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REARING OF *SEBASTES* LARVAE (SCORPAENIDAE) IN STATIC CULTURE

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TECHNICAL REPORT

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Director

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INTRODUCTION

Rockfishes (genus *Sebastes*) are an abundant and speciose group of fishes in the northeast Pacific. About 35 species are found in the Puget Sound area (Hart 1973), many of which are of sport and commercial interest.

Their reproductive biology, a primitive viviparity (Boehlert and Yoklavich 1984), is unusual among marine fishes. Developing embryos receive nutrition during gestation by assimilating and consuming ovarian fluid enriched by reabsorbtion of unfertilized ova and dead embryos. The larvae are born well-developed with the ability to initiate feeding immediately. After several months in the plankton, they are recruited into adult habitats.

Rockfishes exhibit a seasonal plasticity in reproduction over a wide geographic distribution. Wyllie Echeverria (1987) reported that most rockfishes in central California have major periods of larval release during winter (November-March) or spring (April-July). The spring period for larval extrusion seems to occur later off Washington and British Columbia (Kendall and Lenarz 1987).

Seasonal overlaps in reproduction and distribution for many species of *Sebastes* have confused the larval taxonomy. Literature descriptions of single stages of yolk exhaustion or preflexion larvae are usually inadequate for identifying postflexion larval or juvenile stages. Westrheim (1975) found that meristic and morphometric characters in preextrusion larvae are not developed enough to permit accurate identification of field-caught specimens. While different stages of preflexion development for some 50 species have been illustrated, complete larval descriptions exist for only 8 species of northeast Pacific rockfish. Kendall and Lenarz (1987), in comparing interspecific pigmentation patterns of yolk sac larvae from published illustrations, concluded that preflexion larval pigmentation, together with intraspecific and developmental variation, is probably not well established enough to serve as a criterion for field identification. Rearing efforts to establish developmental series will probably be required to establish criteria for identifying field samples.

This report summarizes preliminary efforts to rear larvae of several species of *Sebastes* from extrusion through metamorphosis. Two major goals were to achieve a reliable methodology for culture and provide a developmental time series from known larvae for reference. Larvae from *Sebastes caurinus, S. nebulosus, S. ruberrimus*, and *S. auriculatus* were reared in the laboratory for varying lengths of time; cultures of the latter three species lived beyond 30 days, providing developmental series through metamorphosis. This study also provides results of the first rearing attempts for larvae of *S. nebulosus* and *S. ruberrimus*.

MATERIALS AND METHODS

Gravid *Sebastes* were captured at the Seattle Aquarium by SCUBA diving or from local fisherman. Fish were transported in aerated 150-L tanks at constant temperature and kept in darkened

300-L tanks with flowing seawater. Females not extruding larvae at time of capture were usually catheterized to determine the extent of larval intraovarian development. Larvae extruded during capture were placed in plastic bags or otherwise held separate from adults during transport. Those extruded in holding tanks were transferred in large beakers to culture tanks to minimize handling stress.

Extruded larvae were introduced into black 100-L (fiberglass) or 20-L (polycarbonate) tanks filled with 1.0-µm filtered seawater. Temperature was maintained at 10.1 ± 0.4°C. Constant illumination was provided by a dim overhead fluorescent light and ranged from $0.40\text{-}0.45 \times 10^{14}$ quanta sec^{-1} cm^{-2} within the tanks. Initial stocking densities for larvae were not quantified but estimated at 15-20 larvae L^{-1}. Approximately 33%-50% of the water in the tanks was exchanged each day and circulated by minimal aeration in the center of the tank. Dead larvae and food organisms were siphoned off the tank bottoms before the water exchange to limit bacterial growth.

Food organisms for larvae were the rotifer, *Brachionus plicatilus*, and field-caught zooplankton. *Brachionus* were reared in the laboratory at 18°C on a mixed algal diet of *Isochrysis galbana* and *Thallasiosira weissflogii*. Zooplankton were obtained by gentle plankton tows and seived to different size fractions. Wild zooplankton consisted almost entirely of early copepodite and naupliar stages of the copepod, *Acartia clausi.*

Newly extruded larvae were fed an approximate 2:1 mixture of rotifers and 35- to 100-µm zooplankton, the latter comprised of early-stage nauplii. As the larvae grew, larger food sizes were added and the percentage of rotifers decreased. Late copepodite and adult stages of *Acartia* (>202 µm fraction) were provided for larvae after they had reached a standard length of 6.0 mm. Prey densities were monitored every other day by subsampling triplicate 10-ml volumes with a Hansen-Stempel pipette. Sufficient food was then added to bring food levels near 10 prey ml^{-1}.

Developing *Sebastes* larvae were subsampled every 3-5 days, anesthetized with MS-222, and total lengths measured with a dissecting scope and ocular micrometer. Samples were then preserved in phosphate-buffered formalin following procedures outlined by Markle (1984).

RESULTS AND DISCUSSION

Larvae from four species of *Sebastes* were reared in static culture for varying lengths of time. In general, cultures were maintained until there were no remaining larvae. The single exception was the culture of *Sebastes auriculatus*, from which 23 juveniles were released after 62 days when it was evident that they were outgrowing their food source and would require larger prey. Length versus age data for *Sebastes auriculatus*, *S. caurinus*, *S. nebulosus* and *S. ruberrimus* are presented in Table 1 and Figures 1-4.

Table 1. Age and total length of four species of *Sebastes* larvae reared in culture.

Species	Age (d)	Total length (mm)	± std. dev.	n
S. auriculatus	0	5.45	.14	5
	3	5.99	.14	5
	7	6.35	.16	5
	10	6.47	.30	8
	15	6.60	.18	5
	20	7.08	.52	5
	25	6.91	.24	4
	30	7.85	.46	5
	35	8.16	.15	3
	62	13.87	1.70	3
S. caurinus	0	6.34	.11	5
	10	7.05	.07	2
	18	7.97	.12	3
S. nebulosus	0	5.90	.07	5
	5	6.07	.21	6
	11	6.53	.55	3
	17	7.47	.06	3
	24	7.33	.42	3
	32	7.15	.49	2
	34	7.80		1
S. ruberrimus	0	4.16	.06	5
	3	4.39	.12	5
	7	4.76	.13	10
	10	4.70	.11	10
	15	5.32	.19	5
	20	5.68	.11	5
	25	5.75	.13	3
	30	6.21	.14	3

Larval survival appeared to depend upon several factors in addition to the abundance and size of prey items. The greatest single determinant of initial larval survival was the degree of development at the time of extrusion. Gravid *Sebastes* subjected to stress during transport invariably extruded dead larvae from 12 to 96 h from the time of handling. Near-term larvae generally had fewer initial mortalities than more premature larvae. In several cases, near-term larvae extruded at the time of capture and transported separately survived with few mortalities, while those remaining *in utero* were extruded dead less than 24 h later. These dead larvae were opaque, and their surrounding ovarian fluid was milky.

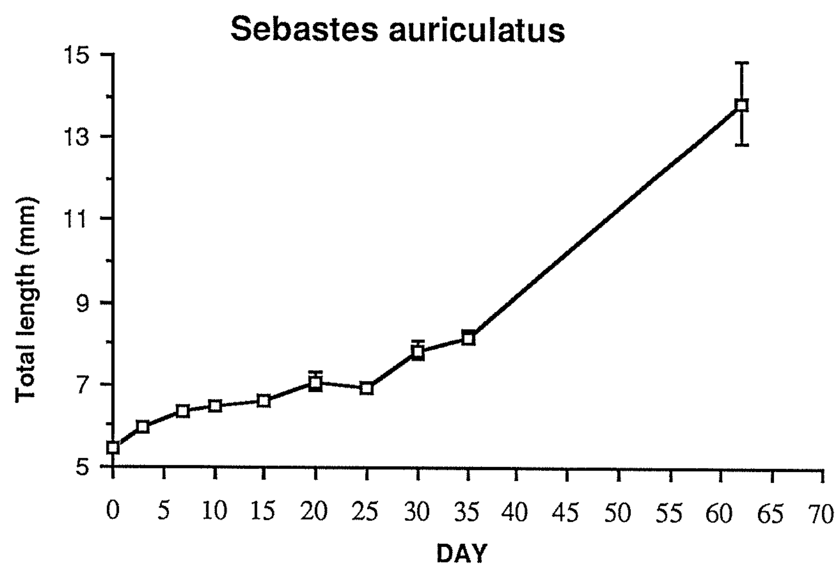

Figure 1. Age versus length relationship for *Sebastes auriculatus*. Vertical bars represent standard error of the mean for each sample.

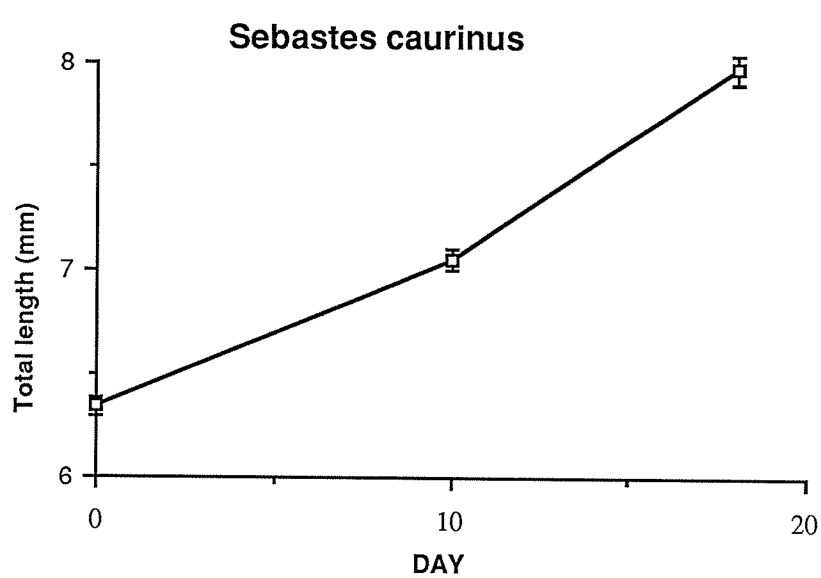

Figure 2. Age versus length relationship for *Sebastes caurinus*. Vertical bars represent standard error of the mean for each sample.

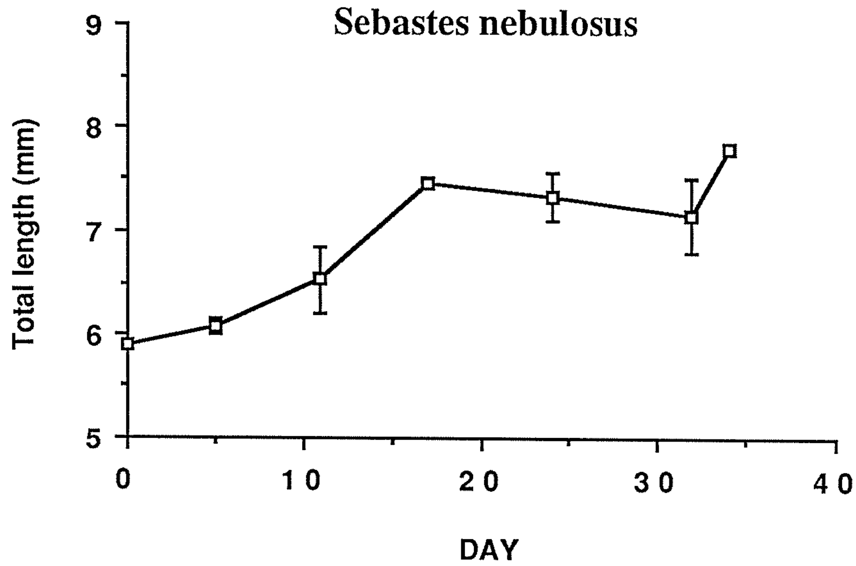

Figure 3. Age versus length relationship for *Sebastes nebulosus*. Vertical bars represent standard error of the mean for each sample.

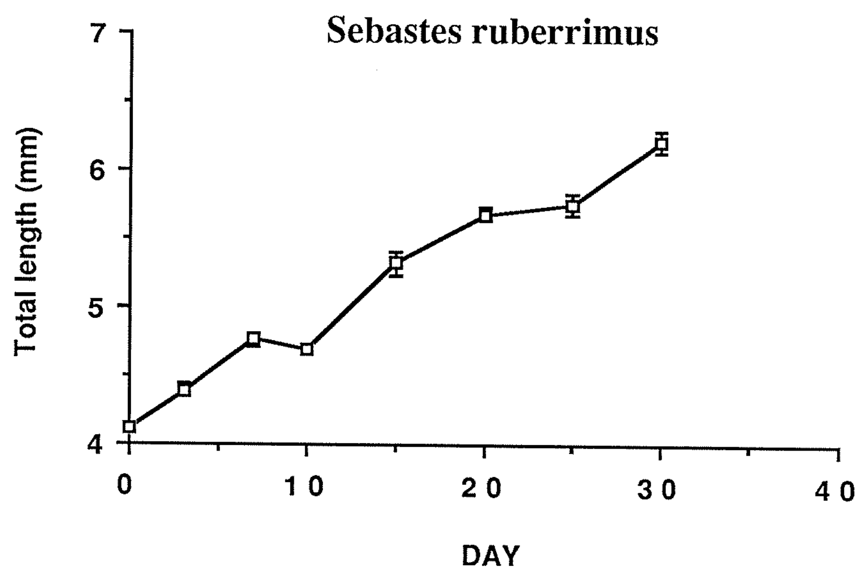

Figure 4. Age versus length relationship for *Sebastes ruberrimus*. Vertical bars represent standard error of the mean for each sample.

Criteria for evaluating the stage of larval development were based on those outlined by Stahl-Johnson (1984). Full-term larvae have a yolk sac volume roughly equal to the lipid droplet, and the yolk sac conforms more to the body contours than in premature larvae.

The sizes at birth of *Sebastes caurinus* and *S. auriculatus* reared in culture are comparable to those found in other studies (Table 2). Near- and full-term larvae were observed to feed shortly after extrusion. Rotifers appeared to be a preferred food item, at least during the first week of development. Their relatively slow, continuous swimming may make them easier to catch than copepod nauplii of similar size. A diet of copepod nauplii alone was not sufficient to rear one group of larval *S. caurinus* beyond 14 days. Stahl-Johnson (1984) reported that 60% of newly extruded *Sebastes* larvae fed on rotifers within 2 hours after these prey were introduced and preferentially consumed them over *Artemia* nauplii and different stages of harpacticoid copepods. However, Moser and Butler (1987) found that feeding behavior was not observed in six species of reared *Sebastes* larvae fed high densities of *Brachionus*.

The mixed diet of rotifers and copepod nauplii at densities near 10 prey ml^{-1} sustained initial larval development. Whether the addition of larger food sizes during the first 2 weeks significantly affects growth and development is not clear. *Sebastes caurinus* larvae reared on a combined diet of rotifers, *Artemia* nauplii and cultured copepods (Stahl-Johnson 1984) averaged 7.4 mm total length after 18 days of culture at 18°C. Those reared in this study on a rotifer and copepod (<202 μm fraction) diet averaged 8.0 mm total length after 18 days at 10°C. *Sebastes auriculatus* larvae grew from day 30 (7.85 mm) to day 64 (13.87 mm) on a diet of adult *Acartia clausi*, which reaches a maximum length of about 1.2 mm. In comparing summaries of *Sebastes* rearing experiments (Moser and Butler 1987; Tables 1 and 2), a mixed diet of rotifers, copepods or wild plankton appears to provide better larval survival, on average, than a diet of *Artemia.*

Light levels had a profound influence on larval distribution in the tanks, especially with young larvae (Stahl-Johnson 1984; L. Wold, personal communication, Moss Landing Marine Laboratories). Increased light levels (above 4.0×10^{13} quanta sec^{-1} cm^{-2}) caused larvae to swim downwards into the bottom of the tank and contributed to some initial larval mortality. Newly extruded

Table 2. Comparison of lengths at birth for *Sebastes auriculatus* and *S. caurinus*.

Species	Mean total length (mm)		
	DeLacy et al. 1964	Stahl-Johnson 1984	This study
S. auriculatus	5.84	5.6	5.45
S. caurinus	4.81	5.5	6.34

S. auriculatus larvae tended to have a uniform distribution just above the bottom of the tank. In contrast, *S. ruberrimus* larvae aggregated mostly at the surface and along one reflective side of the tank. These distributions were most pronounced during the first days after extrusion, particularly when the tanks were cleaned and freshwater introduced. These behaviors diminished within a few days, perhaps because of the constant stimuli of culture. These marked differences in newborn larval behavior suggest that interspecific responses may have a role in larval dispersal patterns.

Larval mortalities were approximately 70% for all species between 7 to 12 days after extrusion. Mortalities of surviving larvae remained low but relatively constant thereafter. Moser and Butler (1987) reported similar mortalities occurring from day 11 to day 15 for species in culture, usually several days after the yolk and oil globule were absorbed. Stahl-Johnson (1984) found that the lipid reserves of *S. caurinus* larvae were exhausted after 10 days of starvation at 13°C. Starved animals had to initiate feeding within 7 days after extrusion in order to survive.

Temperature changes also affected larval mortalities. A power outage caused the temperature of the cultures to rise from 10.0-12.5°C over a period of 11 hours. Approximately 50% of the water in the tanks was exchanged three times during this period in an attempt to reduce temperatures. Over 75% of *S. ruberrimus* larvae died, while *S. auriculatus* larvae were mostly unaffected. Mechanical shock from turbulence during water exchange may have damaged delicate neuromast structures of the larvae, but the mortalities may also indicate that *S. ruberrimus* larvae are more stenothermal than *S. auriculatus*.

SUGGESTIONS FOR REARING *SEBASTES* LARVAE

The developmental stage of *Sebastes* larvae at extrusion is critical to survival and development. Gravid females appear very sensitive to stress and usually abort premature or dead larvae shortly after handling. Broodstock should be obtained during early gestation and kept in darkened tanks until parturition. Fish that are not obviously gravid may be gently catheterized according to procedures outlined by Boehlert and Yoklavich (1984) to determine the degree of larval intraovarian development.

Extruded larvae should be removed from brood tanks as soon as possible with minimal handling. Larvae should be transferred in beakers, culture dishes, etc., and not pipetted. Culture tanks should be dark colored and non-reflective. However, other investigators have found that a white tank bottom does not disturb the larvae, facilitates cleaning, and allows better enumeration of larvae (L. Wold, personal communication, Moss Landing Marine Laboratories).

Illumination should be minimal enough to permit feeding and not elicit escape responses in the larvae. The effect of tank volume on larval mortalities was not quantified in this study, but represents a compromise between optimal larval densities and rearing (or capturing) large numbers of prey items. *Sebastes* larvae reared in 20-L and 100-L tanks did not show any appreciable difference in mortalities.

A mixed diet seems to enhance larval survival. Newly extruded larvae do not seem capable of catching large copepods. Larvae less than 6.0 mm in total length may be fed rotifers and wild plankton less than 200 µm. Guts should be examined to determine larval success in capturing the prey. Larger prey should be added as the larvae grow. Too little is known about food size selection and natural diets of *Sebastes* larvae to determine whether the prey sizes or densities used in this study had significant effects upon larval survival and growth.

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