The effect of Spatial distribution and wave action on the proportion of tetrasporophytes and gametophytes of Mazzaella splendens in the San Juan Islands.

Connor Trentman

University of Washington, Friday Harbor Labs

05/26/2022

Acknowledgments

Partially funded by the Mary Gates endowment.

Abstract

The proportion of life history phases is an important factor for assessing population dynamics. This paper describes the proportion of tetrasporophyte to gametophyte blades of the isomorphic Rhodophyte (red seaweed) *Mazzella splendens* in the San Juan Islands, WA. Focusing on potential effects of spatial distribution, wave action, and a comparison to the outer coast. the proportion of tetrasporophytes to gametophytes was similar at all nine locations. No difference was observed when comparing this study to similar studies on the outer coast of Washington and British Colombia. No difference was observed at the two sites selected for comparing the effects of wave action. The findings of this study are consistent with previous studies on the proportion of life history phases in Mazzella splendens.

Introduction

The Gametophyte and tetrasporophyte are the two independent phases of the triphasic life cycle observed in most red algae (Rhodophyta). The third phase not considered in this study is the carposporophyte. This phase is microscopic and grows in the blade of the female gametophyte. The proportion of isomorphic tetrasporophytes to gametophytes in *Mazzaella* (previously called *Iridaea)* is known to change depending on species, abiotic factors, and season (Scrosati and DeWreede 1999).

Annual blades of *M. splendens* arise from a perennial cushion hold fast. The blades that arise are seasonal and fall off from an abscission layer (Dyck LJ and DeWreede 2006). The perennial holdfast give rise to a set life history phase. The cushion hold fast of a tetrasporophyte

will grow a tetrasporophyte Blade. Observations made by this study in the spring neglect the cushion hold fast, may not reflect the true ratio of the cushion substrate. The G:T ratio reported in this paper reflects the true G:T ratio of only the blades in May.

The proportion of tetrasporophyte and gametophyte phases (G:T) has been studied in *Mazzella splendens* (Dyck and DeWreede 1985; May 1986; Phillips 1994; Dyck and DeWreede 1995), *M. Parksii*, (Hughey and Silva 2001), and *M. oregona* (Mudge and Scrosati 2003). These studies concerning *Mazzella splendens* were focused on the outer west coast of north America, particularly on the Canadian coast.

Several factors determine the G:T ratio in natural population of *Mazzella* (Scrosati and DeWreede 1999). However, there is no species for which all the factors have been determined. A few factors that have been explored were wave action. For *M. parksii*, a species found in the high intertidal on very exposed sites, wave action does not determine the G:T ratio (Scrosati 1998). Wave action has been observed to determine the G:T ratio for *Mazzella splendens* on the outer coast (Dyck and DeWreede 1985; Phillips 1994).

The G:T ratio for *M. splendens* has not been studied in the San Juan Islands. In general, the San Juan Islands do not receive as much wave action as the outer coast. The variation in wave action within the islands may not be sufficient to cause a significant difference in the G:T ratio. However, the differences in other abiotic factors between the outer coast and the San Juan Islands could result in a different G:T. I set out to describe the G:T ratio of *Mazzella splendens* in the San Juan Islands and identify differences in the proportion between the San Juan Islands and the outer west coast of the United States.

Methods

30 blades of *M. splendens* were haphazardly collected from 9 different beaches: Bowman Bay (BB), Cattle Point exposed (CPE), Cattle Point protected (CPP), Deception Pass (DP), Eagle Cove (EC), Friday Harbor Labs (FH), Lime Kiln (LK), Reuben Tarte County Park (RT), San Juan County Park (SJC) (Figure 1). The samples were stored in ambient temperature flowing sea water for no more than 5 days at FHL. Cattle Point was divided into two sites: exposed and protected (Figure 2). The exposed Cattle Point receives more wave action than protected the

Cattle Point. Cattle point is protected from wave action and the prevailing winds (Figure 3) by nearby land masses.

Resorcinol stock solution was prepared by dissolving 150mg resorcinol in 100ml RO (reverse osmosis) water, 9ml of which was then added to 100ml concentrated HCl. Resorcinol stock solution keeps for one week. Acetal stock solution was prepared by adding 0.1ml acetal to 10ml RO water, 1 ml of which was then added to 25ml RO water. Acetal stock solution keeps for three weeks. Resorcinol-acetal was made by diluting 1ml of Acetal stock solution to resorcinol stock solution. Resorcinol Acetal is made fresh daily. (Garbary and DeWreede 1988).

Sample disks are prepped for resorcinol-acetal test by using a 10mm cork bore to punch a hole in the blade, The blade is numbered and retained for later observations. One disk is placed into a numbered 10ml test tube. 2.0 ml of resorcinol-acetal was added to the test tube, and the tube placed into an 80°C-90°C hot water bath for 60 seconds. Any color change is recorded. A color change to dark red or pink indicates the presence of kappa-carrageenan, found only in the blade of haploid gametophytic tissue (Garbary and DeWreede 1988).

The blade was qualitatively observed for pigmentation, elasticity, strength, thickness, and texture. The observations are compared to the corresponding test disk and gametophyte or tetrasporophyte identification.

Data analysis and visualization is done by coding a 2-sample test for equality of proportions with continuity correction, a logistic regression, and a binomial test in R studio.

Results

I found that there is no significant difference in the proportion of tetrasporophytes to gametophytes between any of the locations sampled. Significantly more gametophytic blades were detected than tetrasporophytes at all sites, excluding CPP and CPE. (Figure 4, Table 1). The proportion of tetrasporophytes to gametophytes of all the samples taken was calculated to be 0.253 tetrasporophytes. The proportion is significant ($p<<0.05$) and has a 95% confidence interval of 0.202 to 0.309 (Table 2).

I found that there a marginally significant difference (p=0.06) in the proportion of tetrasporophytes to gametophytes between the exposed and protected Cattle Point locations (Table 3). These two locations had the largest measured difference in the G:T ratio.

I qualitatively observed the tetrasporophyte blades to be thicker and more elastic than the gametophyte phase. And had some dark pigmentation on the outer edge of the blade. The gametophyte blades were thinner and less elastic than the tetrasporophyte blades. Some of the gametophytes were observed be cystocarp (raised dark bumps) (Figure 5).

Discussion

The difference in the proportion of tetrasporophytes to gametophytes between protected and exposed Cattle Point was the largest measured of all the sampled sites. The difference in proportion was marginally significant (p=0.06 and conforms to previously published findings (Mudge and Scrosati 2003; Scrosati and Mudge 2004). The difference in wave action at the two sites may be sufficient to cause a significant difference in the proportion of tetrasporophytes.

The proportion of gametophytes to tetrasporophytes did not differ among the sites sampled. This finding suggests that variation in wave exposure, salinity, sea surface temperature, and nutrient availability between the outer coast and the San Juan Islands did not play a significant role in the proportions observed. A higher proportion of gametophytes in a similar species to *M. splendens* has previously been observed to be proportional with an increase in height in the intertidal (Scrosati and Mudge 2004). Height in the intertidal was not a variable that was assessed in this study.

I did not find a significant (p=0.12) difference when comparing our results to similar studies conducted on the outer coast (Table 4). The similar proportion of tetrasporophytes observed in this and other studies in late spring suggest that two main determining factors for the proportion in *M. splendens* are seasonality (Dyck and DeWreede 1995) and wave action (Mudge and Scrosati 2003; Phillips B 1994; Scrosati and Mudge 2004).

The presence of K-Carrageenan in the tetrasporophytes resulted in some doubtful results. A Resorcinol-Acetal test is used to detect the presence of K-Carrageenan in the cells of the blade. K-Carrageenan is produced exclusively in the gametophyte blade (Waaland 1975), resulting in clear results. Resorcinol-Acetal test for *M. splendens* were not as clear as desired due

to the tetrasporophytes containing low amounts of K-Carrageenan in the haploid tetraspores (Waaland 1975).

The proportion of tetrasporophytes was measured over a period of one week in May. The tetrasporophyte blades were qualitatively observed to be thicker and more elastic than the gametophyte phase. The elasticity and thickness of the tetrasporophyte phase of *M. splendens* could allow for a greater capacity to survive increased wave action and water velocity (Shaughnessy and DeWreede 1996). A difference in capacity for desiccation between tetraphosphates and gametophytes could also be a possible driving factor in the G:T ratio, however there is a lack of studies on the topic. A difference in survivability in different conditions of the two life history phases could make the tetrasporophyte phase more viable for overwintering. The proportion of tetrasporophytes has been observed to be lowest in spring and summer, and higher during the fall and winter (Dyck and DeWreede 2006).

The results of this study are consistent with the finding of previous studies on *M. splendens*. No significant difference was found between any of the sites with the exception of Cattle Point (Dyck and DeWreede 1995). A marginally significant difference in proportion of tetrasporophytes was detected at Cattle Point, the site selected to look at the effect of wave action. This finding is constant with previous studies on the effect of wave action on the life history phases of *M. splendens* (Mudge and Scrosati 2003; Scrosati and Mudge 2004).

Figures and Tables

Table 1: Logistics regression of data from all nine sites

```
Coefficients:
                            Estimate Std. Error z value Pr(>|z|)
(Intercept)                -1.23676    0.37864  -3.266  0.00109 **
siteCattle_Point_exposed    0.69022    0.53564   1.289  0.19754
siteCattle_Point_protected -1.06582    0.71406  -1.493  0.13554
siteDeception_Pass         -0.04844    0.49150  -0.099  0.92150
siteEagle_Cove              0.22516    0.56020   0.402  0.68773
siteFHL_beach              -0.55500    0.61376  -0.904  0.36586
siteLime_Kiln_State_park    0.38946    0.61763   0.631  0.52831
siteRuben_Tart_County_park -0.49046    0.53907  -0.910  0.36291
siteSan_Juan_County_park   -0.61954    0.61206  -1.012  0.31144
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Table 2: Exact binomial test of all data, showing a significant for the true proportion of tetrasporophytes to gametophytes, and a 95% confidence interval.

```
data:  68 and 269
number of successes = 68, number of trials = 269, p-value < 2.2e-16
alternative hypothesis: true probability of success is not equal to 0.5
95 percent confidence interval:
 0.2019741 0.3091390
sample estimates:
probability of success
             0.2527881
```

Table 3: 2-Sample test for equality of proportions with continuity correction comparing the tetrasporophyte to gametophyte proportion between Cattle Point protected and Cattle Point exposed.

```
data:  c(19, 29) out of c(28, 32)
X-squared = 3.5198, df = 1, p-value = 0.06064
alternative hypothesis: two.sided
95 percent confidence interval:
 -0.46146819  0.00611105
sample estimates:
   prop 1    prop 2
0.6785714 0.9062500
```

Table 4: Test for equality of proportion between collected data (201:296) and proportion reported in a similar study (83:100) (Dyck and DeWreede 1995).

```
        2-sample test for equality of proportions with continuity correction

data:  c(83, 201) out of c(100, 269)
X-squared = 2.3706, df = 1, p-value = 0.1236
alternative hypothesis: two.sided
95 percent confidence interval:
 -0.01416887  0.17974508
sample estimates:
   prop 1    prop 2
0.8300000 0.7472119
```

Figure 1: Map of study area

Figure 2: Cattle Point sample sites

Figure 5: Wind rose, based on May data from the Friday Harbor Labs weather station

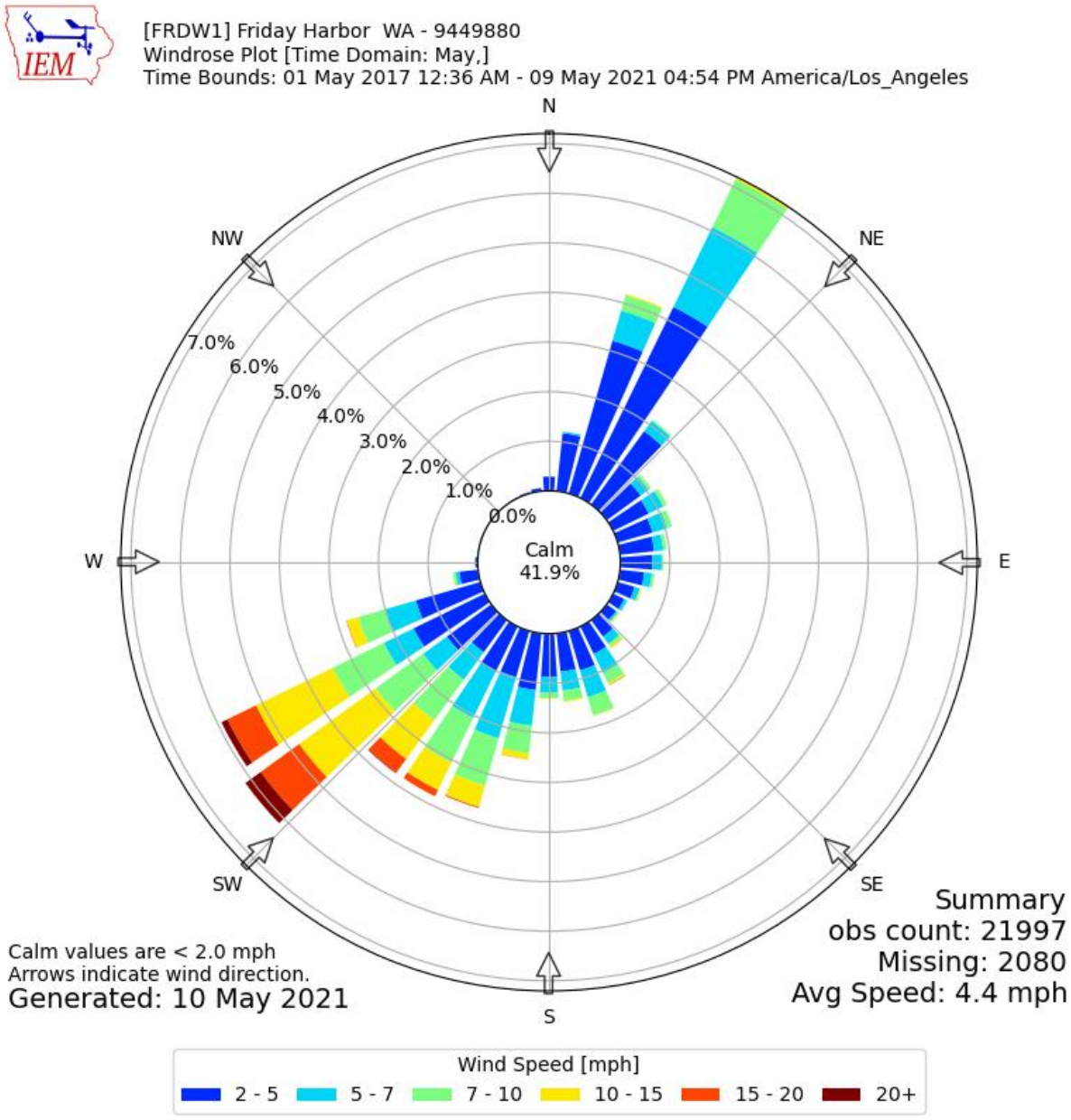

Figure 4: Plot of proportion of gametophytes (# gametophytes/total sample #) at each location visited.

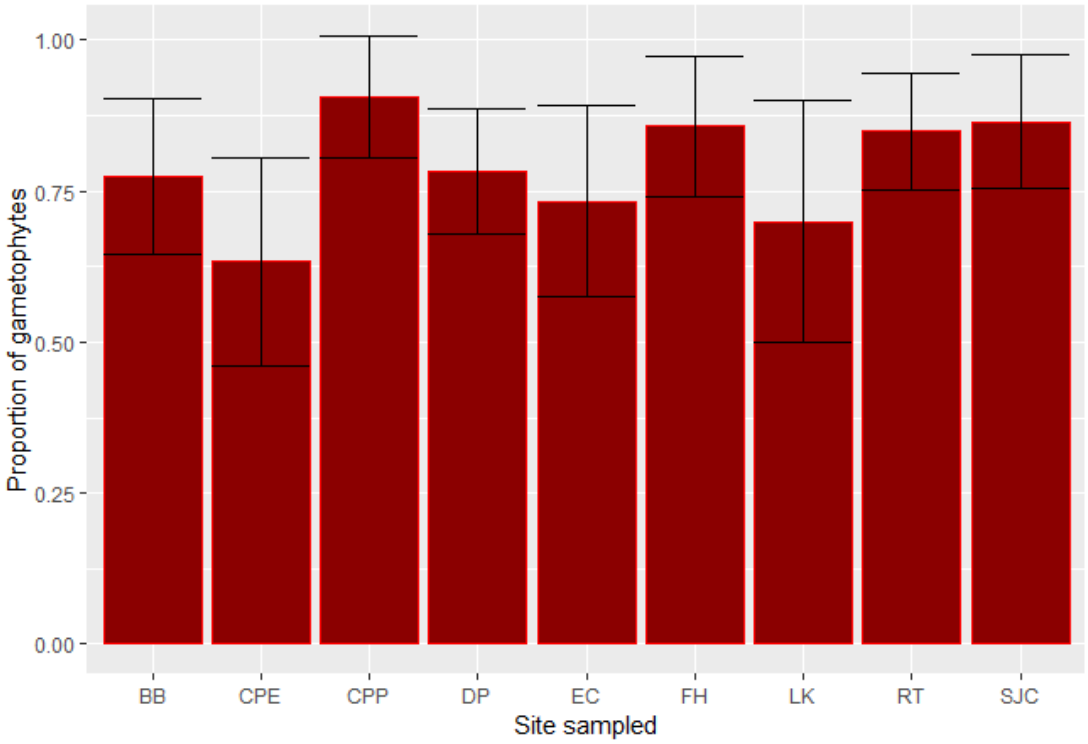

Figure 5: mature gametophytic blade of *Mazzella splendens*

References

Dyck, LJ, DeWreede, R.E. & Garbary, D., 1985. Life history phases in Iridaea cordata (Gigartinaceae): relative abundance and distribution from British Columbia to California. Japanese Journal of Phycology, 33, 225^232

Dyck LJ, De Wreede RE. 1995. Patterns of seasonal demographic change in the alternate isomorphic stages of Mazzaella splendens (Gigartinales, Rhodophyta). Phycologia (Oxford). 34(5):390–395. doi:10.2216/i0031-8884-34-5-390.1.

Dyck LJ, DeWreede RE. 2006. Reproduction and survival in Mazzaella splendens (Gigartinales, Rhodophyta). Phycologia (Oxford). 45(3):302–310. doi:10.2216/04-80.1.

Garbary DJ, DeWreede RE. Life history phases in natural populations of Gigartinaceae Rhodophyta quantification using resorcinol. In: Experimental Phycology: A Laboratory Manual. New York, New York: Cambridge University Press; 1988. p. 174–178.

Hughey, J.R., Silva, P.C. & Hommersand, M.H., 2001. Solving taxonomic and nomenclatural problems in Pacific Gigartinaceae (Rhodophyta) using DNA from type material. Journal of Phycology, 37, 1091^1109.

May, G., 1986. Life history variations in a predominantly gametophytic population of Iridaea cordata (Gigartinaceae, Rhodophyta). Journal of Phycology, 22, 448^455.

Mudge B, Scrosati R. 2003. Effects of wave exposure on the proportion of gametophytes and tetrasporophytes of Mazzaella oregona (Rhodophyta: Gigartinales) from Pacific Canada. Journal of the Marine Biological Association of the United Kingdom. 83(4):701–704. doi:10.1017/S0025315403007665h

Phillips, B., 1994. Ecological deference between the isomorphic phases of Mazzaella lilacina (Rhodophyta, Gigartinaceae): 1) spore production, 2) recruitment specialization, 3) resistance to removal by wave action. MSc thesis, University of British Columbia, Vancouver, Canada.

Scrosati R. 1998. Population Structure and Dynamics of the Clonal Alga Mazzaella cornucopiae (Rhodophyta, Gigartinaceae) from Barkley Sound, Pacific Coast of Canada. Botanica marina. 41(5):483–494. doi:10.1515/botm.1998.41.1-6.483.

Scrosati R, DeWreede RE. 1999. Demographic models to simulate the stable ratio between ecologically similar gametophytes and tetrasporophytes in populations of the Gigartinaceae (Rhodophyta). Phycological Research. 47(3):153–157. doi:10.1046/j.1440-1835.1999.00167.x.

Scrosati R, Mudge B. 2004. Effects of elevation, wave exposure, and year on the proportion of gametophytes and tetrasporophytes in Mazzaella parksii (Rhodophyta, Gigartinaceae) populations. Hydrobiology. 520(1-3):199–205. doi:10.1023/B:HYDR.0000027839.48584.6c.

Shaughnessy F, DeWreede R, Bell E. 1996. Consequences of morphology and tissue strength to blade survivorship of two closely related Rhodophyta species. Marine ecology Progress series (Halstenbek). 136(1/3):257–266. doi:10.3354/meps136257.

Waaland, J.R., 1975. Divergences in carrageenan in gametophytes and tetrasporophytes of red algae. Phytochemistry, 14:1359-1362.