A Predictive Occurrence Model for *Elatine gussonei* based on Environmental Factors

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Declaration of authenticity

I, the undersigned, declare that this dissertation is my own original work, except as acknowledged in the text, and that it was carried out under the supervision of Dr. Sandro Lanfranco and Prof. Dr. Bram Vanschoenwinkel. I have clearly indicated where I have used the published or unpublished work of others and I have provided the source of such work. I have acknowledged all main sources of help. Any conclusions, suggestions or assumptions are mine unless otherwise stated or attributed.

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Declaration of ethics compliance

I declare that I have abided by the University's Research Ethics Review Procedures.

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Declaration of Supervision

We, the undersigned, confirm that this dissertation has been undertaken under our supervision and that we approve of its submission for final assessment by the Board of Examiners.

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30th September 2021

Declaration of Corrections

We the undersigned confirm that all changes and corrections as required by the Board of Examiners have been included in a satisfactory manner.

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February 2022

To those who stood by me through adversity.

The cure for boredom is curiosity.
There is no cure for curiosity.

~Dorothy Parker

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Abstract

Andrea Francesca Bellia

The occurrence of the amphibious plant *Elatine gussonei* in Mediterranean Temporary Ponds (EU priority habitat 3170* and Natura 2000 site) is patchy, with a strong stochastic component. The specific environmental conditions and tolerance ranges that determine its presence in a pool are not known. This represented the knowledge gap that this study aimed to address. The chemical, morphometric and ecological conditions in a sample of rockpools were collected and used to construct a preliminary occurrence model.

Throughout this study, *ca*. 170 pools from 10 pool landscapes were surveyed from the Maltese Islands. Data collected and used in analyses included species lists, water quality (pH, Electrical Conductivity and Oxidation Reduction Potential), and basin morphometry (dimensions, surface area, maximum water and sediment depths of the basin and depths at which *E. gussonei* was present). Analyses carried out included correlation plots, CCA, RDA, t-tests, linear regressions (lm) and binomial logistic regressions (generalized linear models – glm).

Elatine gussonei occurrence in the model was based on dichotomous presence-absence data of the species. Therefore, binomial glms were carried out for environmental factors. Only zwm and surface area (both negatively correlated with occurrence) were statistically significant ($p<0.05$) and were used to model the species occurrence in a given pool. Once the presence of the species was confirmed via glm, lm were used to model the specific depths at which it occurs. The dependent depths (zwe and zse) were significantly positively correlated with independent maximum basin depths (zwm and zsm). Constraining the lms to pass through the origin, however, increased model efficiency by increasing R^2 (0.54 to 0.72 and 0.44 to 0.84 for water and sediment depths), indicating better model fit.

Literature states that its phenotypic plasticity and rapid response to environmental changes make it a good sentinel species on which to model climate change and predict further environmental changes and habitat status. The species and its habitat are both protected, entitling them to monitoring and conservation.

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Frequently Used Abbreviations

Abbreviation	Term
BBG	Birżebbuġa
DNG	Ħad-Dingli
GRG	Għargħur
MNX	Munxar
MST	Mosta Tal-Qares
PBK	Pembroke
QLA	Qala
SLN	Salini
SPT	San Pawl tat-Tarġa
WHS	Wied Ħas-Saptan
WQ	Water quality
SA	Surface area
SAV	Surface area to volume ratio
EC	Electrical Conductivity
DO	Dissolved Oxygen
ORP	Oxidation Reduction Potential
zm	Maximum Morphometric Depth
ze	Maximum depth at which *Elatine gussonei* is present
zw	Water Depth
zs	Sediment Depth
zsm	Maximum Basin Sediment Depth
zwm	Maximum Basin Water Depth
zse	Maximum Sediment Depth at which *Elatine gussonei* is present
zwe	Maximum Water Depth at which *Elatine gussonei* is present
lm	Linear regression
glm	Generalized linear model

1 INTRODUCTION

1.1 *ELATINE GUSSONEI*

1.1.1 Morphology & Structure

Elatine gussonei is a small (3-5mm in diameter), amphibious, annual plant that tolerates temporary flooded conditions, but that cannot persist without a subsequent dry phase (Zedler 1987). Its growth habit allows it to form dense mats (Figure 1) of glossy, oppositely arranged leaves. The plant produces tetramerous, pale pink flowers that are polypetalous and actinomorphic, with eight stamens oriented in two whorls (Figure 2) (Molnár et al., 2014; Razifard et al., 2017; Takács et al., 2017).

In a genus that is highly variable in morphology and has high phenotypic plasticity, *E. gussonei* is distinguishable from other species by its pink flowers (compared to *E. macropoda*'s shorter white petals), long peduncle (compared to *E. hydropiper* which is sessile), highly curved seeds (compared to *E. macropoda*, but less so than *E. hydropiper*) and testae with hexagonal reticulation (Molnár et al., 2014; Popiela et al., 2017; Takács et al., 2017). Prior to Takács et al. (2017), the most suitable feature of seed morphology identified by Sommier (1908) to distinguish *E. gussonei* from *E. macropoda* had been largely ignored. This being the regular as opposed to elongated hexagonal reticulation of *E. gussonei*'s seed coat.

Figure 1: Dense mats of *Elatine gussonei* in flower in a drying rockpool at Wied Ħas-Saptan, taken 23rd February 2021, with visibly stalked tetramerous flowers and opposite leaves

Figure 2: Seeds of *Elatine gussonei* collected during April 2019 towards the beginning of the dry season with visible hexagonal reticulation and variable size and curvature (left). Solitary flower of *Elatine gussonei* (3mm diameter) with 4 semi-circular, pale pink petals, alternating with 4 glossy green sepals, and eight stamens visibly arranged in two whorls (right).

1.1.2 Taxonomy & Nomenclatural History

Elatine gussonei (Sommier) Brullo & al. (1988) as it is known today was likely to have been first recorded in the Maltese Islands by Grech Delicata (1853) over 150 years ago, when recording *Elatine macropoda* Guss. (1827) from Wied Balluta (St. Julian's). *E. gussonei*, however, was first described by Sommier (1908), as *Elatine hydropiper* var. *gussonei* before being elevated to species level by Brullo et al. (1988). Despite its close relation and similar phenotype to *E. macropoda*, all local populations of *Elatine* sp. have been genetically identified by Kalinka et al. (2014) as *Elatine gussonei* (Sommier) Brullo & al. (1988) due to a diploid number of 54 chromosomes (as opposed to 36 in most other species of the same genus). Little is known about the taxonomy and phytogeography of the family Elatinaceae (Takacs et al., 2013)

1.1.3 Systematics

Elatine gussonei is one of 30 species of the cosmopolitan genus of waterworts: *Elatine* L. The genus *Elatine* is one of only two genera of the herbaceous, annual waterwort family Elatinaceae Dum., with the other genus being *Bergia* L. (POWO, 2021). *E. gussonei* is classified in the subgenus *Elatine*, within the section Elatine as depicted in Figure 3. Species in Section Elatine of the subgenus *Elatine*, were resolved as a clade via phylogenetic analyses from genetic and combined morphological data by Razifard et al. (2017), as depicted in Figure 3. Species of this subgroup are characterised by being tetramerous and have 6 or 8 stamens.

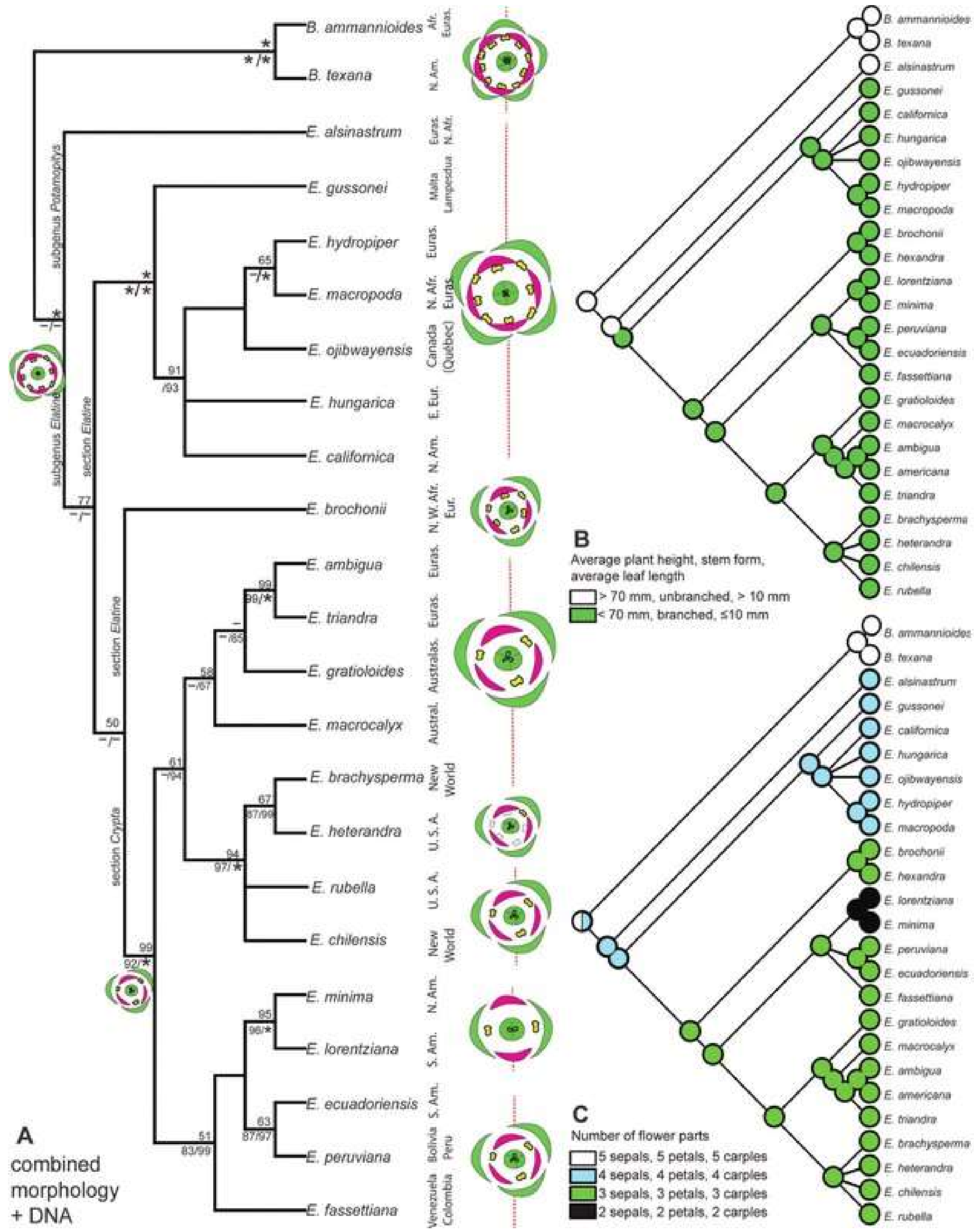

Figure 3: Phylogenetic reconstruction of the genus *Elatine*. Dendrogram (left) and cladograms (right) of the species resolved from combined morphology and DNA, along with floral diagrams, corolla characteristics, and average morphometric dimensions, to indicate characteristic similarities between species. Reproduced from Razifard et al. (2017).

1.1.4 Phytochorology

All species from the genus *Elatine* are herbaceous annual plants that are restricted to ephemeral freshwater habitats, with *E. gussonei* being restricted to the Mediterranean region (Brullo et al., 2020b; Popiela & Lysko, 2010; Razifard et al., 2017). Species of this genus are able to complete their life cycle being either fully or mostly submerged, allowing them to behave both as aquatic and amphibious plants (Brinkkemper et al., 2008; Molnár et al., 2014; Molnár et al., 2015; Popiela et al., 2017; Razifard et al., 2017). Their ability to colonize habitats with fluctuating water levels means that they can complete their life-cycle in areas of shallow-water, or in damp, exposed sites (Brinkkemper et al., 2008).

The different species are distributed mostly in the northern hemisphere, predominantly in areas with a temperate climate. Their known geographical ranges, however, vary from widespread (e.g. *E. alsinastrum*), to very restricted (e.g. *E. gussonei*). *E. gussonei* has been noted to occur in temporary and limestone rockpools, along muddy lakeshores, marshes, oxbows, ditches, and temporary inundated depressions (Molnár et al., 2014; Sommier, 1908; Takács et al., 2017). The distribution and habitat requirements of different species however still remain unclear (Takács et al., 2017).

Initially, *E. gussonei* was thought to be endemic to the Maltese Islands and Lampedusa (Kalinka et al., 2014; Sommier, 1908), and eventually also Sicily (Minissale & Sciandrello, 2016; Molnár et al., 2014). However, Takács et al. (2017) documented findings from 293 specimens of the species from herbaria across Portugal, Spain, France, Algeria, Egypt, Cyprus and Israel; and from field surveys in Cyprus, Morocco, and Spain. These new records indicate that the supposed endemic species; *Elatine gussonei* is more widely distributed than previously thought, suggesting that it is in fact not endemic to the phytochorological 'Dominio Siculo' (Pignatti et al., 2017), but is regionally restricted to the Mediterranean basin.

Observations indicate that the species has a compressed annual lifecycle that begins with germination slightly after the first rains of the Mediterranean wet season around September/October, with flowering ensuing upon the onset of the dry season in March/April. However, apart from the fact that *Elatine* seeds have been known to retain the ability to germinate after over 50 years in the case of *E. triandra* (Takacs et al., 2013), little is as yet known about the germination triggers and requirements for *E. gussonei*.

1.2 THREATS & IMPLICATION FOR CONSERVATION

Of the 30 species within this genus (POWO, 2021), six have been reported as threatened, endangered, or whose population is decreasing in size according to the IUCN (IUCN 2016; USDA, NRCS 2016). Apart from *E. gussonei*, the other five species are *E. alsinastrum*, *E. americana*, *E. brochonii*, *E. macropoda*, and *E. minima* (Razifard et al., 2017). *E. gussonei* is a rare and threatened amphibious-aquatic ephemerophyte, restricted to temporary freshwater Mediterranean habitats, whose population trend is decreasing, and occupancy area is unlikely to exceed $100km^2$ Takács et al. (2017). Despite its global, European and EU status being listed as Least Concern (LC), according to the Habitats Directive Annex II*, E.* gussonei is listed as Critically Endangered (CR) and threatened in Italy and the Maltese Islands according to the Italian Red List (Rossi et al. 2013) and the Maltese Red Data Book (Lanfranco 1989) respectively. is also characteristic to the endangered priority habitat within the Natura 2000 network of the European Union. The habitat is classified by the Habitats Directive as Mediterranean Temporary Ponds (special habitat code 3170*) (Ernandes & Marchiori, 2013; Molnár et al., 2014).

Threats faced by the habitat and its species may result in the extinction in of several rare or endangered characteristic pond species, while endangering such vulnerable habitats. Such threats are mainly a result of inadequate information or management. These include urbanisation (housing, road developments), inadequate environmental management (soil extraction, drainage, overgrazing), agricultural pressures (water used for irrigation and livestock, and pollution from pesticides and fertilizers via runoff), trampling, and fires (Barosa et al., 2014; Ernandes & Marchiori, 2013; Zacharias & Zamparas, 2010). A summary of threats, impacts and interactions as summarised by Zacharias et al. (2007) is visualised in Figure 4.

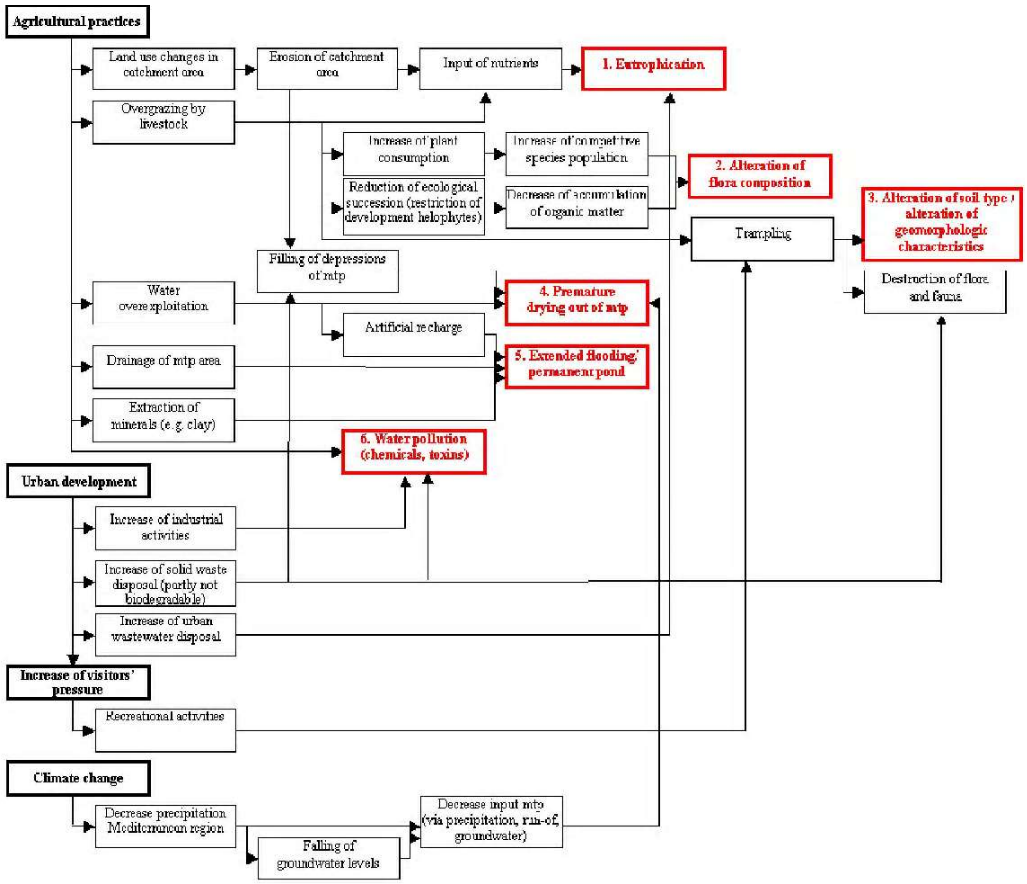

Figure 4: Threats to Mediterranean Temporary Ponds (MTPs). Reproduced from Zacharias et al. (2007)

In general, excessive trampling may modify pond beds' geomorphology, and if prolonged, may also affect soil type. The latter may consequently impact or prevent germination of certain species or alter floral composition to favour more common species respectively. Vegetation communities as well as seed and propagule banks may also be damaged by fires as a result of anthropogenic interference and inadequate protection measures. Pond water use in agriculture along with climate change; however, both pose the greatest threats to such systems. By reducing the inundation period, this may result in shorter hydroperiod durations, which may consequently affect certain species' reproduction and development. Reduced rainfall and increasing temperatures may result in shorter or a lack of pond inundations due to lack of groundwater or runoff. Therefore, any rare or endemic species present are also threatened and may face extinction (Ernandes & Marchiori, 2013; Zacharias & Zamparas, 2010).

A major problem faced by such habitats and conservation management practices is that environmental legislation is not applicable to countries in which ponds are present, but that are not within the European Union. Therefore, appropriate political and managerial measures must be taken to ensure the safety and longevity of the habitat and its species (Zacharias et al., 2007; Zacharias & Zamparas, 2010).

1.3 Mediterranean Temporary Freshwater Habitats

Of the 10 standing freshwater habitats present in the Mediterranean priority habitat 3170* Mediterranean temporary ponds is the only endorheic, standing freshwater body fed solely by rainwater (Evans, 2006). Such habitats are seasonal and undergo a dry and wet season. The latter is colonised by specialised flora and fauna, whose propagules are able to resist the harsh conditions of the hot dry season throughout the summer months, where soil temperatures may reach up to *ca*. 50°C (Lanfranco & Briffa, 2019).

1.3.1 EU Priority habitat 3170* Mediterranean Temporary Ponds (MTP)

The Interpretation Manual of European Union Habitats – EUR28 describes the habitat as “Very shallow temporary ponds (a few centimetres deep) which exist only in winter or late spring, with a flora mainly composed of Mediterranean therophytic and geophytic species”. Temporary ponds are seasonal, ephemeral, freshwater habitats and are subjected to extreme and unstable ecological conditions (Pinto-Cruz et al., 2011). Such self-contained habitats are generally small and characterised by alternating flooded and drought phases. The inundated endorheic depressions remain submerged throughout the wet season and are colonised by specialised resident flora and fauna adapted to the ephemeral and volatile nature of the habitat (Ernandes & Marchiori, 2013).

1.3.2 The Maltese Islands

Though not technically ‘ponds’ in the sense of large expanses of wetlands as in other Mediterranean countries such as the Camargue in France, ponds in the Maltese Islands are more similar to the cupular ponds in Italy, Spain and Portugal. Locally, however, such ponds are more commonly referred to as temporary freshwater rockpools (TFRs) as a ‘subset’ of Mediterranean Temporary Ponds (MTPs). Malta’s TFRs are classified as and fall under EU priority habitat 3170* Mediterranean temporary ponds according to the Interpretation Manual of European Union Habitats – EUR28. They are small, cupular, endorheic waterbodies formed in karstic limestone landscapes and fed by rainfall (Sommier & Caruana Gatto, 1915). The temporary freshwater rockpools are seasonal, consisting of inundation during the wet season, where rainwater fills depressions to support a resident and visiting freshwater biota, and a dry season. Such habitats are the only seasonal lentic waterbodies present in the Maltese Islands (Lanfranco & Schembri, 1986; Schembri, 1997). The specialised resident flora and fauna restricted to such a habitat, as well as the scarce nature of the habitat itself contributes to their local conservation interest and importance.

1.4 Environmental Factors

Temporary pond vegetation responds to changes in hydroperiod more rapidly than to changes in water quality (Zacharias et al., 2007). Environmental factors may be divided into biotic and abiotic, as in Pinto-Cruz et al. (2011), whereby abiotic factors may be further subdivided into morphometric and physicochemical factors, while biotic factors focus on resident pool biota. The following are some studies conducted to illustrate how environmental biotic and abiotic factors affect plant community composition.

Pinto-Cruz et al. (2011) analysed various biotic and abiotic factors to distinguish between different types of Mediterranean temporary ponds (Portugal). Criteria for pond classification included floristic composition based on presence-absence data, whereby ponds were distinguished using climatic, geographic, and geological variables, and a combination of physical and chemical soil properties (texture, nitrogen, and pH). Two pond types – marshlands and Mediterranean temporary ponds (EU habitat 3110 and priority habitat 3170*) were defined using characteristic or indicator species, while the third group was that of disturbed ponds. Habitats 3110 and 3170* were distinguished based on their floristic compositions. These were determined based on basin hydroperiod, and soil texture and pH, whereby the latter habitat present in the Maltese Islands favoured more alkaline, clay-like soils, which had better water-retention properties, and subsequently longer hydroperiods.

Ecological features investigated by Minissale and Sciandrello (2016) on their effect on plant community and composition in Malta, Sicily and Lampedusa included water depth, pH, and conductivity, as well as altitude, soil depth and floristic richness and diversity. Analyses established four distinct communities, each represented by a different dominant species following an ecological gradient related to basin size and water depth. The four indicator species for the categories were: *Callitriche truncata*;

Elatine gussonei; *Tillaea vaillantii* and *Lythrum hyssopifolia*, whereby the former is the most ‘aquatic’, and the latter is the most ‘terrestrial’. Minissale and Sciandrello (2016) also noted that basins with greater water depths and longer hydroperiods had reduced floristic richness and diversity.

Lanfranco et al. (2020) analysed the effect of various morphometric factors on the community composition of 30 plant species, grouped into 3 functional forms (aquatic, terrestrial and amphibious). Morphometric factors included water depth, sediment depth, surface area, shading, distance from the coast, and elevation above sea level. Results suggested that while location was deemed an important determinant of community composition by explaining almost 50% of community variation, it is the landscape characteristics that determine morphometry. Therefore, morphometry may be deemed the most important factor in structuring plant community composition, with the most influential in this case being basin surface area as an indicator of hydroperiod and subsequently, dependent functional form. Basin water and sediment depth however had a greater influence on specific species. Therefore, location was important in predicting community structure and composition, whereby composition was found to be dependent on morphometry, favouring functional forms over species and being location-dependent. Vanschoenwinkel, Hulsmans, et al. (2009), however, state that basin depth is often better correlated with hydroperiod than surface area.

1.5 Predictive Ecological Modelling

van Oijen (2020) defines a model as “a function f of predictor variables x and parameters θ”. *E. gussonei* has a relatively simple life cycle, with distinct, non-overlapping generations. This, coupled with its sensitivity and rapid response to changes in climate makes it an ideal sentinel species and subject for a simple occurrence model.

Linear regression (lm) and generalized linear regression (glm) are the two most common approaches for transforming or analysing data, respectively. Lm, however, has been classified as the more reliable in terms of “maintain[ing] control of type I error rates in tests for no association, while seemingly losing little in power” (Warton et al., 2016). It is worth noting, however, that glm may be used in preference to lm if count data is fit correctly and type I error rates are taken into account and corrected accordingly. Given their strong type I error control, lm may also be used for counts in complex models and difficult diagnostics, however this may result in some loss of power and subsequent interpretability (Warton et al., 2016).

In lm, van Oijen (2020) states that “the linear predictor is a simple regression of the predictor variable(s)”, whereby “the error term has a zero-mean Gaussian distribution”. In glm, however, the linear predictor “is wrapped in a transformation function”, whereby the error may be of a “distribution in the exponential family of probability distributions”. Some examples besides Gaussian distribution include, but are not limited to Poisson, binomial, and gamma distributions. In the case of this study, however, the dependent variable of *E. gussonei* occurrence was based on dichotomous presence-absence data. Therefore, a binomial error distribution was utilised for glms carried out with independent environmental factors.

1.6 Previous Work

So far, the majority of work previously carried out on Mediterranean pool vegetation and their communities are those of Aponte et al. (2010); Bagella et al. (2009); Bagella and Caria (2012, 2013); Bagella et al. (2011); Bliss and Zedler (1997); Bornette and Puijalon (2011); Bouahim et al. (2014); Carta (2016); Deil (2005); Dimitriou et al. (2009); Ernandes and Marchiori (2013); Fernández-Zamudio et al. (2016, 2018); Florencio et al. (2014); Fraga i Arguimbau (2008); Pinto-Cruz et al. (2009); Rhazi et al. (2012); Rocarpin et al. (2016); Rouissi et al. (2014). Those carried out in the Maltese Islands, however, are far fewer and are limited to those of Brullo et al. (2020a, 2020b); Lanfranco and Schembri (1986); Lanfranco et al. (2020); Lanfranco and Briffa (2019); Lanfranco et al. (2000); Lanfranco et al. (2016); Minissale and Sciandrello (2016). With respect to predictive-occurrence models, similar work has not been traced in literature for this genus or for temporary freshwater rockpool flora.

1.7 Knowledge Gaps

The occurrence of *Elatine gussonei* is patchy, and it is as yet unknown what affects its presence in a given pool. The range of conditions within which it survives and reproduces has also not yet been fully characterised. This represents the knowledge gap that this study aims to address.

1.8 AIMS OF THE STUDY

This study aims to:

1. determine whether abiotic environmental factors vary between pools with and without *Elatine gussonei*,
2. determine the morphometric and water chemistry tolerance ranges of pools in which *Elatine gussonei* is present, and
3. construct a preliminary model, comprising environmental factors that significantly affect the presence of *Elatine gussonei*, to predict its occurrence in a pool.

1.9 HYPOTHESES

1.9.1 Hypothesis 1: Basin morphometry determines the presence of *Elatine gussonei* in a given pool

Rationale: Being an amphibious species, *Elatine gussonei* has a higher fitness in pools with a low value for surface area to volume ratio where water and sediment depth are not high enough to result in an aquatic or terrestrial environment respectively.

Prediction: *E. gussonei* will be absent from pools with high water and sediment depths due to competition with better adapted aquatic and terrestrial species respectively.

1.9.2 Hypothesis 2: Water quality determines the presence of *Elatine gussonei* in a given pool

Rationale: *Elatine gussonei* is only able to withstand specific ranges of pH, Electrical Conductivity and Oxidation Reduction Potential in water.

Prediction: *E. gussonei* is only present under specific water quality parameters and will otherwise be excluded from pools in which water quality is deemed unsuitable.

2 MATERIALS AND METHOD

2.1 DESIGN OF THE STUDY

Throughout the wet season 2020/2021, 173 TFRs were surveyed from 10 out of the 22 pool landscapes in which *E. gussonei* has been recorded by Kalinka et al. (2014). For each pool, GPS coordinates, lists of resident macroflora, along with morphometric and water chemistry data were collected. Pool basin morphometry and water chemistry were then used to identify the conditions that favour the growth of *E. gussonei*, and to subsequently assist in the construction of a predictive occurrence model for the plant.

2.2 SELECTION CRITERIA

The general objective was to survey as many pools from as broad a range of landscapes as possible. Landscapes and pools considered for the study included ones followed in past studies, as well as new ones encountered in recent explorations which have not yet been studied. This was done to increase the size of the database for TFRs, and augment information on their morphometry, water chemistry and distribution in the Maltese Islands.

For the purposes of the study, valley bed pools were not considered due to the water being intermittently lotic, as opposed to lentic. This hydrodynamic distinction affects all aspects of the ecology of a TFR. Therefore, only karstic landscapes with lentic waters that are characteristic of the Habitats Directive priority Habitat 3170* Mediterranean temporary ponds, that are enclosed within a karstic limestone basin and that are fed by rainwater and runoff were sampled and analysed for the study. Pools that were deemed as being 'too terrestrial', whereby the basin was dominated by opportunistic terrestrial flora were not included in the study due to the fact that the pool hydroperiod was far too brief to sustain any resident aquatic or amphibious pool flora as a result of high sediment and low water and morphometric depths.

One limitation to the number of study sites and pools sampled was accessibility, whereby pools present on private land, or which were otherwise inaccessible were not sampled. Pool selection was conducted regardless of the state or abundance of pools per landscape, or the proportion of pools having *E. gussonei*, however, as many pools as were encountered and which had a hydroperiod capable of sustaining aquatic and/or amphibious life were surveyed for the study. The dataset collected aimed to be as inclusive as possible, providing sites with a variety of species richness, morphometry, and exposure, irrespective of whether *E. gussonei* had been previously noted in a particular landscape or not. Other locations were also considered but were not included due to time constraints and other considerations such as the shorter wet season compared to previous years. Therefore, a trade-off was made with regards to the number of landscapes and the number of pools surveyed throughout the study.

2.3 DATA COLLECTION

Data collection commenced during the week starting 6th September 2020; the day following the first rains, indicating the start of the wet season for 2020/2021. Meteorological data was recorded from 6th September 2020 till 6th April 2021 (the day following the last rains of the wet season). Data about pool morphometry, water chemistry and species were also collected throughout the wet season and were compiled in a spreadsheet pending analysis.

Water chemistry and morphometry were collected following rainfall events for pools to be completely inundated. Species data were observed, noted and updated at the beginning, middle, and towards the ed of the wet season for a comprehensive dataset to allow for varying germination periods of different species.

In the case of the study site Qala (QLA), due to time constraints and COVID travel restrictions to Gozo, data from studies in previous years was used. Data collected for this site however was incomplete, and not all coordinates for each pool were available, hence the inconsistencies between Table 1, and Figure 5 and Figure 14.

2.3.1 Study Sites

Individual pool coordinates were obtained using a Garmin GPSmap 60CSx receiver. These data were used with geographical information systems software (QGIS) to illustrate the frequency and distribution of pools and pool landscapes sampled throughout the duration of this study as depicted in Figure 5.

The frequency and distribution of individual pools within their respective landscapes are shown in Figure 7 – Figure 17. The main characteristics of each pool landscape are summarised in Table 1.

Table 1: Main study site characteristics for the pool landscapes surveyed. Surface Bedrock: Upper Coralline Limestone (UCL) for all except QLA - Lower Coralline Limestone (LCL).

Landscape (Code)	**Co-Ordinates of Centre of Site**	**Approximate Study Site Area (m^2)**	**Number of pools surveyed**
Birżebbuġa (BBG)	35.806921, 14.516121	1,500	6
Ħad-Dingli (DNG)	35.851658, 14.386357	7,500	8
Għargħur (GRG)	35.931425, 14.453426	2,000	6
Munxar (MNX)	36.032865, 14.229358	8,000	15
Mosta Tal-Qares (MST)	35.915594, 14.425309	6,000	26
Pembroke (PBK)	35.929744, 14.485845	18,000	23
Qala (QLA)	36.029271, 14.320945	2,500	13
Salini (SLN)	35.945683, 14.420609	5,000	4
San Pawl tat-Tarġa (SPT)	35.926516, 14.440718	30,000	31
Wied Ħas-Saptan (WHS)	35.835849, 14.515074	6,000	41

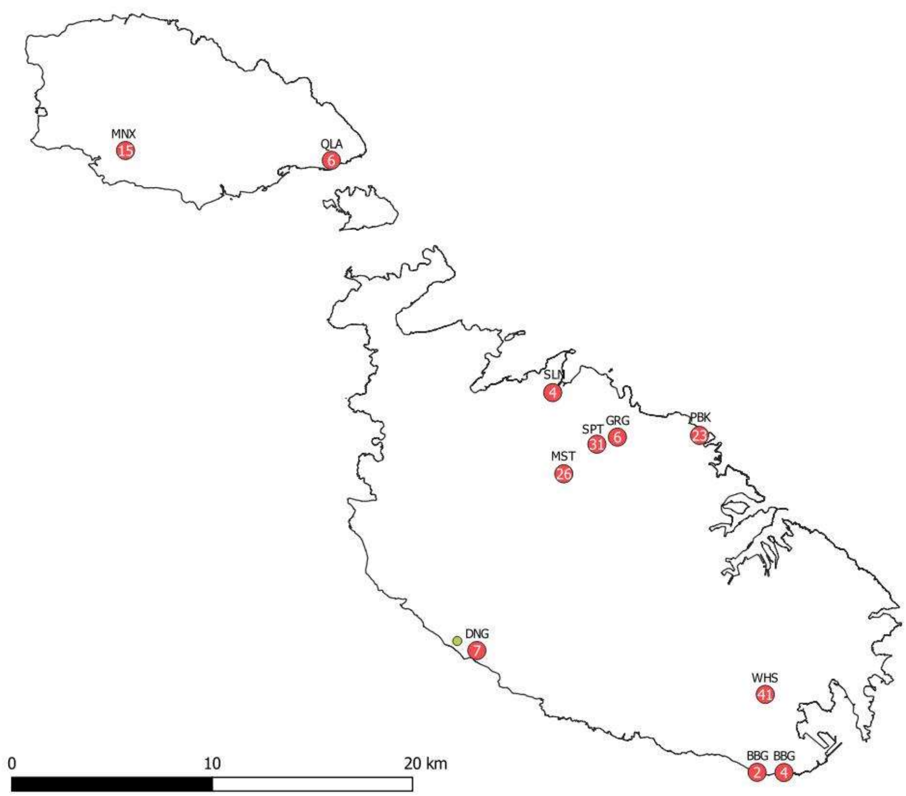

Figure 5: Map of the Maltese Islands (North towards the top of the image) showing the frequency and distribution of pools and landscapes surveyed for the period of study during the wet season 2020/2021. Image source: QGIS v.3.12.3.

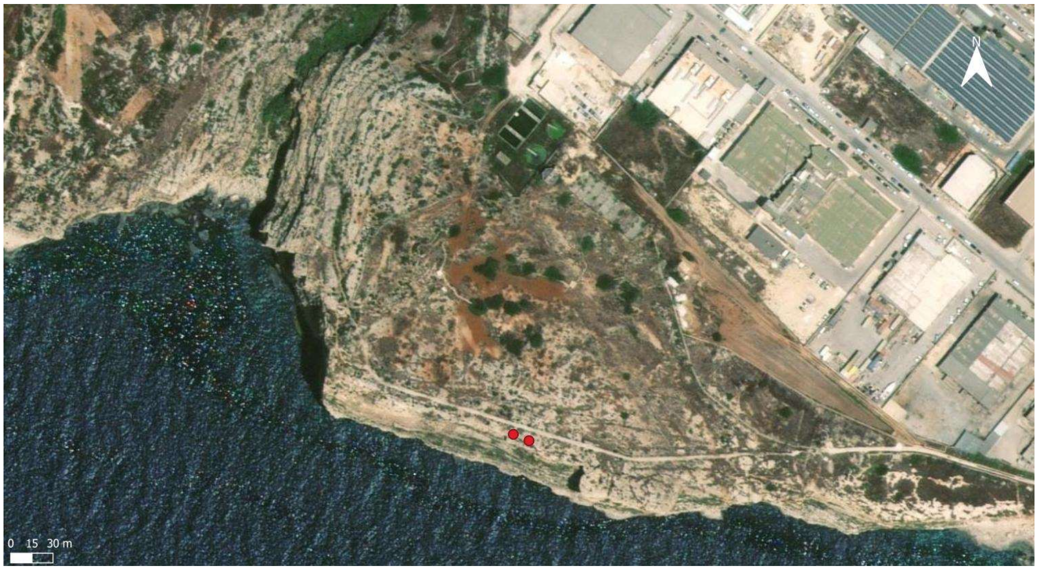

Figure 6: Pool landscape at Birżebbuġa (BBG) (left side), indicating the approximate position of 2 of the 6 individual pools represented by red points.

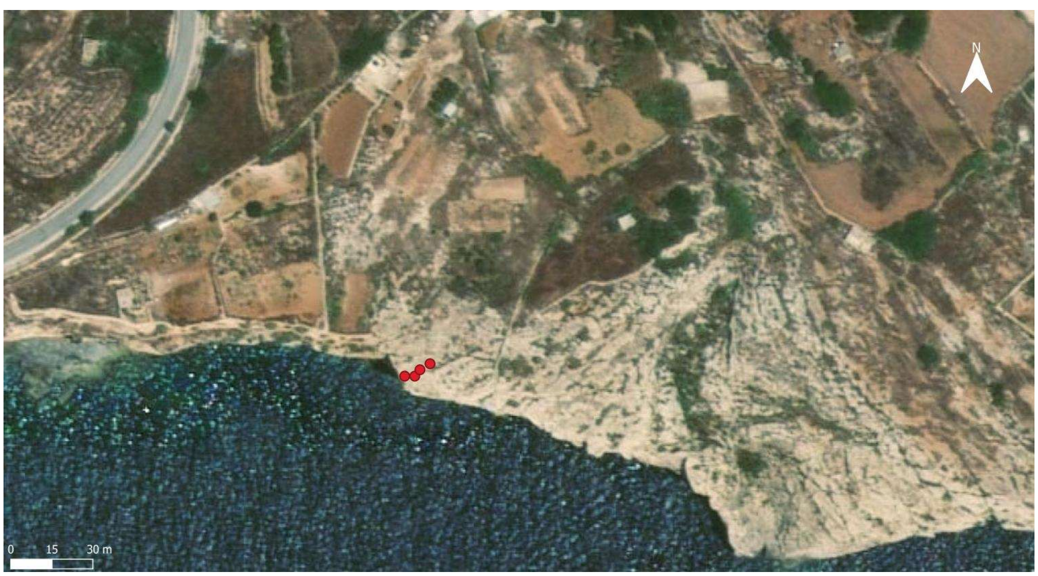

Figure 7: Pool landscape at Birżebbuġa (BBG) (right side), indicating the approximate position of 4 of the 6 individual pools represented by red points.

Figure 8: Pool landscape at Ħad-Dingli (DNG), indicating the approximate position of the 8 individual pools represented by red points.

Figure 9: Pool landscape at Ħad-Dingli (DNG), indicating the approximate position of 7 of the 8 individual pools represented by red points.

Figure 10: Pool landscape at Għargħur (GRG), indicating the approximate position of the 6 individual pools represented by red points.

Figure 11: Pool landscape at Munxar (MNX), Gozo, indicating the approximate position of the 15 individual pools represented by red points.

Figure 12: Pool landscape at Mosta (MST), indicating the approximate position of the 26 individual pools represented by red points.

Figure 13: Pool landscape at Pembroke (PBK), indicating the approximate position of the 23 individual pools represented by red points.

Figure 14: Pool landscape at Qala (QLA), Gozo, indicating the approximate position of 6 of the 13 individual pools represented by red points.

Figure 15: Pool landscape at Salini (SLN), indicating the approximate position of the 4 individual pools represented by red points.

Figure 16: Pool landscape at San Pawl tat-Tarġa (SPT), indicating the approximate position of the 31 individual pools represented by red points.

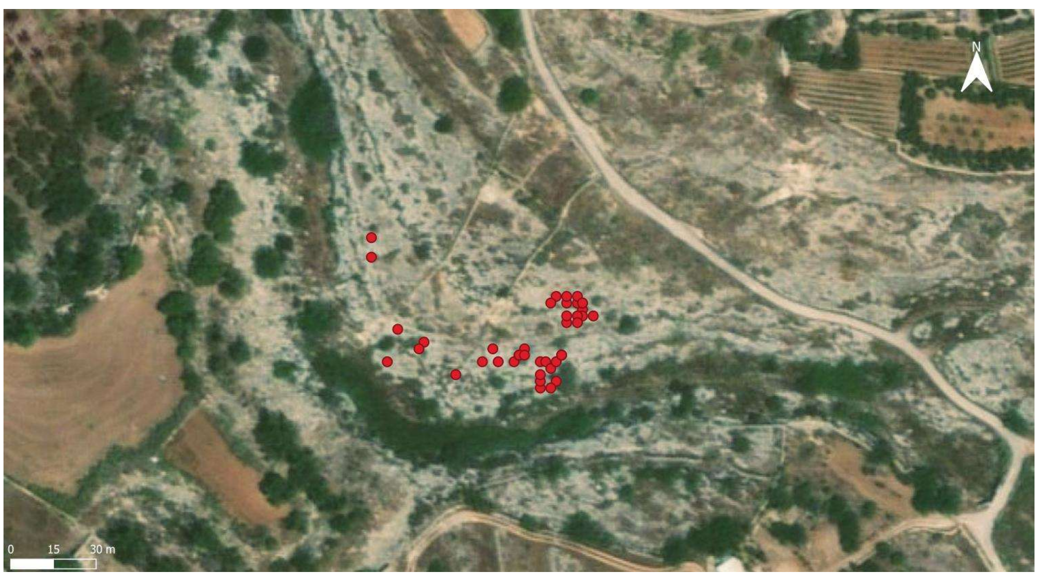

Figure 17: Pool landscape at Wied Ħas-Saptan (WHS), indicating the approximate position of the 41 individual pools represented by red points.

2.3.2 Morphometry

To obtain accurate basin morphometry, the length of the primary and secondary axes (r_1 and r_2 respectively) were measured using a tape measure accurate to the nearest millimetre. These were subsequently used to calculate an estimate of the surface area (SA), assuming an elliptical surface ($SA = \pi r_1 r_2$) (Figure 18).

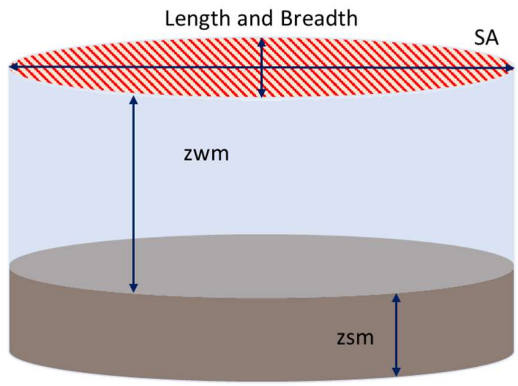

Figure 18: Simplified diagrammatic representation of basin morphometry, assuming elliptical dimensions based on field observations. Top arrows indicate the primary and secondary axes (length and breadth respectively), surface area (SA), maximum water depth (zwm), maximum sediment depth (zsm)

Water and sediment depth were measured using a thin, metal metre ruler, with readings taken at eye level and at the meniscus to minimise parallax errors. Measurements were noted and recorded with a precision to the nearest millimetre, with data subsequently being expressed in centimetres. For water depth data, the metre ruler was submerged into the water at various points in the pool until the basin's maximum water depth (zwm) was noted and recorded. The maximum water depth at which *E. gussonei* was present (zwe) was also measured and noted in the same manner. The same process was subsequently repeated for the maximum basin sediment depth (zsm), and maximum sediment depth at which *E. gussonei* was present (zse). Sediment depths were measured from the bedrock to the sediment surface, and water depths were measured from the sediment surface to the water surface.

In some cases, *E. gussonei* was obscured by filamentous algae, or other species such as *Chara vulgaris*, *Ranunculus saniculifolius* or *Zannichellia melitensis*. Therefore, flora obscuring any *E. gussonei* had to be displaced in such a way as to expose the bottom of the basin to determine whether any *E. gussonei* was in fact present.

The rationale for collecting data for the specific water and sediment depths at which *E. gussonei* occurred was to determine whether there was a significant difference in the range of depths at which it was present. The maximum water and sediment depths at which *E. gussonei* was present (zwe and zse respectively) were also noted to quantify the species' tolerance ranges compared to the morphometric maxima. This is due to the fact that given the maximum morphometric basin water depth (zwm) and actual water depths at which *E. gussonei* is present (zwe) in a given pool may indicate a slight variation in hydroperiod. Therefore, if zwe is lower than zwm, it would indicate a preference of *E. gussonei* to a shorter hydroperiod than that experienced in the deepest part of the basin. Variations in maximum basin and actual species' maximum ecological depth ranges are illustrated in Figure 19.

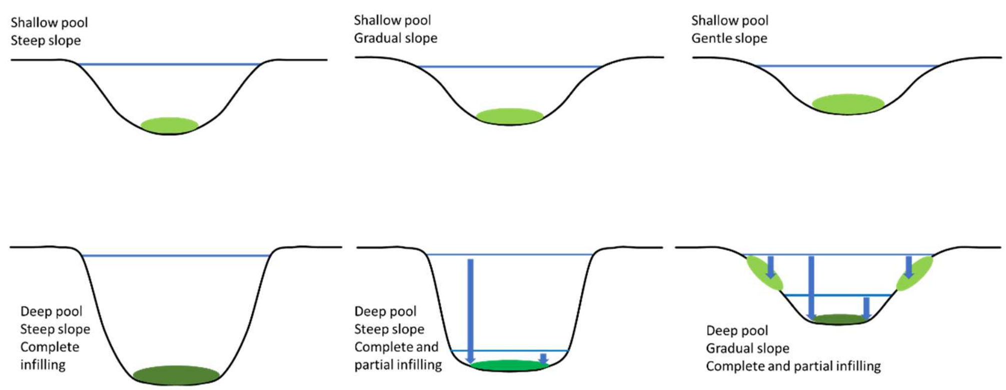

Figure 19: Diagrammatic representation of shallow (top) and deep (bottom) basin profiles, with decreasing angle (left to right), indicating complete and incomplete inundation. Light green ellipses and teal ellipses in incompletely filled deep basins indicate the presence of *Elatine gussonei*, while darker ellipses and deep basins with complete inundation indicate the presence of specialized aquatic flora

This would suggest that *E. gussonei* is present in shallow pools, and shallow parts of deep pools having a gentle to gradual slope (light green ellipses bottom right Figure 19), or deep pools that do not experience complete inundation (teal ellipse bottom centre and right of Figure 19). The bottom central and right basins in Figure 19 indicate complete and partial inundation respectively, implying differences in hydroperiod duration. This is reflected in the consequent species composition, whereby maximum basin water depth (zwm) is representative of the maximum hydroperiod experienced by a given pool, but zwe is representative of the hydroperiod experienced by *E. gussonei*. In the case of shallow pools, zwe may be equal to zwm or slightly less.

2.3.3 Water Quality

Water quality data were collected using a handheld HI98194 Hanna Instruments multiparameter meter. Collection took place when the basins were sufficiently inundated on the day following a rainfall event. This was done to ensure maximum inundation in all basins. Water quality data was taken once per pool between December 2020 and January 2021 between 10:00h and 16:00h. It should be emphasised that the recorded values are point measurements. Boven et al. (2008) state that while nutrient concentration and EC vary throughout the season depending on basin volume as a product of maximum basin water depth, water's high buffering capacity renders pH comparable and almost constant throughout the wet season. In the case of pH, however, values may vary slightly depending on the time of day at which they were recorded due to diurnal fluctuations (Goulder, 1970; Menéndez et al., 2001; Scholnick, 1994; Talling, 2010) as a result of photosynthetic activity (Axelsson, 1988).

To measure water quality, the probe was inserted into the water and allowed to stabilise prior to taking readings. Once the readings had stabilised, three consecutive readings were taken, whereby the third and final reading was used for analysis. In cases where pools were too shallow or did not have enough water in which to submerge the probe, water was collected in a collection jar, and the probe inserted into it. Data collected were water temperature (°C), pH, Electrical Conductivity (EC) (µS/cm), Oxidation Reduction Potential (ORP) (mV) and Dissolved Oxygen (DO) (%). Not all water quality data were utilised in analyses due to incomplete datasets as a result of a shorter wet season, however, all data were utilised for data visualisation.

2.3.4 Species Lists

A species list of resident macroflora and macrophytes was collected from each pool once communities had started becoming established, a few weeks following the start of the wet season (*ca*. late October/November). Further data was collected once communities had become further established and were monitored again at the middle and end of the wet season to enable compilation of a comprehensive species list, without having excluded species that may have been present earlier or later during the wet season. Species lists collected were based on presence-absence data as opposed to phytosociological plant community survey abundance. This made the process easier and less time consuming, while also increasing practicality and reducing ambiguity (Pinto-Cruz et al., 2011).

2.4 DATA EXPLORATION & ANALYSIS

Statistical tests selected were decided upon after data exploration in RStudio (Team, 2020), following the methods recommended by Zuur et al. (2010); Zuur et al. (2009). Initial data visualisation was conducted using raincloud plots, violin plots and boxplots, and scatter plots for morphometry and water chemistry for pools with and without *E. gussonei*. These aided in the identification and selection of relevant factors to be included in the predictive model. Normality of the data and presence of outliers were evaluated graphically using raincloud, violin, and box plots (Allen et al., 2019).

Linear regressions (lm) were used in subsequent analysis of certain scatterplots. Such models are based on dependent response y-axis variables, and independent explanatory x-axis variables, with the assumption of a linear relationship between the explanatory and response variables (Warton et al., 2016). In this case, linear regressions were used to analyse the relationship between the maximum morphometric basin water and sediment depths with the actual depths at which *E. gussonei* was present.

Multivariate analyses were carried out on environmental factors, as well as pool flora using PAST v4.06 (Hammer et al., 2001) and Canoco 5 (ter Braak, & Smilauer, 2012). Unconstrained ordinations (PCA and CA) were used solely for descriptive purposes to visualise data and were not utilised in subsequent analyses. Constrained ordinations (RDA and CCA), however, were used for hypothesis testing and analyses to build and test the effects of predictors on multivariate responses. This was done by testing multivariate statistical relationships between predictors (following normalisation) and their responses.

Following data exploration and subsequent visualisation, data analysis was conducted on the environmental factors measured, along with the species presence-absence data collected in accordance with the recommendations of Zuur et al. (2010). Univariate comparisons, correlations and modelling were carried out using RStudio (Team, 2020), and PAST v. 4.06 (Hammer et al., 2001). Student’s t-test was used to compare the means between independent samples from the same group, such as the maximum basin depths in pools where *E. gussonei* is present with the actual maximum depth at which *E. gussonei* is present in a given pool (zsm and zwm, and zse and zwe respectively). Generalized linear models (glm) were used to predict the probability of occurrence of *E. gussonei* in a pool. This was done based on continuous independent predictor variables (environmental morphometric and water quality factors) to model presence-absence response as a probability between 1 or 0 (Warton et al., 2016).

A correlation plot was also carried out for species and environmental factors separately and together using PAST v4.06 (Hammer et al., 2001). Data collected were used to construct a binomial model to predict the occurrence of *E. gussonei*. The threshold of statistical significance was always taken as the 95% level.

2.5 Predictive Model Construction

Given the convenience of linear models, regardless of the fact that nature itself is nonlinear, these models behave well and facilitate data analysis. Such behaviour and the fact that output is linearly dependent on its parameters makes linear models very useful in preliminary data exploration. Results must, however, be interpreted with caution when used to predict outcomes. This is especially so when predictor variables are extrapolated beyond values for which observations are available (van Oijen, 2020). In this case, a combination of linear statistical models, also referred to as linear regressions (lm), along with generalized linear models (glm) (logistic regressions) and range values were used in the construction of preliminary predictive models for *E. gussonei* using RStudio. For glms, binomial log linear models were carried out based on significant factors ($p<0.05$) which affected the presence of *E. gussonei* in pools. Lms were modelled to compare the significant effect of maximum morphometric basin depths (zm) on depths at which *E. gussonei* is present (ze), with the modelled and forced 0 intercepts. The R^2 values for the two regressions were then compared.

3 RESULTS

Morphometric and water chemistry data collected during the wet season 2020/2021 were visualised and analysed in order to characterise and determine the environmental tolerance ranges of *E. gussonei* in relation to the data. The analysed data were subsequently used to model the probability of the species' occurrence in a given pool.

3.1 DISTRIBUTION AND OCCURRENCE OF *ELATINE GUSSONEI*

Figure 20 and Figure 21 show the number of landscapes and pools surveyed per landscape in the Maltese Islands, along with the relative proportions of pools with and without *E. gussonei* per landscape. The number of pools in each landscape ranged from 4 at SLN to 41 at WHS. The proportionate presence of *E. gussonei* in pools varied widely across landscapes and followed no discernible pattern, ranging from >80% occupancy at WHS to zero in three landscapes (BBG, GRG, QLA).

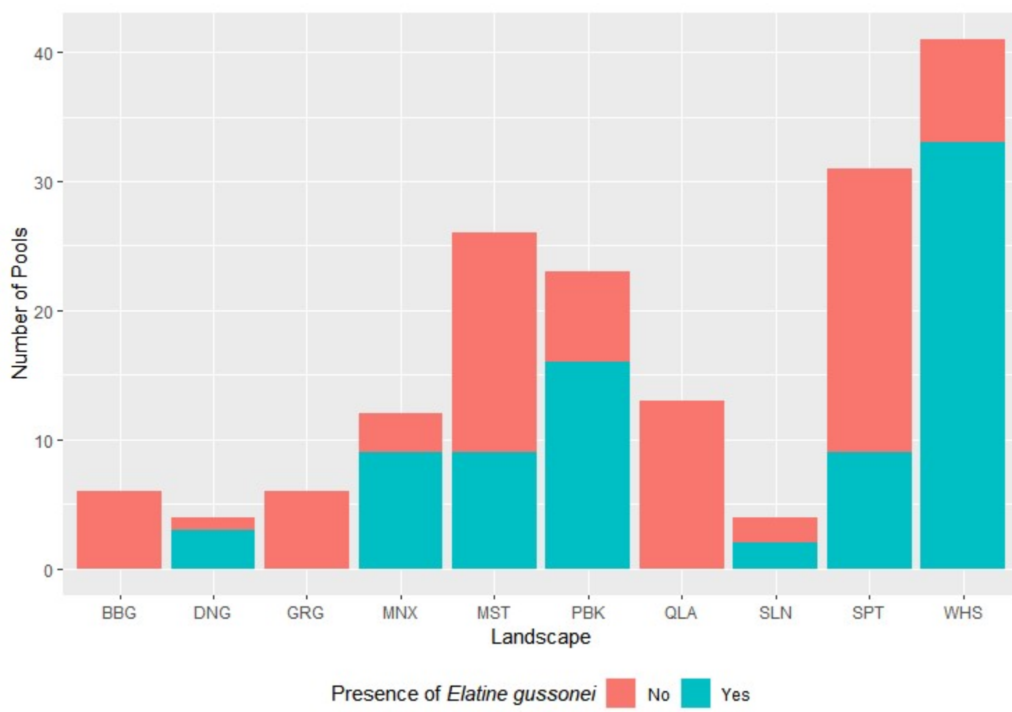

Figure 20: Cumulative number of pools with and without *Elatine gussonei* per Landscape. Landscape Codes: Birżebbuġa (BBG), Ħad-Dingli (DNG), Għargħur (GRG), Munxar (MNX), Mosta (MST), Pembroke (PBK), Qala (QLA), Salini (SLN), San Pawl tat-Tarġa (SPT) and Wied Ħas-Saptan (WHS)

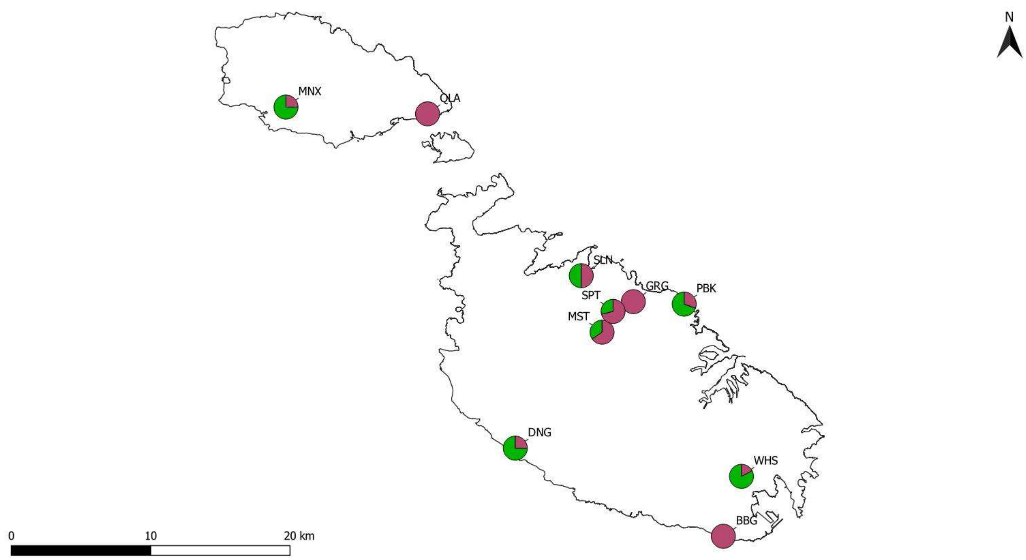

Figure 21: Relative proportion of pools in all 10 pool landscapes surveyed for the current study with (green), and without E*latine gussonei* (pink). Landscape codes: Birżebbuġa (BBG), Ħad-Dingli (DNG), Għargħur (GRG), Munxar (MNX), Mosta (MST), Pembroke (PBK), Qala (QLA), Salini (SLN), San Pawl tat-Tarġa (SPT) and Wied Ħas-Saptan (WHS).

Nonetheless, 'Pool number' was plotted against 'number of pools colonised' by *E. gussonei* (Figure 22) to observe any trends between the two variables. The graph suggests an approximately linear relationship between the two factors. This suggests that the percentage (%) occupancy is an approximately stable proportion of the number of pools (given the gradient of landscapes plotted). This trend, however, should be interpreted cautiously as the two variables are not independent as 'pools colonised' is a component of 'pool number', and not all pools in a given landscape were surveyed. Therefore, the graph below is only representative of the sample collected in the wet season 2020/2021.

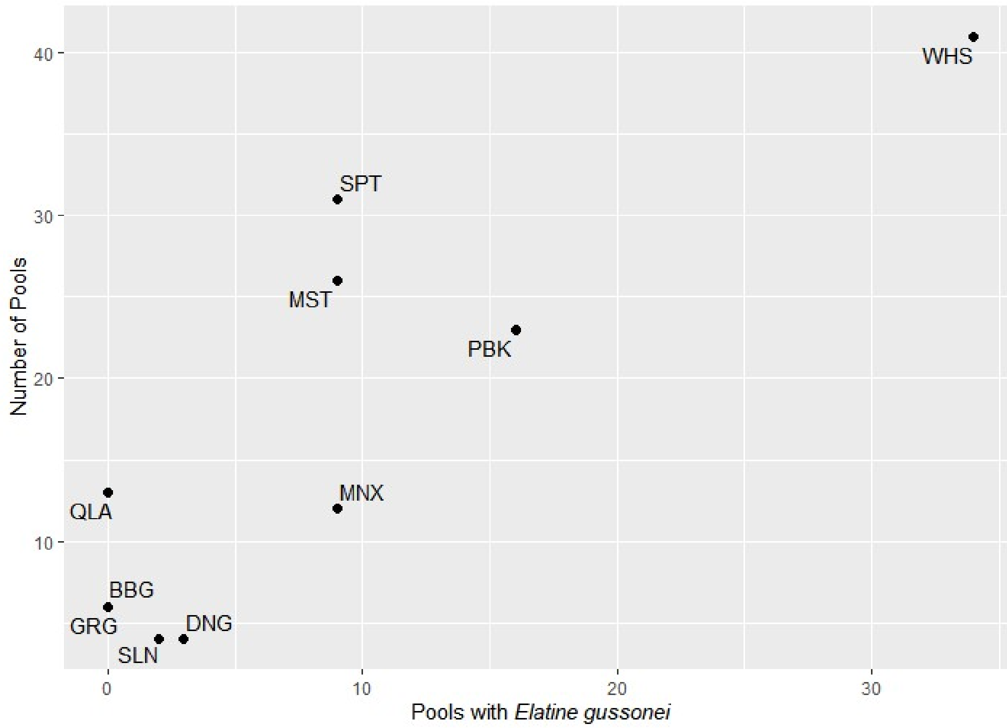

Figure 22: Scatterplot of the total number of pools per landscape against the number of pools with *Elatine gussonei* per Landscape. Landscapes: Birżebbuġa (BBG), Ħad-Dingli (DNG), Għargħur (GRG), Munxar (MNX), Mosta (MST), Pembroke (PBK), Qala (QLA), Salini (SLN), San Pawl tat-Tarġa (SPT) and Wied Ħas-Saptan (WHS)

3.2 Temperature-Rainfall Data

The rainfall and temperature data obtained from the Meteorological Station of Malta International Airport during the period of study (September 2020 – April 2021) is shown in Figure 23. The total rainfall recorded during this period was 357mm, making it *ca.*35% lower than the thirty-year average of 553.12 ±156.99mm from 1961 till 1990 as calculated by Galdies (2011). A pluviothermal diagram of rainfall and temperature during the period 2001-2008 is included for comparison with that during the period of study and that of the preceding season (Figure 24).

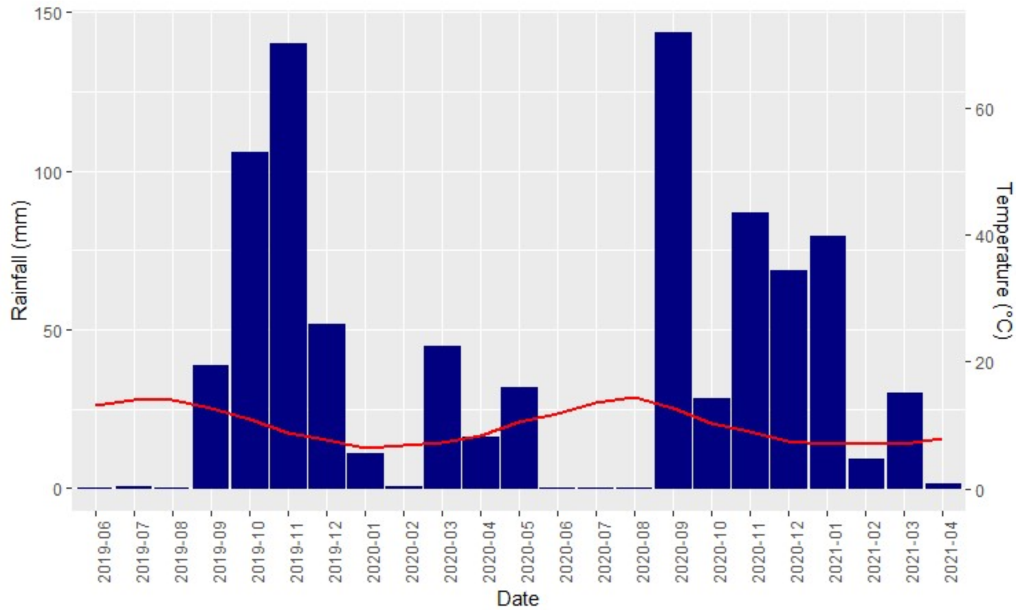

Figure 23: Pluviothermal diagram of monthly Rainfall (mm) (blue columns), and mean Air Temperature (°C), (red line) during the period of study (September 2020 – April 202), and the preceding wet and dry season. Weather data obtained from Malta International Airport plc.

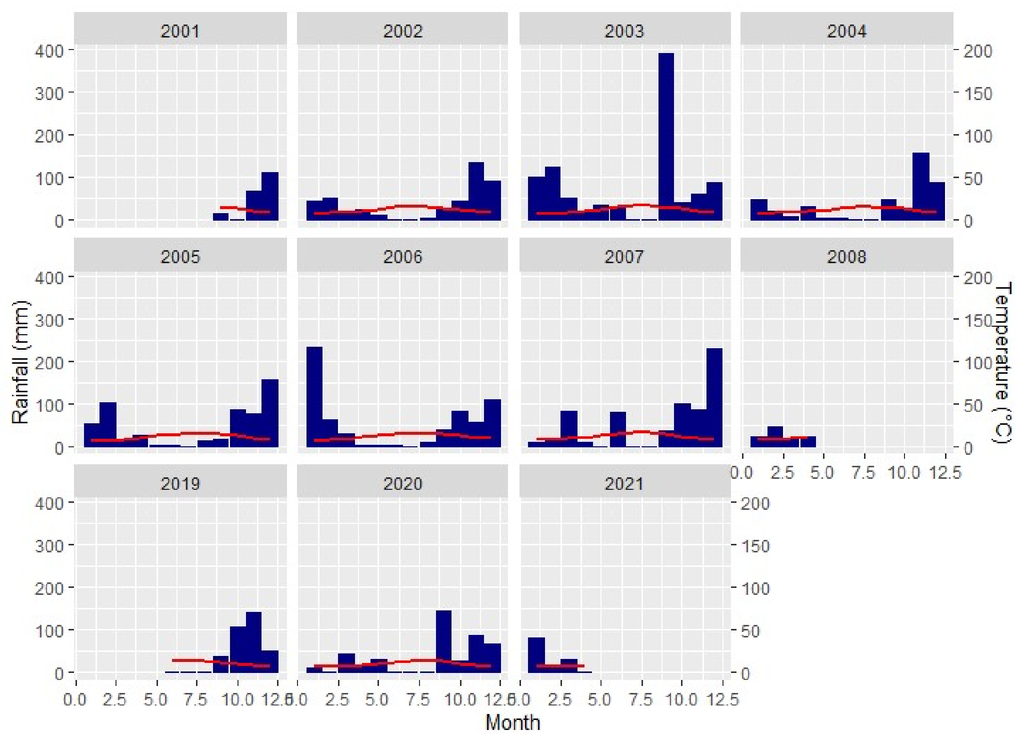

Figure 24: Pluviothermal diagram of monthly Rainfall (mm) (blue columns), and mean Air Temperature (°C) (red line) per year for 2001-2008 (obtained from Malta Weather Services and made available by Sandro Lanfranco) and 2019–2021 obtained from Malta International Airport plc.

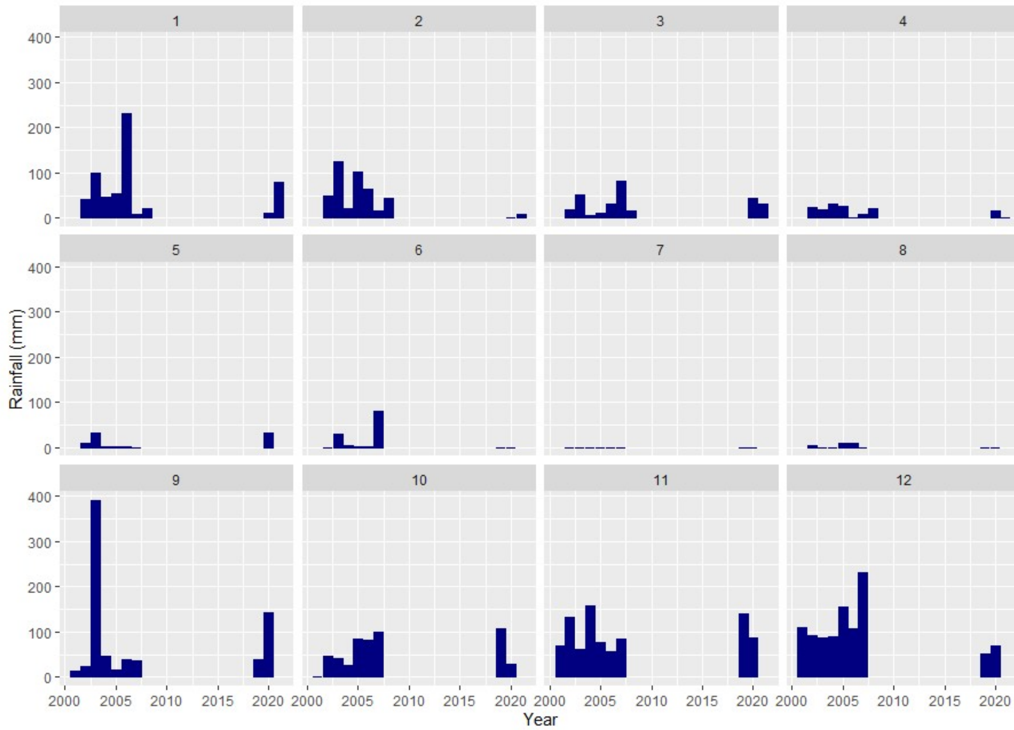

Figure 25: Monthly rainfall for 2001-2008 and 2019-2021, indicating any variation in monthly rainfall across the years. Headers 1-12 represent the months January till December in chronological order. Years indicating no rainfall as blue bars represent periods of missing rainfall data.

In general, the wet season 2020-2021 was characterised by heavy rainfall in September followed by a drier period in October. This was followed by regular rainfall episodes throughout the period November 2020 till January 2021 after which the wet season effectively ended. Temperature appears to remain relatively stable and comparable across the years (Figure 24). Monthly rainfall however was comparable for March, May, September, and November. Data for October, December, January, February, and April for the last two wet seasons 2019/2020 and 2020/2021 (Figure 25), however, were lower than the period 2001– 2008.

3.3 SUMMARY OF ROCKPOOL ABIOTIC FACTORS

The variation and presence of any landscape effects in basin morphometry and water quality were explored through conditional violin plots and boxplots in Figure 26 to Figure 31. The range and mean values of basin morphometry and water quality for all the pools surveyed throughout the duration of the study are summarised in Table 2.

Table 2: Summary of the range, mean and standard deviations of all environmental factors measured for the current study. Codes: SA = Surface Area (cm^2); SAV = Surface Area to Volume Ratio (cm^{-1}); zwm = Maximum Water Depth (cm); zwe = Maximum *Elatine* Water (cm); zsm = Maximum Sediment Depth (cm); zse = Maximum *Elatine* Sediment Dept (cm); EC = Electrical Conductivity (µS/cm); and ORP = Oxidation Reduction Potential (mV)

Environmental Factor	Minimum	Maximum	Mean (±SD)
SA (cm^2)	50.27	76149.61	6920.08 (±110934.99)
SAV (cm^{-1})	0.022	1	0.13 (±0.13)
zwm (cm)	1	55	14.33 (±9.78)
zwe (cm)	2	20	9.16 (±5.77)
zsm (cm)	0.2	51	8.34 (±6.79)
zse (cm)	0.5	15	4.68 (±2.65)
pH	7.31	10.69	8.80 (±0.73)
EC (µS/cm)	9	1552	436 (±248.48)
ORP (mV)	-6.6	267.2	182.2 (±41.46)

3.3.1 Basin Morphometry

In terms of the surface area, this ranged from 50 cm^2 to 7.61 m^2, with a mean size of 2.2 ±19.68 m^2 of bedrock. Any variations in basin size by pool landscape are visualised in Figure 26 – Figure 27. These show that pool size tends to vary across landscapes, suggesting that differential erosion across landscapes gives rise to different morphometric ratios. The sediment and water depth in pools across landscapes is depicted in Figure 28, with values ranging from 0.2 to 51 cm, and 1 to 55 cm respectively.

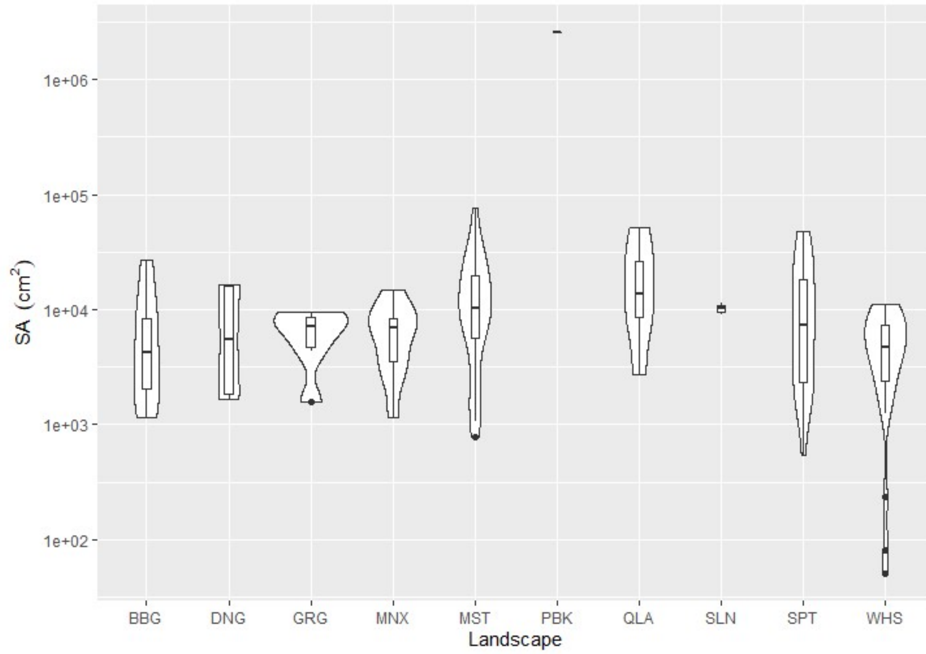

Figure 26: Surface Area (cm²) of pools in each pool landscape. Codes: Birżebbuġa (BBG), Ħad-Dingli (DNG), Għargħur (GRG), Munxar (MNX), Mosta (MST), Pembroke (PBK), Qala (QLA), Salini (SLN), San Pawl tat-Tarġa (SPT) and Wied Ħas-Saptan (WHS)

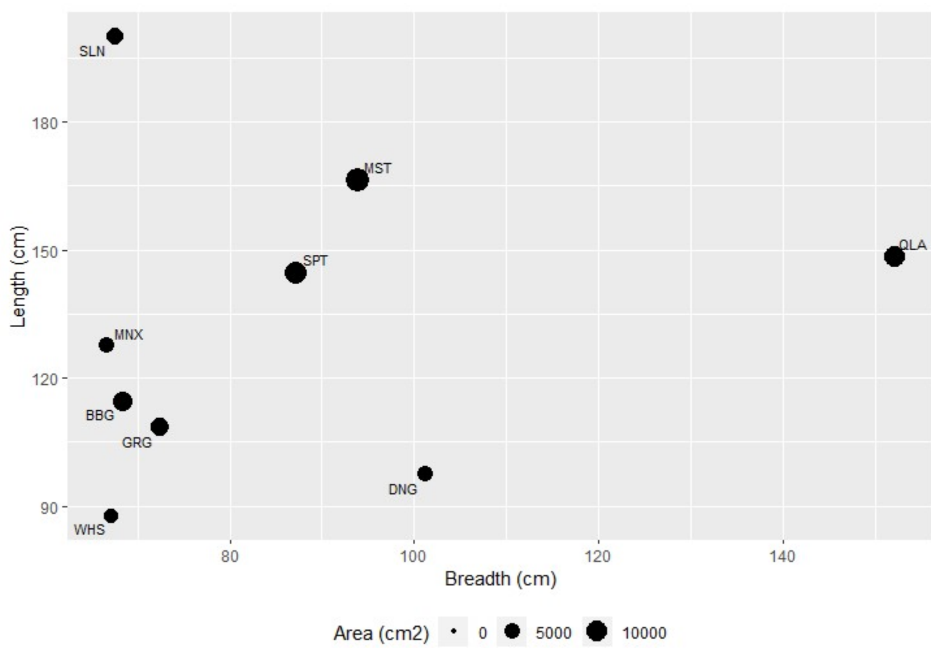

Figure 27: Bubble plot of mean pool axes dimensions and resultant mean pool surface area (cm²) represented by plot size per landscape. Codes: Birżebbuġa (BBG), Ħad-Dingli (DNG), Għargħur (GRG), Munxar (MNX), Mosta (MST), Qala (QLA), Salini (SLN), San Pawl tat-Tarġa (SPT) and Wied Ħas-Saptan (WHS)

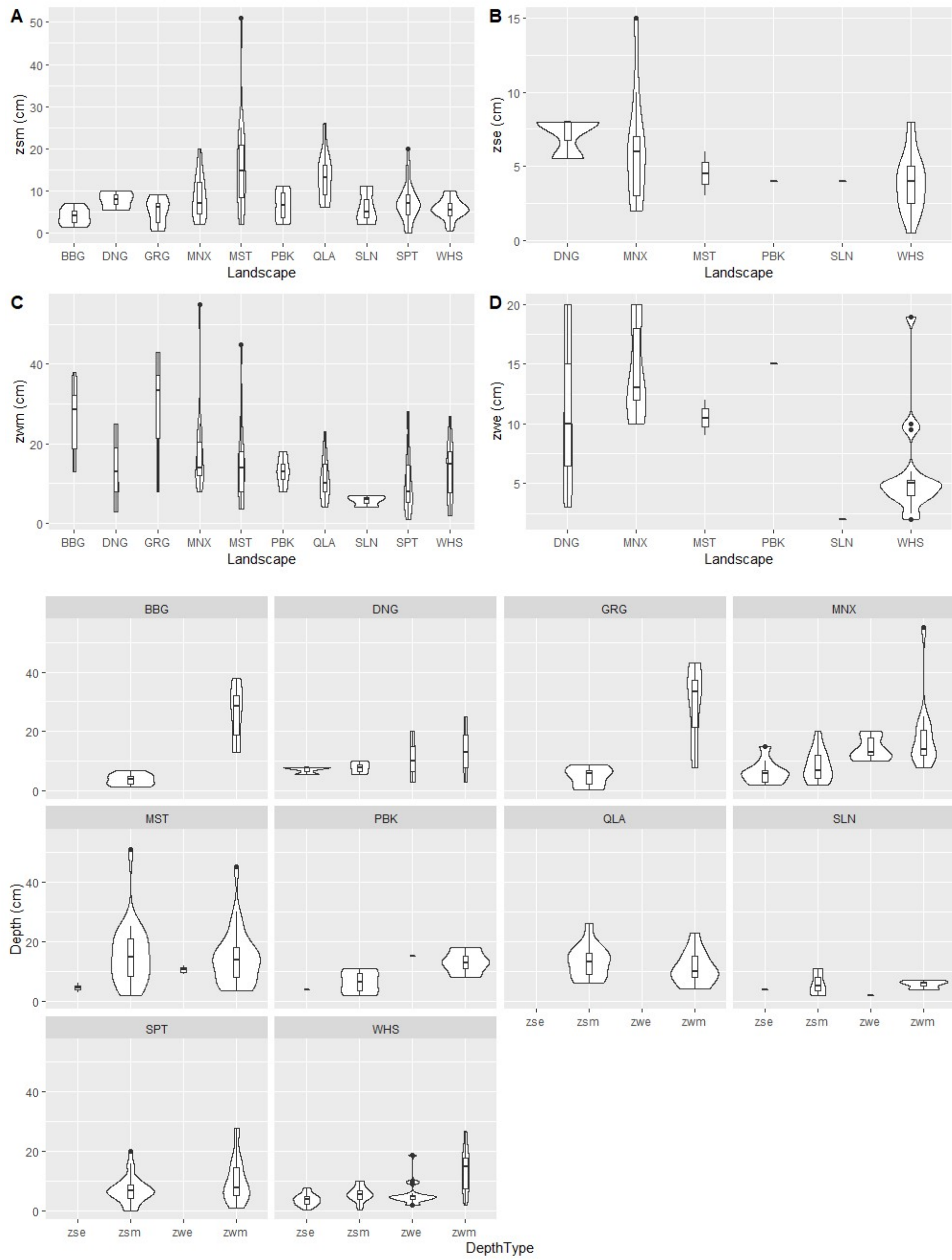

Figure 28: Water and sediment depths for pools across pool landscapes (A-D, with landscape on the x axis) and within pool landscapes (faceted graph with landscape as grouping variable). Codes: maximum basin water and sediment depths (zwm and zsm), maximum water and sediment depths at which *Elatine gussonei* is present (zwe and zse). Landscapes codes: Birżebbuġa (BBG), Ħad-Dingli (DNG), Għargħur (GRG), Munxar (MNX), Mosta (MST), Pembroke (PBK), Qala (QLA), Salini (SLN), San Pawl tat-Tarġa (SPT) and Wied Ħas-Saptan (WHS)

3.3.2 Water Quality

The water quality parameters measured during the study and utilised for analyses were pH, Electrical Conductivity (EC) and Oxidation Reduction Potential (ORP). These are summarised in Figure 29 – Figure 31 to visualise any landscape effects. The pH of the water in the study pools (Figure 29) ranged from 7.31 to 10.69 with a mean of 8.80 ±0.73, while the EC of the water in the study pools (Figure 30) ranged from 9 to 1552 µS/cm with a mean of 436 ±248.48 µS/cm. ORP is being reported from temporary freshwater rockpools in the Maltese Islands for the first time in this study. The ORP in the study pools (Figure 31) ranged from -6.6 to 267.20 mV with a mean of 182.2 ±41.46 mV, which are comparable to those measured by Liu et al. (2018); Yavuzatmaca et al. (2018).

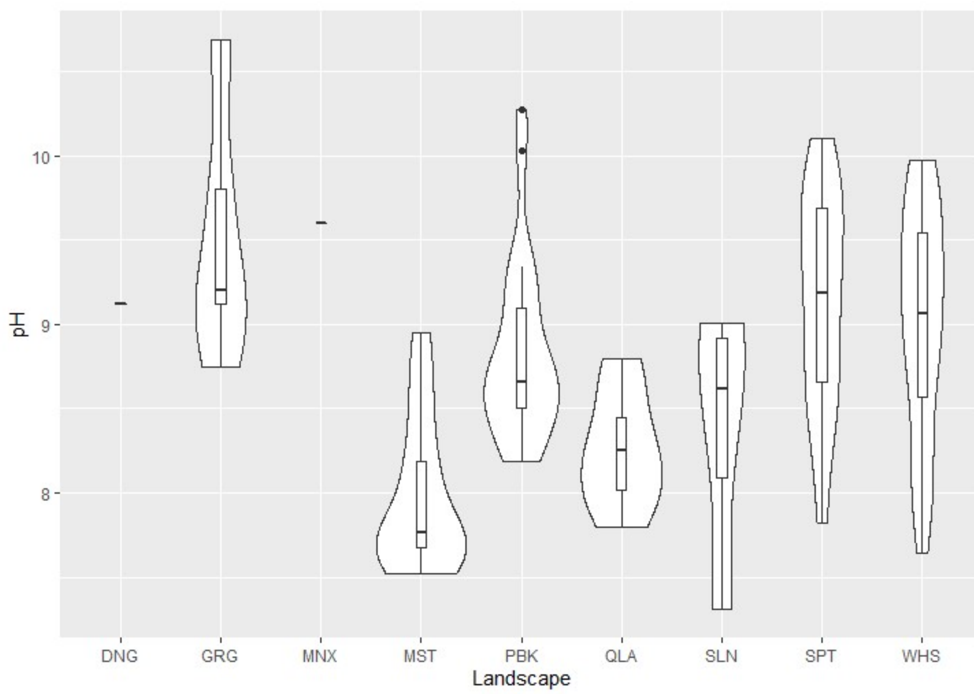

Figure 29: Violin plot of variation in pH across Landscapes. Codes: Ħad-Dingli (DNG), Għargħur (GRG), Munxar (MNX), Mosta (MST), Pembroke (PBK), Qala (QLA), Salini (SLN), San Pawl tat-Tarġa (SPT) and Wied Ħas-Saptan (WHS)

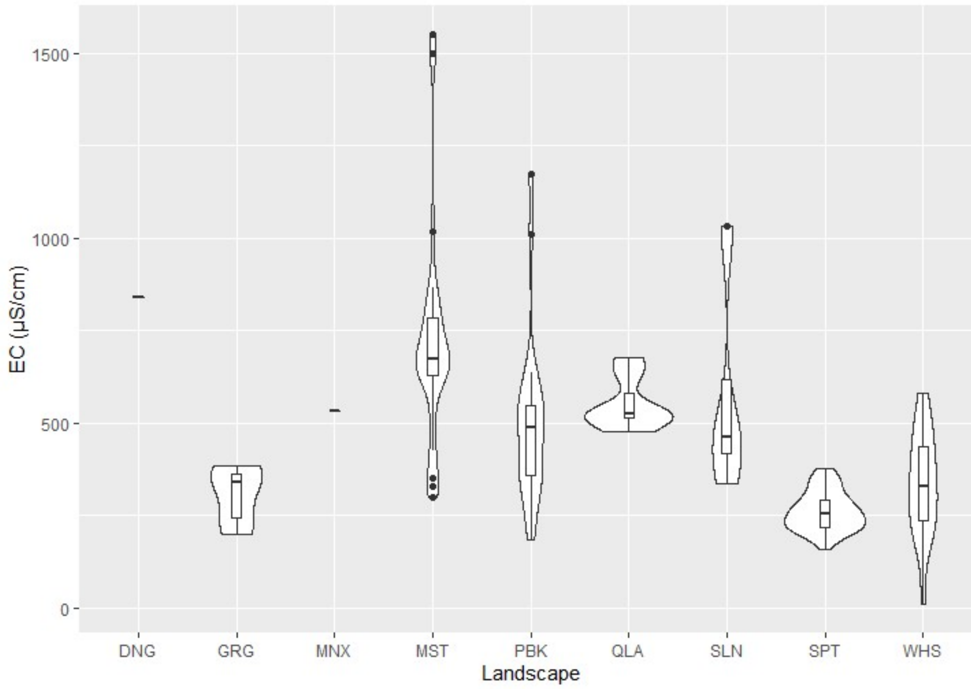

Figure 30: Violin plot of variation in Electrical Conductivity (µS/cm) across Landscapes. Codes: Ħad-Dingli (DNG), Għargħur (GRG), Munxar (MNX), Mosta (MST), Pembroke (PBK), Qala (QLA), Salini (SLN), San Pawl tat-Tarġa (SPT) and Wied Ħas-Saptan (WHS)

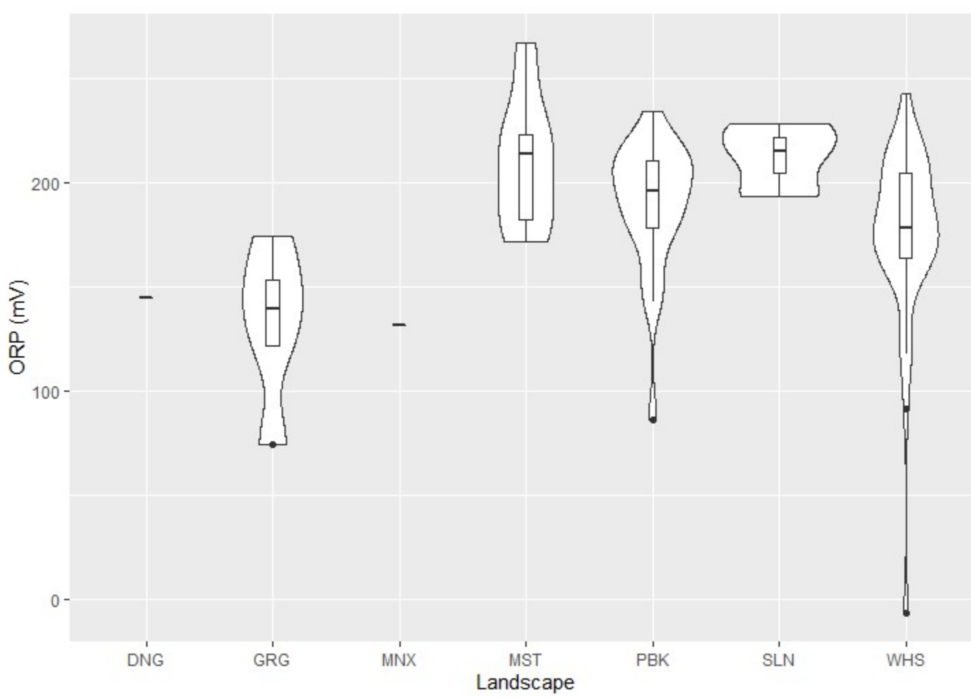

Figure 31: Violin plot of variation in Oxidation Reduction Potential (mV) across Landscapes. Codes: Ħad-Dingli (DNG), Għargħur (GRG), Munxar (MNX), Mosta (MST), Pembroke (PBK), Salini (SLN), and Wied Ħas-Saptan (WHS)

3.4 Data Exploration & Visualisation

As described in the previous chapter, the environmental factors measured during this study included basin morphometry and water chemistry. Data were analysed and compared based on the presence or absence of *E. gussonei* in a given pool.

3.4.1 Morphometry

Pool basin dimensions and sediment and water depth for pools with and without *E. gussonei* are summarised in Table 3 and visualised in Figure 32 – Figure 37.

Table 3: Minimum, Maximum, Mean, and standard deviations of morphometric parameters and water and sediment depths for pools with and without *Elatine gussonei*. Codes: SA = Surface Area (cm^2); SAV = Surface Area to Volume Ratio (cm^{-1}); zsm = Maximum Sediment Depth (cm); zwm = Maximum Water Depth (cm); zse = Maximum *Elatine* Sediment Dept (cm); and zwe = Maximum *Elatine* Water (cm)

Morphometric Parameter	**Minimum**		**Maximum**		**Mean (±SD)**	
Presence/Absence of *Elatine gussonei*	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**
SA (cm^2)	50.3	541.9	3300000	96,956.7	5070.07 (±7695.69)	9302.71 (±13297.75)
SAV (cm^{-1})	0.03	0.02	0.5	1	0.16 (±0.12)	0.11 (±0.14)
zwm (cm)	2	1	30	55	11.1 (±6.9)	16.4 (±10.8)
zwe (cm)	2		20		9.2 (±5.8)	
zsm (cm)	0.5	0.2	23	51	7.8 (±5.2)	9.3 (±7.8)
zse (cm)	0.5		15		4.7 (±2.7)	

The scatterplots with trendlines and linear regression in Figure 32 – Figure 33 show trends between basin morphometry of pools with and without *E. gussonei*, as well as the specific morphometric depths at which the species is present when compared to the basin maxima.

The scatterplot in Figure 32 (left) shows a negative correlation for SA and zwm in pools with and without *E. gussonei*, whereby the latter is almost zero. However, SA and zwe (right) are positively correlated.

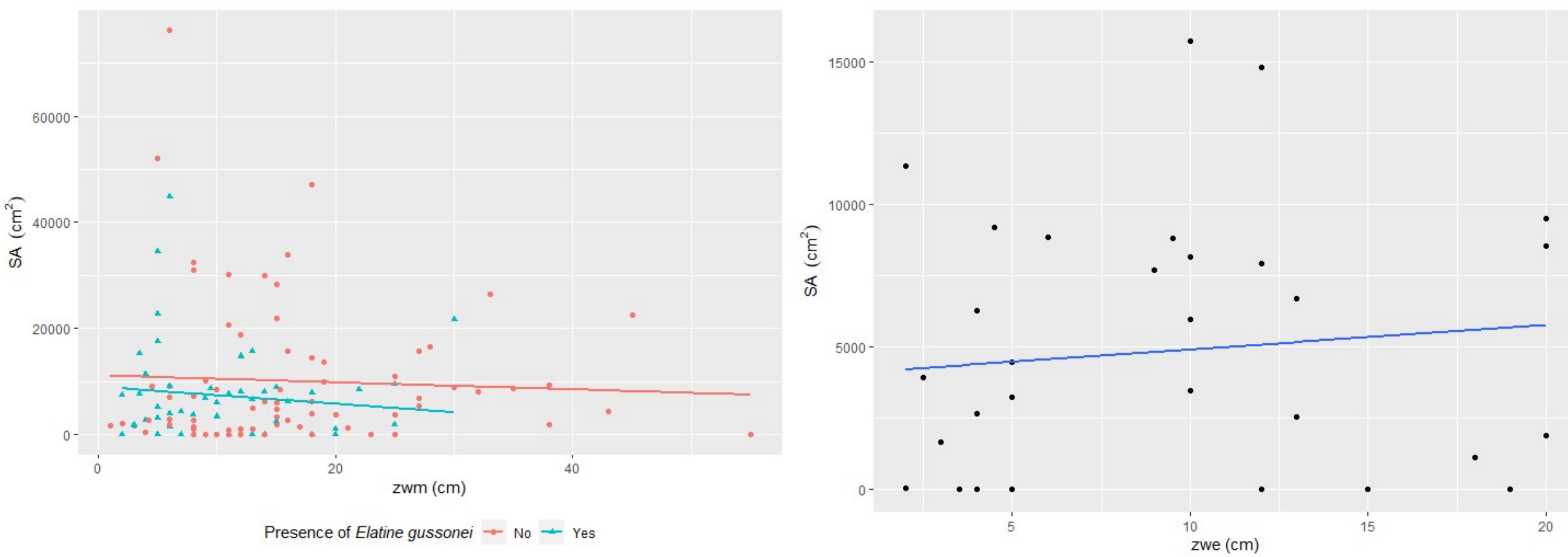

Figure 32: Scatterplot and superimposed trendline of Surface Area (cm²) against Maximum Water Depth (zwm) for pools with and without *Elatine gussonei*, (R^2 = -0.00589 and -0.1096; p = 0.4008 and 0.6577; df = 47 and 73 respectively (left), compared to Surface Area (cm²) against Maximum Water Depth at which *Elatine gussonei* is present (zwe), where R^2 = -0.02087, p = 0.5497, df = 30 (right)

The scatterplot in Figure 33 (left) shows a negative correlation for zwm and zsm in pools without *E. gussonei*, but a positive correlation for pools with *E. gussonei*, with the gradient being almost double. The scatterplot in Figure 33 (right), however, shows that zse and zwe are also positively correlated, with a gradient almost 4 times that of zwm and zsm for pools with *E. gussonei*.

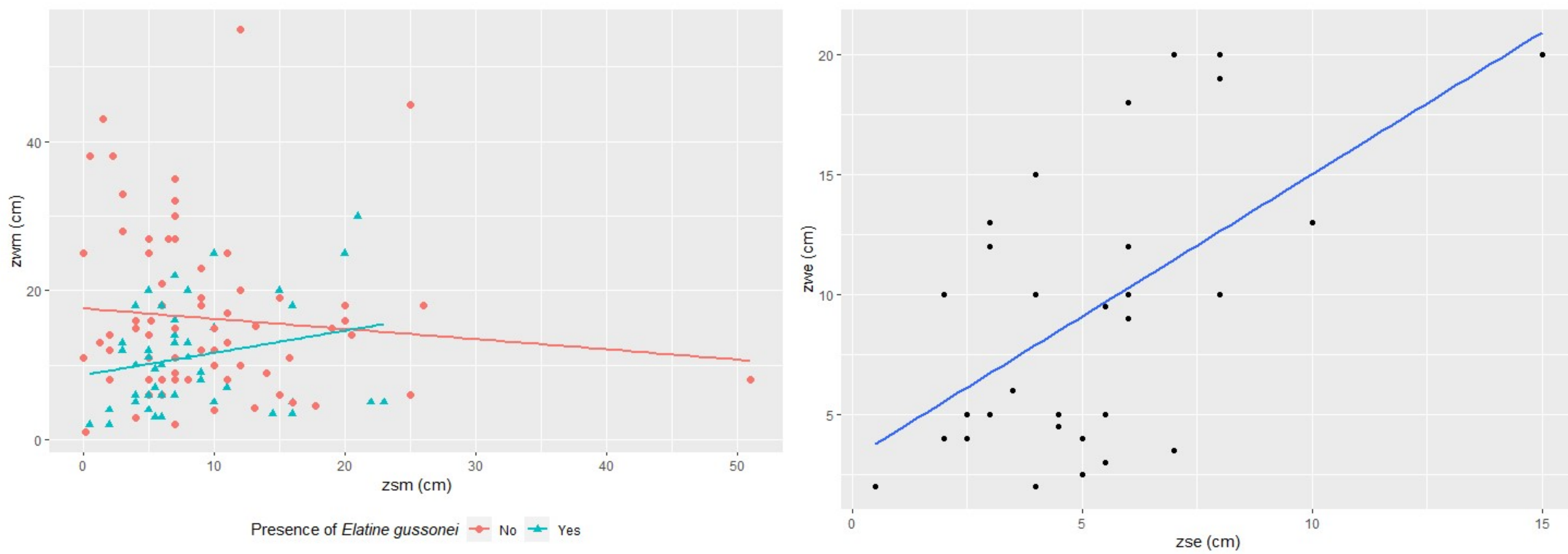

Figure 33: Scatterplot and superimposed trendline of Maximum Water Depth (zwm) (cm) against Maximum Sediment Depth (zsm) (cm) for pools with and without *Elatine gussonei* (R^2 = 0.03362 and -0.004096; p = 0.1138 and 0.4048; df = 45 and 72 respectively) (left), and Maximum Water Depth (zwe) (cm) against Maximum Sediment Depth (zse) (cm) at which *Elatine gussonei* is present (R^2 = 0.3068, p = 0.0005973, df = 30) (right)

The lm in Figure 34 shows an overall positive correlation between maximum and actual water and sediment depths, with $p<0.05$, indicating that zwe and zse are dependent on zwm and zsm respectively. The equations for the regression lines for water and sediment depth are:
zwe = 0.5603 + 0.6668zwm and zse = 1.75079 + 0.46732zsm respectively.

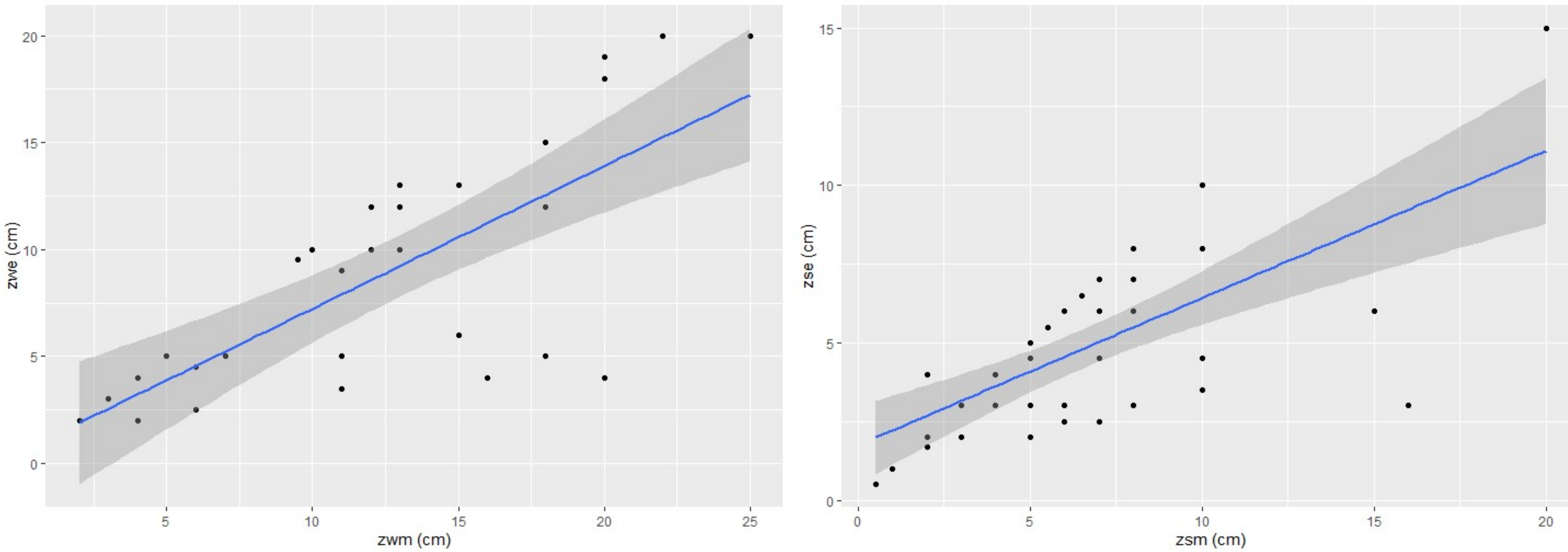

Figure 34: Linear regression with 95% confidence interval for the maximum morphometric water and sediment depths at which *Elatine gussonei* is actually present (zwe (cm) (left) and zse (cm) (right)), against the maximum morphometric water and sediment depths in pools with *Elatine gussonei* (zwm (cm) (left) and zsm (cm) (right)), whereby y~x in the linear model, with R^2 = 0.54, p = 1.6e-06, df = 30 (left) and R^2 = 0.44, p = 1.09e-06, df = 41 (right)

The equations obtained from Figure 34 are not biologically possible for zm values below 1.68 (zwm) and 3.29 (zsm), at which zm and ze are at a minimum of a 1:1 ratio. As a result, the model was rerun, constraining the y intercept to 0. This was done so that zm could never exceed ze, as was the case in the lm in Figure 34 for zm values below 1.68 (zwm) and 3.29 (zsm). The subsequent equations obtained from the regressions for water and sediment depth in Figure 35 with y-intercept as c = 0, are zwe = 0.70199zwm and zse = 0.66974zsm respectively. This ensures that ze can never exceed zm, rendering it biologically feasible.

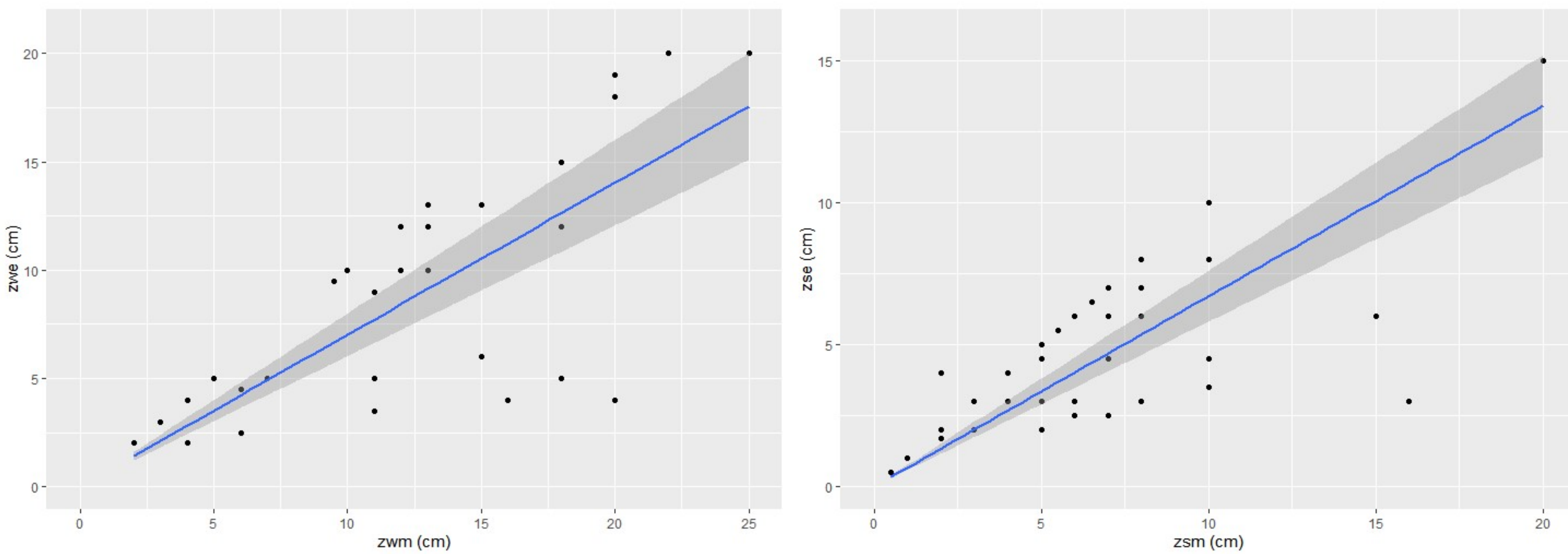

Figure 35: Linear regression with y intercept = 0 and 95% confidence interval for the maximum morphometric water and sediment depths at which *Elatine gussonei* is actually present (zwe (cm) (left) and zse (cm) (right)), against the maximum morphometric water and sediment depths in pools with *Elatine gussonei* (zwm (cm) (left) and zsm (cm) (right)), whereby y~x in the linear model, with R^2 = 0.87, p = 2.21e-15, df = 31 (left) and R^2 = 0.84, p = 2.2e-16, df = 42 (right)

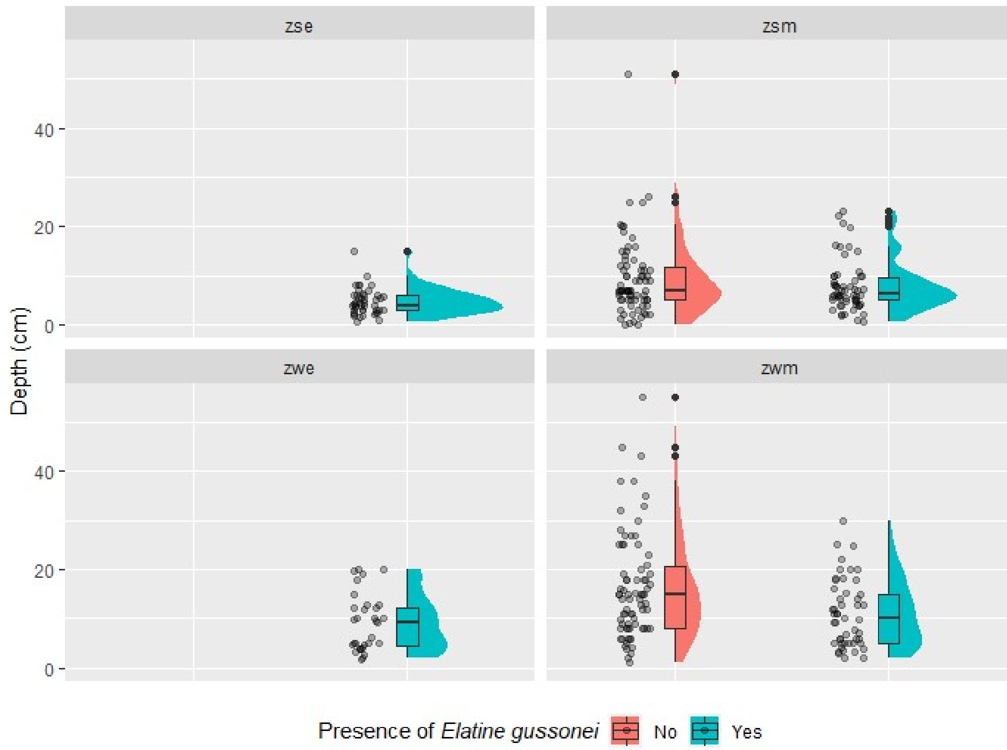

Figure 36: Maximum morphometric sediment and water depths (cm) for pools with and without *Elatine gussonei* (zsm and zwm respectively), compared with the maximum sediment and water depths at which *E. gussonei* is actually present in a given pool (zse and zwe respectively). Codes: zse = Maximum *Elatine* Sediment Dept; zsm = Maximum Sediment Depth; zwe = Maximum *Elatine* Water Depth; and zwm = Maximum Water Depth

The distribution of basin maximum water and sediment depths (zwm and zsm respectively) for pools with and without *E. gussonei* are visualised in Figure 36, along with the range and distribution of the actual maximum depths at which *E. gussonei* is present in a given pool. Pools with *E. gussonei* have lower maximum zm depths (zwm = 30cm, zsm = 23cm) than pools without *E. gussonei* (zwm = 55cm, zsm = 51 and 26cm), but are higher than the ze maximum depths (zwe = 20cm, zse = 15cm).

3.4.2 Water Quality

Water quality of pools in each landscape is depicted in Figure 37 and compared between pools with and without *E. gussonei*. A summary of each water quality parameter for pools with and without *E. gussonei* is summarised in Table 4.

Table 4: Minima, Maxima, and Mean and standard deviations of water chemistry parameters for pools with and without *Elatine gussonei*. Where EC = Electrical Conductivity (µS/cm) and ORP = Oxidation Reduction Potential (mV)

Water Quality Parameter (Unit)	**Minimum**		**Maximum**		**Mean (±SD)**	
Presence/Absence of *Elatine gussonei*	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**
pH	7.31	7.52	10.03	10.69	8.82 (±0.7)	8.78 (±0.8)
EC (µS/cm)	9	158	1552	1171	430.78 (±266.1)	441.56 (±230.2)
ORP (mV)	-6.6	74.3	230.9	267.2	181.98 (±39.8)	182.52 (±45.0)

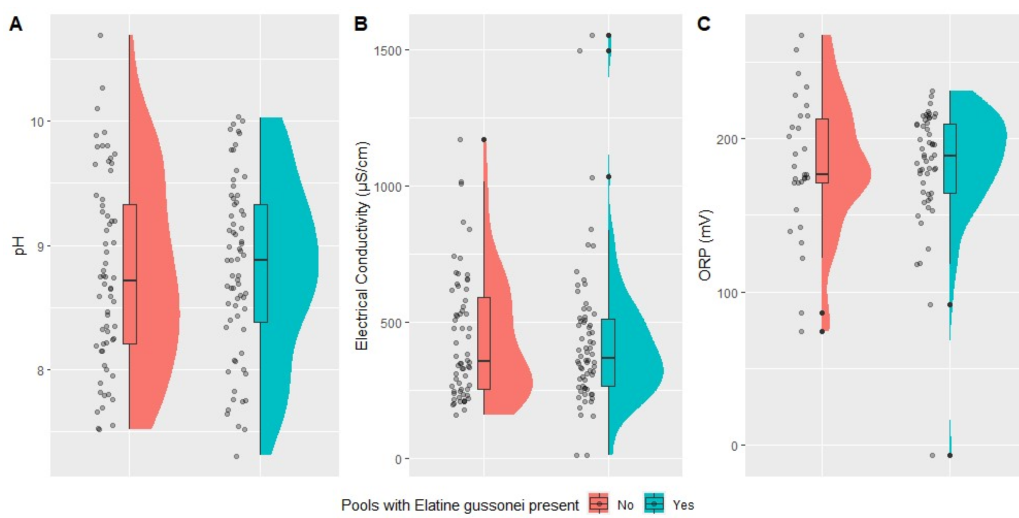

Figure 37: Raincloud plots of water quality in pools with and without *Elatine gussonei*, where EC = Electrical Conductivity (µS/cm) and ORP = Oxidation Reduction Potential (mV).

From Figure 37, means and ranges of water quality for pH, EC and ORP in pools with and without *E. gussonei* all appear to be comparable. Means and distributions for pH are almost identical, with pools having *E. gussonei* appearing to be slightly more tolerant to lower pH values, and less so at higher values. Pools with *E. gusseonei*, however, seem to have a slightly higher EC and ORP averages, with lower maxima as indicated by the boxplots and violin plots. In the case of pools with *E. gussonei*, the majority of pools appear to be clustered at a slightly higher value for all three water quality parameters, as is visible in the violin plots and point cloud density. Therefore, while pools without *E. gussonei* may have and be more tolerant to higher values of pH, EC and ORP, most pools are clustered at a lower value than those in which the species is present.

3.4.3 Pool Flora

Figure 38 summarises the correlation between resident pool flora in terms of presence/absence and excludes terrestrial species. Positive correlations are in blue while negative correlations are in red, whereby the greater the correlation, the larger the circle. Correlations that are significant with a p value less than 0.05 are highlighted and isolated in grey boxes.

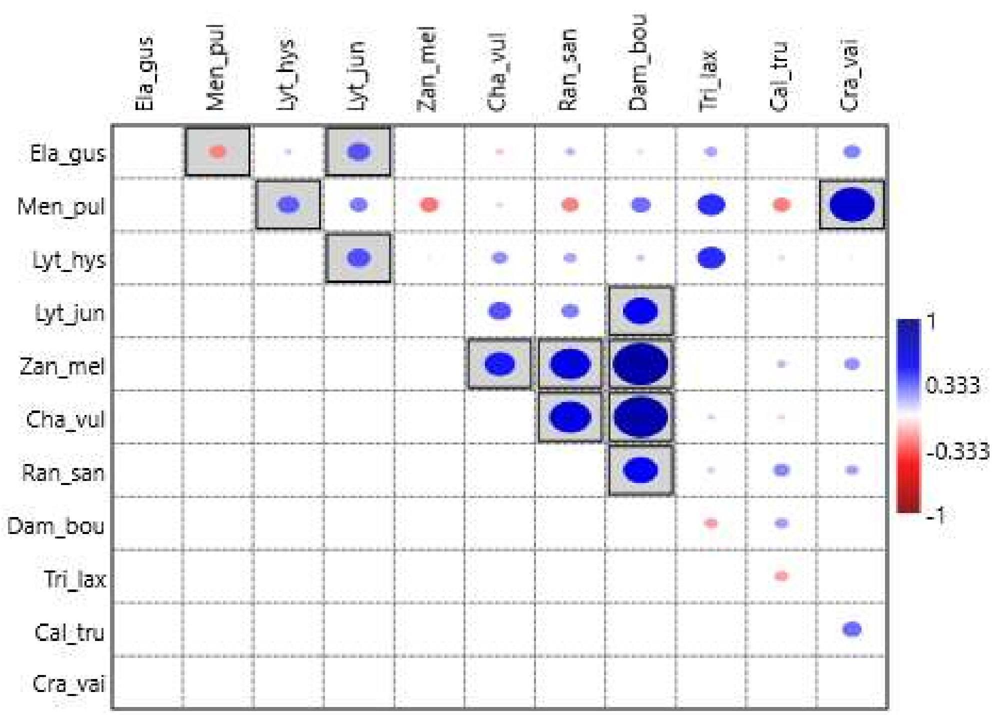

Figure 38: Correlogram species matrix based on presence-absence data, whereby positive correlations are blue and negative correlations are red, and only the significant correlations where p <0.05 are isolated in boxes.

3.4.4 Environmental Factors

The plot in Figure 39 shows the ordination of pools with respect to environmental factors, and with the presence of *E. gussonei* as the grouping variable. The horizontal axis is termed 'Axis 1' and represents the direction of maximum variability in the data. 'Axis' 2 is perpendicular to and independent of Axis 1. In this analysis, Axis 1 explains 8.02% of the variability within the data. Axis 1 encompasses pH, EC, SA and zsm. SAV and zwm on Axis 2 account for the remaining 4.23%. Pools with *E. gussonei* are seen to have high values on the vectors representing high SAV and low zwm.

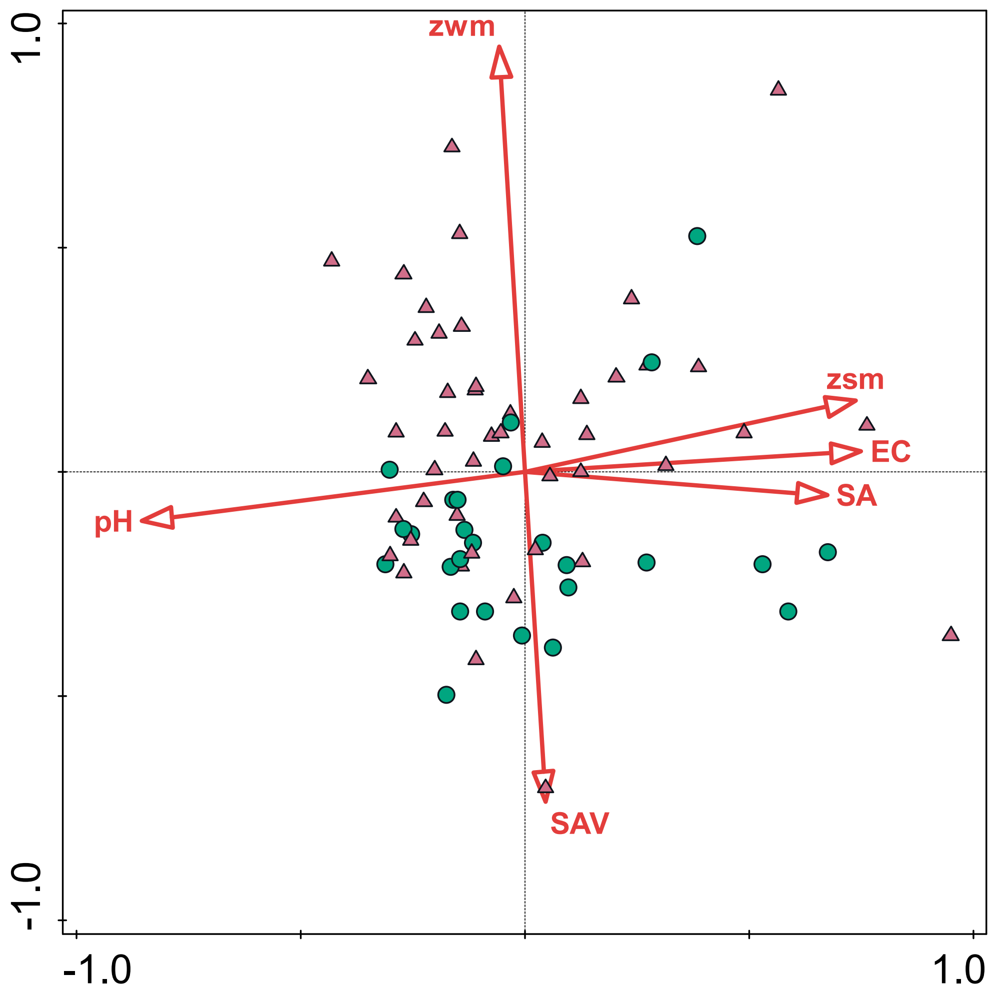

Figure 39: CCA biplot of environmental factors and presence-absence points of *Elatine gussonei*, with axes 1 and 2 explaining 8.02% and 4.23% of the variability within the data respectively. Codes: Surface area (SA), Surface area to volume ratio (SAV), maximum sediment depth (zsm), maximum water depth (zwm), Electrical Conductivity (µS/cm) (EC). Green circles and pink triangles correspond to pools with and without *E. gussonei* respectively.

The plot in Figure 40 summarises the multivariate relationship between the physical and chemical factors, and plant species observed in the basin, with species being constrained by environmental factors. While vectors in Figure 40 correspond to the same Axes as in Figure 39, Axes 1 and 2 account for 10.35% and 3.63% of the variability within the data respectively in Figure 40. Here, zwm appears to be the main controlling factor that distinguishes hydroperiod for aquatic, amphibious and terrestrial species. More terrestrial species are favoured by high SA and zsm, with aquatic species favouring higher zwm, and lower SA and zsm. Basins with low SA, high zwm and high SAV result in high evaporation rate, which provides a more 'terrestrial' environment that is further accentuated by higher zsm.

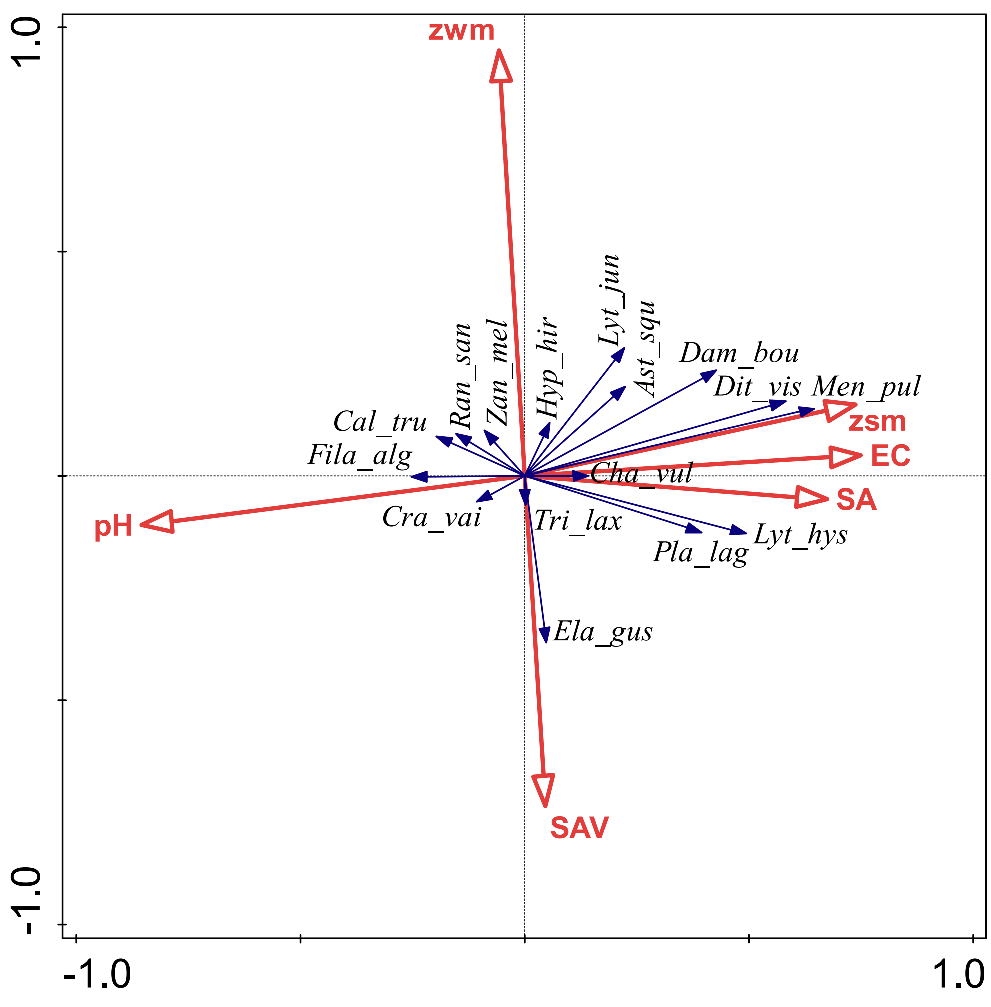

Figure 40: RDA biplot of species and environmental vectors, with Axes 1 and 2 explaining 10.35% and 3.63% of the variability within the species data respectively. Codes: Surface area (SA), Surface area to volume ratio (SAV), maximum sediment depth (zsm), maximum water depth (zwm), Electrical Conductivity (µS/cm) (EC).

The correlogram in Figure 41 provides more detail than correlogram in Figure 38 by incorporating environmental factors, and the RDA biplot in Figure 40 by including the extent and significance of correlations between species and environmental factors. Out of all 7 factors visualised, *E. gussonei* is negatively correlated with 4 (DO, SA, zsm and zwm), of which only zwm is significant.

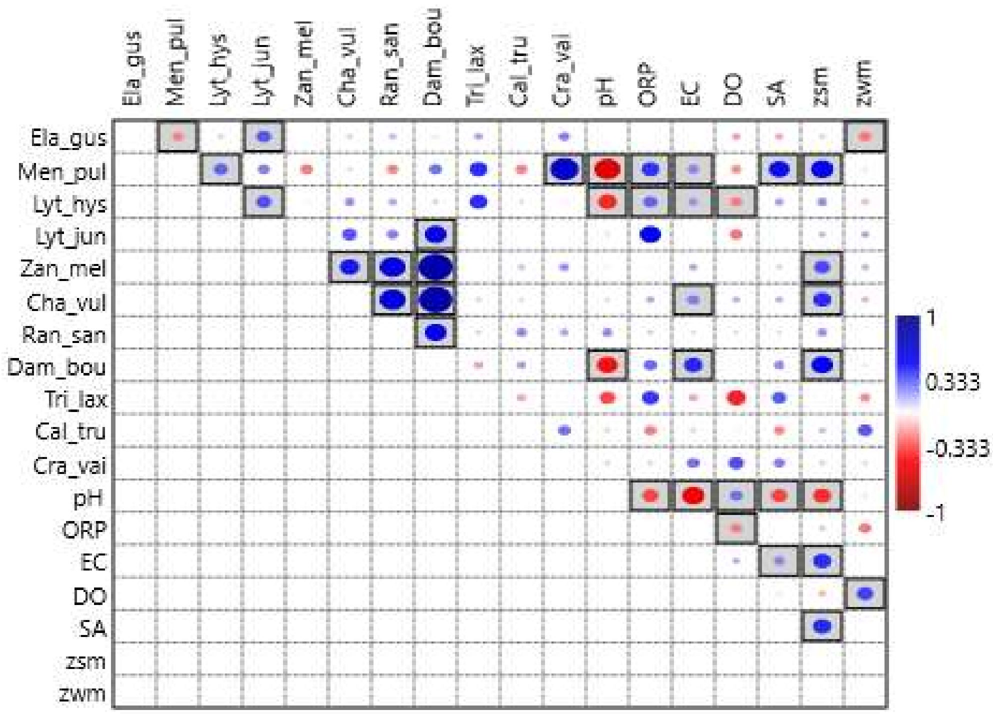

Figure 41: Correlogram matrix of species and environmental factors based on presence-absence data, whereby positive correlations are blue and negative correlations are red, and only the significant factors with a p value of <0.05 are isolated in boxes.

3.5 DATA ANALYSIS

To test the influence of morphometric factors and water chemistry on the presence of *E. gussonei* in a given pool, a binomial glm regression was constructed. T-tests were used to compare means for statistically significant (<0.05) differences between independent means of maximum morphometric basin depths, and the specific maximum depths at which *E. gussonei* was present (zm v ze).

3.5.1 Morphometry

The means of the basin morphometric parameters for the presence and absence of *E. gussonei* are summarised and compared in Table 5, and are compared via glm as described in section 2.4. Combined predictors for larger models were also investigated, but none were found to be statistically significant. Therefore, only the main and significant morphometric parameters are noted in Table 5.

Pool surface area and maximum basin water depth were found to be the only morphometric factors to significantly affect the presence of *E. gussonei* in a given pool due to their influence on pool hydroperiod. The resultant glms for pool surface area and maximum basin water depth were subsequently plotted and visualised in Figure 42 and Figure 43 respectively.

Table 5: Minimum, Maximum, Mean, and standard deviations of basin morphometry and water and sediment depths for pools with and without *Elatine gussonei*. Codes: SA = Surface Area (cm^2); Surface Area to Volume Ratio = SAV (cm^{-1}); zsm = Maximum Sediment Depth (cm); zwm = Maximum Water Depth (cm); zse = Maximum *Elatine* Sediment Dept (cm); and zwe = Maximum *Elatine* Water (cm), along with values of significant differences between groups and their respective degrees of freedom

Morphometric Parameter	**Mean (±SD)**		**glm**	
Presence/Absence of *Elatine gussonei*	**Present**	**Absent**	**p**	**df**
SA (cm^2)	5070.071(±7695.69)	9302.71 (±13297.75)	**0.0202**	164
SAV (cm^{-1})	0.16 (±0.12)	0.11 (±0.14)	0.10740	98
zwm (cm)	11.09 (±6.87)	16.44 (±10.81)	**0.00438**	122
zsm (cm)	7.84 (±5.20)	9.27 (±7.78)	0.236	130

Figure 42 and Figure 43 indicate the binomial distribution of *E. gussonei* dependent on Surface Area (cm^2) and water depth respectively. Since the results are constrained to be binomial as species presence or absence, depending on a morphometric factor, the result obtained from the glm equation gives a probabilistic result. Therefore, the resultant plots indicate that independent values which yield a y-axis value ≥0.5 will likely result in the presence of *E. gussonei* in a pool.

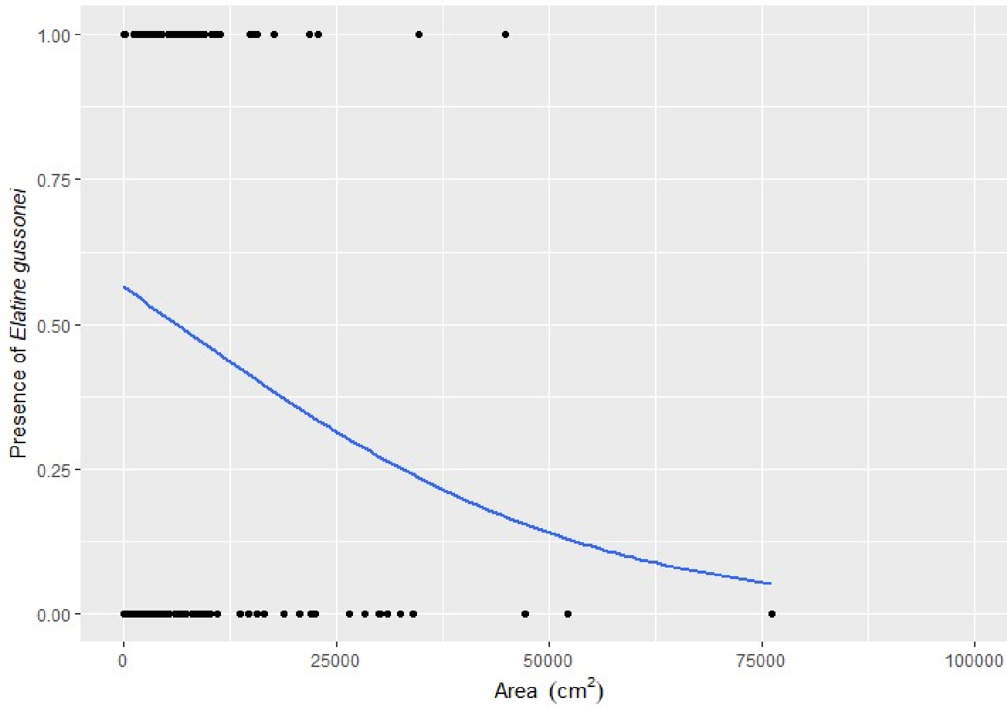

Figure 42: Binomial generalized linear model (glm) of presence/absence of *Elatine gussonei* at a given basin surface area (cm²)

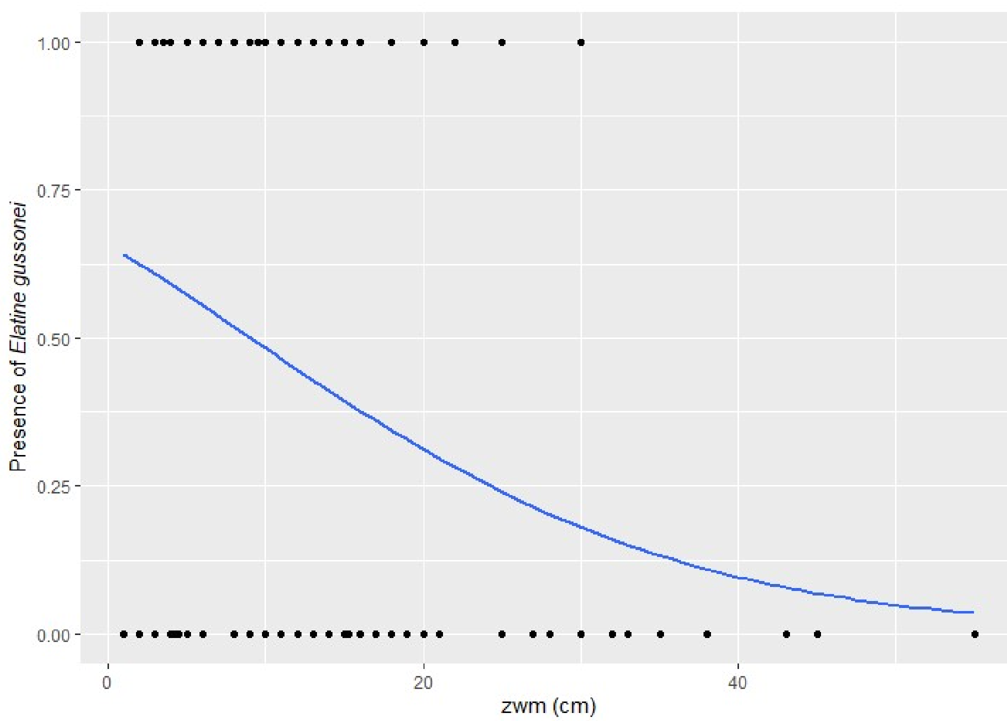

Figure 43: Binomial generalized linear model (glm) of presence/absence of *Elatine gussonei* at a given maximum basin morphometric water depth (zwm)

The results of the tests carried out on the data visualised in Figure 36 are noted in Table 6. These clearly indicate that there is a statistically significant difference between the maximum water depth (zwm) as a predictor of pools with and without *E. gussonei* ($p<0.01$). Maximum basin water and sediment depths (zwm and zsm) at which *E. gussonei* is present were also significant predictors of the actual depths at which the species present (zwe and zse) ($p<0.001$ and $p<0.000001$ respectively).

Table 6: t-test and glm results for pool morphometry where: Surface Area (SA); Maximum Sediment Depth (zsm); Maximum Water Depth (zwm); Maximum *Elatine* Sediment Dept (zse); and Maximum *Elatine* Water Depth (zwe)

Parameter/s	Test	p	df
SA for pools with *E. gussonei* **v** **SA for pools w/o** *E. gussonei*	**glm**	**0.0202**	164
SAV for pools with *E. gussonei* **v** **SAV for pools w/o** *E. gussonei*	glm	0.10740	98
zsm for pools with *E. gussonei* v **zsm for pools w/o *E. gussonei***	glm	0.2360	130
zsm (for pools with *E. gussonei*) v zse	**t-test**	**9.843e-08**	171.91
zwm for pools with *E. gussonei* v **zwm for pools w/o *E. gussonei***	**glm**	**0.00438**	122
zwm (for pools with *E. gussonei*) v zwe	**t-test**	**0.0002403**	82.508

The resultant analyses of the data depicted in indicate that sediment to water depth ratios do not contribute significantly to the presence of *E. gussonei* as data analysis on the data indicate p values greater than 0.05 in Table 7.

Table 7: t-test and glm results for pool morphometry ratios where: Maximum Sediment Depth (zsm); Maximum Water Depth (zwm); Maximum *Elatine* Sediment Dept (zse); and Maximum *Elatine* Water Depth (zwe)

Parameter/s	Test	p	df
zsm:zwm for pools with *E. gussonei* v **zsm:zwm for pools w/o *E. gussonei***	glm	0.4485	122
zsm:zwm (for pools with *E. gussonei*) v **zse:zwe**	t-test	0.09154	72.404

3.5.2 Water chemistry

The resultant analysis of the data shown in Figure 37, indicate that none of the water quality parameters have a statistically significant effect ($p>0.05$) on the presence or absence on the target species; *E. gussonei*. As a result, none of the water chemistry parameters were included in the predictive model.

Table 8: glm results of water chemistry for pools with and without *Elatine gussonei*. Codes/Where: *E. gussonei* = *Elatine gussonei*; EC = Electrical Conductivity (µS/cm), ORP = Oxidation Reduction Potential (mV) / Summary of water chemistry parameters for pools with and without *Elatine gussonei*, and significant difference z value between the two groups

Water Quality Parameter (Unit)	**Mean (±s.d.)**		**glm**	
Presence/Absence of *E. gussonei*	**Present**	**Absent**	**p**	**df**
pH	8.82 (±0.704)	8.84 (±0.770)	0.856	123
EC (µS/cm)	431 (±266)	430.8 (±230)	0.95	123
ORP (mV)	182 (±39.8)	182 (±45)	0.954	82

3.5.3 Pool Flora

The correlogram in Figure 38 indicates that *E. gussonei* is positively correlated with the presence of *Lythrum hyssopifolia*, *L. junceum*, *Ranunculus saniculifolius*, *Triglochin laxiflora*, and *Crassula vaillianti*. It is also shown to be negatively correlated with *Mentha pulegium* and *Chara vulgaris*. From the resident pool flora, only *M. pulegium* and *L. junceum* were found to be significantly negatively and positively correlated ($p<0.05$) with *E. gussonei* respectively.

The correlogram in Figure 41 however gives a broader picture by incorporating all environmental abiotic factors. It is indicated that the presence of *E. gussonei* also appears to be negatively correlated with Dissolved Oxygen concentration (%), surface area, and maximum basin water and sediment depth. However, of the aforementioned factors, only maximum basin water depth was found to have a statistically significant effect. Figure 40 also gives further insight into species life-forms from surface area and maximum basin water and sediment depths. Aquatic species are shown to be significantly positively correlated with higher zwm, while more terrestrial species are more significantly positively correlated with higher surface areas and zsm, while being negatively correlated with zwm.

3.6 SUMMARY

The results in Table 3 and Table 4 indicate the morphometric and water quality tolerance ranges at which *E. gussonei* is present. The data analysis summarised in Table 6 – Table 8 indicated that only 2 morphometric factors had a significant effect on the presence of *E. gussonei* in a given pool (dependent on SA and zwm via glm), with 2 other factors narrowing down the tolerance range of the species (zwe and zse via lm, dependent on zwm and zsm).

Glms indicated that basin surface area (SA) and maximum basin morphometric depth (zwm) were statistically significant influences and were therefore used to model the occurrence of *E. gussonei* (p=0.0202, df=164, and p=0.00344, df=115 respectively). The rationale of the significance of these factors is that pool hydroperiod is dependent on them. Basin surface area to volume ratio, however, was not found to significantly affect the presence of the species (p=0.10740, df=98), though this may also be due to the smaller sample size available.

The ratio of basin maximum water to sediment depth was not found to be statistically significant in pools with *E. gussonei* when compared to those without (glm p=0.4485, df=122). However, the ratio of maximum basin water to sediment depth when compared to the actual ratio of water to sediment depth at which *E. gussonei* is present, though not statistically significant (t-test p=0.09154, df=72.404), given more data may actually prove to be significant.

t-tests comparing maximum basin water (zwm) and sediment depths (zsm) with the actual water (zwe) and sediment depths (zse) at which *E. gussonei* was present were also found to be statistically significant (p=2.4e-403, df=82.508, and p=9.843e-08, df=171.91 respectively). However, while zwe and zse were not included in the glms, they were found to be statistically significant and correlated when conducting linear regressions (df = 30, p = 1.6e-06, and df = 41, p= 1.09e-06 for maxima and actual water and sediment depths respectively). Therefore, zwe and zse are to be taken into account when considering the depth ‘tolerance ranges’ of the species during the species modelling process.

3.7 MODELLING SPECIES OCCURRENCE

Data visualisation and subsequent analyses assisted in the selection of factors to be utilised in the modelling of the occurrence of *E. gussonei* in a given pool. Only factors with a significance of 95% or greater ($p<0.05$) were utilised. Preliminary equations obtained from glms and lms are summarised in Equation 1 – Equation 6.

Equation 1: Binomial general linearized model (glm) equation corresponding to Figure 42, where EG is the dependent response variable for the presence of *Elatine gussonei*, and must be constrained to 0 or 1, and SA is the basin surface area in cm^2

$$EG = \frac{\exp\left(-3.1934e^{-5}SA\right)}{1 + \exp\left(-3.1934e^{-5}SA\right)}$$

Equation 2: Binomial general linearized model (glm) equation corresponding to Figure 43, where EG is the dependent response variable for the presence of *Elatine gussonei*, and must be constrained to 0 or 1, and zwm is the maximum basin water depth in cm

$$EG = \frac{\exp\left(-0.070276zwm + 0.5171\right)}{1 + \exp\left(-0.070276zwm + 0.5171\right)}$$

It should be noted that the dependent response y variable (presence of *E. gussonei*) in Equation 1 and Equation 2 are expressed as a probability, and must be constrained to be either 0 or 1. This is due to the binary response of species presence-absence for the glm. It is also worth noting that for Equation 2, while *E. gussonei* may be present in pools with given maximum water and sediment depths (zwm and zsm), the actual depths at which the species is present (zwe and zse) are dependent on the aformentioned maxima.

The relationship between the actual dependent depths (zm) and independent maxima (ze) are summarised in the linear regressions in Equation 3 and Equation 4 for water and sediment depths respectively.

Equation 3: Linear regression (lm) equation corresponding to Figure 34, where zwe is the water depth at which *Elatine gussonei* is present, while zwm is the maximum basin water depth

$$zwe = 0.5603 + 0.6668zwm$$

Equation 4: Linear regression (lm) equation corresponding to Figure 34, where zse is the sediment depth at which *Elatine gussonei* is present, while zsm is the maximum basin sediment depth

$$zse = 1.75079 + 0.46732zsm$$

However, it is worth noting that unless zm≥ze, values below 1.68 and 3.29 for zwm and zsm respectively, zwe and zsm values do not make sense biologically. This is because the positive y-intercept implies that if zm = 0cm, ze > 0cm.

Therefore, the regressions were carried out again (Figure 35), but this time with the intercept was constrained to c = 0. The resultant equations for water and sediment depths are in Equation 5 and Equation 6 respectively.

Equation 5: Linear regression (lm) equation corresponding to Figure 35 with y-intercept constrained to be c = 0, where zwe is the water depth at which *Elatine gussonei* is present, while zwm is the maximum basin water depth

$$zwe = 0.70199zwm$$

Equation 6: Linear regression (lm) equation corresponding to Figure 35 with y-intercept constrained to be c = 0, where zse is the sediment depth at which *Elatine gussonei* is present, while zsm is the maximum basin sediment depth

$$zse = 0.66974zsm$$

4 DISCUSSION

Throughout this study, 173 pools from 10 pool landscapes were surveyed. Data collected included water quality, basin morphometry and species lists, whereby only 3 factors were found to significantly affect the occurrence and specific maximum depth tolerance range of the species in a pool. Only two factors (surface area – SA and maximum basin water depth – zwm) were subsequently used to model the occurrence of the species in a given pool via glm. The two factors that aided in determining the maximum water and sediment depth at which the species is actually present in a given pool (zwe and zse respectively) were maximum basin water depth and maximum basin sediment depths (zwm and zsm respectively). However, this is only possible after having confirmed the presence of *E. gussonei* in a pool via the aforementioned glms for surface area and maximum basin water depth.

Due to the relatively short wet season, such data was only collected for pools in landscapes which still retained enough water to obtain such measurements, and whose sediment layer was humid enough for a ruler to be inserted. This resulted in a smaller sample size of complete data on which to build the model. Dissolved oxygen (DO) (%) was not included in data visualisation or analysis since being a small water body, the water would be reoxygenated upon agitation caused by wind as well as insertion of the probe itself. As a result, the values may not reflect the actual situation, making it uninformative to include in analysis.

4.1 BIOTIC FACTORS

While maximum basin water depth is a good proxy for complete basin hydroperiod (Vanschoenwinkel, Hulsmans, et al., 2009), reduced rainfall may result in incomplete basin inundation. It is worth noting that while the maximum basin water depth of pools with *E. gussonei* was negatively correlated with surface area (Figure 32 left), the actual depth at which the species is present was positively correlated with basin surface area (Figure 32 right). This indicates that maximum basin water depth may not be an accurate representation of the hydroperiod experienced by the species.

If basins are not adequately inundated due to reduced rainfall, *E. gussonei* present in shallower parts of the pool may not germinate as their seeds would not have enough water. Incomplete inundation may therefore result in a decrease in maximum basin depth that fit with the tolerance range of *E. gussonei*. However, this is conditional on whether seeds of *E. gussonei* are present in the seedbank at said new depth in order for germination to occur and result in a shift in dominant pool life-forms.

From the correlogram in Figure 41, *E. gussonei* was shown to be positively correlated with amphibious and amphibious-aquatic species such as *Lythrum hyssopifolia* and *L. junceum*, *Ranunculus saniculifolius*, *Triglochin laxiflora* and *Crassula vailliantii*. Of these species, 3 are negatively correlated with maximum basin water depth, indicating an inclination towards shorter hydroperiods, and positive correlation with surface area. These observations indicate a preference of high surface area to volume ratio, which is characteristic of amphibious species. The presence of deeper sediments in pool basins, however, also allows for moisture retention within the rhizosphere. This subsequently promotes the completion of amphibious species' lifecycles (Lanfranco et al., 2020). Figure 41 also indicates a negative correlation of *E. gussonei* with more terrestrial obligate pool flora such as *Mentha pulegium*, and more aquatic flora and macroalgae such as *Damasonium bourgaei* and *Chara vulgaris*. The more terrestrial nature of *Mentha pulegium* is indicated by a significant positive correlation with surface area and zsm, and negative correlation with aquatic and amphibious-aquatic pool flora such as *Zaninchellia melitensis* and *Ranunculus saniculifolius*, which agree with the findings of Lanfranco et al. (2020).

Another possible cause that may result in a shift in pool life-form is infilling of soil. Such infiltration may result in a gradual encroachment of terrestrial species into amphibious 'territory'. While the range of maximum sediment depth at which *E. gussonei* was present was 0.5 – 15cm, germination success and burial depth are strongly negatively correlated. Deeper seed burial results in a sharp decrease in germination capacity due to the absence of environmental cues required to trigger germination (Galinato & van der Valk, 1986; Bonis & Lepart, 1994; Jurik et al., 1994). Jurik et al., (1994) state that sediment loads over as little as 0.25cm may significantly reduce species richness, and population abundance of individuals recruited from wetland seedbanks. Seed germination and subsequent seedling emergence from seed banks of such habitats, however, are dependent on sediment load. 22–98% of seeds that germinated in two temporary marshes in the Camargue were within the top 2cm of sediment (Bonis & Lepart, 1994). Rhazi et al., (2007) also stated that *Elatine brochonii* germination was impeded when loaded with >2cm of sediment. Therefore, germination is seldom recorded below 2cm burial depths (Bonis & Lepart, 1994; Galinato & van der Valk, 1986; Jurik et al., 1994, Rhazi et al., 2007).

The correlogram in Figure 41 indicates that higher sediment depths (zs) favour more terrestrial species such as *Mentha pulegium*, *Lythrum junceum* and *Lythrum hyssopifolia* (Lanfranco et al., 2020), which are both hemicryptophytes with the latter being a therophyte, as opposed to a hydrophyte. Their larger seeds and deeper root systems, therefore, allow them to better colonize and outcompete obligate pool hydrophyte species. Such flora also have higher transpiration rates that may further reduce hydroperiod duration (Lanfranco et al., 2020). This is especially so at higher surface areas which dry out quicker, indicating a more terrestrial environment. As regards water depth, the drying out of a pool, even if for a temporary period may eliminate certain competition to favour more amphibious species such as *Ranunculus saniculifolius*, *Damasonium bourgaei*, and *Elatine gussonei*, while eliminating obligate aquatics such as *Chara vulgaris*, *Zannichellia melitensis*, and *Callitriche truncata*.

Prior to establishment and germination, however, the seeds must first be present and have an established seed bank within the sediment. When it comes to species occurrence and presence of propagules within the seed bank, it is worth noting that pools with many close neighbouring populations promotes richness and increases likelihood of dispersal and chances of establishment and subsequently, occurrence. To this effect, dispersal between pools within landscapes has a greater effect on species occurrence in a given pool than dispersal between landscapes (Vanschoenwinkel et al., 2007; Vanschoenwinkel, Gielen, et al., 2009).

4.2 THE MODEL

Since it is impossible to control and account for all the variables at play within a biological system due to its complexity, it is possible to obtain approximations by making use of significant factors. Such models require large datasets and years of data against which to compare (Engler et al., 2004; Ortiz-Rodríguez et al., 2019; Williams et al., 2009). In the case of this study, however, only one year of data is available, and data from only 10 out of the 22 landscapes in which the species has been recorded has been collected. Therefore, independent model calibration and validation was not possible given the reduced sample size and number of observations available.

The resultant equations obtained from lms and glms aided in determining significant factors affecting the presence of the species in a given pool, as well as possibly where in a given pool from the morphometric tolerance ranges and lms. Positive aspects of the models are that they are very simple and have very few parameters that are enough to explain some variance, despite that it is very hard to explain a high proportion of variance in such complex living systems. The glms constructed also have the added advantage of yielding a binomial result as to whether the species is either present or not. This provides better margins of tolerance. The lms simply further informed at what depths *E. gussonei* may be found in a given pool given the basin maxima, once its presence has been confirmed via glms.

These equations, however, simply inform the species distribution and range and are merely speculative as several other biotic factors were excluded from the study. Such factors which may be deemed significant in the species occurrence are: the seedbank; dispersal and connectivity; depth of seed burial, as opposed to the range of sediment depths at which the species is peresent; and the inclusion of a separate hydroperiod model combined into the predictive occurrence model, utilising morphometric and rainfall data. Other factors such as competition, predation and other biological factors may also affect and contribute to the model, but are not straightforward to quantify, and the significance of their contribution may also not merit such inclusion.

Such factors in biological models would result in it being too complex and cumbersome. Therefore, a more simple model which is not too complex, but that also gives a good approximation is ideal, as it is impossible to control and account for all variables included in a model. As a result, only the most significant factors are put forward for inclusion when modelling the species occurrence.

The glms in Equation 1 and Equation 2 are conditional in that the result is binomial and is constrained to being either 1 or 0 to indicate the presence or absence of the species at given surface areas or maximum basin water depths. Results y values obtained from the equation give the probability of the occurrence of the species given the morphometric x values plugged into the equation. Therefore, x values that yield a resultant y value of 0.5 or greater (≥50%) would indicate the minimum value required for *E. gussonei* to occur in a pool.

While both maximum water depth and surface area determine basin hydroperiod and were used to model the occurrence of *E. gussonei*, SAV was not deemed significant enough to be considered as a factor to be used in the predictive model. While this may be due to the fact that less data was available for the ratio to be determined, it may also be due to the variation in hydroperiod experienced by *E. gussonei*, which is not equal to the maximum basin hydroperiod on which the ratio is based.

The condition for a plausible result of Equation 3 and Equation 4 is that maximum morphometric depths must be greater or equal to the specific depths at which *E. gussonei* is present. Their subsequent validity was tested by setting values which are at their plausible limits. With maximum morphometric values of 0cm (i.e. basins with no water or sediment), *E.* gussonei depth values yielded were 0.56cm for water and 1.75cm for sediment, both of which are biologically/ecologically impossible. Therefore, Equation 3 and Equation 4 only provided "plausible" "biologically/ecologically" meaningful results for depths of *E. gussonei* when maximum morphometric depths were greater or equal to the depths at which *E.* gussonei was present at a minimum depth ratio of 1:1. The resultant minimum morphometric depth values for plausible *E. gussonei* depths are 1.68cm and 3.29cm for water and sediment respectively. However, such equations are only valid for the range of depths encountered in this study (Table 3).

Constraining the intercept in the linear regressions to 0 in Equation 5 and Equation 6, making them RTOs (regression through the origin) (Eisenhauer, 2003) resulted in an increase in R^2 from 0.54 and 0.44 to 0.72 and 0.84 for water and sediment depths respectively. This increase in R^2 has no biological significance, but is simply a consequence of the mathematics behind the regression. However, it may be worth further exploring other types of regressions that would account for the restriction of the species to specific depth tolerance ranges, which are significantly different from the basin maxima. As a result, tobit regressions such as censored or truncated regressions (Breen, 1996) may be carried out, as opposed to simply a linear regression, or one with a forced zero intercept. This may subsequently account for specific depths at which *E. gussonei* occurs depths exceeding the morphometric maxima. A mixed model incorporating landscape is also an option that may be carried out in a future study with a larger sample size and more homogeneity in pool samples per landscape. This was not conducted in the current study since not all landscapes in which the species is listed according to Kalinka et al. (2014) were surveyed, and would therefore result in an incomplete picture.

Glms were used to model the occurrence of *E. gussonei* in a given pool, while maximum morphometric depths effectively predicted the actual depths at which *E. gussonei* is present (Table 6). While maximum basin sediment depth was not used as a predictor for the presence of *E. gussonei*, it was used to determine the specific depths at which it occurs. Maximum morphometric water depth, however, was used to predict both the occurrence of *E. gussonei* in a pool, as well as the specific depth at which it is present. This finding fits with the proposed hypothesis that *E. gussonei* is dependent on an individual hydroperiod at which it is present within a basin, as opposed to the maximum basin water depth, and its subsequent hydroperiod as visualised in Figure 19.

4.3 Summary

When addressing the aims and hypotheses put forward, it may be concluded that the measured water chemistry has no effect on the presence of *E. gussonei*. Basin morphometry, more specifically, surface area and maximum water depth (zwm), however are essential in predicting its occurrence by determining basin hydroperiod. However, other less obvious factors were also investigated.

The specific depths at which *E. gussonei* actually occurs were also noted and were found to differ significantly from basin depth maxima. Therefore, specific morphometric depths at which *E. gussonei* is present give further insight into the plant's tolerance range. If the water is too shallow and the surface area too large, the area where the species is present will dry up too quickly. This would make the environment too terrestrial to sustain amphibious species and will be dominated and outcompeted by more terrestrial species. If the water is too deep on the other hand, it will favour specially adapted aquatic species. While present in as little as 0.5cm of sediment (maximum of 15cm and mean of 5cm), deeper sediment would favour more terrestrial species with deeper root systems that may outcompete *E. gussonei* for resources. Such terrestrial species would also take up more water and dry the sediment up quicker due to their larger and more extensive root systems.

While maximum morphometric water depth is indicative of, and a good proxy for complete basin hydroperiod, the depths at which *E. gussonei* was noted is indicative of the hydroperiod experienced by *E. gussonei*, as visualised in Figure 19. Such variance in basin inundation by rainwater or runoff will subsequently affect basin hydroperiod. Given complete inundation of deep pools, these will likely be colonised by obligately aquatic flora which are adapted to such conditions. Partial or incomplete inundation, resulting in shorter hydroperiods may result in a shift in the dominant life-form strategy of flora in a given pool from aquatic to amphibious (Fernández-Zamudio et al., 2016). This phenomenon has already been observed on a shorter temporal scale by Bagella et al. (2009), where rockpools displayed a succession in dominant community assemblage from aquatic (85% *Callitriche stagnalis*) to ephemeral (52% *Crassula vaillantii*).

Given the predicted shorter, drier, and warmer wet seasons in the long term due to climate change (Zacharias & Zamparas, 2010), deeper basins with a higher maximum morphometric water depth may not undergo complete inundation as depicted in Figure 19. If maximum water depth in deeper basins falls within the tolerance range for *E. gussonei*, it may be possible for it to occupy and colonise previously 'uninhabitable' areas of the pool, which were dominated by obligately aquatic flora. This may therefore result in the presence of *E. gussonei* in morphometrically deeper basins with a higher water depth maxima, but which due to reduced rainfall as a result of climate change only reach a maximum water depth within the tolerance range of *E. gussonei*. The occurrence of the species in deeper basins however is conditional on whether its seeds are actually present and well established in the basin sediment and have a persistent and established seedbank (Cross, Turner, Merritt, et al., 2015; Cross, Turner, Renton, et al., 2015) as a result of dispersal and connectivity within a given landscape (Tornero et al., 2018).

Therefore, it may be concluded that *E. gussonei* requires an environment that is neither too terrestrial nor too aquatic in order for germination to occur, and so as not to be outcompeted by specialists. Ideally, the species favours pools with less than 20cm of water and a maximum sediment depth of 15cm. Basin surface area however is dependent on water depth given their positive correlation with each other, and the fact that they determine pool hydroperiod. If conditions are favourable and the hydroperiod is neither too aquatic nor too terrestrial, then it is possible that *E. gussonei* may occur and germinate if basin inundation is suitable and within the species' tolerance range of 2-20cm. However, other factors such as competition that may arise as a result of any changes in hydroperiod, along with morphometric sediment depth have not been taken into account, making these conclusions merely speculative. It is also unknown if and how any changes in shading index as measured by Lanfranco et al. (2020) of morphometrically deeper basins with lower morphometric water depth maxima may affect species germination (Bliss & Zedler, 1997; Carta, 2016). The specific germination triggers and requirements for *E. gussonei* however remain inconclusive and require further study both in and ex situ.

4.4 Limitations

The main limitation in the current study was the short duration of the wet season, which lasted effectively only 5 months, with some distributed rainfall for a further 2-3 months. This however was not enough to sustain a hydroperiod in most pools. As a result, not all pools per landscape were surveyed, nor each of the 22 landscapes in which *E. gussonei* has been recorded. Data for certain landscapes was also incomplete for the same reason, as some pools dried up faster than others, and experienced more hydroperiods in the short wet season, as well as a shorter overall hydroperiod. Another limitation to the study was the fact that it was only conducted over one wet season, therefore factors such as temperature and rainfall were not taken into account and could not be compared to previous years, along with the fact that only maximum water depth was taken for basins, as opposed to regular monitoring of all pools to take hydroperiod into account.

Given the overlap of flora, only presence-absence data was collected as opposed to percentage cover for each species. While percentage cover may give better insight into the community establishment in a given pool, the estimated percentage cover may have not been representative due to obfuscation and overlap of flora. While a preliminary species list was taken during the study to construct a correlation matrix (Figure 38 and Figure 41), competition and competitive exclusion were not included.

4.5 Recommendations for Further Work

While some pool data was utilised from previous years to enhance the dataset and improve the model, individual pool data from only one season was used for analysis. As a result, any subsequent building and testing of the model would not account for interannual variation in pools between different years. Though the dataset compiled was incomplete given the short wet season when compared to previous years, it provided the largest dataset of individual pools and their respective physical and chemical parameters to date, encompassing *ca.* 170 pools from 10 landscapes, whereby complete morphometric and water chemistry data for *ca.*100 and *ca.* 120 pools respectively was collected. Any further improvements that may be made to this would be to conduct the study in each pool over multiple seasons.

Estimate values for percentage cover as opposed to presence-absence data may also improve the model, since presence-absence does not distinguish between very well-established populations and sparse cover and distribution. However, given that in some cases *E. gussonei* was obscured by filamentous algae or other macrophytes, this may not prove too easy, especially in deeper pools, where the sediment surface is obscured. It may also be useful to determine the significance in diurnal variation of water chemistry. This would establish whether taking point measurements at similar times of day, as was done in the present study, would yield comparable results.

Further recommendations for future work may be to conduct germination and growth experiments on *E. gussonei*, to identify any specific triggers such as flooding time, duration and persistence, temperature, seed burial depth and scarification (Carta, 2016). Soil texture may also be worth noting due to its influence on seed percolation and resultant burial depth. Other *ex situ* experiments however may be of competitive exclusion, to identify if *E. gussonei* will not grow in the presence of specific species as indicated in the co-occurrence analysis (Figure 38), or if the results obtained were simply by chance due to the species' stochastic nature. It may also be worth following how species richness and cover, dependent on life-form, affect the presence of *E. gussonei*. Germination and growth experiments along with detailed morphometry and basin dynamics would also contribute to more detailed phenological studies on the species. Such studies would subsequently better aid in the understanding of its sensitivity and triggers at its varying life stages for germination, flowering and seeding. This is especially so given that several pools in which *E. gussonei* was present underwent a minimum of two hydroperiods, of sufficient duration to complete two flowering cycles in a single wet season.

Determining individual pool hydroperiod duration and frequency during the wet-season as demonstrated by Hulsmans et al. (2008) may also aid in providing further insight into the macrophyte's ecology. In order to obtain a holistic picture of basin dynamics, it would be worth systematically mapping basin profile by measuring water and sediment depths to obtain a 3D image of the basin, while also collecting species data per segment and correlating species presence to morphometric basin depths. In this way, basin heterogeneity within pools would give insight into the various clustering of lifeforms at varying depth classes. This would subsequently aid in determining any shifts in community dynamics due to incomplete basin inundation, whereby one may observe a shift from aquatic to amphibious to terrestrial flora as a result of shifts to shorter hydroperiods.

A final comment would be to include any of the other landscapes visited, but not surveyed due to the comparably short, dry wet season, and compilation of a more robust and complete dataset. Other landscapes scoped for further study include Selmun, Xemxija, Manikata and Tal-Wej, as well as those listed in Kalinka et al. (2014).

4.6 Implications for Species Conservation, and Habitat Monitoring and Restoration

Temporary ponds and their surrounding areas contain rare and characteristic pond species. According to several studies, the belt around the ponds is considered as the favoured habitat for characteristic Mediterranean temporary pool species that are rare and unstable (Zacharias et al., 2007; Zacharias & Zamparas, 2010).

Temporary ponds/rockpools may be considered to be good bioindicators of climate change due to their ephemeral nature and rapid response to the impact of meteorological conditions on basin inundation and subsequent hydroperiod duration (Ernandes & Marchiori, 2013). While aiding to understand the habitat itself, pools may also be used for hypothesis testing in conservation biology, ecology, and evolutionary biology research, making them excellent model systems (Brendonck et al., 2010; De Meester et al., 2005; Jocque et al., 2010). Various characteristics are attributed to pools to classify them as attractive model systems in such areas.

Their size and abundance make them ideal ecological laboratories as testing grounds due to their well-defined borders and simple food webs, making them easy to replicate and manipulate. Their cosmopolitan distribution and ephemerality also have a similar effect on biotic processes in other similar patchy, temporary habitats, with certain aspects closely resembling metapopulation and metacommunity models. Being home to and a breeding ground for various organisms of medical importance and vectors of disease is another important aspect of such habitats. The final and most resounding aspect of their importance is the drastic decrease in their quality and quantity by anthropogenic means, which necessitates the protection of the various unique species depending on them. While such habitats aid in developing ecological theories, these should in turn aid in resolving problems and shortcomings of the habitat and its conservation and protection (Blaustein & Schwartz, 2001; Brendonck et al., 2010; De Meester et al., 2005).

Given that pool species and life forms are dependent on hydroperiod duration, these will also be indirectly impacted (Bagella et al., 2009; Bagella & Caria, 2012, 2013;

Bagella et al., 2010; Ernandes & Marchiori, 2013; Minissale & Sciandrello, 2016). By refining the model to account for inter- and intrannual variation in rainfall and its effect on basin hydroperiod duration and number of hydroperiods per wet season, this may be applied to other ephemeral freshwater flora, and improve our understanding of community dynamics based on basin morphometry to better predict species occurrence to aid in their monitoring and protection. Therefore, model species may also subsequently be used to model and possibly also predict further environmental changes, depending on the status of their presence according to the IPCC. *Elatine*'s phenotypic plasticity (Molnár et al., 2015) makes it a good sentinel for climate change due to its rapid response to environmental changes in its physical phenotypic properties (Molnár et al., 2015).

More detail on vegetation distribution patterns and dynamics are required in order to inform conservation management plans to establish the habitat's priority for conservation measures (Bagella & Caria, 2013). Consensus is also required among stakeholders to implement effective conservation measures and activities to protect ecologically important pond areas. Issues pertaining to such management practices that should be considered are that hydroperiod and water quality are the main target of protection and management in the long and short term. It is therefore also vital to consider ponds of varying morphometry and hydroperiods, as well as decaying pool vegetation or leaf litter in order to account for species and species habitat and microhabitat variability (Zacharias et al., 2007; Zacharias & Zamparas, 2010). Proper implementation of such measures, however necessitates the increase of public awareness. Therefore, the habitats, its importance and conservation value must be publicised to count towards the implementation of their protection and conservation (Zacharias et al., 2007).

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6 APPENDICES

6.1 APPENDIX I: SPECIES LIST AND CODES

Table A 1: List of species observed and their respective codes

Species	Code
Aster squamatus	Ast_squ
Bellis annua	Bel_ann
Bellis sylvestris	Bel_syl
Callitriche truncata	Cal_tru
Chara vulgaris	Cha_vul
Crassula vaillanti	Cra_vai
Damasonium bourgaei	Dam_bou
Dittrichia viscosa	Dit_vis
Drimia maritima	Dri_mar
Euphorbia helioscopia	Eup_hel
Filamentous algae	Fila_alg
Hypparhenia hirta	Hyp_hir
Juncus acutus	Jun_acu
Limbarda crithmoides	Lim_cri
Lythrum hyssopifolia	Lyt_hys
Mentha pulegium	Men_pul
Plantago lagopus	Pla_lag
Polypogon monspeliensis	Pol_mon
Ranunculus saniculifolius	Ran_san
Salsola soda	Sal_sod
Triglochin laxiflora	Tri_lax
Zannichellia melitensis	Zan_mel

6.2 APPENDIX II: RAW DATA

6.2.1 Pool Landscapes and Basin Locations and Codes

Table A 2: Coordinates and Codes for Pool Landscapes and their respective pool basins

Landscape	Basin Code	Basin Coordinates (N Latitude, E Longitude)
Salini (SLN)	SLN1	35.9448, 14.419683
	SLN2	35.944817, 14.419717
	SLN3	35.945617, 14.4208
	SLNP1	35.94525, 14.420111
Gharghur (GRG)	GRG1	35.929033, 14.44915
	GRG2	35.928967, 14.4491
	GRG3	35.92895, 14.4491
	GRG4	35.928933, 14.4491
	GRG5	35.928983, 14.449067
	GRG6	35.929009, 14.448997
San Pawl tat-Tarġa (SPT)	SPT11a	35.926233, 14.4402
	SPT1	35.9268, 14.440767
	SPT2	35.926833, 14.440767
	SPT3	35.92685, 14.4408
	SPT4	35.92685, 14.44085
	SPT5	35.926767, 14.4408
	SPT6	35.9268, 14.440767
	SPT7	35.9268, 14.44075
	SPT8	35.926783, 14.44075
	SPT9	35.9268, 14.440717
	SPT10	35.926783, 14.440667
	SPT11	35.926367, 14.44045
	SPT12	35.9264, 14.44045
	SPT13	35.926433, 14.440467
	SPT14	35.926433, 14.4404
	SPT15	35.92645, 14.440367
	SPT16	35.926467, 14.440317
	SPT17	35.92645, 14.440317
	SPT18	35.926433, 14.4403
	SPT19	35.926633, 14.44035
	SPT20	35.926617, 14.440367
	SPT21	35.9266, 14.4405

Landscape	Basin Code	Basin Coordinates (N Latitude, E Longitude)
	SPT22	35.92665, 14.440517
	SPT23	35.9268, 14.440433
	SPT24	35.9258, 14.438233
	SPT25	35.925667, 14.437867
	SPT26	35.925733, 14.437717
	SPT27	35.9256, 14.437417
	SPT28	35.925633, 14.4374
	SPT29	35.925533, 14.437117
	SPT30	35.925617, 14.437467
Mosta Tal-Qares (MST)	MST1	35.9159, 14.425083
	MST2	35.9159, 14.42505
	MST3	35.915917, 14.425017
	MST4	35.915967, 14.425017
	MST5	35.915983, 14.42505
	MST6	35.916017, 14.42505
	MST7	35.916017, 14.425117
	MST8	35.916033, 14.425117
	MST9	35.916167, 14.424983
	MST10	35.916167, 14.42495
	MST11	35.915933, 14.425033
	MST12	35.916033, 14.424967
	MST13	35.916017, 14.424983
	MST14	35.916033, 14.424983
	MST15	35.916033, 14.424967
	MST16	35.916017, 14.424967
	MSTP1	35.914958, 14.424794
	MSTP2	35.915067, 14.424878
	MSTP3	35.914978, 14.424939
	MSTP4	35.915061, 14.425056
	MSTP5	35.915111, 14.4251
	MSTP6	35.915144, 14.425139
	MSTP7	35.915219, 14.425233
	MSTP8	35.915306, 14.425225
	MSTP9	35.915528, 14.425258
	MSTP10	35.915258, 14.425108

Landscape	Basin Code	Basin Coordinates (N Latitude, E Longitude)
Munxar (MNX)	MNX1	36.032917, 14.22955
	MNX2	36.032867, 14.229333
	MNX3	36.03285, 14.229383
	MNX4	36.032867, 14.229383
	MNX5	36.03285, 14.229367
	MNX6	36.03285, 14.22935
	MNX7	36.032733, 14.229267
	MNX8	36.03255, 14.228667
	MNX9	36.032633, 14.228083
	MNX10	36.0327, 14.228033
	MNX11	36.0327, 14.227967
	MNX12	36.03275, 14.227833
	MNX1x	36.032767, 14.227817
	MNX2x	36.0328, 14.227733
	MNX3x	36.033083, 14.227183
Pembroke (PBK)	PBK1	35.929117, 14.484583
	PBK2	35.929083, 14.484567
	PBK3	35.930139, 14.486173
	PBK3a	35.930183, 14.4861
	PBK3b	35.9302, 14.486233
	PBK4a	35.930417, 14.48625
	PBK4b	35.930433, 14.486233
	PBK5	35.9305, 14.486233
	PBK6	35.9305, 14.486367
	PBK7	35.93055, 14.486383
	PBK8	35.929033, 14.48565
	PBK9	35.929017, 14.485633
	PBK10	35.929033, 14.485683
	PBK11	35.92905, 14.485683
	PBK12	35.928933, 14.485617
	PBK13	35.928883, 14.485633
	PBK14	35.928817, 14.4856
	PBK15	35.928817, 14.485533
	PBK16	35.928767, 14.4856
	PBK17	35.92875, 14.48565

Landscape	Basin Code	Basin Coordinates (N Latitude, E Longitude)
	PBK18	35.928717, 14.48575
	PBK19	35.930917, 14.48695
	PBK20	35.930983, 14.487017
Wied Ħas-Saptan (WHS)	WHS1	35.835717, 14.515567
	WHS2	35.835733, 14.515533
	WHS3	35.835717, 14.515533
	WHS4	35.8357, 14.515483
	WHS5	35.835717, 14.515483
	WHS6	35.835717, 14.515517
	WHS7	35.8357, 14.515517
	WHS8	35.83575, 14.515533
	WHS9	35.83575, 14.515483
	WHS10	35.83575, 14.515517
	WHS11a	35.835767, 14.515517
	WHS11b	35.83575, 14.515533
	WHS12	35.835767, 14.515483
	WHS13	35.835767, 14.51545
	WHS14	35.83575, 14.515433
	WHS15	35.8356, 14.515217
	WHS16	35.835633, 14.51525
	WHS17	35.8356, 14.515267
	WHS18	35.835567, 14.515133
	WHS19	35.8356, 14.515317
	WHS20	35.835617, 14.515333
	WHS21	35.835633, 14.51535
	WHS22	35.835617, 14.515333
	WHS23	35.835617, 14.51535
	WHS24	35.8356, 14.5154
	WHS25	35.83555, 14.51545
	WHS26	35.835533, 14.515433
	WHS27	35.835533, 14.5154
	WHS28	35.83555, 14.5154
	WHS29	35.835567, 14.5154
	WHS30	35.8356, 14.515417
	WHS31	35.835583, 14.515433

Landscape	Basin Code	Basin Coordinates (N Latitude, E Longitude)
	WHS32	35.8356, 14.51545
	WHS33	35.835617, 14.515467
	WHS34	35.835617, 14.515467
	WHS35	35.835683, 14.51495
	WHS36	35.83565, 14.515033
	WHS37	35.835633, 14.515017
	WHS38	35.8356, 14.514917
	WHS39	35.835917, 14.514867
	WHS40	35.835867, 14.514867
Qala (QLA)	QLAP1	36.029161, 14.321067
	QLAP2	36.029125, 14.321114
	QLAP3	36.029342, 14.320731
	QLAP4	36.029317, 14.3207
	QLAP5	36.029275, 14.320706
	QLAP6	36.029275, 14.320667
Birżebbuġa (BBG)	BBG1	35.8073, 14.5237
	BBG2	35.8073, 14.523733
	BBG3	35.807317, 14.52375
	BBG4	35.807333, 14.523783
	BBG5	35.807367, 14.51175
	BBG6	35.8074, 14.51165
Ħad-Dingli (DNG)	DNG1	35.851533, 14.385983
	DNG2	35.85165, 14.38605
	DNG3	35.851683, 14.38605
	DNG4	35.85145, 14.38625

6.2.2 Proportion of Pools per Landscape with *Elatine gussonei*

Table A 3: Proportion of pools in each respective pool landscape with *Elatine gussonei*

Landscape (Code)	Co-Ordinates of Centre of Site	Proportion of pools
Birżebbuġa (BBG)	35.806921, 14.516121	0/6
Ħad-Dingli (DNG)	35.851658, 14.386357	3/5
Għargħur (GRG)	35.931425, 14.453426	0/6
Munxar (MNX)	36.032865, 14.229358	9/12
Mosta Tal-Qares (MST)	35.915594, 14.425309	9/26
Pembroke (PBK)	35.929744, 14.485845	16/23
Qala (QLA)	36.029271, 14.320945	0/13
Salini (SLN)	35.945683, 14.420609	2/4
San Pawl tat-Tarġa (SPT)	35.926516, 14.440718	9/31
Wied Ħas-Saptan (WHS)	35.835849, 14.515074	34/41

6.3 APPENDIX III: SUPPLEMENTARY GRAPHS

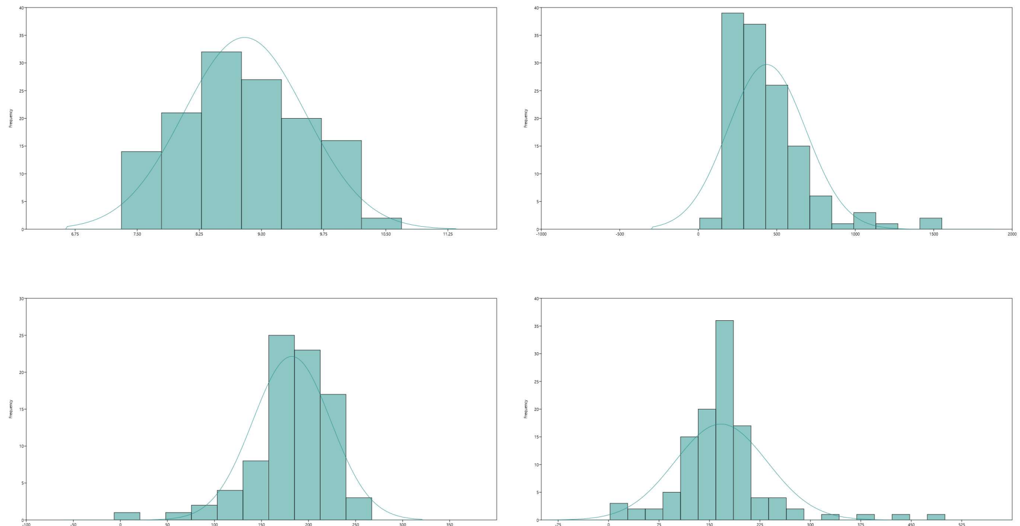


Figure A 1: Histogram of water quality parameters. pH (top left), EC (top right), ORP (bottom left) and DO (bottom right)

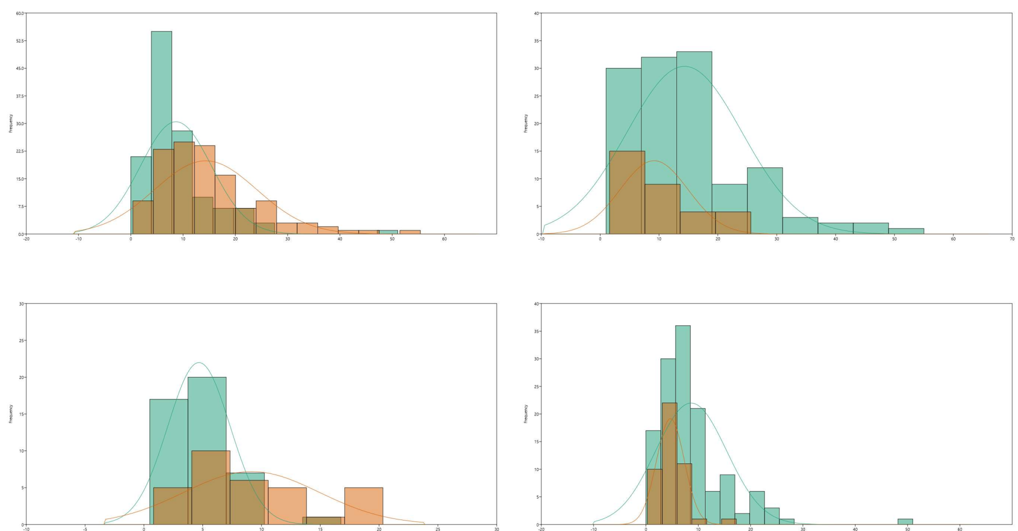


Figure A 2: Histogram of basin morphometric depths. zsm (green) and zwm (orange) (top left), zwm (green) and zwe (orange) (top right), zse (green) and zwe (orange) (bottom left), and zsm (green) and zse (orange) (bottom right)

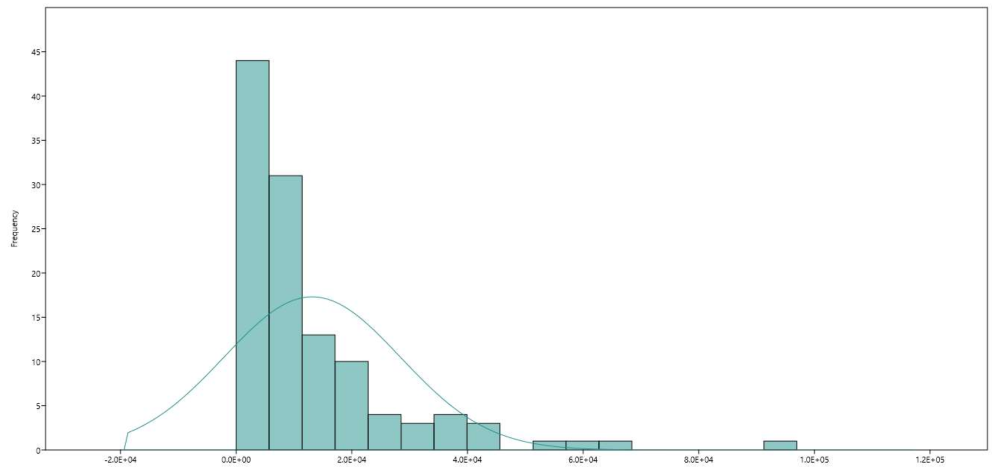
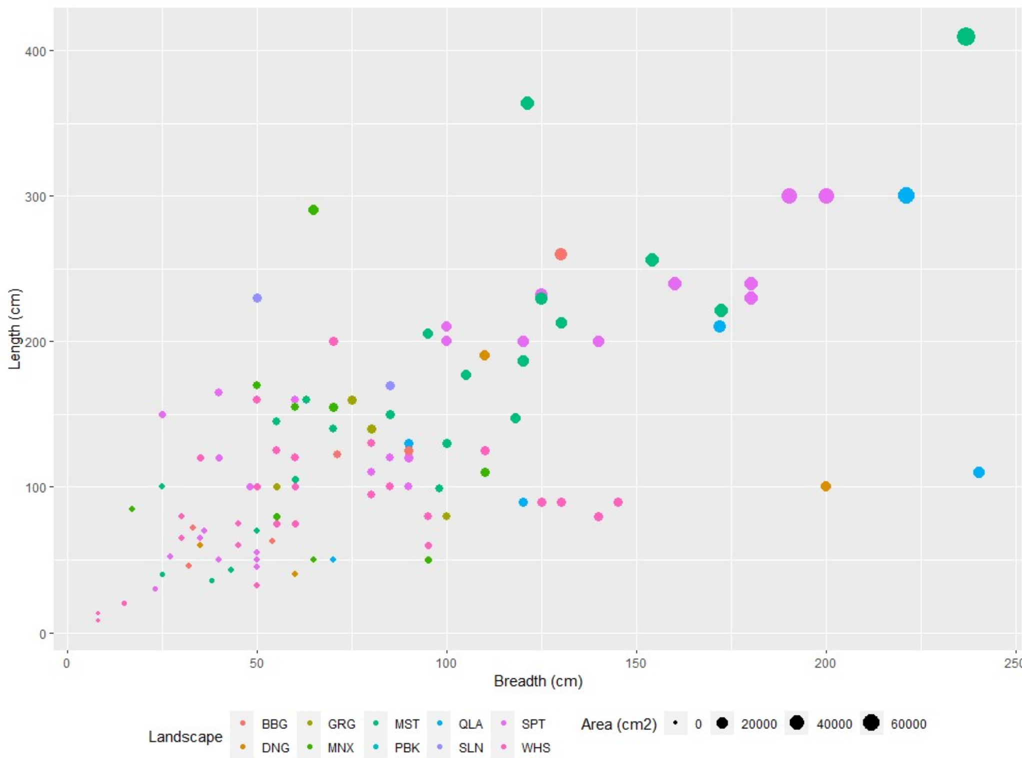


Figure A 3:Histogram of basin Surface Area

Figure A 4: Water quality in pools with and without *Elatine gussonei* in each Pool Landscape, where EC is Electrical Conductivity, and ORP is Oxidation Reduction Potential. Landscape codes: Ħad-Dingli (DNG), Għargħur (GRG), Munxar (MNX), Mosta (MST), Pembroke (PBK), Qala (QLA), Salini (SLN), San Pawl tat-Tarġa (SPT) and Wied Ħas-Saptan (WHS)



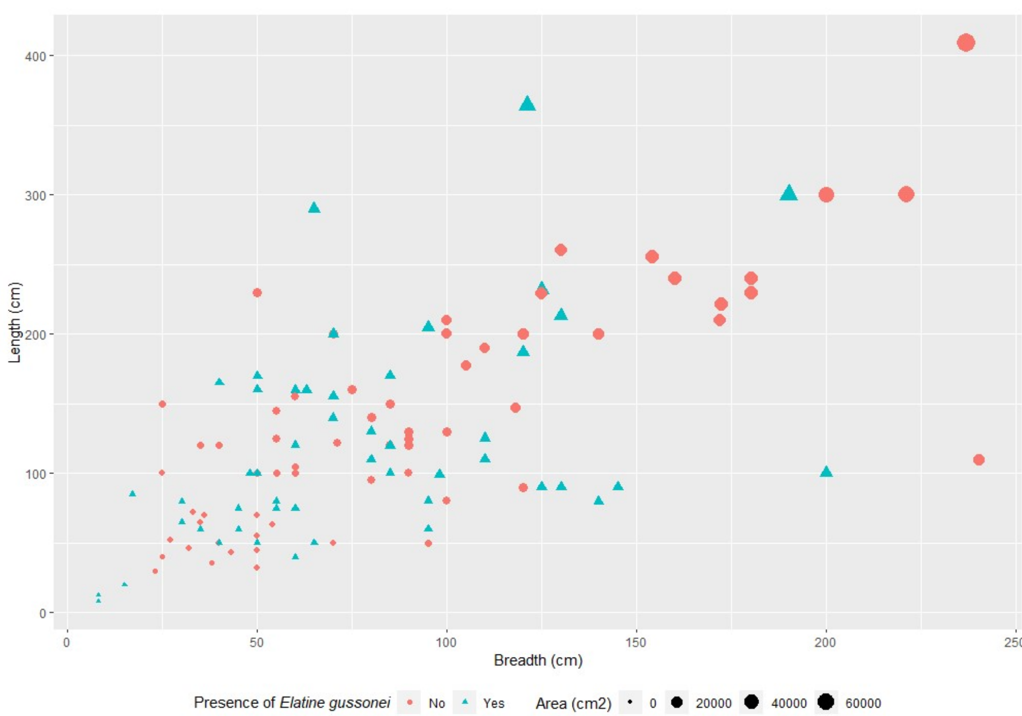


Figure A 5: Bubble plot of individual pool axes, and resultant surface area (cm^2) represented by plot size for pools per landscape (top), and with and without *Elatine gussonei* (bottom). Codes: Birżebbuġa (BBG), Ħad-Dingli (DNG), Għargħur (GRG), Munxar (MNX), Mosta (MST), Pembroke (PBK), Qala (QLA), Salini (SLN), San Pawl tat-Tarġa (SPT) and Wied Ħas-Saptan (WHS)

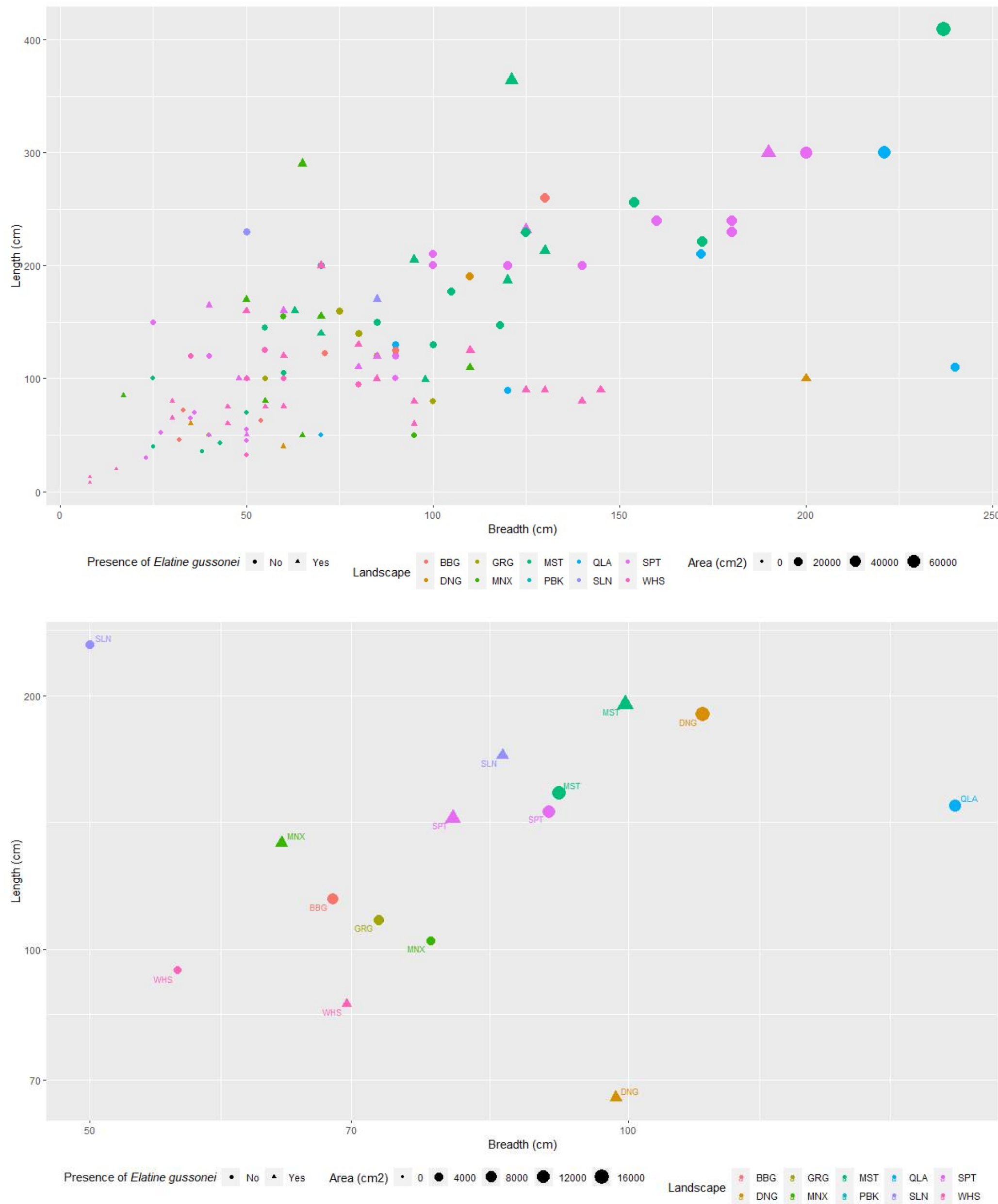


Figure A 6: Bubble plot of individual (top) and mean (bottom) axes dimensions and resultant surface area (cm^2) per landscape for pools with and without *Elatine gussonei*. Codes: Birżebbuġa (BBG), Ħad-Dingli (DNG), Għargħur (GRG), Munxar (MNX), Mosta (MST), Pembroke (PBK), Qala (QLA), Salini (SLN), San Pawl tat-Tarġa (SPT) and Wied Ħas-Saptan (WHS)

Figure A 7: Comparison of water and sediment depths in pools with and without *Elatine gussonei* across (top) and within (bottom) Pool Landscapes, where the maximum basin water and sediment depths are zwm and zsm respectively, and the maximum water and sediment depths at which *Elatine gussonei* is present are zwe and zse respectively. Landscape codes: Birżebbuġa (BBG), Ħad-Dingli (DNG), Għargħur (GRG), Munxar (MNX), Mosta (MST), Pembroke (PBK), Qala (QLA), Salini (SLN), San Pawl tat-Tarġa (SPT) and Wied Ħas-Saptan (WHS)

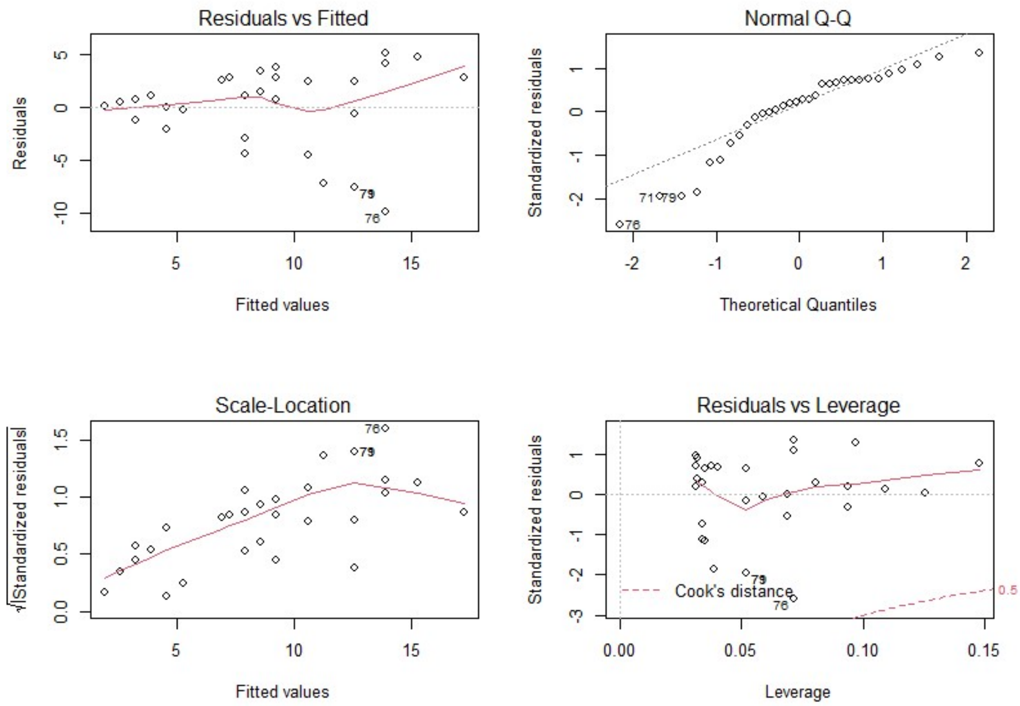


Figure A 8: Plots for fitted linear regression model for water depth

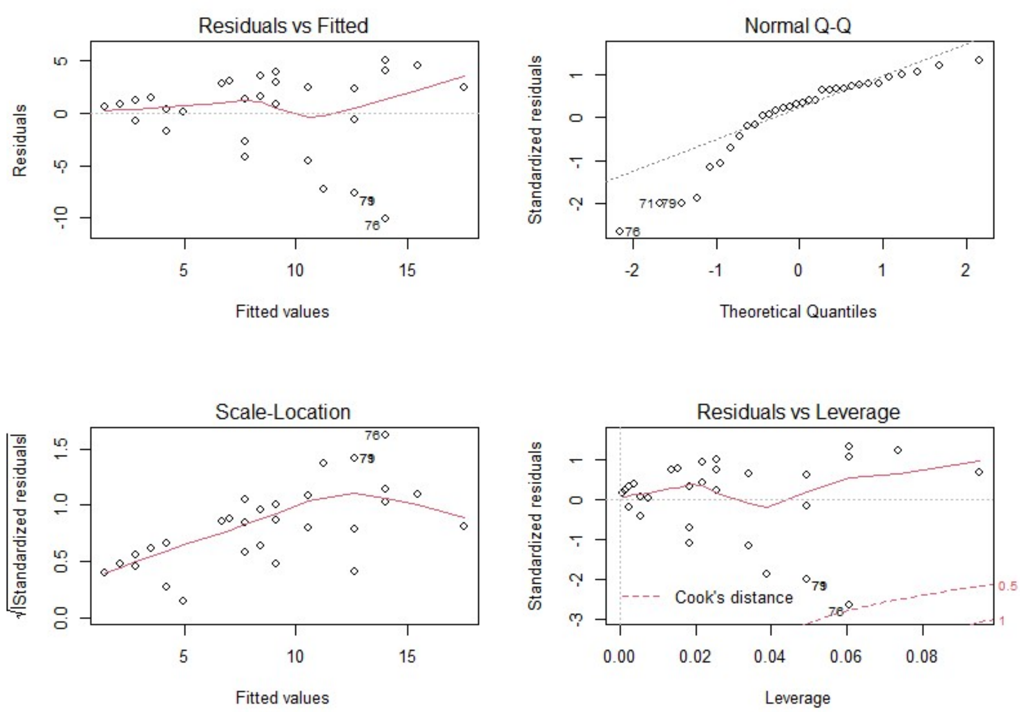


Figure A 9: Plots for fitted linear regression model for water depth with forced 0 intercept

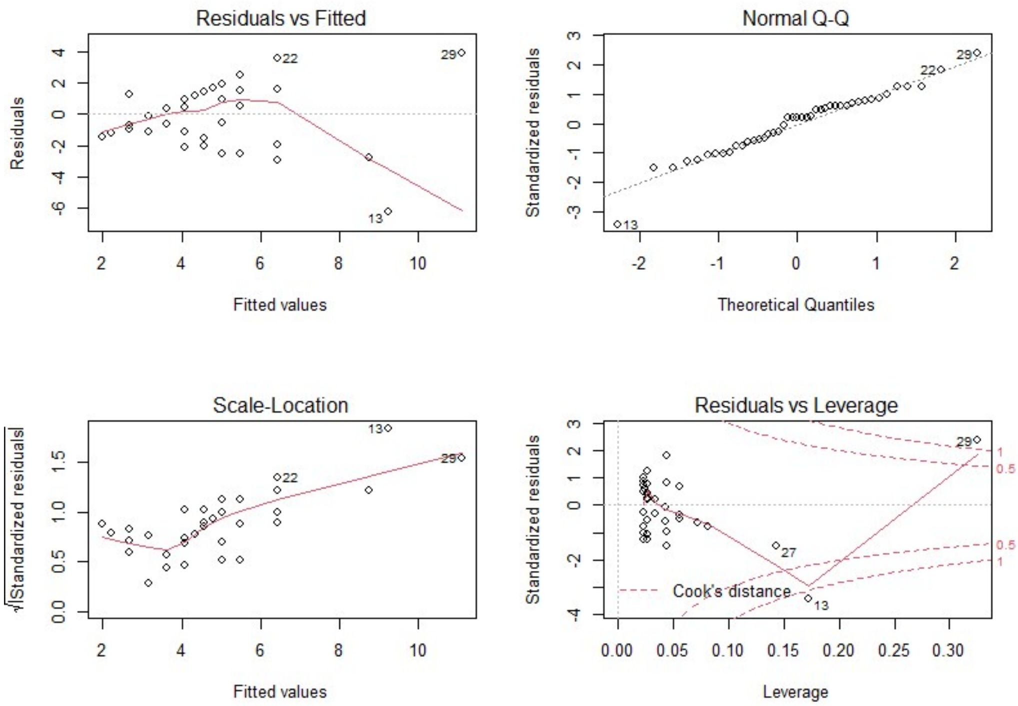


Figure A 10: Plots for fitted linear regression model for sediment depth

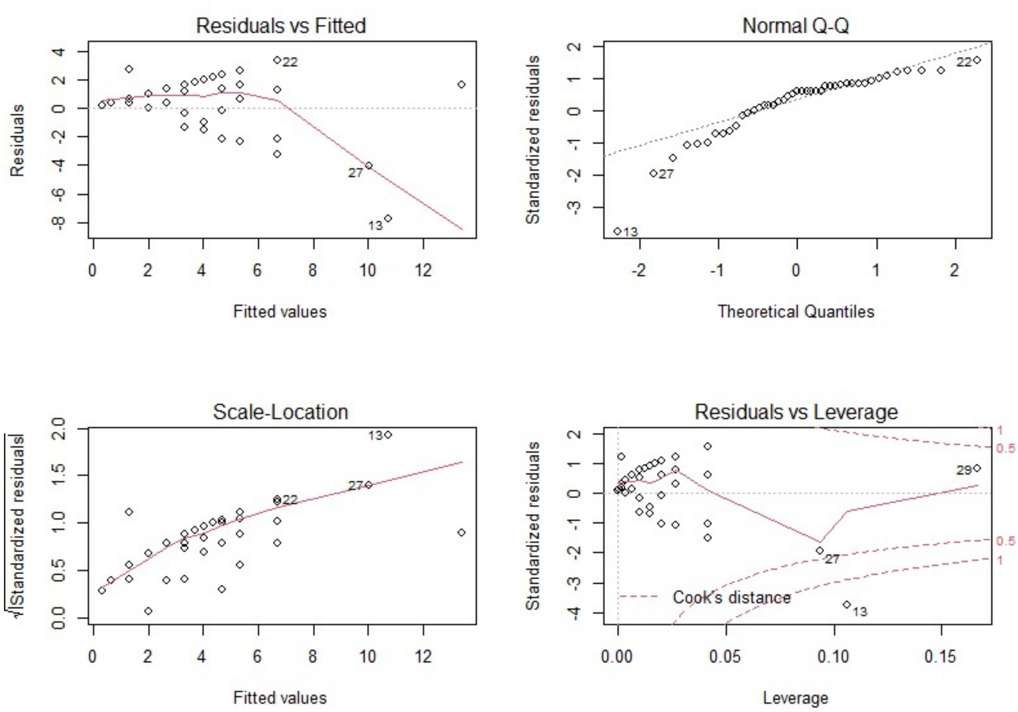


Figure A 11: Plots for fitted linear regression model for sediment depth with forced 0 intercept

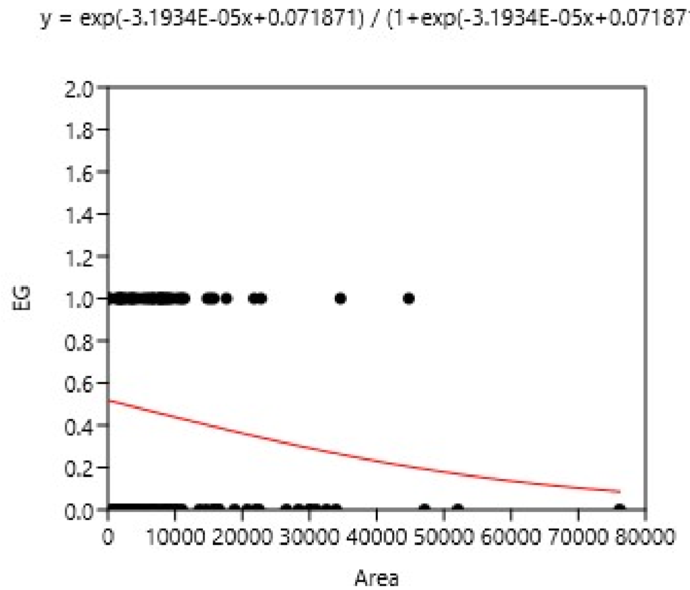


Figure A 12: Glm output from PAST v.6.02 for maximum basin Area (cm^2)

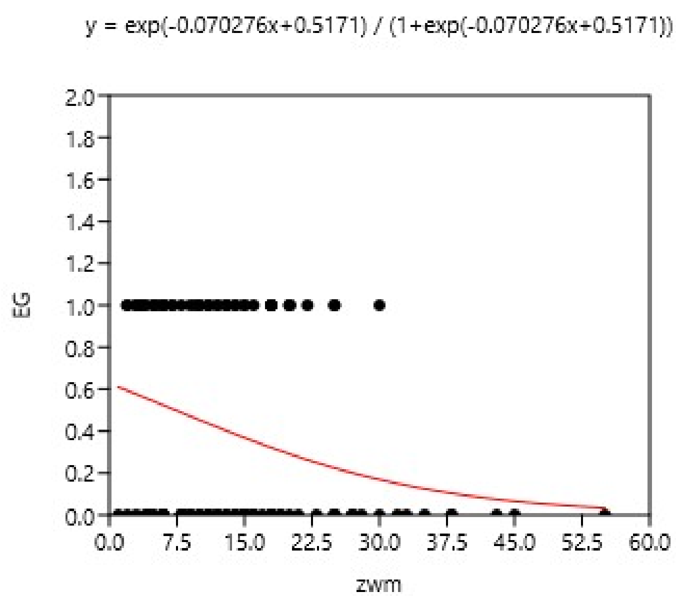


Figure A 13: Glm output from PAST v.6.02 for maximum basin water depth (zwm) (cm)

6.4 APPENDIX IV: ELECTRONIC SPECIFICATIONS

6.4.1 Multiparameter Meter

Table A 4: Hanna instruments multiparameter meter Professional multiparameter handheld meter for pH, conductivity, dissolved oxygen - HI98194 Specifications

pH/mV	
Measuring range	pH 0.00 to 14.00 / ± 600 mV
Resolution	pH 0.01; 0.1 mV
Accuracy	pH ± 0.02; ± 0.5 mV
Calibration	One, two or three points from a selection of 5 standard puddles (pH 4.01; 6.86; 7.01; 9.18; 10.01) or a user-defined buffer
Redox potential	
Measuring range	± 2000 mV
Resolution	0.1 mV
Accuracy	± 1.0 mV
Calibration	Automatically at a user-defined point (relative mV)
Conductivity measurements	
Measuring range	0 to 200 mS / cm (0 to 400 ms / cm absolute conductivity without temperature compensation)
Resolution	Manual: 1 µs / cm; 0.001 mS / cm; 0.01 mS / cm; 0.1 mS / cm; 1mS / cm; automatic: 1 µs / cm from 0 to 9999 µS / cm; 0.01 mS / cm from 10.00 to 99.99 mS / cm; 0.1 mS / cm from 100 to 400 mS / cm; automatic mS / cm: 0.001 ms / cm from 0.000 to 9.999 mS / cm; 0.01 mS / cm from 10.00 to 99.99 mS / cm; 0.1 mS / cm from 100 to 400 mS / cm
Accuracy	± 1% of reading or ± 1 µS / cm whichever is greater
Calibration	Automatic one-point calibration with six standard solutions (84 µS / cm, 1413 µS / cm; 5.00 mS / cm; 12.88 mS / cm; 80.0 mS / cm; 111.8 mS / cm) or a user-defined point
Total Dissolved Solids (TDS)	
Measuring range	0.0 to 400.0 ‰ (the maximum value depends on the selected TDS conversion factor)
Resolution	Manual: 1 ppm (mg / L); 0.001 ‰ (g / L); 0.01 ‰ (g / L); 0.1 ‰ (g / L); 1 ‰ (g / L); automatic: 1 ppm (mg / L) from 0 to 9999 ppm (mg / L); 0.01 ‰ (g / L) from 10.00 to 99.99 ‰ (g / L); 0.1 ‰ (g / L) from 100.0 to 400.0 ‰ (g / L); automatic ‰ (ppt): 0.001 ‰ (g / L) from 0.000 to 9.999 ‰ (g / L); 0.01 ‰ (g / L) from 10.00 to 99.99 ‰ (g / L); 0.1 ‰ (g / L) from 100.0 to 400.0 ‰ (g / L);

Accuracy	± 1% of reading or ± 1 ppm, whichever is greater
Calibration	Based on the conductivity calibration
Resistance	
Measuring range	0 to 999999 Ω • cm; 0 to 1000.0 kΩ • cm; 10.0 to 99.9 kΩ • cm; 0 to 1.0000 MΩ • cm
Resolution	Depending on the measured value
Calibration	Based on the conductivity calibration
Salinity (NaCl)	
Measuring range	0.00 to 70.00 PSU (practical salt content scale)
Resolution	0.01 PSU
Accuracy	± 2% of reading or ± 0.01 PSU, whichever is greater
Calibration	Takes place through the conductivity calibration
Sea water σ	
Measuring range	0.0 to 50.0 σ t, σ 0, σ 15
Resolution	0.1 σ t, σ 0, σ 15
Accuracy	± 1 σ t, σ 0, σ 15
Calibration	Takes place through the conductivity calibration
Dissolved Oxygen (DO)	
Measuring range	0.00 to 50.00 ppm (mg / L); 0.0 to 500.0% saturation
Resolution	0.01 ppm; 0.1% saturation
Accuracy	0.0 to 300.0% saturation: ± 1.5% of the reading or ± 1% saturation, whichever is greater; 300% to 500.0% saturation: ± 3% of reading; 0.00 to 30.00 ppm (mg / L): ± 1.5% of the reading or ± 0.10 ppm (mg / L), whichever is greater; 30.00 ppm to 50.00 ppm: ± 3% of the reading
Calibration	Automatically with one or two points at 0 and 100%; custom calibration at one point
Barometric pressure	
Measuring range	450 to 850 mm Hg; 600.0 to 1133.2 mBar; 60.00 to 113.32 kPa; 17.72 to 33.46 in Hg; 8.702 to 16.436 psi; 0.5921 to 1.1184 atm
Resolution	0.1 mm Hg; 0.1 mbar; 0.01 kPa; 0.01 in Hg; 0.001 psi; 0.0001 atm
Accuracy	± 3 mm Hg within ± 15 ° C of the temperature at calibration
Calibration	Automatically at a custom point
Temperature	
Measuring range	-5.0 to 55.0 ° C; 23.00 to 131.00 ° F; 268.15 to 328.15 K.
Resolution	0.01 ° C; 0.01 ° F; 0.01 K
Accuracy	± 0.15 ° C; ± 0.27 ° F; ± 0.15 K

Calibration	Automatically at a custom point
Other data	
Temperature compensation	Automatically from -5 to 55 ° C
Data recording	45,000 data records (continuous recording or recording of all parameters if required)
Recording interval	1 second to 3 hours
PC connectivity	Via USB with Hanna PC software
Battery type / service life	1.5 V AA batteries / approx. 360 hours of continuous use without backlighting (50 hours with backlighting)
Environmental conditions	0 - 50 ° C, max. 100% rel. Humidity IP67
Mass weight	185mm x 93mm x 35.2mm / 400g

6.4.2 Handheld GPS

Table A 5: Garmin GPSmap 60CSx specifications.

General	
Physical Dimensions	2.4" x 6.1" x 1.3" (6.1 x 15.5 x 3.3 cm)
Display Size	1.5" x 2.2" (3.8 x 5.6 cm)
Display Resolution	160 x 240 pixels
Display Type	256 level colour TFT
Weight	7.5 oz (213 g) with batteries
Battery Type	2 AA batteries (not included)
Battery Life	18 hours, typical
Water Rating	IPX7
High-Sensitivity Receiver	✓
Maps & Memory	
Ability to Add Maps	✓
Basemap	✓

Automatic Routing (Turn by Turn Routing on Roads)	Yes (with optional mapping for detailed roads)
External Memory Storage	64 MB microSD™ card (included)
Waypoints/Favourites/Locations	1000
Track Log	10,000 points, 20 saved tracks
Routes	50
Sensors	
Barometric Altimeter	✓
Compass	✓
Outdoor Recreation	
Area Calculation	✓
Hunt/Fish Calendar	✓
Sun and Moon Information	✓
Geocaching-Friendly	✓
Additional	
Additional	This USB device is compatible with Windows® XP or newer and Mac® OS X 10.4 or later.