Food Webs in the 21st Century: Exploration of New Enabling Technologies to Understand and Predict Changes in Aquatic Food Webs and Impacts on Ecosystems

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Final Report

Overall project summary

The overall goal of this project was to explore application of a new enabling technology to aquatic food web dynamics. The technology is a chromatographic separation system linked to a mass spectrometer and the resulting data gave rise to the new field of metabolomics. Metabolomics can be simply defined as separation and identification of all small molecular weight chemicals (< 1000 daltons) found in plant or animal tissues. The initial hypothesis tested was can metabolomic analysis distinguish algal and zooplankton samples? Stated another way, do organisms within trophic levels have unique chemical/nutrient compositions? The answer, described below, is yes, taxa within trophic levels have distinguishable chemical/nutrient compositions. A secondary goal was identification of those chemical/nutrient categories within taxa using the more robust analytical approach of metabolomics. A wide range of nutrients varied significantly across the samples tested, including amino acids, vitamins and lipids. These data serve as the foundation for pursuit of food web dynamics in a more detailed manner than before and offer the potential of addressing challenging research questions.

Distinguishing algae and zooplankton

Algae

Figure 1 shows the results of principal component analysis of algal samples, using the total metabolites aligned. Slightly over 84% of the variation in samples was accounted for in the 3-way plots, leading to the conclusion that, using the samples and methods chosen, pure and mixed algal samples can be distinguished by their chemical/nutrient composition.

Algal samples acquired and analyzed were two groups of Isochrysis sp. (I and I-198), Pavlova sp. (P), Tetraselmis sp. (TS), Thalassiosia sp. (TS) and 2 mixtures of algae (25% I, 20% P, 20% TS, 30% TH (Rfe) and 5% N and 90%N and 10% T (SD)). Table 1 depicts a summary of metabolites from the 7 algal samples analyzed. A total of 4,010 metabolites were aligned among the 7 samples (with 8 replicates of each sample), with 2,676 occurring in 5 out of 8 replicates which were retained for statistical analysis. From the initial filtered sample, 1,979 metabolites were statistically different among the samples, 131 of which were di- or tri-peptides, which were removed from further consideration. Within the final pool of statistically significant metabolites, 353 could be tentatively identified using the METLIN metabolomics library (Scripps Institute, La Jolla, CA). Using a combination of databases (Kyoto Encyclopedia of Genes and Genomes, Human Metabolome Database, KNApSAcK, PubChem (NCBI), European Bioinformatics Institute (ChEBI) and the Japan Chemical Substance Web Service (Nikkaji), functional categorization of metabolites was accomplished and metabolites grouped by chemical/nutritional categories.

Within the algal samples, metabolites identified as drugs was the largest category of chemicals (101), followed by lipids (51), amino acids (43), steroid hormones (19), unknowns (19), vitamins (19), purines/pyrimidines (17), phospholipids (12), flavanoids(12), and prostaglandins (10). Drug metabolite and biomarker discovery is one of the predominant early uses of metabolomics technology and appears to yield a high number of false positive identifications. Those data, and the unknowns, were discarded from further consideration. Figure 2 depicts the categories of metabolites from the algal samples.

Five of the ten essential amino acids in higher organisms were significantly different in the algal samples tested (Table 2). Isoleucine (ile), methionine (met), leucine (leu), lysine (lys) and tryptophan (trp) varied across algal samples (Note: tabular data are comparisons of spectral intensity values, which will vary as a function of several technical characteristics. These data should not be viewed as absolute changes in concentrations among samples, but as representative, or qualitative, changes across treatments). When all amino acid metabolites are assigned to respective metabolic pathways, there is a clear focus on the aromatic amino acid pathways; phenylalanine (phe), tyrosine (tyr) and trp (Figure 3). Twenty-seven metabolites were associated with the aromatic amino acid pathways, followed by 11 in the basic amino acid pathways (lys and histidine (his)) and 7 in the sulfur amino acid pathways (met and cysteine (cys)).

Three vitamins considered essential in higher organisms were significantly different among the alga samples analyzed (choline, niacin and pantothenic acid, Table 3). Additionally, two metabolites of vitamins D and A were identified as differing among treatments. Excretory products of vitamin C, thiamine and biotin were also identified in the samples as varying significantly. The purines adenine, guanine, and hypoxanthine, the pyrimidine uracil, and the nucleosides guanosine and inosine were significantly different among algal samples analyzed, as well as 11 other purine or pyrimidine metabolites (Table 4). Twelve flavonoids were identified as significantly different among algal samples analyzed (Table 5). A wide range of lipid metabolites were significantly different among algal samples (Table 6). There were no significant differences in essential lipids identified.

Zooplankton

Initial zooplankton samples extracted and analyzed were Calanus finmarchius (ACo), Tigrippus californicus, Pseudodiaptomus (fed and unfed), Tisbe sp. (fed and unfed) and a Great Salt Lake strain of Artemia sp. (enriched (OF) and unenriched (GSL)). The only samples that yielded discernible results were ACo, OF and GSL. Several of the samples analyzed appeared to have undergone autolysis during acquisition, despite having been shipped frozen and on dry ice.

Principal component analysis of the samples is shown in Figure 3. Using the total metabolite dataset, slightly greater than 97% of the variability was accounted for, suggesting zooplankton have distinguishable chemical/nutrient composition.

From the three samples, a total of 2,342 metabolites were aligned, with 1,642 occurring in 5 of 8 replicates. From this subset, 672 metabolites were identified as significantly different among zooplankton samples and 147 could be assigned a name, using the approaches described above (Table 7). Functional categories were assigned as described above.

Within the amino acids, 3 essential amino acids (arginine, leucine and tryptophan) were significantly different (Table 8). Similar to the algal samples, most of the significantly different metabolites were aromatic amino acids (Figure 3). Within the vitamins, choline, vitamin D, niacin and pantothenic acid varied within the samples analyzed (Table 9).Within the purines and pyrimidines, adenine, guanine, guanosine, hypoxanthine, inosine, and uracil were identified as significantly different among algal samples (Table 10). Seventy-three lipid-based metabolites varied among the samples tested (Table 8), 19 steroid hormones derivatives (Table 11), and 12 flavanoids were significantly different among samples (Table 12). While these metabolites are not considered essential nutrients in animals, consumption of prey containing these compounds may elicit beneficial effects.

Implications

These data indicate algae and zooplankton have distinguishable metabolite concentrations and those metabolites include a wide range of essential nutrients necessary for consumers. The flow of these nutrients to consumers is not well understood, nor are the implications of varying nutrient concentrations. However, these data will serve as the foundation for continued work in this area, specifically the flow of essential nutrients through food chains in face of the continual changes in environmental conditions.

Students

Two graduate students worked on this project, Christine Keller (Ph.D.) and Noman Siddiqui (M.S.).

Publications

There are no publications from this project, yet.

Figure 1. Principal component analysis of metabolites from seven algal samples.

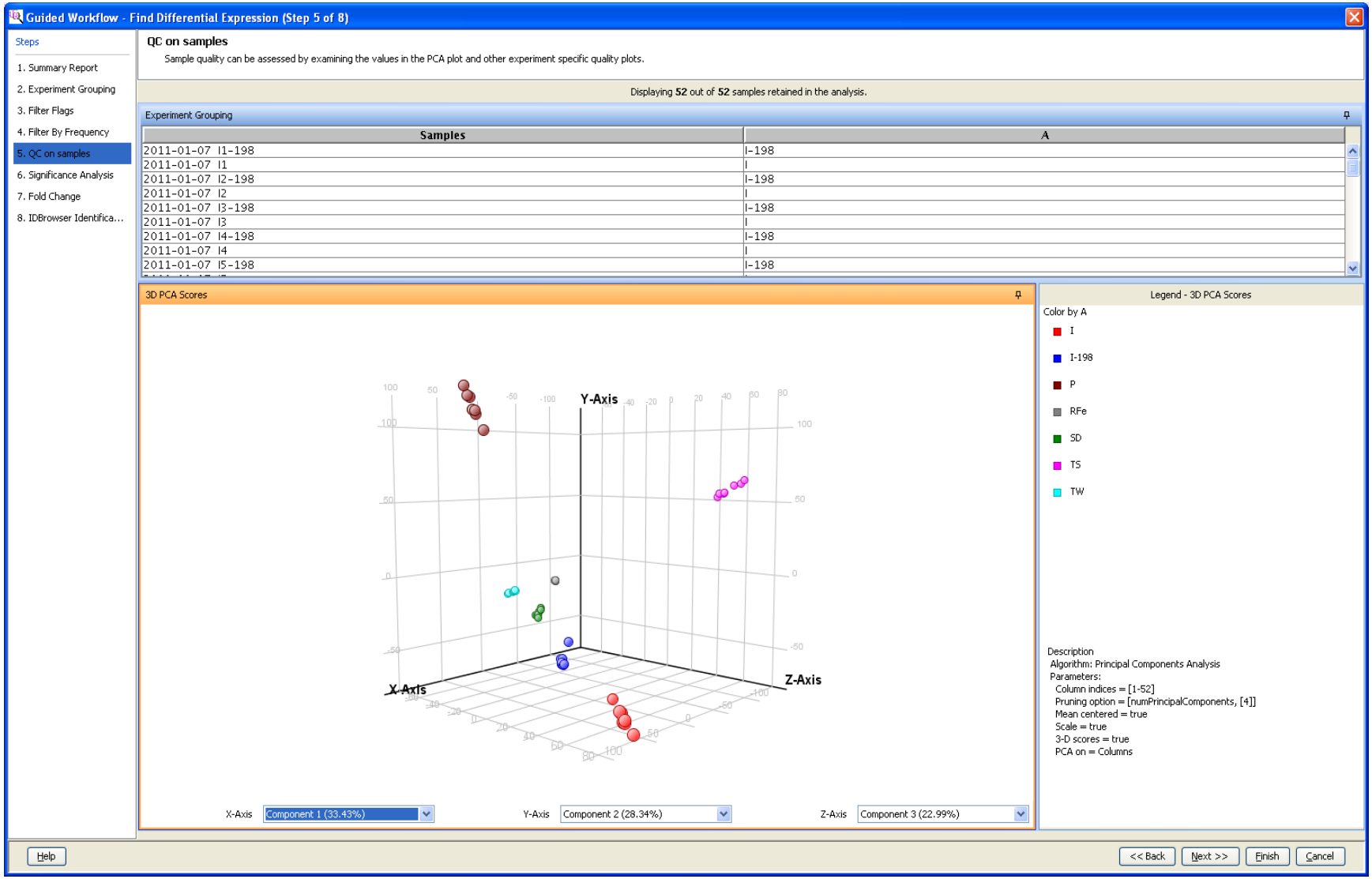

Figure 2. Categorized metabolites from algal samples.

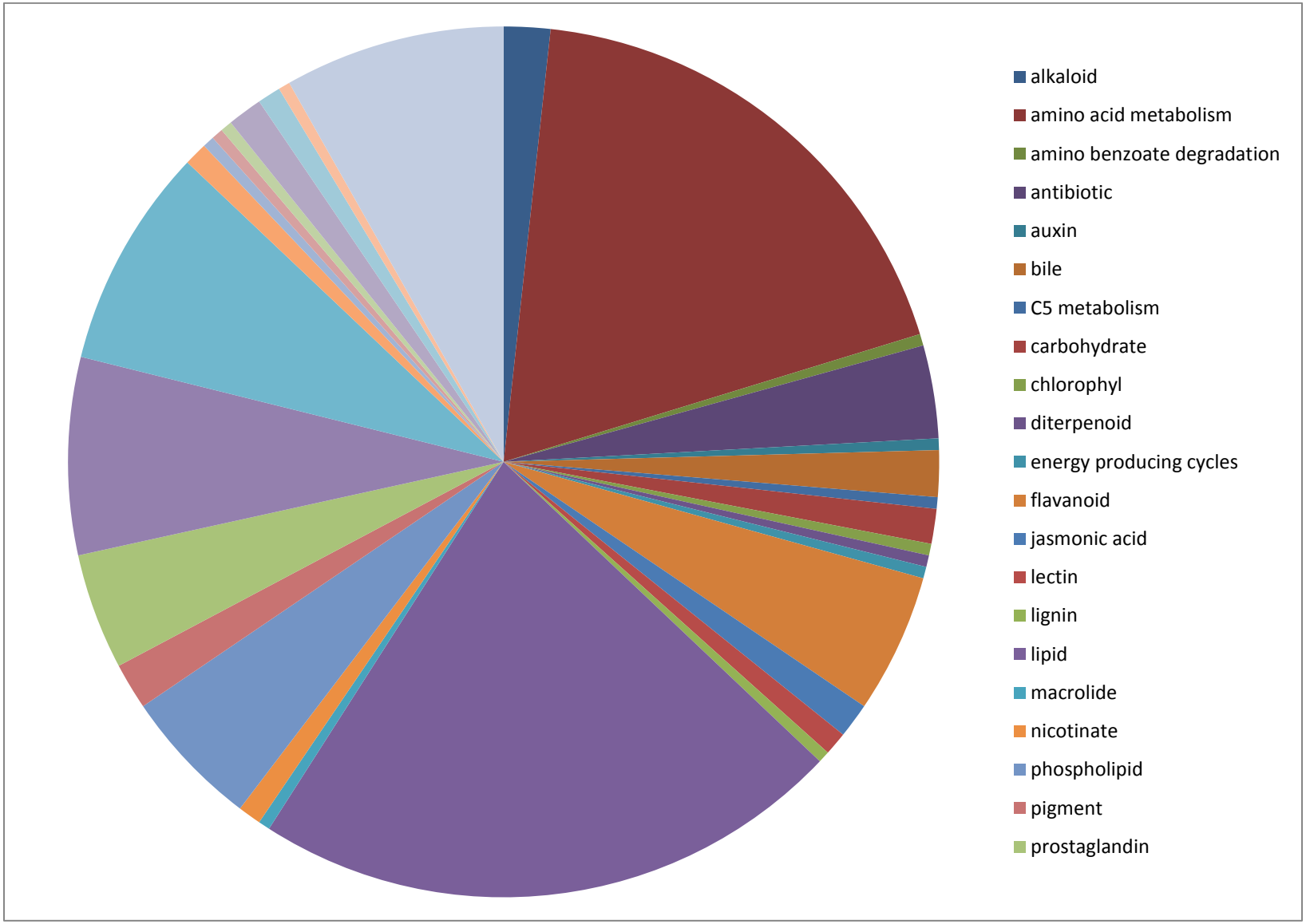

Figure 3. Grouping of amino acid metabolites from algal samples.

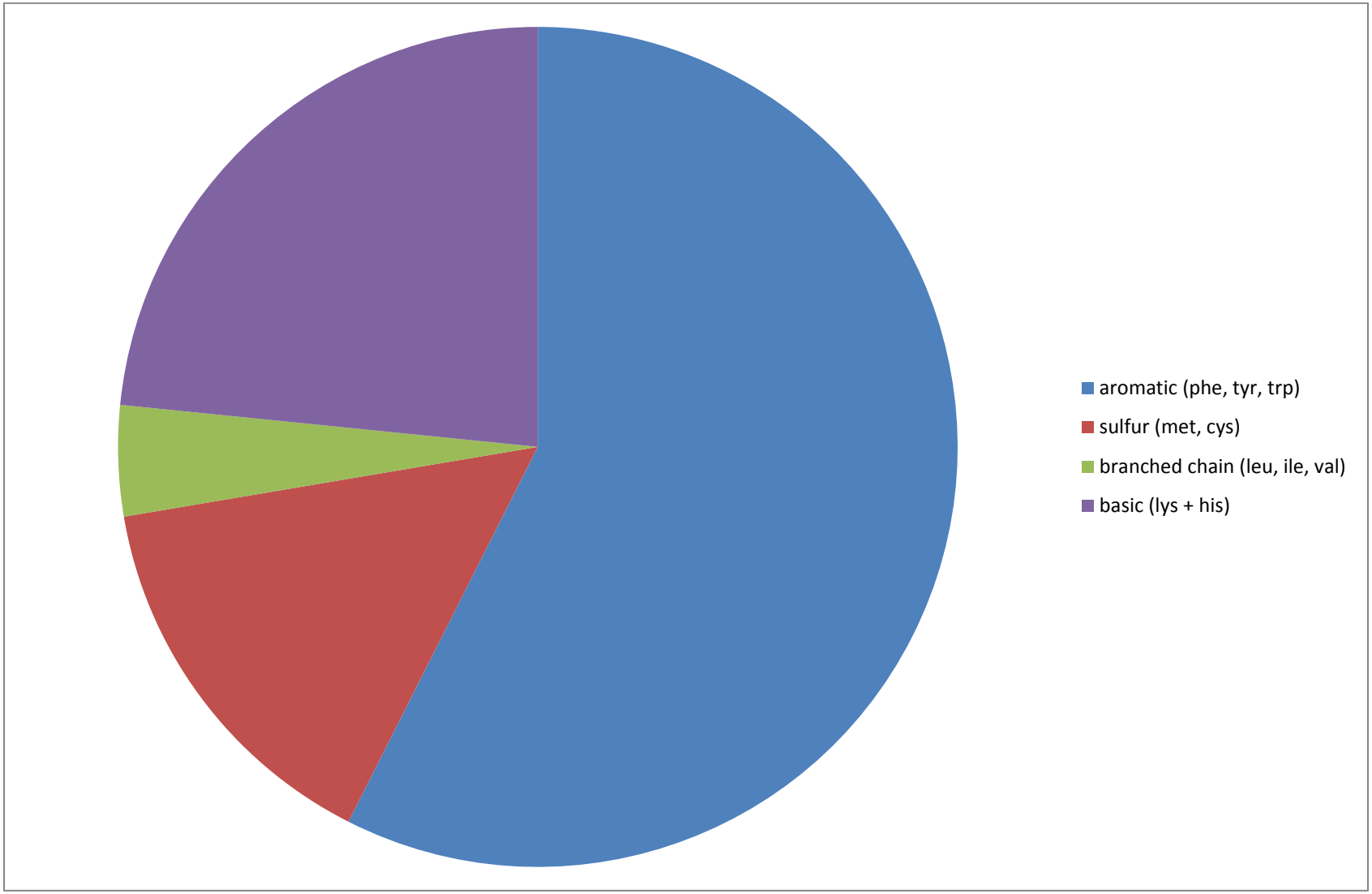

Figure 4. Principal component analysis of zooplankton samples.

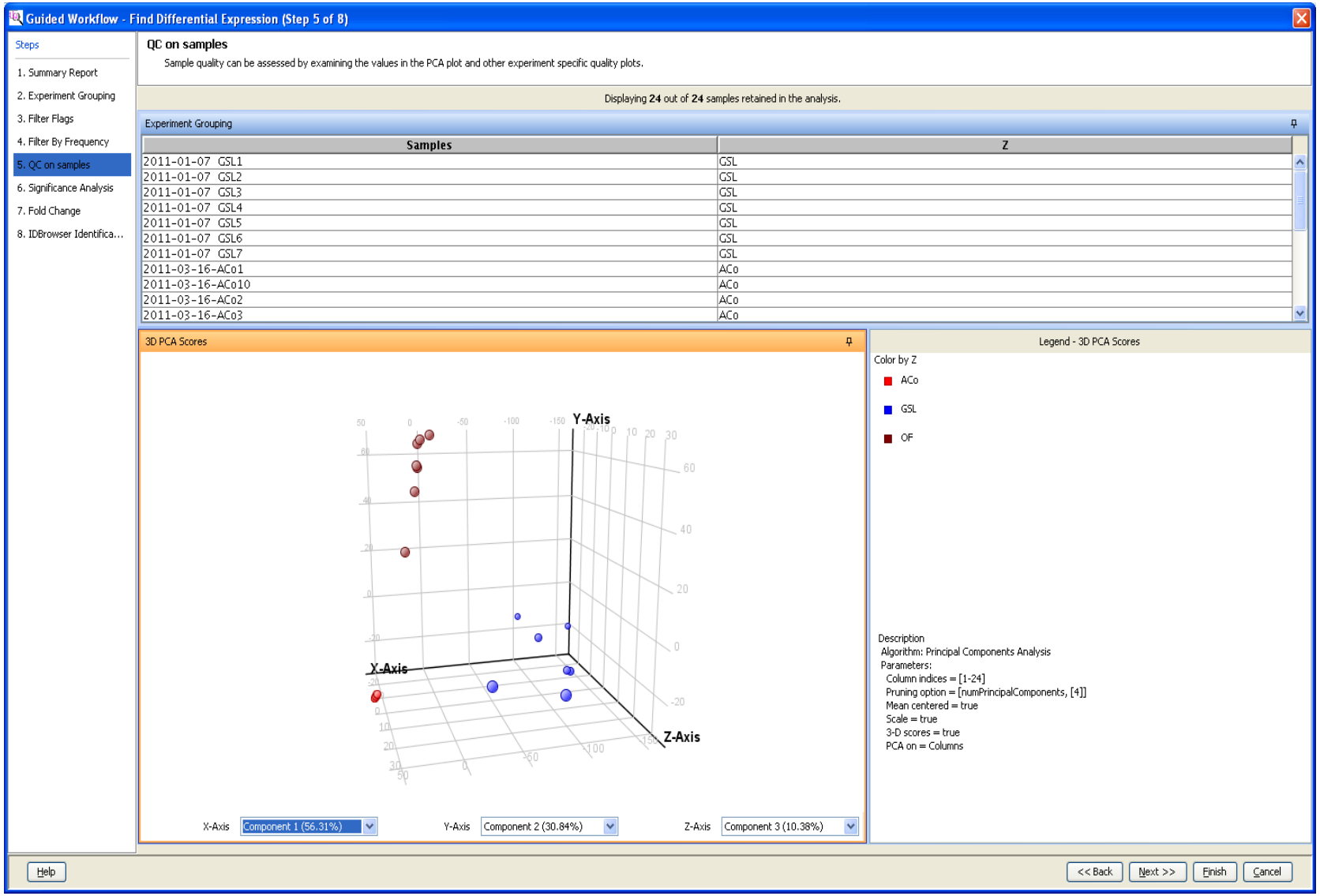

Table 1. Summary of metabolites aligned, filtered and significantly different among algal samples.

Total number of aligned metabolites	4,010
Total number of metabolites occurring in 65% of samples (5/8) within treatment	2,676
Total number of metabolites that were significantly different	1,979
Di- and tripeptides	131
Named metabolites without peptides	353

Table 2. Fold changes in amino acids and amino acid metabolites in algae.

Metabolite	I vs TS	I-198 vs TS	P vs TS	RFe vs TS	SD vs TS	TW vs TS
(3-Methylcrotonyl) glycine methyl ester	1	1	95499 ↑	1	1	1
Indole	2.4 ↓	3.0 ↓	1260487 ↓	1260487 ↓	13.1 ↓	1.30 ↓
L-pipecolic acid	273260 ↑	1	1	1	1	1
L-isoleucine	1268 ↓	1391 ↓	38808 ↓	3445395 ↓	11.8 ↓	3445395 ↓
2,5-diaminohexanoate	78068 ↑	1	103.4 ↑	1	1	1
L-Methionine	225785 ↑	25.2 ↑	161945 ↑	1	4.9 ↑	1
Octopamine	1	1	1	1	1	215238 ↑
Tryptamine	158194 ↑	28.0 ↑	1	1	1	1
5,6-Indolequinone-2-carboxylic acid	137394 ↓	137394 ↓	441.2 ↓	137394 ↓	2.6 ↓	2.0 ↓
3-Amino-L-Tyrosine	1	1	46538 ↑	1	1	1
N-Hydroxy-L-tyrosine	1	1	54588 ↑	1	1	1

N-Acetyl-L-Histidine	51683	↑	9151	↑	4.2	↑	1		1	↓	1	
Hercynine trimethyl his	1	↓	73205	↑	1		1		4.7	↑	1	
2,3-Diaminopropionic acid	178078	↑	1		1		1		1		1	
Discadenine	2430	↑	1	↓	1	↓	1	↓	1	↓	1	↓
Glutathionylspermidine	111437	↑	1		3024	↑	1		1		1	
4-aminohippurate	1		1		1		1		122447	↑	158812	↑
4-Hydroxy-3-methoxymandelic acid	1		1		1		1		49660	↑	1	
5-Aminopentanoic acid	75853	↓	75853		17.7	↑	82.7	↑	13.4	↑	1500	↓
5-Phenylvaleric acid												
Benzoic acid	1		1		1		213004	↑	1		1	
L-Arogenate	664380	↑	1		231284	↑	1		1		1	
Acetyl tyrosine ethyl ester	113200	↑	1		115187	↑	1		1		1	
Capsaicin	184338	↓	184338	↓	184338	↓	184338	↓	184338	↓	184338	↓
Carnitine	60680	↑	1		33753	↑	159914	↑	1		1	
Carnosine	1554	↓	1554	↓	1554	↓	1554	↓	1554	↓	1554	↓
D-1-Piperideine-2-carboxylic acid	1		1		1		1		19.1	↑	51256	↑
hydrocinnamic acid	1		1		1	↓	1	↓	1		253777	↑

Isobutyryl carnitine	1		1		404132	↑	1		1		1	
Leucine	370.1	↑	57550	↑	2804	↑	1		1		1	
Lysine	30.5		30.5	↓	30.5	↓	30.5	↓	30.5	↓	448.9	↑
N-Seryltyrosine	1		1		45990	↑	1		1		1	
Palmitoyl-carnitine	1		1		1995367	↑	1		1		1	
p-Coumaric acid	1		1		1		514322	↑	1	↓	1	
Phenylacetic acid	221778	↑	325186	↑	659275	↑	1		501094	↑	1	
Proline	8241	↑	1		139182	↑	1		1		1	
S-Pyruvyl-glutathione	18223	↓	18223	↓	18223	↓	18223	↓	18223	↓	18223	↓
Tryptophan	58.3	↑	24300	↑	1874	↑	77.9	↓	30412	↑	22480	↑
Tyrosine	1		1		1		579451	↑	1		1	

Table 3. Fold change in vitamins and their metabolites in algae.

Compound	I vs TS	I-198 vs TS	P vs TS	RFe vs TS	SD vs TS	TW vs TS
(23S)-23,25-dihydroxy-24-oxovitamin D3 23-(beta-glucuronide)	93719 ↓	93719 ↓	93719 ↓	93719 ↓	1.1 ↓	93719 ↓
Choline	7.8 ↓	5.9 ↓	227.4 ↓	7.3 ↓	2.1 ↓	7636656 ↓
Threonate	695470 ↑	114770 ↑	1	1	1	1
1alpha,25-dihydroxy-26,27-dimethyl-20,21,22,22,23,23-hexadehydro-24a-homovitamin D3	19068 ↑	46.9 ↑	29412 ↑	1	624.8 ↑	1 ↓
1alpha,25-Dihydroxyvitamin D3 25-trimethylsilyl ether	48963 ↓	48963 ↓	48963 ↓	48963 ↓	48963 ↓	48963 ↓
1-Cyclohexene-1-acrylic acid, 2,6,6-trimethyl-3-oxo-	1	1	115006 ↑	1	1	1
Thiamine aldehyde	40747 ↑	1	1	1	1	1
4-Ketoretinoic acid	1	1	451575 ↑	1	1	1
8-18 bonded retinal	1	1	187482 ↑	1	1	1
Biocytin	4760 ↑	5.2 ↑	1	1	1	1

Choline	1		1		1			1		1		7187010	↑			
Dethiobiotin	1		1		8715	↑		1		1		1				
Niacin	6243	↑	10.7	↑	3845	↑	261.84	↓		37.3	↓	261.8	↓			
Pantothenic Acid	220352	↑	5.3	↑	216401	↑		1		1		1				
Retinoyl beta-glucuronide	1		1		185353	↑		1		1		1				
Threonate	1542016	↓	1542016		↓	139.4	↓	1542016	↓	1542016	↓	1542016.8	↓			

Table 4. Purine and pyrimidine metabolites in algae

Compound	I vs TS	I-198 vs TS	P vs TS	RFe vs TS	SD vs TS	TW vs TS
Adenine	17398 ↑	1	1	1	1	1
1-Methyladenosine	140084 ↓	140084 ↓	140084 ↓	140084 ↓	140084 ↓	140084 ↓
1-Methylguanosine	391180 ↓	391180 ↓	391180 ↓	391180 ↓	391180 ↓	391180 ↓
3-Oxo-3-ureidopropanoate	26.7 ↓	2647 ↑	4474 ↑	26.7 ↓	3133 ↑	26.7 ↓
5'-Adenylyl sulfate	30530 ↑	1	1	1	1	1
5-Aminoimidazole-4-carboxamide	563. ↑	1	201891 ↑	1	1	1
5-Amino-4-imidazole carboxylate	5173084 ↓	5173084 ↓	5173084 ↓	5173084 ↓	8860 ↓	5173084↓
6-Mercaptopurine	71184 ↑	116.5 ↑	1	1	1	1
Allantoic acid	1	1	1	1	157483 ↑	1
Deoxyadenosine	77717 ↓	77717 ↓	77717 ↓	77717 ↓	77717 ↓	77717 ↓
Guanine	263205 ↓	263205 ↓	2.7 ↑	263205 ↓	263205 ↓	263205 ↓
Guanosine	11953 ↓	11953 ↓	26.0 ↑	11953 ↓	11953 ↓	11953 ↓
Hypoxanthine	6426 ↓	6426 ↓	133 ↑	6426 ↓	6426 ↓	6426 ↓
Inosine	1	1	257664 ↑	1	1	1

N2,N2-Dimethylguanosine	72521 ↓	72521 ↓	72521 ↓	72521 ↓	72521 ↓	72521 ↓
Succinoadenosine	1	1	1	106547 ↑	1	1
Uracil	1	1	1	454864 ↑	1	1

Table 5. Flavanoids and their metabolites in algae.

Compound	I vs TS	I-198 vs TS	P vs TS	RFe vs TS	SD vs TS	TW vs TS
Duartin, dimethyl ether 1663	↑ 1	1	1	1	1	
Isogemichalcone B	1	1	1	5345 ↑	1	1
Isorhamnetin 3-(6''-galloylglucoside)	1.3 ↓	1.7 ↓	2.2 ↓	71913 ↓	9.3 ↓	71913 ↓
Leucodelphinidin 3-O-(beta-D-glucopyranosyl-(1->4)-alpha-L-rhamnopyranoside)	1	1	1	1	1	13448 ↑
Triphyllin A	1904837 ↓	1904837 ↓	1904837 ↓	1904837 ↓	1904837 ↓	33.7 ↓
Vitexin 3''',4'''-Di-O-acetyl 2''-O-rhamnoside	79710 ↓	6.4 ↓	79710 ↓	79710 ↓	928 ↓	1.1 ↑
Acacetin 7-(2G-rhamnosyl)-rutinoside	1	1	1	1	1	13617 ↑
Malvidin 3-gentiotrioside	39328 ↓	39328 ↓	39328 ↓	39328 ↓	39328 ↓	39328 ↓
Alatanin 2	31449 ↓	31449 ↓	31449 ↓	31449 ↓	31449 ↓	31449 ↓
Purpuritenin B	5.0 ↑	1	3540 ↑	1	1	1
Tephrowatsin A	1	1	372053 ↑	1	1	1

Tephrowwatsin C	1289	↓	1289	↓	1289	↓	1289	↓	1289	↓	1289	↓

Table 6. Fold changes in lipid and lipid metabolites among algae samples.

	I vs TS	I-198 vs TS	P vs TS	RFe vs TS	SD vs TS	TW vs TS
(+/-)11,12-EpETrE	1	1	1300403↑	1	1000059.2↑	1
(+/-)-11-HDoHE	1	1	760524.1↑	1	1182.1553↑	1
tiglic acid - trans form of crotonic acid	726.41974↓	726.41974↓	726.4197↓	726.41974↓	726.41974↓	726.41974↓
4-hydroxy-crotonic acid - organic acid	235498.42↑	377276.53↑	1	1	201.62177↑	1
12-oxo-9-octadecynoic acid	38974.766↑	1	5.275319↑	1	1	1
2-methylene-4-oxo-pentanedioic acid	877354.1↓	877354.1↓	877354.1↓	877354.1↓	10.856756↓	877354.1↓
16-hydroperoxy-9Z,12,14E-octadecatrienoic acid	304059.7↑	1	1	1	4.9114957↑	1
9-hydroxy-5Z-nonenoic acid	1	1	1	1	3351.8237↑	1
2,4,6,8-decatetraenal	1	1	267977.7↑	1	1	1
2,4,6-octatrienal	31961.244↓	31961.244↓	31961.24↓	31961.244↓	31961.244↓	31961.244↓
2,4-heptadienal	1	1	1	1	25.956703↑	381771.22↑
2,4-pentadecadienal	1	1	159453.7↑	1	1	1
2,5-undecadienal	1	5.2141886↑	175013↑	1	155045.33↑	1
Sebacic acid	1	156881.47↑	1	1	2992.563↑	1
2-amino-8-oxo-9,10-epoxy-decanoic acid	429537.12↓	2.044858↑	429537.1↓	429536↓	3.104669↑	3.5076587↑

2E,4E-hexadecadienoic acid	23.830015↑	1	175284.4	1	1	1
2E,6Z,8E-decatrienoic acid	1	1	230062.2	1	1	1
2E,6Z,8Z,12E-hexadecatetraenoic acid	1	1	149559.4↑	1	765.45636 ↑	6877.0713↑
2S-hydroxy-10-undecenoic acid	1	105174.3↑	1	1	1	1
2-tridecene-4,7-diynal	17708.074↑	1	14466.27↑	1	1	1
3,5-hexadienoic acid	1	1	1	409350.4↑	1	1
3,6-octadecadiynoic acid	92.97265↑	56.84706↑	14.96805↑	54778.26↓	4.5330315↑	54778.26↓
3,9,15-Docosatriynoic acid	6.9798613↑	1	13845.81↑	1	1	1
(±)14(15)-EET-SI	1	1	1	1	1	12271.671↑
3E-undecenoic acid	1	1	154289.6↑	1	22088.521↑	1
4,7,10,13,16-docosapentaenoic acid	2.043134↓	1.8443028↑	273637.2↓	273637.22↓	1.6189193↑	273637.22↓
4,7,10,13-Docosatetraynoic acid	1173679.6↑	1	887976.8↑	1	1	1
6-bromo-5,9-hexacosadienoic acid/ 6-bromo-5E,9Z-hexacosadienoic acid	2237.8967↑	1	1	1	1	189091.95↑
5(6)-EpETrE-EA	1	1	1	1	1	11687.456↑
5,8,11-octadecatriynoic acid	219479.52↑	180251.05↑	151.193↑	1	249490.78↑	1
5,8-heptadecadiynoic acid	18346.516↑	1	1	1	1	1
5-octadecylenic acid						

6,8,10,12-pentadecatetraenal	33301.25↑	5.338814↓	5.338814↓	5.338814↓	5.338814↓	5.338814↓
6,9,12-hexadecatrienoic acid	411795.75↑	1	689110.9↑	1	1	1
GlcNAcβ1-3Galβ1-4Glcβ-Sp glycospingolipid	5495.486↓	5495.486↓	5495.486↓	5495.486↓	5495.486↓	5495.486↓
8,9,16-trihydroxy palmitic acid	35330.098↑	46613.496↑	264631.7↑	1	80109.31↑	1
8-hydroxy-17-octadecene-9,11-diynoic acid	1	1	65335.3↑	1	1	1
8-hydroxy-9,11-octadecadiynoic acid	1	4.8343544↑	225555.8↑	1	481027.9↑	933487.75↑
beta-eleostearic acid	43.891296↑	782852.3↑	1	1	490934.3↑	1
HELENINE/alantolactone - isoprenoid	509996.84↑	1	357126.4↑	1	1	1
C17 Sphinganine	24.815065↑	14289.318↑	3749.504↑	27.519838↓	16513.893↑	27.519838↓
C17 Sphingosine	1	1	1	1	2633.8926↑	1
DL-PDMP (hydrochloride)/DL-threo-PDMP	1	72502.46↑	1	1	63096.336↑	1
4-hydroxy Nonenal Alkyne - lipid peroxidation product	1	1	371662.5↑	1	374188.78↑	493526.94↑
DL-2-Aminooctanoic acid	310143.97↓	310143.97↓	310144↓	310143.97↓	310143.97↓	310143.97↓
Eicosanedioic acid	76972.51↑	1	1	1	1	1
N-(3-oxo-hexanoyl)-homoserine lactone	6085407.5↓	742564.7↓	6085408↓	6085407.5↓	6085407.5↓	6085407.5↓
Resolvin D1	1	1	13975.69↑	1	1	1

sebacic acid	418062.44↑	1	1	1	1	1
sodium chlorovulone III	1	1	94129.53↑	1	1	1
Timnodonic acid	1	1	384578.7↑	1	296000.78↑	1
LysoPE(0:0/18:4(6Z,9Z,12Z,15Z))/LysoPE(18:4(6Z,9Z,12Z,15Z)/0:0)	26149.188↑	1	1	1	1	1
PI(18:1(9Z)/0:0)	1185.8055↑	1	1	1	1	1
PI(18:0/0:0)	425913.53↓	425913.53↓	425913.5↓	425913.53↓	425913.53↓	425913.53↓
PC(18:3(6Z,9Z,12Z)/20:5(5Z,8Z,11Z,14Z,17Z))	4.8778176↑	1	41080.42↑	1	1	1
PC(18:2(9Z,12Z)/24:1(15Z))	68993.69↓	68993.69↓	68993.69↓	68993.69↓	68993.69↓	68993.69↓
PIP2(16:0/18:2(9Z,12Z))	1	1	18914.33↑	1	1	1
Arachidonyl lysolecithin	28016.918↓	28016.918↓	28016.92↓	28016.918↓	28016.918↓	28016.918↓
N-Methylethanolamine phosphate	1	1	5.60578↑	311.38562↑	1	276733.25↑
Glucosylsphingosine	1	1	929293.8↑	1	1	1
GPCho(8:2(2E,4E)/8:2(2E,4E))	1	1	43527.38↑	1	1	1
GPEtn(12:0/0:0)	1	1	1	1	1	116298.94↑
Linolenoyl lysolecithin	819898.75↓	819898.75↓	819898.8↓	819898.75↓	819898.75↓	819898.75↓
1a,1b-dihomo-15-deoxy-delta-12,14-PGD2	1	1	76896.33↑	1	1	1
Eicosanedioic acid/ PGF1α Alcohol/MEDICA 16	4.8641276↓	4.8641276↓	4.864128↓	4.8641276↓	3391.3118↑	4.8641276↓

C22-PGF4alpha/ 1,9-dideoxyforskolin/8-iso-16-cyclohexyl-tetranor Prostaglandin E2	1	1	19559.04↑	1	1	1
17-phenyl trinor Prostaglandin E2 serinol amide	1	1	1	1	1	44239.26↑
9,15-dioxo-11R-hydroxy-2,3,4,5-tetranor-prostan-1,20-dioic acid	3879.2375↓	3879.2375↓	3879.238↓	3879.2375↓	3879.2375↓	3879.2375↓
Alprostadil(15-keto-13,14-dihydro-PGE)	1	1	1	1	1	25533.24↑
bicyclo-PGE2	1	1	232821.2↑	1	1	1
PGB3	1	1	295509.7↑	1	1	1
PGE2-EA	113231.04↑	136912.16↑	141545.7↑	1	183334.83↑	1
PGJ3	1	1	437092.6↑	1	151549.42↑	601489.2↑

Table 7. Summary of the total number of aligned, filtered and significantly different metabolites from zooplankton samples.

Total number of aligned metabolites	2,342
Total number of metabolites occurring in 65% of samples (5/8) within treatment	1,642
Total number of metabolites that were significantly different	672
Di- and tripeptides	114
Named metabolites without peptides	147

Table 8. Fold changes in amino acids and amino acid metabolites among zooplankton

	ACo vs GSL	OF vs GSL
2-Methylbutyroylcarnitine (leu,ile,val)	101,209 ↓	1
4a-Carbinolamine tetrahydrobiopterin (phe,tyr,trp)	1	104,981 ↑
4-aminohippurate (phe)	20,602 ↓	20,602 ↓
5,6-indolequinone-2-carboxylic acid (tyr)	182 ↑	18.4 ↑
5-Aminopentanoic acid (lys,arg/pro)	80,351 ↓	2,345 ↓
Arginine	2.2 ↑	1.5 ↓
Cinnamic acid (phe)	576,515 ↓	1.2 ↓
Dihydroxyphenylacetic acid (tyr)	148,715 ↓	9.3 ↓
Elaidic carnitine	284,759 ↓	284,759 ↓
Glutathione, oxidized	5,864,209 ↓	1
homotrypanothione disulfide (gluta excretory)	1	2,722 ↑
Indole (phe,tyr,trp)	283,315 ↓	1.2 ↑
Indole-3-carboxylic acid (trp)	154,194 ↓	222 ↓
Indoleacrylic acid (trp)	5,905,569 ↓	1.3 ↑
Isobutyryl carnitine (leu,ile,val)	150,409 ↓	7.6 ↓
L-DOPA (tyr)	9,838 ↑	1

Leucine	218,720 ↓	218,720 ↓
N-Acetyl-8-O-methyl-Neuraminic acid (SAM)	63,964 ↑	1
Proline (arg/pro)	9.8 ↑	1.4 ↑
Pyroglutamic acid (5-oxoproline)	296,316 ↓	1
S-Adenosylhomocysteine (SAM)	97,179 ↓	1.7 ↑
Spermidine (arg/pro,ala,gluta)	2,344,313 ↓	1.3 ↑
Tryptophan	2,357,707 ↓	1.5 ↑

Table 9. Fold changes in vitamins and vitamin metabolites among zooplankton samples

	ACo vs GSL	OF vs GSL
1,25-dihydroxy vitamin D3	1	39,762
2,3-Dioxogulonic acid (C, excretory)	10,225	1
7,8-Diaminononanoate (Biotin precursor)	46,426	1
Dihydropteroic acid (Folate precursor)	1	215,666
Niacin	3.8	7,608
Pantothenic acid	932,412 ↓	2.3

Table 10. Fold changes in purine and pyrimidines and their metabolites among zooplankton samples

	ACo vs GSL	OF vs GSL
1-Methylguanine	1	306,861 ↑
4-Methoxycinnamic acid	8,354 ↓	4.0 ↓
5'-Methylthioadenosine	374,987 ↓	1
Adenine	150.2 ↓	1.5 ↑
Cytarabine	150,706 ↑	1
Guanidylic acid	879,045 ↓	1.1 ↑
Guanine	5,102,322 ↓	1.4 ↑
Guanosine	4,127,015 ↓	1.5 ↑
Hypoxanthine	4,000 ↓	1.5 ↓
Inosine	1,013,585 ↓	1.1 ↓
Queuine	48,944 ↓	1.6 ↑
Succinoadenosine	465,216	1.4 ↑
Uracil	122 ↓	19.9 ↑
Ureidoglycine	395.7 ↓	19.9 ↑

Table 11. Fold changes in lipids and lipid metabolites among zooplankton samples

	ACo vs GSL	OF vs GSL
10-nitro,9Z,12Z-octadecadienoic acid	1	35,180 ↑
17,18-dehydro-clavulone I	56,243 ↓	1.1 ↓
1-heptadecanoyl-sn-glycerol 3-phosphate	1	187,573 ↑
1-Linoleoylphosphatidylcholine	143,850 ↓	143,850 ↓
1-Palmitoyl lysophosphatidic acid	1	276,574 ↑
20-carboxyarachidonic acid (mass 334.2079)	1,245 ↓	1,245 ↓
4,7,10,13-hexadecatetraenoic acid	27,353 ↓	27,353 ↓
Arachidonyl lysolecithin	143,527 ↓	143,527 ↓
C17 Sphinganine	26,951 ↓	1,285 ↓
Dodecanamide	201,306 ↓	211 ↓
Heneicosane	9,193 ↓	9,193 ↓
Glycerophosphocholine(24:1/24:1)	1	3,059 ↑
Glycerophosphocholine(O-2:0/O-1:0)	4,813 ↓	4,813 ↓
Glycerophosphoethanolamine(18:0/0:0)	34,554 ↓	34,554 ↓
Heptatriacontanoic acid	1	2,722 ↑
Isobutyryl carnitine	150,409 ↓	7.6 ↓

Linolenoyl lysolecithin	62,824 ↓	62,824 ↓
Lysophosphatidic acid(0:0/18:2)	5.3 ↓	400 ↑
Lysophosphatidylethanolamine(0:0/18:3)	39,754 ↓	39,754 ↓
Lysophosphatidylethanolamine(0:0/20:5)	7,044 ↓	7,044 ↓
Lysophosphatidylethanolamine(0:0/20:4)	44,486 ↓	44,486 ↓
Myristoyl L-a-lysophosphatidylcholine	66,272 ↓	66,272 ↓
Phosphatidic acid (14:0/0:0) (mass 382.6832)	2,822 ↓	2,822 ↓
Phosphatidic acid (12:0/13:0)	14,993 ↓	2.904 ↓
Phosphatidylcholine (16:1/0:0)	17,533 ↓	17,533 ↓
Palmitoylcarnitine	35,733 ↓	35,733 ↓
Phosphocholine (mass 183.0676)	59,932 ↓	59,932 ↓
Phosphatidylinositol(16:0/20:1)	4.2 ↓	23,474 ↑
Phosphatidylinositol(16:0/16:0)	204,513 ↓	1.1↓
Tetranor-PGFM	4,799 ↓	4,799 ↓

Table 12. Fold changes in steroid hormones and their metabolites in zooplankton

	ACo vs GSL	OF vs GSL
18-Oxocortisol	168,315 ↓	1.1↓
21-Hydroxypregnenolone	29,642 ↓	29,642 ↓
4-Hydroxyestrone	1,977 ↓	1,977 ↓
b-D-Glucopyranosiduronic acid, (3a,5b,7a,12a)-24-[(carboxymethyl)amino]-1,12-dihydroxy-24-oxocholan-	78,619 ↓	5,860 ↓
Cucurbitacin S	5,141 ↓	5,141 ↓
Diginatin (mass 796.4458)	443,500 ↓	443,500 ↓
Hellebrin	1	32,097 ↑
Tetrahydrodeoxycorticosterone	34,999 ↓	34,999 ↓