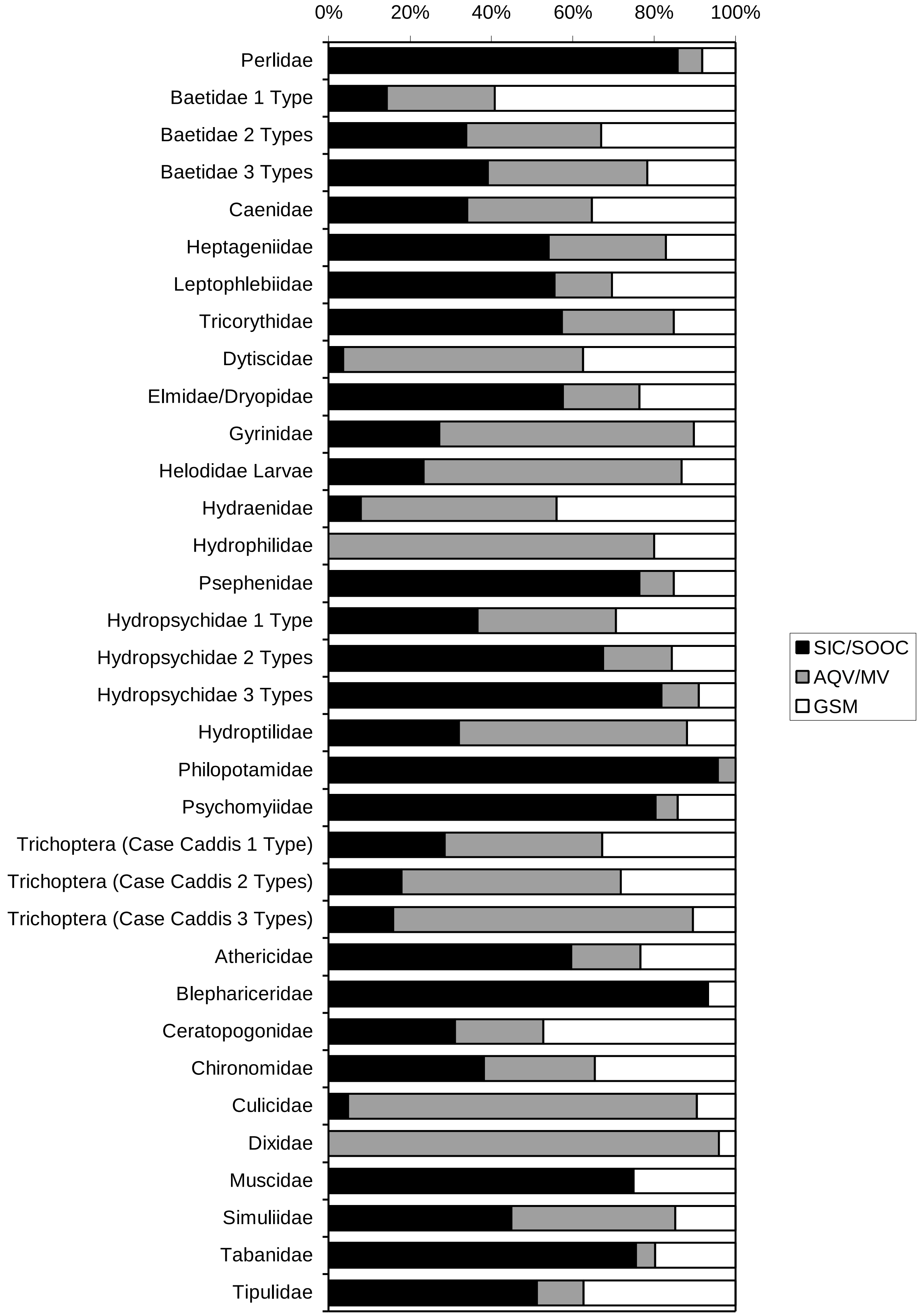

**Figure 4.14. Relative percentage occurrence of each SASS taxon within each biotope-group. Taxa within the following groups are presented: Plecoptera, Ephemeroptera, Coleoptera, Trichoptera and Diptera.**

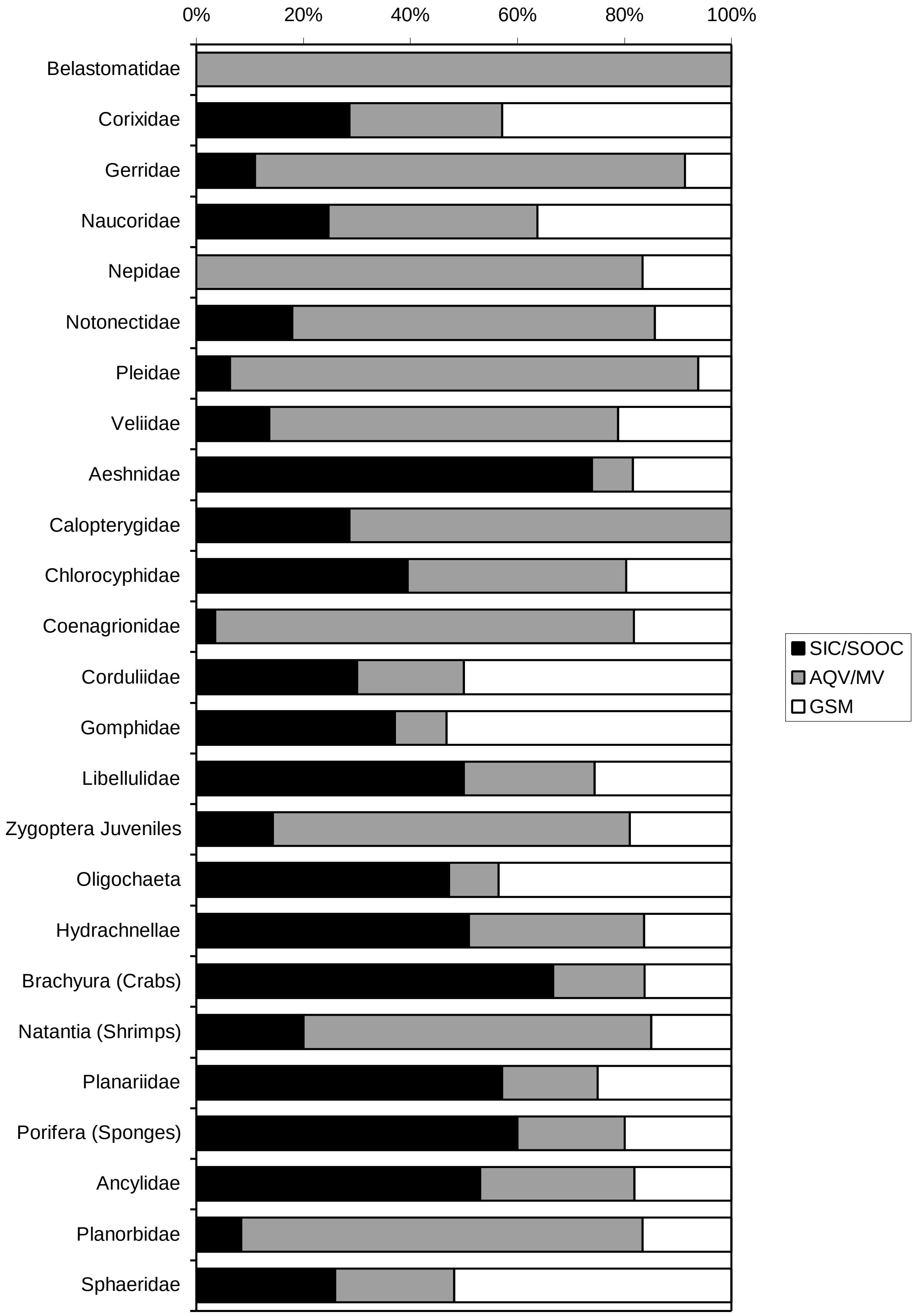

**Figure 4.15. Relative percentage occurrence of each SASS taxon within each biotope-group. Taxa within the following groups are presented: Hemiptera, Odonata, Annelida, Crustacea, Platyhelminthes, Porifera and Mollusca.**

#### *4.4.3.3 SASS4 Scores, number of taxa and ASPT*

The availability of biotopes for sampling may affect SASS Scores. The influence of biotope availability has been examined by calculating the relative percentage contribution of each biotope-group to that for the site.

***Relative percentage contribution***

SASS Scores calculated for each biotope-group were compared with those calculated from the combined-biotope site assessment. Only those sites at which all three biotope-groups were sampled were included in calculations. The percentage contribution of taxa within each biotope-group to SASS4 Score, number of taxa and ASPT for a site is given in Figure 4.16. Because certain taxa are found in more than one biotope the summed percentages from the biotopes do not equal 100%. Instead the percentage given for each biotope-group is that percentage relative to the total calculated for the site (i.e. biotope-groups combined). Because ASPT is calculated by dividing SASS4 Score by number of taxa, subsequent calculation of the percentage contribution of ASPT often resulted in an ASPT greater than 100%. The mean (+ standard deviation) of the percentage contribution of SASS4 Score, number of taxa and ASPT to SASS Scores for the site are given for each Reference Group. Based on these data:

- Taxa present in the SIC/SOOC biotope-group constituted 71-83%, 68-83% and 100-108% of the SASS4 Score, number of taxa and ASPT for a site in Reference Groups 1, 2 and 3 and sub-group 2a. For sub-group 3a, taxa present in the SIC/SOOC biotope-group constituted 30%, 33% and 94% of the SASS4 Score, number of taxa and ASPT for a site. The number of observations for this group is however low.
- Taxa present in the AQV/MV biotope-group constituted 55-68%, 53-67% and 95-103% of the SASS4 Score, number of taxa and ASPT for a site in all Reference Groups. This biotope-group appeared to be particularly important in sub-group 3a sites.
- Taxa present in the GSM biotope-group constituted 36-54%, 40-60% and 83-95% of the SASS4 Score, number of taxa and ASPT for a site in all Reference Groups.

With the exception of sub-group 3a, the SIC/SOOC biotope-group always had the highest percentage contribution to SASS4 Score and number of taxa, followed by AQV/MV and GSM. The differences were most pronounced in sub-group 2a and least pronounced in Reference Group 3. Variation in ASPT between both the biotope-groups and the Reference Groups was less pronounced, particularly between SIC/SOOC and AQV/MV biotope-groups. The percentage contribution of ASPT was often > 100% in the SIC/SOOC biotope-group, suggesting that more of the sensitive and high scoring taxa are present in this biotope-group.

***Numerical differences in SASS Scores***

Percentages were translated to actual values, expressed as the difference between the biotope-group SASS Scores and the combined-biotope SASS Scores, for each Reference Group separately and for Reference Groups 1, 2 and 3 and sub-group 2a together. Mean, median, standard deviation, minimum and maximum differences in SASS4 Score, number of taxa and ASPT between each biotope-group and the combined-biotope group are presented in Table 4.11. Examination of results revealed the following:

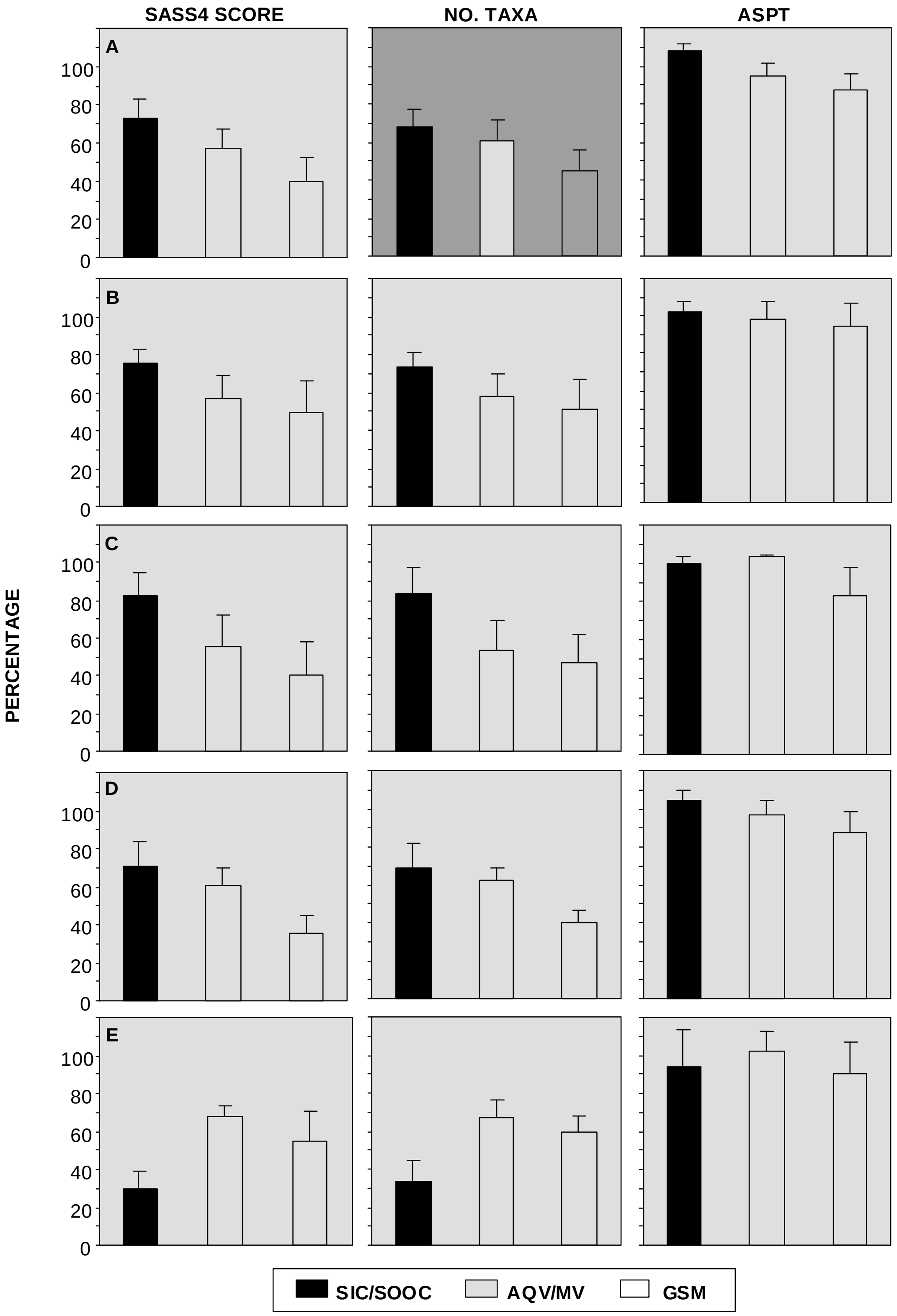

**Figure 4.16** **Mean (+ SD) of percentage contribution of SASS4 Scores, number of taxa and ASPT for SASS samples collected in three separate biotope-groups to SASS4 Scores, number of taxa and ASPT calculated for the site. Mean values have been calculated for each Reference Group, including the sub-groups, as follows: A = Group 1 (n = 10), B = Group 2 (n = 24), C = Group 2a (n = 3), D = Group 3 (n = 9) and E = Group 3a (n = 3). Biotope-groups are: SIC/SOOC = stones-in-current/stones-out-of-current, AQV/MV = aquatic/marginal vegetation and GSM = gravel, sand and mud.**

- *SASS4 Score:* The mean (± standard deviation) difference in SASS4 Score between separate biotope-groups and the combined-biotope group was 59 (± 20) for SIC/SOOC, 99 (± 32) for AQV/MV and 129 (± 39) for GSM.
- *Number of taxa:* The mean (± standard deviation) difference in number of taxa between separate biotope-groups and the combined-biotope group was 9 (± 4) for SIC/SOOC, 14 (± 4) for AQV/MV and 18 (± 5) for GSM.
- *ASPT:* The mean (± standard deviation) difference in ASPT between separate biotope-groups and the combined-biotope group was -0.23 (± 0.38) for SIC/SOOC, 0.17 (± 0.58) for AQV/MV and 0.63 (± 0.81) for GSM.

**Table 4.11 Mean, median, standard deviation, minimum and maximum differences in SASS4 Score, number of taxa and ASPT between each biotope-group and the combined-biotope group. Values are calculated using data from all sites in Reference Groups 1, 2 and 3 and sub-group 2a. SIC/SOOC: stones-in-current/stones-out-of-current; AQV/MV: aquatic and marginal vegetation and GSM: gravel/sand/mud (n = 48).**

| | SASS4 SCORE | | | NUMBER OF TAXA | | | ASPT | | |
|---|---|---|---|---|---|---|---|---|---|
| | SIC/SOOC | AQV/MV | GSM | SIC/SOOC | AQV/MV | GSM | SIC/SOOC | AQV/MV | GSM |
| **Mean** | 59 | 99 | 129 | 9 | 14 | 18 | -0.23 | 0.17 | 0.63 |
| **Median** | 60 | 105 | 125 | 10 | 14 | 18 | -0.27 | 0.15 | 0.58 |
| **SD** | 20 | 32 | 39 | 4 | 4 | 5 | 0.38 | 0.58 | 0.81 |
| **Min** | 7 | 39 | 53 | 0 | 7 | 7 | -1.11 | -1.06 | -2.09 |
| **Max** | 113 | 203 | 201 | 17 | 29 | 28 | 0.68 | 1.32 | 2.56 |

Translation of percentages to actual differences in SASS Scores supports the percentage contribution data in that the SIC/SOOC biotope-group has mean values most similar to the combined-group SASS Scores. With respect to ASPT, the mean difference was negative, again suggesting that more of the sensitive and high scoring taxa are present in this biotope-group. Differences in mean SASS Scores were greatest in the GSM biotope-group, in particular the mean SASS4 Score. Interestingly, differences in mean and median ASPTs were always <0.65, suggesting that when comparing sites with different biotopes available for sampling, emphasis should be placed on the ASPT rather than the SASS4 Score or number of taxa. This is dealt with further in chapter 5.

### *Mean (± standard deviation) and median values*

Median and mean SASS Scores for each biotope-group were calculated and compared statistically using the Kruskal-Wallis test statistic. The SASS4 Score, number of taxa and ASPT values for sites in Reference Groups 1, 2 and 3 and sub-group 2a were incorporated in this analysis. Sub-group 3a was excluded as it was considered substantially different from the other groups with respect to the relative contribution of the biotope-groups to SASS Scores for the site. All three metrics were significantly different amongst biotope-groups ($p < 0.05$; SASS4 Score: Kruskal-Wallis test statistic H = 67.18; number of taxa: H = 33.72 and ASPT: H = 56.69; Figure 4.17).

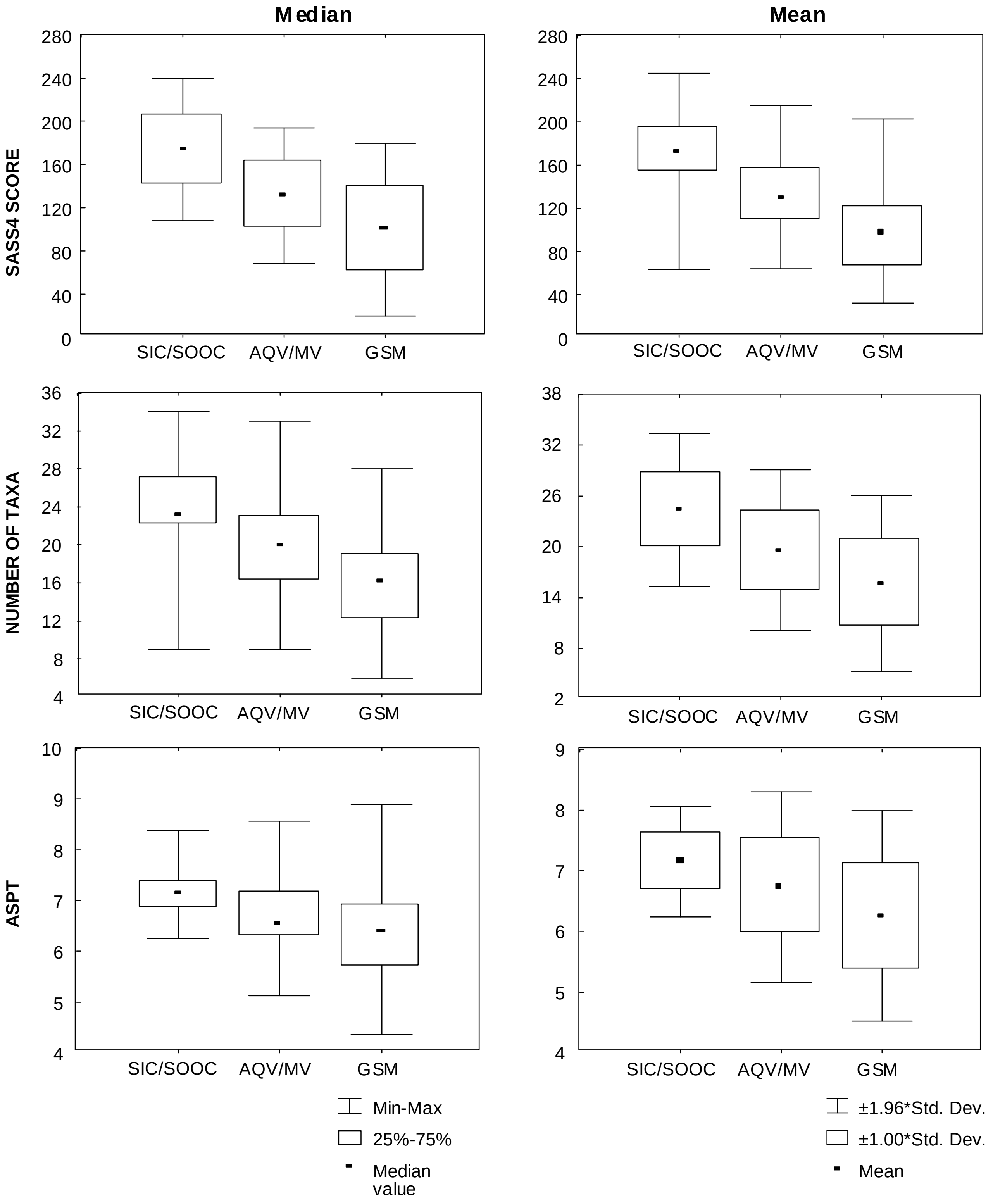

**Figure 4.17.** **Median and mean values for each biotope-group (SIC/SOOC: stones-in-current/stones-out-of-current , n = 54, AQV/MV: aquatic/marginal vegetation, n = 51, GSM: gravel/sand/mud, n = 49). Values are calculated from all sites in Reference Groups 1, 2 and 3 and sub-group 2a.**

Median, 25 and 75 percentiles values, and mean ± standard deviation are also given for each biotope-group but did not differ considerably from the mean values. The SIC/SOOC biotope-group had significantly higher median and mean SASS4 Score, number of taxa and ASPT than either the AQV/MV or GSM biotope-groups.

***Additional taxa recorded per biotope-group***

The number of additional taxa recorded per biotope-group was assessed by comparing the number of taxa (mean ± standard deviation) recorded in a single biotope-group with the number of additional taxa recorded if a second biotope-group was sampled, followed by a third biotope-group. Data from final reference sites (n = 57) at which all three biotope-groups were sampled (n = 49) were used for calculations. Analysis was run twice, first with the SIC/SOOC biotope-group assessed first (Figure 4.18A) and then with AQV/MV assessed first (Figure 4.18B). Results are as follows:

- If SIC/SOOC biotope-group was assessed first, then the mean number of taxa recorded in the SIC/SOOC biotope-group was 15.9 (SD ± 16.2). Adding the AQV/MV biotope-group resulted in an additional 6.1 (SD ± 8.2) taxa and adding the GSM biotope-group an additional 1.4 (SD ± 2.4) taxa.
- If AQV/MV biotope-group was assessed first, then the mean number of taxa recorded in the AQV/MV biotope-group was 13.7 (SD ± 13.0). Adding the SIC/SOOC biotope-group resulted in an additional 8.5 (SD ± 9.9) taxa and adding the GSM biotope-group an additional 1.5 (SD ± 2.5) taxa.

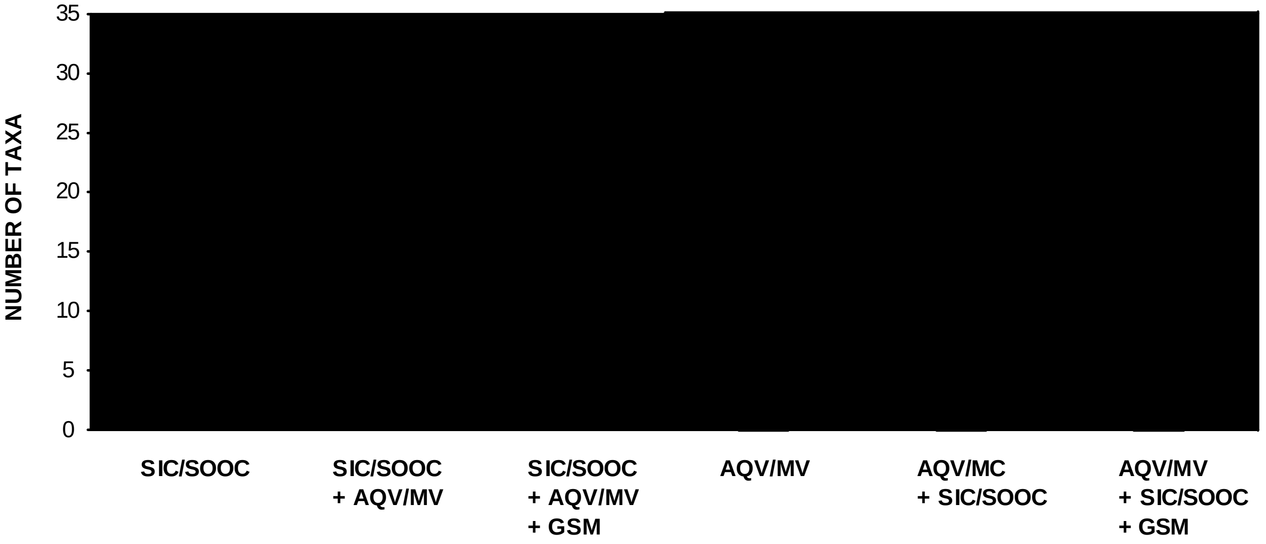

**Figure 4.18 Number of taxa (mean ± standard deviation) recorded in a single biotope-group, showing the number of additional taxa recorded when a second and third biotope-group are included. A: SIC/SOOC, (SIC/SOOC+AQV/MV), (SIC/SOOC+AQV/MV+GSM); B: AQV/MV, (AQV/MV+SIC/SOOC), (AQV/MV+SIC/SOOC+GSM).**

On this basis, both the SIC/SOOC and AQV/MV biotope-groups are important, with the SIC/SOOC biotope-group supporting a higher mean number of taxa than the AQV/MV biotope-group. The GSM biotope-group contributed relatively few additional taxa, and most taxa recorded in this biotope-group were also recorded in SIC/SOOC or AQV/MV. Figures 4.14 and 4.15 indicated that certain taxa are either exclusively or largely restricted to one biotope-group. It is therefore not surprising that additional taxa will be recorded when a second biotope-group is sampled. The standard deviation for the single biotope-group assessments were high illustrating that the frequency of occurrence between sites (n = 49) was high, with some taxa only being recorded in a single site (i.e. rare) with others recorded at > 95% of the sites (i.e. ubiquitous).

#### *4.4.3.4 Invertebrate Habitat Assessment System (IHAS)*

The IHAS was developed to assist with the interpretation of SASS Scores particularly in respect of variability in the number and quality of biotopes available for sampling. The method is still in its developmental and testing phase and its accuracy in reflecting the quality, quantity and diversity of habitat is currently being assessed.

IHAS is divided into two sections:

- Section 1 focuses on sampling biotopes and assesses the quantity and quality of the stones-in-current, vegetation and other biotopes (includes stones-out-of-current, gravel, sand and mud). The quality of each biotope, in terms of potential habitat for invertebrates, is assessed and expressed as a score. The scores for each biotope are then summed to give a total Habitat Score.
- Section 2 assesses stream characteristics and attempts to appraise the site in terms of its suitability for SASS sampling and account for disturbances present at the site. Aspects such as stream width, depth and velocity are included as a means of characterising the stream.

The ultimate goal of IHAS is for the IHAS score to adequately reflect the quantity, quality and diversity of biotopes available for habitation by invertebrates.

Interpretation of the IHAS scores is done by comparing the Total IHAS Score (i.e. Habitat Total Score and Stream Characteristics Score) with the maximum possible score, expressed as a percentage. Three categories have been formulated to assist with data interpretation of Total IHAS Score (McMillan 1998).

- If Total IHAS Score is > 80 %, then habitat is considered to be more than adequate and able to support a diverse invertebrate fauna (category = good);
- If Total IHAS Score is < 80 and >70 %, then habitat is considered to be adequate and able to support invertebrate fauna (category = adequate);
- If Total IHAS Score is < 70 %, then habitat is considered to be limited and unable to support a diverse invertebrate fauna (category = poor).

For the purposes of analysing data collected during the current project, these categories were expanded to enable the Habitat Total Score and Stream Characteristics Score to be assessed individually, i.e. categories were generated for interpreting Habitat Score, Stream Characteristic Score and Total IHAS Score.

The proposal of McMillan (1998) also includes a means of adjusting actual SASS4 Scores on the basis of the IHAS scores such that restricted or limited habitat conditions are accounted for. In this way the effect of the absence of one or more biotope-groups could be accounted for, and if the SASS4 Score at a site was lower than expected, it could be adjusted. If the adjusted SASS4 Score was still lower than expected, one could then conclude that the site was impacted with respect to water quality.

### *Habitat quality as reflected by IHAS scores*

Total IHAS Score (as a percentage), Stream Characteristic Score and the Habitat Score for each sampling occasion (n = 218) at 57 final reference sites are presented as relative percentages within the suggested categories for data interpretation (Figure 4.19). On the basis of IHAS results, >60% of sites in Reference Groups 1 and 2 had adequate or good habitat, whilst sites in Reference Group 3 and sub-groups 2a and 3a had <20% suitable habitat, suggesting that habitat at lowland sites is limited.

### *SASS4 Scores and Habitat Scores*

Reference sites are selected on the basis that they are minimally impacted with respect to both water quality and physical habitat destruction. At some reference sites, particular habitats or biotopes are naturally absent (e.g. some mountain streams in the western Cape which do not have marginal or instream vegetation). SASS is sensitive to biotope diversity (Dallas 1997) and therefore, assuming that SASS4 Scores at reference sites are the highest possible, there should be a linear relationship between SASS4 Scores and Habitat Scores since the higher the Habitat Score the more suitable the site for habitation by invertebrates. Habitat Score was selected for testing rather than Total IHAS Score since the latter incorporates Stream Characteristic Score which relates more to anthropogenic activities than biotope availability. The relationship between SASS4 Score and Habitat Score was examined for the 57 final reference sites on a per sampling occasion basis (n = 170, Figure 4.20).

Unexpectedly, neither SASS4 Score or the adjusted SASS4 Score, were correlated with Habitat Score. This suggests that the values within each habitat section of the IHAS are not a true reflection of the actual quality of the biotope available in terms of its potential for habitation by riverine macroinvertebrates. Correlations between other components were also examined, namely SASS4 Score versus Total IHAS Score, number of taxa versus Total IHAS Score, number of taxa versus Habitat Score, and SASS4 Score from AQV/MV biotope versus Vegetation Score of IHAS. None of these relationships were significant and no trends were evident. The established sensitivity of SASS to habitat diversity suggests that the IHAS is not adequately reflecting habitat quality. In concept IHAS appears to be potentially very useful, but testing and validation are required before it is usable as a tool for aiding the interpretation of SASS Scores within the RHP.

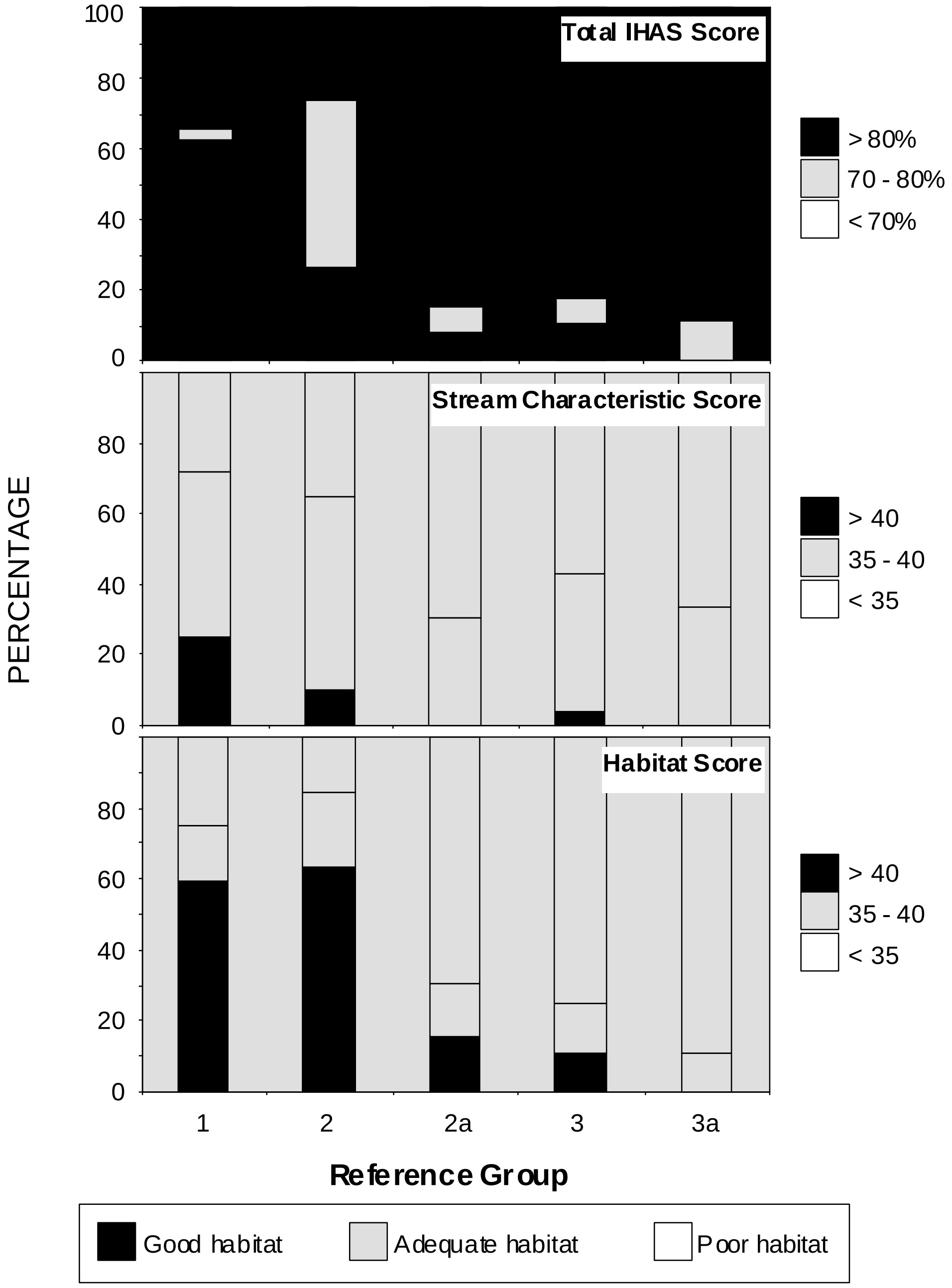

**Figure 4.19** **Relative percentage of reference sites (calculated per sampling occasion) within each IHAS band (McMillan 1998), calculated on the basis of Total IHAS Score, Stream Characteristic Score and Habitat Score. Percentages are given per Reference Group and sub-group.**

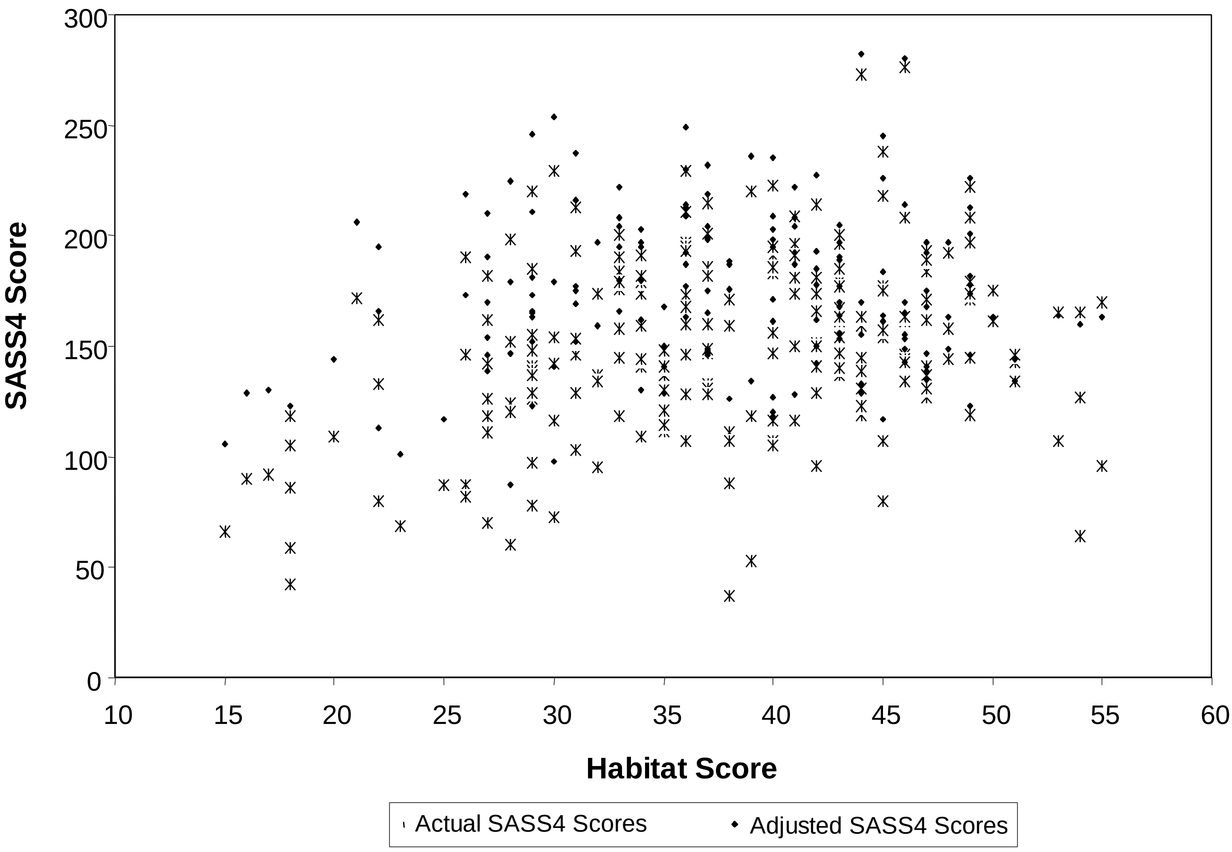

**Figure 4.20 Actual and Adjusted SASS4 Scores plotted against Habitat Scores for 57 reference sites on a per sampling occasion basis (n = 170).**

### *4.4.3.5 Summary of separate- versus combined biotope sampling*

On the basis of the above analyses, separate- versus combined-biotope sampling is an important consideration when establishing reference conditions for riverine macroinvertebrates and for biomonitoring in general. The following aspects were established:

- Multivariate analysis of separate- versus combined-biotope faunal samples from reference sites revealed that, in general, there was agreement between the separate- and combined-biotope site classifications. Several sites were however classified differently when separate- and combined-biotope classifications were compared. This aspect would need to be examined in more detail, particularly if predictive models are employed in the future.
- Certain taxa were exclusively or more commonly present in one or two biotope-groups. Others were regularly recorded in all three biotope-groups, showing virtually no biotope preference. This has implications for biomonitoring when not all biotope-groups are available for sampling at a monitoring site.
- Taxa present in the SIC/SOOC biotope-group contributed the most to overall SASS Scores at a site, followed by the AQV/MV biotope-group. Variation in ASPT was lower than either SASS4 Score or number of taxa, indicting the importance of using this metric in interpretation of SASS data.

- Translated to actual scores, as opposed to percentages, mean differences in SASS4 Scores between individual biotope-groups and combined-biotope-group was as high as 129 points. In terms of number of taxa, mean differences were a maximum of 18, whilst ASPT values differed the least between biotope-groups and was ≤ 0.63.
- SASS4 Score, number of taxa and ASPT calculated for each biotope-group were significantly different amongst biotope-groups. The SIC/SOOC biotope-group had significantly higher median and mean values for SASS4 Score, number of taxa and ASPT than either the AQV/MV or GSM biotope-group.
- The effect of sampling one, two or three biotope-groups revealed that sampling a single biotope-group resulted in the capturing of a mean number of taxa of between 13.7 and 15.9. Sampling a second biotope-group added a mean number of between 6.1 and 8.5 taxa, and a third biotope-group added a mean number of approximately 1.5 taxa.
- IHAS and its reliability in assisting with the interpretation of SASS data needs to be verified.

### 4.4.4 Seasonal variation in invertebrate communities and SASS Scores

In developing a classification system for reference sites it is recommended that data from at least two, and preferably three seasons, be combined for a site and analysis run on the combined data-set. In this way temporal variability is taken into account and taxa present in only one or two seasons will be recorded and thus included in the group of expected taxa for a particular reference site or group of reference sites. When subsequent monitoring is undertaken, the invertebrate community and SASS Scores recorded at the site are compared to the appropriate single or multiple-season reference condition. SASS data from a monitoring site assessed on a single occasion is therefore likely to not record all taxa, and this may be reflected in reduced SASS Scores. Since one of the key objectives is to establish the degree to which a monitoring site has been impacted relative to a reference condition, it is important to reduce or eliminate the potential influence of temporal or season variability. Predictive systems such as RIVPACS and AusRivAS models are often developed for each season and the user has the choice of specifying which single, or combination of seasons should be incorporated in the model. Since it is outside the scope of the current project to develop such predictive models, other methods for understanding and potentially incorporating temporal variability into the interpretation of biomonitoring data have been examined.

#### *4.4.4.1 Single-season versus multiple-season sampling*

Cluster analysis (Figures 4.21, 4.22 and 4.23) and MDS ordinations (Figures 4.24, 4.25 and 4.26) were undertaken at 57 sites for combined-biotope, invertebrate communities for each season. Analyses were run separately for each season such that the resultant site classification could be compared to the three Reference Groups and two sub-group obtained when seasonal data were combined (see Figures 4.5 and 4.6).