飼育条件下におけるクロソイ(Sebastes schlegeli)仔魚の生残と成長に対する水温と給餌条件の影響

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Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat
Effects of Feeding and Temperature on Survival and Growth of Larval Black Rockfish *Sebastes schlegeli* in Rearing Conditions

Byungsun CHIN¹, Masahiro NAKAGAWA² and Yoh YAMASHITA¹

**Abstract:** Effects of feeding and temperature on survival and growth of black rockfish larvae were studied in four rearing experiments. In the feeding regime experiment, growth and survival rates were not significantly different between the combined rotifers and Artemia feeding regime and the Artemia only regime. In the feeding level experiment, total length at 20 days after birth was significantly different by feeding level as $\frac{(1.5)}{(1.0)} > \frac{(0.5)}{(0.1)}$. In the different temperature experiments, survival rates at the intermediate temperatures of $14^\circ C$, $18^\circ C$ and $22^\circ C$ were significantly higher than those at $10^\circ C$ and $24^\circ C$. Growth rate tended to increase as temperature increased within the examined range from $10^\circ C$ to $24^\circ C$. When larvae were reared under diel temperature change ($14^\circ C \sim 18^\circ C$), survival and growth rates were between 14 and $18^\circ C$ of fixed temperatures showing no beneficial effects of a diel change of temperature. The most efficient rearing method for hatchery rearing was concluded to be: using $(1.0)$ feeding level of Artemia only during the larval period at a temperature range approximately $16 \sim 20^\circ C$.

**Key words:** Black rockfish; Rearing condition; Temperature; Feeding

Numerous exogenous factors such as temperature, salinity, dissolved oxygen (DO), food abundance, food quality, food size, fish density, intra- and inter-specific competition, and predation pressure affect the growth rate of fish. Undoubtedly, temperature is the most fundamental abiotic factor for all organisms because of its effects on physiological activities, and hence on virtually all aspects of metabolism (Jobling 1993; Mommsen 1998; Yamashita et al. 2001).

Black rockfish (*Sebastes schlegeli*) is well known for its fast growth and large size among rockfish (*Sebastes spp.*) and aquaculture of black rockfish has been attempted in Japan and Korea to make this fish more commonly available in markets (Kusakari 1995; Myeong et al. 1998; Nakagawa et al. 2006). For this purpose broodstock management (Nakagawa and Hirose 2004), morphological development (Yamada and Kusakari 1991; Nagasawa and Domon 1997), growth and survival under rearing conditions (Kusakari 1995; Myeong et al. 1998) have been studied. However information is still limited. Black rockfish is also a target species for stock enhancement. Nakagawa et al. (2007) reported high market return rates ranging from 9.3 to 15.4% in Yamada Bay, Iwate Prefecture showing that release of hatchery-cultured juvenile fish can effectively enhance local populations. However, because of the high cost in seed production and low market value, hatchery Aquaculture and seed production have some difficulties from the point of economic efficiency (Yamada and Kusakari 1991; Myeong et al. 1998; Nakagawa et al. 2006, 2007). The aim of this study is to examine methods to increase hatchery seed production efficiency of black rockfish by determining the optimum feeding regime and temperature.

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Materials and Methods

Four experiments were conducted on black rockfish, Exp. 1 in 2006 and Exp. 2 in 2004 on feeding regimes, Exp. 3 in 2005 and Exp. 4 in 2006 on influence of temperature. All the rearing experiments were carried out in the Miyako Station, Fisheries Research Agency, NCSE (National Center for Stock Enhancement), Iwate Prefecture, Japan during the year of 2004 ~ 06.

Management of broodstock

Thirteen to fifteen year old female black rockfish, 42.8 ~ 50.5 cm in standard length (SL), 21.6 ~ 23.83 kg in body weight, were used as broodstock in the present study. Female fish that mated with males in aquaria in November were transferred to two 20 kl delivery tanks (10 ± 1°C (SD) in Exp. 3 and 13 ± 1°C in Exp. 1, 2, 4, and 32 ± 1%, 3 weeks before parturition.

Basic rearing conditions

On the day of birth 1,200 healthy larvae were selected and were transferred to each polycarbonate (100 l) container (12 inds/l). Photoperiod was natural (light:dark=15:9) during experiments. Dissolved oxygen was maintained > c.a. 6.0 mg/l by aeration (70 ml/min) and rearing seawater circulated 0.5 times/day for larvae from 0 to 10 days after birth (DAB) and 3 times/day for those after 10 DAB. Containers were cleaned by siphon and deceased individuals were collected, counted and observed under a dissecting microscope every day to calculate the survival rate. In each experiment, 20 ~ 25 individuals were collected from all containers every five days and fixed with 90% ethanol after measuring total length (TL), wet body weight and observation of the developmental stage. Developmental stage was determined by following Nagasawa and Domon (1997) (Table 1). Temperature was controlled using a controller, heater and cooler.

In the feeding experiments (Exp. 1 and 2), the temperature of the birth tanks was fixed at 13°C and it was elevated 1°C/day until it reached 18°C, then larvae were reared at 18°C. Water temperature was measured 3 times each day. Feeding experiments were all carried out until black rockfish reached the juvenile stage. As food organisms of larval fish, rotifers Brachionus plicatilis cultured with yeast and Chlorella and brine shrimp Artemia spp. nauplii 48 h after hatching (22 ~ 25°C) enriched with Aquaran (BASF Japan, 5 ppt) were fed.

Feeding regime experiment (Exp. 1)

In Exp. 1, larvae were reared under two feeding regimes. In Exp. 1 (RA), larvae were fed rotifers from 0 to 10 DAB and Artemia nauplii from 5 DAB to the end of the experiments in 3 containers. In Exp. 1 (A), larvae were fed only Artemia nauplii from 0 DAB to the end in the 3 containers. The amount of rotifers and Artemia nauplii given to black rockfish was determined based on the body weight of the larval black rockfish, a method which had been empirically used in the Miyako Station (Fig. 1).

<table>
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<th>Developmental stage</th>
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<tr>
<td>Pre flexion larva</td>
<td>No notochord flexion</td>
</tr>
<tr>
<td>Flexion larva</td>
<td>Notochord flexion occurs</td>
</tr>
<tr>
<td>Post flexion larva</td>
<td>Dorsal and anal fin rays appear and full complement of pectoral fin rays</td>
</tr>
<tr>
<td>Transforming larva</td>
<td>Notochord flexion is completed and melanophores spread over the body</td>
</tr>
<tr>
<td>Juvenile</td>
<td>Faint saddles of melanophores appear</td>
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Fig. 1. Amount of food introduced in the rearing containers for Exp. 1.
Feeding level experiment (Exp. 2)
In Exp. 2, Artemia nauplii were fed at 5 food levels of feeding; 0 [Exp. 2 (0)], 0.1 [Exp. 2 (0.1)], 0.5 [Exp. 2 (0.5)], 1.0 [Exp. 2 (1.0)] and 1.5 [Exp. 2 (1.5)] times of the control in the two containers at each level. The amount of Artemia nauplii given in the control level container Exp. 2 (1.0) was the same as that in Exp. 1 (A).

In this experiment, the number of missing larvae due to cannibalism was estimated at the end of the experiment by subtracting the number of survivors and the total number of deceased individuals found in the container from the initial total number. Because of black rockfish swallowing their prey whole, these missing larvae were all counted as acts of cannibalism.

Fixed temperature experiment (Exp. 3)
Larvae were reared at 5 temperatures 10, 14, 18, 22, 24°C in three replicate polycarbonate containers at each temperature. Because the birth tank temperatures were fixed at 10°C, temperatures of experimental containers were raised 1°C/day from the day of birth until it reached the set temperature for each container. Water temperature was measured 3 times a day. In the temperature experiments (Exp. 3 and 4), Artemia nauplii were fed at the level of Exp. 1 (A) mentioned above from 1 DAB until the end of the experiments.

Diel temperature cycle experiment (Exp. 4)
In the Exp. 4, three different water temperature regimes were set, constant at 14°C [Exp. 4 (14°C)], at 18°C [Exp. 4 (18°C)] and a diel cycle of changing from 14 to 18°C [Exp. 4 (14 ~ 18°C)]. Each temperature regime was carried out in triplicate containers. Diel temperature change of Exp. 4 (14 ~ 18°C) containers was maintained in a 2 kl water-bath. Water-bath temperature was controlled by steadily pumped seawater from a 5 kl tank which was heated to 18°C from 4:00 am and cooled to 14°C from 12:00 pm. The temperature of the 5 kl tank water reached the set temperature in 2 h after onset of heating or cooling.

Because the temperature of the birth tanks was fixed at 13°C, the temperature was elevated 1°C/day until it reached 18°C, then diel temperature cycling was initiated. Water temperatures were measured using a thermo recorder (Ondotori TR-50C, T&D Japan) every 30 minutes.

Statistics
Survival rates were statistically treated using X² test and total length Kruskal-Wallis test. In the case that Kruskal-Wallis test indicates a significant difference, Scheffe’s multiple comparison method was performed for making a pair wise comparison of the means.

Wet weight of samples measured in Exp. 3 and 4 were used to estimate the instantaneous rate of growth (G) from the following equation.

\[
G = \frac{\ln W_t - \ln W_0}{t}
\]

Where \(W_0\) is the initial wet weight (mg) and \(W_t\) is the wet weight at time \(t\) (in days). Daily specific growth rate is defined as \(e^{G} - 1\) (%) (Ricker 1975).

Results
Feeding regime experiments (Exp. 1)
There was no significant difference in survival rate between Exp. 1 (RA) and Exp. 1 (A) on 35 DAB (\(P > 0.05\)) (Fig. 2). However there was a tendency that survival rates in Exp. 1 (A) decreased at higher rate compared to those in Exp. 1 (RA) from 7 DAB, then became a similar level around 13 DAB. Mean TL at birth was 6.73 ± 0.01 mm and reached 26.89 ± 0.24 mm in Exp. 1 (RA) and 25.83 ± 0.24 mm in Exp. 1 (A) at 35 DAB. There

![Fig. 2. Daily mean survival rate of black rockfish larvae in the two feeding regimes. Exp. 1 (RA) indicates feeding regime of rotifers + Artemia and Exp. 1 (A) Artemia only. Values for triplicates are combined.](image-url)
was no significant difference ($P > 0.01$) in TL between Exp. 1 (RA) and Exp. 1 (A) up to 35 DAB (Fig. 3).

**Feeding level experiment (Exp. 2)**

In the non-fed replicates all individuals died by 8 DAB. There was no difference in survival rate between Exp. 2 (0.5), Exp. 2 (1.0) and Exp. 2 (1.5), but survival rates of these three feeding levels were significantly higher ($P < 0.05$) than that of Exp. 2 (0.1) (Fig. 4).

In TL there were three groups which were significantly different ($P < 0.01$) after 10 DAB, Exp. 2 (1.5) = Exp. 2 (1.0) > Exp. 2 (0.5) > Exp. 2 (0.1). However, the TL of Exp. 2 (0.5) caught up with those of Exp. 2 (1.0) and Exp. 2 (1.5) on 40 DAB (Fig. 5).

Cannibalism in Exp. 2 (0.1) and Exp. 2 (0.5) showed higher rates than those in Exp. 2 (1.0) and Exp. 2 (1.5), 7.4%, 6.2%, 2.7% and 3.7% on 40 DAB, respectively. Cannibalism was first observed on 29 DAB in Exp. 2 (0.1), on 20 DAB in Exp. 2 (0.5), and from 22 DAB in Exp. 2 (1.0) and Exp. 2 (1.5). Some of the deceased individuals were coupled with a small individual (prey) in the mouth of the large individual (predator) which had its jaws opened to the maximum, but was unable to swallow the prey. This was assessed as a failed attempt of cannibalism.

**Fixed temperature experiment (Exp. 3)**

High mortality occurred from 5 to 14 DAB at all temperatures. Survival rates of intermediate temperatures of 14°C, 18°C and 22°C were significantly higher ($P < 0.05$) than those of the highest (24°C) and lowest temperatures (10°C) at 40 DAB (Fig. 6). Larval growth was faster at higher temperatures within the examined range of 10°C ~ 24°C (Fig. 7), while there was no significant difference between 18°C, 22°C and 24°C ($P > 0.05$) at 40 DAB.
Temperature also affected the relationship between developmental stage and TL. At the same TL, larvae tended to reach a more advanced stage at higher temperatures (Fig. 8). For example, the mean TL at the 5th stage at 10, 14, 18, 22 and 24°C was 25.9, 23.9, 24.1, 20.4, and 21.5 mm, respectively (Fig. 8).

**Diel temperature cycle experiment (Exp. 4)**

Diel change of temperature in Exp. 4 (14 ~ 18°C) is shown in Fig. 9. The same cyclical temperature pattern was observed every day.

Survival rates in Exp. 4 (18°C) and Exp. 4 (14 ~ 18°C) were significantly higher (P < 0.05) than that in Exp. 4 (14°C) (Fig. 10). However the
growth rate of Exp. 4 (14 ~ 18°C) was between Exp. 4 (14°C) and Exp. 4 (18°C) (Fig. 11).

Combining all the data in the present study, relationships between the growth rate and temperature and that between the survival rate and temperature were expressed as quadratic equations (Fig. 12). These relationships showed that the highest growth and survival rates would be obtained at 21.0°C and 15.9°C, respectively.

**Discussion**

**Feeding regime**

The survivorship of Exp. 1 (A) in which only *Artemia* was fed indicated that larvae which could not catch *Artemia* started to die from 7 DAB (Fig. 2). However, such less efficient larvae in feeding gradually died until around 15 DAB even in Exp. 1 (RA) in which rotifers and *Artemia* were fed and there became no difference in survival rate between (A) and (RA) treatments after 15 DAB.

As well as the survival rate there was no significant difference in TL between the feeding regimes. Rotifer production is costly for labor, light and heat (Myeong et al. 1998; Nakagawa et al. 2006). Although some hatcheries adopt a rotifer + *Artemia* feeding regime at present, this study indicates that we can skip the rotifer feeding process, which will reduce the rotifer production and staff labor. In hatcheries artificial feed is also fed from approximately 7 DAB for weaning. However, artificial feed was not fed in the present study and further examination of the role of artificial feed is required.

**Feeding level**

There were no significant differences in survival rate and TL at 40 DAB between the feeding levels of Exp. 2 (0.5), Exp. 2 (1.0) and Exp. 2 (1.5) times treatments. However, it is notable that the TL at the level of Exp. 2 (0.5) was significantly smaller than those at levels Exp. 2 (1.0) and Exp. 2 (1.5) up to 35 DAB (Fig. 5).

In this study, larvae at the feeding level of Exp. 2 (0.1) had a slow growth and low survival rate. However some individuals at this low feeding level were of almost a similar size as larvae in the normal feeding level of Exp. 2 (1.0). Food shortage might have induced heavy cannibalism. TL in Exp. 2 (0.5) treatment quickly increased from 35 to 40 DAB resulting in no difference in TL between Exp. 2 (0.5), Exp. 2 (1.0) and Exp. 2 (1.5) treatments on 40 DAB. This is also considered to be caused by cannibalism, since the cannibalism rate was higher in Exp. 2 (0.5) than in Exp. 2 (1.0) and Exp. 2 (1.5) feeding levels.

The deceased individuals with the maximum opened jaw in Exp. 2 were likely to be results of failed cannibalism. When cannibalism occur prey sometimes gets stuck inside the mouth of the predator. And when the predator can not swallow nor vomit the prey from the mouth, the predator dies with prey in its mouth. But these deceased individuals from failed cannibalism were not included in the cannibalism rate, since it is difficult to distinguish attacked and vomited deceased larvae from the other deceased ones.

Cannibalism in Exp. 2 (0.1) feeding level began to occur late (29 DAB) compared to the other feeding levels (20 ~ 22 DAB). This is because larvae reached 10 mm TL around 30 DAB due to the slow growth. In the wild, black rockfish are reported to prey on fish from 10 mm TL (Nagasawa and Domon 1997).

Size variation is thought to be the major cause of cannibalism, and inadequate feeding
practices and high stocking density increase the intensity of this problem. Reducing the size variation by routine size grading is an effective method to reduce cannibalism (Hecht and Pienaar 1993; Hseu et al. 2003). Cannibalism also decreases with sufficient food, relatively low stocking density and darkness for biting and swallowing feeding type fish (Dou et al. 2000). Black rockfish seems to act on object glittering by sunlight (unpublished data). Considering this behavioral characteristics, green water with *Nannochloropsis* and shading with a cover on the tanks which lowers sunlight intensity may also reduce cannibalism.

**Temperature**

The lowest survival rate was found at 10°C in the present rearing experiment. *Artemia* which were transferred from the 23°C container might have not been active in a 10°C container. It is likely that larvae did not actively consume sluggishly moving *Artemia* resulting in a low feeding rate. At 24°C the survival rate was also low probably because energy budget efficiency is lowest due to high metabolic costs. The survival rates at 14, 18 and 22°C were high indicating these were in the physiologically appropriate temperature range for larval rockfish. Especially the survival rate at 14°C was highest after 20 DAB (Fig. 6).

Generally fish reach the juvenile stage at a larger size when reared at a lower temperature (Seikai et al. 1986; Komaki 1996; Aritaki et al. 2004; Aritaki and Seikai 2004; Aritaki and Kumakari 2005). In this study black rockfish showed the same tendency. The size at metamorphosis from larval stage to juvenile stage increased with decreasing rearing temperature (Fig. 8). It is noteworthy that the duration of stage 3 (post-flexion larval stage) and stage 4 (transforming larval stage) at lower temperatures were longer than at higher temperatures.

It is well known that whole body concentration of thyroid hormone (thyroxine, T₄) is elevated just before metamorphosis and T₄ is thought to enhance fish transformation from larval to juvenile stages (Inui and Miwa 1985; Tagawa et al. 1990; Tanaka et al. 1995; Perez-Dominguez et al. 1999; Kang and Chang 2005). The metabolism of T₄ was low at low temperatures and probably this caused a longer period from the post-flexion to transforming stage in spotted halibut *Verasper variegatus* (Hotta et al. 2001a, b).

**Diel temperature change**

Simulations of juvenile red drum *Sciaenops ocellatus* growth performance made under time-varying environmental regimes indicate that given an unrestricted food supply, growth may be much faster under diel-cycling regimes than under constant optimal temperature regimes (Neill et al. 2004). Diel cycles of environmental parameters may contain intervals of nearly ideal conditions for growth that compensate for time spent under less optimal conditions. This may result in an overall greater metabolic scope for growth (Neill et al. 2004). However, diel cycling temperature treatment did not improve survival and growth of rockfish larvae in the present study as found in red drum larvae by Perez-Dominguez et al. (2006). There is a possibility that the range of diel temperature cycle (4°C) adopted in our experiment was too small to stimulate a growth potential. Effect of diel cycling temperature on growth rate is an important subject for future Aquaculture studies.

This study showed that the optimal feeding condition of black rockfish larvae is using only *Artemia* for food with a feeding level of (1.0). The combination of two relations from growth rate-temperature and survival rate-temperature indicated that the highest growth rate was found around 21°C and the highest survival rate around 16°C (Fig. 12). To ensure near the maximal growth and high survival, the optimal water temperature range is 16 ~ 20°C.

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飼育条件下におけるクロソイ（Sebastes schlegeli）仔魚の生残と成長に対する水温と給餌条件の影響

陳 炳善・中川雅弘・山下 洋

飼育実験により、クロソイ（Sebastes schlegeli）仔魚の生残と成長に対する水温と給餌条件の影響を調べた。飼餌開始期からアルテミア幼生のみを給餌した群とシオミズツボワムシからアルテミアに切り替えた群では、生残、成長に差は認められなかった。水研センター宮古栽培漁業センターの従来の給餌量を1としたとき、20日齢では成長に(1.5) = (1.0) > (0.5) > (0.1)の差がみられた。また、10℃、14℃、18℃、22℃、24℃の水温条件下では、40日齢までの生残率は14℃〜22℃において10℃、24℃区よりも有意に高く、成長率は高水温ほど高い傾向が認められた。また、14℃〜18℃の水温日周期を与えた飼育区では、生残率、成長率ともに14℃飼育区と18℃飼育区の間に位置し、日周期変化の効果は認められなかった。仔魚期の平均飼育水温との関係から、生残と成長に最適な水温は、それぞれ15.9℃、21.0℃であった。