Supplementary Material

Workflow for the Targeted and Untargeted Detection of Small Metabolites in Fish Skin Mucus

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Table S1. Complete overview over reference compounds including Sigma-Aldrich product number and solvent.

Reference compounds	ID #	Stocks were prepared in	Reference compounds	ID #	Stocks were prepared in
3-Hydroxydecanoic acid	H3648-5MG	MeOH	Adenosine 3′-monophosphate	A9272-100MG	1M NaOH
Butylated hydroxyanisole	78943-500MG	MeOH	Glucose	47829	H_2O
3-Hydroxyoctanoic acid	H3898-5MG	H_2O	N-Acetylneuraminic acid	19023-10MG	H_2O
9-Hydroxynonanoic acid	CDS018651-10MG	H_2O	L-Cysteine	C7352-25G	H_2O
2-Hydroxyisocaproic acid	219819-1G	H_2O	L-glutathione, reduced	G4251-10G	H_2O
Niacinamide	PHR1033-1G	H_2O	Creatine	C3630-100G	H_2O
3-Furoic acid	163392-5G	H_2O	Nα-Acetyl-L-arginine	A3133-5G	H_2O
DL-2-Aminocaprylic acid	217700-5G	1M NaOH	Ascorbic acid	95210	H_2O
Uracil	U0750-5G	H_2O	L-α-Aminoadipic acid	A7275-100MG	H_2O/MeOH (1:1)
N-Acetyl-L-alanine	A4625-1G	H_2O	L-Glutamine	G3126-100G	H_2O
Pyrrolidone	Y0000659	H_2O	Taurine	T0625-10MG	H_2O
Propionyl-L-carnitine	42602-10MG	H_2O	α-Hydroxyadipic acid	92826-10MG	H_2O
Uridine	U3750-1G	H_2O	Uridine 5′-monophosphate	U1752-1G	H_2O
Hypoxanthine	H9377-1G	1M NaOH	L-Citrulline	C7629-10MG	H_2O
Orotic acid	O2750-10G	H_2O	Succinic anhydride	239690-50G	H_2O
O-Acetyl-L-carnitine	A6706-1G	H_2O	O-Phosphoethanolamine	P0503-1G	H_2O
Inosine	I4125-1G	H_2O	Cytidine 5′-monophosphate	C1131-500MG	H_2O
4-Aminobenzoic acid	01973-100MG	H_2O	Malic acid	PHR1273-1G	H_2O
Cytosine	C3506-1G	H_2O	Fumaric acid	47910-5G	H2O/MeOH (9:1)
Glycolic acid	94815-100MG	H_2O	Guanosine 5′-monophosphate	G8377-500MG	H_2O
Xanthine	X0626-5G	1M NaOH	L-glutathione, oxidised	G4376-1G	H_2O
Azelaic acid	95054-100MG	MeOH	Citric acid	251275-5G	H_2O
Guanine	PHR1243-500MG	1M NaOH	*N*-Acetylputrescine hydrochloride	A8784-25MG	H_2O
Guanosine	G6752-1G	DMSO	Piperidine	76046-100MG	H_2O
Erythronic acid	75025-10MG	H_2O	Butylamine	90893-1ML	H_2O
Uric acid	U2625-25G	H_2O	Amino acid standard (+other small molecules, total 37)	A9906-5ML	diluted with H_2O

Table S2. Liquid Chromatography–High-Resolution Mass Spectrometry characteristics of all 86 small metabolites

Metabolite	Ion	Accurate	RT*	Confirmed	Metabolite	Ion Species	Accurate	RT*	Confirmed
3-Hydroxydecanoic acid	$[M-H]^-$	187.1340	3.7	+	L-Cysteine	$[M-H]^-$	120.0125	15.1	
Butylated hydroxyanisole	$[M-H]^-$	179.1078	3.9		L-Anserine	$[M+H]^+$	241.1295	15.3	+
3-Hydroxyoctanoic acid	$[M-H]^-$	159.1027	4.2	+	L-glutathione, reduced	$[M+H]^+$	308.0911	15.5	
9-Hydroxynonanoic acid	$[M-H]^-$	173.1183	4.7	+	Hydro-L-proline	$[M+H]^+$	132.0655	15.5	+
2-Hydroxyisocaproic acid	$[M-H]^-$	131.0714	4.9	+	Creatine	$[M+H]^+$	132.0768	15.7	+
Niacinamide	$[M+H]^+$	123.0553	6.4	+	L-Threonine	$[M+H]^+$	120.0655	15.7	+
3-Furoic acid	$[M-H]^-$	111.0088	7.4		Nα-Acetyl-L-arginine	$[M-H]^-$	215.1150	15.7	+
DL-2-Aminocaprylic acid	$[M-H]^-$	158.1187	8.1		L-Histidine	$[M-H]^-$	154.0622	15.8	
Uracil	$[M+H]^+$	113.0346	8.4	+	Ascorbic acid	$[M-H]^-$	175.0248	15.8	
N-Acetyl-L-alanine	$[M+H]^+$	132.0655	9.0		L-Alanine	$[M+H]^+$	90.0550	15.9	+
Pyrrolidone	$[M+H]^+$	86.0600	9.0		L-Glutamic acid	$[M+H]^+$	148.0604	16.1	+
Propionyl-L-carnitine	$[M+H]^+$	218.1387	9.6	+	L-α-Aminoadipic acid	$[M+H]^+$	162.0761	16.3	+
Creatinine	$[M+H]^+$	114.0662	9.8	+	L-Glutamine	$[M-H]^-$	145.0619	16.3	+
Uridine	$[M-H]^-$	243.0623	10.1	+	β-Alanine	$[M+H]^+$	90.0550	16.5	+
Hypoxanthine	$[M-H]^-$	135.0312	10.5	+	L-Aspartic acid	$[M-H]^-$	132.0302	16.5	+
L-Phenylalanine	$[M+H]^+$	166.0863	10.5	+	γ-Amino-n-butyric	[M+H]+	104.0706	16.6	+
Orotic acid	$[M-H]^-$	155.0098	11.1	+	Taurine	$[M+H]^+$	126.0219	16.5	+
L-Leucine	$[M+H]^+$	132.1019	11.2	+	α-Hydroxyadipic acid	$[M-H]^-$	161.0455	16.7	
O-Acetyl-L-carnitine	$[M+H]^+$	204.1230	11.2	+	Uridine 5′-	$[M-H]^-$	323.0286	16.9	+
Inosine	$[M-H]^-$	267.0735	11.5	+	L-Carnosine	$[M+H]^+$	227.1139	17.0	+
4-Aminobenzoic acid	$[M+H]^+$	138.0550	11.5		Glycine	$[M+H]^+$	76.0393	17.0	+
Cytosine	$[M+H]^+$	112.0505	11.7	+	L-Citrulline	$[M+H]^+$	176.1030	17.2	+
L-Isoleucine	$[M+H]^+$	132.1019	11.8	+	L-Serine	$[M+H]^+$	106.0499	17.2	+
Glycolic acid	$[M-H]^-$	75.0088	12.1		Succinic anhydride	$[M+H_2O-H]^-$	117.0193	17.3	+
L-Methionine	$[M+H]^+$	150.0583	12.2	+	O-	$[M+H]^+$	142.0264	17.3	
Xanthine	$[M-H]^-$	151.0261	12.3	+	Cytidine 5′-	$[M-H]^-$	322.0446	17.6	
L-Tryptophan	$[M+H]^+$	205.0972	12.4	+	L-Homocystine	$[M-H]^-$	267.0479	17.8	
Azelaic acid	$[M-H]^-$	187.0976	12.4	+	L-Cystine	$[M-H]^-$	239.0166	17.9	
Guanine	$[M+H]^+$	152.0567	13.0	+	Malic acid	$[M-H]^-$	133.0142	18.3	+
1-Methyl-L-histidine	$[M+H]^+$	170.0924	13.1	+	Fumaric acid	$[M-H]^-$	115.0037	18.4	+
L-Valine	$[M+H]^+$	118.0863	13.2	+	Guanosine 5′-	$[M-H]^-$	362.0507	18.7	+
Guanosine	$[M-H]^-$	282.0844	13.4	+	L-Cystathionine	$[M+H]^+$	223.0747	18.7	+
L-Proline	$[M+H]^+$	116.0706	13.5	+	L-glutathione, oxidised	$[M+H]^+$	613.1592	19.0	+
Erythronic acid	$[M-H]^-$	135.0299	13.7/14.1		Citric acid	$[M-H]^-$	191.0197	19.8	+
Uric acid	$[M-H]^-$	167.0211	14.0		N-Acetylputrescine	$[M+H]^+$	131.1179	24.0	
3-Methyl-L-histidine	$[M+H]^+$	170.0924	14.0		δ-Hydroxylysine	$[M+H]^+$	163.1077	26.1	+
Adenosine 3′-	$[M-H]^-$	346.0558	14.1		L-Ornithine	$[M+H]^+$	133.0972	26.3	+
L-Tyrosine	$[M+H]^+$	182.0812	14.1	+	L-Lysine	$[M+H]^+$	147.1128	27.6	+
Glucose	$[M-H]^-$	179.0561	14.4	+	Urea	$[M+2H]^+$	62.0475	28.1	
N-Acetylneuraminic acid	$[M-H]^-$	308.0987	14.7		Ethanolamine	$[M+H]^+$	62.0600	28.2	+
L-α-Amino-n-butyric acid	$[M+H]^+$	104.0706	14.7	+	L-Arginine	$[M+H]^+$	175.1190	29.4	+
L-Sarcosine	$[M+H]^+$	90.0550	15.0	+	Piperidine	$[M+H]^+$	86.0964	30.1	
D,L-β-Aminoisobutyric acid	$[M+H]^+$	104.0706	15.1		Butylamine	$[M+H]^+$	74.0964	30.2	

*Retention time

Table S3. MZmine 2 processing parameters.

	Peak detection			
	Mass detection	Chromatogram building	Chromatogram deconvolution	Deisotope
Mass detector	Centroid			
Noise level	5.00E+04			
MS level	1.0			
Min time span (min)		0.2		
Min absolute height		5.00E+05	5.00E+05	
m/*z* tolerance		0.001 (or 5 ppm)		0.001 (or 5 ppm)
Chromatographic threshold (%)			1.0	
Search minimum in RT* range (min)			0.4	
Minimum relative height (%)			5.0	
Min ratio of peak top/edge			5.0	
Peak duration range (min)			0.3–2.0	
RT tolerance (absolute, min)				0.1
Maximum charge				2.0
Representative isotope				Lowest *m*/*z*
	Peak Alignment and identification			
	Join aligner	Gap filling	Filtering (Peak list rows filter)	Adduct search
m/*z* tolerance	0.001 (or 5 ppm)	0.001 (or 5 ppm)		0.001 (or 5 ppm)
Weight for *m*/*z*	20			
RT tolerance (relative, %)	5.0			
Weight for RT	10			
Minimum peaks in a row			6.0	
Minimum peaks in an isotope pattern			1.0	
m/*z* range			58–870	
RT range			1.5–32	
Peak duration range (min)			0.3–2.0	
RT tolerance (absolute, min)				0.2
Adducts				ESI (+): Na, K, NH_4 ESI (−): formateESI (+/−): MeCN+H
Max relative adduct peak height (%)				50

*Retention time

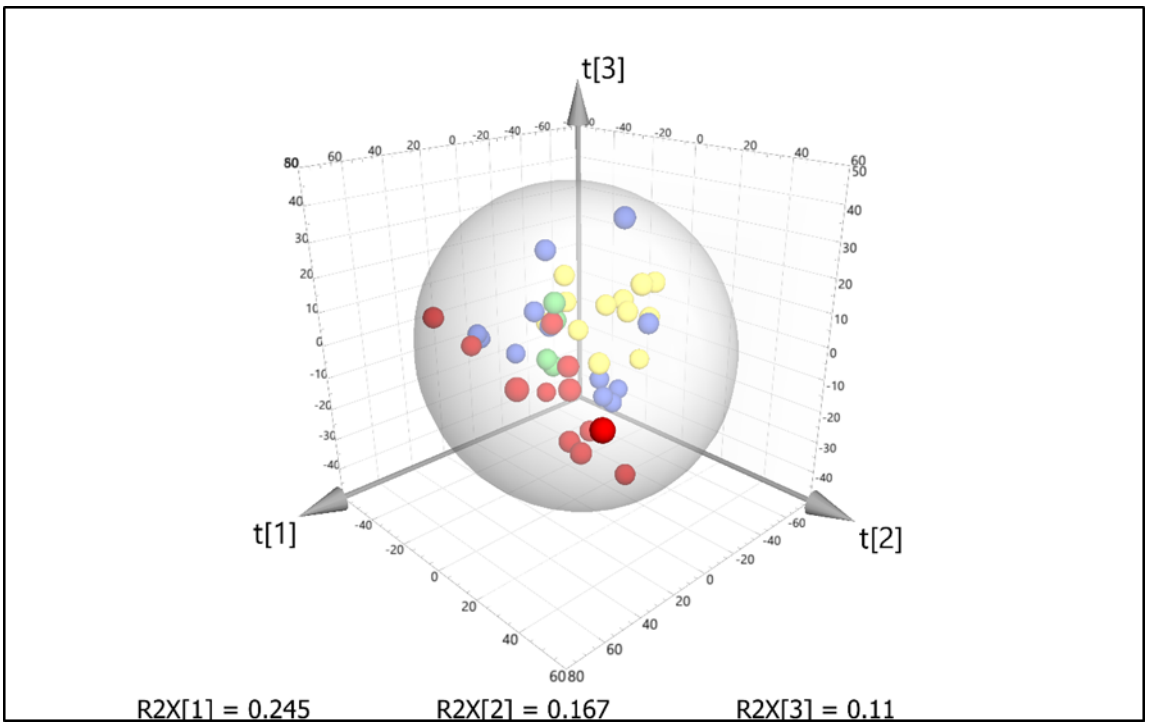

Figure S2. 3D-Score plot from principal component analysis (PCA) (unit variance scaled, first three components) of the metabolite profiles obtained from untargeted LC–HRMS; quality control samples in green, scraped mucus samples in red, wiped mucus samples in blue, and filter absorbed mucus samples in yellow.

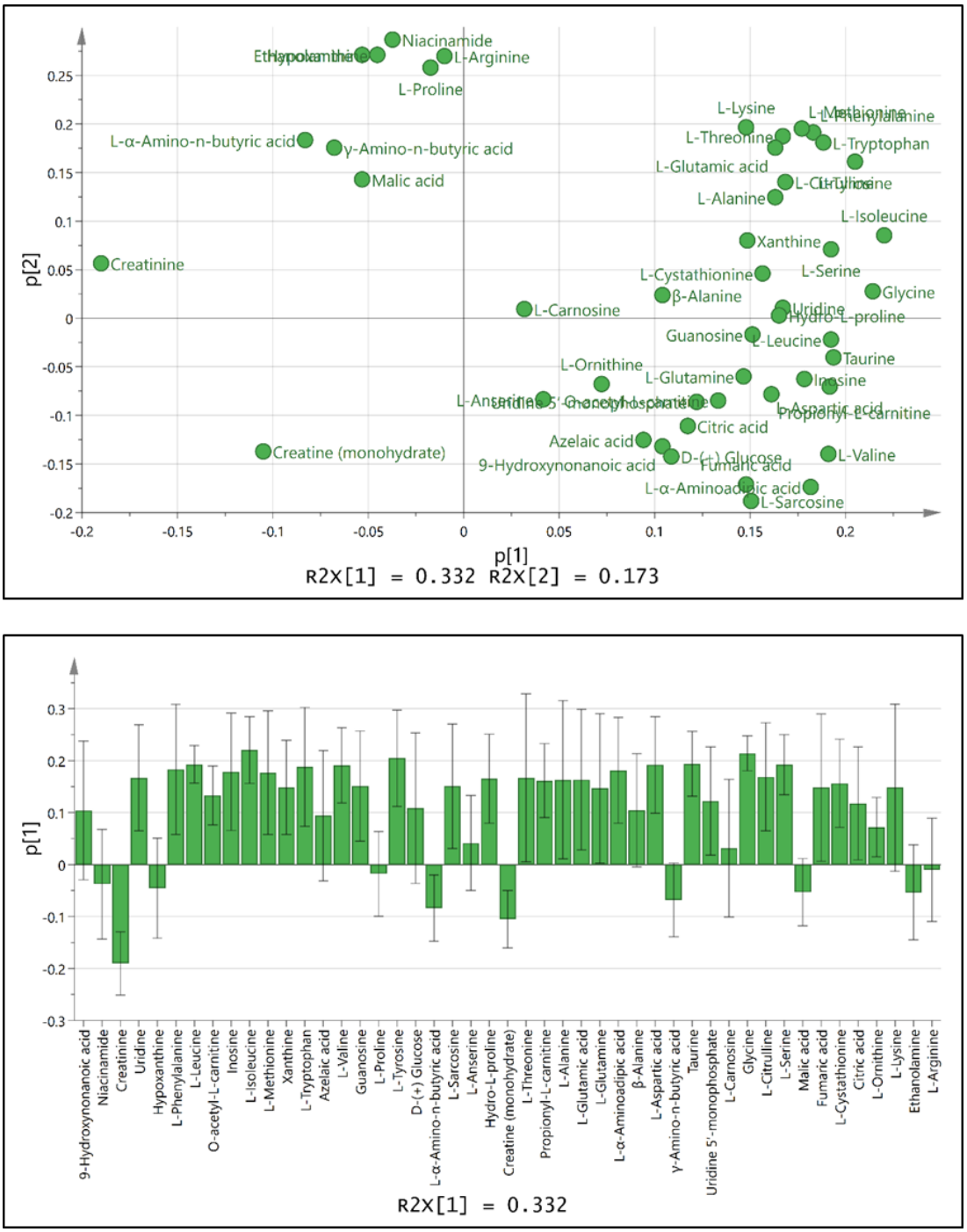

Figure S3. Loading scatter plot (above) and loading column plot (below) from PCA of the targeted metabolite data either processed using Xcalibur or MZmine. The plots show the variation of individual metabolites in relation to each other. MZmine 2 detected all the negatively correlated metabolites in the loading column plot as ^{13}C isotope peaks.