MODELING OF SEMELPAROUS / ITEROPAROUS POLYMORPHISM
IN BOTRYLLUS SCHLOSSERI

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER'S OF SCIENCE

in
THE FACULTY OF GRADUATE STUDIES
MATHEMATICS
INSTITUTE OF APPLIED MATHEMATICS

We accept this thesis as conforming
to the required standard

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C. U. Clark
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The University of British Columbia
September 1991
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Abstract

This thesis describes and models the age of first sexual reproduction in *Botryllus schlosseri*. *B. schlosseri* is a sessile, colonial fouling organism that lives mainly in the low intertidal zone of temperate waters. In Eel Pond, Woods Hole, Massachusetts, semelparous (reproduces only once) and iteroparous (reproduces several times) morphs apparently co-exist.

A survey of previous life-history models is given, but none of them can be used very effectively on *B. schlosseri*. Instead, a dynamic programming model is presented that models, with a good fit to field data, the age of first sexual reproduction in each morph separately. The model makes several predictions. First, the life history characteristics, particularly the age of first sexual reproduction, are near equilibrium. Secondly, the growth reduction after winter affects the optimal age of sexual reproduction throughout the year, suggesting that colonies can tell the time of year through the water temperature or the photoperiod. Thirdly, the primary cause of variance in the age of first sexual reproduction is the spatial variation in the environment. Finally, iteroparous colonies continue reproducing every generation once they begin, not necessarily due to a physical constraint, but simply because it is optimal for them to do so.

Next, the coexistence of the morphs was looked at. An important factor is *Botrylloides leachi*, a closely related competitor, that in the summer and early fall overgrows semelparous colonies but not iteroparous colonies. However, since *B. leachi* was only introduced into Eel Pond about 30 years ago, an interesting question is whether the current conditions (i.e. coexistence) is stable or not. A survey was taken of previous models that look at the coexistence of species (the two morphs are treated as separate species). Although these models indicated that the two morphs can co-exist, none of
them are accurate enough to be able to confidently determine if the coexistence is stable. Although a dynamic programming model would be precise enough, too much unavailable data would be required.
Table of Contents

Abstract ii

List of Tables vii

List of Figures viii

Acknowledgement x

1 Introduction 1

2 *Botryllus schlosseri* 2

2.1 Basic Morphology 2

2.2 Semelparous/Iteroparous Polymorphism 5

2.2.1 Cause of Polymorphism 6

2.3 Growth 11

2.3.1 Substratum Size 12

2.4 Self/nonself-recognition and Fusion 13

2.4.1 Inbreeding Considerations 15

2.5 Senescence 15

2.6 Mortality 15

2.6.1 Predation 17

2.7 Life-History Correlations 18

2.7.1 Semelparous Colonies 18

2.7.2 Iteroparous Colonies 18
2.7.3  Semelparous/Iteroparous Colony Differences  
2.7.4  F₁ Correlations with Parents  
2.7.5  Energy Reallocation  
2.8  Competition  
   2.8.1  Botrylloides Description  
   2.8.2  Competitive interactions  
   2.8.3  Coexistence of Polymorphism  

3  Age of sexual reproduction in B. schlosseri  
   3.1  Comparative Studies  
   3.2  Genetic Models  
   3.3  Life-History Theory  
      3.3.1  The Characteristic Equation  
      3.3.2  Discrete forms of Characteristic Equation  
      3.3.3  Principle of Allocation  
      3.3.4  Fisher’s Reproductive Value  
      3.3.5  Cole’s Model and Extensions  
      3.3.6  Stochastic Models  
      3.3.7  Discussion  
   3.4  Dynamic Programming  
      3.4.1  Definition and Assumptions  
      3.4.2  Solution Techniques  
      3.4.3  Advantages and Disadvantages  
   3.5  The Model  
      3.5.1  Background Information and Assumptions  
      3.5.2  Equation and Parameter Values  

v
3.5.3 The Model Cases ........................................... 70
3.5.4 Density and Reproduction Amounts ......................... 88
3.5.5 Lack of Time Dependent Mortality .......................... 91
3.5.6 Conclusions and Possible Enhancements ....................... 91

4 Modeling of Semelparous/Iteroparous Coexistence ............... 94
  4.1 Previous Modeling ......................................... 94
    4.1.1 Lotka-Volterra Equations .............................. 94
    4.1.2 Gause(-Volterra) Principle ............................ 95
    4.1.3 Interference Competition .............................. 96
    4.1.4 Spatial Heterogeneity ................................ 97
    4.1.5 Temporal Variation .................................. 97
  4.2 Dynamic Programming ...................................... 99
    4.2.1 Environmentally Stable Strategies (ESS) .............. 99

5 Discussion .................................................. 101

Bibliography .................................................. 102
List of Tables

2.1 Life table of September 1-15 iteroparous colonies .................. 16

3.2 Temperature and generation length schedule....................... 53

3.3 Approximate dates for the end of each generation.................. 54
List of Figures

2.1 Habit sketch of *B. schlosseri* on glass ........................................ 4
2.2 Bivariate plots based on field data. ................................................. 7
2.3 Frequency distributions of four life-history traits. ............................ 8

3.4 The proportion of a cohort that survives from settlement to sexual maturity. 40
3.5 Growth trajectories of eight cohorts. .................................................. 50
3.6 *Mean duration of an asexual generation and temperature.* .................. 51
3.7 Seasonal mean daily settlement of *B. schlosseri* ............................... 52
3.8 Frequency distributions of age at first sexual reproduction. .................... 67
3.9 Field data summaries of Fig. 3.8 ..................................................... 68

3.10 Iteroparous first sexual reproduction age cases (a) to (f) with \( k = .001 \) .... 71
3.11 Semelparous first sexual reproduction age cases (a) to (f) with \( k = .001 \) .... 72
3.12 Iteroparous first sexual reproduction age cases (g) to (l) with \( k = .001 \) .... 73
3.13 Semelparous first sexual reproduction age cases (g) to (l) with \( k = .001 \) .... 74
3.14 Iteroparous first sexual reproduction age cases (m) to (r) with \( k = .001 \) .... 75
3.15 Semelparous first sexual reproduction age cases (m) to (r) with \( k = .001 \) .... 76
3.16 Iteroparous first sexual reproduction age cases (a) to (f) with \( k = .002 \) .... 77
3.17 Semelparous first sexual reproduction age cases (a) to (f) with \( k = .002 \) .... 78
3.18 Iteroparous first sexual reproduction age cases (g) to (l) with \( k = .002 \) .... 79
3.19 Semelparous first sexual reproduction age cases (g) to (l) with \( k = .002 \) .... 80
3.20 Iteroparous first sexual reproduction age cases (m) to (r) with \( k = .002 \) .... 81
3.21 Semelparous first sexual reproduction age cases (m) to (r) with \( k = .002 \) .... 82
3.22 Iteroparous colony density and rep. amounts ................. 89
3.23 Semelparous colony density and rep. amounts ................. 90
3.24 Age of first sexual reproduction without time dependent mortality .... 92
Acknowledgement

First, I would like to thank my advisor, Dr. Colin Clark, for his patience with how long it took for me to write a thesis and for my continually bypassing my own deadlines. Next, Dr. Don Ludwig helped by giving me a little nudge when things slowed down too much for me, and also helped me present a lot of graphic information in an intelligible fashion. Dr. Brian Seymour deserves thanks for taking on the task of being the second reader of this thesis on short notice. Last, but definitely not least, I owe most of the background and data in this thesis to the PhD thesis of Dr. Richard Grosberg. Also, Dr. Grosberg has my thanks for giving me some help and advice despite how busy he is at U.C., Davis.
Chapter 1

Introduction

Among living organisms, the most common, basic, and important function is reproduction. A fundamental classification is whether the organism reproduces only once (semelparity) or several times (iteroparity). A clonal organism, *Botryllus schlosseri*, has the unusual distinction of having one genetic morph that is semelparous and one that is iteroparous.

The next chapter will discuss the basic biology and life-history characteristics of *B. schlosseri* in Eel Pond, Woods Hole, Massachusetts where the two morphs apparently co-exist. Chapters 3 and 4 attempt to explain some of these characteristics using life-history models. Chapter 3 examines modeling the optimal age of first sexual reproduction for each morph. First, previous models are investigated, but none are sufficiently general to accurately handle *B. schlosseri*. Instead, a dynamic, state-based model is presented and solved using the dynamic programming technique. Chapter 4 studies the co-existence of the two morphs. Previous models show that co-existence is possible, but none account for all of the important factors affecting possible co-existence. As in chapter 3, a dynamic, state-based model is required. However, problems with the model complexity and lack of required data prevent giving and solving a model. Finally, chapter 5 provides discussion and conclusions about the models discussed.
Chapter 2

*Botryllus schlosseri*

This chapter describes the characteristics of the colonial ascidian *Botryllus schlosseri*. It discusses the basic biology, two life-history morphs, growth, sexual reproduction, senescence, mortality, and competitive interactions among the two morphs and the closely related species *Botrylloides leachi*.

*Botryllus schlosseri* (Phylum Chordata; Subphylum Tunicata: Class Asciacea: Order Pleurogona: Suborder Stolidobranchia: Family Styelidae) is a fouling organism that lives mainly on hard substrata in the low intertidal zone in protected waters. In more exposed locations, it dwells in secure areas like the undersurface of rocks. The species is limited to temperate waters of the Atlantic Ocean, the Mediterranean, the Adriatic, the Black, and the Baltic Seas (Grosberg 1982, 1987). This thesis concentrates on the studies performed at Eel Pond in Woods Hole, Massachusetts.

2.1 Basic Morphology

Colonies of *Botryllus schlosseri* are founded by a sexually produced swimming tadpole larva (about 1.2 mm long) which settles onto any hard surface such as rocks, algal blades, floating docks, or even the surface of another ascidian (Grosberg 1982). The larva does not feed, but a metamorphosis occurs producing the first zoid of the colony, the oozoid, which filter feeds using a siphon. Small protrusions appearing on the oozoid become the palleal buds, the primordia of the asexually produced blastozoooids (to distinguish them from the founder zoid, the oozoid). The blastozoooids bud off still more zooids.
Chapter 2. Botryllus schlosseri

(the primary buds). The primary buds have palleal buds (called the secondary buds) (Grosberg 1988). The blastozoids are morphologically identical to one another and, apart from the presence of gonads, are also almost identical to the oozoid. Due to asexual reproduction (called blastogenesis) occurring synchronously among all zooids in a colony (Milkman 1967; Sabbadin 1971), there is a concurrent maturation of zooids to the next stage (i.e., blastozoids being resorbed, primary buds becoming blastozoids, secondary buds becoming primary buds, and a new set of secondary buds forming). This process is termed takeover. The synchronous cycle is called an asexual cycle or generation (Grosberg 1982). Each generation thus behaves as a single individual since the zooids originate at the same time (Sabbadin 1977). The length of the asexual generation varies inversely with the water temperature (Sabbadin 1955, 1958 cited in Grosberg 1988) except during colony starvation (Grosberg 1982).

The oozoid secretes a tunic (or test) which consists of proteins and carbohydrates. Embedded in the tunic, the zooids are connected to each other by a complex vascular system. The zooids use the blood-vascular system to remain in physiological communication. The blood circulation, among other functions, synchronizes the asexual cycle (Sabbadin and Zeniolo 1979; Sabbadin 1977). The vessels terminate along the periphery of the colony in distally expanded dead-end vessels termed the ampullae. The ampullae serve several purposes: (1) they regulate blood pressure; (2) they allow the colony to move along and adhere to the substratum; (3) they play a crucial role in recognition of contacting organisms; (4) they may function to allow or prevent overgrowth of one colony by another (Grosberg 1982).

The Botryllus colonies are of irregular shape. They may be over a foot in diameter, although they are usually much smaller. When small or medium colonies have ample space, they grow isometrically with a circular encrusting surface (Brunetti 1974). However, most colonies (Fig. 2.1) are composed of rosette-like systems (called "pendants")
Figure 2.1: Habit sketch of *B. schlosseri* on glass. This caption and figure is from Fig. 1 in Milkman, 1967.

of generally 5-18 blastozoids with each pendant being like a solitary ascidian in form (Milkman 1967; Brunetti 1974). The pendants are connected to each other via the vascular system (Grosberg 1982). This level of organization makes *B. schlosseri* one of the most highly integrated ascidian colonies (Milkman 1968).

After five to ten asexual generations, a colony reaches sexual maturity. The sexual reproductive cycle is synchronized with the asexual cycle. However, in a process called protogyny, ovaries with mature ova appear first with the testes ripening synchronously across the colony several days later. Thus, the rapid fertilization of ova is presumably by

Grosberg (1982) contains more detailed descriptions of the colony morphology. In the next section, the presence of two different morphs will be described and discussed.

2.2 Semelparous/Iteroparous Polymorphism

Two distinct clusters of observations are present in bivariate plots (Fig. 2.2) of first sexual reproduction age (in asexual cycles), number of clutches, number of buds per zooid per asexual cycle, and number of embryos per zooid per clutch. The distribution remains weakly bimodal even for the later first reproduction age of members of the last cohort that fail to reproduce until the following spring. The two types are termed semelparous and iteroparous as per the classification of Cole (1954) on the basis of the number of clutches. Semelparous colonies are characterized by: (1) death immediately following the production of a single clutch; (2) early first sexual reproduction age (at the fifth or sixth asexual generation); (3) rapid growth to first sexual reproduction (at rates approaching 4.5 buds per zooid); and (4) high reproductive effort (10-12 embryos per zooid in the clutch). Iteroparous colonies, however: (1) produce at least three clutches before dying; (2) postpone sexual reproduction until they are almost twice the age of semelparous colonies (at least nine asexual generations); (3) grow at about half the rate of semelparous colonies (at rates between 1.5 and 2.5 buds per zooid); and (4) invest roughly 75% less in reproductive effort than semelparous colonies (between two and four embryos per zooid). Only rarely do morphs intermediate in characters appear. The relative abundance of the iteroparous and semelparous phenotypes depends on the season as shown in Fig. 2.3. Early in the summer, most colonies are of the semelparous type while by midsummer the two phenotypes are almost equal and by the end of the settlement season iteroparous
type colonies predominate (Grosberg 1982, 1988).

2.2.1 Cause of Polymorphism

The two morphs are interfertile since semelparous and iteroparous colonies have been successfully cross-bred (Grosberg 1982). This interfertility plus the seasonal change of abundance suggests that the polymorphism is of a phenotypic nature. Two potential sources of environmentally induced variation are:

1. The parental environment - does the parental environment influence the phenotypes of F1's?

2. The F1's environment - is the phenotype determined solely by the progeny's environment during development?

Likewise, three potential contributions to genetically determined variation are:

1. the maternal genotype

2. the paternal genotype

3. the F1 genotype

To determine the relative influence of phenotypic and genotypic factors, Grosberg (1982) performed several laboratory experiments. These experiments involved a series of defined single-pair crosses among several strains of Botryllus schlosseri with parents and progeny reared under a variety of environmental conditions. Seven traits were recorded for each of the parental strains and their progeny: (1) first sexual reproduction age (in days and asexual generations); (2) size at first sexual reproduction; (3) number of clutches; (4) mean number of embryos per zooid per asexual generation (before sexual
Chapter 2. Botryllus schlosseri

Figure 2.2. Bivariate plots based on field data showing the relationships among 1) age at first reproduction, 2) number of clutches, 3) number of embryos per zoid per clutch, 4) number of buds per zoid, and 5) age at first reproduction, 6) number of clutches, 7) number of embryos per zoid per clutch, 8) number of buds per zoid. Numbers in parentheses represent overstrides. This figure is taken from Groberg, 1988.
Figure 2.3: Frequency distributions of four life-history traits: 1) age at first sexual reproduction (asexual cycles), 2) number of clutches, 3) number of buds per zoid per asexual cycle (growth rate), and 4) number of embryos per zoid per clutch (reproductive effort). The distributions are based on colonies that recruited in the field during the intervals shown in a-h. Solid bars represent data from 1979 cohorts; open bars represent data from 1980 cohorts. This caption and figure is taken from Fig. 1 in Grosberg, 1988.
reproduction); (5) the mean number of embryos per zooid per clutch; (6) colony life span (in days and asexual generations); (7) the total reproductive output of each colony.

Crosses between like phenotypes produced progeny with phenotypes like the parents and crosses between semelparous and iteroparous parents produced roughly equal fractions of F₁'s with high and low reproductive effort. There were only two exceptions to this: over the five replicates of semelparous colonies (1-5) and five replicates of iteroparous colonies (6-10): (1) in crosses among semelparous colonies with strain 4 as one of the parents, an occasional iteroparous progeny appeared, and likewise in crosses among iteroparous colonies involving strain 9 an occasional semelparous progeny appeared; (2) crosses between strains 4 and 9 produced the only 6 intermediate F₁ phenotypes detected.

To determine if the life-history type of a particular genotype persisted in a variety of constant controlled conditions, Grosberg (1982) performed a factorial experiment in which he manipulated two factors: temperature and density of food. There were three levels of each factor, thus nine treatments in all. The three temperatures were 15, 20, and 25 °C which encompasses the in situ temperature range during the reproductive season of Botryllus schlosseri. Colonies were fed a commercially manufactured synthetic diet (Marine Invertebrate Food). The food seemed acceptable since at the middle and high feeding levels used, colonies grew as rapidly in the laboratory as in the field. The three feeding levels were: (a) low (1×) - 0.1 ml of Marine Invertebrate Food per 1000 zooids every other day, (b) medium (2×) - double the low level, and (c) high (3×) - triple the low level. Replicates of five semelparous and five iteroparous colonies were used in each of the nine combinations of temperature and food levels. Despite the range of the conditions, none of the genotypes changed phenotypes. The analysis of variance shows that regardless of the culturing conditions, all semelparous colonies remain distinct from all iteroparous genotypes for all seven life-history traits mentioned above. In addition, under the constant laboratory conditions, no significant differences arose among genotypes
(nested within life-history type).

A limited number of $F_1$ backcrosses and $F_2$ crosses were made at 20 °C and feeding level 2×. $F_1$ backcrosses between semelparous progeny and parents uniformly produce semelparous progeny and likewise for iteroparous colonies. Similarly, crosses among phenotype $F_1$'s yield $F_2$'s that are phenotypically equivalent to their parental $F_1$'s. Crosses between unlike $F_1$ phenotypes produce mixed clutches with, on average, roughly equal numbers of semelparous and iteroparous progeny. Matings among the rare intermediate phenotype $F_1$'s and between these $F_1$'s and their parents (strains 4 (semelparous) and 9 (iteroparous)), do not result in an increased frequency of intermediate phenotypes, at least not over the span of the few generations of crosses performed.

The factorial experiment of temperature and food levels described above implies that the polymorphism is not due to the $F_1$'s environment, and the back-cross results imply that the polymorphism is not due to the parents’ environment. On the other hand, all three potential contributions to genetically determined variation have an effect. It is possible that a single locus (or a few tightly linked loci) pleiotropically controls the life-history polymorphism through developmental switching or a regulatory locus. However, even if such a locus does exist, the $F_1$ data show that it does not obey simple Mendelian inheritance. Another possible cause for this genetic effect is gene dosage whereby just simply the number of genes, regardless of their location, determines the phenotype (Grosberg 1982). Then the determination of semelparity and iteroparity may depend on the number of certain genes at several to many loci. Whether above or below a certain threshold, the phenotypic expression of the genotype is canalized so that a variety of sub-threshold combinations produce nearly identical phenotypes (Rendel 1967). This argument is consistent with some of the life-history inheritance patterns among $B. schlosseri$. For instance, if most semelparous and iteroparous colonies have dosages well above or below threshold, then most progeny from within life-history type crosses will
also be clearly above or below threshold. This is not unreasonable due to the predominance of semelparous morphs during the spring and of iteroparous during the fall making mid-summer the only time when there would likely be a significant number of crosses. Most of the resulting progeny from the crosses would be above or below the threshold, although some may be very close. Strains 4 and 9 (mentioned in the experimental results above) may represent these marginal individuals. When a colony that is near threshold dosage is mated with a colony that is well away from threshold, one would predict that relatively more progeny would be phenotypically like the parent away from the threshold. This would explain the lower proportions of like phenotypes produced from crosses involving strains 4 and 9. It would also account for the F₁ backcross results. However, the presence of intermediate phenotypes remains difficult to explain (Grosberg 1982). Assuming that the intermediate phenotypes result from being essentially at the threshold level, one would expect a larger proportion of F₂ intermediate phenotypes when the F₁ intermediate phenotypes were mated among themselves or with their parents. This, however, was not the case in Grosberg’s experiments.

In the next sections, the various life-history parameters will be discussed, starting with colony growth.

2.3 Growth

Colonies can increase their size in two ways. They may secrete tunic or they may produce zooids at a rate greater than the zooids are lost. Secretion of tunic only increases the surface area of the colony. Most of the growth is due to the addition of blastozooids. Thus, “growth rate” is best described in terms of the number of zooids per blastozooid per asexual generation. Various researchers (e.g. Brunetti 1974; Boyd et al. 1986) have noted a large variability in growth rate, although this could be due mostly
to the different life-history types. During the ‘change of generation’, some buds could be resorbed together with their parent so the total number of zoonids of a colony can either increase, remain constant or decrease (Brunetti 1980). However, growth-rate data (Grosberg 1982; Yamaguchi 1975; Brunetti 1974) suggest that growth rates per individual zoonid is constant, resulting in exponential colony growth, until the start of sexual maturity. Nonetheless, Brunetti and Copello (1978) found that the maximum growth is between the 2nd and 4th asexual generation with the rate then falling until it stabilizes around a constant value. Although they do not present any values, I assume that this effect is minimal. The abundance of the colonies and the exponential growth means that during the summer and fall the colonies effectively cover all surfaces. By mid-July in Eel Pond, there is virtually no open space on hard substrata, with Botryllus schlosseri coverage at 50-100% (Grave 1933; Grosberg 1982). This means that often colonies will lack substratum space and/or encounter another colony. The next section discusses the semelparous/iteroparous responses to this situation.

2.3.1 Substratum Size

Grosberg (1982) manipulated the substratum size to study its influence on growth and sexual reproduction. For semelparous colonies, substratum size affected neither first sexual reproduction age nor reproductive effort. With insufficient substratum space, the colony will throw itself into convolutions until it reaches a size of \( \approx 200 \) zoonids before sexually reproducing. However, when the colonies are so convoluted, they may pry themselves off the substratum and eventually die. A larger substratum results in only a minimal increase in size at sexual maturity. Sexual maturity size for iteroparous colonies, however, increases in roughly direct proportion to substratum size, except that colonies will initiate sexual reproduction when they are \( > \approx 800 \) zoonids even if they have not reached the bounds of the plate (Harvell and Grosberg 1988). Notably, the age of
first sexual reproduction is not affected by the substratum size. Instead, the confined iteroparous colonies make significantly more embryos per zoid than unconfined colonies (Grosberg 1982). Semelparous and iteroparous colonies encountering conspecifics behave in an analogous manner to insufficient substratum space (Brunetti 1974; Grosberg 1982; Harvell and Grosberg 1988). Despite the general lack of space (or perhaps because of it), larvae tend to settle near the parents as discussed in the next section.

Local Settling of Colonies

Grosberg (1987) found minimal dispersal, with greater than 80% of the marked recruits located within 25 cm of the parental colony. He also studied fertilization and hatching success as a function of distance between mating colonies. Across all distances studied, between 90% and 95% of the ova were fertilized. Individuals separated by \( \leq 1 \) m had the greatest success but this declined only slightly for more distant matings. However, considering the subset of fertilized ova that hatched into normal larvae, the success rate remained over 95% for distances \( \leq 1 \) m but dropped to 80% for colonies separated by 3 m or more.

2.4 Self/nonself-recognition and Fusion

Related colonies not only settle near one another, but sometimes fuse together. Tanaka and Watanabe (1973) (cited in Scofield 1983) have shown that the fusion/rejection reaction is controlled by contact responses between the ampullae. In addition, Taneda and Watanabe (1982a,b,c) (cited in Scofield 1983) firmly established that humoral and cellular elements in the blood are the actual allorecognition elements that control the fusion/rejection reaction. The histocompatibility system that controls fusion and rejection is controlled by a single Mendelian locus with many codominant alleles such that two
colonies sharing at least one allele can fuse (Grosberg and Quinn 1986, Scofield 1982). Grosberg and Quinn (1986) found through their studies on the Marine Biological Laboratory supply dock at Eel Pond that only 4.2% of the colonies fused, showing that *B. schlosseri* has many different alleles.

Fusion provides several possible advantages. It increases colony size. A larger size may increase survivorship, decrease first sexual reproduction age and affect growth rate (Grosberg and Quinn 1986). Finally, germ cells or stem cells of the germinal line are exchanged between the fused colonies. Zooids of successive generations mature these germ cells even if one or more of the component colonies subsequently perishes (Sabbadin 1977).

Rinkevich and Weissman (1987) found that the offspring suffered deleterious effects by fusing with the parents or even just contacting them. The progeny which remained in contact with the parental colony suffered growth failure and poor survivorship, but those progeny that later disconnected from the parental colony both survived and grew. They suggested that colonies nonetheless cosettle due to interspecific competition such as from *Botrylloides leachi*. The costs due to loss of growth potential by settling close could be less than that due to increased mortality from interspecific competition when the colonies settle further away. Despite the apparent connections between gregarious settlement and self/nonself recognition, these two phenomena (or strategies) may have evolved independently and are still in the process of adaptation.

However, Rinkevich and Weissman (1987) might have misinterpreted the results. Due to the transfer of germ cells, the genome of the progeny would be preserved in the parental colony. The subsequent resorption benefits the progeny since they do not have to make a somatic contribution (Grosberg, personal communication). The germ cell transfer could also prove beneficial by maintaining the genetic variability within communities of reduced size (Sabbadin 1979) as discussed in the next section.
2.4.1 Inbreeding Considerations

Since one possible consequence of close kin association is increased inbreeding (Grosberg and Quinn 1986), an important function of self/nonself-recognition is avoidance of inbreeding depression. When two colonies fuse, the vascular systems join. The common circulation results in synchronization of asexual and sexual cycles which, due to protogyny, prevents the fused colonies from interbreeding. Outbreeding is thus favoured since the probability of fusion increases with the relatedness of the colonies (Sabbadin 1977).

2.5 Senescence

After iteroparous colonies reproduce several times (3-4 generations) they go into a period of senescence. This senescence seems to consist of essentially a reduction of the renewing capacity of the tunic, and is probably related to the intensity of sexual reproduction (Brunetti and Copello 1978). However, no obvious reduction of the blastogenic potential occurs over time (Sabbadin 1979).

2.6 Mortality

During the first week after settlement, juvenile mortality can approach 90% (Brunetti 1974; Grosberg 1982). However, apart from after overwintering, the proportion that survived from first recruitment to sexual maturity ranged from 7.7% to 21.8% over the period 1979-1980 at Eel Pond. Thus, most of the mortality is during the colony’s first week. Most of the colonies that recruited at the end of the season (September 15) did not sexually reproduce until the following spring. These overwintering colonies had a survivorship in excess of 90% in both 1979 and 1980 (Grosberg 1982, 1988).

As the iteroparous colony produces more clutches, its mortality rate increases (Table 2.1). However, sexual reproduction does not represent the end of the life cycle but actually
Table 2.1: Life table on the cohort of iteroparous colonies recruited in the field during 1-15 September 1979. Iteroparous colonies do not, in general, reproduce sexually before the ninth asexual cycle. Hence, survivorship data prior to the ninth asexual cycle are omitted. Estimates of survivorship do not include planktonic mortality of the larvae. Decreasing age-specific fecundity results from decreasing colony size with age and not from decreasing reproductive effort. The above caption and the table below are taken from Table 2, Grosberg 1988.

<table>
<thead>
<tr>
<th>Age ($x$) (number of asexual cycles)</th>
<th>Survivorship ($l_x$) (proportion of cohort)</th>
<th>Fecundity ($m_x$) (mean clutch size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0.207</td>
<td>1,596</td>
</tr>
<tr>
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<td>0.201</td>
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<tr>
<td>13</td>
<td>0.110</td>
<td>1,843</td>
</tr>
<tr>
<td>14</td>
<td>0.053</td>
<td>1,639</td>
</tr>
<tr>
<td>15</td>
<td>0.021</td>
<td>1,704</td>
</tr>
</tbody>
</table>

represents a difficult period when elevated metabolic activity influenced by environmental conditions, especially temperature, causes the increased mortality (Brunetti 1974).

Semelparous colonies die after the production of a single, large clutch (9-12 embryos). Actually, the colony will often cease functioning before the embryos are mature. Since this occurs after the eggs are already fully provisioned with yolk, the parental colony is then merely a receptacle. Although allocating all of the colony's energy to the embryos could cause the mortality, the parental mortality also could be due to enlarging embryos mechanically interfering with respiration and somatic activities (Grosberg 1982). To determine the mortality causes, Grosberg (1982) performed a series of embryo manipulation experiments. He manipulated ten clonal replicates of three semelparous and three iteroparous colonies, with ten clonal replicates of each strain used as controls. The first manipulation involved an early embryo stage removal of embryos from all zooids. In the second manipulation, all the embryos were removed as before but each was replaced with
a glass bead of similar size. In the third manipulation, performed only on iteroparous colonies, the clutch sizes were augmented, with glass beads, to the normal semelparous clutch size.

80% of semelparous colonies from which embryos had been removed survived to produce a second clutch compared to only 3.3% (1 out of 30) for the unmanipulated control colonies. However, the replacement of embryos with glass beads only allowed fewer than 7% to live to make another clutch. This data, especially the glass bead replacement experiment, is consistent with the hypothesis that mechanical impairment is the primary cause of death among semelparous colonies. The enhancement of survival of colonies after embryo removal shows that the surgical procedure did not have gross deleterious effects.

For iteroparous colonies, there were no conspicuous differences among the control, embryo removal, and glass bead replacement manipulations. However, the augmentation of embryos with glass beads caused the death of 90% of the colonies. This provides further evidence that the number of embryos per zooid is the primary cause of semelparous colony mortality rather than the energetic investment. Furthermore, after the removal of an entire clutch of nearly mature embryos, during the next generation the semelparous colonies revives and produces a clutch of nearly the same size as the one removed.

2.6.1 Predation

*Botryllus schlosseri* colonies also perish from predation. Predators include the snail *Mitrella lunata*, bacteria and probably some flatworms and nematodes (Milkman 1967). Bancroft (1903) (cited in Millar 1971) found that crabs eagerly attacked colonies of *Botryllus schlosseri*. Further predators include *Cyclopoerus papillosus* and a number of nudibranch molluscs (*Goniodoris nodosus* (Montagu), *G. castanea* Alder and Hancock, and *Ancula cristata* (Alder)) (Millar 1971). In Eel Pond, the sessile stage of jellyfish occasionally
heavily consumes the larvae, but it does not have an appreciable effect on the recruitment rate. Also, there is some minimal predation from nudibranches and fish grazing (Grosberg, personal communication).

2.7 Life-History Correlations

Despite a lack of significant differences among genotypes within life-history morphs, the life-history type had a highly significant effect on all the dependent variables (Grosberg 1988). Thus, the two morphs (semelparous and iteroparous) each have distinct life-history characteristic values. The next sections discuss both the intra and inter life-history morph correlations.

2.7.1 Semelparous Colonies

In semelparous colonies, first sexual reproduction age is positively correlated with per zooid fecundity and negatively correlated with the growth rate. Also, a marginally significant negative correlation exists between the growth rate and the total reproductive effort. These correlations imply that semelparous colonies that grow slowly will postpone reproduction and have a slightly higher reproductive effort (Grosberg 1982). This pattern is similar to that of the iteroparous/semelparous colony comparison (see section 2.2).

2.7.2 Iteroparous Colonies

Iteroparous colonies have negative correlations between the number of clutches and both the growth rate and the reproductive effort per clutch. Also, colonies which make more clutches invest less in each one. Thus, for iteroparous colonies the reproductive “cost” of high growth rate is paid for by producing fewer, rather than smaller, clutches. In contrast,
among the semelparous colonies, the negative correlation between growth rate and total reproductive effort suggests that rapid growth exacts its toll by a lower reproductive effort (Grosberg 1982).

2.7.3 Semelparous/Iteroparous Colony Differences

From the feeding level experiment described in section 2.2.1, middle (2x) and high (3x) effects are not significantly different from each other. However, semelparous and iteroparous colonies react differently to the lack of food at feeding level 1x. The following compares the 1x feeding level effects to that of feeding levels 2x and 3x, with semelparous values first and iteroparous values in brackets: first sexual reproduction age: +60% (+35%); size at first sexual reproduction: +10% (−1%); buds per zooid: −45% (−20%); clutch size (number of embryos per zooid): 0% (−35%); clutch number: 0% (−15%); fecundity: +10% (−45%); and longevity: +60% (+22%) (Grosberg 1982). When starved, semelparous colonies will grow much more slowly but will wait until they have reached their normal size and can produce their normal clutch size. Iteroparous colonies also grow to their normal size, but relatively quickly thereby sacrificing some of their potential sexual reproductive output (see section 2.7.5 about energy reallocation between growth and reproduction).

Analysis of semelparous and iteroparous colony differences from field studies reveals a positive correlation between growth rate and reproductive effort. This appears to imply that no reproductive price is paid for rapid growth. However, semelparous colonies are smaller at first reproduction and reproduce only once thus resulting in lower total reproductive output (Grosberg 1982).
2.7.4 F₁ Correlations with Parents

The maternal and paternal variance components for parental life-history type and genotype are very similar. This suggests both that F₁ dependent variables are genetically determined and that the maternal and paternal contributions to the phenotypes of progeny are roughly equivalent. In addition to the absence of any parental male by female interactions, this shows that both parents in a cross additively determine the phenotype of the resultant progeny (Grosberg 1982).

2.7.5 Energy Reallocation

To determine how energy can be reallocated, Grosberg (1982) performed a series of experiments where he prevented colonies from allocating resources to either growth or sexual reproduction. During the sexual reproduction process prior to ovulation, the parent supplies the developing oocyte with a substantial quantity of yolk. In the first experiment, Grosberg removed oocytes before the provisioning begins to prevent significant parental energetic investment in sexual reproduction. To test for allocation from somatic growth to sexual reproduction, in the second experiment, Grosberg severed all but one secondary bud from each primary bud.

During the first experiment, the number of buds maturing one and two asexual generations later was recorded. For semelparous colonies, no significant difference existed between the experimental colony and control colonies before they sexually reproduced. However, control colonies die after sexual reproduction whereas the manipulated colonies live as long as their oocytes are removed each generation. Thus, it is possible that the oocyte removal procedure of itself inhibits growth in semelparous colonies. However, after oocyte removal, iteroparous colonies had significantly enhanced growth compared to control colonies (18% one generation later and 50% two generations later). This implies that
the experimental procedure has at most a minimal negative impact on colony growth. Thus, this experiment indicates that iteroparous colonies can reallocate resources from yolk provisioning to growth while semelparous colonies cannot.

During the second experiment, the resulting fecundity was noted and analyzed. While iteroparous colonies increased fecundity in the two asexual generations following the bud removal, semelparous colonies showed no fecundity increase. These two experiments together imply that semelparous colonies cannot reallocate their energetic resources between growth and sexual reproduction, whereas iteroparous colonies can reallocate their energy.

2.8 Competition

As mentioned in section 2.2, over the summer semelparous colony density decreases while iteroparous colony density increases. Semelparous and iteroparous colony fecundity does not change significantly and thus cannot be the cause. Instead, overwintering iteroparous colonies reproducing and increased semelparous colony mortality are partly responsible for the seasonal change. The increasing semelparity mortality occurs before the additional recruitment of iteroparous colonies, so larger inter-morph competition is not the mortality cause. In fact, semelparous/iteroparous density change is mediated primarily by the density of a closely related colonial ascidian, Botrylloides leachi. Botrylloides leachi is competitively dominant to semelparous B. schlosseri but not the iteroparous morph (Grosberg 1982, 1988). As such, semelparous colony mortality increases as the Botrylloides leachi density increases over the summer. Semelparous colonies predominated both in tidal ponds without Botrylloides leachi and in Eel Pond before the introduction of Botrylloides leachi about thirty years ago (Grosberg 1981, 1982).
2.8.1 *Botrylloides* Description

*Botrylloides* is so closely related to *Botryllus* that it has often been relegated to the status of a subgenus (Berrill 1947). The minor differences include its gonad arrangement, larval incubation and some digestive tube morphological aspects (Brunetti et al. 1980).

*Botrylloides* has a central stalk with adhesive organs. This stalk is surrounded by a ring of eight epidermal ampullae that aid in maintaining colonial circulation (Bancroft 1899) and form the permanent organ of attachment on the substratum (Berrill 1947).

In the Venetian lagoon, sexual reproduction occurs mainly during the 2 months from the second half of May to the first half of July. Assuming that water temperature provides the controlling influence, it appears that sexual reproduction occurs in the temperature range 17°C to 25°C (Brunetti 1976). At Woods Hole, this corresponds roughly to the period of July to September.

The colonies are always in an encrusting form with well developed colonies even forming surface folds that resemble a brain surface. The colonies assume a spherical shape with no cases observed of pendant type growth present in *B. schlosseri* (Brunetti 1976).

As the temperature falls below 10°C, *B. leachi* goes into hibernation. First, there is a large reduction in general activity in feeding and blastogenic development. Not all the buds reach the stage of filtering zooid. Many in fact degenerate together with the parent zooids until there are no functional filtering zooids left. With only small rounded ampullae remaining, the colonies resemble a carpet (Brunetti 1976).

*Botrylloides* has many predators in common with *Botryllus* including the posobranch gastropod *Erato voluta* (Montagu), nudibranch molluscs such as *Goniodoris nodosus* (Montagu), *G. castanea* Alder and Hancock and *Ancula cristata*, and finally *Cycloporus*
papillosus. However, although crabs enjoy Botryllus, they quickly learn to avoid Botrylloides (Millar 1971).

2.8.2 Competitive interactions

Myers (1988) describes the competitive effects of other organisms on Botrylloides at Woods Hole. The presence of Bugula decreases the Botrylloides growth rate. This is most probably caused by food competition because Bugula cannot outcompete Botrylloides for space. The solitary tunicate Mogula spp. overgrows Botrylloides thereby causing a high mortality rate. Bugula has a strong positive settlement effect on Mogula.

As mentioned previously, Botrylloides competitively interacts with B. schlosseri. Grosberg (1982) performed a series of experiments to investigate the effect of Botrylloides on Botryllus survival. He used five experimental plates mixed with five control plates. He performed weekly removals of all newly recruited Botrylloides leachi on the experimental plates. For Botryllus colonies that died, he noted the type, extent (in terms of length of contacting margins), and competitive outcomes of contacts involving the B. schlosseri colonies. In a second experiment, Grosberg performed a natural, though controlled, experiment in Green Pond. This pond, located about 10 km northeast of Eel Pond, is similar, but not identical, to the Eel Pond. Most importantly, it does not have Botrylloides. Grosberg placed five clonal replicates each of four strains of semelparous colonies and of four strains of iteroparous colonies at both Eel Pond and Green Pond. This was done on June 1, 1981 before any significant Botrylloides recruitment at Eel Pond and again on July 24, 1981 during the first large Botrylloides recruitment at Eel Pond. In the final experiment, clonal replicates of four iteroparous and four semelparous strains were placed in symmetrical pairwise competition with 3 colonies of Botrylloides randomly collected from the field. All possible pairwise competitions among the four iteroparous and four semelparous strains were performed. In all the above experiments,
a colony with greater than 50% of its area covered by another colony was considered overgrown.

In the first experiment, the proportion of semelparous Botryllus colonies on the control plates began decreasing at the end of July, reaching only 10% at the end of August. On the experimental plates, in the absence of Botrylloides, the semelparous proportion did not begin declining until the end of August. This one month delay is about the life span of a semelparous colony. After mid-October, on both the experimental and control plates, the semelparous colony proportion began to rise. At this time, the growth rates, and hence contact frequencies, of all colonial ascidians have declined resulting in few semelparous colonies being overgrown by Botrylloides.

In the second experiment, in Eel Pond, the semelparous colonies initiated on June 1 had a significantly higher survival rate to sexual reproduction than did the colonies initiated on July 24. However, in Green Pond, the semelparous colonies initiated on both dates had a similar survival success rate. On the other hand, at Eel Pond, the iteroparous colonies initiated in June had a significantly lower survival rate to first sexual reproduction than the July initiates. In Green Pond, iteroparous colonies from both dates had a low survival rate. The above results suggest that the Botrylloides presence hinders semelparous survival but aids iteroparous colony survival.

In the third experiment, all Botrylloides/semelparous contests resulted in Botrylloides overgrowing the semelparous colony before it could sexually reproduce. In contrast, in only one of the twelve Botrylloides / iteroparous competitions did the Botrylloides overgrow the iteroparous colony, with the remaining eleven resulting in a mutual cessation of growth along the interspecific contact region. Most other pairings of dissimilar genotypes also resulted in growth ceasing at the contact margin. The two exceptions involved the same semelparous colony overgrowing two different iteroparous colonies. In summary, the above data shows that Botrylloides consistently overgrows semelparous, but
not iteroparous, colonies. Also, intraspecific contacts usually result only in cessation of growth rather than death of one of the interacting colonies.

The data above strongly support the hypothesis that the increased semelparous colony mortality is primarily caused by the increased competition from the seasonal increase of *Botrylloides leachi* (Grosberg 1982). On the other hand, the increase of iteroparous colonies probably results from the decrease in intraspecific competition after the semelparous colony decline.

The cause of the morph specific difference in *Botrylloides* competition appears to be related to the ampullar density. Contacts between botryllid ascidians first involve epidermal contact and then interactions among the ampullae. *Botrylloides* overgrowth of semelparous colonies involves the interdigitating of the ampullae followed by the *Botrylloides* ampullae pulling their colony edge over the now underlying *Botryllus* colony. This could be due to the *Botrylloides* colony possessing an ampullar density often double or triple that of the semelparous colony. Iteroparous colonies, on the other hand, have an ampullar density about double that of the semelparous colonies and thus similar to that of *Botrylloides*. This similar ampullar density could be why *Botrylloides* is incapable of extending its ampullae over those of iteroparous colonies. However, this remains tentative at best until pertinent experiments are performed (Grosberg 1982). Also, at least part of the reason for the iteroparous *Botryllus/Botrylloides* outcome could be *Botrylloides* recognizing, through some sort of chemical recognition at the ampullae, the iteroparous colonies as being the same species. However, this would not be evolutionarily stable since a random mutation in a semelparous colony to produce these chemicals should quickly spread (Grosberg, personal communication).
2.8.3 Coexistence of Polymorphism

Semelparous colonies continue to coexist with iteroparous colonies despite a severe drop in semelparous colony numbers in the late summer. However, few semelparous colonies die during the winter when shrinkage and death of colonies disrupts the competitive process. The resulting empty space becomes filled with semelparous progeny since overwintering semelparous colonies reproduce about 4 weeks before iteroparous colonies. Finally, substantial numbers of *Botryllus* likely arrive in Eel Pond on boat hulls (Grosberg 1982). Considering the predominance of semelparous morphs in local areas, the majority of the hitchhikers would be semelparous morphs.

The next two chapters will use mathematical models to examine *Botryllus schlosseri*. Chapter 3 will study various life-history characteristics of *B. schlosseri* while Chapter 4 will investigate how the competitive interactions between the two *Botryllus* morphs and *Botrylloides* can result in coexistence.