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Analysis of stygofaunal associations with groundwater salinity and potential salinity tolerances based on observations for the Mulga Downs Iron Ore Mine

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1. Summary

The use of managed aquifer recharge (MAR) through reinjection as a means of disposing excess mine dewater for the Mulga Downs Iron Ore Mine (MDIOM) has the potential to change the groundwater salinity. Within the extent of the predicted groundwater changes, the increase of the salinity is predicted to be up to 7,031 µS/cm (equivalent to 4,500 mg/L TDS). Based on the subterranean fauna data collected from surveys, certain stygofauna were only recorded within the predicted change to salinity extent (referred to as restricted). While these stygofauna are not considered to be truly restricted, a precautionary approach was applied as part of the mitigation to any potential impacts. An initial investigation into inferred salinity tolerances of the stygofauna was found to have limitations; the main was that the vertical profile of the aquifer was not considered. To address this, the approach was to use statistical analyses to investigate the likely distribution of stygofauna based on the inferred baseline salinity data at depth. The focus of the study was on the potentially restricted stygofauna. The outcome from this study was inferred baseline salinity tolerances for the stygofauna (i.e. the maximum salinity ranges which these taxa would occupy). The majority of the stygofauna were found to have ranges in excess of the predicted salinity change of 7,031 µS/cm. Given the low explanatory power of the statistical models, a conservative approach was taken to estimate the salinity tolerances of the genera within which the key stygofauna occur. This information has been used to develop the triggers and thresholds for the MDIOM Water Management Plan to manage the impacts to the stygofauna from any changes to groundwater salinity as a result on the reinjection of mine dewater.

2. Purpose and Scope

This memorandum (memo) has been prepared to inform the environmental impact assessment of the MDIOM (the Proposal). The purpose of this memorandum is to present the analytical work which investigates the association between stygofauna distribution and baseline groundwater salinities using statistical evaluation.

Specifically, this memorandum is to:

- Document the analyses performed and the strengths and limitations of the findings;
- Present the key outcomes of statistical analyses conducted to characterise stygofauna associations with groundwater salinity; and
- Provide information that inform salinity thresholds to manage potential impacts to the stygofauna from changes to the groundwater.

The focus of the work was on the stygofauna considered to be restricted to the areas of predicted salinity changes and other areas of impact. The outcome of this work has been applied to the MDIOM Water Management Plan and the basis for the inferred tolerance ranges and thresholds.

3. Background

3.1 The Proposal

Hancock Prospecting Pty Ltd (HPPL, the Proponent) is proposing to develop the MDIOM, located approximately 210 kilometers (km) south of Port Hedland and 180 km northwest of Newman in the Pilbara Region of Western Australia. The Proposal is located within a 16,848.53 hectare (ha) Development Envelope and requires clearing of up to 4,339.16 ha of native vegetation. The mine will produce up to 12 million tonnes per annum (Mtpa) of ore.

The following key elements of the Proposal were identified as direct impacts to the environmental factor Subterranean Fauna:

- The development of a series of above and below water table mine pits;
- Abstraction of up to 12 giga liters per annum (GL/a) of groundwater (as mine dewatering to facilitate the recovery of ore below water table in the mine pits and to supply the mine); and
- Disposal of up to 11 GL/a of the mine dewater primarily via managed aquifer recharge (MAR) and/or pit infiltration.

The Pilbara Region is recognised as supporting significant subterranean fauna communities. As such, significant effects are considered possible on the subterranean fauna from implementation of the Proposal based on the above key elements.

This memo has been prepared to address the need for appropriate salinity triggers and thresholds to ensure the continued persistence of the stygofauna at MDIOM.

The predicted changes in groundwater salinity from MAR are not considered to have any significant effects on troglofauna, given their habitat is above the water table. Therefore, only the stygofauna are considered in this memo.

3.2 Context of Study for the Environmental Impact Assessment

The level of assessment for the Proposal (under Part IV of the *Environmental Protection Act 1986*) was set as Public Review Period (PER) with a 6-week public review period. The public review period ended in June 2025.

Item 3 of the Environmental Scoping Document (ESD) for the Proposal included a requirement to: "*Conduct detailed habitat characterisation and ecological requirements of relevant subterranean fauna*".

To address ESD item 3, subterranean fauna habitat assessment models were developed by AQ2 using mapped lithologies and the Leapfrog Geo (3D) modelling software (AQ2, 2024). With respect to stygofauna, the habitat assessment identified the extent and continuity of geological units considered to be potentially habitable to stygofauna. The areal extent of the habitat showed continuity across the landscape.

Potential salinity changes within the groundwater at depth were not part of the assessment and were identified as a gap in knowledge during the public review period. As such, in addition to the lithological habitat assessment, salinity was chosen as an ecological criterion for assessment of impacts on stygofauna of the Proposal.

The changes most likely to impede movement within the aquifer are associated with the presence of haloclines, which are distinct layers of water characterised by a rapid change in salinity over a relatively short vertical distance. The hydrological studies completed for this Proposal have provided inferred baseline salinity values up to 30 meters below groundwater level (mbgl). This depth was chosen based on the groundwater studies for the MDIOM (AQ2 2025). At depths greater than 30 mbgl there was a noticeable increase in groundwater salinity. These are presented in Appendix A of this memorandum.

Groundwater studies for the MDIOM have predicted the extent of change to groundwater salinity from reinjection of mine dewater into the aquifer. The extent of the predicted salinity change is presented in Chapter 8 of the MDIOM Environmental Review Document and is also shown in Figures 5 to 8 of this memo.

The salinity tolerance of stygofauna was initially investigated in the Bennelongia (2024a) *Memo 675: Salinity tolerance of stygofauna at Mulga Downs Iron Ore Mine* (memo 675). Groundwater quality data was collected in situ during stygofauna surveys and limited to the first meter below groundwater level (the surficial groundwater; -1 m below standing water level (SWL)). The standard method for sampling stygofauna collects stygofauna through a column of water. Variations in the salinity at depth were not measured. Variations may identify microhabitats and specific niches for stygofauna. The constraints in memo 675 work were summarised by Biologic (2025a) in *MDIOM Stygofauna salinity tolerance memo peer review.* The constraints were identified in the peer review as primarily:

- Limited contextual information on the complexities of stygofauna communities and the spatial and vertical variability in salinity;
- Limited consideration (or supporting information) on the potential for different stygofauna taxa to occupy a variety of salinity niches; and
- Limited consideration that method of collecting water quality may bias the data by measuring only the surficial water quality and relating this to the presence of the stygofauna in only that range.

The outcome of the Biologic (2025a) review has been applied in the impact assessment for the Proposal. This memo provides the outcomes of further studies as suggested in the Biologic (2025a) peer review.

In the absence of point source data (i.e. water quality collected at the same depth that the stygofauna occupy) the approach taken was to apply statistical analyses. Using data from groundwater modelling and the stygofauna records the association between salinity and the distribution of stygofauna, including at depth, was investigated.

4. Datasets and analysis methods

4.1 Stygofauna records

The subterranean fauna dataset includes the records of stygofauna (and troglofauna) collected for the MDIOM and was presented in Bennelongia (2024b) *Mulga Downs Iron Mine: Subterranean Fauna Survey 2019-2024 (Report 595)*. A high level of inconsistencies with respect to the subterranean fauna identifications were found in Report 595. As stated by the author, this is not unusual when reconciling data where identifications are based on limited information such as morphological features and comparing with molecular analyses. To overcome this, a peer review of the data in Report 595 was commissioned by the Proponent. The review is presented in Biologic (2025b) *Peer Review of the Subterranean Fauna Survey Report 2019-2024 (Bennelongia 2024 report 595).* The outcome was an updated dataset with greater confidence in the MDIOM EIA on the subterranean fauna. The statistical analyses for this memo were undertaken using the updated dataset.

The updated data included a compilation of 1,548 records of the stygofauna collected for the Proposal. The dataset also included records from earlier collections in areas that are not currently part of this Proposal.

However, these records were primarily well-known stygofauna and not in the area of impact for the MDIOM. Their inclusion does not appear to affect the outcome.

4.1.1 Filtering of records

The dataset included the following information: collection date, specimen abundance, borehole code, sampling/collection method.

The records were filtered for stygofauna with sampling / collection codes of “net” or “scrape”. Both apply the use of a phreatic haul net as per the *Environmental Protection Authority 2021 Technical guidance – Subterranean fauna surveys for environmental impact assessment, EPA, Western Australia* (Technical Guidance). Using this method, a sample is collected from the entire water column, the range being the top of the water table to the base of the borehole or when the weighted phreatic net stops. While a “scrape” sample is technically above the water table as the method is for the collection of troglofauna; stygofauna records are often assigned to this sampling method. The technique is not accurate, and the net will at times intersect the water table, resulting in the yielding of aquatic specimens (stygofauna). As mentioned previously, given this collection technique, the depth at which the stygofauna were collected at the time of sampling is unclear, especially for the deeper boreholes.

The resulting filtered dataset consisted of 1,406 records which included 169 operational taxonomic units (OTUs), representing five phyla, 10 classes, 15 orders, and 28 families. Sixty-four genera were recorded within the dataset although 39 OTUs remained a high-level classification.

These specimens were collected across 314 unique boreholes (out of the 433 bores included in the wider surveys), and over 73 different sampling dates up to 2024. Each OTU was observed (recorded) between one and 117 times, with 56 (33%) OTUs recorded only once (singletons), and 113 (67%) recorded less than five times throughout the dataset. Abundances varied from one to 556 specimens per sample (average = 7.8).

Note - the term short-range endemics (SRE) is used interchangeably with restricted taxa for the purpose of this study. Taxonomic uncertainty remains high for subterranean fauna and many of the SRE classifications can be explained by the state of knowledge for this group rather than true restricted ranges because of limited dispersal capabilities.

4.1.2 Functional Groups and Selected Genera

To facilitate robust statistical analyses, functional stygal groupings were created by grouping OTUs with limited observational records based on functional attributes and taxonomic relatedness. Figure 1 shows the number of records in each group, and Table 1 shows the functional groups which include the SREs (restricted) stygofauna. Note that despite the use of functional groupings to increase record numbers per analysis group, some groups still have low numbers of records. They could not be grouped further due to taxonomic distance from other groups, however the groups which include SREs all have at least nine records. Analysis outcomes from groups with very low sample number, for example the Acari and Platyhelminthes, are generally considered to be less robust because of sample size, which is not unexpected. These groups are not considered to be restricted for the purpose of the assessment at MDIOM.

The use of functional groups, and in some cases genera, to group data for analysis was deemed necessary by the fact that the vast majority of OTUs observed were observed too infrequently to allow for robust analysis. Having many infrequently observed OTUs is not necessarily considered to indicate rarity but rather is likely to be an artifact of sampling (EPA, 2012; Framenau, McMains, & Campos, 2021). The approach of combining sparse datasets of groups of similar species is recommended in the EPA’s Technical Guidance (EPA 2021). The approach is also commonly applied in the literature, based on the premise that species coexisting within the same communities and/or sharing similar ecological niches are likely to respond similarly to environmental covariates and exhibit comparable spatial patterns (Zhang, Chen, Xu, & Xue, 2020; Bellvé, 2025; Brooke, 2022). In this way, inference of niche requirements for infrequently recorded species may benefit from a statistical ‘borrowing of strength’ from more common species (Erickson, 2023; Qiao, Peterson, Ji, & Hu, 2017; Smith, Godsoe, & Rodríguez-Sánchez, 2019). In addition to the functional groups, three genera were selected from the potentially restricted taxa that occur within the predicted extent of salinity changes. These genera were the *Areacandona* (within the Podocopida functional group which are ostracods), and *Billibathynella* and *Pilbaranella* (both within the Syncarida functional group). These three genera were also chosen as they had adequate records to facilitate analysis.

Where an OTU, genus or functional group was not recorded from a sampled borehole (defined as a unique combination of borehole, date and method), it was designated as absent for the sake of the analysis. The proportion of absences (zero counts) ranged from 55% to 99% across the functional groups.

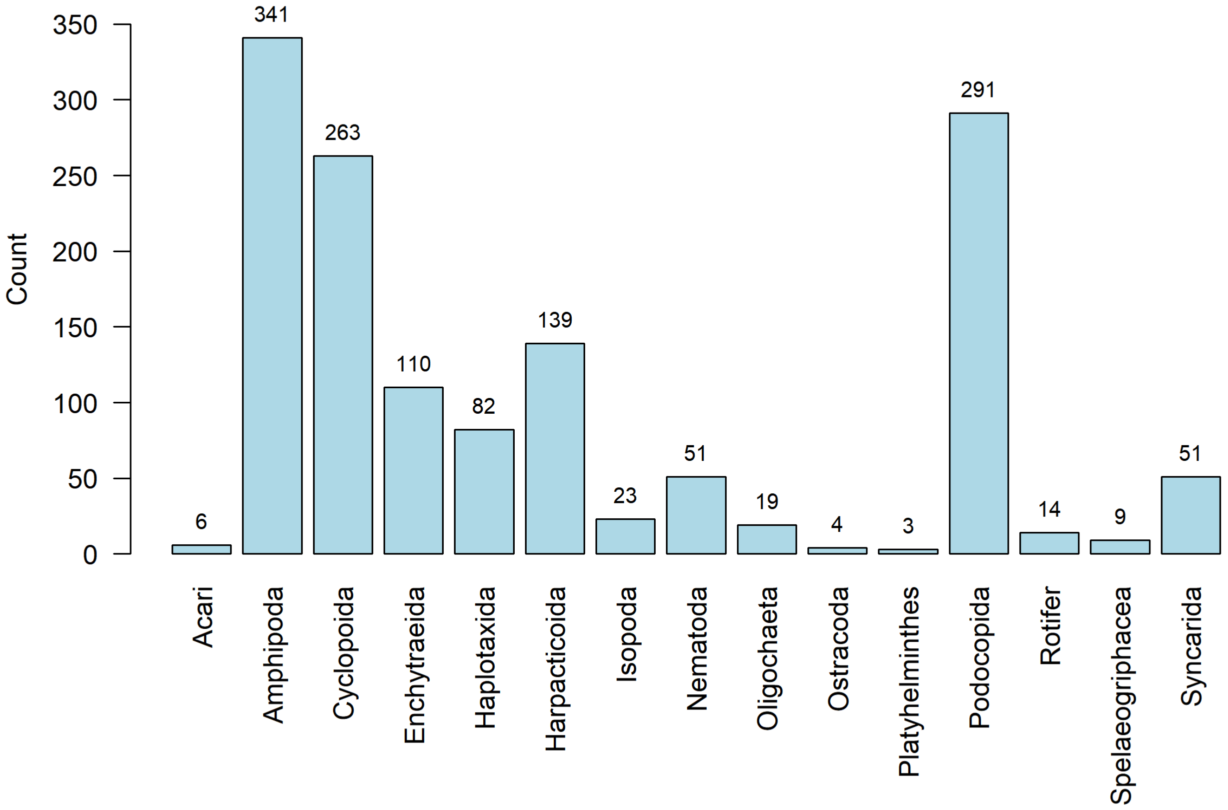

Figure 1 Number of records in each functional group.

Table 1 Functional groups and the SRE stygofauna within the groups

Functional group	Total No. of OTUs	Total records	No. of SREs	Taxa within the groups	No. of SRE records
Amphipoda	24	341	6	*Bogidiella* `BAM221` | *Maarrka* `BAM182` | *Neoniphargidae* `BAM176` | *Paramelitidae* `BAM244` | *Paramelitidae* sp. B42 | *Pilbarana* `sp. new OTU 1`	8
Isopoda	4	23	1	*Pygolabis* `MH1`	2
Cyclopoida	24	263	1	*Diacyclops einslei*	9
Harpacticoida	29	139	2	*Dussartstenocaris* `BHA335` | *Schizopera* `BHA277`	2
Enchytraeida	8	110	1	*Enchytraeidae* `BOL081` (2 bundle long thin)	6
Podocopida	35	291	2	*Areacandona* `BOS1381` | *Humphreyscandona* `BOS1435`	3
Spelaeogriphacea	1	9	1	*Mangkurtu* `BSPE004`	9
Syncarida	23	51	12	*Atopobathynella* sp. B09 (Parabathynellidae `MH1`) | *Bathynellidae* `BSY246` | *Billibathynella* `BSY238` | *Billibathynella* sp. `BSY244` | *Billibathynella* sp. B10 | *Billibathynella* sp. B11 | *Brevisomabathynella* `BSY233` | *Brevisomabathynella* `BSY247` | *Hexabathynella* `BSY234` | nr *Billibathynella* `MH2` (Parabathynellidae `MH2`) | *Parabathynellidae* `MH3` | *Pilbaranella* `BSY380`	19

4.2 Observed and inferred borehole data

Electrical conductivity (EC, measured in µS/cm) was provided as a proxy for salinity, and is used throughout this document as the measure of salinity. All salinity data was scaled and normalised for the statistical analyses. Inferred salinity data for was calculated using a block model (a conceptual and numerical representation of the subsurface, built by dividing the aquifer system into a three-dimensional grid of discrete volumetric cells – 'blocks') (AQ2, 2025).

Borehole data from sampling events was provided separately and standing water level (SWL) and end of hole (EoH) data were incorporated into the above dataset.

As stated above, the sampling methodology does not readily allow for salinity measurement within the depth profile of each bore. Instead, a separate set of groundwater quality measurement bores were used to measure salinity; these informed a model of inferred salinity values for varying depths over the entire area which was modelled by AQ2 (AQ2, 2025). Salinity and lithology values were extracted from this model at 1 m depth intervals through to 30 mbgl (from the surface of the groundwater table), capped by the maximum depth of the bore (EoH) if this was within the first 30 m of the groundwater table.

Rasters (a grid of cells where each cell contains a value representing information) were also generated from the block model showing the minimum, maximum and mean salinity for the first 30 m of the groundwater table (again clipped by end of hole). All spatial data was in GDA94 MGA zone 50 projected coordinate reference system (EPSG:28350) as per HPPL/HanRoy requirements. The potential range of salinity extent was included in maps for reference only.

4.3 Statistical analyses

The key steps for the statistical analyses were:

1. Data compilation, cleaning, and construction of matrices at the level of class, family, functional group, genus and OTU.
2. Plotting salinities and lithologies by depth for each bore to assess for the presence of haloclines (Appendix A).
3. Basic data exploration to quantify coarse trends, likelihood of zero-inflation and overdispersion and develop appropriate methodological approaches.
4. Testing for the presence of random effects from data structure from both borehole (given multiple sampling events at many boreholes) and sample methodology using generalised linear mixed-effects models (GLMMs). Insufficient statistical support for the inclusion of these random effects was found, likely to be due to the similarity of the sampling methodologies included, and the relatively low number of within-bore replications; as such random effects were not included in further steps.
5. Testing for over- and under-dispersion and zero-inflation in the data by assessing preliminary abundance models with residual diagnostics for the most appropriate error distribution out of:
    a. Poisson,
    b. Negative binomial 1 (with linear overdispersion; NB-1),
    c. Negative binomial 2 (NB with quadratic overdispersion; NB-2),
    d. Zero-inflated Poisson (including a ZIP),
    e. Zero-inflated NB1 (ZINB-1), and
    f. Zero-inflated NB2 (ZINB-2).

    Log links were used for the Poisson, NB1 and NB2 models and logit links were for the Bernoulli component used to model the zero-inflation. A binary modelling process is used to account for structural absences in the zero-inflated models.
6. Testing for linear relationships between functional group pooled abundance and salinity variables using generalised linear mixed models (GLMs). Salinity variables (min, max and mean for the top 30 m of the water table) were alternated and model performances compared using Akaike Information Criterion (AIC), a model performance measure that rewards fit and penalizes complexity.
7. Testing for non-linear relationships between functional group occurrence and salinity variables using generalised additive models (GAMs) in comparison with the GLMs mentioned previously. The smooth parameter k = 3 or 4 was used, and a smoothing penalty was included to decide the effective degrees of freedom, such that if the data do not support non-linearity, the smooth is shrunk to almost linear, and if the predictor has almost no effect, the relationship is effectively shrunk out of the model (to effective degrees of freedom ~ 0).
8. Inclusion of additional predictor variables (‘lithology at surface,’ dominant lithology over top 30 m of water table, standing water level from bore observations, and top of the groundwater table based on extractions from the salinity block model) to ascertain if they considerably improve model explanatory power.
9. Calculation of greatest values for each salinity variable and for selected genera and SREs and mapping of the areas in which baseline values do not exceed those values.

4.3.1 Analysis software

All data exploration and analysis were performed in R statistical software version 4.5.1 (R Core Team, 2025). The key packages used for statistical analyses were:

- ‘dplyr’ for data wrangling and creating observation matrices;
- ‘terra’ for working with spatial data;
- ‘stats’ for generalised linear models; for modelling exponential- linear relationships;
- ‘glmmTMB’ for generalised linear mixed-effects models that can incorporate a diversity of error distributions (Poisson, Negative Binomial 1);
- ‘mgcv’ for generalised additive models and generalised additive mixed models; for modelling non-linear relationships; and
- ‘DHARMa’ for simulation-based residuals, dispersion and zero-inflation checks for GLM/GLMM fits.

5. Overview of Findings

The generalised linear models of stygofauna abundance found many statistically significant associations with groundwater salinity. The explanatory power of both linear and non-linear statistical models was generally very low, ranging from 0.2% to 11% of deviance explained with one outlier (the OTU Ostracoda) with only four records.

Alternative model configurations were therefore explored for additional explanatory power. This included occurrence versus abundance modelling, linear versus non-linear associations, and the inclusion of additional predictor variables (lithology and groundwater table depth). Various versions of the predictor variables were used. Where there were sufficient observation records, analyses were also run on genera selected for their SRE status (i.e. *Areacandona*, *Billibathynella* and *Pilbaranella*).

While explanatory power of the models increased with the additional predictor variables and alternative model configurations, it generally remained low, meaning that while some relationships are apparent, the majority of variability in the data is not explained by the models. Result plots from the models including lithology are not presented here as the lithology variable is considered too coarse to produce reliable conclusions given that it is not known what depth, and therefore inferred lithology, the samples come from.

As over- and under-dispersion have been accommodated and non-linearities have been tested for, the low explanatory power indicates that there may be other key ecological drivers missing, and/or there is a lot of random variation (‘noise’) in the data. Compared to similar studies this is not unusual.

While other physicochemical factors (environmental variables) can influence biotic distributions in groundwater; it is accepted that salinity is one of the more important measures to assess distribution of stygofauna (i.e. presence) (Strayer 1994). While the influence of other variables may provide greater explanatory power, lack of repeat records of the OTUs is a strong factor. The high number of singletons, often because of taxonomic uncertainty and an artefact of stygofauna sampling is widely recognised (Eberhard et al, 2009; Framenau, McMains, & Campos, 2021; EPA 2012; 2021).

A study by Reeves et al (2007) on the distribution of ostracods in the Pilbara analysed 13 variables, including pH, alkalinity, TDS, pH, altitude and Eh – oxidising potential, found a low explantory power due to the high number of zeros and singletons within the dataset. The main determining variable was found to be pH and carbonate saturation of the host water. Clear distributional patterns were associated primarily with the extent of the surface water catchment or aquifer.

Challenges to model robustness include stygofauna detectability. This is expected to be low to variable, although repeated sampling of boreholes is required for detectability to be estimated or incorporated into the models. Stygofauna detectability is likely to contribute to the high levels of unexplained variation. Additionally, the grouping of OTUs into functional groups (and to a lesser extent, some at genus level) may have contributed some noise into the data, despite the statistical benefits of having larger sample sizes. Taxonomic uncertainties are inherent in this dataset and some of these may have contributed to the unexplained variation.

5.1.1 Indicative stygofauna – salinity relationships

Notwithstanding the low explanatory power of the statistical models, the indicative relationships for the different groups analysed provide insight into the potential effects of salinity changes. Table 2 provides a summary of the outcomes of the various statistical models, based on the top-performing models (where Δ AIC ≤2) and the greatest levels of variance explained within those, for each analysis group (functional groups and selected genera).

In eight of the functional groups, the modelled association between salinity and stygofauna occurrence and/or abundance was positive, indicating greater predicted occurrence probability and/or abundance with higher salinity, though some of these associations were not statistically significant. In five functional groups, the modelled association between salinity and stygofauna occurrence and/or abundance was negative, indicating that reduced occurrence probability and/or abundance are associated with increases in salinity over the ranges observed.

Some of these associations were not statistically significant. In the Syncarida functional group, which includes the *Atopobathynella*, *Billibathynella*, and *Pilbaranella*, there were conflicting associations across the abundance and occurrence data. However, neither were statistically significant nor explained much variation. A contributing factor here may have been the large random 'noise' in the data from the high proportion of singletons.

Figure 2, Figure 3, and Figure 4 show the indicative relationships between occurrence and/or abundance for groups where a statistically significant relationship was found. Both occurrence and abundance relationships for Nematoda are shown to allow for comparison; the abundance relationship is the significant one but it is difficult to see the slope in the line given the sheer extent of the y axis (reflecting count data). The occurrence association is not significant but is easier to see as the y-axis is reduced. Where statistically significant relationships were found for both occurrence and abundance, only the occurrence-based relationship is shown.

Table 2 Summary of statistical model findings for stygofauna associations with salinity.

Level of analysis	Analysis group (n= number of records)	Salinity variable with most explanatory power	Direction of association (filled = significant)	Significance of salinity variable (p value)	Deviance explained (salinity-only model)	Most appropriate error distribution family (for abundance models)	Is best fit linear or non-linear? (based on occurrence models)	Inclusion of lithology variable in top-performing models (Δ AIC ≤2)	Inclusion of water table depth (Δ AIC ≤2)
Functional groupings (broader)	Acari (6)	Min	△	N.S.	<0.1%	NB-2	Non-linear	✓(surface)	✓
	Amphipoda (341)	Max	▲	Sig (<0.001)	14.5%	NB-1	Non-linear	✓(surface)	✓
	Cyclopoida (263)	Min	▲	Sig (<0.001)	6.0%	NB-1	Linear	✓(dom)	✕
	Enchytraeida 110)	Min	▼	Sig (<0.01)	1.1%	NB-2	Linear	✓(dom)	✓
	Haplotaxida (82)	Min	▲	Sig. (0.048)	0.99%	NB-1	Non-linear	✓(dom)	✓
	Harpacticoida 139)	Min	▲	Sig (<0.001)	8.8%	NB-1	Linear	✓(dom)	✓
	Isopoda (23)	Max	▲	Sig (<0.001)	15.8%	NB-1	Non-linear	✕	✓
	Nematoda (51)	Min	▼	Sig (<0.01): abundance N.S.: occurrence	0.68%	NB-2	Linear	✕	✕
	Oligochaeta (19)	Mean	▼	Sig (<0.01): abundance N.S.: occurrence	1.9%	NB-2	Linear	✓(surface)	✓
	Ostracoda (4)	Mean/Max	–	N.S.	<0.1%	ZIP	Non-linear	✕	✓
	Platyhelminthes (3)	Min	▲	Sig. (0.023)	11.9%	Poisson	Linear	✕	✓
	Podocopida (291)	Min	▲	Sig (<0.001)	9.5%	NB-1	Non-linear	✓(surface)	✓
	Rotifera 14)	Min	▽	N.S.	5.7%	NB-1	Linear	✕	✓
	Spelaeogriphacea (9)	Min	▽	N.S.	0.1%	ZINB-2	Linear	✓(dom)	✓
	Syncarida (51)	Max	△: occurrence ▽: abundance	N.S.	<0.1%	NB-2	Linear	✓(dom)	✓
Genus	*Areacandona* (88)	Min	▲	Sig (<0.01)	2.9%	Not assessed	Non-linear	✓(surface)	✓
	Billibathynella (24)	Max	▲	Sig (0.028)	2.4%	Not assessed	Linear	✓(dom)	✓
	Pilbaranella (10)	Mean	△	N.S.	11%	Not assessed	Non-linear	✓(both)	✓

Min / Mean / Max: inferred minimum, mean or maximum salinity of the first 30 m of the groundwater | Sig = significant at p ≤ 0.05. N.S. = not significant at p ≤ 0.05 | Surface = inferred lithology at groundwater surface. Dom = dominant lithology over the first 30 m of the groundwater table. Both = equal effect.

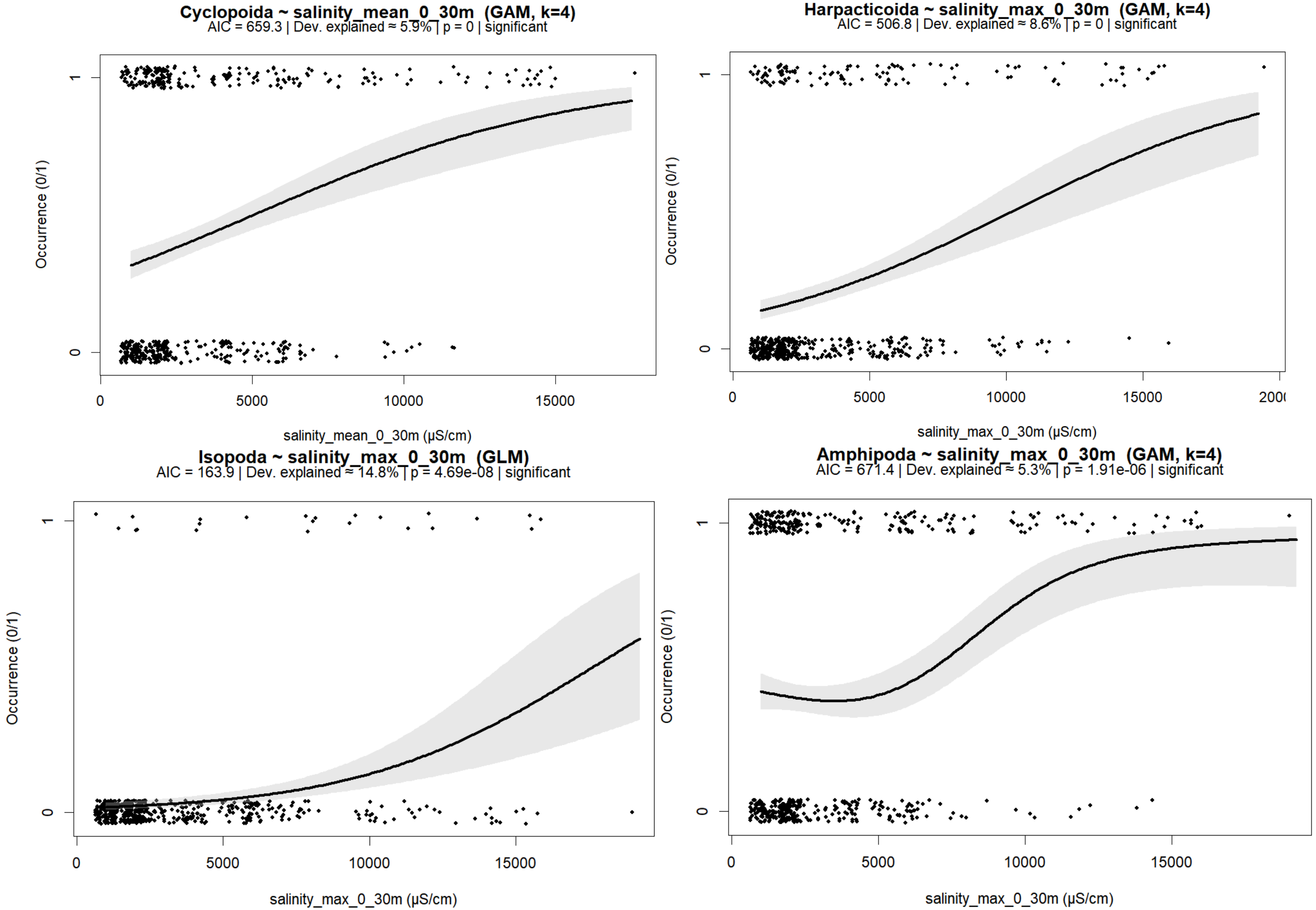

Figure 2 Indicative salinity relationships for, Cyclopoida, Harpacticoida, Amphipoda and Isopoda. Shading shows 95% confidence band for mean prediction.

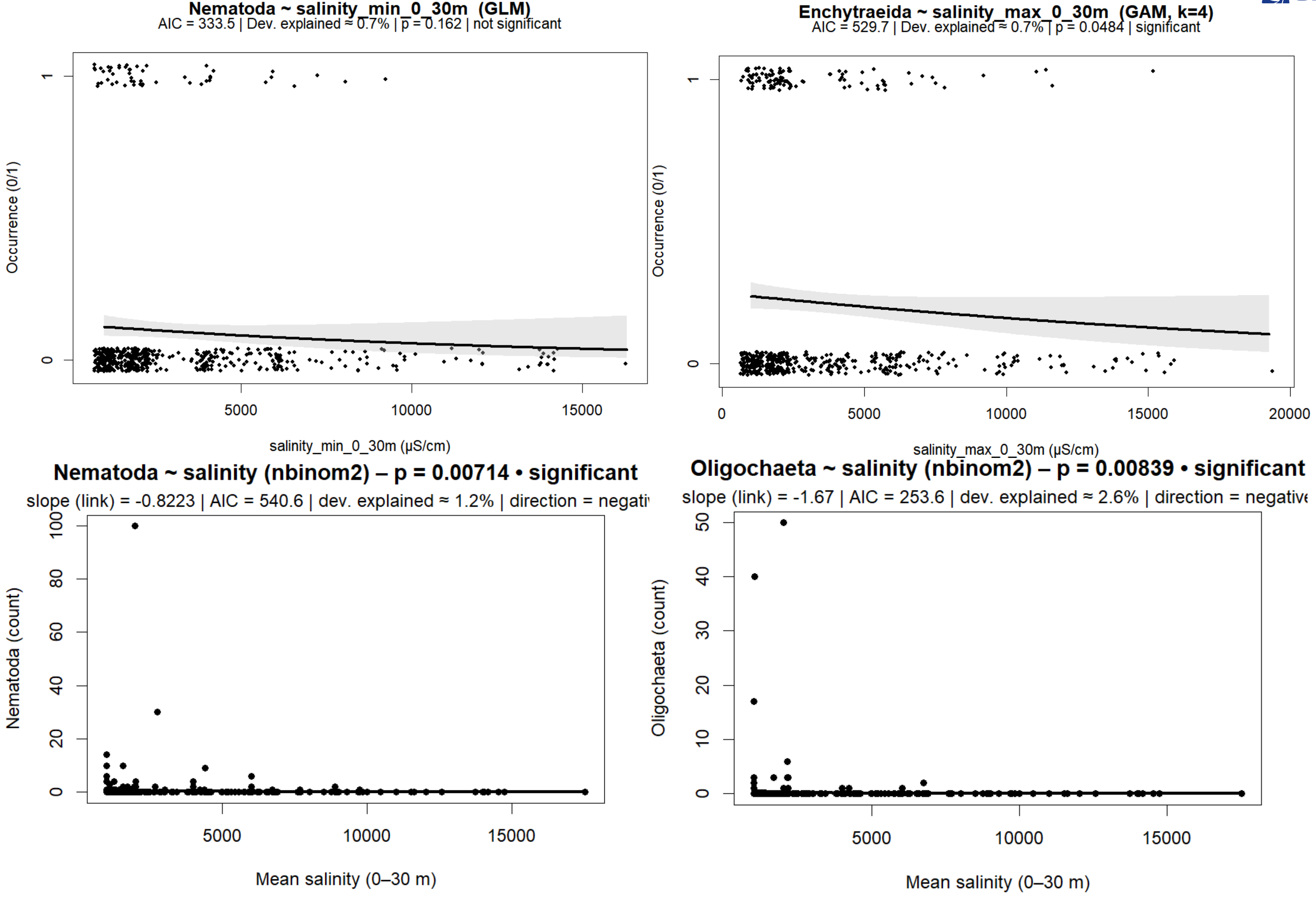

Figure 3 Indicative salinity relationships for Nematoda, Enchytraeida (occurrence model followed by abundance model) and Oligochaeta. Shading shows 95% confidence band for the mean prediction.

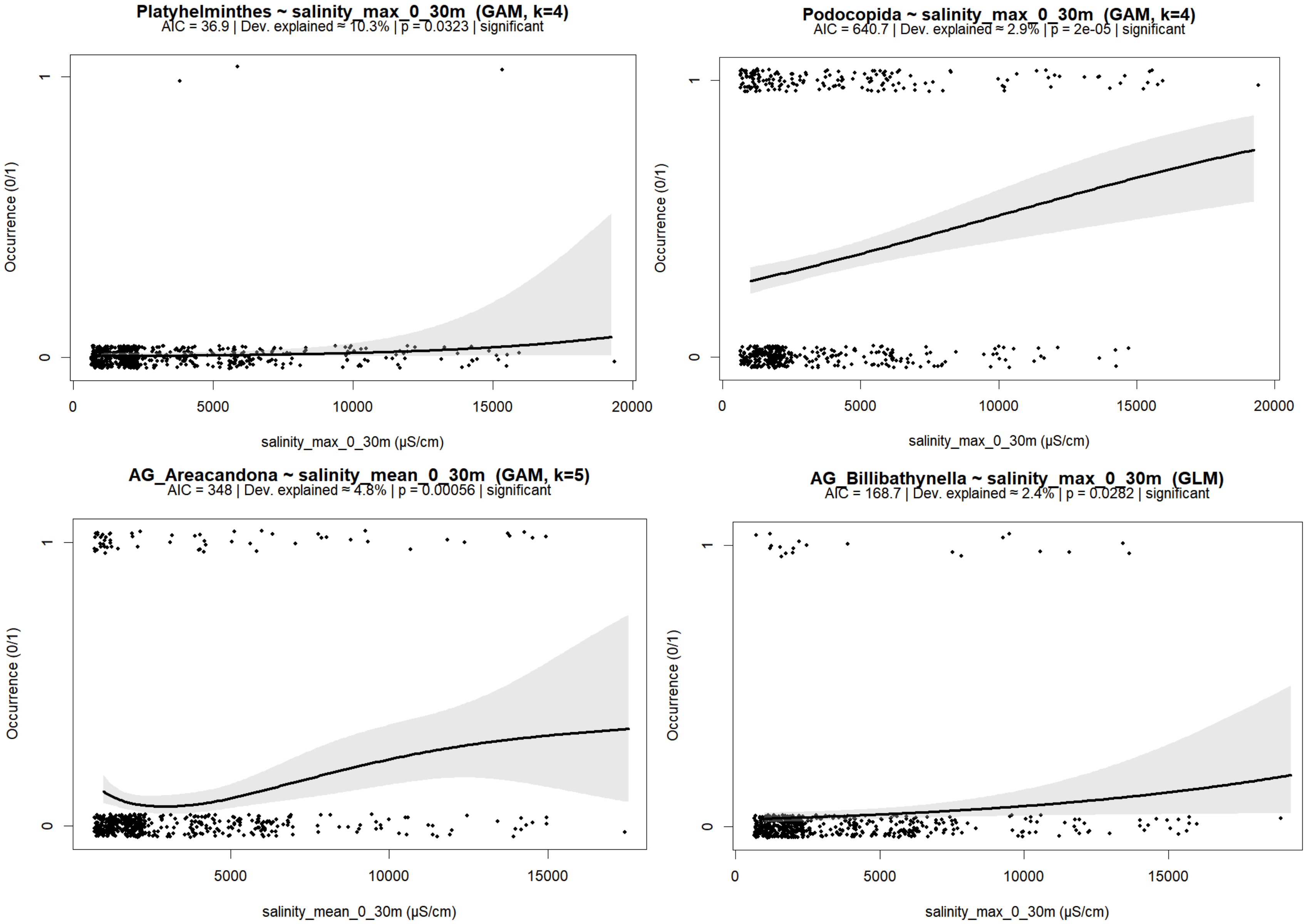

Figure 4 Indicative salinity relationships for Platyhelminthes, Podocopida functional groups and the genera *Areacandona* and *Billibathynella*. Shading shows 95% confidence band for the mean prediction.

6. Mapping of baseline salinity tolerance areas

Given the low explanatory power of the statistical models, a conservative approach has been taken to estimate the salinity tolerances of the genera within which the key stygofauna occur. The stygofauna referred to as SREs alone have very low replication, this is not uncommon of stygofauna surveys. For this study sample numbers varied from one to nine records and averaging 2.2 records per SRE in the current dataset. The SREs are not considered sufficiently surveyed for salinity tolerances to be based on them alone – instead the observed salinity tolerance of their genus was used. This is justified based on existing evidence that taxonomic association is commonly reflected by similar niche requirements in stygofauna (EPA, 2021), and similar approaches used in ecological studies facing the same challenge, as previously mentioned. Additionally, many SRE stygofauna present as having a limited area of occupancy due to the artefact of sampling, current state of knowledge for stygofauna and poor taxonomic resolution (EPA, 2021).

The range of salinities where each selected genus was observed to occur, based on inferred salinity summary data, are presented in Table 3. The areas where mean baseline salinities for the first 30 m of the groundwater are equal to or lower than the top of this range are mapped in Figure 5, Figure 6, Figure 7, and Figure 8.

For the purpose of EIA, these maximum tolerated salinities shown in these figures are considered conservative. It is assumed that the highest salinity at which a genus was mapped was the highest suitable salinity for that taxon (the extent of its salinity range). It is possible that these taxa exist in higher salinities. Over the past decade increased sampling effort has shown resulted in stygofauna records in areas where they were not found in earlier surveys, including aquifers with higher salinities. Additionally, Figures 5 to 8 assume that the stygofauna in question would have a negative relationship with an increase in salinity. This assumption has some (limited) support from the statistical analysis presented in section 4 above for Enchytraeida, Nematoda, Oligochaeta, Rotifera and Spelaeogriphacea. It is not supported by the analyses for the functional groups: Amphipoda, Cyclopoida, Haplotaxida, Harpacticoida Isopoda, and Podocopida. It was also not supported for three of the four genera selected for this baseline tolerance assessment: *Areacandona, Billibathynella*, and *Pilbaranella*. This work for the MDIOM aligns with the findings in Reeves et al. (2007) which found the *Areacandona* to favour chloride dominated waters (alluding to higher salinities). It should be noted that sampling within the MDIOM area in the Reeves et al (2007) study was limited. Since this paper was published the MDIOM sampling program has yielded a higher diversity of *Areacandona* species, expanding their area of occupancy.

Trends for the genus *Atopobathynella* could not be analysed as it only consists of one record. This taxon sits within the functional group Syncarida, for which no significant association with salinity was found. It is noted however that *Atopobathynella* was collected in low salinity groundwater. Recent studies on the distribution of *Atopobathynella* species found, in a complex setting, this group has narrow distribution ranges (Perina et al., 2024). This study also found that despite considerable sampling effort no *Atopobathynella* species were found in the regional aquifer of the Fortescue Valley, with some records in GenBank listing this genus as Parabathynellidae sp. (Perina et al., 2024).

The limited explanatory power of the models should be taken into consideration. Some uncertainty remains regarding how representative a genus is of a particular SRE’s tolerances, but this is difficult to mitigate given existing data limitations.

Table 3 Range of inferred salinities at which the selected stygofauna were observed. Genera are in bold

Genus (in bold) / SRE	No. of records	Salinity in upper 30 mbgl (EC = µS/cm)					
		Minimum		Mean		Maximum	
		Low	High	Low	High	Low	High
Areacandona	**88**	**1000**	**14069**	**1000**	**14746**	**1000**	**15648**
Areacandona \`BOS1381\`	2	1835	4647	1848	4983	1863	5396
Atopobathynella	**1**	**2000**	**2000**	**2000**	**2000**	**2000**	**2000**
Atopobathynella sp. B09 (Parabathynellidae \`MH1\`)	1	2000	2000	2000	2000	2000	2000
Billibathynella	**24**	**1000**	**14000**	**1000**	**14000**	**1000**	**14000**
Billibathynella \`BSY238\`	1	6321	6321	6728	6728	7265	7265
Billibathynella sp. B10	2	1077	2000	1103	2000	1129	2000
Billibathynella sp. B11	1	1405	1405	1447	1447	1513	1513
Billibathynella sp. \`BSY244\`	1	8415	8415	9020	9020	10344	10344
nr *Billibathynella* \`MH2\` (Parabathynellidae \`MH2\`)	4	1720	2160	1801	2634	1894	3145
Schizopera	**2**	**8000**	**14021**	**8000**	**14738**	**8000**	**15646**
Schizopera \`BHA277\`	1	14021	14021	14738	14738	15646	15646

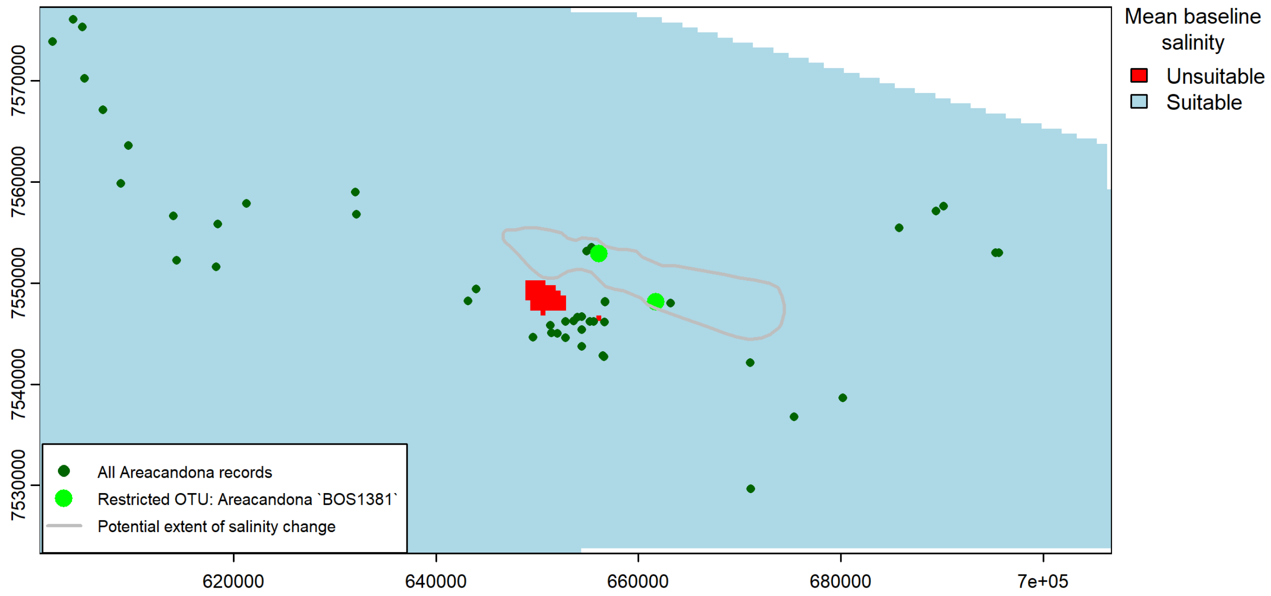

Figure 5 Potential areas of *Areacandona* salinity tolerance, based on the highest salinity at which OTUs from this genus were observed: mean EC 14,745.8 µS/cm.

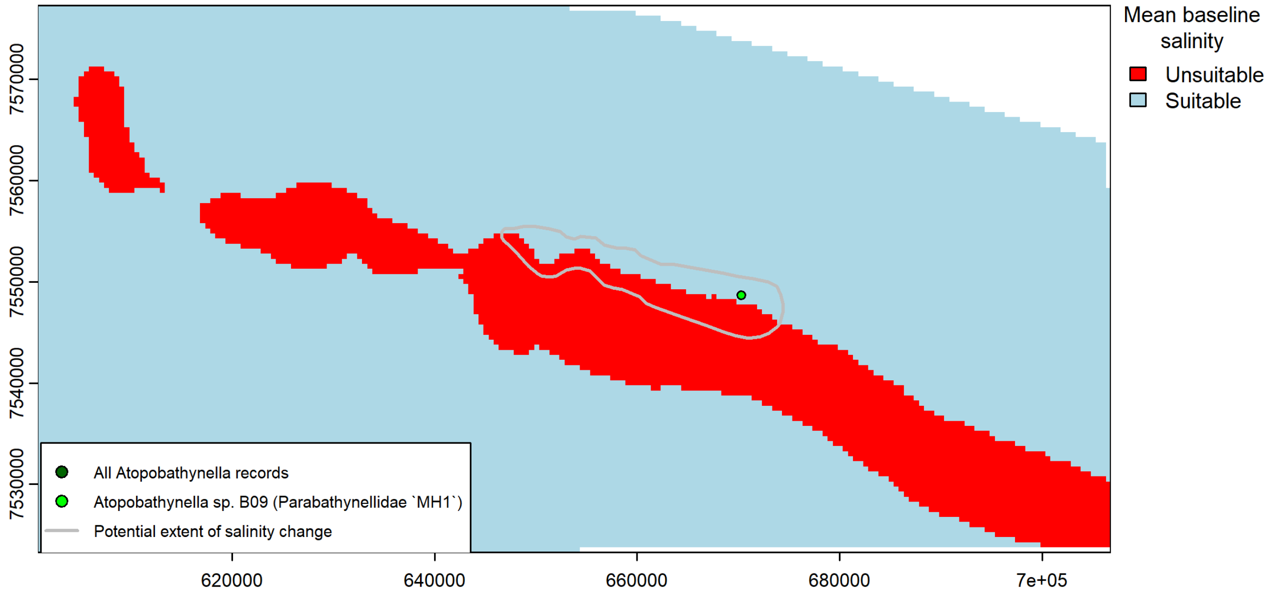

Figure 6 Potential areas of *Atopobathynella* salinity tolerance, based on the highest salinity at which OTUs from this genus were observed: mean EC 2,000 µS/cm. Note that this is in fact based on the SRE *Atopobathynella* sp. B09 Parabathynellidae MH1, with it being the only record of this genus in the current dataset.

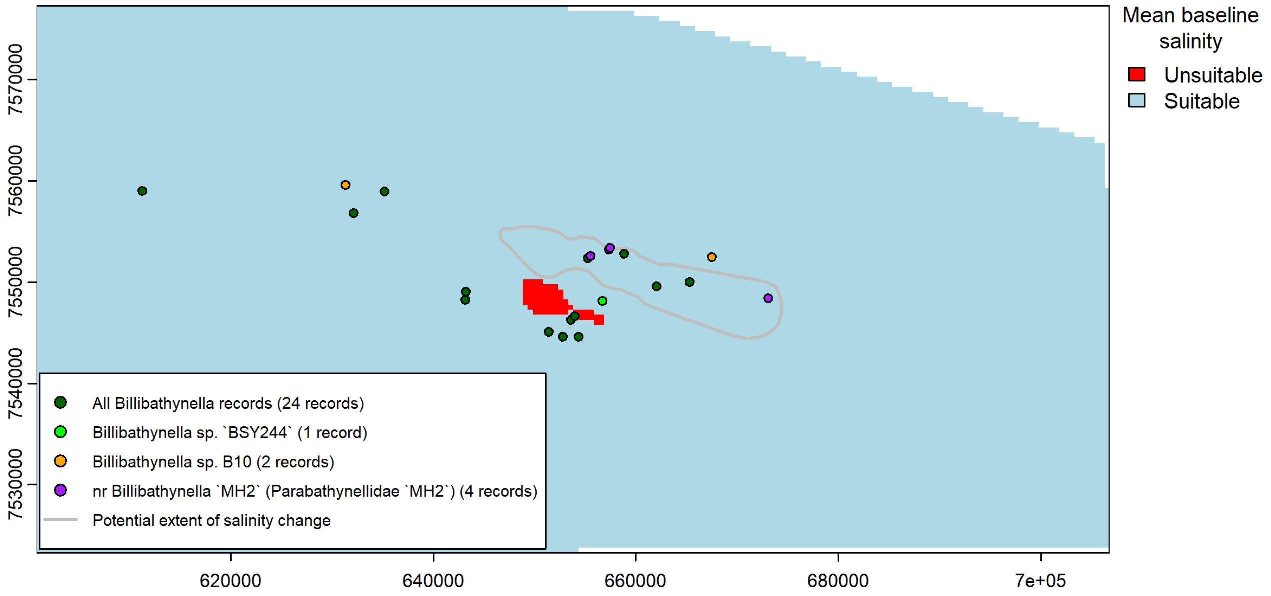

Figure 7 Potential areas of *Billibathynella* salinity tolerance, based on the salinity at which OTUs from this genus were observed: mean EC 14,000 µS/cm.

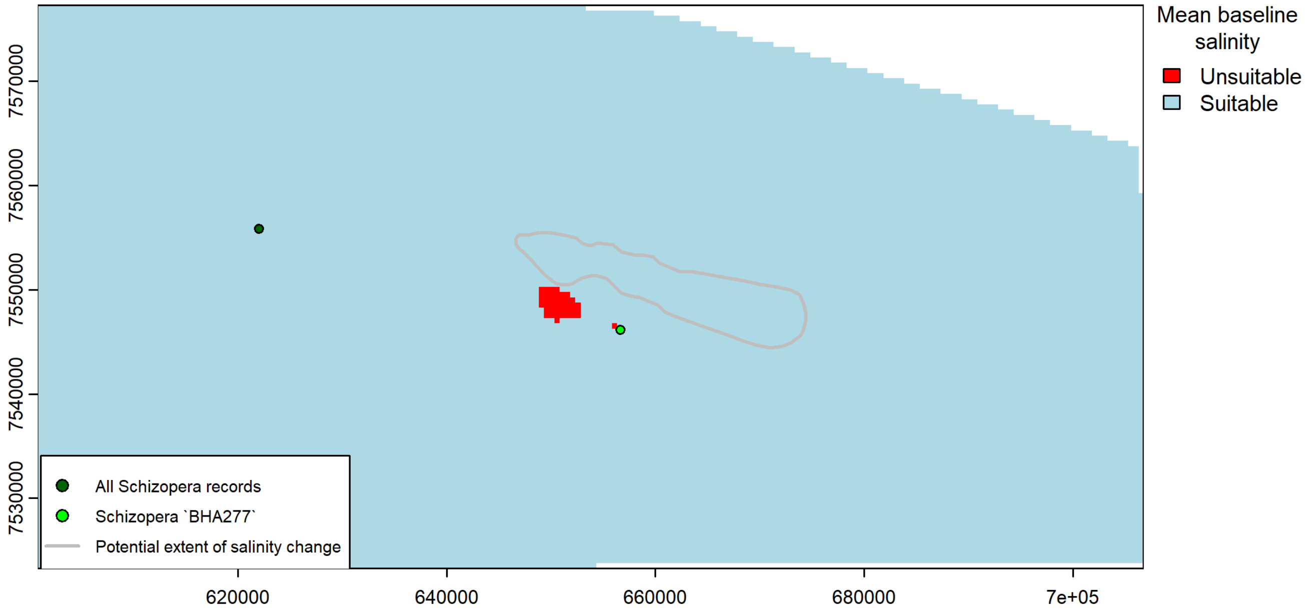

Figure 8 Potential areas of *Schizopera* salinity tolerance, based on the greatest salinity at which OTUs from this genus were observed: mean EC 14,737.5 µS/cm. Note that the SRE *Schizopera* 'BHA277' was recorded in the location with the greater salinity level relative to the single other OTU in the genus within this dataset.

7. Conclusion

Extensive statistical modelling has yielded some insights into relationships between stygofauna groups and salinity. These have largely been consistent across linear and non-linear model configurations, error distribution assumptions, response variables (abundance versus occurrence), and with or without addition of coarse lithology and standing water level into the models, as well as for the different salinity summary statistics (min, max and mean). The low explanatory power of the models limits the confidence with which these findings can be applied to practical impact mitigation applications. Therefore, a more robust and conservative approach has been adopted, based on the observed salinity tolerances at the genus level.

It is unlikely that better explanatory power and/or more robust insights into the stygofauna responses to groundwater salinities can be achieved with the existing dataset. More specific point source sampling including collection of samples at depth to allow attribution of lithology, salinity and other environmental variables, and/or more environmental data would provide for more robust insights into the environmental drivers of species occurrence and distribution.

In summary, this study found:

- There was a positive relationship between salinity and stygofauna occurrence and/or abundance of the eight modelled stygofauna groups, indicating greater predicted occurrence probability and/or abundance with higher salinity,
- While the analyses had low explanatory powers, given the limited sample numbers and high number of singletons, a relationship was observed.
- The stygofauna presented within functional groups showed distribution across a greater landscape. The inferred baseline salinity tolerance ranges for the functional groups which include a number of the stygofauna identified as potentially restricted / SREs are presented in this study.
- This information has informed the MDIOM Water Management Plan with triggers and thresholds developed based on the outcome of this work. These application of the triggers and thresholds will allow for any impacts from the reinjection of mine dewater to be appropriately managed.

8. Analysis limitations

The following limitations were relevant to the analyses documented herein:

1. Available stygofauna sampling methods do not provide information regarding the depth at which individuals were sampled from, given that both ‘net’ and ‘scrape’ methods used here sample the entire depth of the bore as one. This means that the depth, lithology and inferred salinity of each record is not known and analyses are limited to testing for associations between stygofauna indicators and summary statistics per borehole. The summary statistics used here are: minimum salinity (EC) for the first 30 m of the groundwater (in the vast majority of cases this aligns with the salinity at the top of the groundwater table, maximum salinity for the first 30 m, mean salinity for the first 30 m of the groundwater, lithology at the surface of the groundwater table, and dominant (most common) lithology within the first 30 m of the groundwater table.
2. The sampling methodology does not readily allow for salinity measurement along the depth profile of each bore. Instead, a separate set of groundwater quality measurement bores are used to measure salinity; these inform a model of inferred salinity values for the entire study area (AQ2 2025), and values for bore locations at 1 m depth intervals are extracted for each bore. Consequently, actual salinities relevant to each record may differ somewhat from the inferred salinities used in the analysis.
3. In addition to the above point, the inferred values are deemed an average of the ‘recent monitoring period’ and are not assigned to a particular date. This means that any variability in salinities over time,

which may be reflected in sample results from different dates (the monitoring period extends from 27/04/2008 to 20/04/2024 after pre-2008 data was excluded) could not be accounted for in this analysis.

4. Model performance measures gave equal weighting to observed presences and absences in the occurrence modelling, and to zero versus positive integer counts in the abundance modelling. However, it is likely that there are false absences throughout the datasets given that bore samples are expected to have low/variable detection probabilities given that they are only sampling a very small part of the surrounding area, and there is insufficient repeated sampling of boreholes to provide assurance that all species present at a site have been detected. A species distribution modelling approach could handle this limitation better by placing more weight on correctly predicting presences (sensitivity) than correctly predicting absences (specificity); but has not yet been applied here.

5. Data reflecting other environmental drivers such as dissolved oxygen and organic carbon were not available at the scale required for this analysis and these potential drivers could therefore not be considered.

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Appendix A Inferred baseline bore salinities and lithologies

EC by Depth — Bores 1–20 of 433 (1475 –> LF−MB0041) | Page 1 of 22

EC by Depth — Bores 21–40 of 433 (LF−PB004 −> MD0247) | Page 2 of 22

EC by Depth — Bores 41–60 of 433 (MD0253 –> MD0397) | Page 3 of 22

EC by Depth — Bores 61–80 of 433 (MD0398 –> MD0483) | Page 4 of 22

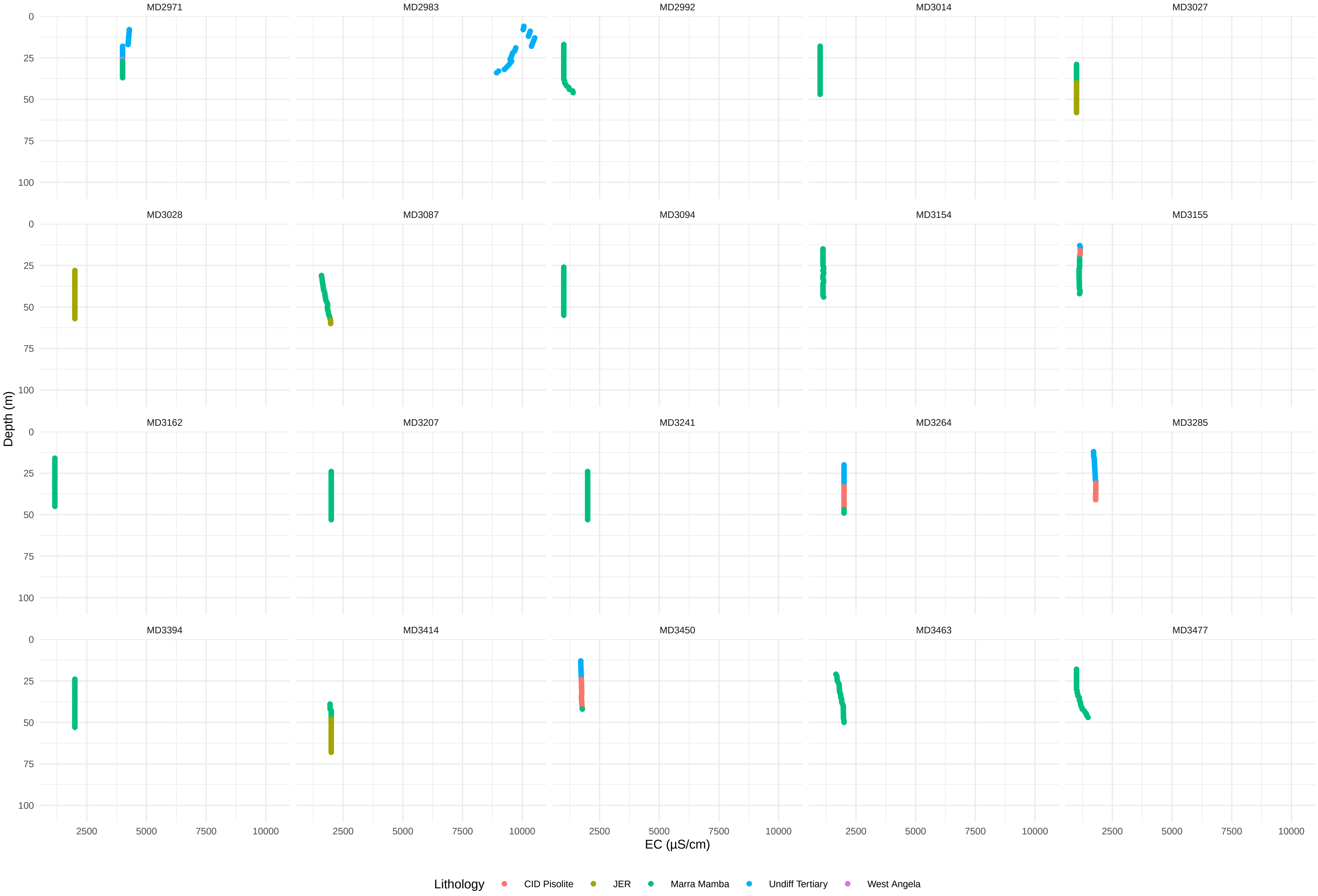
EC by Depth — Bores 81–100 of 433 (MD0486 –> MD0609) | Page 5 of 22

EC by Depth — Bores 101–120 of 433 (MD0612 −> MD0963) | Page 6 of 22

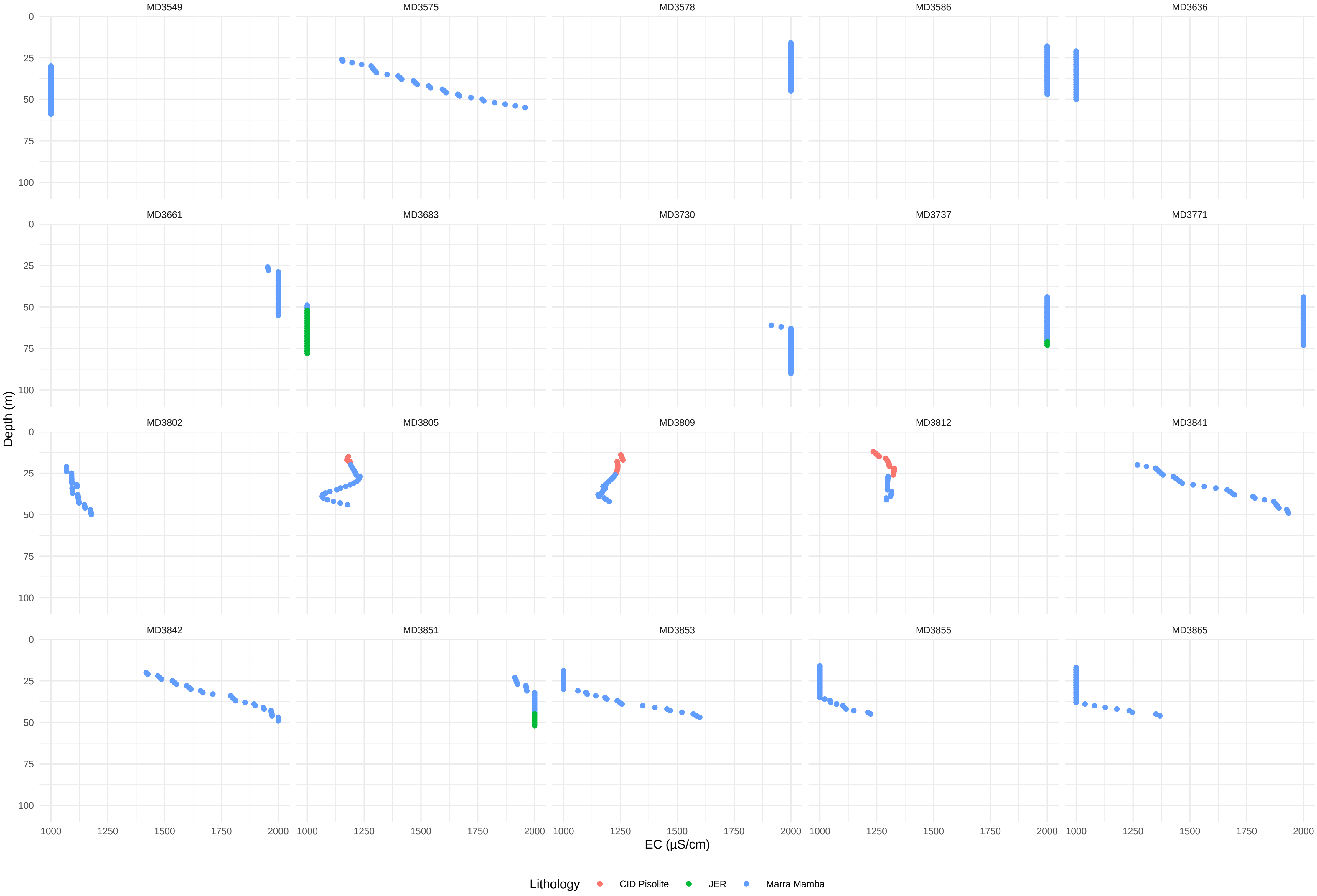
EC by Depth — Bores 121–140 of 433 (MD0974 –> MD2040) | Page 7 of 22

EC by Depth — Bores 141–160 of 433 (MD2059 –> MD2970) | Page 8 of 22

EC by Depth — Bores 161–180 of 433 (MD2971 –> MD3477) | Page 9 of 22



EC by Depth — Bores 181–200 of 433 (MD3549 –> MD3865) | Page 10 of 22



EC by Depth — Bores 201–220 of 433 (MD3874 –> MD4512) | Page 11 of 22

EC by Depth — Bores 221–240 of 433 (MD4542 –> MD5368) | Page 12 of 22

EC by Depth — Bores 241–260 of 433 (MD5382 –> MD6225) | Page 13 of 22

EC by Depth — Bores 261–280 of 433 (MD6304 –> MDCMB09) | Page 14 of 22

EC by Depth — Bores 281–300 of 433 (MDH0092 –> MDPZ7458C) | Page 15 of 22

EC by Depth — Bores 301–320 of 433 (MDPZ7459 –> MDRC1351) | Page 16 of 22

EC by Depth — Bores 321–340 of 433 (MDRC1883 −> MDWB0033) | Page 17 of 22

EC by Depth — Bores 341–360 of 433 (MDWB0036 –> Mt King Well) | Page 18 of 22

EC by Depth — Bores 361–380 of 433 (MULGA1 –> Thieves Well) | Page 19 of 22

EC by Depth — Bores 381–400 of 433 (Tuckanoona Well –> WF0127) | Page 20 of 22

EC by Depth — Bores 401–420 of 433 (WF0129 –> WF0167) | Page 21 of 22

EC by Depth — Bores 421–433 of 433 (WF0172 –> Yampire Bore) | Page 22 of 22