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# Ecological studies of the flame shell (*Limaria hians* (Gmelin 1791)) Focusing on habitat restoration.

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## Abstract

The flame shell, *Limaria hians* (*L. hians*) is a reef forming bivalve mollusc of conservation importance. These bivalves form biogenic reefs of byssus threads supporting highly diverse communities. Flame shell beds are considered a rare habitat and are almost exclusively distributed in shallow, sublittoral areas with high tidal flow on the west coast of Scotland. *L. hians* reefs were once more widely distributed and have seen recent decline which is thought to be due to anthropogenic factors. Substrate preference experiments were conducted which confirmed *L. hians* preference for rugose substrates ( $p < 0.05$ ) observed by other researchers with a new balanced experimental design. The results also showed a preference for substrates containing *L. hians* byssus nest material ( $p < 0.05$ ). Additional comparisons were conducted to confirm this preference for nest material. Between light coloured vs dark coloured substrates and biologically active vs non-biologically active substrates these were concluded to not affect the other comparisons. Nest building observations were conducted revealing much greater nest building activity at night ( $p < 0.01$ ) and rapid rebuilding of the nest after disturbance with much of the basic structure rebuilt after 24 hours. Surveys carried out at Port Appin revealed likely further decline of the bed since the last surveys conducted in 2017. Samples were also collected from a location which had not previously been sampled at the North of the port Appin bed. These animals were used in the laboratory experiments and for a size frequency. The size frequency showed similar results to those from animals collected in The Shian bed in 2006/7 and differed from previous Appin surveys.

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## 1.0.0 Introduction

### 1.1.0 Biology of *Limaria hians*

The “flame shell” *Limaria hians* (Gmelin 1791) is a bivalve mollusc of the family *Limidae*. Its common name is reference to its bright orange pallial tentacles which line the mantle edge (figure 2). Although they are mostly of bright orange colour, some are pale almost ghostly white (Trigg, 2009; Robertson-jones, pers. obs). Their other common name, “gaping file shell” is derived from the approximately 50 radiating ridges originating from the umbo resembling a file and its inability to completely close the valves of the shell. *L. hians* is one of two British representatives of the genus *Limaria*, the other being *Limaria loscombi* (Sowerby) (Hayward & Ryland, 1990).

Their range extends from the Lofoten Islands in Northern Norway down to the Canary Islands in the south and is distributed throughout much of the Mediterranean (Tebble, 1976; Tyler-Walters *et al*, 2016). They are absent from the east coast of England and Scotland and are mostly known from the west coast of Scotland (Seaward, 1990). *L. hians* generally occurs on mixed muddy sandy gravel and is often found in the strong tidal flows at the entrances of sea lochs. High tidal flows export much of the nitrogenous waste away from the beds. Therefore, the water quality in these areas tends to be high. It may be found associated with *Modiolus modiolus* (*M. modiolus*)(Connor *et al*, 2004; Younge & Thompson, 1976) as in

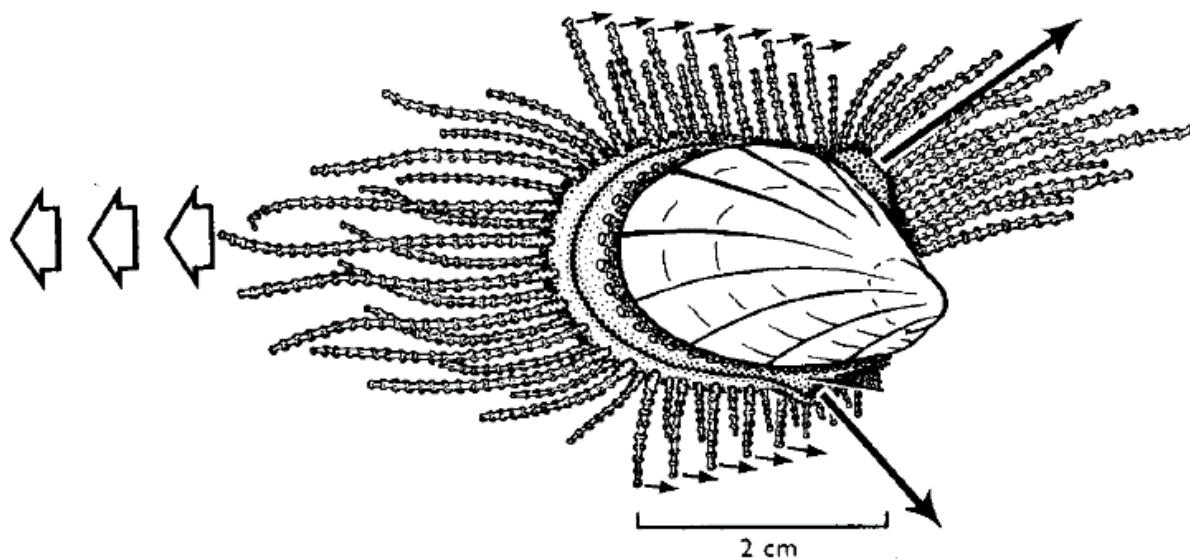


Figure 2. One method of locomotion used by *Limaria hians*. Opening and closing of the valve's pumps water out of the shell in the direction indicated by the black arrows producing a propulsive force in the opposite direction, shown by the white arrows. Adapted from (Gilmour, 1967)

the newly discovered bed in the upper basin of Loch Creran (pers. obs). Maerl beds also often contain *L. hians* (Hall-Spencer & Moore, 2000b). Despite the varied forms flame shell beds can take there is still just one biotope classification for *L. hians* beds, whereas *M. modiolus* is present in 6 and maerl in 7 (Connor *et al*, 2004). They can be found from just below the low water mark to approximately 100m depth (Hayward & Ryland, 1990)

*L. hians* has several defensive adaptations to compensate for its inability to completely seal shut the valves of the shell. Firstly, they construct galleries of byssus threads which offer refuge from predators (Robertson, 1895; Gilchrist, 1895). Secondly, they can autotomise tentacles. Tentacles have many septa along which they can be broken. The tentacles are often said to have repellent properties however this is the subject of much debate. Gilmour (1967) suggested that once exposed to *L. hians* tentacles velvet crabs (*Necora puber*) would avoid contact with *L. hians* in the future however this was from a small number of observations. Thirdly *L. hians* is able to swim to evade predators (Gilmour, 1967). Swimming is performed by opening and closing the valves producing jets of water from the animals anterior (Donovan *et al*, 2004; Gilmour, 1967) analogous to that seen in many scallops (Joll, 1989) (figure 1). Another method of locomotion is through affixing the foot to the substrate and

using it to pull the animal along (Gilchrist, 1895; Gilmour, 1967). They can even use the foot to ascend the vertical glass walls of an aquarium (Gilchrist, 1895; Robertson-Jones, pers. obs).

Trigg (2009) showed that recruitment occurs in the summer, between May and September, peaking in July. Whether this is typical of all flame shell beds is not clear. Some regional differences have been observed in size, with animals from the south reportedly being smaller (Forbes & Haneley, 1853) and it is likely there



Figure 2 *L. hians* specimens in the research facility at Heriot-Watt University, Edinburgh campus. (Photo A. Robertson-Jones)

are similar differences in the timing of spawning and settlement of spat. The largest recorded flame shell was 44 mm shell length (umbo to furthest edge of shell) (Trigg, 2009). The oldest *L. hians* found in Trigg's aging studies was 10 years old but the majority of animals were between 2 and 4 years old. Larvae are reported to remain in the plankton for several weeks before settlement. They then take approximately 2.5 months to attain a size of 2mm (Lebour, 1937).

### 1.2.0 Biogenic reefs

*L. hians* secretes large amounts of byssus fibres from its foot. The foot is highly mobile allowing it to use the fibres to bind together the substrate and form cocoon like galleries above sediments (Merrill & Turner, 1963; Gilchrist, 1895; Gilmour, 1967; Robertson, 1895). This stabilises the substrate allowing for the settlement of epifauna and infauna. High biodiversity occurs both within the galleries and in the stabilised sediment below (Hall-Spencer & Moore, 2000a). One study found 282 species from 16 phyla in cores taken from flame shell beds (Trigg *et al*, 2011a) and another showed similar results with 280 species recorded (Hall-Spencer & Moore, 2000a). Trigg *et al* (2011) concluded that *L. hians* beds are amongst the most important marine biodiversity hotspots in UK waters. Both of these studies are likely an underestimation of the true diversity of flame shell beds as larger animals and meiofaunal animals were not surveyed.

It has long been recognised that biogenic reefs are promoters of high biodiversity (Holt *et al*, 1998; Lenihan, 1999). Biogenic reefs are defined as "Solid, massive structures created by accumulations of organisms" They must also "clearly form a substantial, discrete community or habitat which is very different from the surrounding seabed" (Holt *et al*, 1998). *L. hians* beds were previously excluded from the biogenic reef classification and annex 1 of the EU habitats directive; Holt *et al* (1998) argued that because *L. hians* beds are not raised significantly above the sea floor that they should not be considered reef forming organisms like the polychaete (*Serpula vermicularis*) or the horse mussel (*Modiolus modiolus*). Hall-Spencer & Moore (2000) however, made a compelling case that *L. hians*

aggregations meet the Holt *et al* (1998) definition of a biogenic reef under some circumstances. Regardless of the semantics of this debate it is clear that flame shells are ecosystem engineers which profoundly influence benthic communities. An ecosystem engineer is defined as "a species which causes structure formation in the abiotic environment, creating a new structural state relative to a baseline, unmodified state" (Jones *et al*, 1994). For this reason they should be treated with equal conservation importance as the associated biodiversity they support can be just as great (Trigg *et al*, 2011b) and they are also highly susceptible to dredging and other fishing activities (Moore *et al*, 2018; Hall-Spencer & Moore, 2000b). As of 2016 (Tyler-Walters *et al*, 2016) Scottish Natural Heritage (SNH) recognised that *L. hians* is capable of forming reefs.

The structural complexity of a habitat has been shown to positively correlate with biodiversity and abundance of individuals (Tilman, 1999; Cocito, 2004). As early as 1961 a correlation between bird species diversity and foliage height diversity was observed (MacArthur & MacArthur, 1961). Since then many more examples of habitat complexity correlating with species diversity have been reported in terrestrial, freshwater and marine systems (August, 1983; Roberts & Ormond, 2007; Kalacska *et al*, 2018). This is because complex habitats have a higher ecological niche diversity and niche partitioning which in turn increases biodiversity (Huston & DeAngelis, 1994; Levins, 1979). The nature of the complexity of a particular habitat is also thought to strongly influence the communities present. For example the size of sites available for refuge from predators affects the size of organisms found in these habitats (Nash *et al*, 2014). Habitat structure is thought to be one of the two main drivers of body size distributions alongside resource availability (Holling, 1992). Therefore, the decline of ecosystem engineers such as *L. hians* is likely to displace or wipe out species dependant on the unique habitat they create (Bruno & Bertness, 2001).

This was reported in a study which showed the number of taxa within core samples taken in an area that the reef had declined was less than a

third of the number before the decline (MacLeod, 2012).

### 1.3.0 Threats and impacts

The primary source of damage to *L. hians* beds is thought to be from activities related to fishing. The best documented case of which occurred in loch Carron in 2017 (Moore *et al*, 2018). A scallop dredger passed over the flame shell beds in loch Carron leaving many long dredge scars in the seabed. In response to this the Scottish government placed an emergency marine conservation order (MCO) on the 19<sup>th</sup> May 2017.

Observations of the freshly dredged Loch Carron site showed that the displacement or damage of *L. hians* led to rapid predation by a wide variety of predators. The recovery and damage to the sea bed has been and continues to be monitored but it is difficult to estimate how long a full recovery will take due to a lack of previous data.

Estimates of recovery time are scarce but some sources indicate it could take up to 100 years in heavily fished sites. An experimental study involving simulated dredging of the seabed on a flame shell bed was conducted by Trigg & Moore (2009). The authors reported that regrowth of the bed generally occurred from the edges of the experimental plots and occurred at a rate 3.2 cm per year. None of the nest regrowth was of comparable thickness to before the experiment. They estimated that in heavily dredged sites it could take up to a century to recover, or potentially be beyond recovery if there is no source population left for settlement of larvae or a suitable substrate for them to settle on.

Slow recovery rates were also observed by Minchin (1995), there was a large scale mortality of *L. hians* due to the use of tributyltin (TBT) as an antifoulant on ships hulls in Mullroy Bay, Ireland. The author showed a settlement failure of new spat which had still not recovered 7 years after the cessation of TBT use. Although this may be compounded by the persistent nature of TBT in the environment (Bangkedphol *et al*, 2009).

Flame shell beds are thought to have declined dramatically throughout much of their previous

range. Studies of maerl and associated *L. hians* found that in a dredged site there was evidence of dead *L. hians* shells but no live animals or byssus nests. Live *L. hians* and byssus nest material were found at a nearby undredged maerl bed (Hall-Spencer & Moore, 2000b). There were also reports of dense beds in Millport at the "Tan buoy" site (Gilchrist, 1895) however *L. hians* is now absent from this area with only the shells of dead animals found (Hall-Spencer, 1998). The same is true for the "Skelmorlie Bank" site where Ansell (1974) and Gilchrist (1895) collected their samples (Hall-Spencer & Moore, 2000a, 2000b). Seaward (1990) also found that there are areas where only *L. hians* shells can be found including locations in Orkney and the Scilly Isles.

The records and studies mentioned above suggest that at present, the greatest threat to *L. hians* reefs is benthic fisheries, predominantly scallop dredging. In many cases even after many decades' reefs have not recovered from scallop dredging has ceased.

### 1.4.0 *L. hians* reef restoration

There has never been a large-scale restoration attempted of flame shell reef. There have however, been smaller scale trials conducted.

Cook (2016) attempted several restoration techniques. Firstly, sediment stabilisation where netting was laid over the sediment which significantly increased recruitment of *L. hians* spat despite many of the experimental plots not being recoverable. Secondly, stock enhancement was attempted. Two types of spat settlement collectors were used. conventional spat collectors and crushed scallop shell bags. When placed at the same sites *L. hians* spat showed a preference for the crushed shell bags. Thirdly translocation of *L. hians* reef material to new sites. This was not successful and showed very low survival of translocated adults. Trigg (2009) also successfully achieved settlement of spat on monofilament and crushed shell based spat collectors however no attempt to translocate these and restore reef was made.

### 1.5.0 Aims and hypotheses

Substrate preference experiments were conducted using adult specimens. These experiments were conducted because

observations from Cook's (2016) reef restoration trials showed that some animals in the spat collectors were too large to have settled as spat and must have migrated into the collectors as post settlement fully metamorphosed animals. This showed that the adults are not sessile and can to some degree move to new habitat. Observations of fishing disturbance suggest that viable animals are often displaced from the nest material (Moore *et al*, 2018). Therefore, knowledge of substrates which may promote the settlement of post settlement animals could be valuable in restoration efforts.

Firstly, Preference for a variety of substrates differing in their physical structure would be compared. It was hypothesised that a similar preference for structurally complex substrates would be observed, as seen by previous authors (Taylor, 2018; MacLeod, 2012). This study focused on simplifying the methodology used by previous authors and apply a balanced experimental design to overcome some of the shortcomings of the previous work.

Secondly, a hypothesis that animals would show a preference for byssus nest material vs a suitable control was considered. These experiments were conducted because many aspects of marine invertebrate behaviour are controlled by chemical signals from members of their own species (McClintock & Baker, 2001).

Nest building observations were conducted in order to estimate how long recovery from disturbance may take in the wild. A full spectrum camera was used so animals could be observed day and night over multiple days. We aimed to confirm previous observations of nest building behaviour (Gilchrist, 1895; Robertson, 1895; Gilmour, 1967) and assess whether their behaviour changes during the night. We also aimed to provide data on the rate of construction by quantifying byssus thread placements over time.

Qualitative observations of *L. hians* in captivity showed when removed from nest and dropped they had a tendency to swim back in the direction from which they came. A homing behaviour experiment was designed in an attempt to validate this observation. This was considered important to enhance understanding of how *L. hians* behaves when disturbed.

The final aim of this study was intended to analyse the size frequency of the port Appin

population at the margin of a bed and at the centre and compare the results. Diver observations had indicated that the animals at the margin of the bed tended to be smaller than those at the centre (K. Tulbure, pers. com). If this could be confirmed it would suggest the bed is expanding. It proved difficult to find a sufficiently large area to sample from and it appeared the bed had declined further. In light of these events the aims of this study were adapted to assess the size frequency of the animals in the small patch that was found in order to determine if the area had been recently colonised or if it was an established bed in decline. A diver drift was also conducted to investigate whether the bed extended beyond the extent observed in 2017 (Taylor, 2018). It was hypothesised that there has been further decline as previous work has repeatedly shown (Moore *et al*, 2012; Cook, 2016b; Taylor, 2018).

## 2.0.0 Methods

### 2.1.0 Substrate preference

#### 2.1.1 Collection and housing of specimens

*L. hians* specimens and nest material were collected from Port Appin (56° 33.93564', -5° 24.75684') at a depth of 8.6m. on 28/10/18 by divers from the RV *Serpula*. All diving work was conducted in accordance with the scientific and archaeological approved code of practice (ACOP) from the diving at work regulations 1997 (HSE, 1997). Appropriate permission to sample specimens was obtained from Scottish Natural Heritage (SNH). Clumps of nest material containing live *L. hians* and associated fauna were placed into 25 L buckets by divers on the seabed. The *L. hians* specimens were then separated from the nest material once on the deck of the boat. The *L. hians* specimens and nest material (with associated fauna) were placed in separate cool boxes which were aerated with battery powered air stones for transport. Once the animals and nest material reached the laboratory based at Heriot Watt University, Edinburgh campus, the nest material was sorted through more thoroughly to ensure all individuals were found. The new specimens were left in the cool boxes to acclimate to the ambient temperature. They were then mixed with 60 already captive animals which were in apparent good condition.

All experimental and holding tanks were situated in a temperature-controlled room set to 13°C. 50 L (length = 45 cm, width = 40 cm, height = 30 cm) plastic tanks were used to house animals during the trials. The tanks were

integrated into a closed-circuit system containing two other glass holding tanks (length = 82 cm, width = 32 cm, height = 40 cm and length = 60 cm, width = 55 cm, height = 35 cm).

In order to replicate the expected high water quality found on *L. hians* beds a high capacity filtration system was designed. This was done to maximise the chances of observing as close to natural behaviour in captivity as possible. The seawater was filtered through a large sump trickle filter (length = 100 cm, width = 50 cm, height = 50 cm), with an additional fluidized sand filter of 1500L filtration capacity (V2 Bio 1500 fluidized sand filter). There were also two UV sterilizers (SunSun CUV-318) and a protein skimmer (TMC REEF-Skim 1500) in the loop. A higher capacity filtration system was used for this study which improved the health of the animals throughout the trials compared to previous work (MacLeod, 2012; Trigg, 2009).

The water temperature ranged between 12°C and 14°C. Photoperiod was set at 8am to 6pm illumination daily. Lighting was provided by florescent tubes. The specimens were fed a mixture of *Isochrysis galbana*, *Tetraselmis suecica* and *Chaetoceros calcitrans* at a concentration of between 1,000,000 and 3,000,000 cells/ml. The temperatures and feeding regime were chosen in order to mimic summer conditions, it is thought the animals would be more active under these conditions.

### 2.1.2 Experimental design

Tanks containing two substrate types split down the middle parallel to the direction of water flow. This was done to create identical flow patterns on both sides of the tank (excluding any differences induced by the particular substrates used). A florescent strip light was placed in front of the 4 tanks creating an even distribution of light. They were fed 500ml of *Isochrysis galbana* (exclusively *Isochrysis galbana* during trials) at a culture concentration of between 1 and 3 million cells per ml daily. The flow was turned off for 1.5h during feeding and an air stone was placed in the centre of the tank to delay the settling out of algae. Table 1 lists the substrate comparisons made, the numbers on this table will henceforth be used to refer to the comparisons. All substrates were allowed to soak for at least 24h in seawater to allow formation of a biofilm except comparison 7 (figure 7 table 1) where presence of biological activity was being controlled. Substrates were ~4cm or greater in depth.

Each substrate comparison was conducted in 2 rounds of 4 replicates totalling 8 replicates for each comparison. For the second round of four replicates the positions of the substrates in the tanks were flipped (figure 3). the positions of the substrates differed between comparisons 2, 6 and 7, and comparisons 1, 3, 4 and 5 (figure 3). This was not done for any particular reason and the experimental design was still balanced in terms of each substrate spending equal time in each position.

The tanks and substrates were cleaned of any byssus material between replicates in order to prevent influence on the next round of the experiment. To remove substrates from the shell material a power washer and a mesh container were used.

For the first round of the experiment medium crushed scallop shell (3-6 cm fragments) was tested against sand. As the flame shells could not bury themselves in the sand they could be counted without disturbance. The number of flame shells on each side was counted every 24h after the start of the experiment for 4 days. This allowed us to determine a suitable end point for settlement on a preferred substrate. There was no significant difference in the number of animals on each side from 24h to 48h ( $p > 0.05$ ). There was also no difference in the numbers of animals on each substrate between 48h and 72h ( $p > 0.05$ ) or 72 and 96h ( $p > 0.05$ ). With no significant difference in the number of animals in either half of the tanks after 48 hours. It was therefore decided to conservatively end the experiments after 72h.

Each trial was 72h long. A total of 20 specimens were used for each replicate, ten were placed on each substrate as close to the centre of each substrate as possible. In total 8 replicates were

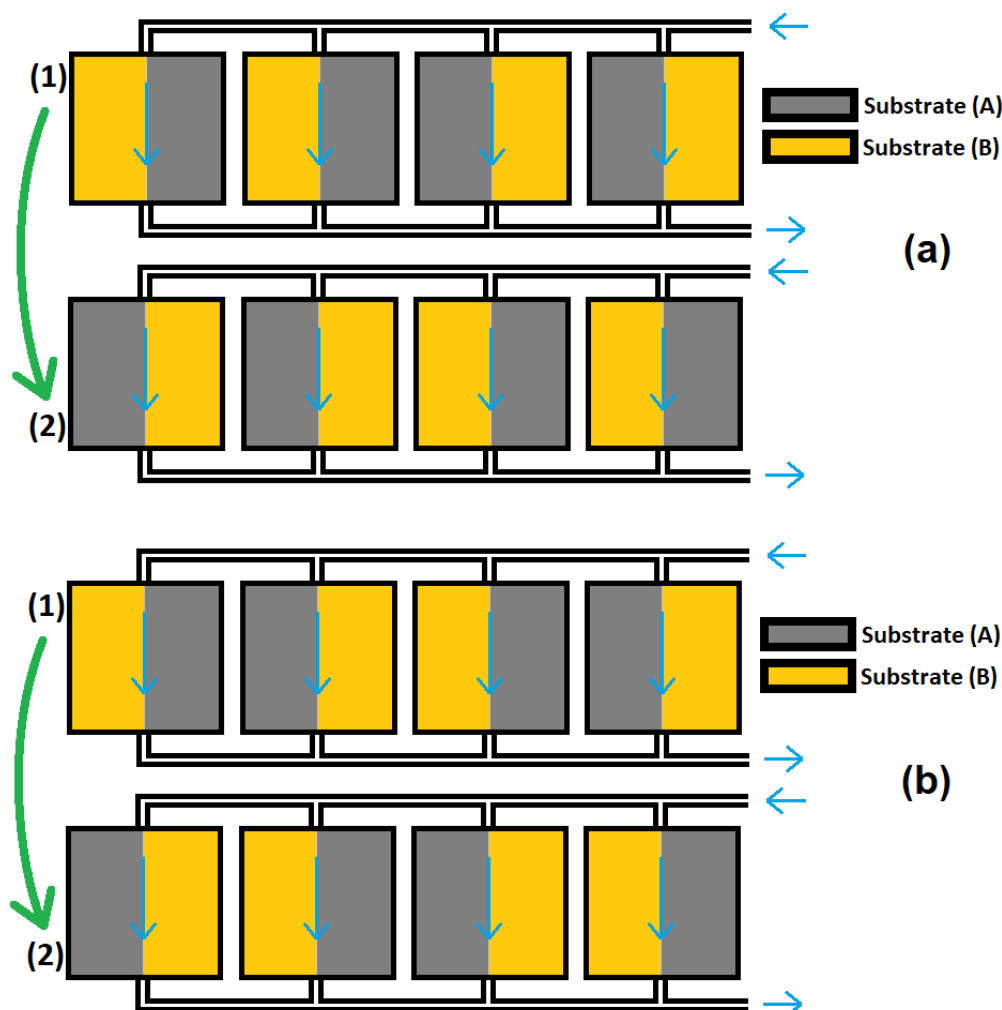


Figure 3 The orientation of substrates on each side of tanks used in substrate experiments. (1) indicates the first 4 replicates and (2) the second four. The green arrow between (1) and (2) indicates how the substrates were switched over between sets the of 4 replicates. Configuration (a) was used for comparisons 1,3,4 and 5. Configuration (b) was used for comparisons 2, 6 and 7 (See table 1 for key to comparisons). Blue arrows Show flow of water through the system.

conducted for each substrate pair. A total of seven substrate pairs were compared (table 1). The mean  $\pm$  SD ( $n = 42$ ) size of the specimens collected was  $21.9 \pm 7.65$  mm, measured from umbo to the furthest edge of the shell. Specimens as close to this value as possible were used in substrate preference trials. Due to limitations on the number of animals we could collect animals were used more than once. Two groups of 80, (20 per experimental tank) were rotated between the experimental tanks and holding tanks for comparisons 1, 3 and 4 (table 1). After these three trials the same group of 80 specimens were used between sets of four replicates.

### 2.1.3 Collection of data

Substrate selection was determined by the number of animals present on each substrate

after 72h. Animals were recorded as present on a particular substrate type if 50% or more of their body lay on it. For trials involving the use of tape to hold mesh down a third substrate type "tape" was introduced and the numbers of animals on the tape was also recorded for each substrate. If an animal died during the trial it was replaced with another specimen placed at the centre of the substrate where the death occurred. This was however a rare occurrence (only 2 recorded over between all 7 comparisons)

### 2.1.4 Statistical methods

A significant difference was accepted at an alpha value of  $p < 0.05$  for all tests. All statistics were performed using R (version 3.5.1 - 2018-

07-02) and RStudio (Version 1.1.456 – © 2009-2018 RStudio, Inc.) Data was visualised using the R packages ggplot2 and cowplot (Hadley, 2016; Wilker, 2019).

Shapiro-Wilk tests ( $p > 0.05$ ), inspection of frequency histograms and QQ plots indicated that we could not be confident that our data were normally distributed. Therefore, Wilcoxon ranked sum tests were conducted between the

counts of *L. hians* on each substrate after 72h using all 8 replicates for each comparison.

Wilcoxon ranked sum tests were also carried out for the positioning of different substrates within individual replicate tanks. We tested for differences between the left and right side of individual replicate tanks for each substrate within the comparisons. This gave a sample size of 4 for the left and right for each substrate type.

Table 1 The seven different substrate comparisons. Numbers correspond to those in figure 1 and Table 2





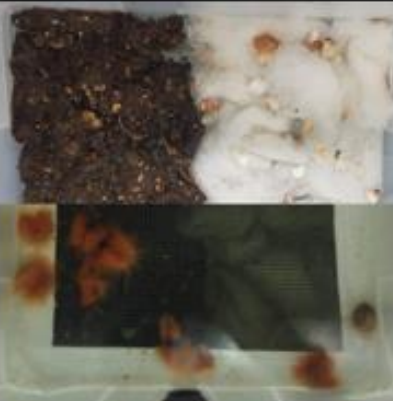


Substrate Pair	Description	Picture
1) Sand VS Medium Shell	Coarse well-sorted sand of approximately 1mm median grain size was compared against crushed scallop shell (1-3cm).	
2) Dark VS light substrate	Black duct tape was compared to white duct tape	
3) Medium shell VS Whole shell (Flat)	Crushed Scallop shell (3-6cm) was compared to whole scallop shell laid flat.	



Table 1 part 2

Substrate Pair	Description	Picture
4) Small shell VS Whole shell (Vertical)	Crushed Scallop shell (1-3cm) was compared to whole scallop shell laid vertical.	
5) Nest material VS Polyester under mesh	Nest material was compared to Polyester fibres under mesh (4mm). The polyester fibres contained small rocks and shell fragments to better mimic the nest material texture. The mesh was held in place with white duct tape. Picture shows substrate before (top) and after (bottom) the layer of mesh was placed on top.	
6) Nest material clumps VS Polyester fibre clumps on top of a layer of medium shell over sand	Nest material clumps ~12x5cm were compared to clumps Polyester fibres. Small pebbles and shells clean of byssus threads were placed in the Polyester clumps to better mimic the complexity of the nest material substrate clumps.	
7) Biologically active substrate VS non biologically active substrate under Medium Shell	Biologically active sand from the upper basin of Loch Creran was compared to Sand under a layer medium shell (3-6cm) approximately 3cm deep. Picture shows substrate before (top) and after (bottom) the layer of medium shell was placed on top.	

### 2.2.0 Nest building observations

Due to time limitations only one nest building observation of significant length (5.5 days) was recorded, featuring only a single specimen. The top of the nest was removed but the animal was left in its original location so it would still be in frame of the camera. Footage was recorded using a Raspberry pi V2 single board computer with the Pi NoIR V2 infrared Camera module. The NoIR camera has no infrared or ultraviolet cut filter so colours under normal lighting conditions were affected. However the light output from florescent tubes does not contain a large proportion of wave lengths outside the visible spectrum (Wang *et al*, 2013). Florescent tubes were the only light source in the room during the day, so the footage was not drastically different from that of a normally (IR/UV cut) filtered camera. At night they were illuminated by an 850nm led light cluster which activated via a light dependent resistor (LDR) when the main light was deactivated. The camera recorded 8 mega pixel photos at a frame rate of 2 FPS. These were combined into a video using ffmpeg (version 4.0.3-1~18.04.york0 Copyright (c) 2000-2018 the ffmpeg developers) adobe premiere pro CC 2018 version 12.0.0 (build 223). The camera module was programmed using python 3.7 and controlled from a Linux (ubuntu 14.14lts) laptop connected via ethernet outside the CT room.

In our analysis of the video, activity was quantified by counting the number of times the animal moved during day and night periods. A movement was defined as movement of the animals' shell and not movement of the foot or pallial tentacles. This was repeated with counts of byssus thread placement for each day and night period. A byssus thread placement was defined as the movement of the foot to and placement of it on the substrate or glass.

### 2.3.0 Disturbance response and homing behaviour

To test the response to disturbance and to see if *L. hians* displays any homing behaviour 20 animals of mixed sizes were placed in one of the holding tanks split with a divider (length = 55 cm, width = 30 cm, height = 35 cm). The substrate was mixed small gravel and nest

material. They were then allowed to settle and construct galleries for at least two weeks prior to any disturbance.

Once galleries were present *L. hians* were removed from their galleries with a pair of kitchen tongs and placed in random positions around the tank whilst being recorded by two cameras (GoPro™ hero 4 silver). One camera on time lapse (1 frame per second) and one recording in real time (30 frames per second). The two videos could then have their time codes synced in order to keep track of which animals were placed where. After the animals had been disturbed, the time-lapse was left running for 2h 40mins. The video was then analysed to see if animals returned to the location they were removed from.

### 2.4.0 Port Appin surveys

#### 2.4.1 Drift surveys and site information

As mentioned in the introduction plans for the field work in Port Appin were adjusted because it proved difficult to find a suitable area of reef to conduct our planned surveys.

Our specimen collection (see above) and transects were both conducted on the Port Appin flame shell bed which has seen significant decline since studies conducted in the early 2000s (O'Malley, 2004; Forrest, 2005; Trigg *et al*, 2011a; Trigg & Moore, 2009; Cook, 2016a; Taylor, 2018). Figure 4 shows the general area where surveys were carried out.

Surveys followed the same methodology outlined by Moore *et al* (2012) and by subsequent studies (Cook, 2016a; Taylor, 2018). The diver drifted along the sea bed tethered to a surface marker buoy with GPS attached. Divers recorded the angle of the line and compass bearing. This enabled the diver's position on the sea bed to be calculated via trigonometric calculations. The divers watch was synchronised with the time code of the GPS, this enabled the individual stations to be identified from the GPS track data later. ArcMap (Esri®ArcMap™, version 10.6.1.9270) was used to visualise this data and previous survey data used in this study (Moore *et al*, 2012; MacLeod, 2012; Cook, 2016a; Taylor, 2018; Trigg, 2009)

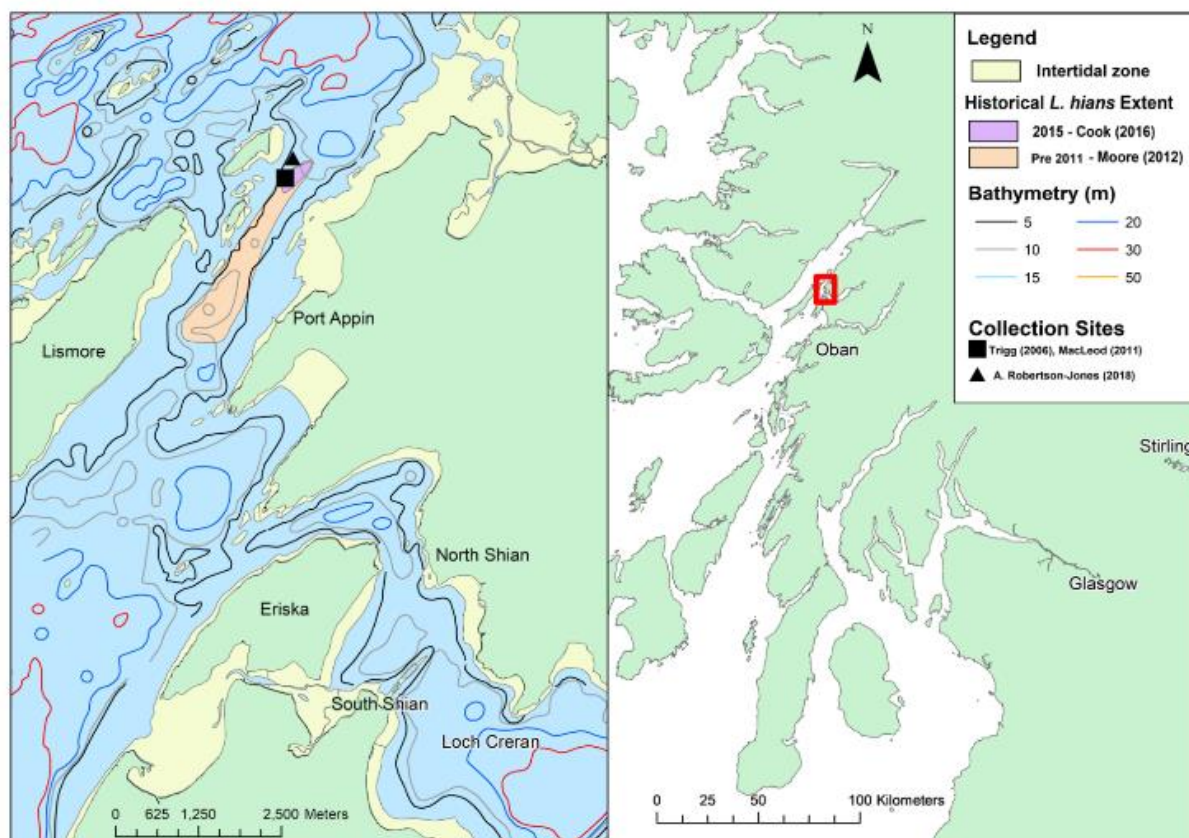


Figure 4 The study area including the historical extent of the bed (orange, from Moore (2012)) and the estimated extent in 2015 (purple, from Cook (2016)). Can be seen in greater detail in figure 8. Collections sites for size frequency also shown (see legend).

### 2.4.2 Size frequency

A total of 141 specimens were obtained from the northern area of the port Appin bed (see above for collection details). Some specimens were damaged during collection, if damage was sustained to both valves making a measurement impossible, they were omitted from the analysis. Animals were measured from umbo to the furthest point on the opposite edge of the shell using digital callipers. Only a single valve was measured as previous work (Trigg, 2009) showed that there was no significant difference in left and right valve length.

The mean for other data sets could not be calculated due to the original data being unavailable. The available data was only available binned into 1mm size categories. Therefore, our data had to be handled in the same way

## 3.0.0 Results

### 3.1.0 Substrate preference

The comparison of sand vs medium shell (1 in table 1 and figure 7) showed a significant difference ( $p < 0.0001$ ). The number of individuals on the Medium shell was significantly higher than the sand with the median number of individuals on the sand side after 72 hours being  $0.5 \pm \text{IQR}$  and  $19.5 \pm \text{IQR}$  on the shell (**figure 7**).

There was no significant difference in the number of flame shells on each side of the replicate tanks when comparing the medium crushed scallop shell and whole scallop shell laid flat (3 in table 1 and figure 7) ( $p > 0.05$ ) after 72 hours (fig 7). The median values for whole shell laid flat and medium crushed shell were both  $10 \pm \text{IQR}$ . There was however a significant difference between small crushed scallop shell and whole shell laid vertical (4 in table 1 and figure 7) after the same time ( $P = 0.00086$ ), the median on the small shell side was  $2.5 \pm \text{IQR}$  and  $17.5 \pm \text{IQR}$  for whole shell laid vertical.

Polyester fibres were compared to *L. hians* nest material under mesh (5 in table 1 and figure 7). For this comparison a third substrate category was added, the tape around the edges of the

mesh. There was no significant difference ( $p > 0.05$ ) between the numbers of animals settled on the tape on either side of the tank ( $p > 0.05$ ). The number of animals on the nest material side was significantly higher ( $p < 0.05$ ) than the polyester fibres when the animals on the tape were included and when they were not. Substrate comparison 5 in Figure 7 shows the number of individuals on each side minus those settled on the tape. Polyester fibres were also compared to nest material in clumps which the animals could directly contact (6 in table 1 and figure 7). In this scenario there was a smaller difference in the numbers of animals on each side but there was significantly more on the side containing nest material clumps ( $p < 0.05$ ).

The comparison of Black Tape vs White tape (2 in table 1 and figure 7) showed a significant difference ( $p = 0.045$ ). The median  $\pm$  IQR number on the black side was significantly lower than on the white. However, the difference between these two medians was only 4 and the  $p$  value was close to our 0.05 alpha value. Biologically active substrate was compared to non-biologically active sand under a layer of medium shell (7 in table 1 and figure 7). There was no significant difference in the number of animals on each side ( $p > 0.05$ ).

Efforts were made to determine whether preference was influenced by the position of the substrate in the tanks (left or right). Wilcoxon ranked sum tests were conducted between counts of animals the left or right of replicate

tanks for individual substrates (see methods). All showed no significant difference ( $p > 0.05$ ) indicating there was no preference for either side of the replicate tanks, results shown in Table 1.

### 3.2.0 Nest building observations

Animals were observed in the reconstructing nests for a maximum of 6 days the initial rate of reconstruction was rapid with most of the galleries rebuilt after the first 24 hours, this then slowed over the next few days. Animals were also observed using the foot to move around the nest and turn on the spot when reconstructing their nests.

Counts of body movements for each light and

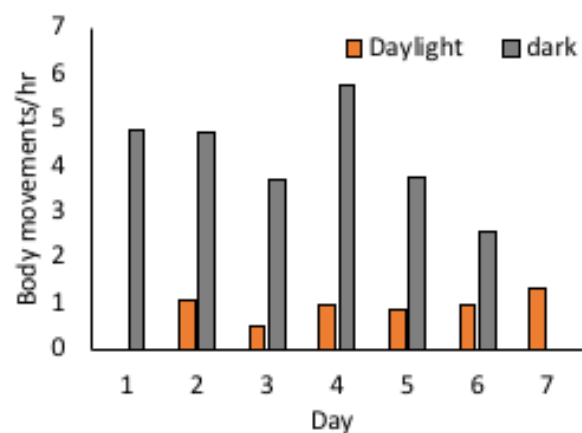


Figure 5 Body position movements per hour for each daylight and dark period. Observations were recorded for a total of 6 days starting at 6pm (first dark period) and finishing at 7pm end of last day period.

Table 2 The resultant  $p$  values of Wilcoxon ranked sum tests performed for each substrate comparing the counts of animals on each substrate grouped by whether the substrate was on the right or left of the individual replicate tank or whether the substrate was in the right or left two of the four replicate tanks. Numbers of substrate pairs relate to the numbers in Figure 7 Table 1.

Substrate Pair	Substrate	Left or right of replicate tank
1) Sand VS Medium Shell	Sand	0.44
	Medium Shell	0.44
2) Dark VS light substrate	Dark	0.15
	light substrate	0.15
3) Medium shell VS Whole shell (Flat)	Whole shell (Flat)	1
	Medium shell	1
4) Small shell VS Whole shell (Vertical)	Whole shell (Vertical)	1
	Small shell	1
5) Nest material VS Polyester under mesh	Nest material	0.3
	Polyester under mesh	0.88
6) Nest material clumps VS Polyester fiber clumps	Nest material clumps	0.28
	Polyester fiber clumps	0.28
7) Biologically active substrate VS non biologically active substrate	Biologically active substrate	0.77
	Non biologically active substrate	0.23

dark period showed that the animals were much more active at night ( $p < 0.05$ , Wilcoxon ranked sum). Activity during the day remained relatively consistent (mean of 0.92 movements/hour) but the activity at night (mean of 4.22 movements/hour) appeared to decrease from day 4 onwards (figure 5).

Thread placement was significantly higher ( $p < 0.05$ , Wilcoxon ranked sum) during dark periods than during the day. The mean hourly rate of threads placed at night was 7.9/hr. During the day this was 0.5/hr with most of these being placed soon after the light was turned on. Thread placement rate was highest during the first dark period at observed 13.5 per hour. This declined across the remaining days to a minimum of 5.2 per hour at night 5 (figure 6)

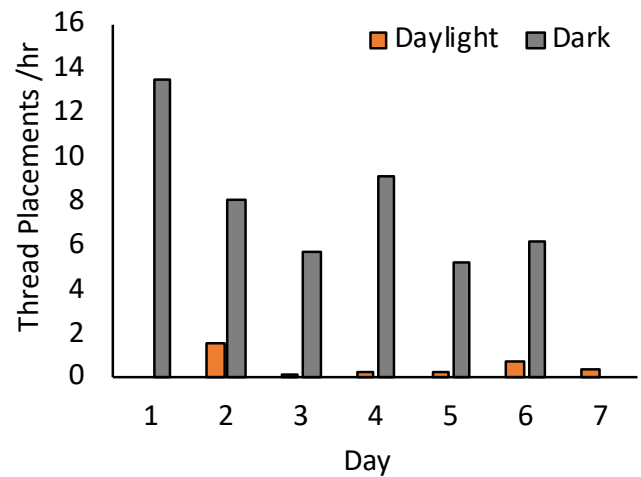
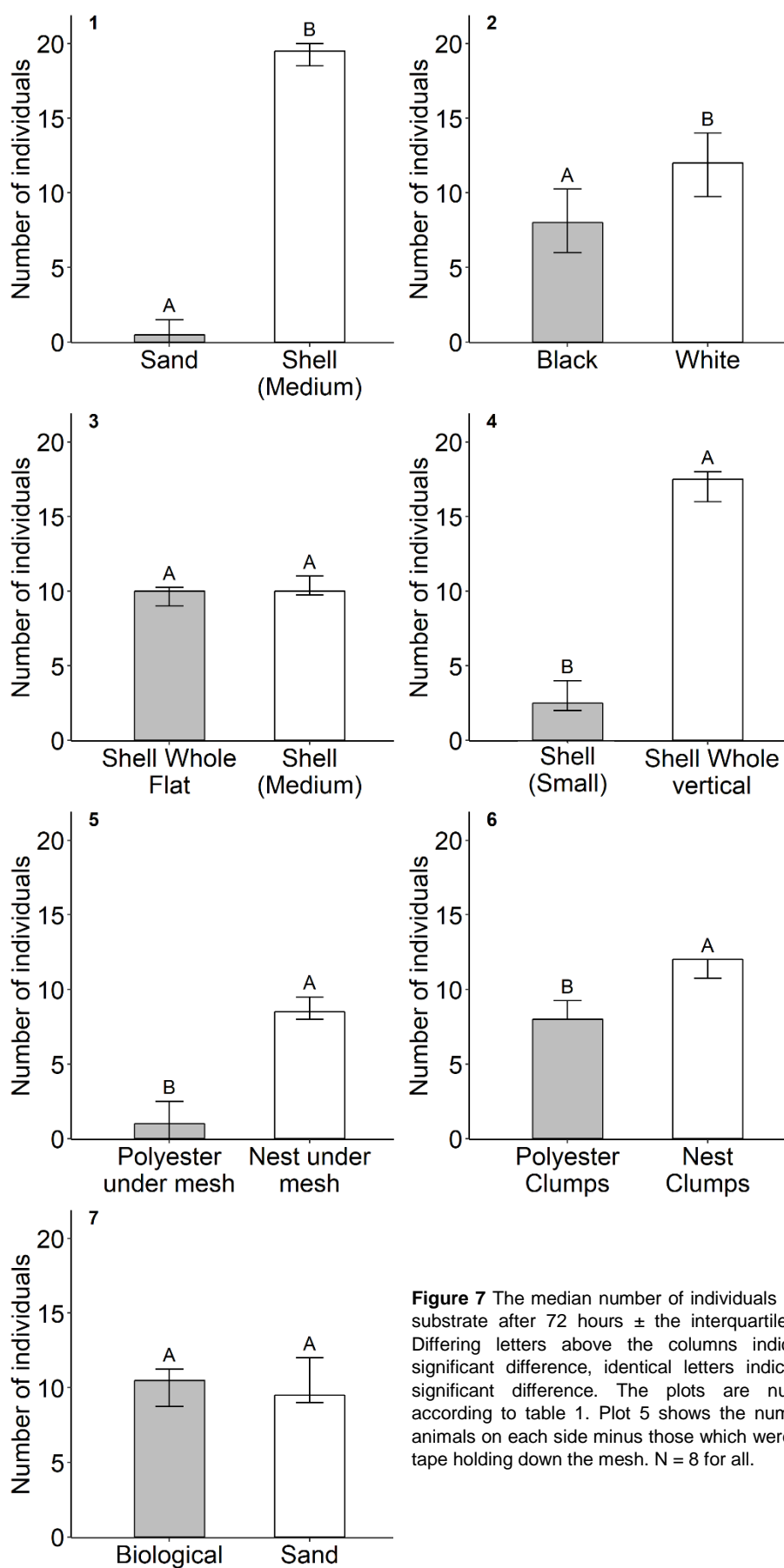


Figure 6 Byssus thread placements per hour for each daylight and dark period. Observations were recorded for a total of 6 days starting at 6pm (first dark period) and finishing at 7pm end of last day period.



**Figure 7** The median number of individuals on each substrate after 72 hours  $\pm$  the interquartile range. Differing letters above the columns indicates a significant difference, identical letters indicates no significant difference. The plots are numbered according to table 1. Plot 5 shows the numbers of animals on each side minus those which were on the tape holding down the mesh. N = 8 for all.

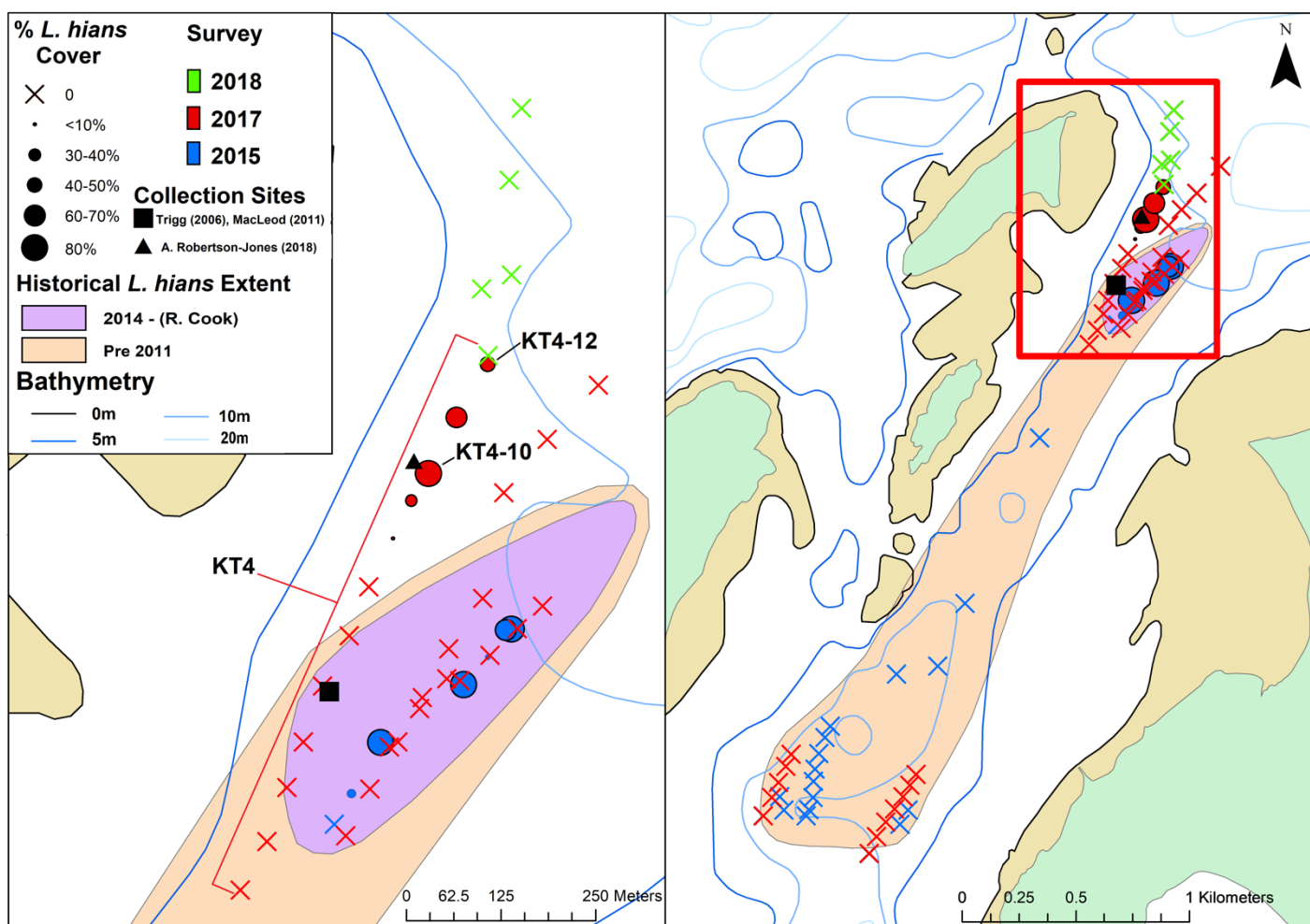


Figure 8 The historical extent of the bed (orange, from Moore (2012)) and the estimated extent in 2015 (purple, from Cook (2016)) overlaid with stations from our drift survey (green), The 2015 survey (blue, from Cook (2016)) the KT4 drift (red, from Taylor (2018)). Crosses indicate no *L. hians* present, where *L. hians* were present points are scaled to reflect the % cover (see legend). Collections sites for size frequency also shown (see legend).

### 3.3.0 Disturbance and homing behaviour.

Specimens were recorded for three hours; no homing behaviour was observed during this time. They largely did not move from the location to which they were moved during this time.

### 3.3.0 Port Appin surveys

#### 3.3.1 Appin drift survey

A drift transect was carried out north of the known extent of the Appin flame shell bed, no *L. hians* or byssus turf material was recorded at any station (figure 8). The first station was recorded 11m from the station 12 of the KT4 transect from 2017.

Our sample collection point was 25.6m from station 10 of the KT4 transect from 2017. On

the collection dive divers were dropped in close to the KT4-10 station from Taylor (2018) (see discussion for context). The divers covered a large search area before encountering a patch of reef (30-40% cover) bed 25.m NE of the KT4-10 station. The data collected for this study

#### 3.3.2 Size frequency results

The shell size distribution of animals collected from Port Appin (56° 33.93564', -5° 24.75684') in late November 2018 was compared to other data sets collected in November from previous years (Trigg, 2009; MacLeod, 2012 at -5° 24.817 , 56° 33.845) (figure 8). Trigg (2009) also compared size frequency distributions between the Appin and Shian (figure 9b), in this case in September after the summer recruitment. The mean  $\pm$  SD (n = 42) size of the specimens collected was 21.9 $\pm$ 7.65 mm.

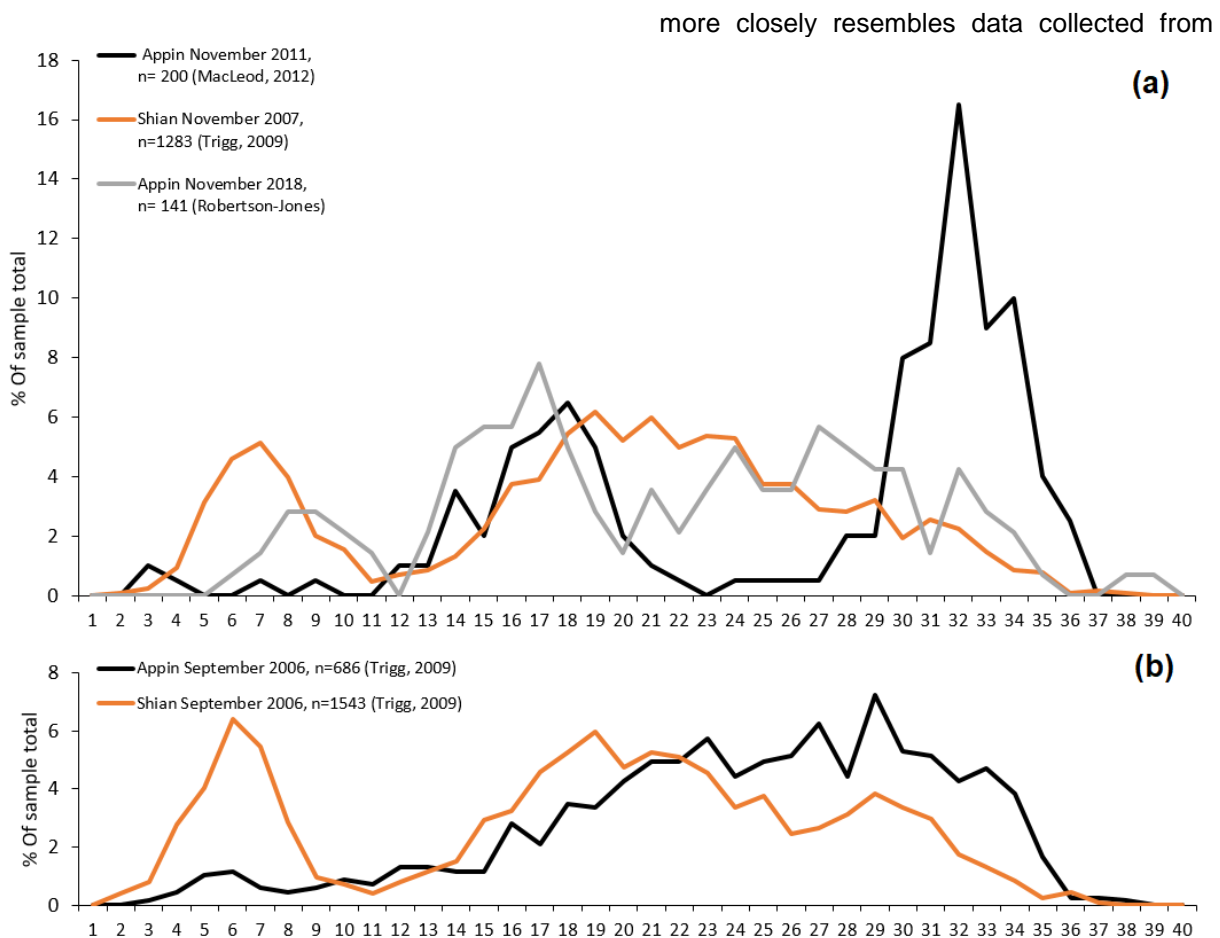


Figure 9 (a) Size frequency distribution of *L. hians* collected in November 2007 (Trigg, 2009), 2011 (MacLeod, 2012) and November 2019 (This study, Robertson Jones 2019) as a percentage of the total of each sample. Shell length was binned into 1mm size classes. (b) Data from Trigg (2009), Size frequency of *L. hians* collected from Shian (left) and Appin (right). Values are a percentage of the total number of specimens in each sample.

In the Shian population during September 2006 the major mode of the distribution was that of that of the new recruits (individuals of 6-6.9mm comprising 6.4% of the total sample). The minor mode was not far below this occurring at 19-19.9mm making up 6% of the total sample. This in contrast to the population in Appin (September 2006) where the minor mode was the new cohort. The new cohort also had a mode of 6-6.9mm but only comprising 1.1% of the total sample. The major mode in Appin occurred at 29-29.9mm

It was observed that the Appin population in September 2006 was predominantly composed of animals over 21mm in length (70%). This is similar to the proportion of animals over 21mm found by MacLeod (2012) in Appin November 2011 (65%). This is in contrast to data collected in Appin November 2019 for this study, which found only 50% of animals over 21mm. This

Shian in November 2006 which showed 42% over 21mm (figure 9a).

#### 4.0.0 Discussion

##### 4.1.0 Substrate preference

*L. hians* specimens showed a significant preference for medium shell when compared to sand ( $p < 0.05$ ). This is in line with previous work by Taylor (2018) and MacLeod (2012) who showed that *L. hians* showed a significantly lower preference for sand compared to the other coarser, more structurally complex substrates. The substrates tested by Taylor were (pebbles (no size given), small crushed scallop shell (3cm) and large crushed scallop shell (6-8cm)).

The comparisons of whole and crushed shell in different orientations showed that *L. hians* preferred vertical whole shell to small crushed shell ( $p < 0.05$ ) (4 in table 1 and figure 7) and



showed no preference for whole shell laid flat compared to medium crushed shell ( $p > 0.05$ ) (3 in table 1 and figure 7). It is suspected that the change in the size of the crushed shell caused a preference for the whole shell rather than the orientation of the whole shell. This is due to ours and other (Gilmour, 1967; Robertson, 1895) observations that *L. hians* when introduced to a new environment will seek immediate refuge. The small crushed shell did not provide large enough spaces in which the animals could easily shelter with minimal effort like the medium crushed shell and both configurations of the whole shell did. *L. hians* is known to exhibit digging behaviour (Gilmour, 1967) however given a choice they seem to choose the path of least resistance which is presumably the quickest refuge from predators and the small shell did not provide this as the spaces between the larger pieces were generally filled with smaller fragments. The vertical shell is also likely preferable to horizontal shell as it offered more spaces and they were more immediately available. Repeating these comparisons and only varying one factor at a time would confirm this speculation.

Our nest material preference tests both showed a significant preference for nest material ( $p > 0.05$ ). When the control material was placed under a mesh to remove the effect of differing substrate texture there was a significant ( $p < 0.05$ ) preference for the nest material (comparison 5 in table 1 and figure 7). When the animals were able to contact the nest material (comparison 6 in table 1 and figure 7) the result was less pronounced. There was a significant preference ( $p < 0.05$ ) for the nest material but by a smaller margin, the median on the nest material side was only 4 greater than that of the control. Compared to the aforementioned comparison the median number of animals on the nest material was 7.5 greater than those on the control material. This suggests that the nest material may have chemo attractive properties which were more obvious when the animals were unable to immediately seek refuge within the substrate. Gilmore (1967) observed that *L. hians* will attempt to immediately shelter in available crevices as opposed to building a nest in the open. This is supported by our results in the substrate preference experiments involving different sizes of crushed shell and general observations of the animals in captivity (pers. obs).

Our results showed that there was a significant difference between the number of animals on the light and dark substrate with a greater number on the light substrate (2 in figure 7). However, the difference was small (4), the IQR large and the  $P$  value was close to our alpha value at 0.045. preference for light substrates was also not our hypothesised result given that *L. hians* has shown photonegative behaviour (Crozier, 1921; pers. obs). The light levels were not measured and the results may have been different with a brighter light source. This experiment could be repeated using light levels equivalent to those typical of a *L. hians* bed at differing depths. This would help inform decisions on appropriate substrates to use in reef restoration projects and confirm whether the colour could impact preference. This also relates to the tests of the nest material preference which used white polyester fibres as a control material. Under our test conditions it seems that any preference for the nest material was not influenced by colour.

When comparing biologically active substrate vs biologically inert sand no significant preference was observed (comparison 7 in figure 7 and table 1). This suggests that the byssus material is responsible for the observed preference for nest material mentioned above, rather than other biological activity in the substrate.

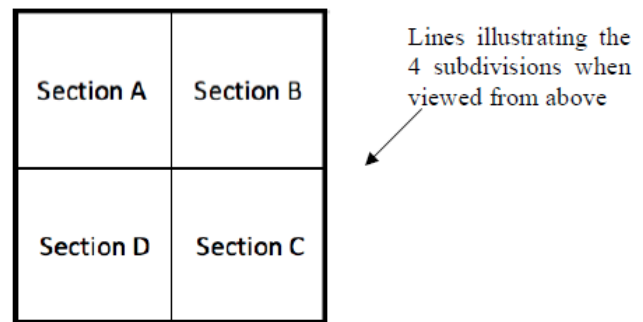
Observations by Gilchrist (1895) that *L. hians* coats its galleries with mucus secretions from the more elongate and glandular of its pallial tentacles. Whether these secretions could be responsible for the observed attraction to the nest material would require further investigation. Another open question is to whether the presence of *Modiolus modiolus*, or other bivalve byssus material could encourage settlement of adults or spat. *Modiolus* and *L. hians* are commonly found in the same habitats, (Connor *et al*, 2004).

*L. hians* like many other bivalves are gregarious (Connor *et al*, 2004; Gilchrist, 1895; Robertson, 1895; Tyler-Walters *et al*, 2016). The factors that cause such aggregations of *L. hians* are so far undocumented. Other marine animals which show aggregative behaviour such as the blue mussel *Mytilus edulis* (*M. edulis*) have been shown to do so; at least in part due to chemical cues from the tripeptide glycyl-glycyl-L-arginine (GGC), a synthetic analogue of similar signalling peptides. This was shown in adult *M. edulis* which migrated towards a source of GGC in a flow through tank (De Vooy, 2003). In

order to conduct similar experiments in *L. hians* a flow through system would also be required in order to maintain a diffusion gradient in the water. Many more species have been demonstrated to have elements of their life history controlled by exogenous signalling peptides. GGC has been shown to act as a larval settlement cue in the barnacle *Balanus Amphitrite* (Browne & Zimmer, 2001; Tegtmeier & Rittschof, 1988) and oyster *Crassostrea virginica* (Turner *et al*, 1994). Signalling peptides are also implicated in the settlement of Pacific sand dollar *Dendraster excentricus* (Burke, 1984). It is likely these adaptations evolved to enable larvae to settle where adults had already established in a suitable habitat (McClintock & Baker, 2001). It seems reasonable to speculate that *L. hians* adults and spat may also respond to similar chemical cues. Experiments on the influence of substrate characteristics on spat settlement may have greater implications for reef restoration attempts however spat have never successfully been reared in captivity. Taylor (2018) induced the release of gametes but no fertilisation was achieved.

While previous authors saw similar a similar preference for complex substrates their results were not conclusive weaknesses in experimental design. Taylor (2018) Did not account sufficiently for differing flow rates in the experimental tanks opting to place 4 different substrates in each creating a greater flow over substrates A and B (figure 10). The substrates in each position were randomly assigned, the manner in which they were randomly positioned is not clear, but the end result was that the substrates which the animals showed the greatest preference for were at the positions A and B most often. This made it hard to conclude which effect was causing the preference. It appears MacLeod (2012) used a similar experimental design however there is insufficient detail in the methods to confirm this.

It should be noted that for the substrate comparisons 2, 6, 7 and 4 (table 1 figure 7) the assumption of independent samples (Wilcoxon Ranked Sum test) was not met as there were no longer enough specimens to use a different set of animals for each round of four replicates (figure 3). For comparisons 1, 3 and 4 (table 1 figure 7) separate groups of animals were used for each round of four replicates therefore these were independent and did not violate the assumptions of the statistical tests.



**Figure 10** Shows the configuration of the different substrates used by Taylor (2018). Positions A and B were influenced by a higher flow rate over the substrate due to the position of an inflow pipe.

The balanced experimental design of this study should have adequately controlled for any left/right bias in the substrate comparisons. Our statistics seem to confirm this (table 2). A more robust control could have used a uniform substrate and then tested for a left right bias in the absence of any other factors with more statistical power than the current method.

The outcomes of this study could inform predictive modelling of suitable habitat, when combined with information on tidal flow preference and possibly settlement a general additive model (GAM) could be applied. This has been done with scallop species on the south coast of Tasmania (Mendo *et al*, 2014). A very large amount of additional data and ground truthing information would be required however if these models are to be accurate.

Data from Cooks (2016) experiments on settlement of spat onto spat collectors showed the presence of animals of a size which based on aging studies (Trigg, 2009) could not have grown to the size they were recovered at in the time since deployment.

Observations from the newly discovered flame shell bed in the upper basin of Loch Creran found a large number of adults sitting outside galleries on the sea bed. This differs from observations of the Appin bed in which animals were rarely visible without digging up nest material (pers. obs). This raises the possibility that some populations are more likely to be mobile than others. Disturbance could also lead to animals relocating to a different area of suitable habitat. Dredging is documented to cause the displacement of flame shells from their galleries (Moore *et al*, 2018). Dragging of creels along the seabed is also speculated to have a similar effect (Moore *et al*, 2012).

Natural causes of disturbance may also induce dispersal of animals to an alternative habitat such as foraging of predators. For example, if removed from the gallery by a crab the animal could autotomise tentacles, escape and settle in a new location. The substrate preference results indicate that animals displaced may preferentially settle in an area with rugose substrate and potentially in response to chemical cues from *L. hians* byssus nest material.

#### 4.2.0 Nest building -

The nest building behaviour of *L. hians* has been documented as far back as the late 1800s (Robertson, 1895; Gilchrist, 1895). Inexpensive modern technology has now enabled the recording of this behaviour. Specimens were observed replacing byssus threads when disturbed. When the majority of the gallery surrounding the animals was removed the animal repaired the basic structure within the next 24 hours. The damage was not repaired to the same condition as before, but the structure would have provided some refuge from predators and tidal currents. This may not be fast enough to allow animals to shelter in all situations after disturbance however as rapid predation has been observed of *L. hians* after dredging events (Moore *et al*, 2018). Tidal flow could also act to rapidly displace *L. hians* and erode the seabed they live on when exposed by dredging activity before they have a chance to rebuild.

Observations indicate that *L. hians* can rapidly (over a few days) rebuild their nest if they are not damaged along with their nests. These observations differ from observations by Trigg (2009) who reported a sizable nest within 5 weeks of introduction to glass tanks. This could be due to the set up in which Trigg's specimens were maintained in which there was a high mortality of animals. Alternatively, a different definition of what qualifies as a rebuilt gallery could explain the discrepancy.

This suggests that when recovery of a bed takes much longer than a few weeks large scale mortality of individuals has likely occurred. This is supported by observations made after the Loch Carron scallop dredging incident of 2018 where large quantities of dead and broken shell material were observed along dredge tracks (Moore *et al*, 2018). Evidence that Dredging

causes mortality of *L. hians* also comes from (Hall-Spencer & Moore, 2000b)

Experiments on the interactions between *L. hians* and its known predators would help give more significance to these findings. For instance, observations using the full spectrum camera set up of disturbance in the presence of a predator such as the velvet crab *Necora puber* (*N. puber*). Gilmour (1967) states that *N. puber* finds the tentacles of *L. hians* distasteful however observations in the field (pers. obs) reveal that this is not enough to deter predation which was frequently observed by *N. puber* and *Liocarcinus depurator*, although these could have been scavenged rather than caught whilst alive.

A striking difference in nest building activity during the day and night was observed. This could be emphasized by the likely higher light levels during the day than would be seen in the wild. Further work could use light levels equivalent to those found on a natural bed.

Further laboratory-based studies could investigate whether the feeding rate is reduced at night compared to the day when less time is spent nest building. This would allow greater accuracy of filtration rate estimation of flame shell beds in terms of the ecosystem services they may provide in reducing water turbidity. Studies of oyster reefs (zu Ermgassen *et al*, 2013) have quantified the loss of filtration by removal of oysters. Similar studies could be conducted in *L. hians*.

Animals were observed to rapidly contract their pallial tentacles in response to the fluorescent light turning on. Once the light turned on the animal would usually continue to place threads for a few minutes and then cease nest building until darkness was resumed. This indicates the infrared light was not detected by the animals as there was no such response to this light source.

The animal was occasionally observed to close both valves as far as possible and hold position for a few seconds then re-open the valves. Investigation into the possible reasons for this behaviour could be of interest to future researchers.

Ideally future investigations would conduct longer observations and conduct independent replicate observations to allow for statistical

analysis. This would indicate whether the observations made in this report are typical of all individuals within a population. The camera system in its current state at full resolution would be capable of recording for ~11 days and much longer at reduced resolution or with a higher capacity hard drive. There is also potential for in-situ observations of animals in the wild during the night as it appears there has been no such data recorded at night. This would however require substantial modifications to the camera system to water proof it and allow it to run on battery power overnight.

#### 4.3.0 Disturbance and homing behaviour

The homing behaviour experiments conducted did not reveal any homing behaviour. The animals appear to move a great deal from where they were placed. This could be due to the fact that when moved to a new location they were placed in an area of identical substrate type (mixed small gravel, shell fragments and nest material). This substrate, as shown by the substrate preference experiments is likely to be a desirable substrate so they may be less likely to move back to their original position as the conditions were the same.

In future attempts at homing behaviour

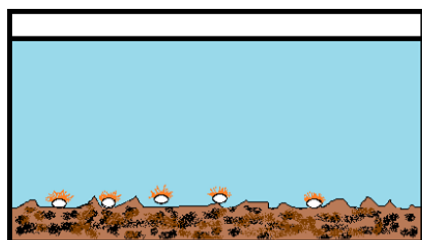
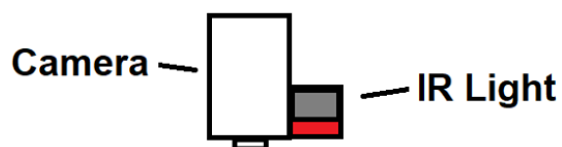


Figure 11 Diagram of the proposed disturbance experiment set up showing the position of the full spectrum camera and the IR light.

experiments the full spectrum camera system used in the nest building observations could be used to observe the animals for much longer

than was possible using the GoPro cameras (figure 11). This would also enable observations the animals at night. The animals may show more activity at night as was shown in the nest building observations.

#### 4.4.0 Port Appin surveys

##### 4.4.1 Historical decline at port Appin

The continued decline of the Port Appin flame shell bed has been reported since 2011 (Moore *et al*, 2012). An 89% reduction in bed area was shown with only three patches of *L. hians* bed present, one in the north and two Southern patches covering a total area of only 2.73 hectares. The northern and Southern patches were now separated by approximately 2.2 km where there had once been contiguous reef. Subsequent work by Cook (2016) showed that the two Southern patches were no longer present and there had been a slight reduction in the northern patch. Finally in 2017 Taylor (2018) conducted diver drifts over the northern part of the bed, from these results it appears there had been a further decline since 2015 with no *L. hians* present at any station within the previously estimated 2015 extent. There were however *L. hians* present at stations between 93m and 250m north of the previously known extent (figure 8). It was not clear whether this is a newly formed patch of reef as the area had never previously been surveyed. There is currently not enough data to estimate its extent.

##### 4.4.2 Drift surveys

The dive conducted for this report shows that the flame shell bed does not extend further than the 2017 survey showed. The first station (0% cover) was recorded 11m from the last station of the KT4 transect (KT4-12) which had 40-50% nest cover in 2017 (Taylor, 2018). The new transect largely followed the edge of the channel at between 8.6 and 9.8m. Moore *et al* (2012) found that flame shell beds were only found between 8.7 and 12.5m at Appin. Therefore, there is an area to the East of the new transect which is of a suitable depth and has never been surveyed (figure 8). Future survey work should focus attention on this area and giving a better estimate of the extent of the Northern patch.

For the size frequency divers were dropped in on the KT4-10 station which had 80% nest cover in 2017 (Taylor, 2018). After searching for

~15 minutes a small patch of 30-40% nest cover was found 25m from the KT4-10 station (figure 8).

The size frequency data from this site (figure 8) revealed a different distribution than was seen at a previous site in Appin which has since disappeared. (figure 9a). These differences suggest that this site may have healthier population and perhaps be less susceptible to decline. This is discussed further in the next section.

Two sources of error were considered when evaluating whether a decline in the Port Appin bed had in fact observed. GPS coordinate error and previous over estimation by the diver. Both of these were dismissed once the original GPS and video data was re-examined.

#### 4.4.3 size frequency

Trigg (2009) observed that the most frequent size class found at Appin (September 2006) was 29mm - 29.9mm, over 7% of the sample total. This is similar to the most frequent size class observed in November 2011 of 31-31.9mm, 16.5% of the sample total (MacLeod, 2012). This differs from the most frequent size class in Shian (September 2006) of 19-19.9 mm, 6% of the sample total. Trigg speculated that the tendency of the Appin population towards larger individuals compared to Shian could be due to lower population densities allowing faster growth rate as in the Atlantic surf calm (Cerrato & Keith, 1992).

The population in Shian (September 2006 see figure 9b) showed vastly more recruitment than Appin individuals of ~2-9 mm peaking at 6.4% for 6mm individuals than Appin which was discernible but small (mode 1.1%). Our data for November showed higher proportion of the population in the new year's cohort when compared to previous years data from Appin (Trigg 2009 and MacLeod 2012). This bears more resemblance to data from the Shian bed.

The data collected in 2019 showed that Appin was not dominated by larger individuals with few smaller individuals, as seen in the previous data from Appin (figure 9a), and more closely resembled the data from Shian in November 2007. This indicates the northern patch has levels of recruitment which closer match those of a healthy bed however the percentage nest cover recorded was not as high (30-40%) as would be expected from a healthy reef. This

could be because the centre of the bed was not found and only an area on the margin. This indicates that there has not been a recent recruitment failure at the sample collection site. Also, the presence of larger adult animals, in similar proportions to Trigg's 2006/7 data indicates that this is not a newly settled area and has been present for at least 5 years based on Trigg's aging studies.

All previous size frequency data from Appin, and the data collected for this study used single replicates. This could affect our ability to draw conclusions regarding the state of the bed as it is unknown how variable the size distribution of animals within a flame shell bed is. There could be highly localised differences in recruitment in this area. The comparisons made in figure 9a and 9b data comparisons somewhat account for temporal variability by comparing datasets collected in the same month (different years).

Specimens for the size frequency collected by MacLeod (2012) and Trigg (2009) were collected from the same location (56°33.845'N 5°24.817'W). It is not known if the specimens were taken precisely the same location as Trigg's original specimens. Subsequent surveys indicate that the site where Trigg and MacLeod collected their samples has likely disappeared (Taylor, 2018; Cook, 2016a). The fact that this area of bed has likely disappeared, and low recruitment of juveniles was seen preceding this suggests that recruitment failure could be responsible. This is in contrast to the results from the northern patch in which higher recruitment was seen. Similar to that in Shian 2006/7.

It appears that the mean size of the animals collected at Appin for this study would have been lower than previous years at Appin. Unfortunately, as previously stated, the original data was unavailable therefore the mean could not be calculated. Future work could compare the means of the different samples however the original data would have to be acquired. Alternatively write an R function to "un-bin" the data however this would reduce the resolution of the data as it is in 1mm size classes.

#### Conclusion

This study confirms previous reports that *L. hians* prefers structurally complex substrates. It was also shown that the ratio of body size to available refuges is an influential factor on a preference for a given substrate. A series of further experiments revealed that there is likely

an attraction of adults to nest material produced by their conspecifics. Nest building observations revealed a much higher level of activity at night. This raises many questions about which other behaviours may be influenced by photoperiod. The findings in this study suggest there has likely been further decline of the Port Appin bed however further investigation would be required to verify this. This follows a trend of continuing decline observed since 2011. The size frequency data collected as part of this study shows more recruitment than previously seen at Appin indicating that there has not been a recruitment failure. Size frequency also suggested that the newly discovered patch has likely been present for at least 5 years and is not a recently settled area.

### Acknowledgements

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APPENDIX

North Appin Drift

Time	Layback (deg)	Depth (m)	Limaria present?	limaria nest %	Substrate	Diver	Date	Latitude	N/S	Longitude	E/W
13:55:00	90	9.8	N	0	10% red algal turf, pebbles 70%, cobbles 20%, on shell gravell, 1-9 kelp /meter	Lisa Kamphausen	27/10/2018	56.566284	N	-5.411730	W
14:05:00	90	8.6	N	0	10% red algal turf, pebbles 70%, cobbles 20%, on shell gravell, 1-9 kelp /meter	Lisa Kamphausen	28/10/2018	56.566721	N	-5.411808	W
14:09:40	22	9.6	N	0	10% red algal turf, pebbles 70%, cobbles 20%, on shell gravell, 1-9 kelp /meter	Lisa Kamphausen	29/10/2018	56.566813	N	-5.411453	W
14:17:04	90	9.8	N	0	Shell sand, 10% red algal turf, 1-9 kelp /meter	Lisa Kamphausen	30/10/2018	56.567434	N	-5.411480	W
14:23:50	90	11.7	N	0	Muddy shell sand, 1-9 kelp /meter	Lisa Kamphausen	31/10/2018	56.567903	N	-5.411333	W