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EFFECT OF HIERARCHICAL STRUCTURE AND ORIENTATION ON WATER-
REPELLENT LEGS OF WATER-WALKING INSECTS

By

Georgia N. Hurchalla

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

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In Materials Science & Engineering

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This thesis has been approved in partial fulfillment of the requirements for the Degree of
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Author Contribution Statement

The fire ant images (Figures 34 - 38) used within this thesis were prepared in a previous exploratory study by Dr. Jaroslaw Drelich at Michigan Technological University.

Abstract

Some insects have the ability to walk on water surface due to hierarchical leg structure and wax coating. This work presents studies of water strider and fire ant leg immersion force profiles to measure resistance of legs to submersion and show orientation effects. A high-sensitivity microbalance measured force during immersion of insect legs at various angles into water droplets. Legs oriented parallel to water surface could support three to five times as much force before immersion, compared to legs in a perpendicular orientation. Water pressure affects the setae structure differently at parallel and perpendicular approaches, and complete wetting is more difficult in the structure observed during parallel approach. Once wetted, perpendicularly oriented legs experienced greater adhesion forces during retraction. Immersion and retraction force profiles were modelled as functions of leg dimensions and angle of approach, with strong correlation to experimental results. The hydrophobic wax coating on water strider legs was also found to decrease adhesion force with little effect on immersion force. Overall, strider legs that are oriented parallel to the water surface, coated in a mildly hydrophobic wax, and have coned setae with nanogrooves to facilitate removal of water, are excellent models for legs of a biomimetic aquatic robot.

1 Background

1.1 General Introduction

Nature has been a source of inspiration for many modern materials and phenomena. These materials based on nature are referred to as biomimetic materials. Some general examples of biomimetic inspiration include burrs (Velcro), humpback whale tubercle fins (improved turbine blades), and shark skin (frictional reduction for aquatic vehicles). A very interesting subset of these materials is naturally occurring superhydrophilic or superhydrophobic surfaces. One of the most well known is the lotus leaf effect, also known as the self-cleaning effect. Lotus leaves have micropapillae with branchlike nanostructures that result in a contact angle with water larger than 150° but a sliding angle below 5° [1]. This creates a superhydrophobic surface with extremely low adhesion to water. Self-cleaning and non-fogging windows and mirrors have been manufactured with coatings modelled after this surface structure and chemistry. Similarly, rose petals have been studied for their hydrophobic nature and spider webs for their hydrophilic nanofibrils that collect and condense water droplets [2]. Rice leaves utilize patterned surfaces to directionally repel water: a longitudinal arrangement of microsetae with nanoscale wax features forms a contact angle over 150° with water and funnels the resultant droplets longitudinally down the leaf [3].

The two biomimetic inspirations discussed in detail in this work are water striders and fire ants, both of which are insects capable of moving on the surface of water, albeit in different ways. Colonies of fire ants can collectively travel on water surface by forming an interconnected raft from their bodies, allowing them to adapt and survive in various weather conditions [4]. Individual water striders travel in a more natural manner on the water surface, and have been studied as inspiration for boat designs, aquatic robots, and hydrophobic coatings [5-10]. As the main focus of this report, a detailed description of strider features can be found in the following subsections.

Figure 1. Individual strider stands on water surface, fire ants form buoyant raft [11].

These naturally hydrophobic materials typically have some common features, including hierarchical rough microstructures and low surface energy due to a wax film [1, 12]. Potential benefits of superhydrophobic surfaces on large scale include reduced flow resistance and drag for aquatic vehicles [1]. This technology could also be used to selectively remove water from oil in order to clean marine oil spills, or to collect water using directional wettability [13].

1.2 Fire Ants

Red imported fire ants, or *Solenopsis invicta*, originated in the wetlands of Brazil [14]. As such, the colonies are well adapted to handling floods: they have the ability to self-assemble into hierarchical hydrophobic structures [11].

An individual fire ant is denser than water and thus relies on surface tension forces rather than buoyancy to stay on water surface. Its legs are structured with a typical hierarchical structure of macro- and microsetae covered with nanogrooves, and the leg surface itself has a wavy plated pattern [15]. Due to this, a single ant is mildly hydrophobic, with contact angle around 102° . Additionally, each ant can trap a plastron, or layer of air, within its macrosetae that provides extra buoyancy force [11].

When threatened by water, fire ants link together and form floating rafts with eggs and larvae at the center [14]. The individual ants rotate positions within the raft so that no ant is under water long enough to drown. In a recent study, the success of these rafts depended on the presence of older larvae: colonies with no larvae could only maintain

rafts for twelve hours, and colonies with young larvae could maintain rafts for just a day. On the other hand, rafts lasted up to twelve days if older larvae were present in the center [14]. The crucial difference between younger and older larvae is the development of forked and curved setae that increase buoyancy by trapping air bubbles [14].

These rafts have been observed to be as large as 45 cm in diameter, and thus are too large to simply rely upon surface tension forces [11, 14]. Two components assist in raft flotation: the plastron and a pseudo-textured surface. The plastrons of the individual ants work collectively to generate a larger buoyancy force and are what allows this conglomeration to truly be termed a raft. Without the plastron the rafts are denser than water and will slowly sink [11]. Additionally, the self-assembled surface is highly textured due to irregularity of linkages and ant bodies, as well as the individual ant leg structures. The area fraction of ant-water contact can be estimated as 0.35, given that the contact angle of water on an ant raft is approximately 133° [11]. This contact angle is 30% higher than that of a single ant.

It is also interesting to note that fire ants form self-assembling structures for feeding purposes, not just in emergency situations. In a sugar water feeding study, ants were observed to assemble a linked layer to cover a sucrose-water droplet and feed. This was noted to be the most efficient method of consumption, and no ants drowned in the study [16].

1.3 Water Striders

Gerris remigis, or water striders, are small insects that can walk and jump on water surfaces. They are capable of leaping at speeds greater than 130 cm/s [7]. Figure 2 shows a representative image of a water strider, with a thin body approximately 15mm long and six legs typically longer than 15 mm with radius 140-180 μm [1, 7, 17, 18]. Water striders in equatorial regions can be much larger than this, but the majority are approximately 1-2 cm in length. The insect is very lightweight (10 mg [19]) and each leg has a contact angle of $\sim 170^\circ$, which allows it to stand on water without breaking the surface [18, 20]. Entomological studies have concluded that the front two legs are devoted to hunting, the middle legs provide propulsion, and the rear legs are rudders for steering while also providing balance and support [17]. Each leg has three joints, resulting in three segments per leg. Only the end segment contacts water for the front and middle legs, but the middle and end segments of the back legs contact water. The front legs are equipped with a hunting hook approximately the same size as the leg diameter. The front legs are also used to dewet the middle and back legs [21]. The entire insect is coated in an organic wax, which is typically assumed in literature to have contact angle $\sim 105^\circ$ with water [1, 18, 22].

Figure 2. Two views of water strider standing on water surface. Note the formation of dimples where legs contact water.

The legs themselves are covered in very small tapered hairs termed microsetae, and each microsetae has nanometer-scale grooves. Combined, these features form a hierarchical structure on the strider legs that improves resistance to water penetration and helps keep the striders on the water surface.

However, there are two critical life stages at which striders must cross the air/water interface. In most regions, females lay their eggs underwater; the adult females and the newly hatched striders must both be able to puncture the free surface [7]. Some water walkers have retractable hydrophilic claws at the ends of their legs for this purpose, although water striders have not been confirmed a part of this group [7].

1.4 Mechanics of Water Strider Propulsion

Bush and others have extensively studied the hydrodynamic propulsion mechanisms utilized by water striders. This section will briefly summarize main findings, but a detailed discussion is beyond the scope of this thesis.

When standing, striders are small enough to be supported by curvature force generated by distortion of the free surface [23]. Buoyancy force has a lesser effect than this curvature force because water striders are denser than water. Impressively, curvature force can maintain strider flotation even in the presence of variable vibrations and flows [24].

A reaction force is needed for forwards propulsion, but striders do not encounter much resistance when walking on water. Thus energy is transformed between muscular strain, gravitational potential, strider kinetic energy, and water kinetic energy [7]. The strider's relatively long legs provide a distinct advantage in this regard. The large length enhances the shift from bioenergy to kinetic energy by reducing energy dissipation [18]. A single leg can support a force fifteen times the weight of the strider, and each stroke of the leg is approximately ten times the body weight [1, 8, 24]. Each driving leg is often accompanied by a small volume of air in the plastron, which results in added mass and increased force [7].

Hierarchical structure was also found to have an effect in this matter: micro and nanoscale structures reduce fluidic drag and allow striders to walk rapidly on the water surface [1]. However, the orientation of the leg relative to the water surface was found to have the most drastic impact on the weight of water repulsed during movement. The largest force occurs when the leg is less than ten degrees from the water surface [1]. In this condition the leg can reject water 300x its volume [1, 8]. As the stepping angle increases, the supporting force decreases. Beyond 28° , the tips of water strider legs were observed to pierce the surface upon contact [1]. Once the leg is partially submerged in the water the supporting force is calculated as the weight of water displaced by the dimple. Contact angle also has a small effect on supporting force [18]. Increase in contact angle slightly increases leg pressing depth as well as pierce force, but much more significantly decreases detaching force and energy [18]. Other work has shown that a change in contact angle from 100° to 167° had little effect on maximum supporting force [25, 26].

The microsetae have an impact on resistance force as well. Each setae can be treated as an individual elastic element, which together have a cumulative effect [20]. Because of morphology variations, a conservative range assumes anywhere between 1540-15400 setae exert a force on the water surface at any point. Given a total force of 1.54 mN per leg before penetration, a deflection force of 0.1-1 μN is required per setae [20]. However, the contributions of each setae may not be uniform due to the close spacing and angled positions of the setae – after some force is applied, setae may be in contact with each other.

Baudoin created a margin of safety index for water walkers, termed Ba. Ba is proportional to the characteristic insect size, squared. The relatively large size of the water strider, and thus its large Ba number, is what allows it to perform behaviors such as leaping off the water surface without penetrating. In fact, this margin of safety estimation suggests that insects smaller than 0.5 mm are not able to manipulate the free surface [7].

1.5 Hierarchical Structure of Water Strider Legs

One very important feature of strider legs is their hierarchical structure: several components of the leg structure combine to increase the non-wetting capability of the legs and insect overall. A typical strider leg is 100-150 μm in diameter [27]. Each leg is covered in tapered 'hairs' called microsetae [17], each 40-55 μm in length and angled at

20-35° from leg surface [8, 18-20, 27, 28]. These hairs are spaced $\sim 10\ \mu\text{m}$ apart, all point in the same direction, and have a base diameter approximately 1-3 μm [1, 8, 19, 27, 28]. The apex angle of taper is approximately 5° [27]. The setae do not adhere to each other, even when pushed together by external forces [29]. Figure 3b shows these microsetae at 900x magnification. Literature provides a hair density ranging from 15,000-20,000 setae per mm^2 for larvae, to 20,000-26,000 setae per mm^2 for adults [29]. On a nanometer scale, each hair has regular longitudinal or quasi-helical grooves [1, 27] with average width ranging 100-400 nm and average depth 80-100 nm [1, 20, 28]. These nanogrooves can be seen in Figure 3c. Combined, these two features create a beneficial hierarchical structure. Air is trapped in the gaps created between the grooves as well as between the setae; this stable air cushion helps prevent wetting [1, 7, 30].

The abdomen and back of water strider are also covered in microsetae, with length only 10-20 μm [28]. Both are convex, with the most extended point in the center. The abdomen surface is very rough and covered with a nodular structure, and has a contact angle with water 161.5°. The back has micron scale pitting and contact angle of 156.3° with water [28].

Figure 3. Hierarchical structure of water strider legs. A) Strider on water surface. B) Microsetae on back leg. C) Nanogrooves on microsetae.

Hierarchical structures are smart designs in that they allow one material property to be adjusted by variance of size levels, independent of other material properties [31]. In the case of water strider legs, the hierarchical structure is directly responsible for much of the beneficial non-wetting properties. To be clear, a hierarchical structure refers to a multiscale system with gradual size transitions between different structural levels. This necessarily results in surface heterogeneity, which shifts the wetting state from ideal to

non-ideal. Two possible scenarios arise: if the air film can or cannot withstand water pressure and resist water penetration. Structural hierarchy increases the energy difference between these two states, which reduces adhesion to water [18]. The difference in Helmholtz energy (total interface energy) between the states is ten times larger for double level structures than for single level structures [18].

Structural hierarchy also reduces water adhesion by reducing contact area and total length of triple-phase contact line [18]. The total real contact area is obtained by multiplying the area fraction of contact of all structural levels – so the more levels, the smaller total contact area. This effect greatly improves water repellency by increasing from one to two structural levels; however, only a small further improvement is noted when hierarchy is increased from two to three levels [18].

The functional hydrophobicity, or resistance of water penetration, of the leg also depends on the relationship between setae density and setae energy of adhesion [32]. Setae with lower energy of adhesion can be spaced further apart and still function as a superhydrophobic surface, because they can support a larger pressure difference between the air and water interface. This sustainable pressure difference, directly proportional to hair density, is defined in the following equation:

$$\Delta P = \frac{\gamma \cos(\theta_c + \varphi)}{\frac{l}{2} - r \cos \varphi} \quad (1)$$

Where γ is the energy of adhesion, θ_c is the Cassie contact angle, l is the center-center distance between hairs, φ is the angle between center-center line and location where air-water interface contacts hair, and r is the average setae radius [32].

The angle of water strider setae also impacts the sustainable pressure, as in Eq. 2 [33]:

$$\Delta P = -\frac{2\pi r \csc \alpha}{l^2 - \pi r^2 \csc \alpha} \gamma \cos \theta \quad (2)$$

Where α is the angle between setae and leg, r is the setae radius, and l is the characteristic distance between setae:

$$l = (\text{number of hairs per sq. cm.})^{-\frac{1}{2}}$$

A positive value of ΔP indicates protection from water penetration, as the excess pressure is on the water side of the interface. If the setae are hydrophobic, angling them provides more efficient defense against water protection by reducing the free space between them. As with Eq. 1, smaller distance between hairs always increases functional hydrophobicity.

Alignment and orientation of the setae along the leg also play a role in the overall insect wetting properties. When water penetration occurs along setae direction, adhesion is very

different than when penetration occurs against setae direction. Immersion opposite setae, as in Figure 4b, results in larger resistance during immersion and easy retraction from water, due to increased contact between water and setae [19]. In contrast, much less resistance is recorded when leg is immersed in setae direction, as in Figure 4a, attributed to a decrease in the triple contact line length [19]. Overall, a larger average wetting force occurs when setae point in opposite direction as immersion. This condition is also more similar to water strider locomotion. Xu et al. showed this behavior by sliding a water droplet back and forth on a single strider leg in opposite directions [19]. They observed a difference in adhesion of approximately seven degrees between the directions. When the droplet was rolled along the setae, it rolled off at 11.1° ; when the droplet was rolled against the setae it was pinned at 18.6° [19].

Figure 4. Water strider legs inserted into water droplets during testing. White arrows indicate setae direction. (a) Leg inserted in setae direction. (b) Leg inserted opposite setae direction.

The hierarchical structure of the water strider's legs has more effect than the wax coating on increasing water resistance [1, 8, 33]. Although it may be tempting to believe that a hydrophobic wax coating is what keeps the insects from sinking into the water, the wax is actually not hydrophobic enough – a typical polyethylene wax has a contact angle ~ 85 - 90° . The legs of water striders are superhydrophobic due to both hierarchical structure and wax coating, with water contact angle as high as 160° or above [18, 20]. Researchers at James Cook University showed the importance of nanotopography by thickly coating setae with PDMS (contact angle 105°) and observing penetration into water. Uncoated insects, and very thinly coated insects, did not penetrate water at forces up to $2.0 \mu\text{N}$. Thickly coated insects with no nanotexture did penetrate at these forces. As the coating contact angle was consistent between thin and thick coatings, water repellency must be due to hierarchical structure [20].

However, a wax coating has been assumed to play some role in the waterproofing of many aquatic insects, and may help the striders clean water off themselves, or assist in some other way besides directly preventing water penetration. Although water strider wax in particular has not been studied, the waxes of other aquatic insects are typically composed of long chain fatty acids, alcohols, and esters, with chain length on the order of C_{30} [34]. A large percentage of the constituents are polar, with only a small amount of nonpolar paraffins [34]. The top layer of waxy material sometimes lies above a layer of lipid chemically bound to protein. In general, the outermost wax layer is approximately 0.2-0.3 μm thick [34]. Beament found that there was a critical temperature, usually just below the melting point, at which the water-permeability of wax-coated insect cuticles abruptly increased. He postulated that at this temperature, the optical properties and therefore crystal structure of the wax changed. Thus, orientation of the wax structure has an effect on the permeability of the insect cuticle [34].

For many studied aquatic insects, some areas of the body are somewhat hydrophilic, seemingly contrary to the mainly superhydrophobic majority. This could have evolved for several reasons, including enhanced adhesion to prey surfaces during hunting, reduced susceptibility to pathogen spore binding, and easier locomotion on smooth surfaces [32]. These differences have been attributed to differences in topical structure, such as varied setae length and distribution.

1.6 Wetting

Wetting refers to the ability of a liquid to spread over a solid surface. This is often measured with a contact angle, or the angle between the solid surface and a tangent drawn from the base of a droplet, as demonstrated in Figure 5. A more wettable material has a lower contact angle and strong adhesive forces. These strong adhesive forces make it energetically more favorable for the liquid to spread over the surface.

Figure 5. Contact angle schematic for water droplet on hydrophobic surface.

Several categories of materials are evident in terms of wetting, as summarized in Table 1. Classification as superhydrophobic also requires that hysteresis is less than 10° [35]. This

work is primarily concerned with superhydrophobic surfaces, as water strider legs have contact angle $\sim 170^\circ$ with water [8, 18, 20].

Table 1. Wetting categories [36, 37].

Categorization	Contact Angle Range
Superhydrophilic	$\theta \sim 0$
Hydrophilic	$\theta < 90$
Hydrophobic	$150 > \theta > 90$
Superhydrophobic	$\theta > 150$

The contact angle, and thus wettability, is determined by a balance between the cohesive forces within the liquid and the adhesive forces between the liquid and solid. More specifically, the droplet behavior can be predicted by the competition between liquid-vapor, solid-liquid, and solid-vapor interfacial tensions [35]. A spreading coefficient S can be defined in terms of these three tensions:

$$S = \gamma_{SV} - \gamma_{SL} - \gamma_{LV} \quad (3)$$

If S is greater than zero, the liquid will spontaneously and completely spread on the surface; if S is less than zero, the liquid will only partially spread on the surface. The latter is more realistic for many cases and is what results in contact angles.

Young's relation provides a quantitative connection between contact angle and surface energies [36]:

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos \theta_Y \quad (4)$$

Where γ_{SV} is solid-vapor surface energy, γ_{SL} is solid-liquid surface energy, γ_{LV} is liquid-vapor surface energy, and θ_Y is Young's contact angle. However, this relation is limited in its applications. It only holds true for ideal surfaces: surfaces that are flat, rigid, inert to liquid, smooth, and chemically homogeneous [35]. It also assumes zero hysteresis, or no difference between contact angles measured after a liquid is advanced over dry solid surface and receded from wetted solid surface, respectively referred to as advancing and receding contact angles.

Non-ideal rough surfaces do exhibit hysteresis due to metastable contact angles. The advancing contact angle is related to the maximum stable angle while the receding is related to the minimum stable angle. There are also many metastable contact angles between these two points [36]. These metastable contact angles can arise from heterogeneity or surface roughness. Roughness and structure can induce effects such as line pinning, porosity pressure buildup, or surface curvature that effect observed contact angle. Roughness can also help minimize contact angle hysteresis, and thus makes it easier for droplets to be removed [35].

Two possible wetting regimes exist on rough surfaces: homogeneous and heterogeneous wetting. Whichever state has a lower contact angle has lower Gibbs energy and is more stable [35]. These cases can be considered in terms of the hierarchical roughness of water strider legs. In homogeneous wetting, liquid penetrates microsetae surface roughness, as shown in Figure 6a. This is termed Wenzel wetting, and is considered homogenous because only solid, not air, is in contact with the liquid. Note that the liquid may fill the first order of roughness, microsetae, without filling the second order, the nanogrooves. An adjusted contact angle θ^* may be calculated from the following equation, where r refers to the ratio of true area of solid surface to the apparent area of solid surface:

$$\cos \theta^* = r \cos \theta_Y \quad (5)$$

This is the most stable contact angle when the liquid completely penetrates roughness grooves. Figure 6b shows the second regime, Cassie-Baxter wetting. In this case, the wetted surface is heterogeneous; both air and microsetae are in contact with the liquid. An additional correction is needed to account for this, so Cassie-Baxter contact angle θ^* is given by [35]:

$$\cos \theta^* = r_f f \cos \theta_Y + f - 1 \quad (6)$$

In this equation, r_f is the roughness ratio of wet surface area, and f is the fraction of solid surface wet by the liquid. Note that if $f = 1$, this becomes the Wenzel regime equation.

Figure 6. Two possible wetting regimes on non-ideal rough surfaces. First and second order roughness represent strider hierarchical structure.

Wetting typically begins in the Cassie-Baxter state and remains heterogeneous until the air pockets beneath the droplet are no longer thermodynamically stable. The Cassie-Baxter state is more stable when the Young's contact angle is greater than θ_{crit} , where r is surface roughness and f is solid fraction [38]:

$$\cos \theta_{crit} = -\frac{1-f}{r-f} \quad (7)$$

When the Cassie-Baxter state is no longer more stable, liquid nucleates from the middle of the droplet to minimize surface energy and wetting transitions to a Wenzel regime. This transition is often sped along by nucleation of small droplets within surface structure. These drops remain trapped in the structure and aggregate, essentially forming a Wenzel state and destroying superhydrophobicity [27]. Water striders' nanogrooves provide them a unique ability to remove nucleated droplets on the order of femtoliter to microliter from their leg texture. At such small contact scales, the adhesion force is not able to overcome the kinetic energy of mobilization and the droplets self-propel down the setae toward the leg surface. As they travel they coalesce and grow, until they deform the setae array and are expelled. This allows water striders to maintain Cassie-Baxter wetting state in conditions such as high humidity [27].

Transitions from the Cassie-Baxter state to Wenzel state can occur even when Cassie-Baxter is energetically favorable, due to external triggers such as mechanical compression, water submersion, or vibration. But these transitions are reversible if only one roughness tier is wetted, such as water penetration into microsetae but not nanogrooves [38]. Reversal can only occur if the receding contact angle of the drop on a partially wetted Wenzel surface is larger than the critical angle where unstable droplet pinch-off occurs. Thus, a system with a large receding contact angle, such as a two-tier hierarchical structure, is necessary [38].

The water strider hierarchical structure behaves as a heterogeneous surface of solid and air in contact with water. In this state, the contact angle of strider legs is given by the following relation [8]:

$$\cos \theta_1 = f_1 \cos \theta_w - f_2 \quad (8)$$

Where f_1 is area fraction of microsetae with microgrooves, f_2 is area fraction of air on leg surface, θ_1 is the apparent contact angle of leg, and θ_w is the wax contact angle with water. Gao and Jiang calculated that f_2 is equal to 96.86% [8]. This indicates that the leg is primarily contacted by air rather than water, so an air cushion must be formed at the leg-water interface. This strongly correlates with the plastron observed in other aquatic insects. Other experiments have found that the strider leg contact angle ranges from 167° in the Cassie-Baxter state to 60° in the Wenzel state [24].

Several factors strongly affect surface wetting properties, such as chemical composition and surface roughness. A surface with low free energy should theoretically be highly hydrophobic, but may be only moderately hydrophobic due to other factors. For instance, a CF₃-terminated surface has low free energy but a contact angle of only 120° [20]. The "lotus effect" mentioned earlier in this thesis is an example of combined effects of

chemistry and texture: the wax surface captures air and increases the water-air interface area, causing water to form near-spherical droplets [20].

Increasing surface roughness increases contact angle of a hydrophobic material by contorting the three phase contact line [35]. For a smooth hydrophobic surface, solid-vapor surface energy is less than solid-liquid surface energy. Thus, a very high contact angle is energetically favorable to minimize solid in contact with liquid. By the same principle, air trapped beneath the droplet lowers the surface energy by forcing more solid-vapor contact through a composite interface. A textured superhydrophobic surface thus requires both roughness and a favorable texture to effectively trap air [39].

Surface structure alone is not enough to create a superhydrophobic surface. The solid must also surpass a critical contact angle for air pockets to be thermodynamically stable. That condition is given by:

$$\cos \theta_Y < \frac{\varphi_s - 1}{r - \varphi_s} \quad (9)$$

Where φ_s is the fraction of solid/liquid interface below the droplet [39].

Another variable to consider is adhesive force. Two surfaces can both be considered hydrophobic, but if both are inverted, droplets may cling to one but fall from the other. This is a reflection of variable water penetration into texture. If water cannot penetrate first order texture such as microsetae, adhesive force is low. Conversely, if water is able to penetrate microsetae but not nanogrooves, adhesive force is high. High adhesive force is more common for larger scale texture [39].

In the case of water striders, it has been shown that both orders of roughness in the hierarchical structure must be present in order for the strider leg to be physically capable of achieving the measured 170° contact angle [1]. The following equation incorporates setae features into the Cassie-Baxter wettability equation [1, 21]:

$$\cos \varphi_1 = (\pi - \varphi_s) f \cos \varphi_s - (1 - f \sin \varphi_s) \quad (10)$$

$$f = \frac{d}{s} \quad (11)$$

Where φ_1 is leg contact angle, φ_s is setae contact angle, d is setae mean diameter, and s is setae mean spacing.

Assuming typical strider geometry, f is in the range $0.05 - 0.1$. Thus, for φ_1 to be 170° , setae must have contact angle greater than 125° . A smooth setae would have contact angle equal to that of the coating, which can be assumed $\sim 90^\circ$; a contact angle greater than 125° is not achievable for a smooth setae, indicating that the nanogrooves are necessary for the extra water repellency effect [1].

Additionally, the wetting of individual setae has been modelled as a combination of valleys and circular arcs [20].

$$\cos \theta_c = (\pi - \theta_w)f \cos \theta_w - (1 - f \sin \theta_w) \quad (12)$$

$$f = \frac{2d}{s} \quad (13)$$

Where θ_c is the resultant contact angle of setae, θ_w is the corresponding contact angle on a flat surface of the same chemistry, $\frac{d}{2}$ is the radius of curvature between setae, and s is the spacing between setae.

1.7 Objectives

Published work on water striders and other water-walking insects dates back to the mid 1800's, when Johann Eschscholtz discovered three species of the water strider family and noted their ability to walk on water. Since then, a great deal of research has been done on propulsion mechanisms and details of strider movement [6, 7, 9, 10, 23, 24], as well as analyses of hierarchical structure related to wetting [1, 3, 8, 12, 13, 20].

This work aims to supplement what is understood about water striders by adding contributions in several key areas.

First, very little is known about the wax covering the striders' bodies and legs. Many researchers state that it is an organic wax with contact angle ranging from $80^\circ - 105^\circ$ [6, 9, 18]. However, literature review revealed no further investigation. This work aims to bolster this gap by extracting and characterizing wax, as well as studying unwaxed legs to assess the contribution of wax to hydrophobicity.

Detailed orientation studies are another objective of this work. Although some researchers have reported studies at various angles, the effect of orientation on immersion resistance and adhesion resistance is underexplored and studied inconsistently. Previous work has also only studied immersion force for oriented strider as a whole, not for individual legs at orientations. This report analyzes the effect of orientation on buoyancy forces, as well as providing immersion force models for both parallel and perpendicular orientation.

Last, it is within the scope of this work to draw comparisons between several different species of insects that travel on the surface of water. Other works have compared between different varieties of the same species, but not between water striders and fire ants.

2 Experimental Methods

2.1 General Sample Preparation

Water strider specimens were gathered from streams and ponds in the Houghton, MI area. Several live samples were maintained in a freshwater aquarium for real-time observation of locomotion and leg orientation. The majority of specimens were fluid dehydrated by preservation in a sealed jar of ethanol for at least three weeks.

Individual legs were removed from specimens with a razor blade and mounted vertically on thumbtacks with Loctite super glue in preparation for immersion testing. Samples were allowed to dry for at least six hours before contact with water.

2.2 Force Measurements

2.2.1 Wilhelmy Method

The Wilhelmy method was proposed by F.L. Wilhelmy in 1863 as an indirect measure of surface tension, and is now one of the most reliable techniques for measuring contact angles on fibers of known dimension [36]. The basic premise is simple: a sample is immersed in a liquid and the force changes are measured during this process, and related to the surface tension by Eq. 12 [40]:

$$F(h) = mg + P\gamma \cos \theta - \rho gV \quad (14)$$

Where F is the force measured as a function of immersion depth, m is the sample mass, g is gravity, P is the wetted perimeter, γ is surface tension, θ is the contact angle between the sample and liquid, ρ is the liquid density, and V is the wetted volume. Note that immersion depth is defined with respect to the undisturbed liquid level, so the meniscus height may need to be added or subtracted from the machine incremented depth to obtain immersion depth.

In this test, a sample was held vertically above a dish of water or other liquid, and the liquid container was moved vertically upwards by a low-vibration motor at a very slow, controlled pace. The speed used should be inversely related to the liquid viscosity. There are several considerations to keep in mind for this test [40]:

1. Samples must be rigid enough that they do not deform during the test. This is especially important for single fibers.
2. The sample should be at least 5 mm from liquid container edges to prevent capillary bridging.
3. The liquid container should be large with respect to immersion depth so that liquid level varies only minutely during testing.

4. The liquid container should be perfectly wettable to prevent border menisci that may affect liquid level.

The use of a symmetric shape, such as a cylinder or parallelepiped, simplifies analysis because horizontal forces are then balanced and only vertical forces need be considered. Similarly, the sample should be perfectly vertical when entering the liquid to maintain consistent cross section during testing. If the sample is inclined, the cross sectional area will be increased and thus the angle of the immersion slope will be increased [40]. In theory, the advancing and receding slopes should be perfectly parallel.

2.2.2 Modified Testing

Submersion resistance and adhesive force were measured during immersion with a high-sensitivity microelectronic mechanical balance DCAT system. Tests were performed using DCATS Module 37: Adhesive Force.

Mounted legs were oriented at varying angles to a water droplet held within a ring, as shown in Figure 7, and placed on a motorized platform below the ring. A 4 μL droplet of HPLC-grade water was deposited in the ring for each test run. After droplet stabilization, the motorized platform was incremented upwards at 0.03 mm/s until contact, as determined by a sudden increase in force. Upon contact, the leg was raised a further 0.8 – 1.0 mm into the droplet. The leg was then retracted from the droplet until complete separation was observed. A force profile was recorded throughout this range, where a negative force indicates resistance between leg and water [1]. An attached iDS camera captured 22 frames per second throughout the range of movement.

Figure 7. Multiple leg orientations relative to ring holder.

Additionally, a pseudo-Wilhelmy method was used to analyze thin metallic cylinders to aid in model development. Test specimens were mounted in the ring holder and incremented downwards into a dish of water, as in Figure 8. Care was taken to ensure the

water depth exceeded the immersion distance by a factor of two to mitigate changes to overall water surface height. Figure 9 shows how this process would look for a vertical water strider leg. At first contact, the meniscus reaches above the water level due to contact with setae. As the leg is pressed into the water, meniscus curves downward, until wetting and immersion occur. Figure 9(c) shows a simplistic view of this moment. The meniscus then curves upwards again during retraction due to adhesive forces, until final snap-off.

Figure 8. Metallic cylinder immersion into water dish. (a) Vertical cylinder. (b) Horizontal cylinder.

Figure 9. The pseudo-Wilhelmy analysis method using a hydrophobic strider leg. Each subfigure shows a sequential portion of the test, from first contact (a) to adhesive force during retraction (e).

For both methods, three elements were mainly considered on each force curve: the maximum force before leg immersion in droplet, the maximum adhesive force during leg withdrawal, and the slope of immersion. The immersion slope (from initial contact to penetration) was taken as an indirect measure of hydrophobicity. A steeper immersion slope, or a slope with larger magnitude, indicates a sample that is more resistant to penetrate water and thus more hydrophobic.

Because samples were not immersed at perfect verticals, the wetted perimeter and wetted volume changed as functions of immersion depth. This is discussed further in Appendix A: Theoretical Analysis.

2.3 Immersion Curve Analysis

Several important points of interest were noted for each curve. As mentioned previously, the immersion slope was taken from the advancing period, until the leg snapped into the water droplet and was considered immersed. This snap-in moment was determined as the point prior to retraction where the force decreased very sharply. Figure 10 shows this selection process. A linear regression was performed to obtain a slope for the segment.

Figure 10. Details of immersion slope determination.

The maximum force was taken as the maximum positive force, and indicated adhesion force. The immersion force was taken as the maximum negative force, and was also the endpoint of the immersion slope segment. See Figure 11 for visual explanation. These forces were normalized to immersion and adhesion pressures by measuring contact areas just prior to immersion and at maximum adhesion force with ImageJ. Immersion pressure was then calculated by dividing force just before immersion by the leg area supporting water at that point; similarly, adhesion pressure was obtained by dividing maximum adhesion force by the leg area in contact with the droplet just prior to snap-off.

Figure 11. Force immersion curve.

2.4 Wax Extraction

Wax was removed with dichloromethane solvent. This was chosen due to its non-polar nature and low boiling point.

Legs were removed from a number of insects to obtain approximately 15 legs total. These legs were placed on a glass slide and covered with dichloromethane. Immediate agitation helped remove wax from legs, then legs were removed from slide before solvent evaporation. Once dichloromethane evaporated, wax remained on glass slide.

2.5 Scanning Electron Microscopy

To prepare samples for scanning electron microscopy, care was taken to dehydrate legs. Insects were soaked in ethanol to remove internal water, and any clinging ethanol quickly evaporated at room temperature. Legs were mounted on metallic stubs with double sided carbon-impregnated tape. For SEM imaging, mounted legs were coated with carbon and imaged on a JEOL JSM-6400 research grade tungsten source microscope. This enabled imaging of the legs and microsetae, but not clear resolution of nanogrooves. To obtain such high resolution, legs were coated with Pt-Pd and imaged with Hitachi S-4700 cold field emission scanning electron microscope. An accelerating voltage of 1 keV and working distance of 4 mm was used.

2.6 ImageJ Measurements

Morphology measurements such as setae linear density, area density, sizing, and spacing were performed using ImageJ, an image analysis software.

SEM and FSEM images were uploaded to the software and a number of measurements were taken for each datum. Figure 12 shows an example of setae linear density calculations: eleven straight lines were drawn across the sample and the number of intersected setae were counted per each line.

Figure 12. Linear density calculations in ImageJ.

2.7 X-Ray Photoelectron Spectroscopy

XPS was performed to determine the elemental composition of the wax coating of water strider legs.

A clean glass slide and the glass slide coated with wax were both desiccated for two hours prior to analysis. For all measurements, the instrument was set at a tilt of 45° and a z adjustment of -0.966 mm.

The standard glass slide was analyzed with a high resolution scan using 23.5 eV pass energy, 0.1 eV per step, and 100 ms per step. Five sweeps were performed for carbon and oxygen regions, and ten sweeps for silicon and sodium regions. A Mg anode was used for all sweeps.

The wax coated glass slide was first analyzed with a Mg anode at the same parameters as the standard. Five sweeps were performed for carbon and oxygen regions, ten sweeps for silicon, sodium, and nitrogen regions, and thirty sweeps for chlorine region. The sodium region was suspect, as it appeared in the same region as chlorine Auger peaks. A lower signal Al anode was used to investigate with additional six sweeps of carbon and thirty sweeps of sodium.

All results were charge corrected to the appropriate carbon data, assuming a carbon bond energy of 284.8 eV.

2.8 Cylinder Preparation

Small metallic cylinders were coated in liquid polyethylene and allowed to dry to simulate an untextured insect leg. Care was taken to ensure as even a coating as possible by rolling samples on wax paper during the setting process.

3 Results and Discussion

3.1 Water Striders

3.1.1 Morphology and Structure

General images of water strider legs can be seen in Figure 13 and Figure 14. Figure 13 shows the tapered nature of the leg tips; although most of the leg averages approximately 95 μm , the very end quickly narrows to about 60% this diameter. The regular distribution of setae can also be seen in these images.

Figure 13. Tapered water strider leg with dimensions. SEM image.

Figure 14. Water strider back leg. FSEM image.

An interesting feature to note is the hook found on some water strider legs. The hook was only observed on front legs, as seen in Figure 15, but not on every front leg. This could be a tool to break the surface tension and allow female striders to dive beneath the surface to lay eggs.

Figure 15. Hook on water strider front leg. SEM image.

The first level of hierarchical structure, microsetae, is the focus of Figure 16. The high setae area density can be observed. Also note the tapered setae tips that have the tendency to bend over perpendicular relative to the shaft, which ranges from 40 to 50 μm in length.

One individual microsetae is also shown in Figure 17. The nanogrooves are visible in this image, and appear to extend down the shaft until approximately two μm from the setae tip.

SEI frontleg_hairs_1700x

Figure 16. Setae on water strider front leg. SEM image.

Figure 17. Individual setae on water strider back leg. FSEM image.

The slightly helical nature of the nanogrooves is visible in Figure 18. The grooves are regularly spaced and run along the longitudinal direction of the setae shaft. The more magnified view in Figure 19 also shows the presence of some nanoscale texture on the setae surface. This may contribute to the overall roughness of the material and increase hydrophobicity.

Figure 18. Nanogrooves on setae, water strider back leg. FSEM image.

Figure 19. Nanoscale plated structure on setae, water strider back leg. FSEM image.

The abdomen and back of the water strider are also covered in microsetae, but these setae are much shorter and appear stiffer. Literature presents an abdomen setae length 10-20 μm , and Figure 20 presents setae approximately 20 μm in length [28]. The surface of the abdomen also appears quite textured and rough.

As a contextual reference, the size of the abdomen is indicated in Figure 21.

SEI rearbody_outside_750x

Figure 20. Microsetae on water strider abdomen. SEM image.

SEI rearbody_27x

Figure 21. Water strider abdomen with dimensions. SEM image.

The morphology of water strider legs and body agreed well with literature values. Table 2 lists notable results for various measurements of strider legs. Literature presents a range 100-150 μm for strider leg diameter [26], which agrees with the collected value of 98.7 μm . Moreover, this work found the setae diameter to range from 2.10 μm at base to 0.44 μm at tip; literature provided a base diameter range 1-3 μm [8, 26, 27]. A setae apex angle of approximately 5° was confirmed, as well as an inclination of approximately $30\text{--}35^\circ$ from leg surface. On strider legs, setae were spaced 13.8 μm apart in a fairly regular pattern. Setae were not observed to be in exact horizontal lines, but rather to be offset somewhat, as in Figure 22.

Figure 22. (a) Setae arrangement on water strider leg. (b) Clear view of setae arrangement.

The studied strider legs had a linear density of 10.51 setae per 100 μm of leg. Area density was calculated as 6.02×10^5 hairs per 100 mm^2 . This is an order of magnitude higher than the literature values of $1.5 - 2.6 \times 10^4$ hairs per 100 mm^2 . This difference could be attributed to documented variations in strider size and morphology dependent on region.

Nanogrooves, the second level of hierarchical structure, were also investigated. Average width was 360 nm. This correlates well with literature width range 100-400 nm [1, 20]. The grooves were observed to be very nearly parallel, with deviations less than five degrees from parallel axis.

Table 2. Collected results for various strider leg structure measurements.

Strider Leg Diameter		Setae Base Diameter		Setae Tip Diameter	
Avg [μm]	98.7	Avg [μm]	2.10	Avg [μm]	0.44
Std Dev [μm]	13	Std Dev [μm]	0.3	Std Dev [μm]	0.08
Std Error [μm]	4.2	Std Error [μm]	0.1	Std Error [μm]	0.03
Distance Between Microsetae		Nanogroove Width		Linear Density: Microsetae Per 100 μm	
Avg [μm]	13.8	Avg [μm]	0.36	Avg [μm]	10.51
Std Dev [μm]	3.7	Std Dev [μm]	0.03	Std Dev [μm]	1.1
Std Error [μm]	0.3	Std Error [μm]	0.003	Std Error [μm]	0.06

3.1.2 Leg Behavior During Wetting

Figure 23. Force profile for water strider leg. Letters on graph correspond to images below.

A basic force profile is demonstrated in Figure 23. Force magnitude increases during advancement until water penetrates setae and leg immerses in droplet, just after point C. At that point of immersion, there is a sharp decline in magnitude of resistance force, as from point C to point D. After immersion the force remains fairly constant until retraction begins. During retraction, force increases linearly until only the tip or edge of leg is still within droplet, until point F in this example. Force then continues to increase but at a slower rate, until maximum adhesion force is reached at point G. Capillary forces are overcome and the leg releases from the droplet, and force drops to zero. The distance

between the beginning of retraction and the end of initial retraction slope determines the magnitude of hysteresis.

There is very little noticeable snap-in force at initial contact, which may be due to the microsetae: these likely contacted the water surface before the leg and created miniscule interactions that could not be detected.

Small force fluctuations are attributed to changes in both diameter and topography as the leg is inserted further into the droplet – contact line may pin on these barriers. As these are natural samples, homogeneity cannot be assured.

3.1.3 Orientation Studies

Force immersion profiles were measured as described in Methods. Angle of contact was considered the independent variable, not to be confused with contact angle. Angle of contact refers to the observed angle between water droplet surface and strider leg upon first contact during testing. This orientation study aimed to measure angles between zero (Figure 24, Left) and ninety degrees (Figure 24, Right) in constant increments; however, exact manipulation of leg in relation to droplet was not a simple task. As such, angles of analysis ranged from 5.4° - 99.4° , in varying increments. Table 3 features a compilation of orientation study results.

Figure 24. Examples of two extreme strider leg orientations. (Left) Approximately 0° angle of contact, termed parallel. (Right) Approximately 90° angle of contact, termed perpendicular.

Table 3. Forces and pressures observed at various angles of contact for water strider back legs. Asterisks (*) indicate that these data points could not be calculated due to fragmented data.

Angle of Contact	Adhesion Force (mN)	Adhesion Pressure (N/m ²)	Immersion Force (mN)	Immersion Pressure (N/m ²)	Immersion Slope (N/m)
5.4	0.0571	1.36	0.118	1.63	-0.16
5.4	0.0541	0.854	0.335	4.71	-0.48
5.4	0.0645	1.53	0.266	3.67	-0.56
5.4	0.0554	2.67	0.114	1.54	-0.12
6.1	0.0160	2.16	0.185	7.17	*
10.2	0.0704	0.455	> 0.255	4.55	-0.24
10.2	0.0639	0.412	> 0.191	4.12	-0.24
20	0.0140	2.53	0.0867	3.53	-0.11
20	0.0101	1.82	0.104	4.23	-0.13
20	0.0110	1.98	0.0898	3.65	-0.12
20	0.00170	0.305	0.0961	3.91	-0.14
21.7	0.0439	*	0.117	*	-0.14
21.7	0.0464	*	0.113	*	-0.16
21.7	0.0456	*	0.0877	*	-0.17
25.6	0.0315	1.01	0.0362	1.62	-0.054
26.7	0.00961	0.678	0.103	2.06	-0.13
26.7	0.0122	0.858	0.0867	1.74	-0.12
26.7	0.0145	1.02	0.102	2.05	-0.13
39.8	0.0443	2.53	0.0442	0.692	-0.096
39.8	0.0483	2.76	0.0433	0.678	-0.094
39.8	0.0477	2.72	0.0412	0.644	-0.10
45	0.0458	*	0.0469	*	-0.010
45	0.0189	*	0.0661	*	-0.010
45	0.0125	*	0.0602	*	-0.0092
45	0.00922	*	0.0692	*	-0.010
49.2	0.0226	2.70	0.0372	2.22	-0.10
51.8	0.00568	*	0.0624	*	-0.011
51.8	0.00167	*	0.0702	*	-0.011
51.8	0.00304	*	0.0729	*	-0.011
51.8	0.00412	*	0.0700	*	-0.011
54.2	0.0133	0.895	0.0939	*	-0.13
54.2	0.00981	0.658	0.0893	*	-0.15
54.2	0.0149	1.00	0.0883	*	-0.13
54.2	0.00834	0.559	0.0928	*	-0.13
56.8	0.0316	1.48	0.0458	4.39	-0.086
56.8	0.0304	1.42	0.0459	4.40	-0.086

Angle of Contact	Adhesion Force (mN)	Adhesion Pressure (N/m²)	Immersion Force (mN)	Immersion Pressure (N/m²)	Immersion Slope (N/m)
56.8	0.0371	1.73	0.0456	4.37	-0.086
56.8	0.0379	1.77	0.0499	4.78	-0.086
56.8	0.0360	1.68	0.0431	4.13	-0.081
64.1	0.0307	3.28	0.0491	5.24	-0.069
64.1	0.0289	3.09	0.0325	3.47	-0.069
64.1	0.0302	3.22	0.0222	2.37	-0.040
64.1	0.0284	3.04	0.0240	2.56	-0.11
65.1	0.0346	1.37	0.0158	1.37	-0.050
65.1	0.0307	1.21	0.0184	1.08	-0.048
65.1	0.0442	1.75	0.0225	1.01	-0.050
65.1	0.0308	1.22	0.0181	1.07	-0.048
67.6	0.0392	2.47	0.0387	3.83	-0.097
67.6	0.0352	2.22	0.0414	4.09	-0.10
67.6	0.0365	2.30	0.0396	3.91	-0.078
70.6	0.0316	6.46	0.0154	3.15	-0.0070
70.6	0.0300	6.14	0.0130	2.66	-0.0074
70.6	0.0305	6.24	0.0122	2.50	-0.0072
94.2	0.0384	5.41	0.0386	4.73	-0.081
94.2	0.0372	5.25	0.0387	4.75	-0.089
94.2	0.0365	5.15	0.0373	4.57	-0.082
99.4	0.0551	4.57	0.0796	7.19	-0.16
99.4	0.0581	4.73	0.0760	8.50	-0.15
99.4	0.0599	5.04	0.0748	5.67	-0.11

Methods of determination for each dependent variable are detailed in Methods.

A correlation analysis was run to determine relations between each variable. Results are tabulated in Table 4. For each relation, the upper cell gives the Pearson correlation coefficient and the lower cell provides the p-value. Assuming 95% confidence, the highlighted cells indicate statistically significant correlations. Again considering angle of contact as the independent variable, every dependent variable has a statistically significant correlation with the independent variable except adhesion force. Additionally, it is interesting to note the positive correlation between adhesion pressure and immersion slope ($p=0.014$). A less negative immersion slope indicates less resistance to water immersion. Thus, there is a correlation observed between higher adhesion during retraction and lower water protection ability during immersion. This relation makes sense, as both observations are indications of less hydrophobicity. There is also a positive relation between adhesion pressure and immersion pressure, indicating that legs that are able to maintain larger pressures may also be strongly attracted to water once immersed.

Table 4. Correlation analysis for adhesion and immersion variables. Green boxes indicate statistically significant correlations.

	angle	adhesion force	adhesion pressure	immersion force
adhesion force	0.012 0.928			
adhesion pressure	0.645 0.000	0.220 0.133		
immersion force	-0.602 0.000	0.130 0.337	-0.365 0.015	
immersion pressure	0.331 0.032	0.115 0.469	0.308 0.048	0.271 0.083
immersion slope	0.471 0.000	-0.471 0.000	0.357 0.014	-0.872 0.000

This subsection is particularly concerned with the effects of angle of contact on the various responses. The following figures demonstrate the relationships between angle of contact and each dependent response.

Figure 25 presents fitted regression lines for adhesion force and adhesion pressure, both as a function of angle of contact. Adhesion force is almost constant across the range of angles, which is to be expected due to variations in contact area. A positive correlation is observed between angle and adhesion pressure. At higher angles, or when the leg is more perpendicular to the droplet surface, the adhesion pressure is greater. This indicates a greater attraction to water once immersed at perpendicular orientations. This makes sense based on the setae morphology: the setae are being directly inserted into the droplet and thus fully fill after immersion and transform to a complete Wenzel state. As discussed in the Wetting subsection, once complete Wenzel transformation occurs, the regime cannot shift back to a Cassie state and the surface behaves in a more hydrophilic manner. Legs immersed parallel to the droplet may not fully fill and thus have the potential to shift back to a Cassie state, resulting in lower adhesion pressure.

Additionally, Figure 26 shows that this fit satisfies the assumptions of regression. Residuals versus fits is distributed fairly evenly about zero, the normal probability plot is a straight line, and residuals versus order is randomly scattered.

Figure 25. Regression lines for adhesion force and pressure as function of angle of contact.

Figure 26. Residual plots for adhesion pressure as a function of angle of contact.

The fitted regression lines for immersion force and pressure are presented in Figure 27. Although these trend lines are affected by a large variance at low angles, they can be fitted in a statistically significant way. Bottom left of the figure shows a linear regression fit, with $p\text{-value}=0.032$. However, the R-squared value increases when the data is fitted with a linear regression line, and $p\text{-value}=0.000$. Based on this fit, strider legs are excellent at preventing water penetration both when parallel and perpendicular to the droplet surface. At angled approaches the legs support the least pressure before immersion.

When the leg is parallel to the droplet surface, the added pressure during immersion presses the setae closer together against the leg surface. This helps prevent wetting in two ways. First, the setae tips are closer together: more energy is needed to displace an interface with a smaller radius so the setae are more resistant to penetration [33]. Also, the gaps between setae at rest are angled approximately 30° from the leg surface, and thus would be slightly angled towards the droplet before contact. The droplet pressure shifts these gaps much more parallel to the droplet, effectively reducing the water's access to

the gaps. Essentially, the presence of water pressure helps the hierarchical structure resist water penetration better when the leg is parallel to the water surface.

When the leg is perpendicular to the droplet, higher immersion pressures are observed for two possible reasons. First, the contact area in this state is approximately half that observed in parallel orientations. Note that immersion force in a perpendicular orientation is much less than immersion force in a parallel orientation. This shows that a perpendicular orientation can hold its own when normalized against a contact area, but the parallel orientation is objectively a better support tactic for the water striders.

Another reason for higher pressures than expected in perpendicular orientations could be related to a change in setae shape. The direct downward pressure on the setae may bend the tips of the setae to a right angle with the stalk. The tips have diameter approximately two thirds that of the main setae stalk, and so are more flexible. As noted by Crisp and Thorpe, this vertical setae structure with bent tips is very efficient at water protection in hydrophobic surfaces [33].

When the leg is moved towards the droplet at an angle of contact between parallel and perpendicular, the smallest immersion pressure is observed. The gaps between setae are oriented in such a way that droplet pressure pushes setae further apart, rather than minimizing the radii as in parallel orientations. As such, the force needed to displace the air-water interface is minimized.

The residual fits in Figure 28 confirm that the quadratic regression is more appropriate than the linear fit. The residuals versus fits is much more evenly distributed when a quadratic regression is used, and the normal probability plot is more linear.

Figure 27. Regression lines for immersion force and pressure as function of angle of contact. (Top) Immersion force fitted linearly. (Bottom left) Immersion pressure fitted linearly. (Bottom right) Immersion pressure fitted quadratically.

Figure 28. Residual plots for immersion pressure as a function of angle of contact. (Left) Linear regression fitting. (Right) Quadratic regression fitting.

The immersion slope is fitted as a function of angle of contact, as both a linear and quadratical regression, in Figure 29. Both are statistically significant ($p=0.000$). The high variation at low angles may contribute to the success of the quadratic fit, but decreasing values are observed in a repeatable manner at high angles. Both these fits indicate that the slope magnitude decreases as angle of contact increases, perhaps until perpendicular orientation. This is expected, as a larger magnitude means a stronger resistance to water immersion. This agrees with the immersion pressure finding.

The residual versus fit plot is much more evenly scattered about zero when the immersion slope is fit as a quadratic regression.

Figure 29. Regression line for immersion slope as function of angle of contact.

Figure 30. Residual plots for immersion slope as a function of angle of contact. (Left) Linear regression. (Right) Quadratic regression.

To explore the reasons for each of these variations in responses based on angle of contact, immersion force profiles of various angles were analyzed in depth.

Figure 31 shows a force profile for an approximately parallel angle of contact, with associated images from the experimental run. The strider leg is incremented upwards into the droplet for 1 mm, and does not immerse until around 0.95 mm. This occurs just after image D. This immersion point correlates to a relatively large force magnitude of approximately 0.35 mN.

Figure 31. Force profile for water strider leg that approaches droplet with $\sim 0^\circ$ angle of contact. Letters on graph correspond to images below.

Figure 32 shows a force profile for an approximately perpendicular angle of contact, with associated images from the experimental run. The strider leg is incremented upwards into the droplet for 1 mm, and immerses around 0.2 mm. This occurs just after image B. This immersion point correlates to a relatively small force magnitude of approximately 0.035 mN, or only 10% of the immersion force sustained by the parallel strider leg.

Figure 32. Force profile for water strider leg that approaches droplet with $\sim 90^\circ$ angle of contact. Letters on graph correspond to images below.

Modeling of these parameters as a function of angle of contact has been begun in Appendix A. See this Appendix for analysis of change in wetted perimeter and wetted volume, as well as comparison between model and experimental results for smooth hydrophilic cylinders.

3.1.4 Immersion Studies

A series of subsequent tests were also performed to determine the effect of prior wetting on strider leg performance. For each of the three angles in Table 5, legs were continuously immersed three to four times without drying between. For each series, no

statistical difference was observed between the results for each run. This could indicate that the structure of strider legs is excellent at removing water in seconds, or that only partial wetting occurred and allowed the legs to remain in Cassie-Baxter wetting state.

Table 5. Continuously wetted immersion series.

Angle of Contact	Adhesion Force (mN)	Adhesion Pressure (N/m ²)	Immersion Force (mN)	Immersion Pressure (N/m ²)	Immersion Slope (N/m)
54.2	0.0133	0.895	0.0939	*	-0.13
54.2	0.00981	0.658	0.0893	*	-0.15
54.2	0.0149	1	0.0883	*	-0.13
54.2	0.00834	0.559	0.0928	*	-0.13
64.1	0.0307	3.28	0.0491	5.24	-0.069
64.1	0.0289	3.09	0.0325	3.47	-0.069
64.1	0.0302	3.22	0.0222	2.37	-0.04
64.1	0.0284	3.04	0.024	2.56	-0.11
70.6	0.0316	6.46	0.0154	3.15	-0.007
70.6	0.03	6.14	0.013	2.66	-0.0074
70.6	0.0305	6.24	0.0122	2.5	-0.0072

A close look at the leg tip in the moments following retraction reveal that the setae spring back remarkably quickly. Figure 33(a) shows the wetted setae immediately after removal from droplet. Note that several setae are adhered with a small amount of water. In Figure 33(b), the setae have recovered and are distinct hairs once again, only 1.4 seconds after removal. Strider legs have been previously documented to be excellent at removing condensed water droplets, although not this quickly.

Figure 33. Water strider leg tip after retraction from water droplet. a) Immediately after snap-off. b) 1.4 seconds after snap-off.

3.1.5 Wax Analysis

Wax was extracted from water strider legs and studied both directly and indirectly.

The extracted glass film was analyzed using x-ray photoelectron spectroscopy. Table 6 tabulates the resultant concentrations observed in a wax film. The wax is composed of carbon, nitrogen, oxygen, and a small amount of chlorine.

Table 6. Relative concentrations observed in a standard glass slide and wax extracted from water strider legs.

Atomic Percentage (%)						
	C 1s	N 1s	O 1s	Na 1s	Si 2p	Cl 2p
Glass	54.58	0	33.42	4.86	7.14	0
Strider Wax Film	64.51	7.16	24.61	0.6	2.81	0.3

The wax was also studied indirectly by using stripped strider legs for experimental runs. As noted in Table 7, the wax removal stripped an average of 0.82 μm from each microsetae. This is a fairly significant size difference, and the increase in radii between setae due to this would be expected to decrease water protection.

The stripped back leg had a greater adhesion force than coated back legs, as expected. The removal of the hydrophobic coating resulted in a slightly more hydrophilic leg, especially during retraction when setae wetting has already occurred.

Table 7. Change in setae dimensions after wax removal.

Setae Base Diameter		
	Coated Back Leg	Uncoated Back Leg
Average [μm]	2.1	1.28
Std Dev [μm]	0.3	0.2
Std Error [μm]	0.1	0.07

Table 8. Difference in adhesion force between coated and uncoated strider legs.

	Coated Back Leg	Uncoated Back Leg
Adhesion Force [mN]	0.022	0.035

3.2 Fire Ants

3.2.1 Morphology and Structure

Fire ant legs have a similar morphology to water strider legs. Leg diameter ranges from 95-110 μm , and each leg is covered with an arrangement of microsetae, each of which is decorated with nanogrooves. An overall image of ant leg is given in Figure 34. There is a significant diametric variation at ant leg joints.

Both macro- and microsetae are present. Macrosetae have an average base diameter of 11.5 μm , and microsetae have an average base diameter of 3.1 μm . These can be seen in Figure 35 and Figure 36. Fire ant setae are also decorated with helical nanogrooves, much like water striders. The average spacing between nanogrooves is 0.5 μm .

The setae density is much lower on fire ant legs than on water strider legs, resulting in much larger radii between setae to support pressure at interface.

Figure 34. Fire ant leg.

Figure 35. Macrosetae and microsetae on fire ant leg.

Figure 36. Macrosetae on tip of fire ant leg. Nanogrooves visible on setae.

While some texture was observed on water strider legs, a much more distinctive and regular texture can be seen on the fire ant leg in Figure 37. The leg area is plated in a very

regular pattern, with the exception of the setae base where the pattern is interrupted. Note in Figure 38 that each plate has an additional wavy texture to it. Thus the leg surface has a hierarchical structure even without considering the setae and nanogrooves.

Figure 37. Plated structure on surface of fire ant leg.

Figure 38. Texture on fire ant leg plated surface.

3.2.2 Water Resistance

Fire ant legs were analyzed in the same manner as water strider legs. Data from two ant leg orientations is tabulated in Table 9 and visualized in Figure 39. Note that the parallel leg can support a larger immersion force, has a steeper immersion slope, and similar adhesion force as compared to the leg oriented at 78°. This corroborates what was seen in water striders, as to be expected from their similar morphologies.

Table 9. Ant leg results.

Angle of Contact	Adhesion Force (mN)	Adhesion Pressure (N/m ²)	Immersion Force (mN)	Immersion Pressure (N/m ²)	Immersion Slope (N/m)
0	0.0177	2.18	0.0523	1.68	-0.15
78	0.0176	4.2	0.0395	9.44	-0.095
78	0.0207	4.95	0.0377	9.01	-0.11
78	0.0201	4.95	0.0389	9.29	-0.13

Figure 39. Overlaid immersion curves for ant legs immersed at 0° and 78° orientations.

A correlation analysis was run to determine relations between each variable. Results are tabulated in Table 10. For each relation, the upper cell gives the Pearson correlation coefficient and the lower cell provides the p-value. Assuming 95% confidence, the highlighted cells indicate statistically significant correlations. Both adhesion pressure ($p=0.037$) and immersion pressure ($p=0.001$) have statistically significant correlations with angle of contact.

There is also a positive correlation ($p=0.048$) observed between adhesion pressure and immersion pressure. This is a much stronger correlation than that seen with water strider legs. The setae of fire ant legs is less dense than that of water strider legs, which means that less energy is required to fill the gaps between setae with liquid. This same feature also implies that liquid cannot be removed from between setae as effectively in fire ants.

Table 10. Correlation analysis for ant leg adhesion and immersion variables. Green boxes indicate statistically significant correlations.

	angle	adhesion force	adhesion pressure	immersion force
adhesion force	0.542 0.458		top cell: Pearson correlation bottom cell: p-value	
adhesion pressure	0.963 0.037	0.745 0.255		
immersion force	-0.994 0.006	-0.617 0.383	-0.979 0.021	
immersion pressure	0.999 0.001	0.508 0.492	0.952 0.048	-0.988 0.012
immersion slope	0.845 0.155	0.165 0.835	0.701 0.299	-0.83 0.17

The fitted line regressions and residual analyses for adhesion force and adhesion pressure are shown in Figure 40. Note that the adhesion force seems fairly consistent between angles of contact, but the adhesion pressure is greater when the leg is perpendicular to the

water surface. This echoes what was observed for water striders, and is likely due to the greater wetting that occurs when setae are perpendicularly inserted into droplet.

Figure 40. Fitted line regressions for adhesion force (left) and adhesion pressure (right).

The fitted line regressions and residual analyses for immersion force and immersion pressure are shown in Figure 41. The immersion force is much greater when the leg is inserted parallel to water droplet surface, as with water strider legs. However, the immersion pressure is much greater at perpendicular orientations due to the much smaller area of contact. So when normalized to supporting area, a perpendicular leg can support slightly more force per area than a parallel leg; however, the larger area of the parallel leg overcomes this advantage and is able to support much more total force.

Figure 41. Fitted line regressions for immersion force (left) and immersion pressure (right).

The fitted line regression and residual analysis for immersion slope is shown in Figure 42. Although there appears to be a larger magnitude slope at parallel orientation, this is not statistically significant.

Figure 42. Fitted line regression for immersion slope.

4 Conclusions

The strong effect of orientation on immersion pressure, adhesion pressure, and immersion slope has been shown. Changing orientation from perpendicular to parallel results in a twofold increase in immersion and a decrease in adhesion. These changes may be due to the arrangement of setae structure dependent on the orientation of immersion force.

Additionally, the removal of strider leg wax results in an increase in adhesive force. While there is always an attractive force between water and leg surface, it is in conflict with wetting energy when the wax is present. Without the wax, wetting energy is changed and the surface becomes more hydrophilic and therefore adhesive.

Water strider legs are also very efficient at self-drying. Within less than two seconds, legs return to unwetted properties. This is necessary as the striders are so small that only a fraction of time passes between steps.

Overall, strider legs that are oriented parallel to the water surface, coated in a mildly hydrophobic wax, and have coned setae with nanogrooves to facilitate removal of water, would be excellent models for legs of a biomimetic aquatic robot.

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A Theoretical Analysis

Theoretical force profiles were generated to model wetting of smooth hydrophobic cylinders by water. As discussed in Methods, the cylinders were coated with polyethylene to mimic the contact angle of water strider wax coating; the advancing contact angle of polyethylene was measured as 84°.

During immersion, both wetted perimeter and wetted volume change as a function of immersion depth. For vertical cylinders, or cylinders with perpendicular angle of approach, the wetted perimeter is constant and depends only on cylinder diameter:

$$P = \pi D \quad (\text{A1})$$

The wetted volume is dependent upon cylinder radius and immersion depth, h :

$$V = \pi R^2 h \quad (\text{A2})$$

For a vertical cylinder with diameter 3.5 mm, the wetted perimeter and volume are modelled in Figure A 1 and Figure A 2, respectively.

Figure A 1. Modelled change in wetted perimeter as vertical cylinder is immersed.

Figure A 2. Modelled change in wetted volume as vertical cylinder immerses in liquid.

For horizontal cylinders, or cylinders with parallel angle of approach, the wetted perimeter depends on both the cylinder diameter, D , and length, l :

$$P = 2 \left[D \sin \left(\frac{\theta}{2} \right) \right] + 2l \quad (15)$$

Where Θ refers to:

$$\begin{aligned} \theta &= 2 \left(\frac{\pi}{2} - \tan^{-1} \left(\frac{R-h}{R} \right) \right) \text{ when } h < R \\ \theta &= 2 \left(\frac{\pi}{2} - \tan^{-1} \left(\frac{h-R}{R} \right) \right) \text{ when } h > R \end{aligned} \quad (A4)$$

Before the cylinder is wetted past its midpoint, the wetted volume is dependent on Θ of Eq. A4:

$$V_{h<R} = l[\frac{R^2}{2}(\theta - \sin \theta)] \quad (\text{A5})$$

After the cylinder is wetted past its midpoint, the wetted volume is dependent on Θ of Eq. A4 as:

$$V_{h>R} = l[\pi R^2 - \frac{R^2}{2}(\theta - \sin \theta)] \quad (\text{A6})$$

For a horizontal cylinder with diameter 3.5 mm and length 6 mm, the wetted perimeter and volume are modelled in Figure A 3 and Figure A 4, respectively.

Figure A 3. Modelled change in wetted perimeter as horizontal cylinder is immersed.

Figure A 4. Modelled change in wetted volume as horizontal cylinder immerses in liquid.

The force profile can then be simulated as a function of wetted perimeter, P , and wetted volume, V :

$$F = P\gamma \cos \theta_Y - \rho g V \quad (A7)$$

Where γ is liquid surface tension, ρ is liquid density, and g is gravity.

Assuming a Young's contact angle of 84° , immersion and retraction slopes for a perpendicular angle of approach are modelled in Figure A 5, along with experimental slopes. Note that the modelled immersion and retraction slopes are identical as the effects of hysteresis are not accounted for. Although magnitudes are slightly varied, the slopes of all four regression lines are very similar (no statistical difference, $p=0.433$).

Figure A 5. Modelled immersion and adhesion slopes (same line) compared to experimental immersion and adhesion slopes, for a perpendicular angle of approach.

Immersion and retraction slopes for a parallel angle of approach are modelled in Figure A 6, along with experimental force profile. In this case, the experimental results indicate a much greater resistance to immersion, both in larger force magnitude and in steeper slope, than predicted by the model. This is worth further investigation, as there may be meniscus effects confounding the buoyancy.

Figure A 6. Modelled immersion and adhesion slopes (overlaid) compared to experimental force profile, for a parallel angle of approach.

Given the same size leg at perpendicular and parallel orientations, the models predict that the leg parallel to water surface can support approximately double the force before immersion. This correlates well with the experimental observations for both water strider and fire ant legs.

Figure A 7 shows that smooth hydrophobic cylinders had a significantly greater adhesion force at parallel orientations ($p=0.000$). Moreover, cylinders at parallel orientations sustained three times more force than cylinders at perpendicular orientations ($p=0.000$), as seen in Figure A 8.

Figure A 7. Fitted regression line for smooth cylinder experimental results. Adhesion force as a function of angle of contact.

Figure A 8. Fitted regression line for smooth cylinder experimental results. Immersion force as a function of angle of contact.