

Appendix B

Ecotoxicological data

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Species

Summary

- In total, we have 12 species when conducting the preferred SSD (AVS < 1 µmol/g + marine AVS < 4.5 µmol/g)
- 4 marine species (4 taxonomic groups)
- 8 freshwater species (5 additional taxonomic groups + amphipod already represented in marine species)
- The species represent different feeding and living conditions from both the marine and the limnic environment.
- Statistical two tailed t-test show **no** significant difference in freshwater and marine NOEC/EC10 values (p=0.36 non-normalised data and p=0.64 for OC-normalised data). T-test on two samples assuming unequal variances in Excel (variance tested with f-test). Pooling can be conducted.
- Also supported during WS3 where pooling was voted the preferred method for deriving a HC5 value for the Baltic Sea Area (Figure 2 and Appendix B).

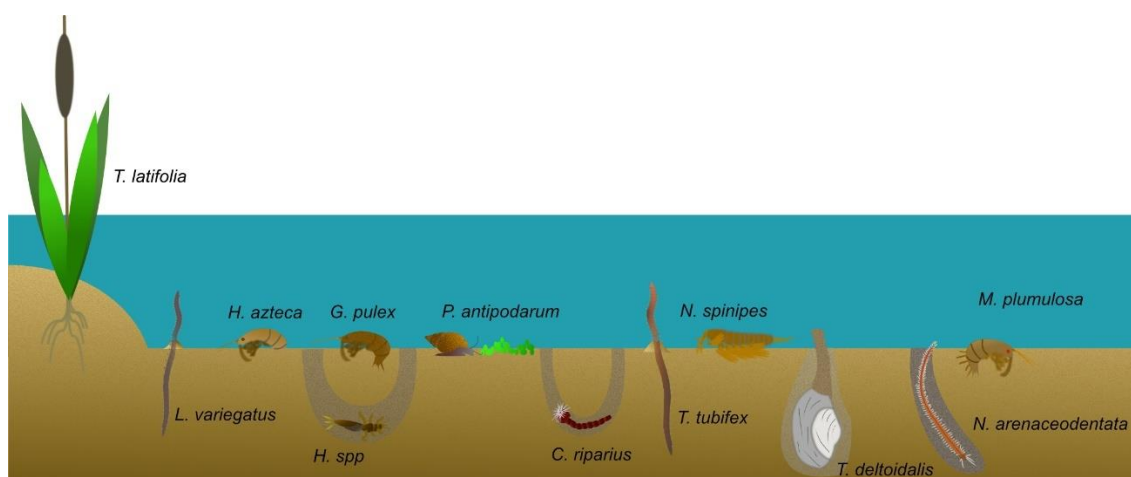


Figure 1: Living and feeding conditions of the 12 species included in the final SSD. More information on each species are found in the text.

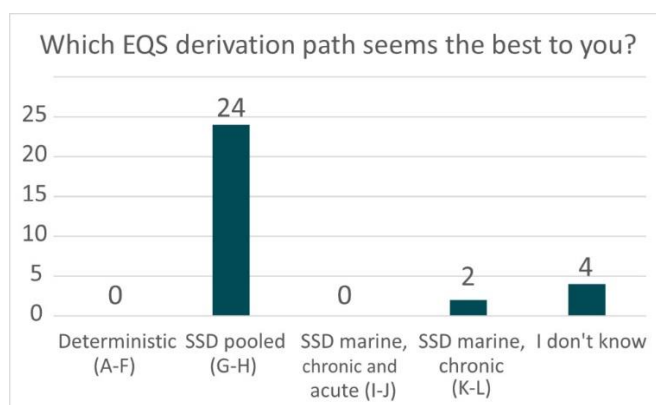


Figure 2: Results from a vote during workshop 3 where the participants were asked to vote on "Which EQS derivation path seems the best to you?". More information on the workshops are found in Appendix B.

Marine species included in the SSD (bold indicates they might appear in the Baltic Sea).

- ***Melita plumulosa*** (Arthropoda – Crustacea – Malacostraca – Amphipoda)
 - epibenthic and intertidal (King et al., 2005)
 - deposit-feeding (King et al., 2005)
 - ingestion of sediments has been shown to be an important source of metal uptake (King et al., 2005)
 - sediment as both a habitat and a food source (King et al., 2005)
 - recorded in most estuaries along the New South Wales coast, Australia (Hyne et al., 2005)
- ***Neanthes arenaceodentata*** (Annelida – Polychaeta – Phyllodocida)
 - Synonym: *Nereis (neanthes) arenaceodentata* (WoRMS, 2021)
 - Benthic (*Moore and Dillon, 1993*)
 - tropical Pacific and Atlantic Oceans and the Caribbean Sea (*Moore and Dillon, 1993*)
 - sub-surface deposit feeder (*Moore and Dillon, 1993*)
 - tubes in upper 2-3 cm of sediment (*Moore and Dillon, 1993*)
 - ingest sediment particles up to 70 µm (*Moore and Dillon, 1993*)
- ***Nitocra spinipes*** (Arthropoda – Crustacea – Copepoda – Harpacticoida)
 - *N. spinipes* is a harpacticoid copepod, which is widely distributed around the world (Lang, 1948)
 - It lives mainly on sandy bottoms, feeding on bacteria or particles. Owing to its ability to acclimatise to fluctuations in salinity (0–30‰) and temperature (0–26 °C) (Wulff, 1972)
 - Found in Baltic Sea Area
- ***Tellina deltoidalis*** (Mollusca – Bivalvia – Cardiida)
 - estuaries and coastal lagoons from southern Queensland to Tasmania and southern Western Australia (King et al., 2010)
 - It lives buried in the top 10–20 cm of sandy or muddy sediments and, like other tellinids, is a deposit feeder, collecting organic material and particles from surface sediments (King et al., 2010)
 - *T. deltoidalis* has been demonstrated to take up metals from both dissolved and particulate exposure routes (King et al. 2005)
- ***Hydrobia Ulvae*** (Mollusca - Gastropoda – Prosobranchia – Mesogastropoda)
 - Mud snail (synonym *Peringia ulvae*)
 - Feeding: surface deposit feeder, sub-surface deposit feeder (Detritus, periphytic microalgae) (MarLIN, 2021)
 - Mobility: Swimmer, Crawler, Burrower, Drifter (MarLIN, 2021)
 - Epifaunal (MarLIN, 2021)
 - Inhabits Baltic Sea Area (and more) (MarLIN, 2021)
 - *Only included in SSD if AVS is not considered.*

Freshwater species included in the SSD (bold indicates they might appear in the Baltic Sea).

- ***Chironomus riparius*** (Arthropoda – Insecta – Diptera)
 - Harlequin flies, midge
 - Chironomus larvae are also sediment ingesting deposit feeders and construct U-shaped burrows, which they irrigate with oxygenated water (Warren et al., 1998).
 - Larvae survive in low oxygen conditions such as at the bottom of a lake or in areas with high organic pollution

- Exist in Europe
- Same genus present in Baltic lagoons (Kornijów et al., 2019)
- ***Gammarus pulex*** (Arthropoda – Crustacea – Malacostraca – Amphipoda)
 - bottom dwellers, mainly feeding on algae and detritus (De Schamphelaere et al., 2004)
 - It does not ingest sediment and does not construct burrows. detritus (De Schamphelaere et al., 2004)
 - exposed mainly via the overlying water, although exposure via resuspended particles (e.g. detritus) may not be excluded detritus (De Schamphelaere et al., 2004)
 - Distributed all over Europe detritus (De Schamphelaere et al., 2004)
- ***Hexagenia spp.*** (Arthropoda – Insecta- Ephemeroptera)
 - Mayfly
 - Burrower mayflies as they create u-shaped tunnels in the aquatic substrate where they reside
- ***Hyalella azteca*** (Arthropoda – Crustacea – Malacostraca – Amphipoda)
 - bottom dwellers, mainly feeding on algae and detritus
 - It does not ingest sediment and does not construct burrows.
 - *Hyalella* mainly feeds on periphyton, algae and detritus located at the sediment-water (Stephenson and Turner, 1993; Warren et al., 1998)
 - North and Central America and Caribbean Islands (WoRMS, 2021)
- ***Lumbriculus variegatus*** (Annelida – Clitellata – Lumbriculidae)
 - Californian blackworm
 - common freshwater oligochaete, found in rivers, ponds, lakes and marshes
 - deposit feeder (Leppänen and Kukkonen, 1998).
 - sediment ingesting behavior, oligochaetes are excellent test organisms for studying bioaccumulation of hydrophobic sediment-bound contaminants (Leppänen and Kukkonen, 1998).
 - Although less detail is known about *Lumbriculus*, they are assumed to behave similarly as *T. Tubifex* (head down feeding in anoxic conditions) (De Schamphelaere et al., 2004)
 - *L. variegatus* has been used as a model organism for studies on the uptake and toxic effects of organic and inorganic pollutants in aquatic systems
 - North America and Europe (Sweden included) (Gustafsson et al., 2009)
 - Also, a marine according to WoRMs taxon details (WoRMS, 2021)
- ***Potamopyrgus antipodarum*** (Mollusca – Gastropoda – Littorinimorpha)
 - Small aquatic mud snail native in New Zealand (ISC, 2021)
 - Now introduced to Europe, North America, Australia, Iraq, Turkey and Japan.
 - Invasive due to abundancy (ISC, 2021)
 - “...in its non-native range, it can be found in either in freshwater, brackish and even salty water, and has been recorded in streams, rivers, lakes, reservoirs, channels, isolated coastal lakes, shallow lakes, estuaries and open seas (Alonso and Castro-Díez, 2008; Alonso and Castro-Díez, 2012).
 - *P. antipodarum* has a broad diet that includes organic matter (detritus), living plants (macrophytes) and microorganisms (periphyton) (ISC, 2021)
 - Can bury sediment to protect from cold (ISC, 2021)
- ***Tubifex tubifex*** (Annelida – Clitellata – Haplotaxida)
 - Sludge worm

- deposit feeder constructing I-shaped burrows which it doesn't irrigate with oxygenated water (De Schampheleere et al., 2004)
- feed head-down, decomposing organic material present in the ingested sediment (organic detritus and its associated microflora) (Warren et al., 1998). By doing so they can bring sediment from deeper layers to the surface, making (metal)-sulfides susceptible to oxidation at the surface.
- Can exist in marine environments (WoRMS, 2021)
- Exist around the Swedish coast (havet.nu)
- It is especially abundant in polluted sediments and marginal habitats not occupied by many other species, e.g. upper estuaries where interstitial salinity is less than 5 psu (MarLIN, 2021).
- *Typha latifolia* (Aquatic plant – macrophyte - Poales)
 - Common cattail
 - *T. latifolia* is a cosmopolitan species, with its native range encompassing large regions on all continents, except Antarctica, Africa and Oceania (ISC, 2021 and references within)
 - Fibrous roots grow from rhizomes produced at base of leaves (ISC, 2021 and references within)
 - “*T. latifolia* grows in a wide variety of wetland habitats. Niches include marshes, wet meadows, lakeshores, roadside ditches, seacoast estuaries, pond margins, bogs or fens as well as rice paddies. Salt tolerance is limited, but it does grow in marine wetlands with moderate salinity, and likewise can tolerate acidity.” (ISC, 2021 and references within)

Other marine ecotoxicological studies

- *Alitta Virens* (polychaeta)
 - Watson et al. (2018); Watson et al. (2021) determined the DNA damage threshold after long-term exposure (up to 9 months).
 - DNA.DT=68 mg/kg dw
 - OC not known (0.5%?)
- *Leptocheirus plumulosus* (amphipoda)
 - In a study by Ward et al. (2015), exposure testing resulted in NOEC for growth, mortality, and reproduction. The mortality in the control was considered too high and the study was excluded.
- *Zostera marina* (eelgrass)
 - Study by Nielsen et al. (2017) compared Cu uptake through leaves vs. rhizomes/roots (most important uptake through sediment)
 - LOEC (growth rate) = 280 mg/kg dw
 - OC unknown
- *Scrobicularia plana* (marine burrowing clam)
 - Study by Scola et al. (2021) showed no effect at 74 mg/kg dw @ 6% POC (=62 mg/kg dw @ 5% POC)
 - CuCl₂ spiked sediment
 - Only 2 exposure levels, low (74 mg/kg dw) and high (444 mg/kg dw) with the primary target to compare Cu nanoparticle exposure to Cu ion exposure.
 - Condition index, bioaccumulation, and mortality as endpoints.
- *Cylindrotheca closterium*, formerly *Nitzschia Closterium* (marine benthic diatom)

- Study by Moreno-Garrido et al. (2003) presenting development of method of measuring sediment toxicity on benthic marine diatom
- Provide EC50 for growth for three metals (Cd, Cu and Pb)
- EC50 Cu=26 mg/kg
- Particle size (sediment) important and problematic factor when testing toxicity on microalgae.
- OC content not mentioned.
- *Paracorophium excavatum* (tube dwelling marine amphipod, New Zealand)
 - In a study by Marsden (2002), *P. excavatum* was exposed to 5 different concentrations of copper (5, 14, 20, 35 and 46 mg/kg)
 - Survival LOEC=20 mg/kg and NOEC=14 mg/kg
 - Recruitment LOEC=14 mg/kg and NOEC=5 mg/kg
 - Tissue copper concentration increase with increasing sediment concentrations
 - *present study suggests that low concentrations of sediment copper can affect specific life-history traits of tube-dwelling amphipods sufficient to alter the population structure of field populations*
 - OC not mentioned
- *Gmelinoides fasciatus* (Baltic amphipod)
 - Berezina et al. (2019) conducted whole sediment testing on embryo development with contaminated sediment diluted with clean substrate to reach different levels of contamination. Sediment contained high levels of metals, organotin and PAHs.
 - 10-days test: Survival rate decrease at sediment dilution 1:256 (=Cu concentration 59 mg/kg dw (LOEC); NOEC=23 mg/kg Cu)
 - TOC approximately 0.6%
 - 28-days testing: morphological malformation at same dilution.
 - *“Given the known high toxicity of Cu and Pb for amphipods (Hyne and Everett 1998; Conradi and Depledge 1999; Sharyn et al. 2006; Borgmann and Norwood 2011) it is likely that they contributed to the overall toxicity of the test sediments (although the concentrations of PAHs and organotins were also very high).”*

Other freshwater ecotoxicological studies

- *Hyaella Azteca*
 - Study by Costello et al. (2019) compared fresh and aged sediment for Ni, Cu and Zn.
 - EC10 (growth) Dow sediment (0.68% Total carbon)
 - Aged = 57 (22-90) mg Cu/kg (approx. 419 mg Cu/kg 5% OC)
 - Freshly spiked = 29 (16-44) mg Cu/kg (approx. 213 mg Cu/kg 5% OC)
 - EC10 (growth) Ocoee sediment (3.6% Total carbon)
 - Aged = 217 (120-310) mg Cu/kg (approx. 301 Cu mg/kg 5% OC)
 - Freshly spiked = 238 (137-343) mg Cu/kg (approx. 330 Cu mg/kg 5% OC)
 - EC10 (freshly and aged combined) in Dow and Ocoee normalized to AVS and OC (=bioavailable fraction) was 42 µmol/g (26-61) and 13 µmol/g (8-22) respectively corresponding to 133 mg Cu/kg sediment (5% OC) and 41 mg Cu/kg sediment (5% OC).

Mesocosm, microcosm and field studies

- Jeppe et al. (2017)
 - Microcosm study with spiked sediment

- *Potamopyrgus antipodarum* (snail); *Physella acuta* (snail); *Chironomus tepperi* (chironomid)
- Glynn's wetland (Cu background 11 mg/kg)
- Total abundance declined with increasing Cu concentration and EC50=133 mg/kg. Most sensitive genera were *Paratanytarsus* (EC50=89 mg/kg)
- *Potamopyrgus antipodarum* reproduction EC50=121 mg/kg
- *Physella acuta* juvenile production EC50=298 mg/kg (limited power to detect patterns)
- *Chironomus tepperi* larval dry weight EC50=238 mg/kg. Also looked at gene expression and observed significant changes with elevated copper levels.
- Species relying on sediment and algae for food, such as *Paratanytarsus* or *Chironomus spp.*, were comparatively more sensitive to copper.
- OC content not mentioned.
- Ho et al. (2018)
 - Exposed field collected marine benthic communities to Cu spiked sediment (single dose of an average of 235 mg/kg dw at approximately 2% OC).
 - Total abundance was significantly reduced compared to sediments containing 7.7 and 25.5 mg/kg dw).
- Gardham et al. (2014a)
 - Studied the benthic community distribution (total abundance, taxonomic richness and diversity) in mesocosm studies, spiking the sediment with copper.
 - Taxonomic richness decreased with increasing copper concentrations.
 - Benthic Chironominae, a species of Ostracoda, Cladocera, and *P. acuta* were particularly sensitive to copper.
- Gardham et al. (2014b)
 - 1.5-year mesocosm study (outdoor).
 - Describe the “spiking phase” where the sediment was equilibrated for 61 days
 - freshwater-spiked sediments may be longer than marine sediments
- Gardham et al. (2015)
 - Mesocosm study with a focus on growth of *V. spiralis* and periphyton growth.
 - *Vallisneria spiralis* (Aquatic plant – macrophyte – Alismatales)
 - Eel grass, tape grass
 - Reduced shoot density at 97 mg/kg dw.
 - Growth decreased with increased Cu concentration. NOEC values changed with season but varied between 60-300 mg/kg dw (2% OC?)
 - Periphyton increased
 - Perhaps due to indirect effect of reduced grazing as it was shown that the snail population (*Physa acuta*) decreased with increased Cu concentration.
- Further compilation of microcosm, mesocosm and field studies can be found in Table 8 of Sahlin and Ågerstrand (2018).

Supporting short-term (acute) testing of copper exposure

- *Americamysis bahia* (estuarine crustacea)
 - Ho et al. (2018)
 - Single species 7-d testing with Cu spiked sediment (CuSO₄)
 - LC50=708 mg/kg dw (0.65% OC)
 - Parks et al. (2018)
 - Compared exposure to different forms of Cu (salts, nano particles etc)

- 2% OC and 50 mg Cu/kg dw in collected sediment
 - CUSO₄ salt generated lowest LC50 (708 mg/kg dw)
- *Ampelisca abdita* (marine/estuarine amphipoda)
 - Ho et al. (2018)
 - Single species 7-d testing with Cu spiked sediment (CuSO₄)
 - LC50=325 mg/kg dw (0.65% OC)
 - Anderson et al. (2008)
 - Compared the species sensitivity to several compounds, exposure range Cu: 36, 65 and 200 mg/kg dw
 - LC50=61 mg/kg dw (TOC=0.78%)
 - Parks et al. (2018)
 - Compared exposure to different forms of Cu (salts, nano particles etc)
 - 2% OC and 50 mg Cu/kg dw in collected sediment
 - CUSO₄ salt generated lowest LC50 (258 mg/kg dw)
- *Eohaustorius estuaries* (burrowing amphipod)
 - Anderson et al. (2008)
 - Compared the species sensitivity to several compounds, exposure range Cu: 327, 590 and 978 mg/kg dw
 - LC50=61 mg/kg dw (TOC=0.78%)
- *Gammarus locusta* (marine amphipod)
 - Correia and Costa (2000)
 - 10-day assay in Cu spiked sediment with different fine fraction (FF) and TOC.
 - 0% FF and 1%TOC: LC50=2 mg Cu/g C (=100 mg/kg dw @ 5% C)
 - 25% FF and 2%TOC: LC50=8 mg Cu/g C (=400 mg/kg dw @ 5% C)
 - no copper toxic effects (mortality) were observed on the 75% FF and 4% TOC (402 mg/kg dw)
- *Ptilohyale barbicornis* (amphipoda)
 - Study by Wu et al. (2021).
 - LOEC at first exposure @ 34 mg/kg dw (NOEC=control=10 mg/kg dw). LC50=24.56 mg Cu/kg.
 - OC content not known but 2% cellulose to artificial sediment suggest 2%?
 - All dead at 64 mg/kg dw
 - CuCl₂ spiked artificial sediment
- *Tubifex tubifex*
 - Thit et al. (2020)
 - Studied conventional endpoints (mortality and growth) plus bioturbation and feeding rate during 7-day exposure testing (uncontaminated or spiked sediment).
 - LC50 48.4 ± 90 mg/kg dw (mean ± SE)
 - “we believe that exposure to particle-associated Cu was the main route of entry to *T. Tubifex* in line with previous studies”
 - “increased Cu concentration in the sediment decreased worm feeding and growth” (effect already around 16-37 mg/kg dw).
 - OC content not mentioned but organic content = 0.28%.
- *Melita plumulosa*
 - Strom et al. (2011)

- Studied the influence of sediment particle size and organic carbon on toxicity of copper to benthic invertebrates in oxic/suboxic surface sediments
 - 10-day exposure, mortality
 - Calculated LC50 for silt (806 mg/kg dw OC=4.5%), silty-sand (212 mg/kg dw, OC=2.1%) and sand (198 mg/kg dw, OC=3.3%).
- *Telina deltoidalis*
 - Strom et al. (2011)
 - Studied the influence of sediment particle size and organic carbon on toxicity of copper to benthic invertebrates in oxic/suboxic surface sediments
 - 10-day exposure, mortality
 - Calculated LC50 for silt (980 mg/kg dw OC=4.5%), silty-sand (455 mg/kg dw, OC=2.1%) and sand (391 mg/kg dw, OC=3.3%).
- *Spisula trigonella*
 - Strom et al. (2011)
 - Studied the influence of sediment particle size and organic carbon on toxicity of copper to benthic invertebrates in oxic/suboxic surface sediments
 - 10-day exposure, mortality
 - Calculated LC50 for silt (997 mg/kg dw OC=4.5%), silty-sand (708 mg/kg dw, OC=2.1%) and sand (582 mg/kg dw, OC=3.3%).

Additional information and related studies

- Stewart et al. (2021) compared bivalve strengths in two sites around Isle of Man (one polluted with Zn, Cu and Pb and one not polluted) and concluded that there was a significant difference between the site-specific populations.
- Capparelli et al. (2020) tested dietary exposure to mudflat fiddler crab (*Minuca rapax*) for 12 days (0, 100 or 500 Cu $\mu\text{g g}^{-1}$). Spiked food resulted in osmoregulatory effects, but it was difficult to connect to sediment toxicity
- King et al. (2006) compared the Cu sensitivity of two amphipod species (one epifauna and one infauna). The focus was however on water concentration and no concentrations were provided for sediment
- King et al. (2010) conducted whole sediment toxicity tests on *T. deltoidalis* (bivalve) but provided only LOEC and NOEC of water concentrations. There was a poor correlation between Cu concentrations in particles and bioaccumulated Cu.
- de Haas et al. (2004) investigated the combined effect from food supply and Cu exposure on *C. riparius*. They concluded that at high feeding levels (recommended by OECD guideline) the food could partly compensate for the toxic effects and result in underestimation of sediment toxicity. Starvation, on the other hand, can lead to overestimation of toxicity. At a certain threshold, i.e. Cu concentration, the compensating effect disappears. No toxic effect observed for Cu concentration < 50 mg/kg, but bioaccumulation was observed at the lowest exposure (25 mg/kg dw).
- Hook et al. (2014) investigated the gene expression from different exposure routes (through water and/or ingestion) of *M. plumulosa*. Study suggest a different mode of toxic action when Cu is ingested as compared to taken up from the water and it is therefore not enough to consider water concentrations when assessing toxicity.
- Holan et al. (2018) compared sensitivity in different invertebrates and concluded that “for the bivalve *Gaimardia trapesina*, adults appeared to be more sensitive than young adults”.
- Soroldoni et al. (2020) investigated the toxicity of sediment spiked with antifouling paint particles (APPs) in estuarine conditions using three species in a whole sediment 10-day

testing. *Monokalliapseudes schubarti* (crustacean - Tanaidacea) LC50 = 0.16% APP (correspond to approximately 600 mg/kg); *H. Azteca* LC50 = 0.45% APP (corresponding to approximately 800 mg/kg). *M. schubarti* more sensitive, potentially because of infauna habit of burrowing and potentially ingesting sediment

Arguments for removing AVS > 1 µmol/g

- As it has been shown that high levels of acid volatile sulphide (AVS) decrease the bioavailability, and thus increase the apparent tolerance during testing, most studies where AVS > 1 µmol/g were excluded from the dataset. This is in accordance with TGD No. 27 (EC, 2018) that recommends to only include studies with AVS < 1 µmol/g. Because many of the ecotoxicology studies on marine species were conducted under aerated conditions where AVS was defined as being < 4.5 µmol/g (but not specified further), these were also included to allow for a more extensive dataset for marine species, also including the most sensitive *T. deltoidalis*.
- Where no information on AVS is provided, expert judgement considering oxygen supply, redox potential and sediment origin determined whether or not the test results were included in the final SSD (see table with raw data at the end of appendix).

Arguments for normalising to organic carbon

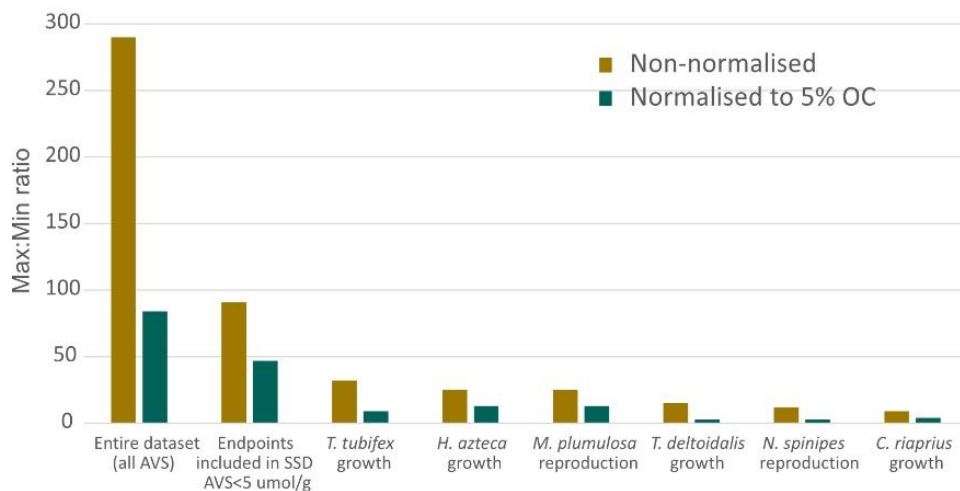


Figure 3: The Max:Min ratios of the entire dataset, the “low AVS” data and the most sensitive end-points of species with more than 3 NOEC/EC10 values. The bars represent data that has not been normalised (mustard) vs. normalised to 5% organic carbon (teal).

Freshwater data

- Within species variation – max/min ratio decrease for *H. Azteca* (from 25 to 13 for growth endpoint) and for *T. tubifex* (from 32 to 9 for growth endpoint) and for *C. riparius* (from 9 to 4 growth endpoint). AVS > 5 µmol/g has been removed prior to comparison.
- For all species with endpoints included in SSD max/min ratio decrease from 41 to 21.
- *T. tubifex* (reproduction end-point) is not showing a decrease when normalizing to OC (rather an increase in max: min ratio from 32 to 84), this is however **not the most sensitive end-point** and it is therefore not included in the SSD analysis.

Marine data

- Within species variation – max/min ratio decrease for *M. Plumulosa* (from 25 to 13 for reproduction end-point) and for *N. spinipes* (from 12 to 3 for reproduction end-point) and for *T. deltoidalis* (from 15 to 3 for growth end-point). AVS>5 µmol/g has been removed prior to comparison.
- For all marine species max/min ratio is similar with/without OC norm (43 non normalized and 41 if normalized).
- OC content of the different tests: min=0.4; max=7.4; average=2.77; std=2.24; 95% confidence interval=0.94.

Pooled dataset

- For all species included (All AVS and several endpoints): Max/min ratio decrease from 290 to 84.
- For all species included (AVS < 5 µmol/g and several endpoints): Max/min ratio decrease from 91 to 84.
- For all species (AVS < 5 µmol/g and only SSD included endpoints): Max/min ratio decrease from 91 to 47.

Comparison of the relative standard deviation (RSD)

Similar to the Max:Min ratio, a comparison was conducted looking at the relative standard deviation (RSD) of 6 species and their most sensitive endpoints. Results are shown in the table below and all RSD are reduced when the data is normalized to 5% OC, supporting the decision to normalize the data prior to the SSD.

Table 1: The relative standard deviation (RSD) of normalised vs non-normalised data.

Species - endpoint	RSD not normalised	RSD normalised 5% OC
<i>C. riparius</i> - growth	0.87	0.48
<i>H. Azteca</i> - growth	1.2	0.65
<i>M. plumulosa</i> - reproduction	0.66	0.53
<i>N. spinipes</i> - reproduction	0.52	0.39
<i>T. deltoidalis</i> - growth	0.97	0.48
<i>T. tubifex</i> - growth	1.4	0.59
All data included in SSD No. 1 (see below)	1.1	0.89

Workshop discussion (more in Appendix B) and poll result

- During workshop 1, OC normalisation was not supported if it could not be proven that variability in the dataset would decrease. No consensus on OC normalisation was reached during this workshop.
- During workshop 3, Marnix Vangheluwe (ARCHE consulting) presented freshwater data from the revisited vRAR (ECI, 2008) where he had re-done the variability analysis and concluded that OC-normalisation (and removing high AVS) resulted in lower Max:Min ratios and that OC normalization could explain most of the variabilities in the original dataset.
- These new insights, plus presentation of Max:Min ratios of the dataset used in the workshop exercise (where Max:Min ratios decreased) resulted in a poll vote where a majority voted that OC normalization could be conducted.



Figure 4: Results from a vote during workshop 3 where the participants were asked to vote on "For the SSD approach, should OC normalisation be applied?". More information on the workshops are found in Appendix B.

Comparison SSD derivation

General method:

- US-EPA SSD toolbox software used for analysis (US-EPA, 2020)
- Maximum likelihood fitting method was used as recommended by Fox et al. (2021) and to allow for AICc (weight) comparison.
- Initially, all distributions are considered
- The distributions with too low P-value (around 0.05/0.1 and lower) are removed
- All remaining distributions are compared with AICc (weight) and QQ-plots (fit)
- Plot all Q-Q, remove distributions with poor fit of the lower values (left tail of SSD curve)
- Plot interactive curves of the remaining distributions.
- Compare distribution models and determine one (argumentation for that specific one)

Table 2: The results (=HC5 value and the 95% lower and upper limit) from the different SSD analysis based on different selection criteria for data (see further description in text). Nr 1 is the approach forming the basis of the EQS derivation.

Nr	Origin	OC norm.	AVS ($\mu\text{mol/g}$)	HC5	Lower limit	Upper limit
1.	Pooled	5%	<1 + marine <4.5	61	33	124
2.	Pooled – only lowest	5%	<1 + marine <4.5	19	7	53
3.	Pooled	5%	<1	74	40	139
4.	Pooled	5%	No limit	75	44	143
5.	Marine	5%	No limit (<5 $\mu\text{mol/g}$)	40	7	231
6.	Pooled	No	<1 + marine <4.5	12	4	33
7.	Pooled – only lowest	No	<1 + marine <4.5	6	2	15
8.	Pooled	No	<1	7	2	27
9.	Pooled	No	No limit	20	7	50
10.	Marine	No	No limit (<5 $\mu\text{mol/g}$)	17	3	94

1. SSD pooled data; AVS<1 $\mu\text{mol/g}$ (plus marine <4.5 $\mu\text{mol/g}$ AVS); 5% OC norm. (preferred approach)
 - 49 test results from 12 species. Range toxicity values (geometric mean): 50-1513 mg/kg dw. Ratio Max:Min=Max/Min=30.
 - Normal, Logistic, Gumble and Burr III have high p-values (indicate a good fit) and logistic has the highest weight in the AICc table (0.5)
 - Burr cannot generate lower confidence interval - removed
 - **Logistic** distribution fits best lower values (Q-Q plot below, $R^2=0.97$)

- All fitting alternatives generates a HC5 value with a confidence interval that does not cover the most sensitive species/endpoint of *M. plumulosa* (40 mg/kg), *H. Azteca* (51 mg/kg), *T. tubifex* (32 mg/kg) and *T. deltoidalis* (37 mg/kg).
- HC5=61 mg/kg dw (Lower limit 33 mg/kg; upper limit 124 mg/kg) **Logistic fit**

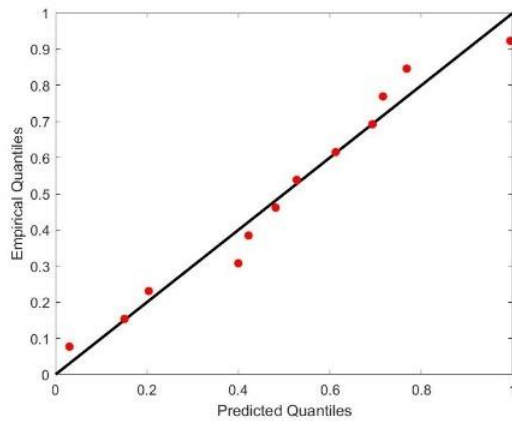


Figure 5: Q-Q plot of the Logistic distribution fit comparing the predicted vs. the true geometric mean (or single value) of each species (red dots). $R^2=0.97$.

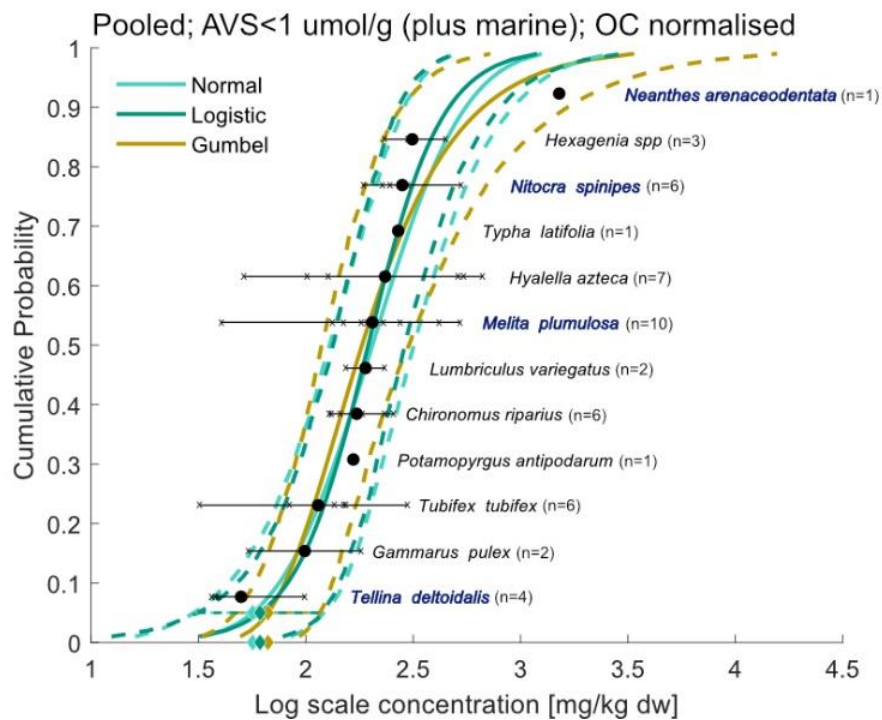


Figure 6: The SSD curves of the pooled dataset normalised to 5% organic carbon where AVS<1 $\mu\text{mol/g}$ for freshwater species (or if AVS has been defined as <4.5 $\mu\text{mol/g}$ for marine species). Black dots represent the geometric mean of the NOEC/EC10 value of the specific species (name to the right of each dot, marine species are bold dark blue) and the lines show the full range (X marks every discrete NOEC/EC10 value included in the analysis; n=number of NOEC/EC10 values). The different colours represent different distributions (Normal, Logistic and Gumbel) with the full line showing the fitted curve and the dashed lines the upper and lower confidence interval of that curve. The diamonds represent the calculated HC5 values with the horizontal dashed lines showing the 95 % confidence interval of the HC5 value. Diamonds are plotted again on the x-axis for improved reading.

2. SSD pooled; only lowest end-point value (discrete); data normalized to OC 5%; AVS<1 $\mu\text{mol/g}$ (plus marine <4.5 $\mu\text{mol/g}$ AVS)
 - 12 test results from 12 species
 - Range toxicity values: 32-1513 mg/kg dw. Ratio Max:Min=Max/Min=47.
 - All but Burr have p-values ≥ 0.05 (but Gumbel p=0.06 and Logistic p=0.06 and is also removed)
 - **Weibull** results in the more conservative HC5 value but might be overprotective, removed. Gumbel has the highest weight but Logistic has better fit.
 - All HC5 values (Figure 7) are protective of the individual NOEC/EC10 values.
 - HC5=19 mg/kg dw (Lower limit 7 mg/kg; upper limit 53 mg/kg) **Logistic fit**

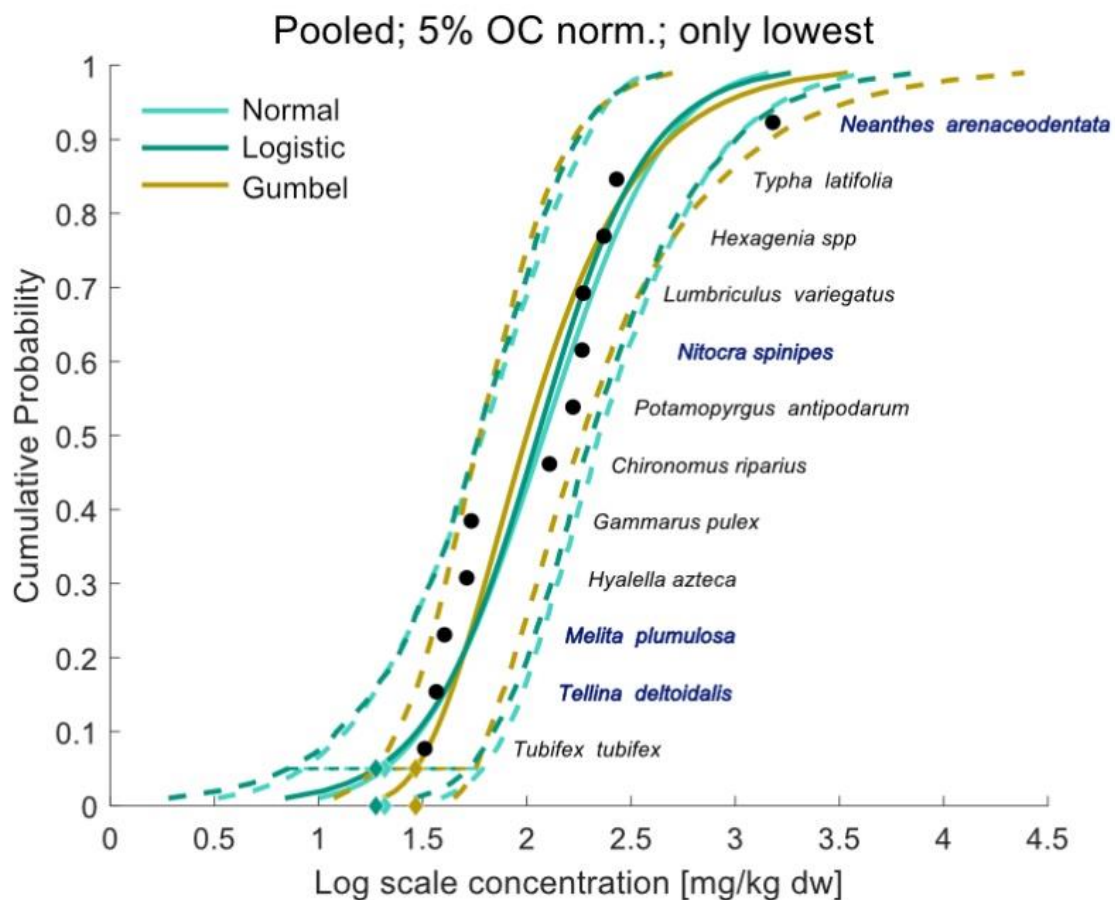


Figure 7: The SSD curves of the pooled dataset normalised to 5% organic carbon where AVS<1 $\mu\text{mol/g}$ for freshwater species (or if AVS has been defined as <4.5 $\mu\text{mol/g}$ for marine species). Black dots represent the lowest NOEC/EC10 value of the specific species (name to the right of each dot, marine species are bold dark blue). The different colours represent different distributions (Normal, Logistic and Gumbel) with the full line showing the fitted curve and the dashed lines the upper and lower confidence interval of that curve. The diamonds represent the calculated HC5 values with the horizontal dashed lines showing the 95 % confidence interval of the HC5 value. Diamonds are plotted again on the x-axis for improved reading.

3. SSD pooled data; AVS < 1 $\mu\text{mol/g}$; 5% OC normalization
 - 31 test results from 11 species
 - Range toxicity values (geometric mean): 99-1513. Ratio=Max/Min=15.
 - Normal, Logistic, Gumbel and Burr have p-values > 0.1 and Gumbel has highest weight (0.67)
 - **Logistic** has better fit (lower values) in Q-Q.
 - Generates a HC5 value with a confidence interval that does not cover the most sensitive species/endpoint of *H. Azteca* (51) and *T. tubifex* (32).
 - HC5=74 mg/kg dw (Lower limit 40 mg/kg; upper limit 139 mg/kg) **Logistic fit**

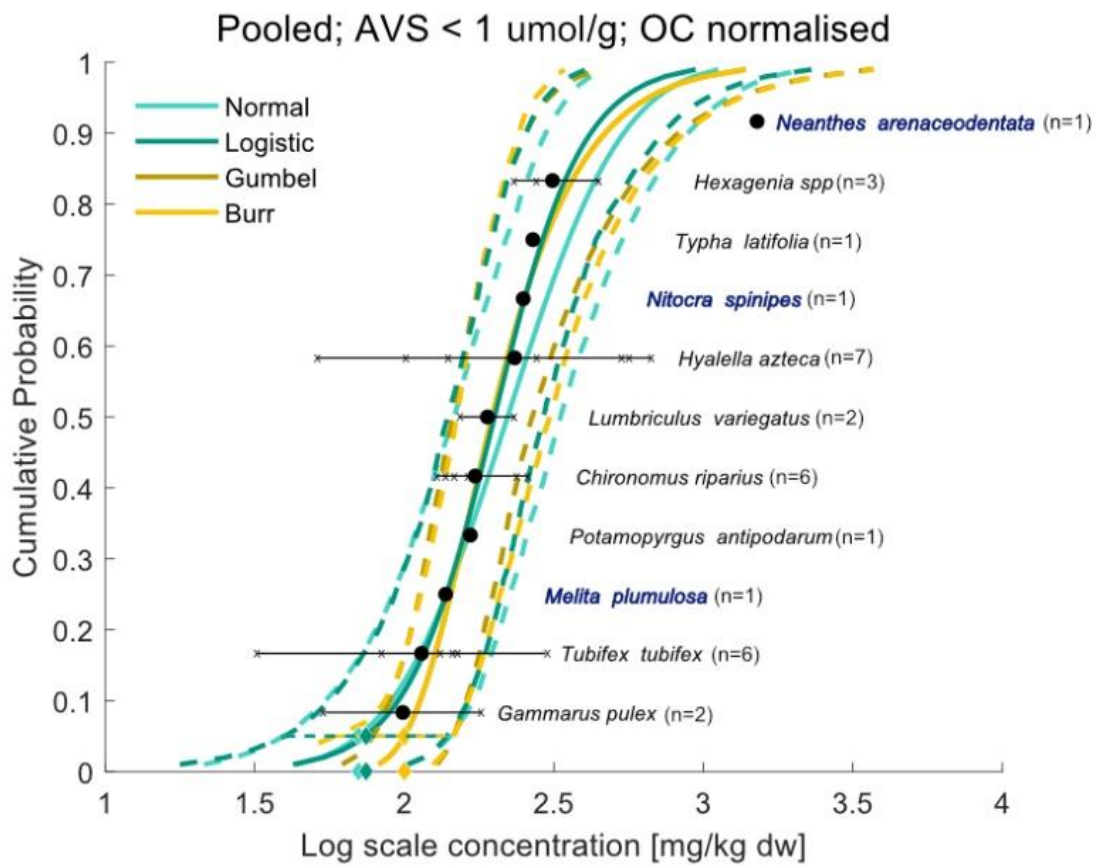


Figure 8: The SSD curves of the pooled dataset normalised to 5% organic carbon where AVS < 1 $\mu\text{mol/g}$ for all species. Black dots represent the geometric mean of the NOEC/EC10 value of the specific species (name to the right of each dot, marine species are bold dark blue) and the lines show the full range (X marks every discrete NOEC/EC10 value included in the analysis; n=number of NOEC/EC10 values). The different colours represent different distributions (Normal, Logistic, Gumbel and Burr) with the full line showing the fitted curve and the dashed lines the upper and lower confidence interval of that curve. The diamonds represent the calculated HC5 values with the horizontal dashed lines showing the 95 % confidence interval of the HC5 value. Diamonds are plotted again on the x-axis for improved reading.

4. SSD pooled; all AVS and normalization to OC 5%
 - 68 test results from 13 species
 - Range toxicity values (geometric mean): 50-1513. Ratio=Max/Min=30.
 - Gumbel and Weibull $p < 0.1$ and are removed
 - Logistic has the highest weight (0.67) while all Q-Q plots look similar with slightly better fit for logistic and burr, normal is the more conservative HC5 value
 - The generated HC5 value from normal fit (more conservative) with the lower confidence interval does not cover the most sensitive species/end-point of many of the species
 - Data varies with more than an order of magnitude (factor of 16 *T. tubifex*).
 - Not possible to calculate lower CI with Burr
 - HC5=75 mg/kg dw (Lower limit 44 mg/kg; upper limit 143 mg/kg) **Normal fit**

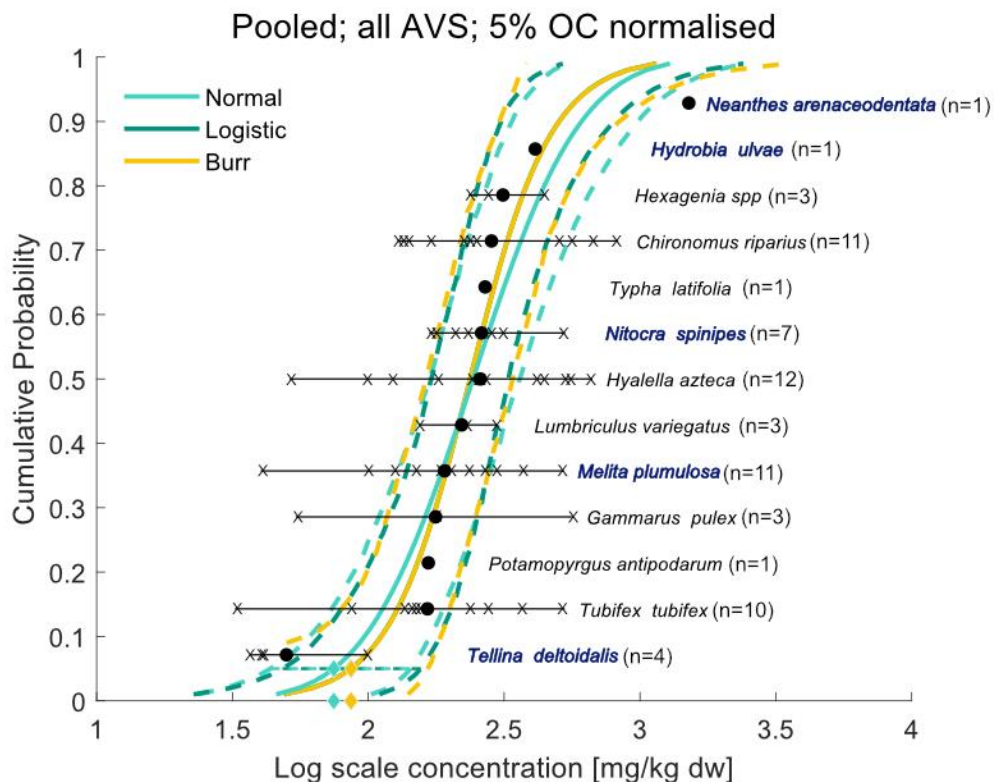


Figure 9: The SSD curves of the pooled dataset normalised to 5% organic carbon where no data has been excluded due to high AVS. Black dots represent the geometric mean of the NOEC/EC10 value of the specific species (name to the right of each dot, marine species are bold dark blue) and the lines show the full range (X marks every discrete NOEC/EC10 value included in the analysis; n=number of NOEC/EC10 values). The different colours represent different distributions (Normal, Logistic and Burr) with the full line showing the fitted curve and the dashed lines the upper and lower confidence interval of that curve. The diamonds represent the calculated HC5 values with the horizontal dashed lines showing the 95 % confidence interval of the HC5 value. Diamonds are plotted again on the x-axis for improved reading.

5. SSD only marine; normalized to 5% OC; all AVS
 - 23 test results from 5 species
 - Range toxicity values (geometric mean): 50-1513. Ratio=Max/Min=30
 - All have p-values>=0.9
 - **Weibull** results in the more conservative HC5 value (=21 mg/kg) but might be overprotective? Both lower interval of logistic and normal covers all species. For HC5-50, only Weibull covers all species.
 - Gumbel does not provide a fit where the most sensitive species is protected at the lower CI and are excluded.
 - Hard to evaluate the better fit.
 - The generated model averaged HC5 value from Weibull, Normal, Burr and Logistic (=36 mg/kg dw; SE=33) corresponds to the most sensitive species/end-point *M. plumulosa*.
 - HC5=21 mg/kg dw (Lower limit 1 mg/kg; upper limit 242 mg/kg) **Weibull fit (most conservative of the four distributions that were left)**
 - HC5=40 mg/kg dw (Lower limit 7 mg/kg; upper limit 231 mg/kg) **Logistic fit**

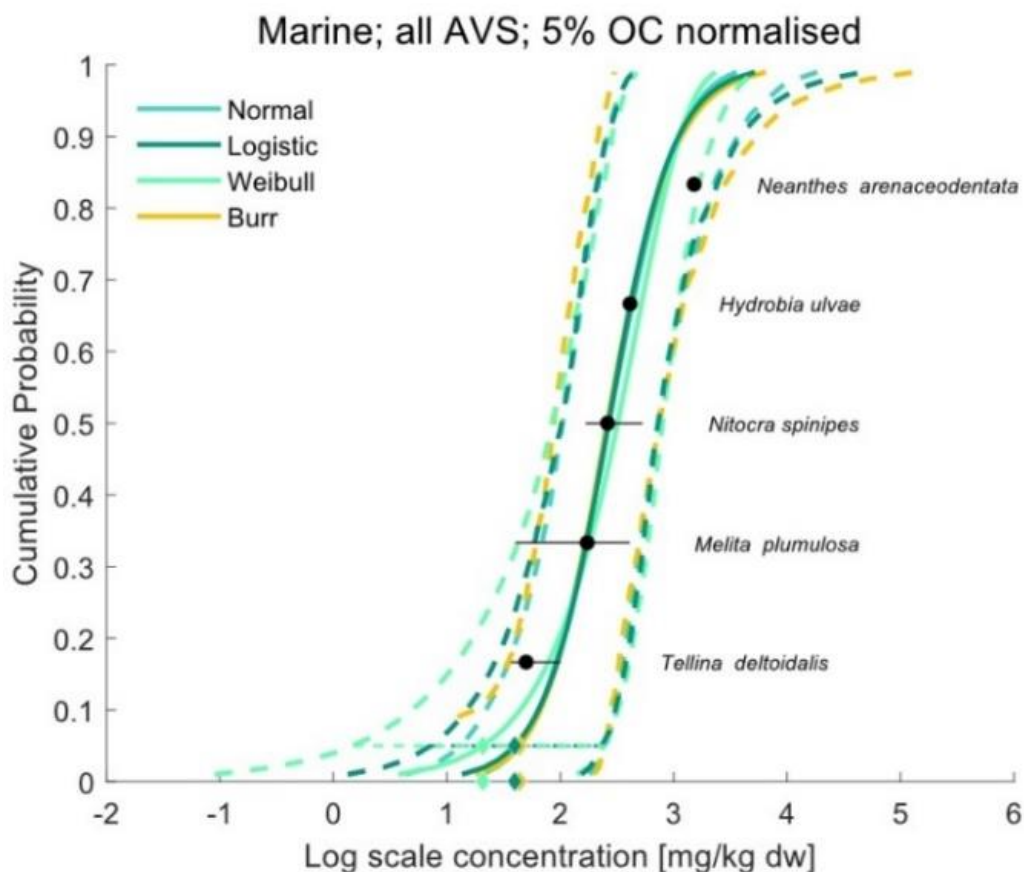


Figure 10: The SSD curves of only the marine dataset normalised to 5% organic carbon where no data has been excluded due to high AVS. Black dots represent the geometric mean of the NOEC/EC10 value of the specific species (name to the right of each dot, marine species are bold dark blue) and the lines show the full range. The different colours represent different distributions (Normal, Logistic, Weibull and Burr) with the full line showing the fitted curve and the dashed lines the upper and lower confidence interval of that curve. The diamonds represent the calculated HC5 values with the horizontal dashed lines showing the 95 % confidence interval of the HC5 value. Diamonds are plotted again on the x-axis for improved reading.

6. SSD pooled data; AVS<5 $\mu\text{mol/g}$; no other normalization
 - 49 test results from 12 species
 - Range toxicity values (geometric mean): 14-230. Ratio=Max/Min=16.
 - Normal, Burr and Weibull have p-values>0.1 and similar weight (Burr a bit lower)
 - **Weibull** has slightly better fit (lower values) in Q-Q plot
 - Generates a HC5 value with a confidence interval that almost covers the most sensitive species/endpoint of *H. Azteca* (22), *M. plumulosa* (11) and *T. deltoidalis* (6.5).
 - HC5=12 mg/kg dw (Lower limit 4 mg/kg; upper limit 33 mg/kg) **Weibull fit**

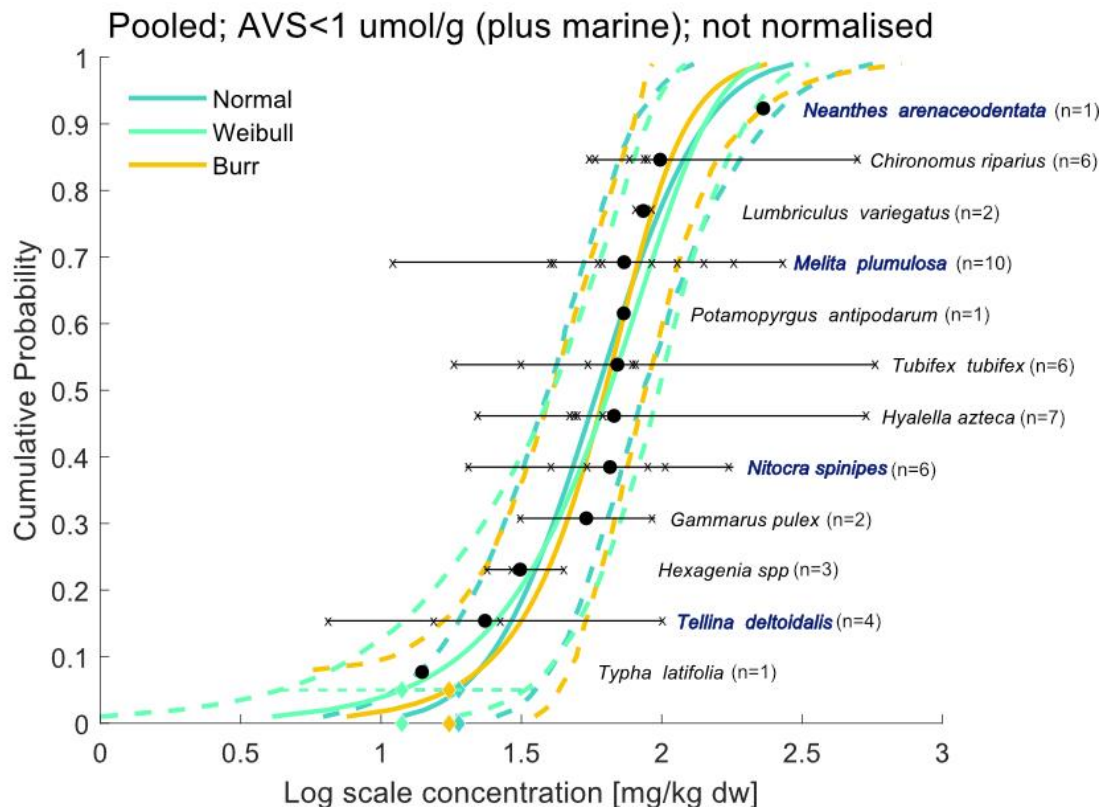


Figure 11: The SSD curves of the pooled dataset not normalised organic carbon where AVS<1 $\mu\text{mol/g}$ for freshwater species (or if AVS has been defined as <4.5 $\mu\text{mol/g}$ for marine species). Black dots represent the geometric mean of the NOEC/EC10 value of the specific species (name to the right of each dot, marine species are bold dark blue) and the lines show the full range (X marks every discrete NOEC/EC10 value included in the analysis; n=number of NOEC/EC10 values). The different colours represent different distributions (Normal, Weibull and Gumbel) with the full line showing the fitted curve and the dashed lines the upper and lower confidence interval of that curve. The diamonds represent the calculated HC5 values with the horizontal dashed lines showing the 95 % confidence interval of the HC5 value. Diamonds are plotted again on the x-axis for improved reading.

7. SSD pooled; only lowest end-point value (discrete); data NOT normalized to OC; AVS < 1 $\mu\text{mol/g}$ (+ marine AVS < 4.5 $\mu\text{mol/g}$).
- 12 test results from 12 species
 - Range toxicity values (geometric mean): 6-230. Ratio=Max/Min=38
 - All have p-values ≥ 0.1
 - **Weibull** results in the more conservative HC5 value but has the lowest p-value and might be overprotective?
 - Logistic fit has the best fit for lower values (Q-Q-plot)
 - The generated model averaged HC5 value corresponds to the most sensitive species/endpoint of many of the species.
 - HC5 value of the Gumbel distribution exceeds the lowest NOEC/EC10 value of *T. deltoidalis*
 - HC5=6 mg/kg dw (Lower limit 2 mg/kg; upper limit 15 mg/kg) **Logistic fit**

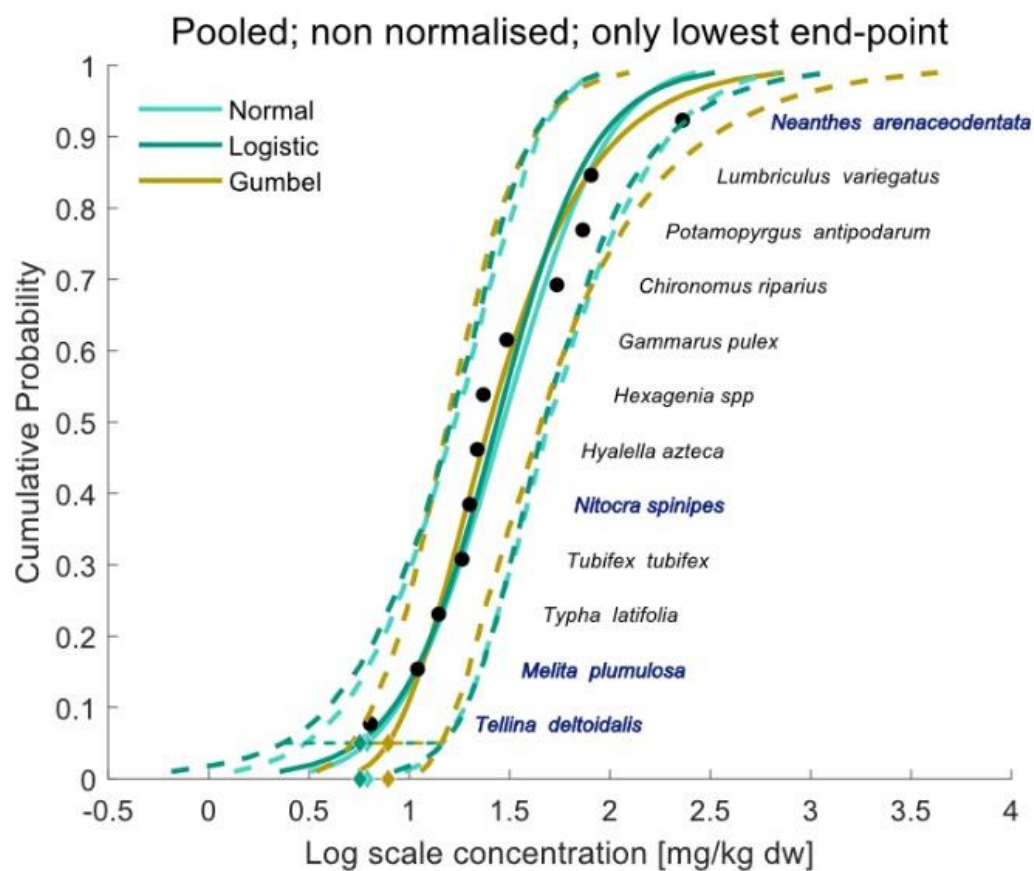


Figure 12: The SSD curves of the pooled dataset not normalised to organic carbon where AVS < 1 $\mu\text{mol/g}$ for freshwater species (or if AVS has been defined as < 4.5 $\mu\text{mol/g}$ for marine species). Black dots represent the lowest NOEC/EC10 value of the specific species (name to the right of each dot, marine species are bold dark blue). The different colours represent different distributions (Normal, Logistic and Gumbel) with the full line showing the fitted curve and the dashed lines the upper and lower confidence interval of that curve. The diamonds represent the calculated HC5 values with the horizontal dashed lines showing the 95 % confidence interval of the HC5 value. Diamonds are plotted again on the x-axis for improved reading.

8. SSD pooled data; AVS < 1 $\mu\text{mol/g}$; no other normalization
 - 31 test results from 11 species
 - Range toxicity values (geometric mean): 11-230. Ratio=Max/Min=21.
 - Normal, Logistic, Burr and Weibull have p-values > 0.1 and similar weight
 - **Weibull** has slightly better fit (lower values) in Q-Q plot.
 - Generates a HC5 value with a confidence interval that covers the most sensitive species/endpoint.
 - HC5 = 7 mg/kg dw (Lower limit 2 mg/kg; upper limit 27 mg/kg) **Weibull fit**

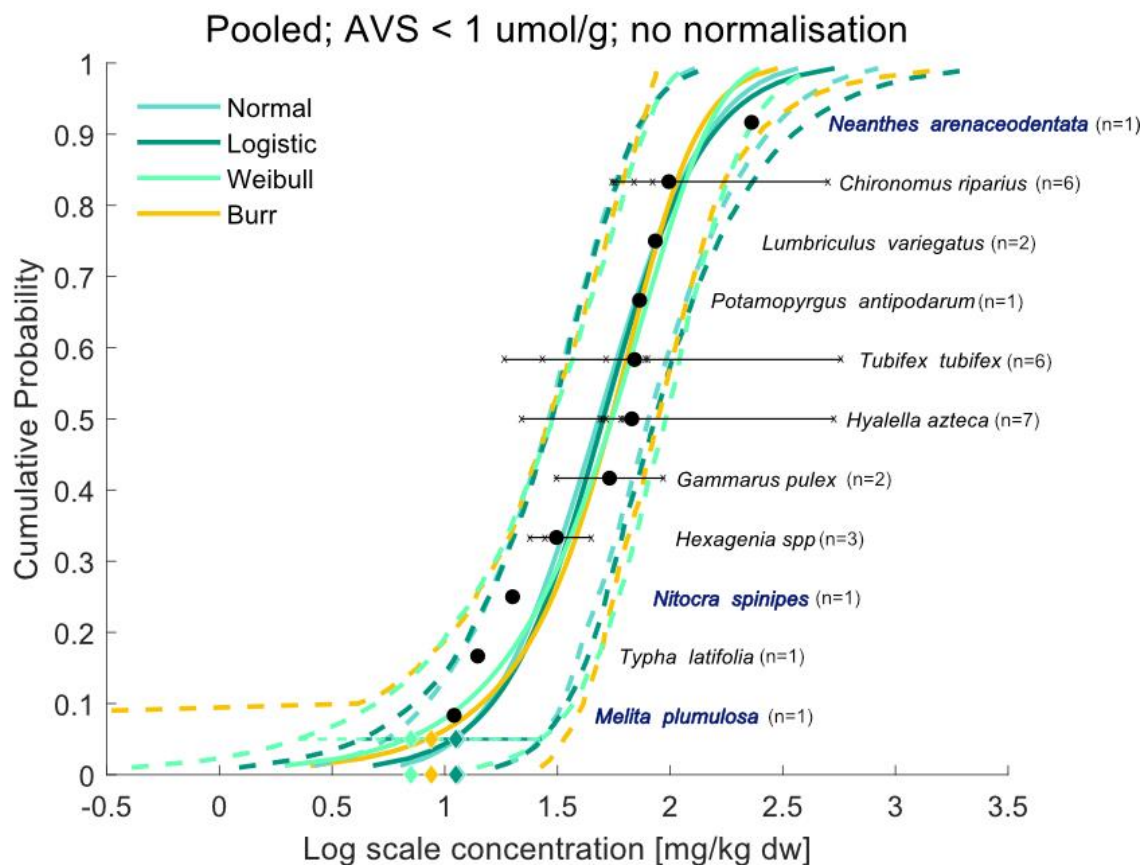


Figure 13: The SSD curves of the pooled dataset not normalised to organic carbon where AVS < 1 $\mu\text{mol/g}$ for all species. Black dots represent the geometric mean of the NOEC/EC10 value of the specific species (name to the right of each dot, marine species are bold dark blue) and the lines show the full range (X marks every discrete NOEC/EC10 value included in the analysis; n=number of NOEC/EC10 values). The different colours represent different distributions (Normal, Logistic, Weibull and Burr) with the full line showing the fitted curve and the dashed lines the upper and lower confidence interval of that curve. The diamonds represent the calculated HC5 values with the horizontal dashed lines showing the 95 % confidence interval of the HC5 value. Diamonds are plotted again on the x-axis for improved reading.

9. SSD pooled; all AVS and no normalization to OC
- 68 test results from 13 species
 - Range toxicity values (geometric mean): 14-247. Ratio=Max/Min=18.
 - Normal, Logistic and Weibull have p-values ≥ 0.1 and Weibull have higher weight than the others (0.7) and the highest p-value (0.3)
 - **Burr** has the better fit of low values in Q-Q plots (but HC5=0) but is removed, and Weibull results in the more conservative HC5 value compared to the rest.
 - The generated HC5 value from **Weibull fit** (more conservative) with the lower confidence interval does not cover the most sensitive species/endpoint of many of the species
 - Data varies with more than an order of magnitude (2 orders of magnitude for *T. tubifex*).
 - HC5=20 mg/kg dw (Lower limit 7 mg/kg; upper limit 50 mg/kg) **Weibull fit**

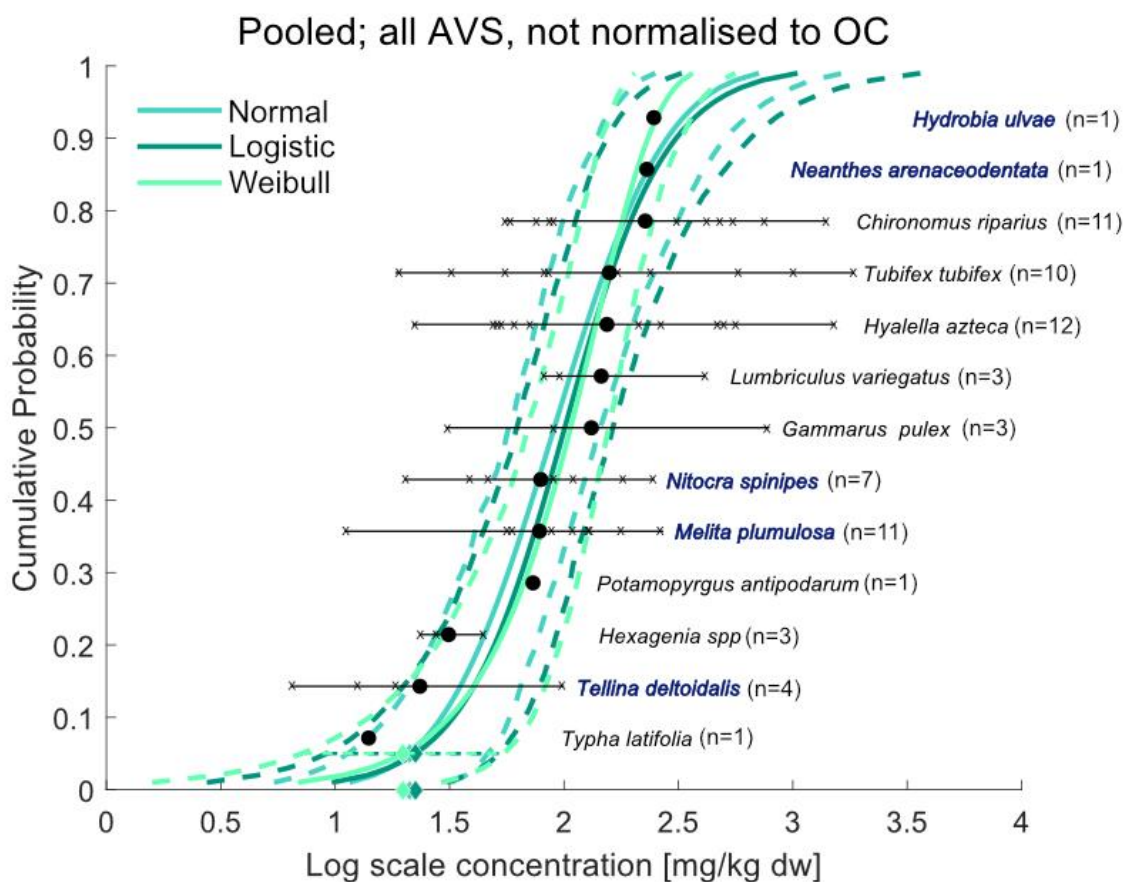


Figure 14: The SSD curves of the pooled dataset not normalised to organic carbon where no data has been excluded due to high AVS. Black dots represent the geometric mean of the NOEC/EC10 value of the specific species (name to the right of each dot, marine species are bold dark blue) and the lines show the full range (X marks every discrete NOEC/EC10 value included in the analysis; n=number of NOEC/EC10 values). The different colours represent different distributions (Normal, Logistic and Weibull) with the full line showing the fitted curve and the dashed lines the upper and lower confidence interval of that curve. The diamonds represent the calculated HC5 values with the horizontal dashed lines showing the 95% confidence interval of the HC5 value. Diamonds are plotted again on the x-axis for improved reading

10. SSD only marine; not normalized to OC; AVS<5 µmol/g (=all)

- 23 test results from 5 species
- Range toxicity values (geometric mean): 23-247. Ratio=Max/Min=11
- OC content of the different tests: min=0.4; max=7.4; average=2.77; std=2.24; CI; 0.94.
- Burr yielded value=0 → removed
- All have p-values>=0.2
- **Weibull** results in the more conservative HC5 value (=17 mg/kg). For HC5-lower interval, only Weibull covers all species.
- Hard to evaluate the better fit.
- The generated model averaged HC5 value from Weibull, Normal, Burr and Logistic (=20 mg/kg dw; SE=14) does not correspond to the most sensitive species/end-point *M. plumulosa*.
- HC5=17 mg/kg dw (Lower limit 3 mg/kg; upper limit 94 mg/kg) **Weibull fit (most conservative of the four distributions that were left)**

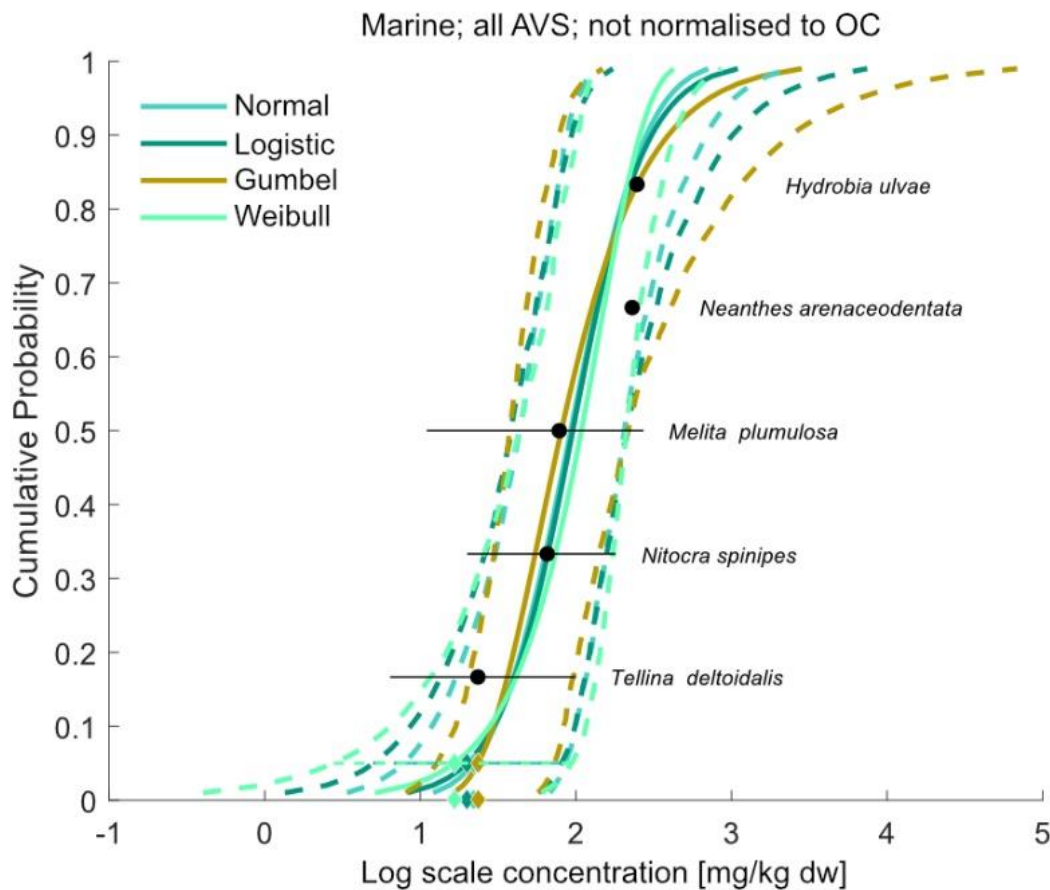


Figure 15: The SSD curves of only the marine dataset not normalised to organic carbon where no data has been excluded due to high AVS. Black dots represent the geometric mean of the NOEC/EC10 value of the specific species (name to the right of each dot, marine species are bold dark blue) and the lines show the full range. The different colours represent different distributions (Normal, Logistic, Weibull and Burr) with the full line showing the fitted curve and the dashed lines the upper and lower confidence interval of that curve. The diamonds represent the calculated HC5 values with the horizontal dashed lines showing the 95 % confidence interval of the HC5 value. Diamonds are plotted again on the x-axis for improved reading

Compilation figure, all SSD plots

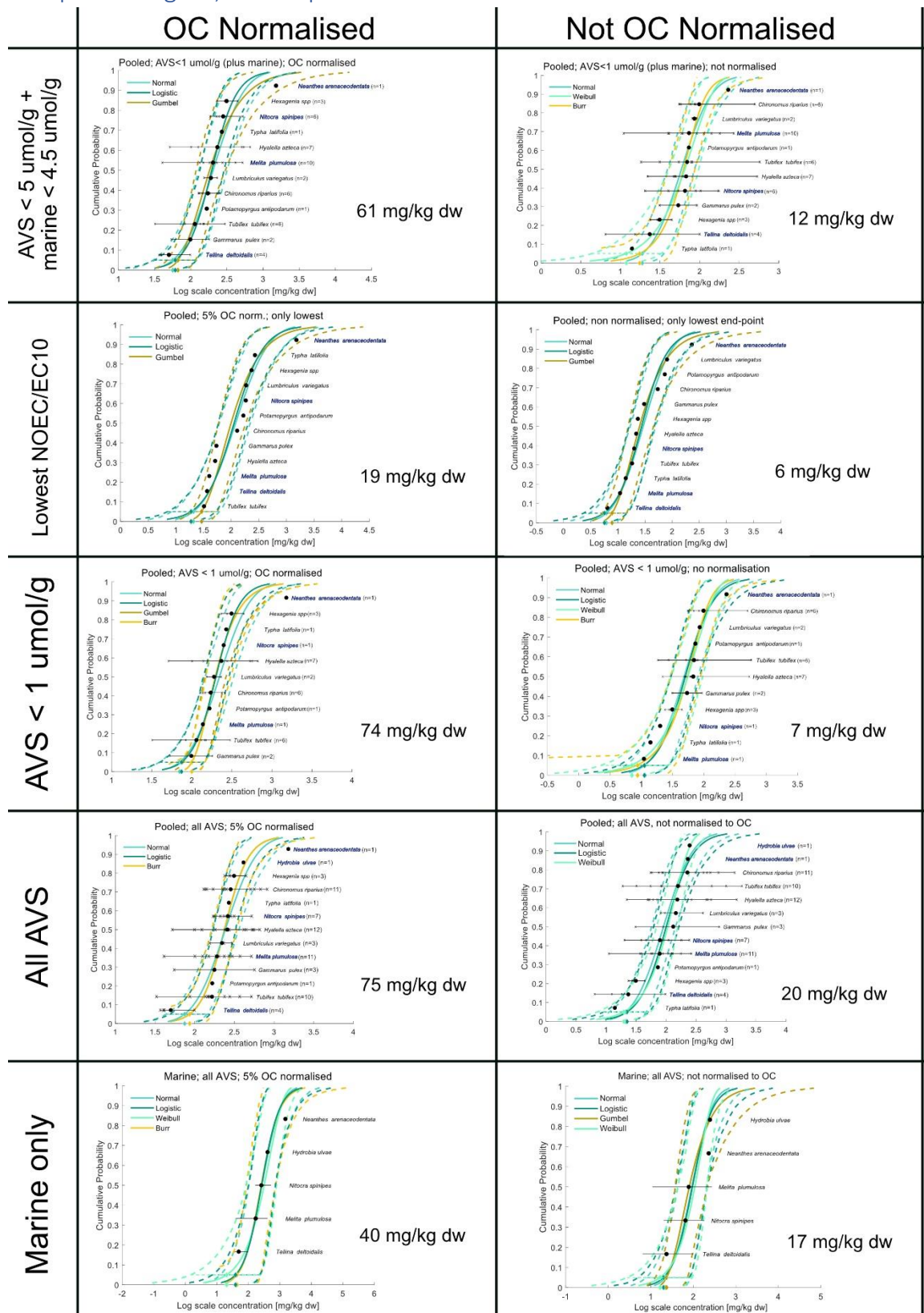


Figure 16: Compilation of all SSD curves (Figure 6-15) with their corresponding HC5 value (based on the most appropriate fit). The 1st column shows results from when the data was normalised to 5% organic carbon and the 2nd column shows the corresponding results (same selection criteria) without normalisation to organic carbon.

Data used in SSD for determination of HC5

Table 3: All data used in the SSD analysis with Genus and species stated together with the non-normalised and normalised (to 5% organic carbon) toxicity value (i.e. NOEC or EC10 value). The organic carbon (OC) content of the sediment is given in % and the acid volatile sulphide concentration in $\mu\text{mol/g}$. If no AVS is defined, more information can be found in the "Comments" column. The relevant endpoint and water type (i.e. freshwater or marine water) for the ecotoxicity test is found in column 7-8 and the reference in column 9. Column 10-13 defines the selection criteria that are being fulfilled and in what SSD analysis the data is used. The results from the reliability and relevance analysis is found in column 15 where Q1 represent highest quality from the reliability analysis conducted by ECI (2008) and R2 means reliable with restrictions for the studies that have been analysed with the CRED tool (Moermond et al., 2016).

Genus	Species	Toxicity value (not OC normalised)	Toxicity value (5% OC normalised)	OC content (%)	AVS ($\mu\text{mol/g}$)	Endpoint	Water type	Reference	Comment	Included in all AVS SSD	Included AVS<1 $\mu\text{mole/g}$ + marine <4.5 $\mu\text{mole/g}$ SSD	Included AVS<1 $\mu\text{mole/g}$ SSD	Included in "only lowest OC norm"	Reliability relevance analysis (CRED) or Q1 (vRAR)
Chironomus	riparius	54.4	128.3	2.12	0.28	Growth	Freshwater	De Schampelaere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	Yes	Q1
Chironomus	riparius	55.5	141.6	1.96	0.1	Growth	Freshwater	De Schampelaere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	No	Q1
Chironomus	riparius	75.4	133.2	2.83	0.15	Growth	Freshwater	De Schampelaere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	No	Q1
Chironomus	riparius	89.2	170.2	2.62	0.05	Growth	Freshwater	De Schampelaere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	No	Q1
Chironomus	riparius	90.0	248.6	1.81	-	Growth	Freshwater	Péry et al. (2005)	Artificial sediment, probably no AVS.	Yes	Yes	Yes	No	R2
Chironomus	riparius	318.0	477.5	3.33	4.02	Growth	Freshwater	De Schampelaere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1
Chironomus	riparius	452.6	230.7	9.81	4.05	Growth	Freshwater	De Schampelaere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1
Chironomus	riparius	505.9	257.8	9.81	0.3	Growth	Freshwater	De Schampelaere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	No	Q1
Chironomus	riparius	553.6	831.2	3.33	16.21	Growth	Freshwater	De Schampelaere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1
Chironomus	riparius	776.5	557.0	6.97	15.57	Growth	Freshwater	De Schampelaere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1
Chironomus	riparius	1417.0	722.2	9.81	12.6	Growth	Freshwater	De Schampelaere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1

Gammarus	pulex	30.6	54.1	2.83	0.21	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	Yes	Q1
Gammarus	pulex	94.7	180.7	2.62	0.05	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	No	Q1
Gammarus	pulex	789.0	566.0	6.97	17.5	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1
Hexagenia	spp	23.4	234.0	0.5		Growth	Freshwater	Milani et al. (2003)	AVS not given but study considered as Q1 in the Cu vRAR (2008) and OC=0.5% indicate low AVS. OC 0.5% is an estimate.	Yes	Yes	Yes	Yes	Q1
Hexagenia	spp	29.2	292.0	0.5		Growth	Freshwater	Milani et al. (2003)	AVS not given but study considered as Q1 in the Cu vRAR (2008) and OC=0.5% indicate low AVS. OC 0.5% is an estimate.	Yes	Yes	Yes	No	Q1
Hexagenia	spp	44.9	449.0	0.5		Growth	Freshwater	Milani et al. (2003)	AVS not given but study considered as Q1 in the Cu vRAR (2008) and OC=0.5% indicate low AVS. OC 0.5% is an estimate.	Yes	Yes	Yes	No	Q1
Hyaella	azteca	21.8	51.4	2.12	0.28	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	Yes	Q1
Hyaella	azteca	49.9	127.3	1.96	0.1	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	No	Q1
Hyaella	azteca	52.3	523.0	0.5		Growth	Freshwater	Milani et al. (2003)	AVS not given but study considered as Q1 in the Cu vRAR (2008) and OC=0.5% indicate low AVS. OC 0.5% is an estimate.	Yes	Yes	Yes	No	Q1
Hyaella	azteca	53.2	101.5	2.62	0.05	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	No	Q1
Hyaella	azteca	244.8	188.9	6.48	18.25	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1
Hyaella	azteca	59.3	593.0	0.5		Growth	Freshwater	Milani et al. (2003)	AVS not given but study considered as Q1 in the Cu vRAR (2008) and OC=0.5% indicate low AVS. OC 0.5% is an estimate.	Yes	Yes	Yes	No	Q1

Hyalella	azteca	66.9	669.0	0.5		Growth	Freshwater	Milani et al. (2003)	AVS not given but study considered as Q1 in the Cu vRAR (2008) and OC=0.5% indicate low AVS. OC 0.5% is an estimate.	Yes	Yes	Yes	No	Q1
Hyalella	azteca	292.5	444.5	3.29	4.87	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1
Hyalella	azteca	492.7	255.0	9.66	5.3	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1
Hyalella	azteca	512.2	265.1	9.66	8.97	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1
Hyalella	azteca	538.6	278.8	9.66	0.27	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	No	Q1
Hyalella	azteca	1531.0	405.0	18.9	58.6	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1
Lumbriculus	variegatus	80.5	153.6	2.62	0.05	Biomass	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)	Same study as presented in (Roman et al., 2007)	Yes	Yes	Yes	Yes	Q1
Lumbriculus	variegatus	91.8	234.2	1.96	0.1	Biomass	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)	Same study as presented in (Roman et al., 2007)	Yes	Yes	Yes	No	Q1
Lumbriculus	variegatus	416.3	299	6.97	16.5	Biomass	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)	Same study as presented in (Roman et al., 2007)	Yes	No	No	No	Q1
Potamopyrgus	antipodarum	73.2	166.4	2.2		Growth	Freshwater	Pang et al. (2013)	Oxygenated sediment, AVS probably low.	Yes	Yes	Yes	Yes	R2
Tubifex	tubifex	18.3	32.3	2.83	0.27	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	Yes	Q1
Tubifex	tubifex	32.2	75.9	2.12	0.28	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	No	Q1
Tubifex	tubifex	53.0	135.2	1.96	0.1	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	No	Q1
Tubifex	tubifex	78.3	149.4	2.62	0.05	Growth	Freshwater	REACH registration ref 005	Could be same as 79.3 at Ghent University?	Yes	Yes	Yes	No	Q1
Tubifex	tubifex	163.0	244.7	3.33	8.04	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1

Tubifex	tubifex	79.3	151.3	2.62	0.05	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	No	Q1
Tubifex	tubifex	271.6	140.6	9.66	15.15	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1
Tubifex	tubifex	580.9	296.1	9.81	0.59	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	Yes	Yes	No	Q1
Tubifex	tubifex	1036.5	528.3	9.81	5.43	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1
Tubifex	tubifex	1855.6	374.1	24.8	56.4	Growth	Freshwater	De Schampheleere et al. (2004) (Ghent University, 2004 Cu vRAR)		Yes	No	No	No	Q1
Typha	latifolia	14.0	269.2	0.26		Seedling root elongation	Freshwater	Muller et al. (2001)	8 mg/L O ₂ in sediment, saturated. AVS probably very low. "An oxidizing environment was indicated by a positive oxidation-reduction potential in all amended sediment exposures; therefore, acid volatile sulfides were not significant for regulating copper bioavailability"	Yes	Yes	Yes	Yes	R2
Hydrobia	ulvae	247.0	411.7	3	-	Reproduction	Marine	Campana et al. (2013)	Very low redox potential so could be high AVS	Yes	No	No	No	R2
Melita	plumulosa	146.0	98.6	7.4	4.4	Reproduction	Marine	Campana et al. (2012)		Yes	No	No	No	R2
Melita	plumulosa	11.0	137.5	0.4	<0.1	Reproduction	Marine	Campana et al. (2012)		Yes	Yes	Yes	Yes (no norm)	R2
Melita	plumulosa	111.0	146.1	3.8	<4.5	Reproduction	Marine	Campana et al. (2012)		Yes	Yes	No	No	R2
Melita	plumulosa	146.0	235.5	3.1	<4.5	Reproduction	Marine	Campana et al. (2012)		Yes	Yes	No	No	R2
Melita	plumulosa	39.0	278.6	0.7	<4.5	Reproduction	Marine	Campana et al. (2012)		Yes	Yes	No	No	R2
Melita	plumulosa	91.0	303.3	1.5	<4.5	Reproduction	Marine	Campana et al. (2012)		Yes	Yes	No	No	R2
Melita	plumulosa	41.0	410.0	0.5	<4.5	Reproduction	Marine	Campana et al. (2012)		Yes	Yes	No	No	R2

Melita	plumulosa	59.5	40.2	7.4	<4.5	Reproductio n	Marine	Campana et al. (2015)		Yes	Yes	No	Yes (OC norm)	R2
Melita	plumulosa	185.0	185.0	5	-	Reproductio n	Marine	Campana et al. (2015)		Yes	Yes	No	No	R2
Melita	plumulosa	58.7	195.7	1.5	<4.5	Reproductio n	Marine	Campana et al. (2015)		Yes	Yes	No	No	R2
Melita	plumulosa	272.0	523.1	2.6	<4.5	Reproductio n	Marine	Campana et al. (2015)		Yes	Yes	No	No	R2
Neanthes	arenaceodentat a	230.0	1513.0	0.76	0.76	Growth	Marine	Ward et al. (2015)		Yes	Yes	Yes	Yes	R2
Nitocra	spinipes	248.0	167.6	7.4	4.4	Reproductio n	Marine	Campana et al. (2012)		Yes	No	No	No	R2
Nitocra	spinipes	114.0	183.9	3.1	<4.5	Reproductio n	Marine	Campana et al. (2012)		Yes	Yes	No	Yes (OC norm)	R2
Nitocra	spinipes	180.0	236.8	3.8	<4.5	Reproductio n	Marine	Campana et al. (2012)		Yes	Yes	No	No	R2
Nitocra	spinipes	20.0	250.0	0.4	<0.1	Reproductio n	Marine	Campana et al. (2012)		Yes	Yes	Yes	Yes (no norm)	R2
Nitocra	spinipes	40.0	285.7	0.7	<4.5	Reproductio n	Marine	Campana et al. (2012)		Yes	Yes	No	No	R2
Nitocra	spinipes	90.0	300.0	1.5	<4.5	Reproductio n	Marine	Campana et al. (2012)		Yes	Yes	No	No	R2
Nitocra	spinipes	53.0	530.0	0.5	<4.5	Reproductio n	Marine	Campana et al. (2012)		Yes	Yes	No	No	R2
Tellina	deltoidalis	100.0	100.0	5	-	Growth	Marine	Campana et al. (2015)		Yes	Yes	No	No	R2
Tellina	deltoidalis	16.9	36.7	2.3	<4.5	Growth	Marine	Campana et al. (2015)		Yes	Yes	No	Yes	R2
Tellina	deltoidalis	6.4	40.0	0.8	<4.5	Growth	Marine	Campana et al. (2015)		Yes	Yes	No	No	R2
Tellina	deltoidalis	28.0	42.4	3.3	<4.5	Growth	Marine	Campana et al. (2015)		Yes	Yes	No	No	R2

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