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Yolk Utilization and Growth of Larvae of Japanese Flounder *Paralichthys olivaceus* at Different Temperatures

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Abstract

The effect of temperature on the egg hatching rate, yolk utilization and growth of larval Japanese flounder *Paralichthys olivaceus* was studied to determine optimal rearing conditions. During the yolk sac phase (from spawning to total yolk absorption) rearing temperatures were maintained at 12, 15 and 18℃. Larvae kept at 12 and 15℃ presented higher viable egg hatching rates, while survival to total yolk absorption was higher for larvae kept at 15℃ ($p<0.05$). Total length at hatching was significantly longer for larvae maintained at 18℃ than for those kept at 12 and 15℃ ($p<0.05$). This was due to the higher yolk absorption rate during incubation observed for the 18℃ group. At the end of the yolk sac phase however, larvae kept at 12 and 15℃ were longer than those at 18℃ ($p<0.05$). Efficiency of yolk conversion was estimated at 25.56, 24.93, and 23.13 mm/cal for the 12, 15, and 18℃ groups, respectively. As longer larvae should result in more successful initial feeding, a temperature regimen of 15℃ is expected to improve the growth and early development of *P. olivaceus* through a more efficient use of its yolk reserves.

Key Words : fish larvae • yolk utilization • growth • temperature • Japanese flounder

Introduction

The Japanese flounder *Paralichthys olivaceus* is one of the most important commercial species among Pleuronectid fish, with juveniles being artificially produced in large scale for cultivation to market size and for releasing at coastal areas in Japan[17,21].

As most marine fishes during their embryonic and larval stages, *P. olivaceus* also depends on the absorption of yolk reserves to provide its nutritional needs until it is able to start feeding. Growth during this period of yolk dependence is considered an important determinant of later survival because larval size and condition might affect the ability to begin exogenous feeding[7,8]. Larger fish are expected to be stronger, to swim better, to search a greater volume of water for food[6,14], to be less susceptible to damage and predation[4], to ingest larger food particles and to be less affected by competition[13] and starvation[1].

Fish size and growth during the yolk sac phase (from fertilization to complete yolk absorption) are functions of the amount of yolk, the rate of yolk absorption, and the efficiency with which yolk

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is converted into larval tissue. Although the amount of yolk present is basically determined by ovum size[11], both the rate and efficiency of yolk absorption largely depend upon environmental parameters, especially temperature. In general, the rate of yolk absorption varies directly with rearing temperature. The influence of temperature on yolk conversion efficiency however, is much more variable. While in some species conversion efficiency is found to increase with increased rearing temperature, in others efficiency decreases at higher temperatures[5], and in still others efficiency reaches a maximum at intermediate temperatures[18]. For culture purposes, it is desirable to adopt a temperature regimen that maximizes the utilization of energy reserves, resulting in the largest possible first-feeding larvae. To our knowledge however, no work dealing with the yolk utilization and larval growth has been conducted with this species. Therefore, this study was designed to determine the influence of temperature on the larval development of *P. olivaceus*.

Materials and Methods

Eggs were obtained from natural spawns produced by broodstock kept in an outdoor 200-ton tank, on Feb., 1989. At spawning time, water temperature was 10.2℃. Within four hours of spawning, only eggs found to be floating were placed in 1.0 liter beakers filled with 800mℓ of seawater collected at spawning time. Developing embryos were incubated at 12, 15 and 18℃ using a multiple temperature incubator. The experimental design consisted of three treatments with four replicates. Throughout incubation, density was kept at 1.5 eggs per mℓ and no aeration was provided. Every 6-8 hours during the incubation period, the developmental stage of the eggs was determined. After hatching, observations and measurements were made at 8-12 h intervals beginning with just hatched larvae and continuing throughout the developmental period to yolk sac absorption.

At spawning a sample of around 500 eggs was fixed in 4% $CaCO_3$ buffered formalin for further analysis. Yolk sac volumes were estimated using formulae appropriate for their shape. The volume of yolk in the eggs could be measured only once since later staged embryos curved over the yolk mass making precise measurements impossible. The one yolk measurement made prior to hatching was at spawning time, when eggs that had not started cleavage yet, and also probably some unfertilized ova, were collected and measured. The volume of yolk at this time was considered to be that of a sphere :

$$V = \pi / 6 \times d^3$$

where "d" is the diameter.

The yolk sac and oil globule of larvae had an ellipsoidal shape and their volume was calculated as in ALDERDICE *et al.*[2] :

$$V = 4/3\pi \ [(1/2d_1)^2 \times (1/2d_2)]$$

where d_1 is equal to the minor axis and d_2 to the major one. In a few cases the formula of the sphere was applied.

Egg samples were thoroughly washed in distilled water, dried to constant weight in an oven at 50℃ for

48 hours and weighed on an electronic balance to an accuracy of 0.1㎎. Fixation on formalin was considered to have no significant effect on dry weight[5]. Eggs were then combusted in a calorimeter (SHIMADZU CA-4P). Due to the minute size of the eggs, it was not possible to perform dechorionation, so the yolk was considered as having a caloric value of 89.09% of the egg total value, according to JOHNS and HOWELL[15] data on *Paralichthys dentatus*.

Total length of the larvae was measured from the tip of the snout to the end of the caudal fin fold, and the standard length to the end of the notochord. Total and viable hatching rates were estimated by incubating in triplicate an average of 50 eggs in test tubes filled with 50mℓ of seawater. Viable hatch is defined here as the percentage of larvae hatched without any abnormalities such as backbone and / or tail deformities.

Yolk conversion efficiency from spawning time to its total absorption was estimated by adapting the methodology of RYLAND and NICHOLS[19] :

$$\text{Conversion efficiency (mm / cal)} = \frac{\text{Growth rate (mm/day)}}{\text{Energy consumption (cal/day)}}$$

Survival rates were determined every 24 hours when the bottom of the beakers were siphoned and one third of the medium water was exchanged.

Observations on morphological and behavioral development deal especially with those characters concerning feeding and swimming functions, such as mouth / digestive tract, eye and fin development.

Standard treatment of data included analysis of variance (one-way ANOVA) and DUNCAN's Multiple Range Test. Differences present at the 5.0% level of probability ($p<0.05$) were considered to be significant. Percentage data were arcsine-square root transformed before analysis, but only untransformed values are presented. All data are presented as means (±SD).

Results

Eggs were pelagic and spherical in shape, and their diameter averaged 0.957 (±0.017) mm. Yolk within the egg occupied a volume of 0.375 (±0.026) mm^3, or approximately 81.7% of the egg volume. A single oil globule in the posterior portion of the yolk sac was present and its initial volume was 3.289×10^{-3} ($\pm0.713\times10^{-3}$) mm^3. The eggs collected at spawning time had a caloric value of 0.165 cal per egg or approximately 0.147 cal per yolk.

Mean temperatures observed during the experimental period were well in accordance with the levels previously designed. Mean values obtained for the designed levels of 12, 15 and 18℃ were 11.9 (±0.1), 15.3 (±0.1), and 18.3℃ (±0.3), respectively.

Developmental sequences were observed earlier in higher temperatures (Table 1) but regardless of temperature larvae presented the same morphological characters at hatching. A widely extended marginal fin surrounding the trunk and caudal areas, and no pectoral fin buds were evident. The eye lenses were already formed but not pigmented. A partially formed alimentary canal (mouth not formed and anus closed) could also be observed. As for pigmentation, chromatophores were spread over the entire body, except on the posterior part of the yolk sac, caudal area and eyes. Before total yolk absorption however, fully pigmented eyes, a functional mouth, an apparently functional digestive tract, and pectoral fins (though still lacking rays) were observed and therefore larvae were considered able to start first-feeding.

During development, yolk and oil globule volume decreased as a result of their utilization as energy source for maintenance and conversion to tissue (growth). Fig. 1 clearly demonstrates that before hatching the yolk utilization was rather low at 12 and 15℃. After hatching however, high yolk utilization rates were observed for these groups. For embryos maintained at 18℃, 48.1% of the yolk initial volume was absorbed before hatching, as compared to only 16.1 and 13.4% for the 12 and 15℃ groups, respectively.

Table 1. Mean period of time (hours) from spawning to selected developmental stages of *Paralichthys olivaceus* at different temperatures

DEVELOPMENTAL STAGE*	TEMPERATURE		
	12℃	15℃	18℃
APPEARANCE OF EMBRYO	44	28	23
CLOSURE OF BLASTOPORE	60	36	28
MOTILITY OF EMBRYO	92	58	44
50% HATCHING	114	66	52
PECTORAL FIN FORMED	180	120	94
MOUTH OPENING	238	144	108
TOTAL YOLK ABSORPTION	282	166	127

* Observations were made in 30 embryo and larvae (n=30)

Viable hatching rates presented significant differences between treatments (Table 2). Results obtained with incubation at 15℃ were not different from 12℃, but both were superior to 18℃ ($p<0.05$).

At hatching, larval length was significantly different between treatments ($p<0.05$). Larvae kept at 12℃ reached 2.42 mm (±0.18) and were smaller than those at 18℃ (2.72±0.09mm). Size of larvae held at 15℃ (2.60±0.13mm) was not significantly different from the other two treatments. At total yolk absorption, an inversion of the total length values was observed. Larvae kept at 12 and 15℃ were not different between themselves but were longer than those at 18℃ ($p<0.05$). Moreover, survival to total yolk absorption was significantly higher at 15℃ (Table 2). Growth rates at 12, 15 and 18℃ were estimated at 0.317, 0.526 and 0.687mm / day, respectively (Table 3). Growth and energy consumption rates were directly proportional to temperature (Table 3). Yolk conversion efficiency however, presented an opposite trend.

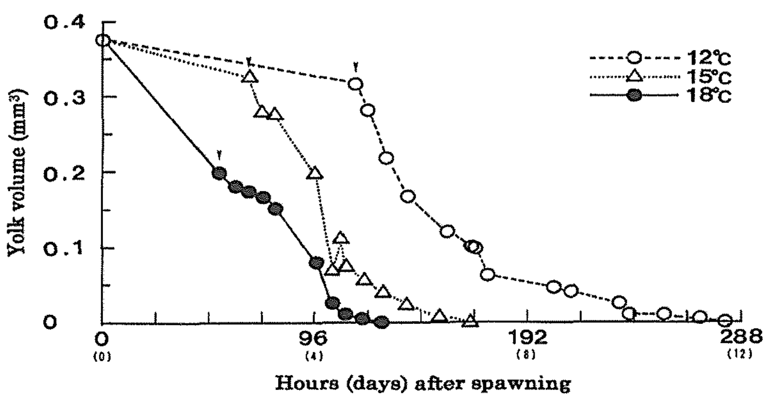

Fig. 1. Yolk utilization by the Japanese flounder (*Paralichtys olivaceus*) at different temperature levels. Arrow indicate 50% hatching.

Table 2. Mean (±SD) percentage of total and viable hatching rates, and survival to total yolk absorption of *Paralichthys olivaceus* larvae at different temperatures

OBSERVATIONS*	TEMPERATURE**		
	12℃	15℃	18℃
TOTAL HATCHING	70.2[ab] (2.3)	77.3[a] (3.2)	63.1[b] (5.6)
VIABLE HATCHING	65.8[a] (2.8)	70.3[a] (1.9)	53.9[b] (6.9)
SURVIVAL TO TOTAL YOLK ABSORPTION	62.1[b] (3.8)	77.5[a] (5.1)	49.1[c] (4.9)

*Observations were made in 50 eggs for total and viable hatching (n=50), and in 4 groups of 700-1,100 eggs for survival to total yolk absorption.
**Within rows, distinct superscripts represent significant differences ($p<0.05$).

Table 3. Mean (±SD) length at hatching and at total total yolk absorption, growth rate, energy consumption, yolk conversion efficiency of *Paralichthys olivaceus* larvae at different temperatures

OBSERBATIONS*	TEMPERATURE**		
	12℃	15℃	18℃
LENGTH AT HATCHING (mm)	2.42[b] (0.18)	2.60[ab] (0.13)	2.72[a] (0.09)
LENGTH AT YOLK ABSORPTION (mm)	3.69[a] (0.71)	3.63[a] (0.13)	3.38[b] (0.12)
GROWTH RATE (mm / day)	0.317	0.526	0.687
ENERGY CONSUMPTION RATE (cal / day)	0.0124	0.0211	0.0297
YOLK COMSUMPTION EFFICIENCY (mm / day)	25.56	24.93	23.13

*Observations were made in 30 larvae
**Within rows, distinct superscripts represent significant differences ($p<0.05$).

Discussion

Like most marine fishes with pelagic eggs, *P. olivaceus* hatches out with its organs in a precocious state. Newly hatched larvae present no eye pigmentation, lack a functional digestive tract, and the pectoral fin buds are not in evidence. Similarly, SEIKAI *et al.*[20] reported that flounder larvae hatch out ontogenetically earlier than other species do. Though their mouth is not formed at hatching, jaw and buccal cavity development is rapid during the yolk sac phase, suggesting a rapid development of predatory abilities. The high rates of differentiation and growth post-hatching are believed to be essential determinants of later survival. Many authors[3,12,16] have reported identical early growth patterns in other species and concluded that this is a compensatory mechanism, for smaller-sized larvae, considered that larvae that do not achieve a rapid growth and differentiation would present reduced survival rates.

Temperature is known to exert one of the most potent influences on the rate of development. The reduction of hatching and developmental time clearly demonstrates that temperature indeed affected the biological processes occurring during early development. Higher rearing temperatures reduced both the duration of the yolk sac period and the energy available for growth during that period, specially prior to hatching. Eggs incubated at 18℃ produced the longest larvae at hatching. The analysis of this single datum would lead us to the conclusion that 18℃ is the optimum temperature for the early development of flounder. However, it must be taken into consideration that such a "maximum length at hatching" was obtained due to the absorption of almost 50% of the initial yolk volume. At 12 and 15℃ embryos consumed only about 15% of their yolk and after hatching, despite their smaller size, they disposed of a greater amount of yolk. Consequently, at total yolk absorption larvae at 12 and 15℃ were significantly larger than those at 18℃. It seems that at 18℃ embryos present a reduced yolk conversion efficiency probably as a result of increasing maintenance costs. This has already been demonstrated in salmonids[9,10].

In some fish species efficiency of yolk utilization reaches a maximum within their range of thermal tolerance, decreasing towards both lower and upper limits. Other species, respected their range of thermal tolerance, present a thermal independence, *i.e.*, the ability to compensate physiologically for changes in temperature due to the following interrelated processes : the rate of increase in metabolic requirements over the temperature range and the decrease in developing time with rising temperature[5]. In case both processes change at the same rate, the yolk utilization efficiency would remain constant. JOHNS and HOWELL[15] found that the summer flounder *Paralichthys dentatus* presents such a thermal independence and therefore gross conversion efficiencies were similar within the range of temperatures studied. The present results show that yolk utilization and growth are similar in *P. olivaceus* larvae reared at 12 and 15℃, while those held at 18℃ were inferior in most aspects examined. This suggests that *P. olivaceus* is not able of maintaining thermal homeostasis within its range of thermal tolerance and also that its maximum yolk utilization is around 15℃.

At total yolk absorption, the longest larvae were obtained at 12 and 15℃. If it is assumed that longer larvae present a more successful initial feeding, then a better post-feeding growth and survival is expected. This assumption however, must be further investigated. According to the present results we conclude that the maintenance of a temperature regimen of 15℃ might improve not only the egg hatching rate and larval survival, but also the growth of *P. olivaceus* larvae.

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異なる水温で飼育したヒラメ仔魚の卵黄利用と成長

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ヒラメの卵および仔魚の飼育における最適条件を明らかにするために，受精卵と卵黄仔魚を水温 12, 15 および 18℃で飼育し，これらの温度が卵の孵化率および仔魚の卵黄利用と成長に及ぼす影響について検討した。卵の正常孵化率は 12℃と 15℃において高く，卵黄吸収完了までの仔魚期の生残率は 15℃ において最も高かった（$p<0.05$）。また，孵化時の仔魚の全長は 18℃のものが 12℃と 15℃ のものより大であったが（$p<0.05$），卵黄吸収完了時の仔魚の全長は反対に，12℃と 15℃の方が大であった（$p<0.05$）。12, 15, 18℃における卵黄転換効率はそれぞれ 25.56, 24.93, 23.13mm / cal であった。卵黄を吸収して摂餌を開始する仔魚にとって，そのサイズが大きいということは摂餌戦略の面で有利である。以上の結果の総合的判断から，ヒラメの卵および仔魚の飼育における最適温度は 15℃ と推定される。

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