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Drivers of Sex-specific Trade-offs in the Macroalga *Ascophyllum nodosum*

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Abstract: Little is known about reproductive trade-offs in seaweeds, but sex-specific differences in mortality, production of metabolites, threshold size for reproduction, and susceptibility to herbivory have been reported. The macroalga *Ascophyllum nodosum* exhibits sex-specific trade-offs at sites where wave-action and herbivory are stronger, because females increase their investment into reproduction at the expense of chemical defences. Females may do this at stressed sites because of high germling and juvenile mortality, or to compensate for lower fecundity due smaller adult sizes at these sites. This study aimed to determine which is the case by comparing *A. nodosum* in an area where stressors (wave-action and herbivory) impacted upon both adult performance and juvenile mortality, to one where only adult performance was impacted (by ice-damage). Seven populations of the algae were compared at both the regional (> 1000 km) and local scales (< 50 km), to assess the presence of sex-specific differences in algal size, sex-ratio, and the chemical defences and tissue condition of both vegetative and reproductive structures. Taking a multi-scale approach is a useful way to determine which abiotic variables are driving biological patterns, because variability in the latter mirrors variability in the former. Sex-specific trade-offs were more common at both the regional and local scale when herbivory and wave-exposure were high. Other factors caused differences in physiology at both scales, but did not drive sex-specific differences. Furthermore, sex-specific differences were consistent in the defence of reproductive tissues at all sites, suggesting that this was not driven by stress at all. Therefore, sex-specific differences in *A. nodosum* are caused by some stressors, not caused by others, and are present in some fashion regardless of stress. This is the first study to directly quantify sex-specific trade-offs at different spatial scales in populations of either plants or algae, and as such it reveals novel insights into the driving forces behind them.

Keywords: Chemical Ecology, Dioecy, Intertidal Ecology, Herbivory, Resource Allocation, Stress Responses

1. Introduction

Limited data exists on sex-specific disparities in seaweeds, but by-sex differences in mortality [1], production of metabolites [2], threshold size for reproduction [3], and susceptibility to herbivory [4-5] have been reported. In accordance with many terrestrial plants, the females of some seaweeds put more energy into reproduction [6], although sex-specific differences in reproductive effort are not always present [3, 5]. Sexual dimorphism and other sex-specific trade-offs (e.g. higher reproductive effort at the expense of anti-herbivore defence) arise in plants and algae because

stressors act on males and females differently, owing to sex-specific costs associated with reproduction [7]. Stress therefore increases trade-offs in many plants [8], although only one investigation has so far examined the manifestation of sex-specific trade-offs with stress in a marine alga [5].

In accordance with results on many higher-plant species, sex-specific-trade-offs positively correlate with stress in *Ascophyllum nodosum*, with females investing more into reproduction and therefore being lesser-defended than males at sites of high wave-action and herbivory [5]. Because eggs are limited compared to sperm in fucoids [6], and female reproductive tissue is both more valuable and often more expensive to produce, there are two non-mutually exclusive

hypotheses that explain the pattern of reproductive trade-offs in this species. Firstly, wave-stress and herbivory reduce the survivability of young algae [9-11]. Germling mortality in areas impacted by even moderate wave-action can be 85-99% [12], juveniles can experience 50-85% mortality in wave-exposed populations [13], and 99.99% of recruits can be consumed in their first year by herbivores [9]. Therefore, females may be selected for which prioritise reproduction since increasing fecundity at the local scale most likely requires more eggs but not more sperm, because the latter outnumber the former by orders of magnitude in fucoid seaweeds [6]. Expenditure in reproduction comes at the cost of defence, and so sex-specific trade-offs manifest in these highly stressed populations [5]. Secondly, wave action and herbivory cause substantial frond damage, often resulting in the loss of the upper half of the frond [13, 14]. As fecundity correlates with individual size in *A. nodosum* [15], the loss of receptacles (fruiting-bodies) and the lack of space upon which to bear them may be more problematic to females, which produce more expensive and better-defended fruiting-bodies. Each reproductive structure lost is consequently more detrimental to female fitness [8, 16-17]. Therefore, sex-specific trade-offs may be the result of recruit mortality, detriments to adult performance, or both, in *A. nodosum*.

Determining the cause of sex-specific trade-offs in *A. nodosum* therefore requires an assessment of populations under stressors that impact adult performance, whilst causing minimal juvenile and germling mortality. This investigation aimed to compare the occurrence of by-sex disparities in *A. nodosum* from a region predominantly stressed by wave-action and herbivory (Anglesey, North Wales), to one predominantly stressed by ice-damage (Tjärnö, Western Sweden) at two different spatial scales, the regional (> 1000km) and local (< 50km). Taking a multi-scale approach is a useful way to determine which abiotic variables are driving biological patterns, because variability in the latter will mirror variability in the former (discussed by [18]). For example, if a physical parameter varies between regions, but not within regions, biological factors driven by this parameter will only be detectable at the regional scale. Likewise, if a physical parameter varies between local sites in one region but not others, biological factors driven by it will only vary within that region.

Ice-damage presents a very different stress to wave-action or herbivory, being more stochastic and absent entirely for much of the time. The degree of wave-action upon a site does of course change through time, and storms can dislodge considerable amounts of standing biomass [19]. Likewise, grazer-stress on algae can increase with temperature over summer [20]. Both, however, are consistent pressures across the year when compared with ice-damage, which is entirely confined to winter in sub-arctic/ northern-temperate regions, and in many localities such as western Sweden, is absent for years at a time [21-22]. The germlings of *A. nodosum* settle in early spring and grow into juveniles by their first winter [23]. Germlings are therefore highly unlikely to be directly impacted by ice-damage, even if the sea freezes in their first

year. In contrast, juvenile (established recruits) mortality has been estimated to be 60-80% during ice-years in *A. nodosum* populations near Tjärnö, depending on severity [22]. However, in years without ice this figure drops to 10% [22], and because the sea remains ice-free in 75% of years, juvenile mortality has been estimated to be only 24% in this region [21]. Because of comparatively low-levels of wave-action and herbivory at Tjärnö therefore, germling and juvenile mortality of *A. nodosum* is likely to be far less than that on Anglesey.

Ice-damage still impacts upon adult performance, however. Non-fatal cropping due to ice-damage usually affects the upper 50% of a frond [24] and is therefore similar to the impacts of wave-action [13] and herbivory [14] which also result in *A. nodosum* fronds being around 50% smaller. Because of *A. nodosum*'s slow growth, the impacts of disturbance events can persist for many years [25], meaning that even in Tjärnö where the sea freezes only one in every four years, *A. nodosum* individuals remain smaller because of the recurrent disturbance [22]. As fecundity correlates with individual size in *A. nodosum* [15], individuals experiencing greater ice-damage must invest more into reproduction to compensate for their smaller sizes [21]. Complete losses of stands in Western Sweden, with its small tidal-range and sheltered geography, are far less common than in areas where tides and waves combine to buckle and drag ice floes across the benthos [26]. Consequently in this area, adult performance is impacted to a similar degree by ice-damage than by wave-action and herbivory elsewhere. This leads to the hypothesis that if germling and juvenile mortality drives sex-specific trade-offs, they will be more common on Anglesey. However, if detriments to adult performance are the cause, sex-specific trade-offs will be equally common in both regions. To test this, four populations near Tjärnö and three on Anglesey were sampled for algal size, sex-ratio, and the living biomass (energy) content and phlorotannin abundance of both vegetative and reproductive tissues in mid-summer, late-summer, autumn, and the following spring. Sampling populations of *A. nodosum* separated at different spatial scales reveals much about the environmental variables driving disparities between them (*sensu* [11]) and assessing reproductive trade-offs in this regard is a novel approach to research both in algae and higher plants.

2. Materials and Methods

2.1. Study Organism

Ascophyllum nodosum is an abundant and often dominant fucoid, which grows on the mid-intertidal of sheltered rocky shores in temperate latitudes [27]. It displays apical, dichotomous, branching growth, reaching sizes of up to 3 m in some habitats, but is more typically around 1 m in length. Every year after it matures (at around 2-5 years of age), it produces a buoyant vesicle at the tip of its apical meristems [13]. Receptacles (fruiting bodies) are produced in pairs along the length of its fronds between mid-summer and the

following spring, when externally-fertilised gametes are released over a two-week period towards the end of April [13, 16, 23]. The dry mass of the whole alga can double once the receptacles are ripe [15, 16] and because of the dense nature of established populations, intraspecific competition for light is strong [13]. Although fronds can break due to wave-action, herbivory, and ice-damage, they continue to grow provided that the holdfast remains attached to the substrate [28].

Like other brown algae, *A. nodosum* produces phlorotannins to impede herbivory and limit damage by UV radiation [29]. Phlorotannins can be induced by light, gastropod herbivory, and by waterborne cues from nearby *A. nodosum* under attack. However, they are not produced in response to crustacean herbivores, or by simulated herbivory [30]. Phlorotannins are expensive to generate and there is a trade-off between their production and both growth and reproduction [31, 5]. Phlorotannins are therefore produced only as required, and their abundance is independent of growth-limiting nutrients, such as nitrogen [14].

2.2. Site Selection

The study was undertaken at seven locations bearing established *A. nodosum* populations between the summer of 2012 and the spring of 2013. Four of these were in the archipelago west of the Sven Lovén Centre for Marine Sciences, Tjärnö in south-western Sweden, and three were on the Isle of Anglesey in North Wales. Western Sweden experiences very small tides (~0.3 m) and has an average salinity of approximately 25 (ranging from 15-30), owing to large freshwater input into the Skagerrak Strait [22]. The four Swedish locations were un-named uninhabited islands designated here, S1) 58.893467°N, 11.123100°E. S2) 58.895333°N, 11.124367°E. S3) 58.851933°N, 11.134983°E. and S4) 58.856894°N, 11.139860°E; chosen to represent typical stands of *A. nodosum* in the area with a proportionate mix of aspects and wave-fetches. Anglesey, by contrast, experiences large (6 m) tides and has a salinity more typical of open seawater (~34). The Anglesey locations were named for towns nearby; Menai (near Menai Bridge); 53.226793°N, 4.157054°W. Cemlyn (in Cemlyn Bay); 53.414750°N, 4.511564°W. Bull (in Bull Bay); 53.422543°N, 4.368959°W; again chosen to represent typical *A. nodosum* stands in the region. Potential wave energy was estimated from the distance of open water at 10° compass intervals around each site [32], using high-resolution coastline data from Google Earth. Greater fetch allows larger waves to develop and is a useful quantitative indicator of 'wave exposure' [33].

Herbivore abundance at each site was estimated at the date of first sampling (June 2012), by haphazardly placing ten (sufficient for the running means to stabilise) 0.25 m² quadrats within the *A. nodosum* stand and carefully collecting all gastropods by hand [14]. Three known herbivores of *A. nodosum* were found to be present at all sites (*Littorina littorea*, *Littorina fabalis*, *Littorina obtusata*) and *Patella vulgata* was also present at Cemlyn and Bull on Anglesey. *Littorina fabalis* was included in surveys because although it

has been previously shown to subsist primarily on algal films [34], it does also consume macroalgae including *Sargassum muticum*, *Fucus serratus*, and *A. nodosum* [35].

2.3. Sampling Protocols

Sampling procedures differed slightly between regions due to the logistics of travelling to Sweden from Anglesey. In June 2012, 24 genets per Tjärnö site (N = 96, identified as per [36]) were selected haphazardly and tagged with cable-ties around the holdfast, since determining their sex at this time was not possible. Only individuals with at least four vesicles present on the fronds were included, because those younger than this are likely to be immature [31]. Length and circumference were measured to the nearest half-centimetre, allowing an estimation of dry mass [37]. Apical meristems (4-5) were torn by hand from each genet to minimise phlorotannin induction [29], returned to the laboratory, and frozen within 12 hours of sampling. These same individuals were then resampled in August, October, and the following April, but because of mortality throughout the experiment, sample size (N) decreased from 96 to 82. Only individuals that survived the entire experiment were included in analysis, leaving sample sizes of n = 22 at S1, n = 19 at S2, n = 23 at S3, and n = 18 at S4.

In April 2013, the conceptacles (sites of gamete production) were visible within the receptacles, and this allowed differentiation of sexes by a combination of light-microscopy [6] and by the colour of the conceptacles [10]. Receptacles (~100 per genet) were collected from the same reproductive laterals as that month's vegetative samples, to avoid bias by intra-individual variation in tissue condition [13]. Because sexes were not known at the time of initial tagging, the numbers of males and females ultimately shown to be tagged in April, provided an estimate of sex-ratio in Tjärnö.

Sampling on Anglesey differed due to greater access, 10 males and 10 females per month were sampled from each site exactly as per Tjärnö algae, except that genets were different in each month due to an excess of males and females being haphazardly tagged during the previous spring. Unlike in Tjärnö, equal numbers of each sex had been specifically tagged and therefore a separate survey was needed to assess sex-ratio as a means to estimate sex-specific differences in mortality [3]. This was undertaken non-destructively in April 2013. 100 individuals were selected haphazardly at each site, and their sexes differentiated [10].

2.4. Phlorotannin Analysis and Energy Content

To assess sex-specific disparities in defence and energy content, both vegetative material and the reproductive material collected in April were cleared of epiphytes, washed with distilled water, freeze-dried to constant mass, and ground until homogenous. A known amount (200 mg) was then assessed for phlorotannin abundance using a modified variation of the Folin-Ciocalteu method [38]. A further subsample was assessed for energy content by estimating the

living-biomass (ash-free dry mass per unit dry mass) of the tissues [39]. A known quantity (~1000mg) was weighed to the nearest milligram, ashed-off at 450 °C for 3.5 hours, and reweighed to the nearest milligram [40]. This quantification of the organic fraction of the tissues is a useful measure of condition and energetic cost of tissue-production, since organic carbon is used extensively in algal metabolism [41], and AFDM per unit DM correlates almost exactly with energy content in algae [42].

2.5. Statistical Analysis

Sex-ratio of *A. nodosum* at each site was tested for significant difference from 1:1 with a one-proportion binomial analysis. Prior to analysis of means, all data were Levene's-tested for equal variance. Differences in herbivore abundances between sites were tested by one-way ANOVA if variances were homogenous, and Welch's ANOVA if they were not. Differences in phlorotannin and living-biomass abundance were assessed with 3-way repeated measures nested ANOVA's, with site nested in region. Differences in whole-algal dry mass, receptacle phlorotannin abundance, and receptacle living-biomass were analysed with two-way nested ANOVA's, with sex and region as fixed orthogonal factors, and site nested in region. All means were compared post-hoc with Tukey's HSD tests.

3. Results

3.1. Stress

Tjärnö sites were similar in their fetch (Table 1), and all four sites had similar total abundances of grazers (Figure 1), although there were differences in species composition between sites. S4 hosted the most *L. obtusata*, whereas S3 had more *L. fabalis*. *Littorina littorea* were the most common

grazer, and abundances of this snail were similar between sites (Figure 1). In contrast, Anglesey showed large differences in both fetch and grazer density, with both being lowest at Menai and highest at Bull (Table 1, Figure 1). Both the average fetch and total grazer abundances were similar between Menai and all four Tjärnö sites (Table 1, Figure 1). *Patella vulgata* were more abundant at Cemlyn than Bull but their densities were low compared to *L. fabalis* and *L. obtusata*, which were extremely abundant at Bull (Figure 1).

Table 1. Descriptive statistics for the fetch of four Swedish sites (S1-S4) and three Welsh sites (Menai, Cemlyn, Bull) bearing established stands of *Ascophyllum nodosum*

	Mean	S. E.	Sum	Maximum
S1	193.6	44.6	6969.7	1022.0
S2	221.8	51.0	7985.3	1002.0
S3	144.5	62.7	5200.9	1680.0
S4	296.0	81.7	10654.9	1500.0
Menai	209.4	47.8	7539.6	1042.1
Cemlyn	16724	6894	602076	130977
Bull	29449	8941	1060178	181133

Overall, Anglesey was significantly more exposed to wave-stress, with an average fetch distance of 15.4 km (S. E. 3.9 km) compared to 0.2 km (S. E. 0.03 km) at Tjärnö. Densities of grazers were also higher on Anglesey, with an average of 146 individuals m⁻² (S. E. 26.7), compared to 9 m⁻² (S. E. 0.64) at Tjärnö. Both *L. obtusata* and *L. fabalis* were common on Anglesey (≈ 10 m⁻², S. E. 1.71, and ≈ 18 m⁻², S. E. 4.06 respectively) but both were rare at Tjärnö (< 1 m⁻², S. E. 0.19), whereas *L. littorea* were equally common in both regions (Anglesey ≈ 7 m⁻², S. E. 1.81, Tjärnö ≈ 6 m⁻², S. E. 0.63). The limpet *Patella vulgata* was present on Anglesey, albeit rarely (≈ 2 m⁻², S. E. 0.66), but absent entirely from Tjärnö (Figure 1). *Ascophyllum nodosum* from both regions had similar total dry masses (Two-way ANOVA SS = 1572, $F_{1,310} = 0.11$, $p = 0.737$).

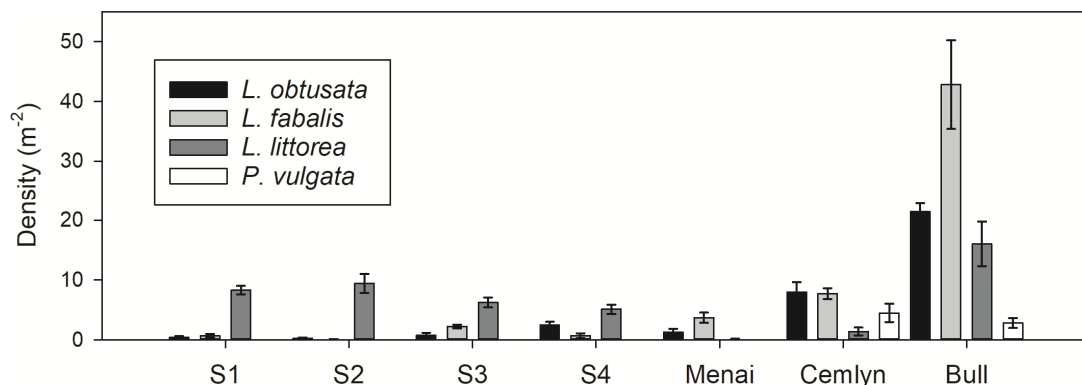


Figure 1. Densities of the known gastropod herbivores of *Ascophyllum nodosum* in each of seven established stands of the alga. S1, S2, S3, and S4 are near Tjärnö in Western Sweden. Menai, Cemlyn, and Bull are on Anglesey in North Wales. Error bars show +/- 1 S. E.

3.2. Responses to Stress

There were significant differences in the mean phlorotannin abundance between sites (Table 2, Figure 2a), although these did not relate to grazer abundance (regression analysis phlorotannin abundance v grazer abundance; $p = 0.134$). There were also significant differences in living

biomass abundance between sites (Table 3, Figure 2b), which showed a weak negative relationship with grazer abundance (regression analysis; gradient = -0.01, intercept = 64.84, SS = 920, $F = 46.92$, $p < 0.001$). On Anglesey, both dry mass and living-biomass content negatively associated with higher stress (wave-exposure and grazer abundance), but there were

also differences in both dry mass and living-biomass abundance between Tjärnö populations despite stressors being broadly similar between these sites. S3 *A. nodosum* were larger than those from S1 and S2 (Figure 2c), and living-biomass was higher in *A. nodosum* from S1 and S3 than that from S4 (Figure 2b), although these differences were variable over time (Table 3). Dry-masses of *A. nodosum* from S3 and Menai were greater than those from S1 and Bull (two-way ANOVA term for site nested in region, $SS = 194753$, $F_{5,310} = 2.81$, $p = 0.017$), but algae from all other sites were of a similar size (Figure 2c).

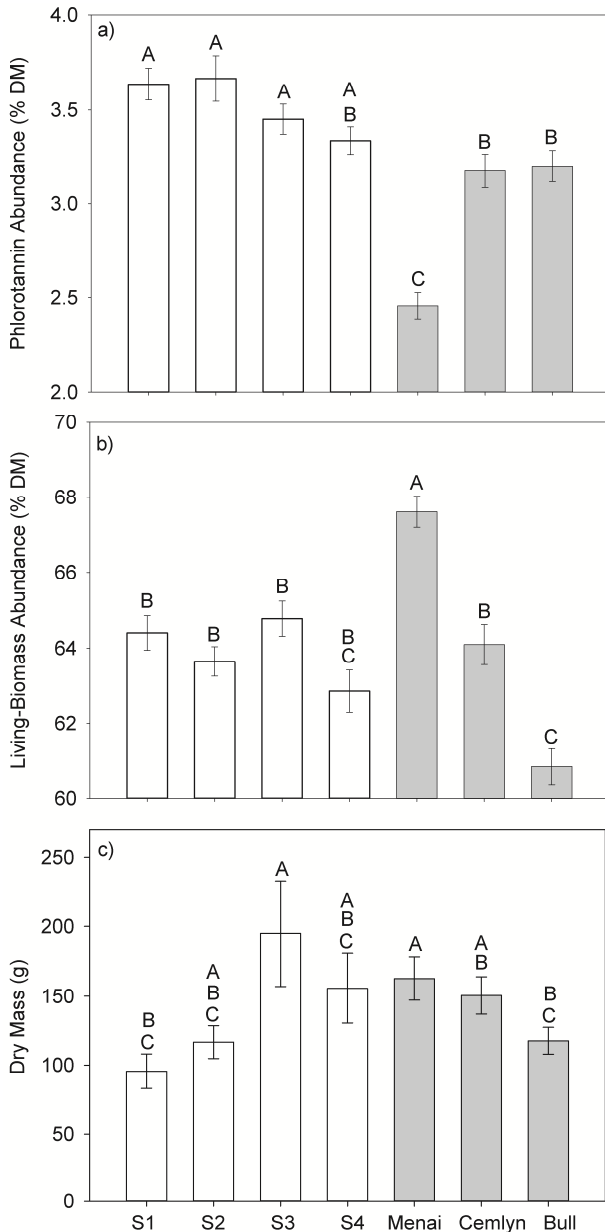


Figure 2. Abundance of a) phlorotannin (percentage dry mass), and b) living-biomass (percentage dry mass) in the vegetative tissues of *Ascophyllum nodosum*, and the total dry-masses of those same algae ($N = 569$) from seven established populations, collected in June, August, October, and April. S1, S2, S3, and S4 are near Tjärnö in Western Sweden (white bars), Menai, Cemlyn, and Bull are on Anglesey in North Wales (grey bars). Error bars show ± 1 S. E., groupings based on Tukey's post-hoc analysis.

Table 2. Three-way repeated-measures nested ANOVA for the phlorotannin abundance in *Ascophyllum nodosum* meristematic tissue samples ($N = 569$), from four established populations near Tjärnö in Western Sweden, and three established populations on Anglesey in North Wales, sampled in April, June, August, and October.

	DF	MS	F	P
Month	3	37.78	112.94	< 0.001
Sex	1	1.62	4.86	= 0.028
Region	1	43.38	129.66	< 0.001
Month x Sex	3	1.42	4.25	= 0.006
Month x Region	3	5.11	15.28	< 0.001
Sex x Region	1	3.04	9.08	= 0.003
Site	5	6.61	19.77	< 0.001
Month x Sex x Region	3	1.18	3.54	= 0.015
Month x Site (Region)	15	1.10	3.28	< 0.001
Sex x Site (Region)	5	0.98	2.92	= 0.013
Month x Sex x Site (Region)	15	0.31	0.93	= 0.531
Residual	514	0.33		

Table 3. Three-way repeated-measures nested ANOVA for the living-biomass abundance in *Ascophyllum nodosum* meristematic tissue samples ($N = 569$), from four established populations near Tjärnö in Western Sweden, and three established populations on Anglesey in North Wales, sampled in April, June, August, and October.

	DF	MS	F	P
Month	3	339.82	24.54	< 0.001
Sex	1	0.436	0.03	= 0.859
Region	1	8.14	0.59	= 0.444
Month x Sex	3	19.52	1.41	= 0.239
Month x Region	3	321.73	23.23	< 0.001
Sex x Region	1	14.86	1.07	= 0.301
Site	5	396.85	28.65	< 0.001
Month x Sex x Region	3	30.81	2.22	= 0.084
Month x Site (Region)	15	32.49	2.35	= 0.003
Sex x Site (Region)	5	21.59	1.56	= 0.170
Month x Sex x Site (Region)	15	35.03	2.53	= 0.001
Residual	513	13.85		

3.3. Sex-specific Differences in Vegetative Tissues

Sex-specific differences in phlorotannin abundance were significant in the vegetative tissues of *A. nodosum* only at Bull, and only in June, August, and April (Table 2, Figure 3a). Sex-specific differences were not evident in size or sex-ratio at any site, suggesting equal competitive ability and mortality between males and females. Although Cemlyn females had higher abundances of living-biomass than males in April, and lower abundances in October, no other incidences of sex-specific differences occurred in living-biomass abundance (Table 3, figure 3b). By region there were no sex-specific differences in living-biomass abundance of *A. nodosum* at any time, although that of Anglesey *A. nodosum* was higher than that from Tjärnö in April, but lower in October (Table 3, figure 3b).

With all sites pooled together, male *A. nodosum* ($N = 273$) had more phlorotannin in their vegetative tissues ($\bar{x} = 3.32\%$ DM, S. E 0.05) than female *A. nodosum* ($N = 296$, $\bar{x} = 3.24\%$

DM, $S. E. 0.05$), although by region this was significant on Anglesey only, and by month in June and April only (Table 2, Figure 3a). Phlorotannin abundance increased in both regions over summer, and was higher in Tjärnö *A. nodosum* than that

from Anglesey in April, June, and August, although in October *A. nodosum* from both regions bore similar abundances (Figure 3).

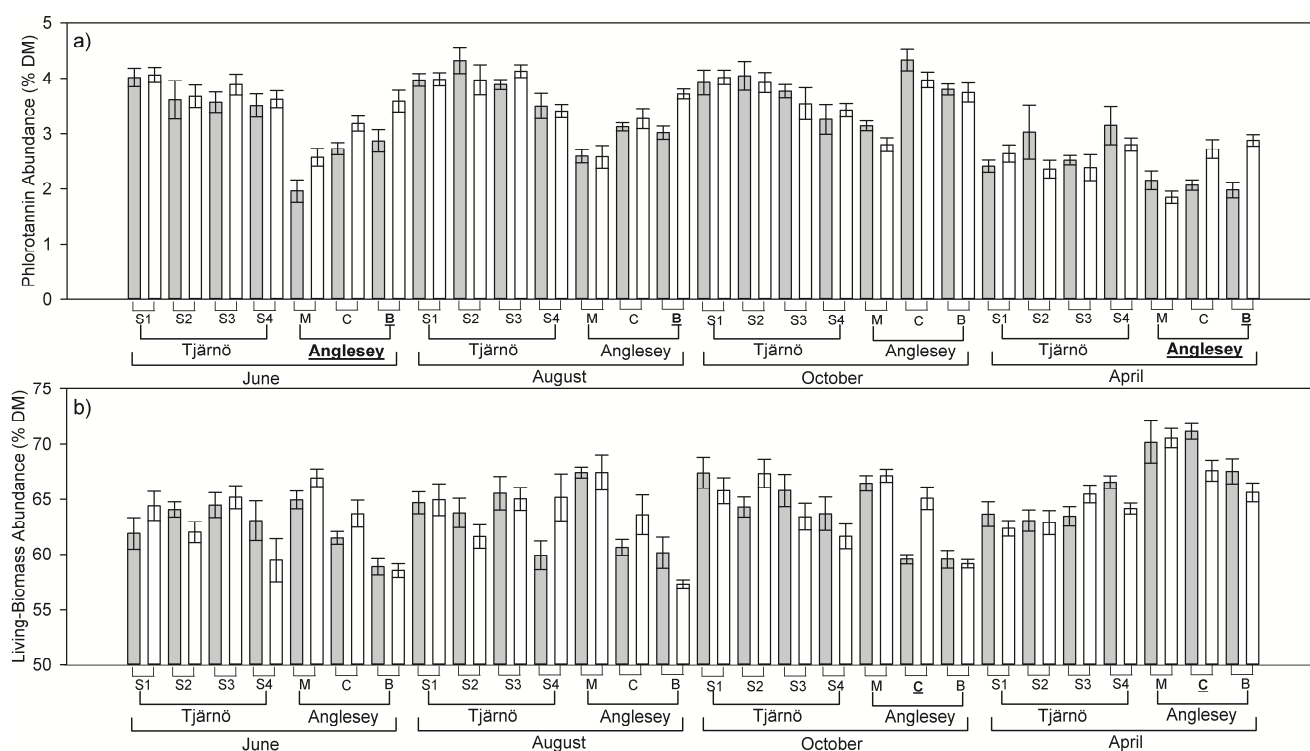


Figure 3. a) Abundance of phlorotannin (% DM) and b) Abundance of living-biomass (% DM) in the vegetative tissues of male (white bars) and female (grey bars) *Ascophyllum nodosum* ($N = 569$) collected from seven established populations. S1, S2, S3, and S4 are near Tjärnö in Western Sweden, Menai, Cemlyn, and Bull are on Anglesey in North Wales. Error bars show $\pm 1 S. E.$, highlighted text indicates where sexes were significantly different (based on Tukey's post-hoc analysis)

3.4. Sex-specific Differences in Reproductive Tissues

Female *A. nodosum* defended their receptacles more than males and this was consistent across all seven sites, despite there being between-site differences in overall phlorotannin abundance (Table 4).

Table 4. Two-way nested ANOVA for phlorotannin abundance in the reproductive tissues of *Ascophyllum nodosum* ($N = 137$), sampled from four established populations near Tjärnö in western Sweden, and three established populations on Anglesey in North Wales, in April.

	DF	MS	F	P
Sex	1	0.63	9.51	= 0.003
Region	1	1.62	24.32	< 0.001
Sex x Region	1	0.005	0.07	= 0.787
Site (Region)	5	0.64	9.56	< 0.001
Sex x Site (Region)	5	0.13	2.01	= 0.082
Residual	123	0.06		

There were no sex-specific differences in the living-biomass abundance in receptacles, and *A. nodosum* from all sites showed similar total abundances, except for Menai *A. nodosum* which showed ~6% more living-biomass abundance in its receptacles, than that from other sites (Table 5).

Table 5. Two-way nested ANOVA for living-biomass abundance in the reproductive tissues of *Ascophyllum nodosum* ($N = 137$), sampled from four established populations near Tjärnö in western Sweden, and three established populations on Anglesey in North Wales, in April.

	DF	MS	F	P
Sex	1	39.77	1.97	= 0.163
Region	1	308.51	15.31	< 0.001
Sex x Region	1	31.74	1.58	= 0.212
Site (Region)	5	83.15	4.13	= 0.002
Sex x Site (Region)	5	13.04	0.65	= 0.664
Residual	123	20.15		

Ascophyllum nodosum from both Cemlyn and Bull displayed sex-specific differences in receptacle phlorotannin abundance relative to vegetative phlorotannin abundance (Table 6, Figure 4). Females from both sites invested ~20% more phlorotannin into their receptacles relative to their vegetative tissues than males, whereas this difference (non-significant) was ~4% elsewhere (Figure 4). The degree of sex-specific disparity in relative receptacle defence was not significantly different between Cemlyn and Bull.

When pooled, Tjärnö *A. nodosum* defended their receptacles more than Anglesey *A. nodosum* (Table 4). Also, when considered relative to vegetative defence, females defended receptacles more, with receptacle phlorotannin

abundance being 64% that of vegetative phlorotannin abundance, whilst males invested only 56% as much phlorotannin in their receptacles as in their vegetative tissues (Table 6). These sex-specific differences were similar between regions, and both groups of *A. nodosum* invested comparable amounts phlorotannin into their receptacles relative to that in their vegetative tissues ($\approx 60\%$). There were no sex-specific differences in the living-biomass content of receptacles in *A. nodosum* from either region, although living-biomass abundance was higher in Anglesey *A. nodosum* than that from Tjärnö (Table 5).

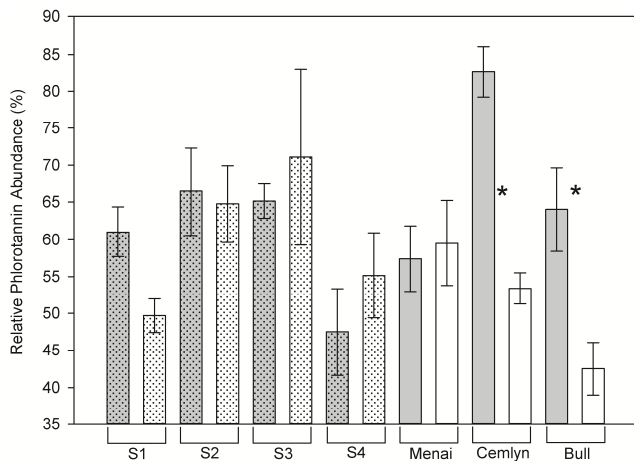


Figure 4. Relative phlorotannin abundance of the receptacles of female (grey bars) and male (white bars) *Ascophyllum nodosum* from seven established populations, sampled in April. S1, S2, S3, and S4 are near Tjärnö in Western Sweden (speckled bars), Menai, Cemlyn, and Bull are on Anglesey in North Wales (plain bars), $N = 137$. Abundances in the receptacles are expressed as a percentage of abundance in the vegetative tissues, error bars show ± 1 S.E., * indicates where significant differences exist between the sexes (determined by Tukey's post-hoc analysis)

Table 6. Two-way nested ANOVA for receptacle phlorotannin abundance expressed as a fraction of vegetative phlorotannin abundance for *Ascophyllum nodosum* ($N = 137$), sampled from four established populations near Tjärnö in western Sweden, and three established populations on Anglesey in North Wales, in April.

	DF	MS	F	P
Sex	1	0.21	10.15	= 0.002
Region	1	< 0.001	0.01	= 0.938
Sex x Region	1	0.21	10.51	= 0.002
Site (Region)	5	0.11	5.43	< 0.001
Sex x Site (Region)	5	0.07	3.66	= 0.004
Residual	123	0.02		

4. Discussion

Sex-specific differences were more apparent in *Ascophyllum nodosum* from Anglesey than in those from near Tjärnö. Male *A. nodosum* were better defended than females on Anglesey for two months, and specifically at the Bull site for three months, whereas no sex-specific differences in vegetative defence ever occurred in Tjärnö algae, at any site. Females from both regions defended their receptacles more than males, both when raw phlorotannin abundances were

compared, and when they were considered relative to phlorotannin in the vegetative tissues. However, sex-specific differences in the relative defence of receptacles manifested significantly in Cemlyn and Bull only. Sex-specific differences in living-biomass also manifested twice at Cemlyn, with both males and females displaying a better condition than the other sex for one month.

Differences between population demographics of *A. nodosum* are apparent at some scales but not others [11]. For example, abundances of juveniles differ considerably at spatial scales less than 1.5 m but not over larger scales [11]. This is to be expected, since recruitment is impacted by micro-scale heterogeneity in shelter and abundance of grazers, which differ at small spatial scales [23, 43]. Abundances of adults however, differ only at large spatial-scales (hundreds of km, [11]), suggesting abiotic factors such as light levels, ice-damage, and available nutrients are more important to adults. This is the first study to assess sex-specific trade-offs in algae at different spatial-scales, and therefore reveals interesting insights into the potential driving-forces behind them.

Differences in sex-specific trade-offs in *A. nodosum* occurred at the regional scale (> 1000 km) but were more prevalent at the local scale (< 50 km), suggesting that multiple stressors can be responsible for sex-specific trade-offs, but critically that not all are. As discussed elsewhere, wave-action and herbivory appear to drive sex-specific differences in both vegetative defence and relative defence of receptacles, which occur only at sites heavily stressed by these environmental variables [5]. Menai had similar wave-stress and herbivory to the Tjärnö sites, and *A. nodosum* from there displayed similar sex-specific differences. Ice damage, however, appears not to drive sex-specific trade-offs at all. Despite there being a considerable sea-ice event prior to April 2013 sampling (Gunnar Cervin, *pers. comm.*), vegetative defence never differed between the sexes at Tjärnö sites, and sex-specific trade-offs in reproductive tissues were similar to those found on Anglesey. Therefore, it is likely that sex-specific trade-offs occur because of high germling and juvenile mortality selecting for higher reproductive investment in females, and not because of direct stress on adults. Phlorotannin levels were considerably higher in *A. nodosum* from Tjärnö, despite grazers being fewer in number there, and differences occurred between Tjärnö sites and Menai despite abundances of herbivores being similar between them. This contrasts with previous local-scale investigations into phlorotannin abundance in *A. nodosum*, which suggested that grazer abundances were the primary reason for between-site differences [14]. Although salinity was lower in Tjärnö, low salinity actually causes a reduction in phlorotannin production [44], and so the cause of this variation is unclear. Whichever physical or biological factors drove this difference in phlorotannin content however, they did not cause sex-specific trade-offs.

In contrast to vegetative defence, sex-specific trade-offs in reproductive defence (phlorotannin abundance in reproductive tissues) occurred ubiquitously, suggesting that

1) These sex-specific trade-offs are independent of stress entirely, and further suggesting that 2) female reproductive tissues are more valuable than male reproductive tissues, because they are better-defended [16]. This is unsurprising since antheridia (sperm) to oogonia (egg) ratios can be between 40 and 400 to one in fucoids [6], although living-biomass abundance and by extension, energy content [42], did not differ between male and female tissues. However, it is worth noting that receptacles were collected in April, and sexual dimorphism in living-biomass abundance has previously been shown to manifest in December to March only in *A. nodosum* [5]. These findings add to the growing body of contemporary literature that suggests considerable flexibility in reproductive trade-offs made by algae in response to environmental factors [45, 46] and raises questions around whether these trade-offs are controlled at the genetic level (i.e. the result of local-scale selective pressures) or at the individual level (e.g. through hormonal control).

5. Conclusions

These findings further our knowledge of sex-specific trade-offs in algae by demonstrating that not all stressors cause them to manifest. Populations experiencing stressors which impact upon germling and juvenile mortality (wave-action and herbivory) demonstrate sex-specific trade-offs more often than those which experience stressors impacting primarily on adult performance and less on recruit mortality (i.e. ice-damage). Some stressors other than herbivory may still necessitate a defensive response by the algae through the production of secondary metabolites (phlorotannins), the production of which impacts negatively upon primary metabolic processes. However, these stressors do not act differently upon the sexes, and therefore do not result in sex-specific trade-offs. This is despite the fact that females invest more heavily into reproduction than males, if only because they produce more expensive defences in their reproductive structures. This study highlights the value of a multi-scale approach to the research of sex-specific trade-offs in both plants and algae.

6. Recommendations

Macroalgae are important parts of coastal marine ecosystems, and understanding their ecology and physiology is of great value. Sex-specific reproductive trade-offs are an important part of plant ecology and more research is needed to appreciate their importance in seaweeds. Future studies should consider both abiotic and biotic stressors, as well as account for seasonality, and in particular the reproductive cycle of the species under study. Stress impacting germlings rather than adults is of the most importance for sex-specific trade-offs in *Ascophyllum nodosum*, but more research is needed in other species – especially monocious ones – where trade-offs associated with reproduction cannot be separated at the individual level by sex.

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