DEVELOPMENT AND ENERGY UTILIZATION IN EARLY LIFE STAGES OF VIVIPAROUS YELLOWTAIL ROCKFISH

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ABSTRACT

Laboratory experiments were conducted with gestating adult yellowtail rockfish and field surveys done of females in late vitellogenesis and gestation to determine the rate of embryonic and larval development, to examine the different measures of egg quality, and to determine the rate of endogenous energy utilization and tissue assimilation. Embryogenesis and larval development followed a predictable sequence and showed rapid development with hatching occurring in 23d and parturition in 29d. Eggs varied in dry weight and energy content with lipid concentrations most directly contributing to the amount of energy per egg. The endogenous energy of yolk and oil globule declined rapidly throughout gestation resulting in limited yolk and oil reserves at parturition. Findings of this research will serve to allow estimation of stock biomass and will contribute to the understanding of the underlying factors that contribute to recruitment variation.

INTRODUCTION

A unique trait common to all *Sebastes* species is their live-bearing reproductive strategy which can be classified as ovoviviparous or viviparous (Wourms, 1991). It is important to know the time course and sequence of embryonic and larval development to enable fishery researchers and managers to estimate adult rockfish biomass by means of the larval production method (Lo et al., 1992). Further, early life stage physiological condition and nutritional and energetic states may play important roles in determining the success or failure of yearclasses, an often cited argument presented to explain recruitment variability (Houde, 1987). A study of a ten year trawl and diving survey of pelagic and newly settled juvenile yellowtail rockfish concluded that population regulation is based on dynamics that influence larval growth and survival (Ralston and Howard, 1995). To address the applied needs for population assessment and to better understand factors effecting early life stage growth and survival a combined field and laboratory study was conducted on yellowtail rockfish (*S. flavidus*) from waters off northern California.

The yellowtail rockfish is a semipelagic, viviparous species that is commercially important through most of its range along the Pacific coast of the United States. Reproductive biology of this species has been the focus of research for approximately the last ten years. The annual reproductive cycle and variability in reproductive effort of the yellowtail rockfish have recently been established (Eldridge et al., 1991, Eldridge and Jarvis, 1995). Maternal nutritional dynamics and their relationship to reproduction and gonadal condition were described and analyzed by Norton and

MacFarlane (1994). Reproduction of this species, and most likely other rockfishes, has been found to be spatially and temporally variable, in both quantitative and qualitative measures, and it is closely related to environmental conditions and to reproductive success.

Previous ontogenetic studies of early life stages of *Sebastes* species are limited. Only Yamada and Kusakari (1991) provide descriptions from controlled experimental conditions using the *S. schlegeli* of Japan as a model. Information of different aspects of embryonic and larval rockfish energetics was reported in studies by Boehlert et al.(1986) and Boehlert and Yoklavich (1984).

The research design of this study combines results from field surveys of adult yellowtail rockfish collected by hook-and-line off northern California with those from laboratory experiments of spawning adults held in captivity. The objectives of this research were to a) describe the time-course and sequence of embryonic and larval development during gestation in the yellowtail rockfish, and b) to examine measures of egg quality, in primarily nutritional and energetic terms, and to determine how endogenous energy stores are utilized during early life stage development.

MATERIALS AND METHODS

For the early life stage study, 21 fertilized female yellowtail rockfish were collected from 1990 - 1993 by hook-and-line from Cordell Bank, approximately 37 km off the California coast, and transported live to the University of California Bodega Marine Laboratory. Fish were maintained in 2000 L circular tanks at ambient light and water conditions for the duration of the study. Temperatures averaged 11.96 degrees Centigrade (SD = 0.88°C) and salinities averaged 32.31 ppt (SD = 0.78 ppt). The average size of the gestating females was 37.8 cm SL (SD = 3.8 cm). At three day intervals throughout the study, which lasted from December through March of each year, fish were anesthetized and a catheter inserted through the urogenital papilla and into the ovary to obtain samples of developing oocytes. Each sample was examined fresh by microscope after removal from the host, and the developing embryo or larva assigned a developmental stage according to the developmental series established by Yamada and Kusakari (1991). This scheme ranges from 1 (unfertilized oocyte) to 33 (larva at parturition).

A corresponding field study was also conducted with adult yellowtail rockfish from 1986 to 1991. From a total collection of 715 adult females taken from Cordell Bank for reproduction studies, 108 fish were found to have fertilized eggs. This latter group was selected for detailed examination of gonadal tissue and the developing embryos and larvae. The adults ranged in standard length from 29 to 46 cm and total weight from 673 to 2083 g. The ovary of each fish was assigned a macrospcopic numerical gonadal stage. The values of this five stage scheme is as follows: 1 = immature, 2 = vitellogenic eggs, 3 = fertilized eggs, 4 = spent or ovaries with recent parturition, and 5 = resting and/or recovering). In this paper, eggs from stages 2 and 3 will be used. Within these stages fractional division from 0.1 to 0.9 was assigned which represented the progressive stage of development within either vitellogenesis or embryogenesis. From each female, samples of the eggs were staged and preserved in 10% buffered formaldehyde, or frozen at -70°C. Eggs were later removed for dry weight determinations (triplicate samples of 20 eggs each, weighed to the nearest 0.0001g). Caloric content was determined by bomb calorimetry in a adiabatic bomb calorimeter. Total lipids were quantified by automated thin layer chromatography/flame ionization detection after chloroform-methanol biphasic extraction. Total protein content was determined by the Lowry method (Lowry et al., 1951) with bovine serum albumin as a standard.

Three gestating females from the experimental group were selected for a study of early life stage energy utilization because they provided samples of eggs ranging from late vitellogenesis, prior to fertilization, through embryogenesis, hatching, and larval release. Formaldehyde fixed specimens at each sampled stage of development were dissected into chorion, yolk, and tissue portions, dried at 100°C for 24 hours, and weighed in triplicates of 20 specimens to the near 0.0001 g. Estimates of the oil globule weight were obtained by subtraction of the combined yolk, tissue, and chorion weights from the total egg weight.

Data from this study were organized into EXCEL spreadsheets, and analyzed for curve-fits and statistical significance by Tablecurve and SYSTAT software.

RESULTS AND DISCUSSION

Embryonic and Larval Development - Of the 21 adult fish successfully held and sampled in the laboratory, 8 provided specimens that spanned from late vitellogenic, unfertilized ova to parturition. The thirteen remaining fish produced specimens that ranged in beginning stages from morula stage to embryos with optic cups and otoliths and extending on to parturition. The developmental sequence followed those of Yamada and Kusakari (1991) and Sanchez and Acha (1988), but the rate of development and the times to hatch and parturition were much more rapid (Table 1). The important developmental periods for field applications in stock assessment can be derived from Figure 1 which has a best predictive equation of $Y = (28.6133 - 0.255935X^2)$, $r^2 = .94$. The time from fertilization to larval release averaged 29.2 d, with a 5d range, most likely due to temperature variation. In contrast, the equivalent time for a much larger *S. schlegali* larva of Japan was 48 d.

Developmental Stage and Gonadal Traits - Field surveys of adult yellowtail rockfish during gestation showed that ovary size did not significantly increase in relative proportion throughout gestation ($r^2 = .09$, N.S.; Figure 2). We conclude that embryos did not demonstrate significant weight increase during gestation despite the fact that MacFarlane and Bowers (1995) did find limited matrotrophic contribution to embryonic and larval nutrition. Gonosomatic indices showed wide variation during gestation, which is probably attributable to the different ages and sizes of the host females.

Egg Quality - Qualitative measures of egg quality varied greatly among field caught females with late vitellogenic and/or early embryogenic eggs (Table 2). Eggs varied significantly in size ($P < 0.01$) and in calories/egg. Since no differences were found in the caloric content per unit weight among the eggs tested, the observed differences in the energy contents of the eggs was due to weight differences. Variation in egg size within species is common among a variety of fish species and has been found to relate to both inherent and environmental factors (Wootton, 1979). Lipids comprise the most energy rich nutrient source for development and Figure 3 demonstrates how energy content of the eggs was directly and positively correlated with the measured lipid content in the ovary ($r^2 = .46$). When the energy content of the egg was analyzed over the course of vitellogenesis and gestation, we found an increase in energy during the period of yolk deposition, prior to fertilization, and a corresponding decrease from fertilization to larval release. This pattern is in agreement with findings of Norton and MacFarlane (1994) for the vitellogenic period and with MarFarlane and Norton (1996) during gestation, both studies using analytical chemical methods.

Table 1. Sequence, time course, and corresponding stages of embryonic and larval development of yellowtail rockfish.

Developmental Stage	**Yamada/Kusakari Stage**		**Days Post - Fertilization**
Newly fertilized oocyte	1		0
Germ disc formation	2		↓
Early cell cleavage	3-8		↓
Morula	9		3
Blastula	10-11		↓
Epiboly	12		↓
Gastrula	13-14		5
Embryonic shield	15		↓
Headfold formation	16		7
Optic vesicle	17		↓
Somite formation	18		↓
Optic cups/auditory vesicle	19-22		11
Otolith formation	23		↓
Retina pigmentation	24-25		18
Blood circulation/mouth and anus open	26-29		21
Yolk depletion	30		↓
Hatching	31-32		23
Yolk and oil globule depletion	32		↓
Parturition	33		29
Developmental Period	**Mean (days)**	**SD**	**Range**
Fertilization to hatching	23.0	1.3	21-25
Hatching to parturition	6.2	1.8	4-10
Fertilization to parturition	29.2	2.4	27-33

Early Life Stage Conversion Efficiency - Gravimetric analyses of endogenous energy utilization (i.e. yolk and oil globule) in embryos and larvae during gestation showed steep linear declines in yolk reserves and gradual declines in oil globule reserves from fertilization to parturition (Figure 5). At the same time, an exponential increase in tissue assimilation was found with the most rapid increase occurring after approximately 14 days post-fertilization. At hatching (i.e. day 23) 40% of the yolk reserves and 26% of the oil globule remained. Overall conversion efficiency by weight from fertilization to parturition was 54%. This compares favorably with the chemical analytical approach used by MacFarlane and Norton (1996) in yellowtail rockfish and with reviews of other fish species during their endogenous feeding periods (40-70%; Blaxter, 1969).

By parturition time when larvae have been incubated for approximately 6 days, only 14% of the original yolk mass remains and 8% of the oil globule remains. These limited reserves indicate that newly released larvae must quickly convert to exogenous food resources for survival. This further supports the Ralston and Howard (1995) finding that dynamic factors influencing the early larval stage, soon after parturition, comprise the most critical time for determining year-class size.

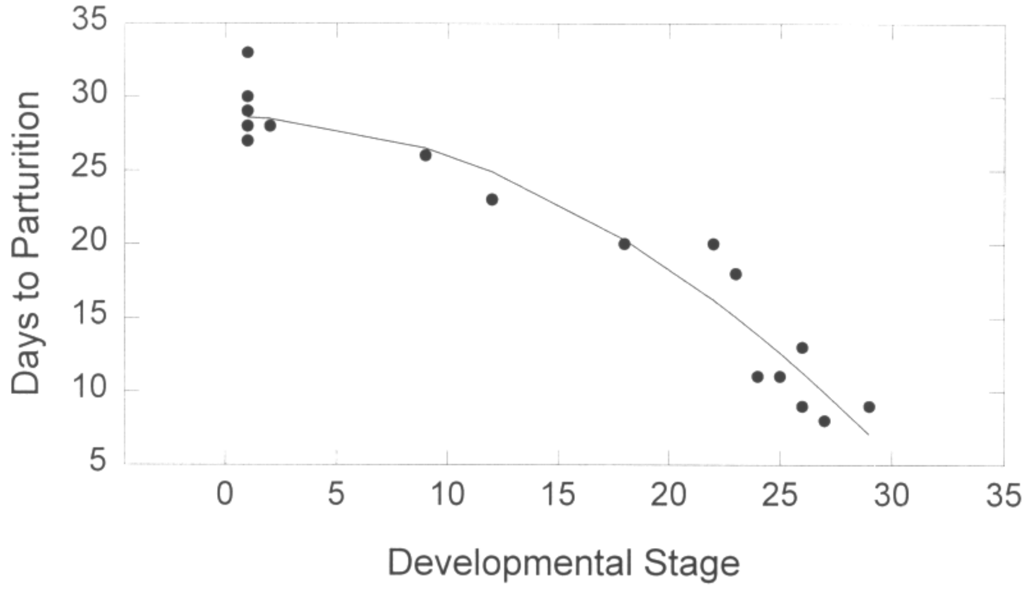

Figure 1. Developmental days to parturition.

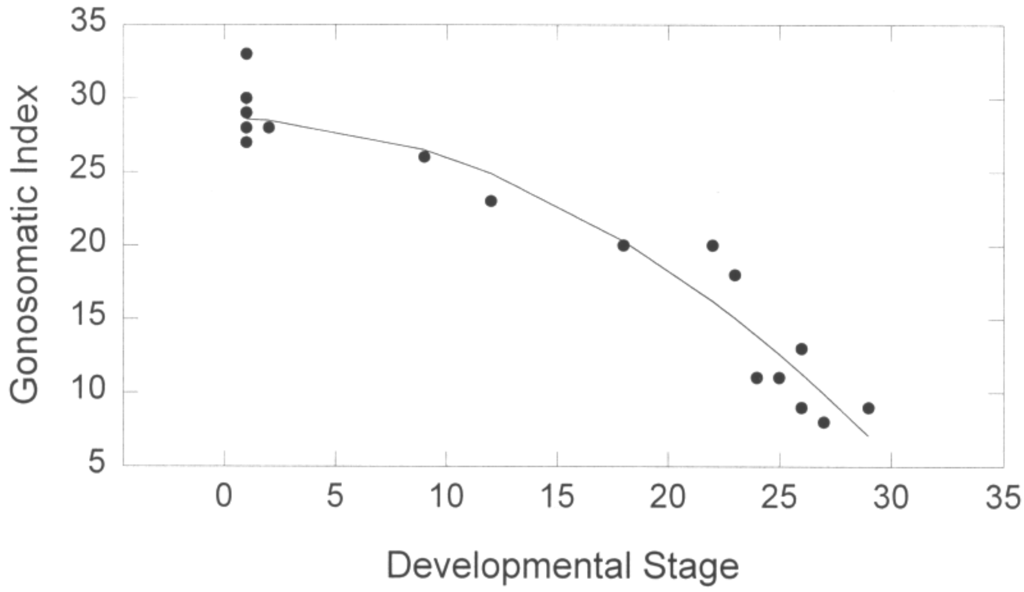

Figure 2. Gonosomatic index by developmental stage.

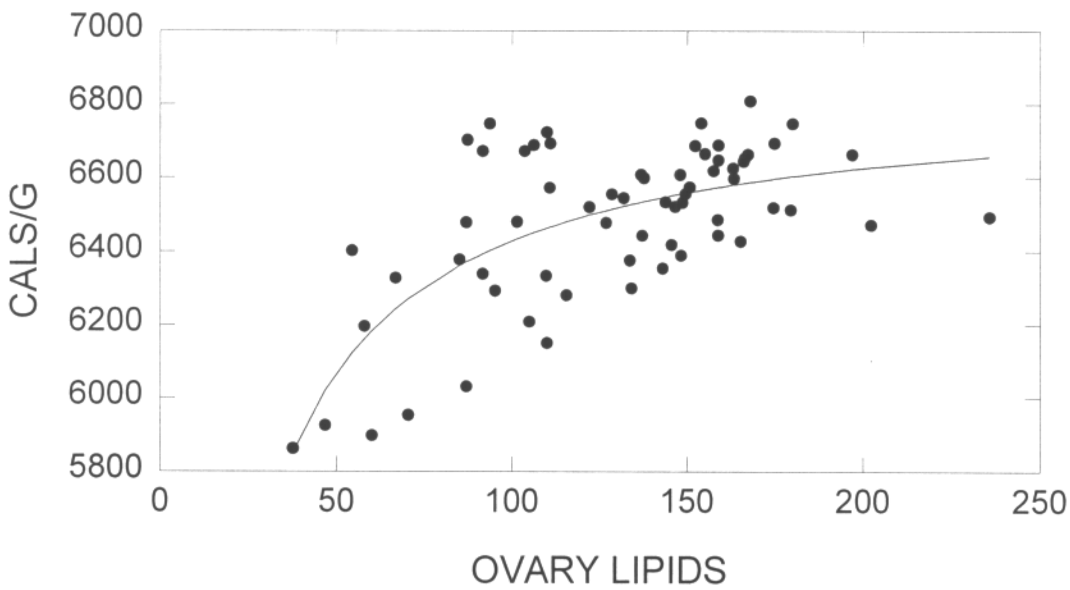

Figure 3. Caloric content and lipid concentrations.

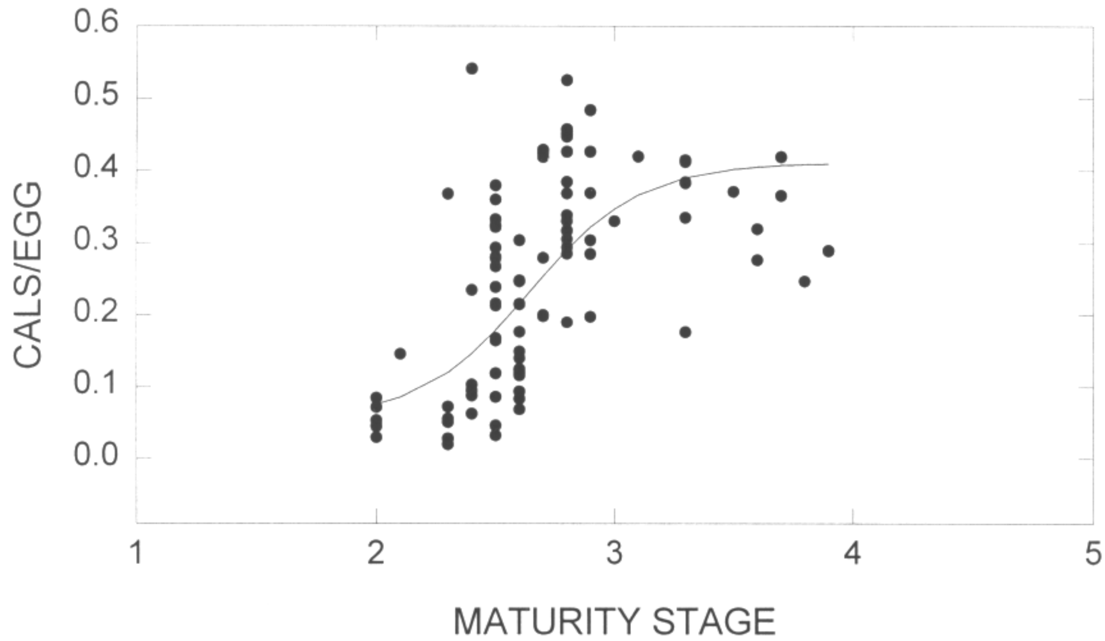

Figure 4. Energy content per egg by gonadal maturity stage.

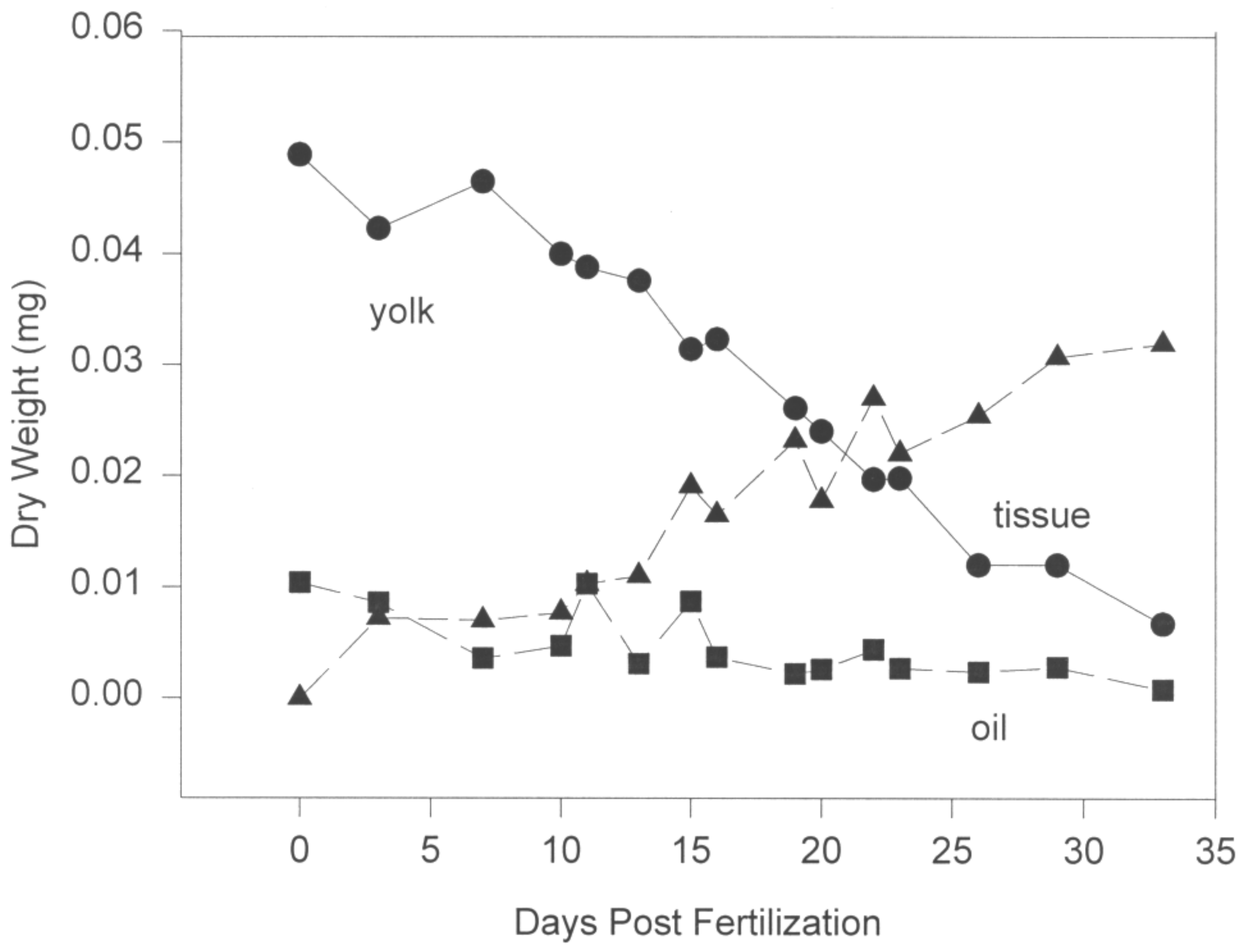

Figure 5. Dry weight of yolk, tissue, and oil globule by the days after fertilization.

Table 2. Measurements of egg quality in yellowtail rockfish.

Variable	Mean	SD	Range
Egg dry weight (mg)	.03766	.02132	.0012-.08500
Calories/g	6,465	259	5,607-6,890
Calories/egg	0.252	0.134	0.020-0.541
Ovary lipid (mg/g)	128.2	41.0	37.7-235.9
Ovary protein (mg/g)	210.2	60.5	73.8-333.0

The findings of this study provide a basis for estimating the time to larval release, essential for estimating stock biomass, and applicable to other *Sebastes* species. Variation in egg size and quality and the pattern of endogenous energy utilization leading up to the time of larval release all factor into the determination of successful recruitment.

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