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Variations in larval density and settlement in space and time: Important determinants of recruitment in sessile marine invertebrates?

Hurlbut, Catherine Jane, Ph.D.
University of Hawaii, 1990
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VARIATIONS IN LARVAL DENSITY AND SETTLEMENT
IN SPACE AND TIME:
IMPORTANT DETERMINANTS OF RECRUITMENT
IN SESSILE MARINE INVERTEBRATES?

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

ZOOLOGY

AUGUST 1990

By

Catherine Jane Hurlbut

Dissertation Committee:

Michael G. Hadfield, Chairperson
Julie H. Brock
Robert A. Kinzie, III
Stephen R. Palumbi
Celia M. Smith
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Recruitment of new individuals can be an important determinant of community structure for sessile marine invertebrates. In order to identify factors influencing recruitment, temporal and spatial variation in settlement and juvenile mortality was measured in the field for the colonial ascidian *Didemnum candidum*, a member of a fouling community encrusting docks in Pearl Harbor, Hawaii. Some comparisons were made with co-occurring species. The hourly pattern of larval release was measured by counting larvae released from adults in traps. Acrylic settling plates were suspended from the dock for varying periods of time and at different angles and depths, and sampled non-destructively to census settlement and monitor juvenile mortality over time. The daily pattern of settlement and mortality differed among seven species. Larval release and settlement of the colonial ascidians *Didemnum candidum*, *Diplosoma listerianum* and *Diplosoma* sp. only occurred during the daytime; larvae thus were settling when light was available as a cue to favorable sites. The effect of light level on recruitment of *Didemnum candidum* was determined by examining larval photo- and geotaxis in the laboratory, comparing settlement intensity between light and dark surfaces in the field, and monitoring survival of juveniles exposed to or protected from direct light. Settling larvae were photo- and geonegative. Settlement and survival were greatest on downward-facing opaque surfaces. The *Diplosoma* species also settled abundantly on downward-facing surfaces, but subsequent exposure to light did not affect survival. For both genera, settlement and survival were greater on downward-facing than on vertical or upward-facing opaque surfaces, probably due to decreased siltation.
Settlement and mortality was more abundant at shallower depths for both genera. However, experimental movement of plates to different depths after settlement revealed that mortality, per se, was independent of depth. Rather, for D. candidum, rate of mortality was a density function, and the higher mortality near the surface was due to the greater number of larvae settling there. For these three ascidians, settlement varied temporally and spatially. The time of larval release and settlement affected recruitment, since light was an important environmental cue used to locate settlement sites on downward-facing surfaces where juvenile survival was enhanced.
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5.9 Density dependence of juvenile mortality. Total percent mortality of juveniles versus number of congeneric juveniles present on the first sampling date..................113
Successful recruitment of planktonic larvae is crucial to the establishment and maintenance of populations of sessile marine invertebrates (Keough and Downes, 1982; Underwood and Denley, 1984; Gaines and Roughgarden, 1985). Both temporal and spatial variability in recruitment have been frequently demonstrated and are considered to be important determinants of community structure (Sutherland and Karlson, 1977; Yoshioka, 1982; Underwood and Denley, 1984; Connell, 1985).

Recent studies have emphasized that larval settling rates plus post-settlement juvenile survival determine recruitment rate; settlement is defined as the number of larvae that leave the plankton and take up a benthic existence, whereas recruitment is defined as the number of the ensuing juveniles that survive until censused by an observer (Keough and Downes, 1982; Keough, 1988).

Temporal variations in larval availability can determine settling rates, and variations in the timing, intensity and location of settlement can affect post-settlement survival rates and hence recruitment. For organisms with short-lived larvae, such as colonial ascidians, the diel time of larval release into the plankton determines the environmental conditions available to larvae as settling cues; the response of larvae to such cues can influence the location of settlement. The settlement location of sessile organisms is critical to survival until reproduction, since location determines the environmental conditions experienced by juveniles and adults (Keough and Downes, 1982). Knowledge of the effects of variations in larval abundance, settlement density and settlement location on recruitment
will lead to a better understanding of the processes underlying the establishment and maintenance of communities of marine invertebrates.

The goal of the present study was to determine the relative importance of factors that cause spatial and temporal variation in settlement and early juvenile mortality in the colonial ascidian *Didemnum candidum*, an abundant member of a fouling community encrusting floating docks in Pearl Harbor, Hawaii.

In Chapter 1, I examine day-to-day variability in the intensity of settlement and juvenile mortality for *Didemnum candidum* and several co-occurring species of sessile invertebrates, as well as determine a sampling regime that accurately monitors juvenile survival of *D. candidum*. In addition, comparisons are made of the timing and intensity of recruitment between species with different patterns of reproduction.

For organisms with short-lived larvae, such as colonial ascidians, the time of larval release into the plankton can directly determine settlement time. Both the seasonal and diel timing of larval release and settlement of *Didemnum candidum* are described in Chapters 2 and 3. The diel pattern of these events is compared to that of the colonial ascidians *Diplosoma listerianum* and *Diplosoma* sp. In addition, for *D. candidum*, the influence of diel variation in larval release on the abundance of larvae in the plankton and on settlement was estimated.

Since larval release was found to only occur during the daytime, larvae were settling when light was available as a cue indicating shaded settlement sites. In Chapter 4, I determine the effects of light level on settlement of *Didemnum candidum* by (1) examining larval
photo- and geotaxis in the laboratory, (2) comparing density of settlement on light and dark surfaces in the field, and (3) monitoring survival of juveniles exposed to and protected from direct light in the field.

Settlement of *Didemnum candidum* and the *Diplosoma* species was most abundant in shaded sites. This behavior could produce abundant settlement on downward-facing surfaces, where mortality from siltation would be low. Therefore, in Chapter 4, I determine the effects of siltation by measuring differences in settlement and juvenile survival with surface orientation for *D. candidum* and the *Diplosoma* species.

In Chapter 5, I examine the distribution of adults of *Didemnum candidum* with depth, and for *D. candidum* and the *Diplosoma* species, I compare the intensity of settlement and juvenile mortality at different depths. To differentiate between the effects of depth, *per se*, and the effects of juvenile density on mortality, experimental plates encrusted with varying densities of settled juveniles were moved to different depths and subsequent mortality monitored.

The results of the research described in this dissertation provide valuable insight into the adaptive value of larval behaviors, as well as into causes of variation in recruitment, distribution and abundance of sessile marine invertebrates.
CHAPTER 1

Community recruitment: settlement and juvenile survival of seven co-occurring species of sessile marine invertebrate

Introduction

Temporal and spatial variability in settlement (Sutherland and Karlson, 1977; Yoshioka, 1982; Gaines et al., 1985) and juvenile mortality (Connell, 1985; Davis, 1988a) are considered to be important determinants of recruitment and hence community structure (Underwood and Denley, 1984; Caffey, 1985; Gaines and Roughgarden, 1985).

Recruitment is defined as the number of initial settlers which survive until some later time when the ensuing juveniles are observed (Keough and Downes, 1982). Settlement rates and patterns can depend on the larval supply and the suitability of substrata for settlement (Connell, 1985). Juvenile mortality can be caused by physical factors, such as desiccation and siltation, which are generally density-independent, or biological factors such as predation and competition which are potentially density-dependent and may vary with juvenile age or size.

Most field studies of fine-scale temporal variation in settlement and mortality have examined individual species, such as barnacles (see Connell, 1985 for review), colonial ascidians (Olson, 1983; Davis, 1987, 1988a) and bryozoans (Keough and Downes, 1982; Keough, 1986; Keough and Chernoff, 1987). However, communities of benthic organisms are composed of species that differ in patterns of reproduction, development and dispersal. Thus, an assessment of settlement and mortality patterns for a single species may not provide representative data for all components of a community, and important interactions among species may be missed.
Furthermore, different types of organisms often show different settlement and mortality patterns. For example, settlement intensity can differ greatly between solitary and colonial organisms; solitary organisms often settle in considerably higher densities than colonial species (Jackson, 1985). Differences in intensity of settlement can influence relative rates of juvenile mortality, because mortality can depend on juvenile density. Looking at barnacles and a colonial ascidian, respectively, Connell (1985) and Davis (1988a) examined mortality of juveniles as a function of settlement density. Both found that mortality was independent of settler density. In contrast, Gaines and Roughgarden (1985) found that intensity of settlement influenced barnacle mortality. When settlement was low, juvenile mortality was density-independent; when settlement was high, mortality was density-dependent. Due to differences in settlement density associated with growth form, juvenile mortality may be density-dependent in solitary organisms and density-independent in colonial organisms.

The purposes of this study were to identify some of the factors which influence rates of settlement and post-settlement mortality in a group of interacting species, and to investigate differences among species in intensities of settlement and of juvenile mortality. I measured daily variation in settlement and juvenile survival on settling plates over a 14 day period for the group of organisms that dominates the fouling community on floating docks in Pearl Harbor, Oahu, Hawaii. These were a serpulid polychaete, *Hydroides elegans*, a bivalve, *Anomia nobilis*, a bryozoan, *Schizoporella unicornis*, three colonial ascidians, *Didemnum candidum*, *Diplosoma listerianum* and *Diplosoma* sp., and a solitary ascidian, *Phallusia nigra* (Edmondson,
1944). The diversity in this group allowed comparisons between solitary and colonial organisms and also between organisms with long- and short-lived larvae. For these species, I asked the following questions: 1) Does intensity of settlement vary from day to day? 2) Can variations in settlement be attributed to differences in the larval supply or to the availability and favorability of substrata? 3) Does juvenile mortality vary with i) time, ii) juvenile age, or iii) juvenile density?

In order to distinguish between the effects of variability in settlement and post-settlement mortality, settlement must be measured before mortality has altered the numerical estimate or the pattern of settlement. Daily samples, as used here, are considered to be a close approximation of actual settlement (Strathmann and Branscomb, 1979; Grosberg, 1981; Wethey, 1984; Davis, 1988a), and are often used when a census immediately after settlement is logistically impossible.

Few studies have simultaneously measured day to day variations in settlement and juvenile mortality for both solitary and colonial species (but see Keough, 1983, 1984). Measurement of settlement and mortality of several species with a range of growth forms and larval types allowed comparisons of settling intensities and mortality rates under similar conditions for a diverse tropical community. The data provide relative rates of recruitment and help explain variations in recruitment among these different organisms.

Methods

Data collection

Settling plates were suspended horizontally from a floating dock in Pearl Harbor, Oahu on 15 August 1985 and sampled daily for the next
14 days. There were three plate treatments, called 1-day, 14-day, and undisturbed 14-day plates. One-day plates were immersed for 24 hours, photographed and then replaced with a clean plate. Fourteen-day plates were immersed for the entire 14 days, but were removed from the seawater and photographed daily. A control series of undisturbed plates was immersed for the entire 14 days and only photographed on day 14. One-day plates were used to measure the number of settlers per day on clean plates. These counts were assumed to reflect daily variation in settlement due to differences in larval supply. Microbial films and juveniles accumulated on 14-day plates; settlement on these plates was used to evaluate the influence of the presence of juveniles and microbial films on settlement intensity. Daily variations in juvenile mortality were recorded on the 14-day plates by following the loss of individuals from day to day.

The plates, measuring 104 x 83 x 3 mm, were made from opaque, black, acrylic plastic which was roughened by light sanding with fine-grit paper. Fifteen plates were horizontally suspended individually 0.3 m below the water surface and 0.3 m apart along a dock, with 5 plates randomly assigned to each of the three treatments. Plates were retrieved and kept immersed in a shallow tray while placed in a frame and overlaid with a grid which designated the area to be photographed. Four central 33 x 22 mm quadrants on the underside of each plate were separately photographed with Kodak Ektachrome 64 color slide film at 1:1. At this size, all settlers could be identified on the slide film.

The slides were projected and the date of appearance and identity of each settler was recorded. The continued presence or absence of each individual was noted on 14-day plates; mortality was scored as
occuring on the date of disappearance (first date not seen). Daily mortality rates were calculated for each species as the percent of individuals present on a given day that disappeared by the next day. Daily mortality rates were calculated similarly for individuals of each age on each day.

Data analyses

Counts of individuals were square-root transformed and percent mortality data were arcsine transformed to ensure independence of means and variances (Sokal and Rohlf, 1981). The following analyses were performed for all seven species except where noted. The statistical tests used to evaluate significance are noted in the Results.

Settlement

To detect daily variability in settlement intensity, counts of settlers on the 1-day plates were compared between days. Differences in intensity of settlement between plates with and without accumulated juveniles were detected by comparing the daily counts of settlers. The spatial pattern of settlement was examined for gregariousness in relation to conspecific and non-conspecific settlers and juveniles. Settler-settler distances were measured on 1-day plates, and settler-juvenile distances on 14-day plates.

Juvenile mortality

Occurrence of juvenile mortality. If no mortality had occurred, there would have been no difference between the total number of settlers and the total number of survivors after 14 days on the 14-day sampled plates. For each day, numbers of settlers of each species were
summed to give cumulative settlement per plate to date and the numbers of these settlers surviving was noted. To determine if significant mortality occurred by the end of the sampling period, the total number of settlers was compared to the number of juveniles present on day 14. Day-to-day differences between the numbers of cumulative settlers and survivors was examined to identify the first day when mortality was statistically significant.

To determine if the sampling regime contributed to mortality, the numbers of juveniles present on day 14 on the undisturbed and 14-day plate series were compared.

**Daily variation in juvenile mortality rate.** Since mortality was very low during the first week, daily mortality rates were compared for only the second week of the study. To avoid the potentially confounding effect of juvenile age on mortality, only counts of 1-7 day old juveniles were used; juveniles of these ages were represented each day during the second week (i.e. from day 8-14). Thus, daily mortality rates of 1-7 day old juveniles (ages pooled) were compared among days 8-14.

**Variation in juvenile mortality rate with age.** Differences in mortality with age were compared during the second week (days 8-14). Daily mortality rates were compared among juveniles of ages 1-7 days old. To compare mortality of all ages, ages were pooled into young (ages 1-7 days) and old (ages 8-13 days) categories, and daily mortality of the two groups compared on day 14, when all ages were present.

**Density dependence of juvenile mortality.** The percent of juveniles that died on any given day was examined as a function of the
number of individuals present at the beginning of that day. For each species, mortality rate was compared to number of conspecific juveniles present, and when species were pooled, to the number of all juveniles present. To avoid the potentially confounding effect of age on mortality, only mortality of 1 day olds was examined. Both date and plate were considered as replicates, giving a total of 35 replicates per comparison (7 days x 5 plates).

Differences between species in intensity of settlement and juvenile mortality

Settlement intensity, recruitment and mortality rates were compared among species. The total number of settlers, the number of juveniles present on day 14, and the total percent mortality were compared between species.

Results

A general pattern of abundance was apparent on all 14-day plates. Initial settlement was slow, but the percent cover of organisms increased up until approximately day 10, when obvious mortality occurred, with organisms removed in broad swaths. This mortality was most noticeable for *Hydroides elegans* and the colonial ascidians. Fish were occasionally observed picking at the undersides of plates and are thus implicated as a mortality source. The only potential predators observed crawling on the plates were flatworms, which were occasionally present for a day. The effect of the clearing of patches, presumably by predators, varied between species. The solitary organisms *Hydroides elegans*, *Anomia nobilis* and *Phallusia nigra* were completely removed, while the colonial organisms *Schizoporella unicornis*, *Didemnum*
candidum, Diplosoma listerianum and Diplosoma sp. were often only partially removed and subsequently regrew. Ascidian colonies were frequently divided into several smaller colonies which then grew as independent colonies. Overgrowth of any organism was rarely observed, and the plates did not become completely covered by organisms during the 14 day study period. Free space decreased over time due to settlement and growth, but patches of space became available as organisms were removed. The plates were casually examined two months after the completion of the study and were found to be completely covered almost exclusively with Hydroides elegans, Anomia nobilis and Schizoporella unicornis.

Settlement

There were significant differences in daily settlement on 1-day plates for Hydroides elegans, Schizoporella unicornis and Diplosoma sp. (1-way ANOVA, P<0.05, Fig. 1.1). Intensity of settlement was higher for Hydroides elegans and S. unicornis during the second week; no such trend was apparent for Diplosoma sp.

Daily settlement was higher on the 14-day plates than on 1-day plates for all species except for Diplosoma listerianum and Diplosoma sp. (2-way ANOVA, P<0.05, Fig. 1.1).

Only larvae of Hydroides elegans and Schizoporella unicornis settled in densities appropriate for nearest neighbor analyses (Clark and Evans, 1954). Settlement by H. elegans was random in relation to conspecific settlers (R=0.96, P>0.05) and noticeably gregarious in relation to previously settled, conspecific juveniles (R=0.72, P<0.01). Recent settlers frequently were found lined up alongside the tube of an
Figure 1.1A. Number of settlers (backtransformed mean + upper confidence limit) per day on 14-day and 1-day plates over a 14 day period.
Figure 1.1B. (Continued)
older juvenile. Recent settlers of *H. elegans* were spaced out in relation to non-conspecific settlers and juveniles (R=1.34 and 1.37, respectively, P<0.01). Recent settlers of *S. unicornis* were aggregated in relation to conspecific and to non-conspecific settlers (R=0.87, 0.01<P<0.05 and R=0.70, P<0.01, respectively) and to non-conspecific juveniles (R=0.66, P<0.01).

**Juvenile mortality**

Significant mortality occurred during 14 days on the 14-day plates. The total number of larvae that settled on the plates over 14 days was significantly greater than the number of surviving juveniles present on day 14 for all species except *Schizonereis unicornis* and *Didemnum candidum* (1-way ANOVA, P<0.001, Fig. 1.2). For most species, daily removal of the plates for photographing did not affect survivorship significantly. The numbers of juveniles present on day 14 differed significantly between 14-day and undisturbed plates only for *Phallusia nigra*, with higher numbers on the undisturbed than on the daily photographed 14-day plates (1-way ANOVA, P=0.0123, Fig. 1.2). *Phallusia nigra* settled in very low numbers, and the apparent difference between 14-day and undisturbed plates may have been due to the small sample size. For all species, cumulative mortality was first significant on or after day 11 (Student-Newman-Keuls, 1-way ANOVA, P<0.05, Fig. 1.3). The rate of daily mortality of juveniles increased throughout the two week period and was highest on day 14 for all species except *Diplosoma listerianum* (juveniles 1-7 days old, ages pooled, days 8-14; 1-way ANOVA, P<0.05, Fig. 1.4).
Figure 1.2A. Total number of settlers (backtransformed mean + upper 95% confidence limit) on 14-day plates, number of juveniles (recruits) present on day 14 on 14-day plates, and number of juveniles present on day 14 on undisturbed plates.
Figure 1.2B. (Continued)
Figure 1.3 A. Cumulative settlement, recruitment and mortality per day of fouling community invertebrates. For each day, total number of settlers to date, number of juveniles present (mean + standard error) and total percent mortality to date (backtransformed mean + upper 95% confidence limit). *: day when total % mortality was first significantly greater than zero.
Figure 1.3B. (Continued)
Figure 1.4A. Daily percent juvenile mortality on days 8-14 (ages 1-7 pooled; backtransformed mean + upper 95% confidence limit).
Figure 1.4B. (Continued)
Mortality rate increased with age for *Hydroides elegans* and *Phallusia nigra* when juveniles of age 1-7 days were compared during the second week of the study (days 8-14 pooled; Student-Newman-Keuls, 1-way ANOVA, P<0.001, Fig. 1.5). There was no correlation between age and mortality rate in *Anomia nobilis*, *Schizoporella unicornis* and the colonial ascidians (1-way ANOVA, P>0.50). In addition, mortality on day 14 varied among ages for juveniles grouped into young and old categories; all ages were present on this day. Mortality was greater in older juveniles of *H. elegans* and *A. nobilis*, whereas mortality was greater in younger individuals of *S. unicornis* (Student-Newman-Keuls, 1-way ANOVA, P=0.02). Mortality rate did not differ between young and old juveniles for any of the ascidians (1-way ANOVA, P>0.05).

Mortality increased with increasing numbers of conspecific juveniles present on the plates for *Hydroides elegans*, *Anomia nobilis* and *Schizoporella unicornis* (Pearson product-moment correlation, P<0.001, Fig. 1.6). For the ascidians, mortality tended to decrease with increased juveniles present, although this pattern was not statistically significant (Pearson product-moment correlation, P>0.100, Fig. 1.6). The same results were obtained when percent mortality was examined as a function of the density of all organisms present.

**Differences between species in intensity of settlement and juvenile mortality**

Species differed in both the abundance of settlers and the number of surviving juveniles on day 14. The species with the highest number of settlers also had the highest number of juveniles present on day 14. Settlers and juveniles of *Hydroides elegans* were most abundant,
Figure 1.5A. Daily percent mortality for each age (backtransformed mean + upper 95% confidence limit).
Figure 1.5B. (Continued)
Figure 1.6A. Percent mortality of juveniles (1 day old) versus number of conspecific juveniles present (all ages pooled). Each point represents data from one plate on one day.
Figure 1.6B. (Continued)
followed by *Schizoporella unicornis*, then *Anomia nobilis* (Student-Newman-Keuls, 1-way ANOVA, $P<0.001$, Fig. 1.2). Settlers and juveniles of the ascidians were less abundant (Student-Newman-Keuls, 1-way ANOVA, $P<0.001$), and no differences were detected between ascidian species (Student-Newman-Keuls, 1-way ANOVA, $P>0.05$, Fig. 1.2). However, mortality rates were not equal between species; they were higher for the ascidians and *A. nobilis* than for *H. elegans* and *S. unicornis* (Student-Newman-Keuls, 1-way ANOVA, $P<0.001$, Fig. 1.3).

**Discussion and conclusions**

The goal of this study was to determine if settlement and juvenile mortality were constant over a short period for a group of co-occurring species, and if not, to identify sources of variation. Both settlement and juvenile mortality varied over time. Juvenile mortality varied with age class and with juvenile density. These factors produced differences in recruitment from day to day and among species.

Differences in larval abundance and substratum suitability can cause variation in settlement intensity (Connell, 1985). Larval abundance can influence both spatial (Gaines et al., 1985; Grosberg, 1982) and temporal (Yoshioka, 1982; Olson, 1983; Davis, 1989) patterns of settlement. Small-scale temporal variations (e.g. daily and hourly) in settlement of short-lived larvae have been attributed to the number of larvae released by nearby parents (Olson, 1983; Duyl et al., 1981; Davis, 1989). In the present study, the number of settlers on clean plates varied, which suggests that larval availability indeed fluctuated from day to day. In addition, variations in settlement were due to the attractiveness of the substrata to larvae. Settlement of
marine invertebrate larvae has been shown to be affected both positively and negatively by the presence of microbial films (ZoBell and Allen, 1935; Crisp and Ryland, 1960; Mihm et al., 1981) and other organisms (Grosberg, 1981; Young and Chia, 1981). In this study, settlement was positively correlated with the presence of previous juveniles or microbial films. *Hydroides elegans* and *Schizoporella unicornis* settled selectively near other organisms. Larvae of the other species may have settled in higher numbers on the plates in response to microbial films or the increased rugosity created by conspecific or non-conspecific juveniles, or their skeletal remains.

Causes of aggregations differ among organisms with long- and short-lived larvae. For organisms with long-lived larvae, such as *Hydroides elegans*, larvae are planktonic for several days (Edmondson, 1944) and are presumably dispersed away from their parents. Larvae must then selectively settle near conspecifics to produce gregarious settlement (Jackson, 1985). In the case of organisms with short-lived larvae, such as *Schizoporella unicornis*, aggregations may arise from larvae actively settling together or from the simultaneous settlement of a group of larvae shortly after being simultaneously released from nearby parents (Jackson, 1985).

Observations made in this study show that significant juvenile mortality occurred in less than two weeks after settlement for most species. Thus, sampling substrata for these organisms at intervals of two weeks or more could not have accurately assessed settlement. However, because virtually no mortality occurred during the first 7 days, sampling one week after a surface becomes available for
settlement should give a reliable indication of settlement intensity in this system.

If post-settlement mortality rates of sessile invertebrates were constant and could be estimated, then potentially the number of settlers at any subsequent time could be calculated from the number of juveniles. However, results obtained here indicate that rate of mortality was variable over time, density and age of juveniles. For some species, the considerably higher mortality rate during the second week appeared to be related to the greater density of juveniles present on the plates. If predation was indeed the major cause of mortality, it apparently was dependent on size and density of the prey. However, the variations in mortality with time, density and age observed here may show different trends if the study had been replicated at another time of year. Typically, settlement is high in August for this community (pers. obs.); mortality patterns may differ when settlement intensity is lower.

The relationship between mortality and animal density in sessile invertebrates varies from species to species, with density dependence demonstrated only when settlement intensity is high. Barnacle mortality increased as a function of settlement intensity (Connell, 1985; Gaines and Roughgarden, 1985), while mortality was independent of density in a colonial ascidian (Davis, 1988a). These studies compared mortality rates with levels of settlement within a single species or group of similar species. In the present study, similar taxon-specific differences were seen within a complex, sessile community. Mortality was density dependent for the three most abundant settlers (Hydroides elegans, Anomia nobilis and Schizoporella unicornis), but not for the
less abundant ascidians. Apparently, the relationship between
settlement rate and density-dependence of juvenile mortality holds true
for comparisons across phyla and between solitary and colonial forms.

Sessile invertebrates are generally considered to have a decreased
probability of mortality with greater size (Jackson, 1985). This
should be particularly true for colonial organisms, because of their
ability to withstand partial predation (Jackson, 1977; Buss, 1979;
Davis, 1988b). Unexpectedly, increased size during the first 14 days
of settlement did not lead to increased survival in the organisms
examined in this study, with the exception of Schizoporella unicornis.
The size range of juveniles examined in this study was small, and no
data were collected on changes in mortality with increasing size after
an age of 14 days. The organisms on the study plates may not have been
large enough to have escaped size-dependent mortality.

Jackson (1985) generalized that solitary species have high
settlement rates and high juvenile survival, while colonial organisms
settle in low densities with low chances of juvenile survival. These
patterns were not clearly demonstrated by the organisms in the present
study. Two solitary species, *Hydroides elegans* and *Anomia nobilis*, had
high rates of settlement and survival. All the ascidian species
examined showed sparse settlement and suffered high mortality rates,
with similar levels for both the solitary and colonial species.
However, the colonial organism *Schizoporella unicornis* also had high
settlement and survival. In general, the densest settlers did have
lower mortality rates, but this pattern did not follow a clear
solitary-colonial dichotomy.
Few studies have closely examined settlement and survivorship in a complex community capable of rapidly and completely covering a substratum. Results of this study indicate that day to day variations in both settlement and juvenile mortality occurred simultaneously in a diverse group of sessile organisms. Species differed not only in intensity of settlement but also in both age- and density-dependence of mortality. Clearly, a pattern of recruitment exhibited by one species or type of organism does not accurately predict similar patterns for other members of the same community, even among related species. To assess factors underlying variation in recruitment in any community, it is necessary to measure settlement and juvenile mortality for the component species over an ecologically relevant time period.
CHAPTER 2

Diel timing of larval release and settlement of colonial ascidians in Pearl Harbor, Oahu, Hawaii

Introduction

Successful recruitment of planktonic larvae is crucial to the establishment and maintenance of populations of sessile marine invertebrates (Underwood and Denley 1984; Connell, 1985). Variations in the timing and location of settlement can affect both larval and juvenile survival and hence recruitment rates (Keough and Downes, 1982; Gaines and Roughgarden, 1985). Diel release time can influence initial survival of larvae due to differences in predator activity (Hobson and Chess, 1978); visual predators may be avoided by night-time release. The diel release time of larvae can also determine the environmental conditions available to larvae as settling cues (e.g. light, current, tidal height), which, in turn, can influence the location of settlement. For sessile organisms, settlement location is critical to subsequent survival (Keough and Downes, 1982).

Many organisms show diel patterns in larval release, with sunrise and sunset frequently controlling release (Branford, 1978). For colonial ascidians, light is known to trigger larval release (Millar, 1971), suggesting that larvae are released in the field shortly after dawn. In contrast, some corals release larvae only at night (Richmond and Jokiel, 1984), while others have maximum larval release at low tide, regardless of time of day (Holloran and Witteman, 1986).

For organisms with short-lived larvae such as colonial ascidians, time of release closely corresponds to settlement time. Most colonial ascidians examined have been observed to release larvae only during the
day (Olson, 1983; Svane and Young, 1989). For these larvae, light may be an important factor in selecting suitable locations for settlement.

The colonial ascidians Didemnum candidum, Diplosoma listerianum and Diplosoma sp. are common members of the fouling community in Pearl Harbor, Oahu, Hawaii. I have examined the diel timing of release and settlement of the larvae of these three species.

Methods

Laboratory

Diel larval release times in the laboratory were examined for five colonies each of Didemnum candidum and the Diplosoma species. Individual colonies were placed in larval traps made of PVC pipe with 125 μm nitex mesh covering the bottom and seawater flowing through from above. Larvae were removed and counted every hour between 0800 and 1800 on 25 June, and 3, 4 and 9 July 1987. Twice colonies were checked for larval release at 2100, then left in traps until 0800 the next morning. In this preliminary work, I did not differentiate between the two Diplosoma species.

Field

Diel settlement times were monitored over 24 hours on 25 July and 1, 8 and 25 August 1987. Settlement was documented on 8 x 10 cm acrylic settling plates which were first ‘conditioned’ by a week of immersion in filtered seawater to allow accumulation of microbial films. Five plates were suspended every 2 hours at 0.25 m from a floating dock and retrieved after 2 hours of immersion. At the end of each 24 hour period, the plates were microscopically examined and settlers identified and counted.
Results

The majority of larvae of *Didemnum candidum* and the *Diplosoma* species were released between 0800 and 1600 (Fig. 2.1). No larvae were released between 1800 and 2200, although colonies of both genera that remained in the traps overnight released larvae prior to 0800. The time of maximum release during the day differed between the two genera (X² contingency table $P<0.05$). *Didemnum candidum* had a bimodal distribution of larval release, with a morning (0800-1100) and early afternoon (1300-1500) peak in release, and small numbers released until 1800. In contrast, the *Diplosoma* species released larvae fairly uniformly from 0800 to 1300, with decreasing numbers released between 1300 and 1600. For both genera, individual colonies released larvae throughout the day, and for several consecutive days.

For all three species, settlement occurred between 0600 and 1600, with the time of peak settlement differing with species (sampling dates pooled, X² contingency table $P<0.05$, Fig. 2.2). *Didemnum candidum* had a bimodal distribution of settlement, with a strong early morning peak (0600-1000) followed by a weak afternoon peak (1200-1600, Fig. 2.2). Settlement of larvae of *Diplosoma listerianum* began early (0600-0800), and was greatest between 1000 and 1400. In contrast, settlement of *Diplosoma* sp. was low between 0600-0800, and was highest between 0800 and 1400. Settlement patterns for both *Diplosoma* species were unimodal (Fig. 2.2).

There were significant daily differences in settlement times within each species (Fig. 2.3). For *Didemnum candidum*, the distribution of settlement times varied significantly among all four days.
Figure 2.1. Number of larvae (mean + standard error) released per colony per hour by Didemnum candidum and the Diplosoma species between 0800 and 1800. **N=5** colonies of each genus. Note difference in scale between genera.
Figure 2.2. Differences in settlement throughout the day of *Didemnum candidum*, *Diplosoma listerianum* and *Diplosoma* sp. Number of settlers (mean + standard error) per 80 cm² acrylic plate for 2 hour periods from 0600 to 1800; data pooled between 1800 and 0600 the following morning.
Figure 2.3A. Differences in settlement abundance on different days. Number of settlers (mean + standard error) for consecutive 2 hour periods from 0600 to 1800 on 7/25, 8/2, 8/8 and 8/15/87; data are pooled between 1800 and 0600 the following morning.
Diplosoma listerianum

Figure 2.3B. (Continued)
Figure 2.3C. (Continued)
(X^2 contingency table P<0.05). For *D. listerianum*, there was an early morning and a early afternoon peak in settlement on 15 August, which was significantly different from the other days which had a single peak in late morning (P<0.05). For *Diplosoma* sp., peak settlement on 25 July was significantly later in the day than on the other three dates (P<0.05).

**Discussion**

Diel variations in larval release and settlement times have been shown for several colonial ascidians (Duyl et al., 1981; Olson, 1983; Gotelli, 1987). Laboratory data indicate that many colonial ascidians begin to release larvae upon first light after a period of darkness. Three general patterns of release times are observed: 1) morning only, 2) all day with a mid-day peak, and 3) continuous over the day-night cycle (see Olson, 1983 for review). Larval release in Hawaiian populations of *Didemnum candidum* and the *Diplosoma* species did not exactly follow any of these patterns. Both *D. candidum* and the *Diplosoma* species released larvae throughout the day but did not exhibit an obvious midday peak. Additional early morning sampling is needed to determine the time of initial release. There were significant differences between the two genera in the timing of release within the daytime. While the ecological significance of these differences in release time is not known, release time does directly influence settlement time.

The extent to which these laboratory data reflect release times in the field can be roughly estimated from settlement times measured in the field. This estimate is affected by the length of the planktonic
period of the larvae. While the larval lifespan of colonial ascidians ranges from 15 minutes to several hours in the laboratory, direct field observations suggest that larvae settle less than 1/2 hour after release (Duyl et al., 1981; Olson, 1983) and that settlement monitored every two hours can provide useful information about release times in the field.

For Didemnum candidum, Diplosoma listerianum and Diplosoma sp., temporal variations in settlement density occurred both within and between days. In order to accurately estimate initial settlement, abundance of settlers must be sampled before significant mortality occurs (Keough and Downes, 1982). Because no significant juvenile mortality occurred between 1 and 10 days after settlement (Chapter 1), I conclude that sampling every 2 hours is adequate to measure settlement unchanged by juvenile mortality.

Settlement of all three species occurred only during the daylight and did not demonstrate a single restricted peak. This is consistent with release times observed in the laboratory. Within daylight hours, the timing of maximum settlement differed among the three species. There were also daily differences in the timing of settlement. These ascidians may be responding differently to environmental factors, or there may simply be random fluctuations in settlement times.

For several species of colonial ascidian that contain symbiotic algae, release and settlement has been found to occur at midday when light levels are high, enabling larvae to settle in locations where light levels favor juvenile survival (Duyl et al., 1981; Olson, 1983). It is thus reasonable to ask if there is a similar fitness component to the diel timing of release and settlement of colonial ascidian larvae
which do not contain symbiotic algae. All three species examined here settled only during daylight hours, strongly indicating that light is used in some way. That is, if light is not necessary for larval release or settlement, then larvae should optimize survival by being in the water column at night, when visual predators, such as fish (Olson and McPherson, 1987), are not a threat. For Didemnum candidum and the Diplosoma species, light level may be an important cue indicating settlement sites where chances of juvenile survival are higher. For example, light levels are generally low on downward-facing surfaces. Many sessile invertebrates settle on undersurfaces (Harris and Irons, 1982) and thus avoid harmful ultraviolet radiation (Jokiel, 1980). In addition, the lower light levels on undersurfaces may be cues to other conditions important to survival, such as low silt levels. For Didemnum candidum, Diplosoma listerianum and Diplosoma sp., I have found that settlement is higher in the field on downward-facing surfaces than on upward-facing or vertical surfaces (Chapter 5). The importance of daytime larval release for these species may be the availability of light as a cue indicating downward-facing surfaces, where chances of juvenile survival might be greater.
CHAPTER 3

Seasonal and diel timing of larval release and settlement of a colonial ascidian

Introduction

Patterns of larval settlement can be primary determinants of community structure for sessile marine invertebrates (Underwood and Denley, 1984; Connell, 1985; Gaines and Roughgarden, 1985; Keough, 1988). Identifying factors that influence these settlement patterns can thus contribute to an understanding of the mechanisms that determine community structure. Both larval supply and larval behavior can influence location and intensity of settlement, which in turn affect survival of juveniles and adults.

Larval behavior has been well studied in the laboratory and is often assumed to affect the spatial distribution of planktonic larvae and of settlement (Meadows and Campbell, 1972; but see Hannan, 1984). Recent work has sought to determine spatial and temporal patterns of larval distribution in the field (see Young and Chia, 1987 for review). Spatial distributions of larvae in the plankton have been shown to influence location of settlement (Bernstein and Jung, 1980; Cameron and Rumrill, 1982; Grosberg, 1982; Yoshioka, 1982; Gaines et al., 1985; Olson, 1985). Large scale temporal variations in settlement have been attributed to seasonal differences in numbers of larvae produced (Sutherland and Karlson, 1977; Yoshioka, 1986; Gotelli, 1987), which are often correlated with temperature or photoperiod (Giese and Pearse, 1974).

Relatively few studies have examined short-term variation in larval supply and the effects of such variation on settlement. For
organisms with short-lived larvae, variation in larval release may determine larval availability and the timing of settlement. For example, colonial ascidians have larvae that usually settle within minutes or hours of release (Millar, 1971; Duyl et al., 1981; Olson, 1983; Stoner, 1989; Davis and Butler, 1989). The number of larvae available for settlement is determined, in part, by the numbers of larvae released on a daily (Davis, 1989) and hourly (Olson, 1983; Stoner, 1989) basis; this variation in numbers released has been attributed to physical conditions such as temperature (Davis, 1989) and light level (Olson, 1983). By controlling release times, adults of species with short-lived larvae may determine the timing of larval settlement.

Larval behavioral patterns that affect the location of settlement may be influenced by the diel timing of settlement. Environmental cues available to settling larvae vary with time of day, particularly light levels. Larvae of many species are responsive to light level at the time of settlement in both the laboratory (Crisp and Chobashy, 1971; Meadows and Campbell, 1972; Miller and Hadfield, 1986) and the field (Olson, 1983). Examining the diel timing of larval release may indicate the importance of both small scale variation in larval supply and of larval behavior to variations in settlement location.

The goal of this study was to examine temporal variability in larval settlement of a colonial ascidian and to identify factors that influence the timing of settlement. For Didemnum candidum, an abundant member of the fouling community on floating docks in Pearl Harbor, Oahu, Hawaii, I documented temporal variability in larval settlement on two scales. First, I examined weekly settlement rates for nine months
and compared these rates to the temperature and salinity of the seawater. Second, I measured the diel timing of settlement and diel variation in light level. In addition, I measured diel patterns of release of larvae of _D. candidum_ in the field and their abundance in the plankton in order to determine how much of the variation in settlement could be attributed to larval release and abundance in the plankton. These measurements allowed me to identify some potential causes of temporal variation in larval settlement.

**Methods**

Seasonal timing of reproduction was documented by photographically censusing weekly settlement on acrylic plates from March to December 1988. I had previously found that significant juvenile mortality does not occur within 1 week of plate immersion during the summer months (Chapter 1). Each week, 5 opaque black, sanded, acrylic, 83 x 106 x 3 mm settling plates were suspended horizontally 0.3 m below the water surface from a floating dock at Rainbow Marina in Pearl Harbor, Oahu, Hawaii. At the end of the week, the central 66 x 44 mm area of each plate was photographed with Kodak Ektachrome 64 slide film. The plates were then scrubbed clean and resuspended. The slides were projected and settlers identified and counted. Plates were lost during June and the first 2 weeks of July through vandalism, so no record of settlement was obtained at that time.

Temperature and salinity data were obtained from Dr. D. Somerton and D. Kobayashi of the Honolulu Laboratory of the National Marine Fishery Service. Measurements were taken weekly from 6/86 to 12/87 with a portable CTD probe (Applied Microsystems, Ltd.) at a station 100
from the study site. The probe took continuous readings while being lowered from the surface to a depth of approximately 2 m. The data were averaged between 1986 and 1987 for comparison to the seasonal settlement data, which were taken in 1988.

While simultaneous measurements of the diel timing of larval release, abundance in the plankton and settlement would have been optimal, this was not logistically possible. Therefore, sampling of these 3 phenomena was split between the summers of 1987 and 1988. Measurements of the bi-hourly variation in abundance of larvae in the plankton and of settlement were taken together in 1987, while larval release and settlement were both hourly assessed in 1988. The diel timing of settlement was thus measured during both years and could be used to evaluate variation in the timing of these events between years.

Diel variation in larval settlement was documented on settling plates during the summers of 1987 and 1988. Bi-hourly variation in settlement was measured over 24 hours on 18 July, 25 July, 1 August, and 8 August 1987. Black acrylic plastic plates, measuring 82 x 103 x 3 mm, were 'conditioned' by a week of immersion in filtered seawater in the laboratory to allow accumulation of microbial films. Five plates were suspended horizontally from the dock at 0.25 m depth every 2 hours, and retrieved after 2 hours of immersion. In addition, settlement was measured hourly during the daytime on 21 July and 26 July 1988. Every hour between 0500 and 1800, 5 unfilmed plates were suspended horizontally at 0.25 m depth and retrieved after 1 hour of immersion. At the end of each sampling day, all plates were microscopically examined and settlers on the undersides of the plates identified and counted.
On 21, 26 and 28 July 1988, light readings were taken once an hour between 0600 and 1800 next to the settling plates at 0.25 m depth with a quantum scalar irradiance meter (Biospherical Instruments, Inc. QSL-100). This light meter has a 4 pi light sensor which measures light coming from all directions.

During the summer of 1988, the number of larvae released per hour by *Didemnum candidum* colonies which were growing on acrylic plates was measured. These plates had been deployed about 2 months earlier and were 100% covered with ascidian colonies. *Didemnum candidum* colonies were most abundant, but the colonial ascidians *Diplosoma listerianum* and *Botrylloides* sp. were also present on the plates. Five larval traps were constructed of semi-translucent plastic sandwich boxes (Fig. 3.1). A bolt was placed through a hole in the lid and an ascidian-encrusted plate was bolted to the lid. The trap was then filled with filtered seawater, the lid attached and the trap suspended vertically from the side of the dock at 0.25 m depth, adjacent to the settling plates. All larvae released by the colonies on the plate were caught in the trap. Every hour between 0500 and 1800 on 21 July, 23 July and 26 July 1988, the water in each trap was poured through a 125 um sieve, and the captured larvae were preserved in 10% formalin for later identification and enumeration. Counts were only made of larvae of *D. candidum*, which glow in cross-polarized light and can easily be distinguished from larvae of the other ascidian species present. No larvae settled inside the trap, that was made of plastic that appeared to be quite hydrophobic.

The abundance of larvae in the plankton was monitored by taking plankton samples with an impellar pump every 2 hours over the same
Figure 3.1. Larval trap containing settling plate encrusted with colonies of adult ascidians.
24 hour periods in which settlement was measured during the summer of 1987. Three replicate 82.5 l samples were taken every 2 hours at 0.25 m depth from the edge of the dock on 18 July, 25 July, 1 August, and 8 August 1987. Samples were filtered through a 125 um mesh, preserved in 10% formalin and larvae identified and counted. Larvae occasionally lost their tails during the passage through the pump but were otherwise intact and readily identifiable.

Temporal variation in the number of larvae captured by each method was examined with Pearson product-moment correlations, regression analyses or analyses of variance (Sokal and Rohlf, 1981). Analyses were performed on raw and log-transformed (log X+1) data. Because the results of the two sets of analyses were the same, the raw data and results are presented. Comparisons of data from all three sampling methods were made with the 1988 data pooled into 2 hour intervals, because samples were taken every two hours in 1987 and hourly in 1988.

Results

Weekly settlement intensity of Didemnum candidum varied between weeks (1-way ANOVA, P<0.0001); it was low during the spring and rose to a peak from August through September and October 1988 (Fig. 3.2). The onset of settlement appeared to follow the increase in water temperature in late spring and early summer, with peak settlement occurring after the apparent peak in temperature. Settlement ended abruptly at the time of the first heavy rainfall in December. At this time, there was extensive mortality in the fouling community on the floats, including Didemnum candidum. Heavy mortality after a
Figure 3.2. Number (mean ± standard error) of newly-settled juveniles of *Didemnum candidum* per 80 cm² plate per week from 3 March to 21 June and 15 July to 14 December 1988 on 5 replicate plates and weekly temperature (°C) and salinity (‰/oo) readings.
particularly heavy winter rain storm also occurred in 1986 and 1987 (pers. obs.). Salinity was lower in December than in other months during both 1986 and 1987 (Fig. 3.2). Settlement only occurred during the daytime. Settlement began at first light, between 0500 and 0600, and showed a strong early morning and a weaker afternoon peak in intensity (Fig. 3.3B). No larvae settled between 1800 and 0500.

Irradiance reached a maximum of 2900 μmol/m²/sec at 1400. In the afternoon, shadows from nearby boats occasionally moved across the traps. Hourly settlement did not closely follow the pattern of increase in light over the daylight hours, although the second peak in settlement was between 1400 and 1500 hours, near the time of peak light level (Fig. 3.3C).

The diel pattern of settlement did not differ between 1987 and 1988 (Pearson product-moment correlation, r=0.8093, P=0.0274). The average number of settlers per day was similar between years (mean ± standard error: 1987, 212±90; 1988, 189±50).

Release, abundance of larvae in the plankton and settlement showed similar diel patterns (Fig. 3.4), with early morning and afternoon peaks in abundance. For all 3 sampling methods, larvae were first captured near dawn, between 0500 and 0600. No larvae were captured in the plankton or settled between 1800 and 0500. Release was not measured at night. The diel timing of release, planktonic abundance and settlement were correlated with each other (Pearson product-moment correlation, release-plankton r=0.9028, P=0.0054; release-settlement r=0.9737, P=0.0002; plankton-settlement r=0.9458, P=0.0013). However, these 3 measurements did not show identical patterns. Larvae were released and captured in the plankton between 1600 and 1800, but very
Figure 3.3. Hourly variation in abundance (mean ± standard error) of larval release and settlement of *Didemnum candidum*. A. Number of larvae released in each larval trap (see text) per hour on 21, 23 and 26 July. B. Density of settlers (number per cm²) per hour on 21 and 26 July 1988. C. Light level (umol/m²/sec) measured hourly on 21, 26 and 28 July 1988. Stippled bars represent readings taken in the direct sun (mean ± standard error); dark bars represent readings taken in the shade (mean).
Figure 3.4. Abundance of larvae released, in the plankton and settling in 2 hour periods (mean ± standard error). A. Number of larvae released in each larval trap (see text) on 21, 23 and 26 July 1988. B. Density of larvae in the plankton (number per liter) on 18 July, 25 July, 1 August, and 8 August 1987. C. Density of settled juveniles (number per cm²) on 18 July, 25 July, 1 August, 8 August 1987 and 21, 26 July 1988. No larvae were captured in the plankton or settled between 1800 and 0400.
few settlers were found at this time, suggesting that larvae released late in the day had low rates of settlement success. When release and settlement are compared in one hour intervals, the same bimodal distribution is apparent (Fig. 3.3A,B); pooling the data into 2 hour samples apparently does not obscure the general pattern (Fig. 3.4A,C).

Variation in the time of larval release accounted for 57% of the variation in the timing of settlement in 1988 (regression analysis T=2.58, P=0.0495), and for 83% of the variation in temporal abundance of larvae in the plankton (T=4.90, P=0.0045). Variation in the temporal abundance of larvae in the plankton accounted for 92% of the variation in settlement times in 1987 (T=7.54, P=0.0006, Fig. 3.5).

Discussion

The most important proximate factor determining the hourly timing of settlement of Didemnum candidum was planktonic abundance, which was, in turn, largely determined by the timing of larval release. The short planktonic period of this larval stage creates a close temporal link between release and settlement; in species with longer-lived larvae, release would less reliably predict settlement.

Short-lived larvae are expected to have low levels of mortality in the plankton and limited dispersal distance (Strathmann, 1985). For Didemnum candidum, the close temporal association between numbers of larvae released, in the plankton, and settling supports both of these generalizations. If either larval mortality or dispersal distance had been large, larval release would have predicted less of the variation in settlement. Most larvae released at this site probably settle near
Figure 3.5. Relationship between number of larvae released, in the plankton, and settling for *Didemnum candidum*. Percentage of variability in each phase which is accounted for by variability in the previous phase.
their parents, making this largely a closed population, with consequences to its genetic structure (Shields, 1982).

Larval release and settlement are less likely to show a similar close temporal association in species with planktotrophic larvae. While larval mortality has seldom been measured directly (see Young and Chia, 1987 for review), long-lived larvae are thought to suffer a high degree of mortality in the plankton. Dispersal distance is also thought to increase with larval lifespan (Strathmann, 1985); a large dispersal distance would weaken the temporal correlation between larval release and settlement at a given site.

In species like colonial ascidians with short-lived larvae, the time when larvae are released largely controls settlement time (Olson, 1983; Davis, 1988a). In the laboratory, larvae of colonial ascidians generally settle within minutes or hours of release, although there is variability both within and between species (Berrill, 1950; Millar, 1971). In the field, larvae of several species have been directly observed to settle within seconds to minutes of release (Olson, 1983; Davis and Butler, 1989; Stoner, 1989). Larvae of Didemnum candidum have been observed to settle within seconds or up to five hours after release in the laboratory (pers. obs). The close temporal link found between larval release and settlement of D. candidum suggests that the planktonic period is brief in the field.

Factors which can affect larval release include temperature and light level. For many invertebrates, gametogenesis is induced by increasing temperatures in the spring (Giese and Pearse, 1974). On a smaller time scale, biweekly (Duyl et al., 1981) and daily (Davis, 1989) variation in larval release of colonial ascidians has been shown
to correlate with water temperature. Recruitment of *Didemnum candidum*
appeared to be correlated with seasonal variation in water temperature,
with peak recruitment following the summer temperature maximum.

The importance of light in stimulating release of short-lived
larvae has been demonstrated in the laboratory for colonial ascidians
(Berrill, 1975), as well as for bryozoans (Ryland, 1974) and sponges
(Amano, 1988). While a variety of release times has been found in the
laboratory (see Olson, 1983 for review), many ascidian species release
larvae shortly after dawn, as was found for *Didemnum candidum* both in
the laboratory (Chapter 2) and the field (present study). However, the
bimodal pattern of larval release of *D. candidum* does not follow any of
those previously observed for colonial ascidian larvae. The second,
early afternoon, peak of release and settlement appears to be unique to
*D. candidum*. One possible function of lower intensity of release and
settlement during midday is avoidance of midday irradiance damage. The
adaptative significance of this particular pattern remains untested.

The ultimate selective pressure determining the time of larval
release in colonial ascidians may be the potential effect of time of
day on larval behavior, as discussed in Chapter 2. In the laboratory,
larvae of colonial ascidians are commonly photopositive upon release
and photonegative prior to settlement (Millar, 1971), leading them to
seek out dark crevices and under-surfaces for settlement (Millar, 1971;
Olson 1983; Cloney, 1987; Young and Chia, 1987). A few studies have
demonstrated settlement in shaded locations in the field (Olson, 1983)
and higher survival in such sites (Olson, 1983; Young and Chia, 1984).
Olson (1983) found that larvae of the colonial ascidian *Didemnum molle*
settled at midday when light levels were high and that the larvae
settled in shaded locations. Using transplant experiments, he demonstrated that juveniles died if exposed to direct light, so larvae were indeed settling in locations where chances of survival were optimized. Similarly, Young and Chia (1984) found juvenile survival was higher on the undersides of opaque surfaces for several solitary ascidian species whose larvae were known to settle photonegatively. The diel timing of larval release in *Didemnum candidum* may have a fitness component because it directly influences settlement times and thus the availability of light as a cue to larvae in determining suitable locations for settlement.

The close temporal link between larval release and settlement indicates that larval availability, as controlled by larval release, is an important determinant of settlement in *Didemnum candidum*. The influence of light on the location of settlement and juvenile survival is discussed in Chapter 4.
CHAPTER 4

The adaptive value of larval behavior: phototaxis, settlement site and juvenile mortality of the colonial ascidian, Didemnum candidum

Introduction

A central process in the study of animal behavior is the evaluation of the adaptive value of behavior, which is accomplished by identifying the consequences of a particular behavior to an individual (Wallace, 1973; Brown, 1975). Larval behavior of sessile marine invertebrates, extensively studied in the laboratory, is thought by many to influence the location of settlement in the field (Meadows and Campbell, 1972), potentially conferring an adaptive advantage. For sessile organisms, settlement location largely determines the environmental conditions experienced by juveniles and adults and is therefore critical to survival to reproductive maturity (Keough and Downes, 1982). While laboratory experiments define potential larval behavior, field observations are needed to determine the extent to which such behavior is typically expressed. In order to determine if larval behavior at settlement has adaptive value, it is necessary to demonstrate that 1) a particular behavioral pattern determines settlement site in the field and 2) that the favored settlement site increases the probability of juvenile or adult survival.

One of the best studied types of larval behavior is phototaxis, which is known from laboratory studies of many groups, such as ascidians (Crisp and Ghobashy, 1971), barnacles (Crisp and Ritz, 1973), bivalves (Bayne, 1964), bryozoans (Ryland, 1960), crabs (Forward and Costlow, 1974), polychaetes (Young and Chia, 1982; Marsden, 1984) and nudibranchs (Miller and Hadfield, 1986). Many species exhibit a change
in phototaxis over the larval lifespan (Thorson, 1964). For example, many colonial ascidian larvae are photopositive upon release which causes them to swim toward the surface; they then become photonegative prior to settlement (Crisp and Ghobashy, 1971; Millar, 1971; Berrill, 1975; Duyl et al. 1981). This photonegativity is thought to cause larvae to swim toward the bottom (Thorson, 1964) or to settle in dark crevices or on downward-facing surfaces (Young and Chia, 1984).

Photic responses at settlement may affect juvenile survival. Many species, including a number of ascidians, tend to settle on the undersurfaces where irradiance levels are low (Crisp and Ghobashy, 1971; Millar, 1971; Olson, 1983; Young and Chia, 1985), a pattern that could enhance juvenile survival directly by protecting the juveniles from lethal ultraviolet radiation (Jokiel, 1980). Light may also indicate other conditions important to survival. For example, predation by visual predators may be decreased in cryptic habitats (Keough and Downes, 1982). Siltation has been shown to be detrimental to juvenile ascidians (Young and Chia, 1984), and settlement on shaded surfaces will aid larvae in avoiding this problem. For ascidians, field observations of greater settlement on shaded surfaces and greater juvenile survival in such sites (Olson, 1983) shows that larval phototaxis influences both settlement location and juvenile survival and thus has adaptive value.

Few studies have documented larval behavioral patterns in the laboratory and then demonstrated the effects of a particular pattern on settlement location in the field. The photonegative settlement of ascidian larvae observed in the laboratory suggests that settlement may be greater on shaded surfaces in the field for some species, where
survival may be enhanced (Olson, 1983; Young and Chia, 1984). The colonial ascidian *Didemnum candidum* is an abundant member of the fouling community on floating docks in Pearl Harbor, Oahu, Hawaii. The larvae settle only during the daytime (Chapters 2 and 3) when light is available as a cue to shaded settlement sites. To determine the extent to which phototaxis influences settlement site and juvenile survival in the field, I documented larval phototaxis in the laboratory and then compared the intensity of settlement and juvenile survival on shaded and unshaded surfaces in the field. Taken together, these data define the importance of light as a cue at settlement for *D. candidum* and assess the adaptive value of patterns of larval behavior observed in the laboratory.

**Methods**

*Laboratory studies*

Colonies of *Didemnum candidum* were collected from floating docks at Rainbow Marina in Pearl Harbor, Oahu, Hawaii and maintained in a running sea water system at the Kewalo Laboratory of the Pacific Biomedical Research Center of the University of Hawaii. Larvae of *Didemnum candidum* are released in the morning in response to light (Millar, 1971; pers. obs.) and thus are easily obtained. Adults were placed overnight in flow-through containers with 125 μm nitex mesh bottoms that retained larvae. In most experiments, I used larvae that had been released within the previous half hour, captured by pipette and added to experimental chambers. In some experiments, an entire colony was added to an aquarium or outdoor tank where larvae were released directly.
Experiment 1: Random versus aggregated settlement

The spatial pattern of larval settlement was examined in the laboratory to determine if settlement was aggregated. If settlement was random, then more than one larva could be used at a time in phototaxis experiments without the potentially confounding effect of gregariousness. A group of sibling larvae was added to a 3.66 liter aquarium in which an 83 x 106 x 3 mm black acrylic plate was suspended horizontally 15 cm below the water surface. The aquarium was positioned on a lab bench lit by an overhead fluorescent room light during the daytime. After 24 hours, settlement was abundant on the underside of the plate. Distances between settled juveniles were measured in mm and a nearest-neighbor analysis was used to test for aggregated settlement (Clark and Evans, 1954). Three trials were run with 1 sibling group, and 1 trial with a second sibling group. The number of settlers per trial were 45, 69, 123 and 160.

Experiment 2: Settlement in shaded and well-lighted sites

Larval selection of settlement location was explored by offering larvae a choice of shaded and well-lighted sites. One larva was placed into the center of a 45 ml stender dish, half of which, including the lid, was covered on the outside with black electrician's tape. The dish was positioned on a lab bench lit by overhead fluorescent room light during the daytime. The location of the settled juvenile was noted after 24 hours. Sixty-five trials were run.

To examine larval preferences for both light level and surface orientation, settlement intensity was compared on clear and black, 83 x 106 x 3 mm, acrylic plates that were positioned either horizontally or
vertically in a 77 liter outdoor tank. Five horizontal and 5 vertical plates of each type were suspended 15 cm below the water surface. During midday, the irradiance level above horizontal plates of both types, next to vertical plates of both types and below clear horizontal plates was 85 \( \mu \text{mol/m}^2/\text{sec} \). The irradiance level below the center of the dark plate was 50 \( \mu \text{mol/m}^2/\text{sec} \) (Biospherical Instruments, Inc. QSL-100). To obtain a large number of sibling larvae, 2 adult colonies were placed on the bottom of the tank at dusk. After 24 hours, the number of settled juveniles on the different plate surfaces was recorded.

Experiment 3: Phototaxis and geotaxis

Settlement was found to be higher on the downward-facing surfaces of both clear and black plates, suggesting that larvae might be geonegative. Larval phototaxis was therefore examined in horizontal chambers to avoid the confounding effect of geotaxis. These 8 x 11 x 8 cm, rectangular, clear plastic chambers were marked with 1 cm gradations along the long axis and filled to a depth of 1 cm. A fluorescent light was placed at one end to produce unidirectional light along the chamber of 33 \( \mu \text{mol/m}^2/\text{sec} \) (Fig. 4.1A). Experiments were run in a photographic darkroom to avoid extraneous light. First, changes in phototaxis over the larval lifespan were examined. Twenty-five newly-released, sibling larvae were pipetted into the center of the chamber, and the location of larvae was recorded in centimeters from the lighted end of the chamber after 1/4, 1/2, 1, 2, 3, 4, 5 and 24 hours. Four replicate trials were run. Second, location of settlement was compared between a chamber which was lighted from one
Figure 4.1. Schematic diagrams of photo- and geotaxis experiments (not to scale). A. Transparent and opaque horizontal chambers with light at one end. B. Clear, dark, and half-darkened vertical cylinders lighted from the side. C. Vertical cylinders lighted only from above or below. After Miura and Kajihara (1984).
end as in the previous experiment, and one that was completely shielded
from light. Twenty-five sibling larvae were placed into the center of
each chamber. After 24 hours, location of settlement was noted in
centimeters from the lighted end wall in the lighted chamber and from
the end wall closest to the light in the darkened chamber. Ten
replicate trials were run in the lighted chamber and 6 in the dark.
Because the quadrants that contained the vertical end wall of the
chambers (1 and 11 cm from end) had a greater surface area available
for settlement, comparisons between treatments were made of settlement
density (number per cm²).

An additional series of experiments was conducted in vertical
chambers to distinguish between photo- and geotaxis, by comparing the
number of settlers at the tops and bottoms of chambers in the presence
or absence of light. These 22.5 cm tall x 2.55 cm diameter glass
cylinders were filled to within 3 mm of the top with seawater and
wrapped with black plastic to create several different light regimes.
Cylinders were either clear, completely darkened, or had the top or
bottom half protected from light; these 4 treatments were laterally
illuminated (Fig. 4.1B). In another set of experiments, the vertical
columns were blackened except at the ends and light entered either from
above or below (Fig. 4.1C). In the cylinders lighted from above, light
levels next to the top and bottom of the cylinders were 200 and 85
μmol/m²/sec, respectively. In the cylinders lighted from below, light
levels next to the top and bottom were 45 and 110 μmol/m²/sec,
respectively. Larvae from several colonies were used together in a
cylinder. Ten to 300 larvae were pipetted into the center of each
cylinder and after 24 hours larvae were scored as settling either
within 1 cm of the top of the cylinder, along the vertical wall or on the horizontal bottom surface. Many larvae settled either on the surface tension or at the miniscus; these larvae were scored as settling at the top of the cylinder. Four to 7 replicates were run of each light treatment.

For the experiments run in the vertical chambers, the raw number of settlers in each location could not be directly compared for two reasons. First, the number of larvae used varied between replicates, and second, the surface area of the 3 locations differed considerably. For each replicate trial, the percent of total settlement that occurred in each location was calculated. This gave the number of larvae that would have settled in each location if there had been 100 larvae in each trial. Settlement density (number per cm²) was then calculated for each location by dividing the adjusted number of larvae by the area of each section of the chamber. Settlement densities in the different locations were then compared between treatments.

Experiment 4: Orientation of settlement

In the vertical cylinders, the only downward-facing surface available to larvae for settlement was the underside of the water surface. To examine the influence of light direction on the orientation of settlement, 3 vertical and 3 horizontal, black, acrylic plates were suspended 15 cm below the water surface in 3.66 liter aquaria which were wrapped with black plastic to create different light regimes. A fluorescent light was used to direct light either from above or below, or light was absent. Larvae from several colonies were used together in an aquarium. One hundred to 250 larvae were pipetted into the
center of each aquarium, and the location of settled individuals noted after 24 hours. The light intensities adjacent to the plates were the same when light was directed either from above or below. Next to the vertical plates, the light level was 50 \( \mu \text{mol/m}^2/\text{sec} \). On the side of the horizontal plates toward the light, the level was 60 \( \mu \text{mol/m}^2/\text{sec} \) and on the side away from the light, the level was 25 \( \mu \text{mol/m}^2/\text{sec} \). Five to 7 trials were run for each treatment.

Field Studies

To document differences in the intensity of settlement and juvenile mortality with light exposure and surface orientation, settling plates were horizontally suspended from a floating dock at Rainbow Marina in Pearl Harbor. These 83 x 106 x 3 mm plates were made of black or clear acrylic plastic and were roughened by sanding (Chapter 1).

Settlement was documented by photographing the central area on the underside of each plate at 1:1 magnification with Kodachrome ASA 64 color slide film; the area photographed was either 33 x 44 mm or 44 x 66 mm, as noted below in the description of the 2 field experiments. Juvenile survival was then monitored by photographing the same central quadrant on the underside of each plate on alternate days for a 2 1/2 week period. The plates were placed in a frame and overlaid with a grid which designated four 22 x 44 mm quadrants. Either 2 or 4 quadrants were photographed, depending upon the experiment. The slides were projected and the continued presence or absence of individuals on each date was noted. Cumulative percent mortality was calculated for each sampling date as the percentage of initial settlers which was
absent by the sampling date. Total percent mortality was calculated as
the percentage of initial settlers which were absent by the last
sampling date.

Settlement was abundant for several organisms in addition to
*Didemnum candidum*. Settlement of the polychaete *Hydroides elegans* was
recorded in some cases. Settlement and juvenile survival of the
colonial ascidians *Diplosoma listerianum* and *Diplosoma* sp. were
recorded on all plates. The larvae and newly-metamorphosed individuals
of the *Diplosoma* appeared to belong to different species, but became
difficult to distinguish once growth began. For this reason, the data
were pooled for the 2 *Diplosoma* species.

For animals with indeterminate growth, such as colonial
ascidians, colony size can be a useful indicator of relative
suitability of conditions. For 3 replicate plates in each treatment,
the outlines of all colonies of *Didemnum candidum* and the *Diplosoma*
species were traced from the slides taken on the first and last
sampling date, and a digitizer was used to calculate the total colony
area of each genus per plate in cm$^2$. For each genus, percent cover on
each plate was calculated then for each date.

Experiment 5: Settlement and juvenile mortality with light exposure

Intensity of settlement and juvenile mortality were compared
between light and dark surfaces of acrylic plates in the field. Five
clear and 5 black plates were individually suspended horizontally at
0.25 m deep from the floating dock on 11 July 1988. One week later, on
18 July, the plates were collected and all settlers identified and
counted. In addition, 5 transparent plates that had their upper
surfaces completely covered with black plastic tape were suspended horizontally on 11 July 1988. Settlement in the central 44 x 66 mm area was documented after 1 week. The tape was then removed from one half of each plate; the juveniles on the uncovered half of each plate were now exposed to transmitted light, while those on the other half remained shaded. Juvenile survival was monitored for the following 2 1/2 weeks (18 June - 5 August).

For 3 of the half-darkened plates, the outlines of all colonies of Didemnum candidum and the Diplosoma species were traced from the slides taken on 18 July, 27 July and 5 August 1988. Percent cover of each genus was then compared between clear and dark halves of the plates.

Experiment 6: Settlement and juvenile mortality with surface orientation

Differences in intensity of settlement with surface orientation were measured on plates attached to a rectangular PVC-pipe rack which held 10 vertical and 10 horizontal black plates. A rack was suspended at 0.5 m depth from the dock on 28 July 1987. Eight days later, on 5 August, settlement was documented in the central 33 x 44 mm area of each surface of each plate. Intensity of settlement was then compared between upward-facing, downward-facing and vertical surfaces. Survival was then monitored for the following 2 1/2 weeks on 5 of the downward-facing plates.

The effect of surface orientation on juvenile survival was examined using 2 additional racks which each held 5 horizontal plates and were deployed at 0.5 m on 28 July 1987. After settlement was
documented on 5 August, plate orientation was altered. Plates on one rack were turned so that the undersides faced upward, and those the second rack were placed in a vertical position. Juvenile survival in the central 33 x 44 mm area on the surface of each plate which had previously faced downward was then monitored. Mortality and percent cover on the upward-facing and vertical surfaces were compared to the downward-facing surfaces of the horizontal plates.

For 3 plates in each surface orientation, the outlines of all colonies of *Didemnum candidum* and the *Diplosoma* species were traced from the slides taken on 5 and 22 August 1987. Percent cover of each genus was then compared between the different surface orientations.

**Data Analyses**

Prior to analysis, percentage data were normalized by arcsine transformation, and counts were square-root transformed to ensure independence of means and variances (Sokal and Rohlf, 1981).

**Results**

**Laboratory Studies**

Experiment 1: Random versus aggregated settlement

There was no evidence for gregarious settlement in *Didemnum candidum*. The number of settled juveniles ranged from 45 to 160 per plate; density ranged from 0.0051 to 0.0182 per mm$^2$. Larvae of 2 sibling groups settled in a random pattern (R=0.99-1.06). The average nearest-neighbor distance was 5.2±0.7 mm (mean ± one standard error).
Experiment 2: Settlement in shaded and well-lighted sites

When larvae were given a choice between light and dark sides of a tender dish, over 95% settled on the dark half (63 out of 65 settlers). When larvae were given a choice between clear and dark plates in an aquarium, 92% of settlement was on the dark plates (2-way ANOVA, P<0.0001). On both clear and dark plates, settlement was greater on horizontal lower surfaces. On the dark plates, settlement was intermediate on vertical and lowest on upward-facing surfaces. On the clear plates, settlement did not differ significantly between vertical and upward-facing surfaces (1-way ANOVA, Student-Newman-Keuls, P<0.0001 for both plate types, Fig. 4.2).

Experiment 3: Phototaxis and geotaxis

The location of larvae in the horizontal chambers changed over time (2-way ANOVA, time x location interaction P<0.0001). Larvae were initially photopositive and were found mostly in the centimeter nearest the light. Subsequently, larvae swam throughout the chamber (Fig. 4.3). Settlement density was highest at the ends of lighted and dark chambers (1-way ANOVA, P=0.0008 and P=0.0103, respectively, Fig. 4.4), suggesting that larvae were indifferent to light direction at settlement (2-way ANOVA, light treatment x location interaction P=0.91). After 24 hours, there was a slight temperature gradient along the length of the chamber. In the lighted chamber, the temperature was 23.6°C at a distance of 1 centimeter away from the lighted end of the chamber, 22.8°C at 6 cm and 22.3°C at 11 cm. This temperature gradient did not appear to significantly affect settlement pattern, since settlement intensity was greatest at both ends of the chamber.
Figure 4.2. Number of settlers of *Didemnum candidum* (backtransformed mean + upper 95% confidence limit) on upper and lower surfaces of horizontal plates and vertical plates in an outdoor tank. Both clear and black plates were used.
Figure 4.3. Number of larvae of *Didemnum candidum* (backtransformed mean + upper 95% confidence limit) in centimeters from lighted end of horizontal chamber at 1/4, 1/2, 1, 2, 3, 4, 5 and 24 hours. Numbering of location starts at lighted end of chamber (1 cm). Data are presented only for odd-numbered centimeters for clarity.
Figure 4.4. Density (number per cm$^2$) of settlers of Didemnum candidum (backtransformed mean + upper 95% confidence limit) in centimeters from end of lighted and dark horizontal chambers. Numbering of location starts at one end of chamber (1 cm); light positioned at this end in lighted chamber.
A consistent swimming pattern was observed in both the vertical chambers and aquaria. Larvae swam toward the surface, then passively sank and usually lay on the bottom for several seconds before swimming upward again. Larvae were never observed to swim downward.

In the vertical cylinders, the density of settlement was greater on the horizontal surfaces at the top and bottom of the cylinder than on the vertical wall in all treatments (1-way ANOVA, Student-Newman-Keuls, \( P \leq 0.0123 \), Fig. 4.5, Table 4.1). Settlement density was greater on the bottom than at the top in the clear cylinder and the cylinder with the bottom half shaded; settlement density at the top and bottom did not differ significantly in the other treatments. At the end of the 24 hour period, the temperature was 23\(^\circ\)C at both the top and the bottom of the clear cylinder.

Experiment 4: Orientation of settlement

When settlement sites were compared in aquaria on dark plates with light coming from above, below, or absent, settlement was greatest on the undersurfaces of horizontal plates in all three treatments (1-way ANOVA, Student-Newman-Keuls, \( P \leq 0.0003 \), Fig. 4.6). The proportion of settlers on the differently oriented surfaces varied slightly between treatments (2-way ANOVA, treatment x location \( P = 0.0320 \)). When the aquarium was lighted from below, settlement was greater on vertical surfaces than on upward-facing surfaces, while when light was directed either from above or was absent, settlement did not differ between vertical and upward-facing surfaces (1-way ANOVA, Student-Newman-Keuls, \( P \leq 0.0003 \) for each treatment, Fig. 4.6).
Figure 4.5. Settlement of *Didemnum candidum* in vertical cylinders that were clear, dark, lighted from above only, lighted from below only or half-darkened. Clear and half-darkened cylinders were lighted from the side. Density (number per cm$^2$ per 100 larvae) of settled juveniles (backtransformed mean $+$ upper 95% confidence limit) that were at the top, along the vertical wall or on the bottom. Student-Newman-Keuls results for differences in settlement intensity between locations given for each treatment. $>$: significantly greater, $-$: no significant difference.
Table 4.1. Raw numbers of settlers of *Didemnum candidum* at top, along wall and on bottom of vertical cylinders. Top = surface tension plus upper 1 cm of vertical wall. Wall = along vertical wall. Bottom = on horizontal bottom of cylinder. A. Clear cylinder. B. Dark cylinder. C. Cylinder lighted from above only. D. Cylinder lighted from below only. E. Cylinder with top half darkened. F. Cylinder with bottom half darkened.

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Figure 4.6. Number of settlers of *Didemnum candidum* (backtransformed mean + upper 95% confidence limit) on upper and lower horizontal surfaces and on vertical plates in aquaria with light from above or below or in the dark.
Field Studies

Experiment 5: Settlement and juvenile mortality with light exposure

Settlement of larvae of Didemnum candidum and the Diplosoma species was much greater on black than on clear plates after 8 days of plate immersion (18 July, 1-way ANOVA, $P<0.001$, Fig. 4.7). For both genera, settlement intensity did not differ between the undersides of plates that were black or were covered on the upper surface with black plastic (1-way ANOVA, $P>0.40$), or between the 2 halves of the undersides of the plates covered with black plastic ($P>0.25$). By the end of the study period (5 August), the abundance of organisms encrusting the 2 halves of the half-darkened plates was quite different, with a clear demarcation visible between the two halves (Fig. 4.8). On the clear half, filamentous green algae and the Diplosoma species were abundant. In contrast, Didemnum candidum was more abundant on the dark half. This distribution of colonies appeared to be produced by differences in both juvenile mortality and growth.

The cumulative percent mortality of Didemnum candidum juveniles was higher on the clear than on the dark half of the plates during the second part of the study period (27 July - 5 August, 1-way ANOVA, $P<0.02$, Fig. 4.9). Mortality of juveniles of the Diplosoma species did not differ between the clear and dark halves (1-way ANOVA, $P=0.1222$).

There were differences between genera in total mortality. During the last week of monitoring (1 - 5 August), the percent mortality of Didemnum candidum was higher on the clear halves of the plates than that of the Diplosoma species (1-way ANOVA, $P<0.0268$). In contrast, the Diplosoma species suffered higher mortality than did $D$. candidum on
Figure 4.7. Number of settlers of Didemnum candidum and the two Diplosoma species (backtransformed mean + upper 95% confidence limit) on the undersides of clear and black horizontal plates in the field.
Figure 4.8. Photograph of underside of half-darkened horizontal plate on 5 August 1988. The half of the plate visible on the left of the photograph was protected from direct light while the half on the right was exposed to direct light.
Figure 4.9. Juvenile mortality of *Didemnum candidum* and the *Diplosoma* species on the undersides of clear and dark halves of horizontal plates. Cumulative percent mortality for each sampling date (back-transformed mean ± 95% confidence limit).
the dark halves ($P < 0.0233$, Fig. 4.9). The temporal pattern of juvenile mortality emphasizes the importance of the timing of sampling to detection of patterns of juvenile mortality. If juvenile mortality had only been monitored through 25 July, there would have been no difference detected in mortality between *D. candidum* juveniles exposed to and protected from direct light, or between species.

There were differences in percent cover over time between halves of the plates for both *Didemnum candidum* and the *Diplosoma* species. Initially, on 18 July, there was no difference in percent cover between halves for either species (1-way ANOVA, $F > 0.26$, Fig. 4.10). Later, on 27 July and 5 August, percent cover of *D. candidum* was higher on the dark than on the clear halves of the plates ($P < 0.0041$), while the area covered by colonies of the *Diplosoma* species was greater on the clear halves; this difference was significant on 27 July ($P = 0.0067$) but not on 5 August ($P = 0.08$).

Percent cover on the dark halves of the plates did not differ significantly between *Didemnum candidum* and the *Diplosoma* species (1-way ANOVA, $F > 0.07$). The percent cover of the *Diplosoma* species was higher on the clear halves than that of *D. candidum* on 27 July and 5 August (1-way ANOVA, $P < 0.0171$). Since initial settlement and subsequent juvenile mortality of the *Diplosoma* species did not differ between halves, the greater percent cover on the clear halves of the plates must have been due to a higher growth rate.
Figure 4.10. Percent cover (backtransformed mean ± 95% confidence limits) of *Didemnum candidum* and the *Diplosoma* species on the undersides of clear and dark halves of horizontal plates on 3 dates in the field.
Experiment 6: Settlement and juvenile mortality with surface orientation

Settlement intensity differed with surface orientation for all species examined. For Didemnum candidum, the Diplosoma species and Hydroides elegans, settlement was greatest on downward-facing, intermediate on vertical, and lowest on upward-facing surfaces (1-way ANOVA, Student-Newman-Keuls, P<0.0001, Fig. 4.11).

Juvenile mortality was monitored on plate surfaces which faced downward initially and were reorientated after settlement was documented on 5 August. Mortality of both Didemnum candidum and the Diplosoma species was 100% on upward-facing surfaces, which was higher than the average mortality on downward-facing surfaces (1-way ANOVA, P<0.0124, Fig. 4.12, Table 4.2). For both genera, mortality was greater on vertical than downward-facing surfaces; this difference was significant for the Diplosoma species (P=0.0372) but not for D. candidum (P=0.11).

While there were no juveniles present on any upward-facing plates on the last sampling date, small numbers of both Didemnum candidum and the Diplosoma species remained on the vertical plates (Table 4.2). For both Didemnum candidum and the Diplosoma species, percent cover was considerably greater on downward-facing surfaces than on vertical surfaces (1-way ANOVA, P<0.0129). Numerous individuals of the polychaete Hydroides elegans survived on all three surface orientations.

The temporal pattern of mortality differed between surface orientations for both Didemnum candidum and the Diplosoma species. On the upward-facing and vertical plates, almost all settlers had died
Figure 4.11. Number of settlers (backtransformed mean + upper 95% confidence limit) of Didemnum candidum, the Diplosoma species and Hydroides elegans on upper and lower surfaces of horizontal plates and on vertical plates.
Figure 4.12. Juvenile mortality of *Didemnum candidum* and the *Diplosoma* species on lower surfaces of horizontal plates and on upper and vertical surfaces of repositioned plates. Cumulative percent mortality for each sampling date (backtransformed mean ± 95% confidence limit).
Table 4.2. Number of initial settlers present and percent cover on 5 and 22 August 1987 and total percent mortality on 22 August on upper and lower surfaces of horizontal plates and on vertical plates (mean ± standard error). Data on 5 August are for undersides of plates. Final orientation of plates noted. Up: upper surfaces of horizontal plates. Down: lower surfaces of horizontal plates.

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<td>22 August</td>
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<td>22 August</td>
<td>100±0%</td>
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within 1 week after repositioning (12 August). On the downward-facing plates, mortality did not reach a similar level until the last sampling date (Fig. 4.12).

Siltation was higher on the upward-facing plates, which were completely covered with silt by 12 August. In contrast, silt did not begin to accumulate until 12 August on the vertical plates and never completely covered them. Filamentous green algae were present on the vertical plates by the end of 2 1/2 weeks (22 August), but were not abundant and did not overgrow the few ascidian colonies present.

Discussion

The goal of this study was to examine the adaptive value of patterns of larval behavior of *Didemnum candidum*, by documenting larval phototaxis and determining its effects, if any, on settlement site and juvenile survival in the field. In laboratory experiments, larvae exhibited both photo-and geotaxis, and these taxes influenced settlement location. In the field, settlement of *D. candidum* and two species of *Diplosoma* was found to be highest on the underside of dark surfaces, the location predicted by laboratory observations (present study; Cloney, 1987). Lastly, light exposure and surface orientation were shown to affect juvenile survival and growth. For *D. candidum*, juvenile survival and growth were greatest on shaded, downward-facing surfaces where larvae predominantly settled. For the *Diplosoma* species, light exposure did not affect survival, and growth was greater when juveniles were exposed to direct light. However, settlement and survival of the *Diplosoma* species were greater on undersurfaces.
Many ascidian larvae are photopositive upon release and become photonegative prior to settlement (Millar, 1971), a pattern that has been shown for the didemnids Diplosoma listerianum (Crisp and Ghobashy, 1971; Cloney, 1987) and Didemnum candidum (present study). The initial distribution of swimming larvae of D. candidum in the horizontal chambers clearly demonstrated that they were photopositive upon release and swam toward light, even at relatively low light intensities. When larvae had a choice between lighted and shaded surfaces, settlement was consistently much greater on shaded surfaces, indicating a photonegative choice at settlement.

Ascidian larvae are able to distinguish surface orientation as well as light level. Several species avoid settling on upward-facing horizontal surfaces (Dybern, 1962; Crisp and Ghobashy, 1971; Young and Chia, 1984; Gote1ii, 1987). Larvae of Didemnum candidum exhibit this behavior in the laboratory, probably as a result of geonegativity; settlement was equally high at the tops of vertical chambers when light was directed into the chamber from above or below.

Apparently, the high settlement of Didemnum candidum larvae on downward-facing dark surfaces is produced by a combination of photonegativity and geonegativity. The switch in larval phototaxis from positive to negative does not serve to bring larvae close to the sea bottom at the time of settlement because strong geonegativity keeps them near the surface, and settlement then occurs in a dark location.

This behavioral pattern of upward swimming and photonegative settlement, in combination with the short planktonic period of the larvae, may explain the distribution of adult colonies of Didemnum candidum at the study site. Colonies are abundant on the sides and
bottom of the floating dock which is less than a meter deep, and are relatively rare at greater depths along the pier pilings (Chapter 5). Because larvae swim upward and settle near the water surface on dark substrata, settlement is probably abundant on the undersides or in dark microhabitats on the sides of the floats. Settlement would be considerably less likely to occur at any great distance below the surface along the pilings.

For selective settlement behavior to be adaptive, survival must be greater in preferred settlement sites. This was found to be the case for selective settlement of Didemnum candidum and the Diplosoma species in relation to surface orientation; juvenile survival of both genera was greater on the undersides of surfaces. However, while larvae of both genera settled predominantly on dark surfaces, juvenile survival was greater in shaded locations only for D. candidum.

There are at least two potential benefits to settling in shaded locations: 1) organisms will be protected from direct sunlight, and 2) seeking out dark locations will be indicative of downward-facing surfaces, where both sunlight and siltation will be absent.

Ultraviolet light has been shown to cause mortality of shallow water invertebrates (Jokiel, 1980), including juvenile ascidians (Dybern, 1962). Olson (1983) found 100% mortality of juvenile ascidians which were exposed to sunlight and protected from silt after only four days; macroalgal overgrowth did not occur in this brief time period.

Additionally, algal overgrowth can kill organisms living in well-lighted areas. Young and Chia (1984) attributed low survival and growth of juvenile ascidians on downward-facing clear surfaces to the
effects of algal overgrowth. Similarly, Goodbody (1963) and Duyl et al. (1981) reported that spatial competition with algae was responsible for mortality of juvenile ascidians, although this was not specifically tested. Day (1983) found lower abundance of bryozoans on artificial substrata in direct sunlight than in dark caves and attributed this difference to the effects of algal overgrowth. While the present study did not distinguish between the effects of algal overgrowth and sunlight itself, survival of Didemnum candidum juveniles was clearly lower on surfaces exposed to light.

Another observation supports the notion that being in a well-lit environment itself is harmful to Didemnum candidum. In the spring of 1988, a rectangular PVC pipe rack holding 10 acrylic plates was suspended 0.5 m deep from the floating dock in Pearl Harbor. This rack was positioned halfway underneath the edge of the dock, so that while the upper surface of the entire rack and the plates was exposed to silt accumulation, one half of the rack was in the shade and the other was exposed to direct sunlight. When the rack was retrieved in August, the undersides of the plates and the rack itself were encrusted with colonies of D. candidum. However, the upper surfaces of the plates and the rack were encrusted with D. candidum only on the shaded half of the rack; there were no colonies growing on the upper surfaces that had been exposed to sunlight. While siltation rate, the initial distribution of settlement and the mortality of individual colonies were not documented, the observation suggests that light is a more important mortality source than silt for colonies of D. candidum on upward-facing surfaces.
In contrast to that of *Didemnum candidum*, growth of juveniles of the *Diplosoma* species was higher when they were exposed to light, suggesting that greater settlement on dark than on clear plates was not due to an avoidance of sunlight. The cause of enhanced growth in direct light is unknown. Because these *Diplosoma* species do not possess symbiotic algae, a mechanism by which sunlight could directly enhance growth is unknown. Alternatively, the greater abundance of the *Diplosoma* species on the clear halves of plates may have been related to decreased competition from *Didemnum candidum*. The lower survival of *D. candidum* when exposed to direct light provided more space for colonies of the *Diplosoma* species on the clear halves of the plates. While the ability to tolerate direct sunlight makes it possible for the *Diplosoma* species to settle in well-lighted sites and thus avoid competition with *Didemnum candidum*, their settlement was higher on dark substrata. The advantage to the *Diplosoma* species of settling in the shade appears to be that darkness indicates downward-facing surfaces where juvenile survival is higher due to decreased siltation.

Surface angle has been shown to influence juvenile mortality of sessile invertebrates (Harris and Irons, 1982). In the present study, juveniles of *Didemnum candidum* and the *Diplosoma* species perished when settling plates were repositioned so that juveniles faced upwards, although the relative effects of silt accumulation and sunlight are not known. These results indicate that for these species, patterns of behavior that led to preferential settlement on specific surface angles had adaptive value. While the selective regimes appear to differ for juveniles of the two genera, similar patterns of larval behavior have evolved.
For *Didemnum candidum* and the *Diplosoma* species, patterns of larval behavior observed in the laboratory are also functioning in the field where they are important in determining settlement sites and, in turn, juvenile survival. Phototaxis in these species thus clearly confers an adaptive advantage.
CHAPTER 5

Effects of variation in settlement intensity and juvenile mortality with depth on adult distribution of the colonial ascidian *Didemnum candidum*

Introduction

The distinctive and characteristic distributions of sessile marine invertebrate species may result from selective settlement patterns, differential mortality after settlement, or both (Gaines and Roughgarden, 1985; Keough, 1988). The location of settlement by planktonic larvae is critical to subsequent survival, since location largely determines environmental conditions subsequently experienced by juveniles and adults (Keough and Downes, 1982). The intensity of settlement at a given location can be influenced by larval availability (e.g. ascidians: Davis, 1989; barnacles: Grosberg, 1982; Gaines et al., 1985; bryozoans: Bernstein and Jung, 1980; Yoshioka, 1982; sand dollars: Cameron and Rumrill, 1982) or active larval behavior (Meadows and Campbell, 1972; but see Hannan, 1984; Butman, 1989).

Settling larvae have been shown to respond to a variety of substratum characteristics in the laboratory (Meadows and Campbell, 1972; Crisp, 1974), and in the field, selective settlement has been shown to produce non-random distributions upon particular tidal heights (Strathmann and Branscomb, 1979; Grosberg, 1982), surface orientations (Gotelli, 1987) and substrata (Keough and Downes, 1982; Sebens, 1983; Keough, 1986). In some cases, settlement has been shown to be preferential on substrata where juvenile survival is high (Knight-Jones et al., 1971; Olson, 1983; Young and Chia, 1984; Davis, 1987).
Numerous studies have demonstrated that site-specific juvenile mortality can affect the distribution of sessile organisms (Keough and Downes, 1982; Connell, 1985). For example, larvae of some barnacles settle in a wide intertidal range, but post-settlement survival is low outside the narrower adult range (Connell, 1961; Strathmann and Branscomb, 1979). By comparing locations of recent settlers, juveniles and adults, it is possible to determine if selective settlement occurs and if post-settlement mortality significantly alters juvenile distribution (Keough and Downes, 1982; Gotelli, 1987; Hurlbut, in press).

Colonies of the ascidian *Didemnum candidum* are non-randomly distributed relative to depth on floating docks in Pearl Harbor, Oahu, Hawaii. Adults are very abundant on the floats, and relatively rare at depths greater than 1 m along the pier pilings (pers. obs.). When observed in the laboratory, settlement is greatest just below the water surface (pers. obs.). Near-surface settlement may be responsible for the observed distribution of adults in the field, but it is unclear if this is due to short-distance dispersal and settlement near parents, or selective larval behavior.

In order to ascertain if larval behavior of *Didemnum candidum* determines the location of settlement in the field, and if selective settlement leads to greater juvenile survival, I asked the following questions: 1) Is settlement selective relative to depth? 2) Does adult distribution determine the depth distribution of settlement? 3) Does juvenile survival vary with depth? To address these questions, I determined if settlement was non-random over depth, if larvae in the plankton and settlers were most abundant at the same depth as adults,
and if juvenile mortality was non-random over depth. In the laboratory, selective settlement can be estimated by comparing the number of larvae which settle on a particular surface to the number of larvae that are exposed to the surface. In the field, it is difficult to determine the number of larvae that contact a surface. For the purposes of this study, selective larval settlement in the field is defined as a non-random distribution of settlement.

Methods

Study site

The study was carried out at Rainbow Marina, Pearl Harbor, Oahu, Hawaii on floating docks moored to fixed pilings. These floats are encrusted with a dense assemblage of filter-feeding invertebrates. At the sampling location the water is 6.5 m deep at high tide, and the bottom substratum is muddy sediment. Pearl Harbor receives agricultural runoff and inflow from streams that causes both salinity and temperature of surface seawater to drop by over 10% during the winter months (Chapter 3).

Adult distribution

Differences in adult abundance of Didemnum candidum with depth and surface orientation were documented with underwater photographs taken with a Canon AS-6 camera and color slide film (Ektachrome 100) in August 1988. To measure differences in abundance with depth, 4 replicate 12 x 17 cm photographs were taken at 1, 2, 3, 4, 5, and 6 m below the water surface at high tide along the side of a piling. To document differences with surface orientation, 4 photographs were taken
of the vertical side of the float at each of 5 sites that were 0.5 m apart, and on the horizontal underside of the float at 0, 0.3, 0.6 and 1 m in from its edge.

The slides were projected onto a uniform grid of points, and percent cover was calculated (Harris and Irons, 1982).

**Larval availability and settlement intensity**

Abundance of larvae of *Didemnum candidum* in the plankton was measured at 3 depths over four 24 hour periods using an impellar plankton pump. Three replicate 82.5 l samples were taken every 2 hours at 0.25, 3 and 6 m depth from the edge of the dock on 18 and 25 July, 1 and 8 August 1987. Samples were filtered through a 125 um mesh, and captured larvae were preserved in 10% formalin. *Didemnum candidum* larvae in the samples were identified and enumerated.

Larval settlement was simultaneously measured so that the effect of variation in larval abundance on intensity of settlement could be calculated. Five black acrylic plates, each measuring 82 x 103 mm (3 mm thick), were suspended horizontally from the dock at 0.25, 3 and 6 m depth every 2 hours over a 24 hour period on 18 and 25 July, 1 and 8 August 1987. The plates and the plankton pump intake were on opposite sides of a float, approximately 2.5 m apart. Plates were retrieved after 2 hours of immersion and stored in filtered seawater. At the end of each 24 hour period, plates were microscopically examined and settled juveniles on the undersides of the plates identified and counted. The same shallow-level (0.25 m) plankton samples and settling plates used in this study were utilized in an analysis of temporal variations in larval settlement in Chapter 3.
Variation in light with depth was measured with an integrating quantum/radiometer/photometer. At 1400 on 25 July and 1 August 1987, irradiance was measured at 0.25, 3 and 6 m depth at the plankton sampling site.

In order to determine if abundance of larvae and intensity of settlement were correlated, replicates were summed for each sampling time, depth and date for both the plankton and settlement samples. This gave a total of 48 data points at each depth for each sampling method (12 times x 4 days). The sums were then compared with a Pearson correlation analysis.

Variation in settlement and juvenile mortality with depth

Settlement

Differences in intensity of settlement with depth were measured on sets of 5 horizontal plates attached to PVC-pipe racks. After either 8 or 10 days of immersion, the identity and abundance of settlers was documented by photographing the central 33 x 44 mm of the underside of each plate at 1:1 magnification with color slide film (Kodachrome ASA 64). I had previously found that significant juvenile mortality does not occur on settling plates at 0.25 m depth within 10 days of plate immersion during the summer months at this site (Chapter 1).

Since adults were very abundant on the float, greater settlement at shallow depths could be due to adult proximity rather than larval behavior. To determine if preferential behavior influenced the depth of settlement, settlement intensity was compared between plates suspended at 2 shallow depths beside the floats. Adult colonies of
Didemnum candidum occurred at both depths. On each of 10 days in August 1988, a rack was suspended at 2 and at 20 cm depth below the water surface. After 24 hours, settlers on the undersides of the plates were identified and counted.

Variation in settlement intensity with depth was examined on a larger scale, from just below the float to near the bottom. On 28 July 1987, racks were suspended at 0.5, 3 and 6 m below the water surface (racks 1, 2 and 3, Fig. 5.1A). Eight days later, on 5 August, the undersides of the plates were photographed to document settlement.

In addition to Didemnum candidum, settlement intensities of the serpulid polychaete Hydroides elegans and the colonial ascidians Diplosoma listerianum and Diplosoma sp. were recorded. Juvenile mortality of the Diplosoma species was also measured. While recent settlers of the 2 Diplosoma species appeared to belong to different species, they became difficult to distinguish once growth began. For this reason, the data were pooled for the 2 Diplosoma species.

Juvenile mortality

After initial settlement was documented, juvenile survival was monitored by photographing the same central quadrant on the underside of each plate twice a week for 2 1/2 weeks. In order to consistently photograph the same area, plates were placed in a frame and overlaid with a grid which designated a 33 x 44 mm quadrant.

Survivorship over time was tallied by projecting the slides of each plate in temporal series and determining the presence or absence of mapped individuals on consecutive days. Cumulative percent mortality was calculated for each sampling date as the number of
A. Settlement series

B. Survival series

Figure 5.1. Diagram of rack arrangement before and after manipulations. 
A. Initial rack array showing plates on which settlement was measured. 
B. Rack array after experimental manipulations showing plates on which survival was monitored.
initial settlers which was absent by that date. The total percent mortality (number of initial settlers dead by last date divided by number of initial settlers) was then compared between plates on the different racks.

To examine variation in juvenile mortality over depth, a rack was deployed at 2 cm and at 20 cm depth on 7 August 1988. After 10 days, on 17 August, the plates were photographed to document settlement. Juvenile survival was then monitored from 17 August to 4 September.

To examine its variation with depth over a larger scale, juvenile mortality was compared between the racks at 0.5, 3 and 6 m depth (racks 1, 2, and 3, Fig. 5.1B). After settlement was documented on 5 August, juvenile survival was monitored from 5 to 22 August. One plate was lost on the rack at 6 m, causing mortality to be monitored on only 4 replicate plates at this depth.

To distinguish between death associated with settlement pattern and that associated with depth, mortality was compared between juveniles on plates which remained at the original settlement depth and juveniles on plates which were moved to a different depth after the larvae had settled. A second rack was suspended at each depth on 28 July 1987 (racks 4, 5, and 6, Fig. 5.1A). After settlement was documented on 5 August, the depth of these racks was altered, as shown in Figure 5.1B. Rack 4 was moved from 0.5 to 3 m, rack 5 from 3 to 6 m, and rack 6 from 6 to 0.5 m. Juvenile mortality was then monitored from 5 to 22 August. For each depth, mortality was compared between the moved racks and the stationary racks.

Percent cover of the colonial ascidians was measured on the racks at 0.5, 3 and 6 m on the first and last sampling date. For 3 plates on
each rack, the outlines of all colonies of *Didemnum candidum* and the *Diplosoma* species were traced from the slides taken on 5 and 22 August, and a digitizer was used to calculate the total colony area of each genus on each plate. Percent cover was calculated (total colony area divided by area of plate) for *D. candidum*, the *Diplosoma* species, and for the 2 genera combined. For both genera, average colony area was calculated (total colony area divided by number of colonies present). Percent cover and average colony area were then compared between depths and between stationary and moved plates.

Because initial settlement and hence juvenile density were found to differ with depth, the possibility existed that differences in mortality between depths were due to the effects of juvenile density, rather than depth per se. To determine if juvenile mortality was density-dependent for each genus, the total percent mortality was compared with the number of 1) congeneric settlers and 2) total of all settlers of *Didemnum candidum* and the *Diplosoma* species pooled that were initially present on each plate. Data for all racks at 0.5, 3 and 6 m (racks 1-6) were used.

**Analyses**

Prior to analysis, count data were square-root transformed to ensure independence of means and variances and percent data were arcsine transformed to normalize their distributions.

**Results**

**Adult distribution**

The white colonies of *Didemnum candidum* were easily visible in the photographic slides of the float and piling. Abundance of the
Diplosoma species was not documented because the thin, gray colonies were difficult to distinguish.

The bulk of the adult population of Didemnum candidum occurred on the float. Percent cover was higher on the vertical side of the float just below the water surface than on the underside of the float (1-way ANOVA, P=0.0092, Fig. 5.2) or at greater depths along the piling (1-way ANOVA, Student-Newman-Keuls, P<0.0001, Fig. 5.2). Abundance on the piling did not differ between 1 and 6 m in depth (1-way ANOVA, P=0.23).

There were no ascidians on the upward-facing surface of the rocky substratum near the shore in shallow water (<1 m deep at low tide).

Larval availability and settlement intensity

Abundance of larvae in the plankton and settlement intensity were significantly correlated (Pearson product-moment correlation r=0.9682, P=0.0001). Both planktonic larvae and accumulated juveniles of Didemnum candidum were most abundant at 0.25 m, with no difference found between 3 and 6 m (times and days averaged; 1-way ANOVA, Student-Newman-Keuls, P<0.0001, Fig. 5.3). There was considerable temporal variation; the number of larvae in the plankton ranged from 0 to 3.35 per liter and the number of settlers from 0 to 0.78 per cm², with greatest abundance in the early morning. Temporal variation in larval abundance and settlement are described in detail in Chapter 3, where the same data for the 0.25 m depth were used.
Figure 5.2. Percent cover (backtransformed mean + upper 95% confidence limit) of *Didemnum candidum* on the vertical side and horizontal underside of the float and along the side of the piling from 1 to 6 m in depth.
Figure 5.3. Density (backtransformed mean + upper 95% confidence limit) of Didemnum candidum larvae in the plankton (number per liter) and settled juveniles after 2 hours of plate immersion (number per cm$^2$) and light level ($\mu$mol/m$^2$/sec) at 0.25, 3 and 6 m in depth.
Variation in settlement and juvenile mortality with depth

Settlement

While settlement intensity of *Didemnum candidum* and the *Diplosoma* species was high at both depths, it was statistically greater at 2 cm than 20 cm below the water surface (1-way ANOVA, $P<0.0001$ and $P=0.0424$, respectively). In contrast, settlement intensity of the serpulid polychaete *Hydroides elegans* did not differ between 2 cm and 20 cm depth (1-way ANOVA, $P=0.27$, Fig. 5.4). The difference in settlement pattern between the ascidians and the polychaete suggests that the greater settlement of the ascidians near the surface was due to directional larval swimming, rather than passive deposition by water currents.

After 8 days of plate immersion, on August 5, settlement intensity of *Didemnum candidum* and the *Diplosoma* species was greater at 0.5 m than at 3 or 6 m (racks 1, 2 and 3, 1-way ANOVA, $P<0.0001$ for each genus, Fig. 5.5, Table 5.1). Percent cover of the settled juveniles was greater at 0.5 than at 3 or 6 m for both genera; this difference was significant for *D. candidum* (1-way ANOVA, Student-Newman-Keuls, $P=0.0048$) but not for the *Diplosoma* species ($P=0.22$, Table 5.1). Settlement of the polychaete *Hydroides elegans* was greatest at 3 m, intermediate at 0.5 m and lowest at 6 m ($P<0.0001$, Fig. 5.5).

Juvenile mortality

For *Didemnum candidum*, juvenile mortality did not differ between 2 and 20 cm in depth (1-way ANOVA, $P=0.51$, Fig. 5.6), while mortality of the *Diplosoma* species was slightly greater at 2 than at 20 cm
Figure 5.4. Number of settlers (backtransformed mean ± upper 95% confidence limit) of Didemnum candidum, the Diplosoma species and Hydroides elegans on the undersides of plates immersed at 2 and 20 cm depth.
Figure 5.5. Number of settlers (backtransformed mean + upper 95% confidence limit) of Didemnum candidum, the Diplosoma species and Hydroides elegans on the undersides of horizontal plates after 8 days of plate immersion at 0.5, 3 and 6 m in depth.
Table 5.1. Number of settled juveniles present and percent cover on 5 and 22 August, total percent mortality on 22 August on each rack for *Didemnum candidum* and the *Diplosoma* species, and percent cover for both genera combined (mean ± standard error). Initial and final depth noted for each rack.

<table>
<thead>
<tr>
<th>Rack</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth initial</td>
<td>0.5</td>
<td>3</td>
<td>6</td>
<td>0.5</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>final</td>
<td>0.5</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Didemnum candidum**

| 8/5   | 86±5 | 33±2 | 33±3 | 108±8 | 45±4 | 15±1 |
| 8/22  | 11±4 | 10±3 | 12±2 | 17±4  | 17±4 | 5±1  |

**Percent cover**

| 8/5   | 10±1 | 7±1  | 5±1  | 15±2  | 7±1  | 2±1  |
| 8/22  | 48±11 | 32±12| 29±10| 45±3  | 39±9 | 33±4 |

**Total percent mortality**

| 8/22  | 87±4 | 67±8 | 63±4 | 84±4  | 64±6 | 61±3 |

**Diplosoma species**

| 8/5   | 39±6 | 31±5 | 37±6 | 96±14 | 40±2 | 16±2 |
| 8/22  | 6±1  | 9±1  | 8±1  | 8±1   | 8±1  | 2±1  |

**Percent cover**

| 8/5   | 22±3 | 14±2 | 11±5 | 27±1  | 9±2  | 3±1  |
| 8/22  | 15±2 | 17±8 | 37±8 | 40±1  | 26±9 | 18±3 |

**Total percent mortality**

| 8/22  | 84±4 | 74±6 | 77±5 | 92±1  | 87±3 | 87±6 |

**Both genera**

| 8/5   | 32±4 | 21±3 | 16±6 | 42±2  | 16±3 | 5±1  |
| 8/22  | 63±10| 49±18| 66±3 | 85±4  | 64±11| 50±1 |
Figure 5.6. Cumulative percent juvenile mortality (backtransformed mean ± 95% confidence limit) over time of *Didemnum candidum* and the *Diplosoma* species on the undersides of plates immersed at 2 and 20 cm depth.
(1-way ANOVA, P=0.0347). Juvenile mortality of *Hydroides elegans* and *Anomia nobilis* was not measured.

Juvenile mortality of *Didemnum candidum* was greater at 0.5 than at 3 or 6 m (racks 1, 2, and 3; 1-way ANOVA, Student-Newman-Keuls, P=0.0325, Fig. 5.7). Mortality of the *Diplosoma* species did not differ significantly between depths (P=0.37, Fig. 5.6). For both genera, despite initial differences in settlement intensity, the number of juveniles present (Table 5.1), percent cover (1-way ANOVA, P≥0.48, Table 5.1) and average colony area (1-way ANOVA, P≥0.10) were similar between depths on the last sampling date.

Predation is a possible source of juvenile mortality. Because fish have been observed picking at the undersides of settling plates at this site, the plates were examined for evidence of fish predation. Examination of the plates on racks at 0.5 m showed that organisms had been removed in broad swaths which appeared to be scrape or bite marks. Individuals of *Didemnum candidum*, the *Diplosoma* species and the polychaete *Hydroides elegans* were removed within a single scrape mark, with no obvious selection for species. The incidence of scrape marks was greater on the plates on the rack that had remained at 0.5 m than on the rack moved from 0.5 to 3 m. On the plates on stationary racks at 3 and 6 m, no such scrape marks were observed. At 3 m, settlement of *H. elegans* was abundant and the plates became covered with tubes of *H. elegans*, which were partially encrusted with ascidian colonies. The thick calcareous layer produced by *H. elegans* tubes may have discouraged predation. At 6 m, colonies of the *Diplosoma* species disappeared while adjacent colonies of *D. candidum* remained.
Figure 5.7. Cumulative percent juvenile mortality (backtransformed mean ± 95% confidence limit) over time of *Didemnum candidum* and the *Diplosoma* species on stationary racks at 0.5, 3 and 6 m (racks 1, 2 and 3).
The effects of altering plate depth varied with species and depth. For *Didemnum candidum*, there was no difference in juvenile mortality, percent cover or average colony area between stationary racks and racks moved from any given depth to a different one (1-way ANOVA, mortality: $P \geq 0.47$, Fig. 5.8; percent cover: $P \geq 0.64$, Table 5.1; average colony area $P \geq 0.18$). For the *Diplosoma* species, mortality did not differ between racks that were initially positioned at 0.5 or 6 m (1-way ANOVA, $P \geq 0.18$, Fig. 5.8). For the racks originating at 3 m, mortality was slightly greater on the rack moved to 6 m ($P = 0.0412$, Fig. 5.8). Percent cover did not differ between racks originally positioned at 3 or 6 m (1-way ANOVA, $P \geq 0.09$), but for the racks originating at 0.5 m, percent cover was greater on the rack moved to 3 m than on the stationary rack ($P = 0.0012$, Table 5.1). Average colony area of the *Diplosoma* species did not differ between stationary and moved racks for any depth (1-way ANOVA, $P \geq 0.15$).

Juvenile mortality increased with increasing numbers of congeneric juveniles present for *Didemnum candidum* ($r^2 = 0.3215$, $t = 3.5760$, $P = 0.0014$), but not for the *Diplosoma* species ($r^2 = 0.0541$, $t = 1.2430$, $P = 0.22$, Fig. 5.9). The same results were obtained when juvenile mortality was examined as a function of the total number of ascidian juveniles present (*D. candidum* and the *Diplosoma* species pooled).

At 0.5 m, the percent cover of juveniles was greater on plates on the stationary rack than on plates on the moved rack, and mortality of *Didemnum candidum* was greater on the stationary rack (1-way ANOVA, $P = 0.0375$, Fig. 5.8, Table 5.1). At 3 m, the plates on the moved rack had greater percent cover than the plates on the stationary rack,
Figure 5.8A. Cumulative percent juvenile mortality (backtransformed mean ± 95% confidence limit) over time at 0.5, 3 and 6 m on racks which remained at each depth and racks which were moved. Stationary: racks which did not have depth altered. Moved to: racks which were moved to a different depth. Data missing for stationary rack at 0.5 m on 8/19.
Figure 5.8B. (Continued)
Figure 5.9. Density dependence of juvenile mortality. Total percent mortality of juveniles versus number of congeneric juveniles present on the first sampling date on racks at 0.5, 3 and 6 m. Each point represents data from one plate on one rack.
and mortality of *D. candidum* was greater on the moved rack on several dates. However, total percent mortality did not differ between the stationary and moved racks at 3 m (P=0.13) or at 6 m (P=0.93). For the *Diplosoma* species, mortality did not differ between racks at 0.5 (P=0.18) or 6 m (P=0.22). At 3 m, juvenile density was greater on plates on the rack moved from 0.5 m than on the stationary rack, and mortality was greater on the moved rack (P=0.0412).

**Discussion**

The goal of this study was to assess the adaptive value of selective settlement behavior of larvae of sessile marine invertebrates, by determining if larvae of a representative organism settle in locations where the probability of juvenile survival is higher. While settlement of the colonial ascidian *Didemnum candidum* was indeed found to be non-uniform over depth, larvae did not always settle at the depth where juvenile survival was subsequently found to be highest. The abundance of *D. candidum* on the floats in Pearl Harbor appeared to result from greater larval availability and greater settlement intensity at shallow depths, despite proportionally greater mortality of juveniles near the float than at greater depths.

The predominance of planktonic and settling larvae of *Didemnum candidum* near the water surface was probably caused by a combination of the effects of adult proximity and larval behavior. For organisms with short-lived larvae, the initial spatial distribution of larvae is determined by the proximity of reproductively active adults (Young and Chia, 1987). Limited dispersal distance may then produce high densities of settlement near parents (Shields, 1982). For example,
larvae of colonial ascidians often settle within minutes of release, and direct underwater observations indicate that larvae typically do not travel far from their parents before settling (Olson, 1983; Davis and Butler, 1989; Stoner, 1989). Such limited dispersal undoubtedly contributes to the high abundance of larvae and settled juveniles of\textit{Didemnum candidum} adjacent to the floats, where adults are most common.

Larval behavior can also influence the spatial distribution of planktonic larvae (e.g. vertical migration, Forward, 1976; Cronin and Forward, 1986) and of settlement (Strathmann and Branscomb, 1979; Grosberg, 1982). It was demonstrated in Chapter 4 that larvae of \textit{Didemnum candidum} are photopositive upon release at light levels similar to those found at 6 m in Pearl Harbor, then swim upward and settle near the water surface. The importance of such behavior in determining settling depth is indicated by the greater settlement of both \textit{Didemnum candidum} and the \textit{Diplosoma} species on the undersides of plates suspended at 2 cm than at 20 cm below the surface. Since these plates were equally close to adult colonies, the more intense settlement closer to the water surface must have resulted from directed larval movement rather than simply adult proximity. The high density of settlement of \textit{D. candidum} on the floats is probably due to the combined effects of the large number of larvae released by adults on the floats and of upward swimming of larvae that are released from adults at greater depths along the pilings.

Enhanced growth and juvenile survival are often invoked as the ultimate factors selecting for site-specific larval settlement. A number of studies have found a positive association between settlement site and survival rate for sessile invertebrates (Knight-Jones et al.,
In the present study, juvenile survival, expressed as a percent of settlers, of Didemnum candidum and the Diplosoma species was not greater near the water surface where settlement was most abundant. First, survival was not greater at 2 cm than at 20 cm, although settlement was greater at 2 cm. However, 20 cm may be too close to the water surface to discern a depth-determined survivorship factor.

Second, juvenile mortality of Didemnum candidum was greater at 0.5 m in depth, although settlement was more abundant at 0.5 than at 3 or 6 m. The tendency of larvae of D. candidum to settle near the surface is so great that they will settle even on fixed surfaces during high tide, where they perish on the ensuing low tide (unpubl. data).

If depth directly affects juvenile survival of a sessile organism, then mortality should differ between juveniles remaining at a depth with favorable environmental conditions and juveniles moved to a less favorable depth. For Didemnum candidum or the Diplosoma species, conditions did not appear to be especially favorable for growth at any one depth. Percent cover and colony size were both similar on all racks, and with one exception, mortality was similar on stationary and moved plates. Taken together, these data suggest that observed differences in juvenile mortality with depth were not caused by variation in environmental conditions associated with depth per se.

For both Didemnum candidum and the Diplosoma species, differences in settlement intensity produced a depth gradient in juvenile density. Juvenile mortality has been shown to be density-dependent when settlement intensity is high for several species of barnacles (Connell, 1985; Gaines and Roughgarden, 1985). Predation is a potential source of
density-dependent mortality, because areas with highest densities of ascidians may attract more predators (Paine, 1974). While predation was not measured in this study, putative bite marks were more frequent on plates originating at 0.5 m, regardless of the depth at which they were later positioned. Since these were the plates with the most juveniles on them, increased predation may have caused the higher mortality.

The abundant settlement of both Didemnum candidum and the Diplosoma species near the water surface was not associated with increased juvenile survival over the period of this study. On a longer time scale, settlement near the water surface at this location appears to lead to particularly low rates of survival. Organisms living on floats near the water surface in bays or estuaries may be subject to seasonal changes in environmental conditions which can markedly affect survival. For example, massive mortality of the fouling community on mangrove roots in Kingston Harbor, Jamaica was observed following heavy rainfall (Goodbody, 1961). Animals near the surface were killed while deeper ones survived, and the community redeveloped within several months. I observed a similar phenomenon in Pearl Harbor in 1986, 1987 and 1988, with extensive mortality of the fouling community on the floats after the first heavy winter rainfall. There was regrowth of the community on the docks during the spring and summer of 1987 and 1988; the docks were not examined during 1989. Presumably, some adult colonies of D. candidum are situated on the piling below the winter halocline and thus survive until spring to provide a source of larvae for the spring recruitment and repopulation of the floats. While I have not measured the life span of D. candidum, colonies on settling
plates at 0.5 m depth are capable of releasing larvae at 6-8 weeks of age (Chapter 3) and can thus reach reproductive maturity and release larvae which settle nearby and grow, resulting in the large population observed on the floats during the summer months.

The results of the present study, where juvenile survival was not greater in sites where settlement was most abundant, contradict the hypothesis that selective settlement leads larvae to settle in locations where chances of survival are high. Why do larvae of Didemnum candidum and the Diplosoma species predominantly settle near the water surface if this behavior doesn't lead to greater survival? The behavioral pattern of upward swimming and settlement near the water surface may have evolved in a very different habitat. In a location which does not experience large seasonal fluctuations in salinity, settling near the water surface may not be maladaptive.

Alternatively, settlement near the surface may be a 'side effect' of other patterns of behavior which do lead to enhanced juvenile survival. I have previously found that larval settlement and juvenile survival of Didemnum candidum and the Diplosoma species are greater on downward-facing surfaces than on upward-facing or vertical surfaces in the field (Chapter 4). The behavior of settling near the water surface may have been maintained despite apparently adverse selective pressure by the advantages of settling on downward-facing surfaces.

In addition, larval behavior that produces settlement near the water surface in Pearl Harbor may not have the same effect elsewhere. In a turbulent environment, upward swimming may not necessarily lead to settlement near the water surface; larvae that reach the surface may be carried to deeper depths by wave action before settlement can occur.
Further upward swimming could result in settlement on downward-facing surfaces that are near the bottom, rather than at the water surface. In an environment with limited water mixing, such as Pearl Harbor, larvae may become concentrated at the surface, despite a possible preference for settlement at greater depths.

The results of this study raise several questions. First, is decreased juvenile survival of *Didemnum candidum* and the *Diplosoma* species near the water surface widespread? The results obtained here may be restricted to bays or harbors, and juvenile survival may be high near the water surface in other locations. Second, what was the natural habitat where these species evolved? Settlement near the water surface may have been more adaptive in the original habitat, or may have been less abundant. Third, will this behavior be lost over time at this location? For this to occur, larvae would need the ability to locate downward-facing surfaces without also settling near the water surface. In addition, the population must be effectively isolated from other populations which are experiencing different selective regimes. It is clear that an investigation such as this one needs to take into account the conditions at a particular study site, which may differ from the original habitat in which a behavior evolved.
EPILOGUE

Larval abundance, settlement and juvenile survival of the colonial ascidian *Didemnum candidum* were found to vary both spatially and temporally. In addition, daily variation in settlement and the effects of age and density on juvenile mortality were found to differ among several co-occurring species. Interestingly, all species showed a similar temporal pattern of juvenile mortality over a two week period. Apparently, while patterns of settlement and juvenile mortality differed among species, some sources of mortality had effects on a community, rather than species, level.

A major focus of this study was determining the function of diel larval release in colonial ascidians, which involved assessing the adaptive value of patterns of larval behavior observed in the laboratory. Release time was indeed found to influence settlement location and subsequent juvenile survival. Larvae of both *Didemnum candidum* and the *Diplosoma* species were released during the daytime when light was available as a cue indicating shaded settlement sites, and settlement was photonegative in both the laboratory and the field. This behavior was clearly adaptive; juvenile survival was greater in preferred settlement sites. For *D. candidum*, settling in shaded locations led to enhanced survival of juveniles due to protection from direct sunlight. For both genera, settlement was abundant on the shaded undersides of opaque surfaces, where chances of juvenile survival were high due to protection from sunlight and siltation.

Settlement of both *Didemnum candidum* and the *Diplosoma* species
was abundant near the water surface. However, a positive function of the observed differences in settlement with depth was not apparent, as juvenile survival was not greater at preferred depths.

This result raises some interesting questions about the adaptive value of patterns of larval behavior of colonial ascidians, and suggests some possible areas for future research. First, what causes larvae to settle near the water surface? Second, is settling near the water surface always maladaptive, or are there locations in which juvenile survival does not vary with depth?

The abundant settlement on the undersides of surfaces near the water surface may be a product of the swimming pattern exhibited by ascidian larvae. In the laboratory, *Didemnum candidum* larvae alternate between upward swimming and downward drifting (Chapter 4). Larvae may be less likely to settle when passively drifting as when actively swimming. *Didemnum candidum* larvae appear to settle more frequently when swimming upward than when sinking; settlement is higher on downward-facing than upward-facing surfaces, even in total darkness (Chapter 4). In addition, many *D. candidum* larvae settle very close to the water surface; settlement on vertical plates is highest within 1-2 cm of the water’s edge in the field, and within 2-3 mm in the laboratory (pers. obs.). This pattern may be produced by only settling when swimming upward.

However, laboratory observations may not accurately reflect larval behavior in the field. Young (1986) observed that larvae of the colonial ascidian *Ecteinascidia turbinata* actively swim both downward and upward in the field. *Didemnum candidum* larvae may also swim
downward in the field, which could affect the pattern of settlement. If D. candidum larvae only settle when actively swimming, and the amount of time spent swimming downward is greater in the field, then the proportion of larvae settling on upper surfaces will be higher in the field than in the laboratory. The effect of larval behavior on settlement location could be determined by comparing the swimming and settlement pattern of larvae of D. candidum in the laboratory and in the field.

Settling close to the surface on artificial substrata may be the result of patterns of larval behavior which evolved in a more natural habitat. The original habitat of Didemnum candidum may have been floating, rather than fixed, objects. Settlement intensity at the water's edge may therefore differ between floating and fixed objects.

For example, in the red mangrove Rhizophora mangle, common in Hawaii, growing prop roots initially float when they reach the water. Larvae settling anywhere on the root will thus continue to be immersed. However, once roots reach the substratum they become anchored and no longer float; larvae settling on the root near the water surface at high tide will then be exposed at low tide. A comparison of settlement and survival rates at the water's edge on floating and fixed roots would determine if larvae are able to distinguish between floating and anchored substrata, and if there are resulting consequences to fitness.

While the discriminatory abilities of larvae have frequently been documented in the laboratory, few direct studies exist of larval behavior in the field. The work described in this dissertation compares field and laboratory observations of larval behavior with
field measurements of variations in settlement and survival. This approach could also be used to identify the causes of settlement near the water surface of *Didemnum candidum*. This would provide valuable information about the influence of larval behavior on settlement location and hence fitness.
LITERATURE CITED


