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Larval Dispersal, Gene Flow and Speciation in the Marine Gastropod Genus *Littorina*

Paul A. Hohenlohe

A dissertation submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

University of Washington
2000

Program Authorized to Offer Degree: Zoology

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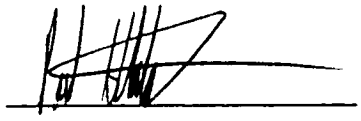
Signature 

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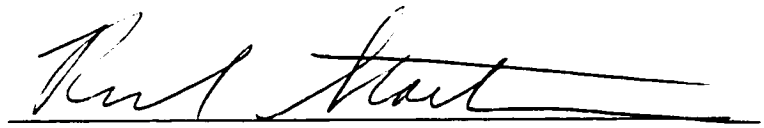

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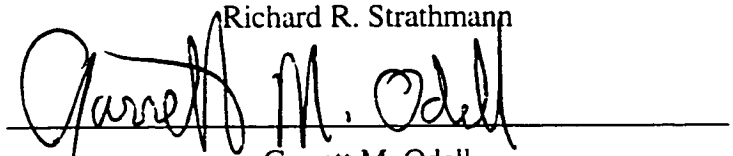

Garrett M. Odell

Date: 11/15/00

University of Washington

Abstract

Larval Dispersal, Gene Flow, and Speciation
in the Marine Gastropod Genus *Littorina*

Paul A. Hohenlohe

Chairperson of the Supervisory Committee:
Professor Alan J. Kohn
Zoology

Many marine animals have a planktonic larval stage, which is expected to increase gene flow and prevent speciation. The sibling gastropod species *Littorina scutulata* and *L. plena* are sympatric in intertidal habitats along the Pacific coast of North America, and both have long-lived planktonic larvae. *L. plena*, but not *L. scutulata*, shows evidence for restricted gene flow.

Restriction enzyme digestion of the mitochondrial gene cytochrome b distinguishes the species, and this tool was used to evaluate quantitative and discrete morphological characters. Though the species differ in most characters, none is completely diagnostic; tentacle coloration is the most reliable. The species overlap broadly along both wave-exposure and tidal-height gradients, though only *L. plena* occupies the most exposed habitats and *L. plena* occurs slightly higher on the shore. The species do not differ in either foot morphology or tenacity.

Both species released planktonic egg capsules from early spring to mid fall in Puget Sound, though previous accounts of capsule morphology distinctions are incorrect. Larvae of both species grew faster at 10^5 cells/mL than at higher or lower food concentrations and required significant growth before metamorphosis in response to a variety of natural materials. Time to metamorphosis in the laboratory and size of larval shells on field-collected animals suggest no species difference in planktonic period. The extended spawning season and 37- to 70-day planktonic period should produce high levels of gene flow, and genetic population structure in *L. plena* remains unexplained.

Spatially explicit, simulation modeling evaluated factors that could restrict gene flow, focusing on southern California and Washington state. Ocean currents can create an

effective barrier to gene flow that is relaxed by temporal variation or an extended spawning season, but not by an extended planktonic period. Larger scales of temporal variation have a greater effect. I suggest a mode of speciation for these *Littorina* species and other marine animals with planktonic larvae, in which short-lived barriers caused by an interaction of physical and biological factors restrict, but do not eliminate, gene flow, and reproductive isolation results from quick divergence in a few genes.

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ACKNOWLEDGEMENTS

I am grateful to my advisor, Alan J. Kohn, for his advice and direction over the past five years, and I wish him a long and fruitful retirement. Other members of my reading committee, Garry Odell and Richard Strathmann, provided valuable help and guidance throughout my research and writing. Thanks also to the rest of my supervisory committee and departmental staff in Seattle and at Friday Harbor Laboratories for facilitating my graduate education. This research was supported by a NSF Graduate Fellowship and a NSF training grant in mathematical biology.

I also thank my parents and my wife Jennifer for their love and support, without which this accomplishment would not have been possible.

CHAPTER 1
Introduction

Many marine animals have a planktonic larval stage that can last for weeks or months, even years (Thorson 1950; Scheltema 1986b; M. Strathmann pers. comm.). The gene flow that is expected to result from the long-distance dispersal of these larvae should swamp genetic differentiation of populations and prevent speciation. Nonetheless, many such species show surprisingly high levels of genetic differentiation among populations (Palumbi 1995), marine lineages with planktonic larvae are often diverse (Kohn 1983; Palumbi 1992; 1994), and diversity may even be currently underestimated (Knowlton 1993). Using the sibling gastropod species *Littorina scutulata* and *L. plena*, I have addressed the following questions: Can basic life history or ecological features account for the apparent difference in gene flow between these species? Given these species' current biology, what mode of speciation most likely produced them? In general, what factors can limit gene flow and allow speciation in marine species with planktonic larvae?

The gastropod genus *Littorina* is well-suited for examining the effects of planktonic larval dispersal. The species in this genus are evenly divided between those with planktonic larvae and those that develop in benthic egg masses or a maternal brood chamber (Reid 1996). The adults are ecologically similar, occupying high intertidal habitats throughout the temperate northern hemisphere and feeding primarily on microalgae (Reid 1996). In a review of population genetic studies of the genus, Ward (1990) found that most species appear to fit the expected pattern of lower rates of gene flow in species without planktonic larvae. The genus also fits the expected pattern of higher speciation rates in lineages without planktonic larvae (Reid 1990b). However, the northeast Pacific species *L. scutulata* and *L. plena* show surprising evidence for population subdivision in allozyme frequencies (Ward 1990). In a study of the mitochondrial gene cytochrome b, Kyle (1998) found evidence for genetic population structure in *L. plena* but not in *L. scutulata*. These species are remarkably similar in life history, habitat, and range, so they provide a model system for finding the factors responsible for limiting gene flow.

L. scutulata and *L. plena* are excellent examples of the cryptic species described by Knowlton (1993), so their taxonomic history is complex and distinguishing them in the field is difficult. Chapter 2 describes a molecular tool used to examine morphological characters that can be used to distinguish the species. Discrete morphological characters are

critical for ecological and life history studies, and the characters analyzed are used for species identification in chapters 3 and 4.

One hypothesis for restricted gene flow in *L. plena* is that populations of this species are restricted to a narrower range of habitats, creating geographically and genetically more isolated populations. To test this hypothesis, chapter 3 examines the relative abundances of the two species along two habitat gradients, wave exposure and tidal height. Chapter 3 also tests the hypothesis that a difference in wave-exposure habitat preference is related to differences between the species in foot morphology or tenacity. Differences in habitat and adaptation also inform speculation on the speciation event that produced these species. The fossil record of *Littorina* is poor, and the divergence of these species is relatively ancient (Reid et al. 1996 gave a mean estimate of 12.8 million years), so direct evidence of the speciation event is not available. Though allopatric speciation has been regarded as the dominant speciation mode (Rice & Hostert 1993; Berlocher 1998), there is evidence for sympatric reproductive isolation through assortative mating in other *Littorina* species (Johannesson et al. 1995b; Takada 1995). This type of divergence, however, depends on some sort of divergent selection according to microhabitat (Johannesson et al. 1995b). Reid (1996) has used degree of overlap of current distributions of sibling *Littorina* species to infer their mode of speciation. Thus sympatry and microhabitat separation in the current species' distributions argue for sympatric speciation, while allopatry and similar niches argue for allopatric speciation.

Several aspects of larval life history may limit gene flow, and these factors are reviewed in chapter 5. Data on the life histories of *Littorina* species with planktonic larvae are limited, however. For example, no planktonic *Littorina* larvae had been raised through metamorphosis in the laboratory until this study. Chapter 4 examines life history characters of *L. plena* and *L. scutulata* that may be relevant to gene flow. In particular, a longer spawning season or planktonic period is expected to increase the seasonal variability of ocean currents that larvae encounter, thus increasing gene flow among populations. Planktonic period depends on pre-hatching development, larval requirements for growth, and settlement cues, so these factors are examined as well. Egg capsule morphology has been used to distinguish these species, so I examine the reliability of previous capsule distinctions.

Chapter 5 uses a mathematical, simulation modeling approach to test factors that could limit gene flow in species with planktonic larvae. Previous models have been criticized for not being solidly based in biological data (Grant 1989), so the research in chapter 4 is intended in part to provide basic parameters for the models. I adhere as closely

as possible to what is known about the life histories of *L. scutulata* and *L. plena* in making assumptions in the models. I use ocean current data from two specific areas, Point Conception in southern California and the Pacific coast of Oregon, Washington, and southern British Columbia. The models predict the effect on gene flow of three factors: ocean current variation, total planktonic period, and spawning season. In particular, the models examine the interaction between physical (ocean currents) and biological (planktonic period and spawning season) factors, as emphasized by Jackson (1986). While these models are rooted in data from two species and from two geographic areas, they are meant to produce general conclusions about the factors that can restrict gene flow in marine animals with planktonic larvae. Conclusions about the mode of speciation that produced *L. scutulata* and *L. plena* can also be extended to a wide range of taxa.

CHAPTER 2

Morphological Characters and a Molecular Tool for Distinguishing the Sibling Species *Littorina scutulata* and *L. plena*

Abstract

The sibling species *Littorina scutulata* and *L. plena* are defined on the basis of reproductive system characters, including penis and egg capsule morphology. They are difficult to distinguish in the field. Here I present a new molecular tool and use it to evaluate the discrete and quantitative morphological characters that have been proposed as diagnostic. PCR amplification and restriction enzyme digestion with Alu I of a 480 bp fragment of the mitochondrial cytochrome b gene was used to distinguish the species. This new molecular assay produces species-specific restriction fragment patterns that correspond with identification of males by penis morphology. To evaluate the usefulness of morphological characters, I scored three discrete shell characters (presence of basal band, presence of basal ridge, and size of checker pattern) as well as tentacle coloration. The four discrete characters differ significantly between the two species, though none is completely diagnostic. Tentacle coloration is the most reliable character and may be combined with the shell characters for successful identification. The two species also differ significantly in overall size and in three out of five size-independent shell shape measurements, with *L. scutulata* having larger, taller-spired shells with narrower apertures. However, shell shape does not separate the species well because of intraspecific variation, and it is unlikely to be useful for species identification. Further analysis suggests that at least some of this intraspecific variation is genetic rather than environmental.

Introduction

The taxonomic history of the *Littorina scutulata* species complex, a group of sympatric intertidal prosobranch gastropods in the Northeastern Pacific, has been complicated by morphological similarity across species and variation within species. Currently two sibling species are recognized, *L. scutulata* Gould 1849 and *L. plena* Gould 1849, which are distinguished on the basis of reproductive system characters, including penis, pallial oviduct, and egg capsule morphology (Murray 1979; Mastro et al. 1982; Reid 1996). The original species descriptions by Gould (1849) depended on shell morphology,

which is usually not sufficient for distinguishing specimens, and the species were subsequently united. Murray (1979) provided evidence for two species on the basis of penis and egg capsule morphology, and Mastro et al. (1982) matched these distinctions to Gould's (1849) specimens. Though hybridization studies have not been done, further evidence that *L. scutulata* and *L. plena* are in fact separate species includes allozyme frequencies (Mastro et al. 1982) and large divergence in DNA sequences (Reid et al. 1996; Kyle 1998). Reid (1996) added copulatory bursa morphology as a defining character in his revision of *Littorina* taxonomy. In chapter 4, I cast doubt on previous egg capsule morphology distinctions. Ultimately, characters used to distinguish these species must be traced back to penis and copulatory bursa morphology. These characters, however, are difficult to use for the non-destructive field identification that is necessary for many ecological studies. Reproductive system characters can not be used for juveniles, and I have also found pallial oviduct morphology to be a difficult character to examine, particularly in small specimens.

Other diagnostic morphological characters have been proposed. Three discrete shell characters have been described: a pale basal band (Murray 1982; Chow 1987a; Reid 1996; Rugh 1997) and a narrow basal ridge (Rugh 1997), both found more often in *L. plena*, and the pattern of checkers on many shells, which tend to be smaller in *L. plena* than in *L. scutulata* (Reid 1996; Rugh 1997). Rugh (1997) was able to use these three shell characters alone to correctly identify 17 male specimens of both species from southern California. Reid (1996) described differences in tentacle coloration: *L. scutulata* individuals tend to have "transverse black bands and flecks," while *L. plena* tend to have a "broad, unbroken black stripe with transverse flecks, or all black." Murray (1982) described a set of discriminant functions of four quantitative shell measurements that correctly classified 96% of specimens. Further principal component analysis by Murray (1982) showed *L. scutulata* shells to be generally taller with narrower spire angles and shorter aperture openings relative to shell height. Chow (1987a) combined three quantitative shell measurements with number of whorls, presence of a basal band, and presence of tesselation in another discriminant function analysis. This analysis correctly classified 92% of specimens, but only when using snails from one habitat; combining specimens from different habitats introduced too much intraspecific variation to allow correct classification. Chow (1987a) also found *L. scutulata* shells to be larger, narrower, and less likely to have a basal band, which agreed with past work. Other characters used with varying success to distinguish these species include spiral sculpture on the shell and radular characters (Reid 1996; Mastro et al. 1982).

Mastro et al. (1982) found eight polymorphic allozyme loci at which the two species differ in their allele frequencies. However, none of these loci was diagnostic. No other molecular studies to date have identified a reliable molecular character at a polymorphic locus that distinguishes the two species.

The previous studies of morphological differences used specimens that were positively identified using reproductive system characters, thus excluding pre-reproductive animals. Here I present a molecular technique for identifying individuals of all ages using mitochondrial DNA. I use this tool to evaluate the reliability of characters that can be observed on intact animals: the three discrete shell characters described above, tentacle coloration, and quantitative shell shape differences.

Methods

I collected 385 snails of both species from 11 areas around Puget Sound and the outer coast of Washington state in January 1998 (see Figure 2.1) and kept them alive until DNA extraction. Animals of all sizes, including juveniles, from within a randomly chosen, small (approx. 1 m^2) area of rocky shore habitat were collected. Individuals anesthetized in 7% $MgCl_2$ solution were scored for three discrete shell characters (Figure 2.2a-d): basal band present or absent, basal ridge present or absent, and checkers large, small, or absent. Large checkers lie in five to 12 spiral rows, depending on shell size, while small checkers number more than 12 spiral rows. Tentacle coloration was scored in one of seven categories: dark transverse bands, spots and bands, spots, dark central stripe, central stripe with bands, no color, or all dark (Figure 2.2e-f; see also Reid 1996). In all further analyses, the first three categories are grouped as "transverse bands," and the last four are grouped as "central stripe." Individuals' sex was also recorded and males scored as *L. plena* or *L. scutulata* penis type. *L. scutulata* penes are relatively short with a terminal bifurcation, while *L. plena* penes are longer, often coiled, with a bifurcation near the base (Reid 1996).

Animals were then sacrificed for molecular analysis. Using the extraction protocol and PCR primers described in Kyle & Boulding (1998), a 480 bp fragment of the mitochondrial cytochrome-b gene was amplified for each individual. These were then digested for two hours using the restriction enzyme Alu I and the digests run on a 2% agarose gel. To predict restriction sites for the two species, I examined 36 *L. plena* and 18 *L. scutulata* haplotype sequences from Kyle (1998; Genbank accession nos. AF077238-AF077291). Identification of these sequences by Kyle (1998), however, depended on a single *L. plena* sequence from Reid et al. (1996; Genbank accession no. U46815), so our

analysis functions also to confirm the identification of those sequences. I expected fragments of 161, 233, and 86 bp for *L. scutulata* and 109, 15, and 356 bp for *L. plena*. Scoring of discrete characters and molecular work was carried out in the laboratory of Dr. Elizabeth G. Boulding at the University of Guelph, Ontario, Canada.

Eight quantitative shell measurements were taken from each individual with a dissecting microscope connected to computer imaging software (Figure 2.3). Since six of these measurements are lengths, they were combined into three non-dimensional ratios to remove the effect of overall shell size as follows: Relative aperture height = Aperture height / Shell height; Whorl ratio = Whorl n-2 / Whorl n-1; and Aperture shape = Short axis / Long axis. Statistical tests and initial discriminant function analysis used these three ratios as well as spire angle and aperture angle (Figure 2.3). Quantitative measurements were taken at Friday Harbor Laboratories at the University of Washington.

In an attempt to improve differentiation of the two species based on shell morphology, further discriminant function analyses were done using different sets of variables. In the first, size was included specifically by adding shell height to the three ratios and two angles, providing six variables for discriminant analysis. In the second revision, the original six linear measurements were not combined into ratios as above, but rather normalized by the method of Clarke et al. (1999). The effects of size were removed from each linear measurement by dividing by the geometric mean of each specimen's measurements:

$$\text{geometric mean} = 10\text{^}((\log_{10}(x_1) + \log_{10}(x_2) + \ldots + \log_{10}(x_6))/6)$$

where x_1 through x_6 are the original linear measurements. This provided two angles and six normalized linear measurements for discriminant analysis

The third attempt to improve the discrimination used only the specimens from six sites at which both species were found (see Figure 2.1) and analyzed the three ratios and two angles as above. Finally, another analysis was performed on data limited to three sites of similar habitat following Chow (1987a). These three sites are the three southern Puget Sound sites shown in Figure 2.1. All are protected shores at which both species were found.

Results

Restriction enzyme digestion with Alu I produced two discrete fragment length patterns as expected: one pattern with three closely spaced bands (*L. scutulata*) and another

with two widely spaced bands (*L. plena*; the 15 bp fragments typically migrated off the gel). Figure 2.4 shows the characteristic banding patterns for each species. These corresponded precisely with identification of males by penis morphology (n = 48 *L. scutulata* and 71 *L. plena*). This supports the identification of sequences in Kyle (1998) and suggests that these restriction sites are consistent across haplotypes within each species. The following analyses are based on these 119 males as well as females identified using only restriction digest and males identified by penis morphology alone. Sample sizes vary also because of damage to some shells during DNA extraction.

The frequency of each of the four discrete characters differs significantly between the species (Table 2.1) consistent with Rugh (1997) and Reid (1996). The differences remain significant following Bonferroni correction of the original p-values from four separate contingency table analyses (Rice 1989). However, no single character completely separates the two species. The shell characters were often not visible because of shell erosion from wave action or fungal or other epiphytic growth, creating a potential bias toward identification as *L. scutulata* from the first two characters. Many undamaged shells also lack any checker pattern. Tentacle coloration, because it was always scorable in live animals, was the most reliable discrete character.

Following Bonferroni correction of the original p-values from six separate two-tailed t-tests (Rice 1989), the species differ significantly in four shell measures (Table 2.2). *L. scutulata* shells are significantly larger than *L. plena* shells. Of the five size-independent shell measures, the two species differed significantly in spire angle, whorl ratio, and aperture shape. These results are consistent with those of Murray (1982) and Chow (1987a), confirming that *L. scutulata* shells are larger, narrower, and taller-spired with narrower apertures. Relative aperture height, though not significantly different, also follows the trend found by Murray (1982). Three of these shell measures, shell height, whorl ratio, and aperture shape, remain significant when considering only the six sites at which both species were considered (Table 2.3). The difference is lost for spire angle. However, the difference in relative aperture height reverses and becomes statistically significant when only these sites are considered.

Combining specimens from all the collection sites, these five shell measures were used in a discriminant function analysis without much success: the function correctly classified only 69% of *L. scutulata* and 67% of *L. plena* specimens. This seems to be the result of overlapping intraspecific variation for all of the characters.

Attempts to improve the discriminant function analysis were marginally successful. Because *L. scutulata* shells were significantly larger, including shell height improved

posterior classification to 76% for *L. scutulata* and 81% for *L. plena*. Normalizing the linear measurements by the geometric mean (Clarke et al. 1999) improved classification of *L. scutulata* to 82%, but reduced successful classification of *L. plena* to 62%. Limiting the analysis to the six sites where both species were found made no improvement over the original dataset: 69% for *L. scutulata* and 68% for *L. plena*. Finally, restricting the analysis to the three protected Puget Sound sites only slightly improved classification: 66% for *L. scutulata* and 74% for *L. plena*. These results are summarized in Table 2.4.

Discussion

L. plena individuals tend to have a pale basal band, basal ridge, and small checker pattern on their shells and a broad central stripe on their tentacles, while *L. scutulata* individuals lack the basal band and ridge, have a larger checker pattern, and have transverse bands and spots on their tentacles. In addition, *L. scutulata* shells are larger and taller-spired with narrower apertures. However, I do not believe the discrete shell characters alone to be sufficient, as did Rugh (1997), nor do I believe shell shape differences to be diagnostic, as did Murray (1982). Though the species differ significantly in these characters, intraspecific variation and shell damage may confound identification.

No method of combining quantitative shell measurements seems to provide reliable identification. The most successful analysis used shell size explicitly, which could potentially bias the identification of different ages of snails, so its utility in ecological studies would be limited. Neither method of removing the effects of size matched the discrimination ability of Murray (1982) or Chow (1987a), suggesting that interspecific differences are truly confounded by intraspecific variation. One attempt to eliminate some intraspecific variation by examining only sites at which both species were found did not improve the results, so this dataset does not show any evidence for character displacement in shell shape. Limiting the analysis to a single habitat type was not successful either. Since this analysis attempted to remove environmental phenotypic variation, the result suggests that at least some of the intraspecific variation observed is genetically based.

One likely explanation for the different results is that these previous studies used only reproductive, hence larger, animals. Species differences may become more apparent as the snails grow (Reid 1996; pers. obs.), and ecological applications typically require identification of animals of all ages. Murray (1982), Rugh (1997), and Chow (1987a) primarily used specimens from California, and geographic differences may also play a role. Chow (1987a) also combined discrete and quantitative characters in a single analysis which was not done here.

I found *L. plena* occupying a wider range of habitats in Washington, from sheltered Puget Sound sites to the exposed outer coast. In contrast, *L. scutulata* was found only on sheltered to moderately exposed shores. This result conflicts with previous work (Reid 1996) and will be investigated further in chapter 3.

These species can be distinguished non-destructively by using the characters discussed here. Male penis morphology can be easily examined by holding the snail upside down, underwater, under a dissecting microscope. For females and non-reproductive males, tentacle coloration is the most reliable character and can be combined with shell characters on undamaged specimens. For positive identification of all ages and both sexes, restriction enzyme digestion of cytochrome b with Alu I is straightforward and provides a reliable character independent of morphology.

Table 2.1: Species differences for four discrete characters. Species were identified by restriction enzyme digest and penis morphology. Data given are number of specimens (percentage) in each category. The p-values are Bonferroni corrections (Rice 1989) of separate χ^2-tests.

Character	State	*L. scutulata*	*L. plena*	p-value
Basal band	present	18 (12.4)	124 (51.7)	<0.001
	absent	127 (87.6)	116 (48.3)	
Basal ridge	present	23 (15.9)	149 (62.1)	<0.001
	absent	122 (84.1)	91 (37.9)	
Checker pattern	large	122 (84.1)	25 (10.4)	<0.001
	small	5 (3.4)	93 (38.8)	
	absent	18 (12.4)	122 (50.8)	
Tentacle color	transverse bands	130 (89.7)	10 (4.2)	<0.001
	central stripe	15 (10.3)	230 (95.8)	

Table 2.2: Quantitative shell measurements. Numbers given are mean (standard deviation), and the p-values are Bonferroni corrections (Rice 1989) of separate two-tailed t-tests.

Measurement	*L. scutulata* (n = 142)	*L. plena* (n = 210)	p-value
Shell height (mm)	8.1 (1.6)	6.3 (1.3)	<0.001
Spire angle (deg.)	54.2 (4.0)	55.5 (5.8)	0.045
Aperture angle (deg.)	24.0 (2.4)	23.8 (2.1)	>0.5
Relative ap. height	0.516 (0.038)	0.519 (0.039)	>0.5
Whorl ratio	0.595 (0.163)	0.555 (0.045)	<0.001
Aperture shape	0.703 (0.038)	0.726 (0.037)	<0.001

Table 2.3: Quantitative shell measurements considering only the sites at which both species were found (6 out of 11 sites). Format and statistical analysis as in Table 2.2.

Measurement	*L. scutulata* (n = 104)	*L. plena* (n = 146)	p-value
Shell height (mm)	7.4 (1.2)	6.3 (1.2)	<0.001
Spire angle (deg.)	54.4 (4.1)	54.5 (5.5)	>0.5
Aperture angle (deg.)	23.9 (2.4)	23.9 (2.2)	>0.5
Relative ap. height	0.528 (0.034)	0.513 (0.039)	0.01
Whorl ratio	0.607 (0.188)	0.568 (0.002)	0.045
Aperture shape	0.697 (0.040)	0.721 (0.037)	<0.001

Table 2.4: Varying success of discriminant function analyses of quantitative shell measurements. Correct posterior classification percentages are given along with total sample size of specimens for each species.

Variable combination	*L. scutulata*	*L. plena*
2 angles, 3 ratios	69 (n=142)	66 (n=210)
2 angles, 3 ratios, shell height	76 (n=142)	81 (n=210)
2 angles, 6 linear measurements normalized by geometric mean	82 (n=142)	62 (n=210)
2 angles, 3 ratios from sites with both species	69 (n=104)	68 (n=146)
2 angles, 3 ratios from protected habitats only	66 (n=38)	74 (n=99)

Figure 2.1: Map of western Washington state showing collection sites in Puget Sound and on the outer coast. Pie diagrams show relative abundance of *L. plena* (dark) and *L. scutulata* (light) with the total sample size for each site.

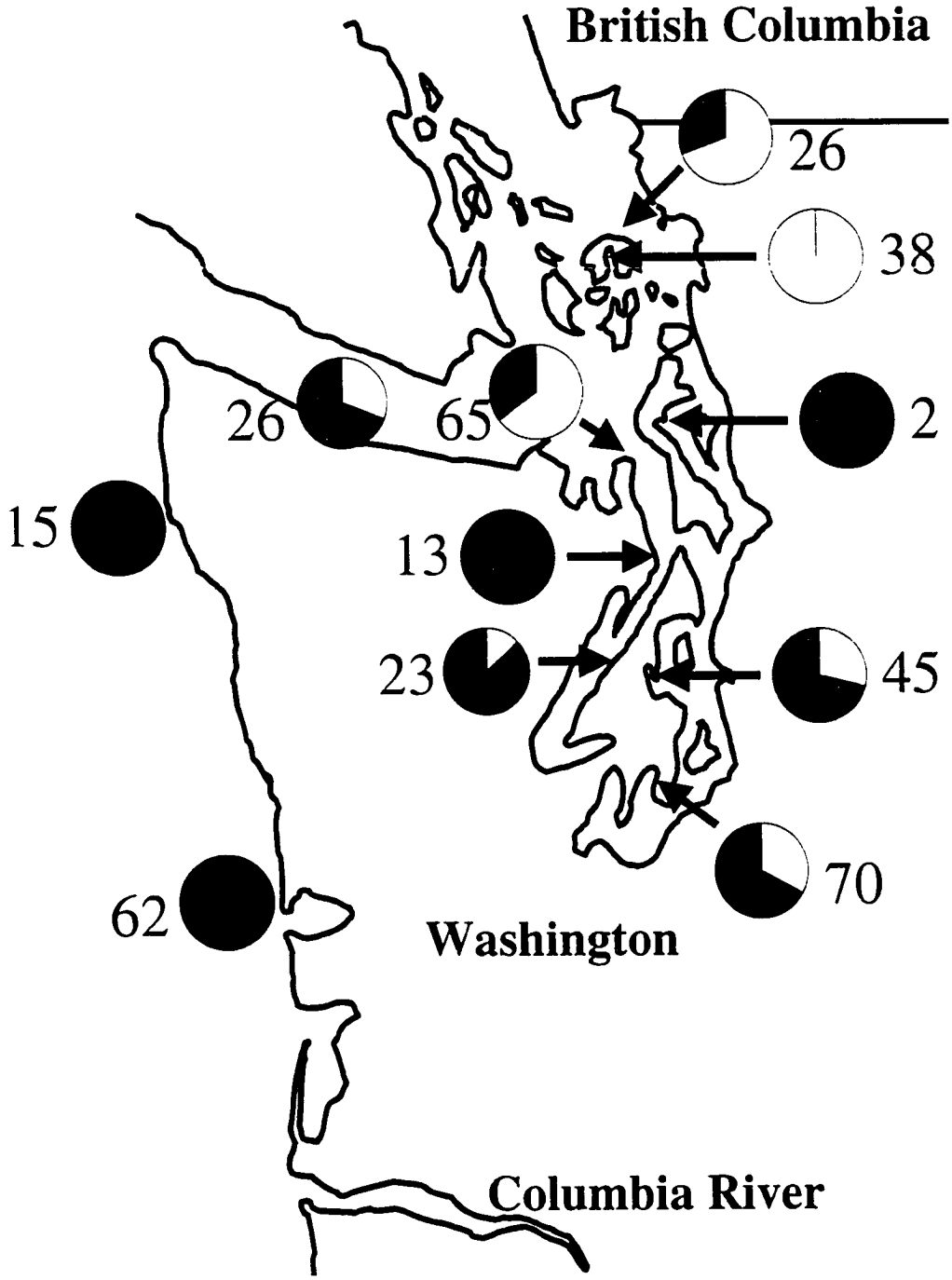


Figure 2.2: Discrete characters. (a) A pale basal band and (b) basal ridge are found more often in *L. plena*. (c) A pattern of large checkers typifies *L. scutulata*, while (d) small checkers are found more often in *L. plena*. (e) Two tentacle coloration patterns found in *L. scutulata*: transverse bands (left) and bands and spots (right). (f) Two tentacle patterns found in *L. plena*: a broad central stripe with bands (left) and without bands (right).

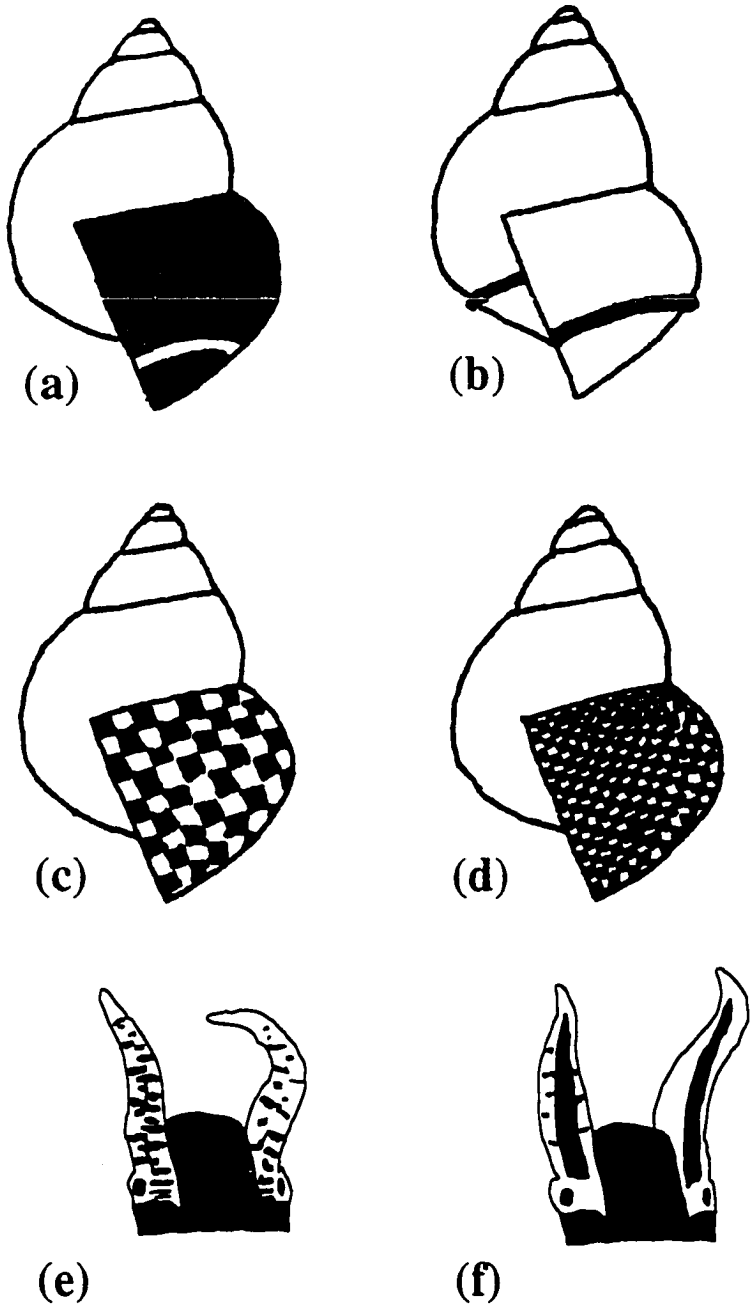
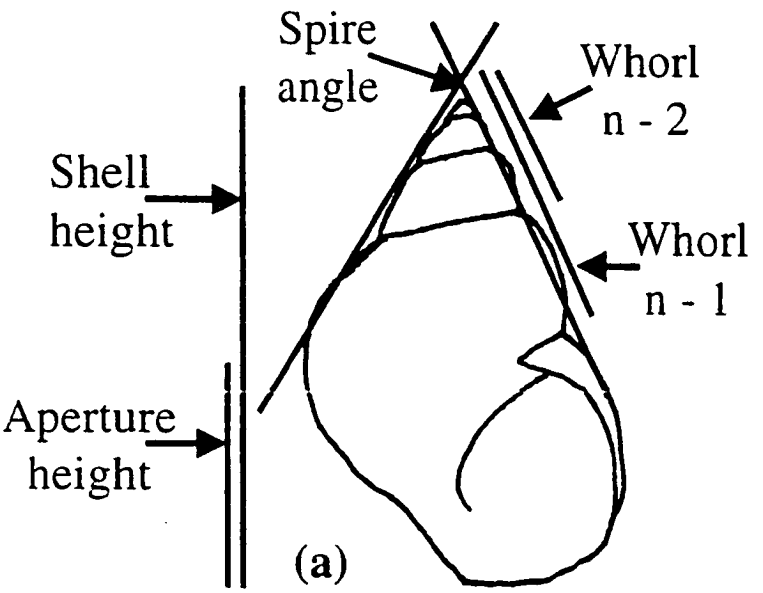
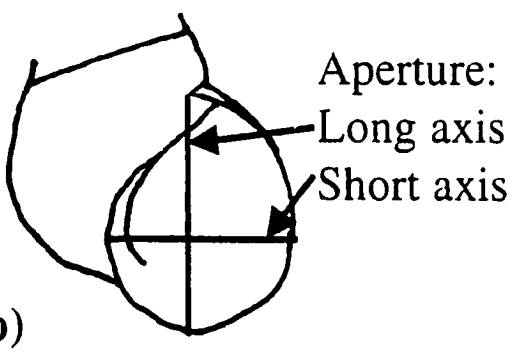


Figure 2.3: Quantitative shell measurements. Shells were viewed through a dissecting scope and video camera connected to a computer, and measurements were taken using imaging software. The two angles were measured in degrees and the six lengths in mm.





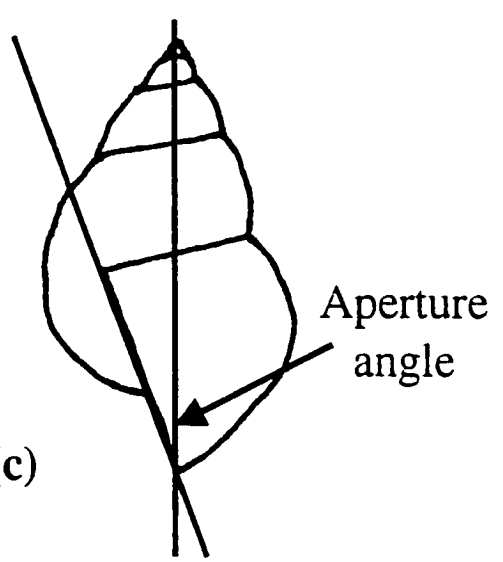
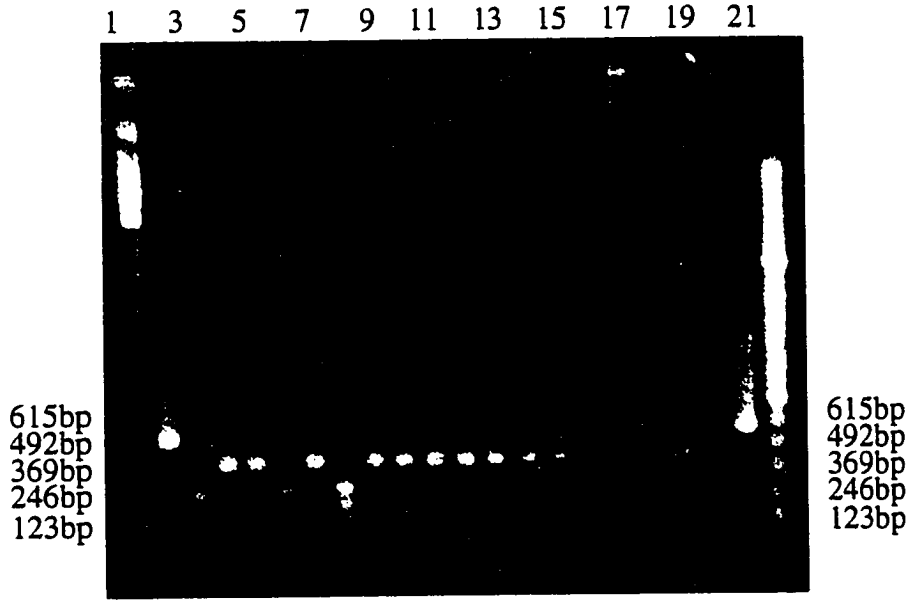


Figure 2.4: Agarose gel electrophoresis of restriction enzyme digestion products. Lanes 1 and 22: 123 bp ladder. Lanes 2 and 21: undigested PCR-amplified cytochrome b gene, showing one band at 480 bp. Lanes 3, 6, 8, and 17: *L. scutulata* cytochrome b gene, digested with Alu I, showing bands at 233 bp, 161 bp, and 86 bp. Lanes 4, 5, 7, 9-15, and 18-20: *L. plena*, with bands at 356 bp and 109 bp.



CHAPTER 3

Distribution and Speciation of the Sympatric Sibling Gastropods *Littorina scutulata* and *L. plena*

Abstract

This study examines the distributions of the sympatric sibling gastropod species *Littorina scutulata* and *L. plena* along the two habitat gradients of wave-exposure and tidal height. The species overlap broadly, but *L. plena* is slightly more widely distributed. Contrary to previous work, *L. plena* was found from wave-exposed, open-coast bedrock shores to protected cobble beaches, while *L. scutulata* was found in moderately exposed to protected habitats. Where they co-occur, *L. plena* extends slightly higher into the littoral fringe than its sibling species. One hypothesis for the occurrence of *L. plena* in more wave-exposed sites, that its foot size and tenacity are better adapted to resist dislodging by waves, is rejected; the species do not differ in either respect.

L. plena has been shown to exhibit higher levels of genetic population structure than *L. scutulata*. The hypothesis that this is the result of a more restricted distribution causing isolated populations is rejected by the wider distribution of *L. plena*. These results also have implications for the mode of speciation that produced these species. Despite their currently overlapping ranges, sympatric speciation is unlikely because of their nearly congruent habitats. Given the relatively ancient estimates for their divergence time (mean 8.47 mya) and potential for rapid range expansion with planktotrophic larvae, vicariant allopatric speciation is the most likely explanation.

Introduction

Taxonomic history. Gould (1849) described three similar species of the intertidal gastropod genus *Littorina* inhabiting the Pacific coast of North America: *L. scutulata, L. lepida,* and *L. plena*. The first two species were collected from Puget Sound and their shells distinguished by the latter's more prominent checker pattern, reddish aperture with white basal band, and more prominent spiral sculpture. *L. plena* was collected in San Francisco and described as being more ovate, with a globose last whorl and smaller aperture.

Carpenter (1864) united these three species into one, *L. scutulata*, and subsequent authors (e.g. Oldroyd 1924; Keep & Baily 1935; Abbott 1954) maintained this view until

Murray (1979) described dimorphic penis and egg capsule morphologies within *L. scutulata*. Discriminant function analysis of shell measurements (Murray 1982) revealed two shell morphotypes corresponding with these differences in reproductive anatomy. Mastro et al. (1982) studied allozyme frequencies and found differences greater than those between other recognized species in the genus, providing strong evidence for genetic divergence and status as separate species. Examining the original type specimens, Mastro et al. (1982) assigned Murray's (1979) type I to *L. plena* and type II to *L. scutulata*. Reid (1996) synonymized Gould's (1849) *L. lepida* under *L. scutulata*. Further morphological characters and a molecular tool for distinguishing the two currently recognized species are discussed in Reid (1996), Rugh (1997), and chapter 2.

Because *L. scutulata* and *L. plena* were long recognized as one variable species and are not easily distinguished in the field, many ecological studies have treated them as one species. This may have obscured differences between the species in food preferences (e.g. Foster 1964; Jensen 1981) and other ecological factors. Chow (1975) found a size gradient of snails by tidal height and attributed it to physical factors and to differential migration of different size classes of snails. However, this study may have included individuals of both species and been confounded by differences between the species in size (Murray 1982; chapter 2) and in tidal height distribution (this chapter). Several studies (Dahl 1964; Bock & Johnson 1967; Behrens Yamada 1992) apparently lumped the two species and found *L. scutulata sensu lato* to have a wider ecological niche than other *Littorina* species considered. This could be interpreted as the overlapping niches of two separate species, a situation which may be a common source of confusion in the marine world (Knowlton 1993).

Phylogeny and speciation. Despite the complex taxonomic history of *L. scutulata* and *L. plena*, a range of evidence supports the following conclusions: 1) They are sibling species; 2) The *L. scutulata/L. plena* clade branched off near the base of the monophyletic genus *Littorina*; and 3) The two species are relatively old, having diverged perhaps over 10 million years ago.

Reid (1986) identified two consecutive spiral loops in the pallial oviduct as an apomorphy uniting the genus *Littorina*. Reid (1989) used *L. plena* and 4 other groups within the genus in a morphological phylogeny of the family Littorinidae. The genus remained monophyletic, with two species (*L. striata* and *L. keenae*) branching off early, and *L. plena* forming a sister taxon to the remainder of the genus. A subsequent morphological phylogeny of 18 species in the genus (Reid 1990a) produced a *L. scutulata/L. plena* clade in the same position. The two species were united by three

synapomorphies: tall-spired shell, egg capsule with two peripheral rims, and more than one egg per capsule. The morphological phylogeny of Reid et al. (1996) added a fourth synapomorphy uniting these two species, the presence of a glandular projection of the penial filament, and produced a phylogeny consistent with that of Reid (1990a).

Molecular cladistic analyses have been largely consistent with morphological results though not as clearly resolved. Rumbak et al. (1994) sequenced the mitochondrial gene 12S rRNA from 11 *Littorina* species, including *L. scutulata* and *L. plena*, and two of three cladistic methods grouped these species as sibling species with weak bootstrap support. Reid et al. (1996) added 16S rRNA and cytochrome-b sequences as well as the remaining 8 species in the genus to this dataset. The *L. scutulata/L. plena* clade received weak support, supported by only one analysis of the 16S rRNA sequences and unresolved in other analyses. However, the consensus tree of Reid et al. (1996), combining morphological and molecular data, places the two species together as sibling species. Analysis of the nuclear genes 18S and 28S rRNA (Winnepenninckx et al. in press) have lent further support to sibling species status and were mostly consistent with Reid et al.'s (1996) consensus phylogeny of the genus.

Molecular data suggest a relatively ancient divergence of *L. scutulata* and *L. plena*. Using the allozyme frequency data of Mastro et al. (1982), Reid et al. (1996) estimated a divergence age of 1.38 to 2.39 mya. However, their estimates based on 12S and 16S rRNA are much older, ranging from 2.48 to 18.63 with a mean of 12.81 mya. Another calculation, based on recently published cytochrome b sequences, is presented here.

The fossil record of *Littorina* is relatively poor. Reid (1996) gives several examples of Pliocene and Pleistocene fossils from California which resemble both species and assigns them to *L. scutulata*. The species *L. remondii* of California has a basal carina and outer calcite layer which suggest an affinity to *L. plena* (Reid 1996). However, the occurrence of *L. remondii* in the Late Miocene postdates the Middle Miocene divergence estimate for *L. scutulata* and *L. plena*, so it is best interpreted as a related species rather than a direct ancestor.

Several factors may have obscured the historical biogeography of the species and make it difficult to determine their mode of speciation: the poor fossil record, the relatively ancient divergence, the dispersal abilities of these planktotrophic developers on a continuous north-south coastline, and climatic fluctuations since speciation (Crame 1993). Reid (1996) suggests that the most parsimonious hypothesis is sympatric speciation, but that allopatric speciation followed by range expansion and overlap must still be considered. Within the entire family Littorinidae, allopatric speciation of the vicariant (large-

subdivision) type appears to dominate on the basis of current distributions (Reid 1994). However, sympatric reproductive isolation as a result of different habitat preferences has been observed in the family (Johannesson, Rolan-Alvarez, & Ekendahl 1995; Takada 1995). If *L. scutulata* and *L. plena* exhibit significant differences in habitat, even on shores where both occur, this could have provided a mechanism for reproductive isolation and speciation in sympatry.

Genetic variation. Ward's (1990) analysis of data from Mastro et al. (1982) showed that *L. plena* has more genetic differentiation among populations than *L. scutulata*, and that both species have higher levels of genetic variation than other *Littorina* species with planktotrophic larvae. Similarly, Kyle (1998) examined population structure from the two species along the coast from Washington to Alaska, using the mitochondrial gene cytochrome b. Thirty-six haplotypes were found in *L. plena*, while only 18 were found in *L. scutulata*. In addition, distribution of haplotypes showed geographic structure in *L. plena* populations but not in *L. scutulata*. These data suggest that despite the species' similarity, *L. plena* has lower levels of gene flow among its populations. One hypothesis for this result is that *L. plena* is more restricted to certain habitats, so that its populations are more isolated from each other and less likely to exchange genes. Burton & Swisher (1984), Black et al. (1994), Hellberg (1995), and Planes et al. (1996) provide examples of marine animals in which patchy populations exhibit reduced gene flow across stretches of unsuitable habitat.

Distribution and Habitat. Because of the taxonomic difficulties in these species, data on their ranges and habitat preferences are minimal. Reid (1996) studied museum collections of both species and found that *L. scutulata* ranges from southeast Alaska to southern Baja California and *L. plena* ranges from Kodiak Island to northern Baja California. On the basis of these collection sites, Reid (1996: p. 67) states that "*L. scutulata* tends to predominate in the more exposed localities, whereas in very sheltered situations and in salt marshes *L. plena* may occur alone." Other workers have found *L. plena* in more exposed sites (S. Behrens Yamada, pers. comm.). Slight differences in exposure preference in species with generally overlapping ranges are common in the genus *Littorina* (e.g. Mill & Grahame 1990; Boulding & Van Alstyne 1993). On a local scale, several sympatric groups of *Littorina* species also appear to separate themselves by tidal height (Underwood 1972; Mill & Grahame 1990; Behrens Yamada 1992; Williams 1995). To date, differences in tidal height distribution have not been examined in *L. scutulata* and *L. plena*.

Tenacity. One important adaptation of organisms living on hard substrates in the intertidal is resistance to dislodging by wave action. Gastropod species differ in tenacity and in foot morphology depending on their adult habitat (Miller 1974a; 1974b); species adapted to high wave-energy habitats tend to have longer, broader feet and greater tenacity. In the genus *Littorina*, tenacity is a factor in microhabitat preferences in sympatric species (Davies & Case 1997) and in local adaptation and phenotypic plasticity within a single species (Trussell 1997). Miller (1974a) found *L. scutulata* to have one of the highest tenacity/foot area measures among the gastropods studied (466+/- 28 g/cm^2 foot area for a force normal to the substrate on a crawling snail), appropriate to its habitat in the high intertidal of relatively wave-exposed shores. However, her study predates the taxonomic resurrection of *L. plena*, and she describes a bimodal habitat distribution for the species along a wave exposure gradient of sampling sites. This suggests that she may have combined *L. scutulata* and *L. plena* in her analysis.

This chapter will examine the large-scale and small-scale distributions of the sibling species *L. scutulata* and *L. plena*. The purposes of this study are four-fold: 1) To review data on the two species' relative abundances in the broad zone of overlap of their ranges; 2) To determine how the species differ in their habitat preferences along gradients of wave-exposure and tidal height; 3) To test whether *L. plena* has a more restricted and patchy distribution that could constrain gene flow among its populations; and 4) To look for clues to their mode of speciation -- specifically to determine whether small-scale habitat differences could be sufficient to produce reproductive isolation in sympatry. I also test the hypothesis that a difference in distribution along a wave-exposure gradient correlates with interspecific differences in foot morphology or tenacity.

Methods

Distribution. Unpublished data were compiled from two sources on relative abundances of *L. scutulata* and *L. plena* at sites along the west coast of North America from southern California to Vancouver Island. In both cases, snails were collected from the intertidal and brought into the laboratory for identification. S. Behrens Yamada identified snails using egg capsule morphology (Murray 1979) and therefore classified only females (collections from Washington and Oregon). E. Mastro used penis morphology (Murray 1979) and therefore classified only males (collections ranging from Vancouver Island to southern California).

In order to study a range of habitats from exposed open coast to sheltered cobble beaches at a similar latitude, further sampling for this study was concentrated on the

Washington coast and in Puget Sound. Animals of all sizes were collected from an area of shore and identified by penis morphology (Murray 1979) or restriction fragment analysis of the cytochrome b gene (chapter 2). These sites were also scored for wave exposure: exposed (facing the open ocean), moderately exposed (five or more km of open water), or protected (less than five km of open water), and habitat: bedrock, boulders (larger than 0.3m in diameter and not moved by typical wave action; includes man-made jetties), or cobbles (smaller than 0.3m in diameter).

Tidal height sampling was performed at two sites in Washington at which the species co-occur: Cattle Point on San Juan Island, a south-facing, moderately exposed bedrock shore, and Kopachuck State Park in southern Puget Sound, a northwest-facing, protected cobble beach. At Cattle Point, three vertical transects were sampled in August and September of 1999. At each of four tidal heights along the transect (1, 1.5, 2, 2.5 m above MLLW), all the snails within a 0.25 m^2 area were collected and brought back to the lab for identification to species by penis morphology and the discrete morphological characters described in chapter 2. Numbers of the sympatric congener *L. sitkana* were also recorded. At Kopachuck, one transect was sampled in April of 2000. Again, all the snails were collected from 0.25-m^2 areas, in this case at five tidal heights (1, 1.5, 2, 2.5, 3 m above MLLW) because of the larger tidal range at this site. Neither species was found 0.5m above or below these transects. For four of the collections from the highest density areas, snails were counted and only a randomly chosen subset identified to species in the interest of efficiency.

Tenacity and foot morphology. Individuals of both species were collected from the same shore at Cattle Point, San Juan Island, Washington, and identified to species using the discrete morphological characters described in chapter 2. Shell heights were measured with an ocular micrometer on a dissecting microscope. To measure foot length, width, and area, the snails were photographed with a digital camera mounted on a dissecting microscope as they crawled upside down on a glass slide. The images were transferred to a computer and measurements were taken with imaging software. Small metal hooks were fixed with epoxy to the shells of 18 *L. scutulata* and 16 *L. plena* individuals, covering a range of shell sizes in each species. Hooks were oriented perpendicular to and centered over the snails' feet.

Tenacity was measured on each individual with the apparatus shown in figure 3.1. Snails were placed on the substrate in filtered seawater and the spring scale attached to their hook. When the animal began to crawl normally, tensional force on the spring scale was smoothly increased until the animal released from the substrate, and the maximum force

was recorded. Each individual was tested in this way ten times on a glass substrate and ten times on smooth rock, and the substrate was cleaned between individuals. Because of the variability of measurements for each individual (as found by Miller 1974a and Davies & Case 1997), including several very low values, the maximum force for each set of ten trials was used in all further analyses.

Results

Distribution. At the largest scale, both species co-occur along the west coast of North America. Figure 3.2 shows proportions of the two species at various sites, including collections by S. Behrens Yamada, by E. Mastro, and for this study. Stars indicate sites at which both species were found but no counts were made. The species co-occur on the same shore at 70% of the 46 sites sampled. Of the 14 sites where only one of the two species was found, only three had exclusively *L. scutulata*. Thus despite the broad overlap of the two species, *L. scutulata* seems to be more restricted in its distribution.

Within Washington, the full range of habitats was sampled within a relatively narrow band of latitude. Figure 3.3 shows the proportion of *L. plena* at each site, total sample size, wave exposure, and substrate. Again the species broadly overlap, but only *L. plena* is found at two of the three most exposed outer coast sites. Both species are found at moderately exposed and sheltered boulder sites and at sheltered cobble beaches. The only site at which *L. scutulata* is found exclusively is Eastsound in the San Juan Islands, a result which is consistent with other samples shown in Figure 3.2. *L. plena* is only rarely found in the moderately exposed and sheltered interior shores of the archipelago but is common on the outer shores, such as Cattle Point.

L. scutulata and *L. plena* differ significantly in tidal height distribution, though they overlap across most of the shore. Figure 3.4 shows densities of these species and the congener *L. sitkana* at four tidal heights along three transects at the moderately exposed Cattle Point site, and five tidal heights along one transect at the protected Kopachuck State Park site. Both species were found at all tidal heights at Cattle Point, though not in all transects, and *L. scutulata* was absent from only the highest tidal level at Kopachuck. The distributions differ significantly along two of the three Cattle Point transects (Kolmogorov-Smirnov test; $p<0.001$ for both; Figure 3.4b,c) and along the Kopachuck transect (K-S test; $p<0.001$; Figure 3.4d). Snail density is much higher overall in the protected cobble beach site (Figure 3.4d), where *L. plena* makes up a greater proportion of the snail numbers. *L. sitkana*, which never reaches large abundances in Puget Sound (A.J. Kohn,

pers. comm.), was found mostly in lower densities than its congeners along these transects.

Tenacity and foot morphology. No difference was found between the two species in foot morphology. Within each species, shell height is highly correlated with foot length, foot width, and foot area ($p<0.001$ for all), but the two species do not differ significantly in any of these relationships (Table 3.1). Figure 3.5 shows the relationship between shell height and foot area for the specimens used.

Snails had greater tenacity on the rock substrate than on glass (paired t-test; $p=0.034$). On rock substrate, *L. scutulata* had an average tenacity of 174.4 +/- 12.4 g/cm^2 (force over foot area +/- S.E.) and a maximum of 273.0 g/cm^2, while *L. plena* averaged 180.2 +/- 11.9 g/cm^2 with a maximum of 258.9 g/cm^2. On glass, *L. scutulata* averaged 172.5 +/- 14.2 g/cm^2 with a maximum of 324.5 g/cm^2, while *L. plena* averaged 164.3 +/- 15.3 g/cm^2 with a maximum of 330.8 g/cm^2. Within each species, tenacity on rock was significantly correlated with both shell height and foot area ($p<0.001$ for all), but again no significant difference was found between the species (Table 3.1). Figure 3.6 shows the relationship between tenacity and foot area for both species.

Discussion

Distribution and Habitat. Along both the wave-exposure gradient and the tidal height gradient, the northeast Pacific sibling species *L. scutulata* and *L. plena* broadly overlap, but in both cases there are significant differences in their distributions. Contrary to previous work (Reid 1996), *L. plena* was found to range widely from protected cobble beaches to exposed bedrock shores on the open coast. The distribution of *L. scutulata* was somewhat more restricted to protected and moderately exposed habitats. As a result, surveys along the west coast of North America found both species co-occurring at most sites, several sites occupied only by *L. plena*, and very few sites occupied only by *L. scutulata*. Similarly, the species' tidal height distributions are mostly congruent, with *L. plena* perhaps extending farther into the littoral fringe. Though the tidal height distributions are statistically different, they do not suggest substantial differences between the species in resource use or habitat preference.

The broad overlap in tidal height distribution suggests that previous sampling (as shown in Figure 3.1) may have incorrectly quantified the relative abundances of the two species where they co-occur, but it was unlikely to have missed one of the species altogether. These data also allow us to reexamine some of the studies before 1979 that may have included both species as *L. scutulata*. Chow (1975) studied animals from Bodega

Head, California, where both species are found (Figure 3.2). He found a general trend of increasing shell size with increasing tidal height, the opposite of what one would expect from combining the larger size (chapter 2) and lower tidal distribution (Figure 3.4) of *L. scutulata* compared to *L. plena*. He attributed this trend to differential vertical migration of different size classes, and it seems likely that his results were not an artifact of interspecific differences.

Behrens Yamada (1992) considered *L. scutulata* and *L. plena* as one species and found it to have the widest ecological niche of the *Littorina* species studied. Was this an artifact of lumping two species together? Here I found *L. plena* occupying the entire length of the two habitat gradients examined, and *L. scutulata* nearly so. Thus *L. plena* appears to occupy the full niche described by Behrens Yamada (1992), and *L. scutulata* occupies a slightly narrower, completely overlapping niche.

Tenacity. One hypothesis for the presence of *L. plena* exclusively in the most wave-exposed habitats is that this species' foot morphology and tenacity are better adapted to withstanding wave action. This hypothesis was rejected here: the species are indistinguishable in both characters.

Though the difference was very slight, snails had higher tenacity on the rock substrate than on glass, which conflicts with previous work. However, the maximum tenacity in each species was recorded on the glass substrate. Davies & Case (1997) found a significant tenacity improvement for *L. littorea* and *L. obtusata* on a polished rock substrate compared to a rougher substrate, and concluded that muscular action was not the limiting factor in attachment. Miller (1974a) reviewed several mechanisms that are likely to play a role in gastropod attachment: suction, simple adhesion (by a thin film of liquid), adhesion with sticky secretions from the foot surface, and muscular action. She found that oxygen is required for attachment, so muscular action cannot be ruled out entirely. Pedal secretions are also probably important (Miller 1974a), and the strength of these may depend not only on the rugosity but also the molecular nature of the substrate. Davies & Case (1997) used rock polished to varying degrees, thus eliminating molecular differences. However, Miller (1972) tested *Nucella* (previously*Thais*) *emarginata* on smooth plexiglass and rock substrata and recorded higher tenacity on plexiglass for a force normal to the substrate. Further investigation is needed into the mechanisms of attachment and their effectiveness on different substrata.

Genetic population structure. Ward (1990) and Kyle (1998) provided evidence of more restricted gene flow among populations of *L. plena* than among populations of *L. scutulata*. One hypothesis is that a more restricted distribution in *L. plena* could lead to

more isolated populations in a stepping-stone gene flow model (e.g. Burton & Swisher 1984; Black et al. 1994; Hellberg 1995; Planes et al. 1996). This hypothesis is rejected here: the two species co-occur in most habitats, but *L. plena* has a slightly wider distribution, the opposite of what is expected.

Speciation. Reid et al. (1996) presented estimates for the age of divergence based on the allozyme frequency data of Mastro et al. (1982; distances recalculated by Ward 1990) and on their own sequences of 12S and 16S rRNA genes (see Table 3.2). The mean of the nucleotide estimates from Reid et al. (1996) is 12.81 mya, which places the divergence in the middle Miocene, predating the 9.60 mya that the same authors estimate for the polychotomy from which the clade arose. To these data I have added an estimate based on sequences of the mitochondrial cytochrome b gene from Kyle (1998). Reid et al. (1996) give calibration values for third-position transitions (0.131 mya per 1% divergence) and transversions (1.358 mya per 1% divergence) based on paleontological and biogeographical evidence in the genus. Using these calibration values on all pairwise comparisons among 36 *L. plena* and 18 *L. scutulata* haplotypes provides mean estimates of 2.98 mya (transitions) and 2.82 mya (transversions) (Table 3.2). This lowers the mean of all estimates to 8.42 mya, but the wide range of estimates (1.38 to 18.63 mya) warrants further study.

In the genus *Littorina*, it is useful to consider the Recognition species concept of Paterson (1978; 1985), in which the Specific-Mate Recognition System (SMRS) defines species. This genus exhibits internal fertilization, and recognition of potential mates often occurs after copulation is initiated (Saur 1990 and references therein). The complementary morphology of the male and female reproductive characters, then, is critical in determining whether fertilization takes place, and so reproductive anatomy constitutes the SMRS. As with the sibling species examined here, many similar, often sympatric, species in the genus *Littorina* are best distinguished on the basis of reproductive anatomy (Reid 1996). While species often exhibit high levels of intraspecific variation in other characters, such as shell morphology (Reid 1996; chapter 2), reproductive anatomy remains relatively constant. This matches the pattern predicted for the Recognition species concept, since change in somatic or "economic" characters (food capture, predator avoidance, etc.) is uncoupled from the features that produce reproductive isolation and define the species (Eldredge 1995; Vrba 1995). Other groups of marine animals also exhibit reproductive isolation without correlated morphological differentiation (Knowlton 1993; Norris et al. 1996). Change in the SMRS is both necessary and sufficient for the reproductive isolation that leads to speciation.

In this case, we would like to know what mode of speciation produced *L. scutulata* and *L. plena*. Bush (1975) defined four modes of speciation: sympatric, parapatric, large-subdivision (vicariant) allopatric, and founder-event allopatric. Parapatric speciation requires limited mobility to maintain a narrow hybrid zone, and selection against hybrids to favor reproductive isolating mechanisms (reinforcement) (Bush 1975). However, these two species have long-lived planktotrophic larvae (chapter 4) and hence the potential for long-distance dispersal. Speciation by reinforcement has also been discounted as an important mechanism in laboratory experiments (Rice & Hostert 1993) and natural populations (Paterson 1978; Vrba 1995 and references therein). Parapatric speciation is therefore not a likely candidate for these species.

Given these species' current overlapping ranges, sympatric speciation must be considered as the most parsimonious hypothesis (Reid 1996). Sympatric speciation also requires some degree of reinforcement and has thus been regarded as unlikely (e.g. Felsenstein 1981) except in extreme cases of habitat specificity, such as parasite-host races (Bush 1994). However, one mechanism important for sympatric isolation is assortative mating, often associated with microhabitat separation, which has been documented in *Littorina* species. Takada (1995) documented seasonal migration in the planktotrophic developer *L. brevicula* that produced two sub-populations during the breeding season. The sub-populations were centered on two different tidal heights and formed nearly, though not completely, discrete groups as a result of different individual migratory behavior. Johannesson, Rolan-Alvarez, & Ekendahl (1995) studied *L. saxatilis*, a highly polymorphic species with brooded development. In this case, two morphotypes occupied different tidal levels, with a narrow zone of overlap in between, and assortative mating is the product both of this microhabitat separation and of mating behavior. Reduced gene flow continues between the two morphotypes, and there do not seem to be any clear variants in reproductive anatomy that correlate with shell morphotypes (reviewed by Reid 1996). In both cases, it is difficult to know whether these situations are equilibrium states or sympatric speciation in progress.

The current distributions of *L. scutulata* and *L. plena* do not show the kind of separation seen between sub-populations of *L. brevicula* or *L. saxatilis*. Along both the wave-exposure and tidal height gradients, the species broadly overlap, with *L. plena* extending slightly farther into wave-exposed and littoral fringe habitats. Both species were found together in 15 of the 17 0.25-m^2 quadrats sampled (Figure 3.4). If these species are the product of sympatric speciation, it must be assumed that microhabitat separation at the time of speciation has been obscured by subsequent reinvasion of each others' niches.

This would require disruptive selection prior to speciation, followed by relaxation of that selection.

The two types of allopatric speciation described by Bush (1975), vicariant and founder-event, form two ends of a continuum. Reid (1994; 1996) views the founder-event model as uncommon in the family Littorinidae on the basis of relative sizes of current ranges of sibling species. Planktotrophic development in the ancestors to *L. scutulata* and *L. plena* also argues against the formation of a small, isolated population. Vicariant speciation in this case requires that one or both species subsequently expanded their range to produce the 80% overlap seen today (Reid 1996). Reid (1996) found no correlation between age of divergence and degree of range overlap for other sister-taxa pairs in the genus, arguing against this model. However, the 8.42 mya divergence estimate presented here for *L. scutulata* and *L. plena* remains older than estimates for the 9 other sister-taxa pairs considered by Reid (1996). These divergence times include clades of up to 5 species and range from 1.32 to 6.42 mya (Reid 1996). Climatic fluctuations have been shown to produce large-scale range shifts in both terrestrial (Davis 1987; FAUNMAP Working Group 1996) and marine (Addicott 1969; Vermeij 1989; Lindberg 1991; Crame 1993; Fields et al. 1993; Barry et al. 1995) taxa. Shuto (1983) showed a positive correlation between species age and range size in Indo-West Pacific *Murex*. Marko (1998) demonstrated a case of allopatric speciation followed by range expansion and overlap in the North American genus *Nucella*. The degree of overlap in these species is much less than shown here for *Littorina*, but their age of divergence is also younger, less than 1 to 2 mya (Collins et al. 1996; Marko 1998), and their offspring develop in benthic egg masses and hence have reduced dispersal potential. These *Littorina* species have two additional advantages for rapid range shifts: the continuous north-south habitat of the Pacific coast of North America, and their planktotrophic mode of development with its potential for long-distance dispersal. Even if *L. scutulata* and *L. plena* diverged as recently as 2 million years ago (Table 3.2), this would provide enough time for their ranges to expand, contract, and shift along the North American coast. In fact, current distributions represent significant shifts since the Pleistocene glaciation. Additionally, the data presented here suggest that these two species occupy remarkably similar ecological niches. The most parsimonious hypothesis, that this similarity reflects the niche of the common ancestor, argues for allopatric speciation. Under the Recognition species model, change in the SMRS and speciation does not require a niche shift, but only allopatry can allow divergence. Such speciation without clear ecological shifts may be common in the marine world (Knowlton

1993). Of the four modes of speciation described by Bush (1975), then, vicariant allopatric speciation seems the most likely for these sibling species.

Table 3.1: Results of statistical analyses on foot morphology measures and tenacity on rock substrate. Separate regression analyses were performed within species. All foot morphology measures are significantly correlated with shell height, and tenacity on rock is significantly correlated with shell height and foot area. Differences between the species in these relationships were tested with a Student's t-test (Zar 1984); the species do not differ.

Dependent variable	Independent variable	Within species			Species differences	
		species	r	p	slope	intercept
Foot area	Shell height	*L. scut.*	0.937	<0.001	>0.5	>0.5
		L. plena	0.886	<0.001		
Foot length	Shell height	*L. scut.*	0.838	<0.001	>0.5	>0.2
		L. plena	0.735	<0.001		
Foot width	Shell height	*L. scut.*	0.973	<0.001	>0.5	>0.5
		L. plena	0.825	<0.001		
Tenacity	Shell height	*L. scut.*	0.868	<0.001	>0.5	>0.2
		L. plena	0.807	<0.001		
Tenacity	Foot area	*L. scut.*	0.896	<0.001	>0.2	>0.5
		L. plena	0.901	<0.001		

Table 3.2: Estimates of the age of divergence of *L. scutulata* and *L. plena*. Where multiple references are given, the first provides the raw data and the second (and third) provide the calculations.

Molecule	Divergence estimate (mya)	Reference
Allozyme frequencies	1.38-2.39	Mastro et al. (1982)/ Ward (1990)/ Reid et al. (1996)
12S rRNA transitions	18.17	Reid et al. (1996)
12S rRNA transversions	2.48	Reid et al. (1996)
16S rRNA transitions	11.94	Reid et al. (1996)
16S rRNA transversions	18.63	Reid et al. (1996)
cytochrome b transitions	2.98	Kyle (1998)/ this study
cytochrome b transversions	2.82	Kyle (1998)/ this study
mean of all estimates	8.42	

Figure 3.1: Diagram of apparatus used to measure snails' tenacity to rock or glass substrate. Force was gradually increased using the micromanipulator, and the maximum force required to pull the snail off the substrate was recorded in grams.

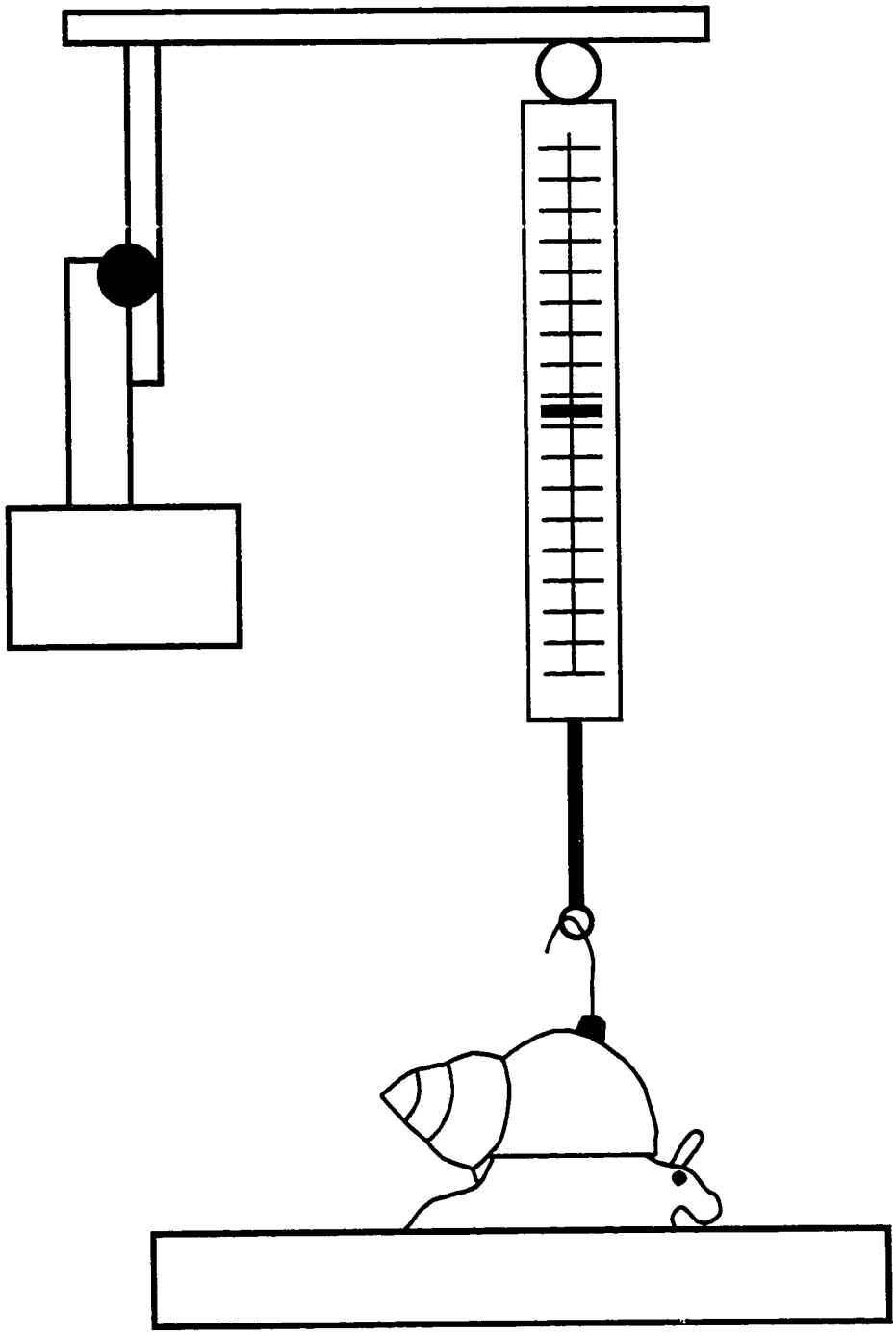


Figure 3.2: Map of the west coast of North America showing proportion of *L. plena* among total sample size of *L. plena* and *L. scutulata*. Collector is indicated by M (E. Mastro), Y (S. Behrens Yamada) or H (the author) following the collection site. Sites at which only one species was found are shown in bold. .

Source	n	*L. plena*
Denman I. (M)	84	0.39
Orcas I. North (H)	26	0.31
Eastsound (H)	38	**0.00**
Friday Harbor (M)	95	0.02
Roche Harbor (M/Y)	90	0.05
Anacortes (M/Y)	150	0.02
False Bay (M)	25	0.60
Tatoosh I. (M/H)	191	0.86
Clallam Bay (H)	26	0.69
Rialto Beach (H)	15	**1.00**
Fort Worden (H)	65	0.35
Termination Pt. (H)	13	**1.00**
Seabeck (H)	23	0.87
Sinclair Inlet (H)	45	0.71
Kopachuck SP (H)	70	0.67
Point Brown (H)	67	**1.00**
Siletz Bay (M/Y)	45	0.91
Newport (M/Y)	87	0.37
Sawyers Landing (M/Y)	30	0.81
Yachats (Y)	3	0.33
Neptune Beach (M)	*	*
Bob Creek (M)	33	**1.00**
Umpqua R. (H)	10	**1.00**
Coos Bay (M/H)	157	0.97
Cape Arago (M)	*	*
Trinidad (M)	*	*
Point Arena (M)	270	0.79
Sea Ranch (M)	156	0.35
Mussel Pt. (M)	164	0.55
Bodega Bay (M)	225	**1.00**
Dillon Beach (M)	23	0.65
Berkeley marina (M)	34	0.97
San Fransisco (M)	205	**1.00**
Santa Cruz (M)	20	**1.00**
Monterey (M)	23	0.09
Morro Bay exposed (M)	39	**1.00**
Morro Bay protected (M)	75	0.89
San Luis Obispo (M)	82	0.61
Gaviota (M)	358	0.94
Goleta (M)	73	0.97
Santa Barbara (M)	205	**1.00**
Oxnard (M)	31	**1.00**
Palos Verde exposed (M)	60	**0.00**
Palos Verde protected (M)	60	**0.00**
Laguna Beach (M)	391	0.13
San Diego (M)	39	0.26

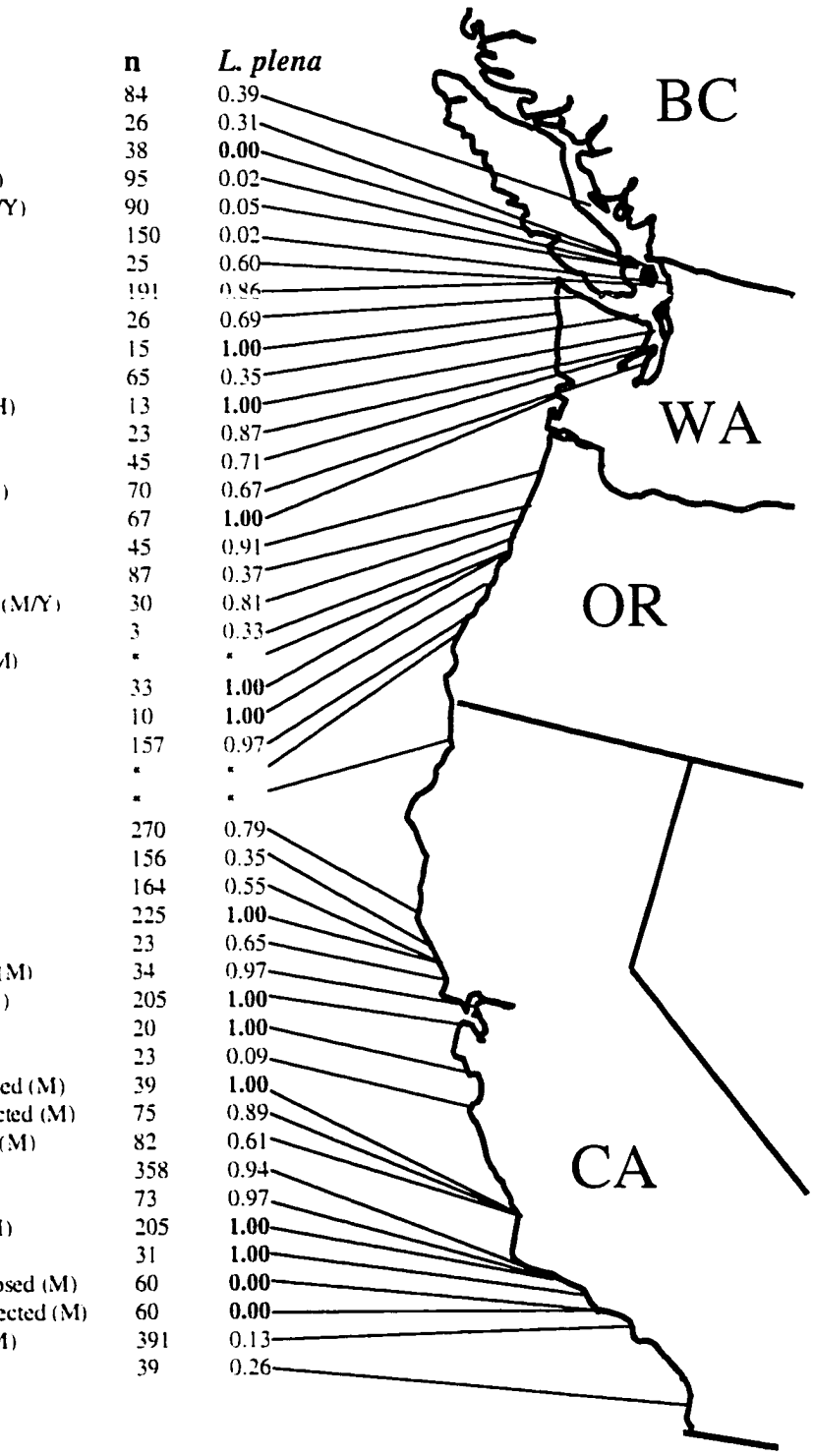


Figure 3.3: Map of western Washington showing proportion of *L. plena* among *L. plena* and *L. scutulata*, with total sample size, wave exposure, and substrate. All collections by the author.

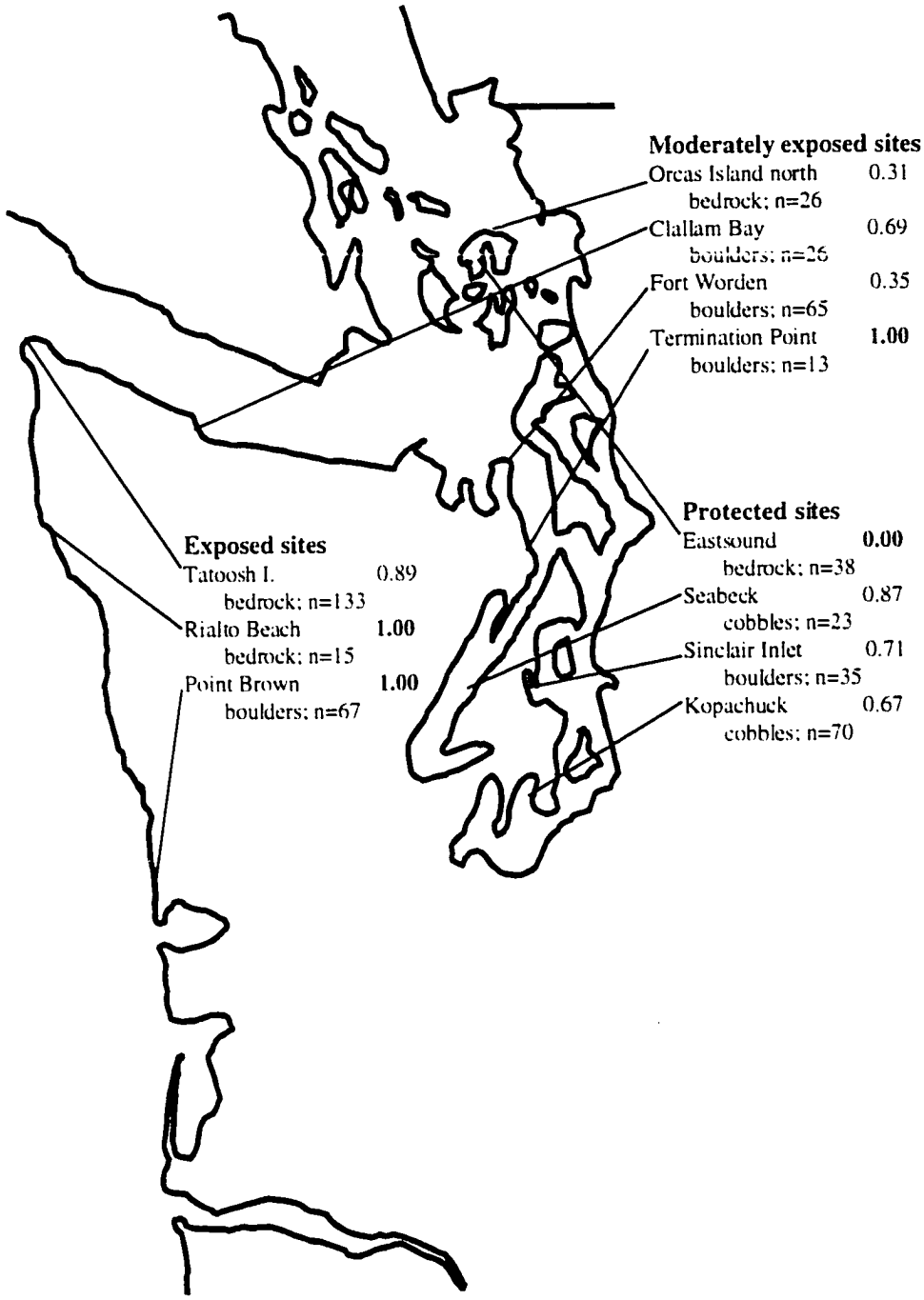
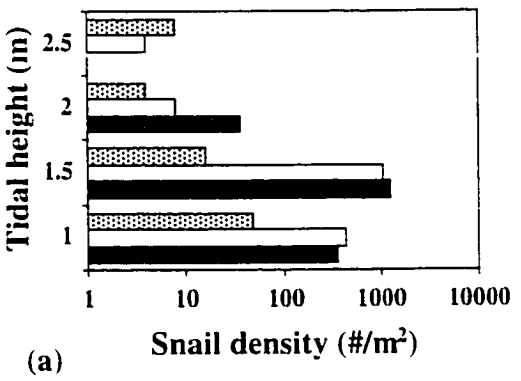
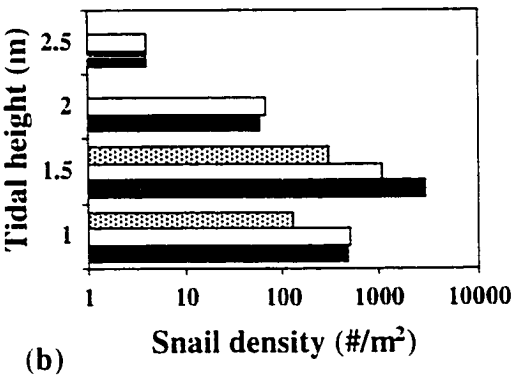


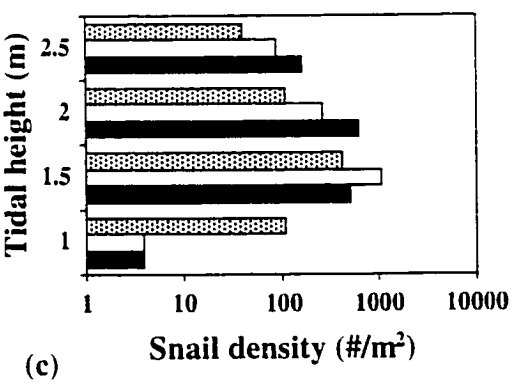
Figure 3.4: Densities of three *Littorina* species according to tidal height along three transects (a-c) at Cattle Point, San Juan Island, Washington, a moderately exposed bedrock shore, and one transect (d) at Kopachuck State Park, Washington, a protected cobble beach. At each site, one 0.25-m^2 quadrat was sampled at each tidal height. Black bars are *L. plena*, white bars are *L. scutulata*, and stippled bars are *L. sitkana*. Tidal heights are measured above MLLW. *L. scutulata* individuals are distributed significantly lower than *L. plena* in b, c, and d (Kolmogorov-Smirnov test; $p<0.001$ for all cases).



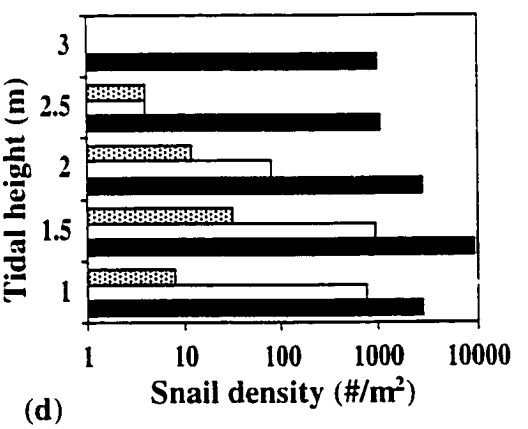
(a)



(b)



(c)



(d)

Figure 3.5: Foot area correlates with shell height in *L. scutulata* (open squares) and *L. plena* (dark squares), but the species do not differ statistically (see Table 3.1).

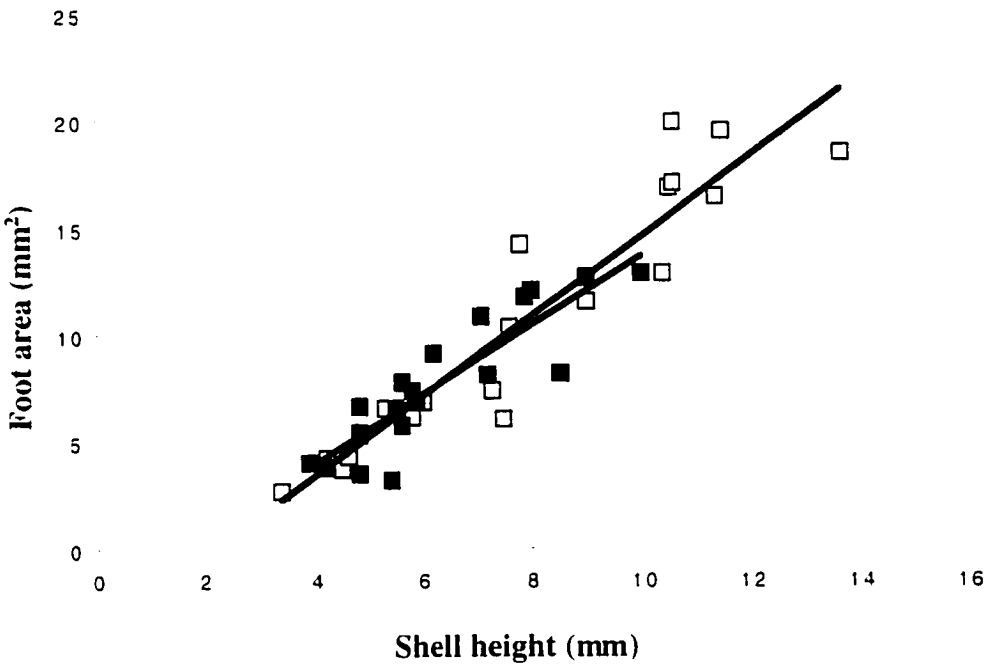
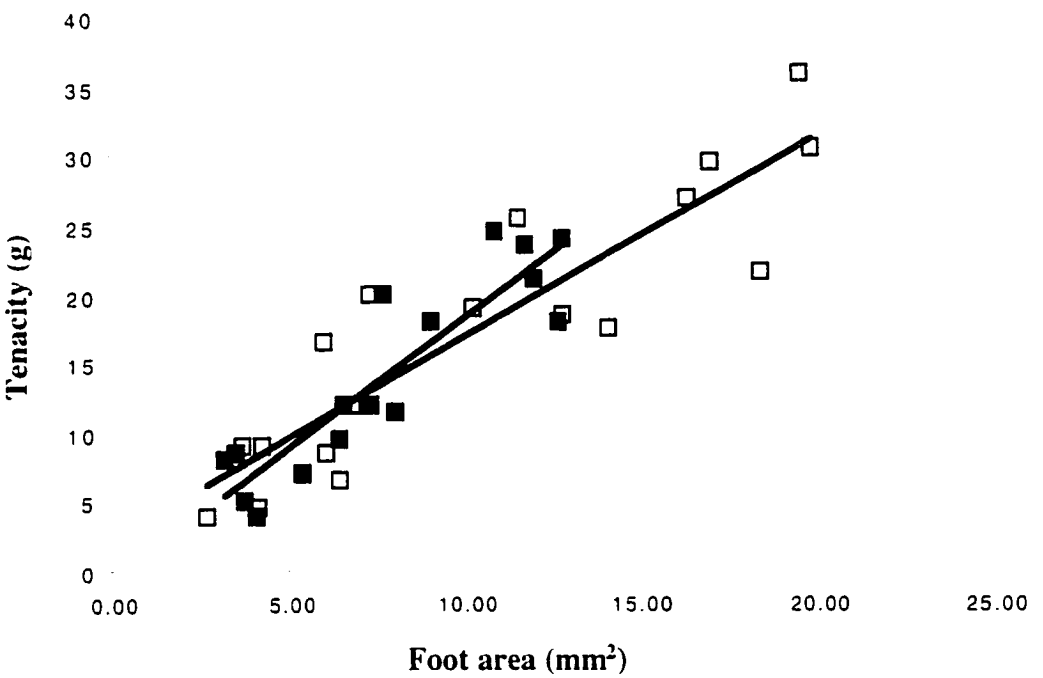


Figure 3.6: Tenacity, measured as the maximum force required to pull a snail off a flat rock substrate. Tenacity correlates with foot area in *L. scutulata* (open squares) and *L. plena* (dark squares), but the species do not differ statistically (see Table 3.1).



CHAPTER 4

Life History of *Littorina scutulata* and *L. plena*, Sympatric Sibling Species With Planktotrophic Larvae

Abstract

The intertidal, sibling species *Littorina scutulata* and *L. plena* (Gastropoda; Prosobranchia) are sympatric throughout most of their ranges along the Pacific coast of North America. Both species release disc-shaped, planktonic egg capsules from which planktotrophic veligers hatch. Here I review existing data and present new observations on these species' life history, including age at first reproduction, maximum fecundity rates, capsule morphology, egg size, pre-hatching development, larval growth at different food concentrations, settlement, and protoconch size. Previous classification of egg capsule morphologies used to distinguish the species is inaccurate; instead, capsules can be categorized into three types of which each species may produce two. *L. scutulata* females produce capsules with either two rims of unequal diameters or one rim. *L. plena* females produce capsules with one rim or with two rims of nearly equal diameter. Females of each species spawn sporadically from early spring to early fall in Puget Sound. *L. plena* larvae hatch one day earlier than *L. scutulata* larvae, and both species grew fastest in the laboratory at intermediate food concentrations. Larvae metamorphosed in response to a variety of materials collected from their adult habitat, including adults, algae, rocks, and barnacle tests. The total planktonic period of 8 *L. scutulata* larvae raised through metamorphosis in the laboratory ranged from 37 to 70 days, and a single *L. plena* larva metamorphosed after 62 days. Protoconch sizes on shells collected from the field range from 256 to 436 μm in diameter and did not differ significantly between the species. Previous allozyme and mitochondrial DNA work has suggested high levels of genetic variability in both species and greater genetic population structure in *L. plena*, despite the long spawning season and teleplanic larvae in both species. The life history differences between the species described here are insufficient to produce consistent differences in gene flow patterns.

Introduction

Thorson (1946; 1950) classified modes of development among marine invertebrates into several types depending on their nutritional mode and site of development. Planktotrophic larvae feed as they develop, while lecithotrophic larvae depend on maternal yolk in the egg for nutrition. Planktotrophic larvae are typically planktonic, developing in the water column, while lecithotrophic larvae may be pelagic, develop in attached benthic egg masses, or be brooded by a parent. With a few alterations, these major categories remain useful (Jablonski & Lutz 1983; Levin & Bridges 1995). The marine gastropod genus *Littorina* exhibits three modes of larval development among its 19 species (Reid 1996). The nine basal members of the clade release planktonic egg capsules containing one or several eggs which develop into planktotrophic veliger larvae. Lecithotrophic development probably arose once in the genus and is currently found in nine species which lay benthic egg masses from which crawl-away juveniles hatch (Reid 1990a; 1996). From these ancestors evolved a third mode of development, found in the single polymorphic species *L. saxatilis*, in which developing offspring are brooded in the pallial oviduct until the juvenile stage. The sibling species *L. scutulata* and *L. plena*, which are sympatric over most of their ranges along the Pacific coast of North America, are members of the planktotrophic group.

Several authors (Scheltema 1971, 1986a; Shuto 1974; Crisp 1978; Grassle & Grassle 1978; Jablonski & Lutz 1983) have made the prediction that gene flow among populations should be higher in species with planktotrophic larvae, because of their greater dispersal capacity. With its various modes of development and ecologically similar adults (Reid 1996), the genus *Littorina* offers a test of this hypothesis. Ward (1990) reviewed molecular genetic work on the genus and found the patterns of genetic population structure to match this prediction, with a few notable exceptions. However, the planktotrophic developers *L. scutulata* and *L. plena* appear to have high levels of genetic variation among populations, similar to that found in some of the benthic developing species (Ward 1990). In addition, Ward's (1990) recalculation of allozyme data from Mastro et al. (1982) and DNA work on the mitochondrial cytochrome b gene (Kyle 1998) indicate that *L. plena* has more population genetic structure, and hence more restricted gene flow, than its sibling species *L. scutulata*. These species are remarkably similar in many respects: their ranges overlap by 80% (Reid 1996), they broadly overlap in habitat preference along both tidal-height and wave-exposure gradients (chapter 3), and both species release planktonic egg capsules from which multiple planktotrophic veligers hatch. These species' overall

similarity and apparent difference in gene flow patterns provide a model system for identifying the factors responsible for limiting gene flow.

There are several examples of marine animals with planktotrophic larvae that exhibit restricted gene flow (Palumbi 1995), and the factors that could account for these exceptions are numerous (Scheltema 1986b; Palumbi 1994). Some of these factors are basic life history traits: length and timing of spawning season, and length of planktonic period, including both precompetent and competent periods. Investigation of spawning season is relatively straightforward, but measuring the planktonic period of marine larvae in nature is more challenging. Two indirect approaches are available: laboratory studies of larval growth and settlement, and examinations of larval structures that are retained in the adult.

Identification of settlement cues has allowed detailed measurement of precompetent and competent periods of other marine molluscs in the laboratory (Hadfield 1984; Coon et al. 1985; Yool et al. 1986; Pechenik & Heyman 1987; Pechenik & Gee 1993), and levels of intraspecific variation are often high (Scheltema 1986b). In the prosobranch *Crepidula fornicata*, slower larval growth can extend the competent period (Pechenik 1980), while faster growth can shorten the precompetent period (Pechenik 1984; Pechenik & Lima 1984). Temperature (Zimmerman & Pechenik 1991) and intrinsic developmental schedules (Pechenik et al. 1996a) also play a role in timing of settlement. Sensitivity to cues increases over time in the oyster *Crassostrea gigas* (Coon et al. 1990) and in the opisthobranch *Haminea callidegenita* (Gibson 1995). Some species exhibit geographic variation in response to settlement cues (Schubart et al. 1995). In an extreme example, larvae of the prosobranch *Fusitriton oregonensis* survived as veligers for four and a half years in the absence of settlement cues, and were still competent to metamophose (M. Strathmann, pers. comm.). Some species, particularly those with a wide range of settlement cues and adult habitats, may have a short and relatively constant competent period (e.g. Davis 1994). However, most species seem to fit the pattern predicted by Jackson & Strathmann (1981) that selection should favor flexibility in timing of metamorphosis and a large ratio of competent to precompetent period (Scheltema 1986b). Intraspecific variation in larval growth rates has also been documented in several species, both with a genetic basis (Newkirk et al. 1977; Stromgren & Nielsen 1989; Hilbish et al. 1993; Pechenik et al. 1996b) and without an apparent genetic basis (Hadfield 1984). These studies were carried out in the laboratory, but they suggest that planktonic period in nature varies widely, depending on temperature, larval food conditions, presence of and sensitivity to settlement cues, and intraspecific genetic variation.

Information about planktonic larval development can also be inferred from adults in a variety of taxa (Jablonski & Lutz 1983; Emlet 1989). In prosobranch gastropods, the larval shell, or protoconch, is typically retained at the apex of the adult shell, or teleoconch, and can illustrate mode of development (Shuto 1974; Bouchet 1990). Hickman (1995a) described four features in which a sudden shift may mark the protoconch/teleoconch boundary: the shape of the outer lip, shell sculpture, shell microstructure, and geometry of shell coiling. Surveys of gastropod species have illustrated these changes effectively using scanning electron microscopy (SEM) (Rodriguez Babio & Thiriot-Quiévreux 1974, 1975a, 1975b; Thiriot-Quiévreux & Rodriguez Babio 1975). Formation of the post-hatching larval shell of planktotrophic species (protoconch II) involves accretionary growth of an organic matrix, but most of the calcification typically occurs after metamorphosis (Hickman 1995b). This sometimes results in delicate sculptures on the protoconch that are not seen on the teleoconch. Hickman (1995a) described a staged metamorphosis in the Hawaiian neogastropod *Carinapex minutissima* in which the mantle retreats at metamorphosis and begins accretionary growth beneath the surface of the protoconch. The aperture lip of the protoconch with two velar notches is underlain by the growing teleoconch with a straight lip. The mantle continues to advance and retreat as it begins to form the teleoconch, producing a pattern of imbricate layers.

If one of these changes marks the protoconch/teleoconch boundary, the size of the protoconch can be measured on adult shells. If larval growth rates are known, planktonic period could be calculated from protoconch size, but the variability in growth rates and timing of metamorphosis discussed above makes such estimates suspect. A significant difference in protoconch size between species with similar early larval development, however, may suggest a difference in planktonic period.

Life history factors, such as spawning season and planktonic period, combine with temporal variation in ocean current patterns to determine the variation in distance and direction that larvae travel from a single location. This interaction will be examined with simulation models in chapter 5. Here I review existing data and present new observations on several aspects of the planktonic life histories of *L. scutulata* and *L. plena*. These features include spawning season, age at first reproduction, short- and long-term fecundity, morphology and variation in eggs and egg capsules, larval growth and development, metamorphosis, variation in planktonic period, and shell growth before and after metamorphosis. I discuss the relevance of these data to gene flow and population genetic structure.

Existing data

The complex taxonomic history of *Littorina scutulata* and *L. plena* makes interpretation of existing life history data more difficult. Until 1979, they were grouped as one species. Buckland-Nicks et al. (1973) and other studies may have included data from both species. Murray (1979) distinguished them on the basis of penis and egg capsule morphology, and subsequent work has identified further molecular and morphological characters (see chapter 2 for a review). However, here I will show that previous egg capsule distinctions are inaccurate.

Egg capsule morphology. Murray (1979) and Mastro et al. (1982) described two egg capsule morphologies that correspond to the two currently recognized species. *L. scutulata* females were found to produce transparent egg capsules with two unequal rims (type A in Figure 4.1). Murray (1979) included in this category the egg capsules described by Buckland-Nicks et al. (1973), which are shaped like saucers with only one rim (type B in Figure 4.1). Reid (1996) considers both of these capsule types to be *L. scutulata,* with diameters of the larger rim ranging from 700 to 1000 µm. Murray (1979) described *L. plena* capsules as slightly larger with two equal rims (type C in Figure 4.1), about 1100 µm in diameter, with usually 17 to 32 eggs in each (Murray 1979).

There may be geographic variation in number of eggs per capsule in *L. scutulata*: From Puget Sound, Behrens (1971) reported egg capsules with 1 to 6 eggs each, and Buckland-Nicks et al. (1973) described capsules with 3 or 4 eggs. Murray (1979) described capsules from southern British Columbia with a mean of 2.9 eggs, but means of 4.2 to 10.4 eggs per capsule for California snails.

Spawning season. Length and timing of spawning season varies widely among marine species (Strathmann 1987) and is often triggered by physical or biological cues (Morgan 1995a). Intraspecific genetic variation in spawning season may also be common (Barber et al. 1991). Within the family Littorinidae, spawning cues include lunar cycles (Berry 1986a), temperature increase (Chase & Thomas 1995), tide cycles (Schmitt 1979; Struhsaker 1966), and light (Struhsaker 1966). Previous work suggests that *L. scutulata* and *L. plena* spawn sporadically from early spring to early fall. Buckland-Nicks et al. (1973) described the spawning season on San Juan Island, Washington, as May through September with a peak in July, though both species may have been included. Behrens Yamada (1989) reported finding egg capsules around southern Vancouver Island from March to October, though again both species may have been included. In northern California, Chow (1987b) distinguished the two species on the basis of penis and egg

capsule morphology, allozyme loci, and discriminant analysis of shell characters. He found each to spawn from March to October. However, never more than 25 percent of females of either species spawned in any single month, so individual females may concentrate their reproduction at different times during the spawning season. Buckland-Nicks (1974) noted that females usually spawn in the laboratory during the morning and evening.

Fecundity. Mastro et al. (1982) reported that *L. plena* females reach sexual maturity at 2 to 3 mm shell height, while *L. scutulata* females reach maturity at 4 to 7 mm. This pattern matches the larger overall size in *L. scutulata* (Murray 1982; chapter 2). These sizes can be combined with growth rates for each species given by Chow (1987b) to conclude that both species reach sexual maturity within a year. Murray (1979) reported that *L. scutulata* eggs are slightly larger (105 µm in diameter) than *L. plena* eggs (96 µm).

Buckland-Nicks et al. (1973) recorded the egg capsule production of three females over two weeks and found an average of 1109 capsules, or about 3900 eggs, per female. Murray (1979) measured egg capsule production for 31 *L. scutulata* females over 2 weeks and recorded a maximum fecundity of 6807 eggs for one female. Chow (1987b) reported peak egg production of 1500 eggs per day in *L. plena*, and 1000 eggs per day in *L. scutulata*. These data suggest lower energy allocation to reproduction in both these species compared to the congener *L. keenae* (Chow 1987b).

Planktonic period. Behrens (1971) and Buckland-Nicks et al. (1973) reported that embryos take 7 to 8 days at 13° to 15°C to develop to hatching as a veliger larva, 160 µm in diameter. Murray (1979) gave developmental schedules for both species and found *L. plena* to hatch after 7 days and *L. scutulata* to hatch after 8 days at 15°C. No one has reported raising any planktotrophic *Littorina* species through metamorphosis, but there is indirect evidence of the planktonic development period following hatching. Buckland-Nicks et al. (1973) raised veligers for 25 days after hatching to a size of 300 to 360 µm. Reid (1996) measured protoconch sizes of 240 µm in "poorly preserved available specimens" of *L. scutulata* and 300 µm in *L. plena*, suggesting that the larvae of Buckland-Nicks et al. (1973) may have been competent to settle at 25 days post-hatching. Chow (1989) observed a peak of settlement of *L. plena* in fall and winter in California, occurring several months after the peak spawning in spring and summer in the same region (Chow 1987b).

Thorson (1946) measured larvae of the congener *L. littorea* caught in several plankton samples off Denmark and concluded that larvae spend a month or more in the plankton and metamorphose at a shell size of 275 µm. Thorson (1946) also found that *L. littorea* larvae raised in the lab under favorable conditions grew to 600 to 750 µm after 6 weeks without metamorphosing, suggesting that larvae of this species may extend their competent period considerably and continue to grow in the plankton. Struhsaker & Costlow (1968) reared the related Hawaiian species *Nodilittorina hawaiiensis* (as *Littorina picta*; see Reid 1989) through metamorphosis in the laboratory. Larvae hatched at 110 to 120 µm after 3 days and metamorphosed at 250 µm after an average of 27 days post-spawning at 25 °C. However, shells of post-veligers collected from local habitats showed greater variation in number of whorls and overall size, sometimes reaching 1000 µm. Struhsaker & Costlow (1969) reported that this species can delay metamorphosis and continue to grow in the laboratory for up to 8 weeks in the absence of suitable substrate for settlement.

Methods

For much of this research, the egg capsule descriptions of Murray (1979) and Reid (1996) were used to identify capsules to species. All capsules of type A or B were classified as *L. scutulata*, until it was discovered that this distinction was inaccurate. In some cases, additional morphological information about the mother was recorded to allow independent species identification. In other cases, however, egg capsules and larvae may have been of either species. These situations are indicated in the results.

Spawning season. Periodically during the spring and summer of 1997 through 2000, snails were collected from sites on the west side of San Juan Island, Washington, where both *L. scutulata* and *L. plena* are found in roughly equal numbers. They were brought into the laboratory and submerged in filtered seawater, either together in a large container or separately in culture wells. Snails were kept for at least a week, and production of egg capsules recorded.

Periodic plankton samples were also taken from April through August in 1997 and 1998, and April through June in 1999, in San Juan Channel, Washington at rising or high tide. A total of 2.9 m^3 of the top 10 m were sampled with a 250 µm net drawn vertically through the water column, collecting egg capsules but not hatched veligers of the two

species. However, *L. scutulata* occurs almost exclusively on the adjacent shores and very few *L. plena* egg capsules were found, so these data provide evidence only for the spawning season in *L. scutulata.*

Fecundity. Thiry-one *L. scutulata* females were collected from three sites on San Juan Island, Washington, and kept submerged with conspecific males in filtered seawater in separate culture wells for 3 months. Snails were fed cultured microalgae, and egg capsule production was recorded periodically. Capsule morphology and maximum diameter were recorded on a sample of capsules from each spawning event.

Larval development. Capsules of both species were spawned simultaneously in July 1999 and maintained at ambient seawater temperature (12-14°C). Timing of prehatching development was measured for 32 *L. scutulata* and 57 *L. plena* eggs.

Three broods of egg capsules were split into four treatments and grown at different concentrations of the microalgae species *Isochrysis galbana* and *Rhodomonas lens:* minimal food with $5x10^2$ cells/mL of each algae species, low with $5x10^3$ cells/mL of each, medium with $5x10^4$ cells/mL of each, and high with $5x10^5$ cells/mL of each. Since cells of *R. lens* are larger than those of *I. galbana*, treatments actually contained more of the former species by volume. For each treatment, approximately 50 larvae were kept in 100 mL of unstirred filtered seawater and kept at ambient seawater temperature (12-14°C). Water was changed and replenished with food every 3 to 4 days. Shell diameters of a sample of up to 9 larvae were measured every 6 to 8 days. Using regression analyses on data from each treatment, four different growth models were tested to determine which best explained the relationship between time and size of larvae. These models included linear, natural logarithmic, and power functions of degree 3 and 5.

Settlement. To determine planktonic period, attempts to induce settlement used larvae from the growth experiments described above, as well as larvae raised under similar conditions and fed concentrations of 10^5 to 10^6 cells/mL of *I. galbana* and *R. lens.* Materials with potential settlement cues collected from the intertidal, including conspecific adults, rocks, empty barnacle tests, and algae, were periodically introduced, starting as early as 12 days post-hatching.

Protoconch size. Snails of both species were collected from two protected sites in southern Puget Sound and one on San Juan Island. Since small specimens were chosen, only males were used to ensure correct identification to species. The soft tissue was removed and the shells prepared in a variety of ways adapted from Hickman (1995a). Methods included soaking in a dilute bleach solution, cleaning in a sonicator, and etching for 1 to 10 minutes in dilute hydrochloric acid, all followed by drying with 95% ethanol.

Specimens were then mounted on SEM stubs with conductive carbon adhesive, sputter-coated, and viewed in a Jeol JSM-35 scanning electron microscope. To measure protoconch diameter, photographs were taken from an apical view.

Results

Egg capsule morphology. Three egg capsule types were produced by these species (Figure 4.1). Type A capsules resembled those pictured for *L. scutulata* by Murray (1979) and Mastro et al. (1982). They had two rims of unequal diameter, with the smaller rim often somewhat upturned as in Figure 4.1. The diameter of the smaller rim ranged from 57 to 83 percent (mean 61 percent) of the diameter of the larger rim. Type B capsules resembled the capsule shown in Buckland-Nicks et al. (1973). They had only one rim, and the diameter of the opposite side ranged from 55 to 73 percent (mean 64 percent) of the diameter of the rim. Type C capsules had two rims of nearly equal diameter (smaller diameter greater than 90 percent of the larger) and resembled capsules shown for *L. plena* by Murray (1979) and Mastro et al. (1982).

L. scutulata females produced capsules of either type A or type B (Figure 4.1). Only one type was produced during a single spawning event, but 20 of the 39 females kept in the laboratory for 3 months produced capsules of each type, sometimes within a week. Of the remainder, 17 females produced only type A capsules and 2 females produced only type B capsules. Egg capsules ranged from 337 to 976 µm outer diameter and contained 1 to 11 eggs, typically 2 to 4 (mean = 2.7; see Figure 4.2). Outer diameter was significantly correlated with number of eggs in the capsule ($r=0.703$; $p<0.001$). Plankton-collected capsules were larger than lab-spawned, and type B capsules were larger than type A capsules (ANCOVA; $p<0.001$ for both factors). However, diameters overlapped broadly across both of these factors (see Figure 4.2). No seasonal pattern was detected in the frequency of the two capsule types, either in the lab or in plankton samples.

L. plena females produced capsules of either type B or type C. Though no *L. plena* females were kept in the laboratory for longer than two weeks, one female produced capsules of each type in two separate spawning events. At least 22 females identified as *L. plena* by tentacle coloration and discrete shell characters produced only type B capsules, and at least 7 *L. plena* females produced only type C capsules. The two types differed in number of eggs and outer diameter (see Figure 4.3). Type B capsules ranged from 730 to 1160 µm outer diameter and contained 2 to 11 (mean = 5.0) eggs. Type C capsules ranged

from 841 to 1340 µm outer diameter and contained 6 to 47 (mean = 19.0) eggs. Within both types, number of eggs per capsule is significantly related to capsule diameter (type B: Y = 725.7 + 30.4X, r=0.71, p<0.001; type C: Y = 756.8 + 17.4X, r=0.682, p=0.001), though the slopes from these two regression analyses differ significantly (Student's t test (Zar 1984); p<0.001). Again, no seasonal pattern was detected in the production of the two capsule types.

Within type B capsules, *L. plena* capsules tended to be larger and have more eggs. The slopes of the two lines shown in Figure 4.3 do not differ (Student's t test; p>0.5), but the intercepts differ significantly (p<0.001). Thus the discriminant function Y = 693 + 15E, where E is the number of eggs, correctly classified 98.6 percent of the 211 type B capsules shown in Figure 4.3, where the identity of the mother was known. Capsules larger than Y were almost exclusively *L. plena*, and those smaller were almost certainly *L. scutulata*.

Spawning season. Figure 4.4 shows densities of *L. scutulata* eggs (in capsules) collected in the plankton and spawning of both species in the laboratory. There was no difference between the species in spawning season. Both species began spawning in early April and continued until early October.

Fecundity. Both species were reproductively mature by 2 to 3 mm shell height: one *L. plena* male measuring 1.8 mm had a fully developed penis, and several female *L. scutulata* measuring under 3 mm produced egg capsules. Total fecundity of *L. scutulata* females was significantly related to shell height (regression analysis on log-transformed data: ln(Y) = 3.83 + 0.37X, where Y=total number of eggs and X=shell height in mm; r=0.508; p=0.004) but varied widely to almost 10,000 eggs over 3 months (see Figure 4.5). *L. scutulata* eggs measured 95.0 +/- 4.3 µm in diameter (n=40) and were significantly larger (t-test; p<0.001) than *L. plena* eggs (90.2 +/- 2.0 µm; n=12).

Larval development. At 12-14°C, *L. scutulata* veligers hatched after 9 days and *L. plena* hatched after 8 days. This trend paralleled the difference found by Murray (1979) at a higher temperature. Pre-hatching development is summarized in Table 4.1. Veliger shells appeared unsculptured, and the aperture lip formed a horn between two velar notches. Sinusigeral growth lines differentiated the post-hatching shell (protoconch II) from the smooth pre-hatching shell (protoconch I) (Figure 4.6). At hatching, the shell was planispiral, but translation down the axis of coiling began about 90° after hatching. As the larvae grew, the right velar lobe became larger than the left, perhaps to support the shell

apex. Velar cilia were 20-30 μm long. Juvenile structures developed gradually as the larvae grew. With medium or high food concentrations, a foot appeared within the first two weeks and continued to enlarge and become muscular and agile. Anterior tentacles with eyespots at their base appeared in the third week. After four weeks the animals began crawling on the substrate with their velar lobes still extended, presumably testing for appropriate settlement cues.

Figure 4.7 shows growth of larvae at four different food concentrations from one representative *L. scutulata* brood. Similar patterns were seen in one brood of *L. plena* and one undetermined brood. Of the four growth functions tested, the one that best fit the data was of the form

$$S^3 = \alpha + \beta d, \qquad (4.1)$$

where S is shell diameter (μm), d is time (days), and α and β are coefficients determined by linear regression for each treatment. The slope β can be considered as the growth rate for each treatment. This function suggests that total mass, which should vary roughly as the cube of this diameter, increased linearly during larval development. Growth rates (β from equation 4.1) for each treatment are given in Table 4.2. Growth rate varied significantly among food concentrations (2-way ANOVA; $p=0.0078$), and no significant growth was observed at the minimal food concentration (see Table 4.2). In posterior Tukey comparisons (Zar 1984), growth rate differed significantly only between minimal and medium ($p<0.025$) and between minimal and high ($p<0.05$) treatments. However, all broods followed the pattern seen in Figure 4.7 that growth rate was highest at the medium food concentration. Growth was faster in *L. plena* than in *L. scutulata*, though the difference was not statistically significant (2-way ANOVA; $p>0.1$).

Planktonic period. A total of 15 larvae metamorphosed in the laboratory: 8 *L. scutulata*, one *L. plena*, and 6 larvae that may have been of either species. The undetermined larvae hatched from type B capsules, and neither the capsules nor the mothers were identified to species. Only larvae fed at the medium or high food concentrations metamorphosed. Larvae settled in response to barnacle shells, intertidal rocks, conspecific adults, and in the absence of any introduced cue. Total planktonic period for *L. scutulata*, including pre-hatching development, varied from 37 to 70 days,

and the *L. plena* larva metamorphosed at 62 days. The undetermined larvae metamorphosed at 48 to 61 days. Sizes of *L. scutulata* at metamorphosis ranged from 320 to 408 μm, with a mean of 355 μm, and there was no relationship between age and size at metamorphosis ($p>0.5$; $n=9$). Figure 4.8 shows a recently metamorphosed *L. scutulata* crawling on the barnacle shell on which it settled. The protoconch/teleoconch boundary is clearly visible. Figure 4.9 shows a newly metamorphosed juvenile shell under SEM, and again the horn of the protoconch aperture lip is clearly visible. As described by Hickman (1995a) for *Carinapex minutissima*, new accretionary growth of the juvenile shell begins beneath the protoconch lip. Even in lab specimens, however, this evidence of the protoconch/teleoconch in the outer shell layers is quickly worn away.

Protoconch size. Most shells of both species collected from the field were very degraded, and the degree of degradation seemed to vary consistently among collection sites. No surface sculpture, such as the boundary shown in Figure 4.9, was ever seen, but some parts of the shell microstructure remained. As in *Carinapex minutissima* (Hickman 1995a), the outer calcified layer, which in *L. scutulata* and *L. plena* is irregular prismatic calcite (Taylor & Reid 1990), showed a clear imbricate pattern (see Figure 4.10). Toward the apex of the shells this layer was typically worn away, but remnants of it were still visible in the sutures, where the imbricate pattern is seen in cross section. On the shell in Figure 4.10, the imbricate pattern begins only at 327 μm shell diameter.

This suggests a model for shell growth in this species: the larval shell is laid down with relatively little calcification (Hickman 1995b). At metamorphosis, as in *Carinapex*, accretionary growth begins beneath the lip of the protoconch and fills in the velar notches. Further accretionary growth involves some advance and retreat of the mantle, which produces the imbricate pattern seen in the irregular prismatic layer that begins only after metamorphosis. The protoconch is calcified simultaneously, not by the mantle edge, but by the entire mantle surface. As the shell continues to grow at the aperture, the protoconch is filled with shell material until it is solid, as seen in these specimens. Since this infilling takes place after metamorphosis and continues into the teleoconch as the snail grows, it should not retain any evidence of the protoconch/teleoconch boundary.

The point where the imbricate pattern in the irregular prismatic layer begins thus marks metamorphosis, and this point was used to measure the size of the protoconch in 8 *L. scutulata* and 25 *L. plena* shells. Protoconch size in *L. scutulata* ranged from 293 to 436 μm (mean = 338.9 μm), and in *L. plena* it ranged from 256 to 405 μm (mean = 330.2

μm). The species did not differ significantly (t-test; $p>0.5$). Protoconch size in field-collected *L. scutulata* also did not differ significantly from lab-raised speciment (t-test; $p>0.5$).

Discussion

The data presented here combine with previous studies (see Table 4.3) to show that the life histories of the sympatric sibling species *L. scutulata* and *L. plena* are very similar. Most of the differences are associated with the egg capsules, but previous descriptions of diagnostic egg capsules are incorrect. As described by Mastro et al. (1982), capsules with two rims of unequal diameter (type A in Figure 4.1), typically containing 2 to 4 eggs, are only produced by *L. scutulata*, and larger capsules with two rims of nearly equal diameter (type C), containing 10 to 40 eggs, are only produced by *L. plena*. However, saucer-shaped capsules with only one rim (type B), described first in Buckland-Nicks et al. (1973) and assigned to *L. scutulata* by Murray (1979) and Reid (1996), can in fact be produced by either species. The diameters of these capsules can still be used successfully to distinguish species. Any functional difference among the capsule morphologies is unclear.

However, the results of this study provide further evidence for geographic variation in the life history characters of these species from the Puget Sound area to California, and capsule morphology may vary as well. Buckland-Nicks et al. (1973) first described type B capsules from Puget Sound. Murray (1979) assigned these capsules to *L. scutulata*, but pictures a capsule with two unequal rims (type A) from his own California collections. Similarly, Mastro et al. (1982) describe California *L. scutulata* capsules as having "two rims of very different diameters" (type A), and do not report finding any capsules with one rim (type B). Further work on egg capsule morphology is needed throughout these species' ranges.

Eggs per capsule and egg size may also vary geographically. Murray (1979) found more eggs in *L. scutulata* capsules from California compared to those from southern British Columbia. The results of Buckland-Nicks et al. (1973), Behrens (1971), and this study, all from the Puget Sound area, are consistent with this result. Murray's (1979) egg size measurements for California snails are larger in both species than those reported here for Puget Sound. Murray (1979) also reported lower fecundity in California *L. scutulata* compared to Puget Sound, though this appears to be based on inaccurate recalculations of data from Buckland-Nicks et al. (1973).

The life history data summarized here do not suggest any differences in dispersal potential that could explain the greater genetic subdivision of populations found in *L. plena* by Ward (1990) and Kyle (1998). Interspecific differences in capsule morphology and diameter are unlikely to affect dispersal. All capsule types are slightly negatively buoyant and should behave similarly as passive particles in the water column. Murray (1979) and this study found *L. plena* larvae to hatch one day earlier than *L. scutulata* larvae. Pre-hatching period may be related to egg size as in *Conus* (Perron 1981). The longer pre-hatching period in *L. scutulata* would prevent siblings in an egg capsule from dispersing away from each other for an additional day. On the other hand, *L. plena* capsules, especially type C, tend to have more eggs, thus keeping larger numbers of siblings from dispersing away from each other before hatching. However, these effects are likely to be minor, since any differences in dispersal of capsules, spread of siblings, or pre-hatching development would affect no more than about 20 percent of the total planktonic period.

Chow (1989) reported higher peak daily fecundity in *L. plena* compared to *L. scutulata* in California. The current study and Murray (1979) both found smaller eggs in *L. plena* which correspond with a 17 to 30 percent difference in egg volume between the species. These data suggest that *L. plena* may invest a roughly equal amount of energy in a larger number of smaller eggs than *L. scutulata*. Accordingly, *L. plena* larvae were found here to hatch at a smaller size, though Murray (1979) found the opposite result. Unfortunately, weekly or seasonal fecundity estimates for *L. plena* are not available for comparison. The high level of variability in *L.scutulata* egg production shown here also makes fecundity comparisons more difficult, but interspecific differences in fecundity appear to be slight. Such differences are unlikely to account for differences in dispersal or gene flow.

One might expect spawning season to affect gene flow among populations by determining the variability in ocean currents encountered by larvae, and thus the geographic range of new recruits from a given source. However, both *L. scutulata* and *L. plena* spawn broadly from spring to early fall, and their planktotrophic larvae presumably encounter the same range of seasonal ocean currents.

One might similarly expect total planktonic period to affect gene flow by determining dispersal potential. For example, Waples (1987) found a significant inverse correlation between planktonic period and population genetic differentiation in 10 species of marine fish. Kohn & Perron (1994) found a significant correlation between minimum planktonic period and geographic range in the gastropod genus *Conus*, though Scheltema (1989) found no such pattern. Here, the single *L. plena* larva raised through

metamorphosis in the laboratory had a total planktonic period within the range of lab-raised *L. scutulata*, though near the maximum value. If these species follow the relationship between egg size and precompetent period found in *Conus* by Perron & Kohn (1985), *L. plena* should have a longer precompetent period. If *L. plena* larvae on average have longer planktonic periods in nature, one would expect higher levels of gene flow and less genetic subdivision in this species, the opposite of what was found by Kyle (1998).

Sizes of protoconchs on snails collected from the field show no difference, suggesting that larvae of both species metamorphose at the same size. Though the difference was not statistically significant, the *L. plena* larvae raised in the laboratory showed higher growth rates than *L. scutulata*. Taken together with the similar sizes at metamorphosis, this could indicate a shorter planktonic period in nature for *L. plena*, resulting in reduced dispersal and gene flow. However, intraspecific variation in planktonic period is the result of temperature, larval food conditions, availability of and sensitivity to settlement cues, and genetic or geographic variation (reviewed above), and nearly two-fold variation was found here within *L. scutulata*. Chapter 5 presents theoretical evidence that differences in planktonic period, when the average time is several weeks as measured here, have only a limited effect on gene flow. Given the general similarity of these two species in the larval characters measured here, plus expected high levels of intraspecific variation, it is unlikely that consistent differences between the species in planktonic period exist that could influence gene flow and produce the different genetic patterns that have been observed.

From the laboratory settlement data presented here, both species can remain in the plankton for over two months, placing them in the teleplanic, or maximum dispersal potential, category of Scheltema (1989; see also Levin & Bridges 1995). This presents the opportunity for dispersal across hundreds of kilometers per generation. In addition, both species spawn over six months, which should introduce high levels of variability into the direction that larvae travel from any population. These traits should produce panmictic populations, rather than the genetic structure seen in both species, especially *L. plena*. Other hypotheses, such as historically more isolated populations whose signature of genetic divergence remains (Palumbi 1995; Hellberg 1995), demography (Benzie & Stoddart 1992), or selection (Boulding 1990; Avise 1994) remain to be tested.

Table 4.1: Early development of *L. scutulata* and *L. plena* at 12-14°C.

Stage	*L. scutulata*	*L. plena*
2-cell (hours)	3	3
4-cell (hours)	5	5
trochophore (hours)	35	35
early veliger (days)	4	4
well-developed veliger (days)	5	5
hatching (days)	9	8

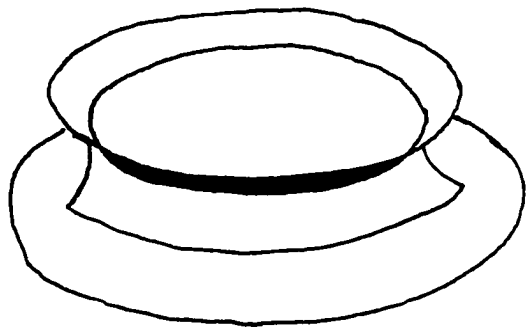
Table 4.2: Growth rates in three broods of larvae, one *L. scutulata*, one *L. plena*, and one undetermined species. Shown are slope (β) times 10^{-3}, a measure of growth rate, and correlation coefficients from regression analyses using equation 4.1. Coefficients followed by a * are statistically significant ($p<0.001$); others are not significant ($p>0.1$).

Food concentrations (cells/mL)	*L. scutulata*		*L. plena*		Undetermined	
	β	r	β	r	β	r
Minimal (10^3)	40.8	0.24			-23.4	0.26
Low (10^4)	350.1	0.75*	427.7	0.92*	330.4	0.78*
Medium (10^5)	794.1	0.86*	1573.7	0.98*	852.5	0.91*
High (10^6)	425.8	0.75*	1333.4	0.98*	818.8	0.85*

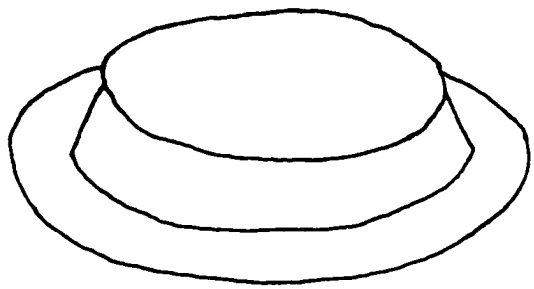
Table 4.3: Summary of current reproductive data on *L. scutulata* and *L. plena*. Asterisks represent differences between the species. References: 1) Buckland-Nicks et al. 1973; 2) Murray 1979; 3) Mastro et al. 1982; 4) Chow 1987b; 5) Reid 1996; 6) this study.

Character	*L. scutulata*	*L. plena*	References *L.s.*	*L.p.*
Size at maturity (mm shell height)	2-3	2-3	6	3,6
Spawning season				
Puget Sound	Apr - Oct	Apr - Oct	1,6	6
California	Mar - Oct	Mar - Oct	4	4
Maximum fecundity (eggs/female)				
(*) daily	1000	1500	4	4
over 2 weeks	7000		2,6	
over 3 months	8000		6	
*Egg capsule morphology	type A,B	type C	2,5	2,5
(see Figure 4.1)	type A,B	type B,C	6	6
*Capsule outer diameter (µm)	700-1000	1100	5	5
	A,B: 337-976	B:730-1100	6	6
		C: 841-1340		6
	correlated with number of eggs		6	6
*Eggs per capsule (range (mean))				
British Columbia	1-4 (2.9)		2	
Puget Sound, San Juan Isl.	A,B: 1-11 (3)	B: 2-11 (5)	6	6
		C: 6-47 (19)		6
Oregon		C: 4-35 (17)		2
California	1-14 (7)	C: 5-41 (23)	2	2
*Egg size (µm)	105	96	2	2
	95	90	6	6
*Hatching at 12-14°C (days)	9	8	6	6
*Hatching at 15°C (days)	8	7	2	2
Size at hatching (µm)	155	169	2	2
	140-150	130-140	6	6
Total planktonic period in lab (days)	37-70	62	6	6
Size at metamorphosis (µm)				
laboratory (range (mean))	320-408 (355)		6	
field (range (mean))	293-436 (339)	256-405 (330)	6	6

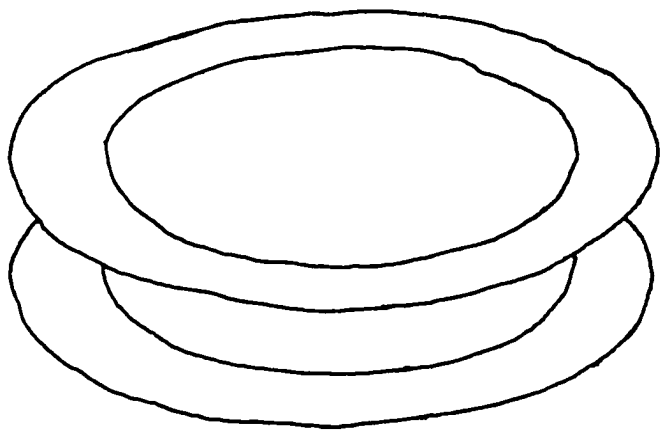
Figure 4.1: Three egg capsule morphologies in *L. scutulata* and *L. plena*. Type A has two rims of unequal diameter, type B has only one rim, and type C has two rims of nearly equal diameter. Murray (1979) and Reid (1996) described types A and B as *L. scutulata*, and type C as *L. plena*. Here I show that *L. scutulata* females produce types A and B, and *L. plena* females produce types B and C.



A



B



C

100 μm

Figure 4.2: Number of eggs per capsule and outer capsule diameter in *L. scutulata*. Bars represent frequencies of capsules (both types A and B) with different numbers of eggs spawned in laboratory (dark bars; total n=11421) or collected in plankton samples (open bars; total n=84). Points above represent mean outer diameter of capsules in each class from the laboratory (type A: filled diamonds; type B: filled squares) and the plankton (type A: open diamonds; type B: open squares). Error bars represent standard deviation. Sample size for each point ranges from 1 to 143.

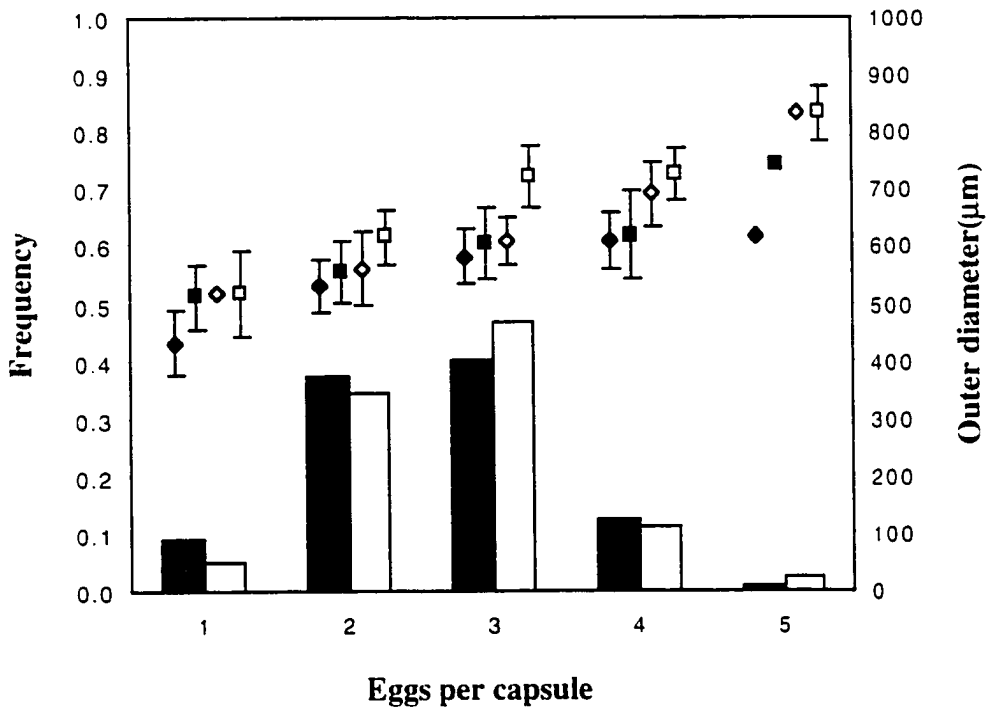


Figure 4.3: Number of eggs and diameter in lab-spawned *L. plena* egg capsules, including type B (open squares) and type C (filled circles). Shown for comparison are type B capsules spawned in the lab by *L. scutulata* (dark bars).

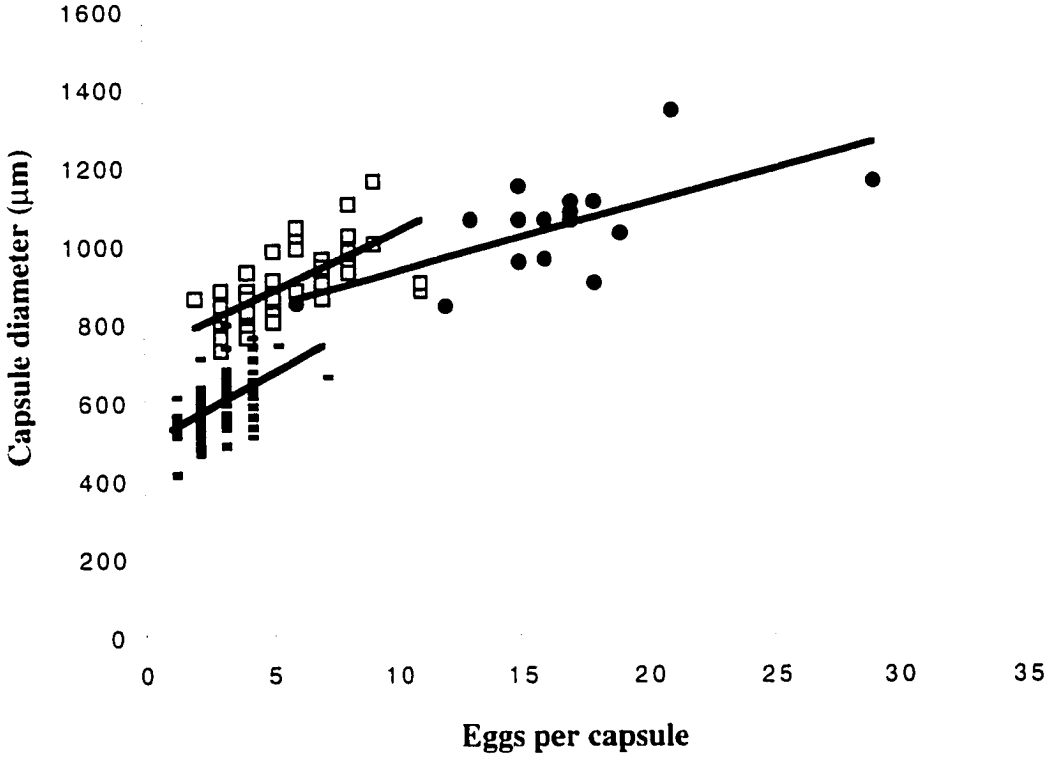


Figure 4.4: Spawning season of *L. scutulata* and *L. plena* in Puget Sound. Lines represent densities of *L. scutulata* eggs in capsules in plankton samples in 1997 (squares), 1998 (diamonds), and 1999 (triangles). Unconnected symbols represent spawning experiments in which at least 50 females of both species were brought into the laboratory and kept for at least 1 week. Shown are spawning events for *L. plena* (closed squares) and *L. scutulata* (open squares), or no capsules of either species (open diamonds).

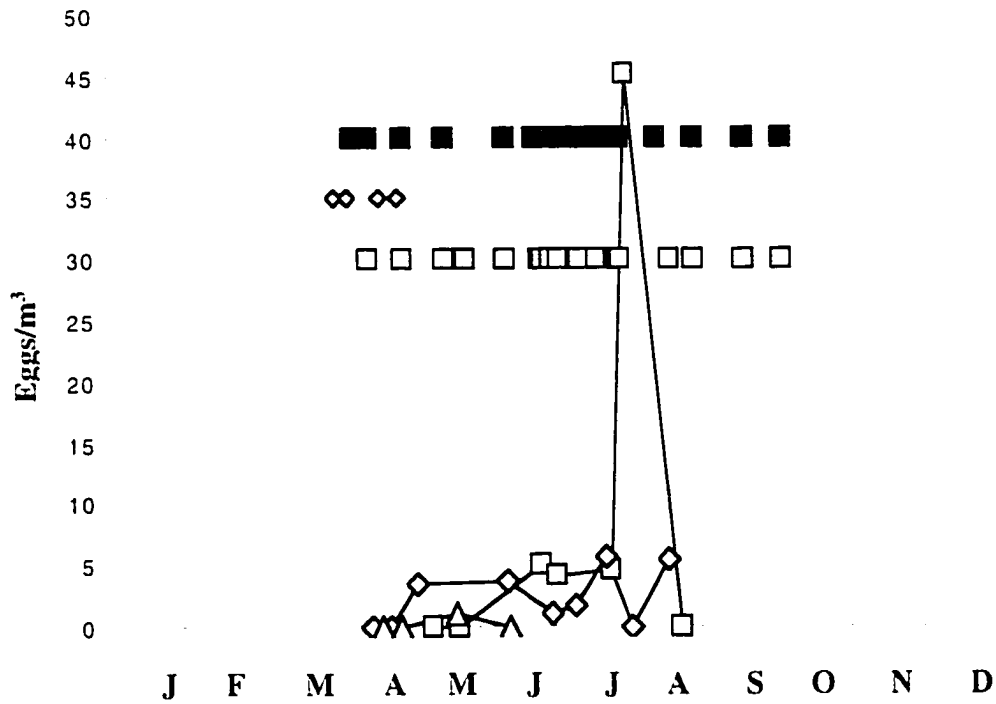


Figure 4.5: Total fecundity of *L. scutulata* females kept in the laboratory for 3 months. Egg production is significantly related to shell size ($r=0.508$; $p=0.004$).

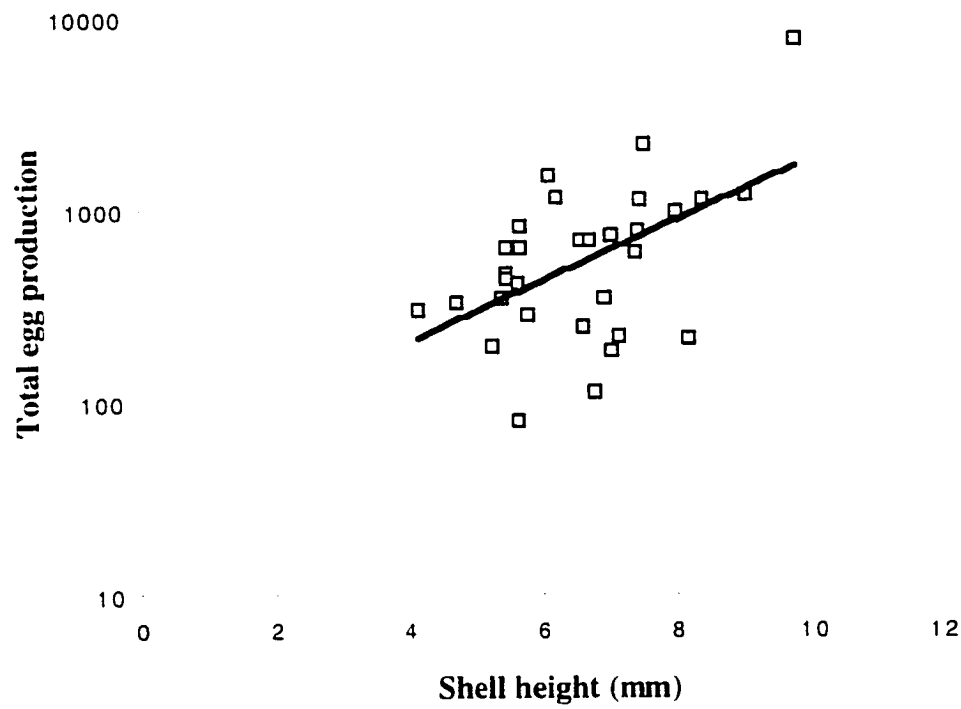
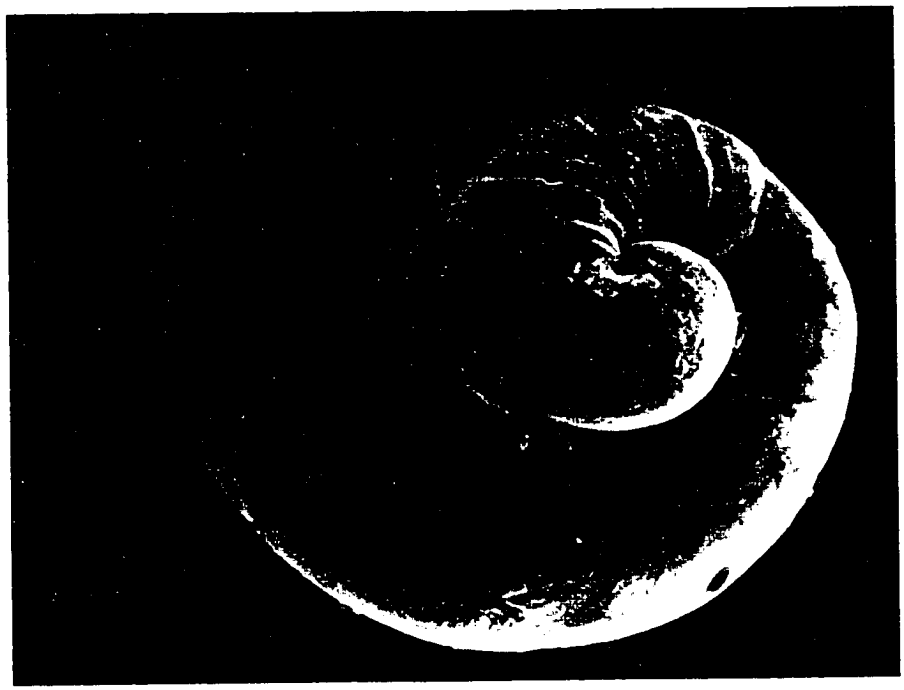


Figure 4.6: Scanning electron micrograph of a *L. scutulata* veliger shell in oblique view. The arrow marks the protoconch I/protoconch II boundary (hatching). Growth lines show the velar notches in the aperture lip.



100 μm

Figure 4.7: Growth of *L. scutulata* larvae from one brood at four food concentrations of the microalgae *Isochrysis galbana* and *Rhodomonas lens* in equal cell concentrations (diamonds = minimal, 5 x 10^2 cells/mL of each algae species; squares = low, 5 x 10^3 cells/mL; triangles = medium, 5 x 10^4 cells/mL; circles = high, 5 x 10^5 cells/mL). The measurement taken on each shell is shown in the lower left. The arrow marks the time at which larvae in the medium food concentration began crawling on the substrate, and the star represents one larva that metamorphosed from the high food treatment on a barnacle shell.

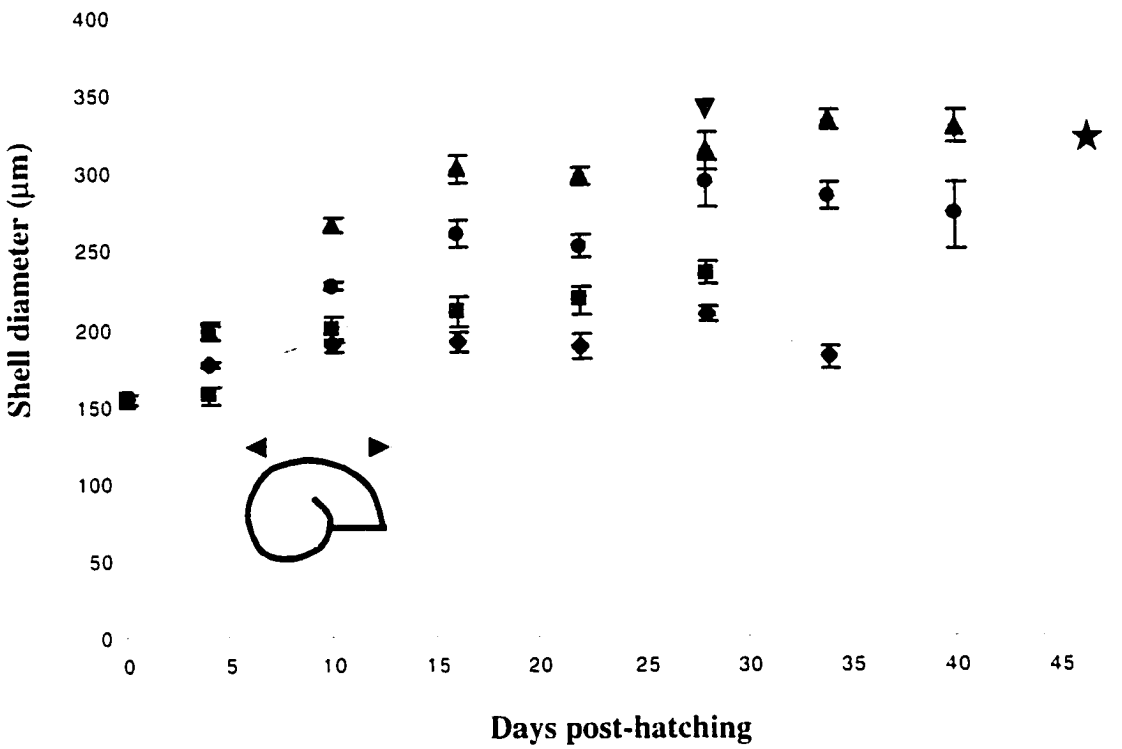
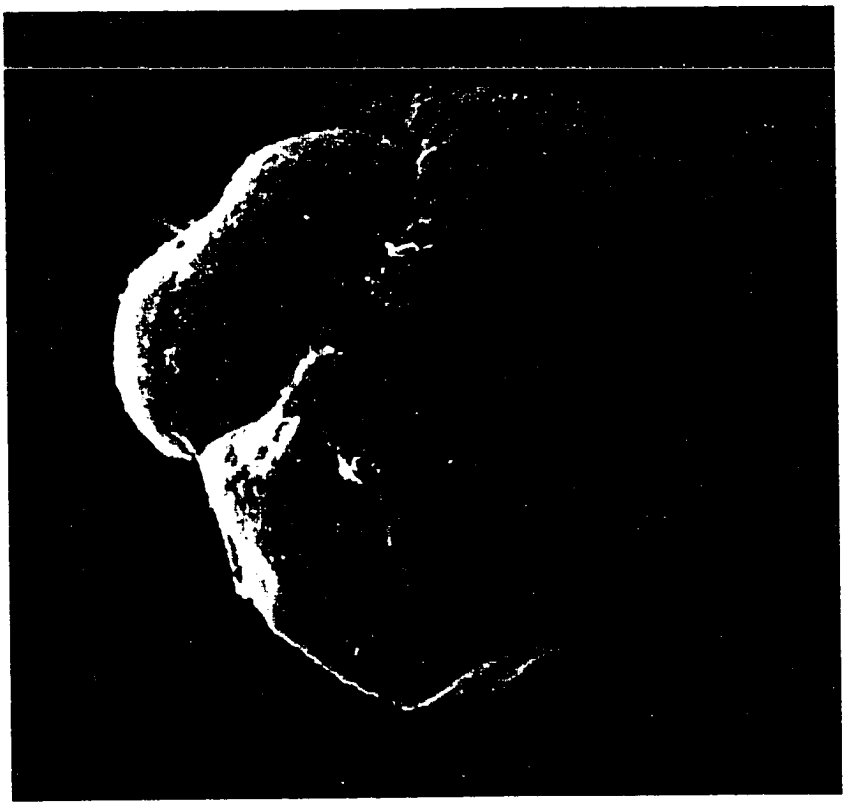


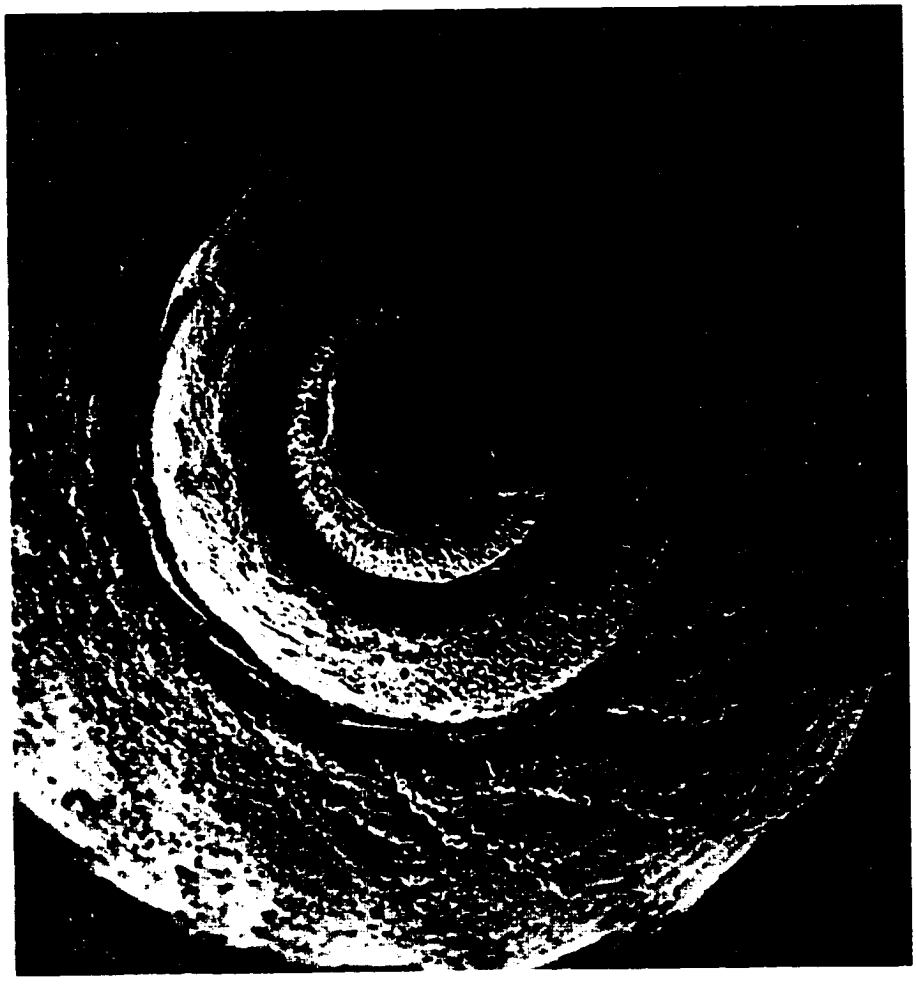
Figure 4.8: Light micrograph of a newly settled *L. scutulata* juvenile on the barnacle shell on which it metamorphosed. The arrow points to the protoconch/teleoconch boundary, which shows the horn and right velar notch of the larval shell. The juvenile right eye and transparent tentacle can also be seen.

Figure 4.9: Scanning electron micrograph of a newly metamorphosed *L. scutulata* juvenile. The arrow shows the prooconch/teleoconch boundary.



100 μm

Figure 4.10: Scanning electron micrograph of a *L. plena* shell collected in Puget Sound, shown in apical view.



100 μm

CHAPTER 5

Limits to Gene Flow and Mechanisms of Marine Speciation: A Modeling Approach

Abstract

Marine animal species with benthic adults and planktonic larvae generally have larger geographic ranges, higher levels of gene flow among populations, lower speciation and extinction rates, and therefore longer-lived species than their relatives without planktonic larvae. Nonetheless, there are numerous examples of population genetic structure and high speciation rates, producing high levels of diversity in animals with planktonic development. Physical factors, such as ocean currents, planktonic environment, and historical climate change, or biological factors, such as spawning season, total planktonic period, predation, larval behavior, and natural selection on juveniles, can limit gene flow among populations.

Simulation models to test factors that can restrict gene flow were based on two areas of the Pacific coast of North America, Point Conception in southern California and the entire coastline of Washington state. They focused on the life history characters of two similar, intertidal gastropod species, *Littorina scutulata* and *L. plena*. The models suggest that ocean currents can create an effective barrier to gene flow that can be slightly relaxed by temporal variation, and that longer scales of temporal variation have a greater effect than shorter scales. Total planktonic period does not affect gene flow above a certain minimum period, which is less than one month in these models. Spawning season can have a large effect on gene flow, and an extended spawning season can eliminate gene flow barriers when currents vary seasonally.

These results fit with a conceptual model of marine speciation, in which barriers restrict, but do not eliminate, gene flow, and these limits may be short-lived on an evolutionary time scale. Divergence in a few genes may act very quickly to produce reproductive isolation, resulting in cryptic species. This mode of speciation is suggested for the focal species of the models.

Introduction

Marine life histories. The life history of many benthic marine animals includes a planktonic larval stage that can last for weeks to months, perhaps even years (Thorson

1950; Scheltema 1986b; Levin & Bridges 1995; M. Strathmann pers. comm.). This allows for dispersal, and potentially gene flow, across ocean basins (Scheltema 1986a; Palumbi 1992). Such gene flow would be expected to produce panmictic populations, preventing the genetic isolation of populations that is necessary for speciation (Mayr 1963; Harrison 1998). Nonetheless, marine lineages with planktonic larvae exhibit high levels of diversity (Kohn 1983; Palumbi 1992, 1994). What factors can restrict gene flow and facilitate speciation in marine animals with planktonic larvae? This study addresses variation in three potential factors, ocean currents, spawning season, and planktonic period, using geographically-based computer simulation models of larval dispersal. The models are based on the life histories of the gastropods *Littorina scutulata* and *L. plena*, sibling species with long-lived planktonic larvae, both of which show high levels of genetic variation (Ward 1990). *L. plena* also shows evidence of geographic structure in mitochondrial haplotypes (Kyle 1998). The models are based on measured ocean currents around Point Conception in southern California, a well-studied biogeographic landmark (Burton 1998), and on the measured currents along the coast of Oregon, Washington, and British Columbia.

Complex life histories with planktonic development are widespread and ancient among marine animals (Wray 1995). Strathmann (1986; 1990) lists several factors that may favor the persistence of planktonic larvae, including the ability to feed and grow, the presence of external fertilization, ventilation of developing embryos, adult size, and reduced predation levels compared to the benthos (discussed also in Signor & Vermeij 1994). Theoretical work supports the hypothesis that dispersal can be adaptive (Strathmann 1974; Hamilton & May 1977; Motro 1983), but the scale of dispersal in many marine animals is much larger than expected as an adaptation for dispersal (Strathmann et al. 1981; Strathmann 1985). The evolutionary consequences of the dispersal of planktonic larvae, then, are decoupled from the selective forces that maintain those larvae.

Consequences of dispersal. Several authors have predicted that lineages with planktonic larvae should have larger geographic ranges (Shuto 1974; Scheltema 1986a, 1989), higher levels of gene flow (Scheltema 1971, 1986a; Shuto 1974; Crisp 1978), lower speciation and extinction rates, and longer-lived species than lineages without a long-dispersing stage (Scheltema & Williams 1983) (all of these predictions: Scheltema 1978; Jablonski & Lutz 1983; Jablonski 1986). The effects of mode of development on speciation and extinction rates may be interpreted as species selection, since genetic population structure and geographic range act as emergent traits, heritable at the species level (Jablonski 1987), that cannot be reduced to selection at the individual level (Jablonski

1986; Grantham 1995). Data have generally supported these predictions, with notable exceptions.

Kohn & Perron (1994) and Scheltema (1989) found a significant relationship between dispersal potential and geographic range in Indo-West Pacific and Atlantic prosobranch gastropods, respectively. A similar relationship is suggested for deep-sea gastropods by patterns of diversity (Stuart & Rex 1994). Emlet (1995) found that echinoid species with feeding, planktonic (planktotrophic) larvae have significantly larger ranges than those with non-feeding (lecithotrophic) or brooded larvae. In the fossil record, gastropods with larval shell morphologies that suggest planktotrophic development have larger ranges than lecithotrophic developers (Hansen 1978, 1980; Shuto 1983; Jablonski 1986). However, both Scheltema (1989) and Emlet (1995) found no relationship between time in the plankton and geographic range when considering only species with planktonic larvae. Vermeij et al. (1990) and Bhaud (1993) found no correlation between mode of development and range in Aleutian molluscs and north Atlantic gastropods, respectively.

Rafting or drifting of adults may be an important mode of dispersal in exceptions to the general pattern (Highsmith 1985; O'Foighil 1989; Johannesson & Warmoes 1990; Johannesson & Johannesson 1995). Studies have observed rafting dispersal by corals on pumice (Jokiel 1989), bivalves on kelp (Helmuth et al. 1994), and ascidians on eelgrass (Worcester 1994). Such rare dispersal events may be more important for non-planktonic developers. In these species a few colonists are better able to establish a sustainable population, because offspring must settle near their parents. Thus distant oceanic islands may have self-sustaining populations of non-planktonic developers (Johannesson 1988), while the offspring of planktonic-developing colonists do not remain (Efford 1970).

Larger geographic ranges and the ability to disperse and recolonize areas quickly may act as a buffer to extinction in species with planktonic larvae, producing longer-lived species in the fossil record (Jablonski et al. 1985). This pattern has been demonstrated by Hansen (1978; 1980), Scheltema & Williams (1983), Jablonski (1986; 1987), and Gili & Martinell (1994). However, Bouchet (1981) found no correlation between developmental mode and species longevity. Chatterton & Speyer (1989) found that trilobite taxa with planktonic larvae suffered lower survivorship through the Ordovician mass extinction, and Valentine (1986) found a similar result for molluscs through the Permian mass extinction. In these events, species with planktotrophic larvae may have been more susceptible to global changes affecting pelagic ecosystems. In addition, Russell & Lindberg (1988) showed that geographic range and species longevity may be confounded in paleontological studies by sampling bias.

Levels of gene flow among populations are expected to be higher in species with greater dispersal potential. Levels of gene flow can be estimated by measuring genetic subdivision of populations. Bohonak (1999) reviewed data from 27 aquatic and terrestrial animal groups and found dispersal ability, inferred from life history, morphology, behavior, or habitat, to contribute significantly to population genetic structure. Similarly, Govindaraju (1988) found the expected pattern in a review of plant species grouped by their mechanism of pollen dispersal. Within marine species, genetic population structure is expected to be more pronounced in species without planktonic larvae than in species with planktonic larvae. This pattern has been found in comparisons of closely related species that differ in mode of development for fish (Waples 1987; Doherty et al. 1995), shrimp (Duffy 1993), solitary corals (Hellberg 1996), sea stars (Stickle et al. 1992; Hunt 1993; Williams & Benzie 1993), sea urchins (McMillan et al. 1992), and polychaetes (Grassle & Grassle 1978). Studies of single species have also fit the expected pattern (an amphipod, Carvalho 1989; a gastropod, Mitton et al. 1989; two sea urchins, Palumbi & Wilson 1990; a rock lobster, Ovenden et al. 1992). However, many of these results are based on allozyme data, and results may have been affected by balancing selection on allozyme loci that masks population structure (Karl & Avise 1992).

The temperate gastropod genus *Littorina* includes species with long-lived planktonic larvae as well as species that develop in benthic egg masses or in a maternal brood chamber (Reid 1996). The intertidal adults are ecologically similar and relatively sedentary, so this genus is suitable for comparative studies. Berger (1973; 1977) and Janson (1987) compared allozyme frequencies for *Littorina* species and found the expected pattern. Populations of the brooding species *L. saxatilis* differ genetically (Janson & Ward 1984; Johannesson, Johannesson, & Lundgren 1995). Ward (1990) reviewed genetic data for the genus and found most species to fit the paradigm. However, Ward's (1990) recalculation of allozyme data from Mastro et al. (1982) showed surprisingly high levels of variation among populations of *L. scutulata* and especially *L. plena*, sympatric sibling species along the Pacific coast of North America with planktonic larvae. Kyle (1998) examined haplotypes of the mitochondrial gene cytochrome b in 4 Pacific species and found genetic population structure in *L. plena*, but not in *L. scutulata*.

One effect of the high levels of gene flow expected for species with planktonic larvae is the inhibition of both phenotypic differentiation and adaptation to local habitats. In direct comparisons of both Atlantic (Behrens Yamada 1987) and Pacific (Behrens Yamada 1989) *Littorina* species pairs with different larval dispersal potential, shell growth rates differed over smaller geographic scales in non-planktonic developers than in planktonic

developers. In a similar comparison of two Atlantic *Littorina* species, Janson (1987) found geographic variation in shell morphology only in the non-planktonic developer. High levels of morphological differentiation are well documented in the brooding species *L. saxatilis* (Janson & Ward 1984; Johannesson, Rolan-Alvarez, & Ekendahl 1995; Reid 1996).

Gene flow, which limits population differentiation, is also expected to inhibit speciation. Several studies have found lower speciation rates in lineages with planktonic larvae in gastropods (Bouchet 1981; Hansen 1982, 1983), bryozoans (Taylor 1988), and several other groups (Jablonski 1986). However, these results may be complicated by the bias in evolutionary transitions from feeding (planktotrophic, and therefore planktonic) to non-feeding (lecithotrophic, and possibly non-planktonic) larvae (Strathmann 1978a, b, 1985, 1993; Strathmann & Eernisse 1994). Lieberman et al. (1993) and Duda & Palumbi (1999) emphasize the importance of phylogeny in these analyses. In the genus *Littorina*, the phylogeny is well established (chapter 3), and speciation rates appear higher in lineages with non-planktonic development (Reid 1990b).

Factors that Limit Gene Flow. Several species with planktonic larvae have significant population genetic structure, and the factors responsible for these exceptions to the general rule may be numerous (Hedgecock 1986; Palumbi 1995). These factors can be divided roughly into physical and biological categories, though the interactions between them may be most important (Jackson 1986). Physical factors, described below, include environmental history, ocean current patterns, physically maintained plankton patches, and characteristics of the larval environment. Biological factors include spawning season, predation, larval behavior, total planktonic period, selection on juveniles, and demography.

History: Present population structure may not reflect current gene flow patterns. Scheltema (1986b) listed four historical factors that can change gene flow patterns: climatic change, transgression and regression of sea level, opening or closing of seaways, and seafloor spreading. Hellberg (1995) found that the forces of genetic drift and gene flow had reached equilibrium at small scales in the solitary coral *Balanophyllia elegans*, but at a larger scale the signature of Pleistocene climatic fluctuations remained. Benzie & Williams (1995; 1997) found patterns of apparent gene flow to be perpendicular to prevailing currents in two species of giant clam. They attribute the discrepancy to historical factors, possibly lower sea level, and suggest that genetic structure may reflect major dispersal events that are separated by thousands of years (Benzie & Williams 1997).

Ocean current patterns: A variety of ocean current patterns may prevent dispersal across an "invisible barrier" (Palumbi 1994) or limit the dispersal distance of long-lived

larvae (reviewed by Okubo 1994). Larvae may be retained in nearshore circulation patterns, especially estuaries or bays (Shanks 1983; Levin 1986; McShane et al. 1988). A number of processes can result in periodic shoreward transport, including Ekman transport (Nelson et al. 1977; McConnaughey et al. 1992), tidal forces (Shanks 1983; Pineda 1991), El Niño events (Connolly & Roughgarden 1999), and other patterns (Shanks 1995). Gaylord & Gaines (2000) have also modeled convergent and divergent alongshore current patterns that can create an effective barrier to dispersal. Rocha-Olivares & Vetter (1999) showed that a sharp genetic break in a rockfish species corresponded with the divergence of the Alaska and California Currents along the Pacific coast of North America. Behrens Yamada (1977) and Efford (1970) documented cases in the same region in which adults can live beyond the northern edge of the species' range, but larvae cannot consistently settle there because of the dominant southward current. A similar situation within the range of species at Point Conception may create directional gene flow (Wares et al. in press), and I explore this mechanism with computer simulation models here.

Planktonic patchiness: Eddies and other complex structures in ocean circulation can create and maintain well-defined patches (Levin & Segel 1976; Koehl et al. 1993; Abraham 1998). Adjacent patches may contain genetically distinct populations of planktonic organisms from widely spaced origins (Bucklin 1991). Such a patch could then be transported to adult habitat by one of the periodic processes mentioned above. This combination of processes may be quite common (Hedgecock 1994), so that successful recruits are typically not a genetically representative sample of a species' total reproductive output.

Planktonic environment: Larvae may be unable to tolerate ocean conditions, such as low temperature, that exist between adult populations. For instance, the warmer water of El Niño events can transport larvae farther north along the Pacific coast of North America than normal temperatures allow (Newman & McConnaughey 1987; Richmond 1990). Colder temperatures may limit northward dispersal of *Littorina keenae* larvae past the species' current range, though transplanted adults can persist north of the range (Behrens Yamada 1977). However, this has not been documented as a barrier to dispersal among established conspecific populations.

Spawning season: Marine animals vary widely in the timing and length of their spawning seasons (Morgan 1995a). This may determine the ocean currents that larvae encounter and thus the variability in the distance and direction that they travel. The best examples are from estuarine systems. The tropical gastropod *Umbonium vestiarium* limits dispersal of larvae by spawning during minimum amplitude tides (Berry 1986b). Levin

(1986) sampled larvae of several species in a California estuary and showed that spawning cued to tidal rhythms can produce dense patches of larvae that are retained close to the parental habitat.

Predation: Planktonic life history stages are subject to high predation rates that can create effective barriers to dispersal. The planktonic copepod *Tigriopus californicus* may rarely disperse among tidepool habitats because of severe predation in the low intertidal, resulting in high levels of between-patch genetic differentiation (Burton & Feldman 1981). Recruitment to intertidal adult habitats of the barnacle *Balanus glandula* may be reduced up to 98 percent by predation by rockfish in adjacent kelp forests (Gaines & Roughgarden 1987).

Larval behavior: Vertical migration is common in marine invertebrate larvae, especially in decapod crustaceans (Young 1995). This behavior may combine with vertical structure in direction and speed of ocean currents, allowing shoreward transport of competent larvae (Shanks 1985). Some estuarine species exhibit a heritable endogenous circatidal rhythm (Zeng & Naylor 1996) or respond to exogenous tidal cues (Tankersley & Forward 1994), and this can retain larvae near the parental habitat (Cronin & Forward 1986) or even transport them farther into an estuary (Young 1995). This movement would not be possible simply by horizontal swimming, since current speeds exceed horizontal swimming speeds of most larvae (Young 1995). Vertical migration, however, is best studied in decapod crustaceans and may not play a role in other larvae, such as those that swim with cilia (Levin 1986; Young 1995). Larvae that remain close to the bottom may also avoid strong currents and spend most of their planktonic period close to the parental habitat (Knowlton & Keller 1986; McShane et al. 1988).

Total planktonic period: The minimum period that a larva spends in the plankton is the precompetent period, which may last several weeks (Levin & Bridges 1995). Total planktonic period may be much longer and more variable (Jackson & Strathmann 1981; Scheltema 1986b), depending on larval encounters with adult habitat. One might expect gene flow to correlate with average planktonic period across species. Waples (1987) and Doherty et al. (1995) found evidence for such a correlation in comparisons of fish species. However, the relationship found by Doherty et al. (1995) predicted panmixis for species with planktonic periods greater than one month. Shulman & Bermingham (1995) found no relationship between planktonic period and gene flow in a similar study of fish species. The results of Scheltema (1989) and Emlet (1995), who examined larval dispersal and geographic range in molluscs and echinoids, respectively, provide indirect evidence against the correlation. While both authors found larger geographic ranges in species with

planktonic larvae compared to those without, there seemed to be no effect of total planktonic period within planktonic developers.

Selection: Strong selection on newly recruited juveniles limits gene flow despite broad dispersal in mussels (Koehn et al. 1984), limpets (Johnson & Black 1984), copepods (Burton 1983; 1986), and barnacles (Hedgecock 1986; Bertness & Gaines 1993). In the brooding gastropod *Littorina saxatilis*, selection among habitats is strong enough to maintain genetic differentiation between tidal levels on a single shore (Johannesson, Johannesson, & Lundgren 1995), and a planktonic disperser is expected to face a wider range of selective environments.

Demography: Benzie & Stoddart (1992) found that gene flow among patches in outbreaking (rapidly expanding) populations of the starfish *Acanthaster plancii* was greater than in non-outbreaking populations. The larvae of this species stay in the plankton for several weeks, but non-outbreaking populations appear to have more localized recruitment or more episodic larval production, leading to stochastic differences in gene frequencies (Benzie & Stoddart 1992).

Species Ranges and Genetic Barriers. The North American biogeographic landmarks Point Conception, on the Pacific coast, and Cape Canaveral, on the Atlantic coast, provide an illustrative comparison. Both have long been recognized as concentrations of species range endpoints (Valentine 1966; Briggs 1974; Hare & Avise 1996; Burton 1998). Avise (1992; Hare & Avise 1996) showed a sharp genetic break at Cape Canaveral for species which range across this location, including the oyster *Crassostrea virginica*, which has a planktonic larval stage lasting several weeks. It appears that both historical vicariant events and ongoing physical or biological barriers to gene flow occur at Cape Canaveral, and that the same set of processes has isolated populations to a varying extent from slight genetic divergence to complete reproductive isolation and speciation (Hare & Avise 1996). In contrast, the large number of species range endpoints at Point Conception do not correspond to genetic breaks in species that range across the landmark (Burton 1998). Previously described genetic breaks at Point Conception in the copepod *Tigriopus californicus* (Burton & Lee 1994) were found to be no more pronounced than at several other sites along the coast, reflecting this species' generally restricted dispersal (Burton 1998). *T. californicus* is a holoplanktonic species, though adults are generally restricted to tidepools (Burton 1986). Despite the convergent currents at this site (discussed below) and a water temperature difference of up to 5°C on either side of Point Conception (Wares et al. in press), neither ocean currents nor selection appear to create a barrier to gene flow (Burton 1998). The faunas north and south of Point

Conception are not phylogenetically related as they are at Cape Canaveral; in other words, there are fewer sibling species pairs whose ranges meet at Point Conception than there are at Cape Canaveral. Thus, Burton (1998) suggests that the concentration of species range endpoints at Point Conception is not the result of in situ genetic isolation and speciation.

Mathematical models. Several types of mathematical models are relevant to larval dispersal and evolution of marine animals. Several authors (Vance 1973; Jackson & Strathmann 1981; Palmer & Strathmann 1981; Roughgarden 1989) have modeled different reproductive strategies, identifying trade-offs among egg size, number of offspring, precompetent and competent periods, and different mortality rates for different modes of development. These models have identified factors other than dispersal per se that maintain planktonic larvae. A few models have examined the interaction between physical and biological factors. Doyle (1975) focused on the increasing likelihood of settlement as larvae age, seen in several species (Gibson 1995), and its relationship to heterogeneity of habitat. Koehl & Powell (1994) showed that the turbulent waters along wave-swept shores overwhelm vertical swimming or sinking of most larvae, maintaining a well-mixed larval population. Smith & Stoner (1993) created models based on two geographic areas that tested the interaction of vertical migration with tidal cycles and turbulent mixing, demonstrating the different outcomes of different migration cues.

Most of the modeling effort has focused on population dynamics of marine species with planktonic larvae, and Gaines & Lafferty (1995) describe two categories. Local population models (Roughgarden et al. 1985; Bence & Nisbet 1989) imagine settlement from an external larval pool, and populations can enter periodic fluctuations or a stable state, depending on space-limited recruitment, time lags, and different types of adult growth. Metapopulation models (Roughgarden & Iwasa 1986; Hanski 1991) consider a series of adult populations that interact with a common larval pool. Improvements to these basic models have included a simple genetic basis to life history parameters (Iwasa & Roughgarden 1985) and incorporation of life history characters measured in the field on particular species (Yoshioka 1982; Hughes 1990). Spatial dynamics have been added by considering advection and diffusion either analytically (Hill 1991) or explicitly with a generic coastline and alongshore currents (Possingham & Roughgarden 1990; Alexander & Roughgarden 1996). These models, combined with field data, have emphasized the importance of recruitment, rather than post-settlement ecological factors, to population dynamics (Gaines & Roughgarden 1985; Roughgarden et al. 1988; Gaines & Bertness 1992).

More specific models incorporate real data on life history, ocean currents, and geography. McGurk (1989) modeled sources of larval mortality for herring in a western Canadian inlet, and showed that starvation, density-dependent predation, and offshore transport were equally important. Craig & McLoughlin (1994) measured current and wind vectors and simulated transport of scallop larvae in Great Oyster Bay, Tasmania. The simulations identified sites that could self-seed and others from which larvae would be swept offshore. Similarly, Roberts (1997) identified upstream and downstream reef areas in the Caribbean to aid management and design of conservation reserves. Gaylord & Gaines (2000) simulated a series of generic ocean current patterns along a coastline that could produce species range boundaries.

Gene flow can be modeled by incorporating genetic variation into larval dispersal models. Planes et al. (1996) analyzed allozyme data from a surgeonfish on ten islands and compared generic metapopulation migration models. As in soft corals (Hellberg 1995), genetic divergence correlated with geographic distance at a small scale but not at a large scale, suggesting isolation by distance within archipelagoes combined with sporadic long-distance migrations, and perhaps a historical component, among archipelagoes.

Models of Dispersal and Gene Flow

The models I present combine several aspects of those discussed above. They use life history data from two similar gastropod species, *Littorina scutulata* and *L. plena*, and ocean current measurements from two areas along the Pacific coast of North America, centered on Point Conception in southern California and on Puget Sound in Washington. The goal is to determine whether barriers to gene flow sufficient to promote speciation can be caused by variation in ocean current patterns, spawning season, and total planktonic period.

Here I present and justify the important life history features and ocean current patterns used in these models; the mathematical and programming details are given in the appendix. The models are based on the sibling gastropod species *Littorina scutulata* and *L. plena*, which inhabit the high intertidal and splash zones of rocky shores along the Pacific coast of North America. These species overlap broadly in their distribution (chapter 3), are remarkably similar in ecology and life history (chapter 4), and have often been inadvertently combined in ecological studies. Therefore data from both species were used in constructing the models. As with the barnacles in previous models (e.g. Roughgarden & Iwasa 1986), using intertidal species allows relatively simple mapping of potential adult habitat. An important difference from those barnacle-based models, however, is that

recruitment is not expected to be space-limited in *Littorina* because adults are mobile. Instead, adult mortality and fecundity are assumed to be density-dependent and recruitment is density-independent.

For the Point Conception models, I covered a rectangle measuring 90 by 110 km (1° longitude by 1° latitude) with a 2-dimensional 1-km mesh (Figure 5.1). For the Washington model, a rectangle of 345 by 820 km (about 5° longitude by 8° latitude) is covered by a 5-km mesh (Figure 5.2). Adult populations are arrayed at meshpoints along the coast. To observe gene flow, two different genotypes originally populate separate sections of the coast in each run of the models (Figure 5.1, 5.2). These genotypes are inherited as mitochondrial haplotypes; that is, there is no recombination, and animals of one genotype produce only offspring of that genotype. In effect, the model ignores males. This type of inheritance is a simplification that still provides a good representation of overall genetic divergence relevant to speciation (Foltz 1997). It also renders the results comparable to population genetic studies of mitochondrial loci (e.g. Kyle 1998). The genotypes are selectively neutral and have no effect on any life history parameters in the model.

At the beginning of each simulated year, each female releases her annual output of offspring (with a few exceptions described below). In reality, these animals spawn broadly from spring to early fall (chapter 4). However, collapsing spawning into a single event allows a precompetent period during which larvae cannot settle, without requiring the computing power to keep track of age structure in the larval population (Gaylord & Gaines 2000 used a similar technique). Below a total adult density (k), fecundity is density-independent, with an annual birth rate of 5000 females per female (chapter 4). Chow (1989) found that food becomes limiting and growth becomes density-dependent at high densities in *L. plena*, so at adult densities above k, fecundity decreases with increasing density. The first 20 days of larval life represent the precompetent period, including the week-long pre-hatching period during which egg capsules drift as passive, slightly negatively buoyant particles (chapter 4). After hatching, some growth appears necessary in these species before metamorphosis (chapter 4). After the precompetent period, larvae that are adjacent to suitable adult habitat settle at a rate that gradually increases, corresponding to the increase in sensitivity to settlement cues described in other animals (Doyle 1975; Gibson 1995). After the 40-day competent period, all larvae that have not settled are considered dead. During both the precompetent and competent periods, larval mortality occurs at a constant instantaneous rate of 0.2 per day, a rough estimate from the wide range (0.016 to 1.01 d^{-1}) given by Morgan (1995b). This includes mortality from starvation,

predation, and UV damage, but not from offshore transport, which is modeled explicitly. Larvae are moved by currents, discussed below, and by turbulent eddy diffusion. The diffusion constant of 10^4 cm^2/s is appropriate at the 1 to 5 km scale of the mesh (Okubo 1971), so that currents, eddies, and other processes smaller than the mesh are subsumed into this diffusion. All boundaries in the models are absorbing; in other words, no larvae can enter from outside the boundaries, and larvae that leave cannot return. The larvae are treated in these models as passive particles. Gastropod veligers swim with cilia and are much less powerful swimmers than crustacean or fish larvae (Waples 1987; Young 1995). From a study of dispersal of bivalve veligers, McQuaid & Phillips (2000) concluded that veligers behave as passive particles and that their movement can be predicted from hydrographic data.

Larvae that settle enter the adult population and are able to reproduce the following year (chapter 4). Chow (1989) found that adult mortality is greatest in winter and becomes density-dependent at high densities in *L. plena*, so in this model all adult mortality occurs outside the breeding season. Below densities of k, adult mortality is set at 0.35 per year, which means that 95 percent of adults die after the 7-year life span described by Behrens Yamada (1992). Above k, mortality rates increase with total density.

All the simulations were run for 100 years, and the degree of overlap of the two genotypes after this period reflected the expected patterns of gene flow along the coast. It is important to note that the models are entirely deterministic; that is, they contain no stochastic processes. Though it is not possible to find the solutions analytically, identical results are produced if the identical model is run twice, so no statistical analyses were used.

Ocean currents. Since these species spawn broadly from spring to early fall (chapter 4), summer current patterns were used. No data were available on the vertical position of *Littorina* larvae in nature, so for several reasons the simplifying assumption was made that they remain close to the surface. They are released from and must return to the high intertidal, and so they must remain high in the water column once competent in order to encounter settlement sites. Except for the pre-hatching period, they depend on phytoplankton for growth, so they presumably spend much of their planktonic life in the photic zone. For each current pattern, a two-dimensional vector field was constructed to reflect average measured currents in the top 10 m.

Around Point Conception, the average summer current pattern includes the southward-flowing, alongshore California Current, which diverges from the coast near Point Conception. The Southern California Countercurrent, like a large eddy, flows northwest along the coast to meet the California Current (Figure 5.3; Brink & Muench

1986; Hickey 1989, 1992, 1998). Two scales of temporal variability have been documented. Intra-annual variability includes currents which cycle among 4 basic patterns (Figures 5.4, 5.5, 5.6, 5.7), spending roughly 4 days in each (statistically resolved by Harms & Winant 1998). The last of these patterns (Figure 5.7) is called the relaxation phase, since it is caused by a relaxation in wind forcing that maintains the southward-flowing California Current (Harms & Winant 1998). Inter-annual variability was observed in 1981 and 1984 by Chelton et al. (1988), when the relaxation pattern persisted throughout the summer.

To test the effects of ocean currents and different temporal scales of variability, the Point Conception model was run under five sets of conditions, each of which included turbulent eddy diffusion:

1) Diffusion only, a control in which turbulent eddy diffusion moved larvae in the absence of any currents;

2) Constant currents, in which average summer currents (Figure 5.3) prevailed throughout the simulation;

3) Intra-annual variation, following the 16-day cycle described above (Figures 5.4, 5.5, 5.6, 5.7);

4) Inter-annual variation, in which one of every 10 simulation years was spent in the relaxation phase (Figure 5.7), and the remaining years were spent in the constant summer pattern (Figure 5.3); and

5) Combined variation, in which one of every 10 years was spent in the relaxation phase (Figure 5.7), and the remaining years were spent in the 16-day cycle (Figures 5.4, 5.5, 5.6, 5.7).

Planktonic period. The effect of total planktonic period was tested in two runs of the Point Conception model. Since Jackson & Strathmann (1981) emphasized the importance of the ratio of precompetent to competent period, this ratio was kept constant. All other conditions of the model match the intra-annual variability run, so the 60-day total planktonic period of that run can be compared to the following conditions:

1) 30-day, with 10-day precompetent and 20-day competent periods; and

2) 90-day, with 30-day precompetent and 60-day competent periods.

Spawning season -- Point Conception. Chow (1987b) observed spawning as early as February in California populations of *L. plena*. To test the effects of such early spring spawning, an additional Point Conception model was developed in which annual reproduction is divided between spring and summer seasons. In spring, the California Current continues around Point Conception and flows along shore to the southeast (Figure

5.8; Hickey 1979, 1998; Halliwell et al. 1981). As in the other models, larvae that settle in the spring are not able to reproduce until the following year. All other features of the models described above are retained here. As a test of comparability, when the constant summer current pattern (Figure 5.3) is used in both the spring and summer seasons, the results are identical to the constant current model described above. Two versions of this spawning season model were run:

1) Constant summer, in which the spring season used the spring current pattern (Figure 5.8) and the summer season used the constant summer current pattern (Figure 5.3); and

2) Variable summer, in which the spring season used only the spring current pattern (Figure 5.8) and the summer season used the 16-day cycle described above (Figure 5.4, 5.5, 5.6, 5.7).

Spawning season -- Washington. The Washington model was also used to test the effect of variation in spawning season. Both species spawn from early spring to mid autumn in Puget Sound (chapter 4). Spring surface currents along the coast of Washington and Oregon flow mostly northward (Figure 5.9), while summer currents flow southward (Figure 5.10). The transition from the spring to the summer regime, called the spring transition, is typically abrupt (Hickey 1989; 1998) and occurs in March or April, with some annual variation (Hickey 1989). To test the effect of timing of the onset of spawning, reproduction was split evenly into spring and summer seasons, as described above. Currents during the summer season remained in the southward pattern (Figure 5.10). Variation in the onset of spawning was tested in 4 versions of the model that differed in current regime for the spring half of the reproductive season:

1) Late spawning, in which all spawning occurs after the spring transition;

2) 20-day, in which the spring transition from northward to southward flow occurs 20 days after the start of the spring spawning;

3) 40-day, in which the spring transition occurs 40 days after the start of spawning; and

4) Early spawning, in which the entire 60-day spring season occurs before the spring transition.

Results

Ocean currents. In most of the models, the entire coastline was populated by adults within 5 years and a quasi-equilibrium was reached within 50 years (figure 5.11). After this point, adult densities and proportion of genotypes remained nearly stable, so that

conditions at 100 years represent a more-or-less steady state. The patterns of gene flow along the coast can be seen in the proportion of genotypes. Proportions of genotype 1 in the adult population at each point along the coast after 100 simulated years for the current variation models are seen in Figure 5.12. When diffusion was the only process moving larvae, a gradual cline of genotypes along the coast was produced. Constant currents in the summer pattern prevented either genotype from dispersing past a point on the coast between Points Arguello and Conception, producing a sharp genetic break. This break was pushed northward, but only slightly relaxed, by temporal variation in ocean current patterns. Inter-annual variation, which spent only 10 percent of the time in the northward-flowing, relaxation phase (Figure 5.7), produced more mixing of the genotypes than intra-annual variation, which spent 25 percent of the time in the relaxation phase. Combining both scales of temporal variation slightly reduced the mixing of the two genotypes compared to the inter-annual variation model.

Planktonic period. Total planktonic period did not substantially affect gene flow in these models. The sharp genetic break produced by the intra-annual variation pattern with a 60-day period remained with both a 30-day and a 90-day planktonic period (Figure 5.13), though the longer period pushed the break approximately 4 km northward. The longer period model was the only one which produced large portions of unoccupied coastline, because higher total larval mortality over the longer 30-day precompetent period rendered some habitats not self-sustaining. These stretches of unoccupied habitat appeared in areas with only one genotype, so they were not associated with the break between the two genotypes.

Spawning season -- Point Conception. Spawning season had a large effect on gene flow in the Point Conception models. In both of the models in which half of the spawning occurred during the southward-flowing spring current phase, genotype 2 was virtually eliminated after 100 simulated years, and genotype 1 occupied the entire coast (not shown). Spreading the spawning season over spring and summer current patterns produced the southward-biased gene flow pattern seen in other species by Wares et al. (in press).

Spawning season -- Washington. The southward-flowing summer current in the larger-scale Washington models produced a similar biased gene flow pattern. Even when spring spawning began 20 or 40 days before the spring transition in ocean currents, genotype 2 remained confined to the southern edges of the habitat by the southward-flowing summer current after 100 simulated years (Figure 5.14). Only when the entire spring spawning season occurred before the spring transition were both genotypes able to spread throughout the habitat. In this model, both genotypes persisted but genotype 2

became dominant along the entire coast while genotype 1 became more common in Puget Sound.

Discussion

Gene Flow. The models suggest that ocean currents and spawning season may play a large role in gene flow patterns, but that variation in total planktonic period is unlikely to play a role in species with generally long-lived larvae. A consistent ocean current pattern like the convergent summer currents near Point Conception can produce an effective barrier to gene flow. Similarly, the simulation models of Gaylord & Gaines (2000) predicted that convergent and divergent currents can create a barrier to dispersal and maintain species range endpoints. This barrier can be shifted, but only slightly relaxed, by temporal variation in the ocean current pattern. The scale of temporal variation also plays an important role, suggesting that infrequent, longer periods of current reversal have a greater effect on gene flow than more frequent, shorter reversals. Thus rare changes in currents, such as El Niño events that persist for a year or more (Glynn 1988), should have a greater long-term effect than intra-annual variation in current patterns. This may make it more difficult in future studies to relate gene flow patterns to ocean currents, unless long-term data on current variation are available.

My models predict that variation in total planktonic period will not have a large effect in species with planktonic larvae. This result is consistent with the genetic data of Shulman & Bermingham (1995) and the geographic range data of Scheltema (1989) and Emlet (1995). It conflicts with the results of Waples (1987) and Doherty et al. (1995). However, both of these studies examined fish species whose larvae may use their swimming ability to retard, rather than enhance, dispersal and gene flow, compared to less powerful ciliated swimmers (Doherty et al. 1995). Doherty et al. (1995) also predicted panmixis for planktonic periods greater than one month. The planktotrophic larvae of the *Littorina* species examined here require a week-long planktonic pre-hatching period plus significant larval feeding and growth before settlement and metamorphosis (chapter 4), so the 10-day precompetent period in the 30-day model is probably an underestimate. A precompetent period of several weeks is probably common among species with planktotrophic larvae (Levin & Bridges 1995), and the models suggest that this minimum planktonic period is sufficient to produce the maximum levels of gene flow permitted by the ocean current regime. This result bolsters previous predictions that extended planktonic development is not an adaptation for dispersal and gene flow (Strathmann 1985), since extending the planktonic period would have little effect. The threshold planktonic period

above which variation does not affect gene flow should depend on life history and behavior as well as geography and ocean currents in the species' range. It may be lower in species like gastropods whose larvae are less powerful swimmers than fish or crustaceans. It is also likely to be lower when ocean currents are faster or the species range is smaller.

Extending the precompetent period does result in greater total larval mortality as expected (Jackson & Strathmann 1981), which may keep some marginal habitats from being occupied because of insufficient larval supply, as in the 90-day model. However, here I assumed an equal reproductive output in all models. If there is a trade-off between precompetent period and parental provisioning, such that species with greater requirements for larval growth are able to produce more offspring with the same total energy allocation, higher numbers of offspring may balance increased total mortality and maintain similar numbers of recruits. This sort of trade-off is predicted (Vance 1973; Christiansen & Fenchel 1979; Strathmann 1985), but has not been observed in these *Littorina* species.

Because of the large effect of current patterns, spawning season also plays a large role when currents vary seasonally. In the Point Conception models, shifting half of the reproductive output to the southward-flowing spring current eliminates the southern genotype after 100 years. In the Washington model, however, the effect of a longer spawning season is seen only when early spawning occurs 60 days before the spring transition. In both cases, extending the spawning season removes any barrier to gene flow and produces similar gene frequencies along the entire coast. Testing this prediction in nature would be straightforward with sympatric, related species that differ in length or timing of spawning season. Spawning season does not seem to play a role in the different population genetic patterns in *L. scutulata* and *L. plena*, since both species spawn from early spring to mid fall in the Puget Sound region (chapter 4).

Burton (1998) did not find any species with a genetic break at Point Conception, despite the current patterns and the predictions of the models presented here. There are several possible explanations for the discrepancy. One is that actual variation in current patterns is much greater than modeled. The complex topography and forcing mechanisms in this area lead to abnormally high levels of variation at all temporal scales (Lynn & Simpson 1987; Hickey 1998), and these models could only address a subset of this variation. A second factor is vertical variation in currents. As an approximation for the larvae of intertidal species, these models used currents from only the top 10 m of the water column. However, larvae that sink as little as 15 m over the inner shelf or 100 m over the outer shelf in summer may move from the southward-flowing California Current to the northward-flowing California Undercurrent (Hickey 1998). Extending these models to 3

dimensions, as in the models of Smith & Stoner (1993), could test the effect of larval behavior in these currents. A third consideration is history; genetic structure in species may not have reached equilibrium under present conditions. Fourth, barriers to dispersal in nature may be created more by other factors. The best-studied Point Conception species, the copepod *Tigriopus californicus*, shows limited dispersal among tidepool habitats because of high predation in the intertidal and strong selection, so the genetic break at Point Conception is no deeper than several other breaks along the California coast (Burton 1998). Finally, these models were limited geographically because of computing time. For example, it is possible in nature for larvae from northern populations to ride the California Current offshore at Point Conception and back onshore farther south, beyond the boundaries of the models. They or their offspring could then continue north or south along the coast.

Perhaps the most important discrepancy between the model results and existing genetic data is resolved in the spawning season models. The results here closely match the southward-biased gene flow found in three species (two barnacles and a sea urchin) by Wares et al. (in press). All three of these species reproduce over several months, and two of them reproduce mostly during the spring during maximum southward flow around Point Conception (Hines 1978; Strathmann 1987). A similar pattern of spawning occurs in several species along this coast (Strathmann 1987). *Tigriopus californicus* reproduces and is able to disperse all year long (Burton et al. 1979), so it experiences the full range of ocean currents around Point Conception.

Marine Speciation. These results combine with previous work to support a conceptual model of marine speciation with two components. First, gene flow is limited, but not eliminated entirely, by some combination of the factors described here that may be short-lived on an evolutionary scale (Palumbi 1996). While the barrier persists, genetic divergence and complete isolation is produced by genetic changes that may operate surprisingly quickly (Palumbi 1994; 1998). This is a more specific version of the divergence-with-gene-flow model described by Rice & Hostert (1993) and modeled by Felsenstein (1981) and others. In their review of laboratory experiments on speciation, Rice & Hostert (1993) emphasize the importance of diminished gene flow combined with divergent selection and the genetic mechanisms of pleiotropy and genetic hitchhiking. They find that complete elimination of gene flow has been overemphasized in traditional views of allopatric speciation.

Most barriers to gene flow in the sea are "leaky." Biological factors, such as spawning season, larval behavior, and selection, do not erect absolute barriers to dispersal

but rather create general patterns and directions of gene flow. Ocean currents also follow predictable general patterns but vary seasonally and annually, which can allow low levels of gene flow as at Point Conception. As Palumbi (1994) points out, cases like the Isthmus of Panama, an absolute barrier to dispersal of marine larvae, are probably the exception in the marine world. In addition, ocean currents vary at millennial time scales along with climate (Sawada & Handa 1998), so many of these leaky barriers to gene flow are transient. Nonetheless, they can leave lasting signatures on the genetic structure of populations (Hellberg 1995; Benzie & Williams 1997). Palumbi (1996) emphasized the role of such transient barriers to gene flow in Pleistocene differentiation and speciation in the sea urchin genus *Echinometra*. The geographical scale of distinct populations varies widely, from large populations like those modeled here or studied by Palumbi (1996), to estuarine populations genetically isolated from the adjacent outer coast (e.g. Bertness & Gaines 1993).

As discussed in chapter 3, the Recognition species concept of Paterson (1978; 1985) is appropriate for the gastropod genus *Littorina* discussed here, and also fits the large number of marine animals with external fertilization (Palumbi 1992). This species concept emphasizes that the features that define species, the Specific Mate Recognition Systems whose divergence produces reproductive isolation and speciation, are uncoupled from somatic or economic characters (food capture, predator avoidance, etc.) (Eldredge 1995; Vrba 1995). In the extreme, the Recognition System in marine animals can depend on very few genes that determine gamete incompatibility (Palumbi 1992; 1998). More genes are likely involved in the differences in reproductive morphology that maintain reproductive isolation in animals with internal fertilization, such as *Littorina* spp (Saur 1990). Nonetheless, growing evidence suggests that cryptic sibling species, which are reproductively isolated but very similar in morphology and habitat, are widespread (Knowlton 1993; Norris et al. 1996). Knowlton (1993) found that important isolating mechanisms between sibling species include behavioral incompatibility, lack of synchroneity in reproductive activity, and fertilization barriers. *Littorina scutulata* and *L. plena* are reproductively active during the same seasons and are broadly sympatric (chapters 3, 4), but the defining differences between the species include aspects of penis and copulatory bursa morphology. Though these species' mating behavior has not been studied, these physical differences are probably involved in reproductive isolation. Other aspects of these species' morphology, habitat, and general ecology are variable within species and similar between the species, and there are not substantial differences in habitat or range. Estimates of the age of divergence of these two species range widely, from 2 to

18 million years, and fossil evidence is scarce (chapter 3). As a result, it is difficult to determine the environmental conditions or ranges of the incipient species at the time of divergence.

I propose that the speciation event that produced *L. scutulata* and *L. plena* follows a pattern common among marine species with planktonic larvae, in which speciation includes a temporary restriction of gene flow, caused by a combination of physical and biological factors, combined with divergent selection and genetic factors like pleiotropy or genetic hitchhiking. Divergence occurs primarily in reproductive characters, perhaps only in a few key loci, and allopatric separation may not persist for long enough to promote significant divergence in other characters. Because of the dispersal potential of species with planktonic larvae, ranges quickly expand or shift when ocean current patterns change. The new species, which diverged in allopatry, may become sympatric and obscure the geographic signature of their separation.

Figure 5.1: Digitized map of the Point Conception area at a 1-km scale, showing initial populations of the two genotypes. Numbers along the coastline represent linear kilometers used in Figures 5.11, 5.12, and 5.13.

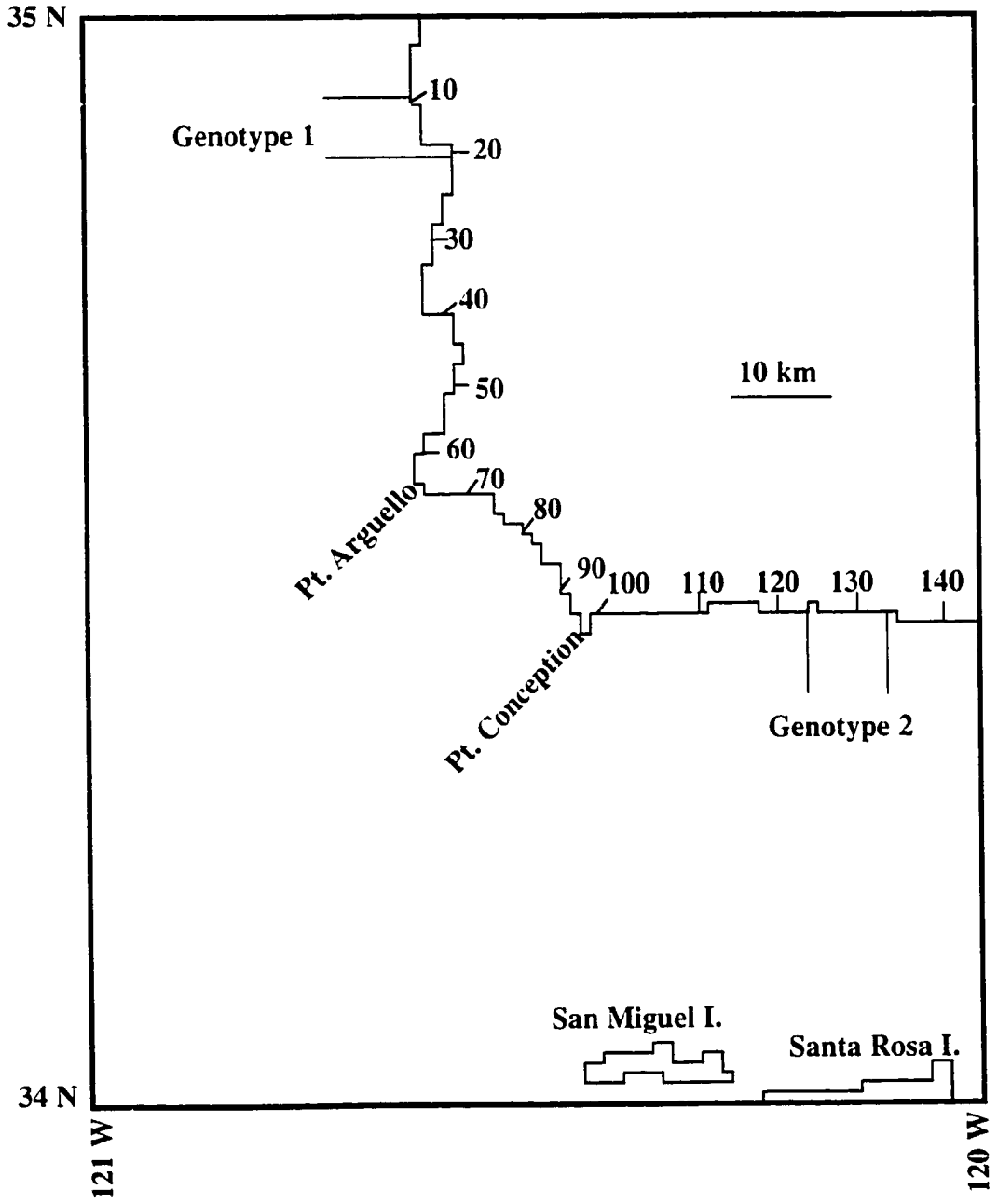


Figure 5.2: Digitized map of the Oregon, Washington, and southern British Columbia coast at a 5-km scale, showing initial populations of the two genotypes and linear kilometers along the outer coast used in Figure 5.14. The dashed line indicates the break in linear kilometers at the entrance to Puget Sound.

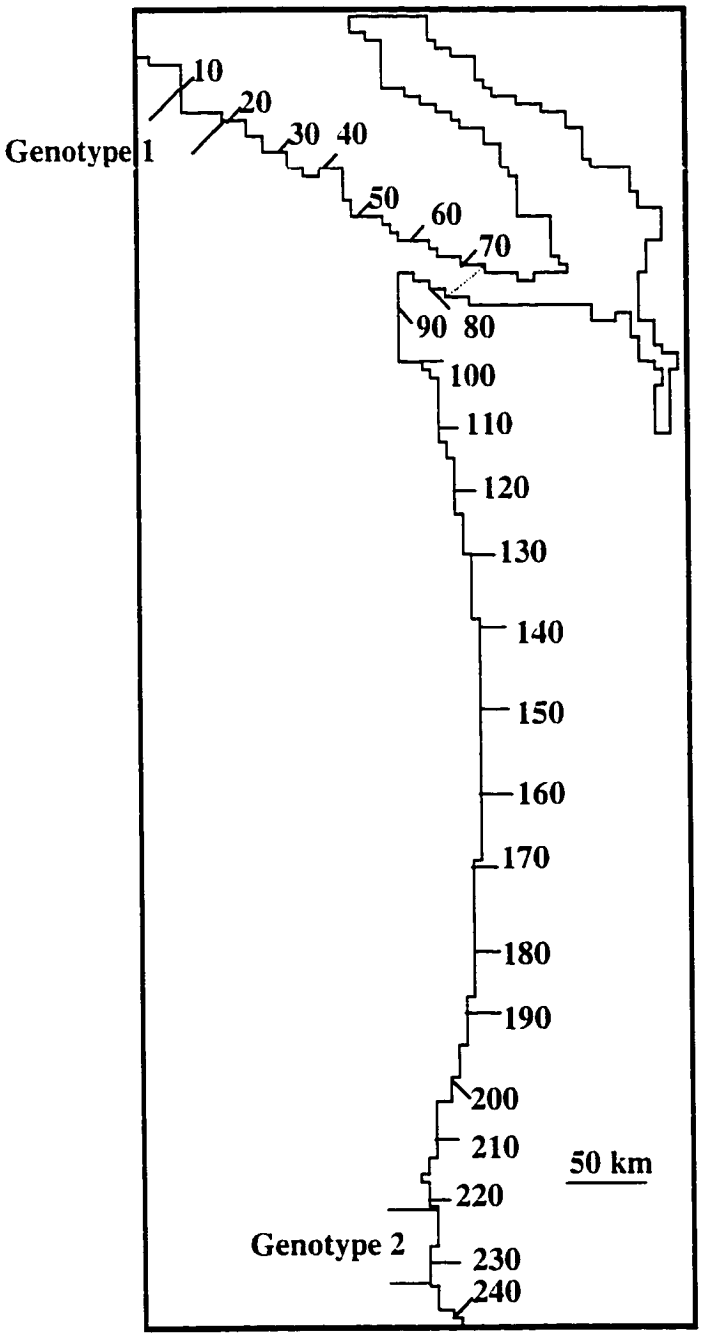


Figure 5.3: Representative current vectors of the average, summer, surface current pattern around Point Conception. Vectors represent speed and direction at their upstream end. Arrows are shown on only a few vectors for clarity. Stars represent current data points from buoys (Harms & Winant 1998) and measured offshore flow (Hickey 1989, 1992, 1998) used for interpolation of current patterns.

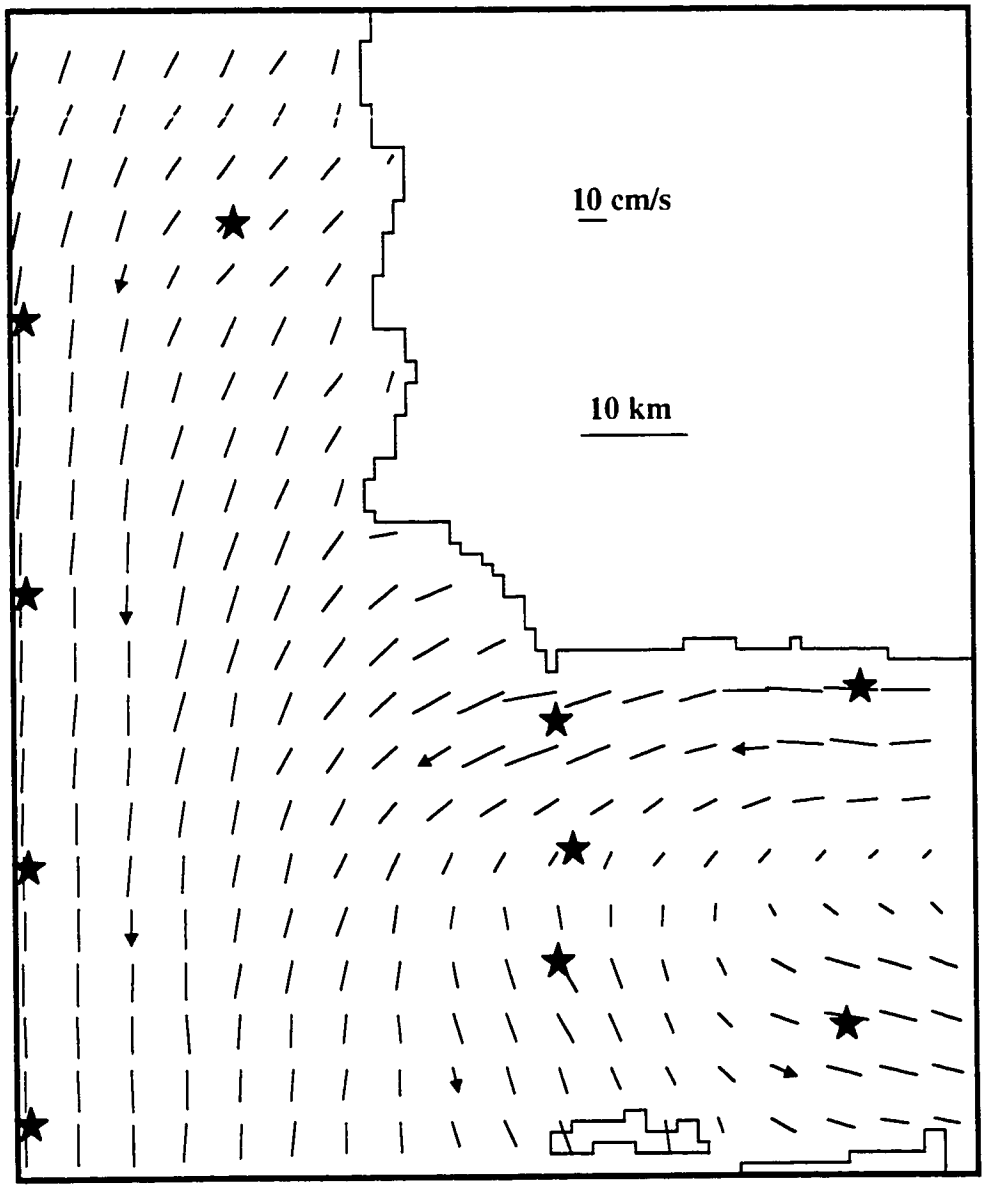


Figure 5.4: The upwelling current pattern described by Harms & Winant (1998), one of 4 current patterns used in the intra-annual variation models.

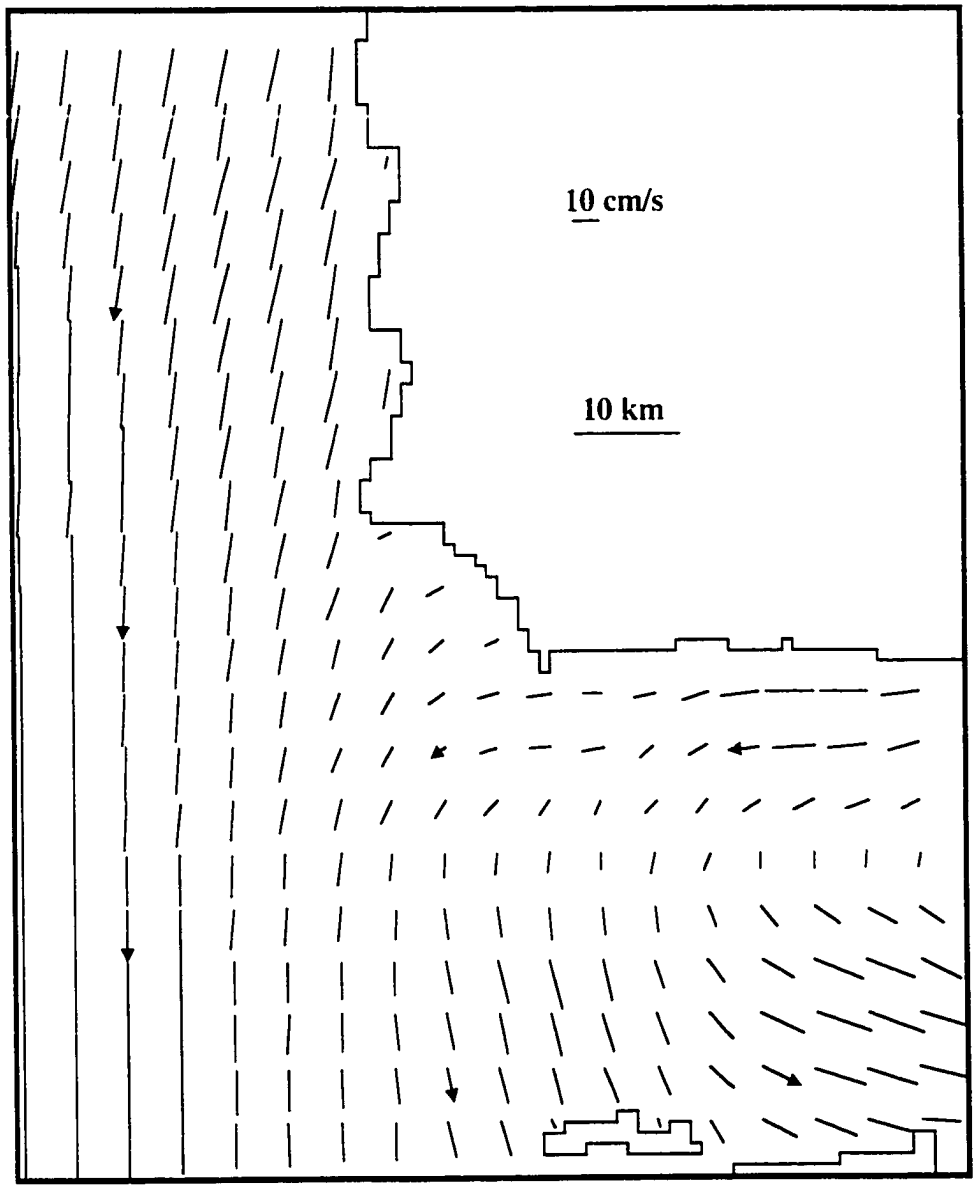


Figure 5.5: The cyclonic current pattern of Harms & Winant (1998), the second of 4 current patterns used in the intra-annual variation models.

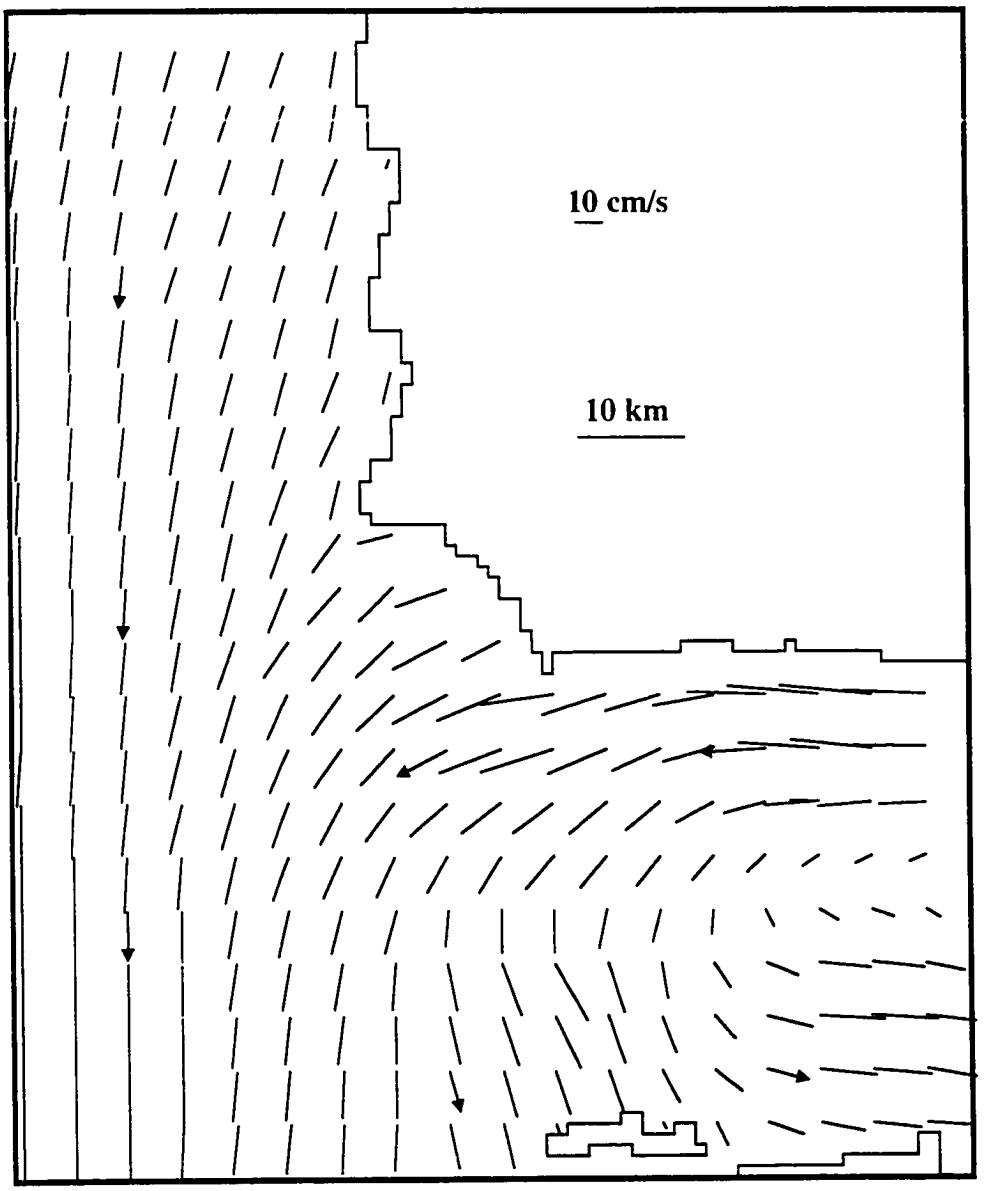


Figure 5.6: The quiescent current pattern of Harms & Winant (1998), the third of 4 current patterns used in the intra-annual variation models.

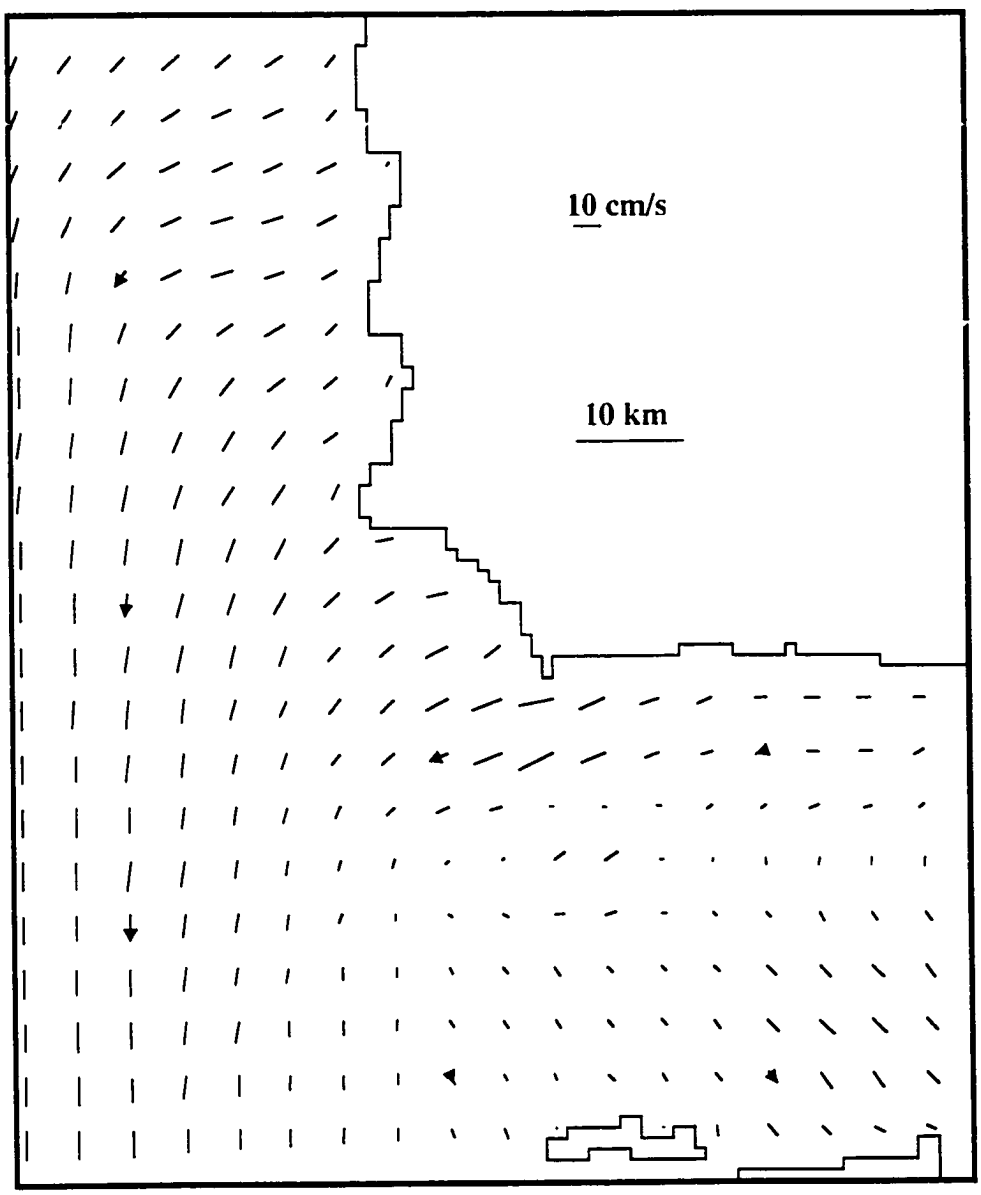


Figure 5.7: The relaxation current pattern of Harms & Winant (1998), the fourth of 4 current patterns used in the intra-annual variation models. This pattern was also used in the inter-annual variation models.

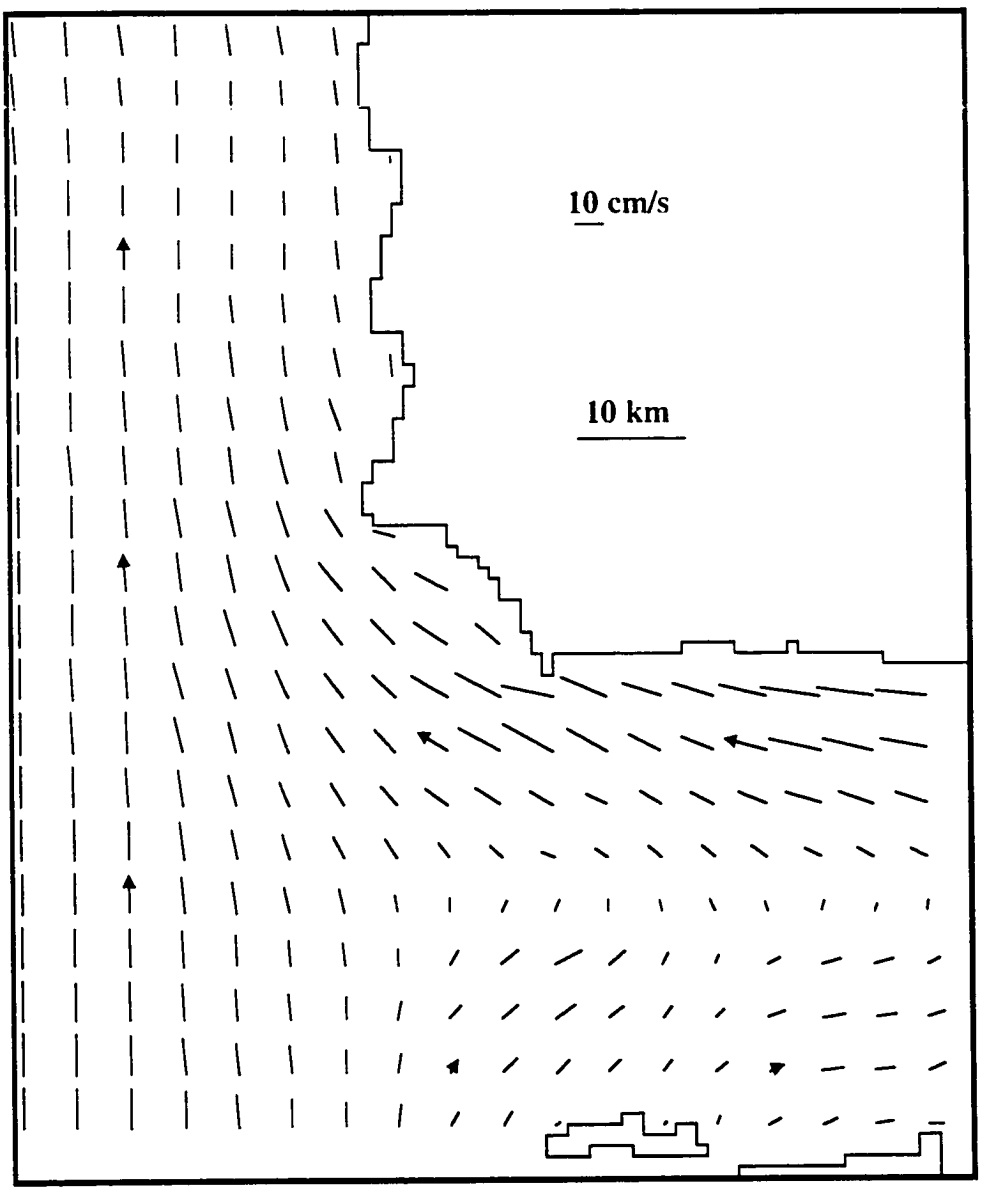


Figure 5.8: The spring current pattern around Point Conception, used in the spawning season models.

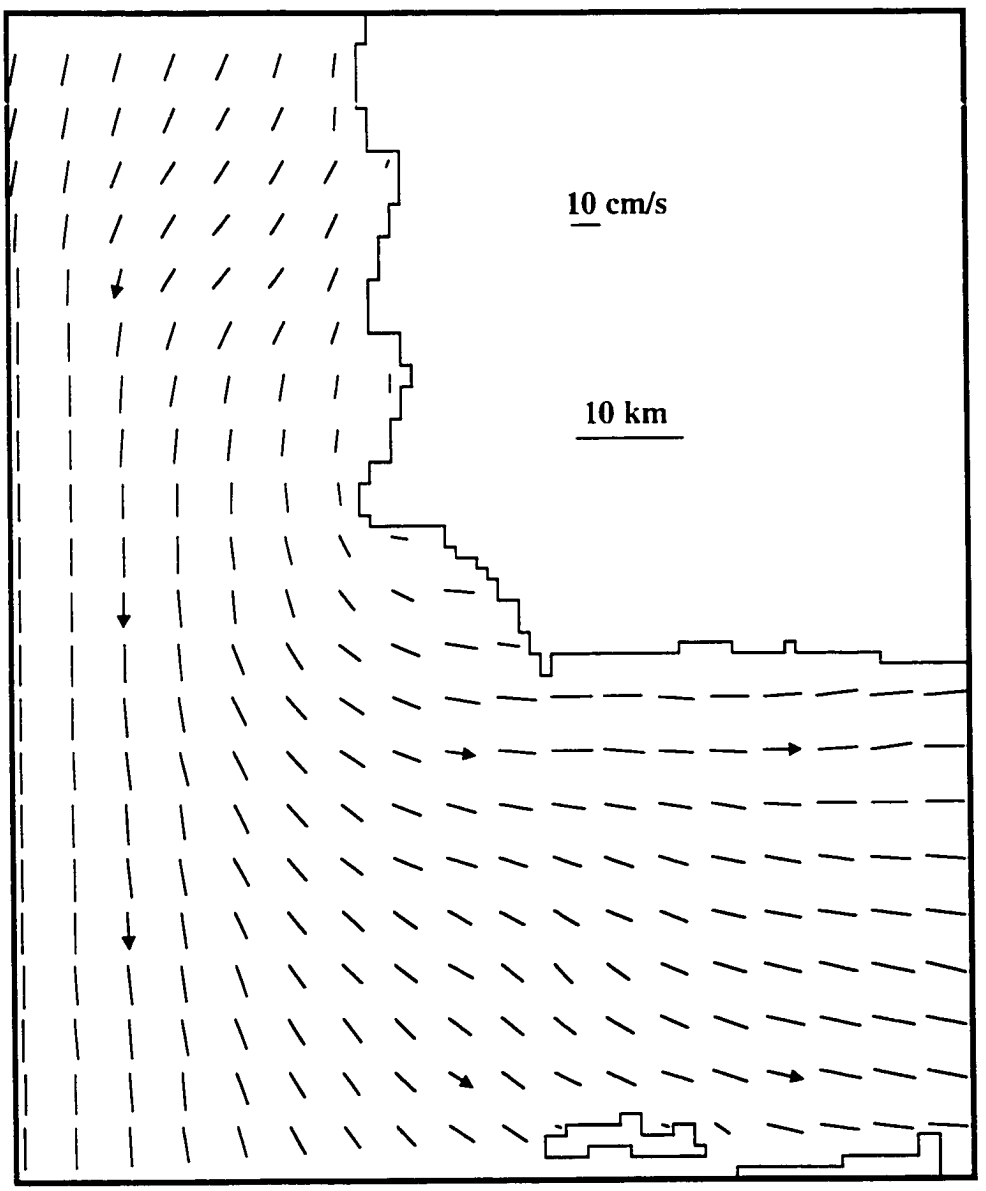


Figure 5.9: The spring current pattern along the Washington coast. Currents are mostly tidal inside Puget Sound, so only turbulent eddy diffusion was used in Puget Sound in the models.

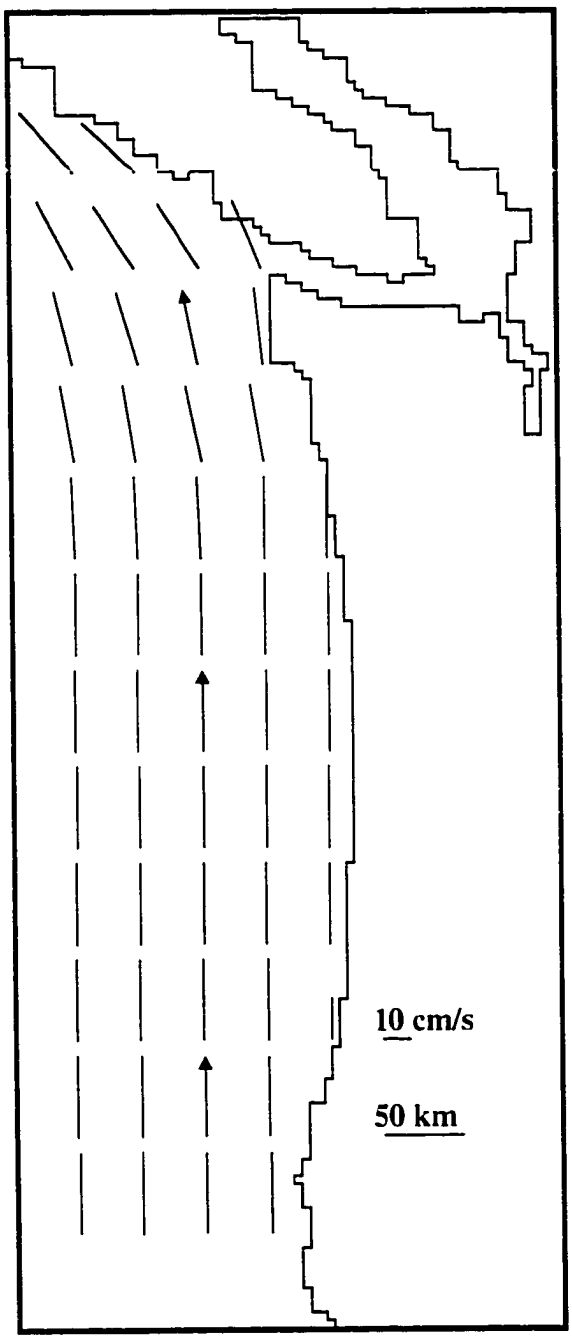


Figure 5.10: The summer current pattern along the Washington coast.

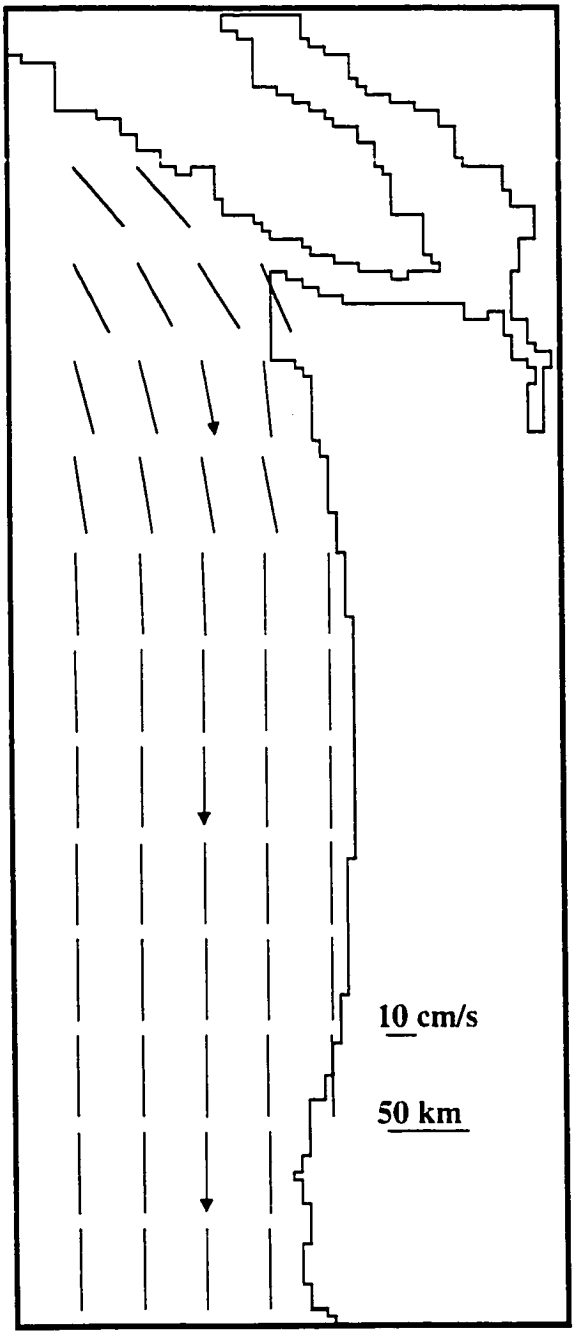


Figure 5.11: Gradual approach to quasi-equilibrium in the Point Conception model with constant currents. Shown are the adult populations along the coast, as a proportion of the final 100-year population, by 5 years (white), 10 years (lighest gray), 15 years (light gray), 25 years (stippled gray), 50 years (dark gray), and 100 years (black). With minor exceptions from kilometer 10 to 15, populations grew monotonically. Positions of the original populations of both genotypes are shown, and a sharp break between the genotypes persisted at kilometer 80 (see Figure 5.12).

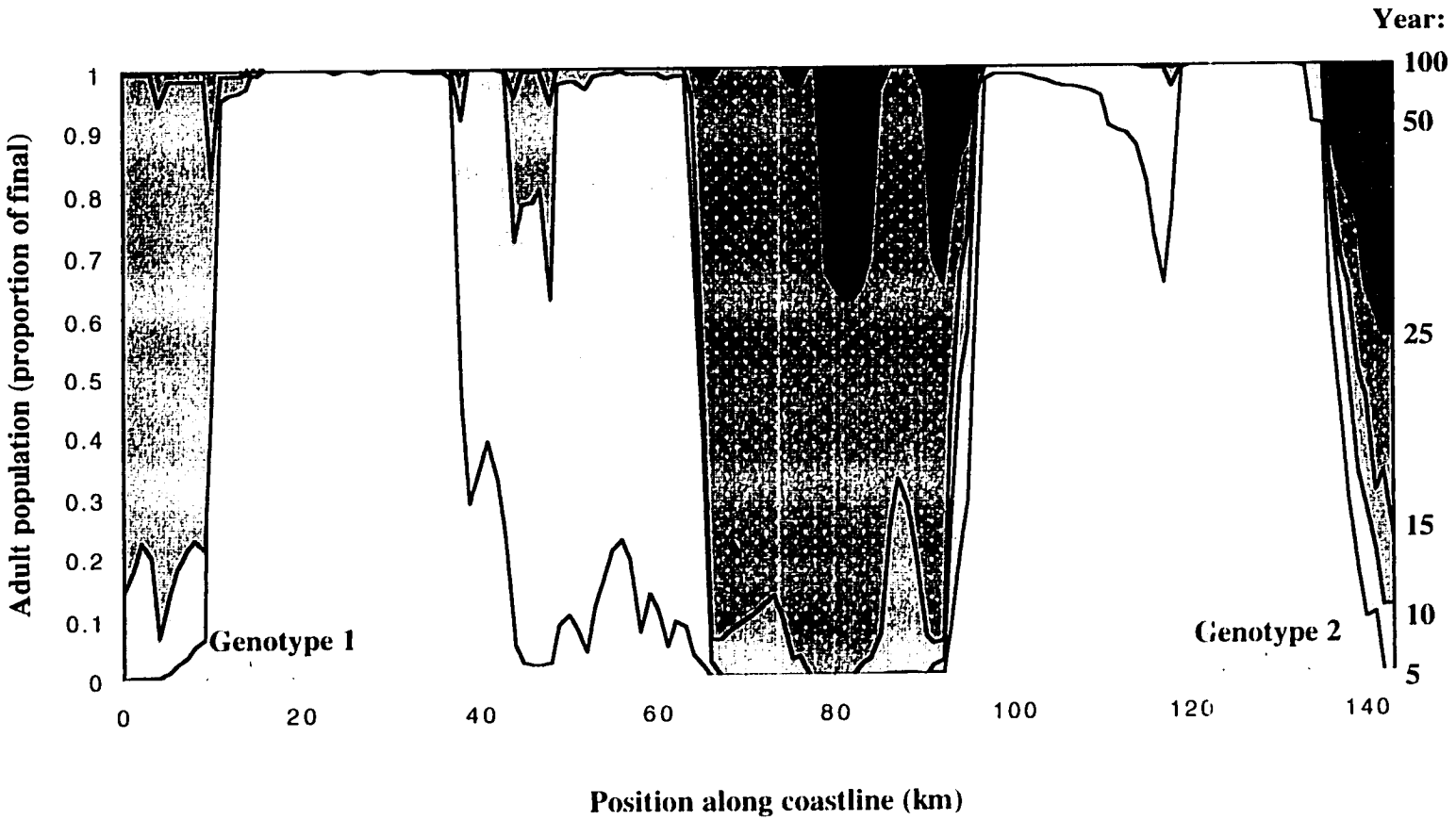


Figure 5.12: Results of the models of ocean current variation around Point Conception. The x-axis represents kilometers along the coast as shown in Figure 5.1. See the text for details of the five treatments.

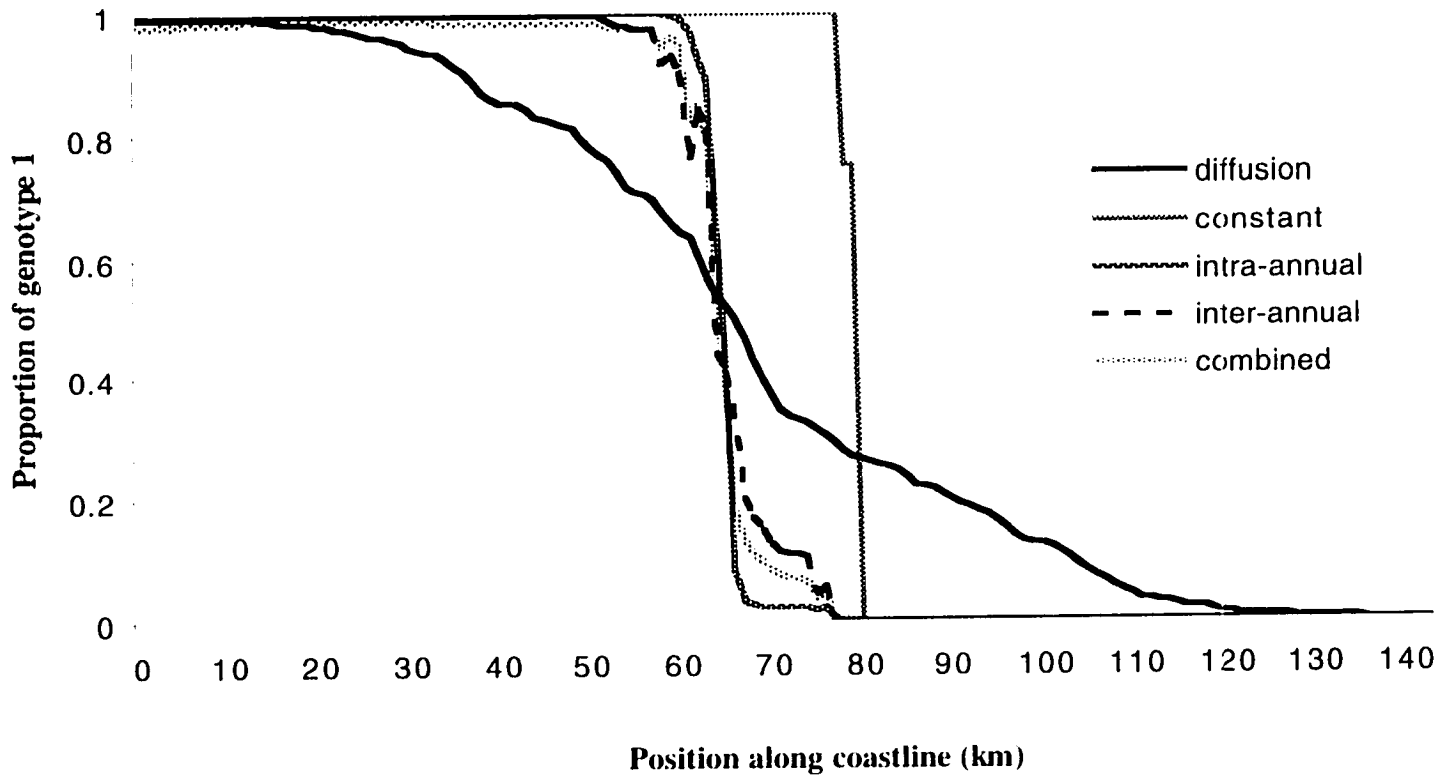


Figure 5.13: Results of the planktonic period variation models around Point Conception. Braces show stretches of unoccupied coast in the long period (90-day) model. The results of the 60-day model, shown for comparison, are identical to the intra-annual model shown in Figure 5.11. See the text for details of the three treatments.

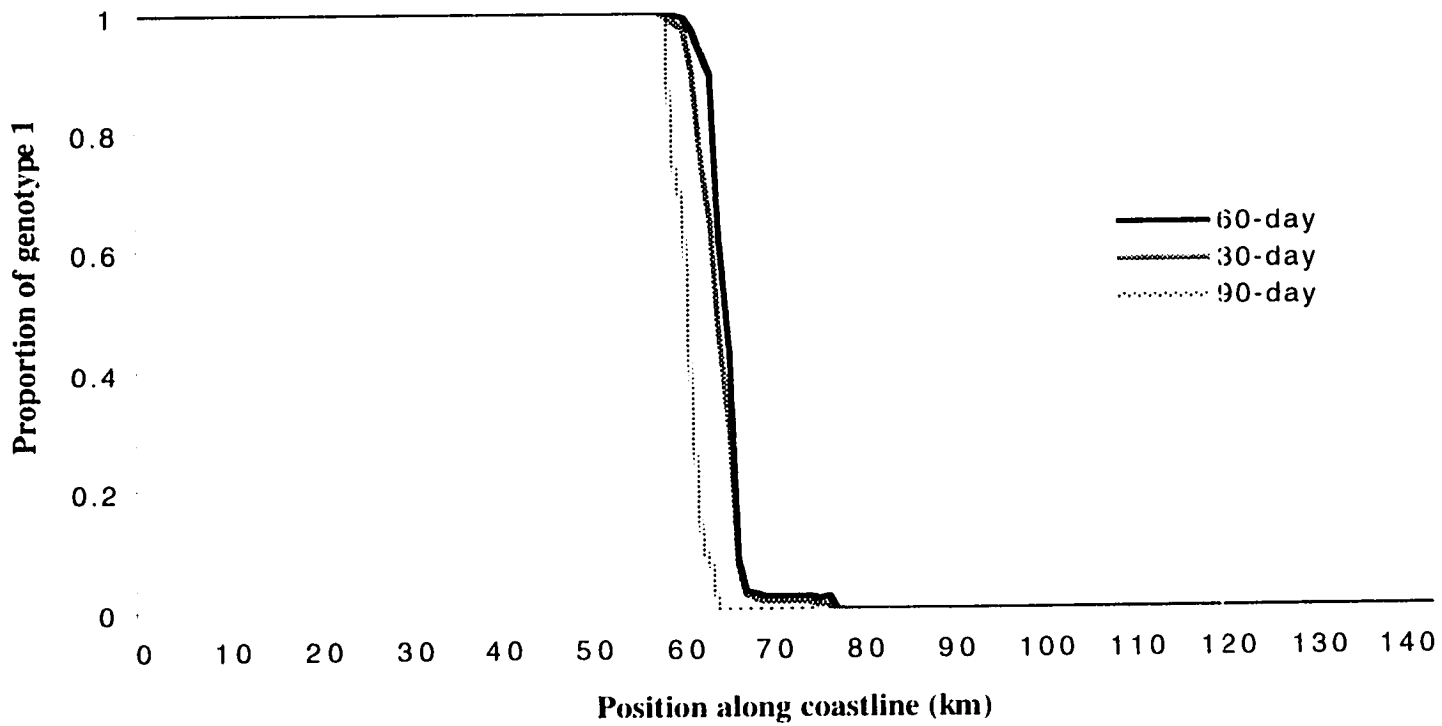
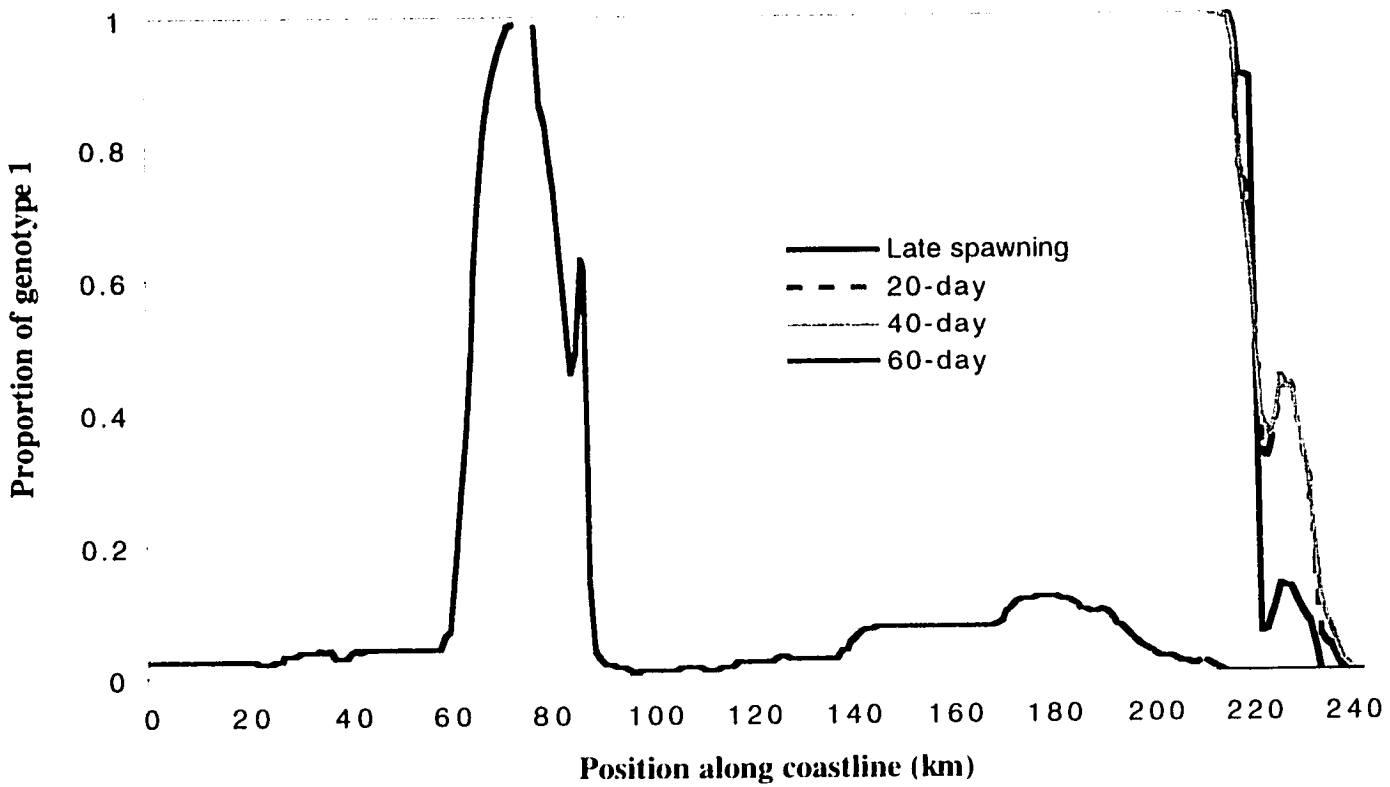


Figure 5.14: Results of the spawning season models along the Washington coast. The x-axis represents kilometers along the outer coast as shown in Figure 5.2. See the text for details of the four treatments. The break in the lines represents the entrance to Puget Sound. In the late-spawning, 20-day, and 40-day models, Puget Sound was occupied exclusively by genotype 1. In the early-spawning (60-day) model, Puget Sound was occupied by genotype 1 at an average proportion of 0.96.



Appendix

The models were written in the computer language C and run on Silicon Graphics Indy computers. The basic form was a system of partial differential equations that were rendered dimensionless by the following conversions:

1 unit of length = 100 m

1 unit of time = 10,000 s = about $1/9^{th}$ of a day

1 unit of female adults = the carrying capacity of one meshpoint of coastline.

The partial differential equations were converted to sets of many ordinary differential equations using a 1-km mesh in the Point Conception models and a 5-km mesh in the Washington models by the method of lines. A diffusion constant of 10^4 cm^2/s was used appropriate to the mesh size (Okubo 1971). A time step of τ=0.1 units, or 1000 seconds, was used, and the ordinary differential equations were solved numerically using the second-order Euler method:

$$Y(t+\tau) = Y(t) + \frac{\tau}{2}\left(F(Y(t)) + \tau F(Y(t))\right).$$

For each ocean current pattern, a map with a current vector at each meshpoint was constructed by interpolation using data from unevenly spaced buoys at which currents were measured (Figure 5.1). For each meshpoint (i,j), the five closest data buoys were ranked from 1 (closest) to 5 (farthest), and the x-component at (i,j) is:

$$\begin{cases} x_{ij} = x_1 & \text{if buoy 1 is at (i,j)} \\ x_{ij} = \dfrac{x_1 + x_2\left(\frac{d_1}{d_2}\right)^2 + x_3\left(\frac{d_1}{d_3}\right)^2 + x_4\left(\frac{d_1}{d_4}\right)^2 + x_5\left(\frac{d_1}{d_5}\right)^2}{1 + \left(\frac{d_1}{d_2}\right)^2 + \left(\frac{d_1}{d_3}\right)^2 + \left(\frac{d_1}{d_4}\right)^2 + \left(\frac{d_1}{d_5}\right)^2} & \text{if buoy 1 is not (i,j),} \end{cases}$$

where d_k is the distance of buoy k from the meshpoint, and x_k is the x-component at buoy k. The y-component at each meshpoint follows similarly. Cross-shore components of current vectors within 5 km of coastline were then reduced by a factor of *0.2 * (5 - d)*, where d is the distance of the meshpoint from the coast. Upstream differencing was used in the approximations at each time step, depending on the current vector at each meshpoint.

In the absence of detailed habitat information, the carrying capacity k was set to 1 unit of females at all points along the coastline. The number of offspring produced by a females at the beginning of the reproductive season is:

$$\begin{cases} ba & \text{if } A \le k \\ \dfrac{ba}{A+1-k} & \text{if } A > k \end{cases}$$

where A is the total adult density.

Larval mortality was modeled as exponential decay during the reproductive season:

$$\frac{\partial L}{\partial t} = -\mu L$$

where μ=0.2 day^{-1}(Morgan 1995b). Adult mortality occurred only outside the reproductive season, and a population of a females was converted to:

$$\begin{cases} (1-\lambda)*a & \text{if } A \le k \\ \dfrac{1-\lambda}{\sqrt{A}}*a & \text{if } A > k \end{cases}$$

where λ=0.35 yr^{-1}, which results in 95% mortality after 7 years (Behrens Yamada 1992).

Larvae adjacent to the coast settle and enter the adult population at the following rate per day:

$$\begin{cases} 0 & \text{during the precompetent period} \\ \dfrac{t}{c}*0.2 & \text{during the competent period} \end{cases}$$

where t is time from the start of the competent period, and c is the total length of the competent period.

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VITA

Paul A. Hohenlohe

Birthdate: Jan. 17, 1973
Birthplace: Washington, DC
Citizenship: USA

EDUCATION

University of Washington, Seattle
PhD in Zoology, fall 2000.

Williams College, Williamstown, MA
B.A. Summa Cum Laude in Biology, 1994.
Advisor: Dr. David Smith. Thesis topic: Niche separation, morphology, and performance in two species of *Peromyscus*

University of Montana, Missoula, fall 1992.

TEACHING EXPERIENCE

Predoctoral Instructor
Zool 433/434: Invertebrate Zoology, University of Washington

Teaching Assistant
Zool 430: Marine Invertebrate Zoology, University of Washington
Biol 454: Evolutionary Mechanisms, University of Washington
Zool 487: Animal Physiology, University of Washington
Biol 101: Introductory Biology, University of Washington
Biol 105: Introductory Biology, University of Alaska Anchorage
Biol 203: Ecology, Williams College

FELLOWSHIPS AND AWARDS

National Science Foundation training grant in mathematical biology, 1998 and 1999
National Science Foundation graduate fellowship, 1995-2000
Rhodes Scholarship regional finalist, 1994
Phi Beta Kappa Honor Society, inducted 1994
Sigma Xi Scientific Research Society, inducted 1994
William C. Grant Jr. Prize in Biology, Williams College, 1994
Highest Honors in Biology, Williams College, 1994
Howard Hughes Medical Institute undergraduate research grant, 1993

PUBLICATIONS

Hohenlohe, P.A. & E.G. Boulding. in press. A molecular assay identifies morphological characters useful for distinguishing the sibling species *Littorina scutulata* and *L. plena*. J. Shellfish Res.

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