A tabular overview of organogenesis in larval turbot (Scophthalmus maximus L.)

Helmut Segner, Volker Storch, Manfred Reinecke, Werner Kloas, and Winfried Hanke


In this overview of the ontogeny of organ structures and functions of larval turbot (Scophthalmus maximus L.) the developmental age of the larvae is determined on the basis of morphologically defined stages. At the onset of exogenous feeding, a functional digestive system is present, with enterocytes capable of protein and lipid absorption, with functional exocrine pancreatic cells, and with intestinal enzymes for nutrient digestion. In addition, key elements for the metabolic utilization of dietary nutrients, such as differentiated liver cells, endocrine factors, and enzymes of key metabolic pathways, exist. During subsequent development, intestine, exocrine pancreas, and liver of turbot larvae undergo mainly quantitative alterations (i.e. growth) but not qualitative alterations (i.e. appearance of new functions). The gills are not functional at first-feeding but differentiate until metamorphosis. The phase of metamorphosis is characterized by the onset of stomach function and by a pronounced increase of glycolytic metabolic activities indicating a change from larval musculature into the adult-type (aerobic) red and (anaerobic) white muscles.

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Introduction

Effective culture of larval fish requires alignment of rearing strategies with the ontogenetic status of the larvae, i.e., with the developing abilities to cope with different environmental and nutritional conditions. The correct assessment of developmental age, however, is difficult. The most widely used markers for development – length and weight – show no constant relationship to ontogenetic steps (e.g., Kamler et al., 1990; Verreth et al., 1993). A more suitable approach appears to be staging of larvae on the basis of morphological and functional criteria (e.g., Ryland, 1966; Al-Maghazachi and Gibson, 1984; Krise and Meade, 1986; Kamler et al., 1990; Verreth et al., 1992, 1993).

Al-Maghazachi and Gibson (1984) subdivided the larval period of turbot (Scophthalmus maximus L.), from hatching to completion of metamorphosis, into a sequence of five morphologically defined stages. The present study aims to correlate the ontogenetic changes of external morphology of turbot with structural and functional alterations of internal organ systems. In order to facilitate comparison with different developmental events, we have chosen a tabular overview for presentation.

Methods

The data given in Table 1 are compiled from findings of our own studies (Berwert et al., 1995; Segner et al., 1993, 1994) as well as of other research groups (Al-Maghazachi, 1983; Cousin and Baudin-Laurencin, 1985; Ueberschär, 1985; Cousin et al., 1987; Munilla-Moran and Stark, 1989; Munilla-Moran et al., 1989; Fukuhara et al., 1990; Padros et al., 1991). In these studies, turbot larvae

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Table 1. Morphologically defined stages* and the corresponding functional organogenesis in the development of turbot.

<table>
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<tr>
<th>Stages</th>
<th>Foregut/stomach/branchial cavity</th>
<th>Intestine</th>
<th>Liver/exocrine pancreas</th>
<th>Other organ systems</th>
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<tr>
<td><strong>Stage 1:</strong> Larvae symmetrical, yolk sac present&lt;br&gt;1a: Head attached to yolk sac; gut almost straight</td>
<td>Mouth and branchial cavity closed (1, 5, 25)</td>
<td>No differentiation into regions, anus closed. Cytodifferentiation of enterocytes completed. Enterocytic enzymes for contact digestion at the brush border present (6, 24, 25)</td>
<td>Cytodifferentiation of liver and pancreatic cells completed; smaller hepatic glycogen reserves. Bile system and gall bladder present. Pancreas: Acinar organization, few zymogen granulae (25), low levels of trypsinogen (14). Protease and amylase activities for luminal digestion present (6, 26)</td>
<td>Functional heart and kidney tubuli (pronephros) present; many chloride cells distributed in the skin (21, 25). Single endocrine islet with insulin-immunoreactivity (4)</td>
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<tr>
<td>1b, 1c: Head free from yolk sac; gut bent ventrally</td>
<td>Mouth and branchial cavity open; esophagus and stomach anlage discernible (5, 25)</td>
<td>Differentiation starts, anus open. Relative intestinal length 26% (1, 25)</td>
<td>Hepatic glycogen contents increased (1, 26); number of zymogen granulae and proteolytic activity increasing (14, 25)</td>
<td>High activities of citrate cycle enzymes (aerobic metabolism) (25)</td>
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<td>1d: Yolk sac very small; active feeding established</td>
<td>Gill arches and taste buds developing (1, 5, 21, 25)</td>
<td>Three distinct regions established; muscular valve separates intestines I and II; lipid absorption in intestine I, protein pinocytosis in intestine II; brush border enzyme activity enhanced (5, 6, 24, 25)</td>
<td>Number of zymogen granulae and of proteolytic activity increasing, allometric increase of liver and pancreas size (1, 6, 14, 25, 26)</td>
<td>Endocrine islets with glucagon-, somatostatin-, IGF-1-immuno-reactivity; one principal islet and additional small ones existing (4)</td>
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<td><strong>Stage 2:</strong> Larvae symmetrical; development of spines and swimbladder&lt;br&gt;2a: First head spines</td>
<td>Esophagus with high mucosal folds, numerous goblet cells; pharyngeal teeth present (5, 25)</td>
<td>Increasing activity of brush border enzymes; continuous increase of relative length, mucosal folds and absorptive surface (5, 6, 25)</td>
<td>Number of zymogen granulae and of protease activities increasing, allometric increase of liver and pancreas size (1, 6, 14, 25, 26)</td>
<td>Levels of NADPH-producing enzymes increasing (15)</td>
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<td>2b: Spines numerous on operculum; gut with a loop; swimbladder small but visible</td>
<td>Caeci developed (1, 5, 25)</td>
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<td>2c: Swimbladder larger</td>
<td>Gill filaments developing (21, 25)</td>
<td>Intensity of lipid absorption enhanced, mucosal leucocytes appearing (6, 25)</td>
<td>Alteration of hepatic morphology (lipid storage in addition to glycogen) (21, 25)</td>
<td>Chloride cells concentrating in branchial cavity (21). Number of islets increasing (4)</td>
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Table 1. Continued

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<tr>
<th>Stage 3: Appearance of fin rays;</th>
<th>3a: First one or two fin rays</th>
<th>Strong increase of stomach size (5). Proliferation of gill filaments and lamellae (25)</th>
<th>Mucosal goblet cells appearing; they contain sialomucins in intestine I and sulphomucins in II (25)</th>
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<tr>
<td>3b: Four to seven fin rays present</td>
<td>Strong increase of stomach size (5). Proliferation of gill filaments and lamellae (25)</td>
<td>Mucosal goblet cells appearing; they contain sialomucins in intestine I and sulphomucins in II (25)</td>
<td>Islets with pancreatic polypeptide-immunoreactivity (4)</td>
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<td>Stage 4: Asymmetry and eye migration; notochord slanted dorsally</td>
<td>4a: Notochord sloped upwards</td>
<td>Stomach with cardia, fundus, pylorus (5, 25). Gill morphology functional (5, 21, 25)</td>
<td>Lipase present (1, 6) Thyroid follicles and corpuscles of Stannius present (21). Glomeruli developed (21)</td>
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<td>4b: Eye migration commences</td>
<td>4c: Right eye positioned further upwards</td>
<td>Stomach glands frequent and well differentiated (5, 25)</td>
<td>Thyroid follicles and corpuscles of Stannius present (21). Glomeruli developed (21)</td>
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<td>4d: Notochord bent straight upwards; caudal fin ray fully developed</td>
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<td>Stage 5: Completion of eye migration; swimbladder and spines resorbed</td>
<td>5a: Half of right eye visible from left side</td>
<td>Pepsin activity present (24, 25)</td>
<td>Strong increase in glycolytic capacity (indicating muscle reorganization into adult-type red and white muscles (25)</td>
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<tr>
<td>5b: Right eye on top of head</td>
<td>Gill rakers developing (1, 25)</td>
<td>Increasing pepsin activity (24, 25)</td>
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<tr>
<td>5c: Right eye situated entirely on left side, but still near upper edge</td>
<td></td>
<td>Increasing pepsin activity (24, 25)</td>
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<tr>
<td>5d: Upper eye placed away from upper edge; swimbladder disappeared</td>
<td></td>
<td>Increasing pepsin activity (24, 25)</td>
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*According to Al-Maghazachi and Gibson, 1984.
were reared under controlled conditions at 2.7–3.5% salinity and at 15–20°C temperature. Rotifers (Brachionus plicatilis) were used as initial food that was gradually replaced by Artemia nauplii. The body size of newly hatched larvae ranged from 2.3 to 3.1 mm length. Body size at metamorphosis greatly varied within individual experiments, as it did between studies from different laboratories, for instance 19.8 mm (Fukuhara et al., 1990), 23 mm (Jones, 1972), 27–39 mm (Jones et al., 1974), 38–45 mm (Al-Maghazachi and Gibson, 1984). Thus, the observations from the different investigations cannot be compared on the basis of body size but only on the basis of morphologically distinguished developmental stages.

Results and discussion

Table 1 provides data on the development of organ structures and functions during the different ontogenetic stages of larval turbot. The presentation concentrates on systems that are necessary for digestion and metabolism of nutrients. The ontogeny of sensory systems is described by Neave (1984a, b, 1986), that of the swimbladder by Al-Maghazachi and Gibson (1984) and Cousin and Baudin-Laurencin (1985), and pigmentation and fin development is discussed in Fukuhara et al. (1990).

From a recent workshop of the Commission of the European Communities, it was concluded that further progress of turbot culture requires improved knowledge of the ontogeny of the digestive system (Anon., 1993). As is evident from the data shown in Table 1, the major digestive structures and functions are present in larval turbot at the onset of exogenous feeding. The only exception is the stomach. The enterocytes, which display the morphological features of functional epithelial cells, are able to absorb lipids and proteins. The microvillous border of the enterocytes possesses digestive enzymes to catalyze final hydrolysis of nutrients ("contact digestion"). The exocrine pancreas, developed in first-feeding larvae, produces proteolytic enzymes and amylase for luminal digestion. The differentiated cytology of hepatocytes suggests the existence of a functional intermediary metabolism. The presence of pancreatic hormones indicates that endocrine control of metabolism is possible. Thus, the frequently expressed view that the digestive system of early-feeding larvae is "poorly developed" is obviously not correct (see also Cousin et al., 1987; Munilla-Moran et al., 1990). The real critical factor may be an insufficient adaptive capacity or the vulnerability of the larval digestive system to inadequate food quantity (e.g., starvation) or quality (e.g., dry food instead of live food) (cf. Eckmann, 1985; Segner et al., 1987; Segner et al., 1993).

After the onset of feeding, intestine, liver, and exocrine pancreas seem to experience primarily quantitative changes, i.e., growth in size or increase of enzyme levels, but not qualitative alterations, i.e., appearance of completely new functions or structures. The shift in liver energy storage from an (almost) exclusive glycogen storage to a mixed glycogen/lipid storage (at the end of stage 2, see Table 1) is most likely not a genetically programmed event but rather the result of the dietary change from rotifers to Artemia at this phase of rearing.

Metamorphosis that takes place during developmental stage 5 brings about major qualitative changes. The stomach becomes functional, as indicated by the appearance of glandular tissue and pepsin production. In parallel, glycolytic metabolic power develops. The shift from the almost exclusively aerobic energy generation of the larva to the mixed aerobic/ananaerobic metabolism of the juvenile reflects the shift from larval all-aerobic muscles to the adult-type aerobic red and anaerobic white muscles (cf. El-Fiky et al., 1987). Gas exchange, which in larval fish is executed via the whole body surface, is transferred to the gills which have finished the differentiation of secondary (respiratory) lamellae (cf. Osse, 1989). All these structural and functional changes prepare the young fish for the pronounced changes of the food spectrum and habitat occurring with the transition to the juvenile stage.

The findings compiled in the present communication suggest that only at two stages of turbot development do pronounced qualitative alterations of the digestive and metabolic system take place: at the transition from endogenous to exogenous feeding, and at the transition from the larval to the juvenile fish. Thus, the occurrence of critical periods with high mortalities, as repeatedly observed during turbot larval rearing (cf. Cousin and Baudin-Laurencin, 1985; Padros et al., 1991), should be related to reasons other than ontogenetic changes of larval physiology.

The observation that turbot larvae possess differentiated structures and functions for diet digestion and metabolism leads to the question what factors limit their capability to utilize dry diets. Is it the absence of stomach digestion, is it an insufficient adaptive capacity of the digestive and metabolic system, or is it a sensory limitation, i.e. the inability to recognize dry feed particles as prey organisms? Only if we gain more insight into larval physiology will we be able to answer these questions and to establish a rational understanding of larval nutritional requirements.

Acknowledgement

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References


