

Onset and development of cannibalistic and schooling behavior in the early life stages of Pacific bluefin tuna *Thunnus orientalis*

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Abstract

Behavioral development was observed in the early life stages of Pacific bluefin tuna in order to provide fundamental information for improving seedling production techniques. Behavioral observations to quantify swimming, schooling and cannibalistic behavior were made at different developmental stages: pre-flexion (5 days after hatching, DAH), flexion (12 DAH), post-flexion (14 DAH) and juvenile (20 DAH). Video recordings of either observation containers or the rearing tank were made to observe swimming and schooling behavior, respectively. Cannibalistic behavior was estimated by frequency of chase behavior. Swimming speed maintained constant values from 6 DAH (9.2 ± 6.0 mm/sec, pre-flexion stage) until 20 DAH (22.4 ± 9.0 mm/sec, beginning of juvenile stage) and increased rapidly thereafter until 29 DAH (85.2 ± 32.5 mm/sec). Schooling behavior was first observed on 25 DAH juveniles (SL 23.5 ± 5.0 mm). Chase behavior was first observed at 14 DAH (standard length, SL 6.1 ± 0.6 mm, transition at flexion to post-flexion stage) and increased thereafter. We propose that practical developmental stage for size grading to reduce the mortality by cannibalism should be between post-flexion and early juvenile (SL 6-23 mm), when the cannibalistic behavior onsets and swimming speed is relatively low.

Keywords: Ontogeny, aggressive behavior, Scombridae.

1 Introduction

Marine fish larviculture involves domesticating wild species of fish. This is problematic as these fish have been subjected to selective pressures which have shaped their response to "fit" their environment (Blaxter, 1986; Munk, 1995). Often our understanding of the natural history and ecology of wild fish, especially at the larval and early juvenile stage is poor, thus we are at a disadvantage when developing protocols and culture technology (Brown et al., 1997). Behavioral development is the key to understand the ecology of fish larvae and juveniles in relation to morphological development and organogenesis (Fukuhara, 1986). Therefore, many studies had been focused on the relation between the development of sensory organs and behavioral development in teleosts to investigate their early life histories (Blaxter, 1986). Behavioral quantification within an experimental design using other performance criteria (survival, growth) allows the evaluation of animal responses to culture conditions and animal welfare assessment (Faleiro et al., 2008). Thus, behavioral quantification can be a valuable aid for improving larviculture production (Brown et al., 1997). For instance, effects of delayed feeding in larval Atlantic cod *Gadus morhua* and haddock *Melanogrammus aeglefinus* is informative to schedule the feeding regime (Laurence, 1978), and the use of feeding behavior observations to specify the timing to wean wolffish *Anarhichas lupus* from live feed to pellets (Brown et al., 1997).

Many fish species develop social responses in their early life stages (Noakes and Godin, 1988). Social behavior includes all behaviors directly related to actual or potential encounters between individuals within a species (Noakes, 1978). Investigating the ontogeny of social behavior is of practical importance to improve the quality of reared fish for stock enhancement and aquaculture (Svåsand, 1993; Olla et al., 1994; Sakakura, 2006). Social behavior includes aggressive behavior as well as

cannibalism, in which one fish attacks its conspecifics. Aggressive behavior, including cannibalistic behavior, can have a significant impact on the early life history of fishes not only in commercial rearing conditions but also in the wild (Smith and Reay, 1991). Observation of ontogeny of aggressive behavior provides the practical information on frequency of feeding and timing of size grading and for aquaculturists in many species, such as yellowtail *Seriola quinqueradiata* (Sakakura and Tsukamoto 1999), Japanese flounder *Paralichthys olivaceus* (Sakakura and Tsukamoto, 2002) and seven-band grouper *Epinephelus septemfasciatus* (Sabate et al., 2009a).

Pacific bluefin tuna (*Thunnus orientalis* Temminck and Schlegel, 1844) has become a target species for aquaculture in Japan (Sawada et al., 2005). The Japanese Fisheries Agency, Kinki University, and the National Center for Stock Enhancement (formerly Japan Sea-Farming Association) started a project to develop rearing techniques for Pacific bluefin tuna for future enhancement trials (Kaji et al., 1996; Miyashita, 2002). Rearing beyond post-flexion was first successful in 1979 (Harada, 1980), and Pacific bluefin tuna was successfully cultured through its complete life cycle in 2002 at Kinki University, Japan (Sawada et al., 2005). However, mass production of Pacific bluefin tuna larvae still fluctuates due to high mortalities during early life stages (60-90% mortality; Kumai, 1998; Miyashita, 2002). Cannibalism and frequency of collision with the rearing tank have been identified as major causes of mortality (Miyashita, 2002).

Several studies have reported on the early development of Pacific bluefin tuna investigating: morphological and physiological development (Miyashita et al., 2001; Sawada, 2006; Nakagawa et al., 2007); morphogenesis of sense organs (Kawamura et al., 2003); and feed selectivity on rotifers (Sawada et al., 2000). However, no study has investigated quantitative behaviors in the early life stages of Pacific bluefin tuna. In

this research, we focused on the onset and development of schooling and aggressive behavior, which is closely related to cannibalistic behavior in this species (Sabate et al., 2009b), and the development of swimming behavior of Pacific bluefin tuna from hatch until the juvenile stage in order to set groundwork for future behavioral studies of this species.

2 Material and methods

2.1 Rearing conditions

Naturally spawned eggs from four years old broodstock maintained in a floating net cage were collected by plankton net on 30 June 2008 at the Amami Station of the National Center for Stock Enhancement Fisheries Research Agency, Japan. Eggs were maintained in 200 L tanks and larvae were transferred into 50 kL rearing tanks. To prevent bottom death, ozonated seawater was supplied from the bottom of the tank at a water exchange rate of 150 % per day in a flow through system (Tezuka et al., 2005; Tanaka et al., 2009). Water temperature ranged from 27.5 to 28.6 °C and the system was illuminated by unfiltered sunlight with a natural photoperiod (13L:11D) and intensity at the surface (70 -1000 lux; measured with an illuminance meter; IM-5, Topcon Corp., Tokyo, Japan).

Fish were fed with L-type rotifers (*Brachionus plicatilis* sp. complex, Kindai strain) from mouth opening (2 days after hatching, DAH) until 20 DAH. *Artemia franciscana* nauplii were provided in addition to rotifers between 12 DAH and 20 DAH. Newly hatched spangled emperor *Lethrinus nebulosus* were fed between 16 DAH and 27 DAH, and this was supplemented with minced sand eel *Ammodytes personatus* from 22 DAH. Rotifers were enriched with Marine Fresh (Mercian Corp., Kumamoto, Japan) and *Artemia* nauplii were enriched with Sujiko emulsified oil

(Nisshin Marine Tech Ltd., Aichi, Japan).

2.2 Behavioral observations

In order to quantify the observed behaviors, we defined four behavioral indices: (i) swimming speed (in mm/sec); (ii) relative swimming speed (swimming speed/standard length, expressed in standard lengths/sec, SL/sec); (iii) schooling behavior, measured by the swimming separation index (SSI) defined by Nakayama et al. (2007); and (iv) aggressive behavior, estimated as the chases/min/fish (during a chase a dominant fish swims after and follows a subordinate for a short time, approximately 1-10sec.). We followed Nakayama et al. (2007) to calculate the SSI. In short, on a still frame of a video image, the position of the snout was marked for all the visible fish and marked again after the videotape was forwarded 0.5 sec. Movement of the snout in 0.5 sec was expressed as a speed vector for each fish. Then a starting point of one vector is parallel-translated to that of the other, and the SSI was calculated as: $SSI = 2d (V_f + V_n)^{-1}$; where d is a distance between the vector endpoints of two adjacent fish, and V_f and V_n are the magnitudes of the vector for the fish, respectively. The SSI represents how far two neighboring individuals are separated from each other in a given time, which is adjusted by an average speed of two individuals. The value of SSI ranges between 0 and 2; 0 when two individuals show perfect parallel swimming with the same speed and direction, about 1.5 when swimming speeds and directions of two individuals are at random, and 2 when two individuals swim in opposite directions. In order to estimate an SSI value for random swimming to compare with our data we run 10,000 simulations of fish pairs swimming at random speeds and directions following Nakayama et al. (2007).

To measure swimming speed of fish, video recordings were carried out in an

enclosed chamber because of the difficulty of making accurate swimming speed measures in the rearing tank due to the effect of water currents. Video recordings were performed at 6, 11, 15, 19, 21, 25 and 29 DAH. In an effort to make representative the observations in the enclosed chamber representative of their behavior the feed concentration and temperature were matched to those in the 50 kL rearing tank by introducing the rearing water directly to the observation container in a water bath (Sabate et al., 2009b). A light intensity of 600 Lux, which was the same light intensity on the water surface of the rearing tank at the observation period, was provided by a fiber optic light source (C-FID, Nikon Corp., Tokyo, Japan) situated at approx. 30 cm over the water surface following Sabate et al. (2009b). Every sampling day, five fish were gently transferred from the rearing tank into an observation container filled with seawater from the rearing tank at 7:00. In order to provide enough space for the fish to swim freely, the size of the observation tanks were increased as the fish grew. According to fish size either a 50 mL container (5 cm length, 4 cm width and 3 cm height), a 350 mL container (10.5 cm length, 8 cm width and 6 cm height), a 5 L container (24 cm radius, 12 cm height) or a 30 L tank (38 cm radius, 32 cm height) was used for the observations. After a 10 minute acclimation period, video recordings of fish behavior were made from above for 60 minutes using a digital video camera (Sony DCRTRV9, Sony Corp., Tokyo, Japan). Swimming speed was determined from the video recordings by analyzing randomly selected 10 second periods of video. Swimming speed was calculated using the computer program DIPP Motion Pro Version 2.0 (DITECT Co., Tokyo, Japan).

To quantify schooling behavior, video recordings of the fish behavior were carried out at the rearing tank on a space with a light intensity of 500-700 Lux provided by unfiltered sunlight in order to clearly identify the fish at 6, 11, 15, 19, 21,

25 and 29 DAH. Every observation day, the video camera was set over the rearing tank at 12:00 (aprox. 2 hours after feeding to prevent the influence of foraging behavior) and one hour of video was recorded. The SSI of all the fish pairs that appeared in the analyzed video recorded period was calculated in the same manner as described above. SSI was calculated until a total of 45 values for the SSI were obtained for each observation day.

Following Sakakura and Tsukamoto (1996), aggressive behaviors were classified as: Aim, a dominant holds its position towards a subordinate for a short period (aprox. 1 sec.); Chase, a dominant bursts towards and follows a subordinate for 1-10 sec. These two phases were observed in a fixed order. Sometimes a dominant attack and bites the body of a subordinate following Chase behavior. Since Chase behavior was observed in every instance of Aim behavior and Chase behavior was easy to observe and record, its frequency was used as an index of aggressive behavior. Chase behavior was directly observed at 7, 12, 14, 16, 20 and 26 DAH in observation containers. According to fish size either a 1 L beaker (11 cm radius, 15 cm height), a 5 L observation container (24 cm radius, 12 cm height) or a 30 L observation tank (38 cm radius, 32 cm height) was used. Four replicates were set in every sampling day. Five fish were gently introduced from the rearing tank into an observation container at 5:30 during sunrise, when fish of all sizes are near the surface to feed. Afterwards, behavior was observed for 10 minutes every 2 hours, from 6:00 (sunrise) to 18:00 (sunset). Twenty minute direct observations to corroborate the onset of chase behavior were made daily every two hours from 7:00 to 19:00 on the rearing tank. Live feed concentration, light intensity (70-1000 Lux at the water surface measured with an illuminance meter and provided by unfiltered sunlight) and temperature were kept similar to the rearing tank during the observations and presence of live feed was

checked at the end of the observations. Frequencies of chase behavior were pooled by hour and age and mean value (per minute per fish) was obtained for each time and age group, respectively. No actual cannibalism (complete consumption of a conspecific) could be observed during our observations.

To determine growth and development throughout the experiment, fish were anesthetized with 0.01 % MS222 (Tricaine; Sigma Chemical Co., St Louis, MO, USA), and standard length (SL) was measured and developmental stage was determined according to Miyashita et al. (2001), and fish were fixed in 10% formalin at the end of the swimming speed and aggressive behavior observations ($n = 5$ and $n = 20$, respectively).

2.3 Statistics

To determine whether there were differences in standard length, swimming speed and relative swimming speed between sampling days, one way ANOVA ($p < 0.05$) was performed and Tukey-Kramer post hoc tests were used to assess differences. To detect the onset of schooling behavior, the SSI values of each sampling day were compared with the expected average SSI (1.4) obtained by running the 10,000 simulations by a two tailed t -test ($p < 0.05$). To determine whether there were differences in chase behavior among observation time and sampling days, Kruskal-Wallis test was used. In case significant difference was detected by Kruskal-Wallis test among observation time ($p < 0.05$), Dunn test was used, and if no significant differences were detected, data were pooled and mean values were calculated for each sampling day. Values throughout this report are given as mean \pm standard deviation.

3 Results

3.1 Growth and development

Fish at the beginning of the experiment (6 DAH) were in the pre-flexion stage with a mean SL of 4.2 ± 0.2 mm. Loss of continuous fin fold was first observed at 19 DAH at which point the mean SL was 10.0 ± 1.2 mm. By the end of the experiment (29 DAH) the fish had grown to 38.1 ± 3.7 mm in standard length (Fig. 1).

3.2 Behavior

Swimming speed did not change from 6 DAH (9.2 ± 6.0 mm/sec) until 21 DAH (juvenile stage, 22.4 ± 9.0 mm/sec; ANOVA, $F = 4.5$, $df = 6$, $p < 0.05$). Swimming speed increased rapidly from the juvenile stage until the end of the experiment (29 DAH, 85.2 ± 32.5 mm/sec; Fig. 2).

Relative swimming speed decreased from 6 DAH (2.2 ± 1.4 SL/sec) until the beginning of the flexion stage (11 DAH, 1.4 ± 0.6 SL/sec; ANOVA, $F = 4.5$, $df = 6$, $p < 0.05$). Afterwards, it increased gradually until the end of the experiment (2.2 ± 0.9 SL/sec; Fig. 2).

For schooling behavior, the simulation of fish pairs of fish swimming at random speeds and directions yielded a SSI value of 1.4. The estimated SSI value for random swimming did not differ with our data between 11 - 21 DAH (t -test; 11 DAH: $t = -1.68$, $p = 0.09$; 15 DAH: $t = 0.26$, $p = 0.80$; 19 DAH: $t = 0.72$, $p = 0.94$; 21 DAH: $t = -1.25$, $p = 0.21$; Fig. 3). At 6, 25 and 29 DAH, the SSI was significantly different from the expected random value (6 DAH: $t = -6.54$, $p < 0.0001$; 25 DAH: $t = -5.33$, $p < 0.0001$; 29 DAH: $t = -16.28$, $p < 0.0001$). The SSI began to decrease sharply on 25 DAH, indicating that the degree of parallel swimming between neighboring individuals became larger. Schooling behavior was also observed to increase (based on

direct observations) in the rearing tank at 25 DAH. At 29 DAH and thereafter, fish in the rearing tank swam circularly in the same direction and at the same speed.

No effect of time of day on the occurrence of aggressive behavior as quantified by mean number of chases/min/fish was detected. Hence, data was pooled and mean values were calculated for each sampling day. No chasing behavior was observed from the beginning of the experiment until 14 DAH (SL 6.1 ± 0.6 mm; Fig. 4) coinciding with the beginning of post-flexion stage. Afterwards, frequency of chasing behavior did not change until the end of the experiment (Kruskal-Wallis test, $H = 4.8$, $df = 5$, $p = 0.44$). Onset of chase behavior was also observed in the rearing tank at 14 DAH.

4 Discussion

Fish growth and development in our experiment are comparable to the results obtained by Miyashita et al. (2001), whose experimental conditions were similar to ours in temperature (24.5 - 27.7 °C), time of the year (July), rearing tank size (20 kL) and feed (rotifers from hatch until post-flexion stage, *Artemia* from flexion stage to juvenile stage and fish larvae from post-flexion stage until the end of the experiment). However, when compared to the findings by Kaji et al. (1996), Pacific bluefin tuna in our experiment had a better growth, probably due to differences in diet (rotifers from mouth open, *Artemia* from flexion and artificial pellets from post-flexion stage) or tank size (5 kL), as other variables were similar (time of the year, 25 °C, 100-1000 Lux).

Swimming speed of Pacific bluefin tuna larvae did not change significantly between hatch and the juvenile stage, but increased rapidly afterwards. However, relative swimming speed steadily increased from the flexion stage to the end of the study. Swimming speed of Pacific bluefin tuna larvae was similar to that of other scombrid larvae at similar body sizes (chub mackerel *Scomber japonicus*; Nakayama et

al., 2007), and was faster than those of comparable size larvae of non-migratory species, such as Japanese flounder *Paralichthys olivaceus* (Fukuhara, 1986) and red drum *Sciaenops ocellatus* (Smith and Fuiman, 2004). Also, juvenile relative swimming speed was comparable to migratory species juveniles, such as chub mackerel (Masuda et al., 2002), yellowtail *Seriola quinqueradiata* (Sakakura and Tsukamoto, 1998), jack mackerel *Trachurus japonicus* (Masuda, 2006), and Japanese anchovy *Engraulis japonicus* (Masuda 2009). Swimming speed is an important factor during early life stages for feeding, predator avoidance as well as change of position in the water column (Fuiman and Magurran, 1994). Japanese flounder larvae change their position in the water column to use tidal currents during their relatively short inshore migration at the metamorphosing stages (Burke et al., 1995). Accordingly, the relatively fast swimming speed in Pacific bluefin tuna suggests that this species may also be migratory.

At 6 DAH, the low SSI value could be interpreted as schooling behavior, but this low value may be an artifact of the relatively strong water currents (aprox. 1 cm/sec) used in the rearing tank to prevent bottom death (Tezuka et al., 2005; Tanaka et al., 2009), not schooling behavior of the fish. Accordingly, an increase in the SSI was observed from 11 DAH, when fish were strong enough to swim against the current, indicating that fish at these stages do not school. In the present study, fish started schooling at 25 DAH, after the transition to juvenile (20 DAH). Pacific bluefin tuna developed schooling behavior later than other species, such as anchovy *Engraulis mordax* (Hunter and Coyne, 1982), Atlantic herring *Clupea harengus* (Gallego and Hearth, 1994), yellowtail (Sakakura and Tsukamoto, 1999) and striped jack *Pseudocaranx dentex* (Masuda and Tsukamoto, 1999), that develop schooling behavior after the post-flexion stage. Schooling behavior requires at least basic locomotor

organs for sustainable swimming (Kohno et al., 1984) and sensory organs, such as eyes and lateral line systems, for adjustment of distance and swimming speed with other shoal members (Hunter and Coyne, 1982). In carangid species, the requirement for juveniles to allow schooling behavior is not only development of locomotor and sensory systems but also development of the central nervous system (CNS; Masuda and Tsukamoto, 1999). Hence, young yellowtail and chub mackerel with underdeveloped CNS do not show schooling behavior compared to those with well-developed CNS at the same age and size in yellowtail (Ishizaki et al., 2001) and chub mackerel (Nakayama et al., 2003). Fishes change morphologically and physiologically as well as their niche, such as habitat and prey preference. Morphological changes, such as pigmentation, and elevated swimming speed may lead to increased conspicuousness to predators (Fuiman and Magurran, 1994), under the conditions where schooling behavior becomes more effective for predator avoidance. Therefore, the relatively late onset of schooling behavior in Pacific bluefin tuna presumably reflect the development of CNS in early juvenile stage and the response to environmental changes during migratory process.

Chase behavior was first observed at the beginning of post-flexion stage, coinciding with an increase in tissue thyroid hormones (Kawakami et al., 2008), in the number and size of upper and lower jaw teeth (Miyashita et al., 2001), and the appearance of the pyloric caeca (Kaji et al., 1996). Although in other species, such as yellowtail (Sakakura and Tsukamoto, 1996) and Japanese flounder (Sakakura and Tsukamoto, 2002), aggressive behavior onsets from the juvenile stages. In Pacific bluefin tuna aggressive behavior seems to be related with the development of feeding capabilities (appearance of teeth and the pyloric caeca). Aggressive behavior in Pacific bluefin tuna appeared earlier than in yellowtail (Sakakura and Tsukamoto, 1998, 1999)

and Japanese flounder (Sakakura and Tsukamoto, 2002) where aggressive behavior onsets from the juvenile stage. Chase frequency of Pacific bluefin tuna at onset was much lower (0.0008 chases/min/fish) than in; yellowtail (around 0.03 chases/min/fish; Sakakura and Tsukamoto, 1999); Japanese flounder (0.05 chases/min/fish; Sakakura and Tsukamoto, 2002); and seven-band grouper (0.05 chases/min/fish; Sabate et al., 2009a). Chase behavior was expressed equally towards individuals of smaller or similar size, never to larger individuals (in this study and Sabate et al., 2009b). Moreover, aggressive behavior in juvenile Pacific bluefin tuna is observed almost exclusively after a period of starvation (Sabate et al., 2009b). Therefore, we theorize that chase behavior of Pacific bluefin tuna is directly related to feeding behavior, namely cannibalistic behavior, which is different from social hierarchy-like behavior of yellowtail (Sakakura and Tsukamoto, 1998, 1999) and Japanese flounder (Sakakura and Tsukamoto, 2002).

Sawada et al. (2005) pointed out that cannibalism of Pacific bluefin tuna can be suppressed by feeding plenty of live fish larvae of other species and size grading. From our results, we propose that the practical time point for size grading to reduce cannibalism during the larval rearing of Pacific bluefin tuna is between the post-flexion stage (14 DAH; SL 9.2 ± 6.0 mm) and early juvenile stage (21 DAH; SL 22.4 ± 9.0 mm) prior to the onset of cannibalistic behavior and when swimming speeds are still low, to allow for easier handling.

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References

- Blaxter, J. H. S., 1986. Development of sense organs and behavior of teleost larvae with special reference to feeding and predator avoidance. *Trans. Am. Fish. Soc.* 115, 98-114.
- Brown, J.A., Wiseman, D., Kean, P., 1997. The use of behavioural observations in the larviculture of cold-water marine fish. *Aquaculture* 155, 301-310.
- Burke, J.S., Tanaka, M., Seikai, T., 1995. Influence of light and salinity on behavior of larval Japanese flounder (*Paralichthys olivaceus*) and implications for inshore migration. *Nether. J. Sea Res.* 34 (1-3), 59-69.
- Faleiro, F., Narciso, L., Vicente, L., 2008. Seahorse behaviour and aquaculture: How to improve *Hippocampus guttulatus* husbandry and reproduction? *Aquaculture* 282, 33-40.
- Fuiman, L.A., Magurran, A.E., 1994. Development of predator defenses in fishes. *Rev. Fish Biol. Fish.* 4, 145-183.
- Fukuhara, O., 1986. Morphological and functional development of Japanese flounder in early life stages. *Bulletin of the Japanese Society of Scientific Fisheries* 52 (1), 81-91.
- Gallego, A., Hearth, M.R., 1994. The development of schooling behaviour in Atlantic

- herring *Clupea harengus*. J. Fish Biol. 45, 569-588.
- Harada, T., 1980. Report on experimental tuna culture. Fisheries Institute, Kinki University, Shirayama, 30 pp (Japanese).
- Hunter, J.R., Coyne, K.M., 1982. The onset of schooling in northern anchovy larvae, *Engraulis mordax*. California Cooperative Oceanic Fisheries Investigations Rep. 23, 246-251.
- Ishizaki, Y., Masuda, R., Uematsu, K., Shimizu, K., Arimoto, M., Takeuchi, T., 2001. The effect of dietary docosahexaenoic acid on schooling behaviour and brain development in larval yellowtail. J. Fish Biol. 58, 1691-1703.
- Kaji, T., Tanaka, M., Takahashi, Y., Oka, M., Ishibashi, N., 1996. Preliminary observations on development of Pacific bluefin tuna *Thunnus thynnus* (Scombridae) larval reared in the laboratory, with special reference to the digestive system. Mar. Freshwater Res. 47, 261-269.
- Kawakami, Y., Nozaki, J., Seoka, M., Kumai, H., Ohta, H., 2008. Characterization of thyroid hormones and thyroid hormone receptors during the early development of Pacific bluefin tuna (*Thunnus orientalis*). Gen. Comp. Endocrinol. 155, 597-606.
- Kawamura, G., Masuna, S., Tezuka, N., Koiso, M., Jinbo, T., Namba, K., 2003. Morphogenesis of sense organs in the bluefin tuna *Thunnus orientalis*, The Big Fish Bang proceedings of the 26th Annual fish larval fish conference (2003). pp 123-135.
- Kohno, H., Shimizu, M., Nose, Y., 1984. Morphological aspects of the development of swimming and feeding function in larval *Scomber japonicus*. Nippon Suisan Gakkaishi 50, 1125-1137.
- Kumai, H., 1998. Studies on bluefin tuna artificial hatching, rearing and reproduction.

- Nippon Suisan Gakkaishi 64, 601-605.
- Laurence, G.C., 1978. Comparative growth, respiration and delayed feeding abilities of larval cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) as influenced by temperature during laboratory studies. Mar. Biol. 50, 1-7.
- Masuda, R., 2006. Ontogeny of anti-predator behavior in hatchery-reared jack mackerel *Trachurus japonicus* larvae and juveniles: patchiness formation, swimming capability, and interaction with jellyfish. Fish. Sci. 72, 1225–1235.
- Masuda, R. 2009. Behavioral ontogeny of marine pelagic fishes with the implications for the sustainable management of fisheries resources. Aqua-BioSci. Monogr. (ABSM). 2, 1–56.
- Masuda, R., Shoji, J., Aoyama, M., Tanaka, M. 2002. Chub mackerel larvae fed fish larvae can swim faster than those fed rotifers and *Artemia* nauplii. Fish. Sci. 68, 320–324.
- Masuda, R., Tsukamoto, K., 1999. School formation and concurrent developmental changes in carangid fish with reference to dietary conditions. Environ. Biol. Fish. 56, 243-252.
- Miyashita, S., Sawada, Y., Okada, T., Murata, O., Kumai, H., 2001. Morphological development and growth of laboratory-reared larval and juvenile *Thunnus thynnus* (Pisces: Scombridae). Fish. Bull. 99, 601-616.
- Miyashita, S., 2002. Studies on the seedlings production of the Pacific bluefin tuna, *Thunnus thynnus orientalis*. Bulletin of the Fisheries Laboratory of Kinki University 8, 1-171 (in Japanese).
- Munk, P., 1995. Foraging behavior of larval cod (*Gadus morhua*) influenced by prey density and hunger. Mar. Biol. 122, 205-212.
- Nakagawa, Y., Eguchi, M., Miyashita, S., 2007. Pacific bluefin tuna, *Thunnus*

- orientalis*, larvae utilize and nutrients of microbial loop. *Aquaculture* 267, 83-93.
- Nakayama, S., Masuda, R., Shoji, J., Takeuchi, T., Tanaka, M., 2003. Effect of prey items on the development of schooling behavior in chub mackerel *Scomber japonicus* in the laboratory. *Fisheries Science* 69, 670-676.
- Nakayama, S., Masuda, R., Tanaka, M., 2007. Onsets of schooling behavior and social transmission in chub mackerel *Scomber japonicus*. *Behav. Ecol. Sociobiol.* 61, 1383-1390.
- Noakes, D. L. G., 1978. Social behavior as it influences fish production. pp. 360-382. *In: S.D. Gerking (ed.) Ecology of Freshwater Fish Production*, Blackwell, Oxford.
- Noakes, D. L. G., Godin, J. G. J., 1988. Ontogeny of behavior and concurrent developmental changes in sensory systems in Teleost fishes. Hoar, W. S., Randall, D. J. (Eds.) *San Diego Academic Press. Fish Physiology*, XI B, 345-395.
- Olla, B. L., Davis, M. W., Ryer, C. H., 1994. Behavioural deficits in hatchery-reared fish: potential effects on survival following release. *Aquacult. Fish. Manage.*, 25 (1), 19-34.
- Sabate, F. de la S., Sakakura, Y., Shiozaki, M., Hagiwara, A., 2009a. Onset and development of aggressive behavior in the early life stages of the seven-band grouper *Epinephelus septemfasciatus*. *Aquaculture* 290, 97-103.
- Sabate, F. de la S., Sakakura, Y., Takebe, T., Nikaido, H., Matsumoto, N., Shiozawa, S., Hagiwara, A., Masuma, S., 2009b. Preliminary observations on the development of aggressive behavior in Pacific bluefin tuna *Thunnus orientalis*. *Aquaculture Science* 57, 329-335.

- Sakakura, Y., Tsukamoto, K., 1996. Onset and development of cannibalistic behavior in early life stages of yellowtail. *J. Fish Biol.* 48, 16-29.
- Sakakura, Y., Tsukamoto, K., 1998. Social rank in schools of juvenile yellowtail, *Seriola quinqueradiata*. *J. Appl. Ichth.* 14, 69-73.
- Sakakura, Y., Tsukamoto, K., 1999. Ontogeny of aggressive behavior in schools of yellowtail, *Seriola quinqueradiata*. *Environ. Biol. Fish.* 56, 231-242.
- Sakakura, Y., Tsukamoto, K., 2002. Onset and development of aggressive behavior in the early life stage of Japanese flounder. *Fisheries Science* 68, 854-861.
- Sakakura, Y., 2006. Larval fish behavior can be a predictable indicator for the quality of Japanese flounder seedlings for release. *Aquaculture*, 257, 316-320.
- Sawada, Y., Miyashita, S., Aoyama, M., Kurata, M., Mukai, Y., Okada, T., Murata, O., Kumai, H., 2000. Rotifer-size selectivity and optimal feeding density of bluefin tuna, *Thunnus thynnus*, larvae. *Suisanzoushoku* 48, 169-177 (in Japanese with English abstract).
- Sawada, Y., Okada, T., Miyashita, S., Murata, O., Kumai, H., 2005. Completion of the Pacific bluefin tuna, *Thunnus orientalis*, life cycle under aquaculture conditions. *Aquac. Res.* 36, 413-421.
- Sawada, Y., 2006. Morphological development and organogenesis of Pacific bluefin tuna *Thunnus orientalis*. *Nippon Suisan Gakkaishi* 72, 945-946.
- Smith, M.E., Fuiman, L.A., 2004. Behavioral performance of wild-caught and laboratory-reared red drum *Sciaenops ocellatus* (Linnaeus) larvae. *J. Exp. Mar. Biol. Ecol.* 302, 17- 33.
- Smith, C., Reay, P., 1991. Cannibalism in teleost fish. *Rev. Fish. Biol. Fish.*, 1, 41-64.
- Svåsand, T., 1993. Are reared juveniles suited for release? ICES Council Meeting F34, 1-13 (mimeogr.).

Tanaka, Y., Kumon, K., Nishi, A., Eba, T., Nikaido, H., Shiozawa, S., 2009. Status of the sinking of hatchery-reared larval Pacific bluefin tuna on the bottom of the mass culture tank with different aeration design. *Aquaculture Science* (in press).

Tezuka, N., Masuma, S., Koiso, M., Takebe, T., Nikaido, H., Ide, K., 2005. Effects of light intensity and water current on the survival of the early life stages of Pacific bluefin tuna *Thunnus orientalis*. *Saibai Gyogyou Center Gihou* 3, 41-44 (in Japanese).

Figure captions

Fig. 1. Growth curve of Pacific bluefin tuna (*Thunnus orientalis* Temminck and Schlegel, 1844) reared from hatch until the juvenile stage in a 50 kL tank at 27.5-28.5 °C with a water exchange rate of 150 %/day under a light intensity of 70-1000 Lux provided by unfiltered sunlight. Numbers in parenthesis above and below each data point indicate sample size.

Fig. 2. Changes in swimming behavior of Pacific bluefin tuna (*Thunnus orientalis* Temminck and Schlegel, 1844). Dots indicate the mean for every age group and bars indicate standard deviations (n = 5). (A): Swimming speed. (B): Relative swimming speed (swimming speed/SL). Significant differences are indicated by letters (Tukey-Kramer test, $p < 0.05$, $a < b < c < d$).

Fig. 3. Changes in schooling behavior of Pacific bluefin tuna (*Thunnus orientalis* Temminck and Schlegel, 1844). Dots indicate the average Separation Swimming Index (SSI; Nakayama et al., 2007) for every age group and bars indicate standard deviations (n = 45). Asterisks indicate significant difference between the mean in an age group and the expected SSI value for fish swimming at random directions and speeds (t -test, $p < 0.05$).

Fig. 4. Mean number of chases per minute per fish \pm standard deviation as an estimate of aggressive behavior in Pacific bluefin tuna (*Thunnus orientalis* Temminck and Schlegel, 1844). Means were calculated from four observation periods per day at 7, 12, 14, 16, 20 and 26 DAH. Behaviors were observed using an observation chamber that was varied in dimensions depending on fish size (see body of text).

Fig.1 Sabate et al.

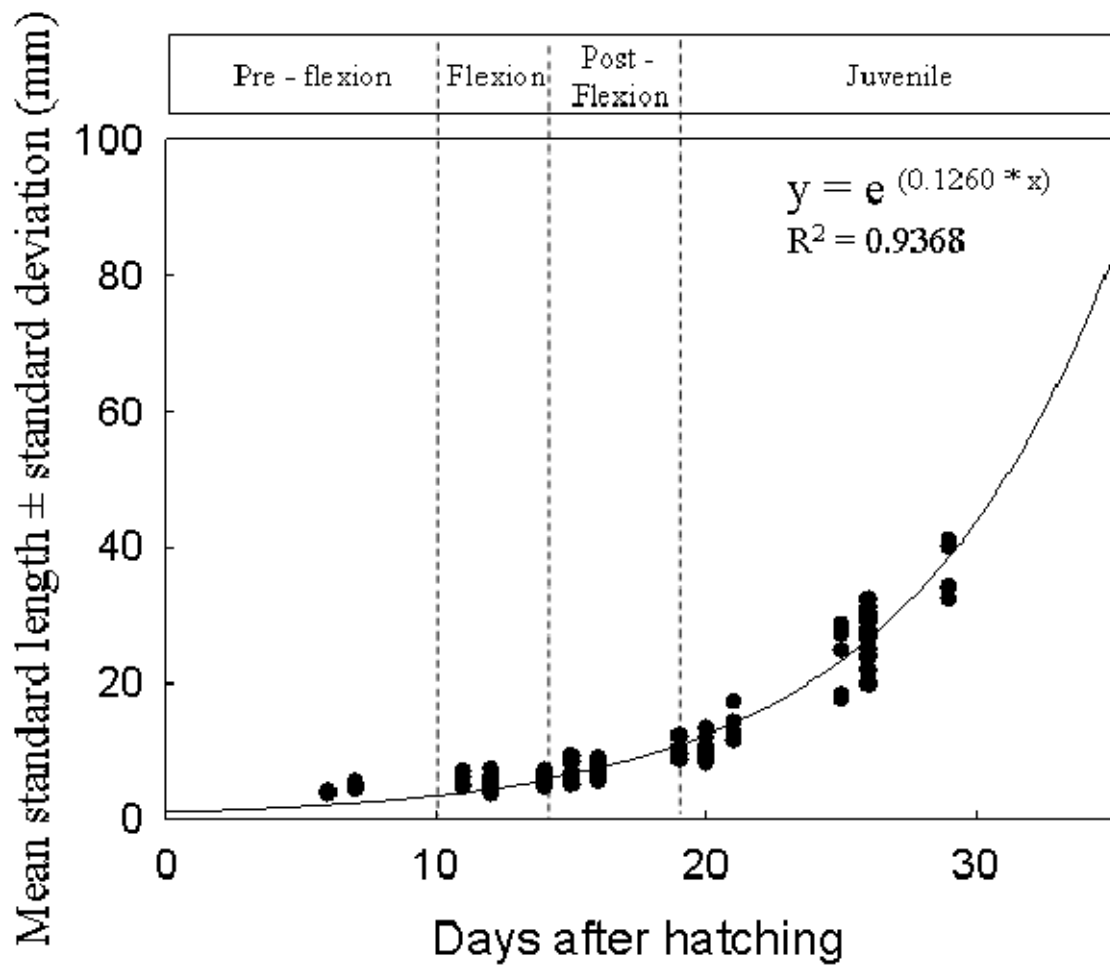


Fig.2 Sabate et al.

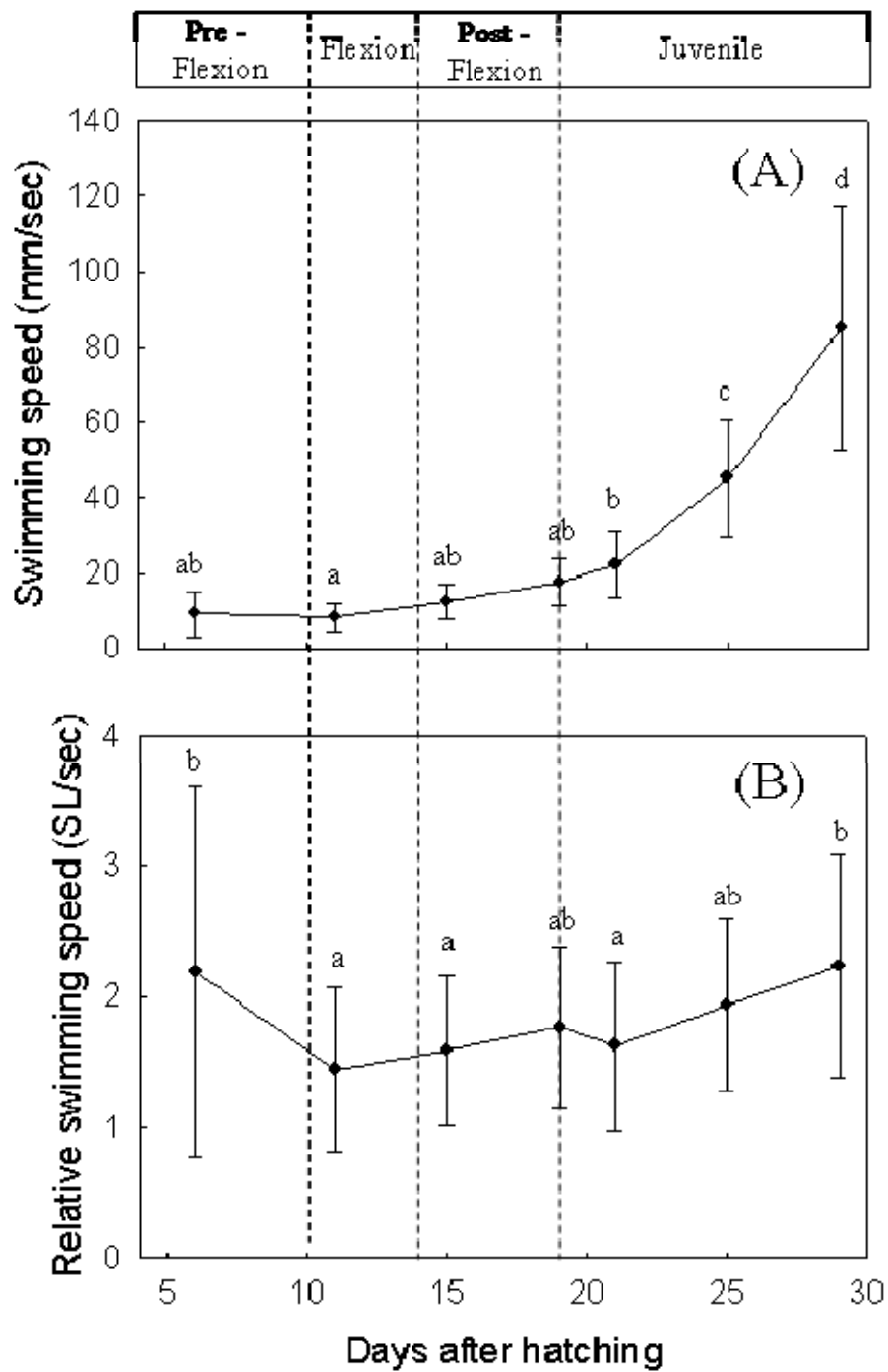


Fig.3 Sabate et al.

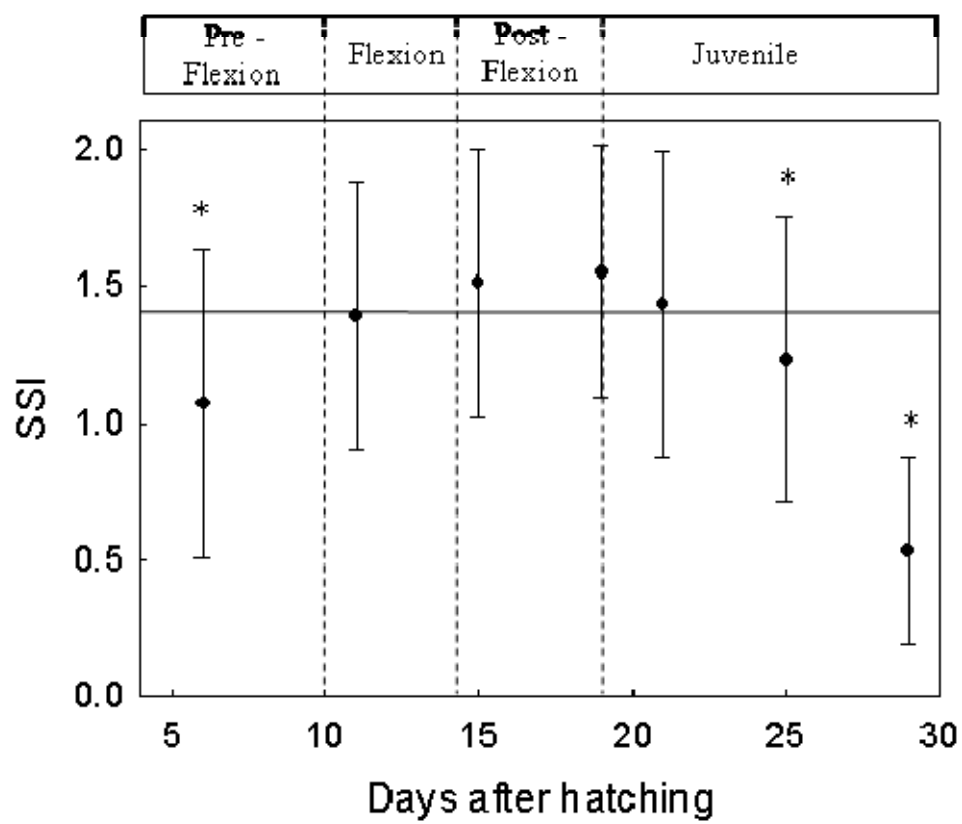


Fig.4 Sabate et al.

