Aquaculture 318 (2011) 479–482

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Short communication

Optimal temperature for growth and condition of an endemic subtropical anemonefish

Kelvin J.W. Rushworth, Stephen D.A. Smith, Kenneth L. Cowden, Steven W. Purcell *

National Marine Science Centre, Southern Cross University, PO Box 4321, Coffs Harbour NSW 2450, Australia

ARTICLE INFO

Article history:
Received 8 November 2010
Received in revised form 1 June 2011
Accepted 1 June 2011
Available online 13 June 2011

Keywords:
Aquarium trade
Climate change
Coral reef fish
Distribution
Modelling
Ornamental

ABSTRACT

The wide-band anemonefish (*Amphiprion latezonatus*), a subtropical endemic of Australia, has strong potential as a cultured ornamental. We investigated the effect of seawater temperature (19–29 °C) on hatchery-reared juveniles over two months. Optimum temperatures for highest specific growth rate (SGR) and condition factor (*K*) were modelled using nonlinear regression. Growth rate increased with temperature to a maximum at 22–23 °C then declined. Optimum SGR, at 22.4 °C, was 0.91% day^{-1}. The condition of juveniles displayed a similar trend but became increasingly variable within groups at higher temperatures and a significant optimum was not elucidated. This finding suggests that dominance behaviour accentuates with increasing temperature. Optimising the temperature for rearing juvenile *A. latezonatus* will improve efficiency of ornamental mariculture. Especially high rises in sea temperatures predicted for its geographical range are likely to impact the growth in juveniles of this species. Given the relative ease of juvenile culture and its apparent sensitivity to variation in sea temperatures, *A. latezonatus* should serve as a useful indicator species for studies on climate change impacts.

1. Introduction

Subtropical reef fishes may be especially vulnerable to rising sea temperatures. They can be lucrative ornamentals in the aquarium trade; however, mariculture technology for many species is, as yet, nascent (Moorhead and Chaoshu, 2010; Olivotto et al., 2010). *Amphiprion latezonatus* is an endemic anemonefish species with a distribution limited to 4° of latitude within the subtropics of eastern Australia. This region experiences broad spatial and temporal variations in seawater temperatures (Malcolm et al., 2011) and is a climate change 'hot spot' in the Southern Hemisphere, where future seawater temperatures are predicted to rise at a much faster rate than the global average (Poloczanska et al., 2007). This species is highly valued in the ornamental aquarium trade, commanding prices of up to US $600 per pair (3reef forum, 2009; RareClownfish forum, 2006). A recent initiative to produce commercial quantities of *A. latezonatus* at the National Marine Science Centre (NMSC), Australia, highlighted the need to further optimise hatchery and rearing technology.

For the rearing of juvenile ornamentals, optimum temperature should be established as it is one of the most important environmental parameters for teleost growth (Jobling, 1996). Up to a species-specific maximum, fish growth rates will accelerate with increasing temperature, after which they sharply decline (Fielder et al., 2005; Jobling, 1996). Identifying this optimum is a prerequisite for examining the effect on growth of other variables such as stocking density and nutritional requirements. For successful commercial production of ornamentals, mariculture must produce fish that are in the best physical condition as this, along with colouration, most influences consumer choice (Avella et al., 2007).

This study aimed to identify a discrete temperature range at which optimum growth rates and condition of juvenile *A. latezonatus* may be achieved. This species is found in association with host anemones *Heteractis crispa* and *Entacmea quadricolor* on offshore subtropical reefs in waters varying from 17 to 29 °C. We employed a regression-based design to model responses in the growth and condition of juveniles over experimentally controlled temperatures between 19 and 29 °C. This approach also provides insights into impacts of rapid climate change on this endemic species.

2. Materials and methods

2.1. Production of juveniles

Two weeks prior to the commencement of the experiment, juvenile *A. latezonatus* were cultured at the NMSC, Coffs Harbour, Australia, using methods modified from Hoff (1996). *A. latezonatus* has been observed to spawn in the wild under a wide variety of sea temperatures (Richardson, 1996). Incubation of eggs and early larval rearing were at 24 °C as pilot studies showed this temperature yielded a high survival rate. All juveniles were from a single spawning of a wild-caught broodstock pair and had been held together prior to the

* Corresponding author at: National Marine Science Centre, P.O. Box 4321, Coffs Harbour NSW 2450, Australia. Tel.: +61 2 6648 3906; fax: +61 2 6651 6580.
E-mail addresses: steven.w.purcell@gmail.com, spurcell@nmsc.edu.au (S.W. Purcell).

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doi:10.1016/j.aquaculture.2011.06.004

experiment. While this had the benefit of standardising the environmental and nutritional history of the experimental fish, we acknowledge that the parentage may have reduced the genetic variability amongst them. 150 F_1 juveniles were held in a 300-L holding system supplied with sand-filtered flow-through seawater at ambient conditions prior to commencing the experiment.

2.2. Experimental design

The flow-through experimental system consisted of 15×8.5-L polyethylene tanks situated in a 220-L water bath, arranged in 5 rows of three tanks with treatments dispersed randomly. The water bath was maintained at approximately 18 °C with a 1-hp chiller and recirculation pump. Three 60-L header tanks were used to precondition sea water closer to the desired temperature and each supplied five tanks. Multiple 100 to 300-Watt heaters were used to thermostatically maintain the desired temperature within each header tank, and float-valves were used to regulate the supply of fresh seawater. All experimental tanks were supplied with a continuous flow of thermally preconditioned seawater, receiving 5 to 8.5 exchanges of total volume daily. Each tank contained a 100-Watt heater placed in a length of 25-mm PVC pipe with an air-diffuser stone mounted in the base. This thermostatically ensured homogeneous water temperature through consistent water movement and maintained dissolved oxygen levels. Excess water from tanks overflowed into the water bath, which in turn overflowed to waste.

Water quality was maintained by the use of flow-through seawater supplied by the seawater system at the NMSC, which is drawn from a beach intake and pre-filtered to 30 μm. A photoperiod of 12 h light:12 h dark was controlled by timer switch to four fluorescent lights, 1.6 m above the tanks.

2.3. Experimental procedures

The growth response of juvenile *A. latezonatus* to varied temperatures was tested over a 60-day period. Fifteen groups of eight individual fish were prepared haphazardly, while attempting to homogenise average size and variance among groups; mean weight of each fish within groups was 514±16 mg; range: 416–614 mg. The groups were randomly allocated to the experimental tanks then, over 3 days, they were progressively brought to their treatment temperatures which ranged from 19 to 29 °C. The remaining juvenile fish were left in the holding system as possible replacements. The stocking density was restricted by the limited number of juveniles available.

At the outset, and then once a month, without the use of anaesthetic all fish were briefly blotted on a damp paper towel and weighed on an analytical electronic balance to ±0.1 mg then immediately returned to their tank. The growth measure at 1 month allowed us to examine whether growth rates, or the optimum temperature for growth, varied over the course of the experiment. Treatment temperature was checked twice per day and all fish were fed to satiation with a 0.8 mm sinking-pellet aquaculture diet for carnivorous fish (Gemma PG, Skretting; nutrient composition: 56% protein, 18% oil, 10% ash), which was readily consumed. Satiation was determined by providing sufficient food so that a small amount was left on the bottom of the tank after each feeding. All tanks were syphoned clean every second or third day, as required.

Inspection of the tanks at every feeding allowed us to remove dead fish and replace them with similar-sized acclimated fish, in order to maintain density. We used replacement fish with unique banding patterns, or fin clipped them on the upper caudal fin, so that they could be identified later and excluded from final data analyses. This ensured all tank means were derived only from original fish.

At the conclusion of the experiment, each fish was weighed again, as described, and was photographed against graph paper to provide a scaled, high resolution image. Using Image Pro Discovery software, the total length of each individual fish was determined to ±0.1 mm by adding the measurements of the length from the anterior point of the mouth to a point on the caudal peduncle and the length from that point to the end of the caudal fin. This procedure circumvented bias in the measurement of fish length due to curvature of the caudal fins at the time of the photo because fish were not anaesthetised.

The temperature in each tank varied on average by ±0.26 °C (SD) of its mean across 114 measurement events during the experiment. The majority of fish appeared healthy and fed well over the entire study. The 24.5 °C tank was removed from the study after a system malfunction caused multiple mortalities. Thirteen other mortalities were recorded among other tanks over the duration of the experiment; nine due to system faults or animals jumping out of tanks and four were unexplained and unrelated to treatments or months. Survival was therefore not analysed as a response variable.

2.4. Statistical analyses

The initial weights, and those at one month and the conclusion of the experiment (two months), were measured and used to calculate the mean specific growth rate (SGR) (% weight gain day^{-1}) of all treatments. Condition factor (K) was also calculated, as outlined below:

$$SGR = \left(Ln\frac{\left(\frac{Wf}{Wi}\right)}{Duration} \right) \times 100, \text{and } K = \left(\frac{W}{L^3}\right) \times 100,$$

where W_i is the weight initial (g), W_f is the final weight (g), L is the fork length of the fish (mm), and Duration is measured in days.

SGR was selected as the most appropriate growth measure as it is relative to initial body weight and this standardisation best mitigates the effects of varying sizes of fish (Hopkins, 1992). Mean initial weight and the coefficient of variation (CV) of each treatment were regressed against final growth rates to determine if initial size of fish, or variation, had biassed the outcomes of the experiment.

Temperature was treated as a continuous independent variable, and SGR and K as dependant variables. For month 1 (1–30 days), month 2 (31–60 days) and overall (1–60 days), the linear and nonlinear regression models for the relationship between the response variables and mean temperature were determined using DataFit 9 software, which fitted the data using 298 standard functions. The sum-of-squares statistics for models that showed best fit with successively increasing numbers of parameters were used to calculate Akaike Information Criterion (AIC) values (Burnham and Anderson, 1998). These models were then compared and the most appropriate function selected based on lowest AIC score. The growth (SGR) rate $\mathbf{y_i}$ was modelled across experimental temperatures $\mathbf{x_i}$ by the equation:

$$\mathbf{y_i} = \frac{\mathbf{a}}{1 + \mathbf{bx_i} + \mathbf{cx_i^2}} + \boldsymbol{\varepsilon_i},$$

where **a**, **b** and **c** are unknown to be determined and $\mathbf{i} = \mathbf{1},\ldots,\mathbf{14}$. The errors ε_i were assumed to be normally distributed. The estimated values of **a**, **b** and **c** are 0.0202 ($P<0.05$), −0.0873 ($P<0.001$), 0.00195 ($P<0.001$), respectively.

We chose not to use a factorial approach for the experimental design or analysis, as this would have required assigning arbitrarily temperatures as levels within the experimental factor and using poor replication within each level due to limited numbers of replicate animals. Such an approach would have precluded identifying a specific optimum temperature for growth, and would not have accounted for actual temperatures in aquaria due to imprecision of heater thermostats, which would introduce experimental error in a factorial design.