



## Ciliary removal of particles by the cold-water coral *Lophelia pertusa*

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### Introduction

*Lophelia pertusa* is a species that often dominates cold-water coral ecosystems and is mostly found on banks, ridges and seamounts at a depth range of 300 to 800 m (Freiwald et al. 2004). Here, *L. pertusa* can be exposed to high sediment concentrations occurring as a result of both strong local seabed currents, and also offshore drilling operations or bottom fish trawling (Freiwald et al. 2004). Although not yet well studied, several recent studies have addressed the impact of sedimentation on *L. pertusa* (e.g. Brooke et al. 2009; Allers et al. 2013; Larsson et al. 2013). A general conclusion is the high resilience of the species to relatively high sedimentation loads. While these studies emphasise the physiological impacts of sedimentation, an understanding of the removal mechanisms is lacking. Shelton (1980) used graphite particles to observe two types of ciliary currents in *L. pertusa*: (1) a ciliary current that moved particles from the base to the tips of tentacles, and (2) pharyngeal cilia, which maintained a current directed inward to the gastrovascular cavity. These types of ciliary currents are well known for many Atlantic and Pacific scleractinians (Lewis and Price 1976; Stafford-Smith and Ormond 1992; Stafford-Smith 1993). More recently, vortical flows induced by epidermal motile cilia were also implicated in actively enhancing mass transport of nutrients and oxygen (Shapiro et al. 2014).

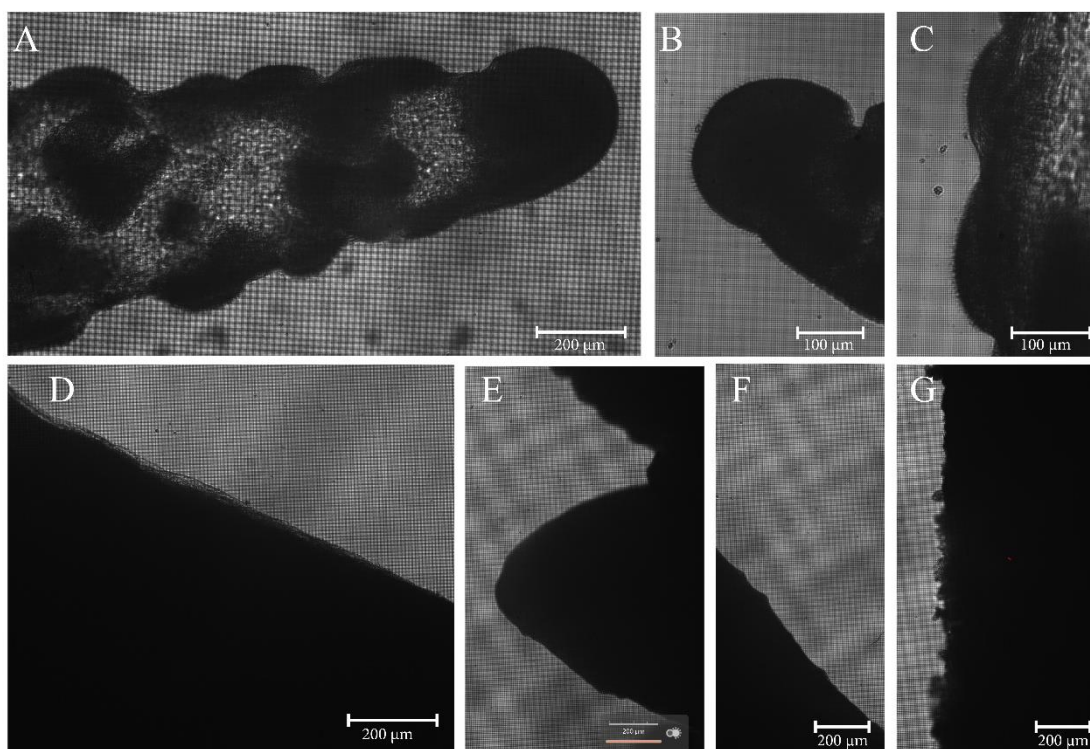
Particle removal mechanisms in warm-water corals frequently involve tissue expansion and ciliary movement as well as mucus release (Stafford-Smith and Ormond 1992; Stafford-Smith 1993; Gass 2006). Tissue expansion has so far not been observed in *L. pertusa*, however, mucus release has been observed

and quantified (Shelton 1980; Allers et al. 2013; Larsson et al. 2013; T. Baussant, unpub.). A recent study has now further confirmed the involvement of mucus in particle removal using a novel technique, so-called digital holographic microscopy (DHM) (Zetsche et al. 2016). This technique enables observations of the behaviour and physiology of *L. pertusa* in vivo at the  $\mu\text{m}$ - to cm-scale, and allows the imaging of otherwise transparent substances such as coral mucus. As a result of the high magnification of DHM, it is also possible to observe the tentacle surface of the individual coral polyps. We hereby present the results of these new observations and discuss their potential implications for our understanding of sediment removal and the resilience to sedimentation effects of the cold-water coral *L. pertusa*.

### Materials and methods

Details of specimen collection, the treatment with particle suspensions, and the imaging set-up and technique are given in Zetsche et al. (2016). Briefly, individual fragments of *L. pertusa*, which were maintained at the IRIS laboratory facilities, were used for this study. The corals were kept in the dark in flow-through seawater conditions ( $7.5 \pm 0.2^\circ\text{C}$ , salinity  $33 \pm 0.5$ ) and fed 2-3 times a week with a solution of live *Artemia salina* nauplii. The fragments were used as part of a larger study (Project HAVKYST-ES460364) investigating the sensitivity of cold-water communities, in particular *L. pertusa*, to drilling mud. This study was conducted in Dec 2013 when fragments were in the recovery phase of an experiment, after an exposure period of 2.5 weeks to different levels of drilling muds (Provan et al. in press). This study used polyps from the control treatment and two treatments with drilling mud, 4 and 14 mg L<sup>-1</sup> respectively.

*L. pertusa* fragments were imaged with a digital holographic microscope (oLine D3HM, Ovizio Imaging Systems, Belgium) in DHM-adapted, flow-through chambers, holding approx. 615 mL of seawater. The water in the chambers was maintained at a temperature of  $7.5 \pm 0.2^\circ\text{C}$  with a flow rate of approx. 10 mL min<sup>-1</sup>. Fragments consisted of 9–15 polyps, which were gently positioned in the chambers avoiding contact with the chamber walls. Fragments were subjected to solutions of suspended particles, either charcoal (average grain size  $\sim 30 \mu\text{m}$ ), or water-based drill cuttings (fine silt particles  $< 63 \mu\text{m}$ , predominantly  $\sim 20 \mu\text{m}$ ; final solution  $26 \pm 11 \text{ mg L}^{-1}$ ).



**Figure 1.** Images are taken from different polyps. A-C: On close inspection cilia are visible along the length of each tentacle. What appears to be a halo in the cilia array (B-C) is an optical artefact caused by the density and 3-D structure of the tentacles and does not indicate the presence of a mucus layer. D-G: No cilia are visible along the theca of the polyps, neither at their base (G) nor close to the oral disc (E).

L-1). Solutions with particles were gently added close to the polyps with a Pasteur pipette, before images and videos were captured with the oLine D3HM.

Compared to bright field microscopy, DHM has the ability to obtain not only light intensity information, but also quantitative phase information (Zetsche et al. 2014). A key advantage is its ability to detect and image transparent substances if the refractive index between two objects is different enough, for example between coral mucus and its surrounding seawater (Zetsche et al. 2016). The D3HM was used with a red LED light (630 nm) and a Leica 4x objective, providing a field-of-view of approx. 1.6 x 1.6 mm, which allowed us to image *L. pertusa*'s polyps at high resolution in vivo and in real-time.

## Results

Cilia were observed on all image records of tentacles, were approx. 4-7 µm in length, and extended in a continuous layer from the tips to the bases of the tentacles (Fig. 1A-C). No cilia were observed on the theca of the polyps, in theca covered with coenosarc or in areas without coral tissue (Fig. 1D-G). Charcoal

particles and drill cuttings only attached to the cilia surface layer of the tentacles as individual particles or small aggregates (Fig. 2). These particles also never reached the actual surface of the tentacles; they always remained at a distance of several µm above or within the layer of cilia. Whether mucus was involved in this entrapment or individual particles themselves were of a sticky nature was not clearly determined. In contrast, particles elsewhere on the polyp with no cilia were indeed

trapped in mucus strings and sheaths (Zetsche et al. 2016).

Most of the individual particles observed along the cilia of the tentacles were also moved along the tentacles, generally towards the tips of the tentacles. On one tentacle, where detailed observations with the DHM were made, sediment rejection of several individual particles and of small aggregates to the surrounding seawater followed the same trajectory, moving along the tentacle's cilia towards the tentacle tip where the final removal took place (Fig. 3).

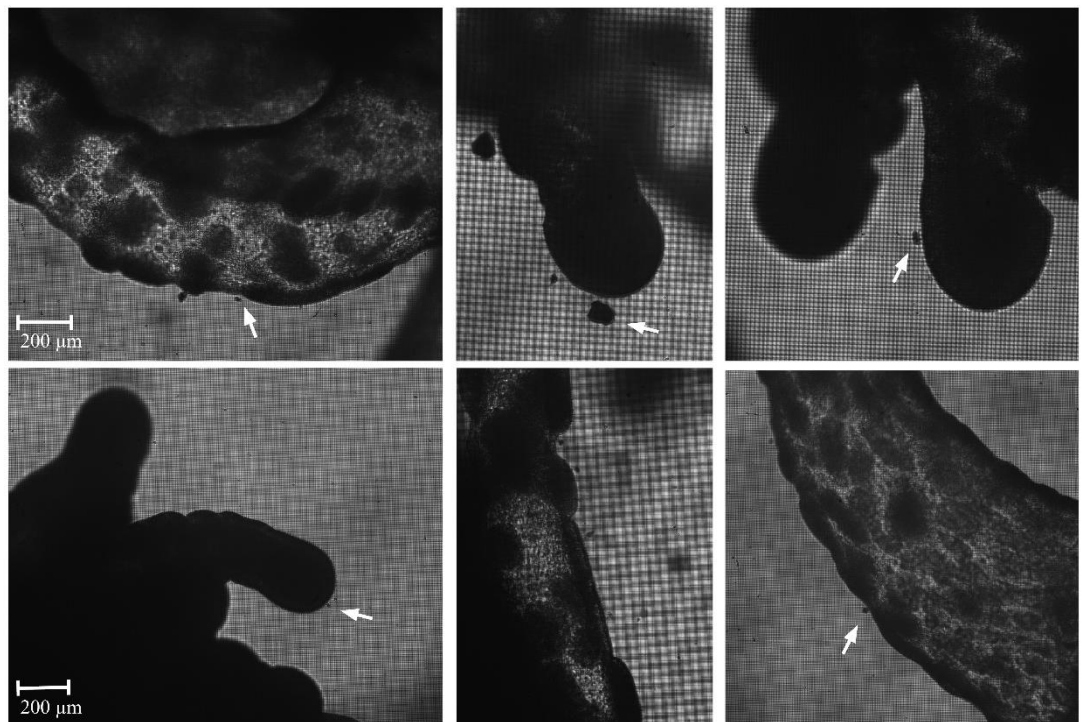
## Discussion

A common feature among scleractinians is the presence of cilia on the epidermis (Lewis and Price 1976; Stafford-Smith and Ormond 1992; Stafford-Smith 1993). Cilia were clearly visible on the D3HM images in a continuous layer (several µm wide) extending from the base to the tip of the tentacles. Shelton (1980) described the presence of motile cilia on the oral disc and tentacles of *L. pertusa*. In this present study, as in others, ciliary movement was suggested, based on movements observed after the

addition of particles. Unprecedented images were obtained with the DHM and visual confirmation of the presence of cilia in *L. pertusa* and of their involvement in the rejection of individual particles clearly obtained.

Particle movement towards and away from the tentacle tip was recorded during several time-lapse observations. The time of particle removal in Fig. 3, for example, varied between 3 sec (Fig. 3 C - E) and ~16 sec (Fig. 3 G - J). It remains unclear whether any mucus was involved in this ciliary movement as mucus was not detectable here with DHM. Movement of mucus-trapped particles along the thecal regions was not mediated by ciliary movement (Zetsche et al. 2016).

Particles do not appear to settle easily onto the tentacles of *L. pertusa*; only few individual particles or small clumps of particles were moved along the tentacles directly (Fig. 2, 3). Shapiro et al. (2014) recently showed that strong vortical flows driven by epidermal motile cilia increased the overall mass transport of solutes in several tropical corals. We speculate that such vortices may also hinder the settling of particles onto the coral's surface. Zetsche et al. (2016) described the occurrence of circular motions of mucus strings and mucus balls containing particles, which suggests that flow patterns with vortices were not only driving mucus transport in *L. pertusa*, but may also influence the settling of particles. Although the magnification used in this study does not allow us to determine whether the cilia were moving passively or actively, it is known that even passive cilia can be used to create self-cleaning surfaces provided there is ambient water flow (Tripathi et al. 2014), as is often the case in *L. pertusa*'s habitat. Future studies using



**Figure 2.** Single particles or small aggregates of particles were observed only at isolated sites along the tentacles of the various polyps. The particles never touched the tentacle surface itself, but remained at a distance above or within the cilia layer. The given scale bars apply to all images.

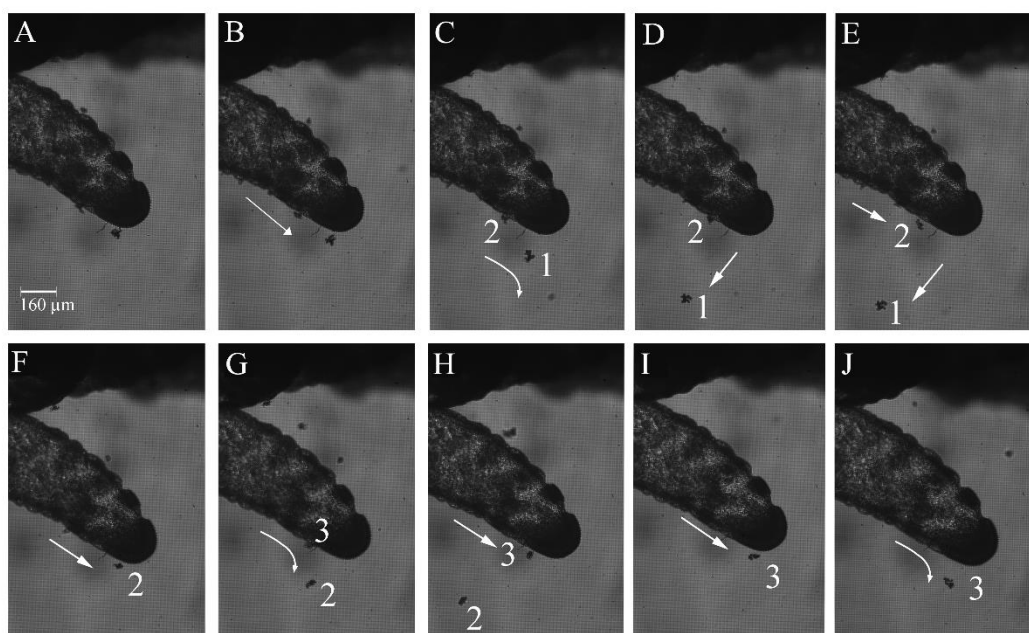
higher magnification may help elucidate this point further.

## Conclusions

The presence of cilia on the tentacles of *L. pertusa* was confirmed, and their involvement in sediment removal observed, by using a novel microscopy technique that maintained polyps in vivo and allowed observations at the μm- to cm-scale in real-time. Recent interest in cilia as a natural defence against biofouling has sparked several studies modelling the behaviour of cilia in terms of (ambient) flows as well as actively or passively settling particles (Shum et al. 2013; Tripathi et al. 2014). These have shed light on the importance of cilia as a protective layer and may explain their role in *L. pertusa* and other coral species as a sediment removal mechanism. These observations may also explain why motile epidermal cilia have been retained by scleractinian corals in their evolutionary history (Shapiro et al. 2014). The movement of cilia requires little energy (<0.1% of the coral's total energy budget; Shapiro et al. 2014) and may partly explain the ability of *L. pertusa* to cope efficiently with sedimentation exposure (Allers et al. 2013; Larsson and Purser 2011; Larsson et al. 2013; T. Baussant, unpub.), at least in the



short-term and below a certain sediment load. Above a given threshold, cilia exhaustion may potentially occur (Stafford-Smith 1993), and their function in cleaning can be disrupted, leading to sedimentation, and in the worst cases death of the coral (Brooke et al. 2009). Further studies are needed to elucidate more explicitly the active role of cilia and their interaction with other sediment removal mechanisms, such as mucus release.



**Figure 3.** The image sequence shows the movement of particles along the cilia of one tentacle and their expulsion at almost the same position each time. Arrows indicate approximate directions and numbers track specific activated charcoal particles. The scale bar shown in A applies to the entire image sequence.

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