A novel adaptation facilitates seed establishment under marine turbulent flows

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Seeds of Australian species of the seagrass genus Posidonia are covered by a membranous wing that we hypothesize plays a fundamental role in seed establishment in sandy, wave-swept marine environments. Dimensions of the seed and membrane were quantified under electron microscopy and micro-CT scans, and used to model rotational, drag and lift forces. Seeds maintain contact with the seabed in the presence of strong turbulence: the larger the wing, the more stable the seed. Wing surface area increases from P. sinuosa < P. australis < P. coriacea correlating with their ability to establish in increasingly energetic environments. This unique seed trait in a marine angiosperm corresponds to adaptive pressures imposed on seagrass species along 7,500 km of Australia’s coastline, from open, high energy coasts to calmer environments in bays and estuaries.

Results

To test our hypothesis, X-ray micro-computed Tomography (X-ray micro-CT) scans were used to digitally dissect seeds for three species of Posidonia (P. coriacea, P. australis and P. sinuosa, Fig. 1A, see Supplementary Movie S1, Tables S1 and S2) to precisely determine the composition, size, surface area and shape of the membranous wing. Scanning Electron Microscopy (SEM) observations revealed that the wing of all three species is unsculptured, smooth with wavy surfaces (Fig. 2) and consists of 3–5 layers of irregular elongated, thickened and flattened cells developed from the ovary wall (Fig. 2). There is no differentiation in the structure of the winged membrane

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covering seeds between the three *Posidonia* species, other than marked differences in the wing width (Fig. 1A,B),
which is also related to the different hydrodynamic regimes where they typically colonize (Table 1). Mean max-
imum width of the wing (Fig. 1B) of 30 seeds was significantly different between the three *Posidonia* species
(ANOVA (ln transformed), $F_{2,87} = 753.7, p < 0.001$). Surface area (Fig. 1C), surface area to volume ratios (Fig. 1E)
($p < 0.01$) and volume (Fig. 1D) ($p < 0.05$) were significantly smaller ($n = 3$, one-tailed paired t-tests, Table 2)
when the membranous wing was removed from seeds suggesting the role of the wing is to increase seed surface
area relative to volume.

A combination of laboratory flume experiments and Computational Fluid Dynamic (CFD) modelling of the
3-dimensional micro-CT scans demonstrated that the large differences in wing width in congeneric species are a
direct adaptation to the hydrodynamic environments where each species are found (Table 1). The membranous
wing that covers the seed, reduced hydrodynamic rotation, drag and lift forces (Fig. 3). There was a tendency for
seeds from all species to rotate in the horizontal plane into a stable position on the bed (Fig. 3A). The greatest
stability occurs when the seeds are pointed into the current or when the membranous wing is pointed into the
current (Fig. 3A).

Seeds maintain contact with the seabed in the presence of strong turbulence: the larger the wing, the more
stable the seed. When all seeds face into the current, drag and lift coefficients (normalised by the seed plan area
and represent the respective forces) were small and converged to similar values for all cases (Fig. 3B, C). However,
the drag coefficient rapidly increased as the seeds were rotated out of this stable position (Fig. 3B). For *P. coriacea*,
the membranous wing could either act to impose lift force on these seeds if they were positioned slightly askew
from their most streamlined position, or act to impose a downward force when the seeds were positioned with
the membranous wing pointed into the current (Fig. 3C). *Posidonia coriacea* seeds have the most effective wing
for reducing hydrodynamic forces that are typical of shallow wave-exposed coastal environment in western and

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**Figure 1.** (A) Micro-CT scans *Posidonia coriacea* (*P. c*), *P. australis* (*P. a*) and *P. sinuosa* (*P. s*) showing size of
wing in relation to the seed (scale = 5000 µm), (B) differences in the width of the wing taken at the widest point
for seeds ($n = 30$) for *Posidonia* species, (C) surface Area, (D) volume and (E) surface Area to Volume ratio
determined from micro-CT are shown for 3 paired replicate seeds for each species with and without the wing.
southern Australia (Table 1). The lift coefficients for *P. australis* and *P. sinuosa* were less influenced by the presence of a membranous wing (Fig. 3C), corresponding to their usual habitat, wave sheltered bays and estuaries, where the seeds are able to settle and colonize less energetic environments with reduced forces that could affect their stability (Table 1).

**Discussion**

Our results demonstrate for the first time that a unique morphology, a thin membrane covering the seed in the Australian species of the marine genus *Posidonia* has evolved to utilize benthic boundary layer physics to settle and attach to the substratum across environmental gradients in wave and current intensity. This thin seed membrane, provides an essential window of stability until the rapid gravitropic growth of the root anchors the seed to the bed. An example of convergent evolution among differing taxa, the asymmetric linear shape of this membranous wing and the seeds of seagrasses is similar in form to similar structures utilized by algae and invertebrate larvae to settle and attach to the substratum in moving seawater. We hypothesize that this unique seed trait in

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**Table 1.** The swell and wind wave climate where seeds of *Posidonia coriacea*, *P. sinuosa* and *P. australis* settle compared to the congeneric *P. oceanica* in the Mediterranean.

| Species      | Region            | Minimum Depth of Meadows                      | Ocean Swells          | Wind waves       |
|--------------|-------------------|-----------------------------------------------|-----------------------|------------------|
| *P. coriacea*| SW Australia      | Exposed coasts, deeper inner shelf regions (>3 m) | 2–3 m mean 8–12 m max | 1.5 m summer     |
| *P. australis*| S Australia       | Estuaries, sheltered coasts                   | 1.5 m mean 7 m max    | 0.5 m summer     |
| *P. sinuosa* | S Australia       | Sheltered coasts, deeper coastal regions (>1 m) | 1.5 m mean 7 m max    | 0.5 m summer     |
| *P. oceanica*| W. Mediterranean  | Sheltered coasts, deep inner shelf regions (>1 m) | <0.5–2 m max          | <0.25–0.5 m summer|

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**Figure 2.** Scanning electron micrographs of surface (A,C,E) and cross-section (B,D,F) of a keel of *P. coriacea* (A,B), *P. australis* (C,D) and *P. sinuosa* (E,F). Bars = 200 µm (A,C,E) or 20 µm (B,D,F). The arrowhead in (A,C,E) indicates base of the membranous wing; W, wing; S, seed. The arrows in (B,D,F) indicate ‘flattened’ cells.
The form of the membranous wing as shown from SEM is quite similar to a winged achene or samara, such as found in maple and ash trees, where papery tissue from the ovary wall develops into a flattened single-sided aerodynamic wing. The wing reduced hydrodynamic rotation, drag and lift forces resulting in the seed being less susceptible to movement on the seabed. Such movement disrupts the gravitropic growth of the root and consequently the success of seedling recruitment at a given location. Seeds of all species rotated in the flow to two weak equilibrium positions parallel to flow direction or where the wing was pointed into the current. Flume experiments with elongated shapes (objects of similar geometrical form to Posidonia seeds) in unidirectional flow have demonstrated that the most stable solution for an asymmetrical elongated shape is parallel to flow direction10 and that elongated shapes are prone to automatically rotate into a pattern that included two weak equilibrium positions11, consistent with our seed rotation results. The wing adds a further dimension, producing a downward force if aligned into the current. Such a downward force has been observed to stabilise flounder on the seabed12 and is also deliberately induced through the careful design of wing features on vehicles13.

The membranous wing in Australian species of Posidonia is a clear demonstration of how form evolves to overcome the physics of settlement and attachment into the benthic boundary layer in the ocean. The need for seeds to maintain a stable position to allow root initiation and growth is similar to the broader issues of settlement and attachment of marine organisms in moving seawater. Some evolved solutions in microbes, algae and invertibrates and other species of seagrass include mucus sheaths in red algae9 and marine snails14, mucus threads in bivalves20, and adhesive hypocotyl hairs in other seagrasses21,22. If the seeds are moved by currents, waves or turbulence the strong gravitropic root response is disturbed. This disturbance can result in roots that develop into the water column or become ‘corkscrewed’ as root growth adjusts to being rotated and tumbled at the sediment surface.

Seagrasses are an unusual ancient group of higher flowering plants that have evolved unique traits to establish, grow, reproduce, and survive in the sea (e.g. tolerance to salt water, submarine pollination, reduced cuticle on the leaf surface, no stomata, chloroplasts in the epidermis). While seagrasses are highly clonal, sexual reproduction and their offspring, seeds, are also important in both ecological and evolutionary connectivity3,4,23,24. Our results demonstrate, for the first time, that species in the marine genus Posidonia have a unique morphology where a thin membrane covering the seed, plays a critical role in its establishment across environmental gradients in wave and current intensity until the seed is able to anchor with rapid growth of the gravitropic root8. The primary root is already growing in the direct developing seeds of Posidonia and rapidly extends into the sediment by 0.5 to 1 cm within 12–24 hours. This reliance on root growth differs from P. oceanica in the Mediterranean where seeds initiate many sticky hypocotyl filaments that attach to rocks and sand grains as a primary root develops8,12. The development of membranous wings appears to have evolved after the isolation of Australian congeneric species from P. oceanica with the closure of the Tethys Sea during the Miocene (20–10 Myr BP)25,26. Sticky hypocotyl filaments like those found in P. oceanica have also been described from other seagrass genera including Zostera27, Halophila28,29 and Thalassia30. That hypocotyl filaments are less abundant in Australian Posidonia increases the importance of the wing-like adaptation of the membrane covering seeds.

Seagrasses are some of the most threatened habitats in the world’s oceans today31,32. Despite providing significant ecosystem services, especially nursery habitat33, as well as many ongoing efforts to ameliorate anthropogenic effects that are the leading cause for seagrass decline34, attempts to restore seagrass remain elusive35. The establishment phase has proved to be a major bottleneck to recovery in many environments35. As seagrass seeds are known to be vitally important in the recovery of seagrass meadows23, a thorough understanding of how seeds of different seagrass species behave in aqueous environments will be one crucial element in the success of managing seagrass ecosystems for future resilience and in developing successful seed-based restoration strategies similar to those found in terrestrial plant restoration36.

### Methods

**Collection of seeds.** Approximately 30 fruits, containing an individual seed were collected from inflorescences of P. coriacea, P. australis and P. sinuosa. These collections were made between 2 and 5 m depths using SCUBA at Rottnest Island (S 32.080625°, E 115.548531°), and Parmelia Bank (S 32.096408°, E 115.728296°), Western Australia. The fruit were transferred to large 300–800 L aquaria with recirculating seawater

| Species | variable | d.f. | t-test | p-value | 95% CI | Mean difference |
|---------|----------|------|--------|---------|--------|----------------|
| P. coriacea | Surface Area (SA) | 2 | 19.316 | 0.0013 | 309.399 | 364.5 |
| P. australis | Volume (V) | 2 | 15.636 | 0.0020 | 41.747 | 51.333 |
| P. sinuosa | SA/V ratio | 2 | 11.942 | 0.0035 | 1.055 | 1.397 |

Table 2. One tailed paired t tests of surface area (SA), volume (V) and surface area to volume ratios (SA/V) between paired seeds with the membranous keel attached and when removed for P. coriacea, P. australis and P. sinuosa.
(salinity = 35.6) in a greenhouse until the seeds were released. Seeds were then taken into the laboratory for processing. Maximum wing width was measured from 30 seeds from *P. sinuosa*, *P. australis* and *P. coriacea* with the use of a pair of calipers and a dissecting microscope.

**Scanning electron microscopy (SEM).** Seeds of each species were fixed in a solution of 2.5% glutaraldehyde and 1.7% paraformaldehyde in seawater that was buffered with 0.1 M phosphate buffer. Seeds were stored at 4 °C until SEM. Segments 10–15 mm² in length were excised from different parts of the wings, washed in deionized water, dehydrated in a graded series of ethanol (30–100% and 100%-anhydrous) and then flooded with

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**Figure 3.** Variation in rotational (A), drag (B) and lift (C) coefficients (normalized by the seed plan area) for *P. coriacea*, *P. australis* and *P. sinuosa* with and without a wing for different positions on the seabed.
The Navier Stokes equations are averaged both in time and in space and the goal is averaged to obtain the turbulent vortices and turbulent motions. No source term is defined over the bed and extended to a height of 5 mm above the bed. We then used the SnappyHexMesh grid generation algorithm to refine the grid over three layers around seed object. To evaluate the grid-size independence of the numerical solution, results for the default computational grid described above and a finer grid (resolution increased by approximately 40%) were compared. Differences between numerical results generated with the two grid resolutions were negligible, indicating grid-size-independent solutions.

A mean pressure gradient was imposed in the streamwise direction to drive the flow at the critical velocity determined from laboratory experiments. At the bed and seed surfaces, a no-slip condition was applied. To avoid the complexity of modeling the free surface, the upper boundary of the domain was treated as a frictionless rigid lid. The water depth in all cases was 0.2 m, which ensured a free-stream flow condition developed above the seed. The simulations were allowed to run until a steady state condition was achieved, which was defined by monitoring the drag force until this force stabilized. A dynamic adjustable time stepping technique was used to guarantee a local Courant number less than 0.5.

To calculate the rotation, drag and lift forces for each seed case as well as for different seed orientations, the pressure and viscous forces acting on the seeds were calculated. The normalized force coefficients were then determined using established drag, lift and rotation equations (see Supplementary Methods). Simulations and calculations were undertaken at 10 degree resolution, by rotating the seed in the horizontal plane about the seed’s centre of mass.

Laboratory experiments. The critical velocity to initiate seed movement was quantified for 30 seeds of each species, with and without a membranous wing, in a reticulating flume at the Indian Ocean Marine Research Centre - Watermans Bay (40 cm × 50 cm × 7.5 m). A sediment bed of beach sand (median grain size of 327 μm) was constructed and smoothed in the flume prior to the commencement of each experiment. In each experiment, six seeds were then placed on the bed and the flume slowly filled with seawater (salinity = 35.6‰) to a depth of 60 cm. The current was slowly increased at 1 cm s⁻¹ increments and maintained for 2 min over a current velocity range of 5–48 cm s⁻¹. The current velocity was measured at 64 Hz using an acoustic Doppler velocimeter (Nortek ADV) positioned 23 cm above the bed and the velocity acting on the seed determined for the steady two-dimensional flow over a hydrodynamically rough bed using the Karman-Prandtl equation. The position of the seeds were tracked using a downward facing camera at 24 Hz. The video data was transformed into Cartesian coordinates, corrected for distortion and the position of the seeds analysed frame-by-frame for the duration of the experiment. The velocity that initiated seed movement along the bed was defined as the velocity where the seed had the greatest instantaneous movement distance.

Data availability
3D reconstructions of the seeds from the micro-CT scans are available on the UWA Research Repository (https://doi.org/10.26182/5df1a8623d1ea). All other data is available in the main text or the Supplementary Materials.

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Author contributions
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Competing interests
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