Effect of Water Temperature on Growth, Survival and Feeding Rate of Humpback Grouper (*Cromileptes altivelis*) Larvae

K. Sugama, Trijoko, S. Ismi and K. Maha Setiawati

Introduction

The primary problem in grouper propagation is high mortality at early larval stage. Some experiments have been carried out concerning this aspect for some species of groupers, such as *Epinephelus akaara* (Tseng and Ho 1979), *Epinephelus salmoides* (Hamanto et al. 1986; Huang et al. 1986; Lin et al. 1986), *Epinephelus tauvina*[1] (Husain et al. 1975; Chen et al. 1977; Husein and Higuchi 1980), *Epinephelus fuscoguttatus* (Supriatna and Kohno 1990), and *Cromileptes altivelis* (Aslianti 1996; Slamet et al. 1996; Kumagai et al. 1998; Sugama et al. 1998). Results of these experiments showed that high mortality was observed during the initial stage up to day-9 old larvae.

In general, the main factors that determine larvae mortality are biotic factors (for example food, disease, parasitism and predation) and abiotic factors (for example oxygen, pH, salinity, toxic substances and temperature) (Kamler 1992). Temperature has a major effect on the development of marine fish. Temperature also influences efficiency of yolk utilisation, growth, feeding rate, time to metamorphosis, behaviour and swimming speed, digestion and gut evacuation rate, and metabolic demand (Blaxter 1988). Kamler (1992) reported that the main factors contributing to the variability in developmental rate during early exogenous feeding of larvae are temperature and food. Therefore information on the effect of temperature on larval developmental is important for marine fish.

The aim of the present experiment was to identify the optimum water temperature for the growth, survival and feeding rate of early stage humpback grouper larvae.

[1] *E. salmoides* is a synonym of *E. coioides*; *E. tauvina* is likely a misidentification of *E. coioides*.

Materials and Methods

Twelve transparent polycarbonate tanks (30 litres volume) were used in this experiment. Rearing tanks were placed in a water bath system with a temperature controller and maintained at 25°, 28°, 31°C and without a temperature controller (control). Eggs were collected from naturally-spawning broodstock and then incubated in a 200 litre transparent tank. One day old larvae (D-1) with a hatching rate of 96% were stocked in each rearing tank at a density of 10 larvae/litre. Light intensity was adjusted to 750–850 lux according to larval stage using TL lamp.

Two types of rotifer, i.e. SS-type and S-type were used as food during larval rearing and fed to the larvae once per day at 8:00 in the morning. Rotifers were enriched with *Nannochloropsis* and a commercial enrichment product (Selco) before feeding to the larvae. SS-type rotifers were fed at day 3–5 at a density of 5 individuals/litre. S-type rotifers were fed at day 6–10 at a density of 10 individuals/litre.

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Feeding rate was determined directly by calculating the number of rotifers in the gut of each larva. Ten samples of larvae were collected from each tank everyday. To calculate the growth of larvae, ten larvae were collected and measured for total length (TL) at the beginning and at the end of the experiment.

Prototype 'backyard' hatchery for grouper, Gondol Research Institute for Mariculture, Indonesia. The prototype hatchery is used to train farmers and extension officers in grouper hatchery technology.

Commerical marine finfish hatchery, Gondol, Bali, Indonesia. The blue-coloured tanks are used for larval rearing of groupers.

Results

Results of the experiment showed that water temperature influenced growth, development of dorsal and pectoral spines, survival, and feeding rate of the larvae.

The effect of temperature on growth of humpback grouper larvae is shown in Table 1. Growth of the larvae reared at 31°C was the best and significantly higher ($P < 0.05$) than other treatments. Growth of larvae reared at 28°C was significantly higher than larvae reared at 25°C and control larvae ($P < 0.05$). These results indicate that the growth rate of the larvae increases with increasing water temperature.

Table 1. Growth (TL) of humpback grouper larvae reared at different temperatures.

Water temperature (°C)	Initial TL (mm)	Final TL (mm)	Growth (mm d^{-1})
Control	2.517 ± 0.082	3.403 ± 0.115[a]	0.099 ± 0.013[a]
25	2.517 ± 0.082	3.385 ± 0.200[a]	0.096 ± 0.022[a]
28	2.517 ± 0.082	3.815 ± 0.074[b]	0.144 ± 0.008[b]
31	2.517 ± 0.082	4.173 ± 0.094[c]	0.184 ± 0.010[c]

Means with the same superscript in the same column are not significantly different ($P > 0.05$).

The effect of temperature on dorsal spine and pectoral spine development is shown in Table 2. Except for the larvae reared at 25°C, the dorsal spine started to develop in most of the larvae on day-7 i.e. 0.001 mm, 0.019 mm, and 0.246 mm for the larvae reared at control, 28°C, and 31°C, respectively. The pectoral spine also started to develop on day-7, but only for the larvae reared at 31°C (0.484 mm). On day-8, development of the dorsal spine and pectoral spine were more significant, except for the larvae reared at 25°C. Dorsal spine and pectoral spine for the larvae reared at 25°C started to develop on day-9, with the length of 0.019 mm and 0.094 mm for dorsal spine and pectoral spine, respectively. These results indicate that development of dorsal spine and pectoral spine of the larvae is more rapid as water temperature increases.

The effect of temperature on feeding rate of humpback grouper larvae is shown in Figure 1. Feeding rate of larvae reared at 31°C was significantly higher ($P < 0.05$) than those of larvae reared at 25°C and control, but was not significantly different ($P > 0.05$) compared with the larvae reared at 28°C. Feeding rate of larvae

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reared at 28°C was higher than larvae reared at 25°C and control. These data indicate that feeding activity of humpback grouper larvae reared at 28°C and 31°C was high.

Table 2. The length of the dorsal spine and pectoral spine of 10 days old humpback grouper larvae.

Water temperature (°C)	Dorsal spine (mm)	Pectoral spine (mm)
Control	0.396 ± 0.131	0.530 ± 0.172
25	0.143 ± 0.033	0.299 ± 0.155
28	0.986 ± 0.108	1.399 ± 0.100
31	1.849 ± 0.210	2.033 ± 0.250

Survival of humpback grouper larvae reared at different temperatures are shown in Figure 2. Survival of larvae reared at 28°C was higher than the other treatments. Survival of larvae reared at 25°C was higher than larvae reared at control and at 31°C ($P < 0.05$).

Counting and grading *Cromileptes altivelis* juveniles in a 'backyard' hatchery in Bali, Indonesia. Hatcheries are an important source of employment for local people in northern Bali.

Discussion

The result of the present experiment showed that growth of humpback grouper larvae increased with rising water temperature. This result agrees with the result reported by Akatsu et al. (1983) for brown spotted grouper *Epinephelus tauvina* (= *E. coioides*). In their experiment, larvae of brown spotted grouper were reared for 12 days at different water temperatures. Total length of larvae reared at high temperature (32°C) was the highest (6.5 mm) compared with larvae reared at 23°C (4.1 mm).

The development of dorsal spine and pectoral spine of humpback grouper larvae increased with rising water temperature. Blaxter (1988) reported that temperature is known to influence growth and time required to metamorphosis of fish larvae. In this experiment, development of pectoral spine was faster than that of dorsal spine, although dorsal spine started to develop earlier than pectoral spine. Slamet et al. (1996) also reported that the development of the pectoral spine was faster than the dorsal spine. Aslianti (1996) found that development of dorsal spine of humpback grouper *Cromileptes altivelis* larvae was started on day-7, the same as the result of the present experiment. Development of dorsal spine of grouper *Plectropomus maculatus* larvae also started on day-7 (Diani et al. 1991).

Feeding rate of humpback grouper larvae increased with increasing water temperature. This data indicates that feeding activity of humpback grouper larvae increases with increasing water temperature. Similar results have been reported by Jobling (1994), who found that feed intake of Baltic salmon increased approximately threefold as temperature increased from 2° to 6°C. In another experiment, Koskela et al. (1997) reported that the feeding rate of juvenile Baltic salmon reared at 6°C was approximately two times higher than that reared at 4°C. Elliott (1991) and Jobling (1994) stated that the metabolic processes of fish are sensitive to the changes of environmental temperature and a decrease in water temperature to the below optimum level results in reduced feed intake and growth. Fish eventually lose their appetite when maintained below the temperature tolerance range.

The highest survival (48.11%) was found for the larvae reared at 28°C that was much higher compared with the survival of larvae reared at 31°C, which was only 4.77%. This data indicates that optimal water temperature for rearing of humpback grouper larvae is 28°C, even though

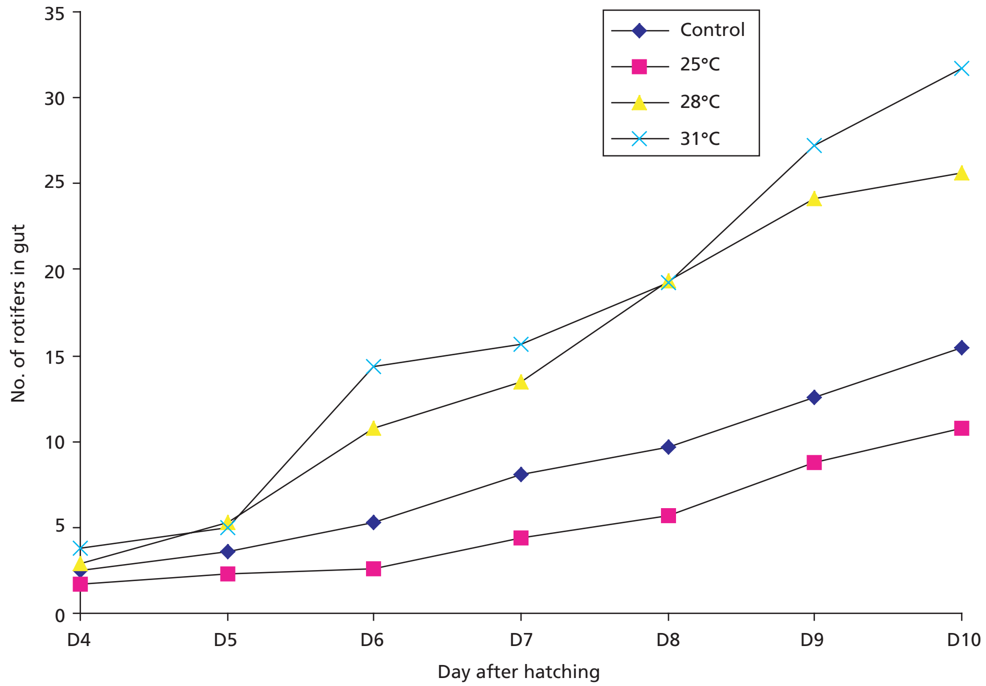

Figure 1. Feeding rate of humpback grouper larvae reared at different temperatures.

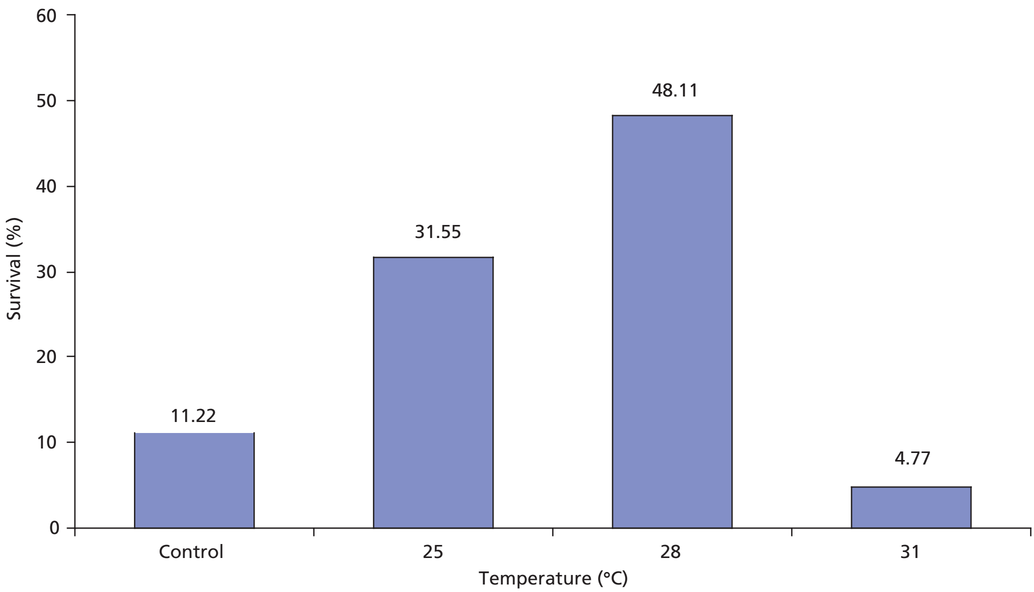

Figure 2. Survival of humpback grouper larvae reared at different temperatures.

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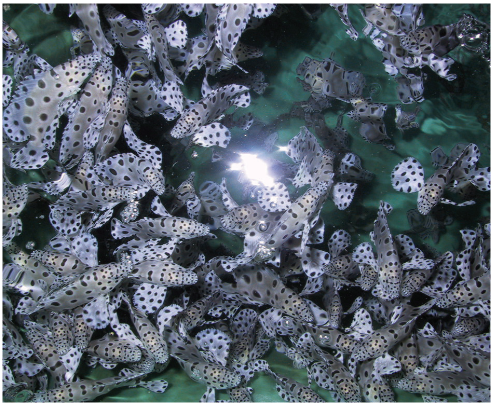
the best growth rate of larvae was at the water temperature of 31°C. This is similar to the findings reported by Akatsu et al. (1998), who found that survival of grouper *E. tauvina* (= *E. coioides*) larvae reared for 12 days at different temperatures, i.e. 18°, 23°, 29° and 32°C were 0%, 0.29%, 9.8% and 0.6%, respectively. The highest survival was at the water temperature of 29°C.



Juvenile humpback grouper/barramundi cod (*Cromileptes altivelis*) reared at Gondol Research Institute for Mariculture, Bali, Indonesia.

Conclusions

- Growth and feeding rate of humpback grouper (*Cromileptes altivelis*) larvae increase with increasing water temperature.
- The optimum temperature for rearing of early stage humpback grouper larvae is 28°C.

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Larval Rearing Tank Management to Improve Survival of Early Stage Humpback Grouper (*Gromileptes altivelis*) Larvae

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Introduction

High mortality during the early larval stages of humpback grouper *Cromileptes altivelis* is one factor hindering the development of mass production of this species (Slamet et al. 1996, Aslianti, 1996). Difficulties in rearing grouper larvae also have been reported by Kohno et al. (1994; 1997). Kawahara et al. (2000) reported that larvae at 0–5 days after hatching are easily trapped at the water surface by surface tension. Those larvae once trapped at the water surface cannot escape from the surface and eventually die. High mortality also frequently occurs at 10–25 days after hatching because of larval entanglement with the spines.

The purpose of the present study was to determine if survival was improved by the addition of an oil film on the water surface and by increasing the percentage water exchange.

Methods

Experiment 1: Effect of oil addition on water surface of larval rearing tank for humpback grouper larvae.

The experiment was conducted using transparent tanks, 200 litres in volume and filled with sea water (34 ± 1 ppt). Eggs of humpback grouper were stocked into each tank at the density of 10 eggs/litre. Starting at day 1 after hatching, squid oil at different concentrations (0, 0.1, 0.2 and 0.3, ml/m2 for Study–1; 0.3, 0.4, and 0.5 ml/m2 for Study–2) was added to each tank. The experiment was a completely randomised design with three replicates per treatment. Survival and growth rate were measured at the end of the experiment (day 6 from hatching). Study–3 was conducted to determine the optimal time for the oil to be added to the rearing water. For this study, oil was added to the larval rearing tanks during the rearing period of day 1–3, day 1–6, day 1–9, day 1–12; the control group had no oil added to the tanks during the rearing period. The experiment was terminated at day 15 and survival of larvae was calculated.

Experiment 2: Effect of different water exchange on initial feeding incidence of humpback grouper larvae.

This experiment was conducted using nine transparent tanks, 200 litres in volume filled with sea water (salinity of 34 ± 1 ppt.). Eggs of humpback grouper were stocked into each tank at a density of 10 eggs/litre. During the experiment, larvae were reared without water exchange (treatment A), 100% water exchange per day (treatment B), and 200% water exchange per day (treatment C). Water exchange was started on day 3 by flow-through system. The experiment was designed in a completely randomised design with three treatments and three replicates for each treatment for 10 days. Samples of larvae were taken every day for observation of larval growth and

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stomach contents. Survival rate was measured at the end of the experiment.

Results and Discussion

Experiment 1

The addition of an oil film on the water surface during larval rearing, influenced survival rate of the larvae. Survival rate of larvae without oil addition was significantly lower ($P < 0.05$) than the treatments with the addition of oil. Addition of oil at the concentration of 0.3–0.4 ml/m^2 resulted in the highest survival (Tables 1 and 2). The highest survival rate occurred with the addition of 0.3 ml/m^2 of oil to the water surface. The total length of larvae in all treatments was the same. Larvae at 0–5 days after hatching were still very weak and slow moving. At this stage, larvae are easily trapped at the water surface by surface tension. Once trapped at the water surface, the larvae cannot escape and eventually die. The larvae trapped at the surface secrete a sticky mucus and this appears to contribute to additional larvae being trapped at the water surface. Finally, a significant number of larvae die in a short time (Kawahara et al. 2000). The addition of an oil film to the water surface reduces the surface tension and therefore the number of larval mortalities was reduced. However, if the amount of oil added is too low (i.e. 0.2 mL/m^2 or lower) the surface tension is still strong enough to trap the larvae leading to surface deaths.

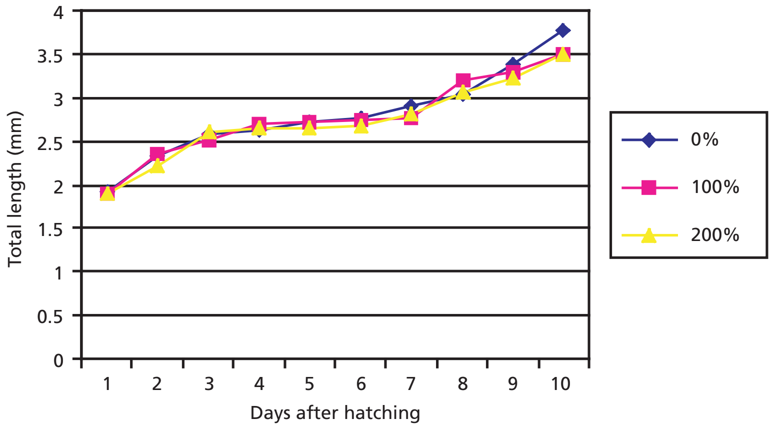

Figure 1. Total length (mm) of humpback grouper larvae reared at different water exchange rates (%/day).

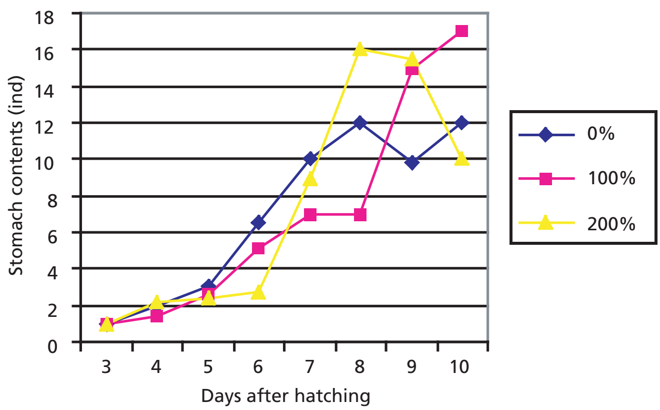

Figure 2. Stomach contents of humpback grouper larvae reared at different water exchange rates (%/day).

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Study 3 demonstrated that the addition of oil up to day-9 post hatch resulted in the best larval survival (Table 3). This result correlates with the morphological development of the larvae. Long spines of dorsal and pelvic fin start to develop in day-9 old larvae. At this time larvae become more active and floating death decreases.

Table 1. Survival and total length of humpback grouper larvae with different concentration of oil addition during the 6 days larval rearing.

Oil addition (ml/m²)	Total length (mm)	Survival rate (%)
0	2.80 ± 0.078[a]	0.30[a]
0.1	2.90 ± 0.073[a]	14.85[b]
0.2	2.99 ± 0.091[a]	42.30[c]
0.3	2.80 ± 0.078[a]	56.25[d]

Values with the same letter within a column are not significantly different ($P > 0.05$).

Table 2. Survival and total length of humpback grouper larvae with different concentration of oil addition during the 6 days larval rearing.

Oil addition (ml/m²)	Total length (mm)	Survival rate (%)
0	2.93 ± 0.067[a]	7.4[a]
0.3	3.03 ± 0.089[a]	52.0[b]
0.4	3.09 ± 0.091[a]	50.0[bc]
0.5	2.97 ± 0.034[a]	48.9[bc]

Values with the same letter within a column are not significantly different ($P > 0.05$).

Table 3. Survival and total length of humpback grouper larvae after addition of oil at different larval rearing period.

Oil addition (day)	Total length (mm)	Survival rate (%)
1–3	5.10 ± 0.045[a]	4.8[a]
1–6	5.54 ± 0.056[a]	4.0[a]
1–9	5.23 ± 0.089[a]	7.9[b]
1–12	4.86 ± 0.023[a]	5.8[a]
No oil	4.35 ± 0.076[a]	0.8[c]

Values with the same letter within a column are not significantly different ($P > 0.05$).

Experiment 2

Results of this experiment showed that the highest survival rate was found for the treatment without water exchange during the first 10 days of larval rearing (Table 4). This result suggests that early stage larvae (days 0–10) are sensitive to fluctuations in environmental factors due to water exchange. However, there was no difference in growth (Figure 1) or feeding activity (Figure 2) at different water exchange rates.

Table 4. Survival and total length of humpback grouper larvae reared with different water exchange rates from day 0 to day 10 post hatch.

Water exchange (%/day)	Total length (mm)	Survival rate (%)
No exchange	3.61[a]	6.48[a]
100	3.54[a]	4.12[b]
200	3.50[a]	4.67[b]

Values with the same letter within a column are not significantly different ($P > 0.05$).

Conclusions

- Addition of an oil film to the water surface improved survival in day 0–day 9 humpback grouper *Cromileptes altivelis* larvae.
- Zero water exchange resulted in the highest survival of humpback grouper *Cromileptes altivelis* for the first 10 days of larval rearing.

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