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Impact of environmental variability on the zoobenthos size structure in North Atlantic and Arctic coastal waters

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PhD Thesis

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Wpływ zmienności warunków środowiskowych na strukturę wielkościową zoobentosu w północnoatlantyckich i arktycznych wodach przybrzeżnych

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Content

Abstract

Body size is a fundamental biological feature that is closely coupled to key biological processes like metabolism or longevity. From the perspective of multispecies assemblages the size of component organisms may also have various implications for these assemblages functioning. Abundance of organisms, competition for space or resources and rate of resources utilisation or trophic interactions are all related to the community size structure. Various environmental drivers may influence the size of organisms and since many of these drivers may interact with each other, complex relationships between the environmental variability and size are possible. Among all environmental factors that can shape the organism size, few are regarded as fundamental: temperature, oxygen availability and food quality and quantity. The relationship between body size and temperature has been extensively studied leading to constitution of ecological rules such as Bergmann’s rule or temperature-size rule, which in general assume that organisms grow larger at lower temperatures. Hence, in the era of climate warming, particular attention should be paid to those temperature-related rules.

Widely used method to investigate the community size structure is to construct the size spectrum that presents the distribution of individuals or biomass among individual size classes (abundance or biomass size spectra, respectively). Size spectra may be powerful indicators of primary production, disturbances and/or stability in the ecosystem, impacts of fisheries or consequences of climate warming. They are also perceived as better indicators of community functioning, especially in terms of trophic interactions and energy flow, than taxonomic composition, diversity or abundance. The term “body size” may in fact refer to various organismal characteristics, such as biovolume, biomass, whole body length, length of body part or even organism age. In this dissertation the term “body size” relates to the dry body mass.

The main aim of this thesis was to determine changes in the size structure of benthic communities in response to temporal (seasonal) and spatial environmental variability in the North Atlantic and Arctic coastal waters (fjords). The objectives of the thesis were: 1) to develop a method of semi-automated measurements and biomass estimation of nematodes; 2) to assess the seasonal (winter versus summer) changes in benthic biomass size spectra in the Arctic fjordic system; 3) to describe patterns of variability in benthic biomass size spectra in response to changes in water temperature and food availability across latitudinal gradient (60-81°N).

First part of this dissertation is focused on the development of a semi-automated method of Nematoda measurements and their biomass estimation. Nematodes are the dominant component of the meiofauna. These invertebrates are an ecologically important group of organisms due to their crucial role in detritus decomposition and nutrient cycling. However, meiofaunal biomass is rarely assessed in marine ecological studies due to methodological constraints: direct methods are inaccurate while indirect procedures are time-consuming and costly. In this dissertation I presented a semi-automated image analysis method for obtaining the widths and lengths of photographed nematodes and estimating Nematoda biomass. Samples of 100 individual nematodes from 10 replicate samples representing two different environments (Artic fjord muddy bottom and Baltic seagrass-vegetated sands) were used for the analyses, which were performed manually and semi-automatically. Manual measurements were taken by three independent analysts to check for individual differences. The automated measurements were performed almost two times faster than the manual ones. The three analysts and the software did not differ in their assessments of nematode length, but significant differences existed among the measurements of maximum width by the three analysts. Those differences arose mainly from different designations of the point of nematode maximum width (required by the manual method) by each analyst. Three different formulas were tested to estimate nematode biomass and a formula using the equation for the volume of cylinder was recommended. The semi-automated method described in this dissertation seems to be less biased and more efficient and cost saving compared with standard manual protocols. The wider application of this method should facilitate more frequent inclusion of meiofaunal biomass assessments in marine benthic surveys.

Second part of the dissertation aimed to document seasonal (summer – winter) differences in the size structure of benthic community in the Arctic fjord. The Arctic system is characterized by strong seasonal variability in environmental conditions and in the pelagic primary production and organic matter fluxes to the sea bottom. Moreover, biotic processes such as recruitment and predation pressure vary seasonally and may have influence on the size structure of benthic fauna. However, most of the studies concerning Arctic biota as well as publications about size structure are based on materials collected in one season only (mostly in summer). Therefore there is a need to recognize how strong Arctic environmental seasonality influences the size structure of benthic organisms and whether the patterns observed in summer are representative for the whole year. In this part of the dissertation I compared summer and winter biomass size spectra and environmental features in the central basin of Kongsfjorden (west Spitsbergen). Evident seasonal differences in water salinity and temperature were observed. Moreover, seasonal changes in the quality of organic matter available to the benthos (indicated by higher chloroplastic pigments concentrations in surface sediments in summer) were noted. Also total abundance and biomass of both macrofauna and meiofauna varied between summer and winter. Despite all those differences, no significant seasonal differences in the size structure were documented by multiple regression models for the normalised biomass. The slope of a linear relationship between normalised biomass and size classes was -0.54±0.02 indicating a productive system, comparable e.g. to estuarine ecosystems. There were seasonal differences in size of dominant taxa but they were inconsistent across the studied taxa and were not translated into a common trend visible at the community level. Summer-winter invariability of size spectra suggests that benthic community functioning in this Arctic system is relatively independent from the seasonality in the supply of organic matter produced in water column. This supports the hypothesis of the existence of a “food bank” in polar sediments.

The third part of the dissertation explores the influence of environmental factors on the spatial variability in size structure of benthic fauna across a wide geographic range. Since global warming is expected to cause reductions in organism body size, possible effects of increasing temperature on biomass size spectra in coastal benthic communities were investigated employing the “space for time analogue” approach. Meiofauna, macrofaunal nematodes and macrofauna samples were collected in the six Norwegian and Svalbard fjords, spanning wide latitudinal range (60-81°N) and bottom water temperature gradients (from -1.6 to 7.7°C). Investigated fjords differed in terms of environmental settings (water temperature, chloroplastic pigments and particulate organic carbon content, $\delta^{13}C$ and grain size composition in sediments). The slopes of normalised biomass size spectra (NBSS) did not differ among the fjords, while the benthic biomass and NBSS intercepts varied and were related to chlorophyll *a* content and $\delta^{13}C$ values in sediments. Both abundance and biomass size spectra remained consistent across the studied locations, regardless of the strong variability in macrofauna taxonomic and functional trait (feeding type) composition. Variable relationships (positive, negative and neutral) between temperature and body size were noted for particular species, genera and families. Results of this part of the thesis indicate that while benthic biomass depends on the nutritional quality of organic matter, its partitioning among size classes is consistent and independent of environmental (temperature, grain size and organic matter) and biological (species and functional trait composition) variability. The observed size structure remains a persistent feature of studied soft-bottom benthic communities and may be resilient to major climatic changes.

The main conclusions of the thesis are: 1) the calculations of nematodes biomass based on semi-automated measurements and with use of formula for the volume of cylinder provide comparable results to those obtained with traditional manual procedures and are less biased and time consuming; 2) the size structure of soft-bottom benthic communities in the Arctic fjord does not differ between summer and winter despite seasonal changes in organic matter supply, what supports the hypothesis of a "food bank" existence in polar sediments; 3) size structure of benthic fauna of undisturbed sediments across a wide geographic range remains constant despite the natural variability in both environmental and ecological characteristics of the benthic systems; 4) responses in organism size to environmental seasonal and spatial variability vary across taxa, these patterns detected for individual taxa do not translate into changes detectable at the community level.

Results of this thesis indicate high constancy of benthic community size structure regardless of environmental (spatial and temporal) variability. The positive relationship between quality and quantity of organic matter and standing stock of benthic fauna was documented. It is predicted that climate change in the Arctic will induce changes in the primary production. Therefore the functioning of benthic systems may be affected by the altered total standing stocks but most probably not by the changes in the distribution of the stocks among different sizes classes of animals.

Streszczenie

Wielkość jest fundamentalną cechą organizmu, ściśle związaną z kluczowymi procesami biologicznymi, takimi jak metabolizm, tempo wzrostu czy długość życia. W przypadku zbiorowisk wielogatunkowych struktura wielkościowa tworzących je organizmów może decydować o sposobie funkcjonowania tych zgrupowań. Zagęszczenie osobników, współzawodnictwo o przestrzeń lub zasoby (np. pokarmowe), tempo wykorzystywania zasobów pokarmowych, zależności troficzne pomiędzy organizmami są zależne od struktury wielkościowej danego zbiorowiska organizmów. Wiele czynników środowiskowych może wpływać na wielkość organizmów, ponadto czynniki te mogą współoddziaływać ze sobą. Spośród czynników środowiskowych, wpływających na wielkość organizmów, kluczowe znaczenie mają temperatura, dostępność tlenu oraz ilość i jakość pożywienia. Zależności pomiędzy temperaturą a wielkością ciała były wielokrotnie studiowane, co pozwoliło na ustanowienie szeregu reguł ekologicznych takich jak reguła Bergmanna, która zakłada, że w niskich temperaturach organizmy osiągają większe rozmiary. W czasach postępujących zmian klimatu badania zależności procesów biologicznych od temperatury nabierają szczególnego znaczenia.

Powszechnie stosowaną metodą analizy struktury wielkościowej zbiorowiska jest przedstawianie rozkładów zagęszczenia osobników lub całkowitej biomasy w wyznaczonych klasach wielkości (odpowiednio rozkład liczebności i rozkład biomasy w klasach wielkości). Potwierdzono, że rozkłady wielkości organizmów mogą służyć jako wskaźniki wielkości produkcji pierwotnej w ekosystemie, obecności/braku zaburzeń, wpływu rybołówstwa lub konsekwencji zmian klimatu. Ponadto są postrzegane jako wskaźniki dokładniej obrazujące funkcjonowanie danego zbiorowiska organizmów, zwłaszcza w kontekście relacji troficznych i przepływu energii, niż tradycyjnie analizowane własności zbiorowisk takie jak skład taksonomiczny, różnorodność biologiczna czy zagęszczenie organizmów. Należy jednak podkreślić, że termin „wielkość organizmu” w zależności od rodzaju badań i studiowanych organizmów może odnosić się do różnych własności morfologicznych. Powszechnie stosowane są takie miary jak: bioobjętość, biomasa, długość całego organizmu lub konkretnego elementu anatomicznego. W tej pracy termin „wielkość organizmu” jest stosowany w znaczeniu suchej masy organizmu.

Celem pracy było określenie zmian w strukturze wielkościowej zbiorowisk bentosowych w odpowiedzi na czasowe (sezonowe) i przestrzenne zmiany środowiskowe w wodach przybrzeżnych (fiordach) północnego Atlantyku i Arktyki. Realizacji celu posłużyło wyznaczenie trzech zadań badawczych zakładających: 1) opracowanie metody określania biomasy nicieni w oparciu o półautomatyczne pomiary; 2) określenie zmian sezonowych (pomiędzy latem a zimą) w strukturze wielkościowej fauny bentosowej arktycznego fiordu; 3) określenie wzorców zmienności struktury wielkościowej fauny bentosowej w odpowiedzi na różnice w temperaturze i dostępności pokarmu w fiordach północnoatlantyckich i arktycznych (w zakresie szerokości geograficznej 60-81°N).

Pierwsza część rozprawy dotyczy opracowania metody pozwalającej na półautomatyczne pomiary nicieni oraz szacowanie ich biomasy. Nicienie są dominującym składnikiem meiofauny, czyli organizmów bentosowych zamieszkujących przestrzenie interstycjalne pomiędzy ziarnami osadu, pełniących kluczową rolę w dekompozycji detrytusu i obiegu pierwiastków w osadach. Pomimo ekologicznego znaczenia meiofauny, badania tej grupy rzadko obejmują określenie jej biomasy. Główną tego przyczyną są ograniczenia metodologiczne; niedokładność metod bezpośrednich określania masy (ważenia) tak małych organizmów, wysoka czasochłonność i kosztochłonność metod pośrednich. W tej części przedstawiam metodę półautomatycznej analizy obrazu pozwalającą na wyznaczanie szerokości i długości nicieni sfotografowanych pod mikroskopem z wykorzystaniem oprogramowania Leica LAS Image Analysis. Uzyskane wyniki są następnie wykorzystywane do szacowania biomasy zmierzonych organizmów. Do analiz wykorzystano po 100 nicieni pochodzących z 10 próbek reprezentujących dwa różne środowiska: muliste dno z fiordu oraz piaski pokryte roślinnością. Pomiary zostały wykonane przy wykorzystaniu procedury półautomatycznej a także ręcznie. Pomiary manualne były przeprowadzone przez trzech różnych laborantów w celu sprawdzenia różnic osobowych. Pomiary w wykorzystaniem procedury półautomatycznej były prawie dwukrotnie szybsze niż pomiary ręczne. Nie zanotowano istotnych różnic w pomiarach długości wykonanych obiema metodami, jednak wykazano istotne różnice pomiędzy pomiarami maksymalnej szerokości nicienia dokonanymi przez różnych laborantów (pomiary półautomatyczne nie dotyczyły pomiarów maksymalnej szerokości). Różnice te wynikały przede wszystkim z różnego wyznaczania miejsca o największej szerokości nicienia (wymaganego przy metodzie manualnej) przez każdego

z laborantów. Przetestowano trzy różne wzory matematyczne do szacowania biomasy organizmów i na podstawie uzyskanych wyników zarekomendowano wykorzystywanie wzoru dla objętości walca. Półautomatyczna metoda opisana w tej rozprawie jest w mniejszym stopniu obarczona błędem ludzkim, szybsza, bardziej wydajna i mniej kosztowna w porównaniu z powszechnie wykorzystywanymi metodami wymagającymi pomiarów dokonywanych ręcznie. Szersze wykorzystanie przedstawionej metody może pozwolić na częstsze szacowanie biomasy meiofauny w badaniach dotyczących fauny bentosowej.

Celem drugiej części rozprawy było udokumentowanie różnic sezonowych (pomiędzy latem i zimą) w strukturze wielkościowej fauny bentosowej arktycznego fiordu. Ekosystem Arktyki charakteryzuje się silną sezonową zmiennością warunków środowiskowych, produkcji pierwotnej w toni wodnej oraz dostawy materii organicznej do dna morza. Ponadto, procesy biologiczne, takie jak: rozród czy presja ze strony drapieżników, również charakteryzują się zmiennością sezonową a także mogą wpływać na strukturę wielkościową fauny dennej. Jednakże, większość badań dotyczących funkcjonowania ekosystemu Arktyki, jak i większość badań dotyczących struktury wielkościowej bazuje na materiałach pobranych w jednym sezonie, zwykle letnim. Zachodzi więc potrzeba rozpoznania w jaki sposób silna zmienność sezonowa warunków środowiskowych wpływa na strukturę wielkościową arktycznej fauny bentosowej a także czy wzorce zmienności struktury obserwowane w sezonie letnim są reprezentatywne dla całego roku. W tym celu w fiordzie Kongsfjorden leżącym na zachodnim wybrzeżu Spitsbergenu, porównano strukturę wielkościową fauny bentosowej oraz parametry środowiskowe w sezonie letnim i zimowym Zaobserwowano wyraźne różnice sezonowe w wartościach zasolenia i temperatury wody oraz istotne różnice w jakości materii organicznej dostępnej dla fauny bentosowej w dwóch badanych sezonach, na co wskazywały wyższe koncentracje barwników fotosyntetycznych w osadach w sezonie letnim. Ponadto liczebność i całkowita biomasa makrofauny i meiofauny różniły się pomiędzy latem i zimą. Pomimo powyższych różnic, nie zaobserwowano znaczących zmian sezonowych w strukturze wielkościowej fauny dennej, co zostało potwierdzone poprzez analizę liniowej regresji wielorakiej dla znormalizowanej biomasy w poszczególnych klasach wielkości. Współczynnik nachylenia krzywej dla regresji liniowej w przypadku zależności pomiędzy znormalizowaną biomasą a klasami wielkości organizmów wyniósł -0.54±0.02 i wartość ta wskazuje na wysoką produktywność ekosystemu porównywalną np. z ekosystemami estuariowymi.

Przeanalizowano również zmiany sezonowe w wielkości osobników dominujących taksonów. Stwierdzono, że różne taksony odmiennie reagują na zmienność warunków środowiska (jedne miały mniejszą masę latem, inne zimą) a te indywidualne zmiany nie przekładały się na zmianę struktury wielkościowej całego zbiorowiska. Brak zmienności struktury wielkościowej fauny dennej Kongsfjorden pomiędzy latem a zimą wskazuje na to, iż zbiorowiska bentosowe w Arktyce funkcjonują niezależnie od sezonowych zmian w dostawie materii organicznej produkowanej w toni wodnej. Otrzymane wyniki potwierdzają hipotezę o obecności „banku zasobów pokarmowych" (ang. „food bank") w osadach rejonów polarnych.

Trzecia część rozprawy zawiera wyniki badań nad wpływem czynników środowiskowych na strukturę wielkościową fauny dennej w fiordach położonych pomiędzy 60°N do 81° stopniem szerokości geograficznej północnej. Skutki zmian klimatu i globalnego ocieplenia w najsilniejszy sposób dotykają rejonów polarnych. Najnowsze badania przewidują, że postępujący wzrost temperatury globu może skutkować zmniejszaniem się wielkości organizmów. W celu zbadania możliwych konsekwencji wpływu globalnego ocieplenia na strukturę wielkościową zbiorowisk bentosowych, wykorzystano podejście „analogii przestrzeni do czasu" i przyjęto założenie, iż fauna bentosowa zasiedlająca cieplejsze wody wybrzeża Norwegii może stanowić odpowiednik przyszłej (po ociepleniu) fauny przybrzeżnej Arktyki. Próbki fauny dennej: meiofauny, nicieni makrofaunowych i makrofauny pobrano na obszarze pomiędzy 60°N i 81°N w sześciu fiordach Norwegii kontynentalnej oraz Svalbardu, reprezentujących gradient temperatury wody przydennej od -1.6°C do 7.7°C. Wykazano, że badane fiordy różniły się pod względem warunków środowiskowych (takich jak: temperatura wody, zawartość barwników fotosyntetycznych i węgla organicznego, skład izotopów węgla $\delta^{13}C$ w osadach oraz uziarnienie osadów). Współczynniki nachylenia krzywej regresji liniowej dla znormalizowanych rozkładów biomasy nie różniły się istotnie pomiędzy badanymi fiordami. Różnice w całkowitej biomasie fauny bentosowej a także wartości wyrazu wolnego równań regresji były powiązane ze zmiennością zawartości chlorofilu *a* oraz składu izotopów węgla $\delta^{13}C$ w osadach. Rozkłady liczebności oraz biomasy w klasach wielkości był podobne w badanych fiordach niezależnie od silnej zmienności składu taksonomicznego makrofauny oraz składu cech funkcjonalnych. Odnotowano także zróżnicowane zależności (dodatnie, ujemne lub neutralne) pomiędzy temperaturą a wielkością przedstawicieli wybranych gatunków, rodzajów i rodzin makrofauny. Uzyskane wyniki wskazują, że o ile całkowita biomasa fauny dennej jest silnie związana z jakością materii organicznej w osadach, to rozkład biomasy pomiędzy poszczególnymi klasami wielkości jest niezmienny i niezależny od zmienności warunków środowiskowych (temperatury, uziarnienia osadów, ilości materii organicznej) oraz od wskaźników biologicznych (skład taksonomiczny i struktura funkcjonalna). Zaobserwowana struktura wielkościowa fauny jest niezmienną cechą zbiorowisk bentosu dna miękkiego i może być źródłem odporności tych zbiorowisk na zmienność środowiskową związaną z ocieplaniem klimatu.

Główne wnioski płynące z tej rozprawy doktorskiej to: 1) procedura szacowaniu biomasy nicieni z wykorzystaniem równania na objętość walca na podstawie pomiarów dokonywanych metodą półautomatyczną zapewnia wyniki porównywalne do tych uzyskiwanych przy pomocy tradycyjnych procedur i pomiarów manualnych, a jednocześnie jest obarczona mniejszą podatnością na błąd ludzki i mniej czasochłonna; 2) struktura wielkościowa zbiorowisk fauny bentosowej dna miękkiego zamieszkujących arktyczny fiord nie podlega zmienności sezonowej (lato-zima) pomimo silnej sezonowości w dostawie materii organicznej, co potwierdza hipotezę o występowaniu „banku zapasów pokarmowych” w osadach obszarów polarnych; 3) struktura wielkościowa fauny bentosowej niezaburzonych osadów miękkich w szerokim zakresie szerokości geograficznych pozostaje niezmienna pomimo zmienności warunków środowiskowych (temperatura, dostępność pokarmu) jak i charakterystyk ekologicznych (całkowita biomasa, skład gatunkowy, struktury funkcjonalnej); 4) zależności między wielkością wybranych organizmów a sezonową i przestrzenną zmiennością warunków środowiskowych różnią się pomiędzy taksonami, co jednak nie przekłada się na zmienność struktury wielkościowej całych zbiorowisk.

Podsumowując, wyniki otrzymane w ramach tej rozprawy dokumentują niezmienność struktury wielkościowej zbiorowisk bentosowych niezależnie od zmienności środowiskowej, zarówno sezonowej jak i przestrzennej. Jednocześnie opisano dodatnią zależność pomiędzy zasobami (biomasą) fauny dennej a jakością i ilością materii organicznej zawartej w osadach. Przewiduje się, że jednym ze skutków zmian klimatu w Arktyce będą zmiany w wartościach produkcji pierwotnej i składzie gatunkowym producentów pierwotnych w tym ekosystemie. W związku z tym funkcjonowanie fauny dennej może także ulec modyfikacji na skutek zmian w całkowitej biomasie organizmów bentosowych, jednakże rozkład biomasy pomiędzy poszczególne klasy wielkości organizmów najprawdopodobniej pozostanie niezmieniony.

Chapter I. General Introduction

1.1. Size as a fundamental organism feature

Body size is a fundamental biological feature - a “master trait” that is closely coupled to key ecological processes like metabolism or development and constrains position of organisms in food webs (Blanchard et al. 2017). Five basic rules supported by correlations of organisms properties with body size, refer to: 1) surface for diffusion, 2) rate of life processes, 3) abundance of organisms, 4) strength, 5) complexity (Bonner 2006).

The relationship between body size and body surface is quite well recognized since it was one of the foundations that steered the evolution of life on earth. The well-known principle that the surface-volume ratio decreases with increasing body size (dimensions), arise from the fact that surface increases as a square while volume as a cube of linear dimension. This has tremendous consequences for diffusion of gasses or heat exchange between an organism and external environment. The heat-conservation hypothesis states that low surface area : volume ratio allows for more efficient heat conservation by large bodied organisms (Bergmann 1847; Blackburn et al. 1999). Regarding gas exchange the surface area : volume ratio has an opposite implication, especially in case of organisms that exchange gasses through the outer body surfaces e.g. invertebrates or amphibians (Ultsch 1973; Spicer and Gaston 1999). With growing body size, surface area (and the gas exchange) increases slower than the body volume and may be insufficient to maintain metabolic processes and limit maximum size of organism (that may be overcome by using special breathing organs like gills or lungs).

The size of organisms directly affects their life processes. The capacity of respiration or production increases with increasing body size, while the rate of these processes decreases with increasing body mass. This relation is described as the “Kleiber’s law” (Eq. I.1) stating that an animal’s metabolic rate B scales to the ¾ power of the animal’s mass M (Kleiber 1932; Figure I.1).

$$B \sim M^{3/4} \qquad \text{(Eq. I.1)}$$

Since energy stored in food is the fuel that powers metabolic processes of heterotrophs (Stryer 2003) it may be expected that the food intake rate should scale with size similarly as metabolic rate (Eq. I.1). This principle is indeed valid, however, the scaling may be different for different groups of organisms like endotherms or ectotherms and may also depend on the efficiency of digestive system. Still, in general the scaling exponent of ingestion rate is similar to the scaling of metabolic rate (ingestion rate ~ $M^{3/4}$, Peters 1983). This relationship has been confirmed by a number of terrestrial and aquatic studies. Reichle (1968) found that the amount of food daily consumed by arthropods scaled to power 0.68 of their body mass, while Cammen (1979) assessed ingestion rate for aquatic deposit feeders and detrivores to scale to the power of 0.74 of body mass.

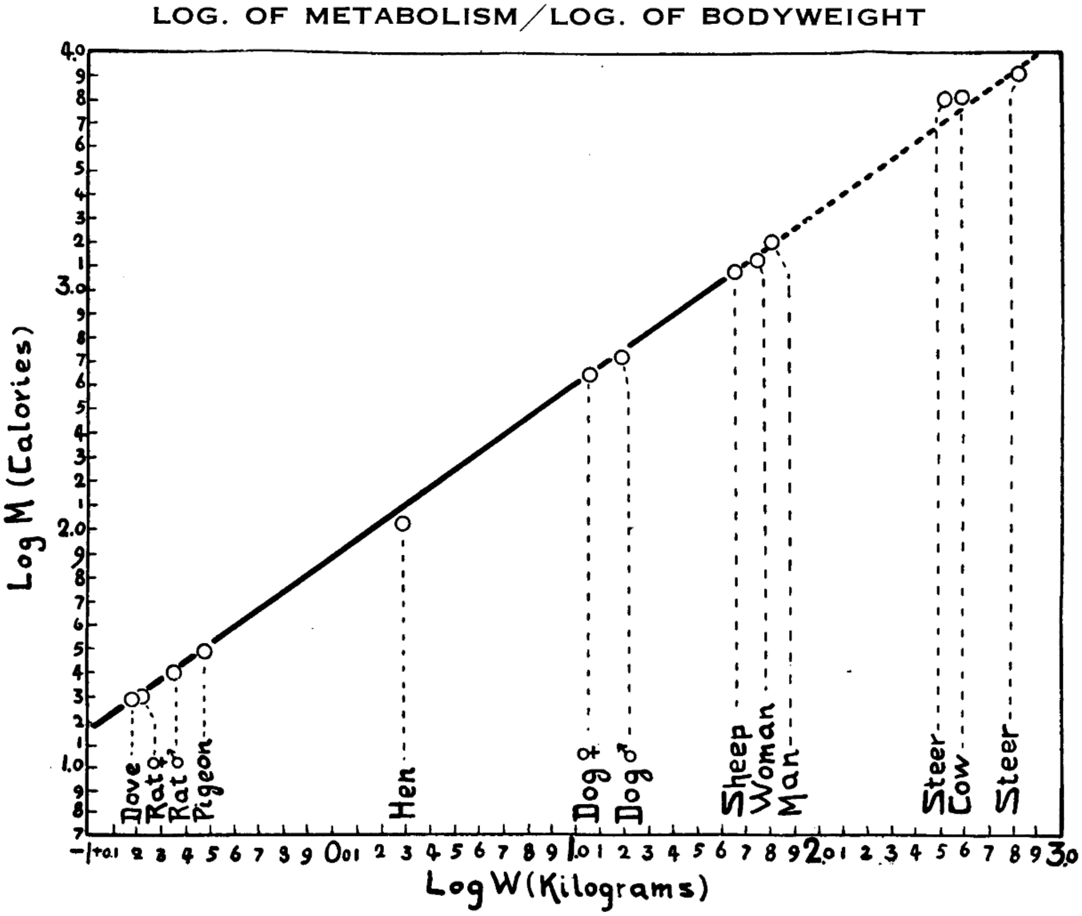


Figure I.1 Relation between metabolism and body weight for various vertebrates. Adapted from Kleiber (1932).

In his concept of "ecological pyramids" Elton (1927), stated that smaller organisms due to their much faster growth (high metabolism rates, productivity and turnover) are able to provide enough food for larger consumers. This concept refers to rates of life processes but also brings another ecological rule, which is the size relation between prey and predator. For many aquatic and terrestrial secondary consumers the prey is selected also based on its morphometric characteristics and the mean size of consumed food is usually smaller than the size of a predator (Cury et al. 2003). This is also related to the ontogeny of organism as the dietary preferences may change over lifetime, when small and young organisms tend to consume different or less variable food than older ones and may occupy different ecological niches (Lucifora et al. 2009).

Size-related trends in the longevity (larger organisms tending to live longer) are often observed among different groups of organisms (Bonner 2006), for example birds and mammals (Figure I.2; Healy et al. 2014). The connection between body size and length of life is also observed in invertebrates e.g., in coleoid cephalopods and may be also related to maturity postponing (Wood and O'Dor 2000). The longevity is naturally highly related to the mortality which can be also a derivative of size. Fenchel (1974) analysed the relationship between intrinsic rate of natural increase r_m (also known as "Malthusian parameter", Malthus 1798) and body mass of unicellular organisms, heterotrophs and endotherms. He estimated that r_m (Eq. I.2) can be described as a power function of body mass W, constant a that differs among unicellular organisms, heterotrophs and endotherms and the constant exponent n of about -0.275.

$$r_m = aW^n \qquad \text{(Eq. I.2)}$$

The exponent parameter n calculated by Fenchel (1974) may be also assumed as an equivalent of mortality rate, thus the mortality may be expressed as approximately 1/4 power of organism body mass (Kelly-Gerreyn et al. 2014). Such relationship between mortality rates and body size were proved in numerous studies on pelagic (Savage et al. 2004) and terrestrial organisms (Marba et al. 2007).

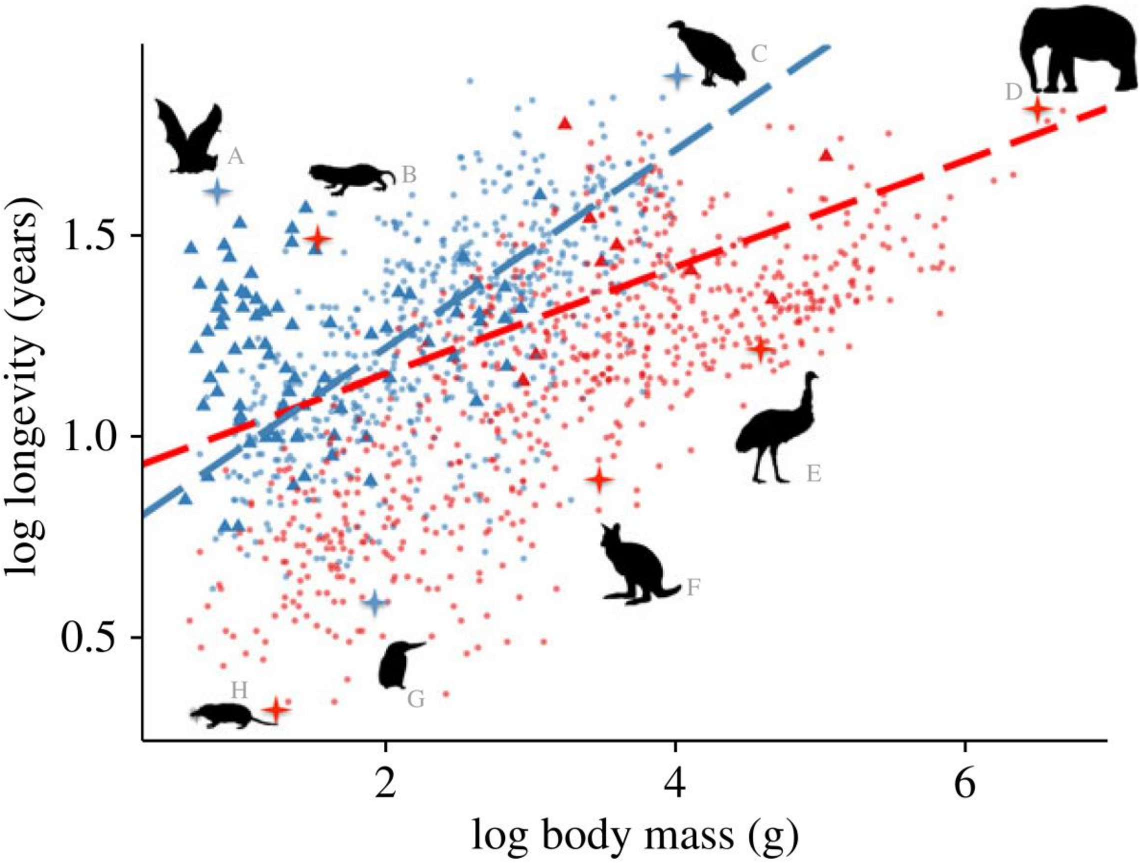


Figure I.2 Relationships between maximum lifespan and body mass in birds (blue dots) and mammals (red dots). Adapted from Healy et al. (2014).

1.2. Implications of size at a community level

Size of organisms may also have various implications for the functioning of multispecies communities. Such features as abundance of organisms or processes as competition for space and resources or trophic interactions may be related to the size structure of organisms comprising the community.

The relationship between body size and abundance of organisms has been extensively studied in ecological research as this link provides information about traits of species, dynamics and structure of ecological communities (White et al. 2007). In a comprehensive study of mammalian herbivores Damuth (1981) described the mean abundance *N* of organisms as a dependent variable of organisms average body mass M with a scaling exponent b (Eq. I.3) of about -0.75:

$$N \sim M^{b} \qquad \text{(Eq. I.3)}$$

Later he confirmed this relationship based on a study of a wide range of vertebrate taxa (Damuth 1987). Also Peters and Wassenberg (1983) studied the relationship between abundance and biomass of various groups of terrestrial and aquatic organisms and found that the b of Eq. I.3 ranged between -0.52 to -0.98 except herbivorous birds where it was 0.25. Also Nee et al. (1991) confirmed that abundance of British birds populations declines with -0.75 power of body mass, however when the data were analysed at low taxonomic level this trend was not confirmed, resulting very often in positive exponent.

Damuth (1981) not only quantified the size – abundance relationship, but also realized that that the scaling exponent b in Eq. I.3 is the inverse of the scaling in the relation between metabolism and body mass (“Kleiber’s law”; Eq. I.3). Thus when those two relationships are taken into account (Eq. I.4) they form the “Damuth’s rule” which states that population energy use is more or less invariant with respect to organisms body size (Damuth 1981):

$$B \times N \sim M^{-3/4} \times M^{3/4} \;=>\; B \times N \sim M^{0} \qquad \text{(Eq. I.4)}$$

The Eq. I.4 allows also to calculate the scaling of total biomass M_{tot} in relation to body size (Eq. I.5; Trebilco et al. 2013):

$$M_{tot} = M \times N, \qquad N \sim M^{-3/4} \;=>\; M_{tot} \sim M^{1/4} \qquad \text{(Eq. I.5)}$$

The “Damuth’s rule” is also known as the “energy equivalence rule”, which comes from work of Nee et al. (1991). This theory was tested and confirmed by Sewall et al. (2013), through direct measurements of energy intake by foragers and indicated that population’s energy use does not vary with the body size. They also concluded that that body size was not an advantageous feature in competition for shared resources among different populations or organisms of different sizes. However in the same study Sewall et al. (2013) also used indirect method to estimate the organisms biomass – energy intake relationship and the results were different showing increasing population energy use with increasing biomass of organisms. This discrepancies were explained by the assumptions and biases of indirect method they used.

The energy equivalence rule holds mostly in populations or communities consisting of individuals that use the same pool of resources, where the available energy determines the possible abundance of organisms. The increase of energy available in the system results in the abundance increase, which is mediated through energy requirements of each single organism (Isaac et al. 2013). However, the energy equivalence may not apply if all considered organisms do not share the same trophic level, or do no exploit the same pool of resources. Such situation is common in size structured systems where higher trophic levels obtain the energy by eating smaller prey. In this scenario the total energy use decreases with trophic level and body size, this will be associated with more rapid (comparing to system where all organisms share the same resource base) density decrease with body size ($N \sim M^{b<-3/4}$) and more rapid biomass decrease with body size (Trebilco et al. 2013). Reverse situation (slower density decrease and biomass increase with body size) may be observed in subsidised system, like deep-sea detritus based seabed communities or estuaries with high allochthonous inputs from land vegetation (del Giorgio and Gasol 1995; Trebilco et al. 2013).

The functioning of ecosystems may be influenced by the presence of some species or groups of organisms. These organisms may act as ecosystem engineers, which modulate the availability of resources to other species (Jones et al. 1994) or habitat-forming species, which provide biogenic structures (substrates) for other species to inhabit (Sellheim et al. 2009). Burrowing macrofaunal worms are an example of ecosystem engineers and studies showed that the amount of resources made available for other species (e.g., oxygen or food) through bioturbation is directly related to their body size. This arise from the fact that rates of nutrient or gas fluxes from sediments to the water relate to the rate of sediment irrigation that scales with bioturbator’s body size (Meysman et al. 2003; Norkko et al. 2013). Size also plays an important role in case of habitat-forming organisms. Buhl-Mortensen and Mortensen (2005) found that diversity of fauna associated with deep-sea gorgonian corals was correlated with number of branches and mass of habitat-forming corals. On the other hand a study of fauna associated with kelp *Laminaria hyperborea* showed that the host size (volume) influenced the abundance of associates but not their species richness (Christie et al. 2003).

1.3. Body size in response to environmental variability

The relation between body size and environmental conditions defies simple explanatory framework and requires multivariate explanations since numerous environmental factors may have influence on the size of organisms (Angilletta et al. 2004; Moran and Woods 2012). Those factors may be temperature, oxygen or food availability, they can invoke biophysical or physiological explanations or involve ecological and biogeographical interpretations.

Theory on links between body size and climate/latitude (environmental temperature in particular) has been first formulated based on observations of endothermic organisms as the "Bergmann's rule" (Bergmann 1847). It stated that endotherms from cooler climate tended to be larger than races of the same species from warmer climates. Further studies led to development of a "temperature-size rule" (Atkinson 1994; Atkinson and Sibly 1997), stating that there is a negative relationship between rearing temperature and organism final body size. All those rules base on a heat-conservation hypothesis, that assumes that larger body size allows for more sufficient heat conservation within body tissues, thanks to lower body surface area to volume ratio and lower heat loss than in organisms with smaller body size (Bergmann 1847; Blackburn et al. 1999; Litzgus et al. 2004). The heat conservation principle concerns especially endotherms that devote a lot of energy to maintain constant body temperature, but there are also proofs that it is valid for ectotherms, as larger body size provides higher thermal inertia for cooling and allows to maintain heat for longer time (Zamora-Camacho et al. 2014). Number of studies showed that most of endotherm species follow the rule of decreasing body size with increasing temperature: 72% of birds and 65% of mammals (Meiri and Dayan 2003). The validity of this rule was also confirmed for numerous ectotherms in large literature survey that showed a negative relationship between temperature and body size of ectotherms in 91 from 109 reviewed publications (Atkinson 1994). However it is worth noticing that during in-situ environmental studies it is almost impossible to assess the influence of temperature alone, as this parameter has also influence on other environmental parameters such as gas solubility, water viscosity or biological parameters like species composition, and in many cases the effect of temperature will be indirect or masked by other effects (Sheridan and Bickford 2011).

Dioxygen (O_2) is regarded as "master environmental variable" responsible for long-term evolution and expansions of biological diversity on Earth as well as short term disturbances in local communities (Sperling et al. 2015). It can also affect the size of organisms, both in terrestrial and aquatic ecosystems. For instance in poorly oxygenated marine basins, the selecting pressure promotes presence of small soft-bodied organisms with a high surface area to volume ratio, which allows sufficient gas exchange (Rhoads and Morse 1971). High oxygen availability may have an opposite effect. According to Chapelle and Peck (1999) high oxygen availability (due to high gas solubility) along with low metabolic rates in cold polar water allows numerous invertebrate species to be larger than in other, warmer/lower latitude regions – this phenomena is called a "polar gigantism" (however, other environmental factors were also proposed to explain this phenomena, Moran and Woods 2012). Regarding crustaceans the mechanism of "polar gigantism" relies on a limited availability of hemocyanin pigments to transport oxygen in dissolved form. In environment of high oxygen solubility, greater quantity of oxygen allows a longer circulation path what supports larger maximum potential size of individuals (Chapelle and Peck 2004).

It is also hypothesized that oxygen availability could have strong influence on the evolution (increase) of size of all organisms, including terrestrial ones (Blankenship et al. 2007; Payne et al. 2011). There is a very good agreement between sizes of the largest organisms and oxygen concentration in the atmosphere through the entire geological record, presenting a stepwise pattern of increase of maximum body size that coincides with stepwise increases of atmospheric pO_2 (Figure I.3; Payne et al. 2009). An example of an extreme influence of oxygen on a maximum size of organisms may be the period of atmospheric hyperoxia (even >30% pO2) in late Paleozoic that was shown to cause gigantism of fusulinoidean foraminifera, with some individuals exceeding their relatives even 6 orders of magnitude in biovolume (Payne et al. 2012). Although the sizes of largest organisms coincide well with high oxygen concentrations through history there are also other environmental circumstances (as sufficient food availability or space in the habitat) that must occur to allow certain organisms to grow to enormous sizes.

In marine environment salinity may be also a very important factor influencing body size, as it is linked to maintaining proper concentration of ions inside body. Many organisms control their internal ions concentrations through osmoregulation process, what, as a metabolic process, generates additional energy consumption (Castro and Huber 2003; Boomer et al. 2016). Good examples of organisms dealing with osmotic problems may be the blue mussel *Mytilus edulis – trossulus*, a marine bivalve, distributed also in the brackish Baltic Sea where it grows much smaller than in the North Sea. The smaller size in Baltic individuals may be explained by higher energy consumption caused by salinity stress (Remane and Schlieper 1972; Tedertgren and Kautsky 1986). Similar response to lower salinity was documented for the prawn *Palaemon elegans* that had much lower body mass in the Baltic Sea comparing to the Mediterranean Sea or waters off Ireland (Janas and Mańkucka 2010). Another good example is the round goby *Neogobius melanostomus*,, which is native to Ponto-Caspian region, and was reported to be about two times larger in marine (salinity 15-17) than in brackish (salinity 5-8) and fresh waters (Curkum et al. 2004).

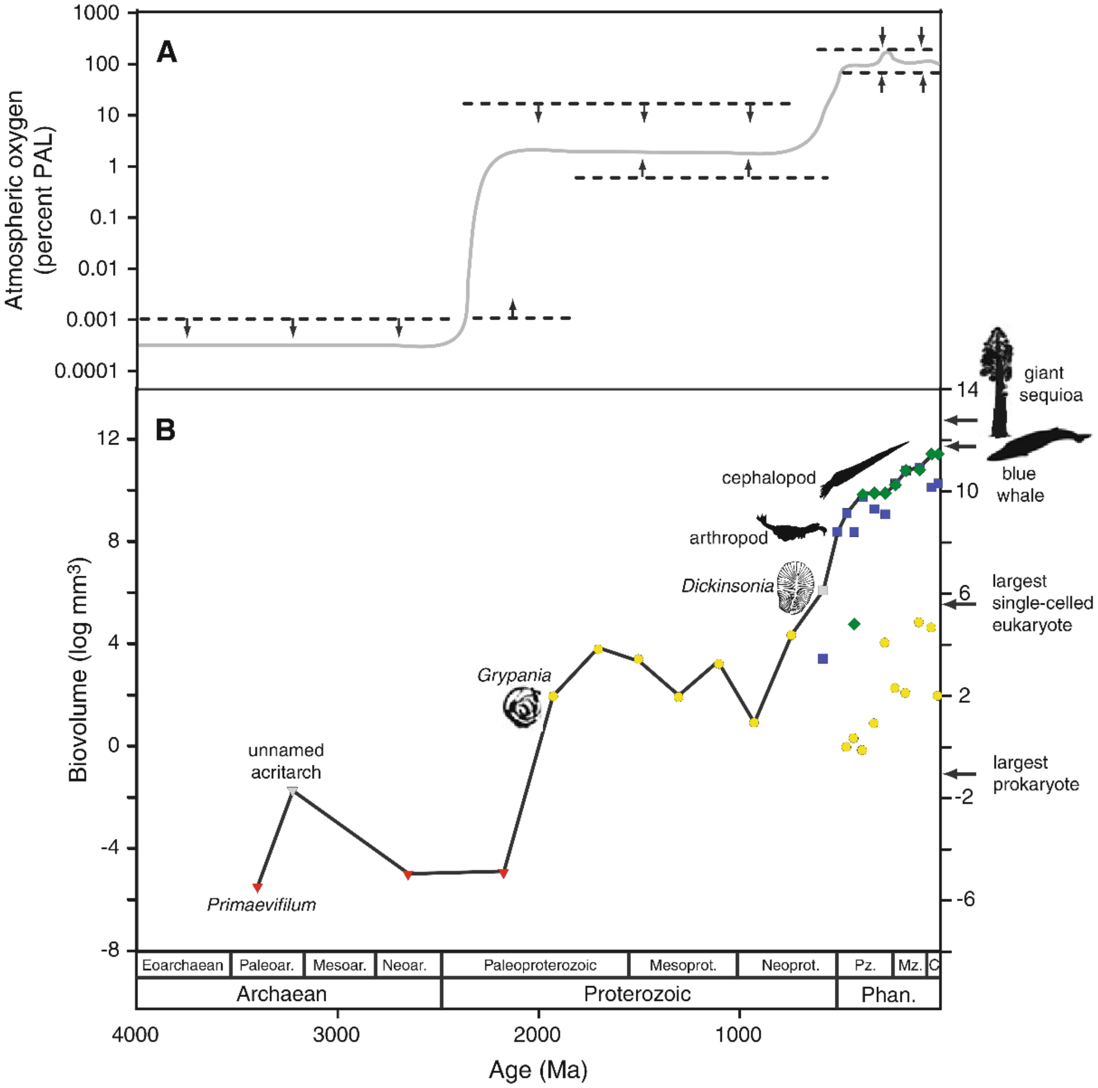


Figure I.3 Percent of present atmospheric levels of oxygen (PAL) and maximum organismal sizes through geological time. Adapted from Payne et al. (2011).

Also food availability or variability in diet is regarded as a factor that may influence size of organisms. Food was also proposed as one of the drivers of “polar gigantism” (Moran and Woods 2012). Studies of Ho et al. (2010) showed that both terrestrial and marine herbivores when reared on a diet comprising high-latitude plants grew larger and/or faster comparing to organisms feeding on plants from lower latitudes. They attributed this phenomenon to higher nutritional quality of high-latitude plants e.g., greater nitrogen content or softer tissues. The study of Arnett et al. (1999) showed that variation in food-availability rather than temperature might influence the growth rate and body size of ant lions (*Myrmeleon immaculatus*). Also studies of Zawierucha et al. (2015) showed that water bears (Tardigrada) inhabiting Arctic tundra characterized by plant communities enriched by the vicinity of birds colonies, had much higher body size index, compared to organisms from regions not influenced by birds. Moreover, exposure to unfavourable conditions, with periods of lower food supply may additionally shape the size structure of organisms. The “starvation-resistance hypothesis” (Cushman et al. 1993), tested on ant assemblages, claimed that large-bodied species were better fitted to resist starvation during periods of disadvantageous food conditions. Larger body size allows storage of bigger amount of energy reserves (usually lipids) and may be promoted in ecosystems with strong seasonality in food supply like polar regions (Moran and Woods 2012). It was shown for the three copepod species from *Calanus* genus from the North Atlantic and Arctic waters that smaller, boreal *C. finmarchicus* had lower percentage contribution of lipids to the body mass, compared to larger Arctic *C. glacialis* and *C. hyperboreus*, what is regarded as an adaptation to survive in more extreme and variable Arctic environment by the two later species (Scott et al. 2000). Unlike planktonic herbivores, benthic species do not tend to store large amount of lipids (Norrbin and Båmstedt 1984). Consequently, resistance to starvation of benthic organisms may even decrease with body size as it was shown for deposit feeding amphipod *Monoporeia affinis* (Aljetlawi and Leonardsson 2006).

1.4. Size spectra as descriptors of aquatic multispecies assemblages

The size spectrum is the representation of the distribution of individuals or biomass among individual size classes (abundance or biomass size spectra, respectively, White et al.

2007). It has been used to describe size structure of aquatic communities in ecological studies for several decades (Trebilco et al. 2013).

The importance of size structure in marine ecology was recognized in 1970s when Parsons (1969) and Sheldon et al. (1972) used particle size spectra (Figure I.4) to provide new insights into plankton communities functioning, and Platt and Denman (1977) published normalized size spectra analyses of organisms in the pelagic ecosystem. Size spectra have been frequently assessed for aquatic communities, since such structural organization may have stronger implications for functionality than commonly reported taxonomic composition, diversity or abundance (Quiroga et al. 2016). The size structure in multispecies assemblages defines interactions among species, including their position in food webs, pathways and magnitudes of carbon flow through the system components and ecosystem engineering effects (Kingsolver and Huey 2008; Norkko et al. 2013). Size spectra may be also powerful indicators of primary production, disturbance and/or stability, impacts of fisheries or consequences of climate warming (Boudreau and Dickie 1992; Jennings et al. 2002; Zhou et al. 2007; Binzer et al. 2016; Quiroga et al. 2016; Sprules and Barth 2016). Therefore, due to the comprehensive information they can provide the size spectra have been commonly used to describe aquatic communities in freshwater (e.g., Sprules and Munawar 1986; Sprules and Goyke 1994), marine pelagic (e.g. Zhou et al. 2004, García-Comas et al. 2014) or benthic (e.g. Schwinghamer 1981, Warwick and Clarke 1984; Quiroga et al. 2005; Kelly-Gerreyn et al. 2014) systems. Nevertheless, pelagic size structure analyses are much more common than those focused on benthic biota due to some technological advantages such as automated particle counters (Laser Optical Particle Counter - LOPC, Coulter counter) or acoustic methods (Yurista et al. 2014; Kerckhove et al. 2016), and faster sample processing.

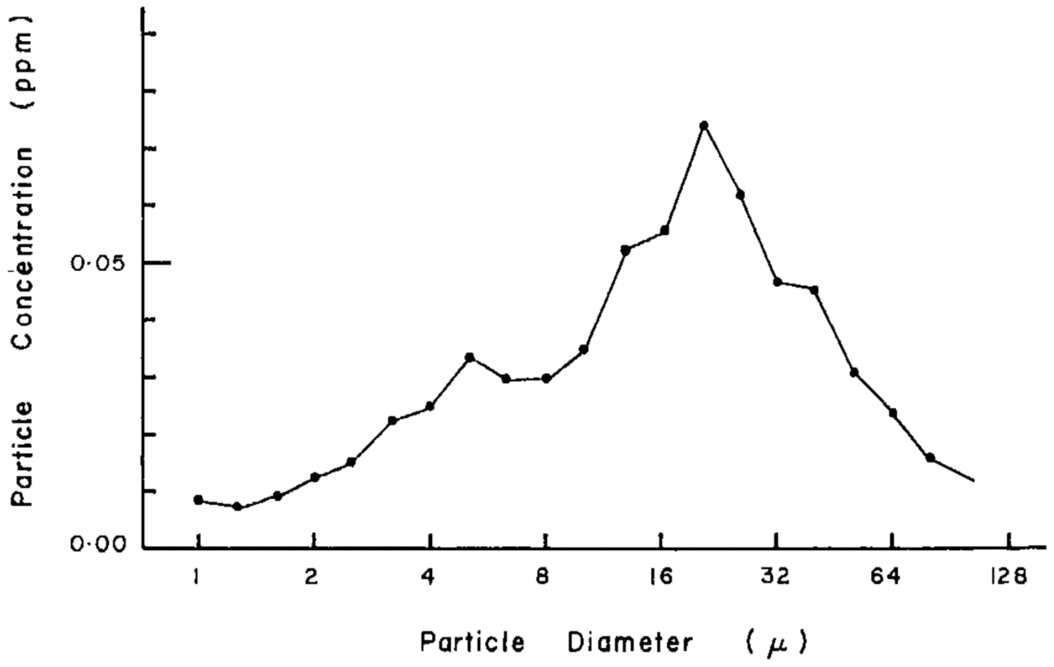


Figure I.4 Size-frequency distribution of marine particulate material presented by Sheldon et al. (1972).

The allometric power law equation that describes abundance-body size relationship (Eq. I.3) or biomass – body size relationship (Eq. I.5) may be easily converted to the logarithmic form as:

$$y = ax^b \;\; => \;\; \log y = b \; \log x + \log a \qquad \text{(Eq. I.6)}$$

This logarithmic form (Eq. I.6) provides much easier analysis since the power exponent b refers to the slope coefficient of the linear regression for log-log relationship between variables (Peters and Wassenberg 1983) and the regression technique is commonly used to compare size spectra in aquatic communities. So consequently according to energy equivalence theory, the relations between abundance and body size of organisms should be described by a slope coefficient of linear regression of about - 0.75, what would indicate equal rates of resource utilization by organisms of different sizes (Akoumianaki et al. 2006; Quiroga et al. 2016).

The Platt and Denman (1978) proposed to normalize the biomass (divide the biomass in each size class by this size class range) when constructing the biomass size spectra. In such normalized biomass size spectra (NBSS) plotted for a community in a an ecosystem in a steady state (lack of disturbance, the flux of energy from smaller to larger organisms) the slope coefficient of linear regression between normalized biomass and size classes should be close to -1. Examples of such steady state marine systems may be the pelagic systems of Sargasso Sea or central gyre in the North Pacific Ocean (Platt and Denman 1978; Rodriguez and Mullin 1986). Deviations from the steady state may be visible in changes of the NBSS regression slope coefficient or in variation of residuals along regression line (Sprules and Barth 2016). In particular, NBSS slope coefficient was shown to decrease with growing trophy of the system i.e., from oligotrophy to eutrophy along with growing linear regression residuals variation indicated (Sprules and Munawar 1986). NBSS parameters may be also sensitive to pollution impacts, ecosystem exploitation, or anoxia (de Bruyn et al. 2002; Quiroga et al. 2005, 2014; Gómez-Canchong et al. 2013). The second parameter of NBSS linear regression is the intercept that is regarded as an indicator of total biomass of the studied community (Sprules and Munawar 1986), or a rate of primary production in the system in case of pelagic systems (Zhou 2006).

Published studies of benthic size spectra often investigate the impact of food availability on size structure, in the context of estuarine zonation (Akoumianaki et al. 2006; Quiroga et al. 2016), depth gradients (Saiz-Salinas and Ramos 1999) or environmental gradients in Arctic glacial fjords (Górska and Włodarska-Kowalczuk 2017). Quiroga et al. (2005) and Duplisea and Drgas (1999) explored the effects of oxygen conditions and sediment grain size, respectively. Yool et al. (2017) incorporated knowledge of benthic biomass partitioning among the size classes in modelling and predictions concerning possible changes in the shelf and deep-sea ecosystems and underscored the importance of studying size structures across a broader range of seafloor environments for predicting future changes. However, hitherto, benthic size structure studies were performed at local scales; benthic size spectra have not been investigated across wider geographical or temperature gradients, despite their high potency to detect changes in functioning of communities and despite the fact that consequences of those changes are of basic interest in the context of climate change.

1.5. Body size in marine studies – units and assessment

The term “body size” may in fact refer to various organismal characteristics, depending on particular study aims or standards accepted in a certain field of study or for a certain group of organisms. A variety of units is being used in biological and ecological studies, these can be: biovolume, biomass, organic carbon content, whole body length, length of body part or even age of an organism. Such variety of units referring to the same term is possible since all of them change during organism growth and covary with each other and the scaling of these relationships may be described thanks to allometric studies (Shingleton 2010).

In marine ecology most common units include: length, ESD (equivalent spherical diameter), biovolume, biomass or carbon content (Sprules and Barth 2016). The ESD is commonly used in plankton studies, it originated from Coulter counter based examination of suspended particles in seawater samples (Sheldon and Parsons 1967). The unit depicts the size as a diameter of a sphere that has the same volume as an examined particle, in plankton studies - an examined individual. Despite the developments of new technologies for automated measurements of suspended particles in the ocean, like development of LOPC (laser optical particle counter) or FlowCAM, the use of ESD is still common in plankton studies, sometimes abbreviates just as a “diameter” (eg. Karnan et al. 2017; Watkins et al. 2017; Trudnowska et al. 2018). Since ESD is related to the volume V:

$$ESD = \sqrt[3]{6V/\pi} \qquad \text{(Eq. I.7)}$$

the use of volume, usually formulated as biovolume, is also very common in plankton size structure studies especially those comprising automated measurements. When studies comprise more narrow group of organisms (than plankton) like phytoplankton, planktonic copepods or benthic molluscs size may be expressed as body length or body part length (e.g., prosome length in copepods or length from anterior to posterior side in bivalves (Thompson et al. 2013; Eklöf et al. 2016)). A special type of length unit may be an echosounder target strength (TS [dB]), also called as acoustic size, that is derived in fish surveys conducted with use of echosounders and is directly correlated with fish size (Burczynski 1982; Kerckhove et al. 2016). Presenting size of organisms in biomass (either wet or dry mass) units is especially popular in benthic studies but it is also frequent in plankton or nekton surveys (e.g., Drgas et al. 1998; Quiroga et al. 2005; Blanchard et al. 2017; Górska and Włodarska-Kowalczuk 2017). Sometimes in benthic size structure studies size is defined by assigning organisms to size classes according to the sieve mesh size on which they were retained (Edgar 1990). The carbon content units are less commonly used in size structure studies and mostly in those concerning pelagic communities (e.g., Quinones et al. 2003; Thompson et al. 2013). In this thesis the term “body size” will refer to an organism body dry mass.

The variety of units used in reference to size arise from diversity in organisms morphological properties (like size or shape), environment their live in (water column or sediments) and available technologies and their limitations. The simplest techniques of size assessment are the manual measurements, done by ruler or calliper or under the microscope (Baguley et al. 2004; Eklöf et al. 2016). However they are limited by the size of organisms, have variable precision and usually involve lots of labour, what is especially bothersome when samples of numerous organisms are proceeded like in case of plankton or meiofauna. Faster and more efficient techniques of measurements require special instruments like LOPC or FlowCAM and/or automated or semi-automated image analysis software (Baguley et al. 2004). Both LOPC and FlowCAM are dedicated to water column measurements and allow for continuous *in-situ* acquisition. In case of LOPC, the instrument provides measurements of 2-dimentionals cross-sectional area of particle that pass through the laser beam and then the objects’ diameter (ESD) is derived by equating the area of circle to the area of measured particle (Herman et al. 2004). In case of FlowCAM particles flow through glass cell and are photographed and then measured (e.g., length, width or depth of field of view) what allows for the volume assessment (Sieracki et al. 1998; Romero-Martínez et al. 2017). Regarding benthic organisms, *in-situ* measurements are possible only in case of organisms that are on a surface of a substrate. Organisms must be photographed and then their dimensions may be assessed (Beuchel et al. 2010; Durden et al. 2016). However, since majority of benthic fauna live inside sediments, they need to be extracted from the medium first and then can be measured manually.

Volume measurements are possible with use of Coulter counter (Sheldon and Parsons 1967), however in most studies volume is assessed indirectly on the basis of organisms dimensions. Organisms are matched to geometric 3D figures (like cylinder or ellipsoid) and the volume is derived from its body dimensions (Hillebrand et al. 1999) or derived from ESD (Eq. I.7).

Regarding biomass assessment, direct measurements are often limited by the readability of available laboratory balance. Thus, for small organisms (e.g. bacteria, meiofauna, zooplankton) indirect techniques are needed to estimate their biomass, however they can be also applied to larger organisms e.g., when a study encompass various size groups and all are to be analysed with use of the same methodology. These indirect methods involve converting the biovolume of individuals (using established conversion factors) into biomass (Andrassy 1956; Brey et al. 2010; Thompson et al. 2013; Górska and Włodarska-Kowalczuk 2017).

Estimation of carbon content may be obtained directly by the combustion or by the elemental analysis of organism or group of organisms and average carbon content assessment (Strathmann 1967). In case of small organisms use of cultures of known size (e.g., length) is essential to estimate relationship between size and the carbon content . The drawback of this direct approach is the destruction of organisms (Baguley et al. 2004). Thus in numerous studies the carbon content is assessed (similarly as biomass) by using conversion factors (Quinones et al. 2003; Yvon-Durocher et al. 2011).

1.6. Aims, objectives and outline of the thesis

The main aim of this thesis was to determine changes in size structure of benthic communities in response to temporal (seasonal) and spatial environmental variability in the North Atlantic and Arctic coastal waters (fjords). The objectives of the thesis were:

1) To develop a method of semi-automated measurements and biomass estimation of nematodes.
2) To assess the seasonal (winter versus summer) changes in benthic biomass size spectra in the Arctic fjordic system.
3) To describe patterns of variability in benthic biomass size spectra in response to changes in water temperature and food availability across latitudinal gradient (60-81°N).

This thesis is divided into three parts. In the first one the general introduction, aims and objectives of the thesis are described (**Chapter I**). The second part consists of three independent chapters that are structured like scientific publications and respond to the main objectives of this study (**Chapters II –IV**). The closing chapter (**Chapter V**) contains general discussion of the thesis findings, conclusions and future perspectives. Finally, the cited literature is listed, followed by lists of all tables and figures.

Chapter II describes a method of semi-automated image analysis for obtaining the widths and lengths of nematodes photographed under stereomicroscope and measured using image analysis software. Nematodes measurements were performed manually and semi-automatically. Manual measurements were taken by three independent analysts to check for individual analyst effects. The duration of automated measurements was compared with time of the manual procedure. Also the results of manual and semi-automated measurements were compared, between methods and among scientists. The individual nematode biomass was estimated using three equations. The results were compared to indicate the equation presenting the closest results to those obtained using manual measurements. Finally methodological recommendations for meiofauna biomass assessments were provided.

The aspects of seasonal variability in size structure of benthic communities (meiofauna and macrofauna) in the Arctic fjord are described in **chapter III** where a comparison of summer and winter biomass size spectra in the central basin of Kongsfjorden (west Spitsbergen) is presented. The seasonal changes in the quality and quantity of organic matter available to the benthos, indicated by chloroplastic pigments concentrations in surface sediments were compared with the differences in total abundance and biomass of macrofauna and meiofauna. Abundance, biomass and normalized biomass size spectra were constructed for each station in each season. Seasonal differences in individual biomass of taxa represented by >30 specimens in each sample were investigated.

In **chapter IV** possible effects of increasing temperature on biomass size structure in coastal benthic communities were investigated. The analysis concerned meiofauna, macrofaunal nematodes and macrofauna samples collected in the six Norwegian and Svalbard fjords across wide latitudinal (60-81°N) and bottom water temperature (from -1.5 to 8°C) gradients. The environmental settings (water temperature, chloroplastic pigments, $\delta^{13}C$, organic carbon content and grain size in sediments) in studied fjords were investigated. Abundance, biomass and normalized biomass size spectra (NBSS) were constructed for each fjord. The relationships between slopes of NBSS and environmental parameters were assessed. The relationships between temperature and body size were assessed for particular species, genera and families. The patterns of similarities in species composition, size classes composition and functional (feeding) traits composition among stations and fjords were compared and related to patterns in benthic size structure.

Chapter II. A semi-automated image analysis method for assessment of nematode biomass in marine sediments

2.1. Introduction

Manual and automated image analyses have been used in many branches of marine research. For instance in physical oceanography they are applied to analyse satellite images to assess information about pigment concentration in the surface layer of oceans or to track hydrogen sulphide emission in the upwelling waters (e.g. Weeks et al. 2004; Woźniak et al. 2011). Raster-based geographic information system (GIS) applications employ image processing for analysis of the distribution of marine flora on the basis of photographs (e.g. Sotheran et al. 1997; Wright 2002; Frederiksen et al. 2004). Underwater images of rocky beds can be used to assess the occurrence and abundance of epibenthic species using graphics software (Beuchel et al. 2010). Image analysis methods are also applicable for the automated quantification of labelled bacteria (Selinummi et al. 2005) or to estimate zooplankton biovolume and biomass (Alcaraz et al. 2003; Di Mauro et al. 2011).

Many image analysis and processing methods were developed in biotechnological studies and experiments of a model nematode species - *Caenorhabditis elegans* (Maupas, 1900). Some procedures focus on automated phenotypic analysis or animal movement tracking and include teaching the image analyses software to distinguish the worm from the background (Mathew et al. 2012) or to proceed on the basis of dedicated algorithms (eg. in MATLAB or C). Processing the images include objects classification, sorting and tracking live animals (Geng et al. 2004; Chung et al. 2008; Ramot et al. 2008; Zhan et al. 2015). It is also possible to analyse live nematodes with use of microchip devices (Jung et al. 2012). Rizvandi et al. (2008) and Ochoa et al. (2007) proposed a method of analysis based on the detection and separation of individual specimens using skeleton analysis and contour energies. For marine ecological studies of meiofauna (benthic invertebrates passing through a 500 μm mesh sieve and retained on a 32 μm mesh sieve) Baguley et al. (2004) proposed a method of estimating the biomass of the two dominant taxa (Nematoda and Harpacticoida) on the basis of image analysis and volumetric approach.

Meiofauna organisms constitute only 3% of benthic biomass, however they play a crucial role in the decomposition of detritus. Recent studies show that meiofauna increases bacterial denitrification processes in marine sediments (Bonaglia et al. 2014). It plays important role in nutrient cycling and in energy flow through the different benthic components and from the sediment back into the water column. Meiofauna makes a substantial contribution to benthic remineralization processes (even as much as 40% of the total benthic carbon demand could be due to nematode respiration; Vanhove et al. 1997). In benthic systems, meiofauna is responsible for 10 to 25% of secondary productivity (Giere 2009) and for up to 25% of total sediment community oxygen consumption (Piepenburg et al. 1995). The relatively high contribution to energy consumption is due to meiofauna specimens high turnover rates, even 5 times higher than those of macrofauna (Gerlach 1971). Moreover, meiofaunal organisms are regarded as good indicators of both natural and anthropogenic environmental gradients due to their high abundances, widespread distribution and sensitivity to environmental variability (Grego et al. 2009). Nematodes are the most abundant and diverse component of the metazoan meiofauna and in most environments they are dominant in terms of both abundance and biomass and usually constitute more than 90% of the metazoan meiofauna (Heip et al. 1982).

Meiofaunal biomass can be determined by direct or indirect methods. To determine biomass by direct methods, large numbers of meiofaunal organisms are needed or only individuals from the top end of the meiofaunal size range can be used. Wet and dry mass of aggregated individuals can then be obtained using a high-sensitivity balance (Feller and Warwick 1988). Direct methods require the aggregation of several individuals and destruction of the sample (in case of dry mass assessment) resulting in loss of the opportunity to perform other analyses (Baguley et al. 2004). Moreover, this method (direct weighting) allows to assess only the total biomass of an aggregation of individuals, but does not provide information on the individual size distribution or biomass structure in the studied community. Therefore indirect methods are usually applied in marine ecology studies. The tube based calculations of biovolume (translated into wet and dry mass with use of conversion factors) or Andrassy formula (biomass estimates that use measurements of animal dimensions) are standard approaches and are commonly employed in benthic studies (e.g. Andrassy 1956; Feller and Warwick 1988; Giere 2009; Danovaro 2010; Somerfield and Warwick 2013). The major drawback of the indirect methods lies in the duration of analyses required to assess the individual dimensions by manual measurements. High costs of this laborious analyses often precludes meiofauna biomass assessment from routine ecological surveys.

The aim of this chapter is to present a semi-automated method based on image analysis for the estimation of biomass of meiofaunal nematodes in ecological samples. The advantage of automatic or semi-automatic methods over manual analyses lies in their more rapid performance and cost reduction. In addition, computer-based analyses are less subjective than those performed by human operators. The analyses' duration time and results were compared for individual nematode sizes and biomasses obtained using the evaluated semi-automatic method and based on manual measurements by three independent researchers. The described new semi-automated method and recommendations for the nematode biomass assessment methodology based on these results can promote a wider use of biomass assessment in ecological studies of marine meiofaunal communities.

2.2. Materials and methods

2.2.1. Sampling

Samples were collected at two localities representing two different marine sedimentary environments: the deep subtidal muddy sediments of a high Arctic glacial fjord and the sands of temperate shallow-water seagrass beds in the Baltic Sea. Five samples were taken at each locality. The Arctic fjord samples were collected in Kongsfjorden (79°N 12°E, west Spitsbergen) at depths of 80, 295, and 305 m in the summer of 2012. Sediments were collected from R/V "Oceania" with use of a box corer. The upper 5 cm of the sediment were subsampled from a box core with a small core (area of 10 cm^2). The Baltic Sea sampling site was located in the Puck Bay (54°42.7'N 18°33.2'E) within seagrass meadows at a depth of 2 meters and sampling took place in July, 2012. The upper 5 cm of sediment were collected by SCUBA divers using a core (area of 3.14 cm^2). Samples were fixed in 4% buffered formaldehyde.

2.2.2. Laboratory analyses and photography

Arctic muddy samples were centrifuged three times in a solution of colloidal silica (Ludox TM-50) with a density of 1.18 g (Heip et al. 1985). A standard decantation technique was used for the Baltic Sea sandy samples (Pfannkuche and Thiel 1988). A sample consisted of all invertebrates passing through a 0.5 mm sieve and retained on a 32 µm sieve. All organisms were stained in a 4% buffered formaldehyde solution with Rose Bengal for at least 24 hours.

The meiofauna samples (consisting mostly of nematodes but also of other meiofauna taxa, organic debris and mineral particles) were placed on square counting dishes (50 x 50mm) with 100 counting areas (5 x 5mm). One after one counting area was checked and 100 Nematoda specimens were photographed using a Leica DFC450 digital color camera (number of pixels / pixel size: 5 megapixel, 2560 × 1920 / 3.4 μm × 3.4 μm) mounted on Leica M205C Microscope equipped with a 1.0x planochromatic objective and connected to a computer with Leica Application Suite (LAS) 4 software enhanced with Interactive Measurements and Image Analysis modules. Depending on the size of the nematodes and their concentration in the sample, from 1 to 20 individuals were photographed in one image. The photographing (i.e. taking pictures of 100 individuals) took from 20 to 140 minutes per sample (time depending on number of individuals included in one image). Only individuals of similar size were chosen for a single image. In the case of contrasting sizes, the invertebrates were photographed separately. Particular attention was paid to keep all individuals separated and avoid overlapping of individuals as well as to minimize the amount of organic debris (which were also stained with Bengal Rose). The pictures were taken at the highest possible magnification from 10x to 160x in a transparent light with manually set strength and automatic image exposure, while the under- and over-exposed levels were corrected manually. Number of analysed pictures ranged from 54 to 90 for Arctic samples and from 10 to 16 for Baltic samples. Lower number of pictures (i.e. processing images with higher numbers of individuals per picture) in the latter case resulted from the higher clarity of the images (lower amount of organic debris).

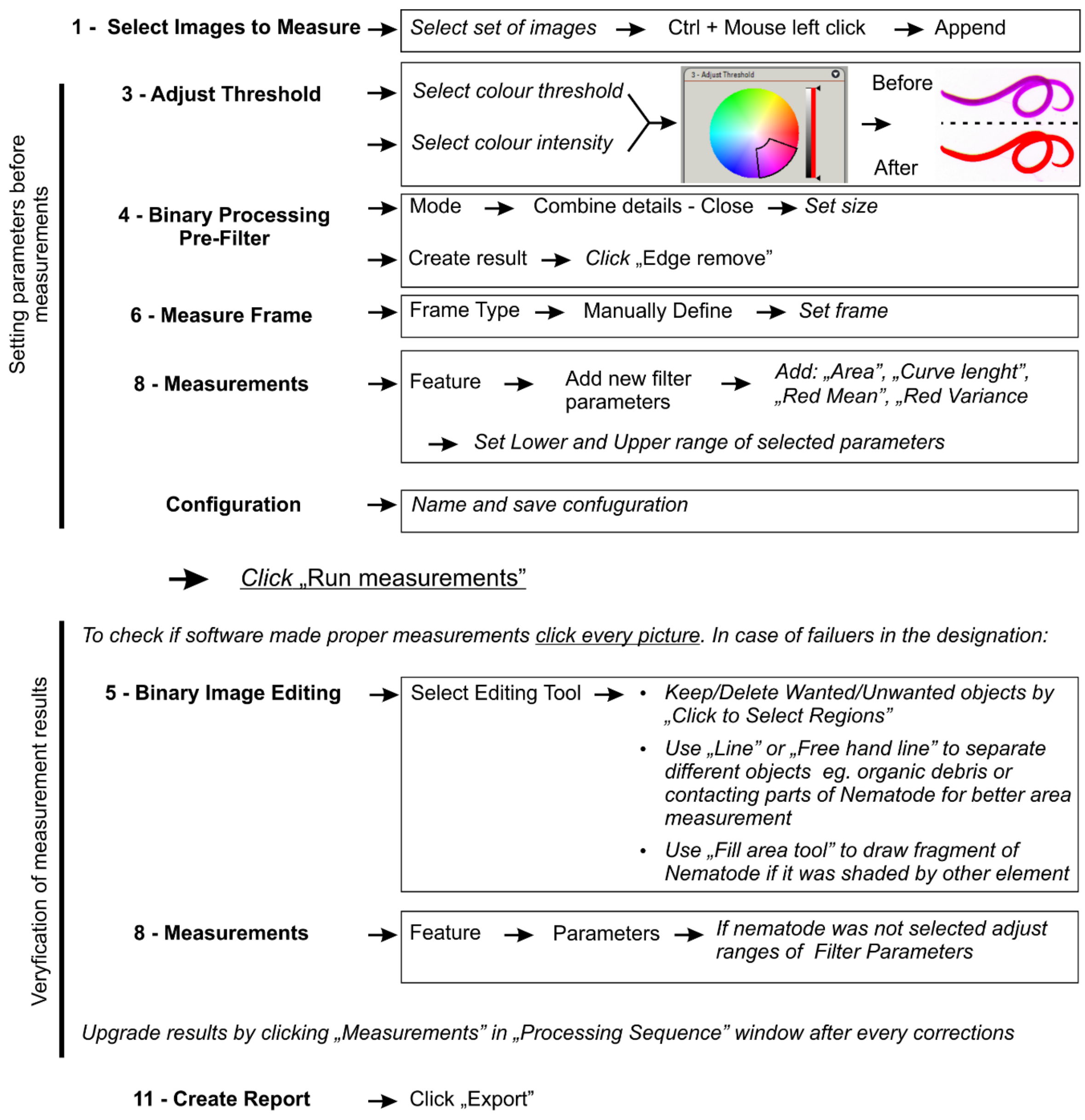
2.2.3. Image analyses

Images were analysed using the Leica LAS Image Analysis module (Figure II.1). The color threshold was selected in a way to fit to the color of the nematodes (stained with Bengal Rose) in the pictures. It was performed with a live preview of original and post-processed pictures, where simultaneously with the selection of the threshold range it was possible to check if the given color selection secured the accuracy of whole nematode designation. To make the nematode images more precise the Binary procession Pre-Filter with the mode of combining close details at strength 3 and the option to remove edge objects were enabled.

To exclude the non-nematode Bengal Rose-stained objects (e.g., organic debris) from the analyses, the parameters of the objects to be considered were selected. Ranges of values for curve length, object area size, red mean and red variance were selected in a way to best fit the nematodes' observed values and to exclude as many unwanted objects from the analysis as possible. This settings configuration was saved and the pictures were analysed in a series of 20 pictures in a row. The parameters measured by the software in the images were the curve length and curve width. After the analysis, the results were checked, and if necessary, corrected using the software's Binary Image Editing tool. This included the manual inclusion of nematodes not chosen by the software, exclusion of unwanted elements, and the checking and manual corrections of nematodes' shapes designated by the software.

The same nematode specimens were also measured manually using a Leica LAS Interactive Measurements module. This was performed by three different persons to check for interpersonal variability, as well as to compare the manual and automated measuring results. The length of the animals was measured using a segment line connecting the ends of an animal (excluding the filiform tails), while width was measured at the widest point of a nematode's body (as identified by the person performing the analysis). Every scientist performed the measurements on a clear picture with no traces of the measurements performed by other analysts.

The method was applied and tested with use of a commercial software – Leica LAS 4. Certainly the same procedure can be performed with use of any other software, provided it offers the automatic scaling of images, objects detection and automated measurements of detected objects. Unfortunately, up to my knowledge, the commonly available freeware programs do not read automatically the scale on the images taken with use of the microscope based image acquisition systems. The manual scaling is possible, however it takes much time and largely prolongs the whole analyses. Also, the brand software that makes part of the whole image acquisition and analyses system, enables to conduct simultaneously the regular meiofauna sample analysis – higher taxa identification, counting of individuals and image acquisition of nematodes for the biomass estimations that further reduces the total time spent on a meiofauna sample analysis.



Setting parameters before measurements

1 - Select Images to Measure → *Select set of images* → Ctrl + Mouse left click → Append

3 - Adjust Threshold → *Select colour threshold* / → *Select colour intensity* →

4 - Binary Processing Pre-Filter → Mode → Combine details - Close → *Set size*
→ Create result → *Click* „Edge remove”

6 - Measure Frame → Frame Type → Manually Define → *Set frame*

8 - Measurements → Feature → Add new filter parameters → *Add: „Area”, „Curve lenght”, „Red Mean”, „Red Variance*
→ *Set Lower and Upper range of selected parameters*

Configuration → *Name and save confuguration*

→ *Click* „Run measurements”

Veryfication of measurement results

To check if software made proper measurements *click every picture*. *In case of failuers in the designation:*

5 - Binary Image Editing → Select Editing Tool →
- *Keep/Delete Wanted/Unwanted objects by „Click to Select Regions”*
- *Use „Line” or „Free hand line” to separate different objects eg. organic debris or contacting parts of Nematode for better area measurement*
- *Use „Fill area tool” to draw fragment of Nematode if it was shaded by other element*

8 - Measurements → Feature → Parameters → *If nematode was not selected adjust ranges of Filter Parameters*

Upgrade results by clicking „Measurements” in „Processing Sequence” window after every corrections

11 - Create Report → Click „Export”

Figure II.1 Procedure for a semi-automated measurement of nematodes including software setting, measuring and results verification, performed with use of LAS 4 software (Legend: bold = main panels of Image Analysis Module; *Italics* = Instructions).

2.2.4. Biomass calculations

The nematode biomass was calculated directly from the measurements of individual dimensions or indirectly from their biovolume. Nematode biovolume was assessed on the basis of automatic measurements (by procedures in the Image Analysis module) and calculated using two different equations. The first equation is for the volume of a cylinder (Eq. II.1) - a widely used equation for nematode biovolume estimations (e.g., McCulloch and Gems 2003; Baguley et al. 2004; Jung et al. 2012):

$$V\ [\mu m^3] = \pi r^2\ L/10^6 \qquad \text{(Eq. II.1)}$$

where r [µm] is the radius (width/2) and L [µm] is the nematode length. The second equation is for the volume of rectangular cuboid corrected for a circular body shape using the conversion factor C_{cf} according to Warwick and Price (1979):

$$V[\mu m^3] = (A\ [\mu m^3] \times W[\mu m])\ \times\ (C_{cf})/10^9 \qquad \text{(Eq. II.2)}$$

where A [µm^2] is the nematode's area, W [µm] is its width and C_{cf} is the conversion factor (which equals 530). Wet mass (WM) was calculated from the biovolume using a specific gravity of 1.13 (Andrassy 1956).

The WM of nematodes measured manually was calculated with Eq. II.3 following Andrassy (1956):

$$WM\ [\mu g] = (L \times W^2)/Cf \qquad \text{(Eq. II.3)}$$

where L [µm] is the nematode's length, W [µm] is its width at the widest point and C_f is a conversion factor that equals 1.6×10^6. These calculations were performed separately for the results obtained by each analyst. Eq. II.3 was also used to calculate the WM of nematodes measured automatically using nematode length as L and nematode width as W. Nematode lengths and widths were recorded by the LAS Image Analysis module as curve lengths and curve widths, respectively. The software measured curve length and curve width according to Eq. II.4 and Eq. II.5 respectively:

$$Curve\ Length = \frac{\left(P - \sqrt{P^2 - 16\ \times A}\right)}{4} \qquad \text{(Eq. II.4)}$$

$$Curve\ Width = \frac{\left(P + \sqrt{P^2 - 16\ \times A}\right)}{4} \qquad \text{(Eq. II.5)}$$

where P is curve (nematodes') perimeter and A is the curve area.

Nematode dry mass (DM) was assessed by converting their WM (obtained for both automated and manual measurements), assuming a DM/WM ratio of 25% following the method of Andrassy (1956).

2.2.5. Statistical analyses

Differences in the measurements, including nematode lengths and widths, as well as analyses of the measurement durations between the manual and automatic methods and among the three persons performing the analyses were tested using univariate PERMANOVA and a model based on a similarity matrix created from the Euclidean distances among the samples. PERMANOVA is a non-parametric permutation-based test that is recommended for analysis of multivariate or univariate ecological data in the context of more complex sampling designs (Anderson et al. 2008). Unlike traditional multivariate statistical methods, PERMANOVA makes no assumptions regarding the distributions of the original variables as it acts on ranks of dissimilarities and uses permutations to obtain *p*-values.

Data on the time spent by an analyst (either a person or a software) on performing measurements (length and width) on 100 nematode specimens in a sample were used to test the differences in measurement times. A two-way PERMANOVA model was constructed (with two fixed factors: Method (manual vs. automated) and Analyst (three persons vs. a software) with Analyst nested within Method). Because of the low number of possible permutations, a Monte Carlo sampling-based method was used for assessing *p* values (p(MC)) as recommended by Anderson et al. (2008).

For length measurements, a three-way model was constructed with three factors: Site (Arctic muddy bottom vs. Baltic sandy seagrass beds), Sample (5 samples from each Site) and Analysts (three persons and an image analysis software) using a nested design with Sample nested within Site). The same design was used to test the differences in maximum width measurements (but with only three persons considered in the factor Analysts, as the software measures average nematode width (Eq. II.5), not the maximum width).

The effects of the method (factor Method - manual vs. automatic), analyst (factor Analyst) taking the manual measurements and calculation method (factor Calc) in the automatic analyses of the individual biomasses (InBiom) and total nematode biomasses (TotBiom, i.e., a summed IndBiom for 100 individuals measured in each a sample) in samples (factor Sample) were tested with the PERMANOVA. Three PERMANOVA tests were performed: three-way tests for differences between methods – (factor Method, automated vs. manual), and among the samples within different localities (factor Sample nested within Site, and Analyst/Calc nested within Method). In the case of TotBiom, two-way tests were performed to assess the differences between sites (factor Site) and the Analyst/Calc (nested within Sample); in the case of IndBiom, only these two factors were possible due to the lower number of replicates with TotBiom.

2.3. Results

All values hereafter are presented as means and confidence intervals (0.95CI). The measurement times (100 individuals per sample) differed significantly between the two methods as indicated by the PERMANOVA tests (PsF = 23.11, df = 1, p(MC) < 0.05), while no significant contrasts were detected among the analysts (factor Analyst, PsF = 0.05, df = 2, p(MC) > 0.05). The automated measurements required on average 26.7 min (0.95CI: 9.4 to 44.0 min), while the manual (MAN) measurements took on average 50.8 min (0.95CI: 38.7 to 62.9 min; Figure II.2a). The time of measurements also differed between sites (factor Site) - in Arctic muddy samples automated measurements (AUTO) were made 1.5 times faster than manual ones, while in samples from Baltic seagrass sandy beds 3.0 times faster (Figure II.2b).

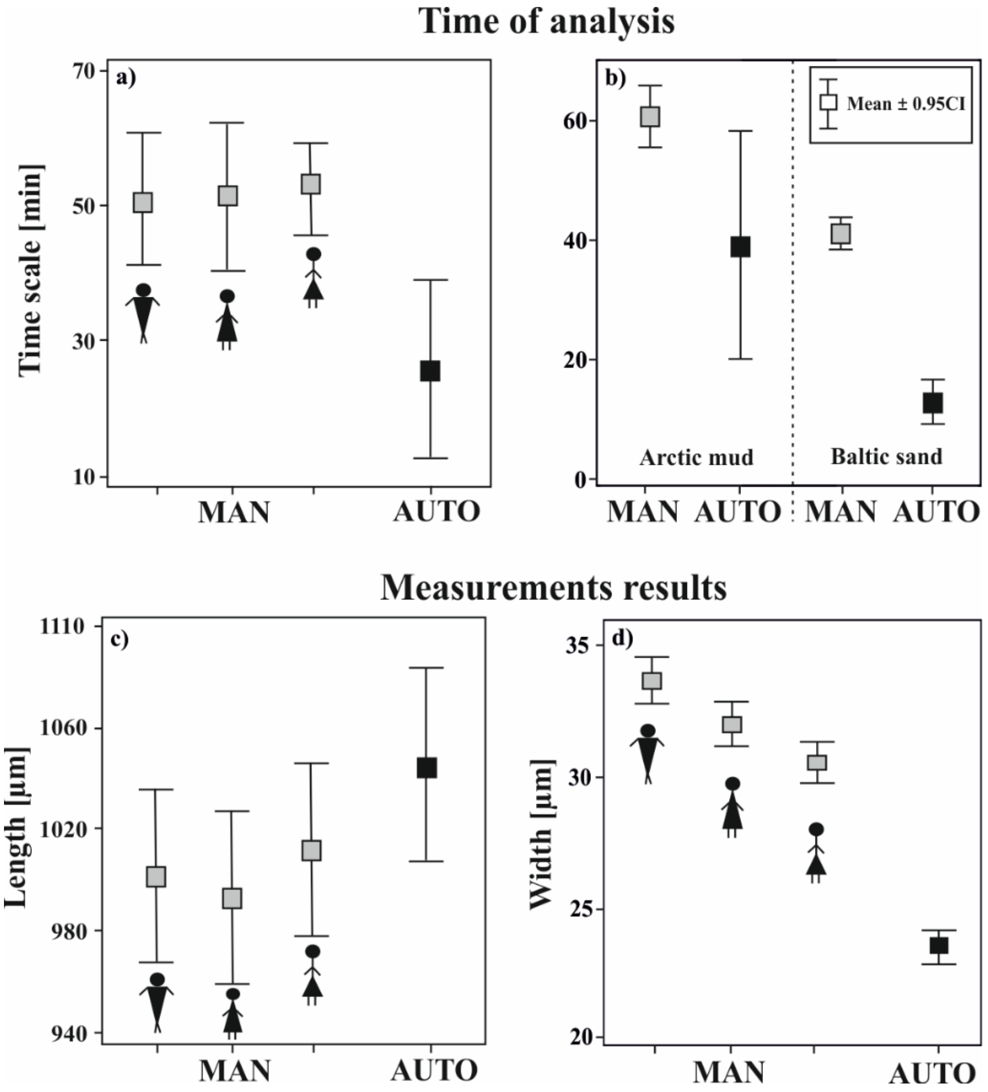


Figure II.2 Comparison of the duration [min] of nematode measurements (100 individuals per sample) between a) the manual (MAN, three researchers) and automatic methods (AUTO) and b) in different sites (Arctic muddy bottom and Baltic sandy seagrass beds) and comparisons of nematode measurements of c) length [µm] and d) width [µm] using manual and automatic methods. The means and 0.95CI are presented. Average width was measured by the automatic methods, while the maximum width was assessed by the manual method.

Average nematodes length was 1044 µm (0.95CI: 1008 to 1081 µm) when measured automatically (AUTO) and while measured manually (MAN) it ranged from 994 (0.95CI: 961 to 1027) to 1013 µm (0.95CI: 980 to 1046 µm; Figure II.2c). Significant differences in nematodes' average length were present among the samples and among the analysts (factor

Analyst, PERMANOVA test, $p<0.05$; Table II.1; Figure II.2c). The *post-hoc* test showed statistically significant differences between every pair of scientists but there were no differences between any scientist and automated (AUTO) measurements (PERMANOVA pairwise *post-hoc* test, $p > 0.05$).

Table II.1 Results of three-way PERMANOVA tests for differences in nematode measurements (length and width). Three factors were considered in a nested design: Site (Arctic muddy bottom vs. Baltic sandy seagrass beds), Sample (Smp) nested in Site and Analyst (An; three persons vs. software). Regarding width, only data from manual measurements were considered.

Length				Width			
Source	**df**	**PsF**	**p**	**df**	**PsF**	**p**	**Perms**
An	**3**	**4.413**	**0.017**	**2**	**29.206**	**0.001**	**998**
Site	1	0.715	0.417	1	3.540	0.087	982
Smp(Site)	**8**	**66.975**	**0.001**	**8**	**67.294**	**0.001**	**999**
An x Site	3	0.592	0.641	2	2.944	0.092	999
An x Smp (Site)	24	0.435	0.999	16	0.575	0.911	997
Res	4008			3006			
Total	4047			3035			

Mean value of maximum nematodes width measured manually ranged from 30.7 (0.95CI: 29.9 to 31.4) to 33.7 μm (0.95CI: 32.9 to 34.6 μm), the mean obtained through automated measurements was 23.7 μm (0.95CI: 23.2 to 24.4; Figure II.2d). Significant differences existed among both the samples (factor Sample) and the analysts (factor Analyst, PERMANOVA test, $p<0.05$), with no differences between locations (Table II.1). The *post-hoc* test showed differences between every pair of scientists (PERMANOVA pairwise *post-hoc* test, $p<0.05$).

Significant differences were found in individual nematode biomasses among the samples as well as between different methods (factor Method) of image analysis: MAN vs. AUTO. Significant differences were also documented for the factor Analyst/Calc (nested within the factor Method), both among the scientists and among the calculation methods (PERMANOVA test, $p<0.05$; Table II.2; Figure II.3). The PERMANOVA test did not indicate significant differences between different localities. The mean individual DM of specimens measured manually ranged from 0.22 (0.95CI: 0.20 to 0.24) to 0.27 μg DM (0.95CI: 0.24 to 0.30 μg DM). From those obtained by the automated measurements the closest were those calculated with Eq. II.1 (Cy) - 0.20 μg DM (0.95CI: 0.18 to 0.22μg DM).

Table II.2 Results of PERMANOVA tests for differences in individual nematode biomass and total biomass of 100 individuals per sample measured between different sites (Site), samples (Smp), between two measurement methods (factor Method (Me); automatic vs. manual), among Analyst/calculation methods (An/Calc; three analysts for manual measurements and three calculation methods for automatic measurements).

Individual biomass				Total biomass			
Source	**df**	**PsF**	**p**	**Source**	**df**	**PsF**	**p**
Site	1	0.003	0.994	Site	1	0.01	0.92
Me	**1**	**21.617**	**<0.01**	**An/Calc**	**5**	**2.38**	**<0.05**
Smp (Site)	**8**	**63.411**	**<0.01**	Site x An/Calc	5	0.05	1.00
An/Calc (Me)	**4**	**14.073**	**<0.01**	Res	48		
Site x Me	1	0.257	0.646	Total	59		
Site x An/Calc(Me)	4	0.590	0.705				
Smp(Site)x Me	**8**	**4.764**	**<0.01**				
Smp(Site) x An/Calc(Me)	32	0.624	0.967				
Res	6012						
Total	6071						

Significant differences in total biomass occurred among analysts and calculation methods (factor Analyst/Calc), but not between the sites (factor Site) (PERMANOVA test, $p<0.05$; Table II.2.). The pairwise *post-hoc* test showed that only values obtained with automated measurements and with use of the equation for the volume of a cuboid (Eq. II.2) and the Andrassy formula (Eq. II.3) differed from those obtained by manual measurements (PERMANOVA pairwise *post-hoc* test, $p<0.05$). The mean total DM based on manual measurements ranged from 22.4±7.6 to 27.6±11.2 μg DM, the one obtained from automated measurements and using cylinder based formula (Eq. II.1) was 20.2±7.3 μg DM while it was much lower when two other equations were used (Figure II.3).

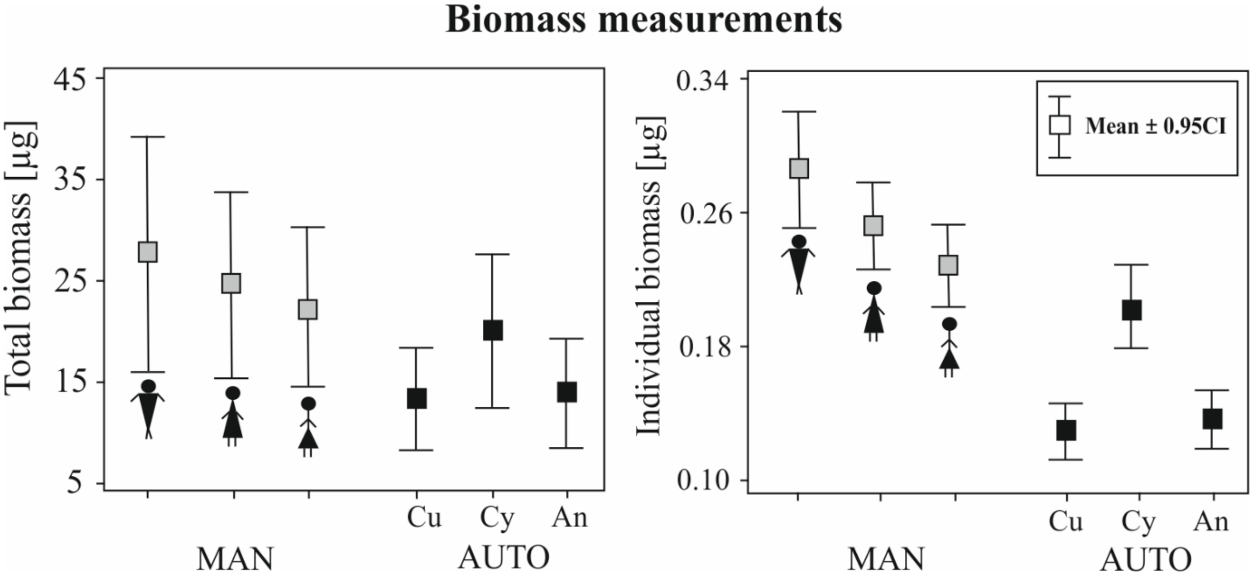


Figure II.3 Comparison of total nematode biomass (100 individuals per sample) and individual nematode biomasses assessed by manual measurements (MAN, three researchers) and automatic measurements (AUTO, using three equations: Cu - for the volume of a cuboid, Cy - for the volume of a cylinder, and An - the Andrassy (1956) formula). Means and 0.95CI are presented.

2.4. Discussion

2.4.1. Biomass of nematodes in marine sediments

The use of manual measurements of the lengths and maximum widths of nematodes to estimate their biomass with the formula proposed by Andrassy (1956) is the most commonly used method in present-day studies of marine meiofauna that document biomass values (e.g., Drgas et al. 1998; Vanaverbeke et al. 2003). The method is relatively simple, but is very laborious and time consuming, as it requires measuring dimensions of each individual, that requires drawing a segment line along every nematode (to assess the organism length) and selection of the widest point and plotting a respective line across the organism body (to assess the width). Therefore, despite the huge ecological importance of assessing biomass, the extensive literature on marine benthic nematodes seldom reports the biomass of the studied biota. This is a major gap in the basic information needed to evaluate the role of these organisms in marine ecosystems. A new semi-automated method presented in this chapter may facilitate and shorten the time required for such analyses and thus foster a wider acquisition of biomass data in meiofaunal ecological surveys.

Few reports about nematode biomass exist in the literature. Ólafsson and Elmgren (1997) reported the dry mass of the Baltic Sea nematodes near Askö at a depth of 37 m, which varies from 0.16 to 0.47 mg 10 cm^{-2} over the entire year, with an average value of 0.29 mg 10 cm^{-2}. On the sandy shallow bottom in the Gulf of Gdańsk the total nematode biomass ranges from 0.27 to 1.14 mg 10 cm^{-2}, with the biomass size spectrum of individuals spreading from 9.95 to 79.05 µg DM (Drgas et al. 1998). In the fine-grained sandy bottom enriched with a small fraction of mud and poorly oxygenated sediments of the Belgian continental shelf, mean annual individual nematode biomass ranges from 0.13 to 0.18 µg DM, while in the medium sand-dominated and well-oxygenated sediments, this value varies from 0.07 to 0.09 µg DM (Soetaert et al. 2009). Nematodes from the Arctic exhibit quite high variations in individual biomass. In the central Arctic Ocean in 1991 at depths 1072–4273 m, Vanreusel et al. (2000) reported mean individual biomasses ranging from 0.03 to 0.15 µg DM and total biomasses spreading from 1.56 to 10.35 µg DM 10 cm^{-2}, while in 1994 at depths of 540–4190 m the individual biomass ranged from 0.01 to ~0.08 µg DM, and the total biomass from 0.6 to 20 µg DM 10 cm^{-2}. In an Arctic fjord (Kongsfjorden) the biomass of nematodes was also reported to vary substantially from 0.22 to 468.26 µg C 10 cm^{-2} (Kotwicki et al. 2004), i.e., approximately from 0.55 to 1170.65 µg DM 10 cm^{-2}. The total biomass in the samples analysed in the study presented in this chapter calculated on the basis of the automated method using the equation for the volume of a cylinder (Eq. II.1) agrees with the values mentioned above, both for the Baltic sands and Arctic muddy bottom; it ranges from 25.04 to 615.53 µg DM 10 cm^{-2} and did not differ between the two environments.

2.4.2. Manual vs. automated measurements

The consistency of the results obtained manually or automatically differed between the two dimensions considered (length and width). The lengths of animals did not differ significantly either among the analysts or between the human and computerized measurements.

This similarity confirms the accurate performance of the software and the relative robustness of the length measurements to the individual analysts' inconsistencies. The width measurements seem to be much more troublesome; the results differed significantly among the three analysts performing the analyses. The manual method requires measuring the width at the widest point of the nematode body. The significant differences among the analysts' results point to a strong subjectivity and inaccuracy embedded in procedures that require selecting the widest point of the imaged nematode. Measurements were often taken at different points by different analysts (Figure II.4) that obviously resulted in different values of "maximum width" for the same individual. Those differences result from trifling variations in the width of the nematode along its body, with the widest point being very hard to distinguish and precisely designate on the image. The accuracy of the widest point selection is rather not dependent on the experience of an analyst and these inconsistencies cannot be totally eliminated. Differences in widths obtained using different methods (manual vs. automatic) are, of course, due to the different measurements - the maximum width was obtained with the manual method and the average nematode width was provided by the computerized analysis. Obviously, the latter approach is much less susceptible to the bias produced by the personal decision of selecting the point of the maximum width and yields more objective results (Schindelin et al. 2012). Hsu et al. (2002), who compared manual and automated methods of analysing magnetic resonance images (MRI), presented similar conclusions. They stated that their automated method is much less time-consuming comparing to manual analysis and less susceptible to the analyst bias during manual tracing; therefore it should be widely adopted in MRI studies. Also Feng et al. (2004) who proposed an automated imaging system for the *C. elegans* behaviour analysis, commented that their approach should eliminate the human scoring bias.

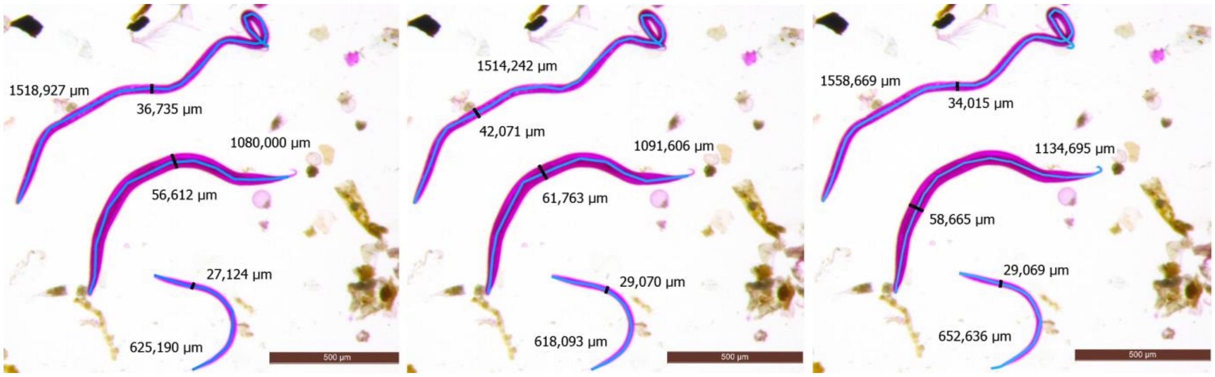


Figure II.4 The same picture with three nematodes measured manually (length and width at the widest point) by three scientists.

2.4.3. Factors influencing duration of measurements

The average time for automated measurements was approximately two times shorter than that for the manual procedure; however, we also found some differences in analysis time between the studied localities (habitats). The automated measurements were approximately 1.5 times faster than the manual measurements for Arctic samples, while three times faster for samples from the seagrass beds. Arctic samples were collected from a muddy bottom after the spring bloom period and contained much more organic debris than the relatively “clean” samples from the sandy seagrass beds. Organic debris slowed the automatic procedure because some of these particles were designated by a program as nematodes, or nematode body parts (e.g. if located too close to an animal) and thereby changed its area; thus, much more work was needed to correct the results of the analyses during the post-procedure. Moreover, because of the clarity of the seagrass sandy samples, more nematodes could be photographed in one image; this also shortened the time of automated analysis, as fewer pictures had to be processed. The dependency of semi-automatic method on the “clarity” of the sample resulted also in the higher variation of the individual sample analyses time in semi-automatic method (Figure II.2) than in manual method that is less sensitive to such constraints.

2.5. Comments and recommendations

The described semi-automated method gave similar estimates of the total nematode biomass as the traditional manual measurements and was much less time-consuming and more objective in making certain required measurements (i.e. individual width). The method described here also has some obvious advantages over the semi-automated method proposed by Baguley et al. (2004). The latter method required that a group of nematodes was placed on slides - a preparatory step that was both quite time consuming and required a high level of precision and experience by an analyst. In the study presented in this chapter pictures were taken when nematodes were placed on a counting dish. The grid marking on the bottom of the dish permits the easy handling of the subsample and continuous imaging of the individuals in the field of view. It is important to ensure that the animals have settled to the bottom of the dish and do not float in the water; thus, only the least possible amount of liquid should be added to the sample. The major difference between the two semi-automatic methods is actually in the measurements that are to be collected. In presented method, software measures the length and width of the animal, while in the approach used by Baguley et al. (2004) the area covered by an animal on the picture was measured automatically; however, the mid-body width (maximum width) was measured by a computer, but in a location that needed to be indicated manually. As it was shown in the study presented in this chapter, the manual selection of the point of maximum width is very subjective and can produce statistically significant differences between the measurements performed by different analysts. The automatically obtained average width is much more objective and choosing this measure eliminates the problem of subjective choice-related analyst bias. A common feature of both methods is their rapidity. The method described here allows to analyse, on average, the same number of nematodes almost 2 times faster than the manual method. Baguley et al. (2004) reported that their automated measurements were 3 times faster than the manual ones, which could also result from the fact that they photographed individuals placed on slides; this eliminated the disturbance produced by organic debris. In the study presented in this chapter, the seagrass samples, which contained very little organic debris, were probably similar to Baguley et al. (2004) slides in terms of clarity, and their automated measurement was also 3 times faster than manually performed. However, the preparation of slides is also time-consuming and duration of this step (it takes about 10 min per slide with 10 nematodes, not including transferring and impregnating them in glycerine solutions) should be added to total time of the analysis using the Baguley et al. (2004) method. Certainly, if the preparation of slides is necessary, for instance for further taxonomic identification, they can also be analysed using presented method. Moreover, in contrast to the methods constructed for worms cultivated on agar plates, which represent "clean" samples (e.g. Mathew et al. 2012; Moore et al. 2013) method presented here works fine with environmental samples that are cluttered with organic debris.

The study presented in this chapter shows that the maximum width of an individual nematode - a measure required by the manual method and the usage of Andrassy (1956) formula - is very subjective, as it requires the analyst to choose for the measurement the point on the nematode body, which is tapered only on ends, while most of the body width changes slightly. The significant differences were shown in the width values obtained for the same individual nematode among the experienced researchers (Figure II.2). Quite probably, even the same person measuring maximum width of the same animal after some period of time could obtain different values, because finding the widest point of the nematode body is not straightforward. Therefore, while using the manual method, it is recommend to measure the animal's width at a several possible "maximum width" points and then calculate an average value or use the highest value to improve the precision and decrease the level of error. Such a routine would require extra time but may improve the reproducibility of the results of manual analyses.

The automated method presented here is also prone to certain errors, or at least prolongation of the analysis time resulting from operator choices in the computer system. For example, the color threshold must be selected manually by the analyst. This step must be performed at the beginning of the analysis and the threshold color range must be selected depending on the color properties of the nematodes in a given sample (set of pictures). An inaccurate selection of the threshold can result in designating too small area of a nematode, so I propose to select little wider threshold exceeding the colors of nematodes. Although, this can be also corrected later by selecting the "Mode and Size" of the Binary Processing Pre-Filter that will fill in missing areas, this will prolong the total time of the analyses. Another potentially time-consuming obstacle may be the stained organic debris that sometimes are wrongly designated by the software as individual nematodes. They need to be excluded in the post-processing in the Binary Image Editing step by selecting the unwanted objects or areas. In some cases, problems can also be generated by dark fragments of organic matter that shade the nematode or by nematodes that overlap other individuals or obscure parts of themselves. In such cases, additional manual treatment is required to ensure the proper automatic measurement. The easiest procedure is to draw a line separating different parts of animal body and then to point to the unwanted material that is not to be taken into account. During manual measurements the human analysts are able to intuitively draw the measuring segment line along the whole nematode, even if some parts of the individual are less visible.

The method described in this chapter is based on Leica software, however the same procedure may be applied using any other image analyses software that allows for: 1) automatic detection of nematodes e.g on the basis of color threshold and specified parameters, 2) automatic measurements of nematodes dimensions, 3) manual correction of objects detected by software. The important prerequisite is that the software automatically scales the image to the metric units, as images are taken with various magnifications (depending on the size and number of nematode individuals in view). This is usually provided by the software that is dedicated to a particular microscope based system of image acquisition (e.g. Leica Application Suite system). In case of using a non-dedicated image processing software (like ImageJ) the scale can be retrieved from the image metadata, that can be assessed e.g. with use of Bio-Formats Java library (http://www.openmicroscopy.org/site/support/bio-formats5.1/).

Three different equations were used in the study presented in this chapter to estimate biomass with use of semi-automated measurements. The biomass computed on the basis of the cylinder volume equation gave the closest results to those obtained using the standard ecological method (i.e. manual measurements combined with Andrassy biomass formula). Baguley et al. (2004) also reported that a use of the cylinder equation combined with the semi-automatic measurements gave estimates that were not significantly different from the direct measurements of biomass, while application of cuboid equation gave much lower values (similarly to the study presented in this chapter). More precise and sophisticated approaches to estimate of nematode biovolume have been developed for genetic dissection of nematode behaviours or analyses of growth phenotypes. These include dividing the organism body into several “segments”, measurement of the dimensions, volume calculation of each marked frustum and then summing them (Moore et al. 2013), or visualization of 3-D models of nematodes from different worm projections and summing intersecting voxels (Kwon et al. 2015).

In conclusion, it is recommended to use described semi-automated method for nematode biomass estimation on the basis of the equation for the volume of cylinder in routine ecological surveys of meiofaunal communities, as well as in more specialized studies focusing, for example, on biomass size spectra in benthic biota. Moreover, semi-automated methods, including the one proposed in this study, can become particularly useful in the studies that apply metagenomic methods to assess the meiofaunal diversity (e.g. Creer et al. 2010). In the future, the combination of metagenomics and semi-automated image analyses can provide a basis of a protocol for rapid assessment of both the diversity and biomass (as well as the size spectra, secondary production and respiration) in the meiofaunal materials. I showed that this method both shortens the duration and secures the objectivity of results of the analyses compared to traditional manual procedures. Compared to the procedure proposed by Baguley et al. (2004), method presented in this chapter does not require any special preparations of meiofaunal samples and nematode images can be taken during routine sample analysis (organisms identification and counting), moreover the nematode measurements can be done almost parallel, just after image acquisition. Herein it was applied and tested on Nematoda, the taxon that is both predominant in marine meiofauna and relatively easy for application of automating methods due to its simple body shape. However, it could be also applied to representatives of other groups, using the measurements and equations recommended for each group in standard ecological methodology (Warwick and Gee 1984; Giere 2009; Danovaro 2010). The application of a semi-automated image analyses to assessment of Harpacticoida biomass was presented by Baguley et al. (2004). Wider application of proposed method should facilitate biomass assessments in ecological studies of marine meiofauna.

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Chapter III. Seasonal constancy (summer vs. winter) of benthic size spectra in an Arctic fjord

3.1. Introduction

The seasonality of environmental parameters like light conditions, air temperature, precipitation, meltwater discharge, iceberg impact, or ice-cover is a major regulator of Arctic marine ecosystem dynamics (Loeng et al. 2005). Those environmental factors influence functioning of the marine ecosystem, which is mostly shaped by both temperature-dependent (e.g. water stratification or metabolism rate) and light-dependent phenomena (Berge et al. 2015a). Seasonality in physical settings influences the timing and rates of primary productivity, and thus, the food supply for consumers forcing organisms to adapt to periods of lower organic matter fluxes (Pawłowska et al. 2011; Juul-Pedersen et al. 2015). The signals of seasonality in benthic communities structure and activity were reported, for example as a rise of benthic oxygen uptake after the seasonal pulse of organic matter sedimentation (e.g., Rysgaard et al. 1998, Renaud et al. 2007, 2008, Link et al. 2011). Studies of Kędra et al. (2011) documented that shallow water communities exhibited seasonal variation in abundance of carnivores and opportunistic species. Also Pawłowska et al. (2011) reported a clear seasonal variation of meiofauna and macrofauna standing stocks and diversity in a glacio-fluvial bay (Adventfjorden, Svalbard) related to temporal variability in primary production and magnitude of meltwater discharges. However, the paradigm of dramatically lower activity of Arctic organisms during the unproductive season of polar night was recently questioned by Berge et al. (2015b). They documented intense biological activities during polar night, implying that, despite the absence of primary production, many biological processes and trophic interactions are maintained on a similar level as in other seasons, based on stored, recycled, or advected energy sources (organic carbon). It was also shown that benthic fauna from Kongsfjorden (Spitsbergen) can be very resilient to seasonal changes in food quality, thanks to the ability to utilize reworked organic material (Kędra et al. 2012). Similar, constantly high standing stocks and activity of the benthic communities observed in all seasons on the Antarctic continental shelf have been explained by the "food bank" hypothesis (Mincks et al. 2005; Glover et al. 2008) that related low benthic seasonality to the persistent availability of labile organic carbon in sediments throughout the year.

Biomass size spectra proved to be useful descriptors of both community structure and its functioning (Sprules et al. 2016). However studies that comprise analyses of benthic size structure in the Arctic are relatively scarce. In the northern polar region they have been used by Soltwedel et al. (2000) to reveal a relationship between mean biomass of deep-sea nematodes and concentration of the photosynthetic pigments in sediments and by Górska and Włodarska-Kowalczuk (2017) who showed that food availability and disturbance may control the total bulk and size structure both in meiofauna and macrofauna in fjords.

Both Arctic publications as well as most of the other published size structure studies have been based on materials collected in one season only (mostly in summer). However the Arctic system is characterized by a large seasonal variability in environmental conditions. Pelagic primary production, organic matter fluxes to the sea bottom and fauna recruitment events are especially variable (Kukliński et al. 2013). Therefore there is a need of the recognition if/how the seasonal variability in environmental features (such as food supply) influences the community size structure (Kelly-Gerreyn et al. 2014). This is crucial to know whether patterns observed in summer are representative for the year-round situation.

The aim of chapter III was to examine seasonal (summer-winter) variability in benthic (meiofauna and macrofauna) community size structure in a high Arctic fjord, which is characterized by high seasonal variability of environmental conditions. Hydrological parameters and geochemical descriptors of organic matter content in sediments, as well as abundance, biomass and taxonomic composition of the fauna were recorded to provide background environmental and biological constraints on this variability. Seasonal variability in the size structure of benthic community would imply seasonal changes in organic carbon processing, nutrient fluxes as result of trophic interactions or recruitment events. According to the current state of knowledge, this is the first study focused on seasonal variability in benthic size structures in the Arctic.

3.2. Materials and methods

3.2.1. Study area

Kongsfjorden (79˚N and 12˚E) is a fjord off the northwest coast of Spitsbergen - the largest island of the Svalbard archipelago (Figure III.1). This 28 km long fjord is oriented along northwest-southeast axis, with a 10 km wide opening connecting the fjord with the Greenland Sea. Four tidewater glaciers flow into the fjord on the eastern and northern sides and are important sources of meltwaters loaded with mineral material, strongly affecting the inner part of the fjord (Svendsen et al. 2002; Włodarska-Kowalczuk and Pearson 2004). The open mouth with no sill allows for the penetration of oceanic, warm water transported by the West Spitsbergen Current (WSC, Svendsen et al. 2002). The sea bottom of Kongsfjorden deep basins is covered with mud sediments (Włodarska-Kowalczuk and Pearson 2004), and the sedimentation rate decreases with increasing distance from glaciers (Svendsen et al. 2002).

The Kongsfjorden ecosystem undergoes strong seasonal and multi-annual changes related to variation in hydrography, driven by impact of the WSC, melting of snow and glaciers, local climate features and global climate changes (e.g., Cottier et al. 2005; Noufal et al. 2016; Wiencke and Hop 2016). These processes are key regulators of ecosystem functioning as they determine timing of the spring bloom, taxonomic composition of pelagic communities or biogenic matter fluxes (e.g., Hop et al. 2006; Hegseth and Tverberg 2013; Lalande et al. 2016). The influence of the WSC (especially in summer) decreases with increasing proximity to the glaciers, due to glacier meltwater discharge (Svendsen et al. 2002). This gradient between oceanic waters and meltwaters, which are highly turbid, is reflected in decreased primary production rates and/or changes in benthic species composition and diversity (Piwosz et al. 2009; Walkusz et al. 2009; Włodarska-Kowalczuk et al. 2012). Benthic community structure in soft sediments of the fjord has been relatively stable for the past 15 years (Kędra et al. 2012).

The strength and timing of the spring phytoplankton bloom is dependent on advection of water from the WSC into the fjord. Inflows along the bottom allow for the convection and mixing, enhancing the bloom, while surface inflows can hinder water mixing and delay the bloom (Hegseth and Tverberg 2013). The spring (April-June) blooms result in increased amounts of zooplankton faecal pellets and chlorophyll *a* concentration in the water column

(Lalande et al. 2016). In summer, as a result of river runoff, precipitation and glacier melting, a surface layer of less saline waters forms, that together with a strong subsurface intrusion of warm and more saline Atlantic waters, causes strong stratification of the water column (Noufal et al. 2016). In fall, when glacier melting weakens and vertical mixing is possible a more favourable conditions for phytoplankton and zooplankton occur and their activity increases enhancing the organic matter fluxes, however they are much lower comparing to the spring bloom period (Lalande et al. 2016). In winter, as a consequence of water cooling the whole water column is well mixed (Noufal et al. 2016). During this time the biological activity, both autotrophs and zooplankton in water column is very low resulting in low organic matter fluxes to the sea bottom (Lalande et al. 2016).

3.2.2. Sampling

Samples were collected in summer (August 2014) from R/V "Oceania" and in winter (January 2015) from R/V "Helmer Hanssen". Sediments, meiofauna, and macrofauna were collected at three stations located in the outer basin of the fjord at depths varying from 260 to 350 m (Figure III.1; Table III.2). Sampling was intended to occur at exactly the same positions in winter and summer, but due to the navigational issues the actual locations of sampling in the two seasons differed by about 450 m (station KB1), 390 m (station KB2) and 220 m (station KB3). Macrofauna samples (one sample per station per season) were collected with use of 0.1 m^2 van Veen grab and sieved on board on a 500 μm mesh. Meiofauna samples (one sample per station per season) were collected with a plastic syringe ($10cm^2$ sampling area) inserted 10 cm deep into sediment collected with a box-corer. Both macrofauna and meiofauna samples were preserved in 4% formaldehyde solution in seawater. Sediment samples were collected with a Nemisto gravity corer. One core per station for grain size analyses was collected only in summer. In both seasons one core for particulate organic carbon (C_{org}) content and three replicates for photosynthetic pigments (chlorophyll *a*, phaeopigments) were collected at each station. Each core was cut into 1 cm slices and frozen (samples for photosynthetic pigment analysis in - 80 °C; other samples in - 20 °C). In both seasons temperature and salinity were measured in the water column at each station (in July with use of CT-Set mounted at Hydro Bios MultiNet, in January with use of a Sea-Bird SBE 911 plus CTD system).

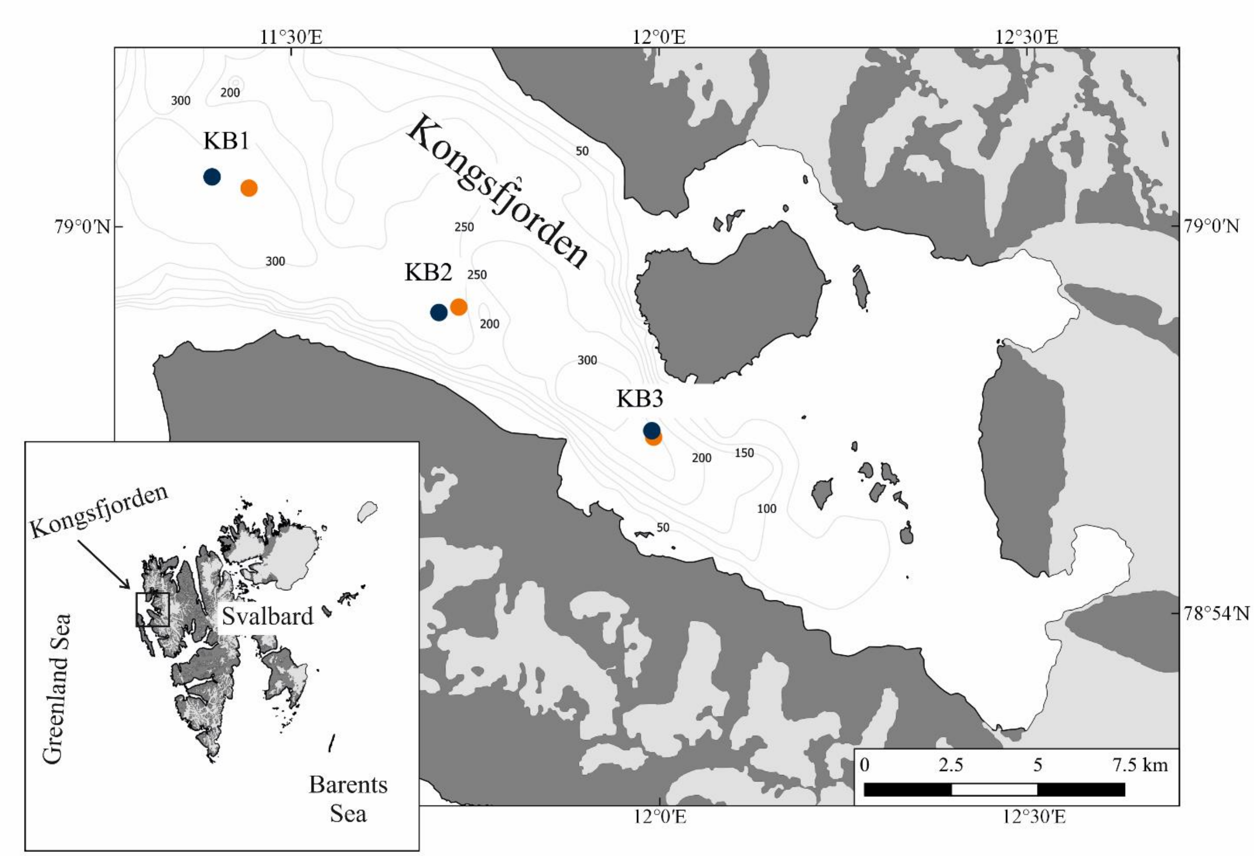

Figure III.1 Location of sampling stations in Kongsfjorden. Orange dots represent locations of sampling station in summer, blue dots – in winter, glaciers are indicated by light grey colour.

3.2.3. Laboratory analyses

Chlorophyll *a* (Chl *a*) and phaeopigment concentrations in the sediment samples were measured using a fluorometric method. Pigments were extracted from freeze-dried sediments in 90% acetone for 24 h at 4 °C (Evans et al. 1987). Measurements were performed with use of a Perkin Elmer LS55 Fluorescence Spectrometer. Emissions at 671 nm and excitations at 431 nm were measured before and after sample acidification with 1M HCl, and used to calculate the chlorophyll *a* and phaeopigment concentrations, according to the method described by (Evans and O'Reilly 1983). The sum of chlorophyll *a* and phaeopigment concentrations is defined as chloroplastic pigment equivalent (CPE). Grain size distribution was determined with a Malvern

Mastersizer 2000 particle size analyser. Grain size parameters were recalculated using the GradiStat 4.0. software. The C_{org} content (%) was determined via continuous flow - elemental analysis - isotope ratio mass spectrometry (CF-EA-IRMS) at the University of Liège with use of a Vario Micro Cube elemental analyzer (Elementar Analysensysteme GmBH, Hanau, Germany). Prior to analysis, sediments were dried at 60˚C for 48h, ground, and acidified with direct addition of 1M HCl to remove carbonates (Hedges and Stern 1984). Then the acid was diluted with distilled water, samples were centrifuged and, after the removal of the solution, dried again at 60°C for 24 h. Subsamples of about 15 mg of the sediment were packed into tin capsules.

All specimens from macrofauna samples were enumerated and identified to the lowest possible taxonomic level. Specimens were photographed with a Leica DFC450 digital camera connected to Leica M205C stereomicroscope. Taxon-specific measurements (Table III.1) were performed with use of Leica LAS Manual Measurements software. In the case of species occurring in numbers higher than 250 per sample, a subsample of 500 randomly picked specimens was measured and these data were extrapolated to the total number of individuals in a sample. In total 6280 macrofaunal individuals were measured.

The meiofauna samples were centrifuged three times in a solution of colloidal silica (Ludox TM-50, density of 1.18 g cm^{-3}) and stained in a 4% buffered formaldehyde solution with Rose Bengal for at least 24 hours (Heip et al. 1985). Then samples were sieved and only specimens that passed through 500 µm mesh and retained on 32 µm mesh were analysed. All specimens were identified to the higher taxa (mostly phylum) level. In each sample five hundred randomly selected individuals of Nematoda (the dominant taxon) and all individuals of other taxa were photographed with Leica DFC450 digital camera connected to Leica M205C stereomicroscope. Nematode lengths and average widths were measured using semi-automated method of image analyses (Mazurkiewicz et al. 2016, Chapter II); in other taxa lengths and maximum widths were measured manually. In total 4241 meiofaunal individuals were measured.

3.2.4. Statistical analyses

Differences in environmental parameters (C_{org}, Chl a, CPE) in surface sediments (0-2cm) between two seasons (Season) and among three stations (Station) were tested with use of two-way balanced analysis of variance (ANOVA). Pairwise *post-hoc* comparisons were performed with use of a Tukey's adjustments of P-values. Prior to analysis data were transformed using power (Box-Cox) transformation to normalize the data and equalize the variance.

The biovolumes of meiofaunal and macrofaunal individuals were calculated based on the measured dimensions. For meiofauna, biovolume was calculated with use of the Feller and Warwick (1988) formula: $V = L \times W^2 \times c$ where V is the volume, L - the maximum length, W - the maximum width, and c - taxon-specific coefficient. Wet mass (WM) and consequently dry mass (DM) were estimated using following equations: wet mass (WM) $= 1.13 \times V$, $DM = 0.25 \times WM$ (Feller and Warwick 1988). For macrofauna – body shapes were matched with geometric figures (Hillebrand et al. 1999; Table III.1). The total lengths of fragmented polychaetes (needed for the individual biovolume estimation) were calculated with use of regression formulas based on relationships between widths of selected chaetigers and animal lengths (Górska 2018). WM was calculated by multiplying the biovolume by a specific gravity factor of 1.13 (Andrassy 1956). For Crustacea and Ophiuroidea WM was obtained from measured dimensions, using published conversion factors (Berestovski et al. 1989). Body mass conversion factors (Brey et al. 2010) were used for obtaining the shell free WM (in case of calcifying organisms) and subsequently DM.

Differences in average individual size (indicated by individual DM) between two seasons (Season) and among three stations (Station) were tested for meiofaunal and macrofaunal taxa represented by more than 30 specimens in every sample. Data were transformed using power (Box-Cox) transformation. Two-way unbalanced analysis of variance (ANOVA type II) was performed to examine the differences according to stations and season. Pairwise *post-hoc* comparisons were performed with a Tukey's adjustments of P-values.

Abundance and biomass size spectra were constructed by using groupings of organisms based on their individual DM (µg) on a $\log_2$ scale. Each size class (SizeClass) is two times larger than the preceding one. For example, size class 5 includes organisms of DM that is $\geq 2^5$ and

$< 2^6$ µg (i.e. ≥ 32 and < 64 µg – the size class covers a range of 32 µg), while size class 4 includes DM values that are $\geq 2^4$ and $< 2^5$ µg (i.e. ≥ 16 and < 32 µg – the size class covers a range of 16 µg). Each specimen was assigned to a body size class and the total abundance and biomass of all specimens in each class were calculated and used to construct abundance and biomass size spectra, respectively (Sheldon et al. 1972; Duplisea and Drgas 1999).

Table III.1 List of taxon-specific morphological features measured during laboratory analysis and geometric figures used for biovolume calculations.

Taxonomic group	Measured feature	Geometric figure
Polychaeta	Length and maximum width of anterior cheatigers	Cone
Oligochaeta	Length and maximum width	Cylinder
Amphipoda, Cumacea, Isopoda	Length along dorsal side	-
Tanaidacea	Length and width	-
Bivalvia	Length, width and thickness	Ellipsoid
Gastropoda	Maximum width and distances from each anterior point to the maximum width line (perpendicular to maximum width line) (Figure III.2)	Cone, double cone or prolate spheroid
Scaphopoda	Length along the longer side and maximum width	Cone
Caudofoveata	Length and maximum width	Cylinder
Ophiuroidea	Disc diameter	-
Priapulida	Length and maximum width	Cylinder
Sipuncula	Length and maximum width (in case of specimens without regular shape, wider and narrower fragments were measured separately) (Figure III.3)	Cylinder or double cylinder
Nemertea	Length and maximum width	Cylinder
Ascidiacea	Length and maximum width	Prolate spheroid
Porifera	Length and maximum width	Prolate spheroid

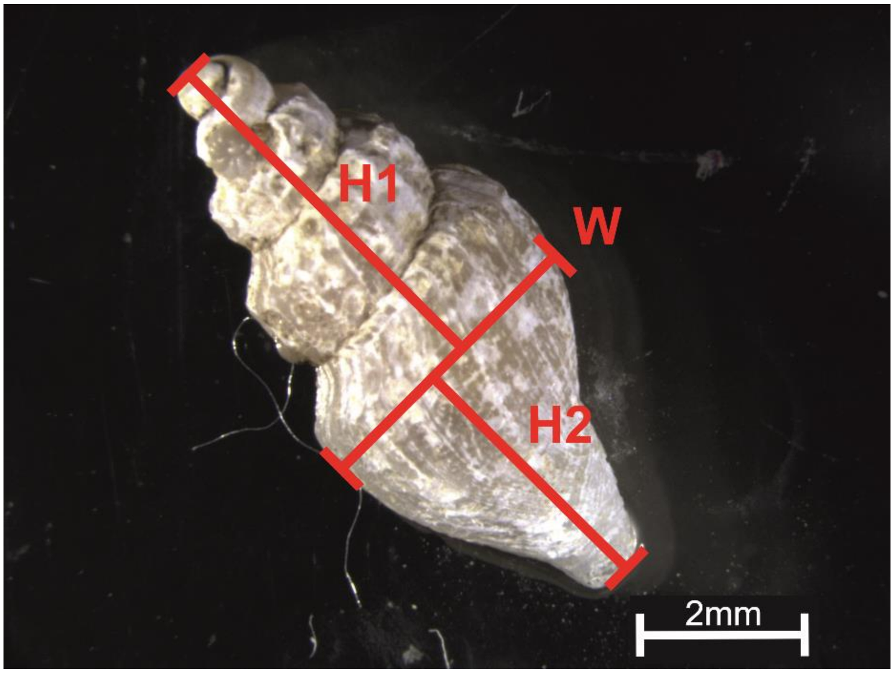

Figure III.2 Measurements conducted on a gastropod to calculate its biovolume as a sum of two cones. H1 and H2 – distances between anterior points and maximum width line W.

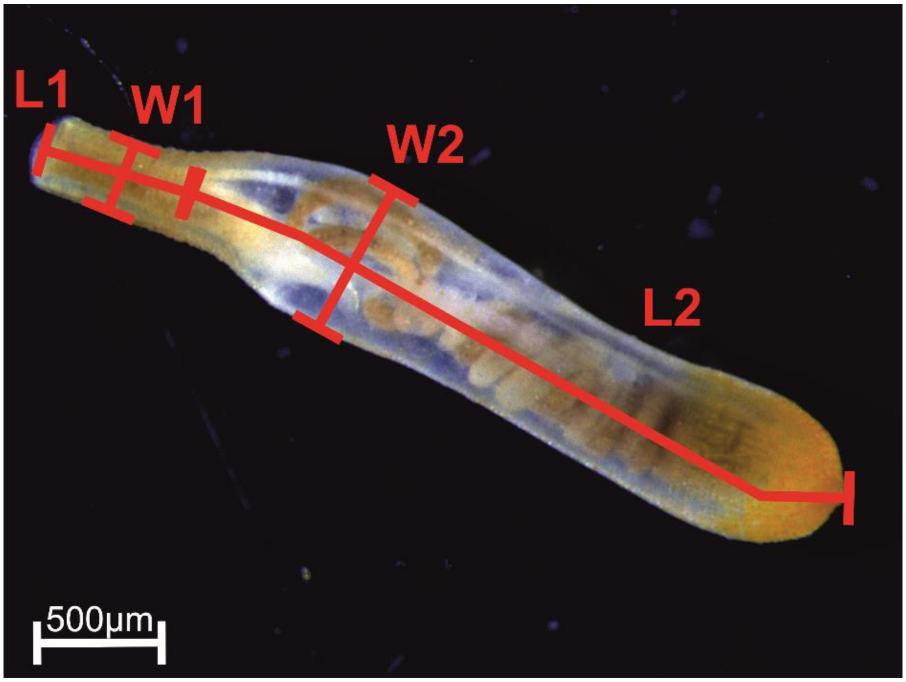

Figure III.3 Measurements conducted on a sipunculid worm to calculate its biovolume as a sum of two cylinders. L1 and L2 – lengths of each separate fragment; W1 and W2 – maximum widths of each separate fragment.

Multivariate analyses were applied to explore the patterns of similarity in taxonomic and taxonomic coupled with size class composition (each taxon spread among size classes) at three stations and in two seasons. Bray–Curtis similarities were calculated for abundance (square-root transformed) and DM (fourth-root transformed) in samples for: 1) meiofauna higher taxa, 2) meiofauna higher taxa/size classes, 3) macrofauna taxa and 4) macrofauna taxa/size classes. Two different data transformations were used due to different magnitudes of variability in biomass and abundance data. The effect of season on these compositions was tested using one-way PERMANOVA model with permutation of residuals under a reduced model, with Monte Carlo sampling used to increase the interpretability of the test due to low number of possible permutations (Anderson et al. 2008).

The normalised biomass size spectra (NBSS, Platt and Denman 1977, 1978) were plotted to correct for the distortion of biomass distribution caused by the logarithmic increase in width of size class bins in biomass or abundance size spectra (Sprules and Barth 2016). The total biomass in every size class was normalized by dividing it by the range of this size class (Δ size class) and $\log_2$ transformed. We determined the parameters of NBSS in every sample using ordinary least squares (OLS) linear regression of normalized biomass (NB) vs. SizeClass.

To assess the influence of Station and Season on the intercepts and slopes of NBSS, the multiple linear regression was carried out with SizeClass as a continuous covariate and Station and Season and as categorical predictors. A stepwise procedure was applied to find the most parsimonious regression model on the basis of Akaike's information criterion (AIC, Akaike 1974). The analysis of covariance (ANCOVA) was performed on final model to assess the significance of predictors. Tukey-adjusted *post-hoc* comparisons were performed to assess significant differences between coefficients of regression model.

The calculation of Bray-Curtis similarities and PERMANOVA analysis were performed in PRIMER with PERMANOVA+ software (version 7; Clarke and Gorley 2015). The other statistical analyses were performed in R statistical environment (R Core Team 2018). The lambda λ for power (Box-Cox) transformations was estimated using *boxcox()* function from *MASS* package (Venables and Ripley 2002). For balanced ANOVA *aov()* function was used and *lm()* for liner regression. Unbalanced ANOVA type II was performed with *Anova()* function from *car* package (Fox and Weisberg 2011). For *post-hoc* pairwise comparisons with a Tukey's adjustments of P-values *lsmeans()* function from *emmeans* package was used (Lenth 2018). The visualization of results was plotted with use of: *ggplot2* (Wickham 2009), and *gridExtra* (Auguie 2017) packages. The map (Figure III.1) was produced in QGIS 3.2 software (QGIS Developement Team 2019) with use of data (land extent, glaciers extent) provided by Norwegian Polar Institute (http://data.npolar.no) and bathymetry provided by Norwegian Mapping Authority (https://www.geonorge.no).

3.3. Results

In summer, salinity was homogenous throughout the water column, except for the uppermost 20 m where it was distinctly lower (possibly due to meltwater discharge), while the temperature was slightly higher in the few upper meters and then decreased with depth to the bottom layers. In winter both salinity and temperature were constant throughout the water column (Figure III.4). Near-bottom temperatures and salinities were higher in summer (on average 2.37˚C and 35.34, respectively) than in winter (1.23˚C and 34.84; Figure III.4).

Sediments at sampling stations consisted mostly of mud (summed clay and silt), that constituted from 60 to 100 % of sample content (Figure III.4). The C_{org} content in sediments decreased slightly with sediment depth, the values differed among the three stations ($F_{(2,10)}=185.2$, $p<0.001$), but not between the two seasons ($F_{(1,10)}=0.1$, $p=0.71$). The highest values in the surface layer (0-1 and 1-2 cm) were noted at station KB1 (mean±SD: 2.18±0.18%), lower at station KB2 (1.76±0.12%) and the lowest at KB3 (1.09±0.05%), values at each station were significantly different from two others (Tukey-adjusted *post-hoc* comparisons, $p<0.001$). Regarding the photosynthetic pigments, their concentrations decreased with sediment depth (Figure III.4). There was no seasonal difference in Chl *a* content in surface sediments (0-1 and 1-2 cm; $F_{(1,30)}=3.2$, $p=0.08$), however there were significant differences among stations ($F_{(2,30)}=9.6$, $p<0.001$). At station KB1 average Chl *a* content in surface sediment was significantly higher (mean±SD: 7.79±4.38 $\mu g\ g^{-1}$, Tukey-adjusted *post-hoc* comparisons $p<0.05$) comparing to KB2 and KB3 stations (3.65±1.58 and 2.93±1.35 $\mu g\ g^{-1}$ respectively). The mean CPE contents in upper sediment layers (0-2 cm) were on average almost two times higher in summer than in winter (mean±SD: 40.98±17.38 vs. 22.12±13.86 $\mu g\ g^{-1}$; $F_{(1,30)}=16.6$, $p<0.001$). CPE concentration also varied among stations ($F_{(2,30)}=5.2$, $p<0.05$). The highest mean concentration of CPE in surface layer (0-1 and 1-2 cm) was noted at station

KB1 (mean±SD: 41.66±17.60 μg g^{-1}), however, it differed significantly only from KB3 (23.09±12.72 μg g^{-1}; Tukey-adjusted *post-hoc* comparisons $p<0.05$). Average CPE concentration in surface sediments at KB2 was 29.90±19.76 μg g^{-1}.

Meiofauna was composed of 13 taxa. The total density ranged from 135×10^3 to 389×10^3 ind. 0.1 m^{-2} in summer and from 131×10^3 to 326×10^3 ind. 0.1 m^{-2} in winter (Table III.2). The biomass ranged from 32 to 62 mg DM 0.1 m^{-2} in summer and from 19 to 147 mg DM 0.1 m^{-2} in winter. Meiofauna was dominated by nematodes, both in terms of abundance (94 - 97 %) and biomass (73 - 92 %). Macrofauna was represented by 132 taxa. The total density varied from 776 to 1410 ind. 0.1 m^{-2} in summer and from 940 to 1563 ind. 0.1m^{-2} in winter, the ratios of winter/summer densities at stations varied from 1.10 to 1.52. The biomass varied in summer from 1211 to 1959 mg DM 0.1 m^{-2}, and in winter from 1726 to 3879 mg DM 0.1 m^{-2}, with winter/summer rations ranging from 1.42 to 2.31. The taxonomic composition of both meiofauna and macrofauna expressed in taxa or taxa/size class abundance or biomass did not differ between the seasons (PERMANOVA, $p>0.5$).

Table III.2 Location, depth [m] and basic benthic characteristics (number of taxa (S), density [ind. 0.1 m^{-2}] and biomass [mg DM 0.1 m^{-2}] of meiofauna and macrofauna for the three stations and in the two seasons.

Station Season	Location	Depth	Meiofauna			Macrofauna		
			Density (ind.×10^3)	Biomass	S	Density	Biomass	S
KB1 summer	79.01˚N 11.44˚E	330	210	71	11	808	1211	51
KB1 winter	79.01˚N 11.39˚E	350	222	81	8	940	1726	54
KB2 summer	78.98˚N 11.73˚E	296	135	32	8	776	1270	50
KB2 winter	78.98˚N 11.70˚E	307	131	19	8	1186	2944	54
KB3 summer	78.95˚N 11.99˚E	274	389	62	11	1410	1959	59
KB3 winter	78.95˚N 11.99˚E	260	327	147	9	1563	3879	73

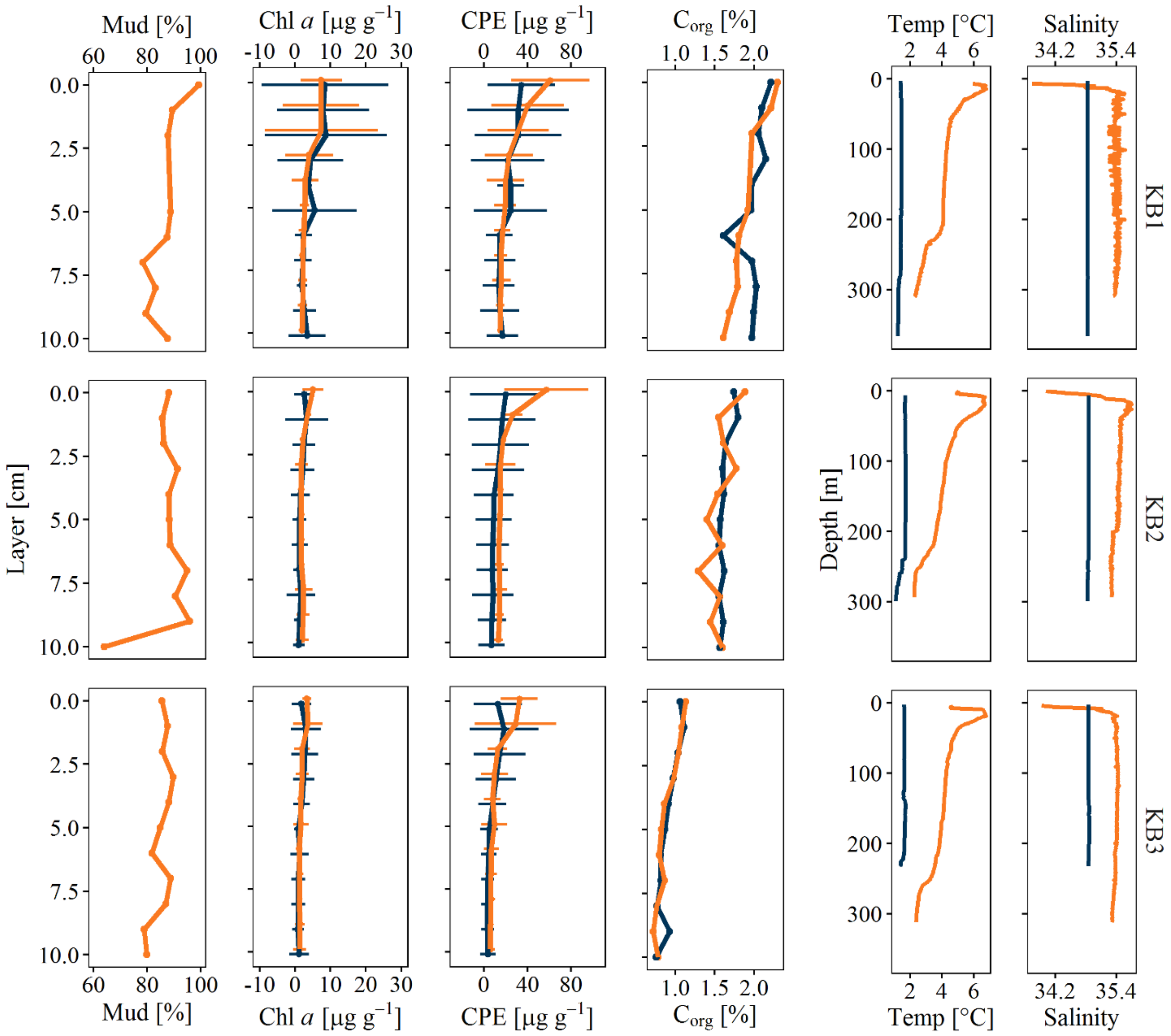

Figure III.4 Environmental variables at three stations and in two seasons (dark blue lines – winter, orange lines – summer). The plots present the mud content (Mud), chlorophyll a content (Chl *a*, mean for three replicates and 0.95CI), chloroplastic pigment equivalent content (CPE, mean for three replicates and 0.95CI) and particulate organic carbon (C$_{org}$) in 10 cm of sediment cores and water column temperature (Temp) and salinity.

Four macrofaunal taxa (polychaetes: *Galathowenia oculata* (Zachs, 1923), *Lumbrineris* spp., *Maldane sarsi* Malmgren, 1865 and *Prionospio cirrifera* Wirén, 1883) and meiofaunal nematodes occurred with > 30 specimens in every sample. The individual DM differed between seasons and between seasons within each station for all dominant taxa (ANOVA, p<0.05; Table III.3). For *G. oculata*, seasonal differences occurred at stations KB1 and KB3 where specimens had higher individual DM in summer than in winter (median = 339 vs. 83 and 236 vs. 93 µg, respectively; Figure III.5). Specimens of *Lumbrineris* spp. collected in winter had higher individual DM than those sampled in summer only at KB3 station (median = 475 vs. 357 µg DM). For *M. sarsi,* seasonal differences were noted at stations KB1 and KB3 where specimens had higher individual *DM* in summer (median = 269 vs. 213, and 276 vs. 228 µg, stations respectively). Specimens of *P. cirrifera* had lower individual DM in winter at stations KB1 (median = 797 vs. 514 µg) and KB2 (median = 1244 vs. 791 µg), while at KB3 in winter they had higher individual DM (median = 1451 vs. 3700 µg). For Nematoda the significant seasonal differences occurred at all stations: summer individual DM was higher at KB1 (median = 0.079 vs 0.058 µg) and KB2 (median = 0.045 vs. 0.038 µg) while at KB3 specimens had higher biomasses in winter (median = 0.028 vs. 0.069 µg).

Table III.3 Results of two-way ANOVA tests for difference in individual biomass (µg DM) of *G. oculata, M. sarsi, Lumbrineris* spp., *P. cirrifera* and Nematoda among stations and seasons. Significant effects of the main test (p<0.05) printed in bold. Stations with significant seasonal effects identified by *post-hoc* tests (Tukey-adjusted comparisons, p<0.05) are listed.

	Galathowenia oculata		***Lumbrineris* spp.**		***Maldane sarsi***		***Prionospio cirrifera***		**Nematoda**	
Factors	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Station (St)	19.26	**< 0.001**	1.87	0.15	4.19	**0.02**	349.02	**< 0.001**	52.65	**< 0.001**
Season (Se)	35.91	**< 0.001**	15.73	**< 0.001**	16.63	**< 0.001**	9.03	**< 0.01**	5.40	**0.02**
St x Se	9.70	**< 0.001**	3.64	**0.03**	3.96	**0.02**	216.46	**< 0.001**	57.55	**< 0.001**
Pairwise Se(St x Se)	KB1, KB3		KB3		KB2, KB3		KB1, KB2, KB3		KB1, KB2, KB3	

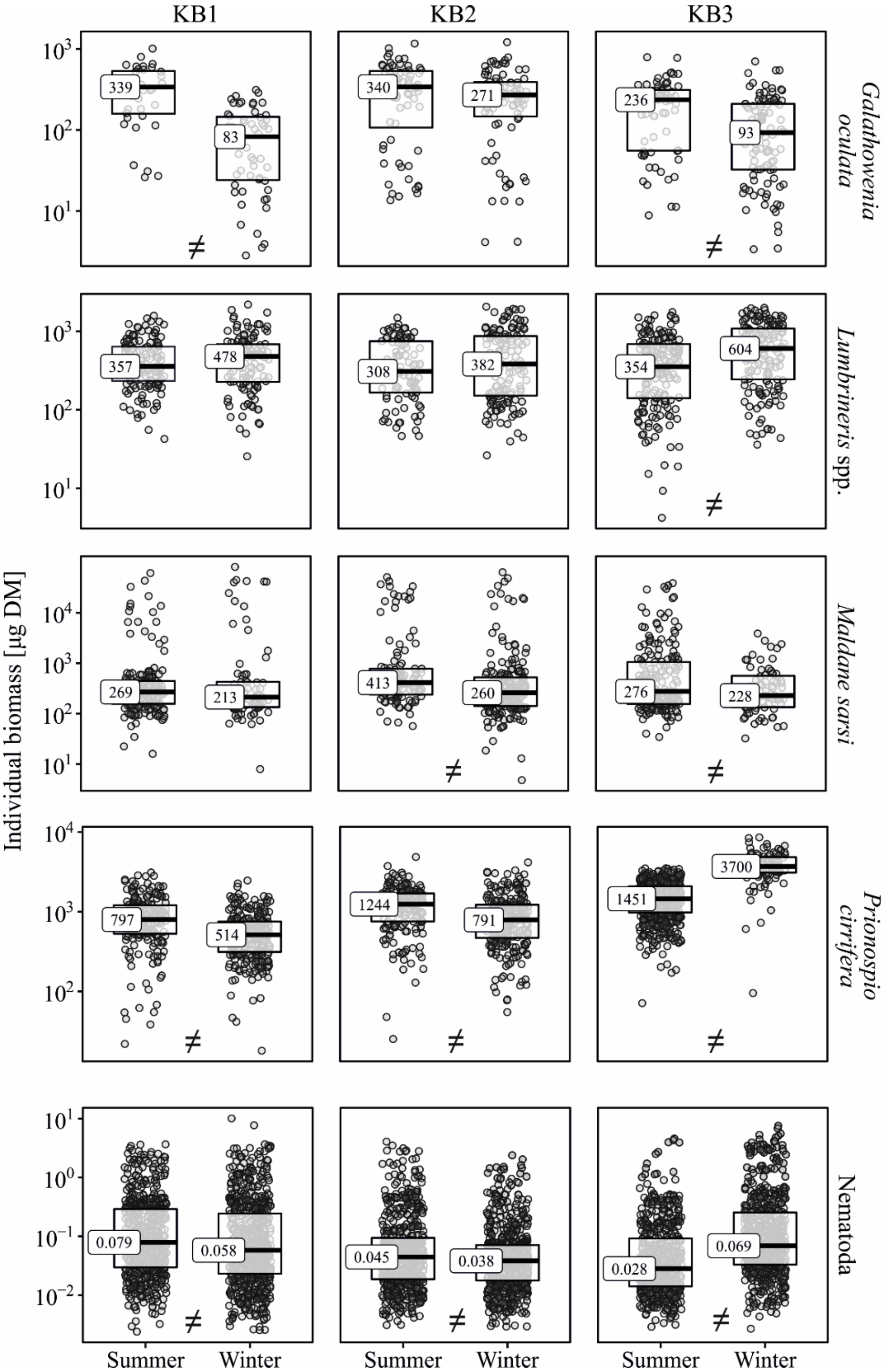

Figure III.5 Individual biomass [µg DM] of the most common macrofaunal species and meiofaunal nematodes at the three stations by seasons. Box - median with 0.25 - 0.75 percentile; dots – single specimen observations, ≠ – statistically significant differences between seasons within station (*post-hoc* pairwise comparison with Tukey's adjustment of P-values $p<0.05$)

Both abundance and biomass size spectra had similar trimodal shapes in summer and winter at KB1 and KB2; the shapes were bimodal at station KB3. Size classes ranged between -9 and 20 (Figure III.6). In abundance size spectra, peaks were observed between size classes -6 to -4, 6 to 9 and 12 to 14. The troughs were observed in size classes 4 and 11 to 12. In biomass size spectra, the peaks were observed at size classes 0 to 2, 9 to 11 and at highest size classes (16 to 20). The troughs in biomass size spectra were present in the same size classes as in the abundance size spectra.

There were some differences in biomass size spectra shapes among stations (Figure III.6). The first peak at KB2 was flatter and wider (from - 5 to 3) compared to KB1 and KB3 where it was steeper and narrower (from -2 to 2). Only at KB2 organisms noted in size class 18 and 20 were noted.

The shapes of abundance and biomass size spectra in the two seasons were consistent at each station. Still, there were some slight differences in peak location (Figure III.6). At all stations in summer the abundance and biomass in the first trough (size class 4) were lower than in winter. At KB2 first biomass peak was less pronounced in winter (-1 to 0) than in summer (0 to 1), size classes 18 and 20 were present only in winter. Moreover, in summer the maximum biomass occurred in size class 16 while in winter it was found in size class 20. At station KB3, the positions of both biomass and abundance peaks were shifted by -1 towards lower size classes in summer compared to winter. Regarding the biomass, both peaks were higher in winter than in summer.

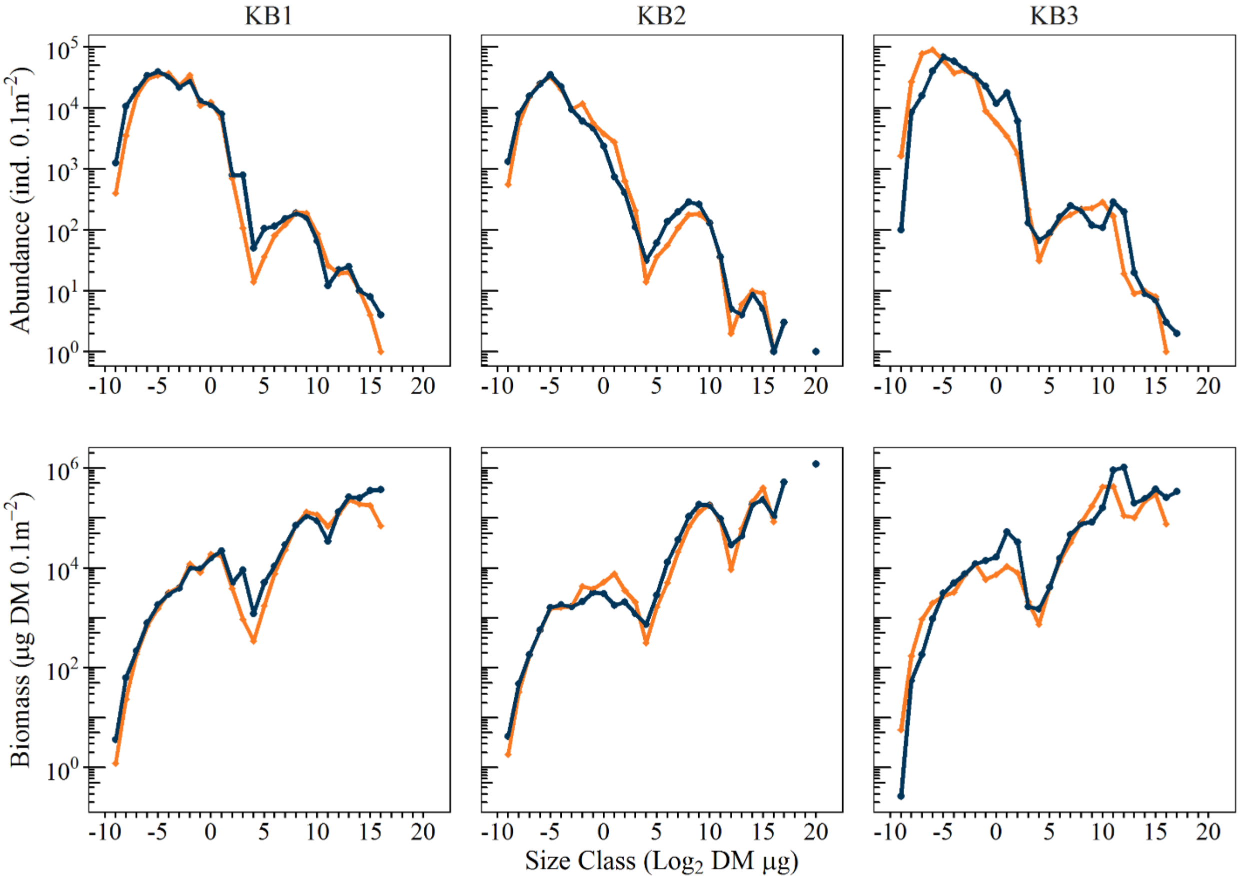

Figure III.6 Abundance size spectra (top row) and biomass size spectra (bottom row) at investigated stations in summer (orange lines) and winter (dark blue lines).

Meiofauna covered 13 size classes (from -9 to 3). Nematoda made up 71-100 % of biomass in size classes from -9 to 2 (Figure III.7). The macrofaunal organisms were spread across 24 size classes (from -3 to 20). Biomass in size classes from 2 to 20 was dominated by Annelida (66 - 100 %). Mollusca had a considerable contribution (11-30 %) to biomass in size classes 13, 14 and 16, and Arthropoda (Crustacea) contributed significantly in size classes 2, 14, 15 (16-17 %; Figure III.7). The shift in dominance from nematodes - meiofaunal dominant taxon - to polychaetes - macrofaunal dominant taxon - was observed between size classes 1 and 3 (Figure III.7).

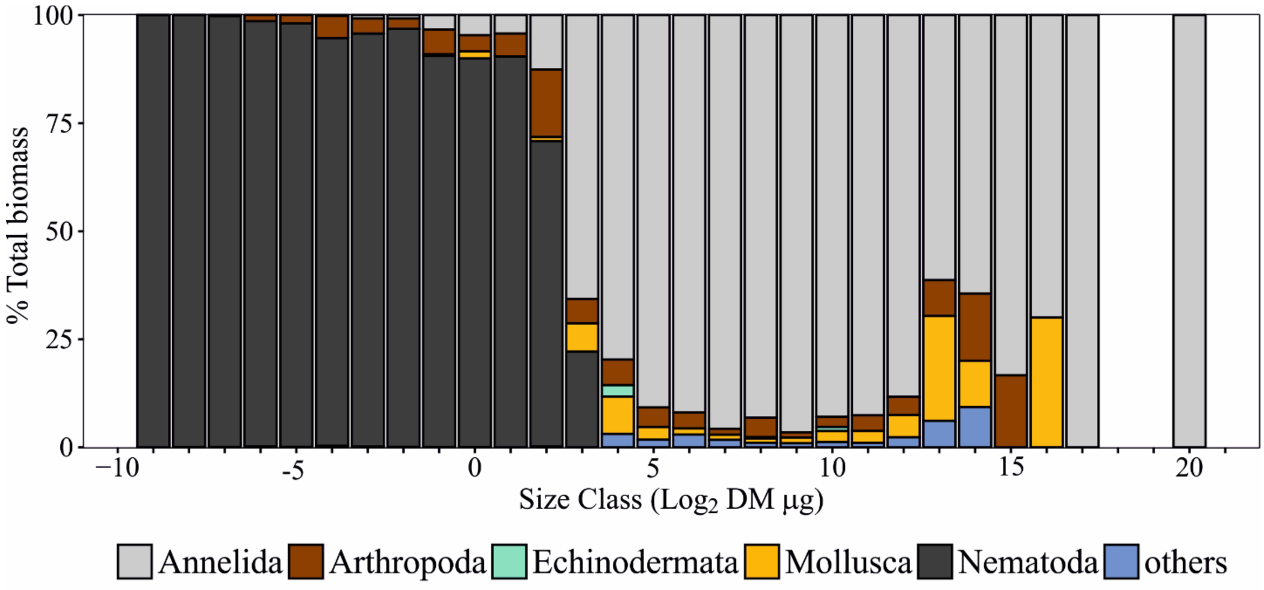

Figure III.7 Contributions of major phyla to total biomass in size classes [%]. Average values based on data from all stations and seasons are presented.

A significant linear relationship between NB and SizeClass was found for all the samples ($p<0.001$: Table III.4). The intercepts and slopes of NBSS varied between 10.72 ± 0.47 to 12.00 ± 0.45 and -0.50 ± 0.07 to -0.58 ± 0.05, respectively.

The most parsimonious multiple regression model describing NBSS incorporated two predictors: SizeClass and Station without interactions:

$$\mathrm{NB_i} = \beta_1 + \beta_2 \times \mathrm{SizeClass_i} + \beta_3 \times \mathrm{Station_i} \qquad \text{(Eq. III.1)}$$

where β_1 is the intercept, β_2 is the slope, β_3 is the effect of Station on the intercept. The significance of these covariates was also confirmed by ANCOVA ($p<0.05$). The intercept in the multiple regression (Eq. III.1) was significantly different (Tukey-adjusted *post-hoc* comparisons $p<0.05$) only between KB2 and KB3 stations (10.77±0.41 vs. 11.89±0.31, respectively) with a common slope = -0.54±0.02 × SizeClass (Figure III.8).

Table III.4 OLS linear regression parameters of the Normalized Biomass Size Spectra calculated for individual samples. The Normalized Biomass (NB) was modelled as a function of SizeClass: $NB_i = \beta_1 + \beta_2 \times SizeClass_i$, where β1 is the intercept, β2 is the slope.

Station	Season	Intercept (β_1) ± SE	Slope (β_2) ± SE	Adj R^2	p
KB1	Summer	11.12 ± 0.54	-0.54 ± 0.07	0.73	<0.001
	Winter	11.65 ± 0.41	-0.54 ± 0.05	0.82	<0.001
KB2	Summer	10.72 ± 0.47	-0.54 ± 0.06	0.78	<0.001
	Winter	10.79 ± 0.38	-0.53 ± 0.04	0.86	<0.001
KB3	Summer	12.00 ± 0.45	-0.58 ± 0.05	0.82	<0.001
	Winter	11.81 ± 0.59	-0.50 ± 0.07	0.67	<0.001

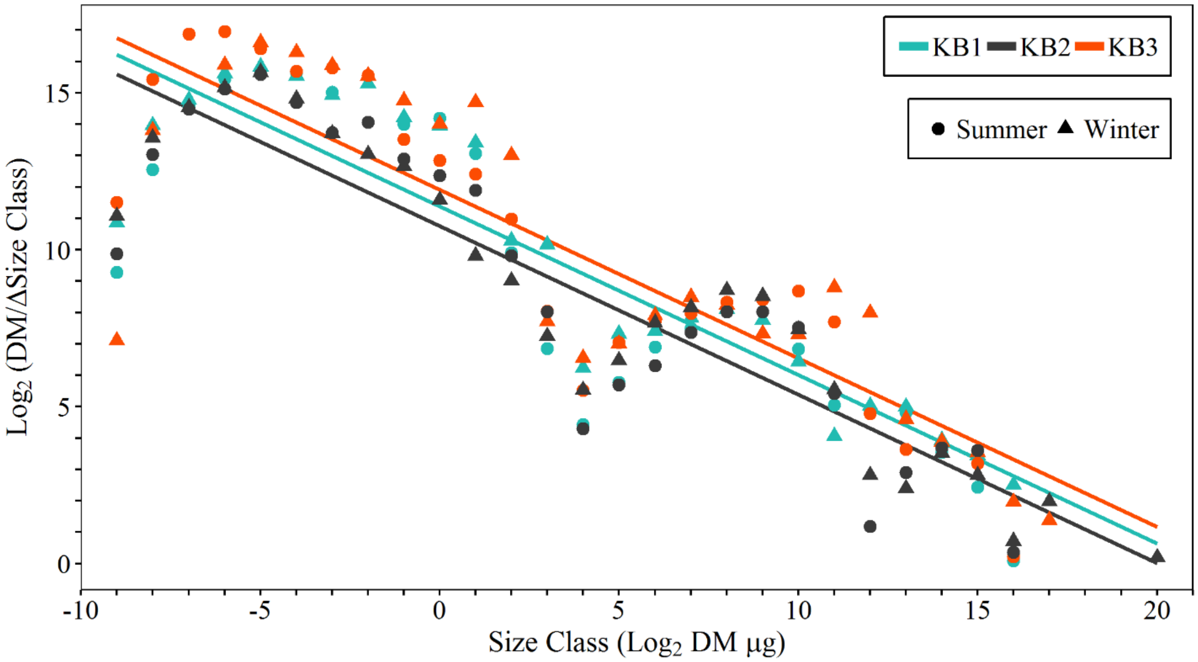

Figure III.8 NBSS with regression lines for the most parsimonious model – Eq. III.1 (the intercepts are significantly different between KB2 and KB3). Colours indicate stations, symbols indicate seasons (summer – dots, winter – triangles).

3.4. Discussion

3.4.1. Benthic response to seasonal variability in Kongsfjorden ecosystem

Despite the strong seasonality in pelagic processes and organic matter supply to the sea-bottom in Kongsfjorden (Lalande et al. 2016), the abundance and biomass size spectra showed little variability between the two studied seasons. Quiroga et al. (2016) stated that benthic biomass size spectra may be useful as an indicator of short-term local dynamics of environmental factors since slopes, and intercepts of estuarine NBSS varied in response to seasonal variability in organic and mineral matter supply to the sea bottom. In Kongsfjorden the seasonal cycle of primary production is strongly pronounced with a marked peak in spring (Svendsen et al. 2002; Hop et al. 2006). The sedimentation of fresh pelagic organic matter in late spring/early summer has been linked to increased values of CPE in sediments (a proxy for the fresh/labile organic matter, García et al. 2010). However, in the study presented in this chapter, no difference between winter and summer was noted for either the C_{org} or Chl *a* content in sediments. The higher CPE content in summer resulting from increased phaeophytin content, product of Chl *a* degradation, may indicate that in the late summer the organic matter produced in the water column reaches the bottom after being grazed by mesozoplankton (Chen et al. 2016). The stable level of C_{org} content indicates that the total pool of organic matter can remain stable throughout the year, even if the pelagic production ceases in autumn. An alternative source of organic matter is kelp detritus, which is delivered to sediments mainly in autumn when kelp blades are being physically destroyed (Shunatova et al. 2018). The lack of seasonal variability in C_{org} content in sediments (contrasting with clear difference in vertical fluxes recorded by Lalande et al. 2016) was also documented by Bourgeois et al. (2016) and explained by relatively stable production of zooplankton fecal pellets over the year.

Lack of seasonal patterns in benthic size spectra is consistent with the study of Włodarska-Kowalczuk et al. (2016), who did not found a clear response of Kongsfjorden benthic standing stocks or diversity to changes of organic matter supply during four seasons. Their findings and the study presented in this chapter support the hypothesis of a “food bank” existence in the sediments of polar regions that was first formulated for the West Antarctic Peninsula by Mincks et al. (2005). According to this hypothesis large amounts of organic carbon produced during the spring blooms and deposited on the bottom may persist in the sediments for a long period of time due to low rates of bacterial mineralization in low temperatures and can maintain benthic communities at constant levels on a year-round basis (Mincks et al. 2005; Glover et al. 2008). It is evident, that Kongsfjorden benthic fauna is not food-limited and dependent on the seasonal pulses of pelagic primary productivity. Renaud et al. (2015) reported that in the Spitsbergen fjords macroalgal detritus can contribute up to 69% to macrobenthic consumers’ diet. The reliance on a food source that is supplied from the shallow rocky banks to the deeper basins year round can buffer benthic communities from seasonal control. However, the patterns observed in Kongsfjorden which is a productive/advective system, with high local primary production, both pelagic (Hodal et al. 2012) and benthic (Woelfel et al. 2010), and strong advection of Atlantic waters that transport organisms and organic matter from the shelf, may differ from the situation in some other Arctic fjords. For example, Morata et al. (2013) reported that in Rijpfjorden (fjord located off northern Svalbard island Nordaustlandet), with a much shorter primary production period, the macrobenthic biomass was lower in winter than in summer and that the benthic communities in January responded with increased activity to experimental addition of fresh organic matter.

3.4.2. Benthic size spectra of an Arctic fjord

The productivity of a system may be reflected in the size spectra characteristics. The intercepts of NBSS plotted for Kongsfjorden fauna were higher than those reported by most other studies (Saiz-Salinas and Ramos 1999; Quiroga et al. 2005, 2012, 2016), but similar to those noted for communities inhabiting highly productive regions and sediments with high organic matter content (Akoumianaki et al. 2006; Hua et al. 2013). The slopes of the NBSS can give information about biomass accumulation across the size classes, with lower slopes indicating higher efficiency of biomass transfer to larger size organisms, and higher accumulation of biomass in these organisms (Sprules and Munawar 1986; Gaedke 1992). It is assumed that a slope of benthic NBSS > -1 reflects highly productive system (Drgas et al. 1998; Saiz-Salinas and Ramos 1999). Slopes found in the study presented in this chapter (~ -0.5) are similar to those found by Akoumianaki et al. (2006) at stations enriched in organic matter near the Spercheios River mouth (Maliakos Gulf, Greece). On the other hand, an oversupply of organic matter can lead to eutrophication and associated disturbance that can result in an opposite effect – the domination of small opportunistic species and low values of NBSS slopes (up to -0.78), as reported from Baker Fjord (Chile) by Quiroga et al. (2012). Also negative effects of low oxygen levels were indicated by slope of NBSS (-0.84) as observed by Quiroga et al. (2005) in the oxygen minimum zone off Chile. However, NBSS slope reported for central basin of Kongsfjorden implicates that this ecosystem is not a subject of perturbations related to organic enrichment or low oxygen levels, probably due to low temperatures, high flow in this unsilled fjord, and thus consistently high bottom-water oxygenation.

The shapes of the observed benthic size spectra were similar to those reported in other studies. Usually, they are bimodal with a well pronounced trough between the modes, corresponding to two major benthic groups: meiofauna and macrofauna (Schwinghamer 1981; Warwick and Clarke 1984; Duplisea and Drgas 1999; Kelly-Gerreyn et al. 2014). However, some studies reported also trimodal spectra, with two peaks within the macrofauna size range (Hua et al. 2013; Górska and Włodarska-Kowalczuk 2017), similar to spectra observed in the study presented in this chapter at stations KB1 and KB2. Still, an obvious trough (region of low biomass) separating meiofauna and macrofauna was observed in every sample. This trough is regarded as a characteristic property of benthic size structure (Warwick and Clarke 1984), and is not related to different sampling procedures for meio- and macrofauna (Schwinghamer 1981; Duplisea and Drgas 1999; Warwick 2014). It is assumed that the shift from interstitial fauna (meiofauna) to burrowing and sedentary macroscopic surface dwellers occurs within the range of size classes corresponding to the trough (Schwinghamer 1981). This is confirmed by the shift in major phyla contributions to size classes observed in the study presented in this chapter, where above size class 3 the dominant meiofaunal component (Nematoda) is replaced by major macrofaunal component (Polychaeta). The size class ranges of meiofauna and macrofauna overlapped between -3 and 3 size classes, but the very high dominance (>80% of biomass) of Nematoda until 2 size class confirms good adaptation of meiofauna to dwelling in the interstitial habitat (Warwick 2014).

3.4.3. Seasonal patterns in size structure of dominant taxa

While there was no seasonal differences in size spectra visible at the whole community level, the size structures of the populations of dominant species varied between the seasons. Similar patterns were observed by Datta and Blanchard (2016) who modelled the seasonal variability in size spectra in fish populations and communities in the North Sea. They reported significant seasonal changes for individual species, but none at the whole community level. In the study presented in this chapter, differences in size of dominating species were not consistent and varied in magnitude and direction of change among stations and species. The decrease of individual biomass in winter was reported for *G. oculata* and *M. sarsi,* while the opposite trend was observed for *Lumbrineris* spp. For meiofaunal nematodes and *P. cirrifera,* in winter the individual DM was lower at stations KB1 and KB2, but higher at KB3. High values of polychaete summer biomass can be explained by their reproduction strategies (Gooday 2002). According to Kukliński et al. (2013), who studied seasonal patterns of meroplankton in Adventfjorden, pulse of planktotrophic larvae occurs from spring to early summer. While the settlement can be prolonged even until early winter, it is the most intense soon after the peak of bloom period. In Adventfjorden the polychaete larval peak was reported to occur from May to late June, and the timing was strongly correlated with the Chl *a* concentrations in the water column (Stübner et al. 2016). This can explain the lower summer biomass of *Lumbrineris* spp., as it was reported to release larvae in May-July (Fetzer and Arntz 2008). Oweniidae and Spionidae were also reported to reproduce in spring/summer time (Blake and Arnofsky 1999; Rouse and Pleijel 2001), but this was not reflected in the size structure of *G. oculata* (owenid) and *P. cirrifera* (spionid) in this study - for both species the average individual DM were higher in summer than in winter. For *G. oculata* there were more small specimens in winter, which indicates that recruitment occurred after summer sampling (in fall or early winter). Obviously, the reproductive strategies of particular species are reflected in seasonal variability of their populations size structures but these effects overlap and are not translated into a tractable pattern at the whole community level as opposite effects may compensate each other. Ambrose and Renaud (1997) did not find the consistent recruitment response to seasonal input of labile organic matter after the spring bloom in the Northeast Water Polynya when analysed the data concerning the whole polychaete group. However, when they looked at the family level, they noted broad peaks of recruitment, that probably could be decomposed to more narrow peaks for certain species. Also very high variability in recruitment of various benthic taxa during whole year was noted in the West Antarctic Peninsula and was explained by persistent food availability (Mincks and Smith 2007).

3.4.4. Spatial variability in size spectra

Benthic size spectra differed more among stations than between seasons. The stations in the study presented in this chapter were located in the central basin of the fjord, area with low sedimentation, weakly affected by local fluvial or glaciofluvial inflows and respective environmental gradients (Włodarska-Kowalczuk et al. 2005). Still, there was a clear trend of decreasing C_{org} content moving into the fjord from KB1 to KB3 indicating gradient of physical factors inducing shift in phytoplankton composition, decrease of primary production and phytoplankton biomass (Keck et al. 1999; Piwosz et al. 2009) and zooplankton fecal pellet supply (Lalande et al. 2016). Akoumianaki et al. (2006), in a study of deltaic macrofauna in the Mediterranean, found evidence of spatial differences in NBSS parameters – the decrease of slopes with growing distance from the river mouth. The decrease of NBSS slopes was also interpreted as a result of impoverished food supply with depth for benthic communities on the Antarctic shelf (Saiz-Salinas and Ramos 1999). In this study an increase of food availability and quality (indicated by increase of Chl *a*) was observed from station KB3 to KB1, but the trends in the total biomass and in the NBSS intercepts values were the opposite. It suggests that the studied communities are not controlled by variability in food availability (indicated by the sedimentary C_{org} and pigments). Other drivers of natural variability (e.g. near bottom currents or bottom topography), possibly including biological synergies (e.g. adult-larva interactions or bioturbation) may play a crucial role in producing these spatial patterns (Ysebaert and Herman 2002; Norkko et al. 2013). Włodarska-Kowalczuk and Węsławski (2008) explored the scales of the spatial heterogeneity in the undisturbed outer basin of Hornsund fjord (similar to study area from this chapter) and reported distinct patches with varying species composition at a distance of 200 m that could not be explained by environmental heterogeneity.

Chapter IV. Latitudinal consistency of biomass size structure despite environmental, taxonomic and functional trait variability

4.1. Introduction

Declining body size has been proposed as one of the three universal ecological consequences of climate warming, alongside changes in species distribution and phenology (Gardner et al. 2011). This assessment is based on numerus rules concerning relationship between size of organisms and temperature such as the "Bermann's rule" (Bergmann 1847) or the "temperature-size rule" (Atkinson 1994; Atkinson and Sibly 1997). Several studies supported predictions of climate warming effects on organism size in aquatic systems. For instance, maximum body size in marine fish assemblages is expected to shrink up to 24%, due to changes in distribution abundance and physiology (Cheung et al. 2013). A meta-analysis of the results of long-term surveys, experimental data and published results concerning the effect of climate change on the body size of aquatic bacteria, phytoplankton, zooplankton and fish showed that global warming has already resulted in an increasing proportion of small species or young age-classes and has caused a decline in size-at-age (Daufresne et al. 2009). One example of a shift in contribution of different size species due to temperature increase may be increasing proportion of small, boreal hyperiid amphipod *Themisto compressa* and decreasing contribution of large, Arctic *T. libellula* in eastern Fram Strait observed between 2000 and 2012 (Kraft et al. 2013). Also (Stempniewicz et al. 2007) predit that climate warming in the Arctic will increase the contribution of small zooplankton species in the plankton communities and impact the composition and functioning of the food web. Furthermore, mesocosm studies in freshwater pelagic ecosystems have shown that a warming of 4°C may cause significant changes in the size structure, i.e., increasing the prevalence of small phytoplankton organisms and alteration of the energy flow in the ecosystem (Yvon-Durocher et al. 2011). In similar experiment on benthic communities, a decline in the total biomass of organisms was noted as a consequence of temperature increase as well as changes of the size spectrum slope coefficient (Dossena et al. 2012).

The decreasing body size at the community level may result from a decline in body size within populations of dominant species or species replacement (species with larger body size replaced with species with smaller body size). These changes may have substantial consequences regarding interactions among organisms and ecosystem functioning. In this case, feeding strategies of organisms may play an important role, and one of the drivers of ecosystem functioning change may be a mismatch in relative sizes of prey and consumer organisms. A decline in the average size of primary producers not accompanied by corresponding changes in consumers can result in the shrinkage of food resources for heterotrophs and then, a reduction in consumer populations and increased susceptibility to diseases or higher mortality (Sheridan and Bickford 2011). Ecosystem functioning is more strongly influenced by size structure of the community level than by its taxonomic diversity (Dossena et al. 2012). The varying proportions of smaller and larger size classes in the total community biomass may lead to changes in secondary productivity (Górska and Włodarska-Kowalczuk 2017). Moreover, Norkko et al. (2013) showed that the presence of large organisms in benthic communities determines the intensity of bioturbation and bioirrigation, that supply deeper parts of sediment with organic matter and oxygen.

The aim of this chapter was to investigate possible consequences of climate warming for Arctic benthic community size structure. A “space-for-time” approach was used, i.e., using of the lower latitude/warm localities as proxies of future/after warming situation in the Arctic. This chapter contains assessment of biomass size spectra of communities representing coastal (fjordic) soft bottom habitats in six localities across latitudinal/thermal gradients, spanning from 60 to 81 °N latitude and from -1.6 to 7.7°C bottom water temperature. The warmer conditions are expected to influence size structure of benthic fauna through size reduction of organisms within dominant species or changing species composition promoting smaller organisms. Two hypotheses are formed: (1) size structure of benthic fauna change along the thermal/latitudinal gradient by increasing contributions of smaller size classes and steepening of the slopes of normalised biomass size spectra in warmer localities; and (2) the NBSS intercept and community biomass are related to system productivity (indicated by sediment organic matter characteristics). At a particular taxa populations level, it is expected that the latitudinal clines in size will be in accordance with the “temperature-size rule” (smaller bodies in warmer fjords).

4.2. Materials and methods

4.2.1. Sampling

Materials were collected in six fjords off continental Norway (Raunefjorden (RAU), Balsfjorden (BSF), Ullsfjorden (ULS)) and off Svalbard (Hornsund (HSD), Kongsfjorden (KGF), Rijpfjorden (RIJP); Figure IV.1). They were selected to span wide latitudinal (60 to 81°N) and thermal (bottom water temperature -1.6 to 7.7°C) ranges (Table IV.1) and to avoid strong anthropogenic impacts, especially fish trawling, aquaculture, pollution inflows or red king crab (*Paralithodes camtschaticus*) predation, which can be detrimental to benthic biota (Falk-Petersen et al. 2011). In each fjord, sampling was conducted at three stations, located at seabed covered with fine sediments in the central part (150-350 m depth) of the outer basin (to exclude the influence of local glacial or fluvial inflows, usually located in the fjords' heads, Włodarska-Kowalczuk et al. 2012).

Material was collected from R/V "Oceania" and R/V "Helmer Hanssen" in 2014 and 2015 (Table IV.1). A set of measurements and samples collected at each station included CTD profiles, sediment samples (three replicates for photosynthetic pigment concentration, one replicate for grain size, $\delta^{13}C$ and particulate organic carbon (C_{org}) content), one macrofauna sample and one meiofauna sample. CTD measurements were performed with the use of a CT-Set mounted at Hydro Bios MultiNet. Samples from the surface (0-1 cm and 1-2 cm) sediment layers were collected with a Nemisto gravity corer and were frozen (samples for photosynthetic pigment analysis at - 80 °C; other samples at - 20 °C). Macrofauna was sampled with the use of a 0.1 m^2 van Veen grab and sieved on board through 500 µm mesh; meiofauna was sampled with the use of a plastic syringe (10 cm^2 sampling area) inserted 10 cm deep into sediment collected with a box-corer. Faunal samples were preserved in 4% formaldehyde.

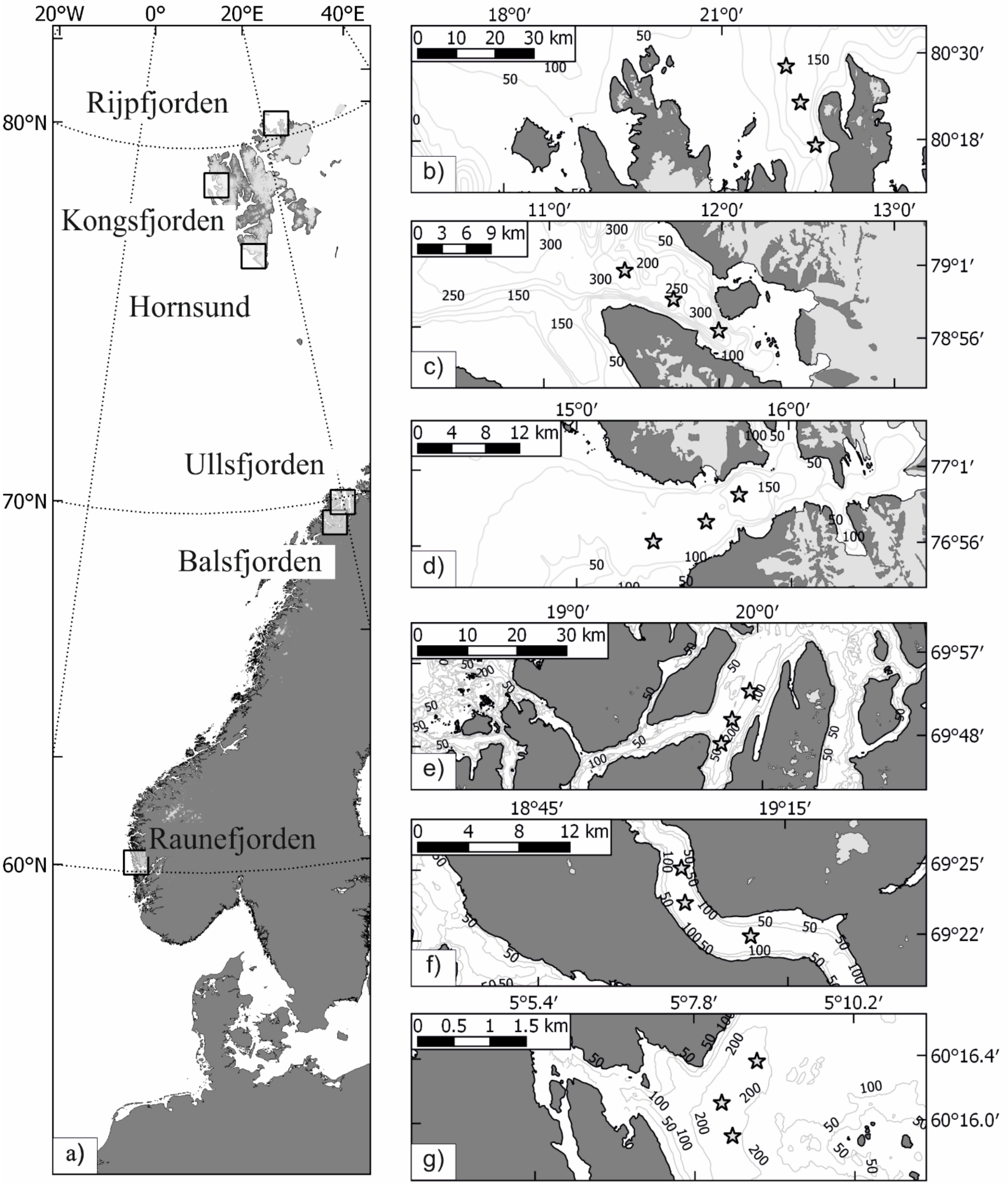

Figure IV.1 A) Sampling locations (squares,) and sampling stations (asterisks) in fjords: b) Rijpfjorden, c) Kongsfjorden, d) Hornsund, e) Ullsfjorden, f) Balsfjorden, g) Raunefjorden.

4.2.2. Laboratory analyses

Chlorophyll *a* (Chl *a*) and phaeopigment (together referred to as chloroplastic pigment equivalent, CPE) concentrations in sediment samples were measured fluorometrically, with the use of a Perkin Elmer LS55 Fluorescence Spectrometer. Grain size composition was determined with a Malvern Mastersizer 2000 particle size analyser, recalculated using the GradiStat 4.0. software. The $\delta^{13}C$ and C_{org} content analyses were performed via continuous flow - elemental analysis - isotope ratio mass spectrometry (CF-EA-IRMS), at the University of Liège, with the use of a Vario Micro Cube elemental analyser (Elementar Analysensysteme GmBH, Hanau, Germany).

The meiofauna samples were centrifuged three times in a solution of colloidal silica (Ludox TM-50, density of 1.18 g cm^{-3}) and stained with Rose Bengal in a 4% buffered formaldehyde solution for at least 24 hours. Then, samples were sieved, and only specimens that passed through 500 µm mesh and retained on 32 µm mesh were analysed. Nematodes retained on 500 µm mesh were termed "macrofaunal nematodes". Macrofauna individuals were identified to the lowest possible taxonomic level, and meiofauna were identified to higher taxonomic levels (mostly phylum). Each specimen was photographed with a Leica DFC450 digital camera, connected to a Leica M205C stereomicroscope and, except for nematodes, was measured with a Leica LAS Manual Measurements software. Nematode lengths and average widths were measured using a semi-automated method of image analysis (Mazurkiewicz et al. 2016, Chapter II). For macrofaunal species occurring in numbers higher than 250 per sample, a subsample of 200 randomly picked specimens was measured. Nematodes occurred in very high abundances, up to over 4000 individuals per sample, therefore a subsample of 500 randomly selected individuals was analysed. Altogether 13772 meiofaunal, 204 macrofaunal nematode and 15054 macrofaunal individuals were measured.

4.2.3. Statistical analyses

The canonical analysis of principal coordinates (CAP) was used to assess environmental parameters (near bottom temperature, C_{org}, $\delta^{13}C$, MUD – silts and clay content in sediments, Chl *a*, CPE; mean values per station) that best discriminated studied fjords.

The biovolumes of meiofauna, macrofaunal nematodes, and macrofauna were calculated based on the measured dimensions. For non-nematode meiofauna, biovolume was calculated with the use of the Feller and Warwick (1988) formula: $V = L \times W^2 \times c$, where V is the volume, L - length, W - width, and c - taxon-specific coefficient. For Nematoda, a formula for a volume of cylinder was used: $V = \pi \times (W/2)^2 \times L$ (Mazurkiewicz et al. 2016, Chapter II). For macrofauna – body shapes were matched with geometric figures (Hillebrand et al. 1999**;** Table III.1). In the case of fragmented polychaetes, L was estimated using empirically determined relationships between widths of selected chaetigers and intact specimen length (Górska 2018). The wet mass (WM) was calculated by multiplying V by a specific gravity factor of 1.13 (Andrassy 1956). For Crustacea and Ophiuroidea, WM was obtained from measured dimensions, using published conversion factors (Berestovski et al. 1989). Dry mass (DM) of meiofauna was estimated as 0.25×WM (Feller and Warwick 1988). Body mass conversion factors (Brey et al. 2010) were used to calculate macrofaunal DM (shell free in the case of calcifying organisms). Organisms were grouped into size classes based on individual DM [µg] on a $\log_2$ scale.

The sum of individual DM of all organisms collected was regarded as total benthic DM at a station. Differences in total benthic DM among fjords were tested using one-way PERMANOVA based on a similarity matrix created from the Euclidean distances among samples (Anderson et al. 2008). Due to the small number of replicates, a Monte Carlo resampling was used to increase the interpretability of the test. To identify the best set of environmental variables (predictors) that accounted for the variation in total benthic DM, a distance-based linear model (DistLM, marginal and sequential tests with forward selection procedure of predictors and adjusted R^2 selection criterion and 9999 permutations) was used.

The patterns of similarity in community structure described by taxonomic composition, size class composition and functional (feeding type) trait composition among stations were explored for macrofauna. Species were classified into feeding types: subsurface deposit feeders, surface deposit feeders, suspension feeders, herbivores, grazers, and carnivores (Fauchald and Jumars 1979; Feder et al. 1980; Macdonald et al. 2010). DM data were square-root transformed, and Bray-Curtis similarities among stations were visualized using multidimensional scaling (nMDS). The relations among the three resemblance matrices were measured using rank correlation (Spearman's ρ), obtained with 9999 permutations (RELATE analysis, Clarke and Gorley 2015).

Total biomass in the n^{th} size class was normalized by the n^{th} size class width (NB). Next, normalised biomass size spectra (NBSS, Platt and Denman 1977, 1978) were constructed (NB as a linear function of size class) for each fjord with ordinary least squares linear regression. To identify spatial variation in NB, an analysis of covariance (ANCOVA) was used with size class as a continuous covariate and fjord as a factor covariate. The ordinary least squares linear multiple regression was used to assess the parameters (slope and intercept) of obtained NBSS. Pairwise *post-hoc* comparisons were performed with a Tukey's adjustment of p-values.

The relationship between NBSS intercepts (calculated for each station separately), total benthic DM at each station, and NB averaged over size classes at each station, was tested using Spearman's ρ rank correlation.

To explore the relationship between near-bottom temperature (NBT) and DM of individual taxa, I applied a generalized linear model (GLM) with a gamma distribution and log-link (Eq. IV.1). The gamma distribution used in GLM can be used for continuous and positive response variable and is usefull when analysing data like biomass, length or density as it allows for a quadratic variance structure of the variable (Zuur et al. 2013).

$$DM_i = e^{(\alpha + \beta \times NBT_i)} \qquad \text{(Eq. IV.1)}$$

where DM_i is the individual DM for the *i*th NBT, α is the constant (intercept), β is the linear coefficient of the NBT predictor. The GLM analyses were performed for each species, genera and families that were observed in all fjords.

The CAP, PERMANOVA, nMDS, RELATE and DistLM analyses were performed in Primer v7 + Permanova (Anderson et al. 2008; Clarke and Gorley 2015). Other statistical analyses were performed in R 3.5.1. (R Core Team 2018), the ANCOVA *post-hoc* tests were performed with Tukey adjusted pairwise comparison, using the *lsmeans()* function from the *emmeans* package (Lenth 2018). The map (Figure IV.1) was produced in QGIS 3.2 software (QGIS Developement Team 2019) with use of data (Svalbard land extent, Svalbard glaciers extent) provided by Norwegian Polar Institute (http://data.npolar.no), Europe extent provided by OpenStreetMap (http://openstreetmapdata.com/) north Europe glaciers data provided by

CryoClim (http://www.cryoclim.net) and bathymetry data provided by Norwegian Mapping Authority (https://www.geonorge.no).

4.3. Results

4.3.1. Environmental variability

Thermal conditions in the water column differed among the studied fjords. The strongest vertical gradients were noted in HSD, BSF and RAU. The maximum values were observed in surface waters in HSD, ULS, BSF and RAU, while warmest temperatures in RIJP and KGF were documented in intermediate water layers (Figure IV.2). The average near-bottom temperature (NBT) in the fjords followed the latitudinal gradient (from 7.7°C in the southernmost fjord to -1.6°C in the northernmost fjord), except for BSF (lower than in ULS) and HSD (lower than in KGF). Near-bottom salinity varied between 33.4 and 35.3 (with no latitudinal pattern) among the fjords (Table IV.1). Average C_{org} in surface sediments was in a range of 1.6-2.0% in most fjords, except for RAU (3.8%; Table IV.1). $\delta^{13}C$ was lower in HSD (-23.6 ‰) than in other fjords (-22.3 to -21.2 ‰). Mean values of Chl *a* were the highest in HSD (9.8 $\mu g\ g^{-1}$), intermediate in BSF and KGF, and much lower in other locations (1.6 - 2.0 $\mu g\ g^{-1}$). Mean values of CPE were also the highest in HSD (54.7 $\mu g\ g^{-1}$), relatively high in KGF (42.8 $\mu g\ g^{-1}$), lower in BSF and RAU (28.6 - 31.3 $\mu g\ g^{-1}$) and very low in ULS and RIJP (15.1 - 16.5 $\mu g\ g^{-1}$). Mud content ranged from 70 to 90% in all localities except for ULS (41%). Two main gradients of environmental variability can be observed from the CAP ordination (Figure IV.2): 1) the difference between RAU and other fjords (defined by C_{org} and water temperature) and 2) a gradient from HSD to ULS (defined by CPE, $\delta^{13}C$ and mud content). Spitsbergen fjords (HSD, KGF and RIJP) form one cluster, defined by high CPE and low temperatures. BSF and ULS can be described as moderately warm fjords, with high $\delta^{13}C$ values and low CPE content. RAU is the warmest and the richest in C_{org}, with moderate CPE and $\delta^{13}C$ values.

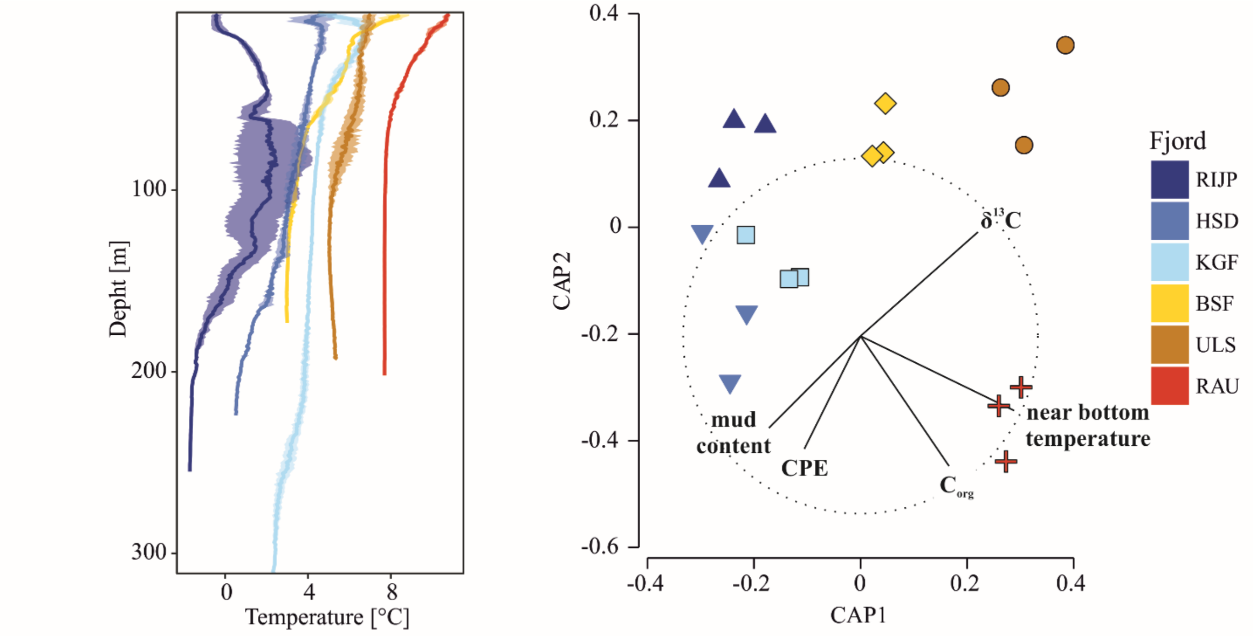

Figure IV.2 Temperature profiles (mean and SD for three stations) in fjords (left), CAP ordination based on sediment characteristics and near bottom temperature (right). Vectors on the CAP plot indicate variables with Pearson correlation to CAP axes >0.7 (CPE – photosynthetic pigments, C_{org} – particulate organic carbon content in sediments).

Table IV.1 Sampling details: fjord coordinates and dates and environmental characteristics: near-bottom water temperature (NBT) and salinity (NBS), particulate organic carbon (C_{org}), carbon stable isotope composition ($\delta^{13}C$), chlorophyll *a* (Chl *a*), chloroplastic pigment equivalent (CPE) and mud (silts and clay) content in sediments. Mean±SD are presented.

Fjord	Lat [°N]	Long [°E]	Date [year.month]	NBT [°C]	NBS	C_{org} [%]	$\delta^{13}C$ [‰]	Chl *a* [µg g^{-1}]	CPE [µg g^{-1}]	mud [%]
RIJP	80.20	22.07	2014.09	-1.578±0.124	34.780±0.054	1.77±0.22	-22.13±0.36	2.00±0.72	13.10±3.02	15.10±3.31
HSD	78.58	15.60	2014.07	1.672±0.839	34.840±0.045	1.82±0.13	-23.61±0.17	9.86±5.57	45.22±12.78	55.08±12.26
KGF	76.97	11.72	2014.08	2.300±0.047	35.345±0.039	1.77±0.51	-22.60±0.30	5.29±2.22	44.90±15.85	50.19±17.50
BSF	69.38	19.04	2015.06	3.000±0.013	33.400±0.014	1.97±0.36	-21.68±0.12	6.81±3.43	21.45±12.82	28.26±15.73
ULS	69.84	19.85	2014.06	5.313±0.072	34.400±0.052	1.63±0.29	-21.16±0.51	1.65±0.56	17.18±18.93	18.83±19.36
RAU	60.27	5.13	2015.06	7.700±0.003	34.869±0.001	3.85±0.23	-22.27±0.04	1.65±0.43	29.63±7.07	31.28±7.48

4.3.2. Benthic standing stocks and size spectra

Total benthic dry mass (DM) differed among fjords (PERMANOVA, $p<0.05$; Table IV.2). It was significantly higher in HSD (42.5 g DM m^{-2}) than in ULS and RAU (5.3 and 9.3 g DM m^{-2}, respectively), as well as higher in KGF (15.4 g DM m^{-2}) than in ULS (PERMANOVA pairwise *post-hoc* tests, $p<0.05$). Macrofauna constituted the majority of benthic DM (91 to 99%). $\delta^{13}C$, Chl *a* and MUD were identified by the DistLM marginal test as significantly related to variability in total benthic DM ($p<0.05$), while Chl *a*, together with $\delta^{13}C$, were selected by a forward selection procedure as the two factors explaining most of the variability (41%).

The organisms in the collected samples spanned a range of size classes from -11 to 21 (meiofauna -11 to 5, macrofaunal nematodes -6 to 6, macrofauna 0 to 21). The highest size classes (> 17) were represented only in RIJP, HSD and BSF. The general shapes of both abundance and biomass spectra were similar among fjords: two distinct modes – one for meiofauna and one for macrofauna, separated by a trough (Figure IV.3). The trough (size classes 2-5) was very prominent in KGF and BSF and less evident in RIJP and ULS. A macrofaunal peak in abundance size spectra was observed between size classes 7 and 10 in all fjords except RAU, where it shifted towards lower size classes (6-7, caused by the very high abundance of polychaete *Pseudopolydora paucibranchiata*). The size class with the highest biomass occurred on the end of each spectrum, except in HSD and KGF, where they were was observed in lower than last size classes (size class 15).

Table IV.2 Abundance, biomass and contributions to total benthic biomass of meiofauna, macrofaunal nematodes and macrofauna in fjords. Mean ± SD values of abundance and biomass are presented. Fjords with significantly lower or higher values (Dunn's test, p<0.05) are listed in brackets.

Fjord	Group	Abundance [ind. $0.1m^{-2}$]	Biomass [µg DM $0.1m^{-2}$]	Contribution to total biomass [%]
RIJP	Meiofauna	224 433 ± 209 435	41 384 ± 27 552	3.2
HSD		302 767 ± 60 368	101 155 ± 13 025 (>BSF, RAU)	2.4
KGF		244 600 ± 130 739	55 021 ± 20 230 (>BSF)	3.6
BSF		37 000 ± 7 671	8 800 ± 3 202 (<HSD, KGF)	0.4
ULS		137 333 ± 69 387	42 952 ± 19 588	8.1
RAU		111 167 ± 43 940	30 423 ± 16 122 (<HSD)	3.3
RIJP	Macrofaunal nematodes	1 033 ± 1 185	3 824 ± 2 746	0.3
HSD		1 367 ± 551	13 864 ± 10 645	0.3
KGF		1 767 ± 2 281	6 086 ± 6 906	0.4
BSF		650 ± 212	5 661 ± 270	0.2
ULS		767 ± 404	3 490 ± 1 293	0.7
RAU		733 ± 306	3 870 ± 1 321	0.4
RIJP	Macrofauna	841 ± 146	1 241 330 ± 775 101	96.5
HSD		1 431 ± 730	4 139 356 ± 2 091 792	97.3
KGF		998 ± 355	1 481 438 ± 414 739	96.0
BSF		569 ± 236 (<RAU)	2 332 508 ± 164 0240	99.4
ULS		586 ± 149 (<HSD, RAU)	481 456 ± 259 653	91.2
RAU		2 086 ± 483 (>BSF, ULS)	898 092 ± 120 558	96.3

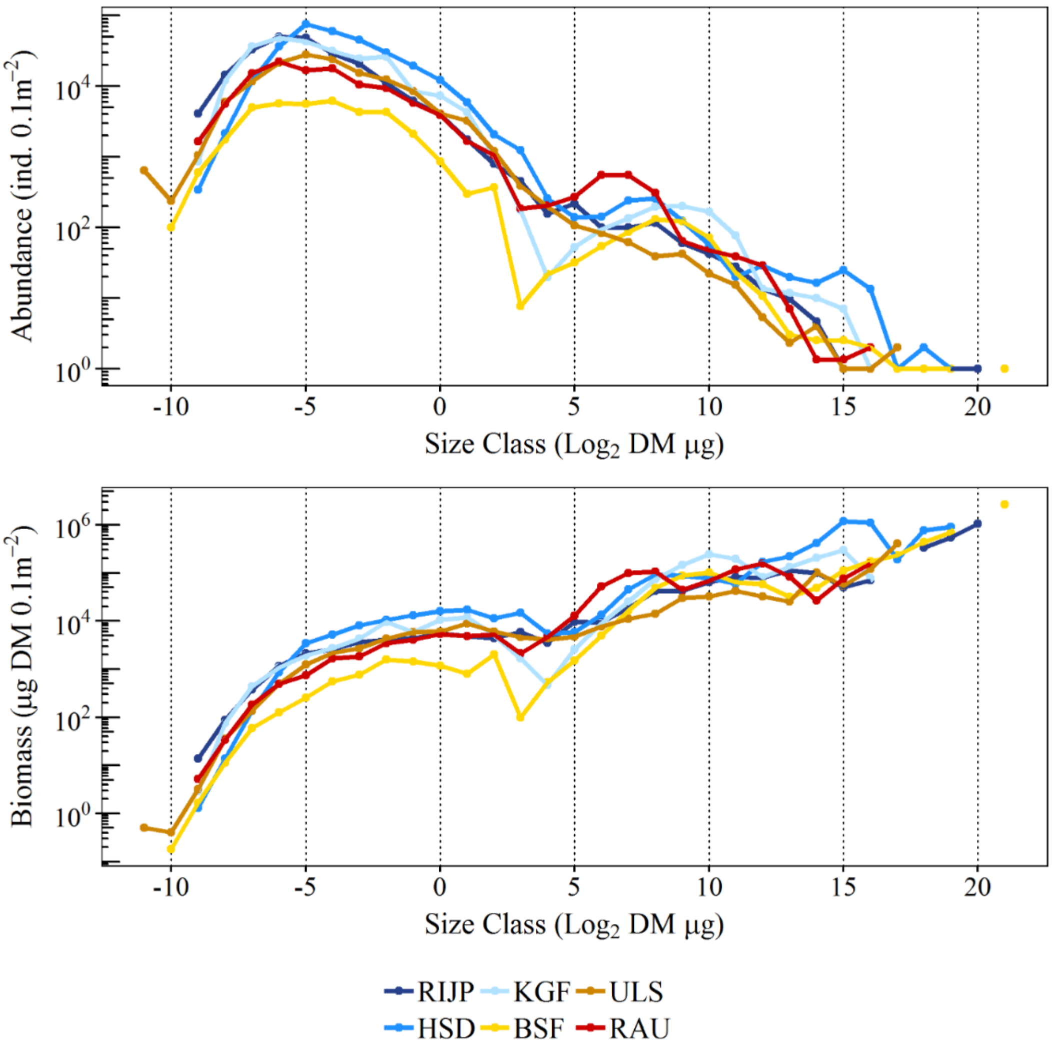

Figure IV.3 Abundance and biomass size spectra (average for three stations) in studied fjords.

A consistent pattern of size structure across stations and fjords was documented by the NBSS plots (Figure IV.4). There was a statistically significant relationship between normalized biomass and size classes (linear regression, $p<0.05$) for every fjord. Slopes of NBSS for each fjord ranged from -0.46±0.03SE in BSF to -0.57±0.02SE in RIJP, with no significant differences among localities (ANCOVA $F_{(5,\ 457)}=1.80$, $p=0.11$); however, significant differences in intercepts among fjords were found (ANCOVA $F_{(5,\ 457)}=12.25$, $p<0.001$). In that case, a multiple linear regression with fixed slope (-0.53±0.01SE) was calculated to find intercept values for all fjords and to study differences among them. The highest intercept was in HSD (11.77±0.24SE), significantly higher than in ULS (10.35±0.27SE) and BSF (the lowest one, 9.45±0.33SE, Tukey-adjusted pairwise *post-hoc* comparisons $p<0.05$). The intercept in BSF was significantly lower than in all other fjords except for ULS.

NBSS intercepts did not correlate with total benthic DM ($\rho=0.22$, $p=0.39$) or mean DM ($\rho=0.15$, $p=0.54$); however, when only the size class range common to every fjord (from – 10 to 16) was considered, a significant correlation to NBSS intercepts was found for both total DM and mean DM ($\rho=0.69$, $p<0.01$ and $\rho=0.52$, $p=0.03$, respectively).

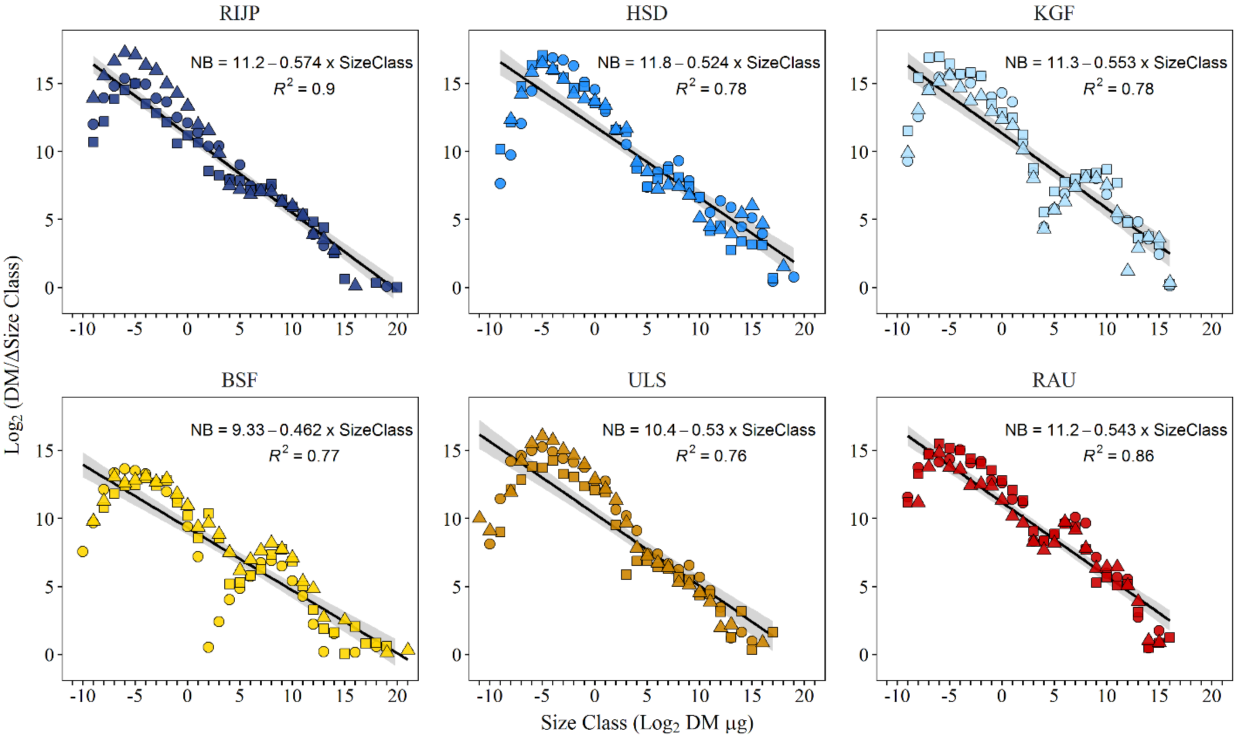

Figure IV.4 Normalized biomass size spectra (NBSS) in studied fjords. Symbols represent three replicate stations. Solid lines represent NBSS regression lines with standard errors.

4.3.3. Taxonomic and functional trait composition in macrofauna

The patterns of similarity among stations differed depending on whether ordinations were based on environmental data (Figure IV.2) or macrofauna biomass data partitioned among taxa, size classes and functional groups (Figure IV.5). The lack of congruence among respective similarity matrices was also confirmed by RELATE analysis. Moderate matching was found only between environmental variability (CAP; Figure IV.2) and macrofauna species composition variability and between functional group variability and size class variability (rho=0.6, p=0.001, in both cases), while the other combinations of similarity matrices exhibited much lower (poor) correlations (rho=0.3-0.4, p<0.05). The MDS plot, based on taxonomic composition, showed four station groups: 1) RAU, 2) ULS, 3) Svalbard fjords (RIJP, KGF, HSD), 4) BSF (Figure IV.5). No clear clusters of stations could be distinguished on ordinations based on size classes or feeding groups, indicating that variability among samples was similar or even higher than variability among fjords.

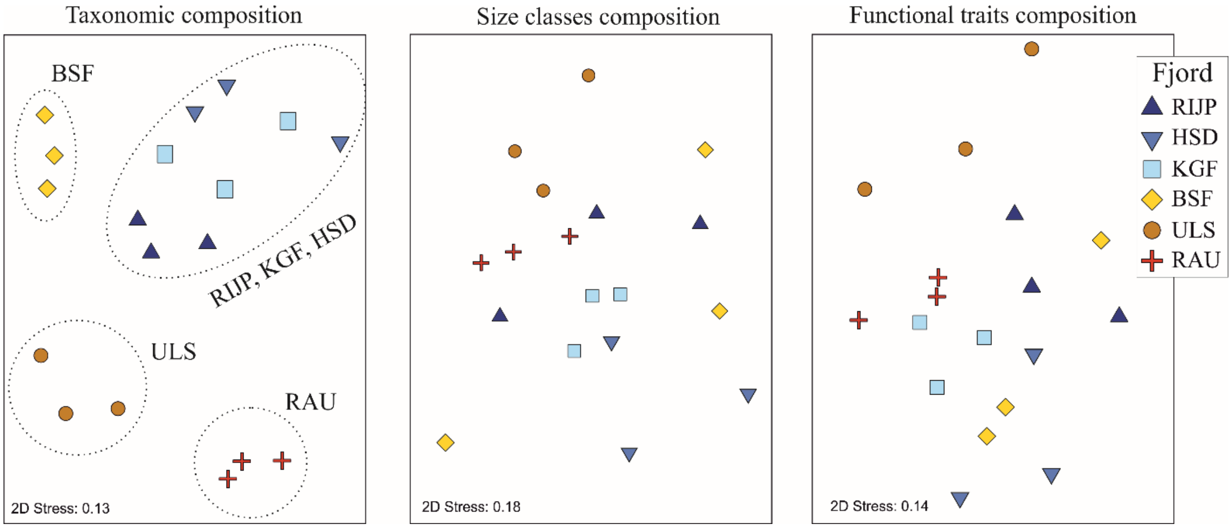

Figure IV.5 Non-metric multidimensional scaling (nMDS) of Bray-Curtis similarities of square root transformed data of macrobenthic biomass partitioned into taxa, size classes, and functional traits.

Polychaeta dominated all macrofaunal size classes, except for the smallest classes (1-5) in RIJP (dominated by Mollusca). Taxonomic composition of polychaetes varied considerably among the fjords (Figure IV.6). In RIJP, four families dominated: Oweniidae (small size classes), Maldanidae (middle size classes), Onuphidae and Nephtyidae (the largest size classes). In HSD, small size classes were dominated by Cossuridae, while large size classes were dominated by Maldanidae. In KGF, the composition of most size classes was diverse, but larger size classes consisted mostly of Maldanidae and Spionidae. In BSF, small size classes were dominated by Paraonidae, middle classes were dominated by Maldanidae, and the largest by Nephtyidae. In BSF, the polychaete composition was the most diverse, and no evident dominance was observed. In RAU, most size classes were dominated by either Cirratulidae or Spionidae.

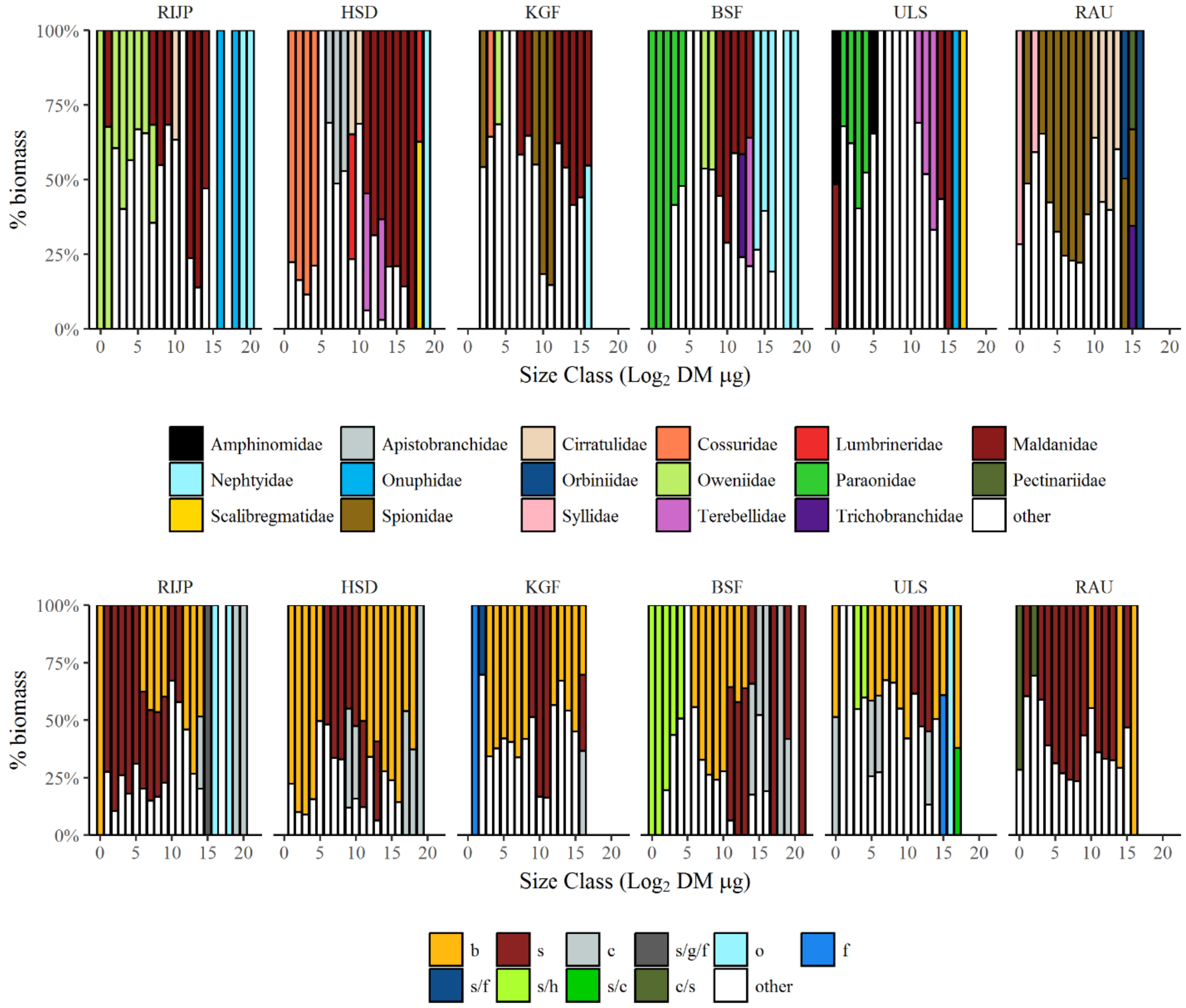

Figure IV.6 Percentage contributions of polychaete families and feeding groups to biomass in macrofaunal size classes in fjords. All groups that constituted <30% in each size class were classified as "other". Feeding groups: b – subsurface deposit feeders, c – carnivores, s – surface deposit feeders, o – omnivores, f – suspension feeders, h – herbivores.

Three feeding types prevailed in macrofauna (subsurface deposit feeders, surface deposit feeders and carnivores) in all locations, with varying proportions among the fjords and size classes (Figure IV.6). Additionally, in BSF, a considerable proportion of surface deposit feeder/herbivores was noted in small size classes. No consistent pattern in the composition of size classes, in terms of feeding types, was noted among fjords. For example, carnivores dominated large size classes in RIJP and BSF, while in HSD they were spread from moderate size classes to the largest, and in ULS they contributed to almost the entire range of size classes (Figure IV.6).

4.3.4. Body size vs. temperature in macrofaunal taxa

Only four macrofaunal species were present in all studied fjords: *Heteromastus filiformis* (Claparède, 1864), *Leitoscoloplos mammosus* Mackie, 1987, *Levinsenia gracilis* (Tauber, 1879), and *Pholoe assimilis* Örsted, 1845. For *L. mammosus* and *L. gracilis*, a relationship of decreasing body mass with increasing temperature was noted (GLM, $p<0.05$; Table IV.3), while for *H. filiformis* the trend was opposite ($p<0.05$); no trend was noted for *P. assimilis.* At the genus level, we noted 9 taxa present in every fjord; positive relationships between DM and temperature were found for three (*Heteromastus, Lumbrineris* and *Yoldiella*), negative relationships were found for two (*Chaetozone* and *Nephtys),* and no relationship was found for four (*Chone, Diplocirrus, Microclymene* and *Pholoe;* Table IV.4). Fourteen families with occurrences in every fjord were noted. A negative relationship between DM and temperature was documented for four families (Capitellidae, Cirratulidae, Lumbrineridae and Yoldiidae), positive for six (Ampharetidae, Maldanidae, Paraonidae, Sabellidae, Spionidae and Terebellidae), and no relationship was found for four (Flabelligeridae, Nephtyidae, Orbiniidae and Pholoidae; Table IV.5).

Table IV.3 Estimated regression parameters, standard errors, t values and p values for the gamma GLM presented in Eq. IV.1 testing the relationship between NBT and individual DM for macrofauna species that occurred in all fjords.

Species	Parameter	Estimate	Std. error	t	p
H. filiformis	α	4.35	0.06	77.85	<0.001
	β	0.03	0.01	2.58	0.010
L. mammosus	α	5.73	0.05	123.96	<0.001
	β	-0.14	0.02	-8.54	<0.001
L. gracilis	α	4.22	0.08	49.78	<0.001
	β	-0.13	0.02	-6.98	<0.001
P. assimilis	α	5.08	0.19	26.16	<0.001
	β	0.04	0.04	0.90	0.369

Table IV.4 Estimated regression parameters, standard errors, t values and p values for the gamma GLM presented in Eq. IV.1 testing the relationship between NBT and individual DM for macrofauna genera that occurred in all fjords.

Genus	Parameter	Estimate	Std. error	t	p
Chaetozone	α	6.59	0.07	92.24	<0.001
	β	-0.07	0.03	-2.96	0.003
Chone	α	4.32	0.12	37.18	<0.001
	β	-0.07	0.04	-1.82	0.072
Diplocirrus	α	7.60	0.19	40.99	<0.001
	β	0.02	0.04	0.63	0.531
Heteromastus	α	4.33	0.05	82.39	<0.001
	β	0.03	0.01	2.82	0.005
Lumbrineris	α	6.10	0.05	113.63	<0.001
	β	0.08	0.02	4.69	<0.001
Microclymene	α	6.05	0.08	71.27	<0.001
	β	0.05	0.03	1.38	0.170
Nepthys	α	12.99	0.49	26.45	<0.001
	β	-0.59	0.13	-4.47	<0.001
Pholoe	α	5.07	0.18	28.50	<0.001
	β	0.05	0.04	1.55	0.123
Yoldiella	α	5.39	0.06	90.01	<0.001
	β	0.09	0.02	3.87	<0.001

Table IV.5 Estimated regression parameters, standard errors, t values and p values for the gamma GLM presented in Eq. IV.1 testing the relationship between NBT and individual DM for macrofauna genera that occurred in all fjords.

Family	Parameter	Estimate	Std. error	t	p
Ampharetidae	α	8.10	0.34	23.52	<0.001
	β	-0.49	0.08	-6.27	<0.001
Capitellidae	α	4.49	0.14	31.76	<0.001
	β	0.08	0.03	2.85	0.005
Cirratulidae	α	6.38	0.06	107.55	<0.001
	β	0.14	0.02	9.21	<0.001
Flabelligeridae	α	7.60	0.19	40.99	<0.001
	β	0.02	0.04	0.63	0.531
Lumbrineridae	α	6.13	0.07	85.63	<0.001
	β	0.08	0.02	3.53	0.000
Maldanidae	α	8.86	0.09	96.51	<0.001
	β	-0.19	0.03	-5.78	<0.001
Nephtyidae	α	11.73	0.38	30.94	<0.001
	β	-0.16	0.11	-1.43	0.160
Orbiniidae	α	5.57	0.11	52.15	<0.001
	β	0.05	0.04	1.55	0.121
Paraonidae	α	5.04	0.15	32.75	<0.001
	β	-0.19	0.03	-6.35	<0.001
Pholoidae	α	5.07	0.18	28.50	<0.001
	β	0.05	0.04	1.55	0.123
Sabellidae	α	5.33	0.26	20.15	<0.001
	β	-0.21	0.08	-2.51	0.013
Spionidae	α	8.03	0.20	40.70	<0.001
	β	-0.26	0.04	-6.92	<0.001
Terebellidae	α	8.95	0.19	47.59	<0.001
	β	-0.17	0.06	-2.73	0.007
Yoldiidae	α	5.54	0.06	97.47	<0.001
	β	0.21	0.02	10.77	<0.001

4.4. Discussion

4.4.1. Benthic size spectra along thermal/latitudinal gradient

The general shape and the slope of benthic biomass size spectra did not vary across the wide geographical and thermal range sampled in the study presented in this chapter. Therefore, the hypothesis of an increase in the proportion of smaller size classes and steepening of the slope of normalized spectra towards warmer waters can be rejected. The invariance in the partitioning of biomass among size classes was observed for both abundance and biomass size spectra and despite differences in species or functional (feeding) group composition and environmental conditions in studied localities. Thus it seems that main characteristics of the size structure (shape and slope of size spectra) remain inherent features of stable and undisturbed marine soft-sediment communities. This provides an evidence of the resilience of these systems to natural environmental variability, including thermal regimes shifts observed at present spatial scales and predicted to proceed in the near future (IPCC 2013).

Significant changes in benthic size spectra have been reported to occur in response to severe anthropogenic and natural disturbances. Anoxic conditions can have a negative effect on the slope of benthic NBSS (Quiroga et al. 2005). Increasing proportions of smaller size classes in benthic size spectra were also reported, due to glacial disturbances (high sedimentation rate of mineral material, sediment instability) in Arctic fjords (Górska and Włodarska-Kowalczuk 2017) and for a fish community in a sewage-enriched river ecosystem (de Bruyn et al. 2002). The reduced presence of organisms from the largest size classes and a steepening of the slope was also observed along the depth gradient (from 32 to 421 m), with a bathymetrically decreasing amount and predictability of food supply (Saiz-Salinas and Ramos 1999). On the other hand, in the absence of a severe stressor, size spectra seem to be insensitive to natural variability. Duplisea and Drgas (1999) showed similar size structures in areas of different grain size composition, despite this being an important driver of variability in taxonomic composition of benthic biota. High consistency of size spectra and their independence from a range of environmental conditions (days since the last storm, carbon and nitrogen content in sediments, grain size) were also reported for benthic communities by Schwinghamer (1983). Sprules (2008) analysed multiannual changes in pelagic biomass size spectra in the Laurentian Great Lakes and did not report any significant differences in size spectra despite substantial changes in species composition, biological invasions or alterations in water quality. He also reported a lack of differences in size spectra among lakes of different geological ages, biological, chemical and physical properties.

Górska and Włodarska-Kowalczuk (2017) suggested a shift in the macrofaunal size spectrum towards higher size classes at lower temperatures/higher latitudes. They compared benthic biomass size spectra, constructed based on their own Arctic collections, with published studies from lower latitudes (Warwick and Clarke 1984; Warwick and Joint 1987; Hua et al. 2013). However, the study presented in this chapter, performed on materials collected over a wide geographical range and analysed in a standardized way, did not confirm such a trend. Organisms in the highest size classes (>17) were present only in colder fjords (RIJP, HSD and BSF). This agrees with theories of organism size clines along thermal gradients (Bergmann 1847; Daufresne et al. 2009). However, in each case there were only few specimens present in the largest size classes, and some inconsistencies to this pattern were observed (e.g., large classes were not represented in KGF in the study presented in this chapter, while specimens of respective size where present in the same area visited in winter (Chapter III). Therefore, conclusions based on these observations cannot be firmly grounded.

Minor differences in size spectra among fjords can be related to local phenomena. For example, in RAU, a peak between size classes 6 and 7 in abundance size spectra was due to the high dominance of the polychaete *Pseudopolydora paucibranchiata*, an invasive species originating from Pacific Japan (Çinar 2013). Individuals of *P. paucibranchiata* can reach very high densities and are able to exclude other tube building polychaetes (Levin 1982).

4.4.2. Benthic standing stock in relation to environmental variability

δ^{13}C and Chl *a* explained a large part of the variability in total biomass and the intercepts of the NBSS model (confirming hypothesis 2). Both parameters are regarded as indicators of organic matter composition and origin. Thus, the results of this chapter support the notion that quality rather than quantity of food determines the level of benthic productivity. Sediment pigment concentration, which represents the “freshly produced” fraction of organic matter in sediments and can be an indicator of ecosystem productivity, was linked with higher biomass, as reported in numerous studies (e.g., Ambrose and Renaud 1995; Soltwedel et al. 2000; Quiroga et al. 2012). The relationship between δ^{13}C and benthic standing stock is less obvious, especially in the study presented in this chapter, where higher standing stock is reported in fjords characterized by lower δ^{13}C values (usually interpreted as indicating a higher contribution of terrestrial organic matter, regarded as less available for marine taxa, Zaborska et al. 2018). However, a mixture of carbon sources and abundance of detrital food sources may be an important buffer of the highly seasonal phytoplankton production for benthic systems capable of assimilating this detritus (Renaud et al. 2015). Studies of Feder et al. (2011) showed that organic carbon from multiple sources (including terrestrial one) supported rich benthic in Chukchi Bight and Kotzebue Sound (Chukchi Sea). Nevertheless, Naidu et al. (2000) and Kuliński et al. (2014) elaborated on the difficulties in defining organic matter source based on stable isotope data in the Arctic. Therefore the sources of organic matter based on δ^{13}C values in this the study cannot be unequivocally assessed. Regardless, the documented strong correlation between δ^{13}C and biomass points to an importance of food origin in promoting benthic productivity.

The intercepts of the NBSS differed significantly among fjords (from 9.45 to 11.77). According to Guiet et al. (2016), the NBSS intercepts decrease with increasing temperature if ecosystem resources are constant. This is due to the imbalance between food assimilation of organisms and their mortality and metabolism that increase with warming. In the study presented in this chapter, the resources differed among fjords (both in terms of quantity and quality, as indicated by C_{org}, sediment pigment concentrations and δ^{13}C), and those differences were not related to the latitude/temperature regime. An intercept in the NBSS model is regarded as an indicator of community biomass (Sprules and Munawar 1986) or ecosystem richness in food reserves (Guiet et al. 2016). However, according to Hua et al. (2013), NBSS intercepts cannot be accepted as a universal indicator of total biomass, as they are sensitive to the size class range. Presented data indicate that intercept can be treated rather as a proxy of the total (or average) biomass only when compared communities are consistent in terms of size class range.

The high contribution of macrofauna to the total benthic DM was similar across the studied fjords (91 - 99%) and agreed with those reported from other soft-bottom systems (e.g., Gerlach 1971). Górska and Włodarska-Kowalczuk (2017) explored the effects of food and disturbance on benthic biomass partitioning among size groups in Arctic fjords and reported a similar range in the contribution of macrofauna (81-94%), regardless of the level of food availability. Only in the presence of strong mineral sedimentation at glacier fronts did this proportion drop strongly, to approximately 60% (Górska and Włodarska-Kowalczuk 2017). Thus, even at this crude resolution (meiofauna, macrofauna), data from this chapter still suggest the partitioning of biomass among groups of organisms defined by size criteria to be a consistent feature of benthic communities, provided stable sediments and undisturbed conditions.

4.4.3. Macrobenthic species and functional trait composition

Species composition varied among the studied locations resulting most likely both from differences in regional species pools and environmental conditions in particular fjords that act as filters, shaping the composition of taxa (Włodarska-Kowalczuk et al. 2012). The regional constrains are visible e.g., in the close location of stations from three Spitsbergen fjords on the ordination. The two north Norwegian fjords are quite different in terms of species composition, despite their close geographic location, most likely due to their different environmental character: BSF is silled and filled with cold water, whereas ULS is open and is filled with warm, oceanic water masses. Interestingly, even if a polychaete family (e.g., Maldanidae) was dominant/subdominant in several locations, it was abundant in different size classes in each location, indicating the independence of taxonomy and size composition

The functional (feeding type) group composition was independent of geographical location, and more reflected the differences in species composition. In addition, no persistent pattern appeared in the distribution of feeding types across the macrobenthic size classes. One of the properties of pelagic food webs is the relatively consistent relationship between predator and prey body sizes (Sprules 2008), suggesting the size structure of pelagic ecosystems can be used to predict the efficiency of predator-prey interactions and the standing stock (Sheldon et al. 1977). In the benthic food web, feeding links can also be size-based, i.e., smaller organisms being consumed by larger ones (Van Oevelen et al. 2011); however, the overall pattern may differ, due to the wide size range of primary consumers of organic matter. Like in this study, where no tendency of increasing (with size class) contribution of predators was noted, e.g., in the USF or HSD fjords carnivores composed mostly small and intermediate macrofauna size classes or like in BSF and RAU fjords, where the largest organisms were deposit feeders. This suggests that energy flow in benthic food webs has more complicated pathways compared to pelagic food webs, which are linked in a chain of progressively larger size classes from planktonic producers to large predators.

4.4.4. Organism body size along temperature\latitude gradient

The concept of increasing body size with increasing latitude/decreasing temperature was proposed more than 150 years ago by Bergmann (1847) and has been supported by numerous marine and terrestrial examples. For instance, ants from the British Isles exhibited increased body size with increasing latitude; however, the strength of this trend depended on the subfamily level (Cushman et al. 1993). Atkinson (1994) found elevated temperature to have a negative effect on body size at a given development stage in 83.5% of 109 reviewed studies of animals, plants, protists and bacteria. Moreover, the decline in body size as a consequence of climate warming is predicted for various organisms (Sheridan and Bickford 2011) and was already observed for the British common toad (*Bufo bufo,* Reading 2007). Temperature effect was also observed for marine ostracods from the genus *Poseidonamicus*, which have increased their size more than 50% during the last 40 million years, corresponding to climate cooling (Hunt and Roy 2006). However, a trend towards publishing only significant trends may distort the accumulated scientific evidence (Matosin et al. 2014; Nissen et al. 2016). In this study a relationship between temperature and body size explored for widely distributed taxa was shown to be inconsistent and equivocal. A similar outcome was achieved for different butterfly families from North America, Australia, Europe and Africa (Hawkins and Lawton 1995). In the case of benthic fauna, the reverse (or lack of) responses of body size to changing latitudes/thermal conditions in particular taxa resulted in an invariance in size structure, explored at the whole community level, as opposite effects were equalizing each other.

Chapter V. Summary, conclusions

5.1. Summary

The dissertation explores the patterns of seasonal and spatial variability of benthic size structure and their environmental controls in the North Atlantic and Arctic coastal waters (fjords). This dissertation also introduces new methodology for nematode biomass assessment that hastens the timely size analyses and thus is likely to promote wider application of size structure analyses in benthic ecology. In this concluding chapter the key findings of the dissertation chapters are summarised to present the overview of the relationships between environmental conditions and the size structure of marine coastal soft bottom communities.

Addressing the **objective 1):** “*to develop a method of semi-automated measurements of nematodes*”, the new methodology was presented in **Chapter II.** Nematoda is a phylum of animals that dominate meiofauna community. They often reach very high abundances of few thousands individuals in a sample (usually from an area of 10 cm^2). The biomass of nematodes or their size structure is usually assessed using estimations based on individual dimensions measurements. These analyses are very time consuming that precludes their wide application in standard ecological surveys. A more rapid, semi-automated method was developed, tested and described in this chapter. The semi-automated method was based on computer detection of nematodes on pictures based on color threshold. The analyses using the new method were almost two times faster than those employing manual measurements. The results of length measurements did not differ between automated measurements and manual ones. The results of maximum width manual measurements differed depending on the analyst (mostly due to different designations of the point of nematode maximum width). For estimating individual nematode biomass, a formula using the equation for the volume of cylinder (one of the three tested in the study) was recommended. The new semi-automated method seems to be less biased (e.g., not prone to the subjective maximum width designation) and significantly time saving compared to standard manual protocols. The wider application of this method should facilitate the more frequent inclusion of meiofaunal biomass assessments and size structure studies in marine benthic surveys.

Referring to the **objective 2):** *“to assess the seasonal (winter versus summer) changes in benthic biomass size spectra in the Arctic fjordic system”,* the summer-winter comparison of benthic size structure in Kongsfjorden (a fjord off west Spitsbergen) was performed and described in **Chapter III.** The Arctic ecosystem undergoes strong seasonal fluctuations, defined mostly by extreme changes in insolation throughout the year that regulate the functioning of the whole ecosystem. Due to logistic restrains most of studies in the Arctic are conducted in summer and the knowledge about biota standing stocks and functioning in winter is limited, especially regarding the size structure of marine communities. In Kongsfjorden, higher concentrations of photosynthetic pigments in the sediments collected in summer than in those collected in winter indicated seasonal changes in fluxes of organic matter produced in the water column. Differences in total abundance and biomass of both macrofauna and meiofauna between two seasons were also detected. The individual biomass (a measure of an organism size) within populations of dominant taxa varied between seasons. However, the differences were equivocal, with individuals being smaller in summer for some taxa, larger for other taxa, or even showing inconsistent patterns for the same taxon depending on the sampling stations. This seasonal variability in particular taxa size (individual biomass) was not translated into a seasonal change in size spectra at the community level. No difference in the community size structure between the seasons was noted when abundance, biomass or normalized size spectra assessed for the two seasons were compared. The summer-winter stability of size spectra in benthic communities supports the hypothesis of the existence of a "food bank" in polar sediments and relative independence of polar benthic biota functioning from the seasonality in the pelagic productivity.

The analyses performed to address **objective 3):** *"to describe patterns of variability in benthic biomass size spectra in response to changes in water temperature and food availability across latitudinal gradient (60-81°N)"* were described in **Chapter IV.** The sampling locations were selected to exclude the effects of anthropogenic impacts like intensive fishing or aquaculture activity or strong natural stressors as glacial inflows in the Arctic fjords. The bottom water temperature in the six studied fjords spanned the range from 7.7°C to -1.6°C, the fjords differed also in terms of organic carbon, chloroplastic pigments content, $\delta^{13}C$ values, and grain size composition in sediments. The total benthic biomass differed among fjords from 5.3 to 42.5 g DM m^{-2} and this variability was mostly attributed to variability in chlorophyll *a* content and $\delta^{13}C$ values in sediments. Despite significant differences in environmental factors and in the benthic standing stocks, the size structure of studied benthic communities showed little variability among inspected localities. The slope coefficient of linear regression calculated for normalised biomass size spectra (NBSS) did not differ significantly among fjords indicating similar energy flow through the community in studied localities. There were significant differences in intercept coefficients of NBSS and these differences were related to the variability in the benthic standing stocks. Moreover, the size structure remained consistent across the studied communities, regardless of the strong variability in macrofaunal taxonomic and functional trait (feeding type) composition. At the species level, the effects of temperature on body size were inconsistent across studied taxa, as variable relationships (positive, negative and neutral) between temperature and body size were noted for particular species, genera and families. The temperature responses of size in individual taxa did not translate into changes visible at the whole community level. Results from this chapter indicate that size structure of coastal benthic communities may be an inherent property, highly unsusceptible to different conditions and will not be affected by thermal regime shifts caused by climate change. This may also represent a form of resilience of benthic communities to consequences of environmental changes that secures the maintenance of ecological functioning, especially in terms of energy transfer in the food web and geochemical processes in sediments.

5.2. Conclusions and future perspectives

The main conclusions of this dissertation are:

- the estimation of nematodes biomass based on semi-automated measurements and with use of formula for the volume of cylinder provides comparable results to those obtained with traditional manual procedures. The semi-automated method is less human error biased and less time consuming compared to the method based on manual measurements
- The size structure of soft-bottom benthic community in an Arctic fjord remains persistent despite the seasonal (summer-winter) changes in organic matter supply (indicated by chloroplastic pigments concentration in the sediments). The summer-winter stability of size spectra in benthic communities in an Arctic fjord supports the hypothesis of the presence of a sediment “food bank” in polar regions (i.e. relative independence of polar benthic biota from the seasonality in the pelagic productivity).

- The size structure of benthic fauna of undisturbed sediments across wide geographic range is a conservative feature of benthic communities, insensitive to natural variability in both environmental (temperature and food availability) and ecological (total biomass, taxonomic composition, and functional trait composition) characteristics of the benthic systems.
- The size responses to environmental seasonal and spatial variability vary across taxa, and these effects perceived at individual taxa level do not translate into changes visible at the community level.

Body size is a “master trait” closely coupled to key ecological processes like metabolism, development or energy flow through food webs (Blanchard et al. 2017). Recent studies alarmed that declining body size might be a universal response of organisms to the climate change, next to changes in species distribution and phenology (Gardner et al. 2011). These predictions were particularly relevant for the Arctic since the climate warming has the strongest effects at the northern high latitudes (ACIA 2005). Results of this thesis show high consistency of benthic community size structure regardless of environmental (spatial and temporal) variability. The positive relationship between quality and quantity of organic matter and standing stock (and the intercepts of the NBSS) of benthic fauna was observed. Since it is predicted that climate change in the Arctic will result in alteration of the primary production, the benthic systems functioning may be affected by the altered total standing stocks but most probably not by the changes in the distribution of the stocks among the groups of animals of different sizes. This should be confirmed by the studies involving analyses of historical time series of benthic samples from regions where the changes in primary productivity were already reported.

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