Response changes with growth in the gustatory receptors of young yellowtails (Seriola quinqueradiata)

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SUMMARY: Laboratory-hatched juvenile yellowtails (mean fork length, 9.3 cm) were reared for 2 months in two groups on different kinds of food, mysid and squid in one group and fish in the other, and the gustatory neural responses to seven chemicals (adenosine-5’-monophosphate, alanine, arginine, betaine, proline, tryptophan, and valine) were recorded in fish before and after the 2-month rearing period. Large variances in the responses to some chemicals were noticed in the juveniles before the rearing experiment. Mann–Whitney U-tests on the neural responses indicated significant changes in the magnitude of the responses to valine and some other chemicals relative to that for proline or tryptophan between the pre- and the two post-rearing groups. No significant differences in the response magnitude for the seven chemicals were observed for the two post-rearing groups suggesting that the response changes during the 2-month rearing might have been intrinsic and not due to specific food items in the diet. The dose–response relationship for some chemicals was also examined in the juveniles before and after the 2-month rearing. A slight lowering of threshold was noticed for alanine and valine after the rearing. Data on the responses of wild yellowtails were in support of the changing responsiveness of gustatory receptors during development of juvenile yellowtails.

KEY WORDS: amino acids, diet, growth, gustatory response, response change, Seriola quinqueradiata, yellowtail.

INTRODUCTION

Yellowtails are known to change their feeding habits during the early stages from larvae to fingerlings while associated with the floating seaweeds in the ocean. Their major stomach contents gradually change from planktonic invertebrates to small fishes when they reach around 3 cm in total length.1 Taste buds are reported to appear earlier at 5.3–7.0 mm in total length when the planktonic feeding has already started, suggesting that the gustatory sense develops at this stage.2 The changing gustatory experience may influence the developing gustatory system, and the responsiveness of gustatory receptors may alter with feeding habits. Developmental changes of gustatory responses have been reported in rat and sheep and their gustatory responsiveness was recognized in perinatal stages, and response changes have been observed during the early stages of development in both species of mammal.3–5 In fishes, we previously noted that the gustatory neural response of captured wild yellowtails to L-alanine (Ala) and L-valine (Val) became greater after the fish were kept for some weeks (Hidaka I, Taishaku H & Kurose N, unpubl. data, 1985). In the present study we recorded gustatory neural responses from laboratory-hatched yellowtails before different animal diets (mysid plus squid and fish), at an average bodyweight of 9.8 g and fork length of 9.3 cm, and 2 months after rearing. We chose these different sets of prey organisms as test diets because of the differences in the contents of free amino acids or ATP-related compounds between invertebrates and fish.6–8 These substances are generally stimulatory to fish taste receptors and the different diets may influence different characteristics of the taste sensation. The results obtained indicated that the relative response to seven chemicals used

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had changed in the 2 months with no significant differences between the two groups of fish fed on different food items. The results were also in accord with the findings in wild yellowtails suggesting that the gustatory response alters during development of juvenile yellowtails.

MATERIALS AND METHODS

Experimental periods, animals and their maintenance

Laboratory-hatched yellowtails

The experiments were performed from July to October 1999 at the Fisheries Research Laboratory, Faculty of Bioresources, Mie University, Zaga Island, Mie Prefecture. Yellowtails used were hatched from eggs of one breeding female and reared to juveniles at the Fisheries Laboratory, Faculty of Agriculture, The University of Tokyo, Maisaka, Shizuoka Prefecture. One hundred and forty fish were transported in a 500 L tank to the Mie University laboratory by car and kept in rearing tanks for a week. They had been fed on a commercial feed developed for juvenile yellowtail (Type Otohime No. 2; Nisshin Shiryo, Tokyo, Japan) and the same artificial feed was fed during the first 1-week after transportation.

Then, 100 fish were reared in two groups of 50 fish each for 1 month by feeding mysid plus squid to one group and fish to the other. At 1 month, all fish were weighed and 30 medium-sized fish in each group were selected and reared for another month. Mysid was obtained commercially in frozen blocks, and squid and fish were freshly caught animals obtained at a local market. Gustatory neural response was recorded from yellowtails at the end of the first week before starting rearing experiments and those in the two test groups at the end of the 2-month rearing. The group of laboratory-hatched juvenile yellowtails immediately before the rearing experiment was named PE (pre-experimental rearing, n=18) and their mean bodyweight and fork length were 9.77 g and 9.34 cm. The mysid plus squid-fed group was named MS (n=23) and their mean bodyweight and fork length were 82.85 g and 17.82 cm. The fish-fed group was named F (n=15) and their mean bodyweight and fork length were 105.89 g and 18.63 cm.

Wild yellowtails

Juveniles were obtained from local fishermen in May 1985. One hundred fish were kept in a net pen and reared for 1 month on a fish diet. The gustatory response was recorded from fish before and after the 1-month rearing.

Fig. 1 Summated neural responses in laboratory-hatched juvenile yellowtails (a) immediately before the experiment and (b) after rearing for 2 months by feeding mysid and squid. The upper tracing in each recording, time marker, in s; sustained downward deflection of the signal line, duration of opening the solenoid valve for stimulus solution flow. The paired spiny deflections in the lower tracing are electrical noises caused by the solenoid valve opening and closing. All chemicals were applied at a concentration of $10^{-2}$ M.
The group of fish from the pre-rearing stock was named PEW (n=16) and their mean bodyweight and total length were 4.35 g and 8.30 cm. The group after rearing for 4 weeks was named FW (n=17) and their mean bodyweight and total length were 42.04 g and 16.64 cm. Warasa (3 kg-size yellowtail) were obtained in December 1983 from Katada Teichi Ami Fisheries Cooperative, Mie Prefecture. They were caught by set nets and transported on shipboard two to three fish in a time in a 1000 L tank to the laboratory and kept in a net pen. They were used for the experiments within a few days after their capture. Eight fish were used for the experiment and their average bodyweight was 3.0 kg.

Recording of gustatory nerve responses

Yellowtails were lightly anesthetized with ethyl m-aminobenzoate methanesulfonate (Sankyo, Tokyo, Japan) and then immobilized with gallamine triethiodide (10 mg/kg of bodyweight) or pancuronium bromide (1 mg/kg of bodyweight) and positioned with the head higher than the tail on a wooden block. Seawater was perfused over the gills throughout the experiment by introducing a tube deep inside the mouth. In small fish of the pre-rearing test, artificial seawater (ASW) was perfused through the palate-stimulating system described below. After removing the eyeball, the ramus palatinus

Fig. 2  Response magnitudes for seven chemicals in fish of pre-experimental rearing (PE) and in those of the mysid and squid-fed group (MS) relative to that for Pro in each fish. In each group 15 individual fish are arranged in order of increasing response magnitude for Trp. Stimulus solutions were applied at a concentration of 10^{-2} M. Note that: (i) the response magnitude of AMP is highly correlated with that of Trp; and (ii) the variance in response magnitudes for the seven chemicals in the MS group was much smaller than those in the PE group.
facialis supplying the anterior palate was isolated and cut. The peripheral cut end of the nerve was placed on a pair of platinum electrodes. Whole nerve activity in response to the stimuli was recorded as the summated response using an electronic integrator (RJG 40225S, time constant 0.02 s; Nihon Kohden, Tokyo, Japan).

For successive application of S.W. and test solutions to the palate a three-way solenoid valve (MVC-3V-M6 for juveniles, or YCV-3V-1/4 for warasa; Takasago Electric, Nagoya, Japan) was used. The test solutions were applied for 3 s with 3 min rest between stimuli. During the resting time ASW was continuously applied. To compare the stimulatory effectiveness of test stimulants, the maximum height of the recorded response relative to that for 10^{-2} M L-tryptophan (Trp) or L-proline (Pro) taking the latter as 100 was measured. The stimulants used were adenosine-5'-monophosphate (AMP), Ala, L-arginine (Arg), betaine (Bet), Pro, L-proline; Trp, L-tryptophan; Val, L-valine.

### Table 1

Correlation between responses to seven chemicals in pre-experimental and mysid plus squid fed groups of laboratory-hatched yellowtails

<table>
<thead>
<tr>
<th></th>
<th>Ala</th>
<th>Arg</th>
<th>Bet</th>
<th>Pro</th>
<th>Trp</th>
<th>Val</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-experimental group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMP</td>
<td>0.303</td>
<td>0.466</td>
<td>−0.188</td>
<td>0.947***</td>
<td>−0.061</td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>−0.490</td>
<td>−0.342</td>
<td>−0.614*</td>
<td>−0.531*</td>
<td>−0.390</td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>0.523*</td>
<td>0.272</td>
<td>0.797***</td>
<td>0.710**</td>
<td>0.324</td>
<td>−0.124</td>
</tr>
<tr>
<td>Bet</td>
<td>0.774***</td>
<td>0.261</td>
<td>0.708**</td>
<td>0.710**</td>
<td>0.424</td>
<td>−0.195</td>
</tr>
<tr>
<td>Pro</td>
<td>0.261</td>
<td>0.708**</td>
<td>0.620*</td>
<td>−0.087</td>
<td>0.261</td>
<td>−0.211</td>
</tr>
<tr>
<td>Trp</td>
<td>0.947***</td>
<td>0.324</td>
<td>0.424</td>
<td>−0.087</td>
<td>0.261</td>
<td>−0.211</td>
</tr>
<tr>
<td>Val</td>
<td>−0.061</td>
<td>−0.390</td>
<td>−0.124</td>
<td>−0.195</td>
<td>−0.211</td>
<td>−0.211</td>
</tr>
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**Mysid plus squid-fed group**

<table>
<thead>
<tr>
<th></th>
<th>Ala</th>
<th>Arg</th>
<th>Bet</th>
<th>Pro</th>
<th>Trp</th>
<th>Val</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>0.383</td>
<td>0.514*</td>
<td>0.480</td>
<td>0.061</td>
<td>0.797***</td>
<td>0.080</td>
</tr>
<tr>
<td>Ala</td>
<td>−0.126</td>
<td>−0.004</td>
<td>0.046</td>
<td>0.681**</td>
<td>−0.033</td>
<td>0.309</td>
</tr>
<tr>
<td>Arg</td>
<td>0.660**</td>
<td>0.420</td>
<td>0.346</td>
<td>0.718**</td>
<td>−0.007</td>
<td>0.443</td>
</tr>
<tr>
<td>Bet</td>
<td>0.313</td>
<td>0.116</td>
<td>−0.028</td>
<td>0.665**</td>
<td>0.089</td>
<td>0.089</td>
</tr>
<tr>
<td>Pro</td>
<td>0.700**</td>
<td>0.112</td>
<td>0.813***</td>
<td>0.630*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trp</td>
<td>0.061</td>
<td>0.797***</td>
<td>−0.033</td>
<td>−0.007</td>
<td>0.089</td>
<td>0.089</td>
</tr>
</tbody>
</table>

Correlation coefficients for pairs of responses to seven chemicals at 10^{-2} M in each group of fish are estimated relative to the response to Pro (upper figure in each column) and to that to Trp (lower figure).

*** Significant (P<0.001); ** significant (P<0.01); * significant (P<0.05).

Ala, L-alanine; AMP, adenosine-5'-monophosphate; Arg, L-arginine; Bet, betaine; Pro, L-proline; Trp, L-tryptophan; Val, L-valine.

### Table 2

Mann–Whitney U-tests for the relative responses to seven chemicals between fish before rearing (PE) and after rearing for 2 months on mysid and squid diet (MS) or on fish diet (F)

<table>
<thead>
<tr>
<th>Standard</th>
<th>AMP</th>
<th>Ala</th>
<th>Arg</th>
<th>Bet</th>
<th>Pro</th>
<th>Trp</th>
<th>Val</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PE vs MS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>Sig.</td>
<td>Sig.</td>
<td>Sig.</td>
<td>No</td>
<td>–</td>
<td>Sig.</td>
<td>Sig.</td>
</tr>
<tr>
<td>Trp</td>
<td>Sig.</td>
<td>Sig.</td>
<td>Sig.</td>
<td>No</td>
<td>Sig.</td>
<td>–</td>
<td>Sig.</td>
</tr>
<tr>
<td>(PE vs F)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>Sig.</td>
<td>Sig.</td>
<td>Sig.</td>
<td>No</td>
<td>–</td>
<td>Sig.</td>
<td>Sig.</td>
</tr>
<tr>
<td>Trp</td>
<td>Sig.</td>
<td>Sig.</td>
<td>Sig.</td>
<td>No</td>
<td>Sig.</td>
<td>–</td>
<td>Sig.</td>
</tr>
<tr>
<td>(F vs MS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>–</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Trp</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>–</td>
<td>No</td>
</tr>
</tbody>
</table>

All stimulants were tested at 10^{-2} M.

* No, insignificant; Sig., significant (P<0.05) by Mann–Whitney U-test.

Ala, L-alanine; AMP, adenosine-5'-monophosphate; Arg, L-arginine; Bet, betaine; Pro, L-proline; Trp, L-tryptophan; Val, L-valine.

facialis supplying the anterior palate was isolated and cut. The peripheral cut end of the nerve was placed on a pair of platinum electrodes. Whole nerve activity in response to the stimuli was recorded as the summed response using an electronic integrator (RJG 40225S, time constant 0.02 s; Nihon Kohden, Tokyo, Japan).

For successive application of S.W. and test solutions to the palate a three-way solenoid valve (MVC-3V-M6 for juveniles, or YCV-3V-1/4 for warasa; Takasago Electric, Nagoya, Japan) was used. The test solutions were applied for 3 s with 3 min rest between stimuli. During the resting time ASW was continuously applied. To compare the stimulatory effectiveness of test stimulants, the maximum height of the recorded response relative to that for 10^{-2} M L-tryptophan (Trp) or L-proline (Pro) taking the latter as 100 was measured. The stimulants used were adenosine-5'-monophosphate (AMP), Ala, L-arginine (Arg), betaine (Bet), Pro, Trp and Val. These were chosen from the potent stimulatory chemicals in a previous study. Room temperature during the electrophysiological experiments was 23.0–27.7°C for the laboratory-hatched fish, 18.2–22.2°C for the wild juveniles and 20.1–21.6°C for the warasa.
Comparisons of dose–response curves for AMP, Ala, Pro and Val obtained from fish before the rearing test (PE) and those from fish reared on mysid and squid (MS). Magnitudes of the neural responses relative to that of $10^{-2}$M Pro, with Pro as 100, are plotted against the log molar concentration of each stimulant. Data for each curve were from six to 10 specimens. (.....), the 95% confidence limit (mean + 1.65 SD) for the response to artificial seawater. *Significant difference ($P < 0.05$) between the two groups of fish.

Statistical treatments

The Mann–Whitney $U$-test was conducted for comparison of data from the pre- and post-rearing groups of fish with a significance criterion of $P < 0.05$, unless otherwise specified.

RESULTS

Gustatory responses in laboratory-hatched juvenile yellowtails

Pre-experimental rearing group responded well to most of the chemicals tested at a concentration of $10^{-2}$M (Fig. 1a). However, large variances were noticed among fish in response magnitude for the stimulants relative to that for Pro or Trp. Figure 2 shows response magnitudes for all the seven stimulants at $10^{-2}$M and ASW in 15 fish of the PE group arranged in the order of increasing magnitude of Trp response relative to that of Pro response. The Trp response varied from 32% to 163% of that of the Pro response. A similar line graph to that for Trp is also seen in Fig. 2 for AMP. The range of the magnitude of the AMP response was 19.0% to 156.3%. The AMP and Trp responses in the 15 fish were highly correlated to each other ($P < 0.001$; Table 1). The variance of the responses to the seven chemicals in the MS group was, in general, much smaller than that of the PE group as shown in Fig. 2 for the Pro standard. A similar tendency toward lower response variance was observed also in the F group. Mean response magnitudes for the six chemicals relative to that for Pro obtained from PE, MS and F groups each with 15 individuals are shown in Fig. 5. Figure 5 shows that Ala, Arg and Val responses in both MS and F groups were larger than those of the PE group, while AMP and Trp responses were both lower than that of the PE group. This difference was also seen in the example of the actual neural recordings shown in Fig. 1. We estimated the significance of response differences between the three treatments by the Mann–Whitney $U$-test rather than use Student's $t$-test for the mean responses because of significant differences in variance-ratios between PE and MS or F groups. The results of the Mann–Whitney $U$-test in Table 2 indicate that there were significant differences for all chemicals except Bet between PE and MS groups or between PE and F groups for Pro or Trp standards, whereas there were
no significant differences found for MS and F groups for either Pro or Trp standards. Dose–response relationships for AMP, Ala, Pro and Val were examined in seven to nine fish in the PE group and six to seven fish in the MS group. Dose–mean response curves for these chemicals are shown in Fig.3. In Ala and Val responses, the response magnitude in the MS group was significantly larger than that in the PE group from $10^{-3}$ to $10^{-2}$ M for Ala and at $3 \times 10^{-3}$ M and $10^{-2}$ M for Val. The threshold of both chemicals decreased by about 0.5 log unit in the dose–mean response curves. In contrast, AMP response in the MS group was significantly lower than that in the PE group for $10^{-3}$ M and $10^{-2}$ M, although no differences were detected at the lower concentrations. For Pro response, no differences in the magnitude of the responses to $10^{-3}$ M and lower concentrations relative to that for $10^{-2}$ M Pro were detected for PE and MS groups.

Gustatory responses in wild juvenile yellowtails

The PEW group also responded well to most of the chemicals tested. Again in this group of wild juveniles, large variances in the relative magnitude of the responses to chemicals were noticed among individuals. The AMP and Trp responses among the 13 fish relative to Pro response ranged from 31.3% to 117%, and 60% to 137.5%, respectively. A high correlation between Trp response and AMP response was also observed in the wild juveniles (correlation coefficient 0.841, $P < 0.001$). The mean magnitudes of the responses to the seven chemicals for PEW and FW groups are shown in Fig. 5 (PEW and FW). In the wild juveniles, like the laboratory-hatched juveniles, the mean magnitude of Ala and Arg responses in the FW group was larger than that in the PEW group. However, no marked differences in the mean responses were observed to the other chemicals between PEW and FW groups. For the same reason as in the laboratory-hatched fish, we estimated the differences by the Mann–Whitney $U$-test. The statistics indicated a significant difference for each of the responses to Ala and Val, but in contrast to the laboratory-hatched yellowtails, no significant differences for the other stimulants between PEW and FW groups were found. Dose–response relationships were examined for Pro, Trp and Val in six fish of the PEW and five fish of the FW groups (Fig. 4). In Fig. 4, the two dose–mean response curves of Val for PEW and FW demonstrate a similar increase pattern for Val response compared to that in the laboratory-hatched yellowtails in Fig. 3, although no significant difference was detected for the data in Fig. 4 by the
Mann–Whitney U-test, presumably due to the small size of the sample. The Trp responses in PEW and FW groups were not significantly different from each other over the concentration range tested. No significant differences were detected in Pro response over the concentration range from $10^{-6}$ to $10^{-4}$ M relative to that of $10^{-2}$ M Pro.

Gustatory responses in warasa yellowtails

Yellowtails of 3 kg size also responded well to most of the seven chemicals with some differences compared with the two stocks of juveniles described above. The mean response magnitudes at a concentration of $10^{-3}$ M obtained from eight fish, weighing on average 3.0 kg, are shown in Fig. 5 (Warasa). The relative magnitude of the Val response was larger than that in the MS, F and FW groups of juveniles, while that of AMP and Arg responses was lower than that in any group of the juvenile fish. The dose–mean response curve for Pro obtained in four fish was quite similar to that shown in Figs 3 or 4, with a threshold of around $3 \times 10^{-6}$ M. The threshold of Val was around $10^{-3}$ M in three fish.

DISCUSSION

The large variances in the response to the seven chemicals in PE and PEW groups in comparison with the post-rearing group suggest that the receptor system at
these juvenile stages is functionally immature and under-developed. Thus, in both the laboratory-hatched and wild juveniles a significant increase in Ala and Val response relative to Pro response was recognized after 1 or 2 months rearing. The most conspicuous is the Val response. While a moderate response was observed to $10^{-2}$M solution in some fish, almost no positive response was elicited in many others. After the 2-month rearing in the laboratory-hatched group such insensitivity decreased and the responsiveness of individual fish was increased, as seen in Fig. 2. However, the AMP and Trp response in the laboratory-hatched yellowtails relative to the Pro response in the MS and F groups was lower than that in the PE group, while that in the wild juveniles showed no significant difference in magnitude between PEW and FW groups. This discrepancy might be due to the heterogeneous development of the receptors or taste units for AMP and Trp and those for Pro. Single-fiber analyses of the gustatory nerve in the yellowtail10,11 showed separate fibers for uridine-5'-monophosphate (UMP) and Trp and for Pro. Since AMP is likely to share the same fibers or taste units with UMP, it seems probable that AMP also shares the Trp fibers that also respond to UMP.12 This is also supported by the high correlation between the AMP response and Trp response in Table 1. Thus the high variance in both AMP and Trp responses relative to the Pro response in both the laboratory-hatched and the wild juveniles suggests the heterogeneous development of the taste units for AMP and Trp and those for Pro in individual fish. The higher mean magnitude of the AMP or Trp response in PE, relative to that for Pro in PEW might be due to an increase in the relative ratio of AMP or Trp fibers to Pro fibers between the sampled fish from the PE group and those from the PEW group or between the two different stocks of juvenile yellowtails. The postlarval growth of the peripheral gustatory system has been investigated in the channel catfish Ictalurus punctatus ranging from 5 to 40 cm in standard length.13 In the channel catfish, both the number and size of gustatory nerve fibers in the recurrent facial nerve increased with growth over the range of fish sizes investigated. In addition, the number of taste buds in the recurrent gustatory system increased at a faster rate than the number of nerve fibers innervating them. The increase of responsiveness to Val and others in the juvenile yellowtails might also be caused by a similar increase in the number of taste buds as well as the development of nerve fibers. In the yellowtail, morphological transformation from larvae to juveniles occurs at about 10 mm in standard length.14 The larvae start feeding on planktonic invertebrates at about 3–4 mm in total length, and the planktonic diet is gradually altered to small fishes starting at around 30 mm in total length after the transition to the juvenile stages accompanied by a rapid development of the alimentary system.1,2 The minimum size of juveniles we studied in the present study was 8.6 cm in fork length for the laboratory-hatched fish and 6.7 cm in total length for the wild fish. Further studies on smaller sizes of juveniles would elucidate more immature stages of the developing receptor system. In contrast, the present results with warasa yellowtails showed that the response to Val in the warasa relative to that for Pro was greater than that of the juveniles after 1 or 2 months rearing, while the response to AMP, Arg and Trp was lower than that in the juvenile yellowtails (Fig. 5). This suggests the possibility that the responsiveness of the gustatory receptors to these substances changes in this size yellowtail.

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