Zebrafish (*Danio rerio*): Feeding and Nutrition

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ZF-MED - ZEBRAFISH AND OTHER AQUATIC MODELS IN MEDITERRANEAN LABS

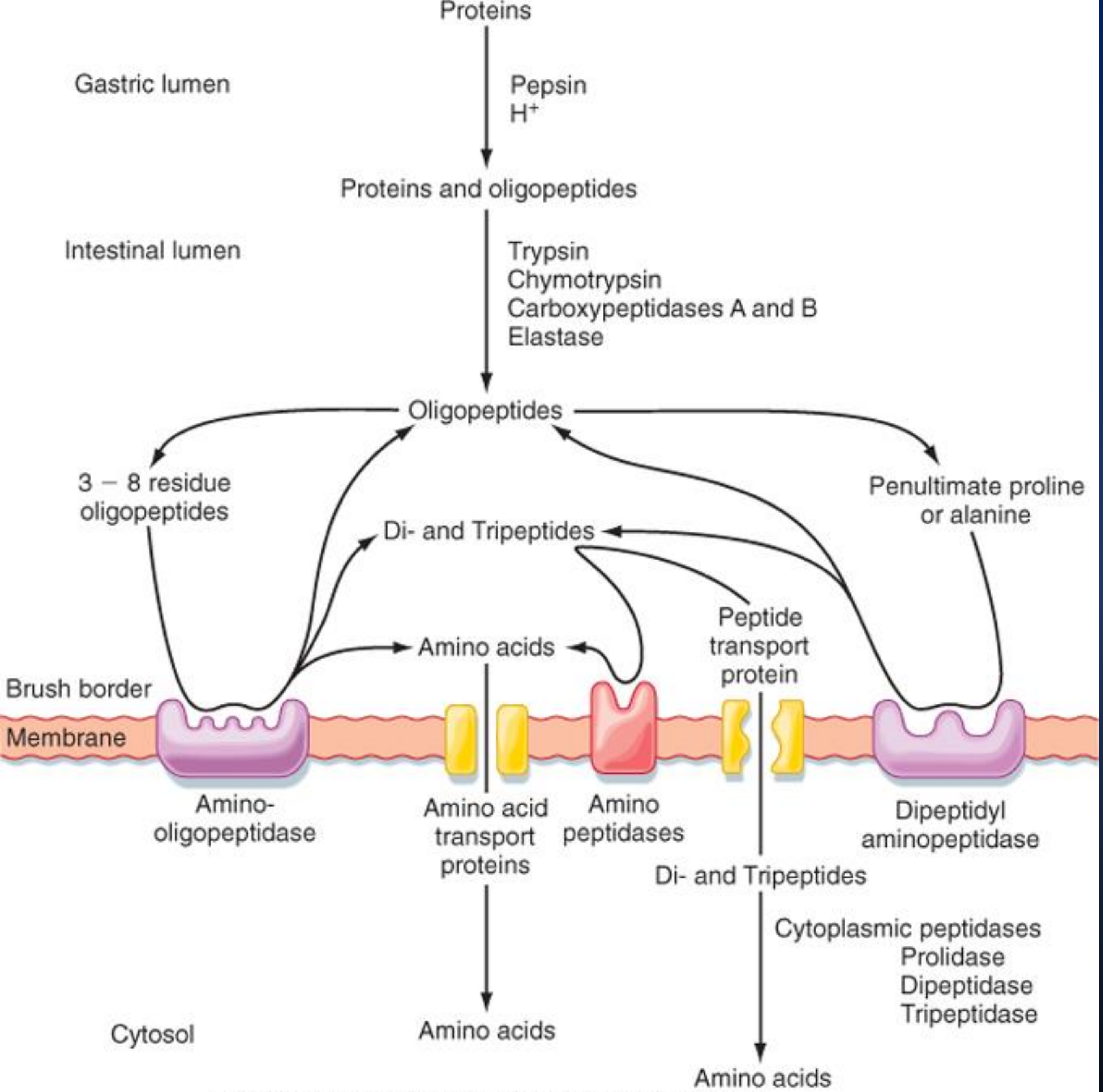
Giornate studio sull'impiego dei Modelli Acquatici a fini scientifici

October 16, 2018

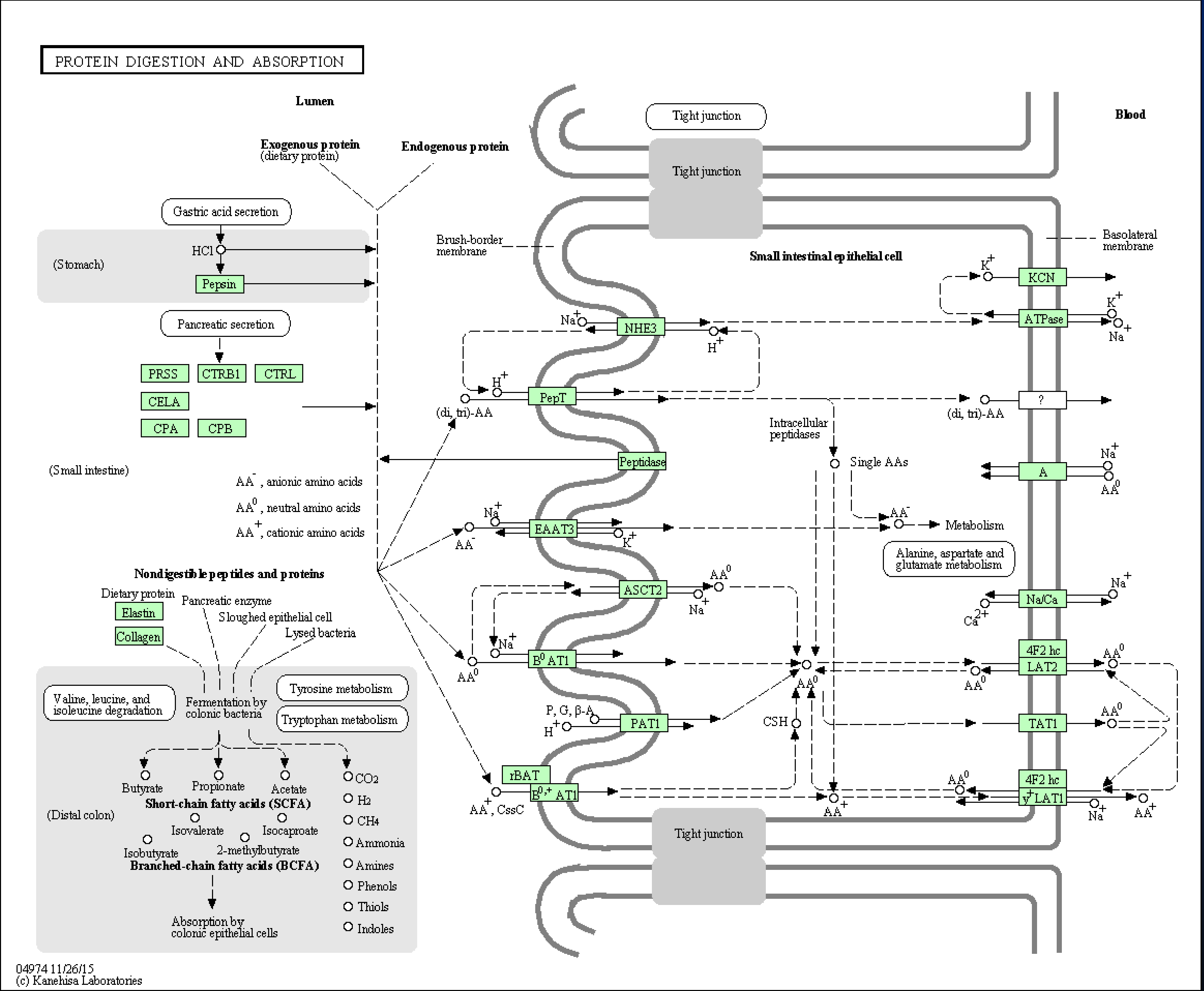
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale' - Teramo

1. Redundancy of transporters involved in amino acid/peptide absorption

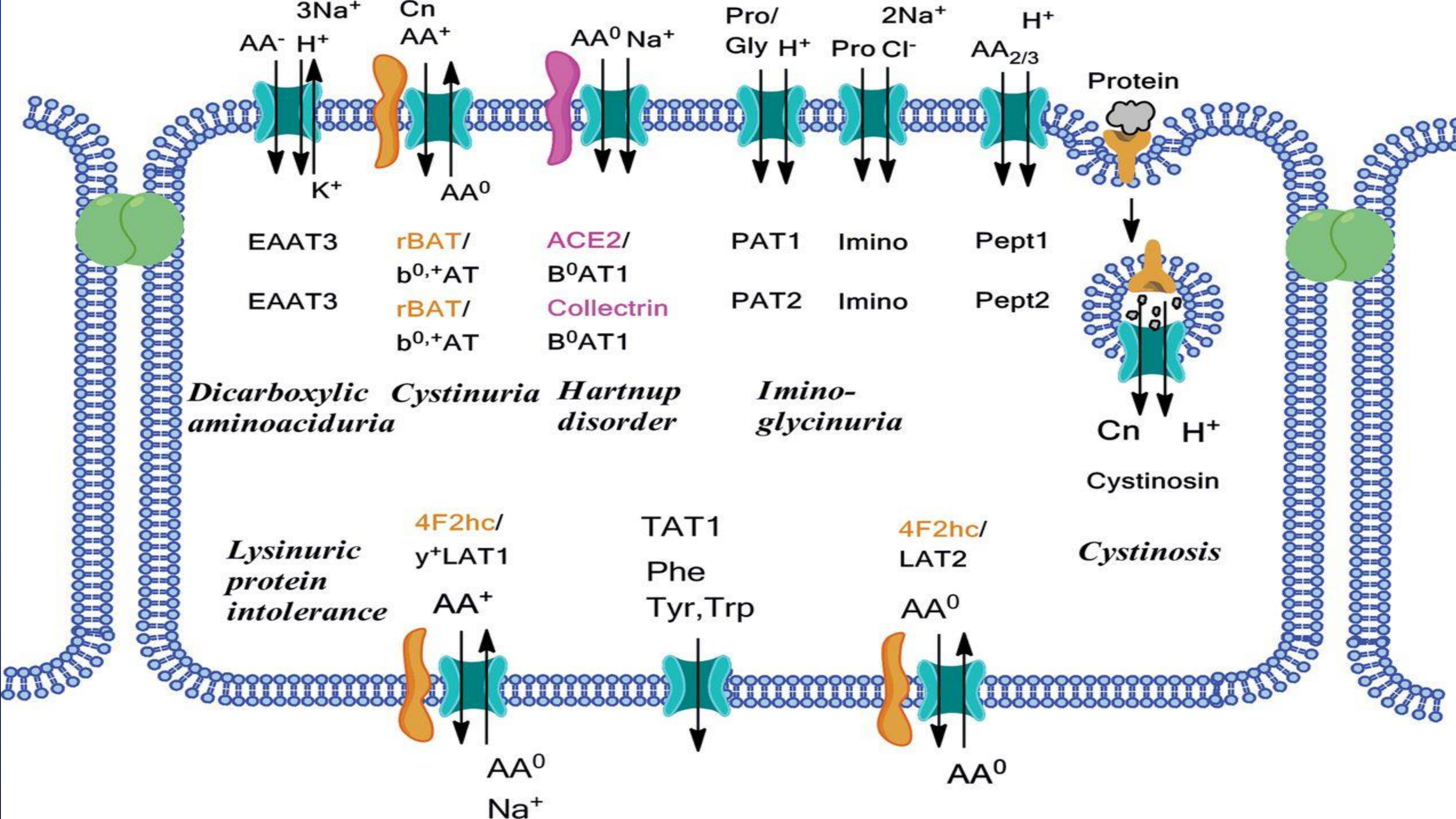
Protein digestion, terminal (membrane) digestion and absorption



Koeppen & Stanton: Berne and Levy Physiology, 6th Edition.

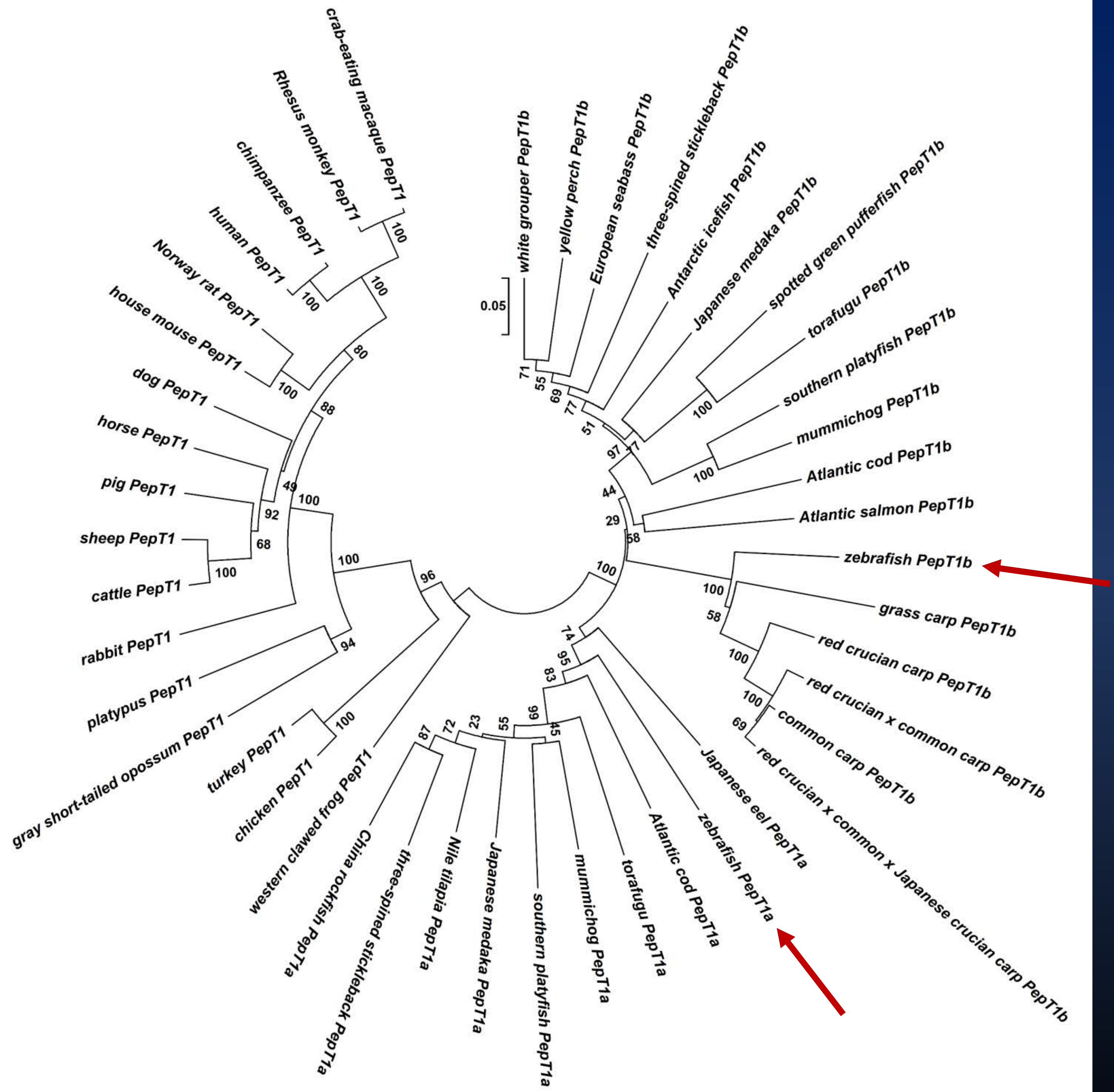


KEGG pathways


3Na+
AA- H+
K+
Cn
AA+
AA0
AA0 Na+
Pro/
Gly H+
2Na+
Pro Cl-
H+
AA2/3
Protein
EAAT3
rBAT/
b0,+AT
ACE2/
B0AT1
PAT1
Imino
Pept1
EAAT3
rBAT/
b0,+AT
Collectrin
B0AT1
PAT2
Imino
Pept2
Dicarboxylic aminoaciduria
Cystinuria
Hartnup disorder
Imino-glycinuria
Cn H+
Cystinosin
Cystinosis
4F2hc/
y+LAT1
TAT1
Phe
Tyr,Trp
4F2hc/
LAT2
Lysinuric protein intolerance
AA+
AA0
Na+
AA0
AA0

SLC34 family - Na^+/phosphate transporters

Higher levels rank[a]	Order[a]	Suborder[a]	Species	slc34a1		slc34a2			slc34a3
Chondrichthyes (Class); Holocephali (Subclass);	Chimaeriformes	-	*Callorhinchus milii*	Yes		Yes			?
Chondrichthyes (Class); Elasmobranchii (Subclass); Selachii (Infraclass); Galeomorphii (No rank); Galeoidea (Superorder);	Orectolobiformes	-	*Rinchodon typus*	?		?			Yes
Sarcopterygii (Superclass); Coelacanthimorpha (No rank);	Coelacanthiformes	-	*Latimeria chalumnae*	-		Yes			Yes
Sarcopterygii (Superclass); Dipnotetrapodomorpha (No rank); Tetrapoda (No rank); Amphibia (Class); Batrachia;	Anura	-	*Xenopus tropicalis*	-		Yes			Yes
Sarcopterygii (Superclass); Dipnotetrapodomorpha (No rank); Tetrapoda (No rank); Amniota (No rank); Mammalia (Class); Theria (No rank); Eutheria (No rank); Boreoeutheria (No rank); Laurasiatheria (Superorder); Cetartiodactyla (No rank);	-	Ruminantia	*Ovis aries musimon*	Yes		Yes			Yes
Sarcopterygii (Superclass); Dipnotetrapodomorpha (No rank); Tetrapoda (No rank); Amniota (No rank); Mammalia (Class); Theria (No rank); Eutheria (No rank); Boreoeutheria (No rank); Euarchontoglires (Superorder); Glires (No rank);	Lagomorpha	-	*Oryctolagus cuniculus*	Yes		Yes			-
	Rodentia	Myomorpha	*Mus musculus*	Yes		Yes			Yes
Sarcopterygii (Superclass); Dipnotetrapodomorpha (No rank); Tetrapoda (No rank); Amniota (No rank); Mammalia (Class); Theria (No rank); Eutheria (No rank); Boreoeutheria (No rank); Euarchontoglires (Superorder);	Primates	Haplorrhini	*Homo sapiens*	Yes		Yes			Yes
Actinopterygii (Superclass); Actinopteri (Class); Chondrostei (Subclass);	Acipenseriformes	Acipenseroidei	*Acipenser oxyrinchus*	Yes		Yes			Yes
Actinopterygii (Superclass); Actinopteri (Class); Neopterygii (Subclass); Holostei (Infraclass);	Semionotiformes	-	*Lepisosteus oculatus*	Yes		Yes			-
Actinopterygii (Superclass); Actinopteri (Class); Neopterygii (Subclass); Teleostei (Infraclass); Osteoglossocephalai (No rank); Clupeocephala (No rank); Otomorpha (No rank); Clupei (No rank);	Clupeiformes	Clupeoidei	*Clupea harengus*	Yes(a)	Yes(b)	Yes(a)	Yes(b)	-	-
Actinopterygii (Superclass); Actinopteri (Class); Neopterygii (Subclass); Teleostei (Infraclass); Osteoglossocephalai (No rank); Clupeocephala (No rank); Otomorpha (No rank); Ostariophysi (No rank); Otophysi (No rank); Cypriniphysae (No rank);	Cypriniformes	-	*Danio rerio*	Yes(a)	Yes(b)	Yes(a)	Yes(b)	-	-
			Sinocyclocheilus rhinocerous	Yes(a)	Yes(b)	Yes(a)	Yes(b)	-	-
			Sinocyclocheilus grahami	Yes(a)	Yes(b)	Yes(a)	Yes(b)	-	-
Actinopterygii (Superclass); Actinopteri (Class); Neopterygii (Subclass); Teleostei (Infraclass); Osteoglossocephalai (No rank); Clupeocephala (No rank); Otomorpha (No rank); Ostariophysi (No rank); Otophysi (No rank); Characiphysae (No rank);	Siluriformes	Siluroidei	*Ictalurus punctatus*	Yes(a)	-	Yes(a)	Yes(b)	-	-
			Pelteobagrus (Tachysurus) fulvidraco	Yes(a)	-	Yes(a)	Yes(b)	-	-
	Characiformes	Characoidei	*Astyanax mexicanus*	Yes(a)	-	Yes(a)	Yes(b)	-	-
			Pygocentrus nattareri	Yes(a)	-	Yes(a)	Yes(b)	-	-
Actinopterygii (Superclass); Actinopteri (Class); Neopterygii (Subclass); Teleostei (Infraclass); Osteoglossocephalai (No rank); Clupeocephala (No rank); Euteleosteomorpha (No rank); Protacanthopterygii (No rank);	Esociformes	-	*Esox lucius*	Yes(a)	Yes(b)	Yes(a)	Yes(b)	-	-
	Salmoniformes	-	*Oncorhynchus mykiss*	Yes(a)	Yes(b)	-	Yes(b.1)	Yes(b.2)	-
			Oncorhynchus kisutch	Yes(a)	Yes(b)	-	Yes(b.1)	Yes(b.2)	-
			Salvelinus alpinus	Yes(a)	Yes(b)	-	Yes(b.1)	Yes(b.2)	-
			Salmo salar	Yes(a)	Yes(b)	-	Yes(b.1)	Yes(b.2)	-
Actinopterygii (Superclass); Actinopteri (Class); Neopterygii (Subclass); Teleostei (Infraclass); Osteoglossocephalai (No rank); Clupeocephala (No rank); Euteleosteomorpha (No rank); Neoteleostei (No rank); Eurypterygia (No rank); Ctenosquamata (No rank); Acanthomorphata (No rank); Euacanthomorphacea (No rank); Percomorphaceae (No rank); Anabantaria (No rank);	Synbranchiformes	Synbranchoidei	*Monopterus albus*	Yes(a)	Yes(b)	Yes(a)	Yes(b)	-	-
Actinopterygii (Superclass); Actinopteri (Class); Neopterygii (Subclass); Teleostei (Infraclass); Osteoglossocephalai (No rank); Clupeocephala (No rank); Euteleosteomorpha (No rank); Neoteleostei (No rank); Eurypterygia (No rank); Ctenosquamata (No rank); Acanthomorphata (No rank); Euacanthomorphacea (No rank); Percomorphaceae (No rank); Carangaria (No rank); Carangaria incertae sedis (No rank);	-	-	*Lates calcarifer*	Yes(a)	Yes(b)	Yes(a)	Yes(b)	-	-
Actinopterygii (Superclass); Actinopteri (Class); Neopterygii (Subclass); Teleostei (Infraclass); Osteoglossocephalai (No rank); Clupeocephala (No rank); Euteleosteomorpha (No rank); Neoteleostei (No rank); Eurypterygia (No rank); Ctenosquamata (No rank); Acanthomorphata (No rank); Euacanthomorphacea (No rank); Percomorphaceae (No rank); Carangaria (No rank);	Carangiformes	-	*Seriola dumerili*	Yes(a)	Yes(b)	Yes(a)	Yes(b)	-	-
			Seriola lalandi dorsalis	Yes(a)	Yes(b)	Yes(a)	Yes(b)	-	-
	Pleuronectiformes	Pleuronectoidei	*Cynoglossus semilaevis*	Yes(a)	-	Yes(a)	Yes(b)	-	-
			Paralichthys olivaceus	Yes(a)	-	Yes(a)	Yes(b)	-	-
			Pseudopleuronectes americanus	?	-	?	Yes(b)	-	-



Evolutionary relationships among Pept1-type transporters in vertebrates

Zebrafish Slc15a1 (Pept1) kinetic analysis by TEVC

Table 2. Kinetic parameters of differently charged dipeptides (neutral, basic and acidic dipeptides) and their pH dependency. The maximal transport current (I_{max}) values are relative to I_{max} of Gly-L-Gln measured at pH 7.5 in the same oocyte.

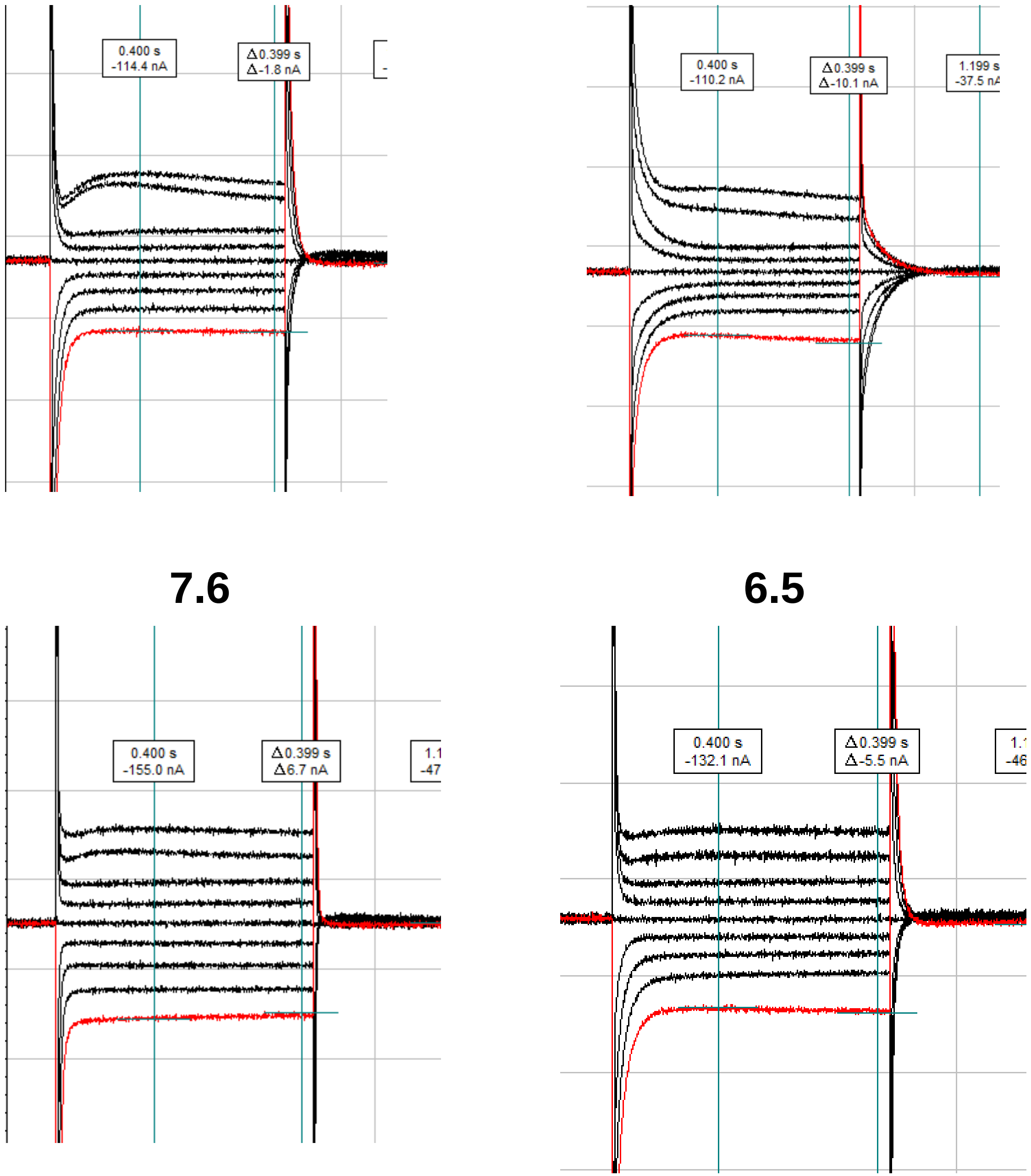
Substrate	pH	$K_{0.5}$ (mM, at –60 mV)	I_{max} (%, relative to Gly-Gln at pH 7.5)	No. of oocytes (*n*)
Gly-L-Gln	8.5	9.6 ± 1.6	1.62 ± 0.10	10
	7.5	1.44 ± 0.18	1.00 (= 200 ± 24 nA)	16
	6.5	0.24 ± 0.07	0.43 ± 0.03	10
	5.5	0.13 ± 0.03	0.36 ± 0.03	16
Gly-L-Lys	8.5	38 ± 8	1.91 ± 0.21	9
	7.5	6.0 ± 1.5	0.94 ± 0.07	17
L-Lys-Gly	8.5	16 ± 6	2.20 ± 0.10	7
	7.5	3.5 ± 0.6	1.26 ± 0.07	16
Gly-L-Asp	7.5	21 ± 5	0.64 ± 0.14	8
	5.5	0.21 ± 0.03	0.47 ± 0.04	15
L-Asp-Gly	7.5	13 ± 1	0.51 ± 0.07	5
	5.5	0.17 ± 0.05	0.36 ± 0.04	5

Electrophysiological data reported in this table were obtained by experiments conducted on *Xenopus laevis* oocytes voltage clamped at –60 mV. Peptide-evoked inward currents were measured in the presence of increasing concentrations of dipeptide (0.5-20 mM) in oocytes perfused with solutions at various pH values (ranging from 5.5 to 8.5). $K_{0.5}$ indicates the apparent concentration of dipeptide that yielded one-half of maximal transport current (I_{max}). Values are reported as means ± SEM of *n*, number of oocytes (see last column).

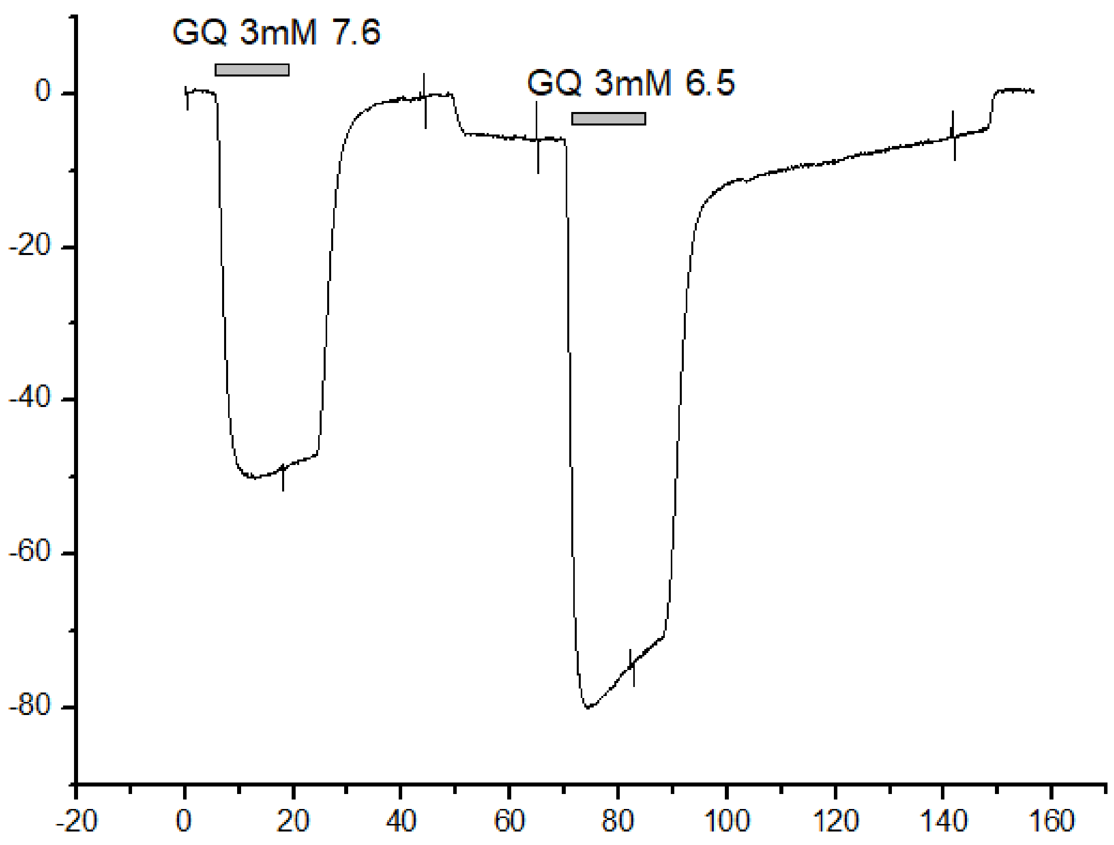
Zebrafish Slc15a1a (Pept1a) is functional

zfPepT1a

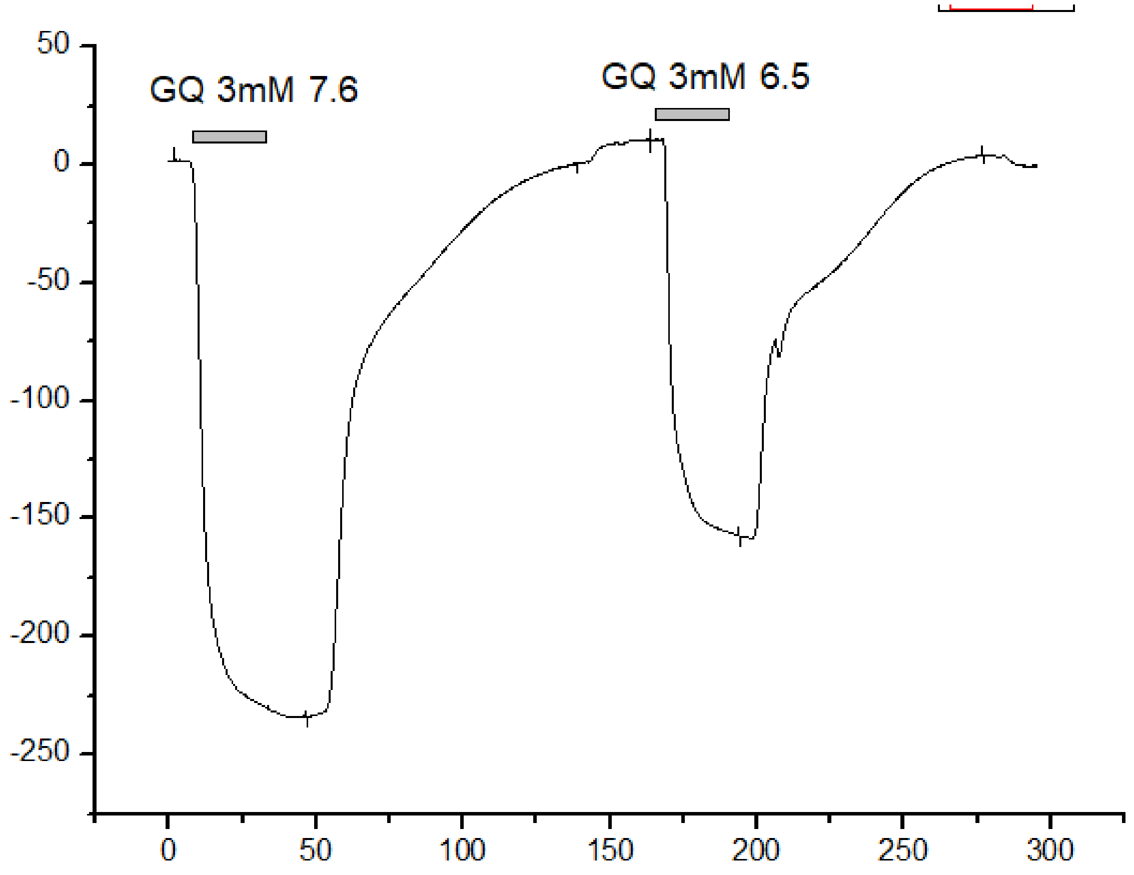
zfPepT1b

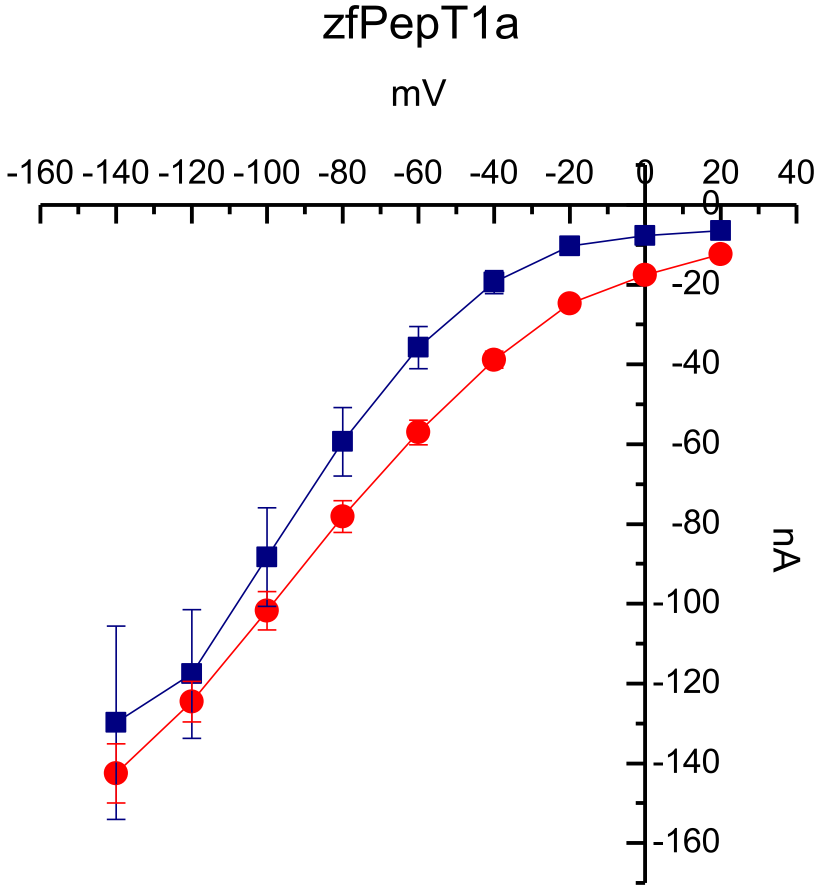


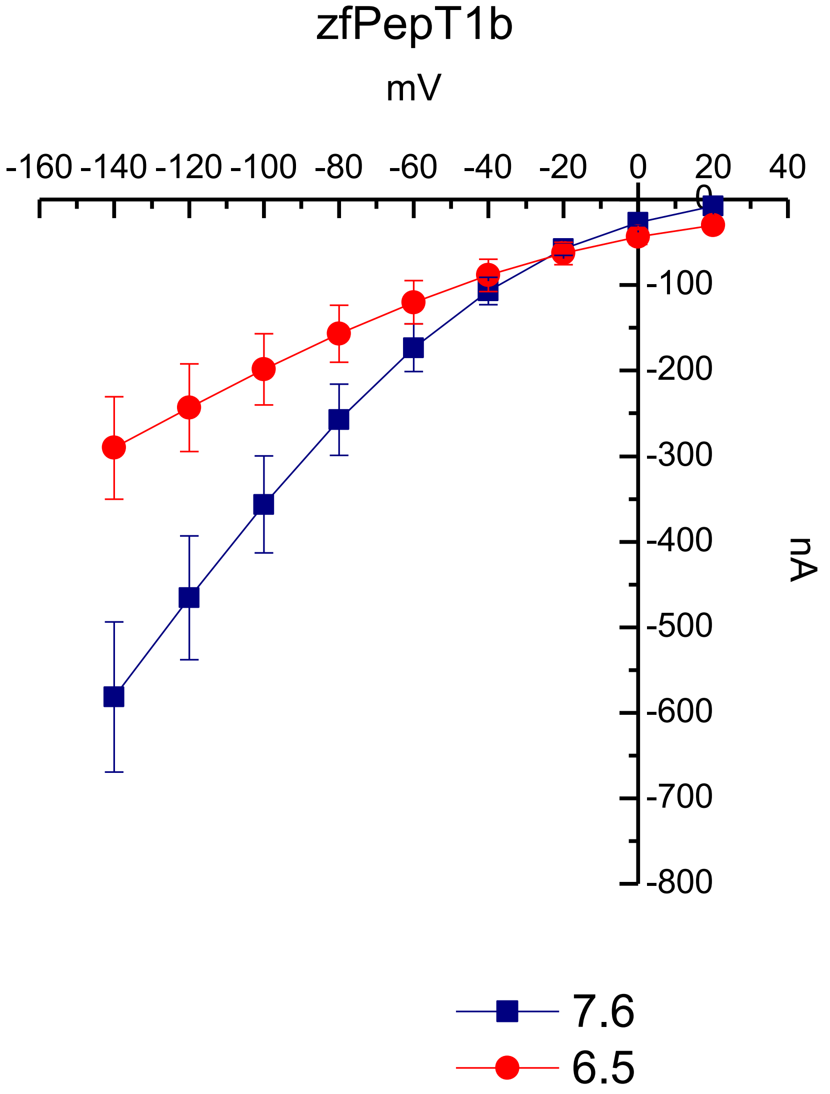
zfPepT1a

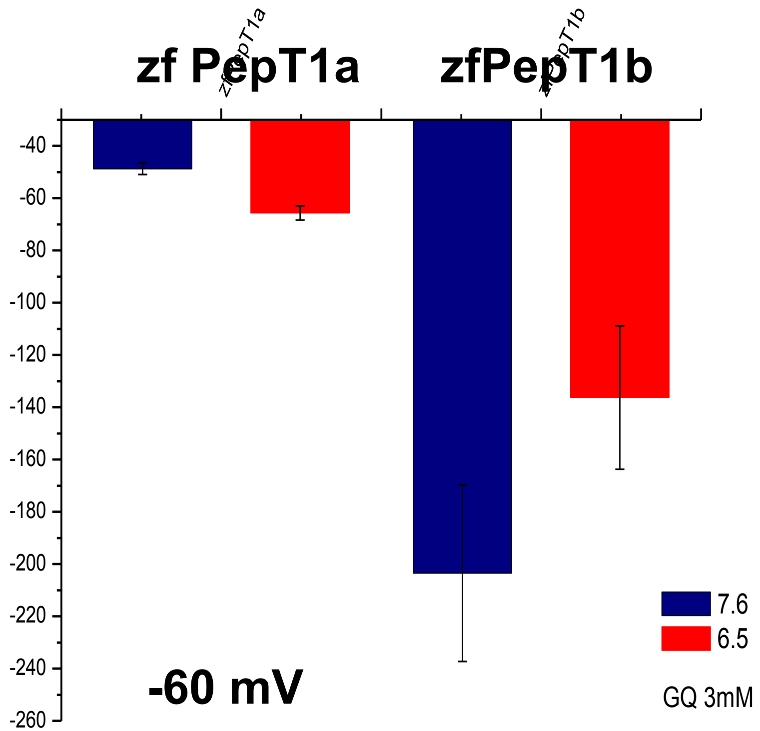


zfPepT1b



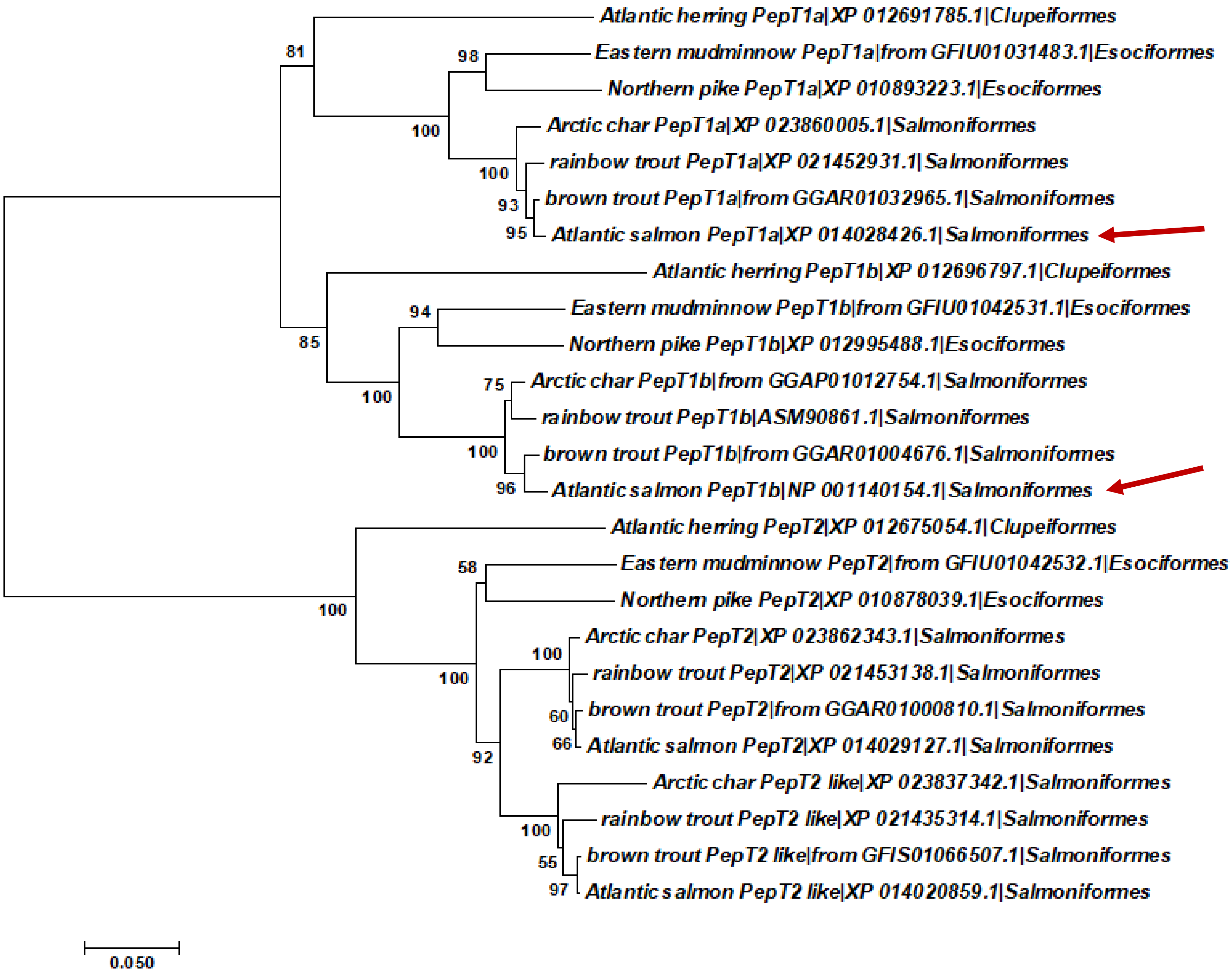






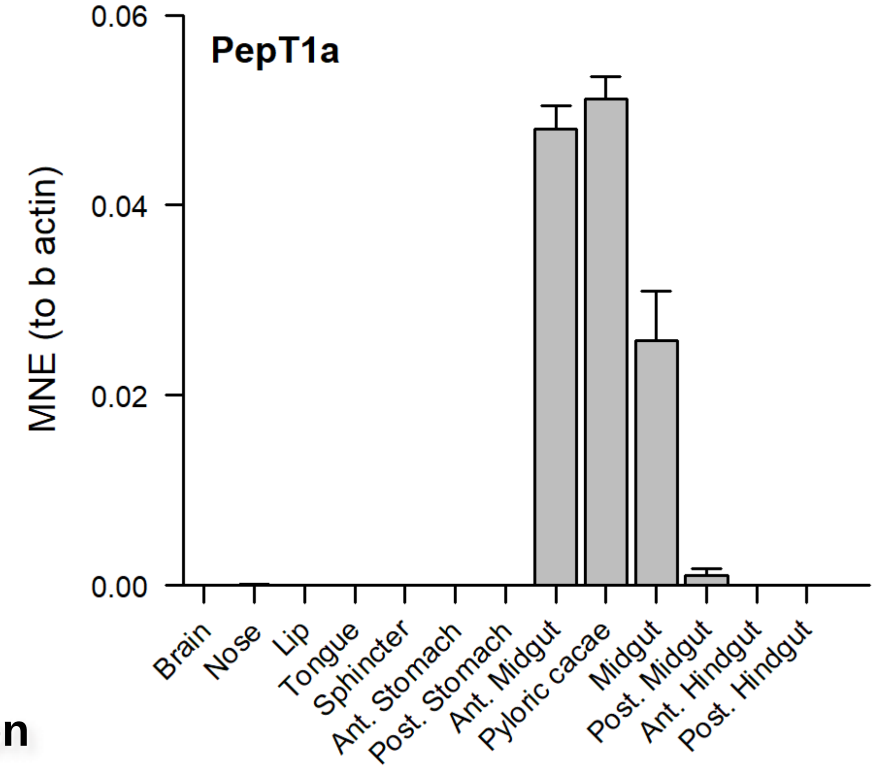
sub GQ 3mM
Vh -60mV

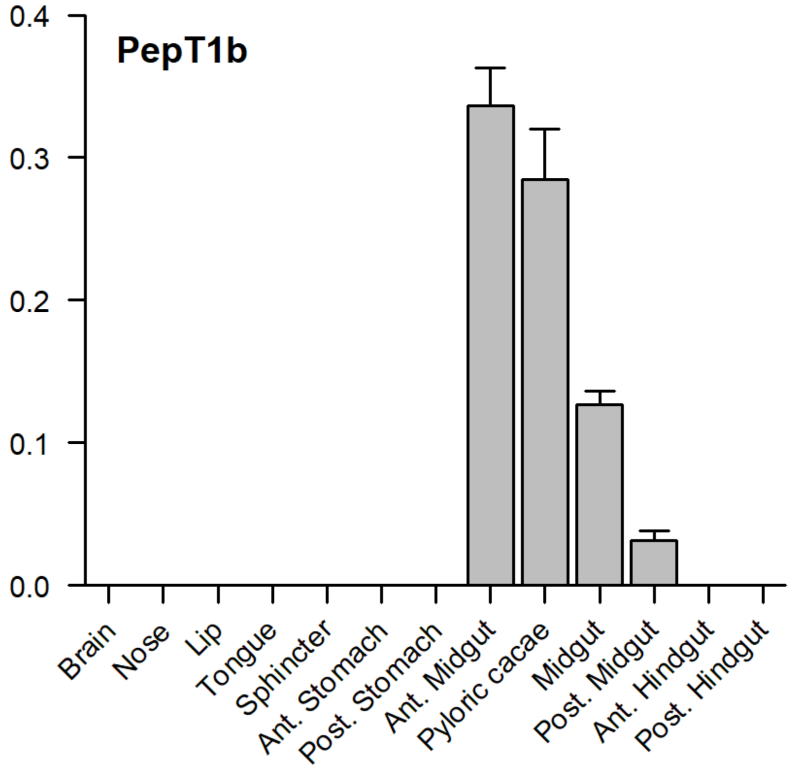
5 oo PepT1a, 4 oo PepT1b



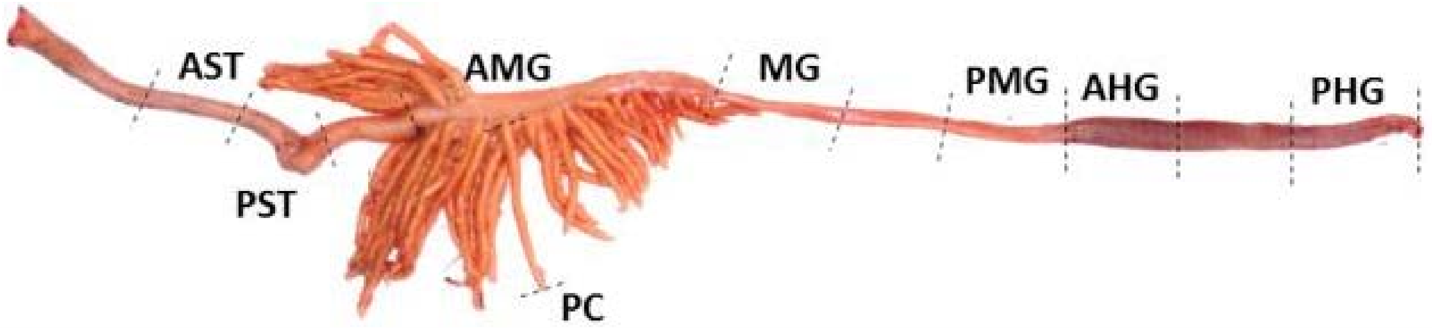
Evolutionary relationships among peptide transporters in salmonids

Spatial distribution of *slc15a1a* (*pept1a*) and *slc15a1b* (*pept1b*) mRNA in the Atlantic salmon digestive tract (RT-PCR)





Atlantic salmon



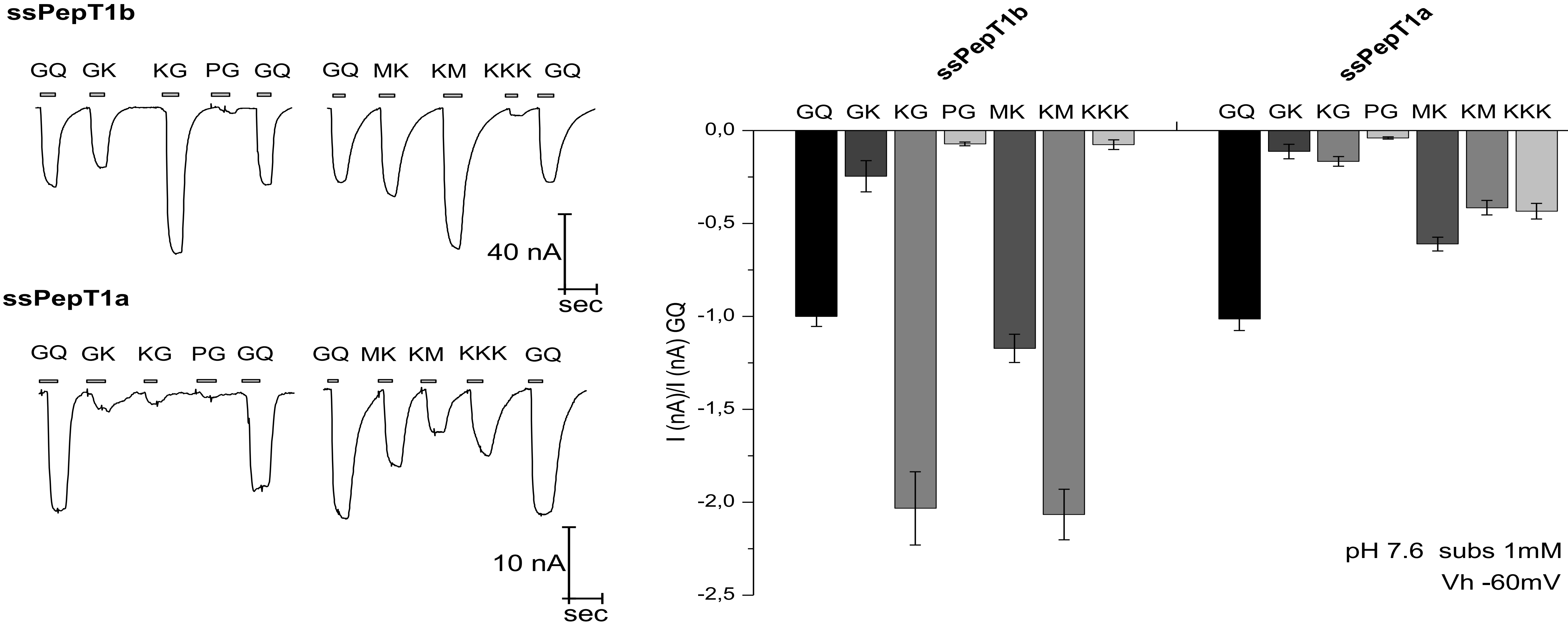
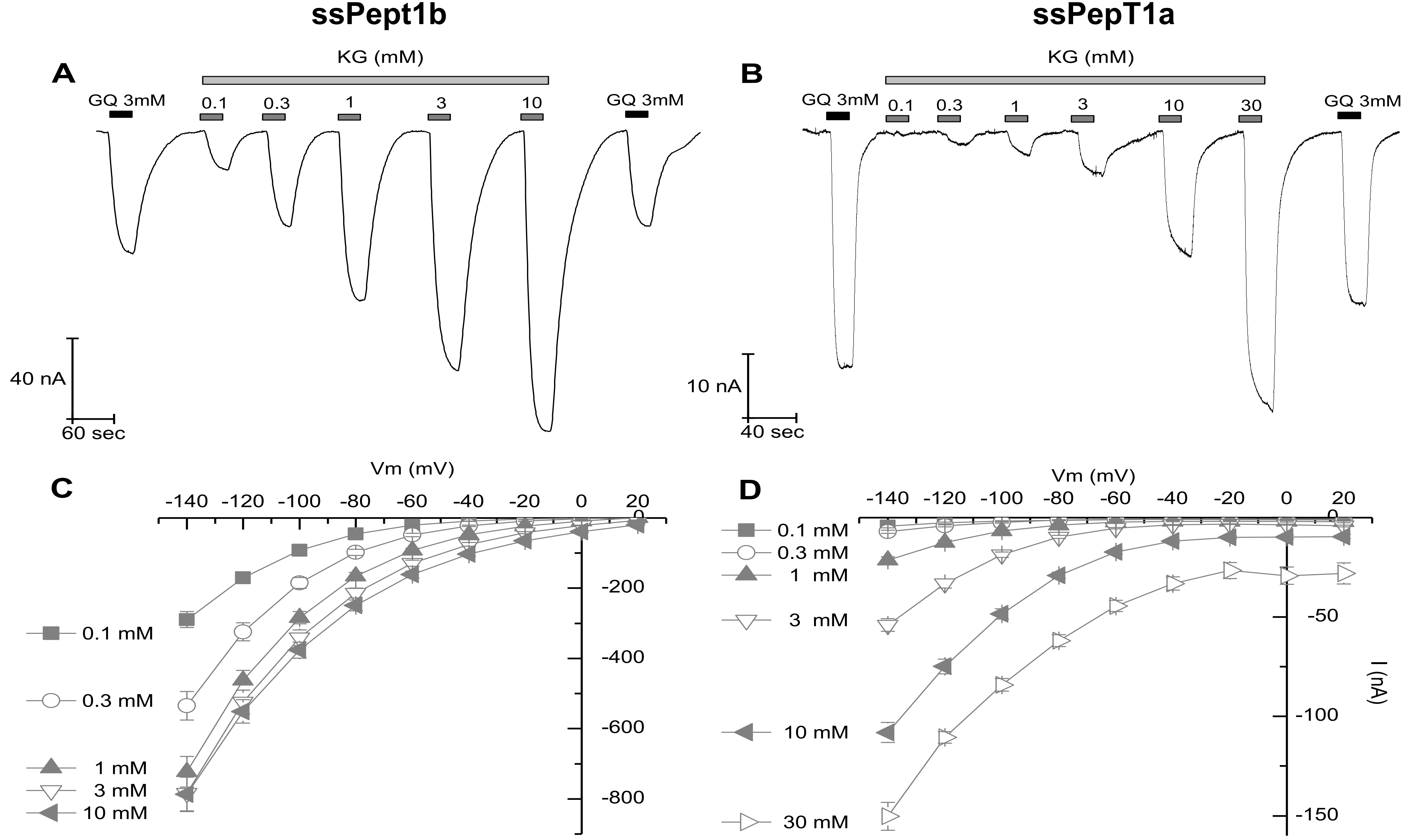


Fig. 4. Transport currents elicited by different Lysine containing substrates. A, representative currents recorded in the presence of the indicated substrates (1 mM) at pH 7.6 in ssPepT1b (upper traces) and PepT1a, the oocytes were clamped at -60 mV. **B**, Mean of transport associated current at -60 mV in the presence of indicated substrates and normalized to the current in Gly-Gln. **C**, current-voltage relationship of transport-associated current in ssPepT1b (top) and in ssPepT1a (bottom) recorded from -140 to +20 mV at two pH conditions: 6.5 (gray symbols) and 7.6 (empty symbols). The current value reported in I/V relationship and in bar histograms are obtained by subtracting from the current in the presence of indicated substrate, the current in its absence.


ssPept1b
ssPepT1a
A
KG (mM)
GQ 3mM
0.1
0.3
1
3
10
GQ 3mM
40 nA
60 sec
B
KG (mM)
GQ 3mM
0.1
0.3
1
3
10
30
GQ 3mM
10 nA
40 sec
C
Vm (mV)
-140
-120
-100
-80
-60
-40
-20
0
20
0
-200
-400
-600
-800
0.1 mM
0.3 mM
1 mM
3 mM
10 mM
D
Vm (mV)
-140
-120
-100
-80
-60
-40
-20
0
20
0
-50
-100
-150
I (nA)
0.1 mM
0.3 mM
1 mM
3 mM
10 mM
30 mM

2. Pept1-type transporters, Lys-Gly-containing peptides & peptide(Lys-Gly)-containing diets

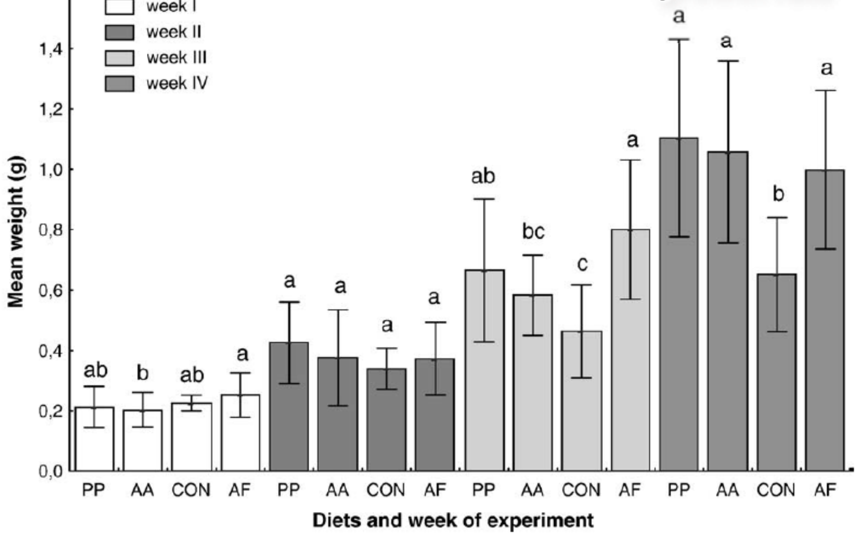
Pept1 and peptide(Lys-Gly)-containing diets (rainbow trout)

Table 1
Composition of feeds used in this experimental study.

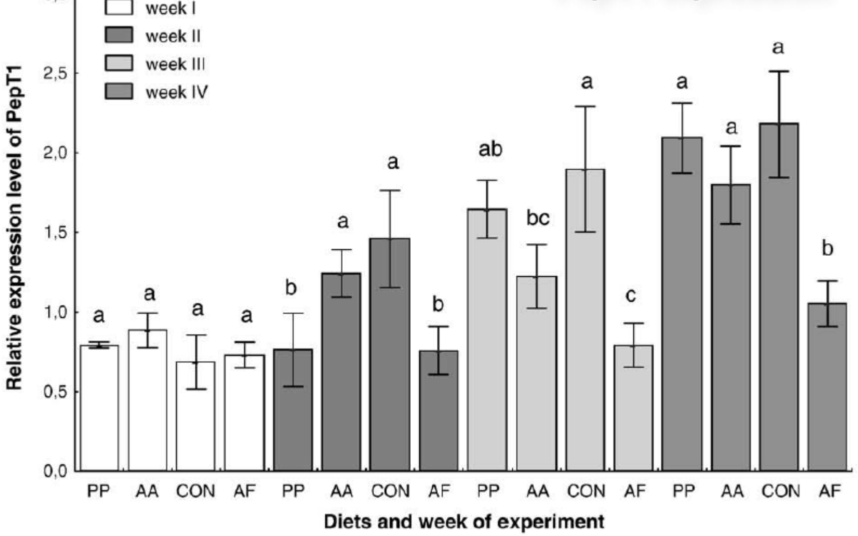
Diet					
Lys–Gly dipeptide based diet (PP)		Free lysine and glycine based diet (AA)		Control diet (CON)	
Ingredient	% in diet	Ingredient	% in diet	Ingredient	% in diet
Fish meal	12.4	Fish meal	12.4	Fish meal	12.4
Wheat gluten*	37	Wheat gluten*	37	Wheat gluten*	37
Wheat meal	17.18	Wheat meal	17.18	Wheat meal	17.18
Fish oil	5	Fish oil	5	Fish oil	5
Lecithin	15	Lecithin	15	Lecithin	15
Mineral mix	3	Mineral mix	3	Mineral mix	3
Vitamin mix	4	Vitamin mix	4	Vitamin mix	4
Lysine–glycine[1]	3.4	Lysine[2]	2.1	Lysine2	0
Cysteine**	0.2	Glycine[2]	1.3	Glycine[2]	1.3
Arginine	0.65	Cysteine	0.2	Glutamate***	2.1
Methionine	0.33	Arginine	0.65	Cysteine	0.2
Threonine	0.29	Methionine	0.33	Arginine	0.65
Ca-mono P	1.5	Threonine	0.29	Methionine	0.33
Vitamin C	0.05	Ca-mono P	1.5	Threonine	0.29
		Vitamin C	0.05	Ca-mono P	1.5
				Vitamin C	0.05

*MP Biomedicals-Solon-OH, **Hara (2006); most potent olfactory stimulating amino acid.
***Hughes (1985); non-toxic level of glutamic acid 1MP Biomedicals and 2Bachem, NY.

growth rate



PepT1 expression



PP, wheat gluten protein-based diet plus Lys-Gly
AA, wheat gluten protein-based diet plus Lys and Gly
CON, wheat gluten protein-based control diet with no Lys
AF, commercial starter (Aller Futura)

Adapted from:
Ostaszewska et al., Comp Biochem Physiol A Mol Integr Physiol. 155:107, 2010

Pept1 and peptide(Lys-Gly)-containing diets (common carp)

PP; wheat gluten protein-based diet plus Lys-Gly
AA, wheat gluten protein-based diet plus Lys and Gly
CON, wheat gluten protein-based control diet with no Lys
Z, frozen zooplankton
AN, commercial starter food Aglo Norse

Table 1
Composition of feeds used in this experimental study.

Diet					
Lys–Gly dipeptide based diet (PP)		Free lysine and glycine based diet (AA)		Control diet (CON)	
Ingredient	% in diet	Ingredient	% in diet	Ingredient	% in diet
Fish meal	12.4	Fish meal	12.4	Fish meal	12.4
Wheat gluten*	37	Wheat gluten*	37	Wheat gluten*	37
Wheat meal	17.18	Wheat meal	17.18	Wheat meal	17.18
Fish oil	5	Fish oil	5	Fish oil	5
Lecithin	15	Lecithin	15	Lecithin	15
Mineral mix	3	Mineral mix	3	Mineral mix	3
Vitamin mix	4	Vitamin mix	4	Vitamin mix	4
Lysine–glycine[1]	3.4	Lysine[2]	2.1	Lysine2	0
Cysteine**	0.2	Glycine[2]	1.3	Glycine[2]	1.3
Arginine	0.65	Cysteine	0.2	Glutamate***	2.1
Methionine	0.33	Arginine	0.65	Cysteine	0.2
Threonine	0.29	Methionine	0.33	Arginine	0.65
Ca-mono P	1.5	Threonine	0.29	Methionine	0.33
Vitamin C	0.05	Ca-mono P	1.5	Threonine	0.29
		Vitamin C	0.05	Ca-mono P	1.5
				Vitamin C	0.05

*MP Biomedicals-Solon-OH, **Hara (2006); most potent olfactory stimulating amino acid.
***Hughes (1985); non-toxic level of glutamic acid 1MP Biomedicals and 2Bachem, NY.

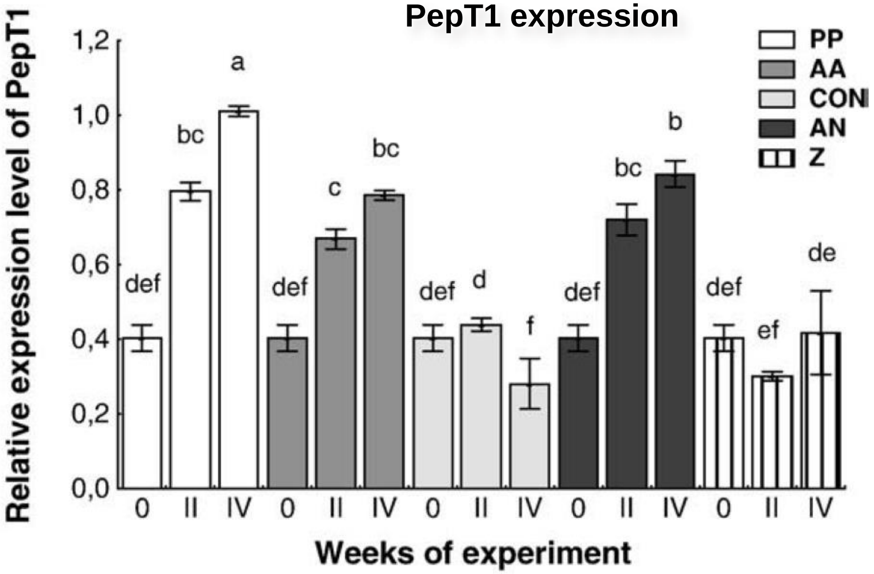
growth rate

Table 3
Survival, final body mass, specific growth rate (SGR), protein efficiency rate (PER), and food conversion rate (FCR) in carp fed experimental diets for 28 days (mean ± s.d., n = 4 tanks).

	PP	AA	CON	AN	Z
Survival (%)	85.3 ± 1.15^b	83.3 ± 5.77^b	85.0 ± 5.77^b	99.5 ± 1.0^a	99.5 ± 1.0^a
Final body mass (g)	0.23 ± 0.06^b	0.22 ± 0.06^b	0.19 ± 0.06^b	0.61 ± 0.28^a	0.12 ± 0.04^c
SGR (%/day)	4.32 ± 0.22^b	4.23 ± 0.19^b	3.73 ± 0.22^c	7.83 ± 0.29^a	2.10 ± 0.16^d
PER (g/g)	1.41 ± 0.15^c	1.24 ± 0.08^{cd}	1.2 ± 0.13^d	2.99 ± 0.15^b	5.37 ± 0.23^a
FCR (g)	1.71 ± 0.16^c	1.94 ± 0.15^b	2.02 ± 0.15^b	0.63 ± 0.09^d	4.41 ± 0.22^a

The statistical analysis was performed using one-way ANOVA followed by Student's t-test. Means with different letters in the same row are significantly different ($p \leq 0.05$).

PepT1 expression



Adapted from:
Ostaszewska et al., Comp Biochem Physiol A Mol Integr Physiol. 157:158, 2010

Pept1 and peptide(Lys-Gly)-containing diets (yellow perch)

Table 1
Composition of feeds used in this experimental study.

Diet					
Lys–Gly dipeptide based diet (PP)		Free lysine and glycine based diet (AA)		Control diet (CON)	
Ingredient	% in diet	Ingredient	% in diet	Ingredient	% in diet
Fish meal	12.4	Fish meal	12.4	Fish meal	12.4
Wheat gluten*	37	Wheat gluten*	37	Wheat gluten*	37
Wheat meal	17.18	Wheat meal	17.18	Wheat meal	17.18
Fish oil	5	Fish oil	5	Fish oil	5
Lecithin	15	Lecithin	15	Lecithin	15
Mineral mix	3	Mineral mix	3	Mineral mix	3
Vitamin mix	4	Vitamin mix	4	Vitamin mix	4
Lysine–glycine[1]	3.4	Lysine[2]	2.1	Lysine2	0
Cysteine**	0.2	Glycine[2]	1.3	Glycine[2]	1.3
Arginine	0.65	Cysteine	0.2	Glutamate***	2.1
Methionine	0.33	Arginine	0.65	Cysteine	0.2
Threonine	0.29	Methionine	0.33	Arginine	0.65
Ca-mono P	1.5	Threonine	0.29	Methionine	0.33
Vitamin C	0.05	Ca-mono P	1.5	Threonine	0.29
		Vitamin C	0.05	Ca-mono P	1.5
				Vitamin C	0.05

*MP Biomedicals-Solon-OH, **Hara (2006); most potent olfactory stimulating amino acid.
***Hughes (1985); non-toxic level of glutamic acid 1MP Biomedicals and 2Bachem, NY.

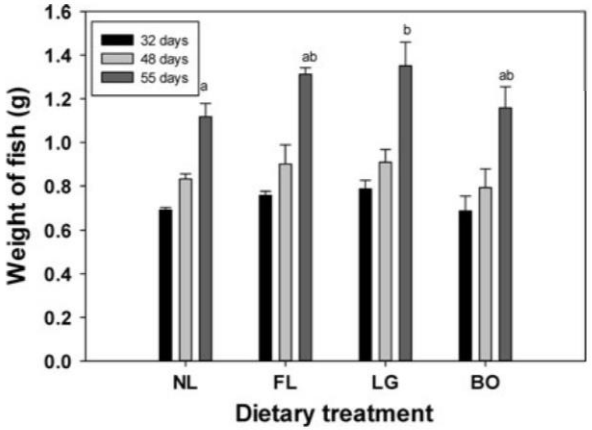


Fig. 2 The growth of juvenile yellow perch after 32, 48, and 55 days of the experiment. *BO* commercial diet, *NL* negative control (no Lys), *FL* diet supplemented with free Lys, *LG* diet supplemented with Lys–Gly dipeptide. *Different letters* indicate statistical difference at $P < 0.05$

growth rate

PepT1 expression

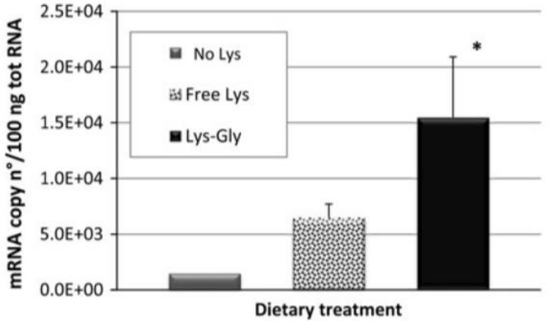


Fig. 7 Expression levels of *PepT1* measured by Real-Time PCR in yellow perch whole digestive tract in the course of the experiment. The *PepT1* mRNA copy number was normalized as a ratio to 100 ng total RNA. The means of six animals in each group are shown (only two in the case of No Lys group). Bars indicate standard error of the mean. The symbol (*asterisk*) indicates statistical difference at $P < 0.05$

LG, wheat gluten protein-based diets plus Lys-Gly
FL, wheat gluten protein-based diets plus Lys and Gly
C, control diet with no Lys
BO; commercial starter Bio Oregon

Adapted from:
Ostaszewska et al., Aquaculture Nutr. 19:100, 2013

Conclusions

- Pept1a and Pept1b operate in fish intestine
- Pept1a and Pept1b are both expressed in fish post-gastric canal
- A functional relationship between Pept1 transporters and dipeptide(Lys-GLy)-containing diets can be assessed under certain conditions

3. Pept1 and compensatory (‘catch-up’) growth

Pept1 and compensatory ('catch-up') growth and the 'sea bass' model)

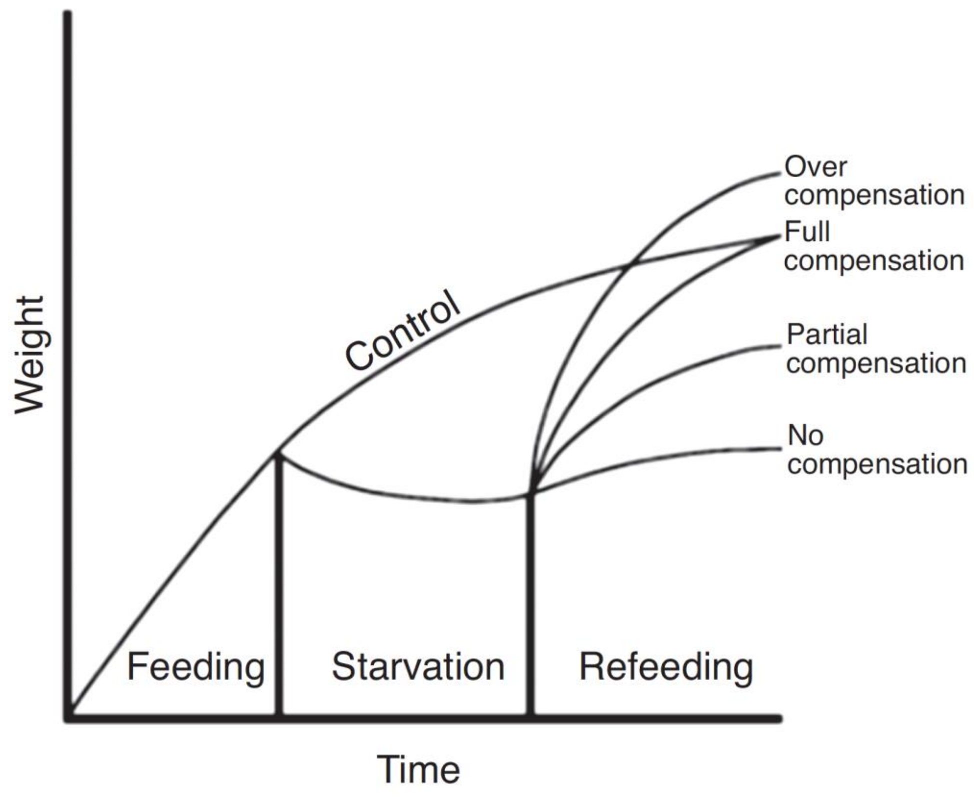
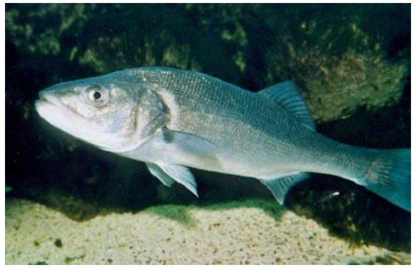


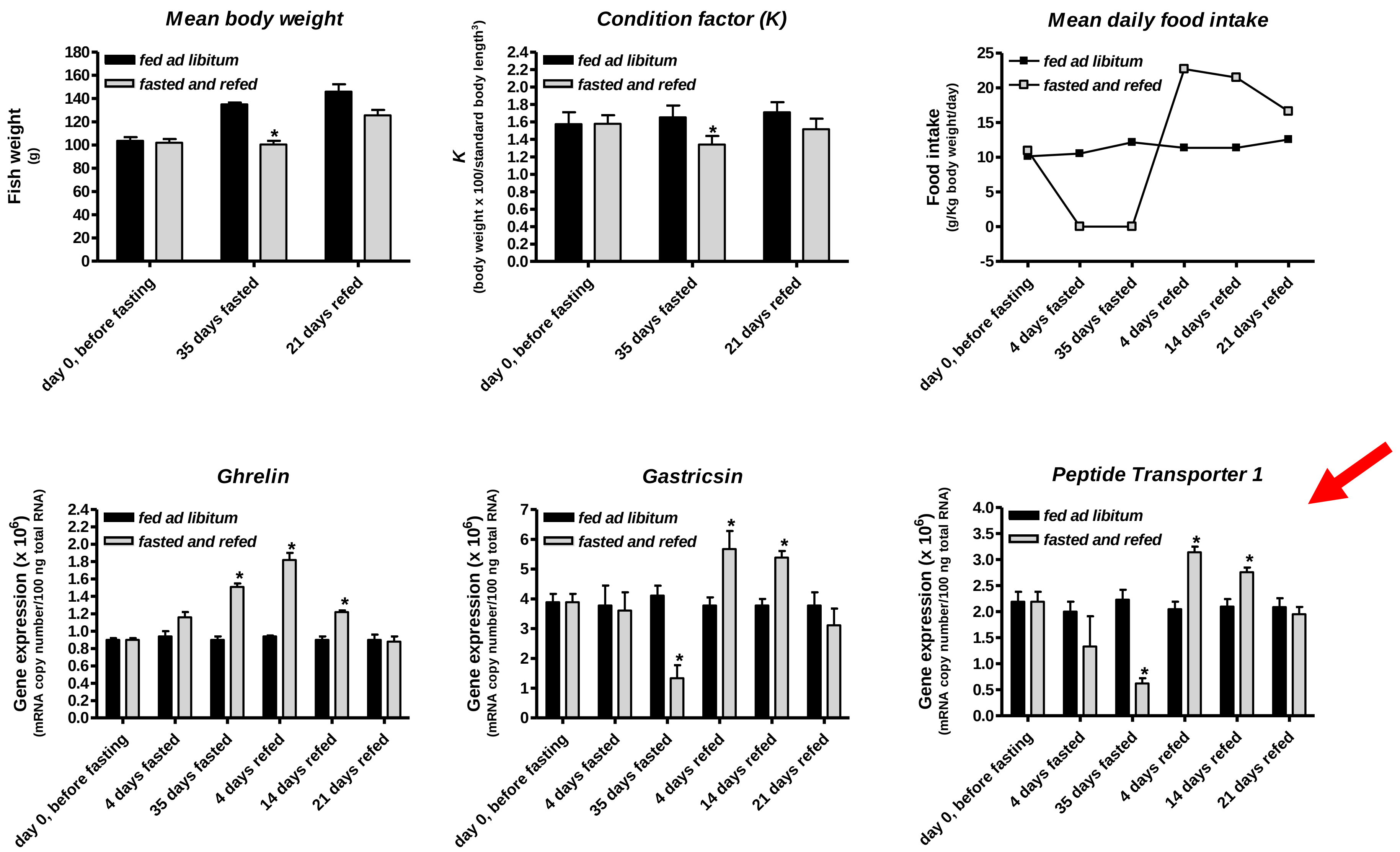
Figure 5.1 Idealized patterns of growth compensation. (From Ali et al., 2003.)

European sea bass

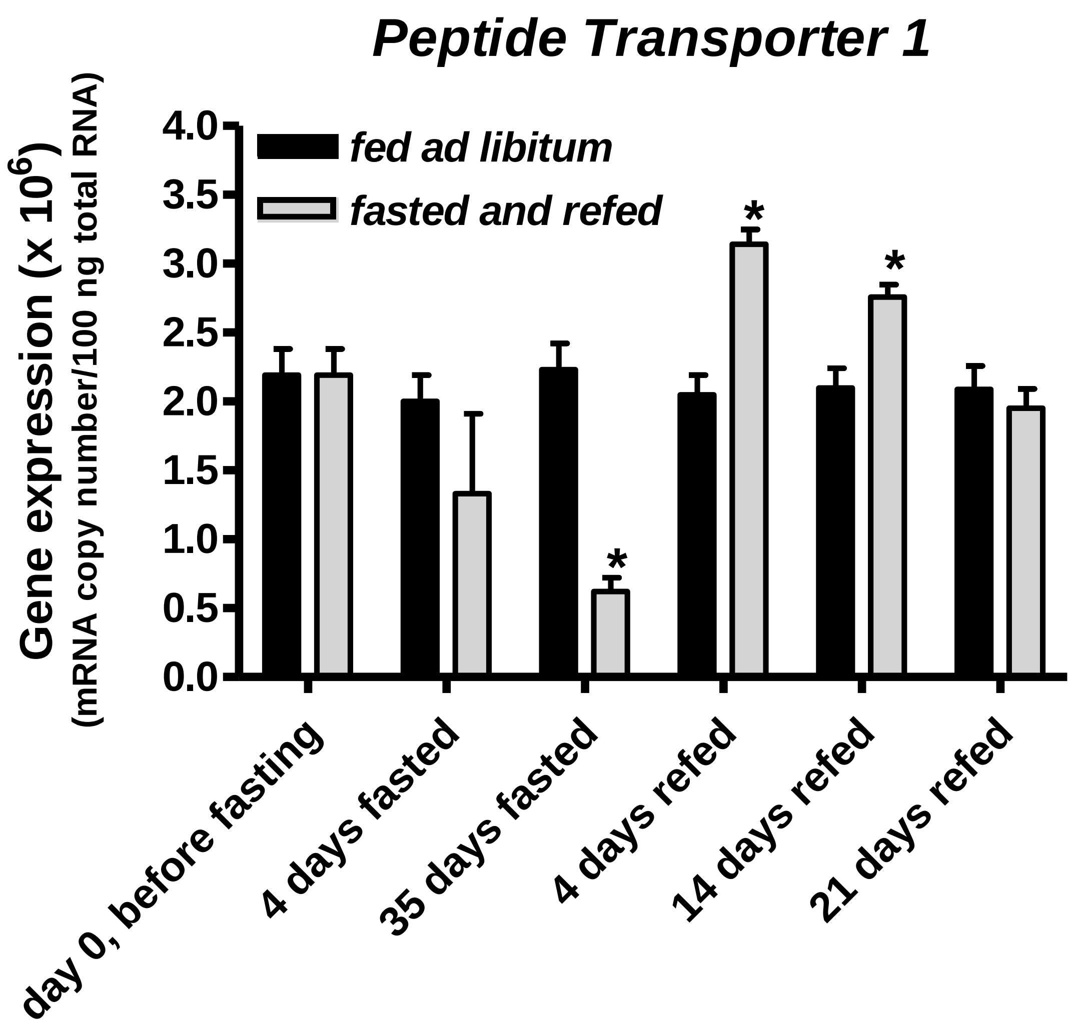


Terova et al., Aquaculture 294:288 (2009)
Verri et al., Biol. Lett. 7:597 (2011)

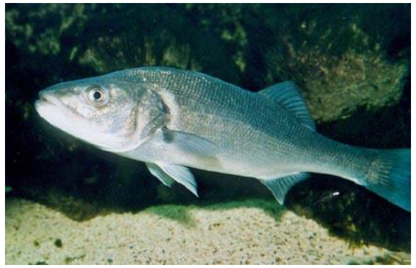
PEPT1 and compensatory ('catch-up') growth (the 'sea bass' model)



Pept1 and compensatory ('catch-up') growth (the 'sea bass' model)



European sea bass

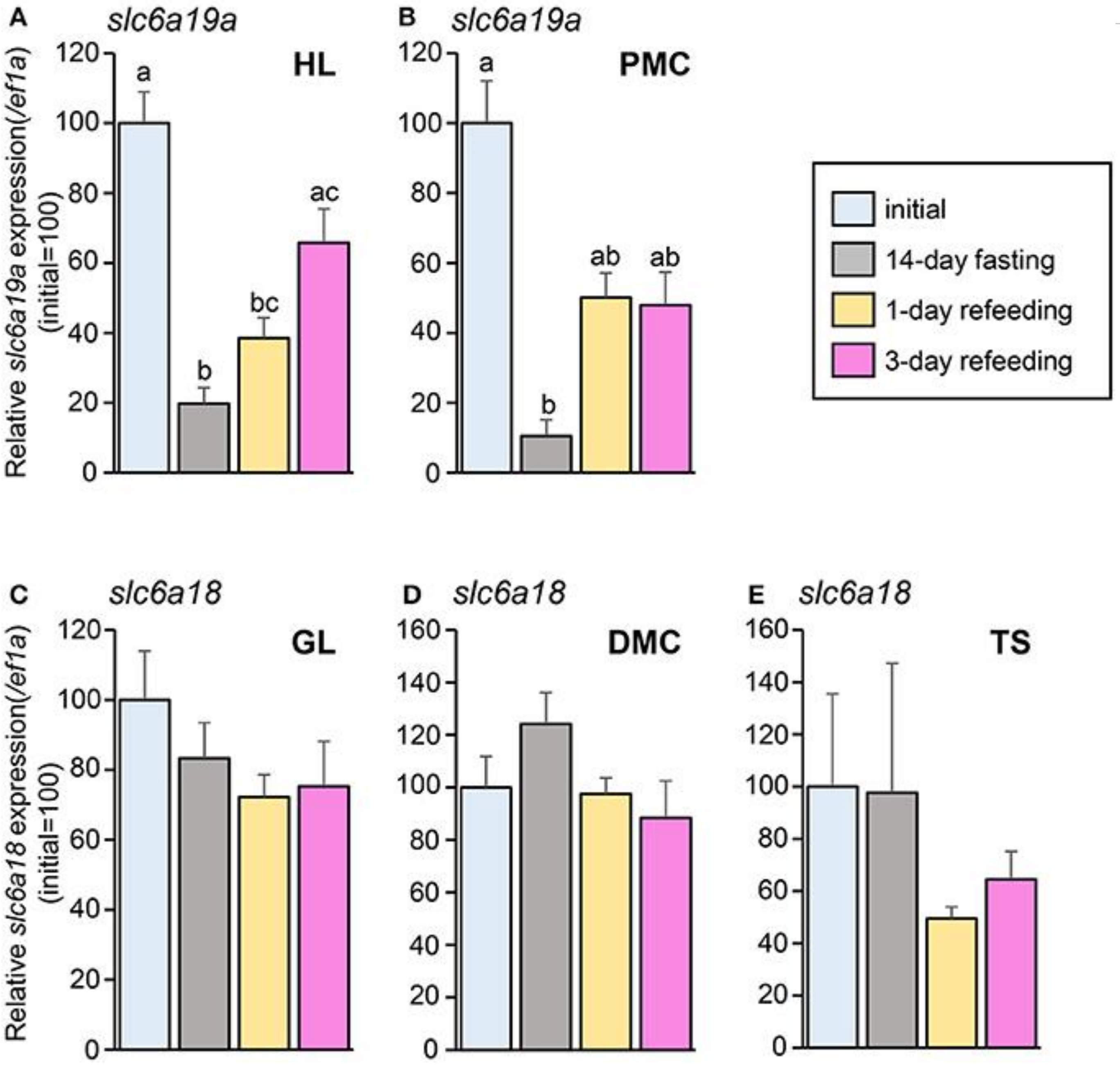


Terova et al., Aquaculture 294:288 (2009)
Verri et al., Biol. Lett. 7:597 (2011)

Pept1 and compensatory ('catch-up') growth (zebrafish)

zebrafish

Koven and Schulte, Fish. Physiol. Biochem. 38:1565 (2013)



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Spatial mRNA Expression and Response to Fasting and Refeeding of Neutral Amino Acid Transporters *slc6a18* and *slc6a19a* in the Intestinal Epithelium of *Mozambique tilapia*

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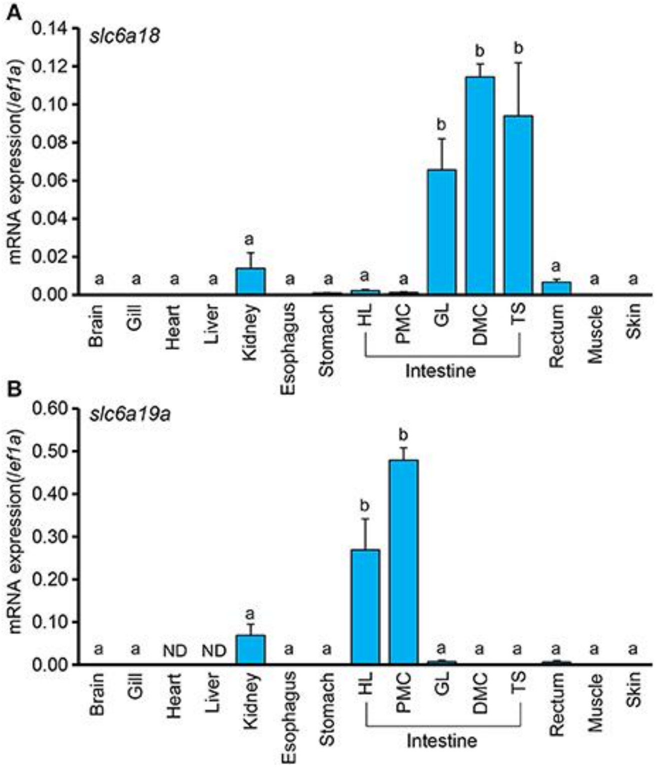

Not only Pept1 but also Slc6a19a responds to fasting and refeeding

Conclusions

- Pept1a and Pept1b operate in fish intestine

- Pept1a and Pept1b are expressed in fish post-gastric canal

- A functional relationship between Pept1's and compensatory ('catch-up') growth exists in fish (… focusing on Pept1 function in mammals during their growth, humans included)

4. Automatic feeding systems

TRITONE: Automatic feeding system

Automated diet delivery system for zebrafish labs

It reduces:

- Fish feeding costs
- High variability associated to ‘more than one person’ feeding

It improves:

- Hygiene at tank level
- Ergonomics during diet distribution
- System water quality

Dottorato di ricerca industriale (2017)

Distribuzione automatizzata di cibo funzionale per zebrafish: verso una migliore alimentazione dei modelli animali acquatici in laboratorio (ZFeedPlus)

- Sviluppo di un sistema per la **distribuzione automatizzata** di cibi funzionali, compatibile con un sistema di allevamento a ricircolo usato per mantenere zebrafish in condizioni di stabulazione.

- Per standardizzare le condizioni di mantenimento di zebrafish in laboratorio migliorando il benessere degli animali, nella prospettiva di rendere più **'certificabile'** il dato sperimentale, il progetto coniuga la necessità di sviluppare nuovi cibi nel rispetto delle esigenze alimentari di zebrafish e di rendere la somministrazione dell'alimento un fatto sempre più controllabile, riproducibile e indipendente dal fattore umano.

- Collaborazione tra **un centro universitario del Sud Italia**, che si occupa di fisiologia dei pesci, **un'azienda del Nord Italia**, leader indiscusso a livello mondiale nel settore della stabulazione, e **un centro universitario in Norvegia**, dove produrre pesce di interesse commerciale è settore industriale strategico nazionale, che è oggi uno dei più importanti centri mondiali di biologia e biotecnologie marine.

Dottorato di ricerca industriale (2018)

Distribuzione automatizzata di cibo funzionale per zebrafish: verso una migliore alimentazione dei modelli animali acquatici in laboratorio (ZFeedPlus)

- In 3 pacchetti di lavoro (WP), il progetto vuole:
- **1. Approfondire l'analisi molecolare e morfo-funzionale del sistema digerente di zebrafish (WP1)**
- **2. Mettere a punto diete speciali (WP2)**
- **3. Analizzare gli effetti delle diete su crescita e, in generale, benessere dei pesci allevati (WP3)**

Dottorato di ricerca industriale (2018)

Distribuzione automatizzata di cibo funzionale per zebrafish: verso una migliore alimentazione dei modelli animali acquatici in laboratorio (ZFeedPlus)

Metodologie (procedure *in silico*, sperimentali e/o di sviluppo tecnologico)

Per l’analisi dei geni espressi nel sistema digerente in zebrafish (WP1):

- Banche dati (*)
- Genomica e trascrittomica funzionale comparata (*)
- Bioinformatica (*)
- Isolamento di acidi nucleici e proteine (*)
- Real-time PCR semi-quantitativa e quantitativa (*)
- *In situ* hybridization
- Western blot (*)
- Whole-mount in situ hybridization (*)
- Analisi ed elaborazione dei dati (*)

- (*) Parte della ricerca sarà svolta presso l’Università di Bergen

Dottorato di ricerca industriale (2018)

Distribuzione automatizzata di cibo funzionale per zebrafish: verso una migliore alimentazione dei modelli animali acquatici in laboratorio (ZFeedPlus)

Metodologie (procedure *in silico*, sperimentali e/o di sviluppo tecnologico)

Per la messa a punto delle diete speciali (WP2):

- Banche dati
- Chemical libraries
- Screening di molecole mediante incubazione con embrioni/larve di zebrafish (vedi ad es. http://www.oecd.org/chemicalsafety/testing/36817242.pdf) (*)
- Preparazione di mangimi contenenti molecole selezionate (***)
- Analisi ed elaborazione dei dati

- (*) Parte della ricerca sarà svolta presso l'Università di Bergen
- (***) I mangimi saranno preparati da SPAROS LDA (vedi www.sparos.pt)

Dottorato di ricerca industriale (2018)

Distribuzione automatizzata di cibo funzionale per zebrafish: verso una migliore alimentazione dei modelli animali acquatici in laboratorio (ZFeedPlus)

Metodologie (procedure *in silico*, sperimentali e/o di sviluppo tecnologico)

Per l’analisi degli effetti delle diete speciali (WP3):

- Ricerca e sviluppo su Tritone (**)
- Implementazione di nuova tecnologia su Tritone (**)
- Standardizzazione di protocolli sperimentali (**)
- Analisi biometriche, fisiologiche e comportamentali (vedi ad es. https://www.ncbi.nlm.nih.gov/books/NBK5216/)
- Analisi ed elaborazione dei dati

- (**) La ricerca tecnologica sarà svolta presso Tecniplast S.p.A.