Original Articles

# Modification of benthic food web structure by recovering seagrass meadows, as revealed by trophic markers and mixing models

Emilia Jankowska[a,*], Marleen De Troch[b], Loïc N. Michel[c,1], Gilles Lepoint[c], Maria Włodarska-Kowalczuk[a]

[a] Institute of Oceanology Polish Academy of Sciences, Powstańców Warszawy 55, 81-712 Sopot, Poland
[b] Ghent University, Biology Department, Marine Biology, Krijgslaan 281 – S8, 9000 Ghent, Belgium
[c] Freshwater and Oceanic Sciences Unit of reSearch (FOCUS), Laboratory of Oceanology, University of Liège, Liège, Belgium

A B S T R A C T

Seagrass meadows are among the most diverse and productive coastal ecosystems in the world. Currently, the accelerating loss of these habitats is recognized worldwide. In the southern Baltic Sea, a natural recovery of *Zostera marina* meadows has occurred after a dramatic reduction within the last century. The aim of this study is to understand if and how the recovering eelgrass meadows affect the functioning of benthic ecosystems. The trophic links within the benthic food webs in the seagrass meadows and bare sandy bottoms were depicted and compared. The trophic connections were examined by combining stable isotope (SI) composition ($\delta^{13}$C, $\delta^{15}$N) and fatty acid (FA) profiles of meio- and macrofauna consumers and of potential food sources (particulate organic matter, surface sediment organic matter, epiphytes, microphytobenthos/bacteria and macrophytes) in a Bayesian mixing model framework (MixSIAR). Significantly higher amounts of the FA bacterial marker (C18:1$\omega$7) were observed in meiofauna (approximately 40%) than in the macrofauna (1% on average), suggesting that bacteria are an important part of the meiofauna diet. The mixing model results indicated that the benthic consumers in the vegetated habitat utilized more food sources (e.g., epiphytes in the diets of meiofauna and macrofaunal grazers) and thus had a more diverse diet. Macrofaunal omnivores relied to a larger degree on animal-derived organic matter in vegetated habitat, which could be linked to higher invertebrate prey availability. The results highlight the importance of recovering seagrass meadows in driving the mechanisms responsible for food web organization. Any type of change to the state of seagrass meadows is crucial to the functioning and stability of marine ecosystems.

## 1. Introduction

Seagrass meadows are among the most diverse and highly productive coastal ecosystems in the world (Hemminga and Duarte, 2000). The plants play an important role as foundation species of seagrass habitats, by creating three-dimensional structures (leaves, rhizomes, roots). Hence, they increase the complexity of the seabed architecture and provide shelter and numerous niches for other organisms (Gartner et al., 2013). Moreover, seagrasses and associated macrophytes or algae can be direct food sources for faunal consumers; thus, they sustain populations of commercially important vertebrate and invertebrate species (Hemminga and Duarte, 2000).

Over the past few decades, significant decreases in seagrass abundance and aerial cover have been recorded worldwide (Waycott et al., 2009). Changes in the state of seagrasses affect coastal systems and may

result in a reduction in fauna abundance and diversity, as well as in modification of food web structures and overall functioning (Boström et al., 2014). The mechanisms driving food web organization within seagrass meadows remain poorly understood as they may depend on local conditions, meadow features and consumer species pools (Boström et al., 2014). The trajectory of eelgrass meadows extent in the southern Baltic Sea off the Polish coast has shown dramatic changes over the past 60 years. Before the 1950s, most of the seafloor of the Inner Puck Bay (103 km$^2$) was covered by the meadows. A significant decrease (most likely caused by eutrophication and massive growth of filamentous algae) of *Zostera marina* in the area was observed in 1987, when the eelgrass area declined to only 16 km$^2$ (Kruk-Dowigałło, 1991).

Recently, the natural recovery of eelgrass-dominated underwater meadows has occured in several locations in the Gulf of Gdańsk

(Jankowska pers. observation). The coverage of the seagrass beds increased rapidly, but the density and biomass of the plants remain low compared to other temperate *Z. marina* meadows (Jankowska et al., 2014).

The aim of the present study is to investigate if and how the recovering eelgrass meadows affect the overall functioning of the benthic systems in the Gulf of Gdańsk (southern Baltic Sea) in terms of food web organization and thus energy flow. The Gulf of Gdańsk serves as an example of a system characterized by both a low level of vegetation development (low density and biomass of macrophytes) and a low faunal diversity (defined by the Baltic Sea species pool). We hypothesize that recovering (low density) eelgrass meadows are capable of shaping the food web structure in similar way as that observed in well-developed meadows, i.e., by increasing the consumer pools and the number of food sources in the diets of benthic consumers.

Most of the effects of seagrass on food webs are reported from well-developed meadows and diverse benthic communities (Lepoint et al., 2000; Vizzini et al., 2002; Leduc et al., 2006, 2009; Baeta et al., 2009; Lebreton et al., 2011, 2012; Michel et al., 2015; Vafeiadou et al., 2013, 2014). The present study documents the structure of the benthic food web in two contrasting habitats: vegetated sediments of recovering meadows and the adjacent bare sands. This study includes all major possible food sources (bacteria/microphytobenthos, epiphytes, macroalgae, seagrass, particulate organic matter, and surface sediment organic matter) and most consumers are analyzed at the species level (meiofauna copepods, macrofauna and fish). Stable isotopes and fatty acids biomarkers are used in Bayesian mixing models (MixSIAR) to estimate the relative contribution of food sources in the consumers' diet. This study is the first field study in a seagrass habitat integrating all components of a benthic system and using both types of biomarkers in Bayesian mixing models to achieve an as accurate as possible depiction of the trophic links in a benthic food web. Moreover, the focus on recovering seagrass meadows is a novel approach that is now crucial because dramatic changes of seagrass cover are occurring, and intensive restoration programs are being undertaken worldwide.

## 2. Materials and methods

### 2.1. Study area

The study took place in the Gulf of Gdańsk, located in the southern Baltic Sea, off the Polish coast where salinity reaches approximately 8 (Nowacki, 1993). A recent inventory of the seabed habitats in the Polish Exclusive Economic Zone documented that areas covered by *Z. marina* meadows are rapidly growing (water.iopan.gda.pl/projects/Zostera/index-pl.html). The areal distribution of eelgrass beds, estimated in 2009, was 48 km$^2$ for inner Puck Bay (Węsławski et al., 2013). Currently, the actual eelgrass-covered area may be even greater, as new locations of seagrass areas in the Gulf of Gdańsk were observed during sampling campaigns in 2012–2014 (Jankowska, pers. observation). Meadow density in the summer seasons reached an average of 202 shoots m$^{-2}$ with a biomass of 40 g dw m$^{-2}$, which is lower compared to other temperate eelgrass meadows in the Northern Hemisphere (Jankowska et al., 2014).

### 2.2. Field sampling

Two sampling stations were selected in the Gulf of Gdańsk – one within the extensive eelgrass meadows and one in the neighboring large unvegetated area; both stations were located at similar depths (1.5–2 m), approximately 2.3 km from each other (Fig. S1, Supporting Information). Sampling (by scuba divers) occured in the summer (August 2014) when seagrass vegetation development is at its maximum in the Gulf of Gdańsk (Jankowska et al., 2014). Six replicates (3 replicates for stable isotope (SI) analyses and 3 replicates for fatty acid (FA) analyses) for each source at each station were collected in the summer

(Table S2, Supporting Information). The same replication protocol was used for the consumers (it was not achieved in case of rare taxa). Sampled potential food sources included: SSOM (surface sediment organic matter, collected using sediment cores), POM (particular organic matter from the water column, 6 liters of water collected at a depth of 1 m), microphytobenthos (upper 2 cm sediment layer, collected using a syringe and extracted in a laboratory), filamentous algae (*Pylaiella littoralis*), benthic macrophytes including eelgrass below- and aboveground structures and epiphytes (on the surface of the eelgrass leaves). Samples of consumers included meiofauna (taxa associated with the seagrass leaves collected with a 42 μm mesh size net and seabed taxa collected in the upper 2 cm of sediments), macrofauna and fish (collected with a sediment corer (upper 10 cm) and a small dredge, respectively). For the meiofauna, only two harpacticoid copepod species – *Paraleptastacus spinicauda* (family Leptastacidae) and *Tachidius discipes* (family Tachidiidae) – were chosen for analysis since only they were abundant enough to collect a sufficient number of individuals.

### 2.3. Laboratory analysis

Samples were processed in the laboratory immediately after sampling. Water was prefiltered on a 320 μm sieve to eliminate large zooplankton and then filtered through GF/F Whatman glass fiber filters (0.7 μm porosity). Macrophytes were identified, and epiphytes were detached from the leaves by shaking using a vortex mixer (10 min) and sonicating (2 × 60 s, using Sonifier Tansonic Labor 2000) the seagrass leaves and macrophytes in prefiltered seawater. Then, the water containing detached epiphytes was filtered through GF/F Whatman glass fiber filters (0.7 μm porosity). Fresh microphytobenthos was collected by transferring the upper 1 cm of the sediment to plastic boxes. Then, the sediment was covered with a 100 × 150 mm Whatman lens cleaning tissue and cover glass, and exposed to artificial white light source, to enable diatom migration. After 24 h, the microphytobenthos were scraped off the cover slides and transferred to vials with prefiltered seawater. However, the FA analyses indicated that these samples contained large quantities of bacteria; thus they were treated as a mixture of bacteria and microphytobenthos.

Adult meiofauna individuals were extracted alive from the sediment using decantation and attraction of positive phototactic copepods with artificial white light. Copepods were placed alive in a petri dish with pre-filtered seawater for a few hours to allow gut clearance. Afterwards, individuals of the two species were picked under the stereomicroscope to make several replicate samples, each replicate sample consisting of 200 adults. Macrofauna and fish were kept for 24 h in prefiltered seawater to purge their gut contents. Then, they were identified to the species level. When individuals were too small to provide enough tissue for one sample, several individuals (from 1 to 60, detailed information presented in Table S2) were pooled to obtain sufficient biomass for a sample. Samples of the fish consisted of parts of their muscles (each sample representing one individual).

All samples were placed in glass vials and stored at −80 °C. Afterwards, samples were freeze-dried and ground for further analysis and then kept at −80 °C. Subsequently, all samples were analyzed for total fatty acids content and stable isotopes ($\delta^{13}$C and $\delta^{15}$N [‰]). Lipid extraction, FA methylation and an analysis of fatty acid methyl esters (FAMEs) were performed in a one-step method according to Abdulkadir and Tsuchiya (2008) and De Troch et al. (2005) at the Marine Biology Research Group (Ghent University). The fatty acid nonadecanoic acid (C19:0) was added (to copepods and microphytobenthos – 20 μl, to the other samples – 40 μl, Fluka 74208) as an internal standard to allow quantification later. The FA shorthand notation A:BωX was used, where A represents the number of carbon atoms, B gives the number of double bounds and X is the position of the double bound closest to the terminal methyl group (Guckert et al., 1985). The results for each FA were expressed as the relative percentage [%] of the total FA content ± standard deviation. Total FA content refers to the FA content detected