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**Preliminary Observations on Ontogenetic Development  
of the Alloimmune Reactivity in Chum  
Salmon, *Oncorhynchus keta***

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**Abstract**

A preliminary study of alloimmune reactivity in the early stages of chum salmon was conducted. Hosts received skin grafts at 5, 14, 21 and 28 days after hatching from donors one month in age. Survival of allografts declined proportionally with age of salmon grafted, from nine out of ten at 5 days to two out of 20 at 28 days after hatching. The results obtained showed that alloimmune reactivity occurs around 14 days after hatching. In histogenesis of the lymphoid tissues, the number of basophilic cells in the kidney increased dramatically from 5 days before hatching to 14 days after hatching. This suggests that the kidney plays an important role in the rejection of allografts.

The chum salmon is commercially important in northern Japan. Returning stocks of chum salmon are maintained mainly by artificial propagation. Juveniles are intensively reared until released to natural rivers. There are sometimes serious problems with diseases in the rearing after hatching.

Many workers have studied vaccination and immunological effects of infectious diseases in salmon aquaculture. In general, exposure of an animal to antigen in fetal of early post-natal life, during which the immune system is not fully developed, results in immunological tolerance (Roitt, 1980). It is supposed that this tolerance occurs in vaccinated fry of chum salmon and therefore vaccination is not effective in preventing diseases.

At present, there is insufficient information on the development of the immune system during the early life stages of Pacific salmon (genus *Oncorhynchus*), although several works have been published on rainbow trout and carp (Manning et al., 1982). In the present study, a preliminary study of alloimmune reactivity in the early life stages of chum salmon was conducted.

**Materials and Methods**

Chum salmon eggs of one brood were fertilized at Oshima Branch of Hokkaido Salmon Hatchery, Yakumo, on November 19, 1984 and maintained at the aquaria of the Faculty of Fisheries, Hokkaido University in Hakodate. The water tempera-

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ture was about 8°C. They hatched on January 11, 1985. The hatched fry were fed daily on commercial dry pellet food after the onset of feeding. Donor fish were aged one month after hatching.

Methods were conducted according to Tatner and Manning (1983). In brief, two fish were used as donors of allografts. After anesthetizing in ice water with MS-222, they were placed on wet gauze under a dissection microscope. A piece of the skin was removed with a sharp knife, without muscle, from the lateral body.

The skin was approximately cut into 0.5 mm<sup>2</sup> section on the gauze and immersed in 0.85% saline solution for about 10 minutes until use.

With a sharp knife, 1 mm<sup>2</sup> of skin of the recipient fish was removed from the trunk dorsal region below the dorsal fin, then a slit was made in this region. These particular points of dissection were selected in order not to damage visceral organs and to permit us to distinguish rejection of grafts easily by the disappearance of pigments.

The skin grafts were inserted into the slit of the recipient fish so as to lie partially under the muscle. No suture was necessary. After the operation each fish was maintained separately in a patrone case (3×5 cm) with water to avoid loss of the grafts by mechanical interference.

The next day after the operation, fish that had lost skin grafts were removed and not used in the experiment. This was done because it was difficult to distinguish whether the loss was caused by the mechanical interference or by immunological rejection.

The water of each patrone case was exchanged every other day.

In the same manner as the allografts, a 1 mm<sup>2</sup> piece of skin was removed with a sharp knife from the trunk and dorsal region below the dorsal fin of the recipient fish and immersed in 0.85% saline solution. The skin was removed from a small area on the opposite side of the trunk region and an autograft was transplanted to the site. The fish that were operated on were maintained in a patrone case as noted above.

Host fry received skin grafts at 5, 14, 21 and 28 days after hatching from donors one month in age. Following the transplantation, host fry were sacrificed.

## Results and Discussion

When graft pigments were clearly evident and the graft looked as if it were a part of the host tissue, the survival of the graft was assured (Fig. 1). Graft rejection was revealed when the pigment was absent in the graft, except in the center where only a dark colored tissue was present (Fig. 1).

All autografts survived without any effect of age (Table 1).

Allografts survival declined in proportion with the age of the fry onto which it was grafted, from nine out of ten at 5 days to two out of 20 at 28 days after hatching (Table 1). Ten % of the allografts were rejected at 5 days, and 60% were rejected at 14 days after hatching. These results suggest that alloimmune reactivity occurs around 14 days after hatching in chum salmon.

This functional change is consistent with histological changes in lymphoid tissues, that is, the number of basophilic cells in the kidney increased dramatically from 5 days before hatching to 14 days after hatching. Therefore, we consider that

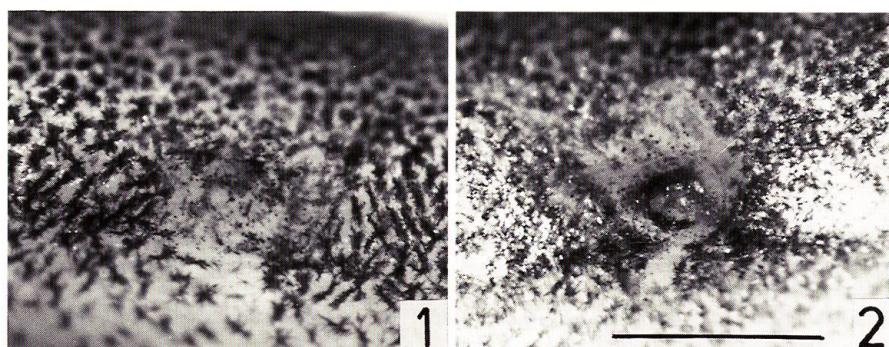


Fig. 1. Photographs showing graft survivals (1) and rejections (2). Bar indicates 1 mm.

Table 1. Number of graft survivals and rejections at 30 days after grafting.

Age in days after hatching	Mean of T.L. (mm)	Grafts	No. of fry grafted	No. of graft survivals	No. of graft rejections	% of rejection
5	19.9	Allo-	10	9	1	10
		Auto-	5	5	0	0
14	22.3	Allo-	20	8	12	60
		Auto-	10	10	0	0
21	23.7	Allo-	20	3	17	85
		Auto-	10	10	0	0
28	25.5	Allo-	20	2	18	90
		Auto-	10	10	0	0

the kidney plays an important role in the rejection of allografts, as suggested by Tatner and Manning (1983) who reported that the organs involved in the rejection of allografts in rainbow trout were the thymus and the kidney.

In comparison with rainbow trout, which completely rejected all allografts within 14 days after hatching (Tatner and Manning, 1983), the rejection age in chum salmon was older than 14 days. This may be due to environmental effects such as water temperature, the rearing water temperature was 14°C for rainbow trout and 8°C for chum salmon.

Whether or not allografts survival on chum salmon 5 days after hatching were immunologically tolerant, will require further study. Ninety % of the allografts were rejected at 28 days after hatching. This suggests that the development of alloimmune reactivity in chum salmon would be complete around one month after hatching. However, further study will be needed to determine the maturation stage of alloimmune reactivity in detail by histologically examining the infiltration of lymphoid cells into grafts and the development of the lymphoid organs or tissues.

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