Effect of prey items on the development of schooling behavior in chub mackerel *Scomber japonicus* in the laboratory

SHINNOSUKE NAKAYAMA,1 REIJI MASUDA,2* JUN SHOJI,1 TOSHIO TAKEUCHI3 AND MASARU TANAKA1

1Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Sakyo, Kyoto 606-8502, 2Fisheries Research Station, Kyoto University, Maizuru, Kyoto 625-0086 and 3Department of Aquatic Biosciences, Tokyo University of Fisheries, Minato, Tokyo 108-8477, Japan

**ABSTRACT:** Chub mackerel *Scomber japonicus* can use both zooplankton and fish larvae as prey items depending on their dietary environment in their larval and early juvenile stages. Here, we compared the development of schooling behavior in chub mackerel fed either *Artemia* nauplii (plankton-fed group) or the yolk-sac larvae of red sea bream (fish-fed group). Video recording was conducted in rearing tanks followed by analysis of separation swimming index as a criterion of schooling behavior. As a result, early juvenile mackerel in the plankton-fed group did not show schooling behavior up to day 22 (at 25 mm standard length (SL)), whereas those in the fish-fed group completed schooling behavior on day 18 (at 19 mm SL). Lack of schooling behavior in the plankton-fed group may be attributed to the low level of highly unsaturated fatty acids, especially docosahexaenoic acid, in their diet, without which a deficiency in the development of the central nervous systems may have occurred. Alternatively, chub mackerel juveniles in the plankton-rich environment may have adapted the balance of feeding and antipredator performance and, thus, delayed the development of schooling.

**KEY WORDS:** behavioral ontogeny, dietary shift, docosahexaenoic acid, schooling behavior, *Scomber japonicus*.

**INTRODUCTION**

Chub mackerel *Scomber japonicus* is a species with a wide range of distribution in subtropical and temperate coastal waters.1 Because of its typical epipelagic large schools, *S. japonicus*, together with *S. scombrus*, has often been used as a good experimental model of swimming and schooling behavior.2–4 In general, schooling behavior provides fish with many advantages, such as a decrease in the risk of predation by confusing predators and the facilitation of prey search efficiency.5 Schooling is now considered to be a highly advanced and yet ubiquitous behavior in fish.

Chub mackerel larvae can use both fish larvae and zooplankton, such as copepods, depending on the dietary environment in their habitat.6 Because they live near coastal areas,1 the composition of prey changes spatially and temporally. Our previous study revealed that chub mackerel fed red sea bream *Pagrus major* larvae were superior in burst and cruise swimming speeds to those fed *Artemia* sp. nauplii.7 Considering that schooling behavior is a more complicated behavior than swimming, the dietary changes may well affect the developmental process of schooling. Here, we compared the development of schooling behavior in chub mackerel fed either plankton or fish larvae. The impact of dietary environment on the behavioral ontogeny is discussed based on the fatty acid composition of each diet.

**MATERIALS AND METHODS**

**Fish husbandry and video recording**

Naturally spawned and fertilized chub mackerel eggs were provided by the Fisheries Institute of Kinki University (Wakayama, Japan) on 29 May 2001. Eggs were transferred to the Fisheries
Research Station of Kyoto University, from which 5000 were stocked in two 500 L black polycarbonate tanks, 2500 eggs in each. One of the two tanks was assigned as the plankton-fed group and the other as the fish-fed group. Eggs were kept at ambient temperature (19.7°C) and hatched on 31 May. Water flow was kept at 1200 mL/min.

Starting from day 2, fish in both groups were provided with rotifers *Brachionus plicatilis* at a density of 5 individuals/mL, enriched with highly unsaturated fatty acids (HUFA) oil (Docosa EM; Akita Jujo Kasei, Akita, Japan). From day 8, fish in the plankton-fed group were provided with rotifers (2.5 individuals/mL) and *Artemia* sp. nauplii (0.1 individuals/mL), both enriched with HUFA oil, whereas fish in the fish-fed group were provided with rotifers (2.5 individuals/mL) and red sea bream larvae (0.01 individuals/mL). Red sea bream eggs were spawned naturally and fertilized in the Kyoto Prefectural Sea-Farming Center, transferred to the Fisheries Research Station of Kyoto University and used as preys at the yolk-sac larval stage (on day 0–2). From day 11, fish in the plankton-fed group were provided with *Artemia* nauplii at a density of 0.3 individuals/mL, whereas fish in the fish-fed group were provided with red sea bream larvae at a density of 0.02–0.06 individuals/mL. Chub mackerel juveniles were reared up to day 22. Water was kept at ambient temperature (19–22°C). The light intensity was approximately 500 lux in the daytime.

The standard length (SL) of 10 fish in each group was measured every 2–3 days from day 8. Those fish were fixed with 10% neutralized formalin and served for the dorsal and pectoral fin ray count.

The fatty acid composition of rotifers, *Artemia* nauplii and red sea bream larvae was analyzed by routine procedures. Lipids were extracted according to the method of Folch *et al.* and were identified by gas chromatography (GC-15A; Shimadzu, Kyoto, Japan). Tricosanoic acid (C23:0; Sigma Chemical, St Louis, MO, USA) was used as the internal standard.

In order to reveal the developmental process of schooling, we recorded the behavior of fish in each rearing tank every 1–3 days from day 11 using a digital video camera (DCR-PC100; Sony, Tokyo, Japan). Two semicircular white reflecting boards (Reflective Panel NT-A041S; Nihon Tentosha, Osaka, Japan) were put on the tank bottom from approximately 15 min prior to recording in order to improve the contrast of the recorded image. Video recording was conducted from 13:00 h for 15 min from above, covering approximately one-quarter of the tank bottom. The video camera focused on 5–10 cm under the surface where fish were most abundant.

### Video analysis

Because our preliminary observations suggested that the early stage of schooling was characterized by parallel swimming of two or more individuals, we defined the separation swimming index (SSI) for further analysis. First, on a still frame of the video image, we focused on a fish that was nearest to the center of the video monitor (defined as the focal fish), as well as on another fish that was nearest to the focal fish (defined as the neighboring fish), and marked the points of their snouts on the monitor. The videotape was forwarded for 1 s and the position of the snouts of the focal and neighboring fish was also marked at that time. The movement of the snout for each fish can be expressed as a vector and approximates its swimming speed (defined as \( v_f \) and \( v_n \) for focal and neighboring fish, respectively; Fig. 1a). Then, the starting point of the latter was parallel translated to the starting point of the former (Fig. 1b). The distance of two vector-endings \( d \) was divided by the average of \( v_f \) and \( v_n \) to give SSI:

\[
SSI = \frac{d}{(v_f + v_n)/2}
\]

Practically, SSI can be calculated without parallel transformation by measuring the angle between two vectors \( \theta \) and transforming \( d \) of the above function as follows:

\[
SSI = \frac{(2\sqrt{v_f^2 + v_n^2 - 2v_f v_n \cos \theta})}{(v_f + v_n)}
\]

The SSI ranges from 0 to 2, the average of which, for each measurement, approaches 1.414 when the swimming speed and direction of two individuals are random and decreases to 0 as the two fish swim together. Measurement of \( v_f \) and \( v_n \) and SSI were repeated in 30 video frames that were sampled at 20 s intervals. The average of \( v_f \) and \( v_n \) was also calculated as an indicator of swimming speed.

The SL and swimming speeds of fish between the two dietary groups were compared by Student’s t-test. Because SSI did not show normal distribution, the Mann–Whitney U-test was applied to compare SSI among different dietary groups and the Kruskal–Wallis test followed by the Nemenyi test was applied to compare SSI among different ages within the same dietary group.

### RESULTS

The average SL between two dietary groups was not significantly different up to day 15 \((P > 0.05, \text{Student’s } t\text{-test})\). On day 18 or later, SL in the fish-fed group was significantly larger than that in the plankton-fed group \((P < 0.01, \text{Student’s } t\text{-test}; \text{Fig. 2})\).
The average swimming speed increased in both groups as they grew (Fig. 3). After day 18, as was the case for SL, the swimming speed in the fish-fed group was significantly greater than that in the plankton-fed group ($P < 0.01$, Student’s $t$-test), although fish in both groups showed smooth and continuous swimming. Dorsal and pectoral fin ray counts revealed that 40% of fish on day 16 and all fish on day 18 in the plankton-fed group attained the same number as the adult (regarded as the juvenile stage), whereas 60% of fish on day 16 and all fish on day 18 attained the juvenile stage in the fish-fed group.

The SSI was consistently high without any significant difference among ages in the plankton-fed group ($P > 0.05$, Kruskal–Wallis test), whereas a significant decrease with age was observed in the SSI in the fish-fed group ($P < 0.01$, Kruskal–Wallis test followed by the Nemenyi test; Fig. 4). The SSI in the fish-fed group was significantly smaller than that in the plankton-fed group on day 16 ($P = 0.016$, Mann–Whitney $U$-test) or older ($P < 0.01$). The SSI...
in the plankton-fed group was always close to 1.414 throughout the observation period, suggesting that the swimming direction and speed of the fish in this group were almost random in neighboring individuals. After day 18, the SSI in the plankton-fed group showed a slightly smaller, albeit not statistically significant, value than 1.414 despite the fact that clear schooling behavior was not observed either on direct observation or in the video recording. Parallel swimming and chasing behavior occurred in this group, but only occasionally. In the fish-fed group, fish swam randomly up to day 14, whereas they started to show occasional parallel orientation on day 16 and started to form a tight school on day 18. The densities in both groups were approximately 1.9 individuals/L on day 8 and 0.2 individuals/L on day 22 and the survival rates in both groups up until that time seemed to be similar, so that survival rate should not have affected the SSI.

Fatty acid analysis revealed that rotifers contained the largest amount of crude fat (2.6%), followed by *Artemia* (1.5%) and red sea bream larvae (1.4%; Table 1). The amount of eicosapentaenoic acid (EPA) in crude fat was highest in rotifers (2.8%), followed by *Artemia* (1.2%) and red sea bream (1.1%). The amount of docosahexaenoic...
acid (DHA) in crude fat was high in red sea bream (2.3%) and rotifers (2.2%), but relatively low in Artemia (0.6%). The fatty acid composition of rotifers, Artemia nauplii and fish larvae is given in Table 2.

**DISCUSSION**

**Separation swimming index as an indicator of schooling behavior**

Visual mutual attraction has been claimed as the primordial behavior of schooling in fish. Early work by Shaw11 on the development of schooling in the silversides Menidia menidia demonstrated that visual mutual attraction to another individual in a glass tube first appeared when the fish was as small as 8 mm, which was ahead of the onset of schooling at 11–12 mm. Mutual attraction to a group of fish in striped jack Pseudocaranx dentex juveniles was observed at 12 mm total length, which was before the onset of schooling characterized by a decrease in both interindividual distance and separation angle.12 Both these studies were conducted by transferring fish into small experimental tanks, which may itself produce handling stress. In contrast, the method we used in the present study does not require the transference of fish. Rearing tanks tend to have background noises, such as that due to water current and aeration, which can be prevented by temporarily stopping the water supply and aeration. Measurement of interindividual distance is generally influenced by the number of fish in the tank. Using our method, the variables for calculating SSI were the angle and the separating/approaching distance of two individuals, but the variables did not include the distance of the two individuals, so that SSI would be less density dependent. The SSI can also be applied to free-swimming fish in the sea if underwater videorecording is conducted for wild fish.

**Developmental timing of the onset of schooling behavior**

Schooling behavior of fish-fed chub mackerel was first observed on day 16 and became more consistent on day 18. Sixty percent of fish in this group on day 16 (approximately 12 mm SL) and all fish on day 18 (approximately 19 mm SL) had the same number of fin rays as the adult, so that the onset of schooling behavior in chub mackerel coincided with metamorphosis (larva–juvenile transformation). The onset of schooling behavior has been observed coincident to or immediately after metamorphosis in other fish species, such as anchovy Engraulis mordax,13 Atlantic herring Clupea harengus,14 yellowtail Seriola quinqueradiata15 and striped jack.16

Schooling behavior in the plankton-fed group was not observed on day 21 (approximately 25 mm SL), even though 40% of fish on day 16 (approximately 11 mm SL) and all fish on day 18 (approximately 16 mm SL) completed metamorphosis. Hunter and Coyne13 suggested the importance of the development of sensory and swimming organs for the development of schooling behavior. In contrast, Kohno et al.17 reported that chub mackerel acquired almost complete swimming ability at approximately 10 mm with the full development of fin rays and fin supports. Swimming speed in plankton-fed chub mackerel was significantly less than that of the fish-fed group on day 18 or later. Fish in the plankton-fed group often zig zagged within the period of 1 s, resulting in an underestimate of swimming speed. Batty et al.18 reported that copepod-fed herring larvae swam faster than their Artemia-fed counterparts, suggesting that having a faster swimming prey improved the swimming ability of the predator. This would not be the case in the present experiment, because the yolk-sac larvae of red sea bream on day 0–2 were less mobile than the Artemia. Our previous work compared the development of swimming speed in chub mackerel between plankton-fed and fish-fed groups using frame-by-frame video analysis in an experimental container.7 The cruise swimming speeds of mackerel juveniles were 4.28 and 5.39 SL/s, both at 17 mm SL, in plankton- and fish-fed groups, respectively, and the difference in these swimming speeds was significant, albeit small. Fish in the plankton-fed group in the present study showed smooth and continuous swimming on day 18 without any sign of schooling. Considering that plankton-fed fish apparently acquired swimming capability to support schooling behavior, the discrepancy regarding the onset of schooling behavior between the two dietary groups should be attributed to factors other than swimming capability.

Neuromasts and lateral line canals are supposed to be the major sensory organs used to detect the movement of water and, it has been claimed, they are important in the maintenance of schooling behavior.19 Development of free neuromasts has been observed earlier than schooling in fish20 and we confirmed that chub mackerel had free neuromasts on day 8 at the latest (S Nakayama et al., unpubl. data, 2001). The possibility remains that buccal and lateral line canals developed differently depending on dietary conditions. Considering that the enriched Artemia contained markedly less DHA (0.6%) compared with the yolk-sac larvae of
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Davis and Olla\textsuperscript{21} reported that the DHA content in wild copepods collected offshore in Oregon fluctuated from 0.08 to 2.01\% over a period of 12 days. Therefore, the DHA content of \textit{Artemia} in the present study (0.6\%) is within the range of natural fluctuation. In the case of yellowtail, replacement of DHA with EPA as the enrichment oil induced a deficiency of mutual attraction and schooling behavior.\textsuperscript{15} It was also demonstrated that dietary DHA is incorporated into the central nervous system.\textsuperscript{22} Relatively low levels of DHA in the diet of the plankton-fed group in the present study may have caused a deficiency in the central nervous system control of parallel orientation.

An alternative, but non-exclusive, explanation is that chub mackerel adapted the development of schooling behavior depending on the dietary environment. The species composition of prey animals should reflect on that of the predator species; planktivorous predators would be abundant in the area where zooplankton prey is abundant, whereas piscivorous predators would be abundant in the area where prey fish are abundant. Mackerel in the former environment would feed actively on zooplankton not to be behind the competitive planktivorous species, whereas in the latter environment they would spend more energy on antipredator behavior (i.e. schooling). Therefore, earlier development of schooling behavior in fish-fed mackerel may reflect the balance between feeding efficiency and predator avoidance.

Ozawa et al.\textsuperscript{23} reported that chub mackerel mainly prey on Appendicularia, crustacean eggs, Cladocera and Copepoda in the open sea off southwest Japan. In contrast, chub mackerel larvae prey on fish larvae from as small as 5 mm SL in the Seto Inland Sea, Japan, where fish larvae as prey are amply available (J Shoji et al., unpubl. data, 1997–99). If the early dietary transition to piscivory directly affects the early ontogeny of schooling behavior, it is deduced that the timing of the onset of schooling would be different between fish in the Inland Sea and those in the open sea, considering that fish larvae are abundant in the former environment, whereas they are scarce in the latter. Alternatively, if it is assumed that the primary factor for the timing of school formation is not food items but DHA content in the diet, then the size of schooling formation would not be different, irrespective of food items or sea area, because copepods in the sea generally contain abundant DHA. In any case, further studies relating food items, the DHA content of these food items and the timing of school formation would be fascinating.

Grave\textsuperscript{24} reported that Atlantic mackerel \textit{Scomber scombrus} larvae mainly prey on Cladocera and Copepoda and that most of their stomach contents shift to fish larvae during the transition period from larvae to juveniles. He also found that there is a high rate of cannibalism during that period, reporting that most of the stomach contents were occupied by conspecifics. He suggested that cannibalism should have occurred in the evening, when mackerel larvae aggregated near the surface. This implies that dietary transition to piscivory synchronizes with the onset of diel vertical migration. Furthermore, the onset of cannibalism could result in promoting the aggregation of the conspecifics and, thus, the onset of schooling. There is, however, no evidence concerning serious cannibalism in chub mackerel \textit{S. japonicus}, not to mention the timing of schooling in the wild. Interspecific comparison of behavioral ontogeny between \textit{S. scombrus} and \textit{S. japonicus}, together with environmental conditions, would be a challenging topic for future study.

In the present study, we demonstrated that chub mackerel fed zooplankton did not show schooling behavior after metamorphosis. Two possible explanations were provided: (i) the inferior nutritional condition of \textit{Artemia} nauplii induced a delay in the development of the central nervous system and, thus, the fish cannot control the parallel orientation of schooling; and (ii) the fish have adapted the development of feeding and antipredator performance to the dietary environment. If the former hypothesis is correct, we suggest that the DHA content of the diets we used is well within the range of fluctuation in the natural diet and that fluctuation of dietary quality may induce antipredator performance and, thus, survival of fish species. Further experiments combining dietary environment with the development of antipredator performance would be required to scrutinize the abovementioned hypotheses.

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