

Development of Swimming and Feeding Functions in Larval Turbot, *Psetta maxima*, Reared in the Laboratory

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Abstract

Developmental sequences of swimming- and feeding-related structures were examined in laboratory-reared larvae of the turbot, *Psetta maxima*. The following five swimming and four feeding developmental stages were recognized. Swimming: 1) primitive swimming stage (from hatching to D-13) - no swimming-related characters developed other than pectoral fin supports; 2) caudal fin swimming stage (D-13 to D-17/20) - notochord flexion begin to develop accomplished, and caudal fin rays and fin supports begin to develop; 3) caudal fin + axial swimming stage (D-17/20 to D-25) - vertebrae with haemal and neural arches and spines developed; 4) well-balanced swimming stage (D-25 to D-30) - dorsal and anal fin-ray complements completed; 5) completed stage (beyond D-30) - larvae considered to have acquired juvenile swimming ability. Feeding: 1) basic sucking stage (D-2 to D-9/13) - mouth opens and oral cavity elements (e.g. suspensorium, hyoid arch and opercular bones begin to develop; 2) functional sucking stage (D-9/13 to D-15/17) - further development and initial ossification of oral cavity elements; 3) sucking + biting stage (D-15/17 to D-30) - jaw and pharyngeal teeth increase in number, some degree of ossification in all oral cavity elements; 4) completed stage (beyond D-30) - larvae considered to have acquired juvenile feeding ability. Previously-recognized critical periods for larval turbot, observed at around D-10 to D-20, corresponded to transformation phases from the primitive, basic modes to stable, more functional modes in both swimming and feeding functional development.

Key Words: turbot, *Psetta maxima*, osteological development, functional development

Introduction

The turbot, *Psetta maxima*, distributed from Norway along the European coast to the Mediterranean and Black Seas, has been a target of aquaculture in several countries due to its high commercial value. Accordingly, the biology of the species has been well studied from the viewpoint of larval rearing (Jones, 1972; Khanaichenko *et al.*, 1994; Moteki *et al.*, in press 2001; Nielsen, 1986; Spectorova and Doroshev, 1976). Morphological development of eggs, larvae and juveniles of the species have been also well investigated (Al-Maghazachi and Gibson, 1984; Cousin and Laurencin, 1985; Gibson and Johnston, 1995). However, because there is no detailed information on larval or juvenile osteological development, this study aimed to clarify ontogenetic intervals, based mainly on the osteological development of swimming- and feeding-related structures.

Materials and Methods

Larvae used in this study came from the spawning and rearing facilities at the Trabzon Central Fisheries Research Institute, Turkey. Fertilization and larval rearing methods followed those described by Moteki *et al.* (2001), the water temperature ranging from 16-19 °C during the experiment. Larvae hatched on 12 May 1998 were examined and samples of 2-5

larvae collected daily from the day of hatching (D-0) to D-11, and on D-13, 15, 17, 20, 25, 30 and 51. A total of 73 larvae (2.66-21.5 mm in standard length, SL) were cleared and stained following the method of Potthoff (1984), and the following measurements and counts made: standard length (SL); angle of notochord flexion (see Kohno *et al.* (1983) for method); caudal, dorsal, anal, pectoral and pelvic fin ray counts; greatest body depth (and position); mouth width; ratio of premaxilla to gape; jaw and pharyngeal teeth numbers; number of branchiostegal rays. Osteological development of the following structures was also recorded: caudal, pectoral and pelvic fin supports (dorsal and anal supports not observed because of incomplete staining); vertebral columns, and neural and haemal arches and spines; jaws; suspensorium and hyoid arch; opercular bones.

Results

Growth

Two larvae collected on the day of hatching (D-0) were 2.66 and 2.77 mm in SL, the mean SL±SD at D-5 being 3.24±0.19 mm (*n*=5) (Figure 1A). Thereafter, the larvae grew rapidly, reaching 4.62±0.46 mm (*n*=3) at D-10, 8.16±0.49 mm (*n*=4) at D-20, 11.7±1.03 mm (*n*=4) at D-30 and 18.9±1.76 mm (*n*=4) at D-51 (Figure 1A).

Swimming-related characters

Flexion of notochord end: The caudal end of the notochord started to flex at 5.84 mm SL (D-13), the angle being 13.5 degrees (Figure 1B). The angle of flexion increased rapidly, the smallest specimen possessing a completely flexed notochord (47.7 degrees) at 7.74 mm SL (D-17). The angle thereafter stabilized between 40 and 50 degrees (Figure 1B).

Caudal fin supports and fin rays: The first visible elements of the caudal complex other than the notochord were cartilaginous hypural plates 1+2 and 3+4 in a 5.84 mm SL specimen (D-13). The cartilaginous buds of the parhypural, haemal arch and spines on future preural centra 2 and 3 (PUC2, 3), and the neural arch and spine on PUC3 occurred first at 6.43 mm SL (D-15). The cartilaginous neural arch and spine on PUC2 were first observed at 7.01 mm SL (D-15). The cartilaginous buds of epurals 1 and 2 and hypural 5 (the final cartilaginous element of the caudal complex) were added at 7.74 mm SL (D-17) and 8.89 mm SL (D-20), respectively.

In the 8.89 mm SL specimen (D-20), ossification had started in the haemal and neural arches and spines on PUC 2 and 3. The bony urostyle and PUC 2 and 3 were also first observed at this time. Hypurals 1+2 and 3+4 and the parhypural began to ossify at 9.05 mm SL (D-25), ossification of all the cartilaginous elements being evident at 10.6 mm SL (D-25). The final caudal-complex elements (bony uroneural) appeared at 12.0 mm SL (D-30). Fusion between hypural plates 3+4 and hypural 5 was observed at 17.7 mm (D-51).

The first discernible caudal fin rays were 4 principal rays at 5.84 mm SL (D-13) (Figure 1C). The full adult complement of 9+8=17 principal rays was attained at 7.89 mm SL (D-20), although completed principal rays were first noticed on D-17 (8.05 mm SL) (Figure 1C).

Dorsal and anal fin rays: Both the dorsal and anal fin rays began to develop anterior to the mid-part of each fin. The smallest specimen with dorsal fin rays (14 in number) was 6.43 mm SL (D-15) (Figure 1D), whereas that with anal fin rays (17) being 7.01 mm SL (D-15) (Figure 1E). The full adult complement of both dorsal and anal fin rays was attained at 9.05 mm SL (D-25) (Figure 1D, E).

Pectoral fin supports and fin rays: A pectoral girdle, including a rod-shaped bony cleithrum and coraco-scapular cartilage, was observed in the smallest specimen examined, 2.66 mm SL (D-0). A cartilaginous fin-plate, representing future actinosts, appeared first at 3.39 mm SL (D-5), and a bony supracleithrum and posttemporal were first observed in a 4.76 mm SL specimen (D-10). The posterior and anterior postcleithra appeared first at 6.43 mm SL (D-15) and 8.05 mm SL (D-17), respectively. Initial

ossification occurred on the coracoid and scapular at 8.89 mm SL (D-20). Three crevices, which appeared on the fin-plate at 9.05 mm SL (D-25), subsequently resulted in the fin-plate being divided into four actinosts, all of which had started to ossify in a 17.7 mm SL specimen (D-51).

The smallest specimen with pectoral fin rays was 12.0 mm (D-30), five rays being visible (Figure 1F). The adult complement of fin rays was attained at 17.7 mm SL (D-51) (Figure 1F).

Pelvic fin supports and fin rays: A cartilaginous basipterygium appeared first at 8.89 mm SL (D-20), beginning to ossify at 10.6 mm SL (D-25). The pelvic fin rays appeared on D-25 (Figure 1F), the smallest specimen of that age (9.05 mm SL) possessing the adult complement (6), although a larger specimen (9.75 mm SL) had five rays only.

Vertebrae: Among the elements making the vertebrae, the two anteriormost neural arches appeared first at 5.30 mm SL (D-11). Haemal arches and spines, 12 in number, were first observed at 5.84 mm SL (D-13). Ossification initially started in the first three neural arches and spines at 5.98 mm SL (D-13), these elements attaining their full complement at 7.01 mm SL (D-15), with ossification evident in the anteriormost 18 haemal arches. The three anteriormost centra initially became visible at 8.05 mm SL (D-17), a full complement of haemal arches and spines being present. In an 8.89 mm SL (D-20), all of the haemal and neural arches and spines had begun to ossify, all of the centra being evident.

Greatest body depth (and position): The greatest body depth (GBD) was not measurable for five specimens from 2.62 to 2.82 mm SL (representing D-0 and D-2) because of damage. The smallest measurable specimen, 2.85 mm SL (D-2), had a GBD of 0.40 mm, some 14.8% of SL (Figure 1G). The ratio of GBD to SL increased abruptly between D-15 and D-17, the mean±SD being 32.1±1.52 % and 43.8±4.95%, respectively (Figure 1G). The ratio increased continuously until D-30, becoming stable thereafter (mean±SD of 67.4±2.07% at D-30, 64.1±2.34% at D-51).

The ratio of GBD position to SL increased rapidly from D-2 to D-10, means increasing 16.9 to 34.8%, but then increased at a slower rate between D-10 and D-17 (Figure 1H). In specimens collected on D-15 and D-17, the ratio clearly fell into one or other of two groups, comprising 36.8 and 37.5% vs 48.7% for D-15, and 35.3 and 37.7% vs 46.4, 46.9 and 48.8% for D-17 (Figure 1H). The ratio stabilized at a higher level thereafter. The changes in ratio indicated a progressive re-positioning of the GBD from the head region to the belly.

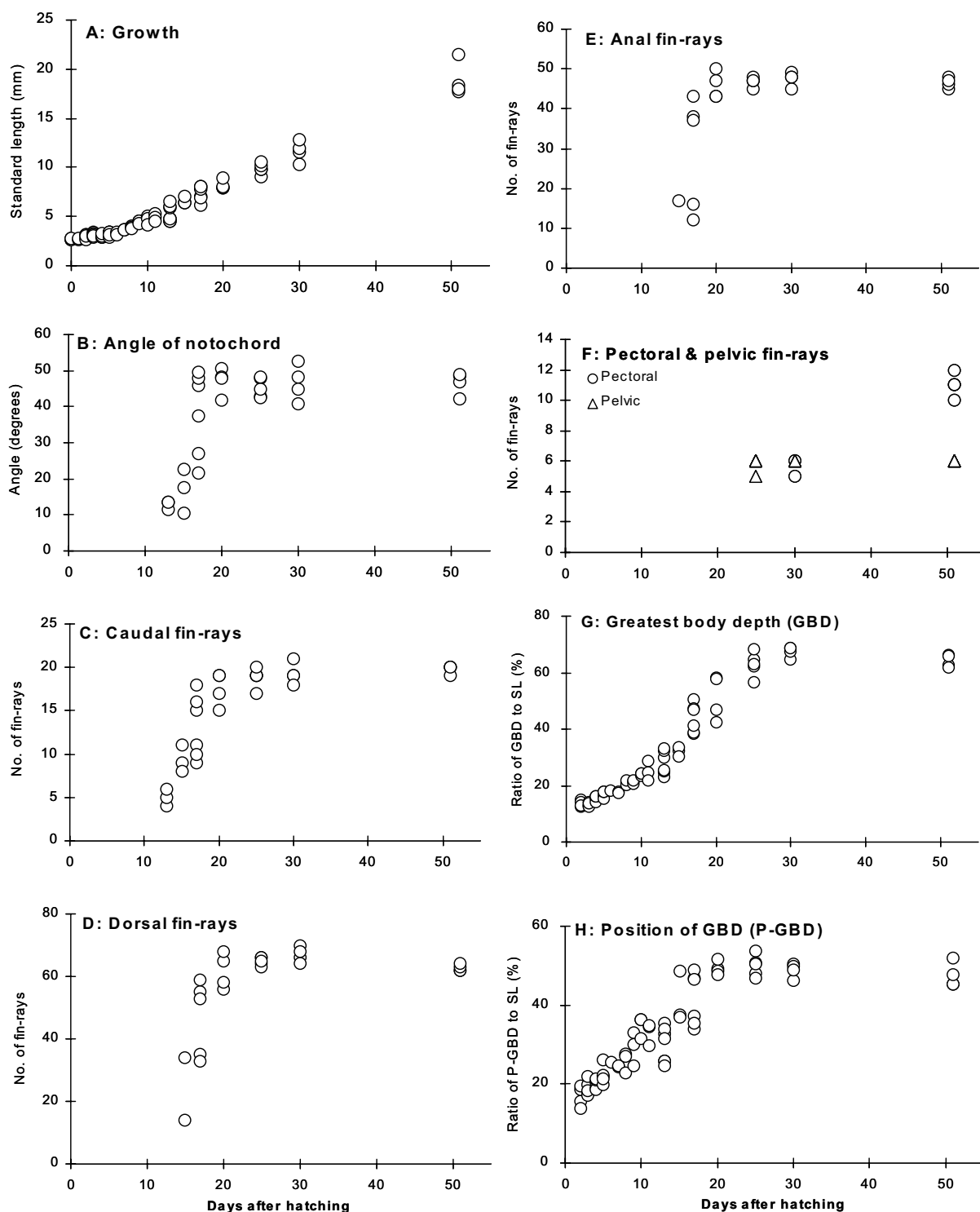


Figure 1. Changes in swimming-related characters with days after hatching in the turbot larvae.

Feeding-related characters

Mouth width: The mouth first opened at 2.69 mm SL (D-2), the width initially being 0.18 mm, but gradually increasing in size to 0.40 ± 0.08 mm at D-8 (Figure 2A). A more or less rapid increase was observed between D-8 and D-30 (width 1.56 ± 0.08 mm). Thereafter, the mouth width stabilized (1.56 ± 0.23 mm at D-50) (Figure 2A).

Jaw structure: A bony maxilla and Meckel's cartilage were first evident in a 2.78 mm SL specimen (D-2), in which the mouth had not opened. A bony dentary and angular first appeared at 4.28 mm SL (D-9) and the premaxilla and retroarticular at 4.76 mm SL (D-10).

Ratio of premaxilla to gape: In the smallest specimen with a premaxilla (4.76 mm SL) (D-10), the ratio of premaxilla to gape was 25.1%. The ratio increased rapidly, reaching $67.4 \pm 3.01\%$ at D-20 and $75.9 \pm 4.06\%$ at D-30, thereafter becoming stable or decreasing slightly ($66.9 \pm 0.71\%$ at D-51) (Figure 2B).

Jaw teeth: The smallest specimens in which upper jaw teeth were observed were both 6.43 mm SL (D-15) (one and two teeth) (Figure 2C), the first lower jaw tooth appearing in a 6.57 mm SL specimen (D-13) (Figure 2D). Both upper and lower jaw teeth increased in number with larval growth, there being no flexion points or abrupt changes observed. The numbers of upper and lower jaw teeth ranged from 11 to 14 and 11 to 15, respectively, at D-30, and from 16 to 23 and 15 to 24, respectively, at D-51.

Suspensorium: The hyomandibular-symplectic and quadrate-metapterygoid cartilages were first seen at 2.69 mm SL (D-2), foramen being observed on the upper, hyomandibular position of the former at 3.70 mm SL (D-7). Ossification started in the hyomandibular and quadrate at 4.76 mm SL (D-10), cartilaginous bud (palatine) appearing and contacting with the anterior part of the quadrate-metapterygoid cartilage, thus forming a triangular plate, at 5.30 mm SL (D-11). At this point, the symplectic had begun to ossify. Bony exo- and ecto-ptyergoids first appeared at 5.84 mm SL (D-13), and ossification started in the metapterygoid and palatine at 7.01 mm SL (D-15) and 8.05 mm SL (D-17), respectively.

Hyoid arch and branchiostegal rays: A cartilaginous hypohyal, ceratohyal-epihyal and interhyal, comprising the hyoid arch, were first observed at 2.69 mm SL (D-2). The ceratohyal and epihyal began to ossify at 4.76 mm SL (D-10), with ossification evident in the interhyal and hypohyal at 5.30 mm SL (D-11) and 8.05 mm SL (D-17), respectively.

The first branchiostegal ray appeared at 4.28 mm SL (D-9) (Figure 2E), the number thereafter

increasing rapidly, and reaching the adult complement (7) at 6.18 mm (D-17) (Figure 2E).

Pharyngeal teeth: The first upper and lower pharyngeal teeth were apparent at 3.92 mm SL (D-8) and 6.43 mm SL (D-15), respectively (Figure 2F). Both upper and lower pharyngeal teeth increased in number thereafter, accurate counts becoming difficult in specimens >D-20 (Figure 2F).

Opercular bones: A bony preopercle appeared first, at 2.69 mm SL (D-2), followed by the opercle (at 3.70 mm SL) (D-7) and inter- and sub-opercles (at 5.84 mm SL) (D-13).

Discussion

The development of swimming- and feeding-related structures in larval *Psetta maxima* observed in this study is shown diagrammatically in Figures 3 and 4, respectively. Peaks of the histograms based on the number of developmental events (Figures 3 and 4) indicate thresholds of developmental intervals (Sakai, 1990; Kohno, 1998). In this study, the larval intervals were determined following consideration of both the histograms and key-character development (e.g., Kohno and Sota, 1998; Kohno *et al.*, 2000), such intervals also being indicated in the figures and elucidated below.

No feeding-related characters had been observed by D-2, at which time the mouth opened and the maxilla and Meckel's cartilage were apparent. Furthermore, characters forming the oral cavity, such as the suspensorium, hyoid arch and opercular bone also first became apparent at D-2, indicating that the initial larval feeding mode was sucking, promoted by the negative pressure of the oral cavity (Gosline, 1971; Otten, 1982).

No conspicuous development of feeding-related structures was observed from D-3 to D-8, the period from D-2 to D-9 therefore being recognized as the "basic sucking" stage (Figure 4).

In the period from D-9 to D-13, development of the following feeding-related structures took place. At D-9, the lower jaw elements started to appear, a flexion point being observed in the increase in mouth width. The premaxilla and retroarticular were first observed, and the suspensorium and hyoid arch started to ossify at D-10. All of the suspensorium and opercular bone elements had appeared by D-13. These developmental events coincided with increasing effectiveness of mouth opening-closing movements and the generation of stronger negative pressures in the oral cavity (Kohno *et al.*, 1996a, 1996b, 1997). Therefore, it was concluded that the larvae increased their sucking ability from D-9/13, such being the "functional sucking" stage (Figure 4).

Mass mortality of turbot larvae during D-6 and 7 (Spectorova and Doroshev, 1976) or D-7 and 13 (Jones, 1972) corresponds to the period during which

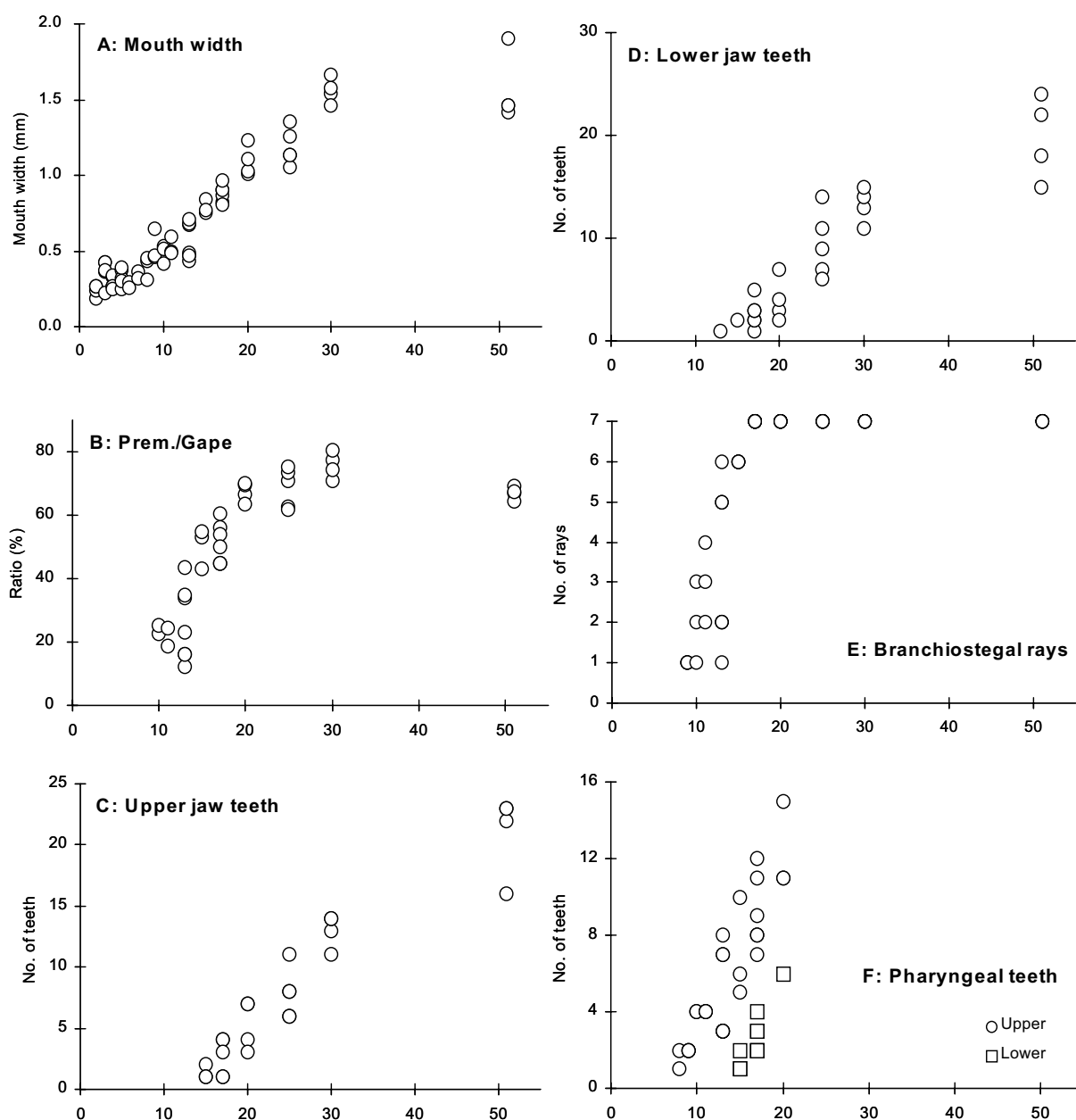


Figure 2. Changes in feeding-related characters with days after hatching in the turbot larvae.

the yolk is exhausted (Khanaichenko et al., 1994; Moteki et al., 2001). Therefore, to survive during this period, the larvae have to change smoothly from endogenous to exogenous nutrient resources (Kohno et al., 1986, 1994) by utilizing their increased sucking ability (describe above), especially since the lack of development of significant structures associated with swimming function indicates that they are still at a “primitive swimming” stage (Figure 3).

The pectoral fin supports were the only swimming-related structures possessed by the larvae until D-10,

at which time a flexion point was observed in the position of greatest body depth. Although the neural arches and spines had started to appear at D-11, caudal fin structures related to swimming started developing only at D-13, notochord flexion and the development of caudal fin supports and fin rays also beginning at that time. Therefore, the larvae were considered as starting to acquire a “caudal fin” swimming mode implied from D-13, the interval developing to the “caudal-fin swimming” stage (Figure 3).

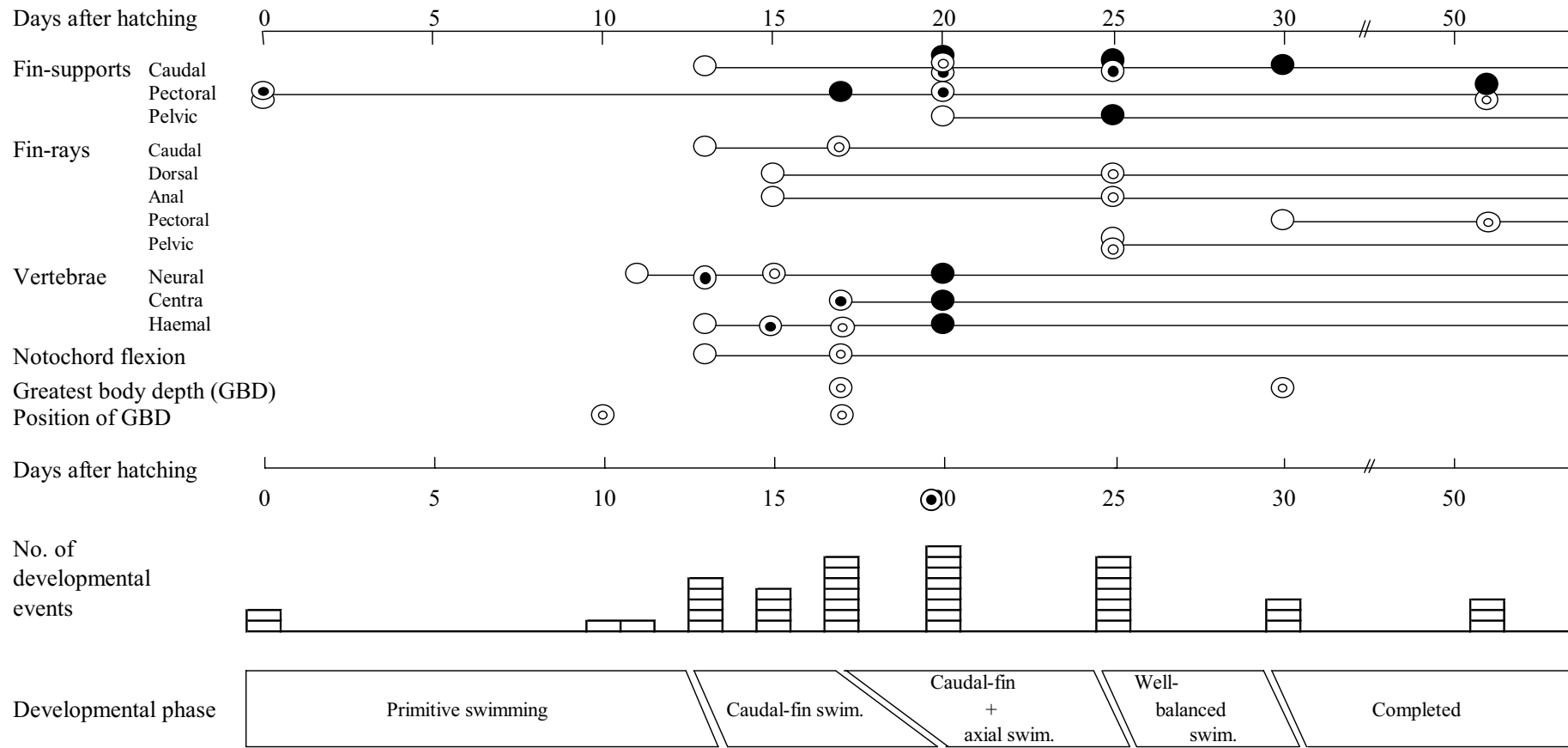


Figure 3. Schematic representation of the development of swimming-related characters with days after hatching in turbot larvae. O, cartilaginous elements or fin rays start to appear, or notochord flexion begins; ⊙, bony elements start appearing, or cartilaginous elements start to ossify; ⊖, all cartilaginous elements start to appear, fin ray numbers become complete, or flexion points of morphometric characters observed; ●, all cartilaginous elements start to ossify, all bony elements start to appear, or notochord flexion completed.

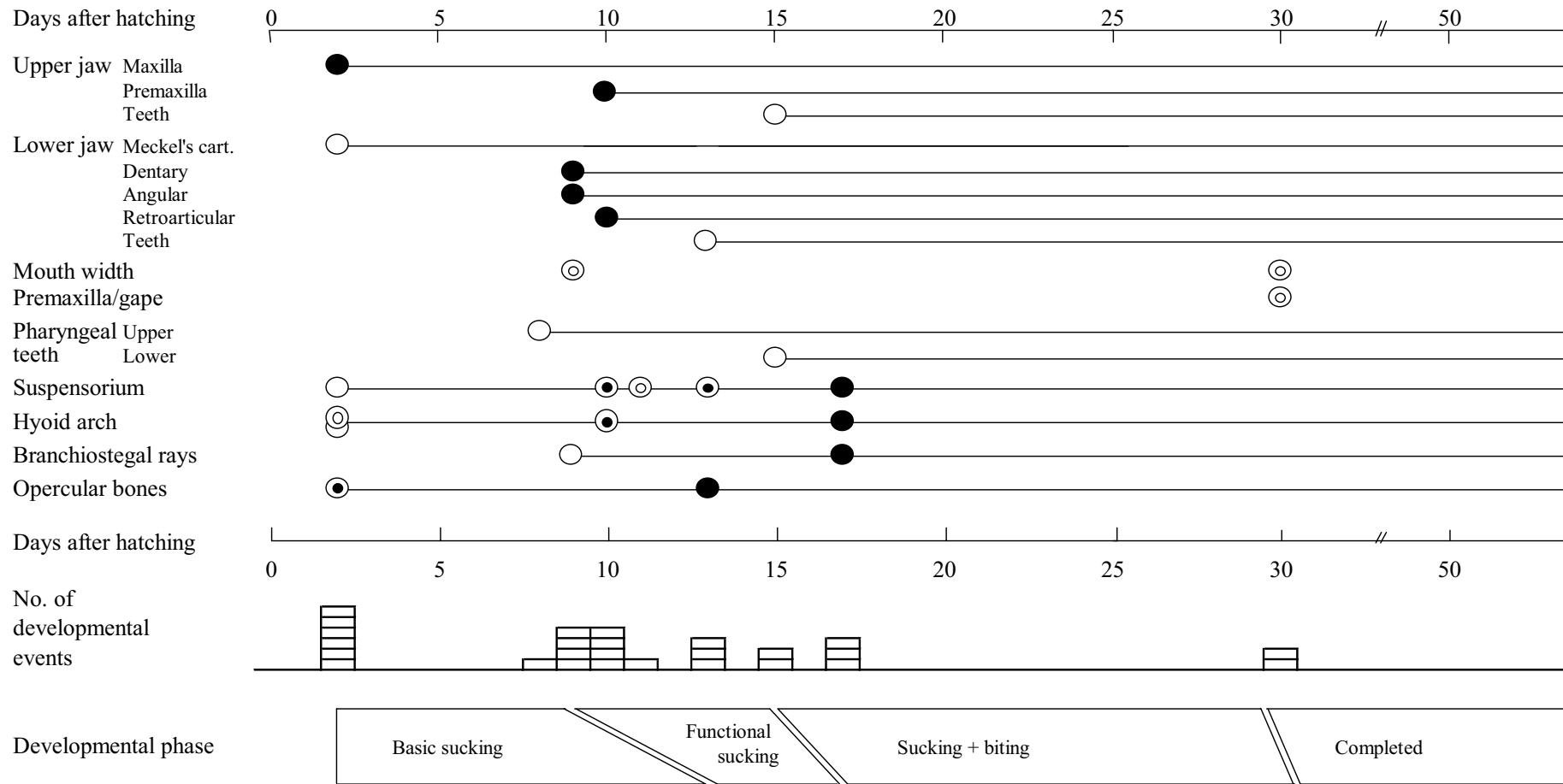


Figure 4. Schematic representation of the development of feeding-related characters with days after hatching in turbot larvae. ○, cartilaginous elements, teeth or branchiostegal rays start to appear, or mouth opens; ◎, cartilaginous elements start to ossify, or bony elements start to appear; ◎, all cartilaginous elements start to appear, or flexion points of teeth number or morphometric characters observed; ●, all cartilaginous elements start to ossify, all bony elements start to appear, or branchiostegal ray numbers completed.

At around D-15, developmental changes in turbot larvae have been reported in several studies. The stomach developed, *Artemia nauprii* being preferred as food at D-10/15 (Khanaichenko *et al.*, 1994), with the formation of gastric glands and the onset of gastric digestion from D-15/16 (Cousin and Laurencin, 1985). Jones (1972) also reported heavy larval mortality at D-15/16.

The period around D-15 is referred to here as the "caudal-fin swimming" stage, although the feeding mode also changed at the same time, from "functional sucking" to "sucking + biting".

At D-15, upper and lower pharyngeal teeth started to develop, although lower jaw and upper pharyngeal teeth were initially observed at D-13 and D-8, respectively. At D-17, all of the suspensorium and hyoid arch elements had started to ossify, and the branchiostegal rays had attained their full complement. These developmental events suggested that from D-15/17 the larvae can generate stronger negative pressure for sucking, in addition to the acquisition of a "biting" feeding mode (Gosline, 1972; Otten, 1982), an interval change to the "sucking + biting" stage.

On the other hand, during the period from D-17 to D-20, elements that resulted in a strengthening of the body axis also started to develop. The vertebral column started to appear at D-17, the full number of vertebrae being attained by D-20. All of the haemal arches and spines were present at D-17, both the haemal and neural arches and spines starting to ossify at D-20. Furthermore, at D-17, the full complement of principal caudal fin rays was attained and notochord flexion completed, flexion points being observed in the greatest body depth and its position. Therefore, "caudal-fin" swimming of the larvae becomes stronger, owing to increasing use of the posterior half of the body, from D-17/20 (Gosline, 1972; Omori *et al.*, 1996), the interval developing to the "caudal-fin + axial swimming" stage.

Spectorova and Doroshev (1976) noted that mass mortality occurred at D-17/18 and Jones (1972) reported that all larvae had died by D-20/30. The period from D-17 to 30 corresponds to the "caudal-fin + axial" swimming and "sucking + biting" feeding stages in this study. Furthermore, Khanaichenko *et al.* (1994) noted that metamorphosis started to occur at D-15/22.

The dorsal and anal fin ray numbers were completed by D-25, resulting in swimming involving both the caudal fin and posterior half of the body becoming more effective because of better overall body balance (Gosline, 1971). Consequently, the swimming mode is considered to change to the "well-balanced swimming" stage at D-25.

Turbot larvae are considered to acquire similar higher swimming and feeding abilities to juveniles by D-30, the developmental stage thus being "completed" (Figures 3 and 4). Nevertheless, the adult complement of pectoral fin ray numbers was not

attained until D-51.

To summarize, two previously-recognized critical periods during larval turbot development (at around D-10 and D-20), in which heavy mortality occurs, are considered to correspond to transitional swimming and feeding modes, the former from "basic" to more "functional" swimming and the latter from "basic" to "functional" sucking, accompanied by the exhaustion of endogenous nutrition and the need to change to exogenous nutritional sources. Consequently, the larvae have to obtain enough food not only to maintain basic metabolism but also to increase in body size and develop functionally.

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