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MANUAL TO STUDY THE RECRUITMENT OF *FICOPOMATUS ENIGMATICUS* (FAUVEL 1923) AT THE CLIVE RIVER, AHURIRI ESTUARY AND RELATED WATER BODIES

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MANUAL TO STUDY THE RECRUITMENT OF *Ficopomatus enigmaticus* (FAUVEL 1923) AT THE CLIVE RIVER, AHURIRI ESTUARY AND RELATED WATER BODIES

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Prepared for Hawke's Bay Regional Council
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1. INTRODUCTION AND OBJECTIVES

Hawke's Bay Regional Council (HBRC) plans to implement a field-based programme to monitor the seasonal recruitment and geographical expansion of the invasive serpulid *Ficopomatus enigmaticus* (Fauvel 1923) in waterbodies in the Napier region. This is important since eradication or control attempts of local populations should not be undertaken within the reproductive season. During this season, individual serpulid worms produce thousands to millions of gametes (Kupriyanova et al. 2001; Wolf 2020). Disturbance associated with removal or treatment activities is likely to cause a so-called 'release-and-die response' (E. Kupriyanova, pers. comm.), during which all gametes are released within minutes. This can lead to an unwanted 'pulse' in fertilisation and re-colonisation of treated areas, undermining the efficacy of control attempts. Recruitment studies also help identify native species whose recruitment and local abundance become suppressed by *F. enigmaticus*.

This document provides support for designing, planning and implementing this recruitment monitoring for *F. enigmaticus*, either independently by HBRC or with assistance from Cawthron Institute. It is one of two reporting outputs of Envirolink Grant 2244-HBRC265.

2. METHODS

In essence, a recruitment monitoring program for sessile species such as *F. enigmaticus* involves the repeated deployment and recovery of experimental 'collectors' at a range of sites and the inspection of these collectors for recruits of the target species. This section describes the various steps involved in this and suitable methods for implementation.

2.1. Selection of monitoring sites

Ficopomatus enigmaticus has a larval phase of at least 5-9 days (Graham 2014; Gabilondo et al. 2013) and potentially up to several months (Thorson 1966; Toonen & Pawlik 2001), enabling dispersal by currents to locations at a considerable distance from parent populations. Since recruitment does not occur homogeneously along estuaries, rivers, and coastlines, we suggest establishing at least three monitoring sites in each waterbody targeted (e.g., the Ahuriri estuary and Clive River) to ensure recruitment events are not missed. As environmental conditions affect recruitment, it is recommended that the monitoring sites are established at locations (1) near, (2) 500–1000m upstream, and (3) 500–1000 m downstream of a 'major' *F. enigmaticus* reef within each waterbody.

If a further objective is to identify the geographic or environmental limits for *F. enigmaticus* recruitment, additional sites should be established further upstream as well as towards the sea and along the coast, with 1–5 km between successive sites (depending on available resources). Regional hydrological models should be consulted to determine a meaningful extent of upstream and along-coast monitoring sites, and to avoid under- or over-sampling. Previous studies have detected *F. enigmaticus* in estuaries and rivers at distances of up to 30 km from the sea (Straughan 1972a; Dittmann et al. 2009).

2.2. Experimental surfaces for attracting recruits

2.2.1. Material

Serpulid worms are known to settle on a wide variety of natural and manmade substrates, including rock, wood, concrete, steel, shells, glass bottles, and algae (Fornós, et al. 1997; Moore et al. 1998; Riedi 2012; Schwindt et al. 2004). This provides a degree of flexibility for the design of recruitment monitoring programs.

The simplest approach for HBRC would be to construct experimental surfaces from a material that is proven in attracting serpulid recruits. From personal experience with native serpulids, I recommend using plates made of sandstone (O'Donnell 1986; Wolf 2021). As an alternative, 'Tufnol' plates (laminated fabric or paper sheets) have also attracted high recruitment of serpulid worms (Thorp 1994). Earlier studies suggest that wood and rubber are less attractive substrates for recruiting individuals of the *Ficopomatus* genus (Straughan 1972b).

Individual experimental surfaces or 'plates' should have a standard size. There is no 'best' size from a biological perspective. However, plate dimensions of 5 x 5 cm to 10 x 10 cm (H x W) are easy to handle and appropriate in relation to the size of targeted recruits.

If HBRC envisage an extended (e.g., multi-year) monitoring program, the initial deployment of multiple substrate types may be a suitable strategy to identify the most cost-effective material for use in the longer term, i.e., one that is highly attractive to *F. enigmaticus*, easy to obtain and affordable. Options beyond sandstone and Tufnol® include PVC, polyethene, fibreglass, or cement sheets such as tiling underlay. Not all substrate types need to be deployed in all program sites. Instead, multi-substrate arrays could be installed only at sites with known *F. enigmaticus* populations/reefs nearby, and/or only once some recruitment has been observed. Once a superior substrate has been identified, the other types can be dropped.

2.2.2. Replication

At least five replicate plates (for each substrate type) should be installed at each monitoring site. All replicates should be attached at a similar tidal height that corresponds to those of existing adult populations in the same water body. *Ficopomatus enigmaticus* often settles just below (0.5–4 m) the intertidal boundary (Weitzel 2021; Bianchi & Morri 1996); the plates should thus not be installed higher than Mean Tidal Level (MTL).

2.2.3. Temperature & Salinity

Water temperature and salinity are important determinants for reproduction, and recruitment of *F. enigmaticus* (see section 3). If resources permit, monitoring of both variables is recommended. The most cost-effective approach is likely to be the use of data loggers. These devices are relatively inexpensive and are able to measure, and record temperature, and salinity at 30 – 60 minutes for periods of weeks or even months, allowing the calculation of averages, and daily variations for both variables. These averages can then be used to make predictions around future reproductive seasons and recruitment periods (see Section 3 & Appendix 2).

2.3. Installation

2.3.1. Sites with hard substrate such as rock or artificial coastal infrastructure

The five (or more) replicates can be attached directly to the rock or artificial structure via a central bolt and a spacer. Replicate plates should be installed at least one plate width apart from one another to avoid edge effects caused by turbulence. A 5-mm gap should be maintained between the plate and the attachment surface as the larvae will preferentially settle on the sun-adverse (shaded) side of the plate. This gap can be achieved via the use of a spacer.

Alternatively, simple holding frames can be constructed from PVC, steel, or aluminium tubing, or larger backing plates. The advantage of these frames is that multiple experimental plates can be attached to them, and that frame attachment may only require 1-2 bolts depending on the design. See Appendix 1 for examples of frames used to deploy recruitment plates of different types.

2.3.2. Soft-substrate sites

In soft-substrate environments such as sand or mud, fixed attachment of recruitment plates or frames is problematic or even impossible. In such situations, the use of 'floating arrays' is advantageous. These can be constructed by suspending the frames (described above) from a surface (or sub-surface) float, and fixing the array in position via use of an anchor or concrete weight. Positioning suspended frames at least 0.3 m below the water surface has yielded high levels of *F. enigmaticus* recruitment in the Lower Lakes and Coorong (near Adelaide, Australia) (Dittmann et al. 2009). A further option for soft-substrate environments is the use of stakes (warratahs) that are driven into the bottom and to which plates or frames can be attached.

In the Napier region, frames should be installed at depths where existing populations are known to exist (this may vary between sites). If determining the vertical range of *F. enigmaticus* were an additional objective, multiple plates (or frames) could be installed from the MTL to 5 m below the water surface near or between existing reefs.

2.3.3. Identifiers

I recommend applying a unique identifier next to each plate attached to the substrate to enable tracking of their location and timing of immersion. There are many options for doing this; an example format could be 'AE3-2-DEC22' (Ahuriri Estuary site 3, plate 2, December 2022), or simply a number (e.g. '001') that corresponds to site and date details logged in a datasheet.

The identifiers need to be applied so they do not fade or wash off the substrate. Products such as 'shellfish glue-on tags' by Hallprint (Australia) have proven useful and can be directly glued to the surface next to the plate using (gel-like) super glue. Alternative, a hole could be drilled into the plates, and cable ties with a tag can be attached.

Again, a datasheet should contain all site information, frame numbers, immersion dates and other information for all plates and their unique identifier. The importance of this cannot be overstated: once a plate has been 'mixed up', it can be impossible to ascertain its correct origin.

2.3.4. Minimizing the risk of plate loss

To avoid losing plates through waves, currents, and winds, plates should be attached securely using mechanisms judged to withstand local conditions. If the plate is made of natural stone and bigger than 50 x 50 mm, it is important to secure the plate with at least two bolts to the substrate to avoid losing the plate through breakage.

Two approaches can be used to avoid vandalism or removal via curious members of the public: (1) 'hiding' frames and plates as best as possible so they cannot be seen, or (2) the use of signage, flyers, and council communications to create public awareness about the project, and the need to avoid interference with any equipment. From a council perspective, option 2 may be the most appropriate.

2.3.5. Dataloggers

If resources permit, single dataloggers (measuring temperature and salinity) should be installed (i) 1 km downstream of the most seaward *F. enigmaticus* aggregation known (or at this aggregation if it is on the open coast), (ii) at a location up the estuary (or river) where salinity is constantly below 5 PSU, and (iii) at 1 km intervals in between these locations. If resources are limited, a single logger can be installed between (i) and (ii).

2.4. Retrieval and exchange

To determine the seasonality of *F. enigmaticus* recruitment, a temporal series of plate deployments needs to be undertaken where immersed plates are exchanged for 'fresh' plates at appropriate intervals. These can be decided on based on the objectives of the study. For example, a reasonable aim may be to determine the months of the year during which recruitment occurs. This necessitates monthly deployment and exchange of plates for at least one year, preferably two. However, during certain times of the year, particularly during spring to autumn, many other sessile invertebrate species undergo reproduction, resulting in elevated 'recruitment pressure' to vacant substrates. Since *F. enigmaticus* recruits are very small in size, monthly immersion events during warmer months may be too long and risk overgrowth or displacement by other species, possibly resulting in non-detection of target recruits. Also, the opposite could occur, where excessive recruitment of *F. enigmaticus* within four weeks could lead to difficulties in quantifying the abundance of recruits.

Therefore, I recommend that plates be exchanged monthly during April–November, but fortnightly during December–March. These intervals can be reviewed and optimised after (or, as needed, during) the first year of monitoring.

Retrieved plates can be placed in zip-lock bags with their unique identifiers, or with a label that states the identifier. The plates can be stored without water for transport. Close spacing and friction should be avoided to ensure no worms get dislodged. If the plates are not examined within 24 hours after collection, it is advisable to store them within their bags in a freezer. Frozen plates can be examined at any later point. For longer transport, e.g. shipping the plates to Nelson, it is advisable to ship them frozen. In this way, the decay of biota on the plates is minimised.

The Dataloggers should be retrieved and read out at least once a year. For better estimates about temperature and salinity, it is advisable to minimise the time when the loggers are out of the water. In the best case, an annual read-out with same-day return of the logger to its deployment location would be ideal.

2.5. Analysis of plates

Plates should be examined using a stereomicroscope so that no target recruits are accidentally missed. Depending on when during the monthly or fortnightly periods recruitment occurred, juvenile *F. enigmaticus* can be expected to have a size of approximately 0.3–1.5 mm.

Ficopomatus enigmaticus on plates can be logged as present/absent to help understand whether recruitment occurred in any given month. However, if resources permit, it is recommended to quantify recruits using direct counts, a logarithmic scale or percentage cover (see below). This provides useful information on the magnitude of recruitment throughout the reproductive period and helps identify when peaks of recruitment can be expected.

For the analysis of plates with a high density of worms, staining (e.g. with methylene blue) is recommended to increase the contrast and make worms more visible. Recruits are likely to be found in particular on the side of the plate, which is facing away from the sun. Some individuals will settle at the front side of the plate near the screw used for attaching the plate and beneath the foliage of attached algae if these are present.

For monitoring purposes, it is sufficient to only quantify recruitment on the side of a plate with the most settlers. If small plates are used (up to 5 x 5 cm), direct counts are realistic. If larger plates (e.g. 10 x 10 cm) are used, subsampling is recommended (Thorp 1994). In this case, a 10 x 10 cm plate could be subdivided into one hundred 1 x 1 cm 'cells'. Ten randomly selected cells can then be counted, the average number of recruits per cell calculated, and this average extrapolated to the total plate by multiplying it by 100.

If counting is too time-consuming, *F. enigmaticus* abundance on plates can be expressed as percentage cover of the plate area that is examined (Dittmann et al. 2009). This can be achieved via the use of a random-dot method, where a set number of randomly placed points (dots) are projected onto the plate. This number should be at least 60. For each dot, it is determined whether it falls onto a target recruit. The percentage cover of *F. enigmaticus* can then be calculated as:

*% cover F. enigmaticus = ((no. dots with F. enigmaticus recruits under them / total no. dots examined)*100)*

However, this method may underestimate the abundance as individual worms can settle on top of one another. Therefore, if only the percentage cover is recorded, storing the plates in a freezer could be beneficial for further investigation.

An even less time-consuming (and hence coarse) method is the use of a logarithmic scale to describe abundance. This involves the description of approximate worm numbers as: none; 1-10; 10-100; 100-1,000; 1,000-10,000 per plate (etc.). This method should only be used following observer training and validation to avoid vast underestimation or overestimation of abundance.

If plates with recruits are not needed for further studies, used plates can be cleaned and redeployed. Following examination, plates should be submerged in ethanol or bleach for 1-2 hours to kill recruits and subsequently cleaned using a wire brush and water.

2.6 Data analysis and interpretation

The seasonality of recruitment and occurrence of reproductive peaks can be described by plotting or tabulating the numbers per unit area, percentage cover, or presence/absence of *F. enigmaticus* observed during the consecutive monitoring events. Recruitment data for a single year is useful but may not be 100% representative of if between-year variation occurs. To capture such variation, at least two years of data are needed.

Visual examination of monthly recruitment magnitude (and associated variation) is adequate for identifying the duration and relative magnitude of *F. enigmaticus* recruitment throughout a year. If empirical assessments are needed, statistical tests such as parametric analysis of variance (ANOVA) or non-parametric alternatives (e.g. PERMANOVA) can be used to determine differences in recruitment between months (or fortnightly periods), waterbodies, sites and other factors included in the monitoring design (e.g., substrate type, deployment depth). Presence/absence records are insufficient for most statistical analyses. If analysis of settlement patterns, recruitment to different substrate types and comparisons to other geographic populations (via the literature) are desired, I would suggest counting the recruits and expressing the data in recruits/day/cm^2.

An additional interesting student project could be a 'nearest neighbour analysis' to investigate if recruits of *F. enigmaticus* settled aggregative or randomly and if this pattern changes with the substrate or potential treatment of plates (Wolf 2020). Such studies on the settlement of this serpulid will also benefit later mitigation efforts.

3. CURRENT KNOWLEDGE OF *F. ENIGMATICUS* REPRODUCTIVE SEASONALITY TO AID PLANNING

This section considers the question: how soon should HBRC's monitoring programme commence, and which areas should be included?

Published information about the reproductive biology of *F. enigmaticus* is conflicting. Water temperature and availability of nutrients are key determining factors for the reproduction and larval survival of many marine invertebrates (Giangrande 1997).

Some publications indicate that *F. enigmaticus* reproduce in water temperature between 18° to 24° C (Obenat et al. 2006; Bianchi & Morri 1996), while others suggest reproductive activity begins at temperatures as low as 10° C (Thorp 1994) (also see Appendix 2). Given the uncertainty about the temperature requirements for the reproduction and recruitment of *F. enigmaticus,* it is advisable to deploy recruitment plates throughout the year.

Using the most widely stated temperature range (18° to 24° C) for recruitment in Napier, I expect a recruitment peak to occur between December and January (Figure 1). Interestingly, monthly water temperature data provided by HBRC suggest a potential for differences in recruitment period and peak in Ahuriri estuary and Clive River (Figure 1). For example, the recruitment period in the Ahuriri estuary could be longer by a month and reach into April. The regional difference in key variables governing reproductive activity highlights the need for a monitoring program that includes all waterbodies where infestations are present.

Gamete production is also influenced by salinity (Bianchi and Morri 1996; Peria and Pernet 2019; Premoli and Sella 1995). As species in the *Ficopomatus* genus seem to prefer water with slightly lower salinity, recruits can be expected at considerable distances upstream (Dittmann et al. 2009; Straughan 1972b). Reviewing the salinity tolerance for *F. enigmaticus,* as well as the salinity range required for larval development and maturation, helps to form the expectations for how far upstream recruitment could be expected. Data for the Ahuriri estuary indicates that the salinity around 11 km upstream is below 5 PSU for most of the year. Based on published tolerance data, this point may represent the geographical limit of the expansion in the estuary. While individuals of *F. enigmaticus* seem to be able to tolerate salinities as low as 0 PSU, gamete maturation and larval development do not occur below 5 PSU (Dittmann et al. 2009). At the HBRC sample station “Quarantine Road”, the salinity peaked in summer 2020/21 (45PSU) and was slightly elevated in January 2022 (6 PSU; data provided by HBRC). Although *F. enigmaticus* may thus be expected to occur at this location, it is uncertain whether the warmer periods with salinities above 5 PSU are long enough for reproduction and recruitment. Therefore, I suggest establishing the final recruitment sample location within 5 km upstream of HBRC’s Quarantine Road sampling station.

Generally, during the peak reproductive season, I would expect (depending on substrate) a daily recruitment of 0.3 to 1 individual per square centimetre on plates deployed near *F. enigmaticus* populations (Bianchi and Morri 1996; O’Donnell 1986; Straughan 1972b; Wolf 2020).

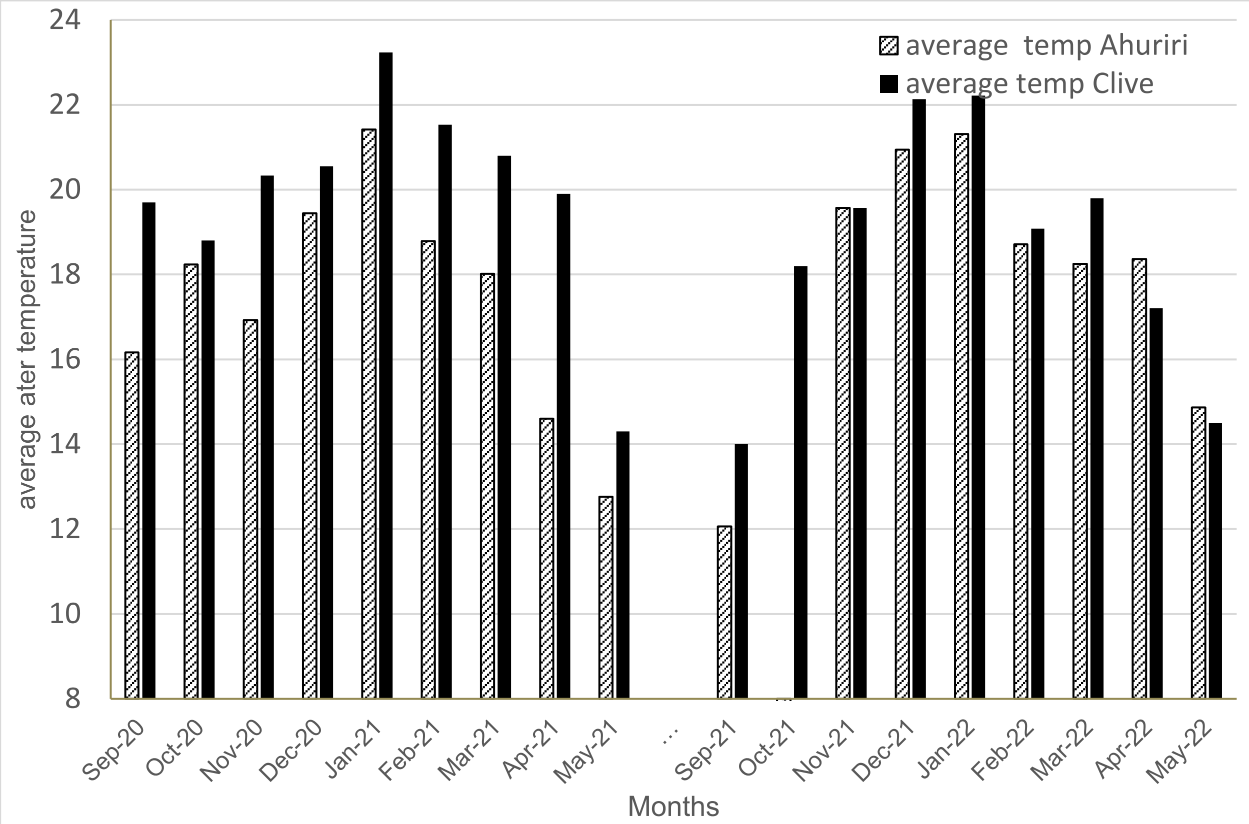

Figure 1. Water temperature (degrees C) at the Clive River and the Ahuriri estuary for the period September to May for 2020 and 2022. Average temperatures are based on single-point measurements provided by HBRC.

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Appendix 1. Examples of frames to hold recruitment panels.

Figure A1.1. Backing plate with replicate plates attached. Image: P. Cahill, Cawthron.

Figure A1.2 Frames made from aluminium tubing holding different types of experimental panels, including 3D printed artificial reef structure. Image: O. Floerl, Cawthron.

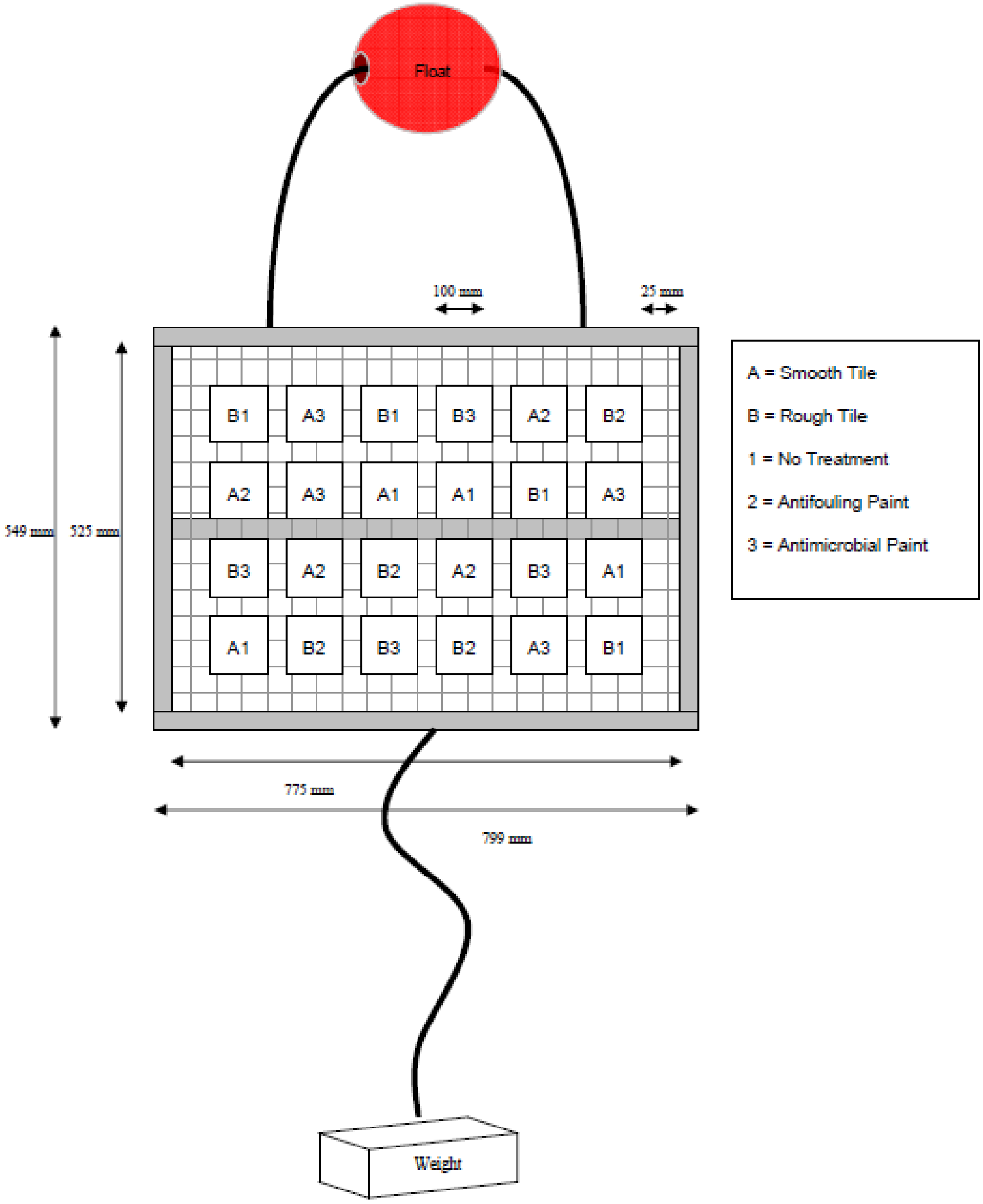

Figure A1.3 Schematic description of settlement array used in studies in south Australia. The frame could be made from piping and lined with a mesh to attach the settlement plates. The frame is kept at a location through weight or anchor and near or below the water surface through a buoy (Dittmann et al. 2009, page 37 Figure 4).

Appendix 2. Additional consideration for determining the reproduction period.

As illustrated in earlier sections (1, 2.5 and 2.6), knowing the season and factors affecting the reproduction is of eminent importance for any mitigation efforts. Therefore, I will briefly describe a few aspects regarding the reproduction of *F. enigmaticus* and potentially other serpulids.

According to literature, recruits can reach maturity in 4 to 16 weeks (Hill, 1967; GISD, 2008), which implies that established individuals can produce gametes within the same timeframe. Subsequently is suggested that *F. enigmaticus* and other serpulids are able to spawn at least twice per season/year (Leone, 1970; Zuraw & Leone 1972; Bianchi & Morri 1996).

In literature, we can find contradicting information regarding salinity and temperature range for the reproduction of *F. enigmaticus* (Bianchi & Morri 1996; Thorp 1994; Dittmann et al. 2009). Amongst other factors, this discrepancy could be explained by exploring the reproductive biological zero point (BZP). This zero point refers to the temperature at which the organism doesn't produce gametes (Bulter et al. 1989; Viana 2005; Wolf & Ruawai 2020). Each degree above the BZP contributes to the maturation of gametes. If the degrees above the BZP per day add up, we receive an estimated accumulative temperature (EAT) (Bulter et al. 1989; Ritar and Elliott 2004). The EAT more the maturation is known for some shellfish species and allows for a more accurate assumption about maturation levels and reproductive season (Leighton 2008; Ritar & Elliott 2004; Wolf & Ruawai 2020). Because the reproductive period for serpulids is often identified through recruitment observation, it is to accept that we only indirect observe the factors affecting reproduction.

Given that salinity is more or less stable and assuming that nutrients are present in excess, temperature could be the major factor determining development of gametes. Hence if we would know the BZP and EAT for *F. enigmaticus* we could make better prediction about the reproductive season. The BZP as well as the EAT could potentially explain the observation of a possible wide range of temperature for the reproduction of this and other serpulids.

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