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Research Article

Comparison of gene expression patterns of key growth genes between different rate growths in zebrafish (*Danio rerio*) siblings

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ABSTRACT. Variable individual growth rate is a phenomenon observed in fish cohorts that influences the aquaculture performance and fish cohort ecological viability. Our aim was to compare gene expression patterns of key growth genes in zebrafish larvae with different growth rate. The body length of sibling zebrafish larvae at 6 days post hatching (dph) was measured. The larvae were reared to 20 dph and measured again. Two body-length groups were clearly observed: 4 mm (small larvae) and 5-6 mm (large larvae). Total RNA was isolated from both groups. Growth hormone (*gh*), growth hormone receptor (*ghr*), insulin-like growth factor 1 (*igf-1*), insulin-like growth factor receptor (*igf-1r* a/b), insulin-like growth factor binding protein 1 (*igfbp-1*), thyroglobulin (*tg*), cholecystokinin (*c*ck), and ghrelin were evaluated by quantitative polymerase chain reaction (qPCR). Glucokinase (*gck*) and *igfbp-1* were included as a gene expression marker of larvae nutritional status. Two genes showed significant differences between the body length groups, *igfbp-1* (P = 0.01) and *igf-1r* (P = 0.02). The *igfbp-1* suggests than growth rate variability was associated with the larvae nutritional status and this condition affect the gene expression pattern of *igf-1r*. Therefore these genes are interesting genes markers for growth rate variabilities studies.

Keywords: *Danio rerio*, zebrafish, growth rate, IGF-1R, IGFBP-1, aquaculture.

INTRODUCTION

Fish larvae stage is a developmental phase after the embryogenesis, in which the metamorphosis process generates a major morphological and physiological changes intrinsically associated with each species of fish (Dufour *et al*., 2012). From a point of view of cohort growth, at hatching the larvae showed similar body length, however within a short period of time is possible observed size heterogeneity among individuals; due a natural phenomenon called growth rate variability (Deangelis & Coutant, 1979; Kestemont *et al*., 2003). Growth rate variability is a natural phenomenon, which influencing individual performance either from an ecological perspective (Pepin *et al*., 2015) or in a fish aquaculture productivity (Goldan *et al*., 1997; Lekang, 2013). In fish farming, size heterogeneity is not ideal, because the subordinate fish have less access to the feed, have more stress and increase the possibility of cannibalism (Lekang, 2013). Size heterogeneity in the cohorts are expressed mathematically as the coefficient of variation (CV) (Weiner & Solbrig, 1984).

The physiological process of growing is mainly regulated by the growth hormone (GH)/insulin-like growth factor 1 (IGF-1) axis (Reinecke *et al*., 2005). Which is a pleiotropic physiological axis of hormones and cellular receptors that regulate: nutrients metabolism, protein synthesis in muscle and in general tissue growth and osmotic balance (Butler & Le Roith, 2001; Reinecke *et al*., 2005). The endocrine activity of GH has two pathways: the direct, in which the physiological effects are mediated by GH binding to its receptor (GHR), and indirect pathway, in which GH induces IGF-1 secretion and promotes biological activities (Canosa *et al*., 2007). Among insulin growth factors: IGF-1, IGF-2 and IGF-3; IGF-1 is the main hormone in the regulation of larvae growth, because IGF-2 expression is only observed at embryogenesis (Wood *et al*., 2005) and IGF-3 is gonad specific (Wang *et al*., 2008). How a counterpart of hormones are the growth axis receptors: the GHR is a single transmembrane glycoprotein that belongs to the class I cytokine receptor superfamily (Pérez-Sánchez *et al*., 2002), and the IGF-1 receptor belongs to the tyrosine kinase superfamily of transmembrane receptor like the

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insulin (Wood *et al.*, 2005). On the other hand, insulin-like growth factor binding protein (IGFBP) is a protein family of six members can bind IGF-1 and IGF-2, their roles are to increase the half-life of IGFs and distribution, which also are been described in fish (Daza *et al*., 2011; Reindl & Sheridan, 2012), the most abundantly in plasma are IGFBP-1, IGFBP-2 and IGFBP-3 (Wood *et al.*, 2005).

Furthermore many peptides or hormones stimulating or inhibiting the GH/IGF-1 axis (Canosa *et al*., 2007; Chang & Wong, 2009), and affect the growth. The thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) have stimulatory effects on GH and IGF-1, and they are the principal factors controlling metamorphosis in fish larvae (Wang & Zhang, 2011; McMenamin & Parichy, 2013). Ghrelin is a peptide hormone mainly secreted in the oxyntic mucosa of the stomach and is the ligand for the growth hormone secretagogue receptor (GHS-R); which is the other endocrine pathway that stimulates the secretion of GH by the pituitary gland in addition to the GH-releasing hormone (Dimaraki & Jaffe, 2006). Additionally, ghrelin is an orexigenic factor that increases food intake and plays an important role in energy and glucose homeostasis (Peter & Chang, 1999; Nakazato *et al.*, 2001; Unniappan & Peter, 2005; Dimaraki & Jaffe, 2006; Arcamone *et al.*, 2009; Pradhan *et al.*, 2013). Cholecystokinin (CCK) is a peptide hormone secreted by the gastrointestinal tract, and its effects include gallbladder and pancreatic secretion, gastric and intestinal motor function, reduced food intake and stimulation of GH secretion (Canosa *et al.*, 2007; Crespo *et al*., 2014; Micale *et al*., 2014; Dalmolin *et al*., 2015).

Many reports have observed that fasting or poor nutritional status in fish alters the mRNA expression of components of the GH/IGF-1 axis, such as: starving *Oncorhynchus kisutch* and *Lates calcarifer* (Duan & Plisetskaya, 1993; Matthews *et al*., 1997) or in fasting *Anguilla japonica*, Dicentrarchus *labrax*, *Ictalurus punctatus* and *Oncorhynchus mykiss* (Duan & Hirano, 1992; Norbeck *et al*., 2007; Terova *et al*., 2007; Peterson *et al*., 2009).

Growth rate variability has been attributed to both biotic and abiotic mechanisms, which have been categorized as either "imposed" or "inherent" (Huston & DeAngelis, 1987; Kestemont *et al*., 2003). Imposed mechanisms include: temperature, day length, food availability, and interactive factors such as food competition. On the other hand, inherent mechanisms have strong genetic influence, so high cohort genetic variability increases the growth rate variability (Nicieza *et al*., 1994; Hutchings & Jones, 1998; Ohlberger *et al.*, 2013). Minimal information is available regarding gene expression patterns of growth factors due the growth rate variability process, as well is necessary define a main mechanism and tested in an isolated fashion. Food competition was the main mechanism proposed for this study, which may influence a poor nutritional status in some individuals; hence larvae growth rate may be associated with key growth gene expressions patters. Our aim was assess the key growth gene patterns observed at growth rate variability process. This study was carried out in environmentally controlled conditions (for the control inherent-non interactive mechanism) and to minimize genetic variability in the zebrafish larvae cohort, we used siblings (for the control imposed genetic mechanism).

MATERIALS AND METHODS

Experimental animals

From a spawning with one pair of adult zebrafish we obtained 100 viable eggs, which were incubated at 26ºC; only those larvae that hatched between 48 to 72 h after spawning were included. In the experimental design proposed, the use hatching siblings sought to reduce the cohort genetic variability. Larvae, were maintained in glass flasks with 2 L of E2 methylene blue media (Westerfield, 2000) under controlled light/dark conditions (14L/10D), with a 30% of water change every day. At 6 days post-hatching (dph) or 156 accumulated thermal units (ATU), the body lengths of co-hatched zebrafish larvae were measured. The larvae standard length measurement was conducted under stereoscopic microscope with a Motic® Images Plus 2.0ML software according to the proceeding proposed by Parichy *et al.* (2009); the larvae were previously anesthetized by benzocaine 20% (0.2 mL L^{-1}). Subsequently, total RNA was isolated from 30 zebrafish larvae (4 mm body length) to establish the initial state of gene expression (reference group); these larvae were grouped into five pools or biological replicates of six larvae each. The other co-hatching siblings larvae were reared for 14 days and were fed with rotifers (*Brachionus plicatilis*), at the rate of 200 rotifers per larva per day (Lawrence *et al.*, 2012). At 20 dph, the body length of each remaining larva was measured, and then 60 larvae were classified into two groups: large larvae and small larvae. The small larvae group was composed of 30 individuals with a body length of 4 mm, and they were divided into five pools or biological replicates with 6 larvae each. The large larvae group was also composed of 30 individuals divided into five pools or biological replicates of 6 larvae each. The large larvae groups were organized in its body length as follow: 4 larvae groups of 5 mm and one larvae group of 6 mm. This study was conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the INTA Universidad de Chile.

Total RNA isolation and reverse transcription

All zebrafish larvae pools (n = 15, small-large reference) were placed in 1.7 mL microcentrifuge tubes; excess liquid was removed, and euthanasia was performed by freezing at -80ºC in liquid nitrogen. Total RNA of all pools was isolated using 800 µL of Tripure® reagent (Roche) (Lan *et al.*, 2009), according to the manufacturer's instructions. RNA was quantified with a spectrophotometry at 260 and 280 nm (Nano-Drop®) and the RNA quality was assessed with 1% agarose gel electrophoresis. They were treated with RQ1 RNase-Free DNase (cat. M6101, Promega®) to avoid genomic DNA amplification, the absence of genomic DNA was confirmed by PCR on the treated RNA. The first-strand cDNA synthesis was performed using the ImProm-II™ Reverse Transcription System (Promega®). Total RNA was combined with 0.5 µg reaction^{-1} Oligo(dT)$_{15}$ Primer (cat. C1101, Promega®) for a final volume of 5 µL and incubated at 70ºC for 5 min. Next, 15 µL of the transcription mix (ImProm-II™ 5X Reaction Buffer 4.6 µL, 2.25 mM of $MgCl_2$, 0.5 mM each dNTP, Recombinant RNasin® Ribonuclease Inhibitor (Promega®) 20 µL and 1 µL ImProm-II™ Reverse Transcriptase (Promega®)) was added. Following the addition of transcription mix, the reaction was maintained at 25°C for 5 min and then transferred to 42°C for 60 min. The reverse transcription reactions were stopped by heating the mixture at 70°C for 15 min.

qPCR analysis

The gene-specific oligonucleotide primers for growth hormone (*gh*), growth hormone receptor (*ghra*), insulin-like growth factor 1a (*igf-1a*), insulin-like growth factor receptor a and b (*igf-1r* a and b), insulin-like growth factor binding protein 1 (*igfbp-1*), ghrelin (*ghrl*), cholecystokinin a (*cck*) and glucokinase (*gck*) were developed using Primer-BLAST (NCBI) (Ye *et al.*, 2012). To test the modulation of T3 and T4 in fish larvae, we assessed the gene expression of their precursor protein, thyroglobulin (*tg*). For normalization of cDNA loading, all samples were run in parallel using the housekeeping gene elongation factor I-alpha (*ef1α*) as the reference gene (McCurley & Callard, 2008). All primers are listed in Table 1. The relative mRNA expression levels of target genes and the reference gene (*ef1α*) were quantified using real-time PCR analysis with AriaMx Real-Time PCR (Agilent Technologies).

Amplification of specific PCR products was detected using the FastStart Essential DNA Green Master® (Roche), according to the manufacturer's instructions. All cDNA examples were analyzed in duplicate. The amplification protocol used was as follows: one initial step of 10 min at 95°C (denaturation and enzyme activation), followed by 45 cycles of 95°C for 10 s, 60ºC for 5 s and 72°C for 15 s. After the amplification, melting curve analysis was performed over a range of 50-95°C to verify that a single PCR product was generated at the end of the assay.

Data & statistical analysis

The cohort's coefficients of variation were calculated based on the formula proposed by Sokal & Rohlf (1995):

$$Cv = \left(1 + \frac{1}{4xn}\right) x \left(\frac{Sx100}{x}\right)$$

where *n* is the number of observations; *s* is the sample standard deviation, and *x* is the sample mean. Density histograms were made using the program R-3.1.2 for Windows (32/64 bit) (R Core Team, 2014).

The relative expression levels of the genes were calculated by the method of Pfaffl (2001), using the reference group as a control group in the equation. The primer PCR efficiency (E) was calculated for each gene fluorescence curve with LigRegPCR 12.18 software (Udvardi *et al*., 2008), and the efficiency rates for the transcripts were as follows: 1.96 for *gh*, 1.87 for *igf-1*, 1.91 for *tg*, 1.8 for *igfbp*, 1.82 for *igf-1r*(a/b), 1.7 for *gck*, 1.88 *cck*, 1.96 ghrelin and 1,84 for *ef1α* over the entire quantification range. The differences in the gene expression levels were analyzed by a Wilcox-Mann-Whitney test (Derveaux *et al*., 2010) between the small and large pools using R-3.1.2 for Windows (32/64 bit) (R Core Team, 2014), *P*-values <0.05 were considered significant. In addition, gene expression was analyzed by principal component analysis (PCA) (Abdi & Williams, 2010). The principal component analyses were made using the FactoMineR packages and the biplot by Factoextra and ggplot2 packages in R-3.1.2 (Ringner, 2008; R Core Team, 2014).

RESULTS

Body size heterogeneity

The distributions of larval body length at 6 dph and 20 dph are presented in the (Figs. 1a-1b). The larvae body length distribution observed in the beginning of the study (6 dph), showed a mode associated with 4.0 mm in body length. This mode represented nearly 72% of the measured larvae, and the remaining larvae were

Table 1. Primers used for the quantification of the mRNA expression by qPCR.

Target gene	Gene symbol	Genbank accession no.	Position	Product length (bp)	Sequence of primers (5´→3´)
Cholecystokinin a	*ccka*	XM_001346104.4	222-302	80	(F) CGCCTGCTGGACAAATCAAC (R) GGCCAGTAGTTCGGTTAGGC
Elongation factor 1 alpha	*ef1α*	NM_131263.1	1414-1516	103	(F) GTGCTGGCAAGGTCACAAAG (R) AGAGGTTGGGAAGAACACGC
Ghrelin	*ghrl*	NM_001083872.1	23-158	136	(F) GCAGCATGTTTCTGCTCCTG (R) TCAGCAGCTTCTCTTCTGCC
Glucokinase	*gck*	NM_001045385.2	952-1119	168	(F) ACGAGAAGCTGATTGGTGGG (R) TGTCCCCTGTGTCACTCTCA
Growth hormone	*gh*	NM_001123676	69-165	97	(F) CTGTTGCAGTTGGTGGTGGT (R) GGTGTTGCACACGGATGACT
Growth hormone receptor (a)	*ghra*	NM_001083578	675-929	255	(F) TGAGTCGTTCAGGGTTGCACTT (R) CGCTGTCGCTGAATTCACCAAA
Insulin-like growth factor 1	*igf-1a*	NM_131825.2	250-405	156	(F) AGTGTACCATGCGCTGTCTC (R) AAAAGCCCCTGTCTCCACAC
Insulin-like growth factor-binding protein 1	*Igfbp-1*	NM_173283	575-716	142	(F) AGTCAACGCGATACGCAAGAA (R) TGTTTGTCGCAGTTTGGCAG
Insulin-like growth factor receptor (a and b)	*igf-1R(a/b)*	NM_152968 and NM_152969	3468-3619	152	(F) AGGCAAAGGGCTGCTGCCGGTG CGCTGG (R) GCTCGTTGGACATGCCCTGGTA GGGCTG
Thyroglobulin	*tg*	XM_689200.5	4274-4455	182	(F) CTCCGACCATTCTCTCGCTC (R) GAGAGCAAAAGACCTGCCCT

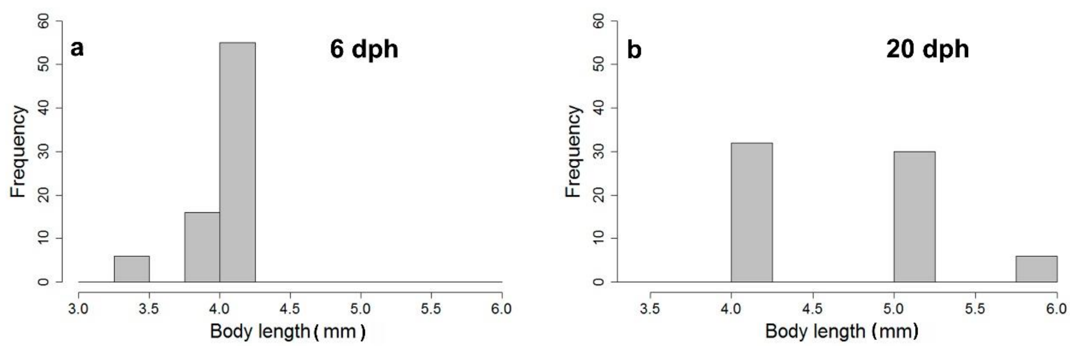

Figure 1. Frequency histograms of body length (mm) zebrafish larvae cohort distribution a) at 6 dph and b) at 20 dph.

shorter than 4 mm. At 20 dph, body length distribution evolved to show two modes, 4.0 mm (46%) and 5.0 mm (44%), and 10% of the larvae were 6 mm long, representing the longest larvae. The coefficient of variation between the cohorts changed from 5.79 at 6 dph to 14.22 at 20 dph. The mortality during the rearing period was 8%.

Modulation of genes related to growth

In the GH/IGF-1 axis, differences in gene expression between small and large larvae are presented in Fig. 2. Only were statistically significant for the *igf-1* (a/b) receptor (Wilcox-Mann-Whitney test, P = 0.02) and *igf1bp* (Wilcox-Mann-Whitney test, P = 0.01), the other genes of growth axis were not statistically significant (P > 0.05) and showed similar levels of gene expression between the groups. The expression levels of the other genes evaluated (*tg*, ghrelin, *cck*, *gck*) were not statistically significant (Fig. 3), although for *gck*, the Wilcox-Mann-Whitney test yielded P = 0.07.

Principal components analysis (PCA)

This descriptive analysis revealed other aspects of variability among individuals (the larvae pools) and variables (genes), enabling us to elucidate the main components of the variability. The PCA results revealed that approximately 84.4% of the inertia was explained by four components or dimensions (PCs): PC1 = 26.6%, PC2 = 23.6%, PC3 = 21% and PC4 = 13.2%. The PCA with PC1 and PC2 (Figs. 4a-4b). Figure 4a shows the correlation among variables (gene expression). There is a positive correlation between *gck*

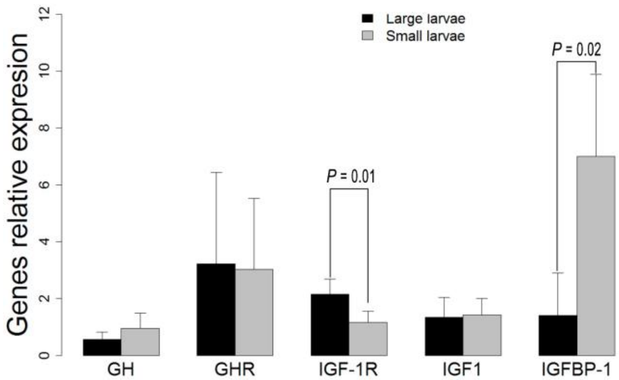

Figure 2. Relative gene expression of: a) Growth hormone (*gh*), b) Insulin-like growth factor 1 (*igf-1*), c) growth hormone receptor (*ghra*) and d) Insulin-like growth factor 1 receptor (*igf-1r*a/b), insulin-like growth factor binding protein 1 (*igfbp-1*) in the zebrafish larvae small and large body length groups assessment by qPCR. Bars represent mean ± SD, n = 5. The statistical significance was determined using the paired sample Wilcoxon Mann-Whitney U signed rank test ($P < 0.05$).

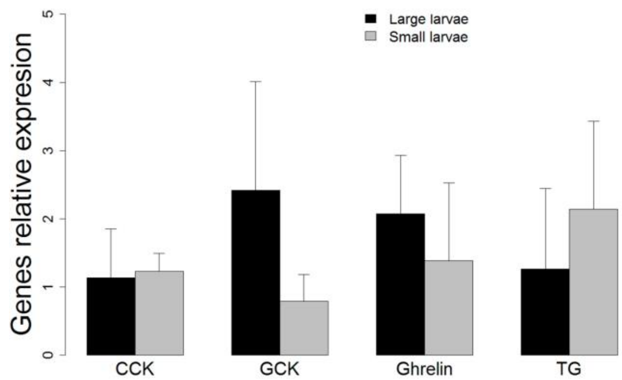

Figure 3. Relative gene expression of: a) Thyroglobulin (*tg*), b) Cholecystokinin (*cck*), c) Ghrelin and d) Glucokinase (*gck*) in the zebrafish larvae small and large body length groups assessment by RT-PCR. Bars represent mean ± and line the SD, n = 5. The statistical significance was determined using the paired sample Wilcoxon Mann-Whitney U signed rank test ($P < 0.05$).

and *igf-1r*, as well as between *tg* and *igfbp-1*. A projection in the first component (PC1) is apparent in both groups and the two projections are negatively correlated. Figure 4b shows the individuals (larvae pools) and circumscribed groups associated with larvae body length. This aggrupation is projected onto PC1, the large larvae are on the negative side and the small larvae on the positive side. The correlations among components and variables are presented in Table 2, which shows that the most important genes correlated with PC1 were *igfbp-1* (0.86, $P = 0.001$), *gck* (-0.67, $P = 0.03$) and *tg* (0.65, $P = 0.03$). Therefore, *gck* was significantly correlated with PC1 and its projection was associated with large larvae, while *tg* and *igfbp-1* were significantly correlated with PC1, although their projections were associated with small larvae (Figs. 4a-4b). In PC2, the most important correlations were *cck* (0.92, $P = 0.0001$) and *ghr* (0.66, $P = 0.03$).

Table 2. Correlation between components and variables.

Variable	PC1	PC2	PC3	PC4
gck	-0.67	0.35	-0.33	0.15
igf-1r	-0.49	0.22	0.11	0.74
ghrelin	-0.36	-0.44	0.75	0.04
ghr	-0.04	0.66	0.29	-0.57
cck	0.02	0.92	0.22	-0.09
igf-1	0.21	0.4	0.77	0.25
gh	0.56	0.51	-0.44	0.39
tg	0.65	-0.13	0.49	0.22
igfbp-1	0.86	-0.09	-0.14	0.05

DISCUSSION

The present study confirms that the growth rate variability phenomenon modulate the gene expression patterns of growth endocrine control genes. The siblings zebrafish cohort presented a growth rate variability after the rearing period, because the larvae body length distribution began with low CV (5.8%) at 6 dph and was raised to 14.22% at 20 dph. The size length variation observed in the study is according to the size variation observed in other studies in *Sparus aurata* (Goldan *et al*., 1997) or in *Sciaenops ocellatus* (Smith & Fuiman, 2003) and this situation is consistent with the report of Kestemont *et al*. (2003) in the growth rate variability phenomenon.

Larvae body length groups not showed significant differences in *gh* and *igf-1* mRNA levels. However, the mean of *gh* mRNA levels was slightly higher in smaller larvae than in large larvae, this trend conforms to the expectations associated with the poor nutritional status (Wood *et al*., 2005; Norbeck *et al*., 2007; Savage, 2013). Conversely, was observed uniformity in *igf-1* mRNA levels between the larvae groups, in contrast to the most fasting reports in different fish species (Wood *et al*., 2005; Norbeck *et al*., 2007; Peterson & Waldbieser, 2009; Reinecke, 2010; Kawaguchi *et al*., 2013; Tian *et al*., 2015; Taniyama *et al*., 2016). However, Wen-Ying *et al*. (2012) in *Carassius auratus gibelio*, Breves *et al*. (2014) and Fox *et al*. (2010) in Mozambique tilapia (*Oreochromis mossambicus*), and Hevrøy *et al*. (2011) in Atlantic salmon (*Salmon salar*) only observed significant differences at the protein le-

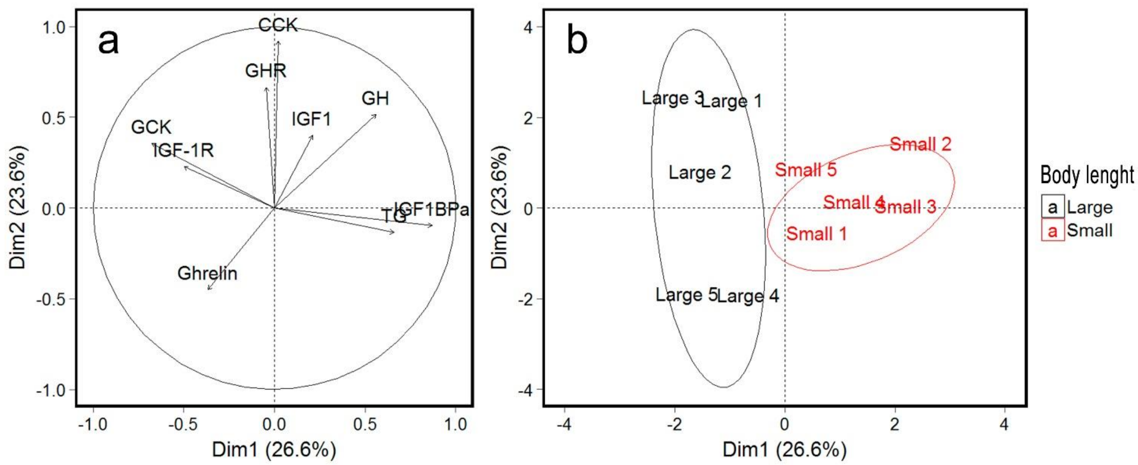

Figure 4. PCA analysis a) PCA-Correlation loadings plot of the variables (gene expression) in the principal components PC1 and PC2, b) PCA-Score plot in the PC1 and PC2 principal components. growth hormone (*gh*), growth hormone receptor (*ghr*), insulin-like growth factor 1 (*igf-1*), insulin-like growth factor receptor (*igf-1r*), insulin-like growth factor binding protein 1 (*igfbp-1*), ghrelin (*ghrl*), cholecystokinin (*cck*) and glucokinase (*gck*), thyroglobulin (*tg*). The black individuals are the large body length zebrafish larvae pool and the red individuals are the small body length zebrafish larvae pool.

vels but not at the mRNA levels by fasting challenge. Less information is available about the *ghr* and *igf-1r* on teleost fish, regarding to the nutritional regulation. Regard *ghr* mRNA levels modulation by fasting challenge, the fish studies showed variable results. In channel catfish (*Ictalurus punctatus*) by increasing feeding levels, *ghr* mRNA levels did not change significantly between the groups (Peterson *et al*., 2008). However in rainbow trout (*Oncorhynchus mykiss*) the fasting reduce *ghr* the mRNA levels (Walock *et al*., 2014); conversely fasting increase *ghr*a and *ghr*b mRNA levels in zebrafish (*Danio rerio*) (Tian *et al*., 2015). Regard to *igf-1r* mRNA levels the fish studies also have been showed variable results. As well, the in *Ictalurus punctatus* and *Oncorhynchus mykiss*, it did not show mRNA levels modulation by fasting (Peterson *et al*., 2009, Gabillard *et al*., 2003); though Norbeck *et al*. (2007) found *igf-1r* up-regulation in gill but not in skeletal muscle in fasting *Oncorhynchus mykiss*. Nevertheless, this studies analyses *igf-1r* mRNA levels by specific tissue, nonetheless this receptor is expressed in all body tissues (Wood *et al*., 2005; Nimptsch & Giovannucci, 2012); therefore, these results give a partial interpretation of the *igf-1r* expression. Conversely, our study used the complete larvae and the *igf-1r* gene expression adds all body tissues. Hence, the significant difference between the body length groups in *igf-1r* suggests that the gene expression of *igf-1r* may be more sensible by the larvae nutritional status than its main ligand *igf-1*.

In fish fasting challenge influence the modulation of gastrointestinal peptides as cholecystokinin and ghrelin. The *cck* mRNA levels decrease by fasting in different fish species (Murashita *et al*., 2006; Feng *et al*., 2012; Ji *et al*., 2015), conversely ghrelin increase mRNA levels by fasting (Amole & Unniappan, 2009; Zhou & Xue, 2009; Tian *et al*., 2015; Volkoff, 2015; Blanco *et al*., 2016). These gene expression modulations were not observed in our results.

The most likely explanation of the key growth gene expression patterns results could be associated to the larvae nutritional status. The *igfbp-1* and *gck* gene expressions are regulated by nutritional status or the glucose levels. The *igfbp-1* is regulated by insulin levels and in consequently with glucose levels (Lee *et al*., 1993) and fasting increase its mRNA levels in fish (Shimizu *et al*., 2006; Hevrøy *et al*., 2011; Kawaguchi *et al*., 2013; Breves *et al*., 2014), according to the our study results. On the other hand, *gck* is a liver enzyme that catalyzes the phosphorylation of glucose to glucose-6-phosphate (Enes *et al*., 2009), is associated with individual nutritional status and showing an up-regulation by feed intake (Caseras *et al*., 2000; González-Alvarez *et al*., 2009; Panserat *et al*., 2014). Likewise, the main significant correlations in PCA analysis were according with this interpretation. The large larvae could be associated with a high food intake or better nutritional status and this increase *gck* mRNA levels; this is according with the high significant correlation observed between these factors. On the other hand, the *igfbp-1* was correlated with small larvae; this outcome was according with the *igfbp-1* modulation by poor nutritional status. Hence, the results suggest that the growth rate variability was associated with the larvae nutritional status, and possibly influenced by food competition (Ruzzante, 1994). However, intensity level of the nutrient restriction was not similar than fasting challege, because the food competition does not prevent small larvae from feeding; rather, they have less access to food. As well, this light fasting condition could prevent observed significant differences in *gck* or *igf-1* mRNA levels between the body length groups.

In conclusion, our results suggest that growth rate variability affect the gene expression of *igfbp-1* and *igf-1r* genes, the increment in *igfbp-1* mRNA levels observed in the small larvae suggest that nutritional status is associated to their growth rate. Future researches have to include protein assess and different feed levels to understand the growth rate variability influence in larvae zebrafish growth rate.

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