

Embryonic Development and Larval Growth and Development of *Tilapia buttikoferi* (Hubrecht, 1881) (Actinopterygii: Cichlidae)

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Abstract: The morphology of mature eggs, fertilised eggs and spermatozoa of *Tilapia buttikoferi* was studied by scanning electron microscopy in order to understand their reproductive and growth characteristics. The embryonic development, growth and development of larvae and fry were observed, photographed and measured, and the growth characteristics of *T. buttikoferi* under artificial culture conditions were studied. The results showed that the mature eggs of the *T. buttikoferi* were non-adhesive sinking eggs. After fertilisation, there were dense micropores on the surface of the egg membrane and adhesive filaments of different length inside the egg. The sperm consisted of three pieces: the head, the mid-piece and the tail, without an acrosomal cone. The fertilised eggs of *T. buttikoferi* were hatched into larvae at 49 h 30 min under 26±1 °C of water temperature. The development of larvae was completed at 16d. The fish reached a total length of 13.59 ± 2.19 mm with scales covering entire body. This study obtained the early life history of *T. buttikoferi*, which could facilitate the breeding of the *T. buttikoferi*.

Key words: *Tilapia buttikoferi*, Egg and sperm morphology, Development, Embryo, Larva

Introduction

Tilapia buttikoferi (Hubrecht, 1881) [Syns. *Chromis buttikoferi* Hubrecht, 1881; *Heterotilapia buttikoferi* (Hubrecht, 1881)] is a popular ornamental fish belonging to the order Cichliformes and the family Cichlidae; it is native to Sierra Leone and Liberia in Africa. In the previous studies of muscle nutrients, *T. buttikoferi* was found to be a fish with higher

nutritional value. It has relatively high protein and amino acid contents. The essential amino acid composition of this fish species is relatively balanced and rich. This fish species has a low fat content and is rich in unsaturated fatty acids such as oleic, linoleic, linolenic, arachidonic and docosahexaenoic. *T. buttikoferi* is a high-quality fish with great edible value and health protective function, and it can be broadly used for development and utilisation. This

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species has the characteristics of rapid growth, delicate meat, delicious taste, no intermuscular thorn and mild fishy odour. It is a freshwater species that has the potential to be developed into an edible fish in aquaculture.

At present, studies on *T. buttikoferi* are limited to resource distribution survey (ERGENS 1981), population genetic structure analysis (MU et al., 2009), karyotype analysis (WANG et al. 2010), anaesthetic drugs suitability (HE et al. 2013). These studies are limited to the preliminary understanding of *T. buttikoferi* and lack of systematic and profound analysis on the reproduction, growth and development characteristics, making the information of *T. buttikoferi* incomplete.

The present study aims to describe, for the first time, egg and sperm morphology, embryogenesis and larval growth to in order to provide a more comprehensive information for the breeding and reproduction of *T. buttikoferi*.

Materials and Methods

Experimental fish

The experimental fish were obtained from the National Freshwater Aquatic Germplasm Resource Centre. Fifteen of each 2-year-old female and male *T. buttikoferi* from natural breeding were used. The female fish weighed 568.35 ± 12.34 g and the male fish weighed 631.25 ± 10.23 g.

Hatching of fertilised eggs and breeding of larvae

Mature fish were selected and separately bred in the aquarium. When the fish displayed obvious oestrus behaviour, the partition boards were removed and replaced with clean tiles. The breeding process was captured on video camera system and the exact time of spawning was recorded. After the observation of spawning, an appropriate amount of fertilised eggs was scraped from the tiles in a timely manner and incubated in an aquarium with constant temperature ($26 \pm 1^\circ\text{C}$). Micro-aerated still water was used and strong light was avoided. Samples were taken daily for the observation of embryonic development. After hatching of the larvae, the water temperature was maintained at $26 \pm 1^\circ\text{C}$, and the larvae were bred in aerated still water. Samples were taken periodically for the observation of the larvae morphology.

Observation of fertilised eggs and embryonic development

The female parental fish were artificially injected with luteinising hormone-releasing hormone analogue $10 \mu\text{g}/\text{kg}$ body weight and domperidon at 10

mg/kg body weight for maturation. Males received a single injection of half female dose. Semen and oocyte collection occurred after 14–18 h and fixed with pre-cooled glutaraldehyde solution for 24 h. For scanning electron microscopy (SEM), the samples were dehydrated using graded alcohol, dried, adhered to a gold plate and scanned with a scanning electron microscope (FESEM Zeiss Ultra-55). To describe the stages of embryonic development, 20 to 30 embryos were collected from incubators every 15 min during the first 3 h and then every 4 h until hatching. The end of each developmental stage was defined as the time at which 50 % of the sampled eggs were at the next stage. The developmental process was observed in vivo under the Olympus SZX16 stereo microscope. The Olympus DP80 imaging system was used for image capture and measurement. Each developmental period was determined based on 50 % of the observed embryos that were exhibiting the typical characteristics. At the same time, the samples were fixed with 5 % formaldehyde for later observation.

Observation on the morphological characteristics of larvae and fry

In the hatching aquarium, 20–30 eggs/time were randomly taken. The development process was observed in vivo under the Olympus SZX16 stereo microscope. The Olympus DP80 imaging system was used for image capture and measurement. Time and characteristics of each developmental period were recorded, and a total of 10 growth indicators including body length, body height and transverse diameter of the eye were measured (Fig. 1).

Statistical analysis

The measured growth data of *T. buttikoferi* larvae and fry were analysed by SPSS 20.0 software. The linear regression model was used to fit the relation-

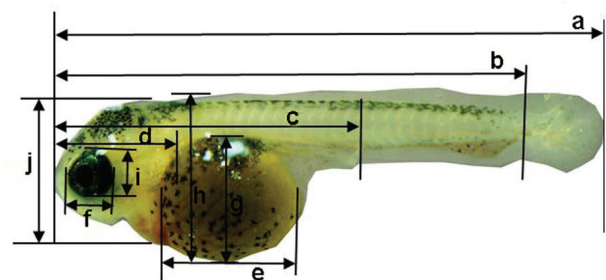


Fig. 1. The measurement model of *Tilapia buttikoferi* larva. (a) Total length. (b) Body length. (c) Preanal length. (d) Head length. (e) Transverse diameter of yolk sac. (f) Transverse diameter of the eye. (g) Vertical diameter of yolk sac. (h) Body height. (i) Vertical diameter of the eye. (j) Head height.

ship between the total length mean and the age, and the fitting curve with the highest R^2 value was taken as the optimal model. The allometric growth model $y=ax^b$ was used to analyse the growth relationship between the transverse diameter of the eye, the vertical diameter of the eye, the head length, the head height, the body height, and the preanal length with the full body length. Value of each growth indicator (mm) in the corresponding different periods was represented by x and y , respectively. In the two equations, a and b were constants.

Results

Egg morphology by SEM

Morphology of mature eggs. The mature eggs of the *T. buttikoferi* were non-adhesive sinking eggs. Under a 50-fold scanning electron microscope, the mature eggs were oval, the vegetal pole was blunt and the animal pole was relatively pointed, the long axis diameter was 1530–1720 μm and the short axis diameter was 1200–1450 μm (Fig. 2a). The surface of mature eggs was smooth with no obvious grooves or ridges, and it was covered with clear mesh patterns, showing an irregular polygon shape (Fig. 2b). No fertilised pores were observed.

The three-layer structure of the egg membrane was clearly visible with a thickness of 35–40 μm (Fig. 2c). The outer layer was the thinnest, at 1.3 μm , and the structure was tightly interweaved; the middle layer was the thickest, at about 28 μm , and it was arranged in a loose columnar shape with irregular filaments distributed in the gap; the inner layer was slightly thicker than the outer layer at about 12

μm , with a dense longitudinal tube that opens to the inner surface (Fig. 2d). Mature eggs were filled with yolk granules of 15–20 μm in diameter (Fig. 2e).

Morphology of fertilised eggs. The fertilised eggs of *T. buttikoferi* did not show a significant difference under a 40-fold scanning electron microscope (Fig. 3a). It was observed under a 500-fold scanning electron microscope that there were adhesive filaments between the fertilised egg and the attached tile residue. The adhesive filaments fixed the fertilised egg on the attachment, making it not easy to fall off (Fig. 3b). The non-attached adhesive filaments of different lengths were distributed on the surface of the egg membrane, and the mesh patterns on the egg membrane disappeared (Fig. 3c). A large number of different sized micropores were distributed on the surface. The adhesive filaments that attached to the objects in the water were protruded from these micropores (Fig. 3d).

Morphology of sperms

Scanning electron microscopy showed that the sperm of *T. buttikoferi* consisted of three pieces: the head, the mid-piece and the tail. The head was round or nearly circular, and the diameter was about $1.55 \pm 0.22 \mu\text{m}$ (Fig. 4a). The mid-piece was right behind the head and consisted of a centriole complex and a sleeve structure. The tail was long and originated from the central space of sleeve with a length of $11.29 \pm 0.34 \mu\text{m}$ (Fig. 4b).

Transmission electron microscopy showed that the sperm consisted of the head, mid-piece and flagellum, without the acrosomal cone. The sperm head was mainly composed of a nucleus, and the

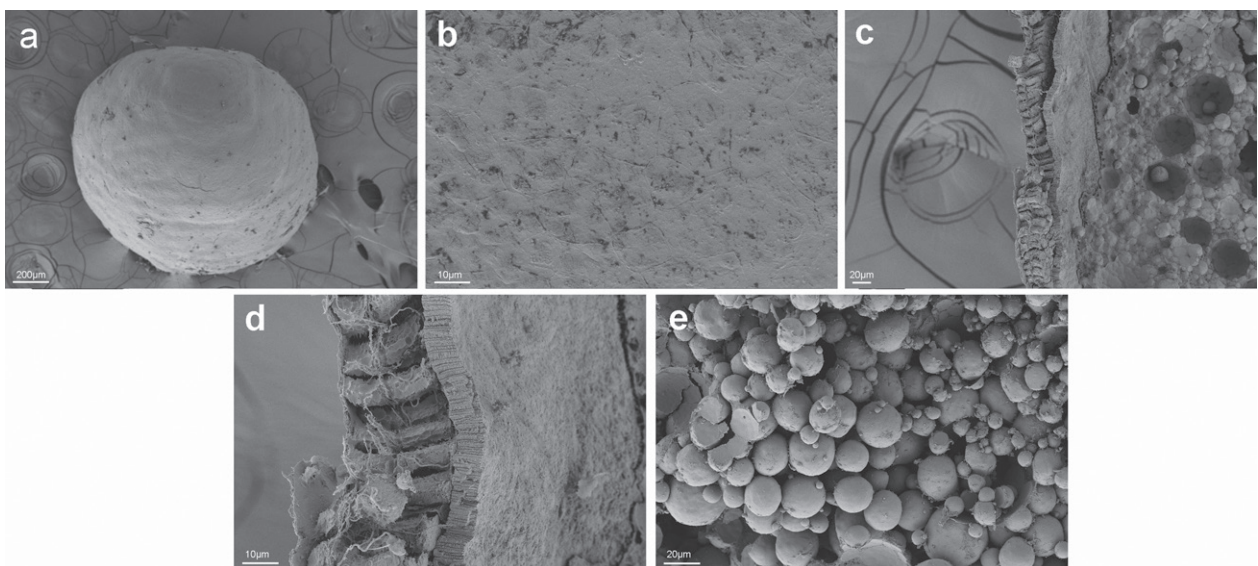


Fig. 2. Scanning electron microscope observation of unfertilised egg. (a) Mature egg. (b) Surface of mature egg. (c) Egg membrane. (d) Three-layer structure of the egg membrane. (e) Yolk granules.

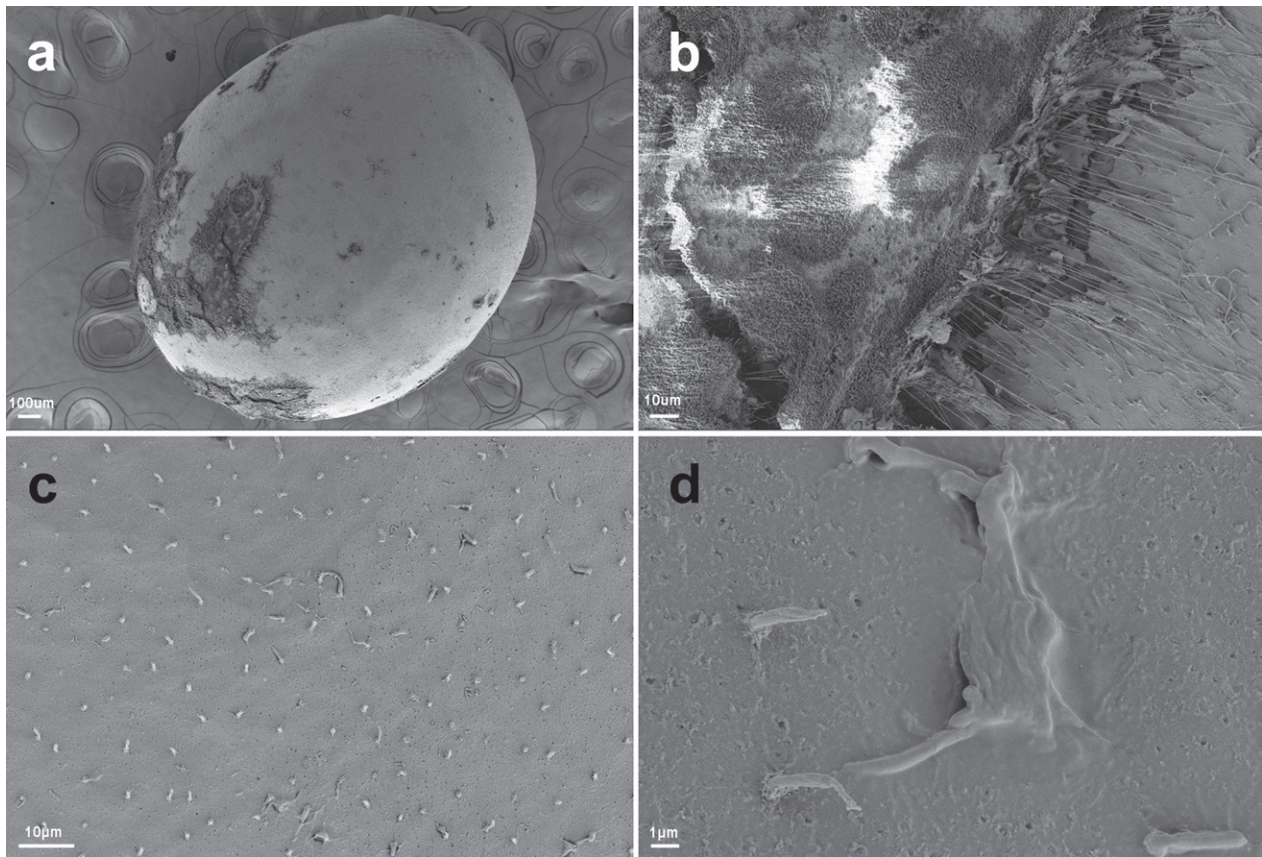


Fig. 3. Scanning electron microscope observation of fertilised egg. (a) Fertilised egg. (b) Adhesive filaments fixed on the attachment. (c) Surface of fertilised egg. (d) Unattached adhesive filaments.

nucleus was surrounded by double layers of nuclear membrane. Except for the bottom of the nucleus, the nuclear membrane was closely connected with the plasma membrane. The chromatin was dense and highly concentrated into clusters, and the near-flagellum end of the nucleus was inwardly recessed into a shallow nucleus pocket. The mid-piece of the sperm consisted of a sleeve and a centriole complex which forms a cylindrical shape. The cavity that connected to the central back of the nucleus was called the central space of sleeve. Both sides of the sleeve were similar in size and contained a small number of mitochondria were randomly distributed on both sides. The centriole complex consisted of proximal and distal centrosomes, the proximal centrosome was located in the nucleus, which was perpendicular to the longitudinal axis of the nucleus, and the distal centrosome was perpendicular to the proximal centrosome, starting from the end of the nucleus pocket and located outside of the nucleus pocket connected to the flagellum (Fig. 4d). The central part of the flagellum consisted of axoneme, which was connected to the kinetosome forming a typical “9+2” microtubule doublet structure. The outside of the axoneme extended from the cytoplasmic membrane to the

sides to form lateral fins, and it did not contain vesicles or mitochondria (Fig. 4c).

Embryonic development

The fertilised eggs of *T. buttkoferi* were elliptical, pale yellow, sinking and had strong viscosity. The fertilised eggs were incubated at a water temperature of 26 ± 1 °C and were hatched to larvae after 49 h 30 min. There was heterogeneity in embryo development because different stages of development were observed at the same time. The developmental process goes through the following periods:

Zygote. After fertilisation of mature eggs, the perivitelline space gradually expanded. At 20 min post-fertilisation, the cytoplasm gradually moved to the animal pole and formed a blastodisc. At 1 h 20 min post-fertilisation, the blastodisc protruded to the highest level, and the height was about 1/6 of the longitudinal diameter of the egg (Fig. 5a).

Cleavage. At 1 h 36 min post-fertilisation, the blastodisc was divided into 2 equal-sized blastomeres (Fig. 5b). After 20 min, it divided again to form four equal-sized blastomeres (Fig. 5c), and the formed cleavage surface was perpendicular to the first time. Eight divisions (Fig. 5d) were completed

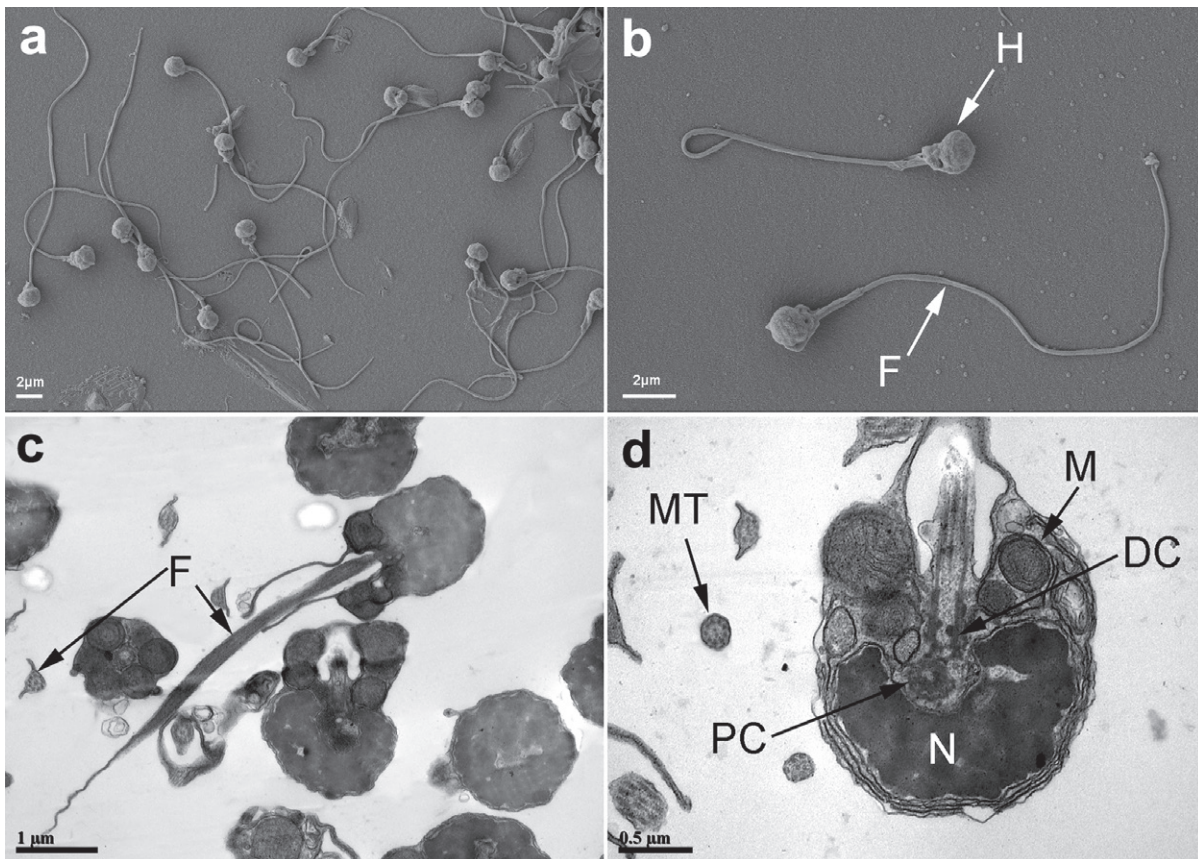


Fig. 4. Scanning electron microscope (SEM) and Transmission electron microscopy (TEM) observation of sperm. (a, b) Sperm, general view. (c) Cross section and longitudinal section of the sperm flagellum; (d) Oblique section of the head; H: head; F: Flagellum; M: Mitochondrion; MT: Microtubule; DC: distal centriole; PC: proximal centriole.

at 2 h 30 min post-fertilisation. A dense amount of blastomeres were protruded at the top of the yolk sac at 5 h 30 min post-fertilisation and entered the blastula period.

Blastula. At 6 h post-fertilisation, the blastoderm was protruded high above the yolk sac, and the height was about 1/4 of the height of the yolk sac. This was the early stage of the blastula period (Fig. 5e). After 28 min, the blastoderm gradually lowered, and the connection between the yolk sac and the blastoderm became smooth and entered the mid-stage (Fig. 5f). At 6 h 40 min post-fertilisation, the blastoderm became low, flattened and fused to the yolk sac, making it looked like a newly fertilised egg. The marginal cells of the blastoderm started epiboly of the yolk sac and entered the late stage of the blastula period (Fig. 5g).

Gastrula. At 12 h 40 min post-fertilisation, the epiboly of blastoderm continued to the yolk sac, the yolk sac was surrounded and the basal margin was thickened to form the germ ring, entering the early stage of the gastrula period (Fig. 5h). The involution of blastoderm gradually continued to reach

1/2-epiboly, and one end of the germ ring formed a thickened ridge, called the embryonic shield, entering the mid-stage of the gastrula period (Fig. 5i). At 24 h post-fertilisation, the epiboly was at 3/4, and the embryonic shield was gradually elongated and thinned, showing the early shape of the embryo and entering the late stage of the gastrula period (Fig. 5j).

Neuroblast. At 28 h 40 min post-fertilisation, epiboly continued, with only a tiny yolk sac uncovered. The embryonic shield prolonged and the anterior segment began to expand, forming the early form of the neural plate (Fig. 5k). The blastopore gradually closed along with the developmental process and was completely closed after about 1 h 10 min. The embryo was curved to wrap the yolk sac, located on the dorsal side, the yolk sac was located on the ventral side, and pigmentation appeared on the yolk sac (Fig. 5l).

Organogenesis. At 30 h 20 min post-fertilisation, the head of the embryonic axis was divided into three parts: forebrain, midbrain and hindbrain. Two enlarged elliptical optic primordia appeared on both sides, and the embryo body wrapped 3/4 of

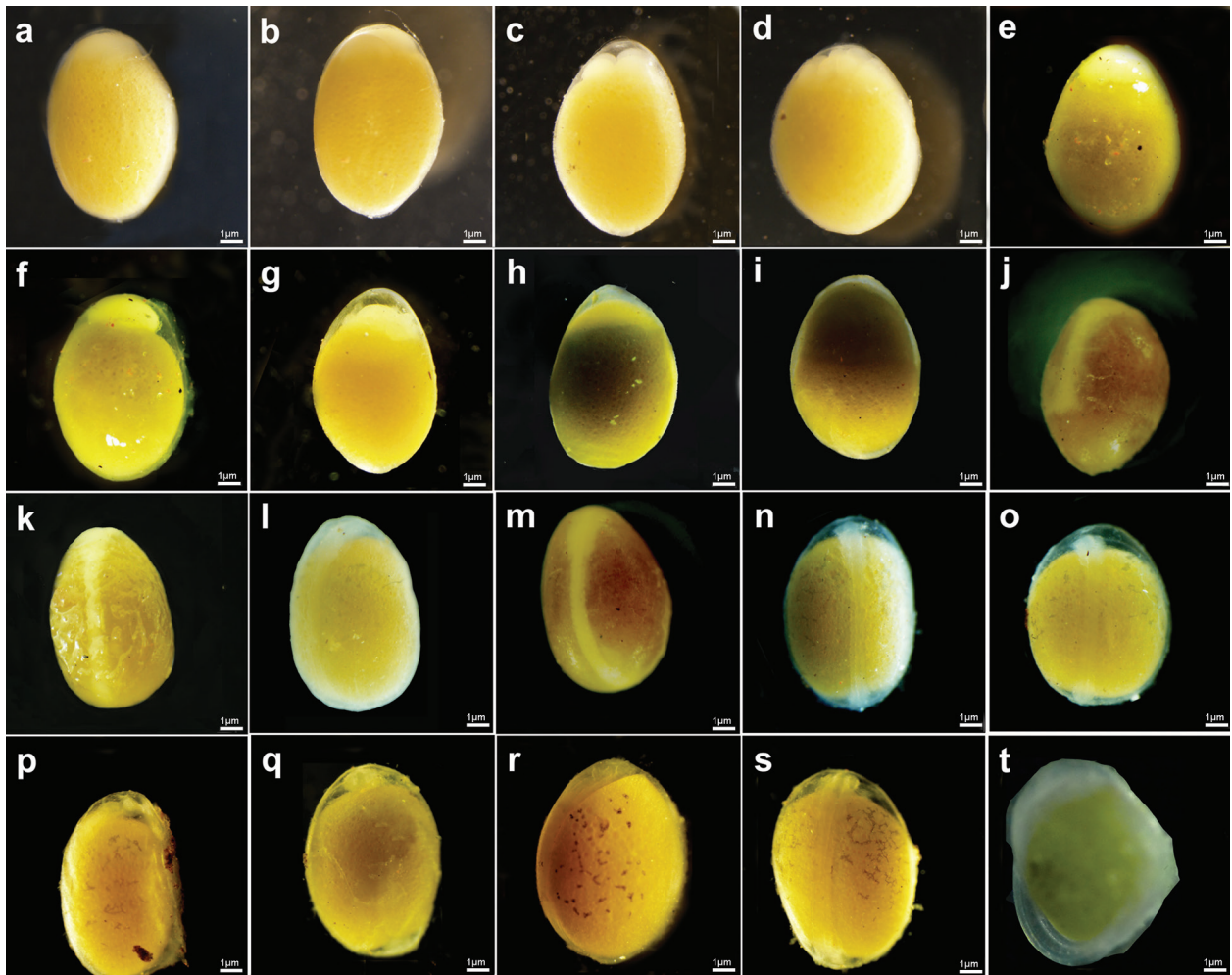


Fig. 5. Embryonic development of *Tilapia buttikoferi*. (a) Blastoderm. (b) 2-cell stage. (c) 4-cell stage. (d) 8-cell stage. (e) Earlier stage of blastocyst. (f) Middle of blastocyst stage. (g) Later period of blastocyst stage. (h) Earlier stage of gastrulation. (i) Middle of gastrulation. (j) Later period of gastrulation. (k) Neurulation. (l) Pigmentation appear on the yolk sac. (m) Somite appearance. (n) Increase of somites. (o) Beano appear. (p) Bulla stage; (q) Otolith formation; (r) Circulation period; (s) Heart beat period; (t) Ail in the membrane.

the yolk sac. Formation of 4 to 6 pairs of somites appeared (Fig. 5m). Then the optic primordia were recessed to form the optic cups and the number of somites was increased to 10–14 pairs (Fig. 5n). At 31 h 15 min post-fertilisation, the tail of the embryo showed bud-like protrusions, called tail bud, and both sides of the yolk sac had cluster-like pigmentation (Fig. 5o). As the body of the embryo continued to elongate, the caudal fin formed at the end of the tailbud, and the otic placodes hollowed at the posterior of the yolk sac, forming the otic vesicles at the back of both sides of the brain (Fig. 5p). At 33 h 15 min post-fertilisation, the tail began to grow, and the embryo began to show a weak arrhythmia. Then, the heart primordia appeared below the optic cup and the otic vesicle, lens placodes appeared inside the optic cups and two visible otoliths appeared in the

otic vesicles (Fig. 5q). At 42 h post-fertilisation, the heart began to beat, the pulsation rate was 110–120 beats/min, and the embryo body wrapped the yolk sac more than 4/5, and frequent twitching occurred (Fig. 5r). At 44 h 30 min post-fertilisation, blood circulation began, the number of heartbeats increased to 135–150 beats/min, the blood vessels branched, blood cells gradually increased, and the colour of blood changed from transparent to pink (Fig. 5s).

Hatching. The hatching period started at 49h 30min post-fertilisation, the egg membrane became thinner, the embryo body was severely twisted, the muscle contraction was obvious, the tail movement was the strongest and it was the first to break through the membrane, and the embryo body was vigorously twisted to pull out the head. There were also a few individuals had the head out first and then the tail (Fig. 5t).

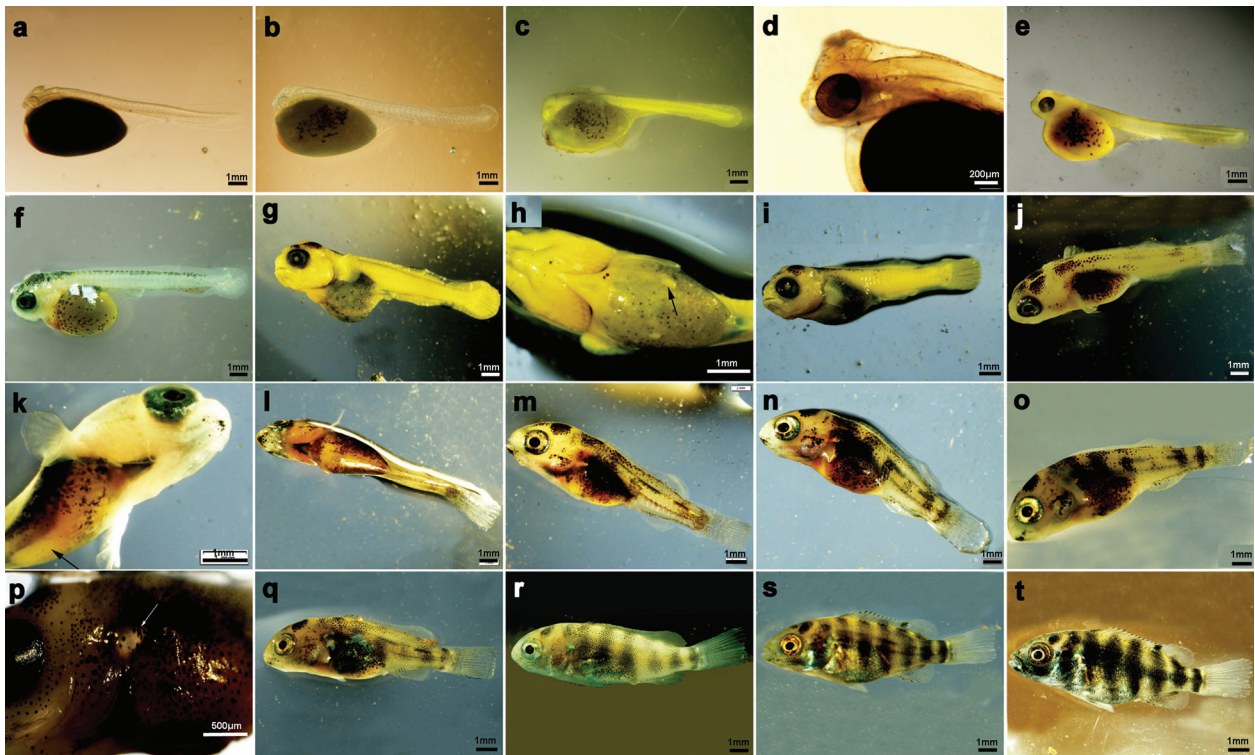


Fig. 6. Development of larvae and fry. (a) Newly hatched *Tilapia buttikoferi* larva. (b) Arrows indicate the primordial anus and perking coccyx. (c) Ear sac and mouth membrane. (d) Larva hatched 32 h. (e) Arrows indicate the differentiation of pterygiophore and branchial arch. (f) Arrows indicate the primordial anal fin. (g) The formation of primordial abdominal fin. (h) Larva hatched 32 h. (i) Larva hatched 5 days. (j) Arrows indicate the clavate yolk sac. (k) Arrows indicate the exhausted yolk sac. (l) Arrows indicate the growing of pterygiophore. (m) Arrows indicate the abdominal fin. (n) Larva hatched 7 days. (o) Arrows indicate the swim bladder cavity. (p) Larva hatched 8 days. (q) Larva hatched 9 days (arrows indicate the bifurcate tail fin and silver belly). (r) Larva hatched 12 days. (s) Larva hatched 14 days. (t) Larva hatched 16 days.

Development of larvae and fry

Under the condition of 26–30°C of water temperature, *T. buttikoferi* completed the development of larvae and fry in about 16 days. The larvae stage can be divided into yolk sac larvae and late larvae. The yolk sac larvae stage lasted for about 7 days from the early stage of hatching to the complete removal of the yolk sac; The late larvae stage lasted for about 6 days from the complete absorption of the yolk sac until the fins were basically developed and completed; the fry period lasted for about 3 days from the completion of the fins to the whole body was covered by scales.

Yolk sac stage larvae. The newly hatched larvae were transparent, with a total length of 7.10 ± 0.25 mm, 22 pairs of sarcomere, the tail was separated from the yolk sac, and the rest was closely attached to the yolk sac. The yolk sac was droplet-shaped, the anterior was oval, and the posterior was tapered. The size was $(2.30 \pm 0.10) \times (1.53 \pm 0.05)$ mm (Fig. 6a).

At 5 h 40 min post-hatching, there was flaky melanin deposited on the sides of the yolk sac along

the fish body. Apparent blood flow was observed throughout the body; the tail vertebrae were upturned, the anus primordium was formed, and there were 3 pairs of gill arcs under the eyes. A transparent digestive tract was formed, and the eyes were transparent (Fig. 6b).

At 32 hours post-hatching, the body showed rainbow colours from the posterior of the head, the eyes were covered with melanin, the yolk sac was reduced in volume, and the operculum was formed (Fig. 6c). Three pairs of transparent rings appeared above the otolith in the otic vesicle, which was the origin for the formation of semicircular canals (Fig. 6d); The mouth was closed, the intestine had a straight tubular shape, there was an opening at the end of the anus, and a trace of melanin was deposited on the dorsal side (Fig. 6e).

At 48 hours post-hatching, a large amount of melanin deposition appeared on the upper edge of the eye; The yolk sac was evenly covered with snow-like melanocytes and blood vessels; rainbow colours appeared in the eyeball, and the operculum could cover 4 pairs of gill arcs; Rays of the fins were

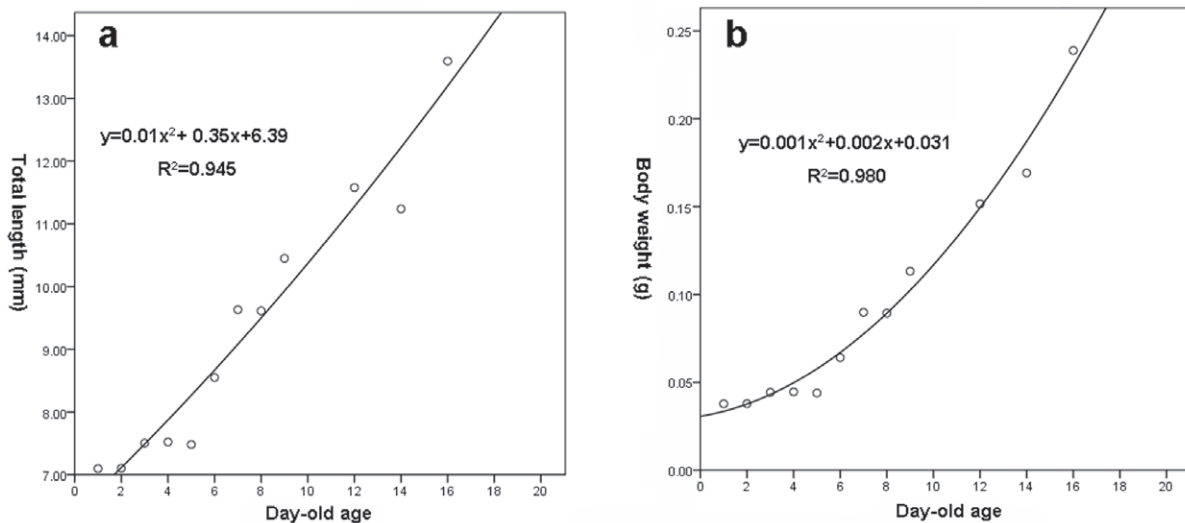


Fig. 7. The relationship between body length and day-old age.

started to become distinguishable at the lower end of the caudal fin (Fig. 6f).

At 72 h post-hatching, the yolk sac volume decreased drastically, the mouth started to open, and the pectoral fins became visible. The melanin deposition in the head and abdomen was ink-like. The rays of the caudal fin increased and the number reached 13–15. The dorsal fin protruded to form the dorsal fin primordium and the posterior of the anal protruded to form the anal fin primordium (Fig. 6g). The anterior of the digestive tract was enlarged, the oesophagus, mouth, intestine and anus were connected, entering the mixed nutrition period.

At 96 hours post-hatching, the number of pectoral fins was increased to 10–15. Dorsal and anal fins became distinguishable, and a pair of bud-shaped pelvic fin primordia was appeared in the abdomen (Fig. 6h); the first, second, third, and eighth transverse stripes became visible on the back; the yolk sac gradually changed from elliptical to slender (Fig. 6i).

At 5-day-old larvae, the number of dorsal fins increased to 15 and the number of anal fins increased to 10; the end of caudal fin changed from arc to straight, and the number of fins increased to 17–20. The fin spine primordium of dorsal and anal fin was formed (Fig. 6j); the yolk sac was rod-shaped at the ventral proximal anus (Fig. 6k).

At 6-day-old larvae, melanocytes appeared in the operculum. The yolk sac was remained in the abdomen as a short rod (Fig. 6l). The number of anal fins and dorsal fins increased significantly, and 10–11 short fin spines appeared at the anterior segment of the dorsal fin. The 5th and 7th transverse stripes became visible (Fig. 6m).

Late larvae. At 7-day-old larvae, the yolk sac disappeared, the intestine was filled with food, and the fish grew by exogenous nutrition; Snowflake-like melanin deposition appeared at the ventral side of the abdomen, dorsal fin and anal fin, and the pelvic fin was formed (Fig. 6n); The fin folds from the dorsal fin to the caudal fin and the anal fin to the caudal fin disappeared, but the fin folds at the anterior of the anal fin remained (Fig. 6o); The swim bladder appeared at the anterior of the abdominal cavity behind the operculum (Fig. 6p). Most of the larvae stayed in the middle and lower level of the water body and were mostly concentrated in the corners.

At 8-day-old larvae, there were 14–16 fins on the dorsal fin and 14 fins on the anal fin. All fin folds disappeared, but no rays appeared. The scales began to appear on the abdomen and the posterior of the operculum (Fig. 6q); six transverse stripes were formed on the body, and the melanin deposition occurred at the end of the anterior dorsal fin spine and at the base of the anal fin.

At 12-day-old larvae, the fish body gradually became opaque, and melanin began to deposit on the dorsal fin. Rays appeared on the caudal fin, dorsal fin, pectoral fin, and anal fin; the development of each fin was basically completed, and melanin deposition appeared at the end of the dorsal fin spine (Fig. 6r).

Fry. At 14-day-old larvae, the fish body was opaque, the total length reached 11.34 ± 0.77 mm, the second transverse stripe on the fish body was formed, and the melanin deposition was distinct at the posterior of the caudal fin; The posterior edge of the eye to the anus had a dazzling silvery white colour. A

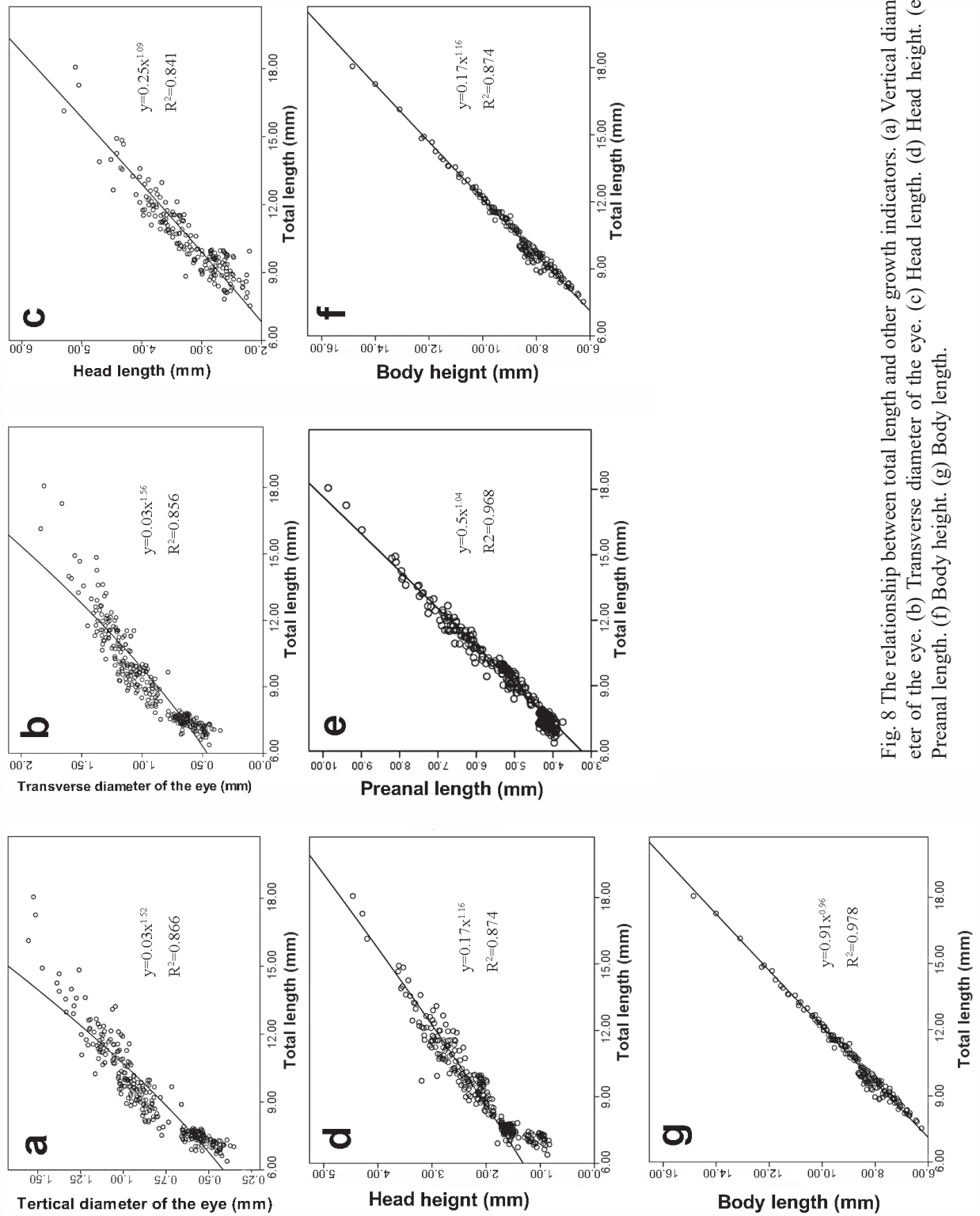


Fig. 8 The relationship between total length and other growth indicators. (a) Vertical diameter of the eye. (b) Transverse diameter of the eye. (c) Head length. (d) Head height. (e) Preanal length. (f) Body height. (g) Body length.

large number of scales appeared in the middle along the body axis, and the scales were evenly arranged; the fins were completely formed, and fish developed from newly hatched larvae to fry (Fig. 6s).

At 16-day-old fry, the total length was 13.59 ± 2.19 mm, the whole body was scaled and the melanin deposition was significantly deepened. The first transverse stripe was formed and the morphological characteristics were similar to those of adult fish. Thereafter, the fish entered the juvenile developmental stage (Fig. 6t).

Growth characteristics of larvae and fry

Relations.hip between total length, body weight and day-old age

According to the analysed data (Fig. 7a), the relationship between the total length mean of the *T. buttikoferi* and the day-old age was in accordance with the quadratic equation $y = 0.01x^2 + 0.35x + 6.39$ ($R^2 = 0.945$). The analysed results showed a significant correlation between the total length and the day-old age. The total length of the newly hatched larvae was 7.10 ± 0.25 mm, and the total length of the scale covered fry was increased to 13.59 ± 2.19 mm. The linear correlation equations between body weight and day-old age was $y = 0.001x^2 + 0.002x + 0.031$ ($R^2 = 0.980$) (Fig. 7b). It can be seen from the fitting curve that the growth rate of total length and body weight of the larvae increased with the day-old age.

Allometric growth of various growth indicators of fish

The allometric growth index b on the vertical diameter of the eye (Fig. 8a), transverse diameter of the eye (Fig. 8b), the head length (Fig. 8c), the head height (Fig. 8d), preanal length (Fig. 8e) and the body height (Fig. 8f) was 1.56, 1.52, 1.09, 1.16, 1.16, and 1.04, respectively. As $b > 1$, which indicated that these body parts grew rapidly during the developmental stage of larvae. The fitted power function growth index of body length was 0.96, which was slightly less than 1 (Fig. 8g), indicating that the body length of *T. buttikoferi* had a slow growth rate during the developmental stage of larvae.

Discussion

The heterogeneity in embryo development observed in this study may be due to the degree of oocyte development. Oocyte size is responsible for prolongation of the time of specific ontogenetic phases (KAMLER 2002). And the size of the oocyte also affects the survival of fish (YIN 1991). However, the diameter of the egg varies differently in different

fish species. The long axis diameter of the eggs of the *T. buttikoferi* is 1530–1720 μm , and the short axis diameter is 1200–1450 μm , which is larger than that of the *Tridentiger trionocephalus* (450–650 μm) in the Yangtze River (ZHANG et al. 2008), the *Epinephelus Moara* (760–820 μm) (GUO et al. 2016), and the *Tilapia nilotica* of the same family (1470 μm) (HUANG 1993). The large egg diameters could prolong the time of larvae to enter the exogenous nutrients stage, ensure the growth and development of important organs in the early developmental stage of *T. buttikoferi*, and make the larvae better adapted to the environment.

It can be seen from the results that the mature eggs of the *T. buttikoferi* had a smooth surface and clear mesh patterns, which were similar to the mature eggs surface morphology of the previously studied *Scophthalmus maxima*, *Paralichthys olivaceus* and *Squaliobarbus curriculus* (SUN et al. 2006). The mature eggs were scattered in water and had no adhesion. After fertilisation, the surface of the egg changed greatly. The mesh pattern on the surface of the fertilised egg disappeared and replaced with many micropores of different pore diameters. A large number of adhesive filaments were observed on the contact surface between the fertilised egg and the object for fixation, which can ensure that after the eggs are discharged into the water, they can be firmly adhered to the nearby objects without drifting by the water flow. These features are accompanied with the highly intensive egg-protecting behaviour of the *T. buttikoferi* and greatly increased their hatching rate.

The egg membrane is not only an important protective barrier for eggs, but it also has a specific recognition function during fertilisation, thereby preventing polyspermy. The egg membrane of the *T. buttikoferi* has a three-layer structure. The interweaved structure of the outer layer and the sparse columnar structure of the middle layer increases the elasticity of the egg membrane and can protect the egg from damage caused by being squeezed or bumped. The middle layer is the thickest part of the egg membrane. Irregular filaments are distributed in the gap and may extend from the egg membrane to adhere to the substrate with which they were in contact and therefore had the task of joining the egg to the support during spawning and the incubation period. No fertilised pores were observed on the egg membrane of *T. buttikoferi*. Since no further observation was made, the type of the fertilised eggs needs to be further determined.

The spermatozoa of the teleost fishes are of the flagellate type, consisting of the head, the mid-piece and the tail. The sperm of the *T. buttikoferi* is also con-

sisted of three pieces, which is morphologically similar to the *Brachymystax lenok* (GUO et al. 2016) and the *Clarias batrachus*, and no acrosome cone was observed.

The embryonic development of the *T. buttikoferi* is similar to most of the fish embryos. There are 7 developmental periods, including: blastodisc formation, cleavage, blastula, gastrula, neuroblast, organogenesis and hatching period. But *T. buttikoferi* have their own developmental characteristics. They enter the gastrula period at 12 h 40 min post-fertilisation, and the development rate is faster than that of the *Oreochromis niloticus* in the same family (FUJIMURA & OKADA 2010). The pigmentation on the yolk sac appears before the organogenesis period, which is different from the *Oreochromis niloticus* that the pigmentation occurs in the optic cup formation stage (FUJIMURA & OKADA 2010), or the *Epinephelus coioides* (ZHANG et al. 2006) and the *Acrossocheilus fasciatus* (JIANG et al. 2012) that their pigmentation occurs in the larval stage post-hatching. During the organogenesis period, the developmental characteristics of the *T. buttikoferi* are the same with *Oreochromis niloticus*, *Erythroculter ilishaeformis* Bleeker (GU et al. 2008), *Brachymystax lenok* (DU et al. 2010), and *Gymnodiptychus pachycheilus Herzenstein*, that the optic primordia appeared before the heart primordium, and the optic cups appeared after the formation of the somite; The embryonic development lasts for 49 h 30 min, then the fish enter the larval stage, whereas the *Oreochromis niloticus* (FUJIMURA & OKADA 2010) and *Symphysodon discus* (MATTOS et al. 2015) of the same family need to take 90–120 h and 50–60 h to complete the whole embryonic development, respectively.

The full body length of the *T. buttikoferi* was 7.10 ± 0.25 mm post-hatching, which is slightly larger when compared with the *Erythroculter ilishaeformis* Bleeker larvae (4.17 mm) in Xingkai Lake (LIU et al. 2012) and the *Scaphesthes macrolepis* Bleeker larvae (6–6.5 mm) (CHEN et al. 2008). The size of the yolk sac is $(2.30 \pm 0.10) \times (1.53 \pm 0.05)$ mm. The volume of the yolk sac not only affects the size of the newly hatched larvae, but also affects the transition time of the larval fish from endogenous to exogenous nutrition (JARDINE & LITVAK 2010). After hatching, vital organs that are related to breathing, feeding and swimming are first developed in the *T. buttikoferi* larvae. At 21 h post-hatching, the eyeballs start to have melanin deposition, which is earlier than the *Epinephelus coioides* (ZHANG et al. 2006) and the *Oreochromis niloticus* (FUJIMURA & OKADA 2010). The melanin deposition of the body stripes is in the order of the first, fifth and seventh, and lastly the cau-

dal fin end stripe. The pigmentation order is middle section first, head next and tail last, which is opposite to the pigmentation order of “rear ends first and middle section last” of the *Acrossocheilus fasciatus*. Only after the *T. buttikoferi* reach the juvenile stage, the body stripe boundary becomes particularly clear. The black and white stripes are contrasted, making the fish to have high ornamental value.

In the developmental stage of the *T. buttikoferi* larvae, the total length was significantly correlated with the day-old age, and the fitting equation was similar to that of the *Oplegnathus fasciatus* (HE et al. 2012). It can be seen from the fitting equation that the total length growth rate of the *T. buttikoferi* during the yolk sac larvae stage is lower than that of the fry stage, and it enters to a low-speed growth stage at 4–6 days of age. Because at this time, the larvae are in a mixed nutrition period. The nutrient absorption from the yolk sac is exhausted, whereas the development of the digestive system is not complete. The larvae also need to swim fast to obtain exogenous nutrition. However, the exogenous nutrients cannot fully meet the requirements for their development. At this time, exogenous diets need to be supplemented to ensure a healthy growth of the larvae (YUFERA & DARIAS 2007).

Allometric growth is prevalent in the early growth and development stage of fish, such as in the larvae of the *Oncorhynchus keta* (MOSS et al. 2016), *Acipenser schrenckii* (MA et al. 2007), *Oplegnathus fasciatus* (HE et al. 2012), *Sebastes schlegelii* (WANG et al. 2017) and hybrid progenies of the *Epinephelus coioides* (♀) × *Epinephelus lanceolatus* (♂) (WU et al. 2014). Allometric growth is to ensure the priority development of the vital organs for survival (RODRIGUEZ & GISBERT 2010), which is a manifestation of fish for the adaptation of the environment and improvement of the survival rates.

During the larvae and fry developmental stages of the *T. buttikoferi*, the organelles in the head had typical allometric growth. Three pairs of gill arcs appeared under both eyes at about 5 h post-hatching, and pigmentation occurred in the eyeballs at 21 h post-hatching. A series of growth indicators such as the head length, head height, fins and digestive system are also rapidly developed to ensure the larvae can quickly adapt to the environment upon hatching, in order to avoid enemies, look for required nutrients, and greatly improve the ability for survival.

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References

- CHEN H. J., JIANG Y. L., SONG J. Y., JI X. S., ZHANG A. C. & TAN J. H. 2008. Study on the embryonic development of *Scaphesthes macrolepis*. *Acta Hydrobiologica Sinica* 32: 926–933.
- DU J., XU G., HAN Y. & MU Z. 2010. Development of embryos, larvae, fry and juveniles in *Brachymystax lenok*. *Journal of Dalian Ocean University* 25: 379–385.
- ERGENS R. 1981. Nine species of the genus *Cichlidogyrus* Paperina, 1960 (Monogenea: Ancyrocephalinae) from Egyptian fishes. *Folia Parasitologica* 28: 205–214.
- FUJIMURA K. & OKADA N. 2010. Development of the embryo, larva and early juvenile of Nile tilapia *Oreochromis niloticus* (Pisces: Cichlidae). *Developmental Staging System. Development Growth & Differentiation* 49: 301–324.
- GU Z., ZHU J. J., JIA Y. Y., PAN Y. J., HUANG X. M. & XU G. X., YANG Y. J. 2008. Research on embryonic and postembryonic development of *Erythroculter ilishaeformis* Bleeker of Taihu Lake. *Journal of Fishery Sciences of China* 15: 204–214.
- GUO W., SHAO J., LI P., WU J. & WEI Q. 2016. Morphology and ultrastructure of *Brachymystax lenok tsinlingensis* spermatozoa by scanning and transmission electron microscopy. *Tissue & Cell* 48: 321–327.
- HE T., XIAO Z. Z., LIU Q. H. & LI J. 2012. Allometric growth in rock bream larvae (*Oplegnathus fasciatus* Temminck et Schlegel, 1844). *Journal of Fisheries of China* 36: 1242.
- HE X., YUAN X., PAN Z. & YU Y. 2013. The anaesthesia effects of MS-222 on juvenile *Tilapia buttikoferi*. *Sichuan Journal of Zoology* 32: 729–733.
- HUANG Y. 1993. Cytological study on fertilization of egg in *tilapia nilotica*. *Acta Zoologica Sinica* 1: 19–22.
- JARDINE D. & LITVAK M. K. 2010. Direct yolk sac volume manipulation of zebrafish embryos and the relationship between offspring size and yolk sac volume. *Journal of Fish Biology* 63: 388–397.
- JIANG J. H., ZHANG D. M., ZHU J. Q., LEI S. Y. & ZHANG Y. M. 2012. The development of embryo, larva and juvenile of *Acrossocheilus fasciatus*. *Oceanologia et Limnologia Sinica* 43: 280–287.
- KAMLER E. 2002. Ontogeny of yolk-feeding fish: an ecological perspective. *Reviews in Fish Biology & Fisheries* 12(1): 79–103.
- LIU D., LINA S. I., ZHANG X. & YIN H. 2012. Research on embryonic and larvae development of *Culter alburnus* of Xingkai Lake. *Journal of Northeast Agricultural University* 43: 110–116.
- MA J., ZHANG L. Z., ZHUANG P., ZHANG T., FENG G. P. & ZHAO F. 2007. Development and allometric growth patterns of larval *Acipenser schrenckii*. *Journal of Applied Ecology* 18: 2875.
- MATTOS D. D. C., CARDOSO L. D., FOSSE P. J., RADAEL M. C., FILHO J. C. F. & MANHÃES J. V. D. A. 2015. Description of the ontogenic and larval period of discus fish (*Symphysodon aequifasciatus*). *Zygote* 23: 460–466.
- MOSS J., MURPHY J., FERGUSSON E. & HEINTZ R. 2016. Allometric relationships between body size and energy density of juvenile chinook (*Oncorhynchus tshawytscha*) and chum (*O. keta*) salmon across a latitudinal gradient. *North Pacific Anadromous Fish Commission* 6: 161–168.
- MU X., LI X., HU Y., WANG X., SONG H. & LUO J. 2009. Isolation of microsatellite DNA and genetic diversity analysis on *Tilapia buttikoferi*. *Biotechnology Bulletin* s1: 236–241.
- RODRIGUEZ A. & GISBERT E. 2010. Eye development and the role of vision during Siberian sturgeon early ontogeny. *Journal of Applied Ichthyology* 18: 280–285.
- SUN J., GUO Y., LI G. & ZHAO H. 2006. An observation on the early fertilization of *Squaliobarbus curriculus* by scanning electron microscope. *Journal of Fishery Sciences of China* 13: 740–744.
- WANG L., WU Z., LIU M., LIU W., ZHAO W. & LIU H. 2017. Length-weight, length-length relationships, and condition factors of black rockfish *Sebastes schlegelii* Hilgendorf, 1880 in Lidao Bay, China. *Thalassas, an International Journal of Marine Sciences* 33: 57–63.
- WANG X., MU X., HU Y. & LUO J. (2010). Morphological character and karyotypes in *Tilapia buttikoferi*. *Journal of Dalian Fisheries University* 25: 187–190.
- WU S., LI J., OU Y., LV G. & LIU J. 2014. Allometric growth of hybrid grouper (*Epinephelus coioides* ♀ × *E. lanceolatus* ♂) larvae and juveniles. *Journal of Fishery Sciences of China* 21: 503–510.
- YIN M. 1991. Advances and studies on early life history of fish. *Journal of Fisheries of China* 15: 348–358.
- YUFERA M. & DARIAS M. J. 2007. The onset of exogenous feeding in marine fish larvae. *Aquaculture* 268: 53–63.
- ZHANG H., LIU X., LIU F., WANG Y., LIN L. & HUANG G. 2006. Embryonic development, morphological development of larva, juvenile and young fish of *Epinephelus coioides*. *Journal of Fishery Sciences of China* 13: 689–699.
- ZHANG L., CHEN L., ZHUANG P., FENG G., XU B. & ZHAO Y. 2008. Observation on spermatozoa, egg and sperm penetration of Tridentiger trigonocephalus by means of scanning electron microscopy. *Marine Fisheries* 30: 308–313.

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