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The health status of mussels, *Mytilus* spp., in Ireland and Wales with the molecular identification of a previously undescribed haplosporidian

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ABSTRACT

Both wild and cultured mussels (*Mytilus edulis*, *Mytilus galloprovincialis* and hybrids), are found along most of the Irish coastline. *M. edulis* is widespread along all Irish coasts and is the only mussel species present on both the east coast of Ireland and the Welsh coast in the Irish Sea. *M. galloprovincialis* and hybrids are found along the Irish coastline except for the east coast. Samples of *Mytilus* spp. were collected from twenty-four sites, encompassing all coasts of Ireland and the Welsh coast, at different times of the year and over several years (2008–2011). In total, 841 mussels were examined histologically to assess their health status and the presence of any parasites or commensals. Mussels from 14 of the 24 sites were screened using polymerase chain reaction (PCR) to determine which mytilid species were present. A range of parasites were observed, generally at low levels. The most diverse community of parasites was observed at a sheltered site with poor water quality. Of significance, a previously undescribed haplosporidian was detected in a single mussel sample in the Menai Strait, Wales, by PCR and was confirmed by direct sequencing and is most closely related to *Minchina chitonis* and a haplosporidian of the Florida marsh clam *Cyrenoida floridana*. While *M. edulis* were infected by a variety of micro- and macro-parasites, only trematodes were observed in *M. galloprovincialis* and hybrids. Habitat description and the environmental factors influencing the study sites, including water quality and exposure, were recorded.

1. Introduction

Mytilus edulis are boreo-temperate in their distribution on both coasts of the Atlantic Ocean and are found in abundance, intertidally and subtidally, in both sheltered and exposed sites, attached to hard substrates or forming biogenic reefs. In the western Atlantic, *M. edulis* is historically found from the Arctic Sea, Canada (Dall, 1889) to North Carolina, United States (Stimpson, 1860: McDougall, 1943) and in the eastern Atlantic occurs from Norway (Christiansen, 1965) to the border of France and Spain (Sanjuan et al., 1994). *Mytilus galloprovincialis* is endemic to the Mediterranean, Black Sea and Adriatic Sea and has expanded its range to the British Isles (Gosling, 1992). Evidence of hybridisation and hybrid zones of *M. edulis* and *M. galloprovincialis* in the south west of England and Ireland were first recorded in the 1970s and subsequent studies have further documented this phenomenon (Gosling and Wilkins, 1977; Skibinski et al., 1983; Gosling and McGrath, 1990; Gardner, 1997; Gosling et al., 2008). Hybrids are commonly thought to have lower fitness (Mayr, 1963), however, certain studies have shown "hybrid vigour" (heterosis) in the first hybrid generation (F1 hybrid) which may allow hybrids to function over a wider range of environmental conditions than the two parental species (Littlejohn and Watson, 1985). Both mytilid species are important commercially in Europe and mussel seed is collected from wild beds or collector ropes and transferred to other areas for on-growing (Fuentes et al., 2002; Kijewski et al., 2009). In Europe, *M. edulis* is harvested extensively from both wild and farmed sources while *M. galloprovincialis* is harvested from cultured stock.

Parasites of mussels can have a detrimental effect on both natural and cultured stocks (Sindermann and Rosenfield, 1967) and it is acknowledged that the prevalence of parasites, mussel condition index and associated mussel mortalities should be examined more

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extensively (http://www.fao.org/fishery/culturedspecies/Mytilus_galloprovincialis/en). Environmental factors such as poor water quality and the presence of parasites have been shown to have had an impact on the health status of mussel stocks in Sweden and the UK (Svärdh, 2003; Bignell et al., 2008). A decrease in water quality can affect the immunological response in aquatic organisms thus making them more susceptible to parasitic infection and increasing parasite prevalence (Khan and Thulin, 1991). Organic pollution is associated with a decrease in dissolved oxygen, which creates a favourable environment for bacteria, while inert suspended solids can damage the gill epethelium and make individuals more susceptible to infection with fungi, etc. (Svobodova et al., 1993). Mussels are commonly used as environmental indicators based on surrounding water quality, which may in turn impact on their susceptibility to disease and parasites.

It has been suggested that high population densities associated with aquaculture may trigger a disease outbreak (Morley, 2010). Several parasites and pathogens have been identified in *M. edulis* and *M. galloprovincialis* (Bayne, 1976; Paul, 1983; Bower et al., 1994; Gosling, 1992; Calvo-Ugarteburu and McQuaid, 1998; Bower et al., 1994; Le Roux et al., 2001; da Silva et al., 2002; Ciacci et al., 2009). The two individual mytilid species, together with their hybrids, are considered to be susceptible to a similar diversity of pathogens (Bignell et al., 2008). A recent study investigating the occurrence of macroparasites in several common intertidal molluscs on the south coast of Ireland detected four trematode species in *Mytilus* spp. and the copepod *M. intestinalis* (Prinz et al., 2010).

The objectives of this study were (a) to investigate the health status of mussels around the entire coast of Ireland and Wales, encompassing both the Atlantic coast and the Irish Sea area, (b) identify the range of parasites and pathogens present at the individual level at different sites, (c) determine if any apparent difference in susceptibility to parasites exists between each mytilid species or hybrids and (d) examine the effect of site-related environmental factors, such as water quality and site exposure on health status.

2. Materials and methods

2.1. Sampling

In this study, a total of 751 mussels from both wild and cultured stocks were sampled from a total of twenty-one sites (including four sites within Cork Harbour on the south coast of Ireland) on all Irish coasts and three sites on the Welsh coast (two in north Wales and one in south Wales), at different times, encompassing all seasons from November 2008 to July 2011. The mussels were collected with sample sizes varying from 14 to 60 individuals. All wild mussels were collected from the intertidal zone and cultured mussels were sampled subtidally (Fig. 1 and Table 1).

A water quality classification system obtained from the Environmental Protection Agency (http://www.epa.i.e./water/watmg/wfd/classification/) was applied to the different study sites based on anthropogenic inputs such as agricultural run-off, leachate from landfills and contaminated sites, untreated waste water and sewage discharge, increased recreational and boating use and industrial run-off in the surrounding catchment area at each site: Class A (very little if any anthropogenic effects), Class B (some anthropogenic effects) and Class C (site influenced greatly by anthropogenic effects).

2.2. Histology

A transverse section of mussel tissue (~1 cm^2) containing mantle, gill, digestive gland and gonad was excised and fixed in Davidson's fixative at 4 °C for 48 h (Shaw and Battle, 1957) before being transferred to 70% ethanol. The fixed tissue was then dehydrated through an ascending ethanol series and embedded in paraffin wax. A 5 μm tissue section was stained using haematoxylin and eosin. Sections were examined at 40× and at 100× under oil.

2.3. DNA extraction and standard polymerase chain reaction (PCR) for *Mytilus* species identification

Gill tissue was excised from 365 individual mussels from 11 of the 21 Irish sites and 90 mussels (from all 3 sites) in Wales and was stored in 96% ethanol (Table 2). DNA was extracted using the chelex-100 extraction method (Walsh et al., 1991; Lynch et al., 2008). A PCR was carried out to amplify the nuclear DNA markers Me15/Me16 to differentiate which *Mytilus* species or hybrid was being screened (Inoue et al., 1995). The PCR mastermix was modified slightly to include 5x green buffer. Amplification was conducted in 25 μl of the reaction mixture containing 14 μl ddH_2O, 5 μl 5× green buffer (Promegal), 2.5 μl of each of the four deoxyribonucleotide triphosphates (dATP, dCTP, dGTP, dTTP), 1.5 μl $MgCl_2$, 0.5 μl

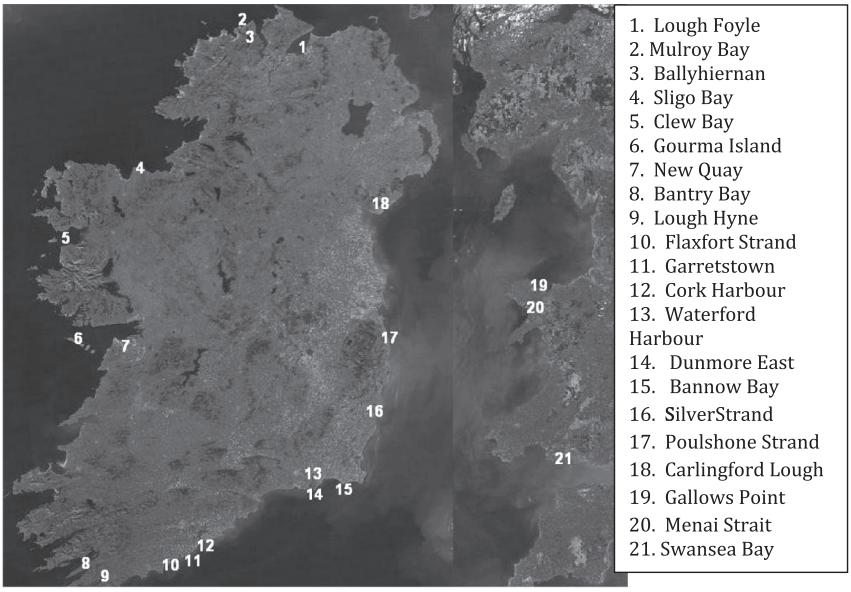

Fig. 1. Map of Ireland and Wales showing *Mytilus* spp. sampling sites.