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# **Investigating invasive species settlement in relation to material properties of concrete.**

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## Introduction:

Harbours, which are used by a wide range of industries including the oil and gas sector, are hubs for biofouling marine invasive species (Marchini *et al.*, 2015), as shipping is the largest vector for the spread of marine invasive species (Molnar *et al.*, 2008).. Biofouling is the settlement of marine organisms on a man-made surface (Callow and Callow 2002). Marine invasive species are species that cause economic and ecological harm. In the UK invasive species cost £1.7 billion annually (Williams 2010). Currently there are no methods to stop settlement of marine invasive species on surfaces in harbours. Concrete is the most used material in the world, including oceans and harbours (Xiao *et al.*, 2017). Concrete has been shown to be biofouled by marine invasive species in harbours (Dumont *et al.*, 2011; Jebakumar *et al.*, 2017; McManus *et al.*, 2018). The surface properties of concrete have however been shown to alter biofouling communities and species. This small grant supported fieldwork and laboratory work as part of a PhD thesis investigating if it is possible to reduce marine invasive species settlement by altering the surface of concrete.

## Funding:

Funding from this grant was spent on equipment for the laboratory spawning study discussed below, and travel to harbours to search for marine invasive species. Currently, some of the money remains, which as due to be used to repeat the laboratory trials and travel harbours, this however was not undertaken due to disruption of covid-19. When possible, the remainder of the money will be used for this purpose.

## Methods and Results:

In September 2019, 5 harbours on the east coast of Scotland were visited and the harbours were searched for marine invasive species. One species identified in 2 harbours and growing on panels which were deployed as part of the PhD thesis, was the tunicate *Corella euymota*. *C. euymota* had been successfully spawned in a laboratory environment (Lambert *et al.*, 1995), and was known to spawn and settle within a time frame (24h to spawn, 11 hours to settle) that allowed for laboratory studies (Lambert *et al.*, 1995). The size of the larvae was also taken into consideration, as settlement trials are usually conducted on glass as clear surface easy to examine under a microscope and light, whereas this study would be occurring on concrete. This study recognised that concrete an opaque surface, would be much harder to identify species on, therefore corella spawn at 0.3mm-0.5mm size on the larger scale of marine invertebrate larvae was chosen in hopes of the best attempt at seeing the settled larvae (Bishop *et al.*, 2015). For those reasons this species was then chosen for laboratory settlement studies to allow for field and laboratory comparison.

Populations of this species were also found in local areas of rockyshore in St Andrews Castle Beach, and individuals were taken from here for the purposes of the study. 6 concrete treatments were taken from the PhD thesis, and made into smaller cubes to be tested in a tank environment. All but 1 treatment were successful in being replicated in smaller sized cubes.

6 tanks were chosen as a trade off between requiring high number of replicates to allow for statistical analysis, and the number of *Corella euymota* individuals estimated to have access to

from the field studies. 6 (24L Pets at Home Kids Aquarium with Filter Tank) tanks were used in the trial. The sides of the tank were lined with acetate sheets, to enable them to be removed from the tank and examined closely under microscope for any individuals who settled on the sides instead of the concrete. 5 concrete blocks, one of each type in each tank, were hung off a frame made out of K'NEX parts (K'NEX Rod 54mm Blue and K'NEX Connector 4-way 3D Silver) (Figure 1), using fishing line to hold the frame level and in place. The position of the 5 concrete treatments on the frame, and therefore to the position of the sea squirts, were chosen using a random number generator and was randomly chosen for each of the tanks to help reduce the impact of distance from the sea squirt as a factor in settlement. Two aerators were connected diagonally at either side of the tanks, to help ensure circulation in order to reduce proximity to sea squirt as an influencing factor by creating a regular flow of water around the tank to allow movement, and also provide aeration for the survival of the sea squirts.

**Figure 1:** 6 trial concrete blocks attached to trial of K'NEX frame.

Two trips to were taken to St Andrews on 18th and 19th of October 2019 to collect 12 individuals of *Corella euymota*. Individuals were collected if they were over the size of 2cm long to give them the best chance of all the specimens collected being sexually mature. Individuals were gently from the surface, or removed on shells if possible to ensure spawning larvae remain inside and the organism is not stressed to ensure survival. The individuals were be placed in a small 150ml tub filled with seawater and sealed. They were then placed into a coolbox, which contained iceblocks and temperature probe. The temperature in the coolbox will be maintained at seawater temperature– to ensure survival of the species during transport.

The 6 tanks thoroughly rinsed and dried twice to remove any contaminants from the surface that may stress the seasquirts. The tanks were then placed in a controlled temperature room, at to match the sea temperature at the time (12.5C). The 6 tanks were filled with 20L of artificial seawater tanks (35.5psu). The concrete was added into the tanks and left to acclimatise due to the possibility of pH changes due to the alkalinity of the concrete surface. 2L of were replaced each day to help regulate the any changes in the water. The pH was measured daily and more water was replaced if alkalinity was getting too high to keep the pH stable around 8.1.

Once the collected sea squirts were returned to the lab, the seasquirts were the moved into artificial seawater tanks (35.5psu), where all organisms were placed, fed and left to acclimatise overnight. The laboratory had no windows, and was completely enclosed, the tanks were all kept in light as corella spawns at dawn, therefore remaining in constant light allowed their spawning cycle to be disrupted until in the tanks. The organism were fed with Varicon Iscochrysis Instant algae mix. A 1ml of Isochrysis mix was diluted into 100 ml of seawater and 20ml of that solution was placed in each tank twice daily.

The seawater and the pots used to transport the sea squirts were sterilized (1 milton tablet per 5L) before being poured down the drain.

After acclimatization, two individuals were placed into each tank, fed and the trial began and lasted 7 days. Once the sea squirts were in the tanks the water temperature was adjusted slowly to go to 15C over a period of 24hours, which is the temperature they spawn at according to Lamberts study. 7 days laboratory run time allowed a day for raising the temperature, a few days for spawning which should occur within 24hours of hitting 15C (Lambert *et al.*, 1995), then a few days for the settled spores to grow in size from 0.3 to 0.55+ in hopes of giving more chance of seeing the spores (Bishop *et al.*, 2015). Each day 2L of water would be changed and the seasquirts would be fed twice daily. After the trial had ended the sea squirts were disposed of during the appropriate waste management. The tanks were sterilized by Milton tablets (1 per 5L), or bleach (200ppm) after which the water was poured into the drains.

Physical examination by eye and by microscope examination (Dinolite AM7915MZTL) was used to determine if and how many settled spores were present. The stain Eosin was also used to try highlight the colours of the spores (0.5% aqueous solution, 0.4ml hydrochloric acid and 100ml Distilled Water). 10 of the 12 sea squirts survived for the entire length of the trials, determined by handling seasquirts at the end of the trial and two were disintegrating, whilst the other remained firm and you could visibly see the siphons moving to feed. At least one sea squirt remained alive in every tank. Despite the survival of the majority of the individuals, no individuals spawned in the tanks. Both concrete and acetate sides, as well at the surface of the water were thoroughly examined for spores. Both the acetate and concrete was also rinsed in the eosin stain to try and enhance the visibility of any spores on the surface and none could be found. Due to all individuals not spawning over the trial it was deduced that it was likely out of spawning season for this species. It was suggested to hold off the study until early spring summer 2020 and try different times throughout the season to find the spawning time. However, due to the covid-19 pandemic this was not possible, and this study has not been repeated.

**Conclusion:**

This project proposed a successful methodology to produce small concrete cubes for biofouling settlement trial. This study also proposed a laboratory set up for settlement of the sea squirt *Corella euymota* on concrete, however this was not a success likely due to difference in spawning time and the time the experiment ran. Future research needs to go into spawning time of these populations within Scotland and repeat of the trial to determine the results of the study. This will be done when possible after covid-19 research disruption.

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