



# 读书报告




汇报人：张隽美 时间：2019年11月17日



**ORIGINAL ARTICLE**

WILEY

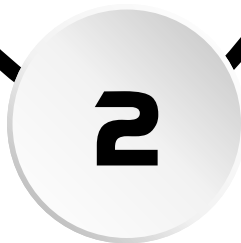
# Transcriptome, enzyme activity and histopathology analysis reveal the effects of high level of dietary carbohydrate on glycometabolism in juvenile golden pompano, *Trachinotus ovatus*

Kang Liu<sup>1,2</sup>  | Beiping Tan<sup>1,3,4</sup> | Wei Zhang<sup>1,3,4</sup>  | Hongyu Liu<sup>1,3,4</sup> |  
Xiaohui Dong<sup>1,3,4</sup> | Qihui Yang<sup>1,3,4</sup>  | Shuyan Chi<sup>1,3,4</sup> | Shuang Zhang<sup>1,3,4</sup> |  
Haitao Zhang<sup>4</sup>

# ● CONTENTS

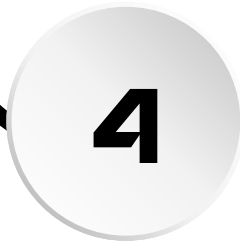
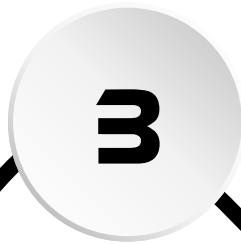
---

Introduction



Materials and methods

Results



Discussion



1

# Introduction

## ● Introduction

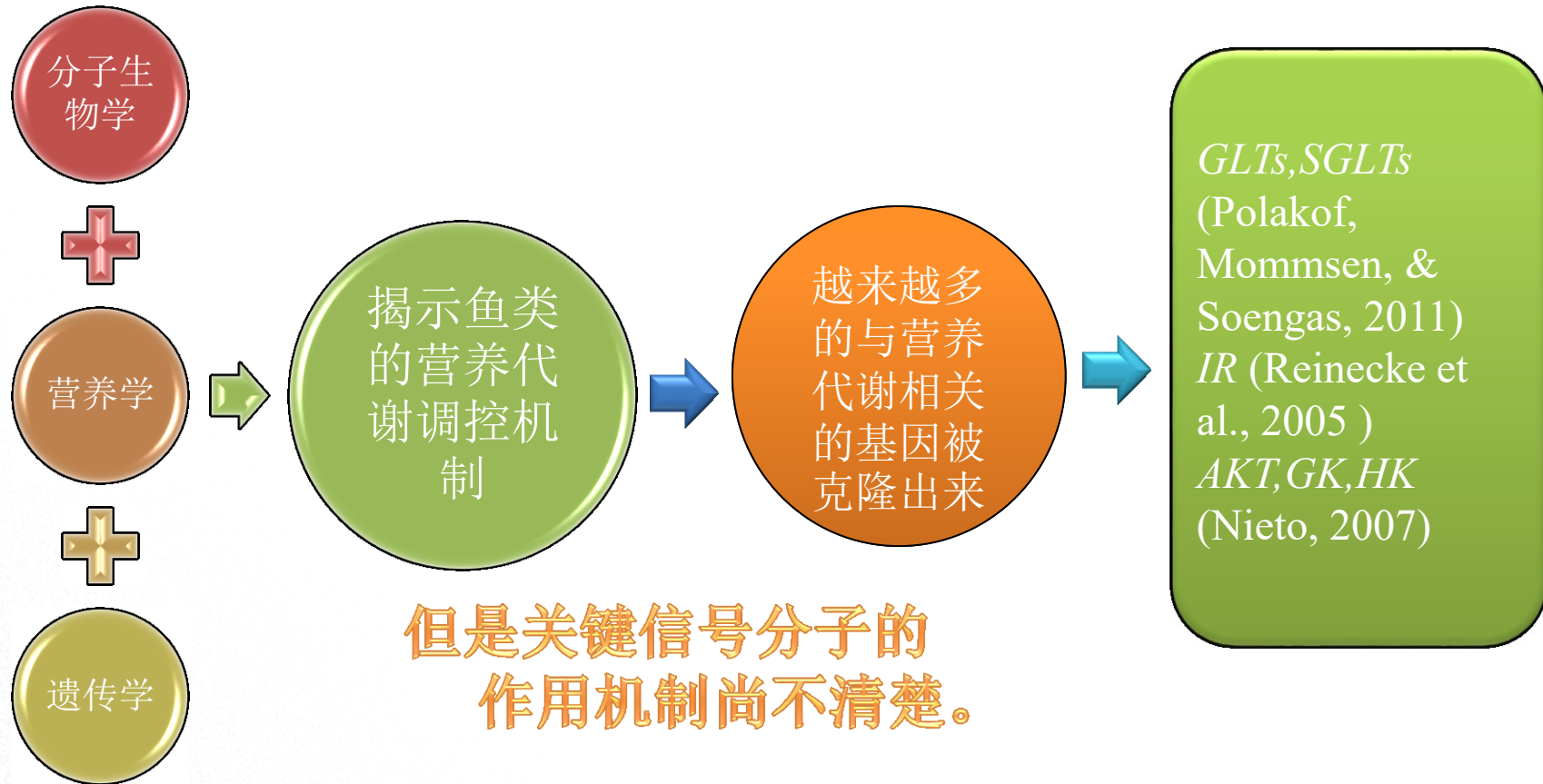
---

鱼被认为具有先天性的“糖尿病体质”，对糖类的利用率较低



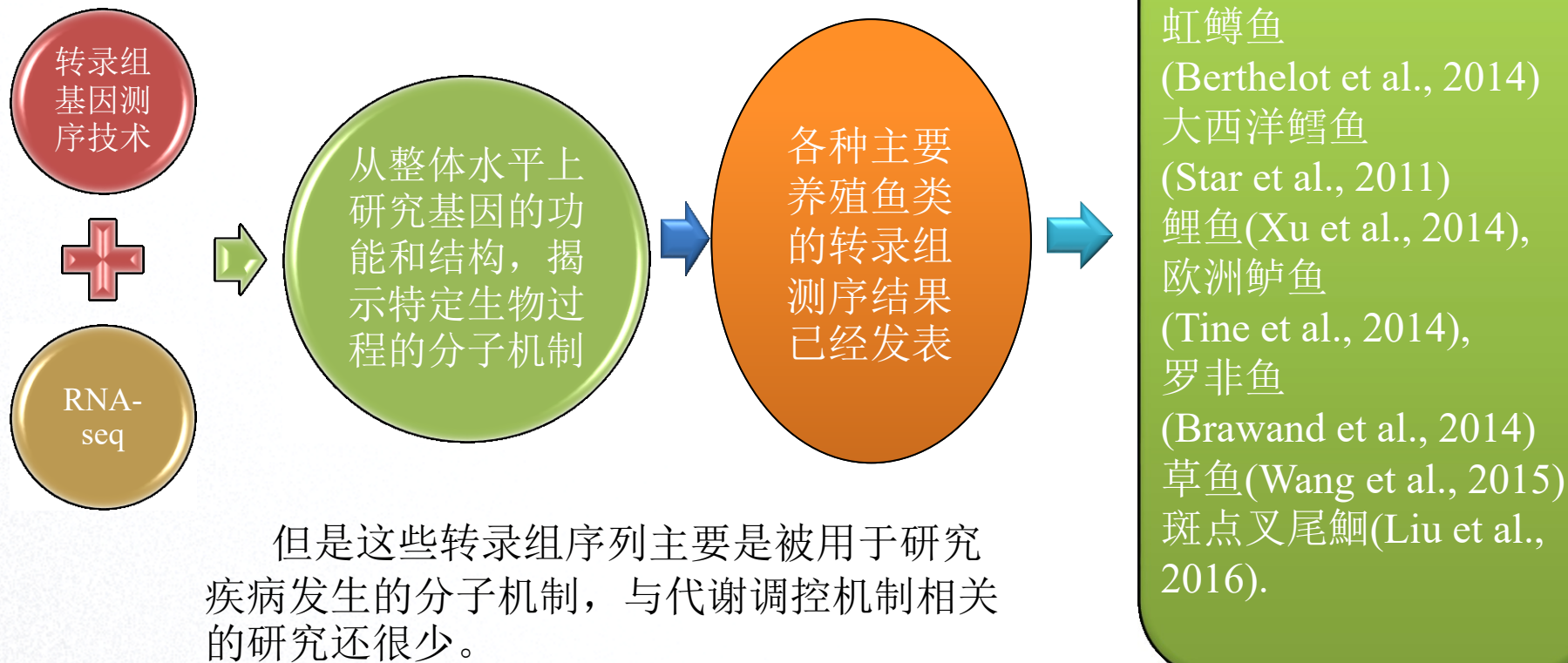
**如何提高对饲料中糖类的利用率是当前鱼类营养研究的一个热门话题！**

# ● Introduction



传统的分子克隆既浪费时间又效率低下，还不能全面的理解代谢通路的调节机制。

# ● Introduction



# ● Introduction



金鲳鱼 Golden pompano  
(*Trachinotus ovatus*)

特点:

1. 整个生长过程都可以用配方饲料投喂;
2. 对高糖(360 g/kg玉米淀粉) 具有较好的耐受性和利用率;
3. 尽管高糖(360 g/kg玉米淀粉)会导致金鲳鱼生长缓慢, 但并未引起明显的营养性肝脏疾病。

**因此, 研究  
金鲳鱼的耐受机制十分有意义。**



# ● Introduction

本研究的主要内容：

● 七种不同  
饲料投喂

● 构建转录  
组文库

● 通过转录  
组序列和  
基因表达  
数据分析

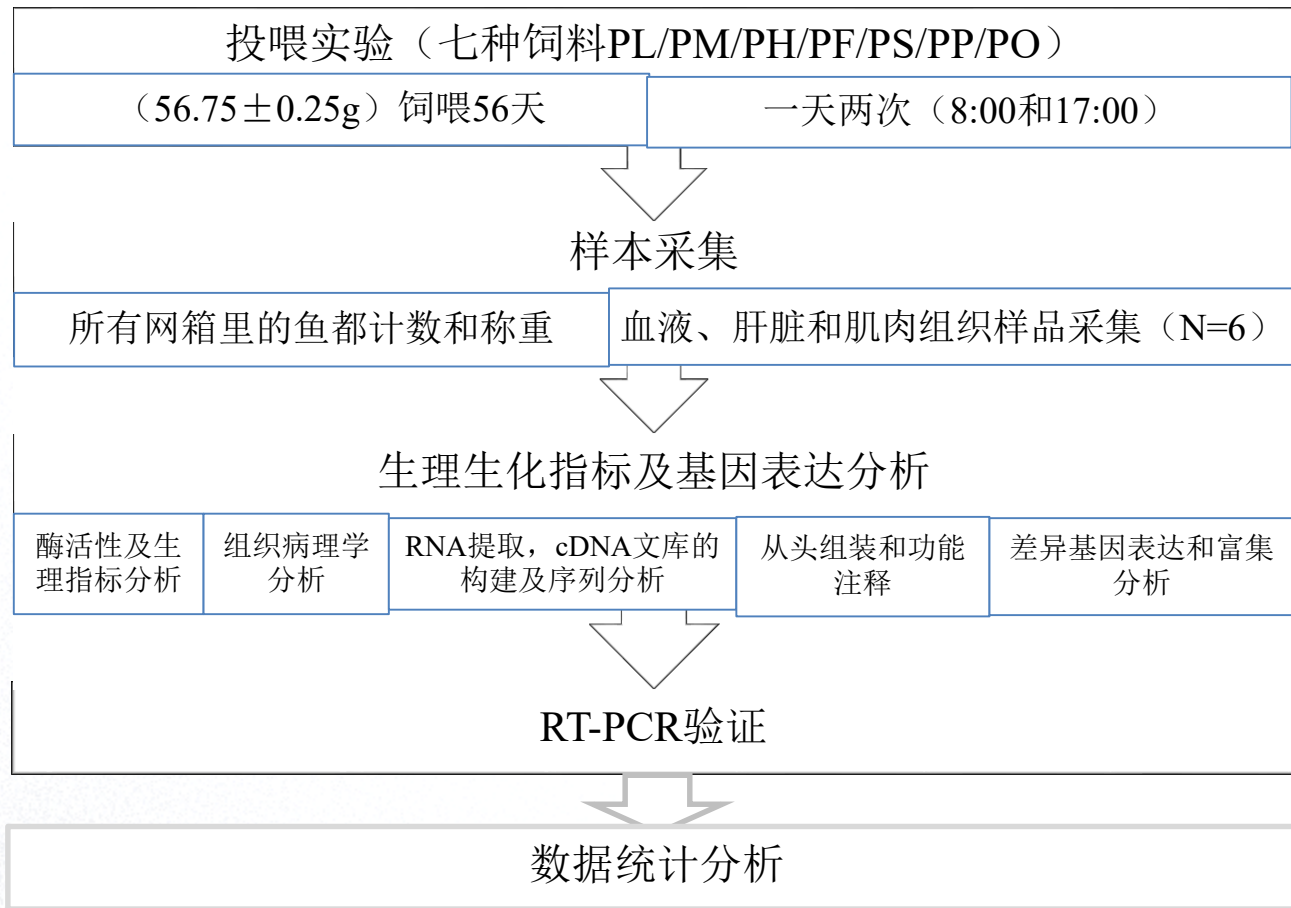
● 研究金鲳  
鱼的高糖  
耐受机制

● 为肉食性  
鱼类糖代  
谢机制的  
研究提供  
参考依据

2

- Materials and methods

# Materials and methods



PL:low level of corn starch, 120 g/kg

PM:middle level of corn starch, 240 g/kg

PH:high level of corn starch, 360g/kg

PF:fish oil, 61.6 g/kg

PS:soybean oil, 61.6 g/kg

PP :lard oil, 61.6 g/kg

PO:oxidized fish oil, 61.6 g/ kg



Results



## The growth and physiological indices 生长生理指标

TABLE 2 Effects of different levels of dietary carbohydrate on growth performance of *Trachinotus ovatus*

Diet	IBW (g)	FBW (g)	WGR (%)	SGR (%)
PM	54.81 ± 1.13	140.92 ± 6.24 <sup>b</sup>	157.08 ± 10.90 <sup>b</sup>	1.48 ± 0.13 <sup>b</sup>
PH	54.85 ± 1.02	102.86 ± 6.50 <sup>a</sup>	87.54 ± 11.54 <sup>a</sup>	0.98 ± 0.19 <sup>a</sup>
ANOVA				
F value	0.088	40.059	42.977	43.445
p value	0.916	0.000	0.000	0.000

PH组饲料中的碳水化合物水平显著降低了金鲳鱼的生长性能( $p < 0.05$ )

## ● The growth and physiological indices

TABLE 3 Effects of different levels of dietary carbohydrate on glycogen content (mg/kg wet weight) in fresh liver and muscle, serum glucose content (mM) in serum and HSI (%) and liver cavity area (%) of *Trachinotus ovatus*

Diets	Liver glycogen content	Muscle glycogen content	Serum glucose	HSI	Liver cavity area
PM	15.05 ± 2.10	2.34 ± 0.40 <sup>a</sup>	7.17 ± 1.36	1.30 ± 0.03	17.47 ± 3.12 <sup>a</sup>
PH	25.38 ± 9.46	3.00 ± 0.09 <sup>b</sup>	8.72 ± 0.64	1.25 ± 0.07	26.45 ± 2.34 <sup>b</sup>
ANOVA					
F value	29.555	2.321	36.401	17.442	89.342
p value	0.796	0.163	0.825	0.094	0.000

PH组肌糖原含量及肝腔面积明显增加( $p < 0.05$ )。但是, PM组与PH组肝糖原含量及血清葡萄糖含量无显著性差异 ( $P > 0.05$ )。

## ● The growth and physiological indices

TABLE 4 Effects of different levels of dietary carbohydrate on the liver enzyme activities of *Trachinotus ovatus*

Diets	G6PDH (U/gprot)	HK (U/gprot)	GK (ng/mgprot)	PFK-1 (ng/mgprot)	PEPCK (ng/mgprot)
PM	2,499.43 ± 1,207.05	102.33 ± 8.69 <sup>a</sup>	24.86 ± 1.89	15.09 ± 3.46	14.14 ± 1.99
PH	3,794.04 ± 1,153.71	157.24 ± 22.3 <sup>b</sup>	28.08 ± 7.59	23.03 ± 13.94	10.61 ± 1.53
ANOVA					
F value	0.174	6.789	5.688	1.385	5.342
p value	0.843	0.027	0.773	0.299	0.476

表4表明，PH组仅肝脏HK酶活性显著升高( $p < 0.05$ )。PH组中G6PDH、GK、PFK-1和PEPCK的肝酶活性与PM组比较无显著性差异( $p > 0.05$ )。

## ● The growth and physiological indices

---

TABLE 5 Effects of different levels of dietary carbohydrate on the serum hormone contents of *Trachinotus ovatus*

Diets	Glucagon (pg/ml)	IGF-I (ng/ml)	Insulin ( $\mu$ IU ml <sup>-1</sup> )
PM	901.47 $\pm$ 71.09	88.32 $\pm$ 35.36	15.12 $\pm$ 2.90
PH	751.16 $\pm$ 114.27	79.71 $\pm$ 41.80	12.44 $\pm$ 1.25
ANOVA			
F value	5.439	0.133	2.235
p value	0.569	0.878	0.188

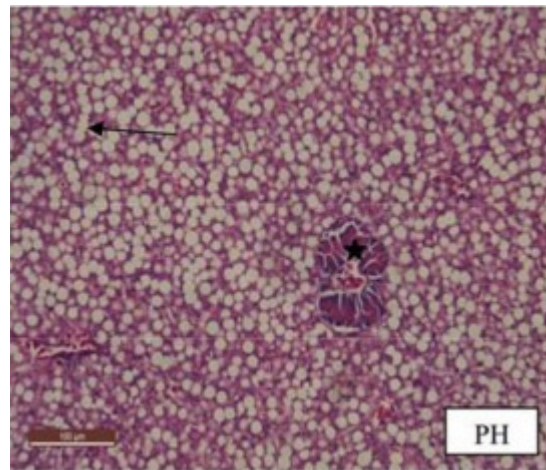
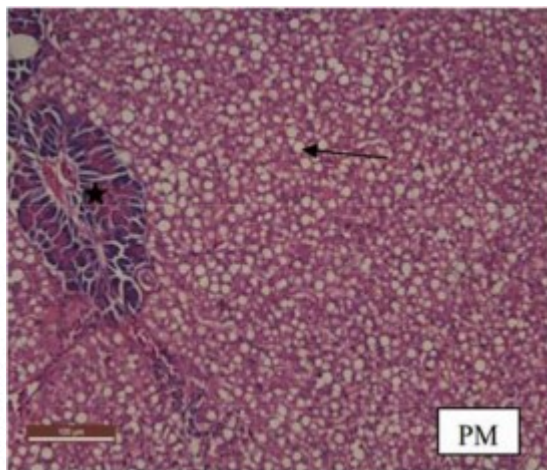
与PM组相比，PH组血清中胰高血糖素、IGF-I和胰岛素的含量均无显著影响（ $p > 0.05$ ）。



## The histological changes of *T. ovatus* liver

### 金鲳鱼肝脏的组织学变化

FIGURE 1 Histologically observed liver of *Trachinotus ovatus* (H.E. staining,  $10 \times 10$ )



PH组与PM组相比，随着日粮碳水化合物含量的增加，肝脏脂肪含量增加。

# Sequencing and de novo assembly of *T. ovatus* liver transcriptome

## 金鲳鱼肝脏转录组的测序和从头组装

TABLE 6 Summary of assembled result by Illumina sequencing

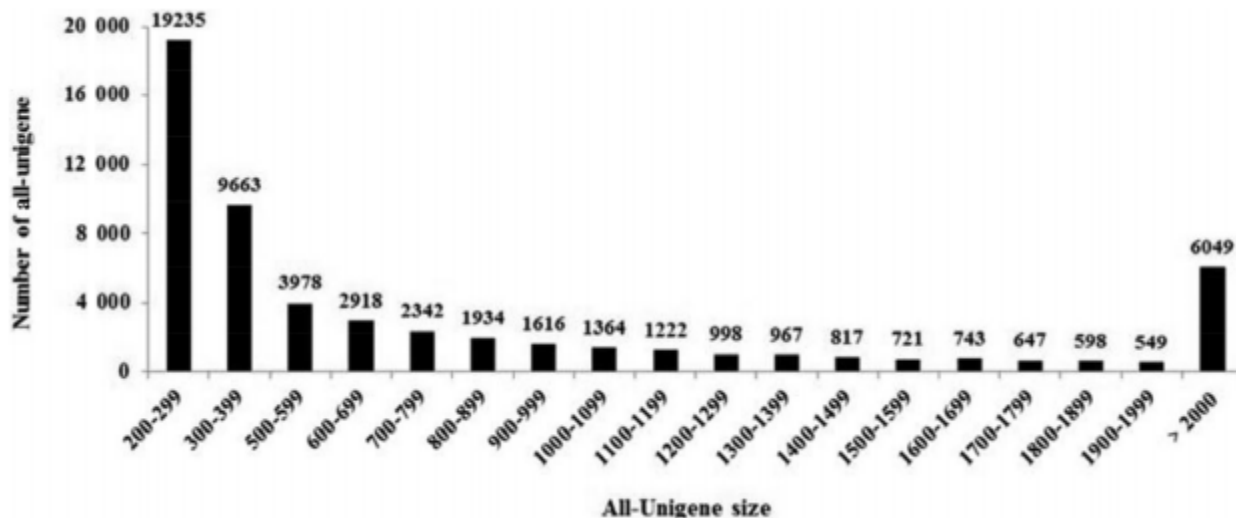
Items	
Total no. of unigenes	62,377
GC percentage	47.05%
Largest length	16,206 bp
Smallest length	201 bp
Average length	822 bp
N50	1,445 bp

用Trinity软件包组装得到62377个Unigenes

## Sequencing and de novo assembly of *T. ovatus* liver transcriptome

### 金鲳鱼肝脏转录组的测序和从头组装

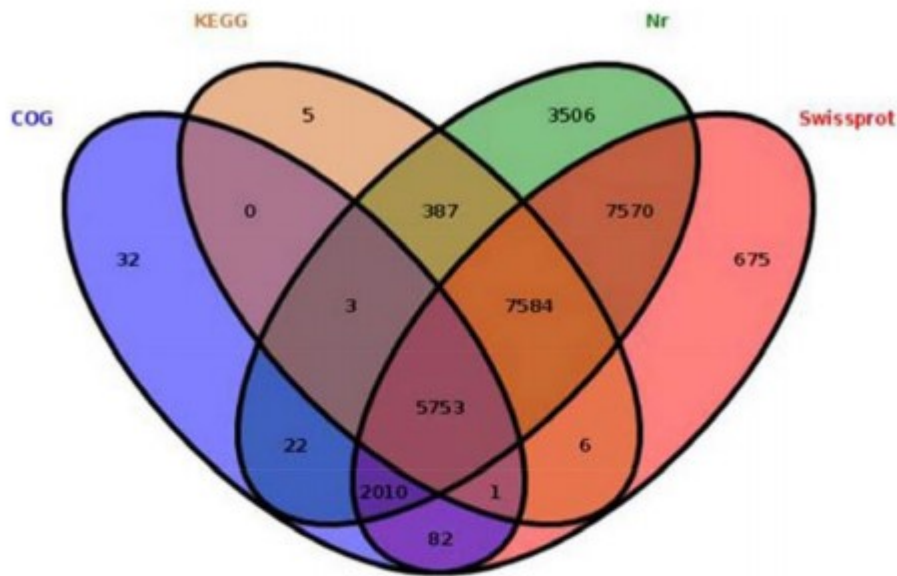
FIGURE 2 Overview of *Trachinotus ovatus* transcriptome sequence length distributions for all unigenes



大部分基因为200~299 bp（30.8%）；其次是300-399 bp（15.5%）。

# Functional annotation and classification of unigenes

## 基因的功能注释与分类



**FIGURE 3** Venn diagram of annotation result in *Trachinotus ovatus* against four protein databases [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

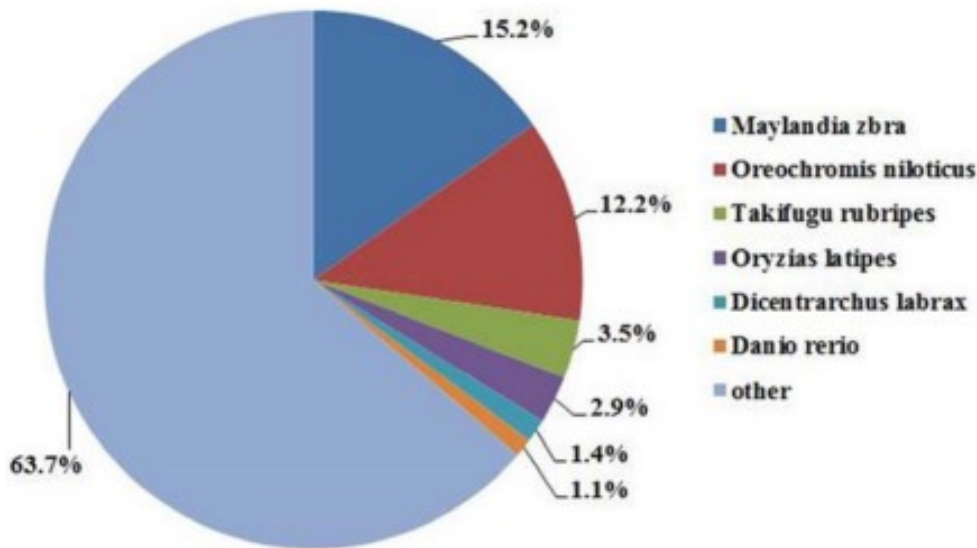
**TABLE 7** Result of annotation on unigenes in different databases for *Trachinotus ovatus*

Database	Annotation	Ration (%)
Annotated in Nr	26,835	43.02
Annotated in SWISS-PROT	23,681	37.96
Annotated in KEGG	13,739	22.02
Annotated in COG	7,903	12.67
Annotated in all databases	5,753	9.22
Total unigenes	62,377	100

参考Nr、SWISS-PROT、KEGG、COG四个数据库对62377个基因进行功能注释。



## Homology comparison analysis of unigenes 基因同源比较分析



- 63.7% : 无鱼类同源基因
- 15.2%: *Maylandia zebra* 同源基因
- 12.2%: 尼罗罗非鱼同源基因
- 1.1%: 斑马鱼同源基因

**FIGURE 4** Species distribution results of a similarity search of unigenes against Nr database [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



# GO annotation

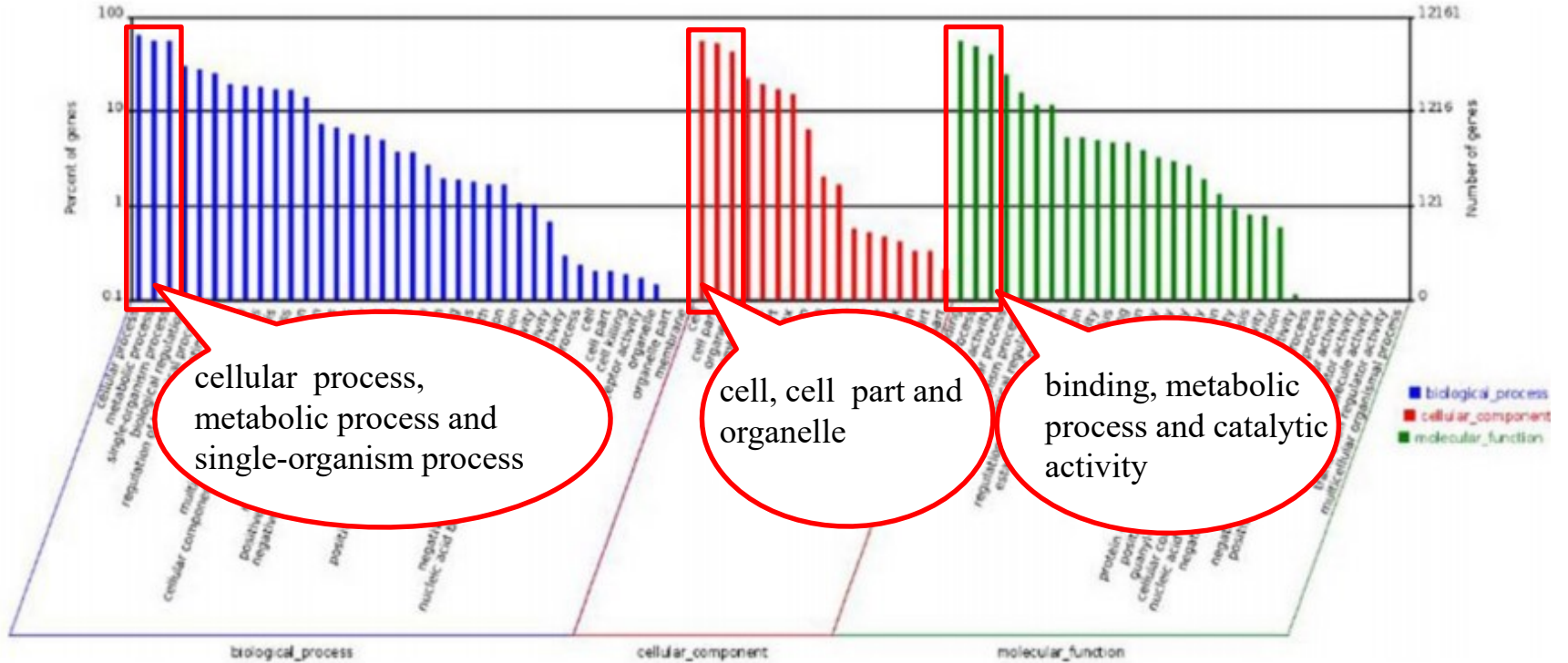
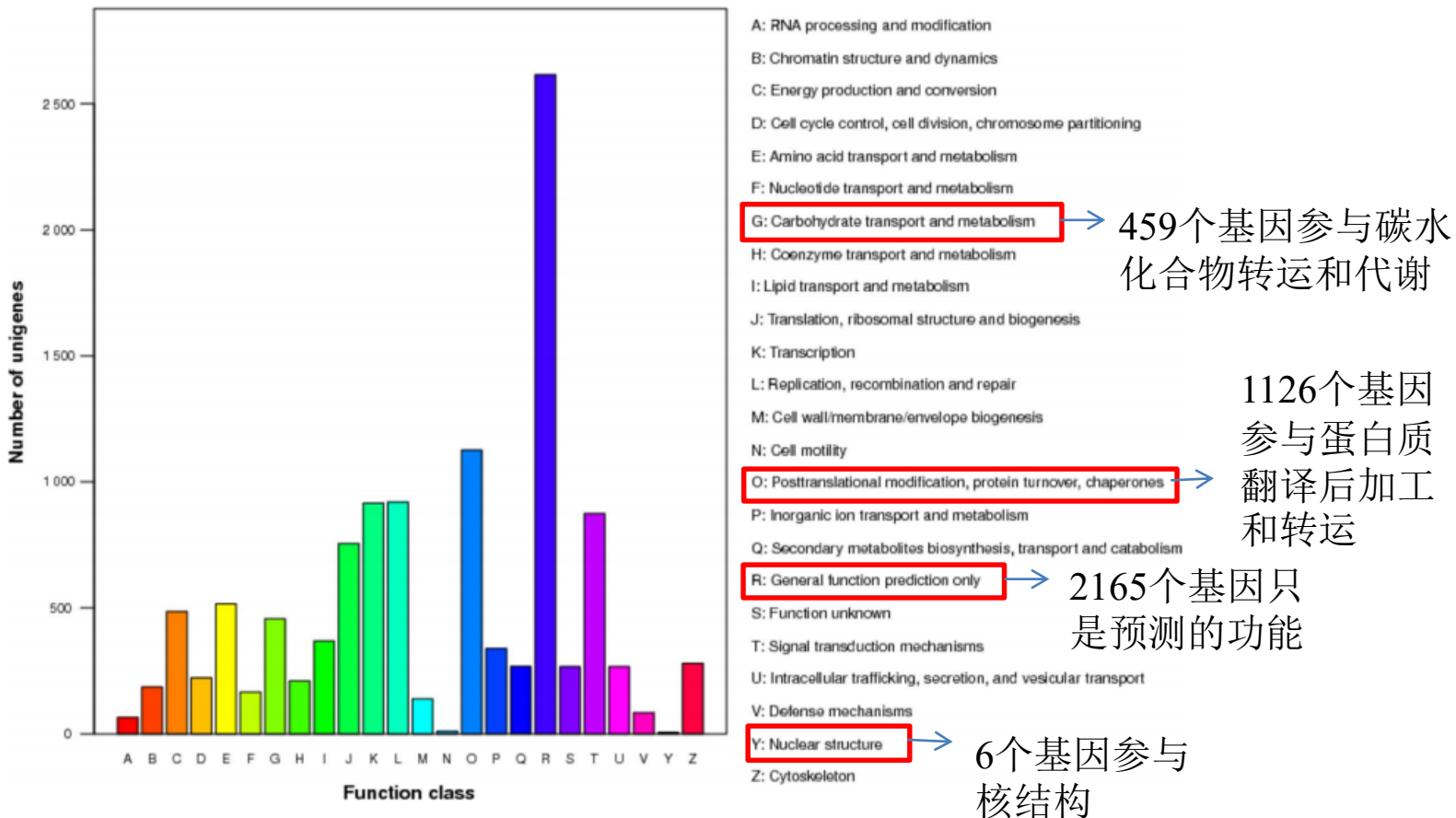


FIGURE 5 Gene ontology classification of assembled unigenes [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

蓝色：生物学过程 红色：细胞定位 绿色：分子功能



# COG annotation



**FIGURE 6** Histogram presentation of COG classification of 7,903 unigenes. The letters on the x-axis indicate different COG categories [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



# KEGG functional classification

**TABLE 8** Top 20 KEGG pathways with the greatest number of annotated sequences

No.	Pathway	Annotation genes (%)	Pathway ID
1	Metabolic pathways	1,874 (13.64)	ko01100
2	Pathways in cancer	676 (4.92)	ko05200
3	Focal adhesion	474 (3.45)	ko04510
4	MAPK signalling pathway	445 (3.24)	ko04010
5	Regulation of actin cytoskeleton	440 (3.2)	ko04810
6	Endocytosis	431 (3.14)	ko04144
7	Huntington's disease	374 (2.72)	ko05016
8	Alzheimer's disease	359 (2.61)	ko05010
9	Chemokine signalling pathway	334 (2.43)	ko04062
10	Tuberculosis	328 (2.39)	ko05152

**TABLE 8** Top 20 KEGG pathways with the greatest number of annotated sequences

No.	Pathway	Annotation genes (%)	Pathway ID
11	Insulin signalling pathway	321 (2.34)	ko04910
12	Protein processing in endoplasmic reticulum	311 (2.26)	ko04141
13	Axon guidance	308 (2.24)	ko04360
14	Influenza A	302 (2.2)	ko05164
15	Ribosome	298 (2.17)	ko03010
16	Tight junction	296 (2.15)	ko04530
17	Wnt signalling pathway	290 (2.11)	ko04310
18	Neurotrophin signalling pathway	284 (2.07)	ko04722
19	Calcium signalling pathway	282 (2.05)	ko04020
20	Purine metabolism	281 (2.05)	ko00230

代谢通路相关基因：  
1847个Unigenes

癌症通路相关基因：  
676个Unigenes

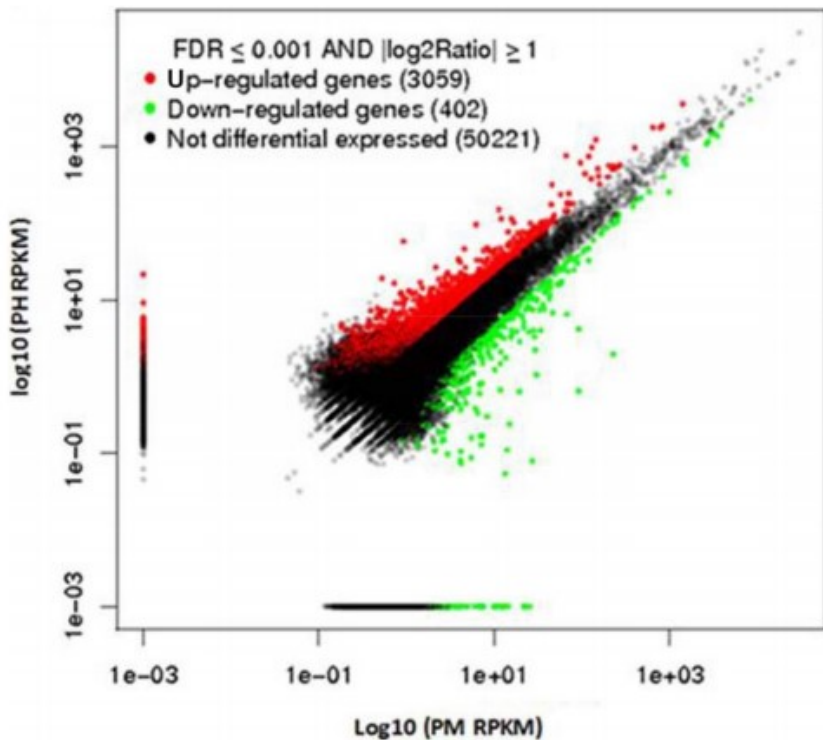
细胞通讯蛋白相关  
基因：  
474个Unigenes

这些注释为研究鱼类特定的生物学和代谢过程、功能和分子机制提供了有价值的信息。



# Identification and analysis of differentially expressed genes

## 差异表达基因的鉴定与分析

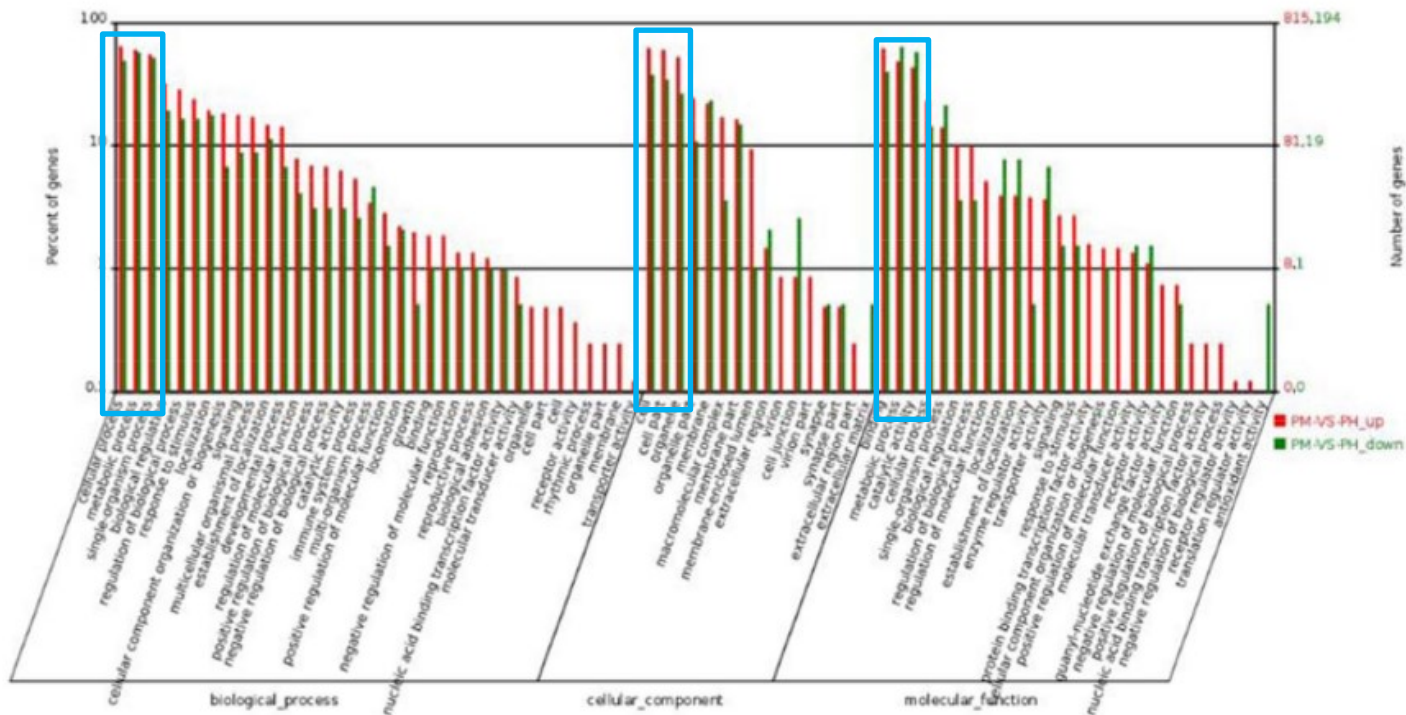


与PM组相比，PH组中共有3461个基因差异表达。其中3059个基因显著上调（红色），402个基因显著下调（绿色）。

FIGURE 7 Overview of different expression genes distribution between PM and PH in the scatter plot

# GO annotation and carbohydrate metabolism pathway analysis of DEGs

## 差异表达基因的功能注释及糖类代谢通路分析



生物学过程

细胞定位

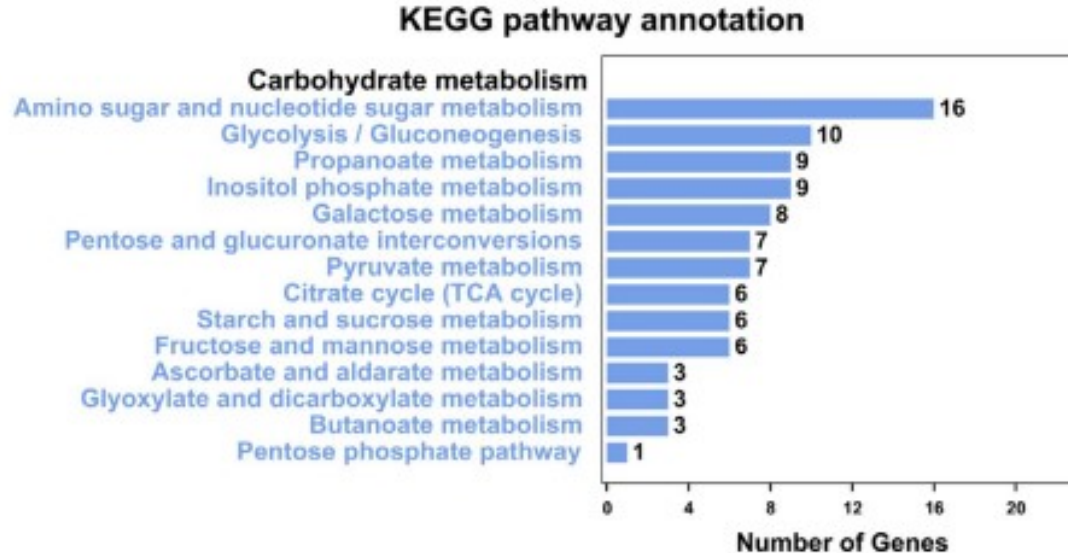
分子功能

FIGURE 8 GO enrichment analysis of the different expression genes in golden pompano between PM and PH



# GO annotation and carbohydrate metabolism pathway analysis of DEGs

---



**FIGURE 9** KEGG annotation of the differentially expressed genes (DEGs) between PM and PH in carbohydrate metabolism pathway [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



# Analysis of the transcriptomic data by qRT-PCR

## 转录组数据的qRT-PCR分析

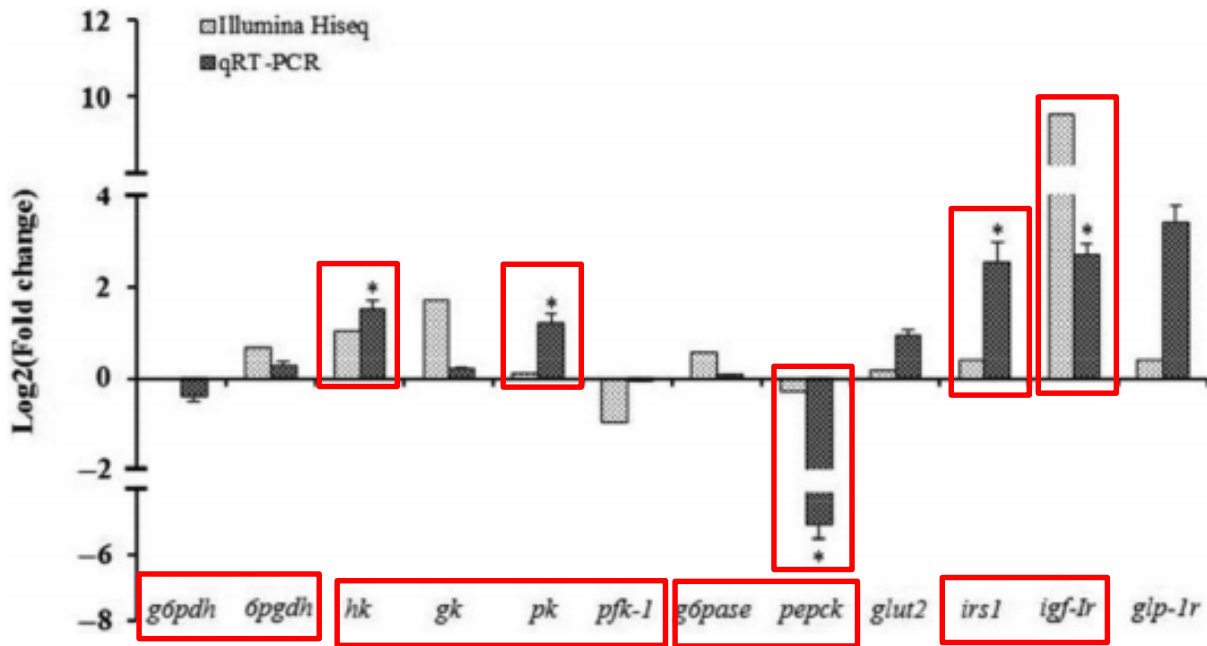


FIGURE 10 Validation of RNA-seq data by qPCR



Discussion



## Discussion

---

### 创新点:

本研究是首次利用转录组技术从整体水平探讨海水鱼的膳食碳水化合物代谢机制。

### 结论:

金鲳鱼对饲料中高水平的碳水化合物具有一定的适应性和需求。但生理代谢机制已经改变，以适应饲料中的高糖，如：糖酵解增加，糖异生减少，糖代谢相关激素和激素受体的表达有维持葡萄糖稳态的趋势。



水产 2019级研究生

恳请各位老师批评指正!



河南师范大学

THANKS

汇报人：张隽美