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Aquaculture 136 (1995) 141–147

Aquaculture

Hatchery study of the effects of temperature on eggs and yolksac larvae of the Nassau grouper *Epinephelus striatus*

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Accepted 30 April 1995

Abstract

The effects of temperature on eggs and yolksac larvae of the Nassau grouper (*Epinephelus striatus*) were examined under controlled, hatchery conditions. Artificially-fertilized eggs, obtained by induced spawning of captive adults, were stocked (36 eggs per l) into 15 500 l cylindroconical indoor tanks at temperatures of 26, 28 and 30°C, with five tanks per treatment. A salinity of 37 g l⁻¹ and a photoperiod of 12 L: 12 D were maintained. Incubation time to hatching was inversely related to temperature, decreasing from 24.9 h post-fertilization (p.f.) at 26°C to 20.4 h p.f. at 30°C, but hatching success (avg. = 82.5%) did not vary with temperature. Survival of pre-feeding larvae declined more rapidly at the higher temperatures to 91.4, 80.7 and 42.2% by Day 1 p.h. at 26, 28 and 30°C, respectively, indicating that early survival was influenced by factors unrelated to feeding. Development time to the first-feeding stage was inversely related to temperature, decreasing from 86 h p.f. (2.54 d p.h.) at 26°C to 71 h p.f. (2.11 d p.h.) at 30°C. Lower temperatures delayed starvation, with survival falling to 32.3, 9.3 and 1.2% by Day 4 p.h. at 26, 28 and 30°C, respectively. A temperature of 26°C is deemed advantageous to higher temperatures for incubating eggs and for rearing first-feeding larvae, although even lower temperatures may be feasible. Temperatures within an ecological range can markedly influence development rates of *E. striatus* eggs and yolksac larvae and hence, dispersal potential, first-feeding and survival in the field.

Keywords: *Epinephelus striatus*; Thermal control

1. Introduction

The Nassau grouper (*Epinephelus striatus*) is a finfish species of considerable commercial importance in many areas of the Caribbean and tropical and subtropical western Atlantic,

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but over-fishing and environmental degradation are depleting natural populations (Sadovy, 1989; Sadovy, 1990). Relatively little is known about the biological and ecological requirements of this species as a basis for fishery management, commercial cultivation and replenishing natural populations.

Tucker et al. (1991) successfully induced spawning in wild-caught *E. striatus* by injection of human chorionic gonadotropin (HCG) and described the development of eggs and early larvae (Powell and Tucker, 1992). Recently, hormone-induced spawnings of a captive *E. striatus* broodstock at the Caribbean Marine Research Center on Lee Stocking Island, Bahamas (Head et al., 1994; Watanabe et al., unpublished data) have provided an opportunity for experimental study of the environmental and nutritional requirements of the early-life history stages of this species under controlled conditions.

Grouper larvae are extremely sensitive to environmental stressors (Hussain and Higuchi, 1980; Tseng and Chan, 1985), particularly temperature (Lim, 1993). In this preliminary study, the effects of temperature on hatching success of *E. striatus* eggs and on survival of yolk sac larvae through first-feeding are examined as a basis for hatchery methods and to evaluate the susceptibility of these stages to thermal control in nature.

2. Materials and methods

This study was conducted 6–16 December 1992 at the Caribbean Marine Research Center on Lee Stocking Island, Exuma Cays, Bahamas (23° 46' N, 76° 6' W). Artificially-fertilized eggs of Nassau grouper (*E. striatus*) were obtained by induced spawning of captive broodstock maintained in 15 m³ flow-through seawater (37 g l⁻¹) tanks. To induce spawning, a female (6.02 kg body wt.) with an average oocyte diameter of 522 µm was injected with HCG at a dose of 1000 IU kg⁻¹ body weight, followed 24 h later by a second injection at a dose of 500 IU kg⁻¹ body weight. Strip-spawning at 18 h following the second injection produced 2.17 × 10⁶ eggs with a fertilization rate of 92.3%. Eggs were incubated in a 500 l tank supplied with seawater at 26°C and with aeration. Approximately 2 h post-fertilization, air flow was discontinued and buoyant, fertilized eggs skimmed for use in the experiment.

To determine the effect of temperature on eggs and yolk sac larvae, fertilized eggs were stocked (36 eggs per l) into 15 500 l cylindroconical tanks supplied with seawater at 26°C. Aeration (approx. 0.1–0.2 l min⁻¹) was supplied from a diffusion ring around the base of a central standpipe. Using immersion heaters (300 W), temperatures were adjusted at a rate of 1°C h⁻¹ to final treatment temperatures of 26, 28 and 30°C, with five tanks maintained at each treatment. Light was supplied from fluorescent sources (400–600 lx) between 06:00 and 18:00 h to provide a 12 L: 12 D photoperiod.

Incubation time to hatching was recorded for each tank and hatching success determined using volumetric methods as the percentage of larvae hatched from incubated eggs.

Beginning on Day 2 post-hatching (Day 2 p.h.), four tanks of each treatment were stocked daily with S-type Hawaiian strain rotifers (*Brachionus plicatilis*) fed phytoplankton (*Nanochloropsis oculata*) and Baker's yeast (1:1) at a density of 20 individuals per ml. *N. oculata* was added at 500 × 10³ cells ml⁻¹. One tank from each treatment was not fed.

Using volumetric methods, survival of larvae in each tank was monitored daily. Time of first-feeding was estimated by sampling ten individuals from each tank at 4 h intervals

during the first 3 days after hatching and at 8 h intervals for an additional 4 days. Larvae were anaesthetized ($60\text{--}100\text{ mg l}^{-1}$ MS-222) then fixed in 2.5% glutaraldehyde and stored at 5°C until examination. Using a dissecting microscope, first-feeding larvae were identified as those having functional (totally pigmented) eyes, mouth and gut.

Static conditions were maintained until Day 2 p.h. Water exchange was approximately 25%/d from Day 3 to Day 4 p.h. Temperature, salinity, and dissolved oxygen were monitored daily, while pH and ammonia were measured on alternate days. Average daily water temperatures during the experimental period were 26.1 ± 0.1 , 27.7 ± 0.1 and $29.5 \pm 0.2^\circ\text{C}$ at the treatment levels of 26, 28 and 30°C , respectively. Salinity ($36.8\text{--}37.3\text{ g l}^{-1}$, avg. = 37 g l^{-1}), total ammonia nitrogen ($0.206\text{--}0.211\text{ mg l}^{-1}$; avg. = 0.209 mg l^{-1}) and pH ($8.02\text{--}8.05$; avg. = 8.04) did not differ significantly among treatments. Average dissolved oxygen increased ($P < 0.001$) with decreasing temperature from 5.26 mg l^{-1} at 30°C to 5.76 mg l^{-1} at 26°C .

Incubation time, hatching success, time to first-feeding and daily survival were compared among treatments by analysis of variance (ANOVA) or Kruskal–Wallis test. For percentage data, arcsin transformation was performed before analysis.

3. Results

Incubation time to first-hatching decreased ($P < 0.001$) with increasing temperature from 24.9 h post-fertilization (p.f.) at 26°C to 20.4 h p.f. at 30°C (Table 1). A curvilinear relationship between temperature and incubation time was evident (Fig. 1). Under all temperatures, hatching occurred over an average duration of 1.72–2.12 h, and incubation time to complete hatching ranged from 26.9 h p.f. at 26°C to 22.1 h p.f. at 30°C (Table 1).

Table 1

Effects of temperature on incubation time, hatching success, time to first-feeding and survival in *E. striatus*^a

| | Temperature ($^\circ\text{C}$) | | |
|------------------------|----------------------------------|----------------|-----------------|
| | 26 | 28 | 30 |
| First-hatching | 24.9 ± 0.2 | 21.6 ± 0.3 | 20.4 ± 0.2 |
| (h post-fertilization) | (24.4–25.7) | (21.1–22.3) | (20.0–20.9) |
| Complete hatching | 26.9 ± 0.3 | 23.0 ± 0.7 | 22.1 ± 0.03 |
| (h post-fertilization) | (26.2–27.8) | (22.2–25.5) | (22.0–22.2) |
| Hatching success | 82.1 ± 5.7 | 88.2 ± 5.4 | 77.2 ± 7.1 |
| (%) | (61.7–96.1) | (70.8–100) | (54.2–97.8) |
| First-feeding | 86 | 81 ± 1 | 71 ± 1 |
| (h post-fertilization) | (86) | (78–82) | (70–74) |
| Survival (%) | | | |
| Day 2 p.h. | 65.4 ± 8.1 | 44.3 ± 9.3 | 42.0 ± 6.9 |
| | (50.3–79.9) | (25.5–62.8) | (23.3–56.4) |
| Day p.h. | 32.3 ± 6.7 | 9.3 ± 2.0 | 1.2 ± 0.3 |
| | (19.9–47.1) | (3.4–12.9) | (0.7–2.2) |

^aValues represent means \pm s.e. (range); $n = 4\text{--}5$.

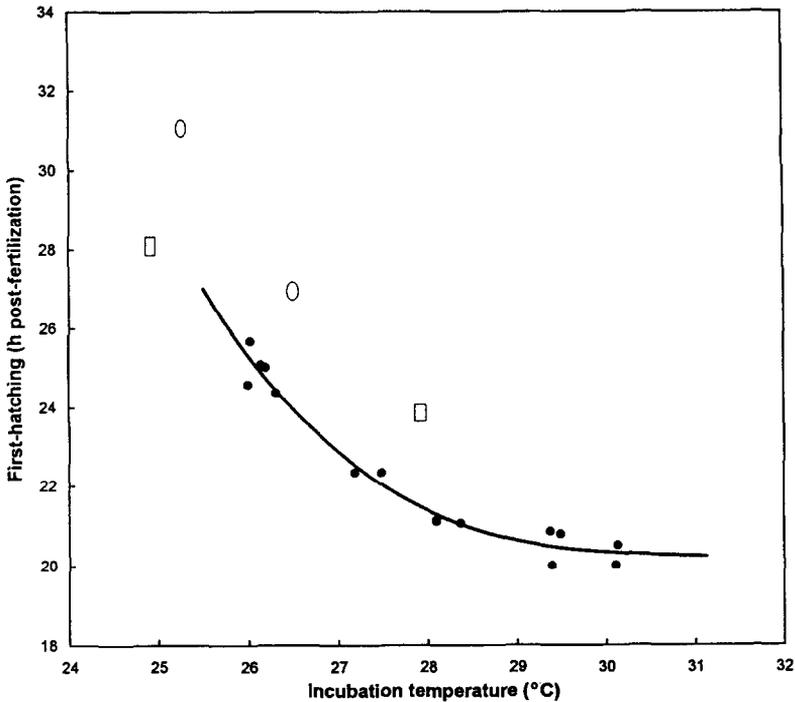


Fig. 1. Relationship between incubation time to first-hatching (y) and temperature (x) in artificially-fertilized eggs of *E. striatus* incubated in 500 l cylindroconical tanks at 37 g l^{-1} . Regression analysis defined this relationship as follows: $y = -0.0438x^3 + 4.0422x^2 - 124.38x + 1296.9$ ($n = 15$; $r^2 = 0.97$). Two data points at 28°C are superimposed. Data from Colin et al. (1987) (ellipses) and Tucker et al. (1991) (rectangles) are shown for comparison and were not used in the analysis.

Hatching success averaged 82.5%, with no significant ($P > 0.05$) differences observed among treatments (Table 1).

Time to first-feeding also decreased with increasing temperature from 86 h p.f. (2.54 days post-hatching) at 26°C to 71 h p.f. (2.11 days post-hatching) at 30°C (Table 1), and this trend could be described by a highly significant ($P < 0.01$) negative regression (Fig. 2). At all temperatures, only remnants of the yolksac were observed by the first-feeding stage.

Under all temperatures, considerable mortalities were observed in yolksac larvae, with survival falling more rapidly at the higher temperatures to 65.4, 44.3 and 42.0% by Day 2 p.h. at 26, 28 and 30°C , respectively (Table 1). Despite the availability of S-type rotifers to larvae from Day 2 p.h., survival declined to 32.3%, 9.3% and 1.2% by Day 4 p.h. at 26, 28 and 30°C , respectively, with significant ($P < 0.01$) differences observed among treatments.

At each temperature, survival of unfed larvae followed a trend similar to that of fed larvae, declining more rapidly at higher temperatures to 24.8%, 6.6% and 0.3% by Day 4 p.h. at 26, 28 and 30°C , respectively.

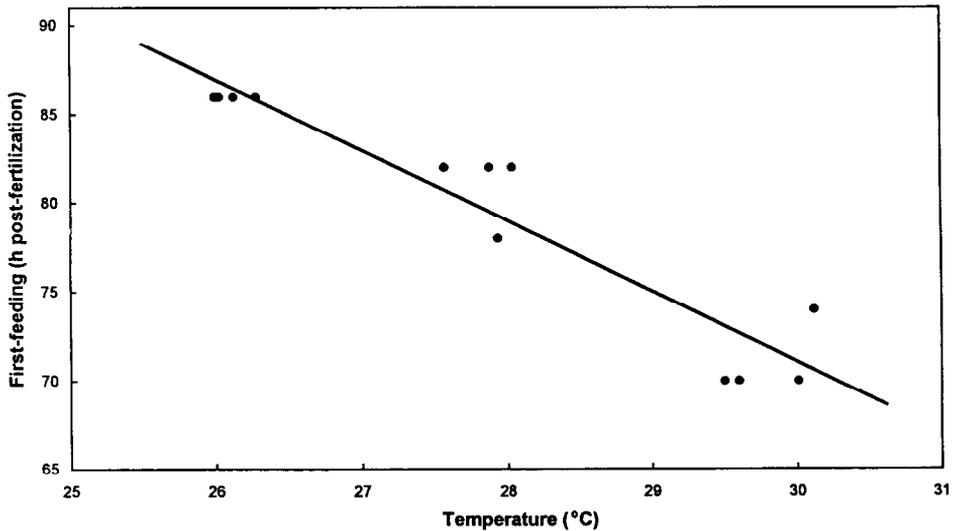


Fig. 2. Relationship between time to first-feeding (y) and temperature (x) in yolksac larvae of *E. striatus* reared in 500 l cylindroconical tanks at 37 g l^{-1} . Regression analysis defined this relationship as follows: $y = -3.97x + 190.3$ ($n = 12$; $r^2 = 0.95$, $P < 0.01$).

4. Discussion

An inverse curvilinear relationship between incubation time and temperature, as observed in *E. striatus* in this study, has been previously reported for other tropical marine finfish, including *Caranx mate* (Santerre, 1976), *Polydactylus sexfilis* (Santerre and May, 1977) and *Mugil cephalus* (Walsh et al., 1991). This curvilinear trend, along with data reported by other workers (Colin et al., 1987; Powell and Tucker, 1992; Fig. 1), suggest that egg incubation times in *E. striatus* may vary markedly with sea temperature within ranges (21–27°C) (Colin, 1992; Wicklund et al., 1993) encountered during the natural reproductive period for *E. striatus* in the Bahamas (Smith, 1972; Colin, 1992; Shenker et al., 1993). Incubation times reported by previous workers (Colin et al., 1987; Powell and Tucker, 1992) for artificially-fertilized eggs of *E. striatus* (Fig. 1) appear generally higher than those observed in this study, which represent time to first-hatching. However, other environmental conditions, including light intensity, aeration and salinity can influence incubation time in this species (Watanabe et al., unpublished data).

Hatching success of *E. striatus* eggs was high (avg. = 82.5%) at all temperatures within the range of 26–30°C, but mortality of yolksac larvae was accelerated at higher temperatures. High hatching success, therefore, may not necessarily indicate temperature conditions conducive to larval survival.

As evidenced by considerable mortality of pre-feeding stage *E. striatus* larvae in this study, early survival was influenced by conditions other than the availability of prey. As these effects were accelerated by increasing temperature, they were apparently linked to metabolism. Biochemical quality of eggs as related to conditions of broodstock husbandry can influence larval survival (Watanabe et al., 1984). Other environmental factors such as

aeration (and circulation) and light intensity may also influence metabolism and survival of yolk sac larvae (Watanabe et al., unpublished data).

Under all treatment temperatures, non-sieved S-type rotifers were inadequate for first-feeding *E. striatus* and did not appreciably improve survival compared to unfed larvae. The large size (mean lorica length = 161 μm , range = 50–264 μm) of these rotifers make them difficult prey for first-feeding grouper larvae which have a relatively small mouth (Hussain and Higuchi, 1980; Tseng and Chan, 1985; Fukuhara, 1989; Lim, 1993). Recent studies have confirmed that small S-type rotifers (mean lorica length = 117 μm , range = 50–200 μm), selected by sieving, produce higher survival than non-sieved rotifers in first-feeding *E. striatus* (Ellis et al., unpublished data).

In *E. striatus*, time to first-feeding at 26°C averaged 15 h later than at 30°C, presumably due to a slower rate of metabolism and development at the lower temperature (Houde, 1974; Santerre, 1976; Buckley et al., 1990). Thus, lower temperatures delayed exhaustion of yolk reserves and starvation as previously observed in other marine finfish larvae (May, 1974; Holt et al., 1981; Gadomski and Caddell, 1991). A temperature of 26°C may therefore be advantageous to higher temperatures for incubating eggs and for rearing first-feeding *E. striatus* when prey concentrations are limiting, although even lower temperatures may be feasible.

Results of this study demonstrate that variations in water temperatures within an ecological range can markedly influence development rates and survival of pre-feeding larvae. This suggests that, eggs and yolk sac larvae of *E. striatus* are susceptible to thermal control in nature. Hence, temporal and spatial heterogeneity in sea temperatures should be considered along with hydrodynamic conditions in estimating source and dispersal potential of eggs and early larvae of *E. striatus* observed in the field.

Acknowledgements

This study was supported by The Oceanic Institute (Hawaii), the George F. Baker Trust and the John H. Perry Foundation, Inc. We thank Kenneth Liu, Garrett Miyamoto and Arturo Rodriguez-Cobacho for technical assistance, Christopher Kelley, Paul Bienfang, William Head and Robert Wicklund for helpful advice.

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