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Effects of Starvation on the Morphometric Characteristics and Histological Changes in Chum Salmon (*Oncorhynchus keta*) Fry

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Abstract : A 26 day experiment was conducted to determine the effects of feeding and starvation on the survival, morphology, and histology in chum salmon (*Oncorhynchus keta*) fry. We included three experimental groups: starved, fed, and initial. The survival and growth rates were lower in the starved group than in the fed group ($P < 0.05$). In the starved group, survival began to decline after 16 days, and all fish had died after 26 days. We determined the effects of starvation on the morphometric parameters using the truss and classical dimensions. The dimensions in the head region were larger in the starved group than in the initial and fed groups. In contrast, the truss dimensions of the fed group were larger than those of the initial and starved groups. Starvation reduced the heights of the hepatocyte nuclei and of the intestinal epithelium ($P < 0.05$). The starved group also showed atrophy of the digestive structures and shrinkage of the foregut and midgut. Starvation led to the degeneration and atrophy of the exocrine pancreas, in which the lumen was markedly diminished and the folds of mucosa were less apparent. The hepatocyte morphology in the starved group was abnormal compared with that of the initial and fed groups, with highly compact, irregularly shrunken nuclei. Melanomacrophages were randomly distributed in the kidneys of the starved group, and their abundance increased rapidly during the experiment. In contrast, neither the initial nor fed group had any melanomacrophages. These results suggest that the nutritional parameters used in this study are useful indices of nutritional status in chum salmon.

Key words : chum salmon (*Oncorhynchus keta*), histological change, morphometric characteristics, starvation

1. Introduction

Fish starvation studies are significant in determining the fish's nutritional and growth characteristics (Love 1980). Many fish species undergo natural periods of starvation during the year, and have consequently evolved the capacity to withstand prolonged food shortages. Such periods may extend from weeks, months, to even years, and may cause extensive loss of energy stores in the body of the fish consumes its own tissues to remain alive (Weatherley and Gill 1987). Larsson and Lewander (1973) observed many fish that suffered from states of starvation but had evolved the ability to tolerate food shortages) Because fish use their tissues as an energy source, survival can entail the excessive loss of energy stores within the body (Weatherley and Gill 1987). Fish starvation studies can contribute to our understanding of the nutritional conditions of natural and cultured fish in relation to their growth (Weatherley and Gill 1987; Park et al. 1998; Park et al. 2001).

Chum salmon (*Oncorhynchus keta*), also known as the 'dog salmon' and the 'calico salmon', is the most widely

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distributed of all Pacific salmon and the most abundant in terms of its total biomass. The chum salmon is a rather large fish, generally growing to a maximum length of 110 cm and a maximum weight of 21 kg (Robert 2002). This species is a staple resource of fisheries, particularly in the USA, Japan, and Canada (Robert 2002). The chum salmon is distributed widely across the North Pacific Ocean and in the Bering Sea, with a high degree of overlap in their distributions between North American and Asian stocks. Importantly, it is a principal catch of major commercial fisheries in the North Pacific and adjacent seas, together with the pink salmon (*O. gorbuscha*) and sockeye salmon (*O. nerka*), which collectively comprise approximately 90% of the annual commercial catches of Pacific salmon by Canada, Japan, the United States, and Russia (Beamish and Bouillon 1993).

The survival rate during the early life stage of the salmonid is known to be highly dependent upon internal factors, which originate from the maternal line, and external environmental factors (Hunter 1981). The point at which the fry absorb the yolk provided by the mother has been shown to particularly affect their external morphometric changes, and also marks a critical period in the fish's capacity to adjust to its external environment (Myoung et al. 1997). Because the ability of the fry to feed under natural conditions is important to its ultimate survival, the initial nutritional supply of the fry is not as relevant (Myoung et al. 1997).

During starvation, the essential processes in the fish are maintained at the expense of accumulated energy reserves, resulting in progressive depletion and wastage (degrowth) of body tissues (Weatherley and Gill 1987). The observed incidence of starvation is essentially the same when it was based on either morphological or histological criteria (Theilacker 1986). The data for the hepatocyte nuclear height in relation to the starvation in fish are generally reported by other researchers. It appears that the onset of irreversible starvation is preceded by structural changes in the hepatocyte nuclear height (Park 2006). Storch et al. (1983) concluded that this parameter is particularly useful as an indication of the nutritional status in fish. Within the fish body, the response to starvation is observed in the digestive organs (Ehrlich et al. 1976). A reduction in the intestinal epithelial cell height and connective tissues has been observed in carp (*Cyprinus carpio*) and pike (*Esox lucius*) larvae (Kostomarova 1962). Teleosts contain abundant kidney melanin. Melanomacrophages (MMs), which are similar to human macrophages, metabolize toxic substances and perform various immune functions in the kidney (Agius 1979), and MMs increase in number in response to various pathological and physiological conditions, such as starvation and vitamin E deficiency (Park 2006). The histology of the pancreatic structures during starvation has generally been considered a good short-term indication of the nutritional condition of larvae (Theilacker 1986). Pancreatic degeneration occurs in the larvae of the Common dentex (*Dentex dentex*) under poor nutritional conditions (Crespo et al. 2001). The purpose of this study was to develop a method, based on morphometric and histological observations, that can be easily applied in the aquaculture industry to diagnose the health status of chum salmon fry.

2. Materials and Methods

Eggs of the chum salmon (*Oncorhynchus keta*) were obtained from the Yeongdong Inland Fisheries Research Institute, National Fisheries Research and Development Institute, Korea, and were fertilized on November 29, 2009, at the Fishery Genetics and Breeding Science Laboratory of the Korea Maritime University in Busan, Korea. The fish were hatched and maintained prior to the experiment at a temperature of 11.5 ± 0.5^{o}C. Hatching was completed on December 11, 2009, and complete yolk absorption (according to Park et al. 1998) was achieved on January 14, 2010. The fry were stored in a rectangular glass tanks (W 109 cm × L 69 cm × H 47 cm), each of which contained 300 fish, with the fed group and starved group housed separately and with three replicate samples of each group.

Filtered and UV-treated water was supplied to each tank at a flow rate of 4 l min^{-1} in a flow-through running-water system, with a water renewal rate once every hour, as per the method of Park and Johnson (2002). The duration of the starvation experiment was selected on the basis of previous studies of the effects of starvation on the fish. The level of dissolved oxygen in the breeding tanks during the starvation experiment were over 9.5 ml l^{-1}, the ammonia level was less than 0.001 ppm, the nitrous acid concentration was 0.08-0.1 ppm, the nitric acid concentration was 2-5 ppm, the pH was 7.3-8.3, and the water temperature was 11.5 ± 0.5^{o}C. The fed group was supplied with sufficient amounts of food (E-Wha Oil & Fat Ind. Co., Republic of Korea; 52% crude protein, 10% crude fat, 3% crude fiber, 16% ash, 1.5% calcium, and 2.7% phosphorous) three times a day.

The survival rate during the starvation experiment was calculated retroactively, with the dead fry counted every

day. The aggregate survival rates of the fed group and starved group during the experimental period were determined for each of the three replicate groups. When the experiment began on January 14, 2010, 20 chum salmon fry were assigned to the initial group, and 20 fry each were assigned to the fed group and starved group on February 9, 2010. The starved group was not fed until the end of the starvation experiment. The fed group was given food continuously. The initial group had absorbed nearly all the yolk and had started to feed. All the samples were replicated three times. The fish were euthanized with an overdose of lidocaine-HCl (300 ppm hydrochloric lidocaine/ 1,000 ppm $NaHCO_3$) at 11.5°C, according to Park et al. (1988).

In this study, we assessed the differences in the external and internal traits of starved and fed fish at the end of the starvation period, using both the truss and classical dimensions and the histological changes in hepatocytes, intestinal epithelial cells, and pancreatic structures. We also measured the accumulation of MMs in the kidney to determine the nutritional status of the fish.

Both the truss dimensions and classical dimensions are used to describe the external morphology of fish (Strauss and Bookstein 1982). Digital Vernier calipers (CD-20CP; Mitutoyo, Japan) were used for all length measurements, in units of 0.1 mm, and an electric balance (JW-1; Acom, Korea) was used for all mass measurements, in units of 0.01 g, for the fry. The external morphometric traits, shown in Table 1 and Fig. 1, were measured in terms of truss dimensions and classical dimensions. All fry within the initial, fed, and starved groups were photographed for the analysis of their external traits using a digital camera (Coolpix 4500; Nikon, Japan). Each of the morphometric trait measurements obtained for the chum salmon fry was arc sin square root transformed about the portion of the fork, after which the relative ratios were determined.

A histological method was then used to determine the effects of feeding and starvation on the hepatocytes, intestinal epithelium, and kidneys, including MMs over a period of 26 days. First, the liver and intestinal epithelium were removed and the tissue samples were fixed in 10% neutral formalin solution (100 ml formalin, 6.5 g $Na_2HPO_4 \cdot 12H_2O$, 4.5 g KH_2PO_4, 900 ml distilled water) for 24 h. The mean hepatocyte nuclear areas and the heights of the intestinal epithelial cells were calculated using the NIH Image (ver. 1.57) system. To investigate the accumulation of kidney MMs, the kidneys were removed and tissue samples were fixed in 10% neutral formalin solution for 24 h. To evaluate the nutritional condition of the fry), the pancreatic tissues were also fixed in 10% neutral formalin solution for 24 h. Samples were prepared as paraffin sections (6 μm thick), stained with hematoxylin and eosin Y-phroxine B, and observed under a microscope (Axioskop; Zeiss, Germany). The asymmetric nuclei of the interstitial cells were measured along their long axes (a) and short axes (b), and examined with an eyepiece micrometer under a microscope at a ×400 magnification. The surface area was measured using the previously demonstrated method of Park (2006), in which $S = ab\pi/4$.

Table 1. Dimensions of body shape used in this study

Dimension	
Truss dimension	
Posterior end of supraoccipital×origin of dorsal fin	2×3
Posterior end of supraoccipital×origin of pelvic fin	2×11
Posterior end of supraoccipital×origin of pectoral fin	2×12
Posterior end of supraoccipital×posterior end of maxillary	2×13
Origin of dorsal fin×origin of adipose fin	3×5
Origin of dorsal fin×origin of anal fin	3×10
Origin of dorsal fin×origin of pelvic fin	3×11
Origin of dorsal fin×origin of pectoral fin	3×12
Origin of adipose fin×dorsal origin of caudal fin	5×6
Origin of adipose fin×ventral origin of caudal fin	5×8
Origin of adipose fin×insertion of anal fin	5×9
Origin of adipose fin×origin of anal fin	5×10
Origin of adipose fin×origin of pelvic fin	5×11
Dorsal origin of caudal fin×ventral origin of caudal fin	6×8
Dorsal origin of caudal fin×insertion of anal fin	6×9
Insertion of anal fin×origin of anal fin	9×10
Origin of anal fin×origin of pelvic fin	10×11
Origin of pelvic fin×origin of pectoral fin	11×12
Classical dimension	
Most anterior extension of the head×posterior end of supraoccipital	1×2
Most anterior extension of the head×origin of dorsal fin	1×3
Most anterior extension of the head×origin of adipose fin	1×5
Most anterior extension of the head×dorsal origin of Most caudal fin	1×6
Most anterior extension of the head×origin of anal fin	1×10
Most anterior extension of the head×origin of pelvic fin	1×11
Most anterior extension of the head×origin of pectoral fin	1×12
Most anterior extension of the head×posterior end of maxillary	1×13
Most anterior extension of the head×most posterior aspect of operculum	1×14
Insertion of dorsal fin×most posterior scale in lateral line	4×7
Most posterior scale in lateral line×insertion of anal fin	7×9

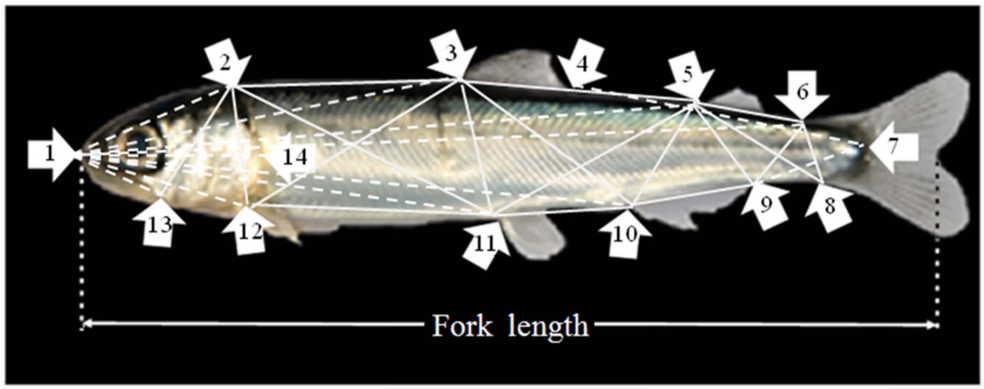

Fig. 1. Truss and classical dimensions measured in the starvation experiment with the chum salmon, *Oncorhynchus keta*. Morphological landmarks are numbered and morphometric distances between the landmarks are shown. 1: Most-anterior extension of the head; 2: posterior end of the supraoccipital; 3: origin of the dorsal fin; 4: insertion of the dorsal fin; 5: origin of the adipose fin; 6: dorsal origin of the caudal fin; 7: most-posterior scale in the lateral line; 8: ventral origin of the caudal fin; 9: insertion of the anal fin; 10: origin of the anal fin; 11: origin of the pelvic fin; 12: origin of the pectoral fin; 13: posterior end of the maxillary; 14: most-posterior aspect of the operculum. ——, truss dimension; – – –, classical dimension

ANOVA and Duncan's multiple range test were used for the analysis in the SPSS statistical package (SPSS 9.0, SPSS Inc., Chicago, IL) to determine whether the values for each set of experimental data were significantly different.

3. Results

After 26 days, the starved group of chum salmon (*Oncorhynchus keta*) fry had rapidly lost vitality, so the experiment was terminated. At the beginning of the starvation experiment, the average fork length was 3.07 ± 0.13 cm and the average weight was 0.30 ± 0.01 g in the initial group. At the end of the experiment, the fry of the fed group measured 5.28 ± 0.33 cm and weighed 1.32 ± 0.22 g, and the fry of the starved group measured 3.34 ± 0.13 cm and weighed 0.29 ± 0.04 g. The average fork length and average weight of the fed group were greater than those of the starved group and the initial group ($P < 0.05$), and the parameters of the starved group were significantly different from those of the initial group.

The survival rates of the fed and starved groups during the 26 days of the experimental period are shown in Fig. 2. In the fed group, the fry were all alive at the beginning of the experiment, but some died during the later stages, so the overall survival rate at the end of the experiment was 98 ± 1.5%. The survival rate in the starved group over the first 14 days was 98 ± 2.3%, but 16 days later, the survival rate had declined significantly to 84 ± 5.9% ($P < 0.05$). Twenty days into the starvation experiment,

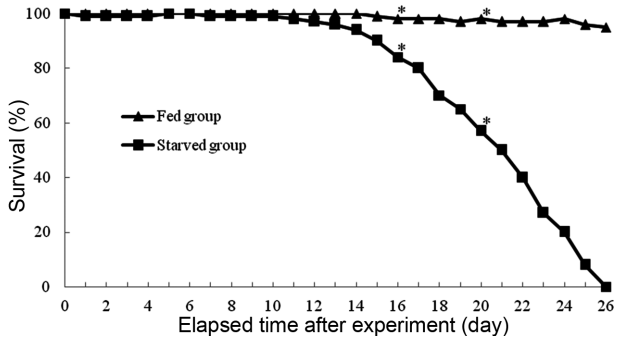

Fig. 2. Survival of fed and starved larval chum salmon, *Oncorhynchus keta*. The asterisks indicates a significant ($P < 0.05$) difference between the fed and starved groups

the survival rate in the starved group was 57 ± 7.4% ($P < 0.05$) and after 26 days, all fry had died (Fig. 2).

The effects of starvation on the truss dimensions and classical dimensions during the 26 days of the experiment are shown in Tables 2 and 3, respectively. During the period of the starvation experiment, several truss dimensions (2 × 3, 5 × 6, 5 × 8, 5 × 9, 6 × 8, 6 × 9, and 9 × 10) and two classical dimensions (1 × 3 and 4 × 7) did not differ between the fed group, initial group, and starved group ($P > 0.05$). In contrast, although the truss dimension (2 × 11) was the same in the initial and fed groups, it was larger in the fed group than in the starved group ($P < 0.05$). The classical dimension (1 × 13) was the same in the initial and starved groups, but was greater in the fed group ($P < 0.05$). The following truss dimensions were

Table 2. Truss dimensions of the chum salmon, *Oncorhynchus keta*, in the initial, fed, and starved groups treated for 26 days

Dimension	Initial group*	Fed group*	Starved group*
2 × 3	25.60 ± 2.396[a]	27.50 ± 1.695[a]	25.53 ± 2.847[a]
2 × 11	35.46 ± 1.550[b]	35.73 ± 1.184[b]	33.67 ± 1.829[a]
2 × 12	12.50 ± 1.119[a]	14.18 ± 0.798[b]	11.66 ± 1.270[a]
2 × 13	15.90 ± 1.487[a]	16.37 ± 1.173[a]	18.83 ± 1.691[b]
3 × 5	26.20 ± 0.965[a]	29.76 ± 1.930[c]	28.02 ± 1.926[b]
3 × 10	23.33 ± 2.565[a]	26.05 ± 1.506[b]	23.66 ± 1.905[a]
3 × 11	12.88 ± 1.621[a]	16.97 ± 1.189[b]	12.94 ± 1.347[a]
3 × 12	24.97 ± 1.529[a]	28.45 ± 1.857[b]	23.81 ± 1.819[a]
5 × 6	11.82 ± 1.425[a]	12.91 ± 1.047[a]	12.09 ± 0.881[a]
5 × 8	13.80 ± 1.188[a]	15.13 ± 1.335[a]	14.25 ± 1.970[a]
5 × 9	9.19 ± 1.413[a]	10.32 ± 1.789[a]	9.62 ± 1.582[a]
5 × 10	14.42 ± 1.104[a]	15.80 ± 0.703[b]	12.89 ± 0.805[c]
5 × 11	25.26 ± 0.955[a]	29.37 ± 1.955[b]	24.03 ± 2.068[a]
6 × 8	6.47 ± 0.433[a]	6.46 ± 0.378[a]	6.12 ± 0.611[a]
6 × 9	12.11 ± 2.743[a]	12.07 ± 1.662[a]	12.17 ± 1.502[a]
9 × 10	13.64 ± 0.938[a]	14.13 ± 1.465[a]	14.11 ± 1.395[a]
10 × 11	13.28 ± 1.113[a]	16.10 ± 1.312[b]	13.34 ± 2.386[a]
11 × 12	25.05 ± 2.354[a]	28.69 ± 1.842[b]	25.15 ± 2.650[a]

*The values are means ± SD (n = 20) of triplicate groups. Means in rows with the same superscript letter are not significantly different ($P > 0.05$)

Table 3. Classical dimensions of the chum salmon, *Oncorhynchus keta*, in the initial, fed, and starved groups treated for 26 days

Dimension	Initial group*	Fed group*	Starved group*
1 × 2	20.36 ± 1.856[a]	20.08 ± 2.633[a]	22.75 ± 1.883[b]
1 × 3	45.45 ± 1.568[a]	46.33 ± 1.287[a]	45.63 ± 2.592[a]
1 × 5	69.91 ± 2.340[a]	74.50 ± 1.991[b]	74.07 ± 6.211[b]
1 × 6	83.90 ± 3.196[a]	89.07 ± 1.926[b]	87.99 ± 2.821[b]
1 × 10	52.17 ± 2.992[a]	53.91 ± 2.756[ab]	56.64 ± 3.757[b]
1 × 11	49.17 ± 2.666[a]	51.08 ± 2.479[ab]	52.54 ± 2.898[b]
1 × 12	23.65 ± 1.977[a]	22.16 ± 1.838[a]	26.02 ± 1.822[b]
1 × 13	11.23 ± 1.414[b]	8.81 ± 1.629[a]	10.76 ± 1.370[b]
1 × 14	23.11 ± 1.607[ab]	22.01 ± 1.446[a]	24.52 ± 1.566[b]
4 × 7	36.51 ± 1.010[a]	38.72 ± 2.118[a]	36.41 ± 4.702[a]
7 × 9	16.48 ± 3.021[a]	15.86 ± 1.113[a]	19.61 ± 2.918[b]

*The values are means ± SD (n = 20) of triplicate groups. Means in rows with the same superscript letter are not significantly different ($P > 0.05$)

identical between the initial and starved groups: 2 × 12, 3 × 10, 3 × 11, 3 × 12, 5 × 11, 10 × 11, and 11 × 12 ($P < 0.05$). The trait showing the procedure in turn- in the fed group, initial group, and starved group was the truss

Fig. 3. Typical external morphology of the chum salmon, *Oncorhynchus keta*: (a) initial, (b) starved, and (c) fed for 26 days

dimension 5 × 10 ($P < 0.05$). The trait showing the procedure in turn- in the starved group, initial group, and fed group was classical dimension 1 × 14, and other traits showing procedure in turn- in the starved group, fed group, initial group classical dimensions were 1 × 10 and 1 × 11 ($P < 0.05$).

Fig. 3 shows the external shape of the fry after 26 days of the experimental period, and demonstrates that the morphological trait that remained unchanged despite starvation being the truss dimension at the posterior end of the adipose fin. The starved group was larger than the initial and fed groups in terms of the truss dimensions and classical dimensions of the head (Fig. 3b). However, the fed group was larger than the initial and starved groups in terms of the truss dimensions of the body (Fig. 3c).

The effects of starvation for 26 days on the nuclear heights of the hepatocytes and intestinal epithelial cells are shown in Table 4 and Fig. 4. The hepatocyte nuclear area in the initial group (122.6 ± 34.33 μm^2) and the fed group (128.7 ± 29.05 μm^2) were very similar ($P > 0.05$). However, the average hepatocyte nuclear height in the starved group was 56.9 ± 10.82 μm^2, which was significantly less than those in the other groups ($P < 0.05$; Table 4, Fig. 4a-c). The nuclear height of the intestinal epithelium in the initial group was 19.8 ± 2.03 μm^2, whereas in the starved group, it was 15.3 ± 2.69 μm^2. The nuclear height of the intestinal epithelium in the fed group was 33.1 ± 2.67 μm^2, which was significantly greater than that in the initial group (Table 4, Fig. 4d-f). The hepatocyte nuclear histologies of the fed, initial, and starved groups after the 26 days of the experiment are shown in Fig. 4a-c, respectively. In the starved group, the hepatocyte nuclear height was greatly reduced as compared with that in the fed group or the initial group, and the distribution density

Table 4. Changes in hepatocyte nuclear areas and nuclear heights of the midgut epithelium in the initial, fed, and starved groups of chum salmon, *Oncorhynchus keta*, treated for 26 days

	Initial group*	Fed group*	Starved group*
Hepatocyte nuclear area (μm^2)	122.6 ± 34.33^b	128.7 ± 29.05^b	56.9 ± 10.82^a
Nuclear height of midgut epithelium (μm)	19.8 ± 2.03^b	33.1 ± 2.67^c	15.3 ± 2.69^a

*The values are means ± SD (n = 20) of triplicate groups. Means in columns with the same superscript letter are not significantly different (P > 0.05)

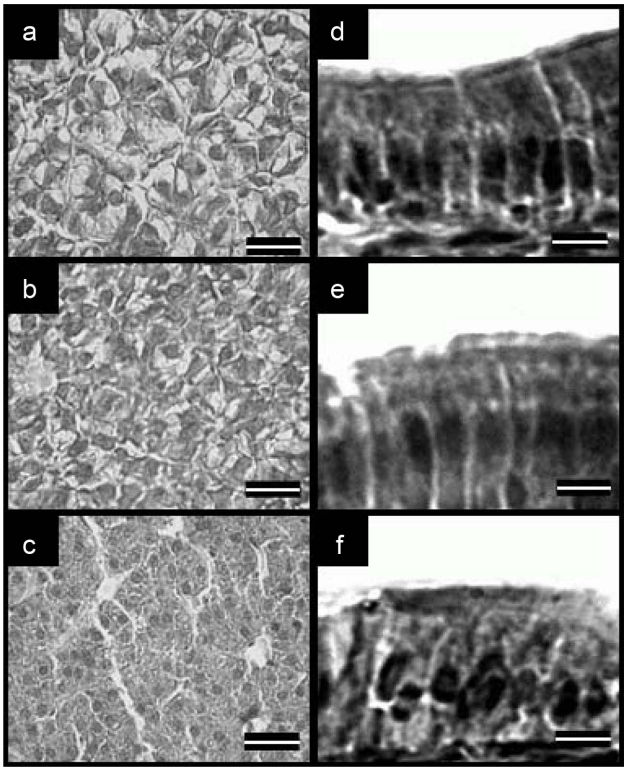

Fig. 4. Histological appearance of the liver and midgut epithelium under starved after the 26 days of the experiment. The livers of (a) the fed group, (b) the initial group, and (c) the starved group. Note the reduction in the size of the hepatocyte nuclei in the starved group. Midgut epithelium of (d) the fed group, (e) the initial group, and (f) the starved group. Note the reduction in the nuclear height of the midgut epithelium in the starved group. a-c: bars = 400 μm; d-f: bars = 10 μm

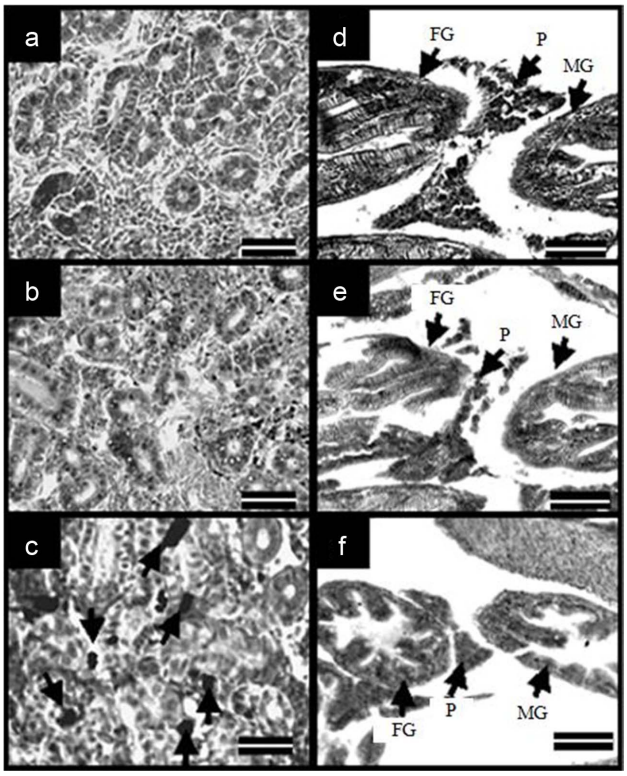

Fig. 5. Histological appearance of melanomacrophages (MMs) in the kidney after the 26 days of the starvation experiment in the chum salmon, *Oncorhynchus keta*. Kidneys of (a) the fed group, (b) the initial group, and (c) the starved group. The panels are shown at ×400 magnification, and the arrows indicate the exact increase in the degree of MM deposition during starvation. Histology of intestinal epithelium cells in relation to the nutritional condition of (d) the fed group, (e) the initial group, and (f) the starved group. Note the atrophy of the exocrine pancreas; the gut lumen is markedly diminished and the folds of the mucosa are less apparent than in the other groups. a-c: bars = 600 μm; d-f: bars = 1,000 μm. FG: foregut; MG: midgut; P: pancreas

of the hepatocyte nuclei was greater in the starved group than in the fed and initial groups. The hepatocyte nuclei of the starved group were irregularly shaped (Fig. 4c). Conversely, a histological analysis showed that the nuclear shapes of the fed group were similar to those of the initial group, and that the hepatocyte nuclei did not overlap (Fig. 4a). The histologies of the nuclei in the intestinal epithelia of the fed, initial, and starved groups after the experiment are shown in Fig. 4d-f, respectively.

The average nuclear height in the intestinal epithelium of the starved group was small, the distribution density was high, and the shapes of the nuclei were irregular compared with those of the initial group (Fig. 4f). Conversely, the

nuclear height in the intestinal epithelium of the fed group was distinctly greater than in those of the starved and initial groups (Fig. 4d).

The histologies of the kidneys of the fed, initial, and starved groups after 26 days of the experiment are shown in Fig. 5a-c, respectively. The fed and initial groups showed normal traits (Fig. 5a-b, respectively), whereas the starved group had dark brown MMs with variously rounded shapes that were distributed throughout the kidney (Fig. 5c). The histologies of the digestive structures (foregut, midgut, and pancreas) in the fed, initial, and starved groups after the experiment are shown in Fig. 5d-f, respectively. The foreguts, midguts, and pancreases of the fed group and the initial group showed the development of intestinal epithelial cells with similar histological shapes (Fig. 5d-e, respectively), whereas in the starved group, atrophy of the digestive organs was observed, and the distinct internal shapes of the intestinal lumen and a wrinkling in the layer of the gut had not developed (Fig. 5f). The pancreas had also degenerated and begun to atrophy (Fig. 5f).

4. Discussion

This experiment demonstrated a decline in growth in response to starvation, and at the end of the starvation experiment, the fed group showed an increase in fork length of 1.58-1.72-fold and an increase in weight of 4.40-4.55-fold relative to those of the starved and initial groups. In a 12-week starvation experiment with the olive flounder (*Paralichthys olivaceus*), starvation was measured in terms of the degree of fatness of the fish body, the rate of growth per day, the growth rate expressed in weight, and the decline in growth. Only the fed group showed growth (Hur et al. 2006a). From the studies of starvation in chum salmon (*Oncorhynchus keta*) and olive flounder, it is clear that the morphometric traits of the fed groups increased significantly relative to those of the starved groups, even when the periods of starvation and the species observed differed. The truss dimensions, in particular, are the primary units of classification of the external anatomical markers normally assessed in fish (Strauss and Bond 1990). The truss dimensions are length measurements that refer to the ratio between the width of the fish's body and the axis of the fish's length. Theoretically, the truss dimensions are a better measurement of the fish's shape than the classical dimensions (Strauss and Bookstein 1982; Currens et al. 1989). When we examined the truss dimensions of the external morphometric traits of the chum salmon fry under starvation conditions, the head, body, and abdomen regions were significantly increased in the fed and starved groups as compared with those of the initial group during the 26 days of the experiment. In contrast, the truss dimension from the point of the caudal fin and on the underside of the latter half of the adipose fin, did not change in either the fed group or the starved group during the experiment. Similarly, the Chinese minnow (*Rhynchocypris oxycephalus*) showed significant increases in the head, body, and abdominal regions in terms of the truss dimensions in both the fed group and starved group after a starvation experiment of 75 days (Park 2004). However, both the fed and starved groups showed reductions in the caudal region.

The truss dimensions were used to evaluate the morphometric traits of starved and fed Chinese minnows under starvation conditions. Based on that study, a determination can be made as to whether or not food should be supplied to the Chinese minnow under different habitat conditions (Park et al. 2001). When different levels of food were given to both the rainbow trout (*O. mykiss*) and chinook salmon (*O. tshawytscha*), the truss dimensions of the head region were generally greater in the fed groups than in the starved groups, whereas the truss dimensions of the body region were smaller in the fed groups than in the starved groups (Currens et al. 1989). Therefore, the differences in the truss dimensions between species should be considered in order to reflect the characteristic differences among those species. In this experiment, when we examined the classical dimensions of the chum salmon fry after 26 days, the head region was reduced in the fed group and increased in the starved group, whereas the dimensions of the body region were increased in both the fed and starved groups. The abdominal region had also increased in the fed and starved groups. However, in contrast to the truss dimensions, the classical dimensions indicated that the caudal region had increased in the starved group but had decreased in the fed group. In this study, classical dimensions of the chum salmon fry in the fed group and starved group differed significantly.

In this experiment, when we examined the external shapes of the initial, starved, and fed groups after the 26 days of the experiment, the head region of the starved group was larger than those of the initial and fed groups, whereas the body region of the fed group was larger than those of the initial and starved groups. In contrast, in a 12-week starvation experiment with the olive flounder, the external shapes of the starved and fed groups were determined by the increase in the width of the fish, according to truss dimensions, and the increase in the

length of the posterior region of the lateral body (Park et al. 2007).

When fish are given sufficient food, the shape and height of the hepatocyte nuclei are regular. However, if food is not abundant, the nonchromosomal protein content of the hepatocyte nuclei changes, and the shape and size of the nuclei are altered (Storch et al. 1983). In this study, histological changes were observed in the livers of the chum salmon larvae under starvation conditions. In the fed group, the hepatocyte nuclei were sparsely distributed and did not overlap, and their average size was greater than in the starved group. In the fed and initial groups, each hepatocyte nucleus was regularly shaped and dark colored. In contrast, within the starved group, the average size of the hepatocyte nuclei was reduced, their distribution density was high, and their shapes were irregular compared with those of the fed and initial groups. The immature rainbow trout showed similar regressive changes in the liver tissue after starvation for three months at a water temperature of 13°C (Robertson et al. 1963). It has also been reported that if larvae do not have sufficient nourishment, they will die of liver degeneration (Theilacker 1978). The mean height of the hepatocyte nuclei in the initial group was similar to that in the fed group, whereas that in the starved group was significantly reduced in this experiment.

A starvation experiment with the olive flounder produced a similar result: the average height of the nuclei in intestinal epithelium under starvation conditions, between weeks 1 and 12, was less than that of the fed group during the same period (Park 2006). The digestive organs of the jack mackerel (*Trachurus symmetricus*), including the intestinal epithelium, were the first affected by starvation (Theilacker 1978). In our starvation experiment with chum salmon, we confirmed the external modification of the digestive organs that contain intestinal epithelium. The fed group showed a distinctly greater growth in terms of the nuclear height of the intestinal epithelium as compared to the starved group. Twelve weeks of starvation had also produced a reduction in the nuclear height of the intestinal epithelium in the olive flounder (Park 2006). These findings suggest that the intestinal epithelium is associated with the storage of energy within the fish, because it responds directly to starvation (Kostomarova 1962; Ehrlich et al. 1976). This study provides a good standard measure with which to confirm the nutrient status of fry over short periods (Theilacker 1986). The study assessed the histological changes in chum salmon, and demonstrated the normal development of the foregut, midgut, and pancreas in fed and initial groups. The foregut and midgut of the starved fish shrank, and the pleating of the mucus layers did not develop. The pancreas also withered in the starved fish, reflecting degeneration. When Crespo et al. (2001) investigated starvation in the common dentex (*Dentex dentex*) for 36 days, the organic structures of the fry appeared to shrink and the pancreas showed degeneration. These histological changes caused by the removal of food are very similar to those observed in our starvation experiment.

After the 26 days of our experiment, we observed normal histological shapes in the kidneys of the fed and initial groups of chum salmon fry, whereas dark brown MMs were scattered throughout the kidneys of the starved group. Similarly, the deposition of MMs increased with starvation in the olive flounder, suggesting that starvation presents a significant physiological burden to fish (Hur et al. 2006b). Increased MM deposition with starvation has also been observed in various organs of different fish species, including the dogfish (*Scyliorhinus canicula*), rainbow trout, plaice (*Pleuronectes platessa*), and tilapia (*Tilapia zillii*) (Hur et al. 2006b). Melanomacrophages were also confirmed in the kidneys of masou salmon (*O. masou*) fry after starvation for 60 days (Mizuno et al. 2002). Agius and Roberts (1981) reported that the MMs proliferate because the tissues of the kidney and spleen are seriously damaged. MMs may also increase rapidly because starvation increases the fish metabolism (Mizuno et al. 2002).

Our results provide information about the morphometric and histological changes associated with the provision of nutrients to larvae during the mass production of salmon. Our results suggest that the nutritional parameters examined in this study are useful indices of nutritional status in the chum salmon. We surmise that the data derived from this study of chum salmon fry under starvation conditions can be used as a basic guide to assist with the regulation and scheduling of feeding and as indices of the nutritional status of chum salmon fry.

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