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Oxygen Consumption of Ayu Larvae in Fasting Condition

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Abstract The oxygen consumption and nutritional condition of ayu (*Plecoglossus altivelis*) larvae under a fasting condition were investigated by the use of the larvae from immediately after hatching (Day 0) until 62 days after hatching (Day 62). In the fasting condition, 50% or more of the larvae at each growth stage survived for 3 to 6 days. During the fasting period, the fasted larvae often showed significantly higher oxygen consumption rates per dry body weight (OCR) for one or two days (the larvae groups fasted from Day 0, 5, 10, 15, 20, 25, and Day 45) and 7 days (Day 55 group) than those before fasting, while the fasted larvae in the Day 5, 40, and Day 50 groups exhibited significantly lower OCRs for one to three days than those before fasting. There were no significant differences in the carbon to nitrogen ratio (C/N ratio) between the larvae before fasting and the live larvae on the last fasting day in any group except the Day 55 group. The moribund larvae exhibited significantly lower (Day 10 and 15 groups) and higher (Day 50 group) C/N ratios than the larvae before fasting. Decrease in glycogen in the liver, atrophy of liver cells, and marked necrosis of the pancreas and the skeletal muscle were observed in the fasted larvae.

Key words: oxygen consumption, C/N ratio, fasting, ayu larvae, *Plecoglossus altivelis*.

INTRODUCTION

Ayu (*Plecoglossus altivelis*) is an important species for aquaculture in Japan. Although ayu larvae are produced widely in Japan, reliable techniques for seedling production of ayu have not been established yet and many technical problems still remain to be solved. More detailed information on the physiological profiles of the larvae is required to overcome these problems.

The oxygen consumption of fish is one of the most useful physiological profile for estimating the nutritional requirement of fish, since it means the rate of energy metabolism (JOBLING, 1994). The oxygen consumption rates of several larval fishes have been reported: carp (KAUSHIK *et al.*, 1982), striped mullet (WALSH *et al.*, 1989), diploid and triploid rainbow trout (OLIVA-TELES and KAUSHIK, 1990), *Labeo rohita* (DIVAKARUNI and SHARMA, 1991), bleak and roach (KECKEIS and SCHIEMER, 1990), sea bream (OIKAWA *et al.*, 1991), Japanese whiting (OOZEKI and HIRANO, 1994), Japanese flounder (KUROKURA *et al.*, 1995), and carp (OIKAWA and ITAZAWA, 1995).

In the present study, the oxygen consumption rate per dry body weight (OCR) and carbon to nitrogen ratio in the body (C/N ratio) were determined to examine the metabolism of larval ayu under a fasting condition. Histological changes in the liver, the muscle, and the pancreas of the larvae were also observed.

MATERIALS AND METHODS

Fish Used

Larval Rearing

The larvae were raised at Hiroshima Prefectural Fish Farming Center feeding on *Brachionus plicatilis* from immediately after hatching to 48 days after hatching (Day 0 to Day 48), egg yolk (Day 5 to Day 13), *Artemia salina nauplii* (Day 6 to Day 55) and formula pellet (Day 9 to Day 55). Water temperature in the culture tanks rose from 17 to 20°C during the raising period.

Experimental Procedure

The larvae used in this experiment were transferred to the Fisheries laboratory of Hiroshima University from Hiroshima Prefectural Fish Farming Center. The larvae were sampled from October to December 1994 from Day 0 to Day 55 at intervals of 5 days. The larvae on Day 0 were carried in fresh water and the larvae on Day 10 to Day 55 were carried in 60 percent diluted sea water to reduce the stress of the transfer. Immediately after the transfer, the larvae were divided and kept in 5 liter plastic beakers in a 30 liter fresh water container. The fresh water was circulated through a cooling machine to regulate the water temperature in the container to be similar to that in the culture tank at Hiroshima Prefectural Farming Center during the raising period. The diluted or normal sea water in 5 liter plastic beakers was aerated continuously and 30 to 50% of it was replaced with normal sea water every day.

Survival Period and Survival Rate

All the larvae transferred were unfed from immediately after the transfer until the day when 50% or more of them starved to death (last fasting day). On each sampling day, 30 to 50 larvae were counted exactly and moved to 5 l different beakers in the container. The dead larvae were counted and removed every day. The survival period was the numbers of fasting days before 50% or more of the larvae had starved to death.

Oxygen Consumption Rate (OCR)

OCR was determined by the improved water bottle method (KUROKURA *et al.*, 1995). One to six larvae were transferred into a 50−100 ml glass syringe that functioned as a respirometer (Fig. 1). The number of larvae closed in the syringe depended on their developmental stage. Eight syringes filled together with the sea water and the larvae and two syringes filled with sea water without the larvae were prepared for one trial of measurement. After the acclimatization period (around 10 minutes), the initial dissolved oxygen in the sea water of the syringes was measured using a Ultra DO Meter (UD-901) and then the glass syringes were sealed at the lip and wrapped with aluminium foil and kept in an incubator for 30 to 60 minutes. The final amount of oxygen dissolved in the sea water of the syringes was measured after the incubation. After measurement of the oxygen con-

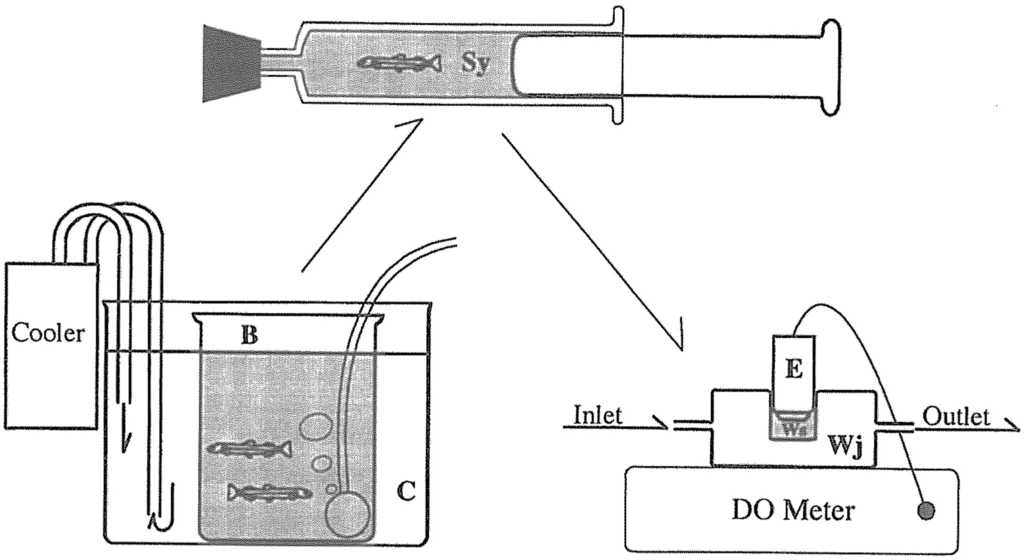

Fig. 1 A diagram showing the apparatus for measurement of oxygen consumption. B, 5-liter beaker (diluted or 100 % sea water); C, 30-liter container (fresh water); E, electrode; Sy, 30−50 ml syringe (diluted or 100 % sea water); Wj, water jacket (fresh water); Ws, water sample.

sumption rate, all the larvae used were dried in an oven at 60°C until their weight became constant and were weighed to a precision of 0.001 mg with an electrobalance (Sartorius 2405). OCR was calculated from the difference between the initial and final amounts of oxygen in the two types of syringes, water volume in the syringe, dry body weight of the larvae, and incubation time. The measurements of OCR in the fasting condition were repeated every sampling day until the day when more than 50% of them starved to death (last fasting day).

Carbon to Nitrogen Ratio (C/N ratio)

Dry samples of the larvae before fasting and both the live and moribund larvae on the last fasting day were used for the carbon and nitrogen determinations. The dry larva weighing 4 mg or less was analyzed whole and the dry larva heavier than 4 mg or more was ground and 2−3 mg of ground material was analyzed using a CHN Coder (Yanaco MT-3). The C/N ratio was calculated from the carbon and nitrogen determinations.

Histological Observation

After every sampling and measurement, some of the larvae were fixed in Gender's fixation, embedded in paraffin, sectioned with a microtome, mounted onto glass slides, stained with PAS Schiff staining, and examined with a light microscope.

Statistical Analysis

The changes in the dry body weight, the oxygen consumption rate, and the C/N ratio with growth and the fasting period were tested by one-way ANOVA. Values are expressed as mean ± standard deviation.

RESULTS

Survival period and survival rate

Under the fasting condition, more than 50% of all the ayu larvae survived for 3 to 6 days (Fig. 2). The survival period changed with the growth. The survival period was 6 days in the group of larvae fasted from Day 0 (Day 0 group), 3 days in the Day 25 group, 6 days in the Day 30 group, 3 days in the Day 40 group, 6 days in the Day 55 group. Of the groups with the longest survival, the Day 0 group showed a sharp decrease in the survival rate between the 6th and 7th fasting day while the others showed a gentle decrease.

Dry Body Weight

The dry body weight and the total body length of the fed larvae (before fasting) used in this experiment increased with the days after hatching (Table 1). Under the fasting condition, the change of the dry body weight did not exhibit any certain tendency in any group (Fig. 3). The dry body weight of the larvae in the Day 0, Day 10, Day 15, Day 20, and Day 30 groups decreased during the fasting period but did not change significantly in the other groups. Furthermore, it occasionally increased even in the fasting condition (4th and 5th fasting day in the Day 5 group, and 2nd fasting day in the Day 35 group) , when compared with those before fasting.

OCR

The OCRs of the fed larvae was highest on Day 5 and then decreased with the day after hatching (Table 1). The OCRs of the fasted larvae changed variously according to the group during the fast condition (Fig. 4). The fasted larvae often showed significantly higher OCRs for one or two days (Day 0, 5, 10, 15, 25, 35, and Day 45 groups) and 7 days (Day 55 groups) than those before fasting during the fasting period. On the other hand, the fasted larvae in the Day 5, 40 and Day 50 groups exhibited significantly lower OCRs for one to three days under the fasting condition than those in the same groups before fasting.

Table 1 Dry body weight, total body length, oxygen consumption rate and C/N ratio of ayu larvae on days after hatching. Data presented as mean±SD.

Days after hatching	Dry body weigth (mg)	Total body length (mm)	Oxygen consumption rate ($\mu l \cdot mg^{-1} \cdot hr^{-1}$)	C/N ratio
0	0.05±0.015	6.6±0.24	10.7±6.00	5.5±0.49
5	0.04±0.030	9.1±0.36	32.6±16.37	4.0±0.62
10	0.3±0.04	12.0±0.51	8.1±1.87	4.0±0.26
15	0.6±0.04	13.8±0.72	5.5±1.21	3.8±0.18
20	0.9±0.15	16.4±0.84	5.1±2.89	3.8±0.25
25	1.2±0.24	17.7±1.70	6.9±2.43	3.8±0.45
30	3.4±1.12	21.2±0.75	5.1±1.89	3.6±0.12
35	3.8±1.98	22.7±2.07	8.7±3.26	3.8±0.05
40	4.3±1.67	21.7±0.81	10.1±3.87	4.0±0.15
45	8.4±2.49	26.0±1.64	4.8±3.18	3.1±0.06
50	18.4±13.80	33.0±3.71	4.0±1.90	4.3±0.08
55	26.9±23.26	33.2±7.23	2.2±1.14	4.3±0.35

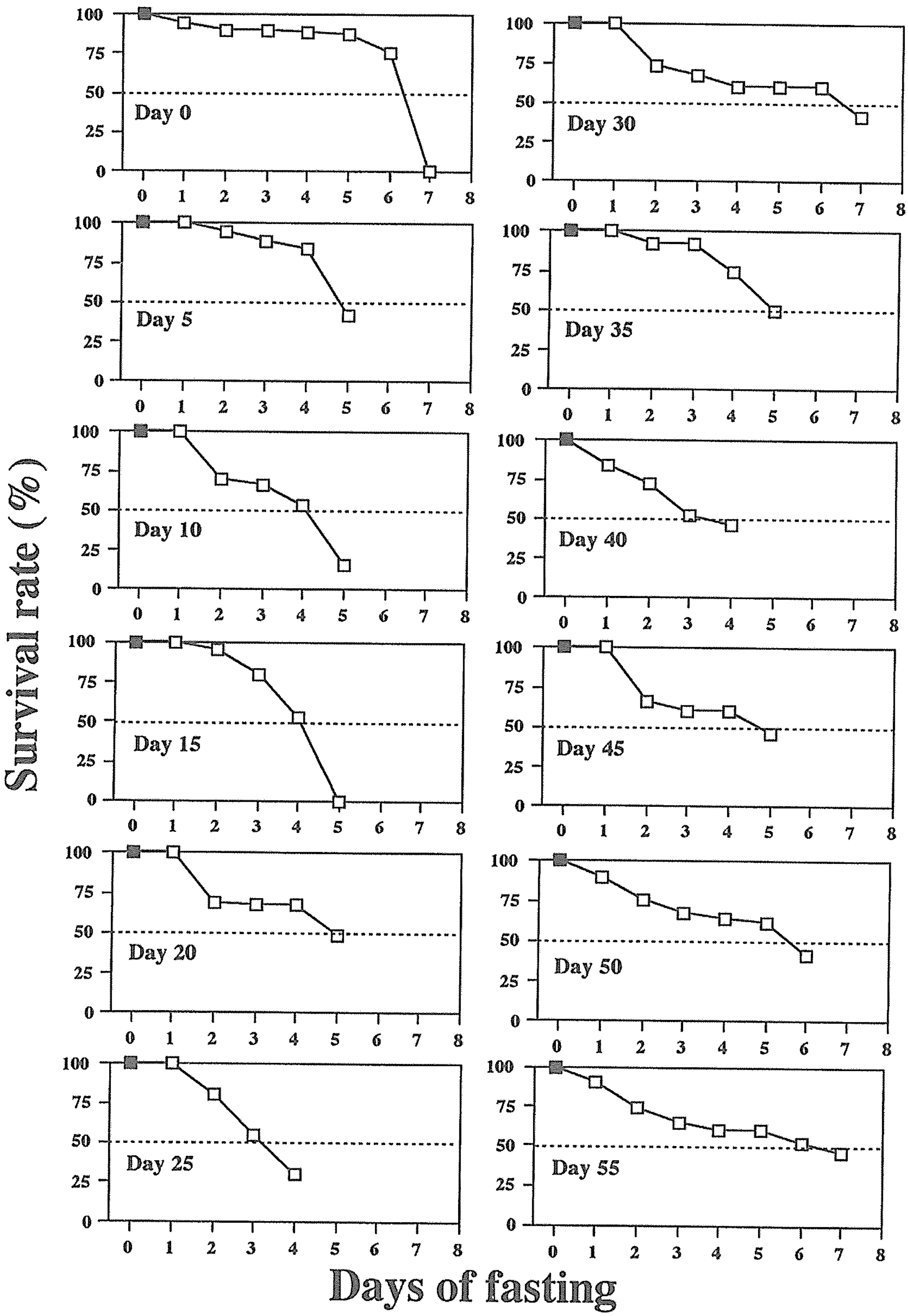

Fig. 2 Survival rate of ayu larvae during the fasting period at each developmental stage.

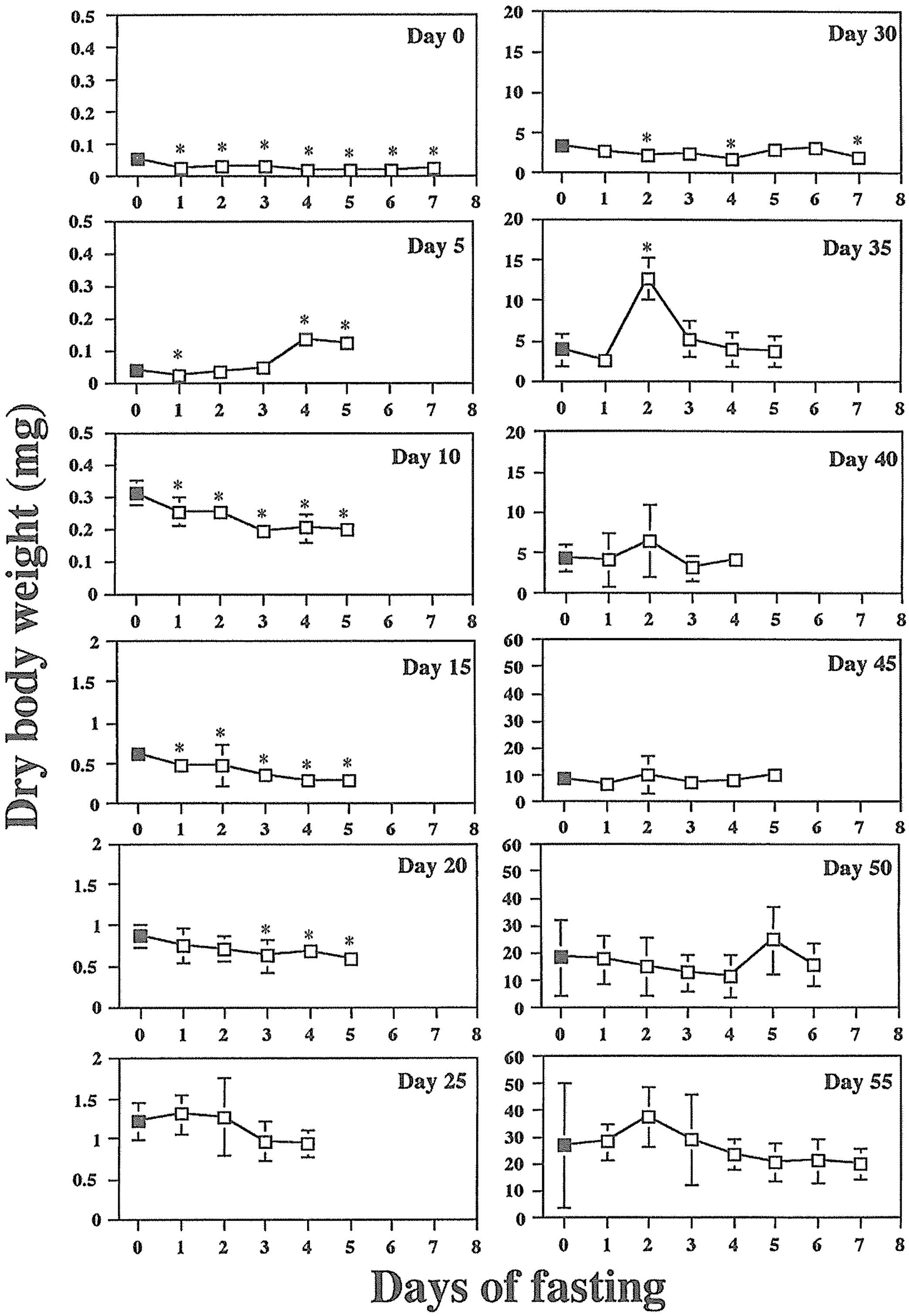

Fig. 3 Changes in dry body weight of ayu larvae during the fasting period at each developmental stage. Vertical bars denote standard deviation. Asterisks indicate statistically significant difference at $p<0.05$ from larvae just after fasting (0 fasting day).

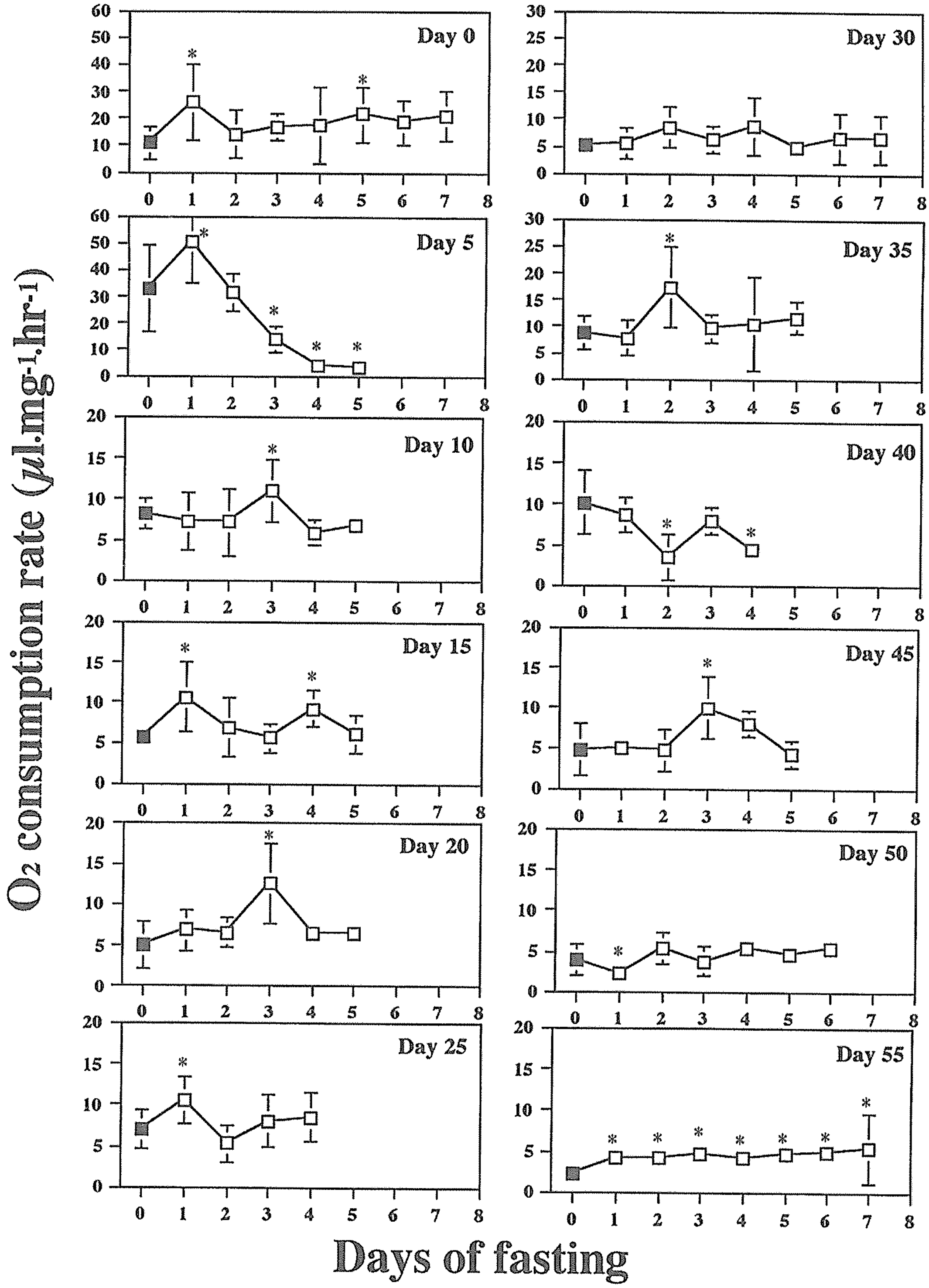

Fig. 4 Changes in oxygen consumption rate per dry body weight of ayu larvae during the fasting period at each developmental stage. Vertical bars denote standard deviation. Asterisks indicate statistically significant difference at $p<0.05$ from larvae just after fasting (0 fasting day).

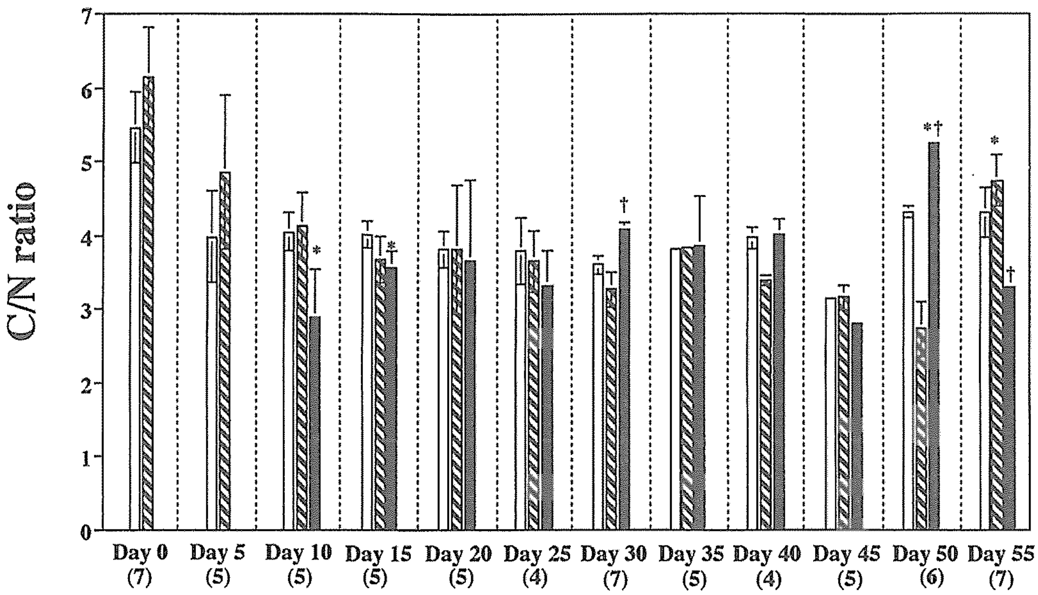

Fig. 5 Comparison of C/N ratio of ayu larvae just before fasting ( ), live larvae ( ) and moribund larvae ( ) on the last fasting day at each developmental stage (Day 0 to Day 55). The number in parentheses indicate the fasting days. Vertical bars denote standard deviation. Asterisks indicate statistically significant difference at $p<0.05$ from larvae just after fasting. Daggers indicate statistically significant difference at $p<0.05$ from live larvae at the last fasting day.

C/N ratio

The average C/N ratio of ayu larvae exhibited the highest value of 5.5 on Day 0 and decreased to 3.1−4.3 in subsequent developmental stages (Table 1). No groups except the Day 55 group showed significant differences in the C/N ratio between the larvae on before fasting and the live larvae on the last fasting day (Fig. 5). The moribund larvae in the Day 10 and Day 15 groups showed a significantly lower C/N ratio than those before fasting, as opposed to those in the Day 50 group. However, the moribund larvae in the Day 30 and Day 50 groups showed a higher C/N ratio than the live larvae in the same groups, contrary to those in the Day 55 group.

Histological Analysis.

Abnormal histological changes in the organs of the fasted larvae were observed in all developmental stages. As the fasting days proceeded, glycogen in the liver decreased gradually. The liver tissue showed wide intercellular spaces, the loss of the cellular cord arrangement, and the atrophy of cells. Furthermore, many regions of the pancreatic tissue and the skeletal muscle exhibited necrosis (Fig. 6).

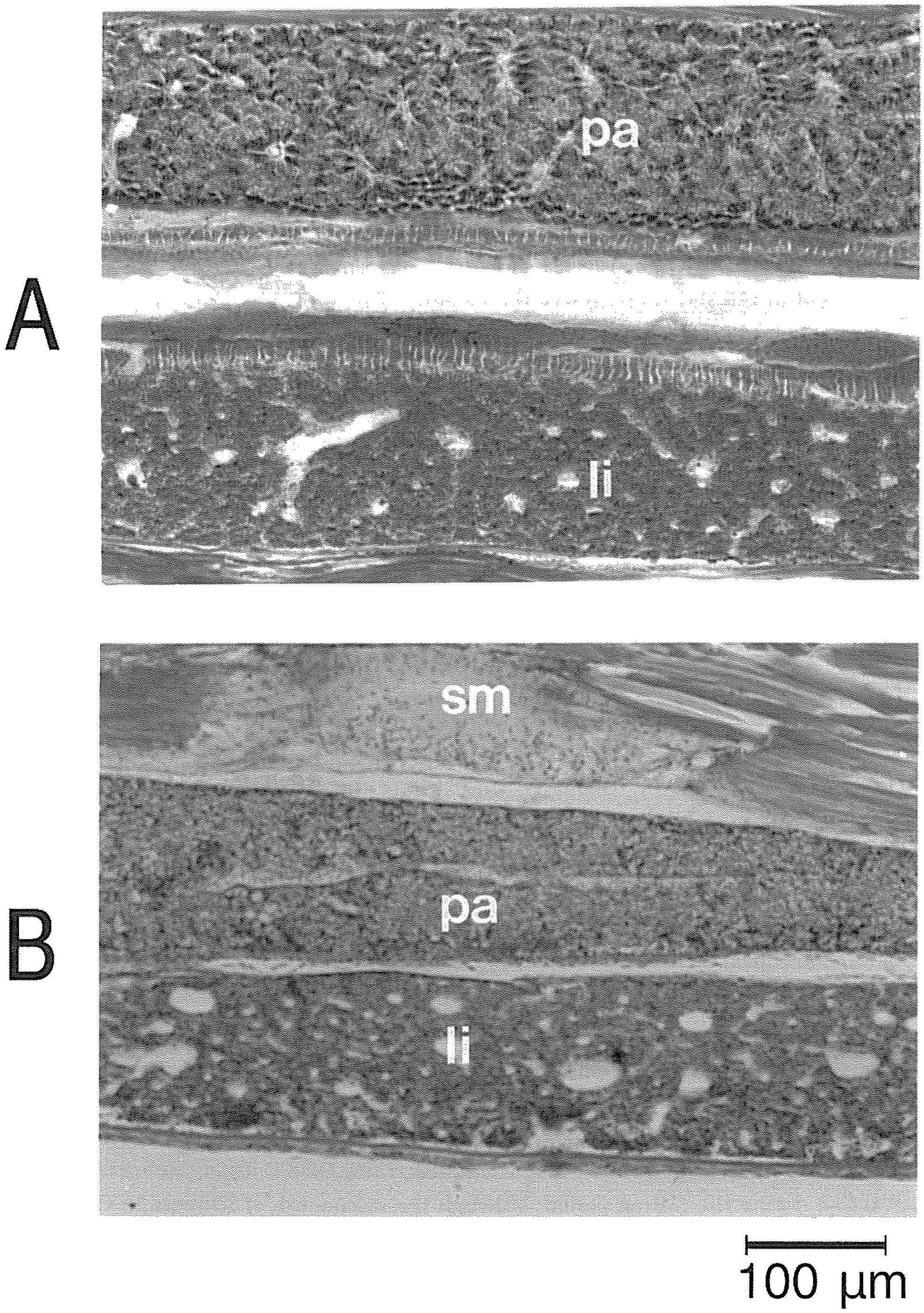

Fig. 6 Microscopic examination of fasted ayu larva. A: Fed larva on Day 44. B: Fasted larva on Day 44 (4 days of fasting) showing marked necrosis in skeletal muscle and pancreas tissues, and atrophy of liver cells glycogen in liver cells almost disappeared. pa, pancreas; li, liver; sm, skeletal muscle.

DISCUSSION

The survival period, i. e., the number of days before 50% of the larvae starved to death, and survival rate of fasted larval fish are determined by the rate of energy depletion resulting from an energy budget for maintaining bodily functions and energy store of the larva. More than 50% of striped jack larvae fasted from Day 0 survived at least for 8 days (KAWABE *et al.*, 1996) and about 75% of the ayu larvae fasted from Day 0 survived for 6 days. Such differences in the survival period and survival rate among larval fishes would reflect a species-specific situation between the energy budget and the energy store during the development. Despite the highest OCR, i. e., the highest metabolic rate in all groups, the survival period in the Day 0 group was the longest. This probably resulted from the large energy store derived from absorption of their yolk. On the other hand, the longest survival periods in the Day 30 and Day 55 groups seemed to be attributed to their lower OCRs in addition to their energy store with growth.

The body weight of larval fish is expected to decrease during the fasting condition. Juvenile perch lost weight during the starvation period (MEHNER and WIESER, 1994). The dry body weight of the fasted ayu larvae was also low in the Day 0, 10, 15, 20 and Day 30 groups, but the dry body weight of the larvae occasionally increased during the fasting period. Such an increase in the dry body weight was most likely caused by the individual variation in body weight which far exceeded the weight loss of individuals, since the different individuals were examined every fasting day.

The oxygen consumption rate of normal larval fish decreased with the developmental stage in sea bream (OIKAWA *et al.*, 1991), Japanese whiting heavier than 0.1 mg dry body weight (OOZEKI and HIRANO, 1994), carp (OIKAWA and ITAZAWA, 1995), and Japanese flounder (KUROKURA *et al.*, 1995). Similarly, the OCR of the fed ayu larvae decreased with growth. Such a decrease in the OCRs probably resulted from the relative growth of organs with different metabolic activities (OIKAWA *et al.*, 1991; OIKAWA and ITAZAWA, 1992; OIKAWA and ITAZAWA, 1993). In addition, MEHNER and WIESER (1994) reported the decrease in OCR of juvenile perch during the starvation period. This means that the juvenile perch could reduce its metabolism during the starvation. Although the ayu larvae in the Day 5, 40, and Day 50 groups exhibited a significant decrease in OCRs during the fasting period, the larvae in other fasted groups did not show any significant decrease in OCRs. This finding indicates that the ayu larvae could reduce their metabolism under the fasting condition only at certain developmental stages. On the contrary, the fasted ayu larvae often showed increased OCRs. The fasted larvae of common carp exhibited increased metabolic activity just before the onset of massive mortality (KAUSHIK *et al.*, 1982). Thus, the increased OCR of larval fish during the fasting period, although the mechanism of increase in OCR is not clear, possibly reflects a more serious physiological condition than a decreased OCR.

In the fasted juvenile perch, proteins in the muscle were catabolized and used as an energy source and glycogen in the liver and the muscle decreased markedly (MEHNER and WIESER, 1994). In the fasted ayu larvae, the live larvae on the last fasting day showed C/N ratios similar to those of the larvae. This implies that the decreasing rates of carbon and nitrogen were similar in the live larvae. However, the moribund larvae on the last fasting day in the Day 10 and Day 15 groups exhibited significantly lower C/N ratios than the larvae before fasting in the same groups. In this case, the moribund larvae seemed to consume carbon in glycogen faster than nitrogen in protein. Furthermore, the moribund larvae in the Day 30 and Day 50 groups showed significantly higher C/N ratios than the live larvae on the last fasting day in the same groups. The increased C/N ratio of the moribund larvae suggest that the protein in the muscle served as a major fuel for energy metabolism after depletion of the glycogen in the liver and the muscle (MEHNER and WIESER, 1994).

The digestive organs of yellow tail larvae on Day 6 to Day 9 strikingly degenerated during 3 days of starvation (UMEDA and OCHIAI, 1975). Early post sac larvae of the northern anchovy showed severe histological changes under the starving condition, i. e., fibrils within the muscle fibers indistinct, basophilic tissue present between muscle fiber layers, disappearance of chromaffin and zymogen in the pancreas, small cells and indistinct nuclei of the liver, *etc.*(O'CONNEL, 1976). In the fasted ayu larvae, similar histological changes such as decrease in glycogen of the liver, atrophy of liver cells, marked necrosis of the pancreas and the skeletal muscle were observed. These histological changes of the fasted ayu larvae were in agreement with the changes in C/N ratio of the moribund larvae.

In the present study, the fasted ayu larvae had low OCRs only in limited developmental stages and often had increased OCRs. Decrease in the OCR in the fasting condition suggests that the ayu larvae in certain stages can adjust their metabolic activity to the fasting condition. The increase of the OCR in the fasting condition means increased metabolic activity which is unfavorable to the fasted ayu larvae, since the energy store is then rapidly depleted. The mechanism of the increase in the OCR of larval fish in the fasting condition is not clear and its elucidation will be a key to the phenomenon of early mortality of fishes.

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絶食時におけるアユ仔魚の酸素消費

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孵化直後から孵化後55日までのアユ仔魚を絶食させ，その代謝の状態を調べた。50%の仔魚が生残する日数は3日から6日間であった。絶食中のアユ仔魚の酸素消費量は減少する場合よりも，増加する場合の方が多く，アユ仔魚の場合は絶食耐性はごく限られた発達のステージのみに現れ，むしろ異常な代謝の亢進を示す場合の方が多いことが明かになった。C/N比は絶食期間の最後まで活力を維持して生き残った個体と摂餌をしている個体との間で有意な差を示さなかった。瀕死の状態にある個体ではC/N比は摂餌個体とは有意に異なり，その変化はエネルギー源としての炭水化物の涸渇と筋肉タンパクをエネルギー源に変えている代謝の状態を反映していた。組織学的検査においても肝臓中のグリコーゲンの消失，筋肉組織や膵組織の壊死が絶食が続くと観察された。

キーワード：酸素消費，C/N比，絶食，アユ仔魚，*Plecoglossus altivelis*