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Inayah Yasir, Jian G. Qin

Institutions: Hasanuddin University, Flinders University

Published on: 01 Aug 2007 - Journal of the Marine Biological Association of the United Kingdom (Cambridge University Press)

Topics: Ontogeny and Yolk

J. Mar. Biol. Ass. U.K. (2007), **87**, 1025–1033
Printed in the United Kingdom
doi: 10.1017/S0025315407054227

Embryology and early ontogeny of an anemonefish *Amphiprion ocellaris*

Inayah Yasir*† and Jian G. Qin*‡

*School of Biological Sciences, Flinders University, GPO Box 2100, 5001 SA Adelaide, Australia. †Department of Marine Sciences, Hasanuddin University, Makassar 90245, Indonesia. ‡Corresponding author, e-mail: Jian.Qin@Flinders.edu.au

The present study describes the embryonic development and early ontogeny of *Amphiprion ocellaris* from fertilization to post hatching. Anemonefish spontaneously spawned at 27–28°C. The newly laid eggs were orange in colour and elliptical in shape (1.8×0.8 mm). Melanin appeared as a black mass situated at the vegetal pole in mature eggs. This is rarely seen in eggs of other fish species. We documented developmental times at 27–28°C to egg activation (0.5 h), cleavage (4 h), blastula (11.5 h), gastrula (20 h), neurula (24.5 h), somite (28.5 h), turnover (72 h), blood formation (113 h) and internal ear and jaw formation (144 h). Hatching occurred 152 h after fertilization. On day 4, the eye buds were pigmented and melanophores formed on the ventral surface of the embryo. Internal ear and gill formation were completed on day 5 and coincided with movement of the opercula and pectoral fins. The mouth formed on day 6 and the digestive tract appeared on day 7. By day 10, the yolk was fully absorbed and a substantial amount of food was observed in the gut. Dark and orange pigments were dispersed and aggregated through muscle contractions by day 14, but red pigments did not appear until the fish were three months old. This study contributes to a further understanding of the embryology and the early ontogeny of damselfish and may help improve the culture of coral reef fish.

INTRODUCTION

Among all species of anemonefish, *Amphiprion ocellaris* is the best known to aquarium traders due to its colour pattern, interesting behaviour and robustness. From 1997 to 2002, *A. ocellaris* was the most common species of marine ornamental fish and made up 15.6% of total number exported worldwide and over 25% into European countries (Wabnitz et al., 2003). Thus, anemonefish are considered the 'goldfish' of marine aquaria (Hoff, 1996).

Most anemonefish species are symbiotic with tropical sea anemones (Allen, 1975; Dunn, 1981; Fautin, 1991; Fautin & Allen, 1997). Both anemonefish and anemone benefit each other through this mutual symbiotic relationship (Porat & Chadwick-Furman, 2004). All anemonefish are protandric hermaphrodite, starting life as a male and later changing to a female (Allen, 1975; Fautin, 1991; Fautin & Allen, 1997). Most anemonefish species form long-term monogamous pairs. The male–female bond only breaks when one member of the pair is lost (Hattori, 1994; Buston, 2003). In most cases, the female is larger making it more vulnerable to predation. In an anemonefish population, the conversion of a functional male into a female, or of an immature male into a mature male typically takes several months to a year (Moe, 1992; Hoff, 1996). Anemonefish are benthic spawners (Allen, 1975; Arvedlund et al., 2000a). Their eggs are highly pigmented and contain carotenoids in the yolk sac resulting in eggs that appear whitish-orange to purple in colour (Kunz, 2004). After spawning, both parents usually attend the nest until larvae hatch. The fanning behaviour of the parent fish brings oxygen to the nest and removes dead eggs (Green & McCormick, 2004).

There have been extensive studies of anemonefish taxonomy (Allen, 1975, 1991), their living habits associated with behavioural ecology (e.g. Brooks & Mariscal, 1984; Fautin, 1992; Arvedlund et al., 1999; Elliott & Mariscal, 2001; Chadwick & Arvedlund, 2005), reproductive biology (e.g. Busle-Sicard et al., 1994; Godwin et al., 2003; Hobbs et al., 2004; Holbrook & Schmitt, 2005) and rearing methodology (e.g. Delbare et al., 1995; Arvedlund et al., 2000c; Johnston et al., 2003). However, little attention has been paid to the embryology and early ontogeny (Green & McCormick, 2004), except for the early development of the olfactory system (Arvedlund et al., 2000b) and one recent preliminary study of the embryology of *A. ocellaris* (Liew et al., 2006). Further studies of the embryology of anemonefish would improve our broader understanding of the embryology of tropical reef fish. Such studies may also improve rearing methods of coral reef fish in general. The objectives of the present study were to examine the embryonic development and ontogeny during early life from fertilization to post hatching. Descriptions were based on observations of different developmental stages using light microscopy on fertilized eggs of anemonefish reared in captivity.

MATERIALS AND METHODS

Brood fish preparation

Six wild caught *Amphiprion ocellaris* from Indonesian waters with a mean total length of 5.5 cm were obtained from an aquarium shop (Sea's View) in Adelaide, South Australia. *Amphiprion ocellaris* has 11 dorsal fin-spines and 17 pectoral

Table 1. *Major stages of development in embryos of* Amphiprion ocellaris *post fertilization (in days).*

Days	Major stages of development
1	Blastula and gastrula stages are complete. The embryo develops from a two-layer blastomere to a sphere shape. The expansion of blastoderm covers 50% of the yolk sac.
2	Neurula stage: cephalization starts with a beak-like structure at the anterior part of the embryo. The brain is formed at the end of the embryonic body close to the stalk. The first two somites appear in the middle part of the embryonic shield. The melanophore is covering the entire blastophore.
3	Body turns over and eye buds appear. Two otoliths inside the otic plate have formed as small granules on each side of the head. The embryo completes the turn-over stage and tail has moved freely. The embryo size is about the same length as the eggshell with 26–30 somites. The heart rate (HR) is approximately 55–60 beats per min (bpm). No blood cell is observed.
4	The pale pigmented blood cells flow through the heart. HR≈110–120 bpm. Two lines of melanophores form at the ventral side of the embryonic body. Eye buds are pigmented.
5	Internal ear and lower jaw form. The blood colour deepens. The gills and the opercula form and the body cavity is seen. The posterior otolith is slightly bigger than the anterior one. Four xanthophores appear on the ventral side of the head. The posterior part of the body detaches the yolksac. The opercula and pectoral fins start to move.
6	The embryo hatches. The pericardium has fully developed. HR≈160–170 bpm. Blood circulation is seen inside the gill. The lower and upper jaws form the mouth, but the mouth was not open. The eye is fully pigmented with the lens popping out. The posterior otolith is 3–4 times bigger that the anterior one.
7	The digestive tract appears as yellowish-silvery and the mouth frequently opens. Body cavity is enlarged and becomes dark. Most melanophores at the surface of the yolksac migrate into the body cavity.
8	The mouth frequently opens and the body movement intensifies. The yolksac shrinks to an oil globule with <½ of the eggshell length. The pericardium is fully formed. HR≈170 bpm.

rays. The sister species, *A. percula* has 10 dorsal fin-spines and 16 pectoral rays (Allen, 1975). The anemonefish used in the present study were identified as *A. ocellaris*. These fish were held communally in a 280-l glass tank with three terracotta pots on the bottom. After co-habitation for a month, spontaneous pairing occurred. Each paired couple swam together and shared one of the terracotta pots. Aggressive behaviour was displayed toward other fish that came close to their pot.

As soon as the pair was established, each fish was transferred into an individual glass aquarium (160-l) without substrates. One terracotta pot and an air-stone were placed inside each aquarium. The three spawning aquaria were connected to a recirculation system filtered by sand. Four submerged 300-watt heaters maintained the water temperature at 28 ±2°C. Salinity was maintained at 28 ±2 ppt by replacing evaporative loss with demineralized water.

The fish were fed a mixture of flesh, mussel, squid, fish roe, *Spirulina* tablets (Synergy) and gelatine powder to cement the ingredients adapted from Hoff (1996). Frozen food was thawed at room temperature and cut into an edible size before use. The fish were fed *ad libitum* three times a day. Fertilized eggs used for this study were produced by these three pairs of fish. Broodfish held under the described conditions produced 100–800 eggs every 11 days.

Observations of the embryology

Eggs produced by the three brood pairs were collected for observations. Eggs were observed from fertilization until eight days post hatching. As soon as the eggs were fertilized, 10–20 eggs were collected with a plastic pipette from the terracotta pot and placed in a glass jar containing filtered seawater from the brood fish tank. The embryology was studied in a plastic Petri dish under a dissecting microscope (Olympus, Japan SZH-ILLD-200) or a compound microscope (Olympus, Japan BX40) connected to a digital camera (Moticam 2000) and a computer with Moticam software. This system was located in a room with temperature of 27–28°C. The newly hatched larvae were transferred to a 4-l glass aquarium containing live foods (*Nannochloropsis* sp. and *Brachionus plicatilis*). Compressed air was injected into the aquarium to produce light aeration.

Images of embryonic development were made at 2 min intervals in the first 3 h, at 5 min intervals on the second day after fertilization and at 30 min intervals until the eggs hatched. The embryonic development time was described as hours post fertilization. During the post-larval stage, development events were studied daily up to eight days after hatching. The embryonic stages were defined based on the same terminology as previous studies (Green, 2004; Arezo et al., 2005).

RESULTS

The eggs were individually attached to the inside surface of the terracotta pot. Each egg was firmly attached to the substrate by a fibrous stalk. This stalk was sticky when the egg was first released into the water, but became hardened after 5–10 min in the water. The major daily developmental events are summarized in Table 1.

Oocytes

The newly laid egg was orange in colour, elliptical in shape (1.8×0.8 mm), and slightly curved around the middle part of the yolk (Figure 1F–O). The chorion was transparent and the egg could be seen through the shell. The yolk was bright orange with an obvious constriction between the animal and vegetal poles, whilst the oil globule was clear.

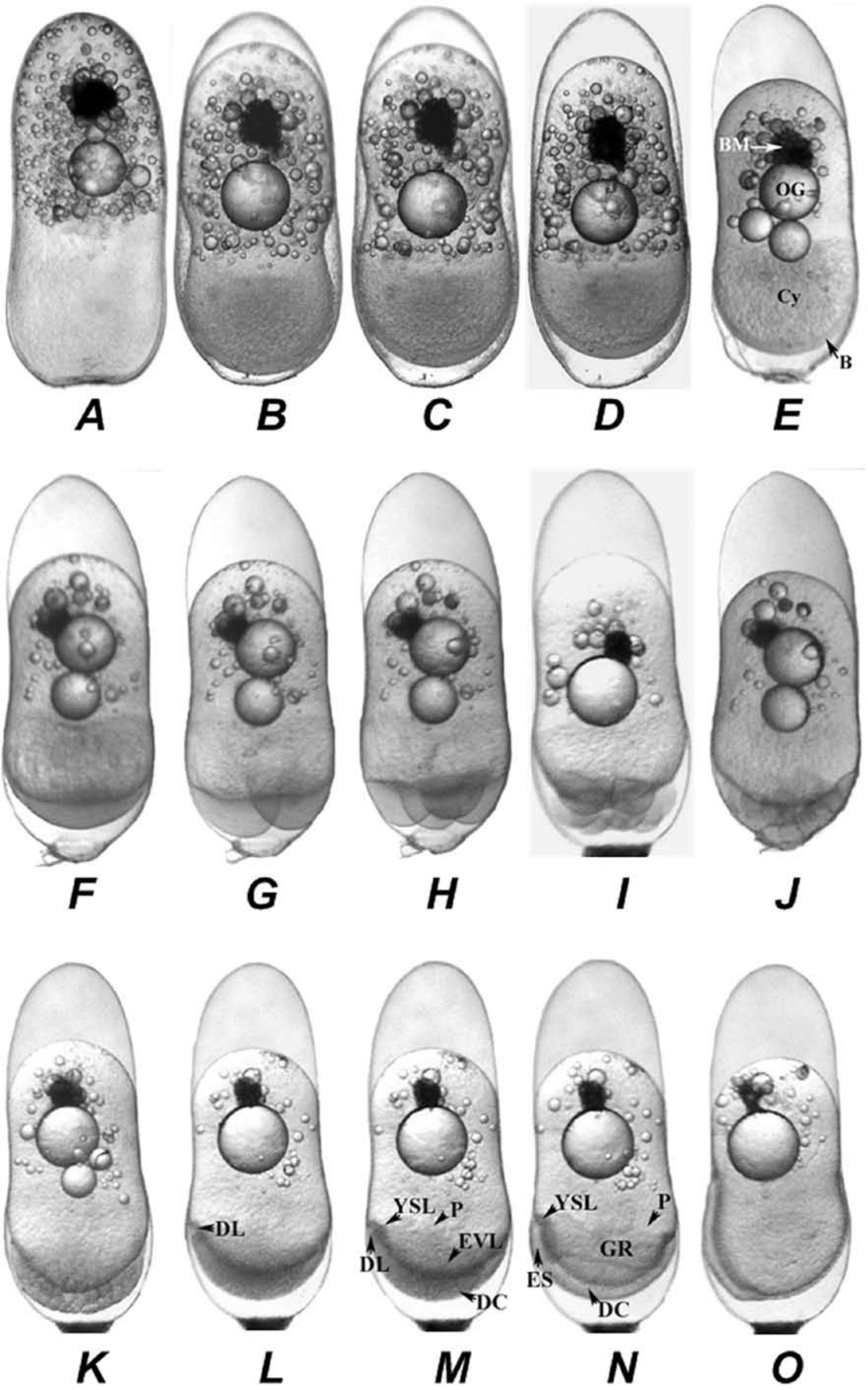

Figure 1. Embryonic development of *Amphiprion ocellaris*, showing black mass (BM), oil globule (OG), cytoplasm (Cy), blastodisc (B), dorsal lip (DL), yolk syncytial layer (YSL), periblast (P), envelope layer (EVL), deep cell (DP), germ ring (GR) and embryonic shield (ES). Panel number letters (A–O) are shown in italics in all figures.

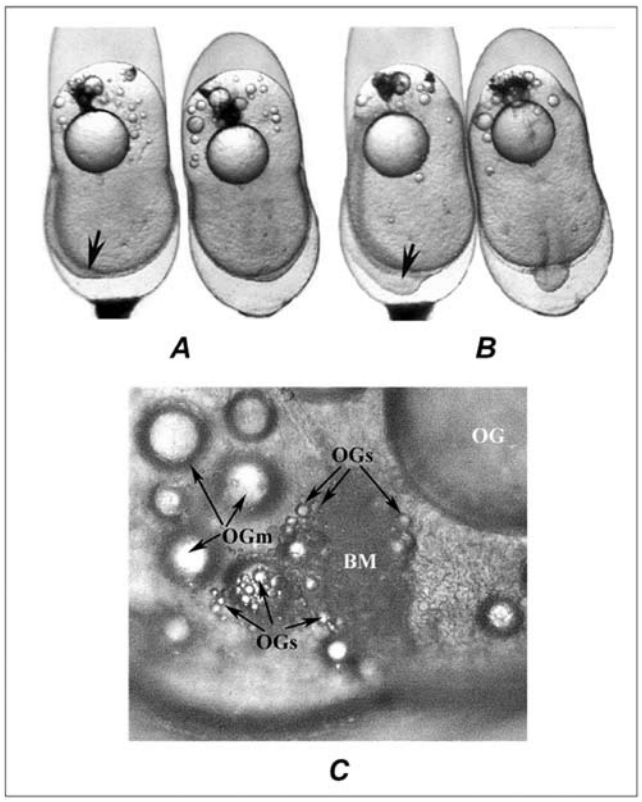

Figure 2. The embryonic development of *Amphiprion ocellaris*, showing (A) 50% epiboly with arrow indicating the formation of the head and (B) the 70% epiboly with arrow indicating the formation of the eye bud. (C) The black mass (BM) was surrounded by numerous small (OGs) and medium (OGm) oil globules.

As soon as the egg was attached to the substrate, a border between the animal pole and the vegetal pole developed. This was due to the accumulation of cytoplasm at the animal pole. Newly laid eggs had one large oil droplet and various small ones. The small oil droplets coalesced with the large ones during development. The perivitelline space was small yet transparent especially at the animal pole (Figure 1A). The blastodisc was barely seen during this stage, but there was a large black mass at the vegetal pole surrounded by oil droplets (Figure 1A–O). This black mass only appeared in eggs within 24 h before ovulation.

Eggs activation (0.5 h post fertilization)

A few minutes after spawning, water was flowing into the space between the cortex and chorion, starting from the animal pole to the vegetal pole. The incoming water suppressed the yolk line (Figure 1B&C) and reached the vegetal pole (Figure 1D) to form the perivitelline space. This process took about 20 min to complete. The perivitelline space at the vegetal pole was larger than that at the animal pole. Immediately after the formation of the perivitelline space, the cytoplasmic area in the animal pole became thicker with an obvious blastodisc (Figure 1E). The yolk sac in fertilized eggs shrank in size after the formation of the perivitelline space but the size of the whole egg did not change. In the activation stage, channels were formed in the cytoplasm that resulted from the spindle fibre activity during mitosis. One hour after spawning, a dome-shaped blastodisc formed, signalling the completion of the activation process (Figure 1F).

Cleavage stage (4 h post fertilization)

The cytoplasm streamed down to the blastodisc, then retracted back, leaving a furrow in the middle (Figure 1G). The groove moved up following the retraction of the cytoplasm and divided into two cells at 1.5 h after fertilization. The cytoplasmic divisions were meroblastic. The embryo was divided into 4 (Figure 1H), 8 (Figure 1I), 16 (Figure 1J), 32 and 64 cells with 0.5 h intervals between divisions. At the 64-cell stage, the blastomeres rearranged the cells into two layers, marking the completion of the cleavage stage. The cleavage stage was completed by 4 h post-fertilization.

Blastula stage (11.5 h post fertilization)

The embryo developed from a two-layer blastomere (Figure 1K) to a sphere where the blastodisc developed into a blastoderm. This stage was completed by 11.5 h post fertilization when the yolk syncytial layer (YSL) and the dorsal lip (DL) formed (Figure 1L&M).

Gastrula stage (20 h post fertilization)

When the blastoderm was flattened, the dorsal lip became obvious (Figure 1L). The envelope layer (EVL) covered the deep cells (DC) and overhung the blastoderm (Figure 1M). The expansion of blastoderm increased the coverage over the yolk sac (Figure 1N). The yolk syncytial layer continued to expand along the embryonic shield (ES) and joined the yolk syncytial layer to form the germ ring (GR, Figure 1N). Prior to the completion of the gastrula stage, the embryonic shield (ES) lengthened and the germ ring disappeared. The periblast (P) and the envelope layer gradually migrated toward the vegetal pole to form the epiboly. At 20 h post fertilization, the blastoderm covered 50% of the yolk sac (Figure 1O).

Neurula stage and somite development (day 2)

At 24.5 h after fertilization, a beak-like structure appeared in the anterior part of the embryo (Figure 2A), marking the process of cephalization. The brain formed at the end of the embryonic body close to the stalk (Figure 2B). Meanwhile, the eye buds appeared at 24.5 h post fertilization. Under high magnification, the black mass was embedded in oil droplets of various sizes (Figure 2C).

At 28.5 h post fertilization, the first two somites appeared in the middle part of the embryonic shield and the blastopore covered 70–80% of the yolk sac. Concurrently, the Kupffer's vesicle occurred at the ventro-posterior side of the embryo. The 4th–6th somites developed in about one hour (Figure 3A). When the blastopore covered 90% of the yolk sac (27 h after fertilization), chromatophores appeared as black dots spreading all over the blastopore (Figure 3B). The structure became more obvious after the blastopore completely covered the yolk sac (Figure 3C). During this stage, 10–12 somites were formed.

Turnover stage (day 3)

The head orientation could be identified by the eye buds (Figure 4A). Next to the big oil globule, a black mass of melanin started to dissolve and darken the area nearby. A few melanophores appeared and migrated across the embryo

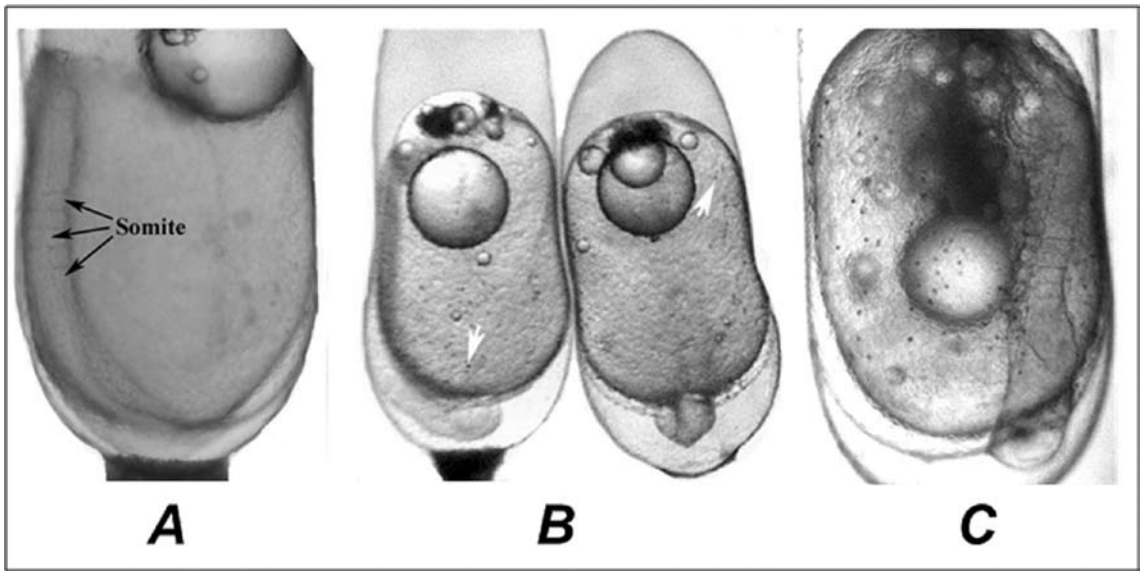

Figure 3. Embryonic development of *Amphiprion ocellaris*, showing somite formation with arrow indicating somites (A) and pigment cells (B) at the early formation and at the end of the epiboly (C).

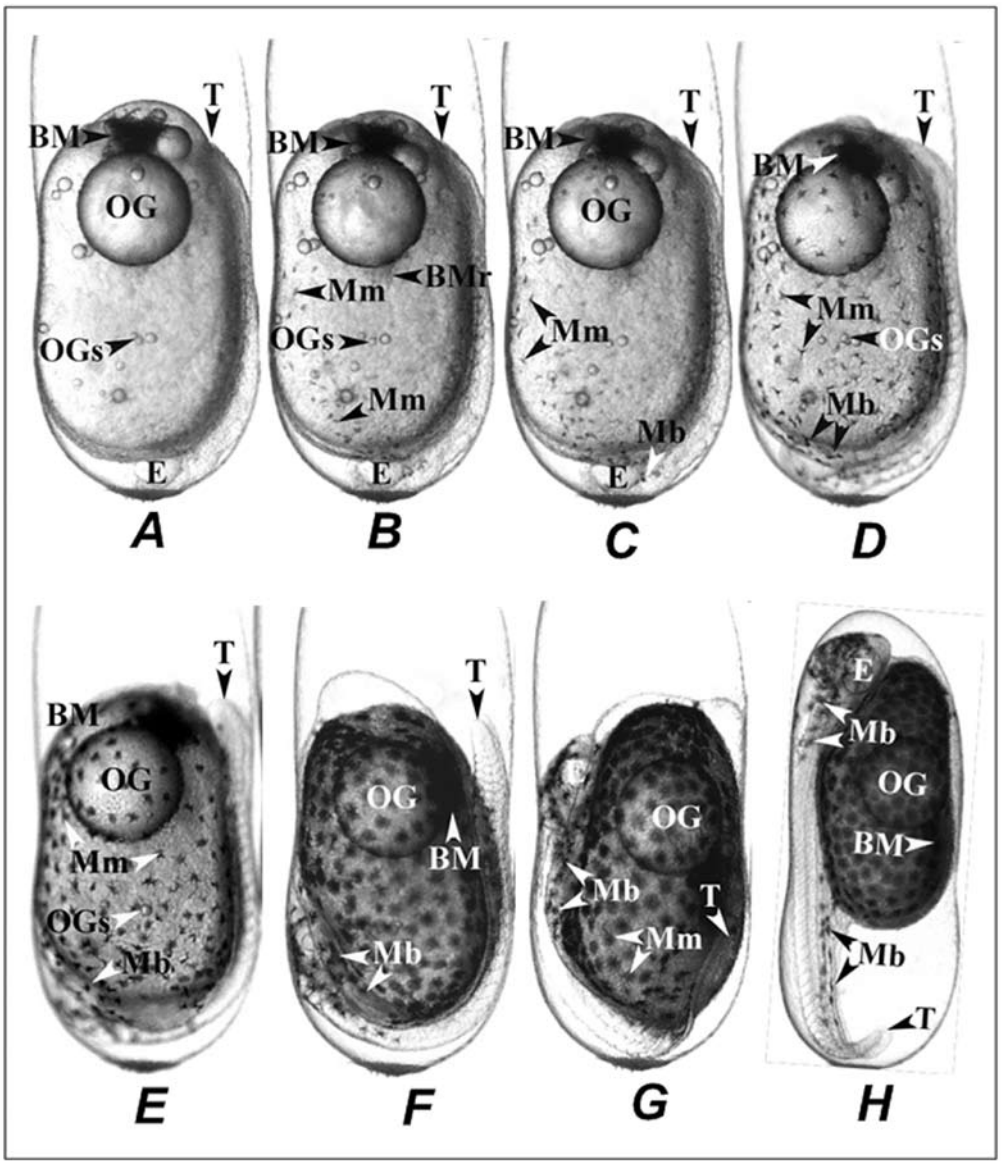

Figure 4. Embryonic development of *Amphiprion ocellaris* at turnover stage, showing black mass (BM), black mass residuals (BMr), eye (E), oil globule (OG), melanophore in the body (Mb), migrate melanophore (Mm) and tail (T).

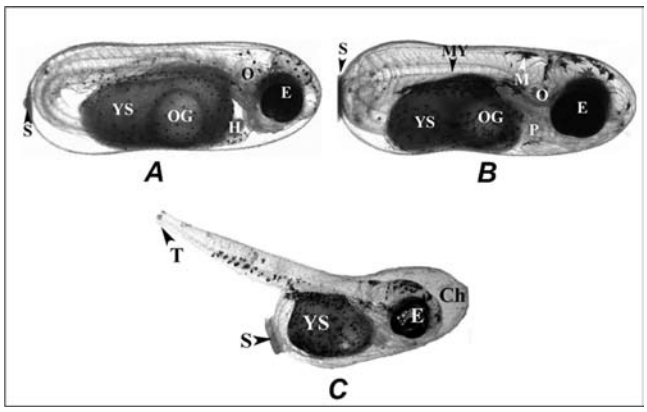

Figure 5. Lateral views of the embryo 4 days old (A), 6 days old (B) and immediately after hatching (C), showing chorion (Ch), eye (E), heart (H), melanophore (M), myomeres (MY), oil globule (OG), otolith (O), pectoral fin (P), stalk (S), tail (T) and yolk sac (YS).

(Figure 4B). While the tip of the tail was still attached to the yolk sac, the embryonic body started to turn over (Figure 4C).

The otic plate (without otolith) and the nasal vesicle were clearly visible on the head. The pigment cells spread to the ear and the ventral side of the body. During this stage, the tubular heart started with very weak beats (55–60 bpm), but no blood cells were observed. Front, mid and hind brains could also be seen through the transparent head, whilst the notochord was observed along the posterior body. The tip of the tail was freed from the yolk sac when the embryo started jerking movements (Figure 4D). By the time the embryo was half way through the turnover stage, tail movements were clearly observed and became intense over time (Figure 4E).

The embryo completed the turnover process when the head was turned from facing the attachment end to the free end of the eggshell. Intense movement of the tail freed 1/3 of the body (Figure 4F). With further tail movement, the whole body moved forward and the rest of the tail separated from the yolk sac (Figure 4G). A pair of otoliths was observed in each ear as small granules of similar size. At the end of the turnover stage (Figure 4H), 26–30 somites were observed in the embryos.

Blood formation (day 4)

Transparent and spherical blood cells were first observed entering the heart chamber 113 h after fertilization. The number of blood cells quickly increased and circulated within the body and in blood vessels on the surface of the yolk sac. At 96 h after fertilization, elliptical blood cells showed pinkish pigments and the heart beat increased to 110–120 bpm. When the eye buds were pigmented the embryo reached 1.25 times the size of the eggshell. Two lines of melanophores ran along the ventral side of the embryonic body. Four orange-yellow casts on the ventral side of the head next to each eye marked the first appearance of xanthophores. By the end of day 4, the peritoneal cavity became dark on the dorsal surface as a result of melanophore migration (Figure 5A).

Internal ear and lower jaw formation (day 5)

The pink colour of the blood cells was intensified by 103 h post fertilization. The length of the embryo was now 1.3

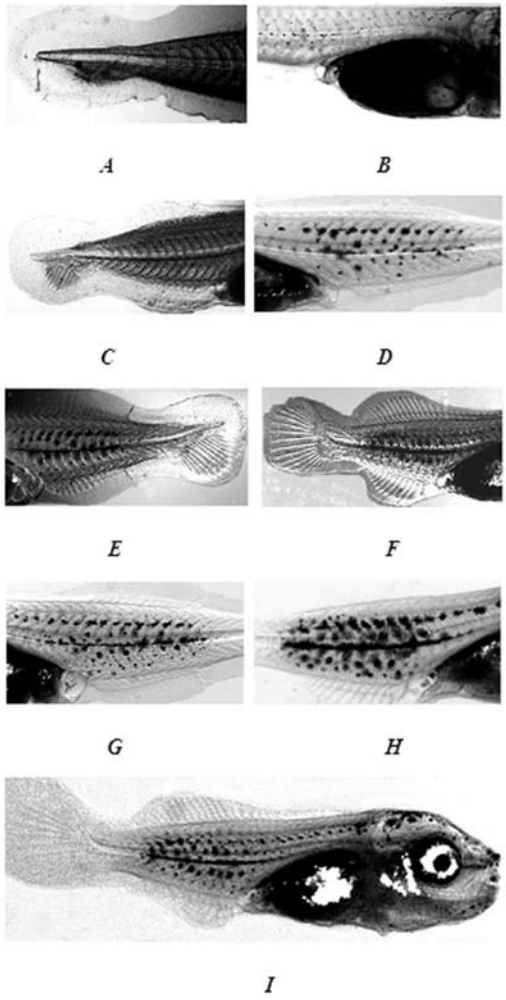

Figure 6. Post hatching larvae, showing the development of the caudal fins (A, C, E, F) and the development of body colour (B, D, G, H, I).

times the length of the eggshell and half of the body length was already separated from the yolk sac. On day 5 after fertilization, the embryo reached 1.5 times the eggshell length. The posterior otolith was slightly larger than the anterior one. The eyes with the retina surrounding the lens were highly pigmented while the choroid fissure was still obvious. On the 5th day, three lines of xanthophores were observed along the middle through to the posterior part of the body. Three to five xanthophores also appeared on the dorsal part of the body. The gill and the opercula were distinct with blood circulation in the gill and pectoral fins. The opercula and the pectoral fins started moving frequently.

Hatching (day 6)

On day 6, embryos were bent inside the eggshell and the tail reached the posterior of the eyes. The pericardium was fully formed and heart beats reached 160–170 bpm. The lower jaw formed when the embryo reached 1.7 times the eggshell length, but the mouth was not yet open. The digestive tract was distinct on the left side of the body. More iridophores created iridescent colour on the digestive tract and the lining of the peritoneal cavity. The eyes were fully

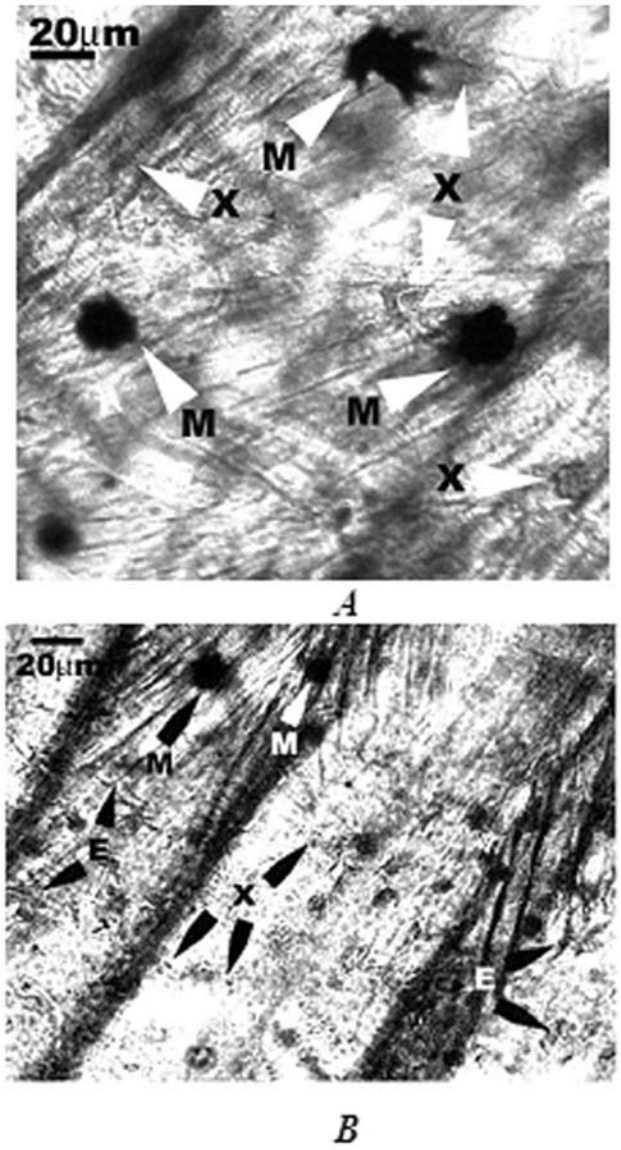

Figure 7. Pigment cells of *Amphiprion ocellaris* larvae 7 days after hatching (A) and 3-month-old juvenile (B), showing erythrophores (E), melanophores (M) and xanthophores (X).

pigmented with iridophores scattered on the retina. A pair of otoliths became obvious with the posterior otolith 3–4 times larger than the anterior one.

At 152 h after fertilization, body movement was intensified and the tail ruptured the area next to the base of the eggshell (Figure 5A). With a few jerking motions, the fish freed its whole body. The yolk sac was less than half of the eggshell length, with only a big oil droplet remaining (Figure 5B).

Post hatching (days 7–14)

On day 7, the digestive tract was yellowish-silvery and the mouth opened frequently (Figure 5C). In some cases where the embryo failed to complete the turnover stage, the hatching process was delayed. Melanophores concentrated on the dorsal side and xanthophores concentrated on the lateral side of the body. The well-developed sensory and olfactory systems enabled the larva to detect and ingest exogenous food on the first day after hatching.

On day 8, the yolk size had reduced to only a half of its original size. The dorsal fin fold at hatching connected the caudal and anal fin folds (Figure 6A). On day 9, the larvae still contained some yolk and one large oil droplet (Figure 6B). The fin rays under the urostyle became obvious while the dorsal and the anal fin rays started to develop on the fin folds (Figure 6C).

The yolk was fully absorbed by day 10 after hatching but the oil droplet still remained. The dorsal and anal fins formed with obvious fin folds. The urostyle developed more rays and started to bend. The digestive tract became green with a substantial amount of food in it. Yellow-orange pigments were spread all over the body (Figure 6D) and the pelvic fin buds became obvious.

On day 11, the urostyle was clearly bent, and the pelvic fins formed (Figure 6E). On day 12, the fin fold gradually disappeared and the anal and dorsal fins separated (Figure 6F). On day 13, pelvic fin rays were clearly observed and melanophores formed within the fin folds. The fish body looked paler when the melanophores retracted (Figure 6G) or darker when they dispersed (Figure 6H). The pigment cells were scattered all over the body except on the fins and the body parts close to the fin base (Figure 6I). On day 14, both dark (melanophores) and orange (xanthophores) pigments were seen to disperse and aggregate through muscle contractions (Figure 7A). By three months after hatching, red pigment cells (erythrophores) were seen on the caudal fin (Figure 7B).

DISCUSSION

Mated pairs of *Amphiprion ocellaris* spontaneously spawned in captivity at 27–28°C. The eggs were elliptical and possessed a black mass at the vegetal pole, which is rarely seen in eggs of other fish species. This black mass is believed to have a close association with the formation of melanophores. The pattern of embryonic development of this species is similar to other anemonefish, but the hatching time is shorter than other species in this genus. During embryonic development, the head turned from the attachment end to the free end upon completing turnover. This seems to be a critical stage for survival since failure to turn the body around would lead to death.

In this study, *A. ocellaris* hatched in slightly over 6 days (152 h) after fertilization, but Liew et al. (2006) reported that this species did not hatch until 7.5 days (180 h) under similar ambient temperatures. In comparison, Green (2004) found that *A. melanopus* did not hatch until 7.5 days again in similar temperatures. Cell cleavage in *A. ocellaris* started at 1.20 h (Liew et al., 2006), but we did not observe cleavage until 4 h after fertilization. In the first 2 days, *A. ocellaris* embryos completed blastula and gastrula. This developmental rate was similar to *A. melanopus* (Green, 2004).

Body movement is a critical part of development between major stages of organogenesis (Patterson & Martin-Robichaud, 1983). Body turnover of *A. ocellaris* embryos occurred on day 3 after fertilization. Similarly, tail movement of *A. melanopus* occurred by day 3 (Green, 2004). Tail-flicking could increase the oxygen circulation within the embryonic capsule especially for benthic eggs (Cronin & Seymour, 2000). In this study, the rudimentary heart chamber formed

in day 3, but the heart rate was only 55–60 bpm, which is much slower than that reported for *A. melanopus* (110–120 bpm; Green, 2004). Similar to *A. melanopus* (Green, 2004), *A. ocellaris* developed a rudimentary heart on day 3 and haemoglobin on day 4. *Amphiprion ocellaris* formed gills and opercula on day 5, but gills did not occur until day 6 in *A. melanopus* (Green, 2004). The development rates of the eye and the digestive system in *A. ocellaris* were similar to those reported by Green (2004) and Liew et al. (2006).

In this study, immediately after fertilization, the perivitelline space in anemonefish eggs started to develop but the space at the vegetal pole was larger than that at the animal pole. Unlike demersal eggs in *Lepistoseus osseus* (Long & Ballard, 2001), where the perivitelline space was formed by the expansion of the chorion, the egg yolk of *A. ocellaris* actually shrank in size without any change in the egg size during perivitelline formation.

The black mass in the embryo has not been reported by other authors who studied the early life of anemonefish (Allen, 1991; Hoff, 1996; Green, 2004; Liew et al., 2006). We found that this black mass on the yolk surface was surrounded by numerous small oil droplets. Hoff (1996) described the *A. ocellaris* egg as a 'clear yellow-orange with a small white dot on the tip of the egg'. This 'small white dot on the egg' may be the black pigment as we observed in this study because the dark pigment could be seen as a white dot under a dissecting microscope with the light source from the top. This black mass in *A. ocellaris* seems to be the melanin mass (J.T. Bagnara, personal communication), but this has not been reported in other fish eggs (Ahlstrom & Moser, 1980; Matarese & Sandknop, 1984; Kunz, 2004).

Once ovulated, fish eggs quickly absorb water. Other materials including vitamins and metals required for enzyme activity in the egg should be obtained from the female (Brooks et al., 1997). Melanin synthesis is carried out by the tyrosinase family protein and is auto-regulated and activated by its own substrate and cofactor tyrosine (Stearns & Wang, 1987). The process of melanin synthesis usually occurs inside melanophores or melanosomes in the dermis, or in melano-macrophage cells situated in the spleen, kidney and liver (Agius & Roberts, 2003). Therefore, the appearance of melanin inside the eggs of anemonefish is unusual. This mass of melanin was only observed within 24 h before spawning, suggesting that the steroid that triggers oocyte maturation and ovulation (i.e. maturation inducing hormone) may also trigger the chain reaction of the melanin precursors in the egg yolk.

According to Ahlstrom & Moser (1980), the melanophores usually first appear along the dorso-lateral surfaces of the embryo and on the yolk sac. Melanophores in teleost fish arise from the neural crest, apart from the cell layer adjoining the retina. During neurula and somite stages, the melanoblasts in other species begin to migrate from the neural crest through the dermis and form the melanophores (Kunz, 2004). When the first melanophores reach the dorsal part of the embryonic head, the stellate melanophores become prominent and start to produce melanin (Bagnara & Hadley, 1973). The present research showed that during the early blastula stage, some black mass dispersed into the yolk. During the late gastrula stage, however, the boundary of the distinctive black mass in the yolk sac gradually dissolved into the yolk. The dissolving process continued to the somite stage and then became stagnant as the melanophores appeared all over the yolk sac. The dermal melanophores on the embryo had a typical stellate form with dark colour, while melanophores on the ventral side of the yolk sac were large brownish and round. During migration, most of the melanoblasts would not synthesize melanin until they arrived at their final sites. This suggests that the melanin mass in the yolk of anemonefish eggs might contribute to the early formation of the melanophores.

The manual rupture of the eggshell could rescue a disoriented larva. Allen (1991) reported that the body turnover in the later stage is an important process for larval hatching in demersal species. However, Kunz (2004) claimed that demersal eggs have the animal pole facing the free end, but we suspect that this author may have only observed the late stages of embryonic development. Olivotto et al. (2003) found that the embryo of *Chrysiptera parasema* could not hatch naturally if fish did not orient properly, since the proximal end of the egg was not easily bent. Recognition of the importance of body turnover in anemonefish would help improve the hatching success in hatchery management. Given the importance of egg orientation in embryo development, it would be a challenge to mass hatch anemonefish in a conventional hatching jar if eggs could not attach to a specially designed substrate.

In summary, the time requirement for each major developmental stage from cleavage to hatching was documented in this study. The comparison of organogenesis between this and other anemonefish species highlights the variation among closely related species in the *Amphiprion* genus. The developmental sequence of major organs such as internal ear, eyes, gills, digestive tract and fins provides an insight into understanding the possible interactions of anemonefish with their environment. The process of pigment formation from the early embryo to later stages would contribute to a further understanding of the ontogeny of coloration in damselfish. The overall understanding of the embryology and ontogeny of *A. ocellaris* may help improve the culture of other coral reef fish.

We would like to thank Ms Sandra Marshall for technical support during the experimental period. The manuscript was substantially improved by comments and constructive suggestions from M. Arvedlund and three anonymous referees. We also appreciate the comments and editing made by M. Bull and C. Meakin on this manuscript and useful discussion with J. Carragher on data interpretation. This study was supported by the Australian Government's overseas aid (AusAID).

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Submitted 3 May 2006. Accepted 16 May 2007.