Do grazers prefer invasive seaweeds?

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ABSTRACT

Sargassum muticum is an invasive alga that affects biodiversity of benthic communities. Its proliferation in recipient communities may be due to several factors including limited grazing effects by native herbivores ("Enemy Release Hypothesis", ERH). Until now, there is no information about grazing preferences exerted by native herbivores over *S. muticum* and native seaweeds on the Galician coast. The aim of this study was to determine whether or not different grazers preferred *S. muticum* compared with native seaweeds. The hypothesis tested was that native meso- and macrograzers would prefer native seaweeds rather than the invasive seaweed *S. muticum*. Concentrations of polyphenols were also examined to see whether or not these compounds correlated with preferences for types of food. Despite variability of responses within and among herbivorous species, results suggested that grazers preferred native seaweeds over the invasive *S. muticum*. In most cases, the gastropods *Littorina littorea* and *Aplysia punctata* preferred to feed on *Ulva* sp., whereas *L. obtusata* fed mostly on *Fucus vesiculosus*. The sea urchin *Paracentrotus lividus*, however, showed no preference, feeding indiscriminately on different seaweeds. It is also important to highlight that in many cases, grazers did not show any preference (i.e. consumption when a choice was available could not be differentiated from consumption when there was no choice). These results, therefore, partially supported our prediction and reinforced the notion that well-designed experiments and analyses must be done before preference can be inferred. We suggest that the invader *S. muticum* is not under substantial pressure from grazers in the area of study.

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1. Introduction

Biological invasions are one of the most important elements of global change, altering biodiversity and function of natural ecosystems (e.g. Dunstan and Johnson, 2004; Vermeij et al., 2009) and causing serious socio-economic problems (Occhipinti-Ambrogi and Savini, 2003; Schaffelke and Hewitt, 2007). To limit and control their negative impacts on ecosystems and to manage correctly and avoid future invasions, it is necessary to know the mechanisms that make non-native species become invaders (Vermeij et al., 2009). Moreover, to effectively explain such invasions and act against them, their ecological implications need more attention (Vermeij et al., 2009).

Seaweeds make up a considerable proportion of invasions in coastal habitats, representing about 5% of the total flora (Ribera and Bouderesque, 1995) and about 10 to 40% of the total alien species (Schaffelke et al., 2006). Herbivory plays an important role in seaweed distribution, abundance and composition of assemblages in temperate or tropical communities (Wessels et al., 2006) and may be an important factor regulating invasions by seaweeds (Parker and Hay, 2005; Strong et al., 2009). Proliferation of invasive seaweeds in

recipient communities is due to intrinsic biological traits of the invasive species and extrinsic factors of the recipient communities. These extrinsic factors include limited grazing effects by native herbivores as proposed by the Enemy Release Hypothesis (ERH, Elton, 1958). The ERH states that invasive species experience reduced negative impact on natural enemies (i.e. herbivores or pathogens) since they are liberated from their coevolved enemies and the native enemies in the new range of the invader fail to recognise them as adequate food or hosts. Contrasting results have, however, been found in experimental studies. In some cases, native predators prey minimally upon invaders (Trowbridge, 1995), whereas in others, native predators prey heavily on invaders (Strong et al., 2009), having little influence on the invasion success or inhibiting the success of invading species, respectively (Byers, 2002).

To establish and persist in benthic communities, invasive seaweeds must escape, tolerate or deter herbivores (Wessels et al., 2006). To tolerate grazing, algal growth must compensate for loss of biomass eaten by grazers (Littler et al., 1995; Wessels et al., 2006) or produce a variety of secondary metabolites (e.g. phlorotannins and polyphenolics) which have been reported to deter herbivores (Pavia and Toth, 2000; Wessels et al., 2006). In general, seaweeds are more susceptible to elimination by herbivores if they are not defended by secondary antifeeding metabolites (Van Alstyne et al., 1999). Several studies have documented a negative correlation between concentration of

polyphenols and phlorotannins and preference by herbivores (e.g., Pavia and Toth, 2000), although the role of these compounds as defence compounds is largely disputed (see Svensson et al., 2007). Polyphenols have been also hypothesised to serve a number of other functions, including protection from pathogens and UV damage (Van Alstyne et al., 1999). Nonetheless, concentrations of polyphenols in marine algae are very variable ranging from undetectable to nearly 20% of an alga's dry mass (Van Alstyne et al., 1999), and they differ greatly among spatial and temporal scales, including among types of tissues in an individual (Van Alstyne et al., 2001). For example, content of polyphenols is higher in fucoids than in laminarians, and meristems have higher polyphenolic content than nonmeristematic vegetative tissues in both fucodis and laminarians (Van Alstyne et al., 2001). Changes in content of polyphenols may also occur at temporal scales ranging from days to weeks in response to grazing pressure or different environmental factors (Van Alstyne et al., 1999; Rohde et al., 2004).

The invasive seaweed *Sargassum muticum* Yendo (Fensholt) was first introduced in west European coasts in the 1970s (Fernández et al., 1990) and it invaded the rocky shores of the northern Spanish coast in the 1980s (Fernández et al., 1990). As a result of its fast growth rate and subsequent rapid spread, it is a strong competitor to the indigenous flora, altering the composition and structure of natural assemblages of species (Britton-Simmons, 2004; Olabarria et al., 2009; Gestoso et al., 2010). Although a variety of grazers can feed on *S. muticum*, there is little evidence to indicate that they are a controlling factor in its abundance or distribution (see Harries et al., 2007). Until now, there has been no information about grazing preferences exerted by native herbivores on *S. muticum* relative to native seaweeds on the Galician coast. This information is necessary in order to evaluate the potential role of grazers in controlling the distribution and spread of this invasive seaweed in the area of study.

In this study, we analysed preferences for food of some key grazers inhabiting intertidal rocky shores on the Galician coast under aquarium conditions. The main goal of this study was to determine whether or not different grazers preferred *S. muticum* compared with native seaweeds (i.e. *Fucus vesiculosus* Linnaeus, *Laminaria ochroleuca* Pylaie, and *Ulva* sp.). Following the predictions of the Enemy Release Hypothesis, we expected that native meso- and macrograzers would prefer native seaweeds rather than the invasive seaweed *S. muticum*.

We also examined concentrations of polyphenols to see whether or not these compounds might be correlated with preferences for types of food. Polyphenols have a concentration-dependent antifeeding activity towards many herbivores (see Van Alstyne et al., 2001), and consequently, choices made by grazers might be more influenced by the chemical composition of food. We expected that (1) the concentration of polyphenols would be higher in Fucales including *S. muticum*, than in the other seaweeds because previous studies have reported such differences (see Van Alstyne et al., 2001), and (2) the content of polyphenols would change over a period of 24 h in response to grazers or physiological condition of seaweeds.

2. Materials and methods

2.1. Algae

Four species of seaweed were chosen, the invasive alga *S. muticum* and the native algae *F. vesiculosus* Linnaeus, *L. ochroleuca* Pylaie and *Ulva* sp. These native species were selected because they coexist with *S. muticum* and they are the most abundant species on mid- and low-intertidal rocky shores in the area of study, Alcabre (42°13′30.7″N, 8°46′14.1″W), located on the southern side of Ria de Vigo (Galicia, NW Spain).

Feeding assays were run using fresh pieces of algae collected from the same area to avoid variations in physical and chemical characteristics among populations. All seaweeds used in the experiments were collected the same day of the laboratory experiments to reduce the risk of possible changes affecting their chemical defences after detachment or under physical stress (Cronin and Hay, 1996a,b) and they were kept in open aerated seawater tanks until they were offered to grazers. To avoid variations among different parts of the plant in nutrient concentrations, chemical defence levels or toughness we always selected the same section of each plant (Pavia et al., 1999; Pavia and Toth, 2000; Martha de Lima et al., 2008). In particular, we selected mid parts of the lamina of similarly sized individuals of *L. ochroleuca* and vegetative blade tissues of *F. vesiculosus*, avoiding the apical meristems or reproductive tissues (Van Alstyne et al., 1999). We also selected the mid parts of unmatured *S. muticum* branches.

2.2. Grazers

We focused on generalist rather than specialist herbivores because generalists (i) commonly have stronger impacts on structure of algal communities in marine systems (Hay and Steinberg, 1992), (ii) are often the relevant consumers in marine ecosystems, where specialist herbivores are rare (Poore and Hill, 2006) and (iii) are more likely than specialists to feed on and thus impact introduced seaweeds. Four species of invertebrate grazers were selected for these experiments, *Littorina littorea* (Linnaeus, 1758), *L. obtusata* (Linnaeus, 1758), *Aplysia punctata* (Cuvier, 1803) and *Paracentrotus lividus* (Lamarck, 1816). The littorinid snails *L. littorea* and *L. obtusata* that inhabit the mid-intertidal zone have been cited as generalist grazers that actively feed on native algae and on the introduced algae *S. muticum* (Harries et al., 2007), although the latter is closely associated with *Ascophyllum nodosum*, *F. vesiculosus* and *F. serratus* (Pavia and Toth, 2000). The sea hare, *A. punctata*, inhabits mid- and low-intertidal and subtidal zones, and has been cited as the most effective grazer of *S. muticum* in the British Isles (Harries et al., 2007). Sea urchins are recognised as having important effects on the establishment, spread and persistence of introduced algal species (Sumi and Scheibling, 2005). The sea urchin *P. lividus* is a common macrograzer that feeds relatively large pieces of macroalgae in many rockpools and at infralitoral levels on the Galician coast.

Grazers were collected from the same area as were the seaweeds 2 (littorinids) or 4 days (sea hares and sea urchins) prior to the experiments and were kept on a diet of the seaweeds among which they were collected. During this acclimatization period animals were fed because starvation may alter feeding behaviour of different herbivores (Cronin and Hay, 1996a,b). Individuals of similar size were used to minimize size-specific differences in preferences (Pavia et al., 1999). Individuals of *L. littorea* had a total body whorl of 19.8 ± 0.1 mm (average $\pm$ SE, $n = 138$) and *L. obtusata*, 11.3 ± 0.0 mm ($n = 56$). *A. punctata* had a mean mantle length of 20.3 ± 1.8 mm ($n = 39$), whereas individuals of *P. lividus* had a mean test diameter of 55.2 ± 0.6 mm ($n = 91$).

2.3. Feeding preference experiments

We defined "preference" as some active behavioural choice that is expressed if different types of prey are consumed in different proportions when presented alone or together (*sensu* Underwood and Clarke, 2005). Experimental demonstration of preference thus requires no-choice and multiple choice feeding tests (see Underwood and Clarke, 2005).

We exposed grazers to food in two stages. Stage 1 in which we estimated the quantity of seaweed that was consumed per individual when only that type of seaweed was offered (no-choice treatment) and Stage 2 in which we estimated the consumption of seaweed by the individual when two different seaweeds were offered (choice treatment). In all choice treatments, grazers were confronted with *S. muticum* and one native seaweed (*F. vesiculosus*, *L. ochroleuca* or *Ulva* sp.). The mass of different seaweeds offered in Stage 2 was the same

as in Stage 1 to avoid confounding effects due to different rates of encounter (Underwood and Clarke, 2005). Rates at which grazers fed were quantified by offering the herbivores pre-weighed pieces of fresh seaweeds and measuring the change in weight of this seaweed over 24 h, relative to the change in weight of an ungrazed piece of tissue from the same individual seaweed (i.e. autogenic change; Cronin and Hay, 1996a).

Feeding assays were conducted from December 2008 to February 2009 using $51 \times 57 \times 50$ cm (approx. 125 l) tanks kept at 17–18 °C in an open aerated seawater system on a 12:12 light–dark cycle. All aquaria had a constant supply of unfiltered seawater from the system throughout the whole experiment. Three replicate tanks were used for all assays. Behaviour by each species of grazer was assessed in separate experiments. Each experiment was repeated 4 times in the case of *L. obtusata* and *A. punctata* and 5 times in the case of *L. littorea* and *P. lividus* to increase the number of replicates. Each replicate of each treatment consisted of a net breeder ($14 \times 13 \times 17$ cm) where seaweeds were offered to each grazer. The net allowed seawater flow and retained the grazer and food within the recipient. In each tank, a total of 28 net breeders, two complete sets of experimental treatments (no choice and choice) and control net breeders (i.e. without grazer), were randomly allocated. With this design, factors such as water temperature or nutrients (especially those released as waste products of metabolism) could affect the control and the experimental units (Prince et al., 2004). In the case of the sea urchins, an internal plastic box with holes was used to prevent the sea urchins from breaking the nets.

Fresh seaweeds were cut into small portions and their wet weights (± 0.0001 g) were determined after drying them with paper towels. The size of the algal pieces was standardised for each species of grazer after doing a pilot experiment to determine natural rates of consumption. In the case of *L. littorea*, approximately 0.20 g of alga was added to the no-choice treatments, 0.02 g to the *L. obtusata* no-choice treatments, 1 g of alga to *A. punctata* and 2.50 g of alga for *P. lividus*. Half of these quantities were added to the no-choice treatments. To make algae equally available to grazers, pieces of seaweed were attached with wooden clips to opposite sides of the net breeders.

Only one animal was used per trial to avoid possible interactions among them (Peterson and Renaud, 1989) and different individuals were used in each assay to get independent replicates (Davis et al., 2005; Wessels et al., 2006).

2.4. Analyses of polyphelonic content

For analyses of polyphenolic compounds, small pieces of tissue were excised from vegetative parts and stored in the freezer until analyses were done. Six replicates of approx. 0.3 g of vegetative tissues from each of the species of seaweed were collected at the beginning and at the end of each feeding assay. Three replicates were weighed, dried at 60 °C for 48 h to constant weight and reweighed to obtain a dry:wet mass ratio. The other three replicates were weighed, ground to a fine paste, placed in 10 ml aqueous methanol (80%) and kept overnight at 8 °C in darkness. Polyphenolic content was quantified colorimetrically using the Folin–Ciocalteu method (Van Alstyne, 1995).

2.5. Statistical analyses

The amount of each seaweed consumed for each replicate was calculated as its loss in biomass (blotted wet weight) as $(T_{pre} - T_{post}) - (C_{pre} - C_{post})$, where T_{pre} and T_{post} were pre-assay and post-assay blotted weights of tissue given to grazers (i.e. treatments) and C_{pre} and C_{post} were pre-assay and post-assay mean blotted weights of controls for autogenic changes per each treatment (Prince et al., 2004; Davis et al., 2005). Changes in biomass of seaweed were expressed as percentage change over 24 h relative to initial biomass. We discarded those few

cases where calculated consumption had a negative value. Since we did not record any growth of the control food items, a negative consumption can only be explained by no or very little consumption in combination with the errors in the weight reading.

Preliminary three-way analyses of variance of seaweed consumed by each species of grazer that included the factors Treatment, Tank and Trial showed no significant effect of the two main factors Trial and Tank nor significant interactions of those with Treatment ($P>0.05$). Therefore, replicates of treatments from the different tanks and trials were pooled to test the null hypothesis.

Under the null hypothesis that selection of seaweed is random, the expected proportions of seaweed consumed of each type were estimated using formulae described by Underwood and Clarke (2005) derived from maximal likelihood estimates. Data from Stages 1 and 2 were randomly paired from all available data and used in analyses applying the formulae. Thus, the null hypothesis of no preference was that the ratio of the porportions of seaweed eaten when choice (i.e. native seaweed *versus S. muticum*) was possible was equal to those calculated when only one type of seaweed was presented:

$$H_0 : q_1 / q_2 = p_1 / p_2 (\text{or } H_0 : q_1 = \theta p_1; q_2 = \theta p_2)$$

where p_1, p_2 are the proportions of each seaweed eaten when they were presented in no-choice treatment (estimates provided from Stage 1); q_1 and q_2 are the proportions of seaweed eaten when they were presented together (estimates provided from Stage 2); θ is a constant, unknown parameter; and $k-1$ the number of degrees of freedom for the resulting χ^2 tests, where k was the number of choices offered to each grazer, i.e. 2. Binomial tests were also used to test whether the proportion of cases in which preference and no preference were shown deviated significantly from a theoretically expected distribution of observations equally likely.

A two-way analysis of variance was used to test for differences in polyphenolic content among seaweeds and sampling times. Seaweed (4 levels: *L. ochroleuca*, *S. muticum*, *Ulva* sp. and *F. vesiculosus*) and time (2 levels: pre and post-assay) were considered as fixed and orthogonal factors. Prior to the analysis, homogeneity of variance was tested by Cochrans' test and, since no transformation rendered the variances homogeneous, we used untransformed data and the level of significance considered was 0.01 instead of 0.05 (Underwood, 1981). Analysis of variance was followed by *a posteriori* Student–Newman–Keuls tests (SNK tests) on appropriate and significant terms of the model.

3. Results

3.1. Preference

Responses by grazers were variable, although there were some clear trends in consumption of seaweed (see Stage 1 *versus* Stage 2 in Fig. 1; Table 1). When some active foraging decisions were made, the preferred seaweed varied with grazer. In most cases the gastropods *L. littorea* and *A. punctata* preferred to eat *Ulva* sp., whereas *L. obtusata* mostly ate *F. vesiculosus*. For example, in the case of *L. littorea* 44.89% of *Ulva* sp. was consumed in Stage 1 and 29.49% in Stage 2, while 24.12% of *S. muticum* was consumed in Stage 1 and only 4.02% was consumed in Stage 2 (Fig. 1). *A. punctata* ate 78.93% of *Ulva* sp in Stage 1 and 47.78% in Stage 2, while 8.25% of *S. muticum* was consumed in Stage 1. In the case of *L. obtusata* 22.01% of *F. vesiculosus* was consumed in Stage 1 and 16.64% in Stage 1, while 9.96% of *S. muticum* was consumed in Stage 1 and only 2.53% was consumed in Stage 2. The sea urchin *P. lividus*, however, showed no preference, feeding indiscriminately on different seaweeds.

In the case of *L. littorea*, when *F. vesiculosus* was presented alongside *S. muticum* preference was detected in 4 cases, but the native seaweed was preferred only once, i.e., the difference from the expected 1:1 ratio
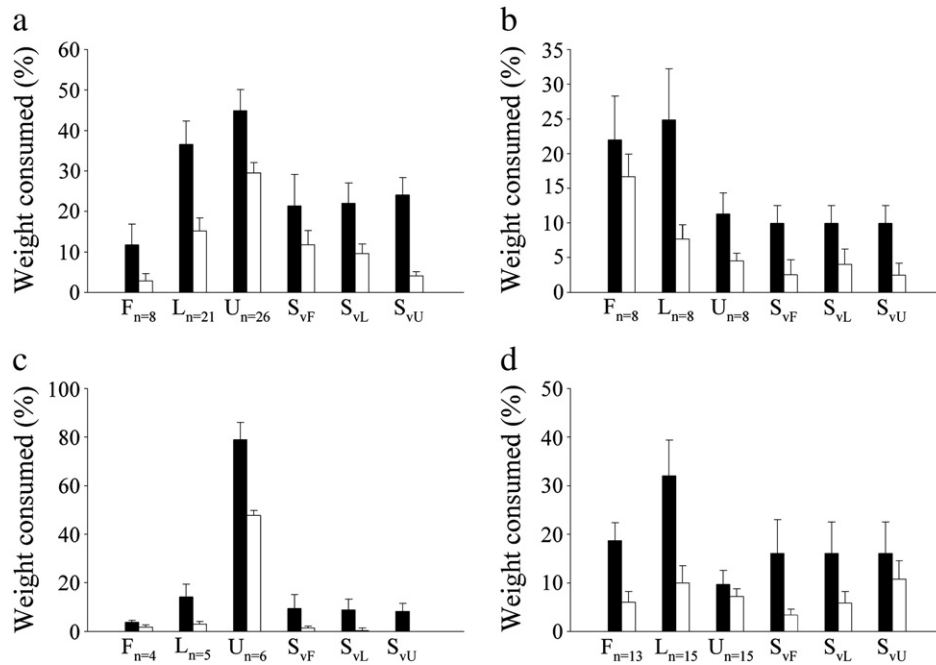
Fig. 1. Mean (%) (+SE) seaweed consumption by the grazers *Littorina littorea* (a), *Littorina obtusata* (b), *Aplysia punctata* (c) and *Paracentrotus lividus* (d). Loss of mass by seaweeds has been adjusted for autogenic changes in control treatments (i.e. no grazers). F = *Fucus vesiculosus*, L = *Laminaria ochroleuca*, U = *Ulva* sp., S_{vF} = *Sargassum muticum* when confronted with *F. vesiculosus*, S_{vL} = *S. muticum* when confronted with *L. ochroleuca* and S_{vU} = *S. muticum* when confronted with *Ulva* sp. Black bars indicate no-choice treatment (Stage 1) and white bars indicate choice treatment (Stage 2).

was not significant (Binomial test, $P=0.250$). Some active decisions were made when choosing between *L. ochroleuca* and the invasive seaweed, but the cases in which *L. ochroleuca* was selected could not be differentiated from the expected ratio (Binomial test, $P=0.209$). The selection of *Ulva* sp. when choice was available (Stage 2) was significantly different from the expected 1:1 ratio ($P=0.001$).

In the case of *L. obtusata*, when consumption of *S. muticum* was compared with that of *F. vesiculosus*, *L. ochroleuca* and *Ulva* sp., the consumption of algae when choice was available could be distinguished from the distribution predicted from Stage 1 in 7, 3 and 3 cases respectively, but differences from the expected 1:1 ratios were not significant (Binomial tests, $P=0.164$, $P=0.375$, and $P=0.375$).

In a total of 8 tests the sea hare *A. punctata* showed active selection of seaweeds when *S. muticum* was confronted with *F. vesiculosus*, *L. ochroleuca* and *Ulva* sp., but only in the case of *Ulva* sp. tests the

difference appeared to be marginally significant (Binomial test, $P=0.063$, Fig. 1c).

P. lividus showed some active foraging decisions when *S. muticum* was confronted with *F. vesiculosus* (3 cases), *L. ochroleuca* (6 cases) and *Ulva* sp. (7 cases), but Binomial tests ($P=0.375$, $P=0.313$, and $P=0.164$ respectively) indicated that the null hypotheses of equality of outcomes (1:1 ratio) could not be rejected.

3.2. Polyphenolic content

Polyphenolic content varied significantly among seaweeds ($P<0.001$; Fig. 2; Table 2), but there was no significant variation between sampling times ($P=0.209$, Fig. 2; Table 2). *F. vesiculosus* had the greatest concentration of polyphenols, compared with the rest of the seaweeds (*Ulva* sp. = *S. muticum* = *L. ochroleuca* < *F. vesiculosus*; SNK tests, $P<0.01$).

Table 1
Percentage of significant preference and no preference cases derived from χ^2 tests applied to no-choice (Stage 1) and choice (Stage2) data for each species.

|	Preference		No preference
	Fucus vesiculosus	*Sargassum muticum*	
Littorina littorea	11.1	33.3	55.6
Littorina obtusata	62.5	25.0	12.5
Aplysia punctata	25.0	25.0	50.0
Paracentrotus lividus	7.7	15.4	76.9
	Laminaria ochroleuca	*Sargassum muticum*	
Littorina littorea	28.6	33.3	38.1
Littorina obtusata	12.5	25.0	62.5
Aplysia punctata	20.0	40.0	40.0
Paracentrotus lividus	23.1	23.1	53.8
	Ulva sp.	*Sargassum muticum*	
Littorina littorea	61.5	7.7	30.8
Littorina obtusata	25.0	12.5	62.5
Aplysia punctata	57.1	0.0	42.9
Paracentrotus lividus	38.5	15.4	46.2
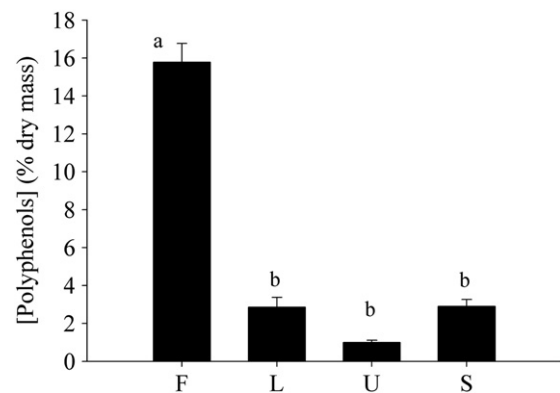
Fig. 2. Mean (+SE) polyphenolic content ($n=12$) of *Fucus vesiculosus* (F), *Laminaria ochroleuca* (L), *Ulva* sp. (U) and *Sargassum muticum* (S). SNK post hoc tests were used for the multiple comparisons between seaweed species. Lower case letters indicate grouping of means.

Table 2
Analysis of variance of polyphenolic content. Seaweed (4 levels: *Fucus vesiculosus*, *Laminaria ochroleuca*, *Ulva* sp. and *Sargassum muticum*) and time (2 levels: beginning and end of each trial) are fixed and orthogonal factors ($n = 12$).

Source	df	MS	F	P
Time (T)	1	14.41	1.35	0.249
Seaweed (S)	3	1086.20	101.50	**0.000**
S×T	3	16.02	1.50	0.221
Res	88	10.70		
Tot	95			

4. Discussion

Knowledge of whether or not observed non-random patterns of association are caused by preference is of great value to ecologists for predicting and understanding responses to changes in availability of resources (Jackson and Underwood, 2007). Some studies of feeding behaviour have demonstrated non-random associations between grazers and different species of macroalgae (e.g. Jormalainen et al., 2001; Britton-Simmons, 2004; Strong et al., 2009; Monteiro et al., 2009). Although most of these studies suggested patterns of prey consumption as a result of preference by herbivores or some behavioural choice, in most cases this could not be demonstrated because consumption of seaweeds when choice was present was not comparable with the consumption when no-choice was available (see Jackson and Underwood, 2007). An important aspect of the experimental design used here was the unconfounding of preference from any other reasons because the consumption of each seaweed species when available alone (i.e. when there was no choice and therefore no preference) was also determined (Underwood and Clarke, 2005; Jackson and Underwood, 2007).

Accordingly with ERH (one of the most cited invasion theories; Elton, 1958), we predicted that grazers would prefer native species of algae rather than the invasive *S. muticum*. This should lead to a competitive advantage of the invader over native species. Despite variability of responses within and among herbivorous species, results suggested that native grazers showed some active selection and preferred native seaweeds over the invasive *S. muticum*. In most cases, the gastropods *L. littorea* and *A. punctata* preferred to feed on *Ulva* sp., whereas *L. obtusata* seemed to prefer *F. vesiculosus*. The sea urchin *P. lividus*, however, showed no preference feeding indiscriminately on different seaweeds. It is also important to highlight that in many cases (see Table 1) grazers did not show any preference (i.e. consumption when a choice was available could not be differentiated from consumption when there was no-choice). These results, therefore, partially supported our prediction and reinforced the notion that well designed experiments and analyses must be done before preference can be inferred (Jackson and Underwood, 2007). Nevertheless, feeding preferences may be affected by a number of factors, including grazing pressure, recent availability of resources and competition (see Cronin and Hay, 1996b, Tuya et al., 2001). For example, starved fauna generally feeds less discriminately than fauna that has been fed (Cronin and Hay, 1996b). Thus, research should be done taking all these aspects into account to ensure that results may be extrapolated to natural systems.

The large intra-specific variability in responses by grazers used in this study might be due in part to the influence of several factors such as past diet, reproductive status and condition of individuals (see Poore and Hill, 2006). For example, periods of starvation have been shown to increase the likelihood of sea urchins consuming seaweeds containing chemical deterrents (Cronin and Hay, 1996b) and of fish consuming artificial diets containing deterrent metabolites (Thacker et al., 1997). Preferences of the amphipod *Peramphithoe parmerong* were dependent on the identity of the alga recently consumed (Poore and Hill, 2006). Similarly, some marine grazers are also known to change diet with size (e.g. the sea hare *A. californica*, Pennings, 1990).

Such results have obvious implications for the conduct and interpretation of assays for feeding and preference in the laboratory (see Cronin and Hay, 1996b).

Previous studies have revealed contrasting results, showing both low (e.g. Britton-Simmons, 2004; Pedersen et al., 2005; Monteiro et al., 2009) and high (Strong et al., 2009) preference by grazers for the invasive *S. muticum* compared to native seaweeds. In our study, some individuals of *L. littorea*, *A. punctata* and *P. lividus* also showed preference for *S. muticum* (see Table 1). This mostly occurred when *S. muticum* was compared with *F. vesiculosus* and may be linked to the presence of large amounts of polyphenols in *F. vesiculosus*. These compounds are considered to be the main defences against herbivores, although they may have a variety of other functions (see Jormalainen et al., 2001). For example, phlorotannins have been found to deter herbivory by fishes, urchins and mesoherbivores, although their effectiveness is highly variable (see Hay and Steinberg, 1992; Pavia and Brock, 2000). In contrast, the presence of high levels of polyphenols in *F. vesiculosus* did not seem to deter *L. obtusata*, which showed a clear preference for this alga. This is not surprising, since this periwinkle is commonly associated with and feeds upon fucoid algae, in particular *Ascophyllum nodosum*, *F. serratus* and *F. vesiculosus* (Watson and Norton, 1987). In addition to chemical defences, other factors such as nutritional content or toughness of algae can also affect feeding behaviour of invertebrates (Tuya et al., 2001; Strong et al., 2009).

The results of this investigation showed large variation in choice of food among and within species of grazers, supporting the assertion that effects of grazers on their host algae cannot be easily generalized (Pavia et al., 1999). Furthermore, in most cases where preference was expressed, native seaweeds were preferred over *S. muticum*. Response by grazers to *S. muticum* in the laboratory may suggest that *S. muticum* is not under substantial pressure from grazers in the area of study. Nevertheless, if preferences can vary depending on past diet, physiological condition of algae, and other local factors, such as grazing pressure or competition (Tuya et al., 2001; Van Alstyne et al., 2001; Sotka, 2003; Poore and Hill, 2006), the likely impacts of herbivores on algal communities might vary over space and time.

We conclude that, in addition to preference for seaweeds other than *S. muticum*, intrinsic characteristics of this macroalga, i.e. fast growth and high fecundity (Arenas and Fernández, 2000), and local characteristics of the environment or recipient assemblages, such as availability of nutrients, diversity and identity of native species and levels of intra- or inter-specific competition (Incera et al., 2009; Olabarria et al., 2009) may facilitate the spread and invasion of this invader in the area of study. Experimental field research to examine top-down control of assemblages of seaweed and its effect on invasibility is necessary in order to elucidate the role of grazers in controlling the spread of *S. muticum*.

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