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Life History and Aging of Captive-Reared California Sea Hares (*Aplysia californica*)

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Although the California sea hare, *Aplysia californica*, is well known from neurobiological studies and is raised in the laboratory for this purpose, various aspects of its life history in the laboratory, such as aging dynamics, are unknown. Therefore we collected life history data on 4 cohorts of eggs from hatchery-reared animals and performed an actuarial analysis of mortality data. Temperature was controlled at 13 to 15 °C, the photoperiod was a 14:10-h light:dark cycle, and the seawater O_2 concentration, pH, and salinity were held at optimized levels. The feeding protocol for 3 cohorts was unrestricted access to the red macroalga *Gracilaria ferox*, whereas the remaining cohort was fed standard hatchery rations of *G. ferox* 4 times per week. Growth was sigmoidal in each cohort and resulted in linear growth rates of 1.25 to 3.62 g/d during the exponential phase; these rates were not influenced by feeding level. Sexual maturity occurred at approximately 160 g, at ages ranging from 144 to 241 d. Egg production was highly variable in the different cohorts. Mean lifespan of cohorts fed ad libitum was approximately 228 d. In contrast, the cohort fed standard rations lived an average of 375 d and showed a lower initial mortality rate, suggesting that calorie restriction on a single-species diet prolongs lifespan in California sea hares.

Abbreviations: t_a, age of cohort when experiment started; G, Gompertz parameter, the aging rate; M, mortality rate; A, initial mortality rate; t, time in days; e, exponent; s, Gompertz survival function; MRDT, mortality rate doubling time

Aplysia californica has served as an important model organism for neurophysiological studies on the cellular basis of behavior for many years due to its relatively simple nervous system which is composed of large, easily identified neurons. Although much is known about the neurobiology of this species, comparatively little is known about some basic aspects of the life cycle of this hermaphroditic opisthobranch mollusc. This deficit is particularly true regarding the transition from sexual maturity to senescence occurring toward life's end in this species.

Adult *A. californica* are found in tidal and subtidal zones along the coast of California and feed herbivorously on seaweeds, with a preference for various species of red seaweeds.[25] The ability to observe the entire lifespan in the laboratory has resulted in a wealth of detailed information regarding the development of *Aplysia* from egg to sexually mature adult.[4,8,9,22–24,32,35] Kriegstein[23] divided the development of this species into 5 phases: embryonic (fertilization to egg hatching), planktonic (veliger larvae feed on phytoplankton), metamorphic (veliger larvae transform into benthic juvenile herbivores), juvenile (from metamorphosis to sexual maturity), and adult (egg laying begins). Recently, it has been proposed that the time period between the appearance of bag cell clusters (approximately 4 mo of age at 15 °C and approximately 2 mo postmetamorphosis at 22 °C) and the time of first reproductive activity represents sexually immature adulthood.[14,15]

Whereas numerous morphological landmarks designate periods of development culminating in sexual maturity,[12,23] it is considerably more difficult to assess aging and senescence in *Aplysia*, because there are no consistently observable morphologic or behavioral characteristics for this part of the life cycle, unlike for early development. As a result, published reports on aspects of aging in *Aplysia* have relied on somewhat subjective determinations of what is an "old" *Aplysia*, particularly if animals collected from the wild were used in the determinations. Several of these reports have used animal size as an index of age.[3,28] However, both seasonal and short-term variations in animal size occur,[2,16,29] making size an unreliable index of age.

Information on the lifespan of this species has been limited in large part to conclusions drawn from 1 field study of wild animals.[2] In this study, lifespan was estimated at approximately 1 year on the basis of the annual variation in animal size and abundance observed at 2 sites on Santa Catalina Island, off the California coast. Observed reproductive behavior and measurements of oocyte diameter suggested that sexual maturity was reached in late spring and was followed by an extended period of copulation and egg-laying lasting through the summer and ending with the death of the mature population in the fall.

Only 1 study has directly investigated the survival of a group of postmetamorphic *Aplysia* in a laboratory setting for the sole purpose of determining lifespan.[18] Because wild *Aplysia* were used in that study, the exact age of the animals was unknown and estimated from the size of the internal shell. Because this technique for estimating age assumes consistent growth rates throughout the animal's life[29]—an assumption that may not hold true, particularly late in the life cycle[34]—the conclusions regarding lifespan were compromised.

Ectothermic organisms, whose metabolic rates are known to be proportional to ambient temperature, generally live longer when kept at lower temperature than when kept at higher temperatures (for review, see 1). Gev and colleagues[17] concluded from a field study on the life cycles of two *Aplysia* species in Israel that the temperature at which the animals develop is more important in setting the lifespan than is any genetically determined, species-specific variable. In particular, all of the long-lived species live through a winter in which temperatures are low, during which time they grow but remain sexually immature. Rising water temperatures in the spring and summer trigger gonadal development and initiate the breeding

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season, after which the animals die. By contrast, the short-lived species, with a 4-mo lifespan, live in regions with relatively warm water year round. The degree to which the lifespan of *A. californica* depends on temperature, as opposed to other factors that affect metabolic rate such as food quantity, is unclear. This determination is complicated by the fact that, in the wild, water temperature and algal food availability generally vary together.[2,17]

It is well established, however, that temperature has profound effects on reproductive activity in *A. californica*. In the wild, animals experience temperatures that range from 13 to 15 °C during the winter to >20 °C during the summer breeding season.[25] It is thought that the rise in temperature in the spring triggers gonadal development and facilitates egg laying,[33] a finding confirmed in the laboratory by Stommes and coworkers.[34] Although in a pair of studies conducted with laboratory animals, rearing at 20 °C compared with 15 °C increased egg-laying frequency,[30,37] this difference does not always result in greater reproductive output for animals maintained at warmer temperatures.[34]

Several studies have investigated aspects of the growth and reproduction of various *Aplysia* species conducted using wild animals of unknown age and environmental history.[10,11,22,29,31] No published studies on *Aplysia* have focused on senescence and lifespan characteristics as the primary goal of the research.

The ability to culture *A. californica* in the laboratory offers a unique opportunity to study aspects of its growth, egg production, time to sexual maturity, and lifespan systematically under controlled environmental conditions. Although there is some normal variation, *Aplysia* cultured in the laboratory at the University of Miami Aplysia Facility grow and mature at fairly consistent rates.[5,6,15,16,34] This consistency makes it possible to study how alterations to standard hatchery conditions affect growth and maturity. Moreover, documentation of these parameters throughout the lifespan of these animals can then more clearly illustrate the assortment of physical and behavioral changes that accompany, and thus signify, senescence in this organism.

Materials and Methods

We studied 45 to 52 animals from each of 4 cohorts of *A. californica* in a hatchery setting, the University of Miami NIH National Resource for Aplysia, between August 2002 and January 2004. The 4 cohorts of animals were raised from 4 egg strands laid by wild-caught brood stock. Animals in each cohort were at least half-siblings to other members of the cohort. The cohorts were maintained in facilities separated from the hatchery population. Juvenile animals were housed at 50 individuals per 8-l cage until weight measurements were begun, at which point animal density was reduced to 5 per 16-l cage. For 3 of the 4 cohorts studied, the age at which animal density was changed to 5 animals per 16-l cage was designated the t_a age; this age varied from 96 to 122 d for these 3 cohorts. For the remaining cohort, t_a was 134 d; this cohort was already housed at 5 animals per 16-l cage when the experiment started. The hatchery is equipped with filtered seawater chilled to 13 to 15 °C, and lighting is maintained on a 14:10-h light:dark cycle. The cages sat submerged in large fiberglass troughs filled with continuously flowing seawater. Seawater flowed through each of the cages via individual valves at a rate of 2 l/min, such that the total cage volume was replaced approximately every 10 min. Animals weighing <2 g were kept in cages equipped with fine mesh filters to prevent animal loss, whereas larger animals were kept in cages with perforated bottoms to optimize water flow. In accordance with standard hatchery procedure, postmetamorphic animals weighing <10 g were fed mainly *Agardhiella* sp. macroalgae, whereas those ≥10 g were fed *Gracilaria ferox*, a diet shown to be second only to a mixed diet in optimizing growth.[7] In this report, the cohorts described as being fed ad libitum essentially received *G. ferox* only, because the period during which *Agardhiella sp.* was offered was comparatively brief. All animals were washed daily with a seawater hose, at which time uneaten algae were removed and replaced with fresh algae.

Baseline observations were made to describe growth and sexual maturity. The live wet weight of each animal was measured to 0.1 g once weekly by using an electronic balance (Mettler, Toledo, OH) after draining excess water from the parapodal cavity by holding the animal tail down and shaking lightly. The age at first spawn for each cohort was recorded, and observations were made daily for mating behavior, the presence of egg masses, and mortalities in each cage.

Unrestricted food access was chosen as the feeding protocol for most cohorts, in order to eliminate food as a variable. For comparison, one cohort of animals was fed standard hatchery rations 4 times per week,[16] such that 3 g animals were fed 430% of their weight per animal per week, with larger animals receiving progressively less food, so that, for example, 90-g animals received 90% of their weight per animal per week. The goal of these observations was to define these life history parameters for different cohorts of hatchery-reared *Aplysia* under conditions of constant temperature but different levels of feeding.

All cages were observed daily for the presence of egg masses to determine whether there were changes in egg production during the transition from sexual maturity to senescence. All egg masses were removed from the cages and weighed. Spawn weight data were used to calculate age-related declines in egg production.

An actuarial analysis of mortality data was performed to quantitate lifespan and aging parameters and to confirm that aging was occurring in these hatchery-reared animals. Therefore feeding and other conditions for each cohort were maintained until the animals died naturally. Age at death (in days) for these individuals was used to calculate lifespan and aging parameters using the SURWBIG survival program.[38] The survival curves were fit by nonlinear regression analysis to the Gompertz survival function, which is derived from the Gompertz mortality rate function, $M = Ae^{Gt}$, where M is the mortality rate, A is the initial mortality rate, G is the Gompertz exponential parameter (which is considered the aging rate), and t is the time in days. The Gompertz function is commonly used to describe the exponential increase in the mortality rate with time that is typical of aging populations. The initial mortality rate, A, is the mortality rate at age 0, which can vary between cohorts and which can be thought of as genetic vigor. G is commonly expressed in terms of the mortality rate doubling time (MRDT), a constant, where $MRDT = \ln 2/G$.

Results

Figure 1 illustrates weekly weight (mean ± 1 standard deviation) beginning at t_a (the age of the animals when they were transferred to 16-l tanks) for each of the 4 cohorts. Table 1 summarizes the relevant parameters of growth, maturation, and reproduction for each cohort. For the 4 cohorts, t_a ranged from age 96 to 134 d. The sigmoidal growth characteristic of this species[16,22,29] is apparent in the growth of cohorts 2 through 4 (Figure 1B–D), in which t_a was sufficiently early in life to capture this feature. The exponential phase of growth was fit by linear regression and used to calculate growth rates (Table 1).

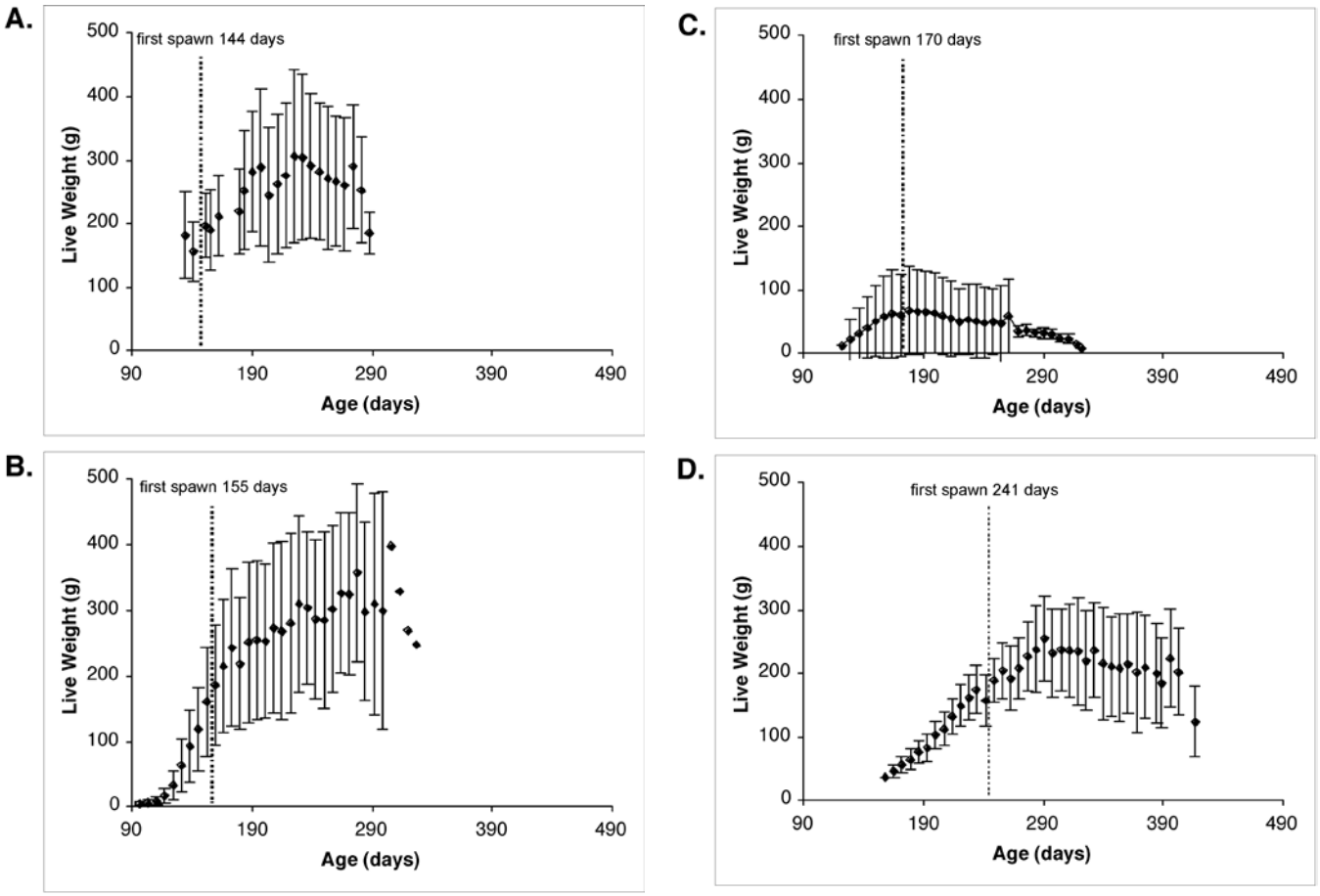

Figure 1. Variation in live weight (mean ± 1 standard deviation) of *A. californica* with age for each cohort, from age t_a (the age at which animals were moved to 16-l tanks) to the death of individual animals. The smaller standard deviations of the last several points were caused by reduced sample size due to animal deaths. Vertical lines denote age at which the first egg mass was laid. (A) Cohort 1. (B) Cohort 2. (C) Cohort 3. (D) Cohort 4.

Growth based on weight determinations varied among the cohorts, ranging from as high as 3.62 g/d in cohort 2 (fed ad libitum) to 1.84 g/d in cohort 4 (fed standard hatchery rations). The mean maximum weight was reached at different times of life in the various cohorts and was itself quite variable, ranging from 67.9 ± 70.5 g in the poorly growing cohort 3 to 358 ± 137 g in cohort 2, both of which were fed ad libitum. Cohort 4, fed standard rations, reached a maximum weight of 255 ± 70.3 g. With respect to weights of individuals within a cohort, a standard deviation of as much as 45% of the mean was noted. Large variations in mass within a cohort are typical for *Aplysia* reared in the laboratory.[5] In 3 of the 4 cohorts, the maximum weight was achieved ≥50 d after the onset of sexual maturation. Subsequently, decline in animal weights was sometimes significant (cohort 1, Figure 1A; $P \leq 0.05$, analysis of variance [ANOVA]).

The age at first spawn was defined as the age the first egg mass was laid. In the 3 cohorts that grew well (cohorts 1, 2, and 4), sexual maturity was reached at mean animal weights of approximately 160 to 170 g, no matter when this weight was achieved (at an age of 144, 155, and 241 d, respectively).

Grouping egg production data into weekly values and plotting these over the entire lifespan allows for the comparison of lifetime spawning patterns between various cohorts. These data are expressed as total weight of egg masses produced per week post-hatch for all cohorts (Figure 2).

Although reproductive output in cohort 3 was less than that for cohorts 1, 2, and 4, total lifetime egg production was highly variable among these 3 cohorts, ranging from 0.673 kg in cohort 1 to 2.24 kg in cohort 2. Egg production per animal peaked for cohort 1 during ages 218 to 273 d, reaching a maximum of 93.3 g eggs laid during week 36, an average of 5.6 g eggs per animal. The last egg mass produced by this cohort was at age 291 d, when survival was only 4%, and this event occurred just 4 d before the final 2 deaths. Thus cohort 1 did not appear to exhibit a prolonged cessation of egg laying before death.

Cohort 2 spawned for 18 consecutive weeks, with egg production peaking at age 176 to 217 d, both in total production (range, 151.7 to 251.7 g/wk) and production per animal (range, 5.4 to 8.4 g/animal). Egg production steadily declined after week 31, with the last egg mass being laid at age 304 d by the sole surviving animal. This final egg mass was produced 26 d before this animal's death at 330 d. Total lifetime egg production for cohort 2 was 2.24 kg, which was 3.32 times that of cohort 1. Cohort 2 produced significantly more eggs than the other cohorts ($P \leq 0.001$, ANOVA with Scheffé post-hoc comparisons; Table 1).

Most animals in cohort 3, which was fed ad libitum, exhibited severely reduced growth compared with the other cohorts, as evidenced by the observed low growth rate of 1.25 g/d. Cohort 3 also exhibited extremely poor egg production. The age at first spawn for cohort 3 was 170 d, when the mean weight was approximately 60 g, which was close to the maximum mean weight achieved in this cohort (67.9 ± 70.5 g). The lifetime reproductive output of this cohort consisted of few, relatively small egg masses found in only 3 of the 9 cages. At least 30 of the 48

Table 1. Growth, maturation, and reproduction data for each cohort

Cohort	1	2	3	4
Feeding (d from t_a)	Ad libitum (134)	Ad libitum (96)	Ad libitum (122)	Standard rations (134)
Growth rate (g/d)[a]	not determined[b]	3.62	1.25	1.84
Maximum mean weight during lifetime (g ± 1 standard deviation) (age in d)	307 ± 137 (225)	358 ± 137 (278)	67.9 ± 70.5 (178)	255 ± 70.3 (291)
Change in weight after achieving the maximum mean weight	Declined[c]	No decline	No decline	No decline
Mean animal weight at first spawn (g)[d] (age in d)	160 (144)	170 (155)	60 (170)	157 (241)
Age at peak egg production (d) (Single week egg weight peak in g; at age in wks)	218-273 (93.3; 36)	176-217 (251.7; 31)	175 (26.8; 25)	301-350 (218; 49)
Total lifetime egg production of cohort (g)	673	2235[e]	61.2	1921
Age at last death (d)	295	330	322	416
n at t_a	47	45	48	52

t_a, age of the animals when moved to 16-l tank.
n, sample size.
[a]Growth rates derived from linear regression fits in which all r^2 values were ≥0.850.
[b]Growth rate not calculated because the experiment began after the exponential growth phase of this cohort.
[c]$P \leq 0.05$, analysis of variance with Scheffé post-hoc comparisons.
[d]Mean weight is approximate because the first egg mass often was produced several days after the weekly mean cohort weight measurement.
[e]Cohort 2 had significantly ($P \leq 0.0001$, analysis of variance with Scheffé post-hoc comparisons) greater egg production than did the other cohorts.

animals (63%) failed to lay any eggs at all. Although egg-laying activity was not ascribed to individual animals during the course of this study because 5 animals were housed per cage and because most egg-laying episodes were not witnessed, each of the 3 cages in which eggs were found contained animals that weighed considerably more than the mean for this cohort. For example, the mean animal weight in the cage where the 1st spawn was observed was 147.7 ± 54.1 g. Because these larger animals were responsible for the eggs observed, the average age and weight of reproductive animals from cohort 3 were consistent with those of first-spawners from the other cohorts fed ad libitum.

Cohort 4, which received standard rations, spawned the latest of any of the cohorts (241 d). Cohort 4 first spawned 97 d later than youngest-spawning cohort 1 (144 d). Cohort 4 spawned for 23 consecutive weeks, and its total egg production was 1.92 kg, a value 2.1 times the total lifetime production of cohort 1 and 63% that of cohort 2. Spawning in cohort 4 peaked during a broad period from ages 301 to 350 d and declined sharply thereafter, as mortalities increased. Egg laying ceased only 1 wk before the final death.

The survival plots for all cohorts, illustrating the fraction of animals in the cohort surviving over time, are shown in Figure 3. Results of the SURWBIG analysis revealed that the survival fractions for all cohorts were best fit by the Gompertz survival function, which is shown plotted as solid or dashed lines in Figure 3. The general rectangular shape of the survival curves is typical for a population exhibiting age-related mortality, where the likelihood of death increases with advancing age.[1] The life history data and aging parameters for all the cohorts are shown together in Table 2.

The mean lifespan for cohort 1 was 231 d (Figure 3; Table 2). The median lifespan, which represents the time when survival was 50%, was 229 d. Deaths were infrequent until approximately 197 d, when they increased to 2 to 6 each week. The final 2 deaths occurred at age 295 d. At the age of first spawn (144 d), survival was 92%, but during the time period of greatest egg production per animal (week 32 to 39), survival fell from approximately 50% to approximately 10%. The last egg mass produced by this cohort was during week 42 at age 291 d, when survival was only 4%. This event occurred only 4 d before the final 2 deaths.

Cohort 2 exhibited several coincident deaths, which resulted in a distinctive step-like shape to the survival curve that was unique to this cohort (Figure 3; Table 2). Furthermore, there were some unusual early mortalities in cohort 2 from ages 95 to 113 d, when 11 animals died (24% of the cohort; data not shown). These mortalities did not appear to be due to senescence, because this cohort had not yet reached sexual maturity, nor were they obviously attributable to water quality, salinity, O_2 concentration, or pH. Therefore the survival curve analysis for cohort 2 reflects removal of these 11 early deaths from consideration, because they very likely were unrelated to aging or senescence. The survival curve for the remaining 34 animals, and the corresponding Gompertz function fit to this curve, are shown in Figure 3. Once altered to exclude the 11 early mortalities, the values of the aging parameters for cohort 2 were more similar to those obtained for cohort 1 and may be more relevant estimates of these parameters. The median lifespan of cohort 2 (228 d) which, by the very nature of the median, was not affected by particularly early or particularly late mortalities, was almost identical to that of cohort 1. The last death in cohort 2 occurred at age 330 d.

Despite the abnormal growth and egg production observed for cohort 3, these animals exhibited survival characteristics comparable to those of cohorts 1 and 2 (Figure 3). The mean lifespan was 224.2 d, with a median of 226.5 d. Like cohort 1, mortality remained low until approximately 175 d, with the last death occurring at age 322 d. At the age of first spawn (170 d),

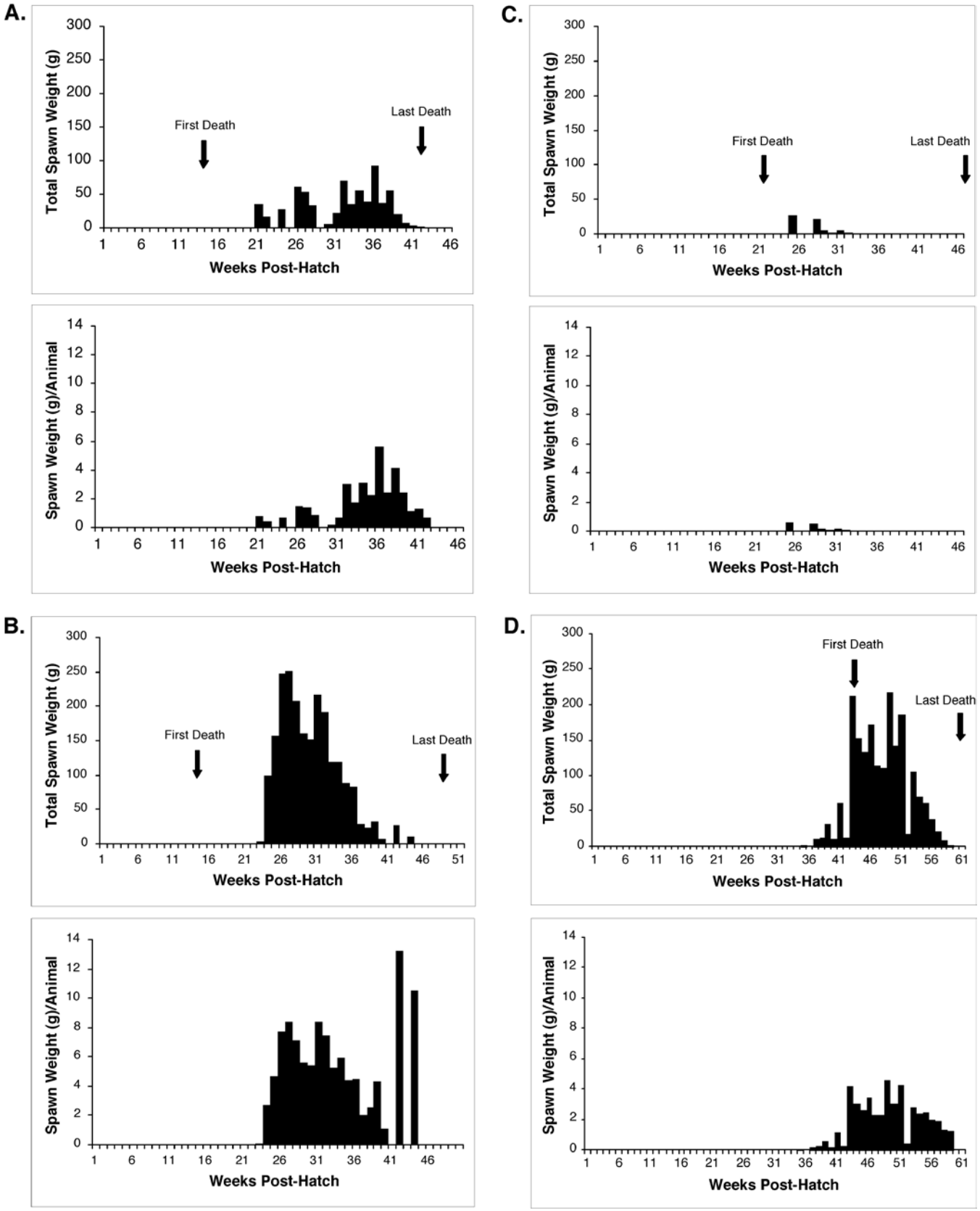

Figure 2. Egg production data over the lifespan of the cohorts. The top graph shows the total spawn weight for entire cohort by week, whereas the bottom graph shows spawn weight per animal during each week of the spawning period. (A) Cohort 1. (B) Cohort 2. (C) Cohort 3. (D) Cohort 4. Cohort 2 laid significantly ($P \leq 0.001$, analysis of variance with Scheffé post-hoc comparisons) more eggs than any other cohort.

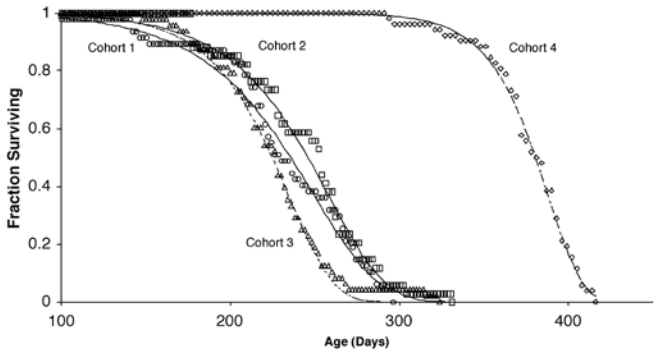

Figure 3. Survival curves for *A. californica* at each temperature. Observations are expressed as a Gompertz survival function, s = exp [(A/G)(1 – e^{Gt})], derived from the Gompertz mortality rate function M = Ae^{Gt}. The data presented for cohort 2 omit the 11 mortalities that were unrelated to senescence.

survival was 94%. One notable feature of the survival of cohort 3 is the pronounced "tail" to the survival curve, which was created by the long survival of the last 2 animals of this cohort.

We compared the survival data and aging parameters of the 3 cohorts fed ad libitum with those of cohort 4, which was raised at the same temperature and animal density but which received standard hatchery rations. The mean lifespan of cohort 4 was 375.3 d and the median was 381 d, both of which were longer than those in the cohorts fed ad libitum. The first death occurred at 291 d, resulting in a rectangular survival curve (Figure 3) marked by a precipitous decline in survival after 354 d.

Each of the mean lifespans of cohorts 1, 2, and 3 were significantly different ($P \leq 0.01$, *t* test) than that for cohort 4. Among cohorts 1, 2, and 3, none of the mean lifespans was significantly different from the others. The aging parameters obtained from the survival data show that each of cohorts 1, 2, and 3 had higher initial mortality rates (A) than did cohort 4. The shape of the cohort 4 survival curve resulted in a Gompertz fit, with a high aging rate (G) of 0.491 and a high MRDT (the time needed for the chance of dying to double) of 14.1 d.

Age-specific mortality rates over time for all the cohorts are shown in Figure 4. The plots in Figure 4A were obtained by inserting the Gompertz survival function parameters, obtained from the fit to the survival data, into the Gompertz mortality rate function M = Ae^{Gt}. The Gompertz mortality rate function is plotted semilogarithmically in Figure 4B. The exponential functions become straight lines, with A as the y intercept and G equal to the slope of the line. In this figure, the lower initial mortality rate (y intercept) for cohort 4 becomes apparent compared with those of the cohorts fed ad libitum.

Discussion

Kriegstein[23] noted that at constant temperature, animal density, and food and water quality, the rate of postmetamorphic growth of *Aplysia* depended solely on dietary amount. In agreement with previous studies documenting the growth of *A. californica* in a hatchery setting[5,16] individual animals in the present study showed variable growth, as indicated by the large standard deviations of the weekly mean weights, and growth rates were higher in the cohorts receiving food ad libitum. The wide ranging growth rates within a cohort may be genetically determined, with ad libitum feeding accentuating this variability. Animals with the highest, genetically determined growth potentials may be able to use relatively more of this potential than animals with lower growth potentials when they are not food-limited. Actual time of metamorphosis during the postlarval phase is another factor we have found important in explaining the variability in growth within a cohort. Animals undergoing metamorphosis sooner may secure a growth advantage that is often maintained throughout life.

Averaging only 1.25 g/d at peak growth, cohort 3, despite larval development of typical duration, had severely reduced average growth when compared with the other cohorts in this study as well as with other published studies on this species.[5,22] Such poor growth is occasionally observed in hatchery-reared *Aplysia*. Possible explanations include a genetic defect, an undiagnosed disease, or an unnoticed problem with some aspect of the culture of these animals.

The ad libitum feeding received by cohorts 1 to 3 shortened the time to sexual maturity compared with that of standard-ration-fed cohort 4. The age at sexually maturity of cohort 4 was comparable with that in other studies in which the animals were raised on standard Aplysia Resource feeding schedules.[15,16] Although the feeding levels affected the age at sexual maturity of the cohorts in this study, they did not appear to affect average animal size at sexual maturity (approximately 160 g). These results suggest that at constant temperature of 13 to 15 °C and identical stocking density (≤5 animals/cage), the rate of maturation depends solely on feeding levels and that animal size may be a good indicator of development until sexual maturity

Table 2. Life history and aging determinations for cohorts 1 through 4

	Cohort 1 (n = 47)	Cohort 2 (n = 34)	Cohort 3 (n = 48)	Cohort 4 (n = 25)
Mean lifespan (d ± SD ± SEM)	231 ± 44.3 ± 6.5	209 ± 70.2 ± 10.5	224 ± 34.4 ± 5	376 ± 27.2 ± 3.8[a]
Median lifespan (d)	229	228	227	381
Age at first spawn (d)	144	155	170	241
Initial mortality rate	3.81×10^{-5}[b]	1.39×10^{-5}	5.83×10^{-6}	2.66×10^{-10}
Gompertz parameter	0.0262[b]	0.0297	0.0374	0.0491
Mortality rate doubling time (d)	26.5	23	18.5	14.1

SD, 1 standard deviation; SEM, standard error of the mean.
[a]Mean lifespan of cohort 4 was significantly ($P \leq 0.0001$, *t* test) longer than those of cohorts 1 through 3.
[b]Gompertz survival function: s = exp[(A/G)(1 – e^{Gt})], where s is the proportion of the population surviving at time t, A is the initial mortality rate (d^{-1}), G is the Gompertz parameter (d^{-1}), exp is e, and t is the time (d).
[c]ln(2)/G = 0.693/G, where G is the Gompertz parameter.

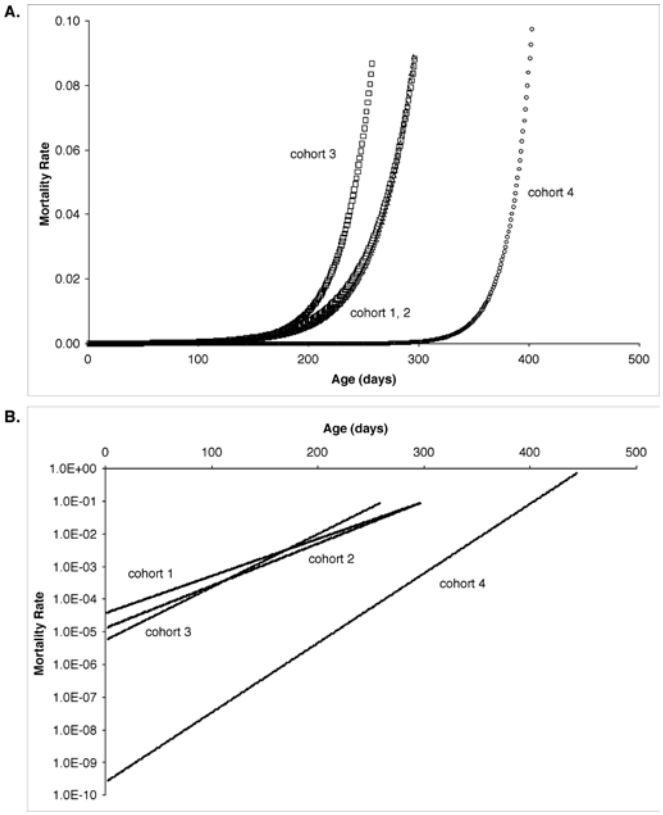

Figure 4. Age-specific mortality rates for the cohorts. (A) Mortality rate versus age, as calculated from the Gompertz mortality rate function $M = Ae^{Gt}$, where M is the mortality rate, A is the initial mortality rate, e is the exponential, G is the Gompertz parameter, and t is the time in days. The values used for A and G were determined from the best-fit Gompertz survival function for each cohort. (B) Semilogarithmic plot of mortality rate versus age for cohorts 1 through 4. For each line, the y intercept was the initial mortality rate, and the slope of the line was the Gompertz parameter.

under these conditions. Unfortunately, size is not of great use in estimating the developmental age of wild *Aplysia*, because temperature, which varies in the wild from 13 to >20 °C during the year[21] is a strong determinant of both growth and development in *A. californica*.[34]

Furthermore, Capo and coworkers[5] noted that animals raised at various stocking densities and fed a mixed algal diet ad libitum starting at approximately 100 d of age varied in size at any given age but reached sexual maturity at the same age regardless of mass from 68 to 238 g. These observations suggested that at some age, *A. californica* in a hatchery environment will spawn to fulfill the reproductive imperative even if the animals have not reached a "critical" somatic weight. In that study, stocking density clearly affected animal growth, and as a result, animal size alone was not a good indicator of development before sexual maturity. High variability in mass at a given age is also likely to be the case in the wild, with variations in food and temperature substituting for stocking density and confounding a relationship between age and size. In the wild, the best predictor of *Aplysia* age is probably the time of year, with size of animals found during a particular season giving, at most, additional insight about their age. These growth studies demonstrate that the rate of growth and maturation of *Aplysia*, even at constant temperature in a hatchery setting, is an extremely complex process and can vary considerably depending on a number of factors, including lifetime feeding levels, diet, stocking density, and genetic variability.

Examination of the changes in mean weight with age show that these cohorts did not undergo a decline in mean cohort weight at the end of life. The large range of animal weight in a given cohort, coupled with increasing mortality as these measurements progressed, obscured aging-related declines in weight that occurred in individual animals during the course of this study. In order to follow these declines in more detail, the weight history of individual animals must be followed (via animal tagging) because mean cohort weight may not reveal senescence-related weight loss, due to the large variation in size at age of members of a cohort.

The lifetime egg production data also revealed a large degree of variability in fecundity between cohorts. The egg production data for cohort 2 (fed ad libitum) were the highest in this study, closely followed by cohort 4 (fed standard hatchery rations). This finding suggests that the total lifetime feeding levels received by a cohort of *Aplysia* may not be directly proportional to the cohort's total lifetime egg production. Egg production of individual animals has been shown to be directly proportional to animal size in *Aplysia*[6,19] and other invertebrates[13,26] and some vertebrates.[36] Therefore the total lifetime egg production of an individual *Aplysia* may depend both on its size and on its health and longevity, which can be enhanced by reducing caloric intake. Although it is not possible from the present study to determine whether food restriction is having separate effects on reproduction and on aging and longevity, aging is capable of regulating reproduction and vice versa,[20] and thus factors that influence aging must affect reproduction.

Relative to the other cohorts, cohort 4 (fed standard rations) had a high aging rate (G) and a high MRDT but the longest lifespan. This apparent paradox is explained by an awareness that the aging rate cannot be measured by lifespan alone.[21] An equally critical parameter to consider is the initial mortality rate, A. Both lifespan and mortality are functions of A and G, and it is not possible to infer which combination will produce a particular lifespan. Whereas A was very low in cohort 4, A and G together yielded a high MRDT *and* a long lifespan. This longer lifespan is in agreement with many other published studies on both invertebrates and vertebrates demonstrating the effects of food limitation on lifespan[27,39] (for review, see 1). The longer lifespan of cohort 4 contrasts with the findings of Capo and colleagues,[6] who reported that 90% of the hatchery reared *Aplysia* in their study, which received food ad libitum beginning at age approximately 100 d, survived longer that 14 mo, suggesting that ad libitum feeding might have prolonged their lifespan compared with that of animals on hatchery feeding schedules that receive restricted calories. One potentially important difference in the culture conditions of animals in the present study and those in the study of Capo and coworkers[6] was diet: animals in the present study were fed only *G. ferox* at ≥10 g, whereas those in the study of Capo and colleagues[6] received a mixed algal diet consisting of 3 algal species (*G. ferox*, *Ulva* sp., and *Agardhiella* sp.) throughout their postmetamorphic life. A mixed algal diet may provide better nutrition to hatchery-reared *Aplysia* and therefore affect the fitness and survival of the animals.

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