TASTE RESPONSES OF CARP FISHES (CYPRINIDAE) TO CARBOXYLIC ACIDS. 1. TASTE PREFERENCES

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The palatability of carboxylic and some other organic acids (10^{-1} M) for dace *Leuciscus leuciscus*, roach *Rutilus rutilus*, and common carp *Cyprinus carpio* was assessed. The conclusion about species specificity of taste preferences in fishes was confirmed. Four out of 17 carboxylic acids stimulate consumption of pellets in dace, of which formic acid has the strongest effect. A significant decrease in consumption is caused by ten acids. For roach, no palatable carboxylic acids were found; most of them (13 out of 15) have a repulsive taste. For common carp, four acids have attractive taste, one (malonic acid) has repulsive taste, and the remaining 11 acids have no effect on pellet consumption. The stimulating effect of the acids persists up to concentrations of 10^{-4} and 10^{-3} M. Among the carboxylic acids, there is not one with the same taste properties for the studied fish. No significant similarity was found between dace, roach, common carp, and other fish species in terms of palatability of carboxylic acids. A direct dependence of pellet consumption on pH of carboxylic acid solutions was observed in roach and dace, while it was absent in common carp. The dependence of the palatability of carboxylic acids on the size of their molecule is weakly expressed. Structural transformations of the acid molecule do not always lead to shifts in taste properties, and in different species, they may not coincide or be opposite. Ascorbic acid (vitamin C) has a repulsive taste for roach, indifferent for dace, and attractive for common carp, which confirms the lack of relationship between physiological needs in essential micronutrients and their palatability, shown earlier on the example of amino acids.

Keywords: carp fishes, Cyprinidae, gustatory system, taste reception, taste preferences, taste attractiveness, carboxylic acids, dace *Leuciscus leuciscus*, roach *Rutilus rutilus*, common carp *Cyprinus carpio.*

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INTRODUCTION

Carboxylic acids and their derivatives participate in the main metabolic processes of animals and plants and are widely represented in fish food objects (Sterry et al., 1985; Daldorph, Thomas, 1991; Liu et al., 2018; Nelson, Cox, 2021). However, these substances are rarely used as stimuli for studying the gustatory system, despite the fact that their effectiveness for fish taste receptors, albeit on a small number of examples, has been established in electrophysiological experiments. It has been shown that caproic, butyric, propionic, and some other carboxylic acids cause significant responses in the taste nerves of juvenile Atlantic salmon *Salmo salar* when stimulating taste buds on the palate (Sutterlin, Sutterlin, 1970). For intraoral taste receptors of the Japanese eel *Anguilla japonica* , all six carboxylic acids used were effective (Yoshii et al., 1979), and for carp taste receptors *Cyprinus carpio* , most of the 14 acids were effective (Marui, Caprio, 1992).

Information about taste properties of carboxylic acids, determined using behavioral tests, is equally limited. It is known that for tench *Tinca tinca* and marble sleeper *Oxyeleotris marmorata* all carboxylic acids used for experiments - 17 and 12 respectively - possess attractive taste (Kasumyan, Prokopova, 2001; Lim et al., 2017). For nine-spined stickleback *Pungitius pungitius* all 17 carboxylic acids used have repulsive taste, while for bitterling *Rhodeus sericeus* (= *Rhodeus sericeus amarus)* among the same acids, 12 have repulsive taste and the remaining five are indifferent (Mikhailova, Kasumyan, 2018; Kasumyan, Isaeva, 2023). The contrastingly different taste properties of carboxylic acids and the limited number of studied species do not allow us to understand whether the conclusions about species-specific taste preferences formulated on the example of amino acids are valid for this group of substances (Kasumyan, 1997; Kasumyan, Døving, 2003; Morais, 2017). For the same reason, it is not possible to determine whether the taste properties of carboxylic acids are determined by taxonomic position, lifestyle and diet of fish, and how universal the relationship between taste attractiveness of carboxylic acids and their molecular weight, pH level of aqueous solutions, and structural features of the molecule can be.

The purpose of this work is to compare the taste attractiveness of carboxylic acids for several previously unstudied species of cyprinid fish (Cyprinidae sensu lato - according to: Tan, Armbruster, 2018), differing in diet and other biological characteristics, as well as to evaluate the relationship between taste properties of carboxylic acids and some of their physicochemical characteristics.

MATERIAL AND METHODS

The experiments were performed on roach *Rutilus rutilus* (average absolute length 7.0 cm, average weight 4.8 g, 16 specimens), dace *Leuciscus leuciscus* (12.5 cm, 11.5 g, 11 specimens) and carp (13.2 cm, 24.8 g, 16 specimens). Young roach were caught in the Vorya River (tributary of the Klyazma River, Krasnoarmeysk, Moscow region), dace - in a small tributary of the Moscow River (near Tuchkovo, Moscow region), carp were obtained from the Freshwater Fisheries Branch of the All-Russian Research Institute of Fisheries and Oceanography (Dmitrov, Moscow region). After delivery to the laboratory, fish of different species were kept separately in common 200-liter aquariums for at least 20 days at a water temperature of 19-23°C and daily feeding with live or freshly frozen Chironomidae larvae.

2-5 days before the start of the experiments, fish were placed in individual 10-liter aquariums with opaque side and back walls. There was no substrate in the aquariums, and changes in illumination corresponded to the natural daily rhythm. Micro-compressors were used for aeration. Partial water replacement in the aquariums was carried out weekly. The fish were fed live or freshly frozen Chironomidae larvae until satiated at the end of each day.

To train fish to grab the provided food, Chironomidae larvae were used, and then agar-agar granules containing an aqueous extract of the larvae (75 g/l). Granules were introduced one by one through a hole in the aquarium lid. For the experiments, granules containing either one of the carboxylic acids or ascorbic or boric acid were used. All tested substances were present in the granules at a concentration of 10^{-1} M. Along with the test substance, the granules contained red food dye (Ponceau 4R, 5 μM). Granules containing only dye were used as a control. All granules had a cylindrical shape; diameter 1.35 mm (roach), 1.50 mm (dace, carp) and length 4 mm. Granules were cut from the agar-agar gel immediately before each experiment.

To prepare the gel, the agar-agar suspension ("Reanal", Hungary, 2%) was heated in a water bath until completely dissolved. A solution of the test substance or Chironomidae larval extract and a dye solution were added to the hot agar-agar solution (60–70°C), mixed, and poured into a Petri dish. The gel with chemical substances was stored at 4°C for no more than 2 weeks, and with Chironomidae extract – for no more than 3 days.

In the experiment, one agar-agar granule was introduced into the aquarium, and its ingestion or rejection after being seized by the fish was recorded. Granule swallowing was determined by the completion of characteristic jaw movements and the restoration of the fish's normal rhythm of respiratory movements of the gill covers. Final rejection was determined by loss of interest and the fish moving away from the rejected granule or the fish orienting in another, often opposite, direction from the granule. The duration of the experiment did not exceed 1–2 minutes. If the fish did not grab the granule within 1 minute, the granule was removed from the aquarium, and the experiment was not counted. Experiments in which fish destroyed but did not swallow the granule, or swallowed less than half of the destroyed granule, were classified as those in which consumption did not occur. The presentation of control granules and granules with test substances was alternated with the presentation of granules containing Chironomidae larval extract. Granules of all types were presented to the same individual in a random sequence with an interval of 10–15 minutes.

The total number of experiments performed was 2200 on dace, 1316 on roach, and 3095 on carp. Statistical evaluation of the results was performed using the χ^2 test, U-Mann-Whitney test, and Spearman's rank correlation coefficient (r_s). The palatability index was also calculated using the formula: $Ind_{pal} = (R - C)/(R + C) \times 100$, where R – consumption of granules with the substance, %; C – consumption of control granules, %.

RESULTS

In dace, four carboxylic acids out of 17 stimulate pellet consumption, with formic acid having the strongest effect, increasing consumption by 1.5 times compared to the control. Ten acids cause a significant decrease in consumption, while the remaining three carboxylic acids (acetic, butyric, and α-ketoglutaric), as well as ascorbic acid, which is not a carboxylic acid, have no effect on pellet consumption (Table 1).

In roach, most carboxylic acids – 13 out of 15 – cause a significant decrease in pellet consumption. Malonic and glycolic acids have the strongest repellent taste, reducing pellet consumption by 2.8 and 3.3 times respectively compared to the control. Ascorbic acid also has a repellent taste. Formic and acetic acids, as well as boric acid, which does not belong to the group of carboxylic acids, have no effect on pellet consumption (Table 1).

For carp, citric, tartaric, and adipic acids are tasteful, causing a significant increase in consumption. Ascorbic acid produces the same effect, second only to citric acid in stimulatory action. Malonic acid has the opposite effect. The remaining 11 carboxylic acids and boric acid have no effect on pellet consumption (Table 1). In an additional series of experiments on carp, it was established that the stimulatory effect of citric and ascorbic acids, when their content in pellets is reduced, persists down to concentrations of 10^{-4} and 10^{-3} M respectively (Fig. 1).

Table 1. Taste responses ($M \pm m$) of common dace *Leuciscus leuciscus* , roach *Rutilus rutilus* and carp *Cyprinus carpio* to pellets with carboxylic acids and other substances

Stimulant	Dace		Roach			Carp		
	Pellet consumption, %	IAR	Pellet consumption, %	IAR	Number of experiments	Pellet consumption, %	IAR	Number of experiments
	Carboxylic acids							
Adipic acid	34.5 ± 4.6***	−25.5	31.5 ± 5.5**	−26.7	73	42.4 ± 3.9*	15.2	157
Valeric acid	70.9 ± 4.4**	9.9	28.2 ± 5.1**	−31.7	78			
Tartaric acid	40.9 ± 4.7***	−17.4	33.8 ± 5.5*	−23.4	74	48.1 ± 4.0**	21.3	158
Glycolic acid	44.5 ± 4.8*	−13.3	16.4 ± 4.4***	−53.7	73			
Glutaric acid						33.5 ± 3.8	3.6	157
Caproic acid	72.7 ± 4.3***	11.2	23.6 ± 5.0***	−39.5	72			
α-Ketoglutaric acid	52.7 ± 4.8	−4.9	25.3 ± 5.1***	−36.5	75	41.1 ± 3.9	13.7	157
Citric acid	32.7 ± 4.5***	−28.0	30.7 ± 5.4**	−27.9	75	70.3 ± 3.6***	38.5	157
Maleic acid	43.6 ± 4.8*	−14.3	26.7 ± 5.1**	−34.2	75	27.2 ± 3.6	−6.9	158
Malonic acid	33.6 ± 4.5***	−26.7	19.4 ± 4.7***	−47.4	72	21.0 ± 3.3*	−19.5	156
Butyric acid	69.1 ± 4.4	8.7				35.0 ± 3.7	5.7	160
Formic acid	86.4 ± 3.3***	19.6	57.3 ± 5.7	2.6	75	31.3 ± 3.7	0.3	150
Propionic acid	70.9 ± 4.4**	9.9				36.3 ± 3.8	7.6	160
Acetic acid	66.3 ± 4.5	6.6	37.8 ± 5.7	−18.0	74	31.3 ± 3.7	0.3	150
Fumaric acid	41.8 ± 4.7***	−16.3	27.0 ± 5.2**	−33.7	74	22.8 ± 3.3	−15.6	157
Oxalic acid	45.5 ± 4.8*	−12.2	29.3 ± 5.3**	−30.0	75	29.9 ± 3.7	−2.1	156
Succinic acid	41.8 ± 4.7***	−16.3	22.5 ± 5.0***	−41.5	71	28.6 ± 3.6	−4.4	157
Malic acid	36.4 ± 4.6***	−23.0	23.3 ± 5.0***	−40.0	73	36.3 ± 3.9	7.6	156
	Other substances							

Ascorbic acid	53.6 ± 4.8	−4.0	23.3 ± 4.9***	−40.0	74	57.3 ± 3.9***	29.5	156
Boric acid			56.6 ± 5.7	2.0	76	36.9 ± 3.8	8.4	160
Chironomidae extract						92.4 ± 1.6***	49.5	277
Control	58.1 ± 3.3	19.6	54.4 ± 6.7		57	31.2 ± 3.7		156

Note. $M \pm m$ – mean value and its error, TPI – taste preference index; concentration of Chironomidae extract –75 g/l; number of control experiments with dace – 220, with each acid – 110; differences from control are significant at p : * < 0.05, ** < 0.01, *** < 0.001. Here and in Table 3: acid concentrations – 10^{-1} M.

DISCUSSION

The study conducted on dace, roach and carp has almost doubled the number of species for which the taste attractiveness of a wide range of carboxylic acids has been evaluated – from four species (tench, marble sleeper, nine-spined stickleback, bitterling) (Kasumyan, Prokopova, 2001; Lim et al., 2017; Mikhailova, Kasumyan, 2018; Kasumyan, Isaeva, 2023) to seven. Information about the taste response to carboxylic acids is also known for some other fish, but these data concern only individual substances of this class. For Atlantic salmon juveniles, of the six acids tested, caproic acid and possibly valeric acid were attractive in taste (Sutterlin, Sutterlin, 1970). For tilapia *Coptodon zillii* citric acid has such taste, while the taste of malic acid, the other of the two used, is indifferent (Adams et al., 1988). Citric, lactic and metacetonic acids stimulate food grabbing in Nile tilapia *Oreochromis niloticus* (Xie et al., 2003). Citric acid has a strong taste for many fish, enhancing food consumption in some of them and causing an opposite reaction in others (Kasumyan, Døving, 2003).

Interspecies Comparisons . The large number of carboxylic acids used for studies of different fish species provides an opportunity to conduct correct interspecies comparisons of taste attractiveness and other types of analysis. The general characterization of taste spectra of the studied fish shows that the response to the taste of carboxylic acids differs among fish of different species, making it easy to distinguish two groups among them. A larger group consists of fish for which many or all carboxylic acids have a repulsive taste – roach, dace, nine-spined stickleback, bitterling (Mikhailova, Kasumyan, 2018; Kasumyan, Isaeva, 2023). The other group includes fish for which all acids have an attractive taste and their presence in food stimulates consumption – tench and marble sleeper goby (Kasumyan, Prokopova, 2001; Lim et al., 2017). For carp, most acids turned out to be tasteless (Table 2). It is quite likely that as the list of studied fish continues to expand, not only will the numerical ratio of these groups change, but species with intermediate positions will also be identified, as observed when comparing amino acid spectra (Kasumyan, 2016).

Table 2. Number of carboxylic acids with different taste properties for fish

Species	Acids												Total number of acids
	monocarboxylic			dicarboxylic			tricarboxylic			all			
	"+"	"+/–"	"–"	"+"	"+/–"	"–"	"+"	"+/–"	"–"	"+"	"+/–"	"–"	
Dace *Leuciscus leuciscus*	4	2	1	0	1	8	0	0	1	4	3	10	17
Roach *Rutilus rutilus*	0	2	3	0	0	9	0	0	1	0	2	13	15
Carp *Cyprinus carpio*	0	4	0	2	7	1	1	0	0	3	11	1	15
Bitterling *Rhodeus sericeus* [1]	0	5	2	0	0	9	0	0	1	0	5	12	17
Tench *Tinca tinca* [2]	6	1	0	9	0	0	1	0	0	16	1	0	17
Nine-spined stickleback *Pungitius pungitius* [3]	0	0	7	0	0	9	0	0	1	0	0	17	17
Marble goby *Oxyeleotris marmorata* [4]	4	0	0	7	0	0	1	0	0	12	0	0	12
Total number of cases	14	14	13	18	8	36	3	0	4	35	22	53	

Note. Taste properties (taste): "+" - attractive, "+/–" - indifferent, "–" - repellent. Monocarboxylic acids: valeric, glycolic, caproic, butyric, formic, propionic, acetic; dicarboxylic acids: adipic, tartaric, glutaric, α-ketoglutaric, maleic, malonic, fumaric, oxalic, malic, succinic; tricarboxylic - citric. Here and in Table 3: according to: [1]Kasumyan, Isaeva, 2023; [2]Kasumyan, Prokopova, 2001; [3]Mikhailova, Kasumyan, 2018; [4]Lim et al., 2017.

Among carboxylic acids, there is not a single one with identical properties for the fish species we studied, but there are many examples when the same acids cause responses of opposite signs, for example, adipic, tartaric, glycolic, malonic, citric, and others. It is necessary to note the good reproducibility of these assessments - the taste responses of carp to citric acid in the present work and in a previously performed study (Kasumyan, Morsi, 1996) completely coincide. Despite the relatively small total number of studied species - seven, the data from comparative analysis confirm the validity of the conclusion formulated earlier based on amino acids regarding the species specificity of fish taste preferences (Kasumyan, 1997; Kasumyan, Døving, 2003; Morais, 2017). As with amino acids, the taste spectra of carboxylic acids in different fish species do not show significant similarity (Table 3).

Table 3. Values of Spearman's rank correlation coefficient of taste attractiveness of carboxylic and some other organic acids between different fish species

Species	2	3	4	5	6	7
1. Dace *Leuciscus leuciscus*	0.08	0.02	0.37	−0.25	−0.22	0.33
2. Roach *Rutilus rutilus*		0.27	0.39	−0.37	0.26	0.22
3. Carp *Cyprinus carpio*			0.08	−0.01	0.14	−0.52
4. Bitterling *Rhodeus sericeus* [1]				−0.89***	0.10	−0.36
5. Tench *Tinca tinca* [2]					0.09	0.32
6. Nine-spined stickleback *Pungitius pungitius* [3]						−0.03
7. Marble goby *Oxyeleotris marmorata* [4]						

Note. For all species, except carp and marble goby, correlation coefficients were calculated based on taste responses to 17 types of pellets (control and containing adipic, valeric, tartaric, glycolic, α-ketoglutaric, caproic, citric, maleic, malonic, formic, acetic, fumaric, oxalic, malic, succinic, and ascorbic acids). For carp, the correlation coefficient with roach was calculated based on taste responses to 15 types of pellets (without pellets containing valeric, glycolic, and caproic acids, but with pellets containing boric acid), with dace, bitterling, tench, and nine-spined stickleback – based on taste responses to 16 types of pellets (without pellets containing valeric, glycolic, and caproic acids, but with pellets containing propionic and butyric acids). For marble goby, correlation coefficients with dace, bitterling, tench, and nine-spined stickleback were calculated based on taste responses to 13 types of pellets (with adipic, tartaric, glycolic, citric, maleic, formic, propionic, acetic, fumaric, oxalic, malic, succinic, and ascorbic acids), with roach and carp – for 12 types of pellets (from the above-mentioned 13, without pellets containing propionic and glycolic acids, respectively); ***correlation is significant at $p < 0.001$.

Among 21 possible variants of pairwise species comparison, a significant correlation was found only between tench and bitterling – −0.89 ($p < 0.001$). The negative correlation means that in the ranked arrays of carboxylic acids by taste attractiveness, their sequence is opposite for tench and bitterling. These fish do not show correlated taste preferences for amino acids (Kasumyan, Isaeva, 2023). The difference in taste spectra between tench and bitterling is unlikely to be adaptive, since in water bodies these fish prefer different biotopes, differ in lifestyle, and therefore cannot be considered as competitors for food (Giles et al., 1990; Lammens, Hoogenboezem, 1991; Froese, Pauly, 2023). There is also no similarity in taste preferences for carboxylic acids and amino acids between roach and dace, and between tench and carp – fish whose ranges and ecological niches largely overlap (Lammens, Hoogenboezem, 1991; Froese, Pauly, 2023). It is quite likely that the discrepancy in taste properties of these substances, which are most common in living organisms, including various fish food objects (Daldorph, Thomas, 1991; Liu et al., 2018; Nelson, Cox, 2021), is aimed at reducing food competition between ecologically similar fish during sympatry.

Thresholds . When studying any taste stimulants, it is important to determine their threshold concentrations. Citric acid – the most palatable for carp – maintains its stimulating effect down to a concentration of 10^{-4} M. In previously conducted experiments, the threshold concentration of citric acid for carp was 5×10^{-3} M, i.e., it was 50 times higher, which apparently was caused by significant differences in the sizes of granules used for testing, as well as the sizes of experimental fish (Kasumyan, Morsi, 1996). The stimulating effect of ascorbic acid, which is less effective than citric acid, is lost after the concentration decreases below 10^{-3} M (Fig. 1).

Similar results for citric and ascorbic acids were obtained for marble sleeper - at a concentration of 10 $^{-1}$ M both acids are equal in effect, but at a concentration of 10 $^{-3}$ M citric acid stimulates pellet consumption much more effectively than ascorbic acid. It is also important that at a concentration of 10 $^{-1}$ M all 13 organic acids are equally effective for marble sleeper, and differences between acids are manifested only when the concentration is reduced (Lim et al., 2017). In dace and roach, and to a lesser extent in carp, the diversity of responses to acids is already manifested at a concentration of 10 $^{-1}$ M (Table 1), which indicates different dose-response relationships in fish. Overall, the results obtained indicate that the level of taste sensitivity of fish to carboxylic acids corresponds to what was established using the same methods for amino acids, including for cyprinid fish (Kasumyan, Døving, 2003; Kasumyan, Isaeva, 2023).

Taste and pH of solutions . The dependence of the taste intensity of acids on the pH value of their aqueous solutions has long attracted the attention of researchers. Organic and inorganic acids and their derivatives are traditional substances used for such studies. In roach, dace, and the previously studied bitterling, there is a direct relationship between granule consumption and the pH of solutions containing carboxylic acids (Kasumyan, Isaeva, 2023). In carp and nine-spined stickleback, this relationship is absent , while in tench it is inverse - as the pH of solutions increases, granule consumption decreases (Fig. 2) (Kasumyan, Prokopova, 2001; Mikhailova, Kasumyan, 2018). In all fish except tench and bitterling, the coefficient of determination of linear regression approximation is small or, as in carp and nine-spined stickleback, close to zero, indicating a weak connection between the variables under consideration or its absence. A significant but opposite correlation between granule consumption and the pH value of carboxylic acids is observed only in bitterling ($r_s = 0.81, p < 0.001$) and tench ($r_s = -0.84, p < 0.001$), which is confirmed by the results of regression analysis. The obtained data indicate that the dependence of taste attractiveness on the pH of carboxylic acids, if it manifests, is expressed differently in fish, which is undoubtedly due to species differences in taste preferences. The same conclusion was obtained when analyzing the relationship between the pH of amino acid solutions and their taste attractiveness for fish (Kasumyan, 2016).

In humans, the relationship between the pH of acid solutions and the intensity of the sour taste they cause is well manifested when using completely dissociable inorganic acids in water, for example, hydrochloric acid. The taste of inorganic acids is determined not only by H^{+} ions formed during dissociation, but also by protonated non-dissociated acid molecules. Therefore, at the same pH value, solutions of organic acids taste more acidic than solutions of inorganic acids (Ganzevles, Kroeze, 1987; Da Conceicao Neta et al., 2007; Frank et al., 2022). Since the same carboxylic acid can have opposite taste properties for different fish, it seems obvious that the taste sensations caused by organic acids, in particular carboxylic acids, are determined not only by the ability to dissociate and protonate, but also by other features, including the structure of the acid molecule and the receptor proteins that provide the sensitivity of animals to acids (Tu et al., 2018).

It cannot be ruled out that the sensory basis of fish response to pellets with acids may be not only the taste system but also the general chemical sense, the receptors of which – solitary chemosensory cells and chemosensitive free nerve endings – are present in the oral cavity of fish along with taste buds (Whitear, 1992). It is assumed that with such bimodal participation in acid reception, the taste system provides enhancement of positive responses in the range of relatively low concentrations. But after the acid reaches a certain concentration, the contribution of the general chemical sense begins to dominate in the formation of the response, and the nature of the response changes to negative (protective), intensifying as the concentration further increases. It is believed that in different animals, the inflection point (bliss point) of the inverted U-shaped dependence corresponds to different concentrations of acids (Roper, 2014; Frank et al., 2022).

Taste, structure and size of the molecule . Finding the relationship between molecular structure and chemosensory effectiveness of substances, olfactory or gustatory, remains a relevant problem in the study of chemoreception. Most studies are performed using amino acids and their derivatives (Hara, 2006). Transfer, removal, or appearance of functional groups or double bonds and other molecular modifications are usually accompanied by changes in the taste properties of amino acids for fish (Caprio, 1975; Marui et al., 1983; Kasumyan, Mouromtsev, 2020). Our data on the taste attractiveness of carboxylic acids, whose structural diversity is no less significant than that of amino acids, confirm these conclusions.

Thus, the neutral taste of acetic acid, upon the appearance of a hydroxyl group (−OH) in the molecule and the formation of glycolic acid, changes to repulsive for dace, roach, and bitterling, and to attractive for tench. But succinic acid in dace, roach, bitterling, and nine-spined stickleback is close in taste attractiveness to malic and tartaric acids, which have one and two hydroxyl groups, respectively. However, for carp and tench, these changes in the molecule are significant, and the appearance of hydroxyl groups significantly enhances taste attractiveness. Similar taste attractiveness is observed in glutaric and α-ketoglutaric acids; in the latter, one of the hydrogen atoms is replaced by a keto group (=O). Replacing one of the single bonds between carbon atoms in the succinic acid molecule with a double bond does not change the taste properties of maleic acid – it retains its repulsive taste for dace, roach, carp, bitterling, and nine-spined stickleback. But for tench, the same molecular transformations sharply increase taste attractiveness – unsaturated maleic acid is more than three times more attractive in taste than saturated succinic acid. The taste properties of maleic and fumaric acids, which are *cis* - and *trans-isomers* of butenedioic acid, are similar for most of the fish studied, except for tench, for which these stereoisomers differ even more than succinic and maleic acids (Fig. 3).

Thus , structural transformations of molecules do not always lead to shifts in their taste properties, and if these changes occur, they may not coincide in different species or may be opposite in nature. Similar results were obtained when comparing the taste attractiveness of amino acid isomers (Levina, Kasumyan, 2024). It should be emphasized that shifts in taste attractiveness in response to structural transformations of acids coincide in roach, dace, and bitterling, in which the dependence of pellet consumption on the pH of carboxylic acids manifests in a similar way. In tench, changes in taste attractiveness in response to the same structural transformations of acids and dependence on pH are of an opposite nature (Fig. 2). Whether the relationship between the considered features is objective or coincidental will become clearer as existing data is supplemented by studies of new fish species.

It is interesting to compare the taste properties of carboxylic acids that differ in basicity - the number of carboxyl groups in the molecule. According to electrophysiological experiments, monocarboxylic acids are, on average, less potent stimulants for carp taste receptors than dicarboxylic acids, and the latter are less effective than tricarboxylic acids (Marui, Caprio, 1992). Our results for carp somewhat correspond to this conclusion: all four monocarboxylic acids have an indifferent taste for carp, among 10 dicarboxylic acids there are two attractive ones, and the only tricarboxylic acid - citric acid - has the same taste. However, there are also contrary examples (dace, roach, bitterling) (Table 2). In humans, the intensity of the sour taste caused by acid solutions decreases with an increase in the number of carboxyl groups in their molecules (CoSeteng et al., 1989).

More rigorous is the use of data on mono-, di-, and tricarboxylic acids with equal carbon chain lengths and without additional functional groups or double bonds for analysis. When comparing such monocarboxylic acids, acetic (C=2), propionic (C=3), and butyric (C=4), with the corresponding dicarboxylic acids, oxalic (C=2), malonic (C=3), and succinic (C=4), it is evident that in dace and bitterling, the latter are significantly less attractive in taste than monocarboxylic acids. In carp, significant differences between the consumption of granules with mono- and dicarboxylic acids were found in only one case, also showing a decrease. In tench, an increase in the basicity of acids in all cases is associated with a significant enhancement of their taste attractiveness, while in the nine-spined stickleback this is observed only when comparing monocarboxylic valeric acid and tricarboxylic citric acid (both C=5). For roach, no differences were detected (Fig. 4).

The dependence of taste attractiveness of carboxylic acids on molecule size is weak and varies in character (dace, bitterling, carp, tench) in some fish, while in other species it is absent (roach, nine-spined stickleback) (Fig. 5). This is confirmed by correlation analysis, which did not reveal a significant relationship in roach, nine-spined stickleback, and carp ($p > 0.05$). Thus, the comparison does not allow us to conclude that there is a general rule or trend characterizing the relationship between the taste properties of carboxylic acids and their structure, basicity, or molecule size.

Ascorbic acid , or vitamin C, is an important regulator of metabolic processes in fish and other animals (Dabrowski, 2000). In teleost fish (Teleostei), unlike more ancient representatives of Actinopterygii (Cladistia, Chondrostei, Amiiformes), as well as Myxini, Petromyzontida, Chondrichthyes and Dipnoi, the ability to synthesize ascorbic acid has been lost (Drouin et al., 2011). The requirements for vitamin C are high, and a deficiency of this essential substance in consumed food leads to various physiological disorders and morphological abnormalities – loss of appetite and reduced growth rate, anemia and hemorrhagic manifestations, functional and morphological pathologies of internal organs, reduced resistance to diseases, and others (Nutrient requirements ..., 2011; Mai et al., 2022).

In carp, roach, and other fish experiencing vitamin C deficiency, spinal deformities (scoliosis, lordosis) develop (Dabrowski et al., 1988, 1989), however, the importance of vitamin C in maintaining functional status and health does not correspond with the taste properties of ascorbic acid for fish. In roach, the inclusion of ascorbic acid in pellets reduces their consumption by more than half, in bitterling – by almost four times, nine-spined stickleback completely refuses such pellets, and for dace it is indifferent (Mikhailova, Kasumyan, 2018; Kasumyan, Isaeva, 2023). In marble sleeper goby, ascorbic acid is inferior in taste attractiveness to eight out of 13 tested acids (Lim et al., 2017). And only for carp and tench was the taste of ascorbic acid highly attractive (Fig. 6).

These data indicate that between the physiological needs for such an important micronutrient as vitamin C and its taste attractiveness, an obvious connection cannot be traced. Apparently, the taste attractiveness of ascorbic acid and other substances for fish is determined not by physiological value or role in metabolic processes, but primarily, directly or indirectly, by the food that fish are evolutionarily adapted to consume, as is assumed for primates and some other animals (Laska et al., 2008, 2009; Breslin, 2013). The absence of a connection between physiological need and taste properties of substances is also confirmed by the fact that the number of tastefully attractive and unattractive amino acids among essential and non-essential ones is approximately equal (Kasumyan, 2016; Levina et al., 2021).

CONCLUSION

The widespread occurrence among living organisms and structural diversity of carboxylic acids allows considering them, along with amino acids, as the most suitable substances for the study of chemoreception in fish and other animals. Our study demonstrates a wide variety of taste preferences exhibited by fish of different species towards carboxylic acids. Despite the small number of fish studied, the obtained information corresponds to the concept of specificity of taste spectra in fish, formulated earlier based on more numerous data on the taste properties of amino acids. New confirmations have been obtained regarding the absence of a direct connection between physiological needs for substances and their taste attractiveness for fish (Kasumyan, 2024). Data on carboxylic acids are certainly important for verification of these and other basic provisions about taste reception.

It is believed that sensitivity to substances that cause a sour taste sensation in humans is evolutionarily the most ancient compared to the perception of substances that cause other taste sensations (Frank et al., 2022). Protein receptors that provide a response to acids are thought to have existed already in the earliest ancestral forms of vertebrates (Tu et al., 2018). Examples of animals losing the reception of sour taste during evolution, unlike the ability to respond to other types of taste substances (sugars, umami), are unknown (Li et al., 2005; Zhao et al., 2010; Jiang et al., 2012; Zhu et al., 2014). Information about the relationship of fish to the taste of carboxylic and other organic acids is important for elucidating the evolutionary pathways and patterns of sour taste reception formation in animals as a whole (Frank et al., 2022). The results of this work show that due to the intraspecific diversity of taste responses to these substances, constructing evolutionary schemes for the emergence and development of vertebrate susceptibility to sour taste based on data from individual (selected, model) acids will with unquestionable probability lead to erroneous conclusions.

In terms of the number of species for which the taste attractiveness of carboxylic acids is already known, fish are ahead of many groups of vertebrates (Kasumyan, Døving, 2003). However, the number of studied fish still remains insufficient to understand not only the evolutionary transformations of sensitivity to acids but also many other aspects of the reception of these substances, primarily the relationships between their taste attractiveness and fish nutrition, features of digestion and metabolism.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors confirm that all experiments were conducted in accordance with current principles and rules for the treatment of animals and did not harm the fish involved in the research. The method used in this study was approved by the MSU Bioethics Commission (application No. 170-zh for research expertise was reviewed and approved by the MSU Bioethics Commission on February 15, 2024, meeting No. 159-d-z).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflict of interest.

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FIGURE CAPTIONS

Fig. 1. Consumption by carp *Cyprinus carpio* of agar-agar granules containing low concentrations of citric and ascorbic acids; difference from control is significant at p : * < 0.05, *** < 0.001. (⊤) – standard error of the mean.

Fig. 2. Dependence of fish consumption of agar-agar granules on the pH value of carboxylic acids (10^{-1} M) contained in them: a – roach *Rutilus rutilus* ($y = 11.31x - 0.23$, $R^2 = 0.41$), b – dace *Leuciscus leuciscus* ($y = 21.08x - 5.86$, $R^2 = 0.51$), c – bitterling *Rhodeus sericeus* ($y = 24.67x - 50.26$, $R^2 = 0.68$), d – carp *Cyprinus carpio* ($y = -1.59x + 40.00$, $R^2 = 0.01$) , e – tench *Tinca tinca* ($y = -54.46x + 193.87$, $R^2 = 0.79$), f – nine-spined stickleback *Pungitius pungitius* ($y = -0.57x + 2.79$, $R^2 = 0.04$). Sources of information on bitterling, tench, and nine-spined stickleback here and in Fig. 3–6, respectively: Kasumyan, Isaeva, 2023; Kasumyan, Prokopova, 2001; Mikhailova, Kasumyan, 2018.

Fig. 3. Fish consumption of agar-agar granules containing carboxylic acids (10^{-1} M) with different molecular structures: a – acetic (■) and glycolic (■); b – glutaric (■) and α-ketoglutaric (■); c – succinic (■), malic (▧) and tartaric (■); d – succinic (■), maleic (▨) and fumaric (■). Differences are significant at p : * < 0.05, ** < 0.01, *** < 0.001.

Fig. 4. Fish consumption of agar-agar granules containing mono-, di- and tricarboxylic acids (10^{-1} M) with different numbers of carbon atoms (C) in the carbon chain: a – dace *Leuciscus leuciscus* , b – roach *Rutilus rutilus* , c – carp *Cyprinus carpio,* d – bitterling *Rhodeus sericeus* , e – tench *Tinca tinca* , f – nine-spined stickleback *Pungitius pungitius* . Acids: monocarboxylic: *1* – acetic (C2), *3* – propionic (C3), *5* – butyric (C4), *7* – valeric (C5); dicarboxylic: *2* – oxalic (C2), *4* – malonic (C3), *6* – succinic (C4); tricarboxylic: *8* – citric (C5); *K* – control; differences are significant at p : * < 0.05, ** < 0.01, *** < 0.001.

Fig. 5. Dependence of fish consumption of agar-agar granules on the molecular weight of carboxylic acids contained in them (10^{-1} M): a – dace *Leuciscus leuciscus* ($y = -0.32x + 85.37$, $R^2 = 0.52$), b – roach *Rutilus rutilus* ($y = -0.09x + 39.11$, $R^2 = 0.12$), c – carp *Cyprinus carpio* ($y = 0.21x + 11.48$, $R^2 = 0.47$) , d – bitterling *Rhodeus sericeus* ($y = -0.31x + 49.75$, $R^2 = 0.49$), e – tench *Tinca tinca* ($y = 0.50x - 7.63$, $R^2 = 0.31$), f – nine-spined stickleback *Pungitius pungitius* ($y = 0.004x + 0.80$, $R^2 = 0.01$).

Fig. 6. Consumption by dace *Leuciscus leuciscus* , roach *Rutilus rutilus* , carp *Cyprinus carpio,* bitterling *Rhodeus sericeus* , tench *Tinca tinca* and nine-spined stickleback *Pungitius pungitius* of agar-agar granules containing ascorbic acid (10^{-1} M) (■), and control granules (■); ***difference from control is significant at $p < 0.001$.

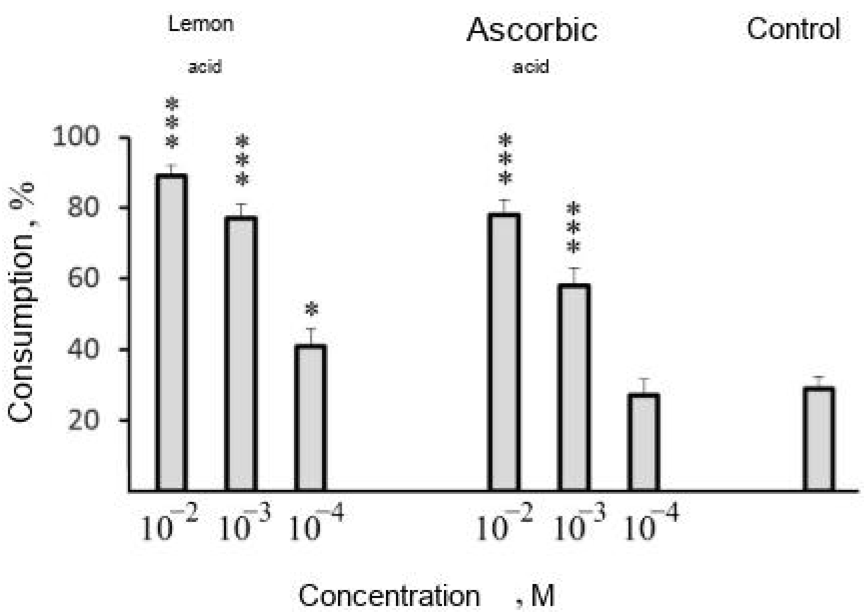


fig. 1

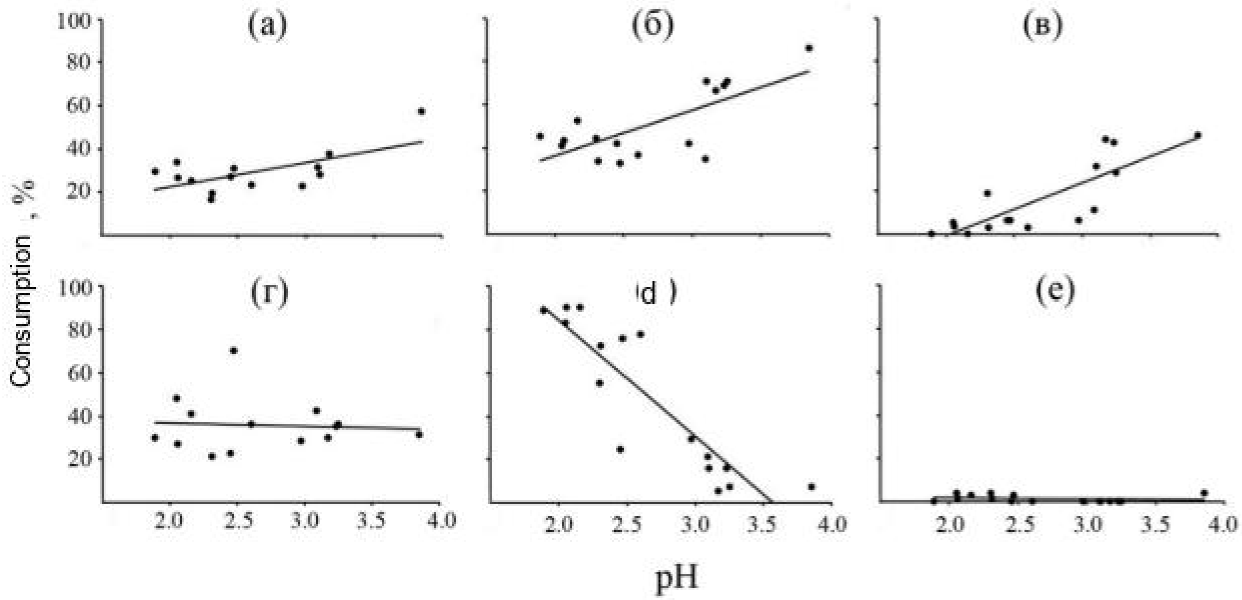
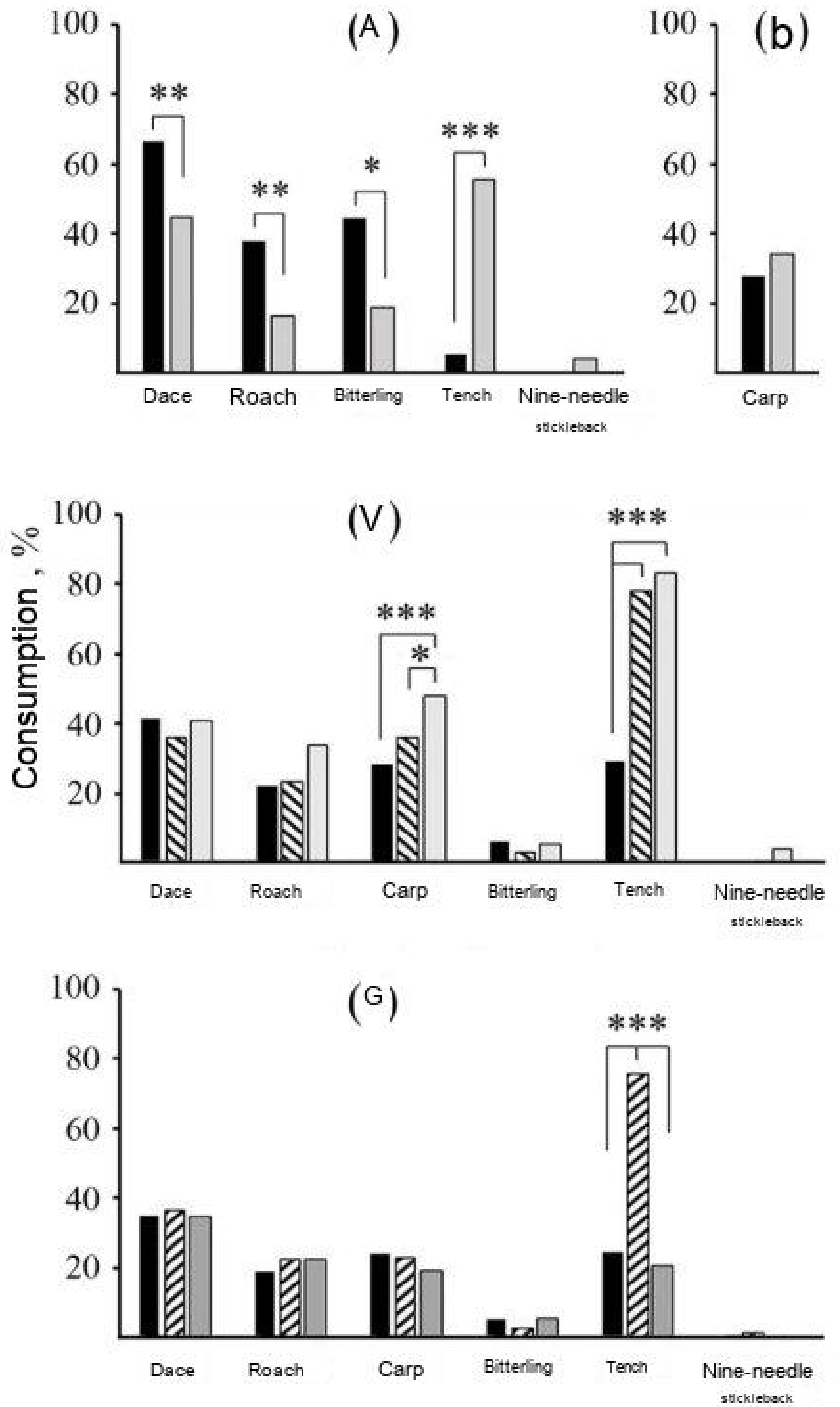


fig. 2



(A)
100
80
60
40
20
**
**
*
***
Dace
Roach
Bitterling
Tench
Nine-needle
stickleback
(b)
Carp
(V)
Consumption , %
***
*
***
Dace
Roach
Carp
Bitterling
Tench
Nine-needle
stickleback
(G)
***
Dace
Roach
Carp
Bitterling
Tench
Nine-needle
stickleback

fig. 3

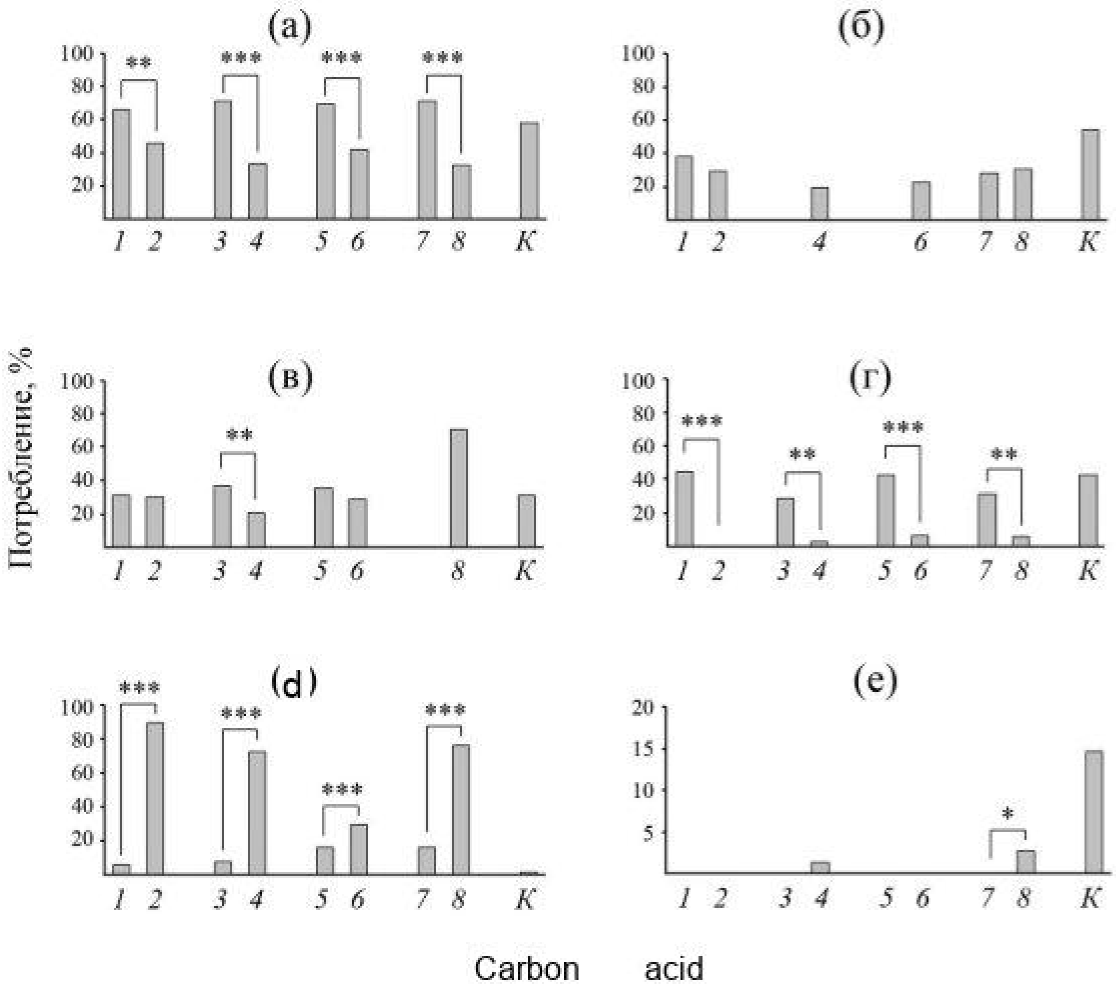
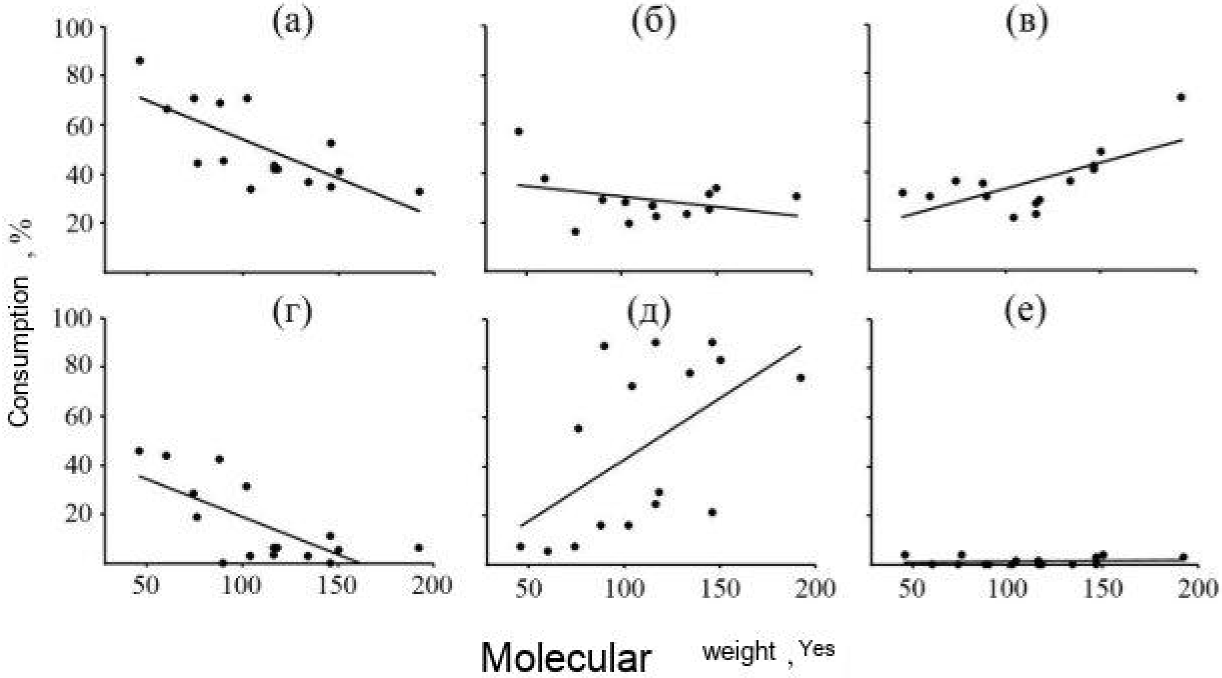
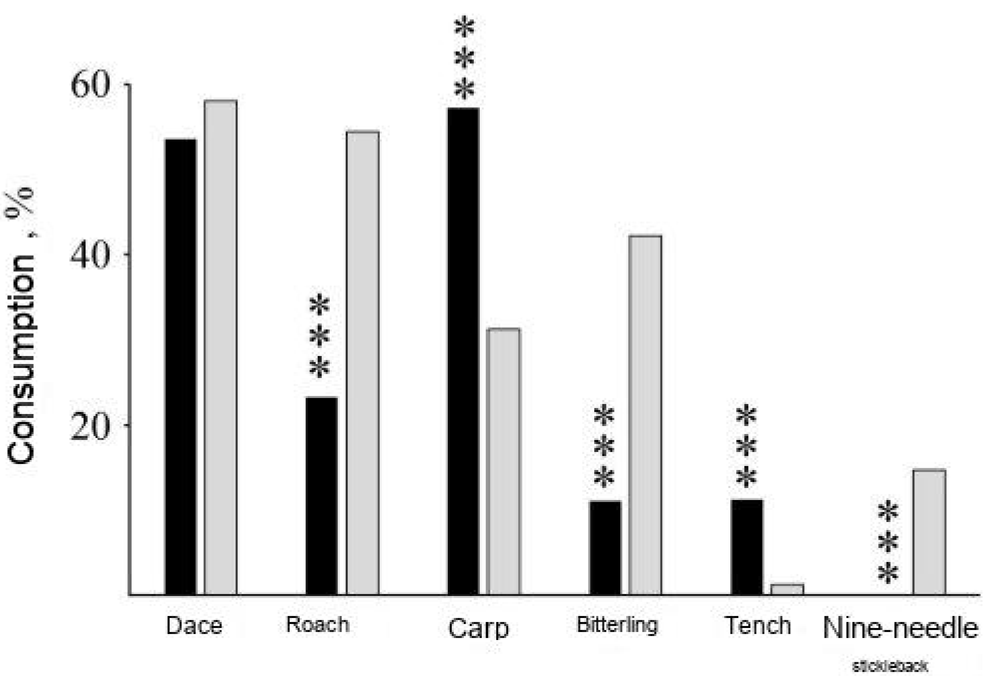


fig. 4



(а)
(б)
(в)
(г)
(д)
(е)
100
80
60
40
20
50
100
150
200
Consumption , %
Molecular weight , Yes

fig. 5



60
40
20
Consumption , %
***
Dace
Roach
Carp
Bitterling
Tench
Nine-needle
stickleback

fig. 6