

Food for Thought

A sea of tentacles: optically discernible traits resolved from planktonic organisms *in situ*

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Trait-based simplifications of plankton community structure require accurate assessment of trait values as expressed *in situ*. Yet planktonic organisms live suspended in a fluid medium and often bear elongate appendages, delicate feeding structures, and mucous houses that are badly damaged upon capture or removal from the fluid environment. Fixatives further distort organisms. *In situ* imaging of zooplankton from a fully autonomous *Zooglider* reveals a suite of trait characteristics that often differ markedly from those inferred from conventionally sampled plankton. *In situ* images show fragile feeding appendages in natural hunting postures, including reticulate networks of rhizopods, feeding tentacles of cnidarians, and tentilla of ctenophores; defensive spines and setae of copepods; intact mucous houses of appendicularians; and other structures that are not discernible in conventionally collected zooplankton. Postures characteristic of dormant copepods can be identified and the presence of egg sacs detected. Intact, elongate diatom chains that are much longer than measured in sampled specimens are resolvable *in situ*. The ability to image marine snow, as well as small-scale fluid deformations, reveals micro-habitat structure that may alter organismal behaviour. Trait-based representations of planktonic organisms in biogeochemical cycles need to consider naturally occurring traits expressed by freely suspended planktonic organisms *in situ*.

Keywords: autonomous instruments, *in situ* imaging, ocean gliders, trait-based approach, zooplankton

Introduction

Trait-based analyses represent a means to simplify the representation of foodweb complexity, where traits are fitness-relevant characteristics that different types of organisms may hold in common, irrespective of species identity or genetic similarity (Litchman and Klausmeier, 2008; Litchman *et al.*, 2013). Some spatial patterns can be represented by traits that transcend species-level resolution (Barton *et al.*, 2013). It is hoped that such a simplified trait space also captures important foodweb characteristics and their ecosystem-level consequences such as C and N export, fish production, and system resilience (Kiorboe *et al.*, 2018). Because modelling of marine ecosystems must *perforce* arrive at simplifications in order to be tractable numerically, the description of community properties in traits appears to be a viable approach to simplification.

For planktonic organisms, the selection of appropriate fitness-relevant traits is complicated by the difficulty of measuring and

accurately assigning trait metrics to fragile, nearly neutrally buoyant organisms that live suspended in a fluid medium. Historically, collection devices such as nets, pumps, water bottles, slurp guns, and dipping containers have been used to remove diverse types of plankton from the fluid environment. Morphological and physiological characteristics are then assessed. However, virtually all collecting approaches also introduce the possibility of structural damage, distortion, loss of appendages, and other biases. If tissues are fixed, this treatment typically leads to shrinkage, further distortion, altered pigmentation, dissolution of mineralized skeletal structures, and other artefacts. The combination of collection and fixation artefacts can limit the ability to accurately measure and assess the functional role of traits as expressed in the ocean and in lakes.

Several decades ago, the advent of systematic blue water scuba diving methods, led by the Hamners (Hamner *et al.*, 1975), opened the door to remarkable insights into the natural history

of diverse types of freely suspended planktonic organisms as observed and photographed *in situ*. Many of the functional attributes and behaviours of multicellular organisms like salps (Madin, 1974), appendicularians (All dredge, 1976), pteropods (Gilmer and Harbison, 1986), ctenophores (Harbison *et al.*, 1978), protists such as rhizarians (Swanberg and Harbison, 1980), and other taxa were revealed for the first time by this approach. However, the depth range of scuba is limited to near-surface waters, and only for short intervals of time. Since the 1960s, occupied submersibles have proven invaluable for *in situ* observational and behavioural studies, resolving such features as giant appendicularian houses (Barham, 1979), remarkable copepod layers (All dredge *et al.*, 1984), jellyfish zonation (Mackie and Mills, 1983), and other characteristics. However, like scuba, occupied submersibles have limited duration underwater. Silhouette photography of organisms flowing through the codend of a plankton net was introduced by Ortner *et al.* (1981). More recently, there has been a rapid expansion of digital submersible imaging devices that are not accompanied by humans, including towed and profiling cameras (Davis *et al.*, 1992; Cowen and Guigland, 2008; Picheral *et al.*, 2010; Schulz *et al.*, 2010; Greer *et al.*, 2013; Bi *et al.*, 2015; Lombard *et al.*, 2019) or those mounted on remotely operated vehicles (e.g. Hull *et al.*, 2011), or fixed platforms like piers or moorings. Holographic imaging has been adapted by Katz *et al.* (1999) and others (see Wiebe and Benfield, 2003 for a comprehensive review of numerous means of sampling and imaging). However, the process of tethering devices to surface vessels, moorings, or the seabed introduces fluid disturbances that often induce avoidance responses and cause physical distortion. Ideally, imaging devices would be untethered to surface vessels or the bottom, extend deep into the ocean water column, image planktonic organisms in their natural habitat unperturbed by fluid disturbances or physical disruption, be deployable autonomously day and night, and be capable of protracted, sustained observations at sea.

The present paper draws upon *in situ* images from a new *Zooglider* that meets many of the requirements above. This is a navigable, yet fully autonomous instrument that images organisms (and marine snow) between the sea surface and 400 m depth (Ohman *et al.*, 2018). It is not tethered to tow cables on surface vessels or to fixed objects like moorings or piers. It generates minimal hydrodynamic and optical disturbances and limited avoidance responses as it moves through the water column (Whitmore *et al.*, 2019). *Zooglider* is routinely deployed for 20 days at a time (with capability to 50 days), performs round-the-clock measurements, and is remotely navigable in order to adaptively sample features of interest. *Zooglider* also includes a dual frequency Zonar (200/1000 kHz).

The objective of the present paper is to reveal optically resolvable traits of planktonic organisms imaged *in situ* by the Zoocam on *Zooglider*. We present images of mesozooplankton (both multicellular and unicellular) because this is the size range of organisms for which *Zooglider* was built, although the Zoocam has also uncovered interesting elements of phytoplankton chain morphology. We will illustrate that fully autonomous devices that image organisms *in situ*, without modifying or deforming structures, can reveal traits and trait characteristics that differ markedly from those deduced from organisms collected by classical methods. We will address traits that have significance for morphological defence (diatom chain length, and copepod spines and setae), encounter rates with visual predators (tissue

transparency), encounter rates with prey (tentacles of cnidarians; tentilla of ctenophores; houses of appendicularians; rhizopods and setae of Rhizopodia; antennular length and setae of copepods), dormancy responses (planktonic copepods), and reproductive investment (egg masses of copepods). We will also illustrate *Zooglider*-resolvable micro-habitat features that provide a key context for trait expression, including the presence of marine snow and of layers of fluid deformation, as well as standard context variables (pressure, temperature, salinity, Chl *a* fluorescence).

Methods

The Zoocam imaging device and autonomous *Zooglider* we developed are described fully in Ohman *et al.* (2018). The Zoocam employs the principle of shadowgraph imaging, creating a cylinder of collimated light 15.0 cm in length and 4.95 cm in diameter, with an imaged volume of 250 ml per frame (slightly less than a perfect cylinder because the uppermost and lowermost portion of the cylinder are partially occluded, Ohman *et al.*, 2018). Plankton, marine snow, and suspended particles passing through the illuminated beam occlude the light and their silhouettes are recorded. The lens is telecentric, implying that the size of the object imaged is independent of its axial position in the illuminated beam, which confers markedly expanded depth-of-focus over conventional lenses and illumination systems (see also Cowen and Guigland, 2008). Pixels are 40 μm on a side and the smallest Region of Interest (ROI) that we segment and retain is 0.45 mm Equivalent Circular Diameter, corresponding to multiple pixels. Images are recorded in 8 bit greyscale at a frame rate of 2 Hz, with exposure times ranging from 30 to 94 μs . Several steps were taken to minimize avoidance (or attraction) responses of organisms to *Zooglider*. The *Spray* glider itself, upon which *Zooglider* is based, was designed as a hydrodynamically efficient, low drag vehicle (Sherman *et al.*, 2001). Illumination for the Zoocam is provided by red light-emitting diodes (LEDs) because crustacean eyes show reduced sensitivity to red wavelengths (see Ohman *et al.*, 2018). Furthermore, the Zoocam light cylinder is recessed 11.1 cm back from the sampling tunnel. The curved sampling tunnel was engineered to minimize hydrodynamic disturbances to the fluid medium, and also serves to minimize detectability of the light source (Ohman *et al.*, 2018). The Zoocam is mounted on a frame projecting from the nose of *Zooglider* into undisturbed water. Whitmore *et al.* (2019) compared abundances and size frequency distributions of organisms sampled by *Zooglider* and MOCNESS. Where differences were detected, they were consistently in favour of live sampling by *Zooglider*. Further evidence of minimal disturbances of organisms by *Zooglider* comes from images of forams in Gaskell *et al.* (2019) and of diverse organisms in the present paper that illustrate elongate diatom chains as well as spines, rhizopods, and tentacles of delicate organisms, and natural drifting, swimming, and feeding postures.

Zooglider is a buoyancy-driven glider that moves through the ocean at an average speed of 10 cm s^{-1} vertically and $\sim 15\text{ cm s}^{-1}$ horizontally, with an average pitch of 16–18° off the horizontal. *Zooglider* measures pressure, temperature, salinity, compass bearing, and Chl *a* fluorescence every 8 s, and acoustic backscatter at 200 and 1000 kHz as described in Ohman *et al.* (2018). Buoyancy is increased by pumping oil to a reservoir external to the pressure case, and decreased by drawing the oil back into the pressure case under vacuum. A typical dive profile entails surfacing, data telemetry at the surface, descent to just below 400 m depth, a drift

phase at depth if desired, then ascent. The Zoocam, Zonar, and other science sensors are activated only on ascent. Once at the sea surface, the sequence of actions is acquisition of GPS coordinates, uploading via Iridium of truncated data acquired on the ascent profile, downloading of new way points or dive parameters (if desired), re-acquisition of GPS, then descent. An ultraviolet LED is activated twice during descent, and a mechanical biowiper is activated once near the surface and once at depth, both designed to minimize biofouling. Technical details of *Zooglider* operation, image segmentation, and image flat fielding for background correction may be found in [Ohman et al. \(2018\)](#). Initial sorting of ROIs is carried out using tuned Convolutional Neural Networks ([Ellen et al., 2019](#)), although the identifications of all ROIs are manually verified.

All images reported here were acquired in the upper 420 m over or near the San Diego Trough, centred on 32.9°N, 117.6°W, between March 2017 and December 2019. The San Diego Trough is a region reaching 1200 m depth, at a distance ~35 km west of the pier of the Scripps Institution of Oceanography in southern California, USA.

Results

Trait of interest: morphological defence

Diatom chain length

At a recent scientific meeting, a polling of the audience revealed that the expected size range of marine diatoms is ca. 10–200 µm, occasionally in longer chains to 1 mm or so. Although taxonomic guides indicate the existence of still longer chains (e.g. [Cupp, 1943](#); [Sardet, 2015](#)), the common expectation is that diatoms are in the microplankton size range. *In situ* imaging by *Zooglider* in the San Diego Trough sometimes shows the occurrence of much larger chains. [Figure 1](#) illustrates diatom chains from 14.0 to 22.2 mm, which are not uncommon in our region. The significance of these elongate chains may be appreciated in [Figure 2](#), where they are illustrated together with co-occurring zooplankton that would otherwise be expected to be grazers, including appendicularians ([Figure 2a](#)) and suspension-feeding copepods

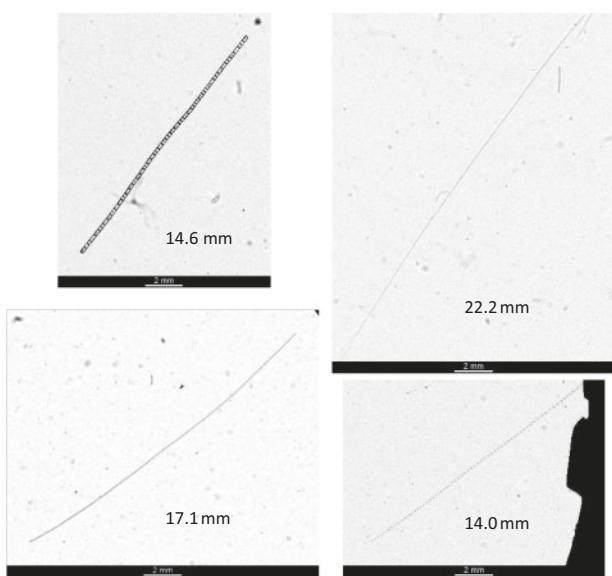


Figure 1. Diatom chains imaged *in situ*. Dimensions indicate chain length (mm).

([Figure 2b–e](#)). Elongate chains often greatly exceed the physical dimensions of the grazers and are essentially unutilizable by these heterotrophs. Appendicularian feeding filters will readily clog. The small-bodied copepod *Oithona* sp. is unlikely to consume the diatom chains visible in [Figure 2e](#) (especially because *Oithona* typically prefer motile prey, [Uchima and Hirano, 1986](#)). Small calanoid copepods ([Figure 2b and c](#)) are dwarfed by the physical dimensions of these diatom chains and even the larger copepod *Calanus pacificus* ([Figure 2d](#), 3.1 mm total length) is unlikely to be able to directly ingest them. The classic “chopsticks” method of chain processing ([Alcaraz et al., 1980](#)), in which herbivores reorient long-chains parallel with their body and remove cells individually, is not feasible with wide, robust cells that exceed the dimensions of the copepod mouth opening. Chain length (in addition to elongate siliceous setae) can be an effective grazer deterrent ([Hargrave and Geen, 1970](#); [Skiver, 1980](#); [Hamm and Smetacek, 2007](#)). Here, *in situ* imaging of undisturbed cells suggests that the prey “available” to suspension-feeders may be only a fraction of the total phytoplankton assemblage suspended in the water column. True chain morphology, and its fitness significance to the diatoms as well as edibility to heterotrophs, may be difficult to assess without imaging of undisturbed chains *in situ*.

Zooplankton spines and setae

Standard collecting devices used for crustacean zooplankton often disrupt extended appendages. Although crustacean exoskeletons may appear to be relatively robust, peripheral appendages such as terminal spines and setae can be vulnerable to mechanical damage during collection and handling. [Figure 3a](#) illustrates a harpacticoid copepod with a long, projecting terminal spine whereas other panels show calanoid copepods with single terminal seta ([Figure 3b and c](#)) or paired caudal setae ([Figure 3d and e](#)) that are relatively rarely seen in net- or pump-collected specimens. Stout spines are structures that likely inhibit prey capture by both visually hunting (e.g. [Miehls et al., 2014](#)) and tactile predators, suggesting that these are effective predator deterrents. Although the caudal setae found on many copepods could play a role in swimming hydrodynamics, they likely also serve as structures that inhibit attack and increase handling time by diverse types of predators.

Trait of interest: predator encounter

Tissue transparency and prey visibility

The first step modulating a successful predator–prey interaction is encounter. Encounter probability can be modified by behavioural and morphological characteristics of both predators and prey ([Ohman, 1988](#)), as well as by ambient environmental conditions. Prey visibility is an important trait that affects predation rates and fitness in interactions with visually hunting carnivores. It is widely appreciated that many types of zooplankton have relatively transparent bodies, in contrast to others that have opaque bodies and appendages (e.g. [Allredge and Madin, 1982](#); [Sardet, 2015](#)). However, when planktonic organisms are treated with fixatives, their tissues often become more opaque. Conversely, pigments can be leached out of tissues or oxidize and the fixed organisms can become more transparent than found in nature. Hence, images of undisturbed organisms *in situ* are highly preferable for assessing this trait. The Zoocam used on *Zooglider* employs the principle of shadowgraph imaging, thus it records silhouettes of organisms rather than recording light reflected off the animals’

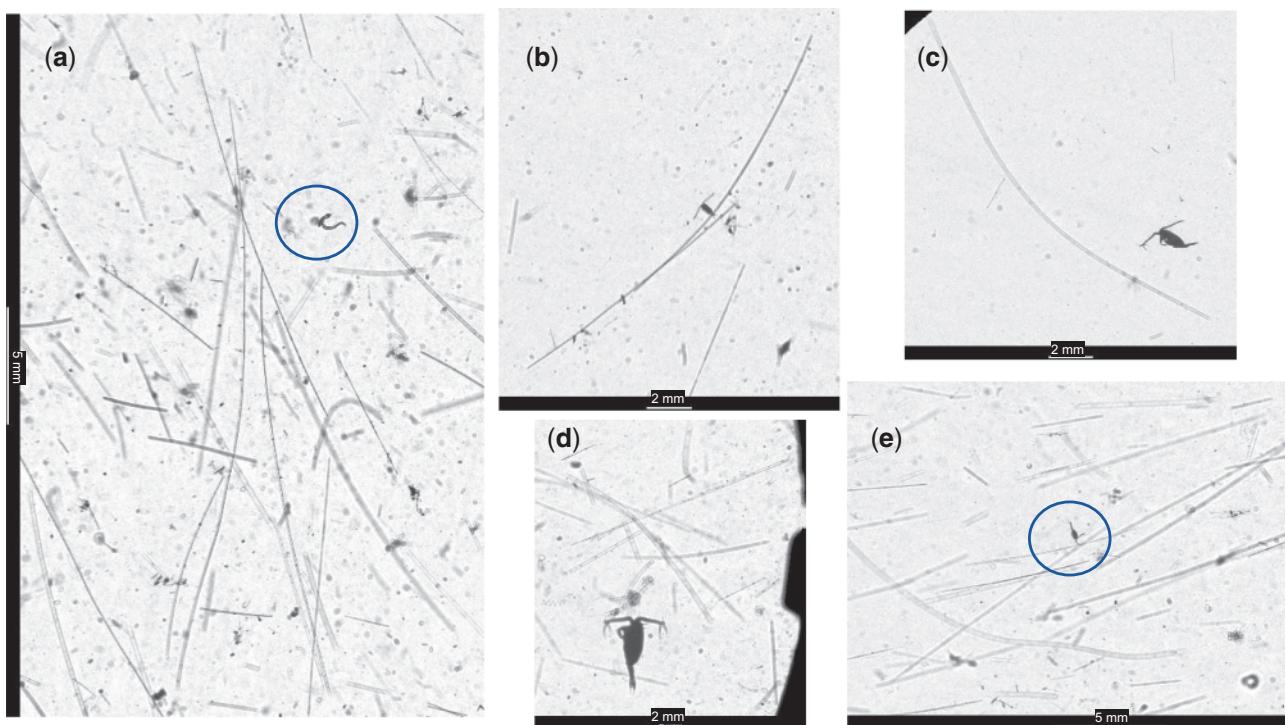


Figure 2. Diatom chains imaged *in situ*, together with suspension-feeding zooplankton. (a) Appendicularian (encircled), (b–c) calanoid copepods, (d) calanoid copepod (*Calanus pacificus*), (e) cyclopoid copepod (*Oithona*, encircled). Note scale bars for each panel.

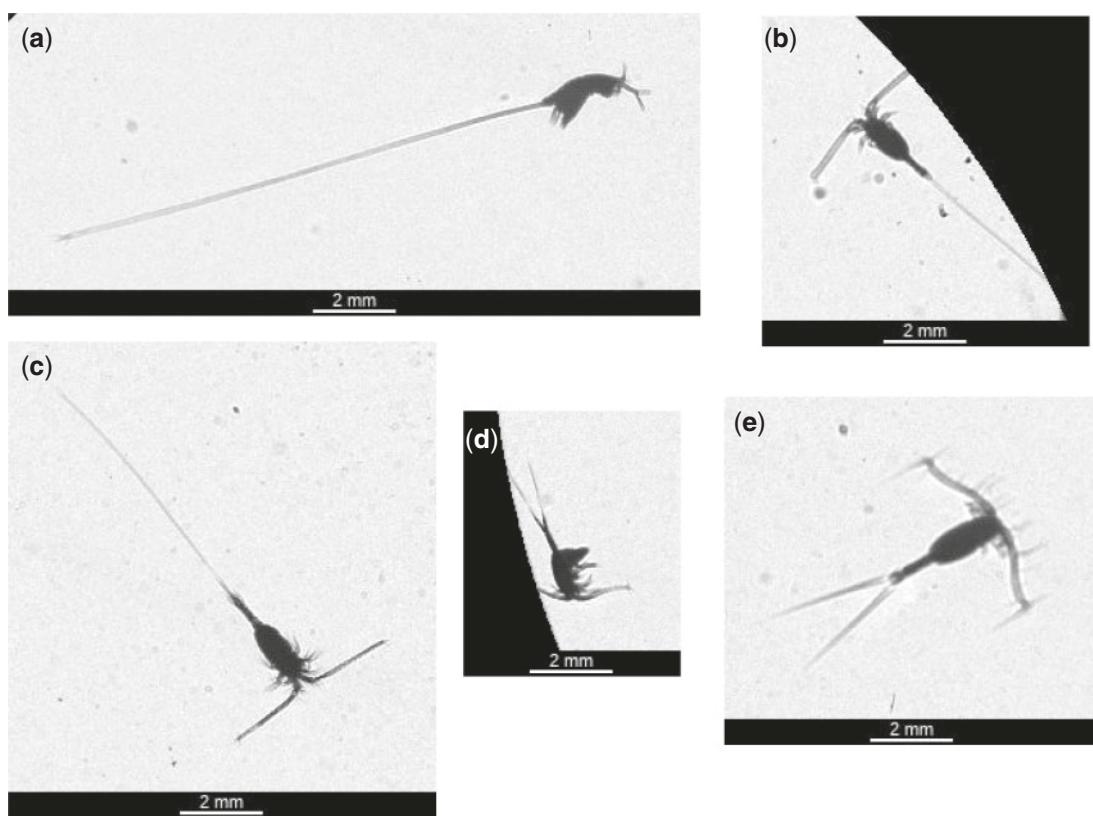


Figure 3. Planktonic copepods imaged *in situ*, illustrating elongate caudal setae and spines. (a) Harpacticoid (*Aegisthus*), (b–e) calanoids.

surface. These silhouettes are not an indication of the true appearance to predators, however they are likely to be an indication, in a relative sense, of differences among organisms in tissue transparency and therefore visibility *in situ* and the risk of detection by visually hunting predators. For example, the copepods and euphausiid to the left of Figure 4 are much more opaque than the chaetognath, doliolid, hydromedusa, and ctenophore shown to the right. Neither are the crustaceans as consistently dark as seen in Figure 4, nor are the perimeters of the gelatinous organisms in Figure 4 as well-defined as seen in Figure 4. However the relative differences in tissue transparency measured *in situ* can nevertheless be informative. By standardizing the background greyscale, the relative transparency of different types of organisms can be assessed.

Trait of interest: prey encounter

Rhizopods and spines of rhizopodia

It has long been appreciated that spines and rhizopods associated with some types of planktonic foraminifera (and other Rhizaria) can significantly increase the surface area of the organism, thus increasing the encounter rate with suspended prey. For forams living in epipelagic waters, such structures also enhance the surfaces available for symbionts. In the case of the mesopelagic foram *Hastigerinella digitata*, the original description by Rhumbler (1911, as *Hastigerina digitata*) from a plankton tow-collected specimen depicted portions of the calcium carbonate spines emanating from the digitate test (Figure 5a). However, not well recognized at the time was the full extent of the rigid calcite spines, as well as the much longer, flexible rhizopods that can extend far from the cell centre. Such structures were depicted in *in situ* imagery by Hull *et al.* (2011) and Gaskell *et al.* (2019). Gaskell *et al.*, who analyse this topic in detail, show that accurately resolved spines + rhizopods in these hastigerinid forams, as imaged by Zooglider, can increase the projected encounter volume with prey

100–1000-fold relative to the same forams with incompletely resolved test extensions. *In situ* imagery quantitatively alters our assessment of encounter volume and suggests that these organisms may be much more important consumers of suspended particulate matter, as well as interceptors of fluxes of particulate organic matter, than previously recognized.

A sea of tentacles—metazoans

Hydromedusae. Small hydromedusae of diverse taxonomic affinities (Wrobel and Mills, 1998) are ubiquitous in the ocean and are frequently found in plankton samples. However, especially after

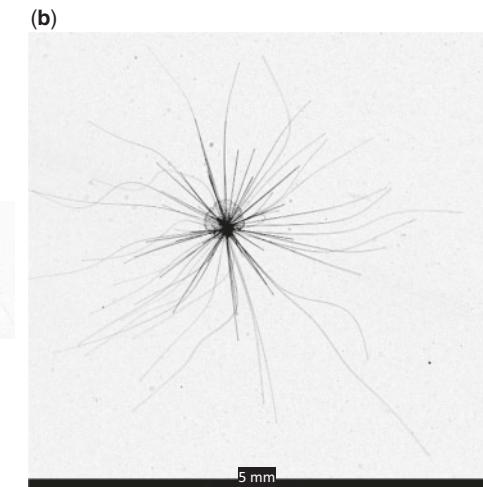


Figure 5. Hastigerinid foraminifera. (a) Type specimen of *Hastigerinella digitata* from plankton tow, from Rhumbler (1911). Reported from 2000 m depth, 5 mm. (b) Likely specimen of *H. digitata* imaged *in situ*, from 389.86 dbar, San Diego Trough, showing elongate spines and still longer rhizopodia.

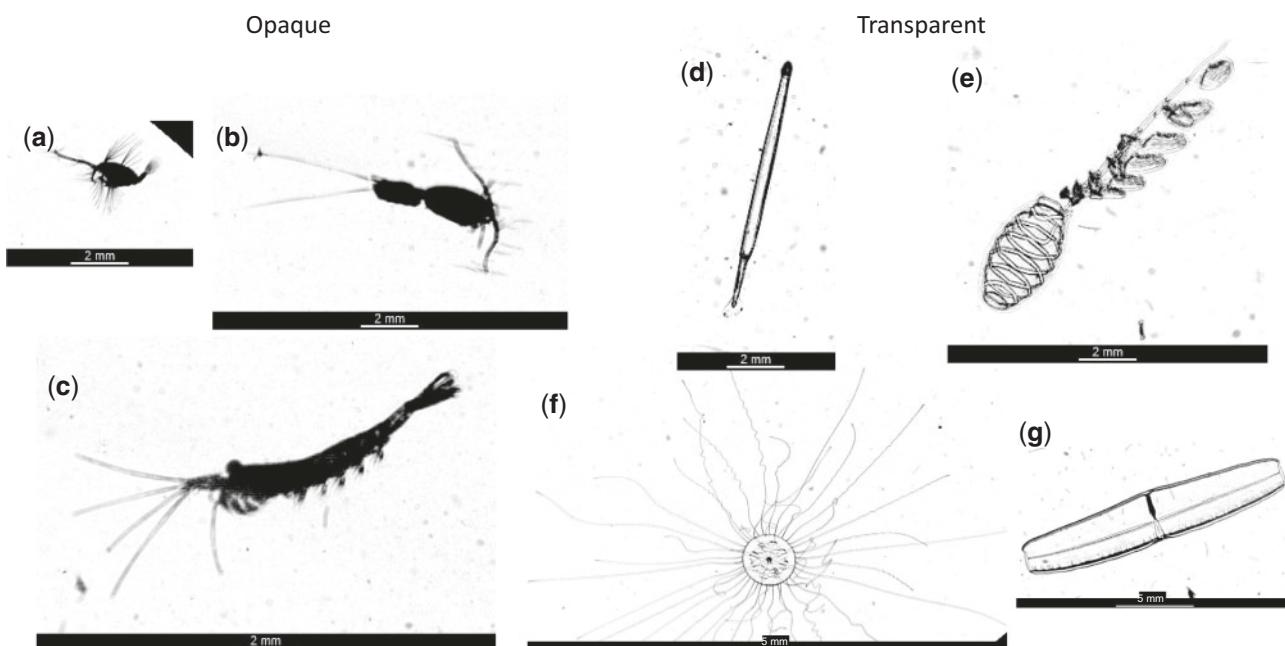


Figure 4. Diverse zooplankton imaged *in situ*, illustrating more optically opaque specimens such as (a, b) copepods and (c) euphausiid, as well as more transparent organisms such as (d) chaetognath, (e) doliolid, (f) hydromedusa, and (g) cestid ctenophore.

fixation, hydromedusae often shrink and their tentacles contract (see Figure 6a). It is difficult to assess their predation impact quantitatively (e.g. Ohman *et al.*, 2008), and is sometimes assumed to be negligible. However, a very different assessment occurs when hydromedusae are imaged undisturbed *in situ*. A small hydromedusa of bell diameter <1 mm can extend feeding tentacles 20 times or more the dimensions of the umbrella (Figure 6b). Somewhat larger hydromedusae (Figure 6c) also have extended feeding tentacles, sometimes not even fully measured across the 39.5 mm opening of the Zoocam window (Figure 6d). Hence, the potential search volume of the living hydromedusae, an important trait influencing their ecosystem role as predators, is substantially larger than inferred from preserved specimens. Their cumulative impact remains to be properly assessed in most ocean provinces.

Siphonophores. Siphonophores are colonial cnidarians that are nearly always fragmented and dismembered during collection. This disruption of colony parts is particularly evident for calyco-phoran and physonect siphonophores. *In situ* imaging by Zooglider reveals not only the intact colonies, but the lateral extent of tentacles and cormidia, in the case of the calyco-phoran siphonophore in Figure 7. Much of the imaging field of the Zoocam is filled with this single colony, which represents a much larger potential prey encounter volume than would be assumed from preserved specimens alone.

Ctenophores. A similar difficulty in accurately quantifying tentacle extent and the trait of prey encounter volume arises with ctenophores, which can degrade rapidly upon fixation, sometimes

leaving behind essentially unidentifiable gelatinous remains. However, in the case of tentaculate ctenophores, in particular, an additional bias arises because of the difficulty in detecting on preserved specimens the very finely branched tentilla that emerge from the primary tentacles. The shadowgraph imaging system on Zooglider resolves these fine tentilla (Figure 8), again quantitatively changing the surface area of prey encounter and thus altering the expected three-dimensional volume of water searched for prey by these widespread predators. The ability to optically

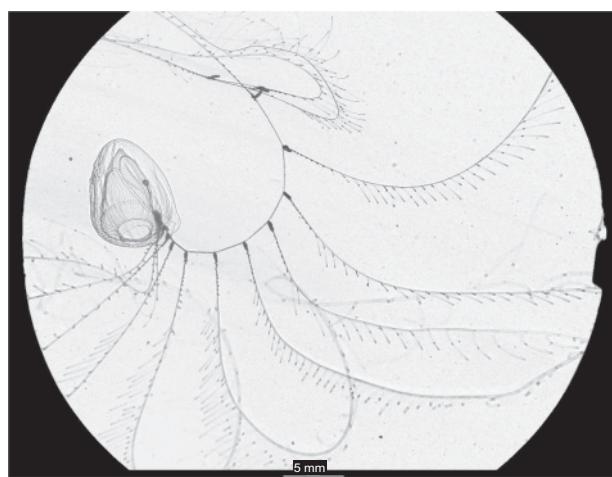


Figure 7. Calyco-phoran siphonophore, imaged *in situ*.

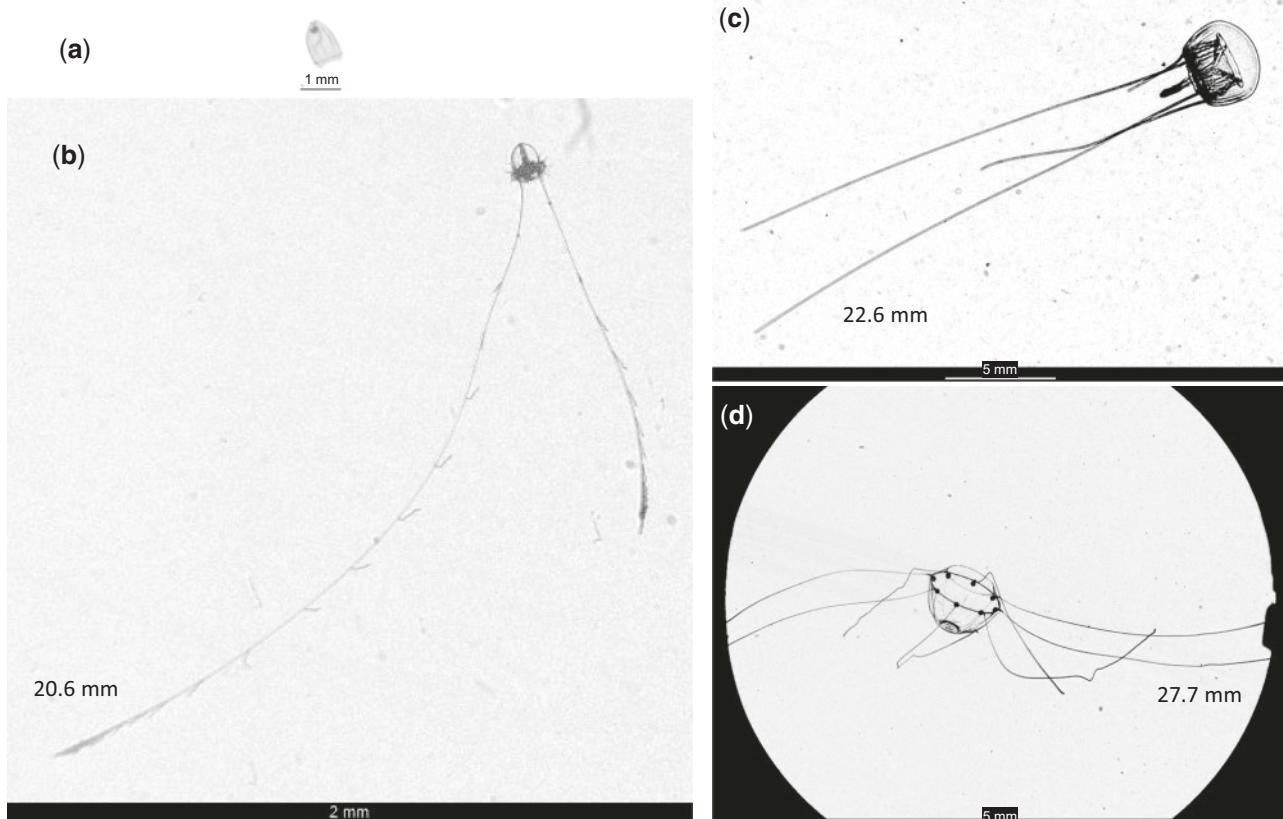


Figure 6. Hydromedusae. (a) Specimen from preserved plankton sample (~1 mm bell height). (b-d) Three other hydromedusae species imaged *in situ* (numerals indicate the length of the longest tentacle).

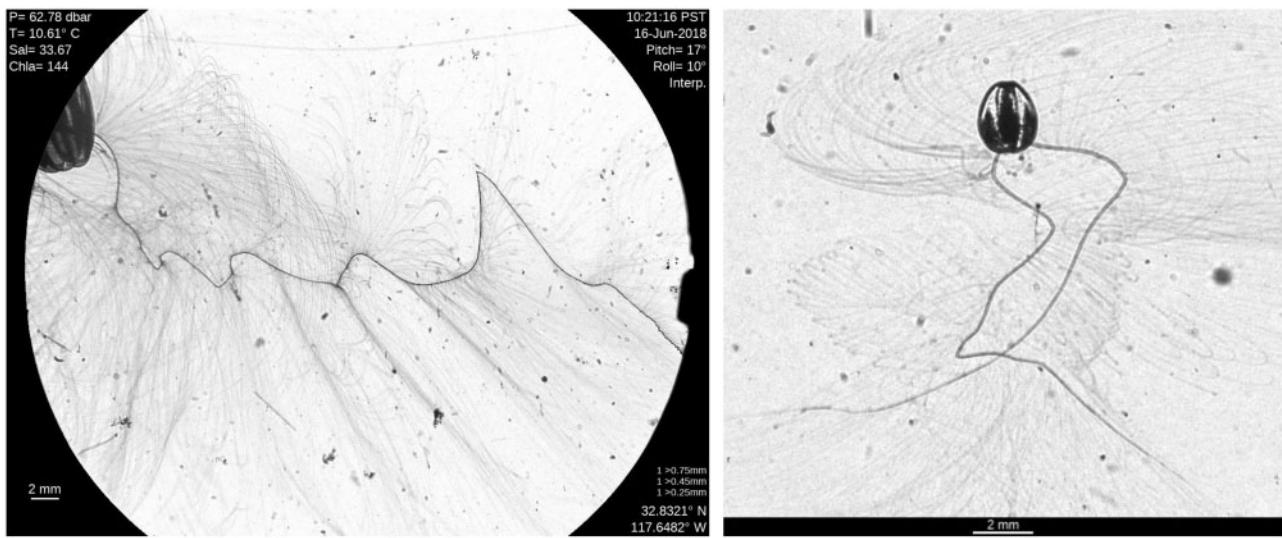


Figure 8. Tentaculate ctenophores, imaged *in situ*. The primary tentacles (black lines) bear large numbers of fine, secondary tentilla (gray strands) that significantly increase the volume of water that is searched by these predators.

resolve fine tentilla changes the expected predation potential relative to that expected from the more easily resolved (and more readily preserved) tentacles alone.

Appendicularian houses

Appendicularians are soft-bodied pelagic tunicates whose external mucous feeding structures, termed either houses or feeding envelopes depending on their structure, are exceedingly difficult to sample intact. Houses readily collapse and sink when abandoned in the ocean (e.g. Alldredge and Madin, 1982; Robison *et al.*, 2005) and are mechanically destroyed during collection by nets. Even when appendicularians are carefully dipped from the ocean, the glandular secretory epithelium on the trunk of the animal can be readily damaged, thus inhibiting secretion of a new house. The grazing impact of appendicularians depends largely on the volume and retention characteristics of their houses, as well as the pumping rate of the adults (e.g. Flood and Deibel, 1998). *Zooglider* images reveal a notable fraction of appendicularians in their houses *in situ*. The images also permit house morphology and sometimes area of feeding filters to be assessed, which can vary greatly depending on the genus (Figure 9).

Length of antennae and sensory setae

In many crustaceans, including planktonic copepods, the paired first antennae (A1) project far into the medium, bearing setae that can be mechanosensory, chemosensory, or sometimes only structural elements that lack innervation (Bundy and Paffenhofer, 1993). The first antennae increase the extension of sensory setae and increase the volume of medium that can be searched for prey, mates, or predators. *In situ* images that illustrate A1s and associated setae in their natural posture (Figure 10) have the potential to act as a proxy for the prey encounter volume searchable by different copepods.

Trait of interest: dormancy

Posture of *Eucalanus*

Dormancy is a critical life-history trait that is particularly widespread among some genera of planktonic copepods (Baumgartner and Tarrant, 2017), especially those dwelling in mid-to-high latitude environments. Dormancy encompasses both physiological quiescence and true diapause (Hirche, 1996) and has implications for many ocean processes, including grazing, prey–predator interactions, and both passive and active components of the biological carbon pump (Steinberg and Landry, 2017). Among the copepod taxa known to enter dormancy in the San Diego Trough region, *Eucalanus californicus* is noteworthy because they show a transient quiescence that appears interruptible (Ohman *et al.*, 1998). Although indirect measures have been used to assess copepod dormancy state, including lipid storage and metabolic activity (Ohman *et al.*, 1998), mandibular morphology (Miller and Terazaki, 1989), moulting hormones (Johnson, 2003), gene expression (Tarrant *et al.*, 2008), and developmental stage structure, another approach that is made possible from direct *in situ* imagery is to assess behavioural state from the orientation of the body, first antennae, mouthparts, and posture of individual copepods, to ascertain whether they are active or dormant in the ocean water column (Figure 11). Dormancy in *Eucalanus* is quite unlike that observed in the genus *Calanus*, where the latter typically enter dormancy as copepodid stage 5 and often remain in a vertically upright posture with outstretched A1s. In *E. californicus*, active individuals maintain extended A1s and extended mouthparts (Figure 11a). In contrast to many species of *Calanus*, individuals of *Eucalanus* can enter transient dormancy as either late juvenile stages or as adults (Ohman *et al.*, 1998), and the main axis of the body can be oriented in different directions. Images of animals that appear to be in a state of dormancy have A1s folded alongside the body, mouthparts folded against the prosome, and the body axis without a preferred orientation (Figure 11b). The orientation of dormant *Eucalanus* is very different from the typical vertical upright posture of *Calanus* copepodid stage 5. *In situ* imagery of live animals may provide an indication of a key life-history trait.

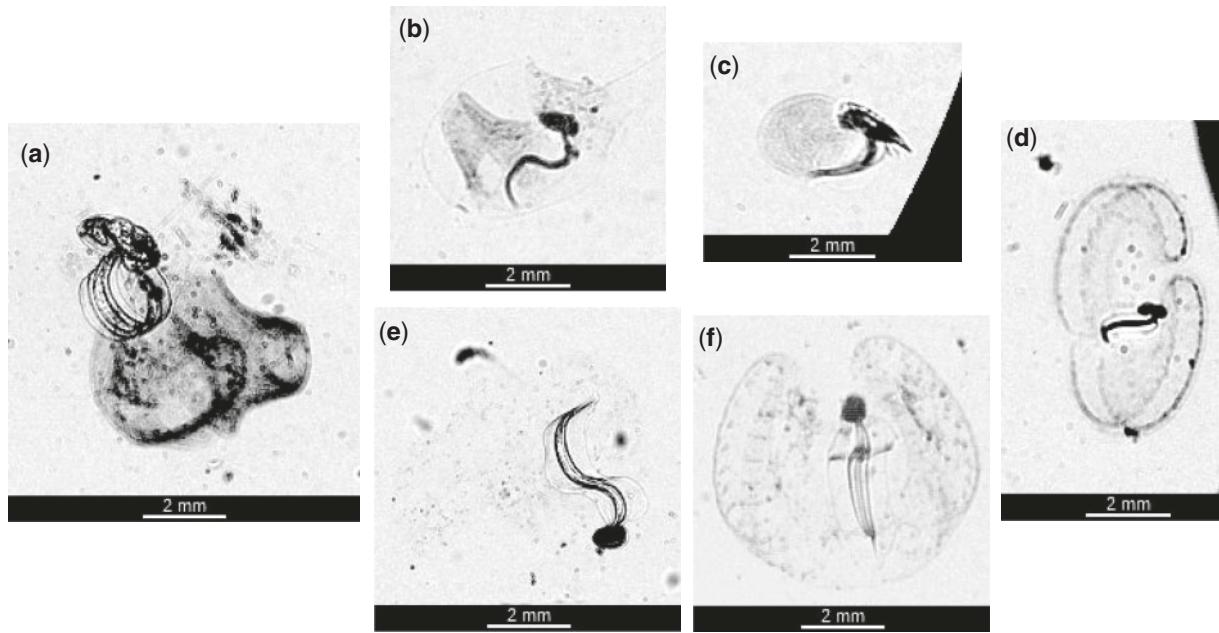


Figure 9. Mucous houses and feeding envelopes of diverse appendicularians, imaged *in situ*. (a) Large appendicularian and house, (b) *Oikopleura*, (c) *Fritillaria*, (d) *Kowalevskia*, (e, f) *Bathochordaeus* with feeding envelopes.

Trait of interest: reproductive investment

Copepod egg masses

Some metazoans brood their embryos in attached egg masses, whereas others freely spawn embryos into the water column. Egg brooding and egg size are important traits, with well-known trade-offs between reproductive investment and mortality (Kiørboe and Sabatini, 1995; Ohman *et al.*, 2002). In the case of cyclopoid copepods, all marine taxa brood their eggs (Ohman and Townsend, 1998), although standard collection methods often result in loss of some attached embryos. We frequently detect cyclopoid copepods of the genus *Oithona* in *Zooglider* images and are able to differentiate egg-bearing individuals from others (Figure 12). This capability makes it possible to assess ocean conditions that are particularly conducive to reproduction of these ubiquitous copepods, and those that are not.

At least three species of *Oithona* are recognizable in our *Zooglider* images (Figure 12) and demographic comparisons should be made within a single species. Also, it is important to distinguish adult males and juveniles from non-ovigerous female copepods.

Trait of interest: mating behaviour

Copepod mating behaviour and courtship

Mating behaviour is a transient activity in marine planktonic copepods and has typically been observed only in the laboratory under specific conditions (e.g. Blades and Youngbluth, 1979; Tsuda and Miller, 1998). The environmental conditions that optimally favour mating are not well understood, although the ability to record animals in the process of mating (e.g. Figure 13a–c) and in apparent courtship (Figure 13d) may make it possible to characterize those optimal conditions *in situ*. The Zoocam on *Zooglider* does not permit resolution of the third (depth) dimension, although based on animal orientation, apparent contact

between individuals, and similarity of blurred outlines of each pair in these images, they appear to be interacting.

Micro-habitat characteristics in the water column

Micro-habitat characteristics of the ocean water column may provide specific conditions that favour expression of some organismal traits over others. A unique aspect of *Zooglider* imaging is that it is possible to resolve some of these habitat characteristics concurrently with, and on the spatial scale appropriate to, the planktonic organisms themselves. Two examples of such micro-habitat characteristics are the presence of suspended marine snow and the existence of micro-scale layers of density anomalies that may influence encounters of prey and predators.

Marine snow

Marine snow, defined as detrital particulate organic matter larger than 0.5 mm (Allredge, 1998), is ubiquitous in the ocean and forms a major part of the available Particulate Organic Carbon. Total detrital material, including marine snow, constitutes the majority of the particles imaged by the *Zooglider* and by other *in situ* imaging devices such as the UVP5. Marine snow (Figure 14a) is highly diverse in size, shape, sinking velocity, age, organic content, and nutritional value. Sometimes Zoocam images reveal direct interactions between zooplankton such as copepods and euphausiids and clumps of marine snow (Figure 14b–d). The snow may provide important sources of nutrition for a variety of suspended planktonic organisms (Dilling *et al.*, 1998; Möller *et al.*, 2012), as well as solid substrates and refuges for others. Some zooplankton function as flux-feeders in the ocean, intercepting the sinking flux of particulate matter (Jackson, 1993; Stukel *et al.*, 2018) rather than the nearly neutrally buoyant, suspended particles. The ability to measure both sinking and suspended particles together with associated organisms *in situ* may help resolve the components of the zooplankton community that

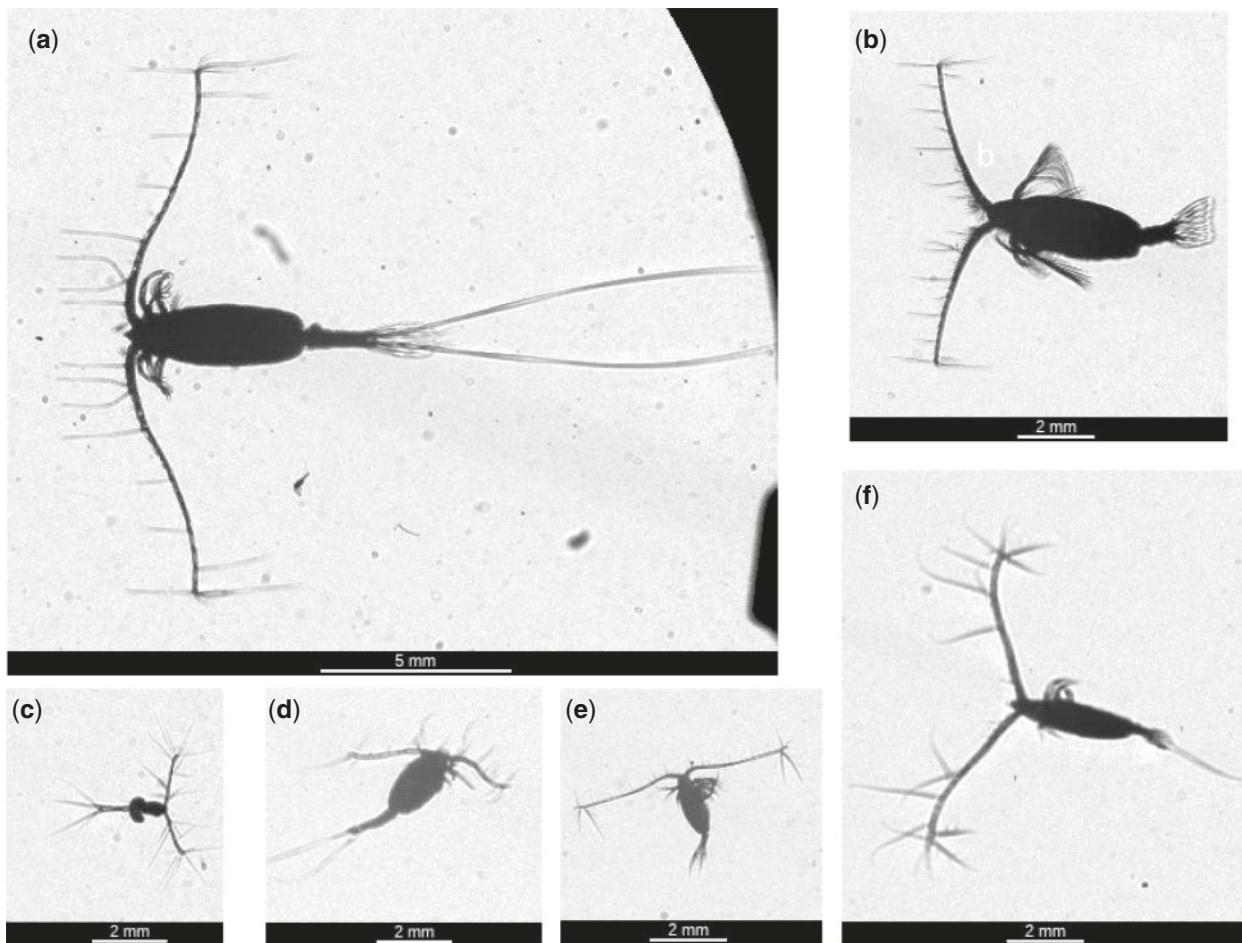


Figure 10. Diverse planktonic copepods, imaged *in situ*, showing the lateral extent and orientation of first antennae (A1) and associated antennular setae. (a) *Euchaeta*, (b) calanoid, (c) *Oithona*, (d–e) calanoids, (f) *Rhincalanus nasutus*.

are particularly important in utilizing and biogeochemically transforming marine snow.

Fluid deformations

Another characteristic of the local environment that may alter the fitness value of different zooplankton traits is the presence of micro-scale density anomalies and fluid deformations that appear to be the result of turbulent stirring. The principle of shadow-graph imaging that is employed on *Zooglider*'s Zoocam has been used since the 17th century to visualize differences in fluid density (Settles, 2001). This imaging method is sensitive to differences in refractive index, which can be caused by density variations. The present paper does not intend to diagnose the physical basis for these density anomalies, but only to point out that they are resolvable by the Zoocam optical system, as shown in Figure 15, and that they may have consequences for planktonic organisms. Figure 15a illustrates a Zoocam frame at 5.58 dbar pressure over the San Diego Trough showing a single appendicularian against a typical quiescent background. Figure 15b illustrates essentially the same pressure surface (5.50 dbar) at the same geographic location 2 months earlier, in more strongly stratified conditions, with clear evidence of fluid deformations throughout the frame. Figure 15c–d shows example images where planktonic copepods

were recorded against a background of such density anomalies. The turbulent flow that likely underlies these spatial variations in refractive index can potentially alter the rates of encounter of zooplankton with their prey (Rothschild and Osborn, 1988) and mates, or perhaps lead to avoidance behaviour (Incze *et al.*, 1996). The ability to simultaneously measure density discontinuities and zooplankton occurrence provides an opportunity to directly test for covariability.

Standard environmental variables

Standard micro-habitat variables (including depth, temperature, salinity, Chl *a* fluorescence) also provide essential environmental context, especially when measured concurrently with zooplankton imaging. Our Zoocam samples at 2 Hz, providing 5 cm vertical resolution, given the *Zooglider* ascent rate of 10 cm s^{-1} . However, physical sensor measurements are recorded every 8 s, thus providing somewhat coarser vertical resolution of such properties, which nonetheless permits identification of habitat variables of relevance to zooplankton fitness. Inclusion of such environmental metadata has also been shown to increase the accuracy of machine learning classifiers of plankton images (Ellen *et al.*, 2019).

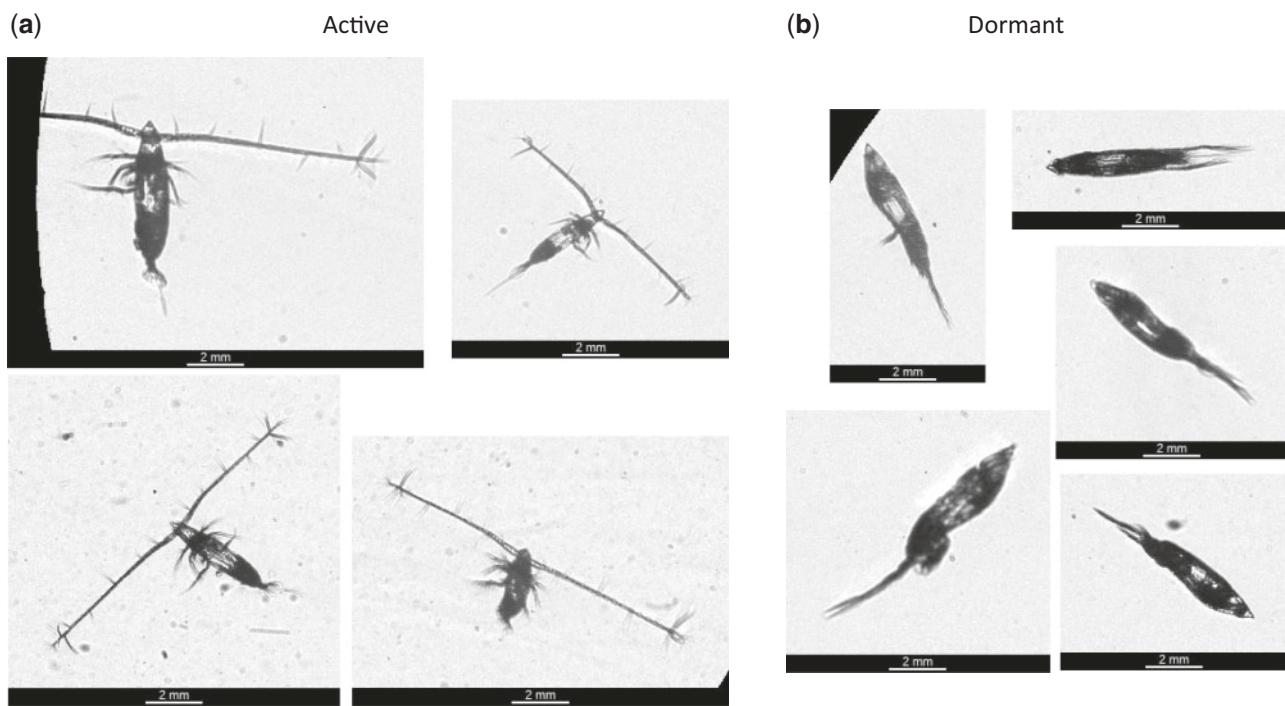


Figure 11. Copepods in the genus *Eucalanus* in (a) an active state, and (b) an apparent dormant posture.

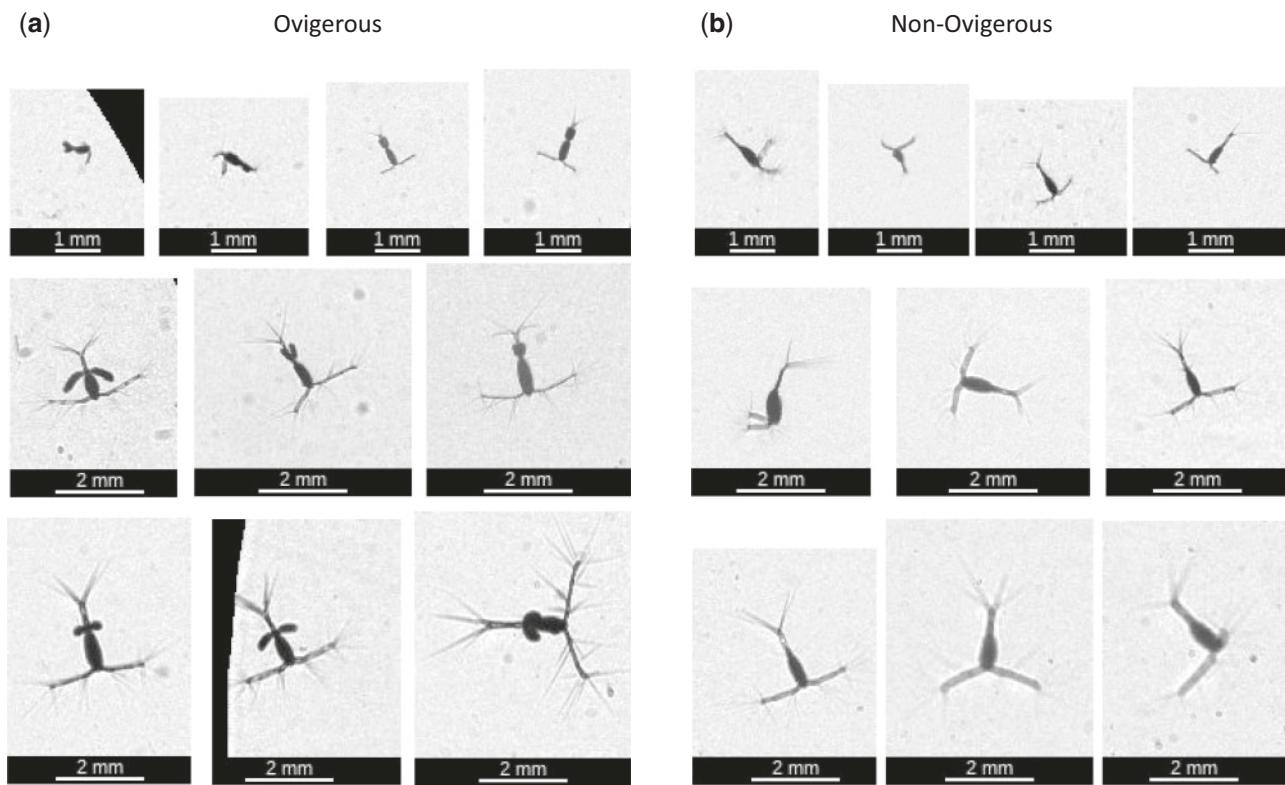


Figure 12. Copepods in the genus *Oithona* that are (a) ovigerous and (b) non-ovigerous. Individuals are arranged in three rows, which probably correspond to three different species in this region.

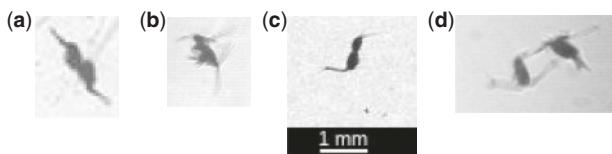


Figure 13. (a–c) Likely mating pairs of copepods, and (d) a possible copepod courtship dance. All imaged *in situ*.

Discussion

Our *in situ* autonomous imaging system successfully records morphological characteristics of both uni- and multicellular zooplankton *in situ* at depths ranging from 400 m to the sea surface. Such images reveal fragile feeding appendages in natural hunting postures (including elaborate networks of rhizopods, feeding tentacles, and tentilla) as well as defensive setae and other structures that are not discernible in conventionally collected zooplankton.

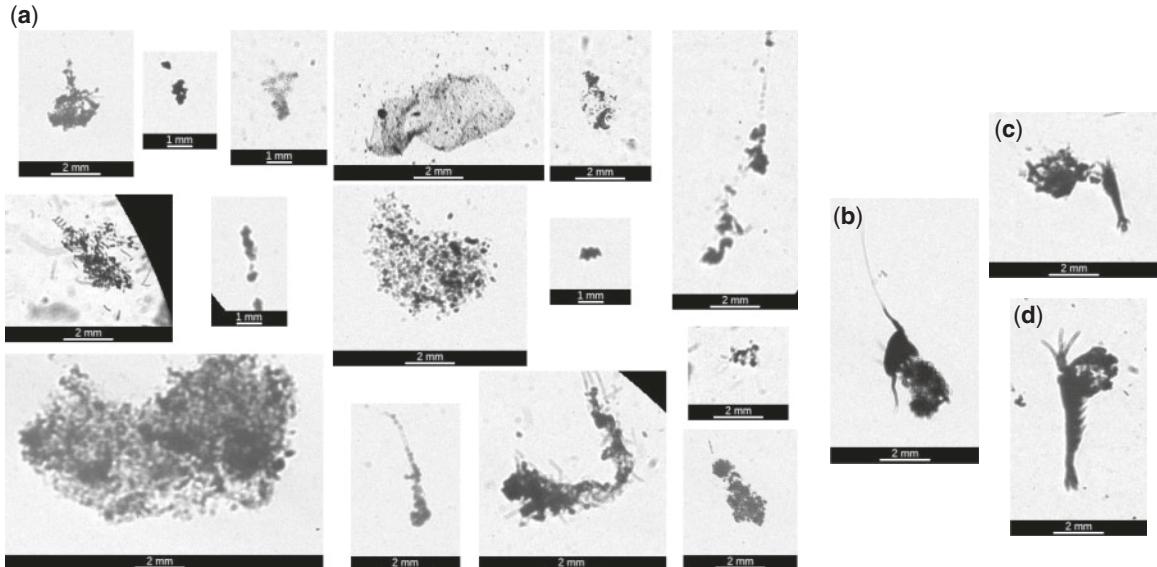


Figure 14. (a) Marine snow of diverse morphologies. Specific associations of marine zooplankton with snow, including (b) a calanoid copepod, (c) a larval (calyptopis) euphausiid, and (d) a euphausiid furcilia. All imaged *in situ*.

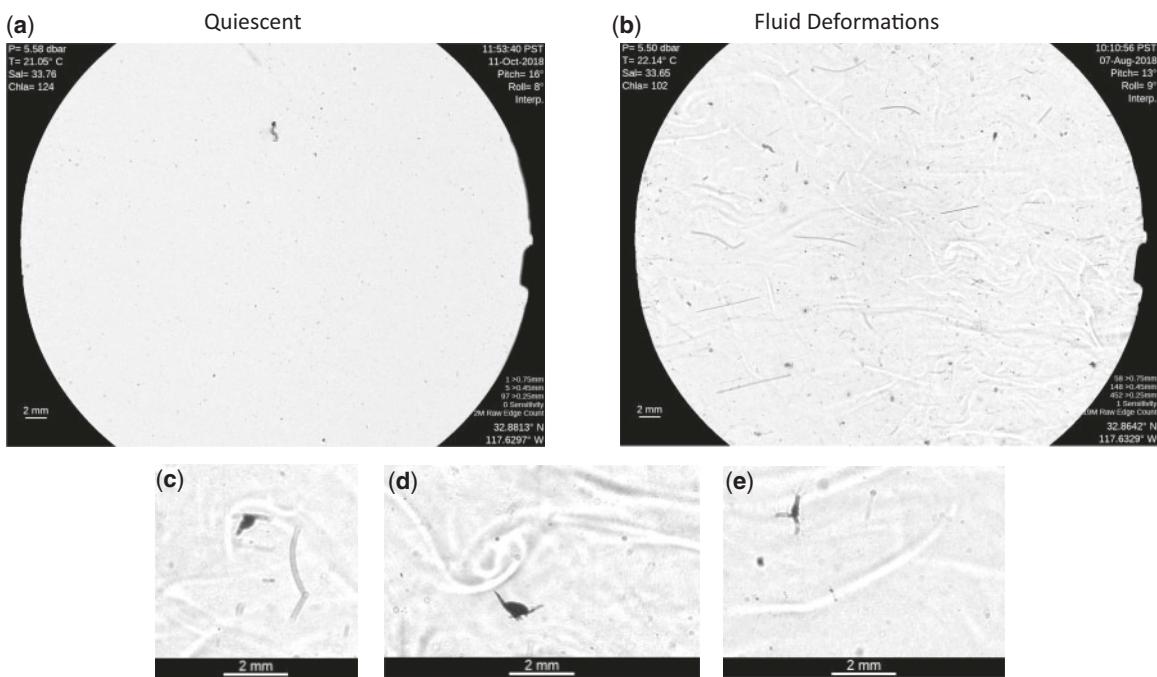


Figure 15. (a) Quiescent background (5.58 dbar, 11 October 2018) with appendicularian, (b) Fluid deformations (5.50 dbar, 7 August 2018), with numerous organisms and particles. (c–e) Individual copepods against a background of fluid deformations. All imaged *in situ*.

Intact mucous houses of appendicularians are regularly resolved. Postures characteristic of dormant copepods can be identified and presence of egg sacs on ovigerous females detected. Intact, elongate diatom chains that are much longer than typically measured are recorded *in situ*.

Although all of these traits are measureable in some manner on organisms sampled by conventional means, the advantage of autonomous *in situ* imaging is that intact structures can be recorded on specimens suspended in their natural environment. The resulting trait characteristics often differ markedly from what is inferred from conventionally sampled organisms removed from the ocean. The hydrodynamic design of the glider hull (Sherman *et al.*, 2001) and the plankton sampling tunnel (Ohman *et al.*, 2018) generate minimal hydrodynamic disruption to the flowfield, permitting imaging of essentially undisturbed organisms. Comparison of *Zooglider*-based with net-based abundances and size distributions of a variety of different zooplankton types showed consistent benefits to *Zooglider* imaging for smaller and more fragile organisms and no significant differences in abundance or size distributions of larger, more robust organisms (Whitmore *et al.*, 2019).

As noted above, diverse high speed towed and tethered profiling imaging systems have been developed that have provided important insights into plankton distributions. Some provide higher resolution images than are currently capable with the *Zoocam*, and can also image in colour, which can facilitate identifications. However towed devices and those lowered on cages like CTD-rosettes or ROVs disturb the fluid environment around the organisms. Stationary, mounted cameras that are associated with physical structures like pier pilings or mooring lines can also generate vigorous hydrodynamic disturbances of the medium and the suspended organisms.

Our autonomous sampling can resolve intact marine snow and the interactions of some organisms with snow clumps. Although such capability already exists with other devices (e.g. Picheral *et al.*, 2010; Taucher *et al.*, 2018), such devices are tethered to surface platforms that transmit surface displacements to subsurface instruments. Drifting Carbon Flux Explorers (Bourne *et al.*, 2019) record marine snow and other detrital particles that settle onto an optical surface, but not necessarily neutrally buoyant suspended material. Another important aspect of the environment that *Zooglider* resolves are small-scale density anomalies, apparently caused by turbulent mixing, that can be readily detected by shadowgraph imaging. These anomalies have the potential to alter encounter rates between organisms and suspended particles, as well as among organisms.

Our imaging system records single frames at a frequency of 2 Hz, which is well suited to analysing morphological structures and the orientation of organisms, but this frame rate is not sufficient to resolve most behavioural traits *in situ*. In addition, because *Zooglider* ascends at ca. 10 cm s^{-1} , each sequential frame images an independent parcel of water and thus records different individual organisms. Hence, it is not feasible to track behavioural changes of the same individual over time. A different approach would be needed to record most behavioural traits *in situ*.

In situ imaging is rarely suitable for species-specific identifications, apart from field situations with very low species diversity or highly distinctive species. Some questions are less amenable to trait-based simplifications than others, e.g. defining oceanic distributional boundaries and evolutionary divergence patterns (e.g. Goetze *et al.*, 2017) are best resolved at the species level. Hence, *in*

situ imaging will not supplant physical sampling, but should be viewed as a complement to it, especially when undistorted traits can be resolved on naturally occurring organisms.

Summary

Characterization of key fitness-related traits in zooplankton – such as morphological defences; elaborate feeding tentacles; mucous houses that increase prey encounter volumes; tissue transparency that affects encounter rates with predators; reproductive investment; and dormancy responses – is important to the development of simplified representations of pelagic ecosystems and prognostic models. Conventional approaches to characterizing these traits that rely on collection and removal of specimens from their three-dimensional environment often provide biased and inaccurate trait assessments. Imaging organisms *in situ*, using autonomous instruments that minimally disrupt the organisms and traits of interest, should improve our ability to advance trait-based simplifications of complex foodweb structure in models representing future states of ocean ecosystems.

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