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Evaluation of barnacle (Crustacea: Cirripedia) colonisation on different fabrics to support the estimation of the time spent in water by human remains

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Highlights

- Barnacle colonization of clothes can aid the estimation of the minPMSI of a corpse.
- Barnacles can colonise neoprene within one month after placement in the ocean.
- Barnacles preferentially colonise neoprene, followed by satin and cotton.
- Barnacles have an inconsistent colonisation rate on velvet.
- Water temperature and type of fabric affect the number of colonising barnacles.

Abstract

The estimation of the time since death (minimum Post Mortem Interval, minPMI) is an essential aspect of forensic investigations. This is particularly complex when a human body is found submerged, floating or beached in a marine environment. When a cadaver is found in a terrestrial environment the minPMI estimation is generally based on the presence of carrion insects. However, when a cadaver is found in an aquatic environment, a correct crime scene reconstruction is more complex and requires the consideration of the time the remains spent submerged underwater (minimum Post Mortem Submersion Interval, minPMSI) and/or floating (Floating Interval, FI). In marine crime scene scenarios, the use of barnacles (Crustacea: Cirripedia) has recently received some attention, due to their permanent settlement on human remains and their accompanying clothing. Previous research considered barnacle growth on human shoes, but the present research is the first to focus on the colonisation of barnacles on clothing materials (fabrics). Polystyrene floats were covered by either cotton, velvet, satin or neoprene and submerged underwater over a period of six months off the coast of Perth, Western Australia. The aims of this research were 1) the identification of marine species colonising the fabrics, with special attention to barnacles; 2) the identification of which fabric type provides the most desirable environment for colonisation; and 3) the identification of factors that affect the growth rate of the different species. Three species of barnacles, *Balanus trigonus* Darwin, *Amphibalanus reticulatus* (Utinomi) and *A. variegatus* (Darwin), were present in varying numbers and sizes. The colonisation process of the

barnacles occurred rapidly, with the first sighting of barnacles observed within the first month on neoprene and control floats. The surface that attracted the largest number of barnacles was neoprene, followed by satin and cotton, while velvet showed an inconsistent colonisation rate. The largest size barnacles were observed on the control floats, while all fabrics showed a similar smaller size. Overall, time spent in water and water temperature had a significant positive relationship with both number and size of the colonising barnacles.

This study is the first to provide information that will aid in the investigation of human remains recovered from Western Australian marine waters, using the barnacle colonisation on different fabric types.

Key words

Barnacles; cotton; neoprene; satin; velvet; PMSI

Introduction

Criminal cases may involve the forensic investigation of human remains discovered in any environment, from the land to sea, from buried in the soil to concealed in a well [1]. To date, research and case studies have mostly considered the investigation of human remains in terrestrial environments, whilst only a limited number have been focused on remains found in aquatic environments, either fresh or saltwater, in natural or artificial basins [2, 3]. However, water covers the vast majority of the Earth's surface, utilized for occupational and recreational activities, and every year a large number of deaths occur in aquatic environments. The 2017-2018 annual report of the Royal Life Saving Society Australia declared that an average of 249 deaths occur in Australian waterways as a result of fatal unintentional drownings [4], with 16% of drowning deaths resulting from boating or watercraft activities. In 2019, 279 people drowned in Australia waterways, 81% males and 19% females, with top locations being swimming pools (11%), sea (26%) and rivers (29%)[5]. To note, 45% of deaths happened in the major cities, followed by inner and outer regional locations (24% and 20% respectively), and only a small percentage in remote and very remote places (6% and 5%). Furthermore, water may cause deaths in cases of mass disasters (e.g. tsunami, boat sinking) or may be used to cover up a crime [6].

From the taphonomical point of view, the typical decomposition process of a cadaver displaced in an aquatic environment is comprised of phases of sinking and floating [7]. As a consequence, the process might potentially involve the action of both aquatic and terrestrial organisms [8, 9]. Unless the cadaver is protected in a vehicle or unable to float for other reasons, birds and terrestrial insects will impact it from above, while fish, crustaceans and aquatic insects will consume or colonise it from below the water surface [10, 11]. At present, due to the sparsity of research on the role that intrinsic and extrinsic factors play in the human decomposition process in water, aquatic forensic cases are a difficult challenge when reconstructing criminal events. For example, when a cadaver is found in an aquatic environment, the estimation of the time since death (minimum Post Mortem Interval, minPMI), must also consider the time the remains spent submerged and/or floating in the water (identified as the minimum Post Mortem Submersion Interval, minPMSI and minimum Floating Interval, minFI, respectively) [2, 8, 9, 12]. Furthermore, in several circumstances, the nature and variability of aquatic environments can be problematic when applying the currently established medico-legal and forensic entomological practices for minPMI estimation [1, 8]. In recent years, efforts to provide a better estimation of minPMI, minPMSI and minFI in forensic aquatic-related cases have explored other avenues [13], such as the use of crustaceans (Arthropoda: Crustacea) [14]. Some areas of research consider the effects of motile crustaceans (e.g. crabs, Crustacea: Decapoda) as colonisers and decomposers of remains, acting as insect-proxy in the ocean [9] or in freshwater [11, 15]. Others have instead focused on the use of barnacles (Crustacea: Cirripedia: Sessilia and Pedunculata), common marine biofouling crustaceans, that colonise the remains settling on shoes, clothing or directly on teeth and bones [14, 16-18].

Unlike motile crustaceans, barnacles are advantageous in the estimation of minPMSI and minFI due to their ability to settle on a substrate in a very short amount of time (48 hours given a desirable surface and optimal environmental conditions [19]. Barnacle settlement is permanent, and their growth is mainly reflective of water temperatures [20, 21]. Additionally, specimens found attached to the remains can be used to locate the primary crime scene, by considering the biogeography of the species and the chemical profile of the shell [22], providing additional data to the investigation.

Despite the clear forensic potential, literature on barnacles at present is focused primarily on taxonomy, distribution, biochemistry (of their glue) and biofouling mechanisms [23, 24]. The use of barnacles in forensic science to date accounts for a small number of case reports [16-18, 25] and only one study based on the colonisation of barnacles on different types of shoes has been published [14]. In many cases, bodies enter the water fully clothed, therefore the present research considers – for the first time – the colonisation of barnacles on fabrics commonly used in clothing.

The aim of this study was to identify the marine fauna (with a specific focus on barnacles) associated with the colonisation of cotton, satin, velvet and neoprene placed underwater in Western Australia. Furthermore, this research identified the fabric which provided the most desirable environment for colonisation and investigated factors that most affected the barnacles' growth rate.

This study is the first to provide data to support the estimation of PMSI of different fabric types recovered from Western Australian marine waters.

Materials and methods

2.1 Site description

The research site, Cockburn Sound (Western Australia), is a natural embayment in the Indian Ocean, approximately 16 km long and 7 km wide (112km^2). This area is open to the North, whilst the western seaward side remains sheltered by Garden and Carnac Island. Stretching approximately 25 km from the South Swan River mouth in Fremantle, reaching to Cape Peron near Rockingham, the research site used was located at 32°15′05″S 115°43′17″E (Fig. 1). At this site, the flow of seawater is restricted by the 4.2 km rock wall making up the causeway – extending northward from Point Peron to the southern side of Garden Island, with small trestle bridges creating a passage for seawater [26]. The causeway and the two islands shelter the Cockburn Sound embayment from the elements, although the northern region is still affected by strong north and north-westerly winds which can generate wind-waves that may sometimes cause rough sea conditions [26]. The water in the embayment ranges from 2-4 m deep in areas such as Southern Flats, with deeper waters in the central channels, that can reach 20-25 m [9]. In the northern part of the Cockburn Sound embayment is a shipping channel hosting a large volume of prominent boating traffic. The shallower areas within Cockburn Sound are covered by dense beds of

foundation seagrass species such as *Posidonia australis, P. sinuosa* and *Amphibolis antarctica*. The deeper basin within Cockburn Sound consists largely of unvegetated bare sand [27]. The mean tidal velocity within Cockburn Sound is less than 0.05 m/s, and the tidal currents observed are minimal, inducing flows of around 0.01 m/s [28].

2.2 Experimental set up

A total of 120 polystyrene floats (Rogue Crab Pot Float®) were used for the purpose of this experiment. The floats were all spherical (150mm diameter), white in colour, made of high-density polystyrene. Each float had a hole (20mm diameter) going through the middle of the structures. Of the 120 experimental floats, 96 were covered with four different clothing materials (referred to as "fabrics" throughout this research), namely cotton (N=24), satin (N=24), velvet (N=24) and neoprene (N=24). In addition, 24 were left uncovered to act as control floats (CF). The fabrics that a victim may potentially wear, were chosen based on their popularity for daily activities (cotton, satin and velvet) and aquatic sports (neoprene), and for their difference in nature, structure, texture and thickness. Each of the 96 floats was covered by one sheet of fabric measuring 52x32 cm. Whilst cotton, satin and velvet are popular fabrics found in various colours within the textile industry, white was chosen for all three fabric types in order to maintain consistency and to allow for an easier visual analysis of the colonising fauna. Neoprene samples were black, as typically wetsuits are in this shade.

The sheet of fabric was attached to each of the floats using three steel sewing pins (birch plastic berry head) and two titanium nails. The fabric was wrapped around each of the floats with the 52 cm longer side running along the top and bottom of the floats, where the hole is located. The fabric was folded over and secured using three sewing pins, along with two 1.6 mm flat head nails. A total of 20 floats, 4 covered by each of the fabrics (a total of 16) and 4 CF, were haphazardly attached to a plastic crate (60x40 cm) (Fig. 2). Each float was attached to the crate by threading the rope through the hole in the middle. A standard knot was tied at each end of the floats and a bowline knot was used to tie the rope to the crate. The rope was 83 cm in length and 6mm in diameter. In order to avoid colonisation occurring inside the hole, two small plastic squares were placed over the openings top and bottom. The maximum

height of the fully assembled structure was 90 cm when measured from the bottom of the crate, to the top end of the floats connected to the crate (Fig. 2). This set up was repeated for a total of six crates.

All six crates were submerged on August 21st, 2018, with the assistance of two SCUBA divers. The crates were placed on the seabed at a depth of approximately 5 m from the sea surface on a day of low/high tide. In line with the requirements of the Department of Transport (DoT), the experimental structure remained at a minimum depth of 2 m below the sea surface for the entire duration of the research. Considering the height of the structure, and the tidal variation affecting the water depth, the chosen experimental site ensured a minimum 2.5 m of water above the structure during low tides for the entirety of the experiment.

Due to the buoyancy created by the floats, the crates were weighted down using railway sleepers and dumbbell weights to allow them to rest on the seabed (Fig. 2). Additionally, each crate was secured with star pickets (1.5 m) on two corners which were tied using both plastic and stainless steel cable ties (370x7.6 mm). The crates were placed with approximately 50 cm spacing between each crate. The small number of crates, the limited space between each and the selection of the specific research site were done in order to limit the difference in the variables possibly affecting any colonisation, e.g. microcurrents, turbulence.

The seawater temperature was recorded over the entire duration of the experiment using a total of 12 Odyssey® temperature loggers (two for each crate). The loggers were secured at the two outer ends of the crate using both stainless steel and plastic cable ties and recorded water temperature at 1-hour intervals. Salinity data were obtained from the Department of Water and Environmental Regulation, Government of Western Australia.

2.3 Crate retrieval

All the crates remained unaltered until the day of their retrieval, allowing aquatic organisms to colonise fabrics and CF. The crates were due to be removed on a monthly basis for six months, but in accordance with the University risk assessment for research (RAMP), the actual retrieval day was chosen considering the conditions of the sea and the safety of the divers. One crate was removed at each sampling date: after 28 days (period referred as “month 1” in this research), 63 days (month 2), 91 days

(month 3), 123 days (month 4), 161 days (month 5), and 185 days (month 5) of submersion. In order to avoid cross-contamination between the floats and to minimise the loss of any colonising organisms, resealable plastic bags (Hercules®) were placed on each float in the underwater phase of retrieval. The bags protected the floats from brushing together as they approached the surface. Once at the surface, the floats were detached from the crate, the bags containing both the floats and their ropes were sealed and stored in an icebox, to prevent degradation from exposure to the elements. At the arrival at the laboratory (approximately one hour after the end of the retrieval operation), the floats were stored at -20°C in a freezer (Westinghouse®), until analyses were conducted.

2.4 Data collection

Following retrieval, each float and their rope were individually photographed before the removal of the fabric. Fabrics were then carefully removed using forceps to dislodge each pin and nail from the pin line, and side cutters were used to remove the cable ties from each end of the float. Each of the fabrics were placed flat on the laboratory bench and photographed before examination.

A 10x10 cm squared grid with a total of 15 squares was placed on top of the sheet of fabrics to facilitate the count and analyses of distribution of the colonising organisms. Colonising organisms were observed, photographed, counted (barnacles only) and measured (basal diameter of the barnacles) using a Dino-lite® Edge 3.0 AM73915 series Microscope. Although a variety of marine organisms were found colonising the fabrics, the focus of this research was specifically on barnacles, therefore, only barnacles were identified to species level. The identification of the barnacles was performed by taxonomists based at the WA Museum in Perth (WA).

2.5. Statistical analysis

In order to evaluate preference in settlement location, the fabrics were divided into three different sections (top, middle and bottom), while the control floats (CFs), due to their spherical structure and the lack of coverage that could be removed for the purpose of the analyses, were divided into only top and bottom sections. A data bar chart produced with Microsoft Excel® was used to create a visual representation of the percentages of barnacle present on the different sections of the fabrics/CF. Due to

the experimental design there was an effect on the independency of the measurements. Hence, the effect of the submersion time and type of fabric on both the colonisation (number of barnacles, all species considered) and growth of barnacles ("barnacle size", based on the measurement of the basal diameter of the most common species of barnacle found throughout the experiment) was analysed using a Kruskal-Wallis rank sum (omnibus) test. In order to analyse the monthly colonization between the different fabrics, the test was used for each month (6 tests) following crate retrieval. Fabrics were the independent variables, and response were either the number or the diameter of the specimens. In order to analyse the colonization within the same fabrics, the test was used five times accounting for each of the substrates, having the months of crate retrieval as independent variables, and response being either the number or the diameter of the specimens. When the Kruskal-Wallis test produced a significant P-value, the Dunn post hoc test was carried out to further compare the differences in response between fabrics and time.

Linear regression analysis was used to investigate the relationship between the number of barnacles colonising the different fabrics and the seawater temperature or the time spent in water. As well, regression analysis was used to investigate the relationship between the size of barnacles and the seawater temperature, or the time spent in water.

All calculations were performed in the IBM SPSS Statistics 22 statistical software package. P-values less than 0.05 were considered statistically significant.

3. Results

3.1 Environmental Data

The seawater temperature showed a gradual increase from winter through to summer, with a minimum of 14.65°C recorded at the beginning of the research period (August) and a maximum of 24.34°C recorded towards the end (February). The average temperature over the six-month research period was 19.68°C. Temperature is shown together with the number (Fig. 3) and size (Fig. 4) of barnacles colonising the different fabrics. The average salinity level during this period was 29.53 practical salinity units (PSU), with a maximum of 32.31 PSU and a minimum of 15.20 PSU.

3.2 Colonisation of barnacles and other marine fauna

The marine fauna colonising the fabrics and the CF was identified up to species level only for barnacles, as the object of this study. Both fabrics and CF were colonised by three different species of barnacles, morphologically identified as *Balanus trigonus* Darwin (Crustacea: Cirripedia: Sessilia), *Amphibalanus variegatus* (Darwin) and *Amphibalanus reticulatus* (Utinomi). The species most consistently and abundantly observed was *B. trigonus*.

Algae and other organisms found adhered to fabrics or CF included, in alphabetical order, ascidians (comm. sea squirts, Chordata: Ascidiacea), bivalves (Mollusca: Bivalvia), bryozoans (comm. moss animals, Bryozoa), motile crustaceans like amphipods (Crustacea: Malacostraca), crabs and shrimps (Crustacea: Decapoda), gastropods (Mollusca: Gastropoda) and their egg sacs (Fig. 5). Barnacles, motile crustaceans, gastropods and bivalves were observed in all months, while bryozoans and ascidians were observed in the last two months of the experiment only. Details of all organisms at each sampling time are provided in Table 1.

3.3 Barnacle settlement location

The analyses of the most common places of colonisation (settlement location) considered all three identified species of barnacles. Overall, barnacles were observed preferentially colonising the middle and bottom regions of both the fabrics (all types) and the CF. Notably, the preferred settlement location on the fabrics was at creases and folds. Toward the end of the research period, barnacles were found colonising the holes of the floats and the polystyrene of floats exposed by damaged fabric (Table 1). Such specimens were not considered in the analyses.

The percentage of the barnacles present on the different sections of the fabrics and the CF is described by the data bar chart (Fig. 6).

3.4 Barnacle settlement abundance (number and size)

The statistical analysis considering the interaction between fabric type, time spent underwater, and water temperature showed that these variables played a role on both the number of barnacles (mean

number of barnacles ± S.E. of the three different species) colonising the fabrics and their size (basal diameter (mm) ± S.E. of of *B. trigonus* only).

Overall, the surface that showed the largest affinity with barnacle colonisation was neoprene, followed by the polystyrene of the CF, then satin, cotton and velvet.

During the six-months spent in water, the number of barnacles increased or remained constant when colonising CF, cotton, neoprene and satin (Fig. 3 and Fig. 1-4 in supplementary material), whilst on velvet they showed an unpredictable colonising pattern (Fig 3 and Fig. 5 in supplementary material). Despite having fewer barnacles colonising the polystyrene CF compared to neoprene, they showed the largest size (Fig. 4 and Fig. 1 in supplementary material). The size of barnacles colonising the fabrics was smaller and statistically similar throughout the experiment (Fig. 4 and Fig. 2-5 in supplementary material 9-12).

3.4.1 Monthly comparison between fabrics

All the results are described in Fig. 3,4 and reported in Tables 2,3. All the P-values produced by the statistical analyses are reported in the supplementary material (Tables 1,2).

Month 1 – Barnacle colonisation was observed on neoprene and on the CF, while cotton, satin and velvet were colonised only by other types of marine fauna (Table 1). The statistical analyses showed that the number and the diameter of barnacles present on neoprene and CF were not significantly different from each other, but were different from all other fabrics.

Month 2 – All four fabric types and the CF were colonised by month 2. The largest barnacle colonisation was observed on neoprene, followed in order by CF, satin, cotton and velvet. The number of barnacles observed on CF in month 2 was much higher compared to month 1 (from 3.75±1.80 to 28.25±6.30), while the other fabrics showed a maximum of 11, 8 and 6 barnacles, respectively. Again, the number of barnacles present on CF and neoprene was not significantly different, while they were different with respect to the colonisation observed on the other fabrics, especially cotton and velvet. In regards to size, the average diameter of barnacles observed on CF was larger and significantly different to all fabrics except neoprene. Neoprene had the second largest average barnacle size, but was not

significantly different to the other fabrics. Barnacles on cotton, satin and velvet were of similar size and not significantly different from each other.

Month 3 – A large barnacle colonisation was observed on both neoprene and CF, however colonisation was minimal on satin and cotton, and absent on velvet. The number of barnacles observed on neoprene and CF were not significantly different from each other, but were significantly different from satin, cotton and velvet. The colonisation on satin, cotton and velvet was not significantly different. Similar to month 2, CF had the largest barnacles, with an average basal diameter significantly different from all the other surfaces, but neoprene. The next largest barnacles were the ones colonising neoprene, significantly larger than cotton and satin; barnacles on cotton and satin showed similar and not significantly different size.

Month 4 – The rate of colonisation remained constant, with a larger number of barnacles still present on neoprene and CF followed by satin, velvet and cotton. Similarly to month 3, the number of barnacles recorded on neoprene and CF were not significantly different from each other, but different from the other substrates. The colonisations on cotton, satin and velvet was not statically different. The size of the barnacles maintained the trend of the previous months, with the largest barnacles observed on CF, and significantly different from the ones colonising the fabrics. The size of the barnacles observed on cotton, neoprene, satin and velvet was not significantly different.

Month 5 – As per month 4, neoprene and CF recorded the highest colonisation with respect to the other surfaces. The number of barnacles observed on neoprene and CF were not significantly different between the two surfaces. The colonisation observed on satin was not significantly different from any other surface. The number of barnacles observed on cotton and velvet were not significantly different between each other, but significantly lower than CF and neoprene. The trend of the barnacle size remained constant, with larger barnacles on CF, significantly different from neoprene, cotton, satin and velvet. Barnacles colonising the fabrics were similar in size and not significantly different from each other.

Month 6 – The surfaces mostly colonised were neoprene and CF, both with a similar and not significantly different number of barnacles, but significantly higher than other surfaces. Satin and cotton attracted a similar number of barnacles, significantly similar between the two, but lower than neoprene

and CF and higher than velvet. Velvet showed the lowest rate of colonisation, significantly different from other surfaces. The average size of the barnacles recorded in month 6 on CF was very similar to month 5, while recorded an increment on the fabrics. The size of the barnacles on CF was therefore not significantly different from cotton and neoprene, while it was from satin and velvet. The size of the barnacles colonising the fabrics was not found significantly different.

3.4.2 Monthly comparison within fabrics

CF (control floats) – The barnacle colonisation over the six-month experiment showed a constant increase in the number of specimens. Such increases were significantly different between months 1 and 4-5-6, from months 2 to 5-6 and from months 3 to 6. There was a significant positive relationship between the number of barnacles on the CF and the time spent in water (R^2=0.94), as well as between the number of barnacles on the CF and the water temperature following the deployment of the buoys (R^2=0.98).

With regards to the size of *B. trigonus*, the basal diameter showed an increase over time, especially significant between month 1 and 5-6. There is a significant positive relationship between the size of the *B. trigonus* specimens observed on the CF and both the time spent in water (R^2=0.81) and the water temperature (R^2=0.74).

All the results are shown in Fig. 3,4 and Fig. 1 (supplementary material), all the P-values produced by the statistical analyses are reported in Tables 3,4 (supplementary material).

Cotton – The barnacle colonisation was minimal and not significantly different for the first four months. During the last two months of the experiment the colonisation showed a constant and statistically significant increase. However, it must be noted that the texture and the integrity of this fabric was affected by the water, with small holes visible from month 3 and massive damage visible by month 6, resulting in the loss of fabric (Table 1, Fig. 7). From month 3 onwards, the number of barnacles recorded from the cotton sheets must be considered as "minimum number of barnacles", as specimens could have been lost because of the wear of the fabric. There is a significant positive relationship between the number of barnacles colonising cotton and the time spent in water (R^2=0.69), as well as between the number of barnacles and the water temperature since the deployment of the buoys (R^2=0.78).

The barnacles size increased over time, showing statistical difference starting from month 3. There is a significant positive relationship between the size of the *B. trigonus* specimens observed on the cotton replicants and both the time spent in water (R^2=0.82) and the water temperature (R^2=0.82).

All the results are shown in Fig. 3,4 and Fig. 2 (supplementary material), all the P-values produced by the statistical analyses are reported in Tables 3,4 (supplementary material).

Neoprene – Barnacles were present on this fabric in large numbers since month 1, but the colonisation remained numerically stable and not significantly different until month 3. Another increase of colonisation significantly different from the months before was recorded in month 5 and 6, whilst during month 4, the colonisation remained stable and not significantly different from month 3.

There is a significant positive relationship between the number of barnacles colonising neoprene and the time spent in water (R^2=0.92), as well as between the number of barnacles and the water temperature since the deployment of the buoys (R^2=0.75).

The barnacles size increased over time, showing statistical difference starting in month 3. There is a significant positive relationship between the size of the *B. trigonus* specimens observed on the neoprene replicants and both, the time spent in water (R^2=0.82) and the water temperature (R^2=0.88).

All the results are shown in Fig. 3,4 and Fig. 3 (supplementary material), all the P-values produced by the statistical analyses are reported in Tables 3,4 (supplementary material).

Satin – The barnacle colonisation was minimal and not significantly different until month 4, and recording a not statistically significant increase in month 5 and 6.

There is a significant positive relationship between the number of barnacles colonising satin and the time spent in water (R^2=0.90), as well as between the number of barnacles and the water temperature since the deployment of the buoys (R^2=0.92).

The barnacles size increased over time, showing statistical difference starting from 3. There is a significant positive relationship between the size of the *B. trigonus* specimens observed on the satin replicants and both the time spent in water (R^2=0.85) and the water temperature (R^2=0.80).

All the results are shown in Fig. 3,4 and Fig. 4 (supplementary material), all the P-values produced by the statistical analyses are reported in Tables 3,4 (supplementary material).

Velvet - The barnacle colonisation was minimal or absent for the first 3 months, while from month 4 the colonisation did not show a not constant growth. No significant relationship was observed between the number of barnacles colonising velvet and the time spent in water (R^2=0.57), as well as between the number of barnacles and the water temperature since the deployment of the buoys (R^2=0.60).

The barnacles size increased over time, showing statistical difference from month 4. However, no significant relationship was found between the size of the *B. trigonus* specimens observed on the velvet replicants and both the time spent in water (R^2=0.63) and the water temperature (R^2=0.61).

All the results are shown in Fig. 3,4 and Fig. 5 (supplementary material), all the P-values produced by the statistical analyses are reported in Tables 3,4 (supplementary material).

4. Discussion

Complexity and variety of aquatic environments, paucity of aquatic forensic scientists, lack of and/or high cost of underwater equipment, time required for the study and hurdles in obtaining safety and biohazard permissions, are the main reasons for this field to be scantily researched [8, 9]. In the event of a forensic investigation involving a cadaver found in water, the presence and the activity of the typical necrophagous insects used to estimate the minPMI is compromised [29]. Furthermore, under the right circumstances, the human remains can develop adipocere, a wax-like organic substance formed by the anaerobic bacterial hydrolysis in corpses [30]. The presence of adipocere alters the typical decomposition pattern – the process may be simply slowed down or even arrested – further complicating or making it impossible to assess the cadaver's minPMI from the forensic pathology point of view [31, 32]. Since both the insects and body of the victim tend to provide unreliable information, research to develop new tools for underwater investigations need to be considered. Instead of focusing on the body displaced in water, such research should consider the objects related to it (e.g. victim's clothing and shoes) and items related the criminal event (e.g. weapons). The forensic analyses of such items could then consider the corrosion process and colonisation by the aquatic organisms to obtain information about time and place of the criminal event [33].

Amongst all aquatic organisms, barnacles have peculiar zoological and ecological characteristics that make them a valuable tool for forensic investigation in marine environments. Barnacles are a foundation

species, that means they are a pioneering organism that can quickly establish colonies on "new" underwater surfaces [34]. Worldwide, barnacles are found throughout different marine environments, inhabiting intertidal and subtidal zones, as well as shallow estuaries [17]. Barnacles are sessile, and their attachment on a surface is permanent [35]. In like manner to insects on land, barnacles' growth rate is based on environmental parameters, especially water temperature. These features make barnacles ideal for forensic investigation with focus on the estimation of the time spent in water, as the settlement on objects related to a crime will be maintained from (short time after) submersion to retrieval [14, 18, 23].

In order to improve the efficacy of such zoological indicators for forensic application, it is necessary to analyse the colonisation of barnacles on items associated with human remains. To date, only a single study has provided such insights, focusing specifically on barnacle colonisation on two different types of shoes placed in the ocean [14]. However, the limitations in relying on shoes is that not all victims that enter the water are wearing shoes, and/or the shoes could be lost whilst the body is adrift. Hence, it is necessary to determine barnacle colonisation on other more common items associated with a human, such as clothing.

The present research considered for the first time the colonisation of barnacles on different types of fabrics commonly used to produce clothing worn for daily activities (cotton, satin and velvet) and aquatic sports (neoprene), placed in Western Australian marine waters.

Out of the 1,220 species of barnacles identified globally [36], 279 species can be found in Australia [37]. However, only three were observed colonising the fabrics in this study, namely *Balanus trigonus*, *Amphibalanus reticulatus* and *Amphibalanus variegatus*. These are three species of WA common acorn barnacles, whose adults are characterized by a calcitic shell made by two fixed calcareous plates growing directly onto a substrate [38]. The central region of the barnacle, the *operculum*, protects the main body, consisting of the reproductive organs, the cement glands, and the feeding apparatuses [24, 38]. Generally hermaphroditic, when mature for reproduction, they use the male sex organ to fertilise close neighbours releasing the sperm into the eggs located within the mantle [39]. The fertilized egg hatches into a *nauplius* larvae which develops over time to swim, feed and grow before metamorphosis into cyprid larvae [35, 37, 40-42]. The cyprid larvae begin the searching phase, consisting of 'wide

searching' and 'close searching', to ensure they locate the most favourable substrate for attachment, survival and growth [40]. The attachment occurs via the cyprid's antennae, excreting a cement-like substance from the cement glands [40]. The survival and the growth of the barnacle – demonstrated by the increase in the size of the basal diameter – is dependent on a successful choice of the attachment substrate, that must be resistant to decomposition and have exposure to water currents from where filter feeding barnacle can capture food particles [21, 43].

Balanus trigonus (commonly known as 'triangle barnacle') is an opportunistic fouling organism, endemic to the Indo-Pacific region [44]. It is typically found on hard substrata including harbour structures, ships' hulls, coral communities, hard-shelled invertebrates, sponges, and whales. The facilitative association with ships and motile organisms, is believed to have contributed to the wide distribution of this species, present in Pacific, Indian and Atlantic Oceans [44, 45]. This species prefers saline, subtidal habitats in warm-temperate, subtropical and tropical seas, and it has been observed in intertidal zones and depths of up to 90 m [44, 45]. The size (basal diameter) of this species is generally between 10-15mm, up to 25 mm [44]. The maximum size of the *B. trigonus* collected in this research was 11.95 mm.

Amphibalanus reticulatus ('reticulated barnacle') [46]) is endemic to the Indo-Pacific region. It was introduced by shipping to tropical and subtropical waters in the Pacific, Atlantic, and Mediterranean areas [45]. *Amphibalanus reticulatus* prefers saline, subtidal habitats in warm seas, it is commonly observed in intertidal regions ranging up to depths of 18 meters, attached to any hard surfaces including, rocks, the hull of ships and the hard shell of other marine fauna [45, 46]. The largest specimen ever examined has been recorded to measure 18mm, although typical basal diameter would be 10-14 mm [46].

Amphibalanus variegatus ('variable barnacle' or 'estuarine acorn barnacle' [46]) is an Indo-Pacific species, endemic to the Indo-Malayan region and Australia, recently also found in Belgian waters [47]. *Amphibalanus variegatus* is typically found in intertidal and upper subtidal regions, on any hard substratum including other marine fauna with hard shells, and the hull of ships [46]. The largest specimens recorded a basal diameter of 20 mm, but the diameter of the average species measure approximately 10-15 mm [46].

Throughout this research, *B. trigonus* was the species most commonly found, colonising every type of fabric and the polystyrene of the control floats, while *A. reticulatus* and *A. variegatus* were present in smaller number and with less consistency. *Amphibalanus reticulatus* and *A. variegatus* are both members of the '*Amphibalanus amphitrite* species complex' and they can easily be confused between each other and to closely related species, highlighting possible errors in the analyses [46]. As a consequence, on order to minimize the risk of inaccuracy in this research, all three species were considered in terms of number of barnacles colonising fabrics and CF, but only *B. trigonus* was analysed in terms of size.

To note, along with the barnacles, several other organisms were observed colonising the different fabrics and CF during the research period. However, no specific organisms were found in constant conjunction with the barnacles, as happened for polychaetes, bivalves, and bryozoans colonising the shoes [14]. As well, no organisms showed an extreme bloom, as occurred for the ascidians [38]. Therefore, considering the location and the environmental parameters of this study, no other species beside barnacles are suggested to have any relevance on the estimation of the time spent in water for fabrics placed underwater.

Overall, the most densely colonised surface throughout the research period was neoprene, followed by the polystyrene of the CF. On these two surfaces, the barnacle settlement occurred during the first month of the deployment of the buoys, while satin, cotton and velvet were colonised during the second month. On every surface but velvet, the number of barnacles showed an increase over time, while velvet showed an unpredictable settlement pattern throughout.

Previous studies have shown barnacles preferentially settle on dark, rough surfaces and regions which can provide a greater degree of protection from sunlight and predators [48, 49]. Accordingly, in the present study, barnacles preferentially colonised the black neoprene, the bottom part of every float, within the float's opening (where the device used to block it failed), the back of the fabric sheets (near the opening), and the areas where fabrics formed creases and folds. The colonisation, growth and survival of barnacles is dependent upon external factors including, but not limited to, the settlement location, temperature of the water, salinity, currents and food availability [21, 48, 50]. Furthermore, negative effects are observed when the population is too dense for a new colony to settle, increasing

competition for food [18, 51]. In this experiment, creases and folds showed large population settlement, that could have affected their growth in terms of both number and size [52].

In choosing the settlement substrate, the cyprid larvae tend to prefer to settle on a surface that has less chance of decomposing or deteriorating [18]. In this research, neoprene provided the ideal substrate for the barnacles' settlement, being a thick synthetic rubber, with a rough surface (especially when compared with cotton, satin and velvet), and the only fabric type chosen in a black shade. Despite the white colour, the polystyrene of the CF also showed to be a surface highly favourable for algal settlement. To note, the CF were quickly colonised by algae and by month 1, becoming darker and rougher, facilitating the subsequent barnacle settlement [49, 53]. Barnacles on the CF were initially fewer in number than on neoprene, but the number increased throughout the experiment, and no significant difference in the number of colonising barnacles was found between the two surfaces.

When considering the texture of the remaining fabrics, cotton, with its rough surface due to the interlocking fibres in a grid-like structure, may be a more favourable surface to be colonised. However, degradation from month 3 onwards, resulted in the loss of large parts of the fabrics. The damage to the substrate reflected an impediment of further colonisation and in the correct count of the colonising barnacles, that could have been lost together with the fabric. For this reason, from month 3 onwards, the numbers of barnacles recorded from the cotton sheets were considered as "minimum number of barnacles". Barnacles were, nonetheless, found settled on the underlying polystyrene where the cotton had disintegrated.

Satin presents fewer interlaces compared to the other fabrics that were used in this study, determining a glossy smooth appearance. While this texture seems opposite to the barnacles typical preferred colonisation surfaces, satin was preferred over velvet and cotton, even when the damages on cotton were limited. Velvet, instead, is a woven tufted fabric in which cut threads are arranged in a dense hair-like manner to create a soft texture. The lack of a hard surface and the small hair-like fibres constantly moving underwater proved to be an unfavourable colonisation fabric for barnacles, with velvet showing the lowest barnacle count and the most inconsistent rate of colonisation. The present results show that for the purpose of an investigation, fabrics with characteristics similar to velvet have less utility for the estimation of time spent in water.

It is well documented that the temperature of the water is a critical factor for barnacle larvae to settle and to grow into juveniles and adults ([18] for review). Generally, optimal growth temperatures range between 10-30°C, while extreme temperatures from as low as 11°C to as high as 36°C have pronounced negative effects on growth and survival [54, 55]. Generally, barnacle species located in regions with warmer waters are not adapted for survival in regions with extremely cold or extremely warm waters [44, 55]. *Balanus trigonus, A. reticulatus* and *A. variegatus*, while observed at depth of 18-90 m, are mostly intertidal, living between the high and low tide lines [44, 46]. Typically, barnacles inhabiting marine shorelines that are exposed to air at low tide and covered with seawater when the tide is high, are more temperature tolerant than subtidal species [56]. At the site of the experiment, no extreme temperatures were observed, and the average temperature was 19.68°C for the whole experiment – within the optimal range for the recruitment, the growth and the survival of barnacles [34, 55]. Being a foundation species, barnacles are expected to show a fast colonisation rate [57]. However, the number of barnacles for all the fabrics and CF were present in a low number and showed no statistical difference between month 1 and 2, and in some cases, until month 3 (cotton, satin, velvet). The experiment took place over spring and summer, therefore the first 2-3 months recorded the lowest temperatures. Considering the results of the barnacle colonisation (Table 2, Fig. 3), it is possible to infer that a water temperature equal to or below 14°C are not ideal for the settlement of *B. trigonus*, and even less for *A. reticulatus* and *A. variegatus*, whilst at 17°C and above, the colonisation of these three species is facilitated. The following increase in both the number and the size of the barnacles (Table 3, Fig. 4) is a direct result of the increase of the water temperature and the right location of attachment, that provided stability and access to a continual food supply [21, 43]. Barnacles are affected by both availability and delivery rate of the food [21]. Currents, waves, tides, wind and rainfall are essential factors affecting the amount and the transport of phytoplankton, because its abundance can be modified by the temperature, salinity, turbidity and nutrient concentrations in the water [35]. It is possible to infer that the typical lower air temperature, wind and rain of the WA spring may have affected the abundance of phytoplankton, which is the primary food source for developing larvae, juveniles and adults [26].

Balanus trigonus, A. reticulatus and *A. variegatus* grow within the same diameter range (10-15mm, 10-18mm and 10-20mm, respectively) [44, 46]. The barnacles observed in this research would have only

been able to achieve a maximum of six months of being sessile, but early life stages have a faster growth rate then the later stages [17, 24, 50, 57]. In the period of the experiment, *B. trigonus* reached a size between a minimum of 5 mm (velvet) and maximum 11 mm (CF). *Balanus improvisus* is affected by currents as low as of 0.20-0.36m/s [58], but Cockburn Sound is a sheltered, natural embayment, with minimal effects of ocean currents of 0.01 m/s [28]. The larger sizes of *B. trigonus* observed on the CF must therefore be a consequence of the type of surface available for the barnacles to grow on [52]. While the CF offered a plain surface, with no restriction on space for the barnacles to attach and grow, the colonisation of the fabrics was mostly concentrated near the creases and folds. These areas provided protection, therefore attracted more cyprids to settle with small interspace between one another, with consequent challenges and limitations in the enlargement of the basal diameter [46, 49, 59].

Previous observations, research and case work did not identify this issue, as both shoes and bones did not provide any creases and folds for any preferential settlement of the barnacles [14, 16, 17, 25, 53]. Furthermore, this issue does not take place in the case studies by Magni et al. (2014) [18], as the human remains were colonised by stalked barnacles (Arthropoda: Cirripedia: Peducolata). Such barnacles attach themselves by means of a muscular stalk, that can extend from the base surface up to 50 cm [24]. At present, the vast majority of the standardised table of growth for barnacles consider the size of barnacles growing under optimal conditions, e.g. settling on the top of typical hard surfaces and with exposure to currents providing a good food supply [1]. Therefore, in the event of a forensic case where barnacles are found on clothing, the specimens that will provide more information will be the one colonising the exposed side of thick synthetic fabrics, while barnacles colonising natural fibers, hair-like surfaces, creases and folds should be avoided.

This research aimed to replicate an investigative scenario in which the clothes of a victim found in the ocean are colonised by barnacles. Due to the limitation in the displacement of organic matter in the ocean, polystyrene floats were used for the experiment. Besides the fact that not all bodies that enter the water are covered by clothing, further limitations to this study are that it was conducted over spring and summer in WA and considered only 4 types of fabric. Furthermore, three of the fabrics were chosen in white colour, while one in black, therefore a question regarding why the barnacles preferentially colonise fabrics because of the texture or the colour remains open. Future studies should be conducted

during other seasons, for longer periods, with different starting points at different times of the year, considering other location and fabrics types and colours. A modification of the experimental design to avoid possible pseudo-replications should be preferred, however when considering such experiments a balance also is required especially when costing, and organising logistics is required. Despite such limitations, this research is crucial in terms of its contribution towards the improvement of the tools available to investigate aquatic forensic cases. This is especially so as this research, the analyses of barnacles colonising human remains contributes to a more accurate estimation of the PMSI.

Disclosure

The permission for this study, the use of the site location, and to install objects in navigational water was granted by the Western Australian Department of Transport (DoT) (20.11.18) and the Western Australian Department of Fisheries (3090/4.4.18).

Authorship contributions

Category 1

- Conception and design of study: PA Magni, J Verduin
- Acquisition of data: E Tingey, J Verduin, PA Magni
- Analysis and/or interpretation of data: E Tingey, NJ Armstrong

Category 2

- Drafting the manuscript: PA Magni
- Revising the manuscript critically for important intellectual content: PA Magni, J Verduin, E Tingey, NJ Armstrong

Category 3

- Approval of the version of the manuscript to be published (the names of all authors must be listed): PA Magni, J Verduin, E Tingey, NJ Armstrong

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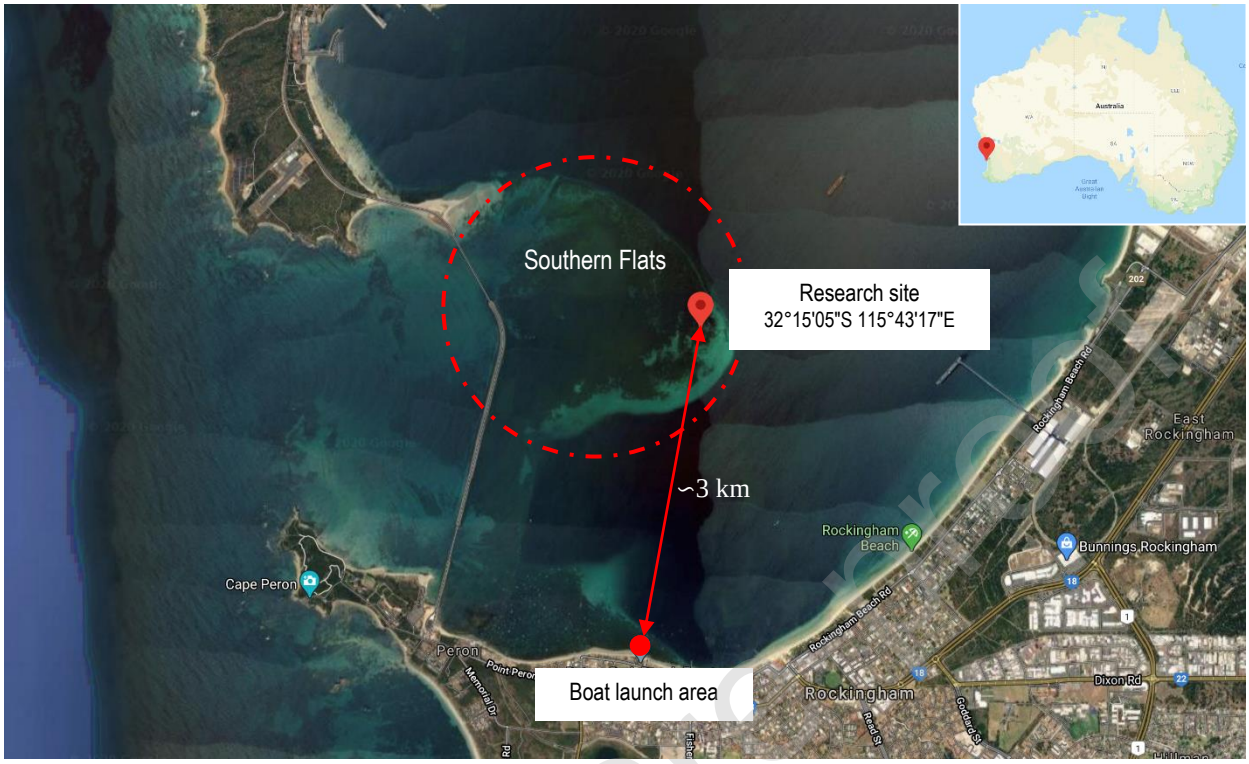

Figure 1. Location of the research site in Cockburn Sound, WA (sourced from Google Earth).

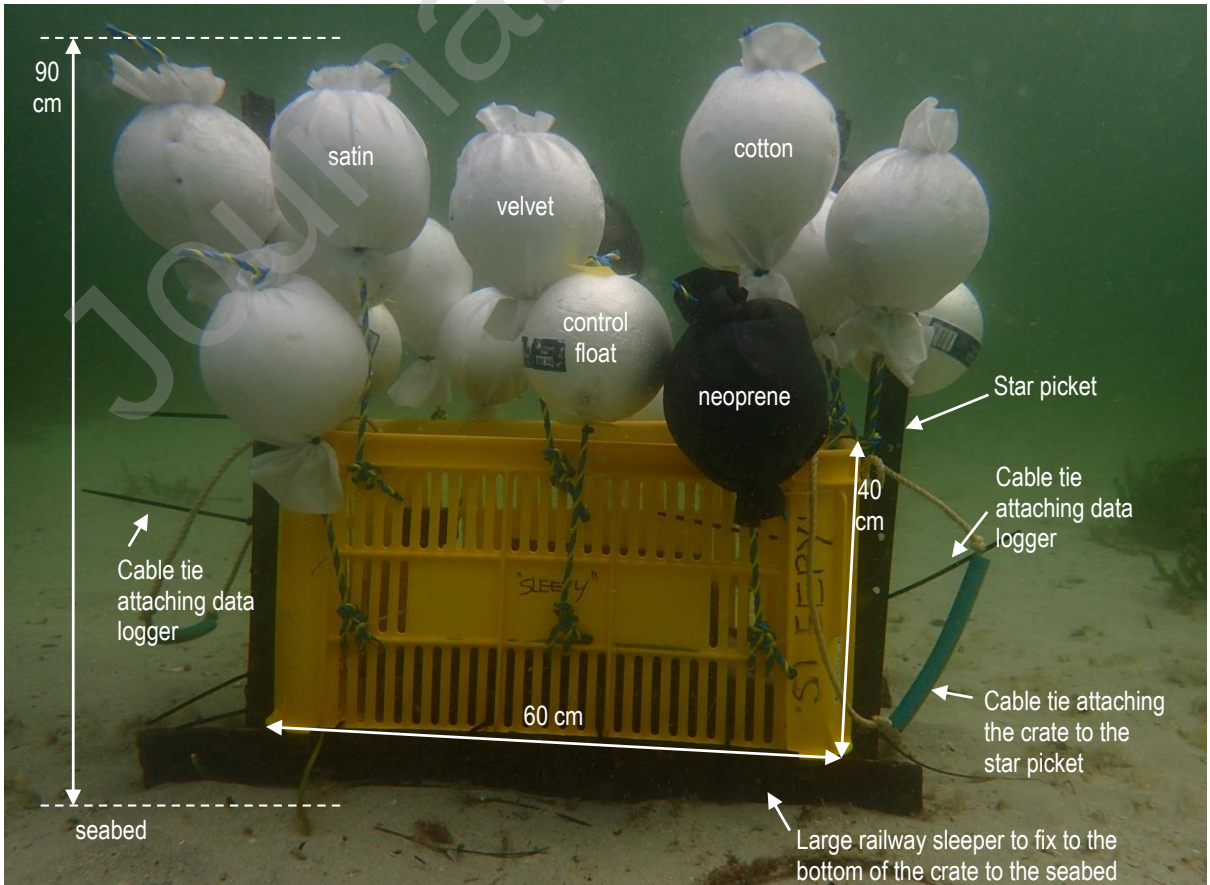

Figure 2. The arrangement of the crate. To each crate, 20 floats covered by cotton (N=4), neoprene (N=4), satin (N=4), velvet (N=4) or uncovered (N=4 control floats). Each float was tied onto the crate by polypropylene rope. The entire structure remained at a minimum depth of 2 m below the sea surface for the entire duration of the research.

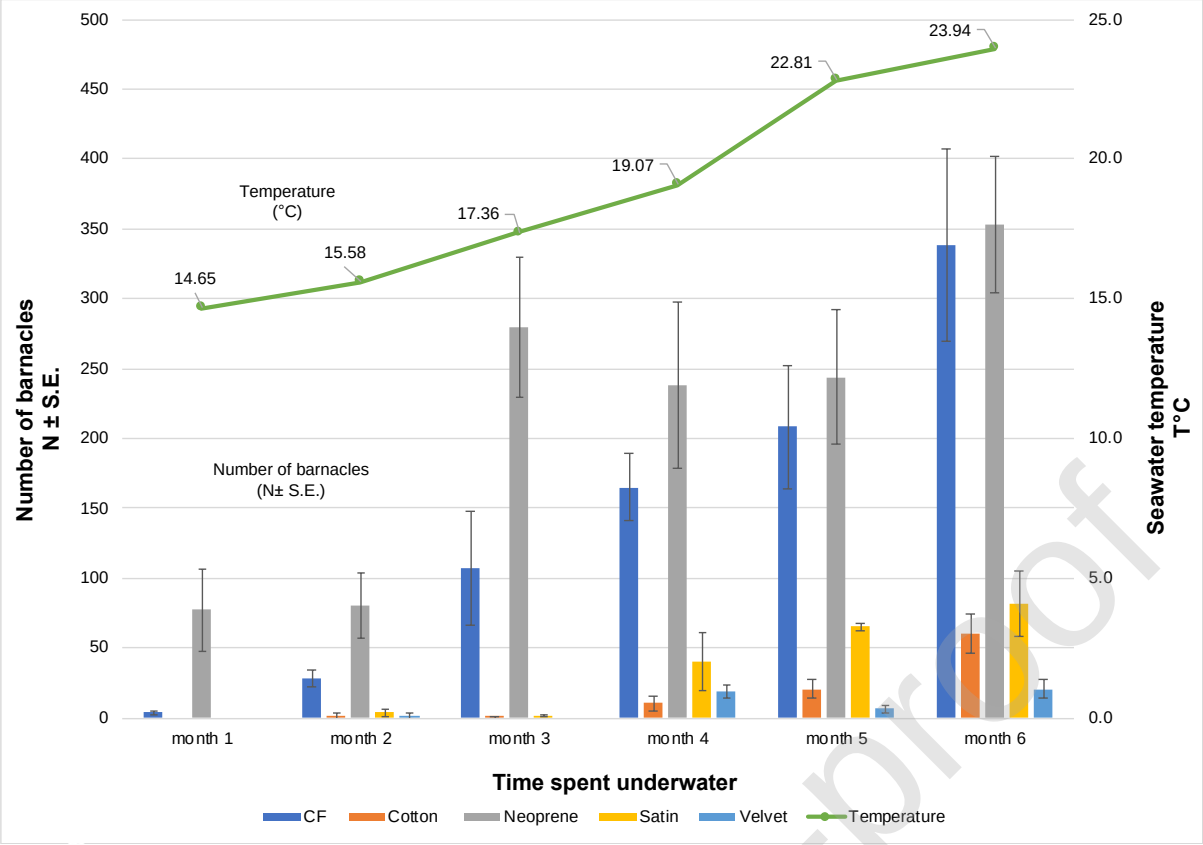

Figure 3. Mean number of barnacles (N±S.E. of the three different species) observed on the fabrics and the control floats (CF) over six months of the experiment. The figure also reports the average seawater temperature.

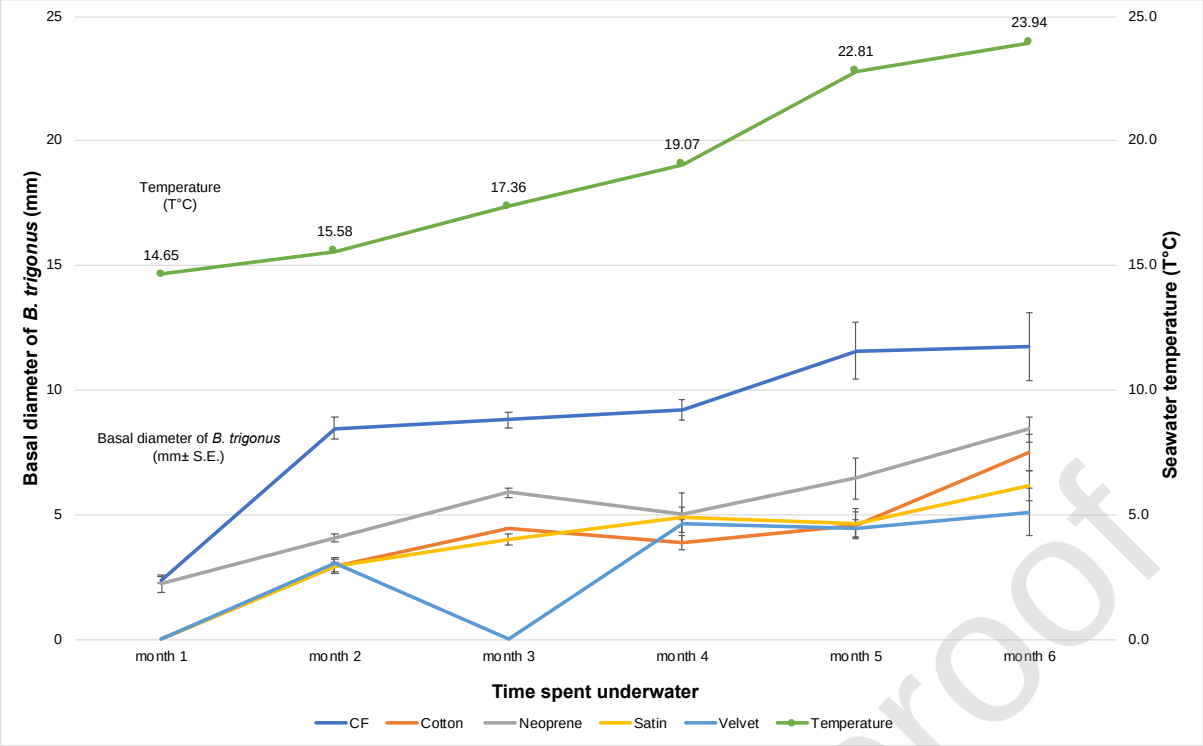

Figure 4. Average size of *B. trigonus* (basal diameter mm±S.E.) observed on the fabrics and the control floats (CF) over the six month experiment. The figure also reports the average seawater temperature curve.

Figure 5. Marine fauna collected on neoprene after 6 months underwater (e). The barnacles *Amphibalanus variegatus* (a), *Amphibalanus reticulatus* (b) and *Balanus trigonus* (c), gastropods adults (d) and egg sacks (f), decapods (g,h,i).

Percentage of barnacle abundance	Section of fabric/buoy	Control floats	Cotton	Neoprene	Satin	Velvet
Month 1	top	26.67%	0.00%	14.56%	0.00%	0.00%
	middle	N/A	0.00%	18.12%	0.00%	0.00%
	bottom	73.33%	0.00%	67.31%	0.00%	0.00%
Month 2	top	48.67%	25.00%	11.49%	26.67%	0.00%
	middle	N/A	12.50%	7.76%	46.67%	0.00%
	bottom	51.33%	62.50%	80.75%	26.67%	100%
Month 3	top	27.35%	34.94%	5.09%	8.40%	23.08%
	middle	N/A	26.51%	29.94%	32.82%	23.08%
	bottom	72.65%	38.55%	64.97%	58.78%	53.85%
Month 4	top	8.88%	0.00%	7.98%	12.50%	0.00%
	middle	N/A	0.00%	29.73%	37.50%	0.00%
	bottom	91.12%	100.00%	62.29%	50.00%	0.00%
Month 5	top	8.88%	34.88%	7.58%	13.50%	15.79%
	middle	N/A	23.26%	16.50%	31.90%	19.74%
	bottom	91.12%	41.85%	75.92%	54.60%	64.47%
Month 6	top	52.18%	5.76%	11.68%	10.15%	26.19%
	middle	N/A	23.46%	21.66%	19.08%	20.24%
	bottom	47.82%	70.78%	66.67%	70.77%	53.57%

Figure 6. Data bar chart reporting the abundance of barnacles (percentage of presence of the three different species combined) observed on the fabrics and the control floats. The barnacles were counted dividing the buoys and the fabrics into different sections, 3 for the fabrics (top, middle, bottom) and 2 for the control floats (top, bottom).

***Figure 7**. The different fabrics retrieved after being submerged for 1 to 6 months in the ocean*

Table 1. Organisms collected at each sampling time on the fabrics and the control floats (CF), and observations of the physical changes of the fabrics and the CF throughout the experiment. Colonising organisms are listed in alphabetical order. N/A = not applicable (no barnacles or other marine fauna observed).

Time	Fabric	Barnacle species	Other marine fauna	Fabric and CF observations
Month 1	Cotton	N/A	Amphipods, bivalves, shrimps	Discolouration of fabric due to algal growth.
	Satin	N/A	Gastropods	Minimal discolouration.
	Velvet	N/A	Amphipods, shrimps	Discolouration of fabric due to algal growth.
	Neoprene	*B. trigonus* *A. variegatus* *A. reticulatus*	Amphipods, shrimps	Minimal algal growth compared to other fabrics in this month.
	CF	*B. trigonus*	Shrimp	Minimal discolouration due to algal growth, small indents in the foam structure.
Month 2	Cotton	*B. trigonus*	Bivalves, crabs, shrimps	Large discolouration of fabric due to algal growth.
	Satin	*B. trigonus*	Bivalves, gastropods adults and egg sacks	Discolouration of fabric due to algal growth.
	Velvet	*B. trigonus*	Bivalves	Large discolouration of fabric due to algal growth.
	Neoprene	*B. trigonus*	Bivalves	Large algal growth compared to month 1.
	CF	*B. trigonus*	Bivalves	Discolouration due to algal growth.
Month 3	Cotton	*B. trigonus*	Bivalves, gastropods	Discolouration due to algal growth, holes observed in fabric.
	Satin	*B. trigonus*	Amphipods, bivalves, shrimps	Discolouration due to algal growth, ~~similar as previous~~ similar to previous month.
	Velvet	N/A	Bivalves, gastropods adults and egg sacks	Discolouration due to algal growth, ~~similar as previous~~ similar to previous month.
	Neoprene	*B. trigonus* *A. variegatus* *A. reticulatus*	Bivalves, gastropods adults and egg sacks	Discolouration due to algal growth, ~~similar as previous~~ similar to previous month.
	CF	*B. trigonus* *A. variegatus* *A. reticulatus*	Gastropods adults and egg sacks	Discolouration due to algal growth, ~~similar as previous~~ similar to previous month.
Month 4	Cotton	*B. trigonus* *A. variegatus* *A. reticulatus*	Amphipods, shrimps	Discolouration due to algal growth, larger holes observed in fabric.
	Satin	*B. trigonus*	Amphipods, bivalves, shrimps	Algal growth on the surface of the exposed surface, not in the folds.
	Velvet	*B. trigonus*	Bivalves	Discolouration due to algal growth, ~~similar as previous~~ similar to previous months.
	Neoprene	*B. trigonus* *A. variegatus* *A. reticulatus*	Gastropods adults and egg sacks	Discolouration due to algal growth, ~~similar as previous~~ similar to previous months.
	CF	*B. trigonus*	N/A	Discolouration due to algal growth.
Month 5	Cotton	*B. trigonus* *A. reticulatus*	Bivalves, gastropods	Discolouration due to algal growth, the fabric is becoming fragile. Barnacles observed on the polystyrene

				of the floats exposed by the damaged fabric.
	Satin	*B. trigonus* *A. variegatus*	Bryozoans, gastropods adults and egg sacks	Large discolouration of fabric due to algal growth.
	Velvet	*B. trigonus* *A. variegatus* *A. reticulatus*	Bivalves, gastropods	Large discolouration of fabric due to algal growth.
	Neoprene	*B. trigonus* *A. variegatus* *A. reticulatus*	Bivalves, bryozoans, gastropods adults and egg sacks	Large discolouration of fabric due to algal growth.
	CF	*B. trigonus* *A. variegatus* *A. reticulatus*	Bivalves	Large discolouration due to algal growth.
Month 6	Cotton	*B. trigonus* *A. variegatus*	Bryozoans, gastropods	Large discolouration of fabric due to algal growth, massive damages of the fabric. Barnacles observed on the polystyrene of the floats exposed by the damaged fabric.
	Satin	*B. trigonus* *A. variegatus* *A. reticulatus*	Bryozoans, gastropods adults and egg sacks	Large discolouration of fabric due to algal growth. Barnacles colonising the hole of the float.
	Velvet	*B. trigonus* *A. reticulatus*	Bivalves, bryozoans, gastropods	Large discolouration of fabric due to algal growth
	Neoprene	*B. trigonus* *A. variegatus* *A. reticulatus*	Bivalves, bryozoans gastropods adults and egg sacks (Fig. 3)	Large discolouration of fabric due to algal growth.
	CF	*B. trigonus* *A. variegatus* *A. reticulatus*	Gastropods, ascidians	Large algal growth, covering the entire CF. Barnacles and ascidians colonising the hole of the float.

Table 2. Mean number of barnacles (N±S.E. of the three different species) observed on the fabrics and the control floats (CF) over the six month experiment. The letters indicated below the numbers (i.e., CF, C, N, S, V) correspond to the fabrics or control whose results proved significantly different ($P<0.05$) from the fabric indicated in the corresponding column.

Average number of barnacles N ± S.E.	**Control Floats (CF)**	**Cotton (C)**	**Neoprene (N)**	**Satin (S)**	**Velvet (V)**
Month 1	3.75 ± 1.80	0.00	77.25 ± 29.06	0.00	0.00
	C, S, V	CF, N	C, S, V	CF, N	CF, N
Month 2	28.25 ± 6.30	2.00 ± 2.00	80.50 ± 23.79	3.75 ± 2.46	1.75 ± 1.44
	C, V	CF, N	C, S, V	N	CF, N
Month 3	107.00 ± 40.59	0.25 ± 0.25	279.75 ± 50.49	2.00 ± 0.71	0.00
	C, V	CF, N	C, S, V	N	CF, N
Month 4	165.25 ± 24.23	10.75 ± 5.38	238.00 ± 59.62	40.75 ± 20.54	19.00 ± 5.20
	C, V	CF, N	C, S, V	N	CF, N
Month 5	208.25 ± 44.44	20.75 ± 6.54	244.00 ± 47.76	65.50 ± 2.60	6.50 ± 2.63
	C, V	CF, N	C, V	-	CF, N
Month 6	338.25 ± 68.49	60.75 ± 14.31	353.25 ± 48.57	81.25 ± 23.35	21.00 ± 6.57
	C, V	CF, N	C, S, V	N, V	CF, N

Table 3. Average size of barnacles (basal diameter mm±S.E. of the barnacle *B. trigonus*) observed on the fabrics and the control floats (CF) over the six month experiment. The letters indicated below the numbers (i.e., CF, C, N, S, V) ~~are~~ correspond to the fabrics or control whose results proved significantly different (P<0.05) from the fabric indicated in the corresponding column.

Average diameter of *B. trigonus* mm ± S.E.	**Control Floats (CF)**	**Cotton (C)**	**Neoprene (N)**	**Satin (S)**	**Velvet (V)**
Month 1	2.39 ± 0.15	0.00	2.21 ± 0.35	0.00	0.00
	C, S, V	CF, N	C, S, V	CF, N	CF, N
Month 2	8.47 ± 0.44	2.96 ± 0.30	4.05 ± 0.16	2.94 ± 0.25	3.07 ± 0.03
	C, S, V	CF	-	CF	CF
Month 3	8.82 ± 0.33	4.47 ± 0.00	5.90 ± 0.18	4.04 ± 0.23	0.00
	C, S, V	CF, V	S, V	CF, N	CF, C, N
Month 4	9.20 ± 0.43	3.88 ± 0.30	5.05 ± 0.26	4.90 ± 0.96	4.64 ± 0.35
	C, S, V	CF	-	CF	CF
Month 5	11.58 ± 1.12	4.61 ± 0.49	6.47 ± 0.82	4.68 ± 0.56	4.44 ± 0.37
	C, S, V	CF	-	CF	CF
Month 6	11.75 ± 1.34	7.51 ± 0.71	8.44 ± 0.50	6.19 ± 0.60	5.11 ± 0.97
	S, V	-	-	CF	CF