ECOLOGY AND LIFE HISTORY OF *NEREOCYSTIS LUETKEANA* IN THE SOUTH SLOUGH ESTUARY

by

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A THESIS

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"Ecology and Life History of Nereocystis luetkeana in the South Slough Estuary," a thesis prepared by Kerri A. Kidder in partial fulfillment of the requirements for the Master of Science degree in the Department of Biology. This thesis has been approved and accepted by:

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An Abstract of the Thesis of

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Nereocystis luetkeana, bull kelp, is an ecologically and economically important species that is found along the Pacific coast. This study describes the epiphytic community associated with drifting *Nereocystis* in the South Slough estuary, the placement and condition of drifters in the South Slough, and a previously undescribed aspect of the *Nereocystis* life cycle. Data from monthly boat surveys of drifting and attached individuals suggest seasonal epiphytic community changes.

The life cycle of *Nereocystis* includes a diploid, macroscopic sporophyte stage and a haploid, microscopic gametophyte stage. Adult sporophytes were found releasing structures that appear to be embryonic sporophytes. The amount of DNA in gametophyte, embryonic sporophyte, and adult sporophyte propoplasts was quantified with DAPI staining and image analysis. The released embryonic sporophytes were diploid and indicate a life history strategy previously unknown in kelps. This has not

been documented before and augments the available knowledge of the life cycle of *Nereocystis*.

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CHAPTER 1—GENERAL INTRODUCTION

Nereocystis luetkeana is a common species of kelp that grows subtidally along the West coast of North America. It is valuable not only as a source of fertilizer, aquaculture feed, and alginates, but also because it creates habitat that is essential for many species of commercially valuable invertebrates and fishes. Culturing *Nereocystis* has been attempted, both for restoration of kelp beds and for harvest of *Nereocystis* itself, but has been largely unsuccessful.

Mariculture of *Nereocystis* requires a complete understanding of the life cycle. Chapter 2 of this thesis documents a new finding relating to the life cycle of *Nereocystis* that is potentially applicable to the mariculture of this species.

Nereocystis is often detached by storms and washes up on beaches. It also drifts in the ocean, and continually drifts into the South Slough National Estuarine Research Reserve in Charleston, Oregon. The epiphytic community of *Nereocystis* is carried in with the kelp, and its members could potentially colonize the Slough. Chapter 3 describes the taxa found on drift *Nereocystis* in South Slough, and seasonal changes in the epiphytic community. Chapter 4 documents the distribution of *Nereocystis* in part of the Slough, and tests the hypothesis that wind speed of the day before a survey affects the number of drifters in the Slough.

CHAPTER 2—DISCOVERY OF EMBRYONIC SPOROPHYTES RELEASED FROM ADULT SPOROPHYTES IN *NEREOCYSTIS LUETKEANA*

Introduction

The bull kelp, *Nereocystis luetkeana* (Mertens) Postels et Ruprecht, is one of the largest kelps, and is found from southern California to Unmak Island, Alaska, and drifts as far as the Kuril and Japanese archipelagos (Miller and Estes 1989). High wave energy and unstable substrata characterize *Nereocystis* habitats (Maxell and Miller 1996). *Nereocystis* is commercially valuable and creates habitat vital for many commercial fish species. Charismatic and easily identifiable, *Nereocystis* has been well studied and has been shown to be ecologically, economically, and culturally important.

Phylogenetically, macroalgae are separated into three distinct taxa, the red algae (Rhodophyta), the green algae (Chlorophyta), and the brown algae (Phaeophyta). Brown algae are characterized by the presence of chlorophylls *a*, c_1 and c_2, the carotenoid fucoxanthin, and flagellated zoospores and gametes (Lee 1999). They are almost exclusively marine, and dominate the rocky intertidal in temperate zones. Kelps are classified within Phaeophyta in the order Laminariales. Characteristics of this order include heteromorphic alternation of generations, absence of an eyespot in the spore stage (with two

exceptions), and intercalary growth from a meristem between the stipe and blade. Growth also occurs from meristems at the base of the blades and on the upper stipe (Nicholson 1970). Most phycologists consider *Nereocystis* an annual. Although some individuals live longer than one year, the upper stipe and blades do not regenerate, and only one stipe is produced during the life of an individual (Nicholson 1970).

Macroalgae generally have complex life histories, and, therefore, have been more difficult to grow commercially than terrestrial plants. For example, prior to Drew 's (1949) discovery of the conchocelis stage, *Porphyra* was "cultivated" only by increasing the available substrate (Kito and Kawamura 1999). Commercial products produced from macroalgae include fertilizers and agar, carageenan, and other thickeners. In addition, the industry of collecting and cultivating algae for direct consumption generates billions of dollars worldwide. Along the west coast of the U.S., however, commercial harvests are fairly small and industrial mariculture is restricted to a few small-scale operations.

Nereocystis and its epiphytes are potentially valuable for harvesting or mariculture. It has been harvested for use in agriculture (Stekoll and Deysher 2003), as food for cultured abalone and to manufacture horticultural products (Roland 1984). The epiphytes of *Nereocystis* are also commercially valuable. *Porphyra nereocystis*, an obligate epiphyte on *Nereocystis luetkeana*, is preferred over other native *Porphyra* species for processing into nori (Woessner

1981). Harvest along a 20 km stretch of the U.S. Pacific coast could yield $23,000 of nori sheets (Woessner 1981) and culturing could generate even more revenue.

Knowledge about algal life histories has been essential not only for successful culture and cultivation, but also for understanding intertidal and subtidal ecology. Kelps provide habitat and nurseries for invertebrates and fish (Norton 1971). *Nereocystis* beds, in particular, seem to provide habitat to many species. For example, in the Gulf of Alaska *Nereocystis* beds harbor more large fish than eelgrass and other kelp habitats (Dean et al. 2000). *Nereocystis* beds may harbor more invertebrates than *Macrocystis* beds (Shaffer 2000).

In addition to habitat, kelps supply particulate and dissolved organic matter to coastal ecosystems. Barnacles and mussels grow much faster in kelp-dominated systems than in low kelp systems (Duggins et al. 1989). *Nereocystis* is a large kelp that creates epiphytic and forest communities, and can greatly impact surrounding rocky intertidal communities.

Life Cycle

The life cycle of *Nereocystis* has been described based on studies of kelps in culture. *Nereocystis* exhibits heteromorphic alternation of generations; its life cycle includes a diploid macroscopic adult sporophyte and haploid microscopic male and female gametophytes (Figure 1). The sporophyte abscises rectangular portions of blade called sori that contain sporangia, which are spore-producing structures. Most sori are abscised in the two hours before

and four hours after dawn (Amsler 1989). As the sori sink, spores are released as a group through the tip of the sporangium (Kemp and Cole 1961), which is essentially a sac of spores.

Although spores photosynthesize, they do not appear to be adapted for a long planktonic life. Even at saturating irradiance, oxygen production due to photosynthesis barely compensates for respiratory demand in spores (Amsler 1989). A dawn release of sori maximizes spore daylight exposure.

While the sorus sinks, some spores disperse long distances and others short distances. Some spores are released near the surface, and some spores are discharged after the sorus has landed on the bottom. Spores settling into low flow conditions would remain near the parent. The spores released into strong currents would disperse further than the ones that are discharged at the bottom.

Spores are presumed to settle on the bottom, and develop into haploid male or female gametophytes. When conditions are favorable, gametophytes produce gametes. Favorable conditions include sufficient nutrients, blue light, and temperatures from 5 to 15° C (Vadas 1972).

Kelp gametophytes, however, have rarely been found in nature (Hubbard et al. 2004). Possibly they are abundant and have not been found because they are microscopic and because less field work is done when they probably occur, in the winter. Although kelp gametophytes may develop along the bottom, gametophyte development has also been observed in red algae and in

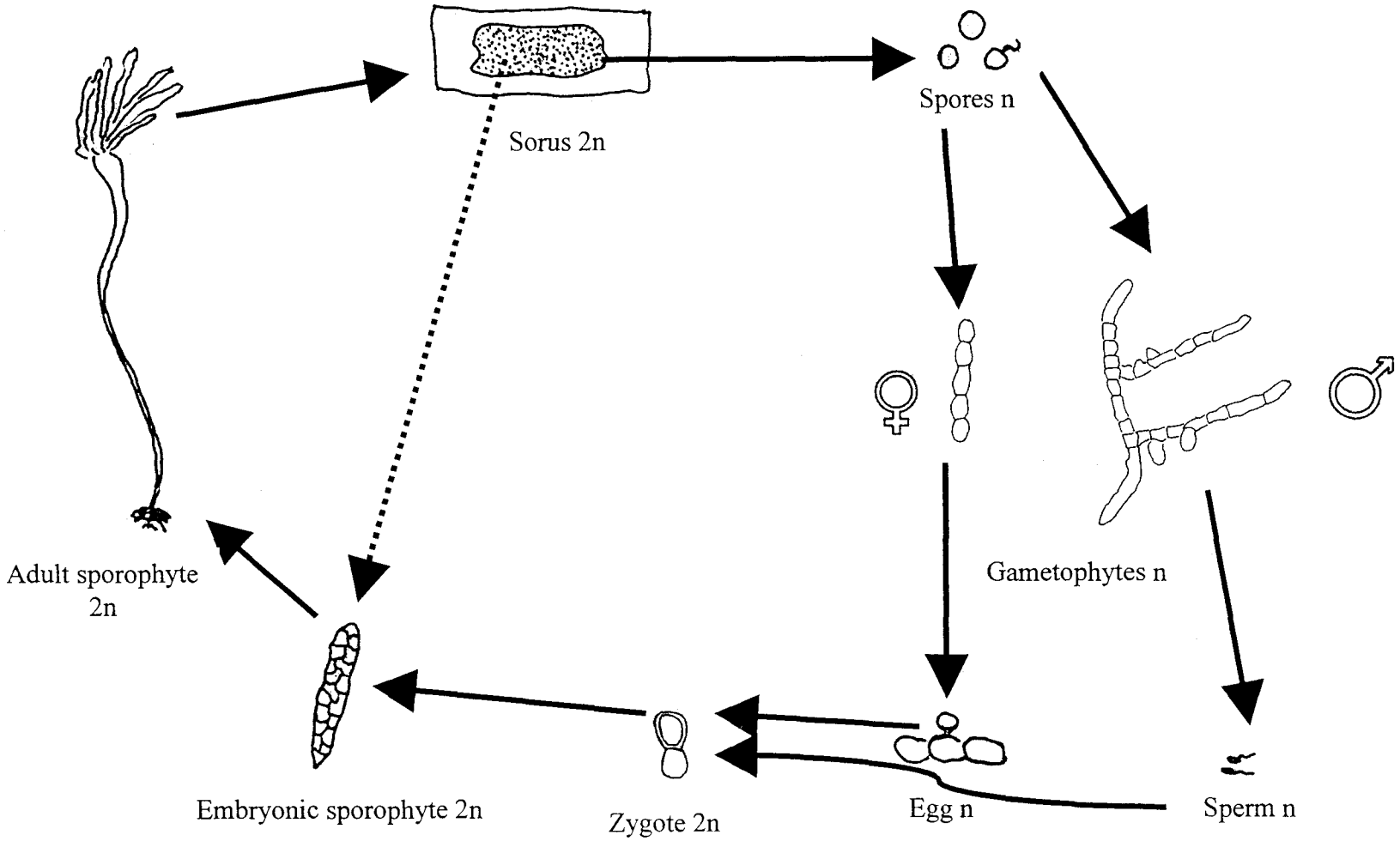


Figure 1. Generally accepted life cycle of *Nereocystis luetkeana*. Dashed line represents new finding reported in this study.

the water column. When they were found growing endophytically in red algae, Garbary et al. (1999) and Hubbard et al. (2004) suggested that gametophytes are not common along the bottom, and that they utilize red algae as substrate instead. Gametophytes of *Pterygophora californica*, another kelp, can also develop freely floating in water (Reed et al. 1992). They can then settle and produce viable gametes.

After gametogenesis, in *Nereocystis*, sperm are released and swim to the egg that remains attached to the female gametophyte. The egg is fertilized and develops into a sporophyte. Mortality is high among *Nereocystis* juvenile sporophytes, and decreases once the bulbs reach the water surface (Foreman 1984). Sporophytes grow very quickly into adults up to 45 m in length (Denny et al. 1997). Once individuals reach the surface, growth is much faster at the blades than the stipe (Kain 1987) and overall growth rates decline (Maxell and Miller 1996). Meristems occur at the holdfast, the upper stipe, and the bases of the blades (Nicholson 1970).

Individuals continue growing through the summer, instead of sequestering storage material for the next year as perennial kelps do. Maxell and Miller (1996), citing data from Miller and Estes (1989) in Alaska and from California (Abbott and Hollenberg 1976 *in* Maxell and Miller 1996), suggest that *Nereocystis* phenology is very similar throughout its range. Phenology is the seasonal timing of biological events, such as the appearance of visible sporophytes, and is related to the seasonal reproduction of organisms. In

contrast, one of the studies they cite (Miller and Estes 1989) suggested that development of individuals in the westernmost bed, in Alaska, appeared to be advanced compared to individuals in the Vancouver Island, Canada region. The condition in summer of those Alaskan *Nereocystis* was similar to the condition of Canadian *Nereocystis* in autumn. Storms in autumn increase mortality and destroy most beds (Foreman 1984), although mortality may vary regionally, because overwintering beds have also been observed (Chenelot et al. 2001).

The life cycle of kelps is known from lab studies; much about kelp reproduction remains unknown. In particular, very little is known about what stages overwinter and how they are adapted to winter conditions. Most kelps seem to release spores in autumn, and young sporophytes are not observed in the field until spring (Markham 1969, Maxell and Miller 1996). The phase that overwinters is unknown. Evidence exists both for the overwintering of embryonic sporophytes, and of gametophytes. *Macrocystis pyrifera* embryonic sporophytes can delay recruitment for up to one month (Kinlan et al. 2003). This suggests that embryonic sporophytes have the potential to survive winter, and that they might overwinter. Others have shown the capacity of gametophytes to grow vegetatively for months, and have suggested that the gametophyte overwinters and produces gametes in spring (Kain 1964; Aleem 1973; tom Dieck 1993; Ødegaard et al. 1998). Additionally, gametophytes seem to develop more slowly in the field than they do in the lab (Aleem 1973).

Kemp and Cole (1961) found haploid "sporophytes" (their quotes) in *Nereocystis* cultures with both male and female gametophytes. They assumed that the "sporophytes" were parthenogenetic and developed from female gametophytes. Development of the structures was irregular and resulted in "sporophytes" with a few multinucleate cells, even after 5 months. The authors suggest that parthenogenesis may be a cultural artifact and that the resulting "sporophytes" do not produce adult sporophytes. Another possibility is that parthenogenetic sporophytes have different requirements than diploid sporophytes, and these were not met in culture.

Alternatives to this life cycle have been studied in more detail with cultures of other kelps. For example, unfertilized eggs of *Laminaria japonica* produced by female gametophytes can develop into parthenogenetic sporophytes, which are haploid for the first generation (Lewis et al. 1993). Haploid parthenogenetic sporophytes can grow into adults and produce haploid spores, but only female gametophytes develop in those cultures. The second generation of parthenogenetic sporophytes can be haploid, diploid, triploid, tetraploid, or hexaploid. Polyploid sporophytes are probably the result of mitotic and cytokinetic irregularities, are abnormally shaped, and do not develop. Lewis et al. (1993) do not mention the likelihood of parthenogenesis occurring in nature, or its possible advantages or disadvantages to the kelp.

Alaria crassifolia displays similar life cycle variations in culture, including parthenogenesis (female gametophytes developing into haploid sporophytes),

apogamy (male gametophytes developing into haploid sporophytes), and apospory (vegetative sporophyte cells developing into diploid gametophytes) (Nakahara and Nakamura 1973).

This study describes a new life history strategy in *Nereocystis*, the release of embryonic sporophytes from adult sporophytes. Structures released along with spores from adult sporophytes were examined, and DNA content of the structures was compared to that of cultured gametophytes and adult sporophytes using DAPI staining and epifluorescence microscopy. Estimates of DNA content were used to establish the ploidy of the three stages of *Nereocystis*.

Materials and Methods

A *Nereocystis* culture was established in the laboratory with sori collected on 1 February 2005 from South Cove, in Cape Arago State Park, Charleston, Oregon (Figure 2). The sori were separated from the *Nereocystis* blades, cut into pieces, and placed in glass containers filled with sterilized filtered seawater and surrounded by running sea water (Chapman 1973) to maintain a temperature of 13 to 14° C. On 2 February 2005 spores released from the sori fragments were settled onto three types of substrate: brick; rock; and tile. Settlement of the spores was facilitated by pipeting the aqueous exudate over the substrates in plastic containers filled with sterile filtered sea water. On February 3, the sea water was removed and an enrichment media

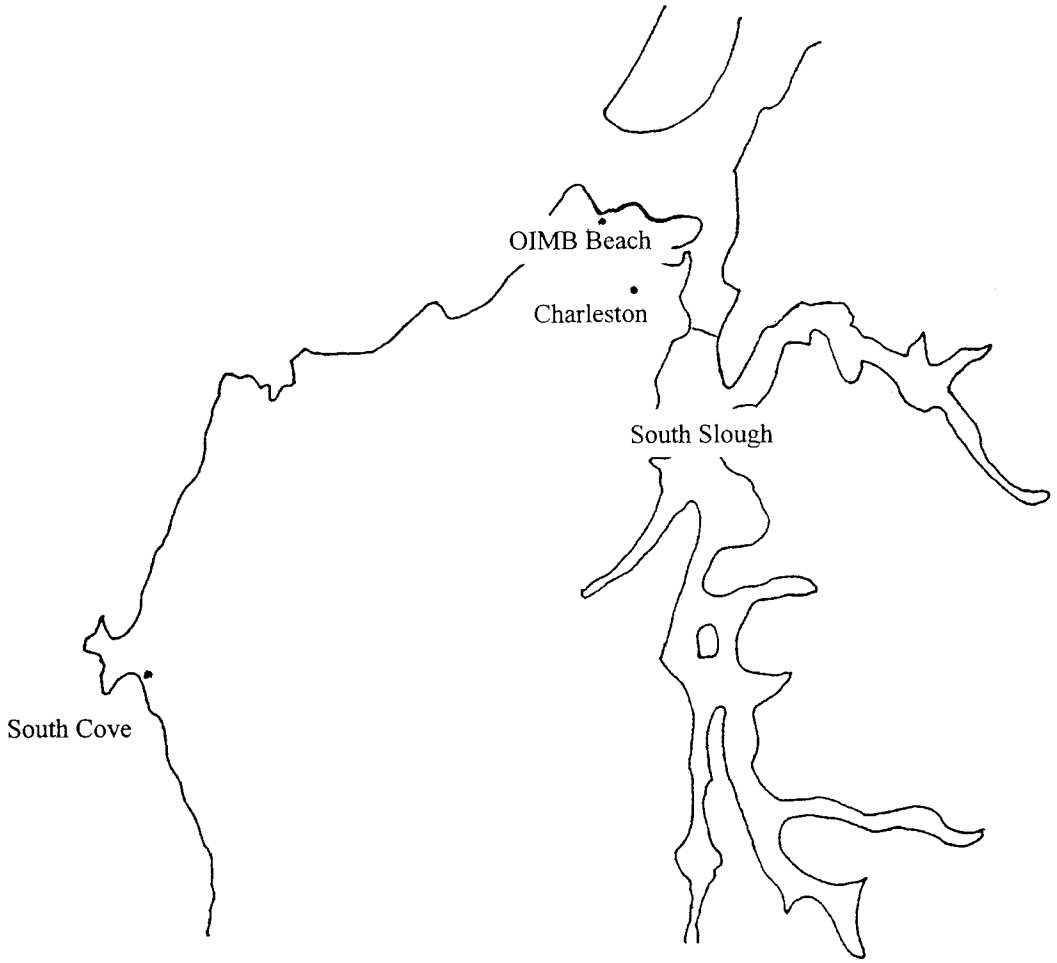


Figure 2. Map of collection areas, OIMB Beach and South Cove.

added. The media used, an enriched sea water, was described by Hruby and Norton (1979) and its components and their amounts are listed in the Appendix (Table 7). A fluorescent light bank on a 12hr:12hr light:dark schedule was placed above the cultures. Media was changed once a month, and added when necessary to keep the gametophytes submerged. An air stone provided aeration. These cultures yielded the *Nereocystis* gametophytes that were used to estimate DNA content.

The morphology of the cellular structures released from sori was observed in spring by light microscopy (Figure 3). Sori were collected from the blades of drift *Nereocystis* 10 May 2005 from South Cove. Whole sori (or whole blades if the sori had not abscised) were placed in beakers filled with sterile filtered seawater, and the beakers were immersed in a running sea water. On May 11, the sori were releasing exudate, and this exudate was examined under a light microscope at a magnification of 400X. Embryonic sporophyte-like structures were observed along with the spores. Following this discovery, sori were collected from South Cove, South Slough National Estuarine Research Reserve, or OIMB beach 2 or 3 times a month until November (Figure 2). The sori were placed in containers filled with sterile filtered seawater in a running sea table. If exudate was released, a sample of the exudate was collected with a pipet, and preserved in 4% formalin, and two additional samples were collected and preserved in Carnoy solution (3 ethanol: 1 acetic acid). The samples preserved in formalin included both sorus and blade tissue.

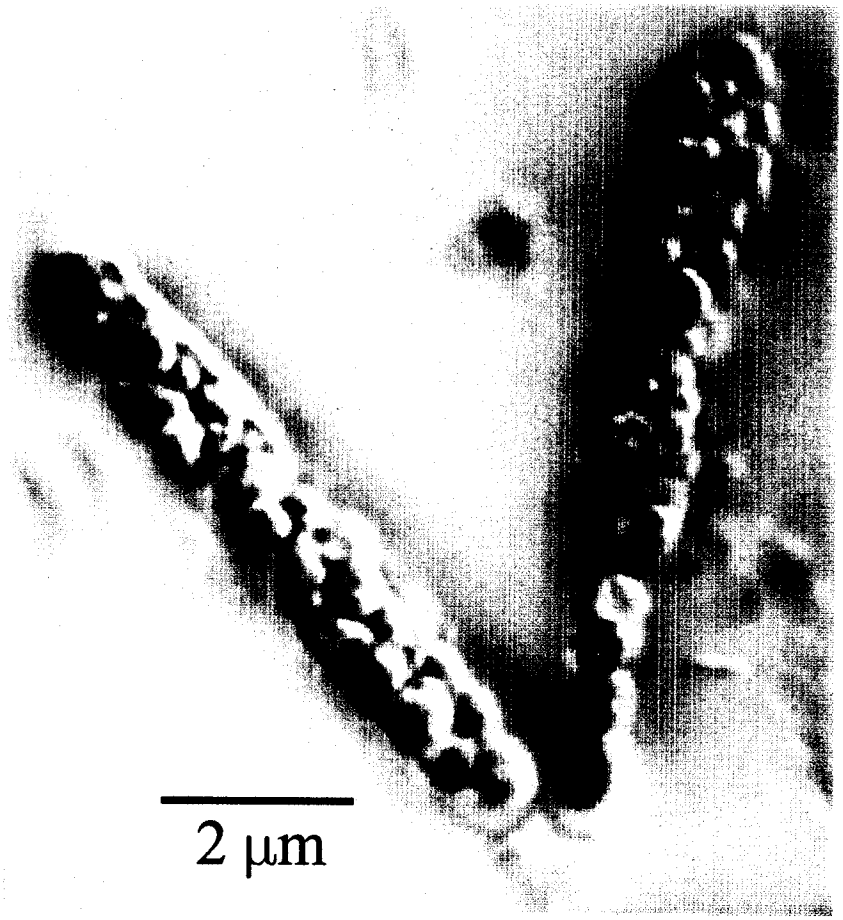


Figure 3. Embryonic sporophytes released from *Nereocystis luetkeana* adult sporophytes

Ploidy of three different *Nereocystis* life history stages was determined by staining protoplasts with DAPI and estimating DNA content. Protoplasts were obtained from preserved adult blade tissue, cultured gametophytes, and preserved embryonic sporophyte-like structures released from sori. Both formalin and Carnoy preserved samples were examined to determine ploidy. The technique of Matsumura (1998) was used to obtain protoplasts but was modified by increasing the incubation times in solutions 1 and 2 from 30 min and 2 hrs to 1 hr and 3 hrs, respectively. The enzymes used in solution 3 were changed from cellulase onozuka RS (Yakult) and abalone acetone powder (Sigma Inc., St. Louis, MO, USA) to cellulase from *Trichoderma reesei* and alginate lyase from *Flavobacterium* sp. (Sigma Inc., St. Louis, MO, USA). All samples were first centrifuged, decanted, and refilled with distilled water three times to rinse. After the last centrifugation, they were refilled with solution 1 (for the contents of solutions see Appendix Table 8) and then incubated for one hour in darkness. Samples were centrifuged to remove solution 1, and were incubated in solution 2 for three hours under gentle agitation. Samples were rinsed with distilled water, and then suspended in solution 3. Presence of protoplasts was confirmed with light microscopy (Letiz Laborlux S, Leica Microsystems, Wetzlar, Germany) at a magnification of 500X.

Protoplasts were dyed and observed on glass microscope slides. The slides were prepared by adding one drop of DAPI (Sigma Inc., St. Louis, MO, USA) solution (1 µg/mL of phosphate-buffered saline) to one drop of protoplast

solution. DAPI stains only DNA, excites at 350 to 365 nm (UV light), and emits 450 to 500 nm (blue light). Photographs were taken of stained protoplasts with a digital camera (Nikon Coolpix 8800, Nikon Corporation, Tokyo, Japan) and microscope adapter and with oil immersion and fluorescence microscopy with DAPI filter set (code number 513596: excitation filter BP 340-380 nm, reflection short pass filter 400 nm, and suppression filter LP 430). Areas of whole cells and stained areas were measured with Uthscsa ImageTool 3.00 software (University of Texas Health Science Center, San Antonio, TX). The ratio of DNA-containing area: whole cell area was calculated.

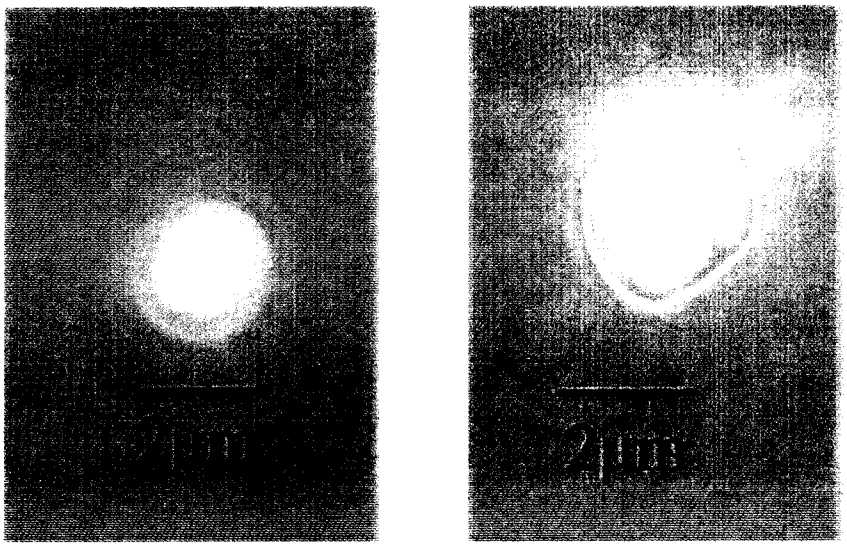
One-way ANOVA and Bonferroni post-hoc tests were used to detect differences in the DNA content ratios between gametophytes, adult sporophytes, and released structures. Statistical tests were performed with Statistica 7.1 software (StatSoft, Inc, Tulsa, OK) and with SAS software (SAS Institute Inc., Cary, NC). Data was square-root transformed to meet the assumptions of normality and homogeneity. Homogeneity was tested with Cochran C and Bartlett's Chi-Square tests ($p>0.07$) and with Levene's Test for Homogeneity of Variances ($p>0.20$). Normality was tested with the Wilk-Shapiro test. After transformations, gametophyte ratios were not normal ($p<0.05$), but, because ANOVA is robust to non-normality, and because embryonic sporophyte and adult sporophyte ratios were not significantly different from normal ($p>0.60$), an ANOVA was performed using SAS, with stage as a fixed factor and individual as a random factor. A post-hoc Bonferroni

test was performed. Outliers (original ratios 0.20 or greater) were removed and tests were run again. The high DNA content outliers were removed because they probably represent dividing cells, a different type of cell than the rest of the data.

Results

Embryonic sporophyte-like structures were released on 11 May 2005. The structures also vaguely resembled sporangia. When ruptured, however, no spores "fell out" of the structure, as would be expected with sporangia. Also, protoplasts were obtained, indicating the presence of multi-cell structures, not sacs of spores. Small spheres completely filled with DNA were occasionally seen in the DAPI-stained samples processed to obtain protoplasts (Figure 4). These are likely spores that were released with the structures and stained along with the protoplasts. Only the nucleus of the protoplast fluoresced. In contrast, the entire cells of spores fluoresced. The spores are also much smaller than protoplasts (Figure 4).

Table 1 and Figure 5 show the results of the DNA content estimation. The haploid gametophytes clearly contain half the amount of DNA, relative to total cell area, that diploid adult sporophytes contain (0.65 and 0.125, respectively). This method of DNA content estimation, therefore, is capable of determining haploids and diploids. Released embryonic sporophytes contain slightly higher relative amounts of DNA than adult sporophytes. After cells with 0.20 ratios were removed, however, the averages of released embryonic



a. b.

Figure 4. Photomicrographs of a spore and a protoplast, both from exudate sample and stained with DAPI under fluorescence. a. Presumed spore. b. Protoplast. White outline around protoplast represents the entire cell area measurement, and yellow outline inside protoplast represents the nuclear area measurement.

Table 1a. Ratios of measured DNA area (μm^2) to total cell area (μm^2) estimate the relative amount of DNA in each cell. Total averages are in bold type. Outliers ≥0.200.

Gametophytes (haploid)n=38	Adult sporophytes (diploid) n=38	Released embryonic sporophytes n=38
0.064	0.073	0.116
0.093	0.135	0.092
0.077	0.106	0.132
0.047	0.131	0.200
0.054	0.127	0.166
0.086	0.156	0.171
0.064	0.154	0.130
0.094	0.174	0.164
0.065	0.070	0.104
0.073	0.148	0.143
0.073	0.111	0.099
0.039	0.125	0.186
0.055	0.202	0.132
0.094	0.141	0.152
0.045	0.068	0.181
0.049	0.111	0.220
0.095	0.065	0.146
0.076	0.090	0.197
0.135	0.080	0.196
0.148	0.122	0.106
0.052	0.162	0.218
0.065	0.104	0.216
0.040	0.066	0.209
0.040	0.165	0.125
0.070	0.123	0.154
0.042	0.132	0.146
0.074	0.098	0.119
0.047	0.119	0.231
0.041	0.187	0.129
0.038	0.107	0.117
0.058	0.175	0.144
0.037	0.105	0.101
0.055	0.224	0.100
0.041	0.230	0.168
0.061	0.123	0.285
0.043	0.138	0.061
0.076	0.088	0.190
0.057	0.235	0.335
0.065	**0.125**	**0.160**

Table 1b. Ratios of measured DNA area (μm^2) to total cell area (μm^2) estimate the relative amount of DNA in each cell. Averages with outliers (≥ 0.200) removed are in bold type.

Gametophytes (haploid)n=38	Adult sporophytes (diploid) n=38	Released embryonic sporophytes n=38
0.064	0.073	0.116
0.093	0.135	0.092
0.077	0.106	0.132
0.047	0.131	0.166
0.054	0.127	0.171
0.086	0.156	0.130
0.064	0.154	0.164
0.094	0.174	0.104
0.065	0.070	0.143
0.073	0.148	0.099
0.073	0.111	0.186
0.039	0.125	0.132
0.055	0.141	0.152
0.094	0.068	0.181
0.045	0.111	0.146
0.049	0.065	0.197
0.095	0.090	0.196
0.076	0.080	0.106
0.135	0.122	0.125
0.148	0.162	0.154
0.052	0.104	0.146
0.065	0.066	0.119
0.040	0.165	0.129
0.040	0.123	0.117
0.070	0.132	0.144
0.042	0.098	0.101
0.074	0.119	0.100
0.047	0.187	0.168
0.041	0.107	0.061
0.038	0.175	0.190
0.058	0.105	
0.037	0.123	
0.055	0.138	
0.041	0.088	
0.061		
0.043		
0.076		
0.057		
0.065	**0.120**	**0.139**

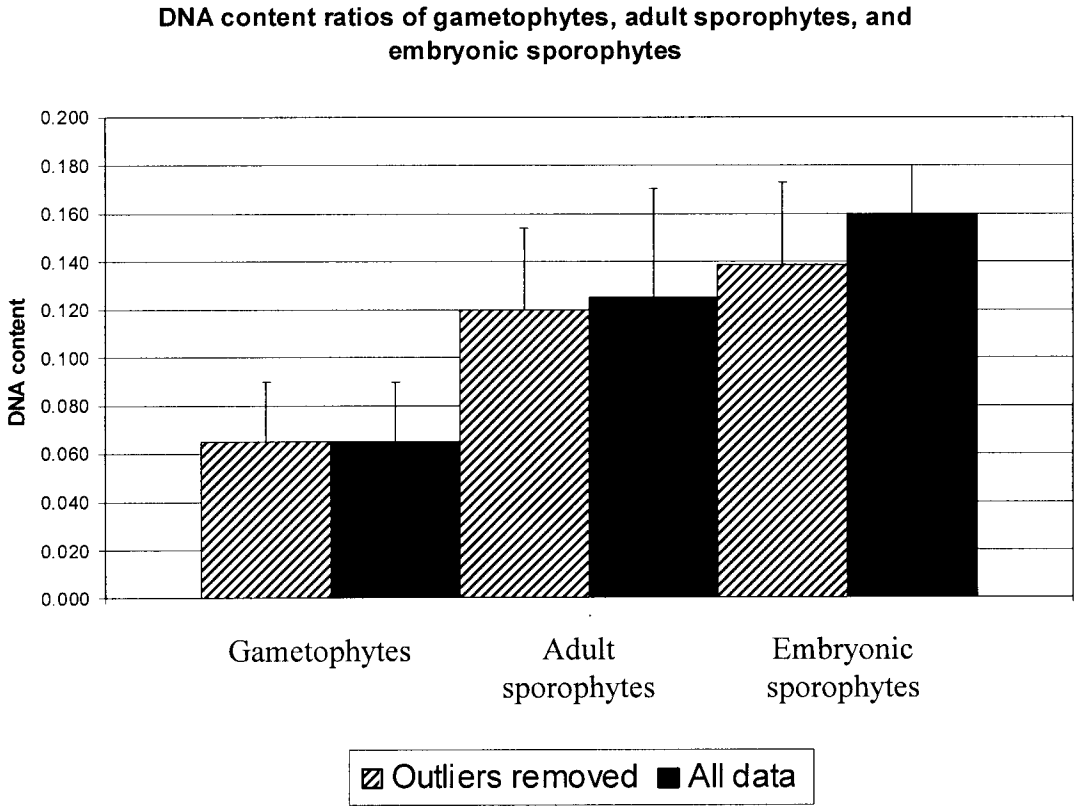


Figure 5. Mean DNA content ratios of gametophytes, adult sporophytes, and embryonic sporophytes. Error bars are one standard deviation from the mean.

Table 2. Results of ANOVA and Bonferroni post-hoc tests. ANOVA indicates that the three stages are different, and the Bonferroni test indicates which stages are significantly different from the other stages. Stage1=Gametophytes, Stage 2=Adult sporophytes, Stage 3=Embryonic sporophytes

ANOVA (including all data)

Source	d.f.	MS	F	p
Stage	2	0.205	55.856	<0.0001
Error	111	0.407		

Bonferroni test (including all data) Probabilities for Post Hoc Tests Error: Between MS = .004, df = 111

Stage	1	2	3
1 (Gametophytes)		<0.000001	<0.000001
2 (Adult sporophytes)	<0.000001		0.003175
3 (Em. sporophytes)	<0.000001	0.003175	

ANOVA (with outliers removed)

Source	d.f.	MS	F	p
Stage	2	0.137	60.638	<0.0001
Error	99	0.023		

Bonferroni test (with outliers removed) Probabilities for Post Hoc Tests Error: Between MS = .002, df = 99

Stage	1	2	3
1 (Gametophytes)		<0.000001	<0.000001
2 (Adult sporophytes)	<0.000001		0.079165
3 (Em. sporophytes)	<0.000001	0.079165	

sporophytes and adult sporophytes were similar, indicating that the released embryonic sporophytes are diploid.

Table 2 displays the results of the ANOVA and Bonferrroni tests. Gametophyte, adult sporophyte, and embryonic sporophyte DNA ratios are significantly different from each other ($p<0.005$). When DNA ratios 0.20 and above were removed, adult sporophytes and embryonic sporophytes were no longer significantly different from each other. DNA ratios of 0.20 and above are roughly equivalent to that expected from tetraploid cells, and likely represent a much smaller, separate group of meristem cells, or those undergoing mitosis that have replicated their DNA but not separated into two cells. The average DNA ratio of gametophytes, presumably equivalent to a haploid state, was 0.065 (Table1). The average DNA ratio of adult sporophytes, presumably equivalent to a diploid state, was 0.125, and that of embryonic sporophytes was 0.160. When ratios 0.020 and over were removed, the average ratio of adult sporophytes was 0.120, and that of embryonic sporophytes was 0.139.

A structure that resembled a gametophyte was observed in preserved exudate/sorus samples (Figure 6). The subject photographed is from a sample taken 5 October 2005 at South Cove that was preserved in formalin. The gametophyte could not have developed after preservation and, therefore, the gametophyte was probably released with the exudate or was within the sorus. Although no gametophytes were ever seen in fresh exudate, they may have been present in sori and released during preservation.

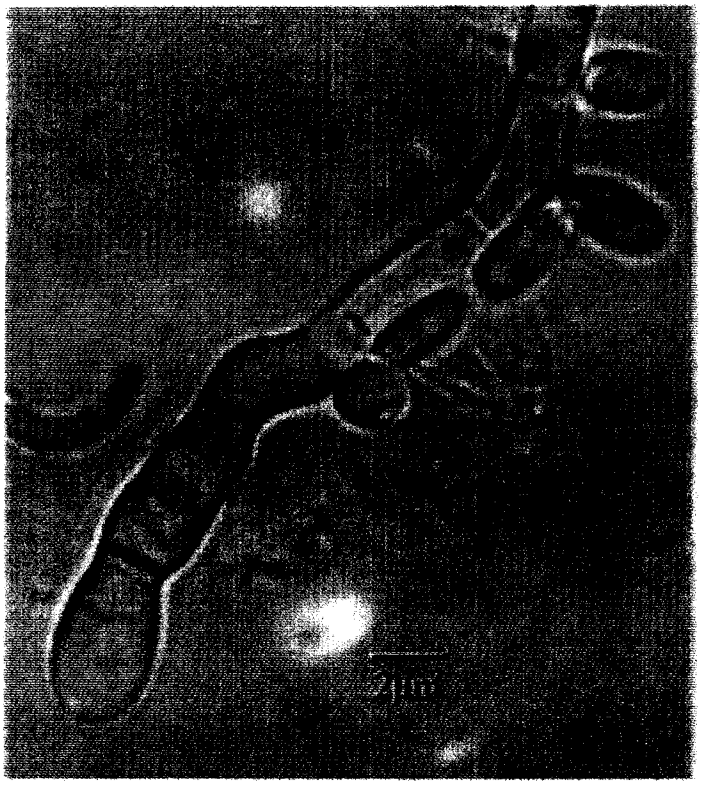


Figure 6. Photomicrograph of gametophyte-like structure from preserved exudate and sorus. Material was preserved in formalin.

Discussion

The structures released by *Nereocystis* adult sporophytes were clearly embryonic sporophytes. Protoplasts of the structures resemble protoplasts of gametophytes and adult sporophytes (Figure 4). The possibility that the structures were sporangia is discounted by the appearance of the obtained protoplasts. Sporangia, under the same conditions, would have appeared to be filled with blue spheres. When stained with DAPI and viewed under fluorescence, only the nucleus of the obtained protoplasts appeared blue. When the structures were ruptured, no spores were released, as would be expected with sporangia.

The embryonic sporophytes released from sori were diploid. The relative DNA contents of the gametophytes and adult sporophytes clearly show that the gametophytes were haploid and the adults were diploid (Table 1). Although the average relative DNA content of the embryonic sporophytes was higher than that of the adult sporophytes, and the three stages were all significantly different from each other, the embryonic sporophytes also appeared to include tetraploid cells. Tetraploid cells may represent meristem cells or those undergoing mitosis after DNA replication but prior to anaphase. After the presumed tetraploid cells were removed from the analysis, the average DNA content of embryonic sporophytes was much closer to the adult sporophytes, and the two sporophyte stages were no longer significantly different from each other. The lack of significance between released embryonic and adult sporophytes does

not prove that they are the same. No statistical test can be performed to test the hypothesis that two groups are the same, because to do so would be proving a null hypothesis. All the results, however, are consistent with the released embryonic sporophytes and the adult sporophytes both being diploid.

The structure shown in Figure 6 appears to be a gametophyte released from a sorus. Other than its resemblance to a gametophyte, no evidence is given to support that it is a gametophyte. Its presence in a sample that was preserved in formalin within a day of collection is intriguing, and suggests that the full life cycle is completed within sori. Embryonic sporophytes could be the result of second generation parthenogenesis, but further evidence against this is the diploidy of the adult sporophytes releasing the embryonic sporophytes. Only haploid adult sporophytes from second generation parthenogenesis (Lewis et al. 1999) or the fusion of haploid gametes has produced diploid kelp embryonic sporophytes.

The embryonic sporophytes could also be the result of asexual reproduction. Nakahara and Nakamura (1973) witnessed what they termed apospory in *Alaria*, but it produced diploid gametophytes, not sporophytes. The aposporous gametophytes did produce gametes, although they were monoecious. Tetraploid sporophytes developed from fertilized eggs in the aposporous gametophyte culture. The embryonic sporophytes released from *Nereocystis* were diploid, so asexual reproduction is unlikely. It has never been

seen in *Nereocystis*, and asexual reproduction in another other kelp produced tetraploid sporophytes.

The release of diploid embryonic sporophytes from adult sporophytes is a new discovery. It changes the known life cycle of *Nereocystis*, and has ecological and evolutionary implications, and well as implications for *Nereocystis* mariculture. The limitations of the results given in this paper are that, although other kelps may release embryonic sporophytes, only *Nereocystis* is known to do so.

Evolutionary Implications

Kelps are morphologically diverse, suggesting a long evolutionary history. Increasing evidence, however, suggests short and rapid divergence of this order (Saunders and Druehl 1992). Intergeneric crosses can produce adult-sized kelps (Sanbonsuga and Neushul 1978), and various divergence estimates indicate that kelp intergeneric differences are equivalent to angiosperm interspecific differences (Fain et al. 1988, Saunders and Druehl 1991, Fain et al. 1992, and Fain and Druehl, unpubl, *in* Saunders and Druehl 1992). The existence of intergeneric hybrids and the relatively low divergence among kelps compared to other groups suggests that the order Laminariales diverged recently. Saunders and Druehl (1992), using small-subunit ribosomal ribonucleic acid sequences for seven kelp genera, estimated that the most distantly related kelp diverged relatively recently, between 16 and 20 million years ago.

Although the kelps appear to be a fairly conserved group, *Nereocystis* has many unique characteristics. Saunders and Druehl (1992) established which specific nucleotide (U, C, A, or G) was present at 27 sites of the small-subunit ribosomal ribonucleic acid sequence for seven genera of kelp. The specific nucleotide varied among the kelps, and at seven sites the sequence present was unique to *Nereocystis*, indicating that it is different from the other kelps tested (Saunders and Druehl 1992). *Nereocystis* is also the only member of its genus, and the only kelp that releases spores via sinking abscised sori. A unique life history strategy among the kelps would be consistent with its apparent divergence from other Laminariales.

A New Life Cycle Strategy

The generally accepted life cycle of *Nereocystis* includes only free-living gametophytes. Kelp gametophytes have been found growing endophytically in red algae (Garbary et al. 1999), and it has been suggested that endophytic gametophytes function as a seed bank, providing a supply of gametophytes to kelp beds after disturbance events (Hubbard et al. 2004). Endophytic gametophytes develop more slowly than free living gametophytes, but may be more protected from herbivory, storms, and other disturbances. Endophytism could also protect gametophytes from predation.

Although endophytism in red algae may occur in *Nereocystis*, the association is difficult to induce in the lab. This study indicates they may be endophytic within adult *Nereocystis*. The discovery of kelp gametophytes in red

algae and the studies that followed have highlighted the fact that gametophytes are very rarely found in the field, and suggested that the reason they have not been found is that they are not there. With *Nereocystis*, they could be growing endophytically within the adult sori. A gametophyte was apparently present in a preserved sorus/exudate sample from October 2005, indicating that gametophytes were present either within sori or in exudate. Spores developing within sori or in exudate would be the only source for such endophytic gametophytes.

Diploid embryonic sporophytes are the result of an egg fertilized with sperm. The only two known ways that diploid kelp sporophytes develop is from a zygote or from second generation parthenogenesis (Lewis et al. 1993). Establishing that the embryonic sporophytes are diploid eliminates the possibility that they developed parthenogenetically because the adults that released them were diploid. Only haploid adults that developed parthenogenetically from female gametophytes could produce parthenogenetic diploid sporophytes.

Embryonic sporophytes of two *Laminaria* species can reattach (Chapman 1984) and it is likely that the sporophytes released by *Nereocystis* can also attach when they contact substrate. Embryonic sporophytes, therefore, are released by adult sporophytes and have the potential to settle and recruit.

Advantages of Releasing Embryonic Sporophytes

An endophytic life cycle has many possible advantages over a free-living one. Spores and gametophytes are likely protected at the surface from sediments and herbivory. Light levels are higher at the surface, and flow is also higher, potentially allowing greater nutrient availability over the boundary layer on the bottom. By releasing both spores and embryonic sporophytes, *Nereocystis* also utilizes a bet-hedging strategy.

The unique sorus abscission of *Nereocystis* creates an opportunity for some spores to be dispersed far distances, and some to remain near the parent. This increases the area of the dispersal shadow. The release of embryonic sporophytes along with spores increases the temporal dispersal shadow. By releasing spores, which need time to develop into gametophytes, produce gametes, and fuse gametes, and releasing embryonic sporophytes, which are ready to begin rapid growth into adulthood, *Nereocystis* is able to take advantage of hospitable conditions, and possibly avoid inhospitable ones.

The release of spores and embryonic sporophytes is a bet-hedging strategy, assuring that young sporophytes are continuously available. Released spores settle and develop into gametophytes. The gametophytes produce and release gametes, the gametes fuse, and form a zygote, which grows into an embryonic sporophyte. The amount of time needed to complete this process is probably weeks or months, during which time the released embryonic sporophytes settle and grow. Favorable conditions are likely

variable in the subtidal, and a constant supply of young sporophytes ensures that when conditions are favorable, young sporophytes can grow quickly.

Another advantage of developing microscopic stages within the blade is that the stages are exposed to higher light levels at the ocean's surface than on the bottom. Light is a potentially limiting factor on the bottom. Under a *Nereocystis* canopy light levels can be very low (Vadas 1972, Aleem 1973) and juvenile survival can also be lower under a kelp canopy than outside of a canopy (Dean et al. 1989). Even in the winter, when the canopy can be reduced, light is likely higher at the surface due to attenuation through the water. *Nereocystis* blades remain at the surface, and therefore have higher light levels compared to the bottom.

Sediment can have a detrimental effect on microscopic kelp stages (Devinny and Volse 1978), although adult sporophytes may not be affected (Deysher and Dean 1986). Endophytic *Nereocystis* spores and gametophytes are able to avoid sediment by remaining at the surface, where sediment deposition and scour are much less likely.

Herbivory may be lower on *Nereocystis* blades than the holdfast or stipe due to faster current speeds. Reducing herbivory is another potential advantage of keeping microscopic stages in *Nereocystis* blades.

Implications for *Nereocystis* Mariculture

Interest exists in kelp culturing due to the commercial value of many kelps and pressure to restore kelp beds that are vital to commercial

invertebrates and fish. The viability of breeding kelps has been discussed for almost fifty years (Lewin 1958 *in* Sanbonsuga and Neushul 1978). Logistical problems, including difficulties in obtaining gametophytes and raising them to adult sporophytes, have been implicated in the failure of culturing kelps in the U.S. (Sanbonsuga and Neushul 1978). Obtaining embryonic sporophytes directly from adult sporophytes could eliminate many of the problems currently associated with *Nereocystis* culturing.

Nereocystis mariculture is possible, and may be easier if embryonic sporophytes released from sori are utilized. Embryonic sporophytes could be seeded onto rope and outplanted. This would reduce the amount of time needed until harvest, and also eliminate the need to keep optimal conditions for gametophytes. Gametophytes need blue light, nutrients, and temperatures from 5 to 15° C in order to produce gametes (Vadas 1972, Ødegaard et al. 1998), and therefore are difficult to culture. Allowing gametophytes to develop in the field, and then collecting the embryonic sporophytes would be more efficient, less costly, and easier than culturing from spores. Attempts during this study to raise them in lab failed, however, and more research is needed to discover the requirements of embryonic sporophytes.

Conclusions

The presence of embryonic sporophytes in adult sporophyte exudate has never been documented before. The embryonic sporophytes released by *Nereocystis* were diploid and probably the result of the "normal" life cycle

occurring entirely within soral tissue. This study documents a life history strategy previously unknown among the brown algae, and one that has ecological, evolutionary, and maricultural implications.

Bridge 1

Chapter 2 documented a new life history strategy in *Nereocystis luetkeana*. The new strategy was discovered while culturing *Nereocystis* as part of a study describing the epiphytic community of *Nereocystis* in South Slough and the apparent lack of *Nereocystis* recruitment to the Slough. The causal factor preventing recruitment was not identified because the new strategy was determined to be more important. Chapter 3, however, is the culmination of the study on the epiphytic community. It describes the taxa found on drifting *Nereocystis* in South Slough, and seasonal changes in abundance of those taxa.

CHAPTER 3—EPIPHYTIC ORGANISMS ON DRIFTING *NEREOCYSTIS LUETKEANA* IN THE SOUTH SLOUGH

Introduction

Drifting algae disperse organisms and can act as nurseries to fish and invertebrates. Although the algae are abundant, relatively little recent work exists on drifting seaweed ecology in the United States; more research has been done off the coasts of Korea (Cho et al. 2001), Japan (Safran 1990, Kokita and Omori1998, Kokita and Omori 1999, Sano et al. 2003), Russia (Tsukion-Lukiana et al. 2001a, Tsukion-Lukiana et al. 2001b), Iceland (Ingølfsson 1998), Chile (Thiel 2002, Thiel 2003), Antarctica (Norkko et al. 2004), the North Sea (Gutow 2003), and the Baltic Sea (Berglund et al. 2003). The research on the ecology of drift algae in the eastern Pacific of the U.S. includes the work of Shaffer et al. (1995), Bushing (1994), Harrold and Lisin (1989), Edgar (1987), and Highsmith (1985). Drift algae have the potential to connect coastal systems to the open ocean and the deep sea, as well as connecting ecosystems on smaller scales. Drifting and attached kelp individuals, for example, are consistently found in the South Slough Estuary in Oregon, although they do not appear to recruit there.

These individuals carry epiphytic algae and animals to the estuary, and may contribute nutrients to the ecosystem. The epiphytes may become established in the South Slough even if the host algae do not.

A note on term definitions-- "Epiphyte" can refer to an alga attached to another alga, or, more generally, to an organism attached to an alga. Otero-Schmitt and Pérez-Cirera (1996) use the term "epiphytic animals" to refer to fauna associated with drifting macroalgae and I have opted to use it along with "epiphytic algae."

Ecological Effects of Drift Algae

Drifting algae in estuarine, coastal, and open ocean ecosystems provide temporary and permanent habitats to invertebrates and fish. They are also among the most commonly reported drifting objects, and can provide food to associated organisms and support diverse communities (Thiel 2003). Their ability to float and to grow while drifting suggests that their communities are long-lived. They can also distribute all the life stages of organisms (Bushing 1994), and function as nurseries for benthic invertebrates (Norrko et al. 2004). Castro et al. (2002) suggest that fish may use drifting objects to increase dispersal and survival of juveniles. Marine invertebrates can, theoretically, increase their dispersal potential by utilizing drifting objects (Highsmith 1985, Edgar 1987). Although they have not been extensively studied, drifting algae likely affect the distributions and abundances of many organisms. Some of the evidence for these effects is reviewed below.

Negative effects of drifting algae include those related to eutrophication and green tides. *Ulva* spp. mats, for example, often cause anoxia and reduce infaunal invertebrate densities (Everett 1991, Franz and Friedman 2002). Macroalgal accumulations in estuaries, however, can also have positive effects on the organisms in estuaries by adding nutrients to the system and releasing nutrients from sediments (Astill and Lavery 2001).

Rafting (on algae or on other material) and floating are two mechanisms that have been proposed to explain the distributions of widely dispersed invertebrates without a pelagic life stage (Highsmith 1985, Thiel 2002). Floating and rafting appear to be important to gastropods and bivalves, regardless of whether the molluscs have a planktonic larval stage (Martel and Chia 1991). Species distributions of epiphytic peracarids along the Chilean coast are related to current regimes. Thiel (2002) concludes that rafting on algae may be an important dispersal mechanism for epiphytic animals without pelagic larval stages, such as peracarids.

Invertebrates also increase dispersal with drift algae by laying eggs on algae while they are attached. Kyle and Boulding (2000) suggest that *Littorina sitkana* populations are more genetically mixed than would be expected from their life history because the snails lay their eggs on *Fucus distichus.* When the algae detach and drift, the eggs hatch in a different region and mix populations. Drift dispersal may be a "side effect" of the snails' preference for

Fucus, which could have evolved because of a benefit to *Littorina* unrelated to dispersal (Ingólfsson 2000). The preference for *Fucus* could also have evolved because the snails were dispersed via drift algae and their populations were less likely to experience inbreeding effects.

Some organisms, however, do seem to preferentially lay eggs on algae that are likely to be detached. Marliave (1976) described *Aulorhynchus flavidus*, the tubesnout, spawning on three kelp species including *Nereocystis luetkeana*. He noted that the fish spawned only on young sporophytes, not on mature adults, and that all of the kelp with egg masses were detached before the eggs began to hatch. Young *Nereocystis* sporophytes are more easily detached because their shape is more vulnerable to chaotic wave forces than the shape of adults (Denny et al. 1997) and because their holdfast is more weakly attached than that of adults (Duggins et al. 2001). Marliave (1976) suggested that *A. flavidus* has developed a preference for unstable kelp as a dispersal mechanism.

The larvae and juveniles of fish are also associated with drift algae. The life cycle of gold-eye rockfish, a commercially valuable species, is seasonally linked to drift algae (Kokita and Omori 1999). The growth pattern of the rockfish suggests a close association with drift algae: the fish grow slowly prior to finding an algal clump, and grow quickly while in the clump. The association is seasonal, and the authors suggest the faster growth rates are related to temperature and not to food availability.

Algal species may also be dispersed by drifting. The most likely explanation for the global distribution of *Acrosiphonia arcta* is that the alga has colonized land after drifting from one pole to the other (van Oppen et al. 1994). *Acrosiphonia* is only found in the Arctic and the Antarctic, but their disjunct populations are genetically very similar (van Oppen et al. 1994). Although drift algal-related colonization events can be difficult to document, they can be supported by analyses of genetics, and they are often the only reasonable explanation for polar distributions.

Colonization by rafting is also likely to occur with clonal organisms, because only one individual is usually necessary for sexual reproduction (Jackson 1986). In the Indo-Pacific and Caribbean Islands, drifting or rafting is the only reasonable explanation for the presence of clonal organisms.

Some species are theoretically more likely than others to be dispersed on drift algae. These include clonal species, those without a pelagic larval stage, those that tend to be found in clumps, those that lay eggs on algae, and those that are small as adults. Quantifying these patterns, however, has rarely been accomplished, because verifying a drift algae facilitated colonization event is difficult.

South Slough Estuary

The South Slough National Estuarine Research Reserve is part of Coos Estuary, a drowned river estuary of 54 km^2 in southern Oregon (Figure 7). It is

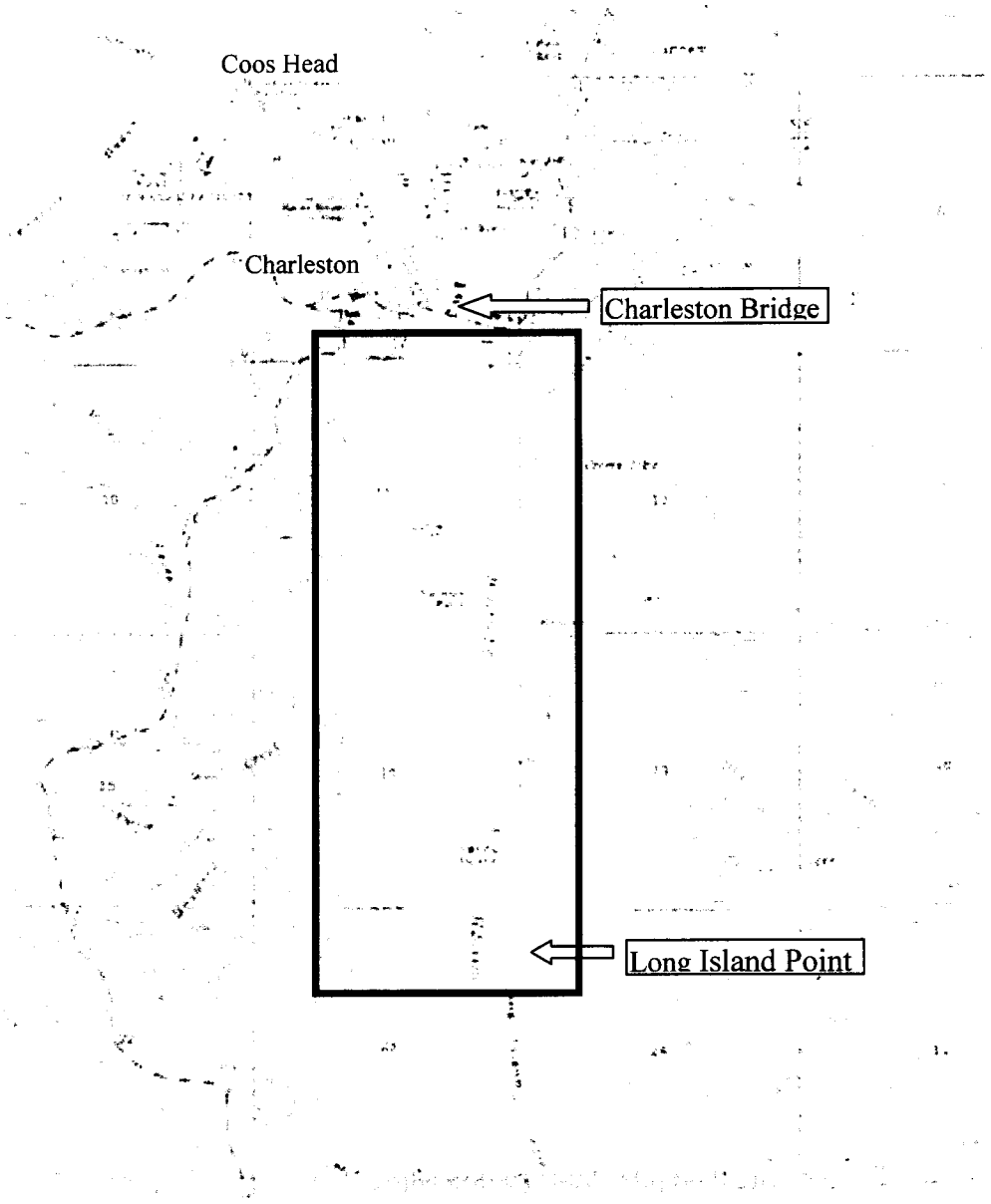


Figure 7. Map of South Slough with box surrounding survey area.

a marine dominated estuary, exhibiting a gradient towards more freshwater input further inland (Rumrill 2003).

The South Slough was altered significantly by European settlers who arrived in the 1850's (Rumrill 2003). Loggers and farmers built dams, dikes, and canals that changed the flow and sediment dynamics in the Slough. Such stream flow changes can make estuaries more vulnerable to invasions. The proximity of a major shipping port, Coos Bay, has facilitated the introduction of many non-native species into the Slough. In 1989, 32 introduced species were known in South Slough, and all were originally from the North Atlantic or Japan (Carlton 1989).

Drifting algae could facilitate introductions. Any drifting object that enters the Slough must first travel through Charleston harbor, where extensive docks are home to many introduced species. *Nereocystis*, in particular, consistently drifts into the Slough. Organisms living on *Nereocystis* are brought into the Slough and could potentially settle there.

This study was designed to describe the epiphytic species that occur on drifting *Nereocystis* in the South Slough and seasonal patterns in their abundances and in the composition of the epiphytic plant and animal communities. *Nereocystis* individuals and their epiphytes continually drift into South Slough. Although the effects on the Slough are unknown, epiphytes are potential colonizers.

Materials and Methods

For one year part of the South Slough was surveyed monthly. Data were collected using a flat-bottom boat from July 2004 to July 2005 in the South Slough from Charleston Bridge to Long Island Point (Figure 7). Surveys were conducted monthly, except for July 2004, when two surveys were conducted, and December 2004 and June 2005, when no surveys were completed due to uncooperative weather, tides, and schedules. The boat was driven from Charleston Harbor to Long Island Point at high tide, and counts of *Nereocystis* individuals were recorded on the return, beginning at Long Island Point and ending at the Charleston Bridge. The data, therefore, only refer to the approximately 2.7 km^2 from Long Island Point to Charleston Bridge, although no individuals were seen further south when a survey was done by kayak from Hinch Road Bridge to Long Island Point. One clump of bull kelp was observed by Hinch Road Bridge (43°16.885', 124°19.117') in October (J. Trainer, pers. comm.), but this was unusual. Surveys were never done on foggy days or at night so the likelihood of seeing floating kelp was similar for all surveys.

Each *Nereocystis* individual found was taken into the boat, tattooed, and the number and taxa of epiphytic organisms was recorded. Nicholson's (1970) method of tattooing was used. A small metal spike was dipped into permanent black india ink and then injected into the bulb to make small dots. Consecutive numbers were tattooed on *Nereocystis* in this manner.

Individuals sampled in July 2004 were not tattooed. The presence of soral tissue was noted, as was whether the individual was attached to the bottom or drifting. Discrete colonies of bryozoans, hydroids, coralline encrusting algae red algal encrusting stage of other red algae, and olive green slime were counted as individuals. Individuals were counted with a minimum of disturbance, and, for the first months of sampling, no vouchers were taken, to avoid disrupting the community for future surveys. After it became apparent that *Nereocystis* individuals were not likely to be resampled, vouchers were taken. Photographs were taken of epiphytic organisms for verification with a Pentax Optio 43WR water-resistant camera. When organisms could not be identified to species, they were identified to the lowest possible taxon. Although *Enteromorpha* species are now classified within the genus *Ulva* (Hayden et al.2003), their morphologies are different and a category of *Enteromorpha*-like *Ulva* was used.

Data were analyzed using PRIMER software (Plymouth Marine Laboratory, Plymouth, UK), a statistics program that analyzes matrices of species. SIMPER (similarity percentages of species contributions analysis) was performed using season as a factor. This analysis was used to determine the contribution of individual taxa to the overall community, and the taxa that were most representative of differences between seasons. January and February were considered winter, March, April, and May were considered

spring, July and August were considered summer, and September, October, and November were considered autumn.

Results

Seasonality of Individual Taxa

Forty-six taxa were found living in or on *Nereocystis* individuals drifting in the South Slough. Categories such as "unidentified worm" and "bushy bryozoan" may include more than one species, thus forty-six is a conservative estimate. The term "taxa" is used to describe these categories because some categories include more than one species. Table 3 lists all the taxa found and their monthly abundances, calculated as the number per individual *Nereocystis,* and Figure 8 depicts the abundances of all taxa graphically. Figure 9 displays monthly abundances of red and green algae. Both epiphytic animal taxa (Figure 10) and taxa with the dominating groups (red and green algae and crustaceans and cnidarians) removed (Figure 11) are also graphed. Epiphytic organisms were found in all months except July 2005.

Thirteen taxa of red algae were found growing on *Nereocystis*. The most abundant taxa for the year surveyed was *Pterochondria woodii*, with an average of 33.47 individuals found per *Nereocystis*, and a peak in April of 386.23 individuals per *Nereocystis* (Table 3a). By far the greatest abundances occurred in February, March, and April, indicating a spring bloom of *Pterochondria*.

Table 3a.-d Taxa and number of individuals found per *Nereocystis* each month surveyed. Yearly total and averages at right, in bold type. a. Red algae.

	Taxa	07/14/2004	07/28/2004	08/30/2004	09/15/2004	10/14/2004	11/28/2004	01/11/2005	02/23/2005	03/23/2005	04/21/2005	05/26/2005	07/01/2005	**TOTAL**
Red algae	*Pterochondria woodii*	0.00	0.00	0.00	0.09	0.88	0.08	0.00	50.55	334.33	386.23	2.00	0.00	**33.47**
	Porphyra nereocystis	0.00	0.00	0.00	0.09	1.27	0.92	0.00	39.09	7.67	30.23	3.64	0.00	**4.92**
	Anththamnionella pacifica	0.00	0.00	0.00	0.00	1.73	0.25	0.00	22.36	80.00	17.08	9.82	0.00	**4.67**
	Cryptopleura ruprechtiana	0.15	0.00	0.00	0.00	1.86	0.17	0.17	0.00	0.00	0.00	0.36	0.00	**0.65**
	Polyneura latissima	1.00	0.00	0.00	0.00	0.22	0.08	0.00	0.00	0.00	0.00	1.09	0.00	**0.27**
	Cryptopleura lobilifera	1.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.18**
	Palmaria sp.	1.04	0.05	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.15**
	Unidentified bladed red	0.00	0.00	0.00	0.13	0.03	0.17	0.50	0.09	0.00	0.00	1.73	0.00	**0.15**
	Microcladia borealis	0.00	0.00	0.00	0.04	0.02	2.08	0.00	0.00	0.00	0.00	0.09	0.00	**0.14**
	Neoptilota sp.	0.00	0.00	0.17	0.00	0.09	0.50	0.00	0.00	0.00	0.00	1.00	0.00	**0.12**
	Encrusting corraline algae	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.54	0.00	0.00	0.00	0.00	**0.04**
	Red algal crust	0.12	0.00	0.00	0.00	0.02	0.08	0.00	0.09	0.00	0.00	0.00	0.00	**0.03**
	Mazzaella sp.	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.01**

Table 3b. Taxa and number of individuals found per *Nereocystis* each month surveyed. Yearly total and averages at right, in bold type. .b. Green algae, unknown, brown algae.

	Taxa	07/14/2004	07/28/2004	08/30/2004	09/15/2004	10/14/2004	11/28/2004	01/11/2005	02/23/2005	03/23/2005	04/21/2005	05/26/2005	07/01/2005	**TOTAL**
Green algae	*Enteromorpha*-like *Ulva*	3.15	0.00	2.50	1.09	16.75	0.00	0.00	12.45	78.00	133.00	3.09	0.00	**16.72**
	Ulva spp.	0.00	0.00	0.00	0.09	8.78	1.75	0.17	0.09	0.00	0.00	1.09	0.00	**3.00**
	Rhizoclonium implexum	0.00	0.00	0.00	0.00	0.00	0.42	0.00	0.00	0.00	0.00	9.09	0.00	**0.53**
	Spongomorpha sp.	0.00	0.00	0.00	0.00	0.06	0.00	0.17	0.00	0.00	0.00	0.00	0.00	**0.03**
Unknown	Olive green slime	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	**0.01**
Brown algae	*Alaria marginata*	0.08	0.00	0.00	0.09	0.02	0.00	0.00	0.00	0.00	0.00	0.18	0.00	**0.04**
	Laminaria setchellii.	0.04	0.00	0.00	0.09	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.03**
	Nemalion helminthoides	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.01**
	Desmarestia lingulata	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.01**

Table 3c. Taxa and number of individuals found per *Nereocystis* each month surveyed. Yearly total and averages at right, in bold type. c. Crustaceans, cnidarians, and bryozoans.

	Taxa	07/14/2004	07/28/2004	08/30/2004	09/15/2004	10/14/2004	11/28/2004	01/11/2005	02/23/2005	03/23/2005	04/21/2005	05/26/2005	07/01/2005	**TOTAL**
Crustaceans	Pelagic barnacle	0.00	0.00	0.00	0.00	0.72	0.00	0.00	0.00	0.00	54.31	0.00	0.00	**3.77**
	Balanomorph barnacle	0.05	0.14	0.00	0.65	0.72	0.13	3.00	13.17	0.00	38.46	0.00	0.00	**3.29**
	Gammarid amphipod	0.00	0.00	0.00	0.30	1.31	2.92	5.33	5.27	41.33	6.85	2.27	0.00	**2.28**
	Caprellid amphipod	0.00	0.00	0.00	0.02	0.00	0.00	0.18	0.00	0.15	0.00	0.00	0.00	**0.03**
	Cancer sp.	0.00	0.05	0.00	0.00	0.72	0.00	0.00	0.00	0.67	0.00	0.09	0.00	**0.02**
	Isopod	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.08	0.00	0.00	**0.01**
	Pugettia producta	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	**0.01**
Cnidarians	Hydroids	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.85	18.18	0.00	**1.91**
	Epiactis prolifera	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.01**
	Unidentified anemone	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.01**
Bryozoans	Encrusting bryozoan	0.31	0.00	0.00	0.13	0.16	0.17	0.50	0.55	0.33	8.62	0.00	0.00	**0.78**
	Bushy bryozoan	0.04	0.05	0.00	0.00	0.02	0.17	0.00	0.36	0.00	0.00	0.00	0.00	**0.05**

Table 3d. Taxa and number of individuals found per *Nereocystis* each month surveyed. Yearly total and averages at right, in bold type. d. Molluscs, unknowns, polychaetes, nemerteans, and fish.

Taxa		07/14/2004	07/28/2004	08/30/2004	09/15/2004	10/14/2004	11/28/2004	01/11/2005	02/23/2005	03/23/2005	04/21/2005	05/26/2005	07/01/2005	**TOTAL**
Molluscs	Herbivorous snail	0.00	0.05	0.00	0.00	0.00	0.08	0.00	0.00	0.67	0.08	0.00	0.00	**0.03**
	Hermissenda crassicornis	0.00	0.00	0.00	0.09	0.05	0.00	0.00	0.00	0.00	0.00	0.09	0.00	**0.03**
	Mytilid mussel	0.00	0.00	0.00	0.00	0.02	0.00	0.17	0.00	0.00	0.23	0.00	0.00	**0.03**
Unknowns	Unidentified worm spp.	0.00	0.00	0.00	4.35	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.51**
	Red strings	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.82	0.00	**0.10**
	Tube worm	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.04**
	White egg	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.67	0.00	0.09	0.00	**0.03**
	Yellow egg mass	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.45	0.00	0.00	0.00	0.00	**0.03**
Polychaetes	*Scale worm*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	**0.01**
Nemerteans	Nemertean	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.01**
Fish	Gunnel-like green fish	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	**0.01**
	Gunnel-like mottled fish	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	**0.01**

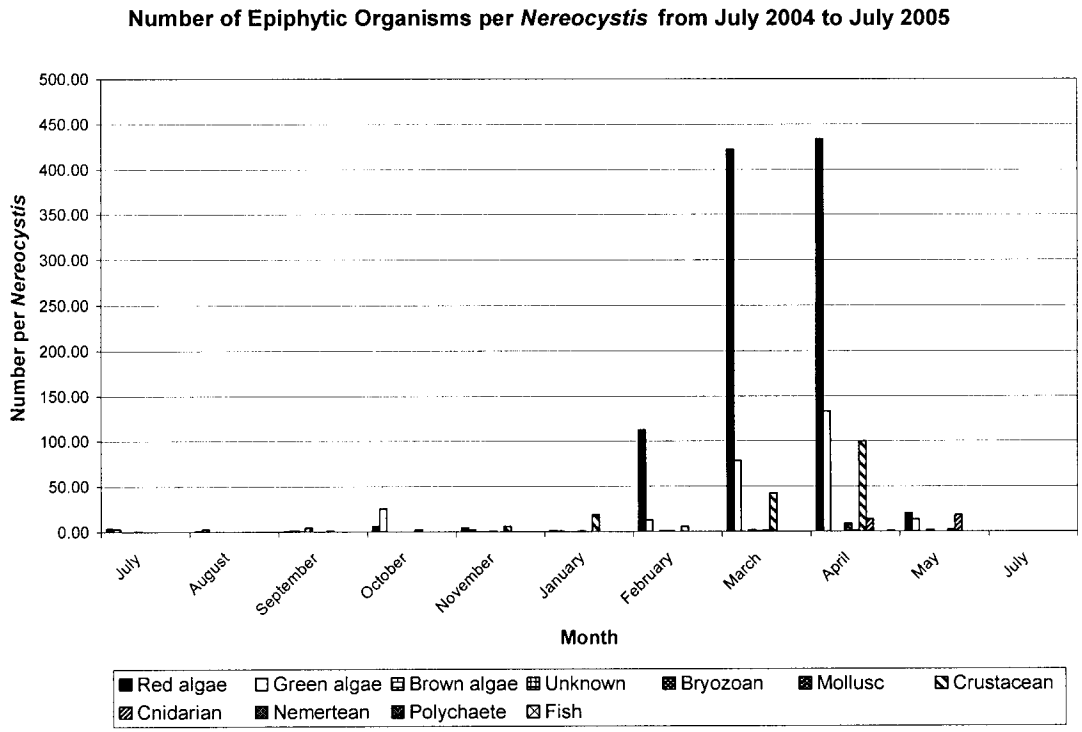


Figure 8. The number of epiphytic organisms, both algae and animals, per *Nereocystis* individual. The most abundant organisms were red algae, green algae, and crustaceans.

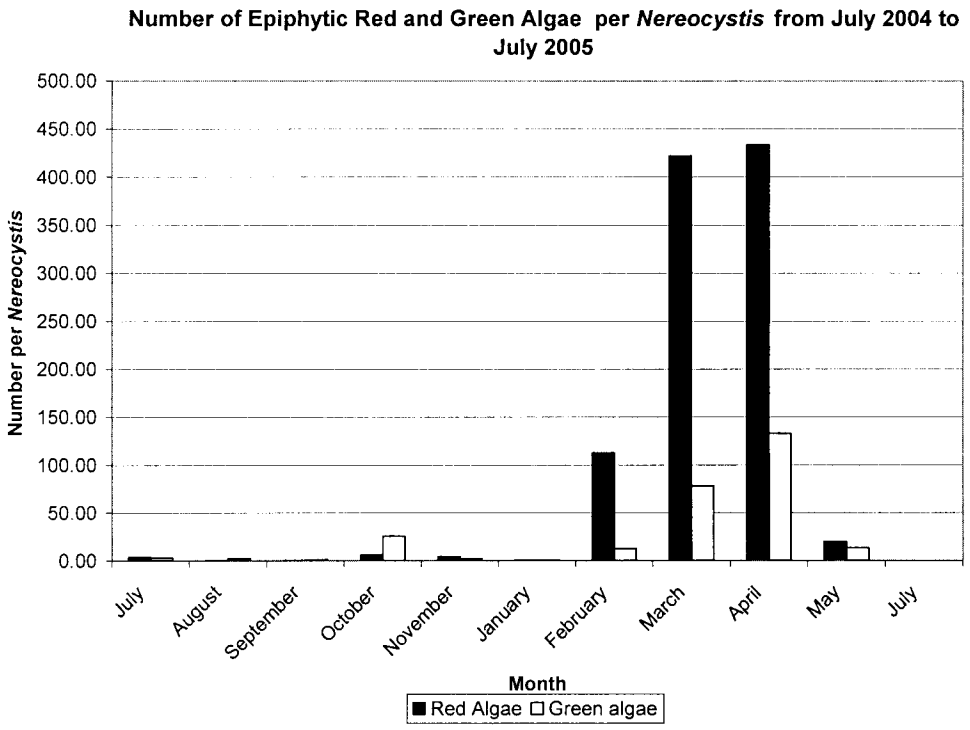


Figure 9. The abundances of red and green epiphytic algae per *Nereocystis* individual.

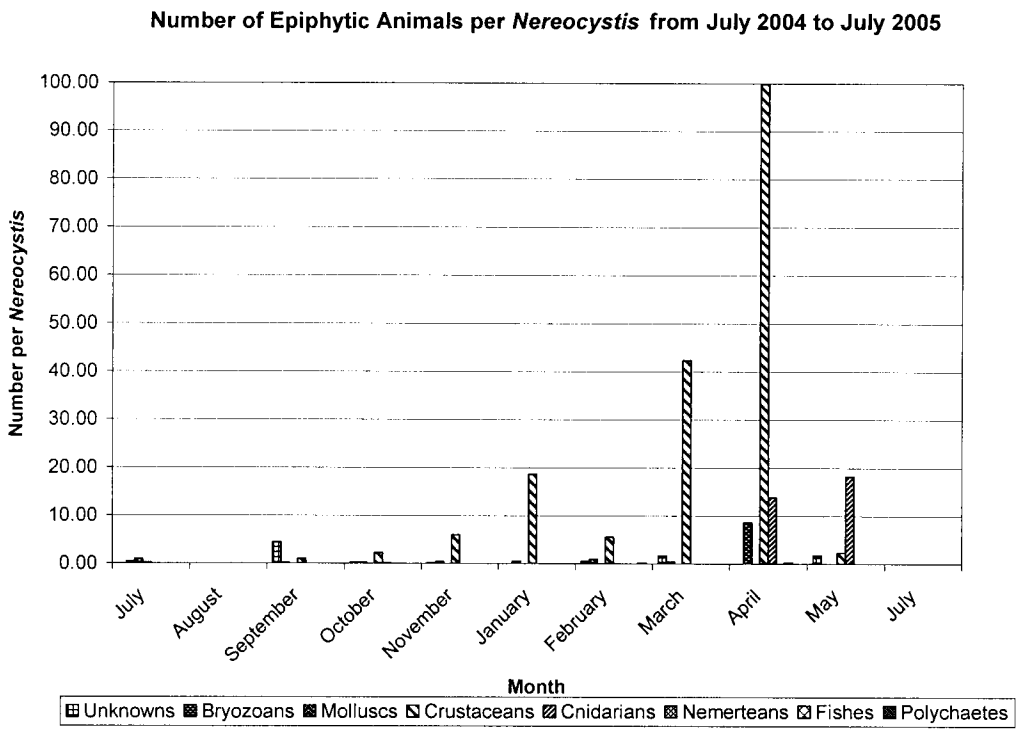


Figure 10. The abundances of epiphytic animals per *Nereocystis* individual. Crustaceans, cnidarians, and bryozoans were the most abundant taxa.

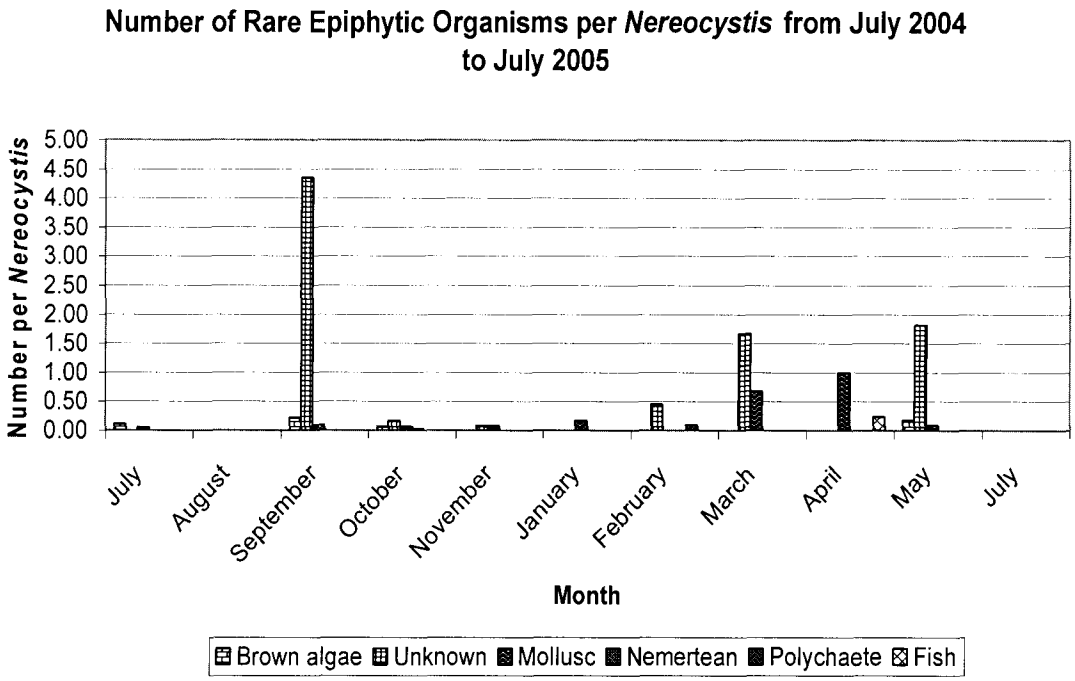


Figure 11. The abundances of rare organisms per *Nereocystis* (red algae, green algae, crustaceans, cnidarians, and bryozoans removed). The most abundant rare organisms were unknowns and molluscs.

Other red algae also bloomed in spring. *Porphyra nereocystis* was found in September, October, November, February, March, April, and May, but abundances were much higher in the spring. *Antithamnionella pacifica* displayed the same pattern of a large peak in spring, presence in autumn and absence in summer.

The presence of other red algae was also seasonal, but they did not appear to bloom (they did not increase greatly in abundance). *Palmaria*, for example, was only found in summer. "Unidentified bladed red alga" was found in autumn, winter, and early summer. *Microcladia borealis* was found only in autumn and early summer.

Apparent seasonal patterns of abundance were absent in many taxa. *Cryptopleura ruprechtiana* was found in July, October, November, January, and May, with no clear seasonal pattern. Others that were found many times, but with no clear seasonal pattern, were *Polyneura latissima, Neoptilota* sp.,and unidentified red algal crust. Coralline algal crust, *Cryptopleura lobilifera,* and *Mazzaella* sp. were found so rarely that no pattern could be detected.

Four taxa of green algae were found on *Nereocystis*: *Enteromorpha*-like *Ulva*, leafy *Ulva*, *Rhizoclonium implexum*, and *Spongomorpha* sp (Table 3b). The clearest seasonal pattern among the green algae was the spring bloom of *Enteromorpha*-like *Ulva*. *Enteromorpha*-like *Ulva* was the second most abundant taxa found, with an overall abundance of 16.72 individuals per

Nereocystis, and a peak of 133 individuals per *Nereocystis* in April (Table 3b). It appeared to bloom in spring, and may have bloomed again in autumn. Leafy *Ulva* was found in autumn, winter, and early summer. The highest abundance occurred in October.

The two other green taxa were found only twice, in nonconsecutive months. *Rhizoclonium implexum* was found in November and in much higher abundance in May. *Spongomorpha* sp. was found in October and January.

Epiphytic brown algae were far less abundant than many of the red and green algae. The species found were *Nemalion helminthoides, Alaria marginata, Laminaria setchelii,* and *Desmarestia lingulata*. The one broad seasonal pattern

to emerge was the absence of epiphytic brown algae from winter to spring, and presence in summer and autumn.

Olive green slime was found only once, in November. The voucher taken was lost, and so its identity is unknown.

Crustaceans were the most abundant epiphytic animals. Pelagic barnacles were the most abundant animal epiphyte (3.77 per *Nereocystis* for the year surveyed) (Table 3c). They only occurred in October and April, and were much more abundant in April. Balanomorph barnacles were the second most abundant epiphytic animal overall (3.29 per *Nereocystis* for the year surveyed) and were found in January, April, July, September, October, and November (Table 3c). The highest abundance of barnacles occurred in April,

although none were found for two months before or after. Gammarid amphipods were found every month from September to May. Their abundance increased in spring and a peak occurred in March (41.33 amphipods per *Nereocystis*) (Table 3c). Less abundant crustaceans found were *Cancer* sp., *Pugettia producta*, caprellid amphipods, and isopods.

Three species of cnidarians were found, *Epiactis prolifera*, an unidentified anemone, and hydroids. The anemones were found in October only. Hydroids were only found in April and May, but were abundant (13.85 and 18.18 per *Nereocystis* for April and May, respectively) when found (Table 3c).

Both encrusting bryozoans and bushy bryozoans were found. Encrusting bryozoans were found in all months except August and May (Table 3c). They
were found in only one of the surveys of July 2004. Their peak abundance occurred in April. Bushy bryozoans were less common and less abundant than encrusting forms. They were found in July, October, November and February.

Molluscs found included herbivorous snails, the nudibranch *Phidiana crassicornis*, and mytilid mussels. No seasonal patterns were apparent (Table 3d).

Several taxa were found only rarely. Nemerteans were only found in October. The one polychaete identified was a scale worm, although the

unidentified worm and tube worms may have been polychaetes. Two vertebrates were found, both gunnel-like fish, living in a thick mat of *Pterochondria* attached to *Nereocystis*. Two green individuals and one mottled individual were observed, and probably represented two species.

Some organisms were only identified to broad categories. Most were only found once. Unidentified worms were very small and could not be identified to a phylum. Tube worms also could not be identified taxonomically. Two types of eggs were found, yellow and white. The white appeared molluscan, but this was not verified. Red strings were noted, but were not identified further. They were not nemerteans, but did appear to be organisms or part of an organism.

Seasonal Community Groups

SIMPER analysis grouped species seasonally according to their contributions to the overall seasonal epiphyte abundance. Instead of the abundances of an individual species, this program yields information about the relative importance of a species to the seasonal epiphytic community.

The summer group consisted of, in order of amount of contribution, *Enteromorpha*-like *Ulva*, *Polyneura latissima*, *Palmaria* sp., balanomorph barnacles, *Cryptopleura lobilifera*, encrusting bryozoans, and *Cryptopleura ruprechtiana* (Table 4a). The importance of contribution is interpreted as the amount that taxa contributed to the overall epiphyte community of that season. Over half of the epiphytes found in summer were *Enteromorpha*-like *Ulva*.

The autumn group, in order of importance, consisted of leafy *Ulva*, gammarid amphipods, *Enteromorpha*-like *Ulva*, and encrusting bryozoans (Table 4b). Leafy *Ulva* contributed almost half of the overall epiphyte numbers.

The winter group consisted of *Porphyra nereocystis*, gammarid amphipods, *Pterochondria woodii*, and *Antithamnionella pacifica* (Table 4c). Almost 40% of the individuals of the winter community were *Porphyra nereocystis*.

The spring group consisted of *Enteromorpha*-like *Ulva*, pelagic barnacles, *Antithamnionella pacifica*, balanomorph barnacles, and gammarid amphipods (Table 4d). *Enteromorpha*-like *Ulva* constituted almost half of the spring community in terms of abundance.

Discussion

This study documents the seasonal abundances of the epiphytic organisms on *Nereocystis* in the South Slough. Seasonal differences may vary from year to year, but the results are still useful and likely describe general patterns. The year surveyed was a fairly typical year in terms of weather, although the winter was drier than average. Epiphytized drifting algae likely have ecological effects on estuaries, but these effects are still unknown. This descriptive study provides a basis for subsequent studies of ecological effects of drifting *Nereocystis* in the Slough.

Table 4a-d. Seasonal taxa groupings based on SIMPER analysis. Average abundance, average similarity, similarity/standard deviation, contribution %, and cumulative % are listed.

a. Summer Average similarity 2.83

Taxon	Average abundance	Average similarity	Similarity/ standard deviation	Contribution %	Cumulative %
Enteromorpha -like *Ulva*	1.96	1.46	0.18	51.44	51.44
Polyneura latissima	0.57	0.46	0.13	16.18	67.62
Palmaria sp.	0.45	0.23	0.12	8.17	75.79
Balanomorph barnacle	0.24	0.21	0.11	7.25	83.04
Cryptopleura lobilifera	0.54	0.10	0.07	3.43	86.47
Encrusting bryozoan	0.12	0.08	0.09	2.92	89.39
Cryptopleura ruprechtiana	0.12	0.08	0.05	2.69	92.08

b. Autumn Average similarity 8.13

Taxon	Average abundance	Average similarity	Similarity/ standard deviation	Contribution %	Cumulative %
Leafy *Ulva*	5.89	3.78	0.34	46.48	46.48
Gammarid amphipod	1.27	2.11	0.26	25.96	72.44
Enteromorpha-like *Ulva*	11.08	1.04	0.15	12.83	85.27
Encrusting bryozoan	0.15	0.45	0.10	5.56	90.83

Table 4 continued. Seasonal species groupings based on SIMPER analysis. Average abundance, average similarity, similarity/standard deviation, contribution %, and cumulative % are listed.

c. Winter Average similarity 13.81

Taxon	Average abundance	Average similarity	Similarity/ standard deviation	Contribution %	Cumulative %
Porphyra nereocystis	25.29	5.11	0.44	37.01	37.01
Gammarid amphipod	5.29	3.02	0.54	21.84	58.80
Pterochondria woodii	32.71	2.75	0.34	19.90	78.75
Antithamnionella pacifica	14.47	1.75	0.34	12.64	91.39

d. Spring Average similarity 15.69

Taxon	Average abundance	Average similarity	Similarity/ standard deviation	Contribution %	Cumulative %
Enteromorpha-like *Ulva*	122.69	7.33	0.65	46.70	46.70
Pelagic barnacle	44.12	2.76	0.27	17.62	64.32
Antithamnionella pacifica	28.88	2.74	0.34	17.44	81.76
Balanomorph barnacle	31.25	0.71	0.27	4.54	86.30
Gammarid amphipod	13.31	0.64	0.34	4.09	90.39

This study was not designed to test why the abundances occur, although some factors will be suggested when they seem probable. Factors that have been documented by others are reviewed. Drifting algae potentially affect regions and ecosystems, and prior knowledge of epiphytic species is essential to understanding ecological effects.

Many factors can affect epiphytic community species compositions. Survival of species can be related to taxonomic groups; Edgar (1987) found that most polychaetes survived drifting for over 100 days, but fewer crustaceans, echinoderms, and molluscs did. Most algal species also survived, particularly small filamentous species. The original ecosystem of drifting algae before they detach can also affect the epiphytic species composition. The epiphytic community on *Cystoseira* spp. individuals from wave-exposed sites were more diverse than those from protected sites (Otero-Schmitt and Pérez-Cirera 1996), possibly due to reduced grazing or predation pressure at exposed sites, or higher availability of propagules at exposed sites due to higher flow rates. Substrate type, particularly the presence of sand, also affects the epiphytic species. The epiphytic algae found on *Nereocystis* are influenced by depth (Markham 1969).

The sources of the drifting *Nereocystis* in South Slough, and the surrounding communities are unknown, but may vary. If wind is the major force causing drift, as Harrold and Lisin (1989) found in Monterey Bay, then the sources may change seasonally because the wind direction along the U.S.

Pacific coast is generally north in summer and south in winter. The shifts occur in autumn and spring. The presence of pelagic barnacles on drifting *Nereocystis* also suggests that some *Nereocystis* may drift for months before entering the Slough.

Succession also could play a role, although distinguishing between succession and seasonal changes can be difficult. Colonization by a bryozoan influences which organisms later colonize the kelp (Kain 1979), and so could be considered a species indicative of succession, but its abundance is also highly seasonal. In a study of drift algae succession, Kingsford and Choat (1985) found that the species found in the drift algal community depends on the length of time drifting and distance from shore. Offshore clumps always had abundant fauna, whereas nearshore clump faunal abundances were more variable. Kingsford and Choat (1985), however, only sampled in December and January, and the changes they document may only occur in winter.

Differences in the host alga itself also influence the associated communities. For example, structural differences between algae, not food value differences, appear to be important for amphipod distributions (Norderhaug 2004). All of these factors interact to influence drift algal community species compositions and abundances.

In South Slough, retention time of the drifters likely influences the effects that the drifters and their epiphytes have. After being tattooed, no *Nereocystis* was found in subsequent surveys. Either the *Nereocystis* drift out

of the sampling area in less than one month (the sampling interval) or they degrade in that time. The flushing time of South Slough is estimated to be 3 days (Rumrill 2003), and so it is likely that many *Nereocystis* remain for that length of time. They were not tracked within the Slough, however, and so it is also possible that they remain and drift to a part of the Slough not surveyed, or that they degrade. Degrading individuals were seen on many surveys, usually trapped by vegetation.

Mobility of organisms would also affect the likelihood of colonization in the Slough. Sessile organisms are probably less likely to colonize because of the apparent short retention time of drifting *Nereocystis*. All of the algae that were found are sessile. The two most abundant epiphytic animals, pelagic barnacles and balanomorph barnacles, are sessile. All the cnidarians and bryozoans are also sessile. The sessile organisms have mobile life stages that could be released into the Slough, but mobile organisms such as amphipods and crabs are still more likely to colonzie the Slough via drifting *Nereocystis*.

Individual Species—Algae

Macroalgal spring blooms are common along upwelling coasts due to the increase in light availability and the increase in nutrients from winter upwelling. Most of the *Pterochondria* individuals were found during a massive peak in abundance in spring. Other algal species that appeared to bloom in

spring were *Porphyra nereocystis*, *Antithamnionella pacifica, and* *Enteromorpha*-like *Ulva*. *Porphyra, Antithamnionella,* and *Enteromorpha* certainly were much more abundant in spring, but, like *Pterochondria*, they were found consistently, but in much smaller numbers, in the autumn. Four species display this pattern of a strong bloom in spring and a lesser one in autumn. The high March and April abundances of *Enteromorpha*-like *Ulva* are consistent with the spring, but not winter, blooms of *Enteromorpha* spp. from the literature (Plaut et al. 1998, Ohno et al. 1999, Pardal et al. 2000).

Relating the results documented here to those in other studies is problematic for other species due to geographic and methodological differences between studies and a lack of data on some species. For example, *Pterochondria woodii,* a filamentous red alga, was the most abundant organism found on *Nereocystis* in the South Slough, but it has not been previously reported growing on *Nereocystis*. Markham (1969) documented the epiphytes of *Nereocystis* in the San Juan Islands and reviewed the literature on *Nereocystis* epiphytes, and mentioned several species that were not found in this study. Several of the species found in the Slough were not mentioned in Markham's study (1969) or those he referenced.

Some differences could also be due to methodological differences between the studies. For example, Markham (1969) only observed *Porphyra nereocystis* in September, but he did not sample from January to June, and

missed the period of peak abundance observed in this study. Hawkes (1981 *in* Miller and Estes 1989) describes *Porphyra nereocystis* appearing first in late November or early December and then becoming reproductive from January to July. His study was conducted on Vancouver Island, and, although *Porphyra* was noted later there than it was found in the Slough, the fall and spring seasonality described is fairly consistent with the results documented in this study.

Few algal species were found in summer. Only 4 out of 12 red algal species were found during any summer survey. Of those that were found, two, *Palmaria* sp. and *Cryptopleura lobilifera*, were found only in summer, and represented a large part of the summer community (see seasonal community composition section). Among the four green algal species, only *Enteromorpha*-like *Ulva* was found in summer. Only the two kelps, *Laminaria* and *Alaria*, were found among the four brown algal species.

Abundances recorded, however, represent the epiphytic species found on drifting or reattached *Nereocystis* in the Slough. Most of the individuals sampled in late spring and summer were probably only months old, because *Nereocystis*

is more vulnerable to detachment when young (Denny et al. 1997). Younger individuals are less likely to be heavily epiphytized because they have been exposed to potential epiphytes for less time than other individuals.

Brown algae and leafy *Ulva* were found most commonly in autumn. Although the seasonal abundances of brown algae and leafy *Ulva* were similar, they have different life history strategies. *Ulva* is considered an ephemeral, opportunistic genus (Fletcher et al. 1990, Everett 1991, Valiela et al. 1997). In contast, three of the brown algal species found are large and probably do not colonize *Nereocystis* as quickly as *Ulva*. Large brown algae are found in the rocky intertidal and subtidal in greatest abundances in the summer. The presence of epiphytic brown algae in the summer and autumn is therefore not surprising.

Seasonal abundances of leafy *Ulva*, however, may change geographically because they are opportunistic and can colonize quickly. A study estimating the amount of leafy *Ulva* biomass available to the Coos Bay estuarine system based on local production in the rocky intertidal indicated that peaks occur in July and August (Hodder 1986). Epiphytic leafy *Ulva* abundance, in contrast, peaked in October. Although comparing biomass to individual count data is not ideal, the seasonal difference between epiphytic and intertidal *Ulva* populations is probably real. No *Ulva* individuals were found on *Nereocystis* during the months of peak biomass that Hodder (1986) quantified. Local intertidal abundances do not
appear to be related to epiphytic abundances, at least of leafy *Ulva*, possibly because the epiphytic leafy *Ulva* may not be from local populations.

Individual Species—Animals

The animal species were most abundant in spring include encrusting bryozoans, peracarid amphipods, balanomorph barnacles, and pelagic barnacles. Hydroids were abundant slightly later, in late spring and early summer. The spring blooms of algae such as *Pterochondria* and *Antithamnionella* probably facilitate the increase in peracarid abundance by increasing the availability of their preferred habitat. The three fish documented were also found in association with *Pterochondria*. The only apparent seasonal patterns among the animal species were high abundances in spring or early summer. They were probably all related to the seasonal increases in phytoplankton and macroalgae.

Brooks and Bell (2001) suggest that drift algae function as "mobile corridors" in estuaries, facilitating movement of organisms such as amphipods between seagrass beds. Amphipods use drifting algae to travel between seagrass beds. Drift algae seem to provide a mechanism for organisms to move within estuaries, and may aid recovery from disturbance events by facilitating movement of organisms and recolonization of seagrass beds (Brooks and Bell 2001). Amphipods, both gammarids and caprellids, were found in this study and may also function as mobile corridors in South Slough.

Many of the epiphytic animals were found rarely and in low abundances. They probably represent chance events, and not seasonal changes. Similarly, bushy bryozoans, although found in five surveys,

displayed no apparent seasonal pattern. Some of the other factors reviewed earlier, such as the original surrounding ecosystem, or the drifting time, may influence bushy bryozoan abundances.

The source of the *Nereocystis* was thought to be local prior to this study, but these results suggest otherwise. The presence of pelagic barnacles and their size (Goldberg and Zahradnik 1983) indicates that some *Nereocystis* individuals were drifting for several months. The difference in timing of the leafy *Ulva* bloom between local the intertidal population and the epiphytic population also suggests that drifting *Nereocystis* were not always local.

Seasonal Community Composition

Understanding the community compositions of drift algae is an essential first step to understanding how the communities function and how they affect the South Slough. Seasonality may be important in determining the composition of the associated fish community of drifting algae (Cho et al. 2001). Densities of animals colonizing floating seaweed clumps can be highly seasonal, with highest densities in summer and lowest densities in winter (Ingólfsson 2000). Seasonal differences in animal densities appeared to be related to the availability of drifting algae.

The most important members of the summer epiphytic community were *Enteromorpha*-like *Ulva*, *Polyneura latissima*, *Palmaria* sp., balanomorph barnacles, *Cryptopleura lobilifera*, encrusting bryozoans, and *Cryptopleura ruprechtiana* (Table 4a). Over half of the epiphytes found in summer were

Enteromorpha-like *Ulva*. Although *Enteromorpha*-like *Ulva* abundance was low in summer compared to spring, it still had a large impact on the summer community. Over 80% of the individuals in the summer community were algae.

Important members of the autumn epiphytic community were leafy *Ulva*, gammarid amphipods, *Enteromorpha*-like *Ulva*, and encrusting bryozoans (Table 4b). Leafy *Ulva* contributed almost half of the overall epiphyte numbers. Almost 60% of the autumn community consisted of algal species.

The important members of the winter community were *Porphyra nereocystis*, gammarid amphipods, *Pterochondria woodii*, and *Antithamnionella pacifica* (Table 4c). Almost 40% of the winter community consisted of *Porphyra nereocystis* alone, and 70% of the individuals in the community were algae.

The spring group consisted of *Enteromorpha*-like *Ulva*, pelagic barnacles, *Antithamnionella pacifica*, balanomorph barnacles, and gammarid amphipods (Table 4d). *Enteromorpha*-like *Ulva* constituted almost half of the spring community in terms of abundance, and all algae species constituted over 60% of the community.

The most important members of the community in every season in terms of contribution to overall epiphytic abundance were algae. In summer, autumn, and spring, members of the genus *Ulva* were the most important. Algae are dominant members of the epiphytic community, but they also

contribute structural complexity. The only vertebrates found were in a mat of *Pterochondria*, and gammarid amphipod abundances are also probably also related to *Pterochondria*.

The seasonality displayed in both species abundances and community species composition suggests that season is an important factor to colonization dynamics in the Pacific Northwest. Although this study does not document colonization of the Slough, the seasonal changes in the abundances of epiphytic organisms suggest that species likely to colonize will change throughout the year. Simberloff and Wilson's (1968) classic study describing the colonization of islands may not be applicable to zones that experience seasonal changes in abundance of organisms. They found three distinct phases of colonization: initial colonization, when organisms began to arrive and competition was probably infrequent; an intermediate phase, when competition began to be more important, and a final equilibrium, when the community composition remained fairly constant. The study was conducted in the Florida Keys, and the authors state that the climate and the availability of colonizers varied little throughout the year. The final state of equilibrium on islands is unlikely to occur in temperate zones, because the species abundances of potential colonizers change seasonally.

Conclusions

Algae dominated the epiphytic community found on drifting Nereocystis in the South Slough. The most abundant species found was *Pterochondria*

woodii, a red alga. Three red algal taxa and one green algal taxon bloomed in spring and, to a lesser extent, in fall, and two red algal taxa bloomed in summer. The four brown algal species and one green algal species were found only in summer and autumn. In contrast, only one apparent seasonal pattern was found among epiphytic animals, a spring or early summer increase in abundance. Encrusting bryozoans, gammarid amphipods, balanomorph barnacles, pelagic barnacles, and hydroids displayed this pattern. Crustaceans were the most abundant epiphytic animals. The organisms documented "hitchhiking" on *Nereocystis* in this study have the opportunity to colonize South Slough. Other possible roles of the epiphytes in the Slough include: nutrient sources, as prey or as detritus; predators,

The drifting *Nereocystis* may not be from local populations, because large pelagic barnacles were found and because leafy *Ulva* peak abundances did not occur at the same time as local leafy *Ulva* abundances from the literature. The taxa likely to be found drifting on *Nereocystis* in the South Slough, and when they are likely to be found, have been documented. *Nereocystis* and its epiphytes consistently drift into South Slough, and possibly other estuaries along the Pacific Coast, and very little is known about how they affect estuaries. This study is the first step of examining the possible ecological effects of epiphytized drifting *Nereocystis* in the Slough.

Bridge 2

Chapter 3 describes the epiphytic community of drifting *Nereocystis luetkeana* in the South Slough. The most abundant taxa were red algae, green algae, and crustaceans. Within the crustaceans, pelagic barnacles were the most abundant taxa. The epiphytes on *Nereocystis* can only colonize the parts of the Slough where they are carried, and may be more likely to colonize areas where *Nereocystis* individuals remain. Chapter 4 documents the distribution of *Nereocystis* in the Slough. This information is used to suggest that *Nereocystis* may have a bigger ecological impact in some areas than others. Many factors contribute to the number of *Nereocystis* found in the Slough. In Chapter 4, the hypothesis that wind speed of the day before a survey was correlated with the number of drifters was tested.

CHAPTER 4—PATTERNS OF DISTRIBUTION OF DRIFTING *NEREOCYSTIS LUETKEANA* IN SOUTH SLOUGH

Introduction

Kelps have evolved many morphological adaptations to living in wave-swept environments. Water movement caused by tidal currents and wind creates a force that breaks stipes, holdfasts, and substrates (Koehl and Alberte 1988). Many organisms in the rocky intertidal reduce the effects of wave forces by having flattened or small bodies, but algae with buoyant floats, like *Nereocystis*, cannot (Koehl and Wainwright 1977). This species has adapted other ways to decrease likelihood of wave-induced mortality.

Variable blade morphology is one adaptation to reduce the negative effects of water forces. *Nereocystis* blades are more narrow and flat at wave exposed sites than those at more protected sites (Koehl and Alberte 1988). Narrow flat blades experience less drag than wider, ruffled blades. At protected sites, however, low flows can inhibit nutrient availability. Wide, ruffled blades increase water movement and so are also adaptive at protected sites.

Nereocystis individuals are very flexible and stretch to avoid breakage (Koehl and Wainwright 1977), but this strategy is only successful once the individuals reach the surface (Denny et al. 1997). Young individuals, particularly those 2 m long, are more vulnerable to breakage from waves than older, longer individuals because they are more likely to experience chaotic, rather than periodic, wave forces, and because they have more mass and surface area per unit length. Other factors, such as current speed, also affect the stress that individuals experience.

Duggins et al. (2001) found that intermediate current speeds were associated with the greatest mortality in *Nereocystis* beds. At protected sites, grazing by *Lacuna vincta* causes extensive damage of *Nereocystis*, but wave action is never strong enough to remove most of the kelp. At exposed sites, grazing is reduced due to wave action, and most *Nereocystis* individuals are able to remain attached. At intermediate sites, wave exposure varies. When wave forces are low, *Lacuna* grazes and weakens *Nereocystis*. When wave forces are high, the damaged *Nereocystis* individuals break.

Models may not accurately predict the likelihood of detachment because they assume the holdfast attachment will withstand more force than the stipe. With a strong holdfast attachment, *Nereocystis* individuals are most likely to fail under wave forces at the thinnest part of the stipe, right above the holdfast (Denny et al. 1997). Logically, therefore, most drifting kelp should have no holdfast (Bushing 1994).

Holdfast failure, however, could occur at the substrate, particularly if the substrate is loose (i.e. cobble or sand) or easily broken (i.e. sandstone or shale). *Nereocystis* is often found in high-energy environments with unstable substata (Maxell and Miller 1996) and probably often detach with intact holdfasts. Koehl and Wainwright (1977) found 55% of drift *Nereocystis* in the San Juan Islands had no holdfast. Conversely, Duggins et al. (2001) found that holdfast failure never accounted for more than 35% of mortality in San Juan Archipelago kelp beds.

Another factor that could affect the number of drift *Nereocystis* with intact holdfasts is season. Holdfast failure may increase in winter when holdfasts degrade (Markham 1969), although an increase would not occur among healthy individuals.

Drift algae accumulations and distance traveled can be affected by wind (Berglund et al. 2003). The distance that detached kelp can travel varies with wind and current speeds and directions. Drifting *Macrocystis* appears to be more influenced by wind than by surface currents, although surface currents become more important when winds subside (Harrold and Lisin 1989). Drift algae can be correlated to storm events (Berglund et al. 2003).

Many factors probably affect the number of drifting *Nereocystis* in South Slough, and those factors likely interact. Wind speed, water motion, including swell, currents, and tides, and the *Nereocystis* population size almost certainly all influence the number of drifting *Nereocystis*.

This study will test the hypothesis that wind speed alone, of the day before surveys, affects the number of drifters in the South Slough. Distributions of *Nereocystis* in the Slough will also be mapped, and the prevalence of intact holdfasts will be described.

Materials and Methods

Boat surveys of drifting *Nereocystis* were conducted from July 2004 to July 2005 in the South Slough from Charleston Bridge to Long Island Point (Figure 7, pg. 37). Two surveys were conducted in July 2004, and none completed in December 2004 and June 2005 due to poor tides and scheduling conflicts, but otherwise, surveys were completed monthly. Counts were taken from Long Island Point to Charleston Bridge. Although no individuals were seen further up the Slough when a survey was done by kayak, monthly surveys were not conducted in the Slough south of Long Island Point. One clump of bull kelp was observed by Hinch Road Bridge (43°16.885'N, 124°19.117'W) in October (J. Trainer, pers. comm.). Surveys were never done on foggy days or at night so the likelihood of seeing floating kelp was similar for all surveys.

GPS coordinates of each *Nereocystis* individual found were recorded. Individuals were then tattooed by Nicholson's method (1970). A metal needle was dipped into permanent black ink, and tattoos were drawn with dots. Tattoos consisted of consecutive numbers. Individuals in July 2004 were

tagged with forestry tape instead of tattooed. Forestry tape tagging seemed to be less effective because the tags could fall off and could increase drag. Several individuals were tattooed and kept in running sea water to ensure that tattoos would remain visible and useful between surveys.

Distributions of *Nereocystis* individuals were mapped with Maptech 1.0 digital mapping software (Greenland, NH). Pearson correlations were performed with Statistica 7.1 (StatSoft, Inc, Tulsa, OK).

Average wind speed and maximum wind speed data for the day prior to surveys were collected from the Centralized Data Management Office of the National Estuarine Research Reserve System and from the South Slough National Estuarine Research Reserve (SSNERR). Drifting *Nereocystis* were assumed to be local, and so wind speed the day before surveys was thought to be possibly correlated with the number of drifters. The SSNERR weather station is located in Charleston on the campus of the Oregon Institute of Marine Biology (43°20.703' N, 124°19.724' W). Wind speeds were collected every five seconds. The average wind speed was the average of those data over twenty four hours and the maximum wind speed was the highest wind speed recorded over twenty four hours. (National Oceanic and Atmospheric Administration, Office of Ocean and Coastal Resource Management, National Estuarine Research Reserve System-wide Monitoring Program. 2004. Centralized Data Management Office, Baruch Marine Field Lab, University of South Carolina http://cdmo.baruch.sc.edu.)

Results

No *Nereocystis* individuals were re-surveyed; individuals were tagged or tattooed, but they were never found in subsequent surveys. Tattoos remained on *Nereocystis* in the lab over three weeks, until the kelps degraded, so the fact that they were not re-surveyed indicates that retention time of *Nereocystis* individuals is less than one month.

The distribution of *Nereocystis* in the South Slough was concentrated around Long Island Point (Figures 12-16), and the only *Nereocystis* observed attached were also there. The *Nereocystis* individuals found from Long Island Point to Charleston Bridge were generally in the middle of the channel. One drifter was observed in the Winchester arm of the southern part of the Slough (J. Trainer, pers. comm., Figure 16).

A Pearson correlation between the number of *Nereocystis* found in the Slough and the average wind speed of the day before the survey was not significant (Table 5 and Figure 17). A Pearson correlation between the number of *Nereocystis* found in the Slough and the maximum wind speed recorded the day before the survey was also not significant (Table 5 and Figure 18).

Seventy three percent of holdfasts were intact throughout the year, and it varied little seasonally (Table 6).

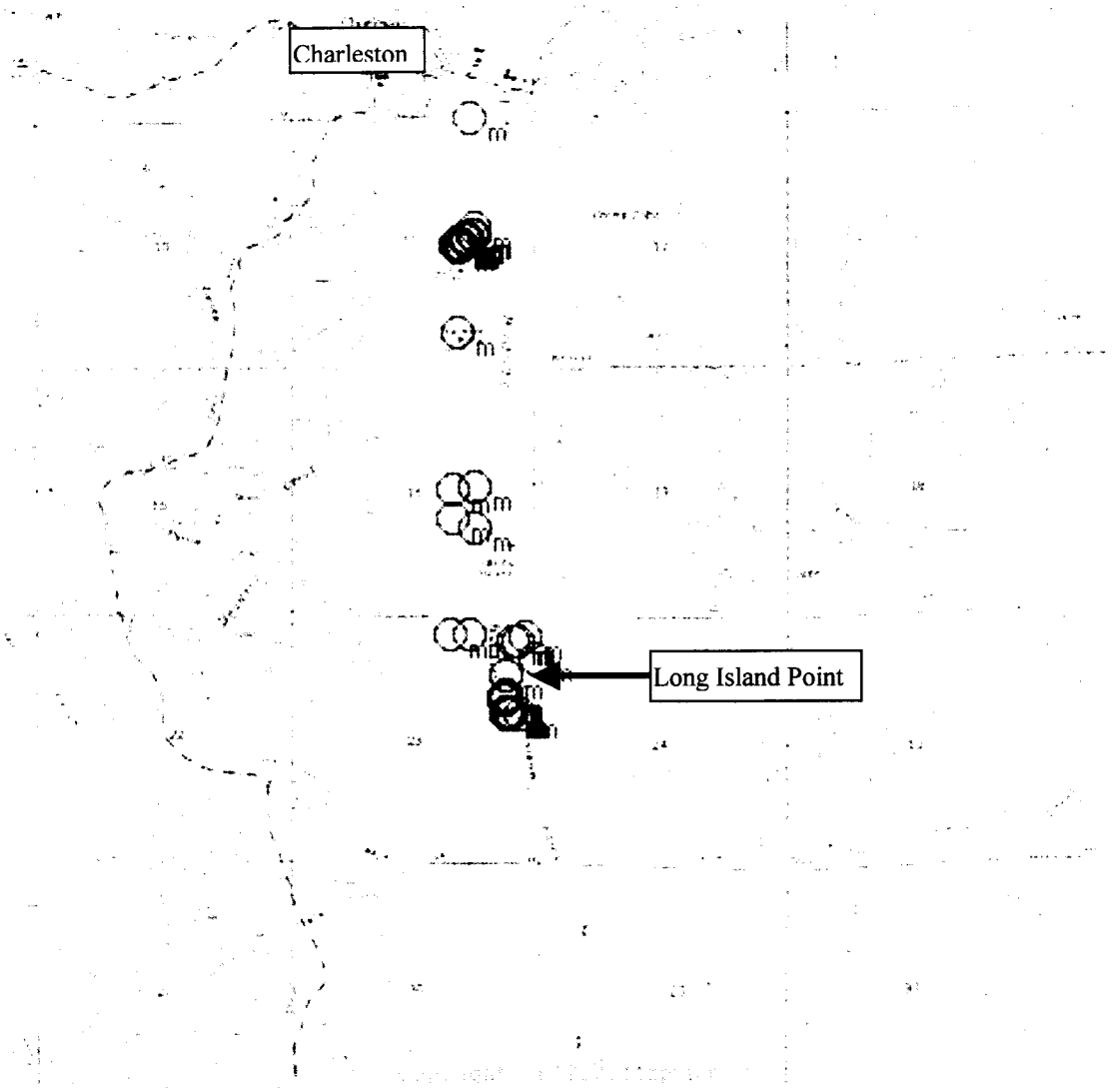


Figure 12. Map of summer distribution of drifting *Nereocystis* individuals. Each drifting *Nereocystis* is represented by a circle.

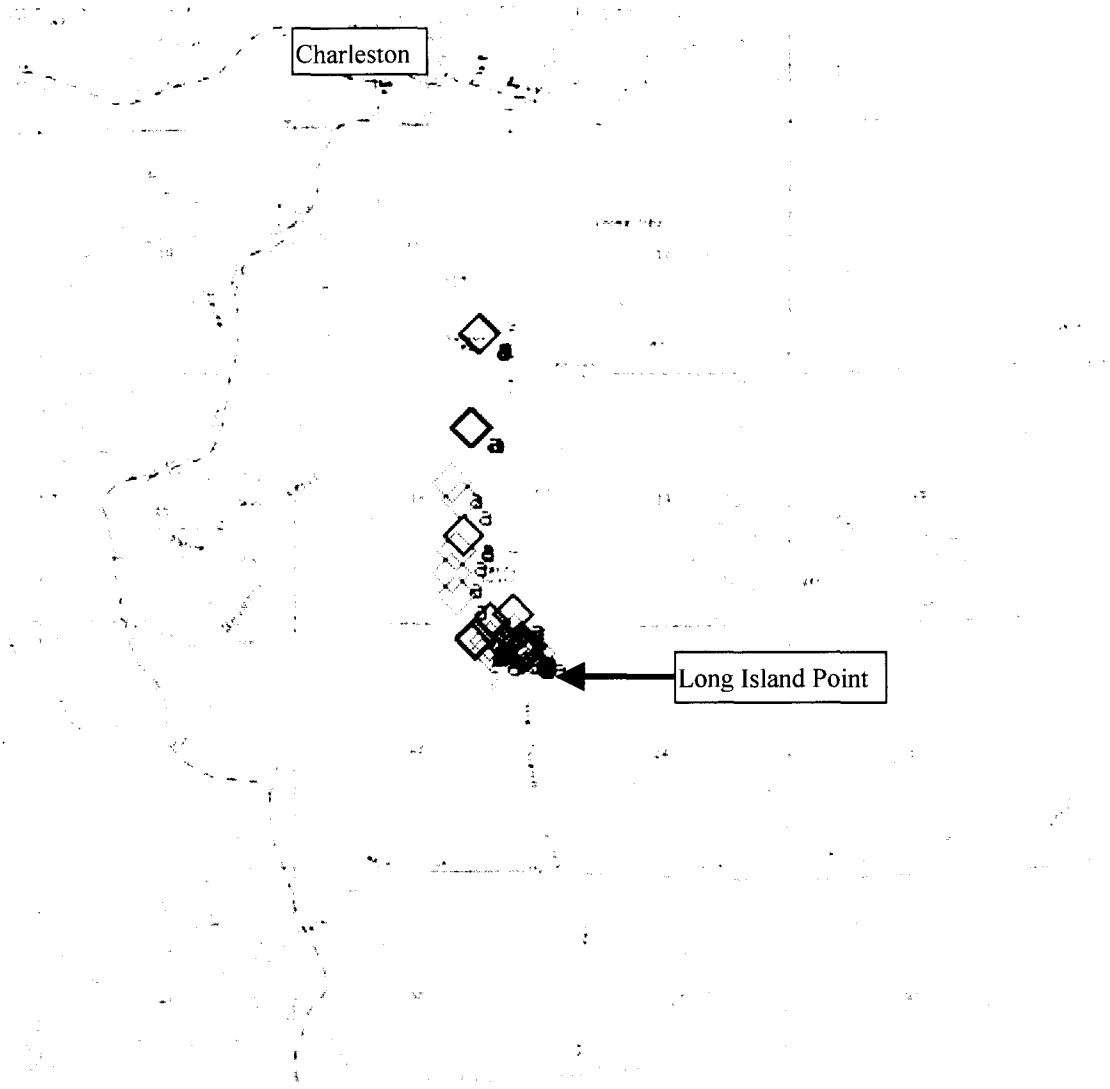


Figure 13. Map of autumn distribution of drifting and attached *Nereocystis* individuals. Each drifting *Nereocystis* is represented by a diamond.

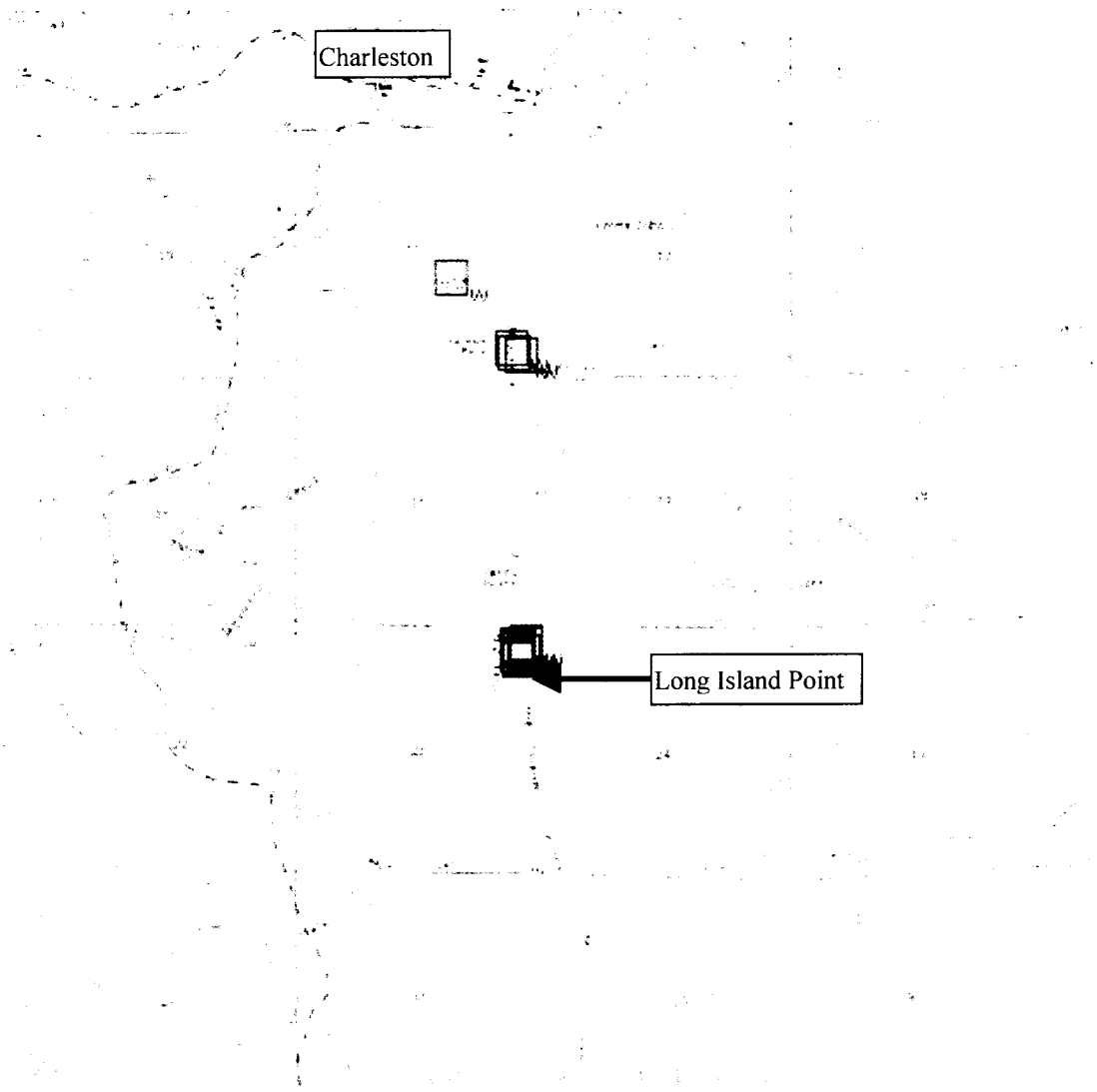


Figure 14. Map of winter distribution of drifting *Nereocystis* individuals. Each drifting *Nereocystis* is represented by a square.

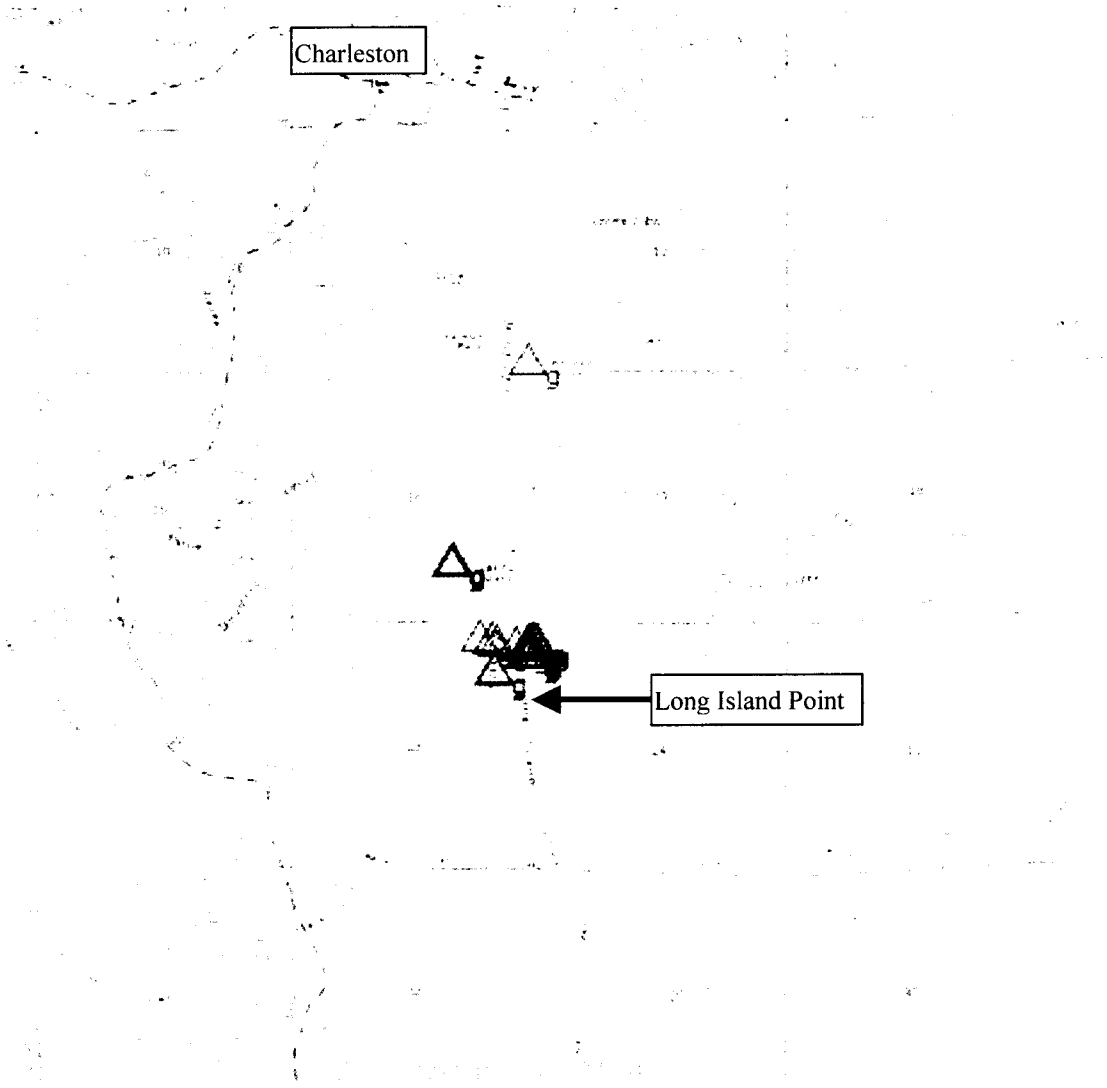


Figure 15. Map of spring distribution of drifting *Nereocystis* individuals. Each drifting *Nereocystis* is represented by a triangle.

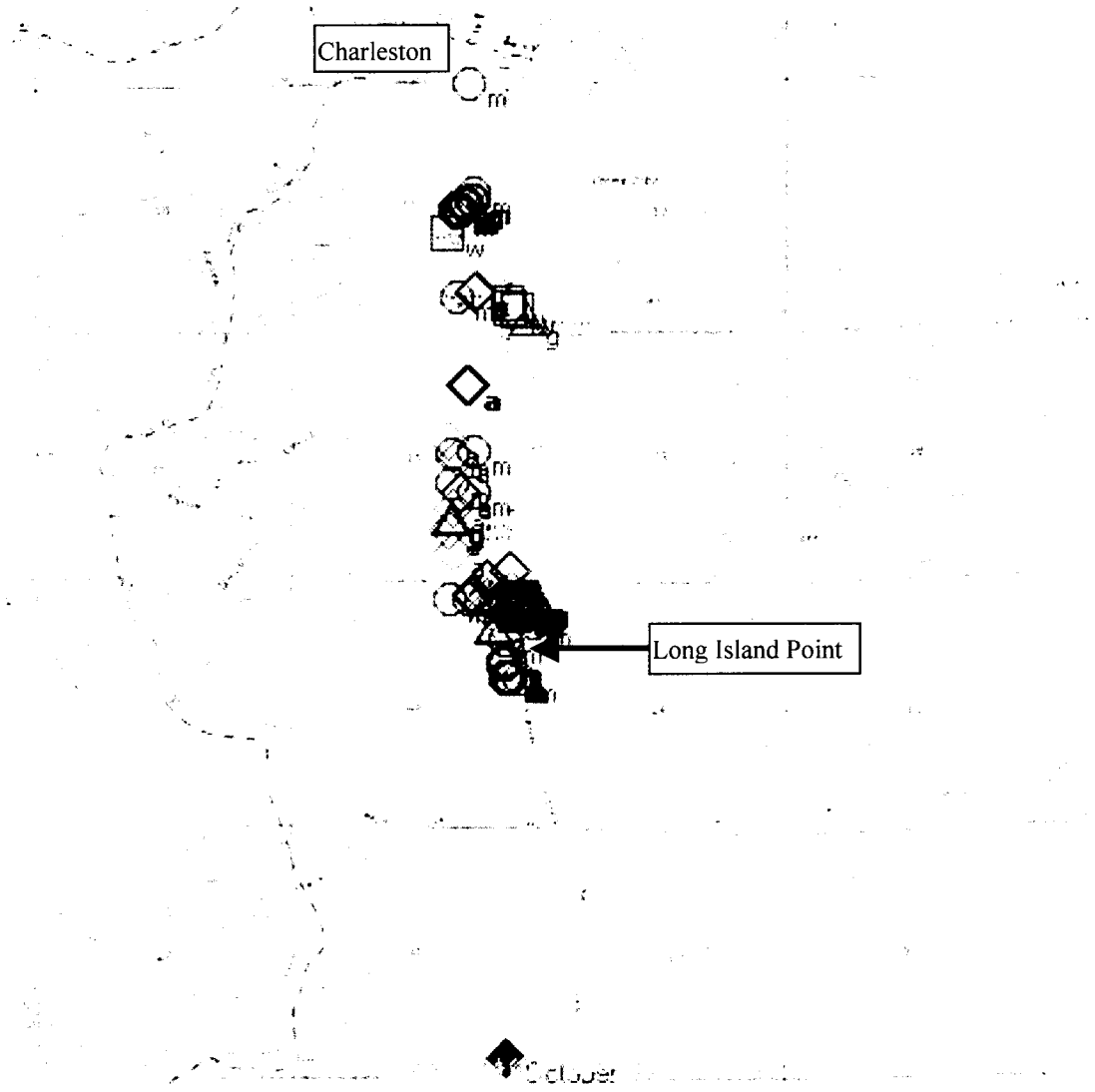


Figure 16. Map of total distribution of drifting and attached *Nereocystis* individuals. =summer individual, =autumn drifting individual, ◇=autumn attached individual, □=winter individual, =spring individual ◆=individual noted by J. Trainer in October.

Table 5. Pearson correlation. Correlations between numbers of *Nereocystis* and the average and maximum wind (Wind max) speed of the day before sampling. Correlations were not significant ($p>0.05$).
r values

	Number of *Nereocystis*	Wind average	Wind max
Number of *Nereocystis*	1.00	-0.07	0.09
Wind average	-0.07	1.00	0.34
Wind max	0.09	0.34	1.00

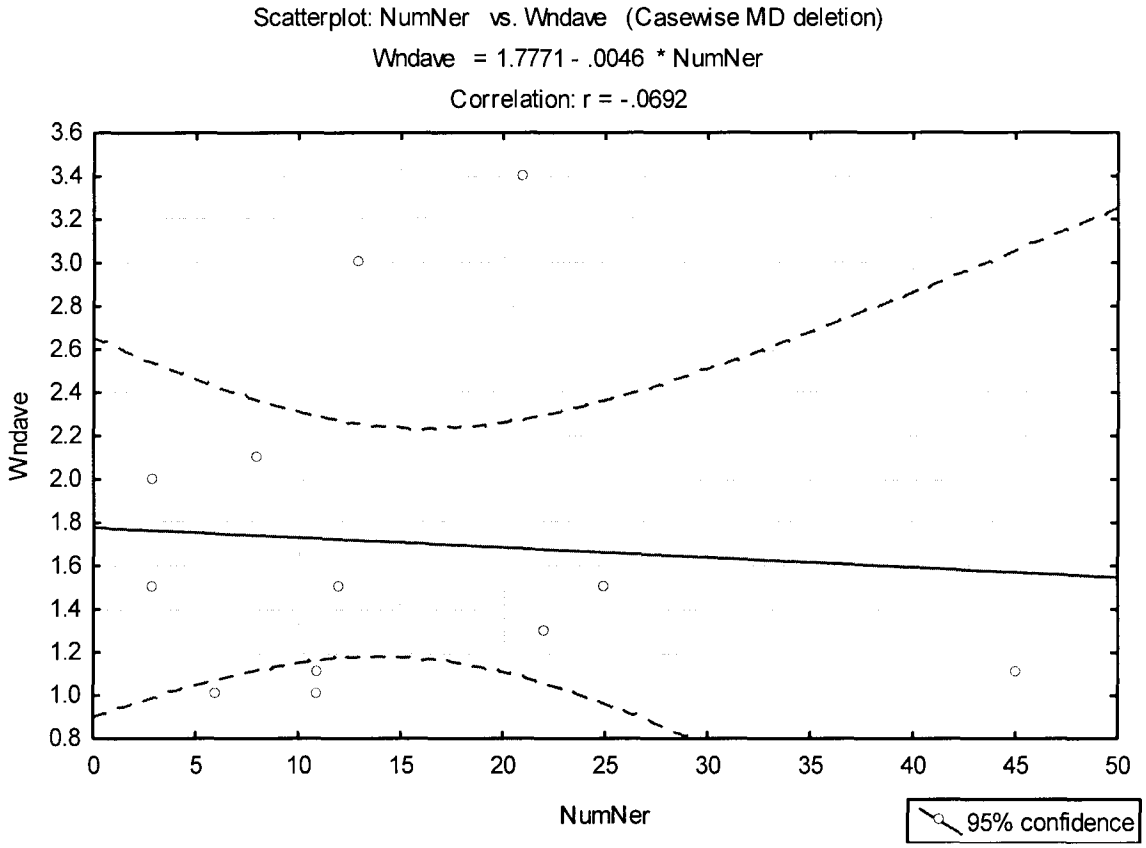


Figure 17. Pearson correlation between average wind speed (Wndave) the day before sampling and the number of *Nereocystis* (NumNer) found.

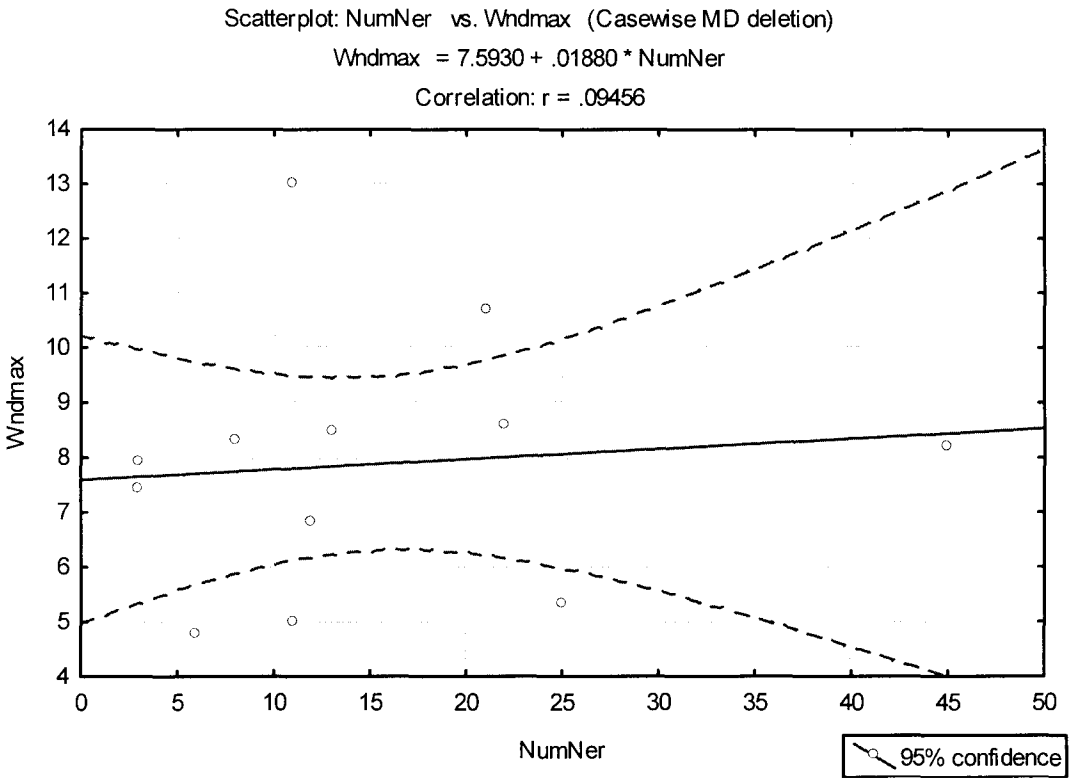


Figure 18. Pearson correlation between maximum wind speed (Wndmax) the day before sampling and the number of *Nereocystis* (NumNer) found.

Table 6. Frequency table of percentages of intact (I) and not intact (N) holdfasts for each season and the year.

Summer

	Count	Cumulative	Percent	Cumulative
Intact	39	39	72.2	72.2
Not intact	15	54	27.8	100.0

Autumn

	Count	Cumulative	Percent	Cumulative
Intact	57	57	74.0	74.0
Not intact	20	77	26.0	100.0

Winter

	Count	Cumulative	Percent	Cumulative
Intact	12	12	70.6	70.6
Not intact	5	17	29.4	100.0

Spring

	Count	Cumulative	Percent	Cumulative
Intact	19	19	73.1	73.1
Not intact	7	26	26.9	100.0

Total

	Count	Cumulative	Percent	Cumulative
Intact	127	127	73.0	73.0
Not intact	47	147	27.0	100.0

Discussion

Nereocystis individuals drift into South Slough and some attach, consistently forming a bed at Long Island Point (Rumrill 2003). *Nereocystis* also concentrated around Long Island Point in this study. Hydrodynamic forces likely cause collection of drifting algae there, although testing that hypothesis is beyond the scope of this study due to the complexity of circulation within the Slough. The surprising result of this study is that wind speed does not seem to affect the number of drifting *Nereocystis* found. Wind creates the force that detaches *Nereocystis* (Koehl and Alberte 1988), and a positive correlation was expected. Percent of intact holdfasts (70.6 to 74%) was higher than in other studies (Koehl and Wainwright 1977, Duggins et al. 2001) and indicates that substrate failure is more common than stipe failure, at least among the individuals that drifted into the South Slough.

Nereocystis individuals appeared to remain in the South Slough Estuary for less than one month, as no individuals were observed in more than one monthly survey. The method of marking them was likely effective because tattoos in the lab were visible on *Nereocystis* until the experiment was ended due to degradation of the kelp. The estimated flushing time of the Slough, three days (Rumrill 2003), is consistent with the short retention time of *Nereocystis* individuals. The process of sampling *Nereocystis* did detach individuals that had reattached. They might have remained attached if they

had not been removed, and the retention time of attached individuals may be longer than of drifting individuals.

The drifting and attached *Nereocystis* in South Slough concentrate around Long Island Point. A bed of *Nereocystis* has consistently been observed there (Rumrill 2003). At Long Island Point the flooding tide splits into two water masses (Rumrill 2003). One flows to the western Winchester arm and the other to the eastern Sengstacken arm. Hydrology, therefore, may cause drifting objects, including *Nereocystis*, to remain at Long Island Point. The one *Nereocystis* observed in southern part of the Slough, in the Winchester arm, is probably an anomaly, but does show that *Nereocystis* can drift there.

The lack of a significant correlation between wind speed and the number of drifting *Nereocystis* found is surprising. High wind speeds were expected to cause high numbers of drifters, and this relationship has been documented with other algae (Berglund et al. 2003). Possibly wind does affect the number of drifters, but the range of wind speeds in this study were not sufficient to detect the effect. The absence of a detectable relationship between local wind speed and the number of drifters could also reflect reality. The majority of drifters were thought to be from local *Nereocystis* beds, and so local wind speeds the day before the survey were thought to be correlated to

the number of drifters. The presence of pelagic barnacles (see Chapter 3), however, indicates that the drifters are not always local, and so the wind speed at other locations could be more closely correlated with the number of drifting *Nereocystis* in South Slough. Many factors probably affect the number of drifters, and those factors could also interact. For example, wind speed, wind direction, wave swell, tidal height, and number of attached *Nereocystis* probably all affect the number of drifters. A more rigorous testing of the hypothesis that wind speed is related to the number of drifters, such as time series analysis, would be necessary to conclude that wind has no effect. The lack of a significant correlation, however, is surprising because it suggests that factors other than wind speed are more important in determining the number of drifting *Nereocystis* in the South Slough. Or, it implies the drifters are not local. The presence of pelagic barnacles on *Nereocystis* indicates that *Nereocystis* can drift for months before entering the Slough.

Conclusions

Retention time of *Nereocystis* in South Slough appears to be less than one month. *Nereocystis* individuals concentrate around Long Island Point, and they only attach at that location. Both daily average and maximum wind speed of the day before a survey was not significantly correlated to the number of *Nereocystis* found. Many factors not quantified in this study, such as the number of available *Nereocystis*, or wind speeds in other areas likely affect the number of drifting *Nereocystis* found in South Slough.

CHAPTER 5—CONCLUDING SUMMARY

The aspects of *Nereocystis luetkeana* described in this thesis include part of its life cycle and life history strategy, and its epiphytic community and distribution in the South Slough National Estuarine Research Reserve.

Chapter 2 documented a bet hedging strategy in *Nereocystis* that also removes one of its microscopic stages from the low-light, low-nutrient conditions along the bottom to the high-light, high-nutrient surface. The finding is also potentially applicable to the mariculture of *Nereocystis*.

The epiphytic community and its seasonal changes are described in Chapter 3. Red and green algae were the most abundant taxa and bloomed in spring. Crustaceans were the most abundant epiphytic animal taxa, and pelagic barnacles were the most abundant taxa within the group. The presence of pelagic barnacles also indicated that not all of the drifting *Nereocystis* individuals were from local populations.

Chapter 4 described the distribution of *Nereocystis* in South Slough and showed that wind speed of the day before a survey was not significantly correlated with the number of drifters found. The retention time of *Nereocystis*

was estimated at less than 1 month because no individuals from a previous survey were ever found. They could, however, have drifted to another part of the Slough or degraded in that time.

APPENDIX

FORMULAS FOR SPORE MEDIA AND SOLUTIONS 1, 2, AND 3 FROM CHAPTER 1

Table 7. Spore media formula.

Component	Amount
$NaNO_3$	20.0 mg
Na_2HPO_4	2.3
$ZnSO_4$	1.0
$MnCl_2$, $CuSO_4$, $CoCl_2$, $NaMoO_4$	1.0
EDTA-$FeSO_4$	0.006
sterile filtered sea water	100 ml

Table 8. Formulas for solutions used for protoplast generation

Component	Solution 1	Solution 2	Solution 3
NaCl	20.454 g	0.430 g	21.477 g
$MgCl_26H_20$	3.050	0.061	3.050
$MgSO_47H_2O$	3.697	0.111	5.546
KCl	0.7455	0.011	0.560
$CaCl_2$		0.001	0.056
MES		0.039	1.952
2-(N-Morpholino)ethanesulfonic acid			
EGTA	3.805		
Ethylene glycolbis			
Cellulase from *Trichoderma reesei* (Sigma)[a]		0.2	
Alginate lyase from *Flavobacterium* sp. (Sigma)[a]		0.2	
pH	5.5	6.5	6.5
Distilled water	500 ml	10 ml	500 ml

[a]The original enzymes (Matsumura 1998) were cellulase onozuka RS (Yakult) and abalone acetone power (Sigma). Cellulase is now available from Sigma, and so it was used, and abalone acetone powder is no longer available, and so alginate lyase was substituted.

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