# 黄条鰤*npy* 基因克隆及其对饥饿再投喂的应答特性[*]

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**摘要**　　为探究神经肽 Y (neuropeptide Y, NPY)在黄条鰤(*Seriola aureovittata*)摄食调控中的作用及机制, 本研究采用同源克隆的方法获得了黄条鰤*npy* 基因的开放阅读框(ORF)序列, 并利用实时荧光定量 PCR 技术分析了 *npy* 基因的组织分布以及其对饥饿再投喂的应答特性。黄条鰤*npy* 基因 ORF 序列长度为 300 bp, 编码 99 个氨基酸的前体蛋白, 其中包括 28 个氨基酸的信号肽、36 个氨基酸的成熟肽。氨基酸序列同源性比对发现, 黄条鰤*npy* 编码的氨基酸序列与斑马鱼(*Danio rerio*)等其他硬骨鱼高度保守(>90%)；系统进化树分析表明, 黄条鰤*npy* 与高体鰤(*Seriola dumerili*)的关系最近。*npy* mRNA 在所检测的 12 种组织中均有表达, 其中, 在脑组织表达量最高, 在垂体和胃中表达量次之。在饥饿再投喂实验中, 饥饿刺激了 *npy* mRNA 的表达, 特别是饥饿 21 d 时, 实验组垂体 *npy* mRNA 表达量显著高于对照组, 再投喂 7 d 后恢复到对照组水平。上述结果表明, *npy* 可能参与了黄条鰤的摄食调控, 在饥饿代谢补偿机制中发挥了重要作用。

**关键词**　　黄条鰤；*npy*；克隆；组织表达；摄食

鱼类的摄食行为不仅与外界因素有关, 还受到多种内源性摄食因子的调控(Volkoff *et al*, 2020)。下丘脑神经内分泌因子, 如神经肽 Y (neuropeptide Y, NPY)、黑皮质素(melanocortin, MCR)及食欲肽(orexin)等在鱼类摄食中发挥重要的调控作用(Bertucci *et al*, 2019; Soengas *et al*, 2018)。其中, NPY 在鱼类中是一种强有力的促食欲因子(Bertucci *et al*, 2019)。

1982 年, NPY 首次从猪脑中提取出来, 因其富含酪氨酸而被称为神经肽酪氨酸或神经肽 Y, 具有显著促进摄食的功能(Tatemoto *et al*, 1982)。在硬骨鱼类中, *npy* 于 1989 年首次在金鱼(*Carassius curatus*)中克隆获得(Kah *et al*, 1989)。目前, 已在大西洋鲑(*Salmo salar*) (Murashita *et al*, 2009)、重口裂腹鱼(*Schizothorax davidi*) (Deng *et al*, 2019)、银鲫(*Carassius auratus gibelio*) (Lei *et al*, 2019)等硬骨鱼中相继克隆获得了 *npy* 基因。已有研究表明, 鱼类 *npy* mRNA 主要在脑中表达(Lei *et al*, 2019; Deng *et al*, 2019), 参与摄食调控、糖脂代谢等多种生理功能

(Horio *et al*, 2021)。

黄条鰤(*Seriola aureovittata*)是一种大型长距离洄游的掠食性鱼类(Booth *et al*, 2022; 柳学周等, 2017)。本团队于 2017 年突破了黄条鰤苗种繁育技术, 并开展了其生长和生殖功能基因的相关研究(Wang *et al*, 2021; 徐永江等, 2019)。黄条鰤食量较大且摄食凶猛, 在养殖过程中, 养殖者需了解黄条鰤的摄食特性及其内在调控机制, 以便对投喂策略做出调整, 在节约养殖成本的同时, 促进养殖鱼类的生长, 从而获得更大的经济效益。NPY 作为一种强有力的促食欲因子(Bertucci *et al*, 2019), 在硬骨鱼中被证明有促摄食的作用(Lei *et al*, 2019; Tolås *et al*, 2021), 但尚未在黄条鰤中鉴定出该基因。本研究拟探究 *npy* 基因在黄条鰤摄食与饥饿补偿机制中的规律, 以期为进一步研究黄条鰤的摄食和能量代谢提供基础。

# 1　材料与方法

## 1.1　实验设计与样品采集

所有动物均经中国水产科学研究院黄海水产研究所实验动物福利伦理和动物实验安全委员会批准。本研究所用黄条鰤购自大连富谷食品有限公司, 克隆及组织表达所用的黄条鰤为 3 龄鱼, 取样 3 尾雌鱼[体重为(5.58±0.21) kg, 全长为(85.67±2.18) cm]和 3 尾雄鱼[体重为(4.28±0.04) kg, 全长为(80.33±1.36) cm]。用 MS-222 (120 mg/L)将黄条鰤麻醉, 分别取脑、垂体、鳃、心、肝、脾、肾、胃、肠、肌肉和性腺等组织样品, 置于液氮中保存备用。

饥饿再投喂实验所用的黄条鰤为 1 龄鱼, 使用 180 尾实验鱼[体重为(616.25±24.23) g, 全长为(41.5±0.65) cm], 设置对照组(持续投喂)和实验组(饥饿再投喂), 每组设置 3 个平行, 每个平行 30 尾鱼, 实验正式开始前驯化 7 d。实验鱼被饲养在容积为 3 m³玻璃钢水槽中, 充气流水养殖(盐度为 27~30, pH 值为 7.8~8.2, 溶氧>6 mg/L, 水温为 24~26 ℃), 日换水率为 300%。饵料为冰鲜玉筋鱼(*Ammodytes personatus*),

每天投喂 2 次(07:00 和 18:00)至饱食, 1 h 后清理底部残饵。饥饿再投喂实验组先进行 21 d 饥饿处理, 随后 7 d 与对照组同步投喂。在实验开始后的第 7、14、21 和 28 天进行取样, 每次每组取样 6 尾。取样前 12 h 统一停止投喂, 采用 MS-222 麻醉实验鱼(身体失去平衡, 腹部向上, 眼睑有反应), 解剖后取出脑、垂体和胃, 置于液氮中保存备用。

## 1.2　总 RNA 提取与 cDNA 反转录

使用 RNAiso Plus Total RNA 提取试剂盒(TaKaRa, 日本)提取黄条鰤的组织总 RNA, 通过 NanoDrop 2000C 分光光度计(Thermo, 美国)测定 RNA 浓度, 当 $A_{260\,nm}/A_{280\,nm}$ 在 1.8~2.0 之间, RNA 可用于后续实验。使用 PrimeScript™ RT reagent kit with gDNA eraser (TaKaRa, 日本)合成 cDNA 第一链, 按照说明书的操作步骤进行, 合成的 cDNA 模板保存于 –20 ℃备用。

## 1.3　基因克隆

根据 NCBI 数据库中预测的 *npy* 序列(XP_023282438.1)设计特异性引物(表 1)。基因克隆以脑 cDNA 为模板, PCR 体系(50 μL)包含 25 μL r*Taq* 酶、ddH$_2$O 21 μL、上下游引物各 1 μL 和 cDNA 模板 2 μL。PCR 扩增条件: 95 ℃预变性 5 min; 95 ℃变性 30 s, 55 ℃退火 30 s, 72 ℃延伸 30 s, 38 个循环; 最后 72 ℃再延伸 5 min, 4 ℃保存。

将符合目的基因大小的条带用 Steady Pure DNA 凝胶回收试剂盒(艾科瑞生物)进行胶回收, 将胶回收产物与 pEASY-T1 Simple 载体、Trans1-T1 Phage Resistant 感受态细胞(TransGene Biotech, 北京)进行连接转化, 37 ℃培养箱中培养 12 h, 挑取阳性克隆产物送至生工生物工程(上海)股份有限公司测序。

## 1.4　基因定量表达分析

基因定量表达分析使用 TB Green Premix Ex *Taq*™ Ⅱ试剂盒(TaKaRa, 日本), 仪器为 Lightcycler

**表 1　本研究所用引物**
Tab.1　Primers used in this study

| 引物名称 Primer name | 引物序列 Primer sequence (5′~3′) | 退火温度 $T_m$ /℃ | 目的 Purpose |
|---|---|---|---|
| *npy* F | ATGCAGCCTAACATGGTGAGC | 55 | ORF sequence amplification |
| *npy* R | TCACCACAATGACGGGTCAT | | |
| *npy* qF | GCGGAGGAACTGGCCAAATA | 60 | qPCR |
| *npy* qR | CAACAGCAGCTCTGAGACCA | | |
| 18S F | TACCACATCCAAAGAAGGCA | 53 | |
| 18S R | TCGATCCCGAGATCCAACTA | | |

480 Ⅱ Real-time PCR 仪(Roche, 瑞士)。PCR 体系 (10 μL): TB Green Premix Ex *Taq* Ⅱ 5 μL, 上、下游引物(10 μmol/L)各 0.4 μL, ddH$_2$O 3.2 μL, cDNA 模板 1 μL。PCR 扩增条件: 95 ℃ 30 s; 95 ℃ 5 s, 60 ℃ 20 s, 共 40 个循环, 反应结束后进行熔解曲线分析以验证产物特异性。所有目的基因和内参基因的标准曲线相关系数($r^2$)和扩增效率($E$): 0.99<($r^2$)<0.999, 0.9<$E$<1.1。18S 为内参基因, 基因相对表达量参照 2$^{-\Delta\Delta Ct}$ 法计算(Livak *et al*, 2001)。

### 1.5 结果分析

使用 NCBI 数据库(https://www.ncbi.nlm.nih.gov/)查找预测的黄条鰤 *npy* 序列, 利用软件 SignalP5.0 Server (http://www.cbs.dtu.dk/services/SignalP/) 分析黄条鰤 *npy* 基因的信号肽, 通过 Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/)进行序列比对和同源性分析, 利用软件 ExPASy (http://web.expasy.org/compute_pi/)分析成熟蛋白的分子量和等电点, 采用 SWISS-MODEL 自动化蛋白质建模服务器 ProMod Ⅱ 程序估计三级蛋白质结构 (https://www.expasy.org/swissmod/SWISS-MODEL.html)。通过 MEGA 7.0 软件, 以邻接法 Neighbor-Joining (NJ)构建系统进化树。

实验数据以平均值±标准误(Mean±SE)表示。采用 SPSS 26.0 统计软件进行 T 检验、单因素方差分析 (one-way ANOVA)和 Duncan 多重比较, 显著性水平 $P$ 设为 0.05, 当 $P$<0.05 时认为差异显著, 当 $P$<0.01 时认为差异极显著。

## 2 结果

### 2.1 黄条鰤 *npy* cDNA 序列克隆、组织分布和系统进化分析

黄条鰤 *npy* 基因的 ORF 序列长度为 300 bp, 编码 99 个氨基酸, 其中, 包括 28 个氨基酸的信号肽, 36 个氨基酸的成熟肽, 32 个氨基酸的 C 端未知功能肽段和 1 个 GKR 蛋白水解位点(图 1), 预测的蛋白质分子量为 11.24 kDa, 等电点为 5.02。

黄条鰤与斑马鱼(*Danio rerio*)、大西洋鲑等物种的 NPY 氨基酸序列比对结果见表 2。黄条鰤 *npy* 与高体鰤(*Seriola dumerili*) (99.0%)、条纹鲈鱼(*Morone saxatilis*) (98.0%)、大口黑鲈(*Micropterus salmoides*) (96.0%)、大菱鲆(*Scophthalmus maximus*) (94.9%)的 *npy* 表现出高度的同源性, 其次是鳜鱼(*Siniperca chuatsi*)和半滑舌鳎(*Cynoglossus semilaevis*) (93.9%)、青鳉(*Oryzias latipes*) (92.9%)。此外, 与其他鱼相比,



图 1 黄条鰤 *npy* 基因 ORF 序列和推导的氨基酸序列
Fig.1 Sequence of open reading frame and deduced amino acid of *S. aureovittata npy* gene

起始密码子加框, 终止密码子用星号表示。
推定的信号肽加下划线, 水解位点用方框表示。
成熟肽标以下划线和粗体字母表示。
The start codon is boxed, and the stop codon is indicated by asterisk. Putative signal peptides are underlined, and proteolytic site is framed. Mature peptides are underlined and indicated in bold letters.

序列相似度也较高(66.3%~80.8%)。*npy* 基因序列的三级结构由 2 个螺旋区组成, 即 α 螺旋和富含脯氨酸的螺旋区。2 个螺旋区是互逆平行的, 都有两性电离的特点, 且靠疏水键维持稳定(图 2A)。与其他脊椎动物相比较, 黄条鰤 *npy* 信号肽和成熟肽的结构非常保守, 都包含 28 个氨基酸的信号肽、36 个氨基酸的成熟肽、3 个脯氨酸和 2 个酪氨酸残基(Pro$^{2/5/8}$ 和 Tyr$^{20/27}$) (图 2B)。系统进化分析表明, 黄条鰤 *npy* 与高体鰤聚为一个小的分支, 且与鲈形目(Perciformes)、鲽形目 (Pleuronectiformes)的鱼类聚为一个大的分支(图 3)。

### 2.2 *npy* 基因的组织表达分析

*npy* 在黄条鰤脑、垂体和胃中表达量较高, 其他组织少量表达。通过对雌、雄黄条鰤 *npy* 的组织表达特性比较发现, 在雌鱼和雄鱼的性腺组织中, *npy* mRNA 表达水平呈现显著性差异($P$<0.05), 在肠组织中存在极显著性差异($P$<0.01) (图 4)。

### 2.3 饥饿再投喂对黄条鰤 *npy* 基因表达的影响

与对照组相比, 饥饿组黄条鰤脑、垂体和胃组织中 *npy* mRNA 上调表达, 恢复投喂后 *npy* mRNA 表达水平下降。脑组织中, 随着饥饿时间的延长, 实验组 *npy* mRNA 表达水平有上升的趋势, 恢复投喂 7 d 后恢复到对照组水平(图 5A)。垂体组织中, 实验组 *npy* mRNA 变化趋势与脑相似, 特别是在饥饿 21 d, 与对照组相比, 实验组 *npy* 显著高表达($P$<0.05) (图 5B)。实验组胃组织中 *npy* mRNA 水平在饥饿 14 d 时略高于对照组, 恢复投喂 7 d 后略低于对照组(图 5C)。

表 2　黄条鰤与其他脊椎动物 *npy* 基因氨基酸序列的同源性分析/%

Tab.2　Homology analysis of amino acid sequences of *npy* gene of *S. aureovittata* and other vertebrates /%

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 100 | | | | | | | | | | | | | | | | | |
| 2 | 83.5 | 100 | | | | | | | | | | | | | | | | |
| 3 | 64.6 | 64.6 | 100 | | | | | | | | | | | | | | | |
| 4 | 61.5 | 62.5 | 65.3 | 100 | | | | | | | | | | | | | | |
| 5 | 63.5 | 64.6 | 65.3 | 97.0 | 100 | | | | | | | | | | | | | |
| 6 | 80.4 | 90.7 | 66.7 | 63.5 | 65.6 | 100 | | | | | | | | | | | | |
| 7 | 63.9 | 61.9 | 64.6 | 79.8 | 82.8 | 63.9 | 100 | | | | | | | | | | | |
| 8 | 61.5 | 61.5 | 64.2 | 91.9 | 94.9 | 62.5 | 77.8 | 100 | | | | | | | | | | |
| 9 | 64.9 | 61.9 | 64.6 | 79.8 | 82.8 | 63.9 | 98.0 | 77.8 | 100 | | | | | | | | | |
| 10 | 62.5 | 64.6 | 64.2 | 93.9 | 97.0 | 65.6 | 81.8 | 91.9 | 81.8 | 100 | | | | | | | | |
| 11 | 64.6 | 64.6 | 65.3 | 96.0 | 99.0 | 65.6 | 81.8 | 93.9 | 81.8 | 96.0 | 100 | | | | | | | |
| 12 | 65.6 | 64.6 | 66.3 | 96.0 | 98.0 | 65.6 | 80.8 | 92.9 | 80.8 | 94.9 | 99.0 | 100 | | | | | | |
| 13 | 63.5 | 63.5 | 93.8 | 67.4 | 67.4 | 66.7 | 66.7 | 66.3 | 66.7 | 66.3 | 66.3 | 67.4 | 100 | | | | | |
| 14 | 62.5 | 62.5 | 92.7 | 66.3 | 66.3 | 65.6 | 65.6 | 65.3 | 65.6 | 65.3 | 65.3 | 66.3 | 97.9 | 100 | | | | |
| 15 | 64.6 | 66.7 | 63.2 | 92.9 | 96.0 | 64.6 | 79.8 | 90.9 | 79.8 | 92.9 | 94.9 | 93.9 | 65.3 | 64.2 | 100 | | | |
| 16 | 80.4 | 76.3 | 61.5 | 58.3 | 59.4 | 74.2 | 58.8 | 57.3 | 58.8 | 60.4 | 60.4 | 61.5 | 59.4 | 58.3 | 59.4 | 100 | | |
| 17 | 85.6 | 94.8 | 65.6 | 61.5 | 63.5 | 92.8 | 60.8 | 61.5 | 60.8 | 63.5 | 63.5 | 63.5 | 64.6 | 63.5 | 62.5 | 78.4 | 100 | |
| 18 | 62.5 | 63.5 | 65.3 | 94.9 | 96.0 | 64.6 | 79.8 | 90.9 | 79.8 | 94.9 | 94.9 | 93.9 | 67.4 | 66.3 | 91.9 | 59.4 | 62.5 | 100 |

注：1. 安乐蜥；2. 牛；3. 斑马鱼；4. 大口黑鲈；5. 条纹鲈鱼；6. 小家鼠；7. 虹鳟；8. 青鳉；9. 大西洋鲑；10. 大菱鲆；11. 高体鰤；12. 黄条鰤；13. 中华倒刺鲃；14. 银鲫；15. 鳜鱼；16. 非洲爪蟾；17. 人；18. 半滑舌鳎。

Note: 1. *Anolis carolinensis*; 2. *Bos taurus*; 3. *Danio rerio*; 4. *Micropterus salmoides*; 5. *Morone saxatilis*; 6. *Mus musculus*; 7. *Oncorhynchus mykiss*; 8. *Oryzias latipes*; 9. *Salmo salar*; 10. *Scophthalmus maximus*; 11. *Seriola dumerili*; 12. *Seriola aureovittata*; 13. *Spinibarbus sinensis*; 14. *Carassius gibelio*; 15. *Siniperca chuatsi*; 16. *Xenopus laevis*; 17. *Homo sapiens*; 18. *Cynoglossus semilaevis*.

# 3　讨论

## 3.1　黄条鰤 *npy* 基因克隆与序列分析

黄条鰤 *npy* 基因编码 99 个氨基酸，这与大口黑鲈(刘浩等, 2016)、牙鲆(*Paralichthys olivaceus*) (Wang *et al*, 2015)一致，而银鲫 *npy* 基因编码 96 个氨基酸 (Lei *et al*, 2019)，大西洋鲑 *npy* 基因编码 100 个氨基酸(Murashita *et al*, 2009)。黄条鰤成熟肽中的 3 个脯氨酸和 2 个酪氨酸残基($Pro^{2/5/8}$ 和 $Tyr^{20/27}$)在脊椎动物中是非常保守的，这些脯氨酸和酪氨酸残基在维持 *npy* 家族的构象中具有重要作用(Cerdá-Reverter *et al*, 2000)。与其他硬骨鱼类相似，黄条鰤 *npy* 的成熟肽 C 端附近也发现 13 个氨基酸残基(ALRHYINLITRQR)，说明这 13 个氨基酸残基高度保守(Deng *et al*, 2019; Lei *et al*, 2019)。在爬行类、两栖类、哺乳类和硬骨鱼类的 NPY 成熟肽中不含半胱氨酸(C)，只在信号肽第 21 个氨基酸位点上有一个半胱氨酸(C)，在重口裂腹鱼(Deng *et al*, 2019)、银鲫(Lei *et al*, 2019)也得到相同结果。通过氨基酸比对发现，黄条鰤与条纹鲈鱼、大口黑鲈分别有 2 个和 3 个氨基酸差异，与高体鰤仅有 1 个氨基酸不同，并且与高体鰤 *npy* 编码的氨基酸序列一致性高达 99%。另外，构建的 *npy* 基因的系统进化树显示，黄条鰤与鲈形目、鲽形目其他鱼类聚为一支，亲缘关系较近。

## 3.2　黄条鰤 *npy* 组织分布特征

黄条鰤 *npy* mRNA 在大部分组织中均有表达，特别是脑、垂体和胃中有较高的表达，这与银鲫(Lei *et al*, 2019)、重口裂腹鱼(Deng *et al*, 2019)、斑马鱼(Kaniganti *et al*, 2021)的结果一致。本研究还发现，黄条鰤雌鱼和雄鱼肠组织和性腺中 *npy* mRNA 的表达有显著性差异，表明 *npy* 基因在黄条鰤的组织表达模式具有明显的性别二态性，这与黄条鰤 *hsp*70 基因的组织表达模式一致(方璐等, 2023)，提示 *npy* 基因在雌、雄性腺发育、消化等生理过程中发挥重要的差异化调控作用，具体的功能差异及可能的作用途径有待深入研究确证。目前，在其他硬骨鱼中尚未见 *npy* 基因的性别二态性相关报道，根据这一特性，可以在产卵前针对性地对雌、雄亲鱼分别进行营养强化，或可
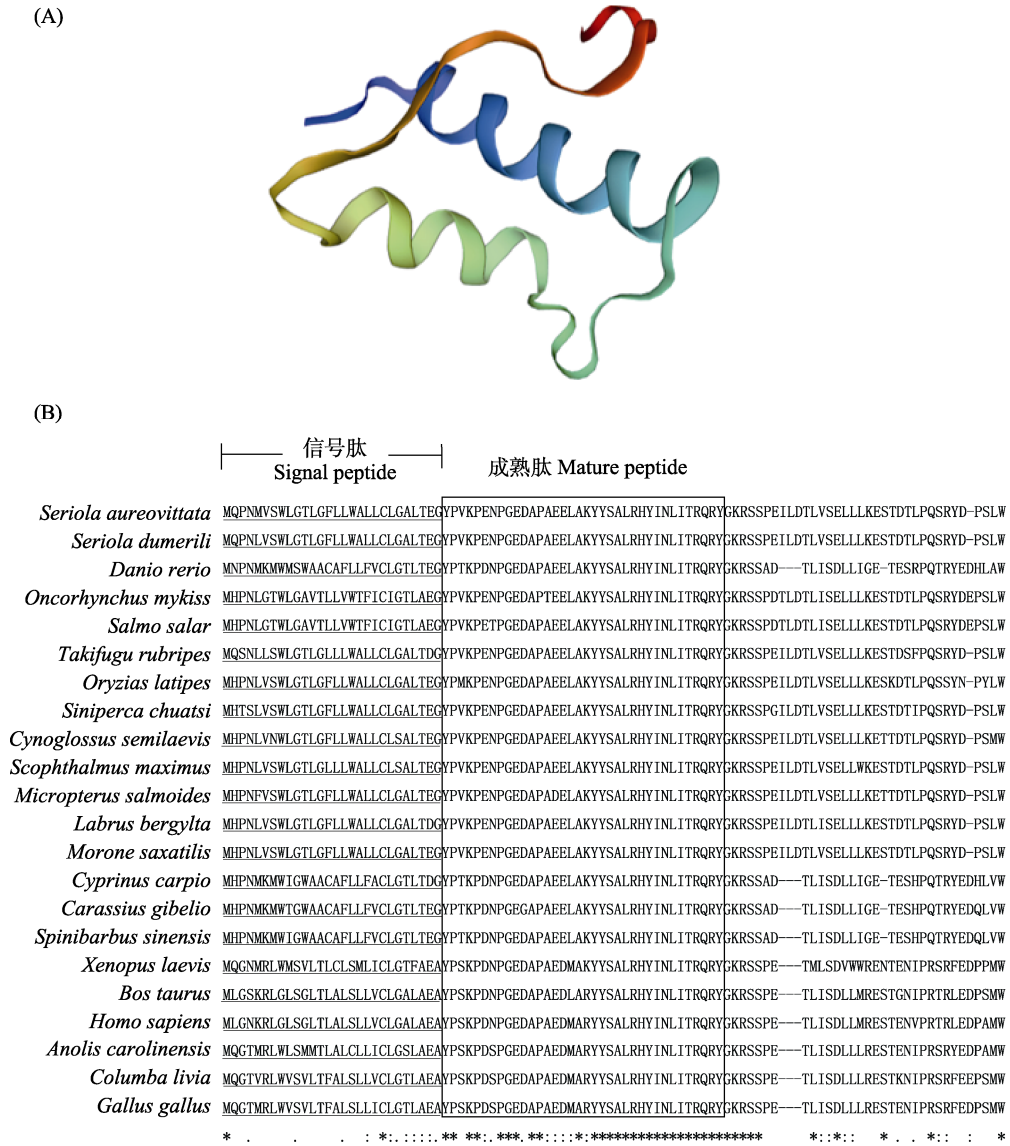
图 2　黄条鰤 NPY 的分子特征
Fig.2　Molecular characterization of *S. aureovittata* NPY

A：预测的黄条鰤 NPY 三级结构；B：黄条鰤 *npy* 基因编码的氨基酸序列与其他脊椎动物的比对分析。
连字符(–)指示在一些序列中引入的间隙以最大化对齐，相同的序列用星号(*)表示，保守氨基酸用点号(.)表示，
高度保守的氨基酸用冒号(:)表示，推定的信号肽标以下划线表示，NPY 成熟肽用方框表示。
A: The predicted tertiary structure of NPY in *S. aureovittata*; B: Alignment of the amino acid sequences of the *npy* gene between *S. aureovittata* and other vertebrates. Gaps introduced in some sequences to maximize the alignment are indicated by hyphens. Identical sequences are indicated by asterisks. Dots denote conserved amino acids, and colons indicate highly conserved amino acids. Putative signal peptides are underlined, the mature NPY peptide sequences are boxed.

为黄条鰤亲鱼提供差异化强化培育的思路。

### 3.3　黄条鰤 NPY 对饥饿再投喂的应答特性

摄食调控是由中枢和外周系统协同完成。已有研究证实，NPY 行使生理功能主要是通过 NPY 受体的直接或间接作用来实现。斑马鱼室旁核中的 NPY 与 NPY Y1、NPY Y2 或 NPY Y5 受体结合引发传出信号，激活 PKC 通路，增加食欲和摄食量(Yokobori *et al*, 2012)。对大西洋鲑的研究表明，弓状核中的 NPY 神经元还可以对进入的外周信号如瘦素、胰岛素等因子做出快速反应，信号向下传递到各个组织，刺激胃等消化系统分泌 *npy* mRNA (Kalananthan *et al*, 2020)；同时上调促摄食因子(*agrp* 和 *ghrelin*)，下调抑摄食因子(*pomc* 和 *leptin*) (Opazo *et al*, 2019; Zhang *et al*, 2020)，从而实现对生物体的摄食调控。
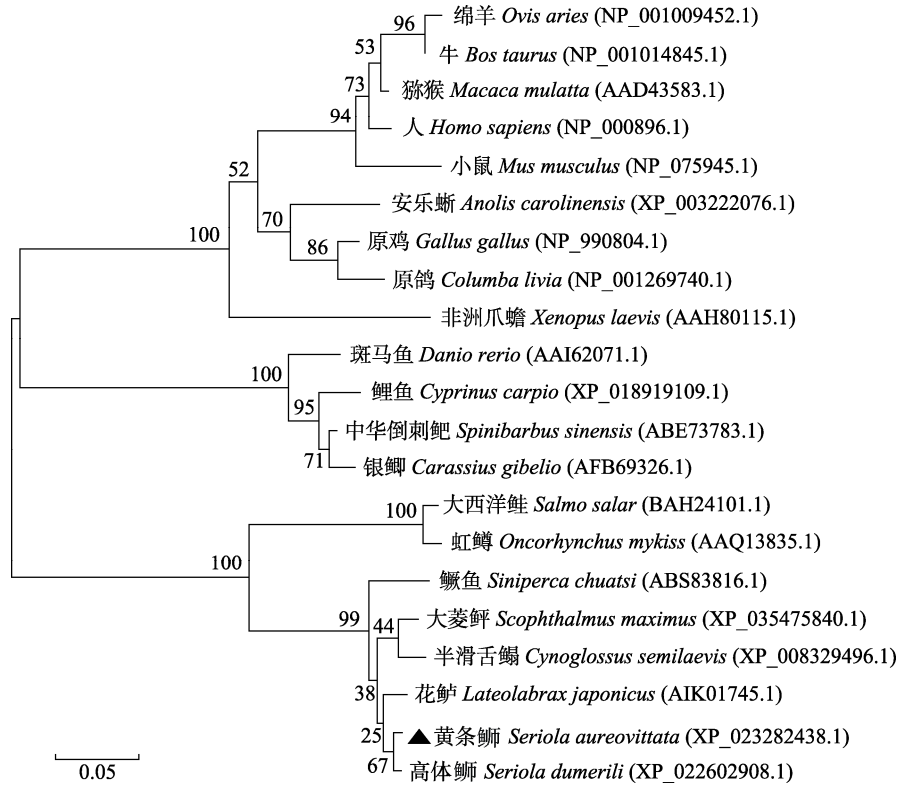
本研究中，黄条鰤在饥饿期间，*npy* mRNA 呈现

图 3　黄条鰤 *npy* 基因与其他脊椎动物的 NJ 系统进化树

Fig.3　The phylogenetic tree of *npy* gene of *S. aureovittata* and other vertebrates using neighbor-joining method
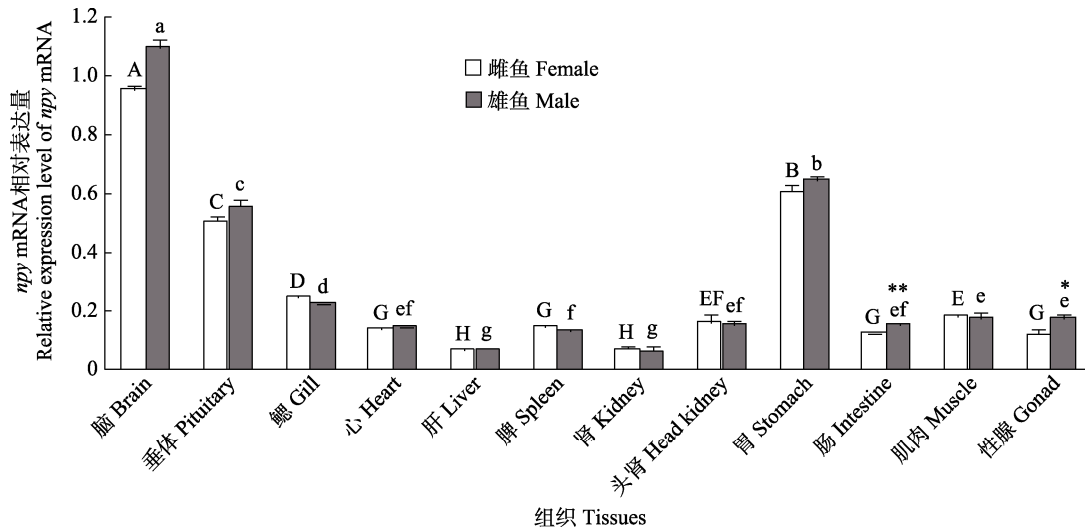


图 4　黄条鰤 *npy* mRNA 的组织表达分布特征

Fig.4　Spatial expression pattern of *npy* mRNA of *S. aureovittata*

柱上方相同字母表示差异不显著($P>0.05$)，不同小写字母表示雄鱼各个组织之间存在显著性差异($P<0.05$)，不同大写字母表示雌鱼各个组织之间存在显著性差异($P<0.05$)。

*表示雌鱼和雄鱼在同一组织内存在显著差异($P<0.05$)，**表示存在极显著性差异($P<0.01$)。

Above the same column, the same letters indicate no significant difference ($P>0.05$), while with different lowercase letters mean significant difference between the various tissues of male fish ($P<0.05$), and different uppercase letters mean significant difference between the various tissues of female fish ($P<0.05$). * indicates significant difference within the same tissue between female and male fish ($P<0.05$), and ** indicates highly significant difference within the same tissue between female and male fish ($P<0.01$).
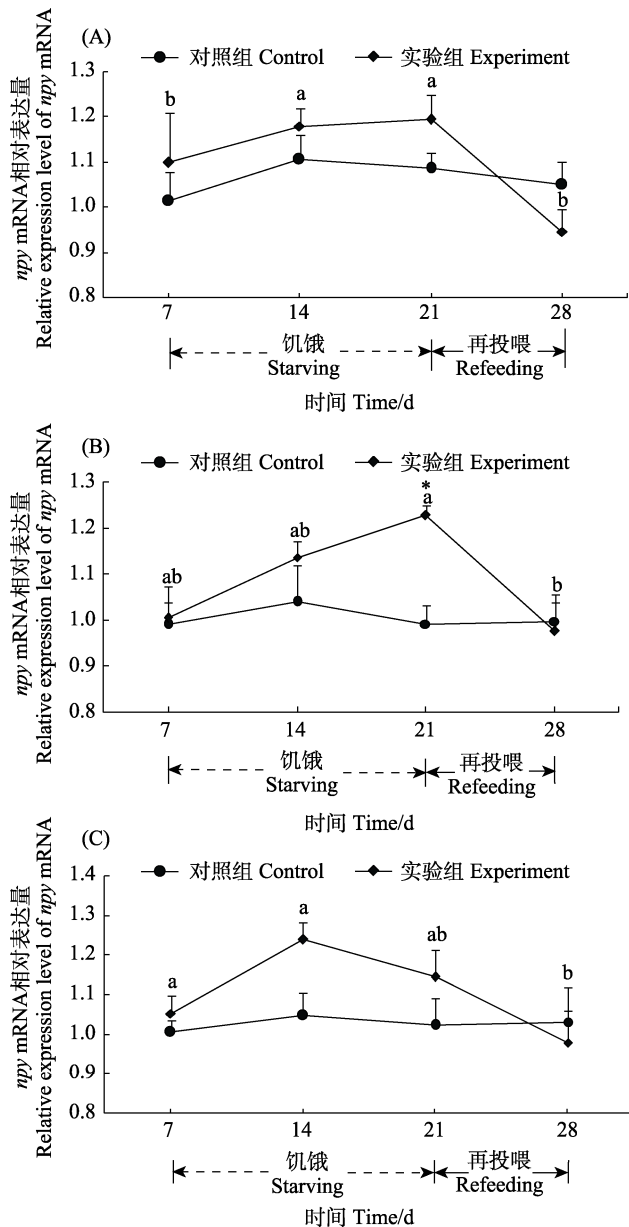
图 5 黄条鰤的脑(A)、垂体(B)和胃(C)中 *npy* mRNA
在饥饿再投喂条件下的相对表达

Fig.5 Relative expression of *npy* mRNA in the brain (A),
pituitary (B) and stomach (C) of *S. aureovittata*
after starving and refeeding

不同字母表示组内有显著性差异(*P*<0.05)，*表示在同一个
时间点，对照组和实验组之间存在显著性差异(*P*<0.05)。
Different letters indicate significant difference within the group
(*P*<0.05), and * indicates significant difference between control
and experimental groups fish at one given time point (*P*<0.05).

为上调表达，恢复投喂 7 d 后，*npy* mRNA 表达水平
恢复到对照组水平，这与银鲫(Lei *et al*, 2019)、重口
裂腹鱼(Deng *et al*, 2019)的研究结果相似，表明与其
他硬骨鱼一样，黄条鰤 *npy* 也参与了摄食调控。

本研究中，饥饿期间，黄条鰤脑和垂体 *npy* mRNA

表达量上升，其原因可能是饥饿刺激神经中枢发出促
食信号，从而启动了 *npy* mRNA 的表达，从而达到增
强食欲的作用(Yousefvand *et al*, 2021)；且在恢复投喂
7 d 后，神经中枢发出饱食信号，使 *npy* mRNA 表达
量略低于对照组。本研究还发现，黄条鰤饥饿 21 d，
脑组织中 *npy* mRNA 的表达量升高(*P*>0.05)；Kehoe
(2007)等对大西洋鳕(*Gadus morhua*)研究发现，饥饿
7 d，其脑组织中 *npy* mRNA 表达变化同本结果一致；
然而，Deng (2019)研究发现，重口裂腹鱼饥饿 5 d 时，
其脑组织中 *npy* mRNA 出现显著变化。上述研究表
明，*npy* 基因在禁食初期出现显著应答的时间不同，
这可能是由于物种特异性和环境差异造成。另外，本
研究仅检测了脑、垂体和胃组织中 *npy* 基因的表达调
控特性，未对 *npy* 信号通路上其他相关基因如 *leptin*、
*ghrelin*、*npyr*、*pomc*、*gh* 和 *igf* 等摄食与生长调控因
子进行检测及互作关系进行分析，因此，未能进一步
揭示 *npy* 基因对黄条鰤摄食调控作用的信号通路，下
一步本实验室将深入开展相关研究，揭示 *npy* 调控黄
条鰤摄食作用的信号通路。

胃是动物重要的消化器官，在摄食调控中扮演了
重要角色(Abdalla, 2017)。本研究中，与脑、垂体组
织一样，黄条鰤胃组织中 *npy* mRNA 也存在着明显的
变化，表明胃中的 *npy* 基因对饥饿再投喂产生了应
答，也参与了黄条鰤的摄食调节。Kalananthan 等(2020)
研究发现，大西洋鲑胃的扩张与肠壁上的内分泌因子
和受体之间的相互作用调节着肽激素的分泌，这些肽
激素将胃和肠道的饱腹程度以及营养含量传递给中枢
系统。本研究中，饥饿再投喂诱导黄条鰤脑、垂体和
胃中 *npy* mRNA 表达的规律一致，均呈现上升再下降
的变化趋势，说明脑、垂体和胃中 *npy* 对于饥饿的响
应机制是一致的。关于 *npy* 基因的调控方式仍需进一
步研究，以探明 *npy* 基因在黄条鰤饥饿再投喂中的机
制，进一步优化黄条鰤的摄食投喂策略。

综上，本研究首次克隆了黄条鰤 *npy* 基因的 ORF
序列并对其结构和表达模式进行了研究。*npy* 转录本
主要在脑中显著高表达，其次是垂体和胃，这与其他
硬骨鱼类似。饥饿再投喂实验表明，黄条鰤NPY 是
具有显著促食欲的内分泌因子，其促摄食的分子调控
机制值得进一步研究。

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(编辑　冯小花)

# Molecular Cloning and Characterization of *npy* Gene and Its Response to Starvation-Refeeding Strategy in *Seriola aureovittata*

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**Abstract**　　　　Yellowtail kingfish (*Seriola aureovittata*), a pelagic marine finfish species with a worldwide distribution, is regarded as an emerging candidate for the aquaculture industry owing to its fast growth, superior flesh quality, and farming suitability in both sea cages and land-based facilities in China. The species has high economic value and is the second most produced *Seriola* species in the world following Japanese yellowtail *Seriola quinqueradiata*. Researchers worldwide have studied the role of regulatory factors neuropeptide Y (NPY) in fish feeding regulation. In recent years, there has been great progress in research on food intake in fish, however, very little attention has been paid to the endocrine regulation mechanism of food intake. Methods on strengthening the production performance of fish through appetite regulation is still a hot research topic.

　　　　The control of food intake and energy metabolism in vertebrates are complex processes involving several neural pathways. Some hypothalamic signals are released by peripheral tissues that are associated with energy homeostasis or nutrient availability. Among the signaling molecules involved, NPY plays a key role. NPY is recognized as one of the most effective appetite regulators, which primarily function as a signaling factor to regulate a variety of biological processes such as food intake and glucose homeostasis. The orexigenic actions of NPY have been well investigated thoroughly over the past decades. Much evidence supports that NPY´s functional role as a regulator of energy homeostasis and appetite control is conserved across vertebrates, including teleosts. In several species, including rainbow trout, Nile tilapia, and grass carp, NPY injections increase food intake, supporting an orexigenic role. In line with this, food deprivation increased *npy* mRNA expression in the brain, such as seen for goldfish and Johnny carp. Moreover, refeeding normalized *npy* mRNA abundance following food deprivation. As *S. aureovittata* feeds heavily and fiercely, the breeding industries need to understand its feeding control mechanism. To make real-time adjustment to feeding strategy, it is necessary to obtain high-quality and high-yield aquatic products with the least input to maximize economic benefits. As a potent appetite stimulating factor, *npy* has been proven to promote feeding, but this gene has not been identified in *S. aureovittata*. Therefore, it is necessary to explore the variable rules of the *npy* gene in feeding and starvation compensation

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mechanism, to provide the special compound feed for breeding.

To gain insight into the existence of *npy* in *S. aureovittata*, we used homologous cloning, RNA extraction and reverse-transcription to obtain the ORF sequence of *npy*. *npy* belongs to the pancreatic polypeptide (PP) family, which plays an important role in appetite regulation and energy expenditure in mammals and fish. The ORF of *S. aureovittata npy* is 300 nucleotides in size and encodes a 99-amino-acid precusor, with a calculated molecular mass and isoelectric point of 11.24 kDa and 5.02, respectively. The precursor protein is composed of a predicted signal peptide of 28-aa in size, 36-aa putative mature peptide, a GKR protein proteolytic site, and a 32-aa C terminus of unknown function. Bioinformatics analysis on the amino acid sequence identities and evolutionary relationships of the *npy* was performed. Comparison of homology of the precursor peptide sequences of *npy* analysis revealed that *S. aureovittata npy* displayed a high degree of identity with the counterparts of *Seriola dumerili* (99.0%), *Morone saxatilis* (98.0%), *Micropterus salmoides* (96.0%), and *Scophthalmus maximus* (94.9%), followed by *Cynoglossus semilaevis* (93.9%) and *Oryzias latipes* (92.9%). Phylogenetic analysis highly supported that the *npy* of *S. aureovittata* was closely related to that of *S. dumerili*. Furthermore, using real-time quantitative PCR, we found that the *npy* mRNA is widely expressed in 12 tissues, with abundant expression in the brain, followed by the pituitary and stomach. In addition, except for the intestine and gonad, *npy* was found to have no significant difference in all other detected tissues of both sexes. To establish the functional link between *npy* and feeding, the expression profiles of *npy* mRNA during food deprivation and refeeding were examined in *S. aureovittata*. We detected the 7 d, 14 d, and 21 d starvation and 7 d refeeding effect on *npy* mRNA levels. Results showed that fasting induced an increase of *npy* mRNA levels in brain, pituitary, and stomach when compared to the control groups. Interestingly, the pituitary *npy* transcripts significantly increased after 21 d of starvation compared with the control group. In addition, refeeding normalized *npy* mRNA abundance following food deprivation in the brain, pituitary, and stomach. These results indicated that *npy* is involved in the regulation of feeding and energy homeostasis in *S. aureovittata*. Collectively, we provided initial evidence for the existence of *npy* in *S. aureovittata* and suggested its involvement in the regulation of feeding, which plays an important role in the starvation compensation mechanism.

In summary, we obtained the ORF sequence of *npy* and clarified its role as a potent orexigenic peptide in feeding regulation of *S. aureovittata*, which would be beneficial for specific feed for this species.

**Key words**　　*Seriola aureovittata*; *npy*; Cloning; Tissue expression; Food intake