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Consumption and feeding preference of *Echinogammarus marinus* on two different algae: *Fucus vesiculosus* and *Ulva intestinalis*

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Abstract

Echinogammarus marinus constitutes the most abundant amphipod species in *Fucus* spp. assemblages from many north Atlantic estuaries. However, there are some doubts about the real use of fucoids by the amphipod. Whilst some studies report the ingestion of *Fucus vesiculosus* by *E. marinus*, others suggest that the amphipod preference for fucoids is mostly related to sheltering rather than feeding, due to the high phlorotannin content of brown algae. The purpose of the present work was to disentangle this issue by checking the consumption rate and feeding preference of *E. marinus* on *F. vesiculosus*, its preferential habitat, and on *Ulva intestinalis*, a green algae abundant in the Mondego estuary (Western Coast of Portugal) and usually considered as highly palatable for herbivores.

In a 2-stage laboratorial setup, fresh discs of the two types of algae were offered to *E. marinus* for three days. Consumption rates were estimated from differences between algal and animal initial and final fresh weights using a control correction factor, while preference was tested by differences in algal consumption rates when no choice was offered (stage I) and when the two algae were offered simultaneously (stage II).

Results showed that *E. marinus* effectively consumed fresh *F. vesiculosus* in much higher amounts than *U. intestinalis* and significantly preferred to consume *F. vesiculosus* over *U. intestinalis*. Therefore, feeding habits must be one of the factors

related to the close association of the amphipod with *F. vesiculosus*, although other factors may also be involved (e.g. sheltering).

Key-words: amphipod, fucoids, green algae, consumption, feeding preference

1. Introduction

Macroalgae and mesograzers are crucial components in coastal areas, frequently displaying important roles in the stability, biodiversity and production of these systems (Duffy and Hay, 2000; Kraufvelin, 2007; Poore et al., 2009; Korpinen et al., 2010) and often exhibiting interactions between each other (Cronin and Hay, 1996; Davenport and Anderson, 2007). However, neither interactions between macroalgae and grazers are straightforward (Van Alstyne et al., 2009), nor is the ecological role of herbivores in coastal systems fully understood (Duffy and Hay, 2000; Davenport and Anderson, 2007; Poore et al., 2009). A better knowledge of such aspects will increase our understanding about the links along marine food-webs and, consequently, about its structure and function (Ruesink, 2000; Kraufvelin et al., 2006; Díaz et al, 2012).

Echinogammarus marinus is a ubiquitous amphipod in marine littoral communities from the North East Atlantic coast (Lincoln, 1979), occurring in high abundances on hard bottoms and rocky substrates covered by the brown seaweed *Fucus vesiculosus* (Maranhão et al., 2001; Guler and Ford, 2010; personal observation). Several studies corroborate *E. marinus* as an important species of the structure and functioning of intertidal communities, contributing with significant values for the local secondary production (Leite et al., in press), acting as an active predator of other macroinvertebrates (Dick et al., 2005) or being an important prey for wading birds (Múrias et al., 1996). Nonetheless, the close association between *E. marinus* and fucoid seaweeds has never been carefully investigated and, consequently, it is not fully understood. In some cases, fresh *Fucus* has been regarded as unpalatable to the amphipod due to its content on phenolic compounds (Maranhão et al., 2001 and references therein), whilst the ingestion of dry and chopped *F. vesiculosus* by *E. marinus* has been reported in laboratory experiments (Maranhão and Marques, 2003). Additionally, the density of *E. marinus* has been positively correlated with the biomass of green macroalgae (*Ulva* spp.), which was attributed to variation of food resources (Maranhão et al., 2001).

The Mondego estuary (Portugal) is a small mesotidal system (7.2 km^2), which nonetheless has significant contributions to the local economy and sustainability (Pinto et al., 2013). In the intertidal area, preferably associated with hard bottoms, *F. vesiculosus* can be found all year-round and within its fronds, the amphipod *E. marinus* displays abundances which can range between 40 and 1350 $ind.m^{-2}$ (Leite et al,. in press). The mudflats of the Mondego estuary are also occupied by rooted-macrophytes (*Zostera noltei*, *Spartina maritima*), *Gracilaria* sp. and *Ulva intestinalis* (Cardoso et al., 2004; Leston et al., 2008; Neto et al., 2013). Being an eutrophic system, blooms of green macroalgae (mostly *U. intestinalis*) may occur and cover substantial extensions of the mudflats (Dolbeth et al., 2003; Patricio and Marques, 2006; Martins et al., 2007). The growth of opportunistic macroalgae depends on the variation of local hydrodynamics, which in turn controls salinity, currents and water residence time (Martins et al., 2001; Kenov et al. 2012).

The purpose of this work was to highlight some aspects of the relationships between *E. marinus* and two of the most abundant macroalgae in the system, *F. vesiculosus* and *U. intestinalis*. In particular, we have quantified consumption rates and checked for the feeding preference of the amphipod on *F. vesiculosus*, its most common habitat, and on *U. intestinalis*, a fast-growing green macroalgae.

2. Material and methods

2.1. Sampling and acclimation

During November 2012, *U. intestinalis* and *F. vesiculosus* fronds with *E. marinus* individuals (visually confirmed) were collected from the intertidal area of the south arm of Mondego estuary (Western coast of Portugal: 40°08' N, 8°50' W) and taken to the lab. Seawater and sand were also collected and taken to the lab for preparation of acclimatization procedures. During acclimation, individuals of *E. marinus* corresponding to 3 different areas were assigned to 3 different aquaria to ensure independency of data (Underwood and Clarke, 2005). Each aquarium, containing 6L of filtered seawater continuously aerated and a sand-bottom layer, was kept at constant temperature and light conditions (20°C and 12h:12h light:dark cycle) for 4 days. During this period, amphipods were fed *ad libitum* with fresh fronds of *F. vesiculosus* and *U. intestinalis*. One day prior to the experiment, 10 amphipods were removed from each of the 3 acclimation aquaria (3 groups of 10 amphipods). Amphipods were individually allocated to 14cm x 14cm aquaria containing 400 mL of filtered seawater, continuously aerated, and kept in starvation for 1 day to equalize the hunger state among individuals (Underwood et al., 2004). In the same day, fresh *F. vesiculosus* and *U. intestinalis* were collected from the previously referred site in the Mondego estuary.

2.2.Consumption and feeding preference experiment

On the day of the experiment, algal discs were cut from the newly collected *F. vesiculosus* and *U. intestinalis* using a metallic cylinder (Ø = 1.8 cm). *Fucus* discs were never cut on the apical part of the alga to avoid bias derived from potentially different inter-individual growth rates. At the beginning of the experiment, which lasted for 3 days, algal discs were distinctly added to the 30 aquaria containing starved *E. marinus* individuals: 10 aquaria were provided only with *Ulva* discs, 10 aquaria were provided only with *Fucus* discs and 10 aquaria were provided both with *Ulva* and *Fucus* discs. 10 controls were also run for each treatment (Total=30 controls) (Fig. 1).

This experimental design allows running stage 1, when animals are separately fed with different food items and proportions eaten are registered (p_1 and p_2 as 2 different items are being tested) and stage 2, when animals are simultaneously fed with different food items and proportions eaten are also registered (q_1 and q_2) (Underwood and Clarke, 2005). The null hypothesis of no preference is:

H_0: $q_1=\theta p_1$; $q_2=\theta p_2$

θ is a constant, unknown parameter.

Proportions of algae eaten were estimated as consumption rates, where consumption of algae by *E. marinus* (g g fresh wt^{-1} d^{-1}) was calculated as in Canhoto and Graça (1995):

Consumption=$(A_i-(A_f.k))/(W_f.d)$ (Eq. 1)

A_i and A_f are algal initial and final fresh weight, respectively. W_f is the animal's final fresh weight, d is the duration of the experiment in days;

$k=C_i/C_f$….(Eq. 2)

C_i and C_f are initial and final fresh weight of algae in controls, respectively.

T-tests were performed with STATISTICA software package (StatSoft).

3. Results

At stage 1, *E. marinus* consumed 0.04 ± 0.04 g g fresh wt^{-1} d^{-1} (average ± stdev) of *U. intestinalis* and 0.15 ± 0.10 g g fresh wt^{-1} d^{-1} of *F. vesiculosus*. At stage 2, *E. marinus* consumed 0.02 ± 0.01 g g fresh wt^{-1} d^{-1} of *U. intestinalis* and 0.29 ± 0.20 g g fresh wt^{-1} d^{-1} of *F. vesiculosus*, respectively (Fig. 2).

T-test results for *Ulva* consumption rate at stage 1 and 2 showed no significant differences (T-test, $t_{0.05\ (2),\ 18} = 1.316$; $p=0.204$), while *Fucus* consumption rate by *E. marinus* at stage 2 was significantly higher than consumption of *Fucus* at stage 1 (T-test, $t_{0.05\ (2),\ 18} = -2.11$; $p<0.05$), which allowed rejecting the null hypothesis.

4. Discussion

According to results, *E. marinus* showed a much higher consumption rate of *F. vesiculosus* compared to *U. intestinalis* and a clear consumption preference of the fucoid over the green algae. At stage 1 of the experiment, when the two algae were offered separately, the consumption rate of *Fucus* by *E. marinus* was about 4-times higher than the consumption rate of *Ulva* (0.15 *versus* 0.04 g g fresh wt^{-1} d^{-1}), while during stage 2, when the two algae were offered simultaneously, the consumption rate of *F. vesiculosus* by the amphipod increased to 15-times more the consumption of *U. intestinais* (0.29 versus 0.02 g g fresh wt^{-1} d^{-1}). These results allowed rejecting the null hypothesis and concluding that *E. marinus* clearly prefers to consume *F. vesiculosus* rather than *U. intestinalis*.

The preference of some grazers by fucoids is well reported in literature. For instance, the isopod *I. balthica* and the gastropod *L. obtusata* prefer to feed on *F. vesiculosus* rather than on *Ulva intestinalis* (Jormalainen et al., 2001; Cacabelos et al., 2010). In case of the isopod, the preference by *F. vesiculosus* is attributed to habitat structure and spatiotemporal stability of the host algae (Jormalainen et al., 2001), while the periwinkle preference by fucoids has been related to the nutritional content or toughness of algae, which besides chemical defences, can also affect the feeding behaviour of invertebrates (Cacabelos et al., 2010 and references therein). In contrast, some grazers seem to prefer opportunistic green macroalgae (Goecker and Kåll, 2003), which is frequently attributed to the high internal nutrient content of this type of algae (Kraufvelin et al, 2006; Van Alstyne et al., 2009). In the case of the present work, it is not possible to conclude about the factors that determine the preference of *E. marinus* by *F. vesiculosus* compared to *U. intestinalis*, as the experimental design was conducted to assess consumption rates and feeding preference of *E. marinus* on two different macroalgae. However, when the algal discs were placed in the aquaria most of the individuals rushed to the *Fucus* discs and hid underneath them, indicating a possible sheltering preference for this alga (personal observation). In fact, *F. vesiculosus* is present all year in the rocky margins of the Mondego estuary whereas other algae have a seasonal occurrence, thus reinforcing the possibility that this alga provides a stable environment for *E. marinus*.

Because feeding preferences have important consequences for community organization (Duffy and Hay, 2000), it would be important to further investigate some questions, such as, identifying the factors that determine the preference of *E. marinus* by *F. vesiculosus*, quantifying the impacts of grazing by *E. marinus* on *F. vesiculosus* assemblages.

According to experimental evidence, grazers can have significant impacts on brown macroalgae (Davenport and Anderson, 2007). In the case of *F. vesiculosus* in the Baltic Sea, grazing pressure is thought to be high (Jormalainen and Honkanen, 2008) particularly at the colonization stage, when grazers can consume up to 80% of the juveniles (Korpinen at al., 2007). None of the research carried out in the Mondego estuary has ever evaluated the effects of grazing by *E. marinus* on local assemblages of *F. vesiculosus*. However, this would be a pertinent question to investigate in a system, which constitutes the southernmost limit of the amphipod distribution.

5. Conclusions

From the present results, it is possible to conclude that *E. marinus* clearly prefers to consume *F. vesiculosus* over *U. intestinalis*. Thus, the close association of the amphipod with fucoids seems to be related with *E. marinus* feeding habits, although other factors may also be involved (e.g. sheltering). In fact, further studies are needed to fully understand the preference of *F. vesiculosus* by *E. marinus*.

Finally, the present work also corroborates the idea that algal preference by macroinvertebrates depends on numerous factors and does not exhibit straightforward patterns.

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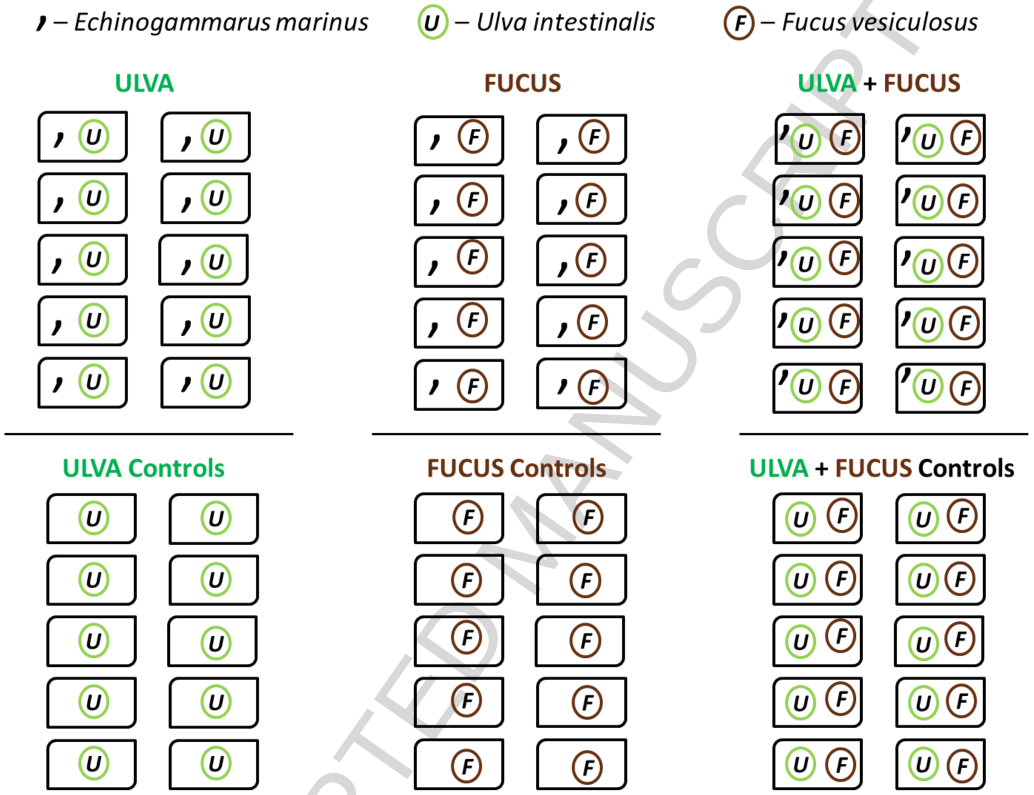
Figure 1- Experimental design of the 2-stage experiment to assess consumption and feeding preference of *F. vesiculosus* and *U. intestinalis* by *E. marinus*.

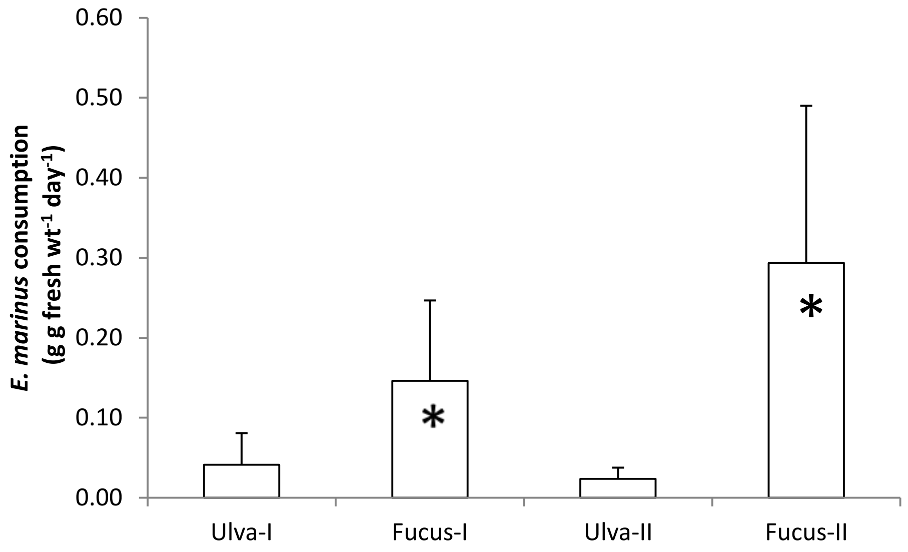
Figure 2- Average consumption rate ± standard deviation (g g fresh wt^{-1} d^{-1}) of *F. vesiculosus* and *U. intestinalis* by *E. marinus* during stage 1 (I) and stage 2 (II). *- Significant differences (t-test, $p<0.05$).

Figure 1



– Echinogammarus marinus
U – Ulva intestinalis
F – Fucus vesiculosus
ULVA
FUCUS
ULVA + FUCUS
ULVA Controls
FUCUS Controls
ULVA + FUCUS Controls

Figure 2



E. marinus consumption
(g g fresh wt-1 day-1)
0.60
0.50
0.40
0.30
0.20
0.10
0.00
*
*
Ulva-I
Fucus-I
Ulva-II
Fucus-II

Highlights

- *Echinogammarus marinus* consumed fresh *Fucus vesiculosus* in high amounts;

- *Echinogammarus marinus* has shown a clear consumption preference of *Fucus vesiculosus* compared to *Ulva intestinalis*.

- The close association of *E. marinus* with *Fucus vesiculosus* must be related with feeding factors, although other factors (e.g. sheltering) may also be involved.