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Bio-inspired design of ice-retardant devices based on benthic marine invertebrates: the effect of surface texture

Growth of ice on surfaces poses a challenge for both organisms and for devices that come into contact with liquids below the freezing point. Resistance of some organisms to ice formation and growth, either in subtidal environments (e.g. Antarctic anchor ice), or in environments with moisture and cold air (e.g. plants, intertidal) begs examination of how this is accomplished. Several factors may be important in promoting or mitigating ice formation. As a start, here we examine the effect of surface texture alone. We tested four candidate surfaces, inspired by hard-shelled marine invertebrates and constructed using a three-dimensional printing process. We screened biological and artificial samples for ice formation and accretion in submerged conditions using previous methods, and developed a new test to examine ice formation from surface droplets as might be encountered in environments with moist, cold air. It appears surface texture plays only a small role in delaying the onset of ice formation: a stripe feature (corresponding to patterning found on valves of blue mussels, *Mytilus edulis*, or on the spines of the Antarctic sea urchin *Sterechinus neumayeri*) slowed ice formation an average of 25% compared to a grid feature (corresponding to patterning found on sub-polar butterclams, *Saxidomas nuttali*). The geometric dimensions of the features have only a small (~6%) effect on ice formation. Surface texture affects ice formation, but does not explain by itself the large variation in ice formation and species-specific ice resistance observed in other work. This suggests future examination of other factors, such as material elastic properties and surface coatings, and their interaction with surface pattern.

Bio-inspired design of ice-retardant devices based on benthic marine invertebrates: the effect of surface texture

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ABSTRACT

Growth of ice on surfaces poses a challenge for both organisms and for devices that come into contact with liquids below the freezing point. Resistance of some organisms to ice formation and growth, either in subtidal environments (e.g. Antarctic anchor ice), or in environments with moisture and cold air (e.g. plants, intertidal) begs examination of how this is accomplished. Several factors may be important in promoting or mitigating ice formation. As a start, here we examine the effect of surface texture alone. We tested four candidate surfaces, inspired by hard-shelled marine invertebrates and constructed using a three-dimensional printing process. We screened biological and artificial samples for ice formation and accretion in submerged conditions using previous methods, and developed a new test to examine ice formation from surface droplets as might be encountered in environments with moist, cold air. It appears surface texture plays only a small role in delaying the onset of ice formation: a stripe feature (corresponding to patterning found on valves of blue mussels, *Mytilus edulis*, or on the spines of the Antarctic sea urchin *Sterechnus neumayeri*) slowed ice formation an average of 25% compared to a grid feature (corresponding to patterning found on sub-polar butterclams, *Saxidomus nuttalli*). The geometric dimensions of the features have only a small (~6%) effect on ice formation. Surface texture affects ice formation, but does not explain by itself the large variation in ice formation and species-specific ice resistance observed in other work. This suggests future examination of other factors, such as material elastic properties and surface coatings, and their interaction with surface pattern.

Keywords: ice, invertebrates, Antarctica, benthic, texture

INTRODUCTION

Ice nucleation remains a serious issue for many industrial applications where spray, contact with, or submersion in liquids in sub-freezing point environments is inevitable. Piping systems, ship hulls, airplane wings, and road surfaces are all situations where the accumulation of ice can compromise safety or strength, reduce friction available for traction, impose additional weight, reduce stability, or alter aerodynamic or hydraulic performance. Though methods of actively removing ice after its formation (chemicals (Hassan et al., 2002), rubber mallets, inflatable boots, heating cables, bleed air, geothermal (Makkonen et al., 2001) or microwave heating (Hansman, 1982) de-icing systems) have been developed to varying degrees of effectiveness, prevention using passive means is preferable (Hassan et al., 2002).

In both plants and animals, previous work has shown that ice formation can adversely affect physiology and function or present severe biomechanical challenges that are likely to be selective (Gusta et al., 2004; Wisniewski et al., 2002; Denny et al., 2011). Biological systems may have evolved features to ameliorate the effects of ice that could be copied in engineered devices. For example, (Gusta et al., 2004) studied ice nucleation within plant leaves and the effects of anti-freeze proteins in systems with internal flow. While such observations are important, they may not be of help in larger scale devices where it is undesirable to

alter chemistry; furthermore, extrinsic sources of ice nucleation may be just as damaging (Wisniewski et al., 2002). As another example, among benthic marine invertebrates in McMurdo Sound, Antarctica (Dayton et al., 1969; Denny et al., 2011; Mager et al., 2013), during subcooled conditions, anchor ice readily forms on some organisms (the sponges *Homaxinella balfourensis* and *Suberites caminatus*), while others are resistant (the rubbery soft coral *Alcyonium antarcticum*, the sea urchin *Sterechinus neumayeri*, the sponge *Mycale acerata*, and the starfish *Odontaster validus*). Accretion of ice can interfere with physiological function and the resulting positive buoyancy can cause removal; as a result, different susceptibility to ice formation affects the community makeup and local ecology (Dayton et al., 1969, 1970; Dayton, 1989; Pearse et al., 1991; Gutt, 2001; Denny et al., 2011; Mager et al., 2013). Denny et al. (Denny et al., 2011) did not test the mechanical determinants of the differences in ice formation, though they pointed out the presence of mucus (in *Mycale*) in contrast with the others, as well as the difference between spongy or rubbery organisms and those with hard surfaces like the urchins and sea stars. (Denny et al., 2011) also did not provide contrast with sub-polar organisms.

There are clearly many variables that can affect the formation and adhesion of ice. Biological systems may make use of many mechanisms of ice inhibition concurrently and may exploit any or all of the following (Gutt, 2001; Denny et al., 2011; Gusta et al., 2004; Wisniewski et al., 2002): shape and mechanical pattern, multi-scale features, internal and surface chemistry, coatings, variation in material properties, animal behavior. Unfortunately, not all techniques used in living systems are amenable to large-scale engineering application with current technology. While it is tempting to model everything to the finest scale possible, it is important also to determine the simplest treatment that might work. Can we narrow the variables of interest, to learn about both the biology and potential applications? We hypothesized that surface texture provides protection from ice formation, i.e. for purposes of manufacturability, an attractive engineering solution is one using mechanical patterning alone. To address if this is possible, we tested four candidate artificial surface textures (grid, valley, cone, stripes) inspired by hard-shelled benthic invertebrates (*Saxidomas nuttalli*, *Crassostrea gigas*, *Pisaster ochraceus*, and *Mytilus edulis*, respectively), with all other mechanical properties (material, stiffness, density, thermal conductivity and specific heat, surface wetting) held constant. To examine sensitivity, an range of surface parameters (feature spacing and height), bounding the range seen in organisms, was tested. Tests included both the ice formation test of (Denny et al., 2011) and a new test to examine ice formation in cold air. Such testing is conveniently also the first step in dissecting the underlying biomechanical causes of inter-species variation described in (Denny et al., 2011); furthermore, it allows comparison between organisms with similar surfaces but different latitudinal distribution (polar versus sub-polar).

METHODS AND MATERIALS

Biological samples and ice formation test

We obtained samples of the hard external surfaces of live sub-polar marine invertebrates from a local grocery store (Ranch 99, El Cerrito, CA) and from material leftover from undergraduate biology teaching labs, including butterclams (*Saxidomas nuttalli*), blue mussels (*Mytilus edulis*), ochre sea star (*Pisaster ochraceus*), and Pacific oysters (*Crassostrea gigas*) (Figure 1). The material was subjected to the ice formation test of (Denny et al., 2011), to contrast it with the Antarctic species reported by Denny et al. Biological samples (< 2 cm maximum dimension) were placed in open, unstirred 250 mL polypropylene tripour beakers and filled with 150 mL of 32 psu artificial seawater (Instant Ocean, Blacksburg, VA). Both sample and seawater were initially at 4 °C. Multiple beakers were placed on a fiberglass tray which was then placed in a -20 °C walk-in freezer and observed for ice formation on the sample within the first 10 min.

Biological inspiration and surface fabrication

Biological samples were observed under a 10x dissecting microscope, measured and sketched. To exaggerate specific physical properties in repeatable patterns to observe phenomena that are a result of surface texture alone, we used a three dimensional printing (3DP) process to create engineered samples mimicking the biological samples. Samples measured 0.03 m × 0.03 m × 0.005 m overall and included patterning of four textures: grid, valley, cone, and stripes (Figure 1). The textures corresponded to *Saxidomas*, *Crassostrea*, *Pisaster*, and *Crassostrea* respectively. The cone texture is also similar to the Antarctic starfish *Odontaster*, while the striped texture is similar to patterning observed on the spines of the Antarctic urchin *Sterechinus*; both are known to be resistant to ice formation (Denny et al., 2011).

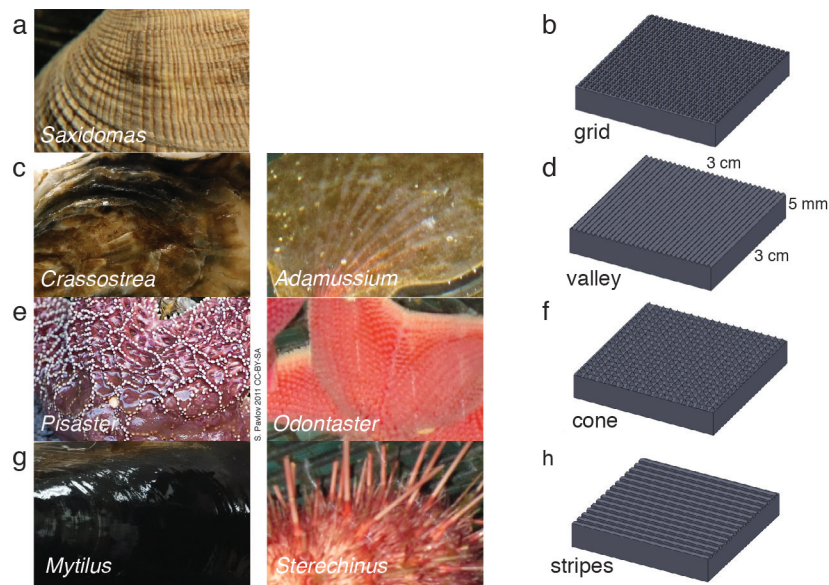


Figure 1. Biological inspiration and surface fabrication. (a-b) *Saxidomas nuttalli* and grid texture; (c-d) *Crassostrea gigas* and valley texture, similar to the Antarctic scallop *Adamussium colbecki*; (e-f) *Pisaster ochraceus* (Pavlov, 2011) and cone texture, similar to the Antarctic starfish *Odontaster validus*; (g-h) *Crassostrea gigas* and striped texture, similar to the patterning on spines of the Antarctic urchin *Stereochinus neumayeri*. Photos scaled to approximately 3 cm width.

The valley texture is similar to the corrugations on the Antarctic swimming scallop *Adamussium colbecki*, although such corrugations are likely linked to weight reduction needed in order to swim (Denny and Miller, 2006), and the presence of epibionts may alter that organism's propensity to form ice.

The observed textures (Figure 1) ranged from 0.25 mm to 0.5 mm high. Features were spaced approximately 0.5 mm to 0.75 mm for the grid-like texture on *Saxidomas*; approximately 1 mm to 3 mm for the groove-like/valley texture on *Crassostrea*; 0.5 mm to 4 mm for the cone texture of *Pisaster*; and 0.5 mm for the stripe texture on *Mytilus*. These measurements were used in setting designing the textures: for initial screening, a height of 0.5 mm was used, with pattern spacing set at 0.5 mm for grid, valley, and stripes and 1.5 mm for cone.

We designed the textures using two solid-modeling programs: Solidworks (Dassault Systems, Waltham, MA) and Blender (Blender Foundation, Amsterdam, Netherlands). Solid models were used to prepare stereolithography (STL) files that were printed in ABS plastic using a ProJet 3000 3D printer (3D Systems, Rose Hill, SC). Newly printed samples were cleaned of support material by gentle heating and use of an 80 °C sonicating warm-oil bath, then washed thoroughly with detergent before testing.

Ice formation test of sample plates

As with the biological samples, we tested the four sample plates using the ice formation test of (Denny et al., 2011) (Figure 2(a)). The onset of ice formation was determined by observing specimens by eye in a walk-in freezer using identical methods to (Denny et al., 2011). Based on the results of this test, the best-performing and worst-performing textures were selected for further optimization of the surface parameters (pattern spacing and height), to examine sensitivity. All plates were found to exhibit ice formation during the test period, so further examination of pattern sensitivity used a new test to more closely examine the freeze time as well as ice formation in intertidal or terrestrial cases.

Droplet tests of sample plates

An additional series of sample plates based on the best-performing and worst-performing textures from the ice formation test was prepared, varying the pattern spacing from 0.5 mm to 4 mm and pattern height from 0.25 mm to 1 mm. In addition, several untextured control plates were also prepared (STL files available for download). Plates were dried and stored in 4 °C walk-in freezer overnight to provide isothermal

starting conditions. Plates were placed randomly in rows on a plastic tray for testing. Five 0.1 mL droplets of 32 psu artificial seawater, also at 4 °C, were placed on the four corners of the plate and the center (Figure 2(b-c)). The plates were then placed in a -20 °C freezer on a flat surface away from overhead fans and observed during freezing. In addition to visual observation of samples with the naked eye, a digital video camera (Hewlett Packard, Palo Alto, CA) was used to obtain time lapse images at 1 frame s⁻¹ (example video available for download). Samples were observed over 30 min or until all of the samples froze. Samples were classified as frozen once the droplet turned opaque white (Figure 2(b)). The resulting freeze times were recorded and analyzed in R (R Core Team, 2014). For the results presented below, freeze times for a given test were normalized by the mean freeze time for the untextured control plates in the test.

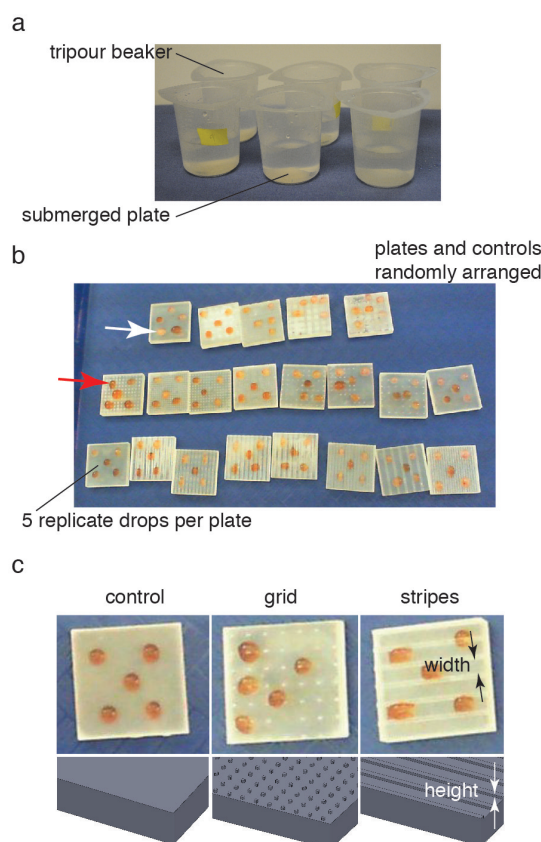


Figure 2. (a) Submerged ice formation test from (Denny et al., 2011). Plates were placed in 250 mL beakers and watched for ice formation in a -20 °C walk-in freezer. (b) Droplet test to test for ice formation in cold air (intertidal or terrestrial case). Plates and controls were randomly arranged on a tray in the same -20 °C freezer. Droplet freezing was identified by color shift from red (middle arrow) to white (upper arrow). Plates 0.03 m square. (c) Textures and control used during droplet test. Feature width and height varied between 0.5 mm to 4 mm and 0.25 mm to 1 mm respectively.

RESULTS

Ice formation test for biological samples and sample plates

Unlike in Antarctic species tested in (Denny et al., 2011), all sub-polar biological samples we tested (hard external surfaces of *Saxidomas nuttalli*, *Crassostrea gigas*, *Pisaster ochraceus*, and *Mytilus edulis*) initiated ice formation during the ice formation test (Figure 3). This is discussed further below.

All sample plates tested in the submerged ice formation test of (Denny et al., 2011) also initiated ice formation, however, there were broad differences in freeze time between the textures (ANOVA,

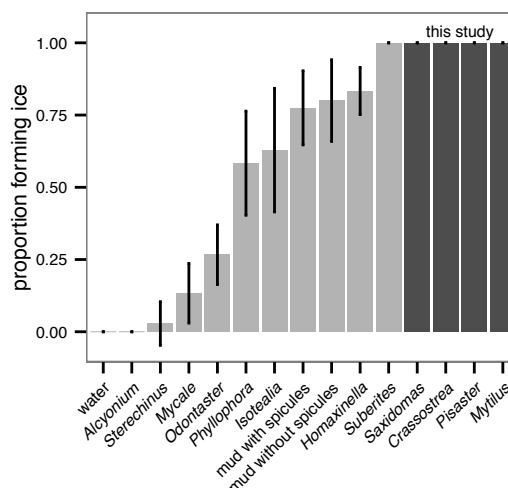


Figure 3. Ice formation on sub-polar organisms (*Saxidomas nuttalli*, *Crassostrea gigas*, *Pisaster ochraceus*, and *Mytilus edulis*, dark gray) compared to Antarctic data reproduced from (Denny et al., 2011) (light gray). All sub-polar samples tested here initiated ice formation during the ice formation test (3 batches, $n = 5$ each).

Table 1. Freeze time (mean \pm s.d.) and wetted area for sample plates during submerged ice formation test. Freeze time does not depend on wetted area (linear regression, $P = 0.223$).

texture	freeze time, s	area, mm ²
grid	375 \pm 92	1800
valley	388 \pm 79	1290
cone	433 \pm 71	1030
stripes	465 \pm 81	1520

$P = 0.019$, Figure 4). The best-performing texture (stripes) increased the time to freeze relative to the worst-performing texture (grid), by 25%. On the other hand, wetted surface area did not appear to affect the freeze time (linear regression, $P = 0.223$, Table 1).

Droplet test and sensitivity of freeze time to pattern spacing

Within both the best-performing texture (stripes) and the worst-performing texture (grid), the pattern spacing appeared to alter the normalized freeze time for droplets up to 28%, though the slowest freeze times were only delayed about 6% relative to the untextured control (Figure 5). Given a texture, there appears to be a (weakly) optimal feature spacing (ANOVA, $P = 0.0004$ for spacing, $P = 0.0389$ for height), however, increases in normalized freeze time obtained by varying pattern spacing or height are small relative to the noise in the measurement. An example time-lapse movie of a droplet test is provided in the supplemental material.

DISCUSSION

Sub-polar species always initiated ice formation

It is perhaps not a surprise that the four species tested (*Saxidomas nuttalli*, *Crassostrea gigas*, *Pisaster ochraceus*, and *Mytilus edulis*) all initiated ice formation (Figure 3). Although all are found in cold water (*S. nuttalli*, *P. ochraceus*, and *C. gigas* range to Alaska), they are not particularly noteworthy as polar species. On the other hand, hard shelled bivalves of closely-related species can be major

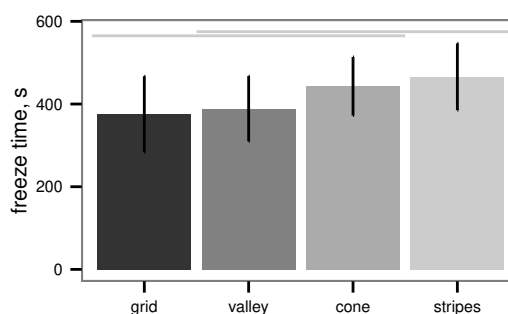


Figure 4. Freeze time (mean \pm s.d.) for sample plates during ice formation test. Differences between textures are significant (ANOVA, $P = 0.019$); light grey lines indicate groups from post-hoc Tukey analysis.

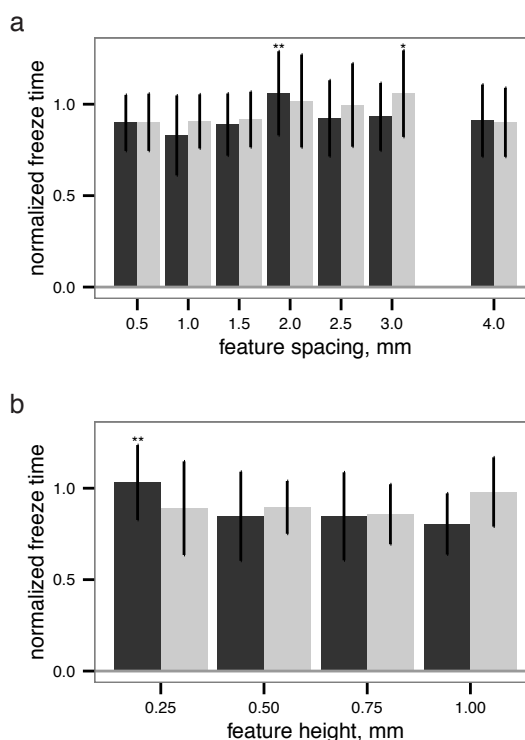


Figure 5. Normalized freeze time versus (a) feature spacing and (b) feature height (see Figure 2(c)), examining sensitivity to surface parameters for two different textures. Grid texture in dark grey; stripes texture in light grey as in Figure 4. Maxima indicated by * for stripes and ** for grid; normalized freeze time does depend on surface parameters (ANOVA, $P = 0.0004$ for spacing, $P = 0.0389$ for height), but increases in freeze time are small relative to the noise in the measurement.

130 components of Arctic communities (Dayton et al., 1994; Gutt, 2001), while echinoderms (e.g. *Odontaster*
and *Sterechinus*) are hugely important in the Antarctic (Dayton et al., 1969, 1994). The relative ease with
which the sub-polar species here initiated ice formation, compared to Antarctic species reported in (Denny
et al., 2011), suggests one potential barrier to polar spread: even mild anchor ice events would likely
135 remove all of the sub-polar species we tested. While *M. edulis* is tolerant of being frozen in ice (Aarset,
1982; Aunaas, 1985; Kanwisher, 1955), (Gutt, 2001) notes that glacier scour and buoyancy effects of ice
can cause removal. Unfortunately, as ice recedes, such barriers may be diminishing.

Implications for device design

Contrary to what we hypothesized, simple surface texture does not appear to be a magic bullet able to
confer a large degree of ice resistance (Figures 4 and 5). However, careful application of a correctly-spaced
140 texture appears to delay the onset of freezing a small amount, though the mechanism is unclear. For
droplet tests in air, one mechanism that may explain the observed maxima is the interaction between
hydrophobicity conferred by finely spaced textures (Cassie and Baxter, 1944) and insulation from entrained
air within the texture. While our tests did not include measurement of contact angles, the ratio of pattern
spacing used in our tests corresponded to the region where surface patterning can increase apparent contact
145 angle (Cassie and Baxter, 1944), potentially increasing the thermal insulation provided by trapped air on
one side of the drop compared to thermal conduction through the features. However, such a mechanism
and its interaction with thermal conduction is uncertain since the minima in these noisy data fall at
intermediate spacing and low height. Biologically, while a Cassie wetting mechanism cannot explain
subtidal differences as observed in (Denny et al., 2011), it could be relevant in the feather or fur coats of
150 animals, in the intertidal, or in trichomes on leaves, perhaps causing extracorporeal ice nucleation as a
means to provide insulation to more sensitive tissues beneath (Duman et al., 1991).

Surface chemistry may have effects on ice resistance; hydrophilic materials reduce resistance to ice
adhesion (Meuler et al., 2010a,b) in air, and many biological materials (notably calcium carbonate in
bivalve shells and echinoderm ossicles and spines) are hydrophilic. It is unclear what the effect of surface
155 chemistry is in subtidal situations, and the ice resistance of *Sterechinus* and *Odontaster* (both echinoderms
with calcareous ossicles) appears contrary to expectations from surface chemistry alone.

Texture has demonstrable, but small, effects on freezing time

While texture does have demonstrable effects on freezing time, the effects are small (Figures 4 and 5).
Mechanical patterning alone cannot explain the large inter-specific differences in ice formation observed
160 in (Denny et al., 2011) for Antarctic species, or the ease with which sub-polar species appear to initiate
ice formation. Thus, we infer that the differences observed by (Denny et al., 2011) are due to other effects
or interactions with surface texture.

Further dimensions that may be exploited in biological systems but were not examined here include
surface wetting (hydrophobic/hydrophilic); surfactants, mucus and slime (as in *Mycale*); and elastic and
165 viscoelastic properties (e.g. rubbery *Alcyonium*); nanoscale pattern, multi-scale shape and behavior. In an
engineering system, these would increase cost; however, perhaps a subset of these may provide synergistic
effects resulting in performance improvement in excess of the cost increase. For example, the combination
of rigid and viscoelastic materials in a micro-scale composite artificial shark skin was effective in drag
reduction (Wen et al., 2014). For ice retardant devices, clearly mechanical designs patterned solely on
170 sub-polar species have not yet provided performance of the same level as the actual polar organisms.
Additional observation is needed of multi-scale shape, behavior, and the material properties of perishable
tissue and secretions that do not preserve well. Further investigation of ice biology in the field, to examine
biomechanical performance in natural environments, along with innovative pairing of materials may
enable more effective anti-ice engineering solutions.

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