# SPAWNING, DEVELOPMENT AND LARVAL REARING OF CLOWNFISH *Amphiprion ocellaris* UNDER CAPTIVE CONDITIONS

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Clownfish species are among the most popular species in marine ornamental trade and play an important role as scientific subject. They are colourful fishes that have high market demands and fetch high prices. In order to fulfill the market demand, most of the fish were caught from the wild by using cyanide, thus destroying coral reef ecosystem and fish population. In order to conserve our coral reef ecosystem and fishes, this research was conducted to study spawning behavior, spawning performance, embryonic and early life development and larval rearing of *Amphiprion ocellaris* under captive conditions.

A total of 15 pairs mature and healthy *A. ocellaris* (5.24±0.44 ♂: 7.67±0.73 ♀) were collected from Bidong Island, Terengganu. After acclimatization under captive conditions, all the pairs were fed on cockles twice per day to satiation. Eight pairs successfully spawned, 3 pairs have eaten their egg while 4 pairs did not spawn.

Observation recorded that some females were also involved in cleaning the substrate before spawning, guarding and agitating the eggs during and after spawning with 8.25, 11.14 and 0.89 times, respectively. *Amphiprion ocellaris* was able to spawin twice per month without being influenced by the moon phase ($P$>0.05). Number of eggs recorded was in the range of 100 – 1300. The eggs took 7.05 days to hatch with 95.9% of hatching rate and 95% of survival rate.

After spawning, the eggs were transferred into a 30 L incubation tank at 27.5±0.5° C to evaluate the embryonic development and yolk volume exhaustion under laboratory-reared conditions for 7 days. A total of 20 eggs were randomly sampled at 24±1 hour intervals. First cleavage occurred one hour after fertilization as a direct indicator of egg viability in *A. ocellaris*. The volume of newly deposited egg was recorded at 0.72 $mm^3$. Blastulation was observed four hours later, followed by gastrulation after 12 hours with a yolk volume at 0.63 $mm^3$. Presumptive pericardial cavity, otic primodium, otic placode, forebrain, midbrain, hindbrain, lens and somite stage had developed well and clearly noticeable 31 hours later. Heart beating and blood circulation occurred at 78 hours. Red pigmentation appeared at 96 hours and yolk volume of embryo was recorded at 0.21 $mm^3$. The pre-hatching stage occurred at 168 hours after insemination with 0.12 $mm^3$ of yolk volume. *Amphiprion ocellaris* larval was hatched at 180 hours at the advance stage.

Hatched larvae were distributed randomly into 30 L rearing tanks with 50 ind./tank to study early morphological development and allometric growth for 40 days with 20 ind./day. Total length (TL), standard length (SL), pre-anal length (PL), head length (HL), upper jaw length (UJL), eye diameter (ED) and body depth (BD) were

measured as morphometric characters. *Amphiprion ocellaris* larvae hatched at the advance stage exhibited fully developed and functional eye, mouth and fins. Pectoral fin was formed at 4 dph. Notochord flexion occurred at 6 dph and caudal fin became rounded at 15 dph. At 25 dph, body pigmentation developed as adult colour. Larvae formed into a small group at 21 – 25 dph. Morphometric growth of standard length, pre-anal length and eye diameter can be described as negatively allometric with a single regression. Head length, upper jaw length and body depth could be fitted into two regression lines. Early ontogeny of body depth growth was positively allometric while upper jaw length growth was nearly isometric.

Larval rearing study was conducted to determine onset of exogenous feeding, suitability of rearing media and types of food as first exogenous feed on survival rate (SR), specific growth rate (SGR) and daily growth rate (DGR). The onset of exogenous feeding in *A. ocellaris* larvae occurred at 16 h after hatched with a yolk reserve at 0.0085 $mm^3$. Yolk-sac exhibited complete exhaustion at 21 h after hatched. The point of no-return of *A. ocellaris* was detected at 2.1 day (50.4 h) after yolk exhaustion. A combination feeding period between endogenous and exogenous was detected at 16 h after hatched or 5 h before completion of yolk exhaustion. Filtered seawater with the addition of *Chlorella* sp. was the most suitable medium with 23.11% of SR, 6.44% $day^{-1}$ SGR and 0.43 mm $day^{-1}$ DGR. *Amphiprion ocellaris* larval only preferred to feed on live food as their first exogenous feed with 73.78% of SR, 6.84% $day^{-1}$ of SGR and 0.42 mm $day^{-1}$ of DGR, respectively. No larvae was able to survive when being fed on commercial food such as artificial plankton, microencapsulated pellet and liquid food as their first exogenous feed. This captive breeding of clownfish is the first successful propagation in Malaysia that can

contribute in conservation of wild population by restocking besides enhancing ornamental fish industry.

Abstrak tesis yang dikemukakan kepada Senate Kolej Universiti Sains dan Teknologi Malaysia sebagai syarat keperluan memenuhi Ijazah Master Sains

**PEMBIAKAN, PERKEMBANGAN DAN LARVA TERNAKAN IKAN CLOWN *Amphiprion ocellaris* DALAM KURUNGAN**

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Spesies clownfish adalah antara spesies yang paling popular dalam industri ikan hiasan air masin dan memainkan peranan penting sebagai saintifik subjek. Ia ialah ikan berwarna-warni yang mendapat permintaan pasaran dan harga yang tinggi. Untuk memenuhi permintaan pasaran, kebanyakan sumbernya adalah ditangkap secara liar dengan menggunkan cyanide yang membinasakan ekosistem batu karang dan populasi ikan. Demi memulihkan ekosistem batu karang dan ikan, penyelidikan ini dijalankan untuk mengkaji tabiat pembiakan, prestasi pembiakan, embrio dan perkembangan hidup awal dan larva ternakan *Amphiprion ocellaris* dalam keadaan kurungan.

Sejumlah 15 pasang *A. ocellaris* (5.24±0.44♂ : 7.67±0.73♀) yang matang dan sihat dikumpul dari Pulau Bidong, Terengganu. Selepas disesuaikan dalam kurungan, semua pasangan diberi makanan kerang sehingga kenyang 2 kali sehari. Lapan

pasangan telah berjaya membiak, 3 pasangan memakan telur manakala 4 pasangan tidak membiak. Pemerhatian telah mencatatkan bahawa ikan betina juga terlibat dalam pembersihan substrak sebelum membiak, menjaga dan mengocak telur semasa dan selepas pembiakan masing-masing sebanyak 8.25, 11.14 dan 0.99 kali. *Amphiprion ocellaris* mampu membiak dengan kekerapan 2 kali sebulan tanpa dipengaruhi oleh fasa bulan ($P > 0.05$). Jumlah telur *A. ocellaris* dicatatkan pada julat 100 – 1300 telur. Telur mengambil masa 7.05 hari untuk menetas pada kadar penetasan sebanyak pada 95.90% dan kadar kemandirian sebanyak 95%.

Selepas pembiakan, telur telah dipindah ke dalam 30 L tangki pengeraman pada 27.5±0.5° C untuk menilai perkembangan embrio dan penghabisan isipadu yolk dalam keadaan makmal selama 7 hari. Sejumlah 20 biji telur disampel secara rawak pada tempoh 24±1 jam. Pembahagian sel pertama berlaku satu jam selepas persenyawaan merupakan satu penunjuk kepada kebolehidupan telur dalam *A. ocellaris*. Isipadu telur yang baru dihasilkan dicatatkan pada 0.72 $mm^3$. Blastulasi berlaku empat jam kemudian, diikuti dengan gastrulasi selepas 12 jam dengan isipadu yolka sebanyak 0.63 $mm^3$. Semua organ berkembang dengan baik dan dapat diperhatikan dengan jelas selepas 31 jam. Denyutan jantung dan pengaliran darah berlaku pada 78 jam. Pigmentasi merah wujud pada 96 jam dan isipadu yolka embrio dicatatkan pada 0.21 $mm^3$. Peringkat pra-penetasan berlaku pada 168 jam selepas persenyawaan dengan isipadu yolka sebanyak 0.12 $mm^3$. Larva *A. ocellaris* menetas pada 180 jam pada peringkat larva yang telah maju.

Larva yang menetas diagihkan secara rawak ke dalam 30 L tangki ternakan dengan 50 ind./ tangki untuk mengkaji perkembangan morfologi awal dan pertumbuhan

allometrik selama 40 hari dengan 20 ind./hari. Panjang kesuluruhan, panjang piawai, panjang pra-anal, panjang kepala, panjang rahang atas, diameter mata, dan kedalaman badan diukur sebagai sifat morfometrik. *Amphiprion ocellaris* larva menetas pada peringkat yang telah maju dengan mata, mulut and sirip-sirip yang berkembang penuh dan berfungsi. Sirip pektoral terbentuk pada 4 hst (hst). Notokod flaksi berlaku pada 6 hari selepas menetas dan sirip kaudal berubah menjadi bulat pada 15 hst. Pada 25 hst, warna badan berkembang seperti ikan dewasa. Larva menbentuk kumpulan kecil pada 21 – 25 hst. Perkembangan panjang badan, panjang pra-anal dan diameter mata boleh diwakili dengan satu regresi yang berkembang secara allometrik negatif. Panjang kepala, panjang rahang atas dan kedalaman badan boleh diwakili dengan dua regresi. Perkembangan awal kedalaman badan adalah secara allometrik positif manakala perkembangan panjang rahang atas adalah hampir isometrik.

Kajian larva ternakan telah dijalankan untuk menentukan masa mula pemakanan luaran, kesesuaian medium ternakan dan jenis makanan sebagai makanan luaran pertama terhadap kadar kemandirian, kadar pertumbuhan spesifik dan kadar pertumbuhan harian. Permulaan pengambilan makanan luaran dalam *A. ocellaris* larva berlaku pada 16 jam selepas menetas dengan baki yolka sebanyak 0.0085 $mm^3$. Yolka habis digunakan pada 21 jam selepas menetas. Titik tiada pulangan (point of no return) *A. ocellaris* dikesan pada 2.1 hari (50.4 jam) selepas kehabisan yolka. Tempoh gabungan di antara pemakanan dalaman and luaran dikesan pada 16 jam selepas menetas atau 5 jam sebelum kehabisan yolka. Air laut tertapis dengan penambahan *Chlorella* sp. ialah medium yang paling sesuai dengan 23.11% kadar kemandirian, 6.44% $hari^{-1}$ kadar pertumbuhan spesifik dan 0.43 mm $hari^{-1}$ kadar

pertumbuhan harian. *Amphiprio ocellaris* larva hanya memilih makanan hidup sebagai makanan luaran pertama dengan 73.78% kadar kemandirian, 6.84% $hari^{-1}$ kadar pertumbuhan spesifik dan 0.42 mm $hari^{-1}$ kadar pertumbuhan harian masing-masing. Tiada larva dapat hidup apabila diberi makanan komersil seperti plankton tiruan, pellet mikroenkapsulasi dan cecair makanan sebagai makanan luaran pertama. Pembiakan ikan clown dalam kurungan ini ialah kali pertama berjaya di Malaysia yang dapat menyumbang dalam pemulihan populasi liar melalui pelepasan semula disamping meningkatkan industri ikan hiasan.