

#### INDICATEURS PHYSIOLOGIQUES DE CROISSANCE

chez l'omble de fontaine Salvelinus fontinalis

MARIA MARTINEZ-SILVA<sup>1</sup>, Aurélie Dupont-Prinet<sup>1</sup>, Louis Bernatchez<sup>1</sup>, Marie Vagner<sup>2</sup>, Céline Audet<sup>1</sup>

<sup>1</sup>UQAR / ISMER <sup>2</sup> LEMAR / CNRS



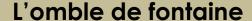




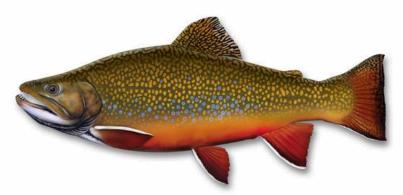
Pêches et Océans Canada











Salvelinus fontinalis

- Salmonidé natif du nord-est de l'Amérique
- Espèce aquacole d'eau douce économiquement importante au Québec
- 50% de la production aquacole totale au Québec
- Ensemencement pour soutenir la pêche sportive
- l'impact économique coûte plus d'un milliard de dollars



#### La croissance dans l'aquaculture

Les frères et sœurs ne sont pas des clones

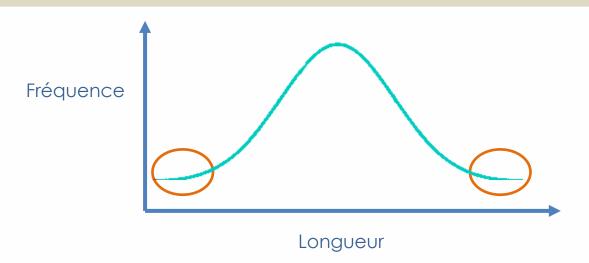


# Plasticité phénotypique intra-familiale





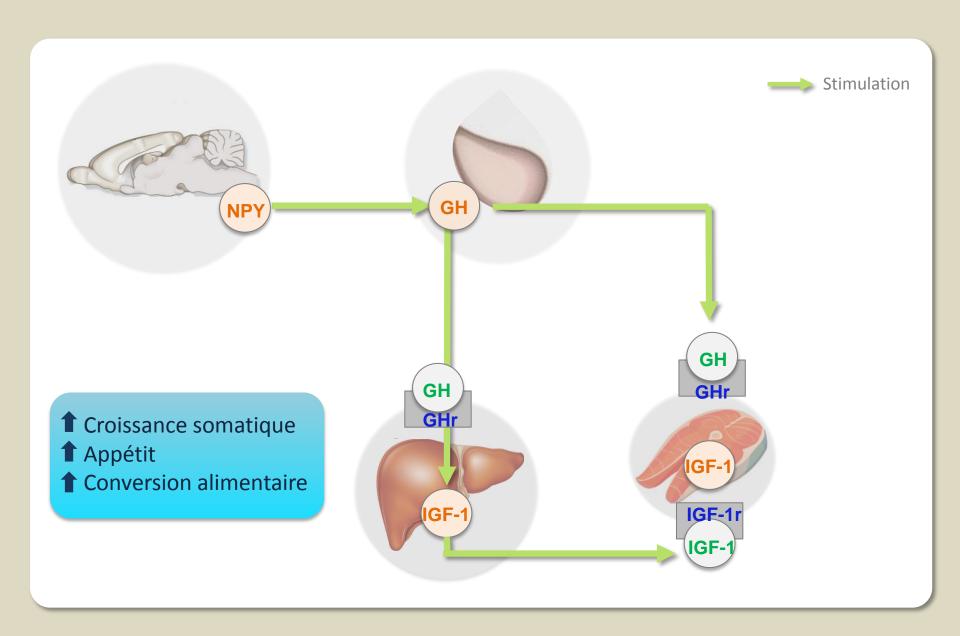
# La croissance dans l'aquaculture







# Cascade endocrine de régulation de la croissance



#### Chez d'autres espèces...







ENDOCRINOLOGY

www.elsevier.com/locate/vgcen

Review

Neuroendocrine control of growth hormone in fish

Luis Fabián Canosa 1, John P. Chang \*, Richard E. Peter 2

nent of Biological Sciences, University of Alberta, Edmonton, Alta., Canada T6G 2E9 Received 6 September 2006; revised 12 December 2006; accepted 22 December 2006 Available online 30 December 2006

Zoological Studies 36(2): 79-89 (1997)

#### Review Article

Neuroendocrine Regulation of Growth Hormone Secretion and Growth in Fish

Chun Peng1 and Richard E. Peter2,\*

<sup>1</sup>Department of Biology, York University, North York, Ontario M3J 1P3, Canada <sup>2</sup>Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada Tel: (403) 492-4757, Fax: (403) 492-7033, E-mail: REPeter@sci.ualberta.ca

Fish Physiology and Biochemistry 27: 227-242, 2002. © 2004 Kluwer Academic Publishers. Printed in the Netherland:

227

Growth hormone endocrinology of salmonids: regulatory mechanisms and

Björn Thrandur Björnsson, Viktoria Johansson, Susana Benedet, Ingibjörg Eir Einarsdottir, Jon Hildahl, Thorleifur Agustsson and Elisabeth Jönsson Fish Endocrinology Laboratory, Department of Zoology/Zoophysiology, Göteborg University, Box 463, S-40530

Göteborg, Sweden (E-mail: thrandur.bjornsson@zool.gu.se)

Key words: growth hormone, GH, insulin-like growth factor I, IGF-I, regulation, secretion, clearance, GH-receptor, GHR, GH-binding-proteins, GHBP, Oncorhynchus, Salmo, review

#### Salmonidés





journal homepage: www.elsevier.com/locate/peptides

#### Effects of neuropeptide Y on food intake and brain biogenic amines in the rainbow trout (Oncorhynchus mykiss)

M. Aldegunde\*, M. Mancebo

Laboratorio de Fisioloxía Animal, Dpto. de Fisioloxía, Facultade de Bioloxía, Universidade de Santiago de Compostela, E-15782 Santiago de Compostela, Spain

British Journal of Nutrition (2005), 94, 353-361 C The Authors 2005

DOI: 10.1079/BJN20051521

#### Regulation of the somatotropic axis by dietary factors in rainbow trout (Oncorhynchus mykiss)

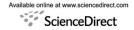
Pedro Gómez-Requeni<sup>1</sup>, Josep Calduch-Giner<sup>1</sup>, Silvia Vega-Rubín de Celis<sup>1</sup>, Françoise Médale<sup>2</sup>, Sadasivam J. Kaushik<sup>2</sup> and Jaume Pérez-Sánchez<sup>2</sup>\*

<sup>1</sup>Instituto de Acuicultura de Torre de la Sal (CSIC), 12 595 Ribera de Cabanes, Castellón, Spain

<sup>2</sup>Laboratoire de Nutrition des Poissons, Unité Mixte INRA-IFREMER, 64 310 Saint-Pée-sur-Nivelle, France

(Received 3 May 2004 - Revised 7 October 2004 - Accented 11 October 2004)





Comparative Biochemistry and Physiology, Part A 145 (2006) 214-224



Effects of aquaculture related stressors and nutritional restriction on circulating growth factors (GH, IGF-I and IGF-II) in Atlantic salmon and rainbow trout

Ryan J. Wilkinson a,\*, Mark Porter b, Hannah Woolcott b, Ryan Longland b, John F. Carragher a

<sup>a</sup> School of Biological Sciences, Flinders University of South Australia, G.P.O. Box 2100, Adelaide, S.A. 5001, Australia b School of Aquaculture, University of Tasmania, Launceston, Australia

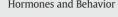
> Received 9 January 2006: received in revised form 14 June 2006: accented 15 June 2006 Available online 20 June 2006

> > Hormones and Behavior 84 (2016) 18-28



Contents lists available at ScienceDirect

Hormones and Behavior





journal homepage: www.elsevier.com/locate/yhbeh

Regulation of feeding behavior and food intake by appetite-regulating peptides in wild-type and growth hormone-transgenic coho salmon



Samantha L. White a, Helene Volkoff b, Robert H. Devlin a,\*

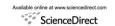
<sup>a</sup> Fisheries and Oceans Canada, 4160 Marine Drive, West Vancouver, BC V7V 1N6, Canada

Departments of Biology and Biochemistry, Memorial University of Newfoundland, St John's, NL A1B 3X9, Canada

#### Chez d'autres espèces...







General and Comparative Endocrinology 151 (2007) 1-26



Review

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Fish Endocrinology Laboratory, Department of Zoology/Zoophysiology, Göteborg University, Box 463, S-40530 Göteborg, Sweden (E-mail: thrandur.bjornsson@zool.gu.se)

Key words: growth hormone, GH, insulin-like growth factor I, IGF-I, regulation, secretion, clearance, GH-receptor, GHR, GH-binding-proteins, GHBP, Oncorhynchus, Salmo, review



journa

#### Effects of neuropep amines in the rain

M. Aldegunde\*, M. Mancebo

Laboratorio de Fisioloxía Animal, Dpto. de E-15782 Santiago de Compostela, Spain

Il y a très peu d'information sur l'omble de fontaine ou sur le processus de sélection, couramment utilisé en aquaculture

ncedirect.com

#### )irect

rt A 145 (2006) 214-224



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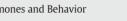
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Regulation of feeding behavior and food intake by appetite-regulating peptides in wild-type and growth hormone-transgenic coho salmon



Samantha L. White a, Helene Volkoff b, Robert H. Devlin a,\*

<sup>a</sup> Fisheries and Oceans Canada, 4160 Marine Drive, West Vancouver, BC V7V 1N6, Canada

Departments of Biology and Biochemistry, Memorial University of Newfoundland, St John's, NL A1B 3X9, Canada



#### L'expérience d'élevage chez l'omble de fontaine

#### Domestication d'une souche sauvage → 5e génération

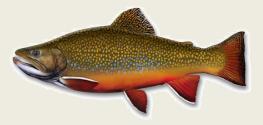
#### Première étape



Familles croisées sans prendre en compte la taille ou la performance des familles

**Familles CONTRÔLES** 

#### Deuxième étape



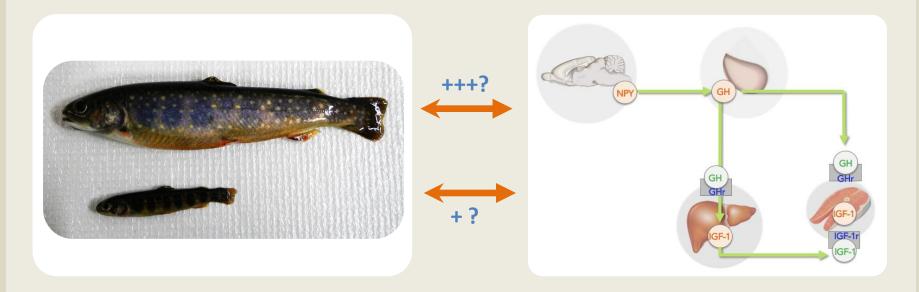
Familles issues d'un processus de sélection par poids et absence de maturité sexuelle

Familles SÉLECTIONNÉES



#### Objectif et hypothèse (première étape)

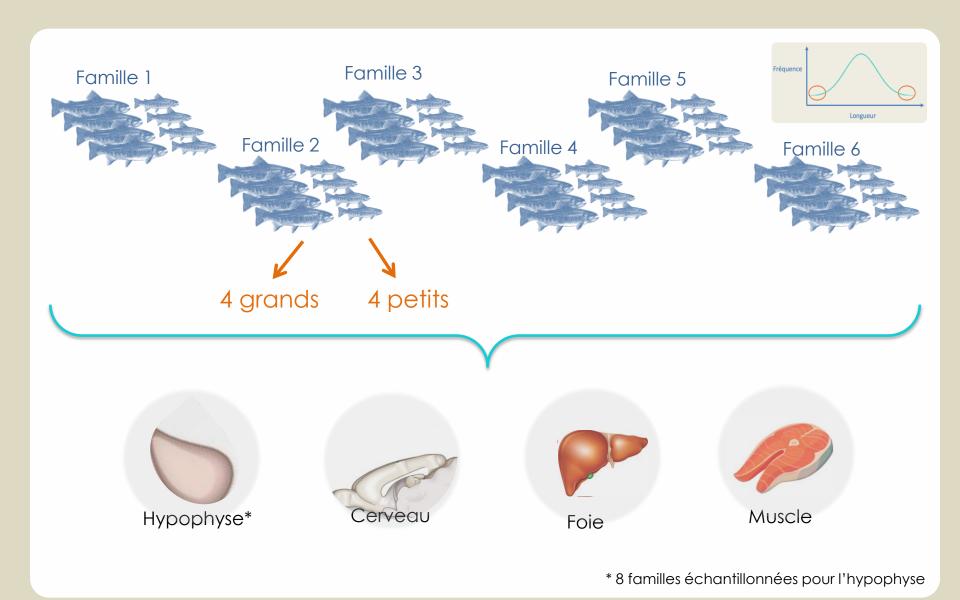
Confirmer la présence d'une **relation positive entre l'expression génique** relative des gènes NPY, GH, GHr, IGF-1 et IGF-1r **avec la croissance** chez l'omble de fontaine



Hypothèse: Niveau d'expression des gènes grand > petit



#### Design expérimental (6 familles d'omble de fontaine control)





**Extraction ARN** 

**Transcription ADNc** 



30 mg tissu



RNeasy Plus Universal Mini Kit RNeasy Fibrous Tissue Kit



Quantitect reverse transcription kit



Retrotranscription Bio-Rad MyiQ iCycler



**Extraction ARN** 

Transcription ADNc

Design amorces



Gene	Amorce (5'-3')	bp	Tm
EF-1 α	F – GCCCCTCCAGGATGTCTACA	20	59
	R – ACGGCCCACGGGTACTG	17	59
18s	F – GATCCATTGGAGGGCAAGTCT	21	59
	R-GATACGCTATTGGAGCTGGAATTAC	25	58
β-actine	F – GGTCGTCCCAGGCATCAG	18	59
	R – CGTCTCCCACGTAGCTGTCTT	21	58
GH	F – CGTCGCTAAGACAGGCTCTTG	21	59
	R – CTGGAGGCCAACTGCACTCT	20	59
GHr	F – CCCACTGCCCCCTGTATCT 1	19	59
	R-CTTCAGAAGGAGGCTGTTTTGC	22	59
IGF-1	F – CAGGCATCCAGATTGTGCAA	20	59
	R-ACCATGTTCTGAGAATTCCTGTGTT	25	59
IGF-1r	F – TCCTCAGTGGGACCCTTCTG	20	59
	R-GGACCATGAAGCCCAGTAGGT	21	59
NPY	F – TGCTGAAGAGCTGGCCAAAT	20	60
	R-TCTGTCTCGTGATCAGATTGATGTAG	26	58

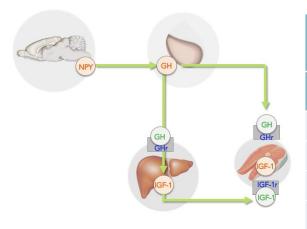


**Extraction RNA** 

Transcription ADNc

Design amorces

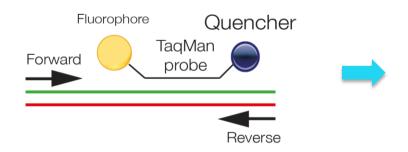
Expression génique



	Axe Principal de la croissance				
Tissu	GH	GHr	IGF-1	IGF-1r	NPY
Cerveau		Х			X
Hypophyse	X	X			
Foie		X	X	X	
Muscle		X		X	



Extraction RNA Transcription ADNc Design amorces Gènes cibles quantification (qPCR)



Quantification relative de l'expression génique Livak and Schmittgen (2001)

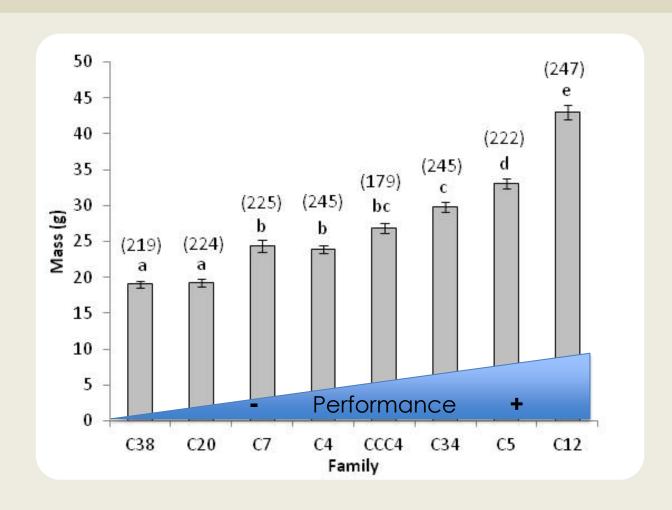
$$2^{-\Delta\Delta C_T} = 2^{-(\Delta C_{Te} - \Delta C_{Tc})}$$

Relative au Groupe Calibrateur

Normalisé avec des Gènes de référence

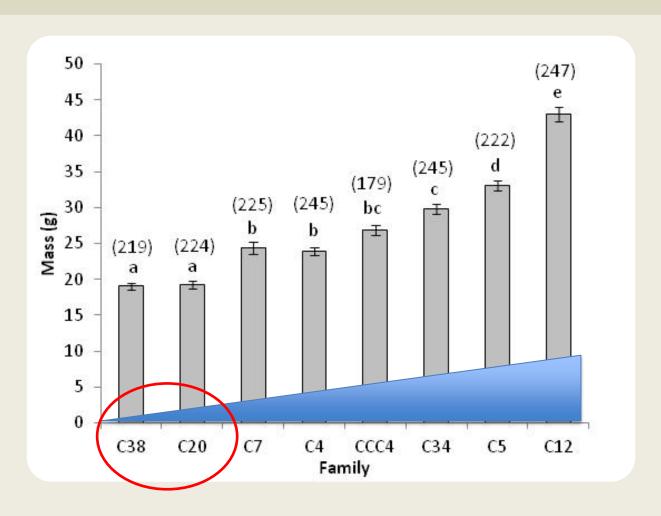


# Groupe calibrateur





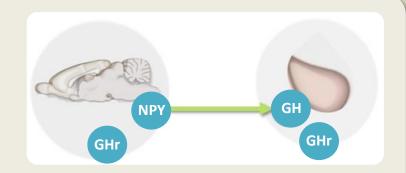
### Groupe calibrateur



Petits C38 et C20 => Groupe calibrateur



# Résultats – Cerveau et hypophyse



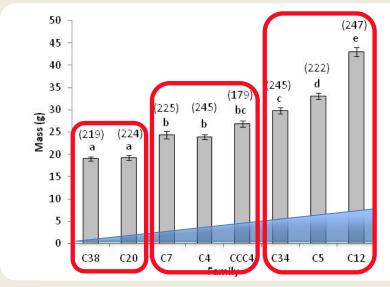
	Gène	Taille	Famille
Cerveau	NPY	F <sub>[1;32]</sub> =0.455; P=0.504	F <sub>[10;32]</sub> =1.395; P=0.226
	GHr	F <sub>[1;32]</sub> =0.070; P=0.792	F <sub>[10;32]</sub> =1.219; P=0.316
Hypophyse	GH	F <sub>[1;14]</sub> =0.017; P=0.897	
	GHr	F <sub>[1;14]</sub> =1.834; P=0.197	

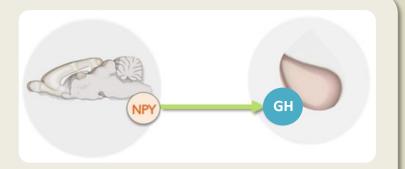
Pas d'effet Taille
Pas d'effet Famille \*

Effet performance?

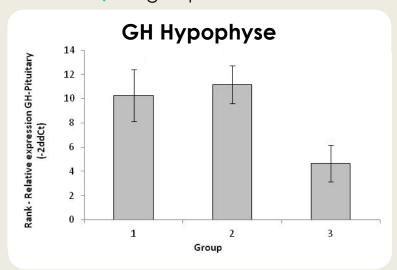


#### Résultats – Cerveau et hypophyse





3 groupes basés sur la masse

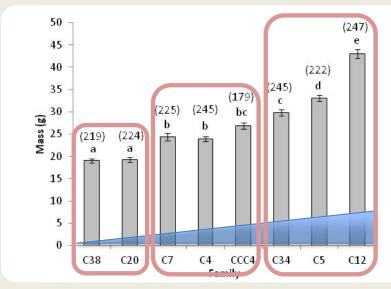


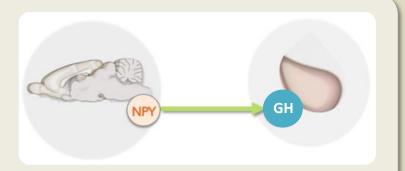
Gène	Groupe
GH	F <sub>[2;13]</sub> =4.72; <b>P=0.03</b>

**Effet groupe (performance)** 

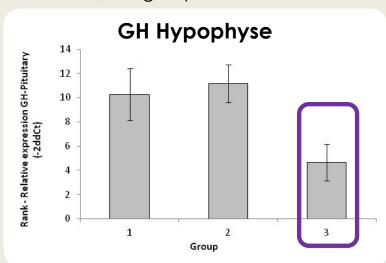


#### Résultats - Cerveau et hypophyse





3 groupes basés sur la masse



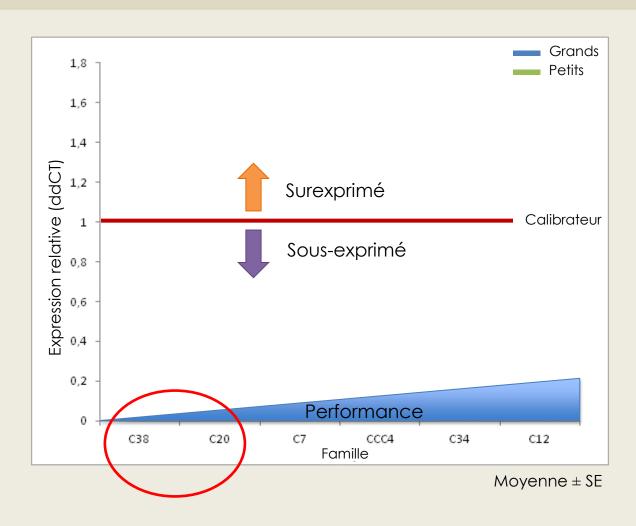


Gène	Groupe
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**Effet groupe (performance)** 

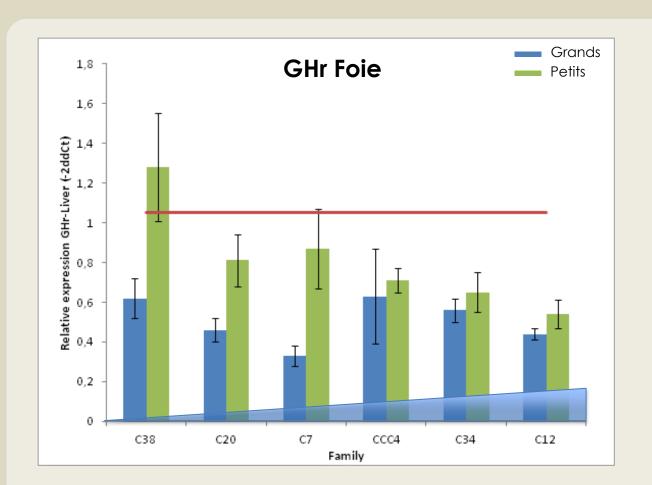


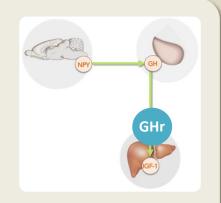
# Résultats - Expression génique



Petits C38 et C20 => Groupe calibrateur

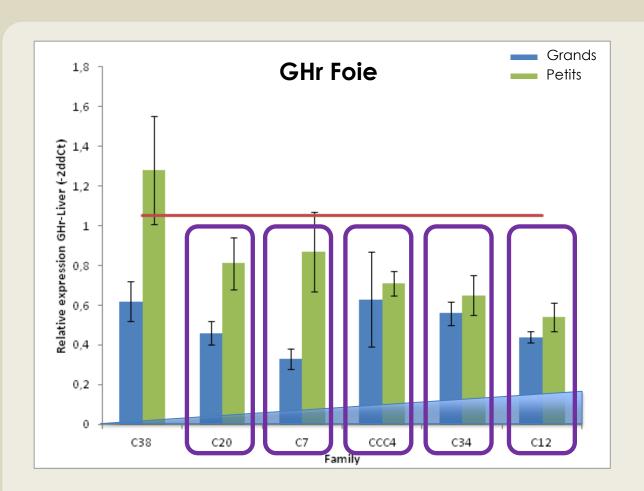


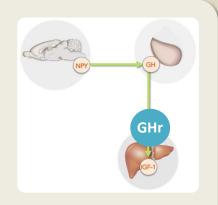




Gène	Taille	Famille
GHr	F <sub>[1;36]</sub> =20.457; <b>P&lt;0.01</b>	F <sub>[10;36]</sub> =2.171; <b>P=0.04</b>







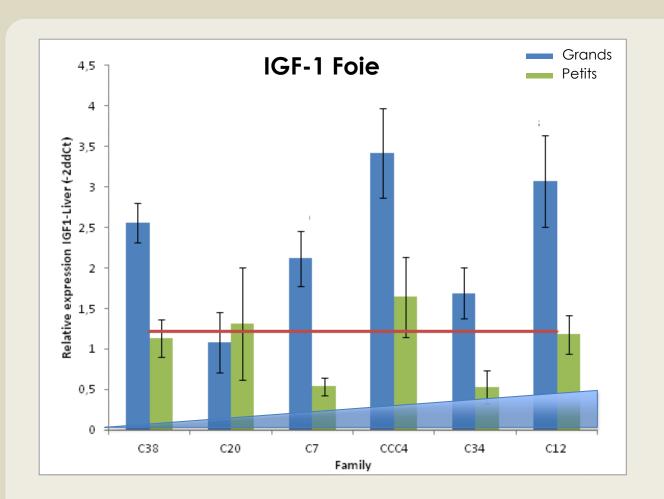
Grands et petits

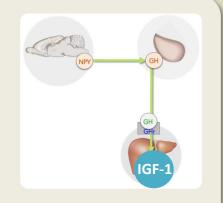
Gène	Taille	Famille

GHr F<sub>[1;36]</sub>=20.457; **P<0.01** 

F<sub>[10;36]</sub>=2.171;**P=0.04** 

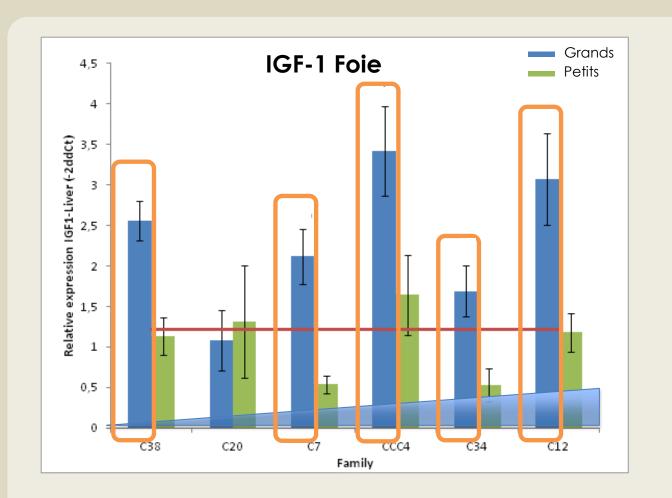


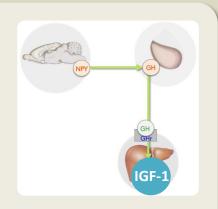




Gène	Taille	Famille
IGF-1	F <sub>[1;36]</sub> =31.052; <b>P&lt;0.01</b>	F <sub>[10;36]</sub> =2.999; <b>P&lt;0.01</b>



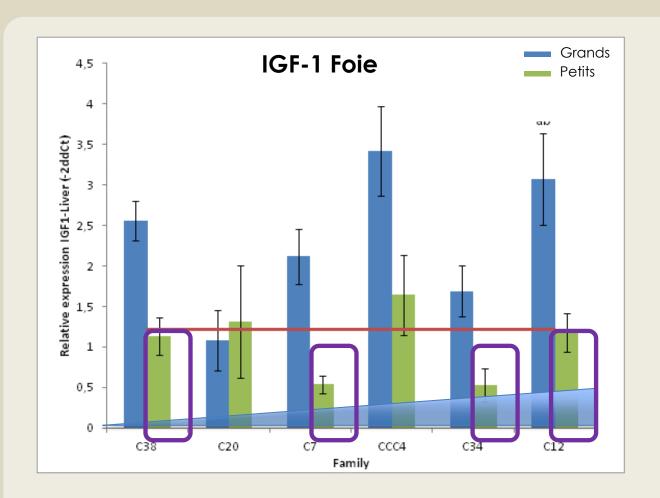


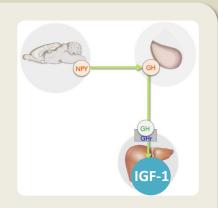




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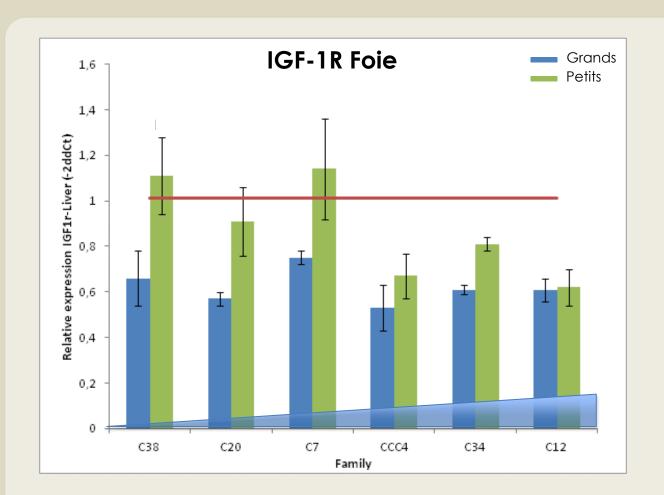


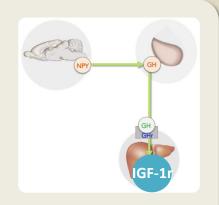




Gène	Taille	Famille
IGF-1	F <sub>[1;36]</sub> =31.052; <b>P&lt;0.01</b>	F <sub>[10;36]</sub> =2.999; <b>P&lt;0.01</b>

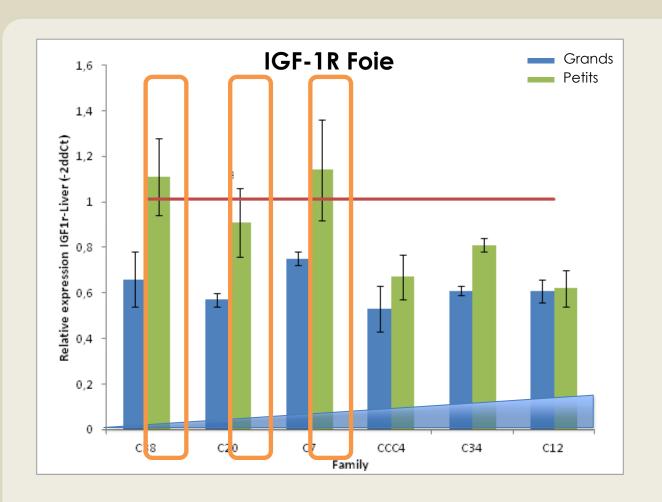


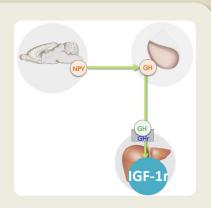




Gène	Taille	Famille
IGF-1r	F <sub>[1;36]</sub> =17.019; <b>P&lt;0.01</b>	F <sub>[10;36]</sub> =2.291; <b>P&lt;0.01</b>



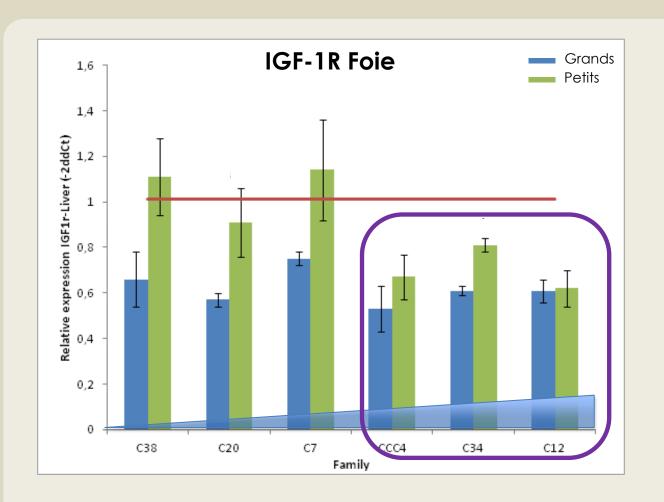


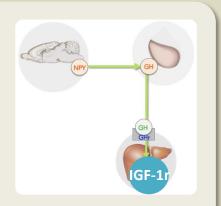


Petits des familles moins performantes

Gène	Taille	Famille
IGF-1r	F <sub>[1;36]</sub> =17.019; <b>P&lt;0.01</b>	F <sub>[10;36]</sub> =2.291; <b>P&lt;0.01</b>





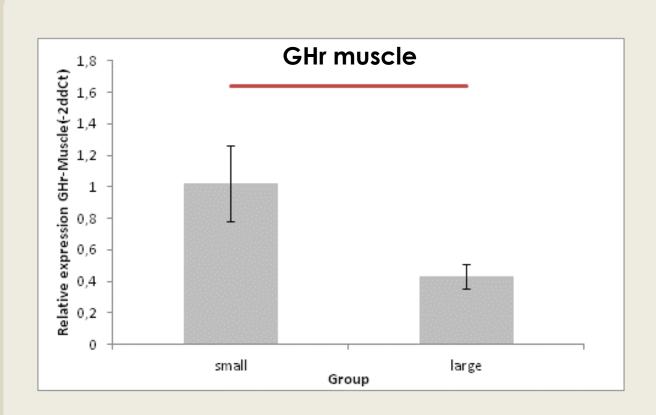


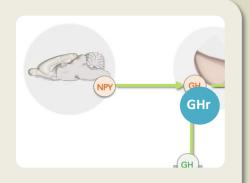
Familles plus performantes

Gène	Taille	Famille
IGF-1r	F <sub>[1;36]</sub> =17.019; <b>P&lt;0.01</b>	F <sub>[10;36]</sub> =2.291; <b>P&lt;0.01</b>







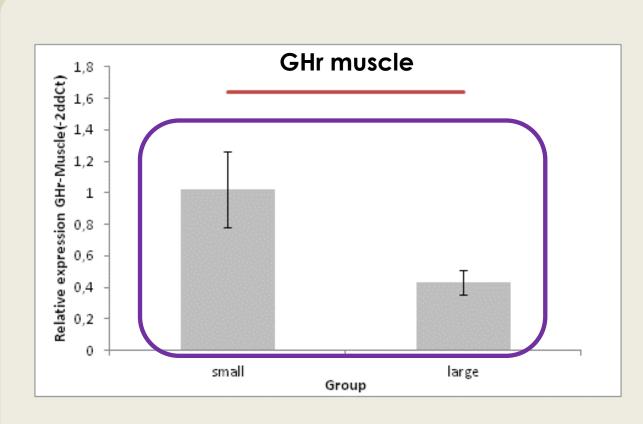


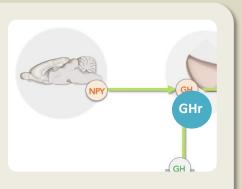
Gène	Taille	Famille
GHr	F <sub>[1;36]</sub> =7.639; <b>P=0.008</b>	F <sub>[10;36]</sub> =1.404;P=0.22

Effet taille







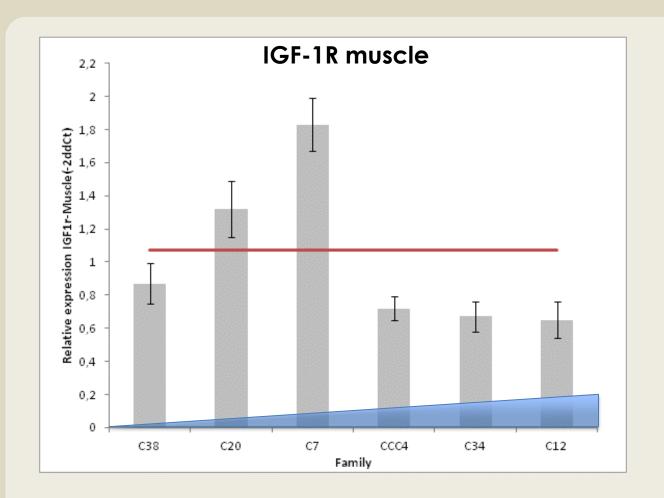


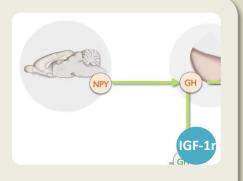
Petits et grands

Gène	Taille	Famille
GHr	F <sub>[1;36]</sub> =7.639; <b>P=0.008</b>	F <sub>[10;36]</sub> =1.404;P=0.22

Effet taille



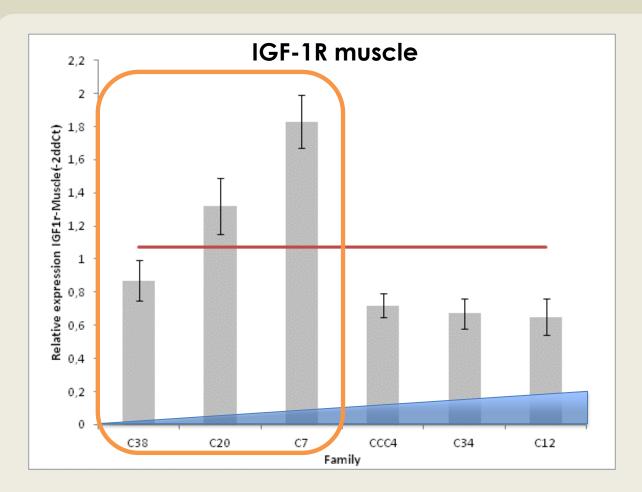


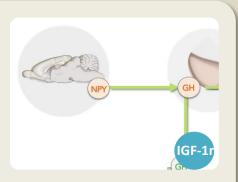


Gène	Taille	Famille
IGF-1r	F <sub>[1;36]</sub> =2.992; P=0.092	F <sub>[10;36]</sub> =5.183; <b>P&lt;0.01</b>

Effet famille





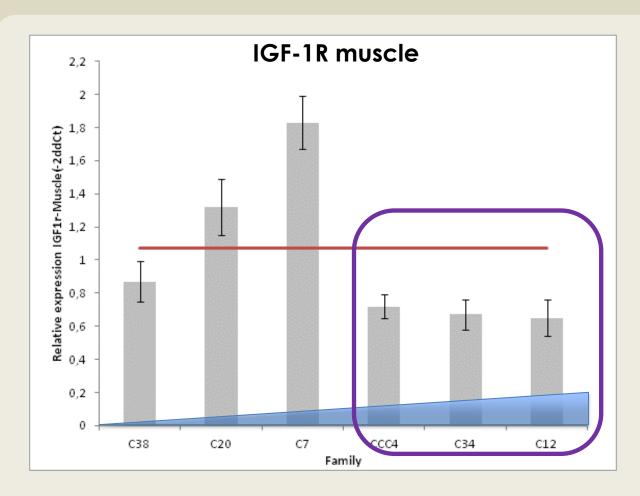


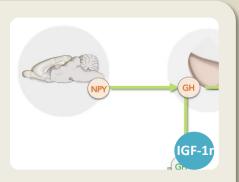
Familles moins performantes

Gène	Taille	Famille
IGF-1r	F <sub>[1;36]</sub> =2.992; P=0.092	F <sub>[10;36]</sub> =5.183; <b>P&lt;0.01</b>

Effet famille







Familles plus performantes

Gène	Taille	Famille

IGF-1r  $F_{[1;36]}$ =2.992; P=0.092

F<sub>[10;36]</sub>=5.183;**P<0.01** 

Effet famille



#### Conclusions – Première étape (familles contrôles)

**Hypothèse:** niveau d'expression des gènes grand > petit



**GH**: pas de différence au niveau des groupes ou des familles

<u>IGF1</u>: surexpression chez les poissons les plus performants au sein des familles, mais pas de différence entre les familles présentant des performances différentes

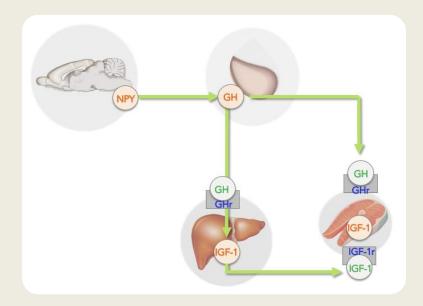
<u>Tous les récepteurs</u>: surexprimés chez les petits ainsi que dans les familles les moins performantes



#### Objectif et hypothèse (deuxième étape)

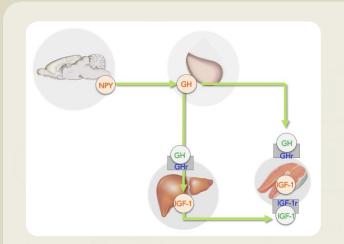
Est-ce que la sélection agit sur les mêmes gènes et de la même façon que dans les familles contrôles ?

Est-ce que la domestication reste, en fait, une forme de sélection ?







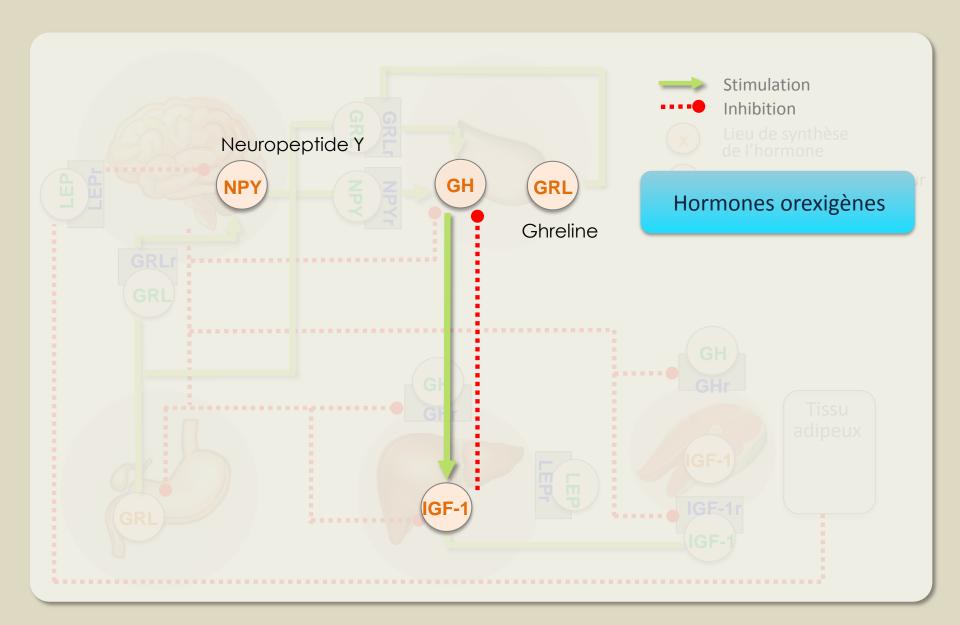






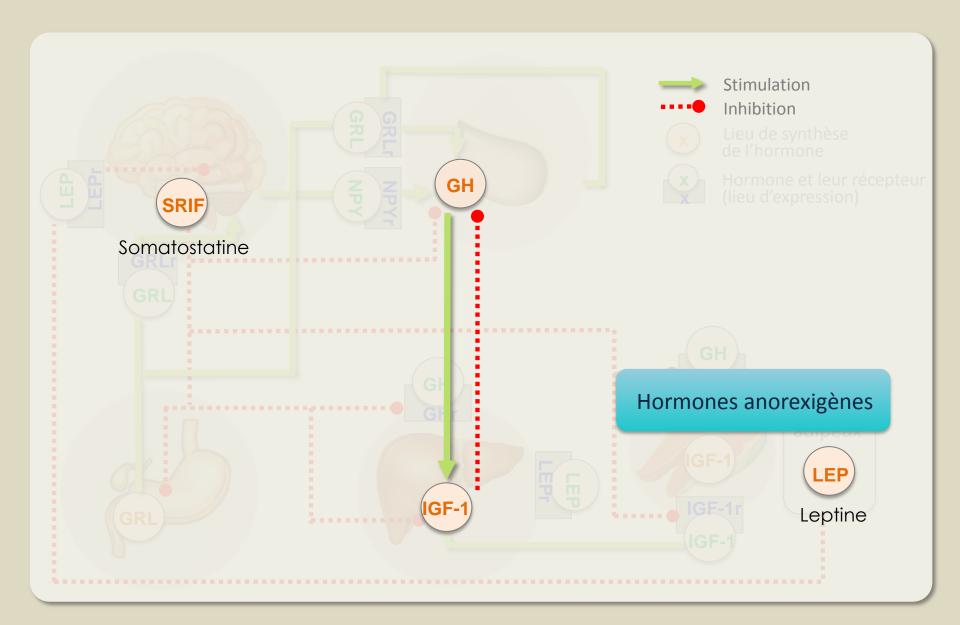


### Boucle de régulation de la croissance



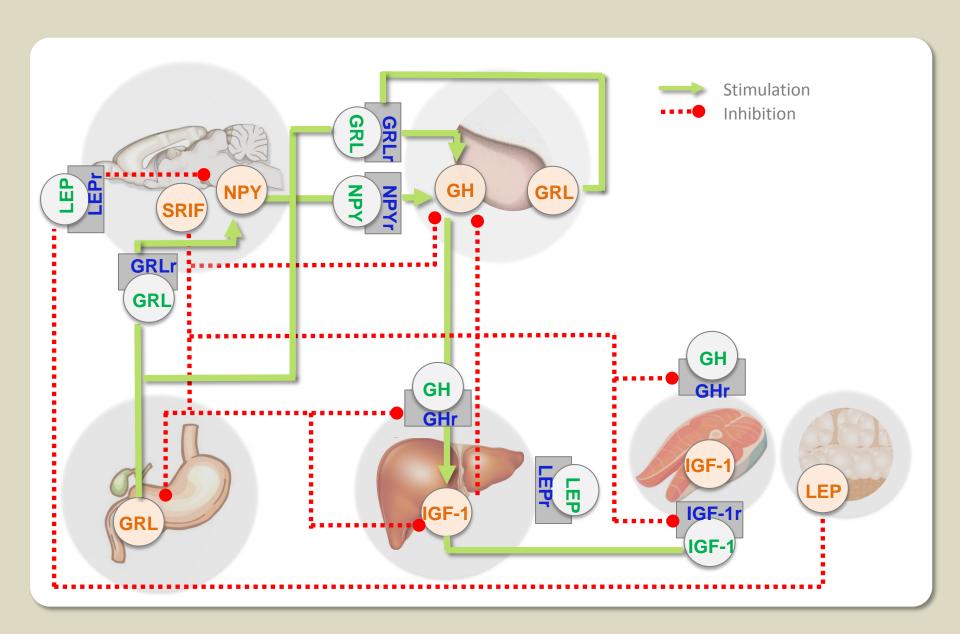


#### Boucle de régulation de la croissance



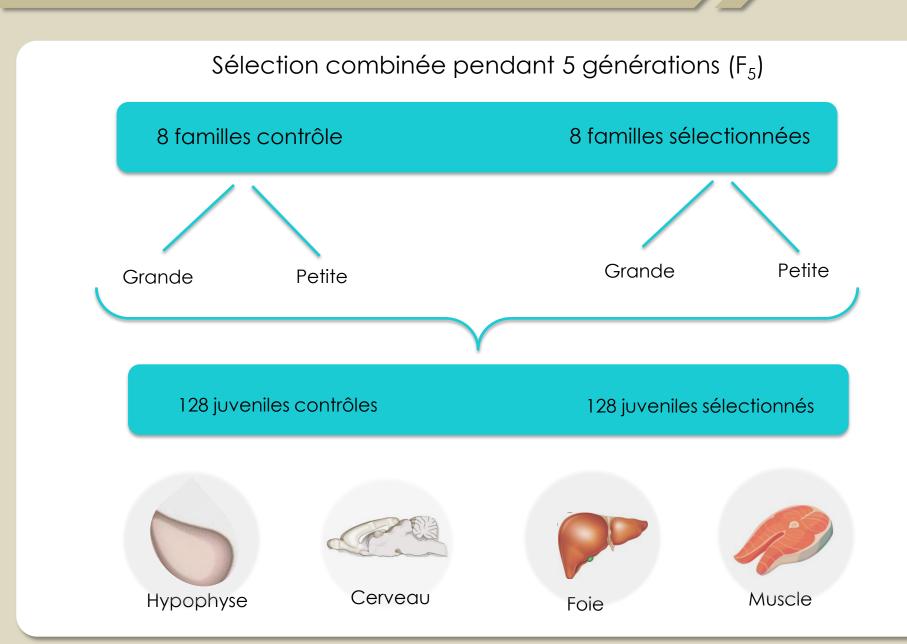


### Boucle de régulation de la croissance





#### La sélection l'omble de fontaine S. fontinalis





#### La sélection l'omble de fontaine S. fontinalis

**Extraction RNA** 

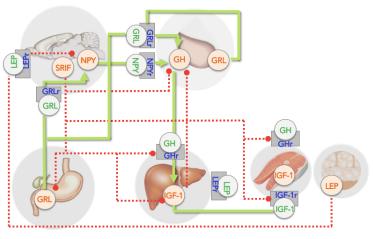
Transcription cDNA

Design amorces

Gènes cibles

Analyse et quantification (qPCR)

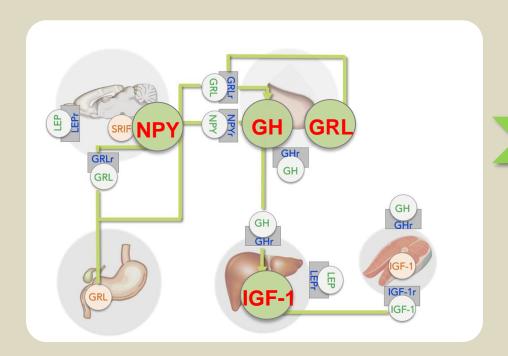
Données en cours d'acquisition



F-)	Axe Principal		Facteurs Orexigènes		Facteurs Anorexigènes		Protein muscular		
Tissue	GH	GHr	IGF-1	IGF-1r	NPY	GHRL	SRIFr	LEPr	MYO
Cerveau					Χ			Χ	
Hypophyse	Χ			Χ		X	X		
Foie		Χ	Χ						
Muscle		Χ	Χ	Χ					Χ



#### Boucle de régulation de la croissance dans la sélection?



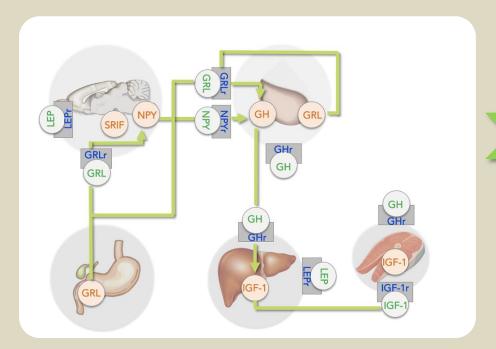
# Processus de sélection

favoriser le chemin de stimulation?

Est-ce qu'on s'attend à une surexpression de l'axe principal GH-IGF1 et des hormones orexigènes?



### Boucle de régulation de la croissance dans la sélection?



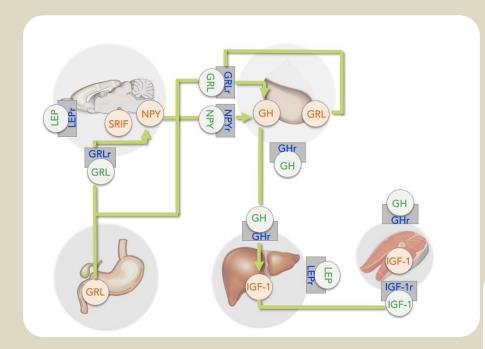


# Les plus gros vs les plus petits...

Est-ce que ce chemin est accentué? C'est un chemin différent?



# Boucle de régulation de la croissance dans la sélection?

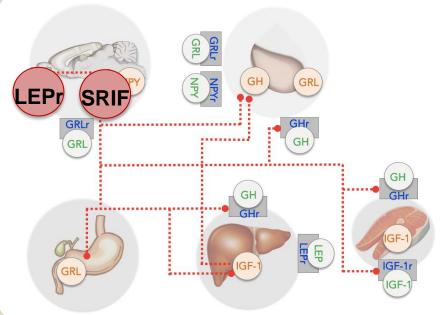


Surexpression d'inhibiteurs de l'axe GH-IGF1 ou d'hormones anorexigènes?



# Les plus gros vs les plus petits...

Est-ce que ce chemin est accentué? C'est un chemin différent?





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