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Gustatory Response in Wild and Cultivated Ayu *Plecoglossus altivelis altivelis*

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Abstract

Gustatory response of wild and cultivated ayu *Plecoglossus altivelis altivelis* was recorded from the facial nerve supplying the anterior palate.

The lowest threshold was noted for arginine at 10^{-6}~10^{-5}M in wild and cultured fish. The threshold for histidine in wild ayu, was similar, though one order higher for cultured fish. ATP-related substances were fairly effective. The threshold values for AMP, IMP and UMP were around 10^{-4}M. NaCl stimulated the receptors weakly. Sugars were not effective at 10^{-2}M.

Relative stimulatory effectiveness at 10^{-2}M of 20 amino acids was compared for wild and cultured ayu. Response magnitude for these amino acids was closely correlated with that for histidine in each group ($p<0.001$). The regression coefficient of cultivated ayu response for wild ayu response was 0.69, suggesting higher histidine response in the cultured group. Student's t-test further indicated arginine and proline response to significantly differ ($p<0.05$). Whether this was due to genetic differences of the two groups of ayu or other ecological or physiological conditions is not known.

Samples of algae on which ayu feed were collected from the river where the ayu were caught, and analyzed for free amino acids. Several amino acids were found at concentrations above the gustatory threshold.

Key words : fish • *Plecoglossus altivelis altivelis* • gustatory response • amino acids • nucleotides • algae

Introduction

Ayu is anadromous fish, breeding in freshwater. After hatching, the larvae drift downstream to the sea in which they overwinter, returning in the spring until sexually mature in the autumn when they spawn and die after spawning[1,2]. Land-locked ayu in Lake Biwa have been transplanted to rivers and lakes of various parts of Japan. The land-locked ayu in Lake Biwa have been reported to differ in genic variabilities from those of the amphidromous race[3-7]. Ayu is well known to feed on animals during their juvenile marine life and change their feeding habit dramatically to feed on algae after

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returning into freshwater[8-11]. Landlocked ayu transplanted in river also is known to change their feeding items from zooplankton to attached algae as they grow[1]. From this unique feeding habit of ayu, it seems of interest to know the sensory capabilities of their gustatory receptors in juvenile and adult life and also to know if there exist differences in the responsiveness of gustatory receptors between the anadromous and the land-locked ayu.

This paper deals with the responses of the gustatory receptors to amino acids and others in adult wild ayu caught in river and cultivated ayu of the land-locked race. The two groups of ayu responded to most of the stimulants in a similar way, but with some differences in sensitivity to some compounds.

Materials and methods

Animals

Wild and cultivated stocks of Ayu, *Plecoglossus altivelis altivelis*, were used. The wild fish were captured at River Miya, Tamaki, Mie Prefecture in September 1985 using gill-nets. The fish obtained were kept for a few days in an indoor tank before use. The wild ayu tested were in total 21, ranging 19.5～60.8 (average 35.80) g in body weight and 14.1～19.6 (16.24) cm in total length. The cultivated fish were reared from wild juveniles which were captured at Lake Biwa, Shiga Prefecture in January to March 1984, '85, and '86. After transporting to the Inland Station, National Research Institute of Aquaculture, they were reared in indoor tanks with running well water until April, and outdoors from May, feeding a commercial feed developed for ayu (Type 2C, Nippun Shiryo Co.,Ltd.). The experiments for the cultivated ayu were performed in August 1984 and in September to October 1985 and '86. The cultivated ayu used in 1984 were in total 10, ranging 15.2～26.4 (average 19.89) g in b. wt. and 12.8～15.2 (13.58) cm in t. l. ; those for 1985 were 16, ranging 14.2～32.7 (21.24) g and 12.0～16.2 (13.80) cm ; those for 1986 were 25, ranging 17.5～52.5 (41.37) g and 13.5～19.4 (17.01) cm.

Recording of gustatory nerve responses

The fish was immobilized by injecting pancuronium bromide (5 μg/10 g of b. wt.) and positioned with its head higher than its tail on a wooden block. Well water was perfused through the mouth over the gills throughout the experiment. After removing the eyeball, the ramus palatinus facialis supplying the anterior palate was isolated and cut. The peripheral cut end of the nerve was placed on a pair of platinum electrodes. Impulse discharges in response to the stimuli was recorded from the whole nerve bundle as the summated response (averaged activity) using an electronic integrator (Nihonkohden, RJG 40225S, time constant 0.02).

Chemicals tested

Nucleosides, nucleotides and ribose were products of Kohjin ; γ-amino-n-butyric acid, Sigma ; phospho-serine and maltose, Wako ; all the other amino acids, betaine and sugars, GR grade from Nakarai. Amino acids used were L-isomers.

Abbreviations for the chemicals used

Ade, adenosine ; Ala, alanine ; AMP, 5'-adenylic acid disodium salt ; Arg, arginine hydrochloride ; Asn, asparagine ; Asp, sodium aspartate ; Bet, betaine ; Cit, citrulline ; CMP, 5'-cytidylic acid disodium salt ; Cys, cysteine ; D.W., distilled water ; EtOH-NH, ethanolamine ; GABA, γ-amino-n-butyric acid ; Gln, glutamine : Glu, sodium glutamate ; Gly, glycine ; His, histidine ; Ile, isoleucine ; IMP, 5'-inosinic acid disodium salt ; Ino, inosine ; Leu, leucine ; Lys, lysine hydrochloride ; Met, methionine ; Orn, ornithine hydrochloride ; Phe, phenylalanine ; Pro, proline ; P-Ser, phosphoserine ; Ser, serine ; Tau, taurine ; Thr, threonine ; Trp, tryptophan ; Tyr, tyrosine ; UMP, 5'-uridylic acid disodium salt ; Val, valine.

Application of test solutions

All the test stimulants were dissolved in D.W. Stimulus solutions were applied to the anterior part of the palate. To apply test solutions and D.W. successively a three-way solenoid valve (MVC-3V-M6, Takasago Electric) was used. The test solutions were applied for 3 s with 2 min or longer rest between stimuli. During the resting time D.W. was continuously flowed over the palate. As a control 10^{-2}M His or Arg was applied at intervals. To compare the stimulatory effectiveness of test stimulants, the maximum height of the recorded response relative to that for 10^{-2}M His or Arg, taking the latter as 100, was measured. The well water perfusing the gills during the experiments was supplied with temperature controlled to 16~17℃. Distilled water for mouth rinse was from a glass container bathed in the well water to adjust its temperature to that of the well water. Glass bottles with test solutions were kept bathed in the running well water during the experiment. The room temperature ranged from 23 to 27℃ during the experimental periods.

Results

pH and temperature effects on the gustatory receptor response

For the application of test stimulants to fish chemoreceptors it is recommended to apply them dissolving in the same water as the fish live in because the receptor membrane is physiologically adapted to that water. Thus artificial river or pond water having a definite composition of inorganic salts has been introduced[12, 13]. However, in the present study, we decided to use D.W. as solvent because we could not prepare artificial pond water suitable for both wild and cultured ayu which had lived in apparently different ionic compositions of water. Since D.W. has no buffering capacity, to begin with the effect of pH on the receptor response was examined using hydrochloric acid and sodium hydroxide. Fig. 1 shows the pH-response curve obtained with 3 cultivated fish. As seen in the figure, the ayu responded to hydrochloric acid below pH around 5 and to sodium hydroxide above pH around 9 and the responses increased sharply with further decrease or increase of pH. Thus, the results in Fig. 1 suggested similar stimulatory effects of pH for the test solutions of stimulants dissolved in nonbuffered D.W. The pHs of solutions at 10^{-2}M of the compounds tested ranged between 5.3 and 7.8 except that for Asn, which was 4.7~4.9, presumably due to dissociation to Asp.

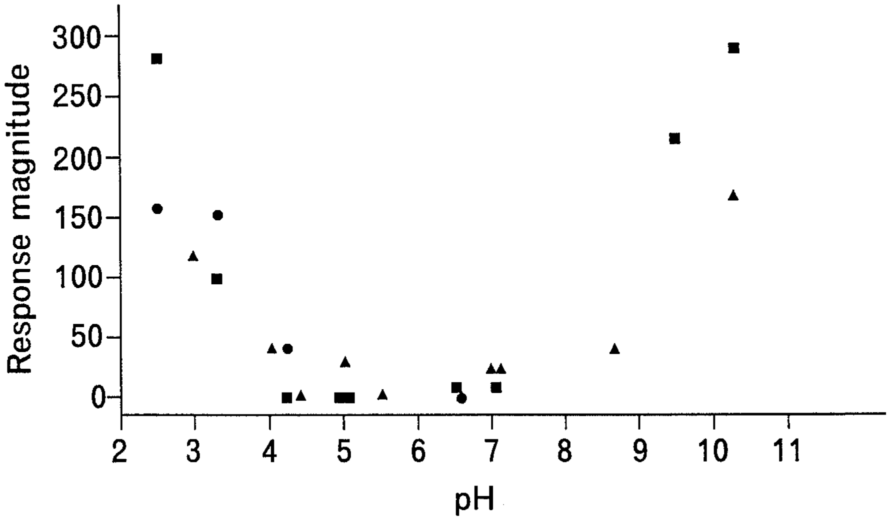

Fig. 1 pH-response relationships for hydrochloric acid and sodium hydroxide. Ordinate, magnitudes of the neural responses relative to that of 10^{-2}M Arg, taking the latter as 100. Different symbols represent different fish. For abbreviations see text.

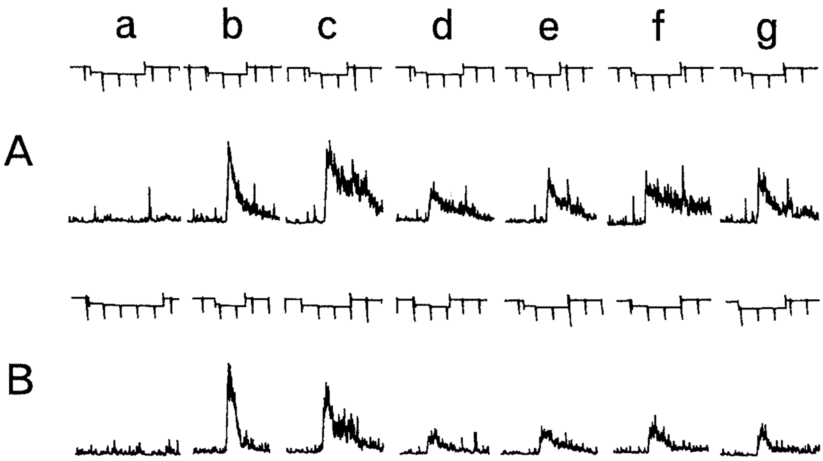

Fig. 2. Summated responses to some amino acids at 10^{-2}M in the wild (A) and cultivated (B) ayu. a, D.W. ; b, His ; c, Arg ; d, Ser ; e,Cys ; f, Lys ; g, Glu. Time marker in s ; sustained downward deflection of the signal line is duration of opening of solenoid valve for stimulus solution flow. The paired spiny deflection in the lower tracing, electrical noises caused by the solenoid valve opening and closing.

Responses to amino acids

The palatal gustatory receptors of the two groups of ayu responded to most of the amino acids in a similar way (Fig. 2) ; they responded well to some amino acids but not so well to many others (Fig. 3). The lowest threshold was for Arg, at 10^{-6}~10^{-5}M in both wild and cultivated fish, next for His, 10^{-6}~10^{-5}M for wild ayu and around 10^{-5}M for cultivated ayu, and then for Lys, Glu for the wild, Glu, Lys, Met, at around 10^{-4}M for the cultivated (Table 1A, 1B). Tau and Bet elicited no appreciable responses at 10^{-2}M in neither wild nor cultivated fish (Fig. 3).

Fig.3 shows the relative stimulatory effectiveness of amino acids and others at 10^{-2}M. The response magnitudes are expressed relative to that for His in each fish taking the latter as 100. In 20 amino acids, the 5 most effective at 10^{-2}M was Arg>His>Glu>Trp>Ala for wild fish and His>Arg>Glu>Lys>Ala for cultivated fish. The responses to Arg and Trp in the two groups of fish were significantly different (Student's t-test, $p<0.05$). When the response magnitudes relative to that of Arg in each fish group were compared between the two groups of ayu, His and Lys responses were found to be significantly different ($p<0.02$). In general, there was a high correlation between the responses of the two groups of ayu : the correlation coefficient was 0.936 ($n=21$, $p<0.001$) as relative responses to that to His in each group (Fig. 4), while the regression coefficient of the wild ayu responses on the cultivated ayu responses was 0.69, suggesting the higher histidine response in the cultured group. When treated with Arg response as standard, the correlation coefficient was 0.937 ($n=21$, $p<0.001$) and the regression line was $y=1.17x+1.79$.

Nucleosides and nucleotides

AMP, IMP and UMP were more stimulatory at 10^{-2}M than Arg both in wild and cultivated fish (Fig. 3). However, the threshold for the three appeared to be located at 10^{-5}~10^{-4}M, about two orders higher than that for Arg (Table 1A, 1B). CMP at 10^{-2}M was also effective, the average response magnitude being 165 in two cultivated fish. Inosine was also highly stimulatory at 10^{-2}M in both groups of fish (Fig. 3). Adenosine was also moderately effective in both groups (Fig. 3).

Sodium chloride

Three individuals were tested for each group. In three cultivated ayu, the response magnitudes to NaCl at 10^{-2}M were 71.4, 32.3 and 28.6 relative to Arg response taking the latter as 100, while for wild ayu, one fish gave 20.5 but no appreciable response were observed in the other two.

Others

Eight sugars and quinine were tested. Fructose, galactose, glucose, lactose, maltose, ribose, sorbose and sucrose were all ineffective at 0.1M in two to four cultivated fish tested. Quinine was effective with a threshold of around 10^{-6}M.

Extracts of algae

Samples of algae attached on the rocks in River Miya which ayu were observed to favorably feed were collected and water extracts of the samples were analyzed for ninhydrin reactive components

Table 1-A Sensitivity of palatal receptors of wild ayu to amino acids and nucleotides. The number of positive neural responses (the first) number and that of fish tested (the second) are shown. The experiments were performed in 1985.

	log molar concentration					
	−7	−6	−5	−4	−3	−2
Arg	0/6	1/6	4/6	5/6	6/6	6/6
His	0/6	2/6	6/6	6/6	6/6	6/6
Lys			0/4	4/4	4/4	4/4
Asp			0/2	1/3	3/3	3/3
Cys			0/1	1/1	1/1	1/1
Glu			0/3	1/3	3/3	3/3
AMP			0/1	1/1	1/1	1/1
UMP			0/1	1/1	1/1	1/1

Table 1-B. Sensitivity of the palatal receptors of cultivated ayu to amino acids, nucleosides, and nucleotides. The experiments were performed in 1984 (*) and 1986.

	log molar concentration					
	−7	−6	−5	−4	−3	−2
Arg	0/1	1/3	3/4	4/4	4/4	4/4
Cit	0/3	1/3	2/3	3/3	3/3	3/3
His		0/5	2/6	6/6	6/6	6/6
Gln		0/2	2/4	3/4	3/4	4/4
Orn		0/3	2/3	2/3	3/3	3/3
P-Ser		0/2	1/2	2/2	2/2	2/2
Glu			0/3	2/3	3/3	3/3
Lys			0/4	2/4	4/4	4/4
Leu			0/1	1/1	1/1	1/1
Met			0/2	2/2	2/2	2/2
IMP*			0/1	1/1	1/1	1/1
AMP*			0/3	2/3	3/3	3/3
UMP*			0/3	2/3	3/3	3/3
Ala				0/2	2/2	2/2
Asp				0/2	2/2	2/2
Cys				0/1	1/1	1/1
EtOH-NH				0/1	1/1	1/1
Ile				0/1	1/1	1/1
Phe				0/2	2/2	2/2
Pro				0/2	2/2	2/2
Ser				0/2	2/2	2/2
Val				0/1	2/2	2/2
Thr				0/2	1/2	2/2
Gly					0/1	1/1
Trp					0/1	1/1

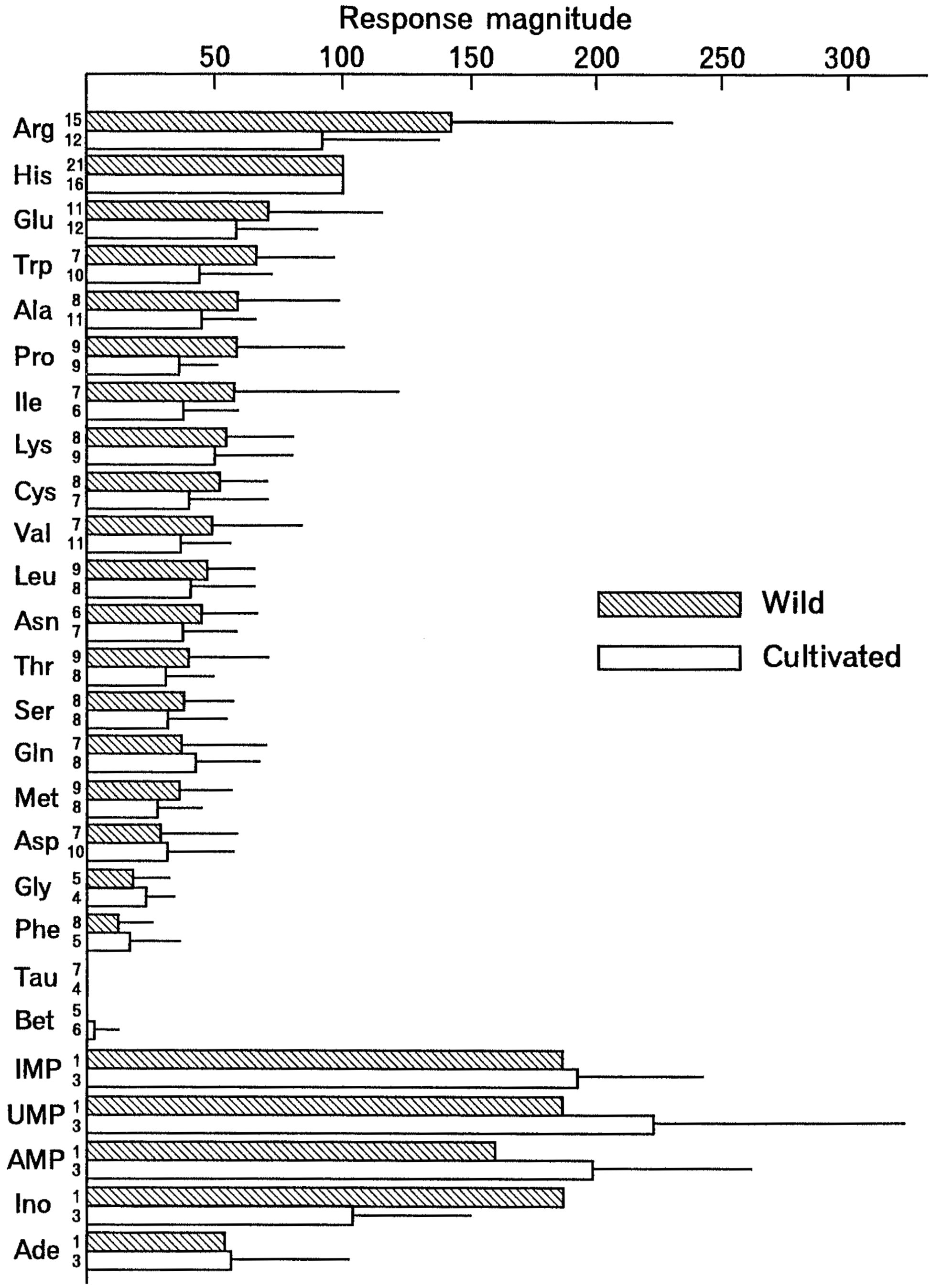

Fig. 3 Relative stimulatory effectiveness at 10^{-2}M of various amino acids and others in wild (filled bar) and cultivated (open bar) ayu. The magnitudes of summated responses are exhibited as a percentage of that of His. The figure on the left of each graph indicates the number of fish tested. The small bar attached to each graph indicates standard deviation.

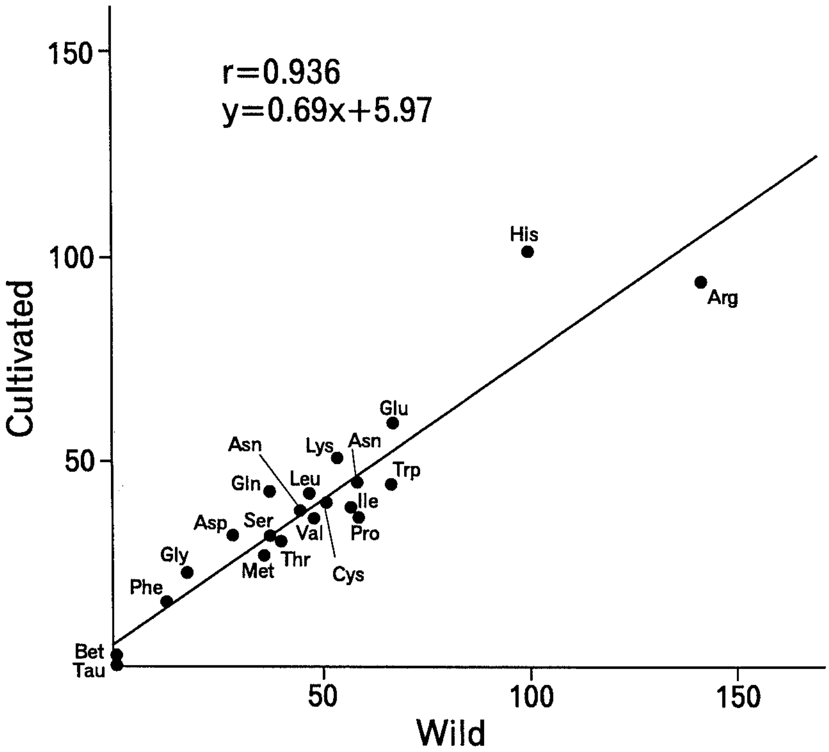

Fig. 4 Correlation between the relative stimulatory effectiveness at 10^{-2}M of amino acids and betaine for the wild ayu and cultivated ayu. The response magnitudes are exhibited as a percentage of that of (a) His and (b) Arg.

(Taishaku et al., in preparation). The samples contained algae of Cyanophyceae, Chlorophyceae and Bacillariophyceae. Among them Cyanophyceae, especially *Oscillatoria* sp. was dominant. In Chlorophyceae, *Scenedesmus quadricauda*, and in Bacillariophyceae, *Cymbella tumida*, *Fragilaria virescens*, *Navicula exogua* and 4 other species were observed. All these species of algae are included in the list of the stomach contents of ayu from River Miya by HARADA and Ito[14]. The compounds found in the aqueous extract of the sample are listed in Table 2. According to this analysis, we additionally examined the stimulatory effectiveness of 5 new compounds for the gustatory receptors with cultivated fish (Fig. 5). In Fig. 5 again the response magnitudes at 10^{-2}M are expressed relative to that for His. Among the 5 compounds Cit, Orn, P-Ser and EtOH-NH elicited responses in the receptors. Of them P-Ser was the most effective and more effective than His or Arg. It showed a threshold close to that for Arg or His. Cit also had a similar threshold, though its effectiveness at 10^{-2}M was not so high (Table 1B).

Table 2. Free amino acids in an aqueous extract of the algae collected at River Miya*.

Compounds	μmol/100g of freeze dried algae	Compounds	μmol/100g of freeze dried algae
Ala	1512.00	Leu	587.20
Ammonia	2040.00	Lys	253.60
Arg	317.00	Met	142.64
Asp	605.40	Orn	605.40
Cit	46.70	P−Ser	43.08
Cystine	26.68	Phe	222.60
EtOH−NH	59.20	Pro	382.40
GABA	58.16	Ser	488.00
Gln	−	Tau	47.42
Glu	982.00	Thr	511.80
Gly	589.60	Trp	45.58
His	58.02	Tyr	185.00
Ile	384.00	Val	556.00

* Taishaku et al., in preparation.

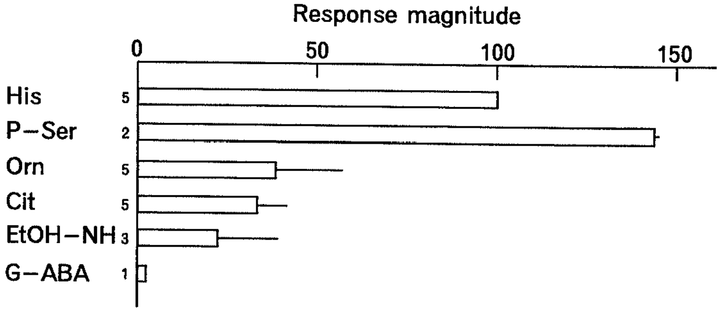

Fig. 5 Relative stimulatory effectiveness at 10^{-2}M of some ninhydrin reacting extracts in algae, which ayu favorably feed on, collected at River Miya. The response magnitudes are exhibited as in Fig. 3.

Discussion

The results in Fig. 3 demonstrate that the wild and cultivated ayu had quite similar response spectra to amino acids and others. The high correlation coefficient (0.936) between the response spectra for amino acids in Fig. 4 also shows the similarity of the two groups of ayu. However, the effectiveness orders of individual amino acids were not quite the same but showed some differences between the two groups (Fig. 3). Thus, the Student's t-test indicated that the relative response magnitudes for Arg and Trp to that for His in the two groups were significantly different ($p<0.05$).

Further, the low regression coefficient (0.69) of the cultivated ayu responses on the wild ayu responses (Fig. 4) suggests the higher His response for the cultivated fish. Whether such a difference arise from genetic differences among races as suggested by isozyme[3], mitochondria DNA[4, 5], microsatellite DNA[6] and AFLP[7] analyses, or other ecological or physiological factors such as different food sources is not known at the present. However, a detailed examination on individual fish showed that the ratio of Arg response to His response differed considerably and even reversed from fish to fish within each group.

The present results in Table 1A and 1B show that the sensitivity of the gustatory receptors of ayu to amino acids is not as acute as the catfish *Ictalurus punctatus*[15], minnow *Pseudorasbora parva*[16], eel *Anguilla japonica*[17], or tigerfish *Therapon oxyrhynchus*[18], but comparable to many other moderate species[19, 20]. Ayu showed the lowest threshold to Arg, and next to His. A high sensitivity to Arg has been reported in some freshwater species, such as the catfish[15], minnow[16], eel[17], tilapia *Tilapia zillii*[21] and euryhaline species, the mullet *Mugil cephalus*[22]. However, in many marine species, Arg is not very effective ; in these species Ala, Gly and/or Pro are often among the most effective[20, 23–25]. It is also a characteristic of ayu that ayu showed a relatively high sensitivity to Glu. A similar high sensitivity to Glu has been reported in herbivorous and omnivorous fishes such as the carp *Cyprinus carpio*[26], tilapia[21] and rabbitfish *Siganus fuscescens*[20]. Glutamic acid is abundant in algae and other plants these fish feed on, but also contained in various classes of invertebrates and fish, though often not the major constituent amino acid[27]. However, carnivorous fishes are often reported not to be very sensitive to this amino acid[17, 20, 28, 29]. The ayu did not respond to Bet at 10^{-2}M. Bet is a highly effective stimulant for the gustatory receptors of many species of fish[19, 20, 23] and has been reported to be a potent feeding stimulant or enhancer in several coastal carnivorous species[30-35].

Like many other species[19, 23], the ayu also responded well to nucleotides and nucleosides (Fig. 3). The thresholds for AMP, IMP and UMP (Table 1A, 1B) are comparable to those of other coastal species[20, 36]. The ayu responded also to CMP. Responsiveness to CMP has also been reported in the minnow[37], whereas the puffer did not respond to it at all though it responded markedly to other nucleotides[36].

We exclusively used D.W. for dissolving stimulants and as rinse water in the present study. However, D.W. has been reported to suppress the responsiveness of olfactory and gustatory receptors of fishes by eliminating ions in the external medium of the receptor membrane, although the relevant ions seem to differ between the two systems[12, 13]. Further studies are needed to know if and how deeply such a suppression is caused by D.W. in the gustatory receptors of ayu.

In the aqueous extract of the algae in Table 2 were contained several amino acids which stimulated the gustatory receptors of ayu. Provided that the water content of fresh algae is 90% or lower[38], the concentrations of Ala, Arg, Glu, Met, Phe and Orn in the algae would be well above the stimulatory threshold (Table 1A, 1B). The original extract solution was highly effective for the receptor : A one twentieth solution of the dried algae extracts (10 g of dried extracts in 200 ml of D.W.) gave an average response of 91.2 (±15.5 SD, n=7) taking the response to His as 100, whereas a one tenth solution of a synthetic mixture of the amino acid fraction in Table 2 gave a much lower value, 26.7

(±14.0, n=5). These results suggest stimulatory compounds other than amino acids were contained in the algae extract. We did not analyze for other substances but we conducted a behavioral experiment in which the feeding stimulatory effect of the algae was examined by adding the total extract and a synthetic mixture of the amino acid fraction to a casein based artificial feed, of which results are in preparation for publication.

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天然および養成アユ *Plecoglossus altivelis altivelis* の味覚応答

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養魚飼料の嗜好性向上のための基礎研究として，アユの味覚器の餌生物エキス成分に対する感受性を電気生理学的に明らかにした。味覚神経応答を記録した結果，アユの味覚器はアミノ酸，核酸関連物質等の餌生物エキス成分に対して高い感受性を示すことがわかった。アミノ酸20種のうち最も閾値の低かったのはArgとHisで，いずれも10^{-6}M付近であった。10^{-2}Mにおける刺激効果の比較では，Arg，His，Glu，Trp，Lys，Ala等に強い応答を示した。核酸関連物質では，AMP，ATP，CMP，IMP，UMP，Ino等に応答し，ヌクレオチドに対する閾値は10^{-5}～10^{-4}Mであった。琵琶湖産種苗を養成したものと，三重県宮川で捕獲した天然アユを比較したが，各物質に対する応答性に大きな差異は認められなかった。宮川で採取した付着藻類のエキスを調製し，その味覚器に対する刺激効果を調べたところ，きわめて高い刺激効果が認められた。アミノ酸分析の結果，Ala，Arg，Glu，Met，Phe，Orn等の刺激成分が閾値以上の濃度で存在することが推定された。しかし，アミノ酸画分のみではエキス自体より刺激効果が弱く，他に有効成分が存在することが予想された。