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Release of dissolved organic carbon from seagrass wrack and its implications for trophic connectivity

Paul S. Lavery

Edith Cowan University, p.lavery@ecu.edu.au

Kathryn M. McMahon

Edith Cowan University, k.mcmahon@ecu.edu.au

Julia Weyers

Mary C. Boyce

Edith Cowan University, m.boyce@ecu.edu.au

Carolyn Elizabeth Oldham

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1 **The release of DOC from seagrass wrack and its implications for trophic connectivity**

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3 Paul S Lavery¹, Kathryn McMahon¹, Julia Weyers², Mary C Boyce¹ & Carolyn E Oldham²

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5 ¹Centre for Marine Ecosystems Research, Edith Cowan University, 270 Joondalup Dve,

6 Joondalup WA 6027, Australia

7 ²School of Environmental Systems Engineering, The University of Western Australia,

8 Crawley WA 6009, Australia

9 *Corresponding author: p.lavery@ecu.edu.au

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11

12 **ABSTRACT**

13 The export of old leaves and stems (wrack) from seagrass meadows provides a mechanism for
14 trophic connectivity among coastal ecosystems. Since little of this wrack is consumed by
15 mesograzers, leached DOC may determine the importance of wrack as a trophic subsidy.

16 However, few studies have examined the effect of seagrass type or age on the release of DOC
17 or its bioavailability. We examined the amount and composition of DOC released from
18 different wrack (*Posidonia sinuosa*, *Amphibolis antarctica* and the alga *Laurencia* sp.). We
19 then examined the effect of age on DOC leaching from *P. sinuosa* wrack. The bio-availability
20 of the DOC was also assessed using a bacterial bioassay.

21

22 The rate of DOC leaching from *Posidonia sinuosa* leaves decreased exponentially with time.

23 According to that exponential model, about 50% of the total DOC release occurred in the first

24 14 days and it would require a further 2.94 years to release the same amount again. Fresh

25 algae (*Laurencia* sp.) leached the greatest amount of DOC in the first 16 h (6.7 mg kg⁻¹ FW

26 wrack), followed by fresh *P. sinuosa* leaves (1.7 mg kg⁻¹ FW), *A. antarctica* leaves (1.1 mg

27 kg^{-1}) and stems (0.6 mg kg^{-1}), 4 wk old *P. sinuosa* (67 mg kg^{-1}) and fine detritus (74 mg kg^{-1}).
28 In all cases, the composition of the DOC was similar and dominated by the hydrophilic
29 component (in *P. sinuosa*, predominantly sugars and amino acids). Leachates from all fresh
30 wrack supported bacterial growth over 24 h. Leachate from older wrack either failed to
31 support bacterial growth, or only supported it for a limited time. Given the exponential decay
32 in DOC release rate, the interacting timescales of transport and leaching will affect the value
33 of wrack as a vector for trophic subsidies.

34

35

36 **INTRODUCTION**

37 Seagrass meadows are conspicuous and highly productive components of coastal ecosystems
38 worldwide (Green & Short 2003). A portion of seagrass production is continually shed as old
39 leaves, which can contribute significantly to wrack (detached macrophyte) accumulations in
40 adjacent coastal habitats (Kirkman & Kendrick, 1997; Mateo, 2010). Export rates of detached
41 leaves from meadows varies enormously but can be as high as 100% of leaf production
42 (Cebrian & Duarte, 2001; Mateo, 2010; Mateo et al. 2006). Given that these older leaves also
43 contain nutrients other than carbon, even after re-sorption prior to shedding (e.g., Prado et al.,
44 2008) this wrack export represents a significant potential loss of nutrients from the habitat and
45 a potential trophic subsidy to adjacent recipient habitats, particularly in oligotrophic
46 environments where alternative sources of nutrient may be limited.

47

48 Despite this potential, there is little published evidence of seagrass wrack being an important
49 source of nutrient to adjacent habitats. Several studies concluded that seagrass wrack was
50 unlikely to be a significant contributor to meso-grazer production in recipient habitats,
51 including unvegetated marine habitats (Hyndes & Lavery 2005), beach ecosystems (Ince et
52 al., 2007), surf zones (Crawley et al., 2009) and within seagrass meadows (Smit et al., 2005;

53 Smit et al., 2006). Wrack typically has a large proportion of macro-algae, and many
54 mesograzers demonstrate a preference for this over seagrass detritus (Doropoulos et al.,
55 2009), likely due to the lower C:N ratio of algae. This suggests that if the nutrients within
56 seagrass wrack are to be recycled within meadows or provide a subsidy to adjacent systems,
57 then mechanisms other than direct consumption of seagrass detritus must be important,
58 microbial pathways utilizing dissolved organic carbon (DOC) being among the most likely.
59
60 Fluxes of DOC to overlying water are higher in seagrass meadows than adjacent unvegetated
61 meadows (Barrón & Duarte 2009). Up to 50% of this DOC is consumed by bacteria (Ziegler
62 & Benner 1999), which can be rapidly transferred to higher trophic levels through
63 consumption by flagellates and ciliates (Robertson et al., 1982). The seagrass leaves
64 themselves are a major contributor to this DOC flux. Through exudation or autolysis and
65 leaching, seagrass leaves typically release 2-10% of net primary production (Moriarty &
66 Pollard 1982, Barrón & Duarte 2009), and this leaf-derived DOC has been shown to support
67 bacterial production (Brylinsky, 1977; Kaldy et al., 2006).
68
69 Because seagrass leaves degrade much more slowly (Klumpp & Vandervalk 1984, Moore &
70 Fairweather 2006) than the rate at which they are transported by currents and storm-driven
71 advection, DOC leaching may occur over extended periods of time and encompass a range of
72 different habitats. This may provide a mechanism of cross-habitat trophic subsidy not
73 dependent on the direct consumption of leaves. Thus, the loss of DOC from seagrass leaves is
74 potentially a key contributor to the total DOC flux from seagrass ecosystems and cross-habitat
75 trophic subsidies.
76
77 Almost all of the studies that have examined the loss of DOC from seagrass leaves have
78 focused on living or fresh leaves (e.g. Brylinsky, 1977; Robertson et al., 1982; Wetzel &

79 Penhale, 1979). Yet there is evidence that the age of detached leaves has a significant impact
80 on DOC leakage and in the composition (and therefore bioavailability) of that DOC (Maie et
81 al., 2006; Velimirov, 1986), which may be crucial for the transfer of seagrass-derived
82 nutrients to adjacent ecosystems. Furthermore, the amount and rate of DOC leaching, and the
83 ability of microbes to utilise the leachate, varies among different vascular plants (Benner et
84 al., 1986; Maie et al., 2006), suggesting that the export and bioavailability of seagrass-derived
85 DOC may be species-dependent. Among the seagrasses, inter-specific differences could
86 relate to the anatomy of the plants (e.g. membranous, leafy species such as *Posidonia* spp.
87 versus heavily lignified species such as *Amphibolis* spp.) or the amounts and forms of soluble
88 compounds within the tissues. These differences among species in DOC leaching and its
89 apparent bioavailability led Maie et al. (2006) to call for more studies into the bioavailability
90 of the DOC fractions that are released from macrophyte leaves. Further, the potential
91 significance of seagrass as a source of DOC to the coastal zone, coupled with the rapid
92 decline in seagrass cover in recent decades (Green & Short 2003), prompted Barrón & Duarte
93 (2009) to call for more studies on seagrasses to understand the export of DOC from these
94 systems and its significance.

95
96 In this paper, we compare the amount, composition and bioavailability of DOC released from
97 different types of seagrass wrack. We also examined the effect of wrack age on the amount of
98 DOC released and its bioavailability. The main objectives were to: 1) determine the amount
99 and functional composition of DOC released from different wrack materials. We tested the
100 hypotheses that the amount of DOC released will vary among different types of wrack and
101 that functional composition will vary among types of wrack; 2) examine whether the amount
102 and composition of DOC released from *Posidonia sinuosa* wrack depended on wrack age. We
103 tested the hypothesis that the amount of DOC released would diminish with age of wrack and
104 that the composition would differ among wrack of different ages; and 3) assess the bio-

105 availability of DOC released from wrack and whether this is affected by wrack age. We
106 hypothesised that bacterial biomass would increase more rapidly when grown in leachate than
107 blank solution, and more rapidly in leachate from fresh wrack than aged wrack.

108

109

110 **MATERIALS & METHODS**

111 **Study region**

112 The study was conducted on wrack accumulations in Geographe Bay (Fig 1), a 100 km wide
113 north-facing embayment, on the south-western coast of Australia. It is a relatively protected
114 bay with extensive beds of seagrass *Posidonia sinuosa* Cambridge & Kuo, and *Amphibolis*
115 *antarctica* (Labillardiere) Sonder *et* Ascherson *ex* Ascherson. *Posidonia* and *Amphibolis* are
116 the dominant meadow-forming genera of seagrasses in south-west Australia. *Posidonia* spp.
117 produce above-ground shoots with 1-3 strap-like leaves that are periodically shed.
118 *Amphibolis* spp. have heavily lignified and persistent stems on which clusters of short leaves
119 are borne (Ducker et al., 1977). The stems are often heavily covered by epiphytes,
120 particularly red (rhodophyte) macro-algae such as *Laurencia* sp (Lavery & Vanderklift 2002).
121 Extensive accumulations of detached seagrass leaves and stems are typical of the region,
122 especially in winter (McMahon et al., 1997). The bay is exposed to the NW-winds that
123 characterise the early phase of storms in the region. This exposure results in the transport of
124 wrack throughout the Bay, providing a high degree of connectivity among seagrass and other
125 habitats in the region. The wrack is typically dominated by *P. sinuosa*, with significant
126 amounts (up to 30% by weight) of *A. antarctica* at times and generally a low amount (but
127 occasionally up to 15%) of red algae (McMahon et al., in review). Geographe Bay is an
128 oligotrophic waterbody and, as such, the decomposition of wrack in sub-tidal and beach
129 habitats may be a vital source of recycled nutrients (Robertson & Lenanton 1984).

130

131 **Release of DOC from different wrack material**

132 The effect of wrack type on the amount and composition of DOC leachate was examined by
133 comparing DOC leached from wrack commonly found in the region: the seagrasses *Posidonia*
134 *sinuosa* (leaves) and *Amphibolis antarctica* (leaves and stems); the red algae *Laurencia sp.*,
135 which is common as both a free-living algae and epiphytic on seagrasses; and the fine
136 particulate organic fraction (>0.1 - <1 mm) of beach-cast wrack that had been on the beach
137 for at least 2 months. Fresh samples of *P. sinuosa*, *A. antarctica* and *Laurencia sp.* were
138 collected from meadows in 0.5 m depth. Beach-cast wrack was collected from Busselton
139 Beach, Geographe Bay (S 33° 39.317', E 115 ° 16.812') and then sieved to separate the fine
140 fraction. Samples were stored at 4°C, for no more than 12 hours, until leachate was collected.

141
142 The effect of wrack age on DOC leaching was examined using one wrack type, *P. sinuosa*
143 leaves. We focused on *P. sinuosa* since this was the dominant component of the beach cast
144 wrack, typically accounting for more than 60% by biomass. Fresh samples of *P. sinuosa* were
145 collected from a meadow in 0.5 m depth. The fresh material was transported immediately to
146 the laboratory and stored in a cool room at 4°C for a maximum of 2 days until leachate was
147 collected. For 'aged' material, approximately 500 g of fresh *P. sinuosa* was placed in nylon
148 mesh litterbags (mesh size < 5 mm) which were then placed on the surface of wrack
149 accumulations on the beach, held in place by pickets driven into the wrack accumulations, and
150 exposed to the ambient weather conditions. Replicate bags (n=3) were removed after 1, 2, and
151 4 weeks and returned to the laboratory for leachate experiments.

152

153 **DOC leachate extraction**

154 Each wrack type and age was incubated to extract DOC. All leaves of both seagrass species
155 and the *Laurencia* thallus were lightly scraped with a razor blade to remove algal epiphytes.
156 For each wrack type, four replicate samples of 100-150 g wet weight plant material were

157 placed in 0.5 L of sterile, artificial seawater (ASW: Red Sea Salt™ at 35 ppt) in acid washed 2
158 L glass beakers. The beakers were sealed with scientific-grade rubber stoppers and incubated
159 for 16 h at 18°C on shaker trays and with periodic, gentle agitation. Blanks were prepared as
160 described above but with no wrack. After 16 h, the leachate was filtered through a series of
161 Whatman GFF filter papers and then a 0.45 µm, hydrophilic polypropylene membrane filter
162 (Pall Life Sciences) and analysed for total DOC with a Shimadzu Total Organic Carbon
163 Analyser 9000. Scraped leaves were used in the experiment to avoid DOC release from
164 epiphytic algae. However, to test for any effect of scraping on DOC release, simultaneous
165 incubations were performed on unscraped leaves with epiphytes gently removed by hand.
166 Even with the removal of epiphytic algae and animals, a microbial biofilm is likely to remain
167 on the leaf surface, which can reduce the flux of DOC to the surrounding waters through
168 direct uptake (Maie et al. 2006). Consequently, the changes in DOC concentration of the
169 incubating water are referred to as Net DOC release.

170

171 The initial leaching experiments indicated high release rates of DOC from fresh seagrass
172 wrack in the first 1-2 weeks of aging. To obtain increased temporal resolution of early
173 leaching rates, the experiment was repeated using *P. sinuosa* leaves to determine the change
174 in release rate over this initial period of high DOC leaching. Three replicate leaf samples (180
175 g wet weight) were incubated in 1.4 L of ASW in acid washed 2 L glass beakers. A blank of
176 ASW was incubated at the same time. Samples of the leachate were collected after 1, 3, 5, 7,
177 10, 14 days of incubation, and the ASW replaced each time. The leachate was filtered and
178 analysed as described above.

179

180 **Characterization of DOC**

181 DOC composition was characterized by fractionation using open column chromatography
182 following modified methods of Chow *et al.* (2004) and Cleveland *et al.* (2004). The leachate

183 was fractionated into three components: 1) hydrophobic DOC (fulvic and humic acids;
184 Hughes, 2007) by retention on DAX-8 resin; 2) transphilic DOC, by retention on the XAD-4
185 resin; and 3) hydrophilic DOC, the eluent passing through the DAX-8 and XAD-4 column
186 (Thurman & Malcolm 1981). The hydrophilic fraction is composed predominantly of low
187 molecular weight compounds, including carbohydrates and amino acids (Cleveland et al.
188 2004).

189

190 DAX-8 Superlite (Sigma-Aldrich) and XAD-4 Amberlite (Sigma Aldrich) resin columns were
191 prepared in a similar manner, following the manufacturer's instructions. The resin was mixed
192 with water (milli-Q) to form a slurry and poured into a glass column (30 x 1.5 cm) fitted with
193 a tap. The bed volume for the resin was approximately 20 mL. The column was conditioned
194 by passing 200 mL Milli-Q water through the column drop-wise, followed by six alternating
195 washes of 0.1 M NaOH (40 mL) and 0.1 M HCl (40 mL), again eluted drop-wise. The final
196 wash was with 0.1 M HCl, to leave the column acidified.

197

198 The DOC leachate (200 mL) was acidified with 35% HCl to pH 2 then passed through the
199 DAX-8 resin (dropwise or at $< 3 \text{ mL min}^{-1}$). The first 10 mL of eluent was discarded (as it
200 was simply displaced acid). When most of the leachate had been applied to the column, 0.1 M
201 HCl (2 bed volumes or 40 mL) was applied to the top of the column. The acid was passed
202 through the column dropwise to elute all but the hydrophobic fraction. The eluent (leachate
203 and acid) was then passed through the XAD-4 column in a similar manner, in this instance the
204 acid eluting the hydrophilic fraction. A sample of de-ionised (Milli-Q) water was passed
205 through the columns and treated in an identical way as the leachates to act as an analytical
206 blank.

207

208 The hydrophobic and transphilic fractions retained on the DAX-8 and XAD-4 resins,
209 respectively, were elute with 0.1 M NaOH. The volume of base added was typically 5 bed
210 volumes (100 mL) or until the absorbance of the eluent at 254 nm was similar to the blank,
211 indicating an absence of DOC.

212

213 The total volume of each fraction was recorded. All pre-filtered leachate samples and
214 fractionated samples were acidified with 35% HCl to pH 2 and analyzed for total DOC with a
215 Shimadzu Total Organic Carbon Analyzer 9000. The UV absorbance for each of the acidified
216 samples was also recorded at 254 nm on a Shimadzu UV-1601 spectrophotometer.

217

218

219 **Wrack composition on beaches**

220 The DOC leaching studies were conducted on wrack collected in sub-tidal habitats and
221 incubated in submerged conditions, typical of sub-tidal seagrass wrack. Initial results showed
222 differences in the net release of DOC from wrack of different ages. Since large amounts of
223 wrack accumulate on beaches, we examined the age of beach-cast wrack to determine
224 whether beach wrack was likely to have arrived while it was ‘fresh’ (and with higher net DOC
225 release rates),and, therefore, whether the bulk of DOC leaching (and potential trophic
226 subsidy) occurs in sub-tidal habitats or on beaches. The composition of beach wrack was
227 determined at three sites on five occasions over the period of maximum wrack accumulation
228 on beaches (May–Oct; McMahon et al., in review). Samples were collected at Forrest Beach,
229 Volunteer Marine Rescue and Geographe Sailing Club (Fig. 1) on 19th - 22nd May, 9th - 11th
230 June, 12th - 15th August, 22nd - 25th September and 20th - 22nd October, 2008. At each site and
231 time, four replicate wrack accumulations were sampled. About 0.001 m³ of wrack was
232 collected from the surface of the accumulation with a quadrat and from the sediment
233 immediately below the accumulation with a corer (90 mm I.D. x 10 cm deep). The wrack was

234 rinsed to remove sand and sorted into categories based on the estimated age of wrack. Age
235 was defined as either old (no green leaves) or new (green leaves or stem) on the basis of their
236 colour: pilot work showed that moist leaves above the surface of the sediment turned brown
237 within 2 weeks (*P. sinuosa*) or 2 - 4 weeks (*A. antarctica*) (Oldham et al., in review).

238

239 **Bioavailability of DOC**

240 We used a bacterial bioassay to test the bioavailability of the filtered DOC leachate produced
241 by *P. sinuosa* leaves, *A. antarctica* leaves and stem, *Laurencia* sp. and fine particulate wrack
242 during the 16 h. incubations, using the methods of Cleveland et al. (2007). The response of a
243 bacterial inoculum to the different DOC leachates was observed as growth rate over a 24 h.
244 period.

245

246 Filtered DOC leachate (200 mL) was combined with a bacterial inoculum (2 mL) in acid-
247 washed glass flasks, wrapped and capped in aluminum foil. A bacterial inoculum was created
248 by combining 100 g of moist beach sediment, 100 g of moist wrack and 800 mL sterile
249 artificial seawater. This was left in the dark for 24 h at 18°C and then filtered through a
250 Whatman 3 filter paper with the filtrate used as the bacterial inoculum. For each DOC
251 leachate, four replicates and four blanks (200 ml ASW + 2 ml bacterial inoculum) were
252 incubated at 25°C. Triplicate 1 mL sub-samples were taken after 0, 3, 18 and 24 h of
253 incubation and fixed with 0.5% glutaraldehyde for 15 min in the dark (Marie et al. 1997) then
254 stored in liquid nitrogen until further processing. Heterotrophic bacterial cell counts were
255 determined on a FACS Canto II flow cytometer. Samples were diluted with TE buffer (1:50
256 dilution) and stained with SYBR Green I for 15 min. at 80°C. Acquisition was run for 2 min
257 at a speed of 1 $\mu\text{l s}^{-1}$. Data were stored as FCS 2.0 files and cell counts (cells mL^{-1}) were
258 calculated using the CYTOWIN 4.3 software.

259

260

261 **Statistical Analysis**

262 A one-way ANOVA was used to test for differences in the total amount of DOC released
263 among different types of wrack, with wrack type as fixed factor. A two-way ANOVA was
264 then used to test for differences among wrack types and DOC fraction on the total amount of
265 DOC released, with wrack type and DOC fraction treated as fixed factors. A one-way
266 ANOVA was used to test for significant effects of wrack age on the total DOC released, with
267 age as fixed factor. A two-way ANOVA was then used to test for effects of wrack age and
268 form of DOC on the amount of DOC released, with age and DOC fraction as fixed factors. A
269 repeated measures ANOVA was used to test for significant effects of scraping on the release
270 of DOC from leaves over time, with scraping a fixed factor.

271

272 The assumption of homogeneity of variances was tested using Cochran's test. When variances
273 were heterogeneous, data were Ln- transformed, or arcsin-transformed for proportions and
274 percentage values. Where significant main effects were detected, post-hoc comparisons
275 (Tukey's) were conducted to determine the sources of significant variation.

276

277

278 **RESULTS**

279 **Amount and composition of DOC**

280 The net DOC leaching from wrack over 16 hours differed significantly among wrack types,
281 with fresh algae (*Laurencia sp.*) leaching about four times the DOC released by fresh *P.*
282 *sinuosa* leaves, six times that released by *A. antarctica* leaves, more than 11 times that
283 released by *A. antarctica* stems and more than 90 times that released by the fine fraction of
284 natural wrack accumulations (Table 1). The recovery of DOC after fractionation into
285 hydrophobic, transphilic and hydrophilic fractions was high, ranging from 78-94% (Table 1).

286 For all wrack types, the hydrophilic fraction dominated the total DOC (37-68%), followed by
287 the hydrophobic fraction (17-31%) and the transphilic fraction (4-11%). Nonetheless, there
288 were subtle, but significant, differences in the percentage contribution that hydrophobic and
289 hydrophilic components made to the total DOC, but not the transphilic component (Table 1),
290 reflected in a significant interaction between wrack type and DOC fraction (2-way ANOVA
291 Wrack Type x DOC fraction d.f.=8,59; $p < 0.001$). The proportion of DOC present as
292 hydrophilic (and presumably the most bio-available) DOC was highest in fresh *P. sinuosa*
293 leaves (68%) followed by *Laurencia* and *Amphibolis* tissues (53-61%) and least in the fine
294 fraction of beach-cast wrack (37%).

295

296 *Influence of aging of P. sinuosa wrack on DOC leaching*

297 The net DOC leaching from *Posidonia sinuosa* leaves declined with increasing age of the
298 wrack (Table 2). Fresh and one week old leaves released similar amounts of DOC ($>1400 \text{ mg}$
299 kg^{-1}) over 16 h, at least 10 times the amount released after 2 weeks of aging and 20 times that
300 released after 4 weeks. The composition of the leachate released by leaves of different ages
301 varied subtly and not systematically (Table 2), with a significant interactive effect of wrack
302 age and DOC fraction (2-way ANOVA, d.f. = 6,35, $p < 0.001$; and Table 2 for post-hoc
303 pairwise comparisons). However, in all cases the leachate was dominated by the hydrophilic
304 fraction (48-67%), followed by the hydrophobic (16-27%) and the transphilic (11-17%)
305 fractions.

306

307 *DOC release during the first 14 days*

308 The rate of net DOC released from fresh *P. sinuosa* leaves during the first 14 days was
309 affected by scraping (Fig 2), with a significant Time x Scraping interaction ($p < 0.05$). For
310 scraped leaves, the leaching rate (A_t) was described by a single-stage exponential decay with
311 a half-life of 1.8 days: $A_t = 752 e^{(-0.317t)}$. For unscraped leaves, the net release rate of DOC

312 was describe by a two-stage model, with an increasing rate of DOC release for the first 5
313 days, after which the leaching rate was describe by a exponential decay with a half-life of
314 1.65 days: $A_t = 1610 e^{(-0.385t)}$, which approached the decay curve for the scraped leaves.

315

316 Despite the differences in initial net DOC release rates, the total mass of DOC released
317 (M_{DOC}) from scraped and unscraped leaves was similar over the initial high release period
318 (first 5 days: 1650 ± 122 vs. $1600 \pm 77.0 \text{ mg kg}^{-1}$) and then the full 14 days (1920 ± 131 vs.
319 $1740 \pm 80.0 \text{ mg kg}^{-1}$) of the experiment, indicating that the effect of scraping the leaves was
320 minimal in terms of quantity of DOC leached.

321

322 The accumulated mass released over the 14 days of incubation approached 2000 mg (Fig 3),
323 with the rate of release dramatically slowing by day 14. Assuming that the mechanism of
324 DOC release remained constant over time, the curve fit to the full dataset (scraped and
325 unscraped leaves, $M_{\text{DOC}} = 716 + 470 \text{ Ln}(t)$) predicts that a further 1100 days (3 years) would
326 be required to release the next 2000 mg.

327

328 **Composition of wrack on beaches**

329 The wrack accumulating on Geographe Bay beaches was typically dominated by old material
330 (Fig 4). In May, the period just prior to the first autumn - winter storms, the wrack on the sand
331 surface and that within the underlying beach sediments was dominated by old material
332 (generally $> 90\%$). During the winter storm period (June - September) the proportion of fresh
333 wrack increased in both zones, reaching 25-30% at the surface of accumulations but was
334 always less than 10% in the sediment layer below accumulations. By October (spring), the
335 proportion of fresh wrack had declined in all accumulations, approaching 5% in the surface
336 layer and negligible in the sediment layer.

337

338 **Bioavailability of DOC leachates**

339 For leachates from all types of fresh wrack material, there were significant exponential
340 increases in bacterial abundance over time following inoculation (Fig 5; Table 3; in all cases
341 $p < 0.001$. The Fine Fraction from beach cast wrack also showed a significant increase in
342 bacterial abundance over time ($p < 0.05$), though the rate of increase was much smaller. In
343 leachate from one month old *Posidonia* leaves, there was no significant increase in bacterial
344 abundance over time. In all cases, when the number of bacteria in the blank incubations was
345 plotted against time the slope was not significantly different to zero, indicating little or no
346 bacterial growth, except for the Fresh *Posidonia* and *Laurencia* leachate incubations, where
347 there was a significant exponential decay in bacterial abundance.

348

349 The age of *Posidonia* wrack affected the ability of the leachate to support bacterial growth.
350 For leachate from fresh *Posidonia* leaf material, the linear increase in bacterial abundance
351 over a 24 h period had an average slope of 2.04×10^5 cells h^{-1} . For the leachate from 4 weeks
352 old *Posidonia* leaves, bacterial abundance increased for the first 18 hours, though the slope
353 over this period was less than half that in the leachate from fresh leaves (9.20×10^4 cells h^{-1}),
354 and declined thereafter.

355

356

357

358 **DISCUSSION**

359 **Effect of wrack type on DOC release**

360 Total net DOC released varied among types of wrack (algae > *Posidonia* leaves > *Amphibolis*
361 leaves > *Amphibolis* stems > fine fraction). This is consistent with studies that found release
362 rates were higher from algae than seagrasses (e.g. Brylinsky, 1977). Algae have less structural
363 carbon, and therefore more storage carbon, per unit biomass, which would account for this

364 difference. Within the different types of seagrass wrack there were also differences in release
365 rates of DOC. Fresh *Posidonia* leaves had the largest release rate of DOC, followed by *A.*
366 *antarctica* leaves and then stems. The stems of *Amphibolis* are vertical rhizomes and serve as
367 a major storage organ for carbohydrates. In the closely related species *Amphibolis griffithii*,
368 soluble sugars account for 15-20% DW, and starches account for 2-3% DW of the rhizome
369 (Lavery et al. 2009). On this basis, we might expect higher fluxes of soluble carbohydrate
370 compounds from the stems than the leaves. However, the lower net release rate for stems may
371 reflect higher levels of other soluble compounds in the leaves, especially proteins associated
372 with photosynthesis, and stronger barriers to diffusion, since stems are highly lignified and
373 contain large amounts of vascular tissue. This may also explain the differences among leaves,
374 since *Posidonia sinuosa* leaves and those of *A. griffithii* typically have similar levels of
375 soluble sugars and starches (*P. sinuosa* = 2-4% DW soluble sugars and 5-10% starches;
376 Collier, et al. 2009); *A. griffithii* = 5-15% soluble sugars and 2-3% starch; Lavery et al. 2009).
377

378 The relatively low release rates from aged *Posidonia* leaves (4 weeks old) and the fine
379 particulate fraction of wrack (at least 2 months old) reflects the effect of aging on DOC
380 release and the significant loss of DOC which occurs in the first few days of leaching.
381 However, despite the low rate of bacterial growth on leachate from the 4 weeks old *Posidonia*
382 leaves, the initial growth over 18 hours confirms that the DOC leachate was bioavailable.
383 The decline in bacterial biomass after 18 hours indicates that it was more likely a function of
384 the mass of DOC in the leachate than the composition that affected bacterial growth.
385

386 The composition of the DOC leached from different species of wrack was similar. This may
387 partly reflect the level of resolution in our chemical characterisation of the leachates. The
388 high % recovery of DOC following fractionation gives confidence that we have not under-
389 estimated a significant portion of the DOC. Maie et al. (2006) found differences in

390 concentrations of sugars and phenols in leachate from a range of aquatic plants they studied.
391 However, the plants they studied covered a wide phylogenetic range, from algal periphyton to
392 freshwater macrophytes, mangroves and seagrasses. In comparison, our wrack was all
393 derived from seagrasses, with the exception of the alga *Laurencia*.

394

395 The similarity in leachate quality from all wrack types, including that of aged wrack, indicates
396 that the quality of DOC that seagrass wrack contributes to recipient habitats is likely to be
397 similar, irrespective of the type or age of the wrack, though the mass contributed will decline
398 rapidly with age. It was surprising that the hydrophilic portion (which contains sugars, amino
399 acids, small molecular weight fatty acids and other compounds likely to be more labile)
400 continued to form a significant proportion (more than 50%) of the DOC leached from aged *P.*
401 *sinuosa* and the fine fraction. It is not clear whether, in the older wrack, the low molecular
402 weight component is derived directly from the wrack, or is contained in exudates from
403 bacteria growing on the wrack or in suspension. In any case, this makes little difference in
404 terms of the potential benefit of the input to recipient ecosystems. If it is derived from
405 exudates of bacteria growing on the wrack, then it is possible that this input of readily
406 bioavailable DOC could persist for months, though at a very slow rate.

407

408 **Mass & Timescale of DOC Release**

409 The release rate of DOC declined rapidly with age of *Posidonia* wrack. About 50% (2000 mg
410 kg⁻¹ FW wrack) was released in the first 14 days. Assuming that the mechanism of DOC
411 release remains constant over the decay period of the wrack, it would take in the order of
412 thousands of days to release the next 2000 mg kg⁻¹ FW wrack. This assumption may not be
413 the case but, nonetheless, it is clear that the rate of DOC release will fall dramatically after the
414 first days. The *Posidonia* leaves used in our studies contained about 33% carbon DW or 11%
415 FW (using a DW;FW ratio of 0.34; unpublished data) so the total mass of carbon released

416 over 14 days was about 1.8% of the leaf carbon, and if we project out to 2000 days, 3.6% of
417 the leaf carbon. These values are similar to the total fixed carbon lost through DOC excretion
418 reported for *Posidonia oceanica* (Velimirov 1986) but much less than the 48% estimated by
419 Kirkman & Reid (1979) for *Posidonia australis*, which has very similar leaf structure to *P.*
420 *sinuosa* (Cambridge & Kuo 1982). Kirkman & Reids' (1979) estimate was likely to have
421 severely over-estimated the leaching of DOC from leaves. They used leaves with necrotic
422 tissue and with a full complement of epiphytes which would have contributed to DOC
423 leakage, and they measured the rates over two hours, which are likely to produce much higher
424 estimates of loss that would occur for aged wrack tissue, as shown by our results.

425

426 Temporal variation in DOC release rates have been reported for other seagrasses. Velimirov
427 (1986) found that the young, green portions of *Posidonia oceanica* leaves released negligible
428 amounts of DOC but high loss was observed for older, brown leaves, while the rate of DOC
429 leaching from *Thalassia testudinum* leaves declined exponentially with age, with 84% of the
430 DOC leached in the first two weeks (Maie et al. 2006). In our case, the initial rate of DOC
431 release was enhanced by scraping leaves to remove epiphytes. This could be due to the
432 removal of epiphytes or damage to the leaf surface. Epiphytic organisms reduce the release of
433 DOC from seagrass leaves to surrounding water (Velimirov 1986, Wetzel & Penhale 1979),
434 presumably through assimilation of the DOC. Scraping is also likely to disrupt the tough
435 cuticle and thick epidermal leaves of *P. sinuosa* (Cambridge & Kuo, 1982), enhancing
436 diffusive losses of cellular DOC. However, this effect was limited mainly to day 1, with the
437 total mass of DOC released over the first 5 days (the period of highest initial release rates)
438 similar in both types of leaf, providing confidence that scraping had little effect on the total
439 amount of DOC released beyond the first day. Despite the potential for scraping to introduce
440 an experimental artefact, it may be representative of the condition of naturally shed leaves
441 which will have damage to the leaf surface, particularly the necrotic upper part of the leaves,

442 through the action of grazers and abrasion by sediments as they are transported in bedload and
443 suspended transport.

444

445 The bacterial growth in the assays demonstrates the bioavailability of the leachate DOC
446 released from fresh seagrass and algae, with exponential increases in abundance indicating
447 bacterial growth. Bacteria grown on seagrass DOC leachate can rapidly be converted into
448 bacterial aggregates that are consumed by ciliates and flagellates at a much faster rate than the
449 residual particulate organic carbon (Robertson et al. 1982). We did not enumerate the bacteria
450 on the surface of the wrack, but these typically are much more abundant than bacteria in
451 suspension.

452

453 **Relative contribution of wrack to DOC in Geographe Bay**

454 While it was beyond the scope of this study to produce a full DOC budget for Geographe Bay,
455 sufficient information is available to compare the potential contribution of wrack with some
456 other sources of DOC (Table 1) to the study area. Oldham et al. (2010) estimated a total
457 annual wrack production of 16,900 t DW of *Posidonia* leaf wrack and 15,700 t of *Amphibolis*
458 wrack from this region (i.e. an area of 60 km² with an average depth of 5 m - the area of
459 seagrass coverage to the 10 m depth contour). At the initial DOC release rates recorded in our
460 study, this mass of wrack would contribute 191 kg of DOC to the study region in one day.

461 Actively growing phytoplankton can leak between 0.0005 - 0.055 pmol DOC cell⁻¹ d⁻¹
462 (Biddanda & Benner 1997). Coastal waters to the north of Geographe Bay typically have
463 about 91,000 cells L⁻¹ of phytoplankton (Hanson et al. 2006). Assuming a similar cell count
464 in Geographe Bay, phytoplankton in the study area would, at most, contribute 0.19 – 21 kg
465 DOC per day, between 1 and 3 orders of magnitude less than seagrass wrack.

466

467 In contrast, living seagrass represents a very large DOC source relative to wrack.

468 Velimirov (1986) compared the DOC release from healthy, living and senescent seagrass
469 leaves; healthy leaves released 0.2% of that released by senescent leaves. However, the
470 biomass of living meadow is much higher than that of wrack. Assuming a release rate
471 comparable to that given by Velimirov for *Posidonia oceanica* ($0.006 \text{ mg DOC g}^{-1} \text{ h}^{-1}$) and
472 using McMahon et al.'s (1997) reported biomass for *Posidonia sinuosa* in Geographe Bay
473 ($115\text{-}470 \text{ g dw m}^{-2}$), live seagrass in the study area would provide 990 – 4000 kg DOC per
474 day to the study area, 10-40 times that of wrack. Of course, this is a fixed source of DOC,
475 compared with the more mobile nature of wrack, which permits inter-habitat connectivity.

476

477 Surface beach sands can be a significant source of DOC to the water column in high-energy
478 environments, with a net flux of $4\text{-}22 \text{ mmol DOC m}^{-2} \text{ d}^{-1}$ (Heymans & McLachlan 1996,
479 D'Andrea et al. 2002, Avery et al. 2012). Geographe Bay has an approximately 0.5 m diurnal
480 tide range and a mean beach slopes of about 0.06 m m^{-1} (Oldham et al. 2010). Using the
481 lower slope estimate, over the 30 km stretch of beach an area of $2.5 \times 10^5 \text{ m}^{-2}$ of beach face
482 would be inundated each day, providing an estimated flux of DOC in the order of 12-66 kg
483 DOC d^{-1} . While significant, this is a smaller source than, and is likely to be most significant
484 to the surf zone adjacent to the beach unlike wrack, which can be transported and gradually
485 release DOC over a wider area.

486

487 **Implications for Trophic connectivity**

488 The exponential-decay model of DOC release from *P. sinuosa* leaves indicates that there will
489 be significant temporal variation in the release of DOC from leaves shed by plants, with
490 significant implications for trophic connectivity. Wrack is constantly being produced in
491 seagrass meadows, but in the case of *Posidonia* and *Amphibolis* leaves significant water
492 velocities, in excess of 0.15 m s^{-1} , are required to suspend wrack, allowing it to be transported
493 away from the meadow (Oldham et al. in review). Typically, this results in wrack

494 accumulating in offshore meadows during quiescent periods (spring through to early autumn
495 in our study site) and leaves being transported to beaches and other habitats during storm
496 events (McMahon et al., in review), typically in autumn and winter. Leaves shed in spring
497 and summer may, therefore, slowly degrade within the meadow for several weeks or months,
498 with the majority of DOC released within the meadow itself. Adjacent habitats will only
499 receive wrack in a high DOC-leaching phase under two scenarios: 1) during unusual storm
500 events which are sufficiently energetic to dislodge and transport living material and when
501 fresh wrack may constitute a significant portion of the total; and 2) during normal autumn-
502 winter storms, when it will only constitute a small proportion of the total wrack exported (i.e.
503 that shed in the previous two weeks).

504

505 The above suggests that in our system and outside of storm events, when the timescale of
506 leaching is typically much faster than the timescale of transport, wrack may be of limited
507 value in supporting trophic subsidies. However, we have noted relatively high DOC
508 concentrations in porewaters beneath wrack accumulations. While fresh wrack was never the
509 dominant component of wrack accumulations, it frequently accounted for 25-30% of the mass
510 during winter (when storm conditions dominate). During this time, beach accumulations can
511 persist for several weeks reaching biomasses of 4 kg m^{-2} under natural conditions but as much
512 as 19 kg m^{-2} in areas affected by coastal structures (McMahon in prep). Assuming 4 kg m^{-2} of
513 wrack with 30% fresh material approximately $4.1 \text{ g of DOC m}^{-2}$ would be released over two
514 weeks, which our data shows is capable of supporting bacterial growth. Thus, while seagrass
515 wrack may have relatively little value as a source of trophic connectivity during periods of
516 quiescent hydrodynamics, it may still be important during periods of higher energy and faster
517 transport, leading to the formation of biogeochemical hot moments (sensu McClain et al.
518 2003). Furthermore, under quiescent hydrodynamics, seagrass detritus may contribute, even if
519 at slow DOC release rates, to the sedimentary organic carbon pool of offshore habitats

520 including oligotrophic unvegetated habitats, as suggested by Ziegler & Benner (1999). This
521 could also apply to beaches and other recipient habitats if the wrack is buried and therefore
522 can persist in these habitats for sufficiently long periods to allow an accumulation of DOC.
523 This demonstrates a complex interaction of timescales of transport (or residence times) and
524 timescales of leaching which must be undertaken into account when considering the potential
525 for trophic subsidies.

526

527 **Conclusions**

528 We conclude that *Posidonia sinuosa* and *Amphibolis antarctica* seagrass wrack leaches
529 bioavailable DOC. We also conclude that, for *P. sinuosa*, there is an initial rapid release of
530 DOC within the first days-weeks followed by an extended period of low release rates. As
531 similar timecourse of DOC release have been shown for other seagrasses such as *P. oceanica*
532 (Velimirov 1986) and *Thalassia testudinum* (Maie et al. 2006), it is likely that wrack from
533 many species of seagrass will demonstrate similar patterns of DOC release. Despite
534 differences in the rate of DOC release from different types and ages of wrack, the
535 composition was similar and it was bioavailable even when released from old wrack, though
536 the amount released would limit bacterial growth. Given the known consumption of bacterial
537 aggregates by higher levels of the foodweb, the leaching of DOC is one means of recycling
538 the nutrients in seagrass detritus. The interaction of the timescales of transport and the
539 timescale of leaching will be critical in determining the value of wrack as a vector for trophic
540 subsidies. When fresh wrack is released during periods of rapid hydrodynamic transport, it
541 has the potential to release most of its DOC into recipient habitats. However, during
542 quiescent periods, the rapid leaching will result in most of the DOC being recycled within the
543 seagrass meadow. Further work is required to determine the importance of bacterial growth
544 on the surface of wrack and in suspension as a sink for seagrass DOC, and the efficiency of its
545 subsequent incorporation into the food web of recipient ecosystems.

546

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552

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676 **Table 1** Dissolved organic carbon composition in leachates derived from different wrack
 677 material (means \pm sd). Within each class of DOC (totals, hydrophobic, transphilic and
 678 hydrophilic), shared subscript letters indicate no significant differences among wrack types
 679 ($\alpha = 0.05$). *Posidonia* = *P. sinuosa*, *Amphibolis* = *A. antarctica*; Fine fraction = 0.1 – 1.0
 680 mm size class of natural wrack accumulations.

Wrack Type	DOC released in 16 h (mg kg ⁻¹ FW wrack; n=4 in all cases)				DOC Recovery (%)
	TOTAL	Hydrophobic	Transphilic	Hydrophilic	
Fresh <i>Laurencia</i>	6 749 \pm 278 _a	1 998 \pm 64 30% _a	675 \pm 95 10% _a	3 554 \pm 213 53% _a	93%
Fresh <i>Posidonia</i> leaves	1 724 \pm 76 _b	298 \pm 14 17% _b	138 \pm 6 8% _b	1173 \pm 58 68% _b	93%
Fresh <i>Amphibolis</i> leaves	1 102 \pm 24 _c	284 \pm 26 26% _b	48 \pm 4 4% _{bc}	676 \pm 21 61% _c	91%
Fresh <i>Amphibolis</i> stems	588 \pm 31 _d	180 \pm 13 31% _c	58 \pm 4 10% _{bc}	312 \pm 29 53% _d	94%
Fine fraction	74 \pm 1 _e	22 \pm 3 30 % _d	8 \pm 5 11 % _c	27 \pm 1 37% _e	78%

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Table 2 Dissolved organic carbon composition of leachate from *P. sinuosa* leaves of different ages. ANOVA revealed a significant interaction of Age and DOC fraction ($p < 0.001$). Shared subscript letters indicate no significant difference in mass of DOC released (Tukey's test; $p > 0.05$) among treatments within each class of DOC.

Age	DOC release over 16 h (mg kg ⁻¹ FW wrack)			DOC Recovery	
	TOTAL	Hydrophobic	Transphilic		Hydrophilic
Fresh	1 419 ± 93 _a	223 ± 18 16% _a	181 ± 17 13% _a	855 ± 45 60% _a	89%
1 week old	1 627 ± 192 _a	417 ± 13 26% _{bc}	277 ± 32 17% _a	783 ± 120 48% _b	91%
2 weeks old	133 ± 17 _b	36 ± 4 27% _b	19 ± 4 14% _a	67 ± 7 50% _b	91%
4 weeks old	67 ± 2 _c	12 ± 1 18% _c	7.2 ± 0.2 11% _a	45 ± 1 67% _a	96%

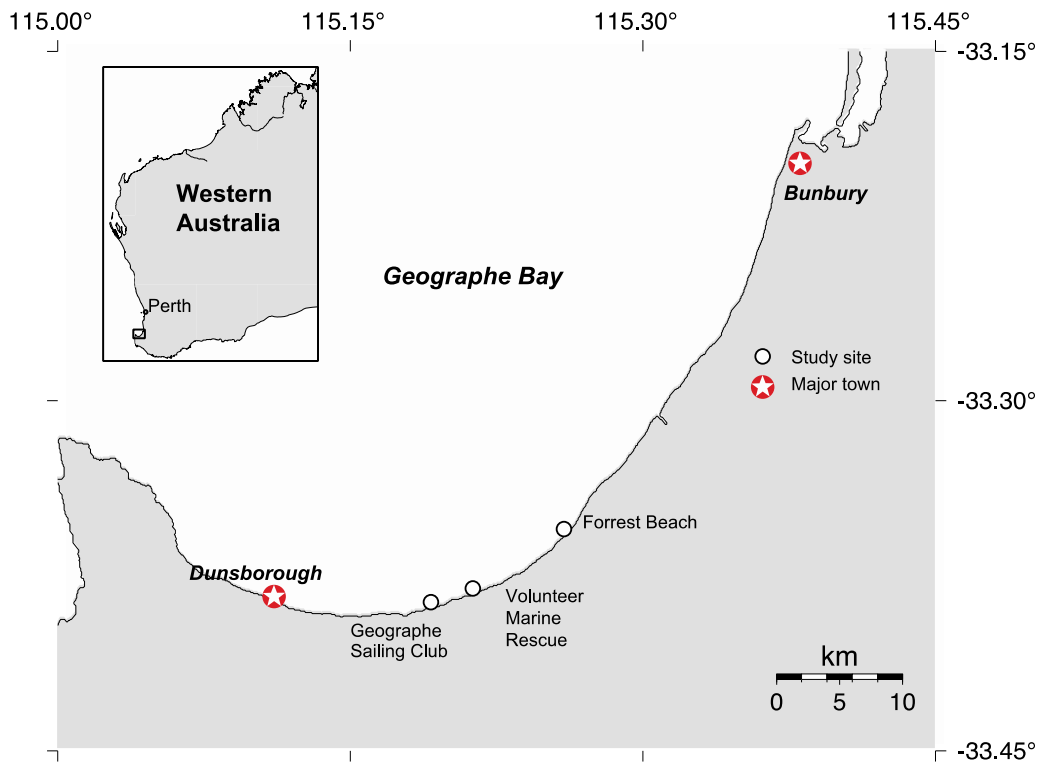
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Table 3 Exponential curve fits describing the change in abundance of bacterial cell abundance over 24 hr in leachates from different types of wrack following bacterial inoculation. * = significant at $p \geq 0.05$; ** $p \leq 0.01$. In all cases, x = time in hours.

Wrack Type	Correlation	r	p
<i>Posidonia</i> leaf (Fresh)	$4.54 \times 10^5 e^{0.109x}$	0.95	***
<i>Laurencia</i> (Fresh)	$1.65 \times 10^6 e^{0.101x}$	0.90	***
<i>Amphibolis</i> leaf (Fresh)	$1.26 \times 10^6 e^{0.076x}$	0.89	***
<i>Amphibolis</i> stem (Fresh)	$1.23 \times 10^6 e^{0.086x}$	0.97	***
<i>Posidonia</i> (Old)	$3.92 \times 10^4 e^{0.129x}$	0.34	nsd
Fine Fraction	$7.64 \times 10^4 e^{0.084x}$	0.51	*
Blank (Fresh <i>Posidonia</i> & <i>Laurencia</i>)	$9.58 \times 10^4 e^{-0.011x}$	-	***
		0.83	

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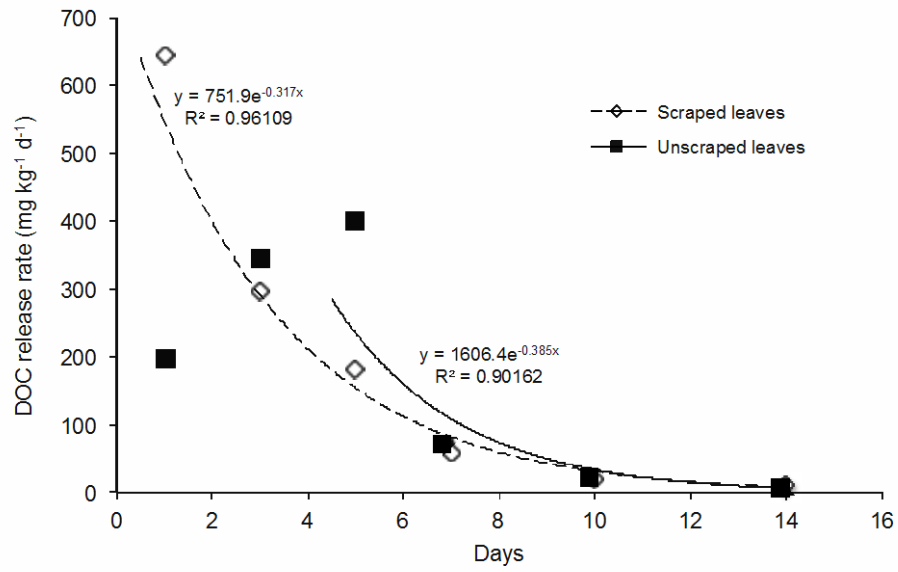
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707 **Figure 1** Map of Geographe Bay, Western Australia, showing the location of the three sites used to
708 sample beach wrack composition.

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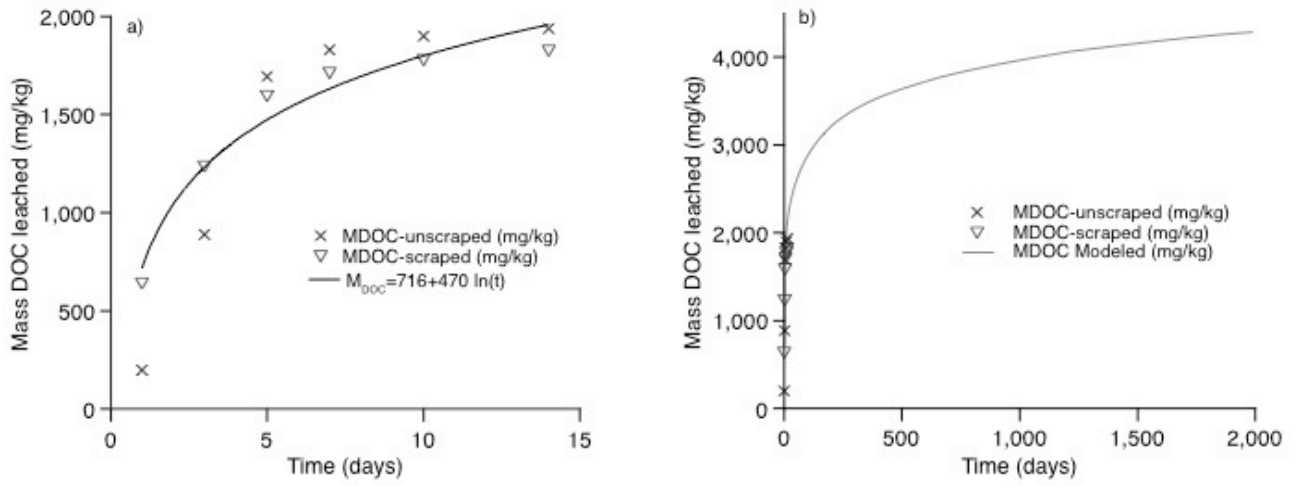
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715 **Figure 2** Net DOC release rates from scraped and unscraped *P. sinuosa* leaves during 14-
716 day incubations. The regression for unscraped leaves is for days 5-14.

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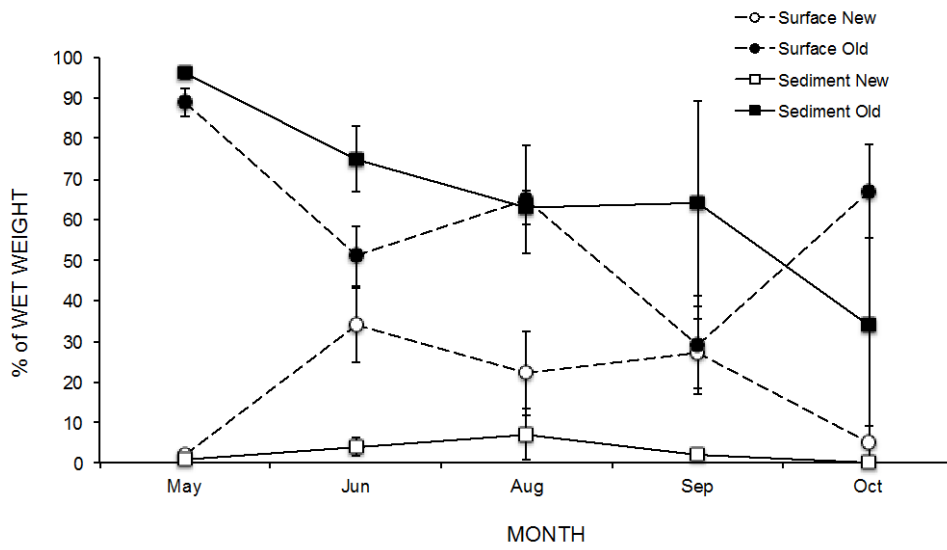
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Figure 3 Cumulative net mass of DOC released over time

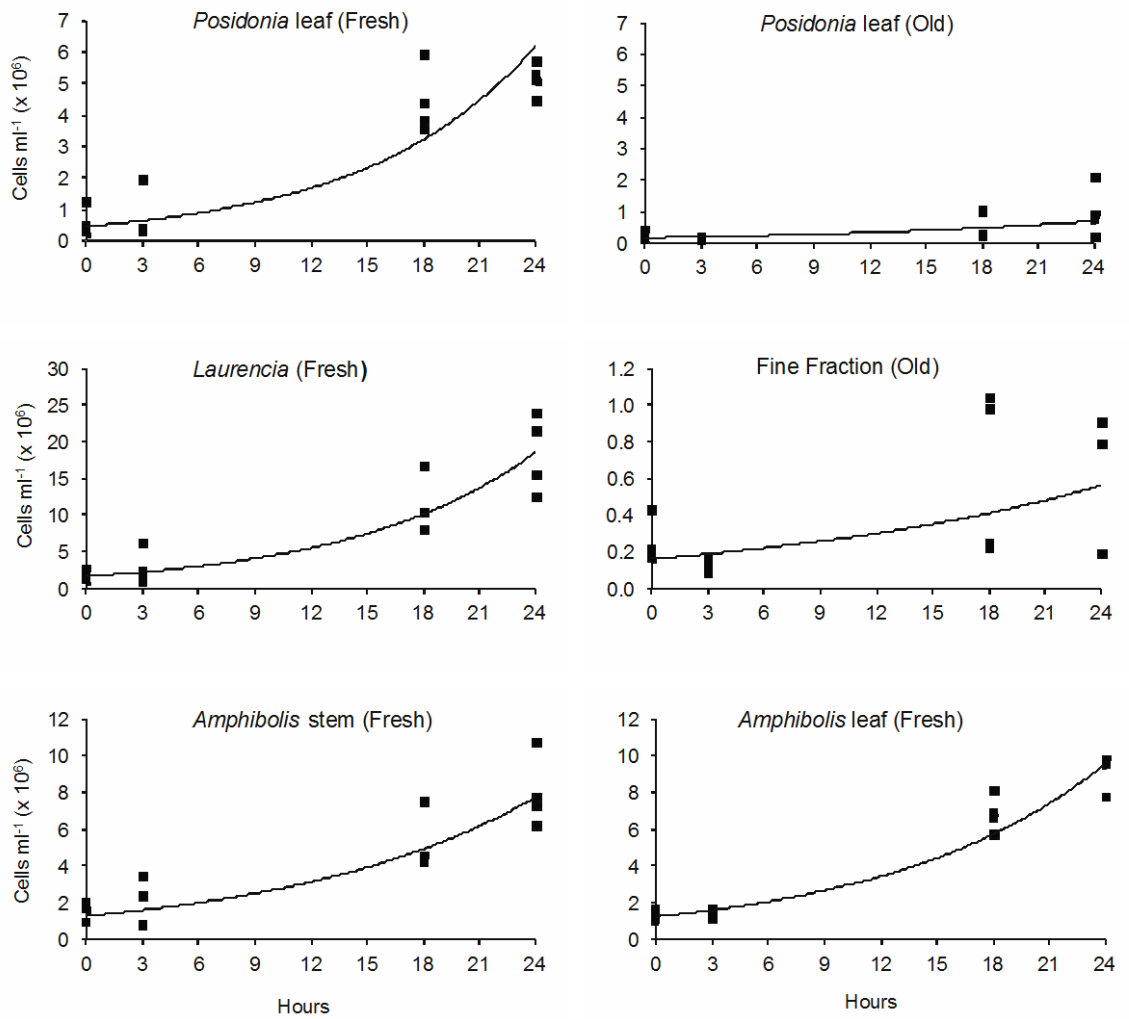
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Figure 4 The composition (new versus old) of wrack on beaches of Geographe Bay from May-October 2008.

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Figure 5 Abundance of heterotrophic bacterial cells following addition of an inoculum to DOC leachates from different wrack types.