Experimental and computational analysis of a novel flow channel to assess the adhesion strength of sessile marine organisms

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Bioadhesives produced by marine macroalgae represent a potential source of inspiration for the development of water-resistant adhesives. Assessing their adhesion strength, however, remains difficult owing to low volumes of adhesive material produced, low solubility and rapid curing time. These difficulties can be circumvented by testing the adhesion strength of macroalgae propagules attached to a substrate. In this paper, we present a simple, novel flow channel used to test the adhesion strength of the germlings of the fucalean alga Hormosira banksii to four substrates of biomedical relevance (PMMA, agar, gelatin and gelatin + lipid). The adhesion strength of H. banksii germlings was found to increase in a time-dependent manner, with minimal adhesion success after a settlement period of 6 h and maximum adhesion strength achieved 24 h after initial settlement. Adhesion success increased most dramatically between 6 and 12 h settlement time, while no additional increase in adhesion strength was recorded for settlement times over 24 h. No significant difference in adhesion strength to the various substrates was observed. Computational fluid dynamics (CFD) was used to estimate the influence of fluid velocity and germling density on drag force acting on the settled organisms. CFD modelling showed that, on average, the drag force decreased with increasing germling number, suggesting that germlings would benefit from gregarious settlement behaviour. Collectively, our results contribute to a better understanding of the mechanisms allowing benthic marine organisms to thrive in hydrodynamically stressful environments and provide useful insights for further investigations.

1. Introduction

Adhesives produced by sessile marine organisms, including macroalgae, exhibit strong adhesion to substrates with different surface properties [1,2]. Adhesion in the marine environment occurs despite heavily biofouled surfaces [3,4] and the strong drag forces that tidal and wave currents generate [5]. Greater understanding of the mechanisms of adhesion present in these marine organisms could lead to the development of bioinspired adhesives with applications in the underwater engineering and biomedical arenas.

Brown algae (class Phaeophyta) include large species such as the kelps Macrocystis pyrifera [6] and Nereocystis luetkeana, and the fucoidean Durvillaea antarctica [7]. Adult plants of these species can be up to 10 m long, weigh more than 50 kg and are able to withstand severe hydrodynamic drag in wave-exposed areas. These features make brown algae particularly interesting in the bioadhesion arena. The adhesive secretions from brown algae are mostly composed of a network of cross-linked polyphenolic compounds and negatively charged polysaccharides [8–12]. Mechanical testing has been performed on adult specimens of...
H. banksii algae, obvious interest in biomedical applications. Among the brown dispersal and settlement of seaweed propagules is, in fact, on adult plants set the grounds for further research, adhesion to withstand pull-out forces up to 300 N. Although experiments ambiguous value of the wall shear stress. In fact, Lu [33] remarked that the actual shear stress felt by the settled diatom attachment across multiple microscope slides using turbulence and low-cost testing method would not require the extraction of significant quantities of adhesives, often an unrealistic task for marine biosurfaces owing to their low solubility, fast curing time and small quantities secreted [10]. Ideally, a more practical and low-cost testing method would not require the extraction of adhesive material while being rapid and repeatable. The use of a water-generated, controllable hydrodynamic force is useful to test the adhesion of sessile marine organisms while minimizing collateral stresses generated by the experimental conditions. Methods employing hydrodynamically generated forces for assessing the adhesion strength of sessile marine organisms have been used in the past, ranging from exposure to naturally generated waves [17] to apparatuses such as radial, annular and straight flow channel designs, water jets [19,20] and turbulence tanks [21]. Among these methods, flow channels have been common, ranging from microfluidic devices using laminar flows to test the adhesion of bacteria and microalgae [22,23] to larger devices used to assess diatom attachment across multiple microscope slides using turbulent flow regimes [24,25]. Flow channel-based adhesion assessment is also common in the study of biofouling-resistant and fouling-release coatings [26–29]. However, these studies do not investigate the actual drag force acting on the settled organisms and propose the wall shear stress generated in the channel as an estimate for the adhesion strength. Conversion between these two forces is not obvious and is likely to be complicated by a number of factors such as the geometry of the flow channel and the relative dimension of the species considered.

Computational fluid dynamics (CFD) represents a useful tool to estimate the drag forces acting on underwater organisms. CFD methods are relatively easy to implement and have been mostly applied to determine the flow field and hydrodynamic drag acting on isolated cells adhering to flat surfaces [30–32], or to walls of parallel plate flow chambers. From a mechanistic standpoint, the forces estimated using a computational approach are more compelling than the ambiguous value of the wall shear stress. In fact, Lu et al. [33] remarked that the actual shear stress felt by the settled organisms could be significantly different from the wall shear stress because of a non-negligible distortion of the flow by the cell. However, the model of an isolated cell is not appropriate for the case of kelp and fucoid propagules, as these often colonize surfaces gregariously, forming random arrays of settled germlings. The presence of neighbouring germlings further distorts the flow field, creating wakes that protect the germlings settled further down the flow channel. Brooks & Tozeren [34] have considered the effect of uniformly distributed arrays of cells on the drag force, while a random distribution has not been taken into account so far.

In this work, we introduce a novel and simple parallel plate flow channel designed to test the adhesion strength of different marine organisms on different surfaces. The flow channel was tested using H. banksii germlings settled on a variety of substrates of biomedical relevance. CFD modelling was used to determine the drag forces acting on H. banksii germlings and to quantify the actual drag force experienced by random arrays of settled germlings.

### 2. Material and methods

#### 2.1. Preparation of the substrates

Seawater was collected from Sumner, Christchurch, New Zealand (43°57’15.31” S, 172°76’56.70” E), and filtered through a 0.2 µm filter. Agar (bacteriological agar; Oxoid Ltd, Basingstoke, UK) was purchased from Thermo-Fisher Scientific. Absolute ethanol was obtained from Nuplex Specialties (Auckland, New Zealand). The ingredients used to prepare the skin model substrates were used as received without further purification: gelatin (type A, 275 bloom porcine skin; Gelita, Sergeant Bluff, IA, USA), lipid (Pro-lipid 141; International Specialty Products, Wayne, NJ, USA) and a cross-linker, microbial transglutaminase (Activa TG; Ajinomoto, Tokyo, Japan). Poly(methyl-methacrylate) (PMMA) flat sheet with a thickness of 2 mm (average roughness 63.5 ± 16.8 nm) was purchased from Dotmar Engineering Plastics (Christchurch, New Zealand).

Carbohydrate-based substrates (agar) were prepared from a 1.5% w/v solution of agar dissolved in purified water (Milli-Q, Millipore) at 70°C. A measure of 100 µl aliquots of agar solution were transferred to clean PMMA slides (25 × 75 mm) and uniformly spread across the slide surface on a horizontal table. The gelatin hydrogels (XL-Gel) were prepared using a 5% w/v gelatin solution in filtered seawater at a temperature of 50°C. Cross-linking of the protein fraction was induced by adding a 5% w/v stock solution of the cross-linker in RO water to give a final concentration of 0.2% of the cross-linker in the gelatin solution. A measure of 100 µl aliquots of the cross-linker and gelatin solution were transferred to clean PMMA slides before the cross-linking reaction completed. The gelatin hydrogels with added lipid (XL-Gel-Lip) were prepared following the same protocol, with the addition of a 20% w/v lipid solution in ethanol to a final concentration of 0.8% lipid prior to the addition of the cross-linker. The thickness of the hydrogel layers was 50 ± 3 µm.

For each substrate, 16 replicate slides were prepared (64 slides in total). These were kept in separate plastic containers (80 × 27 × 22 mm) and covered with 30 ml of filtered seawater to equilibrate the hydrogels. Two additional slides of each substrate were also prepared: one was seeded with H. banksii zygotes and used to monitor germling development while the other was used to measure contact angles.

In the remainder of this paper, the four surface types will be referred to as PMMA, Agar (agarose gel), XL-Gel (cross-linked gelatin) and XL-Gel-Lip (cross-linked gelatin with lipid component).

#### 2.2. Contact angle measurement

Contact angle measurements were performed depositing 2 µl droplets of deionized water on each substrate at 22°C. Advancing
Measurements were repeated four times on each substrate. Contact angles ($\theta_{\text{ABW}}$) were measured using a goniometer (CAM 200; KSV Instruments Ltd, Helsinki, Finland). Ten images of the water droplet were taken over the course of 1 min and the average contact angle was calculated using software (KSV CAM Software v. 4.01) fitting the droplet shape to the Young–Laplace equation.

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2.3. Sample collection, gamete release and fertilization, and settlement of germlings

The protocol used to obtain the germling suspensions is similar to the one previously described by Taylor et al. [18,21]. The H. banksii plants were collected at low tide from Shag Point, along the east coast of the South Island of New Zealand (45°27′35.50″ S, 170°48′47.26″ E), in April of 2014. All plants were thoroughly washed with filtered seawater to remove larger contaminants and stored in a dark environment at 6°C overnight. Gamete release was triggered by placing the plants under halogen lights (two lights, 200 W each) for 30–60 min at a temperature of 25°C. Egg and sperm solutions were prepared by washing female and male plants in separate seawater baths. Egg and sperm solutions were then filtered through 105 μm and 25 μm filters, respectively. The egg suspension was further clarified by allowing the eggs to deposit on the bottom of the container and replacing the supernatant seawater three times. Sperm and egg suspensions were then mixed to allow fertilization to occur. Sperm activity, egg viability and fertilization were checked using a compound microscope (Nikon model SE, Tokyo, Japan) equipped with a 1.3 megapixel USB CMOS camera (ODCM0130C; ProSciTech, Townsville, Australia) controlled using TouView (x64 v3.7.1691). The volume of the suspension was adjusted with seawater to obtain a concentration of approximately 20 000 zygotes per millilitre. Hereafter, we will refer to fertilized eggs in suspension as zygotes, while zygotes attached to a substrate will be referred to as germlings.

Each replicate of the four substrates was seeded with 2 ml zygote solution. Additional slides of each substrate (four) were also seeded to monitor germling development over time. The germlings were cultured in a temperature-controlled room at 15°C under a pair of fluorescent bulbs (light intensity = 40 μmol photons m$^{-2}$s$^{-1}$ PAR) on a 12:L:12 D cycle. Seawater in the trays was replaced every 24 h to prevent nutrient depletion. Germling adhesion on the different substrates was assessed at different post-settlement stages: after 6, 12, 24 and 96 h following the seeding with the zygotes. Four replicates of each substrate were randomly selected to be tested with the flow channel at the different post-settlement times. Germling development was also recorded after 6, 12, 24 and 96 h by measuring the diameter of the germlings settled on the additional slides.

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2.4. Flow channel design and testing of germling adhesion strength

A diagram of the flow channel is shown in figure 1, while a photograph of the actual set-up is presented in figure 2. The internal dimensions of the flow channel were 65 mm L × 4 mm W × 0.5 mm H, with inlet and outlet ports of 2 mm inner diameter. Substrates to be tested were clamped between a 22 mm thick Perspex top and a 10 mm thick stainless-steel base. A 0.7 mm thick silicone gasket maintained water-tightness and defined the channel walls. The gasket was held in place in a 0.6 mm deep groove surrounding the channel in the Perspex top. Seawater used in the flow experiments was filtered at 0.2μm and kept in a temperature-controlled environment at 15°C to minimize the stress caused to the germlings because of temperature variations. Seawater was pumped through the channel using the feed pump block (P-984; four pump heads) of an AKTAcrossflow (GE Healthcare, Uppsala, Sweden) controlled by UNICORN software.

Sample slides were clamped into the flow channel and exposed to a stepwise flow rate, starting at 50 ml min$^{-1}$ and increasing up to a maximum of 300 ml min$^{-1}$ with 50 ml min$^{-1}$ steps, corresponding to average fluid velocities between 0.42 and 2.5 m s$^{-1}$ with 0.42 m s$^{-1}$ step size. Each step lasted 15 s.

Germling attachment to the substrate was monitored in a single field of view using the USB camera mentioned before.
and a Nikon SMZ-1B dissecting microscope (Nikon Corporation, Tokyo, Japan) capable of up to 3.5× magnification. The dissecting microscope’s focal length easily accommodated the flow channel. Viewing at 3× magnification allowed the differentiation of cells as small as 30 μm within a viewable area of 9 mm². The ToupView software was used to control the camera and capture video of the experiments. Selected frames were extracted from the flow channel footage using VLC Media Player (v. 2.1.3). For each replicate, an initial frame was extracted before the germlings were exposed to the flow to calculate their initial density. In the following, density and surface density will be interchangeably used to denote the number of germlings per unit area. Additional frames were extracted at the end of every 15 s flow step. Germling densities were measured using the automatic particle counting feature of the software IMAGEJ (v. 1.47). Percentage survival after each flow step, \( S_v \), was expressed as the ratio between the density of remaining germlings, \( D_v \), and the initial density, \( D_0 \):

\[
S_v = \frac{D_v}{D_0} \times 100. \tag{2.1}
\]

Factorial analysis of variance (ANOVA) was used to test the influence of different settlement times and substrates on the initial abundance of the germlings (i.e. before being exposed to the flow) and on their survival rates recorded at the conclusion of the flow experiment (i.e. following the exposure to all flow intensities). Statistical significance was judged setting \( \alpha \) at 0.05 since two repeated, non-independent measures were taken from each replicate slide. The initial abundance of the germlings was analysed using a two-way ANOVA with the fixed factors: settlement time (four levels: 6, 12, 24 or 96 h) and material (four levels: PMMA, Agar, XL-Gel or XL-Gel-Lip). Data of germling percentage survival recorded at the conclusion of the flow experiment were analysed using a two-way ANOVA with the fixed factors: settlement time (three levels: 12, 24 or 96 h) and material (four levels: PMMA, Agar, XL-Gel or XL-Gel-Lip). The slides cultured for 6 h were excluded from this second analysis because survival rates could not be calculated due to the low starting germling densities compared with the other combinations of treatments (see Results and discussion). Before all analyses, variance heterogeneity was tested with the Cochran’s C-tests and removed with appropriate transformations when required. When homogeneity of variances could not be achieved by transformation, data were analysed nonetheless by judging significance more conservatively (\( \alpha = 0.01 \)). Student–Newman–Keuls (SNK) tests were performed for a posteriori comparisons of the means [35].

### 2.5. Computational fluid dynamics

The software COMSOL MULTIPHYSICS 4.2a (COMSOL Inc., Stockholm, Sweden) was used to reproduce the experimental flow channel and generate CFD models. Minor geometrical imperfections or surface roughness in the flow channel were not considered in the CFD simulations.

**Hormosira banksii** germlings were modelled as smooth spherical bodies randomly placed and rigidly connected to the bottom surface of the flow cell. Average values of germling diameter and settlement density were obtained from the adhesion experiment (i.e. 70 μm and 20 germlings mm², respectively; see Results and discussion). CFD simulations were repeated on five different random arrays of spheres to account for the variability in the velocity profile and drag force acting on different random configurations of settled germlings. Flow rates used in the CFD models were based on those used in the adhesion experiment, corresponding to average velocities in the channel between 0.42 and 2.5 m s⁻¹. Viscosity and density of seawater at 15°C were used in the CFD simulations, i.e. 1.08 × 10⁻³ Pa s and 1026 kg m⁻³, respectively [36].

**Table 1. Contact angles of the substrates investigated (± s.e., n = 4).**

<table>
<thead>
<tr>
<th>substrate</th>
<th>contact angle, ( \theta_{\text{RAW}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA</td>
<td>72° ± 3°</td>
</tr>
<tr>
<td>Agar</td>
<td>31° ± 3°</td>
</tr>
<tr>
<td>XL-Gel</td>
<td>56° ± 2°</td>
</tr>
<tr>
<td>XL-Gel-Lip</td>
<td>65° ± 1°</td>
</tr>
</tbody>
</table>

Additional CFD simulations were also generated for (i) the empty flow channel under the same flow conditions experimentally investigated, (ii) a single isolated cell under the same flow conditions experimentally investigated, and (iii) random arrays of germlings settled at seven intermediate cell densities, ranging from 1 to 22 germlings per mm², at the maximum experimental average velocity of 2.5 m s⁻¹.

The finite-elements method was used to solve the Navier–Stokes equations of fluid motion for a time-independent velocity profile across the domain. A flat velocity profile was assumed at the inlet orifice and a zero pressure was set at the outlet tube of the flow channel. No-slip conditions were considered along both the channel walls and the surface of the germlings. The mesh was refined at a minimum element size of 7.41 μm at the sphere periphery (with an individual sphere consisting of 5438 elements) and was gradually coarsened up to 110.24 μm at the peripheries of the flow channel. This arrangement provided the optimal mesh size as a trade-off between a grid-independent solution and computational time. The overall drag force was calculated as the sum of the elementary forces acting on each mesh element of the periphery of the spherical bodies. To further reduce computational time, mostly associated with the spheres and the fine mesh at their periphery, the array was built only on a limited portion of the flow channel where a fully developed velocity field was established. A preliminary study was conducted considering different areas populated with random spheres at densities of 5 and 20 spheres per mm². This study revealed that a 1 × 1 mm² section provided invariant results while minimizing computational time. Accordingly, all simulations were performed considering random arrays of spherical bodies confined in a 1 × 1 mm² area.

### 3. Results and discussion

#### 3.1. Substrate selection and contact angle measurement

Four different substrates were considered to assess the adhesion strength of the biological glue secreted by *H. banksii* zygotes: untreated PMMA as a reference solid substrate, and three hydrogels based on proteinaceous and carbohydrate components. The substrates were chosen on the basis of three considerations: (i) composition of the biofilm covering the substrate in the natural environment, (ii) relevance in biomedical applications, and (iii) diversity of chemical compositions.

The PMMA is a material widely used in biomedical applications, with particular relevance in dentistry [37], orthopaedic surgery and in contact and intraocular lenses [38]. Human skin, the organ with the largest exposed surface, is mainly composed of collagen and lipids [39]. Cross-linked gelatin is widely applied in the medical field as absorbable haemostats, tissue adhesives and sealants, and scaffolds for tissue engineering [39–41]. Agarose hydrogel approximates the polysaccharide component of extracellular polymeric substances (EPSs).
produced by bacterial biofilms [42] common on substrates available to marine macroalgae [43].

Surface wettability can have a significant impact on settlement rate and adhesion strength of marine organisms [44]. Contact angles were measured to assess the relative hydrophobic/hydrophilic character of the different substrates (table 1). All studied substrates displayed hydrophilic character ($\theta_{\text{AW}}$, 90°). The agar substrate had the most hydrophilic characteristics ($\theta_{\text{AW}} = 31°$) associated with the high content of hydroxyl groups in the carbohydrate backbone [27]. PMMA is inherently hydrophobic, but hydroxyl groups are often introduced in the polymeric structure to decrease its hydrophobicity in biomedical applications [45]. The PMMA material used in our study had a moderate hydrophilic character with a contact angle of 72°. The XL-Gel substrate displayed intermediate hydrophobicity, while the addition of the lipid component into the gelatin hydrogel, even if in relatively small amount, shifted the contact angle from 56° to 65°.

Table 2. Average zygote/germling diameter across all materials and at different stages of development (± s.e., $n = 10$). Zygote diameter was measured immediately after fertilization and before settlement.

<table>
<thead>
<tr>
<th>developmental stage</th>
<th>germling diameter ($\mu$m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PMMA</td>
</tr>
<tr>
<td>zygotes</td>
<td>65.9 ± 0.9 μm</td>
</tr>
<tr>
<td>6 h germling</td>
<td>67.0 ± 1.3</td>
</tr>
<tr>
<td>12 h germling</td>
<td>68.5 ± 0.3</td>
</tr>
<tr>
<td>24 h germling</td>
<td>70.7 ± 0.6</td>
</tr>
<tr>
<td>96 h germling</td>
<td>75.6 ± 1.4</td>
</tr>
</tbody>
</table>

3.2. Germling development

Hormosira banksii germling growth was monitored at 6, 12, 24 and 96 h following settlement as illustrated in figure 3, while table 2 details the diameters of germlings cultured on the different substrates at the different developmental stages. Initial zygote diameter immediately post-fertilization was 65.9 μm, while an average diameter of 75.8 μm was measured at 96 h post-settlement, indicating a size increase around 15%, with negligible differences among substrates. Germling size increased slightly during the first hours after fertilization, but polarization, cell duplication and formation of a pro-rhizoid only occurred after around 24 h. Between 48 and 72 h the germlings shed the primary cell wall that formed immediately following fertilization (pictures not shown), with the rhizoid tip becoming the main point of attachment. While these observations do not necessarily imply that the specific processes involved in the production and secretion of adhesives are identical regardless of substrate, they do indicate that progression...
through the developmental stages is consistent; therefore, primary and secondary adhesion events might be similar across all substrates.

The dimensions of the germlings were always one order of magnitude higher than the roughness of the PMMA slides and the hydrogel coatings. Accordingly, the influence of surface roughness on germling attachment was neglected.

3.3. *Hormosira banksii* zygote settlement

Initial germling densities, measured at different post-settlement stages before the flow experiment, varied with settlement time (figure 4). Substrates sampled after 6 h had significantly fewer germlings than those sampled after 12, 24 and 96 h, which did not differ among each other ($F_{3,48} = 28.71$, $p < 0.001$). There were no significant differences among materials ($F_{3,48} = 2.45$, $p = 0.07$) and no interactive effects of material and settlement time ($F_{9,48} = 2.08$, $p = 0.05$). The average germling density 12, 24 and 96 h after the settlement was around 20 germlings mm$^{-2}$. Reduced densities at 6 h were probably due to the detachment of the germlings as the slides were transferred from their trays to the flow channel. This observation suggests a poor attachment of the germlings in the early post-fertilization stages, probably due to the fact that the adhesion curing process requires longer than 6 h to achieve significant strength. However, there is a suggested, albeit not statistically significant, elevated initial density on the Agar substrate after 6 h settlement. Bearing in mind that this may be the result of uncontrolled variability in forces generated when transferring substrates to the flow channel, it is also worth noting the possibility that this is the result of rapid interactions between the polyphenolic adhesive secretion, the polyanionic moieties of the substrate and positive divalent ions present in seawater, namely Ca$^{2+}$ and Mg$^{2+}$ [3]. Alternatively, increased settlement on Agar may lie in haloperoxidase-mediated cross-linking reactions involving polyphenolic adhesive compounds and the agarose chains of the substrate [8,9,46], though it is unlikely that these processes will have taken place by 6 h settlement. At the other end of the spectrum, initial settlement density after 6 h is lowest on the XL-Gel-Lip surface. Hydrophobic lipid chains embedded in the collagen network may have an influence in preventing the adsorption of the adhesive to the substrate, possibly by limiting the electrostatic forces involved in adsorption and/or by sterically hindering adhesive access to receptive substrate functional groups. It is apparent, though, that exposure to even the lowest flow velocities removes the relative variations in settlement density (figure 5), supporting the idea that any adhesion achieved by the germlings after 6 h is defeated at shear stresses $\leq 5$ Pa regardless of substrate type. This is consistent with the observations reported by Taylor & Schiel [18], who concluded that *H. banksii* zygotes show feeble adhesion between 1 and 6 h post-settlement. In their experiments, cultures of *H. banksii* zygotes were settled on fibre-based cement plates and challenged with artificially

Figure 4. Initial density (+ s.e., $n = 4$) of *H. banksii* germlings settled on the various substrates measured at different post-settlement times.

Figure 5. Surface density of *H. banksii* germlings settled for 6 hours and exposed to increasing linear velocities (+ s.e., $n = 4$).
generated and natural waves, with reported survival rates in the order of 50–60% at 6 h post-settlement.

Average germling settlement density for 12, 24 and 96 h settlement was 20 germlings mm$^{-2}$. The fact that the same initial germling density was observed across all substrates and settlement times after 12 h indicates that the adhesion strength achieved by 12 h is sufficiently high so that any variation in forces generated when transferring the substrates to the flow channel become negligible.

### 3.4. Test of germling adhesion strength

Flow rates used in the flow experiments, $Q$, ranged between 50 and 300 ml min$^{-1}$, corresponding to mean fluid velocities, $v$, between 0.4 and 2.5 m s$^{-1}$. The corresponding Reynolds numbers were calculated using the formula

$$Re = \frac{\rho v D_h}{\mu}, \quad (3.1)$$

where $D_h$ is the hydraulic diameter of the rectangular profile of the flow channel

$$D_h = \frac{4hw}{2(h+w)}, \quad (3.2)$$

where $h$ and $w$ are the channel’s height and width, respectively. Resulting Reynolds numbers ranged between 352 and 2111, indicative of laminar Poiseuille regime in the flow channel across all flow rates tested. The theoretical wall shear stress for laminar flow of Newtonian fluid, $\tau_w$, ranged between 5 and 32 Pa as determined using the following relationship:

$$\tau_w = \frac{6Q\mu}{h^2w}. \quad (3.3)$$

Despite the different geometry, our flow channel is comparable to other existing flow channels in terms of wall shear stresses that can be achieved. For example, the turbulent flow apparatus developed by Hodson et al. [25] is able to create wall shear stresses up to 45 Pa, while the flow channel used by Schultz et al. [24] generates wall stresses between 0.9 and 30 Pa. We remark, once more, that the wall shear stress is not ideal for quantifying the adhesion strength of sessile organisms, mainly because it ignores the size and shape of the adhered organisms as well as the actual flow field present. Nevertheless, we will use shear stress to facilitate the comparison in adhesion strength between H. banksii germlings and other benthic species.

As the substrates sampled after 6 h presented lower germling densities than the other settlement times, it was not possible to compare the survival rates recorded in the 6 h treatment with those observed after 12, 24 and 96 h. Nonetheless, variations in germling densities observed during the flow experiment on the substrates tested after 6 h are shown in figure 5 to provide an indication of germling abundance trends across increasing linear velocities. Despite the large variations in initial density at $v=0$, germling density decreases rapidly once exposed to fluid flow, confirming very low adhesion strength after a settlement period of only 6 h.

Figure 6 presents the experimental survival data of germlings settled on different materials for different settlement times.

![Figure 6. Germling survival (± s.e., n = 4) versus linear velocity and wall shear stress for the different material surfaces and settlement times.](http://rsfs.royalsocietypublishing.org/)
times (12, 24 and 96 h) and exposed to increasing flow velocities. Similar germling survival trends were observed on all substrates. Germling survival decreased more rapidly after a settlement period of 12 h than after the 24 and 96 h treatments. Survival at the conclusion of the flow experiment, i.e. after exposure to all flow velocities, was significantly lower on the substrates tested 12 h after the settlement of the germlings, compared with those examined after 24 and 96 h ($F_{2,36} = 32.23$, $p < 0.001$). Survival rates did not differ among materials ($F_{3,36} = 1.14$, $p = 0.35$) and were not affected by the interaction between materials and settlement time ($F_{6,36} = 0.89$, $p = 0.51$).

Relative adhesion strength is often established in relation to the wall shear stress required to remove 50% of the germlings initially present, i.e. at 50% survival. Even though the shear stresses at the wall generated by our flow channel are of the same order of magnitude as other flow apparatuses currently available, the flow conditions were not sufficient to displace 50% of the germlings from any of the different substrates after a post-settlement period of 12 h or longer.

The lack of variation in survival across the various substrate types suggests the adhesion mechanism used by *H. banksii* propagules is promiscuous enough to engage with a range of substrate types. While all of the surfaces were technically hydrophilic, there was no obvious impact on adhesion strength caused by the relative differences in hydrophobicity of the substrates. Similarities in adhesion strength could be due to the presence of a range of functional groups present in the adhesive material such as glycoproteins and anionic polysaccharides [9,10], allowing interaction with a variety of substrate types. Additionally, the functional groups presented on the surface of the carbohydrate- and protein-based hydrogels, and the polar moieties of PMMA all provide a wealth of locations for adsorption interactions. The large number of hydroxyl groups present in the Agar substrate could result in the formation of metal ion coordination complexes with anionic adhesive components as well as haloperoxidase-mediated cross-linked networks [9]. The high proportion of glycine and arginine residues in gelatin [47] may facilitate the formation of hydrogen bonds between a polar adhesive compound and the substrate. This observation highlights the more consistent adhesion strength of *H. banksii* germlings to different substrates than that achieved by other marine species. For example, the shear stress required to remove 50% of the cells of the diatoms *Navicula perminuta*, *Amphora coffeaeformis* and *Caspodiotus australis* were 25, 10 and 3 Pa, respectively, on glass slides, but more than 53, 24 and 17 Pa, respectively, on poly-dimethylsiloxane elastomer substrate when given 2 h to settle [28]. Strong adhesion in the marine environment is also achieved by mussels, which have been found to bind equally well to a range of inorganic and organic surfaces [48]. The capacity to adhere effectively to a variety of surface types is important to exploit the large range of substrate chemistries presented by the marine environment, including a range of inorganic (e.g. rock) chemistries, and the organic EPSs ubiquitously present due to bacterial biofilms.

### 3.5. Computational fluid dynamics

The calculated Reynolds numbers suggest laminar flow across all experimental conditions. However, both the side walls and the inlet and outlet ports may generate specific disturbances that may cause a mismatch with the ideal fully developed flow in a rectangular channel. Preliminary CFD simulations conducted using an empty channel evidenced that the entrance length, $L_e$, required to achieve fully developed parabolic flow profile was, in the worst case, equal to 23 mm. This result is in line with a calculated entrance length for rectangular ducts of 19 mm, as determined using the empirical relationship proposed by Han [49].

$$L_e = 0.01(D_1Re) \quad (3.4)$$

The CFD modelling also indicates exit effects were confined to within 4 mm of the outlet orifice and, owing to the small height to width ratio of the channel ($h/w = 0.125$), the influence of the two sides on the flow field was limited to 0.3 mm. These results clearly highlight that fully developed Poiseuille flow was always present over the region experimentally monitored.

In general, laminar flow over an object in contact with an infinite plane produces three different external actions on the object: a drag force oriented in the main direction of the flow, $F_D$, a lift force normal to the plane, $F_L$, and a torque with axis parallel to the plane and orthogonal to the main direction of the flow, $T$ [50,51]. In the ideal case of laminar infinite linear shear over a single spherical body, the drag, lift and torque exerted can be computed using the following expressions [52,53]:

$$F_D = 32.0 \tau_w r^2 \quad (3.5)$$

$$T = 11.9 \tau_w r^3 \quad (3.6)$$

$$F_L = 9.257 \tau_w r^2 Re_w \quad (3.7)$$

where $r$ is the radius of the sphere and $Re_w$ is the wall Reynolds number, defined as

$$Re_w = \frac{\rho \dot{\gamma}_w r^2}{\mu} \quad (3.8)$$

where $\dot{\gamma}_w = \tau_w / \mu$ is the wall shear rate. It is important to note that equations (3.5)–(3.7) are valid only when the inertial forces are negligible compared with the viscous forces, i.e. for wall Reynolds numbers lower than 1. This condition was nearly fulfilled in the experimental system investigated, where $Re_w$ ranged between 0.9 and 5.4.

The local stresses present within the adhesive material and at the pad–substrate interface highly depend on the morphology and structure of the adhesive itself. Different approaches have been proposed to model the stress distribution at the interface, with the uniform stress model and the peeling model frequently considered [54–56]. Despite its simplicity, the uniform model, in which all the bonds within the contact area are equally stressed, is able to provide a good estimate of the order of magnitude of the interfacial stresses. According to this model, the mechanical stresses present in the attachment pad serve to counterbalance the acting forces. In particular, the drag force will result in a uniform shear stress, $\tau_D$, the lift force will create a uniform normal stress, $\sigma_N$, while the torque will produce a linear normal stress, $\sigma_T$, zero at the contact point between the sphere and the surface and maximum at the distal parts of the adhesive pad in the direction of the flow [57]. If the adhesive pad has a circular profile with the same dimensions as the sphere (note that the latter assumption is not strictly required and a circular adhesive pad with any dimension could be alternatively used; however, this assumption is used to exemplify the
following relationships) then the stresses present in the substrate–glue interface will be
\[ \tau_D = \frac{F_D}{\frac{D}{2}} = 10.2 \tau_w, \quad (3.9) \]
\[ \sigma_{T,\text{max}}^n = \frac{T}{W} = 15.0 \tau_w \quad (3.10) \]
and
\[ \sigma_L = \frac{F_L}{\frac{W}{2}} = 2.95 \tau_w \frac{R_{\text{sw}}}{\text{c}}, \quad (3.11) \]
where \( W = \pi \frac{D}{2} \) is the elastic section modulus of the circular profile.

Even though equations (3.9)–(3.11) are strictly valid for a single sphere under infinite laminar flow, they can provide a useful estimate of the order of magnitude of the stresses acting on an isolated H. banksii germling present in the parallel plate flow chamber. In particular, it is apparent that, at same flow conditions (i.e. same wall shear stress), and under the experimental wall Reynolds number, all stresses present in the adhesive pad of the settled germlings have the same order of magnitude. Hence, for an isotropic adhesive, calculation of one of the stresses offers a simplified approach for the estimation of the adhesion strength of the adhesive. Accordingly, the drag force has been considered as a reference value in this work and computed in the CFD simulations. A comparable model that can reliably predict the forces/stresses acting on an organism within a parallel wall flow apparatus, i.e. at different specifications of the channel width, average flow velocity and organism dimensions, especially in the light of its wide use in bioadhesion and biofouling [26–29].

Compared with the isolated sphere, the average drag force exerted on the germlings within the random array had a concave downward trend with increasing average velocity. At higher velocities there is a notable reduction in drag force of approximately 40%. The main reason for the lower average drag force is identified as a consequence of the formation of wakes, shielding the germlings settled downstream in the flow channel (figure 8a). The protective action of the wakes reduces the resultant of both the viscous and pressure forces.

Viscous forces, i.e. the forces tangential to the germling surface, are associated with the velocity profile, with higher forces in correspondence with sharper velocity gradients. The highest gradients are observed on the two sides of the frontal germlings, where the velocity transits from 0 in correspondence with the germling periphery to its highest values (coded by the colour red in figure 8a). Germlings in the wake experience a much gentler velocity gradient, indicating a smaller viscous force. On the other hand, pressure forces are directed orthogonally to the spherical surface and are related to the pressure profile. The drag associated with the normal forces is mainly due to the pressure difference between the front and the tail of a sphere. Figure 8c clearly shows that this difference is less pronounced when the germlings are shielded inside the wake.

Given the reduced average drag force affecting the germlings within a colony of conspecifics, it is interesting to quantify the average drag force acting on random arrays of germlings at different surface densities. Figure 8 reported the velocity and pressure profiles for random arrays of 5 and 20 germlings per mm², while figure 9 shows the effect of different surface densities on average drag force. In general, a lower proportion of the settled germlings are shielded by wakes when lower germling densities are present on the substrate. Consequently, a clear decrease in the drag force is observed as the germling density increases, corresponding to lower hydrodynamic stress acting on the settled germlings on both the x and the y directions. This observation indicates a strong degree of germling–germling interdependence in adhesion success, offering interesting ecological insights on the importance of germling density in the face of hydrodynamic forces. The role of density-dependent interactions among seaweed juvenile individuals is highly debated and controversial, with both positive and negative effects being reported [15]. Our results suggest that intraspecific density-dependent facilitation may be key for surface colonization by H. banksii in natural contexts.

We recall that the CFD simulations were performed by approximating the settled germlings with spherical bodies in direct contact with the substrate. This assumption is most accurate for approximating drag forces acting on the younger germlings, before the morphology changes associated with polarization and cell division occur. In addition, adhesion to the substrate progressively shifted from a broad connection underneath the germling thallus to a more localized attachment at the rhizoid tip, where the associated elongation of the rhizoid allowed some germlings to shift into an upright position, offering interesting ecological insights on the importance of germling density in the face of hydrodynamic forces. The role of density-dependent interactions among seaweed juvenile individuals is highly debated and controversial, with both positive and negative effects being reported [15]. Our results suggest that intraspecific density-dependent facilitation may be key for surface colonization by H. banksii in natural contexts.
position. These complications were not taken into account in the current CFD work and represent the object of future studies.

4. Conclusion

In this paper, a novel flow channel to test the adhesion strength of sessile marine organisms was presented. The adhesion strength of the germlings of the brown alga *H. banksii* was assessed on various materials of environmental and biomedical relevance, namely PMMA and protein- and carbohydrate-based hydrogels. Germling development was similar on all the surfaces, with polarization and rhizoid development beginning after 24 h following their settlement. No adverse effects on development were recorded on any of the substrates. Survival rates observed using the flow channel were comparable across the different substrates investigated, with an increase in adhesion strength with settlement time. Survival increased greatly between 12 and 24 h, but did not vary between 24 and 96 h post-settlement. This indicates that, under the experimental conditions investigated in the present study, maximum adhesion strength was achieved approximately 24 h following fertilization.

The CFD simulations were also generated to model the drag forces acting on germlings, both as isolated organisms and as part of colonies of varying surface densities. The simulations showed that, under equivalent flow regimes, germlings within an array experienced, on average, lower drag forces than the drag force acting on a single germling. This suggests that the presence of neighbouring individuals may help the germlings to withstand challenging hydrodynamic conditions. This same behaviour is expected to continue as the germlings develop, where the interactions between nascent holdfasts could also benefit survival. However, as a proportion of the population gets washed out over time, the net result of increasing adhesive strength and decreasing hydrodynamic sheltering become more complicated. Similarly, substrates with topographical features the same order of magnitude as the settled germlings will help reduce the drag force acting on them, even in the case of isolated germlings. The extent of such reduction will ultimately depend on the morphology and surface density of the asperities.

Figure 8. Velocity profiles (a,b) and pressure fields (c,d) with a random array of 20 germlings (a,c) and five germlings (b,d). The profiles are shown in a horizontal section parallel to the flow direction and passing through the centre of the spheres. Average velocity = 2.5 m s⁻¹.

Figure 9. Effects of cell density on drag force at $v = 2.5$ m s⁻¹ (± s.e., $n = 5$).
Future developments of this research will investigate how the chemical, physical and topographical properties of the settlement surface can influence the adhesion strength of marine organisms. The flow channel will be used to study the attachment of H. banksii and D. antarctica on different inorganic materials with varying nano-topographies. In an effort to compare the relative strength of underwater adhesives produced by different marine organisms, other algal species will be considered as well as other marine sessile organisms. These studies can contribute to the characterization of the adhesive strength of the biological glue produced by marine organisms and the assessment of the adhesion force of natural, biomimetic and synthetic water-resistant adhesives. The flow channel and the CFD model presented in this work can also be used to assess the characteristics of antifouling surfaces developed to prevent attachment and colonization of immersed man-made surfaces.

Acknowledgements. The authors thank Tim Huber of the Biomolecular Interaction Centre, University of Canterbury, New Zealand, for his assistance with contact angle measurements.

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