The development of pharyngeal taste buds in *Hucho taimen* (Pallas, 1773) larvae

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Abstract

This study aims to investigate the relationship between the development of pharyngeal taste buds (TBs) and first feeding of *Hucho taimen*. Larvae were fed with live food (water flea and tubifex) for 8 weeks. TBs of larval pharynx were histologically examined using light microscopy during the rearing experiment. The results showed that the first few TBs primordia were visible within the pharynx 27 days after hatch (DAH), which coincides with the onset of feeding, and the first few TBs with open receptor areas appear 45 DAH. TBs of pharynx were well developed 76 DAH. The number and size of TBs were quantified during larval development. The average number of pharynx was 8.63±1.15, 11.29±0.50, 14.50±1.06, and 17.78±0.47 TBs at 27, 36, 45, and 76 DAH, respectively. The number of TBs increased in both the upper and lower pharynx. The ratio of height to width of TBs showed an increase tendency, ranging from 0.81 to 1.11. The height of TBs showed an increase tendency after 29 DAH during the development. However, the width of TBs exhibited a low at 35 DAH. In conclusion, the development of pharyngeal TBs coincides with the first feeding, and this should lead to a better understanding of improvement of larval rearing in *H. taimen* hatcheries.

Keywords: *H. taimen*, Taste buds, Development, Pharynx, Feeding

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Introduction

In fish, taste buds (TBs) are distributed in the mouth, in the oropharyngeal cavity, the gills and often also on the fish's external skin (Gomahr *et al.*, 1992). Pharyngeal TBs are involved in the palatability evaluation and the swallowing reflex (Ezeasor, 1982; Micale *et al.*, 2006) and regarded as the last customs pass of feeding food (Liang and He, 1998; Hachero-Cruzado *et al*., 2009).

The taimen, *H. taimen*, is a member of the family Salmonidae. This species is one of the rare or endangered freshwater fish species in China (Yue *et al*., 1998). The rearing of larvae, however, is still problematic. For instance, larval survival rates in hatcheries are still low. Also, the larvae and juveniles show unusual behavior such as the ability to feed under dark conditions, and thus can be reared under weak-light or dark conditions (Xu *et al*., 2007). This behavior must be due to sensory organs other than the eyes. However, detailed studies about sensory organ development of this species are not known. Information on the morphogenesis of the sensory organs can be used to explain aspects of the early life history in the wild as well as to help develop better larval rearing techniques in the hatchery (Kawamura *et al*., 2003). Thus, whether the development of sensory capacity coincides with the first feeding may provide important information to understand the first feeding of this species.

In adult teleosts, the digestive system has been studied extensively (Barreiro-Iglesias *et al*., 2010; Elsheikh *et al*., 2012; Kapsimali *et al*., 2013), but little is known about the development of TBs in fish. Reutter *et al*. (1995) published preliminary notes on TB development in the turbot *Scophthalmus maximus* based on transmission electron microscopy. Hansen *et al.* (2002) reported the development of TBs at different locations, including the oropharyngeal cavity of *Danio rerio.* More recently, Fishelson *et al*. (2004) described the formation and distribution of TBs and dentition in the oropharyngeal cavity of several Blenniid, Gobiid and Cardinal fish species. Mukai *et al*. (2008) found that two day-old larvae swam horizontally, had sharp teeth, commenced ingesting rotifers and also artificial feed (small-size pellets) under both light and dark conditions; by then the larvae already had many TBs.

This paper examines the development of pharyngeal TBs of *H. taimen* larvae, using light microscopy. We also wanted to investigate the relationship between the development of pharyngeal TBs and first feeding of larvae.

Materials and methods

Experimental materials and procedures

A feeding trial was conducted to feed *H. taimen* larvae when exposed to exogenous nutrients with live food. Development of pharyngeal TBs was evaluated. Larvae originated from induced spawning wild brood stock and larviculture in Bohai Coldwater Fish Hatchery, Chinese Academy of Fishery Sciences. Larvae (initial weight 0.11±0.01 g, 21 DAH) were cultured in a flow-through water system and fed to

apparent satiation for 8 weeks. The tanks (500 L per tank) were randomly allocated into triplicate groups of 5000 fish fed with live food for analysis of histological development of pharyngeal TBs. Larvae fed with water flea *Daphnia magna* from 23 DAH to 27 DAH and tubifex *Limnodrilus claparedeianus* from 28 DAH to 76 DAH. Spring water was filtered through zeolum, corallite and activated carbon, and its quality parameters were monitored daily. The water temperature was 11.0±0.50°C, pH was 7.2±0.1, total ammonia-nitrogen was <0.02 mg $L^{-1,}$ and dissolved oxygen (measurement JPB-607, Shanghai, China, precision ± 0.03 mg L^{-1}) was >6.0 mg L^{-1} across all treatments. Water flow rate started at 0.4 L min^{-1} and then was slowly increased with larvae age until a maximum of 1 L min^{-1}, at 12 DAH. The photoperiod changed to 12 h light: 12 h dark and fish were fed 4 times a day by hand.

Live food preparation

Live food water flea and tubifex were collected daily at a nearby lagoon, disinfected by 2% NaCl, and then placed on a fine mesh screen to remove excess water for feeding. The proximate nutrients are presented in Table 1.

Development of pharyngeal TBs

The examined number and observed proportion of larvae are presented in Table 2. Three larvae per tank were sampled per day during the first 26 days and every 3 days during the other 30 days. Larvae anesthetized with MS-222, then fixed in Bouin's solution for 48 h, dehydrated in ethanol, cleared in the xylene, embedded in paraffin blocks, cut into 6 µm slices with a rotating microtome (Leica, Bensheim, Germany), and then stained with Haematoxylin/easing method. A Motic (China) light microscope and computer program were used for structural observations and analysis. Data are presented as mean±SD.

Results

The following description is limited to the order of appearance of TBs characteristics. It is not possible to give exact time of development with the methods used in this study. The first few TBs primordia were visible within the epithelia of pharynx 27 DAH (Fig.1), which coincides with the onset of feeding. Pharyngeal TBs differentiated and formed two layers of cells 36 DAH (Fig. 2). TBs primordial differentiated into young TBs with receptor areas that are exposed to the environment 45 DAH, which are detectable in the surface epithelium (Fig. 3). Pharyngeal TBs were well developed 76 DAH, and mature-looking open receptor areas were already present (Fig. 4). The overall morphology of the TBs was similar in terms of the shapes and sizes of the receptor and basal cells of pharynx.

The number and size of TBs were quantified during the larval development (Table 2). The average number of pharynx was 8.63±1.15, 11.29±0.50, 14.50±1.06, and 17.78±0.47 TBs at 27, 36, 45, and 76 DAH, respectively.

Table 1: Proximate nutrient composition of live food (dry matter, %).

Diets	Composition			
	Moisture	Crude protein	Crude lipid	Ash
Water flea	84.35	9.02	2.68	3.86
Tubifex	83.88	10.03	5.29	0.69

Table 2: Number of taste buds of observed proportion of *H. taimen* larvae.

DAH	Number of larvae examined	Number of observed larvae	Characteristics of pharyngeal TBs	Basal–apical height (μm) A	Maximum width (μm) B	A/B	Number of lower pharynx	Per mm² of lower pharynx	Number of upper pharynx	Per mm² of upper pharynx
25	9	1	Primordia	53.49	65.84	0.81	3.00	5.00	5.00	7.00
26	9	2	Primordia	53.12±0.24	64.01±0.08	0.83	4.00	6.00	5.50±2.12	7.00±6.00
27	9	8	Primordia	51.86±3.27	61.33±2.07	0.85±0.04	3.50±0.53	5.50±0.53	5.13±1.13	6.88±7.75
28	9	8	Primordia	51.05±3.17	58.34±2.08	0.87±0.04	3.50±0.76	5.38±1.06	6.25±0.89	8.25±7.22
29	9	9	Primordia	51.36±3.12	58.03±3.05	0.89±0.02	4.11±1.05	6.22±1.64	6.00±1.22	7.78±7.50
34	9	1	Two layers of cells	48.21	55.60	0.87	5.00	7.00	6.00	8.00
35	9	3	Two layers of cells	48.84±4.92	55.01±5.23	0.89±0.01	5.33±0.58	7.33±0.58	6.33±1.15	8.33±8.00
36	9	7	Two layers of cells	48.99±3.48	55.04±2.90	0.89±0.03	5.29±0.95	7.43±1.27	6.00±1.15	8.00±8.63
37	9	8	Two layers of cells	49.20±3.42	55.05±2.69	0.89±0.04	5.38±0.92	7.38±0.92	7.75±1.16	10.00±8.22
38	9	9	Two layers of cells	49.16±1.08	55.18±2.58	0.89±0.04	5.44±0.73	7.44±0.73	7.22±1.20	9.33±9.00
43	9	2	Differentiation fininshed	50.53±0.27	56.53±2.72	0.90±0.04	6.00	8.00	7.50±0.71	10.50±0.71
44	9	4	Differentiation fininshed	53.23±5.28	56.82±4.10	0.94±0.03	6.25±0.50	8.50±1.00	7.25±0.96	10.00±1.41
45	9	8	Differentiation fininshed	56.09±4.90	56.91±3.02	0.99±0.06	6.50±0.93	8.75±1.39	8.00±1.31	11.25±2.19
46	9	8	Differentiation fininshed	58.85±4.29	57.73±2.76	1.02±0.04	6.00±0.53	8.13±0.83	8.63±1.19	12.25±1.91
47	9	9	Differentiation fininshed	63.17±3.89	57.55±2.90	1.10±0.06	7.11±0.93	10.00±1.50	8.22±5.00	11.67±1.32
67	9	1	Well developed	65.31	58.62	1.11	8.00	14.00	9.00	15.00
70	9	2	Well developed	64.57±5.64	58.68±2.43	1.10±0.06	8.50±0.71	14.00±2.83	9.50±5.13	13.00±1.41
73	9	5	Well developed	65.12±2.33	59.21±4.74	1.11±0.09	8.60±0.55	14.40±1.67	9.60±6.25	14.80±2.39
76	9	9	Well developed	64.99±3.07	58.35±2.40	1.11±0.06	8.78±0.97	14.33±1.41	9.00±6.00	15.22±1.20

Values are mean±SD (n=3).

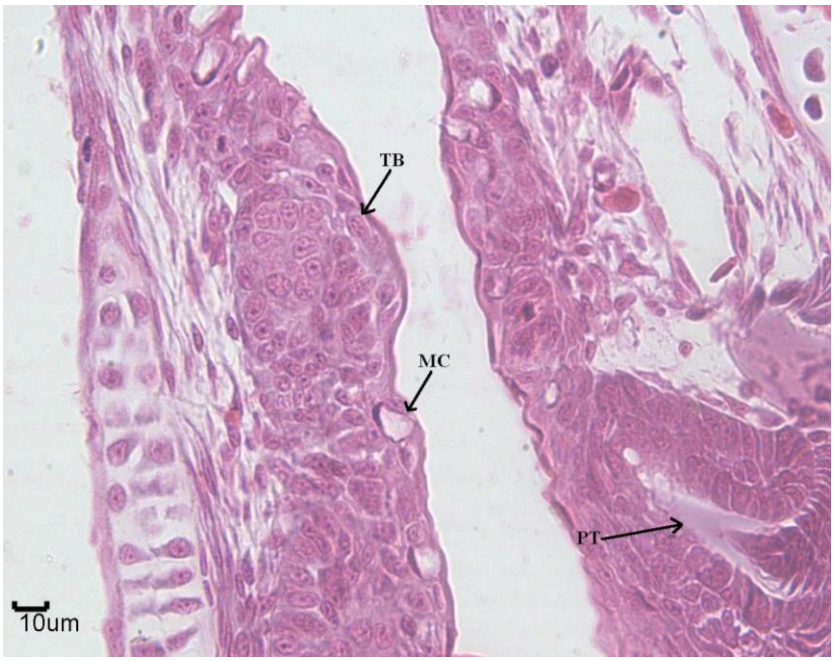

Figure 1: Larval pharyngeal TBs at 27 DAH. TB: Taste buds primordial; MC: Mucous cells; PT: Palatal tooth; Bar: 10 µm.

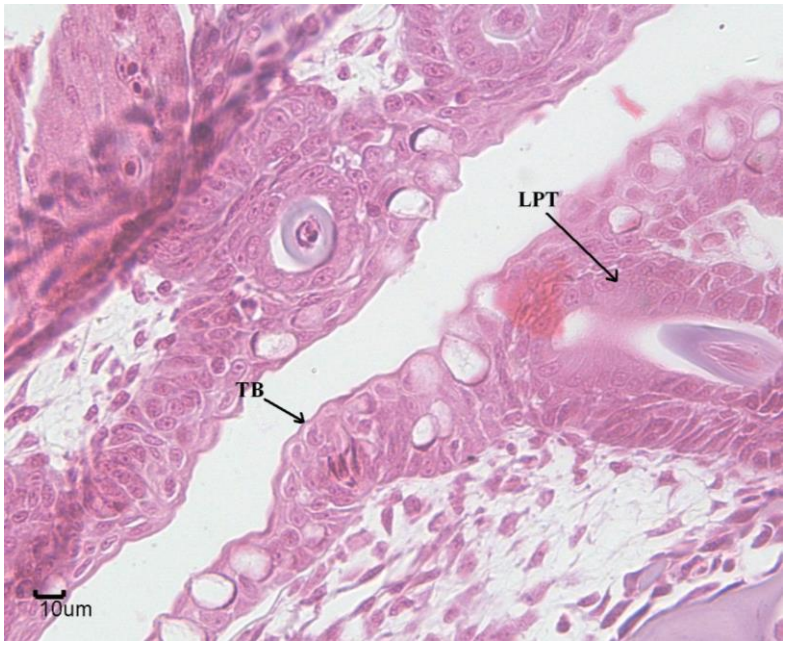

Figure 2: Larval pharyngeal TBs at 36 DAH. TB: Taste buds; LPT: Low pharyngeal tooth; Bar: 10 µm.

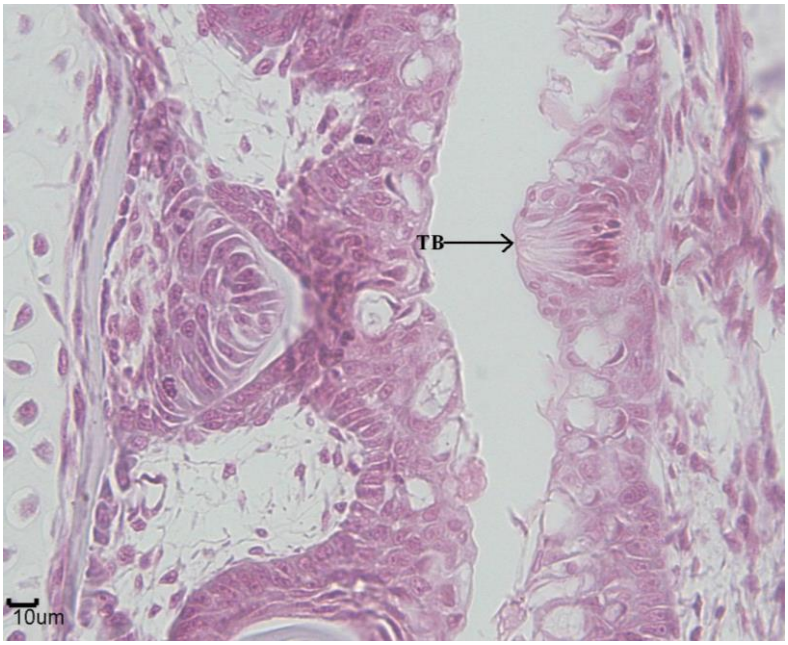

Figure 3: Larval pharyngeal TBs at 46 DAH. TB: Taste buds; Bar: 10 µm.

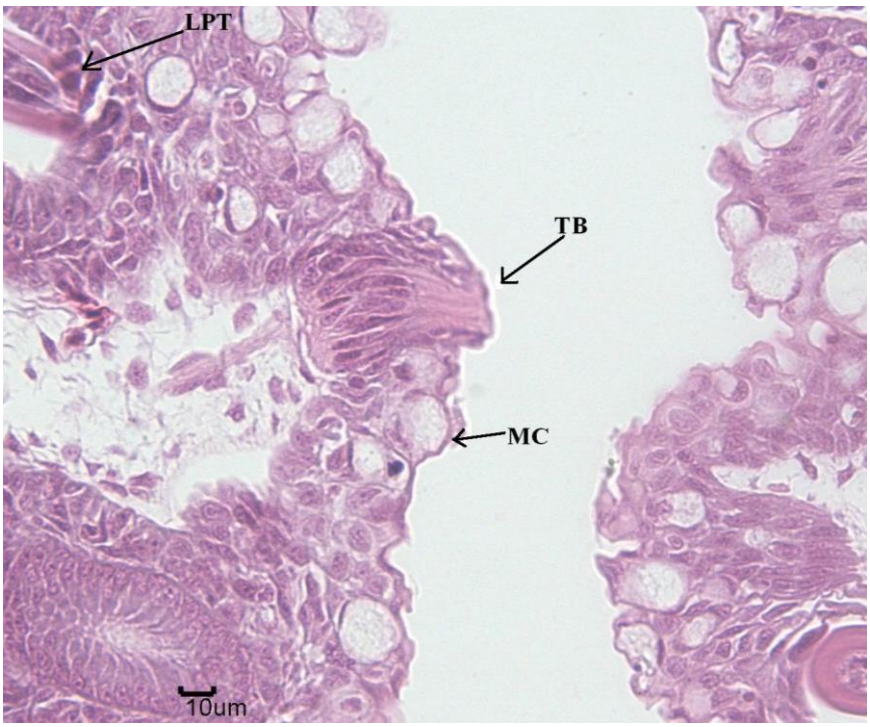

Figure 4: Larval pharyngeal TBs at 76 DAH. TB: Taste buds; MC: Mucous cells; LPT: Low pharyngeal tooth; Bar: 10 µm.

The number of TBs increased in both the upper and lower pharynx. Moreover, the numbers increased more dramatically in larvae, especially in the upper pharynx. The height to width ratio of TBs showed an increase tendency, ranging from 0.81 to 1.11. The height of TBs showed an increase tendency after 29 DAH during the development. However, the width of TBs exhibited a low point. The first few TBs primordial had 65.84 width and the lowest value 55.01±5.23 width was obtained 35 DAH.

Discussion

Sense of taste is an important property in fish for distinguishing between a variety of food available in an aquatic environment (Hara, 1994). However, Khanna (1968) showed that TBs were rare or absent from the highly predacious carnivorous fish, which rely more on their eyesight for detecting prey. During this investigation, *H. taimen* larvae inhabited dark conditions and fed both by sight and taste, had a better gustatory sense. *H. taimen* larvae appear to use their TBs to detect and ingest food under dark conditions. Thus, the presence of TBs could be considered as an adaptation to its benthic water and sluggish feeding behavior compensating for the restricted visibility in the dark conditions, especially for first feeding larvae.

In fish, food items seized, grasped, snapped, or nibbled with jaws, in general, are retained in the mouth cavity. During the retention period, the food is subjected to final sensory judgment and is either rejected or swallowed (Kasumyan and Doving, 2003). In the present study, prominent pharyngeal TBs showed useful in assessing the palatability of the food. Pharyngeal TBs of *H. taimen* was well developed at 45 DAH, and we found that larvae can swallow food or spit it out. Early larvae of *H. taimen* have no capacity of "taste", which spits the food out. This case was also shown for *S. gairdneri* (Ezeasor, 1982). Conspicuous epithelial protrusions of well developed pharyngeal TBs of *H. taimen* may increase probability of contact between the receptors and the food items. Ezeasor (1982) suggested that elevated TBs could have a superior perception of taste rather than non-elevated ones.

By comparing reports in the literature, it seems that in some fishes, feeding begins before the taste system is fully formed, whereas in other species, feeding commences only after the taste system is functional. In rainbow trout, *S. gairdneri*, mature-looking TBs were described as early as 8 DAH (Twongo and MacCrimmon, 1977), although the fish start to feed only 27 DAH (MacCrimmon and Twongo, 1980). However, as described here for *H. taimen*, pharyngeal TBs with primordia are visible 27 DAH (rearing temperature 10.9-11.5°C), and at this time, pharyngeal TBs were seen that coincided with the onset of exogenous feeding. Pharyngeal TBs with open receptor areas appear in taimen larvae 45 to 46 DAH and larvae fed more intensively than early larvae. Although the ontogeny of pharyngeal TBs of *H. taimen* larvae follows a similar pattern as other salmon species, the time span of

each developmental phase was much longer, indicating the slow feeding nature of this fish species. The formation of the functional TBs of pharyngeal was suggested to be the point to wean *H. taimen* onto microparticle feed.

The number of fish TBs greatly differs during the development. For instance, Fishelson (2005) has reported that in *Saurida macrolepis*, in which the number rises from 400 in the oral cavity of 80 mm SL fish, to 1150 in a fish of 260 mm. The present study showed that the number of TBs in the pharynx increased with the development. One possible reason for this is a change to accommodate increase of feeding intake. As for current results, it is unknown whether larvae reach a certain number of pharyngeal TBs typical for *H. taimen* at early stage (Fishelson *et al*., 2010). It is clear that the final number of pharyngeal TBs is species-specific, as observed in other fishes (Fishelson *et al*., 2004; Fishelson *et al*., 2010).

This study indicated that the number of pharyngeal TBs in *H. taimen* was lower than that of other salmonids. The differences in the oropharyngeal cavity of the various fishes seem to reflect evolutionary trends, specifically focused on types of food gustation and consumption and, as stated by Fishelson (2005). To understand the reason for such differences in the numbers of pharyngeal TBs of the various species in the different genera and families, we need to know more about their diet and feeding behavior.

In addition, different with other salmonids, there is a change in the shape of pharyngeal TBs. The ratio of height to width of TBs showed an increase tendency, ranging from 0.81 to 1.11. To some degree, this showed that TBs change with development. Although pharyngeal TBs were well developed 76 DAH, the height to width ratio may further increase with development and arrive at a steady value.

Our findings may lead to a better understanding of the ontogeny of pharyngeal TBs of *H. taimen* larvae. Fish farmers must feed fish according to the development process of pharyngeal TBs. The information gathered about the feeding habits of larvae should be studied further to improve survival during the initial stages of larval development.

This paper demonstrated that under current experimental conditions, the development of pharyngeal TBs coincides with the feeding, and this should lead to a better understanding of improvement of larval rearing in *H. taimen* hatcheries.

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