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MATROTROPHIC VIVIPARITY IN THE YELLOWTAIL ROCKFISH *SEBASTES FLAVIDUS*

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Summary

A capacity for matrotrophic viviparity was established in the yellowtail rockfish *Sebastes flavidus* (Teleostei: Scorpaeniformes). The incorporation of radiolabel into embryos from [^{14}C]phosphatidylcholine present in the maternal serum during gestation provided the first *in vivo* demonstration of matrotrophy of phospholipid for any viviparous teleost and of any nutrient for a member of the genus *Sebastes*. Radiolabel content increased as embryos progressed through ontogeny.

Serum proteins of 170 kDa, present in vitellogenic and embryogenic females, but not in earlier stages, in immature females or in males, indicated the presence of vitellogenin in pregnant females and, thus, the potential for matrotrophic supplementation to yolk sequestered before fertilization. The retention of higher molecular mass proteins and highly phosphorylated proteins and the maintenance of total protein content in yolk during early to mid embryogenesis argue for exogenous maternal supply during gestation. As ovarian development proceeded from the oocyte through successive embryonic stages, the distribution of yolk proteins shifted from higher (67–97 kDa) to lower molecular masses (<70 kDa).

The results of these experimental studies corroborate data from field investigations showing that yellowtail rockfish can matrotrophically supplement embryo nutrients obtained before fertilization. Thus, yellowtail rockfish represent a teleost species positioned within the viviparity continuum and not at its extremes.

Key words: matrotrophic viviparity, phospholipids, vitellogenin, yolk proteins, embryos, yellowtail rockfish, *Sebastes flavidus.*

Introduction

Embryonic modes of nutrition in fishes have traditionally been described as oviparous (egg spawner, nutrients supplied to the embryo before fertilization), ovoviviparous (live-bearer, nutrients provided to the embryo before fertilization) or viviparous (live-bearer, nutrients provided by the mother during gestation). This classification has been modified to present embryonic nutrition as a continuum and not as discrete classes (Wourms, 1981). Thus, the range of embryonic nutrition in viviparous fishes extends from strict lecithotrophy (ovoviviparity) to extreme matrotrophy (maternal supply during gestation only). Although the concept is robust, examples of species falling between the extremes of the spectrum are poorly documented (Wourms *et al.* 1988).

In members of the live-bearing rockfishes, genus *Sebastes* (family Scorpaenidae), gestation occurs intralumenally over the course of 1–2 months. Embryos are enclosed in an egg envelope for most of gestation, but larvae hatch several days prior to parturition (Eldridge *et al.* 1991; Wourms, 1991; Yamada and Kusakari, 1991). Rockfish were once considered to be strictly lecithotrophes (Breder and Rosen, 1966), but recent laboratory studies of several rockfish species have provided energy budget estimates suggesting that some nutrition may originate from maternal sources during gestation (Boehlert and Yoklavich, 1984; Boehlert *et al.* 1986; Dygart and Gunderson, 1991). Thus, rockfish may not be strictly lecithotrophic. Further support for matrotrophy was obtained when *in vitro* incubation of *S. melanops* embryos in media containing [^{14}C]glycine showed uptake of radiolabel into the embryos (Yoklavich and Boehlert, 1991).

During a field study of the reproductive biology of yellowtail rockfish (*Sebastes flavidus*), evidence of matrotrophy was found (MacFarlane *et al.* 1993). Analyses of maternal serum during six annual reproductive cycles indicated that levels of certain nutrients were significantly elevated during gestation. Triacylglycerol, non-esterified fatty acid, phospholipid and calcium concentrations (a surrogate measure of vitellogenin; Hori *et al.* 1979; Tinsley, 1985) were greater in gravid female serum than in male serum during the period of late vitellogenesis and gestation. When considered in relation to the stage of ovary maturation, however, only phospholipid and Ca^{2+} (vitellogenin) levels were significantly elevated during pregnancy. These data suggested that yellowtail rockfish may

supply phospholipids and vitellogenin to developing embryos from maternal sources during gestation.

To distinguish whether a viviparous species is lecithotrophic or matrotrophic, changes in mass or energy content of embryos during gestation have been used (Wourms *et al.* 1988). Strictly lecithotrophic fishes experience embryonic dry mass losses ranging from 25 to 55 % between fertilization and parturition, whereas embryos of matrotrophic species typically gain mass. Changes in ovary mass (−21 %) and energy content (−16 %) during gestation indicate that yellowtail rockfish are primarily lecithotrophic (MacFarlane *et al.* 1993). If *S. flavidus* is capable of supplementing ovaries with phospholipids and vitellogenin matrotrophically during embryogenesis, this would establish one species of the rockfish genus as a live-bearing teleost within the viviparity continuum.

The dynamics of specific nutrients in teleostean viviparity has received little attention. Vitellogenin, the precursor of yolk proteins and lipids, and phospholipids are primary sources of the structural and energetic components for biological syntheses of embryonic tissues (Tocher *et al.* 1985; Mommsen and Walsh, 1988), but their transport and uptake during embryonic development in viviparous fishes have been little studied. By analysing serum and yolk proteins during vitellogenesis and gestation, the transport and metabolism of vitellogenin during embryogenesis can be examined. The transport of vitellogenin and changes in yolk protein distribution during oocyte maturation have been reported for oviparous fishes (Wallace and Begovac, 1985; Greeley *et al.* 1986) but not for viviparous species and, with the exception of studies on the uptake of amino acids in embryos of the matrotrophic *Zoarces viviparus* (Korsgaard, 1992) and the enrichment of phospholipids in the ovarian fluid of the matrotrophic embiotocid, *Cymatogaster aggregata* (deVlaming *et al.* 1983), there are no data on maternal–embryo lipid or protein dynamics in live-bearing fishes.

Matrotrophy *in vivo* has yet to be demonstrated in any members of the rockfish genus *Sebastes*. The purpose of the research presented here was to assess the *in vivo* matrotrophic capability of yellowtail rockfish and to elucidate the maternal transport and ovary dynamics of yolk proteins during gestation.

Materials and methods

Adult yellowtail rockfish, *Sebastes flavidus* (Ayres), were obtained by hook and line from the population at Cordell Bank (38° 01′ N, 123° 25′ W), a marine bank on the edge of the continental shelf 37 km west of Point Reyes, California. Fish were collected monthly from April 1985 to April 1992 at depths of 50–150 m.

Incorporation of [^{14}C]phosphatidylcholine into embryos

In January 1992, inseminated unfertilized females (copulation occurs in August or September, and spermatozoa are stored until fertilization in January or February; Eldridge *et al.* 1991) were placed into holding tanks aboard a chartered fishing vessel and transported to the laboratory for experimental study of *in vivo* phospholipid uptake by embryos.

Fish were maintained in 2200 l circular fiberglass aquaria receiving through-flowing, aerated, filtered ambient sea water at 7.5–15 l min^{-1} at the University of California Bodega Marine Laboratory. For the duration of the holding and experimental period (approximately 40 days), the mean water temperature was 12.0±0.7 °C and the salinity was 32.9±0.3 ‰. The photoperiod was ambient. Fish were fed chopped anchovies to satiety during the holding period, but were not fed during the experimental period.

We removed individuals from holding tanks at 3 day intervals to determine their ovary maturation stage (OMS) according to the classification in Table 1. To assess the oocyte or embryonic stage, females were lightly sedated by immersion in 50 mg l^{-1} tricaine methanesulphonate (MS-222) in sea water; this usually took about 5–10 min. Oocytes or embryos

Table 1. *Ovary maturation stages in* Sebastes flavidus

	Ovary maturation stage	Description
	I	Immature female
Oocyte stages	1	Early recrudescence
	2	Early yolk accumulation
	3	Late yolk accumulation
	4	Migratory nucleus
Embryo stages	5	8-celled
	6	16-celled
	7	32-celled
	8	64-celled
	9	Morula
	10	Early blastula
	11	Late blastula
	12	Beginning of epiboly
	13	Early gastrula
	14	Late gastrula
	15	Embryonic shield
	16	Head fold
	17	Optic vesicles
	18	Somite formation begins
	19	Finfold
	20	Optic cups
	21	Auditory placodes
	22	Lens forms
	23	Otoliths
	24	Pectoral fins
	25	Retinal pigmentation
	26	Heart pumping
	27	Lens transparent
	28	Mouth and anus open
	29	Peritoneum pigmented
	30	Yolk reduction
	31	Prehatching
	32	Hatching
	33	Hatched, preborn larva

Embryo stages derived from Yamada and Kusakari (1991).

(approximately 0.1 ml) were aspirated by gentle suction into 3.2 mm (o.d.) silicone tubing inserted into the ovary through the genital pore. When we observed, using a dissecting microscope, that eggs were fertilized, females were injected with [^{14}C]phosphatidylcholine ([^{14}C]PC) and placed in a separate flow-through experimental aquarium.

To administer the radiolabel, fish were anesthetized by immersion in 200 mg l^{-1} MS-222 for 2.5–3.0 min, placed on an operating table, and continuously irrigated over the gills with 55 mg l^{-1} MS-222. 200 μl of physiological saline containing 16.6 kBq of [^{14}C]phosphatidylcholine (L-α-dipalmitoyl, [dipalmitoyl-1-^{14}C], DuPont Co., New England Nuclear, Boston, MA, product number NEC-682, specific activity 4.3 GBq $mmol^{-1}$) was injected into the efferent branchial artery of the first gill arch on the left side. A three-way Luer stopcock fitted with a 26 gauge needle and two syringes was used to introduce the [^{14}C]PC into the maternal circulatory system (Cech and Rowell, 1976). The physiological saline, prepared according to Houston *et al.* (1985), was modified to match the ionic composition of female *S. flavidus* serum in January (R. B. MacFarlane, unpublished data). Following the injection of [^{14}C]PC, fish were placed in the experimental tank and revived by gently guiding them about the tank until they swam on their own.

To minimize the production of material contaminated with radioactivity, only three females were inoculated with [^{14}C]PC. Aquarium water and water downstream from an activated carbon filter in the effluent line were monitored for radioactivity daily throughout the experiment. No radioactivity was detected.

On days 3, 6, 10 and 18 post-injection, females were lightly anesthetized (50 mg l^{-1} MS-222) until disequilibrium was evident, and embryos (0.5–1.0 g) were withdrawn by gentle aspiration through the silicone catheter. Females were returned to the experimental tank. The embryo samples were washed with physiological saline, weighed and digested in Solvable (DuPont Co., New England Nuclear, Boston, MA).

To evaluate the specificity of [^{14}C]PC uptake by embryos, somatic tissues were assayed for radiolabel content at the end of the exposure. On day 18, females were killed by severing the spinal cord just posterior to the skull. Maternal muscle and liver tissue samples (0.2–0.3 g) were excised, washed with saline, weighed and digested with Solvable.

The incorporation of ^{14}C into embryonic and maternal somatic tissues was determined by liquid scintillation counting using a Beckman LS-7000 LSC. Digested samples were counted in Formula 989 LSC cocktail (DuPont Co., New England Nuclear, Boston, MA) employing Program 4 of the LS-7000 library. This program monitors quenching by the H-number method, using a ^{137}Cs external standard. Disintegrations per minute were computed from the counting efficiency of a sample. Counting efficiency was determined by comparing sample H-number with a standard curve of efficiencies from H-numbers of a series of increasingly quenched ^{14}C standards.

Analyses of serum and yolk protein

To assess the transport of proteins in maternal serum and changes in yolk proteins as embryonic development progressed, protein analyses were performed on females in progressive stages of ovarian maturation from early recrudescence to hatched, intra-ovarian larvae. Additionally, serum from males and immature females was evaluated. Fish were bled immediately after capture at the Cordell Bank and placed in ice until ovarian tissue could be excised within 24 h. Blood was obtained by cardiac puncture using sterile Vacutainers without anticoagulant and stored on ice prior to serum separation. Serum was collected by centrifugation and frozen at −70 °C until analyzed. Ovarian tissue was removed after reproductive stage and morphometric determinations, placed in Whirl bags, purged with N_2 and stored at −70 °C.

Serum and yolk proteins were analyzed on the basis of molecular mass distributions using sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS–PAGE) (Laemmli, 1970). Extracts of yolk proteins were prepared according to the method of Wallace and Begovac (1985). Embryos and oocytes were minced, placed into an equal volume of 0.01 mol l^{-1} phosphate buffer (pH 7.4) containing 0.002 mol l^{-1} phenylmethylsulfonyl fluoride (PMSF), a protease inhibitor, and homogenized with a Polytron. The homogenate was centrifuged at 13 500 ***g*** for 15 min. The yellow yolk supernatant was removed, assayed for protein concentration (Lowry *et al.* 1951), and diluted with sample buffer to the appropriate concentration for electrophoresis. Similarly, serum was analyzed for protein content and diluted with sample buffer. The sample buffer consisted of 0.064 mol l^{-1} dithiothreitol, 0.0016 mol l^{-1} EDTA, 0.01 % Bromophenol Blue, 1 % SDS, 0.1 mol l^{-1} Tris–HCl (pH 6.8) and 10 % glycerol.

Serum and yolk proteins were separated using 10.5 % polyacrylamide gels overlaid with 5 % stacking gels in a Bio-Rad Protean cell (Bio-Rad Laboratories, Richmond, CA). Each slab gel contained 15 sample wells. On all gels, lanes 1, 8 and 15 contained molecular mass standards (broad range, 6.5–200 kDa, Bio-Rad). Diluted serum and yolk protein samples and standards were heated in boiling water for 5 min, applied to gels, and electrophoresed at 30 mA per gel until the tracking dye reached the bottom of the gels. For most runs, two gels were placed into the cell. One gel received protein samples at 50 μg per 20 μl and was stained with the general protein stain Coomassie Blue R-250; the other gel was loaded at 100 μg per 20 μl and stained with Stains All, 1-ethyl-2-{3-[1-ethylnaphtho(1,2-*d*)thiazolin-2-ylidene]-2-methylpropenyl}naphtho(1,2-*d*)-thiazolium bromide, which reveals phosphorylated proteins.

Gels stained with Coomassie Blue were fixed in 45 % methanol, 10 % acetic acid for at least 8 h, then stained with 0.05 % Coomassie Blue in 25 % isopropyl alcohol, 10 % acetic acid for 6 h. They were destained in 5 % methanol, 10 % acetic acid.

Phosphorylated proteins were visualized by staining gels with Stains All according to the techniques of Wallace and Begovac

(1985). Gels were fixed in 45 % methanol, 10 % acetic acid for at least 8 h, immersed twice in 50 % methanol for 30 min, and placed in fresh Stains All solution on a rotary platform overnight. The Stains All solution consisted of 20 mg of Stains All dissolved in 10 ml of formamide, 50 ml of isopropyl alcohol, 1 ml of 3 mol l^{-1} Tris–HCl (pH 8.8) and 200 ml of deionized water. Following staining, gels were transferred to deionized water for 24 h. The water was changed several times. It is important that all procedures, from preparation of Stains All solution to final water washes, are conducted in the dark. Stains All gels were developed by placing them on a light table until the magenta background in the gels had faded adequately to define blue bands diagnostic of phosphoproteins. Since the bands also fade rapidly over time, they were photographed when the optimum contrast between blue bands and gel background occurred, usually about 12–18 min after placing on the light table.

Photographs of both Coomassie Blue and Stains All gels were used to determine molecular mass distributions of protein bands. Electrophoretic mobilities of sample protein bands were compared with those from the closest lane of standards.

Results

Uptake of [^{14}C]phosphatidylcholine by embryos

Eggs of the three females injected with [^{14}C]PC were fertilized between 1 and 10 days prior to the start of the experiment. The characteristics of the females at injection were as follows: fish 950, standard length 33 cm, mass 1034 g, OMS 14; fish 951, standard length 33 cm, mass 932 g, OMS 14; fish 977, standard length 31 cm, mass 816 g, OMS 20.

At 3 days post-injection, the presence of radiolabel was evident in embryos of all females (Fig. 1; Table 2). There was a progressive increase in ^{14}C concentration over the next 15 days of gestation. Radiolabel was enriched 2- to 5.6-fold in embryos as they developed from mid (OMSs 17 and 24) to late (OMSs 25 and 27) embryonic stages. The greatest increase in radiolabel occurred in embryos of female 977, which were at later developmental stages than those of the other females.

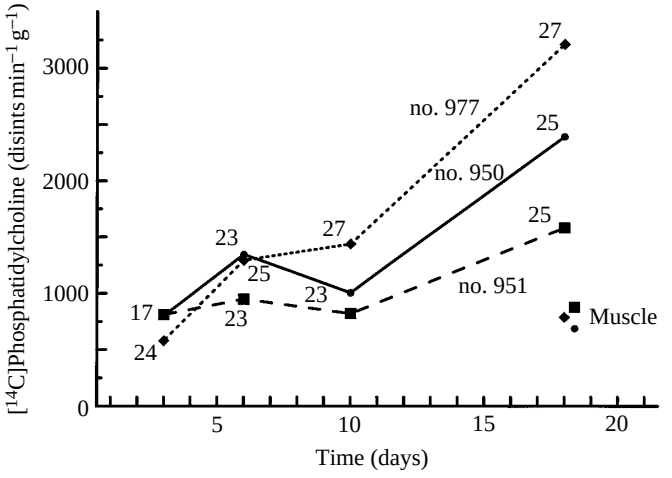

Fig. 1. The incorporation of ^{14}C into embryos of yellowtail rockfish from [^{14}C]phosphatidylcholine ([^{14}C]PC) in maternal serum. Pregnant females were injected on day 1 with 16.6 kBq of [^{14}C]PC in physiological saline. Numbers beside each data point are the ovary maturation stage (OMS) of the embryos (see Table 1 for descriptive key). Each female is designated by an identification tag number, e.g. no. 950. Mean activities of muscle samples on day 18 from each female are presented for comparison.

Embryonic tissue exhibited a significantly (P<0.05, t-test) greater accumulation of [^{14}C]PC than did muscle (Fig. 1; Table 2). After 18 days, the mean activity in embryos was 2396±472 disints min^{-1} g^{-1} whereas muscle contained 879±15 disints min^{-1} g^{-1}, a 2.7-fold greater specificity of embryos relative to muscle. The greatest concentration of radiolabel was in liver, however (Table 2). The mean activity in liver 18 days after intravenous administration of [^{14}C]PC was 7708±1974 disints min^{-1} g^{-1}, more than three times that found in embryos.

Serum and yolk proteins

Serum protein separations by SDS–PAGE were performed on rockfish females spanning all OMSs, immature females and males. The most apparent difference among separations stained with Coomassie Blue was the absence of an intense protein

Table 2. *Incorporation of [^{14}C]phosphatidylcholine (L-α-dipalmitoyl, [dipalmitoyl-1-^{14}C]-) into tissues of pregnant* Sebastes flavidus

			Fish number		
Tissue	Activity	Day	950	951	977
Embryos	disints min^{-1} g^{-1}	3	799	809	577
		6	1341	948	1290
		10	1004	820	1435
		18	2394	1580	3215
			(71)	(66)	(216)
	Σ disints min^{-1} (ovary)	18	35720	76520	50133
	Percentage of Σ disints min^{-1} injected	18	3.5	7.7	5.0
Liver	disints min^{-1} g^{-1}	18	11595	5165	6365
			(385)	(305)	(421)
	Σ disints min^{-1}	18	131830	57854	84712
	Percentage of Σ disints min^{-1} injected	18	13.2	5.8	8.5
Muscle	disints min^{-1} g^{-1}	18	852	904	881
			(34)	(67)	(67)
	Σ disints min^{-1}	18	402078	386789	333031
	Percentage of Σ disints min^{-1} injected	18	40.4	38.8	33.4

Each female was injected with 16.6 kBq of ^{14}C in 200 μl saline into the efferent branchial artery of the first gill arch on the left side.
Standard errors in parentheses, N=3.

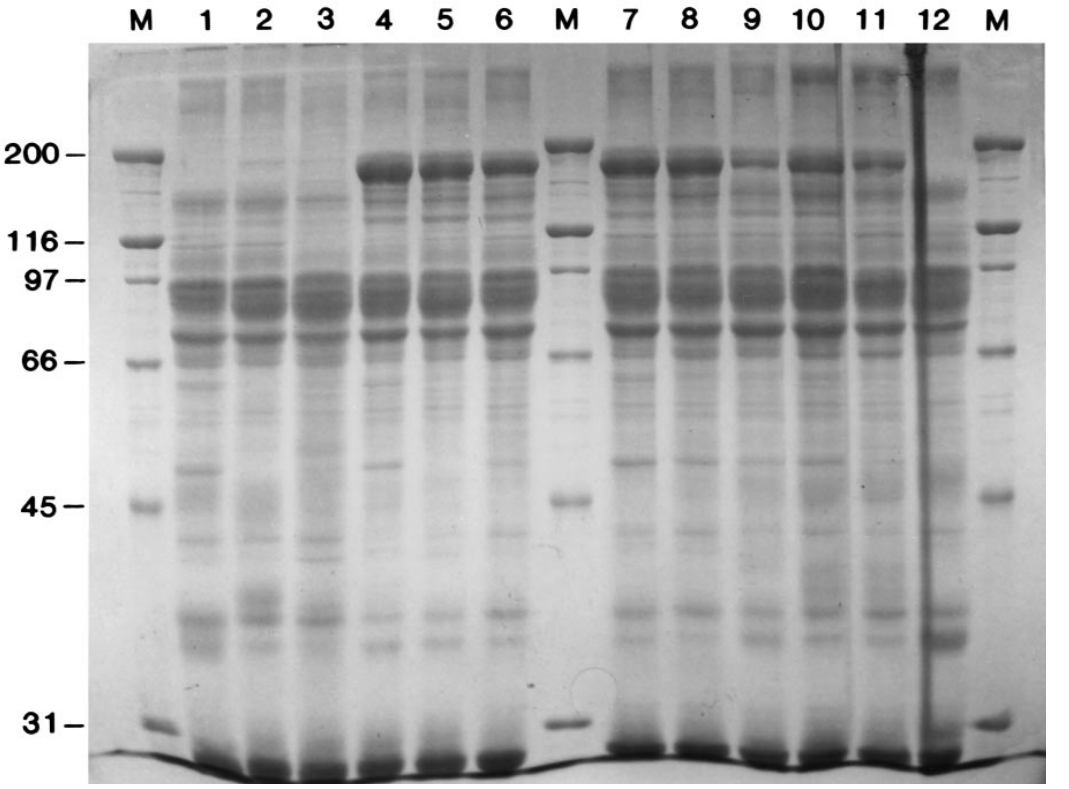

Fig. 2. SDS–PAGE on a 10.5 % gel of serum proteins from yellowtail rockfish. Lanes designated M are molecular mass standards (in kDa). Lane 1 is from immature female, lanes 2–5 are from mature females with progressive oocyte development, lanes 6–11 are from females with progressive embryo development, and lane 12 is from a male. See Table 3 for ovary maturation stage and description of specimens.

band at 170 kDa in sera from males, immature females and the early stages of development within females (Fig. 2; Table 3). All females in vitellogenic (OMSs 3 and 4) and embryonic (OMSs 5–33) developmental stages had a strongly stained protein band at 170 kDa. Other prominent serum protein bands detected by Coomassie Blue in all adult rockfish were found at approximately 145, 127, 83, 75, 66 and <31 kDa.

The distribution of yolk proteins in females during late vitellogenesis and migratory nucleus stages (OMSs 3 and 4) that stained by Coomassie Blue was constant (Fig. 3; Table 4). The most prominent bands were at 97 and 82 kDa. Also prominent was a triplet at 72, 70 and 67 kDa. Bands of molecular masses less than 65 kDa were lightly stained.

As ovary maturation proceeded through the embryonic stages, there was a progressive decrease in the abundance and staining intensity of higher molecular mass yolk protein bands. This coincided with a decrease in the concentration of yolk protein as embryonic development progressed (Table 4). During early embryonic stages (Fig. 3, lanes 5, 6 and 7), a large, intense band was seen at 75–80 kDa. Following the

Table 3. *Biological characteristics of* Sebastes flavidus *used for analysis of serum proteins by SDS–PAGE*

SDS–PAGE lane	Fish number	OMS*	GSI	SL (cm)	Mass (g)	Age (years)
1	1190	I	0.13	32.0	713	7
2	1025	1	0.40	41.0	1679	29
3	1271	2	0.25	32.0	889	7
4	1297	3	9.82	38.5	1651	22
5	1300	4	11.09	40.0	1391	17
6	1313	5	15.77	35.0	1289	12
7	1309	11	17.54	38.0	1460	12
8	1306	16	10.61	33.5	1013	9
9	1311	24	22.07	39.5	1911	22
10	1318	30	20.45	38.5	1642	27
11	1322	33	12.83	32.0	939	8
12	1174	male	0.07	35.0	1059	21

First column refers to lane number on the 10.5 % monomer SDS–PAGE gel shown in Fig. 2.
GSI, gonadosomatic index; SL, standard length; Mass, total fresh mass; OMS, ovary maturation stage.
*See Table 1 for descriptive key.

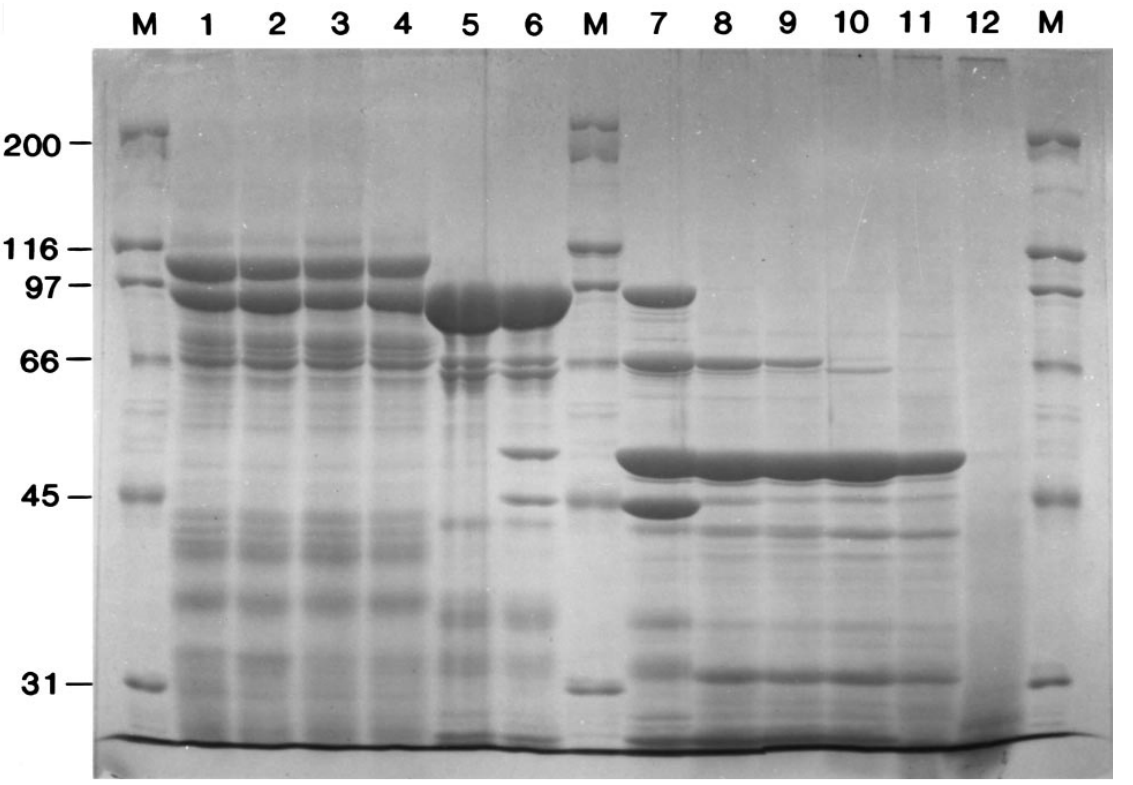

Fig. 3. SDS–PAGE on a 10.5 % gel of yolk protein extracts from mature yellowtail rockfish. Lanes designated M are molecular mass standards (in kDa). Lanes 1–4 are from late-stage oocytes, lanes 5–12 are from progressively later embryonic stages. See Table 4 for ovary maturation stage and characteristics of specimens.

appearance of the head fold (OMS 16), no proteins with molecular masses above 70 kDa were found. Protein bands became evident at 52 and 45 kDa from the late blastula (lane 6) to the yolk reduction (lane 11) stages. The 52 kDa band was particularly strong throughout embryogenesis, whereas the 45 kDa band decreased greatly after the head-fold embryonic stage (lanes 8–11). A protein band at about 32 kDa also became more apparent during embryonic development. Discrete bands <31 kDa were present in all embryo stages but were not clear during oocyte development. Intra-ovarian hatched embryos (larvae) showed no discrete protein bands, except for a few faint bands at <31 kDa (lane 12).

The carbocyanine dye (Stains All) stained protein bands that were not intensely stained by Coomassie Blue. In serum samples, two or three Stains All bands were evident at molecular masses ranging between approximately 48 and 35 kDa (Table 5). Relatively intense bands were found at about 46.5 and 35–40 kDa in serum from females undergoing early oocyte development (OMSs 1–3) and just before parturition (OMS 33), from immature females and from males.

Table 4. *Biological characteristics of* Sebastes flavidus *used for analysis of yolk proteins by SDS–PAGE*

SDS–PAGE lane	Fish number	OMS*	GSI	Yolk protein (mg g^{-1} embryo)	SL (cm)	Mass (g)	Age (years)
1	1301	3	6.48	39.2	35.0	1158	12
2	1297	3	9.82	37.7	38.5	1651	22
3	1305	4	8.41	29.8	36.5	1198	11
4	1300	4	11.09	43.0	40.0	1391	17
5	1313	5	15.77	99.3	35.0	1289	12
6	1309	11	17.54	91.3	38.0	1460	12
7	1306	16	10.61	92.0	33.5	1013	9
8	1314	23	19.39	56.4	37.5	1787	19
9	1311	24	22.07	60.0	39.5	1911	22
10	1319	25	11.16	53.2	32.5	962	8
11	1318	30	20.45	31.2	38.5	1642	27
12	1322	33	12.83	15.3	32.0	939	8

First column refers to lane number on the 10.5 % SDS–PAGE gel shown in Fig. 3.
GSI, gonadosomatic index; SL, standard length; Mass, total fresh mass; OMS, ovary maturation stage.
*See Table 1 for descriptive key.

Table 5. *Serum and yolk protein bands from SDS–PAGE (10.5 % monomer) stained blue by Stains All, indicating the presence of phosphorylated proteins, by ovary maturation stage*

	Ovary maturation stage	Molecular mass (kDa)			
Serum proteins	I	47.2*		34.8*	
	1	46.3*	39.0*		
	2	46.9*	39.1*		
	3	46.8*	42.1*	36.6*	
	4	47.6	39.6	34.2	
	5	48.0	42.1	36.5	
	11	48.3		37.8	
	16	46.3		34.9	
	24	47.0	38.7	34.9	
	30	47.6	38.7	35.7	
	33	46.1*		37.5*	
	Male	46.1*		35.1*	
Yolk proteins	3	>200		35.8*	30.7*
	4	>200		35.8*	30.7*
	5				29.8*
	11				29.8*
	16				29.8*
	23-30	>200			
	33	No bands stained			

See Table 1 for ovary maturation stage descriptive key.
*Indicates the major band.

Few phosphorylated protein bands were detected by Stains All in yolk extracts (Table 5). Two bands, at 35.8 and approximately 30 kDa, stained blue in yolk extracts from vitellogenic females (OMSs 3 and 4). During early embryo development (fertilization to head fold), only the blue-stained band at about 30 kDa persisted. There were no Stains All bands found in yolk protein separations from later embryonic stages. A trace of blue dye was found at the gel origin from yolk samples of vitellogenic and late embryogenic rockfish.

Discussion

The results of the present study provide conclusive evidence that yellowtail rockfish are capable of matrotrophy. The incorporation of radiolabel into developing embryos from [^{14}C]PC in maternal serum is the first *in vivo* demonstration of a matrotrophic supply of phospholipid for any viviparous teleost and of any compound in a member of the genus *Sebastes*. These data corroborate our contention that the elevated serum phospholipid levels in pregnant yellowtail rockfish from the Cordell Bank were due to transport to the ovaries (MacFarlane *et al.* 1993).

The incorporation of phosphatidylcholine into intra-ovarian embryos is not surprising. Phospholipids are commonly the most abundant lipid class in teleost eggs and PC is usually the dominant phospholipid (Tocher and Sargent, 1984; Tocher *et al.* 1985; Henderson and Tocher, 1987). Phospholipids were the only lipids found at high levels in the ovarian fluid of the viviparous embiotocid *Cymatogaster aggregata* (deVlaming *et al.* 1983). Considering the rapid proliferation of biomembranes during embryogenesis and the great fecundity of yellowtail rockfish (approximately 10^6 embryos per female; Eldridge *et al.* 1991), the prodigious phospholipid requirement for membrane synthesis can be more easily met by supply during, as well as before, gestation.

The SDS–PAGE serum protein band of 170 kDa present in vitellogenic and embryogenic females, but not in earlier reproductive stages, immature females or males, strongly indicated the presence of vitellogenin in pregnant females. Teleost vitellogenin typically has monomer subunit masses of 150–200 kDa (Mommsen and Walsh, 1988). The vitellogenin from another acanthopterygian, the striped bass (*Morone saxatilis*), was also shown recently to have a subunit mass of 170 kDa (Tao *et al.* 1993).

The occurrence of the 170 kDa serum protein in all embryogenic females and the elevated serum Ca^{2+} levels found in pregnant females at the Cordell Bank (MacFarlane *et al.* 1993) support the potential for matrotrophy of vitellogenin in yellowtail rockfish. These results contrast with those from a western Pacific congener, *Sebastes taczanowskii* (Takemura *et al.* 1991), where immunochemical techniques showed low levels of female-specific serum protein (vitellogenin) present in pregnant females, leading the researchers to conclude that vitellogenin was not used for embryo nutrition during gestation. Thus, species differences in the transport of vitellogenin may occur within the genus *Sebastes*.

Considering the substantial energy investment in hepatic vitellogenin synthesis, metabolic efficiency dictates that the synthesis and secretion of such a complex molecule should occur only when uptake and utilization ensue. It is possible that the synthesis of vitellogenin persists beyond the active uptake by embryos, however. Since gestation lasts only about 30 days in yellowtail rockfish, the presence of vitellogenin in serum may simply reflect this lag. But, vitellogenin levels were low early in the short gestation of *S. taczanowskii* (approximately 45 days; Takahashi *et al.* 1991) and declined within the first month of a 4 month pregnancy in the matrotrophic blenny *Zoarces viviparus* (Korsgaard and Petersen, 1979). Additionally, a rapid clearance rate from serum, e.g. a half-life of 2 days in *Xenopus laevis* (Wallace and Jared, 1968), would argue that the presence of vitellogenin indicates utilization. Given these considerations, it seems likely that vitellogenin in pregnant yellowtail rockfish indicates matrotrophic supplementation to the yolk accumulation that occurred prior to fertilization (MacFarlane *et al.* 1993).

Yolk protein distributions during embryonic development have not been published previously for any viviparous teleost. Temporal changes in the pattern and intensity of molecular mass bands could have provided insight into protein supply and utilization. Our results revealed no definable relationship to

vitellogenin input. However, the combined increase in protein content, the retention of higher molecular mass proteins and the persistence of highly phosphorylated proteins to mid-gestation (OMS 16) suggest supply from exogenous maternal sources.

The decline of higher molecular mass proteins and the increase in lower molecular mass proteins, seen in yellowtail rockfish as embryonic development progressed, was previously found during final oocyte maturation in oviparous species (Wallace and Begovac, 1985; Greeley *et al.* 1986). Wallace and associates speculated that proteolysis of yolk proteins facilitated oocyte hydration. Proteolytically driven hydration may be operative in yellowtail rockfish as well, since water content increases greatly after fertilization (Norton and MacFarlane, 1995). However, hydrolysis of yolk proteins to supply amino acids and peptides for embryonic membranes, enzymes, etc. was probably the major reason for the shift in yolk protein distributions. The decline in yolk protein concentration during embryogenesis and the absence of protein bands in the greatly reduced yolk of the final ovarian stage, hatched larvae (stage 33, lane 12 Fig. 3), support their utilization for biological syntheses.

Yolk proteins from yellowtail rockfish embryos detected by Stains All were probably highly phosphorylated phosvitins (Wallace and Begovac, 1985) and had apparent molecular masses of 35.8 kDa and approximately 30 kDa. Highly phosphorylated proteins were evident in vitellogenic and migratory nucleus oocytes and in early embryonic stages. Molecular mass distributions of Stains All proteins found in the yolk from oocytes of the marine oviparous species (Greeley *et al.* 1986) were similar to those of the viviparous yellowtail rockfish. Further, after oocyte maturation in the four marine pelagic species assessed by Greeley *et al.* (1986), Stains All bands disappeared from SDS–PAGE gels. The same loss of high-phosphate protein bands in the mid- and later-stage embryos of yellowtail rockfish suggests their involvement in the energy-requiring metabolic processes of differentiation and growth (Craik, 1982).

The failure of Stains All to detect the 170 kDa protein band (vitellogenin) that is stained with Coomassie Blue in yellowtail rockfish serum is not surprising. Vitellogenin, although a phosphoprotein, typically contains no more than 2 % phosphorus (Campbell and Idler, 1980; deVlaming *et al.* 1980) and therefore is not visualized by Stains All, which requires a phosphorus content of about 5 % or greater. Yellowtail rockfish serum did contain other proteins that were stained by Stains All. Phosphoproteins with apparent molecular masses of 46–48, 40 and about 35 kDa were found in serum samples of all adults and immature fish. Since it has been stated that the only phosphorylated protein in adult teleost serum is the high molecular mass vitellogenin (deVlaming *et al.* 1980; Wallace and Begovac, 1985) and we are not aware of published data about other serum phosphoproteins, the identity of the phosphorylated, lower molecular mass proteins in yellowtail rockfish serum is unknown. The relative decrease in staining intensity of serum phosphoproteins during gestation, however, may indicate the utilization of phosphate bond energy for embryogenesis.

The incorporation of [^{14}C]PC into yellowtail rockfish embryos from maternal serum confirms the matrotrophic potential of *Sebastes*. Previously, the presence and extent of matrotrophy in *Sebastes* had been equivocal (Wourms, 1991). Using a combination of respirometry, calorimetry and measurements of embryo mass change in *Sebastes schlegeli* (Boehlert *et al.* 1986), *S. melanops* (Boehlert and Yoklavich, 1984) and *S. caurinus* (Dygart and Gunderson, 1991), calculations revealed the need for maternal contributions during gestation to balance energy budgets. The *in vitro* capacity for matrotrophy in *S. melanops* was demonstrated by Yoklavich and Boehlert (1991). Incubation of embryos in flasks containing [^{14}C]glycine resulted in radiolabel uptake. This was greatest during the later stages of development, when epidermal and rectal cells appear to be capable of ingestion (Shimizu *et al.* 1991).

The occurrence of phospholipids and vitellogenin at elevated levels in the serum of pregnant yellowtail rockfish may be related since teleost vitellogenin contains about 20 % lipid (Campbell and Idler, 1980; Norberg and Haux, 1985), with phospholipids predominating (reviewed by Henderson and Tocher, 1987). Thus, vitellogenin may act as the vehicle for the translocation of phospholipids, as well as yolk proteins, during gestation.

The ability to provide vitellogenin, containing yolk lipid and protein precursors, during gestation would be ecologically beneficial to yellowtail rockfish. As members of the fish assemblage of the California Current ecosystem, they are subjected to a cyclic annual environment with its concomitant productivity cycle. The pattern usually results in the greatest supply of food during the summer upwelling season and very low forage levels during the winter, when gestation occurs (Ainley, 1990; Eldridge *et al.* 1991). Embryo energy and mass losses during gestation (MacFarlane *et al.* 1993) indicate that yellowtail rockfish have adapted evolutionarily to this rhythm by being primarily lecithotropic (Wourms *et al.* 1988). However, the typical annual environmental cycle is perturbed periodically by phenomena such as El Niños (when there is a warming of the eastern tropical Pacific) and by changes in the North Pacific atmospheric pressure patterns, causing dramatic alterations in food supply (Bakun, 1975; Ainley, 1990). In these years, food can be relatively scarce during the summer and more abundant during the winter, when lecithotrophic processes have ceased. By maintaining the capability to synthesize and secrete vitellogenin during gestation, yellowtail rockfish can direct digested food selectively to the ovary for embryogenesis, thereby compensating for reduced maternal inputs during the summer and autumn. Matrotrophic supplementation to yolk nutrients acquired prior to fertilization would be adaptive to the production of maximum progeny under the range of potential environmental regimes occurring in the California Current ecosystem.

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