Original Article

Characterization of the digestive tract of wild ayu

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ABSTRACT: The gut and gut contents of ayu Plecoglossus altivelis caught in the Ohta River in Hiroshima prefecture, Japan were examined. Relative intestine length (intestine length/body length) was almost constant. Except for relative intestine length, characteristics of the gut of wild ayu were not significantly different from those of cultured ayu. The height of intestinal folds increased and their width declined from stomach to anus. Cyanobacteria (Homoeathrix sp. and Calothrix sp.) and diatoms (Cymbella sp., Gomphonema sp., Melosira sp., Navicula sp., and Synedra sp.) were well digested during passage through the gut despite a lack of apparent destruction. In contrast, green algae (Dictyosphaerium sp., Coelastrium sp., and Pediastrum sp.) exhibited little degradation. The pH of stomach contents ranged from 2.8 to 7.4 (mean ± SD = 4.1 ± 1.0), whereas that of the posterior intestine was 7.0–8.5 (7.9 ± 0.4). Gut contents of wild ayu increased with bodyweight, and were more than threefold greater than those of cultured ayu, suggesting that wild ayu compensate for low nutrient content of algae through the continuous ingestion of large quantities of feed organisms.

KEY WORDS: ayu, digestive organ, ingestion, microalgae, Plecoglossus altivelis.

INTRODUCTION

Ayu Plecoglossus altivelis are native to East Asia and are widely cultured in Japan, Korea, and Taiwan. Wild ayu more longer than 5–6 cm in body length are herbivorous, feeding primarily on diatoms (Chrysophyta), cyanobacteria, and green algae (Chlorophyta) that grow on river rocks. Ayu gain weight rapidly while feeding on these microalgae in rivers, but the intestine is relatively short compared to other herbivorous fishes.‡ Food organisms and the feeding behavior of ayu have been described previously by Ueda and Okada,§–⁵ Matsui,⁶ Ishida,⁷ and Kawanabe et al.⁸ Rearing techniques for ayu are well established, but digestion mechanisms are poorly known. Better understanding of digestive mechanisms should be useful for improving the quality of artificial food fed to cultured ayu. In order to understand the contribution of microalgae to growth and digestive functions, the gut and gut contents of ayu were examined.

MATERIALS AND METHODS

Fish

Ayu (bodyweight 21–155 g, body length 13–22 cm) were captured by foul hooks at 13.00–16.00 hours in the Ohta River, Hiroshima prefecture, from July to August in the years 1996–2000. Many artificially produced ayu young are released annually into the Ohta River to enhance the fishery. Because these fish spend part of their life in nature, fish captured in the river were defined as wild ayu regardless of their origin. For comparison, cultured ayu from a facility operated by the Ohtagawa Fisheries Cooperative Association were subjected to gut and gut content measurements 1–2 h after their last feeding to satiation.

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Measurements were made from the tip of a caecum to its base, where it joined either the intestine or a column.

Gut contents were placed in closed glass containers on ice for return to the laboratory. In the laboratory, gut contents were poured into a watch glass and viewed under a dissecting microscope with transmitted light.

Microalgae

Algae were scraped from 10 cm × 10 cm areas of the surfaces of river rocks near other rocks with ayu bite marks. Only exposed and central portions of rock surfaces were scraped to eliminate any edge or shading effects. Algae were dried at 70°C until constant weight on a preweighed filter, and weighed to determine standing crop.

Histology

Sections of the gut fixed in Bouin's solution were dehydrated in ethanol, embedded in paraffin, and stained with hematoxylin–eosin. Height and width of longitudinal folds of the gut and thickness of the basal stratum (circular muscle, longitudinal muscle, and serosa) were measured.

Statistical analysis

Structural and biochemical parameters were analysed for significance using Student's t-test.

RESULTS

Bodyweight, body length, and hepatosomatic index of wild ayu did not differ among date and year, hence, data were pooled for analysis. Except for the IPF ratio and relative intestine length, the parameters of wild ayu were not significantly different from those of cultured ayu (Table 1). As intestine length increased with growth, relative intestine length was nearly constant relative to body length (Fig. 2). Relative intestine length was significantly different between wild and cultured ayu.

The basal stratum of the stomach was thicker than that of the intestine (Fig. 1), and the stomach contained thick-set folds compared with the intestine. A pyloric caecum was lined with lightly folded epithelium (7–10 folds), which resembled that of the stomach. The number of pyloric caeca ranged widely (range 145–358), especially in wild ayu. Nev-
Nevertheless, no correlation was found between the number of caeca and fish size. The pyloric caeca was histologically identical to that of the intestine, and caeca were filled with microalgae.

The diameter of the intestine appeared constant from anterior to posterior in the region behind the pyloric caeca. In this same region, height of intestinal folds and thickness of the basal stratum varied little, although the latter appeared to decrease slightly (Fig. 2). Variance in width of folds was greater in the post-caeca section of the intestine and, while no clear pattern along the intestine was evident, width of folds may have decreased slightly with basal stratum thickness. The section of the intestine from which caeca branched had a distinctly lower fold height and probably a smaller width and thinner basal stratum than the posterior intestine.

Stomach content pH was more acidic than that in other regions of the gut (Fig. 3), with a mean stomach pH of 4.1 (range pH 2.8–7.4). The pH of stomach content varied considerably depending on the quantity of food organisms. Immediately posterior to the stomach, in the section of the intestine with pyloric caeca, pH rose rapidly by more than 3 pH units and then continued to rise to a maximum of pH 7.9 (range pH 7.0–8.5) near the anus. No significant differences were observed in gut content pH between wild and cultured ayu.

Among wild ayu caught by foul hooks, the gut of 6.5% of individuals was empty. Taxa recorded from

Table 1  Measurements (mean ± SD) of gut of wild and cultured ayu

<table>
<thead>
<tr>
<th></th>
<th>Wild</th>
<th>Cultured</th>
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<tbody>
<tr>
<td>Body length (cm)</td>
<td>17.2 ± 2.3 (n=79)</td>
<td>16.0 ± 1.0 (n=36)</td>
</tr>
<tr>
<td></td>
<td>(12.5–21.8)</td>
<td>(14.0–18.3)</td>
</tr>
<tr>
<td>Bodyweight (g)</td>
<td>79.1 ± 30.3 (n=78)</td>
<td>63.1 ± 15.2 (n=36)</td>
</tr>
<tr>
<td></td>
<td>(21.4–155)</td>
<td>(36.6–102)</td>
</tr>
<tr>
<td>Hepatosomatic index (%)</td>
<td>1.14 ± 0.31 (n=68)</td>
<td>1.28 ± 0.35 (n=34)</td>
</tr>
<tr>
<td></td>
<td>(0.47–2.00)</td>
<td>(0.33–2.07)</td>
</tr>
<tr>
<td>Intraperitoneal fat body ratio</td>
<td>0.62 ± 0.55 (n=14)</td>
<td>5.71 ± 1.48 (n=10)*</td>
</tr>
<tr>
<td>(%)‡</td>
<td>(0.30–2.15)</td>
<td>(3.26–7.93)</td>
</tr>
<tr>
<td>Relative intestine length†</td>
<td>0.69 ± 0.11 (n=79)</td>
<td>0.59 ± 0.09 (n=34)*</td>
</tr>
<tr>
<td></td>
<td>(0.48–1.02)</td>
<td>(0.42–0.78)</td>
</tr>
<tr>
<td>No. pyloric caeca</td>
<td>232 ± 63 (n=18)</td>
<td>229 ± 30 (n=5)</td>
</tr>
<tr>
<td></td>
<td>(145–358)</td>
<td>(191–274)</td>
</tr>
<tr>
<td>Pyloric caecum length (μm)</td>
<td>8.65 ± 1.47 (n=15)</td>
<td>7.03 (n=2)</td>
</tr>
<tr>
<td></td>
<td>(6.2–10.4)</td>
<td>(6.9–7.1)</td>
</tr>
</tbody>
</table>

*Significantly different from wild ayu (P<0.05).
†Intraperitoneal fat body/bodyweight.
‡Intestine length/body length.
Values in parentheses indicate the range.
The intestine of wild ayu is rather short compared with those of other herbivorous fishes. Intestine length is thought to be a phenotypically plastic characteristic being variable in its response to feeding conditions. Takesue has suggested that the relatively short intestine of an herbivorous fish such as ayu might be compensated for by a high activity of carbohydrate-splitting enzymes. The protein content of microalgae has been determined to be approximately 4–5% on a wet weight basis (Cleveland A., Nakagawa H. & Nakano T., unpubl. data, 2001). The ingestion of large quantities of food by wild ayu might compensate for nutrient-poor microalgae and a short intestine length. Therefore, active digestive enzymes and a large mass of ingested food suggest an adjustment to poor nutrient conditions. In contrast, the artificial diet used to feed cultured ayu comprises 45–55% crude protein. Cultured ayu fed an artificial diet with a high protein and lipid content might not need to develop the intestine. Diet type influences gut characteristics, including intestine length, suggesting that gut parameters are useful indicators for evaluating an artificial diet.

As to the number of pyloric caeca in ayu, Matsui has reported that ayu of 7–8 cm BL possessed approximately only 160 caeca, and the number increased gradually with differentiation of the alimentary system. Iwai has reported approximately 300 caeca in older, larger fish. In the present study, we counted an average of 232 pyloric caeca (range 145–358) in fish measuring 13–22 cm in BL, but found no correlation between number of caeca and bodyweight. The function of pyloric caeca is consistent with the apparent role it plays in the digestive and absorptive functions in ayu and other fishes. The filaments found in the intestine content, but not the stomach, are clearly food from cyanobacteria. Microscopic observation showed that the intracellular substances of diatoms and blue-green algae appeared to be drawn out completely without apparent disruption by the time food reached the anus. Unlike diatoms and blue-green algae, however, green algae were passed through the gut and or defecated alive or with little apparent damage. When intestine contents were dispersed in water and examined under transmitted light, many filaments were observed that had been absent from stomach contents. The length of filaments ranged from 3.4 mm to 7.6 mm, and was generally 1–2 mm shorter than the length of caeca.

The weight of gut contents increased proportionally with bodyweight ($R^2 = 0.500$, $P < 0.05$). The weight of all gut contents averaged 2.68 g in wild fish and 0.57 g in cultured fish (Table 2), being equivalent to 3.9% of bodyweight of wild ayu, and 1.1% of cultured ayu. Thus, the amount of gut content of wild ayu was markedly higher than that of cultured ayu.

Table 3 shows algal standing crops on rock surfaces on the same sampling site. The values varied markedly, and ranged between 18 g/m² and 78 g/m² on a dry weight basis.

**DISCUSSION**

The intestine of wild ayu is rather short compared with those of other herbivorous fishes. Intestine length is thought to be a phenotypically plastic characteristic being variable in its response to feeding conditions. Takesue has suggested that the relatively short intestine of an herbivorous fish such as ayu might be compensated for by a high activity of carbohydrate-splitting enzymes. The protein content of microalgae has been determined to be approximately 4–5% on a wet weight basis (Cleveland A., Nakagawa H. & Nakano T., unpubl. data, 2001). The ingestion of large quantities of food by wild ayu might compensate for nutrient-poor microalgae and a short intestine length. Therefore, active digestive enzymes and a large mass of ingested food suggest an adjustment to poor nutrient conditions. In contrast, the artificial diet used to feed cultured ayu comprises 45–55% crude protein. Cultured ayu fed an artificial diet with a high protein and lipid content might not need to develop the intestine. Diet type influences gut characteristics, including intestine length, suggesting that gut parameters are useful indicators for evaluating an artificial diet.

As to the number of pyloric caeca in ayu, Matsui has reported that ayu of 7–8 cm BL possessed approximately only 160 caeca, and the number increased gradually with differentiation of the alimentary system. Iwai has reported approximately 300 caeca in older, larger fish. In the present study, we counted an average of 232 pyloric caeca (range 145–358) in fish measuring 13–22 cm in BL, but found no correlation between number of caeca and bodyweight. The function of pyloric caeca is consistent with the apparent role it plays in the digestive and absorptive functions in ayu and other fishes. The filaments found in the intestine content, but not the stomach, are clearly food from cyanobacteria.
materials that have been taken into the caeca, processed, compacted, and then released into the lumen of the intestine; gentle pressure with forceps on the apical tips of individual caeca causes the release of caecal content.

Microalgae ingested by ayu in the Ohta River were similar to those reported by Matsui,6 and Ueda and Okada,2,5 except for the blue-green algae *Homoeaathrix* sp. and the green algae *Dictyosphaerium* sp. reported in the present study. According to Ueda and Okada, ayu food varies considerably among locality and season,2 but the present study did not address this topic.

Herbivorous fishes can digest algal nutrients without any apparent destruction of algal cells.19 The present study revealed that the intracellular substances of diatoms and blue-green algae were well drawn out, but that green-algae appear to pass through the gut with little evidence of destruction. The integrity of algal cell walls may also be compromised by a low pH.20

Stomach contents constituted approximately 2% of bodyweight in wild ayu and 1% in ayu fed an artificial diet (0.67 g and 0.14 g, respectively),7 which is partly similar to the present study's findings of 3.9% of bodyweight in wild and 1.1% in cultured ayu. Ishida demonstrated that the time to digest microalgae was 2.5–5.0 h in wild ayu and 1–2 h in cultured ayu fed an artificial diet.7 Assuming that wild ayu feed continuously on algae during daylight hours (15 h) and need to process three to six full guts of food each day, one ayu might ingest 8.1–16.2 g of wet algae per day. This value is not dissimilar to that estimated by Kawanabe and colleagues,8 who have estimated that 20 g of algae is required for one ayu per day. When the most suitable areas of the river bottom are occupied by territorial ayu, algae in a territory measuring 1.0 m² could maintain the life and growth of 0.73 individuals. Standing crops on rock surfaces in the Ohta River varied from 18 g/m² to 78 g/m² (dry matter) in the present study. The crop volume appears to vary with sunlight intensity and river conditions.

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