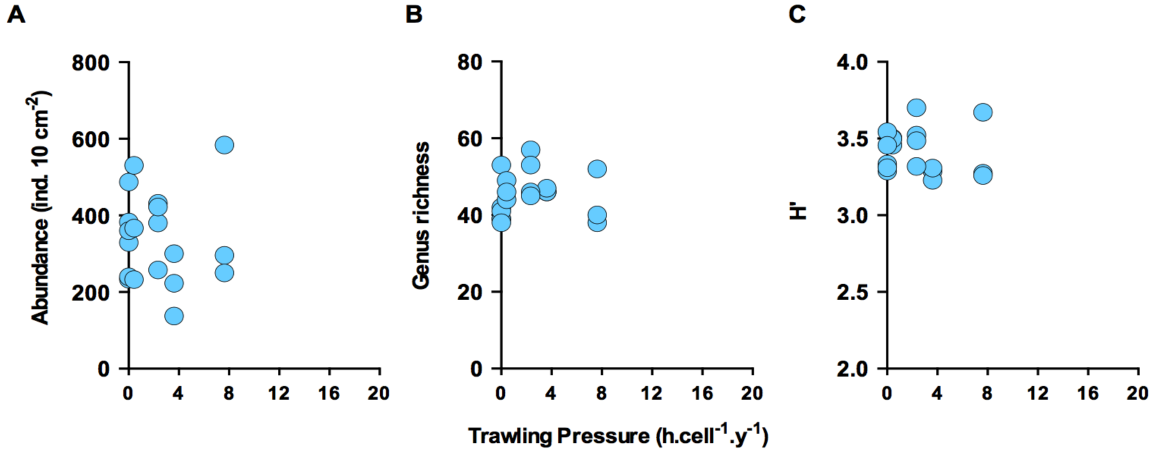
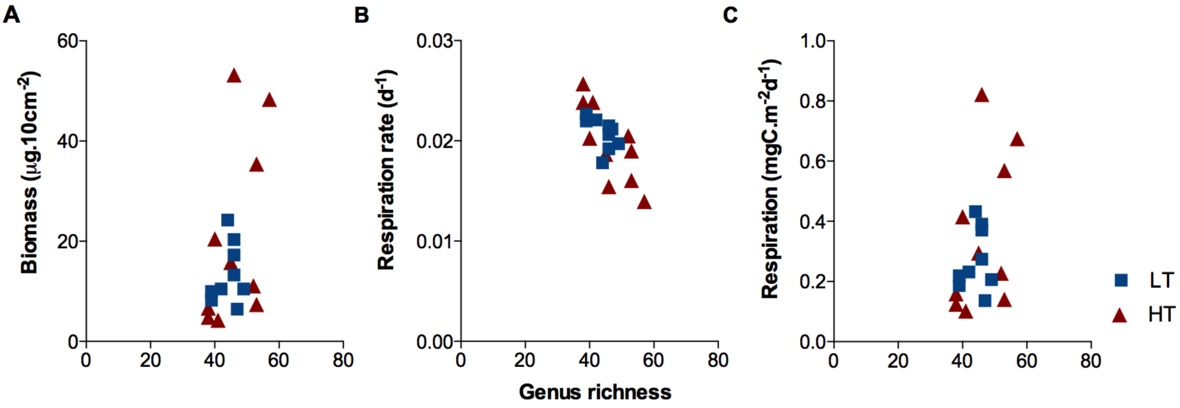
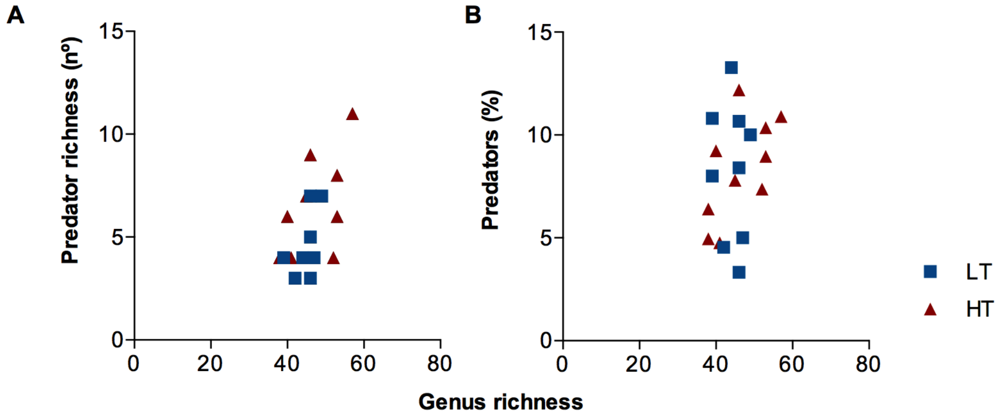
Supplementary material



Supplementary Figure 4.1 Relationship between trawling pressure (h.cell^{-1}.y^{-1}) and Meiofauna abundance (R=-0.1116; p=0.6492), Nematoda genus richness (R=0.2317; p=0.3398) and Nematoda Shannon-Wiener diversity index (H'; R=-0.2565; 0.2891).



Supplementary Figure 4.2 Relationship between Nematoda genus richness and (A) Nematoda biomass (R=0.4217; p=0.0721), (B) respiration rate (R=-0.7173; p=0.0005^{*b}), and total respiration (R=0.3573; p=0.1331). *indicates significant correlation; ^{b} indicates significant correlations after Bonferroni correction.



Supplementary Figure 4.3 Relationship between Nematoda genus richness and (A) predator richness (number of genera which are predators; R=0.5231; P= 0.0216*) and (B) predator relative contribution to the trophic structure (R=0.2858; P= 0.2355).

Supplementary Table 4.1 Metadata of the sediment samples collected for infauna studies (either meiofauna or macrofauna) and environmental characterization (environ.) during the RV Belgica 2017/17 cruise.

Station	**Depl.**	**Date**	**Latitude (N)**	**Longitude (W)**	**Depth (m)**	**Gear**	**Trawling pressure**	**Analysis**
1	4	13/06/2013	37.983433	-9.185117	445	Box	HT_13	Macrofauna/Environ.
1	8	13/06/2013	37.982700	-9.185183	445	Box	HT_13	Macrofauna/Environ.
1	9	13/06/2013	37.982467	-9.184983	445	Box	HT_13	Macrofauna/Environ.
1	6	13/06/2013	37.982450	-9.184717	445	Box	HT_13	Meiofauna/Environ.
1	11	13/06/2013	37.982800	-9.185467	445	MUC	HT_13	Meiofauna/Environ.
1	12	13/06/2013	37.982550	-9.184900	445	MUC	HT_13	Meiofauna/Environ.
1	13	14/06/2013	37.982783	-9.184833	445	MUC	HT_13	Meiofauna/Environ.
2	22	15/06/2013	37.981467	-9.125467	335	Box	LT_13	Macrofauna/Environ.
2	23	15/06/2013	37.981600	-9.125100	335	Box	LT_13	Macrofauna/Environ.
2	24	15/06/2013	37.981567	-9.125233	335	Box	LT_13	Macrofauna/Environ.
2	15	14/06/2013	37.981733	-9.125417	335	MUC	LT_13	Meiofauna/Environ.
2	18	14/06/2013	37.981467	-9.125217	335	MUC	LT_13	Meiofauna/Environ.
2	19	14/06/2013	37.981567	-9.124933	335	MUC	LT_13	Meiofauna/Environ.
2	25	15/06/2013	37.981617	-9.125333	335	Box	LT_13	Meiofauna/Environ.
4	33	16/06/2013	37.852850	-9.115733	325	MUC	HT_13	Meiofauna/Environ.
4	34	16/06/2013	37.853133	-9.116233	325	MUC	HT_13	Meiofauna/Environ.
4	35	16/06/2013	37.852900	-9.115833	325	MUC	HT_13	Meiofauna/Environ.
6	47	17/06/2013	37.926617	-9.116633	296	MUC	LT_13	Meiofauna/Environ.
6	51	17/06/2013	37.926567	-9.116683	298	MUC	LT_13	Meiofauna/Environ.
6	52	17/06/2013	37.926567	-9.116700	298	MUC	LT_13	Meiofauna/Environ.

Deplo.: Deployment; Box: Box-core sampler, MUC: Multiple-core sampler; Trawling pressure groups includes: no (NT), low (LT) and high trawling pressure.

Supplementary Table 4.2 Metadata of the samples collected for infauna (meio- and macrofauna) and environmental characterisation (environ.) and pulse-chase experiment, during the RV Belgica 2014/15 cruise.

Station	Depl.	Date	Latitude (N)	Longitude (W)	Depth (m)	Gear (nº cores)	Trawling pressure	Analysis
1.1	70	09/06/14	37.99915	-9.175467	443	Box	HT_14	Macrofauna/Environ.
1.2	68	09/06/14	37.984417	-9.185717	449	Box	HT_14	Macrofauna/Environ.
1.2	69	09/06/14	37.982817	-9.18785	451	Box	HT_14	Macrofauna/Environ.
1.3	67	09/06/14	37.966833	-9.184083	430	Box	HT_14	Macrofauna/Environ.
2.1	66	09/06/14	37.998367	-9.124233	350	Box	LT_14	Macrofauna/Environ.
2.2	65	09/06/14	37.982817	-9.124667	336	Box	LT_14	Macrofauna/Environ.
2.3	64	09/06/14	37.965917	-9.13255	342	Box	LT_14	Macrofauna/Environ.
4.1	63	09/06/14	37.8492	-9.108717	318	Box	HT_14	Macrofauna/Environ.
4.2	34	04/06/14	37.822733	-9.11495	330	Box	HT_14	Macrofauna/Environ.
4.3	33	04/06/14	37.79995	-9.115183	330	Box	HT_14	Macrofauna/Environ.
6.1	29	04/06/14	37.916283	-9.108233	285	Box	LT_14	Macrofauna/Environ.
6.2	30	04/06/14	37.9265	-9.116617	300	Box	LT_14	Macrofauna/Environ.
6.2	31	04/06/14	37.941633	-9.124767	323	Box	LT_14	Macrofauna/Environ.
6.3	32	04/06/14	37.9445	-9.124767	325	Box	LT_14	Macrofauna/Environ.
6.2	12	03/06/14	37.92425	-9.115567	294	MUC	LT_14	Meiofauna/Environ.
6.2	14	03/06/14	37.9255	-9.117183	296	MUC	LT_14	Meiofauna/Environ.
6.2	10	03/06/14	37.926433	-9.116533	296	MUC	LT_14	Meiofauna/Environ.
7.1	28	04/06/14	37.808133	-9.090783	299	Box	HT_14	Macrofauna/Environ.
7.2	25	04/06/14	37.79330	-9.09160	291	Box	HT_14	Macrofauna/Environ.
7.2	26	04/06/14	37.793067	-9.09155	290	Box	HT_14	Macrofauna/Environ.
7.3	27	04/06/14	37.78070	-9.090617	295	Box	HT_14	Macrofauna/Environ.
7.2	21	04/06/14	37.791567	-9.09070	295	MUC	HT_14	Meiofauna/Environ.
7.2	22	04/06/14	37.79090	-9.090083	294	MUC	HT_14	Meiofauna/Environ.
7.2	24	04/06/14	37.792433	-9.090333	290	MUC	HT_14	Meiofauna/Environ.
9.1	73	09/06/14	38.34175	-9.20140	329	Box	NT	Macrofauna/Environ.
9.2	72	09/06/14	38.33120	-9.194083	326	Box	NT	Macrofauna/Environ.
9.3	71	09/06/14	38.323767	-9.185833	340	Box	NT	Macrofauna/Environ.
10.1	76	09/06/14	38.34115	-9.22740	360	Box	NT	Macrofauna/Environ.
10.2	75	09/06/14	38.33330	-9.217717	550	Box	NT	Macrofauna/Environ.
10.3	74	09/06/14	38.324583	-9.208833	407	Box	NT	Macrofauna/Environ.
6.2	2	02/06/14	37.927717	-9.116233	298	MUC (1)	LT_14	Experiment
6.2	3	02/06/14	37.925483	-9.116283	297	MUC (2)	LT_14	Experiment
6.2	6	03/06/14	37.925483	-9.116283	292	MUC (1)	LT_14	Experiment
6.2	8	03/06/14	37.92715	-9.114833	295	MUC (1)	LT_14	Experiment
6.2	9	03/06/14	37.924833	-9.115817	295	MUC (1)	LT_14	Experiment
6.2	10	03/06/14	37.926433	-9.116533	296	MUC (2)	LT_14	Experiment
6.2	11	03/06/14	37.927167	-9.11820	299	MUC (1)	LT_14	Experiment
7.2	16	04/06/14	37.794317	-9.08670	284	MUC (2)	HT_14	Experiment
7.2	17	04/06/14	37.798017	-9.089333	293	MUC (1)	HT_14	Experiment
7.2	18	04/06/14	37.79010	-9.088550	293	MUC (3)	HT_14	Experiment
7.2	19	04/06/14	37.79105	-9.089283	290	MUC (1)	HT_14	Experiment
7.2	20	04/06/14	37.7908	-9.0905	295	MUC (1)	HT_14	Experiment
7.2	21	04/06/14	37.791567	-9.0907	295	MUC (1)	HT_14	Experiment

Deplo.: Deployment; Box: Box-core sampler, MUC: Multiple-core sampler; Trawling pressure includes: no (NT), low (LT) and high (HT) trawling pressure

Supplementary Table 4.3 PERMANOVA main test results based on the ammonium concentrations (biogeochemical functioning) along the sediment depth profile evaluated at the start of the experiment (T0) and after 3 (T3) and 5 (T5) days under different trawl pressure conditions. PERMANOVA test applied followed a 4-factor layout, with "Trawling pressure (TP)" as a fixed factor and 2 levels: HT and LT; "Time (Ti)" as a fixed factor with 3 levels T0, T3 and T5; "Sediment depth (SedDepth)" as a fixed factor and 10 levels: every centimetre down to 10 cm, and "Replicate" as a random factor nested in "Trawling pressure x Time". Values in bold represent significant values.

Source	df	SS	MS	Pseudo-F	P(perm)	unique perm	ECV
Trawl pressure (TP)	1	5.2716	5.2716	5.3926	**0.0485**	9846	6.39E-02
Time (Ti)	2	0.51529	0.25765	0.26356	0.7657	9946	-1.63E-02
Sediment depth (SedDepth)	8	76.96	9.62	27.609	**0.0001**	9953	0.6181
TPxTi	2	0.37075	0.18537	0.18963	0.8271	9957	-3.62E-02
TPxSedDepth	8	1.7737	0.22171	0.63631	0.7499	9954	-1.70E-02
TixSedDepth	16	6.5243	0.40777	1.1703	0.3114	9925	1.21E-02
Replicate(TPxTi)	9	8.7979	0.97755	2.8056	**0.0071**	9942	6.99E-02
TPxTixSedDepth	16	8.6995	0.54372	1.5605	0.1029	9934	8.03E-02
Res	72	25.087	0.34843				0.34843
Total	134	134					

Supplementary Table 4.4 PERMANOVA pair-wise results test for the significant main test results identified on the ammonium concentrations (biogeochemical functioning) dataset. Values in bold represent significant values. “Trawling pressure (TP)” levels include: HT (high trawl pressure) and LT (low trawl pressure) and “Sediment depth (SedDepth)” include 10 levels: every centimetre down to 10 cm. Values in bold represent significant values.

Pair-wise test - Trawl pressure (TP)

Groups	t	P(perm)	Unique perms
LT, HT	2.3222	**0.0457**	9833

Pair-wise tests - Sediment depth (SedDepth)

Groups	t	P(perm)	Unique perm
0-1, 1-2	0.25348	0.8174	9852
0-1, 2-3	1.0548	0.3187	9824
0-1, 3-4	1.4376	0.1804	9844
0-1, 4-5	4.4285	**0.0017**	9872
0-1, 5-6	6.759	**0.0002**	9826
0-1, 6-7	6.4526	**0.0001**	9838
0-1, 8-9	10.924	**0.0001**	9842
0-1, 9-10	10.14	**0.0001**	9842
1-2, 2-3	0.87049	0.4073	9839
1-2, 3-4	1.1996	0.2613	9835
1-2, 4-5	3.3443	**0.0095**	9834
1-2, 5-6	6.4902	**0.0002**	9839
1-2, 6-7	3.6215	**0.0061**	9844
1-2, 8-9	8.967	**0.0001**	9841
1-2, 9-10	10.139	**0.0001**	9844
2-3, 3-4	1.1539	0.2856	9842
2-3, 4-5	3.4001	**0.0078**	9853
2-3, 5-6	8.2542	**0.0001**	9844
2-3, 6-7	3.8575	**0.0041**	9851
2-3, 8-9	8.5806	**0.0001**	9851
2-3, 9-10	9.997	**0.0001**	9837
3-4, 4-5	2.3753	**0.0418**	9841
3-4, 5-6	6.5698	**0.0001**	9829
3-4, 6-7	3.6299	**0.007**	9833
3-4, 8-9	6.97	**0.0001**	9833
3-4, 9-10	8.4664	**0.0001**	9834
4-5, 5-6	4.5676	**0.0013**	9853
4-5, 6-7	2.0349	0.0701	9836
4-5, 8-9	8.2705	**0.0001**	9837
4-5, 9-10	7.4797	**0.0003**	9859
5-6, 6-7	0.35948	0.7509	9840
5-6, 8-9	4.4273	**0.0018**	9856
5-6, 9-10	7.4503	**0.0003**	9852
6-7, 8-9	2.2951	**0.0366**	9842
6-7, 9-10	2.9459	**0.0118**	9853
7-8, 9-10	2.0013	0.0791	9850

Supplementary Table 4.5 PERMANOVA main test results based on the the^{13}C sediment uptake concentrations (bioturbation) along a depth profile (cm) evaluated at day 3 (T3) and day 5 (T5) under different trawl pressure conditions. PERMANOVA test applied followed a 4-factor layout, with "Trawling pressure (TP)" as a fixed factor and 2 levels: HT and LT; "Time (Ti)" as a fixed factor with 2 levels T3 and T5; "Sediment depth (SedDepth)" as a fixed factor and 2 levels: 0-1 cm and 4-5 cm, and "Replicate" as a random factor nested in "Trawling pressure x Time". Values in bold represent significant values.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**unique perm**	**ECV**
Trawl pressure (TP)	1	0.10994	0.10994	0.50507	0.4882	8726	-9.48E-02
Time (Ti)	1	1.2079	1.2079	5.5494	**0.0454**	8788	8.25E-02
Sediment depth (SedDepth)	1	17	17	68.702	**0.0002**	9842	1.3961
TPxTi	1	0.11908	0.11908	0.54709	0.4711	8752	-1.64E-02
TPxSedDepth	1	0.043987	0.043987	0.17776	0.6817	9835	-0.03391
TixSedDepth	1	0.75949	0.75949	3.0693	0.1236	9842	0.085341
Replicate(TPxTi)	8	1.7413	0.21767	0.87964	0.6109	9946	-1.49E-02
TPxTixSedDepth	1	0.0385	0.0385	0.15559	0.7052	9854	-6.97E-02
Res	8	1.9796	0.24745				0.24745
Total	23	23					

Supplementary Table 4.6 PERMANOVA pair-wise results test for the significant main test results identified on the ^{13}C sediment uptake concentrations (Bioturbation) dataset. The factor "Sediment depth (SedDepth)" includes 2 levels: 0-1 cm and 4-5 cm; while "Time (Ti)" includes the levels day 3 (T3) and day 5 (T5); Values in bold represent significant values.

Pair-wise tests - Sediment depth (SedDepth)			
Groups	t	P(perm)	Unique perm
0-1, 4-5	8.2886	**0.0002**	9929

Pair-wise tests - Time (Ti)			
Groups	t	P(perm)	Unique perm
T3, T5	2.3557	**0.0311**	8896

Supplementary Table 4.7 PERMANOVA main test results based on the the^{13}C uptake concentrations by bacteria (bacteria production) along a depth profile (cm) evaluated at day 3 (T3) and day 5 (T5) under different trawl pressure conditions. PERMANOVA test applied followed a 4-factor layout, with “Trawling pressure (TP)” as a fixed factor and 2 levels: HT and LT; “Time (Ti)” as a fixed factor with 2 levels T3 and T5; “Sediment depth (SedDepth)” as a fixed factor and 2 levels: 0-1 cm and 4-5 cm, and “Replicate” as a random factor nested in “Trawling pressure x Time”. Values in bold represent significant values.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**unique perm**	**ECV**
Trawl pressure (TP)	1	4.2082	4.2082	12.175	**0.0111**	8858	0.35407
Time (Ti)	1	0.9905	0.9905	2.8658	0.1479	9359	5.97E-02
Sediment depth (SedDepth)	1	4.5804	4.5804	11.935	**0.0112**	9836	0.38151
TPxTi	1	0.7125	0.7125	2.0614	0.193	8868	6.88E-02
TPxSedDepth	1	3.7523	3.7523	9.7769	**0.0146**	9857	0.61756
TixSedDepth	1	1.029	1.029	2.6812	0.1444	9859	0.11949
Replicate(TPxTi)	8	2.4194	0.34563	0.90058	0.5991	9947	-1.91E-02
TPxTixSedDepth	1	0.62117	0.62117	1.6185	0.2471	9851	8.90E-02
Res	8	2.6865	0.38379				0.38379
Total	23	23					

Supplementary Table 4.8 PERMANOVA pair-wise results test for the significant main test results identified on the the^{13}C uptake concentrations (bacteria production) dataset. The factor “Trawling pressure (TP)” includes the levels: HT (high trawl pressure) and LT (low trawl pressure); “Sediment depth (SedDepth)” includes 2 levels: 0-1 cm and 4-5 cm; and Trawling pressure (TP) x Sediment depth (SedDepth) interaction. Values in bold represent significant values.

Pair-wise test - Trawl pressure (TP)

Groups	t	P(perm)	Unique perms
LT, HT	3.4893	**0.0033**	8810

Pair-wise tests - Sediment depth (SedDepth)

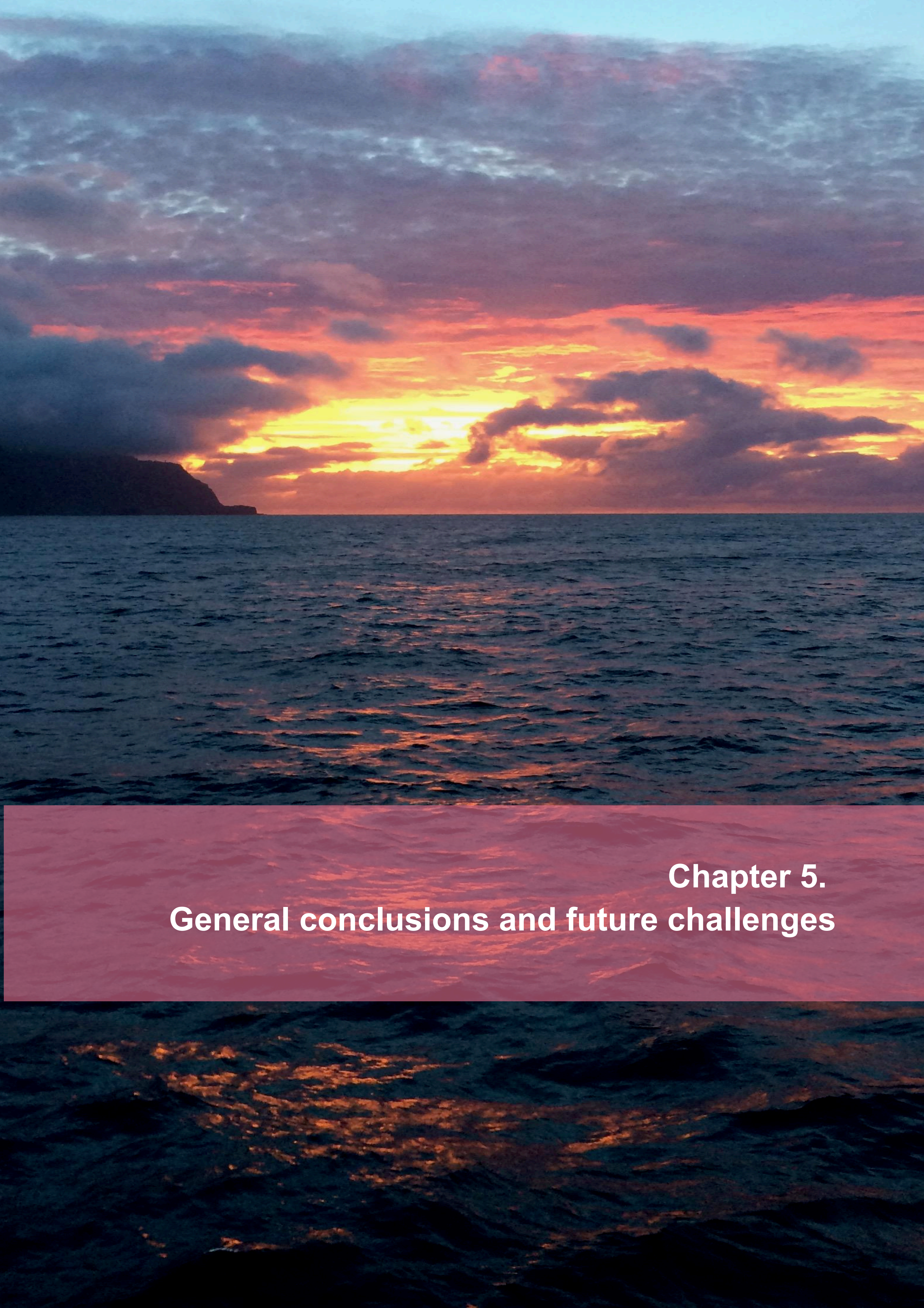
Groups	t	P(perm)	Unique perm
0-1, 4-5	3.4547	**0.0023**	9865

Pair-wise tests – TP x SedDepth

Groups	t	P(perm)	Unique perm
Within level 'LT'			
0-1, 4-5	3.209	**0.0223**	9474
Within level 'HT'			
0-1, 4-5	1.0362	0.4215	4463

Supplementary Table 4.9 Overview of the non-parametric Spearman-rank correlations results for Nematoda genus richness and Nematoda biomass/Nematoda associated ecosystem functions (respiration rates/ total respiration). Values in bold are indicative of significant correlations; ^{b} indicates significant correlations after Bonferroni correction.

		Nematoda genus richness	
		Spearman R	**P-value**
Nematoda Respiration rate	LT	-0.5789	0.092
	HT	**-0.7927**	**0.0079**
	ALL	**-0.7173**	**0.0005**
Nematoda Total respiration	LT	-0.0681	0.8141
	HT	0.4878	0.1545
	ALL	0.3573	0.1331
Nematoda Total biomass	LT	0.05959	0.8878
	HT	0.5732	0.0882
	ALL	0.4217	0.0721
Nematoda Predator diversity	LT	0.4576	0.2196
	HT	**0.6659**	**0.0411**
	ALL	**0.5231**	**0.0216**



Chapter 5.
General conclusions and future challenges

5.1 General conclusions

The increased anthropogenic pressure in deep-sea ecosystems, particularly exploitation practices such as bottom-trawling fisheries, has prompted serious concerns regarding its impacts on biodiversity and maintenance of essential ecosystem functions and services (Loreau, 2008; Ramírez-Llodra et al., 2011; Thurber et al., 2013). Our current understanding on the effects that such exploitation activities induce into deep-sea benthic habitats is flawed, as it is often focused on charismatic hard substrate habitats (i.e. deep-water corals, seamounts) (e.g. Koslow et al., 2001; Hall-Spencer et al., 2002; Fosså et al., 2002; Clark and O'Driscoll, 2003; Gage et al., 2005; Althaus al., 2009; Clark and Rowden, 2009; Clark et al., 2015), while in fact the trawling pressure is concentrated mainly along the sediment continental slopes and submarine canyons. In these habitats, only few studies were carried out to investigate how this activity impacts the seabed structure and its associated fauna (e.g. Gage et al., 2005; Atkinson et al., 2011; Buhl-Mortensen et al., 2015; Murillo et al., 2016; Yesson et al., 2016; Oberle et al., 2016; Almeida et al., 2017). Moreover, most of these studies lack an integrative perspective, by focusing on the effects caused by this physical disturbance on a specific issue, e.g. alteration of seabed structure (Martín et al., 2014; Oberle et al., 2016), or a particular faunal group, e.g. mega-epibenthic assemblages (Althaus al., 2009; Murillo et al., 2016; Yesson et al., 2016).

In this context, the present study explored the effects induced by the long-term history of bottom-trawling physical disturbance on the composition and on the structural and functional (trophic) diversity of soft-sediment benthic assemblages along the SW Portuguese upper continental slope, and how this was translated into the maintenance of several deep-sea ecosystem functions (e.g. nutrient cycling, organic matter transformation, secondary production, ecosystem metabolism). The novelty of this study resides in the incorporation and exploration of the responses of the various components of the benthic assemblages (meiofauna, macrofauna, mega-epibenthic fauna), but also in the integration of the information from proxies of key ecosystem functions. To my knowledge, proxies for ecosystem metabolism in the context of bottom-trawling impacts were only briefly discussed by Leduc et al (2016) in relation to benthos respiration, although the authors found no direct relations between these variables. It is my belief that an integrative approach is crucial to improve our understanding of the actual effects of bottom-trawling fisheries in the deep sea. This is the only way that we may be able to provide scientific evidence to support informed monitoring and conservation measures required for a sustainable exploitation of the current fisheries resources, while preserving the integrity of benthic habitats and the good environmental status of the targeted benthic environments, not compromising the wellbeing of future generations.

In summary, the present thesis demonstrated that the recurrent trawling activities in practice at the SW Portuguese continental slope have compromised the seabed integrity of the areas surveyed, and altered the soft-sediment benthic assemblages. Greater deleterious effects were observed within larger-sized faunal components (mega-epifauna and macrofauna), while no apparent changes were perceived for either meiofauna assemblages or bacterial assemblage's biomass (no community structure nor diversity was assessed here for the latest faunal component) (Fig. 5.1).

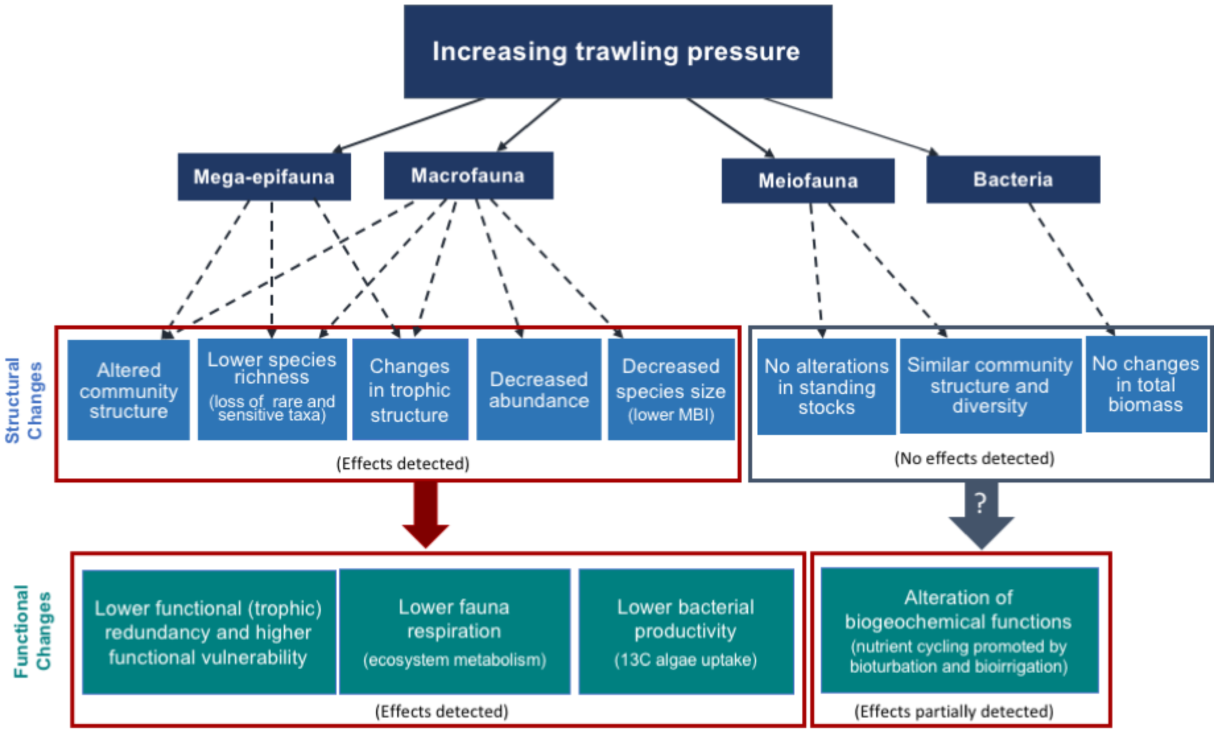


Figure 5.1 Schematics of the major observed effects by the different benthos size-groups under increasing trawling disturbance in the SW Portuguese continental slope.

These results confirm, for some groups of the benthos, the primary hypothesis of this thesis that "*chronic disturbance by bottom-trawling fisheries will induce significant alterations of the benthic communities composition and diversity*". These observed changes were, in addition to a shift of the benthos size structure towards smaller-sized species in highly disturbed areas, linked with the depletion of regulatory ecosystem functions normally mediated by the affected biota (bacterial productivity, nutrient cycling maintained through bioirrigation and bioturbation). Moreover, affected areas showed a decline in faunal ecosystem metabolic efficiency (lower respiration at the highly disturbed areas) and clear changes in trophic structure (Fig. 5.1). Specifically, the observed decline in macrofauna trophic redundancy, which is inevitably associated with a higher functional vulnerability under conditions of high trawling disturbance, suggests that alterations of the food-web may be occurring in the trawl affected areas. This will make such assemblages more susceptible to further intensification of disturbance by trawling, other sources of anthropogenic or natural disturbance (e.g. climate change associated alterations of water conditions) and their putative cumulative or synergistic effects. These results including both field and experimental outputs, also provided evidence to support the second hypothesis raised: "*the alterations of benthic assemblages, particularly within macro-infauna will be reflected in a depletion of important ecosystem functions*" (i.e. inefficiency in carbon mineralization, reduced sediment-water nutrient (nitrogen) fluxes, lower metabolic efficiency).

5.2 Limitations of the study

Even though a shift in the assemblage size spectrum towards dominance of small, fast-growing fauna under conditions of chronic trawling disturbance has been frequently reported (Kaiser et al., 2002; Duplisea et al., 2002), most studies also describe changes in meiofauna, particularly in nematode community structure (Schratzberger et al., 2002; Schratzberger and Jennings, 2002; Lampadariou et al., 2005). In spite of the observed trawling-associated changes in both mega-epibenthic and macrofaunal assemblages, no apparent negative impacts on meiofaunal assemblages and bacterial biomass (small-sized fauna) were observed. This may be simply due to the lack of major differences among the different areas (low and high trawl pressure) investigated for these groups.

Yet, because of evident constraints during the acquisition of the data, that need to be better addressed in future works, this thesis does not allow to confidently state that both meiofaunal and bacterial assemblages remained undisturbed, even under conditions of high trawling pressure along the SW Portuguese margin. First, because time management, limited budget and other logistic limitations during the cruises hindered the collection of a higher number of samples, namely the ones for the characterisation of both meiofauna and microfauna assemblages in undisturbed area where trawling is prohibited by law. This constraint also applies for the experimental set-up, and we may speculate that additional sediments from a reference region, would have improved the clarity of the observed trends in ecosystem function proxies. Secondly, it was not possible to complete the initially planned characterisation and diversity assessments for bacterial assemblages during the timeframe of this thesis. Nevertheless, the observed differences in bacterial productivity (lower in HT areas) suggest a relationship with differences in the trawling pressure regimes. Thirdly, the absence of differences in meiofaunal community structure and its functional diversity were also likely influenced by the differences in taxonomical resolution of the several meiofauna components (e.g. copepods, ostracods), including the identification of nematodes, only performed to genus level, in opposition to macrofauna done to the species level. Moreover, functional diversity within nematode assemblages was only established based on four trophic groups following the Wieser (1953) classification (feeding mode), normally used in most deep-sea studies due to the lack of other alternatives. Such classification does not capture a more refined spectrum of feeding guilds likely present in the study region. In the case of macrofaunal assemblages, the classification of trophic guild codes following MacDonald et al. (2010), not only includes the feeding mode (as Wieser, 1953), but also incorporates both food source and food type, in an attempt to better integrate the niche of the species and their role in the ecosystem function and food-web.

Noteworthy is that determination of functional traits, even within macrofauna, is largely restricted for many deep-sea species, as we lack information on the biology and ecology, and the attribution of traits from closely-related shallow water taxa implies some error or imprecisions, at least until we acquire more information on the biodiversity and biological traits of deep-sea fauna around the globe.

Lastly, because during the ROV surveys, the laser points were inoperative, we lack scale for biomass determination of the mega-epibenthic assemblages, and thus this group was not integrated in the measures of ecosystem metabolism (benthic respiration) in this study. This in addition with the taxonomic resolution obtained for mega-epifauna, morphospieces, implies that structural diversity in the region might be underestimated.

5.3 GES assessment in the West Iberian Margin, importance of integrative studies and future challenges

Overall, the results presented in my thesis suggest that the exploitation of the deep-sea fish and shellfish resources in the SW Portuguese Margin is currently endangering the benthic ecosystems along the upper continental slope, particularly affecting larger-sized fauna that are less tolerant to disturbance. Yet, while I am confident to have demonstrated some of the deleterious effects associated with bottom-trawling disturbance, I also demonstrated that these may be imperceptible when using standard monitoring tools for impact assessment in marine systems, namely community associated univariate indices of diversity (i.e. Shannon-Wiener diversity and Pielou's evenness). The initial report made by the Portuguese government DGRM, under the framework of the European Union's Marine Strategy Framework Directive 2008/56/EC (MSFD; European Commission, 2008), for the assessment of the Good Environmental Status (GES) of the marine environments was completed a few years ago (MAMAOT, 2012). The DGRM report highlights trawling fisheries as one of the most pervasive activities along the Portuguese margin, still it ascribes to the study region, although with a low confidence level, a good environmental condition for both descriptor 1 (biodiversity is maintained) and descriptor 6 (the seafloor integrity ensures functioning of the ecosystem). Specifically, within the descriptor 6, the condition of the benthic assemblages was evaluated through several univarite diversity indices, which, as suggested in Chapter 3, fail in reflecting the existing trawling disturbance in the studied region. The limitations associated with the current scarcity of information regarding biology and ecology of many deep-sea species, hinders the use of some of the indices included in the DGRM report (MAMAOT, 2012): ratio opportunistic/sensitive species and the multimetric index M-AMBI (Borja et al., 2000; Muxika et al. 2005), which need the input of information on the traits and response (e.g. sensitivity and tolerance) of the species under conditions of stress (Borja et al., 2000; MAMAOT, 2012). In fact, the application of these indices will continue to be unfeasible in deep-sea regions until we have a better knowledge on the deep-sea benthic fauna along the West Iberian Margin, and acquire adequate and precise information regarding the biology of many of these species.

Therefore, this thesis advocates the need to reinforce biodiversity studies at the West Iberian margin, particularly urgent in what concerns the deeper regions that are not yet affected by trawling, but are at potential risk of future exploitation activities (Watson and Morato, 2013; Bueno-Pardo et al., 2017). It is crucial to identify such areas, and use the precautionary principle to support their full protection from bottom-trawling fisheries (e.g. through the creation of Marine Protected Areas). The identification and delimitation of areas at risk (present or future) can be carried out, for example, by the application of habitat suitability models (or species distribution modeling), which have proved to adequately identify potential vulnerable marine ecosystems (VMEs) (Rengstorf et al., 2013; Vierod et al., 2014; Anderson et al., 2016). In addition, test surveys should be carried out in areas of interest for crustacean bottom trawlers, by applying Before-After-Control-Impact experimentation tests (BACI: Smith et al., 2011), similarly to those applied in mining prospect regions in the Pacific (Thiel et al., 1992). Such measures may help prioritize areas that require protection. Finally, it is crucial that in future studies, monitoring tools generally applied in estuarine and coastal regions, will be adapted and adequately implemented in deep-sea environments. I recommended, that in the context of the MSFD, monitoring programmes also include multivariate indicators of community composition, ecosystem condition, functional diversity and vulnerability, as well as proxies for ecosystem functions (e.g. production, respiration, food-web structure), which are not necessarily translated by biodiversity indices (van Hoey et al., 2010; Strong et al., 2015). Only then, we can confidently determine, maintain or achieve a Good Environmental Status (GES) of the deep-sea areas within the European margins.

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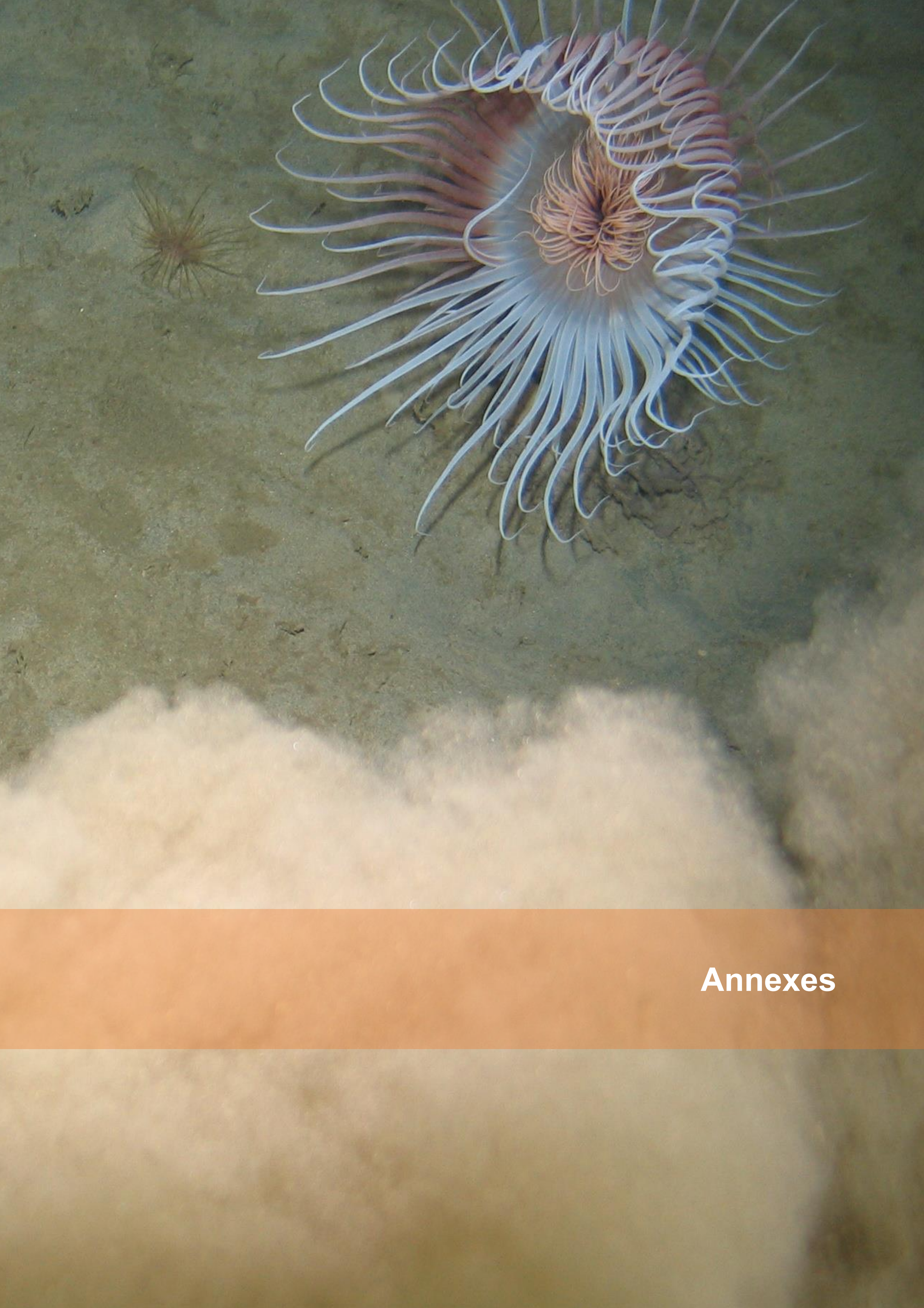


Annexes

Annex 1 List of the morphospecies identified within the ROV surveys. For consistency, the taxonomic classification indicated is in accordance with the World Register of Marine Species (http://www-marinespecies.org). *Indicates morphospecies present in sections with reduced visibility and **indicates pelagic morphospecies, both not included in the main data analysis of this chapter.

Morphospecies taxonomy	Alpha ID
Phylum Porifera Grant, 1836	
Porifera ind. 1	558
Porifera ind. 2	558
Phylum Ctenophora Eschscholtz, 1829	
Ctenophora ind. **	1248
Phylum Cnidaria Verrill, 1865	
Cnidaria ind. 1*	1267
Cnidaria ind. 2*	1267
Cnidaria ind. 3*	1267
Class Anthozoa Ehrenberg, 1834	
Subclass Ceriantharia Perrier, 1893	
Order Spirularia den Hartog, 1977	
Spirularia ind. 1	151646
Spirularia ind. 2	151646
Spirularia ind. 3	151646
Spirularia ind. 4	151646
Spirularia ind. 5	151646
Subclass Hexacorallia Haeckel, 1896	
Order Actiniaria	
Suborder Enthemonae Rodríguez & Daly in Rodríguez et al., 2014	
Superfamily Metridioidea Carlgren, 1893	
Family Hormathiidae Carlgren, 1932	
Genus Actinauge Verrill, 1883	
Actinauge richardi Verrill, 1883	100930
Order Zoantharia Gray, 1832	
Zoantharia ind.	607338
Order Scleractinia Bourne, 1900	
Family Caryophylliidae Dana, 1846	
Genus Caryophyllia Lamarck, 1801	
***Caryophyllia* sp.**	135085
Subclass Octocorallia Haeckel, 1866	
Octocorallia ind. 1*	1341
Octocorallia ind. 2*	1341
Order Pennatulacea Verrill, 1865	
Pennatulacea ind. 1	1367
Pennatulacea ind. 2*	1367
Suborder Subsessiliflorae	
Family Pennatulidae Ehrenberg, 1834	
Genus Pennatula Linnaeus, 1758	
***Pennatula* sp.**	128495

Family Kophobelemnidae Gray, 1860	
Genus Kophobelemnon Asbjørnsen, 1856	
***Kophobelemnon* sp.**	128492
Class Hydrozoa Owen, 1843	
Hydrozoa ind.*	1337
Class Scyphozoa Goette, 1887	
Scyphozoa ind. **	135220
Phylum Nemertea	
Nemertea ind.	152391
Phylum Annelida	
Class Polychaeta Grube, 1850	
Polychaeta ind.	883
Subclass Echiura	
Suborder Bonelliida	
Family Bonelliidae Lacaze-Duthiers, 1858	
Genus Bonellia Rolando, 1822	
Bonellia viridis Rolando, 1822	110363
Subclass Errantia Audouin & H Milne Edwards, 1832	
Order Eunicida	
Family Onuphidae Kinberg, 1865	
Genus Hyalinoecia Malmgren, 1867	
Hyalinoecia tubicola (O.F. Müller, 1776)	130464
Order Amphinomida	
Family Amphinomidae Lamarck, 1818	
Amphinomidae ind.	960
Phylum Arthropoda	
Subphylum Crustacea Brünnich, 1772	
Superclass Multicrustacea Regier, Shultz, Zwick, Hussey, Ball, Wetzer, Martin & Cunningham,	
Class Malacostraca Latreille, 1802	
Subclass Eumalacostraca	
Superorder Peracarida Calman, 1904	
Order Decapoda Latreille, 1802	
Decapoda ind. 1	1130
Decapoda ind. 2	1130
Decapoda ind. 3	1130
Decapoda ind. 4*	1130
Suborder Pleocyemata Burkenroad, 1963	
Infraorder Astacidea Latreille, 1802	
Superfamily Nephropoidea Dana, 1852	
Family Nephropidae Dana, 1852	
Genus Nephrops Leach, 1814	
Nephrops norvegicus Linnaeus, 1758	107254
Infraorder Anomura MacLeay, 1838	
Superfamily Galatheoidea Samouelle, 1819	
Family Munididae Ahyong, Baba, Macpherson & Poore, 2010	
Munididae ind.	562645
Superfamily Paguroidea Latreille, 1802	
Paguroidea ind. 1	106687
Paguroidea ind. 2	106687
Paguroidea ind. 3*	106687

Infraorder Brachyura Latreille, 1802	
Superfamily Majoidea Samouelle, 1819	
Family Inachidae MacLeay, 1838	
Inachidae ind.	148427
Superfamily Portunoidea Rafinesque, 1815	
Family Polybiidae Ortmann, 1893	
Genus Polybius Leach, 1820	
Polybius henslowii Leach, 1820	107399
Infraorder Caridea Dana, 1852	
Superfamily Pandaloidea Haworth, 1825	
Family Pandalidae Haworth, 1825	
Genus Plesionika Spence Bate, 1888	
***Plesionika* sp.**	107046
Suborder Dendrobranchiata Spence Bate, 1888	
Superfamily Penaeoidea Rafinesque, 1815	
Family Aristeidae Wood-Mason in Wood-Mason & Alcock, 1891	
Genus Aristeus Duvernoy, 1840	
Aristeus antennatus Risso, 1816	107083
Phylum Mollusca	
Class Bivalvia Wenz, 1938	
Bivalvia ind.	105
Class Cephalopoda Cuvier, 1795	
Cephalopoda ind. 1	11707
Cephalopoda ind. 2*	11707
Subclass Coleoidea Bather, 1888	
Superorder Octopodiformes Berthold & Engeser, 1987	
Order Octopoda Leach, 1818	
Suborder Incirrata	
Superfamily Octopodoidea d'Orbigny, 1840	
Family Octopodidae d'Orbigny, 1840	
Octopodidae ind.	11782
Subclass Coleoidea Bather, 1888	
Superorder Decapodiformes Young, Vecchione & Donovan, 1998	
Order Oegopsida d'Orbigny, 1845	
Family Ommastrephidae Steenstrup, 1857	
Ommastrephidae ind.	11760
Class Gastropoda Cuvier, 1795	
Subclass Caenogastropoda Cox, 1960	
Order Littorinimorpha Golikov & Starobogatov, 1975	
Superfamily Stromboidea Rafinesque, 1815	
Family Aporrhaidae Gray, 1850	
Genus Aporrhais da Costa, 1778	
Aporrhais serresianus Michaud, 1828	138761
Superfamily Tonnoidea Suter, 1913 (1825)	
Family Cassidae Latreille, 1825	
Genus Galeodea Link, 1807	
Galeodea rugosa Linnaeus, 1771	139024
Family Ranellidae Gray, 1854	
Genus Charonia Gistel, 1847	
Charonia lampas Linnaeus, 1758	141101

Order Neogastropoda Wenz, 1938	
Superfamily Buccinoidea Rafinesque, 1815	
Family Buccinidae Rafinesque, 1815	
***Colus* sp.**	137704
Subclass Vetigastropoda	
Superfamily Trochoidea Rafinesque, 1815	
Family Calliostomatinae Thiele, 1924 (1847)	
Genus Calliostoma Swainson, 1840	
Calliostoma granulatum Born, 1778	141753
Phylum Echinodermata Bruguière, 1791	
Subphylum Crinozoa	
Class Crinoidea	
Subclass Articulata Zittel, 1879	
Order Comatulida	
Comatulida ind. 1	123093
Comatulida ind. 2	123093
Superfamily Antedonoidea Norman, 1865	
Family Antedonidae Norman, 1865	
Genus Leptometra Clark, 1908	
Leptometra celtica M'Andrew & Barrett, 1857	124224
Class Asteroidea de Blainville, 1830	
Asteroidea ind. 1	123080
Asteroidea ind. 2	123080
Asteroidea ind. 3	123080
Asteroidea ind. 4	123080
Superorder Forcipulatacea Blake, 1987	
Order Brisingida Fisher, 1928	
Brisingida ind.	123085
Class Ophiuroidea Gray, 1840	
Ophiuroidea ind. 1	123084
Ophiuroidea ind. 2	123084
Class Echinoidea Leske, 1778	
Echinoidea ind.	123082
Subclass Cidaroidea Smith, 1984	
Order Cidaroida Claus, 1880	
Superfamily Cidaroidea Gray, 1825	
Family Cidaridae Gray, 1825	
Genus Cidaris Leske, 1778	
Cidaris cidaris* Linnaeus, 1758	124257
Phylum Echinodermata	
Subphylum Echinozoa	
Class Holothuroidea	
Holothuroidea ind. 1	123083
Holothuroidea ind. 2	123083
Holothuroidea ind. 3	123083
Holothuroidea ind. 4	123083
Holothuroidea ind. 5*	123083

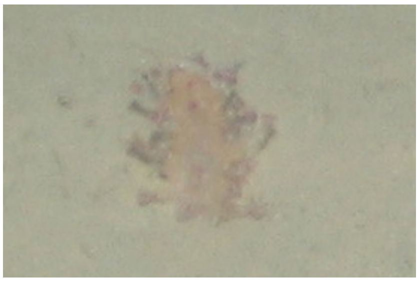
Phylum Chordata Haeckel, 1874	
Subphylum Vertebrata	
Superclass Gnathostomata	
Class Holocephali	
Order Chimaeriformes	
Family Chimaeridae Rafinesque, 1815	
Genus Chimaera Linnaeus, 1758	
Chimaera monstrosa Linnaeus, 1758	105824
Class Elasmobranchii	
Order Carcharhiniformes	
Family Pentanchidae Smith, 1912	
Genus Galeus Rafinesque, 1810	
Galeus melastomus Rafinesque, 1810	105812
Order Carcharhiniformes	
Family Scyliorhinidae Gill, 1862	
Genus Scyliorhinus Blainville, 1816	
Scyliorhinus canicula Linnaeus, 1758	105814
Order Squaliformes Compagno, 1973	
Family Dalatiidae Gray, 1851	
Genus Dalatias Rafinesque, 1810	
Dalatias licha Bonnaterre, 1788	105910
Order Rajiformes	
Family Rajidae de Blainville, 1816	
Genus Raja Linnaeus, 1758	
***Raja* sp.**	105766
Class Actinopterygii	
Order Anguilliformes	
Anguilliformes ind. 1	10295
Anguilliformes ind. 2	10295
Order Gadiformes	
Family Macrouridae Bonaparte, 1831	
Genus Coryphaenoides Gunnerus, 1765	
Coryphaenoides rupestris Gunnerus, 1765	158960
Genus Coelorinchus Giorna, 1809	
***Coelorinchus* sp.**	268809
Family Merlucciidae Rafinesque, 1815	
Genus Merluccius Rafinesque, 1810	
Merluccius merluccius Linnaeus, 1758	126484
Family Gadidae Rafinesque, 1810	
Genus Gadiculus Guichenot, 1850	
Gadiculus argenteus Guichenot, 1850	
Family Gadidae Rafinesque, 1810	
Genus Micromesistius Gill, 1863	
Micromesistius poutassou** Risso, 1827	126439
Order Beryciformes	
Family Trachichthyidae Bleeker, 1856	
Genus Hoplostethus Cuvier, 1829	
Hoplostethus mediterraneus mediterraneus Cuvier, 1829	159409
Order Scorpaeniformes	
Scorpaeniformes ind.*	10329

Suborder Scorpaenoidei	
Family Sebastidae Kaup, 1873	
Genus Helicolenus Goode & Bean, 1896	
Helicolenus dactylopterus Delaroche, 1809	127251
Suborder Platycephaloidei	
Family Triglidae Rafinesque, 1815	
Triglidae ind. 1	125598
Triglidae ind. 2	125598
Order Perciformes	
Suborder Caproidei	
Family Caproidae Bonaparte, 1835	
Genus Capros Lacepède, 1802	
Capros aper** Linnaeus, 1758	127419
Suborder Percoidei	
Family Carangidae Rafinesque, 1815	
Genus Trachurus Rafinesque, 1810	
Trachurus trachurus** Linnaeus, 1758	126822
Suborder Scombroidei	
Family Scombridae Rafinesque, 1815	
Genus Scomber Linnaeus, 1758	
Scomber scombrus** Linnaeus, 1758	127023
Order Pleuronectiformes	
Family Scophthalmidae Chabanaud, 1933	
Genus Lepidorhombus Günther, 1862	
Lepidorhombus boscii Risso, 1810	127145
Family Soleidae Bonaparte, 1833	
Soleidae ind.	125581
Phylum Undetermined	
Undetermined 1*	n/a
Undetermined 2*	n/a
Undetermined 3*	n/a
Undetermined 4*	n/a
Undetermined 5*	n/a
Undetermined 6*	n/a
Undetermined 7*	n/a
Undetermined 8*	n/a

Annex 2. Atlas of mega-epibenthic morphospecies encountered and identified to the lowest taxonomical level possible during the ROV surveys at the Southwest Portuguese margin (details in Chapter 2). Image credits of Vlaams Instituut voor de Zee (VLIZ) and University of Ghent

Taxonomy	Image/Video snapshots
PORIFERA	
Class: n/a Order: n/a Family: n/a Morphospecies: **Porifera ind. 1**	
Class: n/a Order: n/a Family: n/a Morphospecies: **Porifera ind. 2**	
CTENOPHORA	
Class: n/a Order: n/a Family: n/a Morphospecies: **Ctenophora ind.**	
CNIDARIA	
Class: Hydrozoa Order: n/a Family: n/a Morphospecies: **Cnidaria ind. 1**	

Class: n/a
Order: n/a
Family: n/a
Morphospecies: **Cnidaria ind. 2**



Class: n/a
Order: n/a
Family: n/a
Morphospecies: **Cnidaria ind. 3**

Class: Anthozoa (Hexacorallia)
Order: Spirularia
Family: n/a
Morphospecies: **Spirularia ind. 1**

Class: Anthozoa (Hexacorallia)
Order: Spirularia
Family: n/a
Morphospecies: **Spirularia ind. 2**

Class: Anthozoa (Hexacorallia)
Order: Spirularia
Family: n/a
Morphospecies: **Spirularia ind. 3**

Class: Anthozoa (Hexacorallia)
Order: Spirularia
Family: n/a
Morphospecies: **Spirularia ind. 4**

Class: Anthozoa (Hexacorallia)
Order: Spirularia
Family: n/a
Morphospecies: **Spirularia ind. 5**

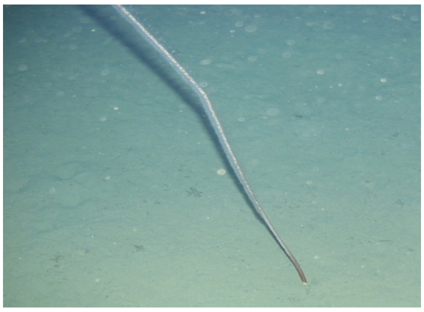
Class: Anthozoa (Hexacorallia)
Order: Actiniaria
Family: Hormathiidae
Morphospecies: ***Actinauge richardi***

Class: Anthozoa (Hexacorallia)
Order: Zoantharia
Family: n/a
Morphospecies: **Zoantharia ind.**

Class: Anthozoa
Order: Scleractinia
Family: Caryophylliidae
Morphospecies: **C*aryophyllia* sp.**

Class: Anthozoa (Octocorallia)
Order: Pennatulacea
Family: n/a
Morphospecies: **Pennatulacea ind. 1**

Class: Anthozoa (Octocorallia)
Order: Pennatulacea
Family: n/a
Morphospecies: **Pennatulacea ind. 2**



Class: Anthozoa (Octocorallia)
Order: n/a
Family: n/a
Morphospecies: **Octocorallia ind. 1**

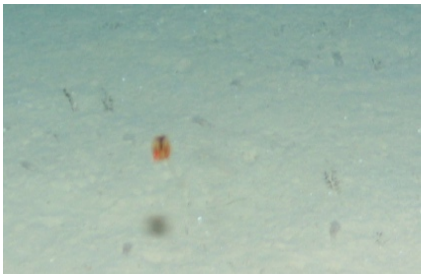
Class: Anthozoa (Octocorallia)
Order: n/a
Family: n/a
Morphospecies: **Octocorallia ind. 1**

Class: Anthozoa (Octocorallia)
Order: Pennatulacea
Family: Pennatulidae
Morphospecies: ***Pennatula* sp.**

Class: Anthozoa (Octocorallia)
Order: Pennatulacea
Family: Kophobelemnidae
Morphospecies: ***Kophobelemnon* sp.**

Class: n/a
Order: n/a
Family: n/a
Morphospecies: **Hydrozoa ind.**

Class: Scyphozoa
Order: n/a
Family: n/a
Morphospecies: **Scyphozoa ind.**



NEMERTEA

Class: n/a
Order: n/a
Family: n/a

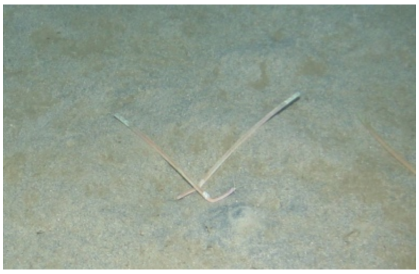
Morphospecies: **Nemertea ind.**

ANNELIDA

Class: Polychaeta
Order: n/a
Family: n/a
Morphospecies: **Polychaeta ind.**

Class: Polychaeta
Order: Echiuroidea
Family: Bonelliidae
Morphospecies: ***Bonellia viridis***

Class: Polychaeta
Order: Eunicida
Family: Onuphidae
Morphospecies: ***Hyalinoecia tubicola***



Class: Polychaeta
Order: Amphinomida
Family: Amphinomidae
Morphospecies: **Amphinomidae ind.**

ARTHROPODA

Class: Crustacea
Order: Decapoda
Family: n/a
Morphospecies: **Decapoda ind. 1**

Class: Crustacea
Order: Decapoda
Family: n/a
Morphospecies: **Decapoda ind. 2**

Class: Crustacea
Order: Decapoda
Family: n/a
Morphospecies: **Decapoda ind. 3**

Class: Crustacea
Order: Decapoda
Family: n/a
Morphospecies: **Decapoda ind. 4**

Class: Crustacea
Order: Decapoda
Family: Nephropidae
Morphospecies: ***Nephrops norvegicus***

Class: Crustacea
Order: Decapoda
Family: Munididae
Morphospecies: **Munididae ind.**

Class: Crustacea
Order: Decapoda
Family: n/a
Morphospecies: **Paguroidea ind. 1**

Class: Crustacea
Order: Decapoda
Family: n/a
Morphospecies: **Paguroidea ind. 2**

Class: Crustacea
Order: Decapoda
Family: n/a
Morphospecies: **Paguroidea ind. 3**

Class: Crustacea
Order: Decapoda
Family: Inachidae
Morphospecies: **Inachidae ind.**

Class: Crustacea
Order: Decapoda
Family: Polybiidae
Morphospecies: ***Polybius henslowii***

Class: Crustacea
Order: Decapoda
Family: Pandalidae
Morphospecies: ***Plesionika* sp.**

Class: Crustacea
Order: Decapoda
Family: Aristeidae
Morphospecies: ***Aristeus antennatus***

MOLLUSCA

Class: Bivalvia
Order: n/a
Family: n/a
Morphospecies: **Bivalvia ind.**

Class: Cephalopoda
Order: n/a
Family: n/a
Morphospecies: **Cephalopoda ind. 1**

Class: Cephalopoda
Order: n/a
Family: n/a
Morphospecies: **Cephalopoda ind. 2**

Class: Cephalopoda
Order: Octopoda
Family: Octopodidae
Morphospecies: **Octopodidae ind.**

Class: Cephalopoda
Order: Oegopsida
Family: Ommastrephidae
Morphospecies: **Ommastrephidae ind.**

Class: Gastropoda
Order: Littorinimorpha
Family: Aporrhaidae
Morphospecies: ***Aporrhais serresianus***

Class: Gastropoda (Caenogastropoda)
Order: Littorinimorpha
Family: Ranellidae
Morphospecies: ***Charonia lampas***

Class: Gastropoda (Caenogastropoda)
Order: Littorinimorpha
Family: Cassidae
Morphospecies: ***Galeodea rugosa***

Class: Gastropoda
Order: Neogastropoda
Family: Buccinidae
Morphospecies: ***Colus* sp.**

Class: Gastropoda (Vetigastropoda)
Order: n/a
Family: Calliostomatidae
Morphospecies: ***Calliostoma granulatum***

ECHINODERMATA

Class: Crinoidea
Order: Comatulida
Family: n/a
Morphospecies: **Comatulida ind. 1**

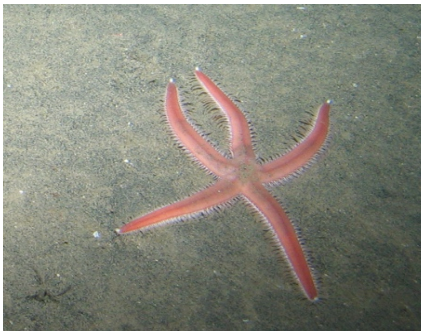
Class: Crinoidea
Order: Comatulida
Family: n/a
Morphospecies: **Comatulida ind. 2**

Class: Crinoidea
Order: Comatulida
Family: Antedonidae
Morphospecies: ***Leptometra celtica***

Class: Asteroidea
Order: n/a
Family: n/a
Morphospecies: **Asteroidea ind. 1**

Class: Asteroidea
Order: n/a
Family: n/a
Morphospecies: **Asteroidea ind. 2**

Class: Asteroidea
Order: n/a
Family: n/a
Morphospecies: **Asteroidea ind. 3**



Class: Asteroidea
Order: n/a
Family: n/a
Morphospecies: **Asteroidea ind. 4**

Class: Asteroidea
Order: Brisingida
Family: n/a
Morphospecies: **Brisingida ind.**

Class: Ophiuroidea
Order: n/a
Family: n/a
Morphospecies: **Ophiuroidea ind. 1**

Class: Ophiuroidea
Order: n/a
Family: n/a
Morphospecies: **Ophiuroidea ind. 2**

Class: Echinoidea
Order: n/a
Family: n/a
Morphospecies: **Echinoidea ind.**

Class: Echinoidea
Order: Cidaroida
Family: Cidaridae
Morphospecies: ***Cidaris cidaris***

Class: Holothuroidea
Order: n/a
Family: n/a
Morphospecies: **Holothuroidea ind. 1**

Class: Holothuroidea
Order: n/a
Family: n/a
Morphospecies: **Holothuroidea ind. 2**

Class: Holothuroidea
Order: n/a
Family: n/a
Morphospecies: **Holothuroidea ind. 3**

Class: Holothuroidea
Order: n/a
Family: n/a
Morphospecies: **Holothuroidea ind. 4**

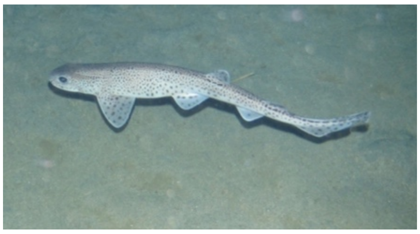
Class: Holothuroidea
Order: n/a
Family: n/a
Morphospecies: **Holothuroidea ind. 4**

CHORDATA

Class: Holocephali
Order: Chimearifoirmes
Family: Chimaeridae
Morphospecies: ***Chimaera monstrosa***

Class: Elasmobranchii
Order: Carcharhiniformes
Family: Pentanchidae
Morphospecies: ***Galeus melastomus***

Class: Elasmobranchii
Order: Carcharhiniformes
Family: Scyliorhinidae
Morphospecies: ***Scyliorhinus canicula***

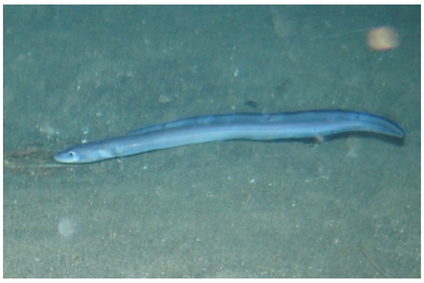


Class: Elasmobranchii
Order: Squaliformes
Family: Dalatiidae
Morphospecies: ***Dalatias licha***

Class: Elasmobranchii
Order: Rajiformes
Family: Rajidae
Morphospecies: ***Raja* sp.**

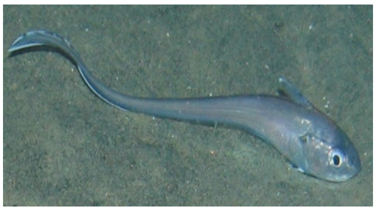
Class: Elasmobranchii
Order: n/a
Family: n/a
Morphospecies: **Elasmobranchii eggs**

Class: Actinopterygii
Order: Anguilliformes
Family: n/a
Morphospecies: **Anguilliformes ind. 1**

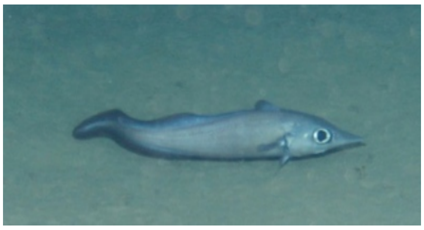


Class: Actinopterygii
Order: Anguilliformes
Family: n/a
Morphospecies: **Anguilliformes ind. 2**

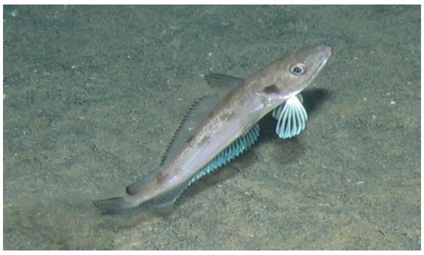
Class: Actinopterygii
Order: Gadiformes
Family: Macrouridae
Morphospecies: ***Coryphaenoides rupestris***



Class: Actinopterygii
Order: Gadiformes
Family: Macrouridae
Morphospecies: ***Coelorinchus* sp.**



Class: Actinopterygii
Order: Gadiformes
Family: Merluccidae
Morphospecies: ***Merluccius merluccius***



Class: Actinopterygii
Order: Gadiformes
Family: Gadidae
Morphospecies: ***Gadiculus argenteus***

Class: Actinopterygii
Order: Gadiformes
Family: Gadidae
Morphospecies: ***Micromesistius poutassou***

Class: Actinopterygii
Order: Beryciformes
Family: Trachichthyidae
Morphospecies: ***Hoplostethus mediterraneus mediterraneus***

Class: Actinopterygii
Order: Scorpaeniformes
Family: Sabastidae
Morphospecies: ***Helicolenus dactylopterus***

Class: Actinopterygii
Order: Scorpaeniformes
Family: n/a
Morphospecies: **Scorpaeniformes ind.**

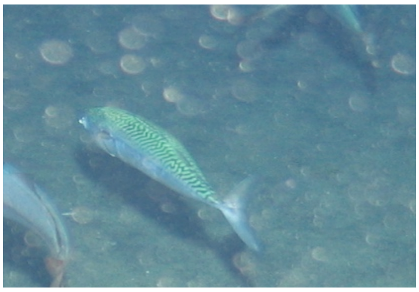
Class: Actinopterygii
Order: Scorpaeniformes
Family: Triglidae
Morphospecies: **Triglidae ind. 1**

Class: Actinopterygii
Order: Scorpaeniformes
Family: Triglidae
Morphospecies: **Triglidae ind. 2**

Class: Actinopterygii
Order: Perciformes
Family: Caproidae
Morphospecies: ***Capros aper***

Class: Actinopterygii
Order: Perciformes
Family: Carangidae
Morphospecies: ***Trachurus trachurus***

Class: Actinopterygii
Order: Perciformes
Family: Scombridae
Morphospecies: ***Scomber scombrus***



Class: Actinopterygii
Order: Pleuronectiformes
Family: Scophthalmidae
Morphospecies: ***Lepidorhombus boscii***

Class: Actinopterygii
Order: Pleuronectiformes
Family: Soleidae
Morphospecies: **Soleidae ind.**

UNDITERMINED

Class: n/a
Order: n/a
Family: n/a
Morphospecies: **Undetermined 1**

Class: n/a
Order: n/a
Family: n/a
Morphospecies: **Undetermined 2**

Class: n/a
Order: n/a
Family: n/a
Morphospecies: **Undetermined 3**

Class: n/a
Order: n/a
Family: n/a
Morphospecies: **Undetermined 4**

Class: n/a
Order: n/a
Family: n/a
Morphospecies: **Undetermined 5**

Class: n/a
Order: n/a
Family: n/a
Morphospecies: **Undetermined 6**

Class: n/a
Order: n/a
Family: n/a
Morphospecies: **Undetermined 7**

Class: n/a
Order: n/a
Family: n/a
Morphospecies: **Undetermined 8**

Annex 3 List of the macrofauna taxa identified within all stations sampled. For consistency, the taxonomic classification indicated is in accordance with the World Register of Marine Species (http://www-marinespecies.org).

	AlphaID
Phylum Platyhelminthes Minot, 1876	
Platyhelminthes sp1	793
Phylum Cnidaria Verrill, 1865	
Class Anthozoa Ehrenberg, 1834	
Subclass Ceriantharia Perrier, 1893	
Order Spirularia den Hartog, 1977	
Spirularia sp 1	151646
Class Hydrozoa Owen, 1843	
Hydrozoa sp1	1337
Hydrozoa sp2	1337
Hydrozoa sp3	1337
Class Scyphozoa Goette, 1887	
Scyphozoa ind.	135220
Phylum Nemertea	
Nemertea sp1	152391
Nemertea sp2	152391
Nemertea sp3	152391
Class Priapulida Théel, 1906	
Priapulida ind.	101063
Phylum Sipuncula	
Sipuncula sp1	1268
Sipuncula sp.2	136021
Phylum Annelida	
Class Clitellata	
Subclass Oligochaeta Grube, 1850	
Oligochaeta sp1	2036
Class Polychaeta Grube, 1850	
Polychaeta ind.	883
Subclass Echiura	
Echiura sp.1	1269
Subclass Errantia Audouin & H Milne Edwards, 1832	
Order Amphinomida	
Family Amphinomidae Lamarck, 1818	
Amphinomidae sp1	960
Family Dorvilleidae Chamberlin, 1919	
Genus *Schistomeringos* Jumars, 1974	
***Schistomeringos* sp1**	129274
Genus *Protodorvillea* Pettibone, 1961	
***Protodorvillea* sp1**	129272
Family Eunicidae Berthold, 1827	
Eunicidae indet.	129280
Genus *Marphysa* Quatrefages, 1866	
***Marphysa* sp1**	129281
Genus *Lysidice* Lamarck, 1818	
***Lysidice* sp1**	129280
***Lysidice* sp2**	129280

Order Eunicida	
Family Onuphidae Kinberg, 1865	
Genus *Hyalinoecia* Malmgren, 1867	
***Hyalinoecia* sp1**	129400
***Hyalinoecia* sp2**	129400
Hyalinoecia tubicola (O.F. Müller, 1776)	130464
Family Onuphidae Kinberg, 1865	
Subfamily Onuphinae Kinberg, 1865	
cf. *Paradiopatra* sp. 1	298365
Genus Aponuphis Kucheruk, 1978	
Aponuphis bilineata (Baird, 1870)	130452
Family Lumbrineridae Schmarda, 1861	
Lumbrineridae indet.	967
cf. *Augeneria* sp1 (Monro, 1930)	129332
Genus *Lumbrineriopsis* Orensanz, 1973	
Lumbrineriopsis sp1	129336
Genus *Lumbrineris* Blainville, 1828	
***Lumbrineris* sp1**	129336
***Lumbrineris* sp2**	129336
***Lumbrineris* sp3**	129336
***Lumbrineris* sp4**	129336
Genus *Ninoe* Kinberg, 1865	
***Ninoe* sp1**	129338
Order Phyllodocida	
Suborder Aphroditiformia Levinsen, 1883	
Family Acoetidae Kinberg, 1856	
Acoetidae sp1	19199
Family Polynoidae Kinberg, 1856	
Polynoidae sp1	939
Polynoidae sp2	939
Polynoidae sp3	939
Polynoidae sp4	939
Polynoidae sp5	940
Family Sigalionidae Kinberg, 1856	
Sigalionidae sp1	943
Order Phyllodocida Dales, 1962	
Suborder Nereidiformia	
Family Hesionidae Grube, 1850	
Hesionidae sp1	946
Family Pilargidae Saint-Joseph, 1899	
Pilargidae sp1	15009
Family Syllidae Grube, 1850	
Subfamily Syllinae Grube, 1850	
Syllinae sp1	152223
Subfamily Eusyllinae Malaquin, 1893	
Eusyllinae sp1	152233
Subfamily Exogoninae Langerhans, 1879	
Exogoninae sp1	152228
Exogoninae sp2	152228
Exogoninae sp3	152228
Exogoninae sp4	152228
Exogoninae sp5	152228
Exogoninae sp6	152228

Genus *Sphaerosyllis* Claparède, 1863	
***Sphaerosyllis* spp.**	129677
Suborder Glyceriformia	
Family Glyceridae Grube, 1850	
Genus *Glycera* Lamarck, 1818	
Glycera lapidum (Quatrefages, 1866)	130123
Glycera* cf. *fallax	336908
Family Goniadidae Kinberg, 1866	
Goniadidae sp1	953
Family Paralacydoniidae Pettibone, 1963	
Genus *Paralacydonia* Fauvel, 1913	
***Paralacydonia* sp1**	22611
Suborder Phyllodociformia	
Family Phyllodocidae Örsted, 1843	
Phyllodocidae indet.	931
Subfamily Phyllodocinae Örsted, 1843	
Genus *Phyllodoce* Lamarck, 1818	
***Phyllodoce* sp1**	129455
Phyllodoce madeirensis (Langerhans, 1880)	130677
Subfamily Eteoninae Bergström, 1914	
Eteoninae sp1	152229
Genus *Mystides* Théel, 1879	
***Mystides* sp1**	129450
Suborder Phyllodocida incertae sedis	
Genus Sphaerodoridae Malmgren, 1867	
Sphaerodoridae sp1	957
Sphaerodoridae sp2	957
Sphaerodoridae sp3	957
Family Nephtyidae Grube, 1850	
Nephtyidae sp.1	956
Genus *Micronephthys* Friedrich, 1939	
***Micronephthys* sp1**	129368
Genus *Nephtys* Cuvier, 1817	
***Nephtys* sp1**	129370
Subclass Sedentaria Lamarck, 1818	
Family Chaetopteridae Audouin & Milne Edwards, 1833	
Chaetopteridae sp1	918
Infraclass Canalipalpata Rouse & Fauchald, 1997	
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Suborder Cirratuliformia	
Family Cirratulidae Carus, 1863	
Cirratulidae spp.	919
Subfamily Raphidrilinae Hartmann-Schröder, 1971	
Genus *Raricirrus* Hartmann, 1961	
***Raricirrus* sp1**	129254
Family Acrocirridae Banse, 1969	
Acrocirridae sp1	920
Family Flabelligeridae de Saint-Joseph, 1894	
Flabelligeridae sp1	976
Family Ampharetidae Malmgren, 1866	
Ampharetidae indet.	981
Ampharetidae sp1	981
Ampharetidae sp2	981

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Family Terebellidae Johnston, 1846	
Subfamily Terebellinae Johnston, 1846	
Terebellinae sp1	322588
Terebellinae sp2	322588
Genus *Pista* Malmgren, 1866	
***Pista* sp1**	129708
Subfamily Polycirrinae Malmgren, 1867	
Genus *Polycirrus* Grube, 1850	
***Polycirrus* sp1**	129710
Family Trichobranchidae Malmgren, 1866	
Trichobranchidae sp1	983
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Family Oweniidae Rioja, 1917	
Oweniidae sp1	975
Family Sabellidae Latreille, 1825	
Sabellidae sp1	985
Order Spionida Rouse & Fauchald, 1997	
Suborder Spioniformia	
Family Magelonidae Cunningham & Ramage, 1888	
Genus *Magelona* F. Müller, 1858	
***Magelona* sp1**	129341
Family Spionidae Grube, 1850	
Spionidae indet.	889
Spionidae sp1	889
Spionidae sp2	889
Spionidae sp3	889
Spionidae sp4	889
Spionidae sp5	889
cf. *Pseudopolydora* sp1	129621
Genus *Aonidella* López-Jamar, 1989	
***Aonidella* sp1**	325170
Genus *Aonides* Claparède, 1864	
***Aonides* sp1**	129605
Genus *Laonice* Malmgren, 1867	
***Laonice* sp1**	129613
Genus *Malacoceros* Quatrefages, 1843	
***Malacoceros* sp1**	129614
Genus *Prionospio* Malmgren, 1867	
***Prionospio* spp.**	129620
Genus *Polydora* Bosc, 1802	
***Polydora* sp1**	129619
Genus *Spiophanes* Grube, 1860	
***Spiophanes* sp1**	129626
***Spiophanes* sp2**	129626
***Spiophanes* sp3**	129626
Family Poecilochaetidae Hannerz, 1956	
Poecilochaetidae sp1	916
Infraclass Scolecida Rouse & Fauchald, 1997	
Family Capitellidae Grube, 1862	
Genus Capitellidae Grube, 1862	
Capitellidae sp1	921
Capitellidae sp2	921
Capitellidae sp3	921
Genus *Notomastus* M. Sars, 1851	
***Notomastus* sp1**	129220

Family Cossuridae Day, 1963	
Cossuridae sp1	908
Family Maldanidae Malmgren, 1867	
Maldanidae sp1	923
Maldanidae sp2	923
Maldanidae sp3	923
Maldanidae sp4	923
Maldanidae indet.	923
Family Opheliidae Malmgren, 1867	
Opheliidae indet.	924
Subfamily Ophelininae	
Genus *Ophelina* Örsted, 1843	
Ophelina cylindricaudata (Hansen, 1879)	130503
Ophelina abranchiata (Støp-Bowitz, 1948)	130499
Ophelina modesta Støp-Bowitz, 1958	130507
Family Orbiniidae Hartman, 1942	
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cf. *Orbinia* sp1	902
Family Paraonidae Cerruti, 1909	
Paraonidae sp1	903
Paraonidae sp2	903
Paraonidae sp3	903
Paraonidae indet.	903
Genus *Aricidea* Webster, 1879	
***Aricidea* spp.**	129430
Family Scalibregmatidae Malmgren, 1867	
Genus *Scalibregma* Rathke, 1843	
***Scalibregma* sp.**	924
Scalibregma* cf. *inflatum	925
Phylum Arthropoda	
Subphylum Crustacea Brünnich, 1772	
Class Malacostraca Latreille, 1802	
Subclass Eumalacostraca	
Superorder Peracarida Calman, 1904	
Order Amphipoda Latreille, 1816	
Amphipoda indet.	1135
Suborder Gammaridea Latreille, 1802	
Gammaridea indet.	1207
Family Ampeliscidae Krøyer, 1842	
Genus *Ampelisca* Krøyer, 1842	
***Ampelisca* spp.**	101445
Genus *Byblis* Boeck, 1871	
***Byblis* sp1**	101446
***Byblis* sp2**	101446
Genus *Haploops* Liljeborg, 1856	
***Haploops* sp1**	101447
Family Amphilochidae Boeck, 1871	
Genus *Amphilochoides* G.O. Sars, 1892	
Amphilochoides longimanus (Chevreux, 1888)	423048
Genus *Gitana* Boeck, 1871	
Gitana* cf. *sarsi	101452
Family Leucothoidae Dana, 1852	
Genus *Leucothoe* Leach, 1814	
Leucothoe* cf. *lilljeborgi	102462
Leucothoe* cf. *incisa	102460

Genus *Lepechinella* Stebbing, 1908	
***Lepechinella* sp1**	101392
Family Melphidippidae Stebbing, 1899	
Genus *Melphidippella* G.O. Sars, 1894	
Melphidippella macra (Norman, 1869)	102860
Family Synopiidae Dana, 1853	
Genus *Syrrhoe* Goës, 1866	
Syrrhoe affinis (Chevreux, 1908)	103186
Family Stegocephalidae Dana, 1852	
Subfamily Stegocephalinae Dana, 1852	
Genus *Stegocephaloides* G.O. Sars, 1891	
Stegocephaloides* cf. *christianiensis	103102
Family Stenothoidae Boeck, 1871	
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Genus *Stenothoe* Dana, 1852	
Stenothoe* cf. *bosphorana	103152
Stenothoe* cf. *marina	103166
Family Oedicerotidae Lilljeborg, 1865	
Oedicerotidae indet.	101400
Genus *Westwoodilla* Spence Bate, 1862	
Westwoodilla caecula (Spence Bate, 1857)	102932
Genus *Perioculodes* G.O. Sars, 1892	
Perioculodes aequimanus (Korssman, 1880)	102914
Perioculodes longimanus longimanus (Spence Bate & Westwood, 1868)	103297
Genus *Synchelidium* G.O. Sars, 1892	
Synchelidium* cf. *longidigitatum	101704
Genus *Deflexilodes* Bousfield & Chevrier, 1996	
Deflexilodes* cf. *acutipes	236537
Family Pardaliscidae Boeck, 1871	
Pardaliscidae indet.	101401
Genus *Nicippe* Bruzelius, 1859	
Nicippe tumida (Bruzelius, 1859)	102944
Genus *Halice* Boeck, 1871	
Halice walkeri (Ledoyer, 1973)	102941
Genus *Pardaliscella* Sars, 1883	
Pardaliscella* cf. *boecki	102950
Family Phoxocephalidae G.O. Sars, 1891	
Subfamily Harpiniinae Barnard & Drummond, 1978	
Genus *Harpinia* Boeck, 1876	
Harpinia antennaria Meinert, 1890	102960
Harpinia crenulata	102963
Harpinia zavodniki	102977
***Harpinia* indet.**	101716
Family Urothoidae Bousfield, 1978	
Genus *Urothoe* Dana, 1852	
Urothoe* cf. *elegans	103228
Superfamily Liljeborgioidea Stebbing, 1899	
Family Liljeborgiidae Stebbing, 1899	
Subfamily Liljeborgiinae Stebbing, 1899	
Genus *Liljeborgia* Spence Bate, 1862	
***Liljeborgia* sp1**	101582
Superfamily Eusiroidea Bousfield, 1979	
Family Eusiridae Stebbing, 1888	
Genus *Eusirus* Krøyer, 1845	
Eusirus longipes (Boeck, 1861)	101380

Superfamily Lysianassoidea Dana, 1849	
Family Aristiidae Lowry & Stoddart, 1997	
Aristiidae sp1	236740
Family Lysianassidae Dana, 1849	
Subfamily Tryphosinae Lowry & Stoddart, 1997	
Genus *Hippomedon* Boeck, 1871	
Hippomedon* cf. *massiliensis	102576
Genus *Orchomene* Boeck, 1871	
***Orchomene* sp1**	101633
Family Uristidae Hurley, 1963	
Genus *Caeconyx* Barnard & Karaman, 1991	
Caeconyx* cf. *caeculus	102543
Suborder Senticaudata Lowry & Myers, 2013	
Infraorder Carangoliopsida Bousfield, 1977	
Superfamily Carangoliopsoidea Bousfield, 1977	
Family Carangoliopsidae Bousfield, 1977	
Genus *Carangoliopsis* Ledoyer, 1970	
Carangoliopsis spinulosa (Ledoyer, 1970)	102074
Infraorder Corophiida Leach, 1814	
Superfamily Aoroidea Stebbing, 1899	
Family Aoridae Stebbing, 1899	
Genus *Autonoe* Bruzelius, 1859	
Autonoe spiniventris Della Valle, 1893	101862
Superfamily Corophioidea Leach, 1814	
Family Corophiidae Leach, 1814	
Subfamily Corophiinae Leach, 1814	
Genus *Apocorophium* Bousfield & Hoover, 1997	
Apocorophium* cf. *acutum	148604
Superfamily Caprelloidea Leach, 1814	
Family Podoceridae Leach, 1814	
Genus *Laetmatophilus* Bruzelius, 1859	
Laetmatophilus ledoyeri (Ruffo, 1986)	103047
Family Caprellidae Leach, 1814	
Subfamily Phtisicinae Vassilenko, 1968	
Genus *Phtisica* Slabber, 1769	
Phtisica* cf. *marina	101864
Subfamily Caprellinae Leach, 1814	
Genus *Pedoculina* Carausu, 1941	
Pedoculina* cf. *garciagomezi	101862
Genus *Liropus* Mayer, 1890	
***Liropus* sp1**	101435
Superfamily Photoidea Boeck, 1871	
Family Photidae Boeck, 1871	
Genus *Photis* Krøyer, 1842	
Photis longicaudata (Spence Bate & Westwood, 1862)	102383
Genus *Gammaropsis* Lilljeborg, 1855	
Gammaropsis* cf. *sophiae	102371
Genus Megamphopus Norman, 1869	
Megamphopus* cf. *longicornis	102378
Family Ischyroceridae Stebbing, 1899	
Ischyroceridae sp1	101389
Infraorder Hadziida S. Karaman, 1943	
Superfamily Calliopioidea Sars, 1895	
Family Calliopiidae G.O. Sars, 1893	
Genus *Apherusa* Walker, 1891	
Apherusa* cf. *vexatrix	102176

Superfamily Hadzioidea S. Karaman, 1943 (Bousfield, 1983)	
Family Eriopisidae Lowry & Myers, 2013	
Genus *Eriopisa* Stebbing, 1890	
Eriopisa elongata (Bruzelius, 1859)	102807
Family Maeridae Krapp-Schickel, 2008	
Genus *Ceradocus* Costa, 1853	
***Ceradocus* sp1**	101668
Genus *Abludomelita* Karaman, 1981	
Abludomelita aculeata (Chevreux, 1911)	102786
Order Cumacea Krøyer, 1846	
Cumacea indet.	1137
Family Diastylidae Bate, 1856	
cf. *Leptostylis*	182066
cf. *Vemakylindrus*	110507
cf. *Makrokylindrus*	110606
Diastylidae sp1	110380
Diastylidae indet.	110380
Genus *Diastyloides* G.O. Sars, 1900	
Diastyloides* cf. *biplicatus	110494
Diastyloides* cf. *serratus	110497
Family Lampropidae Sars, 1878	
Lampropidae sp1	110381
Family Leuconidae Sars, 1878	
Leuconidae sp1	110382
Leuconidae sp2	110382
Genus *Eudorella* Norman, 1867	
***Eudorella* sp1**	110412
***Eudorella* sp2**	110412
Family Nannastacidae Bate, 1866	
Nannasticidae indet.	110383
Genus *Campylaspis* G.O. Sars, 1865	
Campylaspis* cf. *sulcata	110558
Campylaspis* cf. *glabra	110543
***Campylaspis* sp1**	110415
Genus *Nannastacus* Bate, 1865	
Nannastacus* cf. *unguiculatus	110574
Order Tanaidacea Dana, 1849	
Suborder Tanaidomorpha Sieg, 1980	
Tanaidomorpha indet	136152
Superfamily Paratanaoidea Lang, 1949	
Paratanaoidea sp1	136208
Paratanaoidea indet	148687
Family Agathotanaidae Lang, 1971	
Agathotanaidae indet.	237594
Genus *Paranarthrura* Hansen, 1913	
***Paranarthrura* sp1**	136225
Paranarthrura lusitanus (Bird & Holdich, 1989)	136383
Paranarthrura crassa (Bird & Holdich, 1989)	136380
Family Anarthruridae Lang, 1971	
Genus *Anarthrura* Sars, 1882	
***Anarthrura* sp1**	136226
Genus *Anisopechys* Bird, 2004	
Anisopechys crinitus (Bird, 2004)	247428

Family Akanthophoreidae Sieg, 1986	
Genus *Tumidochelia* Knight, Larsen & Heard, 2003	
***Tumidochelia* sp.1**	247031
***Tumidochelia* cf.** uncinata	478776
Family Akanthophoreidae Sieg, 1986	
cf. Akanthophoreidae sp1	599405
cf. Akanthophoreidae sp2	599405
Genus *Akanthophoreus* Sieg, 1986	
Akanthophoreus* cf. *gracilis	136340
Genus *Parakanthophoreus* Larsen & Araújo-Silva, 2014	
Parakanthophoreus inermis (Hansen, 1913)	798746
Parakanthophoreus albus (Hansen, 1913)	798736
***Parakanthophoreus* sp1**	798733
Family Colletteidae Larsen & Wilson, 2002	
Genus *Haplocope* Sars, 1882	
Haplocope angusta (Sars, 1882)	136353
Family Cryptocopidae (Sieg, 1973) Sieg, 1977	
Genus *Cryptocopoides* (Sieg, 1973) Sieg, 1976	
Cryptocopoides* cf. *nobilis	606478
Family Leptognathiidae (Sieg, 1973) Sieg, 1976	
Genus *Leptognathia* Sars, 1882	
***Leptognathia* sp1**	136229
Leptognathia breviremis (Lilljeborg, 1864)	136431
Genus *Leptognathiella* Hansen, 1913	
***Leptognathiella* sp1**	136211
Leptognathiella subaequalis (Hansen, 1913)	136358
Leptognathiella abyssi (Hansen, 1913)	136355
Leptognathiella clivicola (Bird & Holdich, 1984)	136356
Family Nototanaidae Sieg, 1976	
Nototanaidae sp1	136161
Family Paratanaidae Lang, 1949	
Subfamily Paratanaidinae Lang, 1949	
Genus *Paratanais* Dana, 1853	
***Paratanais* sp1**	136240
Family Pseudotanaidae (Sieg, 1973 M.S.) Sieg, 1976	
Subfamily Pseudotanainae (Sieg, 1973 M.S.) Sieg, 1977	
Genus *Pseudotanais* Sars, 1882	
Pseudotanais* (*Pseudotanais*) *jonesi (Sieg, 1973**)**	136246
Pseudotanais* (*Pseudotanais*) *vulsella (Bird & Holdich, 1989)	136518
Pseudotanais* (*Pseudotanais*) *falcicula (Bird & Holdich, 1989)	136503
Pseudotanais* (*Pseudotanais*) *scalpellum (Bird & Holdich, 1989)	136514
Pseudotanais* (*Pseudotanais*) *denticulatus (Bird & Holdich, 1989)	136503
***Pseudotanais* sp1**	136246
***Pseudotanais* indet**	136246
Genus *Mystriocentrus* Bird & Holdich, 1989	
Mystriocentrus serratus (Bird & Holdich, 1989)	136496
Family Tanaopsidae Błażewicz-Paszkowycz & Bamber, 2012	
Genus *Tanaopsis* Sars, 1899	
***Tanaopsis* sp1**	136208

Family Tanaellidae Larsen & Wilson, 2002	
Genus *Araphura* Bird & Holdich, 1984	
Araphura brevimanus (Lilljeborg, 1864)	136344
Araphura **sp1**	136346
Araphura **sp2**	136206
Genus *Tanaella* Norman & Stebbing, 1886	
Tanaella **sp1**	136221
Tanaella **sp2**	136221
Family Typhlotanaidae Sieg, 1984	
Typhlotanaidae indet.	136165
Genus *Meromonakantha* Sieg, 1986	
Meromonakantha irregularis (Hansen, 1913)	247892
Genus *Pulcherella* Blazewicz-Paszkowycz, 2007	
Pulcherella **sp1**	247870
Genus *Torquella* Blazewicz-Paszkowycz, 2007	
Torquella **sp1**	247875
Genus *Typhlotanais* Sars, 1882	
Typhlotanais tenuicornis (Sars, 1882)	478771
Typhlotanais kyphosis Blazewicz-Paszkowycz, Bamber & Cunha, 2011	571692
Typhlotanais spinicauda Hansen, 1913	478769
Typhlotanais **sp1**	136256
Genus Typhlotanoides Sieg, 1983	
Typhlotanoides sp1	247033
Family Paratanaoidea incertae sedis	
Genus *Armaturatanais* Larsen, 2005	
Armaturatanais **sp1**	246898
Genus *Leptognathioides* Bird & Holdich, 1984	
Leptognathioides biarticulata (Bird, 2014)	760841
Genus *Robustochelia* Kudinova-Pasternak, 1983	
Robustochelia longa	136363
Suborder Apseudomorpha Sieg, 1980	
Superfamily Apseudoidea Leach, 1814	
Family Apseudidae Leach, 1814	
Apseudidae indet.	136153
Subfamily Apseudinae Leach, 1814	
Genus *Apseudes* Leach, 1814	
Apseudes grossimanus Norman & Stebbing, 1886	136265
Apseudes **cf.** ***spinosus***	136284
Apseudes **cf.** ***rotundifrons***	136280
Genus *Apseudopsis* Norman, 1899	
Apseudopsis **sp1**	136186
Apseudopsis elisae (Bacescu, 1961)	247073
Order Isopoda Latreille, 1817	
Superorder Asellota Latreille, 1802	
Superfamily Janiroidea G.O. Sars, 1897	
Family Desmosomatidae G.O. Sars, 1897	
Desmosomatidae indet.	118250
Genus *Echinopleura* G.O. Sars, 1897	
Echinopleura aculeata (Sars G.O., 1864)	118548
Genus *Eugerda* Meinert, 1890	
Eugerda **sp1**	118323

Genus *Eugerdella* Kussakin, 1965	
Eugerdella pugilator (Hessler, 1970)	118569
Genus *Desmosoma* G.O. Sars, 1864	
***Desmosoma* sp1**	118319
Genus *Prochelator* Hessler, 1970	
***Prochelator* sp1**	118328
Genus *Chelator* Hessler, 1970	
***Chelator* sp1**	118317
Family Ischnomesidae Hansen, 1916	
cf. *Heteromesus* sp1	118357
Family Macrostylidae Hansen, 1916	
Macrostylidae sp1	118261
Family Munnopsidae Lilljeborg, 1864	
Munnopsidae indet.	118264
Subfamily Eurycopinae Hansen, 1916	
Genus *Disconectes* Wilson & Hessler, 1981	
***Disconectes* sp1**	118339
Genus *Tytthocope* Wilson & Hessler, 1981	
***Tytthocope* sp1**	118346
Genus *Baeonectes* Wilson, 1982	
***Baeonectes* sp1**	118335
Subfamily Ilyarachninae Hansen, 1916	
Genus *Ilyarachna* G.O. Sars, 1869	
Ilyarachna* cf. *longicornis	118677
***Ilyarachna* sp1**	118354
Genus *Pseudarachn*a G.O. Sars, 1897	
***Pseudarachna* sp1**	118355
Subfamily Lipomerinae Tattersall, 1905	
Genus *Lipomera* (*Tetracope*) Wilson, 1989	
***Lipomera* (*Tetracope*) sp1**	249494
Genus *Lipomera* (*Paralipomera*) Wilson, 1989	
Lipomera* (*Paralipomera*) *knorrae (Wilson, 1989)	264194
Family Nannoniscidae Hansen, 1916	
Genus *Hebefustis* Siebenaller & Hessler, 1977	
***Hebefustis* sp1**	118380
Genus *Nannoniscus* G.O. Sars, 1870	
***Nannoniscus* sp1**	118382
Family Paramunnidae Vanhöffen, 1914	
Genus *Pleurogonium* G.O. Sars, 1864	
***Pleurogonium* sp1**	118388
Family Paramunnidae Vanhöffen, 1914	
Genus *Paramunna* G.O. Sars, 1866	
***Paramunna* sp1**	118385
Paramunna bilobata (G.O. Sars, 1866)	118793
Suborder Cymothoida	
Superfamily Anthuroidea Leach, 1914	
Family Anthuridae Leach, 1814	
Anthuridae sp1	118244
Family Hyssuridae Wägele, 1981	
Genus *Hyssura* Norman & Stebbing, 1886	
***Hyssura* sp1**	118298
Family Leptanthuridae Poore, 2001	
Genus *Bullowanthura* Poore, 1978	
***Bullowanthura* sp1**	118303

Superfamily Cymothooidea Leach, 1814	
Family Cirolanidae Dana, 1852	
***Natatolana* sp1**	118404
Genus *Eurydice* Leach, 1815	
***Eurydice* sp1**	118401
Genus Natatolana Bruce, 1981	
Cirolanidae indet.	118273
Family Gnathiidae Leach, 1814	
Genus *Gnathia* Leach, 1814	
***Gnathia* sp.1**	118437
***Gnathia* sp2**	118437
***Gnathia* indet**	118437
Infraorder Epicaridea Latreille, 1825	
Epicaridea sp1	13795
Suborder Valvifera G. O. Sars, 1883	
Family Arcturidae Dana, 1849	
Genus *Astacilla* Cordiner, 1793	
Astacilla* cf. *dilatata	295579
Order Mysida Boas, 1883	
Family Mysidae Haworth, 1825	
Subfamily Erythropinae Hansen, 1910	
Genus *Erythrops* G.O. Sars, 1869	
***Erythrops* sp1**	119856
Genus *Paramblyops* Holt & Tattersall, 1905	
Paramblyops rostratu (Holt & Tattersall, 1905)	446463
Genus *Parapseudomma* Nouvel & Lagardère, 1976	
Parapseudomma calloplura (Holt & Tattersall, 1905)	120165
Genus Pseudomma G.O. Sars, 1870	
Pseudomma affine (G.O. Sars, 1870)	120182
Subfamily Gastrosaccinae Norman, 1892	
Genus *Anchialina* Norman & Scott, 1906	
Anchialina agilis (G.O. Sars, 1877)	119950
Genus *Haplostylus* Kossmann, 1880	
Haplostylus* cf. *normani	148698
Superorder Eucarida Calman, 1904	
Order Euphausiacea Dana, 1852	
Euphausiacea indet.	1128
Order Decapoda Latreille, 1802	
Suborder Dendrobranchiata Spence Bate, 1888	
Superfamily Penaeoidea Rafinesque, 1815	
Family Aristeidae Wood-Mason in Wood-Mason & Alcock, 1891	1128
cf. *Aristeus* sp.	106725
Suborder Pleocyemata Burkenroad, 1963	
Infraorder Astacidea Latreille, 1802	
Superfamily Nephropoidea Dana, 1852	
Family Nephropidae Dana, 1852	
Genus Nephrops Leach, 1814	
***Nephrops norvegicus* Linnaeus, 1758**	107254
Infraorder Brachyura Latreille, 1802	
Superfamily Portunoidea Rafinesque, 1815	
Family Pirimelidae Alcock, 1899	
Genus *Pirimela* Leach, 1816	
***Pirimela* sp1**	106878

Family Goneplacidae MacLeay, 1838	
Genus *Goneplax* Leach, 1814	
Goneplax rhomboides (Linnaeus, 1758)	107292
Infraorder Caridea Dana, 1852	
Superfamily Alpheoidea Rafinesque, 1815	
Family Alpheidae Rafinesque, 1815	
Genus *Athanas* (Leach, 1814)	
***Athanas* sp1**	106979
Subphylum Chelicerata	
Class Pycnogonida	
Order Pantopoda Gerstaecker, 1863	
Suborder Eupantopodida Fry, 1978	
Superfamily Ascorhynchoidea Pocock, 1904	
Family Ammotheidae Dohrn, 1881	
Genus *Paranymphon* Caullery, 1896	
Paranymphon spinosum (Caullery, 1896)	134632
Phylum Mollusca	
Class Bivalvia Linnaeus, 1758	
Bivalvia indet.	15
Subclass Heterodonta Neumayr, 1884	
Infraclass Euheterodonta	
Superorder Anomalodesmata Dall, 1889	
Superfamily Cuspidarioidea Dall, 1886	
Family Cuspidariidae Dall, 1886	
Cuspidariidae indet.	1788
Genus *Tropidomya* Dall & E. A. Smith, 1886	
Tropidomya abbreviata (Forbes, 1843)	139470
Superorder Imparidentia Bieler, P. M. Mikkelsen & Giribet, 2014	
Superfamily Galeommatoidea J.E. Gray, 1840	
Family Montacutidae W. Clark, 1855	
Genus *Kurtiella* Gofas & Salas, 2008	
Kurtiella tumidula (Jeffreys, 1866)	345287
Order Cardiida Ferussac, 1822	
Superfamily Cardioidea Lamarck, 1809	
Family Cardiidae Lamarck, 1809	
Cardiidae sp1	229
Cardiidae sp2	229
Cardiidae indet.	229
Superfamily Tellinoidea Blainville, 1814	
Family Semelidae Stoliczka, 1870	
Genus *Abra* Lamarck, 1818	
Abra longicallus (Scacchi, 1835)	141434
Order Lucinida Gray, 1854	
Superfamily Thyasiroidea Dall, 1900 (1895)	
Family Thyasiridae Dall, 1900 (1895)	
Thyasiridae indet.	219
Genus *Adontorhina* Berry, 1947	
Adontorhina keegani (Barry & McCormack, 2007)	345773
Genus *Axinulus* Verrill & Bush, 1898	
Axinulus alleni (Carrozza, 1981)	875337
Axinulus brevis (Verrill & Bush, 1898)	234163
Axinulus croulinensis (Jeffreys, 1847)	234161
Genus *Mendicula* Iredale, 1924	
***Mendicula* sp1**	152423
Mendicula ferruginosa (Forbes, 1844)	152905

Genus *Parathyasira* Iredale, 1930	
Parathyasira sp1	152893
Parathyasira equalis (Verrill & Bush, 1898)	954693
Genus *Thyasira* Lamarck, 1818	
Thyasira flexuosa (Montagu, 1803)	141662
Thyasira tortuosa (Jeffreys, 1881)	141677
Order Venerida Gray, 1854	
Family Kelliellidae Fischer, 1887	
Genus *Kelliella* M. Sars, 1870	
Kelliella sp1	138093
Kelliella miliaris (Philippi, 1844)	152396
Subclass Pteriomorphia Beurlen, 1944	
Order Arcida Stoliczka, 1871	
Superfamily Arcoidea Lamarck, 1809	
Family Arcidae Lamarck, 1809	
Genus *Bathyarca* Kobelt, 1891	
Bathyarca cf. _pectunculoides_	138799
Order Limida Moore, 1952	
Superfamily Limoidea Rafinesque, 1815	
Family Limidae Rafinesque, 1815	
Genus *Limatu*la S. V. Wood, 1839	
Limatula subovata (Monterosato, 1875)	140243
Order Mytilida Férussac, 1822	
Superfamily Mytiloidea Rafinesque, 1815	
Family Mytilidae Rafinesque, 1815	
Mytilidae sp 1	140458
Order Pectinida Gray, 1854	
Superfamily Anomioidea Rafinesque, 1815	
Family Anomiidae Rafinesque, 1815	
cf. _Heteranomia_ sp.	137651
Superfamily Pectinoidea Rafinesque, 1815	
Family Propeamussiidae Abbott, 1954	
Genus *Parvamussiu*m Sacco, 1897	
Parvamussium permirum (Dautzenberg, 1925)	181289
Subclass Protobranchia Pelseneer, 1889	
Order Nuculanida Carter, J. G., Campbell, D. C. & M. R. Campbell, 2000	
Superfamily Nuculanoidea H. Adams & A. Adams, 1858 (1854)	
Family Yoldiidae Dall, 1908	
Genus *Microgloma* Sanders & Allen, 1973	
Microgloma pusilla (Jeffreys, 1879)	141985
Genus Yoldiella A. E. Verrill & Bush, 1897	
Yoldiella philippiana (Nyst, 1845)	142005
Family Neilonellidae Schileyko, 1989	
Genus *Neilonella* Dall, 1881	
Neilonella latior (Jeffreys, 1876)	600484
Family Nuculanidae H. Adams & A. Adams, 1858 (1854)	
Nuculidae indet.	204
Genus *Ennucula* Iredale, 1931	
Ennucula corbuloides (Seguenza, 1877)	181259
Genus *Nucula* Lamarck, 1799	
Nucula sp	138262
Nucula cf. _nitidosa_	140589
Subfamily Ledellinae Allen & Sanders, 1982	
Genus *Ledella* Verrill & Bush, 1897	
Ledella messanensis (Jeffreys, 1870)	182797

Subfamily Nuculaninae H. Adams & A. Adams, 1858 (1854)	
Genus *Saccella* Woodring, 1925	
Saccella commutata (Philippi, 1844)	236688
Class Caudofoveata C. R. Boettger, 1956	
Caudofoveata sp1	151365
Class Gastropoda Cuvier, 1795	
Subclass Caenogastropoda Cox, 1960	
Caenogastropoda indet.	224570
Caenogastropoda sp1	224570
Order Littorinimorpha Golikov & Starobogatov, 1975	
Superfamily Capuloidea Fleming, 1822	
Family Capulidae Fleming, 1822	
Capulidae sp1	139
Superfamily Rissooidea Gray, 1847	
Family Rissoidae Gray, 1847	
Genus *Pseudosetia* Monterosato, 1884	
***Pseudosetia* sp1**	138454
Genus *Alvania* Risso, 1826	
***Alvania* sp1**	138439
Genus *Onoba* H. Adams & A. Adams, 1852	
***Onoba* sp1**	138451
Superfamily Truncatelloidea Gray, 1840	
Family Iravadiidae Thiele, 1928	
Genus *Hyala* H. Adams & A. Adams, 1852	
Hyala* cf. *vitrea	140129
Superfamily Vanikoroidea Gray, 1840	
Family Eulimidae Philippi, 1853	
Genus *Melanella* Bowdich, 1822	
***Melanella* sp1**	137978
***Melanella* sp2**	137978
Order Neogastropoda Wenz, 1938	
Superfamily Buccinoidea Rafinesque, 1815	
Family Buccinidae Rafinesque, 1815	
Genus *Colus* Röding, 1798	
***Colus* sp1**	137704
Genus *Drilliola* Locard, 1897	
***Drilliola* sp1**	137815
***Drilliola* sp2**	137815
Subclass Heterobranchia Burmeister, 1837	
Heterobranchia sp1	14712
Heterobranchia sp1	14712
Heterobranchia sp2	14712
Heterobranchia sp3	14712
Heterobranchia indet.	14712
Superfamily Pyramidelloidea Gray, 1840	
Family Pyramidellidae Gray, 1840	
Pyramidellidae sp1	162
Pyramidellidae sp2	162
Genus *Turbonilla* Risso, 1826	
***Turbonilla* sp1**	138421
Subclass Vetigastropoda Salvini-Plawen, 1980	
Vetigastropoda sp1	156485
Vetigastropoda sp2	156485
Vetigastropoda sp3	156485
Vetigastropoda sp4	156485

Superfamily Scissurelloidea Gray, 1847	
Family Anatomidae McLean, 1989	
Genus *Anatoma* Woodward, 1859	
***Anatoma* sp1**	138464
Class Scaphopoda Bronn, 1862	
Scaphopoda ind.	104
Phylum Echinodermata	
Subphylum Asterozoa	
Class Asteroidea de Blainville, 1830	
Asteroidea sp1	123080
Class Ophiuroidea Gray, 1840	
Ophiuroidea indet (juv)	123200
Order Ophiurida Müller & Troschel, 1840	
Suborder Ophiurina Müller & Troschel, 1840	
Family Ophiacanthidae Ljungman, 1867	
Ophiacanthidae sp1	123204
Ophiacanthidae sp2	123204
Infraorder Gnathophiurina Matsumoto, 1915	
Family Amphiuridae Ljungman, 1867	
Genus *Amphiura* Forbes, 1843	
Amphiura borealis (Sars G.O., 1871)	125071
Genus *Amphipholis* Ljungman, 1866	
Amphipholis squamata (Delle Chiaje, 1828)	125064
Infraorder Chilophiurina Matsumoto, 1915	
Family Ophiuridae Müller & Troschel, 1840	
Subfamily Ophiurinae Lyman, 1865	
Genus *Ophiura* Lamarck, 1801	
Ophiura* (*Dictenophiura*) *carnea (Lütken, 1858)	125190
Subphylum Echinozoa	
Class Holothuroidea	
Holothuroidea sp1	123083
Holothuroidea sp2	123083
Holothuroidea sp3	123083
Holothuroidea sp4	123083
Class Echinoidea Leske, 1778	
Subclass Euechinoidea Bronn, 1860	
Infraclass Irregularia Latreille, 1825	
Superorder Atelostomata von Zittel, 1879	
Order Spatangoida L. Agassiz, 1840	
Family Brissidae Gray, 1855	
Subfamily Brissopsinae Lambert, 1905	
Genus *Brissopsis* L. Agassiz, 1840	
Brissopsis lyrifera (Forbes, 1841)	124373

This list was compiled in collaboration with several specialists including: Marina R Cunha (Crustacea: Amphipoda, Isopoda, Cumacea, Decapoda), Patricia Esquete (Crustacea: Tanaidacea and Pycnogonida), Mariana Almeida (Crustacea: Euphausiacea and Mysida), Ascenção Ravara (Polychaeta), Luciana Génio (Mollusca: Gastropoda), Clara Rodrigues (Mollusca: Bivalvia; Echinodermata: Ophiuroida).

Annex 4. List of the meiofauna taxa identified within all sampled stations. For consistency, the taxonomic classification indicated is in accordance with the World Register of Marine Species (http://www-marinespecies.org).

Taxonomy		AlphaID
Phylum Annelida		
Class Clitellata		
Subclass Oligochaeta Grube, 1850		
	Oligochaeta ind.	2036
Class Polychaeta Grube, 1850		
	Polychaeta ind.	883
Phylum Arthropoda		
Subphylum Crustacea Brünnich, 1772		
Superclass Multicrustacea Regier, Shultz, Zwick, Hussey, Ball, Wetzer, Martin & Cunningham, 2010		
Class Malacostraca Latreille, 1802		
Subclass Eumalacostraca		
Superorder Peracarida Calman, 1904		
Order Amphipoda Latreille, 1816		
	Amphipoda ind.	1135
Order Cumacea Krøyer, 1846		
	Cumacea ind.	1137
Order Isopoda Latreille, 1817		
	Isopoda ind.	1131
Order Tanaidacea Dana, 1849		
	Tanaidacea ind.	1133
Order Thermosbaenacea Monod, 1927		
Order Trombidiformes	**Thermosbaenacea ind.**	1139
Suborder Prostigmata		
Superfamily Halacaroidea Cunliffe, 1954		
	Halacaroidea ind.	292685
Class Hexanauplia Oakley, Wolfe, Lindgren & Zaharof, 2013		
Subclass Copepoda Milne Edwards, 1840		
Superorder Podoplea Giesbrecht, 1882		
Order Harpacticoida Sars		
	Harpacticoida ind.	1102
Class Ostracoda Latreille, 1802		
	Ostracoda ind.	1078
Phylum Cephalorhyncha		
Class Locidera Kristensen, 1983		
	Locifera ind.	101061
Class Priapulida Théel, 1906		
	Priapulida ind.	101063
Class Kinorhyncha		
	Kinorhyncha ind.	101060
Phylum Echinodermata		
Subphylum Echinozoa		
Class Holothuroidea		
	Holothuroidea ind.	123083
Class Ophiudoidea Gray, 1840		
	Ophiuroidea ind.	123084
Phylum Gastrotricha Metschnikoff, 1865		
	Gastrotricha ind.	2078
Phylum Gnathostomulida Riedl, 1969		
	Gnathostomulida ind.	114710

Phylum Mollusca	
Class Bivalvia Linnaeus, 1758	
Bivalvia Ind.	105
Class Caudofoveata C. R. Boettger, 1956	
Caudofoveata ind.	151365
Class Gastropoda Cuvier, 1795	
Gastropoda ind.	101
Phylum Nematoda	
Class Enoplea	
Subclass Enoplia	
Order Enoplida Filipjev, 1929	
Superfamily Enoploidea Dujardin, 1845	
Family Anoplostomatidae Gerlach & Riemann, 1974	
Genus *Anoplostoma* Buetschli, 1874	
***Anoplostoma* ind.**	2498
Family Anticomidae Filipjev, 1918	
Genus *Anticoma* Bastian, 1865	
***Anticoma* ind.**	2500
Genus *Odontanticoma* Platonova, 1976	
***Odontanticoma* Platonova, 1976**	160852
Genus *Crenopharynx* Filipjev, 1934	
***Crenopharynx* ind.**	2505
Genus *Phanodermopsis* Ditlevsen, 1926	
***Phanodermopsis* ind.**	2509
Family Thoracostomopsidae Filipjev, 1927	
Genus *Enoploides* Saveljev, 1912	
***Enoploides* ind.**	2512
Genus *Epacanthion* Wieser, 1953	
***Epacanthion* ind.**	2514
Genus *Mesacanthion* Filipjev, 1927	
***Mesacanthion* ind.**	2517
Superfamily Ironoidea de Man, 1876	
Family Ironidae de Man, 1876	
Genus *Syringolaimus* de Man, 1888	
***Syringolaimus* ind.**	2526
Family Oxystominidae Chitwood, 1935	
Genus *Cricohalalaimus* Bussau	
***Cricohalalaimus* ind.**	582898
Genus *Halalaimus* de Man, 1888	
***Halalaimus* ind.**	2548
Genus *Litinium* Cobb, 1920	
***Litinium* ind.**	2549
Genus *Oxystomina* Filipjev, 1918	
***Oxystomina* ind.**	2551
Genus *Thalassoalaimus* de Man, 1893	
***Thalassoalaimus* ind.**	2552
Genus Wieseria Gerlach, 1956	
Superfamily Oncholaimoidea Filipjev, 1916	
Family Enchelidiidae Filipjev, 1918	
Genus *Bathyeurystomina* Lambshead & Platt, 1979	
***Bathyeurystomina* ind.**	227167
Genus *Calyptronema* Marion, 1870	
***Calyptronema* ind.**	2557
Genus *Eurystomina* Filipjev, 1921	
***Eurystomina* ind.**	2559

Family Oncholaimidae Filipjev, 1916	
Genus *Filoncholaimus* Filipjev, 1927	
***Filoncholaimus* ind.**	2580
Genus *Viscosia* de Man, 1890	
***Viscosia* ind.**	2570
Superfamily Trefusioidea Gerlach, 1966	
Family Lauratonematidae Gerlach, 1953	
Genus *Lauratonema* Gerlach, 1953	
***Lauratonema* ind.**	2589
Family Trefusiidae Gerlach, 1966	
Genus *Halanonchus* Cobb, 1920	
***Halanonchus* ind.**	2591
Genus *Rhabdocoma* Cobb, 1920	
***Rhabdocoma* ind.**	2592
Genus *Trefusia* de Man, 1893	
***Trefusia* ind.**	2593
Family Tripyloididae Filipjev, 1918	
Genus *Bathylaimus* Cobb, 1894	
***Bathylaimus* ind.**	2586
Order Triplonchida Cobb, 1919	
Superfamily Tobriloidea Filipjev, 1918	
Family Pandolaimidae Belogurov, 1980	
Genus *Pandolaimus* Allgén, 1929	
***Pandolaimus* ind.**	2582
Class Chromadorea	
Subclass Chromadoria	
Order Araeolaimida De Coninck & Schuurmans Stekhoven, 1933	
Superfamily Axonolaimoidea Filipjev, 1918	
Family Axonolaimidae Filipjev, 1918	
Genus *Odontophora* Bütschli, 1874	
***Odontophora* ind.**	2418
Family Bodonematidae Jensen, 1991	
Genus *Bodonema* Jensen, 1991	
***Bodonema* ind.**	227497
Family Comesomatidae Filipjev, 1918	
Comesomatidae ind.	2185
Genus *Cervonema* Wieser, 1954	
***Cervonema* ind.**	2430
Genus *Dorylaimopsis* Ditlevsen, 1918	
***Dorylaimopsis* ind.**	2428
Genus *Laimella* Cobb, 1920	
***Laimella* ind.**	2431
Genus *Metasabatieria* Timm, 1961	
***Metasabatieria* ind.**	227198
Genus *Pierrickia* Vitiello, 1970	
***Pierrickia* ind.**	2432
Genus *Sabatieria* Rouville, 1903	
***Sabatieria* ind.**	2433
Genus *Setosabatieria* Platt, 1985	
***Setosabatieria* ind.**	2434
Family Coninckiidae Lorenzen, 1981	
Genus *Coninckia* Gerlach, 1956	
***Coninckia* ind.**	2435
Family Diplopeltidae Filipjev, 1918	
Genus *Campylaimus* Cobb, 1920	
***Campylaimus* ind.**	2437
Genus *Diplopeltula* Gerlach, 1950	
***Diplopeltula* ind.**	2439

Genus *Pararaeolaimus* Timm, 1961	
Pararaeolaimus **ind.**	2442
Genus *Pseudaraeolaimus* Chitwood, 1951	
Pseudaraeolaimus **ind.**	227199
Genus *Southerniella* Allgén, 1932	
Southerniella **ind.**	2443
Order Chromadorida Chitwood, 1933	
Suborder Chromadorina Filipjev, 1929	
Superfamily Chromadoroidea Filipjev, 1917	
Family Chromadoridae Filipjev, 1917	
Chromadoridae ind.	2162
Genus *Acantholaimus* Allgén, 1933	
Acantholaimus **ind.**	2303
Genus *Actinonema* Cobb, 1920	
Actinonema **ind.**	2283
Genus *Chromadora* Bastian, 1865	
Chromadora **ind.**	2277
Genus *Chromadorita* Filipjev, 1922	
Chromadorita **ind.**	2294
Genus *Dichromadora* Kreis, 1929	
Dichromadora **ind.**	2297
Genus *Endeolophos* Boucher, 1976	
Endeolophos **ind.**	227169
Genus *Hypodontolaimus* de Man, 1886	
Hypodontolaimus **ind.**	2298
Genus *Innocuonema* Inglis, 1969	
Innocuonema **ind.**	2299
Genus *Ptycholaimellus* Cobb, 1920	
Ptycholaimellus **ind.**	2301
Genus *Spilophorella* Filipjev, 1917	
Spilophorella **ind.**	2302
Family Cyatholaimidae Filipjev, 1918	
Cyatholaimidae ind.	2163
Genus *Longicyatholaimus* Micoletzky, 1924	
Longicyatholaimus **ind.**	2309
Genus *Marylynnia* (Hopper, 1972) Hopper, 1977	
Marylynnia **ind.**	834500
Genus *Metacyatholaimus* Stekhoven, 1942	
Metacyatholaimus **ind.**	2311
Genus *Nannolaimoides* Ott, 1972	
Nannolaimoides **ind.**	2316
Genus *Paracyatholaimus* Micoletzky, 1922	
Paracyatholaimus **ind.**	2322
Genus *Paralongicyatholaimus* Schuurmans Stekhoven, 1950	
Paralongicyatholaimus **ind.**	2312
Genus *Pomponema* Cobb, 1917	
Pomponema **ind.**	2318
Family Neotonchidae Wieser & Hopper, 1966	
Genus *Nannolaimus* Cobb, 1920	
Nannolaimus **ind.**	2317
Genus *Neotonchus* Cobb, 1933	
Neotonchus **ind.**	2325
Family Selachinematidae Cobb, 1915	
Genus *Cheironchus* Cobb, 1917	
Cheironchus **ind.**	2326
Genus *Choanolaimus* de Man, 1880	
Choanolaimus **ind.**	2327

Genus *Gammanema* Cobb, 1920	
***Gammanema* ind.**	2331
Genus *Halichoanolaimus* de Man, 1886	
***Halichoanolaimus* ind.**	2332
Genus *Latronema* Wieser, 1954	
***Latronema* ind.**	2333
Genus *Richtersia* Steiner, 1916	
***Richtersia* ind.**	2334
Genus *Synonchiella* Cobb, 1933	
***Synonchiella* ind.**	2335
Order Desmodorida De Coninck, 1965	
Superfamily Desmodoroidea Filipjev, 1922	
Family Desmodoridae Filipjev, 1922	
Genus *Desmodora* de Man, 1889	
***Desmodora* ind.**	2339
Genus *Desmodorella* Cobb, 1933	
***Desmodorella* ind.**	853823
Genus *Molgolaimus* Ditlevsen, 1921	
***Molgolaimus* ind.**	2343
Genus *Paradesmodora* Schuurmans Stekhoven, 1950	
***Paradesmodora* Schuurmans ind.**	2341
Genus *Parallelocoilas* Boucher, 1975	
***Parallelocoilas* ind.**	153342
Genus *Spirinia* Gerlach, 1963	
***Spirinia* ind.**	2350
Superfamily Microlaimoidea Micoletzky, 1922	
Family Microlaimidae Micoletzky, 1922	
Microlaimidae ind.	2171
Genus *Bathynox* Bussau & Vopel, 1999	
***Bathynox* ind.**	227430
Genus *Bolbolaimus* Cobb, 1920	
***Bolbolaimus* ind.**	153204
Genus *Calomicrolaimus* Lorenzen, 1976	
***Calomicrolaimus* ind.**	153207
Genus *Microlaimus* de Man, 1880	
***Microlaimus* ind.**	2366
Genus *Spirobolbolaimus* Soetaert & Vincx, 1988	
***Spirobolbolaimus* ind.**	227177
Order Desmoscolecida Filipjev, 1929	
Suborder Desmoscolecina Filipjev, 1934	
Superfamily Desmoscolecoidea Shipley, 1896	
Family Cyartonematidae Tchesunov, 1990	
Genus *Cyartonema* Cobb, 1920	
***Cyartonema* ind.**	2382
Family Desmoscolecidae Shipley, 1896	
Genus *Desmoscolex* Claparède, 1863	
***Desmoscolex* ind.**	2369
Genus Greeffiella Cobb, 1922	
***Greeffiella* ind.**	2372
Genus *Tricoma* Cobb, 1894	
***Tricoma* ind.**	2379
Family Meyliidae De Coninck, 1965	
Genus *Meylia* Gerlach, 1956	
***Meylia* ind.**	2375

Order Monhysterida Filipjev, 1929	
Suborder Linhomoeina Andrássy, 1974	
Superfamily Siphonolaimoidea Filipjev, 1918	
Family Linhomoeidae Filipjev, 1922	
Linhomoeidae ind.	2191
Genus *Desmolaimus* de Man, 1880	
***Desmolaimus* ind.**	2474
Genus *Didelta* Cobb, 1920	
***Didelta* ind.**	2487
Genus *Disconema* Filipjev, 1918	
***Disconema* ind.**	2488
Genus *Eleutherolaimus* Filipjev, 1922	
***Eleutherolaimus* ind.**	2480
Genus *Linhomoeus* Bastian, 1865	
***Linhomoeus* ind.**	2490
Genus *Metalinhomoeus* de Man, 1907	
***Metalinhomoeus* ind.**	2476
Genus *Terschellingia* de Man, 1888	
***Terschellingia* ind.**	2479
Family Siphonolaimidae Filipjev, 1918	
Genus *Parastomonema* Kito, 1989	
***Parastomonema* ind.**	227191
Genus *Siphonolaimus* de Man, 1893	
***Siphonolaimus* ind.**	2495
Suborder Monhysterina De Coninck & Schuurmans Stekhoven, 1933	
Superfamily Monhysteroidea Filipjev, 1929	
Family Monhysteridae de Man, 1876	
Monhysteridae ind.	2188
Genus *Monhystrella* Cobb, 1918	
***Monhystrella* ind.**	153352
Genus *Thalassomonhystera* Jacobs, 1987	
***Thalassomonhystera* ind.**	2448
Superfamily Sphaerolaimoidea Filipjev, 1918	
Family Sphaerolaimidae Filipjev, 1918	
Genus *Doliolaimus* Lorenzen, 1966	
***Doliolaimus* ind.**	2450
Genus *Metasphaerolaimus* Gourbault & Boucher, 1981	
***Metasphaerolaimus* ind.**	156847
Genus *Sphaerolaimus* Bastian, 1865	
***Sphaerolaimus* ind.**	2451
Family Xyalidae Chitwood, 1951	
Genus *Ammotheristus* Lorenzen, 1977	
***Amnotheristus* ind.**	153198
Genus *Amphimonhystera* Allgén, 1929	
***Amphimonhystera* ind.**	2452
Genus *Amphimonhystrella* Timm, 1961	
***Amphimonhystrella* ind.**	156741
Genus *Capsula* Bussau, 1993	
***Capsula* ind.**	582876
Genus *Cobbia* de Man, 1907	
***Cobbia* ind.**	2454
Genus *Daptonema* Cobb, 1920	
***Daptonema* ind.**	2455
Genus *Elzalia* Gerlach, 1957	
***Elzalia* ind.**	2457
Genus *Enchonema* Bussau, 1993	
***Enchonema* ind.**	582926
Genus *Manganonema* Bussau, 1993	
***Manganonema* ind.**	233963

Genus *Metadesmolaimus* Schuurmans Stekhoven, 1935	
***Metadesmolaimus* ind.**	2461
Genus *Paramonohystera* Steiner, 1916	
***Paramonohystera* ind.**	2463
Genus *Rhynchonema* Cobb, 1920	
***Rhynchonema* ind.**	2465
Genus *Scaptrella* Cobb, 1917	
***Scaptrella* ind.**	2466
Genus *Sphaerotheristus* Timm, 1968	
***Sphaerotheristus* ind.**	227302
Genus *Theristus* Bastian, 1865	
***Theristus* ind.**	2469
Genus *Trichotheristus* Wieser, 1956	
***Trichotheristus* ind.**	2470
Order Plectida Gadea, 1973	
Suborder Desmoscolecina Filipjev, 1934	
Superfamily Ceramonematoidea Cobb, 1933	
Family Ceramonematidae Cobb, 1933	
Genus *Ceramonema* Cobb, 1920	
***Ceramonema* ind.**	2385
Genus *Metadasynemella* De Coninck, 1942	
***Metadasynemella* ind.**	2388
Genus *Metadasynemoides* Haspeslagh, 1973	
***Metadasynemoides* ind.**	153217
Genus *Pselionema* Cobb, 1933	
***Pselionema* ind.**	2389
Genus *Pterygonema* Gerlach, 1953	
***Pterygonema* ind.**	2390
Family Diplopeltoididae Tchesunov, 1990	
Genus *Diplopeltoides* Gerlach, 1962	
***Diplopeltoides* ind.**	2383
Family Paramicrolaimidae Lorenzen, 1981	
Genus *Paramicrolaimus* Wieser, 1954	
***Paramicrolaimus* ind.**	2409
Family Tarvaiidae Lorenzen, 1981	
Genus *Tarvaia* Allgén, 1934	
***Tarvaia* ind.**	2412
Family Tubolaimoididae Lorenzen, 1981	
Genus *Chitwoodia* Gerlach, 1956	
***Chitwoodia* ind.**	2413
Genus *Tubolaimoides* Gerlach, 1963	
***Tubolaimoides* ind.**	2414
Suborder Plectina Malakhov, Ryzhikov & Sonin, 1982	
Superfamily Camacolaimoidea Micoletzky, 1924	
Family Camacolaimidae Micoletzky, 1924	
Genus *Alaimella* Cobb, 1920	
***Alaimella* ind.**	2399
Genus *Procamacolaimus* Gerlach, 1954	
***Procamacolaimus* ind.**	2398
Genus *Stephanolaimus* Ditlevsen, 1918	
***Stephanolaimus* ind.**	2408
Superfamily Leptolaimoidea Örley, 1880	
Family Leptolaimidae Örley, 1880	
Genus *Antomicron* Cobb, 1920	
***Antomicron* ind.**	2400
Genus *Leptolaimus* de Man, 1876	
***Leptolaimus* ind.**	2407

Suborder Plectida incertae sedis		
Family Aegialoalaimidae Lorenzen, 1981		
Genus *Aegialoalaimus* de Man, 1907		
	***Aegialoalaimus* ind.**	2381
Phylum Platyhelminthes Minot, 1876		
	Platyhelminthes ind.	793
Phylum Rotifera		
	Rotifera ind.	14260
Phylum Sipuncula		
	Sipuncula ind.	1268
Phylum Tardigrada Doyère, 1840		
	Tardigrada ind.	1276

This list was compiled in collaboration with Lidia Lins (Nematoda).