The Effect of Food Odor Background on Gustatory Preferences and Gustatory Behavior of Carp *Cyprinus carpio* and Cod *Gadus morhua*

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Received November 26, 2008

Abstract—It was shown that stimulation by food odor (aquatic extract of food organisms, 10^{-2} and 10^{-3} g/l) does not cause shifts in gustatory preferences in carp *Cyprinus carpio* and cod *Gadus morhua* but modifies gustatory behavior. The level of consumption by carp of control granules and granules with attractive, by taste, L-proline (0.1 M) or deterrent L-lysine (0.1 M) (item by item presentation of granules) and by cod of control granules and granules with indifferent, to it, L-asparagine (0.1 M) (presentation of 10 granules simultaneously) is similar prior to and during olfactory stimulation. In the presence of food odor, the duration of taste testing for most types of granules, as well as the number of repeated graspings of granules with an attractive taste do not change in fish. At the same time, granules with indifferent or repulsive gustatory properties are rejected and repeatedly grasped by fish against the background of food odor more frequently than in water without odor. Olfactory stimulation leads to a considerable increase in the average number of graspings per one grasped granule with an indifferent or repulsive taste. Such behavior manifested by fish in the presence of food odor in response to granules with unattractive gustatory properties is apparently caused by the contradiction between the information coming via different chemosensory canals—olfactory and gustatory. The obtained results indicate that food stimulation caused by food odor in nature can lead to an increase in the actual consumption of only those accessible food items that have an attractive taste for fish.

Key words: fish, chemoreception, olfaction, taste, taste preferences, feeding, feeding behavior, carp, cod.

DOI: 10.1134/S003294520906006X

Food behavior of fish has a polysensory basis; all senses, including chemosensory systems—olfaction, taste, and the general chemical sense—are involved in its regulation (Atema, 1980; Pavlov and Kasumyan, 1990). Olfaction in many fish is a leading distant system in the perception of chemical signals and in the search for remote food items (Kleerekoper, 1969; Døving, 1986). The general chemical sense, according to some data, can participate in the near search for food items, as observed, for instance, in gurnards *Prionotus* (Silver and Finger, 1984). The final phase of food behavior when a preliminary and then a conclusive assessment of suitability of the found food are made are considerably based on the function of the gustatory system (Kasumyan and Døving, 2003). Chemosensory systems and other senses closely interact between themselves, providing the reliability of obtaining and an integrated perception by fish of biologically significant information (Kasumyan and Marusov, 2005, 2007).

It is known that many external factors and the inner state considerably affect fish behavior. The manifestation by fish of behavioral responses depends also on the combination of the acting signal with other stimuli that coincide or are opposite in signal value. For instance, food behavior of fish is considerably modified in the presence of an actual predator or its visual or chemical image and species odors (Malyukina et al., 1983; Magurran, 1986; Milinski, 1993; Mikheev, 2006). Multifactor effects on the behavior of fish have, as of yet, been studied insufficiently, and there are only several publications on this issue (Marusov, 1990; Ryer and Olla, 1996; Kolkovski et al., 1997; Pavlov et al., 1997). At the same time, the study of such interactions is key in understanding mechanisms of behavior of fish and other animals.

It is known that the perception by fish of food odors in most cases precedes the discovery and grasping of the food item and increases the feeding activity of fish (Pavlov and Kasumyan, 1998). It is still unknown whether stimulation caused by food odors or food signals of an other nature (visual, seismosensory, electric, acoustic, etc.) affect the assessment by fish of gustatory food properties and, as a result, its consumption. The purpose of this work was to study the effect of food odor background on gustatory preferences of substances of various types and the gustatory behavior of fish.

MATERIAL AND METHODS

Experiments were performed on 12 two-year-old carps *Cyprinus carpio* with a length of 10−12 cm and an average weight of 21.1 g, obtained from the All-Russia Research Production Fishery Association (VNIIPRKh, Moscow oblast) and on six three-year-olds of Atlantic cod *Gadus morhua* with a length of 18.0−20.5 cm and an average weight of 65 g, caught in Velikaya Salma Strait (Kandalaksha Bay, the White Sea).

Carp. Prior to performing experiments, fish, for several months, were kept in common aquariums with a volume of 100 l at a water temperature of 17−19°C. Fish were fed daily with live chironomid larvae (Chironomidae). Experiments were performed in March–April 2007 at the Department of Ichthyology of Moscow State University.

Two weeks before the beginning of experiments, fish were placed singly in aquariums with a size of 30 × 20 × 23 cm. Water was aerated using microcompressors; ground in aquariums was lacking. Each aquarium was covered by a nontransparent cover with an opening in the center for introducing food or experimental granules. Pure water (control) and stimulus solutions (aquatic extract of chironomid larvae with a concentration of 10^{-3} g/l) were introduced to aquariums, using Peristaltic miniflow pump type 304 (Poland) via polychlorvinyl tube with an inner diameter of 2 mm at a rate of 0.017 l/min. The tube terminated with a glass tip, fixed to the lateral wall of aquarium in such a way that the exit opening was 7 cm from the bottom.

After fish were placed in aquariums, they were preliminarily taught to grasp agar-agar granules that contained an aquatic solution of chironomid larvae (175 g/l). For this purpose, they were daily given item-by-item 10−15 granules with an interval of 5−10 min. Three to five days later, fish usually grasped the granule introduced into the aquarium during several seconds after it dropped into water.

After termination of the teaching of fish, experiments were started in which agar-agar granules of three types were used—those containing proline[1] or lysine in the concentration of 0.1 M and control granules without any gustatory substances. The selection of proline and lysine as gustatory stimulants is determined by their different gustatory properties for carp, attractive or repulsive, respectively (Kasumyan and Morsi, 1996). At the first stage of the experiment, the gustatory attractiveness of granules for test fish was assessed in the absence of the delivery to the aquarium of food odor or pure water (series 1). At the second stage of the experiment, the delivery of granules to the aquarium was combined with delivery of pure water during 3 min of food odor—solution of aquatic extract of chironomid larvae. The first granule was introduced

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[1] Here and further, when amino acids are mentioned, their L-stereoisomers are meant.

15 s after the beginning of the delivery to the aquarium of pure water (control) or stimulus solution. A total of one experiment with granules containing proline, lysine, and the control was performed during the delivery of pure water or stimulus solution. After experiments with the extract of chironomid larvae, water in aquariums was replaced by pure water, and repeated experiments were performed with a day interval. The second stage of the experiment was performed twice with an interval of 10 days (series 2 and 3). In the course of each three series, with each test fish, 4−5 experiments with each type of granules were performed. Granules had a cylindrical form, a length of 4.0 mm, and a diameter of 1.35 mm.

During each experiment, the following indices were recorded: (1) whether the granule was swallowed, (2) the number of acts of granule grasping up to the moment of its swallowing by fish or ultimate rejection, (3) the duration of keeping by fish of the granule in the mouth after first grasping, and (4) the duration of keeping the granule in the mouth by fish throughout the experiment. If during 1 min after introduction the granule was not grasped by fish, the experiment was not counted. The rejected or ungrasped granules were removed from the aquarium. The duration of the experiment did not exceed 1 min. Fish were fed on live chironomids once a day ad libitum after termination of experiments.

Cod. Fish after capture were first kept for a week in a small netted tank placed in the sea and then transferred to a flow-through aquarium with a volume of 150 l. Fish were daily fed on live gammaruses *Gammarus* spp. and sand eel *Arenicola marina*. Water temperature was 7−8°C. Experiments were performed in September 2007 at Belomorsk Biological Station of Moscow State University.

Two to four days prior to experiments, fish were enucleated by the removal of the lens by a narrow cross-like cut of the cornea. There was no post-operation fish mortality; fish began to feed several hours after. The operated fish were placed singly in aquariums with a size of 45 × 25 × 20 cm with a small constant inflow of pure sea water (0.25 l/min). Ground in aquariums was lacking. Pure sea water or food extract were delivered from a vessel, fixed above the aquarium through a plastic tube with an inner diameter of 3.5 mm. The tube terminated with a course-grained ceramic sprayer at the bottom in the center of the aquarium; the rate of delivery was 0.02 l/min and the duration was 5 min.

Several minutes prior to the beginning of the experiment, the inflow of sea water through the aquarium was switched off, and the experiment was performed with one type of granules against the background of delivery of pure sea water, and then after an hour interval with the same type of granules against the background of delivery of stimulus solution—an aquatic extract of gammarus with a concentration of 10^{-2} g/l. The delivery of pure water (control) or stimulus solu-

tion was started 15 s prior to the experiment. A simultaneous introduction to the aquarium of 10 control granules or 10 granules that contained indifferent by taste for cod amino acid asparagine (Kasumyan and Sidorov, unpublished data) in the concentration of 0.1 M were taken as the beginning of the experiment. Cylindrical granules with a length of 4.1 mm and a diameter of 4.0 mm were introduced in such a way that they evenly distributed at the bottom in the middle part of the aquarium. Throughout the experiment, using a stopwatch, the time when granules were grasped and whether the granule was eaten or rejected were recorded. After termination of the experiment that continued for 5 min, the uneaten granules were counted and removed; then the inflow of sea water through the aquarium was restored. No more than 4 experiments were performed daily, the interval between experiments with odor stimulation comprised not less than 3 h.

Granules were prepared from agar-agar gel (Reanal, 2%). For experiments with carp, agar-agar gel was prepared on distilled water; the extract of live chironomid larvae or amino acid was included into gel together with Ponceau 4R (5 μM) stainer. Control granules contained only stainer. For experiments with cod, gel was prepared on sea water without using stainer (control granules). For preparing granules with asparagine, the amino acid solution was added to gel during its preparation. Granules were cut out from solidified gel immediately prior to each experiment. A more detailed procedure of the preparation of fish for experiments, methods of preparation and storage of granules, performance of experiments, and keeping of fish in the period of performing work were provided previously (Kasumyan and Morsi, 1996). For preparing stimulus solutions, live chironomid larvae or gammaruses were used. After homogenization in a porcelain mortar, the extract was infused for 30 min, then filtered and diluted to the required concentration using clarified fresh water (extract of chironomid larvae) or fresh sea water (extract of gammaruses). Fresh sea water and clarified fresh water were used for control experiments also.

The total number of experiments on carp and cod was 848 and 84, respectively.

Statistical analysis of results was performed using χ^2 criterion and Student's t-test. The index of taste preference was calculated according to the formula: $Ind_{pal} = (R - C)/(R + C) \times 100$, where R are swallowed granules with substance, in %; and C is swallowed control granules, in % (Kasumyan and Morsi, 1996).

RESULTS

Carp. Consumption by carp of granules with proline in the absence of the introduction into aquarium of food extract or pure water comprised 66.7%, which exceeded by 1.69 times the consumption of control granules. Granules with lysine were consumed by carp almost 4 times less than granules with proline and more than two times less than the control (series 1). The introduction into the aquarium of pure water did not exert a considerable effect on the consumption by fish of granules of all three types—no significant differences were revealed (Table 1). On the whole, close are the values also of other parameters of fish responses recorded in different series when pure water was delivered to aquariums, or there was no such delivery. The exception was made by experimental results in series 2 with granules that contained lysine (fish grasped them repeatedly by 1.38 times more frequently, $p < 0.05$) and with the control granules (the time of keeping them after the first grasping and throughout the experiment was shorter by 1.38 and 1.24 times, $p < 0.001$ and 0.05, respectively), and in series 3 with granules that contain proline (the time of keeping them after the first grasping is shorter by 1.2 times, $p < 0.05$) and with the control granules (the time of keeping them throughout the experiment is shorter by 1.24 times, $p < 0.01$). A comparison of fish responses against the background of the inflow of pure water in series 2 and 3 revealed significant differences only in the number of graspings for granules with lysine (1.29 times, $p < 0.05$) and for control granules (1.14 times, $p < 0.05$).

The introduction into the aquarium of food odor did not cause significant changes in the consumption by fish of granules in both series (Table 1). No statistically significant differences were found in the time of keeping granules after the first grasping either. According to the total time of granule keeping, significant differences were detected only for granules with proline in series 2. The most noticeable changes caused by the introduction of food odor were manifested in the frequency of granule grasping by fish. In both series it increased for granules with lysine and for control granules, but did not change in experiments with granules that contained proline. When comparing responses exhibited by fish against the background of the chironomid extract introduced to the aquarium in series 2 and 3, significant differences were found only for control granules according to the duration of keeping them after the first grasping (1.2 times, $p < 0.05$).

The introduction into the aquarium of food odor in most experiments did not cause in fish a noticeable search for food. Fish, as prior to the presentation of stimulus solution, swam in the expectation of the granule in the center of the aquarium under the opening in the cover through which granules or food during feeding were delivered, and seldom grasped small organic particles in the water column or from the bottom surface. Some fish increased their locomotor activity. The search for food was observed only in some experiments mainly at the beginning of the stimulus period. Fish in an inclined position moved over the bottom near the output opening of the delivery tube and performed testing graspings from the bottom sur-

Table 1. Taste responses of carp *Cyprinus carpio* to granules of various types in the absence of the delivery to the aquarium of pure water or extract of chironomid larvae (series 1) and during the delivery to the aquarium of pure water or extract of chironomid larvae (series 2 and 3)

Type of granules	Consumption of granules, %	Number of grasp-ings granules	Duration of granule keeping, s		Number of experi-ments
			after the first grasping	throughout the experiment	
Series 1					
With proline, 0.1 M	66.7 ± 6.8**/	1.7 ± 0.1	3.0 ± 0.2	3.9 ± 0.3	48
With lysine, 0.1 M	16.7 ± 5.4*/	1.3 ± 0.1	1.6 ± 0.2***/	1.9 ± 0.2***/	48
Control	39.5 ± 7.1	1.5 ± 0.1	2.9 ± 0.2	3.6 ± 0.2	48
Series 2, pure water					
With proline, 0.1 M	62.7 ± 5.9***/	1.7 ± 0.2	2.8 ± 0.2**/	3.6 ± 0.2**/	67
With lysine, 0.1 M	4.8 ± 2.7***/	1.8 ± 0.2	1.2 ± 0.1***/	1.9 ± 0.2**/	62
Control	29.7 ± 5.7	1.6 ± 0.1	2.1 ± 0.1	2.9 ± 0.2	64
Series 2, extract of chironomid larvae 10^{-3} g/l					
With proline, 0.1 M	61.8 ± 6.6***/	1.6 ± 0.1*/	2.4 ± 0.2**/	3.1 ± 0.1/**	55
With lysine, 0.1 M	2.0 ± 2.0***/	2.7 ± 0.3/**	1.0 ± 0.1***/	2.1 ± 0.2*/	50
Control	26.9 ± 6.2	2.1 ± 0.2/*	1.9 ± 0.1	2.8 ± 0.2	52
Series 3, pure water					
With proline, 0.1 M	60.0 ± 6.4**/	1.5 ± 0.1	2.5 ± 0.1	3.4 ± 0.1	60
With lysine, 0.1 M	11.7 ± 4.1*/	1.4 ± 0.1	1.5 ± 0.2***/	2.0 ± 0.2***/	60
Control	31.6 ± 6.0	1.4 ± 0.1	2.4 ± 0.1	2.9 ± 0.2	60
Series 3, extract of chironomid larvae 10^{-3} g/l					
With proline, 0.1 M	59.3 ± 6.4***/	1.5 ± 0.1*/	2.7 ± 0.2	3.3 ± 0.2	59
With lysine, 0.1 M	8.6 ± 3.7*/	2.3 ± 0.2/***	1.2 ± 0.1***/	2.0 ± 0.2***/	58
Control	22.8 ± 5.6	1.8 ± 0.1/***	2.4 ± 0.2	3.2 ± 0.2	57

Note: Differences are significant at *p*: * − < 0.05, ** − < 0.01, *** − < 0.001; before the oblique line is with respect to control; after the line is between variants "pure water" and "extract of chironomid larvae 10^{-3} g/l".

face. Search for food ceased immediately after the introduction of the granule or immediately before its introduction in response to manipulations of the experimenter that accompanied granule introduction, and fish again occupied the position in the center of the aquarium near the site of the granule dropping into the water.

Cod. The enucleated cod usually slowly swims over the aquarium, more frequently near the bottom, seldom touching its surface by the chin barbel or the ends of free anterior rays of the ventral fins. Having touched some uneven site at the bottom or small particle lying on the bottom, fish can return to this site, either moving backwards or turning around. A simultaneous introduction into the aquarium of 10 granules against the background of delivery of pure sea water does not frighten fish and does not affect their behavior. Granules after their introduction in the aquarium almost immediately descend to the bottom. However, because of the specific density close to water, they easily ascend over the bottom or are drifted aside from the fish by small local water currents that arise at strokes of fins

and comparatively rapid turns and movements of fish. Having accidentally touched by the chin barbel, rostrum, or lower head surface the control granule or granule with asparagine, fish can grasp it or not respond and continue swimming. The grasping of the granule provokes a short-time search activity in fish.

The introduction in the aquarium of food extract changes fish behavior and causes in them a well pronounced search for food. The time after which search response for food odor becomes noticeable varies, on average it is from 15 to 40 s and depends on the fact as to how rapidly the cod reaches the odor distribution zone. On getting into the odor zone, fish, with head inclined to the bottom and closely touching its surface with the chin barbel, rays of ventral fins, and rostrum, moves along circular trajectories, searches, moves backwards, and grasps (bites off) from the bottom surface. On the whole, the motor activity of cod at this time is considerably higher than prior to odor presentation. This leads to the fact that granules are periodically drifted from the center to the walls and corners of the aquarium, which makes them temporarily less
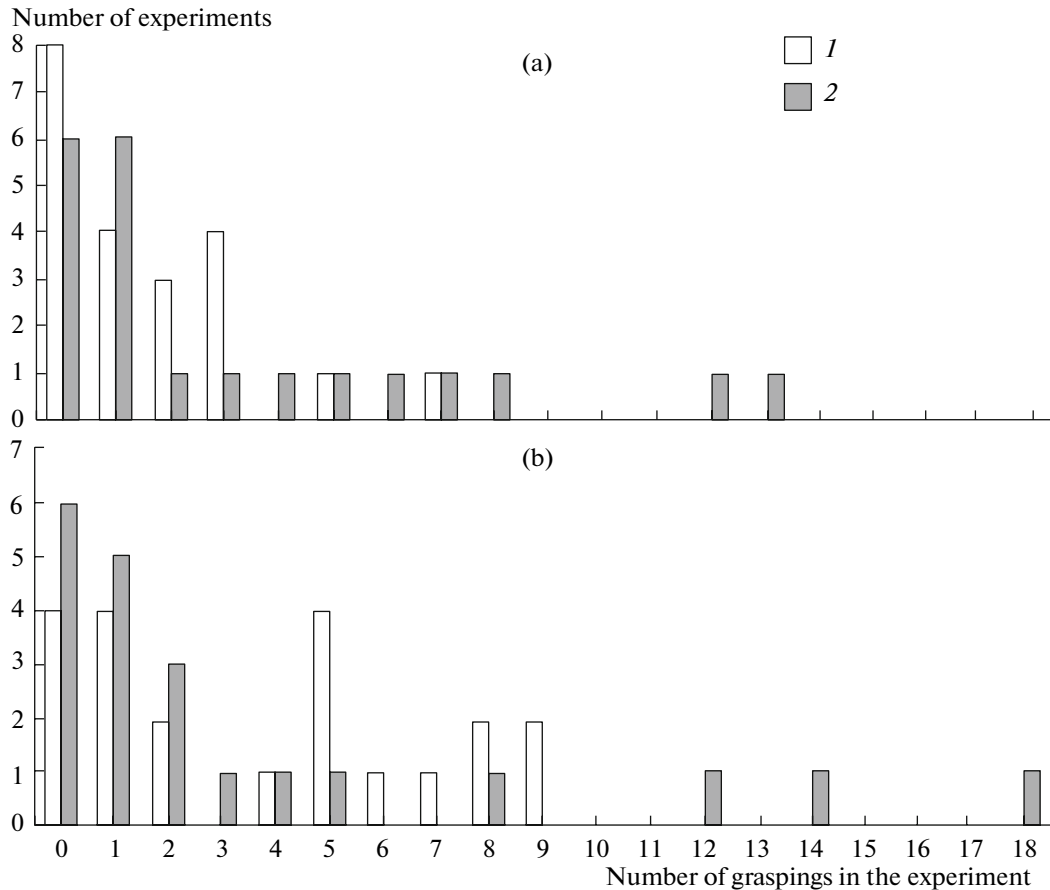
Fig. 1. Distribution of experiments on the number of graspings by cod *Gadus morhua* of (a) control granules and (b) granules with asparagine, 0.1 M against the background of supply to the aquarium of (*1*) pure water or (*2*) gammarus extract, 10^{-2} g/l.

accessible for test fish devoid of object vision. In some cases, fish grasped granules that were raised above the bottom and moved by water microcurrents, if they had touched them accidentally by the rostrum, barbel, lateral and lower head surface, and branchial covers.

Grasping of granules occurred in 60–80% of experiments in different experimental variants. The proportion of grasped granules of their total number presented to fish in each variant (210 items) comprised 17 to 38%. The number of granule graspings in some experiments reached 7–9 in the absence of food stimulation and 13–18 in experiments with the presentation of food odor to the aquarium; however, in most experiments, where graspings were observed, their number did not exceed 2–3 (Fig. 1). The average number of graspings of control granules for 5 min of the experiment with odor stimulation comprised 3.14, which is significantly higher than in experiments with the delivery of pure sea water to the aquarium (1.67, $p < 0.001$). Granules with asparagine, on average, were grasped by fish with equal frequency in experiments with odor stimulation and without it—3.71 and 3.76%, respectively ($p > 0.05$) (Table 2). Against the background of pure water, granules with asparagine

were grasped significantly more frequently than control granules ($p < 0.001$); against the background of gammarus extract, no differences between grasping granules with asparagine were revealed ($p > 0.05$). Graspings were observed throughout the experiment; however, at the beginning, they were performed more frequently. This specific feature, as a more frequent grasping of granules in experiments against the background of the delivery into the aquarium of gammarus extract is more noticeable in experiments with the use of control granules (Fig. 2). The time before the first grasping of the granule in all experimental variants widely varied; on average, it was slightly greater in experiments with odor stimulation than against the background of the delivery of pure water to the aquarium, 89 and 73 s, respectively, for control granules and 102 and 46 s, respectively, for granules with asparagine (differences are insignificant in both cases, $p > 0.05$). The consumption of granules of both types was low in all experimental variants. At the delivery to the aquarium of pure water or food extract, the proportion of granules swallowed after grasping comprised 5.7 and 4.6%, respectively, for control granules and 11.4 and 6.4%, respectively, for granules with asparagine. No

Table 2. Gustatory responses of cod *Gadus morhua* to granules of various types against the background of the delivery to the aquarium of pure sea water or gammarus extract

Type of granules, experimental conditions	Number of experiments with graspings, %	Number of graspings in the experiment ($M \pm m$)	Consumption of granules, % of graspings	Number of experiments
With asparagine, 0.1 M				
—pure sea water	81.0	3.76 ± 0.70	11.4	21
—gammarus extract, 10^{-2} g/l	71.4	3.71 ± 1.14	6.4	21
Control				
—pure sea water	61.9	1.67 ± 0.44	5.7	21
—gammarus extract, 10^{-2} g/l	71.4	3.14 ± 0.86	4.5	21

Note: $M \pm m$ is average index value and its error.

significant differences between the consumption of granules of the same type under conditions of odor stimulation of fish and its absence, as well as between the consumption of granules of various types under similar experimental conditions, were revealed ($p > 0.05$, all variants).

DISCUSSION

Taste preferences in fish are characterized by high species specifics. The spectra of attractive and repulsive, by taste, substances differ in various species, and, at the same time, they are similar in specimens of the same species, but which belong to different populations and generations or having different food experience (Kasumyan and Morsi, 1997; Kasumyan and Døving, 2003; Fokina and Kasumyan, 2003; Kasumyan and Sidorov, 2005a). This indicates a high genetic determination of taste preferences and apparently their low plasticity, slight subjectivity, in particular, to the effects of diverse external factors, against the background of which, the functioning of the gustatory system that provides the selective feeding of fish under varying dwelling conditions occurs. The modifying effect of the environment on taste preferences and gustatory behavior of fish has been studied insufficiently. It is known, in particular, that shifts in taste spectra are caused by water temperature, whose change affects also many other physiological processes and fish behavior, including their food motivation (Kasumyan et al., 1993). The blocking of taste reception is caused by water pollution with heavy metals, whose negative effect is manifested in the disturbance of work of many other sensory systems also, in the disturbance of physiological processes and behavior of fish (Kasumyan and Morsi, 1998; Kasumyan, 2001).

Olfaction is important in the life of fish, in the regulation of various forms of behavior (Malyukina et al., 1969; Kasumyan, 2004). Many odor signals have not only a releasing but also a primary effect and cause changes in the motivation state of fish and their physiological status. Signals of danger, for instance, odor of a predator or alarm pheromone cause the cessation of spawning migration of fish, and change body pigmentation and electrolytic composition of mucosa (Brett and McKinnon, 1954; Idler et al., 1956; Lebedeva and Chernyakov, 1978; Lebedeva et al., 1989, 1999, 2000). The odor image of the predator or food competitor, as well as their visual image or actual presence disturbs feeding of fish or completely blocks their feeding behavior (Smith, 1981; Malyukina et al., 1983, Metcalfe et al., 1987; Magurran, 1990; Marusov, 1990; Jachner, Lanecki, 1999; Brown et al., 2001). A strong stimulus action on the feeding behavior of fish is made by food odors. The odor of familiar or habitual food for fish causes not only a well pronounced, strong, and diverse in manifestation search response, but also sharply increases the food motivation of fish. Observations indicate that food odor makes fish to grasp and subject to odor testing many previously indifferent for them items, even those that in sizes, shape, or coloration only distantly remind of food items (Kasumyan and Ponomarev, 1986, 1989, 1990).

In our experiments on carp, because of the methods of the experiment used, the search activity in response to the administration to the aquarium of food odor was not obviously manifested, except some experiments. Intact carps taught to immediately grasp the presented granules were almost constantly in the center of the aquarium waiting for food and could visually control its entire volume. The concentration of the extract of chironomid larvae used for odor stimulation of fish, by two orders of magnitude exceeded the threshold level for fish at a point administration of stimulus solution, and, under conditions analogous to those that were in our experiments, caused in fish a clearly pronounced food search (Kasumyan and Ponomarev, 1989, 1990; Kasumyan and Marusov, 2005). An increase in the number of repeated graspings of control granules and granules with lysine indicates indirectly the response of fish to odor and an increase in their food stimulation. It is of interest that the number of repeated graspings increased in experiments in which indifferent or unattractive, by taste, granules were used: 1.5—1.6 times for granules with lysine, and 1.3 times for control granules (Fig. 3). The
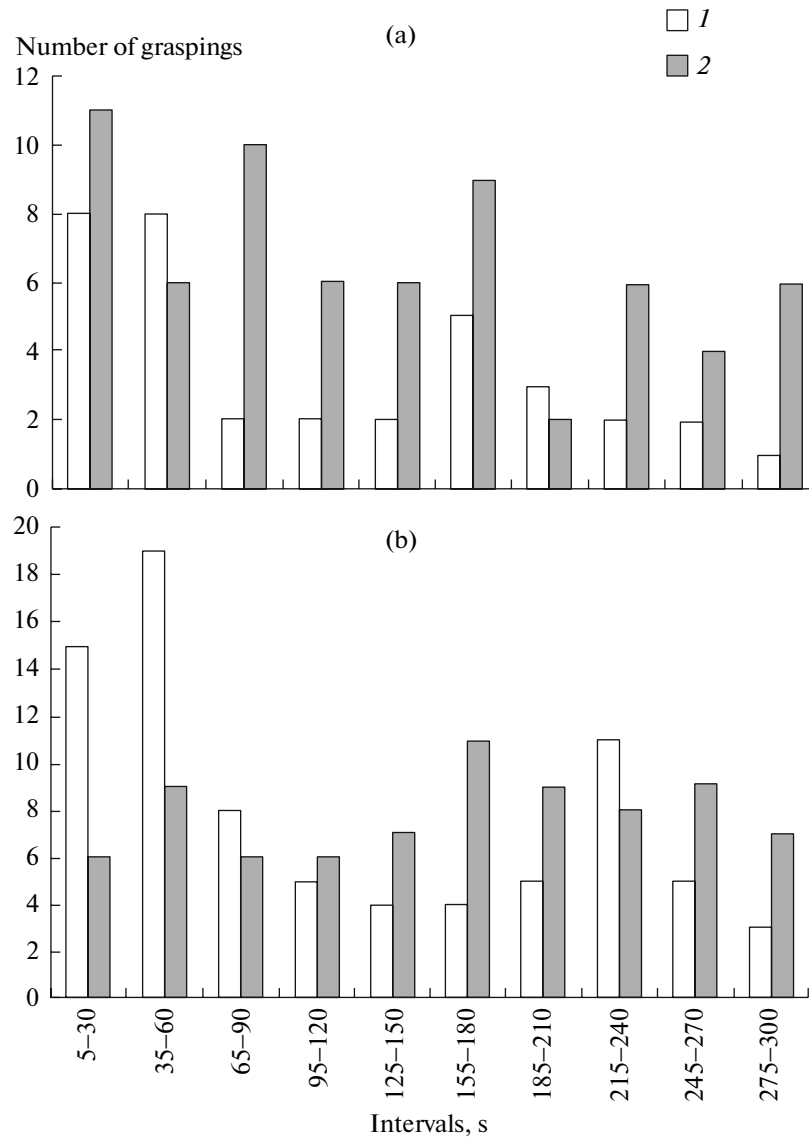
Fig. 2. Distribution of the number of graspings by cod *Gadus morhua* of (a) control granules and (b) granules with asparagine, 0.1 M against the background of supply to aquarium of (1) pure water or (2) gammarus extract, 10^{-2} g/l in different time intervals.

frequency of graspings granules with a highly attractive, for carp, proline was similar prior to the delivery and during the administration of extract to the aquarium. The increase in the frequency of repeated graspings that occurs namely in experiments with granules unattractive by taste is apparently a result of the confrontation of information contained in two chemical signals opposite by sign and different in modality that participate in the regulation of the same form of behavior—a feeding one. Olfactory sensations that arise from the odor of familiar and attractive food do not correspond to the indifferent or slightly attractive odor of the grasped item, and food stimulation caused by odor induces fish to give up the consumption of such an item and to search for something new and more suitable in taste properties. Under experimental conditions when fish are given only one granule, this leads to its repeated graspings and rejections. The absence or not so pronounced contradiction between olfactory and gustatory sensations that, as may be assumed, occurs in fish at presentation of granules with proline does not lead to more frequent rejections and repeated graspings, but, as a rule, decreases the duration of keeping granules (Table 1). This was statistically significant, but small in value (1.16 times) only in one case—in series 2. This parameter of taste responses in carp is closely related to the level of consumption (Kasumyan and Morsi, 1996); therefore its decrease one could consider as some decrease in the taste attractiveness, for carp, of granules with proline under conditions of odor stimulation (despite the fact that actually their consumption in this series almost
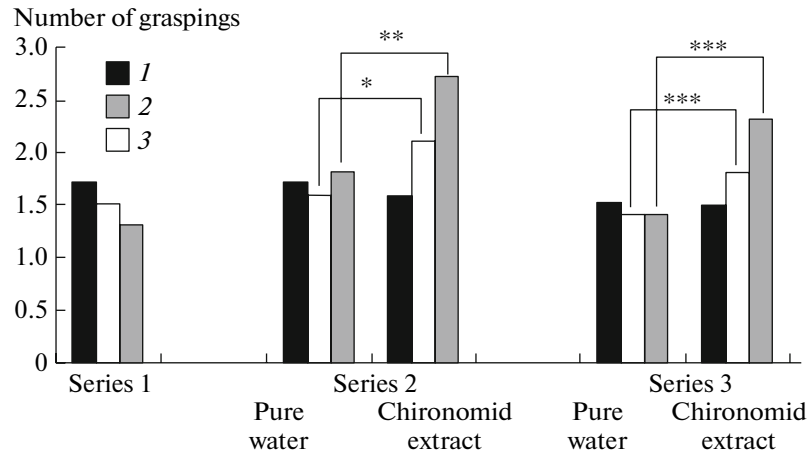
Fig. 3. Average number of graspings by carp *Cyprinus carpio* of (*1*) granules with proline, 0.1 M; (*2*) lysine, 0.1 M; and (*3*) control granules without the supply of water or stimulus solutions (series 1), against the background of inflow to aquarium of pure water or extract of chironomid larvae, 10^{-3} g/l (series 2 and 3).

did not change—62.7 and 61.8% prior to and during the presentation of the extract). A more true explanation of the decrease in the duration of granule keeping is a decrease in time spent by fish on testing, decision making, and swallowing an item with attractive taste properties caused by food stimulation. Many food items for fish are not distributed evenly, but form spatially isolated aggregations or microcommunities, the so called food spots (Kruglova and Bakanov, 1977; Greenblatt, 1982; Omori and Hamner, 1982). Around them, an odor field is formed, the presence of fish in which was imitated by the performed experiments. The less time under such conditions is spent on feeding, the more intensively it will proceed. The adaptive importance of it may be also related to an increase in competitive abilities of fish that lead a group or school mode of life, to a decrease in the vulnerability for predators thanks to a more rapid saturation, and moving to safer sites, etc.

Experiments with cod were performed on preliminarily enucleated fish. As was shown previously, enucleation increases the response of cod and fish of other species (trout *Salmo trutta*, rainbow trout *Oncorhynchus mykiss*, carp, minnow *Phoxinus phoxinus*) to species chemical signals and food odors, decreases fearfulness of fish, and facilitates the recording of behavioral responses (Malyukina et al., 1983; Marusov, 1997; Kasumyan and Marusov, 2002, 2003, 2008; Kasumyan and Marusov, 2005). The response to food extract in experiments on enucleated cod was more obvious than in carp. Stimulus solution caused a well pronounced, for cod, search response—movements of fish near the odor source in an inclined position with touching the bottom by the chin barbel and free rays the of ventral fins (Brawn, 1969; Døving and Selset, 1980; Ellingsen and Døving, 1986; Marusov, 1997). Food stimulation was manifested also in a greater number of graspings, in the case of control

granules, it increased almost twofold. However, this effect is unstable; it was not manifested in experiments with granules that contained asparagine. The cause of a high variability of the number of graspings in experiments is related to the fact that agar-agar granules that are insufficiently heavy with respect to water were easily moved by microcurrents in random directions, their even distribution over the aquarium bottom was disturbed at an increase in the swimming activity of fish. As a result, some granules got into little-accessible sites of the aquarium, which reflected on the efficiency of the search of enucleated fish devoid of object vision. The locomotor activity of fish increased not only during stimulation by food odors, but also in response to the proper introduction of granules to the aquarium, which is testified by more frequent graspings at the beginning of the experiment at the delivery of pure water to aquarium. The effect of odor stimulation was manifested in a slower decrease in the frequency of graspings or its retention approximately at a constant level up to the end of the period of stimulation. The introduction of food odor to the aquarium led also to the fact that the number of graspings in some experiments was 13–18, which considerably exceeded the maximum number of graspings in experiments without odor stimulation: 7–9 (Figs. 1 and 2).

Despite the fact that food motivation increases under the effect of food odor and fish grasp granules more frequently, consumption, as demonstrated by results of the present investigation, does not change. In carp, the relative level of consumption prior to and in the period of odor stimulation was similar for granules of various types—those containing attractive substances (proline), deterrent (lysine), and the control (Table 1). Food odor did not affect consumption in cod either, in experiments with which, control granules and granules with asparagine close to them in taste attractiveness were used (Table 2).

At the same time, as the performed calculations show, the stimulation of fish with food odor drastically changes the efficiency of graspings by them of granules of a certain type. If the average number of graspings per one swallowed granule with a substance attractive by taste (proline, carp) is retained at the same level prior to and in the period of stimulation, swallowing granules with deterrent (lysine, carp) requires many times greater seizings, especially in case of the action of food odor. Granules with indifferent taste substances (asparagine, cod) and control for this index occupy an intermediate position (Table 3).

Data on the effects of odor background on food consumption by fish or other animals are scarce. In experiments on fish (gilthead *Sparus auratus* and genuine porgy *Pagrus (Chrysophrys) major*), feeding with artificial food in combination with an additional odor stimulation by extract or exometabolites of food items (nauplii *Artemia*, polychaetes *Perinereis brevicirrus*, Mussels *Mytilus edulis*) led to a noticeable increase in food consumption (Fuke et al., 1981; Tandler et al., 1982; Kolkovski et al., 1997). This effect was still more pronounced if fish stimulation was performed by olfactory and visual food stimulants simultaneously (Kolkovski et al., 1997). In a situation when stimuli that cause negative responses are used for odor background, food consumption does not change. For instance, feeding of sheep with barley with the simultaneous presentation of odor of *Astragalus bisulcatus*—a pasture sulphur-containing plant that has an unpleasant odor for human and, as assumed, an unpleasant taste and toxic effect for ruminants—found no decrease in consumption, if odor stimulation was not combined with artificially caused toxicosis (injection of lithium chloride) (Provenza et al., 2000).

Comparison of results obtained in the present study with data of earlier investigations indicates that the response of carp to the taste of two amino acids, proline and lysine, remained as before (Kasumyam and Morsi, 1996; Kasumyan and Sidorov, 2005b). The results are close not only by the level of the consumption by carp of granules with these amino acids, but also by the sign and values of the index of taste attractiveness—the relative index that expresses substance attractiveness with regard to the level of consumption of control granules (Figs. 4 and 5). Since test carps were obtained from the same source, but in different years, this confirms the conclusion of the stability of taste preferences in several generations that was formulated previously based on results of studies of other fish species (nine-spined stickleback *Pungitius pungitius,* trout) (Fokina and Kasumyan, 2003; Kasumyan and Sidorov, 2005a). Our experiments can be also still another proof of the fact that methods based on item-by-item presentation to fish of granules with various substances are adequate for the assessment of taste preferences of these substances, and that in the response of fish to granules, olfactory reception either is not involved altogether or this participation is not

Table 3. Average number of graspings per one consumed granule in experiments with stimulation and without stimulation by food odor in carp *Cyprinus carpio* and cod *Gadus morhua*

Type of granules	Number of graspings per one consumed granule	
	without stimulation with food odor	with stimulation with food odor
Carp		
With proline, 0.1 M		
—series 1	2.5	–
—series 2	2.7	2.6
—series 3	2.5	2.5
With lysine, 0.1 M		
—series 1	7.7	–
—series 2	36.7	136.0
—series 3	12.0	22.0
Control		
—series 1	3.7	–
—series 2	5.5	7.9
—series 3	4.4	8.1
Cod		
With asparagine, 0.1 M	8.8	15.6
Control	17.5	22.0

considerable. The level of granule consumption was similar in intact carps in pure water and in water with food odor (our data), as well as in anosmiated specimens (Kasumyan and Sidorov, 2005b) (Fig. 4). The latter two variants are extreme, diametrically opposite by the participation of olfaction in the response of fish to granules. However, similar results were obtained in both cases, which indicates the absence of such participation.

Granules with asparagine, as was already mentioned previously, are consumed by cod in the same way as the control, which makes it possible to relate this amino acid to substances with indifferent taste properties for intraoral taste reception. However, in cod, taste buds are located not only in the buccal cavity, but also in the epithellium of the chin barbel, at the surface of upper and lower lips, rostrum and head, and at the free rays of the ventral fins (Harvey and Batty, 1998, 2002; Devitsina, 2005). Cod devoid of object vision grasps granules not targetly as intact specimens, but after a preliminary touching of the granule namely by those structures that have external taste buds. In pure water, the number of graspings by cod of granules with asparagine was significantly higher than of the control granules; therefore, this amino acid can be considered efficient for external reception and related to a group of the so called insitants (Kasumyan and
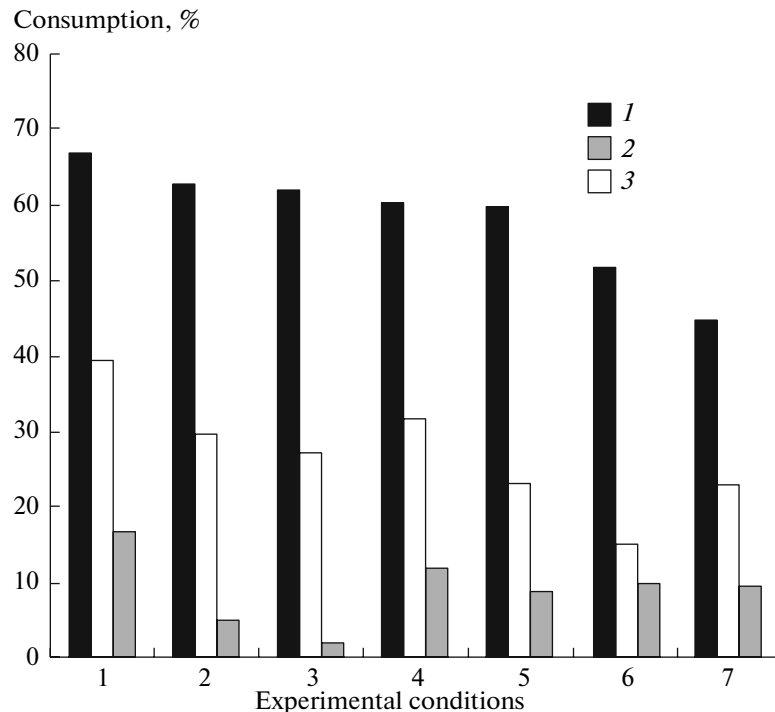
Consumption, %

Fig. 4. Consumption by carp *Cyprinus carpio* of granules with (*1*) proline, (*2*) lysine, and (*3*) control. Experimental conditions: (1) without delivery of water or stimulus solutions (series 1); (2 and 4) against the background of the inflow to the aquarium of pure water (series 2 and 3); (3 and 5) against the background of the introduction to the aquarium of extract of chironomid larvae, 10^{-3} g/l (series 2 and 3); (6) according to data of Kasumyan and Morsi, 1996; (7) anosmiated specimens, according to data of Kasumyan and Sidorov, 2005.

Døving, 2003). In fish, extraoral taste spectra are wider than intraoral and usually involve a considerable number of stimuli that are indifferent for intraoral reception (Kasumyan, 1997; Kasumyan and Døving, 2003).

Food behavior of fish suggests the participation of many senses in the search, discovery, and recognition of prey and the assessment of their properties (Pavlov and Kasumyan, 1990). An integrated food perception is accompanied by a polymodal integration in brain centers of information that comes via different sensory canals and forms a complex perceptual image of the food item (Bult et al., 2007). Simultaneous stimulation by food stimuli of various modality leads also to complex intersystemic interactions that affect final decision making by the consument (Dalton et al., 2000; DeWijk et al., 2006). Many of these processes in fish remain unstudied.

It is known that an increase in food stimulation caused, for instance, by starvation and the, related to this, increase in locomotor activity lead to a decrease in time spent by fish on prey discovery (Pavlov and Kasumyan, 1998). Such a development of events, as follows from the present work, can not always end, as it would seem to be an obvious result, the intensification of feeding of fish and an increase in the consumption of prey accessible to them. For this to occur, the grasped prey should correspond to requirements specific for a particular consumer species, have certain taste properties. Only in this case, a complex, multi-link chain of feeding behavior will end in prey consumption rather than stop earlier at one of the intermediate stages. The results of the work performed indicate that, despite the fact that the efficiency of foraging behavior in fish that are in the zone of food odor field increases, and they grasp a greater number of food items, an increase in the actual consumption will
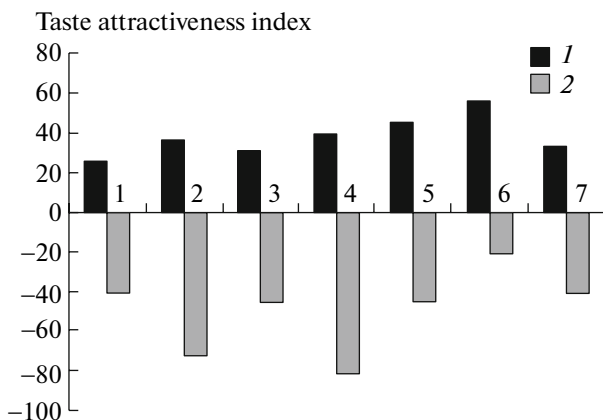
Taste attractiveness index

Fig. 5. Taste attractiveness index of (*1*) proline and (*2*) lysine for carp *Cyprinus carpio*. Designations of experimental conditions are the same as in Fig. 4.

occur in only those who are characterized by a rather high taste attractiveness. The rejection by fish of items that do not meet such requirements, because of an arising contradiction between the information obtained by fish via two different chemosensory canals, will drastically increase

The stimulation of fish by food odors does cause shifts in taste preferences, but modifies gustatory behavior. Under what external conditions and at what physiological state of fish these shifts may occur and, as a result, change the taste attractiveness of the grasped prey for fish remain poorly studied. The investigation of these issues will make it possible to elucidate new specific features of taste reception and gain a more clear idea of the mechanisms that are the basis of trophic relations in aquatic ecosystems.

ACKNOWLEDGMENTS

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