AECOM

Translocation of the little whirlpool Ramshorn snail: Multivariate community analysis 2016

Highways England

14 September 2016

Prepared by: ......[redacted]

Ecologist

Abrehart Ecology

Checke[redacted]

Principal Ecologist

Abrehart Ecology

Approved by: [redacted]

Associate Director

AECOM

Rev No	Comments	Checked by	Approved by	Date
1	Draft	[redacted]	[redacted]	30/08/16
2	Final draft	[redacted]	[redacted]	25/10/16
3				

AECOM, 2 City Walk, Leeds, LS11 9AR, United Kingdom

Telephone: +44(0)113 391 6800 Website: http://www.aecom.com

Abrehart Ecology Limited, Pound Farm, Low Road, Great Glemham, Suffolk, IP17 2DQ

Telephone: +44(0)1728 663282 Website: http://www.abrehartecology.com

Job No 47075202 **Reference** **August 2016**

Contents

1 Introduction

Little whirlpool ramshorn snail *Anisus vorticulus* is small aquatic snail, with a dorsoventrally flattened shell approximately 5 mm in diameter. It is a UK Biodiversity Action Plan Priority Species and the only British non-marine gastropod which is a European Protected Species. It is also listed in Annex II of the EU Habitats and Species Directive and therefore requires the designation of special areas for conservation. In the UK, populations of *Anisus vorticulus* have been declining since the 1960s and although the precise cause is not clear, it is thought that drainage, over-frequent dredging and eutrophication are all likely to be contributing factors (JNCC, 2007; Van Damme, 2012).

Historical records of *Anisus vorticulus* snail exist for the marshes directly adjacent to the A47 between Norwich and Great Yarmouth, also known as the 'Acle Straight'. In 2015, Highways England (formerly the Highways Agency) commissioned a feasibility study (AECOM, 2015a) to review existing literature, current legislation, and consult relevant stakeholders for a conservation translocation of little whirlpool ramshorn snail in and around the grazing marshes adjacent to the Acle Straight. The study considered the ecology of the species, the proposed method of translocation, and steps required to progress the project. A detailed description of the planned conservation translocation pilot study and each of the proposed project phases are described in the previously issued feasibility study (AECOM, 2015a).

An initial non-intrusive scoping survey was carried out in early August 2015 (AECOM, 2015b), to identify ditch systems with the greatest potential to support little whirlpool ramshorn snail. This was followed by a more detailed survey conducted in late August and early September 2015 (AECOM, 2015c). Data collected during the detailed survey included information on mollusc community assemblage, vegetation communities, ditch profiles, disturbance levels, adjacent land use, and some aspects of water chemistry. These data were collated and processed using multivariate analysis to refine knowledge of the habitat requirements of *Anisus vorticulus,* and to determine whether any particular vegetation and/or mollusc communities are associated with the species.

By shedding light on the habitat preferences of *Anisus vorticulus*, the multivariate analysis aided the selection of receptor sites for a pilot translocation of small populations in the spring of 2016 (see accompanying report for further details of translocation work). It is also hoped that, by identifying indicator species and preferred habitat types, the identification of areas likely to support *Anisus vorticulus* can be made more straightforward in the future. This could aid both the discovery of new populations of the species, and the selection of suitable areas for the establishment of new populations in further translocation projects / phases.

2 Methods

1.1 Data Collection

2.1.1 Ditch Assessment

The survey method was adapted from an existing protocol for assessing grazing marsh ditches for the presence of little ramshorn whirlpool snail (as described by Willing, 2014). Data and sample collection was conducted by a pair of surveyors, including an experienced on-site mollusc surveyor [redacted], Ecologist and National Mollusc Specialist) and a second team member responsible for recording ditch features, abiotic variables, and botanical diversity [redacted], Ecologist at Abrehart Ecology). The ditch characteristic and botanical diversity recording sheets were adapted from Buglife's manual for the survey and evaluation of grazing marsh ditch systems (Palmer et al., 2013); examples of the recording sheets used are presented in Appendix A.

At each sample location, ditch characteristics and a range of other environmental features were recorded (as in the 2015 survey, see AECOM 2015c for details). These included exposed and submerged bank profiles, channel width and depth, and levels of grazing, poaching, and shelving. Abiotic parameters were recorded in the surface 10cm of water including pH and conductivity (measured using a HI98129 pH/Conductivity Tester; Hanna Instruments), dissolved oxygen and temperature (measured using a PD0-520 Dissolved Oxygen metre; Lutron). Each sample point was recorded as a 10 figure grid reference using a handheld GPS and recorded on an Archer2 sub metre dGPS.

Mollusc community and botanical diversity were recorded at three points for each sample site, termed subsamples A-C. Subsample B formed the central point. Subsamples A and C were taken 15m on either side.

2.1.2 Aquatic Invertebrate and Mollusc Sampling

Mollusc community samples were collected at each of three sub-sampling points per sample location (as described above in Section 2.2). The mollusc community was assessed and recorded separately for each sub-sample point (thus giving three sets of data for each sample location). This aimed to gauge the consistency of the mollusc community throughout the linear environment of the ditches. A copy of the recording sheet is presented in Appendix A.

Samples were collected using ten-second sweeps of a net with 0.5mm mesh. Sweeps were repeated three times for each subsample in different sections of the ditch profile, i.e. floating vegetation (where present), the benthic layer and the submerged side of the near bank.

The material from the three sweeps was placed in a white gridded tray filled with water from the same ditch area. Molluscs were released from the collected vegetation by agitating the contents of the tray – excess vegetation was then removed. The floating contents of the tray (chiefly vegetation and larger invertebrate species) were poured out into a 1mm mesh net, with molluscs retained in the bottom of the tray; it is accepted that a small proportion of molluscs may be lost at this stage, but previous tests of this method have shown such losses to be negligible (T. Abrehart, pers. obs.). The material remaining was then evenly distributed across the tray for assessment.

Molluscs were identified to species level where possible (although some pea mussels could only be classified to genus level in the field i.e. *Pisidium*), and the relative abundance of each species was quantified using a DAFOR scale (Table 1). The abundance of notable and rare mollusc species, including *Anisus vorticulus*, shining ramshorn snail *Segmentina nitida*, slender amber snail *Oxyloma sarsi*, Desmoulin's whorl snail *Vertigo moulinsiana*, and the pea mussel species *Pisidium pseudosphaerium,* was fully quantified in addition to being categorised with the DAFOR scale. After quantification in the field, all the material was retained for further laboratory analysis.

2.1.3 Botanical Diversity Surveying

The bankside, emergent, floating and submerged flora of the ditch was recorded at each subsample point as per the 2015 survey (AECOM 2015c). The relative abundance of each floral species occurring within 5m of the subsample point was quantified using a DAFOR scale (Table 2). This included vegetation on both the nearside and opposite bank and up to 1 m from the water's edge. The recording sheet, a copy of which can be found in Appendix A, was adapted from Buglife's grazing marsh ditch survey and evaluation manual (Palmer et. al, 2013).

Table 1 DAFOR scale used in mollusc community recording.

Value	Descriptor	Estimation of abundance
D	Dominant	100+
A	Abundant	51-100
F	Frequent	21-50
O	Occasional	6-20
R	Rare	1-5

Table 2 DAFOR scale used in botanical recording

Value	Descriptor	Percentage cover
D	Dominant	>75%
A	Abundant	51-75%
F	Frequent	26-50%
O	Occasional	11-25%
R	Rare	1-10%

2.1.4 Multivariate Assessment of 2015 Datasets.

The data were analysed with the following objectives:

- To identify and define distinct mollusc communities (groups of species) across the study area;
- To identify and define distinct vegetation communities across the study area;
- To assess whether *Anisus vorticulus* is associated with any particular mollusc and/or vegetation communities; and
- To identify any indicator species/environmental factors which could assist with planning future management and surveying of *Anisus vorticulus*.

The composition of mollusc and vegetation communities was investigated using two methods:

- Classification analysis; and
- Ordination (Non-Metric Multi-Dimensional Scaling (NMMDS)).

These analyses use different methods to assess the presence and relative abundance of species at individual sample points, and then compare the species composition between sample points and finally group them into clusters according to similarity. The NMMDS analysis was used for thoroughness to strengthen and confirm the results from the initial classification analysis. Indicator species were identified within each plant and mollusc community – these are species which could be considered “typical” for a certain community i.e. show a particularly strong association with a certain group.

Once communities of plants and molluscs had been identified, an additional twin-axis ordination was used to show any associations (biotic and/or abiotic) with the abundance of *Anisus vorticulus*. This information was then used to refine the selection of receptor sites.

All analysis of the data collected during the 2015 surveys was performed by Physalia Ltd. All figures relating to the multivariate analysis are reproduced with permission from Physalia Ltd.

3 Results

3.1 Multivariate analysis: Mollusc Data

Guidance for interpreting the diagrams of results is provided in Appendix B. Species names have been abbreviated for clarity in figures; a key to abbreviations is provided in Appendix C.

3.1.1 Species-level Interactions

The classification analysis investigated relationships between molluscs at the individual species level and at the wider community level. When analysed at the species level, three main groups were identified. These included:

- A core group of species which were present at the majority of sample sites, including freshwater limpet *Acroloxus lacustris*, whirlpool ramshorn snail *Anisus vortex*, Lister's river snail *Viviparus connectus*, and ramshorn snail *Planorbis planorbis*;
- A more variable group with a patchier distribution, which included little whirlpool ramshorn snail *Anisus vorticulus*, shining ramshorn snail *Segmentina nitida,* slender amber-snail *Oxyloma sarsi,* and *Pisidium pseudosphaerium*; and,
- An additional group of species which were only sporadically present, including *Sphaerium nucleus*, Desmoulin's whorl snail *Vertigo moulinsiana*, marsh pond snail *Lymnaea fuscus,* and marsh whorl snail *Vertigo antivertigo*.

These species groupings may indicate similar habitat/environmental preferences between species.

3.1.2 Community-level Interactions

The analysis of mollusc community data identified eight distinct community clusters (A-H), which formed three main groups; A-D, E and F, and G and H (Fig. 1). The cumulative R^2 value for the ordination analysis was 80%, which indicates a very high level of confidence in the groupings.

Cluster A included some of the highest densities of *Anisus vorticulus*, while many of the most diverse mollusc communities were found in clusters G and H. Clusters E and F were relatively poor in their species richness and abundance, and showed a low level of association to other groups – they were therefore placed far from the other clusters after ordination analysis. *Anisus vorticulus* was placed between clusters A and H in the ordination plots (Fig. 2-3), indicating a level of association with the communities in both clusters. However, it was only identified as an indicator species for cluster H, suggesting that its strongest association is with those communities. *Segmentina nitida* and *Valvata cristata* were also identified as indicator species for cluster H (Table 3), and were closely associated with *Anisus vorticulus* in the species-level classification analysis. *Segmentina nitida* has also been previously suggested as associated with *Anisus vorticulus* (Willing & Killeen 1998; Terrier et al. 2006).

Other indicator species were identified for clusters A, B, C, G and H (see Table 3). However, no indicator species could be identified for clusters D and E; this may indicate a low level of stability in the communities within clusters D and E, potentially arising from environmental disturbance. By contrast, the identification of strong indicator species in the other clusters may be indicative of environmental stability.

When results from the ordination analysis were mapped, the community clusters identified formed spatially discrete areas (Fig. 4). This may indicate areas of the marshes which have similar environmental conditions.

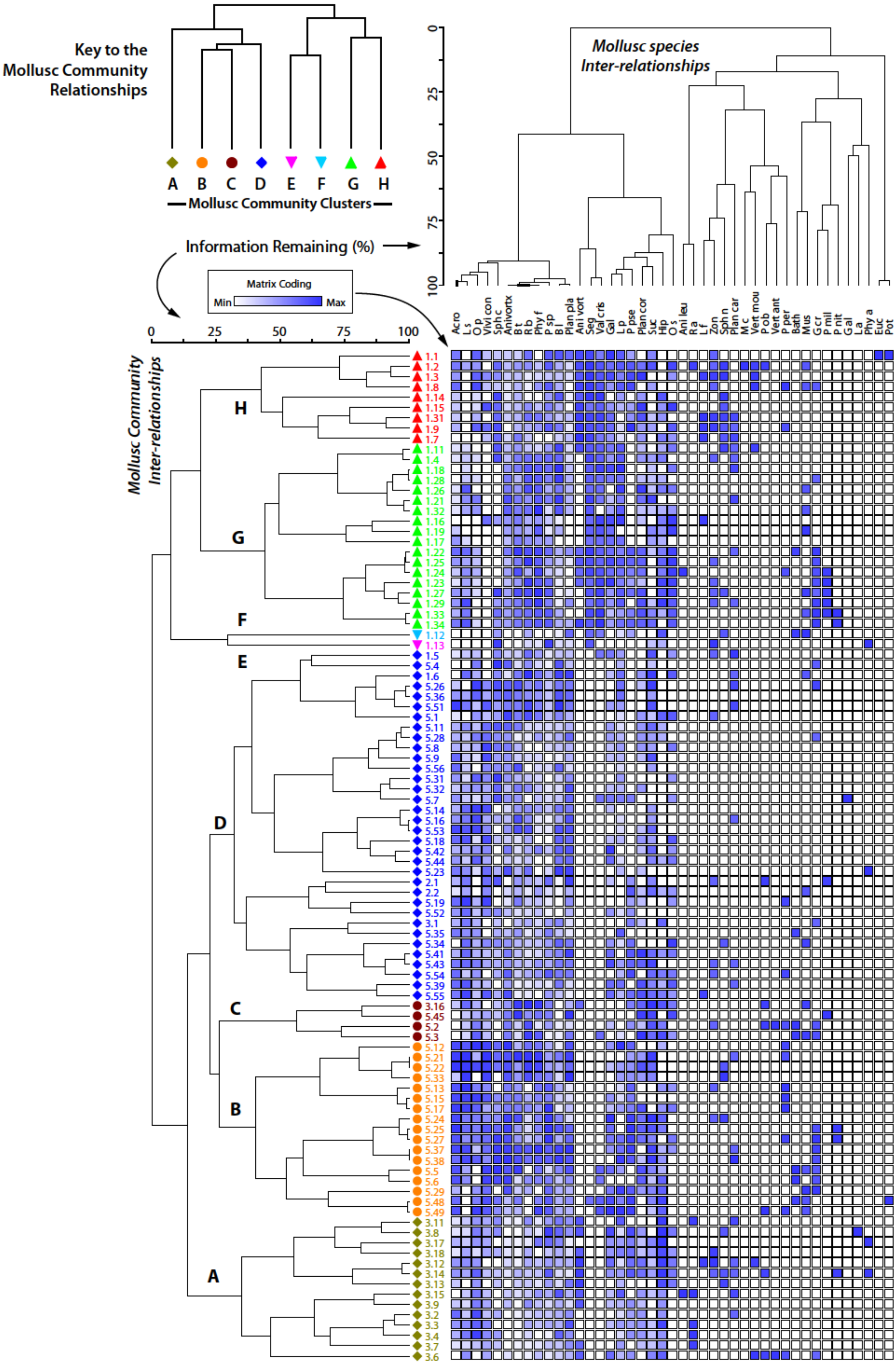


Figure 1. Dendrograms showing the relationships between mollusc species (top) and, separately, their communities (left). The matrix highlights the abundances of species in each community based on the species present and their relative abundances. Darkest blues indicate highest mollusc densities whilst lowest densities/failure to identify species in a given sample are recorded as empty cells. The mollusc community classification dendrogram (left-hand side) identified 8 distinctive assemblages (Clusters A to H)

Ordination Analyses of Mollusc Communities; Axes 1 and 2

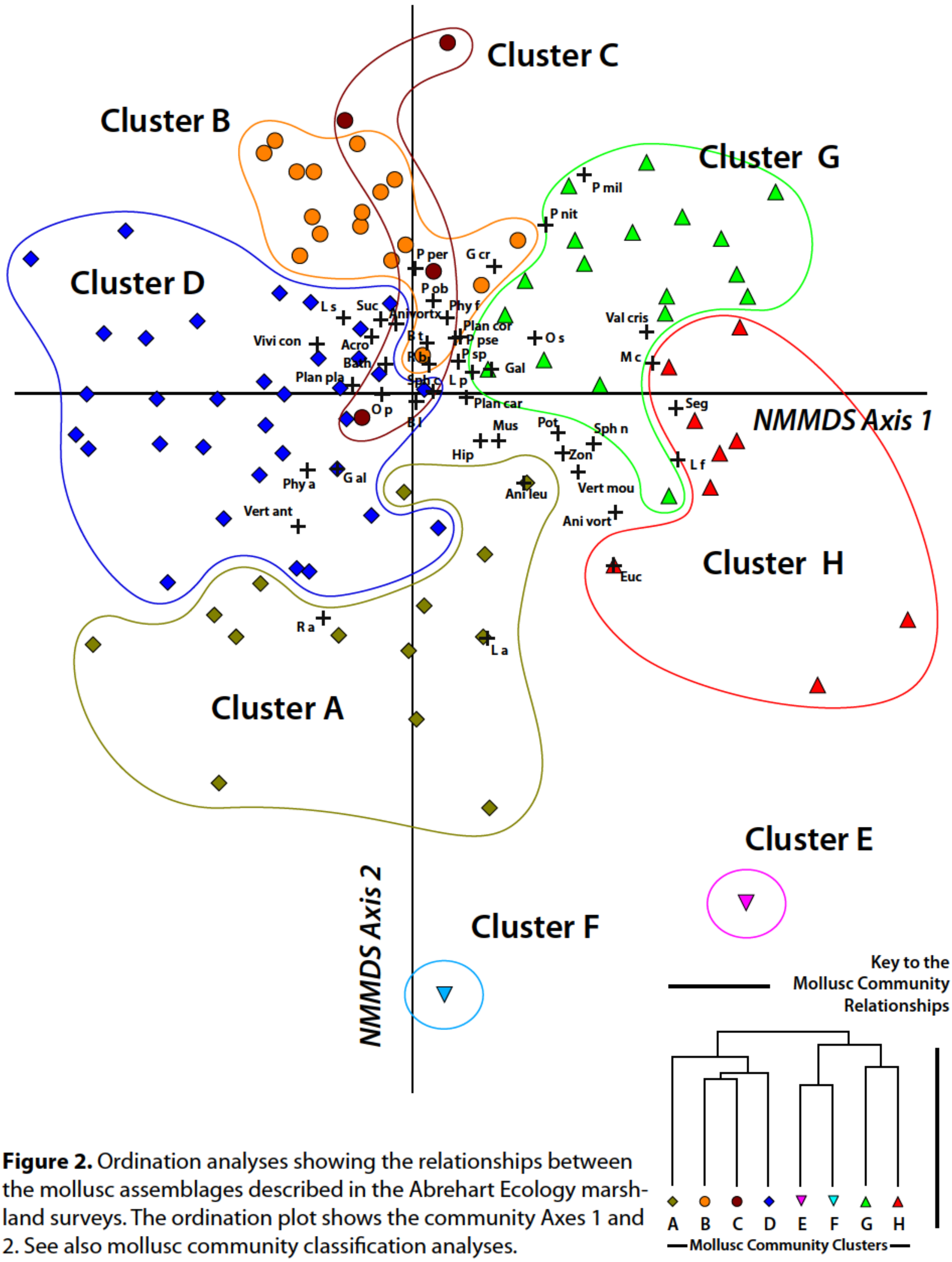
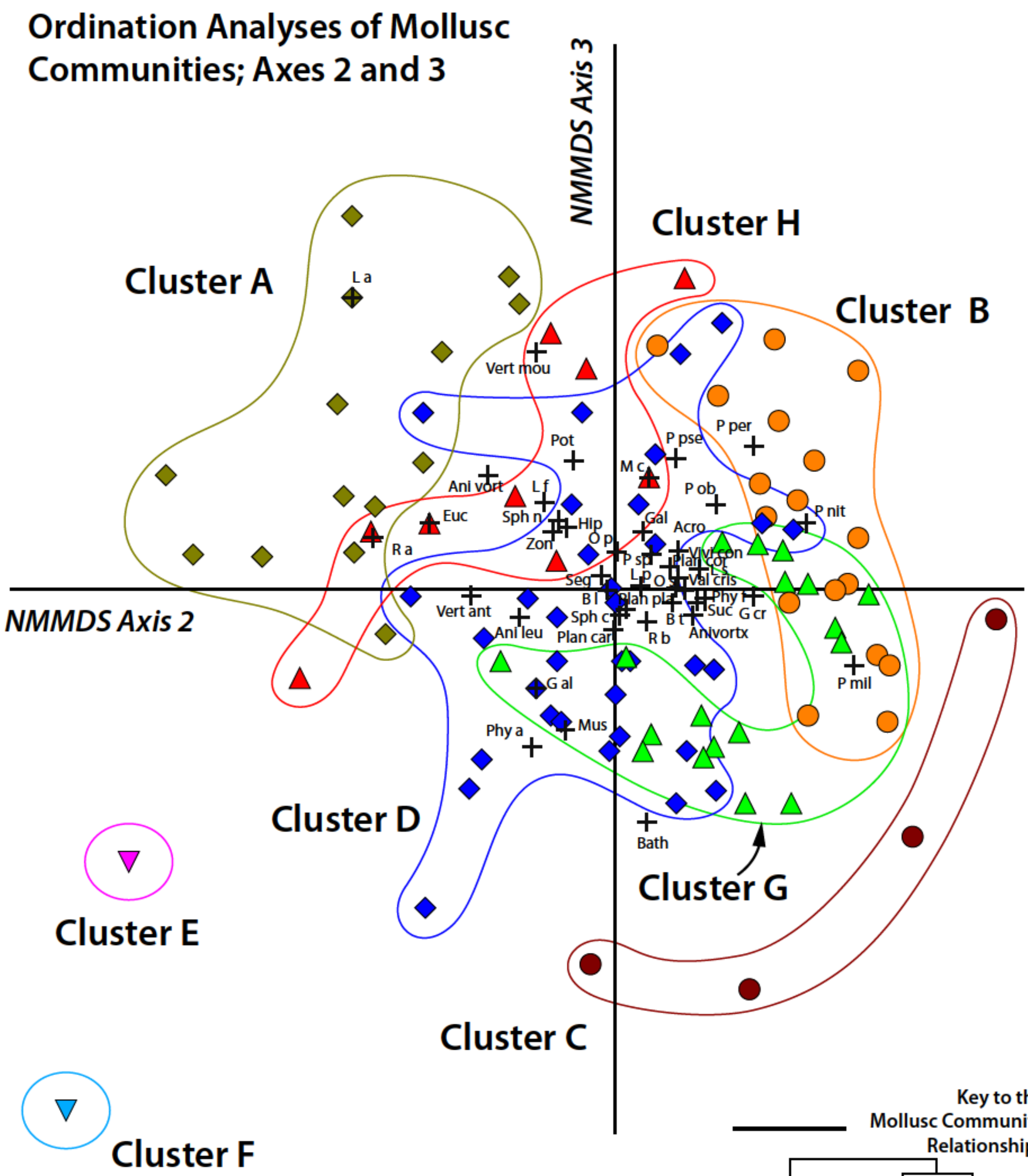


Figure 2. Ordination analyses showing the relationships between the mollusc assemblages described in the Abrehart Ecology marshland surveys. The ordination plot shows the community Axes 1 and 2. See also mollusc community classification analyses.



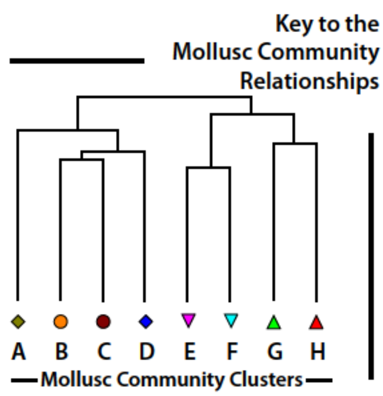


Figure 3. Ordination analyses showing the relationships between the mollusc assemblages described in the Abrehart Ecology marshland surveys. The ordination plot shows the community Axes 2 and 3. See also mollusc community classification analyses.

Species Code	Species Name	Cluster Number	Cluster Letter	IV	Mean	S.dev	*p*-Value
Hip	*Hippeutis complanatus*	35	**A**	36.7	19.1	4.90	0.0086
R a	*Radix auricularia*	35	**A**	29.2	8.6	5.48	0.016
Acro	*Acroloxus lacustris*	52	**B**	37.4	20.5	2.69	0.0002
Vivi con	*Viviparus connectus*	52	**B**	40.8	19.4	3.92	0.0002
Ani vortx	*Anisus vortex*	52	**B**	28.9	19.9	2.06	0.0004
L s	*Lymnaea stagnalis*	52	**B**	27.5	20.5	3.29	0.0378
P sp	*Pisidium* species	52	**B**	23.5	19.9	1.74	0.040
P pse	*Pisidium pseudosphaerium*	52	**B**	29.3	17.9	5.12	0.0418
Suc	*Succinea putris*	40	**C**	41.0	19.8	4.18	0.0002
R b	*Radix balthica*	40	**C**	26.1	19.8	1.51	0.0018
Bath	*Bathyomphalus contortus*	40	**C**	35.3	9.3	6.33	0.011
Plan cor	*Planorbarius corneus*	40	**C**	31.8	19.8	4.32	0.018
P ob	*Pisidium obtusale*	40	**C**	30.1	9.1	6.07	0.0192
Mus	*Musculum lacustris*	40	**C**	26.7	11.5	6.42	0.0354
Phy f	*Physa fontinalis*	2	**G**	25.8	19.9	2.19	0.0112
L p	*Lymnaea palustris*	2	**G**	29.1	20.3	3.03	0.0122
B t	*Bithynia tentaculata*	2	**G**	21.1	19.1	1.05	0.0446
O s	*Oxyloma sarsi*	2	**G**	28.6	17.4	5.49	0.0458
Ani vort	***Anisus vorticulus***	**1**	**H**	**60.0**	**13.6**	**6.02**	**0.0002**
Seg	*Segmentina nitida*	1	**H**	65.1	14.0	6.07	0.0002
Sph n	*Sphaerium nucleus*	1	**H**	51.7	11.4	6.34	0.0014
Val cris	*Valvata cristata*	1	**H**	42.6	14.6	5.64	0.0032
L f	*Stagnicola fuscus*	1	**H**	35.4	8.8	5.60	0.0096
Vert mou	*Vertigo moulinsiana*	1	**H**	22.4	8.8	5.85	0.0286
Sph c	*Sphaerium corneum*	1	**H**	28.8	19.6	4.11	0.0352

Table 3. Summary outputs from the indicator species analyses (ISAs) for the mollusc species identified and described in the Abrehart Ecology marshland surveys. All mollusc species listed here were found to be statistically significantly correlated with their respective clusters (i.e. A, B, C, F and G). Peak indicator values are shown in bold. Note that Clusters D and E were defined by unique combinations of species and did not yield single, cluster-specific indicator species. **p*-Values relate to the associations between indicator mollusc species and their respective clusters. Data derived using Monte Carlo permutation tests.

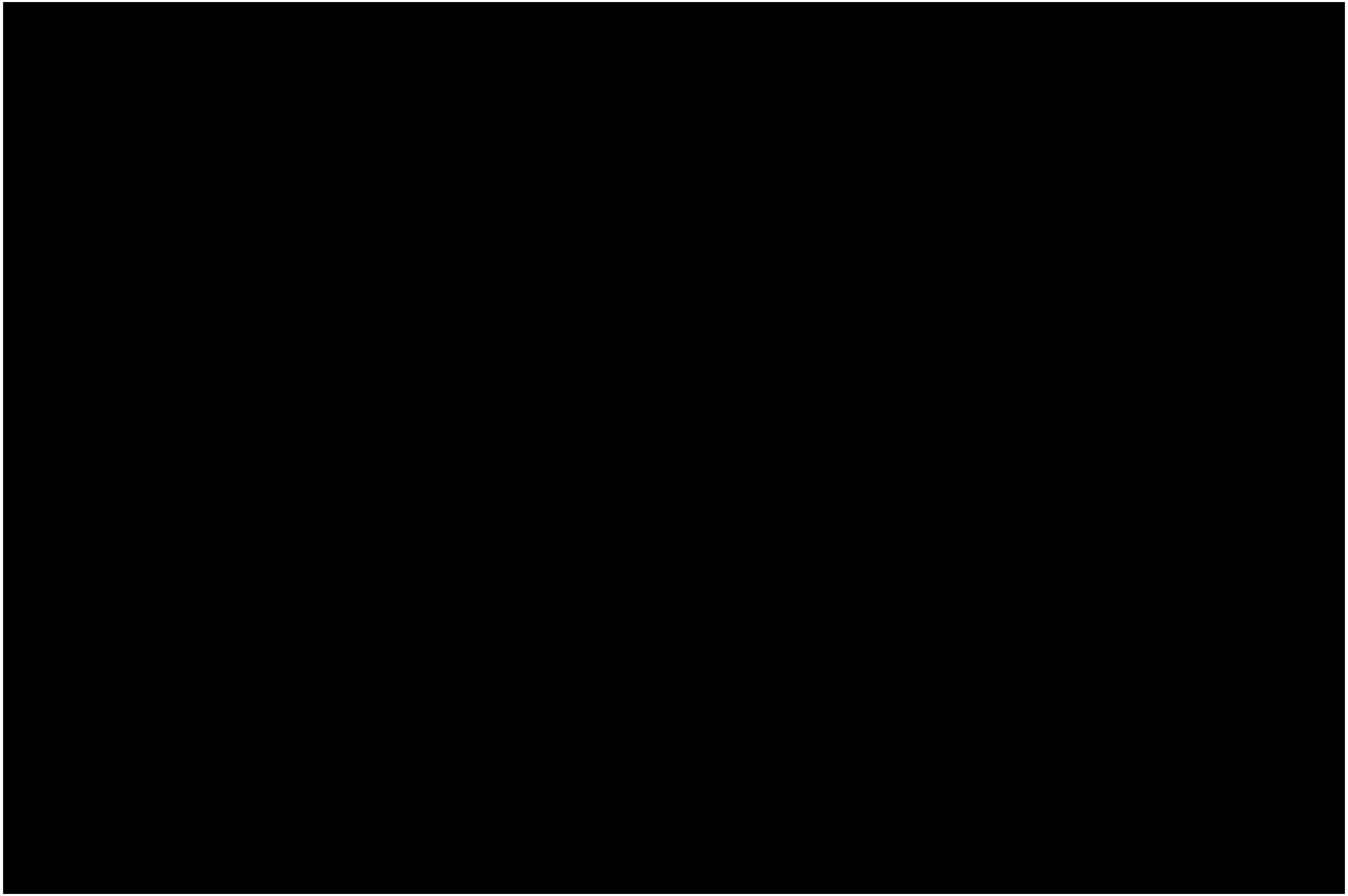


Figure 4. Locations of mollusc community clusters identified by multivariate analysis. Symbols match those shown in the NMMDS ordination diagrams.

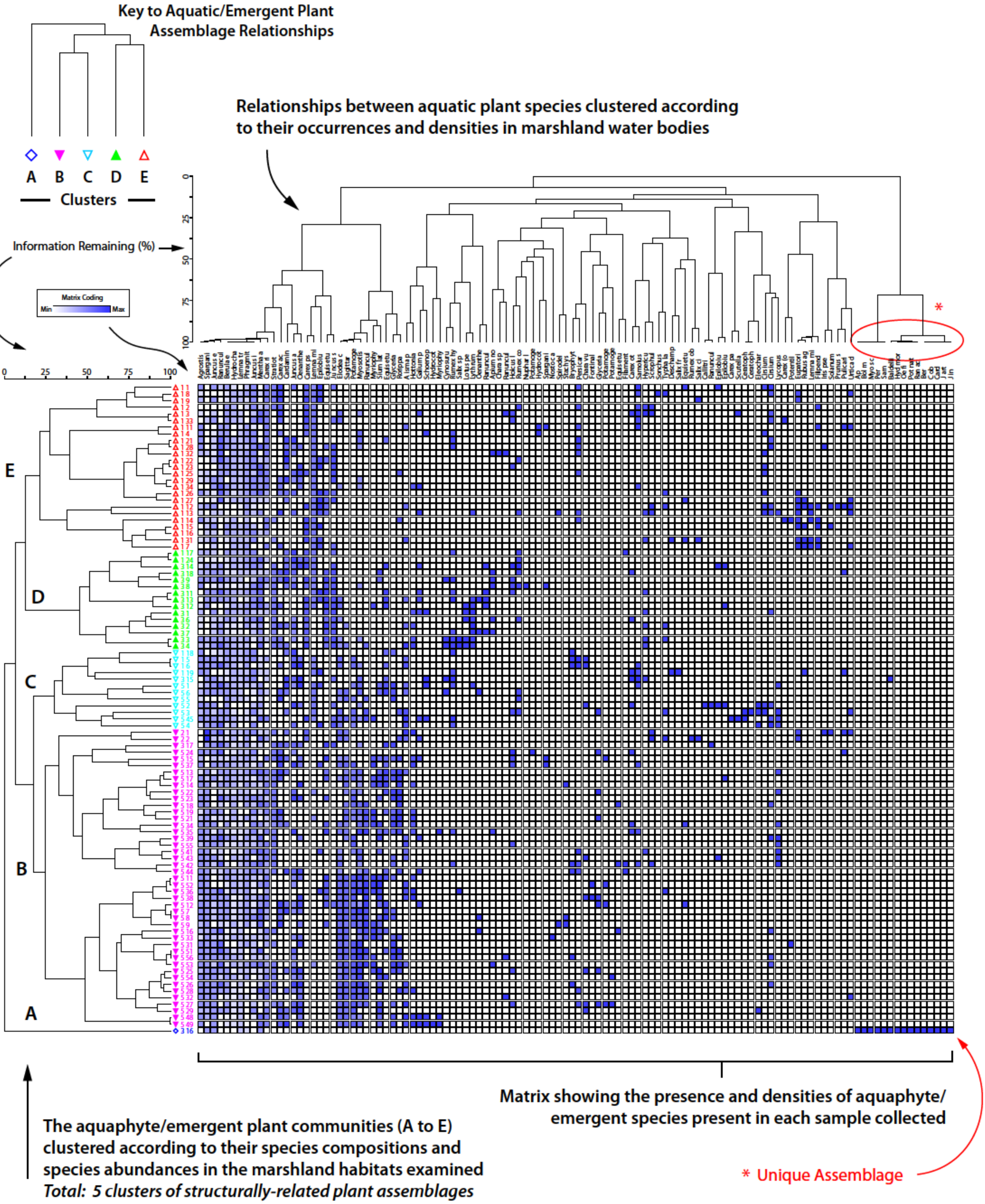
3.2 Multivariate Analysis: Vegetation Communities

Guidance for interpreting the diagrams of results is provided in Appendix B. Species names have been abbreviated for clarity in figures; a key to abbreviations is provided in Appendix C.

The analysis of vegetation communities highlighted five distinct clusters of communities. One of these clusters (Cluster A) contained only a single community with a unique botanical assemblage including fool's water-cress *Apium nodiflora*, lesser water-plantain *Baldellia ranunculoides*, brookweed *Samolus valerandi,* sea club-rush *Bolboscheonus maritima* and jointed rush *Juncus articulates* (Fig. 5).

The remaining four clusters formed distinct areas in the ordination analysis, with the clusters forming two pairs: B and C, and D and E (Fig. 6-7). Clusters B and C were distinguished by the presence of plants in the genera *Elodea, Sagittarius, Ranunculus, Equisetum,* and *Glyceria* among others – these were absent or very infrequent in the communities within clusters D and E. The final twin axis ordination analysis showed a strong association of *Anisus vorticulus* with vegetation community clusters D and E, but little to no association with clusters B and C. Indicator species identified for clusters D and E included blunt-flowered rush *Juncus subnodulosus* and water soldier *Stratiotes aloides,* and lesser duckweed *Lemna minor*, marsh horsetail *Equisetum palustris* and hemp-agrimony *Eupatorium cannabinum*, respectively (Table 4).

As with the mollusc community data, when the vegetation community clusters were mapped they formed spatially discrete areas (Fig. 8), potentially indicating areas of marsh with similar prevailing environmental conditions.


Key to Aquatic/Emergent Plant Assemblage Relationships
A B C D E
Clusters
Relationships between aquatic plant species clustered according to their occurrences and densities in marshland water bodies
Information Remaining (%)
Matrix Coding
Min
Max
0 25 50 75 100
E
D
C
B
A
Matrix showing the presence and densities of aquaphyte/ emergent species present in each sample collected
* Unique Assemblage
The aquaphyte/emergent plant communities (A to E) clustered according to their species compositions and species abundances in the marshland habitats examined
Total: 5 clusters of structurally-related plant assemblages

Ordination Analyses of Aquatic Flora; Axes 1 and 2

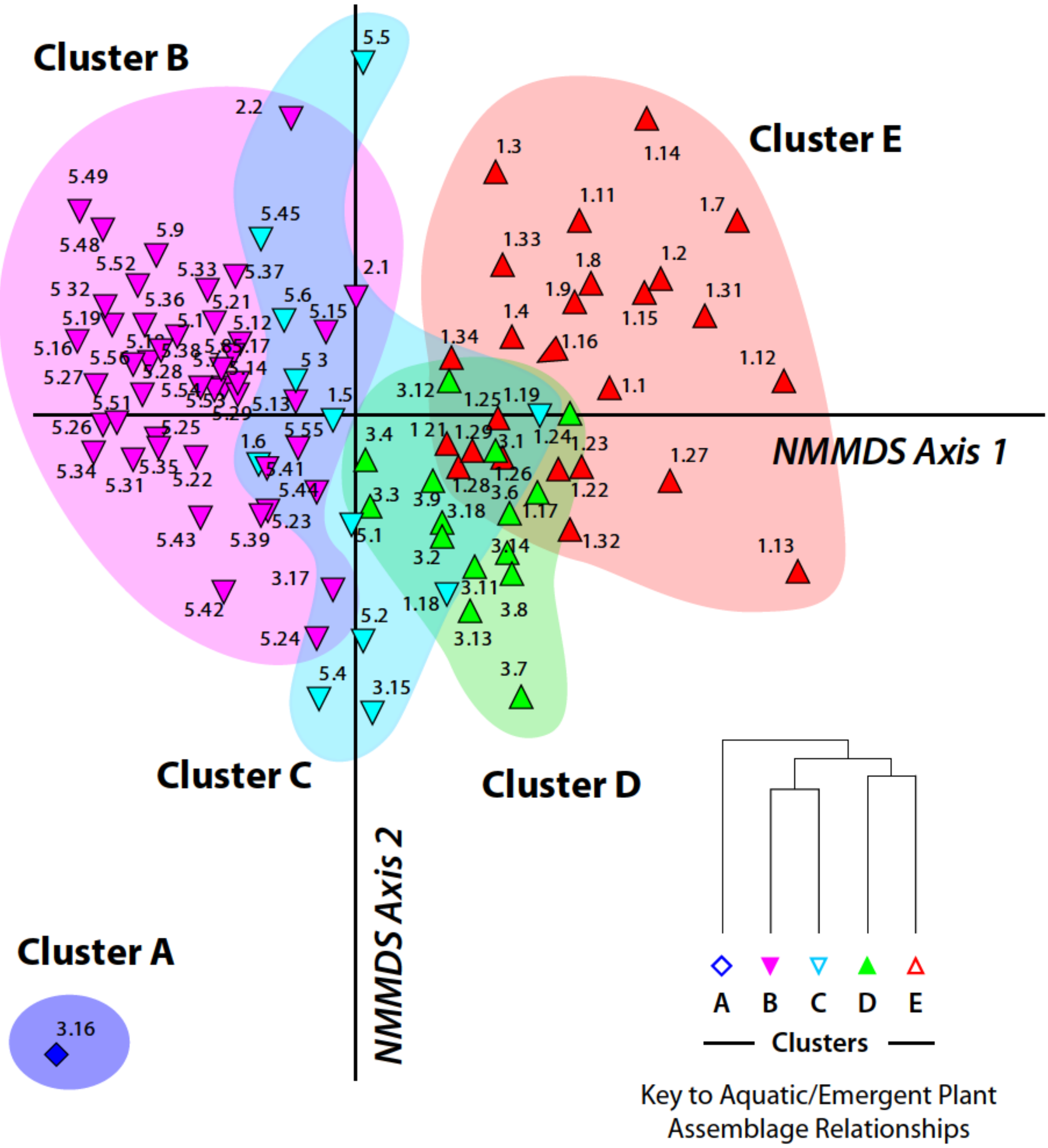


Figure 6. Ordination analyses (employing non-metric, multi-dimensional scaling (NMMDS)) showing the relationships between the aquatic plant assemblages described in the marsh-land mollusc surveys undertaken by Abrehart Ecology. This summary plot shows the community relationships with reference to ordination NMMDS Axes 1 and 2. See also floral ordination analyses.

Ordination Analyses of Aquatic Flora; Axes 1 and 3

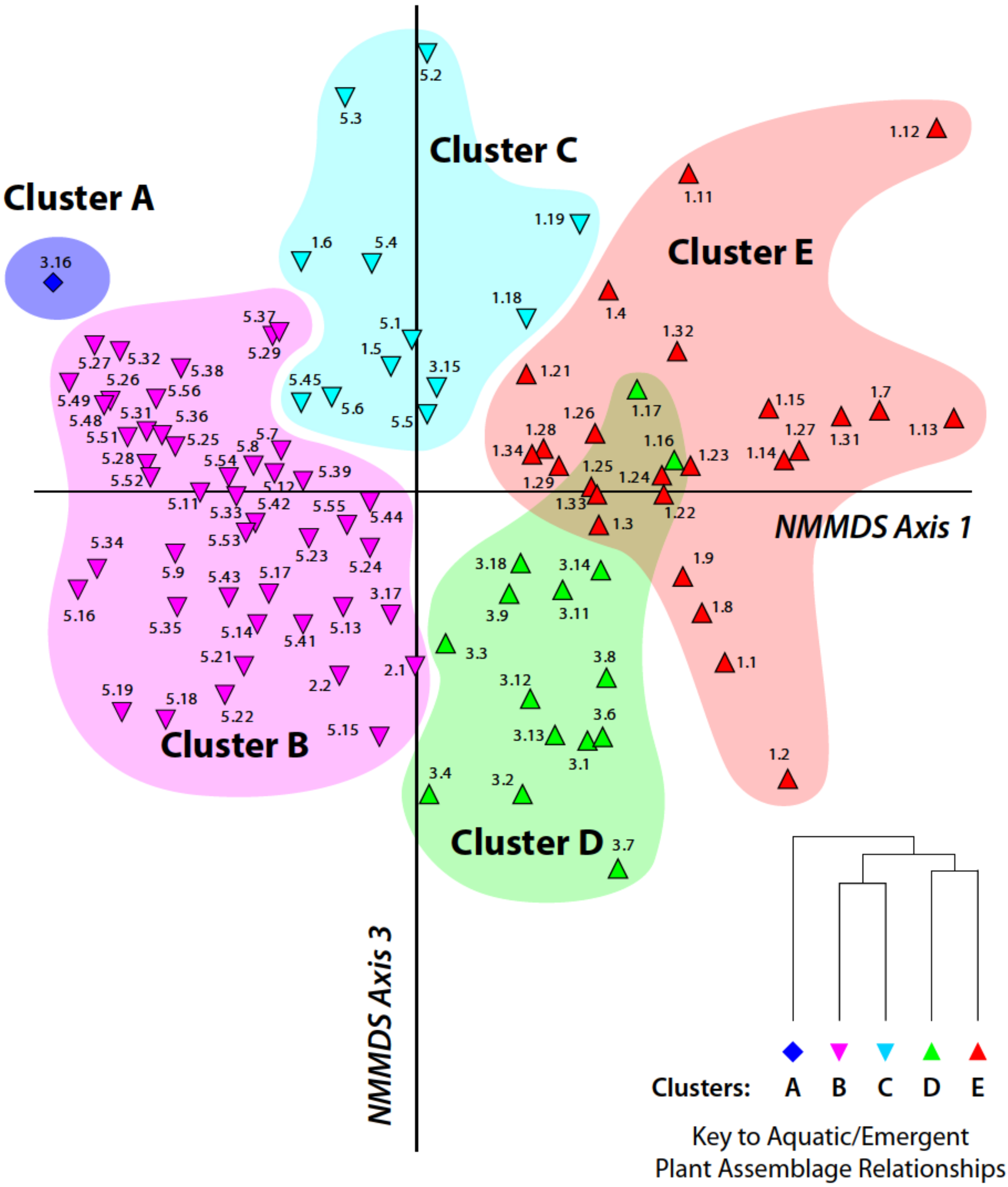


Figure 7. Ordination analyses (employing non-metric, multi-dimensional scaling (NMMDS)) showing the relationships between the aquatic plant assemblages described in the marshland mollusc surveys undertaken by Abrehart Ecology. This summary plot shows the community relationships with reference to ordination Axes 1 and 3. See also floral ordination analyses for Axes 2 and 3.

Species Code	Mathematical Indicator Species	Cluster Number	Cluster Letter	IV	Mean	Std. Dev.	*p*-Value*
Myosotis	*Myosotis scorpioides*	32	**B**	44.1	19.7	3.97	0.0002
Potamoge	*Potamogeton natans*	32	**B**	46.5	17.2	4.37	0.0002
Sagittar	*Sagittaria sagittifolia*	32	**B**	**56.5**	17.9	4.17	0.0002
Spargani	*Sparganium erectum*	32	**B**	36.6	24.2	3.1	0.0008
Sium lat	*Sium latifolium*	32	**B**	41.5	14.9	4.6	0.001
Rorippa	*Rorippa nasturtium-aquaticum*	32	**B**	35.9	12.6	4.48	0.0012
Ranuncul	*Ranunculus circinatus*	32	**B**	34.7	14	4.51	0.0028
Elodea c	*Elodea canadensis*	32	**B**	34.1	19.7	4.01	0.0046
Glyceria	*Glyceria maxima*	32	**B**	31.0	14.3	4.47	0.0062
Myriophy	*Myriophyllum spicatum*	32	**B**	23.1	11.1	4.31	0.0196
Agrostis	*Agrostis stolonifera*	32	**B**	31.4	25.8	2.47	0.0262
Eleochar	*Eleocharis palustris*	9	**C**	23.9	5 7	3.38	0.0022
Bryophyt	Bryophytes	9	**C**	19.3	6.9	3.72	0.0102
Ceratoph	*Ceratophyllum demersum*	9	**C**	16.7	4.9	2.54	0.014
Chara vu	*Chara vulgaris*	9	**C**	19.2	7 5	3.82	0.0148
Alisma p	*Alisma plantago-aquatica*	9	**C**	**27.6**	14.5	4.36	0.0156
Rumex hy	*Rumex hydrolapathum*	9	**C**	23.4	11.2	4.25	0.0162
Schoenop	*Schoenoplectus tabernaemontani*	9	**C**	17.7	7.1	3.96	0.0244
Lycopus	*Lycopus europaeus*	9	**C**	19.2	9 5	4.17	0.038
Carex ot	*Carex otrubae*	9	**C**	16.9	7.9	3.97	0.0408
Carex ps	*Carex pseudocyperus*	8	**D**	45.4	14.8	4.39	0.0002
Equisetu	*Equisetum palustre*	8	**D**	53.3	15	4.29	0.0002
Juncus s	*Juncus subnodulosus*	8	**D**	**56.1**	15	4.33	0.0002
Lotus pe	*Lotus pedunculatus*	8	**D**	33.3	6.1	3.55	0.0002
Lythrum	*Lythrum salicaria*	8	**D**	46.2	8 3	3.9	0.0002
Stratiot	*Stratiotes aloides*	8	**D**	46.3	19.1	4.07	0.0002
Ranuncul	*Ranunculus lingua*	8	**D**	33.3	6 2	3.8	0.0004
Apium no	*Apium nodiflora*	8	**D**	24.4	7 5	3.92	0.0064
Rumex co	*Rumex conglomeratus*	8	**D**	22.3	8.9	3.96	0.0088
Mentha a	*Mentha aquatica*	8	**D**	33.7	25.2	3.06	0.0126
Hypericu	*Hypericum tetrapterum*	8	**D**	17.4	8 5	3.91	0.0382
Salix sp	*Salix* species	8	**D**	12.5	5 3	2.99	0.0428
Lemna mi	*Lemna minor*	1	**E**	**43.5**	18.8	4.09	0.0002
Eupatori	*Eupatorium cannabinum*	1	**E**	38.1	9.1	4.22	0.0006
Carex ri	*Carex riparia*	1	**E**	35.9	24.5	3.03	0.0014
Filipend	*Filipendula ulmaria*	1	**E**	28.0	7.1	3.96	0.0024
Lemna mi	*Lemna minuta*	1	**E**	20.0	6	3 7	0.0122
Epilobiu	*Epilobium hirsutum*	1	**E**	26.2	11.9	4.4	0.0138
Cirsium	*Cirsium arvense*	1	**E**	20.4	8 2	3.91	0.0174
Urtica d	*Urtica dioica*	1	**E**	18.2	7.9	3.96	0.0228
Rubus ag	*Rubus* agg.	1	**E**	15.4	6 5	3.69	0.0314

Table 4. Summary outputs from the indicator species analyses (ISAs) of the plant communities that were identified and described in the Abrehart Ecology marshland snail surveys. All plant species listed here were statistically significantly correlated with their respective clusters (i.e. B to E) and peak values are shown in bold. **p*-Values relate to the associations between indicator plant species and their respective clusters. Data derived using Monte Carlo permutation test methods.

[redacted]

Figure 8. Locations of vegetation community clusters identified in multivariate analysis. Symbols match those from NMMDS ordination diagrams.

1.2 Associations with *Anisus vorticulus*

A final twin-axis ordination analysis tested the densities of *Anisus vorticulus* populations against the five vegetation community clusters that were initially identified. This indicated that the densities of *Anisus vorticulus* were substantially higher in areas containing vegetation communities from clusters D and E (Fig. 9).

When plotted onto a map (Fig. 8), these two vegetation community clusters formed distinct spatial areas which overlapped with mollusc community clusters A and H (Fig. 4). Mollusc community clusters A and H were the most strongly associated with the presence of *Anisus vorticulus* and other closely grouped species such as *Segmentina nitida*. The results therefore indicate that *Anisus vorticulus* can be positively associated with certain vegetation and mollusc communities, and with certain mollusc indicator species such as *Segmentina nitida.*

Donor ditches were based largely on locations where *Anisus vorticulus* was found in the highest densities. However, based on the evidence described here, receptor ditches were selected in areas where suitable vegetation and mollusc communities overlapped, but where *Anisus vorticulus* was currently absent (Fig. 10).

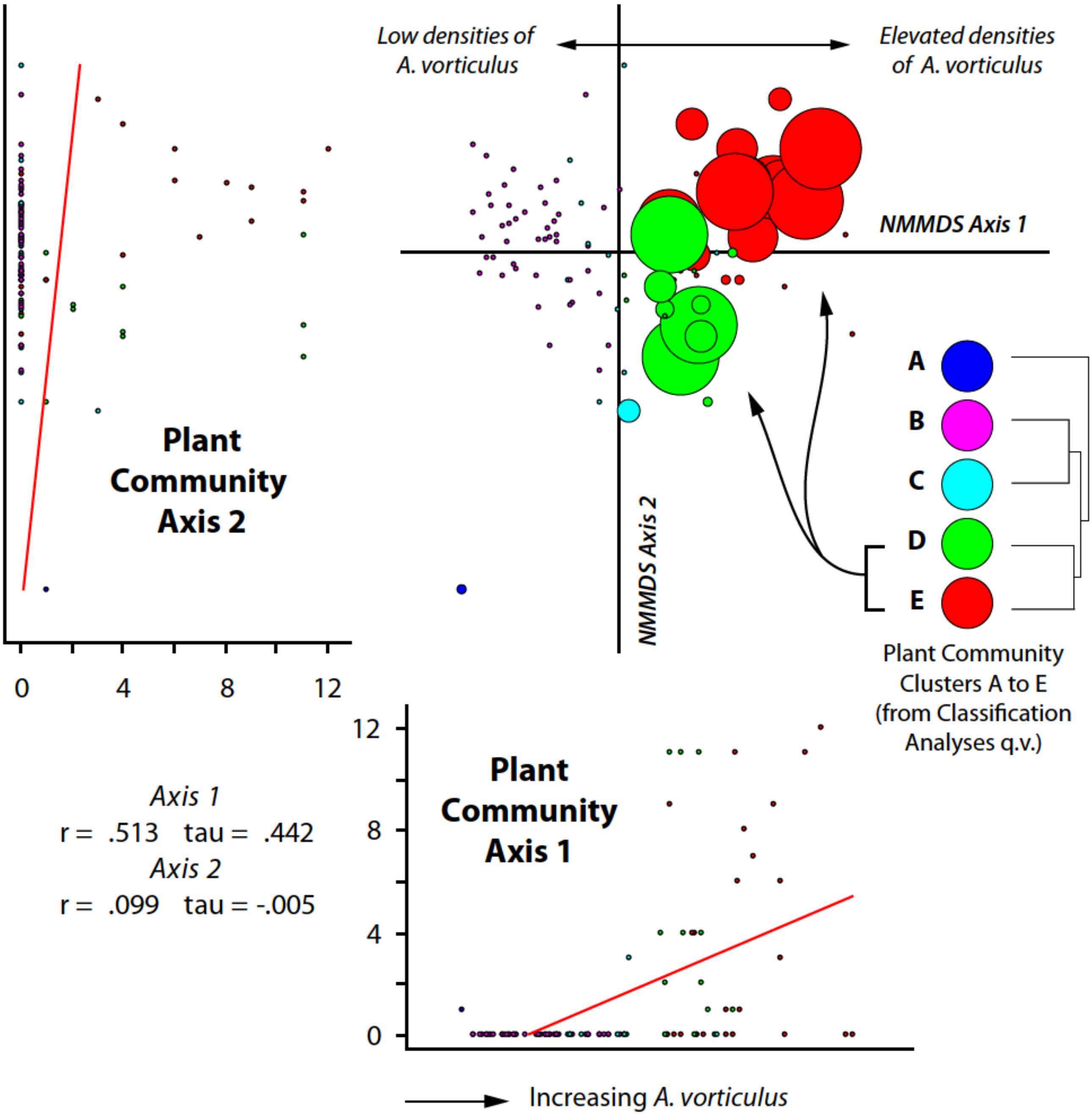


Figure 9. Twin-axis, multivariate ordination analyses of the Abrehart Ecology plant community data identifying 5 structurally distinctive clusters of botanical assemblages (Clusters A to E; based on survey data). Relationships between individual botanical clusters are highlighted in the colours derived from the classification analyses. Here, the circular symbols have also been scaled to reflect the densities of *Anisus vorticulus*. Note that Clusters D and E (green and red symbols, respectively) identify sites with elevated densities of *A. vorticulus* (particularly so in the case of Cluster E). Accordingly, these data analyses should provide a valuable pointer leading to the definition of the biotic and abiotic environmental factors that favour this particular, protected mollusc species.

[redacted]

Figure 10. Final locations of donor and receptor sites

4 Discussion

Overall, the multivariate analyses suggested that *Anisus vorticulus* can be associated with particular mollusc and vegetation communities, and that these communities can be identified using certain indicator species. This facilitated the selection of the most suitable donor ditches for the translocation of three *Anisus vorticulus* populations in Norfolk in the summer of 2016. The results from this analysis will inform selection of sites for future phases of translocation.

The communities of molluscs and vegetation that were most closely associated with *Anisus vorticulus* formed distinct, overlapping spatial areas when plotted onto a map, and it is believed that this may represent areas of habitat with similar and reasonably constant environmental conditions. Further investigation of the vegetation communities contained in clusters D and E, and comparisons with communities from other vegetation community clusters, may shed more light on preferences of *Anisus vorticulus* for certain plants/vegetation densities. This could in turn aid the future rapid identification of site suitable for *Anisus vorticulus,* potentially speeding up the discovery of new populations and identification of receptor sites for future translocation work. Surveys for sites suitable for *Anisus vorticulus* could also focus on mollusc species such as *Segmentina nitida* and *Valvata cristata*, which were statistically found to occur in the same mollusc communities and may therefore have similar environmental requirements.

Preliminary tests relating mollusc communities to environmental variables suggest that factors such as the presence and variety of emergent vegetation, the level of disturbance by trampling (quantified by levels of poaching and shelf formation along ditches), and the type of land use adjacent to ditches, all play a role in determining the types of mollusc and vegetation communities that develop. As stated, this part of the analysis was intended only as a broad indication of which environmental factors may influence mollusc communities (and hence *Anisus vorticulus* distribution). Further, more detailed analyses would give a greater understanding of the implications of disturbance and land use, and could potentially inform management decisions. This would be particularly effective if the data collected in 2016 were included in the analysis, as this would incorporate an aspect of temporal variation that is not currently considered.

4.1 Future Work

The multivariate analysis reported here was achieved using data from the initial 2015 survey (AECOM 2015c), and highlighted some strong associations of *Anisus vorticulus* with particular vegetation and mollusc communities, and identified some potential indicator species which could be used to identify suitable receptor sites for future conservation translocation projects. It would be useful to repeat the analysis to include data from the 2016 survey, as this would allow annual variability in population levels to be taken into account. Initial observations from surveys in 2016 suggest that abundance of *Anisus vorticulus* may fluctuate between years, particularly in the [redacted] (see associated pilot translocation report), but this would need to be confirmed with additional repeat surveys at the same sample points.

A limitation of the surveys conducted in 2015 was that the water chemistry parameters measured were limited to pH, conductivity, temperature and dissolved oxygen - aquatic invertebrates are intrinsically sensitive to water chemistry and it is likely that a greater range of chemical parameters influence their distributions. A fuller analysis of water chemistry in relation to mollusc distributions, particularly for *Anisus vorticulus*, would therefore be highly relevant. For example, it has been suggested that *Anisus vorticulus* is intolerant of eutrophic conditions (reviewed by Terrier et al. 2006) – a better understanding of chemical constraints on the species may therefore inform habitat management decisions for areas around translocation sites and existing populations. Water samples were collected by Abrehart Ecology during the 2016 surveys, and analysis results are expected soon – these data could be included with multivariate analysis, and provide a more detailed picture of the factors determining *Anisus vorticulus* distributions.

Further surveys are planned for September / October 2016, which will include sites in Norfolk and Suffolk known to currently support populations of *Anisus vorticulus,* as well as assessing potential receptor sites for further translocations (see accompanying translocation pilot report for further details of future survey locations). The surveys will use the same data collection methods as detailed in section 2.1.1-2.1.3, and will yield data which could be incorporated with the existing dataset for further multivariate analysis. Using data from additional sample sites will be valuable when considering *Anisus vorticulus* at the entire species level (as opposed to individual population level), as it may highlight differences between populations in terms habitat preferences, chemical tolerances, and species associations. This is highly relevant when making conservation recommendations for the species as a whole.

5 Acknowledgements

Thanks to Physalia Ltd. For assistance with multivariate analysis and figure production. Thanks also to [redacted] for granting access to survey sites, and for their support and interest in the project.

6 References

This report to be cited as: AECOM/Abrehart Ecology, 2016. Translocation of the little whirlpool ramshorn snail: multi-variate community analysis 2016. Report to Highways England.

AECOM.,2015a. Feasibility study on the translocation of the little whirlpool ramshorn snail. Report to Highways

Agency.

AECOM, 2015b. Translocation of the little whirlpool ramshorn snail – scoping survey. Report to Highways Agency.

AECOM, 2015c. Translocation of the little whirlpool ramshorn snail – detailed surveys. Report to Highways Agency.

English Nature, 2000. Norfolk Biodiversity Action Plan Little Whirlpool Ram's-horn Snail (*Anisus vorticulus*), Available at: http://www.norfolkbiodiversity.org/actionplans/speciesactionplans/littlewhirlpoolsnail.aspx. (updated 2012).

Glöer, P. & Groh, K., 2007. A contribution to the biology and ecology of the threatened species *Anisus vorticulus* (Troschel , 1834) (Gastropoda : Pulmonata : Planorbidae). Mollusca, 25(1), pp.33–40.

Palmer, M., Drake, M., Stewart, N. 2013. A manual for the survey and evaluation of the aquatic plant and invertebrate assemblages of grazing marsh ditch systems. Pages 10-11. Version 6. Buglife, Peterborough.

Terrier et al. 2006. Species account for *Anisus vorticulus* (Troschel,1834) (Gastropoda: Planorbidae), a species listed in Annexes II and IV of the Habitats Directive. *Journal of Conchology* 39:2, pp 193-206.

Van Damme, D. 2012. *Anisus vorticulus*. The IUCN Rd List of Threatened Species 2012: e.T155966A738056. http://dx.doi.org/10.2305/IUCN.UK.2012-1.RLTS.T155966A738056.en . Downloaded on 20 October 2015.

Willing, M.J., 2014. A full survey of ditches on RSPB Pulborough Brooks for the Little Whirlpool Ram's-horn Snail *Anisus vorticulus*: (November 2013 / July 2014), Report to RSPB.

Appendix A

Abiotic Data Recording Sheet

Date	
Site ID	
Ditch no.	
Photo(s)	
Grid ref.	
Side A	
Side B	

Water features	
pH	
Conductivity (mS)	
D.O.	
Temp	
Water colour	

	Adjacent Land use	
	A	B
Improved grassland		
Semi-improved grassland		
Unimproved grassland		
Arable		
Swamp/Fen		
Drove		
Cattle/horse grazed		
Sheep grazed		
Hay/Silage		
Stockproof boundary		
Temporary fencing		
Spoil on bank		

	Bank vegetation (DAFOR)	
	A	B
Tall grass/reed		
Short grass		
Bare ground		
Tall herbs		
Overhanging vegetation		
Scrub <1.5m		
Fen		
Woodland ground flora		
Shaded (%)		

	Vegetation cover	
	DAFOR	Absent
Open water surface		
Floating Lemna/Azolla		
Other floating aquatics		
Floating algae		
Lemna trisulca		
Other submerged plants		
Submerged algae		
Open substrate		
Emergent		
Low swamp/Floating mat		
Exposed vegetation		
Exposed mud		
Litter / detritus		
Shaded		
Emergents/floating mat in channel %		

Ditch Features										
Water width (m)	Bank top width (m)	Freeboard (cm)	Water depth (cm)	Silt depth (cm)	Slope - bank A	Slope - bank B	Profile under water A	Profile under water B	Substrate	Turbidity
0-1	0-2	0-25	0-25	0-25	0-15	0-15	0-15	0-15	Clay	Clear
1-2	2-4	26-50	25-50	25-50	16-30	16-30	16-30	16-30	Alluvial	Slight
2-3	4-6	51-100	51-75	51-75	31-55	31-55	31-55	31-55	Peat	Mod
3-4	6-8	100-200	76-100	76-100	56-70	56-70	56-70	56-70	Sand	Heavy
4+	>10	>200	>100	>100	71-90	71-90	71-90	71-90	Gravel	

	Grazing/vegetation structure							
	None		Low		Med		High	
	A	B	A	B	A	B	A	B
Grazing								
Poaching								
Block formation								
Shelf formation								
Tangledness								
Grassy margin								

Management					
Years since last cleared	Not known	1	2-3	4-10	>10
Water relative to normal (cm)	Not known	+	-	Normal?	
Cleared to side	A	B			
Benched profile	A	B			
Cleared by					

NOTES

AECOM Mollusc Data Recording Sheet

Site: Date:

	Subsample (counts)		
Mollusc species	A	B	C
Acroloxus lacustris			
Anisus leucostoma			
Anisus vortex			
Anisus vorticulus			
Bathyomphalus contortus			
Bithynia leachii			
Bithynia tentaculata			
Galba truncatula			
Gyraulus albus			
Gyraulus crista			
Hippeutis complanatus			
Lymnaea fuscus			
Lymnaea palustris			
Lymnaea stagnalis			
Musculum lacustris			
Oxyloma pfeiferi			
Oxyloma sarsi			
Physa acuta			
Physa fontinalis			
Pisidium milium			
Pisidium nitidum			
Pisidium personatum			
Pisidium pseudosphaerium			
Pisidium sp			
Planorbarius corneus			
Planorbis carinatus			
Planorbis planorbis			
Potamopyrgus antipodarum			
Radix auricula			
Radix balthica			
Segmentina nitida			
Sphaerium corneus			
Sphaerium nucleus			
Succinea putris			
Valvata cristata			
Valvata macrostoma			
Valvata piscinalis			
Vertigo moulinsiana			
Viviparous sp.			
Viviparus connectus			
Zonitoides nitidula			

AECOM Vegetation Data Recording Sheet

Site:

Date:

Emergent plants

	Subsample (DAFOR)				Subsample (DAFOR)		
Species	**A**	**B**	**C**	**Species**	**A**	**B**	**C**
Agrost stol				Thal flav			
Alisma lance				Trifol prat			
Alisma plant				Trifol rep			
Alopec genic				Typha ang			
Angelic sylv				Typha lati			
Apium nodif				Urtica dioica			
Apium rep				Veron caten			
Berula erect				Vicia cracca			
Butom umbel							
Carex acutif							
Carex otrub							
Carex pseud							
Carex ripar							
Cirsium pal							
Dactyl glom							
Eleoch pal							
Elytrig repen							
Epilob hirsut							
Epilob parvi							
Equiset fluv							
Eupator can							
Festuc rub							
Filipend ulm							
Galium palus							
Glycer fluit							
Glycer max							
Holcus lanat							
Iris pseudac							
Juncus artic							
Juncus bufo							
Juncus effus							
Juncus inflex							
Lathyr prat							
Lolium pere							
Lotus pedun							
Lycop europ							
Lythrum sali							
Mentha aqua							
Myosot laxa							
Myosot scor							
Oenan aqu							
Oenan fist							
Phragm aust							
Plant lanceo							
Poa trivialis							
Potentil ans							
Ran acris							
Ran flammu							
Ran sceler							
Rorip nas ag							
Rumex hydol							
Rumedx obtus							
Salix ciner							
Salix fragi							
Salix sp.							
Samolus val							
Schoen tab							
Scroph aur							
Scutel galer							
Solan dulca							
Sparg erect							
Stachys pal							

Aquatic plants (submerged-leaves)

	Subsample (DAFOR)		
Species	**A**	**B**	**C**
Callit brut			
Callit obtus			
Callit platy			
Callit stag			
Cerat dem			
Cerat subm			
Chara vulg			
Elodea can			
Elodea nutt			
Filam alg			
Front anti			
Hottonia pal			
Myriop spic			
Myriop vert			
Potam berch			
Potam crisp			
Potam natan			
Potam pect			
Potam pus			
Potam trich			
Ran aqu agg			
Ran circ			
Sagit sag			
Sparg emers			
Sparg erect			
Zannic palus			

Floating leaved plants

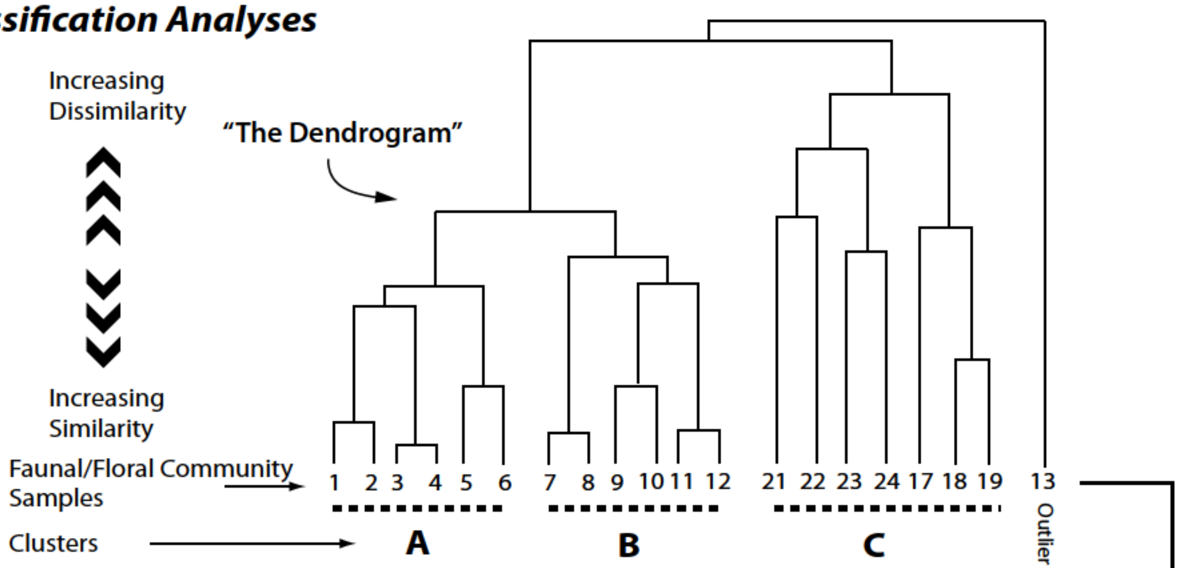
	Subsample (DAFOR)		
Species	**A**	**B**	**C**
Azolla filicu			
Hydroch mor			
Hydroco ran			
Hydroco vul			
Lemna gibba			
Lemna minor			
Lemna minut			
Lemna trisul			
Nuphar lut			
Nymph alba			
Persic amph			
Spiro polyr			
Stratio alo			
Wolff arrh			

Appendix B

A Guide to Summary Outputs from the Multivariate Analyses

Classification Analyses

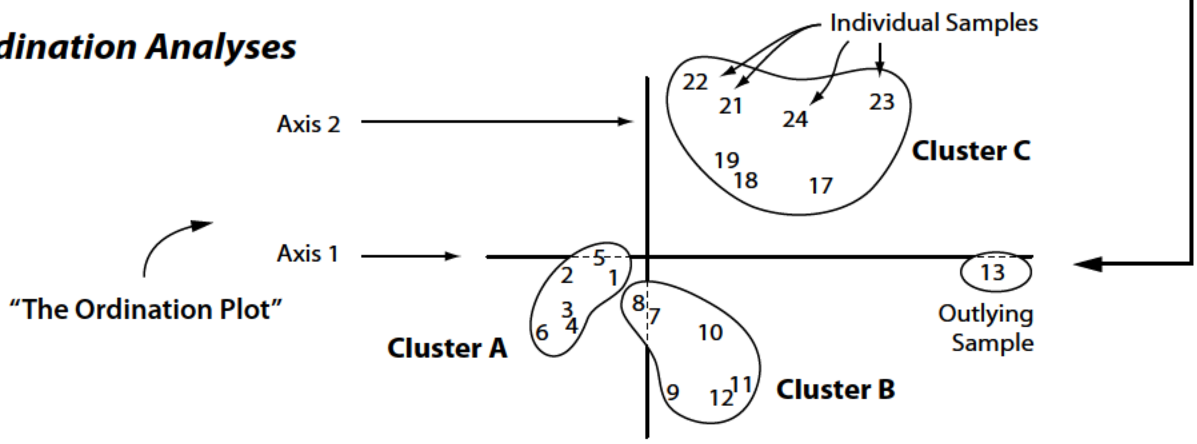
First Cycle of Analyses



Classification analyses, the greater the similarity between the samples (based here on their species and their relative abundances) the closer they are related to one another and *vice versa*. This is shown in the different lengths of inter-connecting lines. Here, the biological samples 1 to 6 form Cluster A and are structurally ("ecologically") more similar to each other than they are to samples 7 to 12 (Cluster B). In turn, Cluster A and B samples are more closely related to each other than they are to those samples in Cluster C. Sample 13 is highly distinctive and forms an "outlier". In this report we use also use <u>two-way classification analyses</u> for biological data. These analyses group the species together based on the samples in which they occur and the densities at which they were present. This produces a matrix that helps in the interpretation of mollusc species distributions amongst samples in the marshland sites.

Ordination Analyses

Second Cycle of Analyses



Ordination Analyses award scores to each sample based on multiple community variables (i.e. species present in each sample and their densities/abundances). The scores are used to spread samples along two (or more) "axes of variation" such that structurally-similar samples are grouped together whilst dissimilar samples are separated by greater distances. This approach uses an entirely different mathematical methodology to the classification analyses. Ordination analyses is therefore used to test the findings of the classification analyses; a process called "coherence testing". Once confirmed, clusters can be plotted onto maps or displayed in 3-dimensional reconstructions to aid interpretation of how controling factors shape communities. This technique is also valuable as a means of tracking changes over time e.g. in post-habitat works surveys or in post-incident investigations.

Fingerprinting

Indicator and Multivariate Correlation Analyses identify measured environmental factors whose presence and concentrations/magnitudes are statistically significantly correlated with community structures. Performed using Monte Carlo permutation tests, these define statistically significant "controlling factors" that can identify/track changes and their causes in biological communities.

Appendix C

Key to the Marshland Mollusc Species Recorded in the Abrehart Ecology Surveys

Species Code	Scientific Name	Species Code	Scientific Name
Acro	*Acroloxus lacustris*	P ob	*Pisidium obtusale*
Ani leu	*Anisus leucostoma*	P per	*Pisidium personatum*
Ani vort	*Anisus vorticulus*	P pse	*Pisidium pseudosphaerium*
Ani vortx	*Anisus vortex*	P sp	*Pisidium* species
B l	*Bithynia leachii*	Phy a	*Physa acuta*
B t	*Bithynia tentaculata*	Phy f	*Physa fontinalis*
Bath	*Bathyomphalus contortus*	Plan car	*Planorbis carinatus*
Euc	*Euconulus alderi*	Plan cor	*Planorbarius corneus*
G al	*Gyralus albus*	Plan plan	*Planorbis planorbis*
G cr	*Armiger crista*	Pot	*Potamopyrgus antipodarum*
Gal	*Galba truncatula*	R a	*Lymnaea auricularia*
Hip	*Hippeutis complanatus*	R b	*Radix balthica*
L a	*Lymnaea auricularia*	Seg	*Segmentina nitida*
L f	*Stagnicola fuscus*	Sph c	*Sphaerium corneum*
L p	*Lymnaea palustris*	Sph n	*Sphaerium nucleus*
L s	*Lymnaea stagnalis*	Suc	*Succinea putris*
M c	*Monacha cantiana*	Val cris	*Valvata cristata*
Mus	*Musculum lacustris*	Vert ant	*Vertigo antivertigo*
O p	*Oxyloma pfeifferi*	Vert mou	*Vertigo moulinsiana*
O s	*Oxyloma sarsi*	Vivi con	*Viviparus connectus*
P mil	*Pisidium milium*	Zon	*Zonitoides nitidus*
P nit	*Pisidium nitidum*		

List of the molluscan species (Gastropoda and Bivalvia) recorded as part of the Abrehart Ecology survey of marshland mollusc species with particular reference to the presence, distributions and relative abundances of protected species.

Key to the Botanical Species Recorded in the Abrehart Ecology Marshland Surveys

Species Code	Scientific Name	Species Code	Scientific Name	Species Code	Scientific Name	Species Code	Scientific Name	Species Code	Scientific Name
Agrostis	*Agrostis stolonifera*	Cynosuru	*Cynosurus cristatus*	Hydrocha	*Hydrocharis morsus-ranae*	Nuphar l	*Nuphar lutea*	Rumex hy	*Rumex hydrolapathum*
Alisma p	*Alisma plantago-aquatica*	Deschamp	*Deschampsia cespitosa*	Hydrocot	*Hydrocotyle ranunculoides*	Oenanthe	*Oenanthe aquatica*	Rumex ob	*Rumex obtusifolius*
Apium no	*Apium nodiflora*	Eleochar	*Eleocharis palustris*	Hydrocot	*Hydrocotyle vulgaris*	Oenanthe	*Oenanthe fistulosa*	Sagittar	*Sagittaria sagittifolia*
Baldelli	*Baldellia ranunculoides*	Elodea c	*Elodea canadensis*	Hypericu	*Hypericum tetrapterum*	Persicar	*Persicaria amphibia*	Salix ci	*Salix cinerea*
Berula e	*Berula erecta*	Epilobiu	*Epilobium hirsutum*	Iris pse	*Iris pseudacorus*	Phragmit	*Phragmites australis*	Salix fr	*Salix fragilis*
Bryophyt	Bryophytes	Epilobiu	*Epilobium palustre*	Juncus a	*Juncus articulatus*	Potamoge	*Potamogeton berchtoldii*	Salix sp	*Salix* sp.
Callitri	*Callitriche stagnalis*	Epilobiu	*Epilobium parviflorum*	Juncus e	*Juncus effusus*	Potamoge	*Potamogeton coloratus*	Samolus	*Samolus valerandi*
Cardamin	*Cardamine flexuosa*	Equisetu	*Equisetum arvense*	Juncus i	*Juncus inflexus*	Potamoge	*Potamogeton friesii*	Schoenop	*Schoenoplectus tabernaemontani*
Carex (o	*Carex* (other)	Equisetu	*Equisetum fluviatile*	Juncus s	*Juncus subnodulosus*	Potamoge	*Potamogeton natans*	Scrophul	*Scrophularia auriculata*
Carex ac	*Carex acutiformis*	Equisetu	*Equisetum palustre*	Lemna mi	*Lemna minor*	Potentil	*Potentilla anserina*	Scutella	*Scutellaria galericulata*
Carex ot	*Carex otrubae*	Equisetu	*Equisetum* spp.	Lemna mi	*Lemna minuta*	Prunus s	*Prunus spinosa*	Sium lat	*Sium latifolium*
Carex pa	*Carex paniculata*	Eupatori	*Eupatorium cannabinum*	Lemna tr	*Lemna trisulca*	Pulicari	*Pulicaria dysenterica*	Solanum	*Solanum dulcamara*
Carex ps	*Carex pseudocyperus*	Filament	Filamentous algae	Lotus pe	*Lotus pedunculatus*	Ranuncul	*Ranunculus acris*	Sonchus	*Sonchus palustris*
Carex ri	*Carex riparia*	Filipend	*Filipendula ulmaria*	Lycopus	*Lycopus europaeus*	Ranuncul	*Ranunculus circinatus*	Spargani	*Sparganium emersum*
Ceratoph	*Ceratophyllum demersum*	Fontinal	*Fontinalis antipyretica*	Lythrum	*Lythrum salicaria*	Ranuncul	*Ranunculus flammula*	Spargani	*Sparganium erectum*
Ceratoph	*Ceratophyllum submersum*	Galium p	*Galium palustre*	Mentha a	*Mentha aquatica*	Ranuncul	*Ranunculus lingua*	Spirodel	*Spirodela polyrhiza*
Chara sp	*Chara* sp.	Glyceria	*Glyceria fluitans*	Myosotis	*Myosotis scorpioides*	Ranuncul	*Ranunculus sceleratus*	Stachys	*Stachys palustris*
Chara vu	*Chara vulgaris*	Glyceria	*Glyceria maxima*	Myriophy	*Myriophyllum spicatum*	Rorippa	*Rorippa nasturtium-aquaticum*	Stratiot	*Stratiotes aloides*
Cirsium	*Cirsium arvense*	Holcus l	*Holcus lanatus*	Myriophy	*Myriophyllum verticillatum*	Rubus ag	*Rubus* agg.	Typha la	*Typha latifolia*
Cirsium	*Cirsium palustre*	Hottonia	*Hottonia palustris*	Nostoc a	*Nostoc algae*	Rumex co	*Rumex conglomeratus*	Urtica d	*Urtica dioica*

List of the plant species recorded by staff members at Abrehart Ecology as part of the assessment programme for marshland mollusc species with particular reference to protected species