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Sediment quality assessment studies with *Corophium* spp. (Amphipoda) in the Gulf of Gdansk, Martwa Wisła and Wisła Śmiała

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Zastosowanie *Corophium* spp. (Amphipoda) w badaniach jakości osadów dennych Zatoki Gdańskiej oraz Martwej i Śmiałej Wisły

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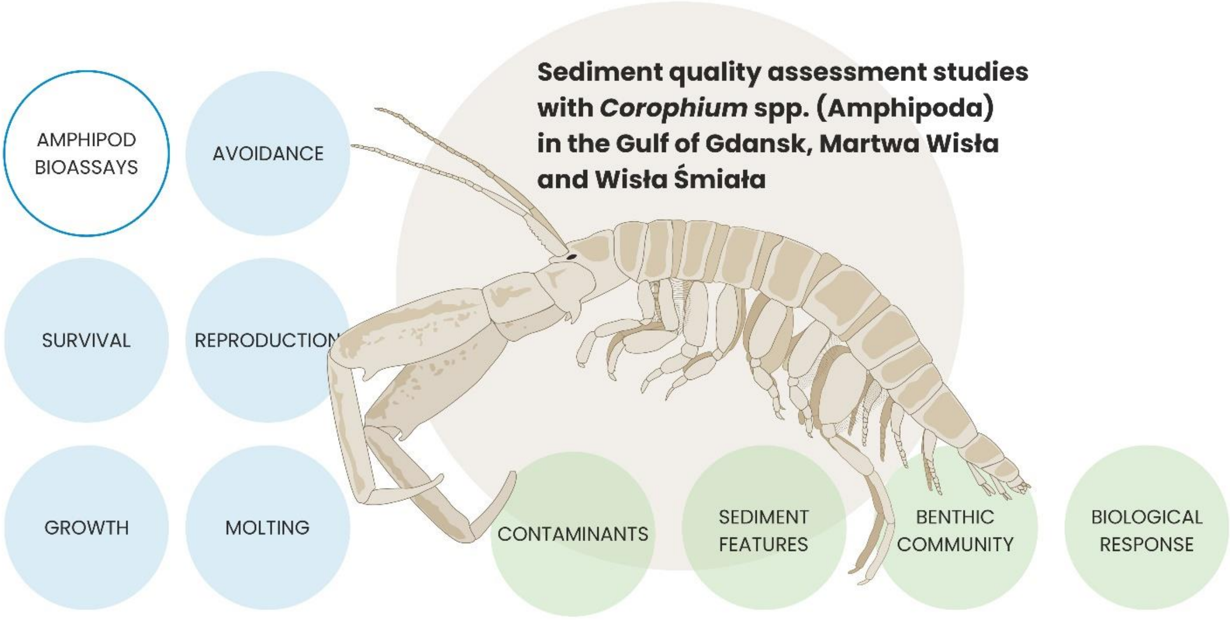
Summary



Crustaceans of the order Amphipoda play a crucial role in marine, estuarine, and freshwater ecosystems. The burrowing amphipod species are extensively used in marine and estuarine sediment toxicity tests. They are particularly well-suited for evaluating the environmental quality of soft-bottom habitats. As endobenthic organisms, they live in direct contact with sediment, which makes them more exposed to sediment-bound contaminants and substances dissolved in the pore water and overlying water. Among five *Corophium* spp. reported to occur along the coast of Poland, two are recommended as indicators for sediment quality assessment in Europe, namely *C. volutator* and *C. multisetosum*. They exhibit sensitivity to a wide range of contaminants, display tolerance to varying salinity levels and sediment particle sizes, and are easy to collect and handle in the laboratory. Yet, the use of those species for the South Baltic area has not been evaluated until now. Organisms collected from local ecosystems may provide more realistic scenarios for bioassays. Moreover, there is a need for rapid screening tests and infaunal amphipods such as *Corophium* spp. are good candidates for short-term behavioral bioassays.

The doctoral thesis aimed to evaluate the suitability of *Corophium* spp. for the assessment of sediment quality in the Gulf of Gdańsk (GoG) and the Martwa Wisła and Wisła Śmiała Rivers (MW&WS). Two hypotheses were tested, namely: 1) *Corophium* spp. are responsive to sediment contamination showing differential severity of responses depending on sediment contamination level, and 2) *Corophium* spp. responses to the MW&WS sediments reflect the status of macrozoobenthos assemblages.

The GoG area is known for its elevated concentrations of legacy persistent hydrophobic organic pollutants (HOCs), emerging contaminants, and metal elements. These pollutants result from extensive urban and industrial development along the coast and the influx of contaminants carried by inflowing rivers. The area around Martwa Wisła is particularly interesting from an ecotoxicological perspective. Martwa Wisła is a historical part of the Wisła, which was redirected to the north by a ditch in 1895. Today, these rivers are divided by the Przegalina sluice, and MW&WS rivers have separate outflows to the Gulf. The MW&WS area was in the past exposed to leakage from a nearby phosphogypsum pile. This leakage included substances with very low pH, high phosphate and fluoride levels, the presence of sulfates, toxic heavy metals, and radioactive elements. The contamination infiltrated both surface and groundwater, posing a significant environmental threat to the environment and local population.

Sediments were collected from 13 sites in the GoG area characterized by low to high contamination, including the seaports in Gdynia and Gdańsk and several other locations within the Gulf area. In the MW&WS area, five sites were selected along the course of the rivers from the confluence of Kanał Wielki with the Martwa Wisła River (above the phosphogypsum pile) towards the mouth of Wisła Śmiała River. From each site, three sediment samples were collected with a Van Veen grab. The uppermost 4 cm layer of each sample was taken for toxicity bioassays, and analyses of sediment characteristics and chemical contaminants. In the MW&WS area, three additional sediment samples were obtained from each site for the determination of the macrobenthos assemblages. The collected sediments were analyzed for the content of fines ($F < 63$ μm), organic matter in the fine fraction (OM_f), organic matter in the whole sediment (OM_w), and HOCs. The MW&W sediments were additionally analyzed for sediment grain composition.

The study involved chronic exposure of *C. volutator* and *C. multisetosum* to the sediments, during which various biological responses were investigated. These responses included survival, growth rate (GR), molting frequency, and emergence (sediment avoidance) of the amphipods. Additionally, the study evaluated reproductive activity by examining the gravidity of female amphipods and the presence of juvenile offspring. To compare the ability to discriminate contaminated and non-contaminated sediments by amphipods, a standardized sediment-response test with ostracod *Heterocypris incongruens* was performed, as well as an overview of benthic assemblages in the Martwa and Śmiała Wisła Rivers.

The test organisms, *C. volutator* and *C. multisetosum*, were sampled from two coastal locations in the Bay of Puck that were known for their relatively low levels of anthropogenic chemical contaminants, distant from pollution sources. Sediment samples from these locations were also used for amphipod acclimation to laboratory conditions and as a control (reference sediment) in the bioassays. The bioassay procedure was based on methods proposed by USEPA (2001). The exposures lasted 28 days, during which sediment avoidance and molting frequency of the amphipods were continually monitored. After the termination of the tests, the survival, length, and gravidity of the females were examined. The bioassay with *H. incongruens* was carried out using the Ostracodtoxkit F kit (MicroBioTests Inc.), following standard procedures. The *Corophium* spp. and *H. incongruens* responses were examined for significant differences from respective controls (t-test or Mann-Whitney

U test) and among the sediment exposures (ANOVA with HSD Tukey test or Kruskal-Wallis test by ranks). Furthermore, their responses were evaluated relative to sediment features using Kendall's tau correlation and Principal Component Analysis (PCA). The macrobenthos in the MW&WS region was examined for significant inter-site differences (ANOVA with HSD Tukey tests or Kruskal-Wallis tests by ranks, as well as ANOSIM analysis). Relationships with environmental variables were explored using DISTLM analysis. The sediment quality scores were prepared based on the biological response of amphipods exposed to the GoG sediments.

The study results showed that the survival and GR of amphipods exposed to the GoG sediments varied markedly. Mean survival was significantly reduced in the Gdynia port sediments. GR was the lowest in the Gdynia and Gdańsk port sediments (inner and outer port areas), with reductions ranging from 1.5 to 6.0 times when compared to controls, indicating the toxic effects of these sediments. However, the GR reduction was not statistically significant due to high coefficients of variation within some treatments. In other GoG sites, GR was 1.9 to 4.8 times greater when compared to controls. Sediment avoidance was higher in the port sediments, although the response was statistically significant in only one of the Gdynia and one of the Gdańsk port sediments. Reproductive processes may have been impaired by the port sediments, as no gravid females nor juveniles were found at the end of the bioassay. In general, the amphipod responses were related to HOC levels and sediment natural features. *C. volutator* and *C. multisetosum* showed some differences in the responses, yet both species identified the GoG port sediments as those of lower quality.

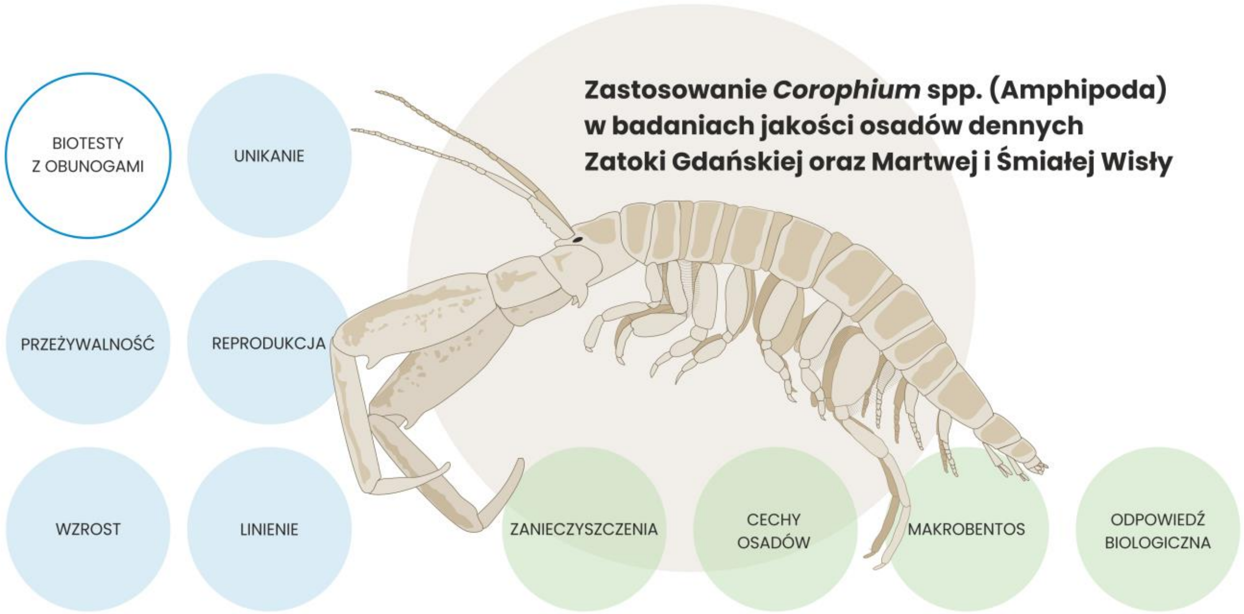
The MW&WS sediments did not exhibit toxicity towards *C. multisetosum* or *H. incongruens*. Responses of *H. incongruens* were consistent with *C. multisetosum*, as both species did not show reduced survival and growth in the tested sediments. The mean survival of each species did not differ significantly from controls, nor was it different among the sediment exposures. Growth of *C. multisetosum* and *H. incongruens* was significantly greater compared to controls in two of the five tested sediments. While significant differences in growth were observed among the sites for both species, the growth trends differed between them. No reproductive activity was observed in *C. multisetosum* in any of the sediments, possibly due to the immature development stage. Similar to the GoG sediments, the amphipod responses were related to sediment natural features. In summary, both *C. multisetosum* and *H. incongruens* bioassays demonstrated similar potential for evaluating sediment quality.

In the case of MW&WS macrobenthos, there were significant differences in abundance and composition among the sites. The ANOSIM analysis of the macrobenthos composition yielded the R statistic of 0.683 indicating significant among-sites dissimilarity. The DISTLM analysis identified several significant ecological factors, including depth, pH, phosphates, OM_w, fine sand, and fines, that influenced the macrobenthos communities. Yet, three environmental variables explained 61% of the variance in the macrobenthos distribution. These were phosphates concentration in the bottom water, OM_w, and the fines. The diversity among macrobenthos was relatively low and uniform, with

Hydrobiidae being the dominant taxa at each site, followed by Oligochaeta, Bivalvia, Polychaeta, and Ostracoda. Among the MW&WS sites, ST3 (the confluence of Młynówka Kanał and the Martwa Wisła River, close to the Pontoon bridge) stood out as it exhibited the lowest macrozoobenthos abundance and taxa richness, as well as the lowest survival, growth, and molting values in the *C. multisetosum* bioassay.

Overall, the study demonstrated that the tested amphipod species could effectively distinguish sediments with elevated levels of contaminants, such as those from harbors, from less polluted areas. The growth rate seemed to be less ambiguous and more efficient in discriminating the sites from survival, although the sediment organic matter content appeared to be an influential factor in this response. The response of amphipods to the MW&WS sediments reflected the abundance of macrobenthos at the sites, but not its rather poor biodiversity. Additionally, the research indicated that behavioral endpoint (sediment avoidance) may be a promising candidate for developing rapid screening tests for sediment quality assessment.

Streszczenie



Organizmy bentosowe, zwłaszcza Amphipoda (obunogi) z rodziny Corophidae, są powszechnie wykorzystywane jako organizmy wskaźnikowe w badaniach jakości osadów w obrębie morskich obszarów przybrzeżnych i estuariów. Wynika to z faktu, że jako organizmy żyjące w osadach, są bezpośrednio narażone na zanieczyszczenia w nich zawarte oraz na substancje rozpuszczone w wodzie porowej i w wodzie nad osadem. Jakość osadów dennych jest zwykle określana na podstawie analiz zawartości priorytetowych zanieczyszczeń chemicznych. Jednak analizy chemiczne wybranej, ograniczonej grupy substancji, nie odzwierciedlają rzeczywistego narażenia organizmów żywych, ze względu na występowanie mieszanin różnorodnych substancji, których współdziałanie, modyfikowane przez naturalne czynniki środowiskowe, jest trudne lub niemożliwe do określenia.

Spośród pięciu gatunków rodzaju *Corophium* występujących wzdłuż wybrzeża Polski, dwa są zalecane i wykorzystywane do oceny jakości osadów w Europie, mianowicie *C. volutator* i *C. multisetosum*. Każdy z tych gatunków charakteryzuje się wrażliwością na szerokie spektrum zanieczyszczeń, tolerancją na szeroki zakres zasolenia i skład granulometryczny osadu. Ponieważ występują w strefie przybrzeżnej, są łatwe do pozyskania, wykazują także dobrą adaptację do warunków laboratoryjnych. Dotychczas jednak nie badano możliwości zastosowania tych gatunków do oceny zanieczyszczenia osadów na polskich obszarach przybrzeżnych.

Celem pracy doktorskiej było zbadanie możliwości wykorzystania gatunków z rodzaju *Corophium* do oceny jakości osadów Zatoki Gdańskiej (ZG) oraz rzek Martwej Wisły i Wisły Śmiałej (MW&WS). Obszar ZG jest znany z podwyższonych zawartości różnorodnych zanieczyszczeń chemicznych w tym trwałych zanieczyszczeń organicznych (TZO), substancji ropopochodnych, powierzchniowo czynnych, pestycydów, metali i innych. Obecność tych zanieczyszczeń w środowisku Zatoki Gdańskiej jest wynikiem intensywnego rozwoju obszarów miejskich i przemysłu na obszarze otaczającym ZG, a także skutkiem napływu zanieczyszczeń z wodami rzeki Wisły i innych rzek. Obszar MW&WS był w przeszłości narażony na wycieki z hałdy fosfogipsów położonej w sąsiedztwie Martwej Wisły. Wycieki zawierały substancje o niskim pH, wysoki poziom fosforanów i fluoru, a także siarczany, toksyczne metale ciężkie i pierwiastki radioaktywne. Zanieczyszczenia te przenikały zarówno do wód powierzchniowych, jak i do wód gruntowych, stanowiąc zagrożenie dla przyrody i okolicznych mieszkańców.

Badania polegały na 28-dniowej ekspozycji *C. volutator* i *C. multisetosum* na osady denne oraz analizę odpowiedzi biologicznych tych organizmów. Obejmowały one przeżywalność, tempo wzrostu (GR), częstotliwość linienia oraz opuszczanie lub unikanie osadów przez obunogi. Badano również aktywność reprodukcyjną skorupiaków oceniając liczbę samic z jajami i obecność młodych osobników w osadzie. Badania osadów z obszaru MW&WS obejmowały dodatkowo standaryzowany test z udziałem małżoraczka *Heterocypris incongruens*, w celu porównania odpowiedzi z *C. multisetosum*.

Testowano dwie hipotezy:

1) *Corophium* spp. reagują na zanieczyszczenie osadów, wykazując zróżnicowane nasilenie odpowiedzi w zależności od poziomu ich zanieczyszczenia;
2) Odpowiedź biologiczna *Corophium* spp. na osady MW&WS odzwierciedla stan zespołów makrozoobentosu w tych rzekach.

Osady zostały pobrane z 13 lokalizacji w obrębie ZG, charakteryzujących się różnym stopniem zanieczyszczenia. Wśród nich znalazły się porty w Gdyni i w Gdańsku. W obszarze MW&WS wybrano pięć lokalizacji wzdłuż biegu tych rzek, od ujścia Kanału Wielkiego do Martwej Wisły (powyżej hałdy fosfogipsów) w kierunku ujścia Wisły Śmiałej do ZG. Z każdej lokalizacji pobrano trzy próbki osadów przy użyciu czerpacza Van Veen'a. Górna warstwa każdej próbki, o grubości 4 cm, została pobrana do biotestów, analiz charakterystyki osadów i zanieczyszczeń chemicznych. Z każdego stanowiska MW&WS pobrano dodatkowo trzy próbki osadu do określenia liczebności i składu taksonomicznego makrofauny bentosowej. Pobrane osady zostały poddane analizie zawartości frakcji drobnej ($F < 63$ µm), materii organicznej we frakcji drobnej (OM_f), materii organicznej w całym osadzie (OM_w) oraz TZO. Osady z obszaru MW&WS zostały poddane pełnej analizie granulometrycznej.

Organizmy testowe, czyli *C. volutator* i *C. multisetosum*, pobierane były z dwóch przybrzeżnych lokalizacji w Zatoce Puckiej, charakteryzujących się stosunkowo niskim poziomem zanieczyszczenia. Osady z tych lokalizacji zostały wykorzystane do aklimacji obunogów do warunków laboratoryjnych oraz jako osad referencyjny (kontrola) w biotestach. Procedura biotestu oparta była na metodach przedstawionych przez USEPA (2001). Czasokres narażenia organizmów na testowane osady wynosił 28 dni. W tym czasie monitorowano zachowanie (unikanie osadu) oraz częstotliwość linienia *Corophium* spp.. Po zakończeniu testów określano przeżywalność, długość każdego osobnika, liczbę samic z jajami i obecność form młodocianych.

Biotesty z *H. incongruens* przeprowadzono przy użyciu zestawu Ostracodtoxkit F (MicroBioTests Inc.), zgodnie ze standardową procedurą. Wyniki uzyskane w biotestach z *Corophium* spp. i *H. incongruens* zostały poddane analizie statystycznej. W celu określenia istotnych różnic w odpowiedzi biologicznej w porównaniu do osadu referencyjnego stosowano test t lub test U Manna-Whitney'a, natomiast różnice pomiędzy stanowiskami analizowano przy użyciu ANOVA i HSD Tukeya lub testu Kruskala-Wallisa. Analizę korelacji tau Kendalla oraz analizę składowych głównych (PCA) zastosowano w celu określenia zależności między odpowiedzią biologiczną (przeżywalność, GR, częstotliwość linienia, częstotliwość unikania osadu, procentowy udział samic z jajami) a parametrami charakterystyki osadów. W odniesieniu do makrobentosu na obszarze MW&WM, istotność różnic między stanowiskami analizowano przy zastosowaniu ANOVA i HSD Tukeya lub testu Kruskala-Wallisa, oraz nieparametrycznej analizy ANOSIM (PERMANOVA). Wpływ czynników środowiskowych na ugrupowania makrobentosu analizowano za pomocą modeli liniowych DistLM.

Wyniki badań wykazały, że przeżywalność i wzrost każdego z dwóch gatunków *Corophium* spp. narażonych na osady Zatoki Gdańskiej znacząco się różniły. Przeżywalność była istotnie mniejsza w osadach pochodzących z portu w Gdyni. Najniższe tempo wzrostu (GR) osobników stwierdzono w osadach z portów w Gdyni i w Gdańsku (w strefie wewnętrznej i zewnętrznej portów). Średnie GR osobników narażonych na działanie tych osadów było 1.5 do 6.0 razy mniejsze niż w grupie kontrolnej, wskazując na negatywne działanie tych osadów. Zahamowanie GR nie było jednak statystycznie istotne ze względu na wysoki współczynnik zmienności w niektórych testowanych osadach. W przeciwieństwie do obszarów portowych, GR było od 1.9 do 4.8 razy większe w porównaniu do kontroli w osadach z innych lokalizacji w ZG. W osadach portowych obserwowano nasilone unikanie osadu, a także nie stwierdzono ciężarnych samic ani młodych osobników, co sugeruje, że procesy reprodukcyjne były zakłócone przez zanieczyszczenia obecne w osadach. Analiza statystyczna wykazała, że odpowiedź biologiczna *Corophium* spp. narażonych na osady ZG była powiązana ze stężeniem TZO w osadach i naturalną charakterystyką osadów. *C. volutator* i *C. multisetosum* różniły się w pewnym stopniu w odpowiedziach biologicznych, ale oba gatunki zidentyfikowały obszary portowe jako najbardziej niekorzystne wśród badanych stanowisk Zatoki Gdańskiej.

Osady z obszaru MW&WS nie wykazywały negatywnego oddziaływania wobec *C. multisetosum* ani *H. incongruens*. Odpowiedzi biologiczne obu gatunków były podobne, tj., ani przeżywalność, ani GR nie były istotnie niższe w porównaniu do kontroli. Co więcej, wzrost *C. multisetosum* oraz *H. incongruens* były istotnie większe w dwóch spośród pięciu testowanych osadów w porównaniu z kontrolą, przy czym trendy wzrostu różniły się pomiędzy gatunkami. Nie zaobserwowano aktywności reprodukcyjnej u *C. multisetosum* w żadnym z osadów MW&WS, prawdopodobnie ze względu na to, że organizmy nie osiągnęły dojrzałości płciowej. Odpowiedzi biologiczne (przeżywalność, GR, częstotliwość linienia) *C. multisetosum* były zależne od charakterystyki osadu, podobnie jak w osadach ZG. Podsumowując, zarówno biotesty z *C. multisetosum*, jak i z *H. incongruens* wykazały podobny potencjał w ocenie jakości osadów.

W odniesieniu do makrobentosu z MW&WS stwierdzono istotne różnice w liczebności i składzie taksonomicznym między stanowiskami, co potwierdziła wynikająca z analizy ANOSIM wartość R (0.683). Analiza DISTLM wskazała istotne statystycznie czynniki ekologiczne, które mogły wpływać na ugrupowania makrobentosu, a mianowicie: głębokość, pH, fosforany, OM_w oraz dwie frakcje osadu (drobny piasek i frakcja drobna F<63 µm). Trzy parametry środowiskowe wyjaśniły 61% zmienności w składzie ilościowym i jakościowym makrozoobentosu. Były to: stężenie fosforanów w wodzie nad osadem, OM_w oraz frakcja drobna osadu. Stanowisko ST3 (ujście Kanału Młynówka do Martwej Wisły, blisko mostu pontonowego) wyróżniało się najniższymi wartościami liczebności makrobentosu i bogactwa taksonomicznego. Obserwacje te były spójne z wynikami biotestów z *C. multisetosum*, ponieważ przeżywalność i GR tych organizmów były najniższe dla tego stanowiska.

Podsumowując, przeprowadzone badania wykazały, że testowane gatunki obunogów, *C. volutator* i *C. multisetosum*, wykazywały zróżnicowane reakcje wobec badanych osadów, ale ich odpowiedź biologiczna wskazywała osady zanieczyszczone. GR było parametrem bardziej jednoznacznym niż przeżywalność w ocenie jakości osadów, przy czym zawartość materii organicznej w osadach okazała się czynnikiem istotnie wpływającym na wzrost organizmów. Odpowiedź *C. multisetosum* na osady MW&WS odzwierciedlała wprawdzie liczebność makrobentosu na badanych stanowiskach, lecz nie jego, raczej ubogą, bioróżnorodność. Dodatkowo badania wykazały, że odpowiedź behawioralna (unikanie osadów) *Corophium* spp. może być obiecującym kandydatem do opracowania szybkich testów przesiewowych oceny jakości osadów przybrzeżnych i estuariów.

1 Introduction

1.1 The amphipods' biology and ecological significance

Amphipoda (Crustacea, Malacostraca) constitute an important biological group of marine, estuarine, and freshwater aquatic ecosystems. They inhabit pelagic and benthic compartments exhibiting different life patterns, habitat preferences, and feeding ecology. They are herbivores, detritivores or scavengers (grazing on algae), omnivores or predators, and represent important links in the aquatic food chains (Jura, 2002). Many amphipod species, considered as herbivorous shredders, show a high diversity in food preferences, consuming various types of plant materials as well as invertebrates (Costa and Costa, 2000; Gerdol and Hughes, 1994a; Kunz et al., 2010). Amphipods can live either close to the bottom (in or on the sediment) or in the water column. Epibenthic species are associated with macroalgae and can occupy seagrass meadows, hide under sheltering structures (stones and shells), or burry in sandy and muddy sediments (Costa and Costa, 2000). Some of the infaunal amphipods burry freely in the sediment and others evolved to be tube-dwelling animals, as in the case of corophiidans (Lowry and Myers, 2003). Species zonation and abundance of amphipods depend on environmental conditions, spatial heterogeneity of substratum composition, and complex interactions in the benthic community (Beukema and Flach, 1995; Jensen and André, 1993). The number of coexisting closely related amphipod species is low, nevertheless, they often cohabit in areas with unrelated deposit-feeding organisms. The competition for food is limited in such species due to morphological differences and different particle-size selection of ingested food, e.g. as in the case of snails from the Hydrobiidae family and *Corophium* spp. (Fenchel et al., 1975a). Bivalves are known to reduce the amount of the available detritus in the sediment and therefore negatively influence the density of detritivorous amphipods. Infaunal polychaetes can induce migration of sediment-dwelling amphipods, mainly due to sediment disturbance, and less significantly due to predation (Bergstrom et al., 2002). Polychaetes alter the substratum by secretion of the mucus, which can occasionally stimulate the abundance of amphipods, as enhanced sedimentation of fine-grained material is favored by some species. This has been indicated by the sympatric occurrence of *N. diversicolor* and *C. volutator in* some intertidal zones (Jensen and André, 1993). On the other hand, the more intense swimming behavior of infaunal amphipods resulting from the polychaetes activity increases the risk of predation and is a limiting factor to *Corophium* spp. abundance (Beukema and Flach, 1995; Cunha et al., 2000a, Podlesińska, unpublished data). Furthermore, the mucus produced by polychaetes might hamper the mobility of amphipods (Podlesińska, unpublished data). Otherwise, temporal variation in amphipod density might depend largely on the water temperature and salinity. Climatic conditions have consequently substantial control over the yearly abundance of oligohaline and mesohaline amphipods (Cunha et al., 2000a). Their abundance may vary seasonally and yearly, from highly abundant to absent. The populations of lower latitudes, at which higher temperatures and lower salinities occur, have a longer breeding period, a shorter life-cycle and longevity, and produce more generations (Cunha et al., 2000a; Nair and Anger, 1979). Tube-building amphipods take significant part in the processes of bioturbation, namely rearrangement of the sediment particles, due to foraging and feeding, as well as tube building maintenance, and ventilation (Queirós et al., 2013).

Amphipods typically have a distinct head with a triangular rostrum, seven-segmented thorax (pereon), and six-segmented abdomen (pleon) terminated with a telson (Figure 1). The body shape in amphipods depends on the habitat and living habits, e.g., in the swimming species like *Gammarus* spp. it is dorsally bent, while in infaunal species like *Corophium* spp. it is elongated and dorsoventrally flattened. A pair of compound eyes (of a complex structure in some mesopelagic species) in amphipods may be located on the head or can be completely absent (Baldwin Fergus et al., 2015; Jura, 2002). The populations of amphipods are usually female-biased and the males are believed to form harems (McCurdy et al., 2000). This is supported by the highly aggressive behavior of males (Forbes et al., 1996) also observed during this study in both *C. volutator* and *C. multisetosum*. A high percentage of females (84 %) was observed in the *C. volutator* summer population in the Bay of Puck as well (Dobrzycka and Szaniawska, 1993). Mature females can be recognized by a brood pouch (marsupium) with setae on the brood plates (oostegites; Figure 3d). Fertilization and development of eggs take place in the marsupium. Eggs hatch directly into a juvenile form and remain in the brood pouch until the next female molt. The development of embryos occurs concurrently with the maturation of a new batch of oocytes in the ovaries in preparation for the next spawning. There is a close link between molting and oogenic cycles, i.e., the onset of molting is delayed until the hatching and release of juveniles. This seems to facilitate the transfer of newly ovulated oocytes through the oviducts into marsupium when the new exoskeleton is still flexible. Mature males can be recognized by the presence of genital papillae at the seventh thoracic segment at the ventral side of a body (Figure 3e) and a well-developed second pair of antennae, which in *Corophium* spp. males can reach the same length as the distance from the tip of the rostrum to the telson. Males are available for mating during most of their molt cycle, while females are sexually receptive for a short period during their molt cycle (Sutcliffe, 1992). Two types of intersexual individuals were observed in *C. volutator*, i.e., those that had non-setose oostegites, external penial papillae, and internal testes, and those with single external penial papilla connected to the internal testicle and an ovary bearing malformed eggs. Intersexes make about 3 % of a population and it has been found that they are able to function as males. Although the brood sizes of females paired with intersexes were observed to be smaller, the intersexes can still be significant for the reproduction success of these amphipods, considering the female-biased sex ratio (McCurdy et al., 2004).

Corophiidae (taxonomic status shown in Figure 2), are well-described and commonly used in sediment quality studies in Europe and other parts of the world. Species like *Corophium volutator, Corophium multisetosum, Corophium orientale,* and *Monocorophium insidiosum* are endobenthic, tube-building species. They inhabit littoral zones of brackish or saline environments where they often occur in extremely high densities, sometimes exceeding 100 000 ind. m^{-2} (Beukema and Flach, 1995; Casado-Martinez et al., 2007; Cunha et al., 2000b; Gerdol and Hughes, 1994a; Jażdżewski et al., 2005; Meadows, 1964; Ólafsson and Persson, 1986). Some closely related species, like *C. volutator* and *C. arenarium*, were observed to occupy the same areas of intertidal mudflats (Beukema and Flach, 1995; Jensen and André, 1993). The corophioid amphipods exhibit three feeding modes i.e., suspension-feeding (particles are filtered from a current generated by the movement of pleopods), deposit-feeding (amphipod leaves its burrow and scrapes detritus from the sediment surface into the burrow with the second antennae), and epipsammic browsing (microbial biofilm is scraped off individual sediment grains) (Gerdol and Hughes, 1994b; Lowry and Myers, 2003). *C. volutator* can efficiently retrieve particles as small as 7 μm in size through suspension feeding (Møller and Riisgård, 2006) using a filtering basket placed on the first and second pair of gnathopods comprised of setae with small bristles (Figure 4). *C. volutator* feeds on benthic micro- and macroalgae, bacteria attached to the small-sized sediment grains, and other forms of amorphous organic matter through epipsammic browsing (Murdoch et al., 1986). These are adaptations to the environment of the intertidal zone, where an amphipod is likely to be subjected to a turbulent, suspended sediment material, increased contact with small fines and organic matter, and to the contaminants bound herein.

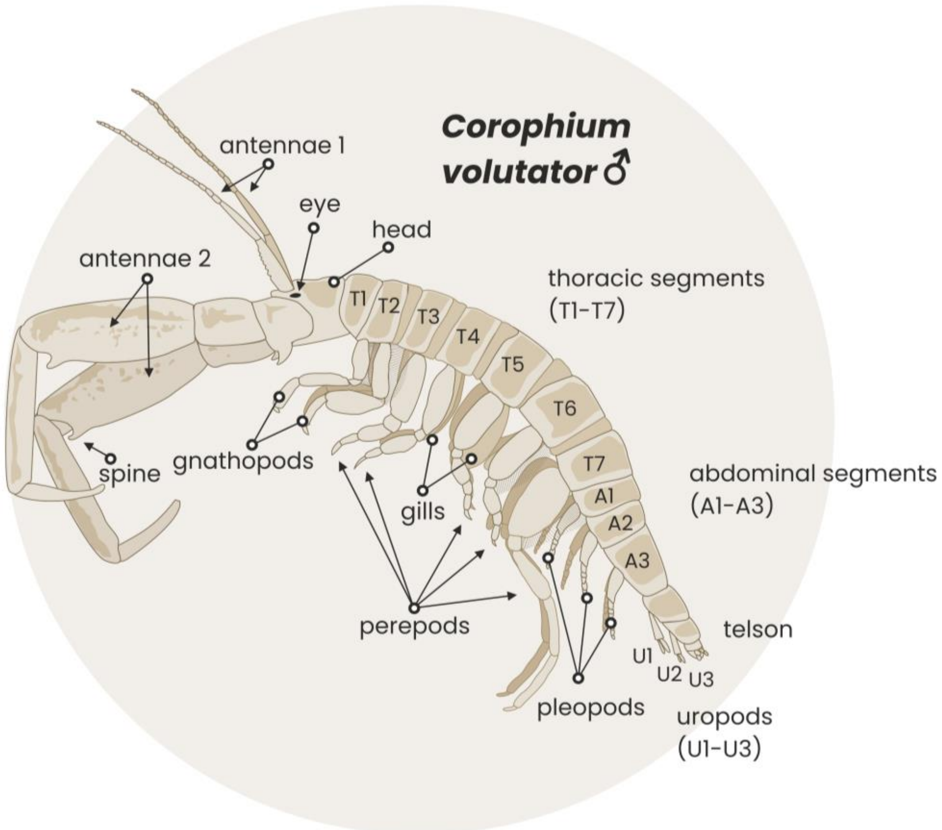


Fig. 1. Schematic drawing of *C. volutator* male (W. Podlesińska).

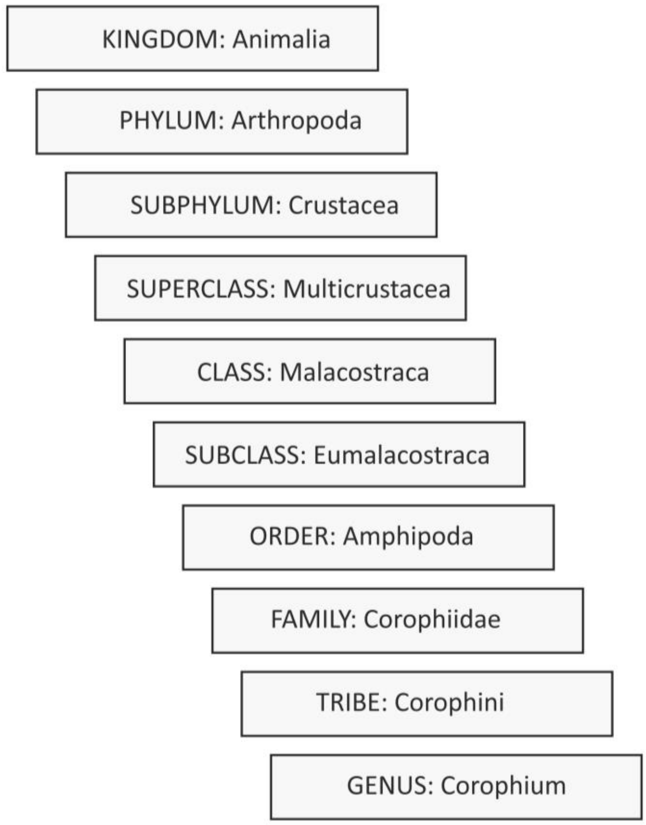


Fig. 2. Current taxonomic status of *Corophium* spp. (Horton et al., 2020).

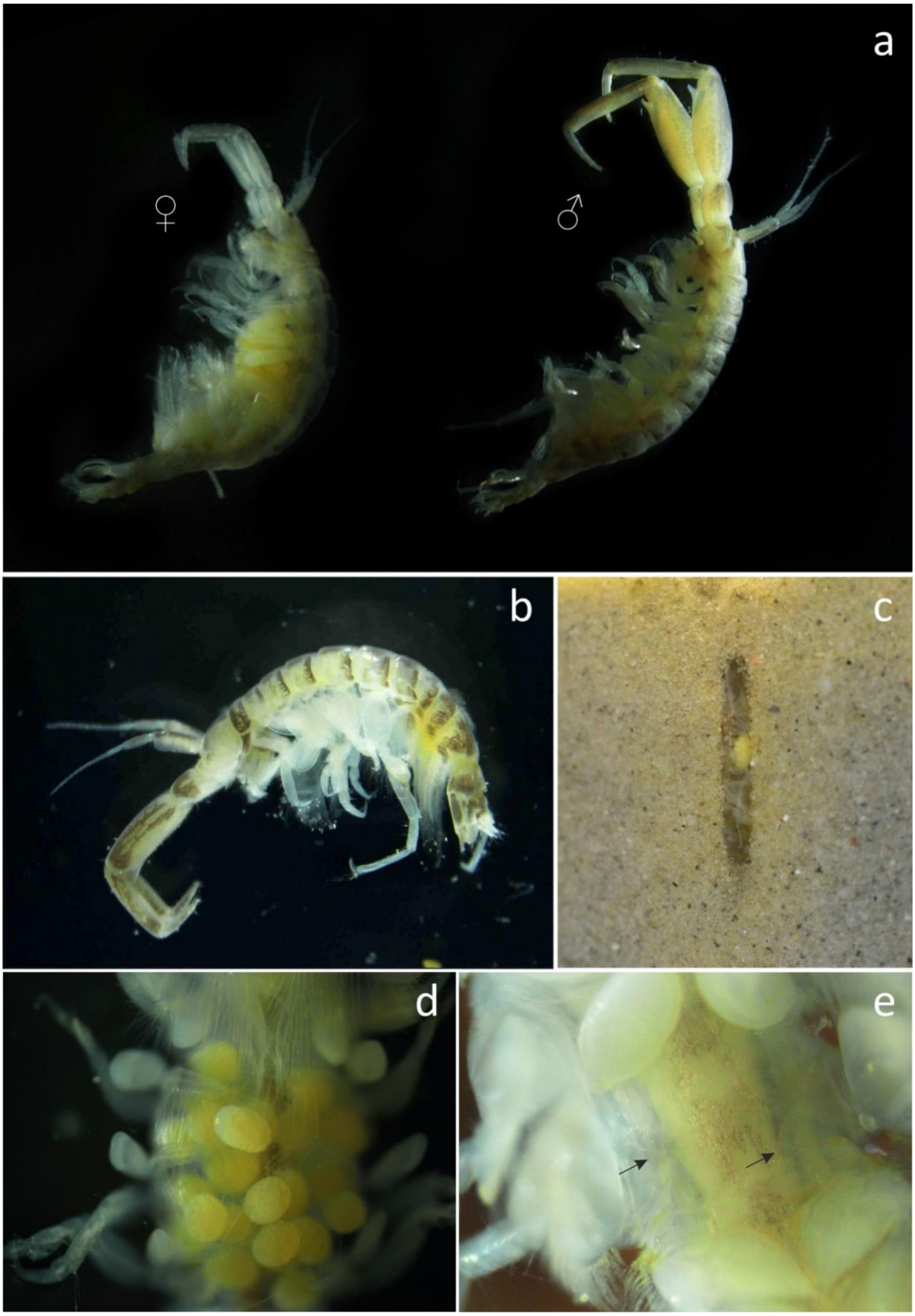


Fig. 3. *Corophium* spp. collected from the Bay of Puck; (a) *C. volutator* female and male, (b) *C. multisetosum* female, (c) *C. multisetosum* female in a tube with visible eggs in the marsupium, (d) marsupium of *C. volutator* female with clearly visible oostegites with developed setae holding eggs; (e) adult male, arrows point to genital papillae. Adopted from Podlesinska and Dabrowska, 2019.

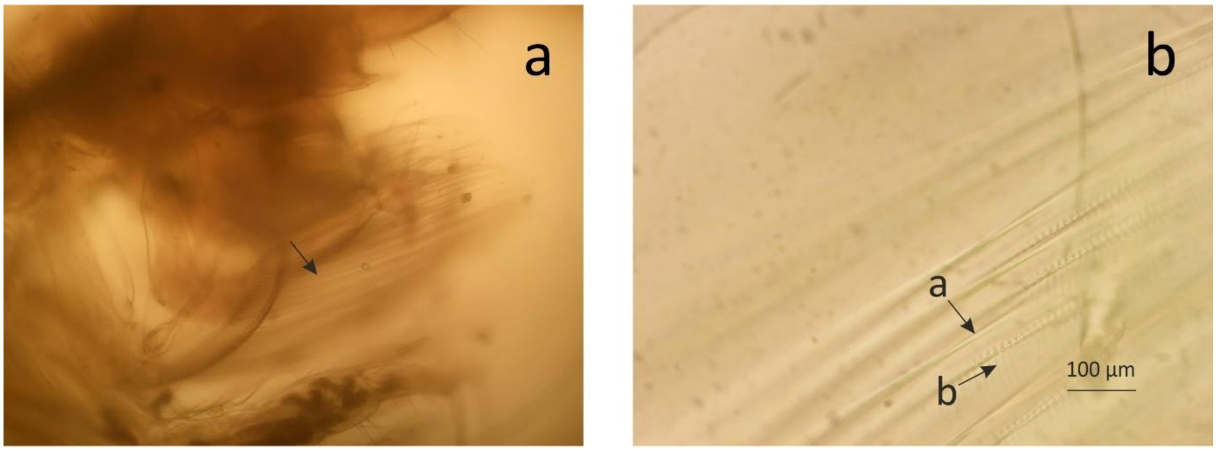


Fig. 4. The filtering basket on a gnathopod in *C. multisetosum*; (a) a gnathopod with setae pointed by an arrow; (b) magnified image, arrow "a" points to seta, and arrow "b" to the bristles (photo by W. Podlesińska).

1.2 The occurrence of *Corophium* spp. In Europe and the coastal area of Poland

Five corophiid species have been reported to occur along the Baltic coast of Poland, i.e. *C. volutator*, *C. multisetosum*, *Apocorophium lacustre*, *Crassicorophium crassicorne*, and *Chelicorophium curvispinum* (Janas and Kendzierska, 2014; Jażdżewski et al., 2005; Kotwicki, 1997; Obolewski and Konkel, 2007; Wawrzyniak-Wydrowska, 1997).

C. volutator (Figure 3a, d, e) is a euryhaline species usually living in silty areas (Beukema and Flach, 1995; Meadows, 1964). It is a species common worldwide. Similarly to other Corophiidae species, it is mostly a deposit feeder whose main sources of nutrition are particulate matter, organic detritus, bacteria, and diatoms (Nielsen and Kofoed, 1982). Its spatial distribution may depend mostly on the availability of food and it was observed to concentrate in the areas of high algal biomass (Weerman et al., 2011). It is an important prey for demersal fish and invertebrates, as well as shorebirds (Wilson and Parker, 1996). Adult individuals show distinct sexual dimorphism, males (Figure 3a) in comparison to females have a considerably larger second pair of antennae (Dobrzycka and Szaniawska, 1993; Drolet and Barbeau, 2012; Peters and Ahlf, 2005).

In Europe, its biology and life history aspects were studied in detail in, e.g., the United Kingdom (Gerdol and Hughes, 1994b; Mills and Fish, 1980), Germany (Meißner and Bick, 1997), Netherlands (Beukema and Flach, 1995; Kater et al., 2000), and Sweden (Jensen and André, 1993). It is widely distributed in the whole area of the Baltic Sea (HELCOM, 2012a). In Poland, it was present in the Szczecin Lagoon, Wolin National Park area, and the Gulf of Gdańsk at 0 - 60 m and deeper (Janas et al., 2004; Janas and Kendzierska, 2014; Wawrzyniak-Wydrowska, 1997; Żmudziński and Andrulewicz, 1997). The average abundance of *C. volutator* in the littoral zone of the Gulf of Gdańsk in 1992 - 1993 was reported to range from 9 to 3401 individuals per m^2 which classified it as one of the most abundant crustaceans in the shallow waters of the Bay of Puck (Kotwicki, 1997).

C. multisetosum (Figure 3b-c) is a species inhabiting oligohaline and mesohaline waters (e.g., canals, rivers, estuaries, lagoons), and various types of soft substrate, preferably sediments with a smaller amount of fines and organic matter content (Pinkster et al., 1992; Queiroga, 1990; Stock et al., 2009; Wijnhoven et al., 2011). *C. multisetosum* is also widely distributed in Europe. Its life history was studied in the Northwestern region of Portugal in Ria de Aveiro, where this species is abundant all year round and its densities were reported to reach over 80 000 ind. per m^2 (Cunha et al., 2000a; Queiroga, 1990). It was observed to occur in a range of salinities, from freshwater to about 30, however, the offspring in the laboratory conditions were produced only at a salinity range of 2 -18 (Cunha et al., 2000c; Queiroga, 1990). It was found to cohabit in the same areas as other corophiids, e.g., *A. lacustre*, *C. curvispinum,* and *C. volutator* (Jazdzewski, 1976; Wijnhoven et al., 2011).

In the Baltic Sea area, it was first reported in the brackish Martwa Wisła River (Poland) in 1962 and was lately listed among the species occurring in the Great Belt, the Little Belt, the Sound, the Arkona Basin, the Wismar Bay, the Greifswald Lagoon, the Rugia Lagoons, Curonian Lagoon and the Darss-Zingst Lagoon (HELCOM, 2012a; Jazdzewski, 1967; Olenin and Leppäkoski, 1999). It was also common in meso- and oligohaline waters of the Netherlands (along with *A. lacustre, C. volutator*) where its range extended inland to almost freshwater reservoirs (Pinkster et al., 1992; Wijnhoven et al., 2011). It is also known to be present in the brackish waters of the United Kingdom, Ireland, and Spain (Wijnhoven et al., 2011). In Poland, it occurs along the Baltic coast, in the Gulf of Gdańsk, and the Szczecin Lagoon (Janas and Kendzierska, 2014; Jażdżewski et al., 2005; Wawrzyniak-Wydrowska, 1997).

Both *C. volutator* and *C. multisetosum* have typically bivoltine reproduction, meaning they produce two generations per year. Still, European populations from colder, northern zones may produce only one generation (Cunha et al., 2000a, 2000c; Peters and Ahlf, 2005; Wilson and Parker, 1996).

C. curvispinum originates from the Ponto-Caspian region and inhabits freshwater, brackish, and saline aquatic habitats (Den Hartog et al., 1992). It was recorded in Sweden, Estonia, Lithuania, Netherlands, and Belgium (Herkül and Kotta, 2007; Leppänen et al., 2017; Noordhuis et al., 2009; Olenin and Leppäkoski, 1999; van den Brink et al., 1993). Following a Sandoz chemical spill in Switzerland in 1986 (which resulted in a decline of the physical, chemical, and biological condition of the river Rhine) this amphipod colonized a large section of heavily eutrophicated Lower Rhine and became the most abundant macroinvertebrate reaching densities of 200 000 specimens per m^2 (van den Brink et al., 1993). The species expanded its range to western Europe (Conlan, 1994; Jażdżewski and Konopacka, 1993; Pinkster et al., 1992). It was also one of the dominating species in the brackish and freshwater reservoirs of Ijsselmeer area (the Northern Netherlands) where it occupied stony bottom and mussel-beds (Noordhuis et al., 2009). In the Baltic Sea area, it was recorded in the Gulf of Finland and the Warnow Estuary (HELCOM, 2012a). In Poland, this species is known to occur in the Szczecin Lagoon and the Odra River, the Vistula Lagoon and the Vistula, the Bug, the Narew, and the Noteć Rivers (Grabowski et al., 2007; HELCOM, 2012a; Wawrzyniak-Wydrowska, 1997).

A. lacustre has been found in the Weser estuary (the North Sea) where it dominated the benthic fauna assemblages inhabiting hard substratum (Wetzel et al., 2014). It occurs in several sites in the Baltic Sea including, but not limited to, the Kattegat, Bay of Mecklenburg, the Sound, Arkona Basin, Bornholm Basin, Gulf of Gdańsk, Vistula Lagoon, Szczecin Lagoon, Greifswald Lagoon, Rugia Lagoons and Curonian Lagoon (HELCOM, 2012a). In the Gulf of Gdańsk, *A. lacustre* and *C. crassicorne* were observed at a depth of 0 - 9 m (Janas and Kendzierska, 2014). *A. lacustre* along with *C. multisetosum*, and *C. volutator* were found in the brackish waters of the Martwa Wisła River in the past, where their distribution depended on the bottom type and salinity (Jazdzewski, 1976; Klekot, 1972). *C. volutator*, *C. multisetosum*, *C. crassicorne*, and *A. lacustre* were also observed in the Puck Bay (Dobrzycka and Szaniawska, 1993; Janas and Kendzierska, 2014, this study).

The literature reporting the occurrence of *Corophium* spp. In the Gulf of Gdańsk is listed in Table 1. Figure 5 shows the occurrence of two species, *C. volutator* and *C. multisetosum,* in the GoG and the MW&WS. It also depicts the reference sites from which the two species were collected for the present study.

Tab. 1. Occurrence of *Corophium* spp. within the area of the Gulf of Gdańsk.

Site number[1]	Site description	Time of collection	References
1	Swarzewo, sewage outfall, 20 m from the shore	1991 - 1992 (each month); 1995;	Dobrzycka and Szaniawska, 1995, 1993
2	Kaczy Winkiel littoral zone	2014 (Jul.); 2015 (Apr., Jun.); 2018 (May);	this study
3	Rzucewo littoral zone	2014 - 2018 (Apr.-Jul.);	this study
4	Kuźnica, littoral zone	2016 (May-Jul.);	this study
5	Chałupy	2016 (May-Jul.);	this study
6	Martwa Wisła	1965 (May, Aug., Nov.)	Klekot, 1972
7	Wisła Śmiała, Martwa Wisła, exact locations not given	1968 - 1971;	Jazdzewski, 1976
8	Gulf of Gdańsk 37 m depth	1994 - 1995 (Mar.-Apr.);	Janas et al., 2004
9	Gulf of Gdańsk 51 m depth	1994 - 1995 (Mar.-Apr.);	Janas et al., 2004
10	Gulf of Gdańsk 60 m depth	1994 - 1995 (Mar.-Apr.);	Janas et al., 2004
11	Kuźnica Hollow	2001 (summer, autumn); 2002 (spring); 2003 (winter;	Szymelfenig et al., 2006

[1] the site number corresponds to the location marked in Figure 5.

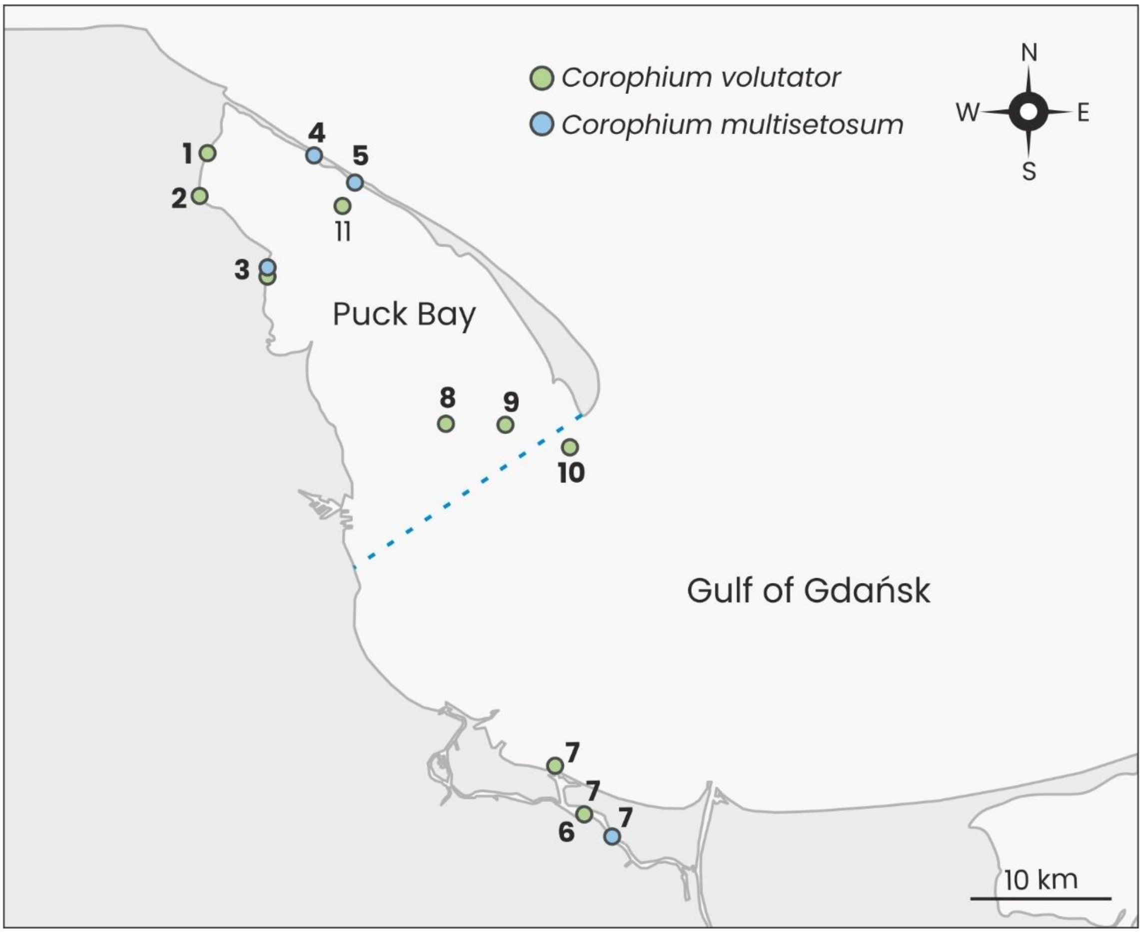


Fig. 5. The occurrence of *C. volutator* and *C. multisetosum* in the Gulf of Gdańsk and the Martwa and Śmiała Wisła Rivers. The numbers correspond to the sites described in Table 1. The numbers 2 and 3 are the reference sites and a source of organisms selected for this study.

1.3 Amphipods in ecotoxicological studies[1]

Amphipods, due to their habitat and lifestyle, are widely used test organisms for the assessment of freshwater, marine, and estuarine sediment quality (Table 2). The burrowing species are highly suitable for this purpose as they spend most of their lives in direct contact with sediment. They can be exposed to contaminants through interstitial and/or bottom waters, as well as through the ingestion of detritus/organic matter and a fine fraction of sediment. As amphipods can accumulate various contaminants they can be themselves a source of contaminants to organisms that feed on them (Viganò et al., 2007). Ecological significance and wide geographic distribution of some *Corophium* spp. allow for comparisons of ecological status throughout regions (Chapman et al., 1992). Other advantages regarding their use for sediment quality assessment include high abundance and availability year-round in the field, broad salinity tolerance in euryhaline species, relatively high and well-studied sensitivity to contaminants, and a tolerance of a wide range of sediment characteristics, which facilitates tests with a variety of sediment types. What is more, laboratory cultures were

[1] Some parts of the chapter 'Introduction' had been published in Oceanologia 2019, 61(2) (Podlesinska and Dabrowska, 2019).

successfully established for some species showing high survival rates (Bat and Raffaelli, 1998a; Kater et al., 2008; Stronkhorst, 2003).

The use of amphipods for sediment toxicity testing was developed in the 1970s (Burton, 1991; Burton and Burton, 1992). In the 1990s, amphipod bioassays were becoming increasingly important in regulation and sediment assessment in North America, less so in Europe since only a few such bioassays had been then developed for European species (Chapman et al., 1992). Since that time, the research progress advanced in this area and many guidelines and numerous papers have been published worldwide (Table 2). Generally, sediment toxicity testing can be performed with relatively simple bioassays, however for in-depth assessment of sediment quality it is recommended that the assessment includes also sediment chemistry and in situ benthic community structure, which comprises the so-called Sediment Quality Triad (Chapman, 1990; Chapman and Wang, 2001). It is an approach that includes three aspects of examined sediments: chemical analysis, toxicity to organisms, and alteration in the benthic community structure (Chapman, 1989). The second component refers to sediment bioassays that test the response of an organism to a given sample. The triad gives valuable and comprehensive information on the physical, chemical, and biological components of an ecosystem (Buruaem et al., 2013; Chapman, 1990). The advantage of bioassays is that they incorporate the interactive effects of the complex contaminant mixtures present in sediments providing an integrative measure of contaminant-induced biological effects. In Europe, the *Corophium* genus has been recommended by ICES as a standard species for marine and estuarine sediment toxicity testing (Roddie and Thain, 2001). It has been also used for regulatory purposes for testing chemical products that are liable to accumulation in sediments (Cesnaitis et al., 2014). OSPAR indicated *C. volutator* for testing chemicals used in the offshore oil industry and the guidelines are also applicable to *C. multisetosum* (OSPAR, 2013). Various species of the *Corophium* genus as well as representatives of other genera such as *Gammarus* and *Ampelisca*, depending on their natural occurrence, availability, and existing methodologies, have been used in studies of sediment quality (Table 2). Some amphipods, such as deposit-feeding benthic, keystone species in the Baltic Sea *Monoporeia affinis* and *Pontoporeia femorata*, have been recommended by HELCOM as indicators of reproductive effects associated with hazardous substances in the marine environment (HELCOM, 2012b; Löf et al., 2016). Guidelines for sediment toxicity testing using amphipods have been developed, as well as procedures for cultivation in laboratory conditions (ASTM, 1991; Costa et al., 2005b; Peters et al., 2002; Roddie and Thain, 2001; Siebeneicher et al., 2013; Stock et al., 2009).

Tab. 2. Selected studies on estuarine and marine sediment quality using amphipod species (adapted from Podlesinska and Dabrowska, 2019).

Test species	Objectives	Test duration and investigated endpoints	Investigated matrix (area)	Reference
Whole sediment exposures				
Leptocheirus plumulosus	Study of effects of sediment-associated weathered slick oil.	28-d survival, growth, reproduction	whole sediment (Gulf of Mexico, USA)	Lotufo et al., 2016
Echinogammarus marinus	Assessment of the short-term in situ assay.	48-h survival; 30-, 60-, 120- min post-exposure feeding rates	whole sediments (the Minho and Lima River estuaries, Spain)	Martinez-Haro et al., 2016
Monocorophium insidiosum [2]	Development of an evaluation tool for sediment toxicity detection	10-d survival	whole sediment (Taranto Gulf, Italy)	Prato et al., 2015
Grandidierella bonnieroides	Assessment of sediment quality	10-d survival	whole sediment (Macaé River Estuary, Brazil)	Molisani et al., 2013
Corophium volutator	Study on burrowing activity as an endpoint.	10-d survival, burrowing	whole sediment, laboratory culture (highly contaminated harbor, Germany)	Siebeneicher et al., 2013
Corophium orientale, Monocorophium insidiosum [2]	Comparison of the sensitivity of two amphipods.	10-d survival	whole sediment, two species sensitivity comparison (Livorno harbor, Italy)	Prato et al., 2010
Corophium multisetosum	Comparison of the sensitivity of laboratory-cultured and field-collected amphipod.	10-d survival	whole sediment	Menchaca et al., 2010
Melita plumulosa	Comparison of in situ and laboratory tests' results.	gametogenesis, fertilization, and embryo development	whole sediment	Mann et al., 2010

Test species	Objectives	Test duration and investigated endpoints	Investigated matrix (area)	Reference
Whole sediment exposures				
Monocorophium insidiosum [2]	Optimization of methodology for a bioassay.	10-d survival	whole sediment	Prato et al., 2008
Ampelisca abidita, Leptocheirus plumulosus	Comparison of acute and chronic toxicity methods.	10-d survival; 28-d survival, growth, reproduction	whole sediment (New York/New Jersey Harbor)	Kennedy et al., 2009
Monocorophium insidiosum [2], *Gammarus aequicauda*	Comparison of the response of the species from different evolutionary levels and habitats.	10-d survival	whole sediments, three species comparison (Ionian Sea, Taranto, Italy)	Narracci et al., 2009
Corophium multisetosum	Assessment of the performance of the amphipod in toxicity testing.	10-d survival	temperature, salinity, cadmium, laboratory culture	Ré et al., 2009
Corophium volutator	Assessment of sediment with the use of a battery of multi-trophic species and multiple exposure phases.	10-d survival	whole sediments (Irish estuaries)	Macken et al., 2008
Corophium orientale	Assessment of sensitivity and applicability of an amphipod in the bioassays.	96-h survival; 10-d survival	water-only and whole sediment (Venice Lagoon)	Picone et al., 2008
Corophium volutator, Ampelisca brevicornis,	Comparison of the sensitivity of amphipods to dredged sediments.	10-d survival,	whole sediment, two-species comparison (Spanish Harbor)	Casado-Martinez et al., 2007
Corophium volutator	Description of methods and confounding factors in a chronic bioassay.	49-d survival, growth, reproduction	salinity, ammonium, nitrate, oxygen, laboratory culture	van den Heuvel-Greve et al., 2007
Corophium multisetosum	Comprehensive estuarine sediment toxicity survey with acute and chronic assessment.	10-d survival; 21-d survival, growth, fecundity	whole sediments (Ria de Aveiro estuary), laboratory cultures	Castro et al., 2006

Test species	Objectives	Test duration and investigated endpoints	Investigated matrix (area)	Reference
Whole sediment exposures				
Melita plumulosa	Development and evaluation of chronic sediment toxicity bioassay.	10-d survival; 42-d survival, reproduction, bioaccumulation	metal-spiked sediments, whole sediments (Cockle Bay, Warners Bay, Nords Wharf, Australia)	Gale et al., 2006
Corophium volutator	Assessment of ammonium toxicity in bioassays at high pH.	10-d survival	ammonium toxicity in bioassays	Kater et al., 2006
Leptocheirus plumulosus	Effects of contaminated harbor sediment in chronic exposure.	42-d survival, growth, reproduction, dry weight,	whole sediment, laboratory culture (Baltimore Harbor, USA)	Manyin and Rowe, 2006
Gammarus aequicauda, Monocorophium insidiosum [2]	Evaluation of toxicity of copper, cadmium, and mercury in 5 species.	10-d survival; 28-d survival, growth	whole sediments	Prato et al., 2006
Melita plumulosa	Development of culturing procedures.	28-d survival, growth, reproduction	optimal conditions for laboratory cultures	Hyne et al., 2005
Mandibulophoxus mai, Monocorophium acherusicum, Haustorioides indivisus, Haustorioides koreanus	Development of bioassay protocols for species native to Korea.	10-d survival, behavior	whole sediments (Sihwa and Onsan industrial complexes, Korea)	Lee et al., 2005
Corophium colo	Assessment and comparison of the species sensitivity to contaminated sediments.	10-d survival	whole sediment (Sydney Harbor, Australia)	McCready et al., 2005
Gammarus locusta	Assessment of moderately toxic sediments in chronic exposures with biochemical endpoints.	28-d survival, reproduction[1], biochemical markers	whole sediments (Sado and Tagus estuaries, Portugal)	Neuparth et al., 2005

Test species	Objectives	Test duration and investigated endpoints	Investigated matrix (area)	Reference
Whole sediment exposures				
Corophium volutator	Study on the laboratory cultures for sediment toxicity testing.	71-d growth, reproduction	laboratory cultures, fecundity, fertility, temperature preference	Peters and Ahlf, 2005
Gammarus aequicauda, Microdeutopus gryllotalpa	The sensitivity of the marine organisms in the assessment of sediments.	10-d survival	whole sediment, two-species comparison (Portmán Bay, Spain)	Cesar et al., 2004
Leptocheirus plumulosus	Evaluation of the relationship between laboratory and field responses to contaminants.	10-d survival, 28-d survival, growth, reproduction	whole sediment (Baltimore Harbour/Patapsco River System)	McGee et al., 2004
Corophium volutator, Ampelisca brevicornis	Comparison of toxicity of sediment contaminated with mining spill to two species.	10-d survival	whole sediment (Bay of Cádiz, Spain)	Riba et al., 2003
Paracorophium excavatum	Assessment of suitability of the amphipod in sediment toxicity assessment.	10-d survival, 28-d survival	whole sediment (Avon-Heathcote Estuary)	Marsden et al., 2000
Corophium orientale	Assessment of suitability of the amphipods in harbor toxicity assessment.	10-d survival	whole sediment (northern Tyrrhenian Sea, Italy)	Onorati et al., 1999
Leptocheirus plumulosus	Study on effects of long-term exposure to copper on survival, growth, and reproduction.	28-d survival, growth, reproduction	Cu-spiked sediment	Ward et al., 2015
Spiked sediment exposures				
Monocorophium insidiosum [2]	Assessment of toxicity of three antiparasitic pesticides.	10-d survival	antiparasitic pesticides - spiked sediments	Tucca et al., 2014

Test species	Objectives	Test duration and investigated endpoints	Investigated matrix (area)	Reference
Whole sediment exposures				
Eohaustorius estuarius	Comparison of the sensitivities of toxicity test protocols.	10-d survival	chlorpyrifos, copper, fluoranthene, permethrin, bifenthrin, and cypermethrin - spiked sediments	Anderson et al., 2008
Corophium volutator	Development of a long-term assay with polychaete and amphipod.	10-d survival; 28-d survival, growth,	toxicity of Ivermectin; UK	Allen et al., 2007
Corophium volutator	Development of a chronic toxicity test method.	28-d survival, growth, reproduction	crude oil - spiked sediment	Scarlett et al., 2007
Corophium volutator	Assessment of suitability of the amphipod in sediment biomonitoring.	behavioral responses	bioban pesticide-spiked sediments	Kirkpatrick et al., 2006
Paracorophium excavatum	Study on the influence of sediment copper on amphipod reproduction.	28-d survival, growth	Cu - spiked sediment	Marsden, 2002
Ampelisca abdita	Evaluation of the ecological significance of the laboratory tests.	10-d survival, 70-d survival, growth	Cd - spiked sediment	Kuhn et al., 2002
Ampelisca abdita	Study on the feasibility of the amphipod for sediment toxicity tests.	10-d survival, avoidance	2,6-DNT - spiked sediment	Nipper et al., 2002
Ampelisca abdita	Determination of body residues associated with acute toxicity.	10-d survival	non-ionic organics-spiked sediment	Fay et al., 2000
Interlaboratory comparisons				
Corophium volutator, Ampelisca brevicornis	Comparison of the sensitivity of amphipods to dredged materials.	10-d survival	whole sediment, inter-laboratory, multispecies, comparisons	Casado-Martinez et al., 2007

Test species	Objectives	Test duration and investigated endpoints	Investigated matrix (area)	Reference
Whole sediment exposures				
Eohaustorius estuarius	Determination of interlaboratory variability in sediment toxicity tests.	10-d survival	whole sediment, inter-laboratory comparisons	Bay et al., 2003
Corophium volutator, Rhepoxynius abronius, Bathyporeia sarsi	Identification of the most useful sediment toxicity tests for regulatory purposes and general assessment.	10-d survival, reburial	whole sediment, inter-laboratory comparisons	Chapman et al., 1992

[1] additional chronic endpoints: whole-body metal bioaccumulation, metallothionein induction, DNA strand breakage, and lipid peroxidation, [2] basionym: *Corophium insidiosum*

1.3.1 Types of bioassays

Two general types of bioassays can be distinguished: acute with exposure time of less than a full life cycle of the test organism, and chronic which covers at least one full life cycle of the test organism (Allen et al., 2007; Chapman, 1989; Prato et al., 2010, 2015; Scarlett et al., 2007). Acute bioassay usually does not differentiate low or moderately contaminated sediments which in natural environments occur more commonly than highly contaminated (Heuvel-Greve et al., 2007; Picone et al., 2016). In the chronic bioassays, in addition to survival, measurements of more sensitive sublethal endpoints are carried out. That allows extrapolating toxicological effects to a population level. Several studies compared acute and chronic exposures to evaluate their effectiveness in sediment toxicity assessment. For example, in the Ria de Aveiro estuary (Portugal), Castro et al. (2006) conducted 10-d and full life-cycle (21-d) exposures of *C. multisetosum* to sediment samples from 144 and 56 sites, respectively. The authors reported that the 10-d exposures yielded high survival and showed almost no toxic areas. While the chronic exposures also showed relatively good survival for most of the sites, the endpoints related to organismal growth and fecundity depicted the more contaminated estuary areas. McGee et al. (2004) presented a study of the Baltimore Harbor/Patapsco River System (MD, USA) which involved 10-d and 28-d exposures of the indigenous amphipod *Leptocheirus plumulosus* to sediment samples from 11 stations. The study showed an overall good agreement in the biological responses between the two types of exposures in depicting spatial differences in sediment contamination. Gale et al. (2006) performed a study of an estuarine amphipod, *Melita plumulosa*, exposed to cadmium-, copper- and zinc-spiked and field-collected natural sediments for 10 and 42 days. It was reported that fertility and length were significantly reduced after 42-d exposure to copper and zinc-spiked sediments. Fertility was the most sensitive endpoint in the amphipods exposed to the field-collected sediments. Kennedy et al. (2009) examined acute and chronic toxicity of the New York/New Jersey harbor sediments collected from nine sites of varying contamination levels to four invertebrate species with an overall objective of comparing the performance of the two tests. The endpoint responses varied among the tests, from low to moderate and high. The 25-d exposure of *L. plumulosus* was the only chronic exposure that differentiated the sediments, and its endpoint magnitudes seemed to be related to sediment chemistry. A study by Allen et al. (2007), designed to develop a long-term sublethal sediment bioassay with *C. volutator,* tested 10-d and 28-d exposures to Ivermectin-spiked sediments. The study showed no significant difference in the LC_{50} values between the exposure periods, indicating that longer exposure periods did not increase the amphipod sensitivity. The performance of *C. multisetosum* was tested in laboratory conditions in different temperatures, salinities, and with different substrates, as well as towards contaminated field sediments. That research indicated that

C. multisetosum was a suitable species to conduct acute and chronic sediment toxicity bioassays due to its tolerance to a wide spectrum of sediment grain sizes, high survival in the control sediment, and feasibility in laboratory cultures (Ré et al., 2009).

Generally, chronic bioassays require greater effort and time than acute bioassays. However, they represent a useful tool for sediment risk assessment in more environmentally realistic exposure scenarios, whereas acute bioassays are useful for identifying highly contaminated sediments or assessing the immediate effect of exposure to specific substances or conditions. It can be assumed that sublethal endpoints, such as growth and fertility, can illustrate sediment toxicity more plainly; however, the response can be contaminant- and species-specific.

1.3.2 Toxicity endpoints

In 10-d acute toxicity bioassays, survival is usually the sole endpoint measured. However, a post-exposure burrowing ability in uncontaminated sediment can be used as a more sensitive sublethal endpoint, as was shown in an extensive study of sediment toxicity from the Sydney harbor and its vicinity (Australia) with an indigenous amphipod, *Corophium colo* (McCready et al., 2005). Similar findings were obtained by Siebeneicher et al. (2013), who tested the burrowing of *C. volutator* and proved that the burrowing activity was related to size and gender, and prior exposure to contaminated sediments. Bat et al. (1998) investigating the accumulation of heavy metals, mortality, and burrowing behavior of *C. volutator,* reported that the metals affected the burrowing behavior in a dose-dependent manner and that burrowing ability was a more sensitive endpoint than mortality. In a study of Halifax harbor sediments (Canada), Hellou et al. (2008) were able to relate the sediment avoidance response of *C. volutator* to PAH bioaccumulation level. Behavioral responses most often pertain to avoidance and/or reburial ability, yet, behavioral endpoints can include a variety of activities (Hellou, 2011). De Backer et al. (2010) reported nine different behavioral activities of *C. volutator*, which can be applied to other tube-building amphipod species. These were: surface inactivity, surface crawling, swimming, scraping, flushing (removing the excess of grain and feces with the pleopods' movement that creates the water current in the tube), subsurface inactivity, ventilating and filter-feeding, subsurface walking, and bulldozing (pushing the sediment grains out of the burrow with pleon). A short-term, novel sediment toxicity endpoint, has been proposed by Martinez-Haro et al. (2016) where post-exposure feeding inhibition was used as an endpoint in a study with amphipod *Echinogammarus marinus*. In a 30-minute bioassay that measured *E. marinus* post-exposure feeding rates, clean and contaminated sites of the Minho and Lima River estuaries (Portuguese coast) could be discriminated. In general, behavioral bioassays represent a whole organism's response and are valued for simplicity, as well as their time- and cost-efficiency.

In chronic toxicity bioassays, in addition to survival, the endpoints most often include growth measurement (mg/individual/d; or length and dry weight) and fecundity (number of neonates per female), sometimes gravidity (presence of eggs in brood pouch) and other reproductive traits (Allen et al., 2007; Gale et al., 2006; Heuvel-Greve et al., 2007; Kennedy et al., 2009; McGee et al., 2004; Ward et al., 2015). These sublethal endpoints bear the importance of the prediction of long-term effects and are ecologically more relevant (Costa et al., 2005a, b). In a study of chronic toxicity of the Saldo and Tagus estuary sediments (Portuguese coast) to the amphipod *Gammarus locusta*, Costa et al. (2005b) examined the reproductive outcome in more detail. The authors considered not only fecundity as an endpoint but also sex ratio (the exposure began with 2 - 4 mm juveniles), the size (diameter and volume), and developmental stages of embryos. Furthermore, the same study included several biochemical endpoints, i.e. metal bioaccumulation, metallothionein induction, DNA strand breakage, and lipid peroxidation (Costa et al., 2005b).

Although molting is not used as an endpoint in amphipod bioassays, it comprises important information on the biological condition of crustaceans, as it can be related to growth, gender, and development stage, since it is a part of the maturation process and preparation for reproductive activities (Hyne, 2011; McCurdy et al., 2000). In the field, molting is also associated with lunar and tidal cycles and is synchronous among females and males (McCurdy et al., 2000). Molting is a process that could increase the response of organisms to contaminants as it was observed that the sensitivity of amphipods increased in the immediate post-molt phase probably as an effect of calcium-contaminant interaction (McGee et al., 1998). Therefore, this endpoint may provide multidimensional information on the organism's response to exposure.

Amphipod embryo malformation rate is an exposure-response that is a sensitive endpoint in field studies (Löf et al., 2016; Sundelin and Eriksson, 1998). In a study of *Monoporeia affinis* females in the Bothnian Bay and the Bothnian Sea with known pollution levels, apart from fecundity, Löf et al. (2016) investigated the developmental stage of embryos and their aberrations which included malformed, membrane-damaged, and undifferentiated embryos with development arrested before gastrulation, as well as dead and partially dead broods. Based on the percentage of females with each type of embryo aberration, the authors found that different types of aberrations were related to elevated concentrations of specific contaminants in sediments. There was a strong correlation between the embryo malformation rate and distance from the source of pollution. The frequency of amphipod embryo malformations has been used in field studies that dealt with sediment quality assessment in the Baltic Sea.

Berezina et al. (2017) investigated this endpoint in the amphipod *Gmelinoides fasciatus* in the Neva River estuary (the eastern Gulf of Finland, the Baltic Sea), whereas Strode et al. (2017) used *M. affinis* in the Gulf of Riga (the Baltic Sea). As all amphipod species show similar embryo development (despite differences in sexual behavior and embryogenesis), the method for embryo staging and malformations can be extended to other species (Sundelin et al., 2008). Further studies regarding associations between groups of contaminants and specific malformations have been encouraged (HELCOM, 2017).

1.3.3 Confounding factors

In sediment toxicity bioassays there are several confounding factors, which can influence the measured endpoints. These factors are related to the water physicochemical characteristics, sediment geochemistry, and the biology of test organisms. Temperature, salinity, and oxygen saturation are the basic factors modulating organismal responses. Some studies point out also toxicants that can be present in sediments, but not always considered in the bioassays, i.e., ammonia (originating from anthropogenic discharges and natural decomposition process) and sulfides, which are common in estuarine sediments (Chapman and Wang, 2001; Neuparth et al., 2002; Postma et al., 2002; van den Heuvel-Greve et al., 2007).

Temperature is one of the key factors influencing metabolic and physiological processes including growth, reproduction, duration of the amphipod life cycle, and sensitivity to contaminants (Cunha et al., 2000c; Prato et al., 2008; Ré et al., 2009). An experimental study by Kater et al. (2008) with *Corophium volutator* showed negligible growth rate at a low temperature of 5 °C, noticeable at 10 °C, and an optimum at 15 °C which did not differ from that at 25 °C. A study involving *Gammarus locusta* reported that at 20 °C compared to 15 °C (reference condition) there was an acceleration and condensation of the species life cycle i.e., faster individual and population growth, reduction in the lifespan, and shorter generation time (Neuparth et al., 2001). Peters and Ahlf (2005) who studied the reproduction of *Corophium volutator* in laboratory conditions at 15, 19, and 23 °C pointed out that the reproductive outcome (number of offspring per female) was significantly greater at 15 °C than at 23 °C in one experimental set-up, while indifferent in another one. Prato et al. (2008) reported reduced mortality and sensitivity of *Monocorophium insidiosum* to contaminants at 10 °C and 15 °C compared to 20 °C and 25 °C, possibly due to decreased oxygen consumption and energy expenditure at lower temperatures. They suggested 15 - 20 °C as the optimal temperature for bioassays with *M. insidiosum.*

Sediment grain size and organic matter content are two variables that, depending on their nature, can not only modify the availability of sediment-bound contaminants but have an influence on the test organism as well. For example, Costa et al. (2005b) reported that

G. lacusta had better growth rates and reproductive traits when chronically exposed to muddy sediments (9 - 11 % of total volatile solids, TVS) than to sandy ones (0.4 % TVS). This positive effect was attributed to a richer and more efficient diet provided by the muddy sediments. Picone et al. (2008), in an extensive evaluation of *Corophium orientale* as a bioindicator for the Venice Lagoon, reported that sediment grain size and organic carbon content (0.4 - 15 %) were not influential factors for the 10-d mortality endpoint. As indicated by Burton (1992), the responses of organisms to test sediments (bioassay endpoints) can be affected by their life stage and health condition, the acclimation to test conditions and exposure duration, the route of contaminant uptake, and the mode of toxicant action. Organisms that burrow freely in sediment compared to tube-building and epibenthic species are more directly exposed to contaminants present in interstitial water and have greater direct contact with contaminated particles, which results in their greater exposure. This has been shown in a laboratory study with two amphipods, *Eohaustorius estuaries* (free-burrowing detritivore) and *Ampelisca abdita* (tube-dwelling feeder), exposed to contaminant-spiked sediments (Anderson et al., 2008a) as well as in field studies of sediment toxicity in the San Francisco Estuary (Anderson et al., 2007).

Organic matter (OM) present in sediment acts as a sorbent for hydrophobic organic contaminants (HOCs; Lewandowski et al., 2014; Staniszewska et al., 2011; Waszak et al., 2019) and constitutes a food source for benthic invertebrates (Gunnarsson et al., 1999). It is considered to be a major factor influencing the bioavailability of sediment-accumulated HOCs. Due to its sorptive features, OM binds the contaminants leading to their reduced bioavailability and limited bioaccumulation by benthic biota (Hecht et al., 2004; Landrum et al., 1997; Landrum and Faust, 1994). Because the types and sources of OM in sediments are diverse (detrital marine and terrestrial plants and animals, fecal matter, phytoplankton cells, humic and fulvic substances), the degree of OM binding strength for HOCs and its nutritional quality as a food source for benthic invertebrates vary as well (Gunnarsson et al., 1999). The sediment OM quality/type may cause significant differences in the toxic effects of contaminants on benthic amphipods (Word et al., 1987). It has been shown that the toxicity and bioaccumulation of sediment-accumulated HOCs by benthic organisms are quite often inversely related to sediment OM content (Hecht et al., 2004; Landrum et al., 1997; Landrum and Faust, 1994). The importance of OM for reducing the bioaccumulation of contaminants has been also reported for mercury and methylmercury in a study with *Leptocheirus plumulosus* which showed that the bioaccumulation factors were inversely related to the sedimentary OM content (Lawrence and Mason, 2001). Organic matter content also influences the distribution of sediment-dwelling amphipods, e.g., *C. multisetosum* inhabits areas known for low OM content (Queiroga, 1990). Considering these facts, sediments used in ecotoxicological studies, are usually characterized with respect to the content of OM or total organic carbon (TOC).

While extrinsic factors influencing the amphipod sediment toxicity endpoints have been widely reported, there have been few studies on biological factors such as body size and gender. Research by McGee et al. (1998) on acute toxicity of aqueous cadmium to the estuarine amphipod *L. plumulosus* assessed the influence of size, sex, and nutritional status on the sensitivity of laboratory-cultured organisms. They reported that sensitivity to Cd decreased (measured as LC_{50}) with an increase in body size, and gravid females were less sensitive than males or mature females. Furthermore, the molting cycle was found a significant factor influencing the sensitivity of individuals, i.e., immediate postmolt organisms were more sensitive than those of an intermolt stage. This aspect was to some extent examined by Kater et al. (2000) in a study of the sensitivity of *C. volutator* to cadmium in the context of their origin (newly field-collected versus long-term laboratory-held organisms) and exposure media (natural versus artificial seawater). The amphipods were larger than 5 mm and no size effect was found. Their study showed significant seasonal variation in sensitivity (lower in the winter than the summer period; expressed as an LC_{50} value) irrespective of changes in the tested variables. In a study that examined the influence of life stage, gender, and a priori nutritional state on the uptake of zinc and cadmium in *Echinogammarus marinus*, it has been shown that life stage and nutritional state were significant factors for bioaccumulation of both metals, whereas only cadmium bioaccumulation was gender-specific (Pastorinho et al., 2009). Another study regarding the effect of seasonality and body size on the sensitivity of *Corophium urdaibaiense* and *C. multisetosum* found that the sensitivity (greater during summer than winter) could be related to reproduction as the percentage of gravid females was significantly inversely related to the LC_{50} values (Pérez-Landa et al., 2008).

2 The area of study

Although the sediment-dwelling amphipods have been used worldwide as test organisms for the evaluation of sediment quality in various freshwater, marine, and estuarine environments, the use of *Corophium* spp. In sediment quality assessment in Polish coastal and estuarine areas known to be polluted with a variety of anthropogenic contaminants, has not yet been addressed. The area of study involved the Gulf of Gdańsk (GoG; extensively investigated over the years) and the Martwa and Śmiała Wisła Rivers (MW&WS). Table 3 lists selected literature regarding the study area. A general map of the study area is shown in Figure 6.

Tab. 3. Selected literature regarding environmental studies in the GoG and MW&WS area.

Research focus	Analyzed material	References
Gulf of Gdańsk		
Legacy and emerging pollutants	review of data on levels, distribution, and sources	Zaborska et al., 2019
PAHs	mussels, sediment	Waszak et al., 2019
HOCs, biomarkers, seasonal variability	flounder	Kopko and Dabrowska, 2018
HOCs and metal contaminants, pollution, and biological effects assessment	flounder, mussels, sediment	Dabrowska et al., 2017
biogens, metals	surface water	Albiniak et al., 2017
HOCs intertissue distribution, gender effect	turbot	Dabrowska and Góra, 2016
organic compounds and metals	sediment	Rogowska et al., 2015
HOCs, Vistula and Duoro estuaries,	flounder, sediment	Waszak et al., 2014
HOCs, biomarkers, spatial variations	flounder	Dabrowska et al., 2014
alkylphenols	sediments	Koniecko et al., 2014
organotin compounds (OTCs; butylin, phenyltin)	sediment	Filipkowska et al., 2014
PBDEs, spatial variations	flounder, sediment	Waszak et al., 2012
organic contaminants (PAHs, PCBs)	sediment	Staniszewska et al., 2011
Dioxin equivalents	cod	Dabrowska et al., 2010
organic contaminants (PAHs)	sediment	Lubecki and Kowalewska, 2010
hazardous substances, pollution sources,	general assessment	HELCOM, 2010
PCBs	fish (herring, sprat, salmon, cod, flounder)	Szlinder-Richert et al., 2009
indicator species for biomonitoring of persistent toxic substances (PTS)	bottom fauna	Galassi et al., 2008

Research focus	Analyzed material	References
Gulf of Gdańsk		
temperature, salinity, depth, nutrients	surface water	Nowacki and Jarosz, 1998
heavy metals	sediments	Szefer, 1998
macrobenthos	sandy littoral zone	Kotwicki, 1997
hydrogen sulfide	sediment	Janas and Szaniawska, 1996
salinity	water	Cyberska, 1990
Port in Gdańsk and Gdynia		
PCDDs, PCFDs, PCP	sediment	Lewandowski et al., 2014
OTCs, heavy metals	sediment	Radke et al., 2013
mercury	sediment	Falandysz, 1999
Puck Bay		
zoobenthos	sediment	Janas and Kendzierska, 2014
depth, temperature, salinity, photosynthetic active radiation, bottom wave velocity, sediment classification	near-bottom water, sediment	Weslawski et al., 2013
the density of macrofauna in the shallow zone	benthic fauna	Obolewski and Konkel, 2007
depth, temperature, oxygen concentration, chlorides, Secchi depth, hydrological conditions, salinity	bottom and surface water	Klekot, 1980
Martwa Wisła and Wisła Śmiała		
hydrographic system	water	Cieśliński et al., 2017
orthophosphate and total phosphorus	surface water	Räike et al., 2015
contamination due to the phosphogypsum heap	surface water, groundwater, air, soil, plant and animal material	Hupka et al., 2006
fauna (amphipods)	sediment	Jazdzewski, 1976
phosphogypsum toxicity	fauna	Pautsch et al., 1975
bottom fauna	sediment	Klekot, 1972
Vistula mouth		
nitrogen and phosphorus	water	Pastuszak et al., 2018
sedimentation processes	sediment	Damrat et al., 2013
organic contaminants (PAHs, PCBs, DDT)	mussels, Mytilus trossulus	Potrykus et al., 2003

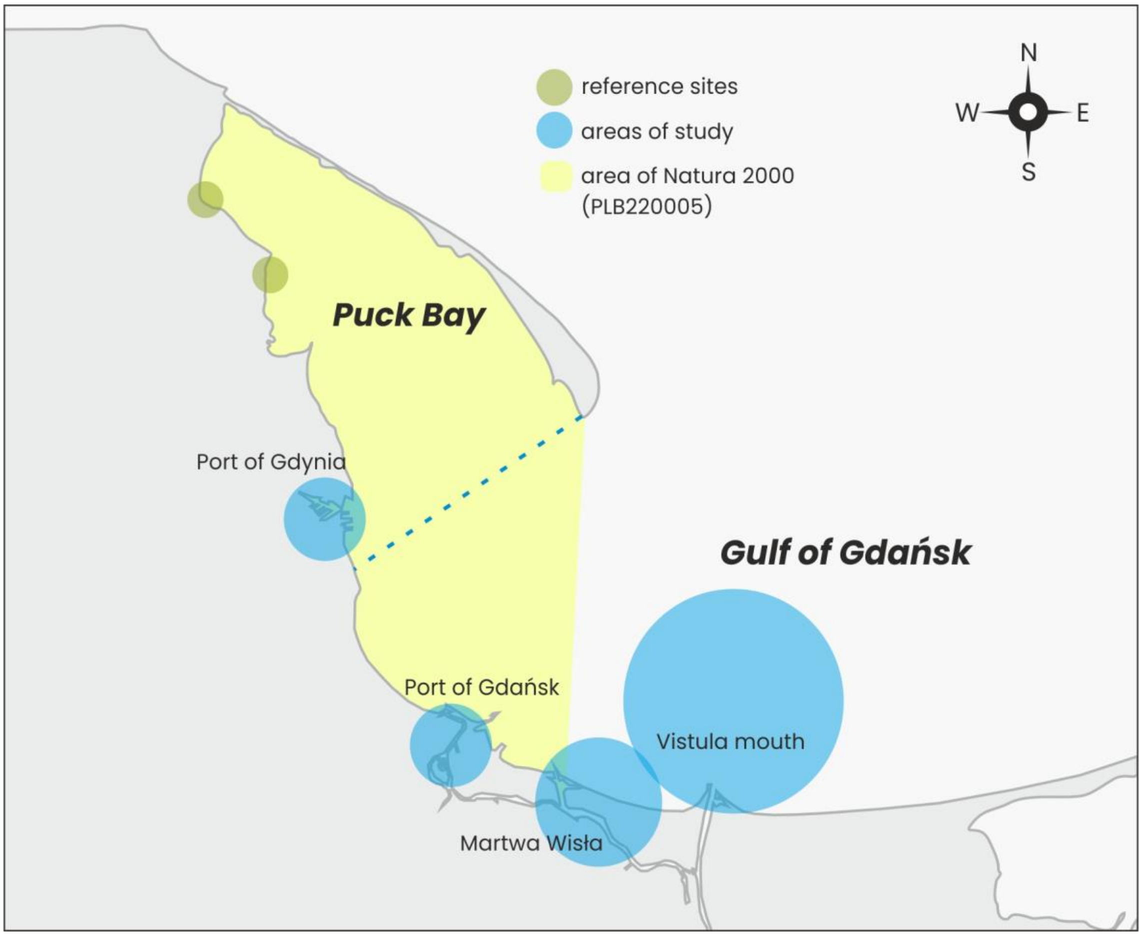


Fig. 6. A general map of the GoG study area with areas of interest marked.

2.1 The Gulf of Gdańsk

The Gulf of Gdańsk (GoG) is located in the southern Baltic Sea within the Polish Maritime Areas. It is the southern part of the Baltic Proper conventionally outlined by an imaginary line between the Rozewie Cape (Poland) and the Taran Cape (Russia). This line goes through the Gdańsk Deep, the deepest point of the Gulf, measuring 118 m. The Hel Peninsula demarks a smaller western area of the Gulf, called the Bay of Puck, an area with a long history of a variety of environmental studies, and a Natura 2000 site.

GoG is an economically and ecologically significant area known for decades to be under great anthropogenic pressure due to an inflow of harmful substances from riverine transport (nutrient loads, HOCs, pesticides, emerging pollutants[2]), atmospheric deposition (transportation, combustion of fossil fuels), wastewater treatment plants, coastal industries (Gdańsk Refinery, shipyards, pharmaceutical companies), point sources of pollution (such

[2] Emerging pollutants are defined as chemicals that are not included in the standard environmental monitoring, but may have adverse effects on the environmental and human health. These include many synthetic or natural substances such as pharmaceuticals and personal care products, hormones, and flame retardants.

as leaking shipwrecks), but also through maritime transport, the activity of harbors and marinas, fisheries, or tourism (Zaborska et al., 2019). These human activities led to the pollution of GoG with a variety of hazardous chemical substances that are toxic, persistent, and accumulate in organisms and sediments (HELCOM, 2010). Such substances have been reported in the GoG water column, sediments, and biota by many studies (Table 3). Selected hydrophobic organic contaminants (HOCs)[3], heavy metals, and radionuclides have been monitored by the Chief Inspectorate of Environmental Protection (Główny Inspektorat Ochrony Środowiska, GIOŚ) in three matrices, namely organisms, sediments, and water column (GIOŚ, 2013). HOCs present in the sediments include legacy pollutants[4] such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCs, e.g. dichlorodiphenyl-trichloroethane - DDT, and hexachlorobenzene - HCB), polychlorinated dibenzodioxins and dioxin-like compounds, polycyclic aromatic hydrocarbons (PAHs), as well as emerging contaminants such as organotin compounds (OTs), flame retardants (polybrominated diphenyl ethers – PBDEs, hexabromocyclododecane – HBCDD), and many others, like phenol derivatives. HOCs are compounds that, due to their physicochemical properties, resist degradation through physical, chemical, and biological processes. These properties make them persistent in the environment and predispose them to accumulation in sediments, soils, animals, and humans to levels that can be a health concern. HOCs can cause cancers, neurological and learning disabilities, hormonal (endocrine) disruption, and subtle changes to reproductive and immune systems.

Regarding the riverine inflow of harmful substances to the GoG, it is mainly the Vistula River which has the second-largest catchment area (192 196 km^2) in the Baltic Sea watershed. The river contributes a major part of the nitrogen and phosphorus inputs (HELCOM, 2010). However, the largest emissions of biogenic substances into the Vistula basin observed at the turn of the 1980s/1990s have been shown to decrease over the last decades mostly due to an effort encouraged by the international regulations and financial support of EU (Pastuszak et al., 2018). The Vistula River carries a considerable load of various organic and metal contaminants that are deposited in the sediments (HELCOM, 2015; Lubecki and Kowalewska, 2010; Szefer, 1998). It also transports sediment particles that settle on the outer prodelta and

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[3] Although, the expression ‘*persistent organic pollutants (POPs)*’ is now commonly used in relation to hydrophobic organic compounds (HOCs), e.g., PAHs, PCBs, and OCPs, the expression ‘*contamination*’ should be used instead ‘*pollution*’. According to Chapman (2007), contamination is defined by the presence of a substance exceeding the background concentration, and pollution is level of contamination that may cause adverse biological effects. Hence, the term ‘contaminants’ rather than ‘pollutants’ is used throughout this work.

[4] Legacy pollutants are chemicals used in the past in various industries that remain in the environment for a long time after being first introduced.

can be transferred to deeper areas, contributing to contaminant dispersion in the Gulf of Gdańsk (Damrat et al., 2013). An increase of sediment contaminants with the distance from the river's outflow indicating the role of water current transport of the riverine material and the significance of sediment type in the accumulation of contaminants has been reported by the studies of Lubecki and Kowalewska (2010) and Staniszewska et al. (2011). Large harbors (the Port of Gdańsk and Gdynia) operate within the GoG area and in the neighborhood (ports in Kaliningrad, Klaipeda, and Baltiysk). The Gdynia and Gdańsk harbor sediments are routinely examined once every couple of years for metals (arsenic, chrome, zinc, cadmium, copper, nickel, lead, and mercury), PAHs, and PCBs (www.port.gdynia.pl; www.portgdansk.pl). Although both ports state that the sediment samples do not exceed the permissible values, these harbors have been known sources of chemical contaminants that harm the surrounding aquatic environment (Falandysz, 1999; Lewandowski et al., 2014; Radke et al., 2013; Wolska and Mędrzycka, 2009). E.g., in the past, in both ports toxic organotin compounds were found, which were used for antifouling paints to prevent the growth of organisms on ships or marine structures (Filipkowska et al., 2011). The sediments collected from the Port of Gdańsk were also found to be contaminated with dioxins (importantly - in acceptable range), although displayed low toxicity in the bioassays with *Aliivibrio fischeri* (Lewandowski et al., 2014). In a study comparing the results of sediment toxicity based on bioassays conducted with *H. incongruens* and *A. fischeri* with the results of chemical analysis of the harbor sediments, it was found that high toxicity of sediment can rarely be justified by an exceedance of the permissible concentrations of contaminants (Wolska and Mędrzycka, 2009).

Wastewater treatment plants that operate in this area include those located in Gdańsk, Dębogórze, Swarzewo, Hel, and Jastarnia. They are not only major contributors of nitrogen and phosphorus delivered into GoG, but they also discharge PAHs, aliphatic hydrocarbons, heavy metals, pharmaceutical residues, microplastics, and surface-active agents with the treated wastewater into GoG (Zaborska et al., 2019).

Other sources of pollution in GoG include shipwrecks that sunk during World War II. In the western part of the GoG, about 2 nautical miles from the Port of Gdynia, remains of shipwreck s/s Stuttgart, which sunk in 1943, are located. In its vicinity, elevated concentrations of PAHs greatly exceeding admissible levels and high concentrations of PCBs and metals (Zn, Pb, Hg, Cu) were detected (Kudłak et al., 2012; Rogowska et al., 2015). Samples of sediments from an area near the shipwreck, tested for toxicity using bioassays with *Aliivibrio fischeri* and *Heterocypris incongruens* revealed that some sediment samples were highly toxic (Kudłak et al., 2012).

Although an effort to identify the threats posed by both legacy and emerging pollutants is reflected in a great number of studies on the GoG environment (Table 3), it is apparent that the determination of the potential sediment toxicity can't be based solely on chemical analysis due to the costs, time effectiveness, the number of pollutants, their varying bioavailability, and interactions. The ecotoxicological assessment of environment quality employing bioassays seems to be a proper tool to support the adequate protection of marine ecosystems.

The study in the GoG area involved 13 sites from which sediment samples were collected (Figure 7; Table 4). They included inner and outer areas of the seaports in Gdynia and Gdańsk, a gradient of distance from the Vistula River mouth towards the Gdańsk Deep, and some other sites along the coastline of GoG. The sites were assumed to differ in sediment toxicity because of spatial differences in contaminant levels and spectrum reported by other studies (Filipkowska et al., 2014; Lubecki and Kowalewska, 2010; Radke et al., 2013; Waszak et al., 2019). Spatial differences in sediment contamination in GoG result from a complex interplay between the sources of contamination and many environmental factors such as the dynamic of water currents and sedimentation processes (Radke et al., 2013; Suplińska and Pietrzak-Flis, 2008). For example, the port areas in Gdynia and Gdańsk show spatial heterogeneity i.e., two zones with prevailing either sea or land influences which is related to storm surges and an inflow of freshwater. The latter is substantial in the case of the Gdańsk port due to the Motława River inflow. The prevailing sea and land influences lead to spatial differences in sediment grain composition, organic matter, and contaminant content in these ports (Radke et al., 2013).

Tab. 4. The locations of the GoG sampling sites.

Site code	Sampling site	Longitude	Latitude
PGDY1	Port of Gdynia, dock IV (the Polish dock)	18.532	54.531
PGDY2	Port of Gdynia, dock V (the USA dock)	18.526	54.534
PGDY3	Port of Gdynia, outside the docks	18.583	54.500
PGDA1	Port of Gdańsk (the Bytomskie dock)	18.682	54.399
PGDA2	Port of Gdańsk (the Defenders of the Polish Post dock; the Dead Vistula River mouth)	18.669	54.360
PGDA3	Port of Gdańsk, outside the docks	18.633	54.450
E57	the Vistula Śmiała River mouth	18.799	54.390
WS	left to the Vistula River mouth	18.814	54.478
ZN2	the Vistula River mouth	18.957	54.383
X1	A gradient of distance from the Vistula River mouth	18.975	54.413
X2	A gradient of distance from the Vistula River mouth	19.000	54.438
395	A gradient of distance from the Vistula River mouth	19.045	54.478
X7	Right to the Vistula River mouth	19.146	54.361

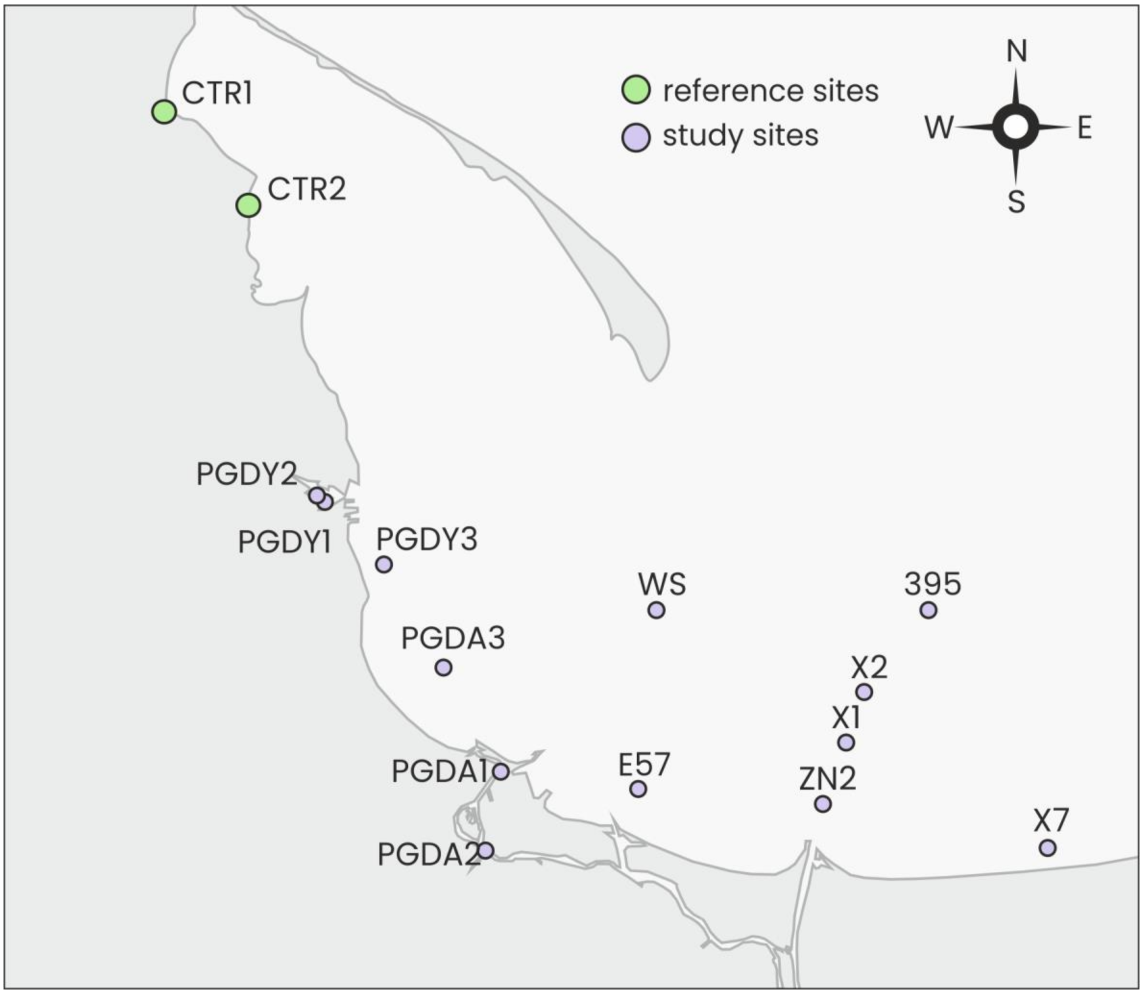

Fig. 7. Map of the GoG with the points marking the study sites and reference points (CTR).

2.2 The Martwa Wisła and Wisła Śmiała River

The Martwa Wisła has two entrances to the GoG, an eastern one through the Wisła Śmiała, and a western one in the Gdańsk Nowy Port district. The MW&WS rivers are characterized by slow water currents with no permanent flow into the sea. The tides in the MW&WS are irregular and depend on the wind direction and strength. Three relatively large canals flow into the Martwa Wisła before its fork with the Wisła Śmiała i.e., Kanał Śledziowy, Kanał Wielki, and Kanał Młynówka. The river also receives water from the surrounding system of 16 polders (Cieśliński et al., 2017). Consequently, the river offers a specific habitat that has some characteristics of the transitional water bodies, as its water salinity depends mainly on the water exchange with the GoG and the inflow of water from surrounding areas. The salinity gradually decreases with the distance from GoG, even up to 3.6 (Kendzierska et al., 2017). The Wisła Śmiała borders with Sobieszewo Island, where there are two nature reserves, Ptasi Raj in Górki Wschodnie and Mewia Łacha near Świbno. Both nature reserves and a 12 km long coastal area in Sobieszewo Island are included in the protected area of the NATURA 2000.

The MW&WS area drew the research interest because of the phosphogypsum landfill located on the Martwa Wisła River bank in the Wiślinka village, which had been of great concern regarding its negative impact on the environment and human health in the past i.e., before its closing in 2009. The phosphogypsum landfill occupies an area of 85 ha including the phosphogypsum heap of 26 ha. It is separated from the Martwa Wisła River by a floodbank (leaky in the past), and there is a short distance to the nearest farms, 350 m to arable fields, and about 3 km to the GoG and the nature reserve NATURA 2000. The concern about the harmful effects of the phosphogypsum was raised already in the 60s and 70s of the last century when the phosphogypsum, experimentally dumped in the GoG, was found to intensify the eutrophication processes, led to the accumulation of fluorine compounds in animal tissues, and caused changes in the composition of the benthic fauna (Żmudziński, 1971). The study by Pautsch et al. (1975) indicated that phosphogypsum caused detrimental changes in the development, metabolism, and behavior of various aquatic organisms. The storage of the phosphogypsum by the Gdańskie Zakłady Nawozów Fosforowych (GZNF) "Fosfory" in Wiślinka began in 1972. In 2009, when the landfill was closed, the heap reached a height of 41 m, a width of 400 m, and contained about 16 million tons of phosphogypsum (Räike et al., 2015). Phosphogypsum is a by-product of phosphoric acid production used for the production of fertilizers. Its dominant components are calcium sulfate dihydrate (85 - 94 %) and phosphates (2 - 10 %). It also contains fluorine compounds (0.5 - 1.5 %), arsenic, cadmium, chromium, nickel, copper, zinc, and lead in quantities from several to several dozen mg kg^{-1} of dry matter, and radioactive elements (Hupka et al., 2006). The heap constituted a major threat to the environment and local population due to its leachate characterized by very low pH, very high concentration of phosphates, and the presence of sulfates, fluorides, toxic heavy metals, and radioactive elements, which penetrated surface and groundwater. Erosion processes caused by wind led to an atmospheric dispersion of heap's particle-bound contaminants over the surrounding areas. The issue of detrimental impact on the surrounding environment and human health was raised repeatedly by various constituents and was the subject of some studies (Hupka et al., 2006; Olszewski et al., 2016; Skwarzec et al., 2010; Stolarska and Wojtasik, 2008). The contamination of groundwater by fluorine and phosphates was confirmed by the Voivodeship Inspectorate for Environmental Protection in Gdańsk (VIEP, Mioduszewski, 2008). After its closing, the heap was secured and recultivated). The VIEP monitoring data from 2011 - 2017 indicate that over the years the concentration of phosphates in the Martwa Wisła River has shown a clear decreasing trend and presently meets the environmental quality criteria (Figure 9; VIEP monitoring data). According to the analysis carried out in 2014 by EkoKonsult, based on the data of the VIEP in Gdańsk from 2008 to 2013 (pH, phosphates, total phosphorus, and fluorides in surface and underground waters), the landfill is no longer a source of significant pollution (EkoKonsult, 2014). Moreover, the Martwa Wisła is considered a stagnant water body, therefore the risk of spreading potentially harmful substances into the Baltic Sea is thought to be doubtful (Pöyry, 2013; Cieśliński et al., 2017).

Fig. 8. The phosphogypsum heap in 2016 (phot. W. Podlesińska).

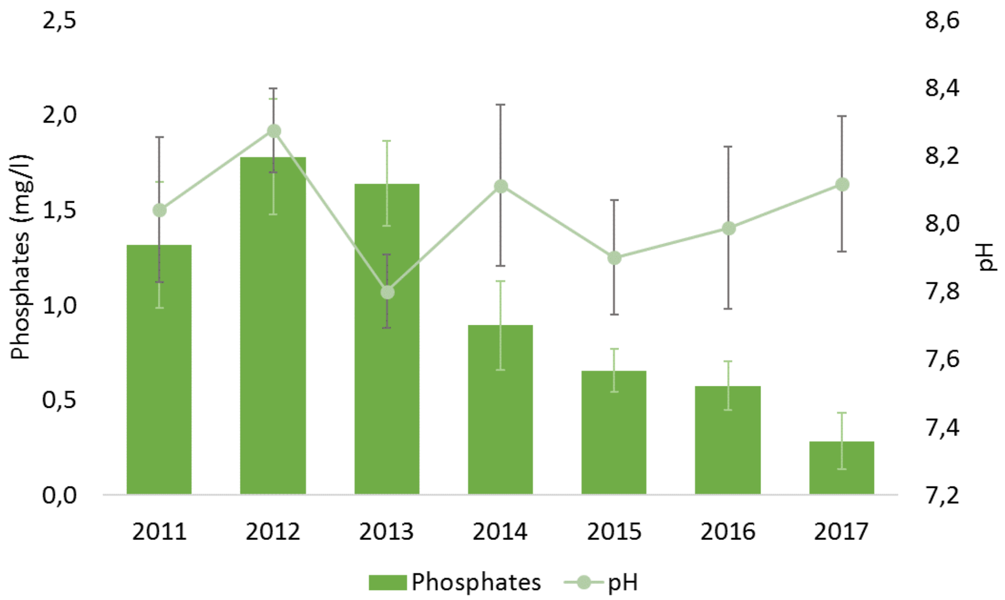

Fig. 9. Phosphates ($PO4^{3-}$) and pH of the surface water of the Martwa Wisła, annual average values with standard deviation (based on data obtained from VIEP in Gdańsk, samples of water were collected in the vicinity of the Sobieszewo pontoon bridge).

Although the landfill is no longer considered a threat to the environment, the leachate from the heap had been soaking into the soils and riverine sediment, and the dust from the heap had been spreading over the surrounding area for decades. The phosphogypsum components and toxic elements released in the past into the environment can remain in the soils and sediments for a long period. It became important to evaluate whether or not the phosphogypsum landfill was a factor influencing the quality of the MW&WS environment. For this purpose, the *Corophium* spp. bioassay was used to evaluate the sediment quality and the benthic fauna was examined since sediments constitute one of the main determinants of the macrobenthos assemblages. There is little information on benthic fauna inhabiting the MW&WS River stretch. One study was found that described the impact of the phosphogypsum on meiobenthos of the Martwa Wisła River (Stolarska and Wojtasik, 2008). However, the benthic fauna in the Wisła Śmiała and the western section of the Martwa Wisła had been investigated in connection with the 2012 - 2014 dredging work in that area coordinated by the Maritime Office in Gdynia (Przewoźniak et al., 2010). The investigation involved monitoring macrobenthos and water parameters over time (before and after the dredging in 2014 - 2017) to evaluate the impact of dredging on the environmental condition and macrobenthos assemblages (Kendzierska et al., 2017). The condition of the MW&WS area was up to now discussed mostly in the context of potential pollution of the GoG and the Baltic Sea with phosphates originating from the phosphogypsum heap (GIOŚ, 2013). Even though the MW&WS water system is connected to the Port of Gdańsk, which has been a major source of pollution in the GoG area, the MW&WS sediments have not been yet studied for contamination with HOCs.

The MW&WM study area involved five sampling sites (Figure 10 and Table 5). The sites were spread along the course of the MW&WS Rivers from the Wisła Śmiała River inflow to GoG up to the confluence of the Kanał Wielki with the Martwa Wisła River above the phosphogypsum heap.

Tab. 5. Locations of the MW&WS sampling sites.

Site code	Sampling site	Longitude	Latitude
ST1	Wisła Śmiała	54.3654	18.7835
ST2	Crossing, Wisła Śmiała - Martwa Wisła	54.3552	18.8013
ST3	Martwa Wisła and Młynówka Kanał confluence, close to the pontoon bridge	54.3444	18.8225
ST4	Martwa Wisła, near the phosphogypsum heap	54.3308	18.8391
ST5	Martwa Wisła, near the Kanał Wielki inflow	54.3255	18.8691

Fig. 10. Map of the studied section of the Martwa Wisła and the Wisła Śmiała with sampling sites marked (ST1-ST5) and a phosphogypsum waste heap location.

3 Study aims and objectives

The overall aim of the doctoral thesis was to evaluate the suitability of *Corophium* spp. for the assessment of sediment quality in the GoG and the MW&WS areas. This was performed through prolonged exposure (28-d) of *Corophium* spp. to the sediments in which their responses were examined. These responses included survival, growth rate (GR), emergence (sediment avoidance), and molting frequency. Additionally, the study evaluated reproductive activity by examining the gravidity of females and the presence of juvenile offspring.

In the testing of the MW&WS sediments, in addition to *Corophium* spp., a standardized bioassay with the ostracod *Heterocypris incongruens* (Ostracodtoxkit FM, MicroBioTests, Inc., Belgium) was applied to compare the responsiveness of both organisms to the sediments. The *H. incongruens* bioassay is becoming increasingly used in sediment ecotoxicological studies (Casado-Martinez et al., 2016; Gonzalez-Merchan et al., 2014; Kudłak et al., 2012). Moreover, in the MW&WM area, the bottom fauna was investigated to evaluate whether or not the bioassay results were indicative of any adverse changes in the macrobenthos community.

The tested hypotheses were:

1) *Corophium* spp. are responsive to sediment contamination showing differential severity of responses depending on sediment contamination level,
2) *Corophium* spp. responses to the MW&WS sediments reflect the status of macrobenthos assemblages.

The research objectives included:

1. Development of method for the *Corophium* spp. cultivation in laboratory conditions and sediment toxicity testing in the Gdynia Aquarium based on published recommendations,
2. Examination of the GoG sediments toxicity using the *Corophium* spp. bioassay,
3. Evaluation of *Corophium* spp. responses to the GoG sediments in the context of contamination and natural characteristics of the sediments,
4. Examination of the MW&WS sediments toxicity using the *C. multisetosum* and *H. incongruens* bioassays,
5. Evaluation of the *C. multisetosum* and *H. incongruens* responses to the MW&WS sediments,
6. Analyses of composition and chemical contaminants of the GoG and the MW&WS sediments,
7. Analyses of benthic fauna in the MW&WS sediments,
8. Evaluation of the MW&WS benthic assemblages in the context of sediment features, environmental parameters, and bioassay results.

4 Methods

4.1 Amphipods, *Corophium* spp.

4.2 Collection and maintenance

Before the bioassays, an exploratory field study was performed in the Puck Bay coastal areas to find natural habitats of *Corophium* spp. The sediments of the Puck Bay coastal area had been recognized as relatively clean, i.e., containing negligible amounts of anthropogenic chemical contaminants compared to other areas of Puck Bay. Two sites were chosen based on accessibility from the shore, *Corophium* spp. population density, and distance to the laboratory. These were Kaczy Winkiel and Rzucewo (see section *Study area*). During the exploratory field study, samples of amphipods were collected and fixed in the 4 % formaldehyde solution for species identification (Section 4.3). The amphipods collected in Kaczy Winkiel and Rzucewo in 2015 were identified as *Corophium volutator* and *Corophium multisetosum*, respectively. These locations were surveyed during May-August in 2015, 2016, and 2018, namely the samples of amphipods were collected and identified. The abundance of organisms was sometimes as high as several hundred individuals per m^2, however, it varied depending on the sediment type, distance from the shore, and season. During the survey in 2018, it was noted that the sediment type in the Rzucewo location was muddier compared to previous years and the population shifted from *C. multisetosum* to *C. volutator*.

Procedures for the collection and cultivation of *Corophium* spp. In the laboratory conditions were based on methods described by other researchers (Heuvel-Greve et al., 2007; Menchaca et al., 2010; Peters and Ahlf, 2005; Scarlett et al., 2007). *Corophium* spp. were collected at about a few to 30 meters from the shore from both locations. The sediments were sieved in *situ* over a 0.5 mm sieve to extract *Corophium* spp. and to eliminate indigenous fauna. The sieved sediment was used as a medium in acclimation aquaria in the Gdynia Aquarium laboratory. The amphipods were transported to the laboratory in plastic containers filled with water and about a 1 cm layer of sediment taken from the amphipods collection site. In the laboratory, the amphipods were transferred to an acclimation system consisting of 16 l aquaria (Figure 11 and Figure 12) filled with a 2 - 3 cm layer of sediment and artificial saltwater [5] to about three-quarters of the volume, with a temperature of about 15°C. According to studies on *C. volutator* by Kater et al. (2008), this temperature assures the optimal growth of amphipods. A small amount of natural seawater was added to the artificial saltwater in the acclimation aquaria to enhance the establishment of a bio-geo-chemical balance before animals were

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[5] the saltwater was prepared according to the standard practices in the Gdynia Aquarium. It consists of a demineralized water and commercial salt (Tropic Marin Sea Salt, Aquaforest Sea Salt).

introduced. The water salinity in the acclimation aquaria was maintained at the same level as in the amphipod collection site. The acclimation aquaria were aerated using silicone aquarium tubing. A natural light regime was maintained during the study of the GoG sediments, and artificial lightning (16:8, light: dark) was introduced in the MW&WS study (Figure 12). During the acclimation, the amphipods were fed every other day with the Microbe-lift Phyto Plus A (diatoms *Phaeodactylum tricornutum)* and with powdered algae (*Chlorella* spp., BioChlorella, Nat Vita) in 2015 and 2016, respectively. The type of food was chosen based on the dietary preferences of *C. volutator* (Gerdol and Hughes, 1994a). Regularly, one day after the feeding about 30 % of the water in the acclimation aquaria was renewed with artificial saltwater. The pH, temperature, oxygen concentration, and salinity were measured twice a week. The levels of ammonia (NH_3^+), nitrates (NO_3^-, NO_2^-), and phosphates (PO_4^-) were measured once a week according to the procedures provided by the Hach company with the Spectrophotometer Hach DR 3900.

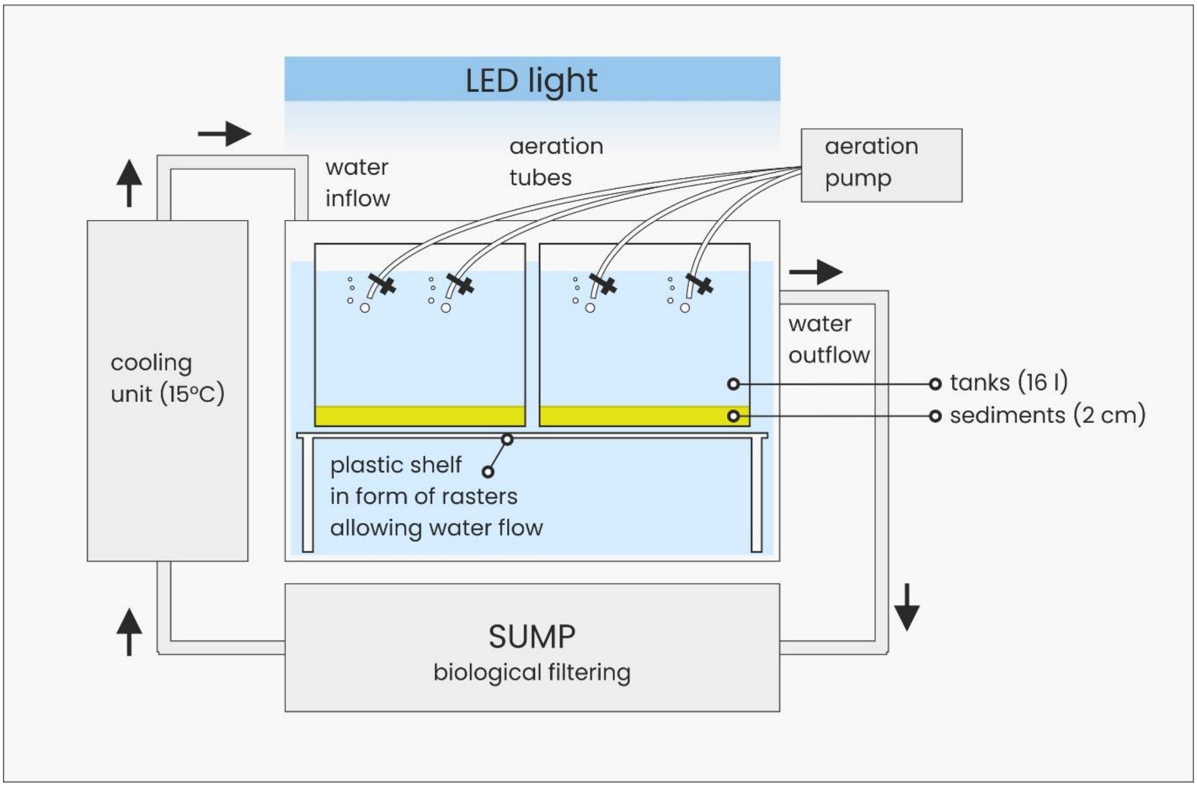

Fig. 11. The scheme of the *Corophium* spp. acclimation system.

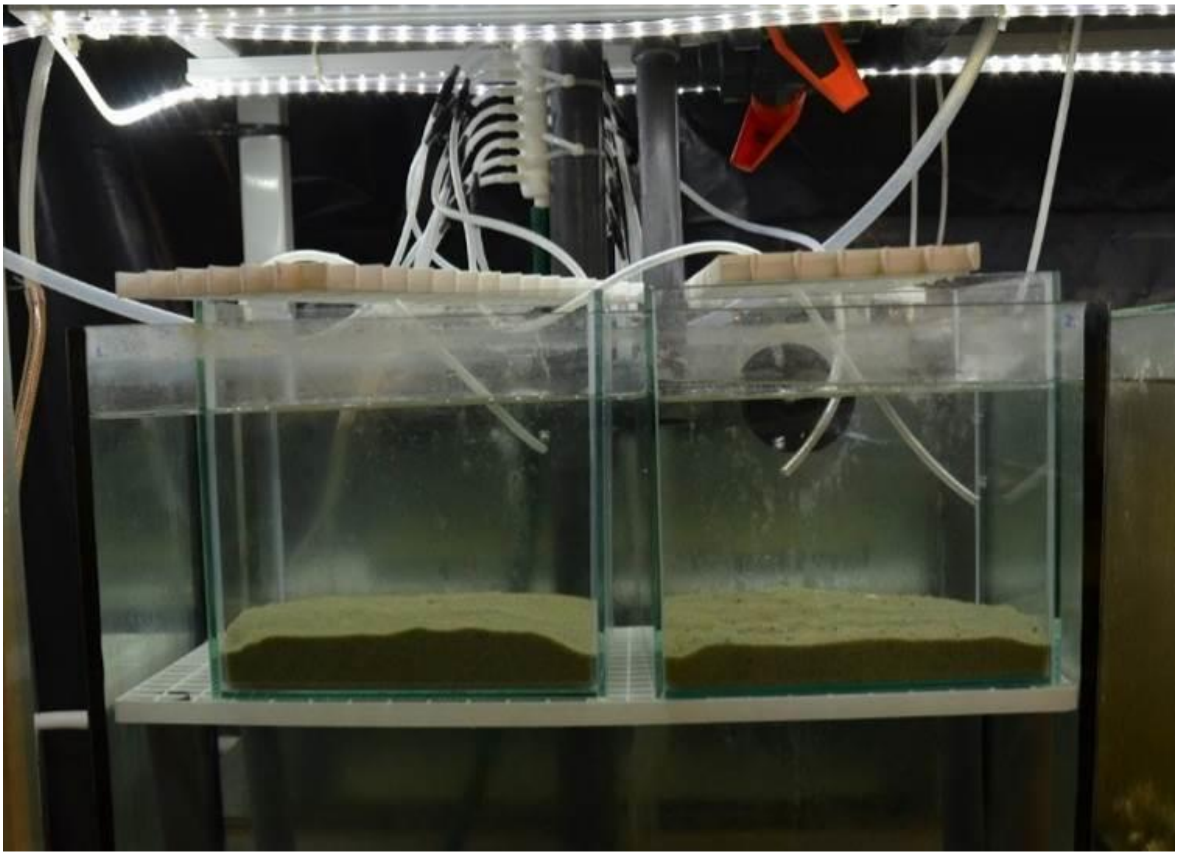

Fig. 12. The amphipod acclimation system.

4.3 Identification of amphipod species

Corophium spp. were identified to a species according to the taxonomic keys and references (Crawford, 1937; Marquiegui and Pérez, 2006; Otte, 1975; Stock, 1952). The main anatomical features that differentiate *C. multisetosum* and *C. volutator* are listed in Table 6. The specimens' gender was identified based on the size of the second pair of antennae (being considerably larger in adult males than in females) and the presence of oostegites or the penial papillae in mature females or males, respectively (Figure 13). The size of the spines on the article I of antennae I (Figure 14) is also gender-specific, being significantly more pronounced in females (Schneider et al., 1994). The latter characteristics allow also for distinguishing the gender of the smaller individuals. However, in this study, the gender of juveniles smaller than 3 mm in length was not identified.

Tab. 6. Morphological features differentiating *C. volutator* and *C. multisetosum* (Ingle, 1963; Marquiegui and Pérez, 2006; Schneider et al., 1994; Stock, 1952).

	C. volutator	*C. multisetosum*
Antenna I ♀ (Figure 14)	The lower edge of segment 1 usually with 2 spines and only 3 setae between them.	The lower edge of segment 1 with 3 spines and about 14 setae between the 2nd and 3rd spine.
Antenna II ♀	The penultimate joint of peduncle without spines, the terminal tooth hardly overreaches the tip of its joint. The angle at the base of the projection is larger than in *C. multisetosum*.	The penultimate joint of peduncle with 1 spine and a terminal tooth is one-third the length of the following joint. The angle at the base of the projection is smaller than in *C. volutator*.
Uropod I ♀	The outer edge of the peduncle with 10 or more unpaired spines.	The outer edge of the peduncle with 8 spines, 6 of them unpaired.
Uropod II ♀	Peduncle with more than 3 - 4 spines and the triangular projection is barely visible.	Peduncle with 3 - 4 spines and a prominent triangular projection.
Uropod III ♀ (Figure 15)	Ramus hardly set off eccentrically. The lateral projection of the peduncle is minor.	Ramus set off eccentrically. The lateral projection of the peduncle as wide as the basal diameter of the ramus.
Antenna I ♂ (Figure 14)	The lower edge of segment 1 without expansion, 2 small spines, and few setae	The lower edge of segment 1 with lamellar expansion, 4 - 5 small spines, a numerous seta

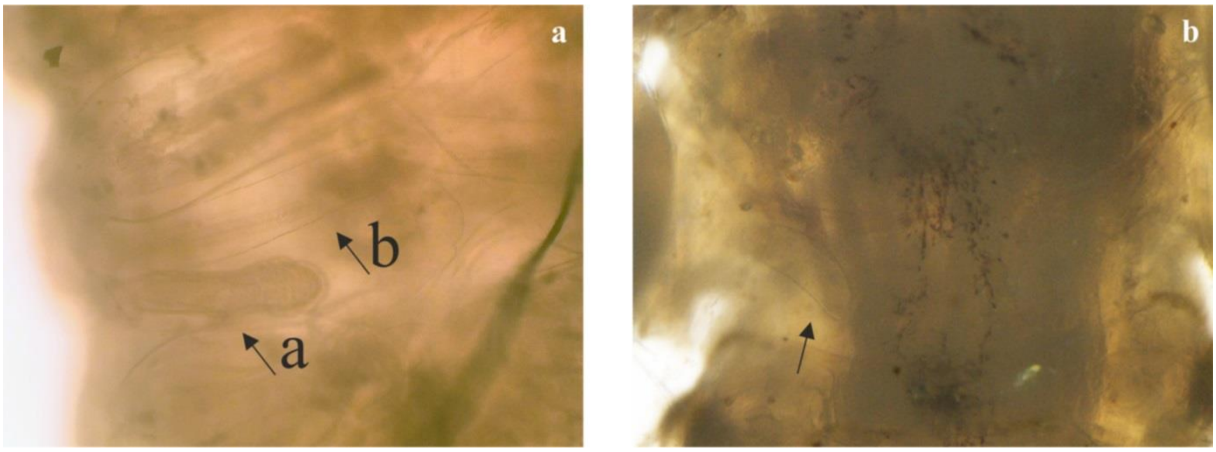

Fig. 13. Gender differences in *Corophium* spp.:(a) the “a” arrow points to a singular gill, arrow “b” to one of eight non-setose oostegites in an immature *C. volutator* female; (b) the arrow points to a penial papilla placed on the 7th thoracic segment in a *C. volutator* male (photo by Weronika Podlesińska).

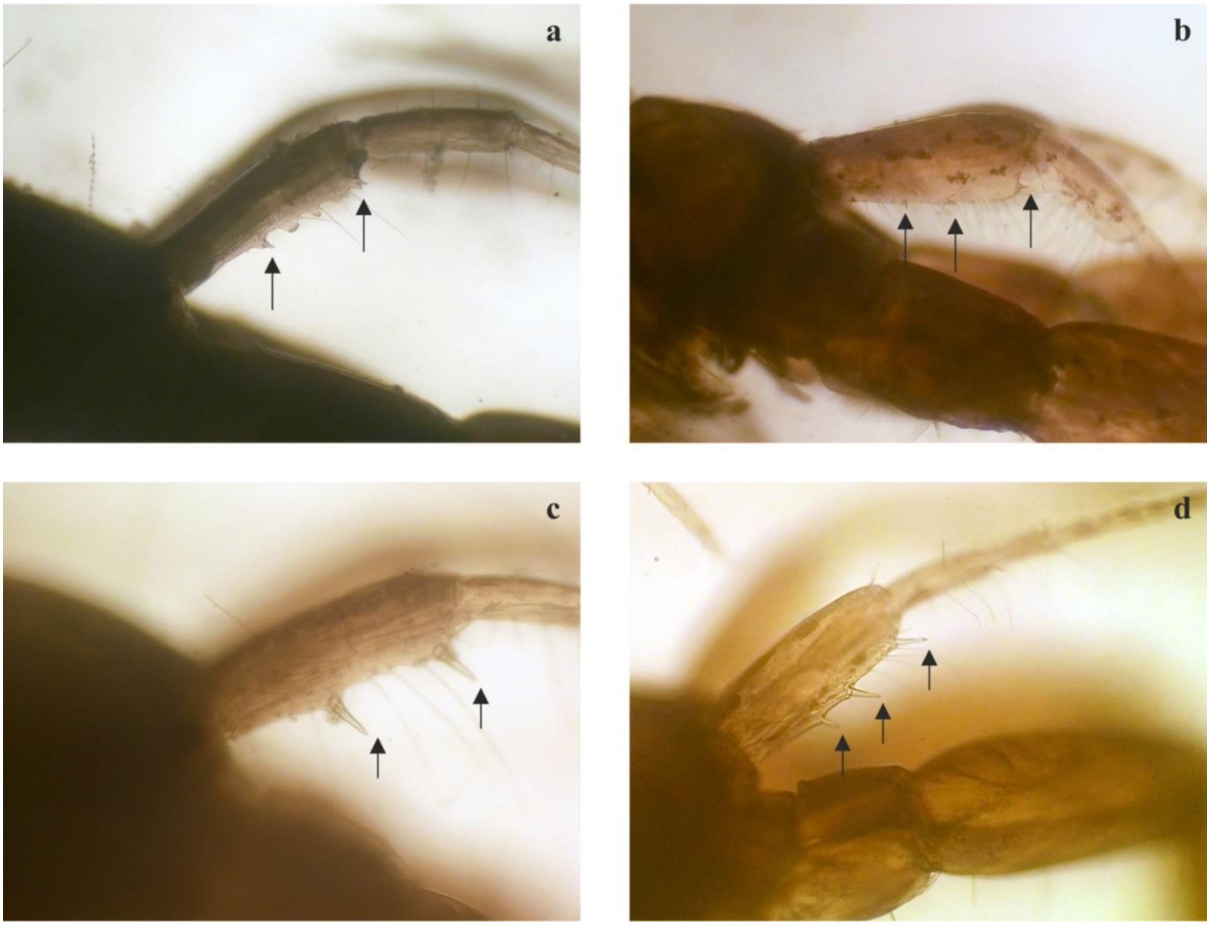

Fig. 14. Antenna I in *C. volutator* male (a) and female (b), and in *C. multisetosum* male (c) and female (d); arrows point to the spines on the ventral side of segment 1 antenna I. The characteristic triangular shape of segment I antenna I in males of *C. volutator* is plainly visible in (b) (photo by Weronika Podlesińska).

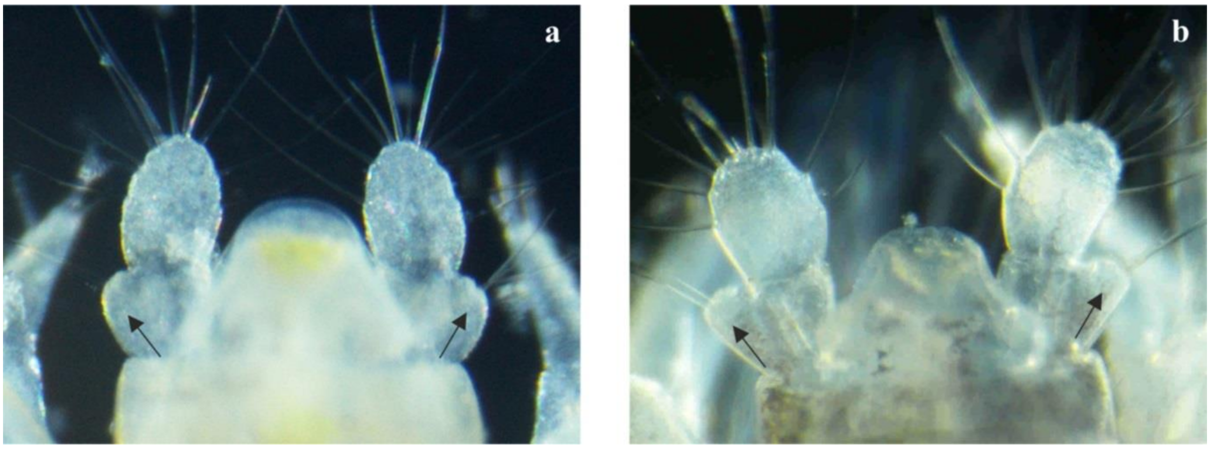

Fig. 15. Uropod III in females of *C. volutator* (a) and *C. multisetosum* (b), arrows point to the ramus, which is eccentric in *C. multisetosum* (photo by Weronika Podlesińska).

4.4 Testing sediment as the amphipod cultivation medium

A pre-study with *Corophium* spp. was performed to test conditions for laboratory cultivation. The requirements regarding water temperature, salinity, pH, and dissolved oxygen have been described in several studies (Castro et al., 2006; Gale et al., 2006; Heuvel-Greve et al., 2007; Menchaca et al., 2010; Peters and Ahlf, 2005; Siebeneicher et al., 2013; Stock et al., 2009). Laboratory cultivation of *Corophium* spp. requires the sediment of good quality to provide a suitable habitat. The test cultivation included two approaches, both with the use of sediment collected from the same location, specifically, the habitat of amphipods. The first approach assumed the use of sediment swept clean of organic chemical contaminants (via chemical extraction with a mixture of dichloromethane and hexane) and the second chemically untreated sediment. After several weeks of cultivation, the experiments were terminated. The chemically treated sediment was evaluated as not suitable for laboratory cultivation of amphipods based on their behavior, mortality, and growth. Most individuals avoided the treated sediment immediately after being introduced into the aquaria, and showed increased mortality and little growth over the experimental period, despite a regular addition of planktonic food with diatoms *Phaeodactylum cornutum* (Microbe-Lift Phyto Plus A). Based on the results of this pre-study, natural sediment from the *Corophium* spp. collection site was chosen as the medium for amphipod cultivation and as reference sediment in bioassays. The chemical analyses showed that it was relatively free from contaminants.

4.5 Bioassays, experimental setup, and procedures

The bioassay design followed the methods proposed by USEPA, (2001) and Roddie and Thain (2001). The test acceptability criteria required the amphipod mortality to be lower than or equal to 20 % in the test controls (USEPA, 2001). The bioassays were carried out in an experimental system consisting of 50L aquaria equipped with mesh shelves of adjusted height and 1L exposure beakers of borosilicate glass (base diameter of 100 mm, the height of 195 mm) placed on the shelves with an aeration system of silicon tubes provided to each beaker (Figure 16 and Figure 17). The 50L aquaria were filled with temperature-regulated saltwater to a level that was exactly in line with the water level in the exposure beakers. This ensured that the water temperature in the exposure beakers was stable. The saltwater delivered to the aquaria was circulating through the sump[6] system, which ensured the biological filtering of the water and maintained temperature. The circulating saltwater was used to exchange the overlying water in the acclimation aquaria and the exposure beakers. The aeration system was set to produce water oxygenation > 80 % without causing any disturbance at the sediment surface. The tested sediment samples were placed in the beakers to form a 2 cm deep layer (about 200 g of sediment) and then the saltwater (described in Section 1.2.1) was added to the level of 1000 ml. Each sediment sample, before use, was thoroughly mixed and sieved through a 1 mm sieve to remove large elements (pieces of wood, shells, stones) and indigenous fauna if present. The beakers with sediment and overlying water were placed in the above-described system and left for sedimentation and equilibration of the media for 24 h at a temperature of 15°C. After that, the water aeration in beakers was turned on for another 24 hours before the *Corophium* specimens were introduced.

In the bioassays with the GoG sediments, two amphipod species were used, *C. volutator* and *C. multisetosum*. The decision was justified by an insufficient number of the laboratory-cultivated *C. volutator* individuals. The number of amphipods placed in each beaker in the GoG study was 10 - 11. In the MW&WS bioassays, solely *C. multisetosum* was used as a large population of this species was available at the time at the Rzucewo site. 15 individuals per beaker were used in the MW&WS study. Upon the transfer of the test animals to the exposure beakers, their behavior was observed. The individuals that did not burry within the first minute were replaced. The bioassays lasted 28 days and the food was administered two times a week. A day after the feeding, 50 % of the overlying water in the beakers had been carefully renewed without disturbing the sediment surface. The salinity, oxygen concentration, pH, and temperature were measured at regular intervals, at least twice a week. The acclimation and exposure conditions during the GoG and the MW&WS studies are summarized in Table 7 and Table 8. The number of individuals presumed dead (present on the sediment surface and not responding to stimulus in form of a gentle stream of water from

[6] Sump is an accessory equipment in a form of container placed under the proper aquarium, with which it is connected. It serves as a system stabilizing the water level and supporting filtration, and is often used to provide other conditions required in the aquaria.

a pipette) and molts at the sediment surface, as well as any amphipods that emerged from the sediment, were recorded daily. The dead individuals and the molts were removed if observed. After 28 days, the tested sediments were transferred from the beakers and sieved through 0.5- and 0.3-mm mesh screens. The retrieved amphipods were counted and preserved in 70 % ethanol (the GoG study) or 4 % formaldehyde solution (the MW&WS study) for the length measurements and the gravidity inspection. Missing amphipods were considered dead.

The GoG and MW&WS sediment testing differed; specifically, the three GoG sediment samples collected from each site were tested separately, whereas the three MW&WS sediment samples collected from each site were mixed to form one composite sample, from which five replicates per site were tested.

Tab. 7. Summary of the acclimation and exposure conditions in the GoG study.

Component	Acclimation	Bioassays
Experimental vessels	16 L aquaria	1 L beakers
Replicates, n	2	3
Individuals, n	<100	10 - 11
Sediment	2 cm layer of sediment taken from the *Corophium* spp. collection site, sieved through 1mm mesh	2 cm layer of sediment collected from various sites in the Gulf of Gdańsk, sieved through 1mm mesh
Aeration	Aeration provided water oxygenation > 80 %	Aeration provided water oxygenation > 80 %
Temperature	15.0 °C	15.0 °C
pH	7 - 9	7 - 9
Water	Artificial saltwater, the salinity of 7	Artificial saltwater, the salinity of 7
Water exchange	Two times a week, 30 % of the volume	Two times a week, 50 % of the volume
Light	natural	natural
Feeding	2 - 3 times a week, Microbe-lift phyto plus A	2 times a week, Microbe-lift phyto plus A
Water monitoring	Once a week: temperature, salinity, pH, oxygen concentration	Once a week: temperature, salinity, pH, oxygen concentration
Duration	25 - 35 days (*C. volutator*), 4 - 5 days (*C. multisetosum*)	28 days

Tab. 8. Summary of the acclimation and exposure conditions in bioassays testing the MW&WS sediments.

Component	Acclimation	Bioassays
Experimental vessels	16L aquaria	1L beakers
Replicates, n	2	5
Individuals, n	<100	15
Sediment	2 cm layer of sediment taken from the *C. multisetosum* collection site in Puck Bay sieved through 1mm	2 cm deep layer of sediment collected from several sites in the Martwa and Śmiała Wisła rivers, sieved through 1mm
Aeration	Aeration provided water oxygenation > 80 %	Aeration provided water oxygenation > 80 %
Temperature	15°C	15°C
pH	7 - 9	7 - 9
Water	Artificial saltwater, the salinity of 7	Artificial saltwater, the salinity of 7
Water exchange	Two times a week	Two times a week
Light	16:8 light:dark	16:8 light:dark
Feeding	2 - 3 times a week, dried Spirulina	2 - 3 times a week, dried Spirulina
Water monitoring	Temperature, salinity, pH, oxygen concentration (twice a week)	Temperature, salinity, pH, oxygen concentration (twice a week)
Duration	4 - 10 days	28 days

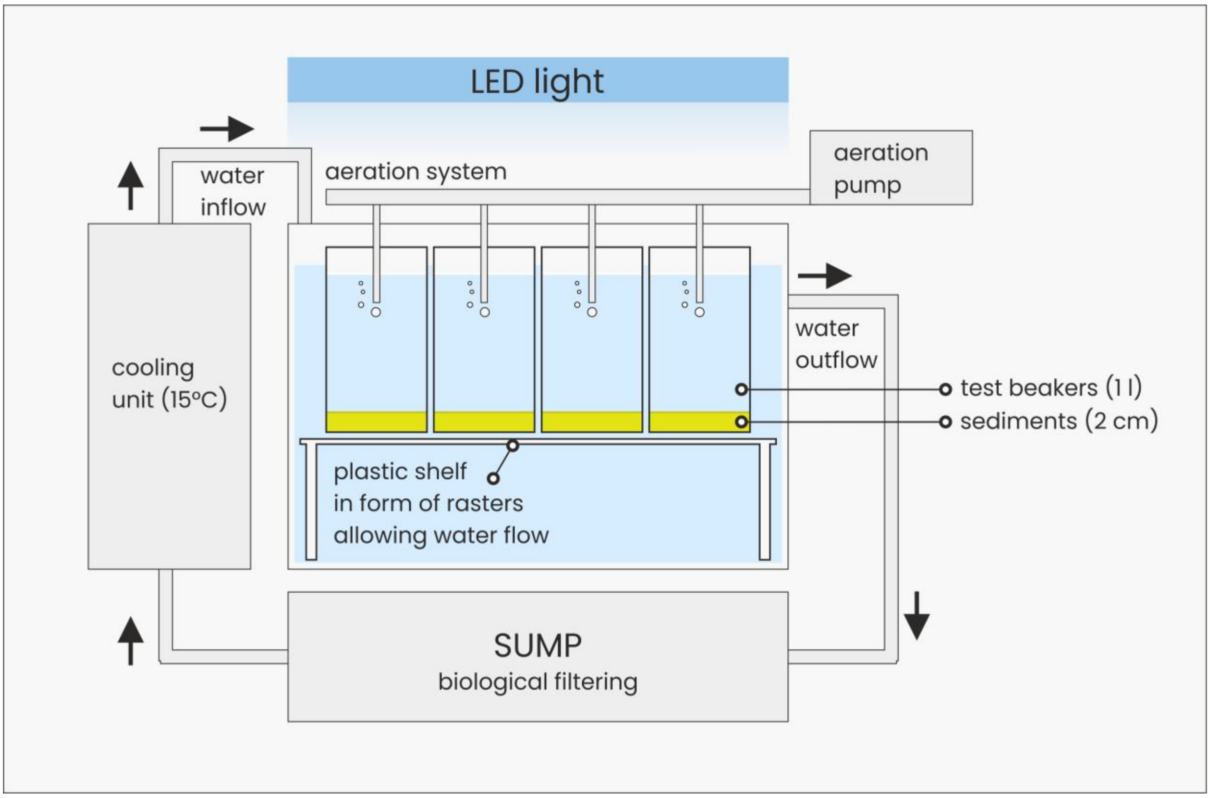

Fig. 16. A scheme of the experimental system for sediment toxicity testing.

Fig. 17. The set-up of the experimental system for sediment toxicity testing.

4.5.1 Examined endpoints

The main endpoints examined were survival and growth. As recommended, additional observations throughout the bioassay were noted (USEPA, 2001), specifically the number of molts and the number of individuals emerging from sediment which is assumed to be a sediment-avoiding response. These endpoints and dead individuals in each test beaker were recorded daily.

The survival (S) in each vessel was determined according to the following formula:

$$S\,(\%) = \frac{N_f}{N_i} \times 100\ \%$$

Where N_f is the number of surviving amphipods, and N_i is the initial number of amphipods.

The initial length of individuals (L_i, the length at day 0) used in the GoG study ranged from 3 to 5 mm. L_i of each amphipod used in the GoG tests was measured to the nearest millimeter with a millimeter paper placed under the Petri dish containing the amphipods. The length range of amphipods used in the MW&WS studies was smaller, namely 2 - 3 mm. At the start of each bioassay, the mean L_i was calculated by measuring the length of 20 randomly selected individuals to the nearest 50 μm, using a microscope, and a micrometer slip. The final body length (L_f, length after the bioassay termination) was determined for each surviving specimen to the nearest mm in the GoG study and the nearest 50 μm in the MW&WS study.

In each case, the body length of amphipods was measured from the tip of the rostrum to the posterior end of the metasomatic segment. The growth i.e., the change in length during the 28-d bioassay, was calculated as a mean growth per beaker/replicate (G, mm individual^{-1} over 28 days) according to the following formula:

$$G = (L_f - L_i)$$

where $\overline{G}$ is a mean increase in length (mm) during 28-d, $\overline{L_f}$ is the final mean length (mm), and the $\overline{L_i}$ is the initial mean length (mm). The G value was used to present the growth data as the mean individual growth rate (GR, µm d^{-1}).

Other examined endpoints included gravidity (the number of gravid females per all living females), the number of males per all living individuals, and the presence of neonates at the bioassay termination. Gender and female gravidity were determined using a dissecting microscope. Survival, gravidity, and the males' share were converted to percentages. The index of molting and emergence from the sediment[7] were calculated as a ratio of collected molts and individuals observed on the sediment surface throughout bioassay, respectively, per initial number of amphipods in each exposure beaker.

4.5.2 Amphipods' sensitivity tests

The sensitivity was evaluated based on mortality resulting from exposure to cadmium chloride ($CdCl_2$). The tests involved *C. volutator* collected from two sites in the Bay of Puck, i.e., Kaczy Winkiel and Rzucewo. Cadmium chloride is a model toxicant used for amphipod sensitivity testing by several authors (Kater et al., 2006; Picone et al., 2008; Prato et al., 2015). The tests included the following water concentrations: 0, 0.5, 2, 5, and 10 mg l^{-1}. They were performed in 1L beakers with a Cd solution of 0.5L, without sediment (Table 9). The stock solution of $CdCl_2$ was prepared in de-ionized water. The Cd-exposure solutions were prepared in artificial saltwater (described in Section 4.1), with salinity adjusted to that at the amphipod collection site. The exposure lasted 72 h. The mortality of amphipods was checked daily. The organisms that did not respond to a stimulus (a gentle stream of water from a pipette) were considered dead.

[7] the ratio of individuals that emerged from the sediments is henceforth referred to as 'emergence'.

Tab. 9. Summary of the acclimation and exposure conditions in the *C. volutator* sensitivity tests.

Component	Acclimation	Exposure
Experimental vessels	16L aquaria	1L beakers
Replicates, n	-	3
Individuals, n	< 600	15
Medium	2 cm deep layer of sediment from the amphipods collection site, sieved through a 0.5 mm mesh screen, about 6 cm layer of overlying water	Water-only exposure, 500 ml volume with increasing Cd concentrations
Aeration	five silicate tubes, the diameter of 4 mm, 3 - 4 air bubbles per second	one silicate tube, the diameter of 4 mm, 3 - 4 air bubbles per second
Temperature	16°C	16°C
Water	Artificial saltwater, the salinity of 7	Artificial saltwater, the salinity of 7
pH	7 – 9	7 - 9
Water exchange	partly every second day	No
Light	continuous	continuous
Feeding	No	No
Water monitoring	every second day: temperature, salinity, pH, dissolved oxygen	No
Duration	9 days	4 days

The LC_{50} values and confidence limits were calculated using the Probit Analysis by Finney (1952). The $CdCl_2$ concentrations were transformed by the common logarithm. The mortality was converted to empirical probits. Empirical probits of less than 1 and more than 7 were ignored as advised by Hayes and Kruger (2014). Empirical probits and log-transformed toxicant concentrations were subjected to regression analysis. Expected probits were derived from the regression curve expressed by:

$$y = ax + b$$

Where y is the corrected mortality expressed in probits, a is y-intercept, x is $\log_{10}$ of the toxicant concentration and b is the slope of the line. Fiducial confidence limits (CL) were calculated with the formula:

$$\text{CL} = antilog(log10(conc.) \pm 1.96 \times SE)$$

4.6 Ostracods, *Heterocypris incongruens*

The sediment toxicity bioassay with the benthic ostracod *H. incongruens* is a standardized test during which the organisms are exposed to the whole sediment over 6 days. The bioassay is based on a comparison of mortality and growth of freshly hatched ostracods in control and test sediments during the 6-day exposure. The control serves to ascertain compliance with acceptability criteria and quantify growth inhibition (growth changes). The bioassay was carried out with the MW&WS sediments using the Ostracodtoxkit F manufactured by MicroBioTests Inc. Gent, Belgium and distributed in Poland by Tigret Sp. z o.o.

4.6.1 Test procedure

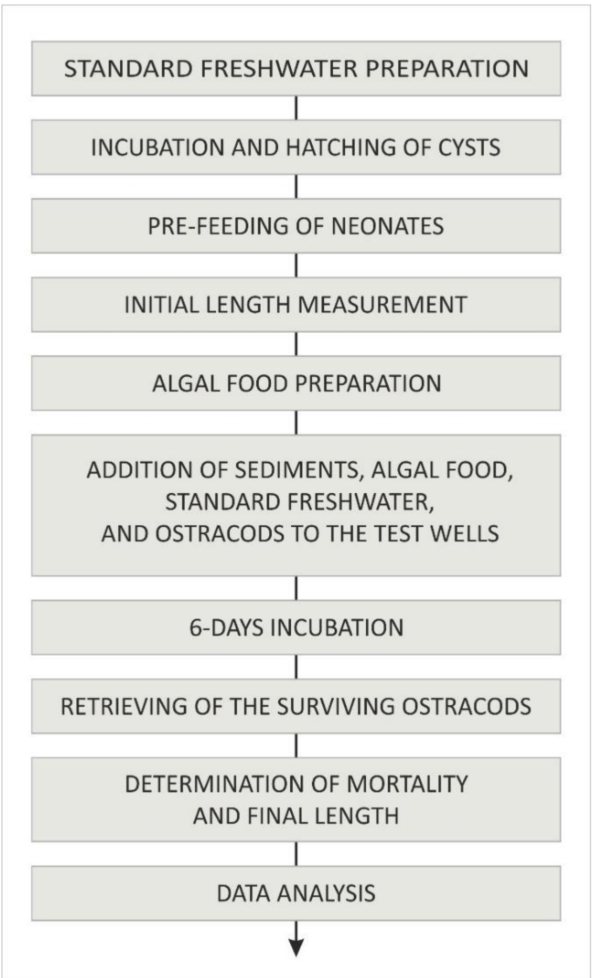

Fig. 18. The general procedure of the *H. incongruens* test.

The Standard Freshwater (SF), being synthetic, moderately hard water, advised as the medium for preparation of algal food, as well as hatching and development of *H. incongruens* was prepared and used throughout the bioassay according to the procedure described in Table 10. The incubator designed and used for incubation and hatching of the *H. incongruens* cysts and then for sediment testing is shown in Figure 19 and Figure 20. It consisted of an aquarium that served as a water jacket system and was equipped with a heater placed at the bottom and a thermostat (Figure 20). The aquarium was equipped with LED lighting and its sides were blacked out. Above the water level, the crate was placed to position the Petri dishes with ostracods. The temperature at the crate level had been monitored until the termination of the test. The algal food was added to all the control and test wells according to the manufacturer's recommendation. The test was considered valid if the mortality in the reference sediment was lower than 20 % and the length of the ostracods increased by 1.5 throughout the test.

Tab. 10. The Ostracodtoxkit test procedure description.

Test step	Description
Standard freshwater (SF) preparation	SF was prepared using the salts solution: 96 mg $NaHCO_3$, 120 mg $CaSO_4$, 123 mg $MgSO_4$, and 4 mg KCl, dissolved in 1 l deionized water. The SF was aerated and brought to room temperature before use.
Incubation and hatching of cysts	Covered Petri dishes with 8 ml of SF and *H. incongruens* cysts were placed in an incubator at 25 ± 1 ºC for 52 hours, under continuous illumination of 3500 lux (Figure 20, Figure 19).
Pre-feeding of neonates	Four hours after the start of incubation, Spirulina powder was added to the Petri dishes with cysts to feed freshly hatched neonates. The neonates were kept in this medium for 48 hours.
Initial length measurement	The ostracods were collected from the Petri dishes with a glass pipette and transferred to multiwell plates. The size of ostracod neonates was determined after 52 h of incubation using 10 randomly selected specimens immobilized with a Lugol liquid. The length was measured with the use of a microscope and a micrometer slip to the nearest 50 µm (Figure 20).
Algal food preparation	The matrix-dissolving medium was added to the tubes with algae beads provided by MicroBioTests Inc. and placed on Vortex until the algae were freed. The tubes with microalgae were centrifuged and the supernatant was replaced by distilled water. Centrifuging was repeated and the supernatant was replaced with the SF. The algal suspension was transferred to a 25 ml volumetric flask and filled with SF to the mark.
Placement of sediment, algal food, SF, and ostracods into the test wells	The bioassays were carried out on multi-well plates, with homogenized sediment samples collected from each station. Each sediment sample, including the reference sediment provided with the test kit, was tested in six replicates (one replicate per well). 2 ml of standard freshwater, 1 ml of the tested or control sediment, 2 ml of prepared algal food (Spirulina), and 10 ostracods were placed in each well.
6-days incubation	The multi-well plates were secured with parafilm, placed in the incubator, and kept at 25 ± 1 ºC in the dark for 6 days.
Retrieving the ostracods	After 6-day exposure, the ostracods were directly collected from the test wells with a glass micropipette. Muddy sediments were sieved through a microsieve provided with the test kit. The ostracods hold to surfaces to resist water currents; therefore, the glass pipette is required to collect the animals.
Determination of mortality and final length	The ostracods retrieved from the sediments were immobilized with a Lugol liquid in the multiwell plate, counted, and their final length was determined with the use of a microscope and a micrometer slip to the nearest 50 µm.
Data analysis	The survival and growth data were processed according to the information presented in Section 4.6.2.

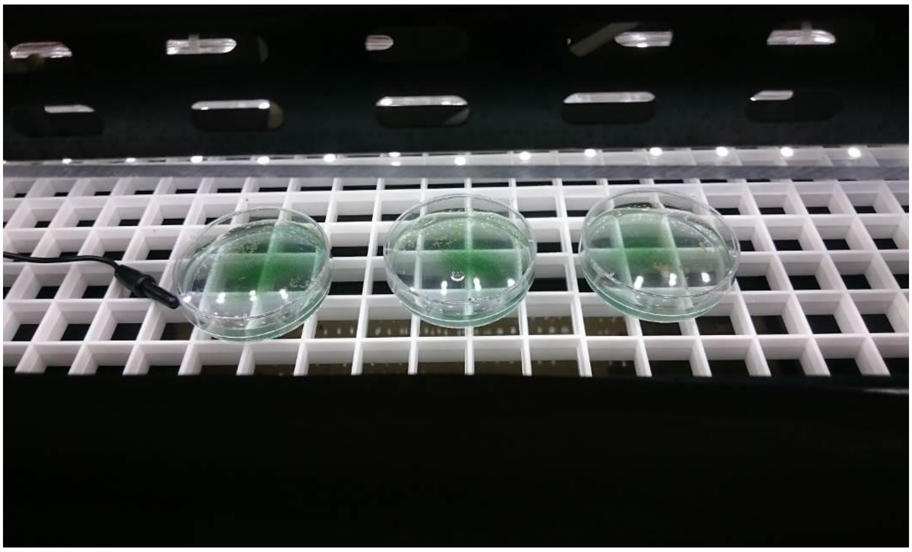

Fig. 19. Petri dishes with the *H. incongruens* cysts in the incubator.

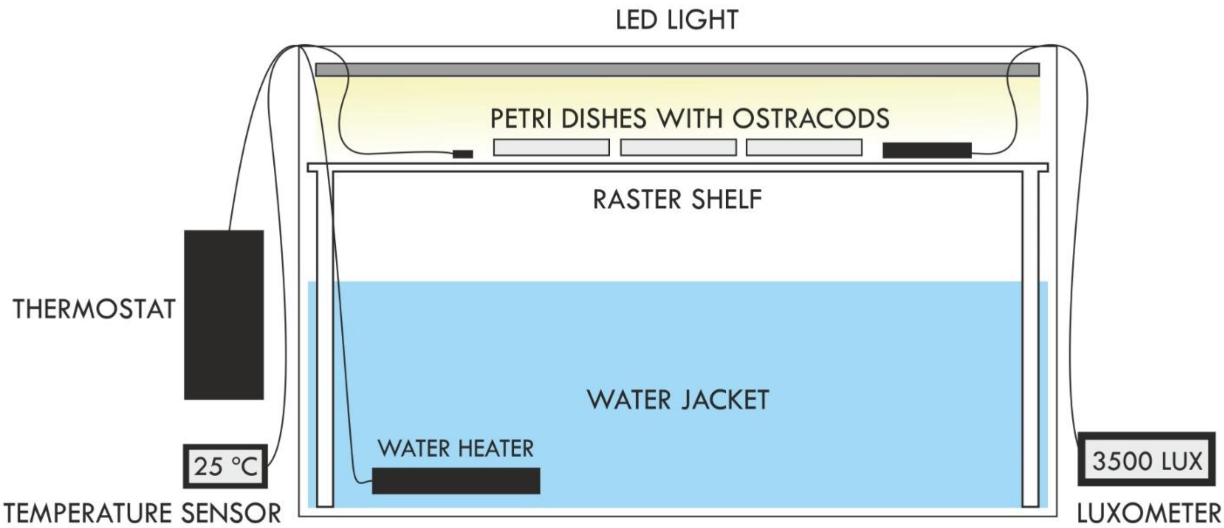

Fig. 20. Scheme of the *H. incongruens* incubator.

4.6.2 Examined endpoints

The endpoints measured in the test were the survival and growth of *H. incongruens* (Figure 21) exposed to sediment samples. After 6 days of exposure, the organisms were separated from the sediments, counted, and measured under a dissecting microscope on the micrometer slip. Missing or immobile animals were considered dead. The results obtained in the treatments were compared to the reference sediment.

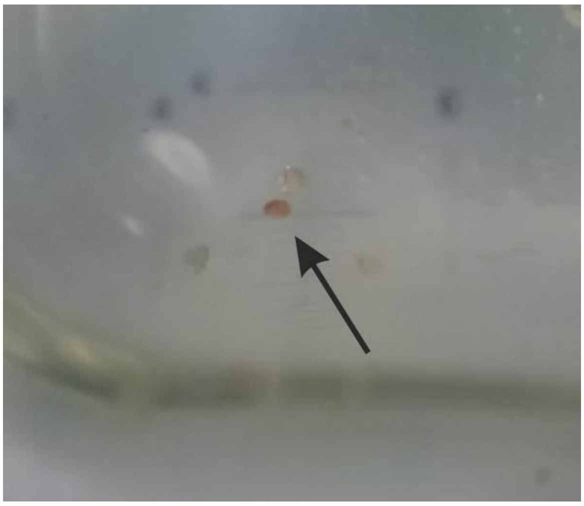

Fig. 2. The microscope photograph with an arrow pointing to a neonate of *H. incongruens*.

The survival (S) in each replicate was determined according to the following formula and expressed as a percentage:

$$\mathrm{S}\ (\%) = \frac{N_f}{N_i} \times 100$$

where N_f was the number of surviving ostracods, and N_i was the initial number of ostracods. The results were presented as the mean survival per sediment treatment.

To assess the growth effect, the length of 10 randomly chosen ostracods was measured under the microscope at the start of the test, and their mean initial length was calculated ($\overline{\mathrm{L_i}}$). The length of each surviving individual was determined at the end of exposure (day 6), and the average final length was calculated per each tested sediment and control ($\overline{\mathrm{L_f}}$). The mean growth ($\overline{\mathrm{G}}$) was calculated according to the following formula:

$$\overline{\mathrm{G}} = \left(\overline{\mathrm{L_f}} - \overline{\mathrm{L_i}}\right)$$

where $\overline{\mathrm{G}}$ is an increase in length (μm) during 6 days, L_f = final mean length (μm), L_i= initial mean length (μm).

4.6.3 The reference $CuSO_4$ test

The Ostracodtoxkit F™ (MicroBioTests, Gent, Belgium) recommends performing reference toxicity tests to assess the sensitivity of *H. incongruens* individuals. This reference test involves the exposure of *H. incongruens to* a range of concentrations of copper sulfate ($CuSO_4 \cdot 5H_20$). Five concentrations were prepared and tested with the use of the reference sediment provided by the manufacturer, i.e., 0, 1.0, 1.8, 3.2, 5.6, and 10 mg l^{-1}. Each concentration was tested in triplicate.

The morality of *H. incongruens* was calculated for each test vessel following the formula:

$$\mathrm{M}\ (\%) = (1 - \frac{N_f}{N_i} \times 100)$$

where M (%) is the percentage mortality of ostracods, N_f is the number of surviving ostracods, and N_i is the initial number of ostracods. The mortality resulting from the $CuSO_4$ exposures was corrected for mortality that occurred in the controls according to Abbott's formula:

$$\bar{p}_{corr.} = \frac{\bar{p}_{exp.} - \bar{p}_{cont.}}{1 - \bar{p}_{cont.}}$$

where $\bar{p}_{corr.}$ is corrected mortality, $\bar{p}_{exp.}$ is mean experimental treatment response, $\bar{p}_{cont.}$ is mean control response. As the requirements for the use of Probit analysis were not met, the LC_{50} values and associated confidence limits were calculated using the Trimmed Spearman-Karber method (Hamilton et al., 1977).

4.7 Investigated sediments

4.7.1 Collection of samples

as shown in Table 4 and Figure 7, there were 13 sites selected for sediment testing in the GoG area. The sediments were collected in July 2015. From each site, three sediment samples were collected with a Van Veen grab of which the uppermost 4 cm layer was taken for toxicity bioassays and analyses of sediment features and chemical contaminants. The bioassays were conducted on each of the three sediment samples taken from each site i.e., there were 3 bioassay replicates per site. Altogether, 15 samples of sediments were tested in triplicates including controls. Some sediment samples were tested against two amphipod species (separately), *C. volutator* and *C. multisetosum.*

In the study of the MW&WS sediments, five sampling sites were selected along the course of the rivers from the Kanał Wielki confluence with MW above the phosphogypsum heap towards the mouth of the Wisła Śmiała (Table 5, Figure 10). The sediments were collected from the middle cross-sections of the rivers in July 2016 with a Van Veen's grab from the board of a small research vessel. From each site, three sediment samples (the uppermost 4 cm layer) were collected for the bioassays, and the analysis of the grain size composition, organic matter content, and chemical contaminants concentration. They were placed in polyethylene bags and transported to the Gdynia Aquarium laboratory in thermostatic boxes equipped with ice. For the amphipod bioassays, the three sediment samples collected from each site were mixed to form one composite sample from which five replicates per site were made and tested.

4.7.2 The grain size composition and organic matter content

A part of each collected sediment sample was freeze-dried and stored pending analyses. The MW&SW sediment grain size fractions were analyzed using a mechanical shaker and a set of sieves of 63 μm, 125 μm, and 250 μm mesh sizes. The material retained on each sieve was transferred to separate containers and weighted. The percentage of each fraction was calculated. In the GoG sediments, only the share of 63 μm fraction was determined. The MW&SW sediment fractions were described using the Wentworth scale (Table 11).

Tab. 11. Wentworth scale of rock particle sizes.

Classification	Particle size (diameter)
Medium sand and coarser fractions	F > 250 μm
Fine sand	125 - 250 μm
Very fine sand	63 - 125 μm
Silt and clay	F < 63 μm

The organic matter content was determined in the dried bulk (un-sieved) sediments and the sediment fine fraction (F < 63 μm) as a loss on ignition (LOI, %) after burning the sediment samples in a muffle furnace at 560 °C for 12 h, according to the standard procedure used in the NMFRI chemical laboratory.

4.7.3 Analyses of chemical contaminants

The concentration of the hydrophobic organic contaminants (HOCs) in the sediments was analyzed by Dr. Ilona Waszak in the National Marine Fisheries Research Institute (NMFRI), Department of Food and Environmental Chemistry, according to the methods developed and applied in the NMFRI. Each sample was analyzed for the content of polychlorinated biphenyls (PCBs), organochlorine pesticides (OCP; a-, b-, g-HCH, HCB, heptachlor, DDT, and its derivatives), polybrominated diphenyl ethers (PBDE), and polyaromatic hydrocarbons (PAHs). PCB measurements included 7 congeners (CB-28, -52, -101, -118, -138, -153, -180). Σ_7PCB was calculated based on concentrations of individual congeners. DDT measurements included op-DDE, pp-DDE, op-DDD, pp-DDD, pp-DDT, which summed concentrations provided the total DDT content (ΣDDT). The PBDE analyses included 7 congeners (IUPAC No. 28, 47, 100, 99, 153, 154, 183), yet the presence of BDE 154 was not found in any sample, and their summed concentrations include 6 congeners (Σ_6PBDE). PAH analyses included 16 native compounds (USEPA priority PAHs) i.e., acenaphthene (Ace), acenaphthylene (Acy), anthracene (Ant), bezno[a]anthracene (B[a]A), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), Benzo[ghi]perylene (B[ghi]P), Benzo[a]pyrene (B[a]P), chrysene (Chr), dibenzo[a,h]anthracene (DB[ah]A), fluoranthene (Flt), fluorene (Flu), indeno[1,2,3-c,d]pyrene (I[cd]P), naphthalene (Naph), phenanthrene (Phe), and pyrene (Pyr). The PCB, OCP, and PBDE analyses were performed using high-performance capillary gas chromatography (HRGC) i.e., GC 6890N apparatus coupled with μ-ECD G1530N (Agilent), autosampler 7683B, and a DB-

5 chromatography column (60 m, 0.25 mm, 0.1μm; J & W Scientific, CA, USA). PAHs were analyzed using a gas chromatograph (GC 6890N) and a mass spectrometer (MS 5975C) operating in SIM (Selected Ion Monitoring) mode, equipped with a DB-5MS column (30 m, 0.25 mm, 0.25 μm).

4.8 Benthic fauna in the Martwa and Śmiała Wisła

4.8.1 Analyses of the river's abiotic conditions

The samples of the near-bottom water were collected with a bathometer at each site. On-site, the temperature, salinity, and oxygen concentration in the bottom water were measured using a salinometer and an oxygen meter. The collected water samples were analyzed in the laboratory for pH and the content of phosphates (PO_4^{3-}) with the use of a spectrophotometer Hach DR 3900 according to the procedures provided by the Hach Company.

4.8.2 Collection of samples

The macrobenthos samples were collected in triplicate from each site at the same time as the sediment samples for bioassays and chemical work. The whole content of the Van Veen grab was sieved initially through a 1mm mesh screen aboard the research vessel, however, this mesh screen was not effective in retaining the fauna. Therefore, a 500 μm mesh screen sieve was applied instead. The fauna that remained on the 500 μm mesh screen was transferred to 0.5L jars and fixed with 4 % buffered formaldehyde solution. The jars were then gently rotated to ensure proper fixation of the organisms.

4.8.3 Analyses of macrobenthos

In the laboratory, the collected macrobenthos samples were sorted using a dissecting microscope and preserved in a 4 % formaldehyde-buffered solution. The macrobenthos individuals were then classified to the lowest possible taxonomic level and counted. The total density and densities of each identified taxon were determined for each sample and expressed as the number of individuals per $1m^2$. The following analyses were conducted with the use of the Primer 6+ software. This software and the methods it offers are designed to handle ecological data in a non-parametric, rank-based approach. This is beneficial for the ecological data that tends to be too overdispersed for parametric analysis (Anderson et al., 2008).

The macrobenthos indices i.e., Margalef index, Shannon index, Pielou's evenness, and Simpson index, were calculated for each site. The formulas used for calculation were as follows:

$$\text{Species richness (Margalef): } d = \frac{S-1}{\ln n}$$

where S is the total number of taxa, and n is the total number of individuals.

Shannon diversity index accounts for both the abundance and evenness of the taxa present.

$$\text{Shannon: } H' = -\Sigma\, p_i \ln(p_i)$$

where p_i is the proportion of observations per taxon relative to all specimens at a site.

Pielou's evenness is a measure of the relative abundance of different taxa contributing to the richness of an area. The greater its value the more evenly individuals are distributed between measured groups.

$$\text{Pielou's evenness: } J' = \frac{H'}{\log(S)}$$

where H' is Shannon index value and S is the total number of taxa.

Simpson's index of diversity $(1 - \lambda)$ measures the probability that two individuals randomly selected from a sample would belong to different taxa. The value of this index ranges between 0 and 1, and the greater the value, the greater the sample diversity. The formula for this index is:

$$\text{Simpson: } 1 - \lambda = 1 - \sum \frac{Ni \times (Ni - 1)}{N \times (N - 1)}$$

where N_i is the total number of organisms of a taxon and N is the total number of organisms of all taxa.

The macrobenthos data was used for evaluating the ecological quality status of the MW&WS area by calculating a multimetric index (B). The index is based on quantitative measurements of species abundance and taxonomical richness and qualitative information on the ecological tolerance/sensitivity of particular taxa (Osowiecki et al., 2012). The B index was calculated according to the formula used by Osowiecki et al. (2012):

$$B = \frac{\sum_{i=1}^{3}(Q_i w_i sens_i)}{\sum_{i=1}^{3} D_i} \cdot \log(1 + \sum_{i=1}^{3} D_i)$$

where D_i – number of taxa in a particular abundance dominance class (D1 > 10 % contribution in abundance; 5 % ≤ D2 ≤10 %; D3 taxa of < 5 %); Q_i – coefficient (Qi = 0 if Di = 0 and Qi = 1 if Di ≠ 0); w_i – the weight of the dominance class (w1 = 3 for D1, w2 = 2 for D2, w3 = 1 for D3); sensi – tolerance/sensitivity (3 – sensitive taxa, 2 – semi-sensitive taxa, 1 – tolerant taxa). The taxa were divided into three groups according to their contribution to samples: dominants, influents, and accessory species if they contributed >10, 5 - 10, and <5 % to total abundance, respectively. Weights were assigned to these groups, as follows: weight 3 – dominants (D1), weight 2 – influents (D2), and weight 1 – accessory species (D3). Each taxon was assigned a sensitivity level (sensi) describing its response to changes in bottom environmental conditions, i.e., sensi 1 – tolerant taxa, sensi 2 – semi-sensitive taxa, sensi 3 – sensitive taxa (Osowiecki et al., 2012). The sensitivity of taxons was assigned based on the literature.

4.9 Statistical analysis and data evaluation

The statistical analysis was performed with the use of Statistica 10.0 software, according to the scheme presented in Figure 22. The variables were first examined with the Shapiro-Wilk test for normal distribution and Levene's test for homogeneity of variance. The variables that did not fulfill assumptions necessary for parametric analysis were subjected to mathematical transformations, such as log10, $\log_e$, square root, arcsin, Box-Cox transform, or raising to different powers. If the assumptions were not fulfilled even after the transformation the data were then analyzed with the Kruskal-Wallis test. Otherwise, one-way ANOVA with post-hoc Tukey HSD was used to examine the significance of differences among the variables (or sites).

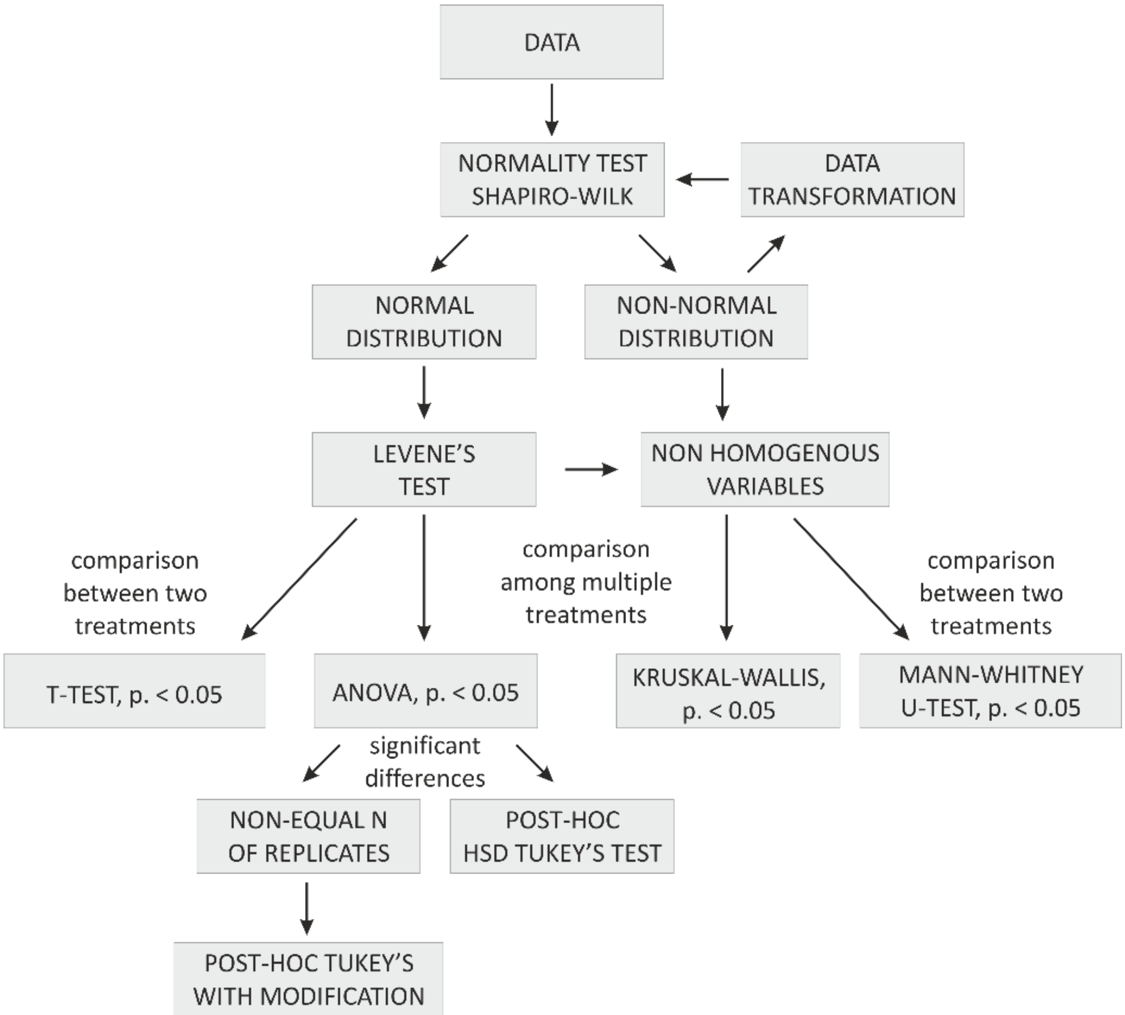

Fig. 22. The approach to the statistical analysis between multiple treatments based on USEPA (1994).

Pairwise comparisons were performed using a t-test ($p < .05$) in cases where the normality and variance homogeneity of the data were confirmed[8]. Otherwise, the Mann-Whitney U test was applied at $p < .05$.

The coefficients of variation (CV) for the biological responses of the sediment-exposed amphipods were calculated for within- and among-treatment responses according to the formulae:

$$\mathrm{CV}\ (\%) = \frac{SD}{\bar{X}} \times 100\ \%$$

where SD is the standard deviation of the grand mean of a specific response, and $\bar{X}$ is the grand mean of the respective response.

Principal Component Analysis (PCA) was applied to explore the relationships between sediment characteristics and bioassays biological endpoints. The analysis was performed on standardized data, which ensured that the use of different units had no influence on the outcomes and that no null values were included in the analysis. In some cases, the associations between sediment features, contaminants, and biological endpoints were also evaluated using Kendall's tau[9] correlation analysis ($p < .05$).

Analysis of similarity (ANOSIM) was performed to examine similarities in macrobenthos community structure, sediment features, and bottom water characteristics among the MW&WS sites. ANOSIM provides a way to test statistically whether there is a significant difference between two or more groups of sampling units. If two groups of sampling units are different in their species composition, then compositional dissimilarities between the groups ought to be greater than those within the groups. The R-statistic in ANOSIM is the ratio between within-group and between-group dissimilarities. It yields the R-value with a maximum of 1. The closer the value is to 1, the more there is similarity within the group and dissimilarity to other groups (Clarke and Warwick, 2001). The DistLM procedure (Distance-based Linear Modeling) in PERMANOVA was employed to investigate how macrobenthos densities relate to certain environmental variables. This analysis aimed to understand the ecological factors influencing the distribution and composition of macrobenthic communities in the MW&WS area. The gathered data, including the macrobenthos densities and the environmental variables (depth, water salinity, oxygen, pH, phosphates, OM_w, OM_f, sediment fractions), were introduced to the

[8] the parametric t-test was applied in some cases, despite that some data sets were very small (n ranged 3-6). The use of the parametric test, in this case, was based on the judgment of Winter (2013), who claimed that although large datasets are always preferred but not always feasible, the t-test can be applied if the effect size is expected to be large. In such cases, a t-test could be used for unequal variances, skewed distributions and extremely low quantity of samples, although with caution.

[9] Kendall's tau is an extension of Spearman's Rho designed for the non-normally distributed data, and according to some authors can draw more accurate results compared to Spearman (Akoglu, 2018).

Primer 6+ software. Based on the resemblance matrix of biological data, the DistLM analysis involved the following steps: 1) the environmental data, transformed by the 4th root and normalized, was chosen as the predictor variables worksheet; 2) the stepwise selection procedure was chosen; 3) the BEST procedure was chosen as the selection criterion; 4) marginal tests were performed; 5) dbRDA plot was created along with the analysis (the chosen number of permutations was 999).

The BEST procedure is a powerful tool that examines the value of the selection criterion for all possible combinations of predictor variables. The common distance metrics were based on resemblance by Bray-Curtis similarity, which is standard procedure in biological samples. Marginal tests were performed to identify significant correlations in biota and each of the environmental variables. The results of DistLM were visualized through an ordination plot.

4.10 Sediment quality evaluation

To evaluate the GoG sediments three responses of *Corophium* were taken into account i.e., survival, growth, and emergence. Each response was evaluated on a four-grade scale and a score was assigned to each grade. The grading of survival was based on an approach presented by Berezina et al. (2013) i.e., 90 - 100 % - *very good* (score of 2), 70 - 89 % - *good* (score of 1.5), 50 - 69 % - *satisfactory* (score of 1.0), < 50 % - *poor* (score of 0.5). The grading of GR and emergence was arbitrary based on expert judgment. For GR it was: *very low* (< 4 μm day^{-1}, score of 0.5), *low (*4 - 12 μm day^{-1}, score of 1.0), *moderate* (18 - 28 μm day^{-1}, score of 1.5), and *high* (36 - 58 μm day^{-1}, score of 2.0). The following grading for emergence was used: *absent* if no emergence occurred (score of 1), *low* if < 0.6 (score of 0.7), *increased* if 0.6 - 0.9 (score of 0.3), and *high* if 1.0 - 1.6 (score of 0.0). The sum of the graded responses, reflecting the overall performance of the amphipods, was used to form a five-grade impact scale upon which the sediments were evaluated.

5 Results

5.1 Sediments of the Gulf of Gdańsk

5.1.1 Environmental parameters

The data on the environmental parameters at the Gulf of Gdańsk (GoG) sites was collected during the sediment sampling by the NMFRI employees (Table 12). Most of the sediments were collected at a relatively small depth (10 - 14 m), and the deepest sites were located in the middle of the GoG, namely WS, X1, X2, and 395 (27, 30, 52, and 66 m, respectively). The salinity ranged from 6.4 to 7.7. The Secchi depth was the smallest at PGDA2 (0.9 m) and the greatest at PGDY3 (4.7 m). The temperature ranged from 13 to 19 °C and was lowest at the deepest sites (395, X2).

Tab. 12. Environmental parameters of the GoG sediment collection sites.

Site	Depth m	Salinity	Secchi depth m	Temperature °C
PGDY1	11.5	7.3	1.8	17.6
PGDY2	11.5	7.2	2.2	17.7
PGDY3	11.5	7.4	4.7	17.8
PGDA1	11.5	6.8	1.3	19.0
PGDA2	10.0	6.4	0.9	19.0
PGDA3	11.0	6.7	3.2	18.7
E57	13.0	6.6	2.5	18.9
WS	27.0	7.3	2.3	16.9
ZN2	14.0	7.1	2.5	16.9
X1	30.0	7.0	2.1	17.0
X2	52.0	7.4	2.1	14.7
395	66.0	7.7	3.2	13.0
X7	13.0	6.7	1.7	17.5

5.1.2 Characteristics of sediments

The characteristics of the GoG sediments i.e., the fine fraction (F < 63 μm), organic matter content in the fines (OM_f), organic matter in the whole sediment (OM_w), as well as the content of hydrophobic organic contaminants (HOCs), varied markedly among the sites (Table 13, Figure 23). The characteristics of the two CTR sediments were similar, thus their mean values were considered in this data evaluation.

The CTR sediments contained a small amount of the fines and OM_w and a moderate amount of OM_f (the mean values were 2.5, 0.7, and 8.1 %, respectively) in comparison to the tested sediments. Specifically, the CTR sediment from Rzucewo contained 2.7 % of the fines, 0.9 % of OM_w, and 8.4 % of OM_f, while that from Kaczy Winkiel was 2.5 %, 0.6 %, and 7.8 %, respectively.

The mean content of the fine fraction in the tested sediments was in the range of 0.2 - 31.0 %, with the greatest level in the PGDY1 (31.0 %) and 395 sites (26.6 %), and the lowest in the E57 (0.2 %) and PGDA3 sediments (0.2 %). The mean OM_w content was in the range of 0.2 - 12.0 % showing the greatest levels in the PGDA2, 395, and PGDY1 sites, and the lowest in E57. The mean OM_f content was in the range of 4.6 - 15.1 %. Generally, the sediments within the ports (PGDY1, PGDY2, PGDA1, and PGDA2) and the deepest sites close to the open sea (X2 and 395) were characterized by the greatest percentage of the fines and OM_w. These sediments showed also the highest levels of contaminants, except for the X2 site. In contrast, sediments characterized by a low content of OM_w and the fines contained much lower contaminant concentrations. These were the PGDY3, PGDA3, ZN2, X1, X7, WS, and E57 sediments. The amount of organic contaminants in CTR sediment was negligible (Table 13).

Spatial differences in the contaminant concentrations are graphically presented in Figure 23. To give an idea of the inter-site differences in contaminant levels, the max/min concentration ratios were calculated. These ratios for $\sum_{16}$PAHs, $\sum_{7}$PCBs, HCB, $\sum$DDTs, and $\sum_{6}$PBDEs were 4207, 4030, 1810, 311, and 80, respectively. Quantitatively at each location dominated $\sum_{16}$PAHs (8 ng g^{-1} d.m. to 33.7 µg g^{-1} d.m.) with the highest level in PGDA2, whereas $\sum_{6}$PBDEs was the lowest (< 0.01 to 0.8 ng g^{-1} d.m.) showing the highest level also in PGDA2.

Of the measured contaminants, selected compounds were assessed against environmental quality criteria i.e., those for which either EAC (Environmental Assessment Criterion) or ERL (Environmental Range Low) have been established. The concentrations of assessed contaminants and respective EACs and ERLs have been shown in Table 14. The criteria were exceeded in four sites i.e., PGDY1 by 4 contaminants (PCB 101, 118, 153, and p,p-DDE), PGDY2 by 2 contaminants (PCB 118 and p,p-DDE), PGDA2 by 3 contaminants (PCB 118, p,p-DDE, and $\sum_{16}$PAHs), and in the 395 site by 2 contaminants (p,p-DDE and $\sum_{16}$PAHs). It should be noted that ERLs for $\sum_{16}$PAHs and p,p-DDE in PGDA2 were exceeded 10-fold.

Tab. 13. The fines, OM content, and the concentrations of organic contaminants in the GoG sediments (mean values ± SD).

Site	Sediment	F < 63 µm [1] %	OM_w [2] %	OM_f [3] %	$\sum$DDTs [4] ng g^{-1} d.m.	$\sum_7$PCBs [5] ng g^{-1} d.m.	$\sum_{16}$PAHs [6] ng g^{-1} d.m.	HCB [7] ng g^{-1} d.m.	$\sum_6$PBDEs [8] ng g^{-1} d.m.
Bay of Puck	CTR	2.6 ± 0.2 ^{ab}	0.7 ± 0.2 ^{ab}	8.1 ± 0.6 ^{ab}	0.1 ± 0.0 ^{ab}	0.3 ± 0.3 ^{ab}	16 ± 1 ^{d}	0.01 ± 0.0 ^{a}	0.01 ± 0.0 ^{a}
Port of Gdynia	PGDY1	31.0 ± 1.7 ^{b}	10.4 ± 0.3 ^{ab}	11.1 ± 0.2 ^{ab}	21.0 ± 7.9 ^{ab}	40.3 ± 4.9 ^{b}	1248 ± 7 ^{ab}	0.19 ± 0.1 ^{ab}	nd [11]
	PGDY2	10.7 ± 5.2 ^{ab}	2.9 ± 0.8 ^{ab}	9.6 ± 0.9 ^{ab}	29.4 ± 19.7 ^{b}	15.3 ± 6.5 ^{ab}	1725 ± 872 ^{ab}	0.4 ± 0.3 ^{ab}	0.3 ± 0.06 ^{b}
	PGDY3	0.9 ± 0.1 ^{ab}	0.6 ± 0.0 ^{ab}	7.8 ± 0.6 ^{ab}	0.1 ± 0.0 ^{ab}	0.2 ± 0.0 ^{ab}	3130 ± 5336 ^{ab}	0.06 ± 0.03 ^{ab}	0.01 ± 0.0 ^{ab}
Port of Gdańsk	PGDA1	8.6 ± 2.2 ^{ab}	2.4 ± 0.8 ^{ab}	8.2 ± 1.6 ^{ab}	1.9 ± 1.4 ^{ab}	3.1 ± 3.5 ^{ab}	2215 ± 1374 ^{ab}	6.9 ± 5.2 ^{bc}	0.3 ± 0.2 ^{ab}
	PGDA2	19.5 ± 2.1 ^{ab}	12.0 ± 1.0 ^{b}	13.0 ± 0.1 ^{b}	31.1 ± 6.7 ^{b}	15.8 ± 0.3 ^{b}	33655 ± 2863 ^{b}	18.1 ± 3.2 ^{b}	0.8 ± 0.02 ^{b}
	PGDA3	0.2 ± 0.1 ^{a}	0.6 ± 0.1 ^{ab}	11.4 ± 1.6 ^{ab}	0.1 ± 0.0 ^{ab}	0.1 ± 0.0 ^{ab}	39 ± 20 ^{c}	0.01 ± 0.0 ^{ac}	0.01 ± 0.0 ^{ab}
Wisła mouth ↓ [9]	ZN2	3.3 ± 0.4 ^{ab}	1.3 ± 0.1 ^{ab}	15.1 ± 1.4 ^{b}	0.8 ± 0.1 ^{ab}	0.3 ± 0.0 ^{ab}	166 ± 25 ^{a}	0.2 ± 0.04 ^{ab}	0.03 ± 0.0 ^{ab}
	X1	3.3 ± 0.3 ^{ab}	1.2 ± 0.1 ^{ab}	9.3 ± 0.1 ^{ab}	1.0 ± 0.2 ^{ab}	0.4 ± 0.1 ^{ab}	228 ± 28 ^{a}	0.1 ± 0.04 ^{ab}	0.05 ± 0.01 ^{ab}
	X2	19.8 ± 3.3 ^{ab}	3.0 ± 0.4 ^{ab}	4.6 ± 0.2 ^{a}	0.5 ± 0.2 ^{ab}	0.2 ± 0.1 ^{ab}	318 ± 46 ^{a}	0.04 ± 0.02 ^{ab}	0.04 ± 0.01 ^{ab}
	395	26.6 ± 0.7 ^{b}	10.6 ± 0.1 ^{b}	8.9 ± 0.2 ^{ab}	23.5 ± 12.0 ^{ab}	2.9 ± 0.8 ^{ab}	5024 ± 933 ^{ab}	0.5 ± 0.2 ^{ab}	0.2 ± 0.07 ^{ab}
GoG [10]	E57	0.2 ± 0.1 ^{a}	0.2 ± 0.0 ^{a}	9.0 ± 1.3 ^{ab}	0.1 ± 0.0 ^{a}	0.01 ± 0.0 ^{a}	8 ± 1 ^{d}	0.06 ± 0.04 ^{ab}	0.01 ± 0.0 ^{ab}
	X7	0.8 ± 0.1 ^{ab}	1.0 ± 0.0 ^{ab}	7.5 ± 0.1 ^{ab}	0.2 ± 0.0 ^{ab}	0.1 ± 0.0 ^{ab}	36 ± 8 ^{c}	0.03 ± 0.01 ^{ab}	0.01 ± 0.0 ^{ab}
	WS	1.6 ± 0.3 ^{ab}	0.6 ± 0.1 ^{ab}	9.4 ± 0.7 ^{ab}	0.2 ± 0.1 ^{ab}	0.2 ± 0.0 ^{ab}	122 ± 90 ^{ac}	0.02 ± 0.02 ^{ab}	0.02 ± 0.0 ^{ab}

[1] sediment grain size fraction less than 63 µm (F < 63 µm); [2] OM in whole sediment; [3] OM in F < 63 µm; [4 - 8] concen tration of organic contaminants; [9] ↓ - indicates the outward direction from the Wisła River mouth (ZN2 site) towards the open sea; [10] GoG - indicates other sites within the Gulf of Gdańsk; [11] nd – not determined. Different letters within a column indicate significant differences among the sites (Kruskal-Wallis and multiple comparisons on ranks, p < .05).

Tab. 14. Concentrations of contaminants in the GoG sediments (mean values, ng g^{-1} d.m.) evaluated against environmental assessment criteria (EAC, ERL).

HOC	PGDY1	PGDY2	PGDY3	PGDA1	PGDA2	PGDA3	ZN2	X1	X2	395	E57	X7	WS	
														EAC [a]
CB 28	0.14	0.12	0.01	0.15	0.74	0.01	0.02	0.04	0.02	0.22	0.004	0.01	0.01	1.7
CB 52	2.51	0.64	0.01	0.13	0.76	0.01	0.02	0.02	0.01	0.24	0.002	0.01	0.01	2.7
CB 101	5.64	1.85	0.02	0.33	0.92	0.01	0.03	0.03	0.02	0.33	0.003	0.01	0.02	3.0
CB 118	6.45	1.76	0.02	0.35	1.89	0.01	0.03	0.04	0.03	0.26	0.003	0.01	0.02	0.6
CB 153	9.57	4.64	0.05	1.00	6.26	0.02	0.07	0.09	0.08	0.79	0.01	0.02	0.04	7.9
CB 138	11.72	4.40	0.04	0.63	2.41	0.02	0.05	0.07	0.05	0.65	0.01	0.02	0.03	40
CB 180	4.28	1.93	0.02	0.55	2.82	0.01	0.03	0.05	0.03	0.37	0.004	0.01	0.02	12
$\sum_6$PBDEs	nd	0.33	0.01	0.27	0.79	0.01	0.03	0.05	0.04	0.20	0.01	0.01	0.02	310 [c]
														ERL [b]
HCB	0.19	0.4	0.06	6.9	18.1	0.01	0.2	0.1	0.04	0.5	0.06	0.03	0.02	20
p,p-DDE	12.4	4.96	0.04	1.33	25.6	0.04	0.16	0.19	0.11	7.27	0.02	0.05	0.07	2.2
$\sum_{16}$PAHs	1248	1725	3130	2215	33655	39	166	228	318	5024	7.5	36	122	3340

[a] EAC (Environmental Assessment Criterion) and [b] ERL (Effects Range Low) values from OSPAR (2009a, 2009b); [c] EAC from ICES (2012).
The bold values show the contaminants that exceeded the EAC or ERL.

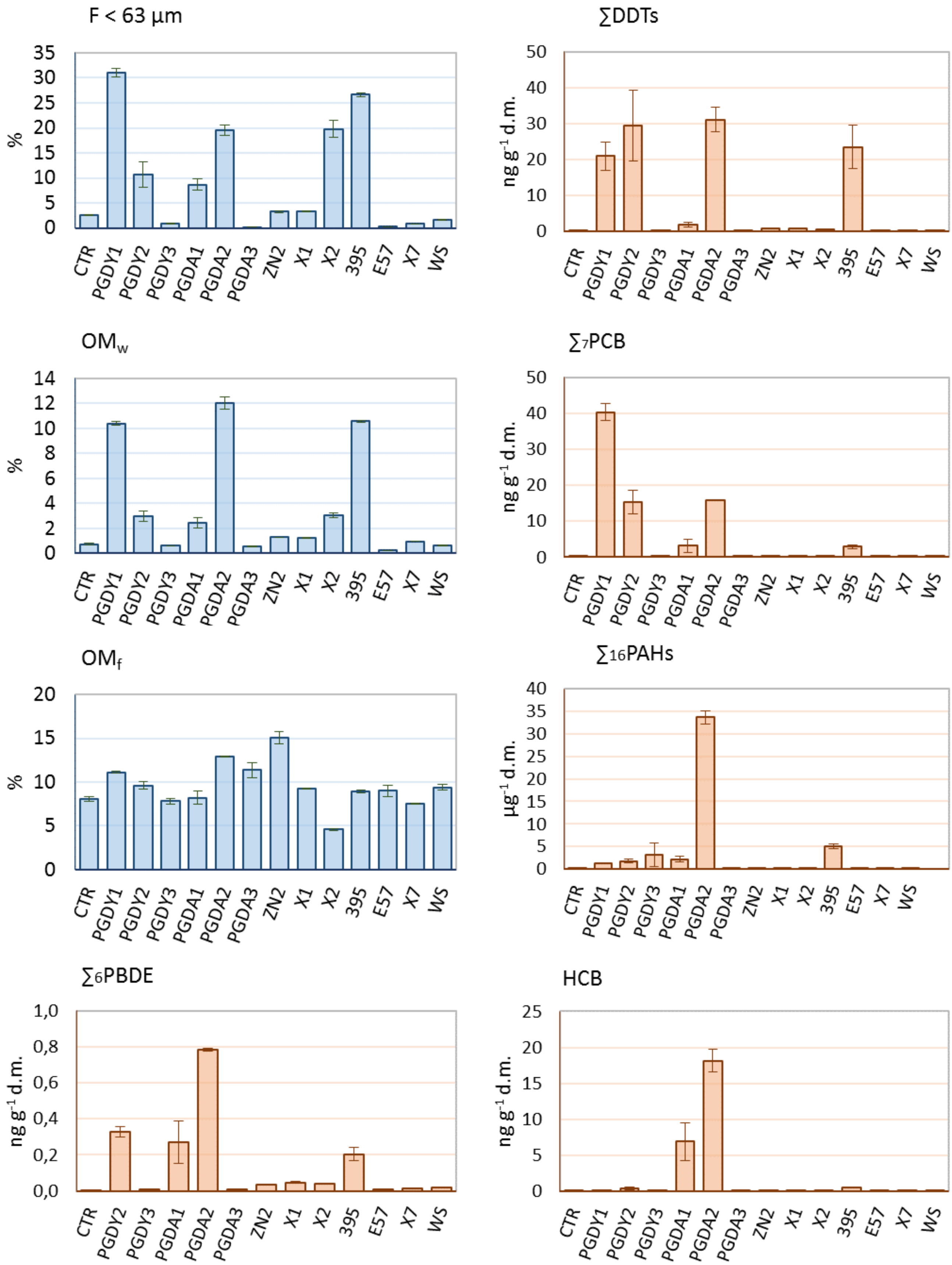

Fig. 23. Content of the fines, OM, and the contaminants in the GoG sediments (mean values ± SD).

PCA, performed to examine the relations between the contaminant concentrations and natural properties of sediments, produced three Principal Components (PCs) with eigenvalues greater or close to 1 that accounted for 90.2 % of the total variance (Table 15). PC1 revealed strong positive relations among the organic contaminants, OM_w and the fines (PC1, 61.9 % of the total variance), and only a moderate association of contaminants with OM_f. OM_f had a highly significant loading extracted with a separate PC (PC3, 10.8 % of the total variance). It is noteworthy, that some contaminants (HCB, Σ_{16}PAHs, Σ_7PCB) and the fines had second loadings extracted with PC2 (17.4 % of the total variance). This might indicate that the characteristics of the fines could play a role in contaminant distribution.

Graphical presentation of the PCA results is shown in Figure 24a and Figure 24b. The sites where the ERL/EAC values for the sediment contaminants were exceeded i.e., PGDY1, PGDY2, and 395 (marked with red) were separated from all other sites and positioned at the left side of the graph. Also, the PGDA2 samples stood out being grouped at the negative side of the PC1 and PC2 axis far from all other sediment samples as they had the greatest levels of $\sum_{16}$PAHs, ∑DDTs, HCB, and $\sum_6$PBDE.

Tab. 15. PCA statistics showing the associations among the sediment fines, OM_w, OM_f, and organic contaminants.

Principal Components	PC1	PC2	PC3
Eigenvalues	5.0	1.4	0.9
Variance explained (90.2 %)	61.9	17.4	10.8
HCB	-0.751	-0.572	0.235
∑DDTs	-0.853	0.217	-0.026
$\sum_{16}$PAHs	-0.808	-0.494	0.217
$\sum_7$PCBs	-0.718	0.476	-0.31
$\sum_6$PBDEs	-0.944	-0.179	0.066
OM_f	-0.416	-0.396	-0.799
OM_w	-0.922	0.23	0.065
The fines	-0.761	0.556	0.135

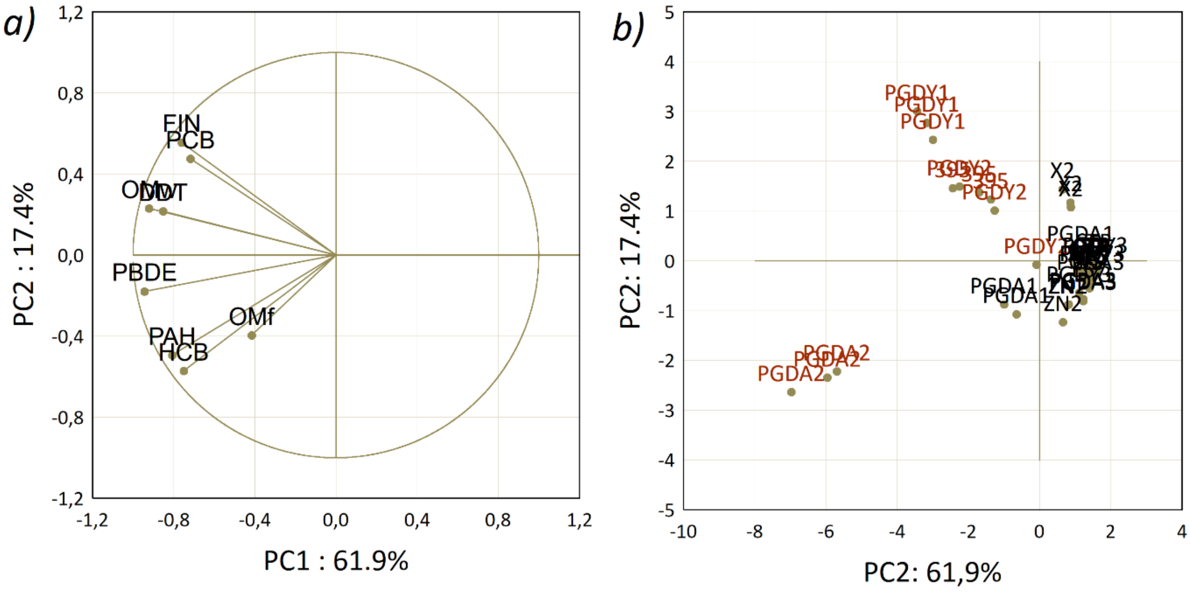

Fig. 24. PCA statistics showing the associations among the GoG sediment characteristics and contaminants: a) relationships among the variables and b) the distribution of the sites on the factor-plane. The sites marked in red exceeded some EAC/ERL values. FIN stands for fines (variables are presented in Table 15).

5.1.3 Bioassays with *Corophium* spp.

5.1.3.1 *Physico-chemical parameters of the overlying water in test vessels*

The physicochemical parameters of the water overlying the tested sediments (Table 16, Table 17) were within ranges recommended for bioassays with the used amphipod species (Postma et al., 2002; Stronkhorst, 2003). The mean values for the parameters were as follows: temperature 15.1 ± 0.2 °C, pH 7.7 ± 0.1, oxygen 8.8 ± 0.1 mg l^{-1}, and salinity 7.3 ± 0.1. The salinity was similar to that in the natural habitat of amphipods from which they were collected (7.2 - 7.5). The levels of ammonia (NH_3), phosphate ions (PO_4^{3-}), and nitrite and nitrate ions (NO^{2-}, NO^{3-}) in the water of the experimental system and the test vessels were negligible (Table 17).

Tab. 16. Properties of water in the test vessels (mean values ± SD).

Species	Sediment	Temperature °C	pH	Oxygen mg l^{-1}	Salinity
C. volutator	CTR	15.2 ± 0.0	7.6 ± 0.0	8.9 ± 0.0	7.2 ± 0.0
C. multisetosum	CTR	15.0 ± 0.0	7.6 ± 0.0	8.9 ± 0.0	7.4 ± 0.1
C. volutator	PGDY1	15.1 ± 0.1	7.7 ± 0.0	8.7 ± 0.1	7.2 ± 0.0
C. volutator	PGDY2	15.1 ± 0.0	7.7 ± 0.0	8.8 ± 0.0	7.2 ± 0.0
C. multisetosum	PGDY2	15.2 ± 0.0	7.6 ± 0.0	8.7 ± 0.2	7.5 ± 0.1
C. multisetosum	PGDY3	15.2 ± 0.0	7.6 ± 0.0	8.8 ± 0.0	7.5 ± 0.0
C. multisetosum	PGDA1	15.3 ± 0.1	7.7 ± 0.1	8.6 ± 0.0	7.4 ± 0.0
C. multisetosum	PGDA2	15.2 ± 0.0	7.7 ± 0.0	8.7 ± 0.1	7.4 ± 0.0
C. multisetosum	PGDA3	15.1 ± 0.0	7.7 ± 0.0	8.9 ± 0.0	7.4 ± 0.0
C. volutator	ZN2	15.3 ± 0.0	7.8 ± 0.0	8.7 ± 0.1	7.5 ± 0.0
C. multisetosum	ZN2	15.2 ± 0.1	7.8 ± 0.0	8.8 ± 0.1	7.2 ± 0.0
C. multisetosum	X1	14.8 ± 0.0	7.7 ± 0.0	9.0 ± 0.0	7.2 ± 0.0
C. multisetosum	X2	14.8 ± 0.0	7.6 ± 0.0	8.9 ± 0.0	7.5 ± 0.0
C. multisetosum	395	15.0 ± 0.0	7.7 ± 0.0	8.9 ± 0.1	7.3 ± 0.1
C. volutator	E57	15.1 ± 0.0	7.6 ± 0.0	8.9 ± 0.0	7.2 ± 0.0
C. multisetosum	X7	14.8 ± 0.1	7.6 ± 0.0	8.9 ± 0.2	7.3 ± 0.0
C. multisetosum	WS	15.0 ± 0.0	7.7 ± 0.0	8.9 ± 0.0	7.4 ± 0.0

Tab. 17. Ammonia, phosphates, nitrites, and nitrates in the water of the testing system (mean values ± SD).

Parameter	NH_3^+, mg l^{-1}	NO^{2-}, mg l^{-1}	NO^{3-}, mg l^{-1}	PO_4^{3-}, mg l^{-1}
Mean ± SD	0.02 ± 0.02	0.02 ± 0.02	2.6 ± 0.1	4.6 ± 0.2

5.1.3.2 *Corophium spp. responses*

As indicated in the Materials and Methods section (section 4.1), two species were used in the bioassays testing the GoG sediments, namely *C. volutator* and *C. multisetosum*. Two approaches were applied for the evaluation of the results. The first approach included the combined response data for the two species, which was justified by the fact that pairwise comparisons of the two species' responses exposed to the same sediments showed no significant differences. The second approach involved the evaluation of the results separately for each species.

The outcomes of bioassays for the two species combined are shown in Table 18 and Figure 25. The mean survival ranged from 55 % to 100 % (PGDY1 and PGDA3 respectively). In two sediments (PGDA3, PGDY3) the mean survival was greater (100 %, 97 %) than in CTR (90 %). In other sediments, it was equal to or lower than 70 % i.e., in groups exposed to the X1, X2, PGDY1, PGDY2, and E57 sediments. The Kruskal-Wallis test found no statistically significant differences in amphipod survival among the sediment treatments. One-tailed Mann-Whitney U tests indicated a significant difference between CTR and two sediments, namely the survival in PGDY1 ($p = .024$) and PGDY2 ($p = .047$) was lower than in CTR. The survival in the port-collected sediments (PGDY1, PGDY2, PGDA1, PGDA2) compared to all other GoG sediments was significantly lower (one-tailed Mann-Whitney U test, $p = .012$). The survival's mean coefficient of variation within treatments (mean CV_{wt}) was 26 % and the variability of the survival among the treatments (CV_{at}) was 18 %.

The mean growth rate (GR) was calculated for both genders together as well as for females only. No significant difference was found between these two data sets (two-tailed t-test on square-root transformed variables), hence GR calculated for all amphipods has been presented. The mean individual length increment during the 28 days was in the range of 0.1 - 1.6 mm, which corresponded to GR of 2 (PGDY1, PGDY3) to 58 (ZN2) $\mu m\ d^{-1}$ (Table 18). The mean GR in CTR was $12 \pm 12\ \mu m\ d^{-1}$. GR less than that of CTR was observed in all port sediments (inner and outer port areas, except PGDY2), however, the difference was not significant (Mann Whitney U test). GR in these sediments was significantly less than that in ZN2 (one-way ANOVA, $p < .001$, and post hoc HSD Tukey test). Furthermore, GR in PGDY1 and PGDY3 was significantly less than in all sites other than ports. In addition, evaluating the effect of site location i.e., inner port sites versus all other GoG sites, and the gradient of distance from the Vistula River mouth versus all other sites outside the ports, it was found that GR was significantly less in the sediments collected from the ports (PGDY1, PGDY2, PGDA1, PGDA2) than that in sediments from all other GoG sites (one-tailed Mann-Whitney U test, $p < .05$), and that there were no significant differences between the sediments collected along the gradient of distance from the Vistula River mouth (the ZN2, X1, X2, 395 sediments) and the areas outside the ports (the E57, X7, WS sediments). Pairwise comparisons between the tested sediments and CTR indicated only one significant difference i.e., significantly greater GR in ZN2 than in CTR (one-tailed Mann-Whitney U test, $p = .002$). The mean CV_{wt} and CV_{at} for GR were 79 % and 83 %, respectively.

Considering the GR differences among the groups, the amphipod growth could be classified into four classes: very low (< 4 μm d^{-1}; PGDY1, PGDY3), low (4 - 12 μm d^{-1}; PGDA1, PGDA2, PGDA3, PGDY2, and CTR), moderate (18 - 28 μm d^{-1}; X1, X7, X2, 395, WS), and high (36 - 58 μm d^{-1}; E57, ZN2).

The mean number of molts per individual collected during the bioassays was in the range from 0.4 (X7, WS) to 1.3 (E57). According to one-way ANOVA, it did not differ significantly among the sediment exposures ($p < .05$). However, pairwise comparisons with CTR showed significantly lower molting frequency in PGDY1, PGDA2, X1, X2, 395, and WS than in CTR (one-tailed Mann-Whitney U tests, $p < .05$). Molting in the port sediments (PGDY1, PGDY2, PGDA1, PGDA2) did not differ significantly from that in all other GoG sediments (two-tailed t-test, $p < .05$). Considering all tested sediments, an individual amphipod molted less than once on average (0.8 molts $ind.^{-1}$). The molting mean CV_{wt} was 79 % and CV_{at} was 40 %.

Emergence, which is indicative of sediment avoidance, was not observed in 5 out of 14 sediment exposures, namely in the PGDY3, PGDA3, X1, 395, and WS sediments. In CTR the emergence was very low (0.1 $ind.^{-1}$). Most frequently it was observed in the PGDY1 sediment (1.6 $ind.^{-1}$), followed by PGDY2, PGDA2, and E57 (0.6, 0.7, and 0.7 $ind.^{-1}$, respectively). Pairwise comparisons between the tested sediments and CTR indicated significantly greater emergence in PGDY1 ($p = .012$), PGDA2 ($p = .012$), and E57 ($p = .024$) than in CTR (one-tailed Mann-Whitney U tests, $p < .05$). Kruskal-Wallis test showed no significant differences in emergence among the treatments. Comparing all port sites versus all other GoG sites, amphipods exposed to the port sediments avoided the substratum significantly more frequently (one-tailed Mann-Whitney U test, $p < .001$).

No significant differences in female gravidity among the treatments were found (Kruskal-Wallis test). Pairwise comparisons with CTR indicated that the gravidity was significantly greater in ZN2 than in CTR (one-tailed Mann-Whitney U tests, $p = .002$). Comparing the inner port sites versus all other GoG sites it was significantly lower in the port sediments (one-tailed Mann-Whitney U tests, $p = .018$). In the sediments where gravid females were found, their mean occurrence was in the range of 3 - 40 % relative to all females at the bioassay termination, with the lowest gravidity in CTR. The length of the gravid females was in the range of 5 - 6 mm (data not shown). No gravid females nor juveniles were observed in the PGDY1, PGDA1, and PGDA2 sediments.

Male specimens were found in all treatments. The mean percentage of the males at the end of the bioassay did not differ significantly among the sediment exposures (Kruskal-Wallis test, $p < .05$). Similarly, pairwise comparisons between the tested sediments and CTR indicated no significant difference in the percentage of males (Mann-Whitney U tests, $p < .05$), which was in the range of 5 - 39 %.

Table 18. Results of bioassays with *Corophium* spp. (mean values ± SD), CV_{wt} in the brackets).

Species	Sediment	n[1]	Survival %	Growth rate [2] µm day^{-1}	Molting [3] n ind.$^{-1}$	Emergence [4] n ind.$^{-1}$	Gravidity [5] %	Males [6] %	Juv. [7]
Corophium spp.	CTR	6	92 ± 16 a (17)	12 ± 12 ^{ab} (98)	0.9 ± 0.3 a (32)	0.1 ± 0.1^{a}	3 ± 8 a	20 ± 16 a	yes
C. volutator	PGDY1	3	55 ± 9 a (17) *	2 ± 4 a (173)	0.5 ± 0.2 ^{a} (29)*	1.6 ± 1.2 ^{a} *	0 ± 0 ^{a}	5 ± 8 a	no
Corophium spp.	PGDY2	6	61 ± 33 a (54) *	12 ± 8 ^{ab} (65)	0.7 ± 0.4 ^{a} (51)	0.6 ± 0.9 ^{a}	9 ± 12 a	16 ± 15 ^{a}	yes
C. multisetosum	PGDY3	3	97 ± 6 a (6)	2 ± 4 a (173)	1.1 ± 0.2 ^{a} (17)	0.0 ± 0.0 ^{a}	4 ± 6 a	31 ± 20 ^{a}	yes
C. multisetosum	PGDA1	3	77 ± 23 a (30)	8 ± 7 ^{ab} (90)	0.9 ± 0.3 ^{a} (39)	0.1 ± 0.0 ^{a}	0 ± 0 ^{a}	18 ± 6 a	no
C. multisetosum	PGDA2	3	77 ± 32 a (42)	4 ± 5 ^{ab} (132)	0.5 ± 0.2 ^{a} (44)*	0.7 ± 0.2 ^{a} *	0 ± 0 ^{a}	14 ± 12 ^{a}	no
C. multisetosum	PGDA3	3	100 ± 0 a (0)	8 ± 4 ^{ab} (49)	0.8 ± 0.3 ^{a} (37)	0.0 ± 0.0 ^{a}	22 ± 25 a	33 ± 6 a	yes
Corophium spp.	ZN2	6	79 ± 15 a (18)	58 ± 17 ^{cd} (30) *	1.1 ± 0.5 ^{a} (50)	0.2 ± 0.2 ^{a}	40 ± 21 a *	32 ± 25 ^{a}	yes
C. multisetosum	X1	3	67 ± 35 a (53)	18 ± 8 ^{bc} (42)	0.5 ± 0.1 ^{a} (29)*	0.0 ± 0.0 ^{a}	8 ± 14 a	32 ± 11 ^{a}	yes
C. multisetosum	X2	3	70 ± 10 a (14)	23 ± 16 ^{bc} (69)	0.5 ± 0.1 ^{a} (29)*	0.1 ± 0.1 ^{a}	0 ± 0 ^{a}	25 ± 11 ^{a}	yes
C. multisetosum	395	3	77 ± 6 a (8)	28 ± 6 ^{bc} (23)	0.6 ± 0.1 ^{a} (24)*	0.0 ± 0.0 ^{a}	8 ± 14 a	35 ± 14 ^{a}	yes
C. volutator	E57	3	61 ± 38 a (62)	36 ± 39 ^{bd} (108)	1.3 ± 0.7 ^{a} (50)	0.7 ± 0.7 ^{a} *	23 ± 22 a	37 ± 23 ^{a}	no
C. multisetosum	X7	3	87 ± 15 a (18)	23 ± 3 ^{bc} (13)	0.4 ± 0.4 ^{a} (101)	0.0 ± 0.1 ^{a}	17 ± 14 a	20 ± 9 a	yes
C. multisetosum	WS	3	77 ± 15 a (20)	27 ± 13 ^{bc} (47)	0.4 ± 0.3 ^{a} (90)*	0.0 ± 0.0 ^{a}	8 ± 14 a	39 ± 34 ^{a}	no
mean CV_{wt} [8]		-	26 %	79 %	44 %	-	-	-	-
CV_{at} [9]		-	18 %	83 %	40 %	-	-	-	-

[1] the number of replicates; [2] the mean growth rate during 28-d; [3] the number of molts per initial number of individuals; [4] the number of amphipods observed at the sediment surface per initial number of individuals; [5] the percentage of gravid females relative to all females at the end of bioassay; [6] the percentage of adult males relative to all individuals at the end of bioassay; [7] Juv. stands for the juveniles, their presence or absence was noted; [8] mean coefficient of variation within treatments; [9] coefficient of variation among the treatments. Different superscripts in the same column indicate significant differences among the sites at $p < .05$ (one-way ANOVA and HSD Tukey test with modification for an unequal number of samples, except for survival, emergence, gravidity, and the percentage of males for which the Kruskal-Wallis and multiple comparisons on ranks was used). An asterisk signifies a significant difference from CTR based on pairwise comparisons (one-tailed Mann-Whitney U test, $p < .05$).

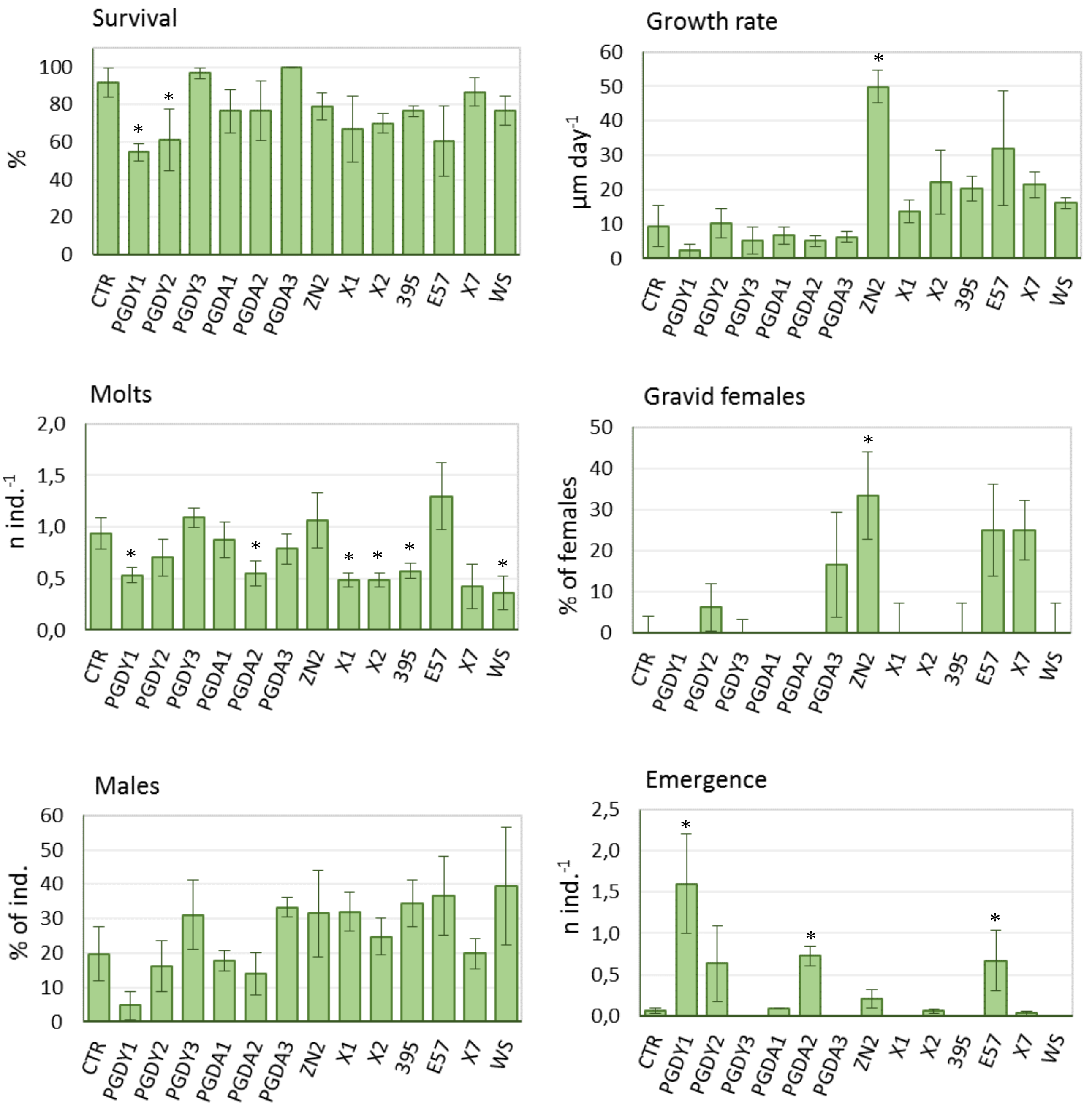

Fig. 25. Responses of amphipods exposed to the GoG sediments (mean values ± SD). An asterisk marks significant differences from CTR.

The *Corophium* spp. responses were evaluated for potential interrelationships with the use of Kendall's tau test. According to the results of this analysis (Table 19), survival was significantly positively related to molting and negatively to emergence. Growth, aside from a negative relation to emergence, correlated positively with the percentage of males and gravid females. The molting, in addition to a positive relationship with survival, was positively related to the percentage of gravid females, and the latter to both growth and molting. Survival, growth, and percentage of males, were significantly negatively associated with the emergence of amphipods from the sediments.

Tab. 19. Kendall's tau (τ) correlation coefficients presenting significant relationships between the responses of *Corophium* spp. exposed to the GoG sediments ($p < .05$).

	Survival	Growth	Molts	Emergence	Males	Gravidity
Survival			0.35	-0.27		
Growth				-0.19	0.20	0.40
Molts	0.35					0.24
Emergence	-0.27	-0.19			-0.23	
Males		0.20		-0.23		
Gravidity		0.40	0.24			

5.1.3.3 *Corophium volutator*

C. volutator was tested against the PGDY1, PGDY2, ZN2, E57, and CTR sediment collected from Kaczy Winkiel i.e., the amphipods collection site. The results of the bioassays are shown in Table 20. No mortalities were observed in any of the CTR replicates. Thus, the test acceptability criteria were fulfilled according to USEPA (2001), which recommended at least 60 % survival in a single replicate and a mean survival at or above 80 % in CTR. The amphipod responses were examined for significant differences from CTR by pairwise comparisons (one-tailed Mann-Whitney U tests, $p < .05$).

The mean survival ranged from 39 % to 88 % in the tested sediments. Compared to CTR, it was significantly lower in PGDY2 (39 %), PGDY1 (55 %), and E57 (61 %). The mean length of *C. volutator* at the beginning of exposure was 3.9 ± 0.1 mm, and it was 4.6 ± 0.9 mm at the end. The mean growth rate (GR) ranged from 2 to 69 µm d^{-1}. The only significant difference in GR was found between ZN2 (69 µm d^{-1}) and CTR (8 µm d^{-1}; $p = .002$). There was no significant difference in molting between tested sediments and CTR. Emergence was significantly greater in PGDY1 and E57 than in CTR ($p < 0.05$), while in other sediments, it did not differ from CTR. Gravid females were absent in CTR, PGDY1, and PGDY2, but juveniles were present in CTR and PGDY2. No reproductive activity was observed in PGDY1. In ZN2 sediments, 40% of females were gravid, with juveniles also present.

Tab. 20. Results of bioassays with *C. volutator* (mean ± SD, CV_{wt} in the brackets).

Sediment	Survival %	Growth rate µm day^{-1}	Molts n $ind.^{-1}$	Emergence n $ind.^{-1}$	Gravidity %	Males, %	Juv. -
CTR	100 ± 0 (0)	8 ± 4 (49)	1.0 ± 0.5 (48)	0.0 ± 0.1	0 ± 0	27 ± 18	Yes
PGDY1	55 ± 9 (17) *	2 ± 4 (173)	0.5 ± 0.2 (29)	1.6 ± 1.2 *	0 ± 0	5 ± 8	No
PGDY2	39 ± 32 (81) *	12 ± 7 (56)	0.5 ± 0.4 (80)	0.9 ± 1.3	0 ± 0	8 ± 14	Yes
ZN2	88 ± 14 (16)	69 ± 17 (25) *	1.5 ± 0.5 (31)	0.3 ± 0.3	40 ± 32	50 ± 20	Yes
E57	61 ± 38 (62) *	36 ± 39 (108)	1.3 ± 0.7 (50)	0.7 ± 0.7*	23 ± 22	37 ± 23	No
mean CV_{wt}	35 %	82 %	48 %	-	-	-	-
CV_{at}	36 %	109 %	46 %	-	-	-	-

An asterisk indicates a significant difference from CTR (one-tailed Mann-Whitney U test, $p < .05$).

5.1.3.4 *Corophium multisetosum*

The results of the bioassays conducted with *C. multisetosum* have been shown in Table 21. This species was tested against 12 sediments including the CTR sediment from the amphipod collection site (Rzucewo). There was a 40 % mortality in one of the CTR replicates, however, the mortality in two other replicates (0 and 10 %) yielded a mean CTR mortality of 17 %. Thus, the mean survival in CTR fulfilled the test acceptability criteria according to USEPA (2001) i.e., the survival of at least 60 % in a single replicate is satisfactory as long as the mean CTR survival is not below 80 %. Moreover, the survival in the PGDY3 and PGDA3 sediments with physicochemical characteristics comparable to those of the CTR sediment was very high (97 - 100 %). Taking into account these facts, the CTR survival was accepted and greater mortality in one replicate was considered incidental. The amphipod responses were examined for significant differences from CTR by pairwise comparisons (one-tailed Mann-Whitney U tests or t-tests at $p < .05$).

No statistically significant differences were found in survival between the tested sediments and CTR. Survival ranged from 67 % to 100 % and was the greatest in the PGDY3, PGDA3, and X7 sediments. The mean individual length of *C. multisetosum* at the beginning was 4.1 ± 0.2 mm and at the termination, it was 4.6 ± 0.4 mm. Regarding GR, the only significant difference from CTR was observed in ZN2, where it reached 47 $\mu m\ d^{-1}$ and was significantly greater than that in CTR. For molting, it was significantly less frequent in PGDA2, X1, X2, 395, and WS than in CTR (one-tailed Mann-Whitney U tests, $p < .05$). In the context of emergence, a significant difference from CTR was found in PGDA2. There was no significant difference from CTR in other studied endpoints i.e., percentage of gravid females, percentage of males, and the presence of juveniles. It should be indicated that no emergence was observed in the PGDY3, PGDA3, X1, 395, and WS sediments. No gravid females were found in PGDA1, PGDA2, and X2, and neither gravid females nor juveniles were present in PGDA1 and PGDA2. The greatest percentage of gravid females was found in the ZN2 sediments (39 %).

Tab. 21. Results of bioassays with *C. multisetosum* exposed to the GoG sediments (mean ± SD, CV_{wt} in the brackets).

Sediment	Survival	Growth rate	Molts	Emergence	Males	Gravidity	Juv.
	%	µm day^{-1}	n $ind.^{-1}$	n $ind.^{-1}$	%	%	-
CTR	83 ± 21 (25)	17 ± 17 (98)	0.9 ± 0.1 (10)	0.1 ± 0.1	12 ± 11	7 ± 12	yes
PGDY2	83 ± 15 (18)	11 ± 10 (89)	0.9 ± 0.2 (20)	0.3 ± 0.5	24 ± 12	18 ± 9	yes
PGDY3	97 ± 6 (6)	2 ± 4 (173)	1.1 ± 0.2 (17)	0.0 ± 0.0	31 ± 20	4 ± 6	yes
PGDA1	77 ± 23 (30)	8 ± 7 (90)	0.9 ± 0.3 (39)	0.1 ± 0.0	18 ± 6	0 ± 0	no
PGDA2	77 ± 32 (42)	4 ± 5 (132)	0.5 ± 0.2 (44) *	0.7 ± 0.2*	14 ± 12	0 ± 0	no
PGDA3	100 ± 0 (0)	8 ± 4 (49)	0.8 ± 0.3 (37)	0.0 ± 0.0	33 ± 6	22 ± 25	yes
ZN2	70 ± 10 (14)	47 ± 9 (18) *	0.7 ± 0.2 (28)	0.2 ± 0.1	13 ± 13	39 ± 10	yes
X1	67 ± 35 (53)	18 ± 8 (42)	0.5 ± 0.1 (29) *	0.0 ± 0.0	32 ± 11	8 ± 14	yes
X2	70 ± 10 (14)	23 ± 16 (69)	0.5 ± 0.1 (29) *	0.1 ± 0.1	25 ± 11	0 ± 0	yes
395	77 ± 6 (8)	28 ± 6 (23)	0.6 ± 0.1 (24) *	0.0 ± 0.0	35 ± 14	8 ± 14	yes
X7	87 ± 15 (18)	23 ± 3 (13)	0.4 ± 0.4 (101)	0.0 ± 0.1	20 ± 9	17 ± 14	yes
WS	77 ± 15 (20)	27 ± 13 (47)	0.4 ± 0.3 (90) *	0.0 ± 0.0	39 ± 34	8 ± 14	no
mean CV_{wt}	21 %	70 %	39 %	-	-	-	-
CV_{at}	13 %	70 %	34 %	-	-	-	-

An asterisk indicates a significant difference from CTR (one-tailed Mann-Whitney U test or t-test, $p < .05$).

5.1.3.5 *Comparison of C. volutator and C. multisetosum responses*

The responses of *C. volutator* and *C. multisetosum* were compared based on bioassays in which each species was exposed to the CTR, PGDY2, and ZN2 sediments. To evaluate potential species-specific differences, the Mann-Whitney U test or t-test was used, with species as a dependent variable (where species represented all replicates regardless of sediment type, N = 18) and sediment as an independent variable. These tests showed no significant differences in survival (U-test, U=34.0, p=0.589), GR (t-test, $t_{(16)}$=0.269, p=0.701), molting (t-test, $t_{(16)}$=-0.121, p=0.905), emergence (U-test, U=39.5, p=0.964), or the females gravidity (U-test, U=23.5, p=0.145) between *C. volutator* and *C. multisetosum* (Table 22).

Furthermore, the *C. volutator* and *C. multisetosum* responses were compared for each sediment separately (n = 6). These statistics, shown in Table 22 and Figure 26, indicated few significant differences between the species in PGDY2 and ZN2, but not in CTR (one-tailed Mann-Whitney U test, p < .05). These were: in PGDY2, a significantly greater percentage of gravid females of *C. multisetosum* than *C. volutator* (in fact there were no gravid *C. volutator* females present in PGDY2); in ZN2, significantly greater GR, molting index, and the percentage of males in *C. volutator* than *C. multisetosum.* Aside from these significant differences, it should be mentioned that in CTR the mean GR of *C. multisetosum* was twice that of *C. volutator* (17 and 8 µm d^{-1}, respectively), while the mean survival was greater than 80 % in both species. Furthermore, in CTR the percentage of surviving males was greater in *C. volutator* than in *C. multisetosum* (27 % compared to 12 %) and, although gravid *C. volutator* females were not observed, juveniles of both species were retrieved from the sediments. In PGDY2, although the only significant inter-species difference was a greater percentage of gravid *C. multisetosum* females, this species showed also markedly greater survival, molting frequency, and the percentage of males than *C. volutator*, while its emergence was notably less frequent than that of *C. volutator*.

Tab. 22. Responses of *C. volutator* and *C. multisetosum* exposed to the same sediments (mean values ± SD).

Sediment	Species	Survival %	GR µm day^{-1}	Molts n $ind.^{-1}$	Emergence n $ind.^{-1}$	Gravidity %	Males %	Juv. -
CTR	C.v.	100 ± 0	8 ± 4	0.9 ± 0.4	0.0 ± 0.1	0 ± 0	27 ± 18	yes
	C.m.	83 ± 21	17 ± 17	1.0 ± 0.1	0.1 ± 0.1	7 ± 12	12 ± 11	yes
PGDY2	C.v.	39 ± 32	12 ± 7	0.5 ± 0.4	0.9 ± 1.3	0 ± 0 *	8 ± 14	no
	C.m.	83 ± 15	11 ± 10	1.0 ± 0.2	0.3 ± 0.5	23 ± 15*	24 ± 12	yes
ZN2	C.v.	88 ± 14	69 ± 17*	1.3 ± 0.4*	0.3 ± 0.3	40 ± 32	40 ± 32*	yes
	C.m.	70 ± 10	47 ± 9*	0.7 ± 0.2*	0.2 ± 0.1	39 ± 10	13 ± 13*	no

C.v. – C. volutator; *C.m. – C. multisetosum*. An asterisk indicates a significant difference between the species response in the same sediments (one-tailed Mann-Whitney U test, p < .05).

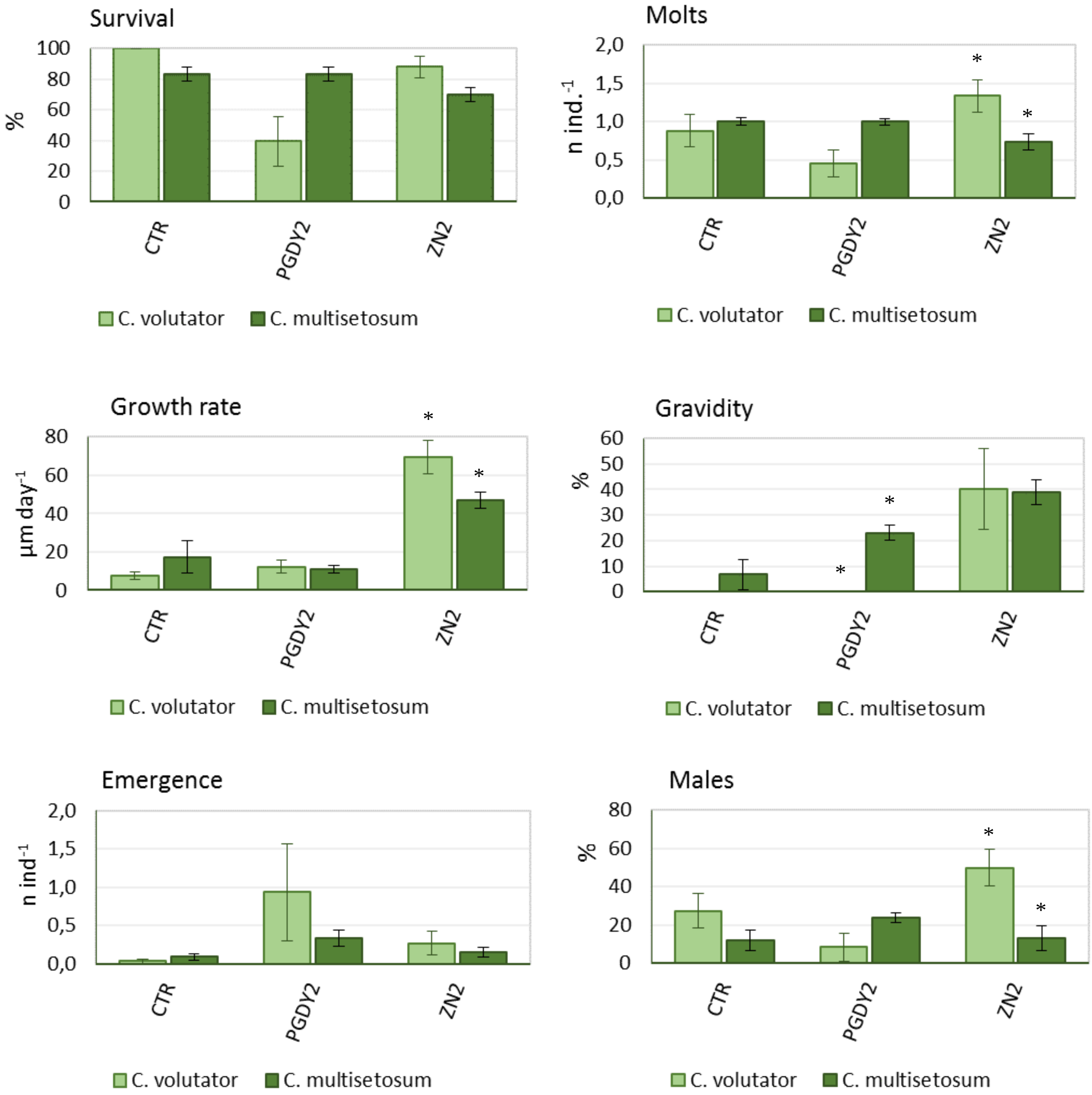

Fig. 26. Comparison of *C. volutator* and *C. multisetosum* responses to two GoG sediments and CTR (mean values ± SD). An asterisk marks significant differences in responses between species.

5.1.3.6 *Associations among Corophium spp. responses and sediment features*

The relations among amphipod responses and sediment characteristics investigated with PCA have been shown in Table 23. PCA yielded four principal components (PCs) with eigenvalues greater than one that explained 78.8 % of the total variance. PC1, PC2, PC3, and PC4 accounted for 40.8, 16.4, 13.5, and 8.1 % of the total variance, respectively. PC1 indicated strong positive correlations between emergence and sediment contaminants (HCB, $\sum_7$PCBs, $\sum$DDT, $\sum_{16}$PAHs, $\sum_6$PBDEs), the fines, and OM_w content. PC1 also showed that amphipod survival, growth, molting, and gravidity, while moderately positively inter-correlated, were all negatively related to those sediment features. These relations are depicted in Figure 27a. PC2, similarly to PC1, indicated moderate positive inter-correlations for survival, growth, molts, and female gravidity and their positive connection with OM_f. However, there was also an indication of a significant positive connection of OM_f with HCB, $\sum_{16}$PAHs, and $\sum_6$PBDEs (Figure 27a). The most significant loadings of survival, growth, molts, and female gravidity were extracted with separate PCs. Namely, growth was extracted with PC3 where it showed a positive correlation with female gravidity and sediment OM_f. Whereas survival and molts were extracted with PC4 indicating a significant positive correlation with each other.

The positive inter-correlation for survival, growth, molts, and female gravidity has been depicted by a small angle between the vectors representing these variables as shown in Figure 27a. Similar small angle occurred between emergence, the sediment fines, OM_w, and some contaminants. The distribution of the sites, based on the variables' scores, is shown in Figure 27b. The closeness of the sites shown in Figure 27b indicates their similarity concerning the sediment conditions and amphipod responses. The inner port sites (PGDA1, PGDA2, PGDY1, PGDY2) along with the 395 site are distributed at the left side of the vertical axis, while on its right side are all other sites i.e., those located in outer port areas (PGDY3, PGDA3), along the distance gradient from the Vistula River mouth (ZN2, X1, X2), other GoG sites (E57, WS, X7), and CTR. The vertical axis marks the separation between negative and positive contributions to PC1, which explains a greater share of the total variance and is mostly determined by the scores related to the contaminants, $OM_{w,}$ and the content of fines, but also to some extent to biological responses, i.e. The emergence, survival, and growth of amphipods. PC2 determines the distribution of sites against the vertical axis, based on the negative scores for OM_f, survival, growth, and gravidity, as well as some contaminants. In general, PCA (Figure 27b) showed that among the sites characterized by elevated levels of contaminants, PGDA2 was distinctly different from the other sites.

Tab. 23. PCA statistics showing the associations among biological responses of *Corophium* spp. and the GoG sediment characteristics and contaminants.

Principal Components	PC1	PC2	PC3	PC4
Eigenvalues	5.3	2.1	1.8	1.1
Variance explained (78.8 %)	40.8	16.4	13.5	8.1
Survival	0.407	-0.393	0.403	0.585
Growth	0.420	-0.419	-0.572	-0.268
Molts	0.393	-0.421	-0.052	0.574
Emergence	-0.602	0.291	-0.496	0.011
Gravidity	0.410	-0.518	-0.526	0.002
HCB	-0.678	-0.527	0.365	-0.192
∑DDTs	-0.832	0.004	-0.179	0.132
$\sum_{16}$PAHs	-0.736	-0.513	0.321	-0.116
$\sum_{7}$PCBs	-0.755	0.234	-0.368	0.342
$\sum_{6}$PBDEs	-0.781	-0.415	0.236	-0.192
OM_f	-0.134	-0.674	-0.503	-0.002
OM_w	-0.881	-0.092	-0.066	0.175
The fines	-0.782	0.208	-0.166	0.243

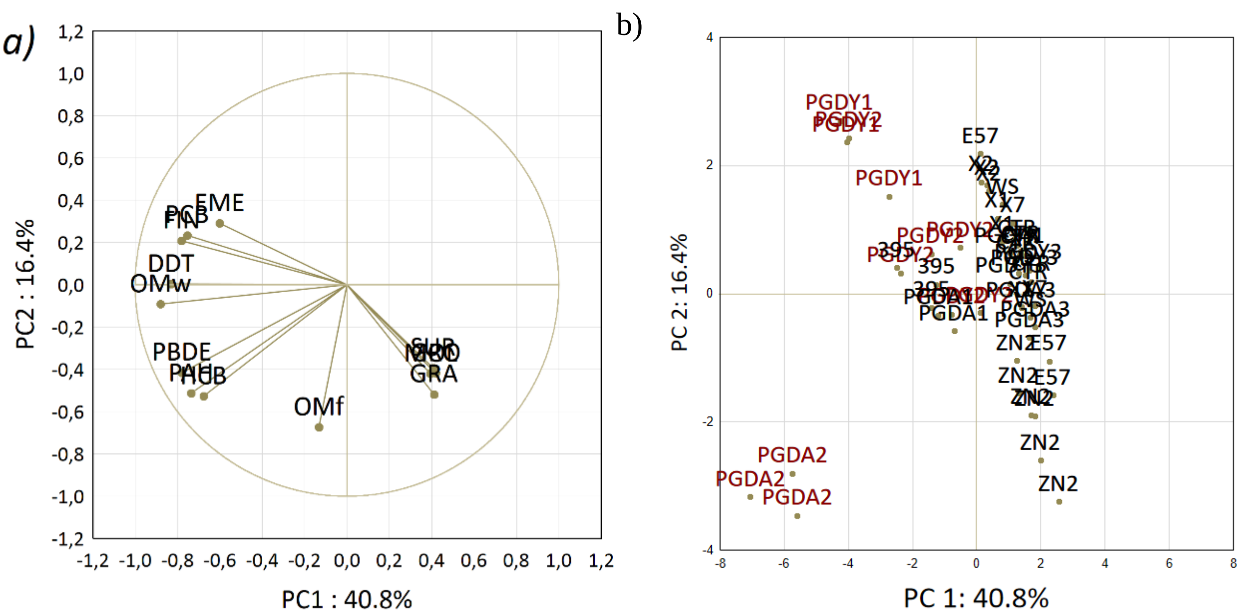

Fig. 27. PCA results showing the associations between the *Corophium* spp. responses and the GoG sediment characteristics and contaminants: a) relationships among the variables and b) the distribution of the sites on the factor plane. The sites marked in red exceeded some EAC/ERL values. Variable abbreviations: SUR – survival; GRO – growth; MOL – molting; EME – emergence; GRA – gravidity; FIN – fines (variables are presented in Table 23).

To investigate the potential influence of the sediment's natural characteristics on *Corophium* spp. responses, the relationships between the measured endpoints and the sediment fines, OM_f, and OM_w were analyzed using data obtained for a selected set of tested sediments that had relatively low contaminant levels, i.e. those in which the EAC and ERL values were not exceeded. The Kendall’s tau test indicated that the growth and gravidity were the only responses influenced by sediment natural characteristics i.e., growth was significantly positively associated with the sediment content of the fines, OM_f, and OM_w, while the gravidity with OM_f (Table 24).

Tab. 24. Kendall’s tau (τ) correlation coefficients presenting significant relationships between the *Corophium* spp. responses, and the characteristics of a selected set of the GoG sediments (PGDY1, PGDY2, PGDA2, and 395 were not included in the data set; $p < .05$).

Sediment feature	Survival	Growth	Molts	Emergence	Males	Gravidity
The fines		0.26				
OM_f		0.30				0.46
OM_w		0.37				

Only significant values are shown.

Kendall’s statistics (Table 24) are in line with the PCA outcome (Table 23) in that they showed contaminants were not the only factors influencing the biological endpoints of bioassays; natural sediment features were also significant.

Another PCA analysis was performed to study the responses of *C. volutator* and *C. multisetosum* separately. The analysis results are shown in Table 25. For *C. volutator*, PC1, accounting for 51.7 % of the total variance, indicated strong significant relationships among all biological variables and their associations with sediment contaminants and natural features, except for OM_f. Survival, growth, molts, and gravidity of *C. volutator* correlated negatively with contaminant concentrations, fines, and OM_w, while emergence showed a positive correlation. PC2 (16.0 % of the total variance) also had significant loadings for growth and gravidity, which correlated positively with each other and with sediment OM_f, and indicated a positive relation between sediment OM_f and some contaminants. PC3 (15.2 % of the total variance) mainly showed positive relations between sediment OMf, OMw, and fines. PC4 (6.8 % of the total variance), similar to PC1, showed a significant negative relation between survival and emergence.

PCA performed for *C. multisetosum* presented similar results, indicating significant positive relationships between emergence and sediment contaminants, fines, and OM_w (as shown by PC1, accounting for 40.8% of the total variance). Biological responses i.e., survival, growth, and molts, were extracted with PC2 (14.2 % of the total variance), demonstrating negative relations for growth with survival and molts. PC3 exposed a highly significant positive relation between gravidity and the content of OM_f, similar to that shown for *C. volutator*, and a moderately negative relationship between gravidity and the sediment fine fraction. PC4 yielded only a moderate positive relationship between the sediment fines content and ∑DDTs. The PCA results are graphically presented on the factor plane in Figure 28a and 28b. Both species were able to discriminate the contaminated sediments (the sediments collected from the harbors and the 395 sites) in which EACs were exceeded.

Tab. 25. PCA statistics showing the associations among biological responses of *C. volutator* and *C. multisetosum*, the sediment characteristics, and contaminants.

	C. volutator				*C. multisetosum*			
Components	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
Eigenvalues	6.7	2.1	2.0	0.9	6.1	1.8	1.7	1.1
Variance explained	51.7	16.0	15.2	6.8	46.8	14.2	13.0	8.2
Survival	0.719	0.117	-0.276	0.539	0.149	-0.668	-0.373	0.272
Growth	0.577	-0.658	-0.207	-0.271	0.344	0.820	-0.113	0.174
Molts	0.773	-0.207	-0.212	0.261	0.107	-0.640	-0.295	0.224
Emergence	-0.763	-0.032	-0.099	-0.506	-0.809	-0.041	-0.253	-0.072
Gravidity	0.564	-0.582	-0.299	-0.203	0.247	0.349	-0.729	0.358
HCB	-0.653	-0.621	0.164	0.229	-0.856	-0.031	-0.044	-0.384
∑DDTs	-0.912	-0.281	0.126	0.131	-0.844	0.010	-0.048	0.406
$\sum_6$PBDEs	-0.500	-0.502	0.621	0.171	-0.962	-0.003	-0.052	-0.101
$\sum_{16}$PAHs	-0.919	-0.318	0.098	0.195	-0.905	-0.029	-0.060	-0.248
$\sum_7$PCBs	-0.857	0.131	-0.474	0.067	-0.860	-0.120	-0.184	0.169
OM_f	0.187	-0.609	-0.622	0.039	-0.313	0.335	-0.748	-0.245
OM_w	-0.763	0.153	-0.610	0.054	-0.863	0.170	0.195	0.227
The fines	-0.815	0.127	-0.542	0.097	-0.668	0.200	0.457	0.471

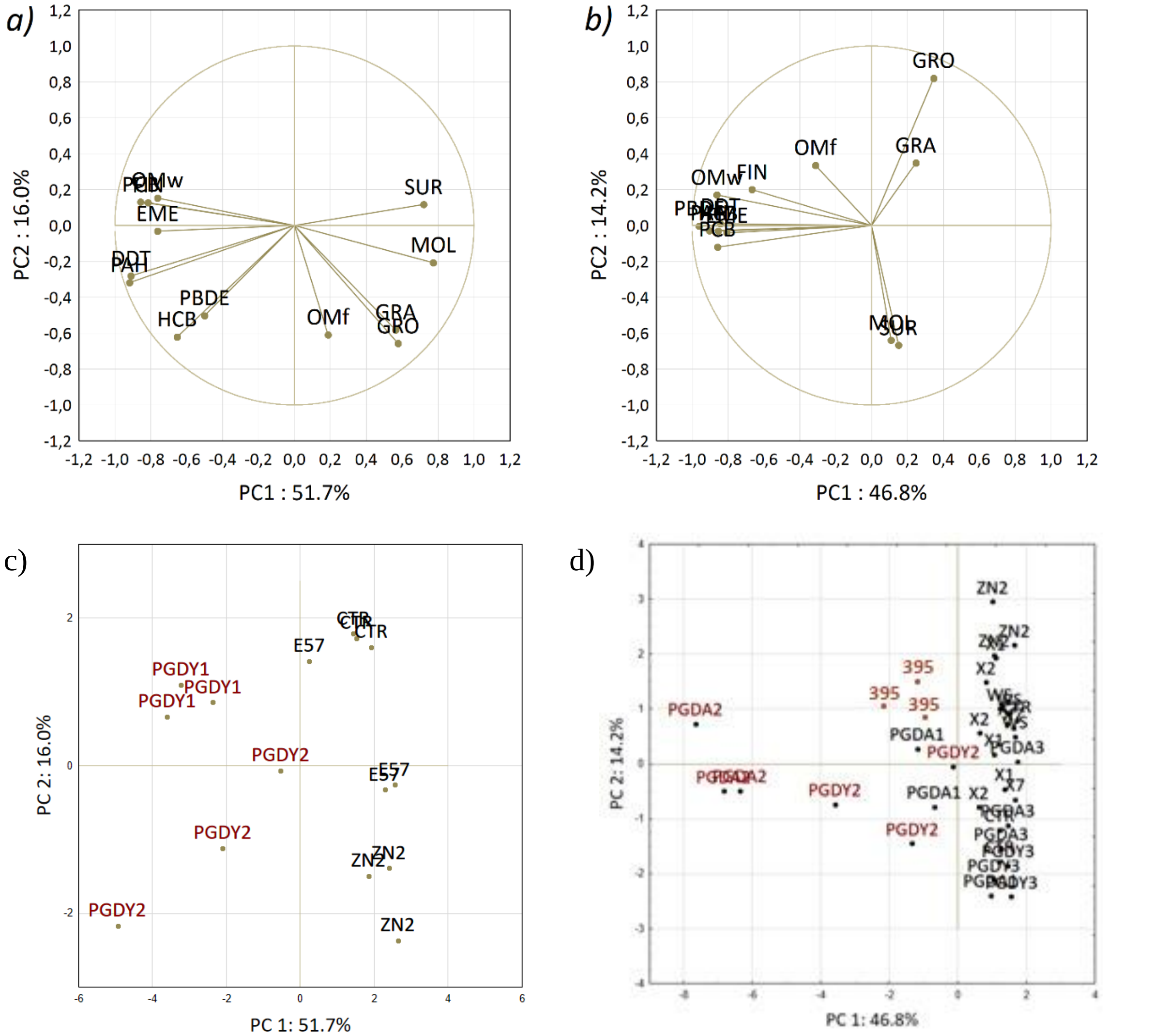

Fig. 28. PCA graphs showing the associations between the amphipod species responses, and sediment features and contaminants: relationships among the variables in a) *C. volutator* and b) *C. multisetosum* (variables are presented in Table 25) and the distribution of the sites on the factor-plane for c) *C. volutator* and d) *C. multisetosum*. Abbreviations: SUR – survival; GRO – growth; MOL – molting; EME – emergence; GRA – gravidity; FIN – fines.

5.1.3.7 *The Corophium sensitivity to reference toxicant*

The results of the 72-h reference toxicant (Cd) tests for two *C. volutator* populations from Kaczy Winkiel and Rzucewo are presented in (Table 26). The LC_{50} value for Kaczy Winkiel individuals was 0.68 mg l^{-1} with confidence limits (CL) of 0.30 - 1.54 mg l^{-1}, while for the Rzucewo individuals LC_{50} was 1.36 mg l^{-1}, with CL of 0.78 - 2.40 mg l^{-1}. The obtained LC_{50} values (calculated with Probit analysis) were not significantly different ($p < .05$).

Tab. 26. Results of water-only cadmium exposure tests with *C. volutator* (mean ± SD).

Code	Concentration Cd, mg l^{-1}	The initial number of organisms N	Mortality, Kaczy Winkiel %	Mortality, Rzucewo %
CTR	0	20	0 ± 0	0 ± 0
S1	0.5	20	40 ± 10	15 ± 9
S2	2	20	100 ± 0	73 ± 3
S3	5	20	98 ± 3	90 ± 10
S4	10	20	100 ± 0	92 ± 3
LC_{50} (mg l^{-1})			0.68 (CL 0.30-1.54)	1.36 (CL 0.78-2.40)

5.1.4 Evaluation of the GoG sediments

Table 27 provides a comprehensive assessment of sediment quality based on features potentially indicative of sediment toxicity, with a comparison to EAC and ERL values (PGDY1, PGDY2, PGDA2, and 395). Sediments collected from four out of thirteen GoG sites exceeded these values. Two of these sediments (PGDY1, PGDY2) exhibited significantly lower amphipod survival, and it was also reduced in E57. Amphipod GR did not indicate toxicity in any of the studied sediments. In fact, growth was significantly higher in ZN2 samples compared to the control (CTR). Emergence was notably higher in PGDY1 and PGDA2 and was also increased in E57. Overall, biological responses to different treatments were often inconsistent across different endpoints. The sediment toxicity scores are depicted in Figure 29.

Amphipod responses were evaluated using the scoring system described in the Materials and Methods section. ZN2 received the highest score (4.16), followed by PGDA3, X7, 395, and WS (4.00). These sediments got better scores than CTR with a value of 3.66. Sediments that exceeded EAC/ERL values (apart from 395) received scores ranging from 2.33 to 2.83, representing the lowest values among all treatments.

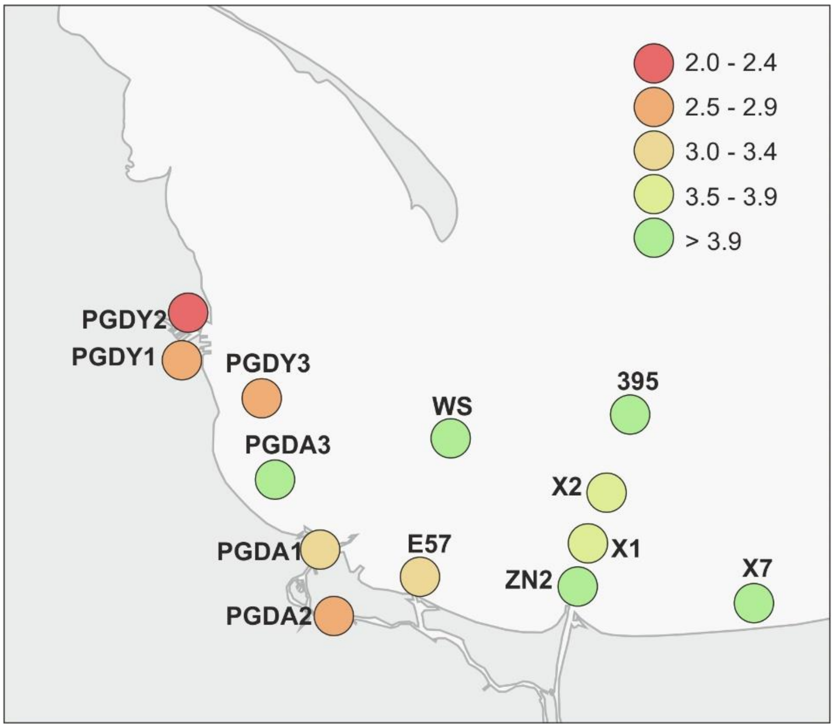

Fig. 29. The visual presentation of sediment toxicity scores based on responses of amphipods, described in detail in Table 27.

Tab. 27. Evaluation of the GoG sediments based on the responses of amphipods (mean values and response grading).

Sediment range, unit	Species	Survival 39 - 100, %			Growth rate 2 - 69, µm day^{-1}			Emergence 0.0 - 1.6, n $ind.^{-1}$			Total score points	EAC/ERL exceeded yes / no
				Score			Score			Score		
CTR	*Corophium spp.*	92	very good	2.0	12	low	1.0	0.1	low	0.66	3.66	no
PGDY1	*C. volutator*	$55^{\blacktriangledown}$	satisfactory	1.0	2	very low	0.5	$1.6^{\blacktriangle}$	high	1.00	2.50	yes
PGDY2	*Corophium spp.*	$61^{\blacktriangledown}$	satisfactory	1.0	12	low	1.0	0.6	increased	0.33	2.33	yes
PGDY3	*C. multisetosum*	97	very good	2.0	2	very low	0.5	0.0	absent	1.00	2.50	no
PGDA1	*C. multisetosum*	77	good	1.5	8	low	1.0	0.1	low	0.66	3.16	no
PGDA2	*C. multisetosum*	77	good	1.5	4	low	1.0	$0.7^{\blacktriangle}$	increased	0.33	2.83	yes
PGDA3	*C. multisetosum*	100	very good	2.0	8	low	1.0	0.0	absent	1.00	4.00	no
ZN2	*Corophium spp.*	79	good	1.5	$58^{\blacktriangle}$	high	2.0	0.2	low	0.66	4.16	no
X1	*C. multisetosum*	67	satisfactory	1.0	18	moderate	1.5	0.0	absent	1.00	3.50	no
X2	*C. multisetosum*	70	good	1.5	23	moderate	1.5	0.1	low	0.66	3.66	no
395	*C. multisetosum*	77	good	1.5	28	moderate	1.5	0.0	absent	1.00	4.00	yes
E57	*C. volutator*	$61^{\blacktriangledown}$	satisfactory	1.0	$36^{\blacktriangle}$	high	2.0	0.7	increased	0.33	3.33	no
X7	*C. multisetosum*	87	good	1.5	23	moderate	1.5	0.0	absent	1.00	4.00	no
WS	*C. multisetosum*	77	good	1.5	27	moderate	1.5	0.0	absent	1.00	4.00	no

▼ response significantly less than CTR; ▲ response significantly greater than CTR at $p < .05$.

5.2 Sediments of the Martwa and Wisła Śmiała Rivers

5.2.1 Environmental conditions

The characteristics of the studied sites during the collection of samples are listed in Table 28. The depth of these sites ranged from 3.0 to 8.0 m (ST2 and ST3, respectively). Salinity ranged from 7.1 to 4.7, gradually decreasing in the river's southward direction with increasing distance from the GoG, to reach its minimum of 4.7 at the site nearest to the heap (ST4). It was significantly greater at ST1 (near the GoG) than at ST4. The dissolved oxygen concentration in the bottom water varied from 4.4 to 8.1 mg l^{-1}. The significantly lower temperature of the bottom water was found at ST3 (19.5 °C) at a greater depth than at ST5 (21.8 °C). The water pH, highest at ST1, was decreasing up the river with a minimum value at ST3. The concentration of phosphates in the bottom water, lowest at ST1, increased in the river's southward direction to reach 8 - 9-fold greater levels at ST4 and ST3 (near the phosphogypsum heap and the Sobieszewo bridge, respectively) and remained elevated at ST5 located above the heap. The water at ST1 was characterized by greater salinity, higher dissolved oxygen concentration, higher pH, and the lowest concentration of phosphates in comparison to the other MW&WS sites. The ST3 site, located just below the Kanał Młynówka confluence, was the deepest (8 m) with a low oxygen level of 4.4 mg l^{-1}. Considering the phosphates and oxygen concentration, ST3 could be characterized by the worst quality of the bottom water among the MW&WS sites.

Tab. 28. Environmental parameters of the MW&WS study sites.

Sediment	Depth m	Salinity	Oxygen mg l^{-1}	Temperature °C	pH	Phosphates (PO_4^{2-}) mg l^{-1}
ST1	6.5	7.1 ± 0.0^{a}	8.1 ± 0.1^{a}	19.6 ± 0.06^{ab}	8.3 ± 0.04^{b}	0.1 ± 0.01^{a}
ST2	3.0	6.1 ± 0.6^{ab}	5.0 ± 0.3^{a}	19.8 ± 0.5^{ab}	7.3 ± 0.07^{ab}	0.5 ± 0.05^{ab}
ST3	8.0	6.4 ± 0.0^{ab}	4.4 ± 0.7^{a}	19.5 ± 0.2^{a}	7.0 ± 0.03^{ab}	0.9 ± 0.04^{b}
ST4	3.6	4.7 ± 0.0^{b}	8.1 ± 0.5^{a}	21.0 ± 0.05^{ab}	7.4 ± 0.03^{ab}	0.8 ± 0.03^{ab}
ST5	4.6	5.4 ± 0.4^{ab}	5.2 ± 0.8^{a}	21.8 ± 0.6^{b}	7.1 ± 0.2^{a}	0.7 ± 0.02^{ab}

Different superscripts in the same column mark significant differences among the sites at $p < .05$ (Kruskal-Wallis and multiple comparisons on ranks).

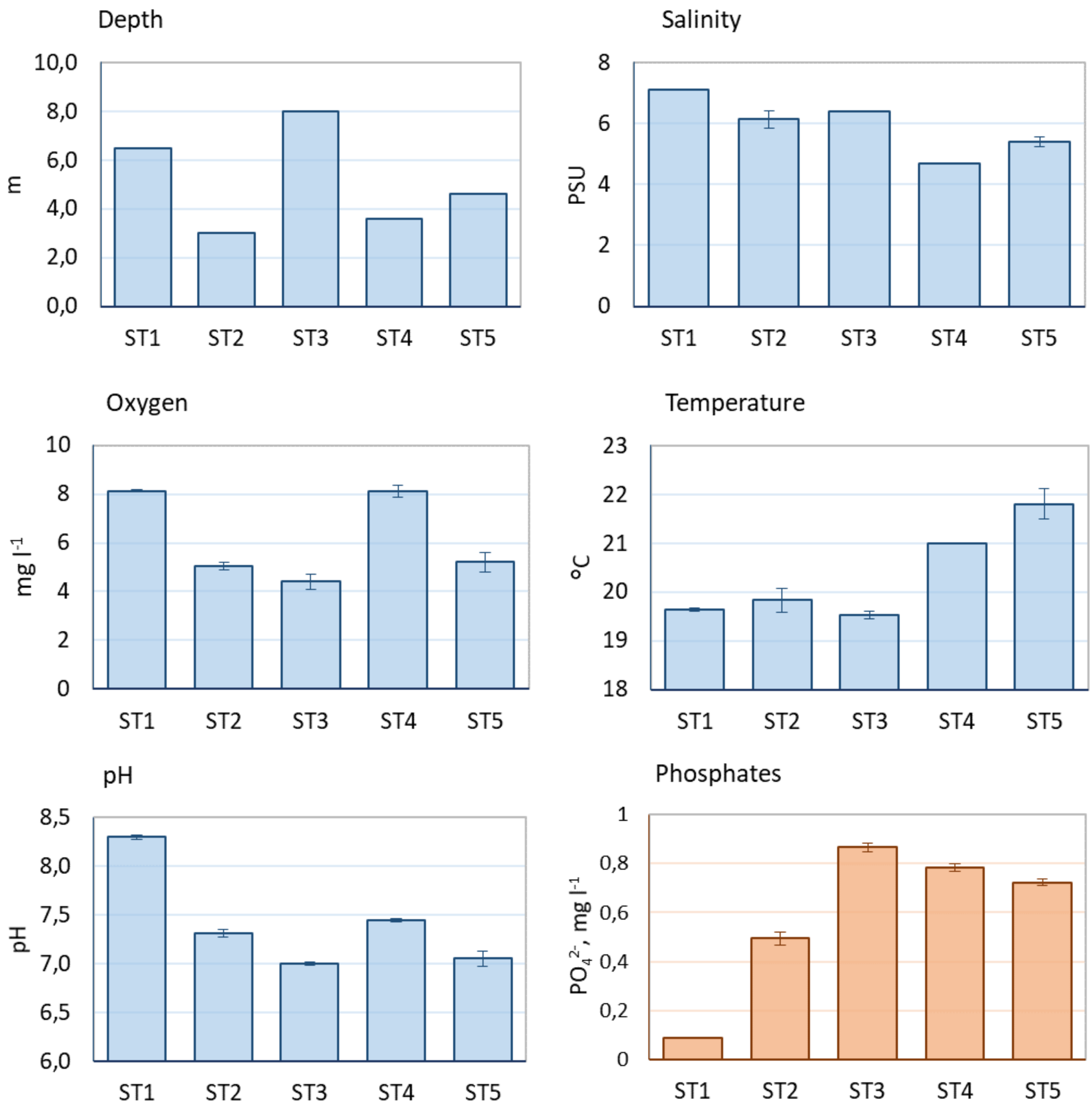

Fig. 30. Sediment collection depth and the bottom water characteristics at the MW&WS sites (mean values ± SD).

Kendall's statistics indicated that phosphates' concentration was negatively related to salinity (τ = -0.398) and pH (τ = -0.476), salinity had a negative relationship with temperature (τ = -0.408), whereas pH was positively correlated to the oxygen concentration of water (τ = 0.632; $p < .05$).

5.2.2 Characteristics of sediments

The sediments of the Martwa Wisła and Wisła Śmiała Rivers (MW&WS) varied considerably in the composition of the grain sizes and OM content (Table 29, Figure 31, and Figure 32). The content of the fine fraction (clay and silt) ranged from 8.2 to 38.7 % (2.3 % in CTR). This fraction was significantly less in CTR than in ST1, ST3, and ST5 sediments, where it constituted 30.4 - 38.7 %. The very fine sand fraction (63 - 125 µm) ranged from 9.1 to 24.6 % (44.8 % in CTR). Its content in CTR was significantly greater than in all examined sediments. The fine sand fraction (125 - 250 µm) ranged from 17.2 to 48.9 % (25.6 % in CTR). In the ST4 sediment, its content was the greatest and significantly different from that in all other sediments. The greater grain sizes (> 250 µm) constituted 9.2 to 49.8 % of the sediments (almost half of the ST2 and ST3 sediments). The OM_w ranged from 4.3 to 13.7 %, and OM_f from 7.4 to 11.7 %. In CTR, the OM content was low i.e., 0.7 % OM_w and 3.0 % OM_f which was significantly less than in other examined sediments.

Tab. 29. Grain size composition and OM content (%) of the MW&WS and CTR sediments (mean values ± SD).

Sediment	F < 63 µm[1]	F 63 - 125 µm[2]	F 125 - 250 µm[3]	F > 250 µm[4]	OM_w	OM_f
CTR [5]	2.3 ± 0.0^{a}	44.8 ± 0.0^{d}	25.6 ± 0.0^{b}	26.8 ± 0.0^{ac}	0.7 ± 0.1^{a}	3.0 ± 0.4^{a}
ST1	38.7 ± 13.3^{c}	24.6 ± 3.2^{c}	26.4 ± 9.1^{b}	9.2 ± 4.9^{a}	7.6 ± 2.1^{bc}	8.1 ± 0.4^{bc}
ST2	20.0 ± 13.6^{ac}	9.1 ± 3.1^{a}	20.2 ± 2.7^{b}	49.8 ± 14.4^{c}	4.3 ± 3.9^{ac}	7.9 ± 0.5^{bc}
ST3	30.4 ± 5.7^{bc}	9.9 ± 3.4^{ab}	17.2 ± 4.4^{b}	42.0 ± 11.0^{bc}	8.1 ± 1.6^{c}	7.4 ± 1.6^{b}
ST4	8.2 ± 0.6^{ab}	16.4 ± 1.5^{bc}	48.9 ± 9.4^{a}	26.2 ± 10.5^{ac}	2.6 ± 0.4^{ab}	10.2 ± 1.2^{cd}
ST5	38.7 ± 10.2^{c}	16.7 ± 2.1^{bc}	22.3 ± 2.4^{b}	21.8 ± 9.9^{ab}	13.7 ± 0.7^{d}	11.7 ± 0.3^{d}

[1] grain fraction less than 63 µm; [2] grain fraction of 63 - 125 µm; [3] grain fraction of 125 - 200 µm; [4] grain fraction larger than 250 µm; [5] CTR was the sediment from the Puck Bay used as CTR in amphipod bioassays. Different superscripts in the same column indicate significant differences among the sites at $p < .05$ (one-way ANOVA and Tukey's HSD test).

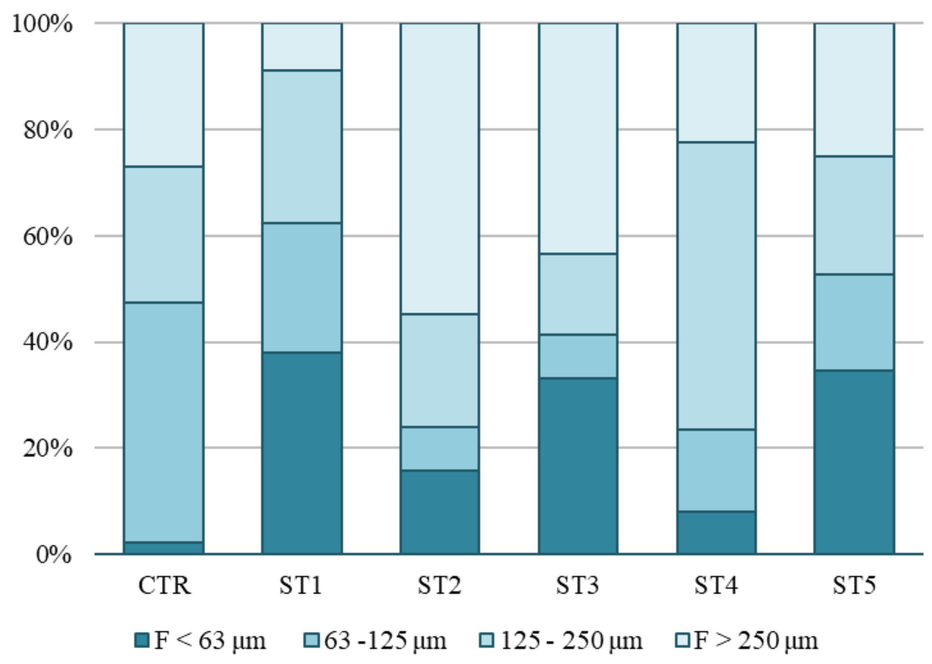

Fig. 31. Content of the grain fractions in the MW&WS and CTR sediments (%, (mean values ± SD).

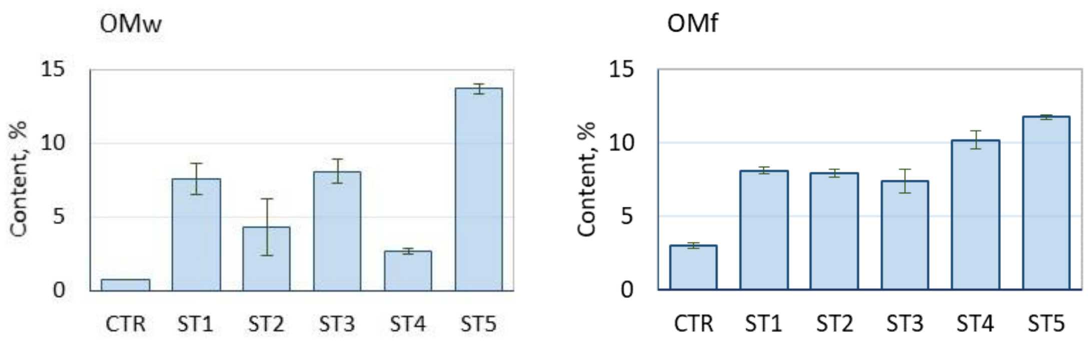

Fig. 32. Content of OM in the MW&WS and CTR sediments (mean values ± SD);
OM_W means OM in the whole sediment; OM_f means OM in the fine sediment fraction).

The concentrations of organic contaminants are shown in Table 30 and Figure 33. Generally, the concentrations were low. They were within the range of values determined in sediments collected in the GoG sites that were distant from the ports. Nevertheless, some significant differences in their levels were found. The ST1 and ST5 sediments had generally greater content of the contaminants than the other sites. These two sediments contained also more than 30 % of the fine fraction. Similarly to the GoG sediments, in the MW&WS sediments $\sum_{16}$PAHs dominated quantitatively, while the content of $\sum_{6}$PBDE was the least and generally negligible. As shown in Table 31, none of the contaminants assessed against respective EACs and ERLs exceeded any criterion, their levels were several to several dozen times lower than the respective criteria.

Tab. 30. Concentrations of organic contaminants (ng g^{-1} d.m.) in the MW&WS sediments (mean values ± SD).

Sediment	$\sum$DDTs	$\sum_{7}$PCBs	$\sum_{16}$PAHs	$\sum_{6}$PBDEs	HCB
CTR	0.2 ± 0.0^{a}	0.03 ± 0.01^{a}	16 ± 1^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
ST1	3.3 ± 0.1^{c}	1.4 ± 0.1^{d}	1113 ± 19^{b}	0.05 ± 0.00^{bc}	1.37 ± 0.08^{f}
ST2	2.1 ± 0.1^{b}	1.4 ± 0.2^{d}	327 ± 5^{ab}	0.02 ± 0.00^{ab}	0.40 ± 0.00^{d}
ST3	1.7 ± 0.2^{b}	1.0 ± 0.1^{c}	266 ± 9^{ab}	0.06 ± 0.00^{c}	0.31 ± 0.04^{cd}
ST4	0.6 ± 0.0^{a}	0.5 ± 0.0^{b}	268 ± 18^{ab}	0.02 ± 0.00^{ab}	0.08 ± 0.01^{b}
ST5	4.8 ± 0.5^{d}	2.2 ± 0.1^{e}	765 ± 20^{b}	0.06 ± 0.02^{c}	0.50 ± 0.08^{de}

Different superscripts within the same column indicate significant differences among the sites at $p < .05$ (one-way ANOVA and Tukey's HSD, except for $\sum_{16}$PAHs for which the Kruskal-Wallis test and multiple comparisons on ranks were applied).

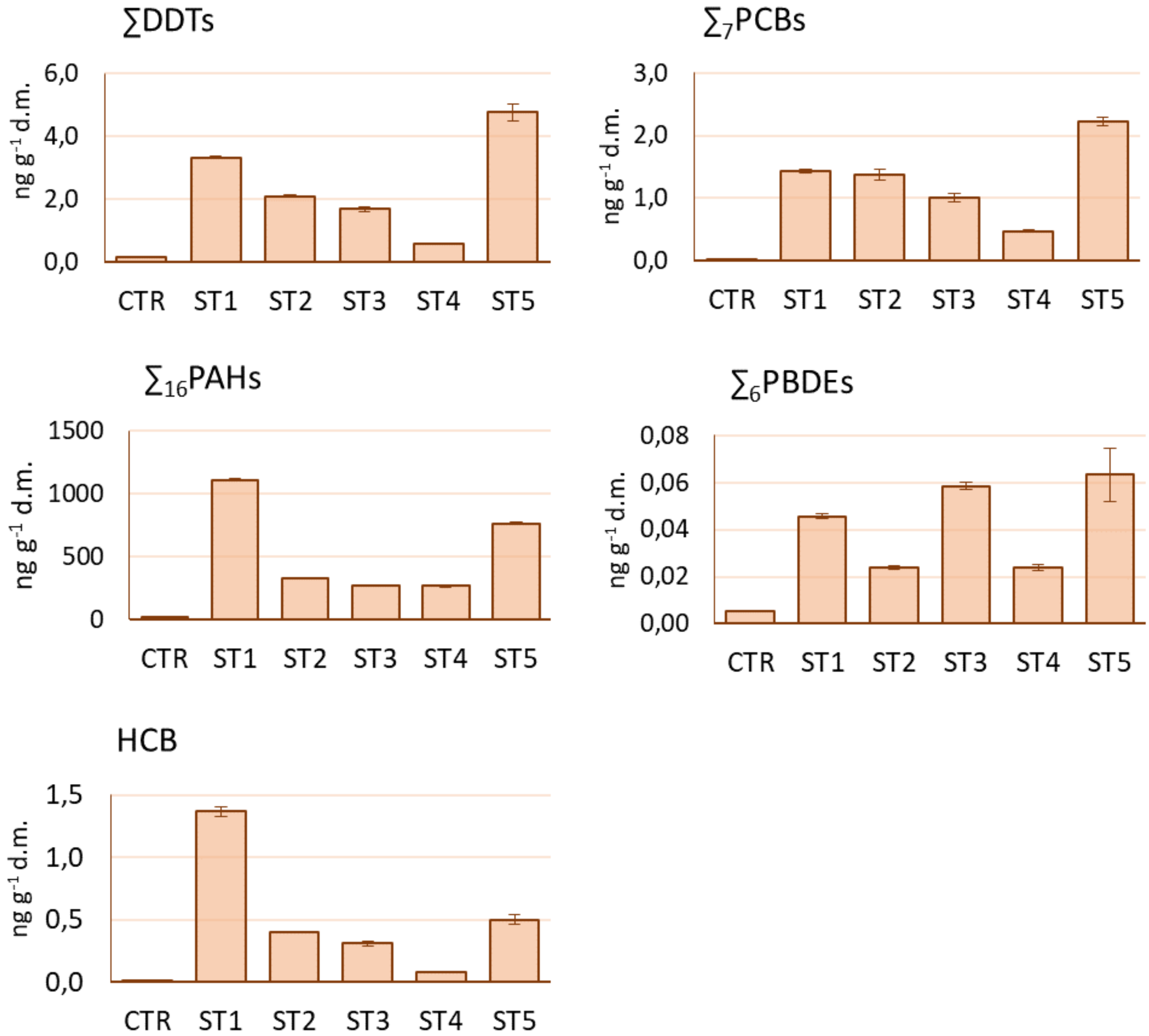

Fig. 33. Contaminants in the MW&WS and CTR sediments (mean values ± SD).

Tab. 31. Mean concentrations of the contaminants in the MW&WS sediments (ng g^{-1} d.w.) evaluated against environmental assessment criteria (EAC, ERL).

HOC	ST1	ST2	ST3	ST4	ST5	EAC [a]
PCB 28	0.12	0.14	0.14	0.06	0.37	1.7
PCB 52	0.11	0.07	0.08	0.09	0.14	2.7
PCB 101	0.14	0.12	0.11	0.03	0.34	3.0
PCB 118	0.14	0.13	0.08	0.03	0.24	0.6
PCB 153	0.37	0.40	0.25	0.13	0.57	7.9
PCB 138	0.35	0.32	0.22	0.09	0.25	40
PCB 180	0.21	0.19	0.13	0.04	0.32	12
$\sum_7$PBDE	0.05	0.02	0.06	0.02	0.06	310 [c]
						ERL [b]
HCB	1.37	0.40	0.31	0.08	0.50	20
p.p-DDE	0.79	0.75	0.63	0.22	1.96	2.2
$\sum_{16}$PAHs	1113	327	266	268	765	3340

[a] EAC (Environmental Assessment Criterion) and [b] ERL (Effects Range Low) values from OSPAR (2009a, 2009b); [c] EAC from ICES (2012).

PCA applied to evaluate the relations between the sediment features (Table 29) and contaminants (Table 30), yielded three PCs that explained the total variance of 87.7 % (Table 32). PC1 (57.0 % of the total variance) showed highly significant positive correlations for the contaminants with the content of the fines, OM_w, and OM_f. The other two PCs, PC2 and PC3 (17.6 % and 13.1 % of the total variance, respectively), were not connected with contaminants. They showed significant relationships between the sediment's natural components. The graph produced by PCA (Figure 34) presenting the distribution of the sites on a factor plane showed their dispersion i.e., ST1, ST4, and ST5 separated from each other and from ST2 and ST3. This depicted the differences among them regarding the sediment characteristics and contaminants, while the proximity of the latter two sites to each other indicated their similarity in this respect.

Tab. 32. PCA statistics showing the associations between the MW&WS sediment characteristics and contaminants.

PC	PC1	PC2	PC3
Eigenvalues	6.3	1.9	1.4
Variance explained (87.7 %)	57.0	17.6	13.1
F < 63 µm	-0.906	0.064	0.211
F, 63 - 125 µm	0.489	0.718	0.327
F, 125 - 250 µm	0.344	0.332	-0.870
F > 250 µm	0.300	-0.887	0.158
OM_w	-0.910	-0.088	0.050
OM_f	-0.681	-0.178	-0.684
HCB	-0.702	0.456	0.170
∑DDTs	-0.949	0.020	0.059
$\sum_7$PCBs	-0.939	-0.170	0.016
$\sum_{16}$PAHs	-0.847	0.433	-0.040
$\sum_6$PBDEs	-0.845	-0.242	-0.022

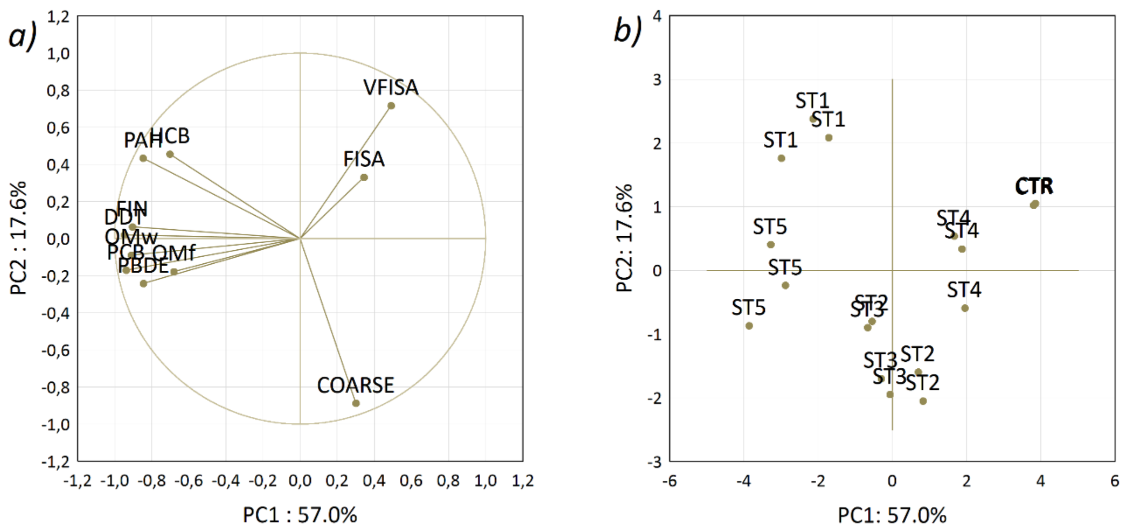

Fig. 34. PCA results showing the associations among the MW&WS sediment features and contaminants: a) relationships among the variables and b) the distribution of the sites on the factor-plane (variables are presented in Table 32). Abbreviations: FIN - fines (F < 63 µm); VFISA - very fine sand (F, 63 - 125 µm); FISA - fine sand (F, 125 - 250 µm); COARSE - coarser sediment fractions (F > 250 µm); OM_f - organic matter in the fine fraction, OM_w - whole organic matter.

5.2.3 Bioassay with *Corophium multisetosum*

5.2.3.1 *Physicochemical parameters of the overlying water*

The mean values of the water parameters in all test vessels were almost constant throughout the tests and similar to those in the bioassays of the GoG sediments. Temperature had mean value of 15.0 ± 0.2 °C, pH of 7.8 ± 0.1, oxygen (mg l^{-1}) - 9.3 ± 0.05, and salinity of 7.7 ± 0.1 (Table 33).

Tab. 33. Properties of water in the test vessels (mean values ± SD).

Sediment	Temperature °C	pH	Oxygen mg l^{-1}	Salinity
CTR	14.7 ± 0.04	7.9 ± 0.05	9.3 ± 0.1	7.6 ± 0.0
ST1	15.3 ± 0.1	7.7 ± 0.04	9.3 ± 0.04	7.8 ± 0.1
ST2	15.0 ± 0.2	7.6 ± 0.02	9.2 ± 0.1	7.7 ± 0.04
ST3	15.2 ± 0.1	7.9 ± 0.02	9.3 ± 0.1	7.8 ± 0.1
ST4	15.1 ± 0.2	7.7 ± 0.02	9.3 ± 0.04	7.7 ± 0.0
ST5	14.8 ± 0.1	7.7 ± 0.1	9.4 ± 0.02	7.7 ± 0.0

5.2.3.2 *C. multisetosum responses*

The results of the bioassay are presented in Table 34 and Figure 35. The mean survival of *C. multisetosum in* CTR was high in all replicates with a mean of 93 % and met the acceptability criteria for the bioassay (mortality < 20 %). The mean survival of amphipods in all tested sediments exceeded 70 %. It did not differ significantly among the sediment exposures (one-way ANOVA, $p < .05$), yet it was the lowest in ST3 (72 %) and the highest in ST2 (89 %).

The final mean individual length of the amphipods during the 28 days varied from 3.2 to 4.7 mm (ST3 and ST5, respectively). The mean length increment was in the range of 0.8 - 2.3 mm ind.$^{-1}$. The mean GR ranged from 28 to 81 μm day^{-1}; in CTR it was 35 ± 8 μm day^{-1}. It was significantly greater in ST5 and ST4 than in ST3, and significantly greater in ST5 than in ST2 (Kruskal-Wallis and multiple comparisons on ranks, $p < .05$).

The number of molts, in the range of 0.5 - 1.8 per amphipod, was the same in CTR as in ST5 (the number of molts indicated that each amphipod molted almost twice during the 28-d bioassays), and significantly greater than in all other tested sediments (one-way ANOVA and HSD Tukey test, $p < .05$). The percentage of males at the bioassay termination was very low in all sediment exposures, 0 - 14 % (no males were found in ST2 and ST3). Gravid females, juveniles, and sediment avoidance were not observed in any sediment exposure.

Tab. 34. Results of bioassays with *C. multisetosum* (mean values ± SD), CV_{wt} in the brackets).

Sediment	Survival %	Growth rate [1] μm day^{-1}	Molts [2] n ind.$^{-1}$	Males [3] %
CTR	93 ± 7^{a} (7)	35 ± 8^{ac} (22)	1.8 ± 0.4^{b} (24)	3 ± 7^{a}
ST1	84 ± 15^{a} (18)	36 ± 7^{ac} (20)	1.0 ± 0.6^{a} (58) *	8 ± 11^{a}
ST2	89 ± 6^{a} (7)	32 ± 4^{ab} (11)	0.9 ± 0.3^{a} (29) *	0 ± 0^{a}
ST3	72 ± 30^{a} (42)	28 ± 2^{a} (6)	0.5 ± 0.3^{a} (61) *	0 ± 0^{a}
ST4	76 ± 17^{a} (22)	61 ± 4^{bc} (6) *	0.9 ± 0.4^{a} (44) *	14 ± 3^{a}
ST5	79 ± 7^{a} (9)	81 ± 4^{c} (5) *	1.8 ± 0.4^{b} (21)	10 ± 10^{a}
mean CV_{wt} [4]	18 %	12 %	40 %	-
CV_{at} [5]	10 %	46 %	46 %	-

[1] the mean growth rate during 28-d (μm day^{-1}); [2] the number of molts during the bioassays per initial number of individuals (n ind.$^{-1}$); [3] the percentage of adult males relative to all individuals at the end of bioassays (%); [4] CV_{wt} - mean within-treatments coefficient of variation; [5] CV_{at} - among-treatments coefficient of variation. Different superscripts in the same column indicate significant differences among the sites; survival and molts - one-way ANOVA and HSD Tukey test, $p < .05$; growth and surviving males - Kruskal-Wallis test, $p < .05$. An asterisk indicates a significant difference from CTR based on pairwise comparisons, one-tailed t-test at $p < .05$, except for survival where U test was performed at $p < .05$.

Pairwise comparisons to CTR (one-tailed t-tests and Mann-Whitney U tests, $p < .05$) indicated no significant differences in survival and the percentage of males between the tested sediments and CTR, whereas some differences were found in the case of GR and molts. Namely, GR in ST4 and ST5 was significantly greater than in CTR, while the molting was significantly less frequent in ST1, ST2, ST3, and ST4 than in CTR.

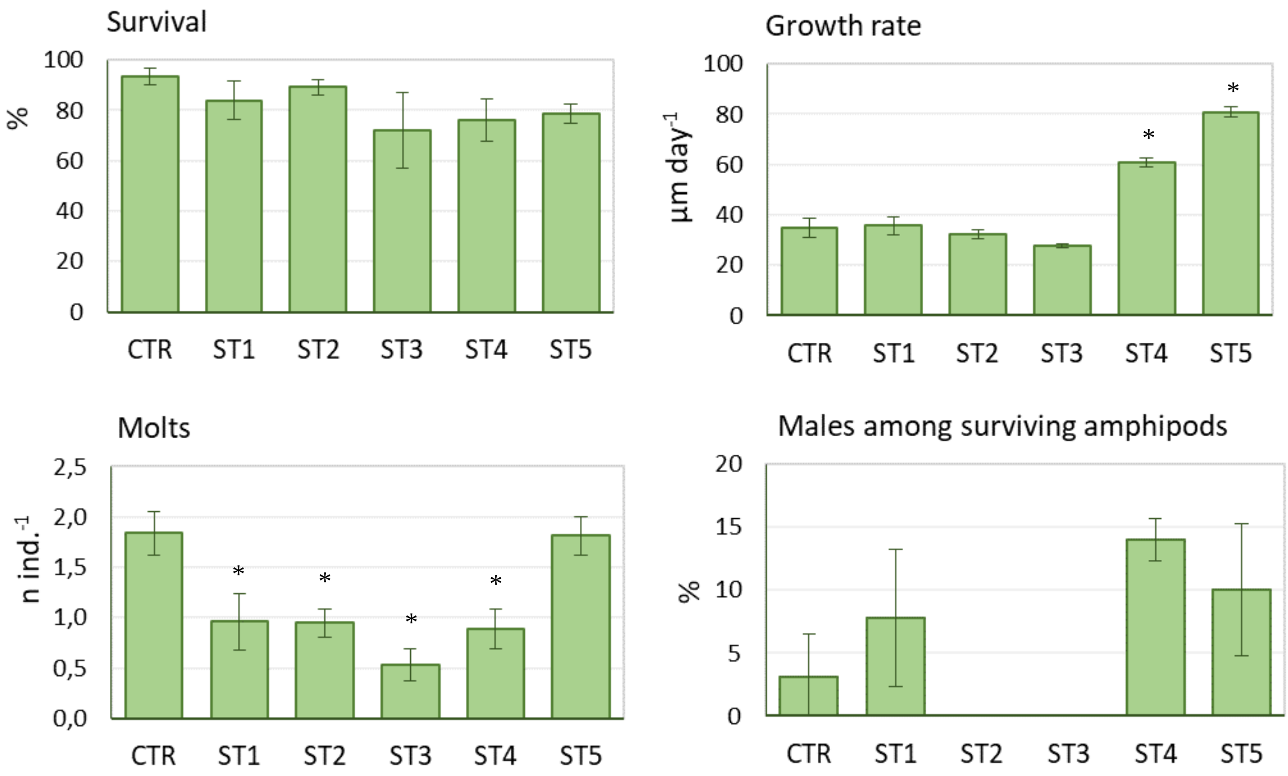

Fig. 35. Responses of *C. multisetosum* exposed to the MW&WS and CTR sediments (mean values ± SD). An asterisk marks significant differences from CTR.

5.2.3.3 *Associations between amphipod responses and sediment features*

There were few correlations among amphipod responses and sediment features indicated by Kendall's tau test (Table 35). The survival was negatively related to the content of OM_f, as well as OM_w. GR correlated positively with the number of molts, the sediment fine sand fraction (F, 125 - 250 µm), and OM_f content. The frequency of molting was positively related to the very fine sand content (F, 63 - 125 µm). Whereas, no significant relationships between amphipod responses and the sediment fines were found.

Tab. 35. Kendall's tau (τ) correlation coefficients present significant relationships between the *C. multisetosum* responses and the MW&WS sediment features ($p < .05$).

	Survival	Growth	Molts
Survival			
Growth			0.36
Molts		0.36	
F > 250 µm			
F, 125 - 250 µm		0.33	
F, 63 - 125 µm			0.37
The fines			
OM_f	-0.35	0.52	
OM_w	-0.38		

The associations between biological responses and sediment components and contaminants examined with PCA have been shown in Table 36. The PCA variables included the *C. multisetosum* survival, growth, and molts, as well as sediment characteristics (sediment fractions, OM_f, and OM_w). Because the bivariate analysis indicated no significant relations between biological responses and the sediment contaminants, the latter were not included in PCA, similarly to the grain fraction > 250 µm. The sediment fine fraction content was included in PCA (despite an indication of no significance for biological responses) because this sediment fraction was found to play an important role in the bioassays with the GoG sediments. The PCA yielded four principal components (PCs) with eigenvalues > 1.0, which explained 93 % of the total variance. PC1 (40.6 % of the total variance) indicated significant positive correlations for growth with sediment OM_w, OM_f, and the fines content, and negative growth relation with the sediment grain size of 63 - 125 µm (Table 36). Furthermore, a positive and highly significant growth correlation with the sediment grain size of 125 - 250 µm was shown by PC2 (21.7 % of the total variance). PC3 (20.6 % of the total variance) extracted high loadings for molts, which seemed to moderately correlate with growth and the sediment fraction of 63 - 125 µm. The amphipod survival was extracted as the only variable with highly significant loading by PC4 (10.1 % of the total variance).

The graphical presentation of the PCA outcome is shown in Figure 36. The distribution of the sites indicated that ST4 and ST5 were distinctively different from each other and from other sites that were grouped. The separation of ST4 and ST5 is related to better GR in these sediments.

Tab. 36. PCA statistics showing the associations among the responses of *C. multisetosum* and the MW&WS sediment characteristics.

PC	PC1	PC2	PC3	PC4
Eigenvalues	3.2	1.7	1.6	0.8
Variance explained (93.0 %)	40.6	21.7	20.6	10.1
Survival	-0.489	0.107	-0.357	-0.766
Growth	0.509	-0.697	-0.441	-0.122
Molts	-0.169	-0.215	-0.900	0.110
OM_w	0.891	0.203	-0.338	0.070
OM_f	0.879	-0.422	0.099	-0.137
F, 125 - 250 µm	-0.147	-0.878	0.362	0.039
F, 63 - 125 µm	-0.695	-0.114	-0.469	0.409
F < 63 µm	0.806	0.437	-0.200	0.067

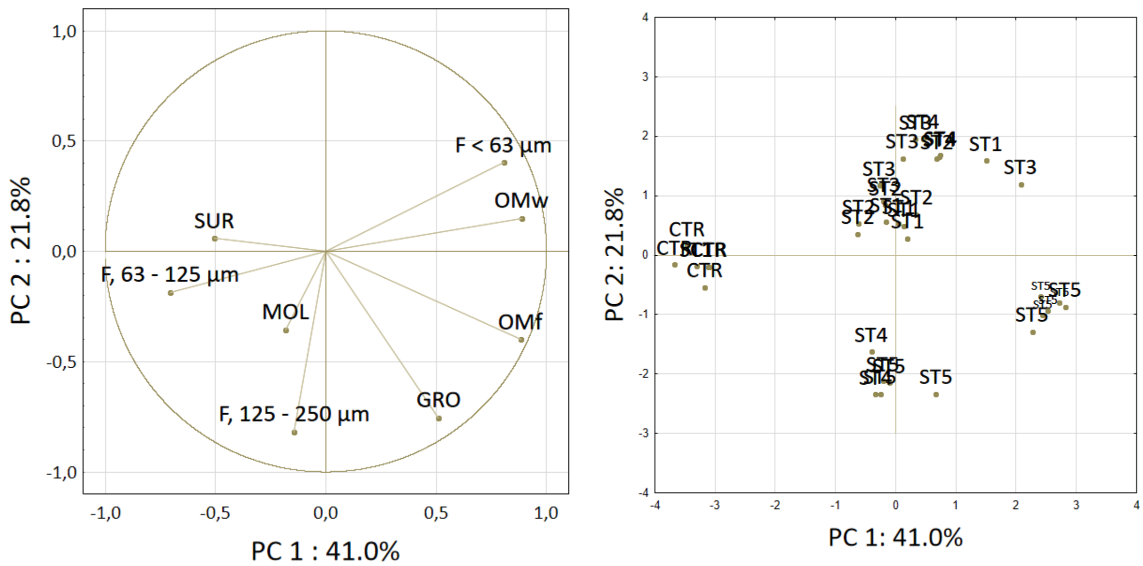

Fig. 36. PCA statistics showing the associations among the *C. multisetosum* responses and sediment features: a) relationships among the variables and b) the distribution of the sites on the factor-plane (variables are presented in Table 36). Variable abbreviations: SUR - survival; GRO - growth; MOL - molting; FIN - fines; VFISA - very fine sand; FISA - fine sand.

5.2.4 *H. incongruens* responses

The test acceptability criteria recommended by the producer of the Ostracodtoxkit test were achieved in the bioassays i.e., the mean mortality of ostracods in CTR sediment was lower than 20 %, and the mean length of ostracods in the CTR sediment increased by a factor of 1.5 compared to the day 0 of the test (the mean length was 200 µm at the beginning of the test). The sensitivity of *H. incongruens* was verified by assessing the lethal concentration (6d LC_{50}) of a reference compound ($CuSO_4 \cdot 5H_2O$). The ostracod survival after 6 days of exposure to various copper sulfate concentrations is shown in Table 37. The calculated 6d LC_{50} value was 11.2 mg l^{-1}, which slightly exceeded the range limit indicated by the Ostracodtoxkit producer (2.21 - 9.37 mg l^{-1}).

Tab. 37. Survival of *Heterocypris incongruens* exposed to copper sulfate for 6 days (mean values ± SD).

$CuSO_4$ mg l^{-1}	Replicates n	Survival %
0	6	83 ± 8
1	3	83 ± 15
1.8	3	67 ± 15
3.2	3	83 ± 15
5.6	3	47 ± 6
10	3	47 ± 21

The results of the ostracod bioassays with the MW&WS sediments are presented in Table 38. The mean survival of ostracods was high in all sediment exposures (87 - 95 %), and no significant differences in this response among the exposures were found in both the Kruskal-Wallis test ($p < .05$) and pairwise comparisons with CTR (one-tailed t-test at $p < .05$). The mean CV_{wt} for survival was 12 %, and CV_{at} was 4 %. The mean growth of *H. incongruens* was in the range of 522 - 650 µm reaching 528 µm in CTR. Pairwise comparisons with CTR indicated significantly greater growth in ST2 (650 µm) and ST4 (620 µm) than in CTR (one-tailed *t*-test at $p < .05$). The growth was smallest in ST5 (522 µm), yet it did not differ significantly among the ostracods exposed to the sediments (one-way ANOVA, $p < .05$). None of the sediment-exposed ostracods had mean growth less than that of CTR. The mean CV_{wt} for growth was 8 %, and CV_{at} was 63 %.

Tab. 38. Results of bioassay with *Heterocypris incongruens* exposed to the MW&WS sediments (mean values ± SD), CV_{wt} in the brackets).

Sediment	Replicates n	Survival %	Growth µm
CTR	6	95 ± 8 (9) [a]	528 ± 80 (15) [a]
ST1	6	87 ± 15 (17) [a]	588 ± 59 (10) [ab]
ST2	6	88 ± 10 (11) [a]	650 ± 37 (6) * [b]
ST3	6	85 ± 12 (14) [a]	556 ± 31 (6) [ab]
ST4	6	95 ± 8 (9) [a]	620 ± 83 (13) * [ab]
ST5	6	87 ± 10 (12) [a]	[1]522 ± 6 (1) [ab]
mean CV_{wt}		12 %	8 %
CV_{at}		4 %	63 %

[1] Two replicates instead of six were considered in the ST5 growth calculations due to technical issues.
Different superscripts in the same column indicate significant differences between the exposures (survival - Kruskal-Wallis test at $p < .05$; growth - one-way ANOVA with post-hoc HSD Tukey test at $p < .05$). An asterisk indicates the value significantly different from the CTR in pairwise comparisons (one-tailed t-test at $p < .05$).

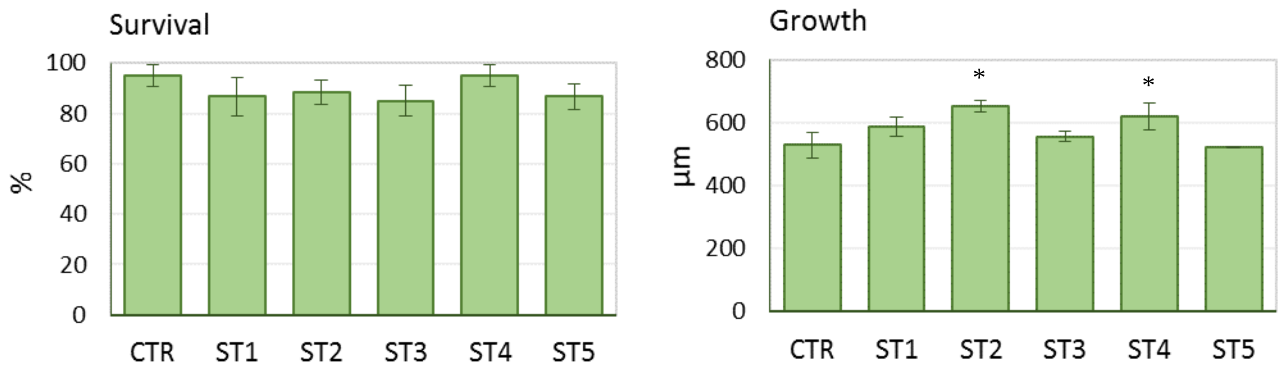

Fig. 37. Survival and growth of *H. incongruens* exposed to the MW&WS and CTR sediments (mean values ± SD). An asterisk indicates a significant difference from the CTR.

5.2.5 Benthic fauna in the Martwa and Śmiała Wisła Rivers

5.2.5.1 *Abundance and diversity*

In the macrobenthos samples, a total of 18 taxa were identified (Table 39). These included 11 species, 2 genera (*Mytilus* sp., *Marenzelleria* sp.), and 5 macrobenthos identified to higher taxa (Calanoida, Oligochaeta, Hydrobiidae, Chironomidae, Ostracoda). The mean total density of macrobenthos ranged from 1307 at ST3 to 9407 ind. m^{-2} at ST5 (Figure 38). The Hydrobiidae gastropods were dominating at each site (593 - 7680 ind. m^{-2}), followed by oligochaetes (243 - 1310 ind. m^{-2}), bivalves (137 - 1553 ind. m^{-2}), polychaetes (277 - 1187 ind. m^{-2}), and ostracods (0 - 1070 ind. m^{-2}). Their abundance constituted 68, 12, 11, 5, and 4 % of the total abundance, respectively (Table 40). Comparison of macrobenthos composition at each site is shown in Figure 39. Significant inter-site differences were found in the density of Hydrobiidae between ST3 and ST1 and ST5, and bivalves between ST3 and ST1. Both bivalves and hydrobiids had the lowest densities at ST3. Of the bivalves, *Limecola balthica* was the dominating species at ST1 (4.9 % of total abundance), while *Rangia cuneata* prevailed at all other sites (5.7 % of total abundance). Among crustaceans, the ostracods were present only at ST3-ST5, calanoids at ST5, and *C. multisetosum* at ST1-ST2.

Tab. 39. The abundance of macrobenthos (species and higher taxa) at the MW&WS sites (ind. m-2, mean values ± SD).

Taxa / Station	ST1	ST2	ST3	ST4	ST5
Bivalvia	1553 ± 665 [a]	267 ± 274 [ab]	137 ± 38 [b]	1060 ± 26 [ab]	397 ± 80 [ab]
R. cuneata	60 ± 66	190 ± 208	137 ± 38	1050 ± 35	357 ± 81
L. balthica	1450 ± 711	53 ± 47	0 ± 0	7 ± 12	10 ± 10
D. polymorpha	0 ± 0	3 ± 6	0 ± 0	3 ± 6	3 ± 6
M. arenaria	3 ± 6	7 ± 12	0 ± 0	0 ± 0	23 ± 40
C. glaucum	17 ± 15	10 ± 10	0 ± 0	0 ± 0	0 ± 0
Mytilus sp.	23 ± 21	3 ± 6	0 ± 0	0 ± 0	3 ± 6
Crustacea	7 ± 6 [a]	17 ± 29 [a]	190 ± 226 [a]	1077 ± 1848 [a]	77 ± 67 [a]
C. multisetosum	7 ± 6	17 ± 29	0 ± 0	0 ± 0	0 ± 0
C. carinata	0 ± 0	0 ± 0	0 ± 0	3 ± 6	0 ± 0
A. aquaticus	0 ± 0	0 ± 0	0 ± 0	3 ± 6	0 ± 0
N. integer	0 ± 0	0 ± 0	0 ± 0	0 ± 0	7 ± 12
Calanoida	0 ± 0	0 ± 0	0 ± 0	0 ± 0	40 ± 53
Ostracoda	0 ± 0	0 ± 0	190 ± 226	1070 ± 1853	30 ± 52
Polychaeta	507 ± 57 [a]	387 ± 95 [a]	13 ± 15 [a]	637 ± 408 [a]	13 ± 6 [a]
H. diversicolor	503 ± 59	353 ± 144	10 ± 10	463 ± 280	7 ± 6
Marenzelleria sp.	0 ± 0	23 ± 40	3 ± 6	120 ± 62	7 ± 12
S. shrubsoli	3 ± 6	10 ± 10	0 ± 0	53 ± 67	0 ± 0
Oligochaeta	1310 ± 450 [a]	243 ± 239 [a]	367 ± 276 [a]	503 ± 612 [a]	1180 ± 868 [a]
Hydrobiidae	5333 ± 1589 [a]	4063 ± 2074 [ab]	593 ± 370 [b]	3587 ± 938 [ab]	7680 ± 2520 [a]
Chironomidae	17 ± 12 [a]	0 ± 0 [a]	7 ± 6 [a]	0 ± 0 [a]	60 ± 56 [a]

Different superscripts in the same row indicate significant differences among the sites at $p < .05$ (Kruskal-Wallis test, except for the Hydrobiidae, Chironomidae where one-way ANOVA and Tukey’s HSD were applied).

Tab. 40. The percentage of each taxon abundance relative to the total macrobenthos abundance at the MW&WS sites (%).

Taxa / Station	ST1	ST2	ST3	ST4	ST5	Total %[1]
Bivalvia	4.97	0.85	0.44	3.39	1.27	10.91
R. cuneata	0.19	0.61	0.44	3.36	1.14	5.73
L. balthica	4.64	0.17	0.00	0.02	0.03	4.86
D. polymorpha	0.00	0.01	0.00	0.01	0.01	0.03
M. arenaria	0.01	0.02	0.00	0.00	0.07	0.11
C. glaucum	0.05	0.03	0.00	0.00	0.00	0.09
Mytilus sp.	0.07	0.01	0.00	0.00	0.01	0.10
Crustacea	0.02	0.05	0.61	3.44	0.25	4.37
C. multisetosum	0.02	0.05	0.00	0.00	0.00	0.07
C. carinata	0.00	0.00	0.00	0.00	0.13	0.13
A. aquaticus	0.00	0.00	0.00	0.01	0.00	0.01
N. integer	0.00	0.00	0.00	0.01	0.00	0.01
Calanoida	0.00	0.00	0.00	0.00	0.02	0.02
Ostracoda	0.00	0.00	0.61	3.42	0.10	4.12
Polychaeta	1.62	1.24	0.04	2.04	0.04	4.98
H. diversicolor	1.61	1.13	0.03	1.48	0.02	4.27
Marenzelleria sp.	0.00	0.07	0.01	0.38	0.02	0.49
S. shrubsoli	0.01	0.03	0.00	0.17	0.00	0.21
Oligochaeta	4.19	0.78	1.17	1.61	3.77	11.52
Hydrobiidae	17.05	12.99	1.90	11.47	24.55	67.96
Chironomidae	0.05	0.00	0.02	0.00	0.19	0.27

[1]The percentage of total number of collected individuals per taxa relative to total macrobenthos abundance.

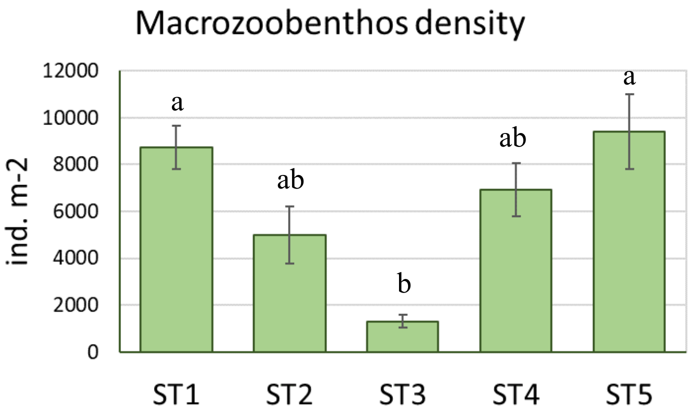

Fig. 38. The mean density of macrobenthos (ind. m-2, mean values ± SD). The letters mark significant differences among the sites (one-way ANOVA and post hoc HSD Tukey test, p < .05).

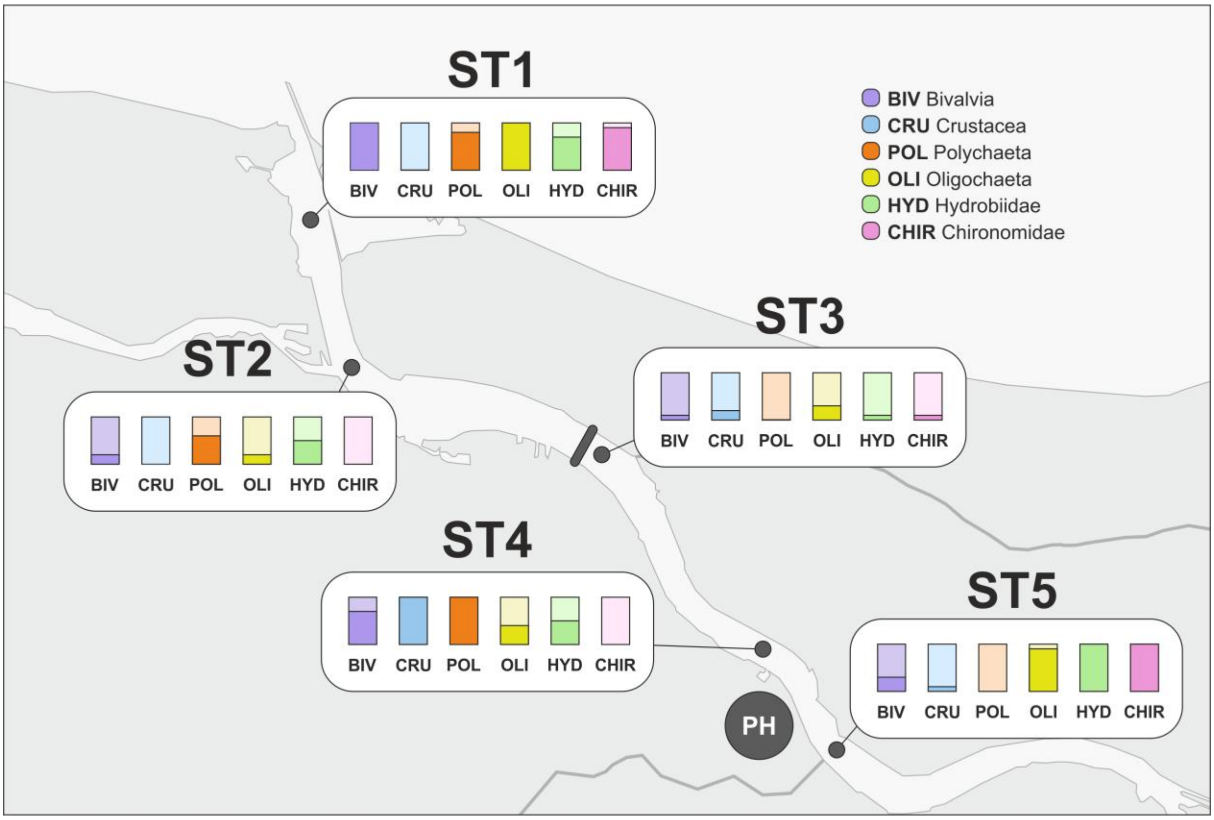

Fig. 39. Comparison of macrobenthos composition at each MW&WS site. The bars represent the density of the individuals of the given taxa referred to its maximum value in the study area. BIV – Bivalvia, CRU – Crustacea, POL – Polychaeta, OLI – Oligochaeta, HYD – Hydrobiidae, CHIR – Chironomidae.

The number of taxa and macrobenthos diversity indices for the MW&SW sites are shown in Table 41 and Figure 40. At each site 8 taxa were identified, except for ST3 where only 5 taxa were found. The mean values for the macrobenthos indices, i.e. The Margalef, Pielou's, Shannon, and Simpson, were in the range of 1.3 - 1.4, 0.3 - 0.7, 0.6 - 1.2, and 0.3 - 0.6, respectively. There were no among-site differences in the Margalef and Shannon indices. The Pielou's evenness was significantly greater at ST3 than ST2 and ST5 indicating that the individual taxa at ST3 were numerically more even i.e., there was less variation in communities between the taxa. The Simpson index was two times lower at ST2 and ST5 than at the other three sites, however, the only significant difference was between ST5 and ST3 (one-way ANOVA with post-hoc Tukey's HSD, $p < .05$). The Shannon, Pielou's, and Simpson indices had the lowest values at ST2 and ST5. It should be noted that these sites were characterized by a greater standard deviation in the number of taxa. The multimetric B index was in the range of 1.11- 2.00 being markedly greater at ST3 than in other sites.

Tab. 41. The number of taxa and diversity indices of the macrobenthos community in the MW&SW area (mean values ± SD).

Site	B index	Taxa, n	Margalef (d)	Pielou's evenness (J')	Shannon (H')	Simpson (1 - λ)
ST1	1.52	8 ± 2	1.4 ± 0.4^{a}	0.5 ± 0.1^{ab}	1.1 ± 0.2^{a}	0.57 ± 0.07^{ab}
ST2	1.77	8 ± 4	1.4 ± 0.6^{a}	0.4 ± 0.1^{a}	0.7 ± 0.2^{a}	0.33 ± 0.09^{ab}
ST3	2.00	5 ± 2	1.3 ± 0.4^{a}	0.7 ± 0.0^{b}	1.2 ± 0.2^{a}	0.64 ± 0.08^{a}
ST4	1.11	8 ± 2	1.4 ± 0.3^{a}	0.6 ± 0.2^{ab}	1.1 ± 0.4^{a}	0.56 ± 0.17^{ab}
ST5	1.50	8 ± 3	1.3 ± 0.5^{a}	0.3 ± 0.1^{a}	0.6 ± 0.2^{a}	0.29 ± 0.14^{b}

Different superscripts in the same column indicate significant differences among the sites at $p < .05$ (one-way ANOVA with post-hoc Tukey's HSD, Pielou's evenness data were transformed to square roots before the analysis).

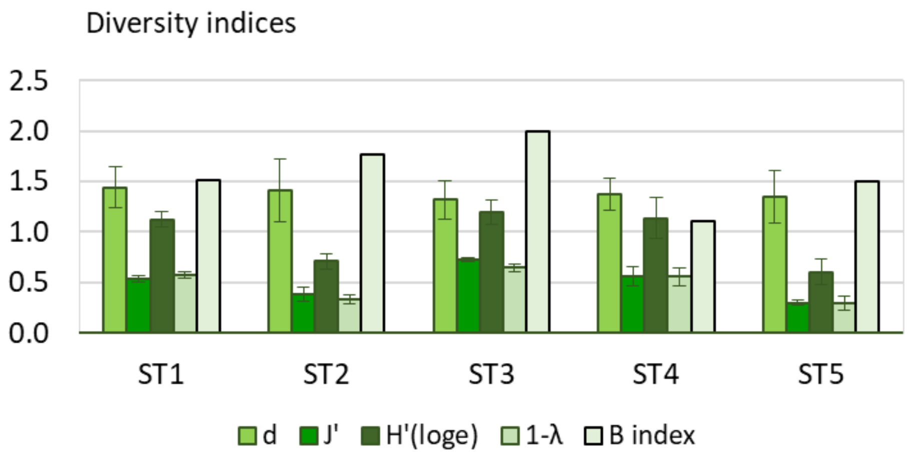

Fig. 40. Macrobenthos diversity indices at the MW&WS sites (mean values ± SD). Margalef (d), Pielou's evenness (J'), Shannon (H'), Simpson (1 - λ) and B index.

The ANOSIM analysis of the macrobenthos composition yielded the R statistic of 0.683 demonstrating significant and relatively high among-sites dissimilarity (Table 42). The R statistic for the benthic fauna diversity indices of 0.188 indicated no statistically significant differences among the sites. This analysis also showed that the sediment features (grain sizes distribution and OM_w and OM_f), as well as the sampling area depth and bottom water characteristics (pH, dissolved oxygen, the content of phosphates, temperature, salinity), were dissimilar among the sites, with significant and high R values close to 1.

Tab. 42. Results of one-way ANOSIM describing the similarities in macrobenthos community structure, sediment features, and bottom water characteristics among the MW&WS sites.

Study component	R	p	Perm
Benthic community composition	0.683	0.001	999
Macrobenthos diversity (Simpson's Diversity, Margalef's richness, Pielou's evenness, Shannon)	0.188	0.086	999
Bottom water characteristics	0.984	0.001	999
Sediment features	0.981	0.001	999

5.2.5.2 *Environmental factors influencing the macrobenthos*

Potential relations between the macrobenthos taxa densities (taxa with ≥ 0.5 % of the total abundance were considered), diversity indices, and environmental variables were initially examined with Kendall's tau test. The sediment contaminants were not included in the analysis, because the bioassays with *C. multisetosum* and *H. incongruens* showed no negative effects of the tested sediments on the survival and growth of any of these species, and contaminant concentrations were determined to be at low, often negligible, levels.

Tab. 43. Statistically significant Kendall's tau (τ) correlation coefficients obtained for the macrobenthos densities and environmental variables ($p < .05$). All identified taxa were subjected to this analysis, but only those showing significant correlations are presented.

Environmental features						
Taxa	Sediment	τ	p	Bottom water	τ	p
R. cuneata	OM_f	0.452	< 0.05	salinity	-0.565	< 0.05
L. balthica				phosphates	-0.663	< 0.01
				pH	0.474	< 0.05
				salinity	0.378	< 0.05
Marenzelleria sp.	F < 63 µm	-0.663	< 0.01	salinity	-0.506	< 0.01
	F, 125 - 250 µm	0.473	< 0.05			
Ostracoda				phosphates	0.462	< 0.05
H. diversicolor				phosphates	-0.446	< 0.05
				pH	0.539	< 0.01
Hydrobiidae	OM_f	0.390	< 0.05	phosphates	-0.502	< 0.01
Total abundance	F, 63 - 125 µm	0.524	< 0.01	phosphates	-0.464	< 0.05
	F, 125 - 250 µm	0.390	< 0.05			
	F > 250 µm	-0.410	< 0.05			

The Kendall's test outcome, shown in Table 43, indicated significant relations between taxa densities and environmental variables (sediment and water features listed in Table 29 and Table 28). Generally, the highest correlations for taxa densities with environmental variables were found in case of salinity, pH, and phosphates concentration in water. Considering particular taxa, the abundance of *R. cuneata* was positively related to the content of OM_f and negatively to salinity. The *L. balthica* abundance was positively related to water pH and salinity, while negatively to the concentration of phosphates in the bottom water. The abundance of *Marenzelleria* sp. was negatively correlated with water salinity and the sediment fines (F < 63 µm), and positively with the fine sand fraction (F, 125 - 250 µm). The ostracods densities showed a moderate positive relationship with the concentration of phosphates, in contrast to *H. diversicolor* (polychaetes) for which the relation with phosphates was negative. The polychaetes abundance was positively related to the pH of the bottom water. Hydrobiidae were positively associated with OM_f content, and negatively with the phosphates in the bottom water. Considering the total abundance of fauna, it correlated negatively with the concentration of the phosphates in the bottom water and coarser sediment fractions (F > 250 µm), and positively with the finer sand fractions (F, 63 - 125 and F, 125 - 250 µm). No significant correlations between the diversity indices and the environmental characteristics were found.

Tab. 44. The results of DistLM analysis (marginal tests) for fitting environmental variables to the macrobenthic assemblages.

Variable	Pseudo-F	p	Proportion
Depth	2.5592	0.0406	0.16448
Salinity	2.2634	0.0666	0.14829
Oxygen	2.0416	0.0959	0.13573
pH	3.8533	0.0057	0.22864
Phosphates	4.713	0.0016	0.26608
OMw	2.7998	0.0266	0.17720
OMf	1.4353	0.2248	0.00994
F > 250 µm	1.0435	0.4034	0.00743
Fine sand	2.3734	0.0473	0.15439
Very fine sand	1.3075	0.2819	0.00913
Fines	2.6126	0.0365	0.16734

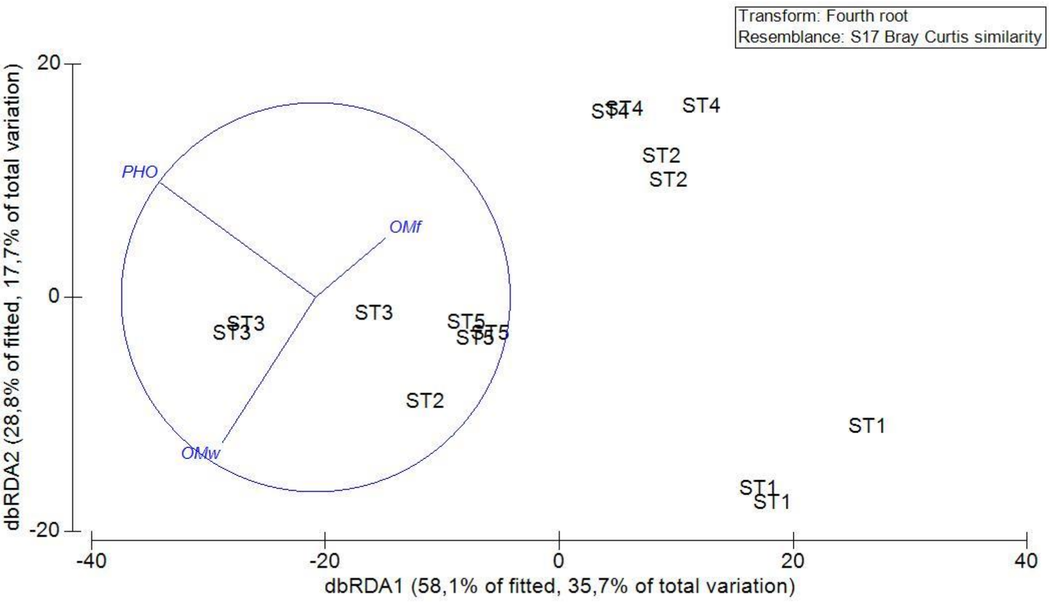

Fig. 41. Distance-based redundancy (dbRDA) plot illustrating the DistLM model based on macrobenthic assemblages and the fitted environmental variables as vectors based on DistLM analysis presented in Table 44.

Relations between the macrobenthos densities and environmental variables investigated using the DistLM procedure in PERMANOVA included the same variables as those in the Kendall's test. Marginal tests resulting from the DistLM analysis indicated depth, pH, phosphates, OM_w, fine sand, and the fines as the ecological factors that significantly influenced the distribution and composition of macrobenthos (Table 44), of which pH and phosphates concentration explained 23 % and 27 % of the variability, respectively. The overall best solution indicated three environmental variables that together explained 61 % of the variance in the macrobenthos distribution, namely the concentration of phosphates in the bottom water and the content of organic matter, both in whole sediment and the fines (Figure 41).

5.2.6 Evaluation of the MW&WS sediments

Neither survival nor growth of *C. multisetosum* or *H. inconcruens* were negatively affected by the exposure to the sediments, indicating that these sediments presented no toxicity. The macrobenthos abundance and composition were found to be related to sediment natural features and phosphate concentration in water. Figure 42 provides the summary of *Corophium* and *Heterocypris* responses in bioassays, and macrobenthos abundance in the MW&WS sediments. The graphs represent the value obtained for the site referred to the maximum observed value in the study area for each category.

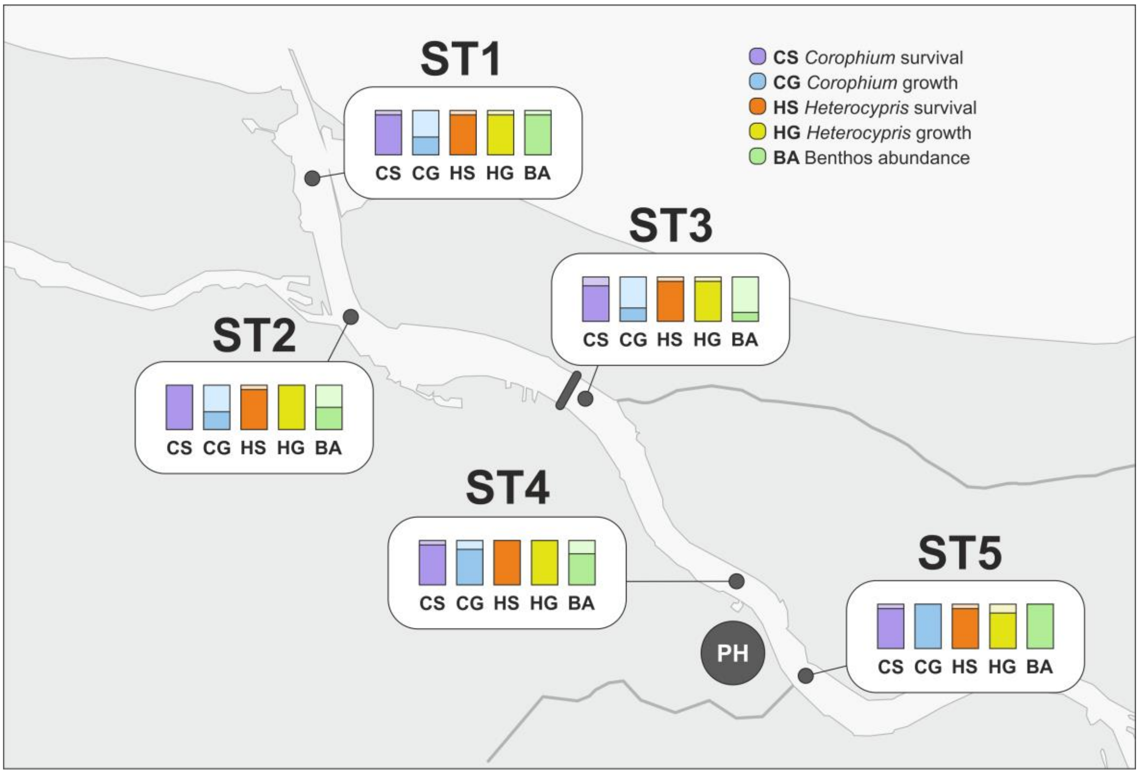

Fig. 42. Comparison of survival and growth of *Corophium multisetosum* and *Heterocypris incongruens* exposed to MW&WS sediments and macrobenthos abundance.

6 Discussion

6.1 Survival

The field-collected *C. volutator* individuals, divided into two populations from Kaczy Winkiel and Rzucewo, showed some differences in sensitivity to the water-only solution of cadmium as a reference toxicant. The obtained 72-h LC_{50} values were 0.68 mg l^{-1} (CL: 0.30 - 1.54) and 1.36 mg l^{-1} (CL: 0.78 - 2.40), respectively. Nonetheless, when Picone et al. (2008) evaluated *C. orientale* as a bioindicator for the Venice Lagoon, they found that up to threefold differences in LC_{50} for Cd exposures can be considered normal variability in laboratory testing. They reported a mean 96-hour Cd LC_{50} value for *C. orientale* of 3.3 mg l^{-1} (CL: 0.87 - 5.80), with seasonal variations ranging from 1.44 to 10.5 mg l^{-1}. Kater et al. (2000) presented results from several years of studying acute Cd toxicity to *C. volutator*, reporting 72-hour Cd LC_{50} values ranging from about 1.9 to 5.8 mg l^{-1}. Re et al. (2009) tested various environmental conditions with *C. multisetosum* that could influence the outcome of Cd acute toxicity tests. They found that higher salinity could decrease amphipod sensitivity to toxicants. Specifically, at 15 °C and a salinity of 18, the 96-hour LC_{50} value was 0.71 mg l^{-1}, whereas, at a salinity of 2, it was 0.47 mg l^{-1}. The LC_{50} values obtained in the present study fall within the range reported by other studies, confirming that *C. volutator* populations from both collection sites were sensitive to the reference toxicant.

Overall in the GoG area, the harmful effects of sediment exposure on amphipods were plainly indicated in two Gdynia port sediments (PGDY1 and PGDY2), as evidenced by significantly reduced survival compared to the control. This corresponds to the levels of HOCs, which in these sediments exceeded EAC and ERL values. However, a statistically significant response was not observed in the Gdańsk port (PGDA2) and the 395 site (Gdańsk Deep) sediments tested with *C. multisetosum*, which were also characterized by contamination with HOCs. While *C. volutator* had significantly reduced survival in PGDY1, PGDY2, and E57 compared to control, *C. multisetosum* showed no statistically significant response to any of the GoG sediments in this endpoint, whether compared between treatments or to control. This outcome could be attributed to relatively high coefficients of variation within some treatments and slightly higher mortality in one of the *C. multisetosum* control replicates. These, along with a low number of replicates (three per treatment), most likely reduced the probability of detecting statistically significant differences. Similar issues were observed by Ward et al. (2015) in a study of the marine amphipod, *Leptocheirus plumulosus,* exposed to copper-spiked sediments and Kennedy et al. (2009) that evaluated acute and chronic toxicity methods for marine sediment testing. Survival of *C. multisetosum* was higher in the contaminated PGDY2 port sediment (83 %) than in other GoG sediments characterized by low (in some cases negligible) HOCs levels (the X1, X2, and ZN2 sediments; ≤ 70 %). This suggests that factors other than organic pollutants played an important role in this species' survival. Moreover, when the response of both *Corophium* species to the same contaminated port sediments (PGDY2) was compared, it was significantly stronger in *C. volutator* treatments than in the case of *C. multisetosum*. Ré et al. (2009) studied *C. multisetosum* survival in various conditions and showed that the species tolerated a range of sediment fines content, but its survival declined in sediments with the fines content above 50 %. In the present study, the content of the fine fraction did not exceed 31 % in GoG sediments and reached almost 39 % in some MW&WS sediments, where the survival of *C. multisetosum* did not fall below 72 %. The direct relationship between the survival of amphipods and the fines content alone is hardly possible. In multivariate analysis of all sediments' characteristics, negative relationships between survival, sediment fines, and OM_w content indicated by PCA were weakly significant for *C. multisetosum* exposures and strongly significant for *C. volutator* exposures. However, these relationships were not confirmed when heavily contaminated sediments were removed from the analysis. This suggests that it was not the fines content alone, but rather the contaminants associated with the fine fraction that negatively influenced the amphipods' survival. In fact, it has been shown that *C. volutator* requires a certain amount of fines in the sediments for efficient feeding (Fenchel et al., 1975).

The two species may differ to some extent in their feeding and living habits. It should be pointed out that sediment natural properties, such as the fines and OM content, can influence the bioassay outcome indirectly as these sediment components bind HOCs shifting their bioavailability for infaunal species (Accardi-Dey and Gschwend, 2002; Harkey et al., 1997; Kukkonen and Landrum, 1998; Meador et al., 1997; Nebeker et al., 1989). The binding of HOCs in sediments depends on many factors such as the quantity and geochemistry of organic carbon (OC), the content of black carbon (BC), and the physicochemical properties of HOCs. In a study of infaunal invertebrates[10] with different modes of feeding, it was shown that the toxicity (and bioaccumulation) of contaminants was controlled by the amount of the sediment OC. Specifically, it influenced the dissolved fraction of contaminants in interstitial water, which is one of the major routes of contaminant uptake in infaunal invertebrates (Meador et al., 1993). OM, especially BC, by binding the contaminants reduces their concentration in interstitial water and thus the availability for uptake by benthic organisms. The role of OM in the binding of contaminants in the GoG sediments could differ since its content varied, and as shown by another study, the BC content in the GoG sediments varies as well (Staniszewska et al., 2011).

[10] the study included amphipods *Rhepoxynius abronius* and *Eohaustorius washingtonianus*.

The survival of amphipods exposed to the GoG sediments could have been, to some extent, influenced by the acclimation period. The *C. volutator* individuals were kept in the laboratory for several weeks before the start of the bioassays, while *C. multisetosum* were acclimated for about a week. The issue of an acclimation period and its relevance to amphipod survival in bioassays has been addressed by several studies, and different results have been reported. Kater et al. (2000), in a study of seasonal changes in acute cadmium toxicity to *C. volutator,* indicated that some variation in sensitivity could occur between tests with animals held in the laboratory and freshly collected from the field. However, no significant difference between the animals that were maintained in the laboratory for 3 - 7 months and those that were tested within 10 days after collection was detected. Ré et al. (2009) reported that laboratory-raised *C. multisetosum* compared to the field-collected ones was neither more sensitive nor more resistant to a toxicant. In contrast, Menchaca et al., (2010) studying *C. multisetosum* exposed to contaminated field sediments reported that cultured amphipods (maintained in the laboratory for about one year) showed much lower mortality, i.e., were less sensitive, than the field-collected animals.

The survival of *C. multisetosum* exposed to the MW&WS sediments indicated no sediment toxicity, as there was no significant difference between the sediment exposures (72 - 89 %) and control (93 %). Although statistically indifferent among the sites, it was slightly lower in the sediments collected in the vicinity of the phosphogypsum heap, just below the confluence of the Młynówka Canal and the Dead Vistula River (the ST3 site), compared to the ones collected down to the Wisła Śmiała River outflow to GoG. The contaminants analyzed in the MW&WS sediments were present in relatively low concentrations compared to the polluted GoG sites. None of them exceeded the EACs and ERL values and, as shown by the statistical analyses, did not influence survival. Multivariate analysis indicated that the survival was independent of sediment natural features. However, judging by moderately negative relationships with the sediment content of OM_w, OM_f, and the fines, the survival could have been to some extent influenced by the content of these sediment constituents, which seems to support the findings of a study by Quieroga (1990). The study surveyed benthic invertebrate macrofauna in the Canal de Mira, Ria de Aveiro (Portugal), and reported that *C. multisetosum* avoids finer sediments reach in particles below 125 μm and organic matter (Quieroga, 1990). The effect of sediment natural properties on *C. multisetosum* survival seems to be unclear and needs further study.

6.2 Growth

The growth of *Corophium* spp. under laboratory conditions has been investigated by several researchers (Allen et al., 2007; Bat and Raffaelli, 1998a; Kater et al., 2008; Peters and Ahlf, 2005; Scarlett et al., 2007; van den Heuvel-Greve et al., 2007). It depends on factors such as temperature, food availability, or season. The studies indicated an average growth rate (GR) of *C. volutator in* the range of 69 - 84 µm day^{-1} at 15 °C (Kater et al., 2008; Peters and Ahlf, 2005), and for the entire life cycle of females, 80 - 100 µm day^{-1} (Van den Heuvel-Greve et al., 2007). Much lower GR was found in the case of a natural population of *C. multisetosum* in the Ria de Aveiro (NW Portugal), namely 19 - 29 µm day^{-1}, and the highest reported GR for *C. multisetosum* juveniles was 100 µm day^{-1} (Cunha et al., 2000c). In the present study, the observed growth rate (GR) was up to 69 µm day^{-1} for *C. volutator* and 47 µm day^{-1} for *C. multisetosum in* GoG sediments, and up to 81 µm day^{-1} for *C. multisetosum* in MW&WS sediments. Generally, the noted GR range was lower in GoG than in MW&WS treatments, but overall the growth observed in both species corresponded to the literature.

The lowest GR was noted in the sediments from Gdańsk and Gdynia ports (inner and outer areas) where it was reduced 1.5 - 6.0-fold compared to the controls, indicating the negative effects of the sediments. An exception was sediment from the Gdynia port (PGDY2) in which the mean GR of both species, *C. volutator,* and *C. multisetosum*, was comparable to that in control sediments. Wolska and Medrzycka (2009) used bioassays with *Vibrio fischeri* and the ostracod *Heterocypris incongruens* to test sediments from the Gdynia and Gdańsk ports. They reported high toxicity in sediments from the Polish and USA docks in the Gdynia port (PGDY1 and PGDY2 in this study) and some areas in the Gdańsk port. These sediments contained elevated contaminant levels, especially in the inner areas, where EACs for selected PCBs and ERLs for p,p-DDE, and/or $\sum_{16}$PAHs were exceeded. Notably, sediment from the Gdańsk port PGDA2 (the Defenders of the Polish Post dock) showed ERLs for $\sum_{16}$PAHs and p,p-DDE exceeded tenfold.

The multivariate analysis (PCA) of *Corophium* spp. responses in GoG sediments indicated moderate negative correlations between GR and sediment HOCs, suggesting that the amphipods' growth was affected by polluted sediments. Unlike in the port sediments, the mean amphipod GR in other GoG sites was 1.9 to 4.8 times greater (18 - 58 µm day^{-1}) compared to the controls, with the highest GR in the ZN2 and E57 sediments. The characteristics of these two sediments were comparable to those of the control i.e., they had low HOCs levels and only slightly differed in the content of fines, OM_w, and OM_f. The higher GR of *Corophium* spp. in the GoG sediments than in the controls suggests that these sites do not present a reason for concern, despite elevated sediment contaminants (particularly $\sum_{16}$PAH levels) in some sites (especially 395, where other HOCs also exceeded ERL/EAC values). Each species responded with higher GR compared to respective controls in ZN2, namely 8.6 (*C. volutator*) and 2.8 (*C. multisetosum*) times greater. The sediment's natural features were undoubtedly of importance for amphipod growth. The higher GR can be attributed to the OM provided by the sediment, which was nutritionally beneficial to the amphipods, as suggested by the PCA results. The stimulating effect of sediment's natural properties was also more clearly observed in bioassays with *C. multisetosum* in the MW&WS study, where the sediments were less polluted with HOCs. A similar effect of sediment OC (with OM as a proxy for OC) on amphipod growth was also reported in studies with *C. insidiosum* exposed to sediments from the Venice Lagoon (Picone et al., 2018) and *Gammarus locusta* exposed to contaminated sediments from the Sado and Tagus estuaries (Costa et al., 2005 b).

GR of *C. multisetosum* in the MW&WS area was also similar to or greater in the tested sediments than in CTR, indicating that these sediments were of good quality. However, similar to survival, GR was the lowest in the sediments collected next to the phosphogypsum heap (the ST3 site). This site was polluted with radioactive elements, metals, phosphates, and phosphorus in the past (Bory et al., 2013; Räike et al., 2015; Skwarzec et al., 2010). However, a study of radioactive elements in the Martwa Wisła by Olszewski et al. (2016) found these levels to be of minor importance. In the MW&WS study, multivariate analysis again showed a high correlation between *C. multisetosum* GR, organic matter (both OM_w and OM_f), and the fines content in the sediments, indicating the same factors stimulating the growth of amphipods as in the GoG study. GR was significantly higher in two sediments from the southernmost sites (ST4 and ST5), where it was about double that of CTR (1.7 and 2.3 times greater, respectively). Although Queiroga (1990) stated that *C. multisetosum* avoids finer sediments rich in OM, it still had a positive effect on this species' growth in this study.

GR was positively linked to the gravidity of amphipods both in multivariate statistics and Kendall's tau (τ) correlations. Lower GR of amphipods chronically exposed to sediment-bound contaminants results in reduced reproduction (Manyin and Rowe, 2006; Scarlett et al., 2007). This has been shown in *C. volutator* exposed for a whole life cycle to sediment spiked with chemically dispersed weathered crude oil (Scarlett et al., 2007). In a study with the burrowing amphipod *Leptocheirus plumulosus* exposed to moderately contaminated sediment from Baltimore Harbor, MD (USA), Manyin and Rowe (2006) reported lower growth and reduced reproduction rates. Based on measurements of bioenergetic parameters in adult amphipods (such as metabolic rate and energy storage), they concluded that contaminated sediments can chronically affect amphipods by diverting energy from production to maintenance pathways, resulting in slower growth and reduced fecundity, and ultimately leading to retarded population growth. Similarly, in an assessment of chronic toxicity of Venice Lagoon sediments characterized by contamination levels ranging from low to moderate with *Monocorophium* *insidiosum* juveniles, Picone et al. (2018) found that long-term exposure to the test sediments negatively affected GR and attainment of sexual maturity of the females. In other extensive studies of chronic sediment toxicity from the Ria de Aveiro estuary (NW Portugal), reduced growth and fecundity of *C. multisetosum* in industrially impacted sediments was also reported; however, it was indicated that the observed reduced fecundity could not be attributed exclusively to reduced growth (Castro et al., 2006). The association of GR with reproductive performance outlined by the above-mentioned studies indicates that growth responses in amphipods have a direct reflection in reproductive performance, which was also observed in the GoG study.

6.3 Reproductive outcome

Although this study focused on *Corophium* spp. survival, GR, and emergence endpoints, reproductive activity was also of interest in light of studies reporting the effects of contaminated sediments on amphipod reproduction (Conradi and Depledge, 1998; Castro et al., 2006; Costa et al., 2005a; Gale et al., 2006; McGee et al., 1993; Mann et al., 2009, 2010; Ward et al., 2015). Reproductive endpoints are highly relevant for assessing risk to field populations since reproductive capacity determines population growth and ultimate survival in the natural environment (Hyne, 2011). Reproductive effects in amphipods have been expressed in various ways. Examples include: mean brood size per surviving adult (Lotufo et al., 2016; Ward et al., 2015) or per surviving female (Ford et al., 2003; Mann et al., 2009, 2010; Scarlett et al., 2007), or mean brood size normalized to female weight (to account for correlation between female size and brood size; Ford et al., 2003). Sometimes the effects have been expressed as the percentage of mature females relative to all surviving females (Picone et al., 2018) or the percentage of gravid females relative to all survivors (Castro et al., 2006). Field and laboratory studies have shown that the amphipod's reproductive potential can be affected by various contaminants (Lotufo et al., 2016; Löf et al., 2016; Scarlett et al., 2007; Sundelin et al., 2000, 2008; Ward et al., 2015) as well as by environmental temperature and salinity (Cunha et al., 2000b).

In the GoG sediments the reproductive outcome, expressed as the percentage of gravid females relative to all females, was affected by the contaminants as evidenced by significant negative relationships between female gravidity and all measured sediment contaminants in *C. volutator*. Although these relationships were less evident in *C. multisetosum*, this species showed no reproductive activity in the Gdańsk port sites, PGDA1 and PGDA2, since neither gravid females nor juveniles were found in these sediment exposures. Based on the study by Lotufo et al. (2016), who reported a dramatically decreased offspring production in the amphipod *Leptocheirus plumulosus* exposed to sediment-amended weathered slick oil, it can be assumed that PAHs could have been the key contributor to the observed reproductive effects in the GoG port sediments. Namely, Lotufo et al. (2016) reported that the offspring production decreased by 80 % with an increase of $\sum_{50}$PAHs concentration from 2.6 to 24.2 mg kg^{-1} and that the concentration of 0.632 (EC20, 95 % CI = 0.11–2.15) mg kg^{-1} was associated with a 20 % decrease in offspring production relative to the reference. PAHs were the dominant contaminants in the GoG port sediments. The concentrations of $\sum_{16}$PAHs were many times greater than the EC_{20} value found by Lotufo et al. (2016) i.e., in PGDY1-PGDY3 in the range of 1.2 -3.1 mg kg^{-1}d.m., in PGDA1 and PGDA2 it was 2.2 and 33.6 mg kg^{-1}d.m., respectively. A negative reproductive effect was also reported in *Monocorophium insidiosum* exposed to Venice Lagoon sediments, which showed a clear decrease in the number of mature females with increasing levels of contamination, with significant effects at PAH concentrations of 6.2 mg kg^{-1} (Picone et al., 2018).

Overall, aside from the lack of reproductive activity in the Gdynia and Gdańsk port sites (PGDY1, PGDA1, PGDA2), the female gravidity in the GoG sediments was relatively low with the highest of 40 % in one site only. The average length of incubating females at the termination of bioassays was 5.2 and 5.1 mm in *C. volutator* and *C. multisetosum*, respectively, whereas the average length of non-gravid females was 4.3 ± 0.7 mm. All females exposed to the GoG sediments exceeded the female maturation size reported for *Corophium* by other studies. Namely, Brown et al. (1999) stated that *C. volutator* matures at a length of about 4.5 mm, and Cunha et al. (2000c) reported a minimum length of gravid *C. multisetosum* females of 4.1 mm. Whereas Nair and Anger (1979) in an extensive study of the *C. insidiosum* life cycle in laboratory culture reported even smaller sizes of matured females demonstrating that the body size at which females were mature was 3.2 - 3.7 mm at 15 °C and 3.0 - 3.3 mm at 25 °C i.e., it depended on temperature and the "status of the brood". It can be assumed that the female maturation and/or reproduction processes in the GoG study were delayed and/or impaired due to the actions of sediment contaminants, which is supported by similar findings of the *C. volutator* study by Conradi and Depledge (1998) where the minimum time for female maturation was markedly increased in animals exposed to sediment amended with sublethal copper concentrations. Yet, the negative effects on female maturation were not distinguishable from those on growth, as a significant positive relationship between female gravidity and growth was shown by Kendall's statistics for both *Corophium* species as well as by PCA. The link between female gravidity and growth was further supported by a positive relationship between gravidity and sediment OM_f content which represent a fine-sized nutrition source. The close relationship between growth and female gravidity does not allow to conclude that the effect on female gravidity was solely the consequence of contaminant actions on female maturation, it could have been an indirect effect on growth rate.

In the MW&WS study, no reproductive activity was observed. This can be explained by the final overall mean female size of 3.9 ± 0.4 mm, which was markedly smaller than that reached by the GoG-exposed females. The smaller final size was a consequence of a small initial size (1.27 ± 0.56 mm) because the amphipods' GR was adequate. Theoretically, the reproduction was possible in two MW&WS sites, ST4 and ST5, where the mean final female size was 4.2 and 4.6 mm, respectively. However, another factor that might have been of influence was the low number of available males. A successful reproduction of *C. volutator in* the laboratory conditions can be achieved at the 2:1 female-to-male ratio (Peters and Ahlf, 2005), yet in the ST4 and ST5 sediments the males amounted to 10 and 14 % at the bioassay termination, respectively.

6.4 Emergence (sediment avoidance)

In a review of behavioral ecotoxicology, Hellou (2011) defined sediment avoidance (described in this study as emergence from sediment) as a rapid response reflecting a defense mechanism against exposure to a detrimental factor. Sediment avoidance seems to be a good candidate as an assessment tool for contaminated sediments. It is an early warning signal of sediment toxicity, relatively fast, simple to perform, and noninvasive (Hellou, 2011). Sediment avoidance by amphipods has been investigated in several ecotoxicological studies (Bat et al., 1998; Kravitz et al., 1999; Siebeneicher et al., 2013; Szczybelski et al., 2018; Ward et al., 2013). Ward et al. (2013), who studied how an amphipod (*Melita plumulosa*), a harpacticoid copepod (*Nitocra spinipes*), and a snail (*Phallomedusa solida*) respond to contaminated sediments, found that *Melita plumulosa* tended to avoid sediments if they caused higher lethality. In bioassays with sediment choice (organisms are allowed to choose between uncontaminated and contaminated sediment), their study demonstrated that each of the tested species responded to chemical cues in the environment and settled in sediment that provided the best chance for survival (Ward et al., 2013). Another study of substrate choice with the amphipod *Eohaustorius estuaries* showed that sediments spiked with high PAHs concentrations were avoided for 2 - 3 days (Kravitz et al., 1999)[11]. In sediment bioassays and behavioral tests, Bat et al. (1998) reported that *C. volutator* avoided metal-spiked sediments and this behavior was more frequent with an increasing degree of contamination. Macken et al. (2008) in an integrated toxicity assessment study of Irish marine sediments, observed an increased re-emergence of amphipods in the case of contaminated sediment of the Alexandra Basin (elevated levels of PAHs, PCBs, and organochlorine pesticides) in the Dublin Bay, Irish Sea. Hellou et al. (2005) investigated whether *C. volutator* showed preference/avoidance behavior

[11] Noteworthy, the amphipods in this study showed mixed avoidance response to the moderately contaminated sediments, presumably due to their varying content of silt and sand.

towards anthropogenic physical or chemical materials in sediments, including PAH-contaminated samples from Halifax Harbor (NS, Canada), both alone and mixed with reference sediment. The amphipods were sensitive to PAHs, however, the avoidance response did not show a clear trend with PAHs exposure levels over a range of higher concentrations. This was attributed to the PAHs bioavailability and the presence of other contaminants in sediments. Nevertheless, the amphipods exhibited an avoidance response to PAH-contaminated sediments, which corresponded to the probable effects level (PEL, > 50 % probability of toxicity) set by sediment quality guidelines. In a subsequent study, Hellou et al. (2008) established the relationship between PAHs threshold effects level based on sediment avoidance and the bioaccumulation of PAHs.

In the GoG study, the amphipod emergence was significantly positively related to the HOCs levels in the sediments. An increased avoiding behavior was observed in three of the four sediments in which HOCs concentrations (some PCB congeners, p,p-DDE, and $\sum_{16}$PAHs) exceeded established environmental quality criteria. Namely, the response was increased in the port sediments (PGDY1, PGDY2, PGDA2), but not in the 395 sediment. It was significantly greater from CTR for *C. volutator* exposed to PGDY1 (Gdynia port, the Polish dock) and for *C. multisetosum* exposed to PGDA2 (Gdańsk port, the Defenders of the Polish Post dock). The emergence was also more recurrent in PGDY2 (Gdynia port, the USA dock). The amphipods emerging from the sediments were most frequently observed within the first few days of the bioassay. A similar time trend was also reported by Bat and Raffaelli (1998a), who studied the response of *C. volutator* to organically enriched sediment. In the present study, the amphipod emergence was related to the toxicity associated with sediments. This has been indicated by negative relationships between the emergence and other biological endpoints (survival, GR, molting, and gravidity), especially in the case of *C. volutator* sediment exposures, less so in the *C. multisetosum* exposures. The negative association of emergence with survival, GR, molting, and gravidity, as well as the positive correlation of emergence with sediment HOCs levels, provide an incentive for developing the emergence index as an early indicator of sediment toxicity. It should be noted, that sediment avoidance was not observed in the MW&WS sediments, which varied markedly in their OM content and grain composition, but were characterized by low content of HOCs.

The avoidance behavior, aside from chemical contamination, may be induced by other factors such as infaunal activity in the substrate or stress due to overcrowding (Kravitz et al., 1999). As observed in the GoG study in *C. volutator* exposed to the E57 sediment, an increased emergence can also be associated with the sediment's poor nutritional value. This sediment had very low contents of the fines and OM_w, and at the same time, negligible levels of contaminants. The fines and OM_w contents were the lowest among all treatments (0.2 and 0.2 %, respectively).

The notion that the lack of a proper source of nutrition caused an increased emergence can be supported by the findings of Fenchel et al. (1975), who showed that efficient feeding *C. volutator* requires a certain amount of fines. Furthermore, it can be supported by the preliminary study conducted before setting up the bioassays, described in Section 4.4. Namely, amphipods placed in a medium with sediment cleansed of organic chemical contaminants avoided the sediment and showed increased mortality and minimal growth despite regular feeding with diatoms. The avoiding behavior did not change even when the chemically treated sediment was submerged in seawater for a few days to allow microflora to develop by the time the animals were introduced to that sediment. Some explanation can be provided by experiments conducted by Meadows (1964) on substrate selection by *C. volutator* and *C. arenarium* which indicated that sediment avoidance can be associated with the absence of a surface biofilm (produced by bacteria and diatoms) on sediment particles.

The sediment avoidance response reduces the exposure of amphipods to negative stimuli present in the substrate. On the other hand, it also reduces the time of feeding and increases energetic expense due to increased motion activity. Throughout the GoG study, the individuals that avoided sediment were observed to swim in an upward direction, and after reaching the water surface, they would motionlessly sink back to the sediment surface. Depending on the tested sediment, this behavior would be repeated numerous times, greatly increasing the energetic expenses of amphipods. Clearly, a continuous emergence can potentially lead to negative consequences in amphipods. Still, Ward et al. (2013) suggested that in natural habitat sediment avoidance has a positive function, as the amphipods migrate to a cleaner site.

The amphipods avoidance behavior has the potential to be used in sediment quality studies, however, it is important to differentiate their natural behavior related to sediment screening or searching for mates from their reaction to negative environmental stimuli such as contamination. *Corophium* spp. males can spend time inspecting surfaces in search of a mate and sometimes they guard the entrance to the female’s tube and even fight potential rivals (Forbes et al., 1996; own observations). However, the percentage of animals crawling on the sediment surface is generally minor. In the Nova Scotia *C. volutator* population, such behavior was performed only by 0.3 - 0.4 % of the animals (Boates and Smith, 1989).

It should be pointed out that there are no standardized protocols for sediment avoidance as an endpoint in sediment toxicity testing. Most of the studies applied bioassays with sediment choice, i.e., the organisms were allowed to choose between uncontaminated and contaminated sediment. Some studies examined the amphipod’s burrowing ability as a response to contaminated sediments by measuring the time it took for the animal from contact with the water surface until it was completely buried in the sediment (Bhuiyan et al., 2022; Siebeneicher et al., 2013). In the present study, the amphipods’ response was simply observed after placing the animals in the vessels with tested sediments. A more refined approach was used by Bhuiyan et al. (2022) in a study of the physiological performance of the estuarine amphipod *Ampelisca brevicornis* related to ocean acidification where the burrowing behavior was investigated among some other physiological responses. In their study, the burrowing behavior was defined as the time it took for individuals to begin burrowing immediately after being placed in a vessel, and the burial rate was defined as the total time it took for the amphipods to bury themselves in the sediment entirely (Bhuiyan et al., 2022).

6.5 Molting

Little is known about the relationship between environmental contamination and molting in amphipods. Molting is a hormonally controlled process that mediates growth as well as reproduction (Hartnoll, 2001; Hyne, 2011; Mykles 2021). Contaminants that interfere with the endocrine hormones can affect these processes. A number of environmental contaminants act as endocrine-disrupting chemicals (EDCs) and may disturb the process of molting. This has been shown, among others, in a study with the amphipod *Gammarus pulex* exposed to two PBDE congeners, which were found to inhibit enzymes associated with molting and chitin synthesis (Gismondi and Thomé, 2014). A detrimental effect of fungicide propiconazole on the molting of the Chinese mitten crab (*Eriocheir sinensis*) was reported by Yao et al. (2023). The species is widely bred in China for its rapid growth and high nutritional value, and raised in rice-crab co-culture systems. Based on gene expressions, the authors showed that propiconazole exerted gender-specific effects on the molting.

In the present study, according to the ANOVA analysis, there were no significant differences in the mean number of molts in exposed *Corophium* spp. However, the bivariate statistics showed that the molting frequency was significantly lower (compared to CTR) in the contaminated PGDY1 and PGDA2 port sediments (the USA dock in the Gdynia port and the Defenders of the Polish Post dock in the Gdańsk port) and the 395 sites. It was also significantly lower in the X1, X2, and WS sediments collected in other GoG sites characterized by low or negligible HOC levels. Thus, it can be assumed that molting was impaired by elevated HOCs levels in the port and 395 sediments. However, it could also be associated with food availability or quality, and/or with sediment unfavorable conditions related to grain composition which can be the case of the X1, X2, and WS sediments in GoG and of four sites in the MW&WS area.

Molting is an essential process for amphipods to grow, i.e., they cannot grow unless they shed their old exoskeleton and secrete a new one. In unstressed environments, the amphipod growth and the duration of the intermolt periods are generally related to temperature and the amount of available food (Hartnoll, 2001). Thus, in the same temperature conditions, the frequency of molting can be indicative of food availability and should be related to GR.

Indeed, a significant positive relationship between the number of molts and GR was obtained for *C. volutator* exposed to the GoG sediments and for *C. multisetosum* exposed to the MW&WS sediments (moderately significant), but less so in *C. multisetosum* exposed to the GoG sediments where the relationship seemed to be inversed. This negative relationship between GR and molting is difficult to explain. The potential influence of gender may be of importance considering that females of both species, *C. multisetosum* and *C. volutator*, are very likely to molt more frequently than males (Cunha et al., 2000c; McCurdy et al., 2000). A significant positive relationship was also found between molting and survival in both species in the GoG sediments as well as in the MW&WS sediments (of moderate significance) indicating that survival was a key factor determining the number of produced molts. Moreover, the molting of *C. volutator* exposed to the GoG sediments, aside from being related to survival and growth, correlated with gravidity. This finding substantiated the study by McCurdy et al. (2000) which reported that the *C. volutator* female breeding propensity was related to timing of the females' moult. The authors reported that no *C. volutator* female became ovigerous unless she had previously molted. The molting and oogenic cycle in amphipods are tightly linked, as the female deposits oocytes into the marsupium after mating, while the exoskeleton is still flexible enough to let the oocytes pass the oviducts (Hyne, 2011). Noteworthy, the mean number of molts produced by amphipods in CTR, which was about one per ind.$^{-1}$ in both species, was in line with the study by McCurdy et al. (2000) who also reported that the molting interval of *C. volutator* females lasted 25 days.

6.6 Comparison of *C. multisetosum* and *H. incongruens* responses

Many studies have used the benthic ostracod *H. incongruens* bioassay as a tool for the ecotoxicological assessment of contaminated sediments (Casado-Martinez et al., 2016; Gonzalez-Merchan et al., 2014; Pandey et al., 2019; Rogowska et al., 2014; Urbaniak et al., 2020). The reliability and sensitivity of this simple ostracod test have been reported by several studies, including toxicity monitoring of river sediments in Flanders, Belgium (Chial and Persoone, 2002), ecotoxicological characterization of sediments in a stormwater detention-settling basin (Gonzalez-Merchan et al., 2014), and the evaluation of selected heavy metals toxicity (Kudłak et al., 2011). The use of this biotest has become popular worldwide and is also recommended as a dredged material assessment tool in Poland (Wolska and Mędrzycka, 2009).

As indicated above, the *H. incongruens* bioassay was applied for testing the MW&WS sediments in addition to *C. multisetosum* to compare the responsiveness of both organisms to the sediments. Both, *C. multisetosum* and *H. incongruens* responded similarly showing no reduction in survival and no inhibition of growth. However, the ostracods showed a different trend in growth in that their lowest growth occurred in the ST5 sediment while that of *C. multisetosum in* the ST3 sediment, which might have resulted from the dissimilar life habits of these two organisms and differences in nutritional preferences. The *H. incongruens* responses were not related to the sediment OM_f content indicating that the fine organic matter did not stimulate the *H. incongruens* growth which was observed with *C. multisetosum*. The *H. incongruens* responses were related to the sediment fines and OM_w content, and these relationships were negative. This finding is in line with the Casado-Martinez et al. (2016) study which reported negative effects of the fine sediment content on ostracod growth. They stated that ostracods placed in sediments with a considerable amount of fines might ingest them instead of the algae that they were fed with during the bioassay. This study and that of Casado-Martinez et al. (2016) indicate that the use of *H. incongruens* for sediment toxicity testing has some limitations due to the possible negative influence of the sediment OM_w and the fines content on the ostracod responses. Therefore, it seems important to provide information on the relations between the ostracod responses and sediment features to facilitate the understanding of the bioassay results.

6.7 MW&WS benthic communities

Diversity, abundance, and spatial distribution of benthic communities are interconnected with environmental conditions, seasonality, habitat properties, and disturbances (Chapman and Wang, 2001; De los Ríos et al., 2016). Key natural factors of importance are sediment features such as grain size, organic matter content, food availability, as well as hydrodynamics and physicochemical characteristics of the water column (Hily et al., 2008; Van Hoey et al., 2004; Włodarska-Kowalczuk et al., 2014; 2016). The MW&WS sites differed with respect to the depth, characteristics of the bottom water, sediment grain composition, and OM content. These differences in environmental parameters carried weight on the macrobenthos abundance and community structure along the studied rivers. Of the bottom water characteristics, salinity is considered the primary factor influencing the structure of benthic fauna in estuarine and coastal ecosystems (Berezina et al., 2017; Chapman and Wang, 2001; Zettler et al., 2014). The salinity gradient along the MW&WS rivers differentiated the distribution of bivalves. *Limecola balthica*, for instance, dominated among bivalves at the river's mouth but was almost absent in the southern sites. Similarly, the bivalves typical of the Baltic coast and the Gulf of Gdańsk, namely, *C. glaucum* and *Mytilus* sp. (Piesik et al., 2009), were predominantly present at the northern sites, although in low quantities. A contrasting effect i.e., a negative influence of salinity, was observed in *Rangia cuneata*, and the polychaete *Marenzelleria* sp.. The distribution of *R. cuneata*, a typical brackish water bivalve that dominated among bivalves at all sites except ST1 (the site closest to GoG), apart from salinity, was influenced by sediment OM_f content. The species migrated to this area a few years before the sampling and was found in the northwestern branch of the Martwa Wisła as one of the dominant species (Dziubińska and Zarzycki, 2017; Janas et al., 2014; Kendzierska et al., 2017). *Marenzelleria* sp. was also reported by Kendzierska et al. (2017) in the northwestern branch of the Martwa Wisła, where salinity ranged from 0.5 to 6.8 PSU. It is a common polychaete in estuaries characterized by lower salinities, and an important component of the benthic fauna in the Gulf of Gdańsk (Kotwicki, 1997). It is a non-indigenous species that has expanded its range in recent years throughout the Polish marine areas and estuaries (Kraśniewski et al., 2018). It has been indicated that *Marenzelleria* sp. can have a beneficial influence on phosphorus retention, leading to lower phosphate concentrations in the water (Maximov et al., 2015).

Of the environmental variables (depth, salinity, oxygen content, pH, phosphate content, sediment grain fractions, sediment OM_w and OM_f content) investigated for relationships with the macrobenthic communities using the DistLM procedure, three variables were identified as potentially significant i.e., phosphates, OM_w and OM_f. Considering the phosphate in the bottom water, its levels were greater in the southern section of the river near the phosphogypsum heap (0.7 - 0.9 mg l^{-1}) compared to the two northern sites closer to the river's mouth (0.1 - 0.5 mg l^{-1}). Another study conducted in this area reported 0.5 and 0.9 mg l^{-1} at two sites (which corresponded to ST3 and ST5, respectively) indicating that the concentration of phosphates in the bottom water can be related to the presence of phosphogypsum heap, because phosphorus compounds may be stored and released from the bottom sediments as a consequence of a long-lasting period of the phosphogypsum heap activity (Räike et al., 2015). Nevertheless, another source i.e., the treated wastewater of the City of Gdańsk, could contribute to elevated phosphate levels in this area as well (Räike et al., 2015). The phosphate levels in the MW&WS area indicated that the rivers did not meet the water quality criteria for the 1st and 2nd class concerning the concentration of phosphates ($\leq$ 0.065 mg $PO_4^{3-}l^{-1}$ for the 1st class of waters and $\leq$ 0.101 mg $PO_4^{3-}l^{-1}$ for the 2nd class), despite that the content of phosphates in the riverine water has been gradually decreasing over the last decade (based on data from GdańskVIEP).

The susceptibility of the benthic fauna to the phosphates, reflected by lower diversity and density in the presence of effluents from phosphate industries, has been reported by other studies (Boudaya et al., 2019; Cheggour et al., 1999). Boudaya et al. (2019) indicated that benthic assemblages in the Gulf of Gabès (south-eastern coast of Tunisia) were related to the proximity of the phosphogypsum outfall as well as linked to edaphic factors such as sediment composition and OM content. In the present study, aside from phosphates, the sediment OM_w and OM_f content were two other significant variables, that shaped the macrobenthic communities. The lowest density of macrobenthos observed at the ST3 site, located just below the Młynówka Canal confluence, was in line with the poor status of the macrobenthos community at this site indicated by the Gdańsk VIEP 2015 report. According to that report, a low MMI[12] and elevated concentration of phosphates were indicative of low ecological potential (class 5 out of 5-grade system). Two sites located close to the phosphogypsum heap (ST4 and ST5) were characterized by greater density of the bottom fauna. Therefore, it seems that the poor environmental quality at ST3 cannot be attributed to a direct influence of the phosphogypsum heap. The confluence of neighboring canals (Kanał Pleniewski, Kanał Śledziowy, Kanał Piaskowy, Kanał Młynówka, Kanał Wielki) carrying the runoff from the local fields may deliver other substances that could affect the benthic fauna.

6.8 Sediment quality evaluation

Sediment quality evaluation may involve an assessment of a comprehensive set of chemical data, results of bioassays with organisms at various trophic levels and benthic fauna (Birch et al., 2018; Chapman, 1990; Chapman and Wang, 2001; Iannuzzi et al., 2008; Romeo et al., 2015). Here, an attempt at sediment quality evaluation was undertaken based on the biological responses of *Corophium* spp. and measurements of a selected set of HOCs in sediments.

The HOCs concentrations in the GoG sediments differed significantly on a spatial scale with high concentrations in six out of eleven sites. Of the measured HOCs, several contaminants exceeded their environmental assessment criteria in the sediments of the Gdynia inner port (dock IV - the Polish dock and dock V - the USA dock), the Gdańsk inner port (the dock of the Defenders of the Polish Post), and the 395 site (close to the Gdańsk Deep). Although the environmental assessment criteria do not pre-judge sediment toxicity but rather indicate a likelihood of adverse effects on biota (O'Connor, 2004), the biological responses of *Corophium* spp. Corresponded to these criteria, except for the 395 site.

The port sediments adversely affected the amphipods' survival, growth, gravidity, and behavior. These responses varied markedly. To comprehensively evaluate all measured responses in assessing the quality of GoG sediments, a scoring system was developed to provide an overall performance indicative of sediment impact. The lowest scores were attributed to the sediments collected from the ports. These sediments were toxic to organisms, which corroborated their high contamination levels and exceeded ERL and EAC criteria. In some cases, a disparity was found between the sediment scores and the contamination level. For example, the 395 site received a better score compared to port sediments, despite the high level of contamination, indicating a lesser adverse impact. On the contrary, sediments from some sites along the gradient of distance from the Vistula River mouth (X1, X2, E57), with

[12] MMI - macrozoobenthos multimeric index

little contaminant burden, received lower scores. This can be mainly attributed to a lower growth due to the sediment organic matter's poor nutritional quality. In general, *Corophium* spp. were responsive to the sediment contamination, however, natural properties of sediments turned out to have a strong influence on the amphipod performance.

In the MW&WS sediments the HOCs concentrations were low and none of the measured contaminants exceeded the ERL/EAC values. The outcome of bioassays with both species, amphipods, and ostracods, showed no detrimental effects on their survival and growth. In two sites the sediments had slightly elevated levels of $\sum_{16}$PAHs (ST1 and ST5), yet it had no influence either on the bioassays' outcome or on the benthic fauna in terms of abundance and diversity. The measures of the benthic community's status were not reduced at any of the two sites. Overall, the bioassays and benthic fauna analysis indicated that the MW&WS sediments were not toxic. However, the natural properties of the sediments, especially the OM content, played a significant role in the bioassay outcome and the benthic fauna assemblages. For the latter, the phosphates concentration in water was an additional significant factor.

7 Conclusions

The overall aim of this doctoral thesis was to evaluate the suitability of *Corophium* spp. for the assessment of sediment quality in the Gulf of Gdańsk and the Martwa and Śmiała Wisła Rivers. The hypotheses assumed that *Corophium* spp. would be responsive to sediment contamination, and the responses would reflect the status of macrobenthos assemblages.

The obtained results indicate that *Corophium* spp. were effective and sensitive indicators of sediment quality, with their responses influenced by both contamination status and sediment natural features. Specifically:

- The amphipods exposed to the GoG most contaminated sediments, namely those from the inner port areas, responded with reduced survival, growth rate, and an increased emergence index. However, the responses were inconsistent, e.g., survival did not correlate with growth rate.
- Both tested *Corophium* species, despite some differences in their responses to the GoG sediments, identified the GoG port areas as the most toxic sites.
- The sensitivity of amphipods to sediment contamination was also indicated by significant negative relationships between survival and growth rate and the contaminants measured in the GoG sediments.
- Based on the amphipod responses, the sediment in the GoG areas of increased ship traffic and industrial activity, such as the ports of Gdańsk and Gdynia, were found of poor quality. In other GoG areas, the sediments seemed to not present any reason for concern.
- The ability of amphipods to distinguish sediments of varying quality was also observed in the MW&WS sediment exposures where no negative effects occurred, but growth was stimulated in some cases.
- The sediment's natural features, i.e., the fines, OM_f, and OM_w content were factors that played significant roles in the responses of *Corophium* spp. This pertains to a positive effect of OM_f and a negative effect of the fines and OM_w on the growth rate. The latter might be associated with positive relationships between the sediment fines and OM_w and contaminants. The stimulating effect of sediment OM_f was also observed in the gravidity endpoint.
- Of the amphipod responses seldom examined in sediment toxicity bioassays (emergence index, molting frequency, and female gravidity), the emergence index turned out to be an easy-to-observe indicator of sediment quality. In the GoG sediment exposures, the emergence index positively correlated with sediment contaminant concentrations and negatively correlated with the survival, growth rate, and molting frequency of *C. volutator*, but showed only moderate correlation with the growth rate of *C. multisetosum*.

Molting frequency and female gravidity were negatively associated with sediment contaminants in *C. volutator*, but not in *C. multisetosum.*

- Although both amphipod species used in the GoG bioassays were able to respond to sediments of varying quality, the results suggest that the two species might differ in the responses possibly due to habitat and feeding habits preferences. This can affect their exposure to sediment contaminants and lead to variation in the bioassay outcome and sediment quality evaluation. It needs further research.
- The ostracods exposed to the MW&WM sediments responded in a similar way as *C. multisetosum* showing no sediment toxicity, with growth stimulation in all but one sediment exposure. Both survival and growth of ostracods correlated negatively with the sediment OM_w and fines content, which might restrain the use of ostracods for sediment toxicity testing in the case of sediments rich in these two components.
- The MW&WS sediment bioassay results corroborated the benthic fauna results. Both indicated that the ST3 site located near the Sobieszewo pontoon bridge presented the least favorable conditions. The survival and growth of both *C. multisetosum* and *H. incongruens* exposed to the ST3 sediment were among the lowest and the native benthic fauna at this site was characterized by the lowest abundance.
- The MW&WS benthic fauna abundance and structure were influenced by several environmental variables of which the most important were the sediment OM_w and OM_f contents and the phosphate concentration in the bottom water.

Abbreviations

CTR – control sediment

CV_{wt} – coefficient of variation within treatments

CV_{at} – coefficient of variation among treatments

EAC – environmental assessment criterion

ERL – effects range low

FIN – fines

FISA – fine sand

GoG – Gulf of Gdańsk

GR – growth rate

HOCs – hydrophobic organic contaminants

MW&WS – Martwa Wisła and Wisła Śmiała

OC – organic carbon

OM – organic matter

OM_f – organic matter in the sediment fine fraction

OM_w – organic matter in the whole sediment

VFISA – very fine sand

PCA – principal component analysis

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