

**LIPID AND PROTEIN CHANGES DURING EMBRYO DEVELOPMENT IN THE
VIVIPAROUS GENUS SEBASTES: APPLICATION TO THE ASSESSMENT OF
REPRODUCTIVE SUCCESS**

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Abstract

Concentrations of total lipid, protein, and lipid classes were determined in oocytes and embryos from two species of viviparous rockfishes; yellowtail rockfish, *Sebastes flavidus*, and shortbelly rockfish, *Sebastes jordani*. Total lipid, protein, triacylglycerols, sterol/wax esters, nonesterified fatty acids, cholesterol, and polar lipids declined during embryo development. Regression analysis of changes in lipid and protein variables depending on the stage of embryo maturation found highly significant linear relationships. By solving regression equations for the final stage of embryo development, estimates of the concentrations of lipids and protein at birth were obtained. These values provide an estimate of the nutritional and structural biochemical composition of larvae at their earliest life history stage in the environment, and thus, an assessment of larval condition and reproductive quality. Data revealed significant differences in the dynamics of lipid and protein depletion during embryogenesis between the two rockfish species and among three populations of shortbelly rockfish located at Ascension, Pioneer, and Bodega submarine canyons. Estimated concentrations of lipids and protein at birth were significantly greater in shortbelly rockfish larvae from Ascension Canyon than in those from Pioneer and Bodega Canyons in 1994, suggesting a greater potential survival during the time period following birth when planktonic food resources are often limited. The technique presented here to assess reproductive quality or the condition of progeny can be applied in field studies of viviparous teleosts and does not require detailed laboratory investigations of embryo development.

Introduction

The assessment of reproductive success includes the determination of both the quantity and quality of progeny. Year-class, or spawn, strength may be influenced by the health of newborns as well as the number produced. The number of offspring produced, or fecundity, is often used as a measure of reproductive success in fish research, and has been related to biological and environmental factors (Blaxter, 1969). Less attention has been given to the assessment of the quality of reproductive

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output. This is due primarily to the difficulty of determining which variables or processes are valid measures of egg, embryo, or larval health. Various measures have been proposed, such as egg size (Blaxter and Hempel, 1963), histological criteria (Theilacker, 1978), and biochemical analyses, including nucleic acids (Buckley, 1984), enzyme activity (Clarke et al., 1992), and biochemical composition (reviewed in Ferron and Leggett, 1994). All have merits justifying their use; however, other factors, such as clear relationships to growth or survival potential, ease and/or cost of analysis, or the ability to assess adequate numbers of replicates for statistical validity, often diminish their utility in routine assessment of reproductive success. Consideration of when during egg or embryonic development valid estimates can be made is also critical to accurate assessment of success. Ideally, evaluation at hatching, or birth, would provide the most accurate determination of the health and survival potential, but this stage is of very short duration and obtaining an adequate number of individuals to characterize a population or species is very difficult.

The determination of reproductive success in marine fishes, especially those that reproduce far from shore, is further complicated by the difficulty and cost of obtaining sufficient numbers of gravid females, or their eggs or embryos. In viviparous species, the complete development from previtellogenic oocyte to hatched larva occurs within the female. This simplifies collection of samples since only one life stage, adult, and not planktonic specimens needs to be evaluated.

We present here a technique that allows estimation of the nutritional status of hatched larvae at parturition, or release into the environment. Nutritional status has a clear relationship to growth and survival potential in that the amount of metabolically available energy establishes the duration of survival in the environment until adequate food becomes available. The method was applied to two species in the viviparous genus *Sebastes*, a taxon well-represented in the northeastern Pacific and commercially and recreationally important from Alaska to Baja California. The assessment of nutritional status involved the determination of protein, total lipid, and lipid class composition in eggs and embryos within ovaries of female yellowtail rockfish, *Sebastes flavidus*, and shortbelly rockfish, *Sebastes jordani*.

Materials and Methods

Female *S. flavidus* and *S. jordani* were obtained during January to March, the period of reproductive development spanning late vitellogenesis to parturition, at locations off the central California coast. Yellowtail rockfish were captured by hook-and-line at Cordell Bank, a marine bank 37 km west of Pt. Reyes, at depths ranging from 50 to 150 m. In 1994, shortbelly rockfish were collected by trawl in the proximity of three submarine canyons: Bodega Canyon (ca. 38°13'N 123°22'W), Pioneer Canyon (ca. 37°24'N 122°52'W), and Ascension Canyon (ca. 37°01'N 122°25'W), at 150 to 200 m depth. Fish were held on ice until examination. Morphometrics were recorded and ovaries were excised, weighed, and stored at -70°C. The stage of oocyte or embryo development was assessed by microscopy according to the classification scheme Yamada and Kusakari (1991) (Table 1).

Lipids were extracted from oocytes and embryos by the method of Bligh and Dyer (1959). Total lipids were quantified using thin layer chromatography with flame ionization detection (TLC-FID) by an Iatroscan TH-10 Mark III (MacFarlane et al., 1993). Lipid classes were separated on Chromarods S-III in a solvent bath of hexane:ethyl ether:formic acid at a ratio of 246:54:0.09. Quantification of separated peaks by TLC-FID was accomplished by comparing peak areas to external standard curves. Lipids were resolved into sterol/wax ester, triacylglycerol, nonesterified fatty acid, cholesterol, and polar lipid classes. Total protein was estimated by the Lowry method using a bovine serum albumin standard (Lowry et al., 1951). Analysis of variance (ANOVA) and linear regression were employed to assess variation in lipids and protein by embryo maturation stage (EMS), rockfish species, or population of shortbelly rockfish by SAS statistical software.

Table 1. Embryo maturation stages (EMS) in *Sebastes flavidus* and *Sebastes jordani*.

EMS	Description	EMS	Description
1	Late vitellogenic/ migratory nucleus oocyte	17	Optic vesicles
2	Formation of germ disc	18	Somite formation begins
3	2 - celled	19	Finfold
4	4 - celled	20	Optic cups
5	8 - celled	21	Auditory placodes
6	16 - celled	22	Lens forms
7	32 - celled	23	Otoliths
8	64 - celled	24	Pectoral fins
9	Morula	25	Retinal pigmentation
10	Early blastula	26	Heart pumping
11	Late blastula	27	Lens transparent
12	Beginning of epiboly	28	Mouth and anus open
13	Early gastrula	29	Peritoneum pigmented
14	Late gastrula	30	Yolk reduction
15	Embryonic shield	31	Prehatching
16	Head fold	32	Hatching
		33	Hatched, preborn larva

Results and Discussion

In yellowtail rockfish, there was a progressive decline in total lipid and protein during embryogenesis (Fig. 1). The concentration of total lipid decreased from a mean of 155.3 mg/g in unfertilized oocytes in the migratory nucleus stage (EMS 1) to an estimated concentration of 26.6 mg/g for fully-formed hatched larvae (EMS 33) at parturition. Although no pregnant females were caught with embryos at EMS 33, the goodness-of-fit of the linear regression of lipid concentration on embryo maturation stage for the stages collected (Table 2) suggested that calculation of total lipid at parturition was valid. Similarly, protein declined from 205.6 mg/g at EMS 1 to 36.0 mg/g at EMS 33 (Table 2).

Total lipid and protein concentrations declined linearly according to stage of development in embryos of shortbelly rockfish, also (Fig. 2). Highly significant linear relationships ($P < 0.0001$; Table 2) between total lipid or protein and EMS allowed estimation of nutrient concentrations at parturition. For all populations of shortbelly rockfish from the 3 submarine canyons, the estimated concentrations of total lipid and protein in larvae at parturition (EMS 33) were 21.5 and 64.7 mg/g, respectively.

The concentrations and rates of metabolism of lipids and protein differed between yellowtail and shortbelly rockfish during their approximately 30 d gestation. The initial lipid and protein concentrations at the start of embryogenesis were greater in yellowtail rockfish than in shortbelly rockfish (t-test: $P < 0.05$ for protein; $P < 0.0001$ for total lipids); however, the rates of depletion were also greater in yellowtail rockfish ($P < 0.0005$). This resulted in similar concentrations of lipids at birth in the two species of *Sebastes*, but greater protein in shortbelly rockfish larvae.

Total lipid concentrations can be considered a component of condition assessment (Ferron and Leggett, 1994), and thus a measure of qualitative reproductive success, but not all types, or classes, of lipids are equal with respect to metabolic availability or energy yield. Therefore, fractionation of total lipids into classes representative of energy-yielding and structural functions provides greater knowledge of the amount of energy available to sustain growth and survival once the larvae are released into the environment.

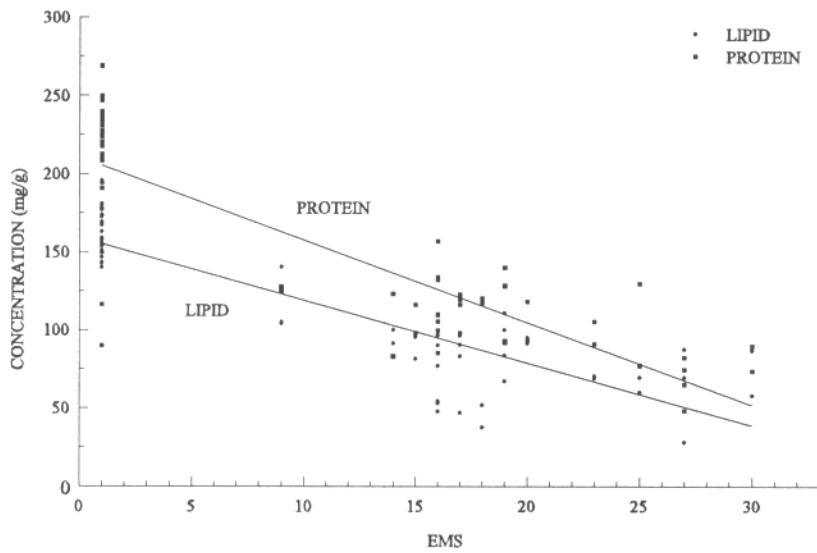


Figure 1. Total lipid and protein concentrations in oocytes and embryos in female yellowtail rockfish, *Sebastes flavidus*, from prefertilized oocytes (EMS 1) through the yolk reduction stage (EMS 30). See Table 1 for descriptions of EMS.

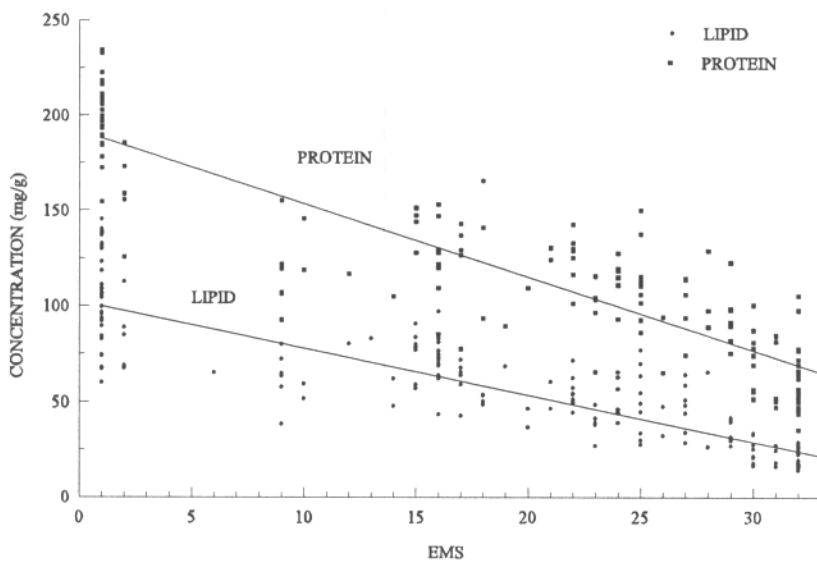


Figure 2. Total lipid and protein concentrations in oocytes and embryos in female shortbelly rockfish, *Sebastes jordani*, from prefertilized oocytes (EMS 1) through the hatched larvae stage (EMS 33). Data for shortbelly rockfish obtained from populations at all 3 submarine canyons are shown. See Table 1 for description of EMS.

Table 2. Linear regression parameter estimates for protein and lipid variables in rockfish embryos in relation to embryo maturation stage (EMS). Data are for yellowtail rockfish (*Sebastes flavidus*) and three populations of shortbelly rockfish (*Sebastes jordani*) from Ascension, Pioneer, and Bodega Canyons.

Variable	r ²	Intercept±SE	P	Slope±SE	P
<i>S. flavidus</i> (n = 60)					
Total lipid	0.774	159.3 ± 4.4	<0.0001	-4.02 ± 0.29	<0.0001
Total protein	0.721	210.9 ± 6.7	<0.0001	-5.30 ± 0.44	<0.0001
<i>S. jordani</i>					
All Canyons (n = 182)					
Total lipid	0.771	103.7 ± 2.3	<0.0001	-2.49 ± 0.10	<0.0001
Total protein	0.793	193.1 ± 3.7	<0.0001	-3.89 ± 0.16	<0.0001
Esters	0.629	9.3 ± 0.3	<0.0001	-0.25 ± 0.01	<0.0001
TAG	0.743	50.4 ± 1.3	<0.0001	-1.32 ± 0.06	<0.0001
NEFA	0.013	0.0 ± 0.1	0.5937	0.01 ± 0.00	0.1224
CH	0.686	4.5 ± 0.1	<0.0001	-0.08 ± 0.00	<0.0001
PL	0.789	39.5 ± 0.7	<0.0001	-0.85 ± 0.03	<0.0001
Bodega Canyon (n = 19)					
Total lipid	0.751	84.9 ± 5.2	<0.0001	-1.67 ± 0.26	<0.0001
Total protein	0.716	190.1 ± 11.9	<0.0001	-3.85 ± 0.59	<0.0001
Esters	0.741	7.5 ± 0.5	<0.0001	-0.17 ± 0.02	<0.0001
TAG	0.708	39.3 ± 2.7	<0.0001	-0.86 ± 0.13	<0.0001
NEFA	ND	ND	ND	ND	ND
CH	0.475	3.4 ± 0.2	<0.0001	-0.04 ± 0.00	<0.001
PL	0.706	34.6 ± 1.9	<0.0001	-0.61 ± 0.10	<0.0001
Pioneer Canyon (n = 102)					
Total lipid	0.805	90.2 ± 2.8	<0.0001	-2.13 ± 0.11	<0.0001
Total protein	0.702	183.3 ± 6.7	<0.0001	-3.65 ± 0.25	<0.0001
Esters	0.730	7.6 ± 0.3	<0.0001	-0.20 ± 0.01	<0.0001
TAG	0.778	45.1 ± 1.7	<0.0001	-1.19 ± 0.06	<0.0001
NEFA	0.187	-0.3 ± 0.1	0.010	0.02 ± 0.00	<0.0001
CH	0.709	4.3 ± 0.1	<0.0001	-0.08 ± 0.00	<0.001
PL	0.834	33.6 ± 0.8	<0.0001	-0.69 ± 0.03	<0.0001
Ascension Canyon (n = 61)					
Total lipid	0.541	107.5 ± 3.6	<0.0001	-2.03 ± 0.24	<0.0001
Total protein	0.671	193.0 ± 5.0	<0.0001	-3.12 ± 0.33	<0.0001
Esters	0.314	9.8 ± 0.6	<0.0001	-0.21 ± 0.04	<0.0001
TAG	0.469	51.8 ± 2.1	<0.0001	-1.02 ± 0.14	<0.0001
NEFA	0.011	0.2 ± 0.2	0.152	-0.01 ± 0.01	0.411
CH	0.445	4.6 ± 0.1	<0.0001	-0.07 ± 0.01	<0.0001
PL	0.589	41.1 ± 1.2	<0.0001	-0.72 ± 0.08	<0.0001

Lipid extracts from oocytes and embryos of shortbelly rockfish were separated into sterol/wax esters, triacylglycerols (TAG), nonesterified fatty acids (NEFA), cholesterol (CH), and polar lipids (PL). TAG and esters are energy-yielding lipids whereas CH and PL are considered to be primarily structural in purpose. PL consists of several groups of lipids including sphingomyelin and cerebrosides, but the great majority of the molecules in this class are phospholipids, the principal component of biological membranes. Although PL can be considered a class of structural lipids, phospholipids have been shown to be metabolized during embryo development in fish (Tocher et al., 1985).

All classes of lipids declined during embryogenesis in shortbelly rockfish (Fig. 3) except NEFA which were at very low concentrations and did not vary linearly with EMS (Table 2). TAG and PL were the most abundant lipid classes and declined the most during development, indicating both classes were significant sources of energy. Esters also declined significantly, but were at very low levels and not a major source of energy. CH was the most stable lipid during embryo development, but showed a slight but statistically significant decrease. The relatively stable concentrations of CH suggest its use as a normalizer of energy-yielding lipid content to correct for differences in size or quantity of embryonic tissue as has been employed previously for larvae of oviparous species (Fraser, 1989).

When lipid class concentrations were assessed in embryos of shortbelly rockfish from separate populations there were differences in the amounts and rates of depletion during intraovarian development (Table 2). Two-way ANOVA determined significant differences among populations at three submarine canyon areas located within about a 1° latitude span off the central California coast. All lipid classes, except NEFA varied significantly by EMS ($P < 0.0001$), population ($P < 0.0001$), and the interaction of population and EMS ($P \leq 0.05$).

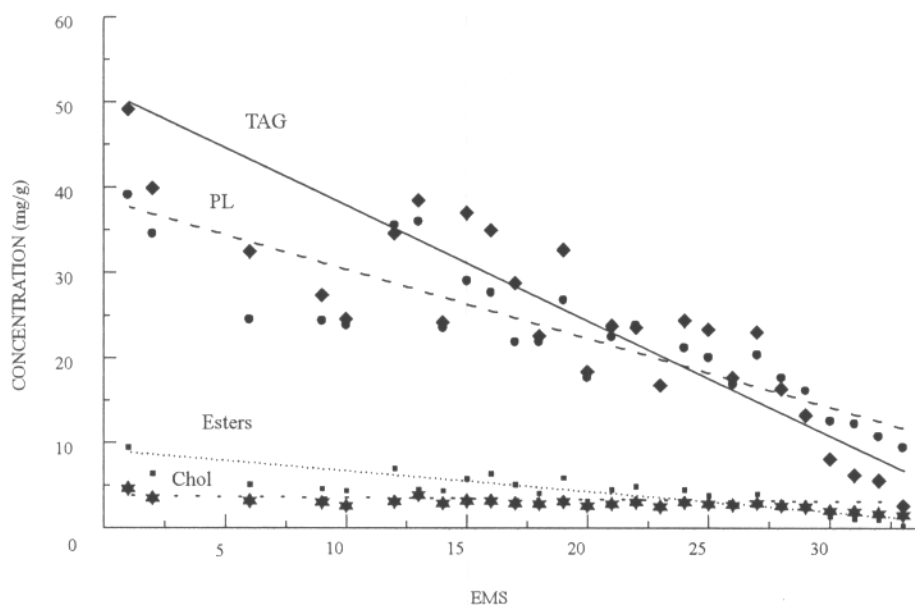


Figure 3. Changes in lipid classes during embryo development in shortbelly rockfish, *Sebastes jordani*. Data from representatives of populations at 3 submarine canyons. See Table 1 for description of EMS.

By solving regression equations for EMS 33 for lipid classes and protein in each population, estimated concentrations at birth can be obtained and compared among the populations (Table 3). These results indicate that there were differences in the condition of embryos at birth among the three populations, despite their relatively close geographical proximity. The estimated concentrations of protein and lipids in hatched, preborn larvae (EMS 33) in shortbelly rockfish at Ascension Canyon were significantly greater than those from the populations at Pioneer and Bodega Canyons. This suggests that the potential for survival during the critical period following birth is greater for the larvae from the Ascension Canyon population and may provide a greater contribution to the year-class of the species along the California coast.

Differences in energy-yielding lipid content may indicate relative survival potential in offspring from the various populations, especially during times of low biological productivity when food for pelagic larvae are scarce. Changes in environmental conditions, particularly those affecting the timing and intensity of upwelling, in the California Current ecosystem have been proposed as a cause of variable year-class strength in rockfishes (Moser and Boehlert, 1991).

The use of lipid and protein data as an indicator of condition at birth for viviparous rockfish larvae extends their application. Lipid class analysis has been employed for condition assessment in pelagic larval (Fraser, 1989; Håkanson, 1989; Lochmann et al., 1995) and juvenile stages (Suthers et al., 1992) of oviparous teleosts, and lipid class composition during embryonic development in Atlantic herring has been documented (Tocher et al., 1985). The assessment presented here provides a method to estimate energy reserves and biochemical composition of larvae at birth for viviparous species from field collections for the first time. Although knowledge of the length of gestation or of specific embryonic stages is not required, it may improve temporal resolution of the embryonic maturation stage scale and, thus, improve lipid and protein estimates at birth.

Table 3. Estimated concentrations of lipids and protein at birth for *Sebastes flavidus* and *Sebastes jordani*. *S. jordani* data are for all populations combined and for each of three populations at Bodega, Pioneer, and Ascension submarine canyons. Values presented as mean \pm SD in mg/g wet weight.

Variable	<i>Sebastes flavidus</i>		<i>Sebastes jordani</i>		
		All	Bodega	Pioneer	Ascension
Protein	36.0 \pm 9.6	64.7 \pm 2.6 ¹	63.1 \pm 9.7	62.8 \pm 2.7	90.0 \pm 7.4 ³
Total lipid	26.6 \pm 6.3	21.5 \pm 1.7 ¹	29.8 \pm 4.2 ²	19.9 \pm 1.2	40.5 \pm 5.5 ³
Esters	--	1.1 \pm 0.2	1.9 \pm 0.4 ²	1.0 \pm 0.1	2.9 \pm 0.9 ³
TAG	--	6.8 \pm 1.0	10.9 \pm 2.2 ²	5.8 \pm 0.7	18.1 \pm 3.2 ³
NEFA	--	0.3 \pm 0.1	--	0.4 \pm 0.0	0.0 \pm 0.2 ³
CH	--	1.9 \pm 0.1	2.1 \pm 0.2 ²	1.7 \pm 0.1	2.3 \pm 0.2 ⁴
PL	--	11.5 \pm 0.6	14.5 \pm 1.6 ²	10.8 \pm 0.4	17.3 \pm 1.8 ³

¹ Significantly different from *S. flavidus* (P < 0.0001)

² Greater than for *S. jordani* at Pioneer Canyon (P < 0.0001)

³ Greater than for *S. jordani* at Bodega and Pioneer Canyons (P < 0.0001)

⁴ Greater than for *S. jordani* at Bodega and Pioneer Canyons (P < 0.001)

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