AN ABSTRACT OF THE THESIS of Lawrence A. Collins for the Master of Science in Biology presented 02 November 1992.

Title: The Effects of Temperature on Oxygen Consumption, Growth, and Development of Embryos and Yolk-Sac Larvae of *Siganus randalli* Woodland (Pisces: Siganidae)

Approved: _____
Stephen G. Nelson, Chairman, Thesis Committee

Eggs collected from laboratory spawnings of the coral-reef fish *Siganus randalli* were incubated at two temperatures (27°C and 30°C). The eggs and larvae were sampled routinely until larval starvation. Changes in oxygen consumption, growth, yolk utilization, and development were monitored routinely. Oxygen consumption, which peaked at hatching, was higher for embryos reared at 30°C than for those reared at 27°C. Rates of oxygen consumption at hatching were approximately the same as those recorded for other temperate and tropical species. Rates of oxygen consumption by yolk-sac larvae were highly variable, and these data suggest that larval oxygen consumption prior to yolk-sac absorption may not be significantly influenced by temperature. A rapid increase in larval length was observed during the first 24 hours post-hatching. After this initial growth, length of larvae kept at 30°C was observed to decrease with age. Rates of yolk depletion were higher for larvae kept at the higher temperature. Egg sizes, egg weights, and maximum notochord lengths of the larvae were significantly different between spawns. Oxygen consumption rates by the embryos for any given age during development varied between spawns, but the regression lines describing oxygen consumption as a function of age did not differ significantly. The initiation and completion of eye pigmentation were used as developmental markers to calculate the amount of yolk present for the larvae at the different temperatures. The larvae maintained at 30°C completed eye pigmentation approximately three hours sooner than those larvae maintained at 27°C, but had less endogenous reserves. This finding indicates

that there is a trade off between rapid development and efficient utilization of the endogenous reserves. The completion of eye pigmentation for larvae reared at the higher temperature occurred at midnight, and, depending on the amount of time that the larvae have to initiate feeding prior to the point-of-no-return, the timing of completion of eye pigmentaion could influence larval survival.

THE EFFECTS OF TEMPERATURE ON OXYGEN CONSUMPTION, GROWTH, AND DEVELOPMENT OF EMBRYOS AND YOLK-SAC LARVAE OF *SIGANUS RANDALLI* WOODLAND (PISCES: SIGANIDAE)

BY

LAWRENCE A. COLLINS

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TO THE GRADUATE SCHOOL:

The members of the Committee approve the thesis of Lawrence A. Collins presented 02 November 1992.

Stephen G. Nelson, Chairman

John M. Lacson

John M. Lacson

Charles E. Birkeland

Charles E. Birkeland

Harley I. Manner

Harley I. Manner

ACCEPTED:

David M. Gillespie
Dean of Graduate School and Research

11-13-92
Date

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INTRODUCTION

Although information exists concerning the effect of temperature on larval developmental biology in temperate fishes, there remains a scarcity of data for fishes inhabiting tropical regions. This work details the effects of two temperatures (27°C and 30°C) on the early larval development of the rabbitfish *Siganus randalli* Woodland (1990) by quantification of: 1) respiration rate; 2) rate of larval growth as quantified by length; 3) rates of absorption of the yolk sac and oil globule; and 4) time to acquisition of selected ontogenetic characteristics.

The influence of temperature on development has been well documented in temperate freshwater species such as chinook salmon, *Oncorhynchus tshawytscha*, (Alderdice and Velsen, 1978); danube salmon, *Hucho hucho* (Jungwirth and Winkler, 1984); brown trout, *Salmo trutta fario* (Jungwirth and Winkler, 1984); and salmon as reviewed by Crisp (1981). Hatching times for commercial salmon and trout species are predictable (±2.3%) given the temperature at which the eggs are kept and the day of spawning in the hatchery (Alderdice and Velsen, 1978; Crisp, 1981; Jungwirth and Winkler, 1984). In general, increased rates of development and shorter times to hatching are the trends for salmon at warmer temperatures (Crisp, 1981).

Similarly, the effect of temperature on many marine fish larvae found in temperate regions has also been well documented. The rate of development for sardine, *Sardina pilchardus*, and summer flounder, *Paralichthys dentatus*, was shown to increase with increased temperature (Miranda *et al.*, 1990; Johns and Howell 1980; Johns *et al.*, 1981). However, neither Johns *et al.* (1981) nor Miranda *et al.* (1990) discussed whether the larvae kept at different temperatures reached the same developmental stage with differing amounts of yolk.

For summer flounder, *Paralichthys dentatus*, Johns and Howell (1980) and Johns *et al.* (1981) reported that there were significant differences in notochord length at

hatching between temperatures, but that there were no significant differences in larval notochord lengths or dry weights after the yolk sac had been absorbed. Fukuhara's (1990) results for four marine fishes (i.e. porgy, *Acanthopagrus schlegeli*; Japanese anchovy, *Engraulis japonica*; red sea breem, *Pagrus major*; and Japanese flounder, *Paralichthys olivaceus*) were consistent with Johns *et al.* (1981) with regard to length after yolk-sac absorption. Both Fukuhara (1990) and Johns *et al.* (1981) reported that yolk utilization increased as experimental temperature regimes increased, although no evidence of statistical testing, or rates of utilization were presented in these studies.

Respiration rates of fish embryos and larvae have been found to increase with age and weight (Houde and Schekter, 1983; Kiørboe *et al.*, 1987; Laurence, 1975, 1978; Giguère *et al.*, 1988). Increased respiration rates of fish larvae are to be expected since larval activity increases, concomitant with growth, as larvae begin to pursue and capture prey. Additionally, Giguère *et al.* (1988) and Laurence (1975) found that respiration was significantly higher with increased temperatures for larvae of Atlantic mackerel, *Scomber scombrus*, and eggs and larvae of winter flounder, *Pseudopleuronectes americanus*, respectively. Such a finding indicated that higher metabolic rates are to be expected as environmental temperatures increase and, therefore, higher metabolic rates may increase the probability of starvation for tropical larvae (Bagarinao, 1986; Houde, 1974; Houde, 1989). Relatively little research has focused on the effects of temperature on larval development of tropical marine fishes. A noteworthy study by Bagarinao (1986) on larval development of tropical marine fishes has documented the transition from endogenous to exogenous feeding of larval milkfish, *Chanos chanos*, seabass, *Lates calcarifer*, and rabbitfish, *Siganus guttatus*. Bagarinao (1986) delayed initial feeding of larvae at varying intervals of time from hatching to demonstrate that tropical larvae: 1) require food sooner after hatching; and 2) reach the point-of-no-return (PNR or the point at which death for the larvae is inevitable even if food becomes available), sooner than for temperate larvae (see also Houde, 1974; Laurence, 1978).

This research provides some of the first information regarding metabolism of tropical fish larvae. It examines not only the effect of temperature on the amount of time required to reach certain developmental stages, but also examines the amount of endogenous reserves remaining at eye pigmentation, as an indicator of a trade-off between accelerated developmental rate and efficient utilization of the endogenous reserves. This information may help to explain how temperature affects rates of *Siganus randalli* recruitment, and may be useful in the development of hatchery production methods.

MATERIALS AND METHODS

Study Species

Siganus randalli is a recently described species of siganid closely resembling *S. vermiculatus* (Woodland, 1990). *S. randalli* has been collected in Kosrae, Papua New Guinea, Solomon Islands, Pohnpei, and Guam. Although Woodland (1990) notes that he collected specimens in clear water over coral reefs, specimens collected by personnel from the University of Guam Marine Laboratory have all come from mangrove habitat. These specimens were juvenile recruits and have been collected throughout the year in Sasa Bay of Apra Harbor. *S. randalli* has been successfully spawned and reared in captivity and produces demersal adhesive eggs which hatch in ≈20 hours at 29°C (Nelson *et al.*, 1992).

Spawning Procedure

Siganus randalli broodstock were maintained in a cement pond (≈210 m^3) supplied with flow-through saltwater from an underground well at the Guam Aquaculture Development and Training Center (GADTC). The broodstock were fed with a commercial pellet food at 3% of their body weight. *S. randalli* were captured from the pond the day of the new moon each month. The female was chosen by visual inspection of the abdomen for the presence of swelling, an indication of reproductive readiness also noted by Juario *et al.* (1985) for *S. guttatus*. The males were chosen if the milt could be easily obtained by lightly squeezing the abdomen. One female (21±0.5 cm standard length) and one male were taken to the University of Guam Marine Laboratory to stock a 2,000-ℓ round fiberglass tank supplied with continuously flowing seawater (≈10 ℓ/min) and moderate aeration.

Results of previous studies revealed that *Siganus randalli* typically spawns around midnight. The flow of seawater was stopped at 2200 - 2300 hours to prevent washing

the eggs out during spawning, and the aeration was turned down low. Square plastic plates (20 cm x 20 cm) were placed on the bottom of the tank to collect the eggs.

Larval Rearing

Within one hour of spawning, four plates were removed from the spawning tank. The egg-covered plates were placed into glass aquaria (10ℓ) filled with 1-μm filtered seawater (31 ppt). Two aquaria were placed in each of two water baths (Blue M-Magni Whirl) that were set at constant temperatures (27°C±0.2°C and 30°C±0.2°C) for development of the embryos and larvae. Preliminary investigations had shown that there was adequate seawater surface area exposed to maintain oxygen levels in the aquaria (>80% saturation) without the use of aeration.

The plates were removed from the aquaria after enough eggs had hatched to provide a larval density of approximately 50/ℓ. The number of larvae in each aquarium was estimated by extrapolating the number of larvae captured in a 100-mℓ beaker to the volume of the aquarium.

Water quality was monitored regularly for salinity and dissolved oxygen, and occasionally for pH, throughout experimental trials. Siphoning and water exchanges were performed as necessary to clean the tank. Larvae were not fed.

Temperature

At least two replicate spawns with two aquaria for each spawn were used for each of the two experimental temperatures (27°C and 30°C). These two temperatures were chosen to represent mid- and high-range sea surface temperatures around Guam, as well as low- and mid-range temperatures found in outdoor cultures on Guam.

Respiration Measurements

A Radiometer dissolved-oxygen probe attached to a Strathkelvin (Model 381 or 781) dissolved-oxygen meter and a Kipp and Zonen chart recorder were used to measure oxygen consumption of the embryos and larvae. The respirometer and chart recorder

were calibrated to zero and 100% oxygen saturation after four measurements of oxygen consumption by the embryos or larvae. The glass respiration chamber (volume from 1.0 to 3.0 mℓ) was maintained at a constant temperature by pumping water from the water bath through the glass jacket surrounding the chamber (Figure 1). The percentage of oxygen in the respiration chamber was plotted by the chart recorder, and the rate of oxygen consumption was determined from the resulting plot. Since there was a slight decline in the amount of oxygen present in the chamber which was not due to the metabolism of the embryos or larvae, the decrease in oxygen of a sample of seawater (blank) was measured after every four samples of eggs or larvae. The value for the blank was then subtracted from the respiration values as a correction factor.

The oxygen content (μℓ/ℓ) of the seawater at saturation in the respiration chamber was taken assumed to be equivalent to oxygen levels given by Richards and Corwin (1956), and the oxygen consumption rate per individual was calculated accordingly. The initial concentration was multiplied by the volume of seawater in the respiration chamber to give the initial amount of oxygen present in μℓ. Multiplication of the initial amount by the percentage of oxygen consumed (determined from the plots made by the strip-chart recorder) gave the μℓ of oxygen consumed. The amount of oxygen consumed was then divided by the time (in hours) taken for consumption. The blank was then subtracted from this value. The corrected value was divided by the number of individuals in the respiration chamber, and multiplied by 1,000. This yielded the amount of oxygen consumed per hour per individual (nℓ O2/hour/ind).

Oxygen consumption rates for three spawns were plotted against time for each temperature, and compared with linear regression analyses. Oxygen consumption rates of the eggs and larvae were plotted and compared separately.

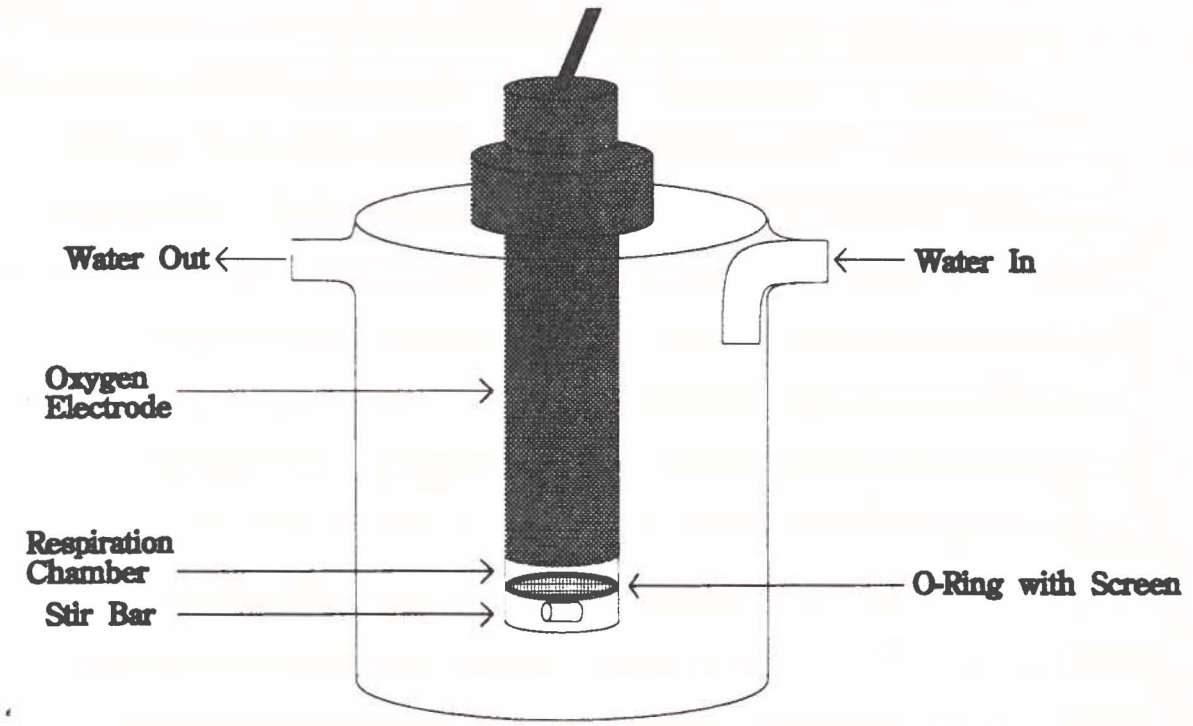


Figure 1. Respiration chamber.

Egg Respiration

Eggs were removed from the aquaria for oxygen consumption analysis. Approximately twenty eggs and 2 mℓ of seawater were removed from the plate in the aquarium and transferred in a pipette to the closed respiration chamber (Figure 1). The respiration chamber was equipped with a moving stir bar to provide adequate mixing of the water and to prevent oxygen gradients from developing around the eggs. The eggs were suspended above the stir bar on a platform made from a piece of 200-µm nylon mesh attached with silicone sealant to an O-ring.

The decrease of oxygen in the chamber due to respiration of the eggs was monitored. After 30 minutes of measuring the decrease in oxygen, the volume of water was measured to the nearest 0.01 mℓ with an one-mℓ pipette, and the eggs were removed and placed into a petri dish. The eggs were then examined with a compound microscope to determine the number of fertile eggs. The respiration chamber was then rinsed with Millipore 18-MΩ water and 0.1-µm filtered seawater and the process repeated until the onset of hatching.

Larval Respiration

The larvae were found to be much more delicate than the eggs and often die when suctioned with a pipette. Therefore, one to five larvae were carefully removed from an aquarium with a 10-mℓ beaker. The larvae and seawater were placed into the closed respiration chamber, and respiration recorded. Stirring was unnecessary for the larvae since they were mobile.

Measurements of Egg Size, Larval Length, and Yolk Sac Volume

After respirometry, the sample of eggs was used to determine whether there was any difference in egg diameters between temperatures or spawns. The diameters of ten eggs from each sample were measured with an ocular micrometer to the nearest .01 mm.

The egg diameters from different spawns were compared for differences between temperatures and spawns with a two-way analysis of variance (ANOVA).

Samples of 8-10 larvae were taken periodically from the aquaria, anesthetized with tricaine methane sulfonate (MS-222), and measurements were made of: 1) notochord and total length (NL and TL), 2) the length of the yolk sac (L), 3) the height of the yolk sac (H), and 4) the diameter of the oil globule (D) (Figure 2).

NL and TL were plotted against larval age. There was a distinct change in the growth pattern between the first 20 hours (post-hatching) and the latter 80 hours; thus, the plots were divided into the two stages and compared separately. The first 20 hours of growth was nonlinear, so nonlinear regression was used to describe the growth in length (NL and TL in millimetres) according to the Gompertz growth curve, where

$$NL=p_1 e^{-p_2 e^{-p_3 t}}$$

and p_1 is the asymptote or L_∞; p_2 is a parameter, such that $p_2 * p_3$ is the instantaneous growth rate when t=0 and $L=L_i$; p_3 is the instantaneous rate of growth at t=0; and t is age (hours) post-hatching (Ricker, 1979; Polo *et al.*, 1991). This formulation followed the methods of Polo *et al.* (1991). The instantaneous growth rates (p_3) were then compared with a one-way ANOVA.

After the initial growth phase, very little subsequent change in length was observed. The lengths of the larvae at each temperature were plotted against time and compared between temperatures with linear regression analyses.

The length (L) and height (H) of the yolk sac were used to calculate the amount of yolk present according to the formula:

$$V_y=\pi/_6 LH^2$$

where V_y was the volume ($\mu\ell$) of the yolk sac; L (mm) was the length of the yolk sac; and H (mm) was the height of the yolk sac (Avila and Juario, 1987; Heming and Buddington, 1988). This formula is described in Heming and Buddington (1988) and was used by

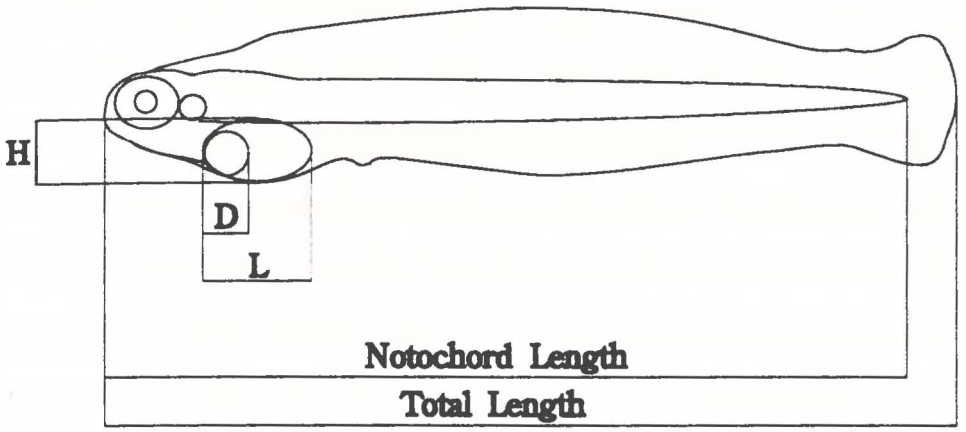


Figure 2. Measurements taken of *Siganus randalli* larva.

Avila and Juario (1987) for another species of siganid. The volume of the yolk platelets was calculated by subtraction of the volume of the oil globule from that of the entire yolk-sac. The yolk-platelet volume was then log transformed, plotted against age, and between temperatures with linear regression analyses. The mean values for each sampling time were used to describe the rate of depletion for the yolk platelets for each temperature. The relationship between yolk volume and age was non-linear and was described as a non-linear regression by an iterative, least-squares method also used in Nelson and deC Wilkins (in review). The formula used was:

$$V=p_1e^{-p_2t}$$

where V was the volume (μl), p_1 was the initial volume, p_2 was the rate of depletion, and t was age (Jennrich, 1990; Heming and Buddington, 1988). The values for p_2 were compared with an ANOVA as were the instantaneous rate of growth values mentioned previously.

The diameter of the oil globule was halved to give the radius (R). The radius (R) was used to determine the volume of the spherical oil globule by the equation:

$$V_{o.g.}=4/_3\pi R^3$$

where $V_{o.g.}$ is the volume (μl) of the oil globule and R is the radius. This formula was described in Heming and Buddington (1988), and has been used by Avila and Juario (1987) for another species of siganid. The volume of the oil globule was log-transformed, plotted against age, and compared in the same manner as for the depletion of the yolk platelets. Likewise, the mean from each sample was plotted against age and this relationship was described as a non-linear regression for each temperature. The depletion rates were compared between temperatures with a one-way ANOVA.

Development

Determination of the effect of temperature on development was obtained by measurement of time required to: 1) 50% hatching of the eggs; and 2) initial and

complete pigmentation of the eyes were recorded for the eggs and larvae. Determination of the effect of temperature on hatching was obtained by removing ten eggs from each aquaria of each temperature regime and placing the eggs into separate test tubes, each containing 10 mℓ of seawater. The test tubes were then placed within water baths, regulated to the experimental temperatures. The time when 50% of the eggs in each test tube had hatched was noted and compared between temperatures with a two-way ANOVA. Times to initiation and completion of eye pigmentation was compared with a two-way ANOVA. Samples of 8-10 larvae were taken from each of the aquaria and examined with a compound microscope. Initiation of eye pigmentation was defined as a distinct dark blotch covering the upper ¼ of the eye. Completion of eye pigmentation was defined as a complete blackening of the eye with a metallic sheen. The time when 50% of the larvae examined had reached initial and complete eye pigmentation was noted. These times were compared with a two-way ANOVA.

Weight

After measurement, the eggs were rinsed with Millipore 18-MΩ water, dried to a constant weight in a drying oven (56°C), and weighed individually to the nearest 0.1 µg on a Cahn C-31 Electrobalance.

Statistics

All statistical analyses were performed with the BMDP package of statistical software (BMDP Statistical Software, Inc.; Los Angeles CA USA) (Dixon *et al.*, 1990). ANOVAs and two-way ANOVAs, as well as Levene's test and the Brown-Forsythe method, were performed with BMDP package 7D (Dixon *et al.*, 1990). ANCOVAs were performed with BMDP package 2V (Jennrich *et al.*, 1990). Linear regression analyses were performed with BMDP package 1R (Dixon *et al.*, 1990). Nonlinear regression analyses were performed with BMDP package 3R (Jennrich, 1990).

RESULTS

Oxygen Consumption

As development progressed, oxygen consumption by the embryos for each spawn increased to rates of between 150-200 nl/hr/ind prior to hatching (Figure 3). The regression lines describing the relationship between age and oxygen consumption were compared for differences in either the slopes or the intercepts between spawns and between temperature classes. No significant differences were found between spawns within temperature classes; therefore, all of the data between spawns were grouped under their respective temperature classes and compared with linear regression analyses. The results of the linear regression were highly indicative that there is a relationship between temperature and oxygen consumption ($F_{2,46}$=3.108, p=.05422). Furthermore, a visual inspection of the data revealed that in paired comparisons, oxygen consumption by the embryos at 30°C was higher than in embryos kept at 27°C in almost every paired comparison (Figure 3). In order to remove the effect of age, an ANCOVA was performed. There were two grouping factors (spawn and temperature) and one covariate (age). The results showed that there was a significant difference in oxygen consumption between temperatures ($F_{1,2}$=4.44, p=0.0409); there was no significant difference between spawns ($F_{2,2}$=2.09, p=0.1367) and there was no significant interaction between spawn and temperature ($F_{2,1}$=0.59, p=0.5573).

The final datum for November at 30°C may be the result of an early hatched larva (Figure 3A & B). If so, the datum would agree well with the findings of Nelson and deC Wilkins (in review) of a post-hatching peak in oxygen consumption by *Siganus randalli* larvae. The single data point was still well below the pre-hatching value for *Chanos chanos* (400 nl/hr/ind) reported by Walsh *et al.* (1991), and since no experimental error is known to have occurred, the datum was retained in the analyses.

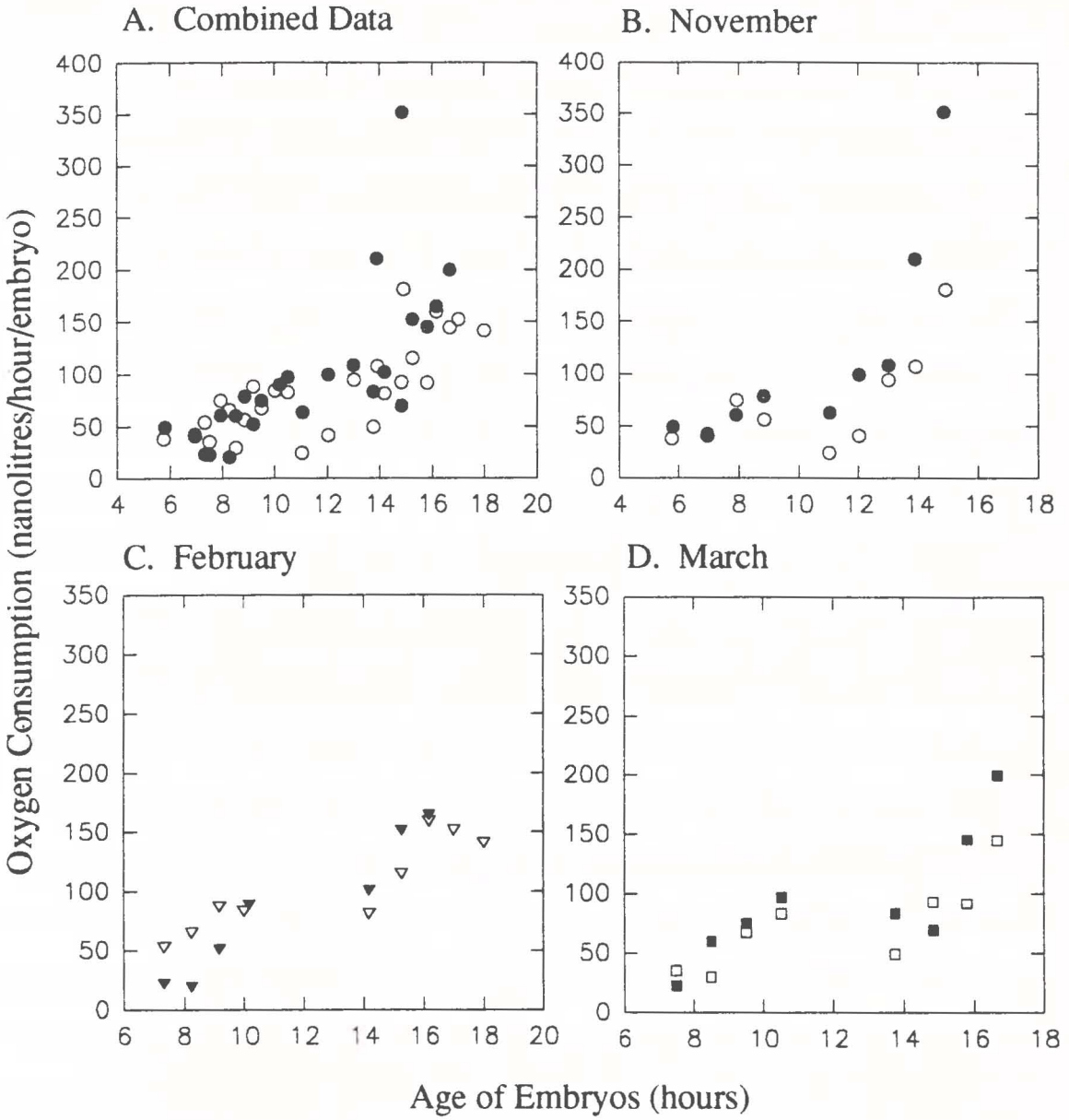


Figure 3. Oxygen consumption of *Siganus randalli* embryos. Open symbols represent embryos kept at 27°C; filled circles, embryos kept at 30°C.

Oxygen consumption of the larvae was also compared between temperatures with linear regression analyses (Figure 4). The slopes of the lines describing the relationship between age and rate of oxygen consumption were not significantly different from 0 at either 27°C ($F_{1,7}$=0.000, p=0.9972) or at 30°C (F1,7=0.051, p=0.8275) (Figure 4). Since there was no relationship between age and oxygen consumption observed, the data were pooled under their respective temperature regime and an ANOVA was performed to test for differences between temperatures. No significant difference in oxygen consumption was observed between the larvae kept at 27°C and 30°C ($F_{1,16}$=2.10, p=0.41706). The mean rate of oxygen consumption for all of the yolk-sac larvae was 164.64±10.13 nl/hr/ind (mean±std. err.).

Eggs

Thirty values for egg diameter were selected at random from each temperature class of each spawn and compared with a two-way ANOVA to test for differences between spawns and temperatures. Egg size differed significantly between spawns ($F_{4,4}$=10.74, p<0.0001) despite maintenance of a constant size for the female. Egg size did not differ between temperatures ($F_{1,4}$=0.12, p=0.7297), and there was no significant interaction ($F_{4,139}$=0.76, p=0.5512). Mean egg diameters for each spawn are given in Table 1.

The times required for 50% hatching of the embryos were compared between temperatures and spawns with a two-way ANOVA. These times did not have homogeneous variances (Levene's test), so the Brown-Forsythe method was used as a subsequent test. The latter method does not assume equality of variances. The times to hatching differed significantly both between temperatures ($F_{1,30}$=2268.77, p<0.0001) and between spawns ($F_{2,30}$=64.07, p<0.0001). The hatching times are given in Table 2.

After five hours of development at the two temperatures, the embryos were observed to be at different developmental stages. The embryos kept at 27°C were

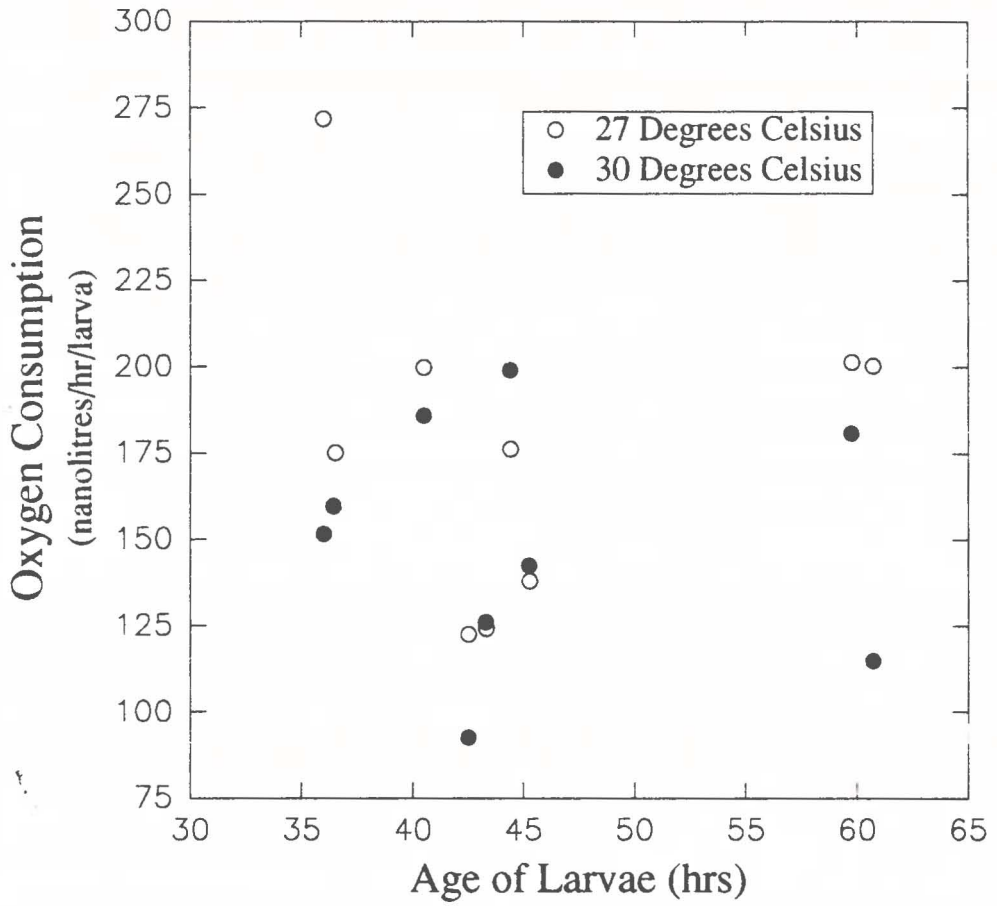


Figure 4. Oxygen Consumption of *Siganus randalli* larvae. Age is post-spawning.

Table 1. Mean egg diameters (±std. err.) (mm) between spawns and temperatures. Letters indicate which groups were significantly different (95% confidence) as determined by Student-Newman-Keuls multiple range test.

	27°C	SNK	30°C	SNK
November 1991	0.580±0.004	a, b, c	0.585±0.004	b, c
January 1992	0.573±0.004	a, c	0.570±0.006	a, c
February 1992	0.597±0.005	b	0.600±0.004	b
March 1992	0.587±0.006	b, c	0.587±0.006	b, c
April 1992	0.574±0.009	a, c	0.563±0.004	a

Table 2. Mean hatch times (±std. err.) (hrs) between spawns and temperatures. Letters indicate which groups were significantly different (95% confidence) as determined by Student-Newman-Keuls multiple range test.

	27°C	SNK	30°C	SNK
November 1991	23.488±0.087	a	19.963±0.095	d
January 1992	22.110±0.075	b	19.651±0.009	e
March 1992	22.718±0.129	c	19.288±0.009	f
Mean Hatch Times	22.772±0.119		19.634±0.060	

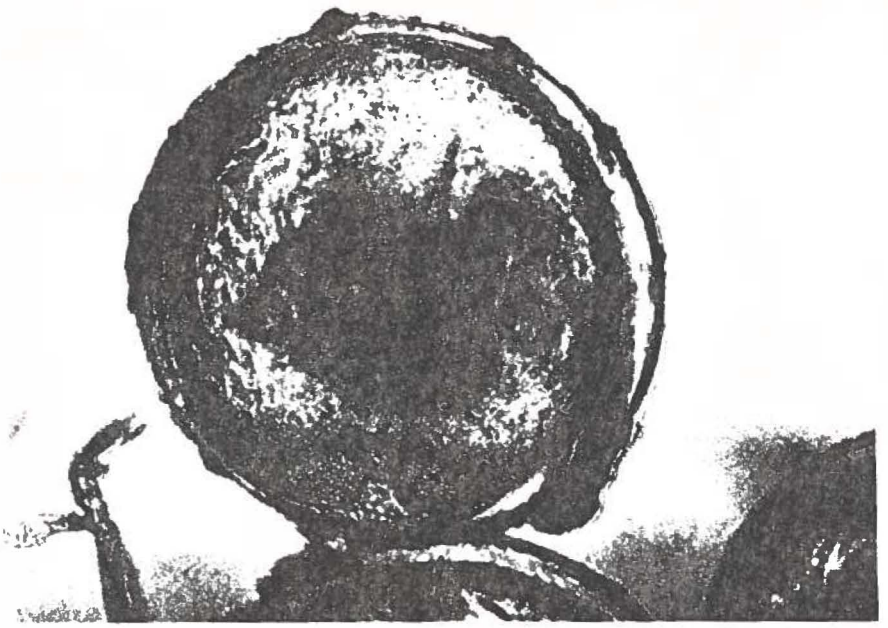
approximately one hour behind those embryos kept at 30°C (Figure 5). After ten hours of development, the larvae kept at 27°C were two hours behind the embryos kept at 30°C (Figure 6).

Larval Length

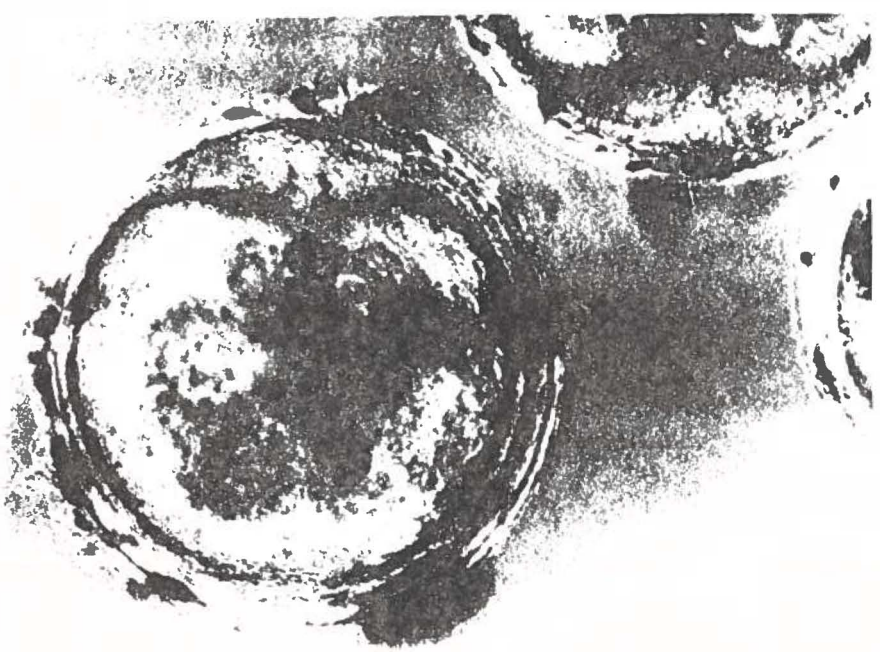
Extremely rapid growth was seen for the larvae during the first 20 hours post-hatching (Figure 7). Mean notochord lengths were plotted against age (post-hatching), and the Gompertz growth curve was used to describe the parameters of this initial growth phase (Table 3). Instantaneous growth rates were calculated, and compared by an ANOVA between the two temperatures. Although the instantaneous growth rates did not significantly differ between the two temperatures, the values were higher for the larvae reared at 30°C than those reared at 27°C (Table 4).

Maximum notochord length attained was also compared between spawns and between temperatures. A two-way ANOVA was performed to determine whether there was a significant relationship between length and temperature or between length and monthly spawns. The variances differed significantly between spawns (Levene's test; $F_{3,72}=3.32$, $p=0.0244$), so the Brown-Forsythe method was used to evaluate differences between the two temperatures ($F_{1,48}=0.00$, $p=0.9645$), and between spawns ($F_{3,48}=9.17$, $p=0.0001$). Table 5 gives the mean maximum notochord lengths (±std. err.) for the different spawns and temperature regimes.

After approximately the first twenty hours of growth, the lengths stabilized for a short period of time. The mean length was then seen to decrease until starvation for larvae kept at 30°C, but this decrease was not as evident for larvae kept at 27°C (Figure 8). The regression lines describing the changes in length during this second stage of growth differed significantly between the two temperatures (Table 6).



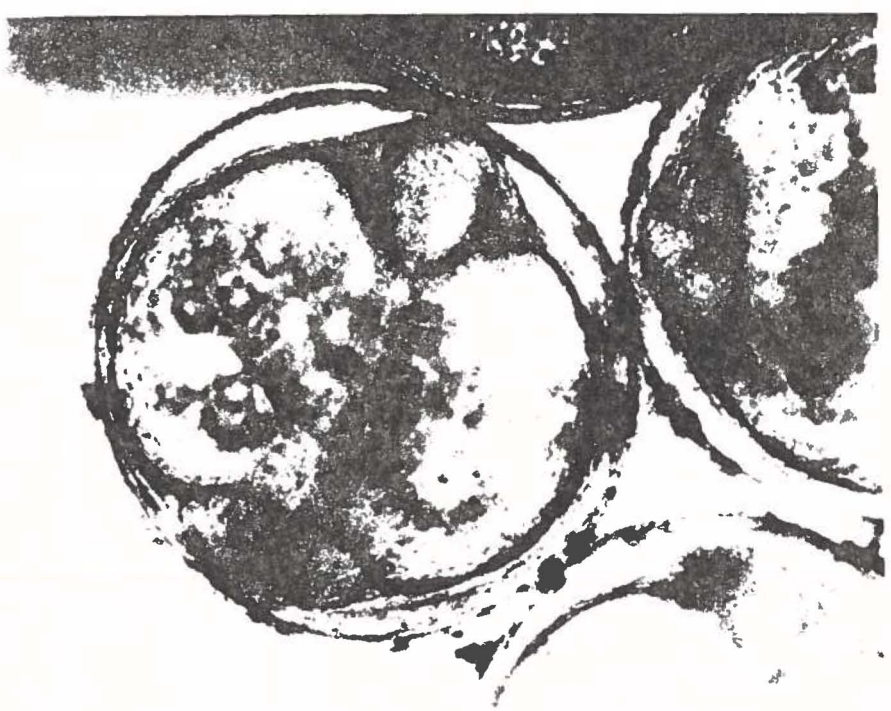
A



B

Figure 5. Embryos of Siganus randalli. Photograph A is an embryo 6.5 hours of age reared at 27°C. Photograph B is an embryo 5.5 hours of age reared at 30°C.

A



B

Figure 6. Embryos of *Siganus randalli*. Photograph A is an embryo 9.5 hours of age reared at 27°C. Photograph B is an embryo 7.5 hours of age reared at 30°C.

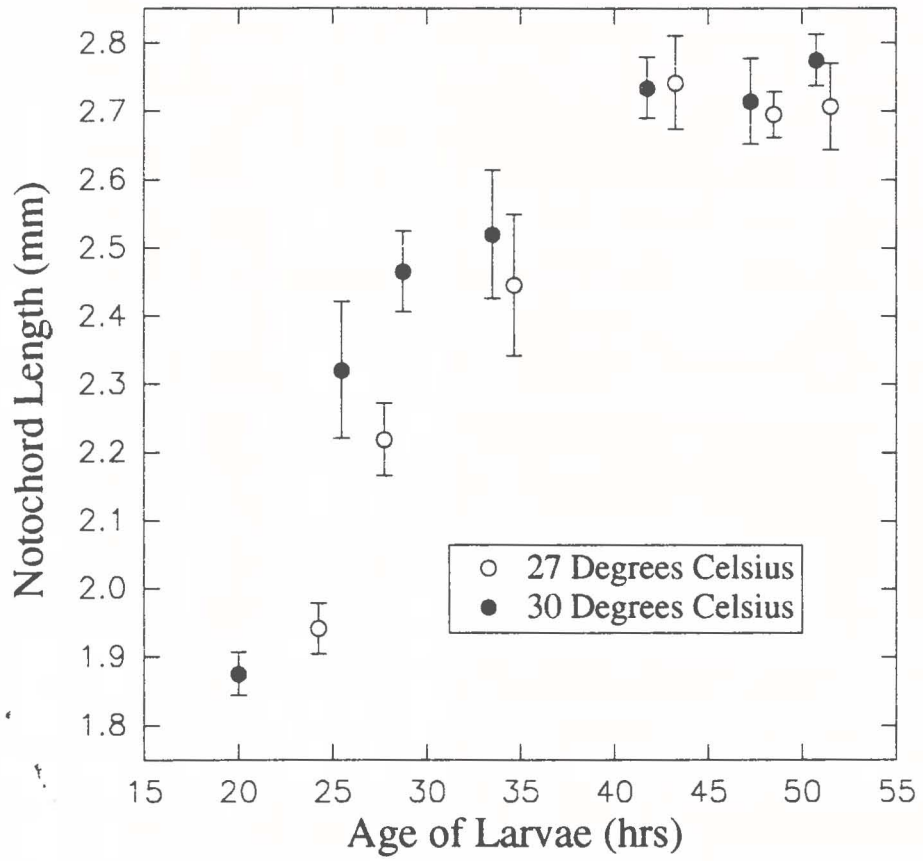


Figure 7. Initial growth of larval *Siganus randalli*. Age is post-spawning.

Table 3. Parameter estimates (±std. dev.) for increase in notochord length . P1 is the maximum length (mm), p2 is a constant, and p3 is the instantaneous growth rate (mm/hr) at t=0 for larvae kept at 27°C and 30°C.

	January		February		March	
	27°C	30°C	27°C	30°C	27°C	30°C
p1	2.730384±.030629	2.766161±.036672	2.908630±.150045	2.776916±.032407	2.642432±.000508	2.688225±.014372
p2	0.526696±.059005	0.494125±.036006	0.483756±.054619	0.449517±.037243	0.442912±.001104	0.377043±.017634
p3	0.133448±.023833	0.128131±.017646	0.107583±.036809	0.177983±.027567	0.246259±.001098	0.230377±.021318

Table 4. Mean instantaneous growth rates (±std. err.) (mm/hr) of length for larvae kept at 27°C and 30°C.

	Mean Instantaneous Rate of Growth		
	27°C	30°C	p-value
Notochord Length	0.162±0.043	0.179±0.030	$F_{1,4}$=0.10, p=0.7674
Total Length	0.159±0.037	0.167±0.039	$F_{1,4}$=0.02, p=0.8949

Table 5. Mean maximum notochord lengths (±std. err.) (mm) between spawns and temperatures. Letters indicate which groups were significantly different (95% confidence) as determined by Student-Newman-Keuls multiple range test.

	Notochord Length			
	27°C	SNK	30°C	SNK
November 1991	2.672±0.028	a, b	2.628±0.039	a
January 1992	2.729±0.034	a, b, c	2.725±0.051	a, b, c
February 1992	2.778±0.018	b, c	2.802±0.020	c
March 1992	2.636±0.028	a	2.664±0.023	a, b

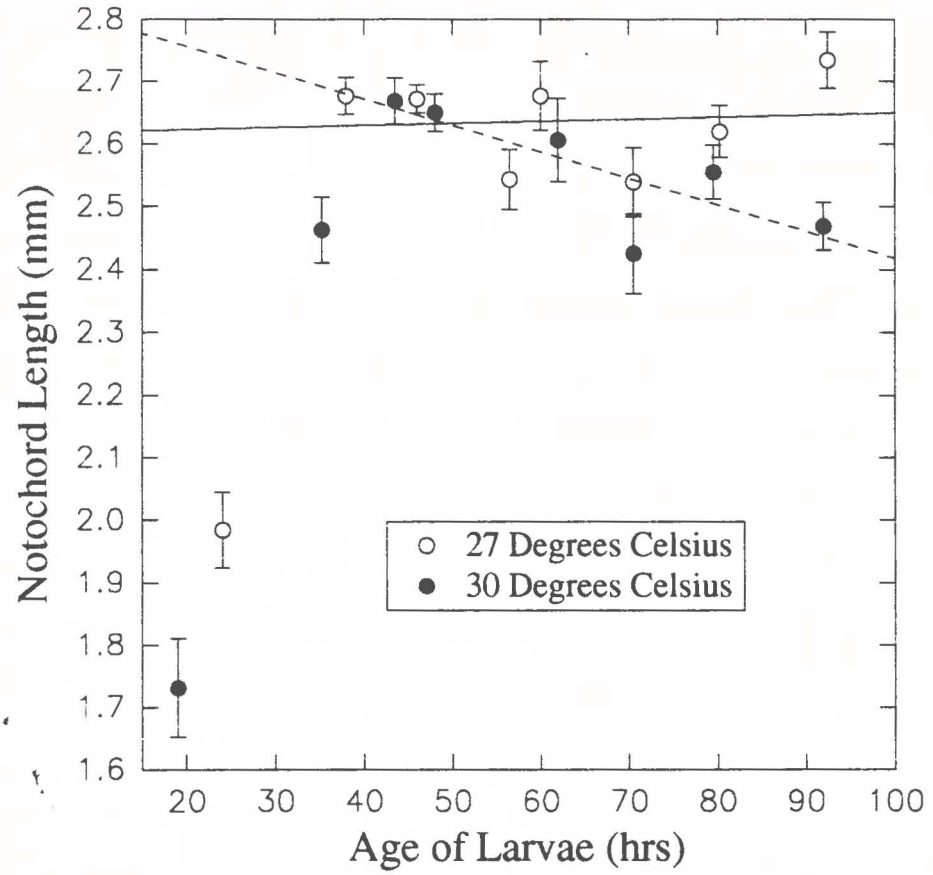


Figure 8. Growth of larval *Siganus randalli* from hatching to starvation. The lines represent the change in length during the second part of the growth phase. The solid line represents the larvae reared at 27°C, and the dashed line represents the larvae reared at 30°C. Age is post-spawning.

Table 6. P-values for tests of significance for regressions of age versus length at the two temperature regimes.

Regression		Test of whether the observed line is significantly different from a line with no slope		Test of significance (slope or intercept) between lines
		27°C	30°C	27°C vs 30°C
Age vs. Notochord Length	November 1991	$F_{1,38}$=0.145, p=0.7057	$F_{1,37}$=11.050, p=0.0020	$F_{2,75}$=6.134, p=0.00341
	January 1992	$F_{1,69}$=1.346, p=0.2500	$F_{1,71}$=7.764, p=0.0068	$F_{2,140}$=4.131, p=0.01807
Age vs. Total Length	November 1991	$F_{1,38}$=1.041, p=0.3139	$F_{1,37}$=10.653, p=0.0024	$F_{2,75}$=7.961, p=0.00073
	January 1992	$F_{1,70}$=0.308, p=0.5805	$F_{1,71}$=7.290, p=0.0087	$F_{2,141}$=4.825, p=0.00940

Yolk Utilization

The yolk platelets and the oil globule were used at different rates and the yolk platelets were utilized completely much sooner than the oil globule. Because of these differences, the yolk platelets and oil globule will be treated separately. An assumption of regression analyses is that the variances are homogenous; however, the variances between groups for these data were not, so age was weighted in order to compensate for the effect of non-homogeneous variances. Additionally, any initial volumes taken within the first two hours of hatching were excluded from the analyses due to an extremely wide range of values. This wide range of values often gave the appearance of net production of yolk between the first sampling time and the second (Figure 9).

Linear regression analyses demonstrated that the yolk platelets were utilized faster at 30°C than at 27°C in both January 1991 ($F_{2,102}$=9.065, p=0.04286) and February 1992 ($F_{2,90}$=20.669, p=0.00124). The mean rate of yolk-platelet depletion was -0.112 μl/hr for larvae kept at 27°C, and -0.134 μl/hr for larvae kept at 30°C. Figure 9 illustrates the decrease in yolk-platelet volume.

The oil globule was also absorbed faster by the larvae at 30°C than at 27°C for all spawns (Table 7). The mean rate of oil-globule consumption was -0.036 μl/hr for larvae kept at 27°C, and -0.048 μl/hr for larvae kept at 30°C. Figure 10 illustrates the decrease in oil-globule volume. Interestingly, a "lag" occurred in the endogenous feeding for many spawns at approximately 40-50 hours post-spawning in both the oil globule and the yolk platelets (Figures 9 & 10).

Larval Development

Times to initiation and completion of eye pigmentation for larvae kept at 27°C and 30°C are given in Table 8. There were approximately three hours difference in the developmental stages between the two temperatures.

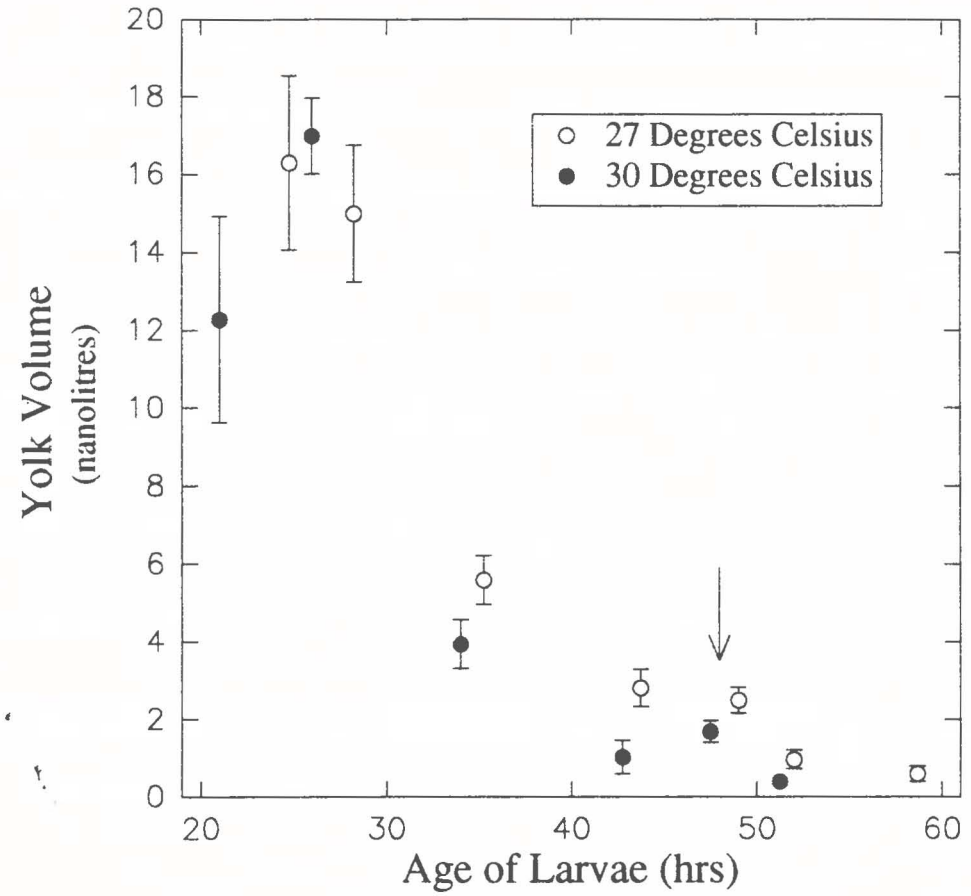


Figure 9. Yolk-platelet volume depletion by larval *Siganus randalli*. Arrow indicates lag in yolk-platelet absorption. Age is post-spawning.

Table 7. Oil-globule rates of depletion as calculated by non-linear regression analyses and p-values for tests of significance between log-transformed data.

	Rates of Depletion (μl/hr)		Test of differences between regression lines
	27°C	30°C	27°C vs. 30°C
November 1991	-0.033586	-0.042124	$F_{1,137}$=6.522, p=0.01175
January 1992	-0.047739	-0.053101	$F_{1,192}$=6.928, p=0.00918
February 1992	-0.026616*	-0.049553	$F_{1,171}$=30.837, p<0.00001
Mean (±Std. Err.)	-0.036±0.006	-0.048±0.003	

* Incomplete data set

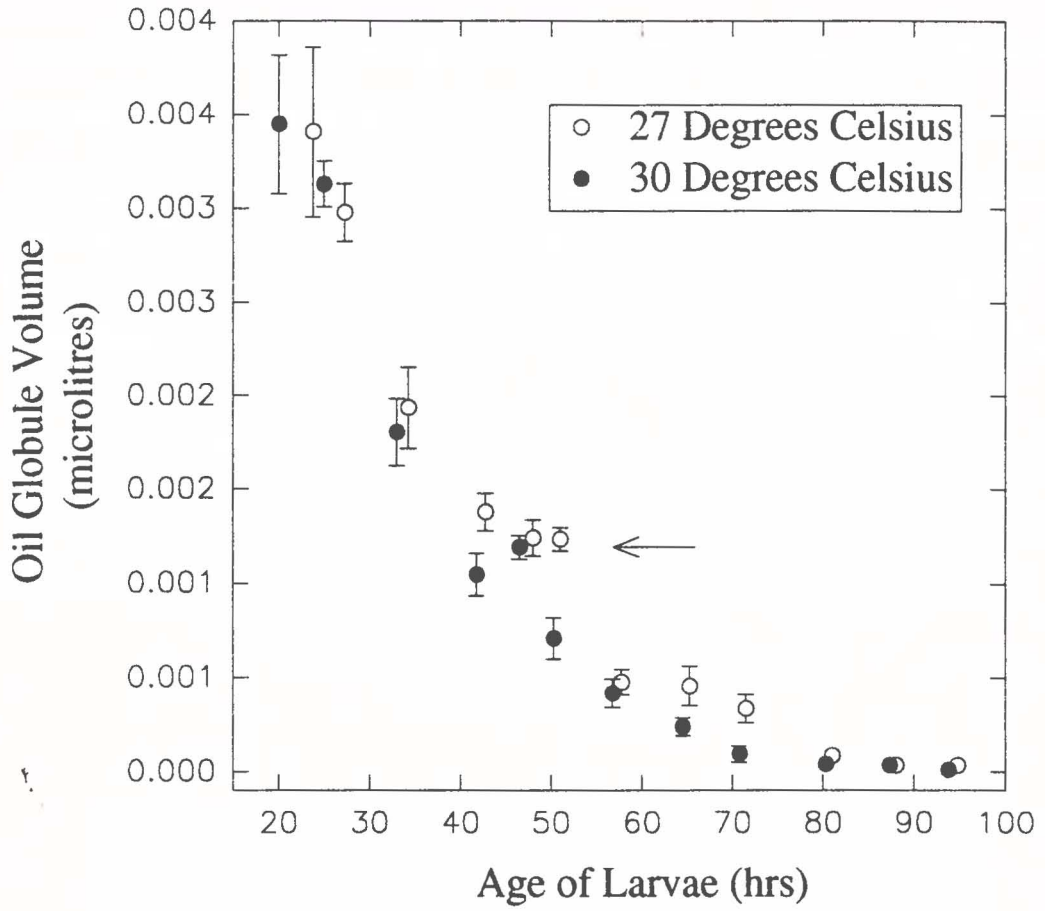


Figure 10. Oil-globule volume depletion by larval *Siganus randalli*. Arrow indicates lag in oil-globule absorption. Age is post-spawning.

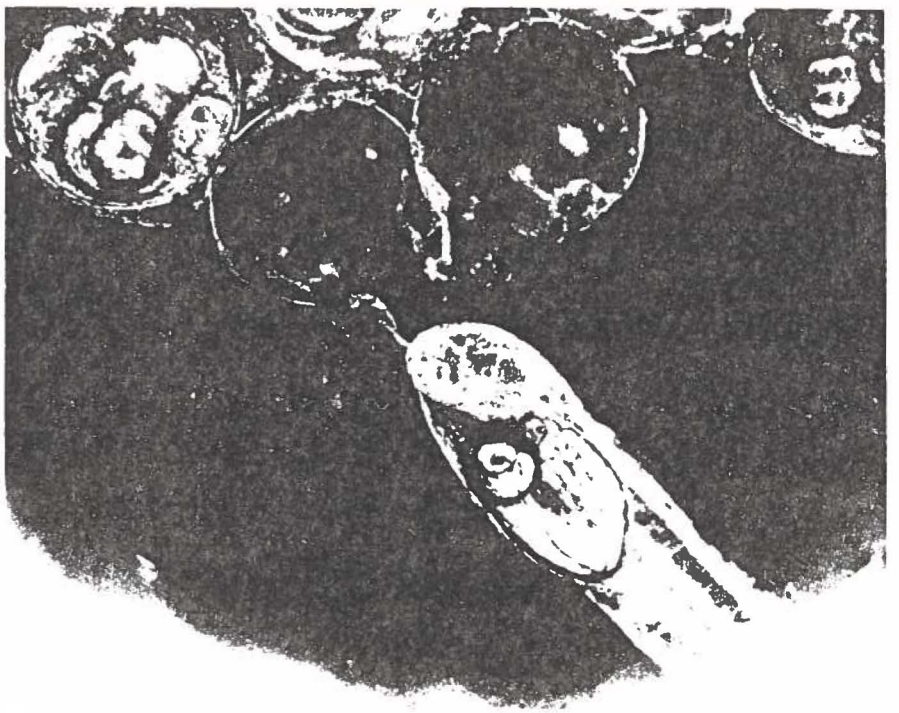
Table 8. Ages (hrs post-spawning) and standard errors around times for initiation and completion of eye pigmentation.

Eye Pigmentation	27°C	30°C
Initiation	44.83±0.67	41.00±0.50
Completion	51.12±0.07	48.06±0.06

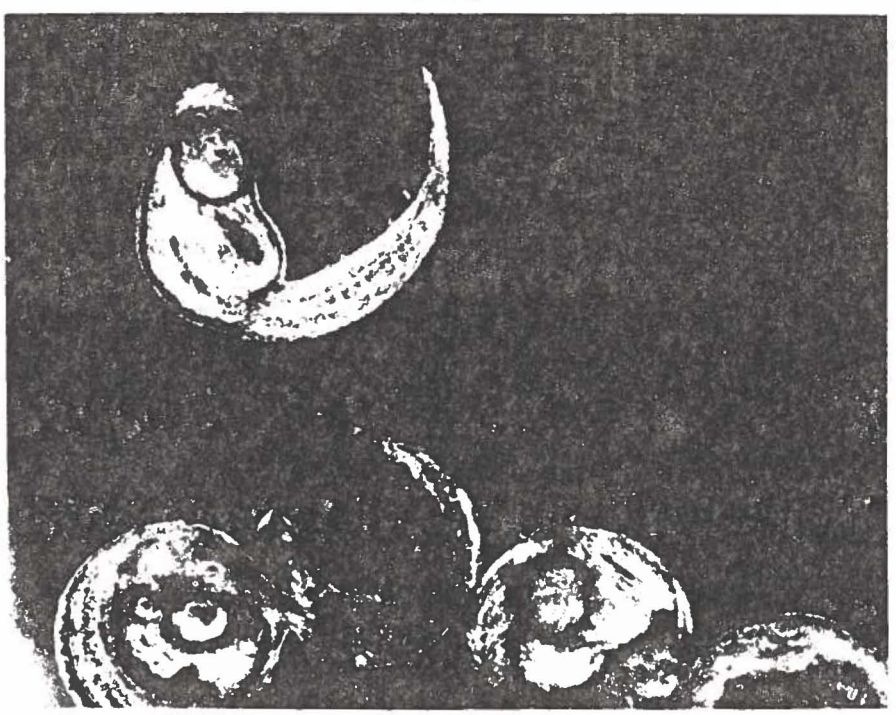
The larvae, when observed under the microscope, were seen to hatch tail first (Figure 11A). In a typical hatching, the shell would rupture, the tail would emerge, and then, with a violent shake of the body, the larva would dislodge the shell from its head. Some larvae were observed to hatch head first, but in the majority of these cases the larvae were deformed (Figure 11B).

Weight

The average dry egg weight was 0.022 mg, with a range from 0.011 mg to 0.031 mg (Table 9). There were significant differences in dry egg weight between spawns ($F_{2,83}$=42.37, $p<0.0001$), but not between temperatures ($F_{1,83}$=2.11, $p=0.1509$).



A



B

Figure 11. Newly hatched *Siganus randalli* larvae. Photograph A represents a larvae that hatched tail first, while photograph B represents a larva that hatched head first.

Table 9. Mean dry weights (±std. dev.) (mg) of eggs of *Siganus randalli*.

	Dry Egg Weight			
	27°C	SNK	30°C	SNK
November 1991	0.024±0.002	b, c	0.025±0.002	c
February 1992	0.023±0.002	b	0.023±0.002	b
March 1992	0.019±0.004	a	0.020±0.003	a

DISCUSSION

As expected, oxygen consumption of the embryonic *Siganus randalli* increased as development progressed toward hatching. Moreover, results of this study provide further evidence for interspecific variation, and perhaps interspawn variation, in oxygen consumption by embryos over time. Linear relationships of oxygen consumption with age have been demonstrated for: striped mullet, *Mugil cephalus* (Walsh *et al.*, 1989); milkfish, *Chanos chanos* (Walsh *et al.*, 1990); and carp, *Cyprinus carpio* (Kaushik *et al.*, 1982). In contrast, apparent nonlinear relationships have been demonstrated for: Atlantic halibut, *Hippoglossus hippoglossus* (Finn *et al.*, 1991); largemouth bass, *Micropterus salmoides* (Laurence, 1969); and cod, *Gadus morhua* (Davenport and Lönning, 1980). The question of whether oxygen consumption during embryonic stages can be generalized to have a linear relationship with age is not further clarified by this study. When the data is combined between spawns, a linear relationship with age is suggested; however, there was considerable variation between spawns (Figure 3). If the spawns are examined individually, rather than as a group, both November and March spawns included data points indicative of a rapid and apparently non-linear increase in oxygen consumption during the final hours prior to hatching (Figure 3B & D); therefore, a non-linear model may also fit the data.

It was expected that rates of oxygen consumption would be higher for *Siganus randalli* than for those reported in temperate fishes at comparable developmental stages. However, this was not evident from comparisons of oxygen consumption values at hatching. Although the time for development of *S. randalli* was much shorter than for many species studied to date, oxygen consumption values at hatching were approximately the same (Figure 10). This implies that the metabolic rate at hatching may be conserved across species at their normal environmental temperature. Both cod, *Gadus morhua*, and largemouth bass, *Micropterus salmoides*, had lower oxygen consumption rates at

Table 10. Oxygen consumption values at hatching for four fishes studied by other authors.

Common Name	Scientific Name	Oxygen Consumption Value at Hatching	Author
Atlantic halibut	*Hippoglossus hippoglossus*	200 nℓ/hr/ind	Finn *et al.*, 1991
Herring	*Clupea harengus*	210 nℓ/hr/ind	Kiørboe and Møhlenberg, 1987
Plaice	*Pleuronectes platessa*	210 nℓ/hr/ind	Davenport and Lönning, 1980
Striped mullet	*Mugil cephalus*	177 nℓ/hr/ind	Walsh *et al.*, 1989

hatching, 62 nl/hr/ind and 79.8 nl/hr/ind respectively, but these values are still within the range observed for *Siganus randalli*, and some interspecific variation is to be expected (Davenport and Lönning, 1980; Laurence, 1969). The values for oxygen consumption near hatching for *Siganus randalli* are lower than those reported by Walsh *et al.* (1991) for the tropical species *Chanos chanos* kept at 28°C, ≈400 nl/hr/ind at hatching.

Houde and Schekter (1983) examined oxygen consumption rates for the embryos of three subtropical species: bay anchovy, *Anchoa mitchilli*; sea bream, *Archosargus rhomboidalis*; and lined sole, *Achirus lineatus*. Although the oxygen consumption rates at hatching were not given, mid-development oxygen consumption rates (30 - 80 nl/hr/ind) agreed well, if a little lower, with the present findings (Houde and Schekter, 1983). A Q_{O_2} value of 4.2 µl/mg/hr for *Siganus randalli* embryos was calculated from the mean values for oxygen consumption and egg weight. This value is three times that found for *A. mitchilli* (1.4 µl/mg/hr) which had a similar egg weight, and almost twice as much as the value found for *A. rhomboidalis* (2.4 µl/mg/hr) which has a mean egg diameter two-thirds larger than *S. randalli* (Houde and Schekter, 1983). The Q_{O_2} value for *Chanos chanos* (3.92 µl/mg/hr) was slightly lower than for *S. randalli* despite results indicating a higher oxygen consumption value at hatching, presumably because *C. chanos* has a larger egg (Walsh *et al.*, 1991).

The oxygen consumption of yolk-sac larvae of *Siganus randalli* was highly variable, and a larger sample size would be needed to determine conclusively whether or not there is a difference in oxygen consumption between the two temperatures. Unfortunately, such efforts were hindered by the fact that the larvae are fragile. Larval fragility and mortality during handling may be attributed to the presence of free neuromasts along the length of the larva. The problems of mortality associated with experimental manipulation increased as the endogenous reserves were depleted. Nelson and deC Wilkins (in review) also noted difficulty in working with the larvae prior to initiation of exogenous feeding. These data suggest a mean oxygen consumption value of

164.6 nl/hr/ind for the period of time between hatching and the initiation of exogenous feeding. Oxygen consumption rates of the larvae were probably higher than low routine values of post-yolk-sac larvae due to the digestion of endogenous reserves (Jobling, 1981). This value is higher than for any of the three species of post-hatch larvae studied by Houde and Schekter (1983).

Egg size and maximum notochord length differed between spawns despite constancy of female parental size. This suggests that there may have been a seasonal effect on egg size, as well as the possibility that one or more factors not taken into account in this study, such as the age, weight, or health of the adult, affects egg size. Differences in maximum notochord lengths and egg weights between spawns were not surprising since initial egg diameters varied between spawns. However, with the limited number of spawns investigated, no correlation between egg size and egg weight or notochord length could be demonstrated, as was demonstrated by McEvoy and McEvoy (1991) for turbot, *Scophthalmus maximus*. An increase in egg size during development was not observed for *Siganus randalli* as has been reported by van der Wateren *et al.* (1990) for plaice, *Pleuronectes platessa*.

Juario *et al.* (1985) reported that, after an initial growth phase, *Siganus guttatus* larvae did not begin growing again until after the initiation of exogenous feeding. This agrees with the results presented here and those of Nelson and deC Wilkins (in review) for *Siganus randalli*. The maximum mean notochord length obtained by *S. randalli* yolk-sac larvae (2.70 mm) was approximately the same as that reported for *S. guttatus* by Juario *et al.* (1985) (2.96 mm) and Bagarinao (1986) (2.5 mm).

The decline in mean notochord and total length over time has been reported by other authors (Polo *et al.*, 1991; Fukuhara, 1990; Rana, 1985). This decline in length for larvae kept at 30°C suggests that, for the maintenance of the larvae through first feeding, the larvae develop optimally at a lower temperature, such as 27°C. In a mariculture facility where high ambient temperatures may be experienced, indoor or well-shaded

tanks may be necessary for optimal year-round production, or production may be limited to cooler months of the year.

The decline in yolk volume for *Siganus randalli* was approximately the same as that reported for other species (Fukuhara, 1990; Juario *et al.*, 1985; Ehrlich and Muszynski, 1982; May, 1974). Juario *et al.* (1985) and Fukuhara (1990) provided graphs that appeared similar to those presented in this study; unfortunately, neither paper provided rates of consumption. Ehrlich and Muszynski (1982) provided rates of consumption for the oil globule of *Leuresthes tenuis* that were considerably lower (-0.013 µl/hr at 25.2°C) than those for *S. randalli* (-0.036 µl/hr at 27°C). May (1974), Ehrlich and Muszynski (1982), and Fukuhara (1990) all reported that larvae utilized their endogenous reserves faster as the experimental temperatures were increased.

The times of initial and complete eye pigmentation were used as markers for determining whether larvae arrived at the same developmental stages with different amounts of endogenous reserves. The remaining yolk volume (yolk platelet and oil globule) was determined from the regression lines of the log-transformed data, using the mean times of initiation and completion of eye pigmentation. Although there was no statistical significance due to the small sample size (n=2 or 3), the larvae kept at 27°C had, on average, 48% more yolk platelets and 27% more oil globule at initiation and completion of eye pigmentation than larvae kept at 30°C (Table 11). This provides another indication that 27°C is optimal for larval survival in the hatchery; maintenance of 27°C in stock tanks may allow for better utilization of endogenous reserves through eye pigmentation.

The period of eye pigmentation also appeared to be transitional for the larvae. It was not uncommon to have a mortality rate of 50% or more during this time. This developmental stage was also the point at which an apparent lag in yolk utilization was

Table 11. Yolk-platelet and oil-globule volume (μl) at initiation and completion of eye pigmentation as calculated from regression lines.

	Volumes remaining at Eye Pigmentation			
	Initiation		Completion	
	27°C	30°C	27°C	30°C
Yolk Platelet				
January 1992	0.002269	0.001630	0.001220	0.000726
February 1992	0.003013	0.002379	0.001635	0.001034
Oil Globule				
November 1991	0.001995	0.001985	0.001414	0.001248
January 1992	0.001168	0.001072	0.000767	0.000637
February 1992	0.002269	0.001657	0.001898	0.001014

observed. If the mortalities consisted of larvae with less yolk than those that survived, this lag in yolk utilization may have been artifactual.

Another potential problem associated with the timing of eye pigmentation may be the larva's ability to feed. Larvae reared at 30°C completed their eye pigmentation 48 hours after spawning, or about midnight; while the larvae at 27°C achieved complete eye pigmentation approximately three hours later (Table 7). Bagarinao (1986) has reported previously that siganids and other tropical larvae require food sooner than temperate larvae. Additionally, Hara *et al.* (1986) has reported that *Siganus guttatus* larvae do not feed at night, and it must therefore be assumed that vision is necessary for first feeding. Since vision is necessary for feeding, and tropical larvae have a shorter period of time to initiate feeding, larvae reared at higher temperatures may be at a disadvantage due to reduced endogenous reserves, and an early timing of eye pigmentation.

Interestingly, although the 30°C temperature regime increased the rate of development of the embryo and produced a three hour difference in hatch time, the increased temperature failed to produce much difference in development for the larvae. Johns and Howell (1980) demonstrated that the time between equivalent developmental stages of *Paralichthys dentatus* larvae reared at two temperatures continued to increase with time. *Siganus randalli* larvae still had only a three hour difference in completion of eye pigmentation 30 hours post-hatching; this indicates that temperature is more critical for embryonic development than it is for larval development.

In conclusion, development and growth of embryonic and yolk-sac fish larvae may be affected by a wide range of environmental factors. This study provides some of the first information regarding the effects of temperature on the metabolism, growth, and development of a tropical fish species. The results suggest that successful hatchery production of *Siganus randalli* may be dependent, at least in part, on proper environmental control of temperature in the larval rearing tanks.

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