LABORATORY OBSERVATIONS ON THE EARLY GROWTH OF THE ABALONE, *HALIOTIS SORENSENI,* AND THE EFFECT OF TEMPERATURE ON LARVAL DEVELOPMENT AND SETTLING SUCCESS

David L. Leighton[1]

ABSTRACT

The influence of temperature on larval development rate and growth of juveniles of the white or Sorensen's abalone, *Haliotis sorenseni,* was investigated using a thermal gradient apparatus. While larvae developed most rapidly at 20°C, most settled juveniles at that temperature did not survive. At 15-16°C, however, the operculate veliger stage was attained in 72 hr and settlement of advanced individuals occurred in 9 days. No settling was observed at 10°C. Juveniles maintained at 15-19°C and provided mixed diatoms as food showed marked variability in growth rate; at 130 days shell length ranged from 4.0 to 8.0 mm (average 5.5 mm). Two distinctly different patterns of shell pigment distribution emerged with continued growth. Approximately 60% of the juveniles were bicolored, red and yellow-green, while the remainder had an even tone of red-violet.

The description by Carlisle (1962) of the trochophore and early veliger stages of the red abalone, *Haliotis rufescens* Swainson, has heretofore been the only published information on larval development of an eastern Pacific species of *Haliotis.* No account of larval development through settlement and juvenile growth of any of the seven American species of abalones exists in the literature. Details of larval morphogenesis and an estimate of growth during the first year of life for the northeastern Atlantic *H. tuberculata* Linnaeus were given by Crofts (1929, 1937). Japanese workers have reported observations on early development and growth in several of their native species; *H. gigantea* Chemnitz (Murayama, 1935), *H. discus* Reeve and *H. sieboldii* Reeve (Ino, 1952), and *H. diversicolor supertexta* Lischke (Ōba, 1964).

Recent interest in mass culture of commercially important species in Japan and the United States has prompted more critical studies of growth and nutrition of abalones. Advances by Japanese workers in the field of abalone culture were reported by Imai (1967) and Ryther (1968). However, no comparable research information has been published heretofore from any abalone culture facility in California. This work, done in conjunction with abalone culture efforts of California Marine Associates, Cayucos, Calif., describes early growth of the white or Sorensen's abalone, *Haliotis sorenseni* Bartsch.

H. sorenseni exhibits an unusual pattern of distribution. Common to southern California and northern Baja California, it appears in abundance along the mainland only at the northern and southern extremes of its range (Santa Barbara to Pt. Conception and Pta. Eugenia to Pta. Abreojos, respectively). Elsewhere it is islandic, occurring at Santa Cruz, Santa Barbara, San Clemente, Santa Catalina, Los Coronados, and Cedros Islands (Cox, 1962). Adults attain a shell length of about 25 cm and are characterized by an orange-tan epipodium with foliose epipodial papillae, brown cephalic tentacles, and a deeply cupped, light-weight, and scar-free shell. Respiratory apertures are strongly fluted. The species is prized by commercial and sport divers for its white and tender edible portion, the right shell muscle.

Sexually mature *H. sorenseni* have been difficult to obtain in good condition since this is a relatively fragile species and losses in transit

[1] Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92037.

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have been common. On several occasions gravid adults have been retained in our tanks and induced to spawn, but such attempts were never successful in obtaining their larvae. It is considered, therefore, of importance to document a first success with the species.

METHODS

Ten adult *H. sorenseni* were collected at Santa Catalina Island and transported by ship to San Diego, February 18, 1971.[2] Four possessed gonads sufficiently mature to respond to spawning stimuli. Spawnings were induced on February 21 and 26 and March 1, using the thermal shock method (Ino, 1952), but in no case did fertilization occur in greater than about 5% of the eggs liberated. Observations of larval development and growth were carried out with progeny (approximately 1,000 trochophore larvae) obtained March 1 from a single male and female pair.

Fertilized eggs were 190 to 200 μ in diameter. Washing of eggs to remove excess sperm, mucus, and other possibly contaminating substances released during or prior to spawning was achieved by repeated suspension in filtered seawater (Millipore[3] filters, pore size 0.45 μ) following settling and decantation. After incubation of the eggs at 12 ± 2°C for 24-36 hr, larvae hatched at the trochophore stage. Because the swimming trochophores exhibited a strong negative geotaxis, they concentrated at the surface where they were easily drawn into a Pasteur pipette for transfer to experimental containers.

The effect of temperature on rate of development, settlement, and growth of recently settled juveniles was investigated using an aluminum thermal gradient block (Thomas, Scotten, and Bradshaw, 1963) with compartments bored to accommodate a series of 100-ml beakers. Each compartment could be illuminated from beneath to permit growth of algae within beakers. Containers, arranged in duplicate, were maintained at 10, 12, 14, 16, 18, and 20°C (each ± 0.5°C). The Pyrex beakers received 80 ml of Millipore-filtered and ultra-violet light treated seawater (Loosanoff and Davis, 1963). Twenty 72-hr veliger larvae were introduced into each together with 1 ml of a suspension of the food organism, *Nitzschia* sp., containing approximately 10,000 cells. To guard against salinity changes due to evaporation or condensation, each beaker was covered with a paraffin sheet, and the entire block insulated with foam styrene. Inspection was made on alternate days to determine the number of larvae surviving, settlement, and growth rate. The water was changed once weekly and new food supplied.

Approximately 500 larvae were distributed among five 1-liter Pyrex beakers at the time the thermal gradient erperiment was begun. These containers received a combination of about 10 species of pennate diatoms collected from illuminated aquarium surfaces in the laboratory (chiefly *Nitzschia*, *Grammatophora*, and *Navicula*). A thermal environment of 15-17°C was maintained.

DESCRIPTION OF LARVAE

The reader seeking details of morphogenesis in haliotids should refer to illustrations and text as provided for Japanese species by Ino (1952) and Ōba (1964), early development of which closely parallels American members of the genus. In this paper an effort is made to indicate distinctive features of *H. sorenseni* larvae.

Trochophores were subcylindrical in outline, bore a distinct prototroch and were yellow-tan in color. At 15-16°C, the roughly symmetrical shape of the trochophore was soon lost and by 48 hr after fertilization the early veliger form was attained with the incipient cap-shaped shell and lobular velum becoming defined. At 72 hr, the larval shell had taken the typical gastropodan snail-form complete with operculum while shell musculature, viscera, eye-spots, and velum were well developed. At the same time the foot and cephalic tentacles began to differentiate (Figure 1). Tissue pigmentation was predominantly beige, velar fringes were yellow, and the

[2] Collections were made at a depth of about 70 ft by T. Tutschulte and were timed to sample the peak of the reproductive season at the Isthmus station.

[3] Reference to trade names in this publication does not imply endorsement of commercial products by the National Marine Fisheries Service.

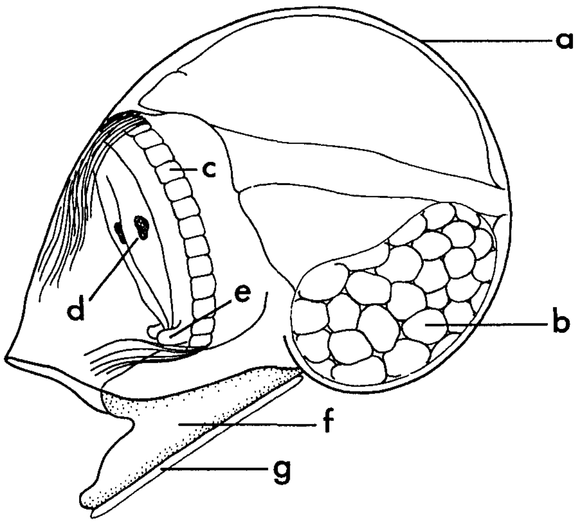

FIGURE 1.—Three-day veliger larva of *Haliotis sorenseni*. (Shell diameter, 270 μ) a. shell, b. digestive gland, c. velum, d. eye spot, e. incipient cephalic tentacle, f. foot, g. operculum.

digestive gland was maroon. Swimming of veligers at this stage carried them throughout the water column.

Settlement occurred at 9-10 days at 15°C. Velar dystrophy, with loss of ciliated fringes, took place gradually, and larvae retained the ability to swim if dislodged for several days after initial settlement.

INFLUENCE OF TEMPERATURE ON DEVELOPMENT RATE

Larvae confined to 100-ml beakers in the thermal gradient block appeared, except for the temperature effects to be described, to be normal in appearance and behavior and were similar to larvae maintained in liter beakers at 15-17°C. Development was most rapid at higher temperatures; some individuals settled as early as the 7th day at 20°C and the 8th day at 18°C. By the 15th day almost all larvae at the two highest temperatures had settled, yet none had settled at 10°C (Table 1, Figures 2 and 3). By the 25th day most larvae at 10°C had succumbed, having progressed only to the late veliger stage. Settling rate varied within groups, and even at 18°C approximately 5% did not settle.

Observations at the end of the 36-day experiment revealed that survival was best at 16° and 18°C, somewhat less at 20° and least at 14° and below. Larvae maintained at 10°C did not survive beyond 25 days and most of those at 12° failed at 25 to 30 days. Size attained by settled juveniles corresponded with survival; at 36 days shell lengths as great as 1.2 mm were attained at 18°C, about 1.0 mm at 20° and 16°C, and only 0.6 mm at 12° and 14°C.

GROWTH OF JUVENILE *H. SORENSENI*

Approximately 100 settled juveniles were obtained from the larvae reared in liter Pyrex beakers at 15-17°C. These individuals were carefully dislodged by a jetting stream of water and teasing needle and transferred to larger containers to observe growth and behavior under a variety of food and water flow situations. Food quality and quantity was not limited to insure maximum growth, hence a wide variety of food organisms was provided (pennate diatoms and filamentous red, green, and brown algae).

TABLE 1.—Number of *Haliotis sorenseni* larvae surviving and settled at 15 days in thermal gradient experiment.

Temperature	Series I			Series II		
	Surviving	Settled	Survivors settled	Surviving	Settled	Survivors settled
°C ± 0.5°	*No.*	*No.*	*%*	*No.*	*No.*	*%*
10	5	0	0	18	0	0
12	7	4	57.2	15	10	66.6
14	15	11	73.4	18	14	77.7
16	12	11	91.5	14	11	78.5
18	9	9	100.0	17	15	88.4
20	7	7	100.0	13	11	84.6

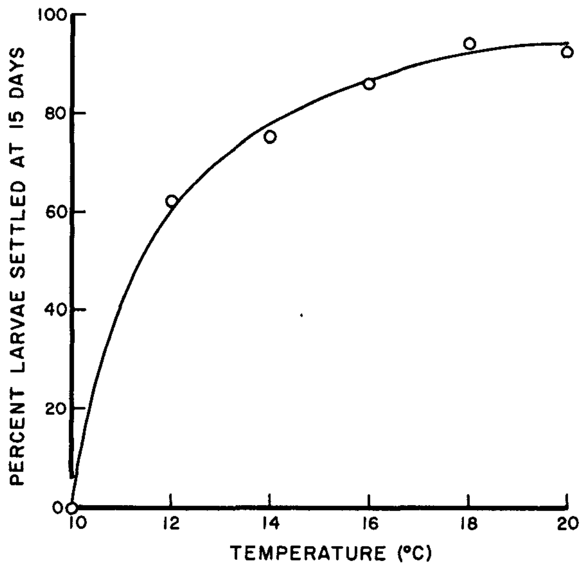

FIGURE 2.—Percentage of larvae firmly settled at 15 days. Points are averages for duplicate observations. Settlement was considered complete when larvae could no longer be dislodged by jetting a stream of water from a pipette.

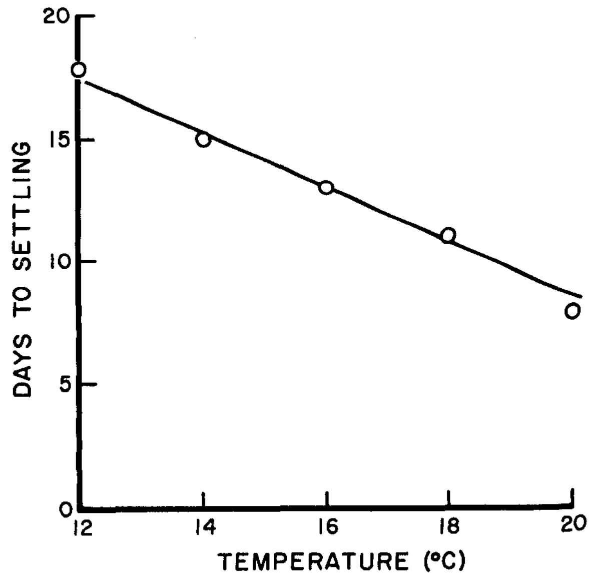

FIGURE 3.—Days to settlement of at least 75% of *Haliotis sorenseni* larvae from groups held at five different temperatures. Not more than 75% of the larvae at 12°C successfully settled, and none of those at 10°C reached this stage.

A marked variation in growth rate existed, even among those individuals which were provided surfaces (such as plastic and glass beakers) on which diatom growth appeared to be quite uniform. In one case, 19 individuals were retained in a liter beaker and provided mixed diatoms. At an age of 85 days some had attained a shell length of 4.5 mm while others were as small as 1.4 mm. The largest had formed four respiratory pores, yet the smallest was just beginning to form the first. Size attained at 100 days (15-19°C) ranged from 3.0 to 5.6 mm (mean, 4.25, SD ± 0.63 mm, $n = 19$). By 130 days the range was 4.0 to 8.0 (mean, 5.53 mm, SD ± 1.01 mm) in the same group (Figure 4).

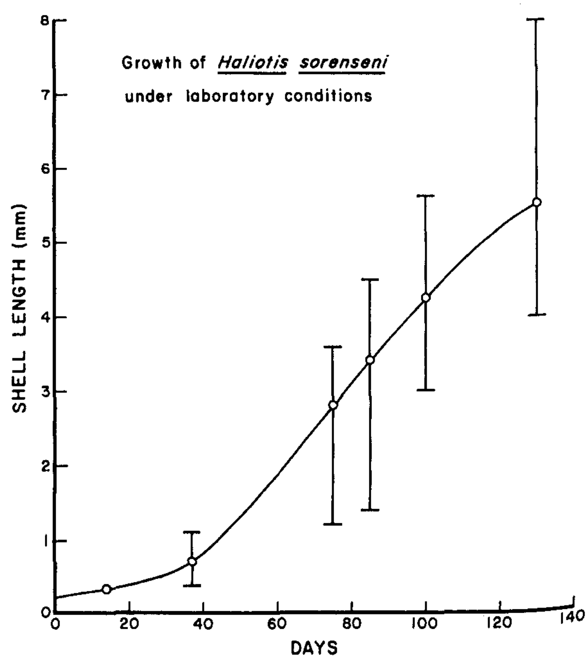

FIGURE 4.—Growth of larval and juvenile *Haliotis sorenseni* in 1-liter beakers provided mixed diatoms as food. Ranges of shell length are shown by vertical lines.

DESCRIPTION OF JUVENILE SHELL FEATURES

The most conspicuous feature of recently settled and minute haliotids (less than 500 μ long) is the asymmetrical outline resulting from dextral growth of the persistomial shell. Through continued growth the asymmetry is reduced and the shell form, as viewed from above, is almost circular until the "notch stage" is reached. Formation of the first respiratory aperture is initiated by separation of two portions of the mantle at the right anterior margin interrupting otherwise uniform marginal shell deposition. The "notch" so formed is a convenient growth mark, reached by the most rapidly growing *H. sorenseni* at an age of 55 days and a shell length of 2.0 or 2.1 mm (Figure 5).

In the juvenile *H. sorenseni* observed, shell pigmentation was a pale violet-pink becoming more vivid as the notch stage was approached. In most cases a cyan-blue flare spread from the apex to the right shell margin. After completion of the first respiratory pore, a dichotomy in pigment pattern emerged. Although all juveniles developed from eggs of a single female, fertilized by a single male, approximately 40% developed an even red-violet shade throughout the greater extent of the shell while the majority became increasingly bicolored with growth. The bicolored pattern consisted of a rich red zone along the left edge of the shell (extending to the aperture row) and pigmentation of blue, green, and yellow over the broader surface right of the aperture row. In individuals of both coloration patterns, a conspicuous ivory-white patch remained at the position of attachment of the right shell muscle until obscured by nacre deposition at about 8 mm. The apex, in turn, became increasingly white.

Elevated apertures, typical of the species, appear even in first-pore individuals. More conspicuous is the ridge bordering the left shell margin. It is this ridge that forms the sharp corner and relatively straight left anterior margin (Figure 6).

DISCUSSION

Establishment of specific characteristics by which larvae and juveniles of the seven cooccurring California species of *Haliotis* may be distinguished must await results of studies of fine structure of larval shells. Examination by scanning electron microscope has revealed sufficient detail of fine crystalline and basement structure

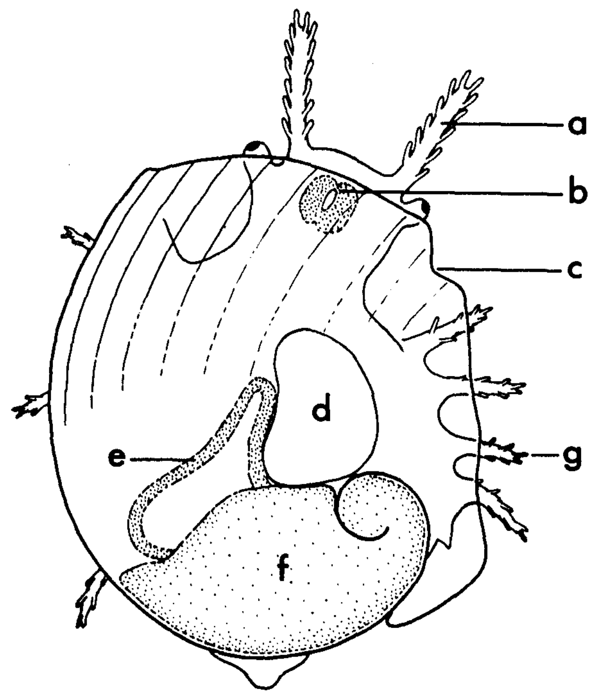

FIGURE 5.—Juvenile *Haliotis sorenseni* at "notch" stage (2.0 mm, 55 days). Shell is still slightly transparent and prominent anatomical features are readily seen from the dorsal aspect. a. cephalic tentacle, b. mouth, c. indentation at point of formation of first respiratory pore ("notch"), d. right shell muscle, e. intestine, f. digestive gland, g. epipodial tentacle.

of larval shells in certain other prosobranchs (Fretter and Pilkington, 1971). Gross shell morphology alone is inadequate to differentiate larvae of even distantly related species. Pigmentation of velar and visceral portions may provide distinctive features for recognition of some species.

Pigments derived from parental yolk appear to be retained by trochophore and veliger larvae of *Haliotis*. Among California species, ovarian tissue is dark green in *H. rufescens*, *H. cracherodii*, *H. walallensis*, and *H. kamtschatkana assimilis*. Correspondingly, larvae of these species are conspicuously green. The remaining three species found locally, *H. fulgens*, *H. corrugata*, and *H. sorenseni*, produce eggs of brown, olive, and beige color, respectively. Their larvae may be expected to reflect these pigments accordingly. I have examined larvae of *H. fulgens* which were generally brown but with green velar margins and those of *H. sorenseni*, which, as described above, were beige with yellowish velar margins. Tissues of larval *H. corrugata* are light yellow-green while velar fringes are a darker shade of green.

Color of the digestive gland in planktotrophic prosobranch larvae has been shown to reflect diet (Fretter and Montgomery, 1968). Since *Haliotis* veliger larvae are lecithotrophic, color of the digestive gland may have diagnostic value. The maroon-colored digestive gland of *H. sorenseni* veligers appears to be distinctive; I have not observed other than green or brown in the digestive glands of other haliotid veligers.

Although development of larvae and growth of settled juvenile *H. sorenseni* were more rapid at higher temperatures, survival was reduced at 20°C. More advanced juveniles, reared initially at 15-20°C, did not appear adversely affected by temperatures as high as 25°C. Possibly thermal tolerance limits are more restricted in larvae and recently settled individuals. Certainly other factors could have influenced success at higher temperatures. Despite weekly changes of water, bacterial, algal, and protozoan growth together with a build up of metabolites and reduced oxygen tensions in the relatively small volume of water used in the thermal gradient study could have influenced the results.

Larval *H. sorenseni* were not successful at 10-12°C. Water temperatures within the bathymetric range of the species may fall to 12° and occasionally to 11°C (e.g., at depths of 130 ft off Santa Catalina Island, T. Tutschulte, personal communication). Therefore the 10° or 12°C bathyisotherms may limit the depth to which larvae of *H. sorenseni* may successfully settle and grow. The results of the temperature block study indicate the range 14-18°C may be optimal for *H. sorenseni* larvae—an outcome not unexpected in view of the prevailing conditions in the natural environment from Pt. Conception to central Baja California.

Information on growth throughout the first days of life is available for several species of *Haliotis*. Ino (1952) reported that *H. discus* attained only 1.25-1.40 mm at 100 days and that the first respiratory pore was not formed until 130 days. In contrast, Ōba (1964) observed rapid early development of *H. diversicolor supertexta*. In that study, trochophores hatched at only 6 hr, veligers developed within 11 hr and settled by 2 days. The first respiratory pore was formed as soon as 23 days after fertilization. Interpolating from his growth curve, 100-day juveniles ranged from 8 to 13 mm (Ōba et al., 1968). In Ino's study, water temperatures declined from 18° to 10°C through the course of observations while Ōba's work was carried out during the summer and fall when temperatures ranged between 20° and 28°C. Whether the different development rates in these two species reflect specific contrasts or thermal influences is not clear. In another study (Kan-no and Kikuchi, 1962), *H. discus hannai* was reared at a relatively constant intermediate temperature (19-20°C). This species also exhibited rapid early development, settling in 3 days. Juveniles reached 11 mm in length at 100 days and 26 mm at 180 days.

The rather extreme variability in growth rate observed in *H. sorenseni* has also been found in *H. rufescens* (Leighton, unpublished data). Comparable variability is reflected in Ōba's growth curve for *H. diversicolor supertexta*. Differences in growth rate may reflect variation in food availability. In the present study, care was taken to provide uniform food distribution and feeding conditions. Yet within a single container, even within sampled subareas, a full spectrum of size variation could be found. The hypothesis may be advanced that gametogenic inequalities (e.g., yolk allotment) may be involved giving greater advantage to some individuals over others. Indeed, mortalities were more common among the smaller and presumably less active members.

Inherent variability in growth rate may be expected in nature to be complicated by differential quality and availability of food. Multimodal size-frequency distributions obtained for juvenile *Haliotis* populations in the field have been concluded to reflect multiple spawnings and recruitment waves (Leighton and Boolootian, 1963; Boolootian, Farmanfarmaian, and Giese,

FIGURE 6.—Photograph of shells of juvenile *Haliotis sorenseni* representing the bicolored (above) and red pattern (below). Shells range in length from 6.6 to 8.4 mm.

1962). The evidence from this study suggests the assumption of uniform growth within a population of juvenile *Haliotis* is untenable and that conclusions regarding settlement date estimated from sizes of members of a sample must be drawn cautiously.

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