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LARVAL BEHAVIOR, PREDATION AND EARLY POST-SETTLING MORTALITY AS DETERMINANTS OF SPATIAL DISTRIBUTION IN SUBTIDAL SOLITARY ASCIDIANS OF THE SAN JUAN ISLANDS, WASHINGTON

by

CRAIG M. YOUNG

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

ZOOLOGY

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled LARVAL BEHAVIOR, PREDATION, AND EARLY POST-SETTLING MORTALITY AS DETERMINANTS OF SPATIAL DISTRIBUTION IN SOLITARY ASCIDIANS OF THE SAN JUAN ISLANDS, WASHINGTON submitted by CRAIG MARDEN YOUNG in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

Supervisor

A N Spencer

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Date July 30, 1982

ABSTRACT

Factors controlling spatial distribution were studied for 13 species of solitary ascidians occurring in hard-bottom subtidal habitats of the San Juan Islands, Washington. Larval responses to physical and biological stimuli, predation, silt, competition with filamentous algae, defense mechanisms and refuges from predation were all considered.

The larvae of some species swam continuously until settlement, but most displayed a "hop and sink" swimming pattern, with amount of time spent resting or sinking being greatest just before settlement. All species responded to shadows by increasing activity.

Most larvae were sensitive to intensities of white light as low as 0.01 uE/m²/sec to 0.05 uE/m²/sec. In the field, *Pyura haustor* larvae were able to detect light at least to a depth of 50 m. None responded to monochromatic stimuli below 425 nm or above 625 nm, and the peak sensitivities of most species were in the green region of the spectrum (500–600 nm).

Settlement distributions with respect to light and surface angle varied substantially among species, and often correlated well with the distributions of adults in the field. Three species predominated in non-cryptic field habitats and the larvae of these were more or less indifferent to light. Larvae of other species all showed a preference for shaded regions, and the adults of these occurred mostly on vertical surfaces or in cryptic habitats. Although many individual larvae settled on downward-facing surfaces, such surfaces were preferred over upward-facing ones in only one species. Unlike compound ascidian larvae, none of the species investigated were photopositive, either at hatching or at settlement.

Tadpole larvae of ascidians almost universally display a shadow response of unknown function in which upward swimming is induced by a sudden decrease in light intensity. In a simple laboratory experiment with 8 species, tadpoles were offered choices of "optimal" and "marginal" habitats while being maintained under conditions of continuous light, continuous dark or alternating light and dark. Larvae induced to swim often by regular shadows were not distributed differently at settlement than those maintained under constant light regimes, and the length of larval life was not significantly different in the various treatments. It was concluded that the mechanism by which larvae

locate shaded habitats and overhangs is independent of the shadow response.

Field transplants with newly settled juvenile ascidians were used to test two hypotheses: 1) Photonegative behavior aids ascidian tadpoles in locating refuges from important sources of juvenile mortality, and 2) Reproduction in ascidians is timed to minimize mortality by seasonal factors. Silt [illegible] mortality on all six species tested. Algal overgrowth was also important [illegible] water, where it not only increased mortality but depressed growth rates during the first two weeks. Common subtidal snails, *Margarites pupillus*, scraped juveniles from the substratum with their radulas while grazing. It was concluded that the juvenile stage is a very vulnerable period of the life cycle, and that by seeking shaded sites, tadpoles are able to avoid several strong sources of mortality.

Juvenile *Pyura haustor* transplanted to the field in three seasons (October, January, June) showed strong seasonal differences in survival and growth. Although growth rates were highest in the June experiments, survival was much higher in January, which is nearer the normal spawning time of the species. In all seasons, shallow (2 m) transplants experienced very heavy mortality in both light and shade.

Most of the rocky sites were dominated by the stolidobranch ascidians, *Pyura haustor, Halocynthia igaboja, and Cnemidocarpa finmarkiensis*, though *Boltenia villosa* and *Styela gibbsii* also occurred at most sites. Besides occasional isolated individuals, phlebobranchs were mostly found on soft bottoms and/or floating docks, where some reached densities as high as 1350 per m².

Field transplants and laboratory feeding experiments demonstrated that the cymatiid gastropod *Fusitriton oregonensis* limits distribution of most phlebobranchs by eliminating them from areas where it is common. *F. oregonensis*, which prefers ascidians over any other prey tested, locates ascidians by random encounter, and feeds by drilling a hole through the tunic with the radula and jaws. Three of the species that co-occur with *F. oregonensis* have effective mechanisms for defending against snail attack. Two other species are able to survive in the rocky subtidal by living as epizooites on the invulnerable forms, and taking advantage of the defenses of their hosts. Chemical defense mechanisms (vanadium and acid) are totally ineffective against *F. oregonensis*. The defense mechanism of *Halocynthia igaboja* consists of long tunic spines with recurved spinelets. *Pyura haustor* strengthens its already leathery tunic by embedding sand grains in it. This species also has small spines, mostly located on the soft siphons. *Cnemidocarpa finmarkiensis* may defend itself behaviorally by flattening against the substratum.

In most species, small-scale distribution correlates well with larval behavior. Negative phototaxis at settlement interacts with substratum preferences to result in the observed field distributions.

Several species occur in discrete, single species aggregations. These aggregations are formed by gregarious settlement of the larvae of *Pyura haustor* and *Chelyosoma productum*, while they are thought to be a consequence of [illegible] life (due to brooding) in *Corella inflata* and high concentrations of larvae in *Ascidia callosa* occurring on floating docks. Larvae of the epizooic species, *Boltenia villosa* and *Styela gibbsii*, settle most readily on the tunic of the prospective hosts, and delay metamorphosis in the absence of these substrata.

Vulnerable species demonstrating no strong substratum preferences (e.g., *Ascidia callosa, Ascidia paratropa, Corella willmeriana*) are found mostly as solitary individuals in the field. It is hypothesized that they persist in the subtidal because of their unpredictable distribution in space, and that the lack of substratum specificity seen in their larvae is an important component of this strategy.

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In addition to those acknowledged in the individual chapters, I wish to thank the following: Dr. Ellie Prepas for help with statistics; Drs. John Holmes, Joe Nelson, John Pearse, John Spence and Andy Spencer for serving on my reading committee; Helen Amerongen and Dr. Johnny Buckland-Nicks for assistance with computer text formatting; Chuck Anderson, Dick DeStaffany, Ron Koss, Randy Mandryk and Ron Seward for help with equipment and techniques; Sandy Branscomb, George Braymore, Dr. Tom Schroeder and Steve Stricker for teaching me S.E.M.; Charlie Eaton and Dr. David Duggins for allowing me to conduct an intensive diving project at Friday Harbor; Drs. Dennis Willows and Eugene Kozloff for always finding me lab space, even during the summer "crunch"; and Tim Pennington for listening to offbeat ideas on the ecology of larvae.

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Finally, I would like to thank my wife, Robyn not only for enduring six years of graduate school, but also for keeping my eyes fixed securely on my goals. Besides providing encouragement and support, she has enumerated tadpoles, measured ascidians, labelled specimens, prepared drawings and graphs, tended boats, sorted dredge hauls, and waded in mud, always with one or two children in tow.

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I. INTRODUCTION

HISTORY AND JUSTIFICATION OF THE PROBLEM

In order to study any animal, it must first be collected, and this activity requires a knowledge of the conditions where the animal is likely to be found. Zoologists of all kinds are therefore concerned with animal distribution. However, the science of Ecology, defined by Andrewartha and Birch (1954) as "the study of distribution and abundance of organisms", claims the bulk of the task of unravelling processes underlying distributional patterns.

Studies of animal distribution in the marine environment have passed through at least three developmental periods, with scientists in each period emphasizing a different controlling mechanism. Early in the present century, much of the observed pattern was attributed to differential survival with respect to physical environmental conditions. Pelagic larvae were viewed primarily as agents of dispersal, and settlement was considered random. Coleman (1933) in an early study on factors producing intertidal zonation verbalized this point of view:

> "...spores of algae and larvae of barnacles are free in the sea, and are apparently away from any influence that could affect their subsequent zonation when settled between tide marks."
>
> "The assumption is made, then, that larvae and spores, given suitable substratum, settle in a manner entirely at random in respect to tide levels. The further assumption is made, that a barnacle, or an alga, once settled, never moves again."
>
> That the settling of a barnacle larva must in some cases be practically instantaneous, and final, and not a matter of 'deliberation' or 'choice', is shown by those individuals of *Cthamalus stellatus* which are found above a predicted level of Extreme High Water Springs. These barnacles can only have settled in rough weather, when the seas were raising the splash zone."
>
> "Zonation in the algae and barnacles, then, is the result of a hit-or-miss method. If a spore or larva settles within the environment which will suit it as an adult, it may survive. If it settles outside this range, it will sooner or later die without maturing."(p. 472)

Even as Coleman was penning these words, a number of workers had already accumulated preliminary data on the behavior of invertebrate larvae which would eventually provide the impetus for reversing the "random settlement hypothesis" (Cole and Knight-Jones, 1939; Day and Wilson, 1934; Nelson, 1924; Wilson, 1928). The activity of documenting settlement behavior gained in popularity during the 1950's and 1960's and has continued to increase ever since (see reviews by Crisp, 1976; Meadows and Campbell, 1972; Scheltema, 1974). Now, larvae in most major groups of benthic invertebrates are known to exhibit specific preferences during the process of site selection. A major objective of the early investigations was that of understanding how behavior helped control distribution. However, very few workers provided more than brief anecdotal descriptions of the patterns they sought to explain, and the interaction of behavior with other controlling factors was seldom considered. Now, as interest has shifted to ultrastructural studies of larval metamorphosis (e.g., Chia and Rice, 1978), the genetics of behavior in larvae (Doyle, 1974), and chemical analysis of the substances to which larvae respond (Hadfield, 1977; Morse et al., 1979), the contribution of larval behavior to distribution remains unclear for nearly all species. Indeed, Moore (1975) has recently resurrected the opinion that behavior is subordinate to currents and other factors in producing the patterns observed in the sea.

In the last two decades, while embryologists and invertebrate zoologists busily studied the movements of larvae in glass laboratory dishes, field ecology experienced a major shift in emphasis. Physical factors and the physiological tolerances of organisms, while always acknowledged, began to receive less attention as determinants of distribution than biological interactions such as predation and competition. Ecologists working primarily in the rocky intertidal zone (reviewed by Connell, 1972; Paine, 1977) and in the shallow subtidal of Britain (Kitching and Ebling, 1967) found that sessile animals were conveniently caged, transplanted, removed, and otherwise manipulated. The isolation of factors by controlled experiments (as opposed to correlation studies) has provided clear explanations for many patterns of distribution commonly seen on the shore. However larval behavior, being nearly impossible to study in the field (but see Young and Chia, 1982), has often been ignored in these studies, and random settlement, as proposed by Coleman (1934) is implicitly assumed in most.

The major purpose of the present study was to fill in some of the gaps between field and laboratory ecology by studying benthic animals during all phases of their life cycles. I chose subtidal subjects, since they are more representative of marine animals on the whole, and their distributions are not complicated by the vagaries of climate characteristic of terrestrial (and hence, intertidal) systems. Ascidians were studied for several reasons. First, preliminary observations indicated that although the 13 subtidal species in the San Juan Islands were similar in mode of reproduction and larval development, their spatial patterns were not all the same. Second, the larvae of ascidians are obtained abundantly and reliably by simple laboratory techniques. Third, the time between fertilization and settlement is short, so large numbers of larvae can be successfully reared through metamorphosis. Fourth, ascidians have seldom been the subjects of detailed ecological studies, yet are among the most common of epibenthic organisms.

Since it was clear from the outset that I could not study all potential determinants of distribution, I chose to emphasize several which, following preliminary observations, seemed to be among the most important. These included larval photoresponse, larval substratum selection, predation on the adults and juveniles, siltation, defense mechanisms, and competition with benthic algae. Tidal currents in the San Juan Islands are very strong because channels between Islands are often narrow and tidal exchange is large. The effect of currents in dispersing larva from dense population centers and in concentrating larvae at specific sites also could be extremely important. Unfortunately, this factor was too complex to deal with in the time available.

This thesis is written in the form of four papers, each dealing with one or more factors which could influence ascidian spatial distribution. The first (chapter 2) contains a brief review of tadpole photoresponse and a comparative study of larval responses to light and other physical factors. It includes the first analysis of wavelength and intensity thresholds in ascidian larvae as well as a study of photoresponses at settlement in 12 species. The second paper reports a simple experiment which refutes the concept that the tadpole shadow response functions in site selection. In the third paper, selective pressures on juvenile ascidians are analysed in controlled field experiments. Hypotheses are proposed and tested on the adaptive values of the photoresponses documented in chapter 2, as well as seasonal reproduction. The last chapter is the largest. In it, I consider predation and larval substratum selection as interactive determinants of distribution, and also discuss strategies (specifically defense mechanisms and the location of refuges) by which ascidians persist in the subtidal habitats where they occur. Quantitative data on distributional patterns of all but one solitary ascidian occurring in the San Juan Islands are divided between chapters 2 and 5. Each paper is written in the format required by the journal to which it will be or has been submitted.

In surveying the recent literature and speaking with marine ecologists, I have become convinced that a resurgence of interest in larval ecology is beginning. It is hoped that the present attempt to integrate life history studies with field investigations of pattern and process will contribute to this movement in some small measure.

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II. LARVAL RESPONSES TO PHYSICAL FACTORS

A Comparative Study of Spatial Distribution and Larval Responses to Light and other Physical Factors in 12 Species of Subtidal Solitary Ascidians from the San Juan Islands, Washington

ABSTRACT

Settlement and pre-settlement responses to light, gravity and abiotic surface features (e.g., cracks) were studied in the tadpole larvae of 12 solitary ascidians, which represent all but three species occurring in the San Juan Islands of Washington State. Some species swam continuously until settlement, but most displayed a "hop and sink" swimming pattern, with the amount of time spent resting or sinking being greatest just before settlement. All species responded to shadows by increasing their level of activity.

Most larvae were sensitive to intensities of white light as low as 0.01 uE/m²/sec to 0.05 uE/m²/sec. In the field, larvae of *Pyura haustor* were able to detect light at least to a depth of 50 m. None responded to monochromatic stimuli below 425 nm or above 625 nm, and the peak sensitivities of most species were in the green region of the spectrum (500–600 nm).

Settlement distributions with respect to light and surface angle varied substantially among species, and often correlated well with the distributions of adults in the field. three species predominated in non-cryptic field habitats and the larvae of these were more or less indifferent to light. Tadpoles of other species showed a preference for shaded regions the adults of these occurred mostly on vertical surfaces or in cryptic habitats. Although many individual larvae settled on downward-facing surfaces, such surfaces were not preferred over upward-facing ones except in one species. Unlike compound ascidian larvae, none of the species investigated were photopositive, either at hatching or at settlement.

INTRODUCTION

The ascidian tadpole, a lecithotrophic larval form which may remain in the plankton for as little as a few minutes or as much as several days, is thought to have evolved primarily for site selection and secondarily for dispersal (Millar, 1966; Berrill, 1950). Many workers, however, consider tadpoles virtually non-discriminating in their choice of substratum, and suggest that tadpole behavior may be generalized in terms of a simple repertoire of photoresponses not unlike those exhibited by the larvae of other subtidal invertebrates (Thorson, 1964). Thus, tadpoles are generally thought to be photopositive at the time of hatching or release from the parent colony, and photonegative just before settlement (Berrill, 1975). In addition, most tadpoles display a shadow response in which they increase their activity, generally by swimming upward, when light intensity decreases abruptly.

The established notion that tadpole photoresponse is virtually identical for all species (Berrill, 1979) is based primarily on work with compound ascidians (Grave, 1920, 1936; Berrill, 1948, 1950; Grave and McCash, 1923; Grave and Woodbridge, 1924; Mast, 1921; Crisp and Ghobashy, 1971; Abbott, 1955) and the cosmopolitan solitary species, *Ciona intestinalis* (Berrill, 1947; Castle, 1896; Dybern, 1963; Yamaguchi, 1970). Besides *C. intestinalis*, the only solitary species that have been studied are *Molgula citrina*, tadpoles of which have abbreviated larval life and no photoreceptors (Grave, 1926), *Styela partita* and *Styela montereyensis*, tadpoles of which have reduced photoreceptors (Grave, 1944; Young and Braithwaite, 1980b), and *Chelyosoma productum*, whose tadpoles display much more variable behavior than the larvae of compound ascidians (Young and Braithwaite, 1980a). The behavior reported most consistently in the literature is the shadow response, which occurs in all species investigated to date with the exception of *Metandrocarpa taylori* (Abbott, 1955) and the blind *Molgula citrina* (Grave, 1926).

The present work documents larval responses to physical factors, particularly light, in solitary ascidians and relates these responses to patterns of distribution observed in the field. Previous workers have used a variety of qualitative and quantitative methods for documenting tadpole photoresponse, making it difficult to separate interspecific differences in behavior from the effects of different experimental regimes.

I have tried to avoid this problem by investigating visual thresholds, wavelength sensitivities and settlement distributions under standardized conditions for a number of different species. Complete data, including both pre-settlement and settlement responses are presented for 8 species, two in each of four families: Corellidae and Ascidiidae in the order Phlebobranchia, and Styelidae and Pyuridae in the order Stolidobranchia. Settlement behavior is documented for four additional species. Behavioral data for two of these latter species, *Chelyosoma productum* and *Styela montereyensis* have been reported in less complete form elsewhere (Young and Braithwaite, 1980a, 1980b). Of the 15 solitary ascidians known to occur in Puget Sound and the San Juan Islands, only the behavior of three rare species, *Pyura mirabilis, Halocynthia aurantium* and *Molgula pugettensis,* is not considered. However, preliminary distributional data are included for two of these.

MATERIALS AND METHODS

COLLECTING SITES AND FIELD SURVEYS

Adult ascidians were collected by SCUBA or dredging at numerous rocky subtidal sites in the San Juan Islands, Washington. A few species uncommon on rocky substrata were taken from floating docks in the town of Friday Harbor or dredged from muddy bottoms at Hankin Point, Shaw Island or the "Potato Patch" between Blakely and Shaw Islands. Animals were maintained in running seawater aquaria prior to use, which was usually within two weeks of collection.

Distributional patterns of ascidians were characterized during dives at a number of rocky subtidal sites. This involved tabulating on slates the number of individuals in three specific habitats (cobble and boulders, cliffs, rock reefs and outcroppings) occurring on various organic and inorganic substrata, at various depths, on surfaces positioned at different angles relative to the horizontal, and in holes, cracks, etc. Substratum composition data from these surveys will be presented elsewhere, together with an account of tadpole substratum preferences (chapter 5); only animals living on rock are included in the tables presented herein. For abundant species, surveys were limited by following transect lines along isobaths or by recording all individuals encountered during one or a few dives. Data for several rare species were compiled from field notes on all animals encountered between 1978 and 1982.

Most of the main study sites were topographically heterogeneous, with more than one habitat type being found within swimming distance. However, surveys were always limited to a single type of habitat on any given dive. The sites where formal surveys were conducted are classified as follows. Cobble and boulders: Shady Cove, San Juan Island (14-19 m); Reuben Tarte Beach, San Juan Island (9-21 m); Elbow Point, Saanich Inlet, B.C. (15-37 m); Limekiln Light, San Juan Island (6-24 m). Rock reefs and outcroppings: Eagle Point, San Juan Island (1-6 m); Cantilever Point, San Juan Island (15-27 m); Pole Pass, Crane Island (3-12 m); Griffin Bay Reef (6-15 m); Bell Island (9 m). Cliffs: Pt. George, Shaw Island (18-24 m); Cantilever Pt., San Juan Island (18-24 m); Shady Cove, San Juan Island (3-14 m); Turn Island (5-15 m); Long Island (28 m).

Two dives were made at Saanich Inlet, B.C. in the research submersible, Pisces IV (Fisheries and Oceans, Canada). On both dives, one to a depth of 200 m at Elbow Point and one to 203 m at McCurdy Point, the submersible ascended cliffs, enabling me to note whether the distributional patterns of ascidians varied at different depths (light levels).

LARVAL CULTURE

During the four years of this study, all but two of the species studied spawned in the laboratory on at least one occasion. I was able to induce spawning of *Chelyosoma productum, Corella willmeriana* and *Corella inflata* seasonally by exposing them to light after 12 h of dark adaptation (Lambert et al., 1981; Young and Braithwaite, 1980a).

Although naturally spawned gametes were used for behavioral experiments whenever possible, at times I obtained gametes by dissection. *Ascidia callosa* collected in the spring, and *Ascidia paratropa* collected in the spring or summer often had gonoducts full of mature eggs and sperm. These were easily obtained by removing the tunic and puncturing the gonoducts with a pipette. After placing the gametes in a bowl of seawater, the water was decanted off and changed repeatedly to remove excess sperm. In these species, nearly all of the eggs developed into normal tadpoles.

Gametes of the stolidobranch species, *Pyura haustor, Styela gibbsii, Cnemidocarpa finmarkiensis, Halocynthia igaboja, Styela montereyensis, Styela coriacea* and *Boltenia villosa* were obtained directly from the ovotestis. The animal was sliced along the mid sagittal plane with a razor blade and each half was removed from the tunic. The gonad was exposed by carefully peeling away the branchial basket and other tissues, removed with a pair of forceps and placed with the gonad of at least one other individual on a piece of 253 or 295 um nitex mesh, resting in a bowl of seawater. It was then pressed through the nitex with the blunt end of the forceps (as suggested by R.A. Cloney). This procedure separated the eggs from one another and also resulted in a very thick concentration of sperm and mucus which had to be removed in order to obtain healthy tadpoles. Immediately after fertilization, most of the liquid in the culture was removed by siphoning it through a filter of 100 um nitex submerged in the dish. Fresh seawater was added and the process was repeated until the water was relatively clear. After this, the eggs were allowed to settle and the water was changed repeatedly during the first 4-5 hours of development. In all, as many as 10 washings were sometimes done. The washing procedure did not seem to harm the embryos in any way. Indeed, tadpoles with abnormal tails and other deformities were seen only in cultures which were not carefully washed.

Cultures were maintained in small pyrex dishes of millipore filtered seawater during the spring plankton bloom and in unfiltered water during the remainder of the year. Culture vessels and all experimental chambers were cooled to ambient seawater temperature (7–14 degrees C, depending on the time of year) by resting them in a shallow aquarium of running seawater. The containers were removed only for brief periods in order to count or observe the animals.

VISUAL THRESHOLD EXPERIMENTS

The shadow response was used as an indicator of sensitivity in experiments with monochromatic light of different wavelengths and white light of different intensities. Intensity thresholds were determined as follows. About 100 to 200 tadpoles, dark-adpated for at least 10 min, were pipetted into a petri dish of filtered seawater. The dish was placed on a white surface under a dissecting microscope. Light from a Bausch and Lomb projecting laboratory lamp, fitted with a general electric "CPR" incandescent bulb was reflected on the specimens from above by means of a mirror.

Cultures were maintained in small pyrex dishes of filtered seawater Intensity was measured with a quantum meter (Lambda Instrument Co., model 185). during the spring plankton bloom and in unfiltered water during the remainder Intensity levels below the sensitivity of the meter (0.1 uE/m^2/sec) were obtained by imposing calibrated neutral density filters in various combinations. The rheostat on the projector was always used at the same setting in order to avoid changing the spectral characteristics of the light. Beginning at the highest intensity level to be tested, I focussed on an individual tadpole (or its shadow, which was often more visible against the white background) resting on the bottom of the dish. I then interrupted the light beam for about 0.5 s with a black card, and noted whether or not the animal moved. Each larva was given two chances to respond. If it did not move after the second trial, I recorded no response. This procedure was repeated with each of 20 larvae at each intensity level. After testing larvae at the lowest intensity (below the ascidian visual threshold) I repeated the experiment once again at an intermediate intensity to assure that the absence of response was not due to tadpole fatigue or adaptation.

The same setup as above was used for testing wavelength sensitivity, except that light of discrete wavelengths was obtained by inserting a diffraction grating monochromator (Bausch and Lomb, model 33-86-02; 9.6 nm bandpass, 350-800 nm) in front of the light source. Sensitivity was tested at a single intensity (1.0 uE/m^2/sec) between 700 and 425 nm, and at the highest intensity obtainable from 425 to 350 nm, which was beyond the range of wavelengths detectable by the light meter. Since it was difficult to see the tadpoles by low intensity light at some wavelengths, we used a laser (Spectra Physics, model 155) as a supplementary light source. The laser produced a concentrated beam at 632.8 nm, which was near or beyond the upper visual threshold of all tadpoles tested, and to which the larvae did not respond. I aimed the laser at the base of the plastic petri dish from a distance of about 1.0 m. The curvature of the dish caused the beam to diffuse somewhat, silouetting the tadpoles. Tests were made at 25 nm increments from 350 to 700 nm, using 20 tadpoles at each wavelength. Intensity was not varied in these experiments because of time and material constraints.

SETTLEMENT EXPERIMENTS

Tadpole swimming activity was observed with a Wild M-5 dissecting microscope. The basic apparatus for settling experiments, as described previously (Young and Braithwaite, 1980a), consisted of 16 ml capacity polystyrene petri dishes (Millipore) with tight-fitting lids. Each dish had a small hole drilled through the top near one side, so that it could be filled completely with seawater and tadpoles by means of a pipette. Black electricians tape covered all sides and half of the top. Dishes rested on a black slate in a shallow seawater table 25 cm below a 100 watt incandescent light bulb in a reflector. The lamp continuously illuminated the dishes at a minimum intensity of 50 uE/m^2/sec; light levels were higher than this during the day, primarily because of sunlight entering the laboratory through large south-facing windows (Young and Chia, 1982b). Larvae were left in the dishes, undisturbed, until most had settled, after which the dishes were opened, and the number settling in the various regions were counted under a dissecting microscope. The regions available to larvae included the light and dark sides of the dish bottom and the underside of the lid. Larvae were also free to select between the "edges" (where top or bottom meets the side) and the "central regions" of the dish. All species were subjected to exactly the same treatment except for *Halocynthia igaboja*, whose tadpoles would not settle on the smooth plastic of the dish. For this species, I sanded the entire inside surface of each dish with fine sandpaper. This rendered the "clear" side of the lid transluscent.

To see if the responses of tadpoles would be similar in the laboratory and under field regimes of light, pressure and temperature, a series of settling experiments was conducted with with larvae of *Pyura haustor* in the field. The usual half-shaded dishes of larvae were held to the top sides of bricks with rubber bands. Small corks plugged the pipette holes so the larvae could not swim out. The bricks were placed on the bottom near the Cantilever Pier of Friday Harbor Laboratories at 5, 15, and 35 m by diving. An additional treatment was lowered to 50 m with a rope. All experiments were recovered and scored after 1 wk.

RESULTS

FIELD DISTRIBUTIONS

In rocky subtidal habitats, most ascidians live on the bottoms of rocks, the sides of rocks, or in holes and cracks (Table I). Two species, *Pyura mirabilis* and *Ascidia callosa* were only found on the undersides of cobble in the subtidal (*A. callosa* occurred more commonly on floating docks), and another uncommon species, *Styela coriacea* was only found in cracks or on the bottoms of cobbles. *Cnemidocarpa finmarkiensis*, and *Pyura haustor* were among the most abundant animals surveyed. Both lived primarily on vertical faces of rock outcroppings and cliffs, where they generally occupied depressions, cracks or the undersides of ledges. Two species were found more commonly on other ascidians than on rock: *Boltenia villosa* and *Styela gibbsii* (Young, 1980). Where they did occur on rock, it was genrally in the same areas as *Pyura haustor*, though free-living *B. villosa* were more common on open rock faces than in cracks.

The distribution of *Corella inflata* was very patchy, and all individuals found were on cliff faces or under large overhangs. All 6 individuals of the congeneric species, *C. willmeriana*, were found as isolated individuals on cliffs. By contrast, the third corellid considered, *Chelyosoma productum*, occupied exposed rather than cryptic sites and only 2 of 62 individuals were attached to rock cliffs, though some did occur on cliffs attached to other species (chapter 5).

The largest ascidians were most commonly found in the open. *Halocynthia aurantium* were on the open sides of large boulders or outcroppings and *Ascidia paratropa* lived on cliffs, boulders and reefs. *Halocynthia igaboja*, though often associated with small cracks on cliffs, was almost never overhung by ledges, and in most cases was effectively exposed. Large body size may inhibit all three of these species from inhabiting the undersides of rocks. In contrast to these subtidal distributions, both *Halocynthia aurantium* and *Ascidia paratropa* were occasionally found on the bottom of the floating breakwater at Friday Harbor Labs, though they were never seen on the sides of the same breakwater.

Styela montereyensis, characteristically an open coast form, seldom occurs in the San Juan Islands. Its distribution (primarily on upward facing surfaces or in surge

Table I.

Field distributions of 13 solitary ascidian species, tabulated by habitat type, surface angle and surface exposure. "Upper" and "Lower" refer to horizontal or slightly sloping surfaces. "Open" indicates a smooth substratum and "Crack" indicates a hole, crack or other surface irregularity.

Species and Habitat Type	Vertical Surfaces		Upper Surfaces		Lower Surfaces
	open	crack	open	crack	
Corella inflata					
cobble; boulders	0	0	0	0	0
reefs	0	0	0	0	0
cliffs	19	0	0	0	12
(TOTALS)	19	0	0	0	12
Corella willmeriana					
cobble, boulders	0	0	0	0	0
reefs	0	0	0	0	0
cliffs	4	1	1	0	0
(TOTALS)	4	1	1	0	0
Chelyosoma productum					
cobble, boulders	0	0	0	0	0
reefs	31	1	23	5	0
cliffs	0	0	2	0	0
(TOTALS)	31	1	25	5	0
Ascidia callosa					
cobble, boulders	0	0	0	0	4
reefs	0	0	0	0	0
cliffs	0	0	0	0	0
(TOTALS)	0	0	0	0	4

(continued...)

Table I. (continued)

Species and Habitat Type	Vertical Surfaces		Upper Surfaces		Lower Surfaces
	open	crack	open	crack	
Ascidia paratropa					
cobble, boulders	0	0	2	0	0
reefs	0	0	1	0	0
cliffs	2	0	2	0	0
(TOTALS)	2	0	5	0	0
Styela gibbsii					
cobble, boulders	0	0	0	0	0
reefs	0	6	0	0	4
cliffs	4	0	0	0	0
(TOTALS)	4	6	0	0	4
Styela coriacea					
cobble, boulders	0	0	0	3	4
reefs	0	0	0	0	0
cliffs	0	0	0	0	0
(TOTALS)	0	0	0	3	4
Cnemidocarpa finmarkiensis					
cobble, boulders	0	0	0	0	1
reefs	13	48	1	9	32
cliffs	3	4	0	0	1
(TOTALS)	16	52	1	9	34
Pyura haustor					
cobble, boulders	31	0	9	0	9
reefs	0	142	0	76	36
cliffs	31	81	0	0	3
(TOTALS)	62	223	9	76	48

(continued...)

Table I. (continued)

Species and Habitat Type	Vertical Surfaces open	Vertical Surfaces cracks	Upper Surfaces open	Upper Surfaces cracks	Lower Surfaces
Pyura mirabilis					
cobble, boulders	0	0	0	0	6
reefs	0	0	0	0	0
cliffs	0	0	0	0	0
(TOTALS)	0	0	0	0	6
Boltenia villosa					
cobble, boulders	3	0	0	0	0
reefs	10	3	2	1	4
cliffs	3	0	0	0	2
(TOTALS)	16	3	2	1	6
Halocynthia igaboja					
cobble, boulders	8	0	3	0	1
reefs	2	3	0	0	2
cliffs	31	81	0	0	3
(TOTALS)	41	84	3	0	6
Halocynthia aurantium					
cobble, boulders	2	0	0	0	0
reefs	2	0	1	0	0
cliffs	0	0	0	0	0
(TOTALS)	4	0	1	0	0

channels) has been discussed elsewhere (Young and Braithwaite, 1980b).

The species composition of ascidians in Saanich Inlet is different from that seen in the San Juan Islands. Therefore, submersible observations were made mostly on animals which I did not study in the laboratory. *Pyura haustor, Ciona intestinalis, Bathypera sp.* and *Ascidia ceratodes* all occurred down to the anoxic zone of Saanich Inlet at about 90 m (V. Tunnicliffe, personal communication). Of these, *Bathypera* was commonly found on open cliff faces, while the others were mostly limited to holes and the undersides of ledges.

PRE-SETTLEMENT BEHAVIOR: GENERAL OBSERVATIONS

At the time of hatching, larvae displayed several different activity patterns. All species developed a shadow response within the first 2 hours, and most showed a marked tendency to swim upward immediately after hatching. In *Boltenia villosa, Pyura haustor, Styela montereyensis* and *Cnemidocarpa finmarkiensis*, swimming generally occurred in short bursts which often carried the larvae to the surface, and which were generally followed by resting periods in which the larvae sank passively, head downward, through the water column to either rest on the bottom or begin swimming once again. *Corella inflata, Ascidia callosa, Ascidia paratropa, Chelyosoma productum* and *Styela gibbsii* often swam continuously during the first few hours. *A. paratropa* demonstrated no apparent photoresponse during this period, but the other four species all accumulated near the dark sides of the bowl. By 24 hours, *Styela gibbsii* and *Chelyosoma productum* tadpoles spent most of their time resting on the dish bottom, though *S. gibbsii* in some cultures continued to swim against the dark side at the surface for several days. *Corella inflata* sometimes swam continuously until settlement, which usually occurred within 24 hours. Surprisingly, *Ascidia callosa* and *A. paratropa,* which delay metamorphosis for a much longer time, may also swim for the duration of their larval life. Several cultures of *A. callosa* were watched carefully for a period of 10 days. Metamorphosis was discouraged in the majority of these animals by maintaining them in very clean dishes of millipore filtered seawater. Although no observations were made at night, most larvae swam continuously during daylight hours. For the first 24 hours, they mostly congregated against the darker side of the dish. When mixed up with a glass rod, they reaggregated in the dark within one hour. Following the first day, the larvae continued to swim up, but their photonegative response was more feeble. Stirred cultures often took several hours to reaggregated against the dark side of the dish. Nevertheless, in undisturbed cultures, the majority of animals avoided the light until day 10, after which they rested on the bottom more frequently. The congener of *Ascidia callosa, A. paratropa,* did not demonstrate such strong negative phototaxis. Though most larvae eventually settled on shaded substrata, the early swimming behavior was characterized by upward swimming without apparent regard for light.

I did not observe a photopositive period in the life of any tadpole species, including *Corella inflata,* for which such a phase has been reported (Lambert and Lambert, 1978).

Ascidian tadpoles rotate as they swim, and seldom navigate an entirely straight path. Animals swimming horizontally in culture dishes do not turn around when contacting the sides. Instead, they generally continue swimming, following the side of the dish to the surface. Consequently, the region near the air-water interface is often crowded with larvae, both before and at settlement.

PRE-SETTLEMENT BEHAVIOR: VISUAL THRESHOLDS

Tadpoles of most species were able to detect and respond to white light intensities as low as 0.02 uE/m²/sec (Fig. 1). Only *Ascidia callosa* was clearly sensitive to intensities below this level. *Styela gibbsii,* which has a reduced photoreceptor like other styelids, had the highest lower threshold, at about 0.05 uE/m²/sec. *C. finmarkiensis,* a styelid whose photolith is similar to that of *S. gibbsii,* however, showed about 40% sensitivity down to 0.02 uE/m²/sec. The response level of both pyurids declined gradually from high to low values, in contrast to those of the phlebobranchs, all of which dropped abruptly below a certain point near the middle of values tested. The differences in the shapes of the curves are partly attributed to the general activity level of the larvae. For example, even under high intensities the larvae of *Pyura haustor* do not respond to every shadow that crosses them. Consequently, the sensitivity curve for *P. haustor* appears more nearly linear than, for instance, that of *Corella inflata,* a species that responds more consistently. Both *Ascidia callosa* and *Corella willmeriana* had curves

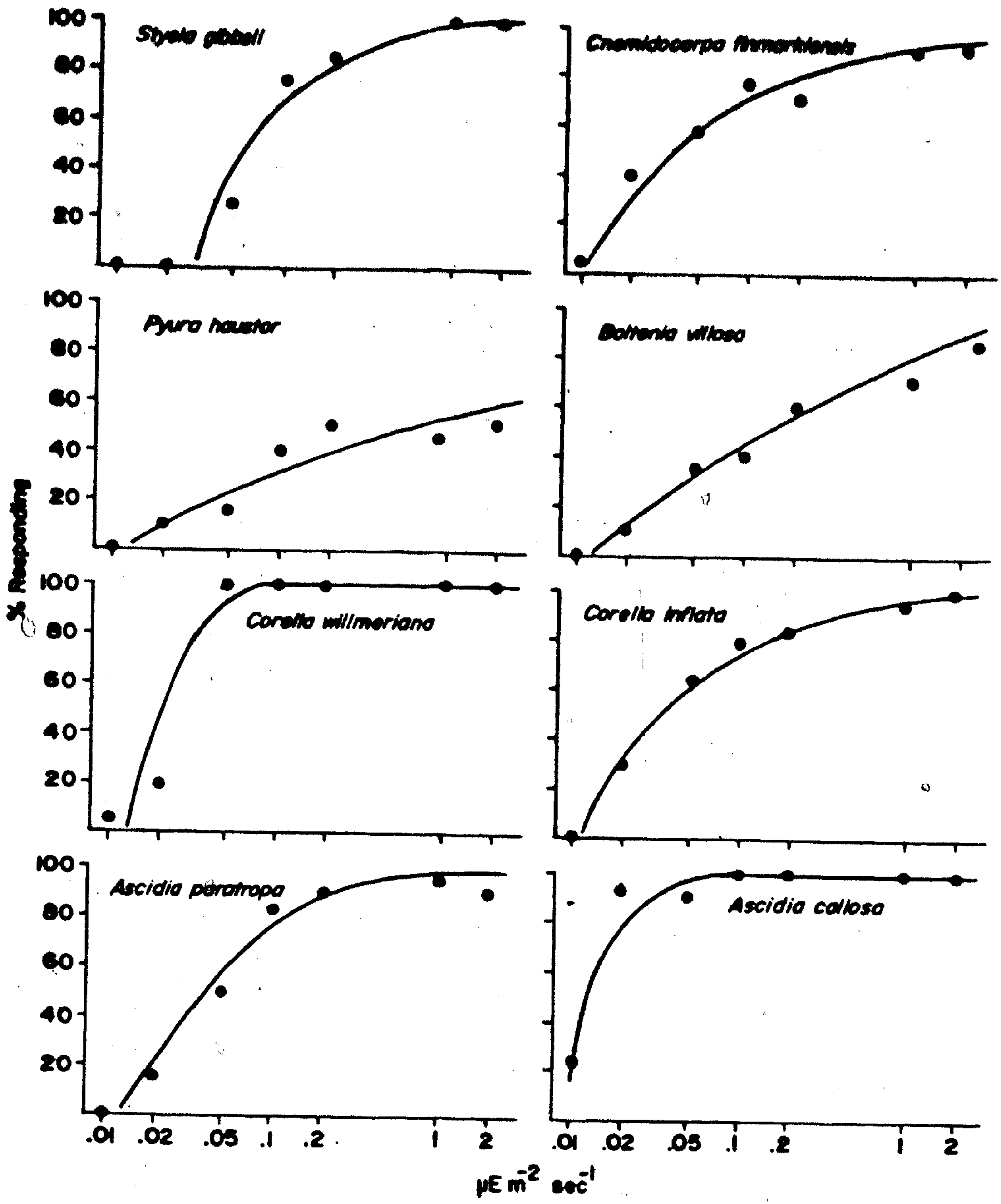

Figure 1. Percentage of tadpoles exhibiting the shadow response at different intensities of white light: eight species of solitary ascidians. Each point represents 20 trials. Note that the horizontal axis is logarithmic, and that graphs are arranged by family (each family is in one row).

with quite distinct shoulders at the lower end. In these species, 100% response was seen down to 0.05 uE/m²/sec; below this, the sensitivity dropped abruptly. The curves of both *A. paratropa* and *C. inflata* declined somewhat more gradually.

Action spectra for the photokinetic response are shown in Fig. 2. The visual spectra of all species tested fell well within the range of the human visual spectrum (400 to 700 nm), though the curves varied somewhat among species. The widest ranges of sensitivity were exhibited by three of the phlebobranch species. In *Corella inflata*, only wavelengths below 425 nm and above 625 nm were not detected, while in *C. willmeriana*, sensitivity dropped abruptly below 450 nm and declined gradually at the upper end, from 575 nm to 650 nm. *Ascidia callosa* was sensitive to virtually the same range as *C. willmeriana*, though *A. paratropa* did not respond to wavelengths longer than 575 nm. The remaining species all had upper thresholds at 600 or 625 nm. *Styela gibbsii* and *Boltenia villosa* both displayed high lower thresholds, the former at 475 nm and the latter at 500 nm. Sensitivity of all species peaked between 500 and 600 nm, with the distribution skewed toward the high end of the spectrum in *B. villosa*, and shifted toward the lower end in the remaining species.

SETTLEMENT BEHAVIOR: LABORATORY EXPERIMENTS

The settlement distributions of larvae in half-shaded, continuously illuminated petri dishes are plotted in Figure 3 and analyzed in Tables II, III, IV, and V, each of which includes species in a single family. The analysis of each species distribution consists of a crossed three factor ANOVA on log-transformed data. Despite the fact that data are in the form of proportions, log-transformation rather than arcsine transformation (Sokal and Rohlf, 1969) was employed, because it rendered the data more homoscedastic. The analyses should be treated with some caution, since the assumption of equal variances, as tested with the F-max statistic (Sokal and Rohlf, 1969), was met for only one species, *Corella willmeriana*. The most serious consequence of violating this assumption in ANOVA is the increased probability of Type I error (Snedecor and Cochran, 1967). Wishing to be conservative in rejecting my null hypotheses, I therefore used the critical values for $p<0.01$ rather than $p<0.05$ in determining significance.

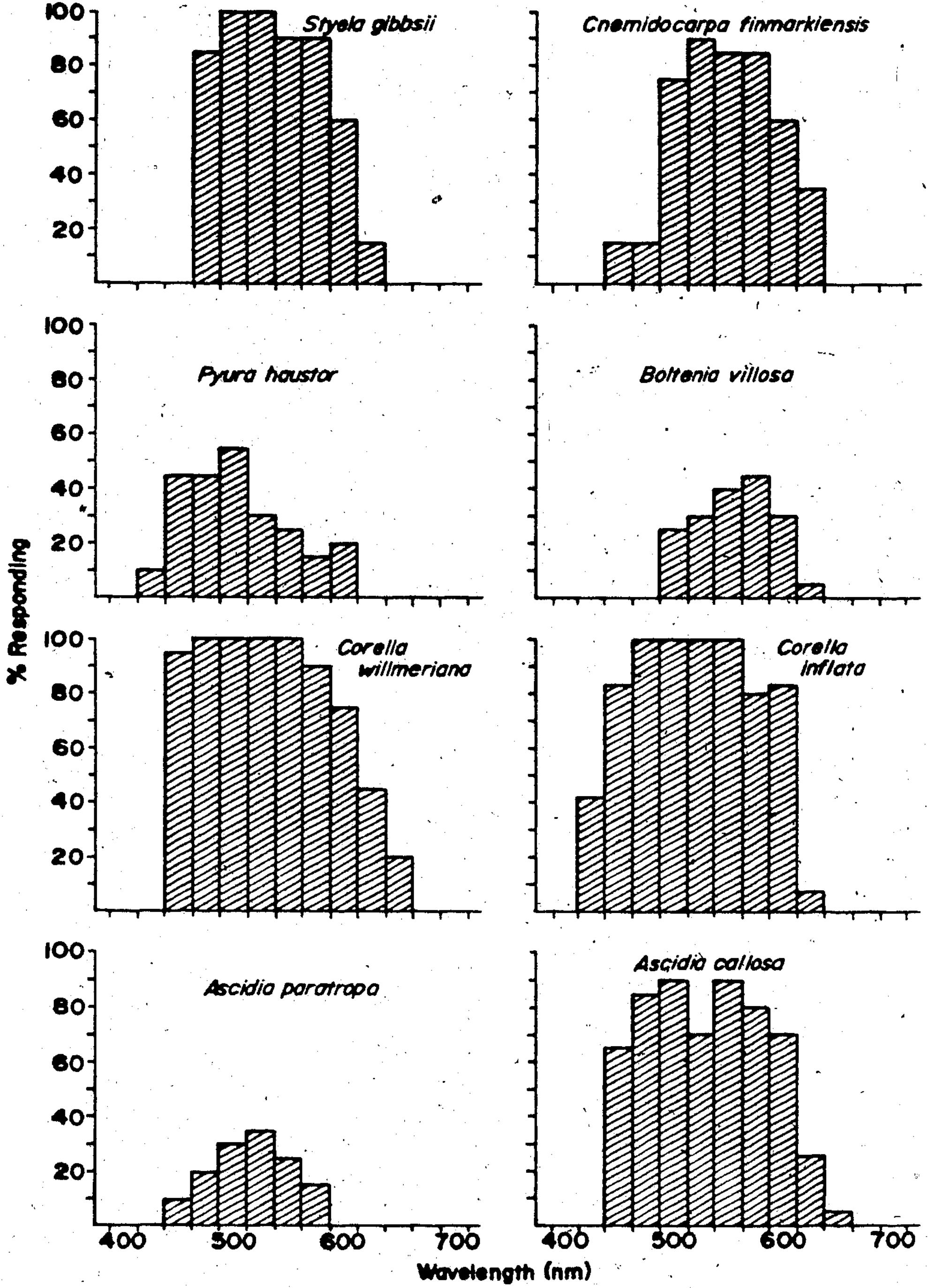

Figure 2. Percentage of solitary ascidian tadpoles (eight species) exhibiting the shadow response when illuminated with various wavelengths of light at an intensity of 1.0 uE/m²/sec.

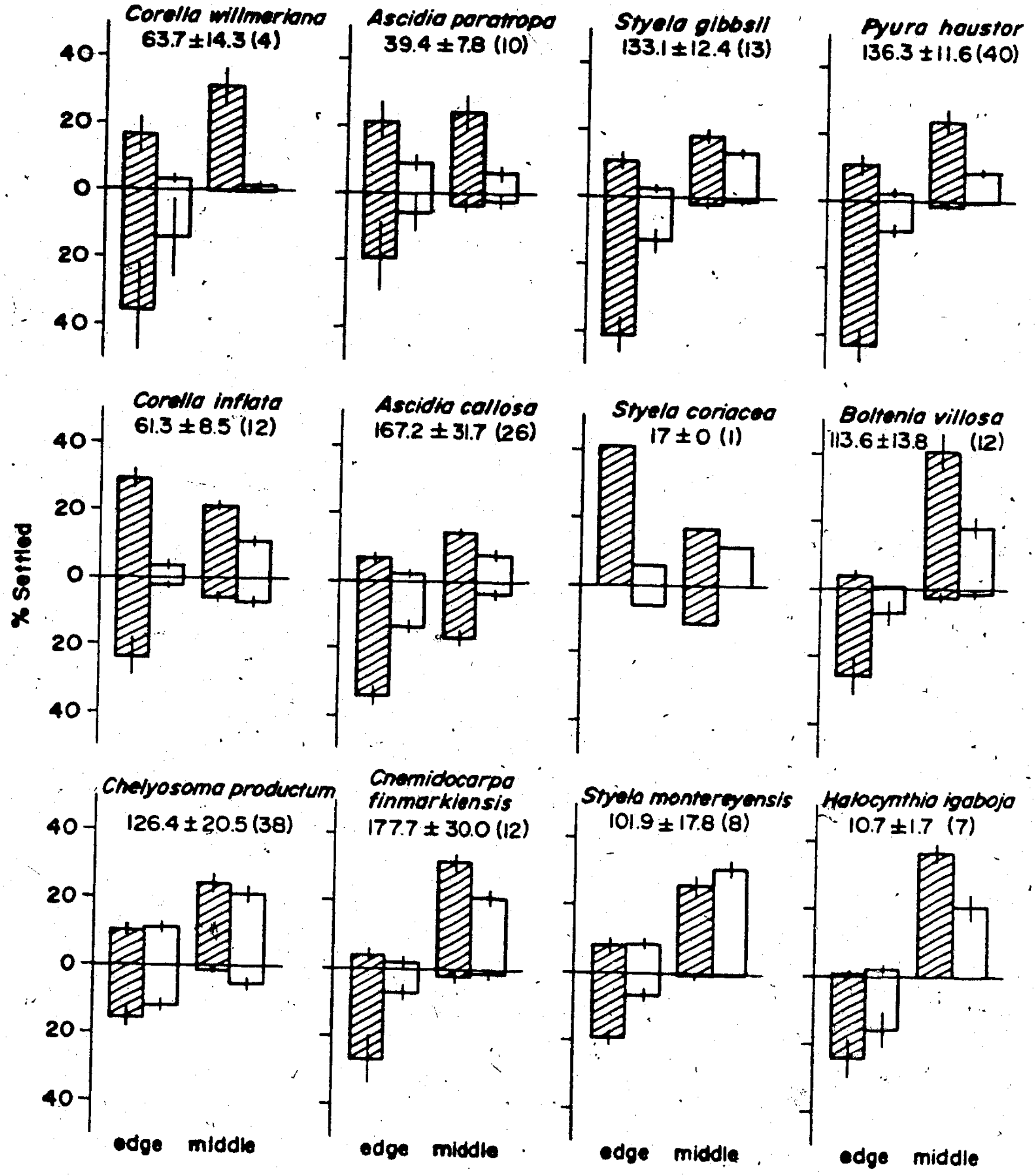

Figure 3. Settling distributions of solitary ascidian larvae (12 species) in filled, covered, half-shaded dishes of seawater. Each histogram bar shows mean percentage plus or minus 1 S.E. Bars below the horizontal axis show proportion of larvae settling on downward facing surfaces (underside of the lid), while bars above the axis represent larvae settling on the bottom (upward-facing surface) of the dish. Cross-hatched bars represent proportions of larvae settled in the shade. Settlement on the edge of the dish is represented by the left set of bars in each graph; settlement in the central region of the dish is represented by the right set of bars. Numbers below species names are average number of larvae per dish, plus or minus 1 S.D., with number of replicate dishes in parentheses.

Table II.

Analysis of ascidian settlement distributions, Family Corellidae. Three factor crossed analyses of variance on Log(y+1) transformed data. Each factor has two levels (see text). Interaction terms abbreviated using the first letters of the main effects.

Source of	SS	df	MS	F	P
Corella inflata					
Lighting	0.05255	1	0.05255	67.26	0.0000
Surface	0.01727	1	0.01727	22.10	0.0000
Position	0.00077	1	0.00077	0.99	0.3220
LS	0.00897	1	0.00897	11.48	0.0011
LP	0.02183	1	0.02183	27.74	0.0000
SP	0.00216	1	0.00216	2.76	0.1002
LSP	0.00067	1	0.00067	0.86	0.3569
Error	0.06875	88	0.00078	-	-
Corella willmeriana					
Lighting	0.03592	1	0.03592	76.83	0.0000
Surface	0.00020	1	0.00020	0.42	0.5219
Position	0.00468	1	0.00468	10.01	0.0042
LS	0.00013	1	0.00013	0.28	0.5987
LP	0.00824	1	0.00824	17.62	0.0003
SP	0.02401	1	0.02401	51.36	0.0000
LSP	0.01538	1	0.01538	32.90	0.0000
Error	0.01122	24	0.00047	-	-
Chelyosoma productum					
Lighting	0.00010	1	0.00010	0.05	0.8278
Surface	0.07101	1	0.07101	33.12	0.0000
Position	0.00101	1	0.00101	0.47	0.4931
LS	0.00011	1	0.00011	0.05	0.8187
LP	0.00041	1	0.00041	0.19	0.6640
SP	0.13311	1	0.13311	62.09	0.0000
LSP	0.00513	1	0.00513	2.39	0.1230
Error	0.63460	296	0.00214	-	-

Table III.

Analysis of ascidian settlement distributions, Family Ascidiidae. Three factor crossed analyses of variance on Log (y+1) transformed data. Interaction terms abbreviated with first letters of main effects.

Source of Variation	SS	df	MS	F	P
<u>Ascidia callosa</u>					
Lighting	0.09048	1	0.09048	60.17	0.0000
Surface	0.05079	1	0.05079	33.78	0.0000
Position	0.00642	1	0.00642	4.27	0.0402
LS	0.01919	1	0.01919	12.76	0.0004
LP	0.00076	1	0.00076	0.50	0.4786
SP	0.06637	1	0.06637	44.14	0.0000
LSP	0.00216	1	0.00216	1.44	0.2317
Error	0.30076	200	0.00150	-	-
<u>Ascidia paratropa</u>					
Lighting	0.01994	1	0.01994	10.21	0.0019
Surface	0.00981	1	0.00981	5.02	0.0276
Position	0.00593	1	0.00593	3.04	0.0849
LS	0.00132	1	0.00132	0.68	0.4128
LP	0.00249	1	0.00249	1.27	0.2620
SP	0.11998	1	0.11998	61.43	0.0000
LSP	0.01457	1	0.01457	7.46	0.0076
Error	0.17188	88	0.00195	-	-

Table IV.

Analysis of ascidian settlement distributions, Family Styelidae. Three factor crossed analyses of variance on Log (y+1) transformed data. Interaction terms abbreviated with first letters of main effects.

Source of Variation	SS	df	MS	F	P
Styela gibbsii					
Lighting	0.03571	1	0.03571	35.70	0.0000
Surface	0.00102	1	0.00102	1.02	0.3153
Position	0.01985	1	0.01985	19.85	0.0000
LS	0.00436	1	0.00436	4.36	0.0394
LP	0.01501	1	0.01501	15.00	0.0002
SP	0.10462	1	0.10462	104.61	0.0000
LSP	0.00976	1	0.00976	9.76	0.0024
Error	0.09601	96	0.00100	-	-
Styela montereyensis					
Lighting	0.00104	1	0.00104	2.10	0.1531
Surface	0.03191	1	0.03191	64.46	0.0000
Position	0.00334	1	0.00334	6.74	0.0120
LS	0.00452	1	0.00452	9.12	0.0038
LP	0.00353	1	0.00353	7.14	0.0099
SP	0.05939	1	0.05939	119.99	0.0000
LSP	0.00124	1	0.00124	2.51	0.1187
Error	0.02772	56	0.00049	-	-
Cnemidocarpa finmarkiensis					
Lighting	0.02001	1	0.02001	10.24	0.0019
Surface	0.00986	1	0.00986	5.05	0.0272
Position	0.00597	1	0.00597	3.06	0.0839
LS	0.00134	1	0.00134	0.69	0.4096
LP	0.00251	1	0.00251	1.29	0.2596
SP	0.11981	1	0.11981	61.33	0.0000
LSP	0.01463	1	0.01463	7.49	0.0075
Error	0.17190	88	0.00195	-	-

Table V.

Analysis of ascidian settlement distributions, Family Pyuridae. Three factor crossed analyses of variance on Log (y+1) transformed data. Interaction terms abbreviated with first letters of main effects.

Source of Variation	SS	df	MS	F	P
<u>Pyura haustor</u>					
Lighting	0.18722	1	0.18722	74.22	0.0000
Surface	0.00060	1	0.00060	0.24	0.6263
Position	0.04887	1	0.04887	19.38	0.0000
LS	0.00655	1	0.00655	2.60	0.1082
LP	0.04506	1	0.04506	17.86	0.0000
SP	0.28545	1	0.28545	113.17	0.0000
LSP	0.07494	1	0.07494	29.71	0.0000
Error	0.78698	312	0.00252	-	-
<u>Boltenia villosa</u>					
Lighting	0.03886	1	0.03886	23.47	0.0000
Surface	0.01351	1	0.01351	8.16	0.0053
Position	0.01286	1	0.01286	7.77	0.0065
LS	0.00071	1	0.00071	0.43	0.5138
LP	0.00000	1	0.00000	0.00	0.9948
SP	0.13366	1	0.13366	80.74	0.0000
LSP	0.02112	1	0.02112	12.76	0.0006
Error	0.14569	88	0.00166	-	-
<u>Halocynthia igaboja</u>					
Lighting	0.00392	1	0.00392	2.67	0.1088
Surface	0.00690	1	0.00690	4.71	0.0350
Position	0.00317	1	0.00317	2.16	0.1484
LS	0.00070	1	0.00070	0.48	0.4936
LP	0.00176	1	0.00176	1.20	0.2792
SP	0.10272	1	0.10272	70.03	0.0000
LSP	0.00610	1	0.00610	4.16	0.0470
Error	0.07041	48	0.00147	-	-

Only one substratum type (polystyrene plastic) was available to the larvae, so settlement distributions in experimental dishes are considered to result from interactions among three simple factors, each with two levels. The factors were named, "lighting", "surface" and "position". Thus larvae were able to choose between the light and dark ("lighting"), between the top and bottom ("surface"), and between edge and the central region of the dish ("position"). With respect to this last factor, it should be noted that because of the way the dishes were manufactured, a slight crack is sometimes present at the top edge, and the sides and top meet at an abrupt 90 degree angle, whereas the bottom curves slightly to meet the side with no break. Animals settling on the sides of the dish were disregarded because they were few in number and difficult to count. Also, addition of an extra level in the "surface" factor would have increased the complexity of the analyses without yielding much extra information.

There was a highly significant preference for dark over light in all but 3 species: *Chelyosoma productum, Styela montereyensis* and *Halocynthia igaboja.* In these species, the responses were variable among replicate runs and often within a given experiment. Thus, in some runs, nearly all individuals settled on the dark side, while in other runs, most individuals selected predominantly the light side of the dish or were dispersed at random with respect to light. Even in strongly photonegative species, there were no instances when 100% of the larvae in a dish selected the dark over the light.

Corella willmeriana was the most strongly photonegative of all species tested (Fig. 4). All but the three species mentioned above showed between 60 and 80% photonegativity. The percentage of larvae selecting the dark correlated with neither taxonomic position nor photoreceptor structure. For example, in the Styelidae, which characteristically have a compound sensory structure (photolith) rather than a separate ocellus (Grave, 1941; Torrence, 1980), one species (*S. montereyensis*) is indifferent to light, while the other two species (*S. gibbsii, C. finmarkiensis*) show fairly strong responses which fall within the same range as those of animals having a distinct ocellus. The most photonegative species and least photonegative species are both members of the family Corellidae. Likewise, in the Pyuridae, two species (*Pyura haustor* and *Boltenia villosa*) are among the most strongly photonegative while *Halocynthia igaboja* is nearly indifferent to light.

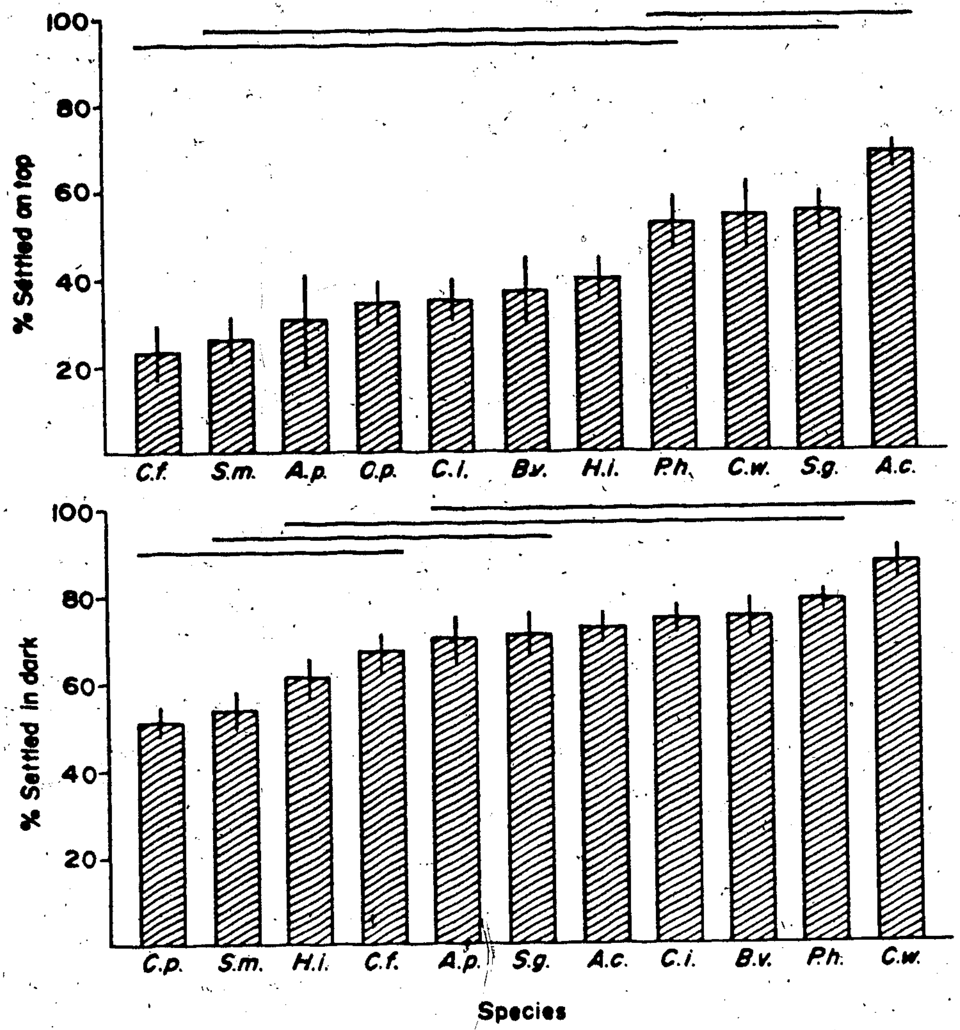

Figure 4. Mean percentage plus or minus 1 S.E. of ascidian larvae (13 species) settled on the top surface of the dish (underside of the lid), and the dark side of the dish, ranked from lowest to highest. Species names are abbreviated with first letter of genus and species name (see Figure 3 for species). Horizontal lines over the bars group non-significant differences ($p<0.05$), as determined by the Student-Newman-Keuls test.

Only *Ascidia callosa* showed a significant preference for the top (downward facing) surface of the dish, though relatively large numbers of all species but *Styela coriacea* did settle on the top. A significant preference for the bottom over the top of the dish was seen in *Corella inflata, Styela montereyensis, Chelyosoma productum* and *Boltenia villosa*. *Cnemidocarpa finmarkiensis, Ascidia paratropa* and *Halocynthia igaboja* settled in larger numbers on the bottom surface, but the preference was nonsignificant. Interspecific differences in the number settling on downward-facing surfaces are summarized in Figure 4. It is noteworthy that the species which swim continuously are among those settling in largest numbers on the top surface.

Two species, *Pyura haustor* and *Styela gibbsii* showed a strong preference for edges of the dishes. The opposite response was exhibited by *Boltenia villosa* and *Corella willmeriana*.

The interaction between surface and position, which is highly significant in every species with the exception of *Corella inflata*, is the most consistent phenomenon appearing in the analyses. The settling patterns (Fig. 3) suggest that the interaction is because most larvae selecting the upper surface (top) also tend to cluster around the edge. Larvae seldom settle in the mid-regions unless on the bottom. The tendency for larvae to follow vertical surfaces upward may explain this strong interaction. *Ascidia callosa* and *Corella inflata* were the only species which settled in relatively large numbers in the top mid-regions. Once again, it seems reasonable to invoke their habit of continuous swimming as an explanation for this. Continuous activity would be expected to result in relatively more encounters with the top surface (and therefore greater probability of attachment there) than the intermittent swimming exhibited by most other species.

The interaction between light and surface angle was significant in *Ascidia callosa, Styela gibbsii, Styela montereyensis* and *Corella inflata*. Inspection of the graphs suggest that the most likely reason for this interaction is that larvae in the dark are more apt to settle on the top than are larvae in the light.

The light/position interaction was significant in 5 species, *Corella inflata, Corella willmeriana, Pyura haustor, Styela gibbsii* and *Styela montereyensis*. In these species, larvae in the dark show a greater tendency to settle at the edge (generally the top edge) than larvae in the light.

SETTLEMENT BEHAVIOR: FIELD EXPERIMENT

P. haustor tadpoles placed in the field at 5, 15, 35 and 50 m showed ability to discriminate between light and dark at all depths (Fig. 5) . The difference among treatments, which included the pooled laboratory settlement data (Fig. 4), was non–significant (p=0.0936) by one–way ANOVA despite a clear trend toward decreased discrimination with depth (Fig. 5). Greater variability was seen in the mean percentage settled on the top surface at various depths; no clear trend was apparent and the among–treatment comparison was significant (p=0.0216). These data suggest that larvae respond to the artificial lighting conditions of the lab in much the same way as they respond under natural conditions of light intensity, wavelength, photoperiod, pressure and temperature in the field.

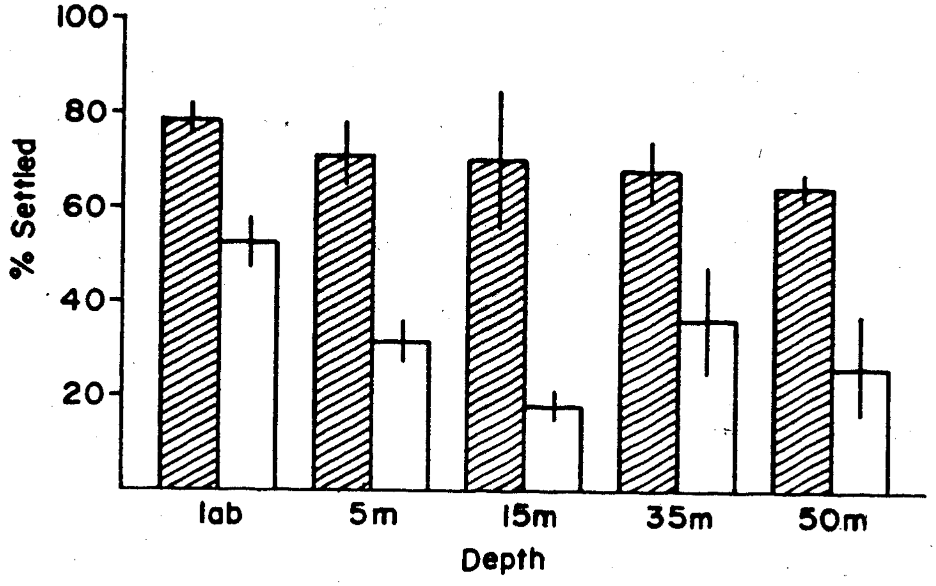

Figure 5. Mean percentage plus or minus 1 S.E. of *Pyura haustor* larvae settled in dark (shaded bars) and on top (open bars) in half-shaded dishes placed in the lab and at four depths in the field.

DISCUSSION AND CONCLUSIONS

GENERAL PATTERNS OF BEHAVIOR

Ascidian eggs are surrounded with follicle cells which presumably help keep them suspended prior to hatching, and which in some cases may cause the egg to float (Lambert and Lambert, 1978). It is likely, therefore, that most of the eggs remain suspended in the water column during the embryonic period. From hatching on, there are numerous small differences in behavior between species. Nevertheless, I would expect "typical" tadpole larvae to display the following movements in the field. Shortly after hatching, the tadpoles of most species swim actively upward. Larvae are not attracted to light at this stage; the response appears to be a negative geotaxis. Some of the larvae, notably those of *Ascidia callosa* and *Corella inflata* swim continually until settlement, so they may actually reach the surface. Most of the other species do not swim for long periods of time. Their behavior can be characterized as a "hop and sink" pattern in which brief upward excursions are followed by head-down sinking through the water column. For these species, it is questionable whether or not the larvae would congregate in the surface layers as the photopositive larvae of other species do (Thorson, 1964). As the larval period progresses, the periods of rest (and sinking) become increasingly longer and more frequent. This resting may be the mechanism by which larvae reach the bottom, as strong negative phototaxis is seldom observed even in the late stage larvae. As the tadpoles drift in the water or sit on the bottom, shadows passing overhead induce them to swim actively for a few seconds, generally upward.

Larvae encountering surfaces tend to follow along them, with their heads in constant contact, rather than turn away. This may be an important part of their search behavior; larvae in dishes seemed to locate the crack at the dark top edge (a preferred settlement spot) largely by this means. Similar behavior has been reported for the polystyelids, *Metandrocarpa taylori* by Abbott (1955) and *Botryllus schlosseri* by Woodbridge (1924) as well as for *Molgula citrina* by Grave (1926).

In the species that swim more or less continuously, the location of dark habitats at settlement is apparently mediated by a phototactic response. These larvae definitely show a negative, directional response to light. The larvae of most other species also prefer to settle in the dark, but the nature of the response remains unclear. It seems possible that the behavior is a type of photokinesis (Fraenkel and Gunn, 1940). Thus, larvae may move more actively under unsuitable light regimes and slow their activity down (or search more locally for a settlement site) when in the dark. Mast (1921), working with compound ascidians of the genus *Amaroucium*, discovered that the photic orientation of those tadpoles depended on the asymmetrical placement of the ocellus. In photopositive larvae, light stimulating the ocellus caused the tail to bend to the abocular side, while in photonegative larvae, the reverse was true. As larvae swim through the water, they spiral, causing an alternate shading and illumination of the ocellus. In this way, they swim toward or away from the light. The relative importance of phototaxis and photokinesis has been investigated in detail for *Diplosoma listerianum* by Crisp and Ghobashy (1971). In this species, larvae demonstrate a clear phototaxis, which constitutes the main photoresponse where there is directional light. Nevertheless, when maintained in a gradient of diffuse horizontal illumination, larvae tended to settle in the region with an intensity of 300 lx (not in the dark, surprisingly), indicating that some form of kinetic response also operates. Further st[illegible]re still needed to clarify the nature of the photoresponse for solitary ascidians.

Larvae of most species settled in relatively small numbers on downward-facing surfaces. Although concrete evidence is lacking, I suspect that many of the larvae settling on the bottom (upward facing) surfaces did so after delaying metamorphosis for a long period of time without locating a chemically or texturally suitable substratum (chapter 5; Young and Braithwaite, 1980a). Given choice of optimal substratum facing down or facing up, I expect that many more larvae would select the downward facing position.

The shadow response, which occurs predictably and immediately each time a tadpole is shaded was a convenient means of assessing sensitivity to various lighting conditions. Tadpoles of all species were able to sense light intensities down to at least 0.05 uE/m²/sec, and in one species, *Ascidia callosa*, over 20% of the tadpoles responded to the lowest intensity tested, 0.01 uE/m²/sec. In the field, larvae are unlikely to encounter white light except in the surface waters. As would be predicted, larvae of all species tested are most sensitive to wavelengths in the blue and green regions of the spectrum. Grave (1936) reported that larvae of several compound ascidian species responded equally to all wavelengths in the human visible spectrum, though he did not provide details on the filters used to obtain these data. Likewise, Mast (1921) commented that larvae of *Amaroucium* responded very well to light from a "ruby lamp" passing through a double filter of ruby red glass. None of the species I tested were sensitive to wavelengths below 425 nm (blue) or above 650 nm (red-orange), so it seems likely that both Mast (1921) and Grave (1936) were making their observations very near the upper end of the ascidian visible spectrum. Several of the species I tested had upper thresholds in the yellow (580-600 nm) region of the spectrum. Although the region of peak sensitivity in ascidian larvae corresponds with that of most other invertebrate larvae investigated to date (reviewed by Young and Chia, in press), many larvae, including those of decapod crustaceans (Forward and Cronin, 1979) and serpulid polychaetes (Young and Chia, 1982a) show greater sensitivity in the ultraviolet and near ultraviolet. Assuming larvae can detect green light down to the same intensities they can detect white light, they should be able to discriminate light and dark or respond to shadows to depths greater than 50 m in the San Juan Islands (Utterbach and Boyle, 1933). This prediction is supported by the field experiment in which *Pyura haustor* larvae were able to choose the dark sides of experimental dishes at a depth of 50 m and also by submersible observations of ascidians occupying primarily the undersides of ledges at even greater depths.

BEHAVIORAL DIFFERENCES BETWEEN SOLITARY AND COMPOUND ASCIDIANS

Prior studies have distinguished compound and solitary ascidian larvae on the basis of size, structure and length of pelagic life (Berrill, 1950; Millar, 1972); the present study suggests that they also differ in certain aspects of their behavior. Compound ascidians generally retain their embryos internally, either in the gonoduct or in a common cloacal chamber, until the larva is fully developed and ready to settle (Berrill, 1979). Consequently, the larvae do not undergo a period of embryogenesis in the plankton. They must disperse, locate a habitat, and settle all within a few minutes to a few hours. Not surprisingly, therefore, the behavioral patterns of compound ascidian larvae change rapidly and predictably during the free-swimming period. The behavior of all solitary and compound ascidians studied to date is summarized in Table VI. Compound ascidians,

Table VI

Summary of asciaian tadpole photoresponses investigated to date. Family classification is according to Monniot and Monniot (1972). Symbols are as follows. C: compound ascidian. S: solitary ascidian. X: response present. 0: response absent. +: response positive. -: response negative. V: response variable. ?: not reported.

Family	Species	C/S	shadow response	Phototaxis		Reference
				early	late	
Polyclinidae	*Amaroucium pellucidum*	C	X	+	-	Grave, 1920; Mast, 1921
	Amaroucium constellatum	C	X	+	-	Grave, 1936; Mast, 1921
Didemnidae	*Diplosoma listerianum*	C	X	+	V	Crisp & Ghobashy, 1971
	Trididemnun Solidum	C	?	+	-	Van Duyl et al., 1981
Polycitoridae	*Distaplia sp.*	C	?	0	0	Berrill, 1948
Cionidae	*Ciona intestinalis*	S	?	+	-	Berrill, 1947; Castle, 1896; Dybern, 1963
Perophoridae	*Perophora viridis*	C	?	+	V	Grave & McCash, 1923
Corellidae	*Corella inflata*	S	X	0	-	this study
	Corella willmeriana	S	X	0	-	this study
	Chelyosoma productum	S	X	0	V	this study; Young & Braithwaite, 1980a
Ascidiidae	*Ascidia nigra*	S	X	+	-	Goodbody, 1963; Grave, 1936
	Ascidia callosa	S	X	0	-	this study
	Ascidia paratropa	S	X	0	-	this study

(continued)

Table VI (Continued)

Family	Species	C/S	Shadow response	Phototaxis early	Phototaxis late	Reference
Styelidae	*Dendrodoa grossularia*	S	?	0	0	Berrill, 1950
	Styela partita	S	X	0	-	Grave, 1941; 1944
	Botryllus schlosseri	C	X	+	-	Grave & Woodbridge, 1924; Woodbridge, 1924
	Symplegma viride	C	X	+	-	Grave, 1936
	Polyandrocarpa tincta	C	X	+	-	Grave, 1936
	Polyandrocarpa gravei	C	X	+	-	Grave, 1936
	Styela montereyensis	S	X	0	V	this study; Young & Braithwaite, 1980b
	Styela coriacea	S	X	0	-	this study
	Styela gibbsii	S	X	0	-	this study
	Cnemidocarpa finmarkiensis	S	X	0	-	this study
Pyuridae	*Pyura haustor*	S	X	0	-	this study
	Boltenia villosa	S	X	0	-	this study
	Halocynthia igaboja	S	X	0	V	this study
Molgulidae	*Molgula citrina*	S	0	0	0	Grave, 1926

unlike solitary ones, nearly always have a photopositive period immediately upon release. They swim actively toward the surface of the water for at least a few minutes. This is also characteristic of bryozoans, hydrozoans, and other animals with short-lived larvae (Thorson, 1964; Young and Chia, in press). Its presumed function is to achieve maximum dispersal away from the adults. Although photopositive periods have been reported for *Ciona intestinalis* (Berrill, 1947), and *Ascidia nigra* (Grave, 1936), both solitary ascidians, such a response does not occur in *Styela partita* (Grave, 1941) or any of the solitary ascidians I investigated in this study. Larvae of solitary ascidians were never observed swarming against the light side of a culture vessel, as is commonly seen in truly photopositive larvae such as barnacle nauplii and compound ascidians. Lambert and Lambert (1978) reported that *Corella inflata* larvae, which hatch in the expanded atrial chamber of the parent, swarm against the upper wall of the atrium at hatching. This behavior was attributed to positive phototaxis. I observed similar swarming many times during the course of the present study. In my opinion, however, *C. inflata* larvae are not attracted to light; as in other solitary ascidians, the behavior seems to be a negative geotaxis.

Assuming that positive phototaxis at hatching functions in maximizing dispersal (Thorson, 1964), it seems reasonable that the response would be lacking in the solitary forms, since most solitary ascidians release small eggs which develop in the plankton and thus disperse for up to 3 days before the tadpole emerges. That some species such as *Ascidia callosa* become photonegative very shortly after hatching supports the idea that tadpoles of solitary species function primarily in site selection rather than dispersal. This assumption would also help explain why many tadpoles are relatively inactive. Unless a species must settle in shallow water, continuous swimming may not be necessary for site selection. Indeed, larvae seeking settlement sites would do well to remain near or on the bottom, swimming only enough to explore locally or to resuspend themselves in the near-bottom currents.

Solitary ascidian tadpoles are stimulated to activity by a sudden decrease in light intensity, as are those of most compound ascidians which have been studied (Table VI). The reverse response (increase in activity when light intensity is increased) was not observed in this study. Such behavior has been reported for several compound ascidians

(Abbott, 1955; Grave, 1936). Where this behavior is seen, the time lag between stimulus and response is from 3 to 10 seconds (Grave, 1936). In the normal shadow response, the latency period is less than a second.

Besides the observation that solitary species tend not to have a photopositive stage, the main distinction between solitary and compound ascidians is a quantitative one: solitary ascidians tend to display more variability in their behavior than has been reported for most compound ascidians. This variability is manifest at species, population and sibling levels. Thus, while in compound ascidians, all or nearly all of the tadpoles will actively seek out and settle in shaded habitats, solitary forms do not display such rigidity in their behavior. In no instance did all larvae choose the dark in a given experiment. Furthermore, the percentage which did demonstrate this behavior often varied widely between offspring of adults collected from different areas or at different times of year. In *Diplosoma listerianum*, the basic pattern of behavior may change with light intensity or at certain levels of other environmental factors including temperature and salinity (Crisp and Ghobashy, 1971). It remains to be seen how much variability in solitary species can be explained by such synergistic effects, and also whether the effects can induce greater behavioral variability in compound ascidian larvae.

THE ROLE OF BEHAVIOR IN CONTROLLING SPATIAL PATTERN

Thorson (1964) popularized the hypothesis that photoresponse is a means by which larvae control their vertical distribution in the water column, and that ultimately, the level at which a larva swims will determine the depth at which it settles. In *Ciona intestinalis*, light is thought to control vertical zonation in land-locked fjords where other physical factors such as current do not overshadow its effect (Gulliksen, 1972; Dybern, 1963). By contrast, Castric-Fey et al. (1978) found little evidence for vertical zonation of ascidians in the subtidal zone of the Glenan Archipelago in Brittany. They attributed observed patterns of distribution largely to the presence or absence of sediment. Very little evidence in the present study supports the supposition that vertical distribution is controlled by larval photoresponse in the San Juan Archipelago. *Ascidia paratropa* and *Halocynthia igaboja*, as characteristically deep ascidians, would be expected to have extremely strong negative phototaxis to keep them in the deeper water strata. This was not the case. Indeed, larvae of ascidians characteristically found on docks (*Corella inflata, Ascidia callosa, Pyura haustor*) were among the most strongly photonegative. Furthermore, the least photonegative species, *Chelyosoma productum*, was found in this same shallow habitat, suggesting that something other than photoresponse is the cause. In the San Juan Islands, complex tidal currents and bottom irregularities cause the water masses to mix thoroughly, top to bottom, on nearly a daily basis (Thompson and Phifer, 1937). For this reason, the feeble swimming of tadpoles would not be expected to have much of an effect on the large scale distribution of the adult ascidians. It seems apparent that photoresponse mainly determines patterns of microdistribution. Activity level, on the other hand may help to determine vertical distribution. The species that swim most continuously are among those found most abundantly on floating docks. This could result from their more frequent presence in the surface waters.

On a small scale, most of the species studied demonstrated field distributions which might be predicted on the basis of tadpole behavior. For example, larvae of all but three species showed a significant negative photoresponse in the lab, and the adults of these species generally occupied the shaded habitats or vertical surfaces in the field. The three species which did not show a consistant avoidance of light in the lab were those which occurred in more open habitats in the field. *Chelyosoma productum*, which occurs only in a few field sites, often settled and survived on the open rock surfaces, facing upward or obliquely upward. *Halocynthia igaboja* occupied similar habitats on occasion, but more often occurred in open areas on the sides of cliffs and large boulders. In these same areas, the strongly photonegative *Pyura haustor* occupied primarily the undersides of ledges and the insides of cracks.

In Saanich Inlet, *Pyura haustor, Ascidia ceratodes, Ciona intestinalis, Chelyosoma columbianum* and *Bathypera sp.* were observed to a depth of 90 m. Even near the lower limits of their vertical distribution, the first 3 species occupied primarily the undersides of ledges. Three alternate hypotheses could explain the occurrence of ascidians in cryptic habitats at very low light intensities: 1) strong differential mortality leaves survivors only in habitats shaded from above (see chapter 4), 2)larvae are able discriminate the dark regions at these depths,, or 3) habitat selection is based on tactile (e.g., rugophyllic) behavior rather than photoresponses. Interestingly, in the same areas where 3 species are cryptic, 2 others, *Chelyosoma columbianum* and *Bathypera sp.* occur commonly on the open rock faces. If we assume that major sources of mortality (e.g., silt: chapter 4) are similar for all species, then this observation favors the second hypothesis.

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III. TADPOLE SHADOW RESPONSE

On the Function of the Ascidian Tadpole Shadow Response

ABSTRACT

Tadpole larvae of ascidians almost universally display a shadow response of unknown function in which incresed swimming activity is induced by a sudden decrease in light intensity. In a simple laboratory experiment with 8 species, tadpoles were offered choices of "optimal" and "marginal" habitats while being maintained under conditions of continuous light, continuous dark or alternating light and dark. Larvae induced to swim often by regular shadows were not distributed differently at settlement than those maintained under constant light regimes, and the length of larval life was not significantly different in the various treatments. It was concluded that the mechanism by which larvae locate shaded habitats and overhangs is independent of the shadow response.

INTRODUCTION

In many respects, the behavior of ascidian tadpole larvae is variable, unpredictable and erratic. However, the shadow response, where an abrupt decrease in light intensity elicits active swimming, occurs almost universally. Only two species investigated to date, *Molgula citrina*, which has no photoreceptor (Grave, 1926) and *Metandrocarpa taylori* (Abbott, 1955) are reported not to exhibit this response. Although such a widespread phenomenon clearly demands a functional explanation, the several hypotheses that have been advanced have not been properly tested experimentally.

The shadow response may be considered a form of photokinesis (*sensu* Fraenkel and Gunn, 1940), inasmuch as the level of activity, and not the direction of movement, changes in response to different levels of an environmental factor. Larvae that are already swimming actively do not exhibit the response, while inactive larvae resting on the bottom, drifting passively, or sinking in the water column do. The response develops with the formation of the larval ocellus and continues until the time of metamorphosis. However, several workers (Grave, 1936; Grave and Woodbridge, 1924; chapter 2) have reported that the larvae respond to shadows less frequently with advancing age. Larvae of most solitary species detect intensities as low as 0.1–0.5 uE/m²/sec (chapter 2).

Many sessile marine invertebrates, including serpulids, echinoids and cirripedes, display shadow responses which are almost always considered defensive in function (reviewed by Steven, 1963). Recently, Forward (1977) has documented a shadow response in a decapod crustacean larva which allows the larva to escape from ctenophores and other predators passing overhead. It seems unlikely that the ascidian response has the same function, since tadpoles would tend to swim toward rather than away from a predator casting a shadow. Mast (1921) and Abbott (1955) both noted that a larva swimming through the water in its characteristic spiral fashion regularly casts a shadow on its own ocellus. Mast (1921) suggested that this was a mechanism for orienting to light. In support of this idea, he noted that the tadpole of *S. gibbsii*, when trapped under a cover slip, would twitch its tail in different directions depending on the direction the ocellus was facing. Woodbridge (1924) proposed that the shadow response enables tadpoles of *Botryllus schlosseri* to locate eelgrass blades, which are suitable sites for attachment and growth. Larvae passing through the shadows cast by the blades would presumably be stimulated to swim upward, thus contacting the undersurfaces of the plant. Woodbridge supported her hypothesis by demonstrating experimentally that more tadpoles would settle on an eelgrass blade suspended diagonally across a culture dish than on the bottom or sides of the dish. Unfortunately, her experiment was not controlled for differences in substratum composition and texture, now known to be important cues used by tadpoles selecting habitats (Young and Braithwaite, 1980; chapter 4).

In the present study, I conducted a simple experiment with eight species of solitary ascidian tadpoles, designed to test the hypothesis that the shadow response helps larvae locate optimal habitats for settlement. The results do not strongly support the hypothesis.

MATERIALS AND METHODS

Collection sites of adults, methods for obtaining gametes, and larval rearing conditions have been described in detail elsewhere (chapter 2). The experimental containers, half-shaded and completely filled petri dishes with tight-fitting lids were also the same as those used in previous work (chapter 2; Young and Braithwaite, 1980). Larvae were able to choose between the dark side and the light side, and also between the top and bottom surfaces.

The Woodbridge (1924) hypothesis was tested by exposing some larvae to alternating light and shade, while maintaining control groups in continuous light or darkness. Alternating light was produced by a 12 cm diameter disk of plexiglas, half painted with mat black paint, and suspended horizontally just above the seawater table. A clock motor rotated the disk at one rpm, 30 cm under a 100 watt incandescent light bulb. Four experimental chambers of larvae placed under the disk were thus exposed to shadows on a regular basis. Control dishes were maintained under the appropriate light regime in adjacent regions of the seawater table. In the dark treatment, experimental dishes were enclosed in a bag of opaque black plastic, submerged in the table. At the conclusion of an experiment, the number settled in each region of the dish was counted, any unmetamorphosed larvae were noted and a small sample (usually 20) of the latter were tested to see if they still responded to shadows.

RESULTS

Field experiments reported elsewhere (chapter 4) suggested that juveniles of all species survive better in dark habitats than light habitats, and better on the undersides of overhangs than on upward-facing surfaces. I thus predicted on an *a priori* basis that if the shadow response helps larvae locate settlement sites, one or both of the following should hold true: 1) larvae in fluctuating light should locate the top surface, the dark side, or the top/dark surface more readily than larvae in continuous light and 2) after a given period of time, more larvae should have settled in fluctuating light than in continous light or darkness. Figure 6 presents distributional data which test the first hypothesis.

More tadpoles reached the top undersurface of the lid in fluctuating light than in the other treatments in *Styela gibbsii*, *Boltenia villosa* and *Corella inflata*, though the difference among treatments, as tested by the Kruskal Wallace statistic, was not significant in any of these cases. Conversely, more animals reached the top in complete darkness in *Ascidia callosa, Ascidia paratropa, Corella willmeriana*, and *Cnemidocarpa finmarkiensis*. The difference was significant only in *C. finmarkiensis*. The extremely low overall settlement on the undersurface of the lid in *Pyura haustor* probably resulted from an unusually inactive culture of larvae, since much greater settlement was seen in similar settling experiments conducted previously (chapter 2).

Larvae in fluctuating light did not locate the dark side of the dish with any greater frequency than larvae in continuous light. Similarly, the number of larvae selecting the top dark region of the dishes was not significantly different in the various treatments.

Table VII gives the percentage of larvae metamorphosed successfully at the end of each experiment. In one species only, (*Styela gibbsii*), a higher percentage of larvae settled in the light dishes than either the dark dishes or the shadow dishes, and in 5 of the 7 species tested, more larvae settled in alternating light than in continuous light. These differences, however, were not significant. Likewise, in the 4 species where settlement was higher in alternating light than in the dark, there were no significant differences.

I expected that larvae stimulated to swim frequently might ultimately become exhausted or unresponsive to shadows. This was not the case. In each species, between 70 and 100% of the unmetamorphosed larvae still responded to shadows at the conclusion of the experiment. The among treatment differences were all non-significant.

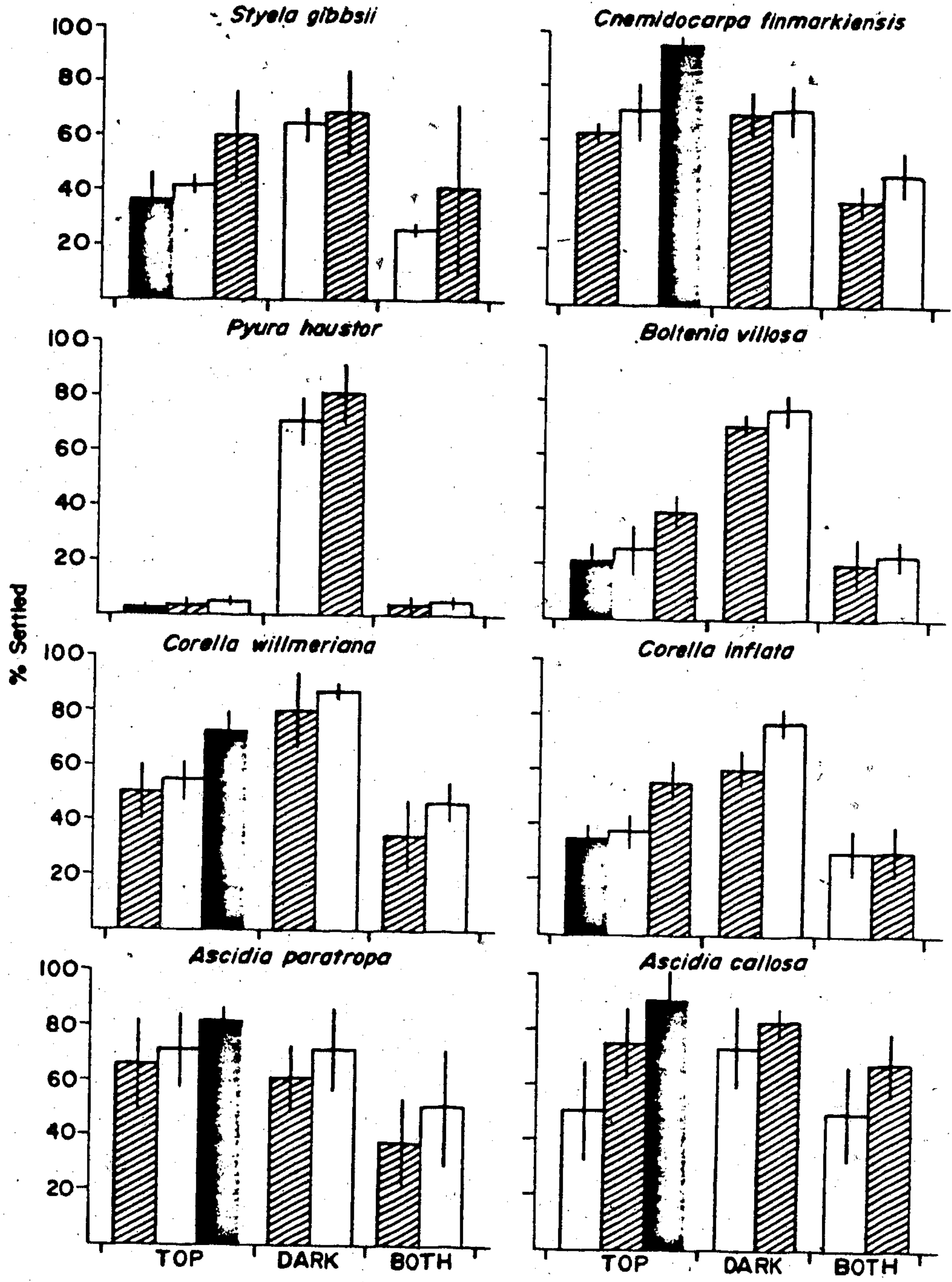

Figure 6. Settlement distributions of ascidian larvae (eight species) under conditions of continuous light (open histogram bars), continuous darkness (black bars) and alternating light in which dishes were shaded for half of each minute (shaded bars). Each bar represents the mean plus or minus 1 S.E. percentage of larvae settled in a particular region of the dish. The left group of bars shows percentage of larvae settled on top surface of dish, the middle group is for larvae settling on dark side of dish, and the right group of bars shows the percentage of larvae in each treatment that located the dark undersurface of the lid.

Table VII.

Percentage of animals metamorphosed at termination of experiment in continuous light, continuous dark and alternating light/dark. Significance tested with Kruskal-Wallace Test.

Species	Duration of Experiment	% Metamorphosed ($\bar{x}$ + S.E.) Light	Dark	Alternating	P
C. inflata	2d	57.8 ± 7.1	49.5 ± 5.6	61.3 ± 6.1	0.329
C. willmeriana	2d	31.3 ± 2.7	53.1 ± 6.3	31.7 ± 6.9	0.059
A. callosa	7d	31.0 ± 7.7	41.3 ± 9.0	43.5 ± 6.4	0.472
A. paratropa	12d	5.9 ± 0.2	10.6 ± 4.4	19.5 ± 15.3	0.925
S. gibbsii	8d	94.3 ± 1.0	87.6 ± 5.8	85.4 ± 1.7	0.208
C. finmarkiensis	10d	85.8 ± 4.7	88.3 ± 4.6	78.9 ± 4.5	0.412
B. villosa	10d	57.1 ± 6.8	62.8 ± 3.5	84.0 ± 6.8	0.037

DISCUSSION

It is possible to envision an attractive hypothetical scenario in which the shadow response would increase a larva's chances of locating a settlement site. A larva drifting along with the currents passively would be stimulated to swim when passing under a ledge or possibly near a surface of low reflectivity. By increasing its activity at this time, even by erratic swimming, the probability of encountering a surface would be greater than if the larva remained motionless. Furthermore, by swimming only when there is high probability of encountering a site, the larva would presumably conserve energy and thus be able to delay metamorphosis for a longer period of time, again improving its chances for successful settlement.

While not definitive for reasons outlined below, the data in the present study do not support the habitat selection hypothesis. Although there was some evidence that larvae would delay metamorphosis longer in alternating light than in continuous light, the differences between treatments were largely non-significant. In comparing the distributions of ascidians under the various light regimes, every possible outcome was seen, and the outcome predicted by the above hypothesis (greater settlement in the "optimal" habitats in alternating light than in continuous light or dark) was seen infrequently. It is conceivable that the absence of effect was an artifact of the laboratory conditions, and that given more normal light regimes and shadows, larvae would locate suitable sites more readily because of the response. One possible source of error derives from the fact that larvae in the "continuous light" treatment were offered a choice of light and dark sides of the dishes. Larvae swimming in the dish may well turn the direction of their swimming upward when passing from the light to the dark (Mast, 1921), thus encountering the underside of the lid. A larger scale experiment, or one in which no shaded areas are offered to the larvae would be required to eliminate this possibility. It might also be of interest to suspend a preferred substratum, such as adult tunic (Young &Braithwaite, 1980) in mid water and repeat the experiment, with the control dish being illuminated from below. Until such experiments are conducted, I must conclude that the mechanism by which dark areas and downward facing surfaces are selected is independent of the shadow response.

An alternative hypothesis worth investigating is that the shadow response is a simple mechanism by which larvae remain suspended in the water column, to be dispersed by currents, without having to swim continuously. Light is among the most predictable environmental factors in the marine environment, and for that reason, it is used as a cue for a large number of different processes, including spawning (West and Lambert, 1968), timing of reproduction (Giese and Pearse, 1974), orientation, vertical migration and entrainment of biological rhythms (Segal, 1970). Shadows are produced regularly not only by organisms in the water, but by meteorological conditions (clouds passing in front of the sun, waves and ripples on the surface, etc.). Since shadows are nearly always present, they could be be good cues for stimulating the larvae to swim on a regular basis. This would result in a "hop and sink" swimming pattern, similar to that of planktonic crustacea. Such a pattern is an energy efficient means of swimming (Haury and Weihs, 1976) and is employed most often by animals using muscular means of locomotion (Chia, Buckland-Nicks and Young, in preparation).

Larvae induced to swim frequently by regular shadows often begin metamorphosis sooner than larvae maintained under constant light conditions (Crisp and Ghobashy, 1971; Grave, 1936). Although Grave (1936) attempted to demonstrate that frequently swimming larvae produced a larger quantity of some metabolic product which induced metamorphosis, it seems equally reasonable that such larvae are forced to settle early because of earlier exhaustion of their yolk reserves. An energetics study on delaying tadpoles similar to those conducted by Pechenik (1980) on gastropod veligers would be required to test this possibility. Even if specific habitats are not located by means of the shadow response, it is conceivable that by accelerating the onset of metamorphosis, the response could result in greater settlement in regions with many shaded habitats, and thus be indirectly involved in the process of habitat selection.

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IV. SELECTIVE PRESSURES ON JUVENILE ASCIDIANS

Survival and Growth of Juvenile Solitary Ascidians: Selective Pressures Favoring Larval Photoresponse and Seasonal Reproduction

ABSTRACT

Field transplant experiments with newly settled juvenile ascidians were used to test two hypotheses: 1) Photonegative behavior aids ascidian tadpoles in locating refuges from important sources of juvenile mortality, and 2) Reproduction in ascidians is timed to minimize mortality by seasonal factors. Silt inflicted heavy mortality on all six species tested. Algal overgrowth was also important in shallow water, where it not only increased mortality but depressed growth rates during the first two weeks. Grazing subtidal snails, *Margarites pupillus,* scraped juveniles from the substratum with their radulas. It was concluded that the juvenile stage is a very vulnerable period of the life cycle, and that by seeking cryptic sites, tadpoles are able to avoid several important sources of mortality.

Juvenile *Pyura haustor* transplanted to the field in three seasons (October, January, June) showed strong seasonal differences in survival and growth. Although growth rates were highest in the June experiments, survival was much higher in January, which is nearer the normal spawning time of the species. In all seasons, shallow (2 m) transplants experienced heavy mortality in both light and shade.

INTRODUCTION

Juveniles of benthic invertebrates are often thought to suffer greater mortality than adults since their small size makes them more vulnerable to environmental factors and to a wider assortment of predators (Thorson, 1966). Although this idea has intuitive appeal, empirical work on the population dynamics of newly settled juveniles is relatively scarce in the literature. To date, successful studies in epifaunal systems have been limited mostly to intertidal barnacles, of which the cyprid larvae and spat are large enough to study on the shore (e.g., Connell, 1961; Strathmann and Branscomb, 1979), and fouling organisms attached to artificial substrata which can be examined in the laboratory (Goodbody, 1963; Lambert, 1968). Repetitive sampling in soft sediment has also yielded indirect data on the population dynamics of juvenile clams (Ayers, 1956; Muus, 1973). Such studies suggest that selection on juvenile stages may have a substantial effect on the overall spatial distribution and population size of a species.

Where sources of mortality are predictable in either space or time, and either detectable by the animals or correlated with detectable cues, species should evolve so as to minimize mortality or improve reproductive success. This may be accomplished by several means, among which are: 1) reproducing during the season when selection is least intense (Giese and Pearse, 1974; Thorson, 1958) or 2) evolving settling responses in which the preferred substratum is a refuge (Buss, 1979; Woodin, 1979; Young and Chia, 1981). Ascidians, like other marine invertebrates, exhibit numerous reproductive and behavioral patterns which are presumably adaptive. Few attempts have been made, however, to elucidate the "ultimate factors" underlying these patterns.

Although some ascidians reproduce all year (Goodbody, 1963; Lambert, 1968), most temperate forms characteristically have a peak spawning season in the spring or summer (Millar, 1952; Svane and Lundalv, 1981). Many hypotheses have been proposed to explain this phenomenon (see Giese and Pearse, 1974), though few have been tested experimentally. The behavior of ascidian tadpoles varies among species (chapters 2,5). However, the majority demonstrate some form of photonegative behavior immediately prior to settlement (Millar, 1972). Furthermore, the same kind of behavior is displayed by subtidal invertebrates from nearly every phylum (Thorson, 1964). Clearly, such a common pattern must have evolved in response to a selective regime which is both widespread and strong.

The above observations suggested two hypotheses, which were tested in the present study: 1) Darkness is a good indicator of sites where the probability of survival is relatively high (i.e., photonegative settling behavior in ascidian larvae may function in reducing juvenile mortality) and 2) Selection on juvenile ascidians is less intense during some seasons than others (i.e., spawning at a specific time of year may increase juvenile survival). In considering the first hypothesis, I designed experiments to identify some of the selective pressures present in shaded and unshaded habitats, and to determine if, during the first weeks after settlement, survival is indeed higher in the dark. Preliminary observations in the San Juan Islands, Washington State, U.S.A. led me to suspect silt, benthic filamentous algae and grazing snails as important sources of mortality associated with lighted substrata. The effect of snails on ascidian juveniles of two species was documented in the laboratory, while the other two factors were studied in a series of field transplants with six species. The second hypothesis was tested in a preliminary manner by transplanting newly settled juveniles of the ascidian *Pyura haustor* to the field during several seasons and monitoring subsequent survival and growth. This approach was possible with *P. haustor* because some members of the population carried viable gametes throughout most of the year.

MATERIALS AND METHODS

Seven species of ascidians in two orders and four families were used in this study: the phlebobranchs, *Corella inflata* (Corellidae), *Ascidia callosa* and *Ascidia paratropa* (Ascidiidae), and the stolidobranchs, *Pyura haustor, Boltenia villosa* (Pyuridae), *Styela gibbsii* and *Cnemidocarpa finmarkiensis* (Styelidae). Adult ascidians were collected from rocky subtidal sites or floating docks in the San Juan Islands, Washington, and maintained in running seawater aquaria at Friday Harbor Laboratories.

CULTURING LARVAE

Corella inflata isolated in dishes of clean seawater generally spawned each morning. Gametes of *Ascidia paratropa* and *Ascidia callosa* were pipetted directly from the gonoducts of ripe individuals after removing them from their tunics. In *Pyura haustor, Styela gibbsii, Cnemidocarpa finmarkiensis* and *Boltenia villosa*, hermaphroditic gonads were dissected from several adults and macerated through 253 um nitex screen into filtered seawater where fertilization occurred. The cultures were then decanted and rinsed repeatedly until the water was clear and free of excess sperm. After hatching, tadpoles were pipetted into small plastic petri dishes of filtered seawater, where they were allowed to settle. The tadpoles of *Ascidia paratropa* did not settle readily on clean plastic surfaces, so this species alone was allowed to settle in glass dishes. After settlement, the juveniles were scraped gently from the dish bottom with a razor blade and placed in plastic dishes, where they reattached within a few days.

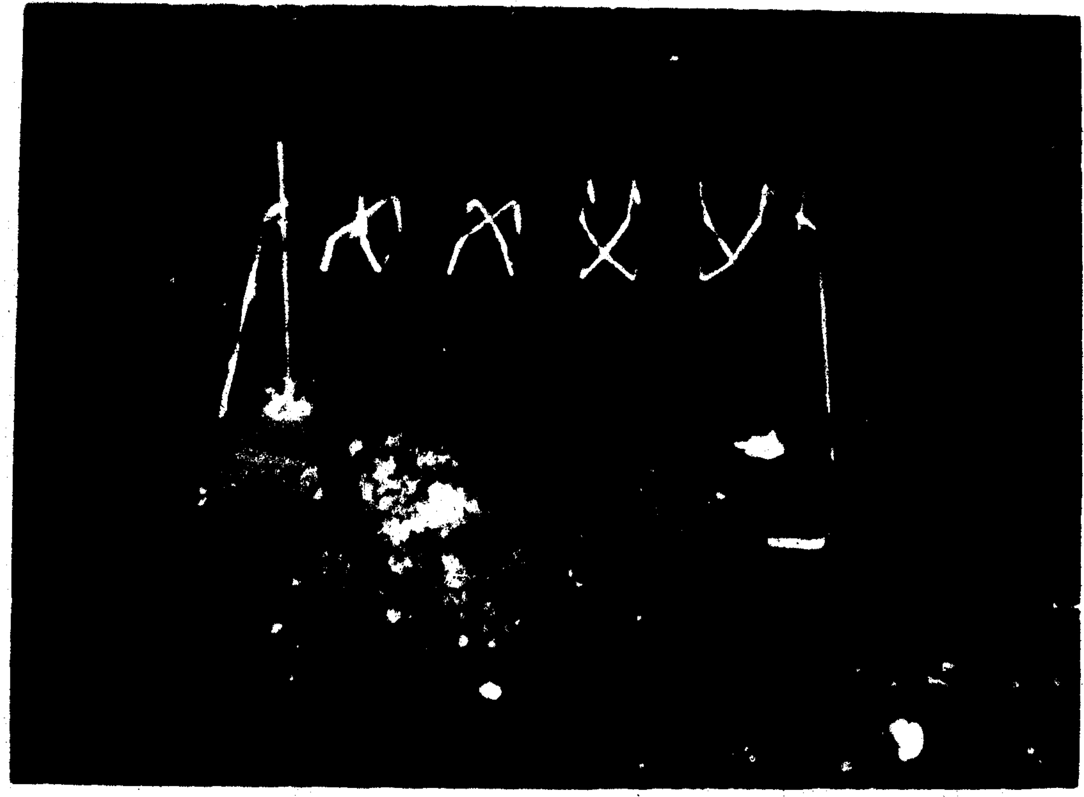
JUVENILE TRANSPLANT EXPERIMENTS

In designing a test for hypothesis 1, it was reasoned that juveniles transplanted to upward facing subtidal surfaces would be exposed to both silt and algae, whereas juveniles transplanted to the bottom sides of transparent surfaces would be subject to algal overgrowth only. Animals on the bottom sides of opaque surfaces, being subject to neither silt nor algae would serve as comparisons. Transplants were carried out at two depths to test the prediction that mortality from both silt and algae would be most intense in shallow water; algae because of favorable light conditions and silt because of greater water turbulence (Schopf et al., 1980).

All transplants were carried out between February and June, 1981. Following settlement, juveniles in petri dishes were maintained in the lab in filtered seawater for about a week to ensure that they were firmly attached. Just before transplantation, they were counted under a dissecting microscope with the aid of an underlying grid. Dishes were randomly assigned to one of the six treatments (three conditions at each of two depths), each treatment consisting of two replicate dishes. The dishes were secured with rubber bands to 6.5 by 28 cm strips of 0.5 cm plexiglas painted black for half their length. Four dishes were attached to one side (ultimately the undersurface), two on the clear half and two on the opaque half, and two more dishes were fastened to the upper surface on the opaque half. To avoid damaging the ascidians during the boat trip to the study site and while carrying them underwater, the assembled experimental strips were transported in a compartmentalized plexiglas box filled with seawater. The box was cooled in a large bucket of seawater while in the boat, and was not opened until after swimming to the transplant site.

In each experiment, one strip of dishes was placed at 4.5 m below MLLW and the other was placed at 21.0 m at Pt. George, Shaw Island, Washington. The strips were held in a horizontal position on racks (Fig. 7), 10 cm above the substratum. Experiments ran for 14 days, after which I recovered the dishes, transported them to the laboratory, and recounted the cohorts. Initially no measurements were taken, but in later experiments, after subjective observations suggested a possible difference in growth rates among treatments, random samples of juveniles from selected dishes were measured. A complete comparison of growth rates among treatments was made only for *Boltenia villosa* and *Ascidia callosa*. In the two other species considered, I measured only shaded and unshaded animals at 4.5 m depth. Lengths of the juvenile ascidians were taken along the mid-sagittal plane (line between siphons) using a calibrated ocular micrometer in a Wild M-5 Dissecting microscope at 25X. In a few cases, where algae obscured the ascidians from above, measurements were made through the transparent bottom of the petri dish. The same method of measurement was used uniformly in all treatments of a given experiment.

Figure 7. Juvenile transplant apparatus in place at 21.0 m depth at Pt. George, Shaw Island, Washington.



SEASONAL VARIATION IN GROWTH AND MORTALITY

Hypothesis 2 was tested by periodically monitoring survival and growth in cohorts of *Pyura haustor* juveniles transplanted to the field during October, 1980, January, 1981 and June, 1981. Because I required ready access to the populations during seasons when diving conditions were unpredictable, the experiments were suspended from a floating breakwater immediately in front of the Friday Harbor Laboratories. As in the Pt. George transplants, dishes were fastened to horizontal, half-painted plexiglas strips. However, because juveniles facing up invariably died after a short time, only downward facing dishes in the light and shade were deployed. The October, 1980 cohort was suspended at 2 m depth, while subsequent cohorts were divided among three depths: 2 m, 6 m, and 10 m. Preliminary experiments had shown that shrimp (*Pandalus danae*) from the breakwater crawled about in the shallowest dishes, damaging the ascidians. All treatments were therefore caged with strips of 1[illegible] VEXAR mesh (Dupont Corp.) held on with rubber bands. On each sampling day, ascidians were transported in seawater to the laboratory, where they were counted, and a sample of randomly selected animals in each dish were measured.

GRAZING EXPERIMENTS

Gastropod grazing experiments were conducted in glass culture dishes containing 300 ml of unfiltered seawater and known numbers of juvenile ascidians. Five *Margarites pupillus* (0.5–1.0 cm spire height) were added to each treatment dish. Adjacent dishes with comparable ascidian densities but no snails served as controls. Water was changed daily to rid the treatments of snail fecal pellets and replenish the oxygen supply. The activities of the snails were observed with a dissecting microscope.

Density of *Margarites spp.* at Pt. George was estimated by counting a series of four 50 by 50 cm quadrats haphazardly positioned at 21.0 m depth in an area where ascidians were common.

RESULTS

SELECTIVE PRESSURES FAVORING LARVAL PHOTOTAXIS

Siltation and algal overgrowth

The experiments at Pt. George were examined routinely while they were running. On every occasion, upward facing dishes contained fine sand and silt, though the absolute amount did not seem to increase substantially over time. I presume that the silt was regularly flushed from and deposited in the dishes by the strong semidiurnal tidal currents. Schopf et al. (1980) measured sedimentation rates near our Pt. George site and found that silt was inversely correlated with depth. As anticipated, algal growth was also strongly related to depth. Following two weeks submersion, diatoms and filamentous algae always blanketed the 4.5 m unshaded treatments, while the 21.0 m treatments were nearly free of algal growth. Dark dishes were likewise free of algal growth at all depths, though they sometimes were colonized by stalked cilitates resembling *Vorticella*.

All six species experienced heavy mortality in upward facing dishes at both depths (Fig. 8), though in the species with some survivors, mortality was generally greater in shallow water as predicted. In downward facing (silt-free) dishes, mortality in shallow water was significantly higher in unshaded than shaded dishes in *Styela gibbsii*, *Boltenia villosa*, *Ascidia paratropa*, and *Ascidia callosa*. In the remaining two species, there was no significance.

Two species, *Ascidia callosa* and *Boltenia villosa* survived equally well in light and dark downward facing dishes at 21.0 m. *Corella inflata*, *Ascidia paratropa*, *Styela gibbsii* and *Pyura haustor*, however, survived much better in unshaded than shaded dishes at the same depth. Although this pattern was not predicted by the hypothesis, diving observations suggested a post-hoc explanation for it. On three different occasions, when recovering the experiments, it was noted that decorator crabs, *Oregonia gracilis*, had taken up residence in the shaded dishes. By wedging their legs into the corners of the dishes, they were able to cling on and hang upside down. I hypothesize that the sharp dactyls of the crabs inflicted damage on the ascidians, causing the observed high mortality. In addition, numerous pandalid shrimp invariably appeared whenever an experiment was introduced (e.g., see figure 34 in chapter 5).

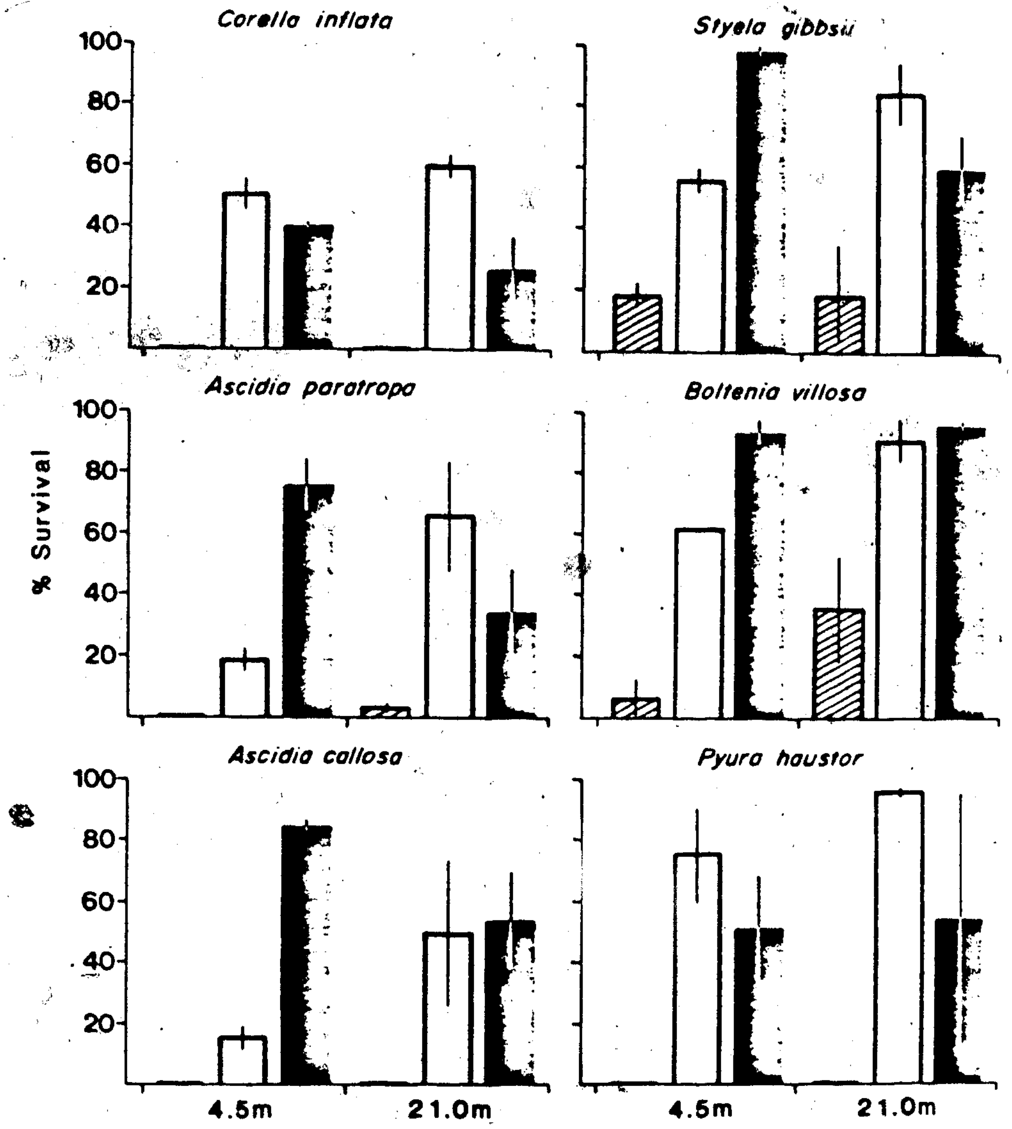


Figure 8. *Corella inflata, Ascidia paratropa, Ascidia callosa, Styela gibbsii, Boltenia villosa, Pyura haustor.* Survival of juvenile ascidians at 4.5 and 21.0 m depths after 2 weeks at Pt. George. Solid bars: shaded, downward-facing treatments; open bars: unshaded, downward-facing treatments; crosshatched bars: upward-facing. Each bar represents mean of 2 replicates. Vertical lines are standard errors.

Between-species comparisons of these survival data should be regarded with caution, since experiments were not all run concurrently, and only two replicates were run per treatment. Moreover, the overall pattern was similar for all species; mortality was greatest in upward facing dishes and generally greater in the light than in the dark. In order to obtain adequate replication for analysis of variance, I pooled data from all species. Since the numbers were in the form of percentages, they were normalized by arcsine transformation (Sokal and Rohlf, 1969). Overall, the difference among treatment means was highly significant. Upward facing treatments at the two depths were not significantly different from one another (Student-Newman-Keuls test), but did differ at the 0.01 level from all other treatments (Fig. 9). The 4.5 m light treatments, which were subject to heavy algal growth, did not differ from the 21.0 m dark treatments which were disturbed by crabs. The deep light treatments and shallow dark treatments however, showed significantly less mortality than the shallow light ones. As predicted, the two downward facing 21.0 m treatments were neither significantly different from one another nor from the shaded, downward facing treatment at 4.5 m depth.

Growth data in the subtidal transplants were collected for only four of the six species; in each species, shallow water animals grew less in the light than in the dark over a two week period (Fig. 10). This difference between light and dark treatments was not observed in the 21.0 m transplants for either *Ascidia callosa* or *Boltenia villosa*. *A. callosa* , though not *Boltenia*, were significantly smaller in the 4.5 m dark treatment than in either deep treatment. On upward-facing surfaces, growth of *B. villosa* was depressed in shallow water but not in deep water. This suggests that while silt causes mortality, algal overgrowth alone has the effect of depressing growth rates. At times mats of algae in the shallow dishes appeared to obstruct the siphons of the young juveniles, suggesting a possible mechanism for this reduction in growth.

Gastropod Grazing

I estimated the density of *Margarites spp.* near the 21.0 m Pt. George transplant site as 49.0 animals per m^2 (S.E: 11.1; n=4). This was on a solid bedrock slope with sparse cover of macroscopic algae. Schroeter (1972) found densities of *Margarites pupillus* as high as 200 per m^2 across the channel at Cantilever Pt., though this figure included those

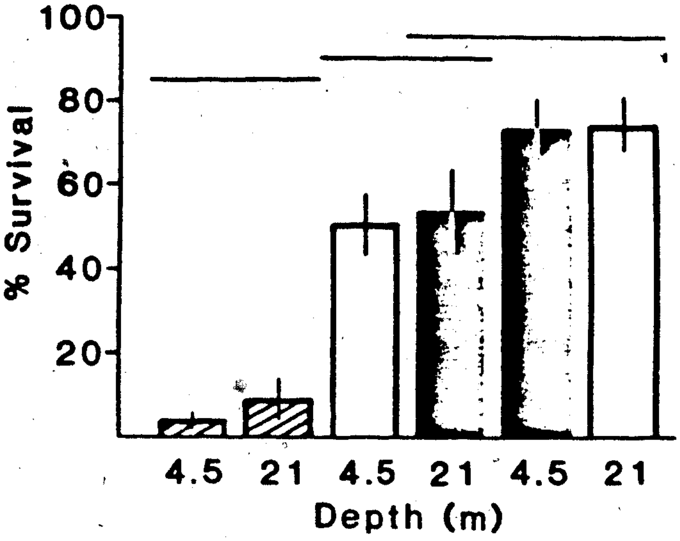


Figure 9. Survival data from Figure 8 pooled for all species and ranked. Horizontal lines span non-significant differences (p<0.01), as determined by the Student-Newman-Keuls test. Shading of bars as in Figure 8.

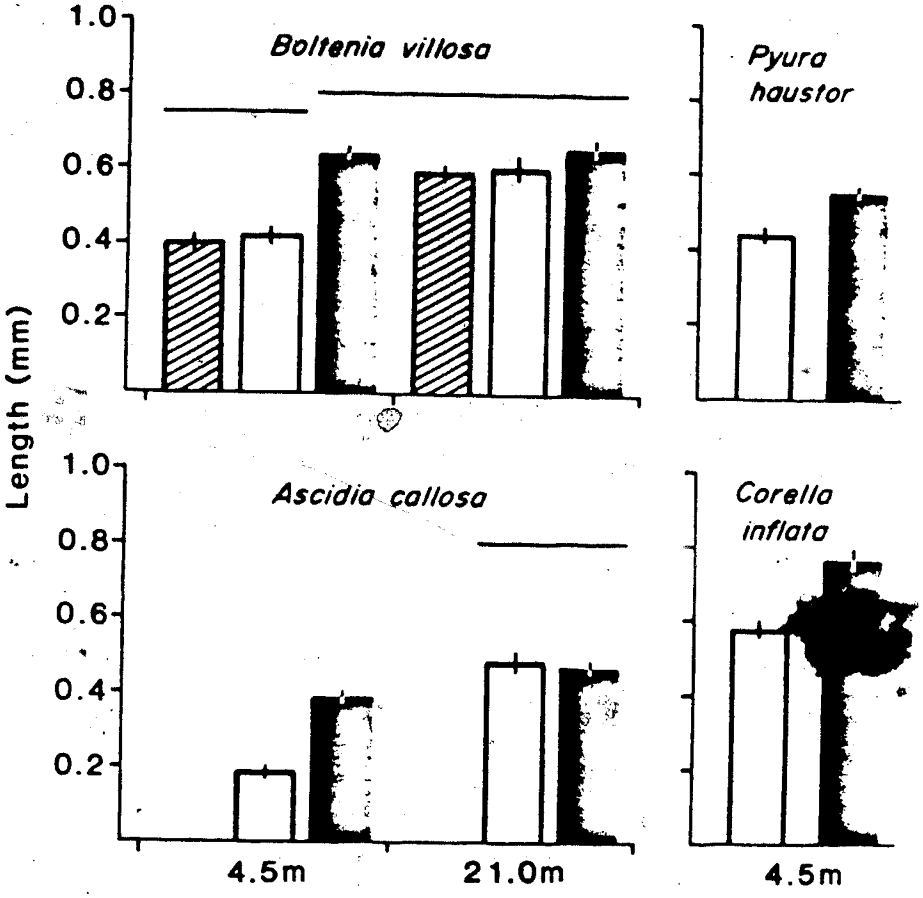


Figure 10. *Boltenia villosa, Ascidia callosa, Corella inflata, Pyura haustor*.. Mid-sagittal lengths of juveniles following 2 week transplant at Pt. George. Shading of bars as in previous figures. Vertical lines are standard errors. Horizontal lines indicate non-significant differences, determined by Student-Newman-Keuls test following one-way Analysis of Variance in *Boltenia* and *Ascidia*, and unpaired t-tests in *Pyura* and *Corella*.

animals occurring on the *Agarum* canopy.

In laboratory experiments, non-feeding snails crawled over established juveniles with no apparent effect. Although they coated the ascidians with a layer of mucus, the ascidians were able to open up and resume filtering after a short time. When foraging, however, the snails removed or damaged many ascidians with their radulas. After 10 days in the presence of *Margarites*, no juvenile *B. villosa* or *C. finmarkiensis* remained alive (Fig. 11). Few animals died in controls run simultaneously.

FACTORS FAVORING SEASONAL REPRODUCTION

Seasonal Variation in Mortality

Although algal growth was clearly higher in the spring and summer months than in autumn, even the unshaded October transplants at 2 m accumulated a heavy blanket of algae after a short period of time (Fig. 12). In both shaded and unshaded treatments, the *Pyura haustor* transplanted on October 6, 1980 experienced high mortality during the first two weeks (Fig. 13). By the seventieth day, all but 2.1% of the juveniles had died.

In January and June experiments, clear differences in survival were seen among cohorts transplanted to three different depths (Fig. 14). Significantly more animals survived in each transplant at 6 and 10 m than at 2 m. The most striking bathymetric differences were seen in the January transplants, where nearly all shallow individuals succumbed in the first month, while all deep and intermediate treatments supported relatively high numbers of survivors for more than seven months. In both January and June experiments, the 10 m transplants showed only slightly higher survival than the 6 m ones during the first three to seven months. However, in January, 1982, one year after the initial January transplant, only 45 animals in the 10 m dark cohorts survived. This represented about 2% of the starting population of 614. In both January and June experiments, the 10 m transplants showed only slightly higher survival than the 6 m ones.

By the first sampling date (7 d in January, 22 d in June), mortality was higher in the light than the dark in several experiments. The difference was most pronounced at 2 m as expected, but also appeared at 6 m depth in the January transplant. It is surprising that greater mortality did not occur in the June transplant at 6 m depth. In this treatment, no difference was seen between light and dark after 22 days, and after 10 weeks, a

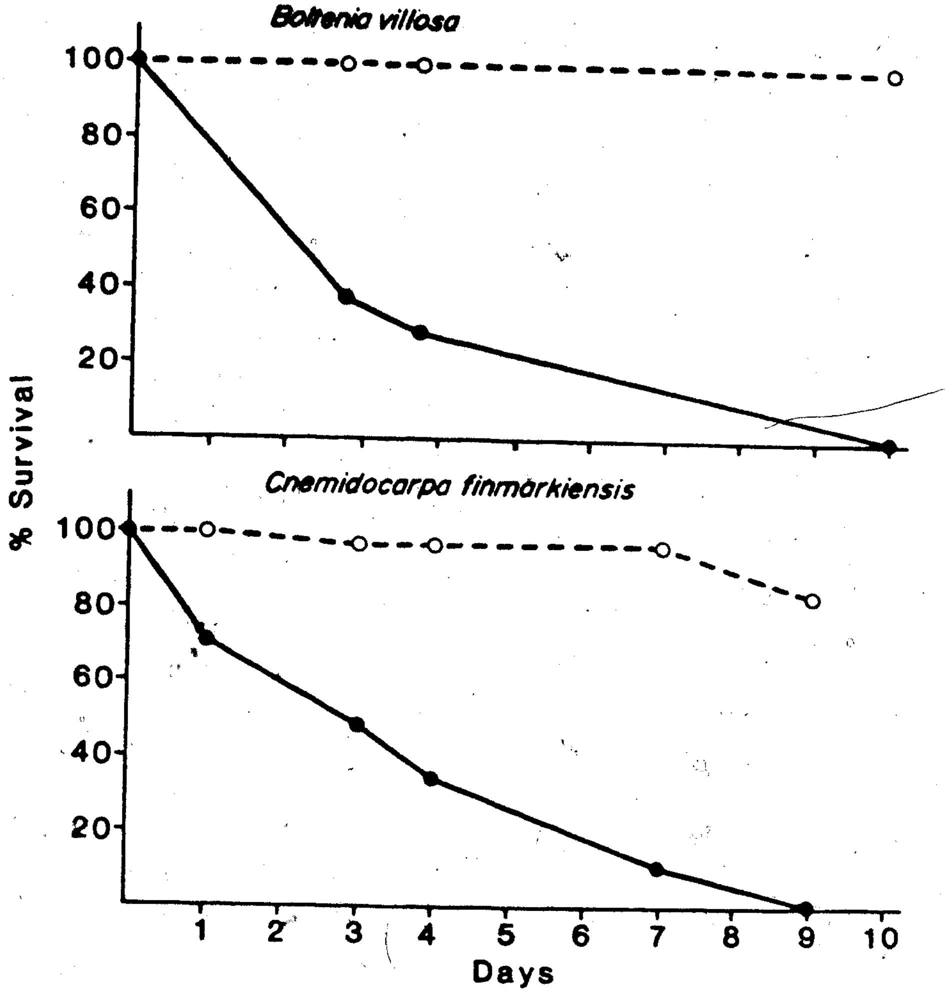
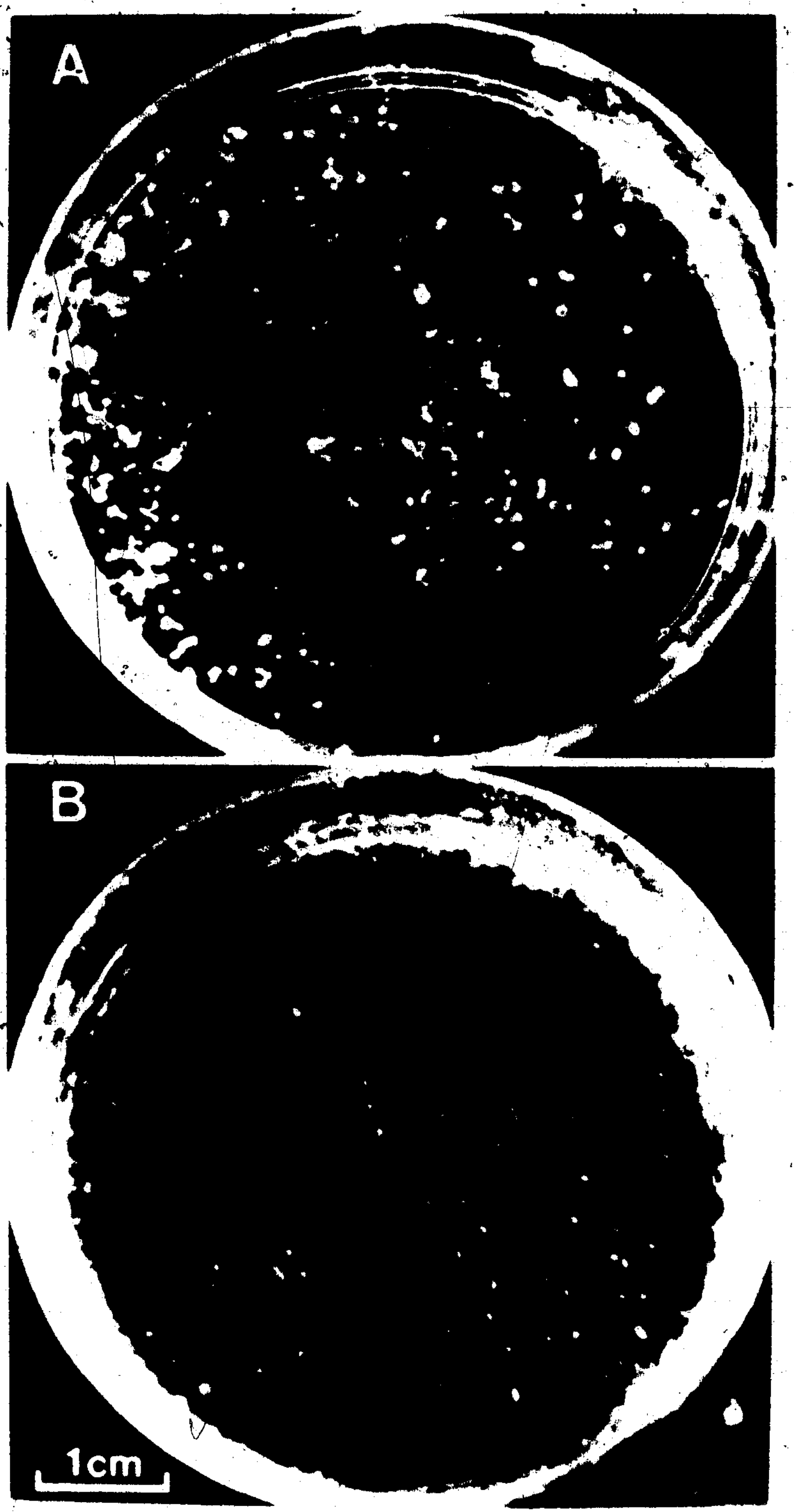


Figure 11. *Boltenia villosa* and *Cnemidocarpa finmarkiensis*. Survival of juveniles in dishes with *Margarites pupillus* (solid lines) and in dishes with no snails (broken lines).

Figure 12. *Pyura haustor*. Juvenile transplant dishes on October 16, 1980 following 10 d immersion at 2 m depth. In dish A (shaded treatment), note larger size of juveniles, absence of algae and barely visible epidermal ampullae. Dish B (unshaded treatment) is heavily colonized with algae, making the juveniles less visible. The juveniles are also smaller in size.



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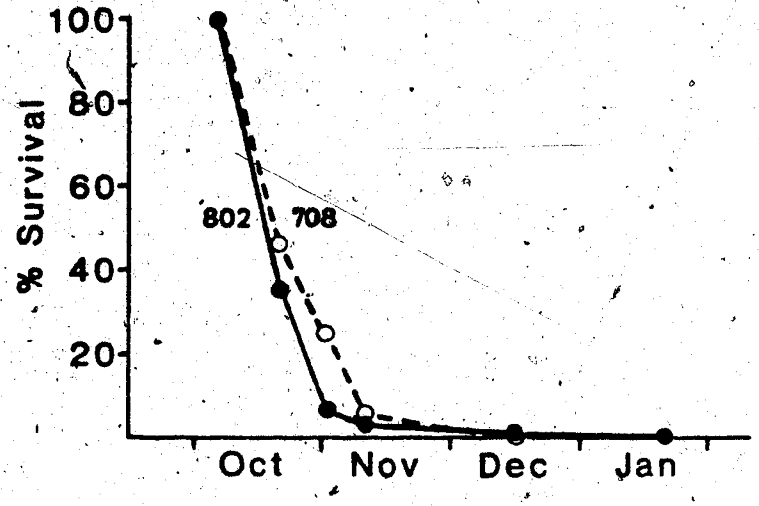

Figure 13. *Pyura haustor*. Survival of juveniles transplanted to 2 m depth in October, 1980. Solid line and solid points: shaded animals. Broken line and open points: unshaded animals. Numbers near the lines indicate starting sizes of the cohorts.

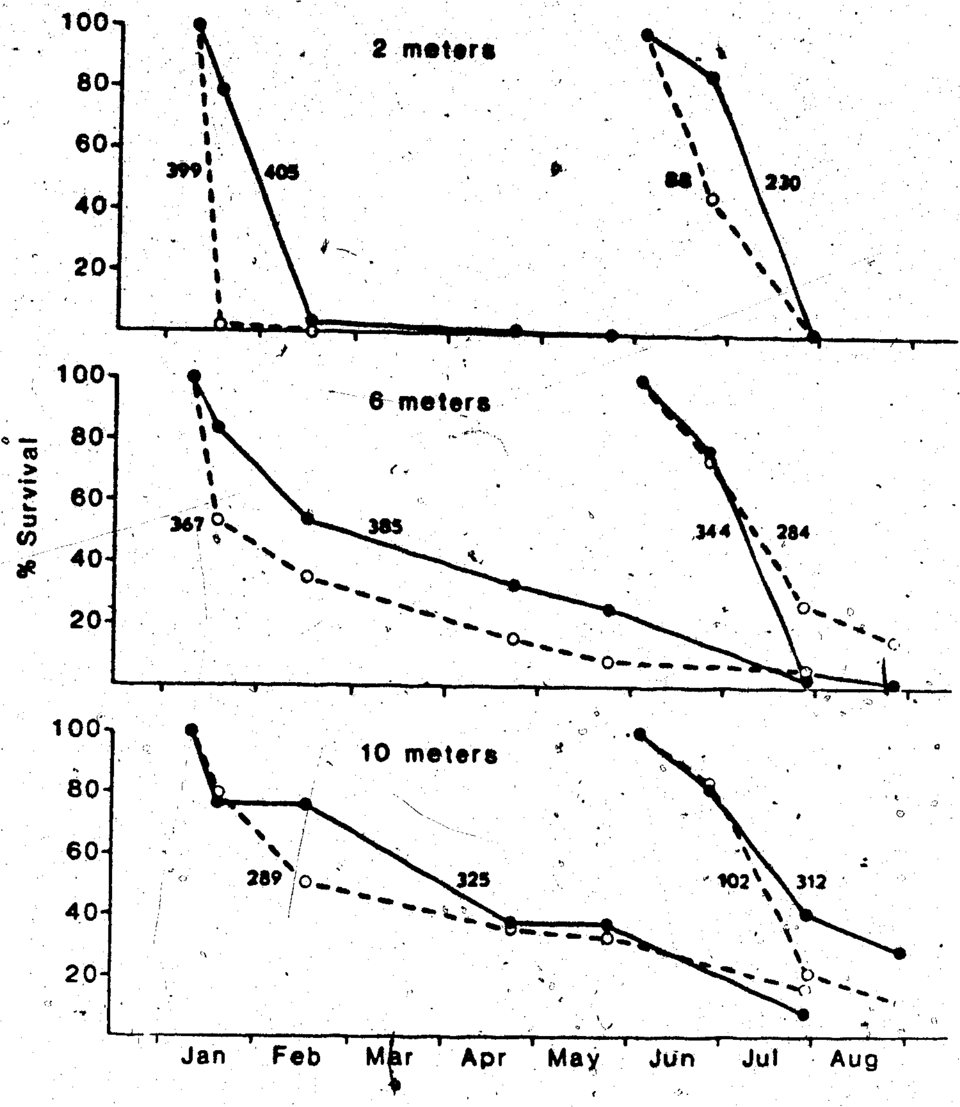

Figure 14. *Pyura haustor*. Survival of juveniles transplanted to 3 depths in January, 1981 and June, 1981. Solid line and points: shaded animals. Broken line and open points: unshaded animals. Number near each line indicates starting size of cohort.

greater proportion of animals had died in the shade than in the light. This unexpected result may be in part because heavy algal growth on the top side of the plexiglas plate blocked light, shading the bottom surface enough to prevent good algal growth. Even in those treatments where mortality was initially higher in the light, the difference did not generally last; eventually, mortality in the dark often equalled or exceeded that seen in unshaded treatments.

Seasonal Variation in Growth

Growth of the October transplants is plotted in Figure 15. Animals in the two treatments showed significant differences in growth during the first few weeks. While average size in the dark cohort increased, animals in the light initially decreased in size. By November, lengths in the two groups were again nearly equal, and both treatments showed slight declines during the month of December. Although it is not known whether the observed decreases in size were due to regression of individual animals, or differential mortality operating on animals of different sizes, the former explanation is most likely, since the average size of light cohort animals in late October was below the lower end of the size range of animals measured at the beginning of the experiment.

January transplants at 2 m depth in the light also showed a slight decline in average size just prior to extinction (Fig. 16). At the first sampling date in each experiment, I used t-tests to compare lengths of animals grown in the light and the dark. Dark-reared animals were significantly larger than light-reared animals ($p < 0.05$) in each experiment with the exception of the 2 m January transplant. In the June experiments, light and dark animals showed a highly significant difference in length ($p < 0.001$) at 2 m and 6 m; the 10 m transplants were significant at a 0.05 level.

Regression analysis was applied to all curves having more than two values of the independent variable (Table VIII). Because of the "negative growth" seen in the October cohort in mid winter, the October data showed poor fit to a linear model. However, all of the 6 m and 10 m transplants in both January and June had relatively high correlation coefficients, indicating little scatter around the regression lines. One-way analysis of covariance (following log transformation to correct heteroscedasticity) demonstrated an overall difference among slopes ($p < .001$). The same procedure was used to make

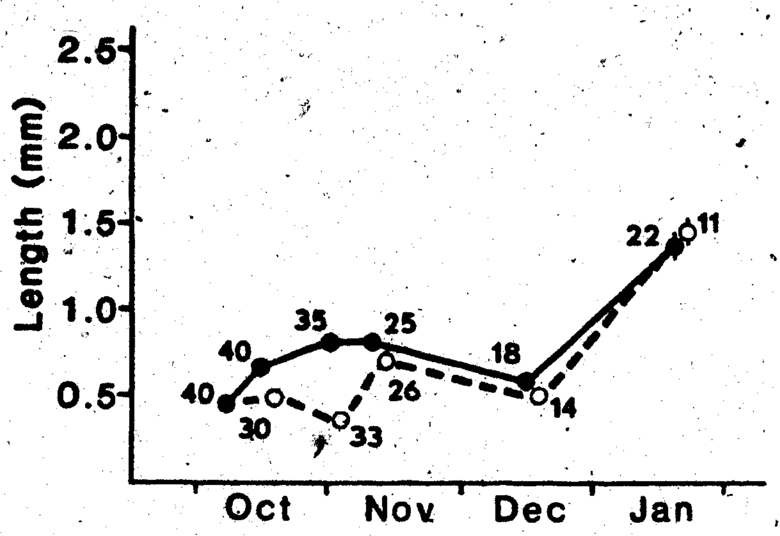

Figure 15. *Pyura haustor*. Growth of juveniles transplanted to 2 m depth in October, 1980. Each point represents mean plus or minus one S.E. (where S.E. bars are not visible, they are smaller than the points). Number near each point indicates sample size on which mean is based. Solid lines and points: shaded animals. Broken lines and open points (displaced slightly to the right to improve resolution): unshaded animals.

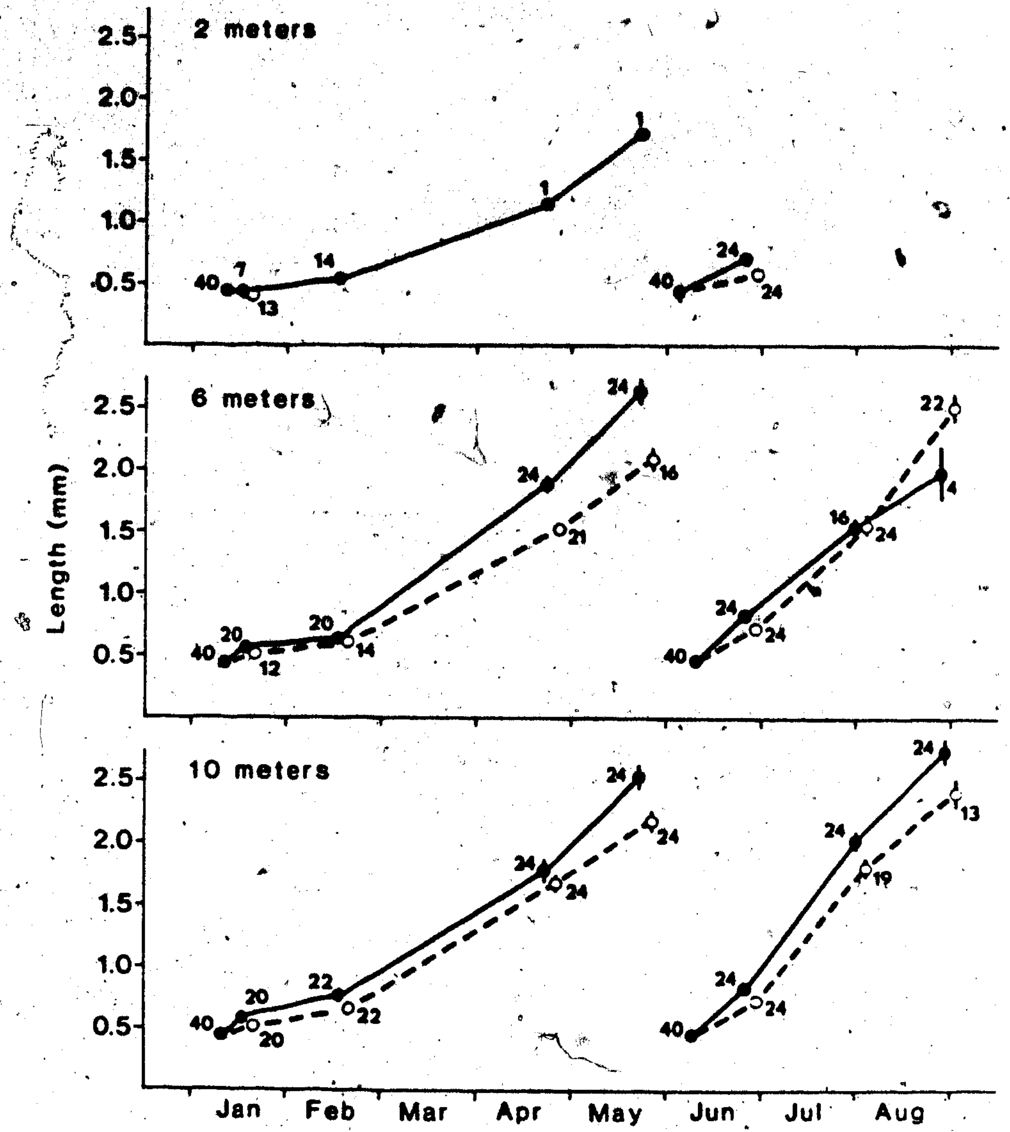

Figure 16. *Pyura haustor*. Growth of juveniles transplanted to three depths in January, 1981 and June, 1981. See Figure 15 for explanation.

Table VIII.

Pyura haustor. Linear regression parameters for juvenile growth curves. All slopes are significant ($p < 0.001$).

Month	Treatment	Regression [illegible]	r
October	2 m Dark	$y = 0.585 + 0.006x$	0.658
	2 m Light	$y = 0.273 + 0.009x$	0.749
January	2 m Dark	$y = 0.415 + 0.007x$	0.835
	6 m Dark	$y = 0.345 + 0.017x$	0.902
	6 m Light	$y = 0.363 + 0.012x$	0.917
	10 m Dark	$y = 0.365 + 0.015x$	0.915
	10 m Light	$y = 0.365 + 0.013x$	0.919
June	6 m Dark	$y = 0.484 + 0.019x$	0.899
	6 m Light	$y = 0.383 + 0.024x$	0.916
	10 m Dark	$y = 0.411 + 0.027x$	0.950
	10 m Light	$y = 0.394 + 0.023x$	0.937

several pairwise comparisons designed to test specific "a priori" predictions based on the experimental design. Light and dark regression lines at each depth/starting month combination showed significant differences in slope (January, 6m: $p<0.001$; January, 10m: $p<0.01$; June, 6m: $p<0.005$; June, 10m: $p<0.005$), with dark-reared animals growing faster than light-reared animals in all but the 6 m June treatment. The single unexpected reversal may be due in part to unexplained mortality in the dark dish, which resulted in a very small sample size on the last sampling date. It would appear that much of the observed difference in growth rate can be explained by the "head start" experienced by the dark animals during the first two or three weeks. Fol[illegible] period, the shaded and unshaded treatments paralleled each other closely in mo[illegible]. Taken overall, these results suggest, in agreement with the Pt. George transplant data, that the major algal-induced stress occurs in the first weeks after settlement, and not later on, despite heavier algal cover.

Seasonal growth rates were compared using data from the dark treatments only. At both depths, growth curves in June showed significantly higher slopes than those in January, ($p<0.001$). Within each month, the difference between the 6 m and 10 m dark transplants was non-significant.

DISCUSSION

ADAPTIVE VALUE OF LARVAL PHOTOTAXIS

Fouling studies have shown that larvae of many benthic invertebrate species, including some ascidians (Dybern, 1962) settle primarily on the bottom or darker portions of panels, rocks, and other substrata in the sea (Meadows and Campbell, 1972; Pomerat and Reiner, 1942). Buss (1979) and others have suggested that the adaptive value of this behavior is that animals occupying cryptic habitats are probably less subject to sources of mortality associated with exposed habitats. Two additional functions for the photonegative settling responses have been proposed. Of these, Thorson's generalization that photonegative behavior allows larvae of benthic species to find the bottom (Thorson, 1964) has enjoyed the widest acceptance (Forward, 1976; Young and Chia, in press). Dybern (1962) also suggested that sunlight itself, and especially the ultraviolet component, may be directly damaging to adult ascidians. Recent controlled experiments with ascidians and other invertebrates (Jokiel, 1980) confirm the importance of this factor in shallow tropical systems.

Thorson's hypothesis is supported indirectly by the observation that regardless of season, there was substantial juvenile mortality associated with shallow (2 m deep) habitats. This mortality was independent of the degree of shading. In my experiments, virtually all ascidians at this depth died within the first few weeks, both in the light and the dark. Tadpole larvae, if they use light as a cue to swim deeper during their pelagic phase, would generally be expected to settle on deeper substrata, and thus avoid some of this mortality.

The data strongly support the idea that shaded substrata are refuges from mortality, and furthermore implicate siltation, gastropod grazing and overgrowth by microscopic algae as selective pressures, or "ultimate factors" which might have favored the evolution of negative phototaxis in larvae. In the rocky subtidal zone of the San Juan Islands, silt appears to be the most important of the three factors. Assuming the upward-facing petri dishes roughly mimic upward-facing rock surfaces, I would predict virtually no survival in such habitats. This is supported by quantitative observations on the distributional patterns of adult ascidians in the San Juans (chapter 2) which show that nearly all individuals of most species occupy cracks, holes, the sides and bottoms of rocks, or vertical rock walls.

Heavy silt loads are often negatively correlated with the abundance of benthic animals (reviewed by Moore, 1977). For example, Mayer (1918) considered silt as the major factor limiting distribution of corals on the Great Barrier reef, an opinion which is supported by recent work on juvenile corals by Bak and Engel (1979). In the shallow subtidal of Lough Ine, Scotland, Round et. al. (1961) demonstrated experimentally that the hydroid, *Sertularia* is unable to survive in areas where suspended or resting silt loads are heavy. Working with boulders in this same region, however, Lilly et al. (1953) did not consider silt an important limiting factor, despite the fact that nearly all species occurred more abundantly on the sides and bottoms of boulders than on the tops. Their rationale was that species occurring predominantly on the bottoms of rocks in sheltered, silty areas would be expected to occur atop boulders in rapidly moving water where silt was minimal; this pattern was not seen. Unfortunately, the authors failed to consider the possible distinction between silt as an "ultimate factor" and silt as a "proximate factor". It seems possible that while the adults may survive on upward facing surfaces in areas free of silt, larvae still prefer to settle on the undersides, since overall, selection has always favored the latter habitat as a refuge from silt. Observational studies have also implicated silt as an important limiting factor for numerous subtidal organisms, including ascidians, hydroids and sponges occurring on rock (Evans et. al., 1979; Kitching et. al., 1934) and epifaunal organisms occurring on algae (Boaden et. al., 1975; Norton, 1971) and hydroids (Hughes, 1975). The present study supports and extends these general conclusions. By monitoring cohorts of known size, I have identified the early juvenile stage as a vulnerable period when silt is an important source of mortality. To my knowledge, such data have not been available previously for any species.

Overgrowth by filamentous algae seems to be important not only in causing mortality, but in suppressing the growth rate of ascidians early in the post-settling period. After two to three weeks, the influence of algae appears to lessen, presumably as the animals become large enough to extend above the algal mat, or create feeding currents strong enough to keep the siphon apertures clear. In my experiments, however, algal colonization began only when the already settled ascidians were transplanted to the field. Thus, under natural conditions where algal mats are already established at the time of ascidian settlement, algal overgrowth may be an even stronger selective agent. The effect of filamentous algae seems to be strong during all seasons. Indeed, in the October transplant, when relatively little algal colonization was expected, I observed not only rapid growth of the algal mat, but also a corresponding regression in size of ascidians exposed to this mat. Perhaps the slow mid-winter growth rates interacted with the algal colonization to produce the observed regression. While algal colonization later in the winter and spring was higher, ascidians were able to outgrow the "vulnerable" stage more quickly, and little or no regression was observed. The effect of algae, as expected, shows a strong negative relationship with depth. In the Pt. George transplants, animals in the 4.5 m treatments (which were even shallower at low tide) in the light grew significantly less and showed much greater mortality than comparable animals maintained at 21.0 m. In the breakwater transplants of *P. haustor*, where treatments floated up and down with the tide and thus were maintained at constant depth, there was strong selection against animals in the light at 2 m, but in the 6 m and 10 m transplants, relatively less effect was observed. Silt in deep water did not reduce growth in *B. villosa* juveniles, though it did produce heavy mortality in all species.

Grazing snails represent an additional hazard to juveniles in some circumstances. In laboratory experiments, the ascidians apparently were not damaged by snails crawling over them, and mucus left by the snails was easily broken by the feeding currents of the ascidians. However, individuals of *Margarites pupillus* did indiscriminately kill ascidian juveniles with their radulas during the course of grazing forays. Snails actually may represent two opposing kinds of selective pressures. Although their direct effect on the juvenile ascidians is clearly negative, they may indirectly render a site more suitable for ascidian growth and survival by removing algae. Additional work is needed to assess the actual relationship between *Margarites* or grazers in general and the distribution of sessile organisms. In particular, information is needed on the activity patterns of the snails. If they graze indiscriminately in cryptic and exposed habitats, ascidian juveniles might not find refuge by settling in the former. If, on the other hand, their grazing activities are more or less limited to the lighted areas where algal food is abundant, then dark regions may truly represent refuges. Lilly et. al. (1953) found that three herbivore species (two snails and a chiton) occurred most abundantly on the top sides of subtidal boulders. In the San Juan Islands, *Margarites pupillus*, is most common on blades of the laminarian alga, *Agarum*, (Schroeter, 1972), where it apparently grazes off diatoms and other microscopic algae.

In designing subtidal transplants, I did not anticipate that crabs and other vagrant animals would use the dark dishes as hiding places. In several instances, mortality was higher in the dark dishes than in certain other treatments, apparently because the decorator crabs and shrimp preferentially colonized the controls. I suspect that these animals killed the young ascidians by scraping them off with their sharp dactyls. Crustaceans could conceivably represent a selective pressure which favors settlement in non-cryptic habitats. That so many sessile animals seek out cryptic sites at settlement, however, argues strongly that this factor is not as important as algae or silt, that its effect is less predictable in time, or (most likely) that the phenomenon is an artifact of introducing unoccupied habitat into the environment.

The technique of allowing larval ascidians to settle on artificial substrata in the lab, then transplanting to the field often has been used to study ascidian growth (Goodbody and Gibson, 1974; Lambert, 1968; Nomaguchi, 1974; Yamaguchi, 1975). However, only Goodbody (1963) transplanted juveniles in more than one experimenal condition. He found that juvenile *Ascidia nigra* grew faster when shaded, apparently because of the absence of diatoms (Goodbody, 1963).

In the San Juan Islands, the distribution of subtidal ascidians is controlled by a number of different factors including predation (Young, 1980; chapter 5) and larval settlement (Young and Braithwaite, 1980; chapters 2,5). The tendency for most animals to occupy cryptic habitats seems to result in part from mortality (due to silt, algae and snails) associated with unshaded areas, and in part from the photonegative responses of the larvae at settlement. It is difficult to distinguish these two factors, since the differential mortality probably favored evolution of the behavioral responses. At this moment in evolutionary time, distribution seems to be controlled largely by the proximate factor (behavior); at some time in the past, the ultimate factors (silt, etc.) documented in the present study were probably more important.

ADAPTIVE VALUE OF SEASONAL REPRODUCTION

Few data exist to support the numerous hypotheses which have been proposed to account for reproductive seasonality in marine invertebrates (Giese and Pearse, 1974). *Pyura haustor* is a convenient animal with which to investigate this question, since like most invertebrates of the Northeastern Pacific, it naturally spawns in the early spring, yet unlike many species, it produces viable gametes, which may be obtained by dissection, throughout the year. Juvenile *P. haustor* of identical age, transplanted in October, January and June, showed striking differences in both growth rate and survival. Although growth rates were highest in June, animals transplanted during this period showed very high mortality; after 7 months all were dead. January transplants grew slower, but showed relatively high survival (except in shallow water); some were still living after a full year. I have observed spontaneous spawnings of *P. haustor* in the laboratory from early February through mid May. The most extensive spawnings seemed to be those in February. At least four other species, *Ascidia callosa* (C. Lambert, personal communication), *Chelyosoma productum* (Young and Braithwaite, 1980) *Styela coriacea* and *Styela gibbsii* (Young, unpublished observations) show spawning peaks during the very early spring; populations of all species have nearly spawned out by April. Interestingly, the spawning season of these ascidians corresponds to the months when surface seawater temperature is lowest in the San Juans (Thompson and Phifer, 1937; D. Duggins, personal communication). We may speculate that algal growth, which may be temperature limited, is also lowest during this time of year. Unfortunately, the data on survival and growth were collected during a single year, and I do not know whether the seasonal phenomena observed are representative of events which occur in a predictable manner year after year. Nomaguchi (1974) carried out a brief study similar to mine with *Ciona intestinalis* in Japan. He also found that juveniles settling in the very early spring survived somewhat better than those settling when the temperatures were warmer. Likewise, Svane and Lundalv (1980) recently demonstrated that adult *Ascidia mentula* in Sweden grew more slowly, and sometimes regressed, during the warm summer months. This species reproduces in the late summer and autumn, a time of year which, in terms of algal growth, could be similar to the early spring.

Palmer and Strathmann (1981) have considered spatially independent, year to year variation in habitat quality to be an important part of a larval dispersal model, and Strathmann et al., (1981) have demonstrated such variability in the habitats available to intertidal barnacles. Similar work is needed in the subtidal zone. Assuming that probability of survival is predictably seasonal, my data strongly support the hypothesis that ultimate factors favoring early spring spawning in temperate ascidians act on the juvenile stage of the life cycle. The spawning peak of *Pyura haustor* appears to coincide with the period of highest survival.

These results contrast in an interesting manner with Goodbody's (1961) study of the tropical ascidian, *Ascidia nigra*, whose reproduction is continuous. Although there is a slight peak in reproductive activity (as indicated by settlement on fouling panels) in some seasons, the peak occurs unpredictably, and may simply reflect chance events occurring in the plankton. *A. nigra* juveniles transplanted to the field showed considerable fluctuation in survival, but the fluctuations were not related to seasonal conditions. The clear contrast between *P. haustor* and *A. callosa*, with respect to both spawning time and survival, supports the idea that seasonal reproduction in temperate ascidians and other invertebrates may be an adaptive response to predictable seasonal variation in those benthic environmental conditions that influence juvenile survival.

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V. PREDATION, LARVAL SETTLEMENT, REFUGES AND DEFENSE

Larval Behavior, Gastropod Predation, Refuges and Defense as Co-determinants of Spatial Distribution in Subtidal Solitary Ascidians of the San Juan Islands, Washington

ABSTRACT

Factors controlling the distribution of subtidal ascidians on hard substratum were studied for a group of 13 species in the San Juan Islands of Washington State, emphasizing the interactive effects of predation, larval substratum selection, defense mechanisms and spatial refuges. Most of the rocky sites were dominated by the stolidobranch ascidians, *Pyura haustor, Halocynthia igaboja,* and *Cnemidocarpa finmarkiensis,* though *Boltenia villosa* and *Styela gibbsii* also occurred at most sites. Besides occasional isolated individuals, most phlebobranchs were found on soft bottoms and/or floating docks, where *Chelyosoma productum,* the most abundant, reached a densities as high as 1350 per m².

Field transplant and laboratory feeding experiments demonstrated that the cymatiid gastropod, *Fusitriton oregonensis* influences the distribution of most phlebobranchs by eliminating them from typical rocky subtidal sites. *F. oregonensis,* locates ascidians by random encounter, and drills holes mechanically, by using the radula and jaws.

Three of the species which co-occur with *F. oregonensis* have effective means of defending against snail attack. Two other species are able to survive in the rocky subtidal by living as epizooites on the invulnerable forms, taking advantage of the defenses of the hosts. Chemical defense mechanisms (vanadium and acid) are ineffective against *F. oregonensis.* The defense mechanism of *Halocynthia igaboja* consists of long tunic spines with recurved spinelets. In *Pyura haustor,* the strong, leathery tunic is rendered more inpenetrable by embedded sand grains. *P. haustor* also has small spines, mostly localized in the siphon region. *Cnemidocarpa finmarkiensis* may defend itself behaviorally by flattening against the substratum.

In most species, small-scale distribution correlates well with larval behavior. Negative phototaxis at settlement interacts with substratum preferences to result in the observed field distributions.

Several species occur in discrete, single-species aggregations. These aggregations are formed by gregarious settlement of larvae in *Pyura haustor* and *Chelyosoma productum,* while they are thought to be a consequence of short larval life (due to brooding) in *Corella inflata* and high concentrations of larvae in *Ascidia callosa*

occurring on floating docks. Larvae of the epizooic species, *Boltenia villosa* and *Styela gibbsii*, settle most readily on tunic of prospective hosts, and delay metamorphosis in the absence of these substrata.

The species without strong substratum preferences are found mostly as solitary individuals in the field. It is hypothesized that they persist in the subtidal because of their unpredictable distribution in space, and that lack of substratum specificity seen in their larvae is an important component of this strategy.

INTRODUCTION

Distributional patterns of organisms are influenced by complex, interactive suites of factors. In most benthic marine species, these controlling factors may act on more than one life history stage; stages often include a period of pelagic embryogenesis and larval development, a settling stage in which larvae interact with benthic organisms while selecting the adult habitat, and wholly benthic juvenile and adult stages. Because of differences in habitat, structure and size, animals in the various stages may experience very different selective pressures. Much of marine ecology involves identifying these selective pressures and determining how they interact to produce the patterns of distribution and abundance observed in the sea (Paine, 1977; Riedl, 1980). However to date, relative contributions of factors acting in different life history stages have been studied for few marine organisms. In the present study, I document spatial pattern of 13 species of solitary ascidians in the San Juan Islands, Wa. and present experiments on the relative roles of predation (occurring in the adult stage) and habitat selection (in the settlement stage) in controlling these patterns.

Several early marine ecologists maintained that the control of benthic distribution was largely dependent on random processes (Nelson, 1928; Yonge, 1937; Coleman, 1933). Larvae were thought to "rain down" on the substratum and survive where conditions were suitable and die where they were not. Although it has now been demonstrated that larvae in virtually every phylum are capable of discriminating among different substrata and of delaying metamorphosis until appropriate habitats are encountered (see reviews by Crisp, 1974, 1976; Meadows and Campbell, 1972a; Scheltema, 1974), the relative importance of dispersal and habitat selection remains in question because of feeble swimming abilities of most larvae (Mileikovsky, 1973; Young and Chia, in press), inability of larvae to move between water masses (Banse, 1964) and velocity of oceanic and coastal currents. Larvae are able to select a habitat only when choices are available to them; the importance of settlement behavior therefore depends on what Moore (1975) calls, "ecological opportunity", which is largely a function of the dispersal processes occurring in the plankton.

Post-settling determinants of distribution have been the most extensively studied on adults, primarily because of the ease with which sessile adult animals may be manipulated experimentally (Paine, 1977). Both physical and biological factors are known to be important in modifying settlement distributions in the intertidal (reviewed by Connell, 1972, 1975; Paine, 1977, 1980) and subtidal (Kitching and Ebling, 1967) zones. Nevertheless, our understanding of larval ecology and recruitment remains inadequate (Jackson, 1979; Thorson, 1966; Woodin, 1976, 1979). Barnacles are nearly unique among benthic organisms in having larvae and juveniles which are visible with the naked eye from the moment of settlement on. Connell (1961), Strathmann and Branscomb (1979) and others have taken advantage of this feature to study the differences between cyprid and adult distributions. Connell's (1961) observation that the newly settled spat of intertidal barnacles had wider bands of vertical distribution than adults of the same species strengthened his demonstration of competition and predation as determinants of the lower limit of barnacle distribution. Unfortunately, this optimal approach is not possible with most species, since the larvae and early juveniles are microscopic. The ecologist is hard-pressed to consider the dynamics of *in situ* populations until after selective pressures in the early growth stages have taken their toll. Consequently, the difference between settlement distribution and adult distribution remains unknown for nearly all species, and correlations between spatial patterns of adults and those of older juveniles have been made in only a few instances (e.g., Bak and Engel, 1979; Barker, 1977, 1979; Birkeland et al., 1971; Nelson, 1979; Sarver, 1979; Stebbing, 1972).

While ascidian population dynamics have been studied in several fouling communities (Goodbody, 1962; Lambert, 1968; Sabbadin, 1957; Sutherland, 1978; Yamaguchi, 1975) and in the Scandanavian subtidal zone (Gullikson, 1980; Svane and Lundalv, 1981), quantitative analysis of ascidian distribution on a small scale has been carried out only by Castric-Fey et al. (1973) in the Glenan Archipelago of Southern France and Dybern (1963) in Denmark. Both of these workers found that ascidians occurred primarily in cryptic subtidal habitats. On a regional scale, Dybern (1969a, 1969b) correlated distributions of several species with salinity and temperature, and Fay and Vallee (1979) noted that distribution of ascidians in Southern California correlated with the mode of reproduction. Insular species were mostly ovoviviparous compound forms with very abbreviated larval periods.

Ascidians in the family Pyuridae often occur in dense aggregations. For example, clumps of *Pyura stolonifera* dominate part of the intertidal zone in certain regions of South Africa (Day, 1974; Stephenson, 1942), as do *Pyura praeputialis* in New South Wales (Dakin et al., 1948), *Pyura chilensis* in Chile (Gutierrez and Lay, 1965), *Pyura pachydermatina* in New Zealand (Batham, 1956), and *Pyura haustor* in Washington State. Subtidally, several species of the pyurid ascidian *Microcosmos* form discrete aggregations in the Mediterranean (Monniot, 1965; Riedl, 1966; Stachowitz, 1980), and both styelid and molgulid ascidians form similar aggregations in the Gulf of Mexico (personal observations). Monniot (1965) and Millar (1971) have hypothesized that such clumps may arise through gregarious settlement of larvae, while Berrill (1950) has attributed similar spatial patterns in *Dendrodoa grossularia* to an abbreviated larval development which, in his words, "keeps the children at home." Although circumstantial evidence for gregarious larval behavior in ascidians has existed for many years (Grave, 1936; Grave and Nicoll, 1940; Bertholf and Mast, 1944), it has been demonstrated experimentally only for one species, *Chelyosoma productum* (Young and Braithwaite, 1980a). Recently, field evidence for larval aggregation has been provided for *Molgula sp.* (Schmidt, 1982), who also demonstrated that adults occurring in aggregations fuse their tunics together.

Millar (1971) has reviewed the literature on ascidian predators; studies not mentioned in his review or which have appeared since, involve the gastropods *Okenia ascidicola* (Morse, 1972), *Trivia arctica* (Gulliksen, 1975), *Bullia digitalis* (Brown, 1964), *Lamellaria rhombica* (McClosky, 1973), *Lamellaria diegoensis* (Lambert, 1980), and 10 species of Cymatiid gastropods from Australia (Laxton, 1971). Asteroids were once considered minor predators on ascidians (Millar, 1971), though in recent years, several studies have documented predation by starfish: *Asterias rubens* (Gulliksen and Skjaeveland, 1973), *Patiriella brevispina, Tosia australis* and *Petricia veruicina* (Keough and Butler, 1979) and *Porania pulvillus* (Ericsson and Hansson, 1974). In the San Juan Islands of Washington, several asteroids feed occasionally on certain solitary ascidians (Mauzey et al., 1968) and a number of specialized gastropods in the genera *Velutina* and *Lamellaria*, all of which prey on compound or solitary ascidians, are found on occasion (McClosky, 1973). *Corella inflata* is preyed upon by a polyclad flatworm, *Eurylepta leoparda* (Lambert, 1968).

In spite of this long list of predators, ascidians are endowed with a number of adaptations which are considered defensive in function. Most defense mechanisms which have been documented are chemical. Tunicates, particularly those in the Order Phlebobranchia, concentrate vanadium (Goldberg et al., 1951; Swinehart et al., 1974; Webb, 1956), iron (Endean, 1955a; Swinehart et al., 1974) and other metals. Vanadium is a metabolic poison at high concentrations, and when occurring above 10 ppm, it renders food unpalatable to small fish and crabs (Stoecker, 1980b). Some compound ascidians have vanadium concentrations as high as 3000 ppm. Stoecker has recently demonstrated the effectiveness of vanadium in preventing predation in several species of tropical ascidians.

Many ascidians concentrate sulfuric acid in specialized cells located in the outer layers of tunic (Lambert, 1979; Stoecker, 1978). This acid may prevent colonization by epizooites and also protect against certain predators (Stoecker, 1978, 1980b).

The predator considered in this study is a member of the mesogastropod family, Cymatiidae, *Fusitriton oregonensis*. *F. oregonensis*, which may reach 13.5 cm in length (J.T. Smith 1970) is easily recognized by its regular rows of long periostracal hairs. It is the most common large gastropod in the rocky subtidal zone of the San Juan Islands. The feeding of this species has been studied by Eaton (1972), who concluded that it is a generalist predator which will accept a wide variety of common subtidal invertebrates as prey and also scavenges dead fish, crabs and other carrion. Eaton also maintained that *F. oregonensis*, like related mesogastropods in the Family Cassidae (Lyman, 1937; Moore, 1956; Schroeder, 1962) prefers sea urchins when offered a choice of foods. The green urchin, *Strongylocentrotus droebachiensis*, which is extremely abundant in the shallow subtidal where *F. oregonensis* is common, is often seen with black spots of necrotic tissue overlying the test. Eaton (1972) found that he could simulate the spots by squirting *Fusitriton* salivary secretions (which are composed largely of sulfuric acid at about pH 3) at the urchins. This observation led to the hypothesis that the urchins with black spots had experienced "near misses" with the predator. Eaton (1972), M.J. Smith (1970) and Avery (1961) all give anecdotal accounts of *F. oregonensis* preying on ascidians.

Fourteen species of solitary ascidians in 2 orders and 5 families occur in the subtidal fauna of the San Juan Islands, Washington. Some are rare, while others are among the most abundant animals of the rocky bottom epibenthos. Preliminary observations revealed a number of clear differences in spatial pattern within the assemblage, suggesting that the system would be amenable to a comparative approach. Additionally, ascidians were attractive for a study of settlement behavior because their larvae are easily obtained and reared in the laboratory, and demonstrate substantial variability in their behavior (Young and Braithwaite, 1980; chapter 2).

In the present study, I documented patterns of aggregation and substratum use in several rocky subtidal habitats, then conducted field transplants and laboratory experiments to assess the relative importance of larval substratum selection and *Fusitriton* predation in controlling these patterns. I found that in many cases, larval behavior was sufficient to explain the small-scale distributions of subtidal species. In general, predation was more important on a large scale. As expected, those species whose larval behavior did not explain the patterns were the ones most strongly influenced by post-settling mortality due to predators. Species that persisted in high numbers where *F. oregonensis* was common posessed either defensive adaptations or the ability to locate and settle in spatial refuges.

MATERIALS AND METHODS

STUDY SITES

Ascidians were observed or sampled quantitatively on over 250 SCUBA dives between January, 1977 and December, 1982. Most sites were on subtidal rocky areas, muddy areas or floating docks in the San Juan Islands (Fig. 17), but these were supplemented by a few dives each in Puget Sound and in Saanich Inlet, British Columbia. Mud and rock bottoms beyond safe diving depths were sampled by dredging from the research vessel Hydah, and additional observations were made in Saanich Inlet from the Fisheries and Oceans Canada submersible, Pisces IV. Table IX gives coordinates, depths surveyed, and a brief description of each site.

All field experiments were carried out at Pt. George, Shaw Island or on the bottom side of the Friday Harbor Labs Breakwater on San Juan Island. The former consists of a gently sloping rock outcropping which extends from the surface to 20 m. Below this, it drops off steeply to form a cliff which ends at 30 m in a sloping field of rubble. Adjacent to the rock outcropping, a cobble field extends from 3 m to at least 30 m. Additional cliffs, boulders and outcroppings in the immediate area provided numerous microhabitats at all depths and within easy swimming distance, all located within a large cove.

The breakwater, which supported a dense population of ascidians, consisted of three floating concrete sections, each approximately 30 m long and 3 m wide. The bottom of the breakwater was continually about 2 m underwater. Although SCUBA was used while diving on the breakwater, all work carried out on the underside was done during short excursions from the sides, during which no bubbles were expelled. As the bottom of the dock was almost perfectly flat, bubbles which were released would pool on the concrete surface indefinitely, damaging the animals.

SPATIAL PATTERN ANALYSIS AND POPULATION ESTIMATES

Spatial pattern was assessed quantitatively for several of the abundant species. However, for species in which only one or a few animals were encountered on any given dive, quantification was deemed pointless. Instead, notes were made on underwater slates each time an individual was encountered.

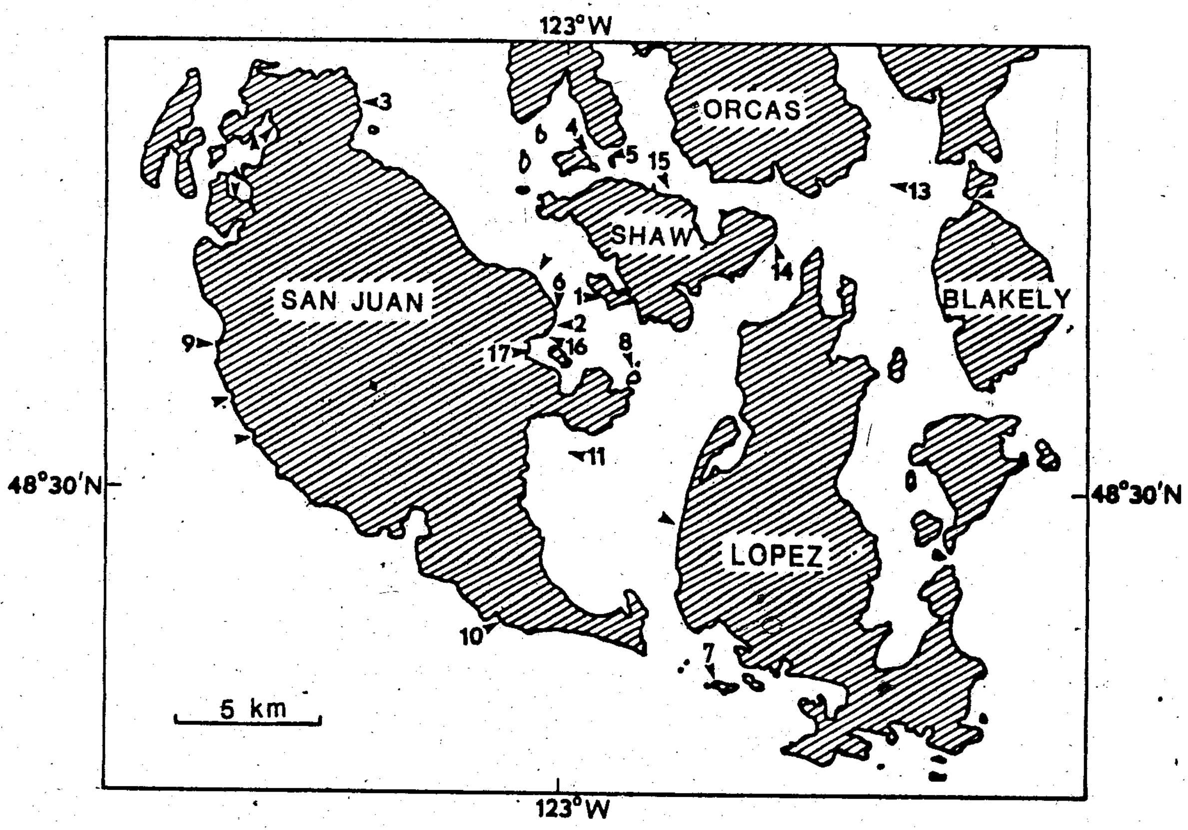


Figure 17. Study areas in the San Juan Islands, Washington State. Sites where formal surveys were conducted are numbered the same as in Table IX. Additional localities where ascidians were collected or observed are indicated by unlabelled arrows.

Table IX.

Coordinates and characteristics of field survey sites.

Site Number and Name	Latitude	Longitude	Depths Surveyed	Substratum
1. Pt. George	48°33'18"N	122°58'42"W	18-24 m	cobble, cliffs, reefs
2. Cantileves Pt.	48°32'48"N	123°0'24"W	15-27 m	cobble, cliffs, reefs
3. Reuben Tarte Pt.	48°36'48"N	123°5'36"W	9-21 m	cobble, reefs
4. Pole Pass	48°36'6"N	122°59'9"W	3-12 m	cobble, reefs, boulders
5. Bell Island	48°35'48"N	122°58'54"W	9 m	cobble, reefs, boulders
6. Shady Cove	48°33'18"N	123°0'30"W	3-19 m	cobble, cliffs
7. Long Island	48°26'36"N	122°55'30"W	28 m	cliffs
8. Turn Island	48°32'12"N	122°58'54"W	5-15 m	cliffs, boulders
9. Limekiln Light	48°31'0"N	123°8'24"W	6-24 m	cliffs, boulders
10. Eagle Cove	48°27'48"N	123°1'24"W	1-6 m	reefs
11. Griffin Bay	48°27'42"N	122°59'42"W	6-15 m	reefs, boulders
12. Elbow Pt.	48°32'48"N	123°28'48"W	15-37 m	cliffs, cobble
13. Potato Patch	48°35'30"N	122°50'36"W	33-55 m	mud
14. Hankin Pt.	48°34'36"N	122°54'54"W	35-41 m	mud
15. Broken Pt.	48°35'30"N	122°56'36"W	23-29 m	mud
16. Lab Breakwater	48°32'48"N	123°0'36"W	2 m	plastic floats
17. Friday Harbor Docks	48°32'12"N	123°0'48"W	0-2 m	concrete floats

Densities of ascidians were measured by various means appropriate to the habitats under consideration. In each case, however, samples used for the estimates were randomly selected, so figures from all sites could be compared statistically. At most subtidal sites, all ascidians were counted in a transect of contiguous quadrats taken along a particular isobath. Quadrat size varied from 25 X 25 cm to 1 m X 1 m, depending on the density of the species under consideration. As many quadrats were taken as possible, within the limitations imposed by bottom time, air supply and water temperature. After returning to the laboratory, a table of random numbers was used to select a subsample of 50% of the quadrats for use in calculating densities. Densities of dock populations were estimated by scraping all animals from randomly selected quadrats 25 by 25 cm or 50 by 50 cm, and counting them in the laboratory.

For pattern analysis of quadrat data, the entire sample was used, since a random pattern should conform to a Poisson distribution even when the sampling scheme is not random (Pielou, 1977). The mean number of animals per quadrat was rounded to the nearest 0.1 and used to obtain individual terms of the Poisson distribution from published tables. Observed and expected values were compared using the chi-square goodness of fit test at n-2 d.f. Expected values lower than 5.0 were pooled, as dictated by convention (Sokal and Rohlf, 1981).

While counting animals, I also noted the substrata to which animals were attached, the numbers of individuals in discrete clumps, the angles (with respect to the horizontal) of surfaces occupied, and whether animals occurred in holes or cracks. Surface angle data for animals living on rocks are presented elsewhere with analysis of tadpole photoresponse (chapter 2). Chi-square analysis was used to determine if the species were independently assorted among the various substrata utilized by ascidians (Greig-Smith, 1957). Expected values were computed as the proportion of total ascidian attachment sites made up of a particular substratum type, multiplied by the total number of individuals in the survey. Data were pooled for all sites of a given habitat type (e.g., rock).

Photographs were used to document pattern on rock walls at Pt. George and Cantilever Pt. An underwater camera with a framing device (25 by 25 cm quadrat) attached in front of its lens was used to photograph grids of 72 or 80 contiguous quadrats, eight quadrats per row (Young and Chia, 1982). The sites were marked with vertical transect lines extending from 18 m to 21 m and marked at 25 cm increments. Wooden clothespins on the lines were used to keep track of position while photographing. The cliffs were photographed with Ektachrome film; slides were later projected, subdivided into 12.5 by 12.5 cm quadrats, and counted for all ascidians.

At a number of sites where quadrat counts were not made, distributional notes were taken on a slate for each ascidian encountered during the dive.

The predator *Fusitriton oregonensis* was always counted when it appeared in quadrats. In addition, I attempted to estimate the size of the snail population on the rock outcropping at Point George where transplant experiments were carried out. The entire portion of the outcropping below 18 m depth (measuring 10 by 15 m) was surveyed on April 15, April 23 and May 7, 1981. Two divers swimming abreast surveyed first the slope at the top of the cliff, then the face of the cliff itself. Each *F. oregonensis* was marked by wrapping a rubber band around its shell spire (Eaton, 1972), then replaced in its original position on the bottom.

TRANSPLANT EXPERIMENTS

Ascidians for use in transplant experiments were collected from areas where they were common; either subtidal rocky habitats or floating docks. Dock ascidians, such as *Corella inflata, Chelyosoma productum,* and *Ascidia callosa* were collected without damaging them by locating specimens attached to sabellid polychaete tubes (*Schizobranchia insignis* or *Eudistylia vancouveri*). The sabellid/ascidian clumps were lashed to bricks or rectangular, brick-sized pieces of 1 cm unpainted plywood with strong monofilament fishing line, taking care that the line did not cross any ascidians, and that it contacted the sabellids only at the strong, basal portions of the tubes. Neither ascidians nor polychaetes were damaged by this treatment. The transplant setups were maintained in running seawater aquaria in the laboratory and in large buckets of seawater both in the boat and while swimming underwater. Just before they were transplanted, all ascidians were counted and measured with calipers.

The bricks were placed on the bottom at 4.5 or 21.0 m depth at Pt. George. Transplants on wood served as non-benthic treatments. Each was tied to two, 1 m lengths of nylon line, one of which was attached to a weight and the other to a 1 gallon plastic jug, filled with air. The weight was placed on the bottom near the benthic transplant, and the float held the ascidians in a vertical position 1 m off the bottom. Thus, these animals were exposed to the same general physical and chemical conditions as the animals on the bottom, but were not subject to factors (e.g. benthic predators) associated with the bottom alone.

Boltenia villosa is a small ascidian which attaches to the substratum by means of a narrow posterior stalk. Two different methods were used to transplant *B. villosa* individuals in their normal, upright position. In the first, 0.5 cm diameter holes were drilled in bricks (2 per brick, 10 cm apart). Stalks of the ascidians were placed in lead screw anchors, where they were secured by crimping the lead slightly and also tying with monofilament line. The anchors were then tapped into the holes in the bricks with a screwdriver so that the ascidians stood upright. In other experiments the same thing was accomplished without using bricks by crimping the ascidian stalks in 5 cm lengths of 1 cm flattened lead pipe. The pipes were bent at right angles near the ascidian end so that the greater mass of the pipe resting on the bottom would hold the ascidian erect.

In one experiment with *B. villosa*, mortality of transplant animals was compared with that of ascidians occurring in natural, *in situ* clumps. The clumps were marked without disturbing them by placing a numbered piece of red terracotta roofing tile about 25 cm away.

Individuals of *Halocynthia igaboja* were secured to bricks by means of monofilament line threaded directly through posterior lobes of tunic. Some transplanted *H. igaboja* individuals had their spines removed with razor blades and others carried naturally occurring epifaunal species such as *Boltenia villosa*.

FUSITRITON OREGONENSIS FEEDING EXPERIMENTS

Experiments on *Fusitriton oregonensis* food preferences and feeding rates were conducted in the laboratory and field. Lab experiments were run in large tanks of running seawater, measuring 127 by 59 cm and filled to a depth of 15 cm. Various combinations of prey items collected from the rocky subtidal zone or from floating docks were placed in a tank with 7 adult snails, randomly selected from a holding tank containing snails which had been collected by dredging or SCUBA. Experiments were of two types. In the first, prey items were replaced each morning after they were eaten, so the availability of prey remained constant for the duration of the experiment. The preference indices "D" and "$\log_{10}Q$" (Jacobs, 1974) were computed from these data. Cock (1978) has reviewed the merits and disadvantages of the numerous measures of preference that have been proposed in the literature. He concluded that Jacobs' (1974) indices have several advantages over more commonly used ones including "Forage Ratio" and Ivlev's (1961) "Electivity". This is especially true when dealing with prey at various densities, as was the case in my field experiments. The formulations for these indices are: $D=r-p/r+p-2rp$ and $\log_{10}Q=\log_{10}[r-(1-p)/p-(1-r)]$, where "r" is the proportion of a given food item in the diet of the predator, and "p" is the proportion of that food item relative to the total available prey. Values of both indices range from 0 to infinity, with high values indicating preference and values near 0 indicating avoidance.

In experiments of the second type, prey items were not replaced as they were eaten, and data were plotted as nests of survivorship curves.

Field experiments were conducted by transplanting *Fusitriton* to the underside of the laboratory breakwater where high density populations of 5 ascidian species were already established and *F. oregonensis* did not occur naturally. Snails were enclosed in cages constructed of galvanized hardware cloth (1 cm mesh size), and measuring 50 cm by 50 cm by 15 cm deep. Twin strips of styrofoam fastened to the "top" of the cages with wire provided flotation, pushing the sides securely against the bottom of the dock. The dock bottom was continuously submerged at a depth of 2.0 m and was about 10 m over the bottom at MLLW. In preliminary runs, I attempted to determine predation rates by photographing the ascidians before and after the snails had fed. However, it proved impossible to differentiate live and dead ascidians in the photographs, and also to count very small individuals. I terminated the experiments reported herein by scraping all animals in each cage into a mesh dive bag, and looking for evidence of predation in the laboratory. Since *F. oregonensis* leaves a characteristic bore hole, this was not difficult. By suspending empty ascidian tests from the breakwater in small cages, I determined that tunic of *Ascidia callosa* and *Chelyosoma productum* remained essentially intact for over 2 weeks and the tests of *Boltenia villosa* and *Styela gibbsii* remained easily recognizable for nearly a month. By limiting the field experiments to 20 days, I probably recovered evidence for nearly all of the attacks which occurred. In the laboratory, all animals, both eaten and uneaten were measured with calipers along whatever linear dimension gave the best correlation with wet mass for that species. A certain amount of subjectivity naturally entered in the size estimates of the ascidians that had been consumed, especially for *Ascidia callosa*, which was often the most decomposed, and for *Styela gibbsii*, the tunic of which contracted somewhat in the dead animals.

Distant chemoreception of *F. oregonensis* was tested in a large plexiglas "Y-maze". The main channel of the maze was 60 cm wide and 90 cm long, and the arms, which were 30 cm across, diverged at a right angle. The potential prey items were held in perforated plexiglas chambers built into the arms. Water from the laboratory seawater system was run into both arms simultaneously at about 2 l/m. Fluorescein and Rhodamine dyes were used as markers to observe the pattern of flow. With the exception of a few "dead regions" near corners, the flow in the arms was nearly laminar. Some mixing occurred in the main channel, but most of the water from the two arms remained segregated until exiting the maze. Six or 10 snails were lined up in the base of the main channel, facing upstream, and allowed to move about for a pre-decided period of time, after which their distribution was noted. In some runs, I monitored movements of the snails for the duration of the experiment to determine how the animals arrived at their ultimate destinations. Since *F. oregonensis* is a nocturnal feeder, short experiments were generally run between 18:00 and 24:00. Longer ones ran until the following morning.

I took advantage of the transparency of some ascidians to observe the mechanism of boring and ingestion in *F. oregonensis*. A large piece of *Ascidia paratropa* tunic was clamped between two pieces of plexiglas having identical 3 cm diameter holes in the middle. The entire assembly, which was held together with brass bolts screwed into threaded holes in the plastic, was placed upright in a small tank of seawater. The body of the ascidian from which the tunic had been removed was placed on one side and a small *F. oregonensis* was placed on the other. When *F. oregonensis* "attacked" the tunic, its drilling mechanism was easily observed from the ascidian side.

MICROSCOPY

F. oregonensis radulas and jaws were removed by dissolving away the soft tissues of the buccal mass with a solution of NaOH. They were then pinned with minute insect pins to paraffin, and air dried prior to mounting for Scanning Electron Microscopy.

Pieces of ascidian tunic were fixed for S.E.M. in 2.5% glutaraldehyde in millipore filtered seawater, rinsed in seawater, and post-fixed in a half-and-half mixture of 2.5% $NaHCO_3$ and 4% OsO_4. They were then dried at the critical point and sputter coated with gold prior to viewing with either a JEOL JSM-35 or a Cambridge Stereoscan-150 Scanning Electron Microscope at 15 to 20 KV.

MEASUREMENT OF TUNIC PROPERTIES

Ascidian tunic pH was determined by the method of Stoecker (1980). A live animal was removed from the water, blotted with a paper towel and bruised by striking it gently. Five different pH papers (Microessential Laboratories, Brooklyn N.Y.), two with broad ranges (1-11, 2-10) and three with overlapping narrow ranges (3-5.5, 5.6-6.8, 4.5-7.5) were applied to the bruised area to estimate the pH.

I obtained a rough estimate of tunic tensile strength with strips of tunic approximately 4 mm wide, trimmed to uniform diameter in the middle (Alexander, 1968). The cross-sectional area of the narrow region was estimated with a calibrated ocular micrometer in a Wild dissecting microscope. The tunic sample was clamped with hemostats on both ends, and suspended vertically from a ring stand. A platform was attached to the lower hemostat, and weights were added to the platform until the tunic broke. Tensile strength was computed as breaking strength/cross-sectional area, expressed in g/mm^2.

The percentage of tunic composed of water was determined by oven-drying tunic samples at 50 degrees C to constant weight, and comparing wet weight with dry weight.

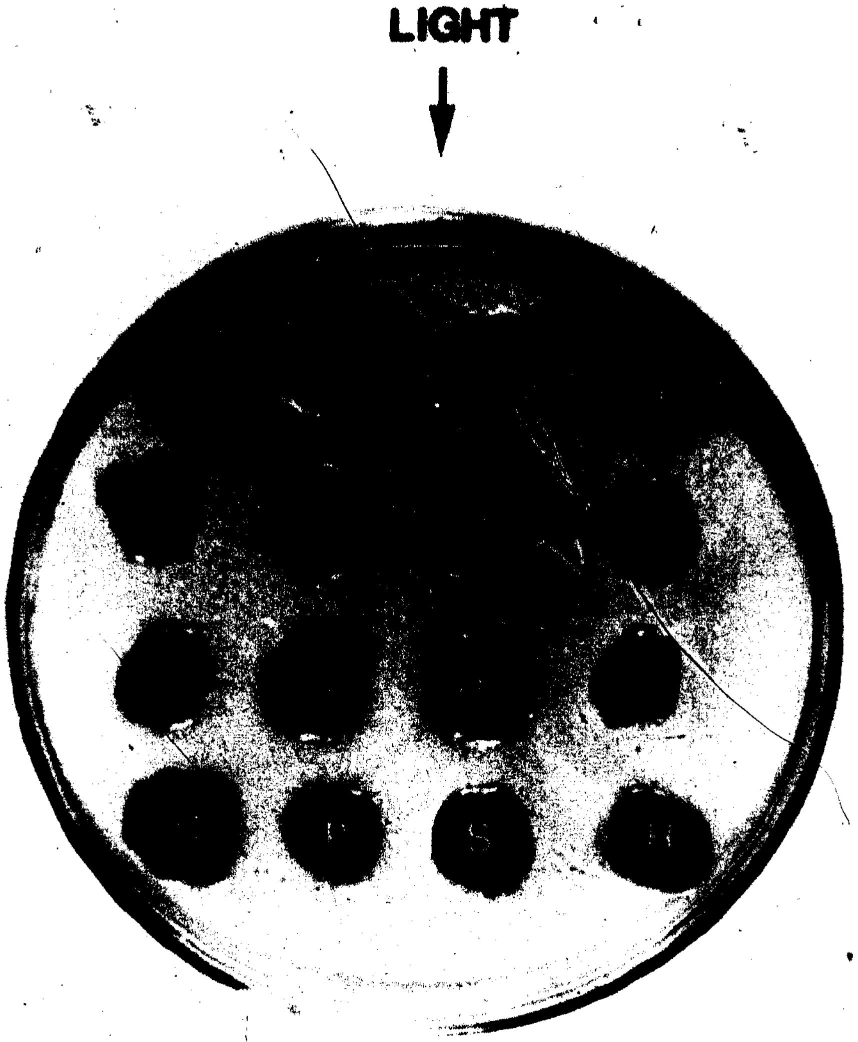
LARVAL SETTLEMENT EXPERIMENTS

Ascidian larvae were reared from gametes spawned in the laboratory or removed by dissection from reproductive adults, according to methods described in detail in chapter 2. Cultures were maintained in dishes of filtered seawater cooled in shallow seawater tables. Only active, normal-looking, tadpoles were used for behavioral experiments.

Three or 4 replicates of each substatum type were offered to tadpoles in each experimental dish. Pieces of ascidian tunic were cut to uniform size (1 cm diameter) with a cork borer, and non-ascidian substrata (e.g., rocks, shell) were either broken to comparable size or collected in the proper size already. Rocks were collected from the low intertidal or high subtidal zone, and except when cleaned off for specific experiments, were fouled with bacteria and small amounts of filamentous algae. Three or 4 replicates of each substratum type were arrayed in a randomized latin square in the bottom of a large petri dish (Fig. 18) and held in place with agar. A 2-3 mm layer of agar (1 g boiled with 30 ml millipore filtered seawater) was poured in the dish first, then substrata were arranged before it gelled. After the dish was cool, seawater and several hundred tadpoles were added and the dish was placed in a shallow seawater table until all or most larvae had settled. A 100 W incandescent bulb, 50 cm overhead, provided constant illumination which was supplemented during the daytime by the normal room-light of the laboratory, most of which entered from large south-facing windows (Young and Chia, 1982). Thus, strong shadows were eliminated by the overhead light, but a definite light gradient was still present in the dish for at least half of each day. The dishes were oriented with replicate rows of substrata aligned parallel to the window, so that the effects of light and substratum could be studied simultaneously. Laboratory light intensities on a typical day were in the range of those measured between 5 and 10 m depths in the field (Young and Chia, 1982).

Data from these experiments were cast into contingency tables and analyzed by goodness of fit statistics in such a way as to test three null hypotheses: 1) larvae are distributed uniformly among the various substrata offered, 2) larvae are distributed uniformly along the light gradient in the dish, and 3) the two factors, light and substratum, are independent in their effect on larval settlement distributions. The first two hypotheses were tested by comparing observed distributions to the appropriate uniform distribution (Snedecor and Cochran, 1967) using the log-likelihood goodness of fit test, "G" (Sokal and Rohlf, 1981). This test was chosen over the conventional chi-square

Figure 18. Setup for substratum choice experiments, showing four replicate rows with four substrata per row, arranged in a latin square and placed in a light gradient. H: *Halocynthia igaboja* tunic disk. P: *Pyura haustor* tunic disk. R: rock. S: *Chlamys sp.* shell fragment.


LIGHT
P
S

statistic because of its additive properties. A typical analysis was run as follows. Each row of substrata in the dish was intitially treated as a single replicate. The total number of larvae settled in the row was divided by the number of substratum choices (3 or 4) to give the expected number of larvae per substratum. A "G" value was computed for the replicate (row) using these expected values; all of the replicate "G" values were summed to obtain an overall value with n(k−1) degrees of freedom, where n is the number of replicates and k is the number of substrata in each row. The same procedure was repeated for each column to obtain a statistic testing the effect of light. Finally, a row by column test of independence was conducted in which expected values were estimated from the marginal totals (Sokal and Rohlf, 1981; Pielou, 1977).

Delay of metamorphosis was documented by offering only one substratum to the larvae at a time and plotting the number settling after various periods. Three types of dishes were run, each replicated twice: rock, ascidian tunic, and no substratum other than the glass dish. Parallel setups were run in continuous light and continuous dark in adjacent regions of the seawater table in order to control for the possibility that larvae delay metamorphosis when shadows (cast by the substrata) were not present. In these experiments, substrata were not embedded in agar. The dishes contained 10 ml of seawater.

RESULTS

ASCIDIAN SPATIAL DISTRIBUTION

Spatial distribution, or pattern of ascidians may be considered on numerous scales, from microscopic to zoogeographical. In order to discuss pattern, it is therefore important to define the scales of intest. I will consider two scales of pattern, to be referred to henceforth as "large scale pattern" and "small scale pattern". Large scale pattern is measured in hundreds of meters to kilometers, and refers to density differences between habitat types or between sites of a given habitat type. Small-scale pattern, or within-site pattern, is on a scale of meters. By this definition, therefore, ascidians occurring at high and low densities on a single underwater cliff would exhibit "small-scale" aggregation, while ascidians occurring abundantly on cliffs and rarely on mud would be clumped on a "large scale". In the present study, the parameters by which spatial pattern will be defined include: 1) density differences between areas of high and low abundance ("intensity" in the terminology of Pielou, 1977), 2) the sizes of aggregations (Pielou's "grain") and 3) the specific substrata used by ascidians. While all 3 parameters are relevant to the discussion of small-scale pattern, only the first is easily considered on a large scale, because of the difficulty of obtaining population estimates from a large enough sampling of sites.

Large Scale Pattern.

Figure 19 shows the proportion of the fauna at each of 14 study sites composed of ascidians in the orders Phlebobranchia and Stolidobranchia. While both phlebobranchs and stolidobranchs are found on soft sediment and on floats, most of the rocky sites are dominated by stolidobranchs. Three sites, Bell Island, Long Island and Shady Cove are exceptions to this general pattern, with the phlebobranch *Chelyosoma productum* appearing at Long Island and Bell Island, and an aggregation of *Corella inflata* being found in the survey at Shady Cove. Dives at a number of additional sites where quantitative data were not taken, however, confirmed the general impression that stolidobranch dominated sites are most typical of the San Juan Islands.

Although eight of the 13 species in the local fauna are stolidobranchs, only 4 of these, *Pyura haustor, Halocynthia igaboja, Boltenia villosa*, and *Cnemidocarpa*

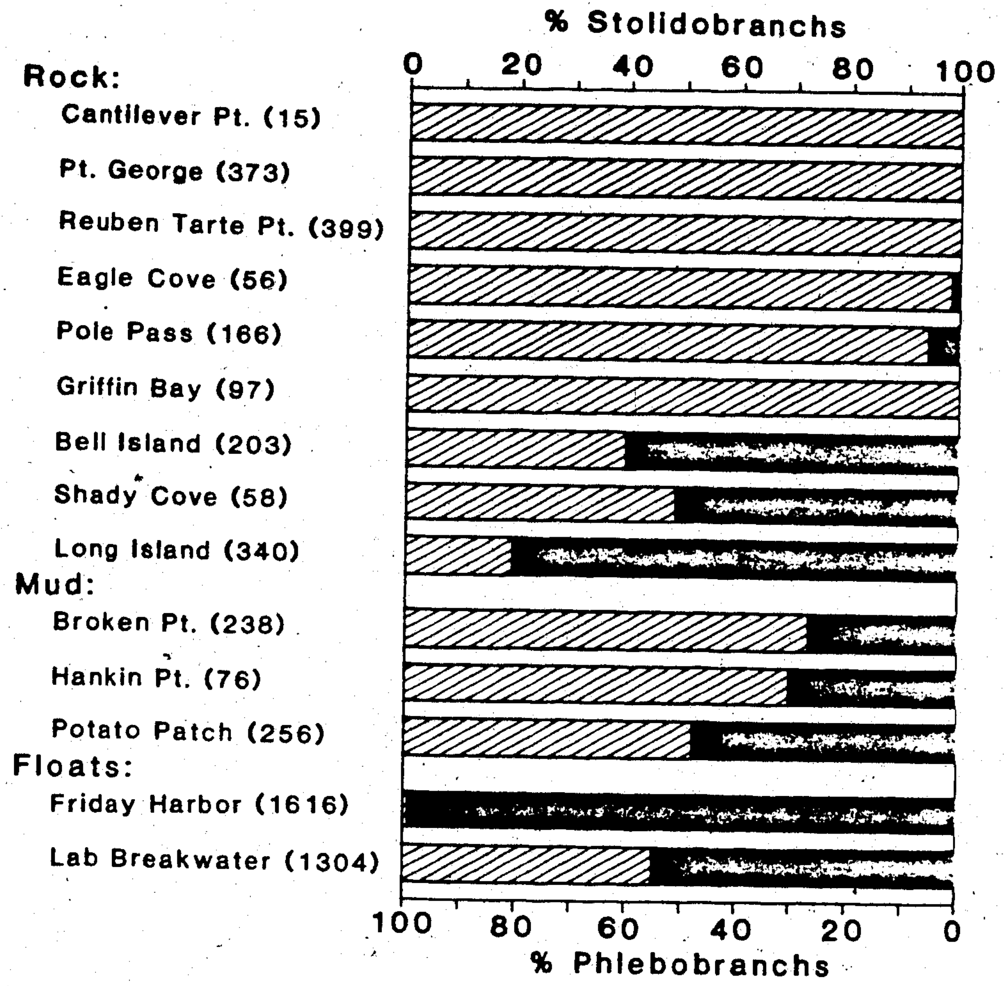


Figure 19. Proportion of ascidian fauna composed of phlebobranchs (black portion of each bar) and stolidobranchs (hatched portion) in subtidal rock, mud and float habitats. Number of ascidians on which each percentage is based is shown in parentheses.

finmarkiensis made up the majority of the rocky bottom fauna (Table X). *Styela gibbsii* were also found in small numbers at most sites. The dominant phlebobranch occurring on rock and mud was *Chelyosoma productum* (Table XI).

On floating docks, two plebobranchs, *Chelyosoma productum* and *Ascidia callosa* dominated the fauna, with total ascidian densities often exceeding 1500 animals per m^2 (Table XI). The difference in species composition between the two docks may seem surprising in light of their proximity (< 500 m apart, in the same bay), but the sites differed greatly in age. While the Friday Harbor Town docks have been in place for decades, the Laboratory Breakwater was installed in October, 1978; less than 3 years before the samples on which density estimates are based were taken. Numerous workers (Sutherland, 1974, 1978; Dean and Hurd, 1980; Mook, 1981) have demonstrated that development of epifaunal communities is largely dependent on the larvae which are in the plankton at the time the substratum is introduced. *Boltenia villosa* and *Styela gibbsii* colonized the breakwater first, and dominated much of the available substratum (Fig. 20). However, at the present time, numerous small *C. productum* are growing, so *Chelyosoma* is now the most common ascidian at this site, as it is on other docks in the region.

More striking than the between-dock difference is the paucity of ascidians at the Cantilever Pt. site, which is situated only a few hundred meters from the laboratory breakwater. Both sites are on the same shoreline, facing the same direction. Nevertheless, ascidians of all common species are as much as three orders of magnitude more abundant on the docks. *Pyura haustor* is the most common species at Cantilever Pt., despite the fact that breeding populations on the nearby docks are much higher for *Chelyosoma productum*, *Ascidia callosa*, *Boltenia villosa* and *Styela gibbsii*. Furthermore, phlebobranchs were not found at Cantilever.

The highest ascidian density at any of the rocky sites studied occurred on the rock outcropping at Pt. George between 15 and 30 m depths. Notwithstanding its relatively protected position in a small cove, the site experienced strong, regular tidal currents. While diving on the outcropping just before or after slack tide, I often noted that the current direction was not the same on all parts of it. The face of the cliff and the middle of the outcropping experienced horizontal currents as expected. However, the cobble fields at the sides of the reef were often swept by currents which ran nearly

Table X.

Population densities ($\bar{x}$ ± S.E.) of stolidobranchs by sites. Genus and species names are abbreviated. Sample sizes as in Table X. +: species present, though not appearing in samples.

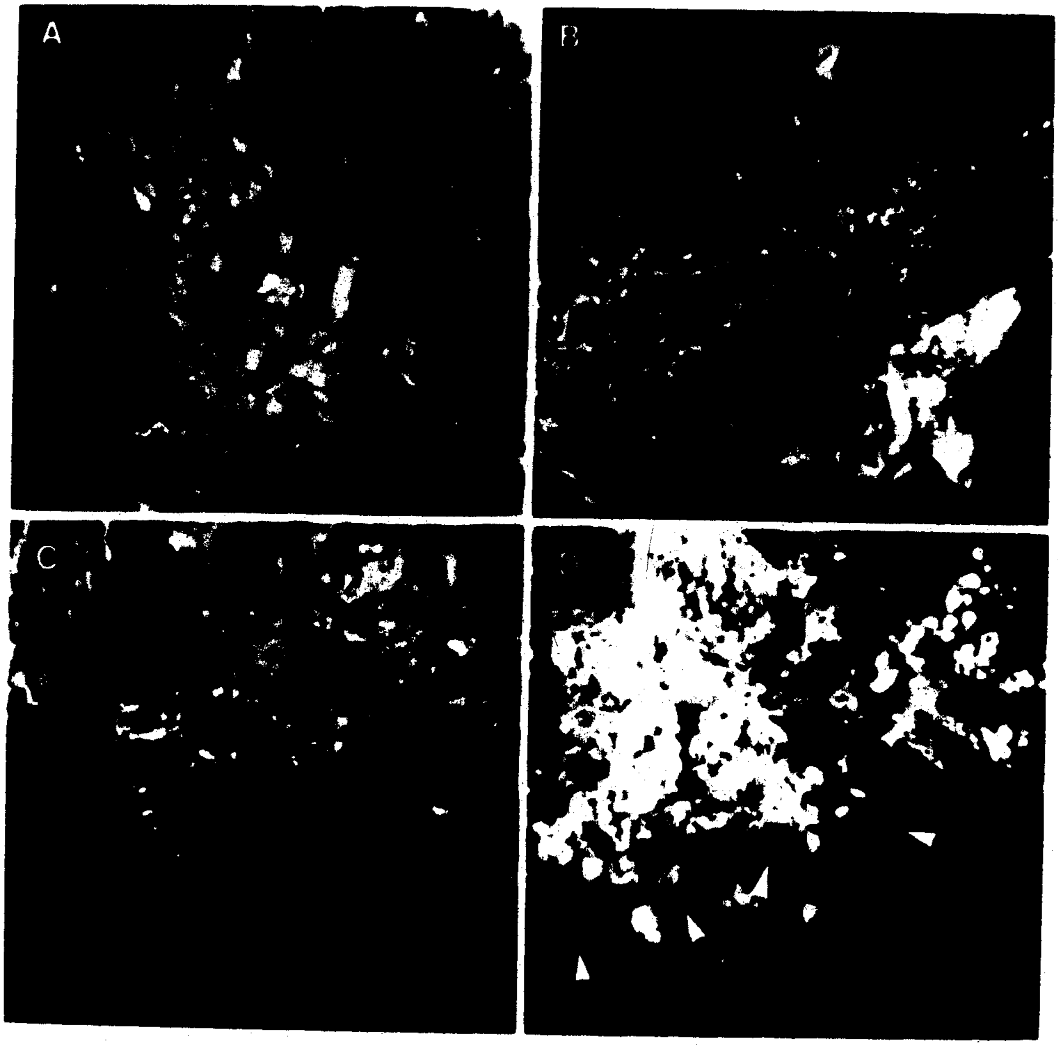
	Species							
Site	Ph	Pm	Bv	Hi	Ha	Sg	Sc	Cf
<u>Rock</u>								
Cantilever wall	2.0 ± 1.3	0	0.8 ± 0.5	0.8 ± 0.5	0	0	0	0.4 ± 0.4
Pt. George wall	58.4 ± 8.6	0	4.4 ± 1.7	8.4 ± 1.9	0	+	0	2.0 ± 1.0
Reuben Tarte reef	32.7 ± 5.9	0	3.4 ± 1.2	1.8 ± 0.6	+	1.4 ± 0.8	0	0.2 ± 0.2
Eagle Cove reef	2.2 ± 0.7	0	+	0	0	0	0	0.1 ± 0.1
Long Island wall	9.1 ± 3.9	0	0.8 ± 0.6	+	0	0	0	+
Bell Island	0	+	0.3 ± 0.2	0.1 ± 0.1	+	+	+	1.9 ± 0.8
Pt. George slope	89.0 ± 14.0	0	10.0 ± 2.7	4.0 ± 1.4	+	1.0 ± 0.7	0	0.5 ± 0.5
Reuben Tarte cobble	6.3 ± 1.3	+	0.1 ± 0.1	0.9 ± 0.3	0	0.3 ± 0.4	+	+
<u>Mud</u>								
Broken Pt.	6.8 ± 1.6	0	1.1 ± 0.4	0	0	1.7 ± 0.8	0	0
<u>Docks</u>								
Friday Harbor	+	0	20.0 ± 7.7	0	0	16.0 ± 6.5	0	+
Lab Breakwater	28.7 ± 8.0	0	76 [illegible] 10.5	0	+	417.0 ± 133.5	0	+

Table XI.

Population densities ($\bar{x}$ ± S.E.) of Phlebobranchs by site. Genus and species names abbreviated. +: species present, though not appearing in samples.

Site	Sample Size	Species				
		Ci	Cw	Cp	Ac	Ap
<u>Rock</u>						
Cantilever wall	40	0	0	0	0	+
Pt. George wall	40	+	0	0	0	+
Reuben Tarte reef	20	0	0	0	0	0
Eagle Cove reef	38	0	0	0.1 ± 0.1	0	0
Long Island wall	15	0	0	20.3 ± 7.2	0	0
Bell Island	32	0.1 ± 0.1	+	4.6 ± 1.9	+	+
Pt. George slope	32	0	0	0	0	+
Reuben Tarte cobble	15	0	0	0	0	0
<u>Mud</u>						
Broken Pt.	33	0	0	5.1 ± 1.0	0	0
<u>Docks</u>						
Friday Harbor	4	68.0 ± 62.8	+	1340.0 ± 244.1	140.0 ± 42.0	+
Lab Breakwater	10	+	+	542.0 ± 88.9	96.2 ± 27.1	+

Figure 20. Aggregations of ascidians at subtidal sites. A: large clump of *Pyura haustor* on the Pt. George Wall at 25 m depth. B: close-up of a juvenile *Pyura haustor* attached to the tunic of a large adult. C: Underside of the Friday Harbor Laboratory breakwater, showing high density assemblage dominated by *Styela gibbsii* and *Boltenia villosa*. Numerous small *Chelyosoma* are also present, though not easily visible in the photograph because of their size and proximity to the substratum. D: Large clump of the barnacle, *Balanus nubilus* supporting an aggregation of *Corella inflata* on its underside. Arrows indicate *Corella* individuals. Pt. George, 15 m depth.


A
B
C

perpendicular to the shore. This suggested the presence of an eddy in the cove, which may be driven by the stronger tidal currents outside the cove. Such an eddy could concentrate ascidian larvae and help account for the much higher densities of ascidians on the cove outcropping than on comparable reefs nearer the edges of the cove.

Pyura haustor, the most abundant ascidian at most rocky subtidal sites, was inexplicably absent from Bell Island, and also rare at some high current sites where formal density measurements were not taken (Turn Island and Shady Cove).

Small Scale Pattern

It was possible to quantify small-scale aggregation only at certain sites and only for those species occurring at relatively high densities. Three major patterns were seen: single species aggregations, multiple species aggregations, and solitary individuals. In the following account, species are described briefly and classified into one of these categories. However, it should be borne in mind that while each species has a characteristic pattern, the pattern may be greatly modified in some circumstances.

Single Species Aggregations

Five species characteristically form single species aggregations. All of these also occur as solitary individuals where population densities are low, and most may also be members of multiple species aggregations.

Pyura haustor.

The commonest ascidian in most rocky subtidal sites is *Pyura haustor*, an irregularly shaped reddish ascidian with long siphons and a tough leathery tunic. Distributions of *P. haustor* at 6 sites are compared with Poisson distributions in Figures 21 and 22. Additional data on spatial pattern have been reported elsewhere (Young, 1978). Mean number of animals per quadrat ranged from 0.7 at Eagle Cove to 8.2 at Reuben Tarte. One common feature was apparent at all densities: many more quadrats were on the upper tail of the distribution (i.e. containing numerous *P. haustor*) than would be expected by chance. The distribution of *Pyura haustor* must therefore be considered strongly aggregated. Although some of this aggregation unquestionably results from a common selection of cryptic sites (chapter 2), a large part of the phenomenon seems to

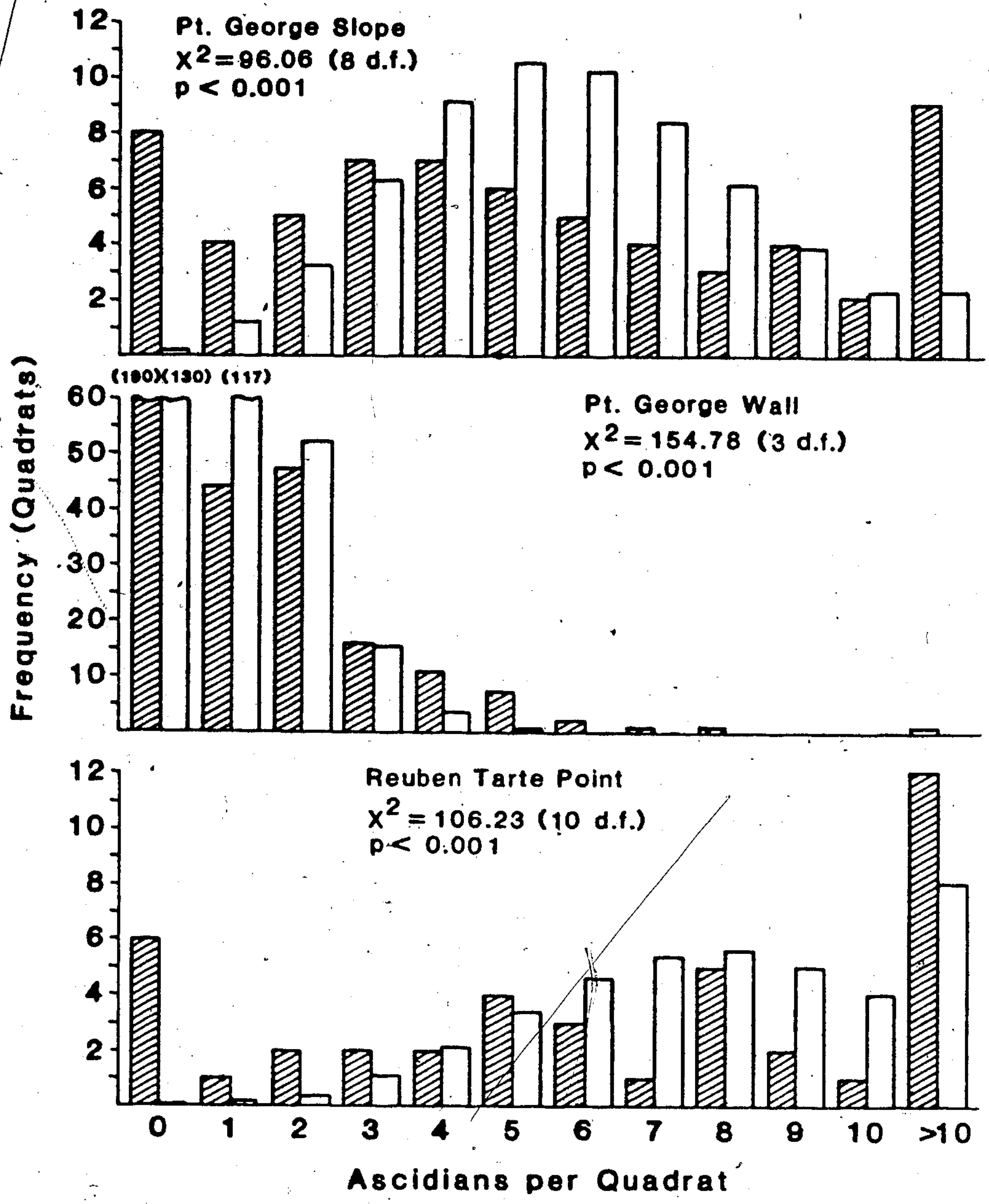


Figure 21. Frequency distributions of *Pyura haustor* per quadrat in transects taken at three rocky subtidal sites. Cross-hatched bars: observed frequencies. Open bars: expected (Poisson) frequencies.

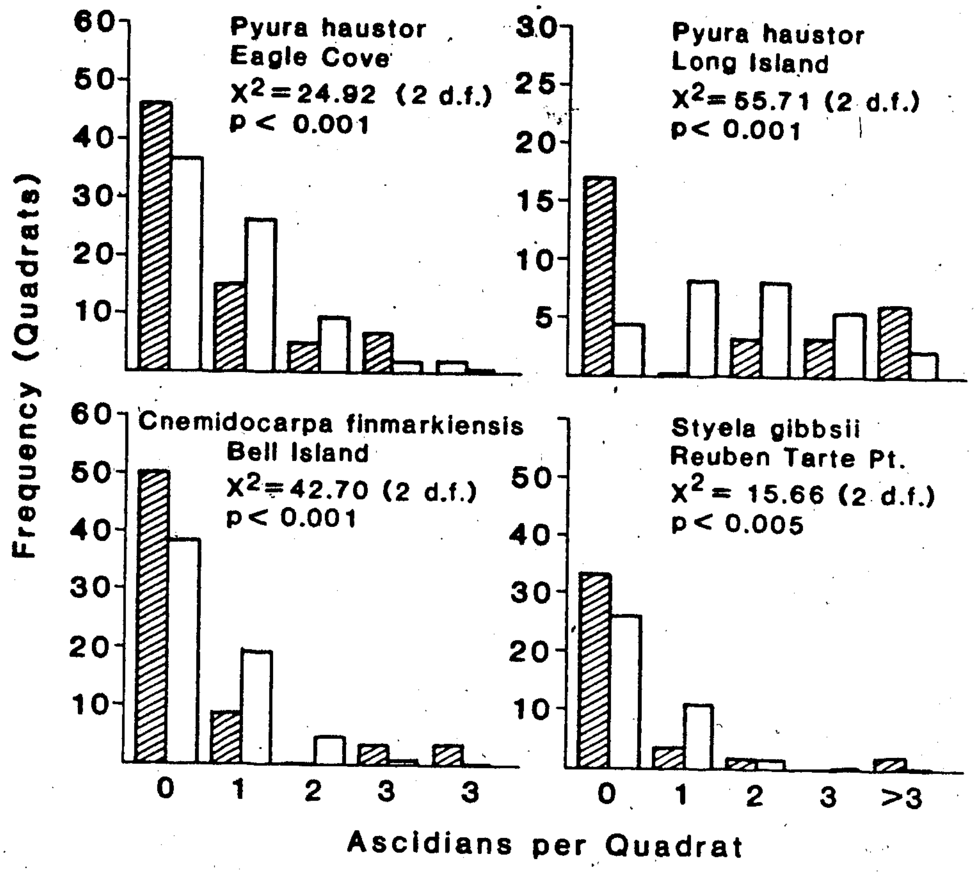


Figure 22. Frequency distributions of animals per quadrat for *Pyura haustor*, *Cnemidocarpa finmarkiensis*, and *Styela gibbsii* in the rocky subtidal. Cross-hatched bars: observed frequencies. Open bars: expected (Poisson) frequencies.

be attributable to gregariousness. Substratum analysis (Fig. 23) showed that a substantial part of the pooled population was attached to conspecifics or related ascidians rather than rock.

Solitary individuals of *Pyura haustor* were most common in cracks between rocks, or under overhangs (chapter 2). When occurring in cobble fields, they may cement together pebbles and cobbles of all sizes, and thereby stabilize the substratum. A typical small clump is formed of one or a few large animals at the base and several smaller animals settled either directly on the tunic of the basal individuals, or around the edges such that they are attached both to the rock and the *P. haustor*. A particularly large clump is shown in Figure 20. Such clumps form discrete masses projecting from the walls of cliffs, or more commonly, from cracks in or between rocks.

A large aggregation may contain well over 50 individuals, with animals of all sizes represented, suggesting that the population consists of several generations of settling larvae. Such aggregations appear on docks, pilings and mud bottoms as well as on rocks. Figure 24 shows the size distribution of animals from two large clumps collected from a dock in Garrison Bay. Although there appears to be a slight mode at about 24 g, all sizes are represented from <1 g to 56 g, and the size class with the most individuals was that composed of recruits, nearly all of which were attached directly to adult *P. haustor*.

P. haustor recruited to the Lab Breakwater about 1.5 years after the breakwater was installed. Barnacles (*Balanus crenatus*) and other ascidians (especially *Styela gibbsii* and *Boltenia villosa*) had already colonized nearly all of the concrete substratum by this time. Consequently, most *Pyura* on the breakwater were attached to barnacle shells (Figure 25). By contrast, *P. haustor* on the much older Friday Harbor Town Docks as well as docks in Mitchell Bay (Snug Harbor Marina), Roche Harbor, and Garrison Bay, were distributed in discrete, single species aggregations as in the rocky subtidal zone. I have followed informally several such aggregations on docks for over 5 years. These clumps have remained in essentially the same place, though some of the older individuals have died and been "replaced" by new recruits. As the animals grew, they spread forth their attachment areas to surrounding substrata, and changed shape to avoid being overgrown by other individuals. Death of the older, basal animals had little effect on stability of the clumps, since most of the smaller individuals were attached to numerous others as well

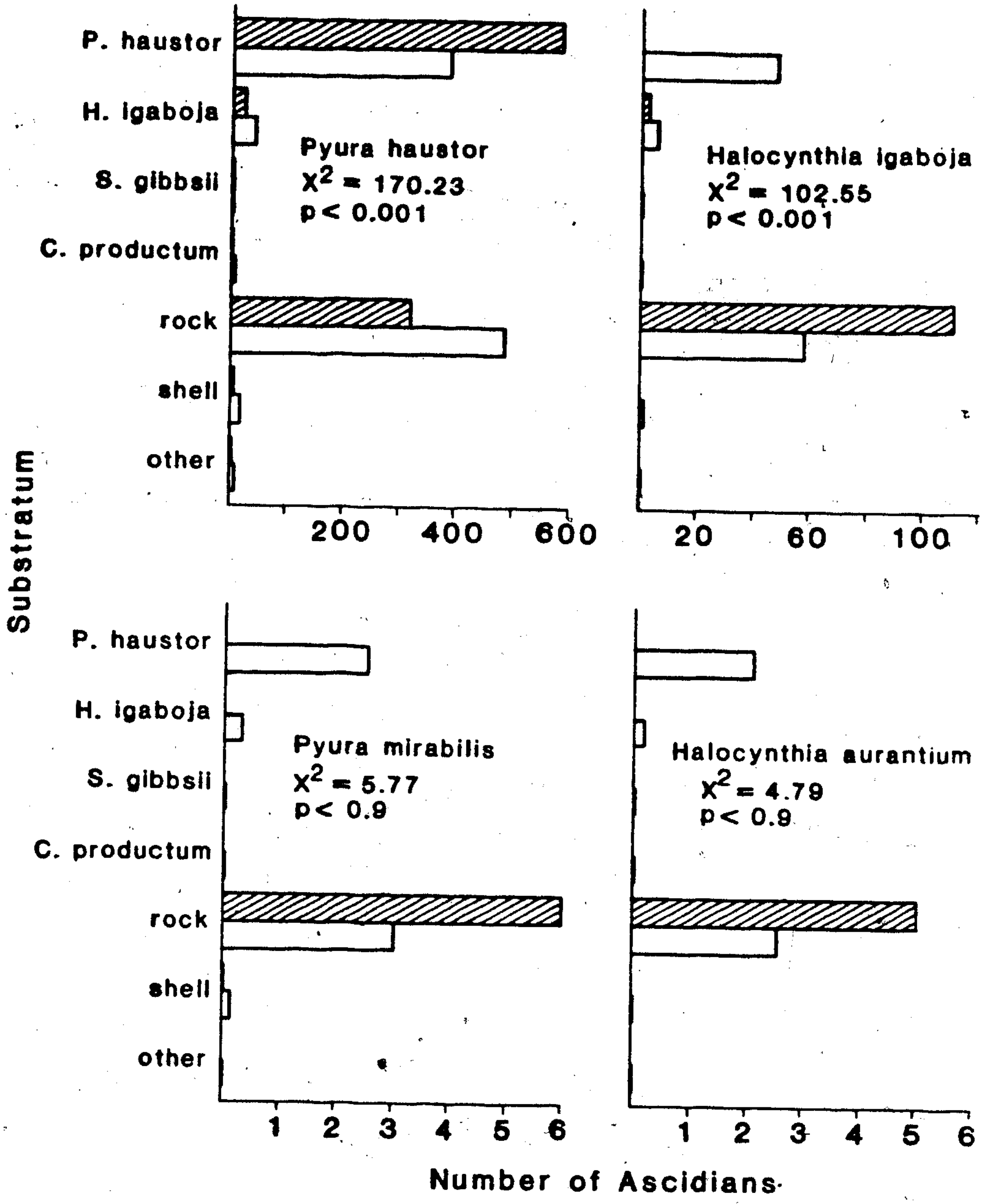


Figure 23. Distribution of ascidians, Family Pyuridae, by substratum, pooled for all rocky subtidal sites. Crossed-hatched bars: observed distributions. Open bars: expected distributions based on substrata used by all ascidian species combined.

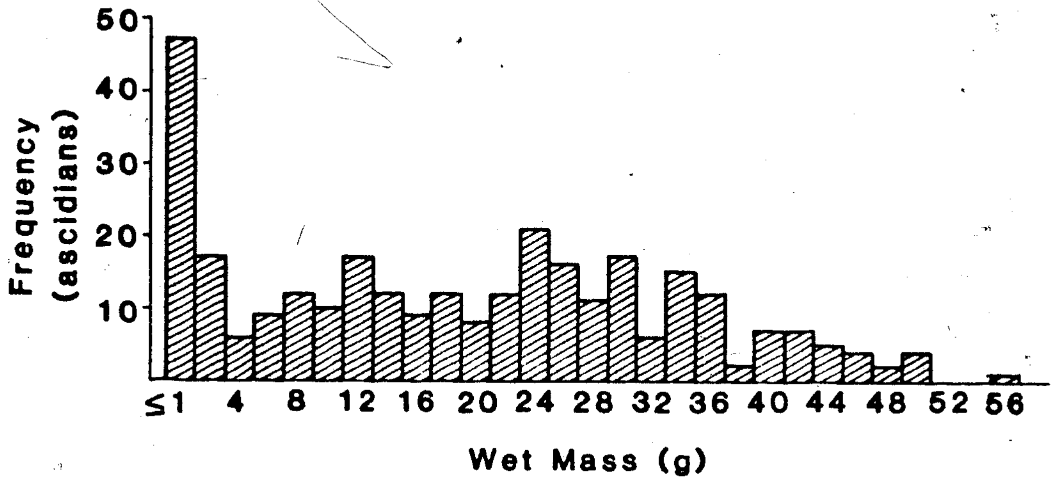


Figure 24. Size-frequency distribution of 301 *Pyura haustor* individuals in two large, discrete clumps collected from a dock in Garrison Bay, San Juan Island, on April 12, 1978. Numbers on the horizontal axis represent upper limits of 2 g size classes. For example, the bar labelled "12" includes all animals weighing between 10.1 and 12.0 g, inclusive.

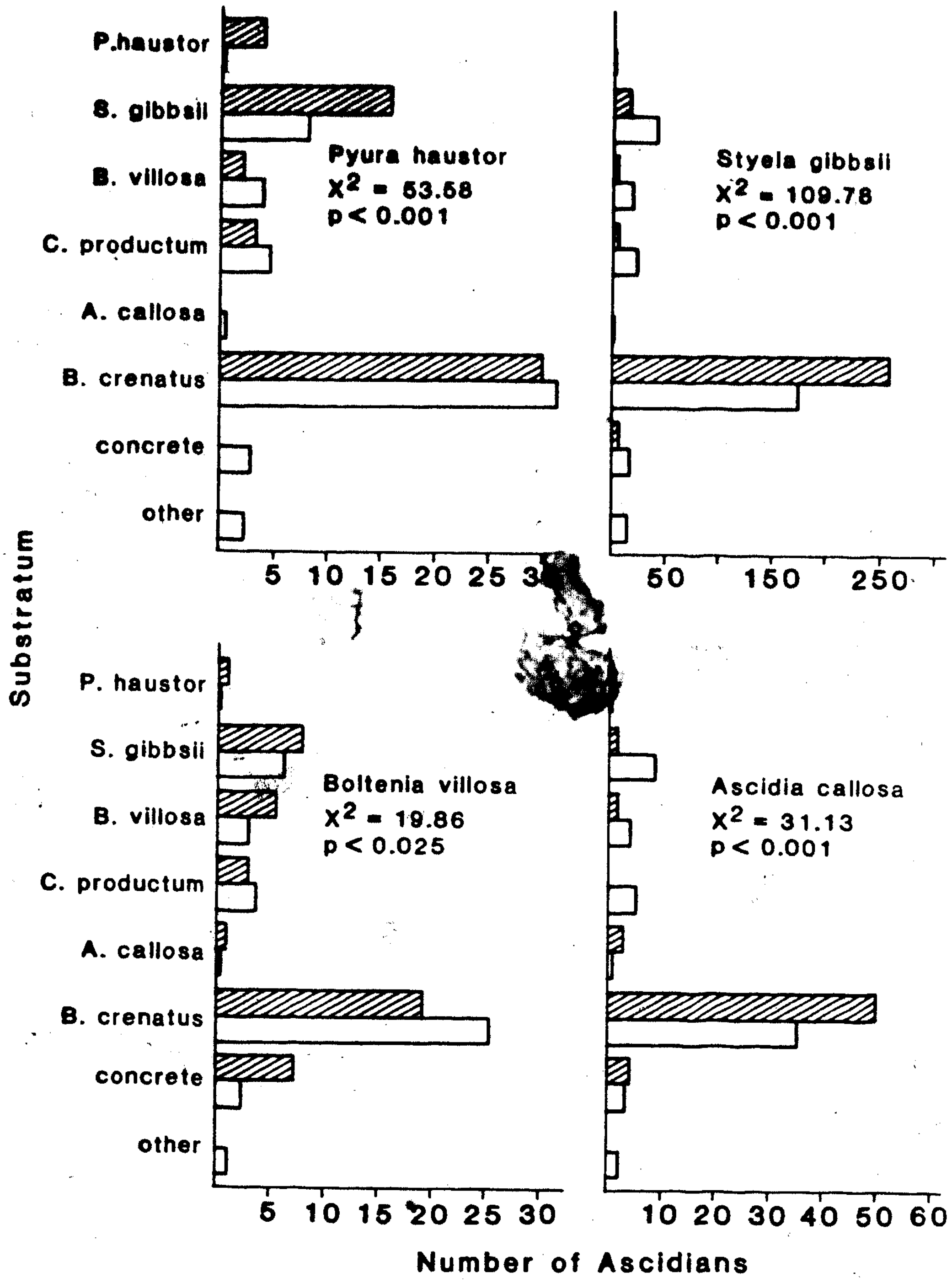


Figure 25. Ascidian distribution by substratum for four species collected from the Friday Harbor Labs Breakwater. Cross-hatched bars: observed distributions. Open bars: expected distributions based on data pooled for all ascidian species in this habitat.

as to the primary substratum.

Chelyosoma productum.

The most common of San Juan Island phlebobranchs, the corellid *Chelyosoma productum* also occurred in discrete aggregations. Frequency distributions of two populations, one at 0.9 animals per quadrat (Bell Island) and one at 9.3 per quadrat (Long Island) both departed significantly from the expected random distributions, and both have many quadrats containing multiple individuals (Figure 26). Substratum analysis for animals occurring at rocky sites (Figure 27) and on floats (Figure 28) are suggestive of gregarious behavior. Although many animals were attached to rock and other substrata, more than expected used conspecifics as attachment sites. A relatively large number of animals were on shells of the giant barnacle, *Balanus nubilus* at the Long Island site. Here, on a wall at 30 m depth, the cover of sessile animals was so dense that virtually no bare rock was exposed, and *Balanus* shells were the most abundant hard substratum. At Bell Island, where epifauna was more sparse and *Chelyosoma* density was lower, nearly all individuals were attached to rock. Much of the aggregation seen in the Bell Island population can probably be attributed to differential suitability of habitat. Along the bell Island transect, *C. productum* and nearly all other ascidians occurred on the larger outcroppings rather than on large cobbles or boulders between outcroppings (Fig. 29).

On floating docks, *C. productum* frequently forms dense aggregations or hummocks, in which only a few individuals are found on the primary substratum, and most individuals use other *C. productum* as living sites (Fig. 28). During each of the last 5 years, recruitment has occurred predictably in February or March, and many recruits settled directly on established adults.

Ascidia callosa.

Like *C. productum*, the brown, nondescript phlebobranch, *Ascidia callosa*, may form dense aggregations or hummocks on the undersides of floating docks. In the breakwater substratum analysis (Fig. 25), this phenomenon was not apparent, since the population was young and the substrata available at the time of recruitment consisted predominantly of the barnacle *Balanus crenatus* which had settled abundantly some months earlier. Based on observations of older populations at Mitchell Bay and Friday Harbor, I predict that in a few years, the substratum use will shift so that most *A. callosa*

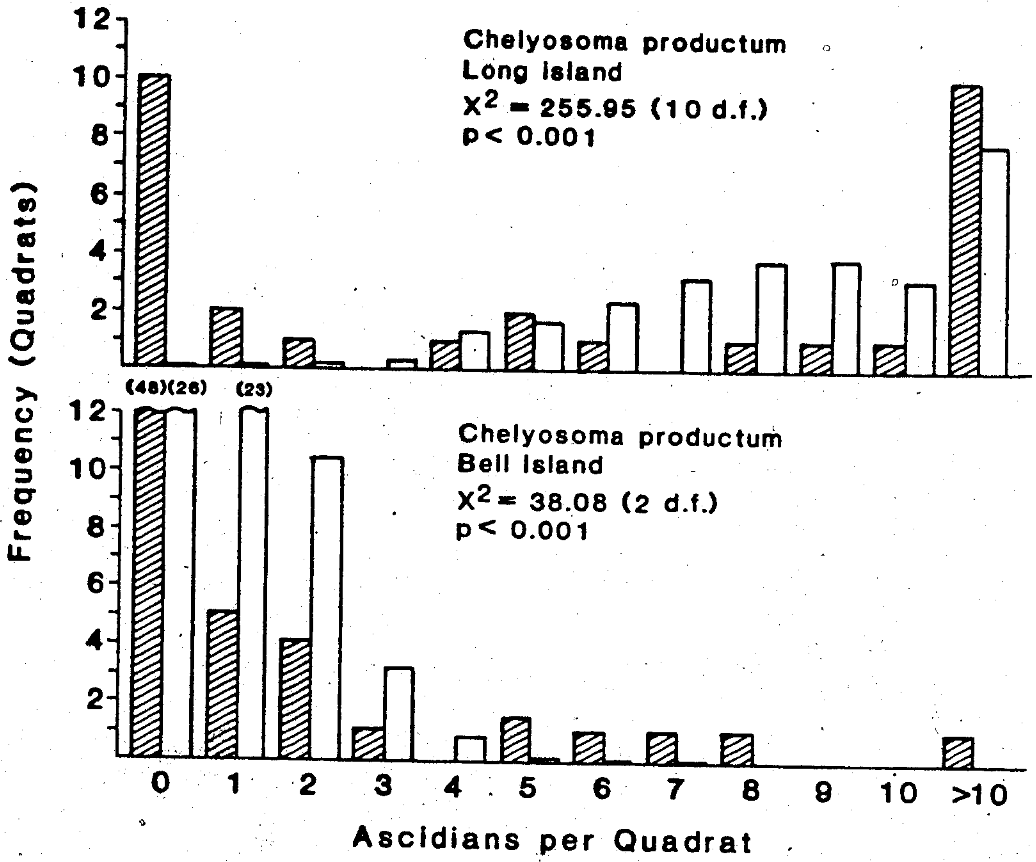


Figure 26. Distribution of *Chelyosoma productum* per quadrat in two subtidal transects. Cross-hatched bars: observed frequencies. Open bars: expected (Poisson) frequencies.

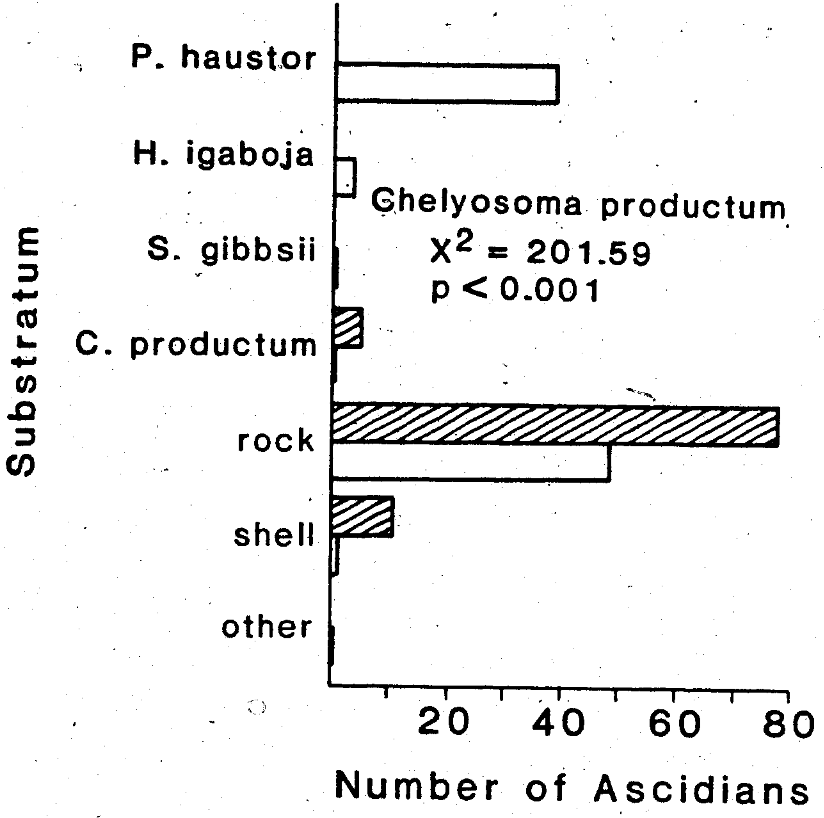


Figure 27. Substrata used by *Chelyosoma productum* at all rocky subtidal sites. Cross-hatched bars: observed distributions. Open bars: expected distributions based on substrata used by ascidians of all species.

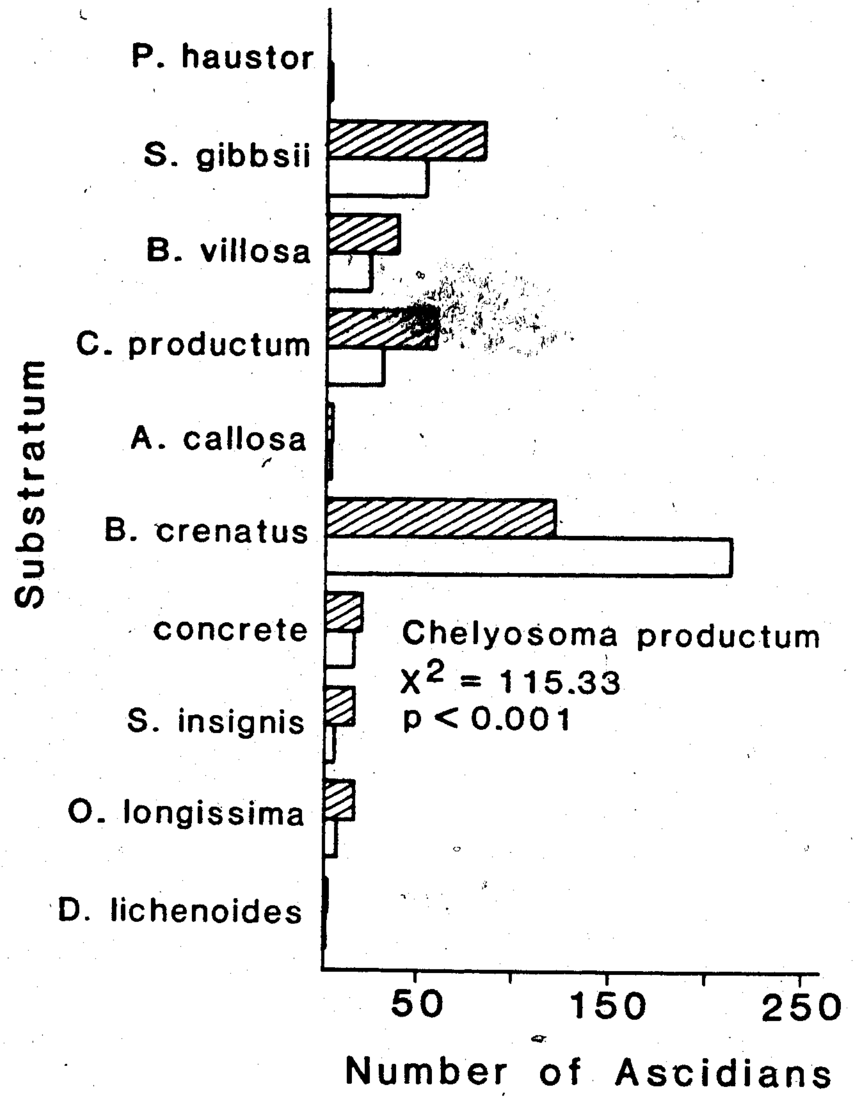


Figure 28. Substrata used by *Chelyosoma productum* on the Friday Harbor Laboratory Breakwater. Cross-hatched bars: observed distributions. Open bars: expected distributions. Non-ascidian species abbreviated in the figure are the balanomorph barnacle, *Balanus crenatus*, the sabellid polychaete, *Schizobranchia insignis*, the thecate hydrozoan, *Obelia longissima*, and the cheilostome bryozoan, *Dendrobeania lichenoides*.

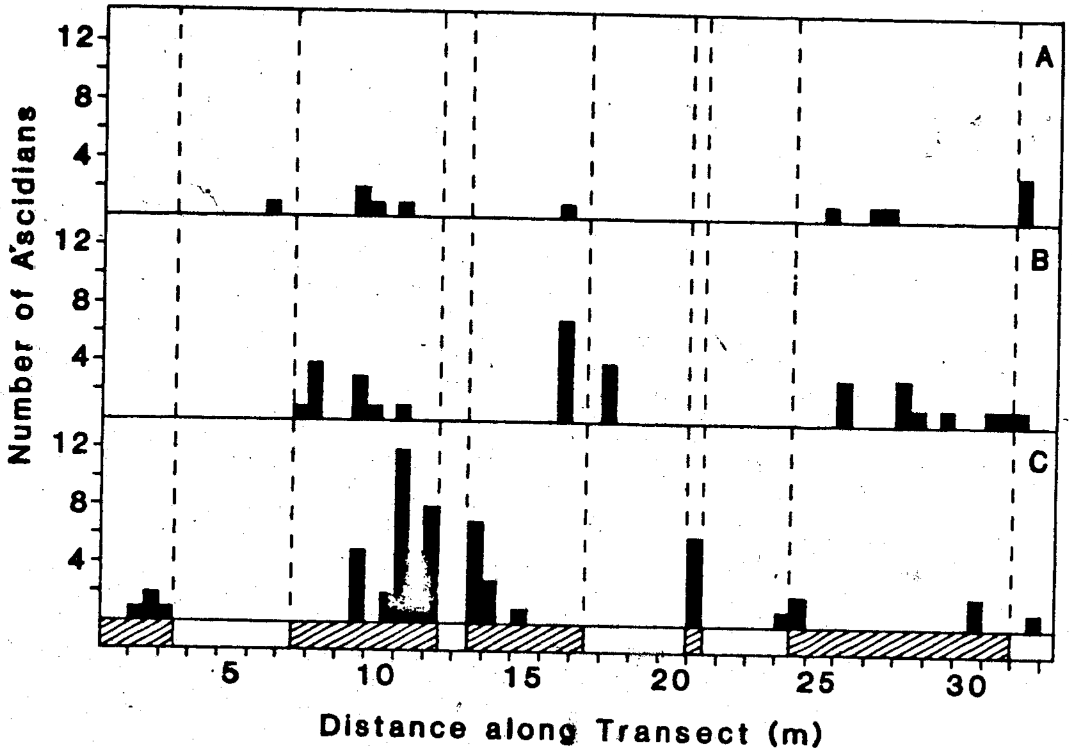


Figure 29. Distribution of ascidians along a transect at 10 m depth on the West side of Bell Island. Crossed-hatched areas along the bottom indicate regions where solid rock completely filled the 50 by 50 cm quadrat; open areas indicate regions of cobble or small boulders. Vertical dotted lines extend the regions of substratum type onto the graphs proper. A: *Boltenia villosa, Halocynthia igaboja, Corella inflata, Corella willmeriana* and *Styela gibbsii*. B: Cnemidocarpa finmarkiensis. C: *Chelyosoma productum.*

are attached to conspecifics or to other ascidians such as *C. productum*.

As indicated in table XI, *Ascidia callosa* are exceedingly rare in the rocky subtidal zone. All of the individuals I found were located on the undersides of cobbles (chapter 2), attached directly to the rock (Figure 30), and occurring as solitary individuals.

Corella inflata.

Unlike the three species discussed above, aggregations of the small transparent phlebobranch *Corella inflata* are not formed by larvae attaching directly to established members of the population. Indeed in no instance did I encounter other ascidians attached to the *C. inflata* test. Where the species was present, however, large numbers were generally found in a small area, with animals being sparse in adjacent regions. Lambert (1968) attributed this clumping to the brooding behavior of the adults. Larvae are retained in the atrial chamber until just before settlement, so extended dispersal does not occur and the larvae settle near the adults. Based on my observations, Lambert's hypothesis seems reasonable. *C. inflata* seem to attach to substrata in proportion to their availability. Thus, in rocky sites, the most common substratum used was rock (Figure 30), and the animals generally occured under overhangs (chapter 2). Since small overhangs were often made of *Balanus nubilus* clumps, barnacle shells were frequently used substrata (Fig. 20). On the dock at Friday Harbor where sabellid polychaete tubes were extremely dense, most individuals were attached to these tubes.

Multiple Species Aggregations.

Most species of solitary ascidians may at one time or another appear in a multiple species aggregations. *Pyura haustor* in particular was often a major constituent of such clumps, either as the basal species or as an epizooite. However the one species most characteristially carrying epizooites and the two species characteristically occurring as epizooites are discussed under this classification.

Halocynthia igaboja.

Halocynthia igaboja is a large, round pyurid ascidian easily recognized by the numerous stiff spines which cover its entire tunic. Although never reaching the high densities seen in *Pyura haustor*, *H. igaboja* was common in the rocky subtidal, and was found at most sites. Even when occurring at relatively high densities (1.8 to 8.4 per m^2),

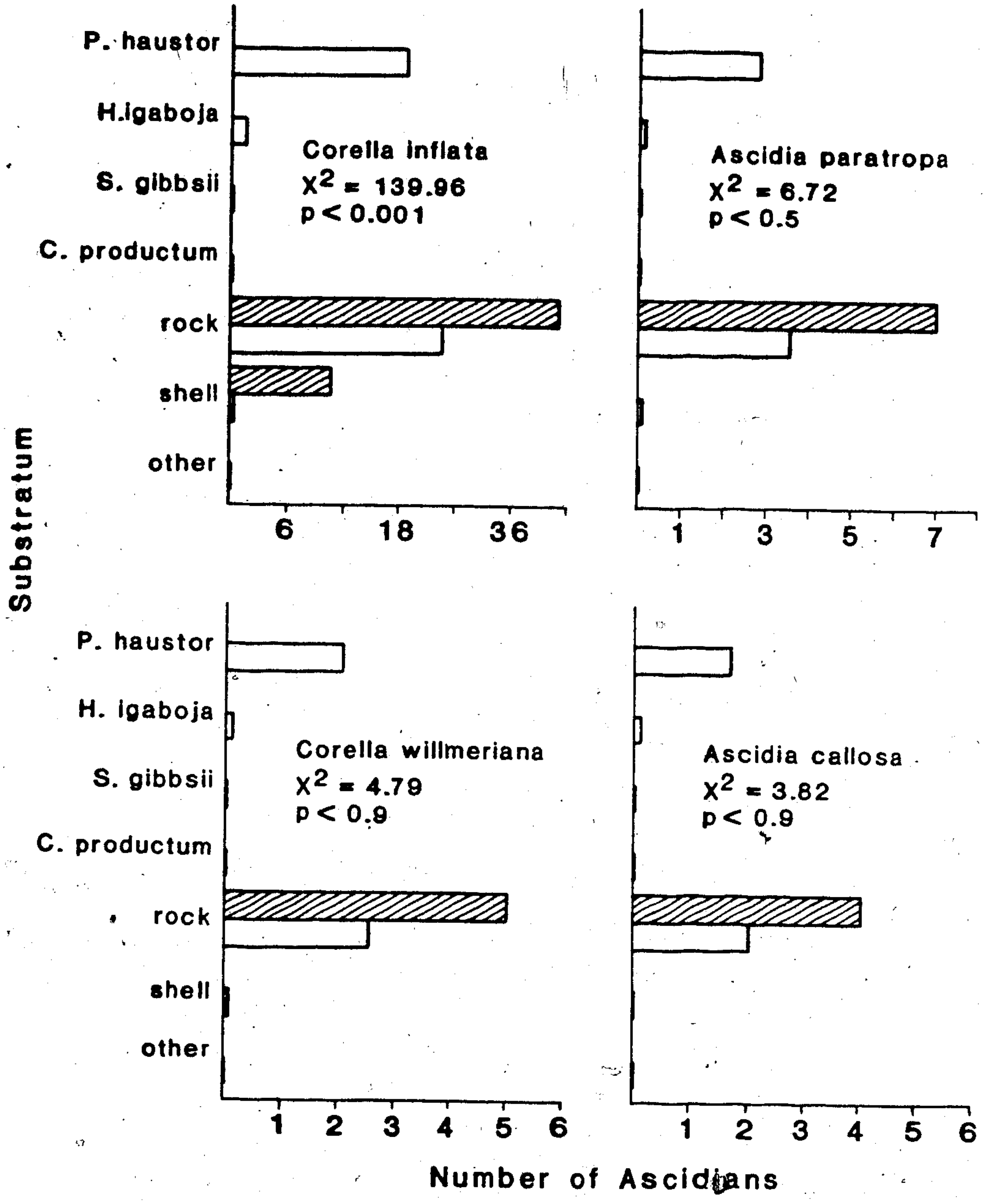


Figure 30. Distribution of four phlebobranchs by substratum, pooled for all rocky subtidal sites. Cross-hatched bars: observed distributions. Open bars: expected distributions calculated as in previous figures

the distribution was essentially random (Fig. 31). *H. igaboja* normally did not occupy cryptic habitats, though it sometimes nestled between cobbles (chapter 2), and was nearly always attached to rock substratum. (Fig. 23). In a few cases, I found individuals attached both to the rock and to another *H. igaboja*, and occasionally, a small individual attached to a larger one.

Halocynthia igaboja, with its dense spines, supported a diverse epifauna which included hydroids, brachiopods, bryozoans, and amphipods (personal observations) as well as a pycnogonid (R. Shimek, personal communication) and a number of ascidians. In July, 1981 I collected 24 individuals at random from Reuben Tarte Point in order to quantify the epizooites. The results are presented in table XII. There was an average of 3.72 epizooites per *H. igaboja*, and only 2 individuals were found which did not support any ascidian epizooites. Variance to mean ratio and the Index of Dispersion were computed for the various species appearing in the Reuben Tarte collection, considering each *H. igaboja* as a discrete habitat unit (Pielou, 1977). The distribution of each species reflected the specific substrata occupied within the clumps. Thus, *Pyura haustor* showed an aggregated distribution, due to its tendency to settle on conspecifics. Likewise, *Boltenia villosa* distribution was clumped because *B. villosa* were most commonly attached to the aggregated *P. haustor*. *Styela gibbsii*, *Styela coriacea*, and *Halocynthia igaboja* epizooites were distributed at random among the basal *H. igaboja* to which they were generally attached. Typical multiple species clumps with *H. igaboja* at the base are shown in Fig. 32. *Pyura haustor* were often members of such clusters. Frequently, they increased the size of an aggregation by several fold and in doing so, helped stabilize it by securing the attachment to rock around the edges. In large multiple species clumps, it was sometimes impossible to tell whether *H. igaboja* or *P. haustor* was the "founding" species.

Boltenia villosa.

This small, hairy, orange and stalked ascidian was commonly found on docks as well as in the rocky subtidal zone. Although it sometimes occurred on rock, a very large percentage of animals were found occurring as epizooites on either *Pyura haustor* or *Halocynthia igaboja* (Fig. 33). In terms of available surface area, rock substrata were nearly always much more common than the ascidians to which *B. villosa* attached,

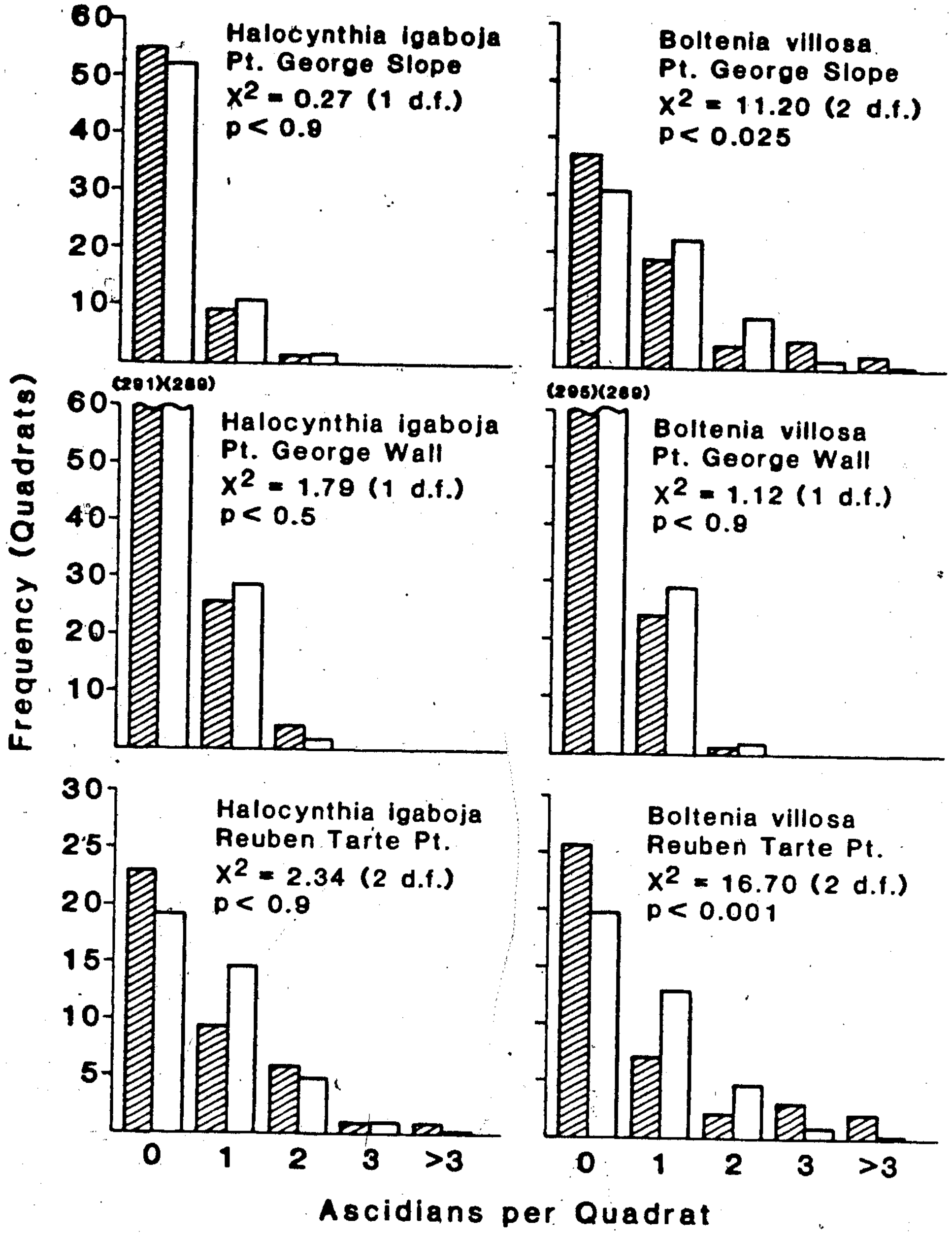


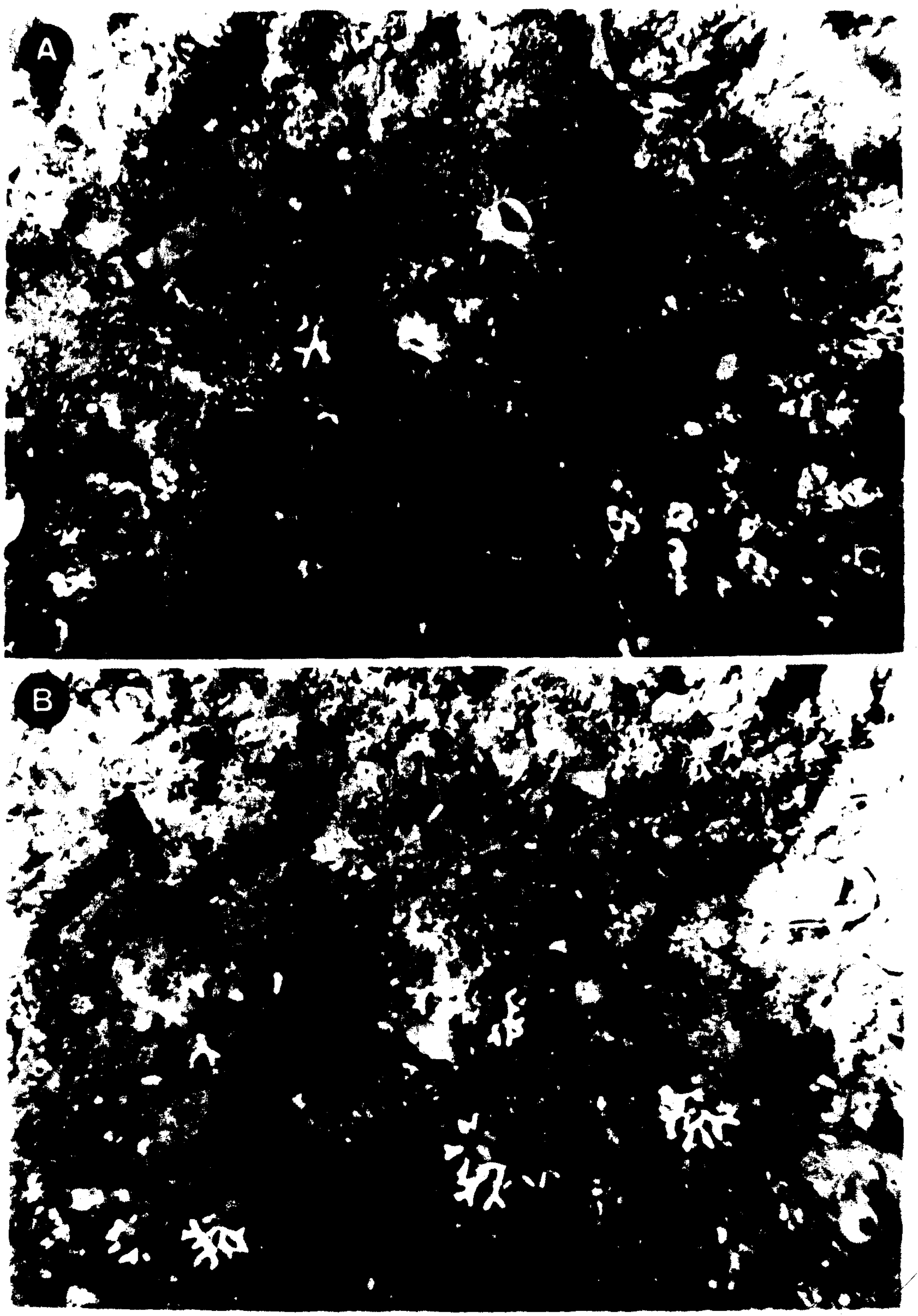
Figure 31. Frequency distributions of animals per quadrat for *Halocynthia igaboja* and *Boltenia villosa* at three rocky subtidal sites. Cross-hatched bars: observed frequencies. Open bars: expected (Poisson) frequencies.

Table XII.

Distribution of epizooic ascidians on 18 *Halocynthia igaboja* collected at Reuben Tarte Pt., July 23, 1981. "I" is the index of dispersion, $s^2/\bar{x}$ (n - 1). Significance levels obtained by comparing "I" with a chi-square distribution at 17 d.f. (Greig-Smith, 1964).

epizooite species	epizooites per clump ($\bar{x}$ ± S.E.)	$s^2/\bar{x}$	I	P
Halocynthia igaboja	0.22 ± 0.10	0.83	14.11	< 0.75
Pyura haustor	1.50 ± 0.53	3.39	57.63	< 0.005
Boltenia villosa	1.40 ± 0.41	2.06	35.02	< 0.005
Styela gibbsii	0.50 ± 0.20	1.47	24.99	< 0.10
Styela coriacea	0.10 ± 0.07	1.04	17.68	< 0.5
(Total epizooites)	3.72 ± 0.94	4.32	73.44	< 0.005

Figure 32. Halocynthia-based clumps at 21–28 m depths on a rock wall at Pt. George. BV: *Boltenia villosa*. PH: *Pyura haustor*. HI: *Halocynthia igaboja*. SG: *Styela gibbsii*. Note that in A, *Pyura haustor* are clustered primarily in the shade created by a large *Halocynthia*.


A
B

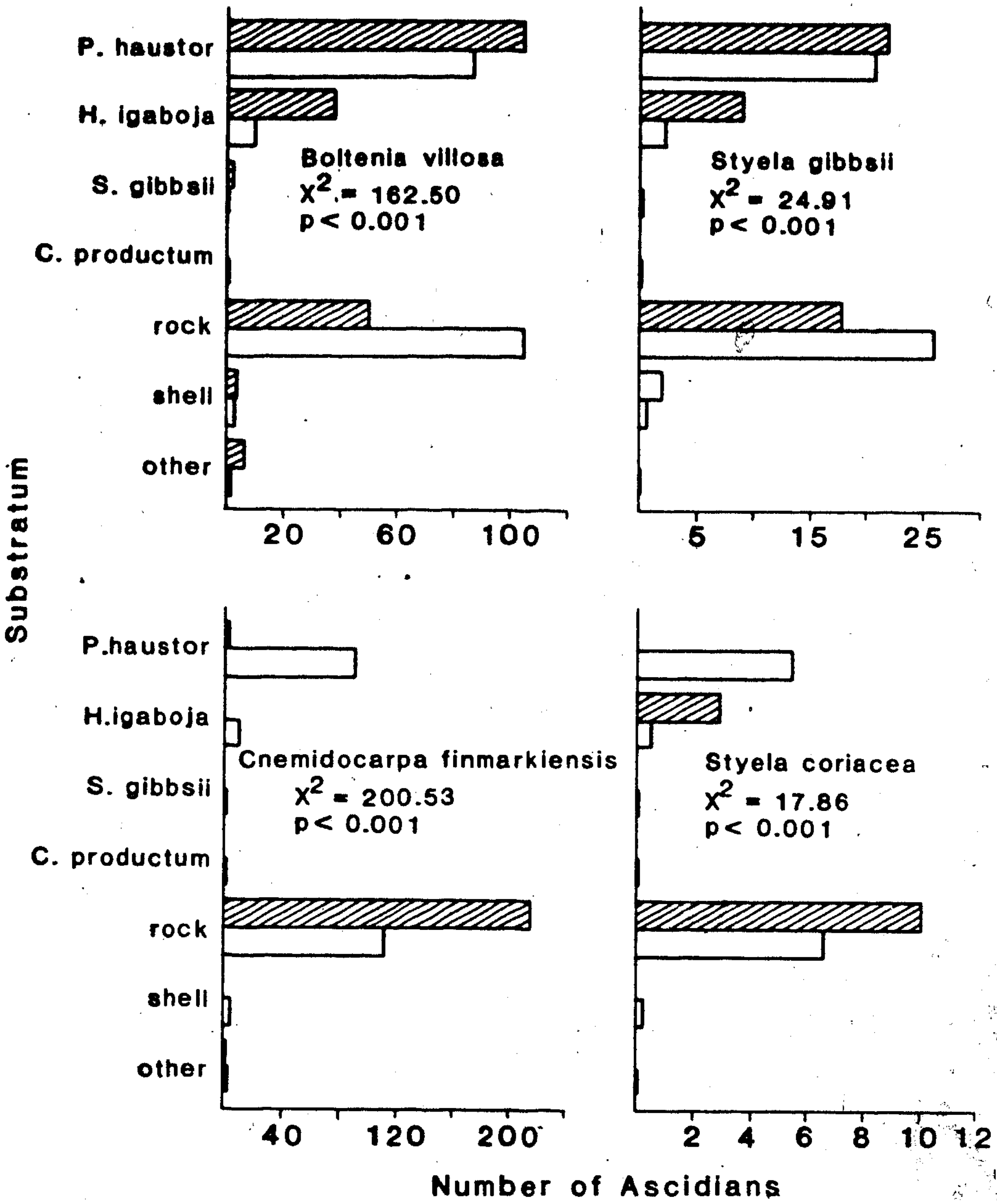


Figure 33. Distribution of four stolidobranchs by substratum, pooled for all rocky subtidal sites. Cross-hatched bars: observed distributions. Open bars: expected distributions.

suggesting that something eliminates established individuals from rock but not other ascidians, that the tadpoles actively select other ascidians as settlement sites, or both. Frequency distributions of *B. villosa* differed significantly from Poisson distributions at Reuben Tarte Point and the Pt. George Slope site, but not at the Pt. George Wall site (Fig. 31). In the first two cases, it seems evident that these patterns simply reflected the distributions of the substrata used. For example, at Reuben Tarte, 19 of 28 individuals occurred on *Pyura haustor*, the distribution of which was strongly aggregated. The non-aggregated pattern seen on the Pt. George Wall may be due to an approximately random distribution of *B. villosa* among clumps, coupled with a nearly random dispersion of *P. haustor* clumps on the uniform rock wall. It is interesting that *B. villosa* distribution was strongly contagious in the same transects where the *H. igaboja* distributions were random (Fig. 31), despite almost identical population densities in the two species. (Table XI).

Boltenia villosa was very common on floating docks, where it sometimes occurred on *P. haustor*, but more often attached to conspecifics, to the numerically more abundant stolidobranch, *Styela gibbsii*, or to the very common barnacle, *Balanus crenatus* (Fig. 25).

Styela gibbsii.

Styela gibbsii is a yellow to brown finger-shaped ascidian common on docks and floats and somewhat less abundant than *Boltenia villosa* in typical rocky subtidal habitats. It occupied essentially the same habitat as *B. villosa*, attaching mainly to *Pyura haustor* or *Halocynthia igaboja* (Fig. 33) in the subtidal and to whatever substrata were available on floating docks where *H. igaboja* were absent and *P. haustor* were relatively uncommon (Fig. 25). It was not unusual to find aggregations of *S. gibbsii*, especially on muddy bottoms. This suggests that it may be a gregarious species like *C. productum* or *P. haustor* under high density conditions.

Spatial pattern of *Styela gibbsii* was quantified only at Reuben Tarte Pt. (Fig. 21). The distribution was aggregated, almost certainly because of the aggregated distribution of the *P. haustor*, to which most of the *S. gibbsii* were attached. This is in contrast to the situation described above in which *Halocynthia igaboja* individuals rather than quadrats were used as sampling units. The two techniques may have given different results because *S. gibbsii* used *H. igaboja* as a substratum when available, and *P. haustor* as a substratum when no *H. igaboja* were present in the clump.

Species Occurring as Solitary Individuals.

All of the rare species and one of the relatively common ones occurred as solitary individuals, either out on the open rock faces, or in cryptic habitats such as the undersides of cobbles.

Cnemidocarpa finmarkiensis.

Individuals of *Cnemidocarpa finmarkiensis*, a brilliant orange ascidian with smooth, clean tunic, occurred at most rocky sites as well as on docks, but reached a high enough density for quantitative sampling only at Bell Island. In this population, the distribution was clearly contagious, with more quadrats containing multiple individuals than expected (Fig. 22). However, this pattern was probably due to the patchy distribution of suitable habitat on the transect, as it was strongly correlated with presence of large rock surfaces as in *C. productum* (Fig. 29). In this same transect, as well as at other sites, most of the *C. finmarkiensis* were found in deep fissures (chapter 2), causing their distribution to be even more strongly localized. This correlation suggests that on a homogeneous surface, distribution would be random, and that the observed aggregation at Bell Island was probably due to common selection of cryptic habitats by the strongly photonegative tadpoles (chapter 2). Although some individuals were attached to large *Pyura haustor*, the overwhelming majority occurred on rock (Fig. 33).

Ascidia paratropa.

Ascidia paratropa is a large translucent phlebobranch, which is encountered unpredictably in rocky habitats. It characteristically occurred on large boulders, cliffs and outcroppings in water deeper than 15 m. Most individuals were on open faces of rocks (chapter 2), and all that I encountered were attached to rock (Fig. 30), with the exception of a small population on the concrete underside of the laboratory breakwater.

Styela coriacea.

This small brown styelid is among the least common of the local solitary ascidians. It generally attaches directly to cobbles (Fig. 33), where it occupies the bottom side (chapter 2), but may at times be found nestled among the spines of the large pyurid, *Halocynthia igaboja*. On soft bottoms, it commonly occurs on the valve of the giant pecten, *Patinopecten caurinus*.

Pyura mirabilis

Pyura mirabilis is a brown ascidian unique in that the [illegible] are located on opposite ends of the body. Only 6 individuals were found during the entire study. As noted in chapter 2, they were all located on the undersides of cobbles, and all were attached directly to rock (Fig. 23).

Halocynthia aurantium.

The largest of local solitary ascidians, *Halocynthia aurantium* is known as the "sea peach" because of its orange color and the microscopic spines which give its tunic the texture of peach fuzz. It was found only rarely in the San Juan Islands. Of the five animals I saw, one was on an upward-facing outcropping and the remainder were on the vertical surfaces of boulders in water deeper than 20 m. All used rock as a substratum (Fig. 23). Several small specimens were collected from the underside of the Lab Breakwater in the summer of 1981.

Corella willmeriana..

This fairly rare species, transparent like its congener, was characteristically found as a solitary individual on the vertical face of a cliff. All individuals I surveyed were attached to rock (Fig. 30) or to concrete docks. It has also been reported to occur on styrofoam floats in the town of Friday Harbor (Lambert et al., 1981).

PREDATION BY FUSITRITON OREGONENSIS

Ascidian Transplant Experiments

The major question suggested by the observed patterns of large-scale distribution was: "Why are most phlebobranch ascidians not found in the rocky subtidal habitats where epifaunal species would be expected to occur?" Initially, I approached the question with a series of transplant experiments to determine if adults were capable of surviving in those habitats where other ascidians (e.g., *Pyura haustor*) were common. Transplants of dock ascidians (*Chelyosoma productum, Corella inflata, Ascidia callosa*) were made so that some animals were on the bottom and others were immediately above the bottom, but out of reach of possible benthic predators (Fig. 34). The difference

Figure 34. Transplant experiments at 21.5 m depth, Pt. George. A: Brick with sabellid tubes and *Chelyosoma productum* on the bottom. Note *Fusitriton oregonensis* (arrow) attached to brick. B: Dead *Chelyosoma productum*, at the end of a transplant experiment, showing typical *F. oregonensis* bore holes. C: Predator-free (floating) treatment 1 m above the bottom. D: Abandoned cable, suspended off the bottom near transplant site. Though *Pyura haustor* is present, the aggregation of ascidians is dominated by *Styela gibbsii* and *Boltenia villosa*.


A
B
C
D

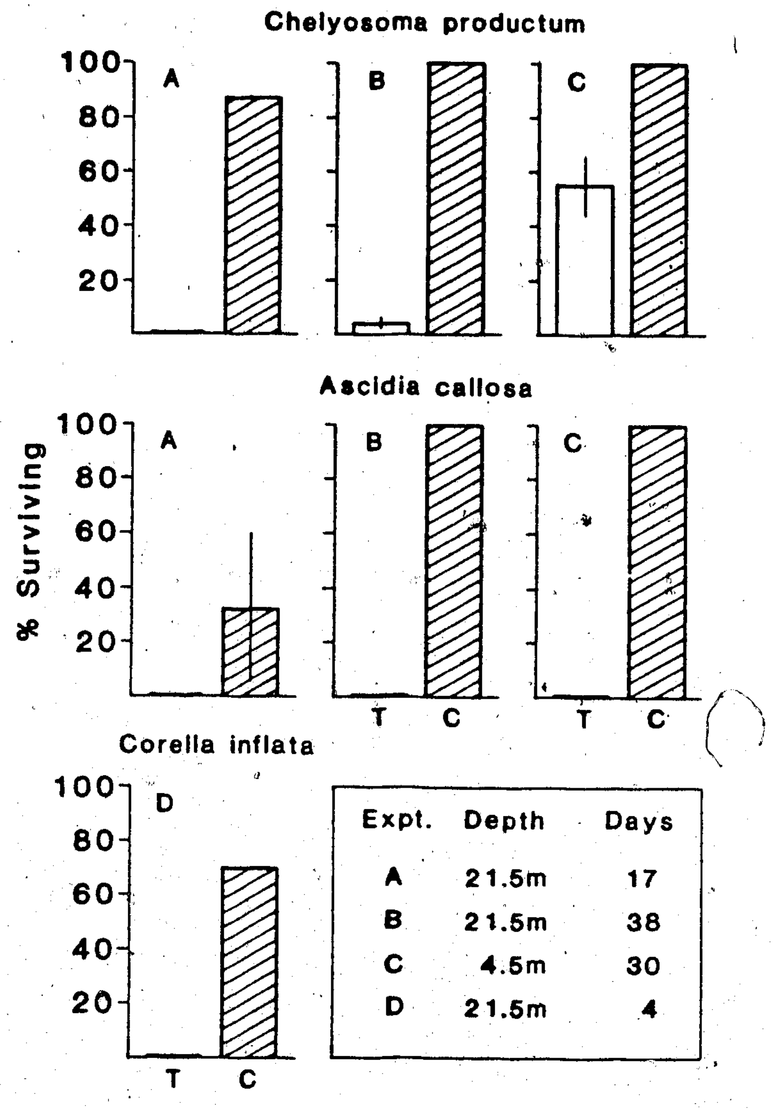
between the two treatments was clear; in each transplant ascidians survived much better 1 m off the bottom than immediately on the bottom (Fig. 35). Mortality of *C. productum* in the 4.5 m benthic transplants (series C) was somewhat lower than in the 21.5 m ones, but was nevertheless substantially greater than in the floating treatment at the same depth. In Series A, which was the first transplant done, the ascidians were lashed directly to bricks or boards, while in the remaining series, only sabellid tubes were crossed by monofilament. The relatively low survival of both *Ascidia* and *C. productum* in the series A experiments is thought to be an artifact of the attachment method.

While monitoring transplant experiments, it became apparent that predation by *Fusitriton oregonensis* was a major cause of the observed mortality. One or two *F. oregonensis* generally appeared at the transplant sites within the first few days, usually remaining on or near the brick until all or nearly all of the ascidians had been consumed (Fig. 34). Panel B of Fig. 34 shows typical damage inflicted on the transplanted *C. productum*. Each animal had a large hole drilled in its side or top plates, and the soft inside tissues had been cleaned out completely. Generally the small scavenger *Amphissa columbiana* was also present, cleaning out small bits of remaining tissue, as were pandalid shrimp.

About 10 m from the transplant sites and at the same depth, I located an abandoned power cable which at one time spanned the channel between Shaw and San Juan Islands. At a place where the cable passes off the top of a cliff, it leaves the bottom for a distance of about 5 m. At this point, there was a dense aggregation of ascidians (mostly *Styela gibbsii*) and sponges, not unlike that seen on some docks. This seems to be a natural replicate of my transplant controls. Large clumps of *S. gibbsii* were never found on the bottom, presumably because they would be consumed by *F. oregonensis*.

Laboratory and Field Feeding Experiments

Table XIII shows the results of a laboratory feeding experiment in which snails were offered a variety of possible foods, including several ascidians and the sea urchin which is its reported preferred prey (Eaton, 1972). As prey items were consumed, they were replaced, so the probability of encountering each item was constant for the experiment, and the same as for each other potential food. Despite the availability of



Expt.	Depth	Days
A	21.5m	17
B	21.5m	38
C	4.5m	30
D	21.5m	4

Figure 35. Survival of *Chelyosoma productum*, *Ascidia callosa* and *Corella inflata* in subtidal transplants at Pt. George. T (open bars): benthic transplants. C (cross-hatched bars): floating treatment. Each bar is the mean of 4 replicates. Error bars are Standard Errors.

Table XIII.

Fusitriton food preference experiment conducted in the laboratory with constant densities of potential prey. Experiment ran for 7 days with 7 snails. "D" and "$\text{Log}_{10}Q$" are electivity indices of Jacobs (1974). "r" and "p" are relative proportions of food item in ration and in total prey population.

Prey Species	Number Available (p)	Number Consumed (r)	No. Consumed per Snail per Day	D	$\log_{10}Q$
Mollusca:					
Tonicella lineata	5(0.08)	0	0	0	0
Calyptraea fastigiata	5(0.08)	0	0	0	0
Echinodermata:					
Strongylocentrotus droebachiensis	5(0.08)	0	0	0	0
Psolus chitonoides	5(0.08)	0	0	0	0
Eupentacta quinquesemita	5(0.08)	0	0	0	0
Ascidiacea:					
Halocynthia igaboja	5(0.08)	0	0	0	0
Pyura haustor	5(0.08)	0	0	0	0
Boltenia villosa	5(0.08)	5(0.21)	0.10	0.507	0.485
Styela gibbsii	5(0.08)	0	0	0	0

(continued...)

Table XIII. (continued)

Prey Species	Number Available (p)	Number Consumed (r)	No. Consumed per Snail per Day	D	$\log_{10}Q$
Chelyosoma productum	5(0.08)	7(0.29)	0.14	0.649	0.672
Ascidia paratropa	5(0.08)	12(0.50)	0.25	0.840	1.060
Corella inflata	5(0.08)	0	0	0	0
(Totals)	60(1.00)	24(1.00)	0.49	-	-

molluscan, crustacean and echinoderm prey, only ascidians were consumed. *Strongylocentrotus droebachiensis* remained unscathed, and no necrotic black spots appeared. After the experiment was terminated, the urchins were added to a holding tank where about 30 *F. oregonensis* were being held without food for use in the feeding experiments. The urchins remained unharmed for a period of 6 months under these conditions. Snails only fed when they were added to tanks where other food items such as ascidians were available.

A second set of experiments with ascidian and non-ascidian prey differed from the previous one in that consumed foods were not replaced (Fig. 36). In Experiment A, two species of sea urchins, *Strongylocentrotus droebachiensis* and *S. pallidus*, a short-spined urchin common in water deeper than 25 m, were offered along with both stolidobranch and phlebobranch ascidians. The urchins survived the experiment, as did the ascidian, *Pyura haustor*. *Halocynthia igaboja* experienced little mortality, but all of the other ascidians were eaten before the experiment was terminated at 12 days. The first to be consumed were the phlebobranchs *Ascidia callosa and Chelyosoma productum*. In Experiment B, only one ascidian, *C. productum* was offered together with a number of common subtidal molluscs, a barnacle, and a sessile holothurian. All of the *C. productum* were consumed quickly. Only several days after the ascidian prey were exhausted did the snails attack another prey item, the chiton, *Tonicella lineata*.

In experiments designed to test for preferences among various combinations of ascidian prey, several features consistently appeared (Fig. 37). The soft-bodied ascidians in the order Phlebobranchia were always the first to be consumed, and these were followed by *Boltenia villosa* and *Styela gibbsii*. Among phlebobranchs, *C. productum* survived better than the other species. Three species, all stolidobranchs, showed high survival in each experiment: *Pyura haustor, Halocynthia igaboja, and Cnemidocarpa finmarkiensis*. In the few cases where *P. haustor* or *H. igaboja* were eaten, they were always attacked on the posterior end; at the point where they had been attached to the substratum prior to collection. The tissue in this region seems to be softer than that normally exposed to predators, and in *H. igaboja* it is the only region of the test not elaborated into spines.

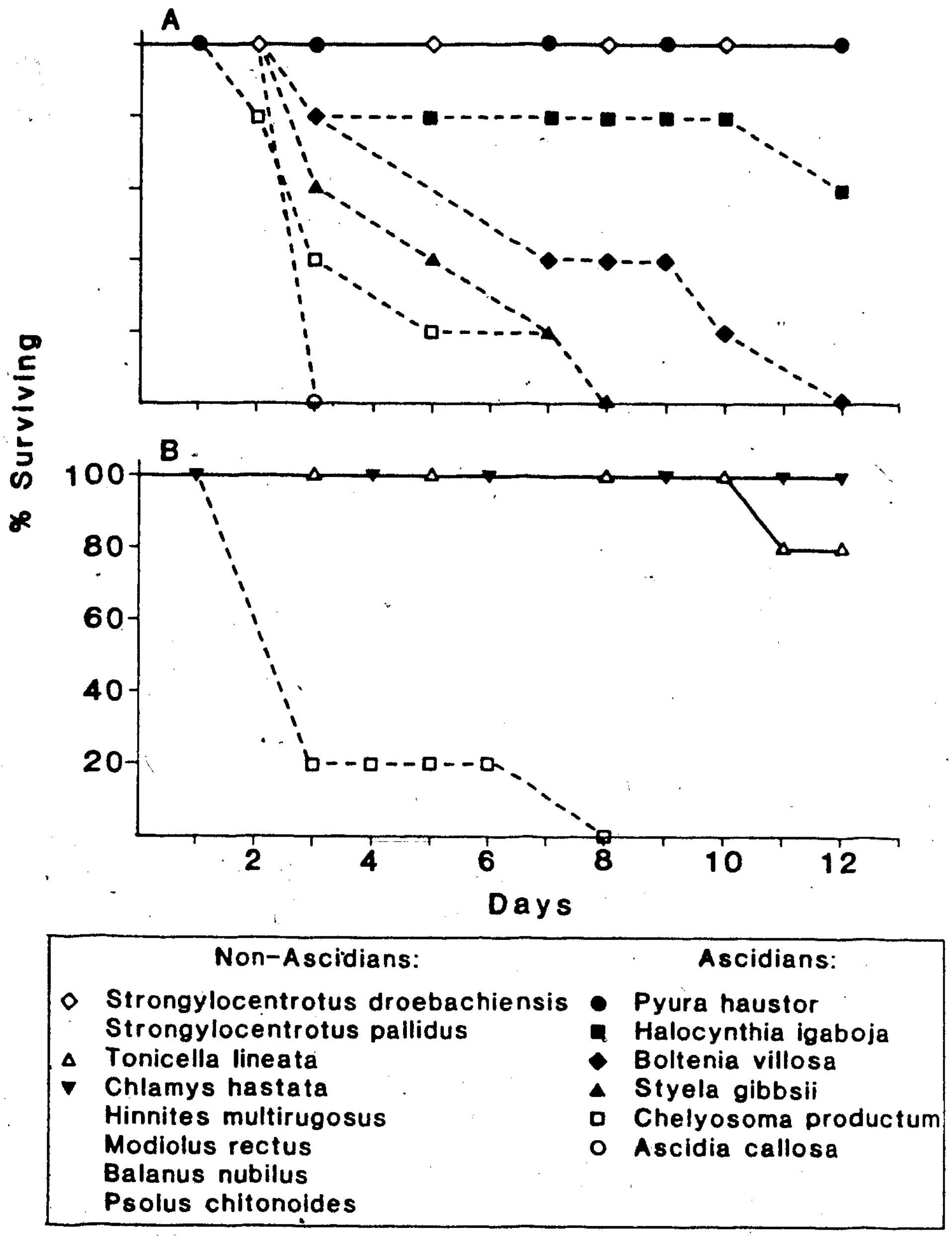
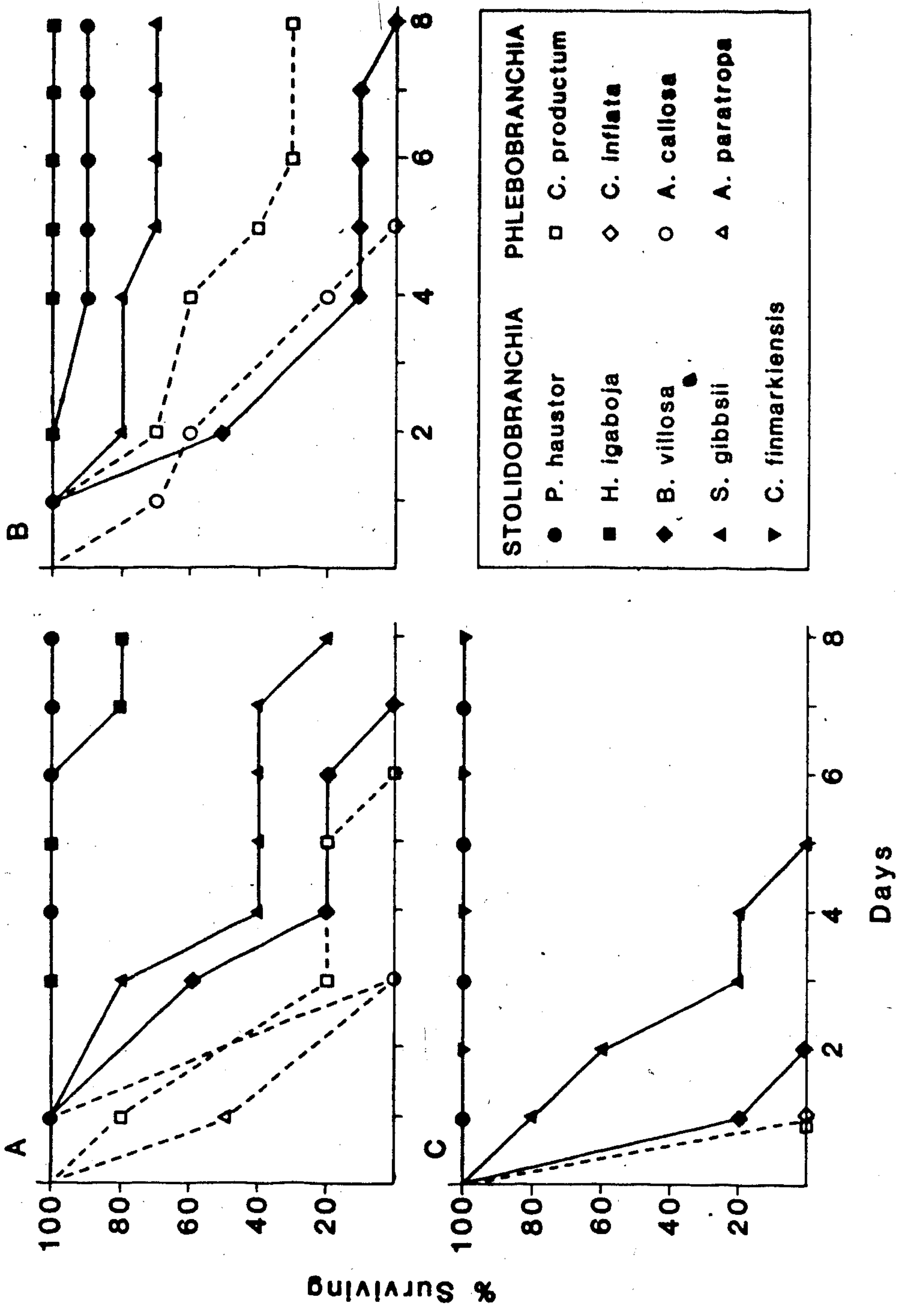


Figure 36. Survival of ascidians and other common subtidal organisms in 12 day laboratory feeding experiments with 7 *Fusitriton oregonensis*. Solid lines: non-ascidians and ascidians never consumed. Broken lines: ascidians. Starting populations: 5 animals per species.

Figure 37. Survival of ascidians in 8 day laboratory experiments with 7 *Fusitriton oregonensis*. Solid lines and points: Stolidobranchs. Broken lines and open points: Phlebobranchs. Starting populations were as follows. Expt. A: *Ascidia paratropa*, 2 individuals; all other species, 5 individuals. Expt. B: 10 individuals per species. Expt. C: 5 individuals per species.


A
B
C
% Surviving
100
80
60
40
20
Days
2
4
6
8
STOLIDOBRANCHIA
● P. haustor
■ H. igaboja
◆ B. villosa
▲ S. gibbsii
▼ C. finmarkiensis
PHLEBOBRANCHIA
□ C. productum
◇ C. inflata
○ A. callosa
△ A. paratropa

I tested the hypothesis that ascidians in aggregations would be less susceptible to predation than solitary individuals by simultaneously offering both clumps and individuals to *Fusitriton* (Fig. 38). The hypothesis was rejected for both *C. productum* and *A. callosa*. *F. oregonensis* encountering a clump of these phlebobranchs invariably remained and fed until all animals had been consumed. The individual *Pyura* consumed again was attacked on the bottom, and this is considered an experimental artifact.

Fusitriton oregonensis were transplanted to floating docks where they do not occur normally in order to assess their potential impact on established, high density ascidian populations. One cage containing no predators was maintained a a control against cage effects. No mortality occurred in this treatment. Likewise, there was no mortality in an uncaged area monitored during the experiment. *F. oregonensis* enclosures were run at 4 densities (Table XIV), all of which were much higher than any observed in subtidal field conditions. As in the laboratory experiments, *Pyura haustor* were never consumed. Individuals of the other four species present were eaten in every cage. At 2 predators per cage and 4 predators per cage, *B. villosa* was the most commonly eaten prey species, while at the higher densities, *A. callosa* was most preferred. This is in contrast to the laboratory experiments in which *B. villosa* were generally attacked only after *C. productum* and *A. callosa*. In three of the four cages, *C. productum* electivity indices are either close to zero or negative. These would be interpreted as showing lack of preference or else active avoidance of these prey items. It should be kept in mind, however, that the figures are based on the total prey available. Not only were *C. productum* the most numerically common ascidians in the cages (Table XIV), but they consisted mostly of very small individuals. Figure 39 shows size-frequency distributions for the total *C. productum* present in the cages, and for the individuals consumed by *F. oregonensis*. The overall size distribution is distinctly bimodal, with peaks at 1.0 cm and 2.0 cm. These represent two cohorts of recruits, one settled 9 to 10 months before the experiment and the other one year earlier (*C. productum* has a discrete spawning season in the early spring; see Young and Braithwaite, 1980a). *F. oregonensis* actively selected only second year individuals as prey. The electivity indices were, however, biased due to the extremely abundant small individuals. If only electivity indices were available, one would conclude that *C. productum* are actively avoided as prey, whereas in reality, the

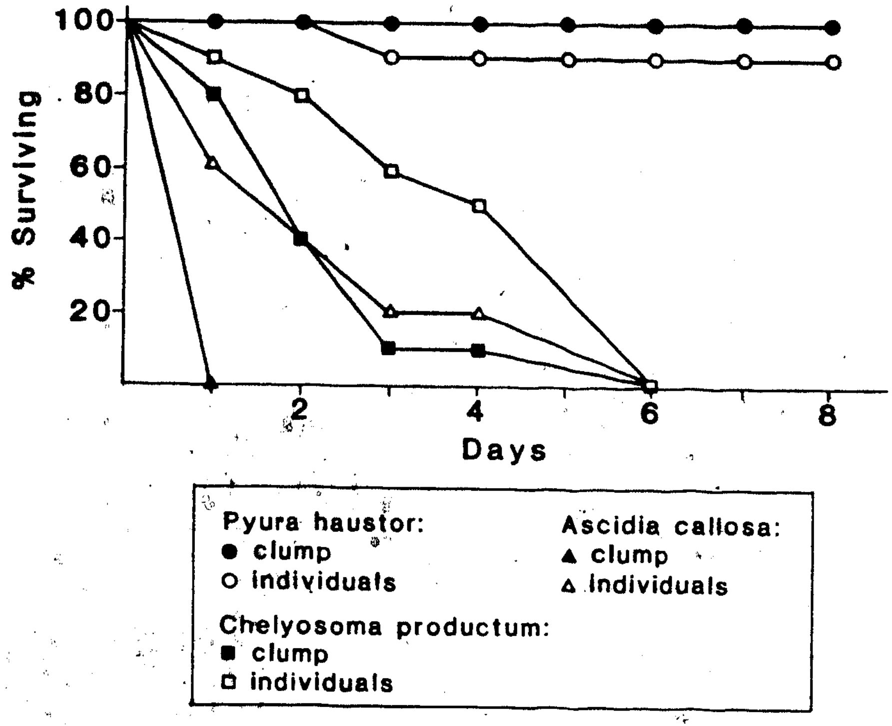


Figure 38. Survival of *Pyura haustor, Chelyosoma productum* and *Ascidia callosa* in clumps and as isolated individuals in a laboratory experiment with 7 *Fusitriton oregonensis*. Starting population: 20 animals per species (10 individuals and a clump of 10) for *Pyura* and *Chelyosoma*, 10 animals (5 individuals and a clump of 5) for *Ascidia*.

Table XIV.

Fusitriton predation experiment conducted under the Friday Harbor Labs Breakwater with 4 predator densities. Experiments ran 20 days. Electivity index notation as in Table XIII.

Prey Species	Number Available (p)	Number Consumed (r)	No. Consumed per Snail per Day	D	$\log_{10}Q$
2 *Fusitriton* ($8/m^2$):					
Boltenia villosa	17(0.0451)	3(0.1428)	0.0750	0.5583	-0.5645
Styela gibbsii	167(0.4429)	4(0.1905)	0.1000	-0.5433	-0.5288
Pyura haustor	17(0.0451)	0(0.0000)	0.0000	0.0000	0.0000
Chelyosoma productum	156(0.4138)	11(0.5238)	0.2750	0.2167	0.1926
Ascidia callosa	20(0.0530)	3(0.1428)	0.0750	0.4972	0.4736
3 *Fusitriton* ($12/m^2$):					
Boltenia villosa	24(0.0759)	5(0.1666)	0.0833	0.4176	0.4091
Styela gibbsii	93(0.2943)	10(0.3333)	0.1666	0.0904	0.0787
Pyura haustor	31(0.0981)	0(0.0000)	0.0000	0.0000	0.0000
Chelyosoma productum	152(0.4810)	12(0.4000)	0.2000	-0.1632	-0.1431
Ascidia callosa	16(0.0506)	3(0.1000)	0.0500	0.3516	0.3190

(continued...)

Table XIV. (continued)

Prey Species	Number Available (p)	Number Consumed (r)	No. Consumed per Snail per Day	D	$\log_{10}Q$
4 *Fusitriton* ($16/m^2$):					
Boltenia villosa	24(0.0750)	8(0.3809)	0.1000	0.7727	0.8804
Styela gibbsii	133(0.4156)	5(0.2381)	0.0625	-0.3909	-0.8223
Pyura haustor	9(0.0281)	0(0.0000)	0.0000	0.0000	0.0000
Chelyosoma productum	140(0.4375)	1(0.0476)	0.0125	-0.8759	-1.1900
Ascidia callosa	14(0.0437)	7(0.3333)	0.0875)	0.8325	1.0389
8 *Fusitriton* ($32/m^2$):					
Boltenia villosa	32(0.0629)	14(0.2333)	0.0875	0.6386	0.6434
Styela gibbsii	162(0.3182)	24(0.4000)	0.1500	0.1764	0.1526
Pyura haustor	7(0.0137)	0(0.0000)	0.0000	0.0000	0.0000
Chelyosoma productum	290(0.5697)	11(0.1833)	0.0687	-0.7101	-1.425
Ascidia callosa	18(0.0354)	11(0.1833)	0.0687	0.7190	0.7781

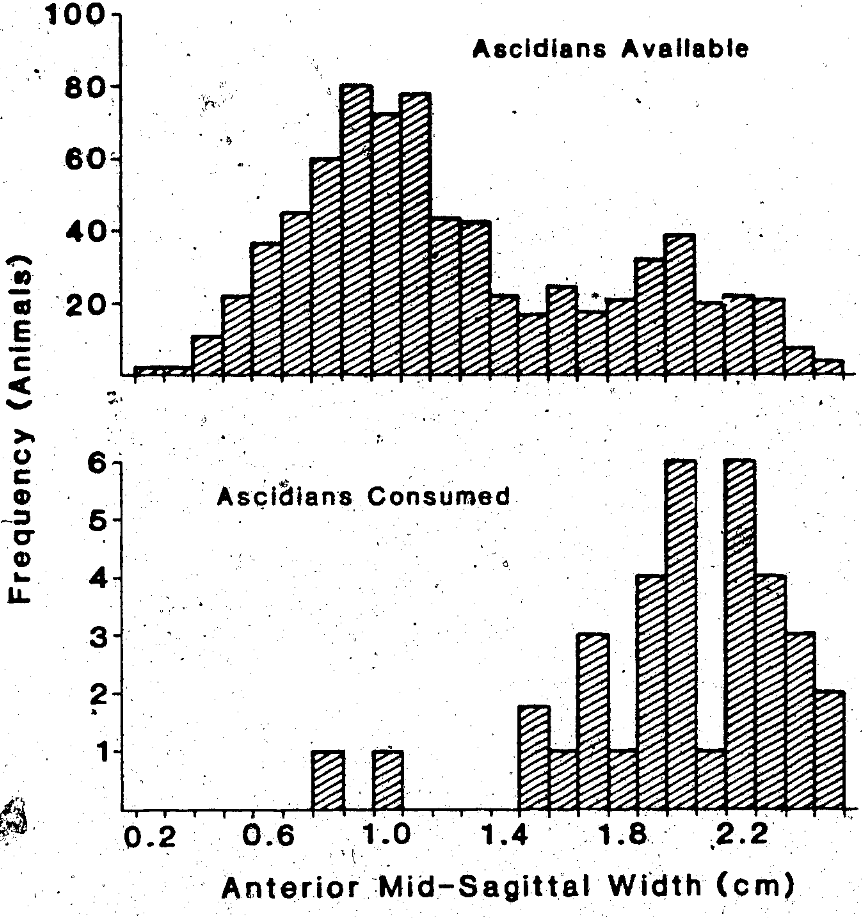


Figure 39. Size-frequency distributions of *Chelyosoma productum* individuals consumed by and available to *Fusitriton* in predation experiments conducted on the underside of the Friday Harbor Labs Breakwater (Table XIV).

larger individuals are preferred.

Ascidia callosa and *Styela gibbsii* had size distributions which were essentially unimodal (Figs. 40, 41), and in both of these species, the predator selected animals of various sizes roughly in the frequency at which they occurred. This was not apparent in the case of *S. gibbsii*, where the peak sizes of prey selected were shifted slightly to the left of the peak on the size distribution. However, this shift could easily be explained by the contractile nature of the *S. gibbsii* tunic in death; I consider the 0.8 cm difference between peaks within the possible error of the estimation technique for this species. No *A. callosa* less than 1.8 cm across were attacked, and only one *S. gibbsii* smaller than 1.4 cm was consumed.

As in *C. productum*, the size distribution of *Boltenia* was skewed toward the lower end because of abundant recruits in the youngest year class (Fig. 42). Again, *F. oregonensis* only attacked the larger individuals. Because of relatively low densities of larger *B. villosa*, a large proportion [illegible]mals (58%) between 1.8 and 5.6 cm were consumed, including all animals which were between 3.2 and 3.8 cm long.

Averaging over all cages, an individual *Fusitriton oregonensis* consumes one ascidian every two days. Thus, in a prey population of unlimited size, and having a dispersion such that search time is minimized (as in the experimental dock population), a single individual would be expected to consume about 180 ascidians per year. In order to predict the potential impact of predation on a field population, it is necessary to have data on both predator and prey population size.

Field Observations of <u>Fusitriton oregonensis</u>

Although *Fusitriton oregonensis* were always counted when they occurred in transects, their density at most sites was so low that they did not occur in the sample. At Long Island, the density of snails was estimated as 0.26 per m² (S.E.: 0.18, n=30 quadrats) and at Eagle Cove, it was 0.32 per m² (S.E.: 1.27, n=75 quadrats). At Pt. George, the large outcropping near the experimental sites was carefully surveyed for the predator on three dives between April 15 and May 7, 1981. The maximum number of animals encountered on a single dive was 24 (0.16 per m² or one animal for every 6.25 m²). If the three surveys are considered independent samples, the snail density averaged 0.1 per m² (S.E.:

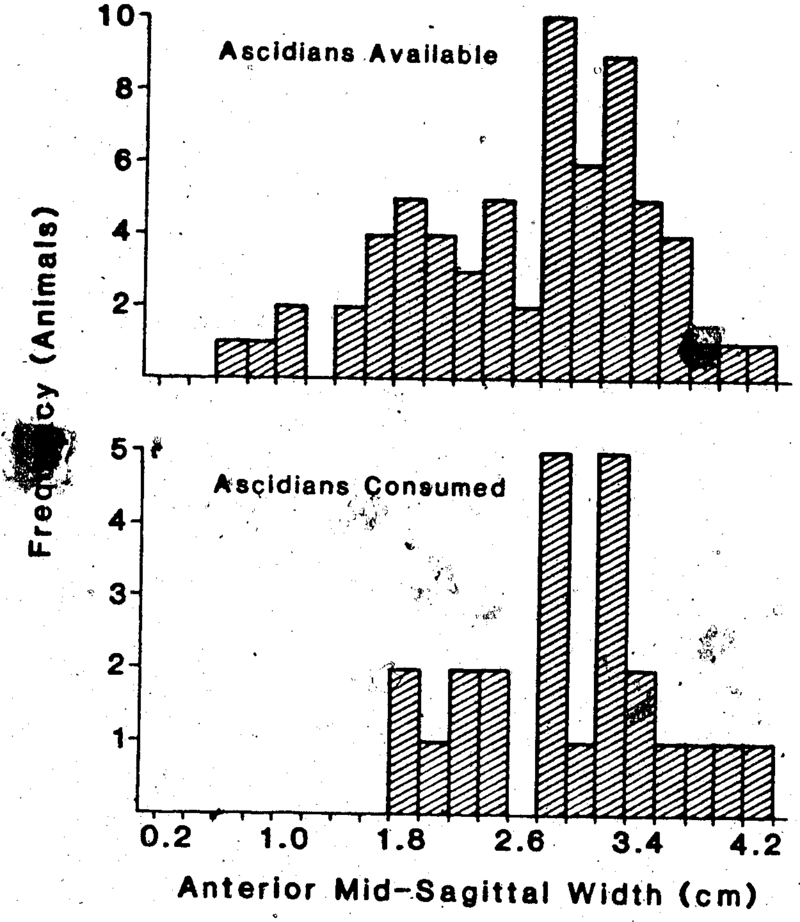


Figure 40. Size-frequency distributions of *Ascidia callosa* individuals consumed by and available to *Fusitriton* in predation experiments conducted on the underside of the Friday Harbor Labs Breakwater.

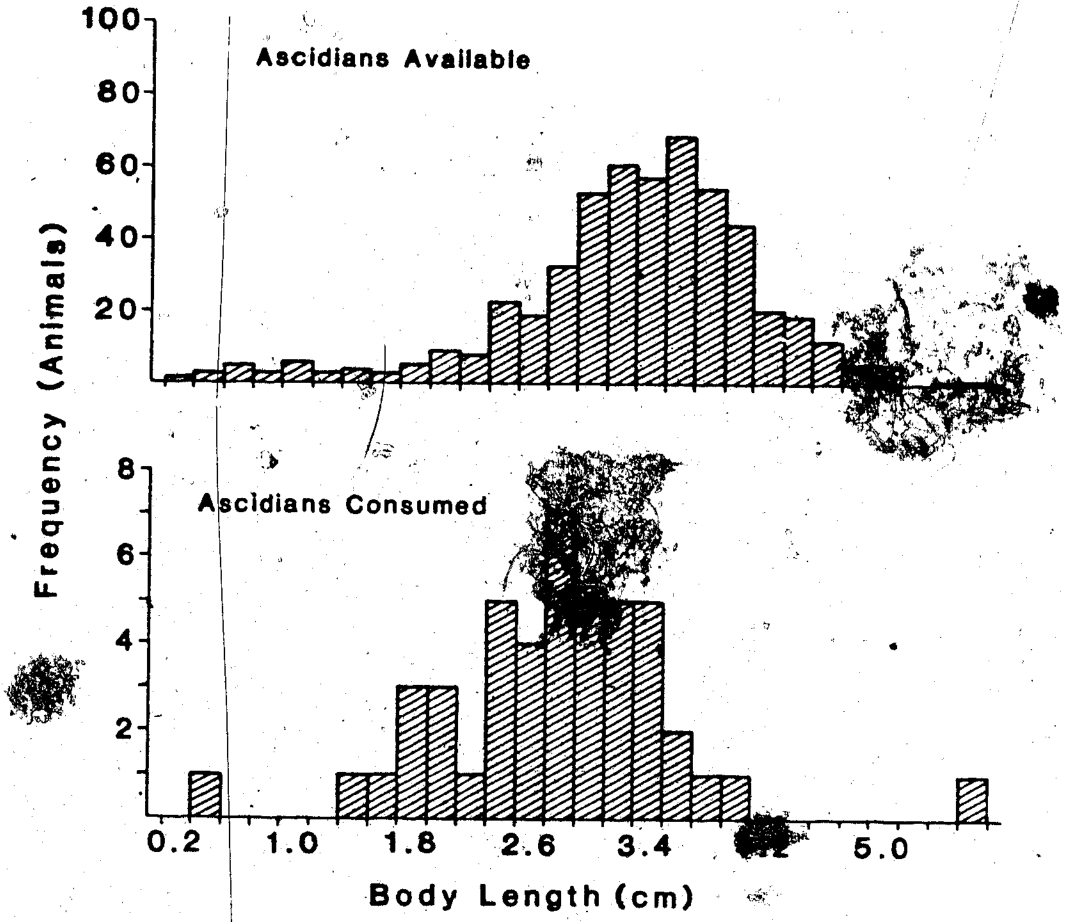


Figure 41. Size-frequency distributions of *Styela gibbsii* individuals available to (top) and consumed by (bottom) *Fusitriton* in predation experiments conducted on the underside of the Friday Harbor Laboratory Breakwater.

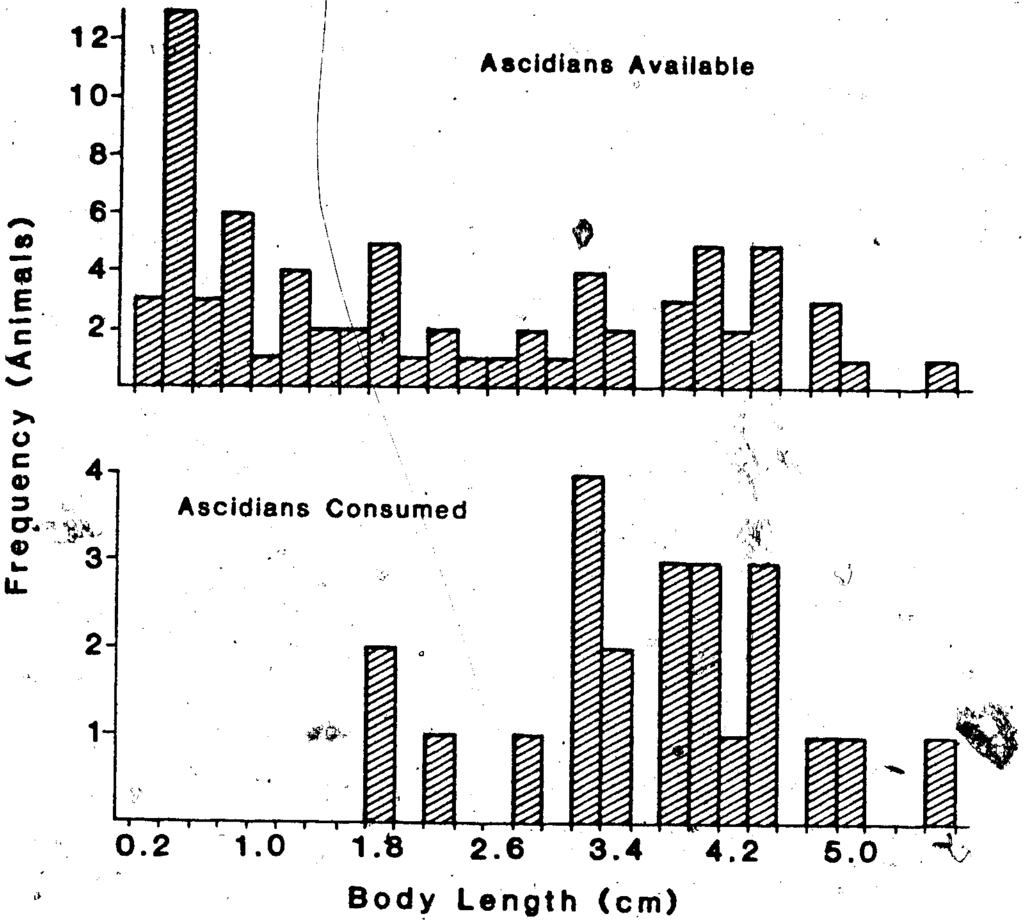


Figure 42. Size-frequency distributions of *Boltenia villosa* individuals available to (top) and consumed by (bottom) *Fusitriton* in predation experiments conducted on the underside of the Friday Harbor Laboratory Breakwater.

0.03). Snails were tagged as they were encountered, and I therefore expected the number of tagged animals to increase with each successive survey. This did not occur. Nine animals were tagged during the first dive. On the second dive, 24 were found and tagged, but none of these had been tagged previously. Of 10 *F. oregonensis* seen on the final dive, only two carried tags. Besides the possibility that the snails lost the rubber bands (this was not a problem in Eaton's 1972 study of egg brooding, in which an identical tagging method was used), two explanations are possible: 1) A large percentage of snails occupy cryptic habitats where they are missed, or 2) The snails are very transitory, and the rock outcropping does not have a permanent resident population. Because the surveys were quite thorough, and the rubber band tags rendered snails more visible underwater, I consider the latter alternative the more likely of the two. During spawning season, I observed an aggregation of 30 *F. oregonensis* laying eggs on this same outcropping.

F. oregonensis feeds primarily at night, so it was relatively rare to observe feeding in the field. Most of the observations I made were on aggregations of snails scavenging carrion, such as urchins which I and other divers had accidently damaged on previous dives. In observing ascidians, however, I encountered a number of dead animals , most of which had bore holes characteristic of *F. oregonensis* attacks (Table XV). The only surprising feature, in light of laboratory experiments is that several dead *C. finmarkiensis* were found. *Cnemidocarpa* was not attacked in the laboratory by *Fusitriton*, and it is possible that another predator was responsible for these deaths.

The Feeding Mechanism of Fusitriton oregonensis

The means by which *Fusitriton oregonensis* locates, attacks and drills ascidians was investigated in the hopes that it would give a clue to the differential predation observed among the various ascidian species. Numerous attacks were observed during the course of the laboratory feeding experiments, and the transparent tunic of some of the prey items made it possible to observe some of the processes occurring inside the bodies of the ascidian prey.

The snails moved about and fed most actively at night. Only starved animals added to aquaria containing ascidians commonly initiated attacks by day. In "Y-maze"

Table XV.

Field observations of dead ascidians.

Date	Site	Species	Observation
March 19, 1980	Pt. George	*Boltenia villosa*	4 hollow tests
April 1, 1981	Bell Island	*Chelyosoma productum*	1 hollow test
		Cnemidocarpa finmarkiensis	1 hollow test
April 9, 1981	Pt. George	*Cnemidocarpa finmarkiensis*	1 injured specimen
August 4, 1981	Shady Cove	*Chelyosoma productum*	1 hollow test
		Corella inflata	1 hollow test with *Fusitriton* nearby
August 24, 1981	Long Island	*Chelyosoma productum*	"numerous" hollow tests, 1 *Fusitriton* eating *Chelyosoma*

experiments, *F. oregonensis* demonstrated some ability to find fish heads (Scorpaenidae) at a distance(Table XVI). However, at the flow rate tested (2 l/min), distant chemoreception was apparently not used in locating ascidians or echinoids, whether damaged or not. When foraging, *Fusitriton* moves about with its cephalic tentacles extended and its siphon held up in the water. Upon touching an edible ascidian with its tentacles, the snail lifts the anterior portion of the foot off the substratum and extends it forward to grasp the prey. The proboscis is then partially everted and moved back and forth across the tunic, apparently in search of a suitable place to drill. The drilling mechanism was watched in detail from the reverse side of transparent (*Ascidia paratropa*) tunic held in a plexiglas clamp (Fig. 43) and submerged in a small tank. *F. oregonensis*, like other cymatiid and cassid gastropods, has very large salivary glands which contain sulfuric acid and empty by means of ducts into the buccal cavity (see Houbrick and Fretter, 1969 for anatomy of a related species). Small, pinkish "accessory salivary glands" are attached to the salivary glands. The radula (Fig. 44) consists of a serated median tooth, blade-like lateral teeth and hooked marginal teeth. The inner surface of the jaw is file-like, and the margin is serrated (Fig. 44). Holes were drilled quite rapidly (about 30 minutes in *Ascidia paratropa* and *Corella inflata*) by using the radula and jaws (Fig. 44). As the mouth is opened, the jaws are extended laterally, where they appear to hold the mouth open until after the radula makes its stroke. At the completion of the radula stroke, the jaws are scraped along the tunic and brought together as the mouth closes. On the average, the snail makes one stroke about every 3 seconds. The proboscis is moved back and forth as the snail drills, so that the radula is continually contacting new regions of the tunic. This enables the snail to drill a hole slightly larger than the proboscis and much larger than the radula or mouth. That the jaws take an active part in the drilling process is also suggested by the worn appearance of their most distal region (Fig. 45). By watching attacks on transparent *Corella inflata*, I was able to observe not only the drilling, but the actual ingestion process. After the hole is drilled, the proboscis is inserted and the tissue is rasped out with the radula. The tunic was never consumed, exept possibly in the drilling process. Occasionally, large bites of tissue are removed by the jaws. *F. oregonensis* is capable of extending its proboscis into the most distant corners of even large ascidians. Avery (1961) reported a maximum

Table XVI.

Y-maze experiments with <u>Fusitriton</u> <u>oregonensis</u>.

Prey Item	No. Fusitriton	Duration	No. in Prey Arm	No. in Control Arm	p
crushed urchin	6	2h	2	2	ns
crushed urchin	6	2h	2	2	ns
crushed urchin	6	2h	1	1	ns
crushed urchin	6	18h	4	2	ns
urchin	10	4h	4	5	ns
urchin	10	4h	3	7	ns
urchin	10	4h	0	0	ns
urchin	10	10h	2	2	ns
urchin	10	4h	4	3	ns
urchin	10	10h	3	4	ns
fish head	10	4h	8	2	[illegible]05
fish head	10	4h	7	1	[illegible]
fish head	10	4h	4	2	ns
fish head	10	10h	6	4	ns
fish head	10	10h	6	3	ns
A. paratropa	10	4h	3	3	ns
A. paratropa	10	4h	5	5	ns
B. villosa (cut)	10	44h	5	4	ns
H. igaboja					
(shaved)	6	4h	1	2	ns
(shaved & cut)	6	20h	0	3	ns

Figure 43. *Fusitriton oregonensis* consuming transparent ascidians. A: Apparatus designed for viewing the drilling process, viewed from above. B: "Inside" view of *F. oregonensis* drilling through a piece of *Ascidia paratropa* tunic clamped in the apparatus shown in A. C: *F. oregonensis* cleaning out the internal tissues of *Corella inflata* with its proboscis. Small black arrows indicate the ascidian tunic. D: Close-up view of the proboscis, showing jaws and radula mid-way through a radula stroke.

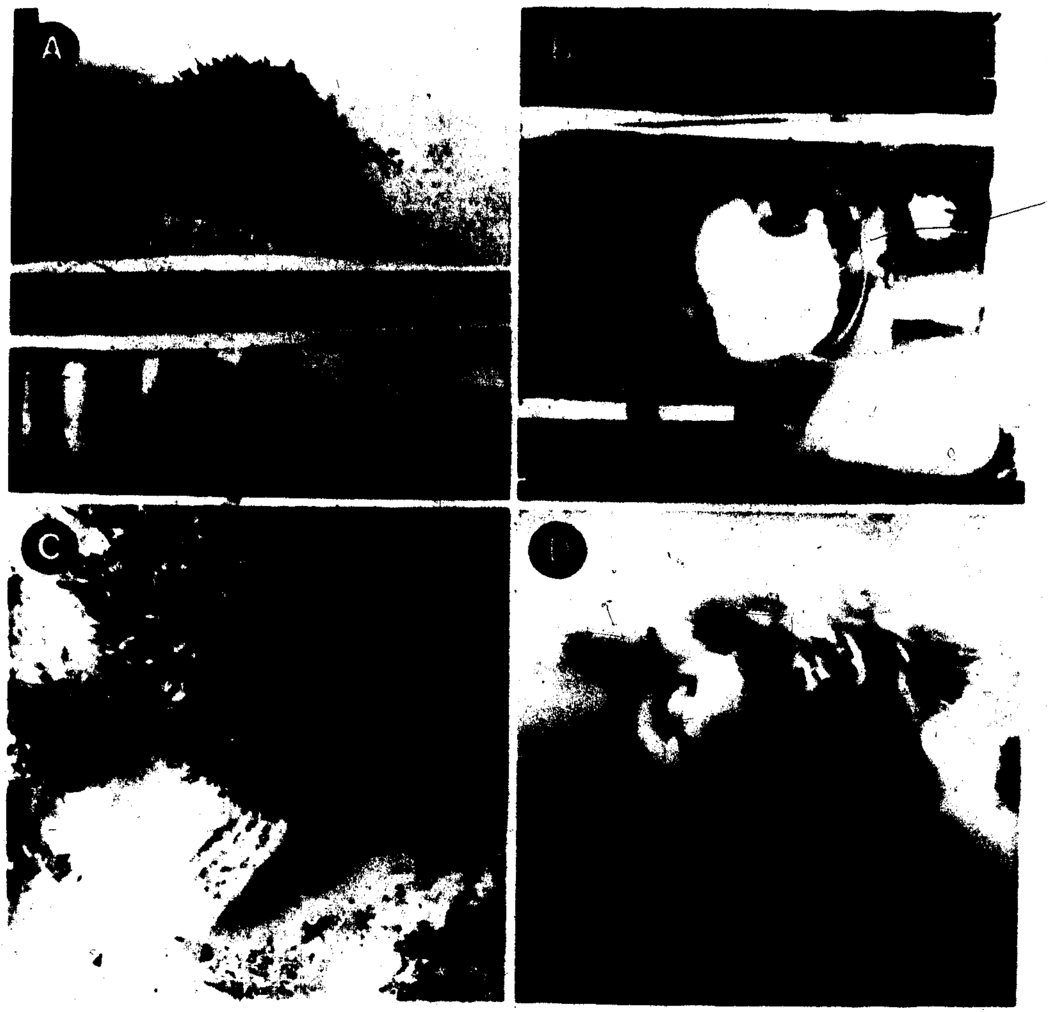

A
C

Figure 44. Scanning Electron Micrographs of *Fusitriton oregonensis* radula (A) and jaw (B).

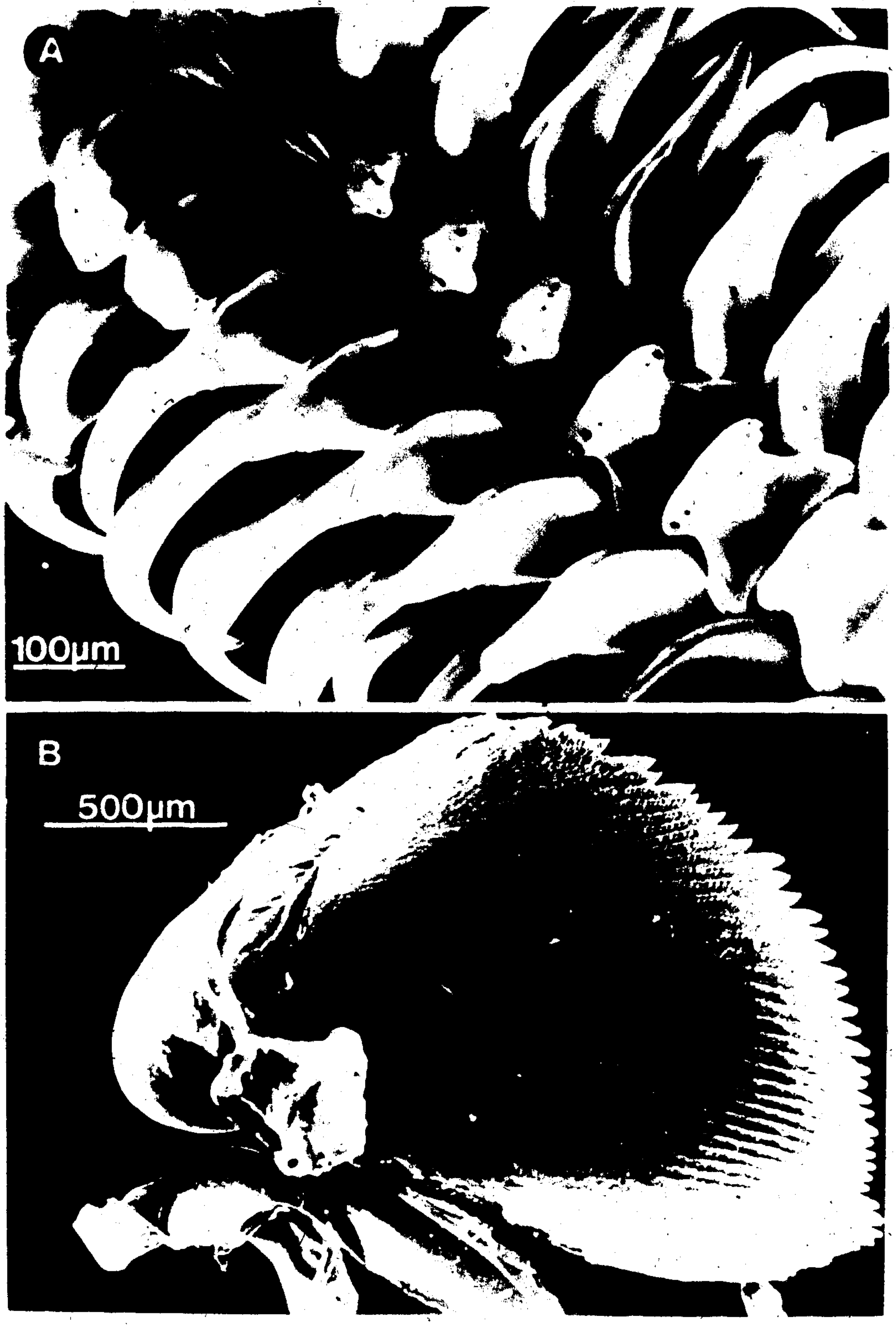
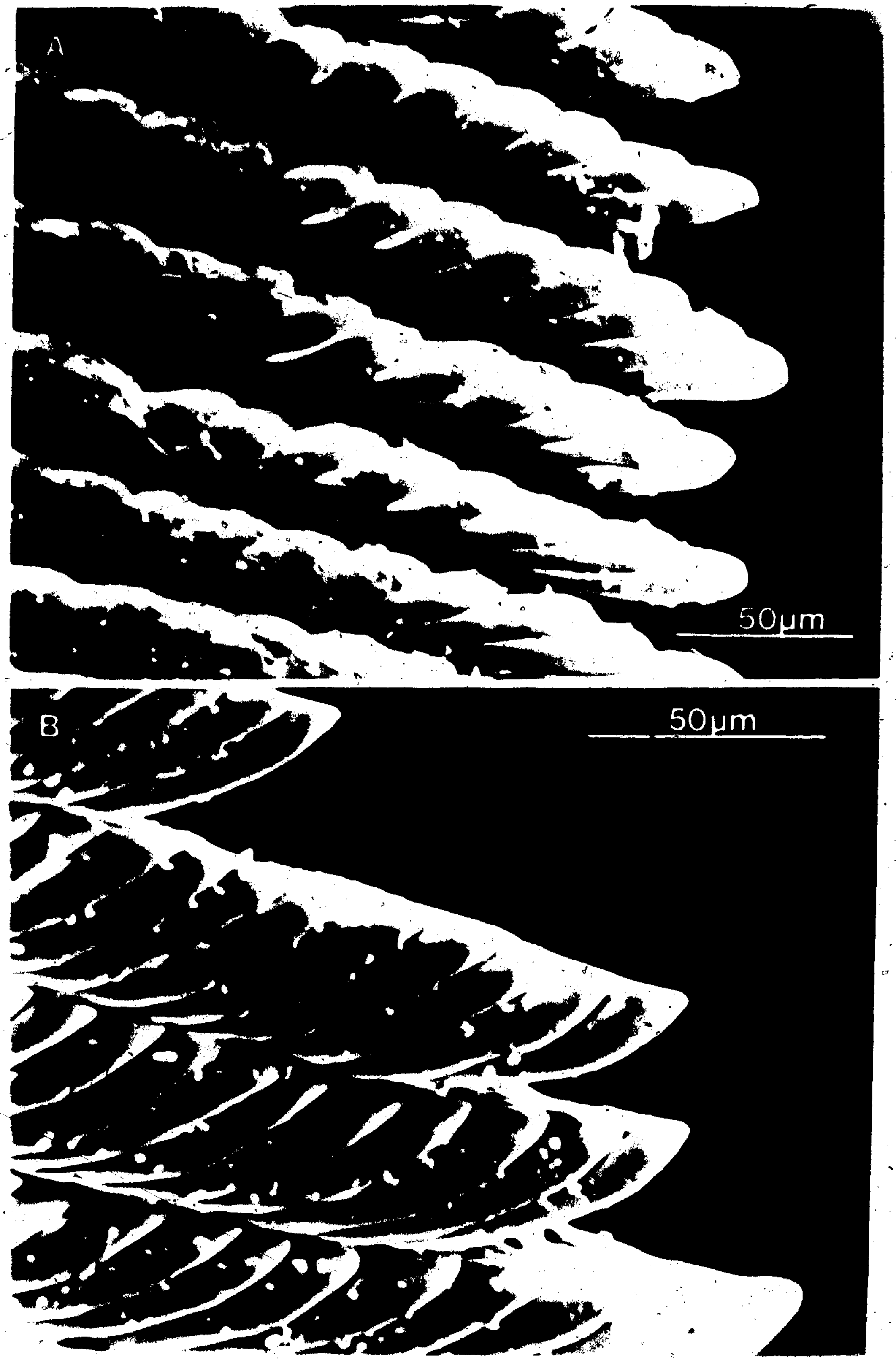

A
100μm
B
500μm

Figure 45. The edge of a jaw in *Fusitriton oregonensis* showing dull region at the distal end (A) and sharp region nearer the proximal end.


A
50µm
B
50µm

proboscis extension of 16 cm in a 9.4 cm long individual.

The possibility that salivary secretions help dissolve tunic during the drilling process was tested by macerating salivary glands in 50 ml of seawater, and adding pre-weighed bits of *Ascidia callosa* tunic. After 24 hours of incubation at ambient seawater temperature, tunic in the salivary juice had not lost any more weight than tunic maintained in seawater as a control.

Although the function of the salivary glands and accessory salivary glands remains enigmatic, my observations suggest that *F. oregonensis* may gain access to the internal tissues of ascidians by entirely mechanical means.

PREDATION BY OTHER PREDATORS

Although I regularly turned over feeding starfish while diving, only *Evasterias troschelli* was ever found eating ascidians. This starfish consumes ascidians almost exclusively on soft substratum (manuscript in preparation) but seems to prefer molluscan prey in hard bottom areas. Table XVII presents feeding observations of *E. troschelli* on hard sediment. *P. haustor* and *C. productum* were sometimes consumed but most of the prey consisted of the clam *Humilaria kennerleyi* in cobble fields and shell bottoms, and sessile molluscs such as *Pododesmus macroschisma* and *Crepidula sp.*, on solid rock substratum. *E. troschelli* was the most common starfish in the areas where *P. haustor* and *H. igaboja* were very abundant (Pt. George and Reuben Tarte Pt.), but they were mostly confined to cobble fields adjacent to the large ascidian populations. Five large *E. troschelli* were collected from the cobble field at Pt. George and offered ascidians (*C. productum, P. haustor, S. gibbsii*) in a large laboratory tank. Over a period of 3 months, only one *C. productum* was eaten. While these starfish starved in the presence of abundant ascidians, conspecifics collected from soft sediment and maintained in an adjacent tank, fed on identical ascidians at a rate of one ascidian per starfish per day. Christensen (1957) reported that *E. troschelli* feeds mostly on the clam, *Protothaca staminea*.

Mauzey et al. (1968) reported predation on ascidians by numerous other local starfish: *Crossaster papposus* (*Ascidia paratropa*), *Pteraster tesselatus* (*Corella inflata*), *Dermasterias imbricata* (*Boltenia villosa*) and *Orthasterias koehleri* (5 species).

Table XVII.

Field observations of *Evasterias troschelli* feeding at hard substratum sites.

Date	Site	Prey Item	No. Starfish
December 11, 1980	Pole Pass	*Pododesmus cepio* (bivalve)	1
		Crepidula adunca (gastropod)	1
		unidentified prey	1
		(not feeding)	(numerous)
February 4, 1981	Bell Island	*Chelyosoma productum*	1
		(not feeding)	(several)
July 23, 1981	Reuben Tarte Pt.	*Balanus nubilus* (barnacle)	1
		Humilaria kennerlyi (bivalve)	3
		Pyura haustor	2
		(not feeding)	10
August 24, 1981	Reuben Tarte Pt.	unidentified prey	2
		(not feeding)	12

However, all of these asteroids characteristically prey on other animals and feed on ascidians only occasionally.

In the ascidian transplant experiments, the small snail *Amphissa columbiana* invariably appeared and fed on ascidian tissues. Initially it seemed possible that *A. columbiana* was attacking the phlebobranchs, especially since some of the dead ascidians had only very small bore holes. However, all such animals were in early experiments where death was probably incurred by careless tieing of the clumps, and it seems most likely that *A. columbiana* was merely scavenging. *A. columbiana* has very good distant chemosensory capabilities and invariably appears on the scene shortly after any type of carrion becomes available (L. Braithwaite, B. Stone; personal communications). I collected several dozen *Amphissa* and maintained them in aquaria with ascidians for over 6 months. During this time, I never observed an attack, and could not attribute any mortality to the small snails. *A. columbiana* apparently fed exclusively on animals which were already dead.

Several small molluscs are specialist feeders on ascidians. Most of these are members of the family Lamellariidae and are specialized for feeding on certain species of compound ascidians (Behrens, 1980; Ghiselin, 1964). One species, *Lamellaria rhombica,* has been seen feeding on *Ascidia paratropa* and *Pyura haustor* in the field and on *Boltenia villosa* and *Chelyosoma productum* in the laboratory (McClosky, 1973). I never encountered this species in the field. The closely related snail, *Velutina laevigata* is sometimes encountered in the San Juan Islands. During all of my dives, I only collected two specimens. These were maintained in the lab for several months, where on two occasions they attacked and partly consumed 2 *Styela gibbsii* previously collected from the breakwater. Clearly *V. laevigata* and *L. rhombica* are predators of interest, and their feeding rates and preferences need to be studied carefully. However since they are found only rarely, their overall impact is probably minimal.

ASCIDIAN DEFENSE MECHANISMS AND REFUGES

Overall, the observed patterns of large scale ascidian distribution are similar to those which would be predicted on the basis of *F. oregonensis* feeding preferences. Thus, low numbers of phlebobranchs at most subtidal sites can be attributed in part to elimination by the predatory snails. Five species, all stolidobranchs, are commonly found in the rocky subtidal: *Pyura haustor, Halocynthia igaboja, Cnemidocarpa finmarkiensis, Boltenia villosa, and Styela gibbsii*. The other species are so rare as to not merit consideration here. Of the common stolidobranchs listed, the first three would be predicted to co-occur with *F. oregonensis*, since the snail does not attack them. The appearance of *Boltenia* and *S. gibbsii* in the subtidal is, however, paradoxical since both are highly preferred prey of *F. oregonensis*. I now consider 1) reasons why three species are not attacked by *F. oregonensis* and 2) how two species maintain subtidal populations in spite of their vulnerability to attack.

Chemical and Morphological Defense Mechanisms

Danskin (1978) found the following vanadium concentrations in ascidians considered in this study: 800 ppm in *Chelyosoma productum*, 3550 ppm in *Ascidia paratropa* (including the blood; 850 ppm in tissues alone), 750 ppm in *Boltenia villosa*, and 175 ppm in *Halocynthia igaboja* . *Corella inflata, Pyura haustor, Halocynthia aurantium* and *Cnemidocarpa finmarkiensis* had no detectable vanadium. The vanadium concentration in *Boltenia villosa* was the highest reported for any stolidobranch to date. Based on these data, some of which are plotted against a measure of *F. oregonensis* feeding preference in Figure 46, it is concluded that there is a large (though probably spurious) correlation between vanadium concentration and the probability of being eaten by *F. oregonensis*; clearly vanadium is not an effective defense.

Tunic pH measurements of ascidians from the San Juan Islands are presented in table XVIII. Three species, all phlebobranchs in the family Corellidae (*Chelyosoma productum, Corella inflata, Corella willmeriana*) had tunic pH in the range of 0.5 to 2.0. All of the other ascidians fell in the 5.0 to 7.0 range. Snail food preference is not correlated with pH (Fig. 46), and the family of ascidians which has low pH are among the most preferred prey of *F. oregonensis*.

Although at first sight, it might seem that the gelatinous tunic of the phlebobranchs contains more water (making it softer) than that of the stolidobranchs, the percentage of water was not correlated with prey preference (Fig. 46). However, a rough measure of tunic "toughness", tensile strength was strongly correlated (Fig. 46). The

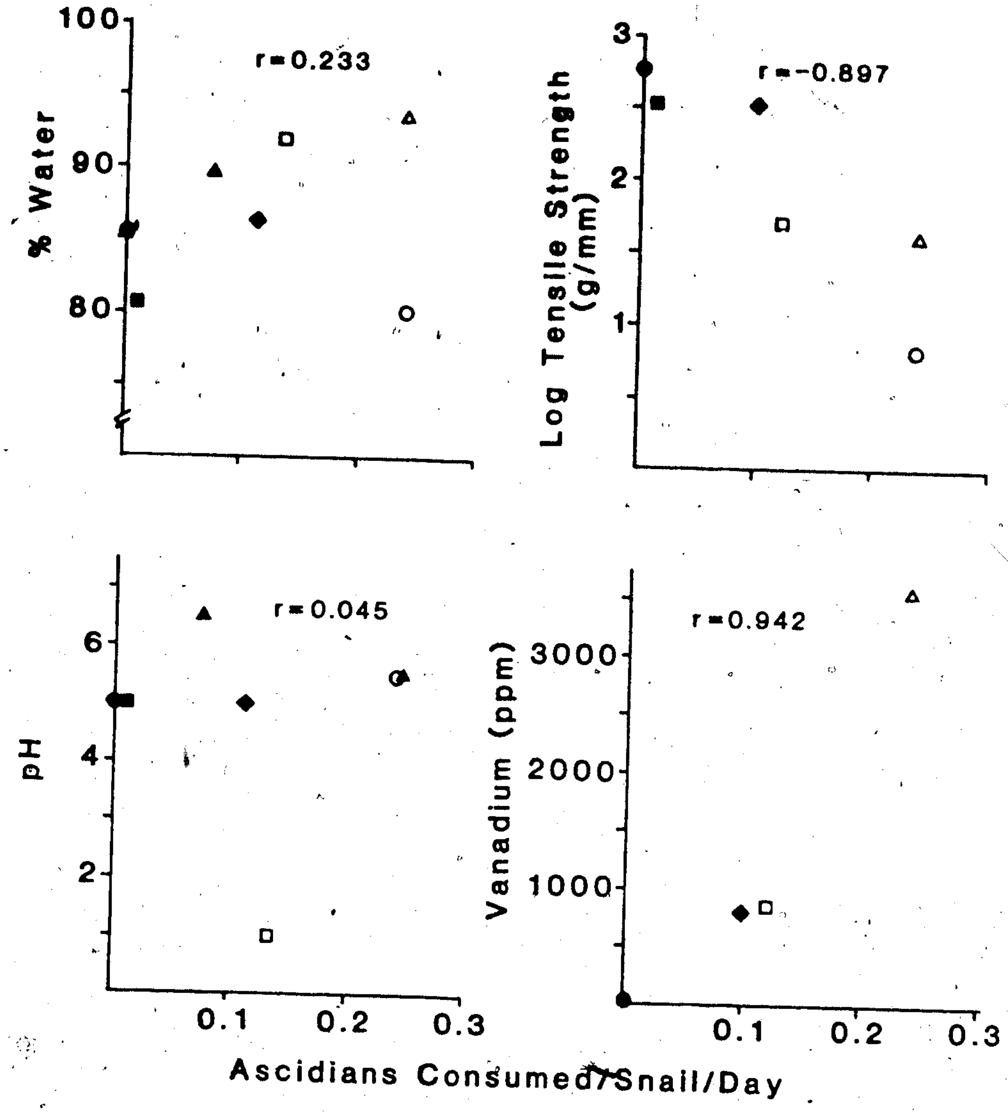


Figure 46. Scatter plots showing the relationship between vulnerability to predation (measured as ascidians consumed/snail/day in Expt. A of Fig. 37) and 4 tunic characteristics: % water, tensile strength, pH and vanadium concentration. Vanadium values are from Danskin (1978). "r" is the Pearson Product-Moment Correlation Coefficient. Tensile strengths have been converted to common logarithms to simplify plotting. Symbols: see key in Figure 37.

Table XVIII.

Ascidian tunic pH and presence (+) or absence (-) of epizooites.

Order	Family	Genus and Sp[illegible]es	pH	Epizooites
Phlebobranchia	Ascidiidae	*Ascidia callosa*	5.5	+
		Ascidia paratropa	5.0	-
	Corellidae	*Corella inflata*	0.5-1.0	-
		Corella willmeriana	1.5-2.0	-
		Chelyosoma productum	1.0	+
Stolidobranchia	Pyuridae	*Pyura haustor*	5.0	+
		Boltenia villosa	5.0	+
		Halocynthia igaboja	5.0	+
		Halocynthia aurantium	5.5-5.8	-
	Styelidae	*Styela gibbsii*	6.5	+
		Styela coriacea	6.5	-
		Cnemidocarpa finmarkiensis	6.5	-

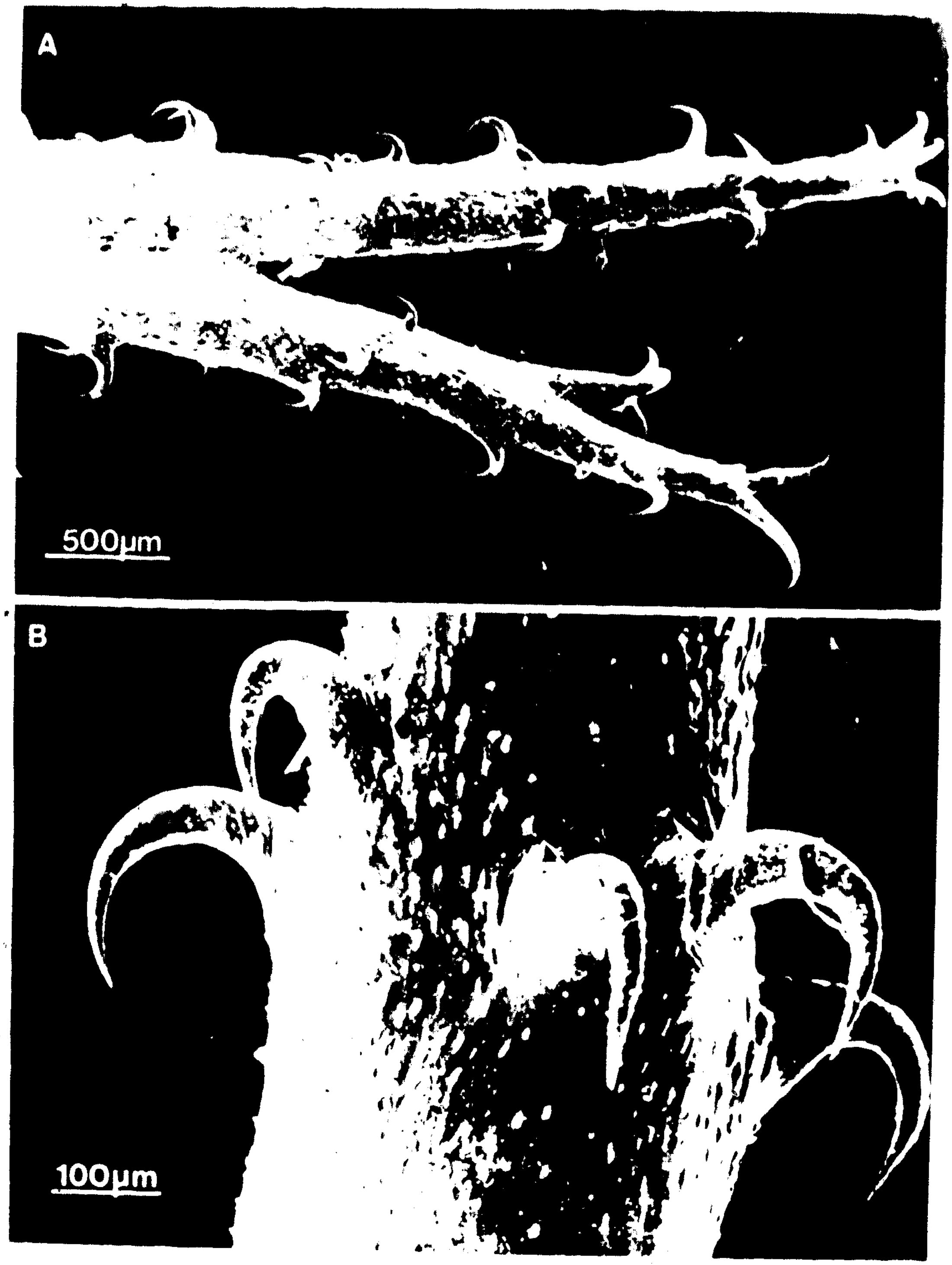
phlebobranchs readily eaten by *F. oregonensis* had the most easily broken tunic (e.g., *Chelyosoma productum*: 160 g/mm^2) while the tunics of stolidobranchs were less easily broken (*Pyura haustor* tunic: 1800 g/mm^2). The correlation is interesting, but it should be kept in mind that tensile strength is not necessarily related to the resistance *F. oregonensis* might encounter in rasping with its radula.

Intuitively, it seemed apparent that the stiff spines of *Halocynthia igaboja* could function as a morphological defense mechanism. The spines, which completely cover the tunic, average about 1–2 cm long and are covered with very sharp spinelets which recurve to point toward the ascidian body (Fig. 47). Small spines growing between the large ones have distal spinelets which splay out in four directions immediately above the tunic. By removing the hairs from some animals, I tested the hypothesis that hairs prevent *F. oregonensis* from gaining access to the tunic with its proboscis. Hairs were shaved off very close to the tunic with razor blades. This treatment did not harm the animals; individuals so treated began filtering normally immediately after being returned to aquaria, began regenerating the hairs within a few days, and lived for months in tanks where *F. oregonensis* was not present. The damage inflicted on the animals also did not attract *F. oregonensis* from a distance. In y-maze experiments, no preference was seen for the side of the maze containing ascidians, either damaged or undamaged, over the side containing no prey (Table XVI).

In laboratory tanks, all shaved animals were consumed by *F. oregonensis* within the first 8 days, while unshaved individuals survived for the duration of the experiment (Fig. 48). In the field, similar results were obtained, with the major differences being that the time required for the experiment was 6 months, and several unshaved animals were attacked in the hairless region where they had been removed from the rock. In any case, it is clear that spines function effectively in defending *H. igaboja*.

Pyura haustor has no structures which can be modified experimentally, so indirect evidence must be used to infer the defense mechanism. Fig. 49 shows the tunic of *P. haustor* in two different regions of the body. Although animals collected from floating docks and quiet bays sometimes have quite soft tunics, individuals from typical subtidal sites have very tough, leathery tunics which are very difficult to cut through and which generally contain embedded bits of sand and rock. I presume that the toughness of the

Figure 47. Scanning electron micrographs of *Halocynthia igaboja* tunic spines, showing recurved spinelets.


A
500µm
B
100µm

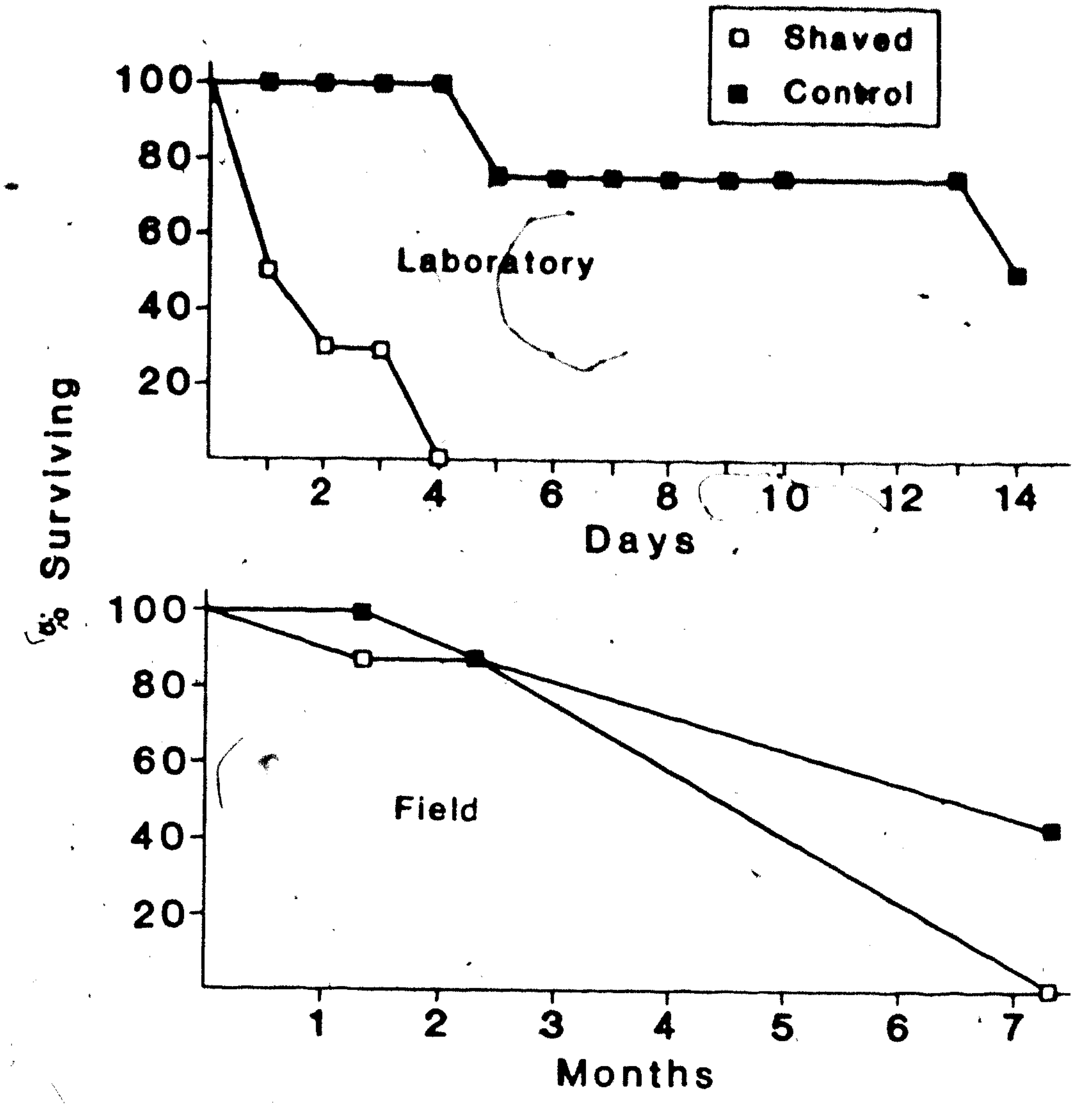
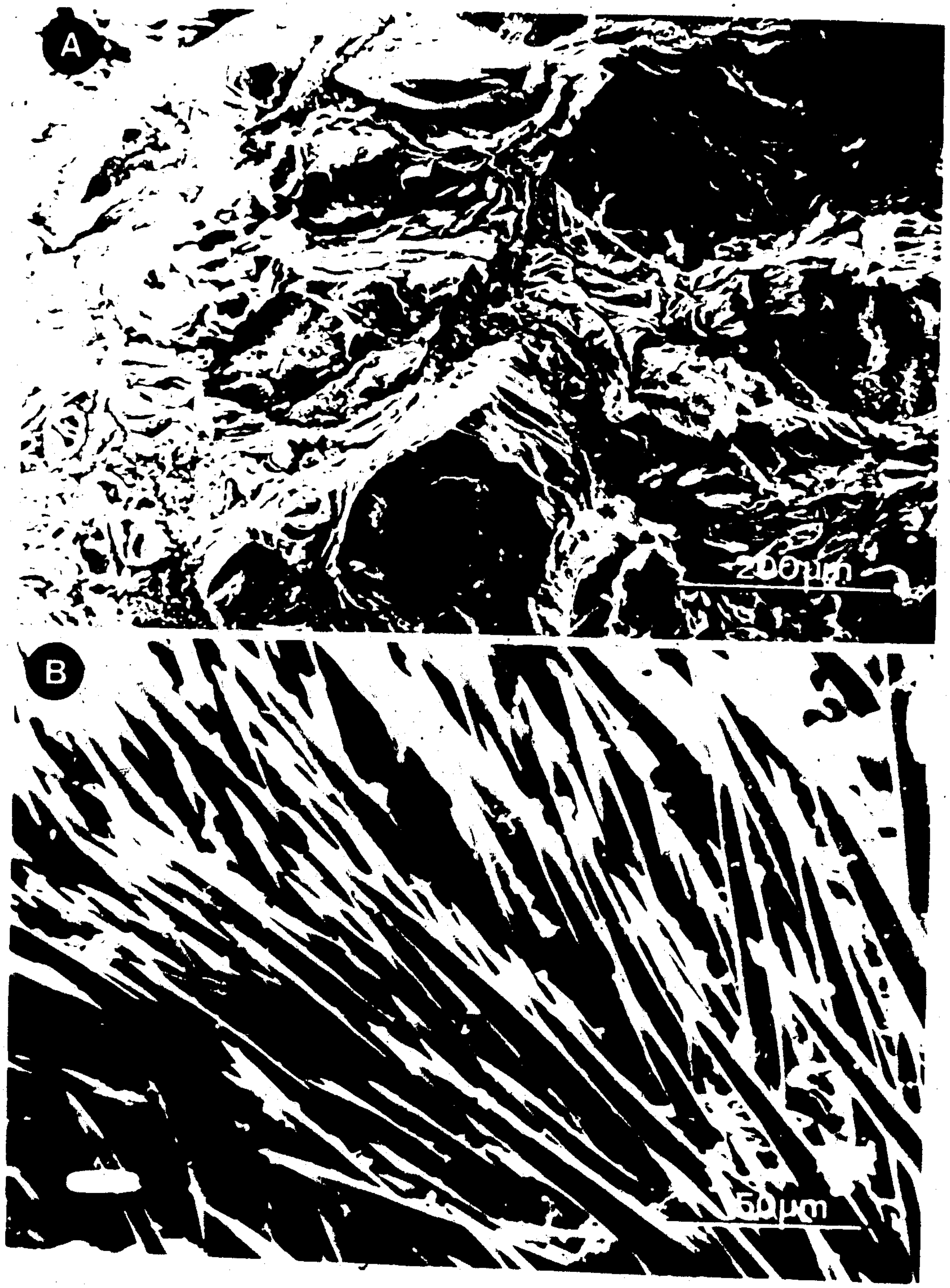


Figure 48. Survival of shaved (open points) and intact (solid points) *Halocynthia igaboja* in laboratory experiment (top graph) and in a field transplant at Pt. George (bottom graph).

Figure 49. Scanning electron micrographs of *Pyura haustor* tunic, showing embedded sand and rocks (arrows, micrograph A) and spines on the contractile region of the siphons (B).

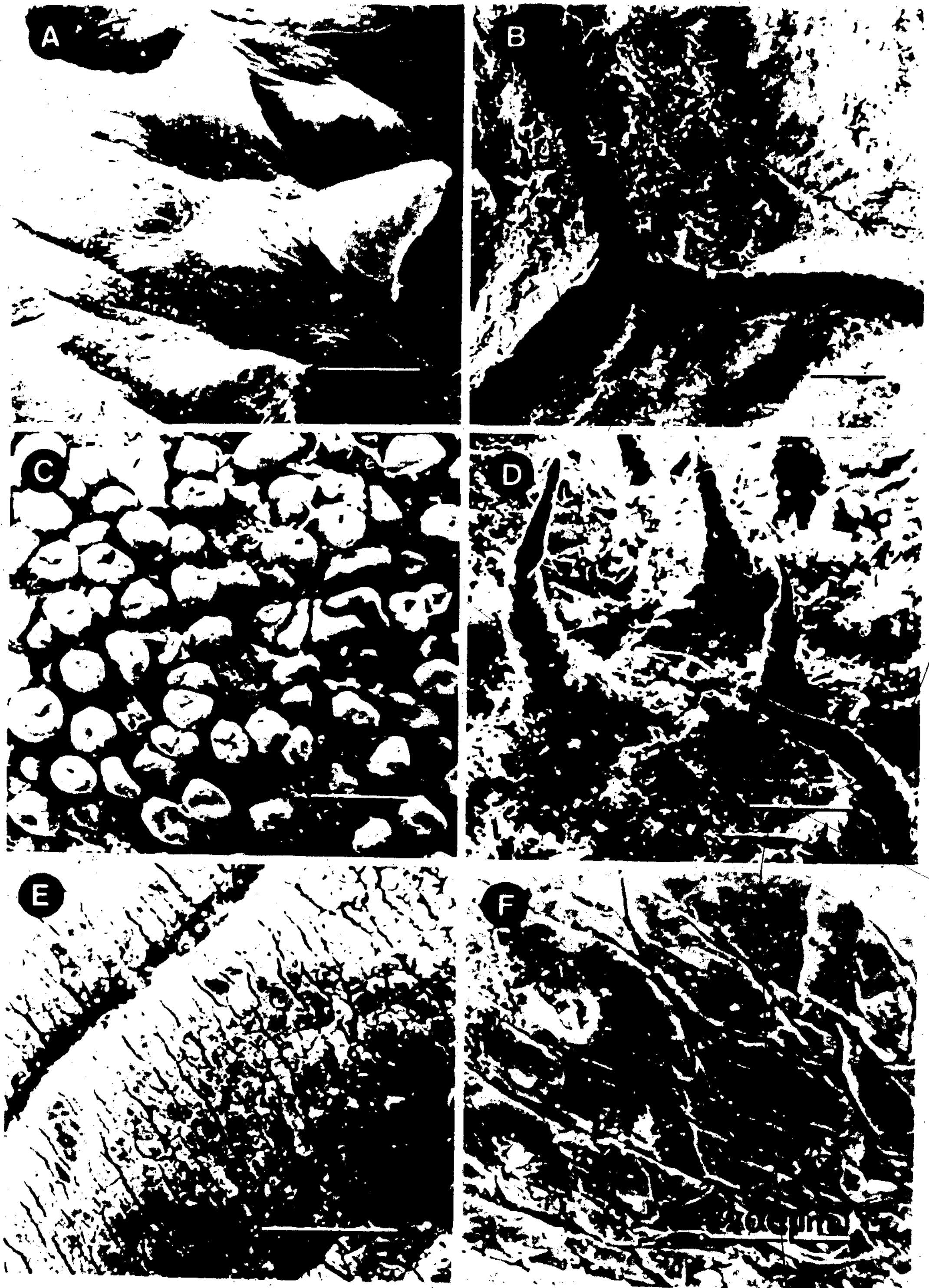

A
200 µm
B
50 µm

tunic coupled with these inclusions is the major defense mechanism. This is supported by the observation made earlier that the *P. haustor* attacked in the laboratory were all attacked on the bottom, where they had previously been attached to the rock. No rocks or sand are generally present in this region of the body, and the tunic here is often thinner and softer. That *F. oregonensis* does not reject *P. haustor* tissues as food (when it can gain access to them posteriorly) indicates that *P. haustor* is not unpalatable, and also that whatever chemicals might be present do not function in defending against the snail. Besides the underside, the only region of the body not protected by sand and tough tunic is the area around the siphons. The siphons of *P. haustor*, being long, flexible, and contractile seem superficially to be a vulnerable place. An enlarged view of the siphon surface (Fig. 49) reveals a dense plush of stiff, sharp spines. While experimental evidence is lacking, these could be defensive structures.

Cnemidocarpa finmarkiensis is not commonly attacked by *F. oregonensis*, and its tunic is smooth and clean, having no macroscopic elaborations of any kind (Fig. 50). Since there is never any other organism attached to the tunic, it seemed reasonable to implicate chemical defense in this species. I tested this possibility by macerating the tunic of several individuals in a waring blender, and soaking 7 *C. productum* in the resultant liquid overnight. These animals were then offered to *F. oregonensis* together with 7 control animals which had not been previously treated. *F. oregonensis* consumed all 14 individuals within the first 24 hours, and did not discriminate between groups even in the order of attack. This experiment neither rules out nor demonstrates the presence of defensive substances; if *Cnemidocarpa* employs such substances, they are not easily adsorbed on the tunic of other species.

Other possible defenses of *C. finmarkiensis* fall in the realm of speculation. One possibility is that this species defends itself behaviorally. When disturbed, *C. finmarkiensis* has the ability, unique among San Juan Island ascidians, of expelling virually all of the water from its branchial basket and atrium, and of contracting down to such an extent that it is nearly flat against the rock. This maneuver may make it difficult for *F. oregonensis* to drill, or to extract the tissues once drilling is completed. Also, since *F. oregonensis* discriminates actively against small ascidians, the contracted *Cnemidocarpa* may be below the size range preferred by the snail.

Figure 50. Scanning electron micrographs of ascidian tunic from 6 species. A: *Corella inflata*. B: anterior plates of *Chelyosoma productum*. C: *Styela coriacea*. D: Boltenia villosa. E: *Cnemidocarpa finmarkiensis*. F: *Styela gibbsii*.


A
B
C
D
E
F
200 µm

Many of the ascidians that are readily attacked by *Fusitriton* have tunic elaborations which might intuitively be considered defensive in function (Fig. 50). These include anterior plates in *C. productum*, soft hairs in *B. villosa*, tubercles in *Styela coriacea* and short spines in *Halocynthia aurantium* . Of these, the very small and rare *Styela coriacea* was never offered to *F. oregonensis* in my experiments, and the other ascidians were attacked in spite of their tunic structures. *Halocynthia aurantium* was not used in the feeding experiments because of its rarity. On one occasion, however, a *F. oregonensis* was accidentally placed in a tank where *H. aurantium* was being held. The snail had consumed it within 24 hours, despite the small, multi-pronged spines (M.J.Smith, 1970) which arise from its tunic. *F. oregonensis* predation on this species also been reported in the Vancouver area where it is much more abundant (M.J. Smith, 1970).

Spatial Refuges

To understand why *Boltenia villosa* and *Styela gibbsii* are able to co-occur with *Fusitriton oregonensis* in the rocky subtidal, while most other vulnerable species do not, their small scale distribution must be considered. As mentioned previously, a large percentage of all individuals of these species found at most of the sites were attached directly to the tunic of *Pyura haustor* or *Halocynthia igaboja*. That these latter species are well defended against snail attack suggested the hypothesis that by living epizooically, the vulnerable species might benefit from the defenses of the host. This was tested in the laboratory by exposing free-living and epizooic (on *H. igaboja*) *B. villosa* to several *Fusitriton* , and in the field by comparing survival of transplanted free-living individuals with that of naturally occurring epizooites, or transplanted epizooites on bricks.

In the laboratory, all free-living *B. villosa* were eaten within 8 days, while the epizooites all survived until the end of the experiment at 20 days (Fig. 51). Upon encountering the spines of *H. igaboja*, *F. oregonensis* often simply turned around and crawled in the other direction. I have observed *F. oregonensis* crawling over individual *P. haustor* in the laboratory, so epizooites on *P. haustor* may be less protected. Nevertheless, the common occurrence of *B. villosa* and *S. gibbsii* on *P. haustor* in the field argues strongly that the predators often avoid crawling on these ascidians. Field experiments produced essentially the same results as the lab experiments, though the

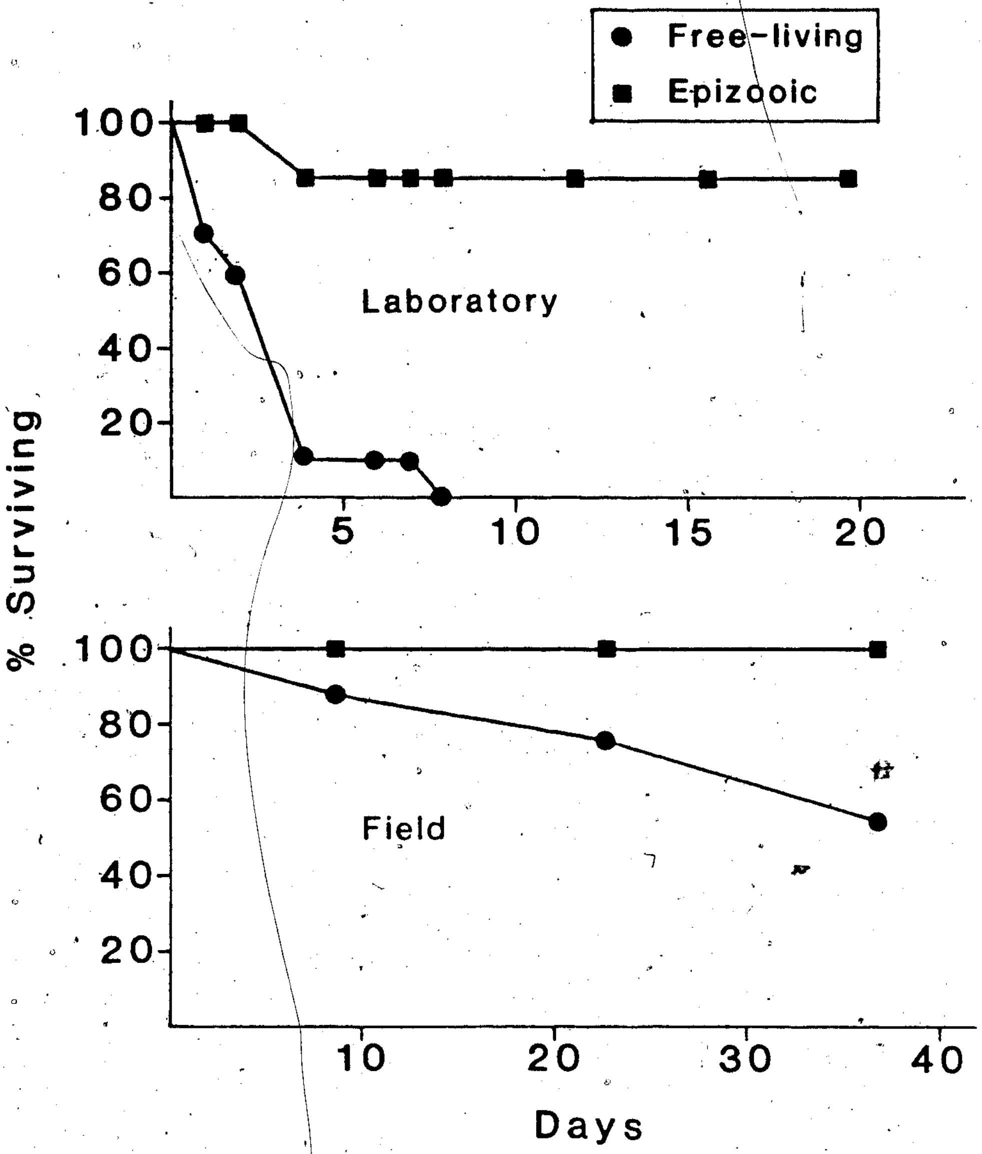


Figure 51. Survival of *Boltenia villosa* as free-living individuals (round points) and as epizooites (square points)in laboratory feeding experiments with *Fusitriton* (top) and in field transplants at Pt George (bottom).

results took much longer to obtain (presumably because of the lower probability of prey-predator encounter under normal *F. oregonensis* densities), and were not as clear cut. In the experiment where epizooites were transplanted together with their host *H. igaboja*, one of the bricks with epizooites was lost within the first months, perhaps to strong tidal currents. At this time, 4 of 8 original free-living animals had been consumed, while of the original 7 epizooites on the 6 *Halocynthia* remaining, 6 were still alive. When the experiment was terminated at 7 months, two of the six *H. igaboja* had died (possibly because of *F. oregonensis* attack to the exposed underside), so the epizooites on these were missing. Only one epizooite remained, and all of the free-living *B. villosa* had died. I attribute much of the epizooite mortality in this experiment to the manner in which the *Halocynthia* were made more vulnerable by their attachment method.

A more satisfying experiment was conducted by laying out a transect line on the Pt. George slope, recording the positions of naturally-occurring epizooites on *P. haustor* clumps near the transect, and comparing mortality of these animals with individual, free-living *B. villosa* transplanted to the immediate area. The experiment, which ran from April 28, 1981 to June 4, 1981, yielded the results plotted in Fig. 51, graph B. No epizooites died in this period, but of the 9 free-living *B. villosa* transplanted, only 5 remained at the end. I observed one of these animals shortly after it had died, and it had the characteristic *F. oregonensis* bore hole. The remaining three animals were decomposed to such an extent that the cause of death was not apparent.

Taken as a whole, the evidence suggests that by living as epizooites on *H. igaboja* and *P. haustor*, the vulnerable species such as *B. villosa* and *S. gibbsii* may find refuge from *F. oregonensis* and thus occur in the rocky subtidal regions where other ascidians without structural defenses are eliminated. Protection from *F. oregonensis* is not 100% effective. When the epizooite occurs near the edge of the host, *F. oregonensis* may encounter and eat it. I observed one instance of this in the field, at Shady Cove. A large dead *Corella inflata* was attached near the base of *Halocynthia*. The animal had clearly been eaten by *F. oregonensis*, and there was a *F. oregonensis* within 20 cm of the ascidians. This phenomenon was also observed on one occasion in the laboratory.

LARVAL SETTLEMENT BEHAVIOR

The distributions of settled juveniles in substratum choice experiments are shown in separate figures for each species, together with the expected values used in the analyses. The tests of significance for all of the experiments are summarized in Tables XIX through XXI. In a comparative study such as this, the number of potential experiments is enormous, because of the large variety of different substrata available to the larvae in the field. I tested only interactions which, on the basis of field distributions, promised to give interesting results. Species forming single species or multiple species aggregations were therefore emphasized, though a few species commonly occurring as isolated individuals on rock substrata were studied for comparative reasons. None of the rare species, with the exception of *Ascidia callosa* (which is common on docks), were studied.

Ascidians in Single Species Aggregations

Pyura haustor.

Preliminary evidence for gregarious settlement in *P. haustor* has been presented elsewhere (Young, 1978). The present experiments were designed to clarify the nature of the response and test preferences of ecological interest. As predicted from the field distributions, *Pyura haustor* larvae are the most gregarious of any species investigated (Figs. 52, 53) Conspecific adult tunic was preferred over rocks collected subtidally in every case and over gastropod shell (*F. oregonensis*) in the one experiment (Expt. 1) where it was offered. Additionally, *P. haustor* tadpoles discriminated against the smooth transparent tunic of *Ascidia paratropa* (Expt. 3,4). Where larvae were offered a choice between tunics of two stolidobranchs, they preferred *P. haustor* tunic over the other species in each case except Expt. 4 (Fig. 53). In this latter experiment, the dish became displaced so that the rows of substrata were diagonal rather than parallel to the window. The single piece of *H. igaboja* tunic which attracted the overwhelming majority of larvae was the substratum most distant from the light.

The importance of light in *P. haustor* settlement has been documented elsewhere (Young, 1978; chapter 2) and is also apparent in the present experiments. In each case, the greatest number of larvae settled in the row farthest from the light. This suggests

Table XIX.

Log-likelihood ("G") goodness of fit tests for substratum choice experiments with ascidians occurring in single species aggregations.

Species	Expt. No.	Factor	G	d.f.	P
Pyura haustor	1	substratum	235.193	6	< 0.001
		light	93.585	6	< 0.001
		interaction	17.877	4	0.001
	2	substratum	317.304	12	< 0.001
		light	304.140	12	< 0.001
		interaction	26.430	9	0.002
	3	substratum	955.403	12	< 0.001
		light	594.855	12	< 0.001
		interaction	83.56	9	< 0.001
	4	substratum	655.716	12	< 0.001
		light	363.501	12	< 0.001
		interaction	141.067	9	< 0.001
Ascidia callosa	1	substratum	6.186	6	< 0.5
		light	7.221	6	< 0.5
		interaction	4.106	4	0.391
	2	substratum	4.738	6	< 0.75
		light	5.813	6	< 0.5
		interaction	2.373	4	0.667
Corella inflata	1	substratum	142.540	6	< 0.001
		light	121.404	6	< 0.001
		interaction	63.055	4	< 0.001

(continued...)

Table XIX. (Continued)

Log-likelihood ("G") goodness of fit tests for substratum choice experiments with ascidians occurring in single species aggregations.

Corella inflata...cont'd	2	substratum	8.354	6	< 0.25
		light	6.545	6	< 0.5
		interaction	5.577	4	0.233
	3	substratum	2.809	6	< 0.9
		light	1.976	6	< 0.95
		interaction	1.890	4	0.756

Table XX

Log-likelihood ("G") goodness of fit tests for substratum choice experiments with ascidians occurring as epizooites in multiple species aggregations.

Species	Expt. No.	Factor	G	d.f.	P
Boltenia villosa	1	substratum	74.295	6	<0.001
		light	27.028	6	<0.001
		interaction	13.994	4	0.007
	2	substratum	9.617	6	<0.25
		light	5.237	6	<0.75
		interaction	5.028	4	0.284
	3	substratum	15.238	6	< 0.025
		light	9.787	6	< 0.25
		interaction	7.634	4	0.106
	4	substratum	35.287	6	< 0.001
		light	25.312	6	< 0.001
		interaction	15.406	4	0.004
	5	substratum	31.236	6	< 0.001
		light	15.908	6	< 0.025
		interaction	11.129	4	0.025
	6	substratum	10.262	6	< 0.25
		light	24.733	6	< 0.001
		interaction	9.733	4	0.045
Styela gibbsii	1	substratum	17.375	6	< 0.01
		light	6.575	6	< 0.5
		interaction	3.275	4	0.513

(continued...)

Table XX. (continued)

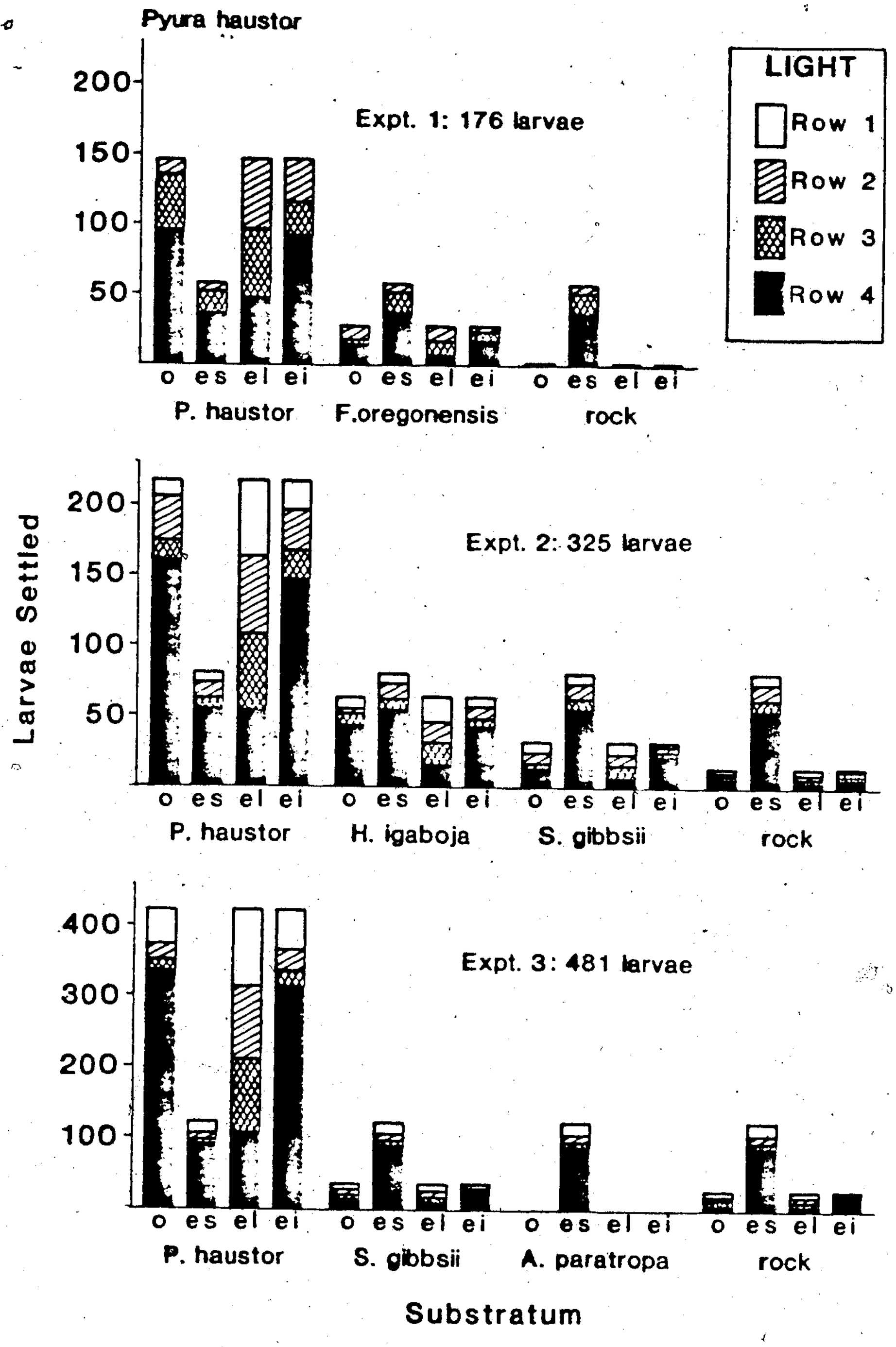
Species	**Expt. No.**	**Factor**	G	d.f.	P
Styela gibbsii...cont'd	2	substratum	2.440	6	< 0.9
		light	9.928	6	< 0.25
		interaction	1.815	4	0.769
	3	substratum	4.013	6	< 0.75
		light	3.394	6	< 0.9
		interaction	2.949	4	0.566
	4	substratum	31.145	12	< 0.005
		light	10.428	12	< 0.75
		interaction	5.230	9	0.814

Table XXI.

Log-likelihood ("G") goodness of fit tests for substratum choice experiments with ascidians occurring as isolated individuals.

Species	**Expt. No.**	**Factor**	G	d.f.	P
Cnemidocarpa finmarkiensis	1	substratum	39.736	6	< 0.001
		light	30.836	6	< 0.001
		interaction	7.161	4	0.127
	2	substratum	32.735	6	< 0.001
		light	32.707	6	< 0.001
		interaction	14.207	4	0.006
Ascidia paratropa	1	substratum	27.507	6	< 0.001
		light	9.638	6	< 0.25
		interaction	3.949	4	< 0.413
	2	substratum	106.439	6	< 0.001
		light	63.55	6	< 0.001
		interaction	26.269	4	< 0.001
Corella willmeriana	1	substratum *	154.748	6	< 0.001
		light	29.369	6	< 0.001
		interaction	25.352	4	< 0.001
	2	substratum	12.516	6	< 0.1
		light	16.223	6	< 0.025
		interaction	9.646	4	< 0.047

Figure 52. Substratum selection experiments in a light gradient with *Pyura haustor* larvae. Each bar is subdivided into three or four regions, which represent identical groups of substratum choices at different light levels; Row 1 or Row 2 is nearest the light, while Row 4 is farthest from the light. For each substratum, four bars are given: "o" is the observed distribution of settled larvae, "es" is the expected distribution based on the hypothesis of uniform settlement among potential substrata, "el" is the expected distribution based on the hypothesis of uniform settlement among rows (light regimes), and "ei" is the expected distribution based on the hypothesis of independence between light and substratum. Genus names are abbreviated with first letter. Significance levels and other details of the analysis are presented in Table XIX.


Pyura haustor
LIGHT
Row 1
Row 2
Row 3
Row 4
Expt. 1: 176 larvae
200
150
100
50
o es el ei
P. haustor
o es el ei
F.oregonensis
o es el ei
rock
Expt. 2: 325 larvae
200
150
100
50
o es el ei
P. haustor
o es el ei
H. igaboja
o es el ei
S. gibbsii
o es el ei
rock
Expt. 3: 481 larvae
400
300
200
100
o es el ei
P. haustor
o es el ei
S. gibbsii
o es el ei
A. paratropa
o es el ei
rock
Larvae Settled
Substratum

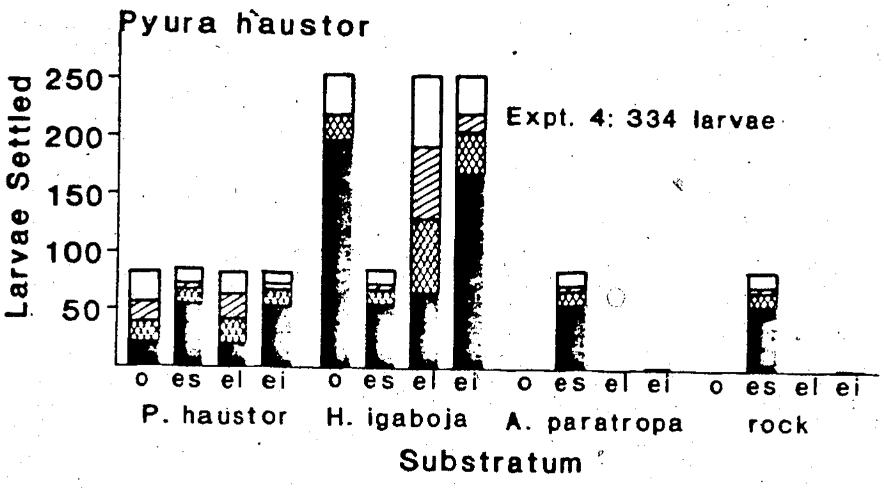


Figure 53. Substratum selection experiments with *Pyura haustor* larvae in which dish was turned 45 degrees so that rows were not perpendicular to the light, and a single piece of *Halocynthia igaboja* tunic was the substratum farthest from the light. See Fig. 49 legend for additional explanation and Table XIX for analysis.

that larvae swim to the dark side of the dish well before metamorphosis and only then seek appropriate chemical cues for settlement.

Pyura haustor larvae delayed metamorphosis in the absence of *P. haustor* tunic (Fig. 54), both when the treatments are maintained in the light and the dark. The dark treatments demonstrated that the preference was for the tunic itself and not for shadows associated with the tunic.

It should be emphasized that the gregarious response of *P. haustor* is not obligatory; larvae will settle on other substrata, especially other stolidobranchs, if *P. haustor* tunic is not available. That the attraction for *P. haustor* is chemical and not due to the wrinkles or surface irregularities is suggested by two main lines of evidence. First, the pieces of *F. oregonensis* shell, with intact periostrocal hairs, of *H. igaboja* tunic, also with hairs present, and of *Styela* with numerous deep wrinkles all presented substantially greater surface areas than the *P. haustor* tunic disks, each of which was generally crossed by only one to a few wrinkles. Second, in the delay of metamorphosis experiments, where both the smooth inner surface and wrinkled outer surface of the tunic were available to the larvae, the inner surface invariably attracted the greatest number of settlers, both in light treatments and dark treatments. Previous experiments on the gregarious response of *P. haustor* larvae (Young, 1978) also support the hypothesis that the response is chemosensory in nature.

Chelyosoma *productum.*

Chelyosoma productum, which often occurs in dense aggregations on floating docks and soft sediment was the first ascidian reported to exhibit gregarious settlement behavior (Young and Braithwaite, 1980a). Larvae of this species prefer to settle on the tunic of the adult or near conspecific juveniles and are capable of delaying metamorphosis for at least a short time in the absence of these stimuli.

Ascidia *callosa.*

Larvae of *Ascidia callosa* are quite non-discriminating in their choice of substratum. Although larvae settled readily on the adult tunic, they attached in larger numbers to the tunic of *Chelyosoma productum* (Fig. 55). The differences, however, were non-significant in each case. Both *P. haustor* tunic and rock attracted fewer settling larvae than phlebobranchs tunic. Neither the effect of light nor the light/substratum

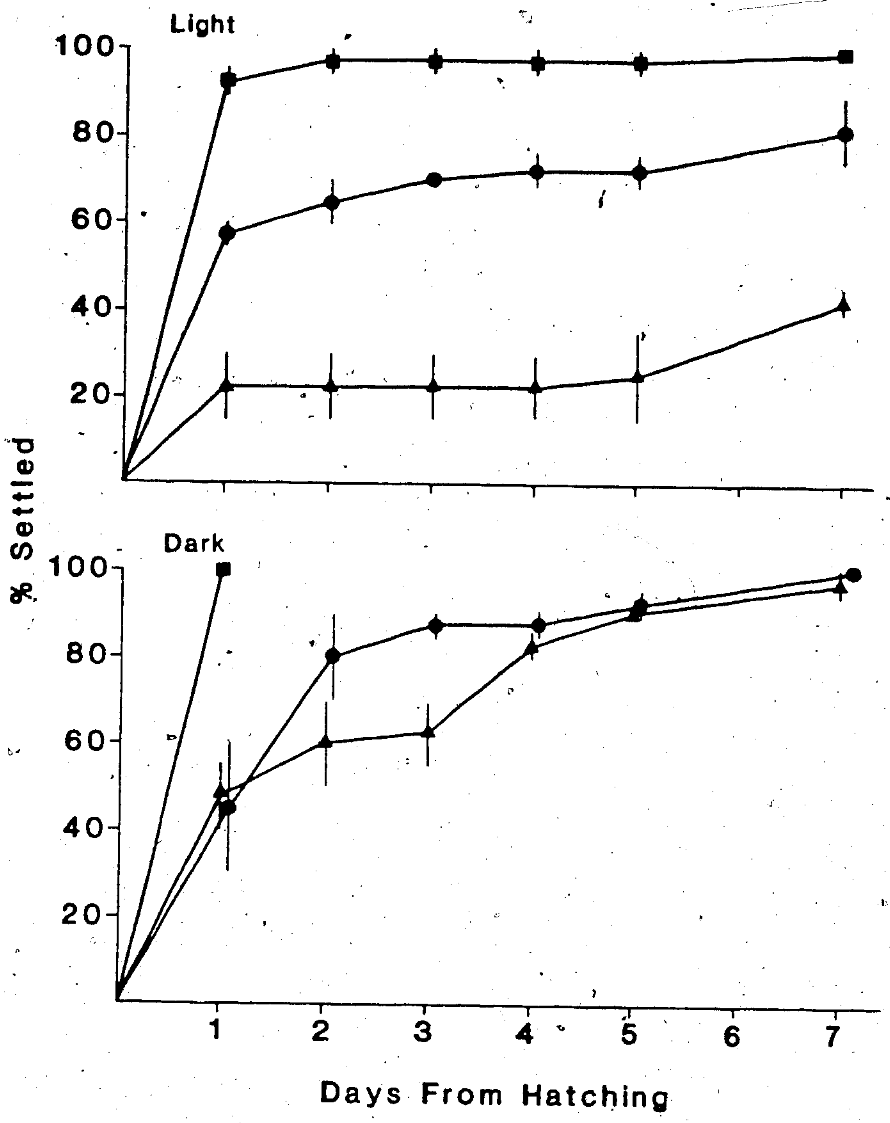


Figure 54. Delay of settlement experiments with *Pyura haustor* larvae in continuous light (top graph) and continuous darkness (bottom graph). Each point is the mean of 2 replicates plus or minus 1 S.E. Square points: piece of *Pyura* adult tunic present. Round points: subtidally collected rock present. Triangular points: no substrata added to the dish. In this and similar graphs, the round points have been displaced slightly and uniformly to the right to allow resolution of S.E. bars.

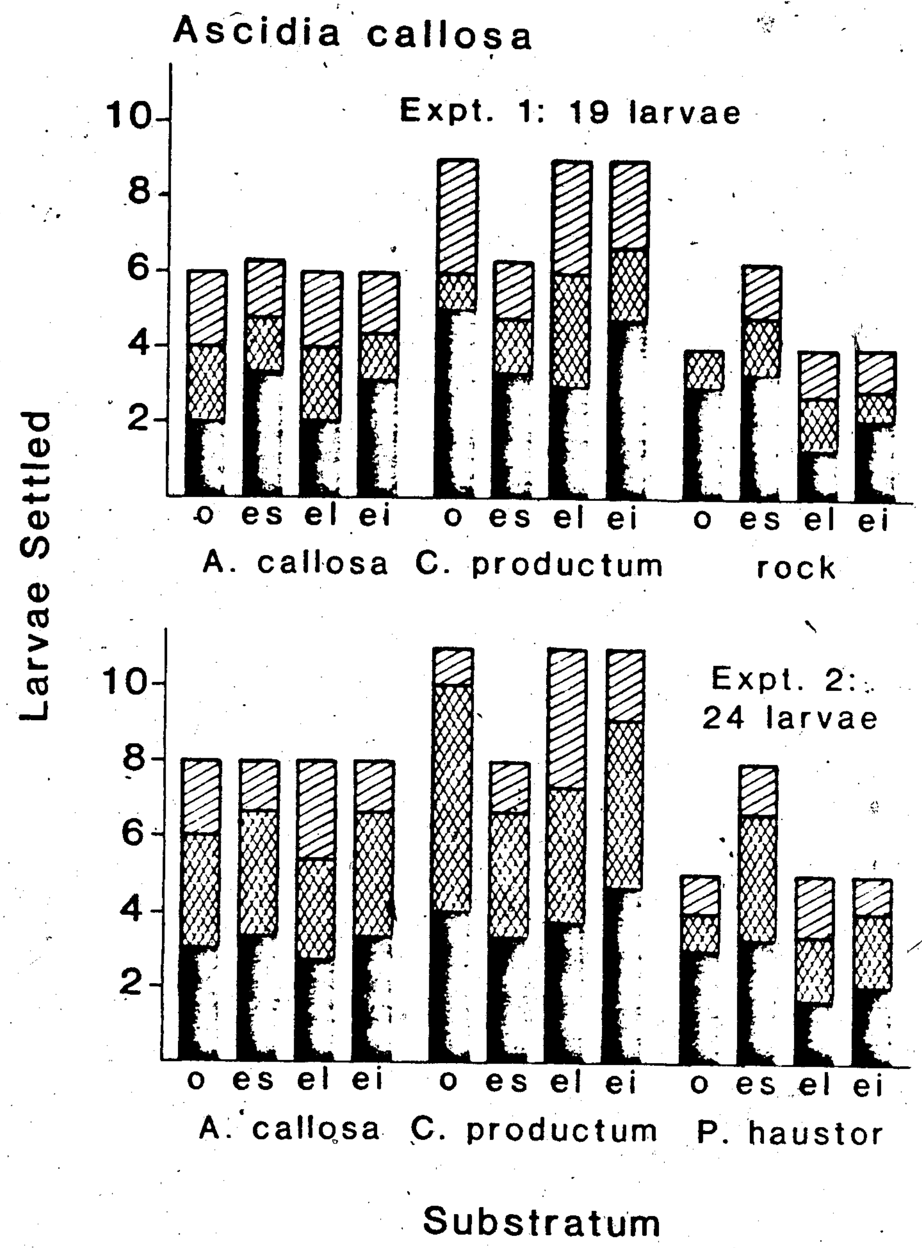


Figure 55. Substratum selection experiments with *Ascidia callosa* larvae. See Fig. 52 legend for explanation of graph and Table XIX for analysis

interaction was significant (Table XIX).

In absence of any substratum other than glass, larvae will delay metamorposis for a full week (Fig. 56; chapter 1), suggesting that they are not entirely indifferent to surface charcteristics. Likewise, they showed a slight tendency to settle sooner in the presence of *A. callosa* tunic than rock.

In summary, the evidence hints that the larvae of *Ascidia callosa* are slightly gregarious, but that they do not discriminate much against other natural substrata. The aggregations observed in the field , which were all on floating docks in relatively quiet bays, are probably as much the result of high larval concentrations as of gregarious settlement.

Corella inflata.

The field distribution of this species suggested that observed aggregations were due not to larvae attaching to adults, but to many larvae attaching to non-ascidian substrata in the same region. Nevertheless, in laboratory experiments, many larvae selected adult *C. inflata* tunic as a settlement site (Fig. 57). In the one case (Expt. 1) where a significant substratum preference was seen, however, the larvae chose rock which had been scrubbed with a toothbrush to remove epiflora. Natural rock with flora intact was less attractive, as was *C. inflata* tunic. The larvae did not discriminate between tunics of *P. haustor* and their own adults, despite obvious differences in texture, reflectance, and composition. In experiment 2, the greatest number of larvae settled on pieces of scallop shell which had numerous small parallel varices. However, the preference was not significant.

Juveniles attached to *C. inflata* tunic in the laboratory always died within 48 hours of settlement. It is not known whether this was due to high bacterial concentrations as the tunic pieces began to decompose, or to chemicals (e.g., acid) in the tunic proper. The latter would seem a distinct possibility in light of the clean appearance of field collected animals and the recent finding by Stoecker (1978) that low pH in ascidian tunic often prevents fouling.

I conclude that the aggregated distribution of *Corella inflata*, unlike those of *C. productum* and *P. haustor*, cannot be attributed directly to larval behavior. It is probably a consequence of brooding; adults retain larvae until just before metamorphosis, so

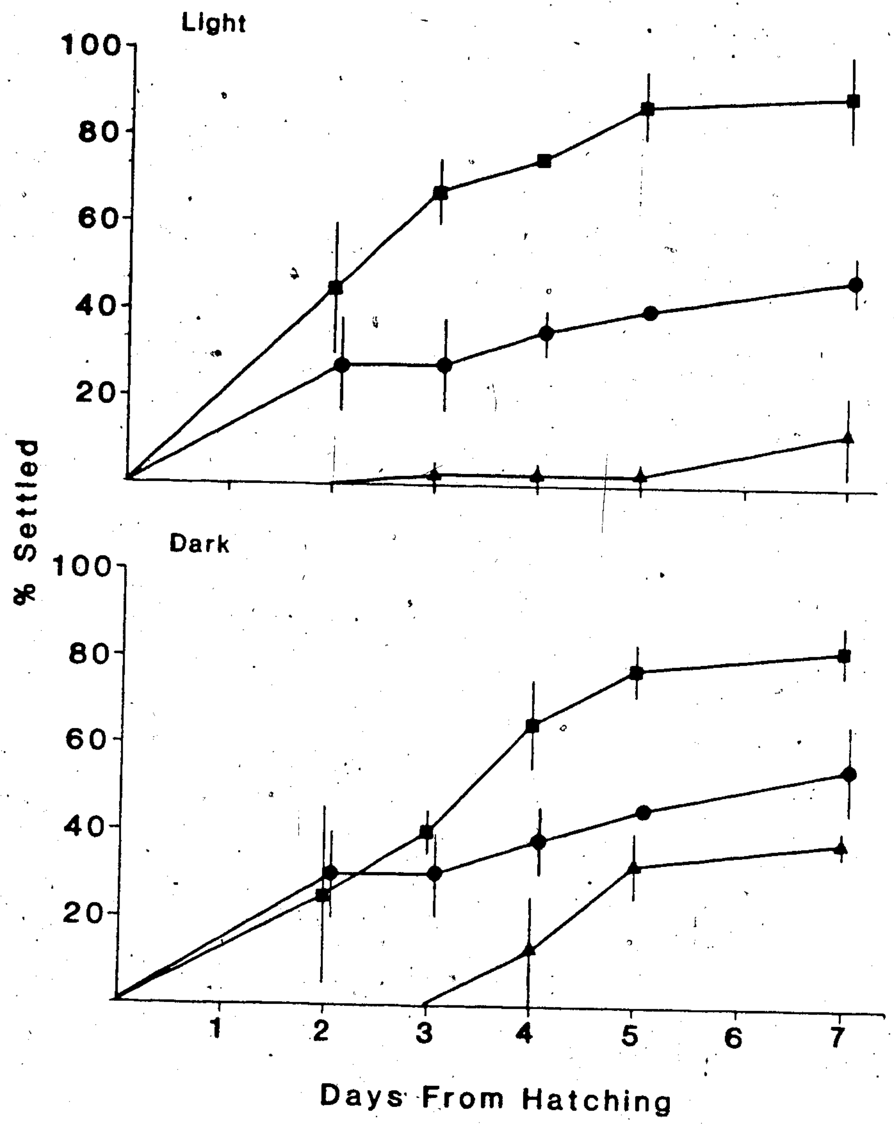


Figure 56. Delay of settlement experiments with *Ascidia callosa* larvae. Square points: *Ascidia callosa* adult tunic. Other details as in Figure 54.

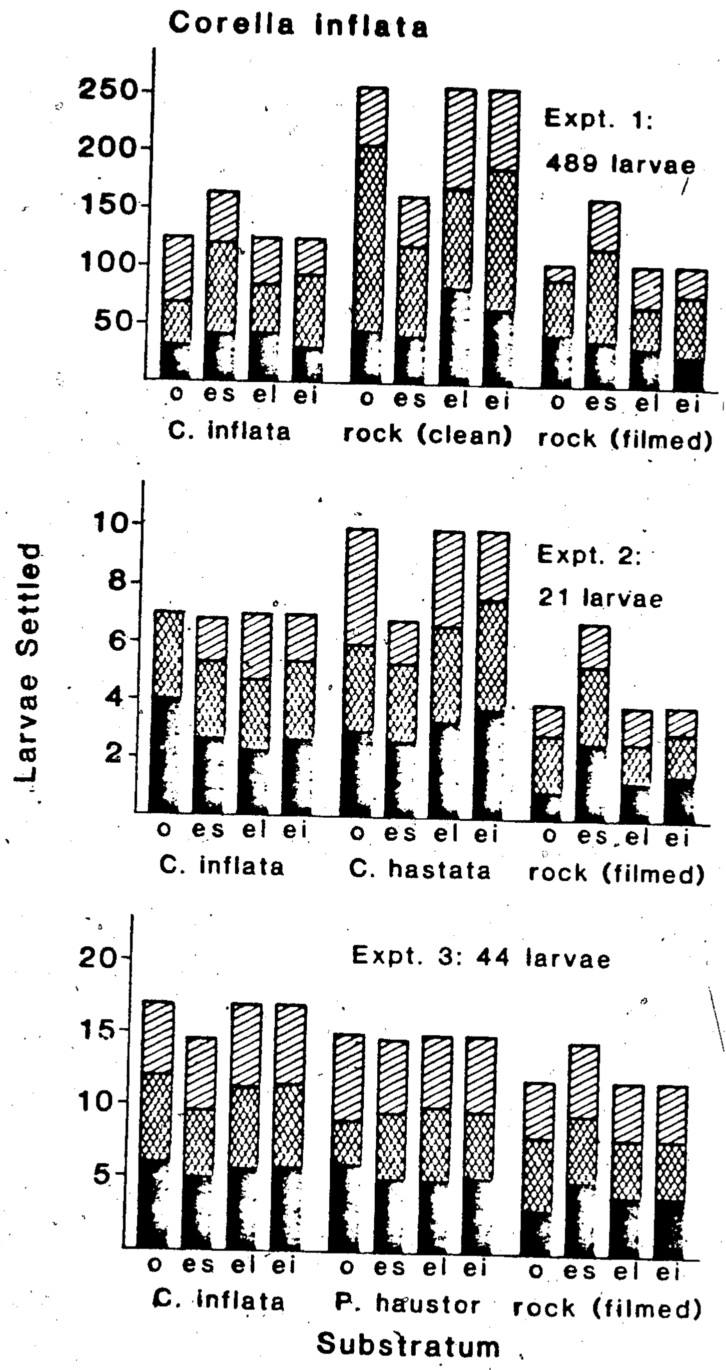


Figure 57. Substratum selection experiments with *Corella inflata* tadpoles. "*C. hastata*" is shell from the scallop, *Chlamys hastata*. Other details as in Figure 52 legend. Analysis is presented in Table XIX.

dispersal is limited.

Ascidians in Multiple Species Aggregations

The behavior of *Halocynthia igaboja* tadpoles was not studied, due to the difficulty of obtaining good cultures. However, since its distribution is unremarkable (random, generally on rock surfaces) it is assumed that the tadpoles are relatively non-discriminating with respect to substratum.

Boltenia villosa

It is predicted that where a refuge occurs, tadpoles should evolve a preference for the refuge, providing predictable cues detectable by the larvae are associated with it (Woodin, 1979). Thus, the larvae of *B. villosa* were expected to discriminate in favor of *P. haustor* or *H. igaboja* tunic and they did (Figs. 58, 59). Whenever offered a choice between tunic of the "invulnerable" stolidobranchs and natural rocks, the former was highly preferred (Fig. 58). Neither *H. igaboja* nor *P. haustor* was preferred significantly over the other (Expt. 1); the slightly higher settlement on *H. igaboja* could easily be a surface area effect. Likewise, the larvae do not differentiate well between the invulnerable species and the tunics of other related stolidobranchs including *Styela gibbsii* (Expt. 5) and their own adults (Expt. 2,6). In Experiment 6, two types of *P. haustor* tunic were offered, some collected subtidally, with embedded sand grains and an irregular shape, and some collected from a quiet bay where individuals had few wrinkels or inclusions. The difference was not significant, suggesting that preference is not based on rugosity. Larvae preferred stolidobranch tunic over *Chelyosoma productum* tunic also (Expt. 5).

The effect of light was significant in 4 of the 6 experiments, and the interaction between light and substratum was significant in these same experiments (Table XX). This suggests that like *P. haustor*, *B. villosa* tadpoles are more likely to settle on an attractive substratum if it is in a shaded area.

In the delay of metamorphosis experiments, larvae delayed metamorphosis for the longest period in the absence of all substrata except glass, and settled most readily when *P. haustor* tunic was present in the dish (Fig. 60). In both dark and light treatments, larvae preferred the cleanest portions of the *P. haustor* test (generally the inside surface),

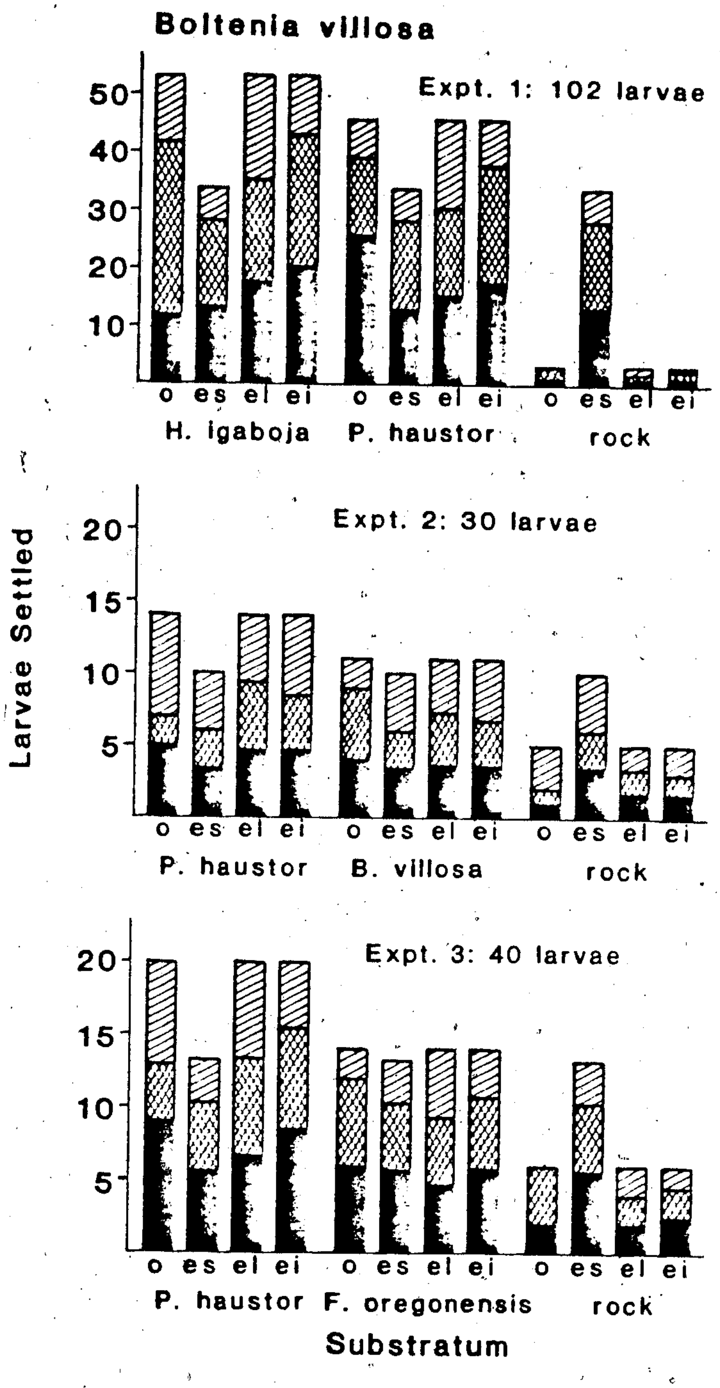


Figure 58. Substratum selection experiments with *Boltenia villosa* tadpoles. "*F. oregonensis*" is shell from *Fusitriton oregonensis*, with hairs on the periostracum intact. See Figure 52 legend for further explanation and Table XX for analysis.

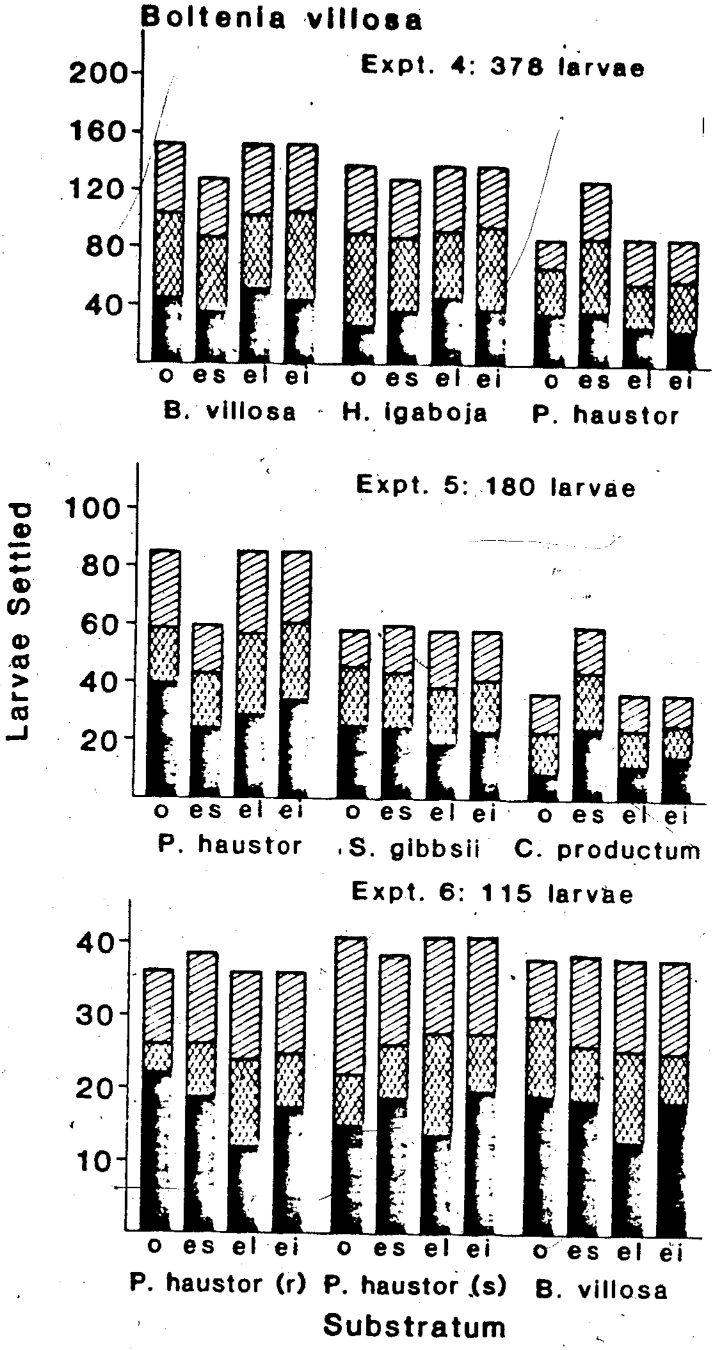


Figure 59. Additional substratum selection experiments with *Boltenia villosa* tadpoles. "*P. haustor* (r)" is *Pyura* tunic which is rough and rugose, collected from current–swept subtidal sites, while "*P. haustor* (s)" is tunic from soft and smooth individuals collected from the protected waters of Wescott Bay on San Juan Island. See Figure 52 legend for further explanation and Table XX for analysis.

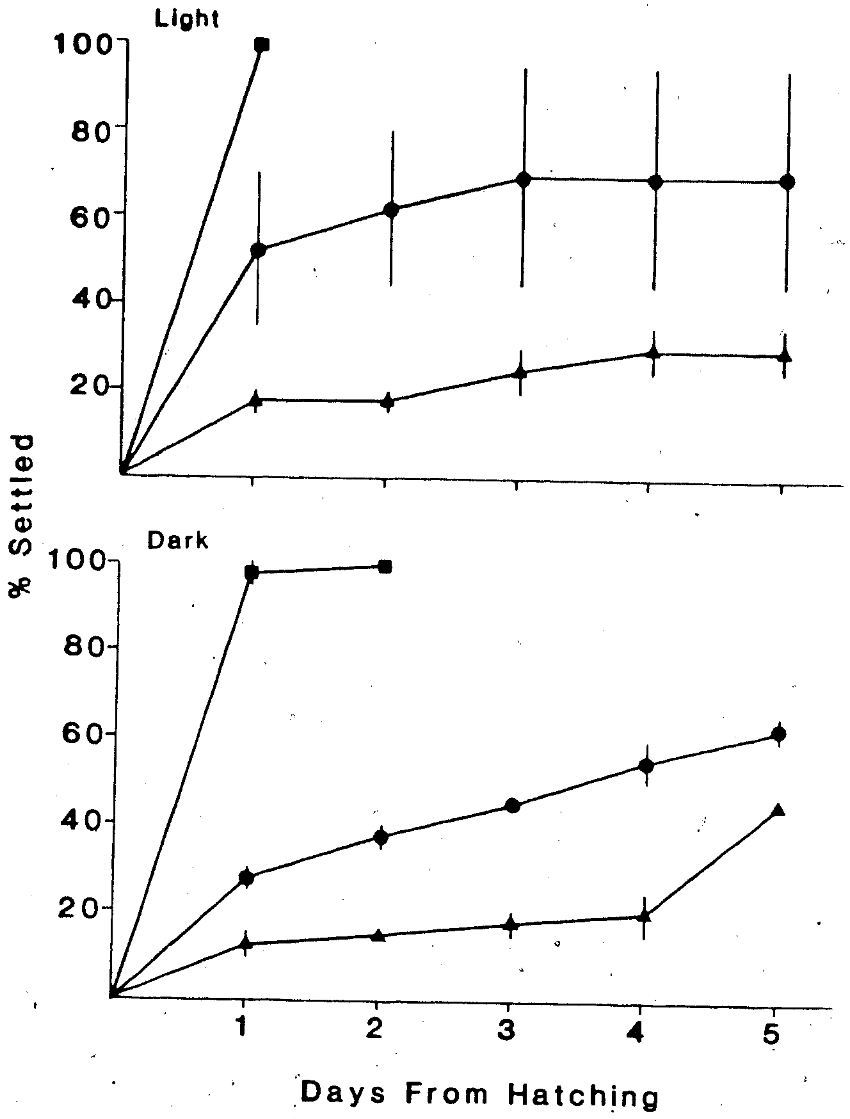


Figure 60. Delay of settlement experiments with tadpoles of *Boltenia villosa*. Square points: adult tunic from *Pyura haustor*. Other details as in Figure 54.

suggesting once again that the factor inducing metamorphosis is a chemical in the tunic, and not simply the irregularity of the surface

Styela gibbsii.

Although the field distribution of *S. gibbsii* is similar to that of *B. villosa*, behavior of *S. gibbsii* in the lab is less definite. Offered a choice between adult tunic and two types of rock, the tunic was strongly preferred (Fig. 61, Expt. 1, Fig. 62 Expt. 4). Pyura tunic was preferred over rock in experiment 4 but not experiment 2. The reason for this discrepency is not known, but experiment 2 was also the one in which larvae showed the strongest preference for the dark side of the dish.

S. gibbsii larvae, like those of *P. haustor*, settle more readily when there is adult ascidian tunic present (Fig. 63).

Ascidians Occurring as Individuals

Cnemidocarpa finmarkiensis.

This species lives almost exclusively on rock in the subtidal zone, where it occupies primarily cryptic sites. Larval behavior correlates well with this observed distribution. The tadpoles preferred rock over adult *C. finmarkiensis* tunic or adult *P. haustor* tunic (Fig. 64). Surprisingly, in Expt. 2, the tunic of *Corella inflata* was preferred over that of *P. haustor*, but once again, was much less preferred than rock. The most attractive substratum in Experiment 1 was shell of the scallop, *Chlamys hastata*. It is suspected that the attractive attribute of this shell type is not its composition, but rather its numerous small ridges. The larvae mostly attached in the cracks where the ridges (varices) were connected to the shell. Once again, this behavior might be expected on the basis of field distribution.

In both experiments, the effect of light was highly significant, while the light/substratum interaction was significant only in experiment 2, apparently because of the much larger proportion of larvae selecting the dark on rocky substrata than on the other substrata offered. This may be in part because of shadows present on the *P. haustor* tunic. Larvae settling on this substratum did not have to be at the dark side of the dish in order to locate a shaded site.

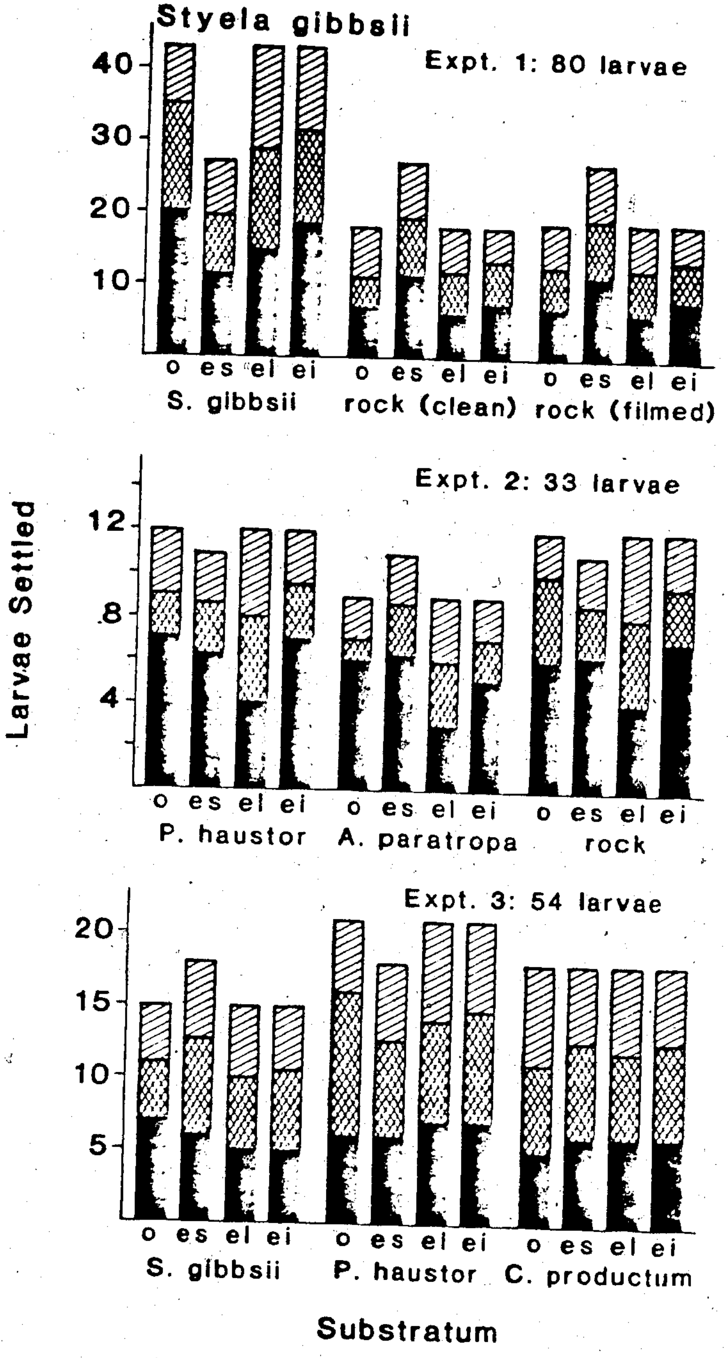


Figure 61. Substratum selection experiments with tadpoles of *Styela gibbsii*. See Figure 52 legend for explanation and Table XX for analysis.

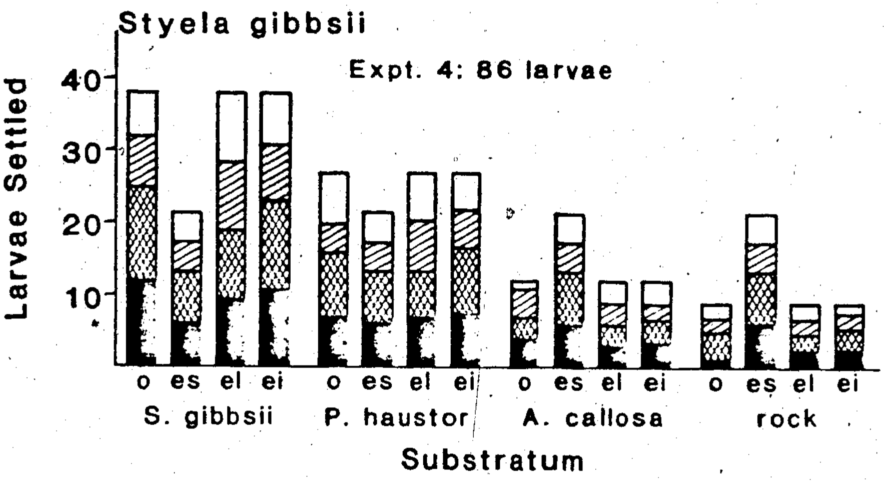


Figure 62. Additional substratum selection experiment with tadpoles of *Styela gibbsii*. See Figure 52 legend for explanation and Table XX for analysis.

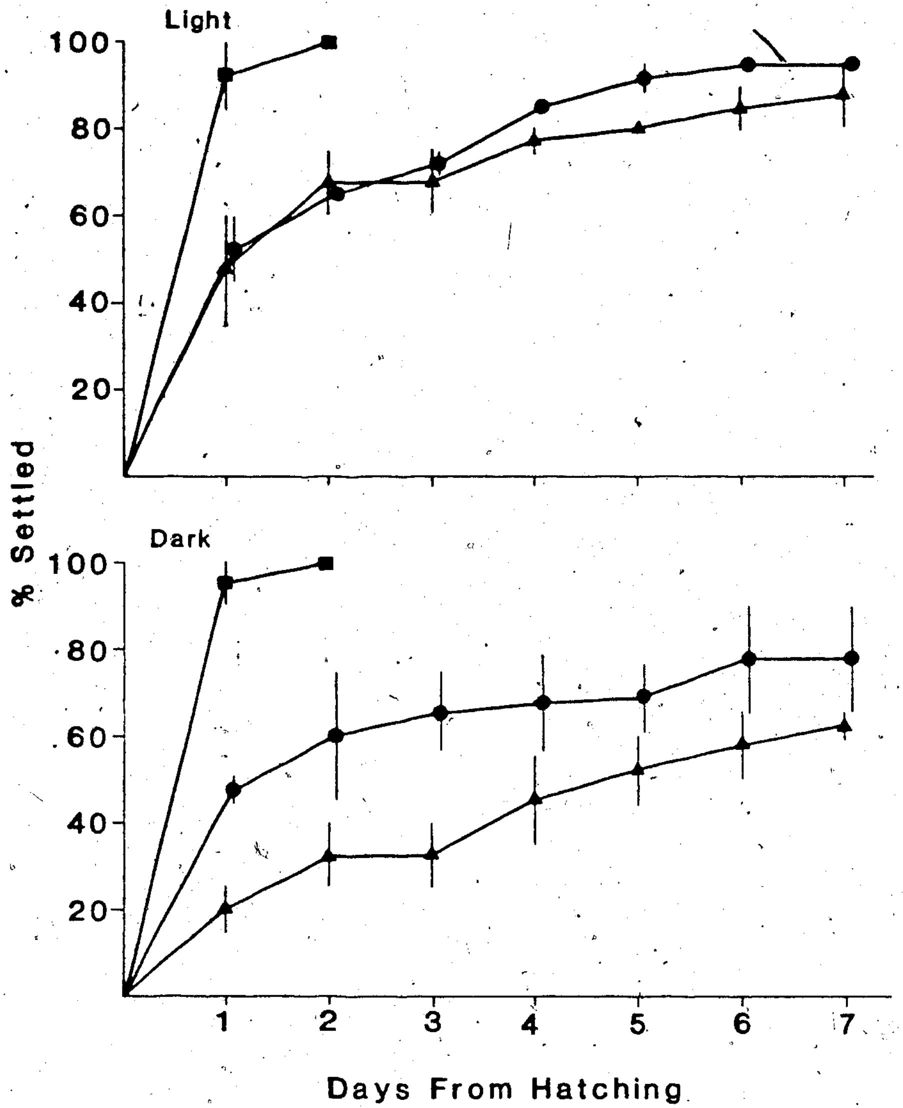


Figure 63. Delay of settlement experiments with *Styela gibbsii* tadpoles. Square points: adult *Styela* tunic. Other details as in Figure 54.

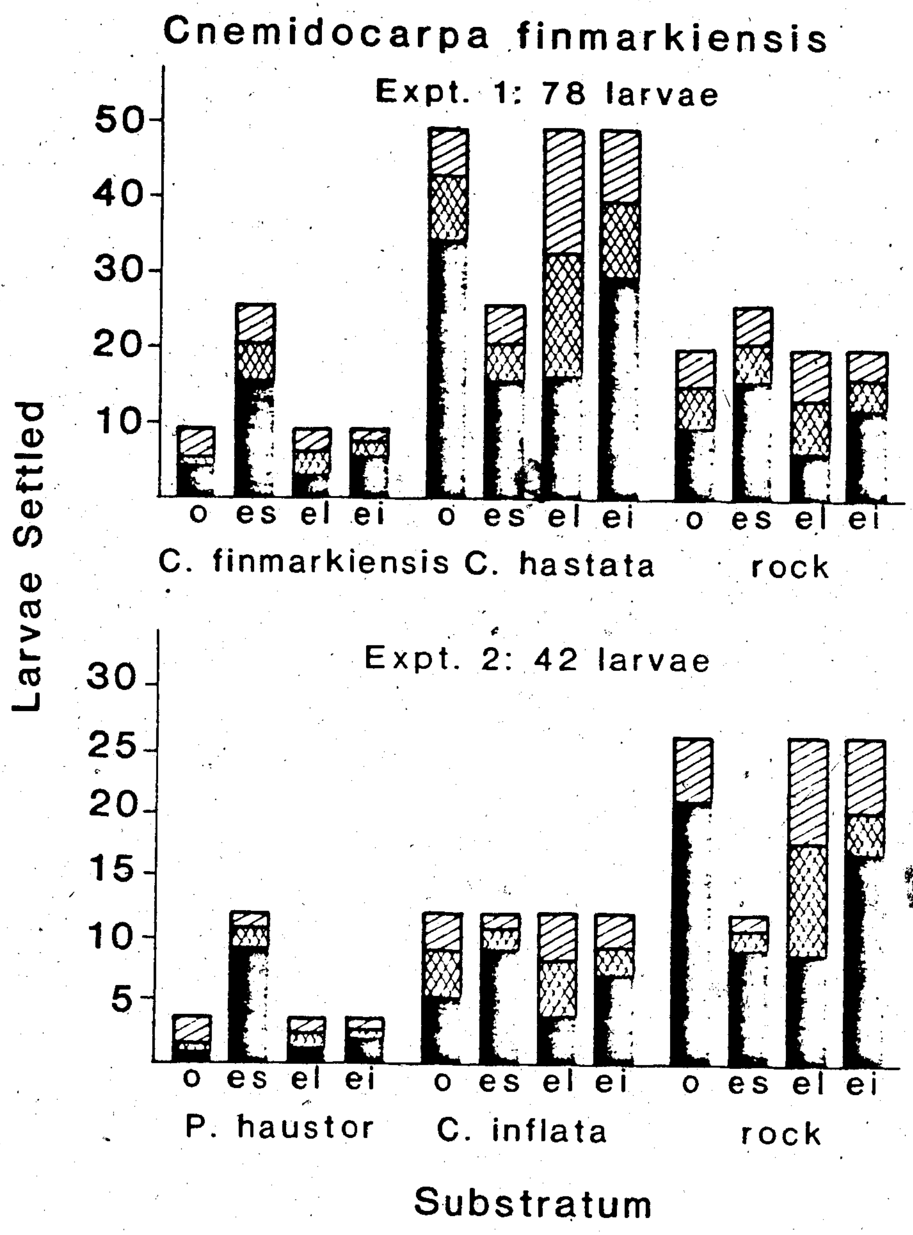


Figure 64. Substratum selection experiments with *Cnemidocarpa finmarkiensis* tadpoles. "*C. hastata*" is shell from the scallop, *Chlamys hastata*. See Figure 52 legend for further explanation and Table XXI for analysis.

C. finmarkiensis larvae demonstrated good ability to delay metamorphosis (Fig. 65). As expected, larvae in the light settled as readily on rock as on adult tunic, unlike the larvae of gregarious species discussed earlier. However, in the dark, larvae in the presence of adult *C. finmarkiensis* tunic demonstrated an unexpected tendency to settle much earlier than larvae in the presence of rock or glass. That there was little difference in settlement between rock and glass groups in the dark suggests that light (or shade) is the dominant cue for habitat selection in this species. This is supported by the studies of photoresponse reported in chapter 2.

Ascidia paratropa.

Only two experiments were run with larvae of *Ascidia paratropa* (Fig. 66). In Experiment 1, tadpoles did not differentiate between rock and the tunic of adult *A. callosa*. However, they did prefer both of these substrata over *Pyura haustor* tunic. Scallop shell, with its rugose surface was preferred over rocks, and no preference was shown for rocks with film over clean rocks.

The effect of light was significant in one experiment, but not the other. In Expt. 2, the interaction between the two factors was highly significant (Table XXI). Inspection of the data (Fig. 66) suggests that this was due to a more or less even distribution of larvae over scallop shells of the different rows, contrasted by a greater settlement on the clean rock furthest from the light than on the other rocks. The effect of adult tunic in inducing metamorphosis was not tested in this species. However, the tadpoles did delay metamorphosis in the absence of rocks (Fig. 67).

Corella willmeriana.

Settlement preferences of *Corella willmeriana* were essentially similar to those of the other individually-occurring ascidians investigated. Rugose scallop shell was highly preferred, but the larvae did not differentiate among any of the other substrata tested (Fig 68). This suggests that while surface texture may be important, larvae will settle on virtually any substratum available. This is consistent with the field distributions, in which rock, the substratum type most available, was the substratum most often used.

The effects of light and the light/substratum interaction were significant in both experiments, though in Experiment 1, it appears that most larvae settled in rows other than that nearest the dark side of the dish.

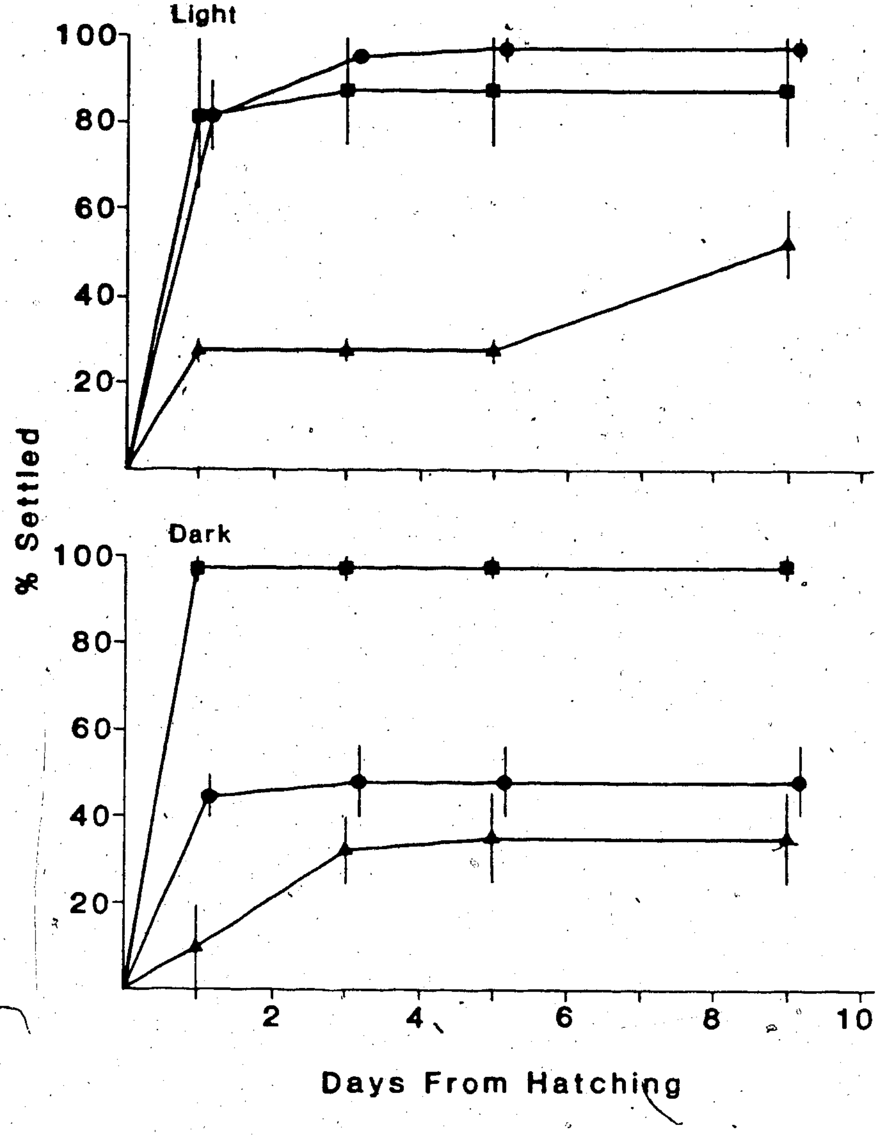


Figure 65. Delay of settlement experiments with *Cnemidocarpa finmarkiensis* tadpoles. Square points: adult *Cnemidocarpa* tunic. Other details as in Figure 54.

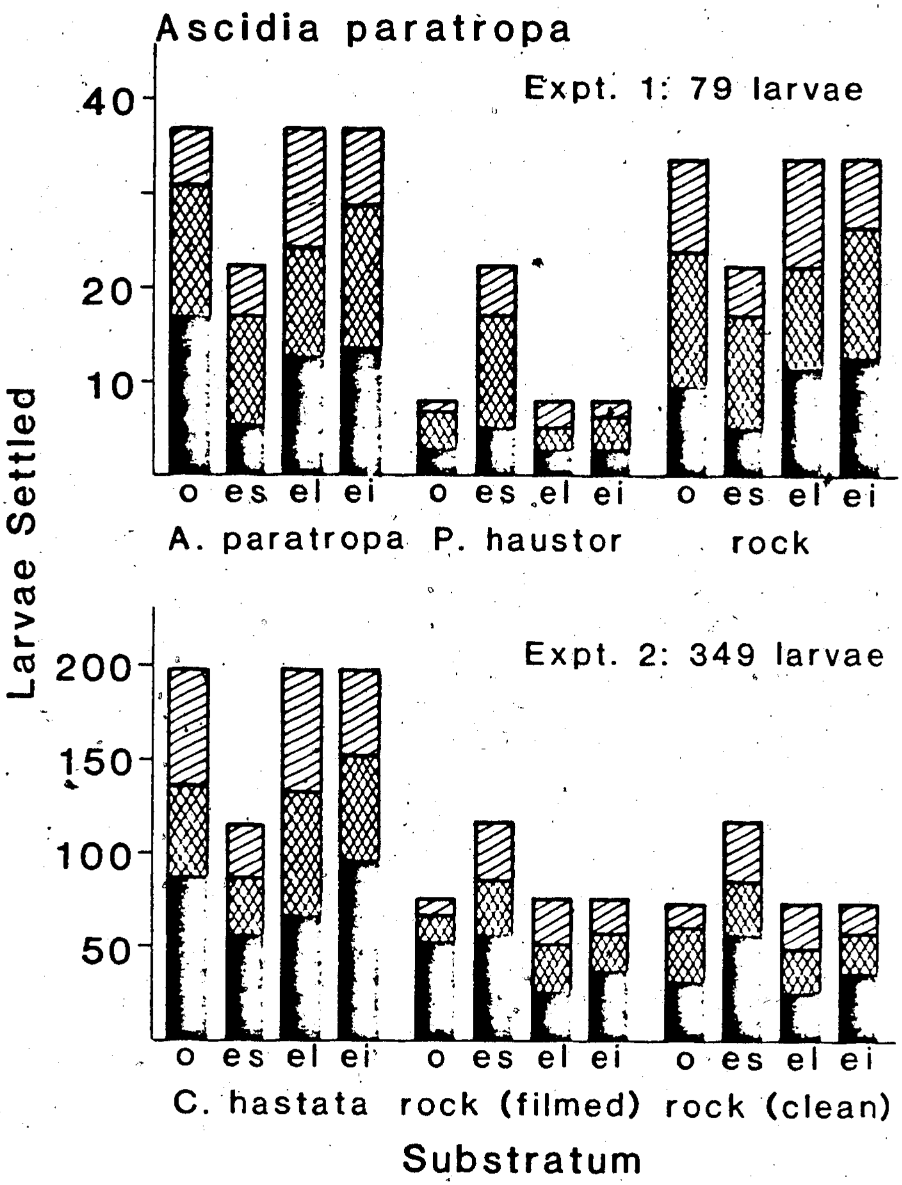


Figure 66. Substratum selection experiments with *Ascidia paratropa* tadpoles. "*C. hastata*" is shell from the scallop, *Chlamys hastata*. See Figure 52 legend for further explanation and Table XXI for analysis.

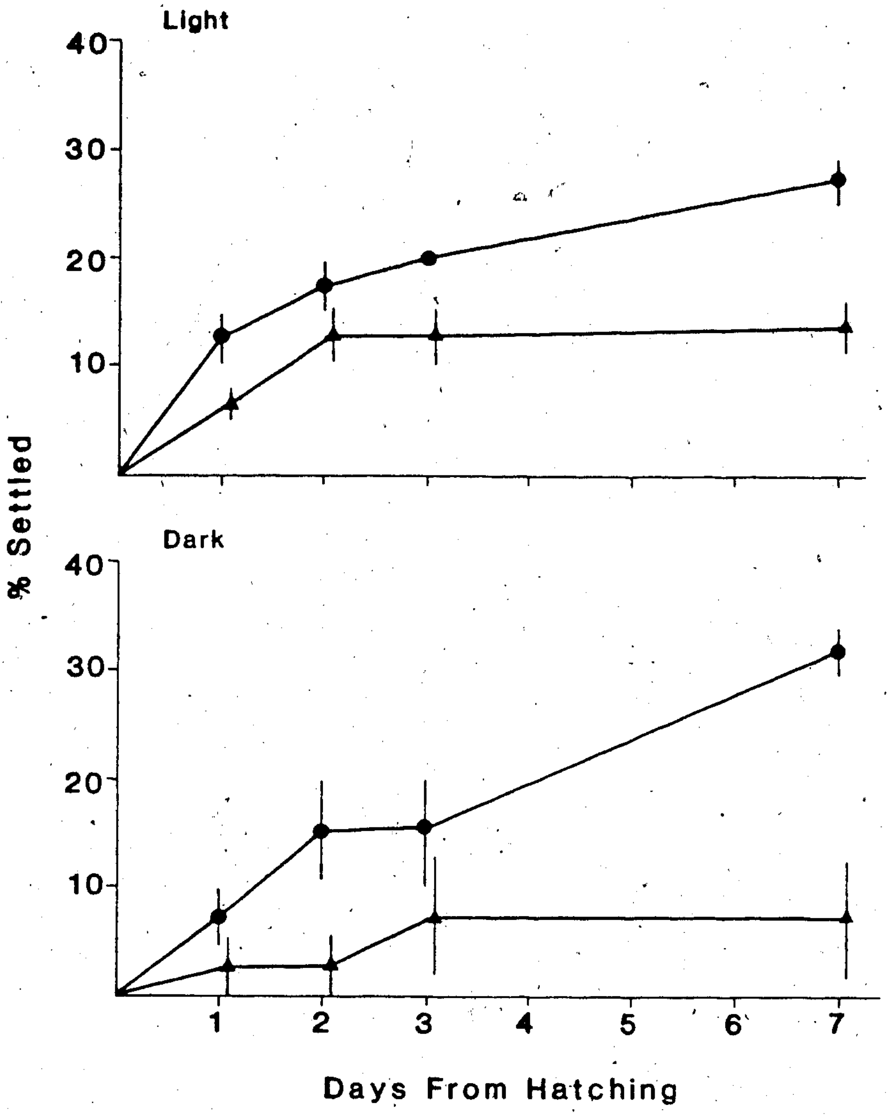


Figure 67. Delay of settlement experiments with *Ascidia paratropa* tadpoles. Round points: rock present. Triangular points: no substratum added. Other details as in Figure 54 legend.

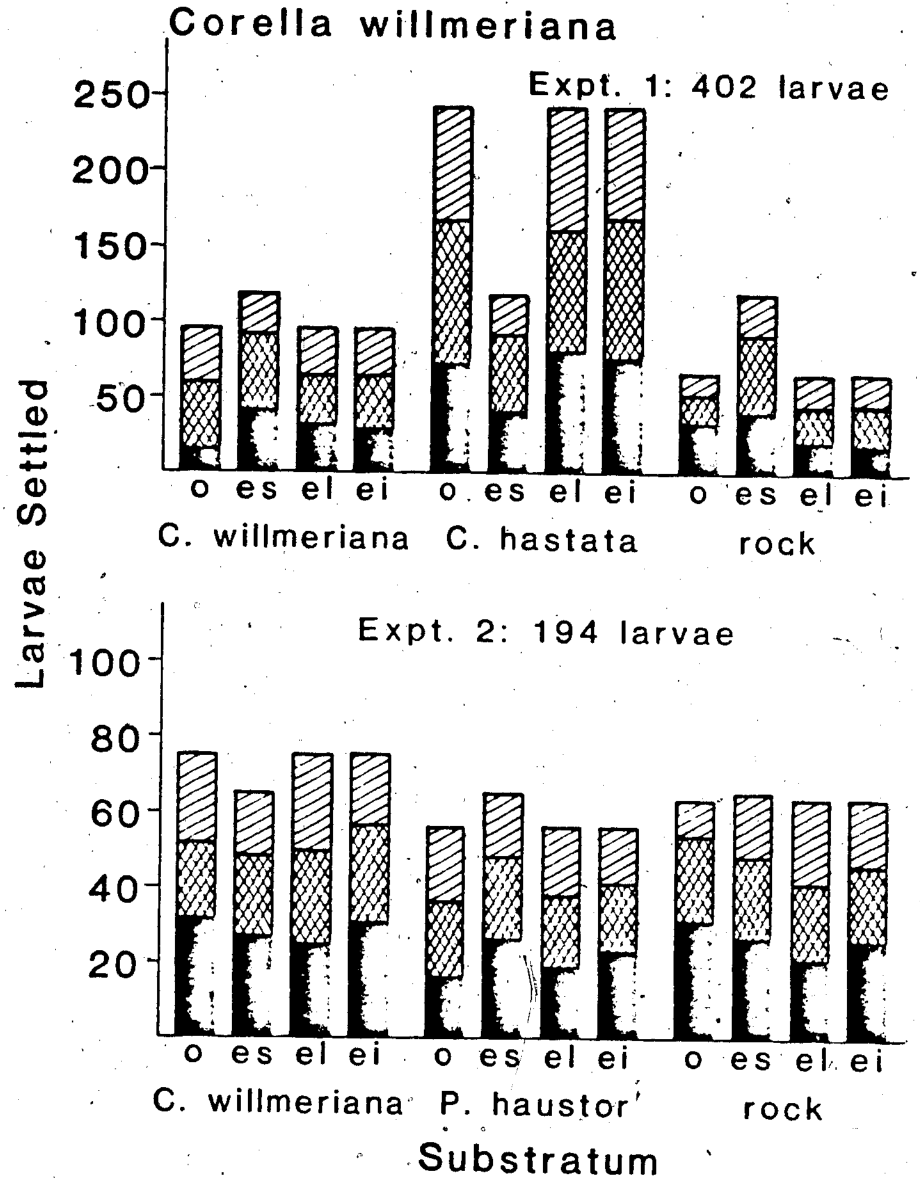


Figure 68. Substratum selection experiments with *Corella willmeriana* tadpoles. "*C. hastata*" is shell of the scallop, *Chlamys hastata*. See Figure 52 legend for further explanation and Table XXI for analysis.

DISCUSSION

All solitary ascidians of the San Juan Islands are primarily subtidal, feed on plankton, require firm substratum for attachment, are simultaneous hermaphrodites, and have short-lived larvae which are roughly the same size. Because of these similarities, it might seem that investigating the ecology of one or two species would give a satisfactory answer to the question posed by this paper, namely, "What are the relative roles of predation, defense, refuges and habitat selection in determining distribution?" While the question seems straightforward, unexpected interspecies differences complicated the answer, vindicating the use of a comparative approach. The determinants of distribution must be considered for each species individually after carefully defining the distributional scale of interest. Nevertheless, the data warrant a few generalizations: 1) A single predator, *Fusitriton oregonensis*, exerts a tremendous influence on the assemblage as a whole, and represents a selective pressure to which ascidians have responded with diverse strategies. 2) Predation affects large scale distribution by excluding from certain habitats those species which lack either defense mechanisms or the ability to locate spatial refuges. 3) The effects of larval behavior are most evident on a small scale, though behavior is not equally important for all species. 4) Tadpole larvae are capable of relatively sophisticated behavior, including delay of metamorphosis and contact chemoreception as well as photoresponses. 5)Concentration and dispersal of larvae by tidal currents, a factor not considered in the present study, appears to be an important determinant of large-scale pattern, and deserves detailed study.

PREDATION

In the subtidal zone, predators may affect invertebrate prey populations by maintaining them at low levels, restricting them to certain areas, changing their age structure or preventing competitive exclusion. That population dispersion may be limited by predators is a foreign concept to many terrestrial ecologists (Hairston et al., 1960; Slobodkin et al., 1967), though the phenomenon is not uncommon in marine systems (Paine, 1977). The best known examples are in the rocky intertidal zone, where animals vulnerable to predators are often restricted to a refuge zone high on the shore which is outside of the predator's range of physiological tolerances (e.g., Hancock, 1960; Connell, 1972; Paine, 1974; Dethier, 1980). Subtidally, where general hydrographic conditions tend to be more homogeneous, and a predator could presumably survive almost anywhere within its geographical range, the situation is seldom so clearcut, and consequently, experiments on the role of subtidal predators have been less common. Nevertheless, distributions of several subtidal invertebrates are suspected to have boundaries imposed by predators. The best documented case is that of the sea otter, *Enhydra lutris*, which is such an effective generalist predator that it eliminates numerous species when it enters an area (Estes and Palmisano, 1974; Simenstad et al., 1978; Duggins, 1980) and consequently has major effects on the overall structure of the community (Estes et al., 1978; Ostfield, 1982). In eastern Canada, the lobster *Homarus americanus* has likewise been implicated as a major controller of *Strongylocentrotus droebachiensis* abundance (Mann and Breen, 1972), though this point of view differs from that of Himmelman and Steele (1971) who noted that even intense predation by lobsters and a host of other predators had little effect on overall urchin abundance. Spiny lobsters, *Jasus ialandii*, are major predators on mussels in South Africa, where they significantly alter the size distributions of species and may also limit spatial patterns under some conditions (Pollock, 1979; Griffiths and Seiderer, 1980). Porter (1972) has suggested that the coral-eating starfish, *Acanthaster planci* causes reefs to be rare in the eastern Pacific. Predation also has important effects on some intertidal soft-bottom communities (Jackson, 1972; Virnstein, 1977; Woodin, 1978; Holland et al., 1980; and others), which are analogous to subtidal communities because of the general homogeneity of physical conditions. Unfortunately, many of the examples of predator-imposed boundaries are inferred from either observed spatial patterns or laboratory feeding experiments rather than field manipulations. Kitching and Ebling (1967), who have documented a number of cases of exclusion in Lough Ine, Scotland, give four criteria for deciding if the absence of an organism is due to the effects of a specific predator: 1) The organism does not survive when transplanted to a site where it doesn't normally occur, unless protected from predators by cages, 2) There is an inverse correlation between predator and prey distributions, and where overlap occurs, the prey animal is inaccessible to the predator. 3) The predator can inflict lethal damage on the prey animal in the laboratory or in field enclosures and 4) There is direct evidence that the predator is responsible for mortality observed in transplant experiments. Ideally, all four criteria should be satisfied before exclusion is attributed to a suspected predator.

In the present study, I observed that a number of ascidian species, mostly phlebobranchs, were found only on floating docks and/or subtidal mud flats, where the predator *F. oregonensis* did not occur. This observation, which corresponds to criterion #2 above, suggested a series of simple laboratory feeding experiments and field transplants which satisfied each of the other 3 criteria and thus demonstrated that predation is important in determining what habitats are occupied by these ascidians. Only three species, *Pyura haustor*, *Halocynthia igaboja* and *Cnemidocarpa finmarkiensis* resisted predation by *F. oregonensis*, though other species, including *Boltenia villosa* and *Styela gibbsii* overlapped the predator's distribution despite apparent vulnerability.

Fusitriton oregonensis, like related mesogastropods in the families Cassidae and Cymatiidae, has previously been thought to prey primarily on sea urchins (Eaton, 1972). Presence of sulfuric acid in the large salivary glands suggests that the snail should have the capability of drilling through calcium carbonate shells. However in my experiments, sea urchins were never attacked, and ascidians were the preferred prey items in every trial. Day (1969) faced a similar paradox, in that the cymatiid she studied, *Argobuccinum argus*, was equipped like *F. oregonensis* but preyed primarily on soft-bodied polychaetes. She speculated that the proboscis mechanism had evolved for drilling calcium carbonate skeletons, but at the present time, abundant worms represented an easier food for the snails to obtain. Laxton's (1971) study of the feeding habits of numerous Australian cymatiids suggests that ascidians may be common prey items for snails in this family; ten of the species he studied fed on some species of ascidian. That the ascidians most preferred by *F. oregonensis* are constantly maintained at a low level in most subtidal rocky communities, indicates that the snail must rely on other sources of prey for much of the time. This is in line with Eaton's numerous field observations of *F. oregonensis* feeding in the lab and field; besides scavenging carrion, the snails fed on a wide variety of molluscs, echinoderms and polychaetes. Day (1969) has suggested that salivary gland secretions may contain a toxic substance which serves to anesthesize such prey animals, making it easier for the snail to gain access to the soft tissues.

A large *Fusitriton oregonensis* consumes about one ascidian every other day. Given this information, it should be possible to predict the impact of *F. oregonensis* at any site by knowing the population density of the snails. *F. oregonensis* densities at my study sites ranged from <0.06 per m² to 0.32 per m². The density at the site where transplant experiments was conducted was estimated as 0.1 per m². Eaton (1972) reported a spawning aggregation near Brown Island in which the egg mass was over 1 m in diameter and snails were clustered all around the perimeter. Shelford et al., (1935) recognized *F. oregonensis* as one of the most characteristic animals of rock bottoms in the San Juans, and reported a maximum density of 4 per m², as estimated by dredging. Taken together, these observations indicate that *F. oregonensis* is sufficiently abundant in some areas to eliminate virtually all vulnerable ascidians that settle.

The ascidians consumed by *F. oregonensis* in dock transplants were generally the largest ones available; small individuals almost always survived the experiments. This suggests that if *Fusitriton* is indeed excluding ascidians from the rocky subtidal small individuals should still be present. This phenomenon was seen at Bell Island, where snails were actively preying on large *Chelyosoma productum* in the summer of 1981, while numerous small ascidians from the previous spring's settlement were not being attacked. Bell Island is near numerous soft-bottom habitats where *C. productum* adults are abundant, and it is assumed that these soft-bottom adults supply the larvae that recruit regularly there. This is the only site where large numbers of juvenile phlebobranchs were encountered. Juveniles may be rare in other areas simply because the local breeding populations are constantly maintained at a low density by *F. oregonensis*.

DEFENSE MECHANISMS

Mechanisms by which prey species co-occur with potential predators are of two main types: defenses and refuges. Several examples of each are seen in the assemblage of ascidians I studied.

Many motile animals use behavioral defense mechanisms. In general, water-borne chemicals from the predator are detected at a distance , enabling the prospective prey animal to either flee (Bullock, 1953; Feder, 1963, 1967; Mackie and Grant, 1974; Margolin, 1964; Phillips, 1976; Ross and Sutton, 1967 and many others) or defend itself aggressively (Branch, 1979; Nielson, 1975). Others have evolved activity patterns that minimize the probability of encountering predators (Nelson and Vance, 1979, Wells, 1980). Defense mechanisms employed by motile and sessile animals alike include: toxic or unpleasant chemicals (Bakus, 1964; Bakus and Green, 1974; Green, 1977; Nance and Braithwaite, 1979; Thompson, 1960), defensive structures (e.g., spines, nematocysts, chelipeds, shells, etc.), and camouflage (Ghiselin, 1964; Thompson, 1973; Vance, 1978). In ascidians, only chemical defense mechanisms have been demonstrated to be effective against predators (Stoecker, 1980a, 1980b) even though numerous ascidians are endowed with spines, tubercles, spicules, hairs, etc. (Monniot, 1970; Van Name, 1945). Though it is possible that chemicals play an important role in reducing fish and crab predation in the San Juan Islands, the major ascidian predator, *Fusitriton oregonensis*, is unaffected by them. Indeed, those animals with the highest concentrations of vanadium (Danskin, 1978) and lowest pH were also those most readily consumed by the predator. Defense mechanisms documented in the present study were all structural. The tunic of *Halocynthia igaboja* is endowed with long spines which have recurved spinelets all along their length. Even though it is not possible to state [illegible] certainty that these spines evolved primarily for defense, experimental evidence demonstrates that they function in that role at the present time. *F. oregonensis* has been present in the Northeast Pacific since the late Oligocene (J.T. Smith, 1970), so predation has probably been a significant selective pressure for at least 25,000,000 years. Kott (1968) expresses the opinion that *Halocynthia igaboja* and several other species in the genus should be synonymized under the name, *H. hispida*. She found almost no internal differences among specimens collected from Australia, Japan, and British Columbia. However, there were major differences in the number, arrangement and size of spines among animals from the various regions. Interestingly, *H. igaboja* collected from the geographical regions overlapping the distribution of *Fusitriton oregonensis* (California through the Bering Sea to Central Japan; J.T. Smith, 1970) are among those with the densest cover of long spines. Several of the Australian specimens illustrated by Kott (1968) are nearly glabrous. Assuming these are indeed the same species, it would be interesting to correlate predation pressure in the various regions with degree of spine development. Unfortunately, neither *F. oregonensis* densities nor presently available information on the distribution of *H. igaboja* morphs is sufficiently detailed to allow this comparison.

Pyura haustor has a very tough tunic, which resembles that described in detail for *Pyura stolonifera* (Wardrop, 1970). In the latter species, the tunic is greatly strengthened by dense bundles of a collagen-like material. It is assumed that similar structures give the tunic of *Pyura haustor* its great tensile strength, and help make it invulnerable to predators. Like many other pyurids and molgulids, *Pyura haustor* incorporates bits of sand and rock into its outer layer of tunic. That this feature helps protect *P. haustor* is suggested by the observation that *Fusitriton* sometimes attacks *P. haustor* on the base, where no sand grains are present. Endean (1955b) also suggested that sand in the tunic serves a protective function in *Pyura praeputialis*. He noted that when tunic and sand were removed from *in situ* ascidians, they became vulnerable to predation by the intertidal crab, *Leptograpsus variegatus*. The siphons of *Pyura haustor* are covered with anteriorly-directed, microscopic spines. It is possible that *F. oregonensis* could penetrate the tunic in the otherwise soft siphon region if these spines were not present.

Cnemidocarpa finmarkiensis is the third species not consumed by *F. oregonensis*. Like *H. igaboja* and *Pyura*, it is commonly found in regions where *F. oregonensis* is common. The defense mechanism of *C. finmarkiensis* has not yet been discovered. Though chemical defense has not been ruled out, it seems unlikely. One possibility worth investigating is that *C. finmarkiensis* defends itself behaviorally by expelling water and flattening itself against the rock.

REFUGES

Animals may find refuge from predators in time (Taylor, 1976; Thorson, 1957), size (Griffiths and Seiderer, 1980; Paine, 1976) or space. Because ascidians rejected by *F. oregonensis* on the basis of size are primarily pre-reproductive individuals, size refuges are probably not important in the San Juans. However, several species appear to take advantage of both temporal and spatial refuges. Woodin (1978) recognizes three categories of spatial refuges, which she classifies according to the distributional patterns of the predators and prey: 1) zones beyond the activity range of the predator (e.g., high intertidal zone), 2)physical heterogeneities in the habitat within the range of the predator which nevertheless buffer the predator's effect or inhibit its ability to locate or consume the prey and 3) biologically generated refuges within the range of activity of the predator.

Ascidians occurring on docks where *F. oregonensis* is not found are occupying a refuge of the first type. However, since docks are only recent additions to the Puget Sound region, it is of interest to consider how these same species persisted or would persist in the absence of these artificial refuges. In other words, "Where are the subtidal spatial refuges for ascidians?"

Chelyosma productum has probably always been the most abundant phlebobranch in the San Juan Islands, both before and after the advent of docks, since large populations exist in the extensive soft bottom areas around Shaw and Orcas Islands. Here, the animals form large clumps with shells or polychaete tubes serving as the initial attachment site. As indicated above, populations occasionally seen on rock substratum are probably recruited by larvae originating in these soft bottom areas. Although the predatory starfish *Evasterias troschelli* takes some ascidians in muddy areas, its impact seems to be minimal, so the soft bottom habitats represent a refuge for *C. productum*. I supect that before the days of docks, *Chelyosoma* was primarily a soft-sediment ascidian. Now, it has exploits the artificial substrata as well, and is thus considered a member of the hard-bottom epifauna by most workers.

The importance of spatial heterogeneity in creating refuges (refuge type #2 in Woodin's classification) has been emphasized by Gaines (1980) for gastropods in Panama, Menge and Sutherland (1976) for intertidal systems and Buss (1979) for coral reef invertebrates. In the San Juan Islands, several species seem to rely heavily on their photoresponses to locate habitats under cobbles and in other cryptic habitats. Although these sites are undoubtedly refuges from silt and other sources of juvenile mortality, they probably protect the animals from predation as well. It seems unlikely that *F. oregonensis* could enter most such habitats because of the large size of its rigid shell. If prey ascidians were detectected from a distance, the proboscis could probably be extended far enough to reach many of them. However, evidence from Y-maze experiments indicates that *F. oregonensis* encounters ascidians in a random manner and detects them by touch. The animal best adapted for living under cobbles is *Pyura mirabilis*, the body of which has become expanded anteriorly so that siphons emerge from opposite sides. Thus, waste water is expelled on one side of the cobble while the feeding currents draw water in from the opposite side. The advantage of this arrangement seems obvious when we consider that food must be extracted from water in the "dead areas" between rocks, and currents do little good at renewing food supplies.

Ascidia callosa is the other major species occupying this habitat. Like *C. productum*, it is now abundant on floating docks. However, before docks were present, it may have been as rare as *Pyura mirabilis*.

Two species, *Boltenia villosa* and *Styela gibbsii*, take advantage of biological refuges in escaping predation and thereby persist in the same habitats as *F. oregonensis*. Their strategy consists of taking advantage of the defense mechanisms of other ascidians. By living as epizooites on *Pyura haustor* and *Halocynthia igaboja*, these animals apparently escape detection by *F. oregonensis*.

An ever-increasing body of literature suggests that refuges generated by other animals and plants are among the most important in subtidal systems. Examples include fishes which live among the tentacles of sea anemones, (Mariscal, 1970; Roughgarden, 1975), anemones that protect hermit crabs from octopus (Ross, 1971), xanthid crabs occupying coral heads (Glynn, 1976), barnacles living on hydrocorals (Young, in preparation) fish nestling among the spines of echinoids or living in holothurian cloacas, young echinoids living under the spines of adult conspecifics (Tegner and Dayton, 1981) clams burrowing into living coral (Highsmith, 1980) and sponges living on scallops (Bloom, 1975). In each case, there is a species which is well defended and an associate which exploits the defense of the host.

Defense mechanisms are not alway involved in biological refuges. For example, algae may increase spatial heterogeneity (e.g., Watanabe, 1980) or create substrata outside the foraging areas of the predators (Lowry et al., 1974;Riedman et al., 1981) in California kelp forests. Woodin (1976, 1978) has demonstrated experimentally the role of *Diopatra* tubes in reducing predation in intertidal mudflats. The onuphids themselves do not possess any remarkable defense mechanisms. They afford protection to associates by creating physical structure which inhibits activities of predators.

Several ascidian species vulnerable to predation are found unexpectedly in rocky regions without having defense mechanisms, without occurring in cryptic habitats and without being associated with biological refuges. These species probably persist by being unpredictable in time and space. The species that has adopted this strategy most effectively is *Corella inflata*. Because the adults reach reproductive size very rapidly (Lambert, 1968) and brood the larvae until they are ready to settle (Child, 1927; Lambert and Lambert, 1978), regions where predator activity is low are rapidly filled up with reproducing individuals. Lambert (1968) refers to this as a "fugitive" strategy. Other ascidians that do not brood are found in low numbers in most subtidal habitats. These include *Halocynthia aurantium, Ascidia paratropa* and *Corella willmeriana*. All of these species are attacked by *F. oregonensis*, so scattered and unpredictable spatial pattern probably accounts for their persistence.

LARVAL HABITAT SELECTION

The correlation between small-scale spatial pattern and larval behavior is good for most species, suggesting that behavior plays a significant role in the ecology of subtidal ascidians. Thus, species without specific substratum preferences as larvae, (*Corella inflata, Corella willmeriana, Cnemidocarpa finmarkiensis, Ascidia paratropa,* and probably *Halocynthia igaboja, Halocynthia aurantium* and *Pyura mirabilis*) utilize substrata roughly in the proportions available to them. Although substratum characteristics influence these larvae very little, several of the species rely heavily on tadpole photoresponse to place them in habitats inaccessible to *F. oregonensis* and free of the effects of silt and benthic algae (chapters 2,4).

Pyura haustor, Chelyosoma productum, Styela gibbsii and *Ascidia callosa* are found in single species aggregations under at least some conditions, and all demonstrate a tend to settle on the tunic of conspecifics. In *Ascidia callosa*, evidence for gregarious settlement is inconclusive, and aggregations of this species on floating docks may be because adult ascidians represent one of the most abundant substrata available for settlement.

The function of the gregarious response remains unclear in these ascidians as in most other invertebrates which display the behavior. Crisp (1979) and Heip (1975) have advanced the idea that proximity to conspecifics is essential for reproduction, especially in animals with internal fertilization such as barnacles. As pointed out elsewhere (Young and Braithwaite, 1980), this explanation is unsatisfactory in the case of *Chelyosoma productum,* since this species is entirely self-fertile and spawns eggs and sperm simultaneously. Another possible function of gregariousness is that the presence of adult conspecifics is indicative of a site where living conditions have been historically good. Scheltema et al. (1981) have suggested that since gregarious responses are most common in species that do not reproduce asexually, settlement in mass may be required in order to successfully compete for space in the epifaunal community.

Aggregations of adults are sometimes refuges from predation for the larvae or young juveniles. For example, the juveniles of the subtidal mussel, *Modiolus modiolus* avoid predators in their vulnerable post-settling stages by nestling among less vulnerable adults (Roberts, 1979). Likewise, larval sand dollars, *Dendraster excentricus* settle most readily in the presence of sand from dense adult beds (Highsmith, 1982). Highsmith was able to demonstrate that the tanaid, *Leptochelia dubia,* which lives only where where adult sand dollars have not disrupted the sediment, is a major predator on settling *Dendraster* larvae. The tanaid is thus envisioned as a selective pressure favoring gregariousness.

The effect of spatial pattern on predator success (and prey mortality) is a subject of some controversy in the literature. A number of studies indicate that aggregation (schooling) in fish decreases a predator's chances of locating the fish or of successfully attacking any given individual (Brock and Riffenburgh, 1960; Cushing and Harden Jones, 1968; Olson, 1964). Taylor (1976) presented a mathematical model which suggested that clumping nearly always benefits the prey and hinders the success of the predator. However, his assumption that the prey animals are capable of fleeing or defending themselves so as to limit mortality to one kill per attack, is not applicable to sessile marine invertebrates. Stinson (1980) supported a mathematical model on the advantages of flocking in shorebirds with empirical observations. An opposing point of view has been presented by Tinbergen et al. (1967) who demonstrated experimentally that eggs arranged in an aggregated pattern were located and eaten by crows much more readily than eggs in a random or regular pattern. Other studies also support the idea that spacing out decreases the chances of a predator encountering any given individual (reviewed by Brown and Orians, 1970). I carried out an experiment in which ascidians were offered to

F. oregonensis in aggregations and as individuals. The data indicate that there is no advantage to clumping in preventing predation. *F. oregonensis* tends to remain near a clump of edible ascidians until all have been consumed. This was true in both the laboratory and the field. At the conclusion of the breakwater experiments, I noted that many of the animals attacked were located in very restricted areas, and indeed were often attached to each other. When clumps of phlebobranchs were transplanted to the field, *Fusitriton* often remained near or on the clumps for over a week, feeding until most or all of the ascidians were eaten. Thus, predator avoidance is not a function of gregariousness in ascidians.

The two most gregarious species, *Chelyosoma productum* and *Pyura haustor* are common on subtidal mudflats, where they attach either to emergent "islands" of hard substratum (clam shells and polychaete tubes) or to other ascidians. If soft bottoms represent a major habitat for these ascidians as suggested above, selective pressures unique to them may have favored the evolution of gregarious behavior. One possibile reason is that animals settling on other ascidians have a better chance of remaining above the soft sediment than animals settling on narrow and flexible worm tubes. Ascidians often form very large clumps which rest on the mud surface. Larger clumps are probably more stable than small ones, so survival of the juveniles may be positively correlated with clump size. This hypothesis could be tested by transplanting juveniles on various substrata to the muddy subtidal and monitoring survival and growth.

It may be significant that species suspected of avoiding predation by means of unpredictable distribution in space and time, do not demonstrate any substratum preferences as larvae, whereas refuge-oriented species display behaviors which enable them to locate refuges. The most intesting example of this latter phenomenon is the case of *Boltenia villosa*. *B. villosa* tadpoles prefer to settle on the tunic of those species which represent a refuge to the adults, and even delay metamorphosis in the absence of these substrata. In deciding whether predation or settlement behavior is most important in determining small-scale distribution of a species such as *B. villosa*, it is necessary to distinguish proximate and ultimate factors. Assuming that predation was the selective pressure favoring evolution of the behavior, the ultimate factor of greatest importance is predation. On the other hand, where settlement behavior operates effectively in the location of refuges, it is the most important proximate factor; in other words, the observed pattern results directly from the behavior of the tadpoles.

Besides the demonstration of gregarious settlement presented by Young and Braithwaite (1980), this study is the first to provide evidence for substratum selection in ascidian tadpoles. The behavioral data support previous experimental (Hirai, 1964) and morphological (S. Torrence, University of Washington, personal communication) evidence that the tadpole oral papillae function as sensory receptors as well as attachment organs.

Major differences in susceptibility to predation, recruitment strategy (including larval behavior) and defense mechanisms in this assemblage of sympatric solitary ascidians confirm the notion that natural systems are complex and varible, and suggest caution when extrapolating concepts from one biological system to another or applying empirical data to general models.

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