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The Effect of Variable Incubation Temperatures on Hatchability and Survival of Goldlined Seabream, *Rhabdosargus sarba* (Forsskål, 1775) Larvae

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Abstract**—**The effect of varying holding temperature on hatching success, occurrence of deformities and mortality rates were investigated for goldlined seabream eggs. Wild broodstock (600 g) were stocked at a 2:1 male-female ratio in a 2 m^3 fiberglass tank supplied with filtered seawater (37 g L^{-1} salinity, temp. range 24±0.5 ^oC [day] and 22±1 ^oC [night], DO_2 in excess of 5.0mg L^{-1}). Females were injected with 200 IU kg^{-1} HCG between 08.00 and 10.00 h and returned to tanks to spawn following which eggs were collected by hand using a 100µm net. Fertilized eggs at the gastrulation stage (120 L^{-1}) were randomly placed into one of 12 experimental 6 L aerated (DO_2 5 mg L^{-1}) plastic containers with water temperatures maintained at 24±0.5 ^oC (ambient), 26±0.5 ^oC, 28± 0.5 ^oC and 30±0.5 ^oC using thermostats. Each treatment was undertaken in triplicate using a 12:12 photophase:scotophase photoperiod. No differences were recorded between eggs reared at 24 and 26 ^oC with respect to viability, deformity, mortality or unhatched egg rates. Increasing temperature reduced the number of viable eggs with those at 30 ^oC returning poorest performance ($P < 0.05$). Mortality levels were lowest for eggs incubated at 24 and 26 ^oC. The greatest level of deformities recorded was that for eggs reared at 28 ^oC.

Keywords**—**Goldlined seabream, Oman, *R. sarba*, deformities.

I. INTRODUCTION

IN many countries the rapid development of aquaculture has been heavily dependent upon the introduction of non-indigenous species. A good example of this is seen with Atlantic salmon farming in Chile. First introduced in 1979, the aquaculture of Atlantic salmon attracted a high level of capital investment and today, Chile is the world's second largest producer of Atlantic salmon with harvests of around 700,000 tons in 2007 [1]. However, along with the positive economic and technological benefits that have attended the growth of salmon culture, there have been reports of negative impacts associated with the industry. These include the usual issues surrounding latent negative environmental effects of intensive aquaculture production [2], [3], such as increased occurrence of floating debris [4], through to the potential displacement of native species via competition for food and space [5]. These, and other consequences, are by no means unique to salmon or fish farming and the published literature teaches that identical problems have been encountered following species introductions around the globe. Most sinister have been the associated transfer of disease with imported eggs, larvae and broodstock [6], [7], [8].

Even given the aforementioned risks, many countries still permit the importation of alien species to support aquaculture development, sometimes with the encouragement of international organizations. Illicit importation of non-native aquaculture plants and animals by producers has also been reported in some countries. In the past many aquaculture research and development projects undertaken in the Sultanate of Oman employed exotic species such as blue, Nile and Mozambique tilapias [9], [10], [11] and gilthead sea bream and sea bass. Today however, a stated goal of the country's Ministry of Fisheries Wealth is to cultivate a tradition of commercial aquaculture based on the farming of indigenous species. This position has been taken in efforts to avoid the many problems connected with imported exotics (*vide supra*) and, due to the embryonic nature of the commercial mariculture industry in Oman this goal is attainable. Moreover, many indigenous plants and animals offer the same or similar production characteristics and qualities as more established cultivated warm-water species (see:[12],[13], [14], [15] for biodiversity listings). An added advantage of using the "indigenous strategy" is that native species may command higher prices in local and regional markets due to product recognition. Before anticipating an industry based on native species however, it is especially important to develop intensive hatchery procedures. This first step is important since determinations can be made regarding the application of existing technologies to the production of local species. Such information assists also in the more rapid expansion of industry.

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The goldlined seabream, silver bream, or tarwhine, *Rhabdosargus sarba*, is an emergent aquaculture species of growing importance to the Western Pacific Rim [16] and has been cultured successfully in India, Japan and France [17]. The species has high potential for both intensive aquaculture and restocking programmes. However, depending on geographical location, differences have been reported in terms of water quality parameters linked to hatchery and natural spawning [18], [19], [20]. Clearly, the most favourable hatchery production of goldlined seabream will rely upon establishing optimal water quality parameters the most important of which are water temperature and salinity, each of which are known to influence hatchability, embryonic development and successive survival and growth potential of marine fish [21], [22]. Reference [22] reported that if incubation conditions are not kept within the optimal range affect the cellular symmetry. Reference [22] suggested that unfavourable conditions during egg incubation negatively influence embryonic development especially during the gastrulation stage.

Since salinity in the Sea of Oman is naturally high due to the influence of the outflow from the Arabian Gulf, the aim of the present research was to examine the effect of water temperature, on the hatchability of the glodlined seabream eggs at ambient salinity.

II. Materials and Methods

Broodstock of *R. sarba* (mean wt 600 g), were collected using traditional traps from a depth of 10 m at fishing grounds located near Muscat, Oman. Fish were transported to Sultan Qaboos University Mariculture Unit, Al-Hail where they were placed into one of three 2000 L fiberglass tanks at a female to male ratio of 2:1. Broodstock tanks were supplied with water derived from a marine well at a salinity of 37g L^{-1} and temperature of 29 ^{o}C. Well water was stored in a 20 m^3 HDPE header tank and prior to use was filtered using sand filter and 5µm particle filter and then passed through a UV unit. The filtered water was pumped to a 20 m^3 concert tank to enable a reduction in water temperature to about 25 ^{o}C overnight. The broodstock tanks were supplied with seawater during the early morning at a rate of 50 L min^{-1}. Dissolved oxygen levels were maintained in excess of 5.0mg L^{-1} using continuous aeration. Temperature averaged 24±0.5 ^{o}C during the day and dropped to 22±1 ^{o}C at night. During daylight hours the tank was shaded using aluminum sheets to reduce incident sunlight. Broodstock were fed fresh chopped cuttlefish and sardines until satiation.

Females were anesthetized with 20mgl^{-1} MS222 and injected with HCG (CHORULON®, Intervet- Lot number: A012A02) at a dose of 200 IU kg^{-1} between 08.00 and 10.00 h and returned to tanks. Following spawning, which generally occurred before sunset, eggs were collected by hand scooping using a 100µm net and transferred to a 50 L glass aquaria. Positively buoyant fertilized eggs were collected from the surface and rinsed with filtered seawater and then re-checked for fertilization. Fertilized eggs at the gastrulation stage were counted under a dissection microscope (Olympus, Tokyo 330549-SZ Japan), and randomly placed into experimental 6 L plastic containers at a density of 120 L^{-1}. Water temperatures were held at 24±0.5 ^{o}C (ambient), 26±0.5 ^{o}C, 28± 0.5 ^{o}C and 30±0.5 ^{o}C. using thermostatically controlled heaters (Resun Aquarium heater 50w (Model: SUNLIKE-50, China). Egg fertilization and image capturing was performed using Digital Biological Microscope (Model: DMWB1-223). Mild aeration, via a diffusion stone, maintained dissolved oxygen at 5 mg L^{-1}. Each temperature regime was carried out in triplicate. A 12:12 photophase:scotophase photoperiod was employed. Viable larvae, deformed animals, mortalities and unhatched eggs were enumerated at study end.

To test for normal distribution the Anderson-Darling normality test was applied. Data were tested for correlation using the Pearson correlation test and means between samples compared using one-way analysis of variance (ANOVA). The differences between means were compared using Tukey's significant difference test at a probability level of $P<0.05$. Statistical analysis was performed using SPSS release 10 [23].

III. Results

Table 1 summarizes the effect of rearing temperature on hatchability, mortality rate and deformity for goldlined seabream. No differences were observed between eggs reared at 24 and 26 ^{o}C with respect to viability of eggs, the rate of deformity recorded, mortality or unhatched egg rates. As temperature increased however, the number of viable eggs decreased, with eggs maintained at 30 ^{o}C returning poorest performance ($P < 0.05$). Mortality levels were similar between the 24 and 26 ^{o}C groups which differed ($P < 0.05$) from the 28 and 30 ^{o}C groups which returned higher mortality rates. The greatest level of deformities recorded was that for eggs reared at 28 ^{o}C (Table 1), which differed ($P < 0.05$) from all the groups.

With increasing temperature, time to hatch decreased ($P < 0.05$) and the rate of development of larvae was hastened, with the yolk sac becoming depleted more rapidly also. In some deformed animals, discrete pigmentation was observed in the eye. A common feature of larvae reared at 30 ^{o}C was the appearance of double tailing (Fig. 1) and by 25 h post-hatch yolk sac nor oil globule could be observed. In larval goldlined seabream reared at 28 ^{o}C a scoliosis-like deformity was often seen and this was accompanied by circular swimming motions. Nevertheless, both yolk sac and oil globule were apparent in such larvae (Fig. 2).

TABLE I THE EFFECT OF VARYING INCUBATION TEMPERATURE ON HATCHABILITY AND THE OCCURRENCE OF MORTALITIES AND DEFORMITIES IN GOLDLINED SEABREAM. VIABLE HATCH = NORMAL SWIMMING LARVAE, NON-VIABLE HATCHED = DEFORMED LARVAE. MEAN VALUES THAT ARE NOT SIGNIFICANTLY DIFFERENT WITHIN A COLUMN (P > 0.05) SHARE COMMON SUPERSCRIPTS

Temp. ^{o}C	Viable hatch	Non-viable hatch	Mortalities	Unhatched
24	$105.0±14.7^{a}$	0^{a}	$1.0±0.0^{a}$	$14.0±14.7^{a}$
26	$110.0±10.0^{a}$	$4.0±1.2^{a}$	$2.0±1.7^{a}$	$10.0±5.2^{a}$
28	$62.3±41.5^{ab}$	$40.0±8.5^{b}$	$11.7±7.5^{ab}$	$29.7±31^{a}$
30	$17.7±16.6^{b}$	0^{a}	$9.3±11.4^{b}$	$92.3±24.1^{b}$

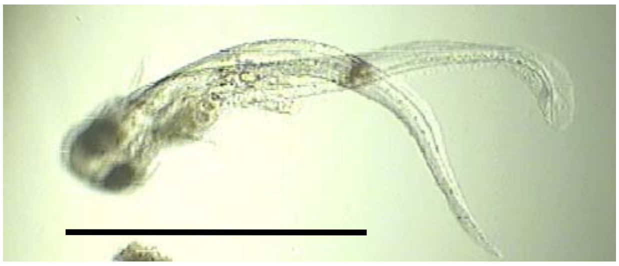

Fig. 1 A deformed *R. sarba* larvae at 25 hours post hatched from 30 °C treatment. Neither yolk sac nor oil globule is visible. The larvae was double-tailed, a commonly observed deformity. Scale bar 1mm, mag. ×4.

Fig. 2 A deformed *R. sarba* larvae at 18 hours post-hatch from the 28 °C treatment. The yolk sac and oil globule remain visible. This larvae which apparently suffered from a scoliosis-like deformity, swam in a circular pattern. Scale bar 1mm, mag. ×4.

IV. Discussion

Temperature considered a key factor affecting the development of embryo during incubation [24], therefore, a temperature of 24 and 26ºC found to be suitable for incubation of *R. sarba* eggs as it give higher viable hatch. Reference [25] reported that the mean sea-surface temperature during the spawning season of *R. sarba* in the Sea of Oman was 24 ºC. Reference [25] observed high mortality of striped trumpeter (*Latris lineata*) due to abnormality at a temperature that was higher than the mean sea-surface temperature during the spawning season of the species.

Reference [27] showed that eggs, yolksac larvae and onset of first feeding of the Nassau grouper (*Epinephelus striatus*) was affected by temperature, the authors stated that the number of viable hatch increases at temperature of 26ºC. Reference [18] recorded spawning activity in goldlined seabream at temperatures varying between 13.8 and 23.5 °C over a 96 day period and survival rate ranged between 7 and 43% at 19.4 ºC indicating that lower temperature is feasible as it was demonstrated in this study at a temperature of 24 ºC.

The deformed larvae of goldlined seabream were observed at higher temperature from the optimal. Reference [28] stated that about 27% of sea bream (*Sparus aurata* L.) larvae at hatching showed different types of axial deformations that were associated with notochord alterations during embryogenesis. Reference [28] demonstrated that from these deformed larvae about 22% died shortly after hatching, and 5% survived and reached juvenile and adult stages and this condition was attributed to unfavorable conditions including temperature during incubation. Similarly, Temperature found to have an effect on embryonic and larval development stages of yellow fin seabream (*Acanthopagrus latus*) [29].

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