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CSG20: Investigating the spawning period of a Priority marine feature (flame shells) using ferry plankton samplers

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Background

Flame shells, *Limaria hians*, are a biogenic bivalve mollusc which build nests on the seabed. When large aggregations of these nests combine, they form a dense biogenic reef known as a flame shell bed. These beds create habitats which support a rich associated community of benthic and epi-benthic species creating biodiversity hotspots (Trigg and Moore, 2009) in addition to providing several ecosystem services, such as seabed stabilisation (Trigg *et al*., 2011). Given their size and global scarcity of the habitat, Scotland's flame shell beds are of international importance. The habitat is classified as a Priority Marine Feature (PMF) in Scottish waters, where they are protected by a suite of Marine Protected Areas (MPAs) (Millar *et al*., 2019).

Like many benthic marine invertebrate populations, flame shell beds are thought to be connected via larval dispersal. Ontogenic shifts in spatial distribution are common in marine systems, particularly in benthic invertebrates, where the pelagic larval phases settle in appropriate settlement sites after drifting with oceanic currents (Chen *et al*., 2014). We refer to these spawning locations as sources and the settlement sites as sinks. Understanding how populations are connected and the directionality of flow between source and sink populations are key to the design and management of effective MPA networks (Cowen and Sponaugle, 2009).

Increasingly, coupled hydrodynamic and particle tracking models, also known as bio-physical models, are being used to model dispersal patterns from source to sink locations (e.g., Elsäßer *et al*., 2013). Information on the timing of spawning, distribution of suitable settlement habitat, and larval behaviour are all required to successfully and effectively model larval dispersal, with the validation of such models heavily depending on the availability and accuracy of such information (Millar *et al*., 2019). Unfortunately, due to the inherent difficulties associated with quantifying spawning behaviour and associated life history traits in benthic invertebrates (Cowen and Sponaugle, 2009), there is limited information available about flame shell spawning characteristics.

Observations by Trigg (2009) suggest that flame shells in Loch Creran spawn between May and July, however, there have been no further surveys to confirm the timing of spawning or the spawning strategy. Flame shell beds are found predominately along the west coast of Scotland with the most northerly beds in Orkney and the most southerly beds located in the South Arran MPA. Studies of flame shell larvae in Ireland and Plymouth suggest that there may be latitudinal variation in spawning within the species (Lebour, 1937; Minchin, 1995), meaning that the timing of spawning could vary between Scottish beds given their widespread distribution.

Field Study Overview

The primary goal of this project was to collect a time-series of flame shell larvae abundance during the reported spawning season to address data deficiencies about the spawning seasonality and strategy of flame shells.

Traditional larval dispersal field studies are typically expensive, labour intensive, and often only provide insight into a single time period at a single location. To overcome these limitations and address data deficiencies about flame shell spawning characteristics, the project designed and commissioned a custom-built zooplankton filtration system which could be fitted onto a commercial passenger ferry to facilitate the routine sampling of zooplankton communities (Figure 1). The concept was based off a previous system used by Stirling (2016) to investigate the spawning and seasonality of the fan mussel, *Atrina fragilis*. The design was modified for installation on the *MV Isle of Mull* (CalMac Ferries Ltd.) by Allan Sloan and the system was constructed by the engineering department at Marine Scotland Science.

Figure 1. Image of the assembled ferry filter system.

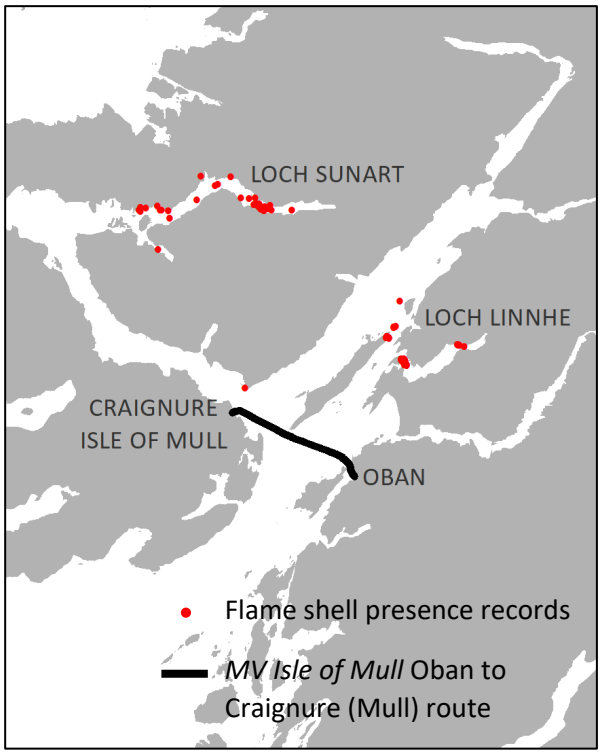

Figure 2. Map showing the *MV Isle of Mull*'s Oban to Craignure (Mull) route (black line) and the nearby presence records of flame shells (red dots) in Loch Sunart and Loch Linnhe.

The system was installed in the *MV Isle of Mull*, which operates in the vicinity of two known flame shell beds within Loch Linnhe and Loch Sunart (Moore *et al*., 2018, Figure 2). The system was operated by the ship's engineering department for 12 weeks between May 2021 and August 2021, during the suspected spawning season, as the vessel conducted its routine schedule between Oban and Craignure, Isle of Mull. Weekly samples were collected and preserved alternately in formalin for morphological analysis of the plankton communities and absolute ethanol for DNA analysis. In total 12 samples were obtained, with 6 being preserved in formalin and the remaining samples preserved in ethanol. From the morphological samples we will calculate the abundance of flame shell larvae in

the water column across the sampling period. The genetic samples will be used to complement ongoing research into the genetic connectivity of flame shell populations.

Use of Funds

Funding from this grant was used to cover fuel expenses and subsistence for two return journeys from Aberdeen to Oban for the delivery and retrieval of the filter system and samples at the beginning and end of the sampling period. Leftover funds were used to purchase phenol-chloroform, a key component of our DNA extraction protocol.

Future Work

Having collected the samples, the next stage of this process will be to analyse them. For the genetic samples this will involve designing primers and trialling them on tissue samples to ensure their efficacy and sensitivity before use on DNA extracted from ethanol-preserved zooplankton samples. Morphologically identifying the larvae will be more challenging. The identification of bivalve larvae down to the species level using morphology alone is often very difficult due to the similarities in morphology of the larvae of many species (Garland and Zimmer, 2002). Samples will be viewed using a light microscope with suspected specimens compared to Lebour's (1937) sketches which are currently the only published images of flame shell larvae. Confirmed specimens will be measured and photographed. The overall counts from the weekly samples will be used to map the commencement, peak and cessation of spawning.

We hope to repeat the study again in the summer of 2022, with modifications to the system to permit the collection of two samples simultaneously. This will ensure that all morphological samples have a genetic counterpart which can be screened for flame shell larvae.

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