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Growth pattern, diet and reproductive biology of the clownfish *Amphiprion ocellaris* in waters of Pulau Tioman, Malaysia ☆

Mei Ling Khoo [a], Simon Kumar Das [a,b,*], Mazlan Abd Ghaffar [c]

[a] Marine Ecosystem Research Centre (EKOMAR), Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor D.E., Malaysia
[b] School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor D.E., Malaysia
[c] Institute of Oceanography and Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

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ABSTRACT

The growth pattern, diet and reproductive biology of the clownfish *Amphiprion ocellaris* collected from waters of Pulau Tioman were investigated. The length-weight relationship showed an isometric growth pattern (b = 3) in *A. ocellaris*. The stomach contents mainly consisted of zooplankton and algae, which showed that the fish is omnivorous and was confirmed by trophic level analysis (2.98 ± 0.29). Fecundity of *A. ocellaris* ranged from 23-1518 with mean egg count of 582 ± 478, and has positive relationships with body length, body weight, eviscerated weight and ovary weight. The gonads were also described and examined histologically. The ovaries showed 4 stages of maturity, displaying different colours for each stage. The different developments of oocytes were also found in each stage of maturity. The males and non-breeders comprised of both testicular and ovarian tissues. In the males, the testicular tissues were more prominent in the ovotestes, whereas in non-breeders the primary oocytes were more prominent. Absence of testicular tissue in ovaries of females showed that the fish is a protandrous hermaphrodite and sex change may not be reversible. Similar observations have been reported in *A. ocellaris* of other countries and other *Amphiprion* species indicating that *Amphiprion* species show consistency in their reproduction strategy throughout their range.

Introduction

Malaysia initially focused on the export of freshwater species, but now is one of the main suppliers of marine ornamental species which are becoming increasingly popular among aquarist (Abol-Munafi et al., 2011; Cato and Brown, 2003). The false clownfish, *Amphiprion ocellaris*, is one of the most commercially exploited species in the aquarium trading as ornamental fishes (Wabnitz et al., 2003). It is unfortunate though that in the present situation with increasing demand for the clownfishes, supplies are mostly dependant on the wild catches (Abol-Munafi et al., 2011; Cato and Brown, 2003). Studies on Malaysian pomacentrids are still lacking (Sin et al., 1994), and concerns have been raised about the declining numbers of this species in Malaysian waters due to the possibility of overfishing and deterioration of its natural habitat caused by destructive collection methods (Abol-Munafi et al., 2011; Livengood and Chapman, 2007). Therefore, it is important to be able to propagate this species in captivity for both commercial and conservation purposes. Although the clownfish *Amphiprion ocellaris* has been successfully reared in captivity in other countries (Juhl, 1992; Moe, 1992), the production in Malaysia is still low (Abol-Munafi et al., 2011). In Malaysia, Liew et al. (2006) has successfully bred the clownfish in captivity but failed to develop broodstocks from F1 generations and still needs to collect broodstocks pairs from the wild.

Amphiprion ocellaris are protandrous hermaphrodites; they have the ability to change sex from male to female at maturity (Thresher, 1984; Fautin and Allen, 1992). Sex change occurs in relation to the social hierarchy, where the two largest individuals (female being the largest of the two) forms a strong monogamous breeding pair while the rest are non-breeders (Allen, 1975; Moyer and Nakazono, 1978; Thresher, 1984; Fautin and Allen, 1992; Hirose, 1995; Liew et al., 2006). When the female dies (or is removed), the male changes sex to become the dominant breeding female and the second largest member from the non-breeders becomes the dominant male (Rosenberg and Cruz, 1988; Fautin and Allen, 1992). This has made the breeding of clownfish

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* Corresponding author at: School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor D.E., Malaysia.

E-mail addresses: simon@ukm.edu.my, skdas_maa@yahoo.com (S.K. Das).

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challenging in captivity; the need for research arose from the lack of detailed information concerning the feeding and reproductive biology of *A. ocellaris* in Malaysian waters, which are important to facilitate effective management in culturing the species to provide for local and global demands. Therefore, this study was conducted to measure important biological aspects of *A. ocellaris*; namely (i) population growth pattern (ii) stomach content analysis (iii) trophic level (iv) fecundity estimates, (v) observations of changes in maturity stages in the gonads and, (vi) histology studies to determine the stages of the reproductive organ.

Material and methods

Fish sampling

A total of 65 fishes were collected from waters of Pulau Tioman using plastic bags while SCUBA diving. The fishes were then killed by a sharp blow on the head or by pithing. The specimens were then measured to the nearest 1 cm (total length TL and standard length SL) and weighed to the nearest 0.1 g (total body weight TW and eviscerated weight EW). Each fish was dissected, the gonads were removed, weighed and photographed.

Length-weight relationship

Population growth pattern was estimated based on length-weight relationship (LWR), expressed by the equation $W = aL^b$ where W is body weight (g), L is total length (cm), a is the intercept and b is the slope. A non-linear regression was used to determine the values of a and b. Curve fitting was carried out by a non-linear iterative method using Levenberg-Marquardt and simplex logarithm using MicroCal Origin™ Version 8.0 programme with a statistical significance of 5%. The degree of adjustment of the model studied was assessed by the coefficient of determination (r^2). A student t-test was applied to verify if the b value presented a significant difference to 3.0, indicating the type of growth; isometric (b = 3.0), positive allometric (b > 3.0) and negative allometry (b < 3.0) (Froese 2006; Simon and Mazlan, 2008; Simon et al. 2009; Froese et al., 2011, 2014; Simon et al. 2014).

Stomach content analysis and trophic level

In the laboratory, the digestive tracts were removed and their content analysed under a microscope and identified. Diet composition data were used to estimate the trophic level of the fish. TROPH value was calculated from the dataset using TrophLab; an application for estimating TROPH and its standard error using weight or volumetric contribution and trophic level of each prey species to the diet, where individuals with a value of 2.0 or lower were considered herbivores, 5.0 and above were carnivores and any value in between those values were omnivores (Pauly et al., 1998, 2001; Simon and Mazlan, 2010).

Fecundity estimates

Fecundity was estimated from 30 females (n = 30) as the total number of oocytes present in the mature gonad. Prepared samples were fixed in Gilson's fluid to loosen the oocytes from the lobe. The total number of oocytes were then counted and reported as total fecundity. Relationships between fecundity and total length, total weight, ovary weight and eviscerated weight were then derived by regression analysis using MicroCal Origin™ software and were analysed using Pearson's correlation coefficient (Simon et al. 2012).

Gonad histology

A total of 30 gonads from all development stages were prepared and placed in cassettes, dehydrated (tissue processing) and embedded in paraffin wax following the procedures as practiced by the National Fish Health Research Centre of Fisheries Research Institute laboratory. Sections were cut at 5μm thickness and then dewaxed, dried and stained with hematoxylin and eosin. They were then mounted on slides before being observed under the microscope (Leica DM1000) equipped with a camera. Diameter of oocytes of all stages were randomly selected and measured to the nearest 0.001mm.

Results

Length-weight relationship

Length-weight relationship was derived from 65 individuals of clownfishes. The length weight relationship was a combination for both sexes and was represented as $W = 0.0197L^{3.06}$ (Fig. 1). Intercept 'a' was 0.0197 ± 0.0037 and the value of exponent 'b' was 3.0674 ± 0.0917, with coefficient of determination 'r^2' value of 0.9429 ($P < 0.001$). The value 'b' was in close proximity of 3, indicating an isometric growth pattern where the body changes form in proportionate to the body weight (following cube law; volume = L^3).

Stomach content and trophic level

The stomachs of 60 fishes were examined, most of which were empty or contained few food item. Those food items were identified to mainly consist of various larvae (barnacle, tunicate, crustaceans and gastropods), copepods, algae, fish eggs and ctenoid scales (Fig. 2). The estimated trophic level ranged from 2.00 to 4.10 with mean values of 2.98 ± 0.29, and remained at the same level as size increased (Fig. 3a and b).

Fecundity estimates

Total fecundity ranged from 23-1516 eggs with a mean egg count of 582±478. An overall positive correlation was obtained with fecundity and each and every comparison including body length (TL), body weight (TW), body eviscerated weight (EW) and ovary weight (Fig. 4) with r values of 0.308, 0.223, 0.154,

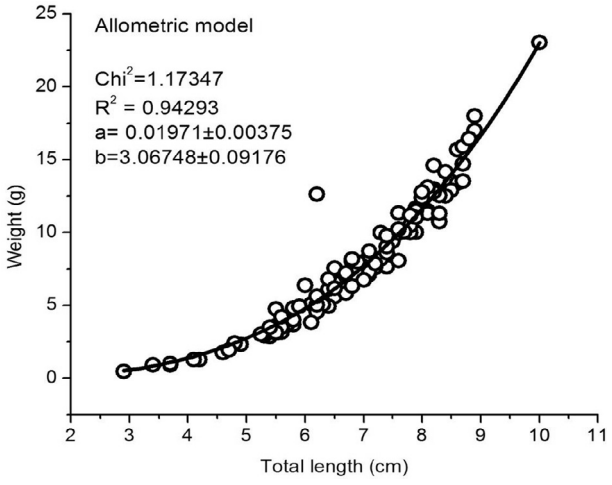

Fig. 1. Length weight relationship of *A. ocellaris* from Pulau Tioman waters. Red line represents a non-linear fit of the *A. ocellaris*, both sexes combined.

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