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SHORT COMMUNICATION

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First detection of gas bubble disease and *Rickettsia*-like organisms in *Paphies ventricosa*, a New Zealand surf clam

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Gas bubble disease (GBD) is a non-infectious condition in aquatic organisms caused by supersaturated levels of total dissolved gas (TDG) in water (Bouck, 1980). GBD is analogous to "the bends" (decompression sickness) in human scuba divers (Barratt, Harch, & Van Meter, 2002). In fish, the disease can manifest externally as bubbles on the eyes, opercula, fins, body and mouth regions, and internally as bubbles in the blood and on gill arches (Espmark, Hjelde, & Baeverfjord, 2010). GBD has also been reported in invertebrates, including in shrimp, blue crab, oysters and clams (Johnson, 1976; Lightner, Salser, & Wheeler, 1985; Malouf, Keck, Maurer, & Epifanio, 1972). The effects of GBD can range from mild to lethal depending on the level of TDG supersaturation, the species affected, life history stage, animal health, depth distribution and water temperature (Beeman et al., 2003; Johnson, 1976; Smiley, Drawbridge, Okihiro, & Kaufmann, 2011).

Gas bubble disease is frequently observed in finfish aquaculture, where poor control of oxygenation can result in extreme saturation of TDG in water (Smiley et al., 2011). Gas bubble disease is less common in the wild, but has been recorded in fish living in the heated water discharges from power stations or below plunge pools from dams and spillways (Gulliver & Groeneveld, 2010; Mcinerny, 1990). Relative to finfish, there are very few accounts of GBD in invertebrates, particularly in the wild (Moiseev, Moiseeva, Ryazanova, & Lapteva, 2013). Malouf et al. (1972) described GBD in oysters and clams held in heated running sea water. In this instance, GBD caused blisters to form on the mantle and valves of oysters (*Crassostrea virginica* and *C. gigas*), and for gas bubbles to form on the gill filaments of both oysters and clams (*Mercenaria mercenaria*).

In November 2016, blisters were observed under the periostracum on the outer valve surfaces of *Paphies ventricosa* (toheroa; Figure 1) at Ripiro Beach on the west coast of northern New Zealand. Although prevalence rates of blisters were not quantified, they were observed on a high proportion of toheroa across approximately 40 km of coastline. Toheroa are a species of large intertidal surf clam endemic to New Zealand (Williams, Sim-Smith, & Paterson, 2013). At the start of the 20th century, extensive toheroa populations were present on exposed west-facing surf beaches of northern and central New Zealand and on the south coast of the South Island. Increased popularity and harvesting pressure from the early 1900s by both commercial and recreational fisheries depleted the resource (Murton, 2006; Williams, Sim-Smith, et al., 2013). By the mid-20th century, toheroa populations declined to levels where their commercial harvest was no longer viable. All commercial harvest ceased by 1969 and regional recreational fishery closures occurred from 1971 to 1980 (Williams, Sim-Smith, et al., 2013). Despite having been protected for between 35 and 45 years, toheroa populations nationwide have, for unknown reasons, failed to recover (Williams, Ferguson, & Tuck, 2013).

Following the detection of blistered toheroa shells at Ripiro Beach, ten live toheroa, ranging in length from 59 to 91 mm, were collected and sent to the Ministry for Primary Industries (MPI) Animal Health Laboratory (AHL) to be examined for the presence of exotic and endemic pathogens or other signs of ill health. After gross examination and dissection of shell blisters, tissue samples were fixed and embedded for histology; DNA extracted and screened; and the mantle and gills swabbed onto agar media for general bacterial culture. This examination revealed that blisters were located both under the periostracum and under the outer calcite layer above the foliate conchiolin, giving the blisters a thin translucent white cap of calcite. There was no evidence of gas bubbles in other tissues. It was concluded that GBD, as described by Malouf et al. (1972), was the cause of the observed blisters. The exact mechanisms by which toheroa may have been exposed to supersaturated levels of TDG in water or the reason that bubbles appear to have only formed on the outer surfaces of toheroa shells are unknown. Current hypotheses regarding exposure to TDG supersaturation include the rapid heating of sea or groundwater percolating through hot intertidal sand (up to

FIGURE 1 Blisters under the periostracum and outer calcite layer of the outer valve surfaces of a toheroa (*Paphies ventricosa*; shell length = 80 mm)

40°C; J. Cope, *unpublished data*) or the heating of sea water retained within the shell and tissues of the toheroa buried in sun-heated sand while the tide is out. For example, if sea water with an initial temperature of 18°C was heated to 30°C within a toheroa, oxygen solubility would drop from 7.48 to 6.09 mg/L, exposing the toheroa to oxygen supersaturation of 122%, a level sufficient to cause GBD-related symptoms in a range of fish species (Geist, Linley, Cullinan, & Deng, 2013; Smiley et al., 2011; Weiland, Mesa, & Maule, 1999).

In addition to the identification of GBD in toheroa, bacterial isolation identified several species of aquatic environmental bacteria. This included *Rhanella* sp., a bacterium associated with gut flora and sand that is not commonly seen in New Zealand shellfish (*pers.obs.*). *Rickettsia*-like organisms (RLOs) were also present in the gills of all ten toheroa examined, where they disrupted gill architecture. RLOs are obligate, intracellular parasites associated with a variety of vertebrate and invertebrate hosts (Gollas-Galván, Avila-Villa, Martínez-Porchas, & Hernandez-Lopez, 2014). Despite their importance as causative agents of severe mortality outbreaks in farmed aquatic species, little is known about their life cycle or host range (Ferrantini et al., 2009). RLOs were first detected in marine bivalves in the 1970s (Harshbarger, Chang, & Otto, 1977). They are typically transmitted directly between hosts via water-borne transmission and may be found free within host cell cytoplasm or within intercytoplasmic vacuoles (Friedman & Crosson, 2012; Travers Boettcher Miller, Roque, & Friedman, 2015). Although RLO infections in teleost fish have been extensively studied (Rozas & Enríquez, 2014; Stride, Polkinghorne, & Nowak, 2014), those affecting molluscs, other than the RLO causing withering syndrome in abalone, have not (Tavers et al., 2015). RLOs have been associated with diseases and mortality in scallops, abalone, clams and oysters (Carvahlo, Poersch, & Romano, 2013; Moore, Robbins, & Friedman, 2000; Sun & Wu, 2004). Although not all RLO infections result in mortality, information relating to the immune responses of molluscs to RLOs is scarce (Gollas-Galván et al., 2014). Since 2015, RLOs have been associated with at least six New Zealand shellfish mortalities events, investigated by the MPI AHL (A. Pande, *pers. obs*). The known New Zealand shellfish hosts of RLOs include scallops (*Pecten novaezelandiae*), pipis (*Paphies australis*), mussels (*Perna canaliculus*), cockles (*Austrovenus stutchburyi*) and now toheroa (Hine & Diggles, 2002; B. Jones, *pers. obs.*).

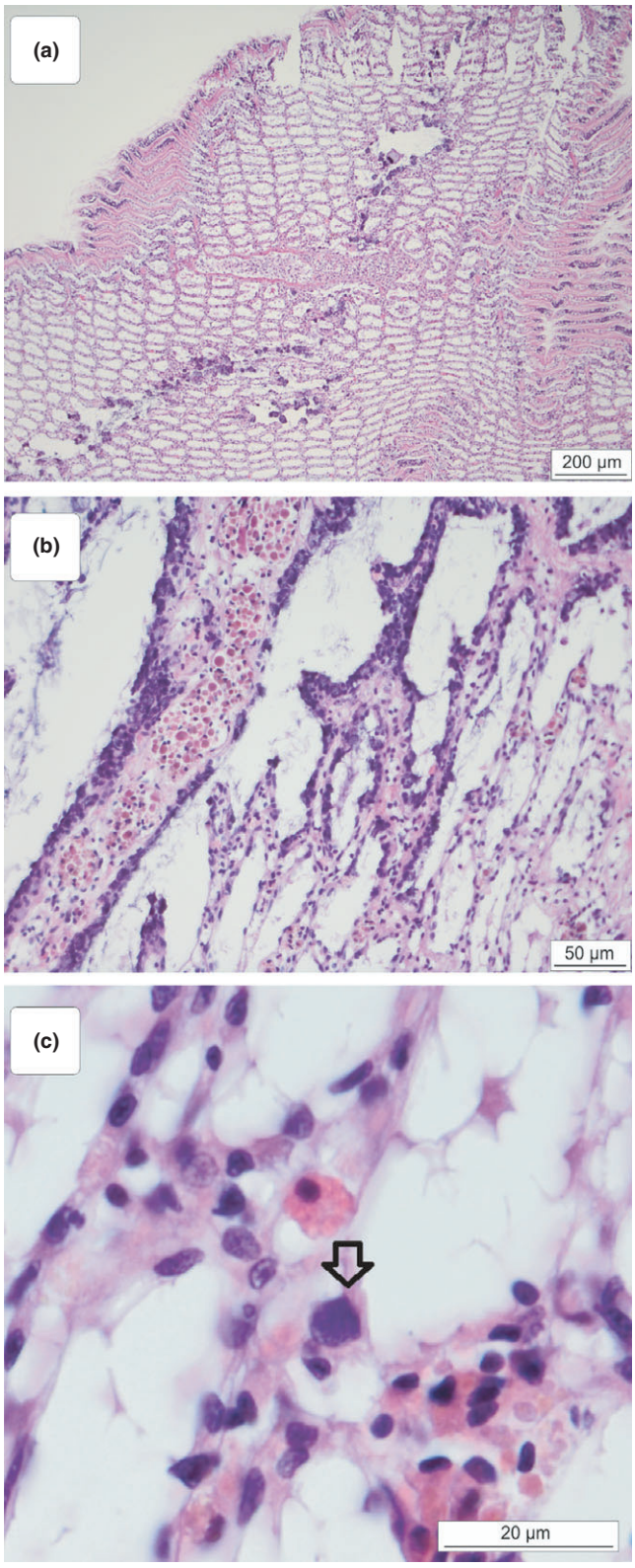

FIGURE 2 Haematoxylin- and eosin-stained toheroa (*Paphies ventricosa*) gill tissues at 10× (a), 40× (b) and 100× (in oil) (c) magnification. RLO-affected cells stain intensely basophilic (dark purple). RLOs are associated with areas where gill architecture is damaged (i.e., gill lattice is broken). Panel c shows an infected epithelial cell (indicated by arrow) immediately below an eosinophilic granulocyte. Two more granulocytes and several hyaline haemocytes are in the vessel to the right of the infected cell. ×100 oil

In toheroa, histology revealed colonies of RLOs within the branchial epithelia (gills) of all specimens and damage to host epithelial cells. The observed damage consisted of broken connections between the lateral ordinary filaments where epithelial cells are focally infected with RLOs leaving holes in the branchial lattice structure (Figure 2). Following the collection of GBD-affected toheroa at Ripiro Beach, there was some evidence of shellfish mortality including: areas of beach with greater than usual numbers of recently deceased (still hinged with intact periostracum) toheroa and tuatua (*Paphies subtriangulata*; J. Cope, *pers. obs.*); observations of recently deceased toheroa shells with blistered periostracum (Figure 1); and in some locations the smell of decaying shellfish emanating from the sand at known shellfish beds (B. Searle, *pers. comm.*).

Because the taxonomy of RLOs is poorly resolved, it is difficult to know at this stage whether RLOs observed in New Zealand molluscs are all the same species, are the causes of mortality events observed in toheroa (or other shellfish species), or whether the toheroa RLOs are native or introduced. Mass mortalities in toheroa have been reported in 1888, 1900, 1917, 1932, 1938, 1956–1959, 1970–1971, 2001 and 2013 (Williams, Sim-Smith, et al., 2013). Few of these events have been thoroughly investigated, and there is only speculation as to the causes of individual events (Akroyd, 2002; Carbines, 1997; Eggleston & Hickman, 1972; Hine & Wesney, 1997; Williams, Sim-Smith, et al., 2013). It is possible that RLOs have contributed to some of these mortality events, potentially in conjunction with GBD or other stressors. Mortalities in aquatic organisms are often multifactorial involving primary and secondary pathogens with effects that may be instigated or exacerbated by stressful environmental conditions. For example, Weiland et al. (1999) reported that chinook salmon with a bacterial infection were more vulnerable to gas bubble disease than healthy fish. In the case of toheroa, it is unknown whether RLOs are related to the detection of GBD, whether one condition is facilitating the other through increased vulnerability to TDG supersaturation or pathogens, or whether there are other factors at play. Targeted investigations will be required to ascertain causative mechanisms.

The discovery of RLOs and gas bubble disease in toheroa is of interest and may ultimately explain observed toheroa mass mortality events and the failure of toheroa to recover from unsustainable harvesting practices of the 20th century (Williams, Sim-Smith, et al., 2013). More work is required to understand the physiological consequences of these two conditions, any interactions between them, and to determine their distribution across species, space (locations) and time (seasonal patterns). The spread of pathogens to unaffected populations should be avoided. Consequently, the detection of RLOs will likely have implications for future toheroa aquaculture and for the translocation of toheroa, and other shellfish, between beaches for aquaculture, reseeding or enhancement purposes.

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