

Supplementary Material

Supplementary Figures

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Human_ENSP00000384364 : ----- : -
Atlantic_salmon_ENSSSAP00000032009 : ----- : -
Atlantic_salmon_ENSSSAP00000023580 : MTAYSDVSVRAPFRNGDLETFLTRPAHHCAAHYKNRAPSVGTLQNAQEQTTPEDSFYLNHPLNPQ : 65
Atlantic_salmon_ENSSSAP00000005847 : -----MCSFGHPPL-SSSKCAPCHRYKTPRMLAAKDSCEHRTVIQRNHLQSSQ : 47
Medaka_ENSORLP00020013686 : ----- : -
Medaka_ENSORLP00020022926 : -----MARADINPSAHCALRRRTETAQEGAQNSAQII : 32
Rainbow_trout_ENSOMYP00000002554 : -----MLAAKHSCEHRTVIQRNHLQSSQ : 23
Rainbow_trout_ENSOMYP00000013188 : ----- : -
Rainbow_trout_ENSOMYP00000027678 : ----- : -
Brown_trout_ENSSTUP00000046804 : ----- : -
Brown_trout_ENSSTUP00000104910 : ----- : -
Brown_trout_ENSSTUP00000080605 : -----MCSFGHPPL-SSSKCAPCHRYKTPRMLAAKDSCEHRTVIQRNHLQSSQ : 47
Brown_trout_ENSSTUP00000081043 : -----MCSFGHPPL-SSSKCAPCHRYKTPRMLAAKDSCEHRTVIQRNHLQSSQ : 47
Coho_salmon_ENSOKIP000005014471 : ----- : -
Coho_salmon_ENSOKIP000005103527 : ----- : -
Coho_salmon_ENSOKIP000005117205 : -----MCSFGHPPLPSPPKCAPCHRYKTHRMLAAKHSCEHRTVIQRNHLQSSQ : 48
Coho_salmon_ENSOKIP000005114415 : -----MCSFGHPPLPSPPKCAPCHRYKTHRMLAAKHSCEHRTVIQRNHLQSSQ : 48
Chinook_salmon_ENSOTSP000005007169 : ----- : -
Chinook_salmon_ENSOTSP000005022142 : -----MGTQNAQEQTTPENN FYLNHPLNPQ : 26
Zebrafish_ENSDARP00000157094 : ----- : -
Common_carp_ENSCCRP00000070566 : -----MTEDLIRDASSRAIARGFCVVRHELRYKTRRRTTQSAENRSSQHPSEKCNQDLIQ : 58
Common_carp_ENSCCRP00000025584 : ----- : -
Three-spined_stickleback_ENSGACP00000009098 : ----- : -
Northern_pike_ENSELUP00000028969 : -----M : 1
Northern_pike_ENSELUP00000026966 : ----- : -
NPYa_Fugu_ENSTRUP00000016766 : ----- : -
NPYb_Fugu_ENSTRUP00000038037 : ----- : -
NPYa_Nile_tilapia_ENSONIP00000046366 : ----- : -
NPYb_Nile_tilapia_ENSONIP00000040820 : -----L : 1
NPYa_Green_spotted_pufferfish_ENSTNIP00000020909 : ----- : -
NPYb_Green_spotted_pufferfish_ENSTNIP00000021590 : ----- : -

Human_ENSP00000384364 : --MLGNKRLGSGLTALSLLVCLGALAEAYPSKFDNPGEDAPAEADMARYYSALRHHYINLITQR : 63
Atlantic_salmon_ENSSSAP00000032009 : --MHPNLGTWLGAVTLLVWTFICIGTAEGYPVKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 63
Atlantic_salmon_ENSSSAP00000023580 : LSHHPNLGTWLGALTLLVWTFICIGTAEGYPVKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 130
Atlantic_salmon_ENSSSAP00000005847 : NKGRPNAAAMSLGM--LAVYSLVCMSTMDAYPSKFWIPAEADAEVDFAKYFYSALRHHYINLITQR : 110
Medaka_ENSORLP00020013686 : --NSPNF-----LALCLLCCFFSGTNAYPAKPPGPRGCAPEELAKYYYSALRHHYINLITQR : 55
Medaka_ENSORLP00020022926 : GNHPNLVSWLGLTLGLLWALCLGALTEGYPMKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 97
Rainbow_trout_ENSOMYP00000002554 : HKGRANAAMSLGM--LAVYLLLCVSNMAAYPSKFWIPAEADAEVDFAKYFYSALRHHYINLITQR : 86
Rainbow_trout_ENSOMYP00000013188 : --MHPNLGTWLGAVTLLVWTFICIGTAEGYPVKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 63
Rainbow_trout_ENSOMYP00000027678 : --MHPNLGTWLGALTLLVWTFICIGTAEGYPVKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 63
Brown_trout_ENSSTUP00000046804 : --MHPNLGTWLGALTLLVWTFICIGTAEGYPVKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 63
Brown_trout_ENSSTUP000000104910 : --MHPNLGTWLGAVTLLVWTFICIGTAEGYPVKKPETPGEDAPAEELAKYYYSALRHHYINLITQR : 63
Brown_trout_ENSSTUP00000080605 : NKGRPNAAAMSLGL--LAVYSLVCMSTMDAYPSKFWIPAEADAEVDFAKYFYSALRHHYINLITQR : 110
Brown_trout_ENSSTUP00000081043 : NKGRPNAAAMSLGL--LAVYSLVCMSTMDAYPSKFWIPAEADAEVDFAKYFYSALRHHYINLITQR : 110
Coho_salmon_ENSOKIP000005014471 : --MHPNLGTWLGAVTLLVWTFICIGTAEGYPVKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 63
Coho_salmon_ENSOKIP000005103527 : --MHPNLGTWLGALTLLVWTFICIGTAEGYPVKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 63
Coho_salmon_ENSOKIP000005117205 : NKGRPNAAAMSLGM--LAVYLLLCVSNMAAYPSKFWIPAEADAEVDFAKYFYSALRHHYINLITQR : 111
Coho_salmon_ENSOKIP000005114415 : NKGRPNAAAMSLGM--LAVYLLLCVSNMAAYPSKFWIPAEADAEVDFAKYFYSALRHHYINLITQR : 111
Chinook_salmon_ENSOTSP000005007169 : --MHPNLGTWLGAVTLLVWTFICIGTAEGYPVKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 63
Chinook_salmon_ENSOTSP000005022142 : LNHPNLGTWLGALTLLVWTFICIGTAEGYPVKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 91
Zebrafish_ENSDARP00000157094 : --NPNMKMMWGWAAACAFLLFACLTGTEGYPTKKPDNPGEDAPAEELAKYYYSALRHHYINLITQR : 63
Common_carp_ENSCCRP00000070566 : GSHPNMKMMWGWAAACAFLLFACLTGTEGYPTKKPDNPGEDAPAEELAKYYYSALRHHYINLITQR : 123
Common_carp_ENSCCRP00000025584 : --MHPNMKMMWGWAAACAFLLFACLTGTEGYPTKKPDNPGEDAPAEELAKYYYSALRHHYINLITQR : 63
Three-spined_stickleback_ENSGACP00000009098 : --QNHHPVVSWSLGLLWVWALCLGALTEGYPVKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 64
Northern_pike_ENSELUP00000028969 : SKMHPNPGTWLGAVTLLVWTFICIGTAEGYPVKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 66
Northern_pike_ENSELUP00000026966 : -----MYLGV--LWVCSLVCMSSVDAYPSKPPASPRGCAPEELAKYYYSALRHHYINLITQR : 55
NPYa_Fugu_ENSTRUP00000016766 : --QSNLLSWLGLTLGLLWALCLGALTDGYPVKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 63
NPYb_Fugu_ENSTRUP00000038037 : --CDSRTRSAAALITCLLAAPSGTDAYPSKPPASPRGCAPEELAKYYYSALRHHYINLITQR : 62
NPYa_Nile_tilapia_ENSONIP00000046366 : --MHPNLVSWLGLTLGLLWALCLGALTEGYPVKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 63
NPYb_Nile_tilapia_ENSONIP00000040820 : IRTCSNAVMSHSI--LALCLLVCHSGINAYPAKPPASPRGCAPEELAKYYYSALRHHYINLITQR : 64
NPYa_Green_spotted_pufferfish_ENSTNIP00000020909 : --QSNLLSWLGLTLGLLWALCLGALTDGYPVKKPENPGEDAPAEELAKYYYSALRHHYINLITQR : 63
NPYb_Green_spotted_pufferfish_ENSTNIP00000021590 : ---CDSRSRSTAAVLITCLLAWAPSDTDAYPSKPPASPRGCAPEELAKYYYSALRHHYINLITQR : 62
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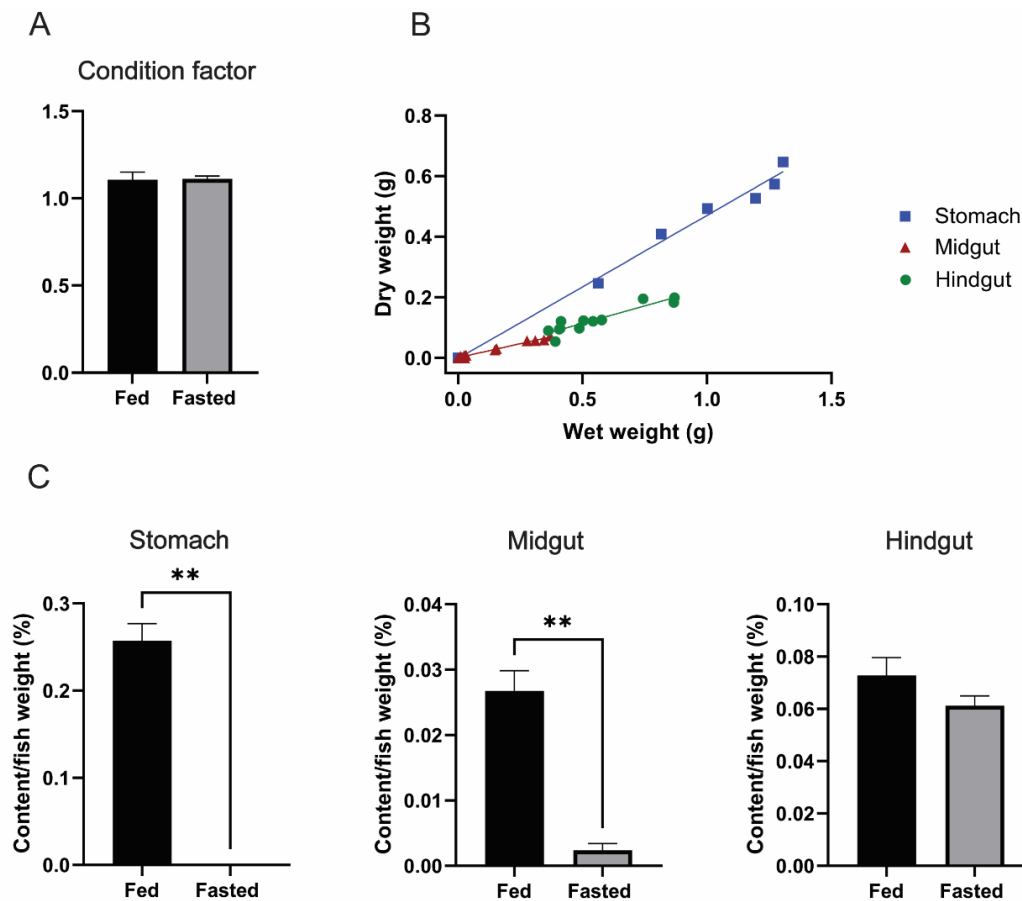
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Human_ENSP00000384364      : YGKRSSF---ETLLISLLMKESSTENVPRRL-----: 91
Atlantic_salmon_ENSSSAP00000032009 : YGKRSSFDTLDTLLISSELLMKESDTLPQSRV-----: 94
Atlantic_salmon_ENSSSAP00000023580 : YGKRSSFDTLDTLLISSELLMKESDTLPQSRV-----: 161
Atlantic_salmon_ENSSSAP00000005847 : YGKRSSF---DTVFSLLQRESSTESVPLASY-----: 138
Medaka_ENSORLP00020013686      : YGKRDNF---DTVFSLLQRESSTESVPGSNV-----: 83
Medaka_ENSORLP00020022926      : YGKRSSFETLDTLVSELLMKESKDTLPQSSV-----: 128
Rainbow_trout_ENSOMYP00000002554 : YGKRSSF---DMVFSLLQRESSTETVPLASY-----: 114
Rainbow_trout_ENSOMYP000000013188 : YGKRSSFDTLDTLLISSELLMKESDTLPQSRV-----: 94
Rainbow_trout_ENSOMYP000000027678 : YGKRSSFDTLDTLLISSELLMKESDTLPQSRV-----RHCVCVHTD: 103
Brown_trout_ENSSTUP000000046804 : YGKRSSFDTLDTLLISSELLMKESDTLPQSRV-----: 94
Brown_trout_ENSSTUP000000104910 : YGKRSSFDTLDTLLISSELLMKESDTLPQSRVLSLSRPNIRAHRRGDEQLTSTTWPPHHGQRPILT: 128
Brown_trout_ENSSTUP000000080605 : YGKRSSF---DTVFSLLQRESSTESVPLASY-----: 138
Brown_trout_ENSSTUP000000081043 : YGKRSSF---DTVFSLLQRESSTESVPLASY-----: 138
Coho_salmon_ENSOKIP000005014471 : YGKRSSFDTLDTLLISSELLMKESDTLPQSRV-----CVCVCVRACDWSRTHIDKV: 113
Coho_salmon_ENSOKIP000005103527 : YGKRSSFDTLDTLLISSELLMKESDTLPQSRV-----: 94
Coho_salmon_ENSOKIP000005117205 : YGKRSSF---DMVFSLLQRESSTESVPLASY-----: 139
Coho_salmon_ENSOKIP000005114415 : YGKRSSF---DMVFSLLQRESSTESVPLASY-----: 139
Chinook_salmon_ENSOTSP000005007169 : YGKRSSFDTLDTLLISSELLMKESDTLPQSRVCCLSRPNTRAHRRGDEQLISPTWPMSENNPN--: 126
Chinook_salmon_ENSOTSP000005022142 : YGKRSSFDTLDTLLISSELLMKESDTLPQSRV-----: 122
Zebrafish_ENSDARP00000157094    : YGKRSSA---DTLLISLLIGSTESRQOTRY-----: 90
Common_carp_ENSCCRP000000070566 : YGKRSSA---DTLLISLLIGSTESRQOTRY-----: 150
Common_carp_ENSCCRP000000025584 : YGKRSSA---DTLLISLLIGSTESRQOTRY-----: 90
Three-spined stickleback_ENSGACP00000009098 : YGKRSSFDTLDTLLISSELLMKESDTLPQSRV-----: 95
Northern_pike_ENSELUP000000028969 : YGKRSSFDTLDTLLISSELLMKESDTLPQSRV-----: 97
Northern_pike_ENSELUP000000026966 : YGKRSSF---DTVFSLLQRESSTENIPRASV-----: 83
NPYb_Fugu_ENSTRUP000000016766   : YGKRSSFETLDTLVSELLMKESDTPQSRV-----: 94
NPYb_Fugu_ENSTRUP000000038037    : YGKRDIET---DSVLTDMLMKESSTESRNS-----: 90
NPYb_Nile_tilapia_ENSONIP000000046366 : YGKRSSFETLDTLVSELLMKESDTLPQSRV-----: 94
NPYb_Nile_tilapia_ENSONIP000000040820 : YGKRDSF---DTVFSDLVRESSTESVPGSSV-----: 92
NPYb_Green_spotted_pufferfish_ENSTNIP000000020909 : YGKRSGEETLDTLVSELLMKESDTPQSRV-----: 94
NPYb_Green_spotted_pufferfish_ENSTNIP000000021590 : YGKRDIET---DSAFETVLMKESSTESVRWNV-----: 90

Human_ENSP00000384364      : -----EDFA---MW-----: 97
Atlantic_salmon_ENSSSAP00000032009 : -----DEES---LW-----: 100
Atlantic_salmon_ENSSSAP00000023580 : -----DEES---LW-----: 167
Atlantic_salmon_ENSSSAP00000005847 : -----GRYEDLGL---WW-----: 148
Medaka_ENSORLP00020013686      : -----IRYEGLIV---W-----: 92
Medaka_ENSORLP00020022926      : -----NRY---LW-----: 133
Rainbow_trout_ENSOMYP00000002554 : -----GRYEDLGL---WW-----: 124
Rainbow_trout_ENSOMYP000000013188 : -----DEES---LW-----: 100
Rainbow_trout_ENSOMYP000000027678 : VCVCTSVNSCFVAS---KV-----: 120
Brown_trout_ENSSTUP000000046804 : -----DEES---LW-----: 100
Brown_trout_ENSSTUP000000104910 : TPMGFMTYASPSLGI---LHPEAR-----: 149
Brown_trout_ENSSTUP000000080605 : -----GRYEDVGL---WW-----: 148
Brown_trout_ENSSTUP000000081043 : -----GRYEDLGL---WW-----: 148
Coho_salmon_ENSOKIP000005014471 : SQSRSGAGSLCRTDERSAL--LL-----: 133
Coho_salmon_ENSOKIP000005103527 : -----DEES---LW-----: 100
Coho_salmon_ENSOKIP000005117205 : -----GRYEDLGL---WW-----: 149
Coho_salmon_ENSOKIP000005114415 : -----GRYEDLGL---WW-----: 149
Chinook_salmon_ENSOTSP000005007169 : ---GFHEYASPSLGI---LHPEAR-----: 144
Chinook_salmon_ENSOTSP000005022142 : -----DEES---LW-----: 128
Zebrafish_ENSDARP00000157094    : -----EDHL---AW-----: 96
Common_carp_ENSCCRP000000070566 : -----EDHL---VW-----: 156
Common_carp_ENSCCRP000000025584 : -----EDHL---VW-----: 96
Three-spined stickleback_ENSGACP00000009098 : -----DEES---LW-----: 100
Northern_pike_ENSELUP000000028969 : -----DEES---LW-----: 103
Northern_pike_ENSELUP000000026966 : -----GRFEDIV---WW-----: 93
NPYb_Fugu_ENSTRUP000000016766   : -----DES---LW-----: 99
NPYb_Fugu_ENSTRUP000000038037    : -----TRYDELGL---W-----: 99
NPYb_Nile_tilapia_ENSONIP000000046366 : -----AFKGATFLSLFLYLDLYTSLSLSFVSVPDMTHQCGDAAITLVSLTAVPPALTF: 147
NPYb_Nile_tilapia_ENSONIP000000040820 : -----TRYDGLGL---W-----: 101
NPYb_Green_spotted_pufferfish_ENSTNIP000000020909 : -----DES---LW-----: 99
NPYb_Green_spotted_pufferfish_ENSTNIP000000021590 : -----IRYDGLGL---W-----: 99

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Supplementary Figure 1. Multiple sequence alignment of NPY. The predicted amino acid sequences of the Atlantic salmon NPY were aligned with those of other teleost species using Multiple sequence alignment in MUSCLE. Conserved amino acids among the species are highlighted in black.



Supplementary Figure 2 – A) Condition factor (K) in fed and fasted Atlantic salmon. **B)** Correlation between dry weight (g) and wet weight (g) content for stomach, midgut and hindgut. Dots represent individual fish ($n = 12$), while solid lines represent the linear regressions. p-values following Pearson’s correlation analysis were < 0.0001 **** for all regions. **C)** Stomach, midgut and hindgut dry content standardized by the fish weight. Results are presented as mean $\pm$ SEM ($n = 6$) and asterisks indicate statistically significant differences; ** $p < 0.01$. For detailed statistical information, refer to Supplementary table 4.

Supplementary tables

Supplementary table 1 - Sequence of the specific primers used for qPCR mRNA expression analysis. Primer sequences, amplicon sizes, R^2 , and qPCR efficiency are indicated for each primer pair

Gene	Ensembl gene acc.no.	Sequence 5' $\rightarrow$ 3'	Amplicon length (bp)	R^2	Efficiency (%)
<i>npya1</i>	ENSSSAG00000040508	F:GAACGCACAGCAGCAGAAAG R:AGGATGCATATTGACTTGAAGGTT	80	0.9979	110
<i>npya2</i>	ENSSSAG00000015791	F:CAGTCCAGGTATGATGAACCGT R:GGCACAGGAGTAACCTCTGG	195	0.9974	103
<i>npyb</i>	ENSSSAG00000002935	F:CCAAACCTGTCATTCCCGCA R:CTTTTCCCATACCGCTGTCTG	109	0.9982	114
β -actin	ENSSSAG00000001782	F: CCAAAGCCAACAGGGAGAAG R: AGGGACAACACTGCCTGGAT	91	0.9995	97
<i>s20</i>	ENSSSAG00000073156	F: GCAGACCTTATCCGTGGAGCTA R: TGGTGATGCGCAGAGTCTTG	85	0.9975	100

Supplementary Table 2: Databank accession number in Ensembl¹ and Genbank², chromosome placement, predicted gene length, predicted amino acid (AA) sequence for full length, without signaling peptide and mature peptide and predicted masses for each of the Atlantic salmon NPYs.

Gene	Protein	Ensembl acc.no	GenBank acc.no	Length (bp)	Chr	Ensembl protein acc.no	Full length (AA)	Predicted mass (kDa)	Without signaling peptide (AA)	Predicted mass without signaling peptide (kDa)	Mature peptide (AA)	Predicted mass mature peptide (kDa)
<i>npya1</i>	AsNPYa1	ENSSSAG00000040508	NM_001146681.1	328	ssa14	ENSSSAP000000032009	100	11.,33	72	8.33	36	4.25
<i>npya2</i>	AsNPYa2	ENSSSAG00000015791	XM_014178359.1	1513	ssa27	ENSSSAP000000023580	167	18.83	72	8.31	36	4.27
				1585	ssa27	ENSSSAP000000023586	191					
<i>npyb</i>	AsNPYb	ENSSSAG00000002935	XM_014202299.1	789	ssa05	ENSSSAP000000005847	148	16.87	73	8.55	36	4.28

Supplementary Table 3: Percent identity between Atlantic salmon NPY paralogs and human NPY for the mature peptide

	AsNPYa1	AsNPYa2	AsNPYb
AsNPYa2	97.22 %		
AsNPYb	75.00 %	75.00 %	
hNPY	83.33 %	86.11 %	77.78 %

¹ <https://www.ensembl.org/index.html>

² <https://www.ncbi.nlm.nih.gov/genbank/>

Supplementary Table 4: Statistical analysis of information pertaining to fish and gastrointestinal content.

	Average fed	Average fasted	Shapiro Wilk	F-test	Test	p-value
Condition factor	1.11 ± 0.04	1.11 ± 0.02	Passed	Passed	t-test	0.9251
Weight (g)	188.9 ± 18.98	178.7 ± 10.10	Passed	Passed	t-test	0.6478
Length (cm)	25.62 ± 0.82	25.22 ± 0.59	Passed	Passed	t-test	0.6998
Stomach (dry content/fish weight (%))	0.2573 ± 0.0196	0 ± 0.0000	Invalid for fasted	Failed	Mann-Whitney	0.0022**
Midgut (dry content/fish weight (%))	0.0267 ± 0.0031	0.0024 ± 0.0010	Passed	Failed	Mann-Whitney	0.0022**
Hindgut (dry content/fish weight (%))	0.0728 ± 0.0068	0.0612 ± 0.0038	Passed	Passed	t-test	0.1652

Supplementary Table 5: Statistical test and p-values for each gene expression per brain region between treatments

Brain region	<i>npya1</i>				<i>npya2</i>				<i>npyb</i>			
	Shapiro-Wilk	n fed;fasted	Test	p-value	Shapiro-Wilk	n fed;fasted	Test	p-value	Shapiro-Wilk	n fed/fastest	Test	p-value
OB	passed	6;5	t-test	0.044*	passed	6;6	t-test	0.809	passed	6;6	Mann-Whitney	0.065
TEL	passed	6;6	t-test	0.154	passed	5**;6	t-test	0.281	passed	6;6	t-test	0.825
MB	passed	6;6	t-test	0.187	passed	5**;6	t-test	0.0007***	passed	6;6	t-test	0.597
CE	passed	6;6	t-test	0.237	passed	6;6	t-test	0.784	passed	6;6	t-test	0.639
HYP	passed	6;6	t-test	0.240	passed	6;6	t-test	0.099	passed	6;6	Mann-Whitney	0.699
SV	passed	4;4	t-test	0.899	passed	4;4	t-test	0.712	passed	4;4	t-test	0.296
PT	passed	6;6	t-test	0.316	passed	5**;6	Mann-Whitney	0.178	passed	5**;6	t-test	0.019*
BS	failed	6;6	Mann-Whitney	>0.999	passed	6;6	t-test	0.246	passed	6;6	Mann-Whitney	0.818

*Statistically significant **Outlier removed following Grubb's outlier test

Supplementary Table 6: Results from the Pearson correlation analysis for the hypothalamic *npy* mRNA expression levels *versus* gastrointestinal tract dry weight content normalized to fish weight (n=12). Correlation coefficient, 95% confidence interval and p-value are given.

Gene	Gut compartment	R²	r (95% confidence interval)	p-value
<i>npya1</i>	Stomach	0.1432	-0.3785 (-0.7824; 0.2497)	0.225
	Midgut	0.0375	-0.1937 (-0.6908; 0.4277)	0.546
	Hindgut	0.1666	-0.4081 (-0.7957; 0.2165)	0.188
<i>npya2</i>	Stomach	0.2154	-0.4641 (-0.8197; 0.1496)	0.129
	Midgut	0.1163	-0.3411 (-0.7652; 0.2895)	0.278
	Hindgut	0.0006	0.0247 (-0.5571; 0.5902)	0.939
<i>npyb</i>	Stomach	0.1429	-0.3781 (-0.7822; 0.2501)	0.226
	Midgut	0.1037	-0.3221 (-0.7562; 0.3089)	0.307
	Hindgut	0.0024	-0.0490 (-0.6058; 0.5401)	0.880