Neural Mechanisms of Mutualistic Fish Cleaning Behaviour: a Study in the Wild

Romeo, Daniele[1]; Ramirez-Calero, Sandra[1,2]; Ravasi, Timothy[3]; Rodolfo-Metalpa, Riccardo[4]; Schunter, Celia[1,5*]

[1]*Swire Institute of Marine Science, School of Biological Sciences, The University of Hong Kong, Pokfulam Rd, Hong Kong SAR*

[2]*Departament de Biologia Marina, Institut de Ciències del Mar (CSIC), Barcelona, Spain.*

[3]*Marine Climate Change Unit, Okinawa Institute of Science and Technology Graduate University, Onna-son, Japan*

[4]*IRD Institut de Recherche pour le Développement, Nouméa cedex, New Caledonia*

[5]*State Key Laboratory of Marine Pollution, City University of Hong Kong, Hong Kong, Hong Kong SAR, China.*

**correspondence to: celiaschunter@gmail.com*

Abstract

One crucial interaction for the health of fish communities in coral reefs is performed by the cleaner fish by removing ectoparasites and other particles from the body of other fish, so called clients. Studying the underlying mechanisms of this behaviour is essential to understanding how species react to social stimuli and defining the drivers of mutualism. Here, we pinpoint the neural molecular mechanisms in the cleaning behaviour of *Labroides dimidiatus* in the wild through an *in-situ* interaction experiment at a coral reef in New Caledonia. Five cleaners and clients (*Abudefduf saxatilis*) were placed into underwater aquaria to interact, while five were not presented with a client. The brain transcriptomes revealed 291 differentially expressed genes in cleaners that were interacting with a client. Among these genes, *grin2d*, *npy*, *slc6a3* and immediate early genes (*fosb*; *fosl1*; *nr4a1)* were related to learning and memory, glutamate and dopamine pathways, which confirm molecular pathways observed in laboratory studies. However, a new potential mechanism was found with *npy* (Neuropeptide Y) as a driver of feeding behaviour. These results show that in-situ experiments are essential for corroborating interpretations inferred from experiments in captivity and identify drivers of interspecific interactions.

Keywords: mutualism, cleaner fish, brain transcriptome, neuroethology, cleaning behaviour

Introduction

Social behaviour affects individual fitness in animal populations, influencing their persistence (1). The interaction between two species can lead to conflictual situations, such as competing for the same limited resources (competition) but can also be a beneficial collaboration (mutualism) where both parties gain an advantage from the interaction (2). Over time, the expression of such interaction behaviours has been shaped by natural selection by finetuning the ability of individuals to maximize the benefits of social interactions (3). This led some species to specialize and evolve sophisticated social behaviours, using interaction with other individuals to cover basic biological needs such as feeding or predator defence (4,5). Mutualistic interactions are frequent in nature and require a high grade of social skills and cognition of the social environment (5–7). Thus, species involved in a mutualistic interaction can exploit the most marginal environments, capitalizing on unoccupied niches and avoiding competition (8). Therefore, mutualisms are drivers of ecosystem complexity and functions (9).

In coral reefs, the blue streak cleaner wrasse *Labroides dimidiatus* relies on social interactions as its main trophic source (10). Its survival is based on the ability to clean other fish (called clients) consuming parasites, mucus, and dead skins from their bodies (11). This cleaner fish can have over 2200 interactions per day and is considered a "dedicated cleaner" due to its persistence in cleaning throughout its life (12). Due to the central role of it social interactions, the cleaner wrasse has evolved decision-making, reputation management, and social skills to better manage its reputation and clientele in every social context (13,14). Indeed, *L. dimidiatus* has the capacity to prioritize different clients based on their ecological patterns (accessibility to only one or multiple cleaning station within the home range) and adjust cooperation levels (the ratio between parasites and mucus eaten) in the presence of a bystander client fish (4,14). Therefore, the ability to use social skills and the phenotypic flexibility shown in cleaning interactions make *L. dimidiatus* an ideal species for examining the drivers of such social interaction behaviour.

Gene expression is one of the main processes involved in the phenotypic response to the social environment, modulating species' plasticity in the short- and long-term (15,16). For instance, differential expression of Vitellogenin drives the division of labour in Hymenoptera (16) and knocking out the Hrh1 gene reduces aggressiveness in mice and zebrafish when exposed to an unfamiliar individual (17,18). For cichlids, *Astatotilapia burtoni*, behavioural responses to intruders or gravid females are regulated by nonapeptides and sex steroid gene expression (19). For the cleaning behaviour in *L. dimidiatus*, glutamatergic receptors, immediate early genes (IEGs), isotocin, estrogen and progesterone receptors as well as dopaminergic pathway genes play a central role in the brain

(20). Processes of learning and memory in the cleaner wrasse are suggested to be mediated by glutamate through different expression of both ionotropic and metabotropic receptor genes, while partner recognition, which is a key factor influencing the interaction behaviour, may be driven by IEGs (20). In addition, reduction of dominance towards the client and promotion of prosocial behaviour have been associated with the expression of estrogen and progesterone (20). Thus, different gene expression patterns modulate the species' response to social stimuli, influencing their cleaning success by promoting their capacity to react to the social environment.

Most of our understanding of molecular drivers underlying social behaviours comes from mechanistic experiments conducted in captivity, and the lack of in-situ experimental data is evident. Gathering data in natural settings eliminates a potential distortion in phenotypic signals caused by captivity conditions in lab-based experiments (21,22). Thus, in-situ experiments are essential to corroborate or contest conclusions observed in ex-situ experiments. Therefore, our aim is to unravel the neural molecular drivers involved in the interaction behaviour of *L. dimidiatus* and its client through an in-situ experiment in the wild. As observed in previous studies in captivity, we may expect to find differential expression of Immediate Early Genes and genes related to neurotransmitters such as dopamine, isotocin and glutamate as main molecular drivers of cleaning interaction. Understanding the underlying mechanisms of wild cleaner's interactions with clients will elucidate the drivers that prompt two species to engage in a mutually beneficial relationship.

Methods

Sampling and behavioural experiment

This experiment was conducted in southwestern New Caledonia (-21.953529, 166.004627) between 12th and 14th March 2020. Ten individuals of *L. dimidiatus* and five individuals of the clients *Abudefduf sexfasciatus*-were collected on SCUBA using barrier nets and hand-nets. The experimental set-up included three experimental tanks (20 x 20 x 30 cm) that were placed underwater on the seafloor, filled one-fifth with sand and a weight to avoid buoyancy at approximately 30 meters from the coral reef (fig. 1). A video camera, GoPro Hero 6 Black, was placed in front of each tank.

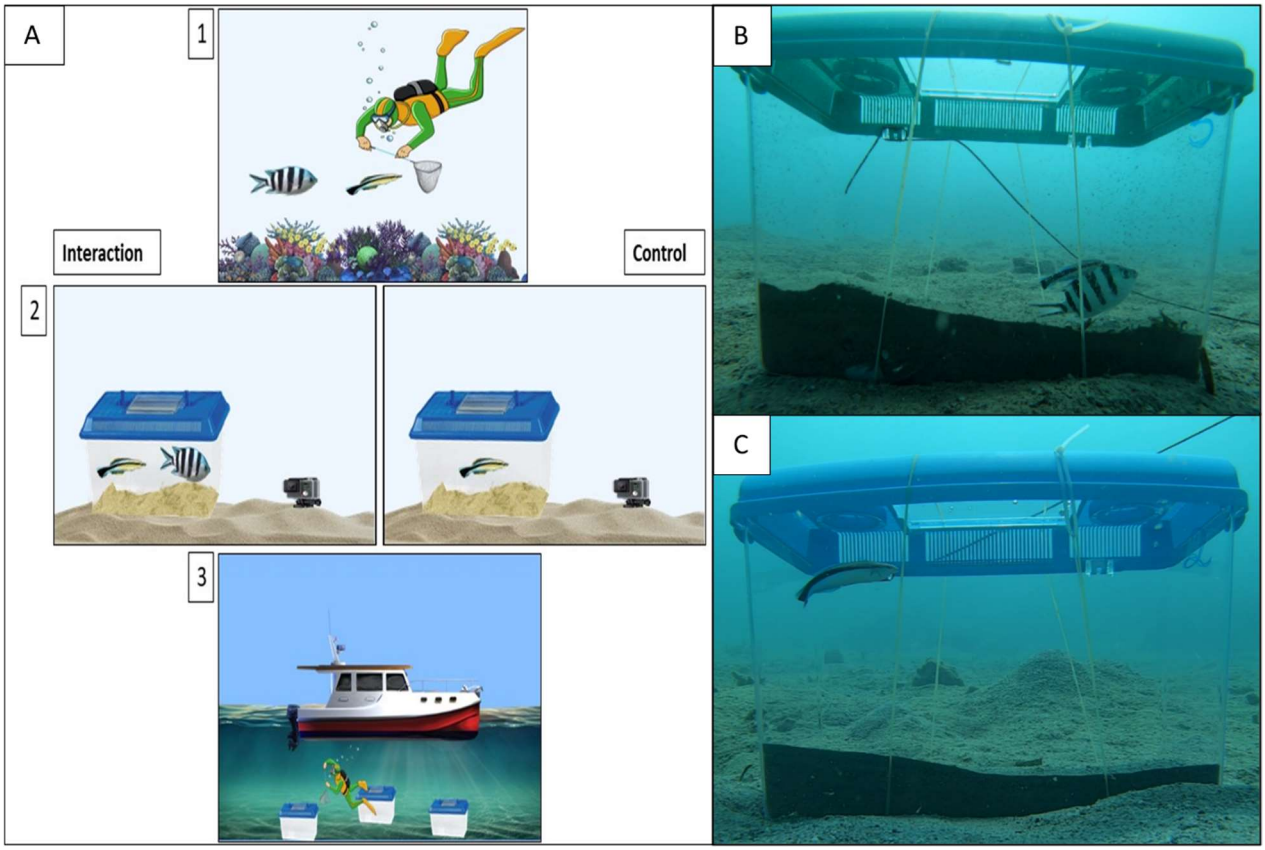

Figure 1: A) In-situ experiment: Labroides dimidiatus *are collected and placed in aquaria underwater whether with a client (Interaction) or without (Control). After the experiment, the diver brought the cleaner to the surface for brain collection. B) Experimental set up for both condition (interaction (B) and control (C)).*

Collected cleaner fish were placed first into the experimental tanks, followed by the client shortly after. To isolate the gene expression signal in cleaning interaction, a control condition was adopted where cleaner fish were placed in the tanks without clients (figure 1A2). The recording was immediately started as the fish were placed in the tank, and their behaviour was recorded for 50 minutes. Directly after, the fish were collected and brought to the water surface, where the brains

were dissected immediately on the boat, stored in RNAlater (Invitrogen), and frozen after 24 hours. Subsequently, the samples were shipped to The University of Hong Kong for further processing and kept in the -80° freezer.

Behavioural analysis

To determine if the cleaner fish interacted with the client and to detect any abnormal behaviour in the control cleaner fish, videos were analysed using the software Boris v.8.22.17 (23). The first five minutes were considered as acclimatization and were not considered in the analysis. For the interaction treatment, five different behaviours, previously standardized and calibrated in laboratory conditions (20), were evaluated: Interaction, Dance, Tactile Stimulation, Inactivity and Jolt. The time of occurrence and the duration of the first four were counted in seconds; while for Jolts only the time of occurrence was counted. Analysis parameters were considered as follows: Interaction: each time the cleaner fish approaches and inspects the client or exhibits the typical behaviour of cleaning interaction such as dancing, tactile stimulation or biting the surface of the client's body. Dance: broad and smooth symmetrical longitudinal movement to capture the attention of the client, directly linked with its willingness to interact (24). Tactile Stimulation: the cleaner places itself over the dorsal part of the client and through slightly inclination rapidly moves the pelvic fins on the back of the client (25). Inactivity: the time spent by the cleaner lying down without moving for more than two seconds, which is directly linked to stress (26). Jolt: a sudden movement of the client in response to a bite of the cleaner during an interaction which is considered cheating behaviour (breaking of the mutualism) (27).

RNA Extraction and Transcriptome Analysis

Total RNA was extracted from cleaner fish whole brain tissue using RNeasy Mini Kit (Qiagen), and the quality and quantity was checked on a 4200 TapeStation (Agilent) and a nanodrop respectively. High-quality samples (RIN>8) were used, and cDNA libraries were prepared by KAPA mRNA HyperPrep Kit and sequenced paired-end 151bp on an Illumina NovaSeq 6000 at the Centre for PanorOmic Sciences (CPOS) of the University of Hong Kong. An average of 38 (±1.23) million reads per sample were obtained, and the quality was checked by FastQC v. 0.12.1. Illumina adapters and low-quality reads were removed using Trimmomatic v0.39 (28), using the parameters: SLIDINGWINDOW:4:30; MINLEN: 40; threads: 32; 2:30:15:8:true (surviving reads 96% (±0.13)). Subsequently, to map the reads to the reference genome (29), the software HISAT2 v.2.2.1 (30) was used adopting default parameters, with a mapping rate average of 83% (±10.05%). To count the number of reads mapped to each gene in the reference, we used FeatureCounts with default parameters (31).

To statistically assess the differential gene expression between the cleaner fish that interacted with the client and the control group (cleaner fish alone), we employed the package DESeq2 v.3.18 (32) with a Wald Test statistic while adopting FDR p-adjusted significance value of 0.05 as a cut-off. Furthermore, to analyse networks of genes with significantly correlated expression patterns, weighted correlation network analysis was carried out with WGCNA v1.72-5 (33). Gene module networks were created with the command blockwiseModules by using "signed" as topological overlap measure (TOM) by setting 2000 and 30 as the cut-off for the highest and lowest number of genes in the network and an elevated power value of 21 due to the relatively low sample size. Then, eigengene values for each module were correlated with the time (in seconds) spent by the cleaner interacting with the client by using the Pearson correlation. The expression patterns of the modules of which eigengenes resulted significantly correlated with the trait (p-value < 0.05) were further analysed with paired t-tests between interaction and control. Functional enrichment on the DEGs and on the genes of the modules with significantly correlated expression patterns between interaction and control was carried out by using OmicsBox v. 3.1 using Fisher's exact test with a cut-off of FDR 0.05 (34).

Results

Behavioural analysis

The cleaner fish placed in the tank with the client on average spent 32.5 ± 20% (1069 ± 672 seconds) of the time interacting and 8 ± 18% in an inactivity state. Behavioural results are reported in detail in Supplementary Table 1. Control cleaner fish did not show any abnormal behaviour, except for one fish, which showed stereotyped movement (recurring circular displacements) and long inactivity time (2048 seconds; 39% of the total time). Therefore, this fish LD9 was excluded from the transcriptomic analysis.

Transcriptomic analysis

The cleaner fish interacting with the client exhibited 291 differentially expressed genes (DEGs) compared to the cleaner fish in the control group (Supplementary Table 2). In total, 154 enriched functions emerged from these DEGs, 114 of which were biological processes (Supplementary Table 3). Several differentially expressed genes (*gria4*, *grin3a*, *grin2d*) were related to glutamatergic synapse, and glutamate-gated receptor activity GO terms (fig. 2A, Supplementary Table 3). Several immediate early genes (IEGs) (*fosl1*, *fosb*, *foxo1a, nr4a*(*1 & 3*), *egr2b*) (fig. 3) and second cellular messengers such as *zfp36l3*, *cnga3* and *map4k2* related to learning and memory processes were also differentially expressed (fig. 2B, Supplementary Table 3). Dopamine was further altered by the interaction behaviour, reflected in dopamine biosynthetic process and regulation of dopamine metabolic process with genes *slc6a3, npy, nr4a1* and *nr4a3* underlying these functions (fig. 2C). Furthermore, the gene *npy* encoding for neuropeptide Y is involved in feeding behaviour function among other genes (fig. 2D; fig. 3). Considering the WGCNA analysis, 193 gene modules were produced, of which 12 significantly correlated with the interaction trait (Supplementary Table 4, Supplementary Figure 1). Functional enrichment analysis produced results for two modules (Sky Blue and Dark Red) (Supplementary Figure 2, 3). The module Sky Blue showed enrichment in functions related to tRNA processing and regulation of chromosome segregation (Supplementary Table 5; Supplementary Figure 2), while the module Dark Red was related to metabolism, such as lipids, protein, pyrimidine-containing compounds, regulation of glucose import and catabolic processes (Supplementary Table 6; Supplementary Figure 3). Metabolism was also a predominant function of genes in two other modules such as Powder Blue (*apoo*, *ddhd1*, *trmt61b* and *pgs1*) and Chocolate (*bdh1*, *mmut*). Genes in the Powder Blue module are also related to glutamate pathway (*frrs1l*, *dglucy*), neurotransmitter GABA (*slc6a13*), glucocorticoids (*gmeb1*), vision (*opn3*) and epigenetic processes (*setd7*) while in the module Chocolate genes are involved in synaptic plasticity *(arf1 and sstr5)* and circadian rhythm *(prkg1, per2, and nfil3)* (Supplementary Table 4).

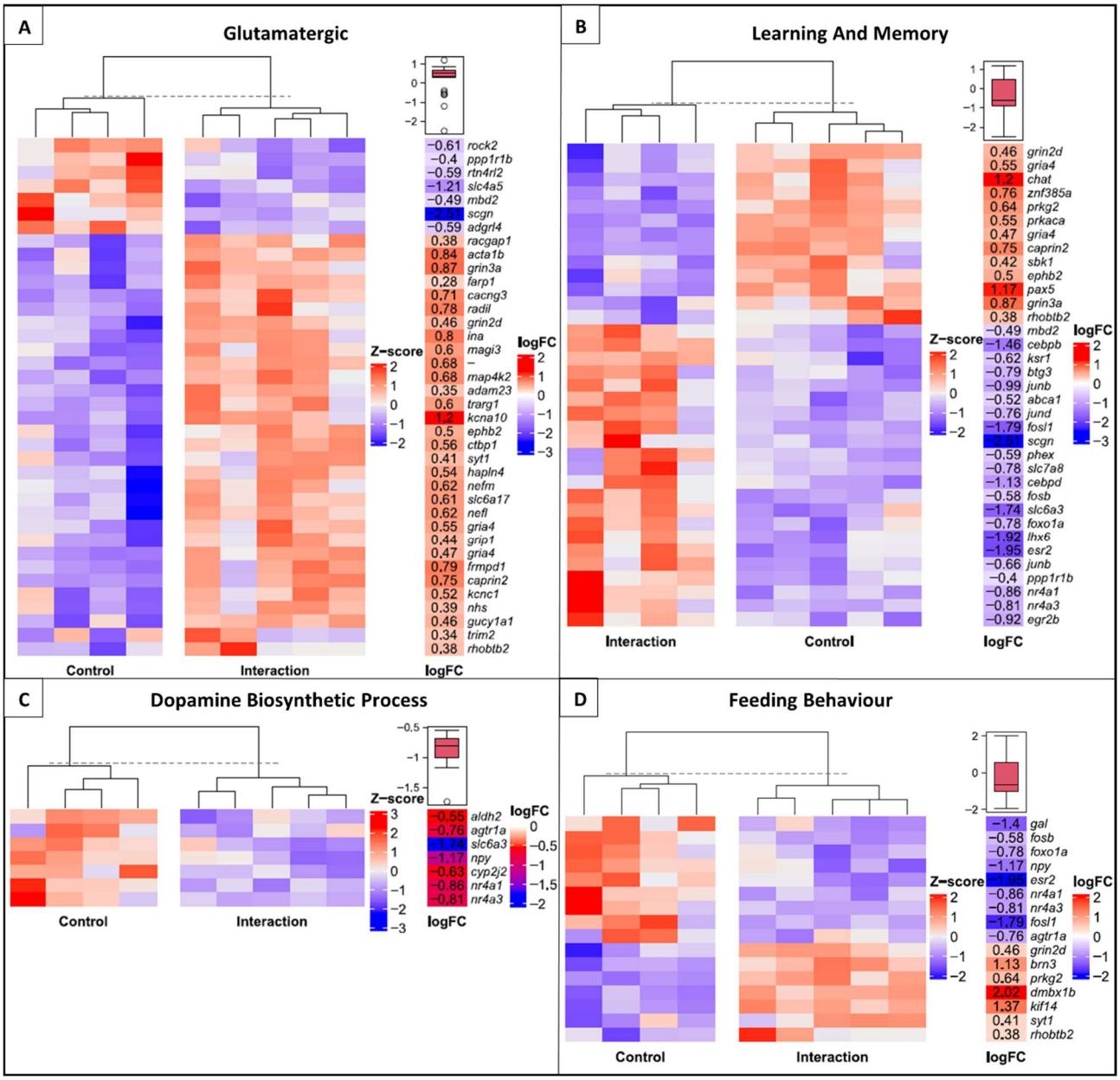

Figure 2: Enriched functions in the differentially expressed genes between cleaner fish that were not exposed to the client (control) and cleaner fish that were exposed (Interaction) for A) glutamatergic synapse, B) learning and memory function, C) dopamine biosynthetic processes and D) feeding behaviour. For each enriched function genes names are indicated and the Z-score related to the heatmap indicates the level of differential expression of each gene, while the column logFC refers to the log2fold change of each gene. The boxplot above logFC column shows the average of the gene expression related to the function in the cleaner fish that interacted with the client.

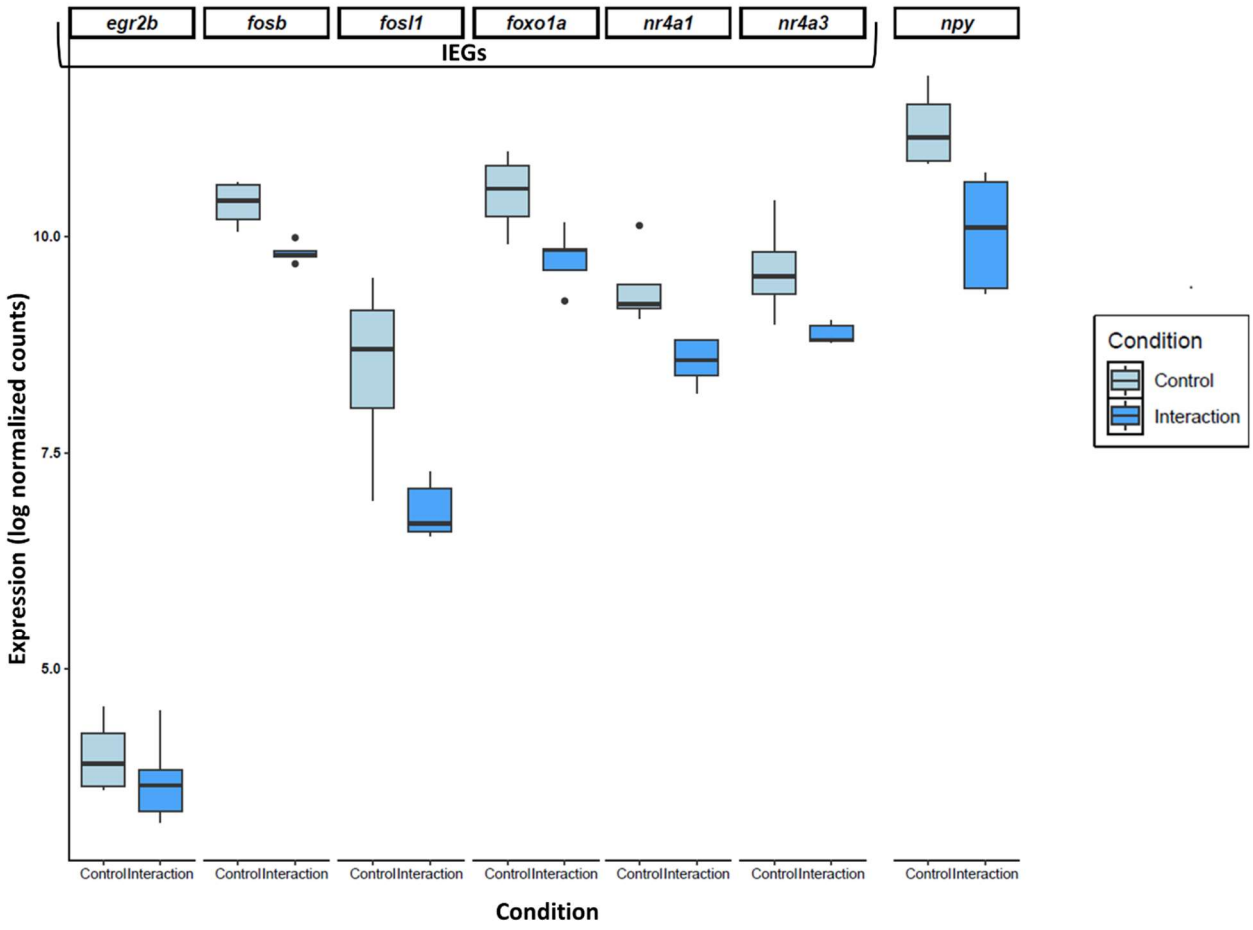

Figure 3: Differential gene expression of Immediate Early Genes (IEGs) and npy gene. In light blue the control cleaner fish and in blue the cleaner fish that interacted with the client. The differential gene expression is showed as log normalized counts.

Discussions

This study reveals the transcriptional drivers in *L. dimidiatus* when interacting with a client in the wild. The interaction behaviour provoked changes in genes related to glutamate pathway, immediate early genes and dopamine pathways, but did not detect changes in genes related to isotocin as found in studies in captivity (20). However, we discovered novel gene expression changes, such as the neuropeptide Y. Therefore, our study corroborates previous findings in *L. dimidiatus,* but also adds additional molecular drivers underlying the interaction behaviour.

We found differential expression related to glutamatergic synapsis and glutamate receptor activity that mediate learning and memory processes, confirming previous findings on this species in which glutamate is one of the main molecular drivers in the cleaning interaction (20). Glutamate ionotropic receptors (NMDA and AMPA) are known to drive the social recognition memory consolidation after a social stimulus in rats (35), improving their social recognition potency of familiar or unfamiliar individuals when treated with NMDA (36). For *L. dimidiatus*, the upregulation of genes related to these receptors (*gria4*, *grin3a*, *grin2d*) may allow the recognition of clients, which is a key feature as it interacts over 2000 times daily and can adjust the outcome of the interaction through partner prioritization or adjusting service (13,37). Moreover, we found upregulation of gene *grip1* in the cleaners that interacted with the clients, which encodes for AMPAR-binding protein GRIP1 and promotes synaptic plasticity by inserting AMPARS into synapses that may enhance learning and memory processes (38), also important for cleaning interactions. Furthermore, synaptic plasticity and circadian rhythm genes showed correlated expression patterns with clock genes being able to influence neuronal activity and excitability, affecting memory consolidation and recalling learned behaviour (39,40). Therefore, no matter if in a wild or a laboratory setting, glutamatergic pathways are one of the main molecular drivers in the cleaner wrasse brain, and together with clock genes, regulate synaptic plasticity and learning and memory processes.

Immediate Early Genes (IEGs) are modulators of social behaviour in the social brain network, a highly conserved neural network in the telencephalon and diencephalon (41), and were differentially expressed when the cleaner interacted with the client. These genes are the first that react to extracellular stimuli and are associated with neuronal activity (*fosl1*, *fosb*, *egr2b, foxo1a*) and neural plasticity (*nr4a1, nr4a3*) that affect social behaviour (42–45). They drive the social decision-making in *A. burtoni,* whether to cooperate with another male to defend his territory from an intruder or to exploit the social opportunity to ascend as a dominant male (19,46). Furthermore, the processes involving IEGs are mediated by second cellular messengers such as mitogen-activated protein kinase (MAPK) and cAMP pathways, as shown in the fighting fish *Betta splendens,* where the brain-

299 transcriptomic changes during a fight with a conspecific are associated with IEGs and MAPK
300 pathway genes (47). *Labroides dimidiatus* upregulated the gene *map4k2*, amongst other genes
301 involved in cAMP pathways such as *zfp36l3* and *cnga3*. Therefore, when exposed to social stimuli,
302 IEGs together with cAMP and MAPK pathways could synergistically lead to a downstream molecular
303 response cascade that can drive the decision-making process of *L. dimidiatus* on whether to exploit
304 the social opportunity to approach the client and choose which behaviour to perform (dancing,
305 cleaning, tactile stimulation or cheating). Thus, IEGs mediate *L. dimidiatus* cleaning behaviour in the
306 wild, influencing its decision-making on whether and how to interact with the client.

307 Genes involved in dopamine pathways were also altered with the interaction behaviour in *L.*
308 *dimidiatus*. An important regulator of synaptic dopamine availability is *DAT1* (Dopamine Transporter
309 1) encoded by *slc6a*, which was downregulated in interacting cleaner wrasses. Lower expression of
310 *slc6a*, which is involved in the uptake of dopamine and extracellular clearance, would suggest higher
311 dopamine levels in cleaner fish that interacted with the client (48). Induction of higher dopamine
312 levels in rats or primates increases social interactions and an exaggeration of behaviours related to
313 social rank, such as subordinates becoming more subservient in their social interactions (49,50).
314 During the cleaning interaction, the client may impose such partner control mechanisms (chasing the
315 cleaner) to avoid cheating behaviour by the cleaner, and therefore, downregulation of *slc6a3* may
316 show submissive behaviour in the cleaner fish through increasing extracellular dopamine levels.
317 Furthermore, pharmacologically blocking the D1 and D2 receptors promotes the willingness of the
318 cleaner to interact with the client and provide tactile stimulation (51). Here, we detected changes in
319 genes (*nr4a1* and *nr4a3*) that are linked to D1 and D2 receptor activity. In fact, in mice,
320 overexpression of *nr4a1* impairs D1 and D2 receptor signalling (the effect of *nr4a3* is still unclear)
321 (44,45). Therefore, changes in *nr4a(1,3)* could alter D1 and D2 receptor pathways and regulate
322 cleaner fish behaviour by mediating submissive behaviour via dopamine extracellular concentrations.
323 Thus, the dopaminergic pathway is another molecular driver of cleaner fish interspecific social
324 behaviour in the wild.

325 Interestingly, *npy*, a gene encoding for neuropeptide Y (NPY) known for its role in food intake (52–
326 54), was downregulated in the fish that interacted with the client. NPY is one of the brain's most
327 abundant and effective orexigenic peptides (53). In sturgeon fish, for instance, *npy* brain expression
328 decreased after a meal and in goldfish, injection of Y1 and Y5 receptor agonists increased food intake
329 while food deprivation increased hypothalamic expression of *npy* mRNA(55–57). Hence, fasting
330 leads to an increase in *npy* expression promoting food intake behaviour, while following a meal,
331 expression levels decrease in numerous teleost fish (58–61). In fish, NPY can negatively affect food

intake behaviour by inhibiting dopamine neurons through pre-synaptic and post-synaptic mechanisms in the ventral tegmental area (VTA), the brain region where the mesocorticolimbic dopamine system controls food intake, food reward and feeding-related behaviours (62). Therefore, we may hypothesize that upregulated expressed of *npy* in the control cleaner fish, could drive to seek out clients and initiate cleaning interactions, while a low level of *npy* can prevent cleaner fish from interacting, modulating the mesocorticolimbic dopamine system through food reward processes. Moreover, the differences in genes related to metabolism observed in gene networks correlated with the interaction behaviour show support for physiological mechanisms triggered after a meal. Thus, overexpression of *npy* may be a promoter of cleaning interaction in *L. dimidiatus*, making this gene one of the molecular drivers in cleaning behaviour.

The brain transcriptomic profile involved in cleaning interaction found in this study corroborate previous studies in *L. dimidiatus* cleaning behaviour where glutamate, IEGs, and dopamine were shown to be molecular drivers. Moreover, we indicate *npy* as an additional regulator of this interspecific interaction revealing that while in-situ studies provide a mechanistic approach to studying interactions, ex-situ experiments allow important additional insights into wild animal behaviour. The molecular processes in the cleaning interaction direct the regulation of partner recognition and memory consolidation to recognize clients and retain information for future interactions. This allows social decision-making on whether and how to interact with the client, feeding and submissive behaviour to promote interspecific interaction and honesty while cleaning. All these features, driven by the molecular pathways, affect the efficiency of cleaner fish behaviour and thus the persistence of one of the most crucial interspecific interactions for the balance and health of the coral reef ecosystem.

Data accessibility

The raw sequencing data can be found NCBI Bioproject number PRJNA1120171. Reviewer link can be accessed here:
https://dataview.ncbi.nlm.nih.gov/object/PRJNA1120171?reviewer=n10bqpe73p6t48tsvn5r4qvi23

Authors' contributions

C.S. designed the experiment and sample collection was conducted by S.R.C., C.S., T.R. and R.R-M. D.R. performed RNA extractions, analyzed the behavioural videos and transcriptomic data with input from C.S. D.R. and C.S. wrote the first draft of the manuscript and all the authors revised and approved the final manuscript.

Acknowledgments

We are thankful to Erina Kawai, Michael Izumiyama and Billy Moore for aiding with the sample collection. We want to acknowledge the Schunter lab members at The University of Hong Kong Dr. Sneha Suresh, Arthur Yan Chi Chung, Jade Sourisse, Maxine Cutracci, Dr. Lucrezia Celeste Bonzi and Maddalena Ranucci for their feedback and comments provided to this work. We also thank our collaborator Dr. José Ricardo Paula, for the stimulating discussions on the topic and the feedback on data analysis.

Funding

D.R. is funded by a Hong Kong PhD Fellowship (HKPF) (by the Research Grants Council (RGC)). The project was financially supported by a research seed fund from the University of Hong Kong; the Excellent Young Scientist Award by the National Natural Science Foundation of China (AR225205) to C.S and by Flotte Oceanographique Francaise for using the R/V Alis (project SuperNatural 2020 granted to RR-M https://doi.org/10.17600/18001102).

Ethics statement

The experiment was performed under the permits granted from Province Sud (New Caledonia), project SuperNatural N. 34314-2019/3-REP/DENV.

References

1. Hamilton WD. The Genetical Evolution of Social Behaviour. II. Vol. 7, J. Theoret. Biol. 1964.
2. Forsman JT, Seppänen JT, Mönkkönen M. Positive fitness consequences of interspecific interaction with a potential competitor. Proc R Soc Lond B Biol Sci. 2002 Aug 7;269(1500):1619–23.
3. Oliveira RF. Social plasticity in fish: integrating mechanisms and function. J Fish Biol. 2012 Dec 27;81(7):2127–50.
4. Bshary R. Building up Relationships in Asymmetric Co-operation Games between the Cleaner Wrasse Labroides dimidiatus and Client Reef Fish [Internet]. Vol. 52. 2002. Available from: https://www.jstor.org/stable/4602153
5. Bronstein JL. The evolution of facilitation and mutualism. Journal of Ecology. 2009 Nov 13;97(6):1160–70.
6. Peacock KA. Symbiosis in Ecology and Evolution. In: Philosophy of Ecology. Elsevier; 2011. p. 219–50.
7. Zander CD. Cleaner Fish: from Mutualism to Parasitism-or vice versa? Putzerfische: vom Mutualismus zum Parasitismus-oder umgekehrt? Vol. 18, Bulletin of Fish Biology. 2018.
8. Six DL. Climate change and mutualism. Nat Rev Microbiol. 2009 Oct;7(10):686–686.
9. Hale KRS, Valdovinos FS, Martinez ND. Mutualism increases diversity, stability, and function of multiplex networks that integrate pollinators into food webs. Nat Commun. 2020 May 1;11(1):2182.
10. Vaughan DB, Grutter AS, Costello MJ, Hutson KS. Cleaner fishes and shrimp diversity and a re-evaluation of cleaning symbioses. Fish and Fisheries. 2017 Jul;18(4):698–716.
11. Grutter AS, Bshary R. Cleaner fish, Labroides dimidiatus, diet preferences for different types of mucus and parasitic gnathiid isopods. Anim Behav. 2004 Sep;68(3):583–8.
12. Grutter A. Parasite removal rates by the cleaner wrasse Labroides dimidiatus. Mar Ecol Prog Ser. 1996;130:61–70.
13. Bshary R, Grutter AS. Asymmetric cheating opportunities and partner control in a cleaner fish mutualism. Anim Behav. 2002 Mar;63(3):547–55.
14. Pinto A, Oates J, Grutter A, Bshary R. Cleaner wrasses labroides dimidiatus are more cooperative in the presence of an audience. Current Biology. 2011 Jul 12;21(13):1140–4.
15. Bukhari SA, Saul MC, Seward CH, Zhang H, Bensky M, James N, et al. Temporal dynamics of neurogenomic plasticity in response to social interactions in male threespined sticklebacks. PLoS Genet. 2017 Jul 13;13(7):e1006840.
16. Weitekamp CA, Libbrecht R, Keller L. Genetics and Evolution of Social Behavior in Insects. Annu Rev Genet. 2017 Nov 27;51(1):219–39.

17. Kárpáti A, Yoshikawa T, Naganuma F, Matsuzawa T, Kitano H, Yamada Y, et al. Histamine H1 receptor on astrocytes and neurons controls distinct aspects of mouse behaviour. Sci Rep. 2019 Nov 11;9(1):16451.

18. Yao Y, Baronio D, Chen YC, Jin C, Panula P. The Roles of Histamine Receptor 1 (hrh1) in Neurotransmitter System Regulation, Behavior, and Neurogenesis in Zebrafish. Mol Neurobiol. 2023 Nov 20;60(11):6660–75.

19. Weitekamp CA, Hofmann HA. Neuromolecular correlates of cooperation and conflict during territory defense in a cichlid fish. Horm Behav. 2017 Mar;89:145–56.

20. Ramírez-Calero S, Paula JR, Otjacques E, Rosa R, Ravasi T, Schunter C. Neuro-molecular characterization of fish cleaning interactions. Sci Rep. 2022 Dec 1;12(1).

21. Calisi RM, Bentley GE. Lab and field experiments: Are they the same animal? Horm Behav. 2009 Jun;56(1):1–10.

22. Mason GJ. Species differences in responses to captivity: stress, welfare and the comparative method. Trends Ecol Evol. 2010 Dec;25(12):713–21.

23. Friard O, Gamba M. <scp>BORIS</scp> : a free, versatile open-source event-logging software for video/audio coding and live observations. Methods Ecol Evol. 2016 Nov 28;7(11):1325–30.

24. Horton S. Factors affecting advertising in Indonesian adult and juvenile bluestreak cleaner wrasse (Labroides dimidiatus). Bioscience Horizons. 2011 Mar 1;4(1):90–8.

25. Losey GS, Margules L. Cleaning Symbiosis Provides a Positive Reinforcer for Fish. Science (1979). 1974 Apr 12;184(4133):179–80.

26. Portz DE, Woodley CM, Cech JJ. Stress-associated impacts of short-term holding on fishes. Rev Fish Biol Fish. 2006 May 5;16(2):125–70.

27. Bshary R, Würth M. Cleaner fish *Labroides dimidiatus* manipulate client reef fish by providing tactile stimulation. Proc R Soc Lond B Biol Sci. 2001 Jul 22;268(1475):1495–501.

28. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014 Aug 1;30(15):2114–20.

29. Kang J, Ramirez-Calero S, Paula JR, Chen Y, Schunter C. Gene losses, parallel evolution and heightened expression confer adaptations to dedicated cleaning behaviour. BMC Biol. 2023 Aug 23;21(1):180.

30. Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat Biotechnol. 2019 Aug 2;37(8):907–15.

31. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics. 2014 Apr 1;30(7):923–30.

32. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014 Dec 5;15(12):550.

33. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics. 2008 Dec 29;9(1):559.

34. OmicsBox – Bioinformatics Made Easy, BioBam Bioinformatics, March 3, 2019, https://www.biobam.com/omicsbox.

35. Marcondes LA, Nachtigall EG, Zanluchi A, de Carvalho Myskiw J, Izquierdo I, Furini CRG. Involvement of medial prefrontal cortex NMDA and AMPA/kainate glutamate receptors in social recognition memory consolidation. Neurobiol Learn Mem. 2020 Feb;168:107153.

36. Hliňák Z, Krejčí I. N-Methyl-d-aspartate improved social recognition potency in rats. Neurosci Lett. 2002 Sep;330(3):227–30.

37. Triki Z, Wismer S, Rey O, Ann Binning S, Levorato E, Bshary R. Biological market effects predict cleaner fish strategic sophistication. Behavioral Ecology. 2019 Nov 8;30(6):1548–57.

38. Tan HL, Chiu SL, Zhu Q, Huganir RL. GRIP1 regulates synaptic plasticity and learning and memory. Proceedings of the National Academy of Sciences. 2020 Oct 6;117(40):25085–91.

39. Wang LMC, Dragich JM, Kudo T, Odom IH, Welsh DK, O'Dell TJ, et al. Expression of the Circadian Clock Gene *Period2* in the Hippocampus: Possible Implications for Synaptic Plasticity and Learned Behaviour. ASN Neuro. 2009 May 6;1(3):AN20090020.

40. Parekh PK, McClung CA. Circadian Mechanisms Underlying Reward-Related Neurophysiology and Synaptic Plasticity. Front Psychiatry. 2016 Jan 12;6.

41. Goodson JL. The vertebrate social behavior network: Evolutionary themes and variations. Horm Behav. 2005 Jun;48(1):11–22.

42. Poirier R. Paradoxical role of an Egr transcription factor family member, Egr2/Krox20, in learning and memory. Front Behav Neurosci. 2007;1.

43. Vallone D, Pellecchia MT, Morelli M, Verde P, DiChiara G, Barone P. Behavioural sensitization in 6-hydroxydopamine-lesioned rats is related to compositional changes of the AP-1 transcription factor: evidence for induction of FosB- and JunD-related proteins. Molecular Brain Research. 1997 Dec;52(2):307–17.

44. Cirnaru MD, Melis C, Fanutza T, Naphade S, Tshilenge KT, Muntean BS, et al. Nuclear Receptor Nr4a1 Regulates Striatal Striosome Development and Dopamine D $_1$ Receptor Signaling. eNeuro. 2019 Sep;6(5):ENEURO.0305-19.2019.

45. Eells JB, Wilcots J, Sisk S, Guo-Ross SX. NR4A Gene Expression Is Dynamically Regulated in the Ventral Tegmental Area Dopamine Neurons and Is Related to Expression of Dopamine Neurotransmission Genes. Journal of Molecular Neuroscience. 2012 Mar 20;46(3):545–53.

46. Burmeister SS, Jarvis ED, Fernald RD. Rapid Behavioral and Genomic Responses to Social Opportunity. PLoS Biol. 2005 Oct 18;3(11):e363.

47. Vu TD, Iwasaki Y, Shigenobu S, Maruko A, Oshima K, Iioka E, et al. Behavioral and brain- transcriptomic synchronization between the two opponents of a fighting pair of the fish Betta splendens. PLoS Genet. 2020 Jun 17;16(6):e1008831.

48. Gainetdinov RR, Jones SR, Fumagalli F, Wightman RM, Caron MG. Re-evaluation of the role of the dopamine transporter in dopamine system homeostasis1Published on the World Wide Web on 27 January 1998.1. Brain Res Rev. 1998 May;26(2–3):148–53.

49. Miller MH. Behavioral effects of amphetamine in a group of rhesus monkeys with lesions of dorsolateral frontal cortex. Psychopharmacology (Berl). 1976;47(1):71–4.

50. Rodriguiz RM, Chu R, Caron MG, Wetsel WC. Aberrant responses in social interaction of dopamine transporter knockout mice. Behavioural Brain Research. 2004 Jan;148(1–2):185–98.

51. Messias JPM, Paula JR, Grutter AS, Bshary R, Soares MC. Dopamine disruption increases negotiation for cooperative interactions in a fish. Sci Rep. 2016 Feb 8;6(1):20817.

52. Yuan D, Gao Y, Zhang X, Wang B, Chen H, Wu Y, et al. NPY and NPY receptors in the central control of feeding and interactions with CART and MC4R in Siberian sturgeon. Gen Comp Endocrinol. 2019 Dec;284:113239.

53. Mercer RE, Chee MJS, Colmers WF. The role of NPY in hypothalamic mediated food intake. Front Neuroendocrinol. 2011 Oct;32(4):398–415.

54. Yousefvand S, Hamidi F, Zendehdel M, Parham A. Interaction of neuropeptide Y receptors (NPY_1, NPY_2 and NPY_5) with somatostatin on somatostatin-induced feeding behaviour in neonatal chicken. Br Poult Sci. 2019 Jan 2;60(1):71–8.

55. Narnaware YK, Peter RE. Effects of food deprivation and refeeding on neuropeptide Y (NPY) mRNA levels in goldfish. Comp Biochem Physiol B Biochem Mol Biol. 2001 Jun;129(2–3):633–7.

56. Volkoff H, Canosa LF, Unniappan S, Cerdá-Reverter JM, Bernier NJ, Kelly SP, et al. Neuropeptides and the control of food intake in fish. Gen Comp Endocrinol. 2005 May;142(1–2):3–19.

57. Narnaware YK, Peter RE. Effects of food deprivation and refeeding on neuropeptide Y (NPY) mRNA levels in goldfish. Comp Biochem Physiol B Biochem Mol Biol. 2001 Jun;129(2–3):633–7.

58. Kehoe AS, Volkoff H. Cloning and characterization of neuropeptide Y (NPY) and cocaine and amphetamine regulated transcript (CART) in Atlantic cod (Gadus morhua). Comp Biochem Physiol A Mol Integr Physiol. 2007 Mar;146(3):451–61.

59. Silverstein JT, Breininger J, Baskin DG, Plisetskaya EM. Neuropeptide Y-like Gene Expression in the Salmon Brain Increases with Fasting. Gen Comp Endocrinol. 1998 May;110(2):157–65.

60. Ji W, Ping HC, Wei KJ, Zhang GR, Shi ZC, Yang RB, et al. Ghrelin, neuropeptide Y (NPY) and cholecystokinin (CCK) in blunt snout bream (Megalobrama amblycephala):

cDNA cloning, tissue distribution and mRNA expression changes responding to fasting and refeeding. Gen Comp Endocrinol. 2015 Nov;223:108–19.

61. Wei R, Zhou C, Yuan D, Wang T, Lin F, Chen H, et al. Characterization, tissue distribution and regulation of <*scp*>*neuropeptideY*</*scp*> in *Schizothorax prenanti*. J Fish Biol. 2014 Aug 16;85(2):278–91.

62. Rezitis J, Herzog H, Ip CK. Neuropeptide Y interaction with dopaminergic and serotonergic pathways: interlinked neurocircuits modulating hedonic eating behaviours. Prog Neuropsychopharmacol Biol Psychiatry. 2022 Mar;113:110449.