Comparison of behavioural development between Japanese flounder (*Paralichthys olivaceus*) and spotted halibut (*Verasper variegatus*) during early life stages

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Summary

Behavioural development was compared between two flatfish species (Japanese flounder and spotted halibut) from hatching to settlement (juvenile stage) in order to speculate on the ecology of the early life stages of both species and to provide fundamental knowledge for improving seedling production techniques for stock enhancement. Fish were cultured under the same rearing conditions (500 L tank maintained at 17.8±0.4°C, 34 ppt, 10L:14D light regime and an initial stocking density of 20 larvae/L). Behavioural observations were conducted at about 4-day intervals from hatching to the juvenile stage. Fish were sampled randomly from the rearing tank, and one fish was transferred into a 250 mL observation container. Their behaviour was video-recorded for 5 minutes without food and for an additional 5 minutes with live feed (rotifer or *Artemia*). The observation was repeated 10 times on every sampling day. All behavioural data were sorted according to eight developmental stages, and these parameters were compared among developmental stages and between species.

The average standard length of the spotted halibut was significantly greater than that of the Japanese flounder in all developmental stages, while the development of the Japanese flounder was faster than that of the spotted halibut. For Japanese flounder, feeding, swimming and Ohm-posture (typical shivering behaviour that is seen during early life stages in...
flatfishes) frequency were highest before metamorphosis (mean±SD; 1.0±2.0 attacks/min, 24.0±9.6 actions/min, 1.1±1.1 counts/min, respectively). Spotted halibut expressed feeding behaviour frequently from the beginning of metamorphosis (3.6±5.2 attacks/min), had relatively low swimming activity during all the developmental stages, and showed a peak of Ohm posture frequency during the flexion stage (2.6±1.0 counts/min).

Introduction

The Japanese flounder *Paralichthys olivaceus* is a marine demersal species abundant along the coasts of Japan, Korea and China. It can reach a size of 75 cm (Dou, 1995a) and is commercially important in Japan. In the 1980s, a drastic decrease in the natural stocks of Japanese flounder spurred the creation of stock enhancement programmes (Tanaka et al., 1998). More than 30 million juveniles are artificially produced and released every year, making the Japanese flounder one of the most important species in stock enhancement programmes in Japan (Tanaka et al., 1998). The spotted halibut *Verasper variegatus* is another commercially valuable demersal species distributed in the eastern parts of Asian coastal waters, reaching 53 cm in maximum body length (Dou, 1995a). Since the total catch of *V. variegatus* has been continuously decreasing in Japan, this species became a major target for stock enhancement after Japanese flounder (Wada et al., 2004).

In spite of the stock enhancement programmes of both the Japanese flounder (Furuta et al., 1997, Furuta, 1998) and spotted halibut (Imamura, 1999, Kitada, 1999), the desired effectiveness has not yet been achieved because of the high mortality of seedlings after their release, mainly because of predation, including cannibalism by wild stocks. It has been suggested that differences in feeding behaviour between wild and reared juveniles, which translate into longer residence in the open water column, can cause this high predation on released Japanese flounder juveniles (Furuta, 1998). Detailed study of the development of fish
behaviour can be helpful for predicting the ability of reared fish to survive in the wild environment and for predicting and/or improving stock enhancement effectiveness by producing higher quality fish (Tsukamoto et al., 1997). Such study will also provide fundamental knowledge that will help improve seedling production techniques. Recently, Ohm posture, which is a typical shivering behaviour that occurs during the early life stages in Japanese flounder, has been positively correlated to high growth rates in the juvenile stage (Sakakura et al., 2004) and has been confirmed to be related to higher aggressiveness in the juvenile stage (Sakakura, 2006). Given the apparently important effects of this behaviour on the ecology of the Japanese flounder, we decided to include a study of Ohm posture in our research as well as to confirm its expression in other flatfish species. The present study compares the development of the behaviour of the Japanese flounder and spotted halibut from hatching to settlement (juvenile stage) in order to determine common behavioural patterns and their ecological meanings.

Materials and Methods

Rearing conditions

Two rearing trials were conducted at the Aquaculture Biology Laboratory, Nagasaki University, Japan: one for Japanese flounder from 1 December 2004 to 25 January 2005 (56 days), and another for spotted halibut from 27 January 2005 to 28 March 2005 (63 days). Fertilized Japanese flounder eggs were purchased from a private fish farmer (MBC Kaihatsu Co. Ltd., Kagoshima, Japan). Naturally spawned spotted halibut eggs were transferred from Nagasaki Prefectural Fisheries Experimental Station, Nagasaki, Japan.

Rearing conditions were set according to previous experiments on Japanese flounder (Sakakura and Tsukamoto, 2002, Sakakura et al., 2004, Sakakura, 2006). A 500-L black polyethylene tank was used as a rearing tank. It was equipped with ceramic sand for fish
rearing as a substrate on the bottom (MS-0 Micros Ceramic, Norra Co. Ltd, Kyoto, Japan) and was filled with 34-ppt artificial seawater (Marine artHi, Tomita Pharmaceutical Co. Ltd., Tokushima, Japan). A recirculating system was used, which consisted of a 100-L auxiliary tank and a biological filter composed of ceramic sand for fish rearing (MS-10 Micros Ceramic, Norra Co. Ltd, Kyoto, Japan). Seawater was siphoned from the rearing tank into the 100-L tank using a vinyl hose with 100-μm mesh. The water was then drained by an electrical pump into the biological filter and was poured into the rearing tank. The recirculation system worked at a water exchange rate of 280% daily. Water temperature was maintained at 17.8±0.4°C in a temperature-controlled room and was checked at 9:00 and 15:00. The photoperiod was 10L:14D to simulate winter day length, and the light intensity in the daytime was about 600 Lx on the water surface.

A total of 10,000 eggs were introduced into the rearing tank. Japanese flounder had a hatching rate of 95%, while spotted halibut reached 100% (initial stocking density of 19 larvae/L for Japanese flounder and 20 larvae/L for spotted halibut). Larvae were fed with two types of live feeds, L-type rotifers *Brachionus plicatillis* sp. complex (Hagiwara et al., 2007) and *Artemia franciscana*. The rotifers were cultured with HUFA-enriched *Chlorella vulgaris* (Super Fresh *Chlorella* V-12, SFCV12, *Chlorella* Industry Co. Ltd., Fukuoka, Japan), and the *Artemia* nauplii were enriched with Super Capsule Powder A-1 (*Chlorella* Industry Co. Ltd., Fukuoka, Japan). SFCV12 was added to the rearing tank at an initial concentration of 200,000 cells/mL. Every morning at 9:00 the concentration of SFCV12 in the rearing tank was checked, and was adjusted to the initial concentration when necessary. We fed rotifers to Japanese flounder at a density ranging from 5 to 10 ind/mL from mouth opening (3 days after hatching, DAH; standard length, SL 2.6±0.2 mm, mean ± standard deviation) to the flexion stage (19 DAH, SL 8.0±1.3 mm). Afterwards, they were fed enriched *Artemia* nauplii at a density ranging from 0.1 to 3.0 ind/mL. Spotted halibut were fed rotifers at a density of 10
ind/mL from mouth opening (4 DAH, SL 4.5±0.4 mm) to the climax of metamorphosis (37 DAH, SL 11.2±0.6 mm) and Artemia nauplii at a density of 0.3-1.3 ind/mL from the flexion stage (28 DAH, SL 10.4 ± 0.5 mm) until the end of the experiment. Feed density was maintained twice daily, at 9:00 and 14:00, in both rearing trials.

At hatching, a sample of 40 fish was taken, and thereafter 10-20 fish were sampled every 3 days from the rearing tank to measure SL and determine their developmental stages. These fish were immediately anesthetized with 0.01% of MS222 (Tricaine; Sigma Chemical Co., St Louis, MO, USA) and fixed in 5% formalin solution, and then SL and the developmental stage of each sample was investigated. We defined the developmental stage of the fish (Table 1) based on the previous definitions for Japanese flounder (Fukuhara, 1986) and for spotted halibut (Aritaki et al., 2001). To measure SL, a digital microscope (VH-6300, Keyence Corp., Osaka, Japan) was used.

Behavioural observations

Behavioural observations were conducted at about 4-day intervals after hatching. Ten fish were randomly taken from the rearing tank, transferred to a 1-L beaker containing fresh seawater, and starved for 12 hours at 18°C following Dou et al. (2000). Observations were performed in a black-walled enclosed chamber (100 cm length, 90 cm width and 250 cm height) to prevent interference from the observer and external illumination. Light conditions were set to match those on the surface of the rearing tank (600 Lx, Sakakura and Tsukamoto, 2002). Using a small glass beaker, one fish was gently introduced into an observation tank (10.5 cm length, 8 cm width and 6 cm height, Matsuo et al., 2006) filled with 225 mL of artificial seawater (3.5 cm depth). Following the previous studies on the larval behaviour of marine larvae (Sakakura and Tsukamoto, 2002, Sakakura et al., 2004, Matsuo et al., 2006, Sakakura, 2006), an acclimation time of 10 minutes after the transfer to the observation tank.
was set. Video recordings of the behaviour of the fish were made from above for 5 minutes by
digital video camera (SONY DCR-TRV9, SONY Corp., Tokyo, Japan, Sakakura and
Tsukamoto, 2002, Matsuo et al., 2006). Afterwards, live feed was added (1 rotifer/mL or 0.1
Artemia nauplii/mL, according to the live feed in the rearing tank) and the behaviour was
recorded for another 5 minutes. The live feed density used in the observations was 10 times
lower than that of the rearing tank in order to simulate natural conditions. Immediately after
the recording, the larvae were anesthetized with 0.01% of MS222 and fixed in 5% formalin
solution to determine their SL and developmental stage.

In order to quantify the behavioural observations, three behavioural categories and 9
indexes were defined: (i) feeding behaviour: attacking live feed (number of attacks/minute,
Dou et al., 2000), feeding incidence (proportion of larvae with food in the gut among the
samples, Dou et al., 2000), pre-strike distance (distance from the snout of the larvae to live
feed at the moment of the attack, Hunt von Herbing and Gallager, 2000) and relative pre-
strike distance (pre-strike distance/SL of larvae); (ii) swimming: swimming activity (sum of
the frequencies of turns and moves in a straight line), swimming speed (Fukuhara, 1986) and
relative swimming speed (swimming speed/SL); (iii) Ω-posture: frequency and duration.

Video analysis was performed to measure the frequency of the index behaviours and
the duration of the Ω-posture, and to measure the pre-strike distance and swimming speed.
The swimming speed was determined from the video recordings by analyzing 20 seconds of
swimming behaviour before feeding using the computer programme LARVAE version 0.9,
developed with the cooperation of the Aquaculture Biology Laboratory, Nagasaki University,
and Dr. Nobuyoshi Taguchi at the Technology Center of Nagasaki Prefecture, Nagasaki,
Japan. To measure the feeding incidence a sampling of 10 individuals was taken from the
rearing tank every 2 days.
Statistical analysis

To compare SL, pre-strike distances and swimming speeds between Japanese flounder and spotted halibut, the Student \( t \)-test was undertaken for each developmental stage. One-way ANOVA was performed in order to compare the differences in behavioural indexes among the developmental stages in each species. If a significant difference was detected \((p<0.05)\), the Tukey-Kramer test was used for multiple comparisons. For the rest of the data (feeding attacks, swimming activity, \( \Omega \) frequency per minute and \( \Omega \)-posture duration), the Mann-Whitney \( U \)-test was applied for comparisons between species at the same developmental stage. The Kruskal-Wallis test was used to determine the difference in these parameters among the developmental stages in each species. If a significant difference was found \((p<0.05)\), Dunn’s non-parametric multiple comparison test was performed. Statistical analysis of the data was performed with the computer programme Statview version 5.0 for Windows (SAS Institute Inc., Tokyo, Japan).

Results

Growth and development

The early metamorphosis stage (stage VI, Table 1) appeared at 28 DAH and 35 DAH in the Japanese flounder and the spotted halibut, respectively (Fig. 1). In the Japanese flounder about 95% of the fish reached the juvenile stage at 54 DAH (stage VIII, Table 1) compared to 50% in spotted halibut of the same age. The Japanese flounder showed faster development than the spotted halibut (Fig. 1).

Growth in the present experiment was only used to characterize and compare developmental stages in both species under the experimental conditions. Size at hatching was 2.6±0.2 mm for the Japanese flounder and 3.9±0.2 mm (stage I, Table 1) for the spotted halibut (Fig. 2). According to age, the spotted halibut was bigger than the Japanese flounder.
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\((t\text{-test, } df=18 \text{ to } 78, t=-27.371 \text{ to } -3.779, p<0.05),\) except at 12, 18 and 21 DAH, when no difference was observed, and 15 DAH, when the Japanese flounder was significantly bigger \((t\text{-test, } df=28, t=2.092, p<0.05).\) According to developmental stage, the spotted halibut had significantly bigger body size than the Japanese flounder throughout the experiment \((t\text{-test, } df=89 \text{ to } 159, t=-28.8 \text{ to } -3.8, p<0.05),\)

**Behavioural observations**

Feeding attacks of Japanese flounder were observed from mouth opening (stage II, Table 1; 0.7±2.6 attacks/min, Fig. 3) and showed higher frequencies between the pre-flexion (stage III, Table 1) and early metamorphosis stages (Kruskal-Wallis test, df=7, \(H=37.4, p=0.01\)). The attack frequency of Japanese flounder decreased thereafter. For spotted halibut, feeding behaviour was first recorded in the flexion stage (stage IV, Table 1; 0.2±0.9 attacks/min). However, the feeding incidence of larvae in the rearing tank showed active feeding from the pre-flexion stage and maintained high values from there on until the end of the experiment (Fig. 3). The attack frequency of the spotted halibut increased from the flexion stage onward and remained high until the end of the experiment (0.2±0.9 to 3.6±5.2 attacks/min; Kruskal-Wallis test, df=7, \(H=35.7, p=0.04\)). The Japanese flounder had a significantly higher feeding frequency during the flexion stage than did the spotted halibut \((U\text{-test, } U=172.0, p=0.01).\) However, in the juvenile stage, the spotted halibut had a higher feeding frequency than the Japanese flounder \((U\text{-test, } U=80.0, p=0.03).\)

Pre-strike distance and relative pre-strike distance in the Japanese flounder did not change with developmental stage (Fig. 4, for Japanese flounder, 0.14±0.03 mm and 0.016±0.004 pre-strike distance/SL respectively, and for spotted halibut 0.20±0.03 mm and 0.014±0.003 pre-strike distance/SL respectively). Although in the spotted halibut the pre-strike distance did not change with developmental stages (Tukey-Kramer test, \(p=0.16),\) the relative pre-strike distance decreased after the metamorphosis (ANOVA, df=4, \(F=6.7,\)
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$p=0.001$). Pre-strike distance was significantly longer at early metamorphosis in the spotted halibut than in the Japanese flounder ($t$-test, $df=13$, $t=-4.5$, $p=0.0006$).

The swimming activity of Japanese flounder was relatively high from the yolk-sac stage (4.6±2.9 actions/min, Fig. 5) and showed high frequencies between the mouth-opening (15.1±13.4 actions/min) and post-flexion stages (stage V, Table 1; 24.0±9.6 actions/min; Kruskal-Wallis test, $df=7$, $H=51.0$, $p<0.0001$). Afterwards, swimming activity occurred with low frequency from metamorphosis to the juvenile stage (10.1±7.0 to 7.5±5.1 actions/min).

For the spotted halibut, swimming activity was high between mouth opening and the climax of metamorphosis (stage VII, Table 1), and it decreased in the juvenile stage (Kruskal-Wallis test, $df=7$, $H=46.3$, $p<0.0001$). The swimming activity of the Japanese flounder was significantly higher than that of the spotted halibut during the mouth-opening ($U$-test, $U=324.5$, and $p=0.002$), flexion ($U$-test, $U=68.0$, and $p<0.0001$) and juvenile stages ($U$-test, $U=60.0$, and $p=0.004$).

The swimming speed of Japanese flounder increased at the mouth-opening stage (16.0±3.0 mm/sec, Fig. 5) and did not change in the following stages (ANOVA, $df=7$, $F=2.765$, $p=0.01$). On the contrary, the swimming speed of the spotted halibut decreased from the yolk-sac stage to the flexion stage (16.1±2.5 mm/sec; ANOVA, $df=7$, $F=7.8$, $p<0.0001$). Swimming speed showed a peak at the climax of metamorphosis (35.3±14.2 mm/sec) and decreased until the juvenile stage. The swimming speed of the spotted halibut was significantly faster than that of the Japanese flounder at mouth opening ($t$-test, $df=63$, $t=-3.7$, $p=0.0004$), post-flexion ($t$-test, $df=15$, $t=-2.2$, $p=0.04$), early metamorphosis ($t$-test, $df=46$, $t=-3.9$, $p=0.0003$) and climax of metamorphosis ($t$-test, $df=33$, $t=-2.8$, $p=0.01$).

Relative swimming speed in the Japanese flounder decreased from the yolk-sac stage (6.2±2.1 SL/sec, Fig. 5; ANOVA, $df=7$, $F=33.5$, $p<0.0001$) until the juvenile stage. For the spotted halibut, a similar pattern was observed (ANOVA, $df=7$, $F=21.7$, $p<0.0001$).
Significant differences between the two species were found in the yolk-sac ($t$-test, $df=28$, $t=3.2$, $p=0.003$) and flexion stages ($t$-test, $df=46$, $t=3.9$, $p=0.0003$), when the Japanese flounder was faster than the spotted halibut.

No $\Omega$-posture was recorded in the yolk-sac stage of either species (Fig. 6). For the Japanese flounder, the frequency of fish showing a $\Omega$-posture rapidly increased in the mouth-opening and pre-flexion stages (70.3% and 69.2% respectively), decreased slowly afterwards, and disappeared at the climax of metamorphosis. For spotted halibut, the frequency of fish showing a $\Omega$-posture increased rapidly toward the post-flexion stage. Afterwards, this frequency decreased and disappeared in the juvenile stage.

High frequencies of $\Omega$-posture in Japanese flounder were observed between the mouth-opening stage and the post-flexion stage (Fig. 6; Kruskal-Wallis test, $df=7$, $H=34.5$, $p<0.0001$). In spotted halibut, $\Omega$-posture frequency increased until the post-flexion stage and disappeared at the climax of metamorphosis (Kruskal-Wallis test, $df=7$, $H=74.3$, $p<0.0001$). $\Omega$-posture frequencies were significantly different between the two species at the mouth-opening stage ($U$-test, $U=372.0$, $p=0.01$), when the Japanese flounder had a higher frequency, and in the flexion stage ($U$-test, $U=19.5$, $p<0.0001$), post-flexion stage ($U$-test, $U=11.5$, $p=0.02$) and early metamorphosis ($U$-test, $U=175.0$, $p=0.002$), when the spotted halibut had higher frequencies than the Japanese flounder.

**Discussion**

Since the rearing method in the present study, following other studies (Sakakura and Tsukamoto, 2002, Sakakura et al., 2004, Sakakura, 2006), was designed to maximize the survival of larvae, it may not represent exactly natural conditions. Therefore, there may be differences in behaviour between wild and reared fish. Sensory deprivation caused by the buffering nature of the rearing tank, which may reduce the sensory input of a/biotic factors for
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fish at early life stages to below required minimum levels, leads to the abnormal development of sensory organs (Blaxter, 1970), and high stocking density can produce increases in size range and stress (Blaxter, 1975), all of which produce adverse effects on the quality of the larvae. Consequently, these factors can mask whether the results obtained under rearing conditions apply to wild populations. However, the fish in the observation tank were acclimatized as in other studies on Japanese flounder behaviour (Sakakura and Tsukamoto, 2002, Sakakura et al., 2004, Sakakura, 2006), and the conditions in the observation tank were as similar to the rearing tank as possible (salinity, light intensity, temperature, tank colour and live feed offered); therefore, we presume that the data collected can accurately represent behavioural changes. Moreover, our results were similar to the results from the former studies in terms of growth (Fukuhara, 1986, Aritaki et al., 2001) and swimming speed (Fukuhara, 1986) for both species; hence we can assume that the growth and behaviour of the fish in this study are representative for each species.

Comparison of growth and development between the two species and ecological implications

The Japanese flounder showed faster development than the spotted halibut, and achieved complete settlement of juveniles almost one week earlier than the spotted halibut under the same rearing conditions (Fig. 1). Earlier settlement will reduce the pelagic period of the larvae, the most vulnerable stages of development in these species, while settlement and development of cryptic colouration of the body during the juvenile stage will reduce their exposure to predators.

Although a high feeding incidence (95-100 %, Fig. 3) in the rearing tank was observed during the metamorphosis and juvenile stages, attack frequency in the Japanese flounder was highest just before metamorphosis, and almost no attacks were observed at the peak of metamorphosis in the video recordings (Fig. 3). This contradiction can be explained by the
difference in feed density used in the rearing and observation tanks or as an effect of sensory deprivation, in that fish in a new environment may not receive complete sensory input, and thus conditioned behaviour may be observed (Blaxter, 1970). Hence, we think that the feeding incidence in the rearing tank may indicate that the Japanese flounder is capable of ingesting feed during metamorphosis, while the attack frequency in this stage suggests that larvae will not search for prey actively. For the spotted halibut, the feeding behaviour began to increase just before metamorphosis (Fig. 3), and high attack frequencies were maintained until the juvenile stage. Although in some species, such as the maroon anemonefish *Premnas biaculeatus* (Job and Bellwood, 1996), Atlantic cod *Gadus morhua* L. (Hunt von Herbing and Gallager, 2000) and striped trumpeter *Latris lineata* (Cobcroft and Pankhurst, 2006), the relative pre-strike distance increases with age, it decreased in the Japanese flounder and spotted halibut, indicating that changes occur in their feeding habits during settlement towards the juvenile stage (Dou, 1995b). Although ingestion was observed during the video recordings, feed capture success was not analyzed in this study. As capture success increases with developmental stage (Rosenthal, 1970, Blaxter and Staines, 1971), we consider that further research on feeding success in the spotted halibut and Japanese flounder is needed.

The fast relative swimming speeds during the yolk-sac and mouth-opening stages in both species (Fig. 5) can be attributed to a predator avoidance tactic displayed as dispersion of larvae after hatching (Fuiman and Magurran, 1994). In the mouth-opening and flexion stages, swimming activity was higher in the Japanese flounder than the spotted halibut (Fig. 5). The high swimming activity of the Japanese flounder during pre-flexion and post-flexion may be related to the long migratory movements that the Japanese flounder performs from pre-flexion to the end of the metamorphosis stage (Burke et al, 1995).

Although the swimming speed of the spotted halibut was faster than that of the Japanese flounder beginning at the post-flexion stage, the relative swimming speeds of the
two species were similar throughout the experiment (Fig. 5), so we can conclude that the
higher swimming speed of the spotted halibut was due to the fact that its body size was bigger
than that of the Japanese flounder at the same developmental stage. Moreover, the low
swimming activity of the spotted halibut seems to indicate that the spawning and nursery
grounds are close to each other, and likely coexist, indicating the likelihood that no long
migration occurs in this species, as pointed out by Wada et al. (2004).

Both species settle in coastal waters when they become juveniles (Japanese flounder,
Gibson, 1997, Burke et al., 1995; spotted halibut, Wada et al., 2004). At this stage, their
swimming activity and relative swimming speed decreased to minimum levels (Fig. 5) as a
consequence of the change in habitat. However, the swimming activity of the Japanese
flounder was still higher than that of the spotted halibut. This difference in swimming activity
between the two species can be attributed to the onset of aggressive behaviour in the Japanese
flounder at settlement during the juvenile stage (Sakakura and Tsukamoto, 2002), while no
aggressive behaviour was observed in the spotted halibut in the present study.

More frequent $\Omega$-posture was observed in the spotted halibut than in the Japanese
flounder (Fig. 6). Sakakura et al. (2004) found a positive relation between $\Omega$-posture
frequency during the larval stages and growth during the juvenile stage. Accordingly, a higher
growth rate may be found for the spotted halibut than for the Japanese flounder with further
rearing experiments.

*Implications for seedling production*
The number of feeding attempts indicates periods during which the larvae have high energy requirements. Accordingly, more live feed may be required before metamorphosis in the Japanese flounder and beginning at pre-flexion in the spotted halibut.

During stages of high swimming activity, sufficient water volume and the creation of sufficient water velocity by aeration should be used to allow larval free swimming and to prevent possible stress (Sakakura, et al., 2007). Consequently, water velocity and volume should be increased from mouth opening to the flexion stage in the Japanese flounder and from mouth opening to the pre-flexion stage in the spotted halibut. Afterwards, a constant reduction of water volume until the juvenile stage will synchronize their metamorphosis as indicated in Klokseth and Oiestad (1999) for the halibut *Hipoglossus hipoglossus* L.

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**References**


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Table 1. Definition of developmental stages for 2 flatfish species compared in this study

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>Japanese flounder (Fukuhara, 1985)</th>
<th>Spotted halibut (Aritaki et al., 2001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>yolk sac larva</td>
<td>A</td>
<td>A,B</td>
</tr>
<tr>
<td>II</td>
<td>mouth opening larva</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>III</td>
<td>pre-flexion larva</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>IV</td>
<td>flexion larva</td>
<td>D,E</td>
<td>E</td>
</tr>
<tr>
<td>V</td>
<td>post-flexion larva</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>VI</td>
<td>early metamorphosis larva</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>VII</td>
<td>climax of metamorphosis larva</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>VIII</td>
<td>juvenile</td>
<td>I</td>
<td>I</td>
</tr>
</tbody>
</table>
Fig. 1. Development of Japanese flounder and spotted halibut describing the transition from yolk sac larvae to juveniles in terms of the percent of observed individuals, indicating the overlap of stages within certain size ranges. The numbers at the tops of the columns represent the average SL in mm. The developmental stage of the larvae was defined as: I, Yolk sac; II, Mouth opening; III, Pre-flexion; IV, Flexion; V, Post-flexion; VI, Early metamorphosis; VII, Climax of metamorphosis; VIII, Juvenile. Top: Japanese flounder. Bottom: spotted halibut.
Fig. 2. Growth curve of Japanese flounder and spotted halibut. Open dots indicate Japanese flounder and solid dots indicate spotted halibut. A: Growth curve expressed by age in days (for spotted halibut, n=40 at hatching, n=20 afterwards; for Japanese flounder, from hatching until 18 DAH n=10, after 18 DAH n=20). B: Growth curve expressed by developmental stage. Bars indicate standard deviations. Numbers in parentheses beside each data point indicate sample size.
Fig. 3. Changes in feeding behaviour of Japanese flounder and spotted halibut expressed by developmental stage. Open dots indicate Japanese flounder and solid dots indicate spotted halibut. A: Attack frequency. B: Feeding incidence. Bars indicate standard deviations. Numbers in parentheses beside each data point indicate sample size for that developmental stage.
Fig. 4. Changes in feeding behaviour of Japanese flounder and spotted halibut expressed by developmental stage. Open dots indicate Japanese flounder and solid dots indicate spotted halibut. A: Pre-strike distance of the attacks observed. Numbers in parentheses beside each data point indicate attacks observed for that developmental stage (normal font for spotted halibut and italics for Japanese flounder; same sample size for both graphs). B: Relative pre-strike distance (defined as pre-strike distance/SL of the larvae) of the attacks observed. Bars indicate standard deviations.
Fig. 5. Changes in swimming behaviour of Japanese flounder and spotted halibut expressed by developmental stage. Open dots indicate Japanese flounder and solid dots indicate spotted halibut. A: Swimming activity. Numbers in parentheses beside each data point indicate sample size for that developmental stage (same sample size for all the graphs). B: Swimming speed. C: Relative swimming speed. Bars indicate standard deviations.
Fig. 6. Changes in ohm posture of Japanese flounder and spotted halibut expressed by developmental stage. Open dots indicate Japanese flounder and solid dots indicate spotted halibut. A: Proportion of fish showing ohm posture. Numbers in parentheses beside each data point indicate sample size for that developmental stage (same sample size for both graphs). B: Frequency of ohm posture. Bars indicate standard deviations.