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Settlement patterns of the mussels Mytilus spp. in Moss Landing, California

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SETTLEMENT PATTERNS OF THE MUSSELS *MYTILUS* SPP. IN MOSS LANDING, CALIFORNIA

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ABSTRACT

SETTLEMENT PATTERNS OF THE MUSSELS *MYTILUS* SPP. IN MOSS LANDING, CALIFORNIA

by Shannon B. Johnson

Larval settlement may determine the distribution of marine populations. I compared larval settlement to adult distributions for intertidal mussels in Moss Landing, California. Adult *M. californianus* were most abundant in wave-exposed rocky intertidal areas and adults of Blue mussels (*M. trossulus* and *M. galloprovincialis*) were more abundant inside protected Moss Landing Harbor. Recruitment monitored on fibrous scrubbing pads during 1-2 week intervals for 12 months in 2002-2003 revealed that all mussels settled in greater numbers on the open coast, and that Blue mussels settled in slightly greater numbers there than did California mussels. Settlement was generally low in the harbor environment. California mussels settled mostly in their adult populations. However, Blue mussels settled where adults were rare, thus post-settlement mortality appeared to be the strongest influence on adult distributions.

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Introduction

A great deal of spatial and temporal variation in rocky intertidal communities can be attributed to differential larval settlement patterns (Morgan 2001). Early research concluded that larval behaviors such as settlement preferences, delayed metamorphosis, and decreased discrimination among settlement sites by older larvae were critical in enhancing the survival and reproduction of sessile adults (Thorson 1950; Meadows and Campbell 1972; Scheltema 1974; Crisp 1976; Morgan 2001). Revived interest in larval recruitment during the 1990's revealed that larval settlement was more important for adult population abundances at lower settlement densities, and density-dependent processes were more important at higher settlement densities (Connolly and Roughgarden 1998; Morgan 2001). Larval biology and settlement patterns thus can have important effects on intertidal community structure.

Mussels of the genus *Mytilus* are conspicuous inhabitants of rocky intertidal environments on much of the west coast of North America (Suchanek 1978). Adult distributions vary on a latitudinal gradient: densities of mussels in mussel beds are significantly higher in northern and central Oregon than in California (Connolly and Roughgarden 1998). These differences have been correlated with a latitudinal gradient in recruitment that decreases southward from Oregon. This gradient may be attributable to differences in upwelling, which is stronger in California and may transport larvae offshore, thereby reducing larval supply to nearshore benthic communities (Connolly et al. 2001). More free space is believed to lessen the effects of predation, competition, and disturbance on community structure (Connolly and Roughgarden 1998). Therefore,

larval supply should be more important in California because adult interactions have smaller effects (Connolly et al. 1999).

Three species of mussels inhabit the central coast of California: the California mussel, *Mytilus californianus*; the Blue mussel, *Mytilus trossulus*; and the invasive Blue mussel from the Mediterranean Sea, *Mytilus galloprovincialis* (Suchanek 1978; McDonald and Koehn 1988). Adult *M. californianus* primarily inhabit exposed rocky intertidal areas of the North American Pacific coast from the Aleutian Islands to northern Mexico (Soot-Ryen 1955). Adult *M. trossulus* and *M. galloprovincialis* primarily inhabit protected bays and harbors. *Mytilus trossulus* occurs in the northern Pacific from Siberia to central California, the Canadian Maritimes, and the Baltic Sea. *Mytilus galloprovincialis*, in contrast, occurs in the Mediterranean Sea and the Atlantic coast of Southern Europe, and was introduced in Japan, Hong Kong, Western Australia, Tasmania, New Zealand, South Africa, and California (Seed 1976; McDonald and Koehn 1988).

The Blue mussels, *M. trossulus, M. galloprovincialis* and *M. edulis,* are sibling species and together comprise the *M. edulis* species complex (McDonald and Koehn 1988; Koehn 1991). They are morphologically similar with thinner shells and weaker byssal threads than California mussels (Fankboner 1978). They also have greater motility, enabling them to avoid higher sedimentation rates in bays (Harger 1968; Gosling 1992). Hybridization occurs at all locations where Blue mussel species are sympatric (McDonald et al. 1991; Gosling 1992; Rawson et al. 1999).

In this study, I compare settlement patterns of California and Blue mussels in Moss Landing to adult distributions. Sampling in three areas that varied in adult abundance was used to determine if adult distributions could result from selective settlement by juveniles or if all three species settle opportunistically and adult distributions more likely result from post-settlement processes. These areas were: the exposed side of the entrance to Moss Landing Harbor (rocky intertidal), Moss Landing Harbor beneath Sanholdt road bridge (protected area), and the harbor channel which is somewhat protected by north and south jetties (intermediate area). *Mytilus californianus* inhabit the outer jetty of Moss Landing Harbor and Elkhorn Slough. The jetty provides exposed, rocky intertidal habitat that is subjected to nearly constant high wave action and routine removal of mussels. *Mytilus trossulus* and *M. galloprovincialis* inhabit Moss Landing Harbor and Elkhorn Slough where temperatures fluctuate with tidal cycles, and water has large amounts of suspended sediment (unpublished data, Elkhorn Slough Foundation). Mussels are primarily attached to pier pilings and rocks because the majority of the slough and harbor is soft bottom. The main harbor channel also provides rocky intertidal habitat that is colonized by small patches of *M. californianus.* The channel is subjected to environmental conditions found in the harbor and outside the jetty such as elevated temperatures, changes in salinity, and moderate exposure to waves. Environmental conditions, however, are modified both by mixing of slough and open ocean waters and reduction in wave exposure. These conditions collectively create an "intermediate" environment in the channel (Broenkow and Breaker, *in press*).

Sampling prior to the present study determined that larvae of *M. californianus, M. trossulus,* and *M. galloprovincialis* co-occurred within Moss Landing Harbor (See results). Because *Mytilus* spp. juveniles do not necessarily require chemical cues for metamorphosis (Lutz and Kennish 1992), it is not essential that juvenile mussels find adults and settle among them. Larvae of *Mytilus* spp. vertically migrate and are able to delay metamorphosis until suitable substrate is encountered. These mechanisms, however, are probably used more to select micro scale habitats rather than large-scale environments. Therefore, if settlement occurs within an inhospitable habitat, it is likely that adult distributions of these mussel species are a result of post-settlement mortality rather than specific settlement patterns of juveniles (Heath et al.1996). All three *Mytilus* species should settle opportunistically in all three habitats. If post-settlement mortality explains adult distributions, then mussels may settle uniformly among these three habitats.

Materials and Methods

Adult Mussel Populations

Adult mussels were sampled at all three sites with a one-meter random point contact quadrat (RPC) with ten points. The RPC was used to identify mussels and estimate their proportional abundance and length rather than to estimate absolute percent cover. Intertidal zones were stratified as lower (0.0 to 1.0 m from 0.0 tide) and upper intertidal zones (1.0 to 2.0 m above 0.0 tide). Ten random samples were allocated within each stratum along a 50-m transect. Adults contacted by points (or nearest point) within the quadrat were measured with hand-held calipers to the nearest millimeter and identified as Blue or California mussels according to the criteria of Smith and Carlton (1975).

Larval Populations

Plankton tows were done in February 2002 within the harbor and channel of Moss Landing in order to determine if larvae of all three species were present. The open coast area was not sampled due to large swell. Three tows were conducted in each area with a net with 83-μm mesh size. Mussels were sorted from tows and identified in groups of ten and one hundred mussels with PCR reaction using species-specific primers that target the cytochrome b gene (primers from Geller and Bartl, in prep). Based on the findings that all species of larvae were present within these areas (see Results), a pilot study to design a sampling program for settlement was conducted in March 2002. Three main variables were tested: sample unit size, sample size, and duration of monthly sampling. Flat green

60 cm^2Scotch Brite™ pads were used as sample units rather than bulbous Tuffy™ Pads to avoid the possibility of passive ensnarement of juvenile mussels instead of actual recruitment (Caceres-Martinez et al. 1999). Based on results from the pilot study forty 60-cm^2 pads were deployed for an average of twelve days each month from March 2002 to February 2003 at each location. At the end of sampling, each pad was retrieved from the field and stored individually in small Ziploc™ Bags for transport. On average, on the jetty and channel ten recruitment pads were lost per month due to high wave action.

Diagnostic Molecular Identification

To facilitate byssal thread detachment, juvenile mussels were extracted from individual pads by soaking them in a 10% solution of commercial bleach for five minutes (Ramirez and Caceres-Martinez 1999). Individually, pads were rinsed with fresh water and detached mussels were counted and stored in 1.5 ml microfuge tubes containing 70% EtOH for molecular analysis.

To distinguish Blue and California mussels, juveniles were identified by polymerase chain reaction (PCR). For molecular analyses, 88 juvenile mussels per site per month were randomly chosen. Each pad contributed to these analyses based on total number of pads in field each month. Sample sizes were not always equal due to loss of pads and differing levels of settlement. Mussels stored in 70% EtOH were rehydrated for 15 minutes in autoclaved Nanopure® water and then sorted in 2 µl water into a 96 well PCR plate containing 100µl extraction buffer with 10x Invitrogen/Gibco® PCR buffer (200 mM Tris, pH 8.4, 500 mM KCl), 10 mg/ml Proteinase-K buffer, 5 µl/ml Tween-20,

and water (Li and Hedgecock 1998). Mussels were then incubated at 56°C for three hours, then 95°C for thirty minutes, and stored at 4°C.

Polymerase chain reaction (PCR) was used to differentiate California mussels from Blue mussels with one primer from Martel et al. (1999) and one designed by J. Geller that flank the internal transcribed spacer (ITS1) region of the ribosomal 18S gene cistron and the 5.8S region. Products for California mussels were about 550bp long and Blue mussels were about 600bp long. The 5' primer sequence was: 5'TTGATTACGTCCCTGCCCTTT3' located at the 3' end of the 18s gene. The 3' primer sequence was 5'AGTGATCCACCGCATAGAGTAGT3' located at 5' end of the 5.8s gene. A 2 µl aliquot of DNA was used in 10 µl reactions containing 10x Qiagen PCR buffer (Tris-Cl, KCl, $(NH_4)_2 SO_4$, 15 mM $MgCl_2$; pH 8.7), 200 µM dNTP, 10 pmols ITS-1 forward and 5.8s reverse primers, and 0.5 U/µl HotstarTaq™ Polymerase (Qiagen). Amplification of products was performed in a PTC-100 thermocycler (MJ Research®). To activate Hotstar™ enzyme, the initial denaturing step was lengthened to 15 minutes at 95°C and was followed by 30 cycles of 94°C for 30 seconds, 56° C for 30 seconds, and 72° C for one minute. This program was concluded with a step of 72° C for two minutes. Positive controls of adult genomic DNA isolated from gill tissue using DNAzol™ (Molecular Research Center) were used both as size comparisons and as evidence of successful PCR. Negative control reactions, containing no template, also were performed for every batch of juveniles identified to ensure there was no contaminating template. PCR products were electrophoresed on 1.5% agarose gels and visualized with an ethidium bromide stain.

A blind validation test was performed to ensure PCR products were correctly identified on gels. DNA was extracted and amplified from ten adults of each species using the same protocol as juveniles. Shells were kept as evidence of correct species identification. Samples were then amplified but loaded in a randomized pattern on an agarose gel. The loading order was unknown to the scorer (SJ). All mussels were correctly identified.

Statistical Analyses

Settlement rates were calculated as total number of settlers per cm^2 per number of days in field. Relative proportion Blue and California mussels on each pad were calculated from proportion of Blue (or California) mussels on each pad*total number of settlers per cm^2 per number of days in field. These data were standardized to weekly settlement rates. Monthly averages were then calculated from these data and used as replicates for ANOVA. Harbor samples were excluded from these analyses due to lack of California mussel settlement. A model-I two-factor (species and site) ANOVA was used to analyze species-specific settlement. A model-I, one-factor ANOVA was used to test for differences in Blue mussel settlement among all sites. The α-levels were set at 0.05. A one-sample KS test showed data were not normally distributed for species proportions and average sizes of juveniles and adults. There was no transformation found to normalize data, however, ANOVA is robust to non-normality (Zar 1998), so analyses were performed despite this violation. Assumptions of equal variances (Cochran's test $P > 0.05$), and independence were met.

Results

Planktonic Larval Sampling

Plankton tows and subsequent molecular analyses confirmed the presence of all three species of larvae in harbor and channel waters (Table 1).

Adult Mussel Sizes and Distribution

California mussels were the dominant species sampled on the jetty and the majority was between one to three centimeters long in the upper intertidal zone and two to four centimeters long primarily in the lower intertidal zone. Only a few Blue mussels, one to two centimeters in length, inhabited both the upper and lower intertidal zones of the jetty (Fig. 1a).

The size frequency data from the channel were somewhat compromised. Mussels were non-randomly chosen and measured because they were nearly absent from the channel with the exception of few California mussels in rock crevices in the upper intertidal. No mussels were found living in the lower intertidal zone. Additionally, most California mussels were very small (~1 cm) (Fig. 1b).

Blue mussels dominated the harbor, with only two California mussels found in the upper intertidal zone, both of which were small. On average, adult Blue mussels from the upper intertidal in the harbor were smaller than those in lower intertidal. Blue mussels from this site, however, were larger than mussels found at other sites (Fig. 1c).

Overall Mussel Settlement

Settlement of juvenile mussels occurred during the entire year but varied among jetty, channel, and harbor sites (Fig. 2). Settlement was generally higher from late spring through early fall and reduced during winter months. All settlement pads for the channel and jetty were lost in December during a large storm. There were pulses of settlement in May, August, and November at all sites. However, settlement densities on the jetty were generally higher than the channel and harbor during late spring and summer months, ranging from 0.02 to 1.47 mussels/cm^2/week. Channel settlement was somewhat intermediate compared to the jetty and harbor with settlement densities ranging from 0.001 to 0.29 mussels/cm^2/week. Settlement measured in the harbor was very low yet relatively constant throughout the year, ranging from 0.001 to 0.04 mussels/cm^2/week.

Settlement by Each Species Group

Primers amplified ~600-bp fragments with template from Blue mussels while template from California mussels produced ~550-bp fragments (Fig 3).

Average monthly proportions of Blue and California mussel settlement were not different on the jetty and channel; however, the jetty and channel differed in settlement rates, where more mussels settled on the jetty than on the channel. Interaction between site and species was not significant (Table 2a) (Fig. 4). The harbor site was excluded from these analyses because settlement was low but dominated by Blue mussels, and California mussels were rare or absent in all months (Fig. 4).

Blue mussel settlement was observed among all sites, with the jetty site having the highest rate of settlement in comparison to the channel or harbor (Table 2b). In

addition, for many months, juvenile Blue mussels outnumbered juvenile California mussels settling on the jetty (Fig. 4).

Discussion

Exposed and Semi-Exposed Environments

Patterns of adult mussel distributions in California differ from those of the Pacific Northwest. Mussel bed densities are greater on the outer shores of Oregon and *M. trossulus* regularly inhabits areas where *M. californianus* has been removed (Suchanek 1978; Connolly et al. 2001). In addition, there is a regular and well-defined band of *M. trossulus* living above *M. californianus* in exposed rocky intertidal areas (Suchanek 1978). In California, these relationships are not well defined, and the presence of *M. trossulus* is more sporadic (Connolly et al. 2001). The occurrence of Blue mussels in California has been noted in open coastal areas of moderate wave exposure. In areas of high wave exposure, however, such as that on the Monterey Peninsula, Blue mussels are absent (Harger 1972). Adult distributions measured during this study reflected those previously reported for mussels on the central coast of California (Harger 1968; Suchanek 1978), where adult Blue mussels were absent from exposed and semi-exposed environments. However, juvenile Blue mussels were found to inhabit these areas, signifying recruitment is plausible for these species.

Both larval availability and post-settlement mortality are important in regulating rocky intertidal communities (Underwood and Denley 1984; Connell 1985; Morgan 2001). It has been hypothesized that the differences in distribution to habitats in California and further north of California and Blue mussels is caused by larval supply. Because upwelling is stronger in California than in the Pacific Northwest, more larvae are advected offshore and entrained in offshore upwelling fronts (Connolly et al. 2001). Loss

of larvae subsequently reduces the abundance of adults on exposed rocky shores. Species interactions may become less important with respect to adult distributions because space is not limiting (Connolly and Roughgarden 1998). With lower densities of adults, pulses of larvae settling on substrates are more likely to survive and contribute to the structure of the community (Morgan 2001).

Settlement rates of Blue and California mussels differed among my study sites. California mussels rarely settled in the harbor. Settlement of Blue mussels occurred at all sites. Therefore, in exposed habitats, Blue mussel settlement appeared to be opportunistic because juveniles did not settle only within parental habitats. In fact, on the jetty, juvenile Blue mussel settlers outnumbered California mussel settlers. These results were surprising because adult Blue mussels are rare on the outer shore in California (Harger 1972; Navarrete and Menge 1996; Connolly and Roughgarden 1998). This settlement pattern also occurred on the west coast of Canada where juvenile *M. trossulus* also outnumbered juvenile *M. californianus* (Heath et al.1996). However, in the north Pacific, *M. trossulus* co-occur with *M. californianus* on outer shores. *Mytilus trossulus* is a "fugitive species", adapted to exploit ephemeral environments by maturing early (at length 15-20 mm) and exhibiting a high reproductive output at an early age (Suchanek 1981; Emmett et al. 1987). This contrasts the life history of *M. californianus*, which matures and colonizes more slowly but is better adapted to survive in exposed environments (Suchanek 1981).

During this study, supply of juvenile Blue mussels to the settlement pads was not limiting to potential adult abundance. The absence of adult Blue mussels from exposed

and semi-exposed environments in Moss Landing is thus probably not due to a deficiency in larval supply, or an inability to settle as evidenced by the data presented here. Therefore, plausible explanations for the absence of adult Blue mussels at exposed and semi-exposed sites in Moss Landing may be Blue mussels are competitively inferior to California mussels, and/or are more susceptible to predation or wave shock than California mussels are.

As adults, California mussels often crush Blue mussels in the exposed rocky intertidal (Seed and Suchanek 1992). However, I found no evidence of crushing during this study. Remnant byssal threads, which were common throughout the study period, however, indicated previous occupation of mussels on the jetty. Blue mussels are more susceptible to heavy wave shock and rocky intertidal predators than California mussels because Blue mussels have thinner byssal threads, thinner shells, and larger sizes (Fuller and Lutz 1988). On exposed Washington shores, *M. trossulus* initially settles on algal substrata, barnacles colonize, and then *M. californianus* settles, excluding others. Predators such as snails and gulls differentially reduce the population of early successional species such as barnacles, *Pollicipes polymerus*, and *M. trossulus*. This, in turn, allows *M. californianus* to persist (Wootton 2001). During the current study, numerous young Blue mussel recruits were observed on the jetty. However, adult Blue mussels were not observed. In central California, space may not be as limiting a resource as it is in Oregon and, therefore, density dependent processes may be less influential in driving adult populations (Connolly et al. 2001). Therefore, the absence of Blue mussels from exposed shores may be due to environmental factors such as wave exposure or

differential predation, rather than competition. These results imply that post-settlement factors have important influences on adult distributions even when density of recruits is low (Menge 2000).

Protected Environment

California mussels are competitively inferior to Blue mussels in protected environments, and as a result, generally do not occur in such environments (Harger 1968). In the current study, settlement of California mussels only rarely occurred in protected environments. Although they were present in the February 2002 plankton it is possible that during other times *M. californianus* were not present, did not settle, or died less than one week after settling in this environment. These results were similar to those of Heath et al. (1996) who also did not find settlement of juvenile *M. californianus* in protected near exposed coastal areas. Blue mussels are thought to out-compete California mussels by moving to the outside of clumps to avoid siltation and suffocation (Harger 1968; Suchanek 1985; Heath et al.1996); however, this relationship may be restricted to transplanted adult mussels if recruitment is rare. In addition, this behavior seems unlikely because space was not limiting on settlement pads and no empty shells were found, indicating juvenile mortality. The absence of *M. californianus* from the harbor may be due to pre-settlement barriers, such as obligate settlement of larvae on adult con-specifics (Petraitis 1978; Suchanek 1978, 1981). Mussel larvae readily settle on a variety of filamentous substrates, including the byssal threads of adults in the laboratory and field (Lutz and Kennish 1992). Larvae of *M. trossulus* tend to avoid clumps of adult *M.*

californianus in laboratory experiments, while they preferentially settle on red algae, like *Rhodomela larix* (Petersen 1984). Larvae of *M. californianus*, however, preferentially settled on adults of *M. trossulus* when given the choice of conspecific adults, algae or competitive adults (Petersen 1984). Juvenile mussels all had the same type substrate, settlement pads, in this experiment. Therefore, it is unlikely that juvenile Blue and California mussels are entirely dependent on adult conspecifics. This is further supported by settlement patterns in the intermediate environment where juveniles of Blue and California mussels occurred in nearly equal frequencies, despite the absence of adults.

Settlement patterns of *M. californianus* in protected environments may be a result of larvae and juveniles of *M. californianus* being more sensitive to changes in temperature and salinity than those of *M. trossulus* or *M. galloprovincialis* (Young 1941; Heath et al.1996). Blue mussels are more tolerant of extreme environmental stresses such as desiccation and thermal stress than California mussels (Seed 1969; Harger 1978). Petersen (1984) noted a high incidence of larval mortality among *M. californianus* during experimentation because of unfavorable conditions in experimental containers. Temperature is one of the most important factors with respect to growth of larval Blue mussels (Lough 1974; Bayne 1983; Lutz and Kennish 1992). In addition, temperature, perhaps more than any other factor, influences time required for larvae to reach competency (Strathmann 1987; Lutz and Kennish 1992). Larvae of *M. californianus*, a more stenothermal organism than Blue mussels, experience greater levels of mortality in areas of higher or substantially lower temperature (Lutz and Kennish 1992). Elkhorn Slough and Moss Landing Harbor have higher average monthly temperatures than open

coastal waters of Monterey Bay (Fig. 5). Differential settlement patterns of *M. californianus* may be explained by pre-settlement mortality of larvae and juveniles once advected inside the slough, while Blue mussels are less affected or unaffected. These results add another level of complexity to adult population dynamics. Therefore, lack of supply of larvae or juveniles may be the limiting factor affecting adult distributions; however, environmental conditions may contribute to these patterns.

On the central coast of California, distributions of *Mytilus* spp. may be further confounded by the presence of the invasive species *M. galloprovincialis* and Blue mussel hybrids. Hybrid genotypes may contribute to differential mortality of Blue mussel populations (Fuentes et al. 2002). In addition, hybrid mussels are associated with higher parasitization and diminished levels of stress and heat-shock proteins (Fuentes et al. 2002). Therefore, it is possible that in addition to mortality of Blue mussels caused by environmental factors; there may have been an increased level of mortality of hybrid mussels, partially augmenting differential adult distributions.

Conclusions

Blue mussel settlement may be opportunistic rather than selective. More Blue mussels settled on the open coast than California mussels. Settlement frequencies on the jetty and channel showed environmental conditions rather than larval supply are responsible for adult distributions. Adult populations of Blue mussels on the jetty may be more susceptible to environmental stresses due to possibly large numbers of hybrid

mussels within populations. Because hybrids are relatively less fit than adults with pure genotypes, they may be more susceptible to predation, parasitism, and physical factors. As a result, hybrids experience greater mortality, possibly limiting Blue mussel populations on exposed shores.

Although larval supply can drive adult populations in intertidal environments (Connolly et al. 2001), adult distributions of all three species of mussels found in Moss Landing, CA could have been results of oceanographic conditions rather than settlement patterns of juvenile mussels. Recruitment of mussels is affected by many physical factors, including temperature, salinity, wave force, substrate availability, and currents. Larval behavior, though important, becomes secondary to physical constraints of juvenile populations.

Future understanding would be greatly enhanced by knowing the distributional patterns and physiology of juvenile hybrid mussels. Because of differential fitness of hybrids and pure populations, mussel populations in central California may be more influenced by oceanographic conditions than in areas where hybridization does not occur. In addition, a further investigation into the relative degree of introgression between pure and hybrid genotypes would be useful in determining the relative fitness of hybrid genotypes. It is important to understand how the presence of an invasive species can affect distributions of adult populations, as well as the ecology of an area. In addition, to understand the complexities of the hybrid zone on a larger scale with respect to dispersal and fitness, more sites on the California coast should be examined. Of course, we cannot begin to understand the complexities of intertidal ecology by only examining the effect of

settlement on adult distributions. Reproductive output, larval survival, and transport also need to be closely (and concurrently) examined to determine population dynamics of rocky intertidal organisms (Morgan 2001).

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Table 1. PCR results of larval mussel DNA from plankton tows in Moss Landing Harbor and the channel of Moss Landing Harbor amplified with species-specific primers (Bartl and Geller, pers. com.). PCR was pooled into reactions containing 10 and 100 larvae/reaction. The symbol X denotes the presence of species in reaction, 0 denotes the absence. Negative controls were used to ensure no contamination occurred.

Location		**Species**		
Channel		*M. californianus*	*M. trossulus*	*M. galloprovincialis*
Tow 1	10 Larvae	X	X	X
	100 Larvae	X	X	X
Tow 2	10 Larvae	X	X	X
	100 Larvae	0	X	X
Tow 3	10 Larvae	X	X	0
	100 Larvae	X	X	0
Harbor		*M. californianus*	*M. trossulus*	*M. galloprovincialis*
Tow 1	10 Larvae	X	X	0
	100 Larvae	X	X	X
Tow 2	10 Larvae	X	X	0
	100 Larvae	X	X	X
Tow 3	10 Larvae	X	X	0
	100 Larvae	X	X	0

Table 2. ANOVA results for (a), Blue and California mussel average monthly proportions of settlement on the jetty and channel, and (b) Blue mussel settlement at all sites.

* Indicates significant result at $\alpha = 0.05$.

Source of Variation	**df**	**F**	**P**
a *Settlement By Species*			
Species	1	1.812	0.187
Site	1	4.363	0.044*
Site X Species	1	1.742	0.195
b *Settlement of Blue Mussels*			
Site	2	4.111	0.028*

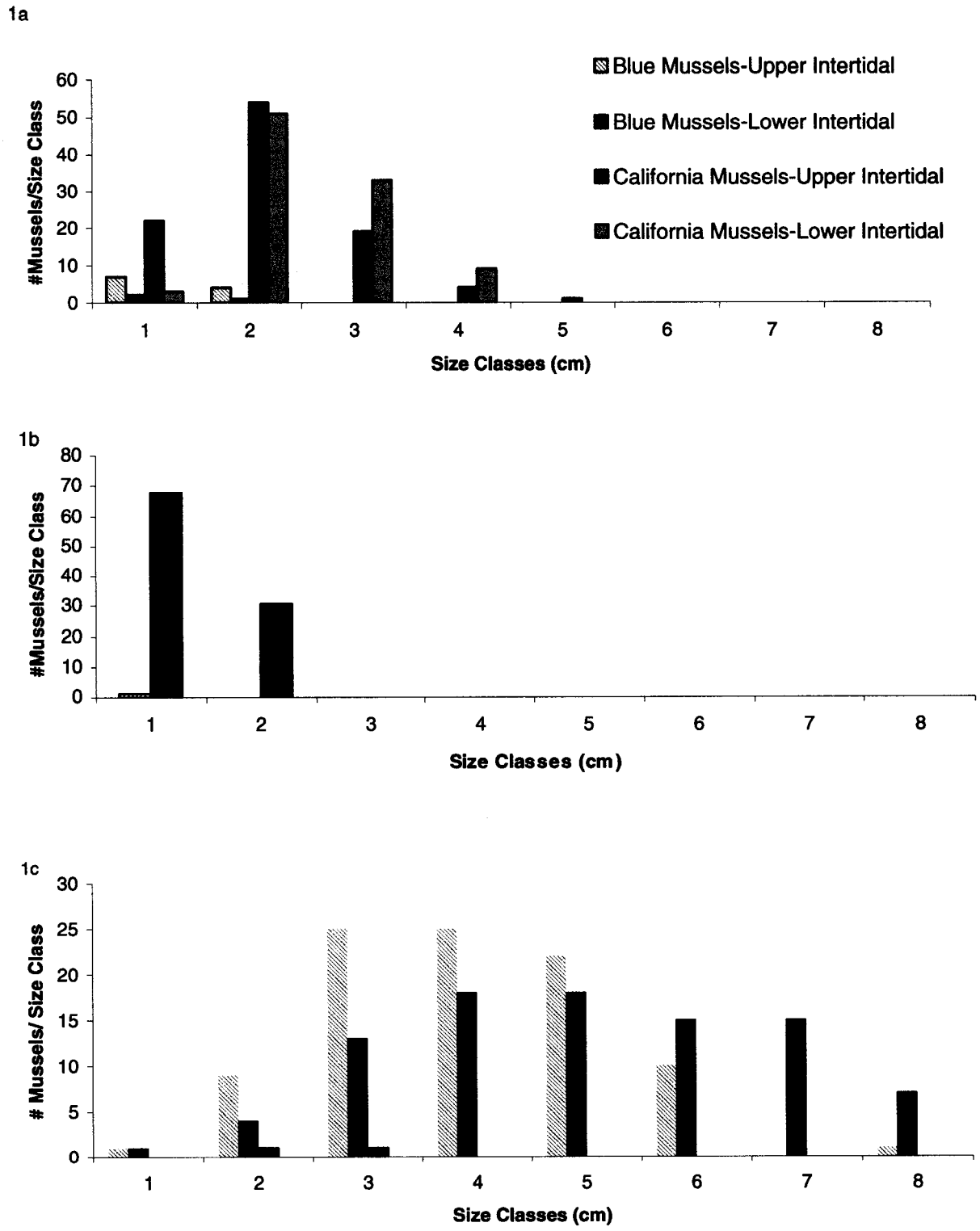


Figure 1. Size frequency distributions for adult Blue and California mussels in upper intertidal zones (~6.0-3.0 ft) and lower intertidal zones (~2.9-0.0 ft) on the (a) North Jetty, (b) Channel, and (c) Harbor. Mussels were absent from lower intertidal of channel.

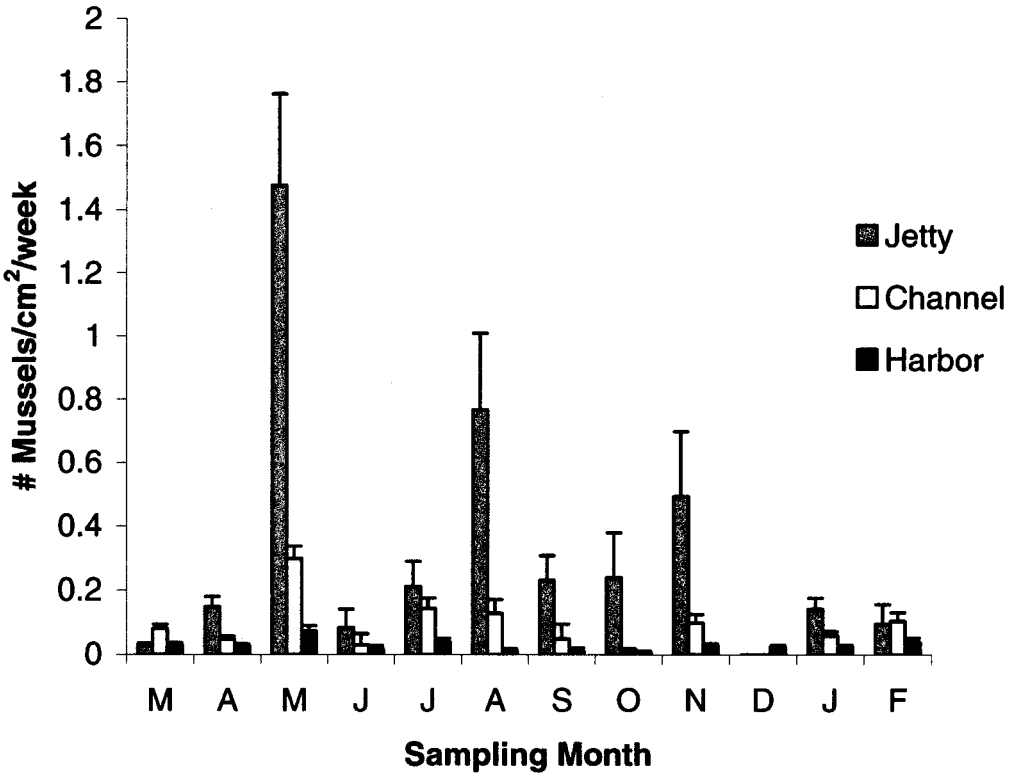


Figure 2. Average rate (μ + SE) of mussels settling on 60 cm^2 recruitment pads at each site between March 2002 and February 2003.

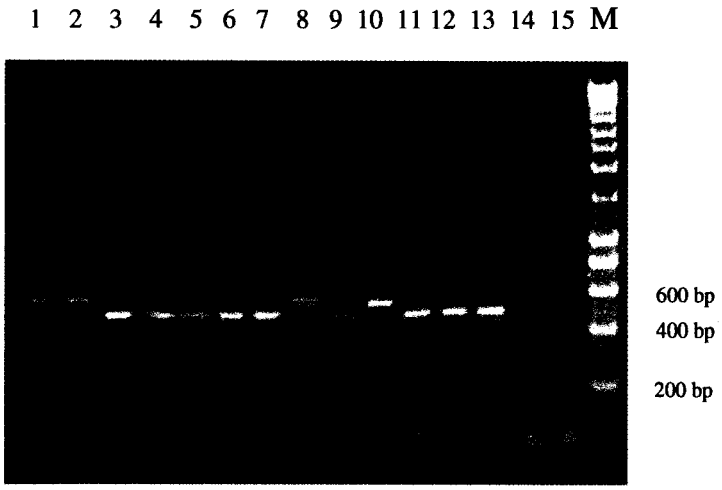


Figure 3. Electrophoretic gel results of juvenile mussel PCR products for the ITS 1 genome. Products for *M. californianus* are 550 bp and are in lanes 3, 4, 5, 6, 7, 9, 11, 12, and 13. Products for *M. trossulus* and *M. galloprovincialis* are 600 bp and are in lanes 1, 2, 8, and 10. Lanes 14 and 15 are failed PCR reactions. GeneChoice™ ladder was used for size comparison in lane M.

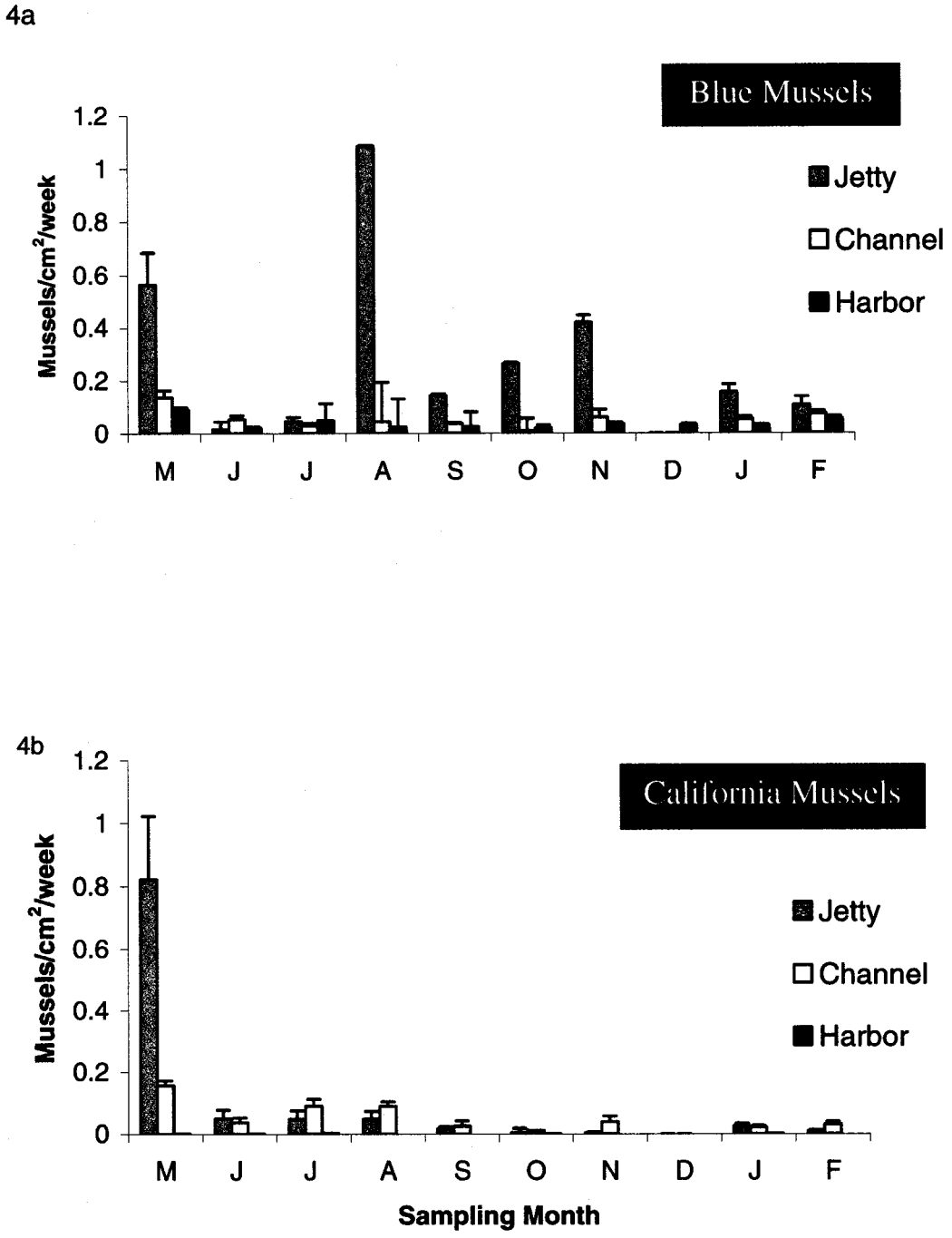


Figure 4. Mean rate (μ + SE) of (a) Blue and (b) California mussel settlers between May 2002 and February 2003.

APPENDIX 1

Results of Pilot Study Conducted in March 2002

To design an optimal and precise study on settlement of juvenile mussels in the slough, the channel, and the jetty, a pilot study was conducted in March 2002. Three main variables were tested; sample unit size, sample size, and duration of monthly sampling. Flat green Scotch Brite® pads were used as sample units rather than bulbous Tuffy® Pads to avoid the possibility of passive ensnarement of juvenile mussels, instead of actual recruitment (Cáceres-Marinez et al. 1999).

Sample unit size: Sample unit size was determined using three Scotch Brite® pads of two different sizes (7.75 cm^2 and 15.5 x 23.25 cm^2) randomly placed on pilings of Sandholdt Road Bridge and broken pilings near the bridge. The pads were left in the water for three different time periods (1 day, 1 week, and 3 weeks). The large pads (15.5 x 23.25 cm^2) are the size produced by the company and small pads (7.75 cm^2) are simply one sixth of a large pad; therefore, sample unit sizes were chosen accordingly. A model-III, two-factor ANOVA was used to test for differences in densities of juvenile mussels on different sizes of pads and differences in density of juvenile mussels during different time periods. Data were normally distributed, Cochran's test for equal variances was insignificant, and samples were independent. This test showed there was a significant difference ($\alpha = 0.05$) between densities of juvenile mussels of different sample unit sizes ($p = 0.046$). These densities were higher on smaller recruitment pads than on the large pads. There was no difference found among time-periods ($p = 0.32$). The interaction term between sample unit size and time period also was insignificant ($p = 0.49$). To increase sample size, time

period samples were pooled, and a one-factor ANOVA was used to test for a difference between sample unit sizes; smaller recruitment pads had a greater density than the larger pads ($p = 0.0013$). Although only two sizes were tested and replication was small, the smaller pad (7.75 cm^2) was chosen for the actual study. The smaller pad seems to have adequate recruitment, is more affordable, easier to work with in the field, and is more durable in the field because there is less surface area to be destroyed by high wave action.

Sample size: To test for the optimal sample size (replication) eighteen 7.75 cm^2 pads were randomly anchored inside the harbor for each time period (1 day, 1 week, and 3 weeks). Means, variances, and standard deviations were calculated from these data (Appendix 2). Because distributions of juvenile mussels are neither random nor homogenous and variances for the data were greater than the means, a negative binomial distribution was used to approximate sample size (Appendix 3). Using settlement data from April, additional power analyses concluded ($f = 0.49$, $u = 2$, $\alpha = 0.05$, and power = 80) about 14 samples are needed (Cohen 1988). Because many samples are lost in the field due to high wave activity, forty independent pads, therefore, were allocated at each location.

Duration of Sampling: Time periods were chosen based on tide level and initial settlement of juvenile mussels. If the pads were left in the water for too long, post-settlement mortality could have become a factor influencing species composition on recruitment pads. Using data from the sample size analyses one-factor ANOVA was used to test significant differences ($\alpha = 0.05$) among the three time-periods (1 day, 1 week, and 3 weeks). Data were normally distributed, Cochran's test for equal variances was insignificant, and samples were independent. There were significantly more juvenile

mussels found to settle on recruitment pads during the time-period of one week ($p = 0.0017$) rather than one day or three weeks. Recruitment pads, therefore, remained in the field depending on tidal cycles for one to two week periods for 12 consecutive months starting in April 2002.

APPENDIX 2. Means, variances, and standard deviations for counts of juvenile mussels from 60 cm^2 Scotch Brite® recruitment pads during the time-periods; 1 day, 7 days, and 21 days used to approximate sample size.

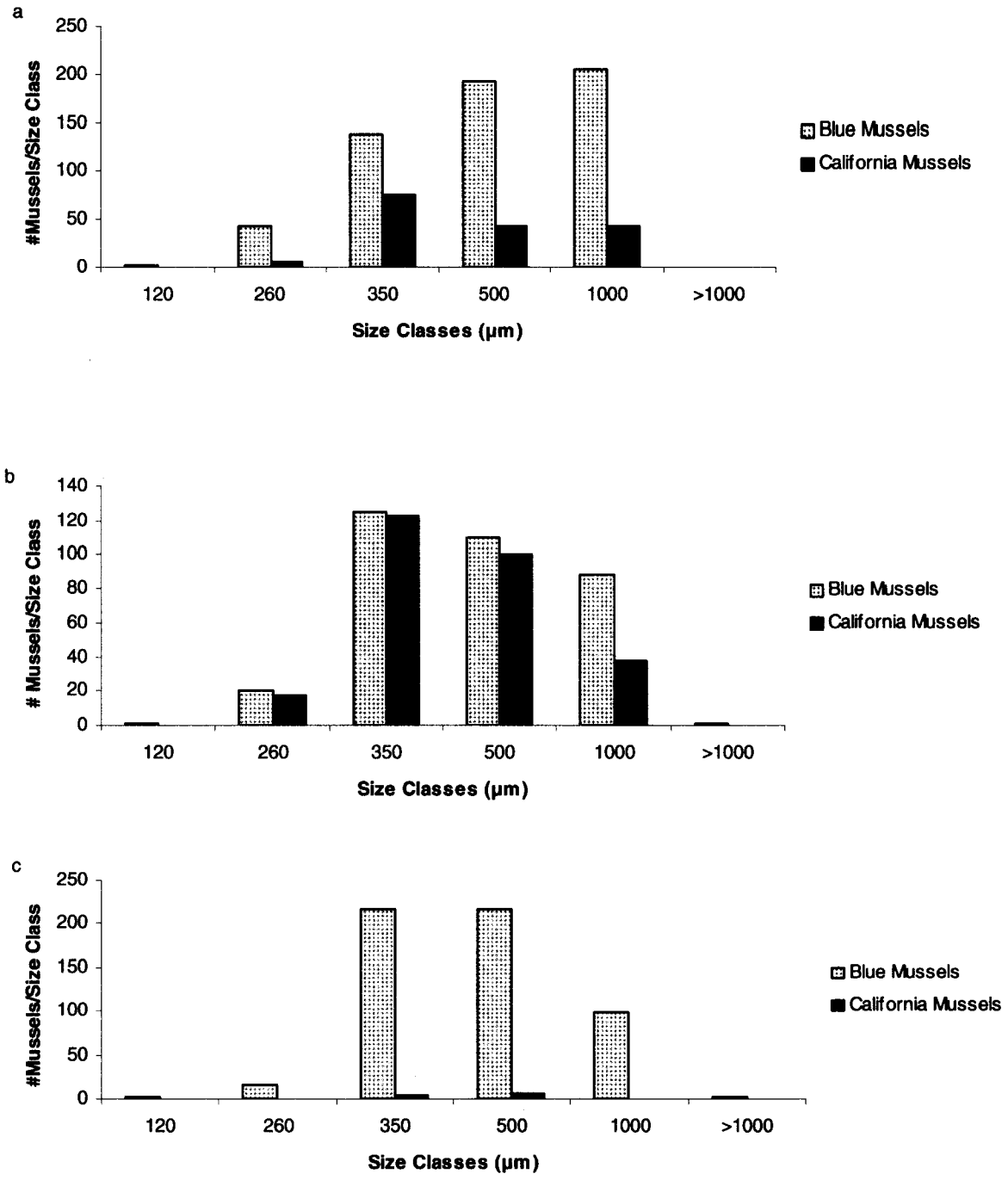
Statistical Data for Sample Size Analyses

n = 18	1 Day	7 Days	21 Days
Mean (x)	1.5556	5.0000	2.9440
Variance (s^2)	8.8497	13.1765	6.0566
Standard Deviation (s)	1.6881	3.6299	2.4608
n = 21			
Mean (x)	1.5714	4.8095	3.1905
Variance (s^2)	3.2571	12.4619	6.6619
Standard Deviation (s)	1.8048	3.5301	2.5811

APPENDIX 3. Number of samples needed to acquire relative levels of precision of 10% and 25% during different time periods of 1 day, 7 days, and 21 days ($\alpha = 0.05$, n = 18 and 21) using data from table 1 and the formula: $n = (100t_\alpha)^2/r^2(1/x + 1/k)$ where n = sample size required for a negative binomial variable, t_α = student's t-value for n-1 degrees of freedom for α probability, x = estimated mean of counts, k = estimated negative binomial count, and r = desired level of error (percent) (Krebs 1999).

Samples Needed for 10 and 25% Precision Levels

n = 18	1 Day	7 Days	21 Days
10%	486	219	287
25%	78	34	46
n = 21			
10%	486	219	266
25%	78	35	43



APPENDIX 4. Size frequency distributions for Blue and California mussels settling for 0-14 days on the jetty (a), channel (b), and harbor (c) of Moss Landing, CA between May 2002 and February 2003 (all months combined).