



The evolution of senescence in natural populations of guppies (*Poecilia reticulata*): a comparative approach

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Abstract

Model organisms like *Drosophila melanogaster* or *Caenorhabditis elegans* have revealed genes that influence senescence and the evolvability of senescence. We are interested instead in evaluating why and how senescence evolves in natural populations. To do so, we are taking the ecological geneticist's perspective of comparing natural populations that differ in factors that are predicted to influence the evolution of senescence and are evaluating whether senescence has evolved in the predicted fashion. We are also manipulating the environment to evaluate more directly the evolution of senescence. Guppies (*Poecilia reticulata*) are found in streams throughout the Northern Range mountains of Trinidad. Natural populations experience large differences in mortality rate as a consequence of the predators with which they co-occur. We have already shown, both with comparative studies and manipulations of the distribution of guppies and their predators, that the early life history evolves very rapidly in response to these differences in mortality. For example, high adult mortality rates select for individuals that develop more rapidly, produce their first litter of young at an earlier age, and devote more of their available resources to reproduction for the remainder of their lives. These changes were predicted by independently derived theory. Aspects of this same theory also predict how the late life history and senescence should evolve. Specifically, theory predicts that the populations that experience low mortality rates should also experience delayed senescence and longer life spans relative to those that experience high mortality rates. We are currently evaluating these predictions with representatives from two high-predation and two low-predation environments. Our presentation will focus on our pilot study, which evaluated life span, lifetime reproduction, and the patterns of aging in our laboratory

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populations. We will also report on the progress in our ongoing comparative studies of senescence in natural populations. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

Our goals in this essay are to outline our rationale for studying the evolution of senescence in guppies.

First, we will explain why guppies present an interesting system for evaluating the evolution of senescence. Second, we will consider how to evaluate senescence. Senescence is most often evaluated in terms of mortality rates and the way mortality rates change with age. However, senescence may also include age-specific changes in reproduction and a diversity of other physiological changes. There are now some proposals for how to combine mortality and reproduction into composite indices of senescence (Partridge and Barton, 1993, 1996; Dudycha and Tessier, 1999). The question of how to evaluate senescence is made more interesting by the argument that the patterns of age-specific changes in mortality and reproduction may be as readily explained by the evolution of the optimal life history as by senescence (Blarer et al., 1995). We will thus detail a pilot study that illustrates the various ways in which senescence is manifested in guppies. The intent of this study was to yield guidelines for what to measure in our comparative study. Finally, we will present progress in our ongoing comparative study of senescence in natural populations of guppies.

The prevailing theories for the evolution of senescence argue that senescence is a byproduct of other processes. Medawar's (1952) mutation accumulation model implies that senescence is a passive byproduct of constant mutations and Darwinian selection that removes deleterious mutations. If deleterious mutations are age specific in their effects, then those that are expressed early in life will have a larger impact on fitness and hence will be more readily eliminated by natural selection. Those that are expressed later in life will have a relatively small effect on fitness because far fewer individuals will live long enough to experience their deleterious effects. These mutations will thus tend to accumulate more readily in natural populations. It is this accumulation of late acting deleterious mutations that is the proposed cause of senescence.

Alternatively, Williams (1957) proposed that senescence is a byproduct of the evolution of the early life history. Natural selection will optimize variables like the age at maturation or the quantity of available resources that are used for reproduction as opposed to other functions, such as growth, storage or maintenance. Williams proposed that the genes that control the early life history will have pleiotropic effects on the late life history. Specifically, the evolution of an increase in the rate of investment in reproduction early in life will cause an earlier onset of senescence.

Both of these theories yield the same predictions for how organisms will evolve in response to mortality rate. When organisms experience a high mortality rate due to external causes, such as predation, few individuals will survive to advanced ages. A consequence of lower survival is weak selection on advanced ages since fewer individuals

survive to those ages (Hamilton, 1966), resulting in a higher probability for the accumulation of deleterious mutations and earlier senescence. A second consequence of high adult mortality rates is that selection will favor those individuals that mature at an earlier age and invest more in reproduction early in life (Gadgil and Bossert, 1970; Law, 1979; Michod, 1979; Charlesworth, 1980). An increase in the rate of investment in reproduction can be manifested in various ways, such as by producing more offspring, reproducing more often or providing more resources to individual offspring. This increased investment early in life comes at the cost of reduced investment in later life stages and hence senescence at earlier ages. Earlier senescence is thus predicted as a byproduct of the evolution of the early life history. Conversely, if an organism has a high life expectancy due to a low risk of predation or disease, it is predicted that natural selection will favor those individuals that mature at more advanced ages, have reduced investment in reproduction and exhibit delayed senescence as a consequence of the evolution of these early life history traits. Kirkwood and Holliday's (1979) disposable soma theory, which can be understood as a special, more narrowly defined case of Williams' hypothesis, makes the same prediction.

Abrams (1993) reconsidered the predictions of Williams' model under different conditions of population regulation. His is the only model that we are aware of that does not necessarily predict that high mortality rates will select for an earlier onset of senescence. He instead predicts that the nature of the outcome will depend upon whether or not the population experiences density regulation. If the population is routinely density regulated, then the predicted outcome of selection depends upon how density regulation is manifested. For example, high population densities can cause a decrease in fecundity or an increase in mortality rates. Furthermore, the increase in mortality rate may be more severe for either the younger or older age classes. All of these conditions turn out to influence how senescence will evolve in Abrams' models.

2. The comparative study of senescence in guppies

The approach that we are taking in our study of the evolution of senescence is the same as that developed, beginning in the 1940s, and popularized under the name "ecological genetics" (Ford, 1971). This approach has been one of the most successful for studying adaptations and the process of selection in natural populations (Reznick and Travis, 1996). One first describes differences among natural populations of organisms in traits of interest then evaluates the correlates of these differences. The existence of correlations between traits and the environment provides clues about potential causes of these differences among populations. These initial descriptions are followed with an evaluation of the genetic basis of observed phenotypic variation and experiments that evaluate more directly the causes of the differences among populations. This is the approach that was used in all of the classic studies of natural selection, including industrial melanism, shell banding polymorphisms in snails, and the evolution of mimicry in butterflies (Ford, 1971). This same approach has already been successfully applied to studies of senescence in Virginia opossums (Austad, 1993), grasshoppers (Tatar et al., 1997) and waterfleas (Dudycha and Tessier, 1999).

Guppies (*Poecilia reticulata*) are small freshwater fish from the northeastern coast of South America and some neighboring Caribbean islands. They are live bearers, but they are egg retainers, meaning that all maternal provisioning happens prior to the fertilization of the egg. Once an egg is fertilized, it is retained in the female while it completes development without the female providing further resources to sustain growth. Female guppies give birth for the first time when 10–20 weeks old and males can attain maturity in less than seven weeks. Sexually mature individuals reproduce continuously thereafter and females produce a new litter every three to four weeks. Our pilot studies indicate that there is no “menopause” or extended post-reproductive lifespan. The maximum longevity of reproducing females is 24–30 months. This life history makes guppies less ideal for studies of aging than *Drosophila melanogaster* or *Caenorhabditis elegans*, but still favorable relative to most vertebrates.

We have concentrated on natural populations of guppies from the Northern Range mountains of Trinidad because earlier research established that there are substantial differences among populations in their exposure to predators (Haskins et al., 1961; Endler, 1980). Our work has dealt primarily with “high” versus “low” predation localities. In high-predation localities, guppies co-occur with pike cichlids (*Crenicichla alta*) and other species of fish that often prey on adults and young alike. These sites also include some avian predators, such as kingfishers and a relatively low abundance of invertebrate predators (Reznick et al., 2001). Low-predation localities are found in the same drainages but are upstream of rapids or waterfalls that serve as barriers to the larger predators. Here guppies are found with the killifish, *Rivulus hartii*. *Rivulus* is an omnivore that feeds on small, immature guppies. These differences in predation are found in several different drainages. Such a repeated geographic pattern means that there are several replicates of high- and low-predation environments and that any adaptation that guppies have to these environments could have evolved independently in each drainage. Because of variation among drainages and localities within drainages in other features of the environment, often independent of predation, it is possible to evaluate the potential importance of these other factors in how guppies evolve. For example, there is considerable variation in stream size, canopy cover, and primary productivity. All of these factors have been evaluated as possible agents of selection (Grether et al., 2001; Reznick et al., 2001).

Guppies from high-predation localities experience far higher mortality rates than their counterparts from low-predation localities (Reznick et al., 1996a). This initial work was based on short-term (12 days) mark-recapture studies in a series of high- and low-predation communities. We have since applied these methods to mark recapture studies of approximately six months duration and found that the differences in recapture probability were magnified in the fashion that one would predict based upon the short-term studies. For example, the average probability of recapture after 12 days was approximately 90% in low-predation localities and 75% in high-predation localities (Reznick et al., 1996a). After six months, we instead obtained a 38 and 22% recapture rate from two low-predation sites and a 1–2% recapture rate from two high-predation sites (Fig. 1); these values are close to what one would predict if the mortality rates in the shorter term studies were constant throughout the longer intervals. Guppies from low-predation localities therefore have a 20- to 30-fold higher probability of surviving for 200 days.

Guppies from high-predation environments consistently mature at earlier ages, produce

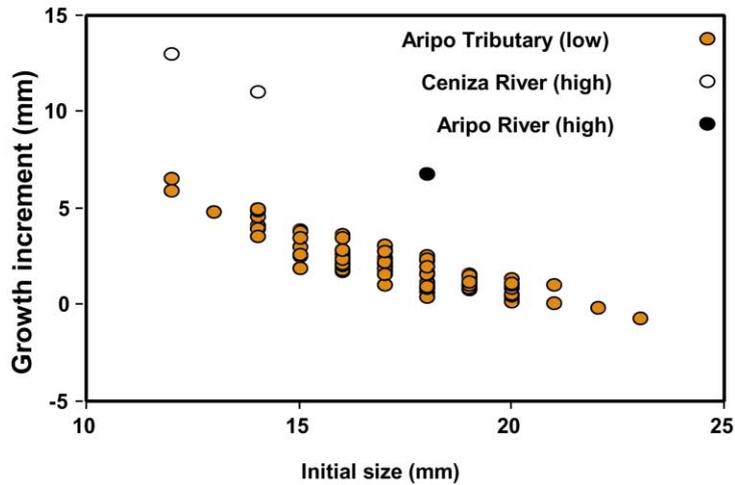


Fig. 1. Recaptures of marked individuals in three different mark-recapture studies. The x-axis represents the size class of the fish when they were originally marked. The y-axis represents the growth increment or the difference between the size when recaptured and the size when released. Fish were recaptured after approximately 6 months. The Ceniza and Aripo Rivers are high-predation localities. In each case, the probability of recapture was less than 2%. The Aripo Tributary is a low-predation locality. The probability of recapture was 38%. These results also illustrate the differences in growth rates of fish from these two different types of localities. The much larger growth increments for the representatives from high-predation sites is similar to the consistently higher growth rates that we have found in shorter-term mark-recapture studies.

more offspring per litter, and reproduce more often (Reznick and Endler, 1982; Reznick, 1989; Reznick et al., 1996b). These differences have a genetic basis (Reznick, 1982; Reznick and Bryga, 1996) and are consistent with predictions from life history theory (Gadgil and Bossert, 1970; Law, 1979; Michod, 1979; Charlesworth, 1980). Furthermore, we have manipulated the mortality rates of guppies with introduction experiments. Some drainages have barrier waterfalls that stop the upstream dispersal of all fish except *Rivulus hartii*. In two such streams, we introduced guppies from a high-predation locality below the barrier waterfall into the previously guppy free, low-predation site above the barrier waterfall. This introduction was first done by John Endler on a tributary to the Aripo River as part of his studies of the evolution of male color patterns (Endler, 1980). The second introduction was done by one of us (D.R.) on a tributary to the El Cedro River. In both cases, guppy life histories evolved very rapidly in the direction that is consistent with the earlier comparative studies and as predicted by life history theory (Reznick and Bryga, 1987; Reznick et al., 1990; Reznick et al., 1997). These results, in combination with our characterization of mortality rates, suggest that mortality has been a significant agent of selection on early life history traits in guppies. These results do not argue that mortality rate differences are the only cause of evolution; we are also characterizing the effects of density dependence and resource availability as factors that may also have shaped the evolution of guppy life histories (Grether et al., 2001; Reznick et al., 2001).

The factor that selects for the evolution of the early life history is also predicted to select

for changes in the rate of aging. First, guppies from high mortality rate environments mature earlier and have higher rates of reproductive investment early in life. Williams (1957) hypothesized that there is a cost to this early investment, paid in terms of an earlier onset of senescence (antagonistic pleiotropy). Second, these populations differ substantially in the probability of survival to an advanced age. Guppies from low-predation environments are much more likely to survive to advanced ages, so the effects of natural selection on late life performance are expected to be greater. Kirkwood and Holliday (1979) predicted that this difference in mortality rate will favor decreased investment in reproduction and greater investment in the maintenance of somatic tissues, relative to the high-predation localities. Medawar (1952) predicted that late acting, deleterious mutations will accumulate more readily in those populations that experience high mortality rates. All of these authors thus make the same prediction, which is that guppies from low-predation environments should have delayed senescence when compared to their counterparts from high-predation environments.

We are evaluating this prediction by comparing guppy populations from natural high- and low-predation environments that are known to differ in mortality rate and the early life history. Are there correlated differences in the late life history? Specifically, do guppies from low-predation environments, that have much higher natural life expectancies in nature, have delayed senescence in a predator-free laboratory setting? In addition to age-specific mortality rates, we are evaluating lifetime reproductive success, offspring quality, and physiological indices of aging. In the future, we will make similar comparisons among our experimental populations for which we have already documented the evolution of early life history traits. We are complementing this laboratory work with long-term demographic studies that will evaluate patterns of senescence in natural populations.

3. How to evaluate senescence?

Traditionally, senescence is evaluated as an age-specific acceleration in mortality rate (e.g. Finch et al., 1990). However, many authors have noted that it is important to consider some combination of reproduction and mortality. For example, Partridge and Barton (1996) argue that, if we are interested in senescence from an evolutionary perspective, then post-reproductive lifespan is irrelevant since everything that contributes to an individual's fitness has already occurred. Patterns of post-reproductive mortality are therefore of no consequence from an evolutionary perspective. The only exception to this argument would be if there is an extended maternal care after the last reproductive event (e.g. Hawkes et al., 1998; Packer et al., 1998). Partridge and Barton (1993, 1996) and Dudycha and Tessier (1999) have suggested indices of senescence that combine reproduction and mortality. A complete evaluation of senescence would thus entail evaluating age-specific changes in mortality rate and reproduction, then combining the two in a single measure. The resulting picture, particularly when comparing different populations, could be different from what you might infer from considering just age-specific change in mortality rate.

Partridge and Barton (1996) proposed using age-specific change in reproductive value

as an index of senescence. Dudycha and Tessier (1999) instead proposed the “...unweighted contribution of each age class to R_0 , the total lifetime reproduction” (Dudycha and Tessier, 1999). For our purposes, these arguments suggest that our comparative study of senescence should include an evaluation of lifetime reproduction and age-specific mortality. Doing so means being able to evaluate senescence from different perspectives and being able to see if conclusions about the comparative rates of senescence are influenced by the index chosen.

4. Pilot study

4.1. Methods

We have based the design of our large scale, comparative study on a pilot study of second-generation laboratory-born fish. The study was based on a small sample of fish retained from an ongoing study of the early life history, which focused on age at maturity and early life reproductive performance. A subset of these fish was maintained under the same conditions for their entire lifespans. The nature of the laboratory conditions is detailed in our earlier publications (e.g. Reznick, 1982; Reznick and Bryga, 1996). Fish were kept in individual, eight-liter aquaria so that we could follow their individual reproductive performance. Females were isolated when 25 days old, which is well before sexual maturity, then mated once per week until they began to reproduce. They were then mated for a day after the birth of each litter since females are especially receptive to mating at this time (Crow and Liley, 1979). Such repeated matings ensured that the female always had a good supply of viable sperm. These females received quantified amounts of food twice a day. There was a “high” and a “low” food treatment and there were six individuals in each treatment group. All of the fish were from the same low-predation locality. The dependent variables in the first study included:

- (1) the female’s age and size when each litter of young was born;
- (2) the number of offspring per litter;
- (3) the size (dry mass) and fat content of the offspring;
- (4) the female’s age at death;
- (5) post-mortem histopathology.

A consequence of recording the age at the birth of each litter is that we were also able to evaluate the regularity of reproduction by considering the time interval between each litter plus we were able to evaluate post-reproductive lifespan. The histopathology results were augmented with some first-generation laboratory-reared fish that were also kept in isolation for their entire reproductive lifespans. This latter group had not been mated until after they had attained sexual maturity and were maintained on unquantified amounts of food throughout their lives.

4.2. Results — life history traits

The sample sizes in this study were far too small to evaluate senescence as an

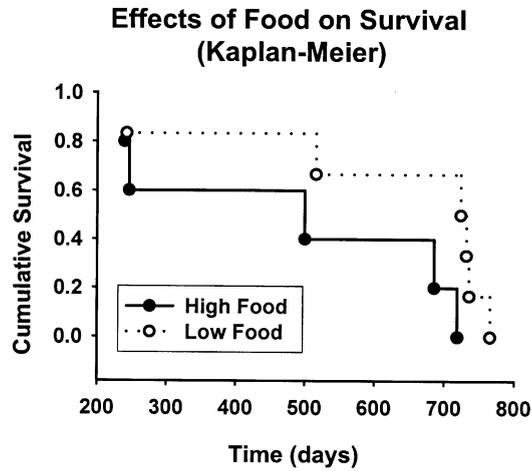


Fig. 2. Ages at death of guppies reared on high versus low levels of food availability in Pilot Study 1.

acceleration of mortality rate; however, they were large enough to reveal that females on low food rations tended to live longer than those on high food rations (Fig. 2). Caloric restriction has been found to extend lifespan in a large number of organisms (Finch, 1990). The low-food fish continued to reproduce with the same regularity as high-food fish.

The number of offspring in each litter initially increased to a plateau (Fig. 3). This pattern parallels individual growth, since size increased, then leveled off as the fish aged. This growth pattern paralleled resource availability, which we increased throughout the early life history, then kept constant thereafter. Fecundity is generally well correlated with body size (e.g. Reznick and Endler, 1982). Females on low rations initially gave birth to

Effects of Food and Time on Offspring Number

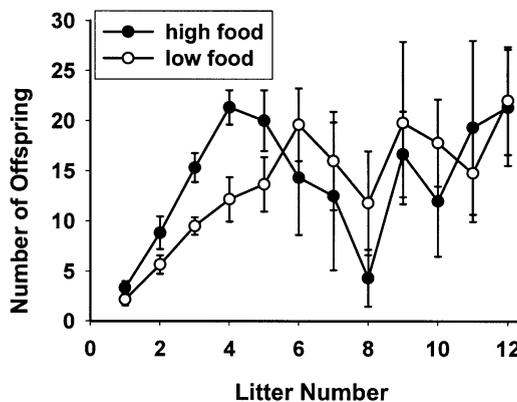


Fig. 3. Fecundity of high versus low food females in Pilot Study 1. Note that the sample sizes declined due to mortality as the litter number increased, so there is an increasing potential of bias in the mean values.

Effects of Food and Time on Offspring Weight

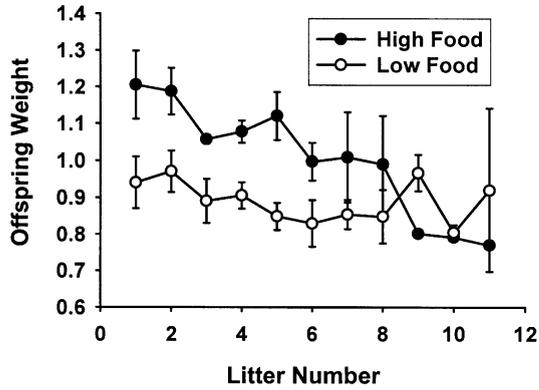


Fig. 4. Offspring weight (mg, dry weight) in high- versus low-food females in Pilot Study 1. Note that the decline in offspring size with advancing age was most evident in fish from the high food treatment.

fewer offspring per litter, which is similar to our results in all the earlier studies (e.g. Reznick, 1982). This trend remains even when differences in body size are accounted for with an analysis of covariance, but disappeared by litter 6. Thereafter, low-food females often had higher average fecundity than their high-food counterparts. The more interesting aspect of these results is that there was not an obvious decline in fecundity with age. It is important to note that there was some mortality, beginning with litter 6, so that the sample sizes became progressively smaller and potentially biased in subsequent litters.

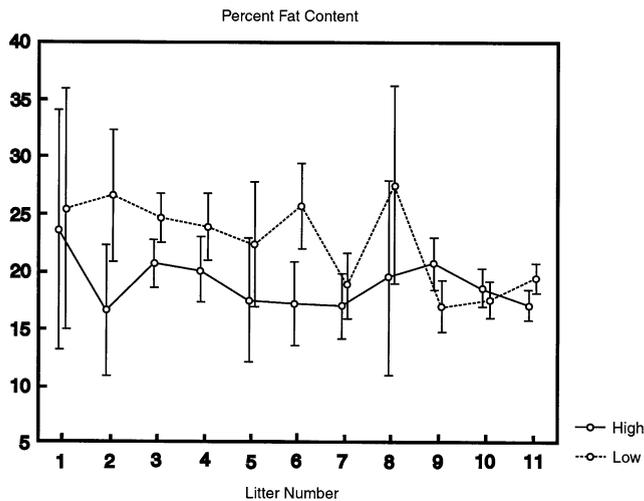


Fig. 5. Fat content (% dry weight that is ether-soluble fat) in offspring from females in the high- and low-food treatments in Pilot Study 1.

Variance in Inter-brood Interval Increases with Age

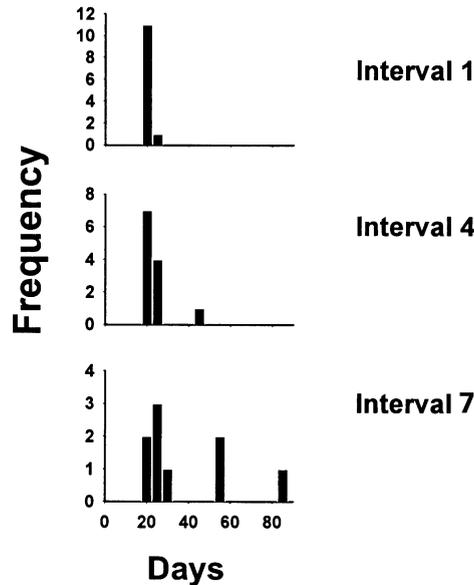


Fig. 6. Interbrood intervals (in days) for the first, fourth, and seventh intervals of all females in Pilot Study 1. Note the outliers in intervals 4 and 7 that have values that are approximately twice or three times the mean for the remaining data points. Such outliers appeared for the first time in the fourth interval and became more common as the fish grew older. The age when a female first skipped a litter was positively correlated with the age at death (see text).

There was a steady decline in the size of individual offspring, evaluated as dry mass, produced by older females (Fig. 4). Females on high-food rations initially gave birth to larger offspring, but offspring size declined more rapidly with age. This decline in offspring size was matched by a steady decline in the percent of dry weight that consisted of ether-soluble fat (Fig. 5); again, the decline was more steep in the offspring from females reared on high rations. Such a decline in offspring size and fat reserves might signal a decline in offspring quality since both size and reserves can enhance survival when resources are scarce (Reznick and Yang, 1993 and references therein).

We also found that there was a tendency for females to reproduce less regularly as they age. Such irregular reproduction is seen as an increase in the interbrood interval. Females normally reproduce on a regular cycle with the interval between litters being approximately 25 days. Beginning with the fourth litter, we began to see individual females whose interbrood intervals were statistical outliers because they were substantially longer than seen in the rest of the population (Fig. 6). These outlying intervals were often on the order of 50 or more days. We interpret such outliers as resulting from skipped litters or the failure of the female to initiate a new litter on schedule after the birth of the previous litter. Are such outliers an index of aging? We found that there was a positive correlation

Table 1

Distribution of pathologies in adults from the pilot aging studies (Group 1), young adults reared in the same environment as in the pilot aging studies (Group 2) and wild-caught adults from the same locality (Group 3). Significance is derived from chi-square tests

	Group 1		Group 2		Group 3		2 vs. 3	1 vs. 2
	–	+	–	+	–	+		
Glomerular nephritis	6	8	3	9	14	0	*	ns
Nephrocalcinosis	3	11	7	5	13	1	*	†
Renal tubular necrosis	10	4	12	0	14	0	ns	*
Renal interstitial inflammation	11	3	8	4	14	0	*	ns
Hepatitis	12	2	10	2	14	0	ns	ns
Oophoritis	8	6	12	0	14	0	ns	*
Hepatic vacuolization	8	6	8	4	14	0	*	ns
Granuloma	4	10	10	2	11	3	ns	*
Enteritis	5	9	11	1	13	1	ns	*
Melano-macrophage hyperplasia	2	12	11	1	14	0	ns	*
Pigment deposition	2	12	11	1	13	1	ns	*
Myocardial vacuolation	6	8	12	0	14	0	ns	*
Pleuroperitonitis	5	9	12	0	14	0	ns	*

* $p < 0.05$.

† $+0.05 < p < 0.10$.

between the age when a female first had such an extended interbrood interval and lifespan ($r = 0.554$; $0.05 < p < 0.10$). Said differently, females that first skip litters at an earlier age also tend to have shorter lifespans. This skipping of litters may thus be a component of the aging process.

Finally, we consider the relationship between age at last litter and age at death to see if there is a prolonged post-reproductive lifespan. There was a strong positive correlation between the age when a female last gave birth and the age at death ($r = 0.958$, $p < 0.01$). Eight of 12 individuals died within one interbrood interval of their last litter, which suggests that there is not a prolonged post-reproductive lifespan.

4.3. Histopathology

We compared three groups of fish to evaluate the relative incidence of pathologies in old fish. First, we considered fourteen of 30 fish from two pilot studies. Second, we evaluated 12 laboratory-reared adults from the same localities that had been preserved at earlier ages and while still apparently healthy. Finally, we evaluated 14 healthy, wild-caught adults from the El Cedro and Aripo Rivers. The purpose of the two control groups was to allow us to separate pathologies that were just associated with old age from those that might be associated with the laboratory environment. Laboratory-induced pathologies might be equally abundant in the old and young laboratory-reared individuals and more abundant than seen in the wild-caught fish. Pathologies that are strictly associated with age should be more abundant in just the laboratory-reared fish from the pilot aging studies. We note that

being more abundant in this class of fish is not by itself evidence that these pathologies are causes of mortality or associated with senescence. If they represent events that are equally probable in all age classes and if these events are recorded as some form of scar tissue, then they would become more common in older age classes. The nature of the pathologies is thus critical if we are to associate them with senescence.

All subjects had either recently died or had been humanely euthanized with an overdose of tricaine methanesulfonate (MS-222, Sigma Chemical, St. Louis) prior to fixation. The internal viscera were exposed via a ventral midline incision prior to fixation of the entire fish in 10% neutral-buffered formalin for a minimum of 48 h. Mid-sagittal sections of the entire fish were routinely processed and embedded in paraffin for sectioning. Tissue sections were cut to 5–6 μm and stained with hematoxylin and eosin (HE) for routine histological examination.

One of the 13 classes of lesions (hepatitis) did not differ in frequency among the three groups of fish (Table 1). The hepatitis was characterized as a mild, multifocal, chronic lesion that was not likely to be related to the morbidity and mortality in these fish.

Three classes of lesions (glomerulonephritis, renal interstitial inflammation, and hepatic vacuolation) were equally common in old and young laboratory fish and more common in these groups than in wild-caught fish, implying that they were in some way associated with the laboratory environment. Chronic glomerulonephritis (GN) is a common lesion in fishes and, as in higher vertebrates, is generally caused by exposure to antigens, especially infectious disease agents, with the subsequent deposition or localization of antigen–antibody complexes within the glomerular membranes. Although exposure to infectious disease agents is assumed to be more common in wild populations, factors such as water quality, density and various stressors may increase the susceptibility of captive fishes to GN. Renal interstitial nephritis is often a consequence of GN or may occur concurrently with GN. Hepatic vacuolization is consistent with a lipid hepatopathy due to the enlarged, single to multiple, discrete, vacuoles that are typical of lipid vacuoles. Lipid hepatopathy is relatively common in fishes and may be secondary to conditions that result in the catabolism of lipids for energy. Such conditions include inanition or anorexia associated with reproductive activity, nutritional deficiencies, morbidity, or any condition that results in a negative nitrogen balance. The cause of an association between lipid hepatopathy and the laboratory environment was not determined.

Of the remaining nine classes of lesions, eight (renal tubular necrosis, oophoritis, granuloma, enteritis, melano-macrophage hyperplasia, pigment deposition, myocardial vacuolation and pleuroperitonitis) were not significantly different between the young lab fish and the wild-caught fish but were significantly more common in the old lab fish than in the young lab fish. The ninth class (nephrocalcinosis) was significantly more common in the young lab fish than the wild-caught fish and was marginally significantly ($p = 0.055$) more common in the old lab fish than in the young lab fish. These nine classes of lesions represent targets of interest for future studies because they appear to increase in frequency with age and cannot be attributed to laboratory conditions alone. Among these lesions, five (oophoritis, coelomitis, pigment deposition, melano-macrophage hyperplasia, and granuloma formation) are common in older or mature fishes. Melano-macrophages are phagocytic

cells that accumulate various products of pigment catabolism and other antigens including particulate matter. Therefore, an excessive amount of pigment products or other antigens generally results in melano-macrophage hyperplasia. Pigment accumulation and melano-macrophage hyperplasia are more common in older fishes and/or with conditions such as inanition and anorexia, poor nutrition, or oxidative states that result in increased cellular degeneration and the production of degenerative cellular products. Likewise, the formation of granulomas occurs within focal accumulations of macrophages or hyperplastic foci of melano-macrophages. Therefore, pigment accumulation, melano-macrophage hyperplasia and granuloma formation are associated lesions. Chronic oophoritis is also a common finding in older or mature fishes that is generally associated with failure of ovulation or aborted development and resorption of ova/embryos. Extension of the ovarian inflammation commonly results in inflammation of the coelomic cavity and the visceral organs that may have been a partial explanation for the coelomitis in these fish.

Renal tubular necrosis (RTN) in fishes may be a consequence of hypoxia, toxicity, or renal inflammation, although none of these factors could explain the cause of the RTN in these guppies. Because RTN can also be a common post-mortem artifact of collection and preservation, it was not considered a likely cause of aging in these fish despite the statistical significance between the young and old laboratory fish. Likewise, the myocardial vacuolization was a mild lesion that was not a likely cause of mortality. The cause of myocardial vacuolization was not determined but may have been related to myocardial degeneration and/or the accumulation of lipids within the myocardium of older fish. The enteritis in these guppies was a mild to moderate, subacute to chronic lesion due to the infiltration of mononuclear inflammatory cells within the intestinal lamina propria and submucosa. The lesion is consistent with an exposure to enteric antigens, although infectious disease agents were not apparent in the tissue sections of the intestine. Nephrocalcinosis is a common finding in fishes that has been associated with factors such as dry feeds, feed binders, excessive selenium concentrations, excessive environmental carbon dioxide concentrations, magnesium deficiencies, and generalized mineral deficiencies. However, the cause of the condition in this study has not been determined.

The patterns in these results suggest that the old females had a chronic inflammatory syndrome affecting the enteric organs, potentially associated with the weight loss and atrophy experienced before death. Another weight loss related pathology was myocardial vacuolization. All of these pathologies are consistent with the suppression of the immune system due to an elevation of cortisol, as seen in the aging of other fish (Finch et al., 1990). They also have a high incidence of nephrocalcinosis, which could be an independent syndrome that caused the other pathologies.

In summary, our pilot studies did not reveal a strong decline in fecundity with age but did reveal that irregularity of reproduction might be a significant indicator of senescence. They also revealed that offspring size and fat content tend to decline with age, which may indicate a decline in offspring quality. Low-food females may have delayed senescence relative to high-food females because they tend to live longer, attain higher fecundities later in life, in spite of their continued lower food rations, and sustain a less dramatic decline in offspring size and fat content.

Table 2

Design of the comparative study of senescence. (1) Second generation laboratory-born females. (2) 100 each from high- and low-predation environments. (3) Smaller cohort of 20 each from high- and low-predation environments initiated nine months after the large cohort

Predation	High	Low
River drainage		
Oropuche River	High food	High food
	Low food	Low food
Yarra River	High food	High food
	Low food	Low food

5. Comparative studies of senescence in guppies from high- and low-predation environments

5.1. Methods

Our comparison of senescence in guppies from high- and low-predation environments has been in progress since January 1999. We report here on our methods and our progress through August 2000. The design and selection of sample sizes for our comparative study were based upon a power analysis of the pilot studies and logistical limitations. We are comparing the life histories of 100 females each from high- and low-predation environments, which is sufficient to detect relatively small differences in mortality rate. The allocation of fish to high- and low-predation sites is further subdivided. Two drainages with both high- and low-predation localities, the Oropuche and the Yarra, are represented with 50 females from each high- and low-predation site for a total of 200 individuals. Other studies indicate that there are substantial genetic differences between guppies from the two drainages (Shaw et al., 1991; Fajen and Breden, 1992). This pattern implies that the life history differences among high- and low-predation sites within each drainage evolved independently. The two drainages are thus likely to represent two independent examples of the evolution of senescence.

Partridge and Barton (1996) argue that studies of senescence must be executed in environments that are as similar as possible to those experienced in nature because the way organisms senesce may be a function of the environment. If populations respond differently to a change in any one feature of the environment, then the differences among populations and treatment groups would be influenced by the conditions under which they are compared. Guppies from high-predation localities have, on average, higher levels of resource availability than their counterparts from low-predation localities (Reznick et al., 2001). More abundant resources result in higher growth rates and larger asymptotic body sizes in guppies from high-predation environments. If the expression of senescence is influenced by resource availability and if populations differ in their response to resource availability, then our perception of differences among populations will be a function of the resource availability under which they are compared. We therefore added high and low levels of food availability to our design. We designed two different levels of resource

Table 3

Early life history traits for the large cohort in the comparative senescence study. The complete analyses included food availability, which had a significant impact on all life history traits. Fish on lower food were older and smaller at first parturition, tended to have longer interbrood intervals, and produced fewer offspring per litter. Because there was never a significant interaction between food availability and other factors, we present means that are averaged across food availability

Drainage	Predation	Age 1st parturition	Weight 1st parturition	Interbrood interval 1	# Offspring litters 1–3	Age 3rd Parturition
Yarra	High	82 days *	142 mg†	25 days *	19 *	129 days *
	Low	91	157	29	13	144
Oropuche	High	87 *	168 *	26 *	15 *	137 *
	Low	99	198	28	12	149

* $p < 0.05$.

† $+0.05 < p < 0.10$.

availability to mimic the growth rates and asymptotic body sizes seen in high- and low-predation environments. The full design is thus a three-way factorial comparison among guppies from high- and low-predation environments crossed with two drainages and crossed with two levels of food availability (Table 2), with 25 females represented in each of eight cells. If no interactions are present between the main effects, then high- and low-predation localities can be compared with almost no loss of statistical power. The experimental subjects are second generation, laboratory-born descendents of wild-caught females. They were reared under the same laboratory conditions as described in our earlier papers (e.g. Reznick and Bryga, 1987). Rearing for two generations in a common environment will remove environmental influences on the life history. Differences that persist after two generations in a common environment are assumed to have a genetic basis.

Because the evaluation of aging is inherently confounded with time and with any features of the laboratory environment that change over time, we have repeated this entire experiment on a smaller cohort of 40 individuals. This smaller cohort was initiated nine months after the larger cohort. Our intention is to evaluate whether or not age-specific trends change in parallel in the two groups, as opposed to changing as a function of time, independent of age. Parallel patterns of change and an absence of differences between the two cohorts would suggest that there was not confounding of temporal changes in the laboratory environment with age-specific changes in the guppies. Under such circumstances, we may be justified in lumping the two cohorts into a single set of analyses and hence increasing the statistical power of our design.

Our dependent variables will be the same as those in the pilot study, but will also include an assessment of offspring quality and an evaluation of a suite of physiological variables, including the C-start, acceleration, and maximum swimming speed. We are assessing offspring quality by rearing four offspring from each female on controlled food for 28 days so that we can evaluate survival and growth rate. This assay was added because our pilot study suggested that offspring quality might decline as the mother ages. Physiological variables were added because they provide an additional assessment of senescence. Offspring quality and physiological variables are being assessed approximately every six months.

5.2. Preliminary results

First, we consider a range of variables that characterize the early life histories of these fish (Table 3). We found that the guppies from high-predation localities attained maturity at an earlier age and smaller size than their counterparts from low-predation localities. They also had shorter interbrood intervals, based upon their first three litters, plus gave birth to more offspring in their first three litters. All of these differences correspond to the results of our prior comparisons of guppies from high- and low-predation environments. These patterns thus show that the differences in the early life history are repeatable. They also establish the rationale, based on Williams' (1957) antagonistic pleiotropy theory of aging, for predicting differences in the late life history. The premises for our comparative study of aging are thus satisfied.

At the present time, over 80% of the fish are still alive and actively reproducing, so it is too early to present an appropriate statistical comparison of senescence. We will instead

Predicted Trend in Fitness Trait versus Age

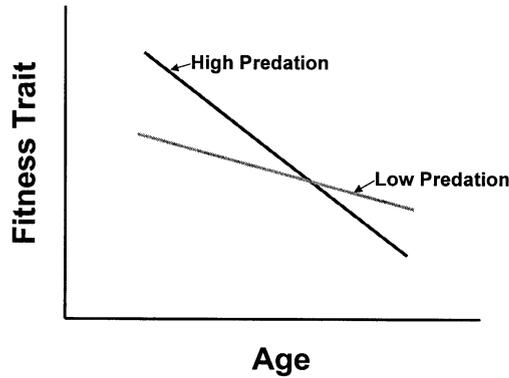


Fig. 7. The predicted pattern of results if guppies from high-predation sites senesce more rapidly than those from low-predation sites. Senescence is interpreted as a decline in fitness related variables with age. Theory predicts that this decline should be more rapid in guppies from high-predation environments.

present some preliminary analyses that depict the current trends in the results. What is summarized is thus a progress report on an ongoing study. We expect that components of fitness will decline as individuals age and that such declines will be characteristic of guppies from all locations. Our hypothesis predicts that the rate of decline will be higher and hence the onset of senescence earlier and more rapid, in guppies from high-predation

Mean Number of Offspring vs. Litter Number

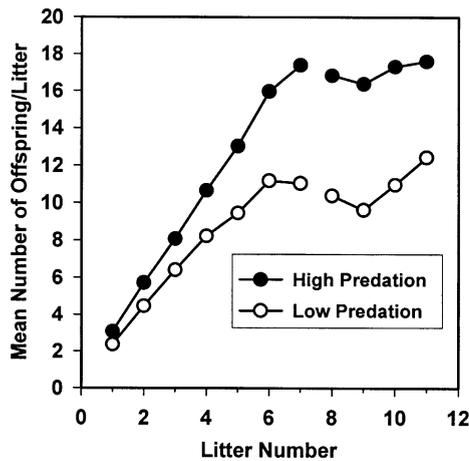


Fig. 8. Age-specific fecundity in females derived from high- and low-predation environments. The means for the first seven litters are derived from a formal repeated measures analysis of variance. The means for litters 8–11 are sample arithmetic means and are included here just to illustrate the continuing trends in the data.

Cumulative Number of Offspring vs. Age of Female

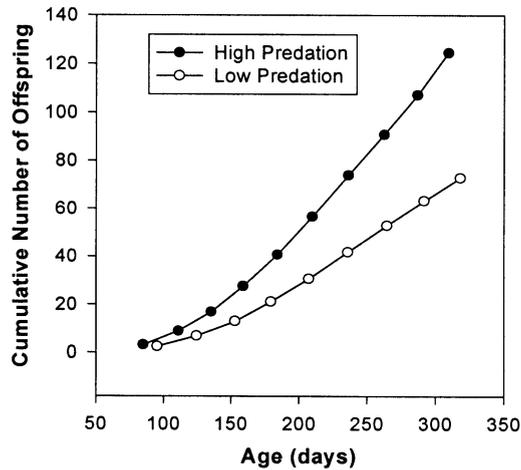


Fig. 9. Cumulative fecundity of females from high- and low-predation localities. Note that the higher fecundity in females from the high-predation sites is greater for two reasons: they produce more young per litter plus they produce litters more often. For the time period illustrated, females from high-predation localities produced 10 litters as compared to nine for females from low-predation localities.

localities (Fig. 7). In the final analyses, these differences in rate will be evaluated as interactions between high- versus low-predation and age.

The trends in fecundity (Fig. 8) are very similar to those in the pilot study. Fish from both types of localities produced progressively larger litters of young through litter 6, then litter size plateaued. These increases in litter size parallel the growth of individual fish. The ration levels were increased until the fish were five months old and remained constant thereafter. When the rations no longer increased, growth rate declined and litter sizes plateaued. The proportional differences in fecundity between high- and low-predation localities are fairly constant through the first 11 litters. There is thus no evidence that fecundity is declining with age in either of the treatment groups. Note that the means for the first seven litters are derived from a formal repeated measures analysis of variance, while those for the next four litters are simply means reported to illustrate the continuing trends in the data. The trends in the data are not consistent with predictions concerning differences between guppies from high- and low-predation environments. In fact, if anything, they appear to be the opposite of predictions, since the high-predation guppies have continued to produce more offspring per litter. This difference is more dramatic if we instead consider the cumulative number of offspring produced by the different types of females (Fig. 9). At an age of approximately 350 days, the high-predation females have produced almost twice as many offspring as their low-predation counterparts, in part because they produce more offspring per litter and in part because they have produced an average of one more litter. They have produced an additional litter because they began having young at an earlier age with shorter time intervals between litters.

Mean Offspring Weight vs. Litter Number

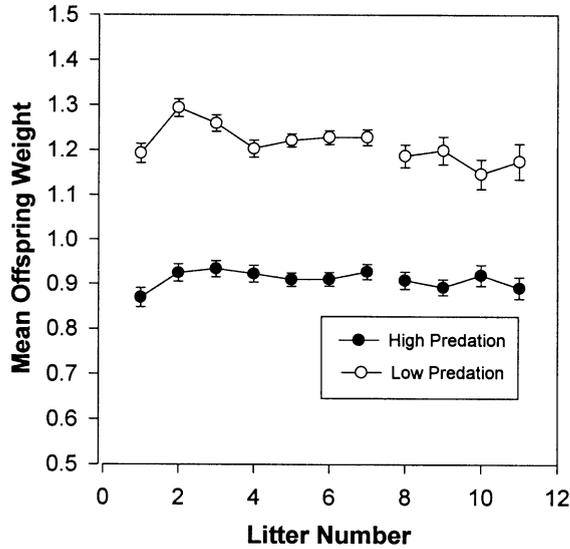


Fig. 10. Mean offspring weight (mg, dry weight) for offspring from high- versus low-predation localities. Note that the means for the first seven litters are derived from a formal, repeated measures analysis of variance while those for litters 8–11 are simple means, included here to illustrate continuing trends in the data.

Percent Fat of Offspring vs. Litter Number

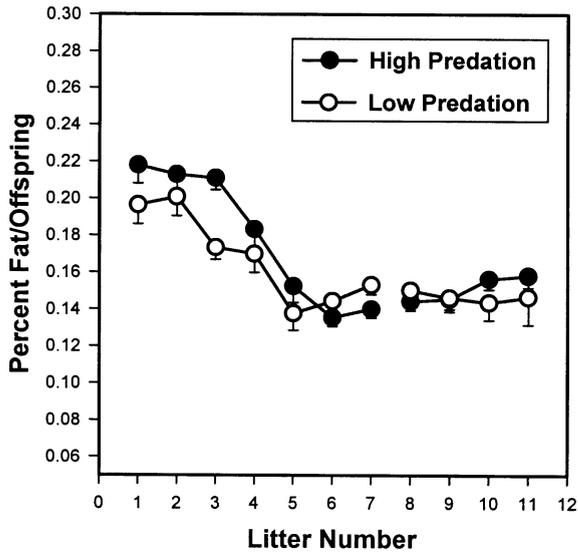


Fig. 11. Percent fat ((%) ether soluble dry mass) in offspring from high versus low mortality rate environments.

Table 4
Forms of product failure

	High predation	Low predation	Total
<i>(a) Skipped litters</i>			
Oropuche	9	14	23
Yarra	4	13	17
Total	13	27	
<i>(b) Mortality</i>			
Oropuche	0	8	8
Yarra	8	11	19
Total	8	19	

There was a tendency for the size of individual offspring to increase over the first few litters, then to remain relatively constant thereafter (Fig. 10). The means for offspring weight in litters 8–11 are slightly smaller than those for litters 1–7 in both types of localities, but the decline with age is much less dramatic than in the pilot study. The guppies from low-predation environments consistently produce larger offspring, which is the same trend as in our earlier comparisons of guppies from high- and low-predation environments. The difference in offspring size in females from the two types of localities is not changing over time.

Finally, we consider the composition of the offspring, which is evaluated solely in terms of their fat content at birth. As in the pilot studies, fat content declines with age (Fig. 11), but the trends so far do not indicate a difference between high- and low-predation localities in the rate of decline.

A second perspective on senescence involves traits that are discontinuously distributed; we refer to these indices of senescence as “product failure”. The first such index is irregular reproduction, which involves either the apparent skipping of litters or the complete cessation of reproduction (Table 4a). Here we are finding that there is a difference among rivers with the Oropuche fish being more likely to skip than the Yarra fish. The more interesting result is that the low-predation fish are more likely to have developed irregular reproduction than the high-predation fish. This trend is the opposite of what is predicted on the basis of standard life history theory.

The second form of product failure is mortality (Table 4b). Here, again, there is a difference among river drainages with the Yarra fish being more likely to die than the Oropuche fish. We also found that the low-predation fish were more likely to die than high-predation fish. Again, this trend is the opposite of our prediction for high- versus low-predation localities.

It is important to realize that the final analyses of all of these data when the experiment is complete will be different from those reported here. Continuous variables will be presented as formal, factorial analyses of variance with planned comparisons for interactions. Mortality will instead be evaluated as age-specific changes in mortality rate and the inference of senescence will be based on age-specific increases in mortality rate. Indices of aging that combine reproduction and mortality will also be considered.

In conclusion, natural populations of guppies experience consistent differences in

mortality rate. Mortality rate selects for earlier maturity and an increased rate of investment in reproduction. Evolutionary theory predicts that high mortality rate populations should also experience earlier senescence. The results of the first year plus of the comparative study are not consistent with this prediction. There are as yet no differences among high- and low-predation guppies in the age-specific pattern of change in continuous traits. The more compelling trend in our data is that low-predation guppies are more likely to skip litters or die.

It is still far too early in our work to conclude anything about the differences in senescence among guppies from high and low mortality rate environments. For that matter, it is also too early to characterize how senescence is manifested. While there are some specific and testable hypotheses for why the results might come out in a fashion that is the opposite of predictions (e.g. Abrams, 1993), we feel that it is premature to consider them at this point because it is so early in the aging process. We instead remind you that this is a work in progress and counsel patience.

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