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Effect of temperature on growth, survival and occurrence of skeletal deformity in the golden pompano *Trachinotus ovatus* larvae

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ABSTRACT

Golden pompano *Trachinotus ovatus* larvae were reared at 23, 26, 29 and 33°C from 3 day post-hatching (DPH) to 18 DPH. Growth, survival, RNA/DNA ratio, as well as jaw and skeleton deformities were used as evaluation criteria on fish performance. Growth of fish held at 29 and 33°C was significantly faster than those held at 23 and 26°C, while survival at 26 and 29°C was higher than those held at 23 and 33°C. Similarly, the RNA/DNA ratio was higher at 26 and 29°C than at 33°C. Jaw deformities significantly increased when the rearing temperature exceeded 29°C, while vertebral deformities were encountered more frequently at 33°C than at lower temperatures. Results of the study indicate that the temperature range of 26 to 29°C is appropriate while temperatures >33°C or <23°C can have adverse impact on rearing of golden pompano larvae.

Keywords: Deformities, Golden pompano, Growth performance, RNA/DNA ratio, Temperature, *Trachinotus ovatus*

Introduction

Temperature is a critical factor during the early development of fish larvae since it can regulate metabolism and feeding behaviour (Kestemont and Baras, 2001; Ma, 2014). In aquaculture, temperature directly influences the size of fish larvae during hatch, yolk absorption, growth, survival, feeding and digestion (Martinez-Palacios *et al*., 1996; Rombough, 1996; Fielder *et al*., 2005; Bustos *et al*., 2007; Jobling, 1997). Besides, several studies have demonstrated high mortality and abnormality in fish larvae due to inappropriate temperature (Lein *et al*., 1997; Ornsrud *et al*., 2004; Ludwig and Lochmann, 2009). Within the tolerance range for fish, increase of temperature accelerates ontogenetic development, but high temperatures may reduce fish survival. At high temperature, yolk-sac absorption of fish larvae is fast but the period of endogenous feeding is shortened (Dou *et al*., 2005; Bustos *et al*., 2007; Ma, 2014). Therefore, maintenance of appropriate temperature is essential to improve growth and survival of fish larvae in hatcheries.

Jaw and skeleton malformation is often associated with poor growth and low survival of fish larvae (Kihara *et al*., 2002; Ma and Qin, 2014). Furthermore, cultivation of fish with morphological deformities is a waste of time and effort as these fish have a poor marketable value (Negm *et al*., 2014). Jaw and skeleton malformations in cultured species such as striped trumpeter *Latris lineata* (Cobcroft *et al*., 2012), Senegal sole *Solea senegalensis* (Gavaia *et al*., 2002), gilthead sea bream *Sparus aurata* (Andrades *et al*., 1996; Prestinicola *et al*., 2013), Japanese eel *Anguilla japonica* (Okamura *et al*., 2011), and yellowtail kingfish *Seriola lalandi* (Cobcroft *et al*., 2004) have been frequently reported. Factors such as the rearing environment (*e.g.,* temperature, salinity, water current, dissolved oxygen, tank colour) (Koumoundouros *et al*., 1999; 2001; Hattori *et al*., 2004; Sfakianakis *et al*., 2004; Okamoto *et al*., 2009; Georgakopoulou *et al*., 2010; Owen *et al*., 2012), parasites and pesticides (Liang *et al*., 2012; Liu *et al*., 2012), and nutrient deficiency (Cahu *et al*., 2003a) have been attributed to be associated with morphological deformities.

Jaw malformations are also associated with poor feeding in many fish species (Suzuki *et al*., 2000; Cahu *et al*., 2003b; Mazurais *et al*., 2009; Sandel *et al*., 2010). Lein *et al*. (1997) observed that temperature was a primary factor triggering jaw development. At unsuitable temperature, significant deformities of gill-cover and skeleton have been reported in gilthead seabream

S. aurata (Georgakopoulou *et al*., 2010) and cranial deformities in European seabass, *Dicentrarchus labrax* (Georgakopoulou *et al.*, 2007). Golden pompano *Trachinotus ovatus* is a candidate species for aquaculture due to fast growth and suitability for cage culture. In golden pompano, over 33% of fish in the stocked population exhibited at least one type of malformation during the larval phase (Ma *et al.*, 2014c; Zheng *et al.*, 2014). Earlier studies have identified the type, position, and frequency of jaw and skeleton malformations in hatchery reared golden pompano larvae (Ma *et al.*, 2014c; Zheng *et al.*, 2014) but there are no reports on factors leading to occurrence of malformations. An attempt was made in this study to understand the influence of ambient temperature on the rearing performance and occurrence of skeletal malformation in the larvae of golden pompano.

Materials and methods

Fertilised eggs of golden pompano, belonging to the same batch were obtained from Lingshui, Hainan Province and transported to the Tropical Fisheries Research and Development Center, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Science, Xincun Town. Upon arrival, all eggs were transferred to 500 l incubators until hatching.

The experimental design included four constant temperatures 23, 26, 29, and 33^{O}C with three replicates each. On 2 day post-hatch (DPH), yolk sac larvae were acclimatised at each desired temperature for 5 h, and then stocked in 500-l fiberglass tanks at a density of 60 fish l^{-1}. All rearing tanks were supplied with filtered seawater with a 5 µm filter from the bottom of each tank with a daily water exchange rate of 200% tank volume. One air stone was used in each tank to maintain dissolved oxygen close to saturation and also to promote even distributions of microalgae, rotifers and *Artemia* nauplii. Light intensity was maintained at 2300 lx under a photoperiod of 13 l : 11D. Salinity was maintained at 33‰ throughout the experiment.

Rotifers (*Brachionus rotundiformis*) were provided to fish larvae from 2 DPH until 13 DPH, three times a day at a density of 10 rotifers ml^{-1}. Rotifers were cultured at 25.5^{O}C. Rotifers fed with microalgae (*Nannochloropsis* sp.) were enriched with DHA Protein Selco (INVE Aquaculture, Salt Lake City, UT, USA) before adding to larval rearing tanks. This DHA Selco product for rotifer enrichment has been reported to result in the highest survival in feeding Florida pompano larvae (*Trachinotus carolinus*) (Cavalin and Weirich, 2009). Instant microalgal paste (*Nanochloropsis* sp., Qingdao Hong Bang Biological Technology Co., Ltd, Qingdao, China) was also added in the larval fish rearing tanks to feed rotifers and also to create a green water system for fish larvae. *Artemia* nauplii enriched with DHA Protein Selco (INVE Aquaculture) were introduced to the rearing tank from 9 to 18 DPH at 5 nauplii ml^{-1}.

In each tank, 10 fish were sampled for size measurements at 1, 3, 5, 9, 12, 18 DPH. Fish were anaesthetised with AQUI-S® (New Zealand Ltd., Lower Hutt, New Zealand) and were measured in a stereo microscope with a micrometer at 10× magnification to the nearest 0.01 mm. Growth was estimated in terms of specific growth rate (SGR) as % day^{-1} using the following equation (Hopkins, 1992): $SGR = 100\,(LnSL_f - LnSL_i)\,/\,\Delta t$, where SL_f and SL_i were the final and initial standard lengths (mm) of fish, respectively, and Δt was the time between sampling intervals. At the end of this experiment, fish from each rearing tanks were harvested and counted for the final survival. Coefficients of variation (CV, %) of fish standard lengths between treatments were calculated from standard deviation (SD) and the mean for each treatment at each sampling time (CV = 100 × SD/mean).

On termination of the experiment, 50 fish were randomly collected from each rearing tank for examination of incidence of malformation if any. Fish were anaesthetised with AQUI-S® and fixed in 10% neutral buffered formalin. Alcian blue and alizarin red were used for staining cartilage and bone (Taylor and Van Dyke, 1985). After staining, samples were examined under a stereo microscope (Olympus SZ40, Tokyo, Japan) for jaw and skeleton malformation. Analyses of skeletal malformations were done following the methods described by Ma *et al*. (2014c) and Zheng *et al*. (2014) which was calculated as: Incidence of malformation (%) = (malformed larvae/total number of larvae) x 100. On each sampling day, the occurrence of malformation was calculated as: Incidence of deformity (%) = (total number of one deformity/total number of all the deformations) x 100.

RNA and DNA samples were taken on termination of the experiment from fishes sampled from each treatment tank. Ten fish were randomly collected from each of the rearing tanks. The anaesthetised fish were pre-washed with distilled water to remove salt from the body surface, and then immediately preserved in liquid nitrogen. Frozen samples were dissected on an ice tray, and muscle tissue was collected from the fish. The RNA:DNA ratio was determined following the method described by Zehra and Khan (2013). Tissue samples from each tank were pooled individually and weighed to the nearest 0.001 g and placed in a test tube in an ice slurry bath. Tissue samples were homogenised in 5% trichloroacetic acid (TCA) at 90^{O}C and then centrifuged at 5,000 x *g* for 20 min. For RNA determination, 2 ml of distilled water and 3 ml of the orcinol reagent were added to 1 ml of supernatant. The reagent mixture was kept in boiling water for 20 min. The greenish-blue colour developed was read at 660 nm on a spectrophotometer. For DNA determination, supernatant (1 ml), distilled water (1 ml) and freshly prepared diphenylamine reagent (4 ml) were mixed. The reagent mixture was kept in a boiling water bath for 10 min. The blue colour developed was measured at 600 nm. Standard curves for RNA and DNA were developed using different concentrations of yeast RNA (Sigma-Aldrich, USA) and calf thymus DNA (Sigma-Aldrich, USA).

All percentage data were arcsine-transformed prior to statistical analysis. However, the data were presented as untransformed values in figures. The data were expressed as mean ± SD, and subjected to one way ANOVA (PASW Statistics 18.0, Chicago, SPSS Inc.). When a significant treatment effect was found, Tukey's test was performed for multiple range comparisons with the level of significance set at $p<0.05$. All data were tested for normality, homogeneity and independence to satisfy the assumptions of ANOVA. The size variations of fish larvae between deformed and normal fish were compared by independent t-test.

Results

Growth performance and survival of golden pompano larvae

Temperature significantly affected the growth and survival of golden pompano larvae ($p<0.05$, Fig. 1). The SGRs of fish reared at 29 and 33°C were 3.64±0.20 and 4.31±0.74% per day, respectively, which were significantly higher than those reared at 23 and 26°C ($p<0.05$, Fig. 1). The SGR of fish was not significantly different when fish were reared at 23 and 26, or at 29 and 33°C ($p>0.05$). On 18 DPH, coefficients of variation of standard length in fish larvae were not significantly different between temperature treatments ($p>0.05$, Fig. 1). The CV of standard length in fish larvae varied from 6.79±2.41 (23°C) to 10.51±2.78% (29°C). The RNA/DNA ratio of fish reared at 26 and 29°C was not significantly different ($p> 0.05$, Fig. 2), but was higher than that at 33°C ($p<0.5$, Fig. 2). In the present study, the lowest and highest survival rates were observed at 23 and 29°C, respectively, with intermediate values at 26 and 33°C.

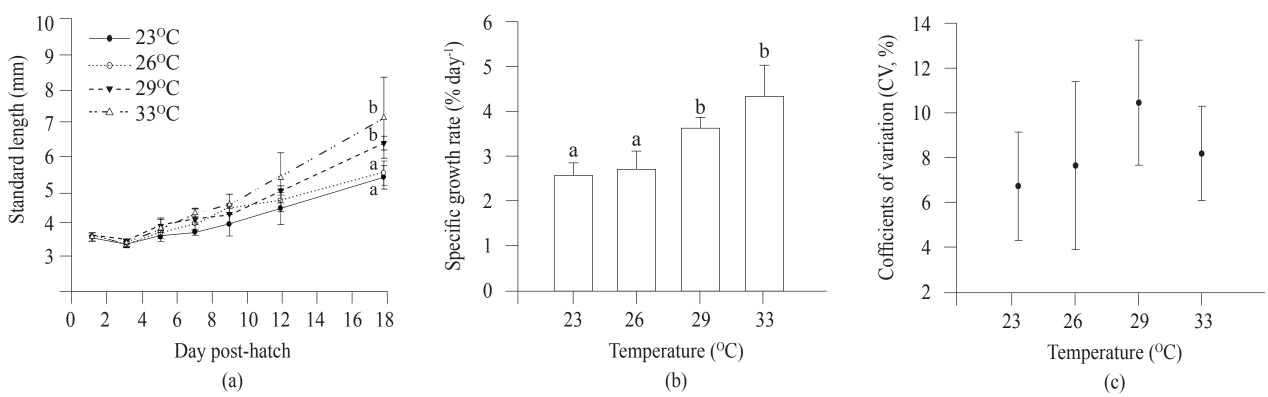

Fig. 1. Standard length, specific growth rate, and coefficients of variation of body lengths of golden pompano larvae reared at 23, 26, 29, and 33°C. Bars and whiskers are the means and standard deviations of three replications. Bars that share a superscript in common do not differ at $p<0.05$ (Tukey post-hoc test)

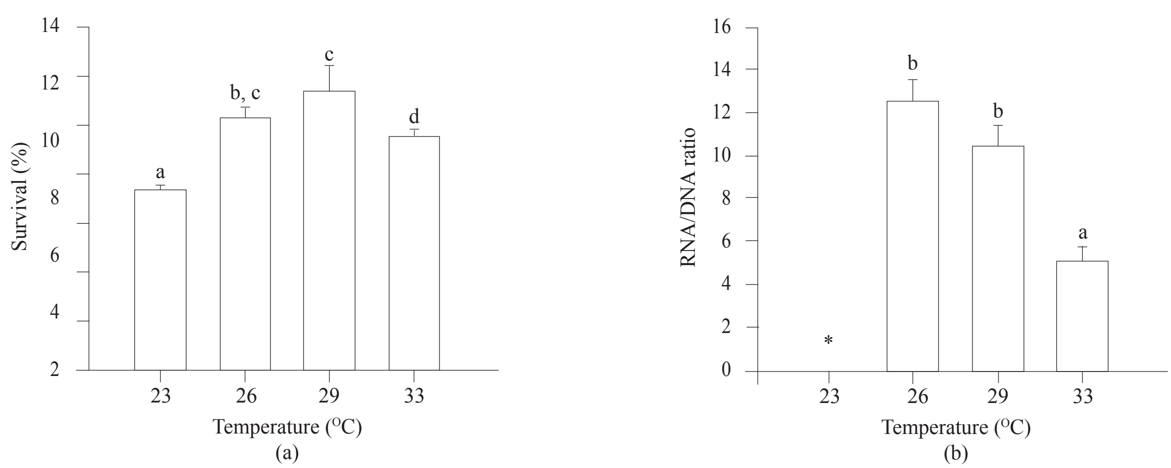

Fig. 2. (a) Survival and (b) RNA/DNA ratio of golden pompano larvae at 18 DPH reared at 23, 26, 29, and 33°C. Bars and whiskers are the means and standard deviations of three replications. Bars that share a superscript in common do not differ at $p<0.05$ (Tukey post-hoc test) *: Due to low survival at 23°C, there were not enough samples for RNA/DNA testing in this treatment

Deformities

The frequency of jaw deformity was highest at 33^{O}C and lowest at 23-26^{O}C, with intermediate scores at 29^{O}C (Fig. 3). At the end of the experiment, 83.34% of fish reared at 26^{O}C showed normal morphology, which was significantly higher than fish reared at 29 and 33^{O}C ($p<0.05$, Fig. 3). Temperature impacted the development of vertebrae and caudal skeleton (Fig. 4). Deformities of the vertebrae, both in the caudal (Vca) and anterior (Vco) regions were more at 33^{O}C than at lower temperatures. The frequency of Vco and Vca malformations at 26 and 29^{O}C did not differ significantly ($p>0.05$, Fig. 4). Malformations observed at hypural (Hy) and epural (Ep) levels were not significantly affected by the rearing temperature at the range of 26-33^{O}C ($p>0.05$). Within the temperature range tested, *i.e.,* 26 - 33^{O}C, the Hy malformation ranged from 18.67 to 30.62%, while the Ep malformation ranged from 11.43 to 17.17%. V-shaped lordosis was the most frequent type of deformity at all temperatures (>75%), but it was proportionally more frequent ($p<0.05$) at 33^{O}C than at lower temperatures (Fig. 5). At 18 DPH, the size of fish was not statistically different between deformed and normal fish in each temperature treatment ($p>0.05$).

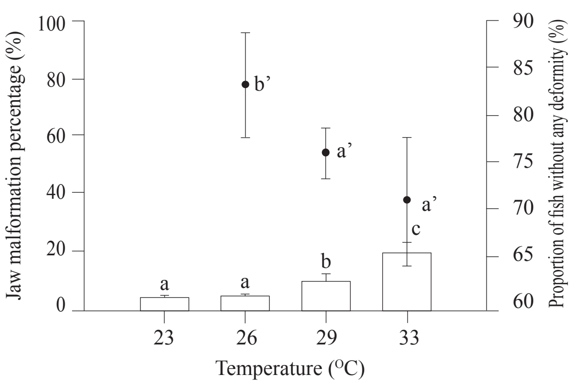

Fig. 3. Incedence of jaw malformation in golden pompano larvae at 18 DPH reared at 23, 26, 29, and 33^{O}C. Bars that share a superscript in common do not differ at $p<0.05$ (Tukey post-hoc test)

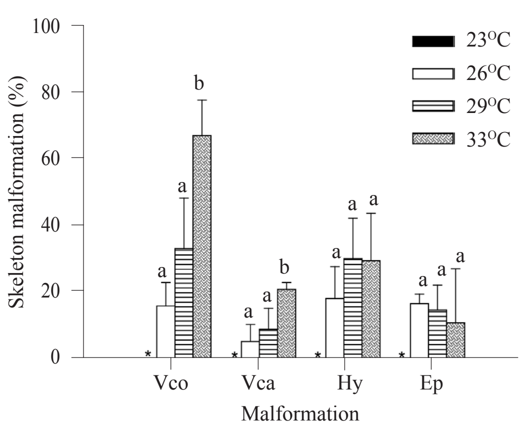

Fig. 4. Malformations in vertebral column (Vco), caudal vertebra (Vca), hypural (Hy) and epural (Ep) of golden pompano larvae at 18 DPH reared at 23, 26, 29 and 33^{O}C. Bars that share a superscript in common do not differ at $p<0.05$ (Tukey post-hoc test)

*: No information available for fish at 23^{O}C, due to very low survival

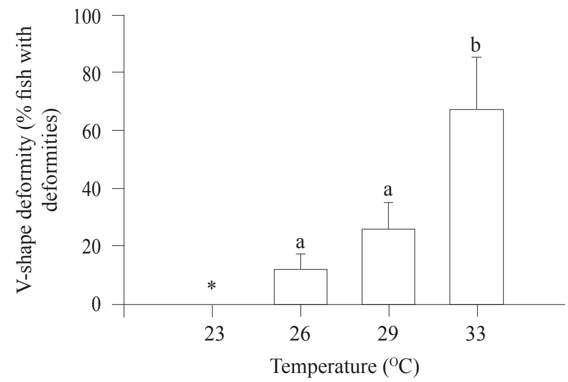

Fig. 5. V - shape deformity (% fish with deformities) of fish larvae at 18 DPH reared at 23, 26, 29, and 33^{O}C. Bars that share a superscript in common do not differ at $p<0.05$ (Tukey post-hoc test).

*: No information for fish at 23^{O}C, due to very low survival

Discussion

Temperature affects fish metabolism, food intake and growth of fish (Jobling, 1997), and the effects of temperature on body growth have been documented in the larvae of many fishes *viz.,* haddock *Melanogrammus aeglefinus* L. (Martell *et al.*, 2005), nase *Chondrostoma nasus* L. (Keckeis *et al.*, 2001), Australian snapper *Pagrus auratus* (Fielder *et al.*, 2005), striped trumpeter *Latris lineata* (Choa *et al.*, 2010), yellowtail kingfish *Seriola lalandi* (Ma, 2014) and Atlantic halibut *Hippoglossus hippoglossus* L. (Lein *et al.*, 1997). In this study, the growth of newly hatched golden pompano was slow in the first 9 days. The size of fish larvae gradually showed differences between temperature treatments after 12 DPH. At 18 DPH, fish growth was clearly affected by temperatures, and the growth was accelerated when temperature increased from 29 to 33^{O}C. The fast growth at high temperature observed in golden pompano may be attributed to the improved food ingestion and digestive function of larvae after 15 DPH as Ma *et al.* (2014b) found that the goblet cells and gastric glands formed in the gut of golden pompano larvae after 15 DPH at the rearing temperature of 27-29^{O}C. Similar to the Florida pompano *Trachinotus* carolinus (Riley *et al.*, 2009), the mouth gape height of about 1.05 mm should allow golden pompano larvae to ingest *Artemia* nauplii and other particles of similar size by 12 DPH. Therefore, the significant differences in fish size between thermal treatments after 18 DPH may be also related to the use of much more energetic food from 9 DPH onwards. Riley *et al.* (2009) found that the growth trajectories of *T. carolinus* could vary substantially between progenies, with some progenies exhibiting a slow growth and others a much faster growth. Similarly, such growth trajectories were also observed in golden pompano in this study.

The RNA/DNA ratio has been proven as a sensitive indicator of growth and nutritional condition in fish larvae and juveniles (Buckley *et al.*, 1999; Islam and Tanaka, 2005; Zehra and Khan, 2013). Previous studies have clearly demonstrated that water temperature and food availability can affect the RNA/DNA ratio of fish larvae (Goolish *et al.*, 1984; Mathers *et al.*, 1992). In the present study, the RNA/DNA ratio of fish dropped significantly when temperature increased from 29 to 33^{O}C at 18 DPH. Reduction in RNA/DNA ratio can be regarded as a result of nutrient deficiency in fish larvae (Tanaka *et al.*, 2008). The reduction of RNA/DNA ratio of fish reared at 33^{O}C indicate poor growth status of fish larvae at 18 DPH. The higher SGR of a few larger fish observed towards the end of the study, could have resulted from cannibalism on smaller fish, but the reason of the lower RNA/DNA ratio at 33^{O}C is not clear, which warrants further study.

Fish mortality frequently occurs at the critical period of nutritional transition from endogenous to exogenous feeding (Otterlei *et al.*, 1999; Ma *et al.*, 2012). During the feed transitional period, when food supply and photoperiod are within the range of fish requirement, temperature can be an important determinant for fish survival (McGurk, 1984; Kamler, 1992; Gardeur *et al.*, 2007; Ma, 2014). For instance, mortality is strongly temperature-dependent in the larvae and juveniles of *S. lalandi* (Ma, 2014), *Pangasianodon hypophthalmus* (Baras *et al.*, 2011), *Inimicus japonicas* (Wen *et al.*, 2013), and *Glyptocephalus cynoglossus* (Bidwell and Howell, 2001). Ma (2014) suggested that there is a temperature-sensitive period where mortality is likely to occur in fish larvae during early ontogeny. In the present study, though survival of fish larvae were low in all the temperature treatments, extremely low survival was observed when fish were reared at 23^{O}C, suggesting a temperature effect on the physical development of golden pompano larvae at this temperature. However, low fish survival may also be attributed to poor egg quality, inadequacy of food supplies and cannibalism (May, 1974; Baras and Jobling, 2002; Baras *et al.*, 2011; Ma *et al.*, 2012).

Being one of the primary physical factors, water temperature can affect the appearance of early morphological abnormalities during the ontogenetic development of marine fish larvae (Seikai *et al.*, 1980; Seikai *et al.*, 1986; Klimogianni *et al.*, 2004). Evidence indicates that high water temperature can lead to high percentage of malformation during the ontogenetic development in fish species such as tilapia *Oreochromis mossambicus* (Wang and Tsai, 2000), Senegalese sole *S. senegalensis* (Dionisio *et al.*, 2012) and gilthead seabream *S. aurata* (Georgakopoulou *et al.*, 2010). The present study demonstrated significant temperature effect on the vertebral column and caudal vertebra malformation. This finding is consistent with the result observed in *D. labrax* (Sfakianakis *et al.*, 2006).

Temperature associated abnormality in the vertebral column of the larval golden pompano was the most prevalent malformation observed in the present study. Additionally, the V-shaped malformation in the vertebral column, accounted for >50% of the whole deformations. Previous studies suggest that the absence or malfunctioned swim bladder is the major cause of V-shaped malformation in the vertebral column (Daoulas *et al.*, 1991), and haemal lordosis can be induced by high velocity of water current (Divanach *et al.*, 1997). Furthermore, swimming dynamics has significant impact on lordosis induction (Kihara *et al.*, 2002; Kranenbarg *et al.*, 2005). Deformities were observed at all temperatures during the present study, suggesting that other factors may also be involved in causing deformity and further research is required to identify these factors.

The caudal fin complex is one of the most sensitive part of the skeletal system in fishes, and deformities can occur even at standard rearing temperature (Takeuchi *et al.*, 1998; Haga *et al.*, 2002; Haga *et al.*, 2011). Incidence of deformities can further increase with increasing temperature, but this is not systematic in other fish such as *S. aurata* (Fernandez *et al.,* 2008; Georgakopoulou *et al.*, 2010). In the present study, however, the proportion of fish with deformed caudal fin complex did not vary significantly with water temperature.

Jaw malformation is a major concern in fish culture because it affects fingerling quality in the growout stage (Von Westernhagen, 1988). It is frequent that the proportion of fish larvae exhibiting jaw deformities increases with increasing water temperature, as was the case here for golden pompano (Alderdice and Velsen, 1971; von Westernhagen, 1974; Bolla and Holmefjord, 1988; Lein *et al.*, 1997). This temperature-dependent pattern is generally attributed to higher oxygen (Rombough, 1997) and nutritional requirements at high temperatures, which may not be fulfilled unless feed with higher energy or protein contents is provided (Cahu *et al.*, 2003a, b; Ma, 2014).

The present study examined the effect of temperature on the rearing performance and malformation of golden pompano larvae and found that water temperature of 26-29^{O}C favoured growth and survival, while 23^{O}C was too low for both growth and survival. Jaw, vertebral column and caudal vertebra deformities significantly increased at 33^{O}C. A reduction of RNA/DNA ratio of fish reared at 33^{O}C was also observed in this study. Therefore, the temperature range of 26-29^{O}C is optimal, and temperature >33 and <23^{O}C may have adverse impacts on fish performance. The present findings add further concerns as regards the impact of global warming on the culture of golden pompano larvae, unless for productions in thermo regulated water systems.

Acknowledgements

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