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Taste preferences and taste thresholds to classical taste substances in the carnivorous fish, kutum *Rutilus frisii kutum* (Teleostei: Cyprinidae)

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HIGHLIGHTS

- Palatability of classical taste substances is assessed for kutum, the teleost fish.
- Threshold concentration for citric acid, the most palatable substance, is 0.01 M.
- Kutum differentiates pellets' taste if citric acid concentration differs twice.
- Kutum retains palatable pellets in the mouth for much longer time than other pellets.
- Carnivorous kutum and omnivorous roach have opposite taste preferences.

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ABSTRACT

The objective of this study was to compare the taste preferences in the closely related sympatric fish species with different feeding patterns. For this purpose, palatability for four classical taste substances was evaluated for carnivorous kutum *Rutilus frisii kutum* and the results were compared with the taste preferences of the omnivorous roach *Rutilus rutilus* which had been studied earlier. In addition, the threshold concentration and the dose–response relationship of the most palatable tastants were evaluated and the ability of kutum to differentiate food with tastants in different concentrations was estimated. It was found that citric acid significantly increases the agar gel pellet consumption within the range of concentrations from 0.01 M to 0.52 M; the pellets with a concentration of 0.026 M were the most palatable. The pellet consumption is significantly different if the concentration of citric acid in the pellets differs more than two times. The absolute threshold concentration is 0.01 M, or 2.74 μg of citric acid per pellet. Sucrose and NaCl have deterrent taste at the highest concentrations tested (0.29 and 1.73 M, respectively). Both substances are palatable at 10 times lower concentrations and become indifferent after further gradual decrease in their concentration. $CaCl_2$ decreases the pellets consumption at 0.9 M but is an indifferent tastant at lower concentrations (0.45, 0.09 and 0.045 M). The number of rejections and repeated grasps of a food pellet is fewness and is not related to the pellet's palatability, while the retention time of pellet in the oral cavity positively and highly correlates with the pellet's palatability. Kutum have opposite taste preferences for most substances tested in comparison with the roach. It indicates that the taste preferences mediated by the oral taste receptors are different in closely related sympatric fish displayed diet divergences.

1. Introduction

All sensory systems are involved in the regulation of feeding behavior in fish and the final steps of this complex behavior are based on the function of the gustatory system [1]. The taste bud is the end organ of the gustatory system in all vertebrates. In fish, taste buds are numerous and cover the oral cavity, and in many species they are also distributed on the fish body surface [2]. The extraoral taste buds mediate the food grasping behavior while the stimulation of the oral taste buds releases the food swallowing reflex and food rejections [3].

Using the standard bioassay method developed to study taste preferences in fish it was found that some substances are highly effective taste stimuli and increase the consumption of flavored food items but other substances make food unpalatable for fish. Comparison of the responses of intact and anosmic specimens do not reveal any significant differences in flavored food pellets' consumption [4,5]. Taste preferences have evident similarity among conspecifics [6]. The taste responsivity to free amino acids and classical taste substances is the same in males and females of the guppy *Poecilia reticulata* and in both the brown trout *Salmo trutta* and the nine-spined stickleback *Pungitius*

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pungitius individuals from different geographically isolated populations [7–9]. The dependence of taste preferences upon feeding experience is weak, as it has been shown in the grass carp *Ctenopharyngodon idella* juveniles reared on different diets for several months [10].

In contrast, the taste preferences seem to be highly species specific in fish. It was found that the same substance dramatically increases the food consumption in one species of fish and has the opposite effect on other fish [6]. As an example, amino acid L-cysteine and citric acid are highly palatable for brown trout *S. trutta* but evoke strong deterrent response in chum salmon *Oncorhynchus keta*, which belongs to the same family Salmonidae [8,11]. The species specificity in taste preferences still remains not clear especially in closely related fish. The comparison of several acipenserids (Russian sturgeon *Acipenser gueldenstaedtii*, Siberian sturgeon *Acipenser baerii*, stellate sturgeon *Acipenser stellatus* and Persian sturgeon *Acipenser persicus*) revealed that the oral taste preferences are highly specific among these fish species, but the specificity of extraoral taste preferences are less pronounced [12,13]. However, oral taste spectra of threespined stickleback *Gasterosteus aculeatus* and ninespined stickleback *P. pungitius* (both sticklebacks belong to family Gasterosteidae) are similar and the list of highly palatable amino acids coincide in both species [14]. The taste spectra in closely related fish having different feeding ecology were not compared.

The threshold concentrations estimated by electrophysiological recordings from the taste nerves are between 10^{-6} and 10^{-9} M for the most potent substances, and the responses increase sharply with a logarithmic increase in their concentrations [15]. The threshold concentrations determined by behavioral assay are usually in the range of 10^{-2}–10^{-4} M [4,16,17]. It was supposed that there might be several reasons for the discrepancy between the threshold concentrations obtained by behavioral and by electrophysiological methods [6]. Behavioral data concerning differential gustatory sensitivity in fish as well as dose–response relationships have not been obtained.

The aim of the present study was to investigate the taste preferences in the closely related sympatric fish species displayed differences in diet. For this purpose, palatability for four classical taste substances was evaluated for kutum *Rutilus frisii kutum* which is a carnivorous fish. The results were compared with the taste preferences of the omnivorous roach *Rutilus rutilus* which had been studied earlier using the same behavioral method [18]. The behavioral method was used also for evaluation of the threshold concentration and the dose–response relationship of citric acid, the most effective taste substances in kutum. In addition, the ability of kutum to differentiate artificial food pellets containing citric acid and other classical taste substances in different concentrations was estimated.

2. Materials and methods

2.1. Fish maintenance

The study was carried out on 20 specimens of the 4–5 month old kutum juveniles with the 5.9 ± 0.8 cm[1] total length (TL) and 1.5 ± 0.5 g[2] body weight reared at the Sijaval fish farm (Turkmen seaport, Golestan province, Iran). The fish were transported to the facilities of the Shahid Fazli Aquaculture Research Station of the Gorgan University of Agricultural Sciences and Natural Resources and were maintained for one month in the 100 l aquaria. The fish were fed manually once a day until an apparent satiation with pellets (0.8 mm diameter; brown color) of the commercial formulated diet containing 56% protein, 18% lipid and 10.4% ash (BioMar Group, Denmark; http://www.biomar.com/).

Three weeks before the trials fishes (n = 20) were placed individually in separate 5-liter aquaria with fresh tap water (water temperature 22–23 °C; oxygen level 7.2–7.8 mg·L^{-1}; pH 7.3–7.4; salinity 0.009–0.011 g·L^{-1}; total hardness 270–300 mg·L^{-1}; electrical conductivity 30–35 μS·cm^{-1}; 16 L:8D photoperiod). Aquaria were equipped with an air pump and did not contain gravel or other material on the bottom. The back and side walls of aquaria were opaque for visual isolation of fish. During the first 2–3 days, the fish were fed daily with thawed freshly frozen *Chironomidae* larvae which were offered to the fish one by one. After several days, when the fish have been adapted to the new conditions and feeding procedure, they were trained for 3–5 days to take the artificial agar pellets flavored with *Chironomidae* larvae extract, 175 g·L^{-1}. Flavored pellets were given to the fish one by one with an interval of 1–2 min.

In the course of the training and experiment the fish were fed ad libitum with *Chironomidae* larvae once a day after the completion of daily session in order to ensure that they have the similar feeding motivation at the beginning of a new session the next day. A part of water in aquaria was replaced daily with fresh water. Observations and video recordings were made through the transparent frontal wall of the aquaria.

2.2. Taste stimuli

Sucrose (0.29; 0.145; 0.029; 0.0145; 0.0029 M), sodium chloride (1.73; 0.173; 0.0865; 0.0173 M), calcium chloride (0.9; 0.45; 0.09; 0.045 M) and citric acid (0.52; 0.26; 0.182; 0.13; 0.026; 0.01; 0.0052; 0.0026 M) and the fresh *Chironomidae* larvae water extract (175 g·L^{-1}; g in wet weight) were used as taste stimuli. Classical taste substances are used often for various studies of taste physiology and taste-evoked behavior in animals and man [19–25]. The concentrations of these substances are relatively high in the hemolymph of a variety of potential food organisms for fish: citrate and sodium chloride concentrations can reach over 3×10^{-2} M and 0.5 M respectively [26,27]. The chemical substances were produced by Merck KGaA (Germany). The *Chironomidae* larvae were purchased from a local aquarium fish shop. The *Chironomidae* larvae were homogenized in a ceramic mortar and the obtained homogenate was mixed with distilled water in the proportion of 175 g·L^{-1} and then was filtered through a cotton cloth after extraction for 5 min at 18 °C.

2.3. Preparation of pellets

The pellets were prepared from 2% agar gel. After dissolving agar (Merck KGaA) in boiled distilled water, a dye (Cr_2O_3,[3] 0.35%; Merck KGaA) and water solution of one of the four classical substances were added into the agar solution. The water extract of *Chironomidae* larvae was added to the cooled (50 °C) agar solution. The agar solution was then mixed and poured into a Petri dish. The blank pellets with the dye only were used as control. The agar gels which contained chemical substances and *Chironomidae* larvae extract were kept at + 5 °C for up to 7 and up to 3–4 days, respectively. The cylindrical pellets with a diameter of 1.1 mm and a length of 1.5 mm were cut from the cold gel with a stainless steel tube just before each trial. In total, 23 types of agar pellets differing by substance and their concentration in the agar matrix were used for trials.

2.4. Experimental procedure

Twenty fish (each fish in individual aquarium) were divided on 5 groups by 4 fish in each. Each fish in a group received the same type of pellets during the day; next day the pellets of another type were offered to each fish in a group. The fish in different groups received pellets with different substances. The blank pellets were used every 5 days. Thus, within 5 days each fish from 20 fish used received 5 different types of the pellets including the control ones. Different types of the

[1] The standard error of the mean.
[2] The standard error of the mean.
[3] Chromium (III) oxide is inert and indigestible green color marker that a commonly used for studies in fish feeding and digestion.