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**Maternal Contribution of Energy to Embryos during
Gestation in the Viviparous Copper Rockfish,
Sebastes caurinus (Richardson)**

by

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ABSTRACT

The energy requirement for developing copper rockfish (*Sebastes caurinus*) embryos was measured through direct respirometry. This was compared with the energy obtained from the egg alone. The difference was ascribed to direct maternal inputs of energy during the development period. The results suggested that copper rockfish are matrotrophic with 11.5% of the energy consumed during gestation being contributed by the mother. Available information pertaining to other members of the genus *Sebastes* suggests that matrotrophy may be the common mode of reproduction, but that the magnitude of the energy contribution varies greatly between species.

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INTRODUCTION

Among viviparous fishes a distinction is made between those that supply energy to embryos (matrotrophic) and those that do not (lecithotrophic). Members of the genus *Sebastes* were formerly presumed to be lecithotrophic (Wourms 1981). Recently, however, Boehlert and Yoklavich (1984) used both energetic and morphological evidence to demonstrate that *Sebastes melanops* (black rockfish) were matrotrophic.

The objective of this paper is to present new information on the reproductive biology of another member of the genus, *Sebastes caurinus* (copper rockfish), and assess the maternal energy contribution made to embryos during gestation.

MATERIALS AND METHODS

The reproductive habits of *Sebastes caurinus* are similar to those of other members of the genus (Moser 1967). Mating and insemination occurs in January and February, but the internal fertilization of eggs does not begin until early March (Washington et al. 1978). Gestation of developing embryos lasts about 6 weeks. Spawning of the pelagic larvae takes place from mid-April to mid-May (DeLacy et al. 1964). At birth the newborn larvae are 5.3-6.0 mm long, and the remnant yolk sac is reduced and contained within the fusiform body. The eyes, jaw, gut and pectoral fins are all well developed and functional. The larvae are active swimmers and capable of capturing and consuming live prey (Stahl-Johnson 1984).

The approach used for determining the maternal energy contribution during gestation was to estimate the difference between the energy lost per embryo, and the energy consumed per embryo, from the time of fertilization to birth. Energy lost was determined as the difference between the energy content per individual at the beginning (fertilized egg stage) and end of gestation. Energy consumed was estimated by developing an empirical relationship between respiration rate ($\mu\text{l O}_2/\text{embryo}/\text{hr}$) and embryonic age. The respiration function was then integrated, evaluated over the length of the gestation period, and converted to energy units using an oxycaloric equivalent.

The fish used for the analysis of embryonic development and respiration rate were collected near Bainbridge Island, Washington, during February 1983 and 1984 using gill nets and hook and line gear. Additional data on the relationships between development stage, embryo dry weight and caloric content were obtained from fish taken by spearfishing in 1984.

The live fish were immediately transferred to the National Marine Fisheries Service lab at Manchester, Washington, where each fish received a prophylactic shot of an antibiotic and was marked with a spaghetti tag. The fish were held in 1.2-m circular tanks and in floating net pens approximately 2.4 x 2.4 x 3.0 m in size. The tanks were supplied with unfiltered flow-through seawater at ambient temperature. The temperature in the tanks increased from 9.1 to 11.5°C during the period when the respiration estimates were made. The fish were given a constant supply of live herring and/or live shore crabs. Food items occasionally disappeared from the enclosures, but total consumption was minimal.

Twelve fish captured in 1983 were divided into 2 groups. Four fish held in the tanks were used for the observation of early larval development (group 1). Eight fish (group 2) were held in the net pens and left undisturbed initially. This second group of fish was used for observing later development and estimating the length of full-term gestation. Six of the eight fish eventually spawned full-term larvae. Three fish taken live in 1984 also completed reproductive development.

Embryos from the fish in group 1, and later group 2, were sampled at approximately weekly intervals to monitor the progress of development. The embryos were extracted by catheterization with a flexible polyethylene tube. To minimize stress, the fish were catheterized quickly and without anesthetic using a modification of the method described by Stahl-Johnson (1984).

The embryos were categorized as to developmental stage using a 13-stage index developed by Stahl-Johnson (1984) for copper rockfish. Embryo staging data were used to estimate the length of the gestation period and the age of embryos at each stage. A total of 50 stage observations were made on 12 different fish during the embryonic development study of 1983. These data were supplemented when necessary with 21 stage observations of 8 *S. caurinus* made during a morphological development study by Stahl-Johnson (1984).

The respiration rate per embryo was measured using a Gilson differential respirometer and standard manometric techniques employing a methodology similar to that of Boehlert and Yoklavich (1984). Extracted embryos were extruded into an isotonic saline solution (325 mOsM/kg, Forster and Hong 1958; Boehlert and Yoklavich 1984) and quickly sorted with a pipette to insure that only undamaged embryos were used. The embryos were then placed in 15-ml respiration flasks containing the same saline solution, attached to the manometers, and allowed to equilibrate for 1 hr before starting the respiration observations. Once the embryos began to develop eye pigmentation, the reaction flasks were wrapped in dark plastic to exclude light. Comparative light-dark respiration experiments showed that earlier embryos were not light sensitive. The temperature was always held at 9.1°C, and runs were generally done in triplicate. Run times ranged between 3

and 10 hrs depending on the observed rate of oxygen consumption. After each run, the embryos were counted, placed in foil pans and used along with additional samples to determine dry weights.

Caloric content of whole gonad tissue containing embryos of known age were determined using a Parr Instruments adiabatic bomb calorimeter. The caloric content of whole gonads is probably not exactly the same as that of the embryos within, since the integumentary and connective tissue does have a slightly different energy content (mean for spent gonads = 5,725 cal/g, n = 4). However, the spent gonad weight was only about 8% of the whole gonad weight at the time of fertilization. Any correction would therefore be small relative to the predominant effects of declining caloric content (see below).

RESULTS

To estimate the length of the gestation period, it was necessary to sum a number of estimates of stage duration. The data were stratified into 3 stage intervals: (1) fertilization to early cleavage (Stages 1-2), (2) early cleavage to a 26-somite stage (Stages 2-8), and (3) 26-somite stage to parturition (Stages 8-13).

Our earliest observations after fertilization were of 8- and 16-celled embryos (Stage 2). Two fish took 5 days to reach stage 5. Stahl-Johnson observed the ovulation and fertilization of one fish and a 4-celled stage of another. Both of these fish took 6 days to reach stage 5. We therefore estimated that it took 1 day to develop from fertilization to the early cleavage stage.

The four group 1 fish provided a mean estimate of development time from early cleavage up to stage 8 of 12.5 days ($s = 1.29$, $n = 4$) (Table 1). Using group 2 fish, the average development time of stage interval 8-13 was estimated to be 27.8 days ($s = 3.19$, $n = 6$). The estimated total gestation period was therefore 41.3 days ($s = 3.44$) (Table 1). Stahl-Johnson (1984) estimated that the gestation length in *S. caurinus* was between 41 and 44 days. Application of the above methodology to her data yielded an estimate of 41.7 days, and the average value of 41.5 days was used as the best estimate of gestation length.

The functional relationship between oxygen consumption (R , $\mu\text{l O}_2/\text{embryo/hr}$) and age was determined using 20 respiration rate observations for 12 different development stages (Fig. 1). The staging data were used to estimate the age of embryos so that respiration rate could be expressed as a function of age (t , days since fertilization). The derived function

$$R = .0093e^{.0830t}$$

Table 1. Estimate of stage interval duration for determining gestation length.

Stage interval	Fish I.D.	Duration (days)	Average duration
Fertilization- early cleavage		1 day*	1 day
Early cleavage- 8	1	12	$\bar{x} = 12.5$ days, $s = 1.29$
	2	13	
	3	14	
	4	11	
8-13	5	31	$\bar{x} = 27.8$ days, $s = 3.19$
	6	27	
	7	25	
	8	28	
	9	32	
	10	34	
			Total 41.3 days, $s = 3.44$

*See text.

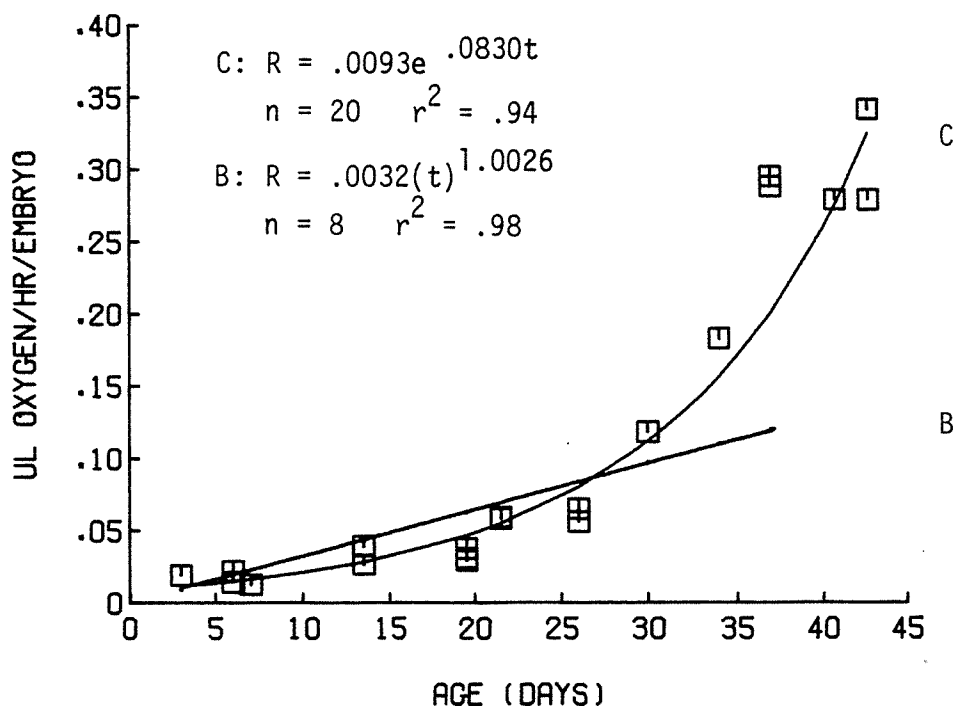


Figure 1. Oxygen consumption rates of copper (C) and black (B) rockfish (Boehlert and Yoklavich 1984) shown as a function of embryo age. (Points plotted for copper rockfish only.)

was then integrated, evaluated over the gestation length of 41.5 days, and multiplied by 24 hr/day. The result (81.68 $\mu\text{l O}_2/\text{embryo}$) was converted to .408 calories consumed per embryo using the oxycaloric equivalent of .005 calories/ $\mu\text{l O}_2$ (Lasker 1962; Boehlert and Yoklavich 1984).

To estimate the number of calories lost per embryo during gestation, it was necessary to account for the decline in both dry weight and caloric content. The weights of ova at fertilization (.160 mg) and embryos at birth (.127 mg) were estimated from an age-embryo weight regression function (Fig. 2). The decline in caloric content was best described by a quadratic equation (Fig. 3). The estimated caloric content of gonads at fertilization and birth was 6,554 and 5,419 cal/g dry weight, respectively. The caloric loss per embryo was therefore estimated to be .361:

	Dry weight (mg)	Calorie content per g dry wt	Calorie content per individual
Fertilization	.160	6,554	1.049
Birth	.127	5,419	<u>0.688</u>
			0.361

The difference between the energy consumed (.408 cal/embryo) and energy lost (.361 cal/embryo) during gestation indicates that .047 calories/embryo, or 11.5% of the energy utilized during gestation, is contributed by the mother after fertilization.

DISCUSSION

Our results can be compared directly with those of Boehlert and Yoklavich (1984) who, using similar methods, found a substantially greater (70%) maternal contribution for black rockfish. Their conclusion concerning the maternal contribution in black rockfish was confirmed with additional histological data that demonstrated that larvae ingest nutrients by mouth during the later stages of development and absorb them through their gut (Boehlert and Yoklavich 1984).

The development of black rockfish embryos differed from that of copper rockfish in that the former lost proportionally less weight during gestation. Black rockfish embryos lost .0043 mg or 6.1% of their initial weight (.071 mg) whereas copper rockfish lost .033 mg or 20.6% of their weight at fertilization (.160 mg). This differential weight loss was the predominant factor responsible for the difference in the estimates of maternal contribution between the two species. Scrimshaw (1945) reported a net weight loss during development of 34% in *S. marinus* (Atlantic

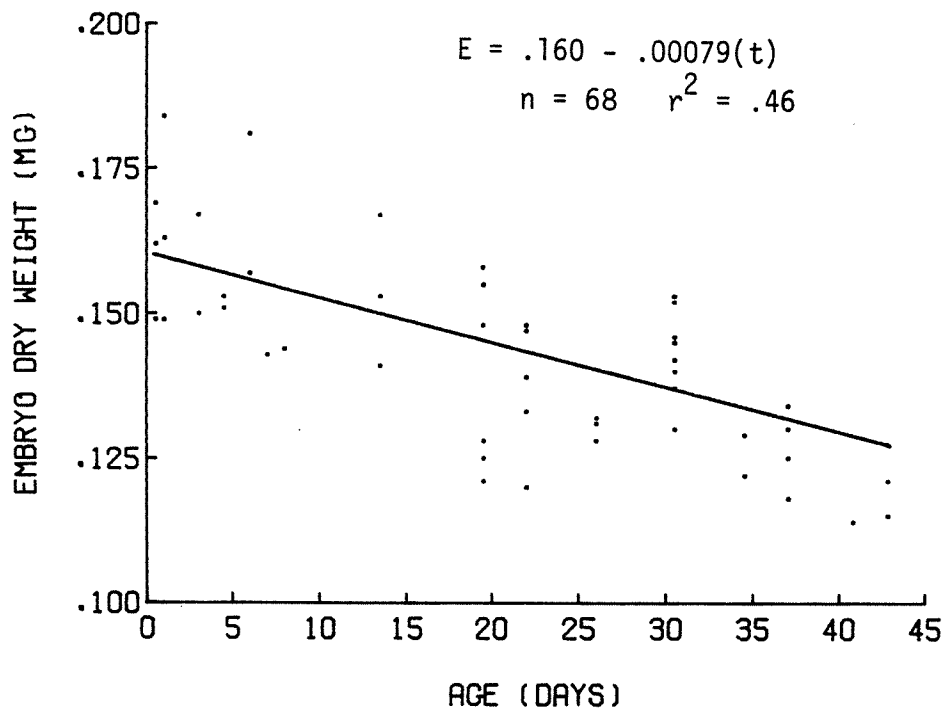


Figure 2. Embryo dry weight (E) shown as a function of embryonic age.

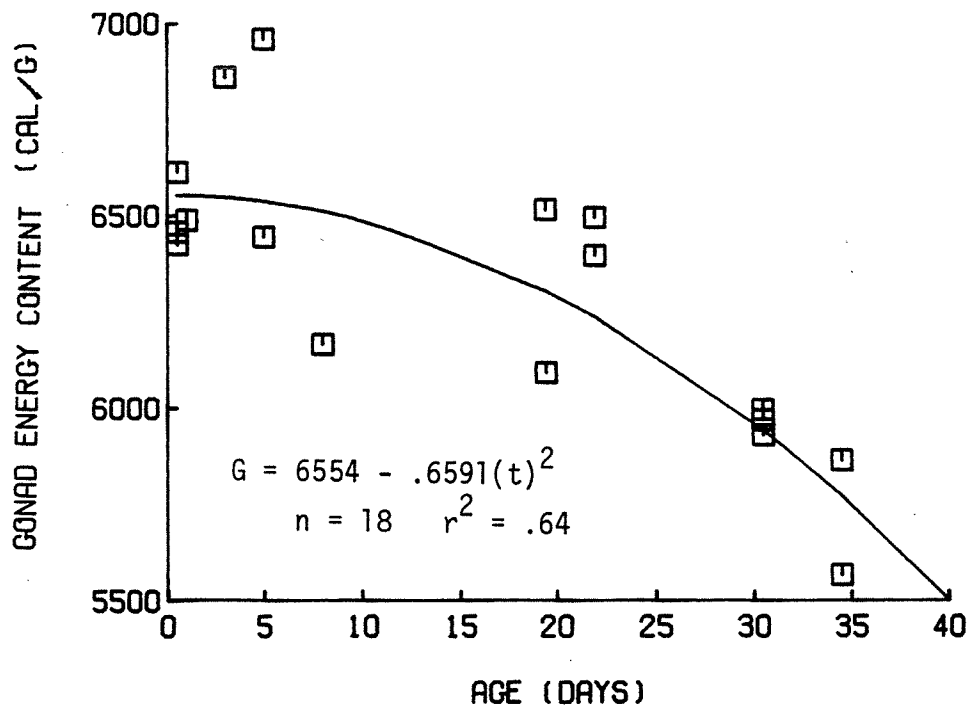


Figure 3. Whole gonad calorie content (G) shown as a function of embryonic age.

redfish) which suggests that some members of the genus may in fact be lecithotrophic as was suggested by Wourms (1981).

There were some major differences in the respiration observations for black and copper rockfish. Integration of the respiration curves led to estimates of 54.67 and 81.68 $\mu\text{l O}_2/\text{embryo}$ for black and copper rockfish, respectively. However, it is important to note that the functional forms of the curves were different (Fig. 1). The results for black rockfish were nearly linear ($\mu\text{l O}_2/\text{embryo}/\text{hr} = .0032t^{1.0026}$), whereas a steeply rising exponential function best fit our data. However, the respiration data presented by Boehlert and Yoklavich only included observations for the first 27 days of the 37-day gestation period. Inspection of Figure 1 with this in mind suggests that they may have missed the dominant inflection point in the respiration data and therefore underestimated total oxygen consumption per embryo. Recent comparable work on *Sebastes schlegeli* has shown an exponential increase in the respiration function (Boehlert et al. 1986) similar to that found for copper rockfish. If the total respiration of black rockfish is underestimated, then the maternal energy contribution estimate may be even greater than previously reported.

Boehlert and Yoklavich (1984) described a trend in the evolutionary development of the subfamily Sebastinae toward an increasing maternal commitment to the progeny. More primitive genera probably spawned eggs shortly after fertilization (Barsukov 1981). Members of the genus *Helicolenus* retain eggs for longer periods of time, but the length of this period varies between species (Graham 1939; Kreft 1961). Species within the genus *Sebastes* are more advanced and release fully developed larvae. Direct examination of the embryonic energetics of *S. caurinus* and *S. melanops*, and additional information pertaining to *S. schlegeli* and *S. marinus*, has shown that matrotrophic viviparity does exist within *Sebastes*, but there is considerable variability in the magnitude of the maternal contribution during gestation.

ACKNOWLEDGMENTS

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