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絶食はサケ稚魚の群れ構造を変化させる(短報)

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Fasting alters school structure in chum salmon *Oncorhynchus keta* fry (short paper)

Mitsuru TORAO*

Salmon and Freshwater Fisheries Research Institute, Hokkaido Research Organization,
Eniwa, Hokkaido 061-1433, Japan

Chum salmon *Oncorhynchus keta* fry were reared for 5 days at three feeding rates (0%, 1%, and 3% of the total fish weight), and the distances between individuals were compared using video analysis to examine the effects of the nutritional status of on school structure. The inter-individual distance was significantly greater in the 0% feed group, suggesting that a decline in nutritional status affected the school structure of salmon fry.

キーワード：nearest neighbor distance, nutritional condition, *Oncorhynchus keta*, video analysis

The term “herd” refers to the spatial aggregation of individuals that behave in a unified manner to varying degrees (Iwasa *et al.,* 2003). In fish, an aggregation of individuals is often described as a “school” or “shoal” and such behavior may provide advantages with regards to predator avoidance, foraging, learning, mating, and migration (Pitcher and Parrish, 1992；Masuda, 2007, 2010；Sakai, 2017).

Chum salmon (*Oncorhynchus keta*) exhibit schooling behavior as soon as they emerge from the gravel after hatching (Torao *et al*., 2014). These schools of fry can be found in slow moving currents during the day (Hasegawa *et al*., 2011；Urabe, 2015), and actively descend rivers at night. (Kobayashi, 1953；1958；Neave, 1955). In long-river systems, the in-stream mortality of chum salmon fry may be greater (Kasugai *et al*., 2014；Morita *et al*., 2015) because of predation by piscivorous fish (Kubo, 1946；Hikita *et al*., 1959；Fresh and Schroder, 1987；Takami and Nagasawa, 1996). Because fry in long river systems can experience a significant decline in their nutritional status during downstream migration (Mizuno and Misaka, 2010；Shimizu *et al*., 2016), they may become more susceptible to predation (Torao *et al*., 2021).

Several factors influence the schooling structure, and various measurements can be used to quantify the structure of fish school. These metrics include measuring the distance between neighboring individuals (nearest neighbor distance [NND]), the angle of separation between individuals (separation angle), and the separation swimming index (SSI) as indices (Masuda, 2010). Among these, the NND is specified by the characteristics of each fish species, such as plankton-feeding and fish-feeding habits (Masuda *et al*., 2003). In addition, the presence of predators, foraging status (Masuda, 2010), differences between wild and reared fish, and water temperature (Tsukamoto and Uchida, 1990) may affect NND in several fish species. When a predator is present, the NND tends to be smaller (Masuda, 2010), which is considered an anti-predator behavior. However, little is known about the effects of nutritional status on school structures.

To examine the effects of nutritional status on schooling structure, we compared the distance between individuals in schools of chum salmon fry reared at different feeding levels.

MATERIALS AND METHODS

Fish stocks and rearing conditions　A total two experi-

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*Tel: 0123-32-2136. Fax: 0123-34-7233. E-mail: torao-mitsuru@hro.or.jp

ments were conducted in 2018 and 2019 at the rearing facility of the Salmon and Freshwater Fisheries Research Institute (SFFR) of the Hokkaido Research Organization, using 2017 and 2018 year classes chum salmon fry. The 2017 year class were obtained from eggs artificially inseminated at the Chitose River Hatchery of the Nihon-kai Salmon Enhancement Program Association in October 2017. The fertilized eggs were maintained at the Chitose River Hatchery, transported to the SFFR rearing facility in December, and incubated in a vertical incubation system until emergence. On February 2018, 5000 fry were moved from the incubators and placed in a plastic rearing chamber (3.26 m [length] × 0.33 m [width] × 0.33 m [height]) supplied with a constant flow of fresh water. The flow velocity in the tank was less than 1 cm/s. The fry were fed a commercial diet (Alpha Crumble EX Masu C1 and C2 ; Nosan Co., Ltd., Yokohama, Japan) *ad libitum* on six times a day until dietary intake was manipulated to control for nutritional status. The 2018 year class was transported to the SFFR immediately after artificial insemination at the Chitose River Hatchery in September 2018 and incubated in a vertical incubator system until emergence. Emerged fry were reared using the same protocols as those described above for the 2017 year class.

Feeding rate-controlled rearing Chum salmon fry were reared with limited feeding for 5 days in both the 2017 and 2018 classes. Once the salmon fry reached an average fork length of 50 mm, 500 fry were placed in each of the three rearing chambers. The fry in each tank were fed a commercial diet at 0% (feeding rate; FR 0%), 1% (FR 1%), or 3% (FR 3%) of their body weight. Members of the 2017 class were placed in three rearing tanks on April 17, 2018, and feed was restricted for 5 days from April 19 to 23, 2018. Similarly, feed for the 2018 class was limited from March 7 to 11, 2019, after an acclimation period of 3 days. The average water temperatures during these feeding experiments were 8.6 ± 0.3 °C and 8.2 ± 0.1 °C (mean ± SD) for the 2017 and 2018 year classes, respectively.

Video recording of schooling behavior Schooling behavior was recorded on the day after feeding restriction treatment, which was at least 15 hours after the last feeding. A circular polyethylene tank (diameter, 48 cm; experimental tank) was used to record the schooling behavior of the chum salmon fry. The water depth the experimental tank was maintained at 3 cm to restrict the behavior of the fry to two dimensions. The amount of water in the experimental tank was approximately 5.7 L. Water temperature was kept constant within the optimal temperature range for chum salmon fry. The video recordings were conducted in a room with a constant temperature of 10 °C to help maintain a constant water temperature. In addition, iced gel packs were placed under the experimental tank to keep the water temperature at 8–9 °C.

Ten randomly selected fry were moved from the feeding rate-controlled rearing chambers to the experimental tank and allowed to acclimatize to their new environment for 10 min. Fry schooling behaviors were then video-recorded for 5 min using a digital camera (Pentax Optio WG-4 GPS, Ricoh Imaging CO. LTD., Tokyo, Japan) placed above the experimental tank. Three experimental groups with three different feeding rates were recorded in triplicates, each with a different group of test fish. In both years, video recordings were conducted between 9:00 and 10:00. The illuminance above the experimental tank ranged from 114 to 138 lx, which is within the range in which fry can recognize others. All fry used in these trials were anesthetized after each recording, their fork length (FL, mm) and body weight (BW, g) were measured, and the condition factor (CF) was calculated.

Measurement of Neighbor Distance Neighbor distance (ND) was calculated to quantify the schooling structure of chum salmon fry. Still pictures were captured from the recorded video every minute, for 5 images per group and 15 images overall. For each image, all inter-individual (10 individuals) were measured using ImageJ (Schneider *et al*., 2012), and the mean value was used as the ND for that group. The total lengths of the fry were also measured from the images. The ND was expressed as a standardized value obtained by dividing the measured inter-individual distance (mm) by the mean body length of the image.

Statistical Analysis Data on FL, BW, CF and ND among the three feeding treatments were compared using Scheffé's *F* Test.

RESULTS

Differences in body size according to feeding conditions In both the 2018 and 2019 experiments, FL, BW, and CF tended to increase as the feed rate increased (Table 1). In the 2018 experiment, there were significant differences in FL, BW, and CF between the FR0% and FR3% treatments (Scheffé's F Test, $p < 0.05$). In 2019, only CF in the FR0% and FR3% groups differed (Scheffé's F Test, $p < 0.05$).

Differences in ND by feeding conditions The ND was greater in the FR0% treatment in both the 2018 and 2019

Table 1 Fork length, body weight and condition factor (mean ± SD, n=10) of chum salmon fry in the different feeding groups in 2018 and 2019 experiments. Values with different letters within the same column in the same year class are significantly different (Scheffé's F Test, $p < 0.05$).

Date of Experiment	Feeding rate (%)	Mean fork length (mm)	Mean body weight (g)	Mean condition factor
Apr. 24, 2018	Fasted (0%)	49.1 ± 1.75 [a]	0.83 ± 0.10 [a]	7.00 ± 0.43 [a]
	Fed (1%)	52.2 ± 3.24 [ab]	1.08 ± 0.21 [ab]	7.46 ± 0.38 [ab]
	Fed (3%)	52.8 ± 3.06 [b]	1.17 ± 0.25 [b]	7.85 ± 0.42 [b]
Mar. 13, 2019	Fasted (0%)	51.1 ± 4.97 [a]	0.98 ± 0.27 [a]	7.15 ± 0.43 [a]
	Fed (1%)	52.7 ± 3.22 [a]	1.12 ± 0.19 [a]	7.58 ± 0.41 [ab]
	Fed (3%)	54.2 ± 5.19 [a]	1.31 ± 0.42 [a]	8.04 ± 0.33 [b]

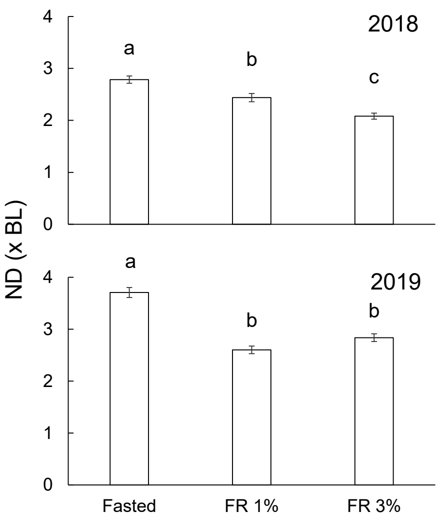

Fig. 1 Neighborhood distances (ND, mean ± SD, n=10) for different feeding groups in 2018 and 2019 experiments. The bars with different letters in the same year class are significantly different (Scheffé's F Test, $p < 0.05$).

experiments. In 2018, ND (mean ± SD) was 2.78 ± 0.07 for the FR0% treatment, 2.44 ± 0.08 for the FR1% treatment, and 2.08 ± 0.06 for the FR3% treatment, with significant differences among all treatments (Scheffé's F Test, $p < 0.05$). In the 2019 experiment, the NDs of the 0% FR, 1% FR, and 3% FR treatments were 3.71 ± 0.10, 2.60 ± 0.07, and 2.84 ± 0.08, respectively. ND was significantly greater in the FR0% treatment than in the FR1% and FR3% treatments (Scheffé's F Test, $p < 0.05$), and there was no significant difference in ND between the FR1% and FR3% treatments.

DISCUSSION

The structure of the fish school was characterized by uniformity and inter-individual distance. In this study, the 3 cm depth of the experimental tank limited the complete freedom of movement of the fry. However, previous reports have shown that changes in schooling behavior can be assessed using this method (Torao *et al*., 2014). Despite the two-dimensional limitations of this study, distances between chum salmon were affected by feeding rates, with increasing distances as feeding declined, suggesting that nutritional status may affect the school structure of chum salmon fry.

Fish schools are dynamic and often form different shapes depending on the situation of the school and the environmental factors. For example, distances between individuals tend to increase during foraging, whereas aggregations can become compact and dense when a predator is present (Masuda, 2007). This may be explained by the psychological state of individuals, such as motivation for feeding and wariness of predators, reflected in their behavior, resulting in changes in inter-individual distance. Similarly, schools of ayu, *Plecoglossus altivelis*, become less dense as the water temperature increases and more compact when it declines (Tsukamoto and Uchida, 1990). In this study, water temperature and illuminance were controlled to eliminated their effects on school structure. However, we observed that the distance between individual chum salmon fry was maintained by following the movement of adjacent individuals within the school. One factor that may have contributed to the expanded ND observed in the school of fasting fry is that their poor nutritional status could have compromised their ability to maintain a distance between individuals within the group. Although long-term fasting (> 20 days) markedly reduces the swimming ability of chum salmon fry (Torao *et al*., 2021), it is possible that relatively short-term fasting can affect the behavioral activity of salmon fry and lead to ND expansion.

Predation is a cause of mortality of salmon fry in rivers, but the specifics regarding how changing the dynamics of school structure help mitigate such threats are unclear. Predation experiments have suggested that a reduced nutritional status can negatively affect swimming ability and increase the risk of predation (Torao *et al*., 2021). Starvation can lead to an energy decline that can reduce activity levels, thereby influencing school dynamics and the ability to protect against predation.

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