Reference: *Biol. Bull.*, **154**: 430–439. (June, 1978)

LARVAL REARING, METAMORPHOSIS, GROWTH AND REPRODUCTION OF THE EOLID NUDIBRANCH *HERMISSENDA CRASSICORNIS* (ESCHSCHOLTZ, 1831) (GASTROPODA: OPISTHOBRANCHIA)

JUNE F. HARRIGAN AND DANIEL L. ALKON

Laboratory of Biophysics, Section on Neural Systems, Intramural Research Program, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

In the eolid nudibranch, *Hermissenda crassicornis* (Eschscholtz, 1831), neural pathways responsive to light, chemosensory stimuli, and gravitational stimuli converge within the circumesophageal nervous system (Alkon, 1974, 1975, 1976). These convergence points, as defined by intracellular recordings, may be important for choice behavior and behavioral modification as demonstrated for this animal. Maintenance conditions, primarily light-dark cycle, temperature, and diet, had to be carefully controlled when analyzing both the behavior and the nervous system of *Hermissenda*. The goal of the present study was to establish a laboratory strain of *Hermissenda* to provide animals of known history for these studies, and for studies on behavioral and neural development in these three sensory pathways.

Hermissenda, a monotypic genus, is widely distributed along the west coast of North America (Lance, 1966; MacFarland, 1966). Field observations (Yarnell, 1972; Birkeland, 1974) indicate that *Hermissenda*, although preying primarily on coelenterates, has a broader diet than most nudibranchs. *Hermissenda* and its egg masses appeared on fouling panels exposed for one month at a time throughout the year in Monterey Bay, California (Haderlie, 1968). Year-round availability of eggs and adults and a relatively broad diet in the adult stage simplify cultivation of *Hermissenda*.

Materials and Methods

Reproductive periodicity

Weekly shipments of *Hermissenda* were obtained from Mr. Michael Morris, Sea Life Supply, Sand City, California. Ten animals (2.5 + cm in length) were removed from each week's shipment from May, 1976, to May, 1977. Animals were incubated separately on a 12L: 12D schedule at an average sea water temperature (± s.d.) of 14.1° ± 1.8° C, which approximates the mid-point of the annual temperature range occurring in the natural habitat (9°–18° C; Haderlie, Mellor, Minter, and Booth, 1975). A daily record was kept of the number of each set of ten animals that deposited an egg mass.

Fecundity measurements

Fifty newly-arrived animals of widely varying sizes (73–3204 mg) were weighed underwater on a Mettler PN323 balance, immediately after each deposited

its first egg mass. The number of eggs per egg mass was estimated by multiplying the length of the egg string in mm by the average number of embryos per mm.

Egg diameter and egg capsule size were measured through a Zeiss Universal microscope with a calibrated ocular micrometer. All computer analyses of data on growth and reproduction were performed on a PDP 11/10 computer, using standard statistical packages.

Diet experiments

Forty individuals (ten per diet) were maintained until death on one of four locally available diets: frozen squid mantle muscle (*Loligo pealii*), mussel (*Mytilus edulis*), tunicate (*Ciona intestinalis*), minus the tunic, and an alternating diet consisting of one day of squid, then mussel, then tunicate, etc.

Weighed animals were placed singly in numbered 7 × 4 cm plastic snap-top vials which were perforated with slits for water exchange, and maintained at a sea water temperature of 12°–14° C. An excess of food was provided fresh daily. Weight, days survived, number of egg masses laid and whether eggs developed normally were recorded for each animal on each diet.

Larval rearing

Egg masses were incubated at 13°–15° C in 0.22 μm Millipore-filtered (MPF) sea water. On day 5 or 6 following oviposition, the veligers were liberated by teasing apart the egg mass. Cetyl alcohol flakes sprinkled on the surface of larval cultures prevented larvae from becoming entrapped in the surface film (Hurst, 1967). The rearing method was adapted from that developed for aplysiid larvae by Switzer-Dunlap and Hadfield (1978). A similar method was employed by Harrigan and Alkon (1978) to rear the opisthobranch molluscs, *Elysia chlorotica* Gould, 1870 and *Haminoea solitaria* (Say, 1822).

Larvae were cultured at a concentration of three per ml in covered one-liter Pyrex beakers filled with 800 ml of culture water (0.22 μm MPF sea water containing 5 ppm chloromphenicol). Cultures were maintained on a 12L : 12D cycle at an average temperature of 13.8° ± 1.2° C. Larvae were transferred three times a week to clean culture water. Cultures were fed daily and stirred to resuspend food and veligers.

Cultures were initially fed equal amounts of *Isochrysis galbana* and *Monochrysis lutheri* at a final concentration of 3.0×10^4 cells per ml and the larger flagellate *Chroococcus salina* (strain 3C) at a final concentration of 7.5×10^3 cells per ml. Food concentration was gradually decreased as the cultures aged. Algal cultures were bacteria-free, and were grown in 100 ml aliquots according to the methods of Guillard (1975).

Juveniles were fed for one week on the hydroid species (provided by Sea Life Supply) on which they metamorphosed. They were then fed only on tunicate (*Ciona intestinalis*) obtained from Cape Cod waters. Body lengths, measured when the animals were fully extended, were taken weekly; body weights were taken occasionally.

Results

Reproductive periodicity

Fertile egg masses, which produced normal veligers, were obtained every week of the year from sets of ten animals collected from Monterey Bay, California. Chi-square analysis of an R × C contingency table indicated no significant interaction between the number of animals laying eggs per week and the month of the year the eggs were obtained ($P > 0.99$, df = 33). *Hermissenda* did not exhibit seasonal periodicity in egg-laying in the laboratory.

Over the one-year sampling period 79% of the total number of animals tested (n = 490) deposited at least one egg mass. Thirty-one per cent of the animals laying one egg mass produced a total of 2, 3, or 4 egg masses within the one-week test period.

Characteristics of the egg mass

Hermissenda deposits its egg masses, while or pink, in a tight counter-clockwise spiral. Structure of the egg mass is further described by Hurst (1967). Diameters of the first egg mass deposited in the laboratory by the adults (73–3204 mg body weight) ranged from 0.24 cm to 3.62 cm. Average egg mass diameter increased linearly with adult weight (polynomial regression, $P < 0.01$, df = 49). The number of eggs estimated per egg mass (see Methods) varied from 6.9×10^3 to 1.0×10^6.

The number of eggs per egg capsule increased with adult weight ($P < 0.01$) from one to an average of nine eggs for adults greater than 500 mg. Eggs are packed one per capsule for adults weighing less than 500 mg. The average egg diameter was 65.4 ± 1.2 μm (n = 70, 7 adults). Egg diameter was not a function of the number of eggs per capsule. Egg capsule length increased significantly with number of eggs per capsule (one-way ANVAR, $P < 0.01$) (Table I).

Larval development

Veligers hatch in 5–6 days at 13°–15° C. Unsculptured shells are of about ¾ whorl and belong to Thompson's Type I (Thompson, 1961). Average shell length and width at hatching is 105.9 ± 6.3 × 75.4 ± 4.8 μm (n = 25). *Hermissenda* has an obligatory veliger stage of at least 34 days. Metamorphosis is delayed

Table I

Relationship between capsule size and number of eggs per capsule. Each number represents average length and width (μm) of 100 capsules, 20 from each of five adults.

Eggs/capsule	Length × width (μm) (±s.d.)
1	110.3 ± 10.0 × 76.2 ± 4.4
2	145.5 ± 5.8 × 102.3 ± 6.1
3	157.0 ± 9.9 × 112.8 ± 3.8
4	175.4 ± 8.5 × 126.4 ± 7.1
5	185.8 ± 10.1 × 141.9 ± 10.9

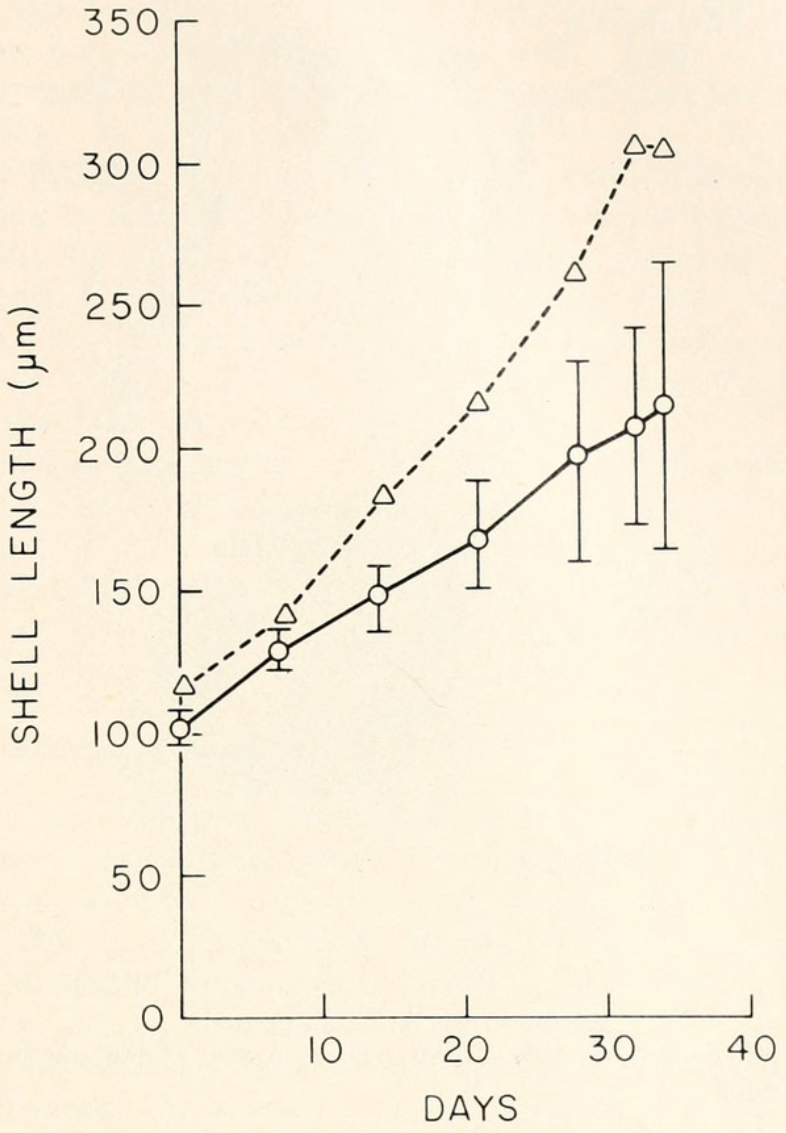

FIGURE 1. Growth of veligers in terms of shell length. Vertical bars represent one standard deviation. Dots represent average shell length; triangles represent the size of the largest individual measured.

by about 2–4 days after maximum shell length (310.4 ± 9.8 μm, n = 11) is attained.

Veligers which were competent to metamorphose were recognized by the following criteria: presence of eyes, shell length of at least 300 μm, enlargement of the foot and development of the propodium, reduced swimming activity, the veliger remaining on or near the bottom, and the presence of a tooth at the base of the shell aperture. The average shell length of a sample of veligers did not accurately reflect the size of the largest individuals. Figure 1 illustrates shell growth in veligers from three replicate cultures.

On day 34 post-hatching, competent veligers crawled immediately on the thecate hydroid, *Obelia longissima,* and on an unidentified thecate hydroid from California. Competent veligers also crawled on the related species, *Obelia geniculata,* collected from Woods Hole, Massachusetts. The velum is lost during the first 12–24 hours after crawling begins. In the next 12–24 hours the larva slowly crawls out of its shell. During shell exit one pair of tentacle buds and two pairs of cerata buds grow out of the dorsal surface. The operculum is discarded at

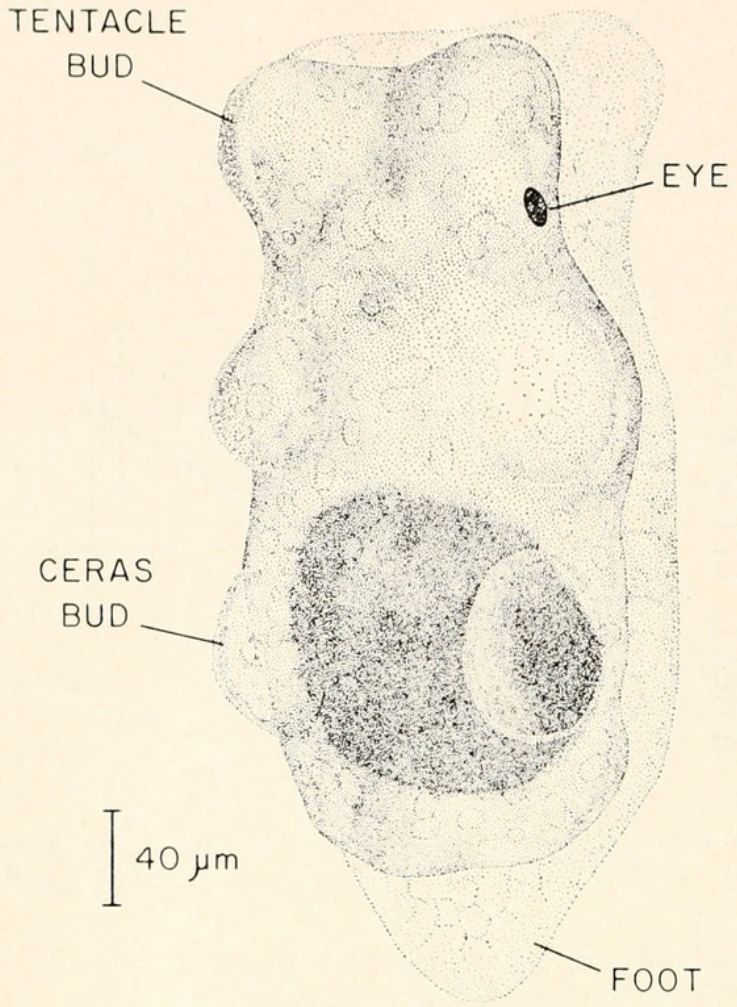

FIGURE 2. Newly metamorphosed *Hermissenda*. A pair of tentacle buds and two pairs of cerata buds are visible.

metamorphosis. The newly-metamorphosed animal measures about 400 μm in length (Fig. 2). The body is still divided into a dorsal visceral mass and a ventral foot, and the larval digestive system is visible. By four to five days post-metamorphosis the distinction between foot and visceral mass is lost and the juvenile has begun to feed on hydroid tissue.

Metamorphosis occurred only in veligers that reached full development between days 34–58 post-hatching, although individuals settling after day 50 soon died. Although larvae may survive up to 76 days, there was little or no shell growth after day 58.

Diet

Survival of *Hermissenda* through metamorphosis was low. Addition to a larval diet of *Isochrysis galbana* and *Monochrysis lutheri* (5 μm cell diameter) of the larger flagellate *Chroococcus salina*, strain 3 C (10–11 μm cell length) increased the percentage of metamorphosis from 1 to 5%. Increasing the concentration of *Isochrysis* and *Monochrysis* did not improve the percentage of metamorphosis, nor did feeding *Chroococcus* alone.

Post-metamorphic stages, however, were easily maintained in the laboratory.

Variation in diet significantly affected both growth rate of adults and number of days survived, but not number of egg masses laid. Diets containing tunicate, either alone or in combination with squid and mussel, gave the best growth and survival (Table II).

Initial average weights of four groups of ten small wild *Hermissenda* each varied from 299 mg to 509 mg. Average weight gains on each of the four diets were: 195.8 ± 362.1 mg (squid); 1218.0 ± 1514.3 mg (mussel); 2680.5 ± 1121.1 mg (tunicate); and 2752.3 ± 1268.4 mg (alternating diet). Animals survived significantly longer on tunicate-containing diets than on either squid or mussel (t-test, $P < 0.01$, df = 19). Mean number of days survival on the two tunicate-containing diets was 63.9 days (range = 34–122 days).

The total number of egg masses produced did not vary significantly between diets (Table II). There was no significant correlation between an individuals growth rate on any diet and the total number of egg masses produced by that individual. However, there was a significant positive regression of number of egg masses produced on days survived, all diets combined (polynomial regression, $P < 0.01$, df = 40) (Fig. 3).

Growth rate and reproduction in five F1 adults

From day 1 to day 70 post-metamorphosis increase in body length (on a tunicate diet) was approximately linear, averaging 0.82 ± 0.11 mm per day. The growth rate slowed to 0.35 ± 0.17 mm per day between days 71–120 post-metamorphosis. The largest individual attained a length of 81.7 mm, nearly equalling the length of the largest *Hermissenda* obtained from the field, 90 mm. After day 120 food intake decreased and the animals began to shrink. Death occurred between 116–137 days post-metamorphosis ($\bar{X}$ = 128 days).

The average life-span of a laboratory-reared *Hermissenda* encompasses approximately 163 days (35 day veliger stage plus 128 day adult stage), confirming that *Hermissenda* is a subannual species.

Hermissenda was not observed to self-fertilize. No egg masses were deposited by F1 adults, which were maintained separately, until three animals were allowed to copulate on day 65 post-metamorphosis (total egg masses = 28 from first copulation to death). Two isolated individuals deposited 2–3 sterile egg masses each between days 95–133 post-metamorphosis.

Fertile egg masses were deposited in the laboratory by wild specimens of *Hermissenda* as small as 73 mg, and motile sperm were observed in squash prepa-

TABLE II

Growth rate, survival, and egg mass production for ten specimens of Hermissenda *on each of four diets.*

Diet	Average growth rate mg/day	Average days survived	Average egg mass production
Squid mantle	10.1 ± 11.0	28.0 ± 11.4	2.0 ± 1.8
Mussel	31.4 ± 26.7	45.4 ± 14.8	3.7 ± 2.4
Tunicate	58.4 ± 35.6	65.0 ± 25.3	3.5 ± 3.9
Alternating	55.3 ± 14.9	62.9 ± 7.0	1.9 ± 1.6

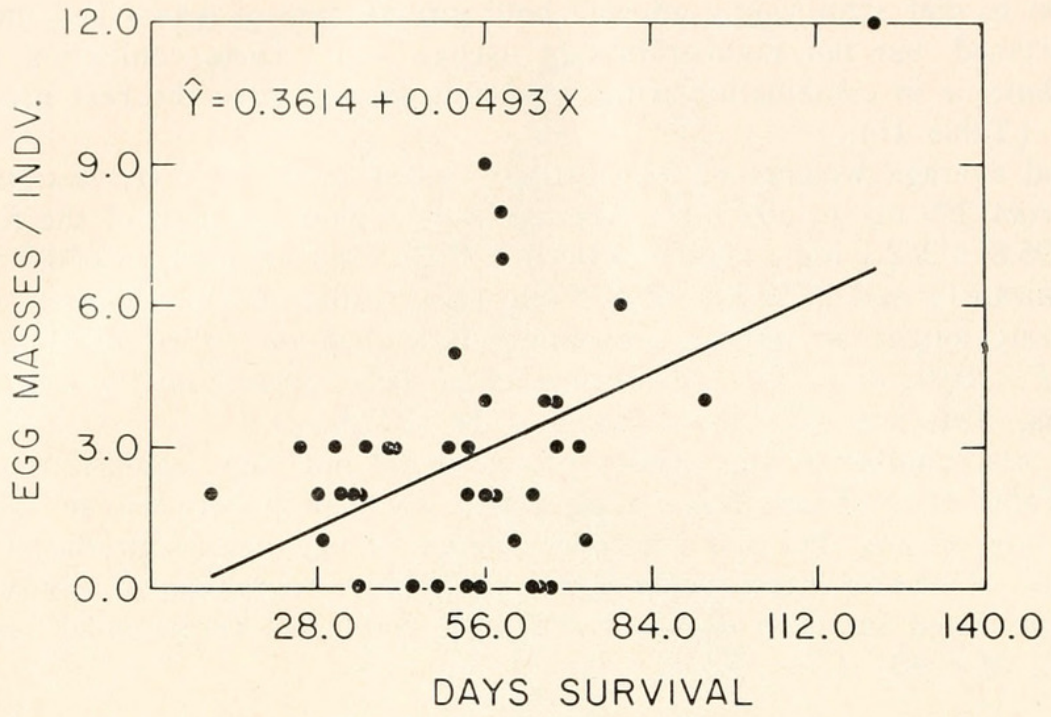

FIGURE 3. Regression of number of egg masses deposited on days survival.

rations from wild individuals weighing 34 mg (1.12 cm body length). Egg production in individuals from wild populations is estimated to begin at about 1.5 months post-metamorphosis and continue until death at 5–8 g, four months post-metamorphosis. Both the total number of egg masses produced and the age at which egg-laying commences depend on age at initial copulation.

DISCUSSION

Hermissenda crassicornis is one of several nudibranch species which have been reared through metamorphosis in the laboratory (Bonar and Hadfield, 1974; Thompson, 1958, 1962, 1967; Tardy, 1970; Perron and Turner, 1977; Harris, 1975). Harris (1975) and Perron and Turner (1977) have successfully reared nudibranch species having planktotrophic (feeding) larvae from egg to egg. Other nudibranch species reared have been either lecithotrophic or direct developers. *Hermissenda* has a longer obligatory planktotrophic stage, 34 days, than either *Phestilla melanobranchia* Bergh 1874 (Harris, 1975) or *Doridella obscura* Verrill (Perron and Turner, 1977).

The length of the veliger stage in *Hermissenda* is similar to that reported for five species of Pacific aplysiid opisthobranchs, 30–34 days (Kriegstein, Castellucci, and Kandel, 1974; Switzer-Dunlap and Hadfield, 1978). Switzer-Dunlap and Hadfield (1978) observed a plateau in shell growth before metamorphosis in four aplysiid species similar to that noted in *Hermissenda*. As adults, three of the four above mentioned aplysiid species were reported to grow to a larger size in the laboratory than in the field. No specimens of *Hermissenda* fed in the laboratory have exceeded the maximum size of wild individuals.

Stages in the life cycle of *Hermissenda* follow in the same sequence as the seven general life history stages listed by Bonar and Hadfield (1974): hatching,

competency to metamorphose, velum loss, shell and operculum detachment and loss, and sinking of the visceral mass into the foot. The seventh stage, the pseudovermis stage, is eliminated in metamorphosing specimens of *Hermissenda* which grow tentacle buds and cerata buds as they crawl out of the shell.

The life cycle of *Hermissenda,* as observed in the laboratory, follows the pattern described for other hydroid-eating nudibranchs by Thompson (1964) and Clark (1975). Animals used in the present study came only from the Monterey Bay population; however, reported sizes of eggs and egg capsules, and structure and size of the egg masses deposited by individuals from other parts of the species range are within the range of values reported here (Hurst, 1967; O'Donoghue and O'Donoghus, 1922).

The most variable factor observed in populations of veligers and adults was growth rate. In the veliger stage part of this variation may have been due to culture conditions. Growth of larvae may have been inhibited by the antibiotic used, chloramphenicol, known to inhibit protein synthesis in eukaryotes as well as bacteria (Pestka, 1975), or the larval diet may have been suboptimal for many veligers.

Large laboratory populations of *Hermissenda* can be maintained on the tunicate *Ciona intestinalis,* which is commonly found in Cape Cod waters. Year-round availability of *Ciona* and ease of collection gives it an advantage over the normal field diet, which consists primarily of numerous coelenterate species, as well as tunicates. A mixed diet did not markedly improve growth or survival over the single item tunicate diet.

In *Hermissenda,* individuals which have the fastest growth rates are also the largest adults. A program of selective breeding of *Hermissenda* will concentrate, at least initially, on selection for fast growth rates. High selection pressure is already exerted on the laboratory population in terms of survival in the specific culture conditions utilized, and because adults are reared on a diet of only tunicate.

We would like to thank Richard Waltz for assistance with the statistical programming, Helen Stanley of Woods Hole Oceanographic Institution for providing the initial algal cultures, Ruthanne Theran for technical assistance, and Dr. Izja Lederhendler for his critical comments on the manuscript.

Summary

1. *Hermissenda crassicornis* is a subannual nudibranch species that reproduces year-round.

2. There is a significant positive relationship between adult weight, diameter of the egg mass, estimated number of eggs per egg mass, and average number of eggs per capsule.

3. There is a planktonic veliger stage of 34 days minimum at 13°–15° C.

4. Larvae metamorphose on at least three species of hydroids.

5. To develop in reasonable numbers to a state competent to metamorphose

veligers require a diet that includes phytoplankton of larger cell size (10–11 μm) than the commonly used *Isochrysis* and *Monochrysis* (5 μm).

6. Although *Hermissenda* feeds on a wide variety of sessile invertebrate species in the ocean, a diet of tunicate alone (*Ciona intestinalis*) promotes good growth and survival in the laboratory.

7. Egg mass deposition is initiated only after first copulation, except in the last month of life, and continues from about one-month post-metamorphosis to death, at about four months post-metamorphosis. Generation time (egg-to-egg) may be as short as 2.5 months.

8. A laboratory strain of *Hermissenda* is being established to provide animals of known history for research on the neural correlates of behavior. Animals, at least initially, are being selected for fast growth rate.

LITERATURE CITED

Alkon, D. L., 1974. Associative training of *Hermissenda crassicornis*. *J. Gen. Physiol.*, **64**: 70–84.

Alkon, D. L., 1975. Neural correlates of associative training in *Hermissenda*. *J. Gen. Physiol.*, **65**: 46–56.

Alkon, D. L., 1976. Neural modification by paired sensory stimuli. *J. Gen. Physiol.*, **68**: 341–358.

Birkeland, C., 1974. Interactions between a sea pen and seven of its predators. *Ecol. Monogr.*, **44**: 211–232.

Bonar, D. B., and M. G. Hadfield, 1974. Metamorphosis of the marine gastropod *Phestilla sibogae* Bergh (Nudibranchia: Aeolidacea). I. Light and electron microscope analysis of larval and metamorphic stages. *J. Exp. Mar. Biol. Ecol.*, **16**: 227–255.

Clark, K. B., 1975. Nudibranch life cycles in the Northern Atlantic and their relationship to the ecology of fouling communities *Helgo. Wiss. Meeresunters*, **27**: 28–69.

Guillard, R. L., 1975. Culture of phytoplankton for feeding marine invertebrates. Pages 29–71 in W. L. Smith and M. H. Chanley, Eds., *Culture of marine invertebrate animals*, Plenum Press, New York.

Haderlie, E. C., 1968. Marine fouling organisms in Monterey Harbor. *Veliger*, **10**: 327–341.

Haderlie, E. C., J. C. Mellor, C. S. Minter III, and G. C. Booth, 1975. The sublittoral benthic fauna and flora off Del Monte Beach, Monterey, California. *Veliger*, **17**: 185–204.

Harrigan, J. F., and D. L. Alkon, 1978. Laboratory cultivation of *Haminoea solitaria* Say, 1822 and *Elysia chlorotica* Gould, 1870. *Veliger*, in press.

Harris, L. G., 1975. Studies on the life history of two coral-eating nudibranchs of the genus *Phestilla*. *Biol. Bull.*, **149**: 539–550.

Hurst, A., 1967. The egg masses and veligers of thirty Northeast Pacific opisthobranchs. *Veliger*, **9**: 255–288.

Kriegstein, A. R., V. Castellucci, and E. R. Kandel, 1974. Metamorphosis of *Aplysia californica* in laboratory culture. *Proc. Nat. Acad. Sci. USA*, **71**: 3654–3658.

Lance, J. R., 1966. New distributional records of some northeastern Pacific Opisthobranchiata (Mollusca: Gastropoda) with descriptions of two new species. *Veliger*, **9**: 69–81.

MacFarland, F. M., 1966. Studies of Opisthobranchiate Mollusks of the Pacific Coast of North America. *Calif. Acad. Sci. Mem.*, **6**: 1–546.

O'Donoghue, C. H., and E. O'Donoghue, 1922. Notes on the nudibranchiate mollusca from the Vancouver Island Region. II. The spawn of certain species. *Trans. Roy. Can. Inst. XIV*, **1**: 131–143.

Perron, F. E., and R. D. Turner, 1977. Development, metamorphosis, and natural history of the nudibranch *Doridella obscura* Verrill (Corambidae: Opisthobranchia). *J. Exp. Mar. Biol. Ecol.*, **27**: 171–185.

Pestka, S., 1975. Chloramphenicol. Pages 370–395 in J. W. Corcoran and F. E. Hahb, Eds.,

Mechanism of action of antimicrobial and antitumor agents. Springer-Verlag, New York.

SWITZER-DUNLAP, M., AND M. G. HADFIELD, 1978. Observations on development and metamorphosis of four species of Aplysidae (Gastropoda, Opisthobranchia) in laboratory culture. *J. Exp. Mar. Biol. Ecol.*, in press.

TARDY, J., 1970. Contribution a l'etude des metamorphoses chez les nudibranches. *Ann. Sci. Nat. Zool. Bio. Anim., Ser. 12,* T., **12**: 299–371.

THOMPSON, T. E., 1958. The natural history, embryology, larval biology and post-larval development of *Adalaria proxima* (Alder and Hancock) (Gastropoda, Opisthobranchia). *Phil. Trans. Roy. Soc. Lond. Ser. B.*, **242**: 1–58.

THOMPSON, T. E., 1961. The importance of the larval shell in the classification of the Sacoglossa and Acoela (Gastropoda, Opisthobranchia). *Proc. Malacol. Soc. Lond.*, **34**: 233–238.

THOMPSON, T. E., 1962. Studies on the ontogeny of *Tritonia hombergi* Cuvier (Gastropoda, Opisthobranchia). *Phil. Trans. Roy. Soc. Lond. Ser. B.*, **245**: 171–218.

THOMPSON, T. E., 1964. Grazing and the life cycle of British nudibranchs. Pages 275–297 in D. J. Crisp, Ed., *Grazing in terrestrial and marine environments.* Blackwell Press, Oxford, England.

THOMPSON, T. E., 1967. Direct development in a nudibranch, *Cadlina laevis*, with a discussion of developmental processes in Opisthobranchia. *J. Mar. Biol. Assoc. U.K.*, **47**: 1–22.

YARNALL, J. L., 1972. The feeding behavior and functional anatomy of the gut in the eolid nudibranchs *Hermissenda crassicornis* (Eschscholtz, 1831) and *Aeolidia papillosa* (Linnaeus, 1761). *Ph.D. dissertation, Stanford University*, 134 pp. (*Diss. Abstr.*, **33B** (6): 2864, order number 72–30,725.)