

Effects of Photoperiod on Growth of Larvae and Juveniles of the Anemonefish *Amphiprion melanopus*

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Abstract

Rearing of anemonefishes is now relatively routine compared to the culture of cardinalfishes (Apogonidae) or angelfishes (Pomacanthidae). However, it is still a labor intensive, time intensive and expensive procedure. To reduce time and cost of rearing anemonefishes, experiments were undertaken to improve the methods for rearing *Amphiprion melanopus*. These experiments were conducted to determine the effect of the length of photoperiod on larval duration, growth to metamorphosis and early juvenile phase. Growth of larvae was significantly faster and the duration of the larval phase was significantly shorter, under a photoperiod of 16 hours light/8 hours dark, compared to the photoperiods of 12 hours light/12 hours dark and 24 hours light/0 hours dark.

Introduction

The tropical marine anemonefishes (Pomacentridae) are important in the trade for ornamental fish (Wilkerson 1998) and are a popular subject of research (Fautin 1991). Over the last 20 years, mariculture centers and scientific laboratories have started rearing these fishes in large quantities (McLarney 1985, 1986; Miyagawa 1989; Hoff 1993; Young 1996; Job et al. 1997). The list of marine fishes reared in captivity today, for purposes other than human consumption, contains more than 84 species (Tables 1 and 2). The fact that 26 different species from the family Pomacentridae are reported to be reared is notable. This is a significantly higher number of species compared to all other families. However, when we look at species that can be reared reliably in large quantities, they include only a dozen anemonefish species, seven species of gobiids (Gobiidae), five species of cardinalfishes (Apogonidae) and eight species of pseudochromids (Pseudochromidae). The last two are only included here as a result of recent work by Gardner (1997) and Job et al. (1997). For all the species within the families mentioned above, larval rearing is still time-consuming and expensive.

This paper describes the growth experiments conducted at the Sir George Fisher Research Aquarium, James Cook University (JCU), to improve the methods for rearing anemonefishes so as to reduce the time and cost of rearing them for scientific studies.

Only two studies have experimented with ways of enhancing the efficiency of larval rearing of anemonefishes. Frakes and Hoff (1982) published a study on the effect of high nitrate-N on the growth and survival of juvenile and larval anemonefish *A. ocellaris*. Alayse (1984) studied the survival rate of *A. ocellaris* larvae fed on enriched food. This study examines the growth rates of larvae and juveniles under different light regimes. This variable is important as anemonefish larvae are visual feeders (Coughlin 1994; Job and Bellwood 1996). Scientists have shown that an extended photoperiod can significantly increase the growth rate of the larvae and early juveniles of a variety of marine fish species (Fuchs 1978; Barahona-Fernandes 1979; Boehlert 1981; Kiyonon and Hirano 1981; Tandler and Helps 1985; Duray and Kohno 1988; Barlow et al. 1995).

Hoff (1996) and Wilkerson (1998) estimate the optimum growing conditions for anemonefish larvae to be

a 16-hour daily light period, while Juhl (pers. comm.) recommends a 24-hour light regime. None of these authors present supporting data. This study investigates the effect of 12 hour, 16 hour and 24 hour photoperiods on the growth and larval duration of the anemonefish *A. melanopus*.

Materials and Methods

FISH MAINTENANCE AND REARING

Breeding pairs of *A. melanopus* were collected from the Cairns section of the Great Barrier Reef and placed in 60-l tanks with gravel filters and 200-l powerheads for the circulation of the water. Rocks were placed as a surface on which the fish could spawn. Water in the tanks was obtained from the JCU aquarium system. It had 33‰ salinity, 27-30°C temperature (daily variation, summer), 21-25°C (daily variation, winter) and a pH of 8.0-8.2. The water in the parental tank was flushed daily with water from the main aquarium system. The JCU system is closed, with coastal water filtered through sand and large protein skimmers.

Spawning occurred approximately every three weeks, and produced

Table 1. List of marine fishes reared in captivity for purposes other than human consumption.

Family	Species	Common name	Reference
Apogonidae (6 species)	<i>Apogon cyanosoma</i>	Yellow-striped cardinal	Job et al. 1997
	<i>Apogon compressus</i>	Split-banded cardinal	Job et al. 1997
	<i>Sphaeramia nematoptera</i>	Pyjama cardinal	Job (pers. comm.)
	<i>Cheilodipterus quinquelineatus</i>	Fiveline-cardinal	Job et al. 1997
	<i>Apogonichthys nigripinnis</i>	Nigripes cardinal	Lange 1989
	<i>Pterapogon kauderni</i>	Banggai cardinal	Marini 1996
Batrachoididae	<i>Opsanus tau</i>	Common toadfish	Schumann 1969
Blennidae	<i>Blennius pavo</i>	Mediterranean blennius	Patzner and Brandstaetter 1989
Carangidae	<i>Trachinotus carolinus</i>	Florida pompano	Moe 1992
Callionymidae	<i>Synchiropus splendidus</i>	Mandarinfish	Gardner 1997
Ephippidae	<i>Chaetodipterus faber</i>	Atlantic spadefish	Walker 1991
Gobiesocidae	<i>Gobiosox strumosus</i>	Skilletfish	Moe 1992
Gobiidae (10 species)	<i>Gobiosoma multifasciatum</i>	Greenband goby	Moe 1992
	<i>Gobiosoma evelynae</i>	Sharknosed goby	Moe 1992
	<i>Gobiosoma oceanops</i>	Neon goby	Moe 1992
	<i>Elactinus xanthipora</i>	Golden goby	Young 1994
	<i>Gobiodon citrinus</i>	Citron goby	Anon. 1997
	<i>Gobiosoma prochilus</i>	West indian cleaner goby	Anon. 1997
	<i>Gobiosoma genie</i>	Genie's cleaning goby	Anon. 1997
	<i>Coryphopterus personatus</i>	Masked goby	Anon. 1997
	<i>Gobiosoma okinawae</i>	Yellow goby	Gardner 1997
	Lutjanidae (2 species)	<i>Lutjanus griseus</i>	Grey snapper
<i>Ocyurus chrysurus</i>		Yellowtail snapper	Moe 1992
Opistognathidae	<i>Opistognathus aurifrons</i>	Yellowhead jawfish	Young 1982
Plesiopidae	<i>Callopleysiops altivelis</i>	Comet-marine betta	Wassink 1990
Pomacanthidae (6 species)	<i>Centropyge argi</i>		Anon. 1997
	<i>Centropyge ferrugatus</i>		Hioki et al. 1990
	<i>Centropyge loriculus</i>	Flame angel	Anon. 1998
	<i>Centropyge resplendens</i>		Anon. 1998
	<i>Pomachantus arcuatus</i>	Grey angelfish	Moe 1975
	<i>Pomachantus paru</i>	French angelfish	Moe 1975
Pomacentridae	(26 species) See Table 2.		
Pomadasyidae (2 species)	<i>Anisotremus virginicus</i>	Porkfish	Moe 1992
	<i>Haemulon plumieri</i>	White grunt	Moe 1992
Pseudochromidae (9 species)	<i>Labracinus cyclophthalmus</i>	Dottyback	Lange 1989
	<i>Ogilbyina novaehollandiae</i>	Australian dottyback	Gardner 1997
	<i>Pseudochromis aldabrensis</i>	Neon dottyback	Gardner 1997
	<i>Pseudochromis flavivertex</i>	Sunrise dottyback	Brons 1996
	<i>Pseudochromis fridmani</i>	Orchid dottyback	Brons 1996
	<i>Pseudochromis fuscus</i>	Yellow dottyback	Gardner 1997
	<i>Pseudochromis olivaceus</i>	Olive dottyback	Gardner 1997
	<i>Pseudochromis sankey</i>	Striped dottyback	Gardner 1997
	<i>Pseudochromis springeri</i>	Springeri dottyback	Gardner 1997
Sciaenidae (4 species)	<i>Equetus acuminatus</i>	High-hat	Moe 1992
	<i>Equetus lanceolatus</i>	Jackknife-fish	Moe 1992
	<i>Equetus punctatus</i>	Spotted drum	Moe 1992
	<i>Equetus umbrosus</i>	Drum	Anon. 1997
Serranidae (3 species)	<i>Grama loreto</i>	Royal gramma	Moe 1992
	<i>Grama melacara</i>	Blackcap basslet	Moe 1992
	<i>Hypoplectrus unicolor</i>	Hamlet	Moe 1992
Syngnathidae (8 species)	<i>Doryrhamphus dactyliophorus</i>	Ringed pipefish	Lange 1989
	<i>Hippocampus erectus</i>	Lined seahorse	Moe 1992
	<i>Hippocampus hippocampus</i>		Lange 1989
	<i>Hippocampus kuda</i>	Spotted seahorse	Lange 1989
	<i>Hippocampus punctulatus</i>		Schumann 1969
	<i>Hippocampus reidi</i>		Anon. 1998
	<i>Hippocampus zostera</i>	Dwarf seahorse	Moe 1992
Tetraodontidae	<i>Syngnathoides biaculatus</i>	Pipefish	Lange 1989
	<i>Spoeroides maculatus</i>	Northern pufferfish	Moe 1992
Labridae	<i>Lachnolaimus maximus</i>	Hogfish	Moe 1992

200 - 300 eggs per clutch. Embryos hatched in nine days. An hour or two before hatching, the rock with the egg clutch (and the host sea anemone) was transferred in a water-filled bucket to the hatching aquarium, where the clutch was left in the dark for approximately 90 min. The water in the hatching tank was gently aerated but not filtered, since the fish larvae are sensitive to currents (Arvedlund, pers. obs.). The sides of the hatching tanks were covered with black plastic to reduce light reflection. The phytoplankter *Nannochloropsis* sp. was used to "green up" the tanks until the bottom of the tank could no longer be

seen. These methods of reducing light stopped the "headbutting syndrome" of the fish and improved water quality, since the algae act as a nutrient sink (Job et al. 1997). Water in the hatching tank came from the breeding tank to ensure constant osmolarity for the fish larvae. Approximately 20% of the water was replaced every second day with water from the parent aquarium.

The larvae were fed the rotifer *Brachionus plicatilis* for the first two days after hatching. Then they were gradually introduced to a diet of *Artemia*. After approximately 30 days, the juveniles were gradually weaned to a mixed diet of finely

chopped sardines, prawns and vitamin supplements, i.e., the same diet as adult fish.

GROWTH AND SURVIVAL

Due to the difficulty of dividing up a batch, larvae from three different batches from the same parents were used for the experiments. To examine variability in growth among different batches of larvae from a single pair of *A. melanopus*, three batches of larvae from the same parents were exposed to a uniform rearing environment (16L:8D). These were the control batches. Five fish were randomly sampled every fifth day, starting from the day of hatching (day 0).

The sample fish were anesthetized by refrigerator chilling, preserved in 70% ethanol and measured to the nearest 0.01 mm total length (TL). For wet and dry weight, all fish were weighed to the nearest 0.0001 g. For dry weight, the larvae were dried in an oven at 60°C for 16 hours.

To examine the effect of extended photoperiods on fish growth, three batches of larvae from the same breeding pair were reared under three different light regimes. All other rearing conditions were replicated for each batch. The light regimes were: (a) 12 hours light/12 hours dark (12L:12D); (b) 16 hours light/8 hours dark (16L:8D); and (c) 24 hours light/0 hours dark (24L:0D). The light was provided by two 40 watts fluorescent light bulbs. Food was made available for 24 hours/day in all three treatments, in densities of 3-5 rotifers (later *Artemia*) per ml. A sample of five fish larvae was collected randomly at hatching (day 0) from each batch, and then every 5 days after hatching up to day 25. The sample fish were preserved, weighed and measured as described above. The test batches were not replicated.

Growth curves were calculated for each photoperiod treatment. The growth curves were compared by first using a test for homogeneity of slopes. If found nonsignificant, an ANOVA test for differences among

Table 2. List of marine fishes of the family Pomacentridae reared in captivity.

Species	Common name	Reference
1) Anemonefishes (subfamily Amphiprioninae)		
<i>Amphiprion akallopisos</i>	Skunk anemonefish	Moe 1992
<i>Amphiprion allardi</i>	Allard's anemonefish	Terver 1975
<i>Amphiprion akindynos</i>	Barrier Reef anemonefish	Fisher (pers. comm.)
<i>Amphiprion bicinctus</i>	Red Sea anemonefish	Young 1990
<i>Amphiprion chrysogaster</i>	Orange-fin anemonefish	Moe 1992
<i>Amphiprion clarkii</i>	Clark's anemonefish	Miyagawa 1989; Moe 1992; Wilkerson 1992
<i>Amphiprion ephippium</i>	Red saddleback anemonefish	Moe 1992; Gardner 1997
<i>Amphiprion frenatus</i>	Tomato anemonefish	Miyagawa 1989; Juhl 1992; Moe 1992; Gardner 1997
<i>Amphiprion latezonatus</i>	Wide-band anemonefish	Moe 1992
<i>Amphiprion leucokranos</i>	White-bonnet anemonefish	Moe 1992
<i>Amphiprion melanopus</i>	Red and black anemonefish	Moe 1992; Gardner 1997; Job et al. 1997
<i>Amphiprion ocellaris</i>	False clown anemonefish	Miyagawa 1989; Juhl 1992; Moe 1992; Arvedlund and Nielsen 1996; Gardner 1997
<i>Amphiprion percula</i>	Clown anemonefish	Moe 1992; Gardner 1997; Job et al. 1997
<i>Amphiprion perideraion</i>	Pink anemonefish	Miyagawa 1989; Moe 1992; Gardner 1997
<i>Amphiprion polymnus</i>	Saddleback anemonefish	Terver 1971; Moe 1992
<i>Amphiprion rubrocinctus</i>	Australian anemonefish	Moe 1992
<i>Amphiprion sandaracinos</i>	Orange anemonefish	Miyagawa 1989; Moe 1992; Gardner 1997
<i>Amphiprion tricinctus</i>	Three-band anemonefish	Moe 1992
<i>Pomacentrus biaculatus</i>	Spine-cheek anemonefish	Moe 1992; Gardner 1997; Job et al. 1997
2) Damsel-fishes other than anemonefishes		
<i>Abudefduf saxatilis</i>	Sargeant major	Moe 1992
<i>Dascyllus albisella</i>	Hawaiian Dascyllus	Danilowicz and Brown 1992
<i>Dascyllus aruanus</i>	Humbug Dascyllus	Danilowicz and Brown 1992
<i>Hypsypops rubicundus</i>	Garibaldi	Moe 1992
<i>Microspathodon chrysurus</i>	Jewelfish	Moe 1992
<i>Neopomacentrus bankieri</i>	Chinese demoiselle	Job et al. 1997
<i>Pomacentrus amboinensis</i>	Ambon damselfish	Job et al. 1997

intercepts was made. The relationship between total length and age was curvilinear and was linearized by natural log transformation prior to analysis. The relationship between dry weight and age was linear.

A gross estimate of the survival rate in all batches was made by counting the fish larvae in each batch at the time of hatching and at the end of the experiment (day 25).

LARVAL DURATION

At the end of their larval stage, anemonefish metamorphose and take on juvenile behavior and morphology (Allen 1975). This involves the development of white bars and a shift from occupying the top to midwater, to the bottom of the tank (Miyagawa 1989; Arvedlund, pers. obs.). The number of days between hatching and the settlement of all of the fish (100%) was recorded and used as a measure of larval duration.

Results

Variability in growth among batches of larvae from the same parents but exposed to different photoperiods was low (Figs. 1a and 1b).

For the three batches reared under different photoperiods, growth in total length differed significantly among treatments (test of homogeneity of slopes: $F_{2,84}=7.71$, $p<0.0008$). Paired comparisons among the slopes found that fish from the 16L:8D and 24L:0D treatments did not differ from one another, but both had significantly higher slopes than the 12L:12D treatment (Fig. 2a, Table 3).

These trends were further accentuated when growth was expressed as change in dry body weight with age. Rates of change in body weight also differed among photoperiod treatments ($F_{2,82}=10.84$, $p<0.0001$). In the case of dry body weights, all curves differed from each other. Fish reared under 16L:8D had the highest growth followed by the 24L:0D treatment, with the 12L:12D fish displaying the lowest growth (Fig. 2b, Table 4).

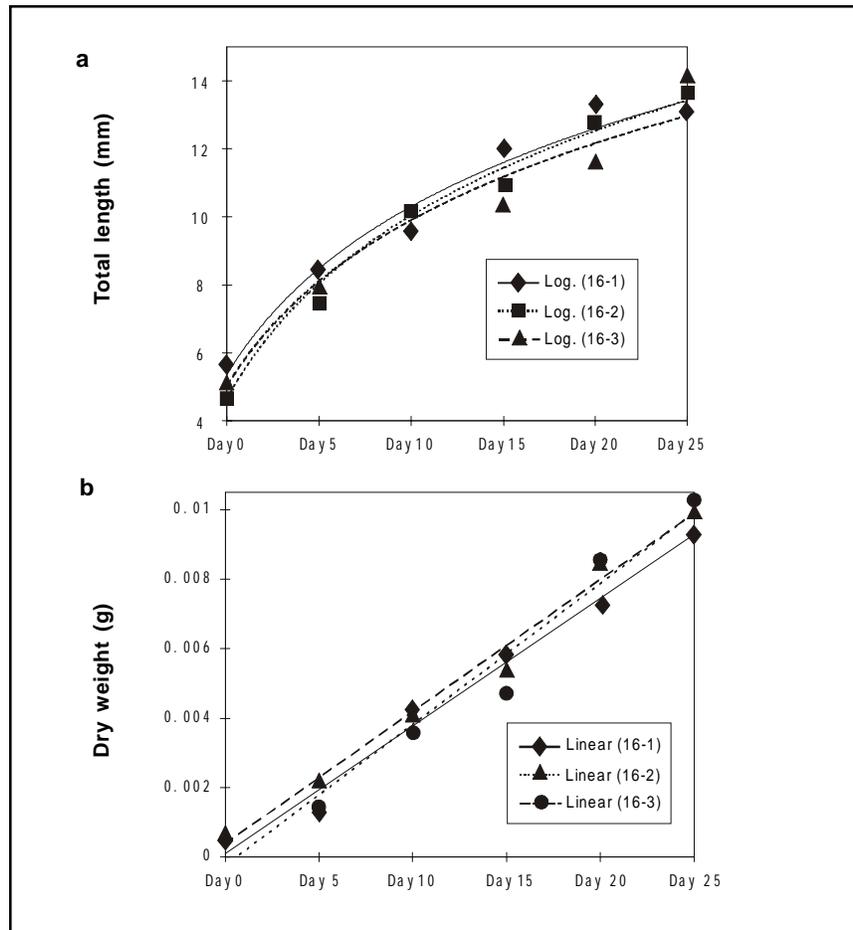


Fig. 1. Regression lines of log transformed total length (a) and dry weight (b) against time for each of three batches of *A. melanopus* (from the 16L:8D group), reared under the same conditions. $R^2 \geq 0.095$ for all equations.

The survival rate was approximately 70% for all three batches.

LARVAL DURATION

For the three control batches, all fish larvae (100%) of each batch settled, i.e. metamorphosed, and gained white bars at day 8 after hatching. For the test batches, the larvae reared in 16L:8D also settled

on day 8 after hatching, while those reared in 12L:12D and 24L:0D acquired the white bars on day 10 after hatching.

Discussion

A. melanopus larvae and juveniles up to 25 days after hatching grew fastest under an extended photoperiod of 16-hour light. The 24-hour light

Table 3. Comparison of growth (total length) versus age for three batches of *A. melanopus* larvae reared under three different light regimes.

Light regime	Equation
12L:12D	$y=4.0823\ln(x)+4.6486$, $R^2=0.9605$
16L:8D	$y=4.8933\ln(x)+4.6543$, $R^2=0.9913$
24L:0D	$y=4.7137\ln(x)+4.5696$, $R^2=0.9952$

Table 4. Comparison of growth (dry weight) versus age for three batches of *A. melanopus* larvae reared under three different light regimes.

Light regime	Equation
12D:12L	$y=0.0013x-0.002$, $R^2=0.9813$
16D:8L	$y=0.002x-0.0023$, $R^2=0.9655$
24D:0L	$y=0.0017x-0.0019$, $R^2=0.9425$

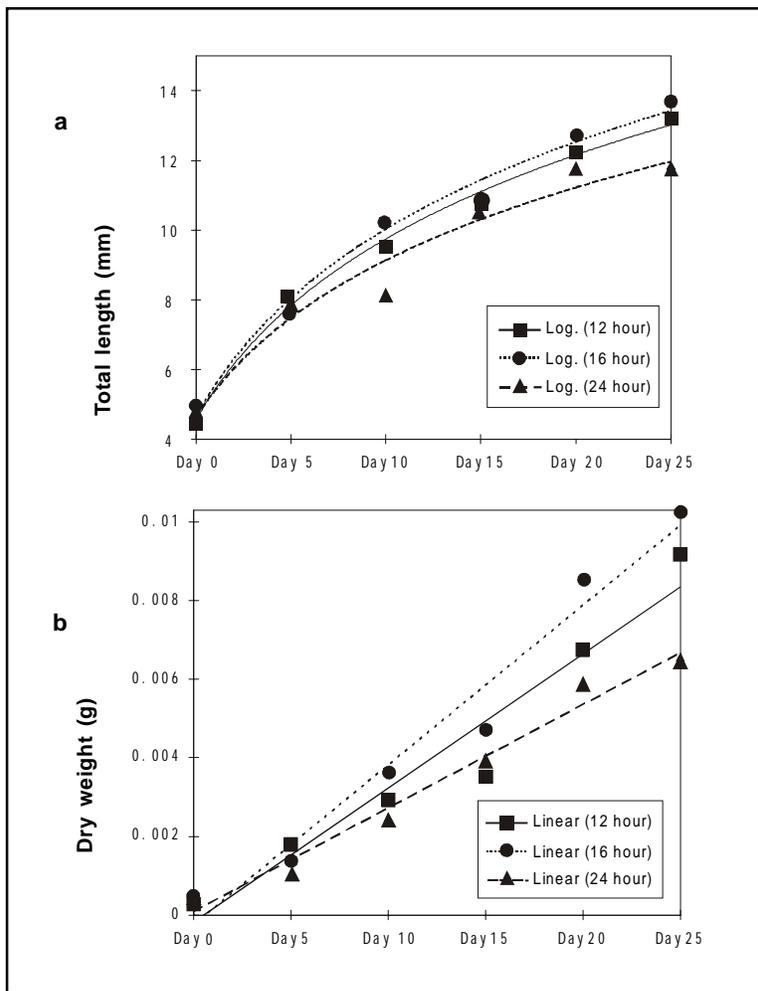


Fig. 2. Regression lines of log transformed total length (a) and dry weight (b) against time for each of three batches of *A. melanopus*, reared under three different light regimes. $R^2 \geq .095$ for all equations.

regime yielded faster growth rates than the 12L:12D regime. However, both were slower than fish in the 16L:8D photoperiod. The authors suggest that fish in the extended light regimes feed for longer periods of time than those reared under 12L:12D photoperiod, thereby yielding higher rates of growth and development. The fact that growth under 24L:0D was slower than under 16L:8D suggests that unless the developing juveniles have a period of inactivity during darkness, their growth is compromised.

Our findings are supported by earlier studies of other fish species. A study of the rockfish *Sebastes diploproa* was reported to have an optimum growth rate with a 16-hour

light period (Boehlert 1981). A study on the sea bass *Dicentrarchus labrax* reported an optimum growth rate with 18-hour light periods (Barahona-Fernandes 1979). Barlow et al. (1995) examined the growth of barramundi larva (*Lates calcarifer*) under different photoperiods, and found that individuals 8-20 days old had significantly higher growth rates with photoperiods of 16-hour and 24-hour light. A similar result was also obtained for sole (*Soela solea*) by Fuchs (1978).

In contrast, Kiyonon and Hirano (1981) reported an optimum growth rate of black porgy (*Mylio macrocephalus*) with continuous light. Tandler and Helps (1985) also reported this for gilthead sea bream

(*Sparus aurata*) and Duray and Kohno (1988) for rabbitfish (*Siganus guttatus*).

The wide range of results suggests that different families of fishes have different feeding patterns and therefore different requirements as larvae. Differences in the quality of the food between each study may also play a role.

The conclusion of this study is that the optimum lighting condition for growth of *A. melanopus* is 16L:8D, given that appropriate food is present for all the 16 hours of light. Using this photoperiod for larval rearing should improve growth rates and decrease larval production time.

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