

**Effects of cigarette butts on marine keystone species (*Ulva lactuca* L. and *Mytilus edulis* L.) and sediment microphytobenthos**

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**Abstract**

Outdoor mesocosms with constantly flowing natural seawater were used to test the effects of littered cigarette butts on the filter feeder *Mytilus edulis* (blue mussel), the macroalga, *Ulva lactuca* (sea lettuce) and sediment microphytobenthos in a semi-natural marine setting. Either conventional, cellulose acetate, or biodegradable, cellulose, smoked cigarette butts were added at densities of 0.25 or 1 butt L<sup>-1</sup>. The clearance rates of mussels exposed to 1 butt L<sup>-1</sup> of cellulose acetate butts were three times less than the controls. The growth of *U. lactuca* was not measurably affected by cigarette butts, however the sediment chlorophyll content was significantly less in mesocosms exposed to 0.25 and 1 butt L<sup>-1</sup> of cellulose acetate butts. These effects occurred despite constant replacement of seawater indicating how hazardous conventional cigarette butts are to marine life. Biodegradable cellulose cigarette butts had minimal effects on the measured variables but should still not be discarded as litter.

**Key words:** smoking, cellulose acetate, green butts, biodegradable, single use plastics, hazardous.

## 1. Introduction

Global consumption of cigarettes has been rising steadily for years and tobacco consumption is currently considered a global epidemic by the World Health Organization (WHO 2019). In 2016, ~5.7 trillion cigarettes were smoked worldwide and it is predicted that by 2025 at least 9 trillion cigarettes will be smoked annually worldwide (Araujo and Costa, 2019). With the majority of smokers littering their used filters (i.e. cigarette butts) (~75%; Patel et al. 2013), it is not surprising that they have maintained their position as the most abundant litter item found in beach cleans for over 30 years (Ocean Conservancy 2019). They are difficult to collect as litter, especially due to their small size, and many remain in the environment even after organised litter picking events (Loizidou et al. 2018). Tourist holiday locations are particularly prone to cigarette litter with densities up to 13.3 butts m<sup>-2</sup> on beaches in Thailand (Kungskulniti et al. 2018) and a mean of 8 butts m<sup>-2</sup> on a beach in Uruguay (Rodríguez et al. 2020). Cigarette butts are also one of the most common litter items caught by floating litter collection devices used in marinas and harbours (Seabins™, The Seabin Project 2020). For example, they account for ~29% of all litter collected by Seabins™ in France (Plastics Europe 2019). Once washed or thrown into water, cigarette butts are only buoyant on the water surface for a short time before they sink (Rech et al, 2014), potentially to be washed back onto shore or further out to sea via waves or currents (Roman et al. 2020). Recently, a citizen-science program: “Dive Against Debris®”, found that cigarette butts were the second most common single use plastic item found on the Mediterranean seafloor (at <30 m depth); accounting for 5.14% and cigar tips 3.4% of total debris (Consoli et al. 2020).

The majority (~90%; Pauly et al. 2002) of cigarette filters are still composed of cellulose acetate and are not readily biodegradable (<15% weight loss per year in seawater; Gerritse et al. 2020), but can fragment and persist as micro- or nano- sized plastic fibres (Chevalier et al. 2018). Even clean, unsmoked cellulose acetate cigarette filters can cause detrimental effects on

plants (Green et al. 2019), marine and freshwater fish (Slaughter et al. 2011) and amphibians (Lawal and Ologundudu 2013). Once smoked, however, cigarette butts present a greater risk to the environment than unsmoked filters due to thousands of chemicals including, for example, nicotine, polycyclic aromatic hydrocarbons and heavy metals which are retained in the butt and can leach into the water (Moerman and Potts 2011; Roder Green et al. 2014; Dobaradaran et al. 2019; Dilip et al. 2021). Such leachate has been found to be lethal for marine fish (Slaughter et al. 2011) and gastropods (Booth et al. 2015).

In response to concerns about plastic cigarette filters, alternative filters, composed of pure cellulose have arrived on the market. Alternative cellulose filters have been described as “green”, “biodegradable” and “environmentally friendly” implying they would be benign as litter (Amos et al. 2017). A recent experiment, however, found that leachate derived from cellulose cigarette butts had the same detrimental effects on freshwater invertebrates as leachate derived from cellulose acetate cigarette butts (Green et al. 2020). The comparative effects of cellulose acetate versus cellulose cigarette butts have not yet been tested in a marine system. Indeed, despite their prevalence as litter, the effects of any type of cigarette butt on benthic marine organisms has seldom been tested. Of the few studies carried out on benthic marine organisms, lethal effects have been found on gastropods (*Austrocochlea porcata*, *Nerita atramentosa* and *Bembicium nanum* exposed to 5 butts L<sup>-1</sup>; Booth et al. 2015), sublethal behavioural (when exposed to leachate from 4 – 8 butts L<sup>-1</sup>) and genotoxic (when exposed to leachate from 8 butts L<sup>-1</sup>) effects on polychaetes (*Hediste diversicolor*; Wright et al. 2015) and alterations to microbial assemblages (exposed to 25 butts L<sup>-1</sup>; Quéméneur et al. 2020). These experiments, however, were conducted using highly controlled, closed aquatic systems which did not simulate the continuous flow and replacement of seawater that occurs in the marine environment.

The aim of the current study was to assess the impacts of conventional versus alternative smoked cigarette filters (butts) in a model benthic habitat with flowing seawater by examining physiological responses of the benthic filter feeder *Mytilus edulis* Linnaeus (1753) (blue mussel), the primary producer *Ulva lactuca* Linnaeus (1758) (sea lettuce) and sediment microphytobenthos. It was hypothesised that butts made of cellulose and cellulose acetate would have similar, but negative (i.e. reducing) effects on the clearance rate and attachment strength of *M. edulis*, growth rate of *U. lactuca* and the concentration of chlorophyll-*a* and -*c* as a proxy for the sediment microphytobenthos.

## **2. Material and methods**

### *2.1. Preparation of cigarette butts*

Cigarettes were rolled by hand using standard, bleached cigarette papers (Rizla, Bristol, UK) filled with an average ( $\pm$  S.E.) of  $0.543 \pm 0.002$  g per cigarette of a leading brand of tobacco. Cigarettes contained either a cellulose acetate (slim size; 5 mm diameter x 14 mm length) or a cellulose (unbleached) filter (slim size; 6 mm diameter x 15 mm length). All cigarettes were smoked using a hand-operated vacuum pump in a fume cabinet with silicone tubing attached to the filter of the cigarettes. After lighting, approximately  $30 \pm 1$  mL of air was drawn into each artificial “breath” and each cigarette was smoked for a total inhalation volume of ~600 mL per cigarette, thereby emulating a similar total inhalation volume smoked by humans ( $549 \pm 166$  to  $585 \pm 245$  mL; McBride et al. 1984). Cigarette butts were added to mesocosms 24 - 28 hours after smoking.

### *2.2. Experimental design and mesocosm set-up*

The experiment consisted of an asymmetric design with 2 fixed factors; Butts (2 levels; cellulose versus cellulose acetate) and Concentration (2 levels; 0.25 and 1 butt L<sup>-1</sup> equivalents). A single Control treatment was also included which consisted of no added butts. Each of the

five treatments was replicated using 6 separate mesocosms (n=6, N=30). The experiment was carried out in an outdoor mesocosm system at the Queen's University Marine Laboratory (QML), Portaferry, Northern Ireland, with natural light conditions (unenclosed system with no roof) and continuously through-flowing, sand filtered seawater pumped from the adjacent Strangford Lough. Mesocosms consisted of opaque polypropylene buckets with a 10 L capacity (height = 25 cm, diameter = 25 cm), each filled up to 3 cm depth with clean coarse sand (autoclaved, median grain size 500 – 1000  $\mu\text{m}$ ) and to a volume of 8 L with seawater and left open at the top to ensure full natural light availability. Five individual *Mytilus edulis* (blue mussel) with an average ( $\pm$ S.E.) length of  $45.6 \pm 0.2$  mm and wet biomass of  $14.17 \pm 0.22$  g were added to each mesocosm onto a square, 25 cm<sup>2</sup> slate settlement plate. Mussels were sourced from Strangford Lough and were acclimatised to the QML outdoor mesocosm system for >3 months before being used in the experiment. In addition, one individual *Ulva lactuca* (sea lettuce) was added to each mesocosm with an average wet biomass of  $4.63 \pm 0.04$  g and secured to a pebble using a piece of cotton string in order to simulate how they were found in the field attached to the substratum. *Ulva lactuca* had been collected from the shore outside QML and maintained within separate outdoor flow-through seawater tanks, for a period of 48 hours prior to commencement of the experiments. When in the mesocosm, *M. edulis* were fed every 2 days throughout the experiment with 100 mL of  $\sim 5 \times 10^5$  cells mL<sup>-1</sup> of the microalga *Nannochloris atomus*. The mesocosms were allowed to settle for 48 hours before introduction of any cigarette butts, and on day 1 of the experiment, treatments were randomly assigned to mesocosms and corresponding butts were added by dropping them onto the surface of the water. Most (~90%) butts sank immediately to the sediment, but some remained floating at the surface for up to 2 hours before sinking. Throughout the experiment, the water in the mesocosms was ~10°C with a pH of ~8.2 and salinity of ~33 ppt and was continuously being replaced via individual hoses at a rate of ~500 mL min<sup>-1</sup> meaning that the water was completely

replaced >3 times per hour. Each mesocosm was a completely independent replicate and wastewater discharged from mesocosms could not leak into any other mesocosm, with a mesh on their outlet to prevent the butts from being inadvertently removed from the mesocosms. In this way, butts were retained within the mesocosms and were added only once.

### 2.3. Measuring responses of *M. edulis* exposed to cigarette butts

After 5 days of exposure in the outdoor mesocosms, clearance rates were estimated using one individual *M. edulis* from each mesocosm. *M. edulis* were held in separate 500 mL glass beakers with an air bubbler and clean sand filtered seawater containing  $\sim 5 \times 10^4$  cells mL<sup>-1</sup> of the microalga *N. atomus*. *M. edulis* began filtering almost immediately and samples of 5 mL were taken after 0, 20, and 40 min and algal cells were counted using a haemocytometer. This time length was chosen because it is below the saturation reduction level for *M. edulis* whereby clearance is reduced when feeding for > 2 hours at  $3 \times 10^4$  or more cells mL<sup>-1</sup> (Pascoe et al. 2009). The dry biomass of each individual *M. edulis* used in the clearance rates was determined by drying at 60°C for 24 hours and weighing to the nearest µg. Clearance rates were expressed as litres of water cleared h<sup>-1</sup> g<sup>-1</sup> dry weight.

Tenacity (or attachment strength) of one mussel per mesocosm was measured after 5 days of exposure using a portable dynamometer (Pesola, Sweden) scaled 0–10 N to measure the maximal vertical force required for the individual to become dislodged (attachment strength, N). The dynamometer had a small clamp attached to it that gripped individual mussels laterally without displacing them. The maximum dislodgement force to the nearest 0.1 N was recorded for one mussel from each mesocosm. The surface area of each mussel was approximated to an ellipse using height and width (measured with Vernier callipers to 1 mm) as major and minor axes

(Bell and Gosline 1997). Tenacity is expressed as dislodgement force (N) per unit mussel area ( $\text{cm}^{-2}$ ).

#### 2.4. Measuring responses of primary producers to cigarette butts

After 10 days, each individual *U. lactuca* was removed and spun dry with a handheld centrifuge for 30 s before weighing fresh biomass to the nearest 0.01 g. Growth rates were calculated as the increase in biomass between days 0 and 10.

The biomass of the microphytobenthos (MPB) was estimated after 10 days by chlorophyll extraction. Approximately the top 1 cm of oxic sediment was sampled and wrapped in tin foil to protect from the sunlight. Chlorophyll was extracted immediately for 1 hour under constant shaking at room temperature in the dark after adding 10 mL of 90% acetone to ~1 g of wet, homogenised sand. Chlorophyll-a and chlorophyll-c concentrations were measured from the supernatant using a spectrophotometer and calculated according to equations by Jeffrey and Humphrey (1975). Concentrations are expressed as  $\mu\text{g}$  chlorophyll  $\text{g}^{-1}$  dry sediment.

#### 2.5. Statistical analysis

The design was asymmetrical (i.e. having a single control group for the two factors “Butt” and “Concentration”), therefore the data were analysed by using the mean squares from two independent ANOVAs (see Green et al. (2016) for an example of the calculations). Briefly, this included partitioning of the variance by calculating (1) a one-way ANOVA with all treatments as separate levels (five treatments  $\times$  six replicates each) and (2) a full-factorial two-way ANOVA of “Butt” by “Concentration” without the Control (two factors  $\times$  two levels  $\times$  six replicates each). The residuals of the 1st ANOVA were used to assess differences between the

levels within the 2nd ANOVA, allowing the variation associated with Control and that of the other treatments to be distinguished (“Control vs. Others”), which is contrasted with one degree of freedom (Underwood, 1997). When a significant effect in the “Control vs. Others” (C vs. O) contrast was found, Dunnett’s test was used to contrast the Control versus each level of the significant term. Post-hoc pairwise comparisons were also computed using Tukey HSD tests when the main terms in the full-factorial ANOVA were significant. Statistical significance was assumed at  $\alpha = 0.05$ . Data were screened for normality of distribution and homogeneity of variance to check that they conformed to the assumptions of ANOVA. All statistical analyses were done using R v3.6.2. (R Core Team, 2019).

### 3. Results

#### 3.1. Effects of cigarette butts on *M. edulis*

No individuals of *M. edulis* died during the experiment. The dry biomass of *M. edulis* did not significantly differ amongst treatments (Tables 1 & 2). Clearance rates of *M. edulis* were significantly reduced by the addition of 1 cellulose acetate butt L<sup>-1</sup>, causing a 2.6 times reduction in clearance rates compared with *M. edulis* in the Control mesocosms or in those dosed with cellulose butts (Table 1a). The tenacity of *M. edulis* was not significantly affected by the addition of cigarette butts (Tables 1a and 2).

#### 3.2. Effects of cigarette butts on primary producers

The growth rate of *U. lactuca* was positive in all mesocosms but was not significantly affected by cigarette butts (Tables 1b and 2). Chlorophyll-*a* content of the sediment in mesocosms exposed to 0.25 or 1 cellulose acetate butt L<sup>-1</sup> was 2.8 times less than that of the Control mesocosms and 2.2 times less than of mesocosms with 0.25 cellulose butts L<sup>-1</sup> (Table 1b, Figure 2). While mesocosms with 1 cellulose butt L<sup>-1</sup> had less chlorophyll-*a* than Control



mesocosms, this was not significantly different (Figure 2). Chlorophyll-*c* content was 3.5 times less in sediment contaminated with cellulose acetate butts than in sediment with 0.25 cellulose butts L<sup>-1</sup> (Table 1b and Figure 2).

#### 4. Discussion

The current study found that even with constant replacement of seawater, simulating a realistic marine environment, cellulose acetate cigarette butts significantly reduced the clearance rates of *M. edulis* and the chlorophyll content of the sediment, whereas cellulose cigarette butts had minimal impact.

Clearance rates of *M. edulis* are used in ecotoxicity testing because they are a sensitive and ecologically relevant sub-lethal endpoint (Abel 1976). Reduced clearance rates have also been found to occur in *M. edulis* in response to other contaminants including mercury (Micallef and Tyler 1990), copper (Al-Subiai et al. 2011) microplastics (Woods et al. 2018) and a range of hydrophobic organic chemicals (Donkin et al. 1989). A reduction in clearance rates of these ecosystem engineers could lead to cascading effects on water quality, nutrient cycling and primary productivity in sedimentary habitats due to their role in benthic-pelagic coupling (van der Schatte et al. 2020; Barbier et al. 2011). A prolonged reduction in feeding could lead to a reduction in health causing a decrease in reproductive output and/or growth performance (Seed and Suchanek 1992). Longer term studies will help to elucidate population level implications of the results of our short-term investigation.

Although there were no measurable effects on the growth rate of *U. lactuca*, the concentration of chlorophyll-*a* and *c* of the sediment was reduced even when exposed to just 0.25 cellulose acetate butts L<sup>-1</sup>. Effects on primary producers are important since they form the base of food webs. The microphytobenthos, for example, deliver an array of vital

ecosystem services including nutrient cycling, primary productivity and sediment stabilisation, and are an essential, but often overlooked, component of sedimentary habitats (Hope et al. 2019). They are also a pivotal food source for heterotrophs in sandy subtidal habitats (Evrard et al. 2012). In our study we quantified the effects on the early colonisation of the sediment by using clean sand as a starting point. It is also likely, however, that cigarette butts will affect established microphytobenthic communities as indicated by the recent work of Quéméneur et al. (2020) who found that leachate from cigarette butts altered established microbial communities in marine sand.

The effects on clearance rates of *M. edulis* and chlorophyll concentrations in the sediment could be due to a combination of the chemicals accumulated in the butt after smoking tobacco and the plastic itself in the cellulose acetate butts. Recently, Dilip et al. (2021) characterised 98 chemicals from smoked cigarette butt leachate, a third of which are classified as very toxic. In addition, leachate from unsmoked cellulose acetate cigarette filters has been found to be toxic to marine and freshwater fish (Slaughter et al. 2011) and to freshwater microalgae (Bonanomi et al. 2020) and unsmoked butts added as whole items have been found to decrease the germination and growth of ryegrass and clover (Green et al. 2019) and to reduce the pH of seawater and alter microbial communities in marine sand (Quéméneur et al. 2020). These effects could be due to plasticizers, such as diethyl phthalate, which in isolation can be toxic to plants (Cheng, 2012) and animals (Liu et al., 2009). It is possible that differences between the effects of cellulose acetate and cellulose cigarette butts in the current study were due to (i) a greater concentration of chemicals retained in cellulose acetate cigarettes after smoking, or (ii) leaching of plasticizers from cellulose acetate cigarette butts. A complete characterisation of the chemical profiles of each type of cigarette butt is needed in order to elucidate these mechanisms.

Although there was a marginal effect of 1 butt L<sup>-1</sup> of biodegradable cellulose cigarette butts on sediment chlorophyll concentrations, there were no statistically detectable impacts on the measured responses in the current study. In a closed system, however, such as a rockpool, biodegradable cigarette butts would likely cause similar effects to non-biodegradable cigarette butts due to the retention of leachate in the water (Booth et al. 2015). Indeed, a recent experiment in a closed freshwater system showed that biodegradable cellulose butts had similar detrimental effects as plastic cellulose acetate butts; causing mortality and a reduction of movement of four invertebrate species (Green et al. 2020). Cigarette butts, regardless of their biodegradability, pose a threat as litter in the environment and need to be disposed of appropriately.

#### *Recommendations and conclusion*

It is likely that littering of cigarette butts occurs due to misconceptions that they are benign, i.e. having no effect on the environment and that they are rapidly biodegradable. The majority (43%) of smokers surveyed in Germany for example, were not aware that cigarette filters are composed of synthetic material (Kotz and Kastaun 2020). Despite most cigarette butts being composed of a type of plastic, cellulose acetate, they are still not widely classified as a single use plastic. There is now evidence that cigarette butts can have detrimental effects on organisms in terrestrial (Green et al. 2019), freshwater (Green et al. 2020) and marine habitats (Booth et al. 2015, Wright et al. 2015 and the current study). To protect the environment, cellulose acetate cigarette butts should be globally classified as single-use plastics as there is urgent need to improve regulation relating to their use, collection and disposal. In addition, there needs to be an increase in campaigns to raise awareness of the impacts of cigarette litter, an increase in fines and smoking bans in areas of conservation importance (Axelsson and van Seville, 2017) and the introduction of extended producer responsibility for tobacco companies to hold

manufacturers responsible for collection, transport, processing and disposal of tobacco product waste (Curtis et al. 2017).

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## Tables and figures

**Table 1.** Asymmetrical ANOVA results for (a) tenacity (Tenacity), dry weight (DW) and clearance rates of *M. edulis* (Clearance rates) and (b) growth of *U. lactuca* (*Ulva* growth), chlorophyll-*a* or -*c* content of the sediment (Chl-*a*, Chl-*c*). F ratios with P-values significant at  $\alpha = 0.05$  are indicated in **bold**. MPB Chl-*a* and MPB Chl-*c* were square root and log (x+0.5) transformed respectively in order to meet the assumption of normality of distribution.

(a)		Tenacity			DW			Clearance rates		
Source of variation	d.f.	MS	F-ratio	P-value	MS	F-ratio	P-value	MS	F-ratio	P-value
One-way	4	0.02	0.57	0.686	0.01	0.62	0.651	23.94	<b>4.75</b>	<b>0.005</b>
Control vs others	1	0.03	0.82	0.374	0.00	0.42	0.521	21.80	<b>4.32</b>	<b>0.048</b>
Butt (B)	1	0.01	0.18	0.679	0.01	1.00	0.327	11.29	2.24	0.147
Concentration (C)	1	0.03	0.77	0.389	0.01	0.70	0.410	23.44	<b>4.65</b>	<b>0.041</b>
B x C	1	0.02	0.52	0.477	0.00	0.37	0.551	39.23	<b>7.78</b>	<b>0.010</b>

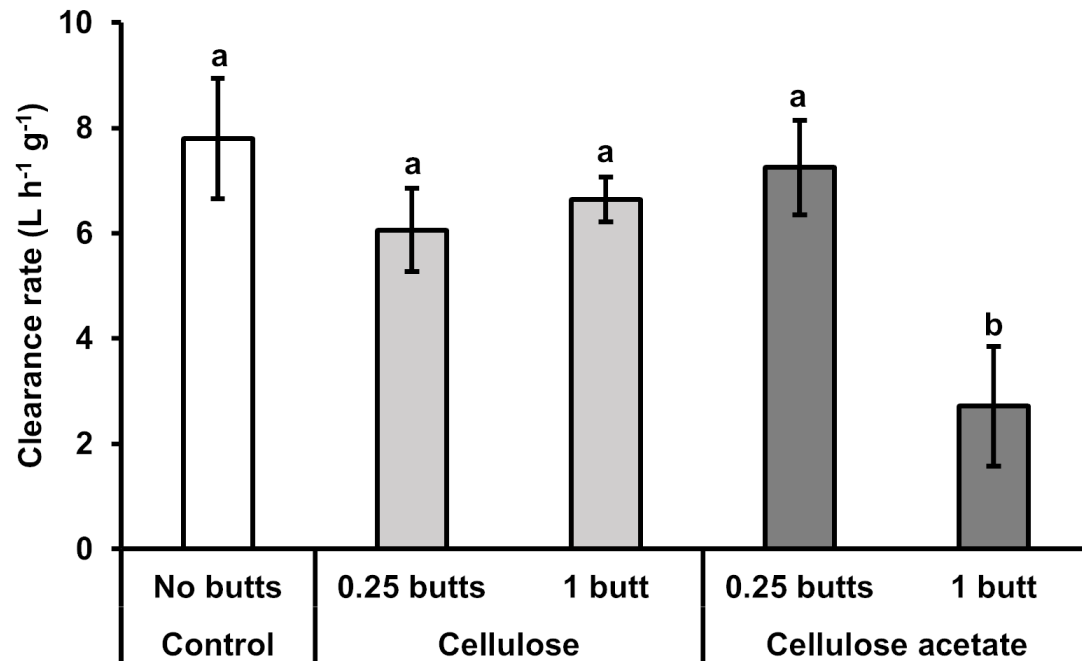
  

(b)		<i>Ulva</i> growth			MPB Chl- <i>a</i>			MPB Chl- <i>c</i>		
Source of variation	d.f.	MS	F-ratio	P-value	MS	F-ratio	P-value	MS	F-ratio	P-value
One-way	4	6.21	1.29	0.299	0.26	<b>4.71</b>	<b>0.006</b>	0.49	<b>2.97</b>	<b>0.039</b>
Control vs others	1	5.11	1.06	0.312	0.30	<b>5.77</b>	<b>0.024</b>	0.35	2.50	0.127
Butt (B)	1	6.66	1.39	0.250	0.43	<b>7.86</b>	<b>0.010</b>	0.94	<b>6.45</b>	<b>0.018</b>
Concentration (C)	1	9.55	1.99	0.171	0.18	3.89	0.060	0.35	1.66	0.210
B x C	1	3.51	0.73	0.400	0.10	1.30	0.264	0.30	1.27	0.271

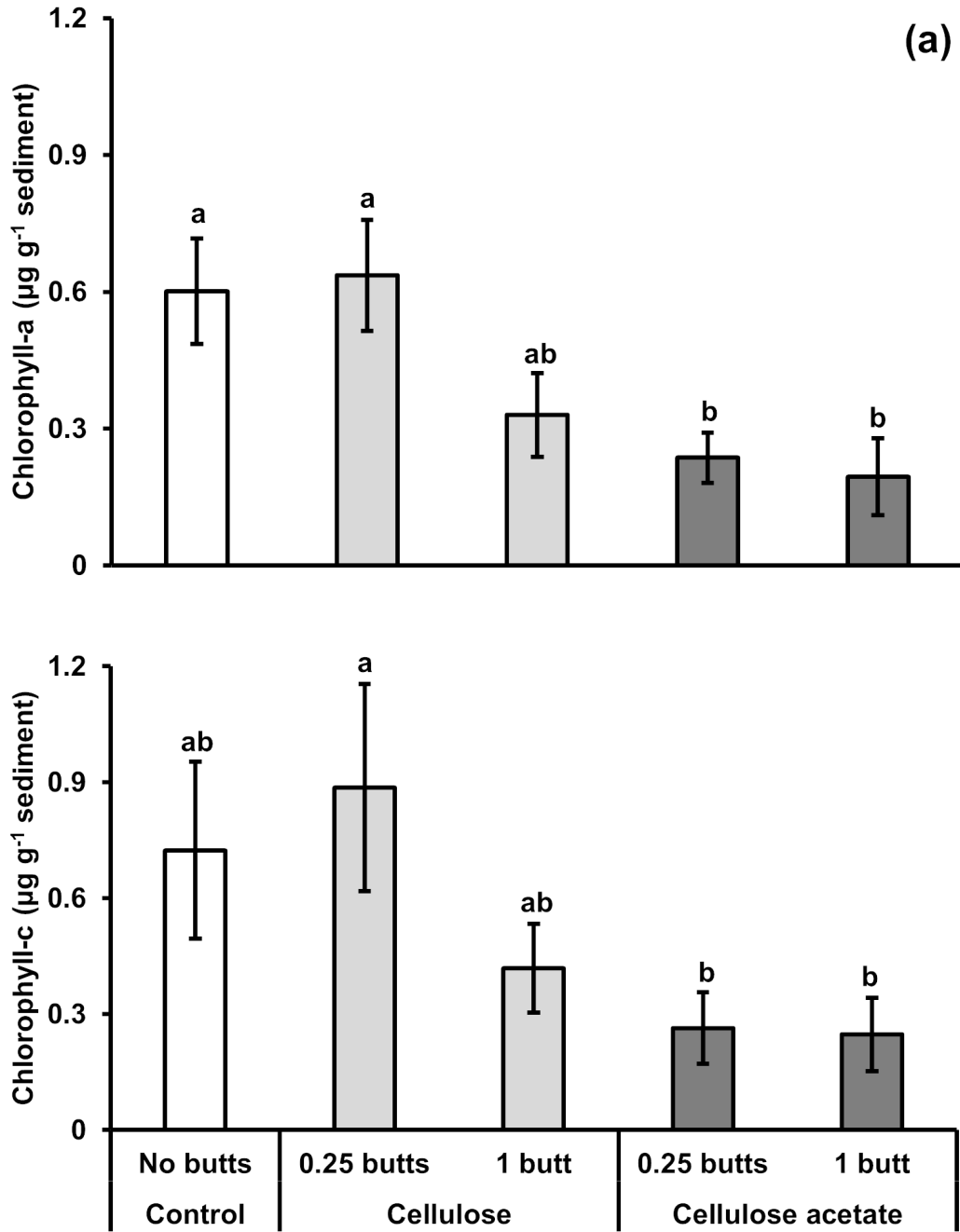


**Table 2.** Tenacity (N cm<sup>-2</sup>) and dry weight of flesh (g) for *M. edulis* and growth (g) of *U. lactuca* after exposure to either no butts (Control) or to 0.25 or 1 butt L<sup>-1</sup> of smoked cellulose (C) or cellulose acetate (CA) cigarette butts. Data are mean ± S.E.M., n = 6.

Response / Treatment	Tenacity (N)	Dry weight flesh (g)	<i>U. lactuca</i> absolute growth (g)
Control	2.2 ± 0.6	0.32 ± 0.04	5.69 ± 0.95
C 0.25 butts L <sup>-1</sup>	1.6 ± 0.3	0.37 ± 0.05	4.38 ± 0.66
C 1 butt L <sup>-1</sup>	1.8 ± 0.3	0.37 ± 0.03	3.89 ± 0.56
CA 0.25 butts L <sup>-1</sup>	0.3 ± 0.1	0.30 ± 0.04	6.20 ± 0.92
CA 1 butt L <sup>-1</sup>	0.2 ± 0.1	0.36 ± 0.05	4.18 ± 1.23



**Figure 1.** Clearance rates of *M. edulis* exposed to no butts or to smoked cellulose or smoked cellulose acetate cigarette butts at 0.25 or 1 butt L<sup>-1</sup>. Data are mean  $\pm$  S.E.M. based on dry weight, n = 6. Different superscript letters indicate a significant difference at  $\alpha = 0.05$ .



**Figure 2.** Chlorophyll-*a* (a) and chlorophyll-*c* (b) content extracted from sand exposed to either no butts (Control), smoked cellulose or smoked cellulose acetate butts at 0.25 or 1 butt  $\text{L}^{-1}$ . Data are mean  $\pm$  S.E.M. based on dry sediment,  $n = 6$ . Different superscript letters indicate a significant difference at  $\alpha = 0.05$ .