Feeding behavior and capture success of turbot *Psetta maxima* larvae during the transition from upright to tilted swimming position

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Abstract – Aquaculture production of high-quality marine fish larvae might be hampered by poor success in larval initiation of exogenous feeding or the lack of appropriate live feed in their first feeding period. The period of larval metamorphosis may further constrain the successful rearing of flatfish larvae. In order to ascertain changes in feeding during metamorphosis of flatfish, we here compared feeding behavior when larvae of turbot *Psetta maxima* were either swimming upright or tilted. Using video recordings, we compared the attack rate and prey capture success between flexion (12–13 days-post-hatch, stage 4b–4c) swimming predominantly in upright position and post-flexion (16–17 days-post-hatch, stage 5a–5b) larvae in tilted swimming mode. Both larval groups were fed on copepod nauplii and copepodites. Our results showed a capture success of <50% during the flexion stage, increasing to 73% in the post-flexion stage, and larvae were more successful when feeding on nauplii than when offered copepodite stages. An ontogenetic shift from intermittent to cruise swimming was observed during the metamorphosis concomitant with improved hunting skills. Thus larvae appeared to be able to successfully complete metamorphosis without compromising their feeding ability on copepod prey.

Keywords: Flatfish / Turbot / Larvae / Capture success / Feeding behavior / Copepods

1 Introduction

Aquaculture production of high-quality marine fish larvae could be impeded by problems such as an unsuccessful larval transition from endogenous to exogenous food (Shields, 2001), or an inability to provide suitable live feed (e.g. Rao, 2003). The process of metamorphosis might be an additional critical phase for larvae of flatfish species due to the profound morphological and physiological changes during this stage. After hatching, larvae of flatfishes have bilaterally symmetric bodies and swim in an upright position. During metamorphosis, one of the former lateral sides becomes the ventral side of the fish and they settle to the bottom. In particular, the migration of one of the eyes to the other side constitutes a dramatic change during development of flatfish larvae (Osse and Van den Boogaart, 1997). This transformation can affect the ability to search, locate and capture prey (Geffen et al., 2007). For example, it has been observed that feeding success of plaice *Pleuronectes platessa* could be reduced during eye migration, suggesting the need for a period of readjustment to the new position of eyes (Osse and Van den Boogaart, 1997; Blaxter, 1986). De La S. Sabate et al. (2008) reported an enhanced attack frequency for Japanese flounder *Paralichthys olivaceus* just before metamorphosis, while almost no attacks were observed at the peak of metamorphosis. On the other hand, the feeding behavior of spotted halibut *Verasper variegatus* began to increase just before metamorphosis and high attack frequencies were maintained until the juvenile stage of this species. For larvae of turbot *Psetta maxima* Person-Le-Ruyet (2010) observed “visible swimming disturbances” during the transition from a pelagic to a benthic status.

In this study, we present an analysis of turbot larval behavior to assess whether the transition period from the
2 Materials and methods

2.1 Production system

The investigation took place in 2012 in a land based fish farm (Maximus A/S) located at Limfjorden, Denmark. The farm uses an outdoor semi-intensive production system where turbot larvae are fed live feed from the yolk stage until end of metamorphosis. Turbot larvae were sampled from outdoor open-top concrete tanks; 2 large tanks (volume: 220 m³, depth: 2.5 m) and 1 smaller tank (volume: 54 m³, depth: 1.5 m). All tanks were filled with pre-filtered water from the estuary (Hydrotech HDF 2007-2H drum filter, Sweden, fitted with 50 μm screens). No water renewal or mechanical mixing was applied and no organic nutrients were added to the tanks.

Copepods were provided prior to the addition of turbot larvae, allowing the development of a planktonic prey community for the turbot larvae. Copepods and adult copepods were collected by a wheel filter (UNIK Rotating Wheel Filter Type 1200, Oslo, Norway, mesh size 250 μm) which filtered the seawater pumped to the concrete tanks from a storage tank where a seeding culture of copepods is maintained to prevent parasites in the culture system (an extensive description of the copepod collection method with UNIK filters is made by van der Meeren et al., 2014). Generally a species succession is seen during the annual production period: Acartia spp. dominates in spring and early summer, whereas Centropages hamatus dominates in late summer (Jepsen et al., 2017). The monitoring of abiotic factors and of the planktonic community in the tanks is described in detail in Blanda et al. (2016).

A batch of yolk-sac turbot larvae (<48 h after hatching) was obtained from Stolt Sea Farm AS (Norway). The larvae were gently oxygenated to keep them in suspension and water from the tanks was slowly added to their transport box to acclimatize them during ~12 h. Then, the larvae were counted and released into the tanks. The density was 0.05–0.06 yolk sac larvae L⁻¹ (12 819 larvae per tank in the 220 m³ tanks and 2700 in the 54 m³ tank).

2.2 Experimental procedure

Table 1. Days-post-hatch (DPH), number of turbot larvae studied for each recording, larval standard length (flexion n=23; post-flexion n=16), larval developmental stages (after Al-Maghazachi and Gibson, 1984).

<table>
<thead>
<tr>
<th>DPH</th>
<th>Number larvae</th>
<th>Standard length (mm ± SE)</th>
<th>Developmental stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>12–13</td>
<td>3 ± 1</td>
<td>8.0 ± 0.2</td>
<td>Flexion (stage 4c–4d)</td>
</tr>
<tr>
<td>16–17</td>
<td>5 ± 0</td>
<td>11.5 ± 1.2</td>
<td>Post-flexion (stage 5a–5b)</td>
</tr>
</tbody>
</table>

Larvae 12–17 day-post-hatch (DPH) were gently sampled from the tanks with a hand-held plankton net. The number of sampled fish larvae varied due to difficulties in capturing them on the given sampling day. We studied and measured in total 13 larvae in the flexion stage (DPH 12–13), and 15 larvae in the post-flexion (DPH 16–17) stage (Table 1). The developmental stages, as described by Al-Maghazachi and Gibson (1984), and pictures of these two stages with their descriptions are provided in Figure 1. Copepod prey (Acartia spp. and Centropages hamatus) was provided at a density of 500 L⁻¹ and consisted of a mixture of mainly nauplii (50%, within the size range 50–250 μm), and copepodites and adults (50%, >250 μm).

For the experiments we used a glass aquarium (15 h × 16 w × 7 d, cm) filled with 45 μm filtered seawater from the production tanks (salinity 29%). The turbot larvae were randomly captured from the tanks and transferred into a 4 L
bucket 3 h before each recording. The sampled larvae were not fed during these 3 h and water was gently air-bubbled during the period. The larvae (3–5 at each experiment; Table 1) were then gently transferred into the experimental aquarium where they were allowed to acclimatize for a period of 30 min before starting the recording. The focus area of the video prior to each recording was calibrated using a ruler vertically positioned in the middle of the aquarium. The copepods were added to the experimental aquarium, where after the recording was started. For each replicate experiment, new larvae were used.

The experiments took place in a dark room, to improve visual contrast and thus facilitate the analysis of the videos. The video camera used was a Sony DXC-101P CCD (Japan) with mounted lens (Tamron EOS c80-210 mm, the range used was 100–120 mm). The camera was connected to a video converter (Grabster AU 350), to provide a close-up view of the aquarium. The frame rate was 25 frames s⁻¹. The camera was set on an optic bench in front of the aquarium, and a collimator lens was positioned between the aquarium and the used IR diode (880 nm), to provide infrared light to the camera. The distance between the diode and the aquarium was 37 cm. A portable programmable power supply (Iso-tech IPS 2010 3 V) was connected to the IR diode. One desktop lamp (halogen bulb 40 W) was positioned above the aquarium at a distance of 25 cm, to provide light to the larvae. Light intensity was measured at the beginning of each recording with a light-meter (Li-COR Model LI-185B, USA) and was throughout ca. 170 µmol m⁻² s⁻¹. The distance between the aquarium and the camera lens was 162 cm. The recorded area was 6.7 ± 1.3 cm wide and 5.5 ± 1.1 cm high. Only larvae in focus were analyzed.

Water temperature was checked at the beginning and at the end of each recording (18 ± 0.5 °C and 19 ± 0.5 °C, respectively). At the end of each recording session which lasted 60 min, the larvae were sedated by gently immersing them in filtered seawater with addition of carbonated water, and pictures were taken with a digital camera (Nikon Coolpix L100, Japan) mounted on a dissection microscope (Olympus SZ40, USA). The standard length of the larvae was measured using the image analysis software ImageJ (https://imagej.nih.gov/ij/).

Developmental stages (1–5) were determined using the scale developed by Al-Maghazachi and Gibson (1984).

### Table 2. Definition of turbot larval categories, or Modal Action Patterns (MAPs, after Brown, 1986).

<table>
<thead>
<tr>
<th>Definition of categories (Larval Modal Action Patterns (MAPs))</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Swim</strong></td>
<td>The larva swims actively</td>
</tr>
<tr>
<td><strong>Pause</strong></td>
<td>The larva is motionless for ≤2 s (similar to non-swimming in Munk, 1995) including when the larva rests immobile on the bottom of the aquarium</td>
</tr>
<tr>
<td><strong>S-shape</strong></td>
<td>The larva makes an S-shape with its body when locating a prey</td>
</tr>
<tr>
<td><strong>Tilted S-shape</strong></td>
<td>The tilted larva (benthic) makes an S-shape with its body when locating a prey</td>
</tr>
<tr>
<td><strong>Attack</strong></td>
<td>The larva performs a short forward burst while opening its mouth (after Munk, 1995)</td>
</tr>
<tr>
<td><strong>Shake</strong></td>
<td>Several rapid sideways movements propagating from head to tail</td>
</tr>
</tbody>
</table>

### 2.3 Behavioral analysis

The video recordings were analyzed according to larval foraging categories (Modal Action Patterns), abbreviated as MAPs (Brown, 1986) (Table 2). A kinematic diagram was compiled of transition rates from one behavioral category to another, describing the probability of a change from one MAP to another. Every single larva was visually tracked from when they entered the field of view of the camera to their disappearance from this view. The first minutes of each recording after the addition of the copepods were excluded from the final analysis due to the temporary high water turbulence.
The attack events were identified as larval burst forward toward a prey. The attack success was assessed from a shake of the head by the larvae and/or disappearance of the attacked copepod.

### 2.4 Statistical analysis

We tested whether the time allocated to the MAPs “Swim”, “Pause”, “S-shape”, “Tilted S-shape”, “Attack” and “Shake” was statistically different between flexion and post-flexion stages. As the individual observations within a track could not be assumed as being independent, we used a non-parametric bootstrap test. For the observed data the standard chi-square statistics (T-obs) was computed for the hypothesis $H_0$: the distribution of time on the six considered MAPs is the same for all days. Then $N=1000$ datasets were bootstrapped by randomly assigning days to the observed tracks. If the $H_0$ was valid those should be equally likely datasets. The chi-square test statistics was then computed for the bootstrapped datasets giving $N$ samples of the distribution of the test statistics $T$. The $p$ value for $H_0$ is now the fraction of the $T$-simulated, which are greater than $T$-obs.

To test whether the developmental stage had an effect on rates of performed S-shapes, Attacks and targeted prey, $t$-tests and $z$-tests were run in SigmaPlot (Version 12.5, 2015). Results were considered significant if $p < 0.05$.

Tests for time spent in individual MAP-categories were set up in the same way, and the Bonferroni correction for multiple testing was applied to counteract the problem that some tests could have been statistically significant by chance. Behavioral transition matrices were constructed for each stage by tabulating the frequencies of changes from one behavioral MAP to another MAP. We also performed a bootstrap analysis to test whether there was a significant difference in behavioral transitions between flexion and post-flexion stages. The criterion for significance was set at $p < 0.05$. These tests were run in R Ver. 3.0.3 (R Development Core Team, 2014).

### 3 Results

#### 3.1 Larval swimming

In the flexion phase (Fig. 1a) turbot larvae exhibited a swimming movement during which both caudal and pectoral fins were continuously moving. This swimming mode has been defined as “caudal-fin” swimming, as the movements of the caudal part of the body are pronounced (Kohno, 2001). The active swimming was interrupted by short breaks, Pause (defined MAP’s are hereafter in italics), in which neither the caudal nor the pectoral fins were moving. During the flexion stage Pause was the predominant category (45%) (Bootstrap test; $p < 0.001$) (Fig. 2).

In the post-flexion stage (Fig. 1b), swimming involved also use of dorsal and anal fins and the larvae switched between the pelagic, upright swimming (Fig. 1c), and tilted swimming, with a variable degree of tilting ($56^\circ \pm 7^\circ$, $n = 6$) (Fig. 1d, e). The Swim category was predominant (69%), while the Pause category was only performed 1% of the time (Bootstrap test; $p < 0.001$) (Fig. 2). The overall bootstrap test showed a significant difference in the distribution of behavioral patterns (MAPs) between flexion and post-flexion stages (Fig. 2).

#### 3.2 Attacks

Turbot larvae in attack posture bend their body in an S-shape posture from which they suddenly straighten and dart forward, as described by Jones (1972). However, not all larvae perform an S-shape during their preparation of an attack. The transition rate from Swim to Attack, without performing an S-shape to prepare the attack, was not significantly different between the flexion and the post-flexion stages (Fig. 3).

The rate of S-shapes decreased significantly from the flexion to the post-flexion stage, from $2.5 \pm 0.8$ to $1.9 \pm 0.6$ S-shapes $\text{min}^{-1}$ ($t$-test; $p < 0.05$) (Fig. 4). The duration of the S-shape was not significantly different from the flexion to the post-flexion stage.

The larvae performed more than one S-shape (an S-shape followed by another S-shape after a 1–2 s pause) 36% of the times during flexion stage against <1% of the times during the post-flexion stage (Bootstrap test; $p < 0.0001$). The performance of an S-shape did not always lead to an Attack. The rate of interrupted attacks, i.e. S-shape followed by a Swim action instead of an Attack, was not significantly different from the flexion to the post-flexion stage (Bootstrap test; $p > 0.05$).

The transition rate from S-shape to Attack increased from the flexion to the post-flexion stage, from 41% to 71% (Bootstrap test; $p < 0.001$) (Fig. 3). The total attack rate increased from the flexion to the post-flexion stage, from $3.3 \pm 0.1$ to $4.5 \pm 0.5$ attacks $\text{min}^{-1}$ ($t$-test; $p < 0.001$) (Fig. 4).

In the flexion stage, attacks were successful in 41% of the cases, while the successful attacks reached 73% in the post-flexion stage ($t$-test; $p < 0.001$). After a successful capture, turbot larvae made a Shake action.
In the post-flexion stage, turbot larvae also performed another kind of preparation to the Attack other than the well-described S-shape. It was a Tilted S-shape, where the body was tilted, bent and stabilized by the movement of the dorsal and anal fins. In this stage, turbot larvae performed $2.5 \pm 0.1$ Tilted S-shapes min$^{-1}$ (Fig. 4). The Attack was performed either with an upright or tilted posture. The rate of successful upright Attacks of total upright Attacks was slightly lower than the rate of successful tilted attacks on total tilted Attacks, but not statistically different ($z$-test; $p = 0.059$).

The prey type (nauplii or copepodites) was determined in more than 50% of the Attacks in the post-flexion stage. Nauplii were targeted in 100% of the attacks during the flexion stage, and in 67% of the Attacks during the post-flexion stage, the difference being significantly different ($z$-test; $p < 0.001$). Rate of total Attacks was higher on nauplii than on copepodites (Fig. 5), and also the rate of successful Attacks was higher on nauplii (63%) than on copepodites (15%) ($z$-test; $p < 0.001$) (Fig. 5).

**4 Discussion**

The transition period from the pelagic to the benthic stage in flatfish larvae, occurring during metamorphosis, is characterized by profound morphological and physiological changes. In the present study, we assessed the behavioral traits related to feeding of turbot larvae during the transition from an upright to a tilted position that could indicate feeding constraints and thus affect the success of turbot larval rearing in aquaculture.

The level of Swim in the flexion stage was significantly lower than in the post-flexion stage. A higher level of activity during the post-flexion phase has been previously observed in turbot larvae (Skiftesvik, 1992). We also observed that turbot larvae shifted from a “saltatory” strategy in the flexion stage, pausing for brief periods, toward a “cruise” predatory behavior in the post-flexion stage, showing a more continuous
swimming. The same was observed in the larvae of the flatfish
witch flounder *Glyptocephalus cynoglossus* (Rabe and Brown, 2001). Intermittent motion is characteristic for early
turbot larvae (Cunha et al., 2007) and often first-feeding
larvae interrupt their swimming by “routine” turns (own
observations). An important difference between “saltatory”
and “cruise” strategies is that during the first, the search for
prey items only occurs during the pauses in larval swimming
The shift from a “saltatory” to a “cruise” predatory behavior
indicates that an ontogenetic shift in swimming mode has
taken place.

The aiming behavior (use of *S*-shape, *Tilted S*-shape)
changed from flexion to post-flexion. In both stages turbot
larvae performed an *S*-shape to prepare the attack. Our results
show, however, a decline in the frequency of *S*-shapes from
the flexion to the post-flexion stage. A decline in the frequency of
*S*-shapes has been observed in other studies, for example, for
larvae of Japanese flounder *P. olivaceus* (Dou et al., 2000) and
of European sea bass *Dicentrarchus labrax* (Georgalas et al.,
2007). However, during the post-flexion stage, turbot larvae
also performed *Tilted S*-shapes. The decrease in *S*-shapes
and the introduction of *Tilted S*-shapes during the post-flexion
stage show that turbot larvae still would have to aim at the prey
when in tilted position, but need to reposition. Interestingly, we
observed that the performance of multiple *S*-shapes declined
significantly from the flexion to the post-flexion stage,
performance of multiple *S*-shapes indicate failure to aim
correctly, and hence it appears that the larvae during this
transition improved their predator skills. *S*-shapes did not
always lead to an attack, and such interrupted *Attacks*
are usually defined “aborted” *Attacks*, and such have been reported
for other species, for example larval cod *Gadus morhua* (Hunt
von Herbing and Gallager, 2000). The aborted *Attacks* could be
related to the challenges in prey capturing due to the prey
moving prior to the *Attack*.

We observed that *Attack* success increased from the flexion
to the post-flexion stage. This improvement would be related to
the morphological and physiological development during
ontogeny. For example, rods appear in the retina at stage 5, and
the presence of this type of photoreceptors increases the visual
capabilities by improving vision in low light conditions (de
Miguel Villegas et al., 1997). Further, the gut develops (Segner
et al., 1994) and the feeding structures become more complex
during ontogeny (Hunt von Herbing and Gallager, 2000). The
increasing attack success during larval ontogeny have been
observed in other species, like larval cod (Hunt von Herbing
and Gallager, 2000) and Atlantic herring *Clupea harengus*
larvae (Une-Palm, 2004).

The significant increase in both attack rate and in capture
success from the flexion to the post-flexion stage show that,
this transition does not hamper feeding of turbot larvae at this
stage. During post-flexion, about half of the right eye can be
seen from the left side, but turbot larvae might still rely on a
binocular fixation on the prey, as when larvae were in their
symmetrical stage (Osse and Van den Boogaart, 1997). A study
on Southern flounder *Paralichthys lethostigma* showed that
tilted behavior can be occur independently of the asymmetric
position of the eye (Schreiber, 2006). Thus, the transition from
flexion to post-flexion stage might be a learning phase, aiming
at prey from a tilted position. Estimates of attack rates during
metamorphosis differ between experiments on flatfish species.
De La S. Sabate et al. (2008) observed that in Japanese
flounder the attack frequency was highest just before
metamorphosis, while almost no attacks were observed in
the middle of the metamorphosis period. De La S. Sabate et al.
(2008) also investigated spotted halibut, for which the feeding
behavior began to increase just before metamorphosis and a
high attack frequency was maintained until the juvenile stage.
A relatively low capture success (< 50%) in the flexion
stage points to difficulties in catching the prey, here copepods.
The type of swimming behavior of the different copepod
species could represent a challenge for turbot larvae. In the
present study, we did not distinguish between *Acartia spp*.
or *Centropages hamatus* during the video analysis, however, we
noticed that larvae captured more nauplii than copepodes.
Studies on the effect of specific prey species and develop-
mental stages could complement the present results. In the
flexion stage of turbot larvae the prey sizes in the range we
offered the larvae does not constitute a limitation to ingestion.
The development of the jaws and sucking system of turbot is
being completed at flexion stage, e.g. around DPH 13 turbot
larvae develop tooth-bearing pharyngeal jaws and a toothless
buccal jaw (Wagemans et al., 1998). These morphological
features, and the obtained mouth size, help the larvae to capture
relatively large prey (500–1000 μm, like *Artemia* nauplii and
metanauplii) (Wagemans et al., 1998). The upper jaws could
extend to create a pre-buccal cavity, and this capability might
be influenced by the asymmetries at metamorphosis (Wage-
mans et al., 1998). At the age of our oldest larvae, 16–17 DPH,
all of the sospensorium and hyoid arch elements start to ossify,
suggesting that at this stage the larvae can generate stronger
negative pressure for sucking, defined as the “sucking +
biting” stage (Kohno, 2001).

In conclusion our results suggest that transition from an
upright to a tilted larva is rather a phase of development than a
critical phase to larval feeding and survival, in contrast to what
was observed in other flatfish species (e.g. Japanese flounder
and spotted halibut). During this transition phase, the larvae
proved to be more efficient in capturing their prey. There is an
ontogenetic shift from intermittent to cruise swimming, and the
hunting skills of turbot larvae are apparently improved. The
feeding processes are inevitably dependent on the type of prey
offered and we encourage complementary studies to investi-
gate the effect of specific copepod species and developmental
stages on the feeding during this interesting ontogenetic phase
in the early life of flatfish.

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