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Ocean acidification impedes gustation-mediated feeding behavior by disrupting gustatory signal transduction in the black sea bream, *Acanthopagrus schlegelii*

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ABSTRACT

Growing evidence suggests that ocean acidification (OA) may affect animal behaviors such as feeding. Although gustation plays a crucial role in evaluating the quality and palatability of food and ultimately influences whether or not teleosts consume the food, the potential impact of OA on gustation-mediated feeding behavior remains unknown. In this study, gustation mediated-feeding behavior, as indicated by the consumption rate (CR) and swallowing rate (SR) of agar pellets with or without feed upon OA exposure was investigated in black sea bream (*Acanthopagrus schlegelii*). Results showed that the exposure to acidified seawater led to significant reductions in the CR and SR of feed-containing agar pellets. In addition, the *in vivo* contents of three neurotransmitters and expression of genes from the gustatory signal transduction pathway were all significantly suppressed by the OA treatment. In general, the data obtained indicated that OA may hinder the gustation-mediated feeding behavior of *A. schlegelii* by disrupting gustatory signal transduction, which may aggravate the issue of food shortage for wild populations of black sea bream.

1. Introduction

Since the industrial revolution, increasing anthropogenic activities such as cement production and fossil fuel utilization have released considerable amounts of carbon dioxide (CO_2) into the atmosphere, which has led to a significant increase in CO_2 partial pressure (Widdicombe and Spicer, 2008). Approximately 1/3 of anthropogenic CO_2 is absorbed by the ocean, resulting in a decrease in seawater pH; this phenomenon is termed ocean acidification (OA) (Caldeira and Wickett, 2003; Doney et al., 2012). Compared with preindustrial levels, the pH of surface seawater has already decreased by 0.1 units and may drop by another 0.3–0.4 units by the end of the 21st century and by 0.7–0.8 units by 2300 according to the predictions of the Intergovernmental Panel on Climate Change (IPCC) (Kleypas et al., 1999; Orr et al., 2005; Sabine et al., 2004).

Since it has been discovered, OA has generated global concern over its potential threats to marine organisms and ecosystems (Egilsdottir et al., 2009; Liu et al., 2016; Sewell et al., 2013; Su et al., 2017). Increasing evidence suggests that OA may adversely affect a number of physiological processes such as fertilization success (Sewell et al., 2013; Shi et al., 2017a, b), embryonic development (Egilsdottir et al., 2009), metabolism and growth (Wu et al., 2014; Zhao et al., 2017), immunity (Liu et al., 2016; Su et al., 2017, 2018), and behavioral responses (Clements and Hunt, 2015; Nagelkerken and Munday, 2016; Peng et al., 2017; Rong et al., 2018) in various marine organisms. Because calcifying organisms such as corals, echinoderms, crustaceans, and bivalve mollusks are considered to be more sensitive to shifts in pH and saturation of calcium carbonate ($CaCO_3$) under future OA scenarios, most of the studies investigating the impacts of OA have been performed with these species (Fine and Tchernov, 2007; Gazeau et al., 2010; Havenhand et al., 2008; Reuter et al., 2011). In the past decade, a substantial number of studies have been conducted investigating physiological and behavioral impacts of OA on teleosts (Cattano et al., 2018; Grosell et al., 2019), however, many questions (i.e. the potential effects on gustation/feeding) remain unanswered.

Feeding, which provides matter and energy for development, growth, reproduction, and adaptation to a changing environment, is crucial for the health and survival of an animal (Kasumyan and Døving, 2003). Feeding consists of a series of behaviors including searching, targeting, capturing, evaluating, and consuming the food; the feeding of teleosts is achieved through the interaction of multiple sensory modalities such as vision, audition, olfaction, and gustation (Kasumyan and

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Døving, 2003). During the feeding process of teleosts, visual, auditory, and olfactory perception play important roles in detecting and identifying potential food sources (Draper and Weissburg, 2019), whereas gustation provides the final evaluation of the quality and palatability of food prior to consumption (Kasumyan and Døving, 2003; Kasumyan, 2019). Therefore, any damage to these sensory modalities will have profound impacts on teleost species.

Recently, several studies have suggested that OA may hinder feeding behavior by disrupting the olfactory perception of marine teleosts (Cripps et al., 2011; Dixson et al., 2015; Rong et al., 2018). For example, compared with the control, brown dottyback (*Pseudochromis fuscus*) exposed to elevated CO_2 levels spent approximately 20% less time in the water stream containing prey odor and showed a shift from preference to avoidance of prey olfactory cues (Cripps et al., 2011). Similarly, smooth dogfish (*Mustelus canis*) exhibited a decreased sensitivity to prey odor cues following OA treatment as indicated by the time spent in the water column with the prey olfactory stimulus and their preference for prey odor (Dixson et al., 2015). Our previous study also demonstrated that black sea bream (*Acanthopagrus schlegelii*) took significantly longer to follow the olfactory cue to the food source and swam in a less direct manner after OA treatment (Rong et al., 2018). In addition, several studies suggest that OA exposure may disrupt visual ability of teleosts as well (Chung et al., 2014; Goldenberg et al., 2018). For instance, it was shown that the maximal retinal flicker frequency was significantly reduced by OA exposure in the damselfish *Acanthochromis polyacanthus* (Chung et al., 2014). One mesocosm experiment carried out with six fish species (juvenile stage) demonstrated that exposure to OA could affect the ability of fish individuals to visually detect potential food, however, the feeding was unaffected probably due to compensation in food detection by other sensory such as olfactory (Goldenberg et al., 2018). Together, these findings indicate that OA may increase the difficulty of locating food for teleosts, and thus they could experience malnutrition under future OA scenarios.

When food is captured by teleosts, the gustatory sense governs whether or not to consume it; therefore, maintaining a robust gustatory response is crucial for the feeding of teleosts as well. However, to the best of our knowledge, whether the gustatory mediated-feeding behavior of teleosts is affected by OA remains unknown. During the gustatory evaluation of food, the binding of taste-stimulating molecules from food particles to corresponding taste receptors such as T1R3 (taste receptor type I member III) on the membrane of type II taste receptor cells (TRCs) triggers the hydrolyzation of phosphatidylinositol (4,5) bisphosphate (PIP2) to inositol triphosphate (IP3) (Hisatsune et al., 2007; Morais, 2017). Subsequently, IP3 binds to its receptor (IP3R) and activates ion channels such as the transient receptor potential cation channel subfamily M member 5 (TRPM5) on the cell membrane, resulting in the influx of cations and membrane depolarization of the type II TRCs (Morais, 2017). The gustatory electrical signal is then transmitted to the brain by nerve fibers; based on this transmission, teleosts decide whether to ingest or reject the food. In this process, neurotransmitters such as adenosine triphosphate (ATP), γ-aminobutyric acid (GABA), 5-hydroxytryptamine (5-HT), and acetylcholine (ACh) play crucial roles in activating the primary afferent nerve fibers and generating the gustatory signal (Dvoryanchikov et al., 2011; Huang et al., 2005; Ogura, 2002). For example, ATP, which is an excitatory neurotransmitter released by type II TRCs upon gustatory stimulation, can activate postsynaptic primary sensory afferent fibers and adjacent presynaptic TRCs, i.e., it can activate type III TRCs through binding to the pyrimidinergic receptor (P2Y4) (Finger et al., 2005; Huang et al., 2007, 2009). Our previous study demonstrated that OA may impair olfaction-mediated foraging behavior of black sea bream (*A. schlegelii*) by altering neurotransmitter levels (GABA and ACh) *in vivo* and subsequently disrupting the olfactory signal transduction pathway (Rong et al., 2018). Given the considerable similarity between olfactory signal transduction and gustatory signal transduction, the latter could also theoretically be affected by OA. However, this hypothesis requires verification with empirical data.

The black sea bream *A. schlegelii* is a euryhaline, omnivorous fish and is one of the major commercial fish species in the Asian Pacific (Ji et al., 2003; Ma et al., 2008). It has been suggested that seasonal variation and an uneven distribution of food in the ocean frequently leads to a periodic food shortage for black sea bream, which presents a major challenge to the wild populations of this species (Nip et al., 2003). As mentioned above, the impact of OA on the gustatory-mediated feeding behavior in fish and the physiological mechanism that mediates this impact, if any, remains unclear to date. Therefore, the present study was conducted to determine whether the feeding behavior mediated by gustatory signal transduction in black sea bream would be affected under near future OA scenarios. In addition, the impacts of OA on the levels of key neurotransmitters (GABA, ACh, and 5-HT) *in vivo* as well as the expression of genes that play a crucial role in gustatory signal transduction (*T1R3*, *IP3R*, *TRPM5*, and *P2Y4*) were investigated to evaluate whether the detected inhibition of feeding behavior is due to the disruption of gustatory signal transduction.

2. Materials and methods

2.1. Ethical statement

This study was performed in accordance with the guidelines of the Animal Ethics Committee of the School of Medicine, Zhejiang University (ethics code permit no. ZJU2011-1-11-009Y).

2.2. Experimental animals and acclimation

Juvenile black sea breams (length 9.06 ± 0.05 cm, weight 21.75 ± 0.38 g) were purchased from the Dongtou fish-breeding farm and immediately transferred to the Qingjiang Station of the Zhejiang Mariculture Research Institute, Wenzhou, China, in June 2018. The individuals were acclimated in a 500 L tank filled with 400 L of flowing seawater (temperature 23.81 ± 0.08 °C, pH 8.11 ± 0.01, salinity 20.76 ± 0.01, and light-dark cycle 12:12) prior to the experiment. During the acclimation, sea breams were fed commercial pellet feed (diameter 2.5 mm, containing >42% crude protein and >7% crude lipid, Tech-Bank, Ningbo, China) to satiation twice daily at 9 a.m. and 5 p.m. After a one-week acclimation, healthy individuals without physical injury were used for the experiments.

2.3. OA treatment and seawater chemistry monitoring

Based on IPCC predictions, pH values of 8.1, 7.8, and 7.4 were adopted in this study to simulate the pH levels at present and in the years 2100 and 2300, respectively. Following the method described by Zhao et al. (2017), seawater aerated with dry air was used as a control, and lower experimental pH was achieved by continuous aeration with a CO_2-air mixture corresponding to the predicted pCO_2. The CO_2-air mixture was obtained by mixing CO_2-free air and pure CO_2 gas at known flow rates using flow controllers (Shengjie Instrument Co. Ltd, Hefei, China).

To ensure the consistency of seawater chemistry parameters throughout the experiment, the pH_{NBS}, salinity, and temperature of experimental seawater were measured daily, and the total alkalinity (TA) was determined weekly. The pH_{NBS} was measured using a pH meter (PB-10, Sartorius) and calibrated with NBS standard buffers. Salinity was determined using a conductivity meter (Multi 3410, WTW), temperature was measured with a mercury thermometer, and TA was estimated using potentiometric titration (Anderson and Robinson, 1946). The carbonate system parameters were calculated from the measured pH_{NBS}, salinity, temperature, and TA values using the open-source program CO2SYS (Pierrot et al., 2006) with the constants supplied by Mehrbach et al. (1973) and refitted by Dickson and Millero (1987); the KSO_4 dissociation constant was taken from Dixson et al. (2010). The

seawater chemistry parameters during the experiment are listed in Table 1.

After acclimation, 150 healthy black sea bream were randomly selected and equally divided among 15 separate experimental tanks (3 treatments × 5 replicates, 10 fish/replicate) containing 50 L of aerated seawater that was pre-adjusted to the corresponding experimental pH values. During the 15-day exposure, fish were fed a commercial pellet feed at the satiation rate twice daily at 9 a.m. and 5 p.m. An hour after feeding, approximately 3/4 of the seawater in each tank was replaced with pre-acidified seawater at the corresponding experimental pH values.

2.4. Feeding behavior experiments and analysis

The feeding behavior experiments were performed following published methods with modifications (Kasumyan and Sidorov, 2010). Briefly, agar (2%) pellets containing commercial feed or blank agar pellets without feed were prepared prior to the experiment. During the preparation of the agar pellets, Ponceau 4R dye (5 μM) was introduced to facilitate visual observation of the feeding behavior. Five days prior to the experiment, the black sea bream from three of the six replicates were trained with agar pellets containing commercial feed 1 h before the routine feeding and used for feeding behavior analysis thereafter. During this training, the agar pellets were offered to the fish one at a time at 1-min intervals. In this study, it was observed that after three days of training, the agar pellets were consumed by fish immediately (within several seconds) after introduction.

After exposure to ambient or acidified seawater for 15 days (including training before routine feeding in the last five days) and food deprivation for 24 h, five fish were randomly selected from each replicate and transferred to a white tray (50 cm × 33 cm × 30 cm) containing 30 L of seawater at the corresponding experimental pH level. After 3 min of acclimation, agar pellets containing commercial feed and blank agar pellets were randomly presented to fish one at a time at intervals of 30 s until satiation. During this period, the number of agar pellets provided, whether the pellet was grasped by the fish, and whether the grasped pellet was swallowed were recorded. The consumption rates (CRs, percentage of agar pellets consumed relative to the total number of pellets provided) and swallowing rates (SRs, the percentage of agar pellets swallowed relative to the total number of pellets grasped) were calculated for pellets containing feed and for blank agar pellets. In total, five replicates (each with 5 fish tested simultaneously as described above) were conducted for the feeding behavior analysis of each experimental group. Fish tested were excluded from further analysis to ensure that no individual was tested more than once.

2.5. Estimation of in vivo levels of three neurotransmitters

After rearing in ambient or acidified seawater for 15 days, 12 fish were randomly selected from each experimental group (the replicates without agar pellet training) and dissected on ice after anaesthetization with eugenol (60 mg/L). The gill tissue, which contains high numbers of TRCs (Ishimaru et al., 2005), was carefully peeled off and used for the determination of *in vivo* levels of GABA, ACh, and 5-HT using commercial ELISA kits (ML086216, ML095412, and ML064340, respectively, MLBIO Biotechnology Co. Ltd., Shanghai, China). Following the manufacturer's instructions, tissue samples were homogenized in ice-cold PBS (0.01 M, pH 7.4) after weighing followed by centrifugation at 2000 rpm for 20 min at 4 °C. A 10 μL aliquot of the supernatant was mixed with 40 μL of the sample diluent and 100 μL of the corresponding enzyme reagent provided with the kit. After incubation at room temperature for 60 min, the corresponding chromogenic reagent was added and the sample mixture was incubated at room temperature for another 15 min. Once the chromogenic reaction was stopped by adding the terminating solution, the absorption value of each sample was measured at 450 nm with a microplate reader (Thermo Multiskan Go, USA). The *in vivo* levels of GABA, ACh, and 5-HT were subsequently estimated by reference to corresponding standard curves constructed with neurotransmitter standard dilutions provided with the kits.

2.6. Expression of key genes from the gustatory signal transduction pathway

After exposure to ambient or acidified seawater for 15 days, four fish were randomly selected from each experimental group (the replicates without agar pellet training) and dissected on ice as described above. The gill tissue of each individual was carefully peeled off and immediately frozen in liquid nitrogen. The total RNA was extracted from the gill tissue using an EASYspin Plus tissue/cell rapid RNA extraction kit (Aidlab, RN2802) following the method described by Peng et al. (2016). The quality and concentration of total RNA were verified using gel electrophoresis and a NanoDrop 1000 UV/visible spectrophotometer (Thermo Scientific), respectively. Following the manufacturer's protocols, cDNA templates were synthesized from high-quality RNA samples using an M-MLV First-Strand kit (Invitrogen, C28025-032).

Quantitative PCR was then performed in a CFX96™ Real-Time System (Bio-Rad) with a total reaction volume of 10 μL containing 5 μL 2 × Super Mix (Bio-Rad, 172-5201AP), 3 μL double-distilled water, 0.5 μL forward and reverse primers (10 μM each), and 1 μL cDNA template using the following amplification cycling parameters: 95 °C for 5 min followed by 40 cycles of 95 °C for 20 s, 61 °C for 20 s, and 72 °C for 20 s. A final extension was carried out at 72 °C for 5 min after the 40 cycle's amplification. The melting curve analysis (MCA) was used to confirm the specificity of the PCR products and amplification efficiency was found to be approximately 93.80%–96.45% in this study. The 18S rRNA was adopted as a reference to calculate the relative expression levels of the genes tested. In total, four genes encoding taste 1 receptor member 3 (*T1R3*), inositol 1,4,5-trisphosphate receptor type 3 (*IP3R*), transient receptor potential cation channel subfamily M member 5 (*TRPM5*), and pyrimidinergic receptor (*P2Y4*) from the gustatory signal transduction pathway were investigated. All primers used in the present study were synthesized by TsingKe Biotech (Hangzhou, China), and the sequence of these primers is listed in Table 2.

Table 1
Seawater chemistry parameters during the 15-day experiment for the control and pCO_2-acidified groups (mean ± SE). The partial pressure of CO_2, dissolved inorganic carbon, and saturation state of aragonite and calcite were calculated from the measured pH_{NBS}, salinity, temperature, and TA values using the open-source program CO2SYS. T: temperature; Sal: salinity; TA: total alkalinity; pCO_2: CO_2 partial pressure; DIC: dissolved inorganic carbon; Ωara: aragonite saturation state; and Ωcal: calcite saturation state.

Target pH	T(°C)	Sal(‰)	pH_{NBS}	TA (μmol/kg)	pCO_2 (μatm)	DIC (mmol/kg)	Ωara	Ωcal
pH **8.1**	23.81 ± 0.08	20.76 ± 0.01	8.11 ± 0.01	2050.55 ± 3.49	348.75 ± 11.34	1848.74 ±3.99	2.62 ± 0.06	4.21 ± 0.10
pH **7.8**	23.81 ± 0.09	20.61 ± 0.01	7.79 ± 0.01	2066.71 ± 3.13	813.71 ± 19.20	1980.30 ±3.99	1.38 ± 0.03	2.22 ± 0.04
pH **7.4**	23.78 ± 0.09	20.74 ± 0.02	7.40 ± 0.01	2080.67 ± 4.30	2118.45 ± 46.48	2098.42 ±5.21	0.60 ± 0.01	0.97 ± 0.02

Table 2
Primer sequences for the genes investigated and the internal reference 18S rRNA (F and R after the dash in the primer name indicate forward and reverse primers, respectively).

Primers	Sequence (5′ to 3′)	Accession no.
18S–F	GCCAAGTAGCATATGCTTGTCT	GU017319
18S-R	AGACTTGCCTCCAATGGATCC	
T1R3-F	TGCTGGAGCCTCTCATCTGACAATA	MH381813
T1R3-R	AGTCGCTCGCTCACAGGTAGTT	
TRPM5-F	CCTTCAGAACCGACAGCAGATGG	MH379649
TRPM5-R	CGTGCAGACTCAGCTTCAGACTC	
IP3R–F	ACACTTCAGCCAGAGACA	MK440592
IP3R-R	ATCCAGATCCGCCTTGAT	
P2Y4–F	CCTGTGGCTGTTCTTGAGGATGC	MH381812
P2Y4-R	TGGCTGCGGTTGGCGTAGTA	

2.7. Statistical analysis

After arcsine square root transformation of the data to meet the requirements of data normality and homogeneity of variance (Su et al., 2017), CRs and SRs were analyzed using a linear mixed effects model with the treatment tank as a random variable using 'R' statistical package lme4 (Bates et al., 2015). The *in vivo* levels of GABA, ACh, and 5-HT in the black sea bream from different experimental groups, which satisfied statistical assumptions, were directly analyzed without transformation. Duncan's multiple range tests (Tallarida and Murray, 1987) were conducted to detect any difference in the expression levels of the genes tested. All data obtained are presented as the mean ± SE and a *p*-value less than 0.05 was accepted as a statistically significant difference.

3. Results

3.1. The effect of ocean acidification on feeding behavior

The gustatory-mediated feeding behavior as represented by CR and SR was significantly affected by the exposure of black sea bream to acidified seawater for 15 days (Table 3). Although no significant difference was detected in the feeding of blank agar pellets among the experimental groups, individuals reared in acidified seawater had significantly lower CRs and SRs of feed-containing agar pellets (Table 3, $F_{2,14} = 22.47$, $p = 3.19E3$ and $F_{2,14} = 52.21$, $p = 3.56E4$ for CR and SR, respectively). Compared with the control, the CRs of feed-containing agar pellets decreased by 11.16% and 21.45% for sea breams exposed to seawater at pH 7.8 and pH 7.4, respectively. Similarly, the SRs of feed-containing agar pellets dropped to approximately 93.44% and 82.65% of the control for OA treatment groups at pH 7.8 and pH 7.4, respectively.

Table 3
The consumption rate (CR) and swallowing rate (SR) of feed-containing and blank agar pellets after a 15-day exposure to control or pCO_2-acidified seawater at pH 8.1, 7.8, and 7.4, respectively. CR: percentage of agar pellets consumed relative to the total number of pellets provided; SR: the percentage of agar pellets swallowed relative to the total number of pellets grasped. Approximately 36.07 ± 4.51 pellets were provided for each experimental trial. Different superscripts indicate a significant difference among the values in the column.

	CR of agar pellets containing feed (%)	CR of blank agar pellets (%)	SR of gar pellets containing feed (%)	SR of blank agar pellets (%)
pH**8.1**	91.09 ± 2.83^{a}	11.65 ± 3.33	95.60 ± 2.18^{a}	14.58 ± 4.54
pH**7.8**	80.92 ± 2.68^{b}	10.93 ± 3.69	89.33 ± 1.94^{b}	13.33 ± 4.38
pH**7.4**	71.55 ± 3.12^{c}	7.80 ± 2.15	79.01 ± 2.33^{c}	11.46 ± 3.29

3.2. The effect of ocean acidification on the in vivo levels of GABA, ACh, and 5-HT

The *in vivo* levels of GABA, ACh, and 5-HT in the gills of black sea bream were all significantly reduced after 15 days of exposure to acidified seawater (Fig. 1, $p < 0.05$). Compared with the control, the GABA content was significantly decreased by 19.37% and 27.23% for black sea bream reared in acidified seawater at pH 7.8 and pH 7.4, respectively (Fig. 1A, $F_{2,35} = 54.400$, $p < 0.05$). Similarly, the *in vivo* levels of ACh in black sea bream were significantly reduced by 31.78% (pH 7.8) and 41.61% (pH 7.4) following OA treatment (Fig. 1B, $F_{2,35} = 71.432$, $p < 0.05$). The 5-HT content of the treatment groups at pH 7.8 and 7.4 were decreased by approximately 4.91% and 8.60%, respectively, compared

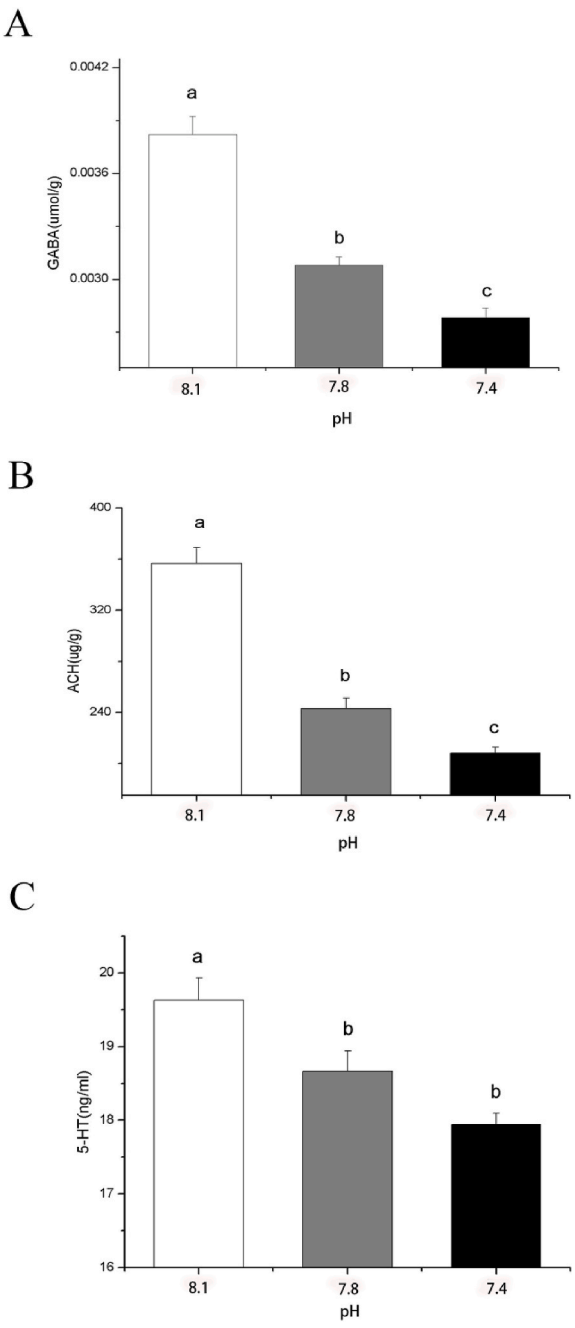

Fig. 1. The *in vivo* levels of GABA (A), ACh (B), and 5-HT (C) in black sea bream exposed to 15 days of control or pCO_2-acidified seawater at pH 8.1, 7.8, and 7.4, respectively. All data are presented as the mean ± SE; groups with different superscripts are significantly different at $p < 0.05$.

with that of the control (Fig. 1C, $F_{2,35} = 11.192$, $p < 0.05$).

3.3. The effects of ocean acidification on the expression of genes from the gustatory transduction pathway

The relative expressions of the four genes under investigation from the gustatory transduction pathway were all significantly suppressed in black sea bream reared in acidified seawater (Fig. 2, $p < 0.05$). Compared with that of the control, the relative expressions of *T1R3* decreased by 28.17% and 69.57% for individuals reared in acidified seawater at pH 7.8 and pH 7.4, respectively. Decreases in the expression of *IP3R* of approximately 88.81% and 90.23% were detected for black sea bream treated with seawater at pH 7.8 and pH 7.4, respectively. Similarly, after 15 days of OA treatment, the expressions levels of both *TRPM5* and *P2Y4* decreased significantly to less than half and less than one-third of those of the control for the experimental groups at pH 7.8 and pH 7.4, respectively.

4. Discussion

Although it has been suggested that various behaviors of some marine animals might be affected under future OA scenarios (Clements and Hunt, 2015; Peng et al., 2017; Rong et al., 2018), to the best of our knowledge, the impact of OA on gustatory-mediated feeding behavior has not been previously investigated. The results of this study demonstrated that the ability of the black sea bream to evaluate the quality and palatability of food through gustatory perception may be significantly impeded under future OA scenarios. The black sea breams reared in acidified seawater for 15 days had a significantly lower CR and SR of feed-containing agar pellets compared with those of the control, which indicates that OA rendered black sea bream less sensitive to the taste of the feed. In a natural environment, this reduction in gustatory perception and subsequent decline in food intake could aggravate the issue of seasonal food shortages and pose a severe threat to wild populations of black sea bream.

Although no study has previously investigated the impact of OA on taste perception in marine organisms, it has been demonstrated that other sensory modalities of teleosts such as olfaction and vision can be disrupted by OA (Chung et al., 2014; Cripps et al., 2011; Dixson et al., 2010, 2015; Ou et al., 2015; Porteus et al., 2018; Rong et al., 2018; Williams et al., 2019; Schunter et al., 2019). Moreover, alterations in taste preference as a result of other environmental factors such as salinity and temperature have been previously reported in fish species (Kasumyan et al., 1993; Kasumyan and Sidorov, 1995; Kasumyan and Mikhailova, 2010). For example, the transfer of three-spined sticklebacks (*Gasterosteus aculeatus*) from seawater to fresh water led to a change in their taste preference for some substances such as citric acid, sucrose, and $CaCl_2$ (Kasumyan and Mikhailova, 2010). After the salinity change from seawater to fresh water, the response to citric acid resulted in attraction rather than indifference, whereas the response to sucrose and $CaCl_2$ became indifferent instead of distasteful (Kasumyan and Mikhailova, 2010). Similarly, compared with those in cold water (12.5 °C), stellate sturgeon (*Acipenser stellatus*) raised in warm water (20 °C) had significantly higher SRs for agar pellets containing amino acids such as cysteine, lysine, serine, histidine, alanine, and glycine, indicating a taste preference for these ingredients at high temperature (Kasumyan et al., 1993). In addition, one study demonstrated that exposure of European grayling (*Thymallus thymallus*) to low pH caused L-cysteine, which is the normally the most preferred amino acid, to become significantly less attractive to this fish species, suggesting a change in gustatory perception as a result of low pH (Kasumyan and Sidorov, 1995). However, because the acidification was achieved by adding sulfuric acid instead of elevating pCO_2, the findings in European grayling have limited application to OA scenarios.

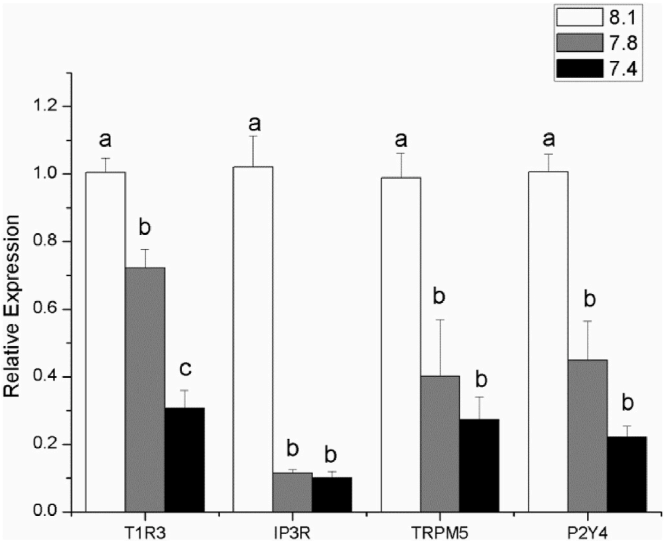

Fig. 2. The expression of *T1R3, IP3R, TRPM5*, and *P2Y4* after 15 days of exposure to the control or pCO_2-acidified seawater at pH 8.1, 7.8, and 7.4, respectively. All data are presented as the mean ± SE; groups with different superscripts are significantly different at $p < 0.05$.

The results obtained in this study suggest that OA may hinder the gustation mediated-feeding behavior of black sea bream by disrupting gustatory signal transduction. Based on the gustatory signal the brain receives, fish evaluate the palatability of food and decide whether to ingest it (Kasumyan and Døving, 2003). Therefore, the successful generation and transmittal of the gustatory signal through a transduction cascade is crucial to the feeding behavior of teleosts. Briefly, the taste stimuli from food activate taste receptors such as T1R3 on the membrane of type II TRCs and result in an increase in IP3 (Sainz et al., 2007). The subsequent binding of IP3 to its receptor IP3R opens ion channels such as TRPM5 and the sodium voltage-gated channel alpha subunit 2 (VGNC) on the membrane, leading to an influx of Na^+ that depolarizes the membrane of type II TRCs (Roper, 2013). Because the expressions of *T1R3*, *IP3R*, and *TRPM5* were all significantly suppressed following OA treatment in this study, the generation of the gustatory signal through the above processes could be obstructed and result in a reduced capacity for gustatory perception under future OA scenarios.

Once generated by the type II TRCs, the gustatory signal is subsequently transduced to postsynaptic primary sensory afferent fibers and adjacent presynaptic TRCs through neurotransmitters such as ATP, ACh, GABA, and 5-HT (Roper, 2013). First, the depolarization of type II TRCs activates calcium homeostasis modulator 1 (CALMH1) and triggers the release of the excitatory neurotransmitters ATP and ACh (Gilbertson et al., 2000; Lindemann, 2001; Liu and Liman, 2003; Margolskee, 2002; Perez et al., 2002). In addition to stimulating postsynaptic primary sensory afferent fibers, the ATP and ACh released by type II TRCs also pass this signal on to adjacent TRCs by binding to P2Y4 and muscarinic AChR on the membrane of type III and type II TRCs, respectively (Eguchi et al., 2008; Finger et al., 2005; Huang et al., 2007, 2009; Ogura and Lin, 2005; Simon and Baggett, 1992). The activation of type III TRCs then triggers the release of GABA and 5-HT, and transmits the signal to sensory afferent fibers via 5-HT (Takeda, 1977). In addition, both the GABA and 5-HT released by type III TRCs can regulate this process through negative feedback by inhibiting further ATP secretion (Dvoryanchikov et al., 2007; Huang et al., 2009; Kaya et al., 2004; Roper, 2013).

In this study, it was shown that exposure of black sea bream to acidified seawater for 15 days led to significant reductions in both *P2Y4* expression and *in vivo* levels of ACh, GABA and 5-HT. Under these circumstances, type III TRCs could be less sensitive to the ATP signal released by type II TRCs due to the suppression of *P2Y4*. In addition, reductions in the *in vivo* levels of neurotransmitters may render both type II and type III TRCs less efficient at transmitting the gustatory signal

to the sensory afferent fibers. The alterations in 5-HT and GABA induced by OA also indicate a disruption in the regulation of the signal transduction process. All these changes could obstruct gustatory signal transduction and may explain the reduction in gustatory mediated-feeding behavior observed in response to OA treatment.

In general, the results obtained in this study indicate that near-future OA may disrupt the gustatory perception of black sea bream and subsequently lead to impeded feeding behavior. Together with other adverse impacts such as interference with the olfactory detection of food, OA may aggravate the food shortage issue and pose a substantial threat to wild populations of black sea bream.

CRediT authorship contribution statement

Jiahuan Rong: Conceptualization, Methodology, Experiment implementation, Writing - original draft. **Yu Tang:** Conceptualization, Experiment implementation. **Shanjie Zha:** Methodology, Experiment implementation. **Yu Han:** Methodology, Experiment implementation. **Wei Shi:** Experiment implementation. **Guangxu Liu:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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