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Chapter

NANO- AND MICROFLUIDICS, RHEOLOGY, EXOPOLYMERIC SUBSTANCES AND FLUID DYNAMICS IN CALANOID COPEPODS

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ABSTRACT

During the last half century, much progress has been made on understanding feeding and encounter in copepods, including observation and modeling of fluid flow around their carapaces, appendages, setae and setules. This modeling has generally assumed a Newtonian medium, and laminar shearing at low Reynolds numbers, with zero velocity at the surface of both the copepod and its prey. This implies on the one hand no slippage of the fluid at surfaces, "*no slip*", and on the other no finite layer of no motion, "*no stick*". This conforms closely to the Derjaguin-Landau-Verwey-Overbeek (DLVO) model, widely used by engineers for over 60 years in nano/micrometer-scale fluidics problems involving surfaces. At the scales of pelagic calanoid copepods (< *ca*. 1 mm) the medium they live in is, at times and in places, functionally non-Newtonian. It presents excess viscosity and elasticity, imparted mainly by exopolymeric substances (EPS) secreted largely by algae and bacteria, and so may be most marked in blooms and chlorophyll maxima. Furthermore DVLO theory is increasingly being recognized as only a convenient assumption, now partly outdated by findings of slip layers up to several micrometers thick, notably on hydrophobic or omniphobic sculptured surfaces. This chapter briefly reviews DVLO theory, and then reviews some notable departures from this theory found in natural waters at scales applicable to copepods. The chapter reviews seawater rheology, and the possible effects of EPS producing stick layers of finite thickness, particularly on copepods' prey. It then considers possible slip layers associated with superhydrophobic drag reduction (SDR), omniphobic drag reduction (ODR) and slippery liquid-infused porous surfaces (SLIPS). Such possible effects include increase

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(EPS layers) or decrease (SDR, ODR, SLIPS) in water velocity and shear rate close to the surface, as well as possible resistance to adhesion (slipperiness) and biofouling. Slipperiness in prey may hinder copepod feeding. Finally the differences are briefly treated between the relatively mucus-free calanoid copepods and more mucus rich harpacticoids, and a suggestion is made for interdisciplinary capacity building activities for rheology and nano/microfluidics to be understood better by teams investigating copepod dynamics and all aspects of plankton ecology and biogeochemistry.

INTRODUCTION

Advances in nano- and microfluidics since about 1990 have driven new surface science that shows that models of surface properties developed in the 19th and 20th centuries have ill-equipped biogeochemists and both microbial and plankton ecologists with engineering tools to understand many of the phenomena observed at the surfaces of living and non-living particles. This chapter exposes the importance of some recent advances in nano- and microfluidics, focusing on a particular area of biogeochemistry and plankton ecology, the feeding and hunting by planktonic, mostly calanoid, copepods.

In the 19th century Physics and Chemistry made enormous progress, driving engineering and the Industrial Revolution. Steam-powered, internal-combustion and electric engines were developed, together with chemical processes and purification, shaping the social, political, and ultimately the ecological and biogeochemical history of the following 20th century. However, the problems of electrical and turbulent boundary layers [1] remained mired in controversy. The 20th century saw arms races develop, first Anglo-US-German-Russian (1890-1945), then principally US-Soviet (1946-1990), driving huge motivation quickly to develop better and faster aircraft, missiles, ships, torpedoes and so on. These arms and machines develop huge physical pressures, so the small forces in soft, non-Newtonian materials tended to be largely neglected [2]. In the early 20th century, models of the tiny boundary layers were also greatly simplified and taught in the engineering schools principally in Europe and North America. In the 1940s, the simplified versions were drawn together to model the stability of colloidal systems in what is now known as the Derjaguin-Landau-Verwey-Overbeek (DLVO) model [3]. See below for further details.

The development of nano- and microfluidics, including nano- and microfiltration, at the end of the 20th century, with high added value in nano- and micro-machines, including lab-on-a-chip, reverse osmosis and desalination techniques, soon showed that DLVO did not suffice to model fluid dynamics at the nanometer, micrometer and even larger scales involved [4-9,19]. The published science has at times lagged behind innovation by trial and error, and the length scales happen to be at least in part those used by nauplii to adult copepods in swimming and prey capture. Measured in the sea at larger length scales (typically larger than 0.35 mm), the rheology, or visco-elasticity of natural waters is now known to be partly controlled by exopolymeric substances (EPS) exuded by algae and bacteria [10-15].

In this chapter, bearing in mind scales relevant to copepod hydrodynamics, I summarize the system of units considered, the length scales, particularly those relevant to nano- and microfluidics, the DVLO model, and observed departures from it, including superhydrophobic drag reduction (SDR) and wall slip as well as some effects that are unclear, or even contested. I subsequently consider the effects of EPS on copepod hydrodynamics and food handling, partly through its known effect on the rheology of the copepods' milieu, as well as by stickiness mechanisms. Copepods' ways of dealing with stickiness are also considered. Within this section, to minimize future confusion, I will list some different meanings of so-called "*sticky water*". The copepod surfaces will be considered, particularly the epicuticle of the feeding appendages, in relation to feeding, and that of the rest of the body in relation to swimming and ambush hunting. The surfaces of calanoid copepods' principal food, phytoplankton, are also considered. I finally treat some of the differences between harpacticoids and calanoids, particularly in relation to mucus.

UNITS

In this chapter I discuss mechanical stress quite explicitly, but mass very little. I follow Jenkinson & Sun [2] and rearrange the familiar framework of S.I. units, length [m], time [s] and mass [kg], into a framework of length [m], time [s] and pressure or stress [kg m^{-1} s^{-2}]. Further details are shown in Table 1.

Table 1. Units used in this chapter

Length	m
Time	s
Stress	Pa (pascals) = N m^{-2} (N = newtons)
Temperature	K (kelvins), °C (degrees Celsius) (0°C = -273.15K)
Salinity	Practical salinity units (psu)
Electric charge	C (Coulombs)
Molecular concentration	M (moles L^{-1}, where 1 mole = 6.02 ㄹ ㅊ 10^{23} molecules)

NANO- AND MICROFLUIDICS IN RELATION TO THE DYNAMICS OF MICRO- AND MESOPLANKTON

The Length Scales of a Few Things

Nano- and microfluidics refer to flows at length scales below about 100 nm and 100 µm respectively. The discipline has recently progressed rapidly, and is being used to build entire micromachines, including microvalves, micro- and nanopumps, gears, cantilevers and other microdevices [16], driving intense research on processes at these scales.

Figure 1 has scale from 10^{-1} to 10^{7} nm. Table 2 also collects some of the scales mentioned in this chapter. At centimeter scale we have giant copepods such as *Bathycalanus* sp. directly subject to flow at the limits of turbulence. Here friction and leverage are important. *Bathycalanus*'s appendages and whiskery bits reach down to smaller scales, hence to different physical constraints. Its feeding parts are millimeter in scale, larger than the entire cephalosome of the adults of many small species and naupliar stages. Descending past the millimeter milestone, diffusion and viscosity become important. The next milestone, marked micrometer, includes the realms of cell biology. Between the micrometer to nanometer scales is where strong capillarity, molecular adhesion and recognition, as well as steric repulsion kick in. Here surface energy effects are important. Down at the nanometer scale we have chemistry, molecular structure, "*water-of-hydration shells*" or "*solvation shells*" [19] around ions, and the multimolecular shells of water itself (Tim Wyatt, *pers. com.)*.

Table 2. Typical molecular and hydrated radii and diffusion coefficients in bulk water at 25ºC. Summarized from Conlisk [3]

Solute	D_A (cm^2 s^{-1})	Molecular radius (nm)	Hydrated radius (nm)
Ions			
Na^+	1.33 ㅊ 10^{-9}	0.12	0.36
K^+	1.96 ㅊ 10^{-9}	0.15	0.33
Ca^{++}	2.1 ㅊ 10^{-9}	0.19	0.33
Cl^-	2.0 ㅊ 10^{-9}	0.16	0.33
Biomolecules			
Albumin	0.061 ㅊ 10^{-9}	-	7.2
Glucose	0.94 ㅊ 10^{-9}	-	1

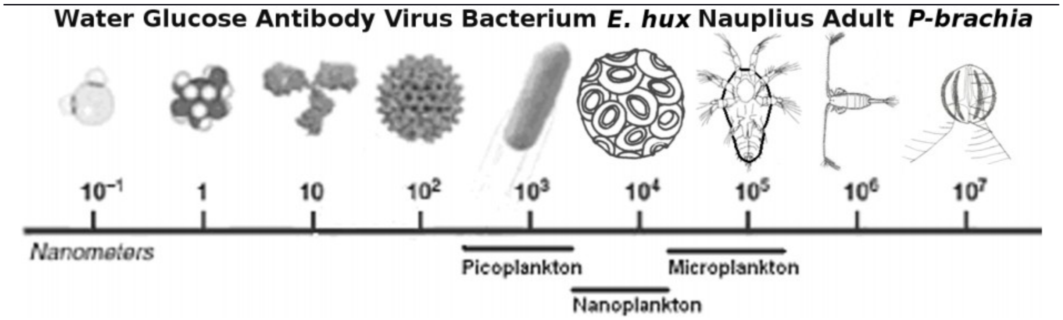

Figure 1. The length scales of a few things, ranging from water molecules to the ctenophore *Pleurobrachia pileus*. are compared to the nano- and microfluidics scales addressed in this chapter. *E. hux*: *Emiliana huxleyi*; *P-brachia*: *Pleurobrachia pileus*. Drawing of *E. huxleyi* and *P. pileus* from Jahnke [130] and Greeve [131], respectively.

Length Scales of Layers: The Derjaguin-Landau-Verwey-Overbeek (DLVO) Model

This section is a description of liquid-solid surface structures based on the Derjaguin-Landau-Verwey-Overbeek (DLVO) model, expanding on Conlisk [3].

Electrical Double Layer

Many solids acquire a negative charge when in contact with an aqueous medium. The development of electrical charge on a solid surface is a combination of physical and chemical processes. The surface charge is acquired through a variety of mechanisms such as ion adsorption, exposure to charged crystal surfaces, and ionization of surface groups. Among these, the ionization of surface groups plays a dominant role. For devices with surfaces made of glass or silicon, surface silanol groups (SiOH) undergo deprotonation, which results in development of negative charges on the walls.

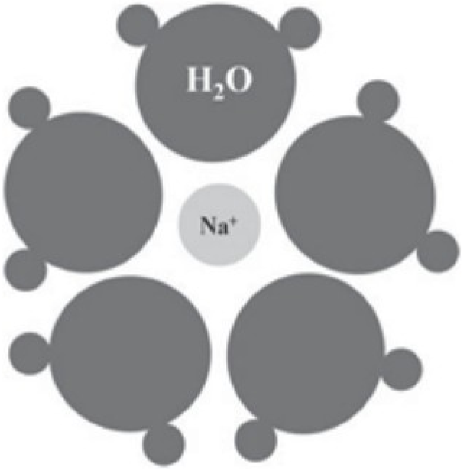

Figure 2. Sketch of the hydration of a sodium ion by water molecules. Values of the hydrated ionic radius a_H can range from 3 to 5 times the unhydrated ion radius a. For example the ratio $H = a_H/a$ is typically 1.83 for Cl^- and H = 3.91 for Na^+. Multivalent ions typically have hydration radii larger than monovalent ions because of the stronger electrical fields around them. Modified from Conlisk [3].

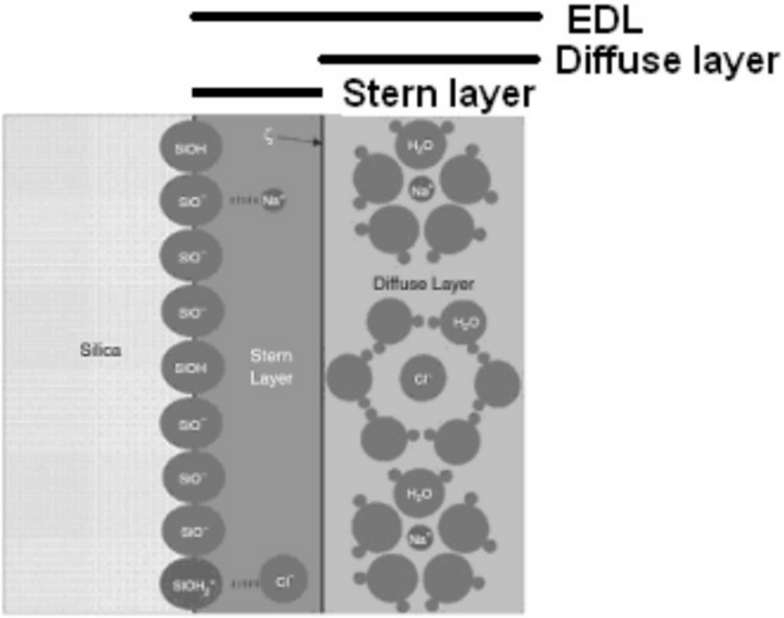

Figure 3. To show the hydration of ions by water molecules near a charged silica surface. The electric double layer (EDL) consists of a layer of counter ions pinned to the wall, the Stern layer, and a diffuse layer of mobile ions outside that layer. Here the wall is shown as being negatively charged, and the ξ-potential is defined as the Stern plane. Modified from Conlisk [3].

If the surface is negatively charged, the positively charged ions (called counterions) in the vicinity of the surface tend to collect near the charged surface, and an inner layer of counterions is attached to the surface. Outside this pinned layer, ions float within the electrolyte solution near the surface. The total system, including the pinned and floating ions or diffuse layer, is called the electrical double layer (EDL). On the basis of the analysis of the EDL surrounding an ion (Figures 2, 3), the local electric field can be in the order of 10^{10} V m^{-1}. Under the influence of this large electric field, the polar ends of water molecules can electrically bond to the ion, creating a "molecule" whose effective radius is larger than the unhydrated one. Several radii of solvated hydrated ions are shown in Table 2, but these are the radii considered to be time averages over a large number of ions.

The following treatment uses published values determined at 20ºC unless otherwise stated. The layer outside the Stern layer consists of mobile ions and is called the diffuse layer, and the total system, including the Stern layer and the diffuse layer is called the Electrical Double Layer (EDL) (Figure 3). The presence of the charged surface and the ions thus creates an electrical field. Conlisk [3] presents parameters for simplified seawater, consisting of a solution of totally dissociated NaCl, *i.e.* Na^+ and Cl^- ions. The ionic strength of this solution

$$I = \sum_i z_i^2 c_i \tag{1}$$

where c_i is the molar concentrations of the electrolyte constituents of species i, z_i is their valence and here i is (Na^+, Cl^-). To obtain an approximate value of I for seawater, let us assume it to be a 3.5% (w/v) (35 g l^{-1}) solution of salt, NaCl. Since the atomic weights of Na^+ and Cl^- are, respectively, 23 and 35.5, their separate concentrations are, respectively, [(35 ㄹ ㅊ 23)/(23 + 35.5), (35 ㅊ 35.5)/(23 + 35.5)] g l^{-1}, or (13.7606, 21.2393) g l^{-1}, (13.7606/23, 21.2393/35.5) M, or 0.598 M each. Since z_i is (1, 1), and c_i is (0.60, 0.60) M, I = 1.2 M.

The permittivity (or dielectric constant) of a medium, such as water

$$\varepsilon_r = \frac{\varepsilon_e}{\varepsilon_0} \tag{2}$$

where ε_0 is the permittivity of a vacuum, 8.85 ㅊ 10^{-12} C^2 N^{-1} m^{-2}, and ε_r is the permittivity of a medium (*e.g.* water) relative to ε_0.

Meissner & Wentz [17] determined ε_r at over a range of salinities at 20°C to be from 78.5 to 72.7 at salinities from 0 to 35, respectively. From Eq. 2, the respective values for ε_e are thus 6.95 ㅊ 10^{-12} and 6.47 ㅊ 10^{-12} C^2 N^{-1} m^{-2}.

The nominal length scale associated with the EDL is the *Debye length*, defined by

$$\lambda = \frac{\sqrt{\varepsilon_e RT}}{FI^{1/2}} = \frac{1}{\kappa} \qquad (3)$$

where F is Faraday's constant (9.65×10^4 C mol^{-1}), ε_e is the electrical permittivity of the medium; R is the universal gas constant (8.31 J mol^{-1} K^{-1}), T is the temperature expressed in °K, I is the ionic strength, calculated above as 1.2 M.

For salinity 35, the calculated value of λ (Eq. 3) for our simplified seawater thus comes to 0.39 nm. The corresponding values for salinities 10, 1.0 and 0.10 are I = 0.34, 0.034 and 0.0034 mol L^{-1} and ε_r, = 75.2, 77.7 and 77.8 [17] and λ = 0.71, 3.0 and 7.3 nm.

Debye length is an important characteristic of interfacial electric double layers. It is a measure of the distance between charged interfaces where the electrostatic component of surface forces is important. Reduction of the Debye length due to increasing ionic strength leads to weaker electrostatic interactions and possible stability loss by dispersions and emulsions (an example of instability is shown when a liter of milk curdles in seconds following the addition of a drop of acidic lemon juice). Transition from the fresh to seawater in estuaries, with the formation of deltas of organic-rich sediment is a striking example illustrating importance of this parameter. As λ becomes smaller the voltage gradient (electric field) across the Debye layer on suspended and colloidal particles becomes greater, resulting in the particles aggregating into stronger and more compact flocs [3] of higher density [18]. This leads to more rapid sedimentation both in and downstream of the sharpest salinity gradients in estuaries with the frequent appearance of a turbidity maximum, mud banks and deltas, rich in organic matter. Some copepod species, particularly of the genera *Eurytemora* and *Acartia*, are specialized, having adapted to live in these stressful areas in and around estuarine turbidity maxima. Here, steep spatiotemporal gradients occur in salinity, and thus also in Debye lengths and in the electrical fields around mineral, non-living-organic and living particles. As a result, steep gradients will also frequently occur in the stickiness, density and size distribution of marine snow, marine organic aggregates and transparent exopolymer particles (TEPs), often giving high loads of recently aggregated and rapidly sinking or rising [18] suspended particulate matter.

Turbulent Boundary Layers on Copepod Surfaces

The Reynolds number is the main dimensionless parameter associated with viscous flow, and for boundary layer flow on a flat plate of length *L*, it is defined by

$$Re = \frac{\rho U_\infty L}{\mu} \tag{4}$$

where ρ is the fluid density, μ is the viscosity, U_∞ is the velocity far from the plate, and *L* is its length [1].

At low values of *Re* (< *ca*. 1), a copepod lives in an immediate fluid environment free of turbulence, while at high values of *Re* (> *ca*. 100) it lives in a fluid that is turbulent at body-length scales, but a shearing, non-turbulent boundary layer covers all its body surfaces.

Slip Layers and Non-Stick Layers

Wall Slip

Rothstein [8] reviews the development of the notion of wall slip. The concepts of wall slip and slip length were formalized by Navier in 1823 [3] as shown in Figure 5.

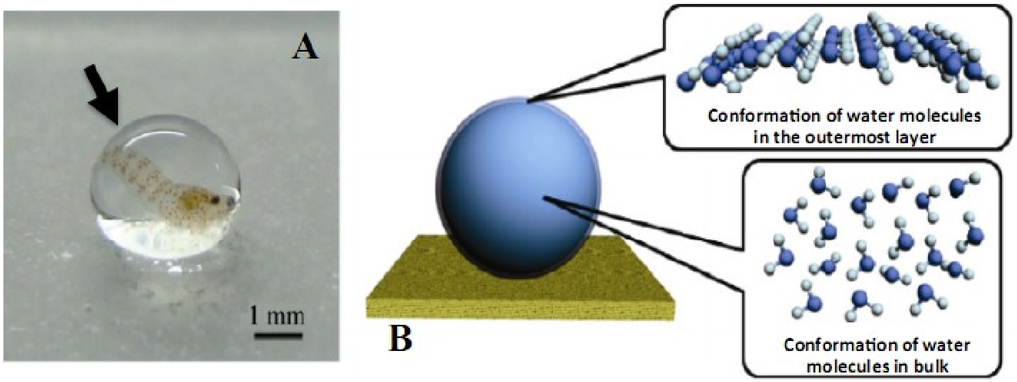

Figure 4. (A) Optical photograph of a several-mm^3 droplet, in which a *Caridea serrata* shrimp was trapped, placed on a superhydrophobic pedestal. (B) Schematic illustration of a droplet on a superhydrophobic plate, showing the H-bonding net around the droplet consisting of a regular array of water molecules. The conformation of water molecules closest to the water/solid surface and water/atmosphere interface is different from that in the bulk. Interfacial molecules form regular structure in order to maximize their H-bonding partners and thereby form a compact H-bonding net. Modified from Su *et al*. [134], and reproduced with kind permission.

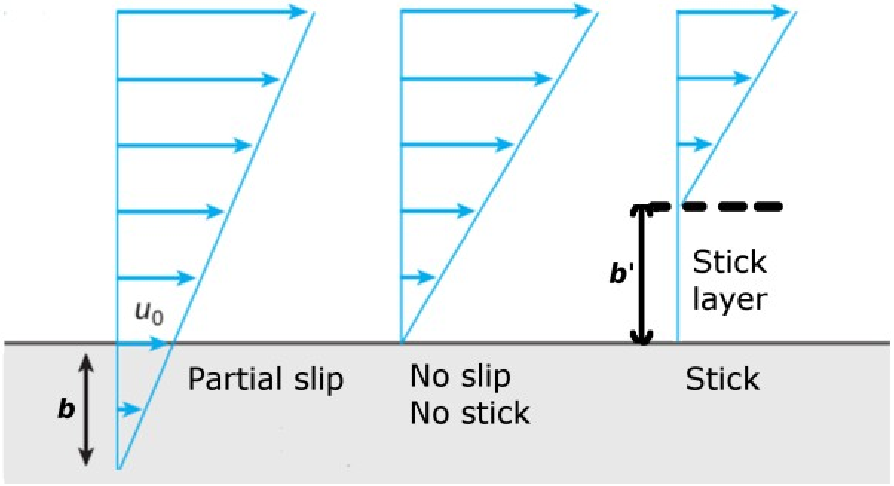

Figure 5. Schematic diagram of slipping and sticking at a fluid-solid interface by a fluid subject to simple shearing. *b* is slip length; *b'* is sticking length; u_0 is slip velocity. Modified from Rothstein [8].

In Navier's model, the magnitude of the slip velocity, u_0, is proportional to the magnitude of the shear rate experienced by the fluid at the wall:

$$u_0 = b\dot{\gamma} \tag{5}$$

where *b* is the slip length and $\dot{\gamma}$ is shear rate such that

$$\dot{\gamma} = \left|\frac{\partial u}{\partial y}\right| \tag{6}$$

where *u* is the fluid velocity at any point and *y* is the distance of that point from the surface. When the value of *b* is negative (*i.e.* notionally within the wall) it is a slip length, but when *b* is positive (*i.e.* in the "fluid") it becomes a stick length (Figure 5) (see "Stick layer" section below).

There followed much debate on the subject during the 19th century. During the 20th century, however, published experimental results generally confirmed that no slip existed at liquid-solid boundaries, so the functional "*no-slip*" idea came to be universally accepted as the normal condition [8,20,21], apart from minor exceptions and dissident voices such as that of Grasso et al., (2002) [22] and Drost-Hansen (2006) [23].

The Lotus Leaf Effect and Superhydrophobic Drag Reduction

Better experimental techniques and the advance of requirements by the micro- and nanofluidics industry, however, led to a wealth of papers in the 1990s finding violations of the no-slip law mainly at hydrophobic walls. More dramatically, the 2000s have seen the discovery of superhydrophobic drag reduction (SDR), and by 2010 Rothstein [8] could authoritatively state that, "*The no-slip boundary condition* [was] *no more than a convenient approximation found to hold under most normal flow conditions*". Slip lengths up to 25 µm have now been found [5].

Until recently, it was considered that SDR could exist when the superhydrophobic surface was in either of two states, the Wenzel state (*i.e.* when liquid occupies the spaces between the surface irregularities) or the Cassie state, in which gas is trapped between the irregularities [8]. The Cassie state tends to show more powerful SDR than the Wenzel state, but a disadvantage to pelagic organisms might be the susceptibility of SDR to compression and dissolution of the trapped gas by hydrostatic pressure. Barthlott & Neinhuis's [4] much cited paper represents a landmark. They investigated the water-repellence and self-cleaning of lotus leaves. Quickly, workers found such surfaces to show SDR, with slip lengths of many nanometers in tubes with hydrophobic coatings [7].

Slip Layers and Hierarchical Microstructuring

As well as the Cassie and Wenzel states, there is a recently reported third drag-reducing state. Cottin-Bizonne et al., [24] modeled that, while regularly spaced and sized nano- and microstructures can give the biggest slip lengths and hence the most drag reduction (DR), the amount of DR can be very sensitive to small changes in structure shape and size. A fractal hierarchy of structure sizes gives the most stable DR. This may explain the generally hierarchical distribution of structure sizes on flowering plants [25]. If microstructuring on copepods also generally occurs at a hierarchy of sizes, this too would be compatible with a function evolved for stable drag reduction. The setules on setae and other parts of the copepod body may function as part of such hierarchical structuring.

The Flower Petal Effect

Water drops stick to many flower petals rather than rolling off. Feng et al., [6] show that these petals bear a hierarchy of nanometer- to micrometer- scale, hydrophobic irregularities. Like on lotus leaves, water drops round up due to repulsion, but because of the particular sizes and shapes of the irregularities, the drops remain pinned to such petals. After the Lotus Leaf effect (which can incorporate SDR and self-cleaning of the surface whether in the Cassie state or the Wenzel state), I class the Flower Petal effect as a second superhydrophobic state.

Slippery Liquid-Infused Porous Surfaces (SLIPS)

A third new state has now been identified on the pitchers of the insect-catching pitcher plant *Nepenthes alata*. Insects are attracted to sweet, scented nectar on the rim (peristome) of the pitcher. But it is a trap. The nectar is slippery and the insects aquaplane on it and slip into digesting fluid in the pitcher. Bauer & Federle [26] showed that the peristome bears *hydrophilic* microstructures, and is lubricated by the aqueous nectar. The nectar prevents the insects' feet getting a grip by resistance to dewetting, or resistance to draining between the foot and the peristome structures, or both. This system inspired Wong et al., [27] to propose microstructures with water-immiscible fluid trapped in the spaces between the microstructures. They are termed slippery liquid-infused porous surfaces (SLIPS). The authors then tested such SLIPS for omniphobic action. To be functionally useful, either in evolved organisms or in manufactured objects, the fluid would have to be resistant to erosion by the ambient shear field, or replenished by reservoirs. Unlike Cassie state surfaces (with their compressible and soluble gas), SLIPS are predicted to be omniphobic, and insensitive to increased hydrostatic pressure. They are now being intensively investigated industrially for commercial applications in marine antifouling [135]. SLIPS properties are potentially useful to aquatic organisms, particularly those covered by replenishable mucus, such as algae, mollusks, tunicates, hydrozoans, and these organisms should be investigated for SLIPS-mediated anifouling properties. Such surfaces might also produce surface laminar and/or turbulent drag reduction. Pelagic copepods seem unlikely candidates to bear SLIPS, as they secrete little or no mucus. As many of their prey do, however, the predatory copepod then has to deal with it. We further consider a hunting copepod below.

Possible SDR on Plankton Surfaces

Jenkinson & Sun [15] found that flow of cultures of harmful algae species and of a bacterium in non-turbulent flow in capillaries of different radii, and at different values of shear stress could be increased (drag reduction) or decreased (drag increase) relative to that of pure seawater or culture medium. Drag increase was probably caused by EPS-mediated increased viscosity now well known in seawater [11-14,28-32] as well as in cultures of both phytoplankton [10] and marine bacteria [33]. Jenkinson & Sun [15] suggested the drag reduction, however, may have been SDR associated irregular hydrophobic surfaces of the cultured organisms and/or on the EPS they were producing.

Stick Layers

Stick layers occur when there is a layer of non-deforming material adhering to the surface, even though such a layer may consist mainly of water. Some examples of stick layers are described hereafter.

Electrical Double Layers

EDLs are treated above. They are of thickness about 0.7 to 7 nm.

Layers of Adhering Materials Such as Polymers, or Sculpturing

In the sea most surfaces, both solid and gaseous, rapidly accumulate a skin of organic material, particularly surfactant matter. Such layers on the outside of copepods seem not to be frequent in pelagic copepods, although a limited layer of what appears to be a 1- to 2-μm layer of bacterial “plaque” was shown by scanning electron microscopy on copepod cuticles [34].

Layers of Epibionts Adhering to the Cuticle

Various epibionts, including diatoms, protozoa and bacteria inhabit the outer surfaces of copepods, details of which follow.

Diatoms

Studies have indicated that high infestations of epibiotic diatoms on copepods fail to change respiration rate or excretion rate of ammonia or phosphate relative to non-infested copepods, yet infested copepods were found to have higher mortality rates. Epibionts are considered to benefit from excretion of nutrients by their hosts, especially those on the uropods, near the anus, or near the mouthparts, where they may benefit from sloppy feeding. The host’s swimming would also bring nutrients past the epibiont, and thin the microlayer/boundary layer around it, allowing faster nutrient uptake. There is no record of hosts feeding on algae attached to their exoskeleton. Pelagic copepods appear to be infested more generally than benthic ones, although the reason is not known [34]. Epiphytic diatoms may not only affect swimming speed but may increase the host’s visibility to predators by increasing or changing their hydrodynamic signal [35].

Protozoa

Carman & Dobbs [34] review protozoan epibionts of the Crustacea, including ciliates of the sub-classes Hypostomata, Suctoria, Hymenostomata, Peritricha and Spirotricha, as well as foraminiferans, naked rhizopods, choanoflagellates and ellobiopsids. The number of epibiont protozoa can range from none (very common) up to 250 per host. The authors cite 13 references (not counting older reports, cited elsewhere) referring to different patterns of distribution on the body that are characteristic of different epibionts as well as of different host species, development stages and sometimes the sex of the host. Infestation intensity may also be a function of time of year. As well as the Suctoria and Peritricha, apostome ciliates (subclass Hypostomata) are widespread epibionts [35]. Ciliates range in the symbiotic relationship from exuviotrophic ectocommensals to parasites, see Carman & Dobbs [34] and references therein.

Bacteria (*sensu lato*)

Bacterial epibiont infestation of copepods can be divided (according to the classification of the authors) into that by *Vibrio* and that by other bacteria. Details follow.

Bacteria other than Vibrio. Non-*Vibrio* bacteria encountered on copepods include *Acinetobacter*, *Aeromonas*, *Alteromonas*, *Bacillus*, *Chromobacterium*, *Cytophaga*, Flavobacterium, *Leucothrix mucor*, *Pseudomonas* and other unidentified rods and cocci (classification following [34]). The incidence of copepods with bacteria is reported to be unrelated to the abundance of free-living bacteria, and may involve the physiological stage of the host copepod. Numbers of epibiotic bacteria are reported to vary from none to 10^5 or even 6 ネ 10^5 per copepod [34]. Epibiotic bacteria are generally unevenly distributed on copepods, favoring areas between segments, around mouthparts, near the anus, and on legs.

On pelagic copepods, filamentous bacteria are not found abundantly [34]. In *Labidocera* sp., a planktonic copepod, filamentous bacteria occur only at the bases of feeding appendages and legs, where they may contribute little to surface drag. Among benthic copepods, however, filamentous bacteria are relatively common. Indeed, fouling by the cyanobacterium *Leucothrix mucor* on the harpacticoid *Tigriopus brevicornis* has been shown to change swimming patterns [37].

Vibrio. Along with other *Vibrio* species, *V. cholerae* has been much studied because it causes diseases and the strain O1 (VCO1) causes cholera in humans [38-40]. Copepod cuticles are comprised essentially of chitin [39,40]. The VCO1 connection with chitin is an excellent example of a successful bacterium-substrate interaction, with a detailed influence on its lifestyle in the different environments it colonizes [38,40]. It has a variety of life forms, which can succeed each other. They show various forms, including (i) a free-living flagellated form and (ii) a multicellular form, that produces layers of bacteria or biofilms on surfaces. This multicellular form can colonize the chitinous cuticles of copepods, other crustacea, and chitin-containing phytoplankton and macrophytes in marine, brackish and fresh waters. Vibrios are attracted to and attach to both chitin and copepods. However, attachment of vibrios to copepods is less efficient than their attachment to chitin particles. Pruzzo et al., [39] suggest that this is because the copepod exoskeleton bears a wax epicuticle that prevents close contact with the chitin until bacterial enzymes (*e.g.* lipase) have digested it. Scars and pits on copepod exoskeletons occur in *Acartia* species [34,41], which result from the activity of chitinoclastic or wax-degrading epibiotic bacteria (possibly *Vibrio*). Based on observations, however, Carman & Dobbs [34] consider that chitinoclastic activity by epibiotic bacteria is rather exceptional.

In contrast to the lack of relationship between abundance of epiphytic (probably non-*Vibrio*) bacteria and that of free-living bacteria in the surrounding water, abundance of *Vibrio alginolyticus* on the rock-pool copepod *Tigriopus fulvus*, was positively correlated with the abundance of *V. alginolyticus* in the water [42].

Glycocalyxes

Glycocalyxes are considered here as they cover many of the prokaryotic and eukaryotic prey of copepods. Most cells, including bacteria [43], protists [44], and the cells of multicellular organisms [45] bear a glycocalyx (also known by terms including, “cell coat”, “extracellular matrix” and “spore mucilage”). It consists of a system of polymer fibers, largely of polysaccharides. They are generally arranged in an intertwining network. These membrane carbohydrates occur usually in combination with proteins or lipids as glycoproteins or glycolipids. Many other carbohydrate compounds, *e.g.* proteoglycans, are loosely attached to the outer surface of the cell as well. One can say that the entire outside surface of the cell often has a loose carbohydrate coat, the glycocalyx.

Glycocalyx molecules manage the recognition of and adhesion to surrounding cells and other structures [46]. Functions of the glycocalyx and its different components are multiple and probably still not well elucidated Many of the carbohydrates moieties of glycocalyx glycoproteins act as *receptor sites* for binding pheromones, and they can communicate such information into the cell as part of two-way cross-talk [47]. When bound to external antigens, this combination activates attached proteins inside the cell that, in turn, activate a cascade of intracellular enzymes. Some carbohydrate moieties enter into immune reactions, based on lock-and-key recognition mechanisms. Some harmful algae, moreover, use their glycocalyx to effect short-range or contact allelopathic action, to harm other members of the plankton community [48], including multicellular potential predators [49], such as copepods. While the glycocalyx serves the protist to detect potentially useful and harmful molecules outside the cell [50], it may also serve potential predators, including Crustacea such as copepods, to better identify their prey. In both prokaryotes and eukaryotes, the glycocalyx also mediates adhesion between their cells in colonies, as well as the initial adhesion of epibionts to their substrates and hosts. Typically, the thickness of glycocalyxes can range from 0.1 µm or less to several µm [2].

Vicinal Water (VW)

Drost-Hansen [23] reviews considerable published evidence for layers of changed properties of water close to surfaces. The causes of "vicinal water" (VW) are often poorly understood, and, Drost-Hansen's ideas notwithstanding could be several. Manifestations of VW seem to vary, sometimes without obvious cause, leading some to doubt their existence. The existence of VW is thus rarely mentioned in physics or engineering textbooks. In the present chapter we are interested in what happens around copepods, not the physico-chemical (molecular) reasons for it. Some discussion of mechanisms is unavoidable, however, in order to consider the evidence.

Viscosity of VW

A succinct summary of properties that have been reported for water close to surfaces is shown in Table 3. In particular, the viscosity has been reported to be increased by a factor of 2 to 10. No characteristic deformation rates or stresses are given for the measurements of such viscosity. A relevant length scale, however, may be the layer thickness. Published values of long-range ordering effects extend from 0.05 to 5 µm from the surface (Table 4) while Grasso et al., [22] lists publications reporting H-bonding effects from a few hundreds of nm.

Evidence that this VW viscosity is fundamentally different from that of the bulk phase is provided by measurements of viscosity of water squeezed between flat horizontal plates 30 to 90 nm apart. Peschel & Adlfinger [51] found that the viscosity not only showed a strong negative relationship with inter-plate separation, but also goes through a number of maxima and minima, corresponding with temperatures 15, 30, 45 and 60ºC (the so-called "Drost-Hansen temperatures"). Other workers cited by Drost-Hansen [23] have found similar increases in viscosity, as well as in other properties (Table 3), in small capillaries at the same temperatures. These anomalies do not occur far from surfaces.

Thermal anomalies measured by differential scanning calorimetry, are reported close to 60°C, a "Drost-Hansen temperature" [23] (see last paragraph) in highly dilute (~0.01%) aqueous solutions of the quaternary amine, cetyl-trimethyl ammonium salicylate. Such solutions form large worm-like micelles, and the author suggests that these results indicate that the micelles are vicinally hydrated, which may explain their unusual rheological properties.

Table 3. Summary of some of the properties of vicinal water. From Drost-Hansen [23]

Property	Bulk water	Vicinal water
Density (kg m^{-3})	$1.00 \text{ x } 10^3$	$0.96\text{-}0.97 \text{ x } 10^3$
Specific heat (cal kg^{-1})	$1.00 \text{ x } 10^3$	$1.25 \text{ x } 10^3$
Thermal expansion coefficient (°C^{-1})	250 ㅊ 10^{-6}	300-700 ㅊ 10^{-6}
Adiabatic compressibility (Atm^{-1})	7 ㅊ 10^{-17}	35 ㅊ 10^{-17}
Heat conductivity (cal s^{-1} °C^{-1} m^{-1})	1.4 ㅊ 10^{-3}	10 ㅊ 10^{-3} to 50 ㅊ 10^{-3}
Viscosity (mPa s)	0.89	2-10
Activation energy, ionic conduction (kcal $mole^{-1}$)	4	5-8
Dielectric relaxation frequency (Hz)	19 ㅊ 10^9	2 ㅊ 10^9

For references, see Drost-Hansen [23].

Table 4. Experimentally determined values for “long range ordering effects” within interfacial water layers at the boundary of solid surface and water. From Drost-Hansen (2006) [23], in which please find the references

Reference	Method	Solid boundary	Penetration depth (µm)
Etzler and Lilies (1986)	Dielectric constant	Sheets of mica	2-5
Drost-Hansen (1976)	Adhesion at glass	Glass	1.5
Henniker (1949)	Disjoining pressure	Mica or steel plates	< 1
Mastro and Hurley (1985)	Surface conductivity	Glass tube	0.3-0.4
Peschel and Adlfinger (1969, 1976)	Conductivity	Quartz particles	0.2-0.3
Falk and Kell (1966)	Viscosity	Glass plates	0.25
Montejano et al (1983)	Conductivity	Pyrex glass	0.05-0.2
Drost-Hansen (1969)	Viscosity	Pyrex glass	*ca.* 0.2
Steveninck et al (1991)	Viscosity	Convex quartz plates	0.16
Braun and Drost-Hansen (1981)	Rigidity modulus	Convex glass plates	0.15
Bailey and Koleske (1976)	Disjoining pressure	Convex fused-silica plates	*ca.* 0.1
Nir and Stein (1971)	Disjoining pressure	Quartz plates	0.1
Derjaguin (1933)	Rigidity modulus	Glass	0.1
Antonsen and Hoffman (1992)	Viscosity	Glass plates	*ca.* 0.1
Clifford (1975)	Air-bubble flow	Glass tubes	*ca.* 0.1

Assuming that VW really does form close to surfaces, what about the surfaces of other suspended particles and dissolved macromolecules? Reviewing considerable evidence, Drost-Hansen [23] concludes that there is a critical size for macromolecules below which no effects of VW are detected, and above which they are. This size corresponds to molecular mass of 1 to 5 kDa. The increased viscosity suggests that other rheological properties might also be changed in such solutions or suspensions. Furthermore VW-type properties are destroyed by fluid shearing, and conditions without shearing for a critical time t_c are required for them to re-establish. Values of t_c are found to range from about 1 s to about 1 day.

Other Properties of VW

Similar manifestations of VW are often reported from the vicinity of quite different types of surface, for example those of polystyrene and silica. Furthermore, the density of VW is reported up to 3 to 4% less than that of bulk water, and the specific heat up to 25% higher. (Table 3). Drost-Hansen [23] considers that VW can extend from surfaces, out to a "*penetration distance*" of *ca.* 0.1 to 5 µm (Table 4), and that they are unaffected by ionic strength, pH, or any surface-specific epitaxial ordering.

VW effects may also be particularly important in intracellular processes, but that is outside the scope of this chapter.

Objections to the Theory of VW

Yaminsky et al., [52] carefully showed that at surfaces of glass or of fused silica, in contact with water, the silica can interact with the water to form a gel. This gel formation is variable and difficult to repeat, and can show complex dependence on time, temperature and other properties. This may lead to variable physical properties being measured in thin water layers that are ascribed to fundamental properties of the water, but are in reality due to the presence of silica gel. Many of the experiments cited to justify the existence of VW were conducted in glass or fused-silica apparatus. So findings used to oppose the now highly unfashionable "polywater" idea [53], appear to weaken the concept of VW as well. More recently, however, new structuring phenomena at surfaces have been proposed [22], and much work is required to deepen understanding of the phenomena that may be responsible for different manifestations of VW.

THALASSORHEOLOGY AND EXOPOLYMERIC SUBSTANCES IN COPEPODS' ENVIRONMENTS

Thalassorheology

The Viscosity and Elasticity of Seawater and Other Natural Waters

Thalassorheology, the rheology of seawater and other natural waters, has recently been reviewed [2], so only a brief outline is given here in relation to copepods. In natural water, the liquid phase consists of water and, particularly in seawater, its dissolved small molecules, mainly salts more or less dissociated into cations and anions. The viscosity of this phase is controlled by temperature and salinity. Its elastic modulus G' is zero, which means that, apart from effects due to gravity waves in surface and subsurface density discontinuities, it has no memory of past conformation and thus no tendency to spring back after deformation.

While it has previously been written [2, and references therein] that the viscosity of the aquatic phase is independent of length-scale, this is strictly true only down to a length scale often considered to be close to the Debye length (Eq. 3), roughly the size of water shells (*ca*. 0.2 – 0.3 nm) [54] (Figures 2 and 3). Increase in viscosity of pure water at larger scales due to VW effects at particle surfaces (nm- to µm-scale, Table 4) may also occur.

Thalassorheology, Lumpiness and EPS

The intra-sample variability found in the rheological properties of seawater led me to suggest centimeter-scale heterogeneity [11]. Excess viscosity was similarly found to be variable at 5-10-cm scale in the sea [13]. This rheological variability has been termed "lumpiness" [2,11,55]. It may be due to physical, chemical and biological mechanisms of aggregation by EPS, as well as direct formation of EPS structures by the producer organisms. An extensive and advanced literature on aggregation by sludge EPS [56] is available to inspire further research on marine and freshwater EPS.

The following types of EPS suspended or colloidal in seawater can all be sticky: marine snow [57], transparent exopolymeric particles (TEP) [58,59], as well as phytoplankton [60-65] and bacterioplankton [66]. Partly through inter-particle bridging by fibers and fibrils of EPS or other matter, much of this stickiness is transferred to length scales larger than the aggregates themselves, and therefore imparts viscoelastic properties to the medium at length scales exceeding aggregate size.

Attraction to and Avoidance of EPS and Its Effects on Swimming

Savage [67] described patches in the North Sea of *Phaeocystis* sp., a sometimes-colonial haptophyte that sticks to fishermen's and plankton nets. The fishermen knew that these patches are avoided by herring and are thus associated with low catches. Later, Seuront and Vincent [32] showed that *Phaeocystis globosa* blooms increase seawater viscosity up to 4-fold, and give it centimeter-scale heterogeneity, even when the colonies had been strained out. These authors showed furthermore that the swimming trajectories of the copepod *Temora longicornis* were more convoluted in *P. globosa* bloom water than in clear water, suggesting that the copepods were avoiding zones of high EPS viscosity. The *Phaeocystis* EPS was constraining copepods' swimming trajectories *in situ*. On the other hand Bochdansky & Bollens [68] found that *Acartia hudsonica* slightly favored patches of the diatom *Skeletonema* sp., but this is nutritious food for them.

Zooplankton sometimes associate with phytoplankton patches and thin layers (TLs), but sometimes avoid them. In a study involving a mucus-rich TL of marine snow, Alldredge et al., [69] found that most zooplankton avoided it. The authors suggested that the abundant TEP within the aggregates may have clogged delicate feeding appendages. In particular, the diatom *Odontella longicruris* occurred abundantly in the aggregates, and appeared to be especially avoided by herbivores, which preferred *Thalassiosira* sp. McManus et al., [70], in contrast, found that zooplankton could form TLs associated with phytoplankton TLs, or else their own TLs, separate from the phytoplankton TLs. In particular, zooplankton preferentially avoided TLs rich in toxic diatoms *Pseudo-nitzschia* spp. It would be expected that, as a general rule, zooplankton including copepods would like to feed in phytoplankton TLs, where the concentration of food is higher than in most parts of the water column. Little is still known, however, about the effects on zooplankton in TLs of toxic and non-toxic algae and their specific rheological properties [71,72].

Various Meanings of “Sticky Water”

The different meanings of “sticky water” found in the literature relevant to plankton are briefly reviewed as follows.

1. DVLO-type EDL of water in contact with a solid surface (Figure 3); with thickness the Debye length λ = O(0.1 to 1 nm) at salinity 10 to 0.1 respectively.
2. The “sticky water” of Strickler [73]. This is DVLO-type boundary-layer water where the streamlines hug a rigid body moving in a stationary fluid, such as that of a sinking copepod [74], or a stationary body in a uniformly moving fluid. This meaning of “sticky” is a synonym for what engineers confusingly call the “viscous regime”, and it is in opposition to turbulent water or the engineers’ “inviscid” regime.
3. Non-DVLO-type layers of vicinal water (VW), with ordering up to ca. 5 µm from a solid surface (Table 4). Such VW may be due to several phenomena, difficult to study because of intermittence or time-dependence [22,23].
4. The “sticky water” referred to by Kiørboe [75]. Such “sticky water” is due to EPS, including mucus, made by organisms such as *Phaeocystis*, certain dinoflagellates and diatoms as well as algal spores and many macroalgae [76]. This EPS structures the water [77], making it more viscous and

sticky, and at least in some cases also lumpy. It appears also to have made swimming trajectories by *Temora longicornis* more compact [32].

5. Water trapped in eddies for example between structures in coral reefs has been described as "sticky" [78,79]. This is an example of a more general phenomenon of water trapped in eddies, for example along the bank of a river, or along an irregular coastline bordered by an alongshore current. This "stickiness" is a function of eddying.
6. Finally we should not forget Maxwell's definition of viscosity [80] (in a Newtonian fluid) as "internal friction", where friction itself is a form or sticking that resists movement between two parallel surfaces in contact with the fluid. So in a sense all fluids are "sticky" internally in that they resist deformation, with a stress, the viscous modulus, *G"* [Pa] proportional to the shear rate [s^{-1}]. Elastic materials, on the other hand are internally "sticky" in that they resist deformation with a stress, the elastic modulus, *G'* [Pa] proportional to the absolute amount of shear [$m\ m^{-1}$ or dimensionless] the material is showing relative to its shape in the absence of external force. This elasticity is a memory of past shape. Ideal liquids have no memory, therefore no elasticity. Kiørboe's [75] "sticky water" (item 4 above) is viscoelastic seawater that will show both increased *G"* and finite *G'*.

Effects of EPS on Feeding by Copepods and *Daphnia*

Prieto et al., [81] investigated copepods feeding on the diatom *Thalassiosira weissfloggii* and its associated abundant TEP. The copepods ingested the diatoms but not the TEP. However, their presence affected the TEP so that it formed larger particles that sank faster.

Malej & Harris [82] found that the presence of TEP reduced feeding on diatoms by copepods, and they suggested that the TEP mechanically interferes with the handling of food and its ingestion. Later, however, Ling & Alldredge [83] suggested that this finding might have been because the copepods were feeding on TEP (with its associated bacteria and algae) instead of on the diatoms. It might be that both effects are at work, and that the dominant one in any particular circumstance might depend on the real or "perceived" (by the copepod) nutritional and toxicological status of the diatoms present [84].

In further support of feeding hindered by mucus and filaments, Young et al., [62] compared *Daphnia* feeding on algae considered "edible" with that on "inedible" algae. The "edible" algae were *Scenedesmus*, while the "inedible" ones were the filamentous *Ulothrix* sp. and *Phormidium tenue* as well as algae that aggregate into lumps, including *Achnathes* and *Palmella*. *Daphnia* did eat the so-called “inedible” algae, but more slowly than the “edible” ones. It was observed microscopically that the physical structure of mucus and filaments impeded food manipulation. Moreover, when given a mixture of “edible” and “inedible” algae, the *Daphnia* ate even the ”edible” algae more slowly than in the absence of the “inedible” ones. They suggested that the presence of the mucus and filaments of the “inedible” algae hindered feeding even on the “edible” algae.

While EPS and mucus thus often hinder copepod feeding, copepods sometimes actually feed on it beneficially. Richman et al., [85] found that *Acartia negligens* fed actively on mucus produced by several species of coral, and assimilated around 50% of its organic matter. Coral mucus, however, may be more attractive than that produced by plankton because its C:N ratio is lower, while it also contains energy-rich wax-ester products of coral metabolism. Feeding is specifically considered in the next section. Effects of EPS on hunting are considered below in the section, “Ambush feeding”.

THE COPEPOD SURFACE IN RELATION TO FEEDING AND SWIMMING

Feeding

In zooplankton, “Smaller predators hunt individual cells, whereas larger ones use feeding currents, mucous nets or elaborate filters to collect them en masse” [86]. Copepods fall between the smaller and the larger categories. Pelagic copepods are among the quickest feeders in the plankton, able to clear around 10^6 of their body volume per day [75]. Like most other crustaceans their bodies, including the appendages and mouthparts they use for feeding are made of chitin, with calcareous inclusions, covered by an exocuticle, the composition of which is discussed below.

Much remarkable, painstaking observation has been made of feeding-appendage morphology and filter-feeding between setules at small length (ca. 5-10 µm) and time (ca. 10 ms) scales, e.g. [73,87-93]. For an example, see Figure 6. From these and similar observations, flow around the appendages and setules was considered laminar, with Reynolds number estimated at 10^{-2} to 10^{-1} [94]. Descriptions and physical modeling of suspension feeding have always been based on implicit assumptions of DLVO-theory with no slipping and no sticking at walls. Jørgensen [92] concluded that such filtering between setules is physically impossible because the cells would be prevented from touching the setules by their surrounding non-turbulent boundary layer. This may have influenced subsequent workers to conclude that the observations of filtering must have been wrong. Recent ideas, however, are coming back to the notion that these observations cannot have been mostly wrong, and that filtering between setules may be important after all [75]. After all, seeing is believing. These observations need to be reassessed theoretically in the light of Lotus-leaf theory and other non-DLVO models of surface slipping and sticking. If the setules are superhydrophobic and self-cleaning, water will pass between them more readily, and even sticky cells will have less tendency to stick to them. A “stickiness arms race” and a “fouling-antifouling arms race” may thus be parts of Smetacek’s [86] “watery arms race”.

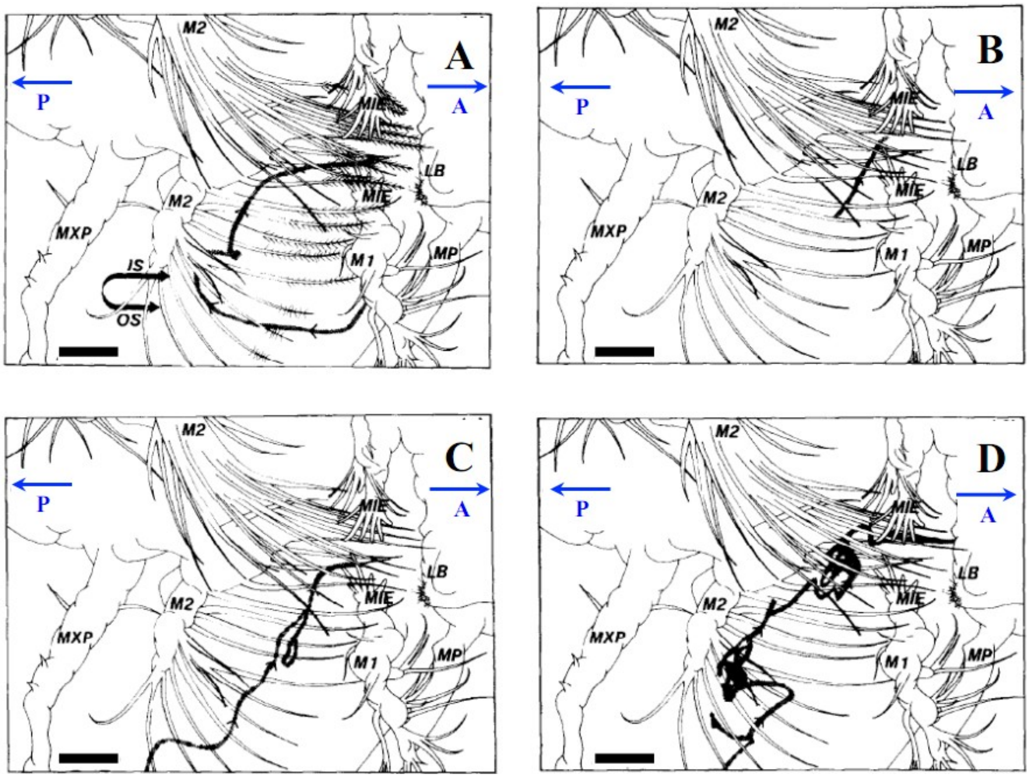

Figure 6. Representative digitized cell paths across the 2nd maxillae during passive capture of *Thalassiosira weissfloggii* cells by *Eucalanus elongatus*. The low amplitude motions of the M2 were omitted for clarity, and the position of the appendage was fixed at midcycle. (A) Cell approaches from outer side of M2, passes between setae at the proximal-distal joint, then is swept across inner surface of the M2 toward the setal tips and MlE without sticking to the setae. (B) Cell path similar to view (A), but cell passes between M2 setae near MIE. (C) Cell approaches M2 from the inner side through the space between the left and right M2. No passage between setae. d. Cell approaches from outer side of M2 and passes between setae of the distal segment, but sticks to the appendage briefly at several locations as it is swept across toward the MlE. A: anterior; P: posterior; LB: labrum; MP: mandibular palp; Ml: first maxilla; M1E: first maxilla endite; M2: second maxilla; MXP: maxilliped; OS: outer surface; IS: inner surface. View is focused on the inner surface of the left M2, which is held at an angle ventro-lateral to the body wall. Not all of the setules are in focus in view A, and all setules were omitted

in views B, C, and D for clarity. The scale bar is 200 µm. Modified from Price & Paffenhöfer [91]. Reproduced with kind permission.

The set of physical parameters associated with solid-liquid boundaries is much richer than just DLVO and Newtonian-water ideas, useful though this simplified version of Physics was for 20th-century product-innovation and engineering. Unfortunately many biologists believed such straightjacketing ideas too literally. From now on, models of small-scale, low-force interactions in aquatic biological systems should be continually reassessed as knowledge of physics progresses, driven by the current revolution in industrial micro- and nanofluidics.

Previously, feeding in plankton copepods was generally classified into: (i) filter feeding and (ii) raptorial feeding [95]. Based on high-resolution (ca. 1 µm) and high frequency (ca. 500 Hz) filming [89], it was concluded, perhaps under the influence of Jørgensen [92], that they were not “filter feeders” in the sense of using the setae on their feeding appendages to filter phytoplankton passively out of their feeding currents. More recently it has been considered they actively use sensory inputs to detect, capture and ingest their prey [91,96]. Kiørboe [75] has reviewed the problem still more recently, and has classified feeding into (i) feeding-current feeding and (ii) ambush feeding. As mentioned above, he tentatively reinstates some filter-feeding as part of the first category, an arrangement I follow below.

Filter Feeding and Raptorial Feeding

The categories, filter feeding and raptorial feeding, are modes of handling the prey. In a short review, Boyd [88] considered that few calanoid copepods can filter particles smaller than 5 µm or larger than 100 µm, and that most grazers filter particles of a much narrower size range. It follows that larger particles are handled individually, and may be broken, pierced or crushed by raptorial feeding. All food particles >100 µm are subject to raptorial feeding [75,88].

Feeding-Current Feeding

Copepods detect some prey in feeding currents [73]. At least part of this detection is done by sensing a cloud, or microzone, of chemicals leaking from the cells. This cloud is elongated into an ellipsoid by shearing and acceleration of the feeding current [75,97], so that part of the cloud arrives at the copepod’s sensors before they prey itself. This prompts the copepod to redirect the feeding current and capture the particle. Presumably the amount and type of the “scent” will help the copepod to select or reject as appropriate. From experimental results, Frost [98] concluded that, below saturating food concentrations, *Calanus pacificus* feeds on both large (*ca*. 80 µm) and small (*ca*. 4 µm) cells, but handles and eats larger cells with greater effectiveness than it handles and eats small cells.

Moreover, other things being equal, larger prey should secrete more chemicals, allowing detection at a greater distance. The clearance rate on “large” prey is indeed larger than on “small” prey, which has been deduced from observed size spectra successfully preyed upon by copepods, relative to the size spectra present in the water [75,88,90].

Furthermore, Hong et al., [99] found that *Acartia tonsa* fed on *Karenia breve* (18-45 µm in length and width) and *Karlodinium veneficum* (< 20 µm long; sizes from [100]) by feeding-current feeding (*i.e.* “*the copepod generates a feeding current to attract and capture prey*”) and not by raptorial feeding.

Ambush Feeding

General Considerations

Kiørboe [75] points out that ambush feeding is mandatory for pelagic cyclopoid copepods, and is also found amongst some calanoids, including *Acartia.* Ambush-feeding copepods wait motionless, then attack prey that move through their perceptive sphere. The jump attack is accomplished in only 2-10 ms. The rapidity of the attack reduces the thickness of boundary layers around the copepod, while enabling it to penetrate the prey’s boundary layers more. If the attack were slower, the thicker boundary layers would push the prey away. Approach of the predator to the prey will tend to subject the boundary layer to squeezing deformation.Ambush feeding with such active attack jumps is restricted to powerful, large (> 250 µm) plankton, with a streamlined body shape, including chaetognaths and some copepods. This streamlining together with the pointed front end with projecting grasping processes will further help the predator to penetrate the boundary layer [75].

EPS As a Defense Against Ambush Feeding

Approach of the predator to the prey will subject the boundary layer to squeezing deformation. The relevant viscosity for squeezing is the 2D squeezing/elongational (SE) viscosity, which is larger than the shearing viscosity by the Trouton ratio, *T*. In Newtonian liquids *T* is always 3, but in different polymer solutions *T* can be as high as 10^3 [102]. Incorporation of loosely bound EPS (LB-EPS) in the boundary layer around mucous phytoplankton (thickness around 10-100 µm) may thus be a powerful protection against ambush feeding. Modeling combined with rheological characterization of such LB-EPS is required. Furthermore, LB-EPS may be difficult for copepods to feed on because their appendages, setae and setules will easily pass through it. Sufficiently strong mucus, however, such as tightly bound EPS (TB-EPS) or some TEP and organic aggregates, would be graspable or filterable.

Thus even small amounts of polymeric thickening in the water, whether diffuse or associated with the prey surface, might make ambush feeding much more difficult, depending on the rheological properties of the polymer, including *T*, at the strain rates and scales involved in the deformation. In principle, this effect could be tested experimentally. However, pointed appendages that could penetrate the boundary layer and grasp the prey would be an additional advantage to the predator.

SDR As a Possible Aid to Ambush Feeding

Asmolov et al., [9] have modeled the hydrodynamic forces (in Newtonian liquid) impeding the approach of a sphere with a hydrophilic surface to a flat superhydrophobic surface. The authors found reduction in the forces impeding approach (SDR). Approach would thus be facilitated. The amount of the reduction is sensitive to details in the parameters, particularly the different length scales involved in SDR. Asmolov et al., [9] point out that for real situations, atomic force microscopy could be used to measure the forces developed. Such forces therefore need to be modeled and measured in future investigations of the nano- and microfluidics of copepod feeding, along with millisecond and micrometer-scale videomicroscopy from the scale of whole-copepod ambush feeding to that of a setule approaching a picoplankton cell [103]. Such studies would provide insight into whether such a type of predation would provide evolutionary pressure for copepods to develop a superhydrophobic surface. Approach may be hindered, however, and some length scales greatly increased by the rheological effects of any tightly or loosely-bound EPS around the prey.

Rheological and Microfluidics Considerations on Feeding

Because of the boundary-layer constraints in ambush feeding, it is not surprising that ambush-feeding copepods are notably clean of mucus, since any mucus would tend to thicken boundary layers, either by increasing squeezing/elongational viscosity or by coating surfaces, or both. Similarly, copepods that use filter feeding (in many cases the same ones) should avoid producing mucus as loosely-bound mucus would tend to increase viscosity generally, and tightly-bound mucus might stick to the setae and setules of the filtering appendages, in both cases increasing boundary-layer thickness. Copepods finding themselves in rheologically thickened water may then feed as best they can by converting from filter-feeding to “fling-and-clap” feeding [73]. Benthic copepods, particularly harpacticoids (see below), are likely not affected by these constraints, and thus have less need to keep clean of mucus. The importance of mucus and EPS in feeding is also considered above in the “Ambush feeding” section.

Copepod Feeding Appendages

For eight species of *Calanus* and *Neocalanus*, Yamaguchi et al., [93] review the inter-seta spacing on the feeding appendage, the second maxilla (Mx 2), as they consider that the inter-seta spacing is indicative of the size of particles that can be removed from the water. Spacing ranges from *ca*. 3 µm in *Neocalanus plumchrus* naupliar stage-1 to -3 to *ca*. 10 µm in *Calanus hyperboreus* stage 6 females (pre-adults) (Figure 7).

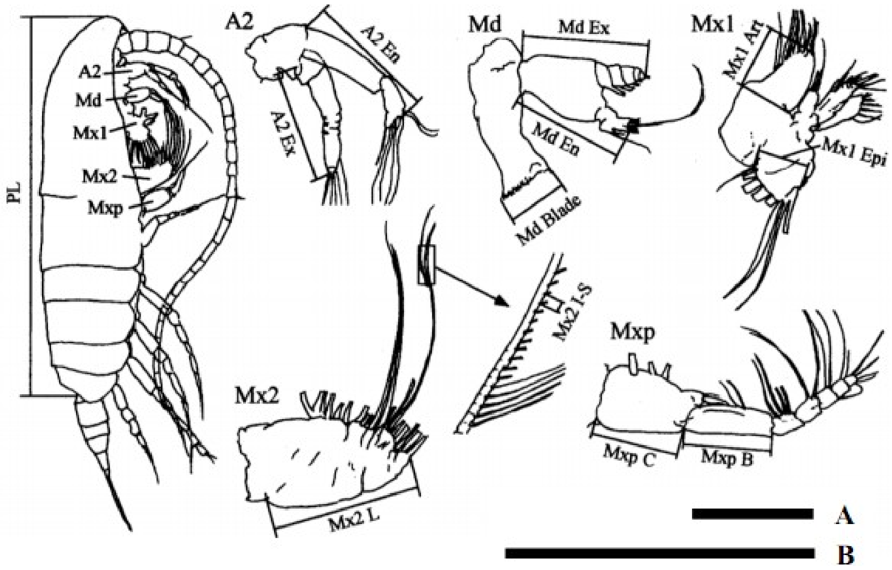

Figure 7. Diagrams showing the prosome length (PL) and 11 parts of feeding appendages measured for interspecific comparisons (abbreviations shown in the parentheses): A2 endopods (A2 En), A2 exopods (A2 Ex), Md cutting blade (Md Blade), Md endopods (Md En), Md exopods (Md Ex), Mx1 arthrite (Mx1 Art), Mx1 epipodite (Mx1 Epi), length of Mx2 (Mx2 L), inter-seta spacing of near the tip of strong spine on Mx2 basis (Mx2 1-S), Mxp coxa (Mxp C), and Mxp basis (Mxp B). Scale bars are both 1 mm, A for whole body (*Neocalanus plumchrus* C5) and B for feeding appendages (except for strong spine on Mx2 basis). Modified from Yamaguchi *et al*. [93]. Reproduced with kind permission.

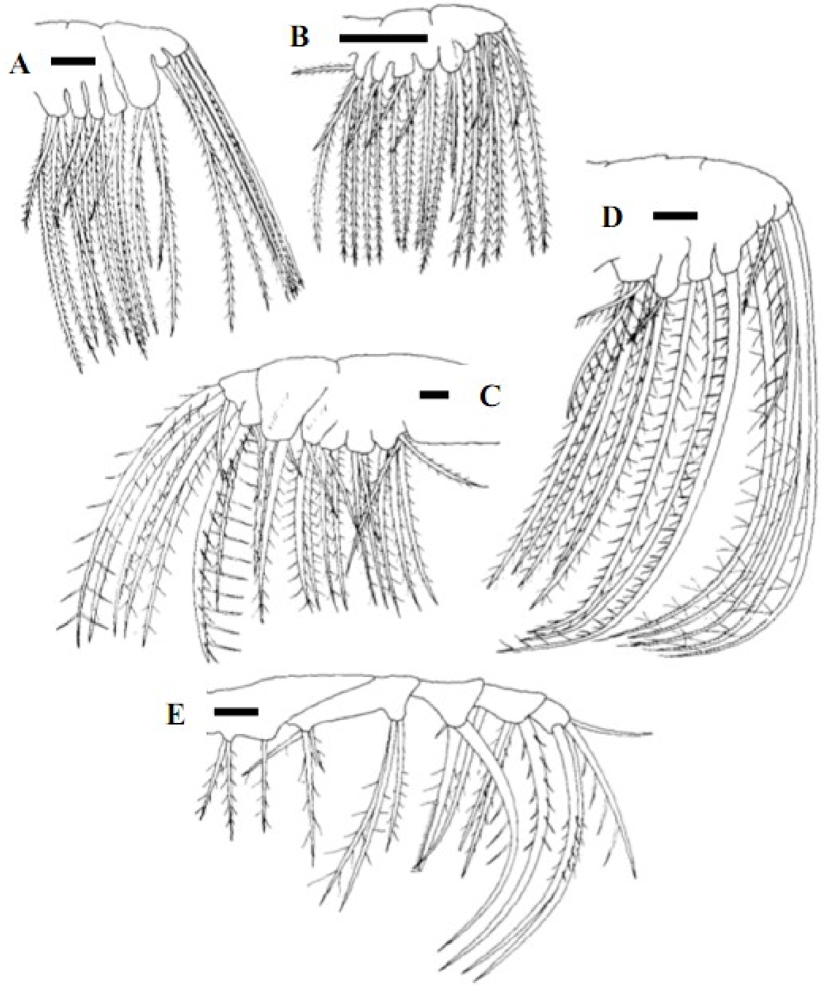

Figure 8. Setulation of maxillae. A, *Pseudocalanus*; B, *Temora*; C, *Centropages*; D, *Acartia*; E, *Oithona*. Modified from Marshall & Orr [87]. Copyright (1986) by Association for the Sciences of Limnology and Oceanography, Inc. Reproduced with kind permission.

Marshall & Orr [86], however, show that each seta bears two rows of setules (Figure 8), and these authors consider that the inter-*setule* distance determines the size of food taken. For smaller copepods of the genera *Pseudocalanus*, *Temora*, *Centropages*, *Acartia* and *Oithona*, Marshall & Orr [87] found this distance to range mostly from 2 to 6 µm, in some cases up to 17 µm. In many copepods, the setae of the maxilliped are longer, thicker, and often pointed, and may be used almost exclusively for grasping. Anraku and Omori [104] also give very useful drawings of marine plankton copepods (not shown here).

The Crustacean Epicuticle

Most free-living crustaceans are covered by a cuticle, which is hard, except just after moulting. The external layer in contact with the water is the epicuticle, and we shall consider it because its surface properties may change the way the copepod interacts with ambient water and other materials. The cuticle of large decapods (crabs) has been extensively studied, but that of copepods much less so. In crabs and copepods, the mass of the cuticle consists of chitin. This is partly calcified in the crabs studied, but apparently not in copepods. The outer layer, or epicuticle, may be rich in hydrophobic wax in plankton copepods. The evidence for this is considered hereafter and discussed in comparison to decapod epicuticle.

The Decapod Epicuticle

The interface between the copepod and its milieu is its carapace, which covers the whole body including setae and setules, although it is thin and flexible at the limb joints. It is thickest where it covers the cephalothorax dorsally, and this part has been the most studied [105]. Much work has been done on larger crustaceans. Beneath the cuticle (or carapace) in the shore crab *Carcinus maenas* is an epidermis. This epidermis is overlain by a cuticular "principal layer", itself overlain by a "pigmented layer", with a final thin wrapping, the epicuticle [106,107]. The organic matrix of the principal and pigmented layers of the cuticle is composed mainly of units of chitin-protein fibers. In crabs, the units are spatially organized according to a twisted-plywood model, characterized by curved "arcs", that may resist predators [108] by providing toughness and protection against fracture propagation. These twisted structures are clearly multiscalar [106-108]. Interfibrillar lacunae start to become calcified with hours of ecdysis. Penetrating the cuticle from the underlying epidermis to the outside are "pore canals", apparently containing cellular material, that likely allow communication to the outside. The edible crab *Cancer pagurus* presents similar structures, in which Fabritius et al., [108] describe the chitin structure and development as "*Molecules of the sugar N-acetylglucosamine polymerize to form anti-parallel chains of a-chitin. Crystallized chitin chains coated with a protein matrix form about 5-7 nm thick nanofibrils. The nanofibrils further assemble to chitin-protein fibers with diameters of 50–250 nm. These fibers arrange with parallel long axes to form horizontal planes. In stacks of these planes, the fiber orientation changes helically and generates a twisted plywood structure, similar to that in* Carcinas maenas*, that forms the three main layers of the cuticle*." In Fabritius's [108] terminology, from proximally to distally, overlying the epidermis is the membranous layer, overlain successively by the endocuticle, the exocuticle and finally the external, thin epicuticle.

In *C. pagurus* [108], the thin epicuticle that covers the whole integument contains no helicoidally arranged fibers and consists of a compact layer and a subjacent layer with numerous pore canals as described earlier. Raman spectroscopy has shown [108] that the compact epicuticle consists mainly of waxes containing C—H stretching bands that appear in hydrocarbons, but little or no proteins or chitin. The pore canals crossing the inner layer, and considered by Girard-Guille [107] to contain cellular material, were found by Fabritius [108] to contain mineral particles that are not calcite. Ultrastructural investigation of the soft membranous layer, which separates the proximal surface of the cuticle from the underlying epithelium, shows regularly layered fibrous planes that consist of chitin and proteins.

Becker et al., [109] measured the surface properties of carapaces from 42 decapod and 3 squillid species. Properties varied from extremely hydrophilic (wetting angle 0º) to moderately hydrophobic (91º). Colonization by fouling organisms was also investigated, but no relationship between fouling and wetting angle was discovered. It would be interesting to make a similar investigation for different copepod species.

The Copepod Epicuticle, Compared with Decapods

Copepod Epicuticle Structure

Like the edible and shore crabs, copepods have a chitinous exoskeleton covered by an epicuticle thought by Pruzzo et al., [39] to be of wax This epicuticle probably protects the cuticle from attack by chitinolytic bacteria, which are ubiquitous in the ocean. Chitinolytic bacteria of the genera *Enterococcus* and *Vibrio*, including *E. faecalis* and *V. cholerae* of human origin, have been found largely associated with copepods in both fresh- and seawater [39]. These same *E. faecalis* continued to be active and culturable much longer when grown with chitin particles, (*ca.* 30 days), than with live freshwater copepods (9-15 days) [39], suggesting that the epicuticle does indeed protect the chitin exoskeleton of copepods from bacterial attack.

In copepods, Raymont et al., [105] showed sections of the cuticle (“integument”) of *Calanus finmarchicus* by transmission electron microscopy (TEM), combined with staining. From this they concluded that the outer zone consists of about six 1-µm layers, and contains basic protein, some mucoprotein, mucopolysaccharides and lipoproteins. The inner zone is also layered and is of similar composition except for a paucity of mucoproteins. It is underlain by an epithelium.

Parts of the cuticle, in particular that part covering appendages, bear setae covered with a similar outer and inner cuticle, underlain not by an epithelium but by, what may be homologous, a cell-containing lumen. Setae in turn bear setules composed of cuticle, but setules have no lumen [105].

Copepod Epicuticle Composition

Because the epicuticle is the copepod's interface with its environment, its surface properties are vitally important to its life style and survival. Organisms as diverse as flowering plants, insects and humans bear waxy epicuticles [110]. Many copepods in salt lakes [111] and in the sea [112,113] produce large amounts of wax for energy storage and buoyancy regulation. Like the cuticle of decapods, that of copepods and their setae consists of chitin [105]. Chitin is a highly hydrophobic polysaccharide but it is easily transformed into a variety of soft, hydrophilic derivatives. In crustacean carapaces it generally occurs as nanofibres encased in protein sheaths [108]. But is the thin epicuticle surface hydrophilic or hydrophobic, and does it really contain any wax as Pruzzo et al., [39] believe? The surface properties must be important not only in harboring or excluding pathogenic bacteria, but also in determining feeding and encounter dynamics. I have been unable to find any clear information as to whether copepod epicuticle is hydrophobic or hydrophilic, or if it is waxy. In natural waters, however, surfaces generally accumulate a coating of hydrophobic or amphiphilic algal or bacterial exopolymers [114,115], which in both cases would generally be hydrophobic to the outside.

Copepod Epicuticle Function

Boxhall [116] remarks that most planktonic copepods have a generally smooth body surface externally, but that some, such as *Centraugaptilus horridus*, are covered with spinules, and reticulate ornamentation occurs on the planktonic harpacticoid *Aegisthus aculeatus*. Spinules and other microsculpturing are borne also on the integument of some planktonic harpacticoid misophrioid copepods, particularly *Benthomisophria palliata*, but not in others. A system of grooves, seen by SEM, decorates the uropods of the freshwater cyclopoid *Acanthocyclops venustus*. The functional significance of these markings is poorly understood, but that the adaptive advantages are presumed [116] to outweigh any disadvantage of increasing hydrodynamic drag in the free-swimming forms. Nano- and microsculpturing on hydrophobic surfaces are now known, however, to sometimes *reduce* drag by superhydrophobic drag reduction (SDR), at both low and high Reynolds-number flow [8]. If hydrophobic, as well as producing SDR, this nano- and microsculpturing may further have an anti-stick or self cleaning role, like that on the petals and leaves of some flowering plants [4,6,8], and thus help to keep the copepod surface clean and free of fouling organisms. Superhydrophobic and sculptured amiphilic surfaces are now being developed industrially to reduce sticking by organisms fouling marine structures [27].

Similar techniques should be used to investigate whether copepods and other plankton use similar mechanisms to reduce biofouling.

Swimming

Some effects of EPS on copepod swimming aspects of EPS on hunting (ambush feeding) have been considered above. Detailed models of copepod swimming and hovering in relation to energy consumption, feeding currents and chemoreception have been made [117-120]. The effect of excess copepod density has also been modeled on feeding and swimming fields as well as suspension feeding currents [121]. The detection of hydrodynamic signals by copepods has also been modeled [122]. The hydrodynamic modeling was done by Computational Fluid Dynamics (CFD) using FLUENT software. This work represents powerful advances to understand the phenomenology and energetics of pelagic copepod life. Yet at that time this work was done, techniques of CFD could not yet model the effects of non-Newtonian fluids, and neither were non-DLVO effects taken into account. This work thus cannot tell us about the subject of the present chapter, but it deserves to provide the basis for future work that will. Already a new version of FLUENT is available that can take into account non-Newtonian liquid [123].

HARPACTICOIDS ARE DIFFERENT

The free-living copepods, calanoids and cyclopoid, abundant in the plankton, show remarkably little mucus secretion to the outside, except for the hardened mucus of egg cases and spermothecae. On the other hand, harpacticoids produce mucus more frequently. Benthic and meiobenthic harpacticoids, including *Diarthrodes nobilis* [124] and *Pseudostenhelia wellsi* [125] build mucous burrows. This mucus may be used "garden" microflora, with the mucus and associated microflora being subsequently ingested [124,125]. Two other harpacticoid species, *Nitocra lacustris* [126] and *Laophonte* sp. [127] do indeed ingest mucus with bacteria. In another mucous-tube-building meiofaunal harpacticoid *Stenhelia palustris*, secretory products, glands and secretory pores have been described [128]. Furthermore, dense concentrations of mucous tubes made on seagrass blades by *Dactylopodia tisboides* attract meiofauna [129].

How is the abundant mucus production in harpacticoids compared to the "clean" lifestyle of most calanoids and cyclopoids related to their respective lifestyles, mostly benthic (or particle-associated) and planktonic? Work is needed to explore this question further.

CONCLUSIONS AND RECOMMENDATIONS

Currently, most aquatic zooplankton and microbe ecologists as well as biogeochemists are poorly informed about many modern engineering models that can help explain nano- and micrometer-scale processes, particularly those observed at surfaces. I hope that this review of rheology, nano- and microfluidics and biofouling will be useful to improve understanding other processes in the lives of pelagic calanoid copepods, including swimming, feeding, protection against biofouling, parasitism and infection. This approach should also be extended to wider areas of pelagic ecology and biogeochemistry.

The disciplines of oceanography, rheology and surface science are all composite, and have of necessity been built from parts of Physics, Chemistry and Biology. I suggest there is now a need to form a multidisciplinary group of experts in pelagic ecology, biogeochemistry, rheology, nano/microfluidics, biofouling and allied fields. Such a group should associate to build capacity by building capacity in this new branch of aquatic science. The first step should be to teach each other what they know. This discipline might be called, *Rheology micro/nanoFluidics and bioFOuling* (RheFFO). The next step would be for the trained experts to build capacity in younger researchers, to make new discoveries.

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