Electronic Supplementary Material (ESI) for Analyst.

Supporting Information For:

NMR Assignment of the *In Vivo Daphnia Magna* Metabolome

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Part A. Assignment Protocol

Assignments were performed in this paper using a rigorous and time intensive manual approach that took over a year to fully perfect. It was found that all automated approaches gave rise to numerous false positives and a multi-tiered approach was required to offer meaningful assignments given the very complex and overlapping nature of the datasets.

The result of the protocols are two categories of assignments (see Table S1), namely "Confirmed Assignments" and "Tentative Assignments" as summarized in Table S2 and Table S3, respectively. Tentative assignments generally indicate "to the best of our knowledge the compound is likely present, but for a range of reasons we cannot be 100% confident", thus, to err on the side of caution we report the assignment as tentative and do not include it with the main assignments.

The following section (stage 1 through 4) outlines in detail the protocols followed to assign both confirmed and tentative assignments in this manuscript.

Table S1. Summary table for confirmed and tentative assignments

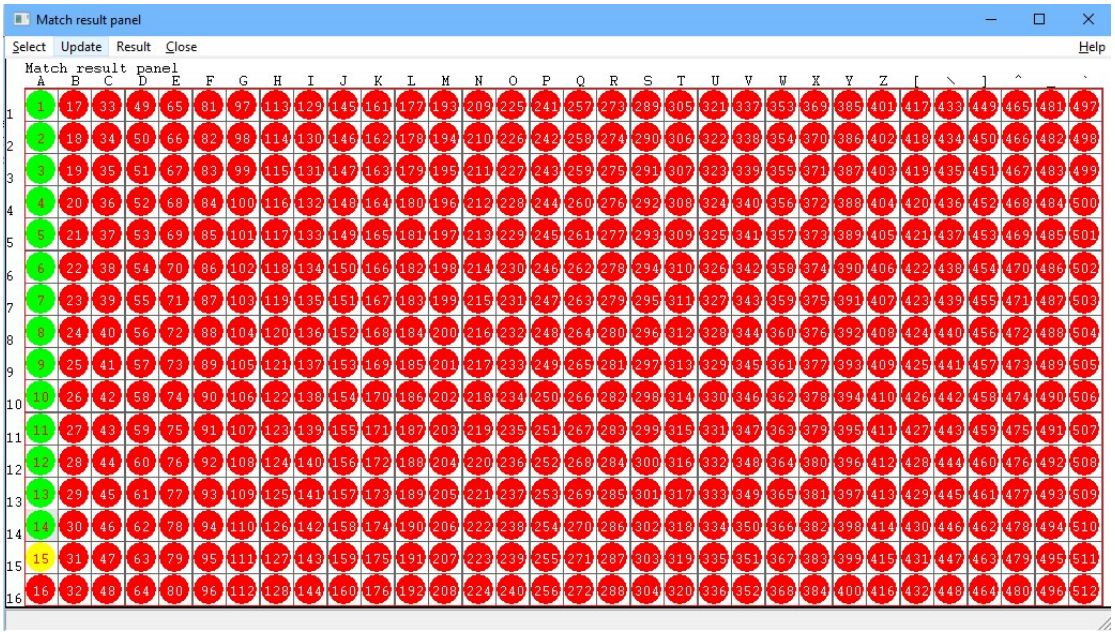
Scenario	Listed in	Description
Confirmed Assignment	Main Assignment, Table S2	Only offered if numerous key peaks can be seen in multiple NMR experiments and these metabolites have been previously confirmed in the literature.
Tentative Assignment A	Tentative, Table S3, as Scenario A	Assignments can only be made in a single NMR experiment, and the literature supports the metabolites being present.
Tentative Assignment B	Tentative, Table S3, as Scenario B	Assignments match with multiple NMR experiments but due to overlap their assignment cannot be made with complete confidence. The literature confirms the presence of the compound.
Tentative Assignment C	Tentative, Table S3, as Scenario C	Assignments are made with confidence from the NMR data, but compound has not been reported in aquatic crustaceans.

Stage 1: Screening of experimental data for database matches

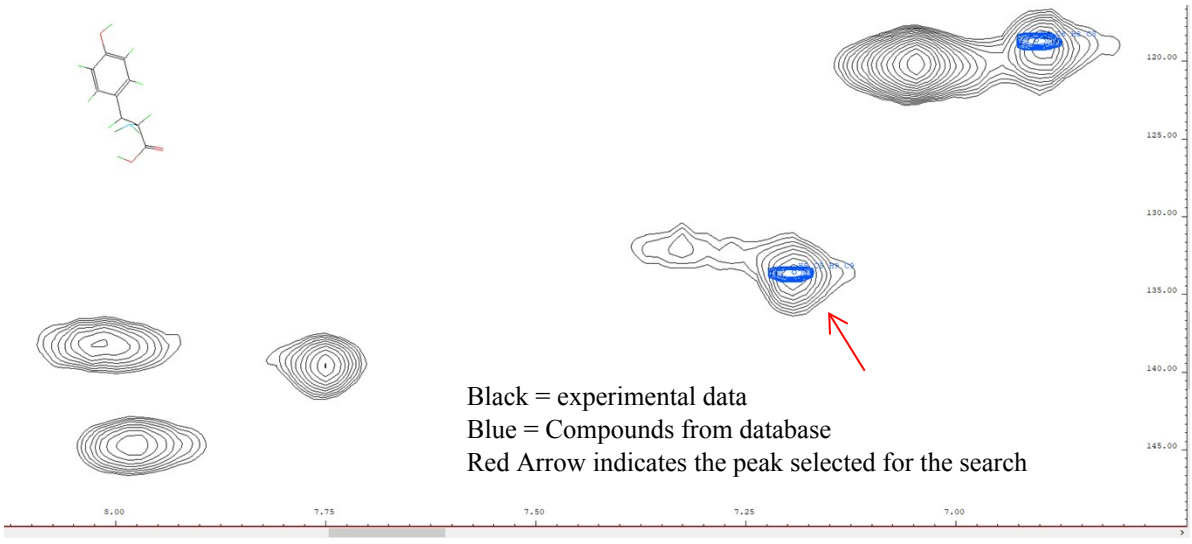
(example given for HSQC data)

1) Carefully calibrate all experimental data against known compounds, for example glucose, alanine, and glycine in the Bruker Bio-reference Databases version 2-0-0 to 2-0-5.
2) Define all peaks using maximum entropy-based deconvolution AMIX version 3.9.15.

3) Select a single peak, using a match search with the following parameters; 80% match threshold, sort results, find “at least one peak” allow variance 1 ppm carbon 0.05 ppm proton. Note that the variance threshold is based on the size of the contours seen *in vivo* and designed such that the peak has to, at least in part, overlap with the database hit (see Figure S1 later).
4) A typical result may look like the panel below. The result below essentially shows 14 compounds in the data, match the chemical shift of the single peak selected.



5) Next each compound is checked against the real data. This is achieved by clicking on the result panel which automatically overlays the selected compound from the database over the real data. The example below shows the match for tyrosine in the aromatic region. Only the peak highlighted with the red arrow was selected for the search.



6) A compound is only considered if all its signals fall in an area of signal intensity in the experimental data. If any peaks from the database compounds fall in a region of the experimental data that has

no signal, then the compound is immediately discarded. In an ideal world all peaks would be clearly defined and be the most intense in all regions. However, in reality, this rarely happens *in vivo* due to overlap with dominant components for example lipids/carbohydrates. As such if a number of clear peaks are present that match with a compound and other peaks are buried under a region of much greater signal intensity the peaks are retained, but as described in Table 1, will require confirmation from an independent second NMR dataset and confirmation from the literature before becoming a "confirmed assignment".

7) Next, the intensity of the various peaks in the compound are considered. For example, if 3 resolved peaks exist, are the 3 peaks roughly at the same intensity in the experimental data (gauged by interactively scaling the experimental data when overlaid onto the database compound spectrum)? If so, it is likely that all these peaks arise from the same compound and this compound dominates in this region giving relatively high confidence in the assignment. However, if one of the peaks is much less intense (for example 10 fold less intense), this suggests either the assignment is not correct (i.e. one of the peaks considered a match, is actually overlap from a different compound altogether) or that the more intense peaks likely have overlap from other compounds. If the intensities are not logical, the potential assignment is only considered as a tentative hit (see later) and must be confirmed again independently in a second NMR dataset and again in the literature to remain on the tentative list.

Stage 2: Multiple confirmation and other considerations

1) If signals clearly identified in more than one independent experiment (i.e. not tentative but confident assignments) they are retained as potential assignments which are then screened against the literature (see next step).
2) If signals appear as tentative assignments on more than one spectrum or confident assignment in only one spectrum, they are retained but only as tentative assignments.
3) *Consideration: Long Chain Aliphatic Species.*
   By NMR it is not possible to accurately determine the length of an aliphatic chain (for example a lipid or alkanes) as peaks from longer (> C_6) chains tend to overlap. As such if a database containing a range of lipids is searched, they will all return positive matches for the aliphatic region. While in fact it is not possible to say the exact number of carbons in the chain present. As such to avoid these false positives, the hits for compounds with chains > C_6 are discarded. Instead, the aliphatic region which is mainly dominated by triacylglycerides and lipids is assigned separately in terms of general structures.
4) *Consideration: Amino Acids.*
   Amino acids can be assigned with great confidence due to the amino acid-only NMR experiment[1] developed specifically for in *vivo NMR* profiling. Because of this, the exact chemical shifts of the α and β positions are known with great accuracy. Due to this, the amino acids resonances are labelled on the HETCOR and HSQC even if in some cases the α-β resonances are buried under other more abundant species. The rationale is that if we know for sure that the amino acids are present and know for sure where they resonate, they should be labelled such that other researchers can gauge exactly where they resonate in common

experiments such as HSQC or HETCOR. In cases where there is considerable overlap with a specific amino acid's peak and a more abundant compounds (i.e. the amino acids resonance is buried), a note is added to the main assignment table to indicate this.

Stage 3: Independent crosscheck

The chemical shifts of the matches from the database are plotted against the chemical shifts observed in the experimental data for both ^{1}H and ^{13}C. An example is shown below (Fig S1). Any peaks that do not fall close to the trend line indicate these database shifts are not a perfect match in comparison to the experimental data. Any peaks falling off the trend line are removed to ensure an R2 > 0.99 between the observed and database chemical shifts. Essentially, this independent check, while extremely laborious, ensures only the most accurate assignments are retained

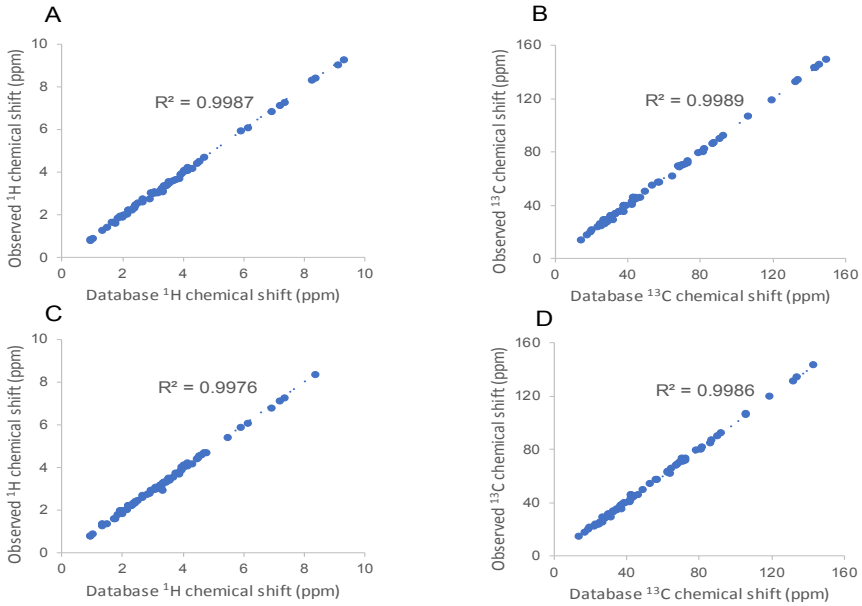


Fig S1. HSQC (A&B) and HETCOR (C&D) ^{1}H and ^{13}C correlation plot of the reference databases vs the observed chemical shifts with R^2 values displayed.

Literature confirmation

1) The above protocols produce the most confident matches of the NMR data against known human metabolites (note: a *Daphnia* NMR database does not exist). However, to be considered a full assignment the literature was searched for a report of the metabolite existing in aquatic crustaceans. While this filter is rather strict and rules out discovering novel metabolites that have to be assigned in *Daphnia*, we decided to stay on the side of caution for the main assignments.

2) To offset this, if specific metabolites, not previously reported in *Daphnia* are detected. They are added to the tentative table as scenario C. The idea is to reduce any false positives in the main assignment table while still offering tentative assignments.

Further considerations

While the protocol outlined above, gave us the highest level of confidence in the assignments, it does lean towards being more conservative, due to the fact that we only considered compounds that are known to be present in *Daphnia* eliminating the possibility of discovering new or unexpected metabolites. By ensuring compounds appear in at least 2 spectra, if one experiment is more sensitive than the other, real peaks are likely detected in the more sensitive experiment but missed in other less sensitive experiments. To somewhat offset this, in addition to the main assignments (see later), a secondary table (S3) covering some tentative assignments and secondary assignments in the case of overlap are discussed. Furthermore, it is important to note that while the assignments offered in this paper are the best we could achieve and represent ~1-2 years of work, assignments especially in the complex overlapping regions can still be somewhat subjective. For example, the targeted amino-acid only experiment assigns amino acids beyond doubt[1] while in more complex spectra (for example HSQC/HETCOR) we know these peaks are present but many of them are hidden under more intense resonances. Finally, it is worth noting that the assigned peaks represent on average only ~55-65% of the total signal intensity in the HSQC/HETCOR datasets which best represent the range of structures *in vivo*. In the long term this is highly promising for *in vivo* NMR and suggests that, as databases become more comprehensive and novel experiments are developed (for example new targeted experiments that reduce overlap),[1–3] further assignment will become possible, strengthening *in vivo* NMR's position to provide unique insight in living molecular processes.

Part B. Supporting Tables and Figures

Table S2. Assigned metabolites of ^{13}C labelled *D. magna* determined by combined *in vivo* NMR approaches.

Metabolites	**Literature reference**	**Biological Role / Comment**	**Most Characteristic ^{1}H, ^{13}C chemical shifts[1]**
Acetylcholine	*Artemov and Metropolitanskaja*[4]	Neurotransmitter; in brain alerts neuronal excitability, and coordinates the response of neuronal networks in brain[5]	3.74, 67.42 3.22, 56.68 [2]
*Adenosine triphosphate (ATP) & *Adenosine diphosphate (ADP)	*Martinez-Cruz et al.*[6]	Intracellular energy transfer[7] Involves in intracellular signal transduction[8] Involves in extracellular purinergic signalling and neurotransmitter[9] *Note the signals of ATP and ADP overlap. In living cells, the ATP concentration is four to ten fold higher than the concentration of adenosine diphosphate (ADP) and adenosine monophosphate (AMP).[10] Therefore, the majority of these resonances are likely from ATP.	6.13, 89.89 [2] 4.28, 67.94 [2]
Adenosine monophosphate (AMP)	*Martinez-Cruz et al.*[6]	Is interconverted to ADP and/or ATP[11] Is a constituent in RNA synthesis[12]	6.13, 89.89 [2] 4.04, 66.41
Alanine	*Stepanova and Naberezhnyi*[13]	One of the most common free amino acid utilized by muscle cells in crustacean for the purpose of osmoregulation[14] Has a key role in recovery from hypoglycemia[15] Rapid cold-hardening can result in increase in alanine[16] Can be converted to pyruvate in TCA cycle, which is an indicator of change in energy metabolism[17]	1.55, 18.76 3.85, 53.30
Arginine	*Stepanova and Naberezhnyi*[13]	Crucial for synthesis of muscle protein, formation of sterol and regulation of cell growth[18–20]	3.25, 43.31 [2] 1.72, 26.71
Asparagine	*Sadykhov et al.*[21]	Is required for the brain function[22]	2.87, 37.35 2.97, 37.36
Aspartic Acid	*Stepanova and Naberezhnyi*[13]	Known energy substrates that is transferred to osmoregulatory organs to provide energy source for osmoregulation[23]	2.79, 39.39

		Major excitatory amino acid of central nerve system[24]	
Betaine	*Wagner et al.*[25]	Serves as organic osmolytes; salt stress in *Daphnia* increases Betaine[25]	3.27,56.29 3.90, 69.14
Choline	*Vandenbrouck et al.*[26]	Precursor of the neurotransmitter acetylcholine; salt stress in *Daphnia* decreases Choline[25]	3.52, 70.25 4.07, 58.41
Citric acid	*Bruno Campos et al.*[27]	Primary substrate for fatty acid and amino acid synthesis which are vital for proliferating cells[28]	2.64, 48.61
Citrulline	*Vandenbrouck et al.*[26]	By-product of the enzymatic production of nitric oxide from the amino acid arginine[26] Is converted to ornithine and ammonia through liver[29]	1.61, 27.70 3.15, 42.06 ***2***
Cysteine	*Stepanova and Naberezhnyi*[13]	Important source of sulfide. Sulfide in nitrogenase is extracted from cysteine[30] Has a regulatory rule in the proliferation and activation of T-cells[31]	3.94, 58.88 3.05, 27.79
D-Glucose	*Smith*[32]	Salt stress in *Daphnia* increases glucose[25] Its increase can indicate glycogen metabolism as a result of stress-induced hyperglycemia[33,34] Primary substrate for the glycolysis[35]	3.48, 78.66 3.41, 72.50
Gamma-Aminobutyric acid (GABA)	*Barry*[36]	Inhibitory neurotransmitter[37] Through GABA receptors affect proliferation, mitigation, and differentiation in cell development[38]	3.01, 42.16 1.90, 26.47
Glutamic acid	*Stepanova and Naberezhnyi*[13]	Key role in recovery from hypoglycemia[15] Major excitatory amino acid of central nerve system[24] Known energy substrates that is transferred to osmoregulatory organs to provide energy source for osmoregulation[23]	2.41, 34.84 2.19, 28.34 ***2***
Glutamine	*Czeczuga*[39]	Involves in neurotransmitter synthesis[40] One of the most common free amino acids utilized by muscle cells in crustacean for the purpose of osmoregulation[14]	2.18, 28.07 ***2*** 2.50, 32.67

Glycerophosphocholine	*Nagato et al.*[41]	Biosynthetic precursor of acetylcholine; delivers choline to the brain[42]	4.32, 62.06 3.67, 68.57
Glycine	*Stepanova and Naberezhnyi* [13] *Czeczuga et al.*[39]	One of the most common free amino acids utilized by muscle cells in crustacean for the purpose of osmoregulation[14] Inhibitory neurotransmitter[43] Biosynthetically linked with serine that are both essential precursors for synthesis of proteins, nucleic acids and lipids[44]	3.56, 44.24
Histamine	*Sadykhov et al.*[21]	Is found in brain and nervous system more than other organs and has roles in hormonal regulation, sleep, photoreception and local neurotransmission[45–47] *Daphnia* has a well-developed histaminergic system, including the visual system and histamine is involved in the control of phototaxis in them[47]	3.31, 41.6 3.06, 26.48
Histidine	*Sadykhov et al.*[21]	Involves in neurotransmitter synthesis[40]	3.32, 29.90 3.29, 29.90
L-Glutathione	*Elendt*[48]	Its decrease in *Daphnia* indicates the presence of detoxification[49]	4.57, 58.46 3.77, 46.35 [2]
L-Isoleucine	*Sadykhov et al.*[21]	Is utilized for energy in skeletal muscle[50] Inhibits vascular endothelial growth factor[51]	0.92, 13.95 1.98, 38.75
Lactic acid	*Sadykhov et al.*[21]	Energy metabolite[52] Indicator of anaerobic metabolism[53]	1.32; 22.83 4.11, 71.24
Leucine	*Stepanova and Naberezhnyi*[13] *Czeczuga et al*.[39]	Crucial for synthesis of muscle protein, formation of sterol and regulation of cell growth[18–20]	0.94,24.79 1.72, 42.59
Lysine	*Vandenbrouck et al.*[26]	Proteinogenesis[54] Has epigenetic regulation role by means of histone modification[55]	1.90, 32.80 1.76 29.26
Methionine	*Vandenbrouck et al.*[26]	Is stored by crustaceans to be used as a metabolic reserve during molting[56]	2.65, 31.64 2.14, 16.76

Myo-inositol	*Vandenbrouck et al.*[26]	Makes neurotransmitters and some steroid hormones bind to their receptors[57]	4.05, 74.78 3.60, 75.23 [2]
Ornithine	*Vandenbrouck et al.*[26]	Stress handling [58] Allows for disposal of excess nitrogen[59]	3.06, 41.66 1.83, 25.59
Phenylalanine	*Stepanova and Naberezhnyi*[13]	Its conversion to tyrosine helps the production of signalling molecules such as melanin and dopamine[60,61] Has a regulatory rule in the proliferation and activation of T-cells and immune responses[31]	7.32, 132.28
Proline	*Stepanova and Naberezhnyi*[13]	One of the most common free amino acids utilized by muscle cells in crustacean for the purpose of osmoregulation[14] Its metabolism plays roles in aging, senescence, and development[62]	4.13, 63.99 3.34, 48.85
Pyruvic acid	*Coen et al.*[63]	Rapid cold-hardening can result in increase in pyruvic acid[16] One of the end products of glycolysis[64]	2.37, 29.17
Ribose	*Vandenbrouck et al.*[26]	Enhances the recovery of skeletal muscle ATP and total adenine nucleotide[65] In the form of ADP-ribose regulates cell survival and cell death programmes, provides energy metabolism, and transcriptional regulation[66]	4.14, 85.87 3.97, 85.06
Serine	*Stepanova and Naberezhnyi*[13]	Its phosphorylation regulates many important biological reactions in eukaryotic cells[17] Biosynthetically linked with glycine that are both essential precursors for synthesis of proteins, nucleic acids and lipids[44]	3.97, 62.99 [2]
Succinic acid	*Vandenbrouck et al.*[26]	A key intermediate in tricarboxylic acid cycle that is used to produce chemical energy[67]	2.41, 36.82
Triacylglycerides (TAG)	*Durand*[68]	Storage lipid that breaks down to free fatty acids when body requires fatty acids[69] Its oxidation during *Daphnia* starvation can provide ATP needed for carbohydrate synthesis[70]	5.24, 71.62 5.27, 133.15
Threonine	*Stepanova and Naberezhnyi*[13]	Its phosphorylation regulates many important biological reactions in eukaryotic cells[17]	1.33, 22.27
Tryptophan	*Vandenbrouck et al.*[26]	*In vivo* precursor for several bioactive compounds such as	7.32, 127.821

		nicotinamide[71] A key regulator of inflammation and immunity[72] An important precursor of the neurotransmitter serotonin[72] Its metabolic pathways are related with sleep and wakefulness[72]	7.73, 121.21
Tyrosine	*Vandenbrouck et al.*[26]	Involves in neurotransmitter synthesis[40] Its phosphorylation regulates many important biological reactions in eukaryotic cells[17]	6.90, 118.65 ***2*** 7.19, 133.58 ***2***
Uridine	*Vandenbrouck et al.*[26]	Plays a role in the glycolysis pathway of galactose[73] Has key role in neuroregulatory processes[74]	5.90, 92.17 4.13, 87.07
Valine	*Stepanova and Naberezhnyi*[13]	Is associated with insulin resistance[75]	0.99, 19.49

*All ATP signals overlap with ADP and AMP signals with one exception of AMP signal (4.04, 66.41). In living cells, the ATP concentration is four to ten fold higher than the concentration of adenosine diphosphate (ADP) and adenosine monophosphate (AMP).[10] Therefore, the majority of these resonances are likely from ATP.

1 Most characteristic ^{1}H, ^{13}C chemical shifts of assigned metabolites that may be most useful to future researchers for identification and quantification.[76] These signals to tend to be the least overlapping signals in *Daphnia* and thus most useful for identification.

2 High risk of overlap with the peaks of metabolites with the similar structure or to overlap with dominant peaks in an overcrowded area. Magnetic susceptibility distortions induced by the living organisms' different parts (cell matrix, membranes, cell walls, etc.) broaden *in vivo* NMR signals compared to NMR signals obtained from filtered buffer extract metabolites.

Table S3. Tentative metabolites of ^{13}C labelled *D. magna* determined by combined *in vivo* NMR approaches.

Metabolites[1]	**Literature reference**	**Biological activities**	**^{1}H, ^{13}C chemical shifts**[2]
Acetoacetic acid***C***	*Polakof et al.*[77]*	An energy source during starvation[67] Brain uses it when the glucose level is low[67]	3.45, 56.07
Agmatine***B***	*Kanehisa et al.*[78]	Decarboxylation product of the amino acid arginine and an intermediate in polyamine biosynthesis[78] Putative Neurotransmitter[79]	1.74, 27.07 ***3***
Arabitol***B, C***	*Li et al.*[80]*	Is associated with ribose-5-phosphate isomerase deficiency[80]	3.57,73.32 ***3*** 3.86, 65.87 ***3***
Creatine***B***	*Scanlan et al.*[81]	A major source of energy stored in skeletal muscles and brain[82,83] In brain provides protection against neurological disorders[84]	3.04, 39.81 ***3***
Cysteic acid***C***	*Kanazawa and Teshima*[85]*	Metabolic precursor of taurine[86]	4.11, 53.95 3.28, 53.28
D-Gluconurolactone***A***	*Vandenbrouck et al.*[26]	Inhibits glucose uptake in adipose tissue[87]	5.46, 105.57 4.74, 71.84
Glutaric acid***B***	*Vandenbrouck et al.*[26]	Produced during the metabolism of some amino acids such as lysine and tryptophan[88]	2.18, 40.15 ***3*** 1.78, 25.82 ***3***
Guanidino acetic acid***C***	*Robin and Marescau*[89]*	Immediate precursor for creatine[90] Is involved in oxidative stress[91] Exists in metabolic pathways of some amino acids such as ornithine and arginine[90]	3.80, 47.85 ***3***
Guanidino succinic acid***C***	*Robin and Marescau*[89]*	Can be biosynthesized through repression of arginine-glycine transamidinase, and on the appearance of a new enzyme, arginine-aspartate transamidinase or, cleavage of arginino succinic acid resulting in formation of carbamyl aspartate, which could be then converted to guanidino succinic acid[92]	2.79, 43.30 2.55, 43.47
*Guanosine 5'-diphosphate****A, 4***	*Oikawa and Smith*[93]	Makes the guanine nucleotide-binding proteins (important signal transducing molecules) off when binds	5.92, 89.81 4.35, 86.44

		to it[94]	
Inosine[4]	*Kim et al.*[95]	Is commonly found in DNA and RNA[96] Antioxidant protecting DNA from oxidative damage[97]	8.33, 143.05 8.22, 149.04
Lipoic acid[B]	*Reed*[98]*	Carries out biochemical reactions for oxidative metabolism and modulates various cellular functions; is an enzyme cofactor in oxidative metabolism and metabolic regulator[99]	2.18, 40.25 [3] 2.01, 42.95
Malic acid[A]	*Vandenbrouck et al.*[26]	An intermediate of the TCA cycle[100] Can be converted to pyruvic acid[100]	2.65, 42.76 2.35, 42.75
N- Acetyllysine[B]	*Kwon et al.*[101]	The most dynamic post translational production[101]	4.15, 57.78 [3] 1.90, 32.58 [3]
Niacinamide[C, 4]	*Gago-Tinoco*[102]*	Critically important part of the structure of Nicotinamide adenine dinucleotide phosphate (NADP) and Nicotinamide adenine dinucleotide (NAD)[103]	8.23, 139.24 8.69, 154.52 [3]
Nicotinamide adenine dinucleotide (NAD)[A]	*Ivnitskii et al.*[104]	Generally, the total pool of NAD in most types of cells is larger than NADP[105] Acts as a coenzyme in redox reactions[106]	9.32, 142.51 [3] 9.15, 145.07 [3]
Nicotinamide adenine dinucleotide phosphate (NADP)[A]	*Martins et al.*[107]	Allows the regeneration of glutathione (GSH) to protect against the toxicity of reactive oxygen species (ROS)[108] Responsible for generating free radicals in immune cells[109]	9.27, 142.49 [3] 9.09, 144.92 [3]
Nicotinic acid (Niacin)[C, 4]	*Kodicek*[110]*	The precursor of the Nicotinamide adenine dinucleotide (NAD) and Nicotinamide adenine dinucleotide phosphate (NADP)[103]	8.26, 140.39 8.93, 151.76 [3]
Oxoglutaric acid[C]	*Krebs et al.*[111]*	One of the most important nitrogen transporters in metabolic pathways and plays a role in detoxification of ammonia in brain[112,113]	2.42, 33.42 [3]
Pimelic acid[B]	*Vandenbrouck et al.*[26]	Its derivatives are involved in the biosynthesis of lysine[88] Precursor of biotin biosynthesis[114]	1.55, 28.56 [3] 2.18, 40.15 [3]
Ribitol[B]	*Vandenbrouck et al.*[26]	Ribitol 5-phosphate is a functional glycan unit[115]	3.82, 65.06 [3]
Threonic acid[B]	*Vandenbrouck et al.*[26]	Is derived from ascorbic acid degradation or glycated proteins[116]	4.02, 75.36

Tyramine[B]	*Ehrenstrom and Berglind*[117]	Octopamine precursor[118] Norepinephrine/noradrenaline neurotransmitter[118]	2.93, 34.92
Uracil[A, 4]	*Vandenbrouck et al.*[26]	Coenzyme and structural unit for the nucleic acid[119] Allosteric regulator[120]	5.79, 103.93 7.52, 146.31
Uridine 5'- diphosphate (UDP)[A,4]	*Roff et al.*[121]	Glucose bound to UDP is a precursor of glycogen[122] In the form of UDP-N-acetylglucosamine, is the immediate precursor of chitin[121]	7.99, 144.39 4.11, 66.72 [3]

[1] Assigned metabolites are tentative. They are included as there is a very good chance that they are present.

[2] Most characteristic ^{1}H, ^{13}C chemical shifts of assigned metabolites that may be most useful to future researchers for identification and quantification.[76] These signals tend to be the least overlapping signals in *Daphnia* and thus most useful for identification.

[3] At high risk to overlap with the peaks of metabolites with the similar structure or to overlap with dominant peaks in an overcrowded area. Magnetic susceptibility distortions induced by the living organisms' different parts (cell matrix, membranes, cell walls, etc.) broaden *in vivo* NMR signals compared to NMR signals obtained from filtered buffer extracts.

[4] Metabolites that were identified *ex vivo* only. They are included in table S3 because they might be seen *in vivo* as different samples are slightly different.

[A] Tentative assignment scenario A

[B] Tentative assignment scenario B

[C] Tentative assignment scenario C

[*] To the best knowledge of the authors, the metabolites have not been reported in *Daphnia Magna*. They are included however, as they have been reported in other living organisms and there might be a potential to be found in *D. magna* in future.

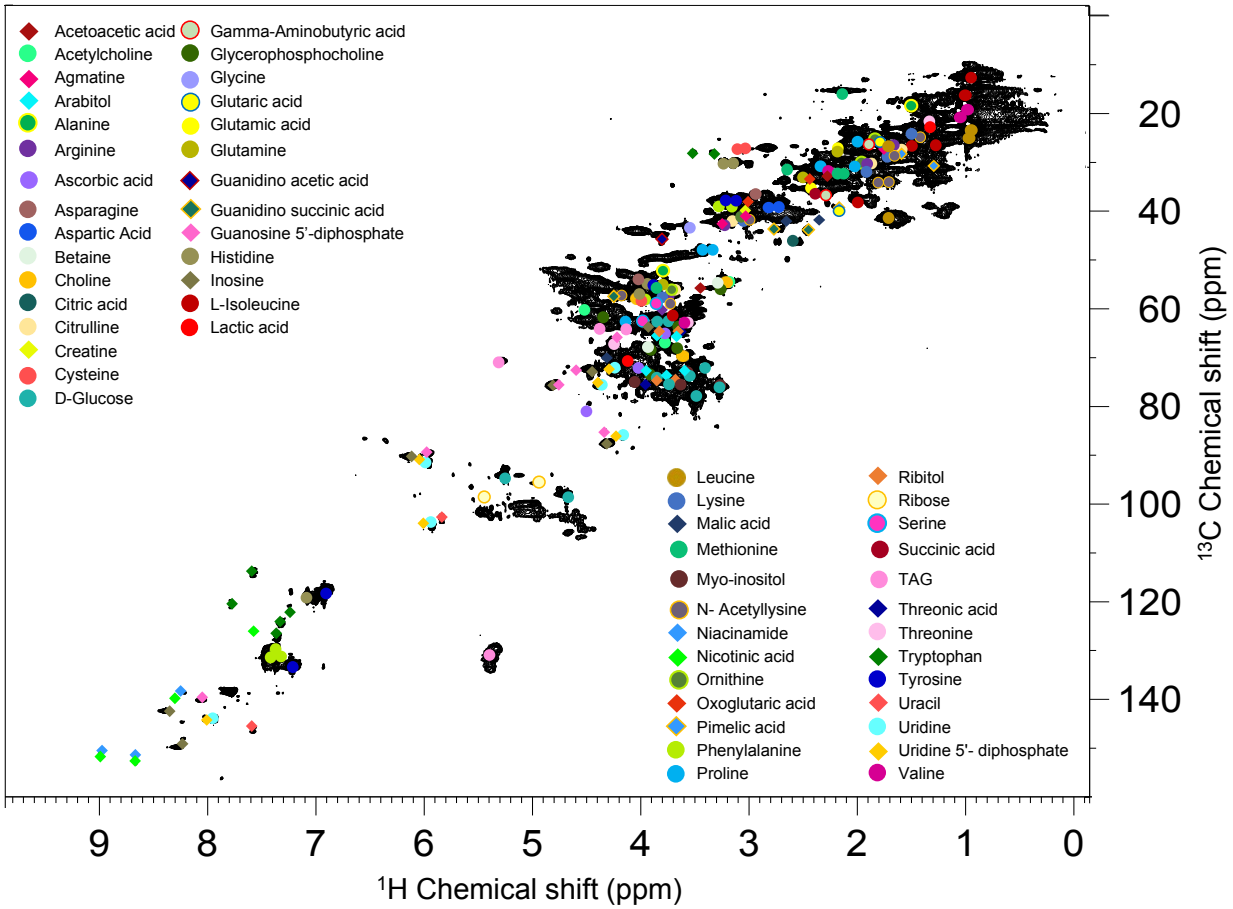


Fig S2. *Ex vivo* 2D HSQC (^{1}H-^{13}C) spectrum of ^{13}C-enriched *Daphnia magna* with color coded metabolites assigned.

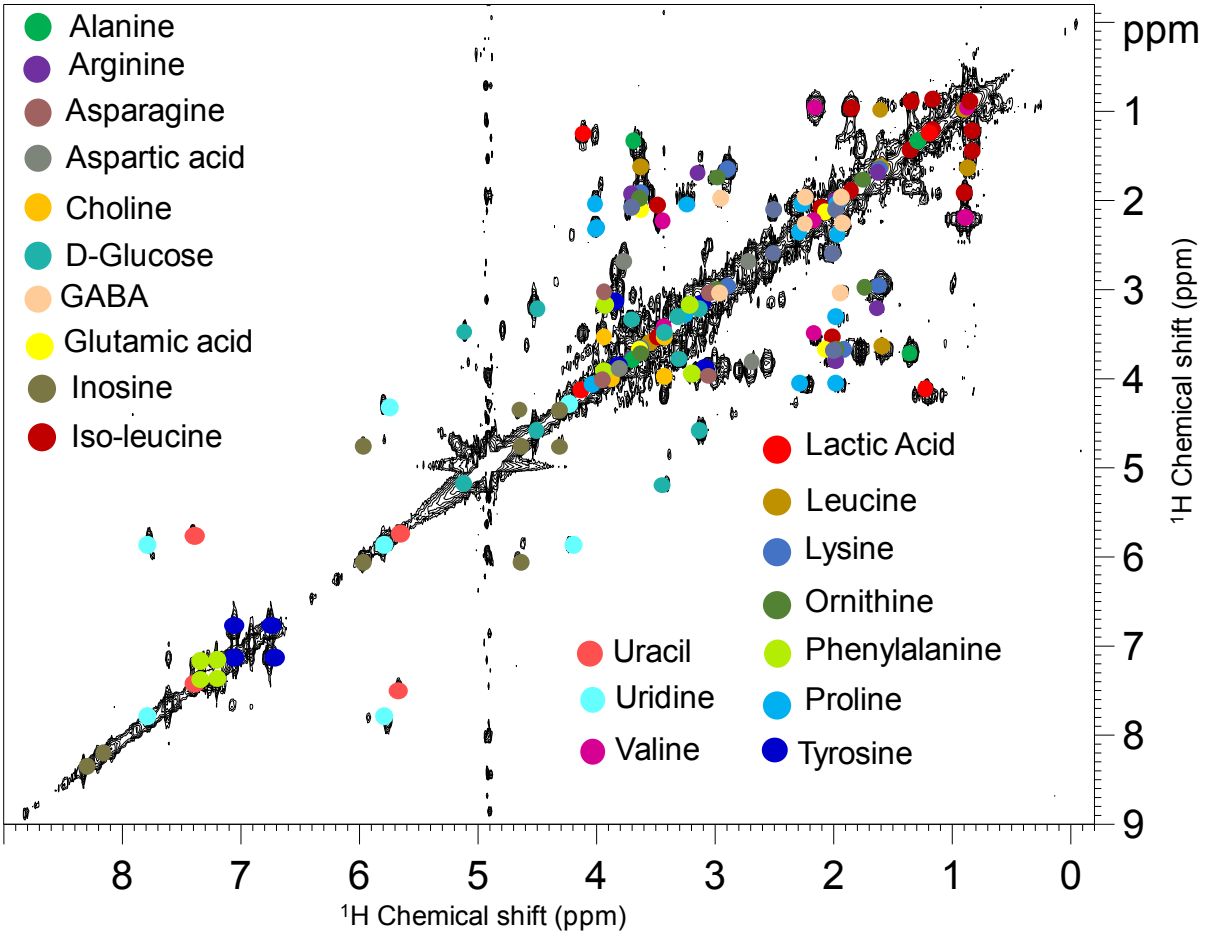


Fig S3. *Ex vivo* ^{1}H-^{1}H COSY spectrum of ^{13}C enriched *Daphnia magna* with color coded determined metabolites.

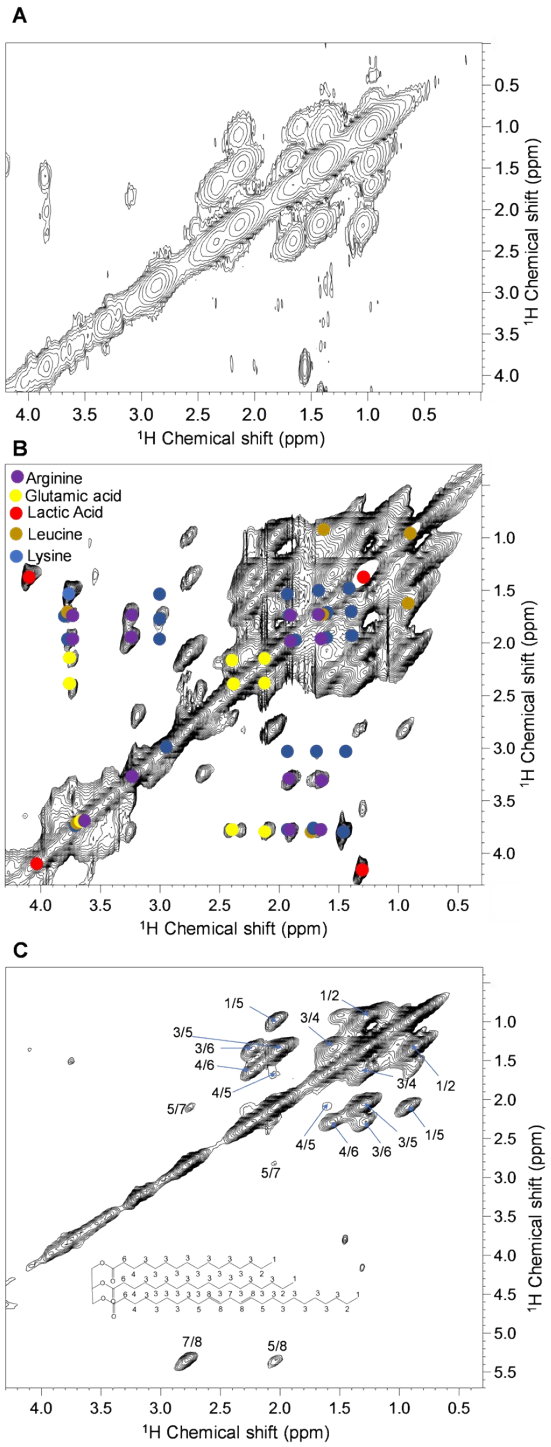


Fig S4. (A) *In vivo* ^{1}H-^{1}H COSY data where the dominate correlations all arise from lipids. (B) *In vivo* ^{1}H-^{1}H ATOCSY spectrum of ^{13}C enriched *Daphnia magna* with color coded determined metabolites. (C) Generic triacylglyceride (TAG) assignment. The spectrum scaled to show assignment of the dominant TAG.

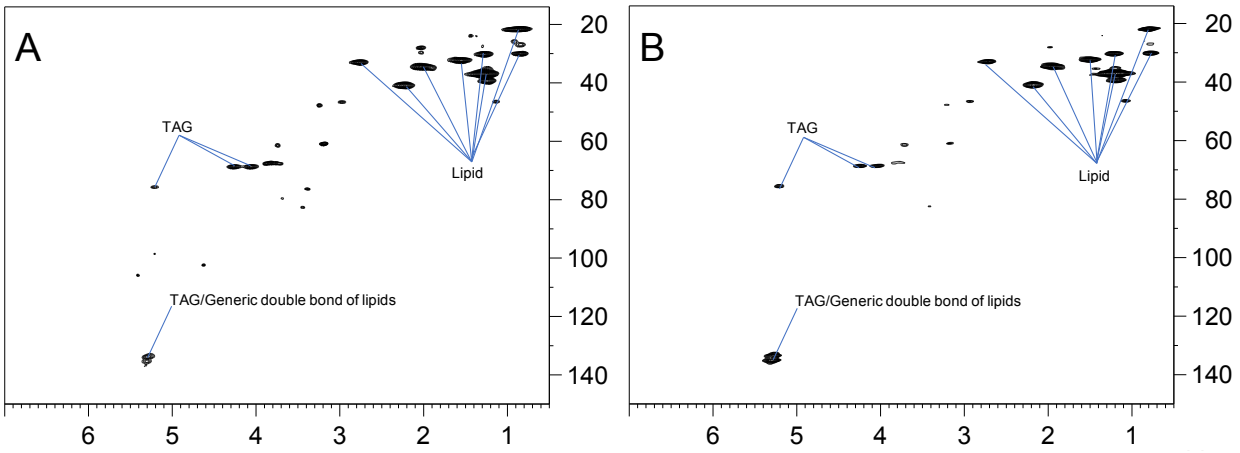


Fig S5. High Threshold Plots *in vivo* 2D HSQC and 2D HETCOR spectra (A and B, respectively) highlighting the most abundant signals that are consistent with TAG and other lipids.

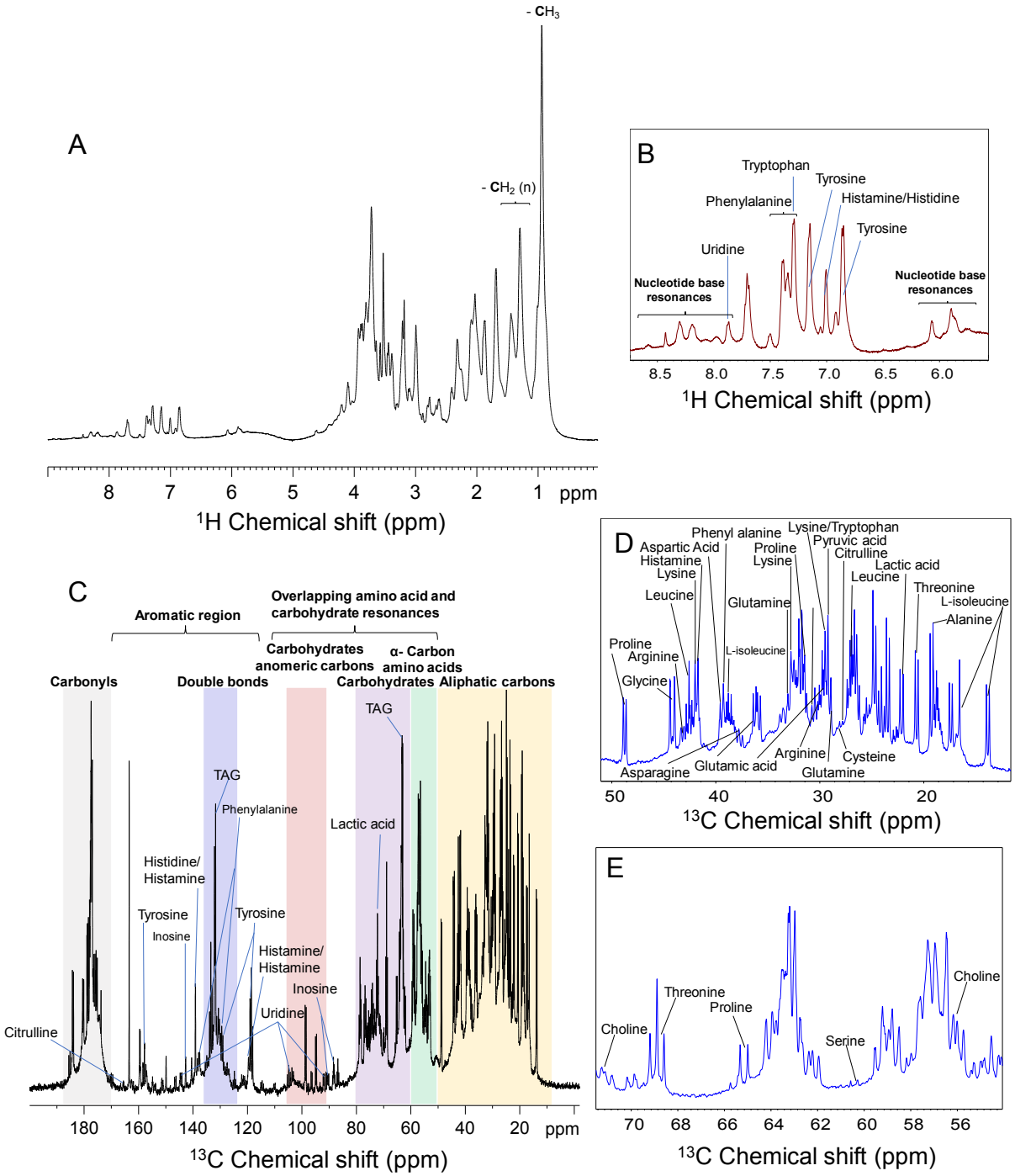


Fig S6. *Ex vivo 1D* ^{1}H (A and B) and ^{13}C NMR spectra (C, D, and E), of ^{13}C enriched *Daphnia magna*.

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