

Comparison of Three Methods for the Identification and Enumeration of Zooplankton Densities

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EXECUTIVE SUMMARY

A study was undertaken to examine the differences between alternative zooplankton sample processing methods (DWR and DOP) and data using a different subsampling and enumeration method from a previous contractor (BSA). 16 samples processed by BSA were re-processed by ICF using DWR and DOP enumeration and subsampling methods. The estimated catch per unit effort (CPUE) of all three methods was then calculated and compared. Additionally, any differences in the community composition using the three different methods were compared using non-metric multidimensional scaling (NMDS) and analysis of similarity (ANOSIM). There were large differences in estimated CPUE calculated from BSA data compared to both DWR and DOP methods for some individual samples (i.e., sampling variance in CPUE was higher for BSA relative to the other methods). However, the average CPUE estimated across samples using BSA, DOP, and DWR methods was not significantly different for either Calanoid, Cladocera, Cyclopoida, or total CPUE (all three taxonomic groups combined). When community composition was analyzed, NMDS and ANOSIM indicate that the BSA method had a high likelihood of generating data with a different composition of species compared to DWR and DOP methods. We conclude that CPUE estimates of all three methods for broader taxonomic groups (e.g. Calanoid, Cladocera, Cyclopoid and total CPUE) are similar, however there are likely issues with the level of taxonomic identification as indicated by NMDS and ANOSIM for samples processed using the BSA method. This may be undesirable when trying to identify changes to zooplankton community structure due to differences in estimated abundances at finer taxonomic scales.

INTRODUCTION

In March 2020, California Department of Fish and Wildlife (CDFW) issued Incidental Take Permit (ITP) No. 2081-2019-066-00 to the California Department of Water Resources (DWR) for the continued operation of the State Water Project (SWP). ITP Condition of Approval (COA) 3.13.1 requires continuation of existing monitoring programs through the Interagency Ecological Program including the Yolo Bypass Fish Monitoring Program (YBFMP) led by DWR. YBFMP has conducted monitoring of the lower trophic food web including zooplankton since 1998. ITP COA 9.1.3 and 9.1.3.1 require DWR to plan and implement the Summer-Fall Habitat Action and discretionary food enhancement actions to improve habitat and food availability. Both habitat and food actions are considered annually, including the North Delta Food Subsidy (NDFS) action. The NDFS action is an adaptive management action that manages flow pulses through the Yolo Bypass to improve food availability in downstream Delta smelt habitat.

Recently, the projects warranted increased zooplankton taxonomic resolution and comparison of contractor results to ensure DWR is utilizing the best scientific practices for producing lower trophic data to ensure effective monitoring around management actions. Confirmation of zooplankton data quality and identifying potential improvements in analyses of samples from the Yolo Bypass will inform the robustness of historical data, as well as inform future practices for the YBFMP and the NDFS action. The purpose of this study is therefore to compare results, based on the same underlying samples, for three different enumeration and identification protocols used to estimate zooplankton densities for habitat monitoring programs.

METHODS

16 samples were provided to ICF by DWR for zooplankton taxa identification and enumeration. These samples were collected during various months between 2019 and 2022. Dates and station codes for the samples used in the comparative analysis are shown in Table 1.

The samples were originally processed by a previous contractor, and the data resulting from that identification and enumeration protocol (“BSA”) were provided in spreadsheet format to ICF for the comparison. The previous protocol, as well as the two other identification and enumeration protocols (“DWR” and “DOP”) that were analyzed for comparison, involve subsampling with replacement, i.e., drawing subsamples from the sample jar with a pipette, performing identification and enumeration on the subsamples, and then returning the subsamples to the sample jar once the data were recorded. Because subsamples were drawn with replacement, the original number and composition of zooplankton in the sample jar was assumed to be unchanged between the separate applications of each enumeration and identification protocol. Therefore the estimates of zooplankton density for each protocol was assumed to be based on the same underlying sampled zooplankton density.

The BSA and DOP processing protocols considered have been used to estimate zooplankton densities for recent monitoring studies. The BSA approach has been used for monitoring studies for the NDFS action (Davis et al. 2022), while the DOP approach has been used for zooplankton samples under the Directed Outflow Project (Schultz et al. 2019, Lee et al. 2023).

The BSA method was applied to the samples in a separate setting, prior to application of the DWR and DOP methods. We did not observe the steps taken when the BSA method was applied. Rather, as noted above, the resulting BSA identification and enumeration results were already tabulated in the data provided to ICF by DWR. The DWR and DOP methods were carried out in the ICF taxonomy laboratory for this analysis. The DWR and DOP processing protocols are described below.

The DWR samples target mesozooplankton and microzooplankton which are sampled with the 150-micron and 50-micron plankton net respectively. In the laboratory, samples were first sieved, excess formalin was rinsed, and the samples were suspended in a beaker with water. Subsamples from each sample were examined under a stereo microscope and organisms were identified and enumerated.

Filtration:

The sample jar was opened under a fume hood and the contents were slowly poured over a 50-micron sieve placed over an empty beaker. The beaker with excess formalin was set aside. The sieve holding the sample was then placed over a new empty beaker. Next, the sample jar and lid were rinsed with water and poured slowly through the sieve to remove any organisms that may have been missed. Once the sample jar was emptied of all contents, the retained formalin was poured back into the original sample jar and set aside. The sample in the sieve was then rinsed into an empty beaker. This step was repeated multiple times to ensure all organisms and debris were rinsed into the beaker from the sieve.

Preparing Sample Dilution:

The sample was suspended in a glass graduated beaker of water with a target concentration of 200 - 400 organisms per 1 mL. The sample was stirred in a random figure 8 motion until the organisms were well mixed and suspended in the beaker. With a calibrated wide bore single channel 1-10mL pipette, a 1 mL subsample was quickly drawn before the organisms began to settle. The subsample was transferred to a clean Ward's plankton wheel. All organisms of interest were counted (see Table 1) using a hand counter. If the organism count was within the 200-400 per 1 mL range, and all organisms were visible in the plankton wheel, the dilution was not altered and was kept as the first subsample.

If the organism count was too high or too low, the following formula was used to estimate the new desired volume:

$$(C / D) * I = V$$

Where the initial count (C) is divided by the desired count (D) (between 200 and 400, depending on amount and size of silt or detritus). This fraction of the desired count was then multiplied by the initial sample volume (I) to get an estimate of the desired sample volume (V); rounding V to the nearest 5 mL.

If the organism count was too high, the subsample in the counting wheel was rinsed back into the sample beaker and then water was added to bring the sample volume to the estimated desired volume. For example, if the initial count was 445 organisms of interest in an 80mL dilution:

$$(445 / 250) * 80 = \sim 140 \text{ mL}$$

then 140mL is the desired sample volume (as rounded to the nearest 5 mL). The initial sample volume in this worked example would be adjusted from 80mL to 140mL.

If the organism count was too low, the subsample in the counting wheel was rinsed back into the sample beaker. For example: If the initial count was 127 organisms of interest in an 80mL of dilution:

$$(127 / 250) * 80 = \sim 40 \text{ mL}$$

The water from the beaker was poured through the 50-micron sieve until the volume in the beaker is lower than the desired volume (V), to allow for rinsing any sample organisms caught in the sieve back into the beaker without exceeding the desired volume. Using a fine-tipped squeeze bottle while holding the sieve at an angle, the organisms were concentrated into the bottom edge of the mesh sieve. Using as little water as possible, while still being thorough, the organisms were gently rinsed back into the beaker. The sieve was then inspected to ensure all visible organisms and debris were transferred. An appropriate amount of water was then added to bring the sample volume up to the desired sample volume, such that the target organism density was achieved.

Sample Processing:

DWR - Once the proper dilution was achieved and the sample information was recorded on a datasheet, 1 mL subsamples were processed one at a time using a Ward's plankton wheel. 400 microzooplankton and 200 mesozooplankton total were targeted per sample. As many subsamples were processed as required to reach target numbers. All taxa and life stages of interest were identified and enumerated. The organism counts were tallied using mechanical tally counters and recorded on a physical datasheet.

DOP - Once the proper dilution was achieved and the sample information was recorded on a datasheet, 1 mL subsamples were processed one at a time using a Ward count wheel, with a minimum of 5 subsamples and a maximum of 10 subsamples processed per sample. Roughly, 1000 to more than 2000 organisms were counted per sample. All taxa and life stages of interest were identified and enumerated. The organism counts were tallied using mechanical tally counters and recorded on a physical datasheet.

After each subsample was processed, using water in a squeeze bottle, completed subsamples were rinsed into a second beaker, until all subsamples were fully processed. When the sample processing was completed, the counted subsamples and the remainder of the sample were poured into a 50-micron sieve, and the sample was transferred back to its original bottle. A label with the taxonomist's initials and sample processed date was put on top of lid. If the sample was stored in more than one container, a second sample tag with the same information as the first was placed in the second container and "1 of 2" was written on one bottle lid and "2 of 2" on the other.

After the resulting DWR and DOP data were recorded, and CPUE (catch per unit effort) in individuals per cubic meter was calculated using the equation:

$$CPUE = \frac{\text{catch}}{\text{volume}}$$

Catch was calculated using the following equation:

$$Catch = Count * \frac{Sample_{Vol}}{Subsample_{vol} * N_{subsamples}}$$

Volume was calculated using the following equation:

$$Volume (m^3) = Distance * Area$$

Distance was calculated using the following equation:

$$Distance(m) = \frac{[(Flow]_{end} - Flow_{begin}) * 26873}{99999}$$

Area was calculated using the following equation:

$$Area_{zoop} = \pi * 0.1^2$$

Results from the three protocols were compiled, plotted, and analyzed for comparison using the R statistical software language (R Core Team 2023). Non-metric multidimensional scaling (NMDS) was used to visualize any differences in community structure between the different methods. NMDS was applied to the matrix composed of CPUE for individual species in each sample with microzooplankton excluded. CPUE values were square-root transformed followed by Wisconsin double standardization. The transformation helps to equalize the emphasis of samples and species with high CPUE values that could otherwise mask patterns of interest. Ordination was projected in two dimensions, based on the Bray-Curtis dissimilarity matrix of the transformed CPUE values. The transformation and ordination were run using the *metaMDS* function of the *R* library *vegan* (Oksanen et al. 2022).

Analysis of Similarity (ANOSIM) was used to statistically test differences in species composition between enumeration methods. ANOSIM is analogous to ANOVA, but instead of a regression test for a difference in a univariate response variable mean between groups, a multivariate permutation test is performed for differences in dissimilarity matrices between groups. In other words, the null hypothesis of ANOSIM in this case is that there is no difference in the zooplankton community structure between enumeration methods.

RESULTS

The total CPUE calculated across all zooplankton taxa varied substantially between the 16 samples; for example, sample RD22_11:41_150 had a mean CPUE of 85 across all three methods/datasets, while sample STTD_12:02_50 had a mean CPUE of 15,129 (Tables 2 and 3). When examining the total CPUE of samples with microzooplankton excluded, CPUE using the BSA method showed higher variance than both DWR and DOP methods (Table 3, Fig. 1). The percentage difference between BSA and DWR estimates of CPUE ranged from 1 % - 78% (Table 3), and the percentage difference between DOP and DWR estimates of CPUE ranged from 1% - 8%.

There were some notable differences for taxa specific CPUE (with microzooplankton removed). Data from the BSA method produced several under and overestimates of calanoid CPUE relative to the DWR and DOP methods (Samples STTD_9:41_150, STTD_9:30_150, STTD_9:22_150, STTD_12:02_50, SHR_8:26_50). There were also a few samples which had relative underestimates of cyclopoids (SHR_8:26_50, RVB_7:13_150, BL5_11:15_150) (Table 3, Fig. 1). Microzooplankton taxa (e.g. calanoid nauplii and rotifers) were removed from analyses since some nauplii could be identified to the genus level (e.g. *Pseudodiaptomus* nauplii) while other nauplii may have been either calanoid or cyclopoid nauplii. This did influence calanoid CPUE estimates (Fig. 1).

Boxplots of CPUE for each taxonomic group and total CPUE with microzooplankton removed indicated that there were no significant differences between the three enumeration methods in the average CPUE calculated across samples (Fig. 2). Variance was higher for calanoid and cyclopoid CPUE for the BSA method. A two-factor analysis of variance (ANOVA) was run accounting for the three taxonomic groups (excluding “Total CPUE” from the comparison) and no significant difference was found in the average expected CPUE between the three enumeration methods ($p = 0.939$). A separate ANOVA was also run with “Total CPUE” as the response variable (i.e., all three taxonomic groups combined, but again, excluding microzooplankton), and no significant difference was found in the average CPUE between enumeration methods in that comparison either ($p = 0.979$).

NMDS involves the application of an iterative numerical minimization algorithm, which may not always converge to a stable solution for the ordination. In this case, NMDS converged on some attempts, but about two-thirds of attempts failed to converge. The reported “stress” metric for all ordination attempts, regardless of convergence, was approximately 0.23 which may not be as reliable to interpret as stress values less than 0.20 (Clarke 1993); however, the interpretation of thresholds for “stress” values may also be case specific (Dexter et al. 2018). So, while some caution is warranted in the visual interpretation of the NMDS patterns shown in Fig. 3., they are generally consistent with patterns between enumeration methods evident in the raw individual sample data shown in Appendix A. The NMDS pattern across the entire set of individual samples shows a higher degree of similarity in species composition for the DWR and DOP methods compared to the BSA method (Fig. 3).

Like the NMDS visualization shown in Fig. 3, the results of the ANOSIM permutation test are consistent with the species composition from the DWR and DOP enumeration methods being similar ($p = 1.0$). However, the comparisons with BSA are more consistent with a significant difference in sampled species composition. The ANOSIM comparisons yielded p-values nearly at the 0.05 level for BSA compared to DOP ($p = 0.06$) and likewise for BSA compared to DWR ($p = 0.06$). This is consistent with sampled zooplankton community structure having a high likelihood of being different under the BSA enumeration method compared to enumeration using either the DWR or DOP methods (Fig. 4).

DISCUSSION

While there were differences between the CPUE estimates calculated using BSA data and DWR and DOP methods, these differences were not consistent across samples nor were they consistent across taxa. BSA data produced larger relative under and overestimates of CPUE, compared to the generally closer estimated CPUE values between the DWR and DOP methods. While some of the differences were within 10% of CPUE estimates using the DWR method, there were notably many samples where the difference was greater than 10% (13 out of 16 samples). Some of these differences were particularly large (78% for sample STTD_9:30_150) but most were in the 10-30% range. The differences between the CPUE estimates calculated using DWR and DOP methods were below 10%. However, ANOVA results indicate that the differences between individual samples did not result in a significant relative bias in mean CPUE as measured across samples. It is not immediately obvious how the higher variability in CPUE estimates using the BSA method might (or might not) impact conclusions made about the effects of flow actions on zooplankton densities, although higher sampling variability is known to decrease statistical power to detect effects, all else being equal.

Because there were more subsamples counted for the DOP method, we expect the DOP estimates of CPUE to be more accurate. The number of subsamples done for each method changed depending on the total number of organisms counted for each method. The DWR method targeted 200 mesozooplankton and 400 microzooplankton while the DOP method targeted 1000 – 2000 organisms total. Analysis by Lund et al. (1958) found an increase in count precision with an increase in counting effort (e.g. more organisms counted, see table 4.11 in Harris et al. 2000) for marine zooplankton samples.

Both NMDS and ANOSIM analyses indicated that there were taxonomic CPUE differences in the BSA data compared with the DWR and DOP method data. The differences in taxa specific CPUE estimates could impact conclusions being made about the efficacy of flow actions and the effects on specific taxa. These differences are likely a result of taxonomic misidentification or using different taxonomic categories (e.g. lumping certain taxa together).

Regarding which method (DWR or DOP) is “better”, our results suggest there is on average less than a 10% difference between the DWR and DOP methods. Both ANOVA and ANOSIM results indicate there is no significant differences in CPUE estimates and taxa composition generated using the respective methods. While the DWR method was expected to be less time intensive since less organisms are counted, it took significantly more time to reach the 200 mesozooplankton and 400 microzooplankton counts in some samples. For example, half of the 150 μ m samples (which were targeting mesozooplankton) required more than 10 subsamples to reach the 400 microzooplankton count. This is likely due to the larger net mesh size allowing for most microzooplankton to slip through, resulting in fewer being caught in the sample. Results from Kayfetz et al. (2020) found the mesoplankton net mesh size is likely under sampling certain microzooplankton taxa and mesozooplankton life stages. Likewise, for the 50 μ m samples, it can take some time to reach the 200 mesozooplankton count since the target taxa are microzooplankton. The DOP method tends to be much faster due to the set amount of organisms per sample with no distinction between mesozooplankton and microzooplankton. Results need to be interpreted keeping in mind biases that arise with using a larger or smaller net sizes and the target size class of zooplankton.

Ultimately, we recommend that there needs to be a rigorous and reproducible methodology when processing zooplankton samples. Not having a clear explanation of the methodology behind how the BSA processing took place left many of the inconsistencies we found when comparing estimated CPUE unexplained. Likewise, we recommend quality assurance/quality control procedures be included as part of any protocol for processing samples. Differences in the NMDS and ANOSIM analyses indicate that taxonomic differences in identification of certain zooplankton taxa (specifically calanoid and cyclopoid taxa) could lead to different interpretations of community structure and dynamics between BSA and DWR/DOP methods. Analyses that include data generated from BSA methods should focus on higher level, broad taxonomic resolution since these counts are likely reliable, however any taxa specific level analysis such as at the genus or species level or if the analysis spans a period where two different enumeration methods were used should be treated more cautiously.

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TABLES:**Table 1.** Sample dates, nets, stations and unique sample IDs used in this analysis.

Unique Sample ID	Sample Date	Net Mesh	Station	Station Name
		(µm)	Code	
STTD_9:53_150	2019-Jan-08	150	STTD	Toe Drain at Screw Trap
STTD_9:22_150	2019-Jun-03	150	STTD	Toe Drain at Screw Trap
STTD_12:02_50	2019-Sep-04	50	STTD	Toe Drain at Screw Trap
STTD_9:41_150	2020-Jan-27	150	STTD	Toe Drain at Screw Trap
RD22_11:41_150	2020-Jul-14	150	RD22	Toe Drain at Road 22
RVB_9:00_150	2020-Jul-27	150	RVB	Sacramento R. at Rio Vista Bridge
BL5_11:15_150	2020-Jul-27	150	BL5	
RYI_9:19_150	2020-Aug-10	150	RYI	Cache Slough at Ryer Island
STTD_9:33_50	2021-Apr-27	50	STTD	Toe Drain at Screw Trap
SHR_9:55_50	2021-May-10	50	SHR	Sacramento R. at Hood
RVB_7:13_150	2021-Aug-03	150	RVB	Sacramento R. at Rio Vista Bridge
PRS_9:28_150	2021-Oct-13	150	PRS	
STTD_9:30_150	2021-Nov-30	150	STTD	Toe Drain at Screw Trap
STTD_10:48_150	2021-Dec-28	150	STTD	Toe Drain at Screw Trap
STTD_12:33_150	2022-Jan-24	150	STTD	Toe Drain at Screw Trap
SHR_8:26_50	2022-Apr-12	50	SHR	Sacramento R. at Hood

Table 2: Comparison of estimated CPUEs by sample and method as plotted in Figure 1. The numbers in this table exclude microzooplankton counts.

Sample ID	Calanoid			Cladocera			Cyclopoida			Total CPUE		
	BSA	DWR	DOP	BSA	DWR	DOP	BSA	DWR	DOP	BSA	DWR	DOP
STTD_9:53_150	357	329	273	2578	2805	2851	1086	1083	1252	4021	4386	4376
STTD_9:22_150	466	243	146	3159	4450	3657	11081	10963	11093	14706	15786	14896
STTD_12:02_50	272	121	131	1672	523	784	2022	1780	1386	3966	2030	2301
STTD_9:41_150	38	86	86	741	1043	988	823	988	958	1602	2087	2032
RD22_11:41_150	0	1	0	70	69	75	16	11	12	86	82	87
RVB_9:00_150	3001	2584	2813	12	24	12	24	24	15	3037	2623	2840
BLS_11:15_150	119	156	153	36	40	40	12	49	48	167	244	241
RYI_9:19_150	2726	1850	1866	9	8	14	18	17	12	2753	1870	1892
STTD_9:33_50	2942	3993	3796	196	210	181	294	368	305	3432	4508	4282
SHR_9:55_50	0	0	0	171	229	270	1113	1270	1338	1284	1567	1608
RVB_7:13_150	238	310	326	11	15	9	4	12	9	253	334	344
PRS_9:28_150	1088	871	937	229	300	288	222	229	228	1539	1399	1453
STTD_9:30_150	32	230	212	76	79	72	122	118	112	230	421	396
STTD_10:48_150	26	37	55	2821	2456	2622	780	600	632	3627	3125	3309
STTD_12:33_150	87	120	145	550	827	766	682	765	764	1319	1711	1675
SHR_8:26_50	16	0	0	114	75	66	0	108	116	130	191	182

Table 3: Total CPUE estimates for using the BSA data, DWR method and the DOP method. The numbers in this table exclude microzooplankton.

Sample	BSA CPUE estimate	DWR CPUE estimate	DOP CPUE estimate	DWR-BSA % Difference	DWR-DOP % Difference
STTD_9:53_150	4020	4256	4387	5.8%	-3.0%
STTD_9:22_150	14706	15656	14911	6.5%	5.0%
STTD_12:02_50	4043	2473	2431	-38.8%	1.7%
STTD_9:41_150	1608	2124	2040	32.1%	4.2%
RD22_11:41_150	86	85	91	-0.6%	-6.0%
RVB_9:00_150	3037	2631	2840	-13.4%	-7.3%
BL5_11:15_150	194	246	241	27.2%	2.1%
RYI_9:19_150	2753	1883	1893	-31.6%	-0.5%
STTD_9:33_50	3874	4742	4404	22.4%	7.7%
SHR_9:55_50	1498	1801	1902	20.3%	-5.3%
RVB_7:13_150	258	338	345	31.1%	-1.9%
PRS_9:28_150	1667	1459	1506	-12.5%	-3.1%
STTD_9:30_150	243	432	408	78.0%	5.9%
STTD_10:48_150	3926	3093	3347	-21.2%	-7.6%
STTD_12:33_150	1353	1712	1678	26.5%	2.0%
SHR_8:26_50	1349	2162	2324	60.3%	-7.0%

FIGURES:

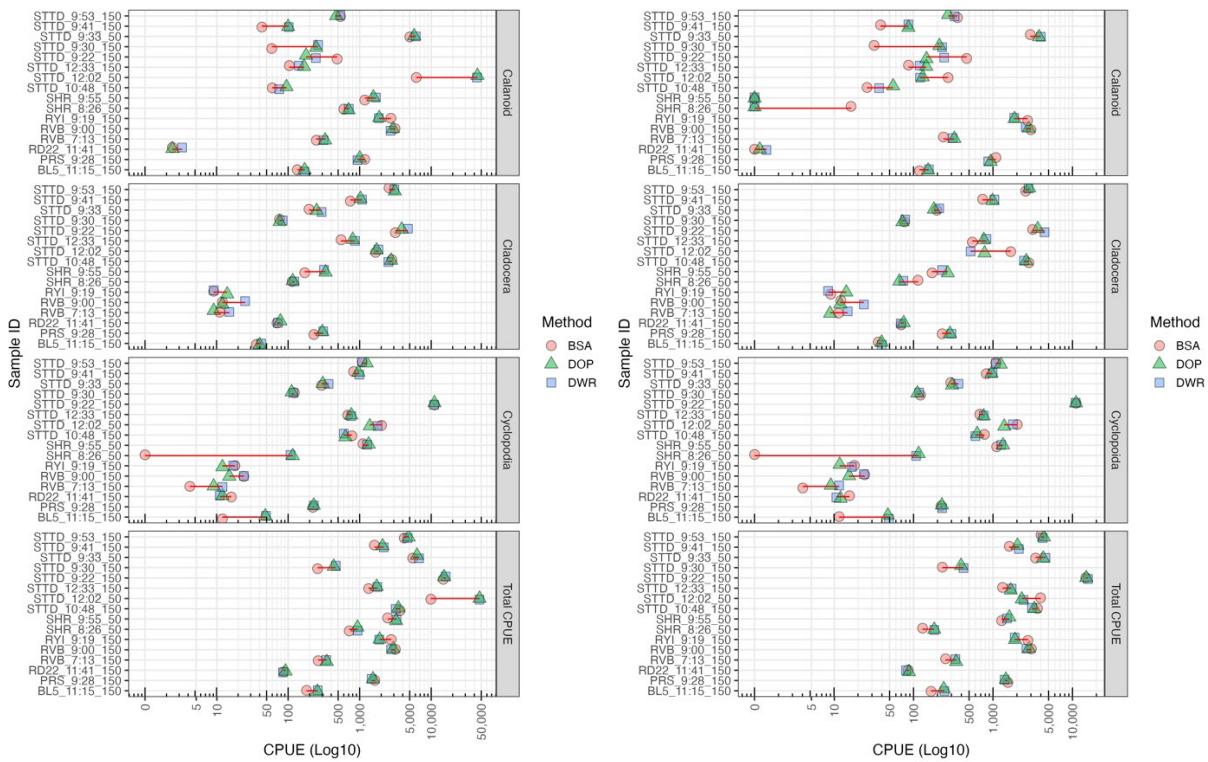


Figure 1. Estimated zooplankton CPUE summing across species counts within each of three major taxonomic groups as well as Total zooplankton CPUE estimated from each method. The left plot shows CPUE including microzooplankton (e.g., nauplii) counts. The right plot shows CPUE after excluding microzooplankton counts. The CPUE values are plotted in Log10 pseudo space (allowing for zero values) to ease visualization. Values are jittered slightly in the vertical direction to minimize overplotting between methods. The red lines connect the lowest and highest estimated CPUE value between methods for each sample. Catch was calculated by summing counts for individual species in each taxonomic order, or across taxonomic orders for the total CPUE. Counts were then divided by the volume sampled through the net to calculate CPUE (individuals per cubic meter sampled in the zooplankton tow).

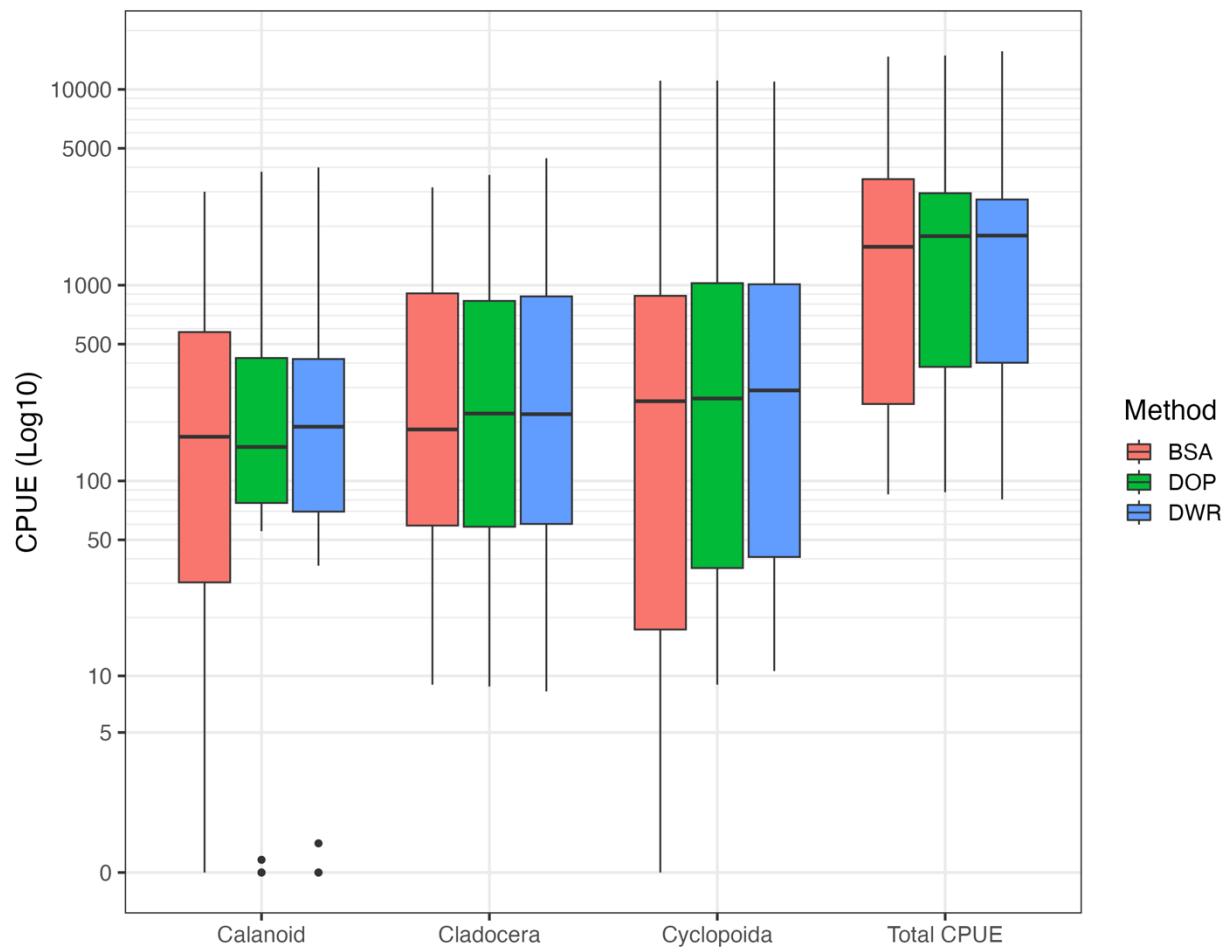


Figure 2. Boxplots are shown representing CPUE across samples for each method and three taxonomic groups, with microzooplankton counts excluded.

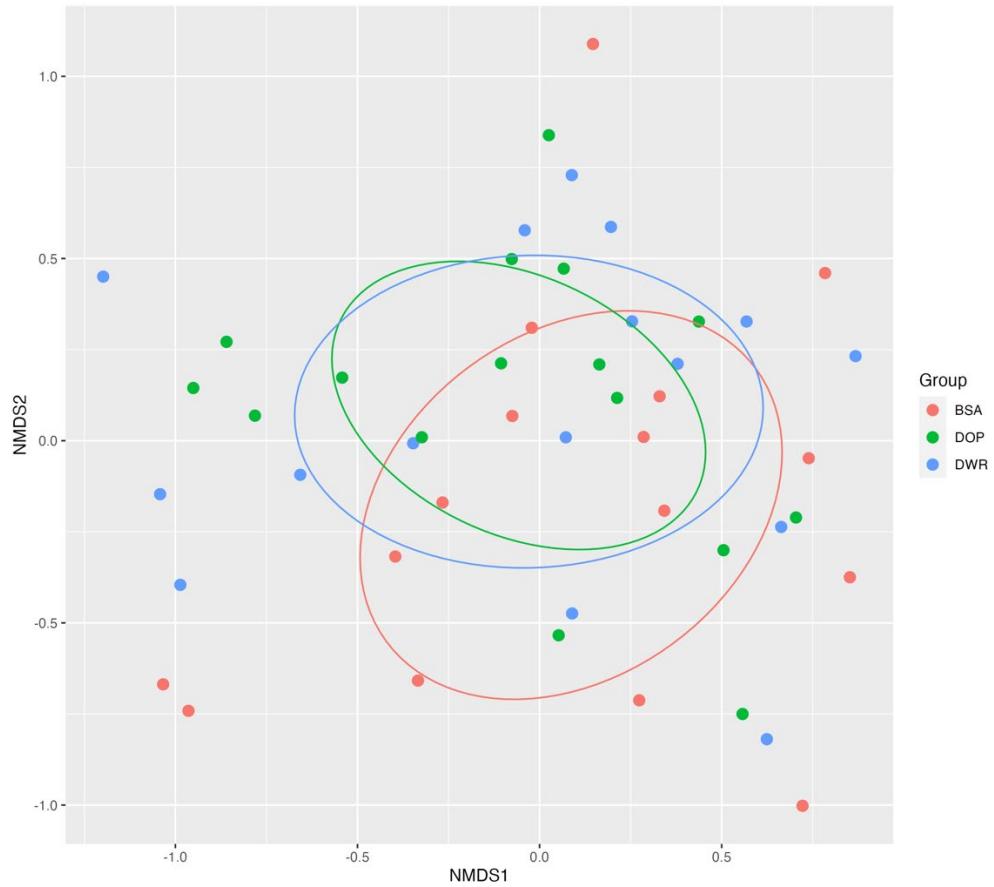
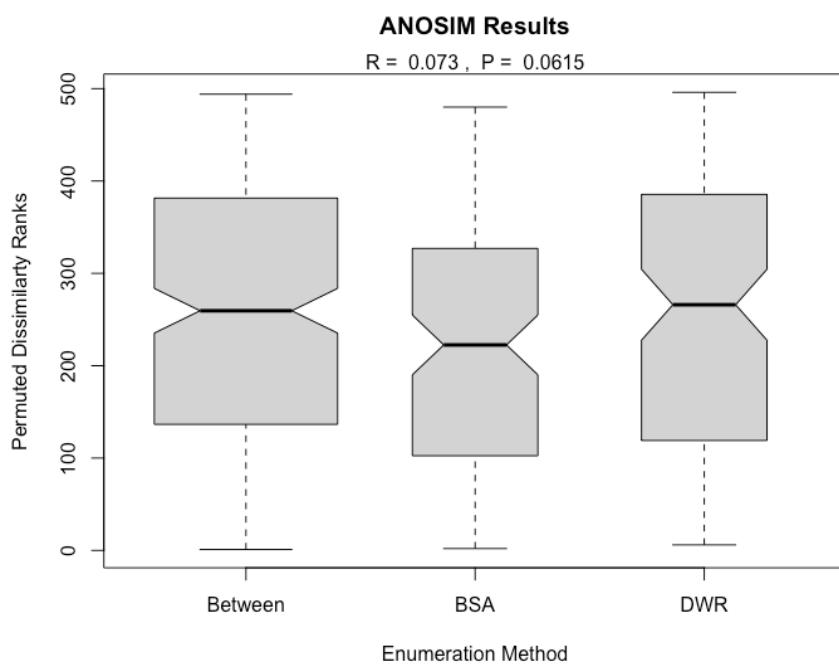
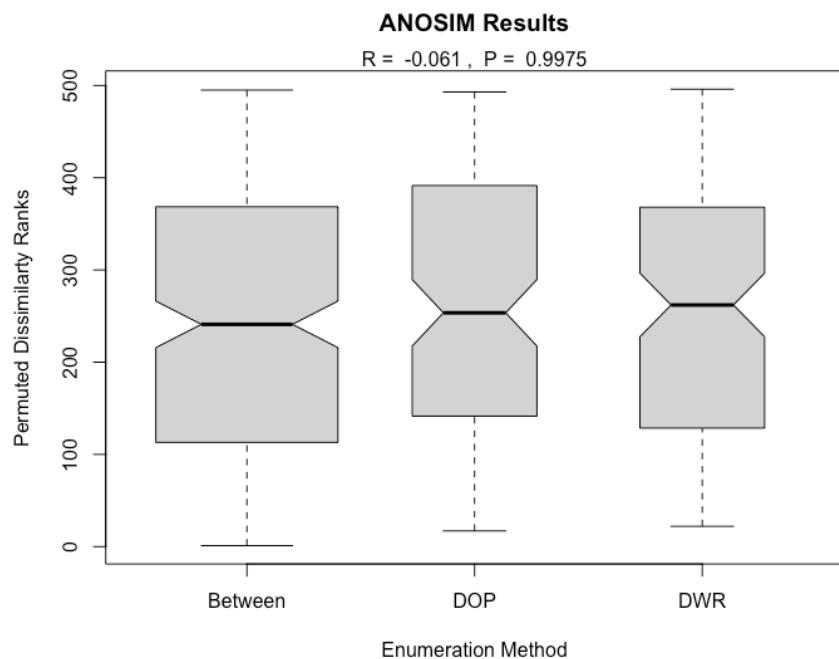


Figure 3. NMDS plot showing the relationship between estimated zooplankton species community structure for each enumeration method. Points are plotted in ordination space for each individual sample. 95% confidence ellipses are shown around the estimated center of the mass of the points for each enumeration method.



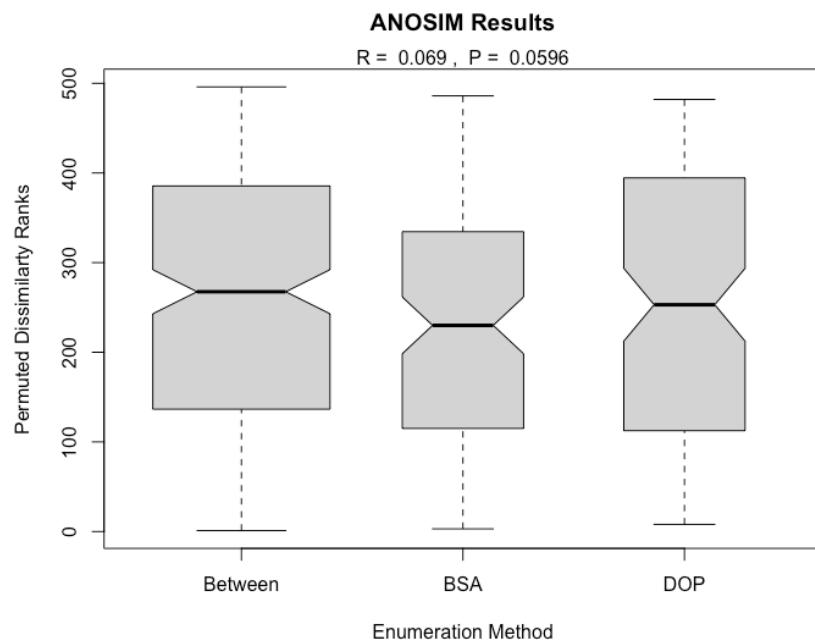


Figure 4. Analysis of similarities (ANOSIM) permutation test results are shown in three plots comparing community structure between the enumeration methods. The “Between” group represents the permuted null distribution for the dissimilarity rank between the enumeration methods in each plot.

APPENDIX A

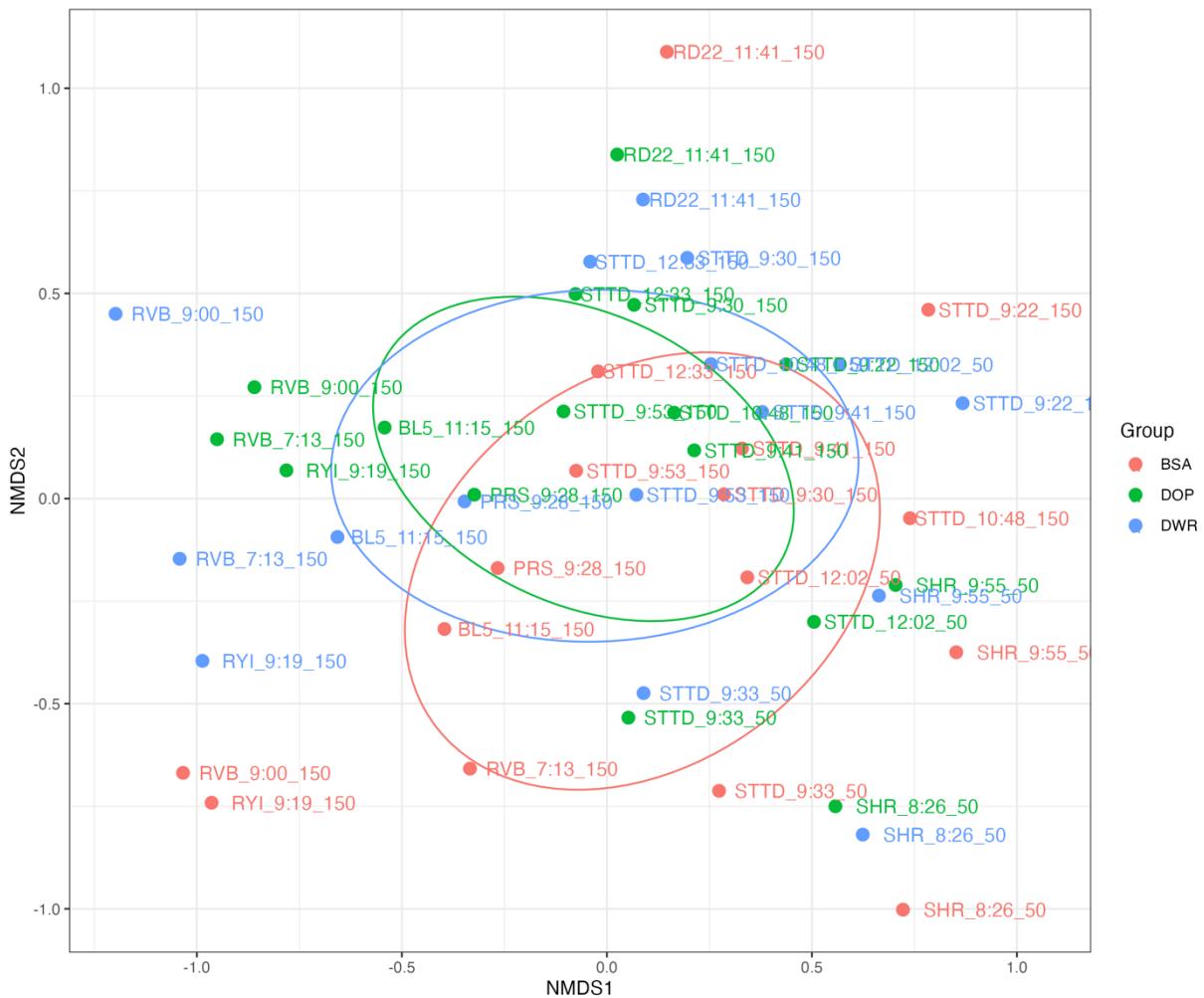
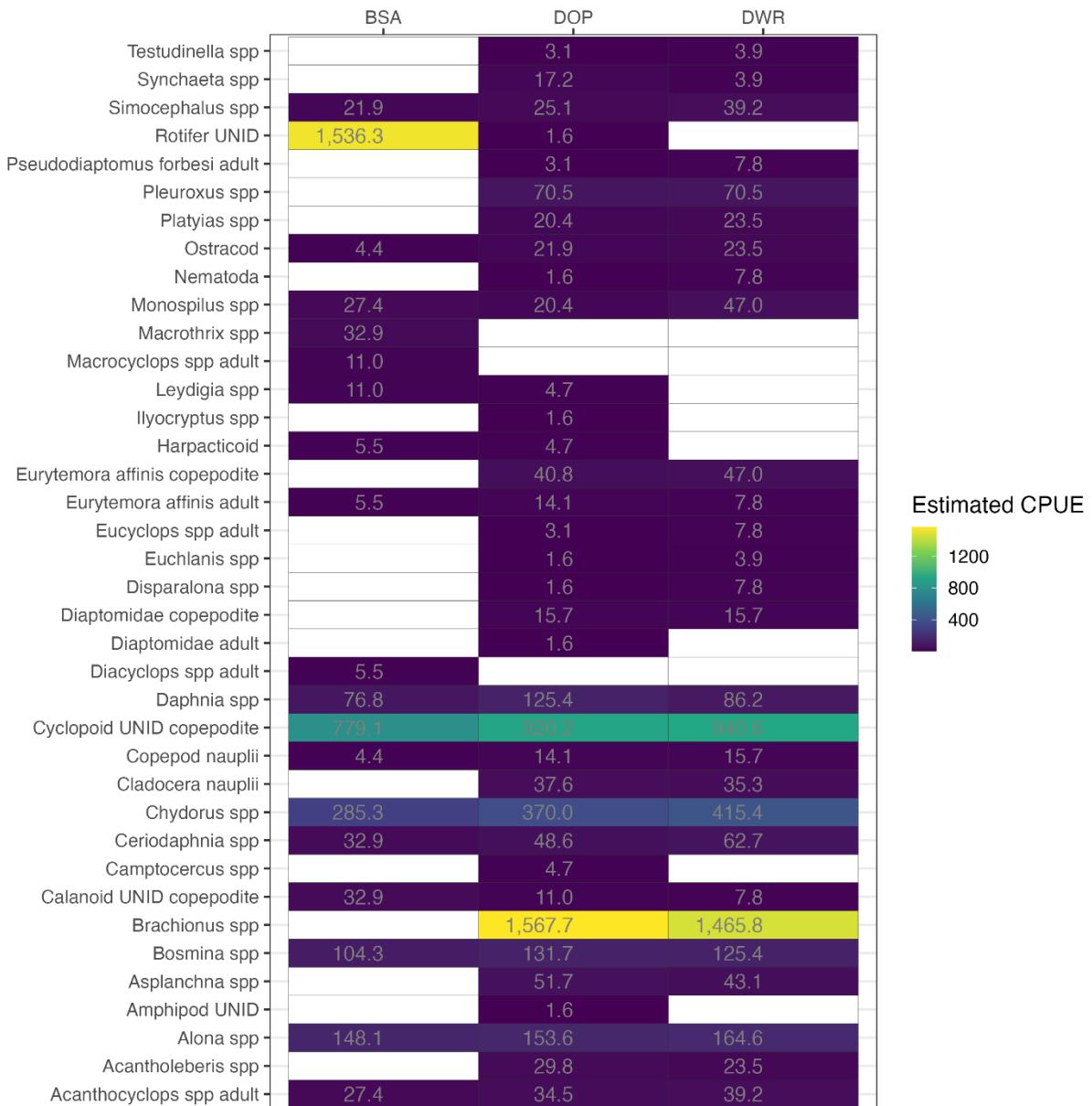


Figure A. NMDS plot as per Figure 3, but with individual sample IDs labeled.

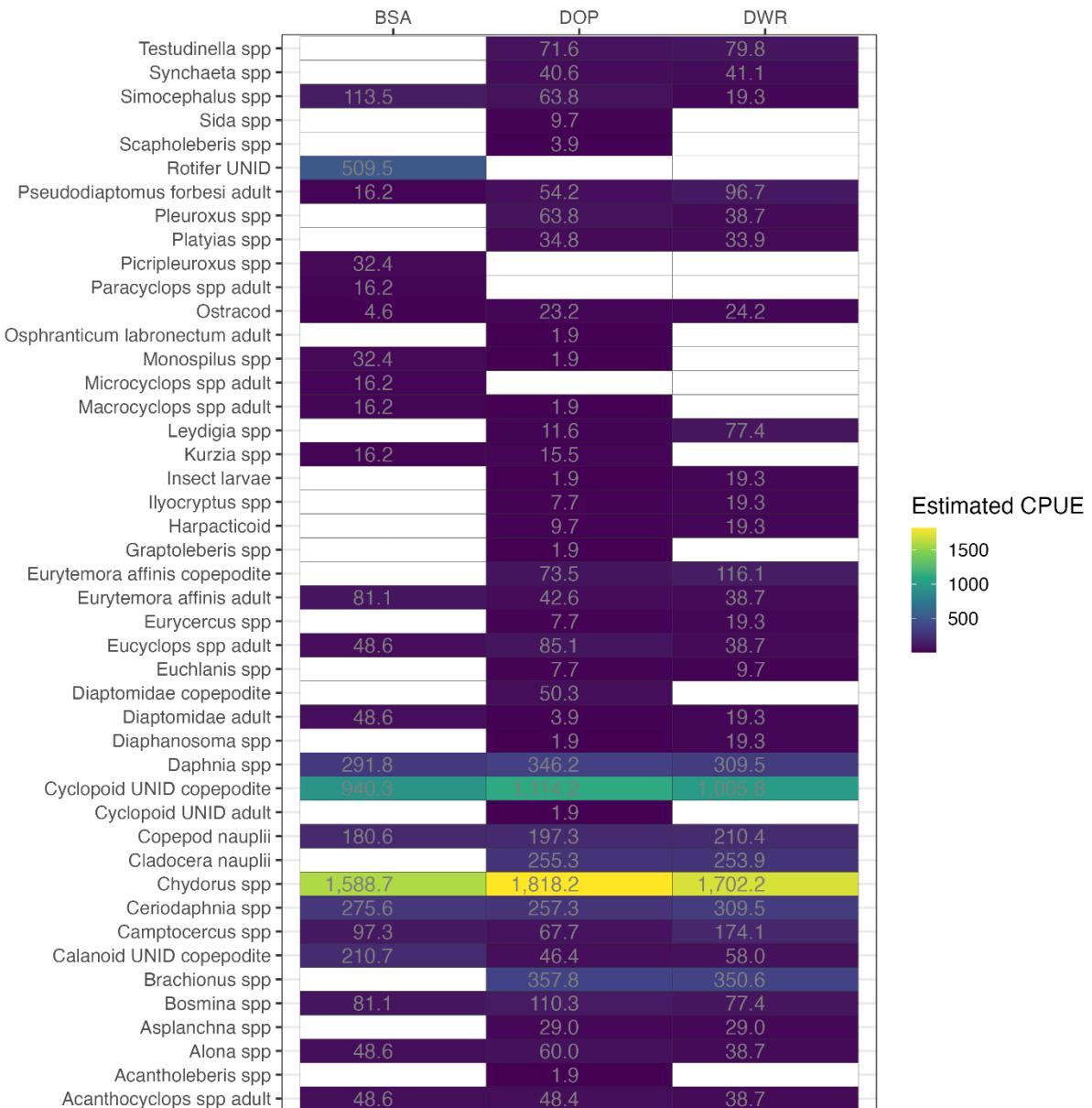
APPENDIX B

Comparison of estimated CPUE for species identified by at least one protocol in each sample.

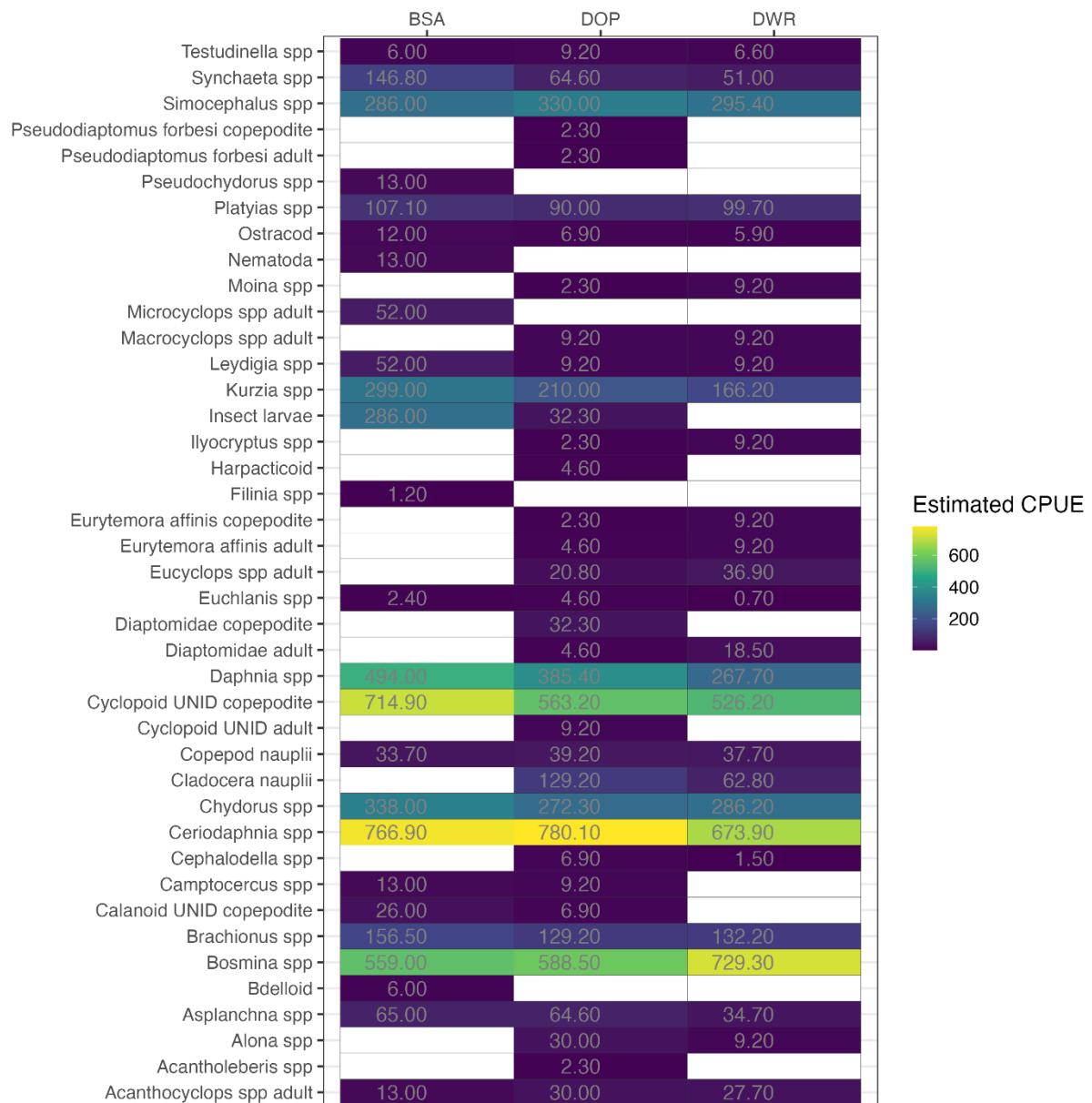
STTD_9:41_150



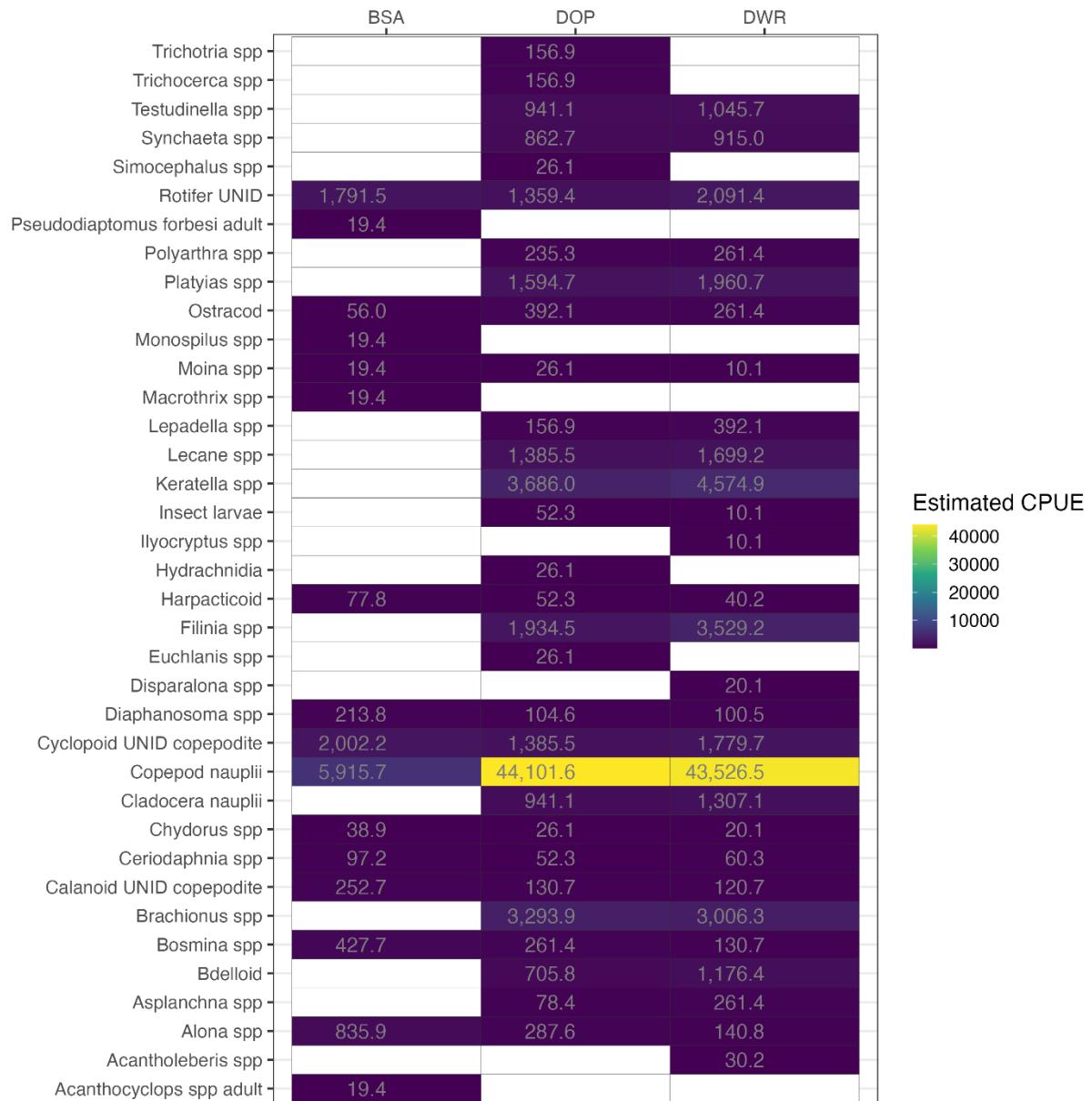
STTD_9:53_150



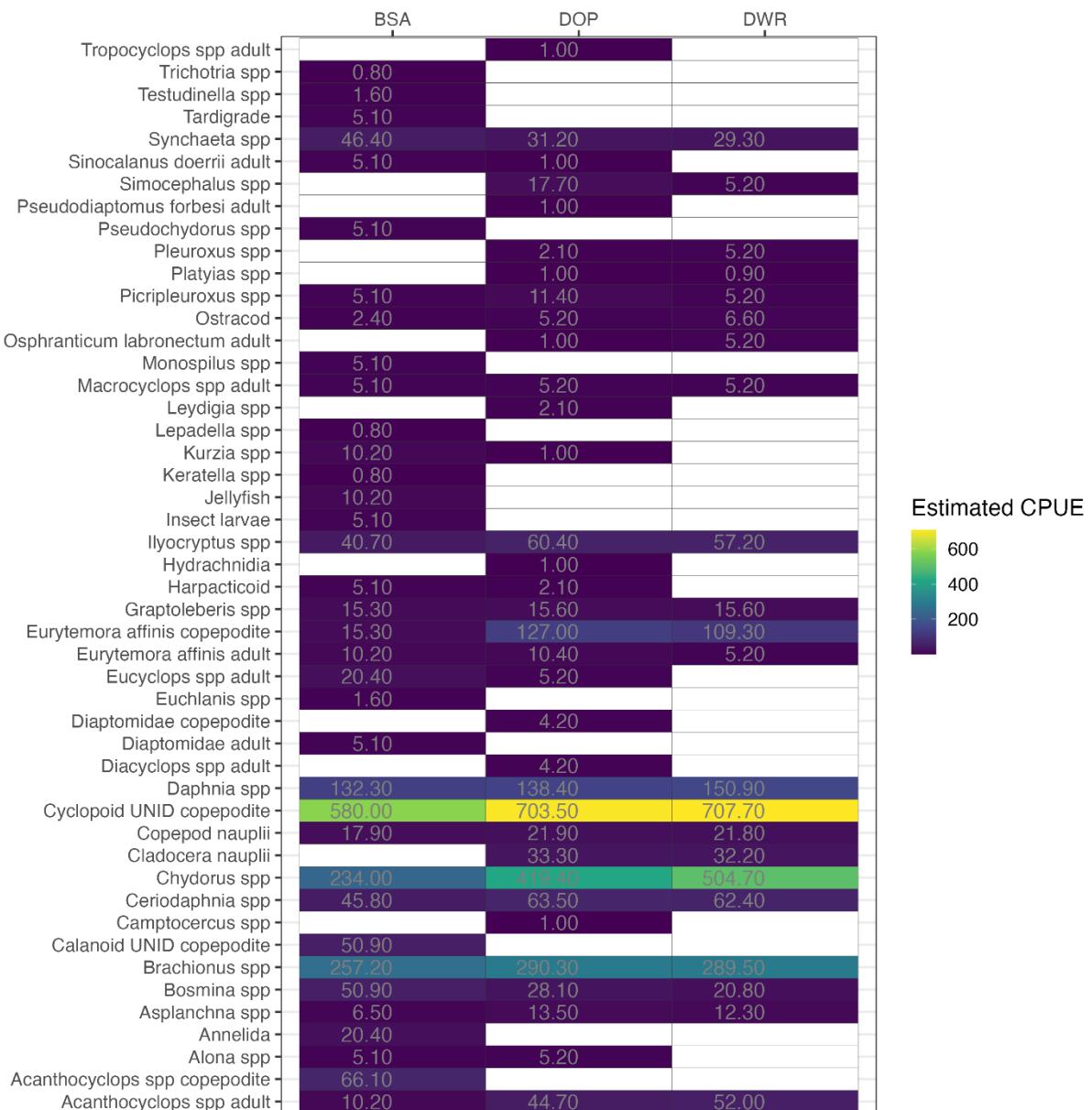
STTD_10:48_150



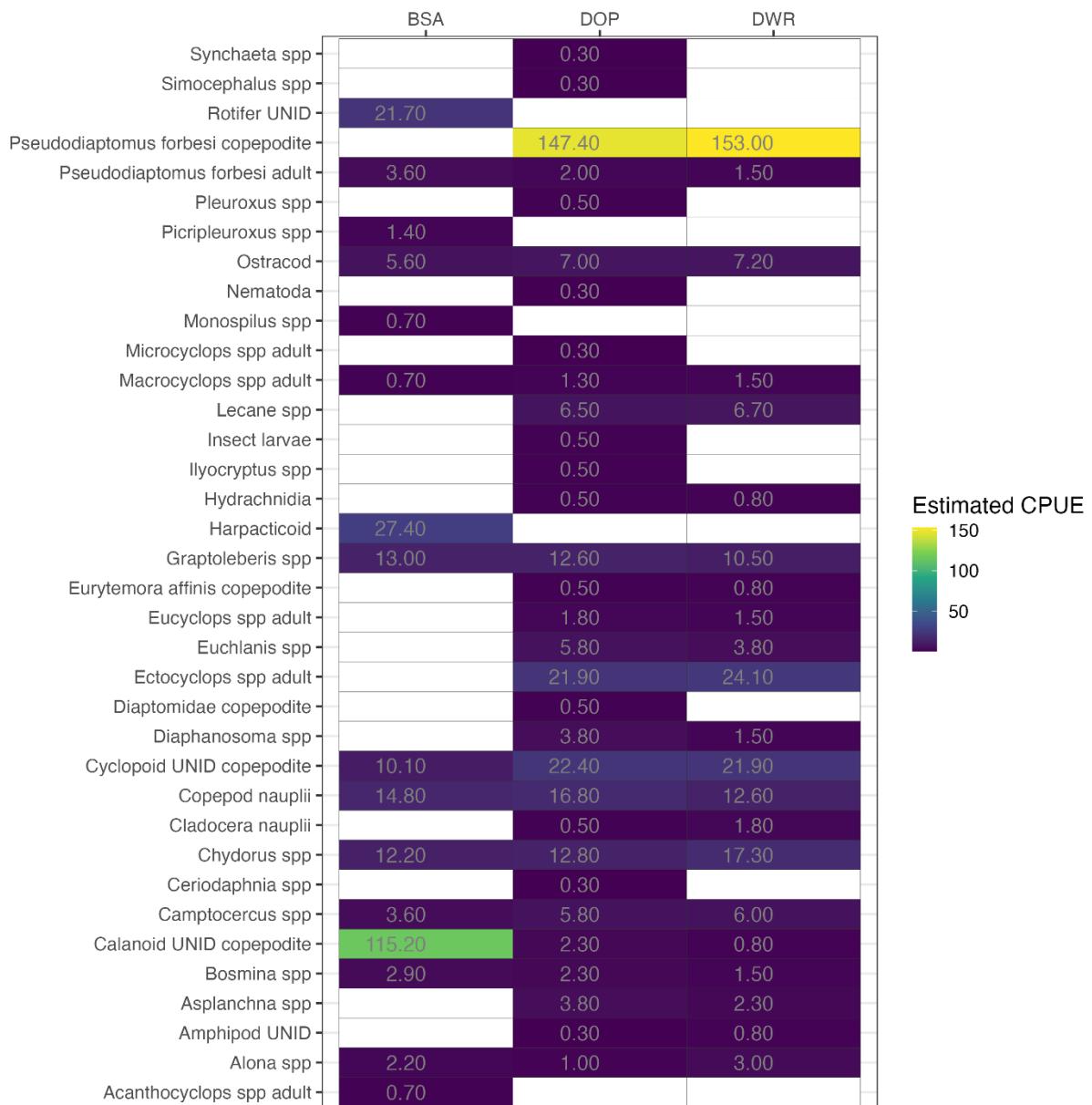
STTD_12:02_50



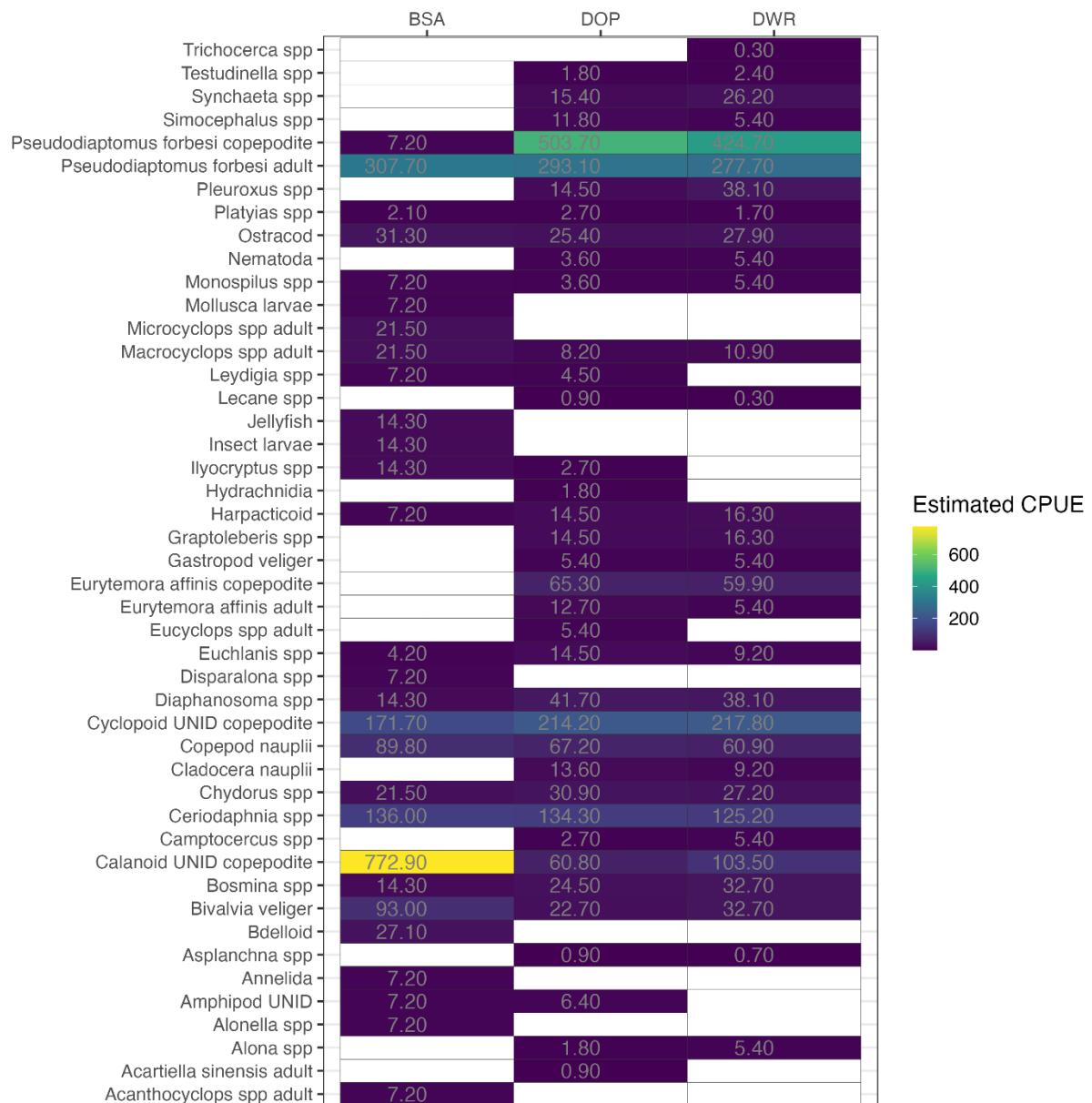
STTD_12:33_150



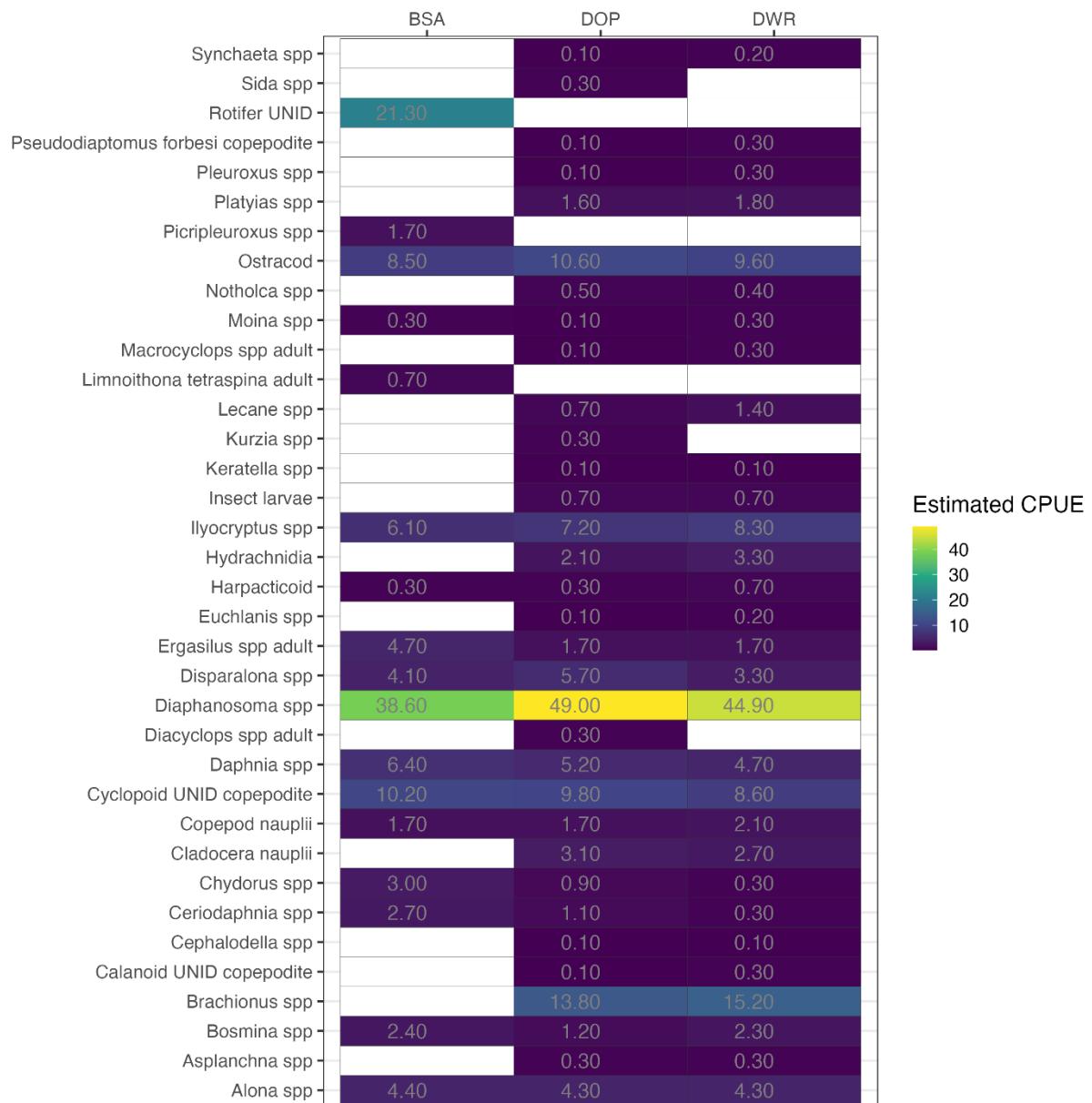
BL5_11:15_150



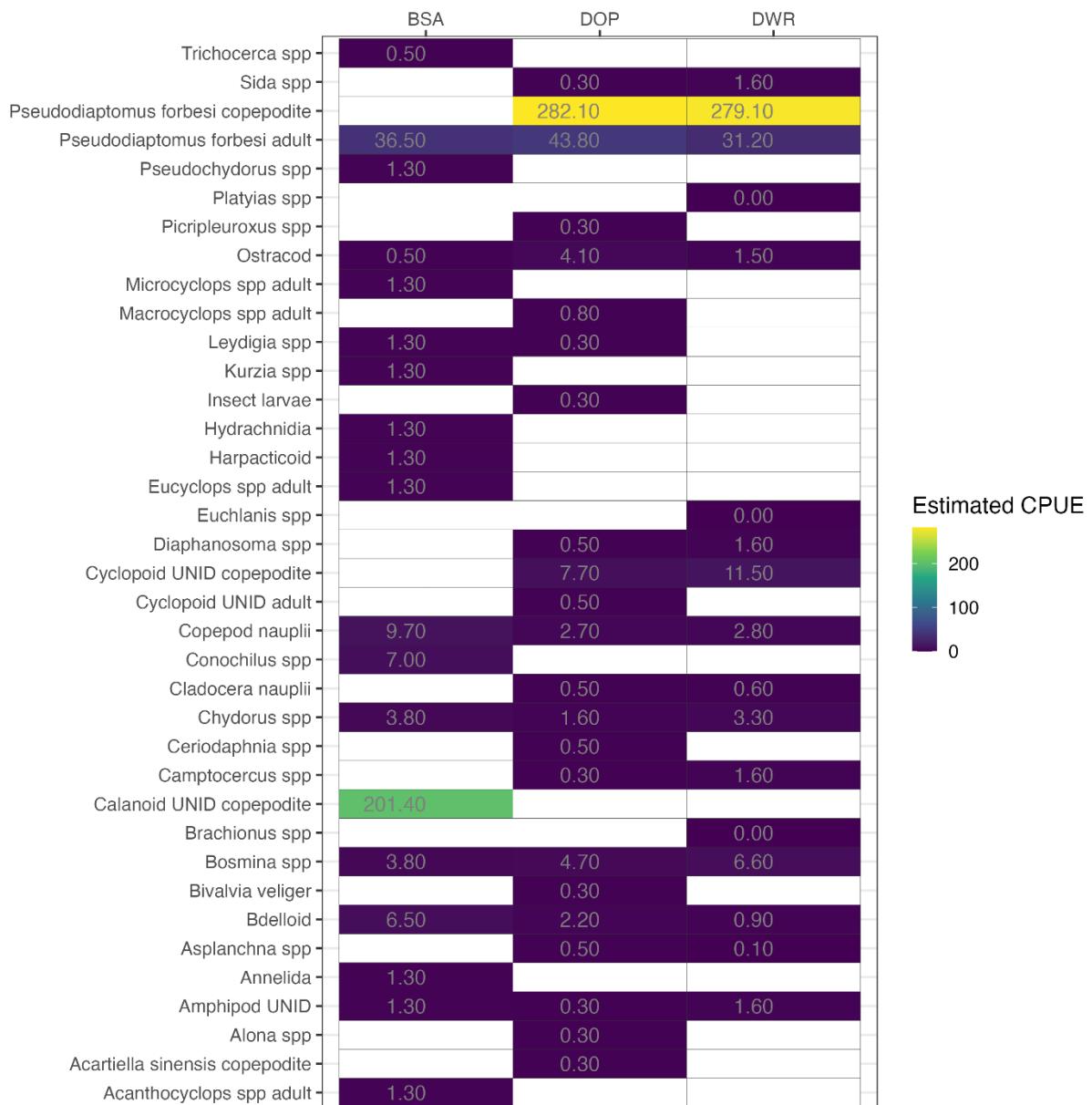
PRS_9:28_150



RD22_11:41_150



RVB_7:13_150



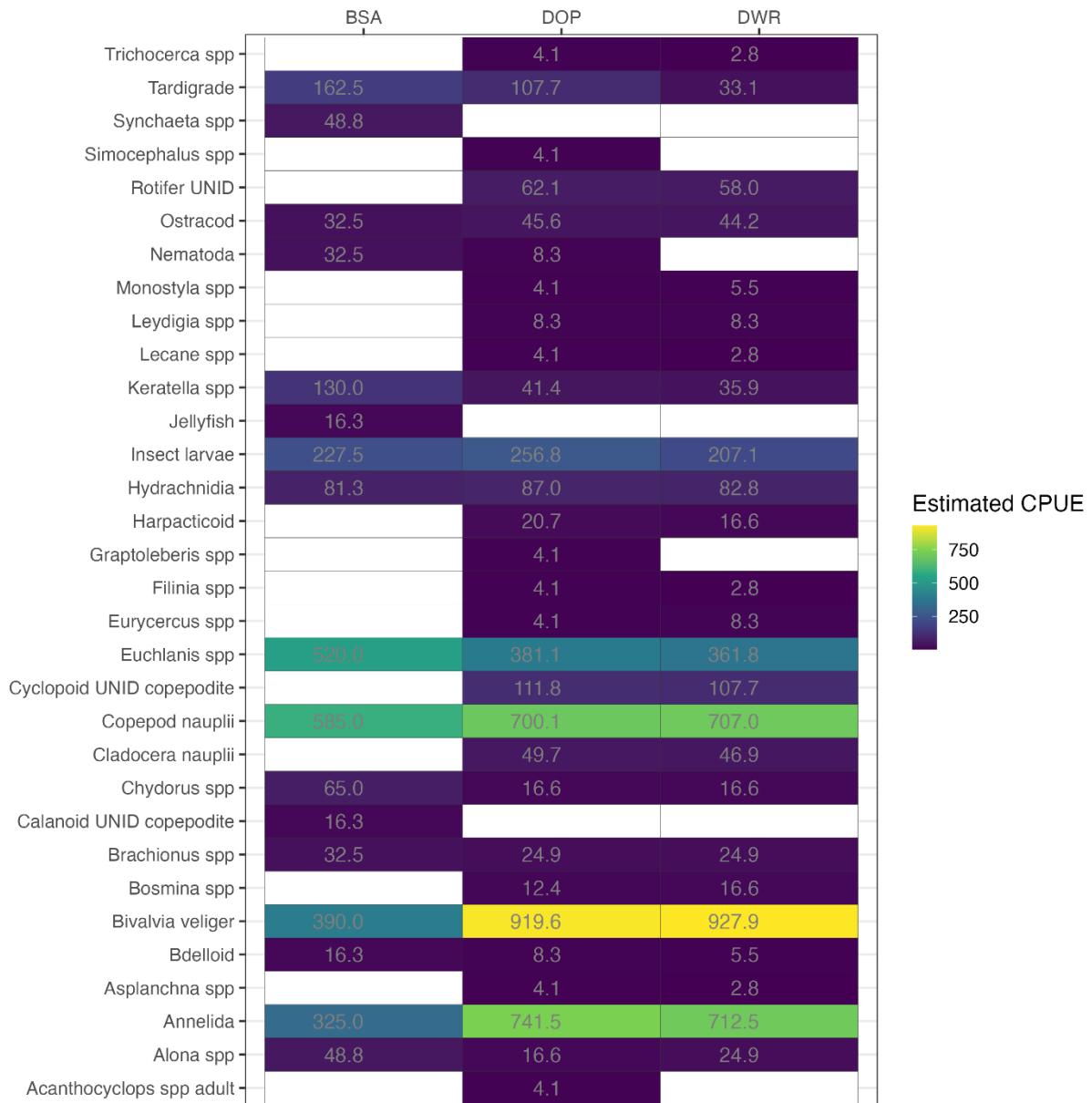
RVB_9:00_150



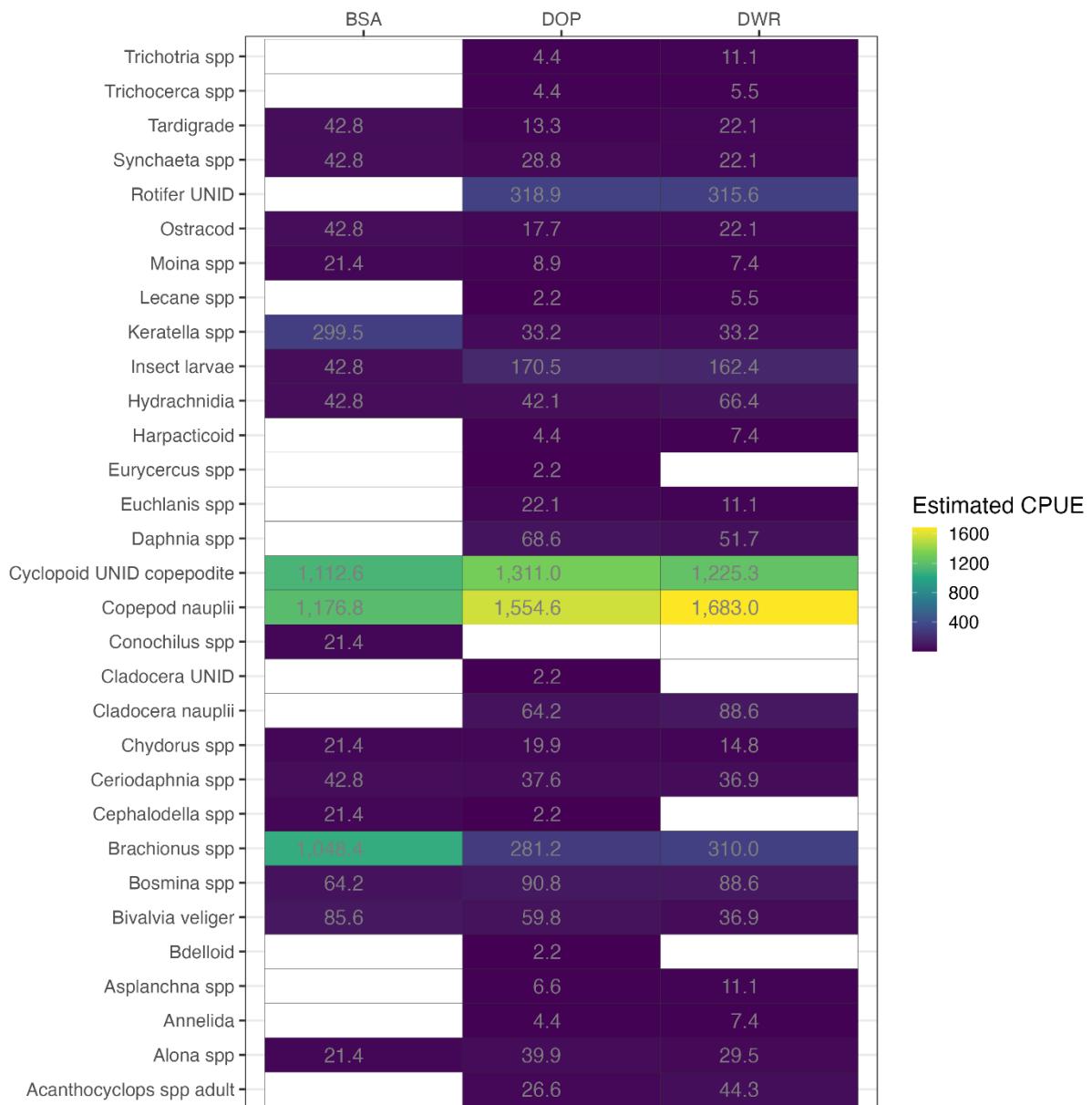
RYI_9:19_150



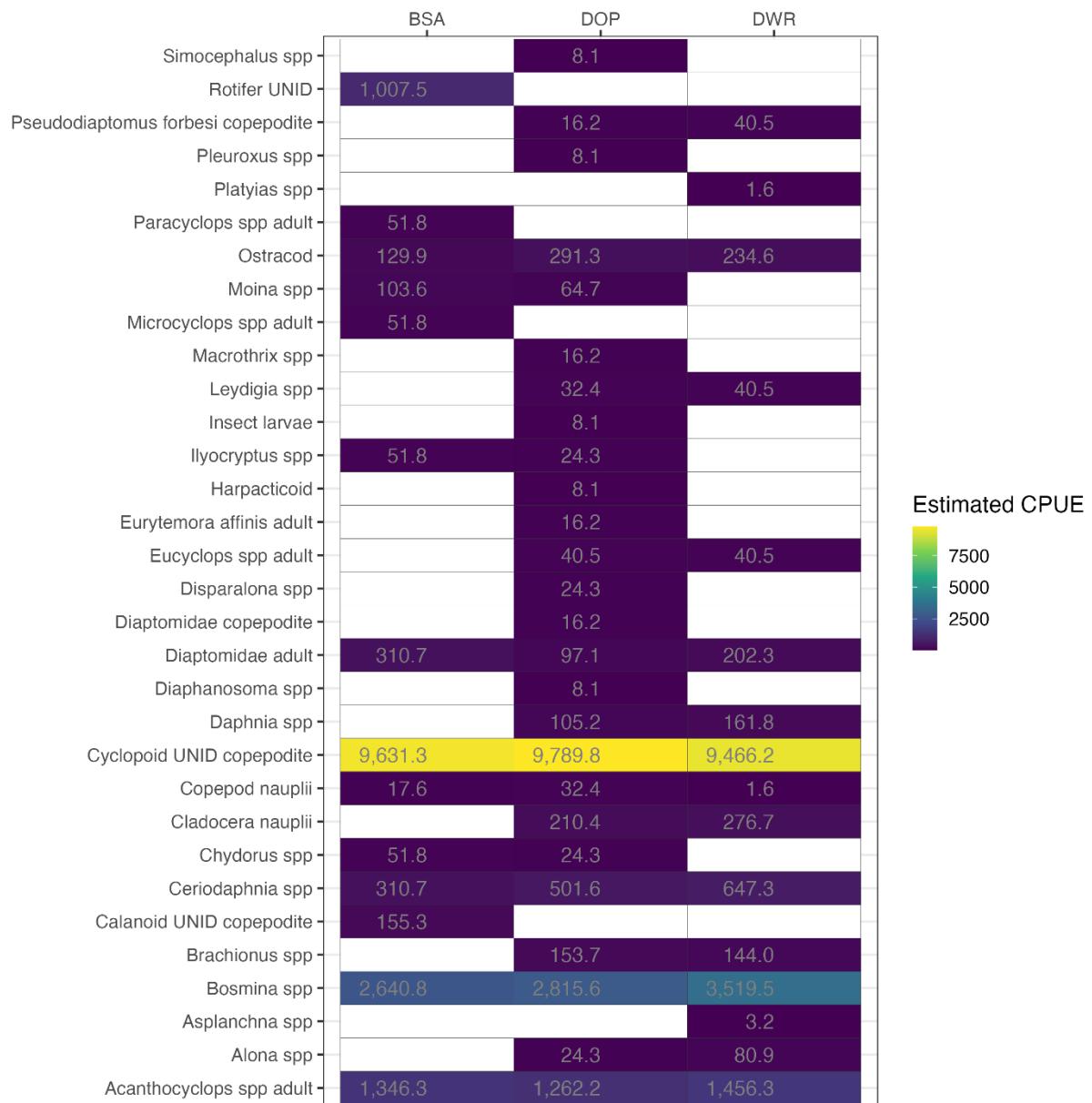
SHR_8:26_50



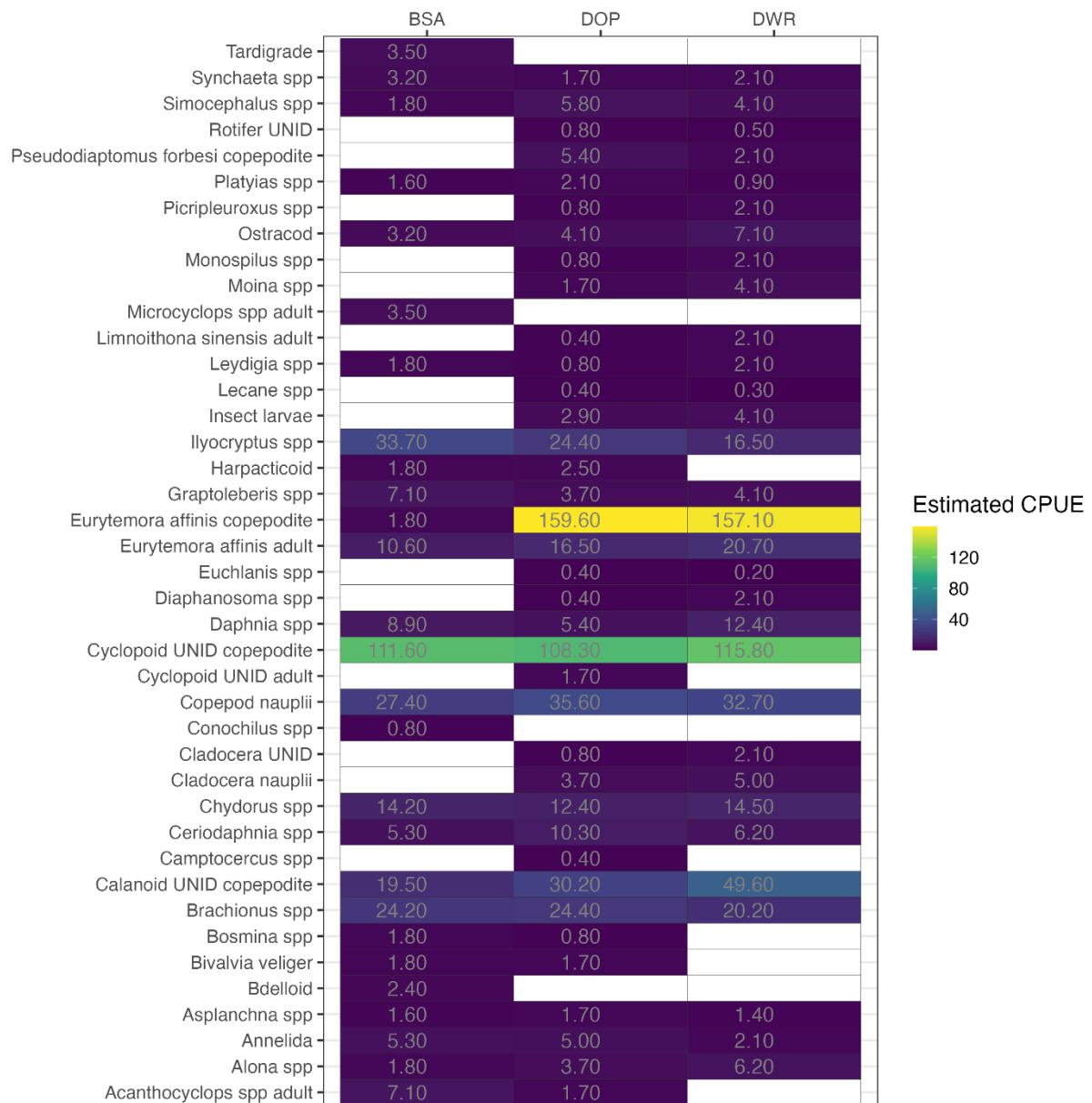
SHR_9:55_50



STTD_9:22_150



STTD_9:30_150



STTD_9:33_50

