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## Energetics during embryonic development in kurosoi, *Sebastes schlegeli* Hilgendorf

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**Abstract:** Studies on the live-bearing scorpaenid genus *Sebastes* have recently shown that embryos of one species receive nutrition in addition to that supplied in the yolk. In this large genus, however, reproductive characteristics may differ among species. Energetics of embryonic development in kurosoi, *Sebastes schlegeli* Hilgendorf, were analyzed to determine the patterns of embryonic nutrition. The egg of this species is larger and contains over three times the energy content of that in *S. melanops*, another species which has been studied. Catabolism during the 51 days of embryonic development required 88% of the original energy in the egg, but the embryo at birth contained 93% of the initial egg energy. Thus the total energy required for development from fertilization to birth requires  $\approx 1.8$  times the initial, endogenous energy supply. Histological studies demonstrate that uptake occurs through ingestion and absorption of ovarian fluid in the hindgut. Protein and nitrogen budgets during development suggest that the primary substance taken up is nitrogenous.

No distinct structures are apparent in the ovarian system to supply nutrients to the developing embryos. Analysis of fecundity-at-length, however, shows that post-fertilization fecundity estimates are significantly lower than pre-fertilization values; the reduction apparently occurs through resorption of ova or early embryos. Along with catabolism, this results in an overall decrease in the energy content of the ovaries during development, but the total amounts of protein and nitrogen remain nearly static. We thus suggest that resorption of unfertilized ova or early embryos which die may enrich the ovarian fluid and supply energy to the surviving embryos. This is a primitive form of embryonic nutrition in viviparous species and may be common in the genus *Sebastes*.

**Key words:** Reproduction; Embryo; Energetics; Respiration; Viviparity; *Sebastes*

### INTRODUCTION

Reproduction in the live-bearing genus *Sebastes* has historically been considered to be lecithotrophic viviparity, with the only parental care provided as a form of embryonic protection (Balon, 1975) rather than a nutritional association between female and embryo (Needham, 1942; Scrimshaw, 1945). The reasons for this description have not been based on energetic studies but rather on inference; initial egg size is small, larvae are small at birth, and fecundity is very high (Phillips, 1964; Moser *et al.*, 1977; Boehlert *et al.*, 1982). Recently, however, Boehlert & Yoklavich (1984) have shown that embryos

of the black rockfish, *S. melanops*, derive some form of nutrition from the maternal system during later stages of the approximately 37-day gestation period. They suggested that the source of nutrition was a nitrogenous substance, because nitrogen content remained static during development, in contrast to oviparous species (Hayes, 1949; Rogers & Westin, 1981). The suggested mechanism of nutrition was through ingestion of ovarian fluid and subsequent uptake in the hindgut (Boehlert & Yoklavich, 1984). This mechanism is suggested to be one of the earliest in the evolution of viviparity (Wourms, 1981).

The genus *Sebastes* has over 100 species (Barsukov, 1981); there is thus a question of how representative the results from a single species are to the reproductive pattern in the genus. In the live-bearing poeciliids, for example, Thibault & Schultz (1978) observed remarkable variation within a single genus. Considering the ratio of dry weight of developed embryo at birth to that of the egg at fertilization in the genus *Poeciliopsis*, they noted values from 0.61 to 19, demonstrating a range from lecithotrophic to matrotrophic viviparity. More recent work has demonstrated both spatial and temporal population-level differences in the type of viviparity in *Poecilia latipinna* (Trexler, 1985).

Considerable differences exist in initial egg size, larval size at birth, and weight-specific fecundity in the genus *Sebastes*. *Sebastes melanops* has an egg diameter of 0.8 mm at fertilization (Boehlert & Yoklavich, 1984), typical of the small ( $\approx 1$  mm) eggs of eastern Pacific species (Moser *et al.*, 1977). Eastern Pacific species were previously considered to be a different genus (*Sebastodes*) to Atlantic and western Pacific species but were combined by Matsubara (1943), whose interpretation was supported by Chen (1971). Western Pacific species generally have larger eggs (to 1.5 mm in *S. pachycephalus*, Fujita, 1957). Advanced viviparous species typically have very small eggs at fertilization and rely upon maternal nutrition for embryonic development (Turner, 1947; Thibault & Schultz, 1978). Thus within a genus it might be advantageous to compare species with different egg sizes; in this study we describe embryonic energetics and development of kurosoi, *Sebastes schlegeli* Hilgendorf, from Japan.

*Sebastes schlegeli* is a coastal species which inhabits rocky reefs and is found from depths of 30 to 100 m. It is relatively docile in aquaria, has an egg size of  $\approx 1.2$  mm, and larvae may be reared; it is thus considered a species with aquaculture potential in Japan (Kusakari *et al.*, 1977; Kusakari, 1978). Shimizu & Yamada (1980) considered ultrastructural aspects of yolk utilization in the vitelline syncytium and suggested that the pattern was similar to that in oviparous fishes. They did note, however, an opaque substance in the hindgut in late stage embryos, a situation analogous to that in *S. melanops* (Boehlert & Yoklavich, 1984). This suggests that a similar mechanism for nutritional uptake may exist in this species.

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## MATERIALS AND METHODS

## EXPERIMENTAL ANIMALS

Experiments were conducted during May 1984, the typical spawning season for this species. Fish were held in 1.1 m by 1.8 m tanks at the Hokkaido Institute of Mariculture, Shikabe, Hokkaido, Japan, where they were subject to ambient temperature. Most fish were laboratory broodstock which had been held for long intervals, but additional females were also captured near Otohe in the Sea of Japan shortly prior to the experiments. Since temperature control was not available for the large holding tanks, females were held throughout the experiments at ambient temperature, which fluctuated between 4.6 and 7.3 °C.

Before experiments, all females were anesthetized in ethane methanesulfonate (MS-222, Argent), weighed, tagged with an identifying number, and embryos removed from the ovary by gentle catheterization for determination of stage of development. Developmental stages were classified according to a modification of Oppenheimer (1937) and Yamada (1963) (Kusakari, unpubl. data).

## LABORATORY PROCEDURES

Laboratory procedures generally followed Boehlert & Yoklavich (1984). Gestation time and duration of different stages of development were determined from several measurements on 55 females held in the laboratory during 1983. Although temperature in the tanks varied between 8.0 and 13.6 °C during this time, the mean temperature was 10.6 °C (SD 1.48 °C). Fish were catheterized at times  $t_1$  and  $t_2$ , and the corresponding developmental stages determined as  $S_1$  and  $S_2$ , respectively. The data points

$$\frac{S_1 + S_2}{2}, \frac{t_2 - t_1}{S_2 - S_1},$$

or mean stage ( $S$ ) and stage duration ( $D$ ) in days, were plotted and a curve fitted to the data. Only those observations separated by 7 days or more were used in these calculations.

Oxygen consumption was determined for embryos at several stages of development. Embryos were removed from the female and placed in isosmotic saline (340 mosM/kg, Wasserman *et al.*, 1953) in a 15-ml respirometer flask; numbers of embryos per flask ranged from 45 to 267. After equilibration of temperature for  $\approx 1$  h, oxygen consumption ( $Q$ ) was measured on a Gilson<sup>1</sup> differential respirometer at 10 °C using standard techniques (Umbreit *et al.*, 1972). Except when readings were taken, lights were kept off during experiments. Experiments were run between 1000 and 2000 h. Values of

<sup>1</sup> Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

oxygen consumption ( $\mu\text{l O}_2$ ) were recorded at approximately hourly intervals and experiments lasted from 3 to 8.2 h. Two or three control flasks were run concurrently with each experiment and control values were subtracted from readings in experimental flasks. At the end of each experiment, the embryos were counted and used for dry weight and carbon and nitrogen determinations, and some embryos were preserved for determination of developmental stage. Ash-free dry weight (AFDW) was determined from groups of 35–100 embryos dried at 58 °C to constant weight (on aluminium foil), cooled to room temperature in a desiccator, and weighed. They were ashed for 4 h at 500 °C and ash weight determined. Carbon and nitrogen content were determined in a Hewlett-Packard Model 185 CHN analyzer. All values were means of duplicate samples. Caloric content was determined from percent carbon using the nitrogen-corrected equation of Salonen *et al.* (1976) following Boehlert & Yoklavich (1984). Protein was determined using Lowry's method (Lowry *et al.*, 1951), and protein nitrogen was determined by dividing this value by 6.25. Embryos were fixed in glutaraldehyde in saline and prepared for scanning electron microscopy using standard techniques (Dobbs, 1974; Boehlert, 1984) and were observed under a JEOL JSM-2F SEM. Transmission electron microscopy followed Shimizu & Yamada (1980) with 1.5 h fixative in a paraformaldehyde–glutaraldehyde mixture (Karnovsky, 1965) and post-fixation in 1% osmium tetroxide in phosphate buffer for 1 h. Ultrathin sections were doubly stained with uranylacetate and lead citrate and observations were made with a Hitachi HU-12 electron microscope.

## RESULTS

Stage duration calculations were based upon initial ( $S_1$ ) stages from 4 to 31, and final ( $S_2$ ) stages from 21 to birth. Earliest stages, which are passed through rapidly, were least represented in our estimates of stage duration ( $D$ ). These data were linearized and fitted to an exponential model with the resulting relationship;

$$D = 0.02535 S^{1.4333} \quad n = 55 \quad r^2 = 0.67 \text{ (Fig. 1).}$$

This curve (solid line) suggests that early stages are passed through very rapidly whereas later stages may take as long as 3.6 days. The area under this curve was integrated to give the dashed line, time since fertilization ( $T$ , days) as a function of developmental stage ( $S$ ). The equation is

$$T = 0.0104S^{2.4333}.$$

This relationship predicts that fertilization to birth (Stage 33) takes 51.5 days. Observations of three females from 1983 suggest a mean gestation time of 46 days but range from 42 to 50 days. This period may therefore be somewhat variable under natural conditions.

Oxygen consumption was determined from embryos at 16 stages of development and

showed an exponential increase with time since fertilization (Fig. 2). Earliest embryos (Stage 8, 1.6 days post-fertilization) showed very low oxygen consumption rates, with a mean value of  $0.0181 \mu\text{l O}_2 \cdot \text{embryo}^{-1} \cdot \text{h}^{-1}$ , whereas the latest stages tested

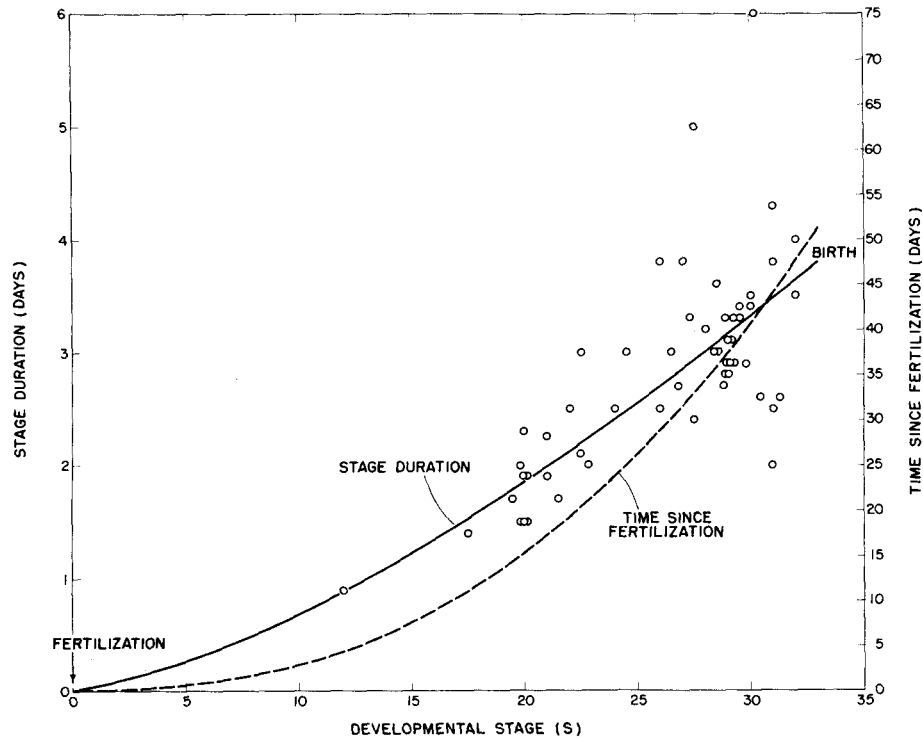


Fig. 1. Gestation time in *Sebastes schlegeli*, showing how long it takes to get to any given stage from fertilization: the calculations are based on the method of successive catheterization developed by Boehlert & Yoklavich (1984); stages follow Kusakari's modification of Oppenheimer (1937); here, Stage 33 represents birth; the estimated time from fertilization to birth is 51.5 days.

(Stage 31.5, 46 days post-fertilization) had mean oxygen consumption of  $0.6688 \mu\text{l O}_2 \cdot \text{embryo}^{-1} \cdot \text{h}^{-1}$ . Groups of the earliest embryos tested, when counted at the end of our experiments, were often characterized by a percentage of eggs which enlarged (and perhaps died) in the saline. To assess potential effects on respiration, eggs were allowed to remain in saline, and groups of enlarged ones placed in respirometers; oxygen consumption did not differ, however, between the "enlarged" and normal eggs. For embryos in the latest stages of development, limited swimming activity occurred in the flasks; thus our values for late stage embryos may be slightly inflated.

The curve fitted to the linearized oxygen consumption data is represented by

$$Q = 0.02188e^{0.07012T} \quad n = 16 \quad r^2 = 0.96 \text{ (Fig. 2) .}$$

This curve was integrated to estimate the total oxygen consumed ( $Q_T$ ) as a function of time since fertilization, resulting in the equation

$$Q_T = 7.4889e^{0.002922H},$$

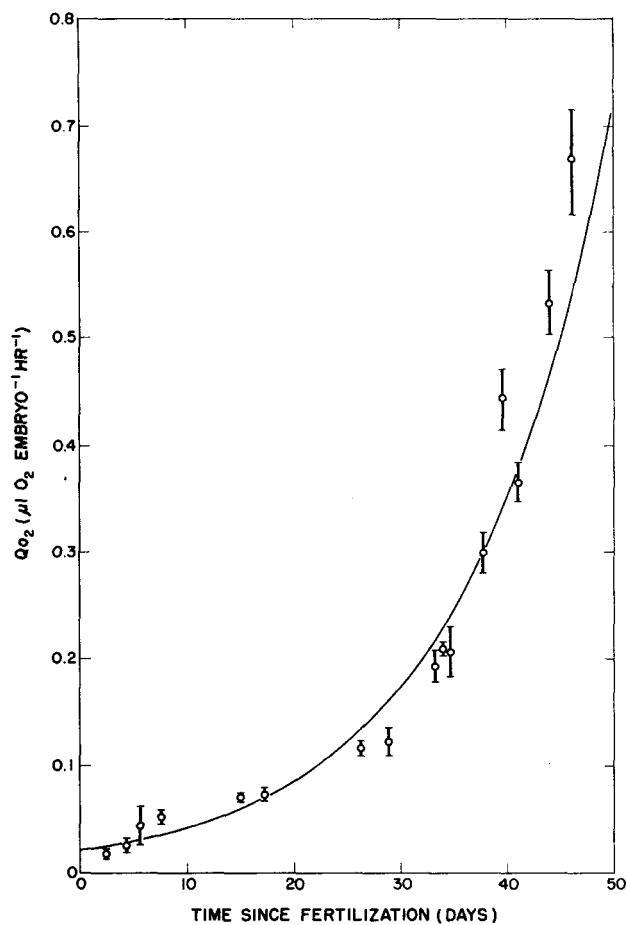


Fig. 2. Oxygen consumption in embryonic *Sebastes schlegelii*; the numbers of embryos varied between 45 and 267; there were generally more used in experiments with early stage embryos; experiments were run in a Gilsón differential respirometer at 10 °C; each point ( $\pm 2$  SE) represents the mean of three or four replicates from embryos from the same female.

where  $H$  = time since fertilization in hours. This relationship suggests that an embryo would consume a cumulative total of 277  $\mu\text{l O}_2$  during development from fertilization to birth. This value, converted to calories using the oxycaloric equivalent of 0.005 cal  $\cdot \mu\text{l O}_2^{-1}$  (Lasker, 1962), suggests that catabolism during gestation requires 1.386 cal.

Embryo weight and AFDW were estimated for 13 stages throughout gestation (Fig. 3A). Ash content generally increased with time since fertilization; the mean value was 8.5% dry weight. Our data suggest increasing AFDW ( $\mu\text{g}$ ) with time since fertilization, with the fitted curve

$$\text{AFDW } (\mu\text{g}) = 255.8 + 1.086T \quad n = 13 \quad r^2 = 0.40.$$

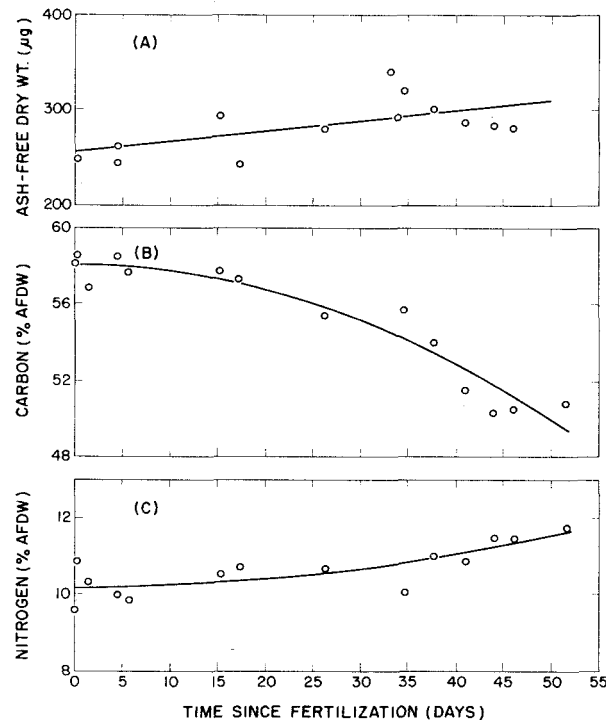


Fig. 3. Changes in ash-free dry weight (AFDW) (A), carbon (B), and nitrogen (C) as a function of time since fertilization (days): carbon and nitrogen are both expressed as percentages of AFDW; the increase in AFDW and nitrogen and the decrease in carbon are all significant.

A concern with this relationship is the source of weight increase. It is possible, for example, that older, larger females have larger embryos at all stages, as observed in some other species (Bagenal, 1971). If this were the case, and if larger females were fertilized earliest in the season, then the observed relation could be an artifact. To test for this, we regressed embryo weight on female length. The relationship was not significant ( $n = 13$ ,  $P > 0.25$ ). We thus concluded that the increase in weight is a function of time since fertilization rather than female size.

Carbon and nitrogen were determined as a percentage of AFDW. Carbon decreases as a function of time since fertilization ( $T$ ) by the fitted curve

$$\% C = 58.09 - 0.003272(T)^2 \quad n = 14 \quad r^2 = 0.92 \text{ (Fig. 3B).}$$

The decrease is highly significant. The carbon content at fertilization was converted to a caloric value of 1.59 cal using the formula from Salonen *et al.* (1976). The opposite trend occurs for nitrogen which shows a significant increase with time since fertilization, described by the curve

$$\% N = 10.16 + 0.000533(T)^2 \quad n = 14 \quad r^2 = 0.64 \text{ (Fig. 3C).}$$

The increasing AFDW and increasing % N combine to result in a marked increase in nitrogen over the course of development.

Protein was determined for embryos at 12 stages and increased during development. The relationship is described by

$$\mu\text{g protein} = 148.52 + 0.862T \quad n = 12 \quad r^2 = 0.75,$$

which predicts an increase of 44  $\mu\text{g}$  protein over the 51.5-day gestation period. Thus protein accounts for  $\approx 80\%$  of the weight gain of AFDW over development (Fig. 3A), suggesting that protein is the major source of maternal nutrition in this species. Protein nitrogen, calculated by dividing protein by 6.25, increases linearly but not as fast as total nitrogen (Fig. 4). Based upon the curves for AFDW and nitrogen (Fig. 3A,C), total

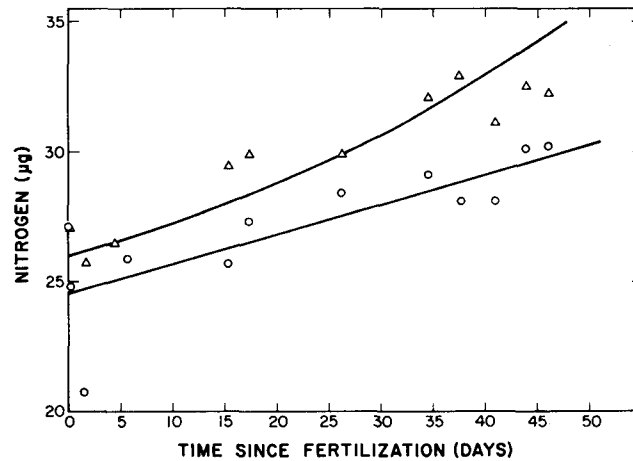


Fig. 4. Changes in nitrogen content of embryonic *Sebastes schlegeli* as a function of time since fertilization: the lower curve and points represent protein nitrogen (protein/6.25); the upper curve represents total nitrogen determined by CHN analysis.

nitrogen increases from 26.0  $\mu\text{g}$  at fertilization to 36.1  $\mu\text{g}$  at birth, whereas protein nitrogen increases from 24.5  $\mu\text{g}$  to only 30.5  $\mu\text{g}$  (Fig. 4). Thus non-protein nitrogen increases from 5.7 to 15.3% during development, suggesting that nutrition from the maternal system may also include amino acids and peptides in addition to protein. The same phenomenon was observed for *S. melanops* (Boehlert & Yoklavich, 1984).



A comparison of catabolic and calorimetric estimates of energetics of embryonic development in *S. schlegeli* shows different patterns (Fig. 5) similar to that observed in *S. melanops* Girard. The catabolic energy usage is calculated by considering the

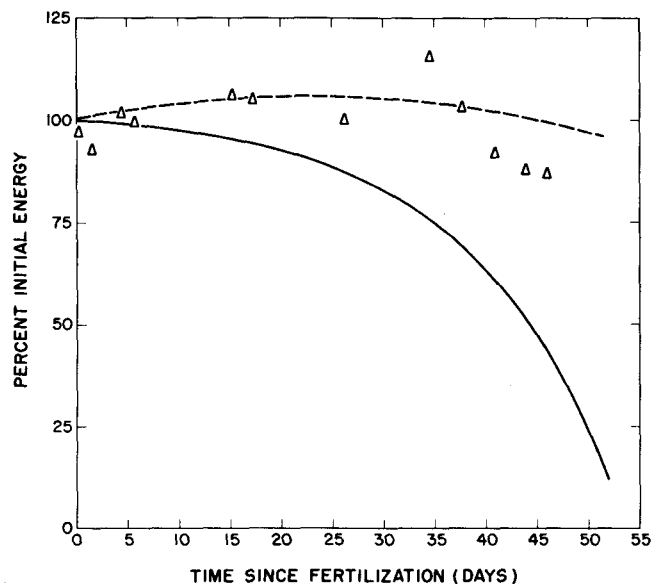


Fig. 5. Summary of the energetics of development in embryonic *Sebastes schlegeli*: the  $y$ -axis is the percent initial energy, where 100% represents 1.59 cal; the solid line represents expected energy remaining with time based on subtraction of cumulative catabolism; the dashed line and data points represent data from actual caloric content of embryos at different stages.

cumulative oxygen consumption (Fig. 2) and converting it to calories; the resulting values were subtracted from the total energy available in the egg at fertilization (1.59 cal). The catabolic energy utilization suggests that  $\approx 12\%$  of the original yolk energy remained at the end of development. The data points represent actual energetic data. Fitting a curve to these data suggests that  $\approx 93\%$  of the original energy remained at birth. The difference between the catabolic calculation (solid curve) and the actual calculation (dashed line) represents the maternal contribution to embryonic development. Our estimate of total energy necessary for each embryo is thus 2.87 cal (1.39 catabolic calories, 1.48 in larva at birth); this represents 1.8 times the initial energy content of the yolk at fertilization.

The mechanism of nutrient uptake in embryos of *S. schlegeli* was investigated by analyzing morphology. The epidermis of this species, when examined with scanning electron microscopy, shows microridges developing in early stages (Figs. 6A-C). They are particularly conspicuous in the dorsal head, yolk sac, and tail regions, scattered over the cell surface as irregularly arranged, discontinuous fragments. With advancing developmental stage, microridge development increases over the entire body

(Figs. 6D–E), but some cells have little or no microridge development (Fig. 6F). At Stage 28, smooth continuous microridges become apparent along the cell border, but nowhere is there evidence of microvilli, which typically characterize absorptive surfaces (Veith, 1979). A very conspicuous cell type, the sacciform cell (Bullock, 1980), is highly abundant in the area underlying the epithelial cells. On the surface, they are evident as

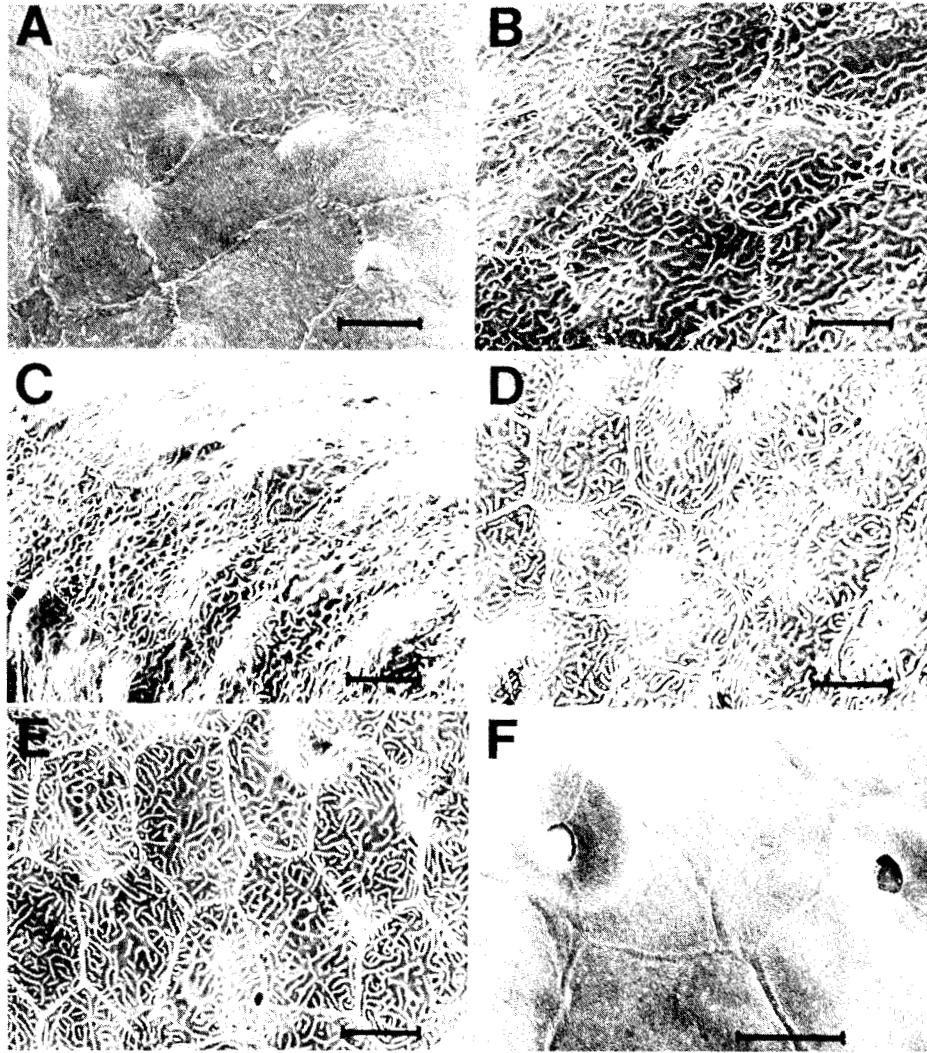


Fig. 6. Scanning electron microscopy of epidermis of embryonic *Sebastes schlegelii*: A, development Stage 24, dorsal surface of head; B, Stage 24, yolk sac surface; C, Stage 24; dorsal side of the tail finfold; D, Stage 28, dorsal surface of head; E, Stage 28, yolk sac surface; F, Stage 27, surface of the posterior part of the yolk sac corresponding to the site of an oil droplet; note the pores associated with the underlying sacciform cells; scale bars = 1  $\mu$ m.

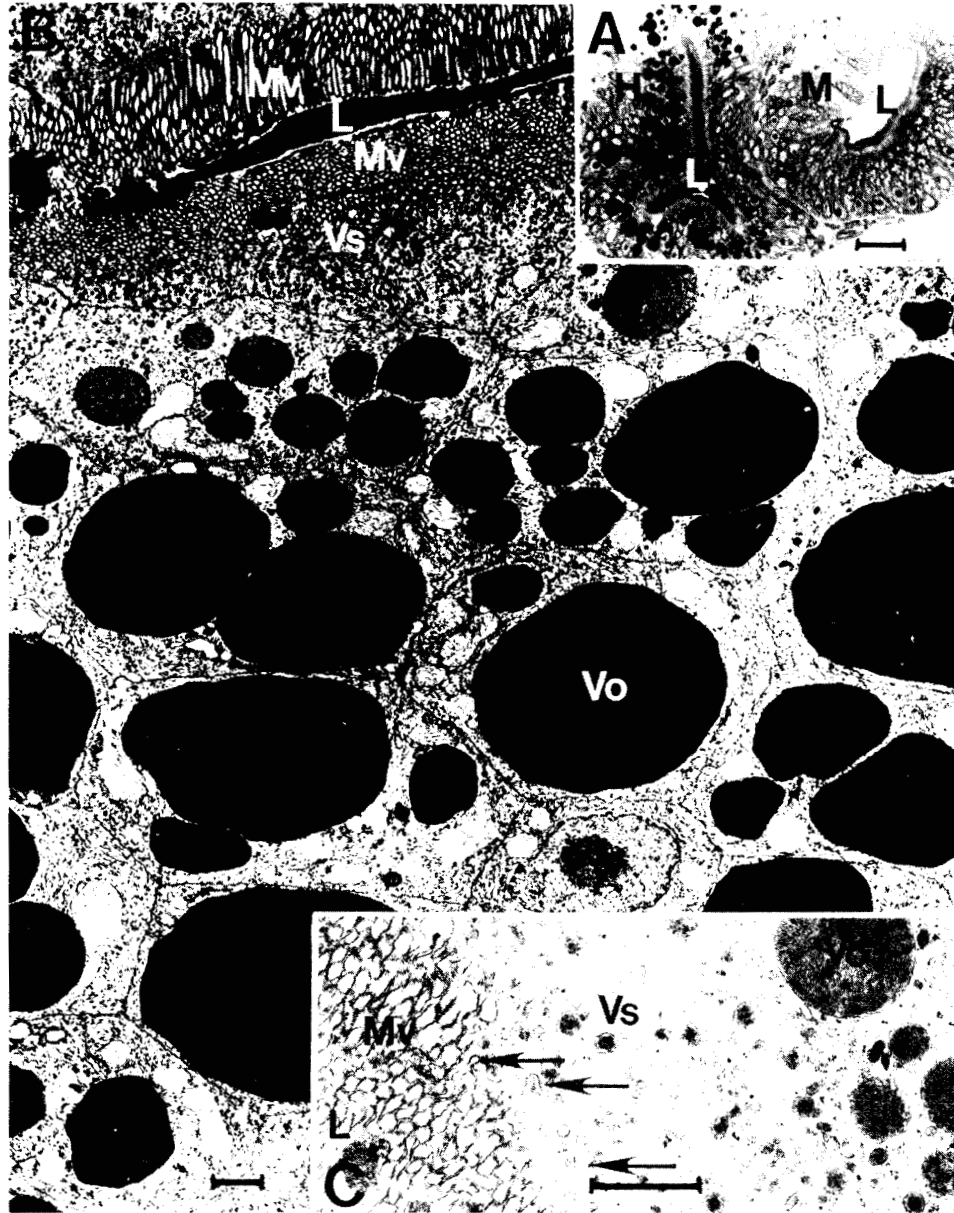


Fig. 7. Structures of the hindgut in embryonic *Sebastes schlegeli* at development Stage 28 (34.5 days post-fertilization); A, light micrograph of a 1  $\mu\text{m}$ -thick Epon section of the hindgut (left) and midgut (right); methylene blue-azur II stain; scale bar = 20  $\mu\text{m}$ ; B, transmission electron micrograph of the apical part of the hindgut cells; scale bar = 1  $\mu\text{m}$ ; C, enlargement of the apical region of a hindgut cell; scale bar = 1  $\mu\text{m}$ ; H, hindgut; L, lumen; M, midgut; Mv, microvilli; Vo, vacuole; Vs, vesicle; arrows indicate pinocytotic invaginations between microvilli.

either swellings with central pits or depressions (Figs. 6A,C,D) or as pores (Fig. 6F). Their function in *S. schlegeli* embryos is unknown.

While we see no evidence of epidermal nutrient uptake, the gut is especially well developed in the hindgut region after opening of the mouth. We observed an opaque substance in the hindgut of developing embryos after mouth opening, as noted previously (Shimizu & Yamada, 1980). Under the microscope we observed movement of this substance within the gut and peristaltic movement in the hindgut region. Gut structure of Stage 28 embryos shows a high degree of development and apparent digestive activity (Fig. 7). The hindgut is characterized by many large vesicles not apparent in midgut tissue (Fig. 7A). Transmission electron microscope observations of hindgut cells show that the vesicles are variably sized and contain an electron dense, homogeneous substance, mostly distributed in the apical cytoplasm (Fig. 7B); it is likely that vacuolar contents in this part of the gut are proteinaceous (Watanabe, 1982). A number of tiny, round, or tubular vesicles are apparent under the microvillous cell surface facing the lumen, apparently forming from pinocytosis (Fig. 7C). These small vesicles merge to form larger ones as distance from the lumen increases (Fig. 7B,C).

#### DISCUSSION

The results of this study demonstrate that *S. schlegeli* embryos receive nutrition beyond that available in the yolk, consistent with the interpretation of Boehlert & Yoklavich (1984), who observed a similar pattern of embryonic energetics in *S. melanops*. The egg of this latter species has only 27% of the energy content of the larger egg of *S. schlegeli* at fertilization, and the gestation time at 10 °C is 37 compared with 51.5 days. Despite greater initial energy content, uptake of additional nutrition and energy in *S. schlegeli* is greater than in *S. melanops*, particularly for protein (Table I). Boehlert & Yoklavich (1984) suggested that the major source of uptake was protein or some other nitrogenous compound because total nitrogen remained static while protein decreased. Nitrogen utilization in early development may be generally true in fishes; Eldridge *et al.* (1982) pointed out that lipid is reserved until feeding begins in striped bass larvae. This might explain the increasing carbon/nitrogen ratio noted during development in this species (Rogers & Westin, 1981).

Our histological studies suggested that the hindgut was the location of nutrient uptake; the presence of an opaque substance in the lumen and the characteristic features of the hindgut cells (Fig. 7) indicated that the substance ingested into pinocytotic vesicles may undergo intracellular digestion. The hindgut region is where protein absorption occurs in fish larvae (Iwai, 1969; Tanaka, 1973; Watanabe, 1982; Govoni *et al.*, 1986). In *S. schlegeli*, nitrogen and protein both increase with development (Fig. 4; Table I), as supported by morphology of the hindgut (Fig. 7). We observed no evidence that epidermal cells are involved in nutrient uptake (Fig. 6), and the pattern and development of microridges were similar to those in medaka, an oviparous fish

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(Yamada, 1966). The presence of a large number of sacciform cells, however, with openings to the epidermal surface deserves more study due to their unknown function. Thus, a similar, but perhaps more advanced mechanism of embryonic nutrition is present in *S. schlegeli* compared with *S. melanops*.

TABLE I

Changes in selected developmental features at the beginning and end of gestation in *Sebastes schlegeli* (present study), *S. melanops* (Boehlert & Yoklavich, 1984), and *S. caurinus* (Dygert, 1986): gestation times are 51.5, 37, and 41.5 days, respectively; PN/TN, protein nitrogen to total nitrogen ratio; C/N, carbon-nitrogen ratio; \* near, but slightly after fertilization.

	<i>Sebastes schlegeli</i>		<i>Sebastes melanops</i>		<i>Sebastes caurinus</i>	
	Initial	Final	Initial	Final	Initial	Final
AFDW ( $\mu\text{g}$ )	255.8	311.7	67.5	60.4	160	127
Caloric content	1.59	1.48	0.429	0.345	1.05	0.69
Nitrogen ( $\mu\text{g}$ )	26.0	36.1	7.0	7.0	-	-
C/N ratio	5.72	4.27	5.74	4.80	-	-
Catabolic energy use (%)	0	88	0	64	0	38.9
Protein ( $\mu\text{g}$ )	148.5	192.9	45.3*	35.3	-	-
PN/TN	0.94	0.85	0.99*	0.73	-	-

A comparison of embryonic oxygen consumption by these two species suggests that it is greater in *S. schlegeli*, as would be expected on size considerations alone, but the relative consumption throughout gestation is also greater (Table I). The data for *S. schlegeli* are more complete than for *S. melanops* and reveal a marked exponential increase in later stages of development (Fig. 2). This agrees with data for *S. caurinus* embryos (Dygert, 1986). Several sources of error may occur in these estimates of oxygen consumption. In the present study, experiments were run at 10 °C but gravid females were held at lower, ambient temperatures. In juvenile *S. diploproa*, Boehlert (1978) distinguished between acute and acclimated changes in oxygen consumption rate between different temperatures and showed that different  $Q_{10}$  values characterize these different temperature changes. Applying the increased oxygen consumption values associated with acute temperature change suggests that values in the present study may be some 12% higher on average than if the females were held at 10 °C and thus acclimated to that temperature.

A second source of error in the measurement of oxygen consumption may be activity in later stages of embryos when removed from the maternal system. Differences in larval respiration rates between "active" and "inactive" Pacific sardine larvae may be from 1.3 to 3.5 times (Lasker & Theilacker, 1962). The third and perhaps most serious source of error in measurements of oxygen consumption for all three species (*S. schlegeli*, *S. melanops*, and *S. caurinus*) may be due to oxygen tension. Oxygen tension in the

Gilson respirometer is initially at saturated, atmospheric levels (Umbreit *et al.*, 1972). Oxygen tension inside the ovary is unknown, but *Sebastes* has a unique system of supplying arterial blood to both ovaries (Moser, 1967). Still, the ovaries in *Sebastes* are very large and later stage embryos must require a great deal of oxygen; diffusion from the vascularized ovarian wall must be important. Also, in the embiotocids *Rhacochilus vacca* and *Embiotoca lateralis*, Webb & Brett (1972) suggested that convection of embryonic fluid must occur. Still, for *S. schlegeli*, a 35-cm female with advanced embryos has estimated fecundity near 120 000 embryos (Kusakari, unpubl. data). Given the oxygen consumption rate of later larvae (Fig. 2), this could require oxygen delivery of some 40 ml/h to each ovary. Thus oxygen tension in the ovary may be reduced, a situation which may lower respiration rate in embryos (Carlson & Seifert, 1974). It is therefore possible that the rate of development of embryos may be under the control of oxygen availability and that the long gestation times and stage durations (Fig. 1) may be a result.

While all the above factors would operate to increase in vitro estimates of oxygen consumption and therefore our estimates of catabolic energy utilization, this does not change our conclusions. That is, the increasing AFDW (Fig. 3), protein content (Fig. 4, Table I), and hindgut development (Fig. 7) confirm that *S. schlegeli* is a matrotrophic vivipare. Nonetheless, it would be interesting to measure oxygen tension in the ovary and oxygen consumption of embryos at these values, if they are lower than saturation, and also to determine the developmental rate at saturation oxygen levels in vitro. Triplett (1960), for example, observed more rapid development, typically at smaller sizes in embiotocid embryos reared in vitro. These embryos come from an ovary with advanced structures for nutrient and respiratory gas exchange (Webb & Brett, 1972) and embryos have specialized spatulate median fins for gas exchange (Dobbs, 1975).

The source of the nutritional substance is an interesting question. *Sebastes* has no known specialized ovarian system for nutrient supply as seen in other viviparous fishes (Amoroso, 1960; Wourms, 1981), although the ovarian granulosa cells hypertrophy in the ovary of *S. paucispinis* (Moser, 1967). Boehlert & Yoklavich (1984) suggested that embryos resorbed after death in the ovary may be a source of such nutrition, since realized fecundity at birth is considerably lower than the fecundity estimates before fertilization (*S. marinus*, Raitt & Hall, 1967; *S. thompsoni*, Yoneyama, 1979; *S. entomelas*, Boehlert *et al.*, 1982). We noted atretic, post-fertilization embryos in *S. schlegeli*, particularly at early stages of development, and estimates of fecundity in *S. schlegeli* similarly differ for pre- and post-fertilization ovaries (Kusakari, unpubl. data), with typically lower fecundity at a given length for females with fertilized embryos. If these resorbing embryos represent the source of nutrition, this mechanism would be similar to the case of oophagy in elasmobranchs (Amoroso, 1960; Wourms, 1977). It would be interesting to note whether the decrease in fecundity provides sufficient energy to fuel the additional energy input to the remaining embryos. In a 35-cm (SL) fish, for example, the predicted difference in pre- and post-fertilization fecundity is  $\approx 25\%$ . Total ovarian embryonic energy content, embryo dry weight, and carbon all show significant

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declines in this situation (Table II). Nitrogen and protein, however, are nearly conserved since they increase in individual embryos during gestation (Figs. 3 and 4). It is thus possible that the resorption of embryos from the reduction in fecundity after fertilization

TABLE II

Estimated energy content of the ova and fully developed embryos of a 35-cm *Sebastes schlegeli* based on the fecundity change after fertilization: the fecundity relationships from 1979 data are as follows: pre-fertilized fecundity (range 25.8–38.8 cm SL, mean 33.6 cm SL):  $\ln(F) = 5.824 + 0.1760(\text{SL})$ ; ( $n = 40$ ;  $r^2 = 0.75$ ); post-fertilized fecundity (range 28.6–41.6 cm SL, mean 34.5 cm SL):  $\ln(F) = 7.935 + 0.1072(\text{SL})$ ; ( $n = 24$ ;  $r^2 = 0.67$ ), (AFDW = ash-free dry weight).

	At fertilization	At birth
Number of embryos	160 171	119 014
Energy (kcal)	254.17	176.28
Embryo AFDW (g)	40.89	37.12
Carbon (g)	23.75	18.34
Nitrogen (g)	4.15	4.30
Protein (g)	23.74	22.98

provides nutrition for surviving embryos. Natural embryo death may be the cause of this reduction in fecundity. Vladimirov (1975) suggested the existence of genetic critical periods in embryonic development where heightened mortality occurs. Longwell & Hughes (1981) provide cytogenetic data supporting this argument in eggs of *Scomber scombrus*, finding chromosomal anomalies presumed to cause mortality. If high embryonic death is generally the case in *Sebastes*, viviparity is especially significant; rather than losing the energy in dead embryos as in oviparous species, it may be recovered by surviving broodmates, in turn increasing their chances of survival. The additional nutrition provided during gestation would thus come from within the ovary itself rather than being provided by new energy from the maternal system.

The evolution of viviparity in the sebastine scorpaenids may involve egg size and, therefore, gestation time. Egg size in *Helicolenus* is relatively small but gestation time is unknown (Graham, 1939). *Sebasticus*, a relatively primitive genus, has a shorter gestation time (20–25 days) and larvae are born immediately after hatching from the chorion (Tsukahara, 1962). Larvae at birth are small, near 3.7 mm total length, but are fully developed and ready to commence feeding (Tanaka, 1973). In species of *Sebastes* on the west coast of North America, the egg size is generally 1 mm or less and gestation time is typically intermediate, estimated as 37 days for *S. melanops* (Boehlert & Yoklavich, 1984) and 41.5 days for *S. caurinus* (Dygert, 1986). With a 1.2 mm egg, gestation time in *S. schlegeli* increases to 51.5 days (Fig. 1) and the characteristics of additional nutrition are similarly greater (Figs. 3 and 4; Table I). The longer gestation period may allow more time for absorption of nutrients. Our results suggest that

viviparity in this group evolved through a passive strategy as suggested by Wourms (1981).

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