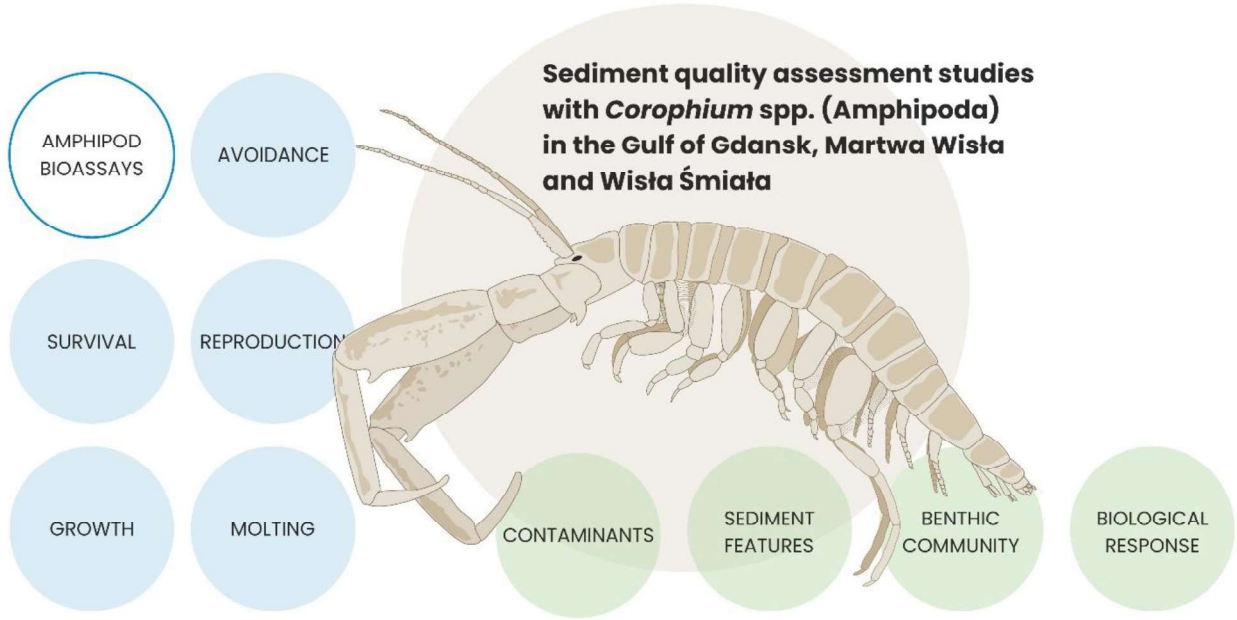
Summary



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.