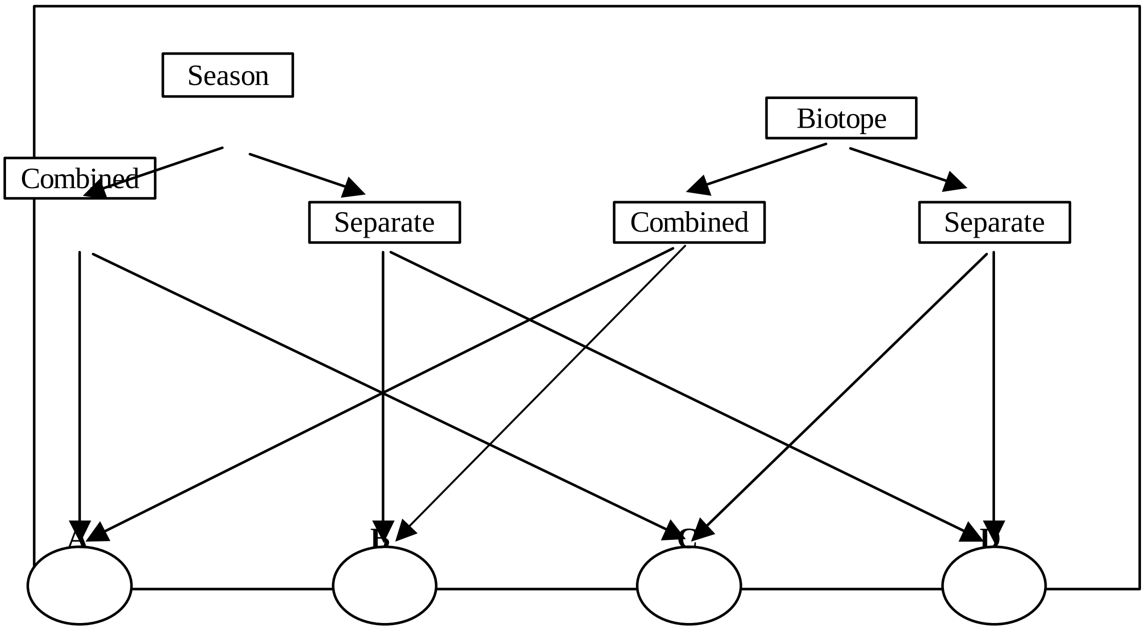

Figure 4.4 Possible combinations for data analysis incorporating biotope availability and season as potential factors of variability.

Three biotope-groups were used for examining the influence of separate- versus combined-biotope sampling on both invertebrate communities and SASS Scores. The biotope-groups were:

- stones-in-current + stones-out-of-current (SIC+SOOC),
- aquatic + marginal vegetation (AQV+MV), and
- gravel + sand + mud (G+S+M).

It was decided to use these biotope-groups as SASS practitioners strongly suggest that these are the maximum number of separate biotope-groups that can realistically be sampled in routine biomonitoring.

Multivariate analysis was run on seasonally-combined, invertebrate communities for each separate biotope-group within each Reference Group. In order to examine the influence of separate-biotope sampling on resultant invertebrate communities and SASS Scores, the following was undertaken:

- Separate-biotope site groups were compared with the Reference Groups established when taxa from all biotopes were combined.
- The frequency of occurrence of each SASS taxon amongst biotope-groups was examined.
- SASS Scores from separate-biotope sampling were compared to those from combined-biotope sampling within each Reference Group.
- Mean and median SASS Scores from each biotope-group were compared.
- The number of additional taxa recorded per biotope-group was calculated so that the effect of the number of biotope-groups sampled could be assessed.
- The Invertebrate Habitat Assessment Matrix (IHAS) and its contribution to the interpretation of SASS Scores was examined.

4.3.6 Seasonal variation in invertebrate communities and SASS Scores

Multivariate analysis was run on combined-biotope, invertebrate communities for each season (autumn, winter and spring) within each Reference Group. In order to examine the influence of separate-season sampling on resultant invertebrate communities and SASS Score, the following was undertaken:

- Separate-season groups were compared with the Reference Groups established when taxa from all seasons were combined.
- The frequency of occurrence of each SASS taxon amongst seasons was examined.
- SASS Scores from each season were compared to those from combined seasons within each Reference Group.
- Mean and median SASS Scores from each season were compared.
- The number of additional taxa recorded per season was calculated so that the effect of the number of sampling visits on invertebrate communities could be assessed.

4.4 RESULTS

4.4.1 Invertebrate data: composite reference communities

During the process of analysing the data as described in section 4.3.3, certain reference sites regularly separated from others both within and across ecoregions and sub-regions. These sites have been excluded in the final site classification. Possible reasons varied from site to site and included unusual substratum composition, poor biotope quality and quantity, undetected water quality impacts and lack of perenniality. Some sites nonetheless appeared to be excellent candidates for reference sites and yet they were classified as outliers on the basis of multivariate analysis. It was considered appropriate to exclude such sites even though the reason for their separation from other sites was unknown. Analysis of composite reference communities for the remaining 57 sites resulted in the classification of sites into three Reference Groups.

4.4.1.1 Cluster analysis and multi-dimensional scaling (MDS)

On the basis of cluster analysis of invertebrate data, all sites were at least 60% similar to each other (Figure 4.5). Three Reference Groups were apparent, with two sub-groups present in two of these Reference Groups:

- Reference Group 1 comprised a mixture of Mountain Stream (MS) and Foothill-cobble Bed (FC) sites within the Central Highlands Ecoregion (H). Sites in this group were at least 70% similar.
- Reference Group 2 comprised mostly MS and FC sites, together with one Rejuvenated Cascade (RC) site, within the Great Escarpment Mountain Ecoregion (E). Three FC sites from H, one MS, 2 FC and 2 RC sites from the Lowveld Ecoregion (L) grouped with Reference Group 2 sites. Sites in this Reference Group were at least 68% similar. A sub-group, 2a, consisting of two FC and two RC sites of L and a FC site of E, split off from Reference Group 2 at the 69% similarity level. They grouped at 73% similarity.
- Reference Group 3 comprised Foothill-gravel Bed (FG) sites, one FC site, one RC site and a Rejuvenated Foothill (RF) site of the Lowveld Ecoregion. Sites in this Reference Group were 63%

similar. A sub-group, 3a, consisting of a three Lowveld sites, a FG, FC and RC site, split off from Reference Group 3 at the 64% similarity level. Whilst sub-group 3a is only represented by three sites, it does appear to have different invertebrate communities to Reference Group 3, and has therefore been considered a separate sub-group in subsequent discussions.

The MDS ordination (Figure 4.6), displayed in two-dimensional space, supports the classification of sites into three groups. Stress level was 0.23 in 2-D space and 0.16 in 3-D space. The sub-groups are interspersed with other sites in the ordination suggesting a less clear differentiation into the sub-groups. This is discussed further in section 4.4.2.

From these multivariate analysis, it seems that in general, sites classified on the basis of ecoregional boundaries, as follows:

- Of the 14 Central Highlands sites assessed, 11 grouped together to form Reference Group 1.
- Of the 26 Great Escarpment Mountain sites, 25 grouped together to form Reference Group 2, whilst one grouped with four Lowveld sites to form sub-group 2a.
- For both the Central Highlands and Great Escarpment Mountain ecoregions, sub-regions appeared not to be important and there was no separation of sites on the basis of sub-regions for these ecoregions. Almost all sites in these ecoregions were in the Mountain Stream or Foothill-cobble Bed sub-regions.
- Lowveld sites were distributed over all three Reference Groups, and classification appeared to be dependent on sub-region, substratum composition, in particular the percentage of bedrock, and biotope availability.
- The single Mountain Stream site, whilst designated a Lowveld site on the basis of ecoregions, was at an altitude of 900 m, had > 90% boulder/cobble substratum and approximately 80% SIC/SOOC biotope. On the basis of the invertebrate community, it grouped with Mountain Stream sites of the Great Escarpment Mountain Ecoregion.
- Foothill-cobble bed sites and Rejuvenated Cascade sites of the Lowveld ecoregion, which had no bedrock, > 60% boulder/cobble, < 40% gravel/sand/mud and > 70% SIC/SOOC biotope, formed a sub-group, 2a, of Reference Group 2.
- The nine Foothill-gravel Bed sites grouped together at 60% similarity and were all at < 600 m altitude, 4^{th} or 5^{th} order, > 50 km from the source, > 10m wide, had a range of substratum types and all biotopes were present. The SIC/SOOC biotope comprised cobble riffle, bedrock rapid or both.
- Three of the Lowveld sites, including one Foothill-gravel Bed site, the Foothill-cobble Bed site and Rejuvenated Cascade site, separated from the other nine sites to form a sub-group, 3a. These sites were bedrock-dominated (≅ 65%), with approximately 10% boulder/cobble, and 20% sand. The SIC/SOOC biotope was scarce and was limited to a bedrock rapid when present, and both AQV/MV and GSM biotopes were present in relatively large percentages.

More detailed information is provided on the environmental variables responsible for group differentiation in section 4.4.2.

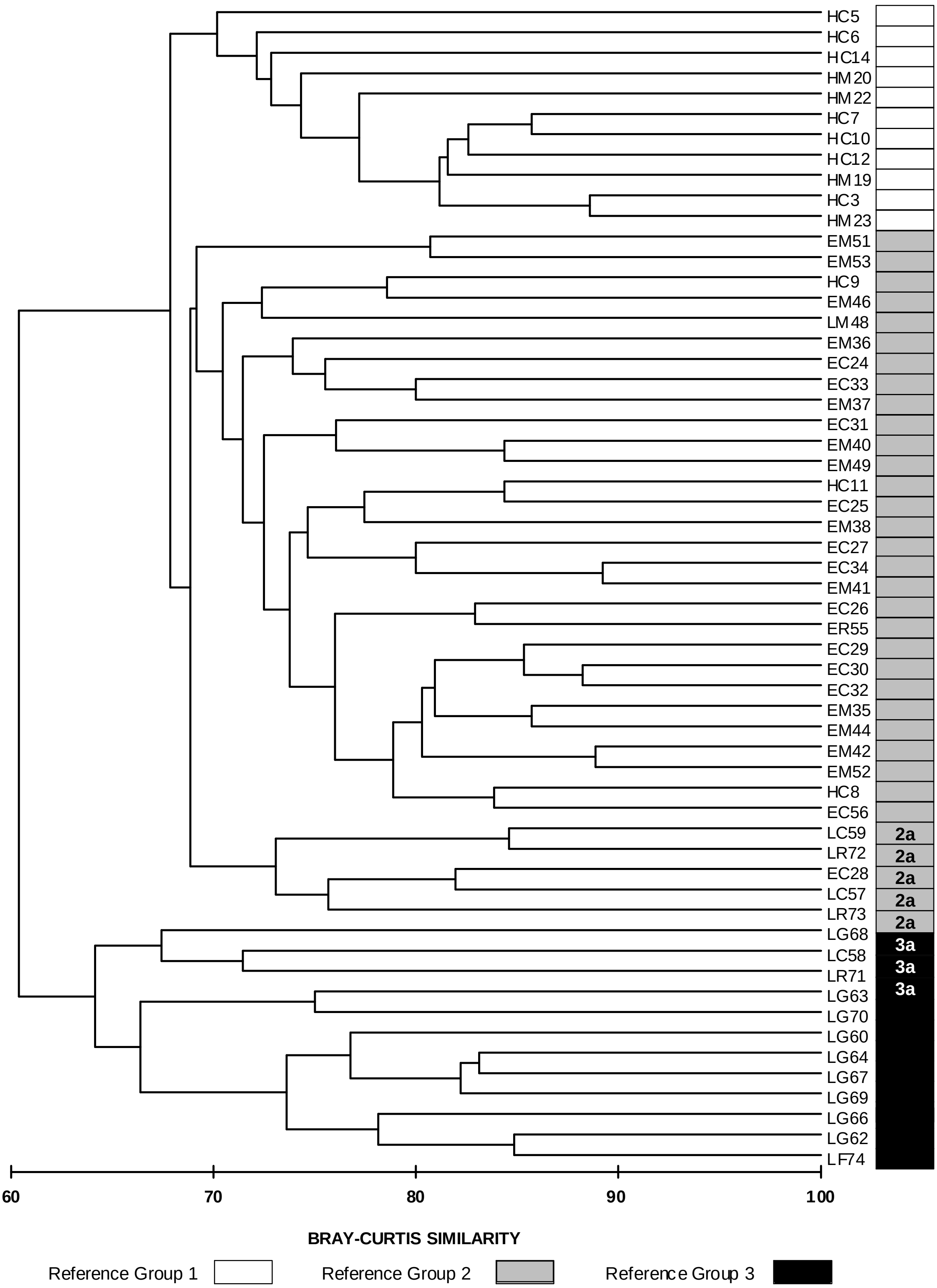

Figure 4.5 Dendrogram showing the classification of 57 composite reference sites in Mpumalanga. HM: Central Highlands, Mountain Stream; HC: Central Highlands, Foothill-cobble Bed; EM: Great Escarpment Mountains, Mountain Stream; EC: Great Escarpment Mountains, Foothill-cobble Bed; ER: Great Escarpment Mountains, Rejuvenated Cascade; LC: Lowveld, Foothill-cobble Bed; LG: Lowveld, Foothill-gravel Bed, LR: Lowveld, Rejuvenated Cascade; LF: Lowveld, Rejuvenated Foothill. The numbers refer to individual sites and are listed in Appendix B.

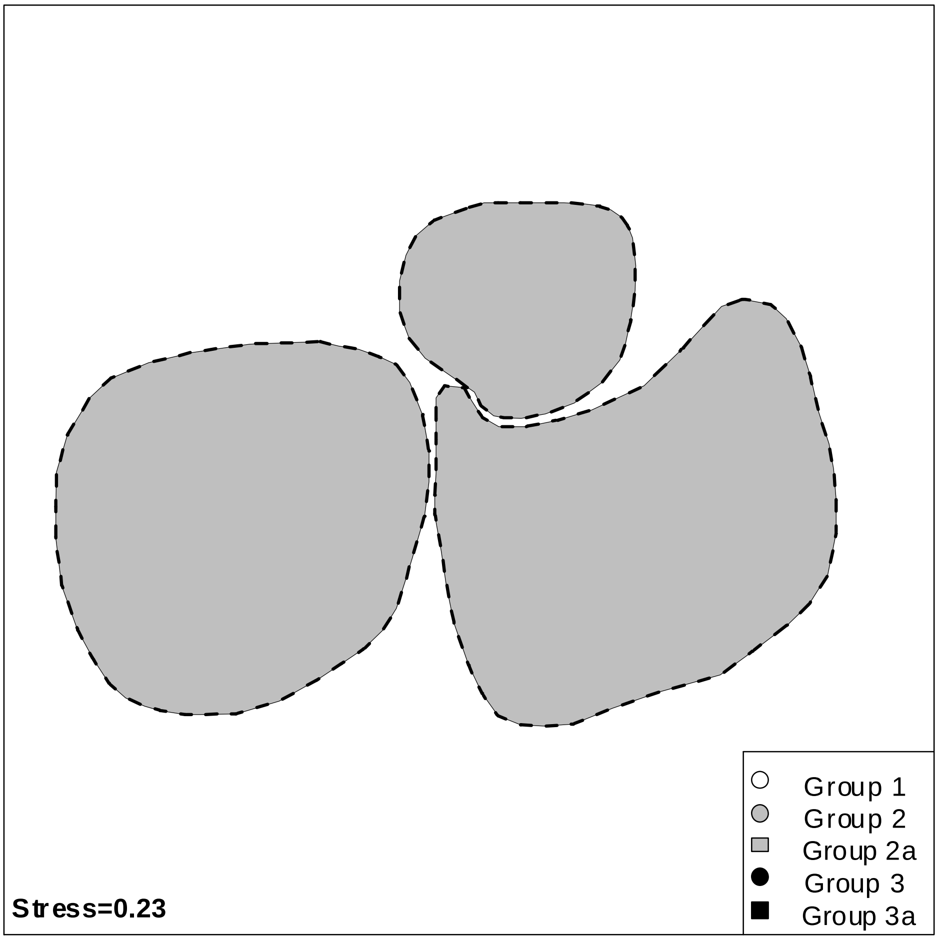

Figure 4.6 Ordination of 57 composite reference sites in Mpumalanga. The groups have been outlined manually on the basis of the cluster analysis.

4.4.1.2 Identification of distinguishing taxa

SASS invertebrate taxa responsible for the similarity within each Reference Group (Table 4.4), in addition to taxa responsible for the dissimilarity between Reference Groups and sub-groups (Tables 4.5 and 4.6), were identified using SIMPER analysis (Primer Ver. 4). Only taxa contributing to a total of 50% similarity or dissimilarity have been included in the tables.

- Average similarity within Reference Groups and sub-groups was between 68.8 and 75.7% (Table 4.5). Several taxa were ubiquitous, including baetids (3 types), chironomids and simulids, and contributed to similarity within all Reference Groups or sub-groups. Certain taxa were absent from one sub-group, namely tricorythid mayflies in sub-group 3a, or did not contribute to similarity within a group, namely heptageniid mayflies in sub-group 2. Each Reference Group or sub-group had at least one taxon noted as uniquely contributing to the similarity of sites within that Reference Group or sub-group. This does not necessarily imply that the particular taxon was absent from sites within other Reference Groups or sub-groups, but it was simply not considered a distinguishing taxon for the Reference Group. Taxa responsible for the similarity within Reference Groups and sub-groups are as follows: Reference Group 1 had 15 taxa responsible for group similarity (75.5%), several of which were also represented in other Reference Groups and sub-groups. Two taxa were unique to Reference Group 1, namely tabanid flies and hydropsychid caddisflies (2 types).

- Reference Group 2 had 12 taxa responsible for group similarity (71.7%). Two taxa were unique to Reference Group 2, namely athericid flies and oligochaete worms. When sub-group 2a was considered separately from Reference Group 2, several additional taxa characterised one or the other group. Crabs, leptophlebid and caenid mayflies, elmid or dryopid beetles and psychomyid caddisflies characterised Reference Group 2, whilst planarians, heptageneid mayflies, chlorocyphid dragonflies and perlid stoneflies characterised sub-group 2a.
- Reference Group 3 had 11 taxa responsible for group similarity (68.9%). Two taxa were unique to Reference Group 3, namely libellulid dragonflies and naucorid bugs. When sub-group 3a was considered separately from Reference Group 3, several additional taxa characterised one or the other group. Tricorythid and caenid mayflies, elmid/dryopid and dytiscid beetles, corixid and velid bugs and libellulid dragonflies characterised Reference Group 3, whilst oligochaete worms, belastomatid bugs, ceratopogonid flies and hydrachnellid water mites characterised sub-group 3a.

The taxa principally responsible for the dissimilarity between Reference Groups 1, 2 and 3, as measured by the Bray-Curtis similarity measure, are presented in Table 4.5.

- Reference Groups 1 and 2 (average dissimilarity = 32.5%) differed in the abundance of 20 taxa, 15 of which were more numerous within sites in Reference Group 1, including sphaeriid, planorbid and physid snails, and philopotamid, hydroptilid and cased (1 type) caddisflies. Psychomyid and cased (2 types) caddisflies, perlid stoneflies, helodid beetles and athericid flies were more numerous within sites in Reference Group 2.
- Reference Groups 1 and 3 (average dissimilarity = 37.2%) differed in the abundance of 20 taxa, 15 of which were more numerous within sites in Reference Group 1, including planorbid, ancylid and physid snails, psephenid beetles, philopotamid, hydropsychid (2 types), hydroptilid and cased (1 type) caddisflies. Belastomatid bugs, cordulid dragonflies, cased (3 types) and hydropsychid (1 type) caddisflies and shrimps (Natantia) were more numerous within sites in Reference Group 3.
- Reference Groups 2 and 3 (average dissimilarity = 40.5%) differed in the abundance of 21 taxa, 10 of which were more numerous within sites in Reference Group 2, including crabs, psephenid beetles, psychomyid and hydropsychid (2 types) caddisflies, aeshnid dragonflies and athericid flies. Belastomatid, naucorid, notonectid bugs, cordulid dragonflies, cased (3 types), hydropsychid (1 type) and hydroptilid caddisflies, and shrimps (Natantia) were more numerous within sites in Reference Group 3.

Examining sub-groups within Reference Groups 2 and 3 revealed the following:

- Reference Group 2 and sub-group 2a (average dissimilarity = 31.5%) differed in the abundance of 16 taxa, 9 of which were more numerous within sites in Reference Group 2, psephenid and helodid beetles, cased (2 types) caddisflies, aeshnid dragonflies and dixid flies. Naucorid and corixid bugs, libellulid dragonflies, philopotamid and hydropsychid (2 and 3 types) caddisflies, and water mites were more numerous within sites in sub-group 2a.

- Reference Group 3 and sub-group 3a (average dissimilarity = 35.8%) differed in the abundance of 16 taxa, 12 of which were more numerous within sites in Reference Group 3, including leptophlebid and tricorythid mayflies, hydraenid beetles, philopotamid, psychomyid and hydropsychid (1 type) caddisflies, perlid stoneflies and shrimps. Ancylid snails, pleid bugs, and dixid and tipulid flies were more numerous within sites in sub-group 3a.

On the basis of SIMPER analysis it is clear that certain taxa have greater significance in particular Reference Groups. This was examined in a complementary manner by calculating the frequencies with which each SASS invertebrate taxon occurred at sites within each Reference Group are presented in Table 4.7. Sub-groups 2a and 3a are represented by few sites and conclusions drawn should therefore be considered preliminary. The number of SASS taxa which occurred > 75% of sites within each Reference Group or sub-group, and which considered representative of each respective group, were as follows:

- Reference Group 1 is represented by 24 taxa, nine of which occurred at all sites (i.e. 100%).
- Reference Group 2 is represented by 22 taxa, six of which occurred at all sites.
- Sub-group 2a is represented by 22 taxa, 14 of which occurred at all sites.
- Reference Group 3 is represented by 21 taxa, 13 of which occurred at all sites.
- Sub-group 3a is represented by 14 taxa, 14 of which occurred at all sites.

All three analyses, i.e. similarity within groups, dissimilarity between groups and frequency of occurrence of taxa, have been used to derive expected invertebrate communities for each composite Reference Group. These are provided in chapter 5 which synthesises biotic and abiotic information and attempts to derive ecological reference conditions for riverine macroinvertebrates for each identified Reference Group.