Marine Biodiversity Hub

National Environmental Science Programme

Conservation of handfish and their habitats – Annual Report

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EXECUTIVE SUMMARY

We have completed and analysed performance assessment surveys at nine local population sites for spotted handfish in the Derwent estuary from 2015-2019. To this time series we have also incorporated historic data for individual sites back to 1998. Local populations generally show stability of occurrence but with some difference in abundance (as measured by estimates of fish densities per habitat) by years. At one site, Ralphs Bay, while there has been a large reduction in population size over the last 15 years, animals continue to persist in low numbers. At another site with low densities, Howrah Beach, no fish were sighted in 2019. At both these sites the densities of animals may be at levels that are currently difficult to detect with our current survey effort (<3 per hectare). Outside of the Derwent Estuary several more potential local populations were identified. In additional to a population discovered in the D' Entrecasteaux Channel in 2016, the National Handfish Recovery Team (NHRT) received notification from Huon Aquaculture (HAC) and Tassal that they had both observed spotted handfish during Remotely Operated Vehicle (ROV) surveys undertaken as part of environmental assessments in Storm Bay. HAC also reported an additional site in the Huon estuary, adding to the near-by site reported in 2016. This brings the total known extant locations for spotted handfish to 13.

In 2018 we tested a new type of ceramic artificial spawning habitat (ASH) for spotted handfish. Fish showed a preference for ceramic ASH but this habitat type had a lower survival rate than plastic. We redesigned the ASH and found it had a much-improved survivorship compared to the old design, though at one site high losses of all ASH types still occurred. As we found that ASH was only used by handfish when densities of natural spawning substrates (ascidians both native and introduced) were below a critical level, in the 2019 surveys we counted ascidians as well as handfish which enabled improved targeting of our ASH plantings

Juvenile red and spotted handfish have continued to survive and grow in captivity, though in two cases groups of 10 and 8 spotted handfish were lost to disease. There were no captive breeding events by the adult spotted handfish in the 2018-2019 season but both the lone captive red handfish and a spotted handfish did lay infertile eggs. We hypothesis, based on their observed natural behaviours that fish may need to be kept in isolation to become gravid. Two more red handfish egg masses were collected and ~50 juveniles have since hatched. Successful captive breeding will require a method to determine sex of handfish with minimal disturbance as well as an improved understanding of breeding biology and behaviours.

Genetic analysis of spotted handfish was conducted on fin clips collected between 1998-2008 across multiple sites in Derwent estuary. Local populations, while genetically diverse, appear to be isolated from each other, with the exception of their closest neighbouring population. This suggests that local populations were not subject to in-breeding effects when these samples were taken. Local populations, or at best groups of nearest neighbours' local populations, should be considered independently for conservation management for if they collapse then it is unlikely they will re-establish naturally from outside recruitment. From opportunistically sourced genetic material, eDNA markers for red handfish have also been developed. This may provide a method to detect and narrow the search for unknown populations of red and possibly other species of handfish.

Four environmentally sensitive (ES) moorings were deployed and one 'before' and two 'after' rounds of assessments conducted. At one site spotted handfish were observed in the recovering scar from the removed chain mooring. Modelling of various mooring designs suggests that ES mooring designs reduce maximum loads in various vessels by between 39% to 57% in extreme weather conditions, when compared to traditional chain moorings.

A website handfish.org and a fundraising campaign to "name a handfish" were also launched as part of the Handfish Conservation Project.

1. SURVEYS

In 2019 we completed the fifth annual round of annual performance assessment surveys for spotted handfish across the 9 Derwent Estuary study sites (Fig 1). This NESP and Threatened Species Commissioner funded work commenced in 2015 and the dataset now allows for the calculation of a total of 45 density estimates (5 per site) with variance. Annual estimates are calculated from search effort by the dive team of 8-12 transects per site, which were of ~250 m x 3 m swath size. In addition to this orthogonal design we have also incorporated 26 more *ad hoc* additional density estimates from previous studies conducted by Mr Mark Green between 1998 and 2012, and a methods development survey conducted at Battery Point in 2014 (Fig 2). This brings the total to 71 estimates of fish densities. Prior to 2015 these estimates are annually uneven in their occurrence across sites.

Estimates between 1998-2019 have been derived with similar methodology and search effort but there are a number of differences between the 1998-2012 and 2014-2019 work. The main difference in survey methods was the use of transect reels prior 2014 to parametrised search effort. Since 2014 search effort has been parametrized with a towed GPS float. This change in method greatly improved search efficiency and has allowed for more sites to be assessed within a season with the same resources. There are also differences in estimation approaches. Since 2014 these have been computed using a GLM with a poisson distribution and a log link offset for search area. Prior to 2014 a gaussian approach was used with average numbers of fish per transect and search area used to extrapolate density estimates. While Figure 2 provides an iterative explanation of spotted handfish local population dynamics, consolidation of the data into one set and global re-analysis using the GLM approach should occur in the future.

Two points of interest from the 2019 surveys were the observation of fish again at the Ralphs Bay site, while no fish were observed at Howrah Beach for the first time in 5 years. For both of these relatively large sites, fish are in very low numbers, which is consistent with what we have observed since 2015. This suggests either a) survey effort does not have the sensitivity to reliably detect fish densities below 3-5 fish per hectare b) that some larger sites require stratification of sampling or truncation to better account for patchiness and/or c) fish can persist in very low numbers.

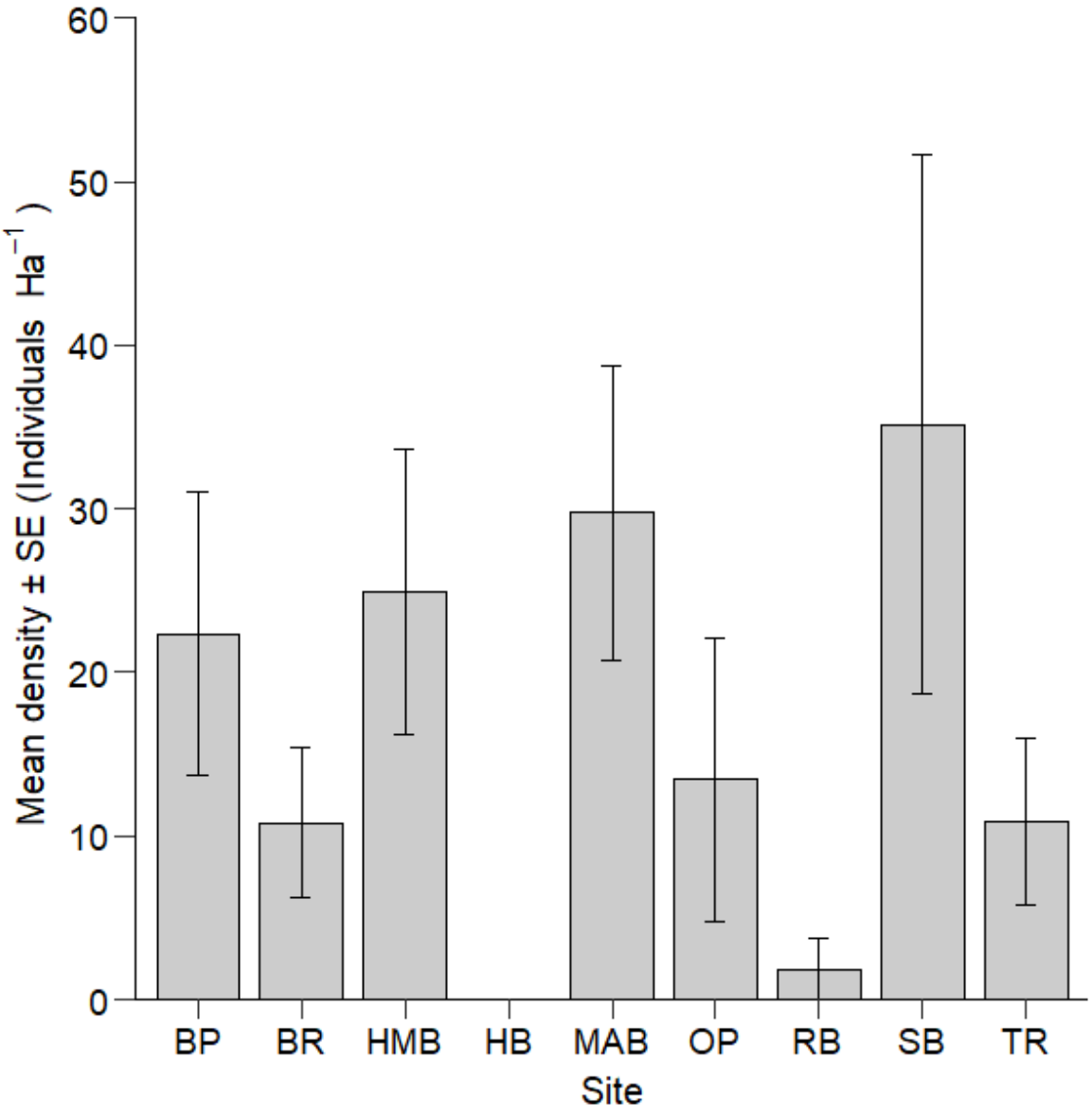

Figure 1 2019 densities of spotted handfish across the 9 Derwent Estuary monitoring sites with their areas: BP = Battery Point (13 Ha), BR = Bellerive (21 Ha), HMB = Half Moon Bay (33 Ha), HB = Howrah Beach (21 Ha), MAB = Mary Anne Bay (14 Ha), OP = Opossum Bay (32 Ha), RB = Ralphs Bay (20 Ha), SB = Sandy Bay (13 Ha), TR = Tranmere (13 Ha).

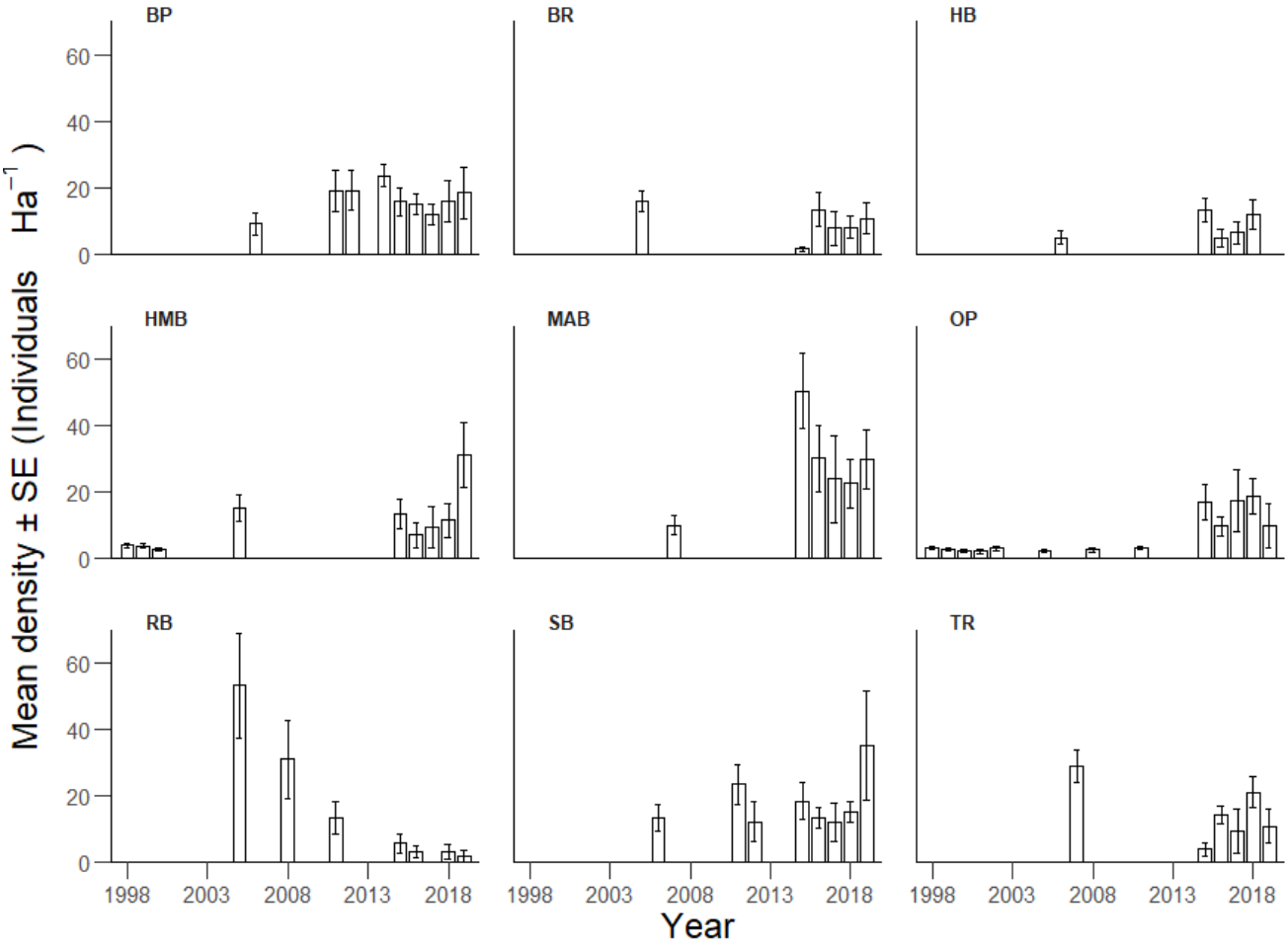

Figure 2 Time-series 1998-2019 of density of spotted handfish at 9 sites in the Derwent Estuary. BP = Battery Point (13 Ha), BR = Bellerive (21 Ha), HMB = Half Moon Bay (33 Ha), HB = Howrah Beach (21 Ha), MAB = Mary Anne Bay (14 Ha), OP = Opossum Bay (32 Ha), RB = Ralphs Bay (20 Ha), SB = Sandy Bay (13 Ha), TR = Tranmere (13 Ha).

1.1 Ascidian count

In 2018 assessment of the use of Artificial Spawning Habitats (ASH) by spotted handfish showed a strong negative correlation with stalked ascidian densities (both the light-coloured native species and the introduced dark species). Hence, if there are abundant natural spawning habitats then planting ASH is a waste of resources as it is not used. To more efficiently guide ASH planting we incorporated an ascidian count into the 2019 performance assessment surveys (Table 1). Based on this analysis, Tranmere and Bellerive were chosen to plant ASH due to their low ascidian densities. Similar observations of ascidian densities by site were recorded in 2018 (Lynch et al. 2018).

Table 1 Average count of ascidians per transect by site.

Site	Dark ascidian	Light ascidians	Combined ascidians
Bellerive	3.0	1.2	4.2
Tranmere	1.3	3.8	5.0
Opossum Bay	0.6	5.0	5.6
Mary Ann Bay	3.6	6.3	9.9
Sandy Bay	7.9	4.9	12.8
Howrah Beach	11.2	2.3	13.5
Battery Point	22.3	0.0	22.3
Ralphs Bay	25.3	2.1	27.4
Half Moon Bay	2.8	69.1	71.9

1.2 ASH plant

The ASH surveys of 2018 indicated a preference by spotted handfish to use ceramic over plastic ASH, so we only planted ceramic ASH in 2019. To reduce breakage, the design of the ceramic ASH was re-assessed and re-designed into two thicknesses – 9 and 11 mm. These are both thicker than the 2018 ceramic ASH design (6mm) and more closely resembled the thickness to the natural spawning habitat (stalked ascidians). The new ASH was also longer (30 cm vs 20 cm), and unlike the 6 mm ASH which replicated the disc of the original plastic design, this was removed from the thicker versions (Fig 3). Advice from the UTAS engineering department was that the disc produced a point of structural weakness with the stalk tending to snap off just above the disc. The 11 mm ASH also had 4 small indentations on either end allowing for easy identification in the field.

As well as the two plantings at Bellerive and Tranmere, which were based on the data analysis of ascidian count, we also conducted a small, handling trail at Sandy Bay. The new design, which was thicker and removed the ceramic disc proved to be much more robust, compact and easy to handle underwater as bundles could be transported easily in dive bags.

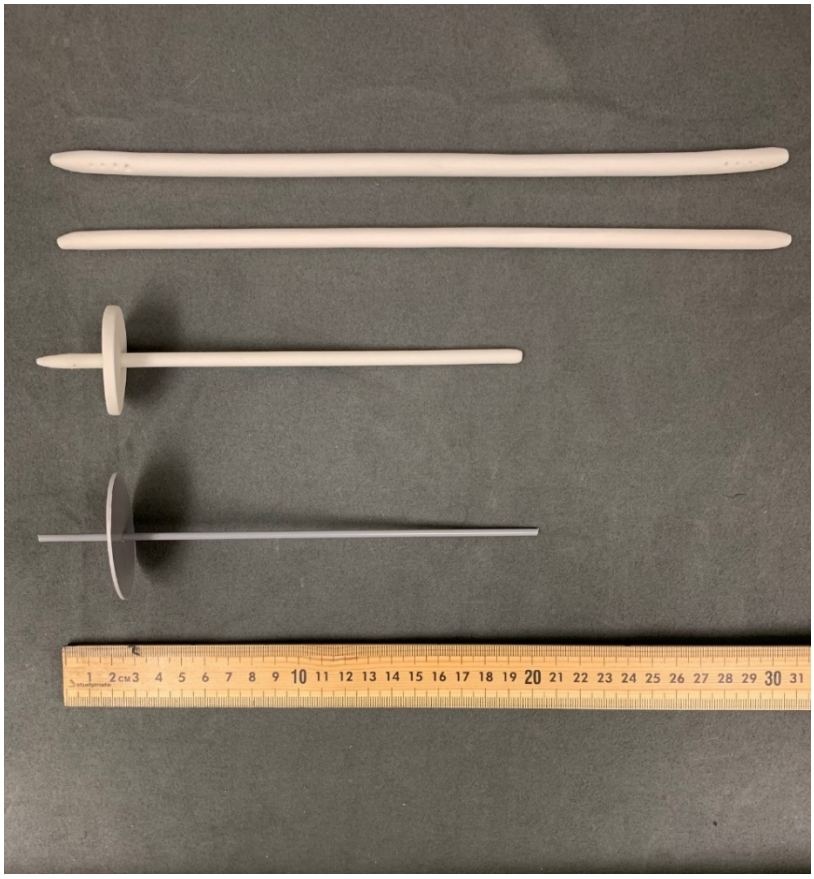

Figure 3 Various ASH designs to scale, top to bottom: 11mm, 9mm, 6mm and plastic.

To choose where to plant the ASH, the spatial distributions of all GPS observations of spotted handfish at targeted sites between 2015 and 2018 were plotted and two 250 m long transect lines fitted through the densest clusters. The coordinates of the start and end points were recorded and then used in the field to place deploy two 250 m transect reels of weighted line from the vessel. Two rows of ceramic ASH were planted along each transect line, each alternating between the two larger thickness (9 mm – 11 mm) and then with a one 6 mm planted every 8th ASH (left over from last year's work) to act as controls. The array was completed prior to the start of the spawning season (Table 2).

Table 2 Sites, dates planted and checked for ASH at Sandy Bay (SB), Bellerive (BR) and Tranmere (TR).

Site	Planted	Checked	Days
SB	26/7/2019	20/11/2019	62
BR 1	16/8/2019	4/11/2019	80
BR 1	16/8/2019	4/11/2019	80
BR 2	30/8/2019	5/11/2019	67
BR 2	30/8/2019	5/11/2019	67
TR 1	3/9/2019	22/11/2019	80
TR 1	3/9/2019	22/11/2019	80

Along all transects at Sandy Bay and Bellerive, there was ≥93% survival of both the 11 mm and 9 mm ASH compared to ~ 52% for the 6 mm ASH (Table 3). The loss rate of 6 mm ASH at these sites was like what occurred in 2018 (Lynch et al. 2018).

Table 3 Number of ASH of various thickness (mm) planted and survived (Surv) at Sandy Bay (SB), Bellerive (BR) and Tranmere (TR) sites by transect.

Site	Transect	Plant 11mm	Plant 9mm	Plant 6mm	Surv 11mm	Surv 9mm	Surv 6mm
SB	1	62	59	16	48	52	5
BR 1	1	62	62	16	62	62	10
BR 1	2	62	62	16	62	62	8
BR 2*	1	62	62	16	59	53	8
BR 2	2	62	62	16	57	53	12
TR 1	1	62	62	16	14	12	1
TR 1	2	62	62	16	13	13	2
TR 2	1	62	62	16	14	21	5
TR 2	2	62	62	16	14	21	4

*****damage was from an anchor

At one site, Tranmere, ASH loss was much higher for all sizes of ASH, with only 19% of 6mm, 27% of 9mm and 22% of the 11mm surviving. Again, this lose rate was similar to ASH loss in 2018 at this site, suggesting a strong site effect, perhaps due to the hard nature of the substrate or interaction with large skates and rays.

ASH was checked for survivability and breeding activity (Table 4). We did this late in the breeding season as we were more interested in survival rates for the ASH, rather than use of ASH by fish, which was established in the previous year. Hence rates of use reported were lower than were observed in 2018, when multiple observations across the season were made by Alex Hormann as part of a Master's thesis. In a number of cases, we suspect ASH had been used for breeding earlier in the season – due to indentations in the sediment around the ASH - but we did not count these as breeding activity.

In 2019 we counted and categorised each type of ASH, fish with eggs on ASH, fish only associated with ASH or eggs only on ASH. In some cases fish had used the ASH, the eggs had hatched, and remnants remained. When we observed remnants we recorded these as "eggs", though in some cases the signs were very subtle. Our limited observations suggest fish prefer the 9 mm, with 11 examples of breeding activity compared to 4 for the11 mm ASH. We observed no breeding activity on the more sparsely planted 6 mm ASH.

Table 4 Use of ASH by site, transect, ASH thickness and whether this included fish guarding eggs, just fish or just eggs/remanets.

Site	Transect	Fish with eggs 11mm	Fish with eggs 9mm	Fish with eggs 6mm	Fish 11mm	Fish 9mm	Fish 6mm
SB	1	0	2	0	0	1	0
BR 1	1	0	0	0	0	1	0
BR 1	2	1	1	0	1	0	0
BR 2	1	0	2	0	0	0	0
BR 2	2	0	1	0	0	0	0
TR 1	1	1	0	0	0	0	0
TR 1	2	0	1	0	0	0	0
TR2	1	0	0	0	0	0	0
TR2	2	0	0	0	0	0	0

Site	Transect	Eggs 11mm	Eggs 9mm	Eggs 6mm
SB	1	0	0	0
BR 1	1	1	0	0
BR 1	2	0	0	0
BR 2	1	0	1	0
BR 2	2	0	1	0
TR 1	1	0	0	0
TR 1	2	0	0	0
TR2	1	0	0	0
TR2	2	0	0	0

1.3 Storm Bay observations of spotted handfish by ROV

The National Handfish Recovery Team (NHRT) received notification from two aquaculture companies Huon Aquaculture (HAC) and Tassal that they had both observed spotted handfish during Remotely Operated Vehicle (ROV) surveys undertaken as part of environmental assessments in Storm Bay. HAC also reported an additional site in the Huon estuary, adding to another site reported in 2016. This brings the total known extant locations for spotted handfish to 13.

In Storm Bay, the HAC ROV observed a single fish at a depth of 35.2 m, outside of their Trumpeter Bay lease, on the 17/6/2019 (Fig 4). Spotted handfish are known from depths between 0-60 m (Last and Gledhill 2009a), so this is within the expected depth distribution.

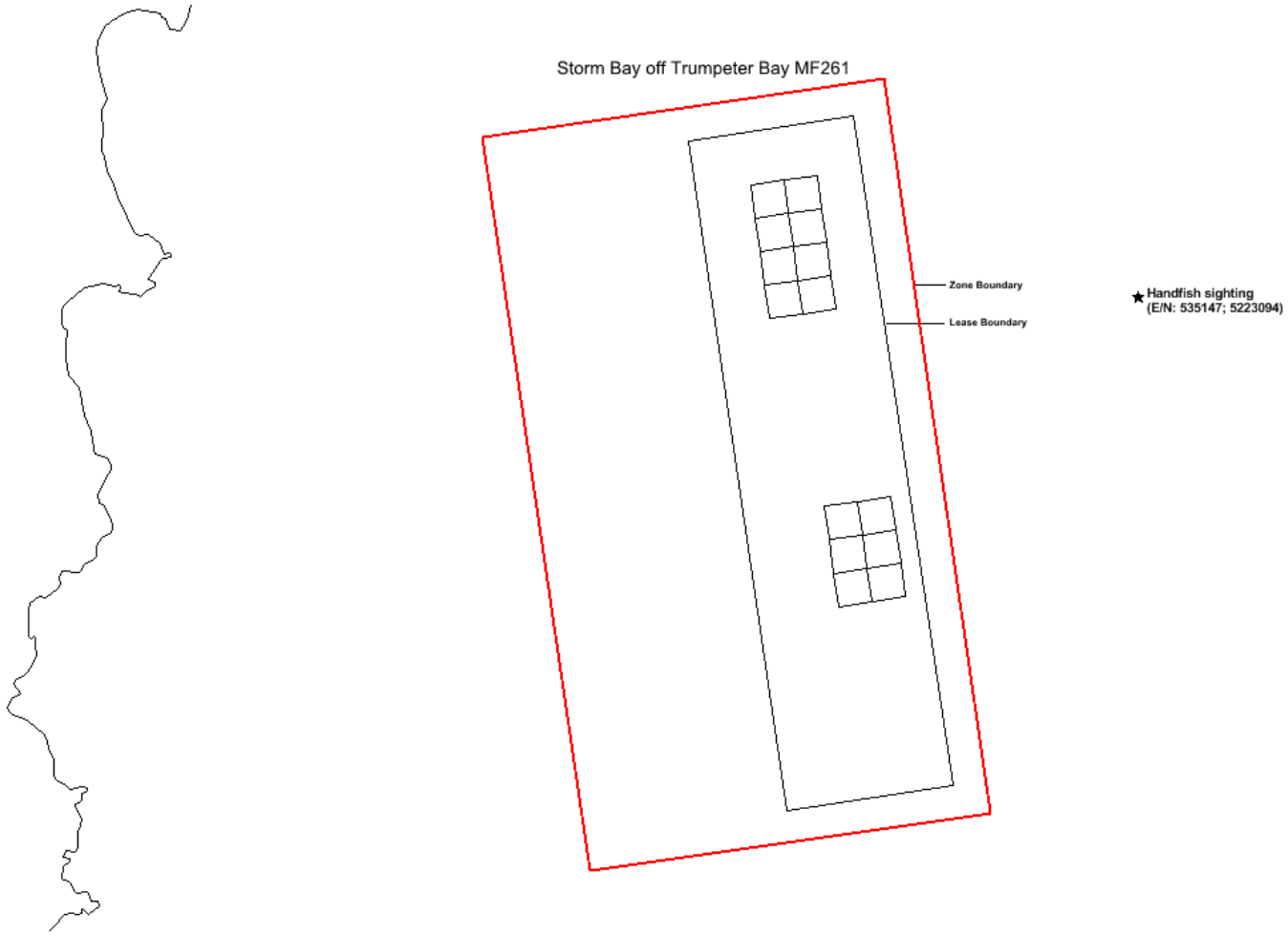

Figure 4 Eastern coastline of Bruny Island, the HAC Trumpeter Bay zone, in red and lease in black and the ★is the location where the HAC ROV observed the spotted handfish.

The fish appear to be spotted handfish (Fig 5), rather than the Australian handfish *Brachionichthys australis*. The Australian handfish is pale with yellow to brown dashes rather than spots. It also has a much larger eye, a smaller mouth more prominent and longer illicium, smaller esca and differences in fin lengths and ray counts. The Australian handfish is usually seen in the catches of trawls taken at depths between 18 m and 210 m.

Figure 5 Close up photograph of a spotted handfish taken by the HAC ROV

Photos were also taken of the micro-habitat (Figs 6a, b, c) and a typical spotted handfish threat display (Fig 6c) towards the ROV. The micro-habitat appears to be sand ripple with material and biota deposited in the troughs, which is a preferred micro-habitat of the fish (Wong et al. 2018). Tassal provided no further information other than to report the presence of a spotted handfish near their lease on the western side of Storm Bay.

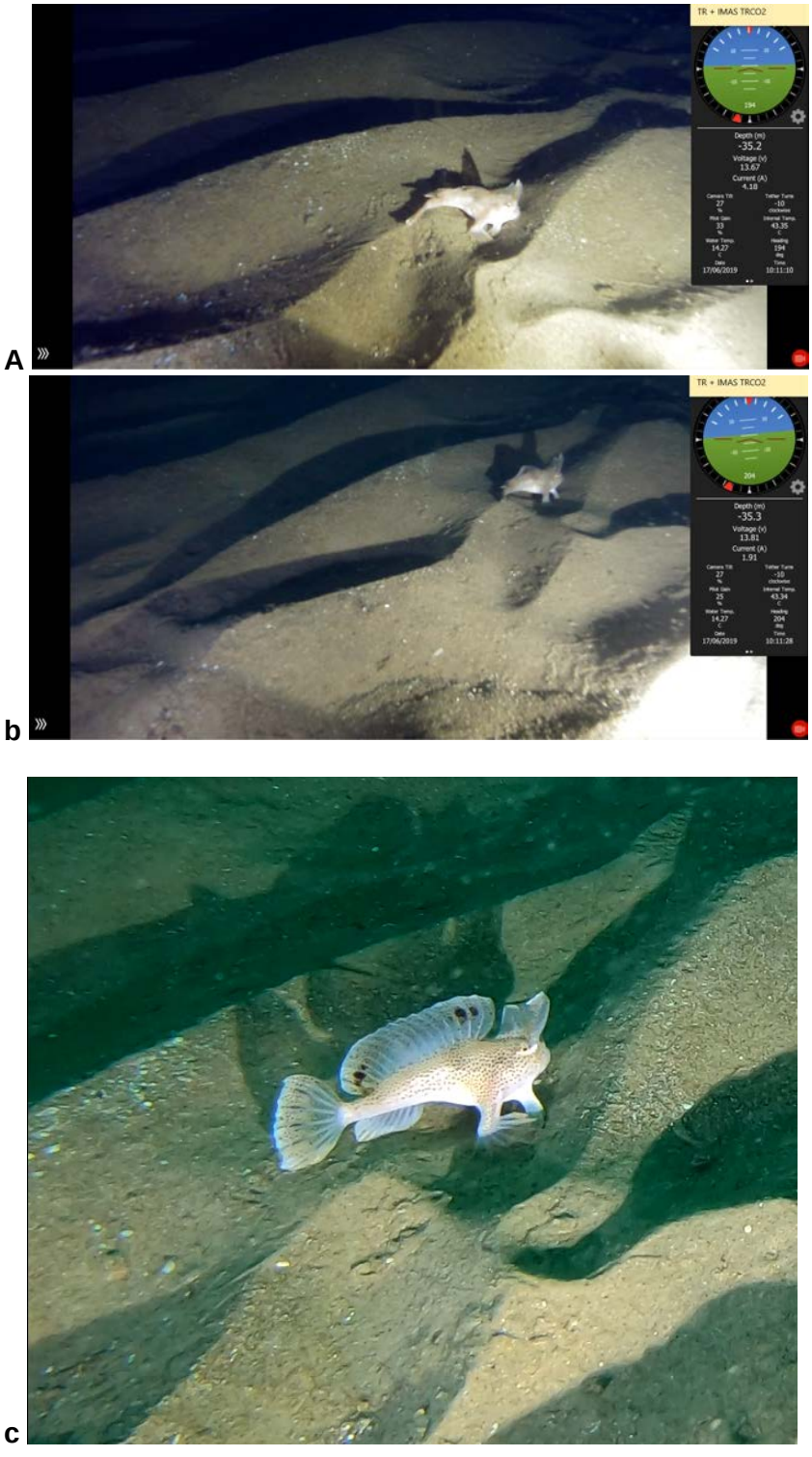

Figure 6 a) and b) pictures taken by the HAC ROV of a spotted handfish in a preferred micro-habitat type and c) showing a typical spotted handfish threat display.

1.4 Discussion

The persistence of low numbers of fish raises the issue of false negatives for local populations thought to be extinct (Barrett 1996) or unknown local populations. It is possible that fish exist

elsewhere in low densities and possibly even high densities. It is interesting to note that this year we identified 3 new locations where presence of spotted handfish have been reported.

There is no evidence, however, that this species is in anyway abundant compared to historical records (Last et al. 1983) or as would be expected from the state of its highly degraded environment. A reasonable hypothesis, based on our growing knowledge of spotted handfish life history strategy, behaviour, reproductive ecology and genetics is that local populations of spotted handfish are fragmented and isolated remnants of what was once a very large, dynamic and more contiguous population. This large historic population was probably associated with a now collapsed but once diverse and extensive nearshore bivalve community.

There is a strong correlation with the historic distribution of spotted handfish and the historic extent of scallop beds (Last and Gledhill 2009a). Across this extent it is possible that more fragmented local populations of spotted handfish are persisting in isolation. Spotted handfish are both camouflaged to blend into a background of scallop shell hash and also prefer complex micro-habitats, particularly those associated with predation of bivalves (Wong et al. 2018). In the mid-20th century, 15,000,000 scallops, per year, were dredged from the waters of Southern Tasmania in the winter months and then canned and exported (Shea 1948). Studies of sediment cores taken across the region show the catastrophic collapse of this bivalve ecological community, which was temporally correlated along a spatial timeline of serial depletion by the dredge fishery for scallops (Edgar and Samson 2004). By-catch of spotted handfish by dredge fishing was identified as a threatening process early in the recovery process (Bruce and Green 1998), but an ongoing impact may have been the destruction of the bivalve community. Recovery of this ecological community has been suppressed (Edgar and Samson 2004), perhaps through predation by the introduced marine pest, the North Pacific Seastar, or other factors such as recruitment over-fishing and changes in the environment.

Several aspects of spotted handfish life histories suggest that they were once much more abundant and are not a naturally rare species. They appear to be relatively short lived, with 90% of the observed population ≤ 5 years of age (Bessell 2018). Spotted handfish are also a mid-trophic species and display a range of predator avoidance mitigation strategies. These include hiding, though when discovered they extend their dorsal fins as a threat and hinderance to being swallowed. Hence, their life-spans and position in the food chain is typical of more abundant species.

Spotted handfish also expend considerable energy on reproduction, both through the production of large eggs and egg masses in relation to their body size and parental care. As a planktonic phase is avoided and juveniles are directly recruited and are highly cryptic, survival of early life history stages may be high and spotted handfish populations may be able to increase rapidly. Over time, we have observed variable inter-annual dynamics in local populations, both with rapid increases to high abundance (>50 fish per hectare), but also rapid declines, as well as stability. This suggests either a species with high degrees of movement – for which we can find no evidence – or one where local population can be dynamic over time either through declines following recruitment failure as cohorts rapidly age and are removed through natural mortality or rapid increases in abundance following successful recruitment events.

Spotted handfish also appear to have strategies to avoid over consumption of resources. Outside of the breeding season they are solitary, and from our captive breeding results there may be a density dependency for fish to becoming gravid – with only fish kept in isolation moving to this reproductive stage. This may be related to the reproductive strategy of direct recruitment and their observed high dynamism in local population densities. Over consumption of resources, within a spatially limited but potentially dynamic habitat, when combined with poor adult dispersal, could lead to starvation, especially for females who must contribute large amounts of energy for reproduction. The fish also seem to have great flexibility in spawning aggregations, which can occur in either high numbers or in parties of two.

There is no evidence to suggest widespread dispersal of adults or juveniles, which would be a pre-requisite for our study populations being spawning aggregations and/or juvenile rearing areas. Aspects of the species life history strategy would also be at odds with this hypothesis. The advantage of direct recruitment is to avoid dispersal away from the habitat at spawning and we also find adults in these habitats all year. The Brachionichthyidae, of which spotted handfish are one of 14 species, is also the most speciose of the marine fish families endemic to Australia (Bruce et al. 1998), but all species, with the exception of the Australian handfish, occupy extremely narrow geographic ranges (Last and Gledhill 2009a). This suggests niche separation through competitive natural selection.

Genetic analysis – detailed later in this report – demonstrated geographically structured and isolated local populations. There is strong genetic connectivity only between close groups of local populations of spotted handfish. These groups are upper estuary (Battery Point, Howrah), middle estuary (Tranmere and Ralphs Bay) and lower estuary (Mary Anne Bay and Opossum Bay). Sandy Bay individuals were shown to be somewhat genetically different to other *B. hirsutus* individuals; even to those at nearby Battery Point. It is also interesting to note that each of these groups has single, tenuous links to its next closest group. This may suggest either some low-level of dispersal – perhaps from egg masses breaking free in floods and moving down the estuary – or is an historic artefact of a once more contiguous population where small movements over generations would have linked groups.

The population dynamism of spotted handfish makes the use of photo recognition to undertake capture-mark-recapture estimation of population size difficult, as they live for too short a time for any economically reasonable sampling program to have enough replication to acquire enough recaptures for modelling. Density estimates, as a proxy for population abundance, requires much lower sampling to track the dynamics of local populations through time. Though, the small number of recaptures we do have suggest low levels of movement by adults (Bessell 2018).

We now hypothesize that handfish population dynamics may be explained by: historical and ongoing habitat modification and fragmentation; their relatively short lifespan; low dispersal and life-history barriers to recruitment; and variable breeding success due to their natural spawning habitats, such as stalked ascidians, being consumed by an introduced marine pest ,the north Pacific Sea star (Ross et al. 2003) or otherwise destroyed. If spawning fails, then population declines may occur over short periods of time as cohorts quickly pass through a limited 2-3 year window for breeding and die. As there appears to be no external recruitment,

isolated local populations are also vulnerable to stochastic events but appear to be able to persist in low densities (<3 fish per hectare). Conversely, if there is a period of successful breeding then populations can increase rapidly. Direct intervention, such as monitoring ascidian density as a proxy for natural spawning habitat and then planting artificial spawning habitats as required, is one way to support local populations. Also critical to their survival are mitigating specific threats, such as replacing destructive chain moorings with more environmentally sustainable (ES) designs (see section 5). Spotted handfish have been observed under an ES mooring in the recovering scar after removal of the chain mooring (Wong pers comm). Guiding planning decisions for coastal developments that are sensitive to handfish conservation as well as receiving feedback from environmental assessments will also be important to maintain populations.

In the future, discovery of unknown local populations or avoidance of false negatives due to sampling sensitivity may be enhanced with the use of new techniques such as eDNA. We expect to find more remnant local populations but recovery to historic levels would be dependent on recovery of the habitat and bivalve populations.

2. CAPTIVE BREEDING

The Ambassador Fish program is well underway, with fish on display at Melbourne SEA LIFE Aquarium and Seahorse World. Both partner institutes held launches with extensive media coverage, including ministerial attendance in Tasmania.

In 2017 we collected 20 adult spotted hand fish captures and 14 adult fish remain alive as well as approximately 30 juveniles. No adult fish have been lost this year. Two mortality events of juveniles occurred. The first was 10 juveniles sent to Woodbridge Marine Discovery Centre. The second was 13 juveniles in a tank at Seahorse World. Though pathology reports were taken, it is unknown what caused these deaths. In general, juvenile fish have continued to survive and grow.

The single red handfish and egg mass was captured in late 2018 and eggs hatched in captivity at CSIRO (Fig 7). These fish were transferred to Seahorse World (Fig 8), and of the 17 juveniles transferred from CSIRO, all but one has survived. Two egg masses were collected in late 2019 and hatched at the IMAS aquaculture laboratory in Taroona (Fig 9). Around 60 individuals have been raised at this site with low mortality.

There were two egg laying events within the captive adult populations in the 2019 season. One for the spotted and one for the red handfish. While potential 'male' mates were collected in the case of the reds (2 animals) or added to the tanks which contained the gravid females from existing stock, they did not fertilise the eggs. The sex of handfish cannot be determined externally, so it is unknown if these were males or females. The animals that became gravid were either alone or in low densities (2 fish in a large tank) and it is likely that stocking densities may influence breeding. Both spotted and red handfish have known dispersal and aggregating behaviours and the dense year-round aggregations in the tanks may not replicate what is required to stimulate the development of eggs or sperm.

More research is needed to understand what triggers breeding behaviour and/or egg and sperm development. A method to differentiate between the sexes in live fish is also required in order to increase chances of mating occurring.

Figure 7 Red handfish and eggs in the CSIRO Aquarium.

Figure 8-9 Captive-bred red handfish at Seahorse World and IMAS Taroona site.

3. ECOLOGY AND CONSERVATION OF RED HANDFISH

3.1 Introduction

The critically endangered red handfish (*Thymichthys politus*) is a demersal reef species found in 1-20 m of water (Last and Gledhill 2009b), a narrower depth range than for the spotted handfish. They are small (usually less than 90 mm) and highly cryptic, preferring to take refuge under the canopies of macroalgae such as *Sargassum sp*. Currently, red handfish are known to exist in only two locations on Tasmania's southeast after suffering a dramatic decline over the past few decades. Previously occupied reefs from where the species has disappeared include the Acteaon Islands, and the Forestier and Tasman Peninsulas (Edgar et al. 2017; Last and Gledhill 2009b). Last and Gledhill (2009b) reported a major population collapse at one site as a result of a native urchin (*Heliocidaris erythrogramma*) infestation. Native urchin overgrazing of macroalgae remains a threat to the species, as do other threats such as nutrient pollution, direct human disturbance and warming seas (Commonwealth of Australia 2015).

There is limited biological or ecological information for the red handfish, with a paucity of published literature specific to the species (e.g. Bruce et al. 1997; Edgar et al. 2017; Last and Gledhill 2009b). Formal population estimates are yet to be determined, as are population dynamics, age and growth, movements, habitat preference, and genetics. Knowledge of basic biological parameters such as these are important to effectively manage any endangered species (Caughley and Gunn 1996; Tella et al. 2013). Due to the limited number of known sites, low number of individuals and their cryptic nature, researching this species remains challenging. Current research is focused on population estimation at the two know locations, and development of environmental DNA (eDNA) techniques and markers for the species in order to provide a method for discovery of new populations.

3.2 Population estimates

In January 2019 divers from the Institute of Marine and Antarctic Studies (IMAS) and Reef Life Survey (RLF) conducted intensive surveys in Fredrick Henry Bay for red handfish at one of the two known sites. This site was known to have the higher density of fish. A total of 42, 1x50 m transects covering a 2 km^2 patch of reef was meticulously searched for red handfish by experienced divers. Photographs were taken of each individual handfish sighted to allow for photo-identification, similar to the protocol described by Moriarty (2012), Bessell (2018), Wong and Lynch (2017) and Wong et al. (2018). The location of each fish was also recorded via a timestamp of photographs and GPS position, recorded via diver-towed GPS units at the surface (Lynch et al. 2015b). Additionally, the length of every fish was recorded, as was the surrounding habitat type.

A total of 149 red handfish were observed and photographically 'marked' using their individually unique skin patterns. The 'fingerprints' of each fish were then processed in the individual identification software, *I^3S Classic* a variant of the software used to identify spotted handfish, as described in (Bessell 2018) and stored within a database. These fingerprints act as a record of every photographed red handfish sighted since late 2018. With this information, if surveys are ongoing and have sufficient replication to provide adequate recaptures, population estimates for the species may be obtained via traditional capture-mark-recapture

approaches, such as Jolley-Seber (Jolly 1965; Seber 1965) and/or Lincoln-Petersen (Lincoln 1930; Petersen 1896) models. Additional information that may be obtained from regular surveys include growth data, movement, habitat use, and insights into the longevity of the species.

The 149 individuals sighted in January 2019 ranged in lengths from 10 to 80 mm (average = 56 mm), with those fish between 40 to 75 mm long comprising 92.0% (137 fish) of all observed fish (Fig 10). Only 4.7% (7) fish were considered juveniles (i.e. < 40 mm in length), with two fish being less than 20 mm long and most probably young of the year from the previous breeding season. The low number of fish in the smaller size class may be the result of difficulty sighting these cryptic small fish. Fish were found between 1.2 and 2.9 m in depth. Habitat usage of fish sighted included both sandy and rocky reef environments, with fish being almost always associated with either seagrass over the sand or *Sargassum* sp on the reef.

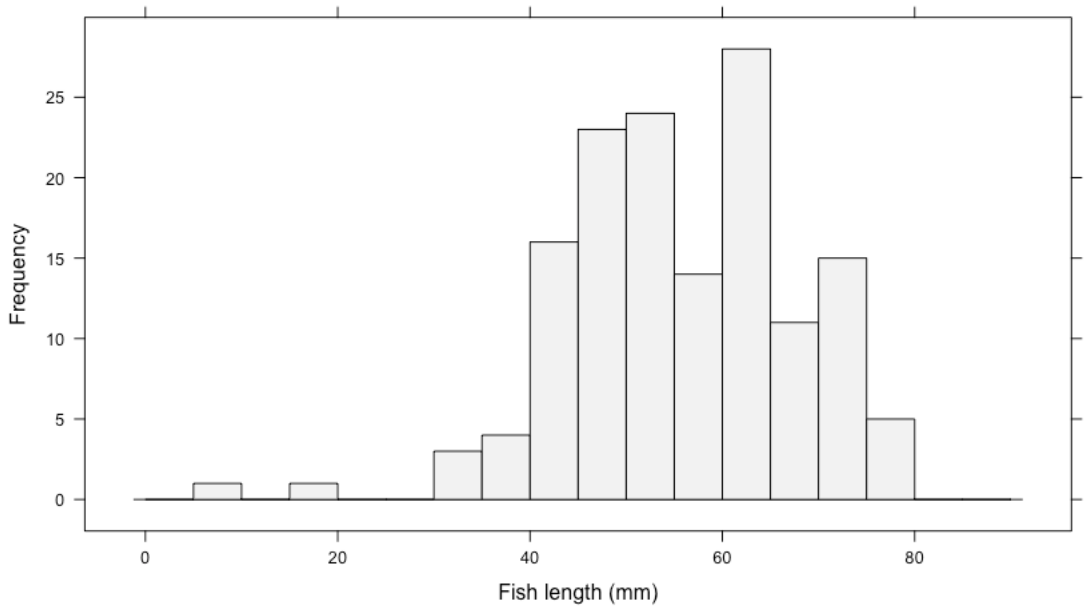

Figure 9 length frequency plot of all observed red handfish (n = 149) during January 2019 surveys at higher-density population.

Prior to the January 2019 surveys, 29 fish were photographed. The length and habitat usage of these fish were not recorded, though 4 of these fish were identified as having been observed in the past. This identification was aided by the program *I*^{3}*S Classic*.

A full census of the second lower-density population within Fredrick Henry Bay is planned for January 2020, along with further surveys to search for resighting's at the higher-density population.

3.3 Environmental DNA (eDNA)

An approach to monitoring that has become increasingly popular in the field of ecology is environmental DNA (eDNA). This technique detects trace amounts of DNA left behind by

organisms in their surroundings in the form of mucus, waste products, respiration, gametes and decaying matter (Taberlet et al. 2012). eDNA is useful for a variety of applications such as in water, soil, sediment, ice and permafrost samples for a wide range of taxa (reviewed by Bohmann et al. 2014). In aquatic environments, eDNA is used to for biodiversity studies to detect presence/absence of species within ecosystems (e.g. Kelly et al. 2014; Thomsen et al. 2012; Zhang et al. 2019), management of invasive species (e.g. Clusa and García-Vázquez 2018; Doyle et al. 2017; Minamoto et al. 2017; Uthicke et al. 2018) and detection of species of conservation importance (e.g. Jerde et al. 2011; Simpfendorfer et al. 2016; Thomsen and Willerslev 2015; Weltz et al. 2017).

One application of eDNA is to detect a species within a water sample, if the DNA of the target organisms (i.e. the template DNA) is known. The purpose of this study is to (1) test the sensitivity and limitations of eDNA to detect the presence of red handfish in both a controlled environment (an aquarium), and in the wild, (2) develop a sampling protocol that can be applied to target searches for red handfish, improving the cost-intensive SCUBA surveys for this highly cryptic fish, and (3) demonstrate the ability of eDNA to be used as a conservation tool for other rare and endangered marine fishes.

During field sampling in January 2019 a deceased red handfish specimen was opportunistically recovered. Additionally, juvenile mortalities/stillborns from captive reared red handfish were preserved. DNA was extracted from four individual specimens, providing template DNA for red handfish. The mitochondrial cytochrome oxidase subunit 1 (COI), 16S ribosomal RNA (16S), 12S ribosomal RNA (12S) and cytochrome b (Cytb) regions were PCR amplified and then cleaned with magnetic particles. Products were then cycle sequenced using the same primers and Big Dye Terminator chemistry, cleaned again and bi-directionally sequenced on an ABI 3130XL Genetic Analyser. The same mtDNA gene regions in spotted handfish (*Brachionichthys hirsutus*) and Australian handfish (*B. australis*) were also amplified and sequenced (using samples from the CSIRO Australian National Fish Collection). To determine any polymorphism within and among handfish species, the COI, 16S, 12S and Cytb sequences were aligned using the software, *Geneious*. The consensus sequences (the combination of both forward and reverse sequences) were then aligned against corresponding sequences for all other known species within the BOLD and GenBank databases. Of the four genes, COI was shown to be the most useful with a larger number of base pair differences observed among the three species than within the species sequences.

The next stage of assay development is several rounds of design, testing and optimisation of red handfish-specific primers and probes (likely TaqMan MGB probes, though SYBR Green may also be tested). After successful optimisation of the primers and probes (using handfish template DNA and a small number of filtered water samples), the red handfish assay will then be screened in the field samples (see below). If a specific red handfish assay cannot be developed, an alternate genus level handfish assay will be assessed.

To test the utility and limitations of the developed assay for detection of red handfish eDNA in water samples in the field, a staged approach is to be implemented (Fig. 11). Firstly, we will sample water collected from a closed-circuit aquarium (system capacity of 170 L) containing a single adult red handfish to test whether presence can be detected using eDNA. We collected 10 L of aquarium water at Seahorse World, Beauty Point on the 13 June 2019. This was later separated into three replicate 2 L water samples, all of which were filtered using Sterivex filters

and stored at -80°C to prevent eDNA degradation before DNA extraction. In the second and third stage we will expand the work attempt to detect red handfish in the field.

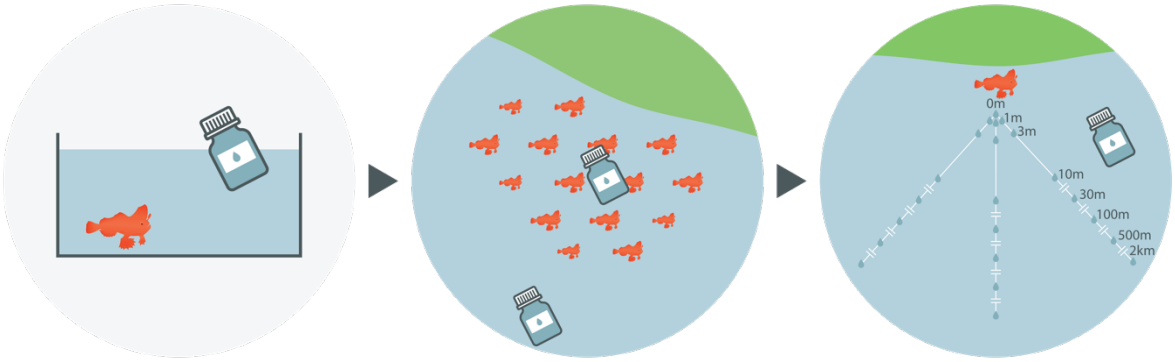

Figure 10 Staged approach to testing detectability of red handfish eDNA. Stage 1 is to test whether red handfish DNA can be detected from eDNA in a controlled environment (aquarium) where presence is known. Stage 2 tests whether eDNA can be used to detect red handfish in the field at a high-density population. Finally, stage 3 assesses the performance in the field in order to demine the limitations of the technique, with water samples taken from a low-density population in the field, at increasing distances (0, 1, 3, 10, 30, 100, 500 m, and 2 km) away from the known population.

3.4 Expected outcomes

Research into the critically endangered red handfish is still in its early stages, and therefore more extensive research is required to gain a better understanding of the species' ecology and required approaches for its conservation. With ongoing monitoring and regular surveys, we expect to have robust population estimates by 2021 for both our high and low density sites and more informed knowledge on habitat usage and movement patterns of the species. Additionally, the successful development of a species-specific assay would provide a method in which red handfish can be accurately screened from water samples to guide searches for more populations, potentially giving pathways for detection of other handfish species in the future. Identifying sites in which red handfish are present, but not yet known, will improve sampling effort and provide higher quality data for improving our understanding of the species, and how best to conserve them.

4. GENOMIC (SNP) STUDIES ON SPOTTED HANDFISH (*B. HIRSUTUS*) FROM THE RIVER DERWENT

4.1 Summary

The population diversity and structure of spotted handfish, *Brachionicthys hirsutus* was investigated for the first time with a genome-wide single nucleotide polymorphism (SNP) approach. This is the first instance of any handfish species being examined with any form of population genetic marker. This study provided a valid method for obtaining the first set of genome-wide variable markers in this highly endangered fish species through next generation DNA sequencing of genetic variation. In addition to SNPs, the study also developed an *in-silico* microsatellite library which could be utilised in the future for captive breeding and parentage studies in *B. hirsutus* and other handfish species.

DNA (extracted from small fin clips) from 262 *B. hirsutus* individuals sampled across eleven collection sites in the River Derwent from 1998 to 2008 provided the basis for diversity examination through Genotype by Sequencing (GBS). While the sample sizes in several collections were sub-optimal (i.e. several collections were represented by N < 5) and resourcing restricted the number of individuals that were genotyped, over 4 170 biallelic SNPs were analysed in 193 *B. hirsutus* individuals. Genetic diversity in the spotted handfish collections was modest (average observed heterozygosity was 0.250) with multiple connectivity and structure estimates demonstrating concordant outcomes.

Spatially discrete *B. hirsutus* collections in the River Derwent (from 2007 & 2008) were significantly different. Genetic proximity analysis, clustered individuals into three main genetic groupings, indicating a reduced level of gene flow among local populations of this threatened anglerfish in the River Derwent. A lack of genetic homogeneity among collected samples in the upper estuary areas compared to the lower areas suggests isolation by distance driven by a) migration barriers (likely a result of a lack of continuous preferred microhabitat in the River Derwent following destruction of spawning habitat by an introduced marine pest); b) species-specific biological attributes (i.e. benthic egg production with direct recruitment and no planktonic phase combined with limited adult mobility) and c) impacts of by-catch and collapse of bivalve communities from historical dredge fisheries.

While contemporary (2018-2019) samples from dedicated collection surveys were not available for analysis in the current study, the inclusion of deceased individuals from the current captive breeding program demonstrated the feasibility of this genomic approach for diversity and connectivity assessments.

These findings highlight the need for ongoing protection of all *B. hirsutus* individuals at each location, as recruitment and gene flow between all but the closest local populations were limited. Spatial locations of spotted handfish should continue to be managed or considered as separate conservation units, even within the River Derwent. This important genomic information for *B. hirsutus* fills a knowledge gap and should be considered in the actions of the recovery plan, particularly given the current captive breeding program.

4.2 Introduction

The successful management of aquatic biodiversity, including endangered species, requires an understanding of the historical and contemporary factors that have, and continue to influence the range and connectivity of species (Lowe and Allendorf 2010; Beheregaray et al. 2017). Obtaining this knowledge however can be challenging for many species in aquatic environments due to seemingly continuous habitats (and lack of physical barriers), varying dispersal potentials of different life stages, ecological differences among species and populations and difficulties of physically tracking or tracing individuals both spatially and temporally (Ward et al., 1994; Avise 1998; Waples 1998; Bargelloni et al., 2000; Lowe and Allendorf, 2010; Junge et al., 2019).

Molecular genetic techniques and population wide data can be utilised to assess genetic diversity, estimate effective population sizes, track movements and assess genetic connectivity among populations (Lowe and Allendorf 2010; Ovenden et al., 2013), thereby determining the degree to which gene flow effects evolutionary processes within populations. Importantly, genetic connectivity is not demographic connectivity, with the two differing primarily in the degree on which population growth rates are affected by dispersal (Lowe and Allendorf 2010; Ovenden 2013). The population growth rate (or survival and birth rates) is intrinsically affected by immigration or emigration in demographically connected populations (and is dependent on population sizes), whereas genetic connectivity is the degree to which gene flow affects evolutionary processes within populations (Waples and Gaggiotti 2006; Lowe and Allendorf 2010; Ovenden 2013). While methods such as capture-mark-recapture and imagery analysis can track fish movement, only genetics can detect whether movement has resulted in reproduction in the subsequent population (Ovenden 2013).

Over the last twenty years several different forms of genetic data (including mtDNA haplotypes and microsatellite genotypes) have been used to assess gene flow through the analyses of *F*-statistics. *F*- statistics or genetic fixation indices describe the expected level of heterozygosity in a population and is a measure of correlation between genes drawn at different levels of a subdivided population. Originally, Hartl and Clark (1997) stated $F_{ST} < 0.050$ represented little genetic differentiation; 0.050 - 0.150 = moderate genetic differentiation and > 0.150 = great genetic differentiation (albeit these measures were based on genetic markers such as allozymes or microsatellites which do not represent genomic scale variation); the significance of these differentiation measures among populations also needs to be calculated. F_{ST} is considered the standard measure of divergence at loci among sub-populations (Wright 1943) with an F_{ST} of 1 indicating separate species. However, different amounts of dispersal result in different levels of genetic connectivity, depending on the evolutionary consequence of interest (Lowe and Allendorf 2010). Wright (1949) stated that very small amounts of gene flow (leading to the one-migrant-per-generation rule) (Mills and Allendorf 1996) are enough to avoid the harmful effects of genetic drift and inbreeding, although Lowe and Allendorf (2010) stated that more migrants per generation could still result in populations being genetically different.

The analysis of many genetic loci (termed genomics - when thousands of loci or markers in the genome are screened) now makes it possible to assign individuals to their 'subpopulation' of origin based on their multi-locus genotypes. However, this is predicated on the existence of genetic divergence among subpopulations. Connectivity assessment (i.e. stock structure) is undertaken through analyses of genetic diversity and aims to identify barriers to gene flow

among populations of the species across its' demographic range. Building on the rapid advancements in genomic analyses and high throughput sequencing (HTS), contemporary studies in organismal population genomics are now likely to be based on Single Nucleotide Polymorphisms (SNPs). SNPs may also provide more fine scale resolution of connectivity, particularly given the informativeness of the thousands of markers across the genome.

A SNP is a substitution or insertion/deletion in a DNA sequence, primarily observed at a nucleotide (e.g. C → T) at a specific position in the genome; SNPs are ubiquitous throughout the genome of most species (Morin et al., 2004; Seeb et al., 2011). As with microsatellites, SNPs are bi-parentally inherited. Recent developments in HTS (via double digest Restriction site-Associated DNA sequencing or ddRAD sequencing) enable the simultaneous screening of high-density genome SNP markers in individuals of non-model organisms (Baird et al., 2008; Peterson et al., 2012; Paris et al., 2017). The aim of such studies is to identify polymorphisms within and between populations (Paris et al., 2017 and refs within). Genotype by Sequencing (GBS, Elshire et al., 2011) is a genetic method for discovering and undertaking SNP genotyping (based on ddRAD) which enables comparative analyses across individuals without the need for a reference genome. Depending on experimental attributes, GBS can be utilised to study both neutral population structure and adaptive potential (Allendorf et al., 2010). SNPs provide the opportunity to analyse large numbers of genome wide loci with improved power to estimate genetic parameters such as gene flow and connectivity in smaller sample sizes above that of other marker types (e.g. mitochondrial DNA and nuclear microsatellites) (Allendorf 2017).

The current genomic investigation was undertaken to gain insights into the genetic diversity, gene flow and connectivity of spotted handfish (*Brachionichthys hirsutus*) in the River Derwent, Tasmania. Population genetic studies have not been published in the literature for any handfish species (although see Lawler 1999, Honours thesis), despite listings as threatened and endangered species. Previous *ad hoc* mitochondrial DNA studies by the Australian National Fish Collection (ANFC) generated mtDNA reference sequences deposited in the BarCode of Life Database (BOLD, http://www.boldsystems.org/), however there are no polymorphic, nuclear DNA population level studies for this endangered species.

Brachionichthys hirsutus is a critically endangered anglerfish species endemic to southeast Tasmania (Bruce et al., 1998; Last & Gledhill 2009). This small (individuals grow to approximately 135 mm, Bessell et al., 2019), rare and cryptic species is found in cool temperate marine waters in Tasmania (Bruce et al., 1998; Lynch et al., 2015; Wong et al., 2018). Between the 1980's and 1990's, *B. hirsutus* experienced significant population declines (Barrett et al., 1996) resulting in the species being the first marine fish to be listed as Critically Endangered under the Australian Environment Protection and Biodiversity Conservation Act (1999) (Conservation advice approved on 20 September 2012), listed as endangered in Tasmania (under the Threatened Species Protection Act 1995) and Critically Endangered by the International Union for Conservation of Nature (Bruce & Last 1996).

Once widespread and locally common from Coles Bay (east coast Tasmania) to the D'Entrecasteaux Channel in the south of the state, studies and underwater surveys in the last ten years indicate the presence of only nine known 'hotspots' of spotted handfish populations in the Derwent Estuary (Green 2005; Green 2007; Last et al., 2007; Last & Gledhill 2009) and

two recently re-discovered locations in the D'Entrecasteaux Channel (Lynch et al. 2018). The extent of connectivity amongst these spotted handfish locations is however unknown.

In these areas, *B. hirsutus* lives on soft sediment, at depths from 1-60 m (Last & Gledhill 2009), with the location of the hotspots likely a result of this suitable habitat (Wong et al., 2018). *Brachionichthys hirsutus* is best described as a habitat specialist, showing strong micro-habitat preferences within soft sediment habitats (Lynch et al., 2015; Lynch 2018; Wong et al., 2018).

Analyses of movement data on spotted handfish showed that individuals move within the hotspot locations but not between locations (Bessell et al., 2019). Fish movements ranged from 32 m (in 13 days) to 567 m (in 585 days) (Bessell 2018; Bessell et al., 2019).

Since 1999, three *B. hirsutus* recovery plans have been implemented (see http://www.environment.gov.au/biodiversity/threatened/recovery-plans/recovery-plan-for-three-handfish-species) with current conservation strategies focusing on assisting fish to breed with deployment of artificial spawning habitats (ASH), visual determination of population estimates, habitat restoration through environmentally-sensitive moorings, and the establishment of an Ambassador fish and captive breeding program (Lynch 2018).

In the absence of any genetic diversity knowledge or genomic connectivity estimates among handfish populations, and to address one of the 2015 spotted handfish Recovery Plan's objectives (i.e. 'increasing understanding of the biology and ecology of spotted handfish in order to conserve and contribute to future recovery of the species'), the current study was developed to fill two major knowledge gaps. The first was to investigate what level of gene flow and connectivity occurred between remnant populations (termed herein 'collections') of spotted handfish within the Derwent estuary, and the second was to assess the levels of genetic variation of these local collections. In addressing these two gaps, GBS was deployed to generate genome wide, co-dominant SNP data in *B. hirsutus* individuals.

Sampling was *ad hoc* and opportunistic, in some instances, (as will be outlined in later sections of this report) impacted the type of analyses that could be undertaken. There was no focus on genetic assessment of population sizes, or on selection/local genetic adaptation as the genome of *B. hirsutus* is not known or available. Robust environmental/categorical data did not accompany individual sampling at the time and microhabitat preferences for *B. hirsutus* were determined later (see Wong et al., 2018). Importantly, however, this is the first genomics study on spotted handfish and indeed the first genetics study on any handfish species.

4.3 Background to the SNP study

Prior to the commencement of the NESP project in early 2018 the meta-population structure of the species was unknown. Researchers in the CSIRO ANFC and O&A had sampled fin clips and preserved whole animals (approximately N = 240) from 1998 – 2008; these clips had either been stored in either ethanol or were frozen at -80°C. As part of an ANFC and Bioplatforms Australia project, in 2016, DNA was extracted from several of these samples as part of a DNA barcoding project - in which co-dominant polymorphic nuclear marker libraries were developed (i.e. for SNPs and microsatellites) in spotted and Australian (*B. australis*) handfish. Subsequent screening of a small number of individuals (N = 24) at the Australian Genome Research Facility (AGRF, http://www.agrf.org.au/) in Melbourne resulted in an establishment library of SNPs (as

characterised by double digest restriction enzymes *EcoRI* and *MspI*) and an *in-silico* library of putative microsatellite loci for batch screening in spotted handfish.

The genomics approach and results outlined below do not include any microsatellite loci genotyping; however, if funds were made available, the *in-silico B. hirsutus* microsatellite library could be used in future studies to develop loci suitable for parentage and sibship analyses for captive breeding programs for spotted handfish. Additionally, it is likely that any primers developed from the *in-silico* microsatellite library may amplify in other closely related handfish species, thereby enabling cross species amplification and genotyping without the need for development of additional handfish microsatellite loci.

4.4 Materials and Methods

As outlined above, the spotted handfish samples included in the current study were obtained through opportunistic and *ad hoc* sampling. Fin clip samples were obtained from studies previously undertaken by Mark Green (CSIRO) and Miles Lawler (CSIRO, University of Tasmania). The samples analysed here were not specifically obtained for the current study. Additionally, three whole specimens (see Figure 12) (deceased individuals, contributed by Mark Green) were sampled for the initial development of the SNP panel at AGRF.

Figure 11 *B. hirsutus* adults and examples of fin clips that were DNA extracted and utilised in the initial SNP screening and batch processing at AGRF

For the population study, individuals were sourced from several known spatial locations/collections in the River Derwent from 1998 – 2018. Sample sizes per 'collection' varied, ranging from N = 3 in the M. Lawler 1998 study to > 70 individuals from Tranmere in 2008. Due to small sample sizes in some collections, individuals were grouped according to location and year. Only seven individuals were sampled in 2018 as part of the current captive breeding program (see Table 1, BPM 2018). This is important to note, as the bulk of the

individuals came from Mark Green's research collections from 2006 - 2008; hence consistent temporal sampling was not undertaken and there was no population sampling for the last ten years. Additionally, there is no paired imagery analyses for any of the pre-2017 individuals.

Following the *in-silico* SNP library developed in 2016, the current study focussed on genotyping the SNPs in a larger number of spotted handfish. For this research, the laboratory work and DNA extractions were undertaken by the author at the CSIRO marine laboratories, while the GBS analyses (for SNP batch screening) was completed by AGRF in Melbourne. Quality control and downstream filtering of SNP data and subsequent population genomics analyses was completed by the author at the CSIRO marine laboratories.

4.4.1 DNA extractions for *B. hirsutus*

In mid-2018, DNA was extracted from 262 spotted handfish fin clips (Figure 12 gives an indication of the size of the fin clips used for extraction; Figure 13 outlines the known locations of *B. hirsutus*, as per Wong et al. (2018); Appendix 1 outlines the total number of spotted handfish samples that were extracted). Up to 25 mg of fin clip tissue per individual was DNA extracted according to a slightly modified Promega Wizard SV 96 well extraction protocol (https://au.promega.com/products/dna-and-rna-purification/genomic-dna-purification-kits/wizard-sv-96-genomic-dna-purification-system/; https://au.promega.com/resources/protocols/technical-bulletins/101/wizard-sv-96-genomic-dna-purification-system-protocol/) (i.e. the standard fish DNA extraction method used by the ANFC).

Tissues were digested overnight with Proteinase K and total DNA was eluted in DNAse free water. DNA was quantified on a Nanodrop 8000 (Thermo Fisher Scientific, Australia) with A260:A280 ratios reflecting DNA quality. Undiluted stocks of archival DNA were plated into 96 well plates and frozen at -80°C.

From the 262 fish, a subset of individuals was selected for SNP analysis. The batch size for genotyping was based on DNA quality and included individuals from each spatial and temporal sampling/location. The final number of individuals that were submitted for SNP analysis (n = 193) was based on available funds for the SNP genotyping. An aliquot of each DNA sample (see Table 5 below for SNP sample details) was placed into 96 well plates and sent to AGRF via courier (at room temperature) for SNP genotyping on an Illumina NextSeq Platform (150 base pair single end reads).

Table 5 Spotted handfish (*B. hirsutus*) samples extracted for DNA and screened for SNPs; sampling sites and dates are shown

Location/collection	Sample size	Sampling data	Collection abbreviation
Primrose Sands	3	1998	PR1998
Half Moon Bay	4	1998	HB1998
Kangaroo Bay	4	1998	KB1998
Opossum Bay	5	1998	OP1998
Howrah Beach	17	2006	HW2006
Manning Reef (Sandy Bay)	15	2006	MR2006
Battery Point	9	2007	BP2007
Mary-Ann Bay	15	2007	MAB2007
Tranmere	31	2007	TR2007
Opossum Bay	20	2008	OP2008
Ralphs Bay	41	2008	RB2008
Tranmere	22	2008	TR2008
Breeding Program morts	7	2018	BPM2018 (LO2018)
Total	193		

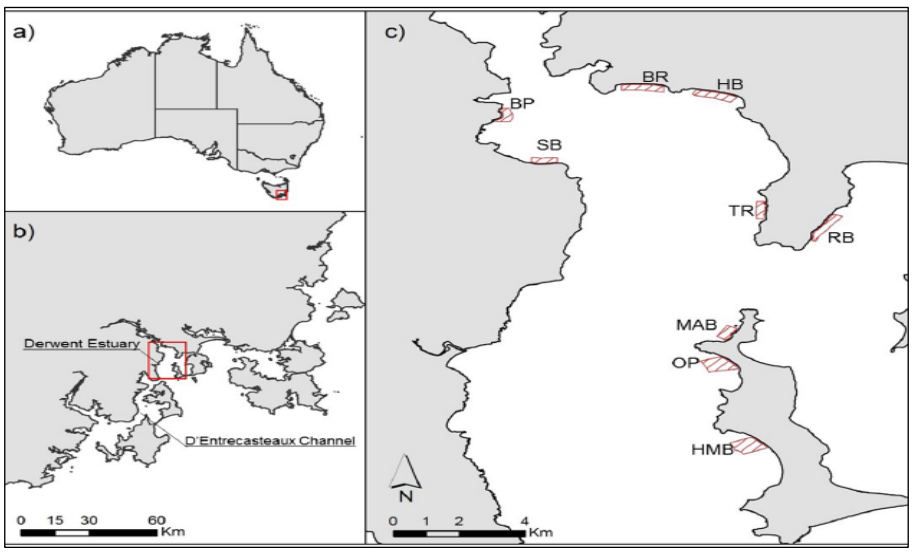

Figure 12 Map (from Wong et al., 2018) of spotted handfish, *B. hirsutus* collection locations A) South-east Tasmania; B) sampling locations in the Derwent Estuary; C) BP = Battery Point; BR = Bellerive Beach; HB = Howrah Beach; HMB = Half Moon Bay; MAB = Mary-Ann Bay; OP = Opossum Bay; RB = Ralphs Bay; TR = Tranmere; SB = Sandy Bay (as per Wong et al., 2018). Note – in current study, Manning Reef = Sandy Bay; Primrose Sands and Kangaroo Bay not shown on map

4.4.2 SNP batch screening at AGRF

In September 2018 the ddRAD libraries (as in Peterson et al., 2012) were prepared and sequenced at the AGRF facility in Melbourne. Briefly, the AGRF process pipeline for GBS consisted of library preparation and sequencing which included:

- DNA digestions with two restriction enzymes (*EcoRI* and *MspI*; determined from the GBS establishment service)
- ligation of barcoded adapters
- size selection of pooled digested-ligated fragments
- amplification of libraries via PCR using indexed primers
- sequencing on an Illumina© NextSeq platform flow cell (Illumina Inc, USA) with 150 cycles in MID-output mode according to their in-house GBS methodology

AGRF then processed the raw reads using their in-house bioinformatic pipeline and Stacks software v1.47, (http://catchenlab.life.illinois.edu/stacks/) (Catchen et al., 2011; 2013). Briefly this included:

- raw sequences were demultiplexed, checked for read quality and restriction site presence and trimmed; RAD-tags were analysed in Stacks (resulting in a separate FASTQ file for each sample) (using 'process_radtags' in Stacks)
- sequence reads were aligned into matching stacks/tags from which loci were formed and SNPs are detected ('ustacks','cstacks' 'sstacks' in Stacks); parameters used to define a 'stack' and resulting subsequent SNPs for each individual from the catalogue included: a minimum depth coverage of two to create a stack; disabling haplotype calls from secondary reads; one mismatch allowed between sample tags when generating the catalogue; a minimum of five reads to call a homozygous genotype and a heterozygote was called when the frequency of the minor allele in a stack was 0.05 - 0.1 across the entire dataset

AGRF provided the post processed SNPs (following the Stacks pipeline) as raw and unfiltered SNP output in a variant call format (VCF) file.

4.4.3 SNP DATA ANALYSIS AND FILTERING

Individuals in the VCF file were renamed (taking out the indexing information from AGRF) using bcftools reheader (Li et al., 2009) and filtered initially using VCFtools v0.1.14 (Danecek et al., 2011) in CSIRO's Galaxy instance (version release 18.01), with initial high-level filtering undertaken by treating all individuals as arbitrarily belonging to one group. Filtering removed sites whose minor allele frequency (maf) was too low (as a result of sequencing or alignment errors) and kept variants that had been successfully genotyped in at least 50% of individuals. When multiple SNPs were detected on the same fragment, a single SNP was randomly chosen for analyses to avoid linkage disequilibrium between loci. The renamed and filtered VCF file, along with the spotted handfish strata (i.e. collection) data and original VCF file from AGRF is lodged on the CSIRO DAP: https://data.csiro.au/collections/#collection/CIcsiro:38857.

The resulting VCF file was further filtered and converted (i.e. prior to population genomic analyses) using R-Packages (R vers 3.5.1 (R Core Team 2018); R-Studio vers 1.1.463

(RStudio Team 2016)); vcfR and dartR (Jombart et al., 2010; Gruber et al., 2018, 2019; Knaus and Grünwald 2017). This consisted of a). filtering out monomorphic loci; b). using one SNP per tag; c). filtering on call rate per individual and population > 0.85; d). ensuring loci with a maf > 0.025 were used; e). using loci in Hardy-Weinberg Equilibrium (HWE).

4.4.4 Genomic variation and population connectivity analyses

As outlined below, the statistical and graphical capabilities of R (including analysis packages) were used to reformat input files, check for duplicates and undertake the post processing (following filtering) analysis of genomic data (i.e. pegas (Paradis 2010); adegenet (Jombart 2008; Jombart and Ahmed 2011), diveRsity (Keenan et al., 2013), radiator (Gosselin 2019), hierfstat (Goudet and Jombart 2015), mmod (Winter 2012), ade4 (Chessel et al., 2004; Dray and Dufour 2007; Dray et al., 2007; Bougeard and Dray 2018), strataG (Archer et al., 2016) and genepop (Raymond and Rousset 1995; Rousset 2008)). Additionally, PGDSpider vers 2.1.1.5 (Lischer and Excoffier 2012) and Arlequin vers 3.5 (Excoffier et al., 2005; Excoffier and Lischer 2010) were also used for file conversions, diversity and connectivity analyses.

The resulting filtered SNPs and population genotype data set was used to calculate population genomic diversity estimates (including allelic richness, percentage of polymorphic loci, mean observed heterozygosity and mean expected heterozygosity (H_O and H_E respectively)) in diveRsity, hierfstat and Arlequin. An estimate of inbreeding across the collections (F_{IS}) at all loci was undertaken in genepop.

Genomic assessments were also undertaken to determine if the collections were structured/differentiated into clusters or groups of closely related individuals. An assessment of genetic diversity/differentiation (G''_{ST}, Hedrick 2005; Meirmans and Hedrick 2011) across the collections was undertaken (where G''_{ST} ranges from 0 to 1 where 0 indicates no differentiation and 1 indicates collections are segregating for differing alleles; G''_{ST} is corrected for the average within collection heterozygosity and the number of collections). Pair-wise collection differentiation estimates (based on F_{ST} (Wright (1949) and based on the Weir and Cockerham (1984) implementation)) were undertaken. F_{ST} values ranged from 0 to 1, with high F_{ST} values implying considerable differentiation among collections. Significance for all tests was assessed following 10 000 permutations and *P*-values for each pair-wise comparison were corrected for multiple comparisons with a sequential procedure (Rice 1989).

An Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992), based on genotypes and matrix of pairwise squared Euclidean distances was undertaken in Arlequin to assess hierarchical structure of the *B. hirsutus* collections. This method detects population differentiation based on covariance components corresponding to different hierarchical levels. The variance components are used to calculate a series of measures called Φ statistics which describe the difference between the mean heterozygosity among the subdivisions in a population and the potential frequency of heterozygotes if members of populations mixed freely (Hartl and Clark 1997). These Φ statistics are analogous to *F* – statistics, being a measure of the correlation between genes drawn at different hierarchical levels in collections (Wright 1949). In panmictic collections most of the genetic variance is expected to arise from individuals within collections; therefore, structure is assumed if most of the variance occurs within individuals within collections or among collections.

Additionally, number of genetic groups in the *B. hirsutus* SNP data set was estimated using Bayesian clustering algorithms implemented in STRUCTURE v2.3.4 (Pritchard et al. 2000) run using admixture models with correlated allele frequencies with K (the number of clusters) set between 2-10 and 10 iterations per K value were undertaken. The optimum K was determined by ΔK using Structure Harvester v0.6.9.54 (Evanno et al. 2005; Earl and Vanholdt 2012).

The above model-based methods of assignment are based on multi-locus genotypes and the expected probability of genotypes occurring in various collections. These models assume that collections are at HWE and linkage equilibrium. However, these assumptions may be invalidated particularly in small sampling collections such as those used for the spotted handfish analyses. Therefore, non-model-based methods, such as the spatial analysis of principal components (PCA), and Discriminant Analysis of Principal Components (DAPC) were used with the *B. hirsutus* genotype data to assess the number of genetic groups.

The PCA multivariate method focuses on the entire genetic variation in the collections and identifies spatial genetic patterns. This was implemented in adegenet (with missing data inputted using 'mean allele frequencies', no *a priori* groups assumed, and Euclidean distances used). Additionally, as a robust alternate to Bayesian clustering methods like STRUCTURE (Pritchard et al. 2000), DAPC in adegenet was also undertaken. DAPC is also a multivariate, non-model sequential method that identifies clusters of genetic variation maximised between clusters of individuals and minimised within clusters (Jombart et al., 2010; Pritchard et al., 2000; Grünwald & Goss, 2011). DAPC provided determination of genetic clusters using synthetic variables (i.e. discriminant functions) and derived probabilities of membership (i.e. the genetic proximity of individuals to the different clusters) into different groups. Data was first transformed using PCA, with clusters identified with discriminant analysis (without making assumptions of panmixia). As outlined by Jombart et al. (2010), one third of the principle components were retained in the current DAPC analysis so that discriminant functions were not overfitted.

4.5 Results

Due to the endangered nature of the species, and as the sampling of spotted handfish was opportunistic from 1998, the sample numbers for each 'collection' did not reach a preferred sampling size of N = 30 individuals per collection. All sampled individuals were DNA extracted; several fish were re-identified as non-spotted handfish and these individuals were removed from the study. Due to limited funds for the SNP analyses, a smaller subset (N = 193) of the total DNA extracted individuals (N = 262) were genotyped.

Resulting sequence data from the Illumina NextSeq was of a high quality (with over 89% of bases above Q30 (a quality control metric)) producing 65.2 GB of data and 431 852 810 reads (average 2 219 351 reads per individual). The number of RAD-tags per individual in the catalogue ranged from 30 to 61 925 with an average of 34 869 and average coverage (tag depth) per individual ranging from 2.6 to 92.1 (with average of 45). Following analysis in Stacks (as per parameters outlined above), 120 763 sites were determined (of these 118 076 sites were polymorphic, 2 687 sites were monomorphic).

Further filtering (for the most informative SNPs across individuals and filtering out individuals with low numbers of RAD-tags and depth) was undertaken in Galaxy and R, resulting in a final

dataset of 4 172 SNPs in 162 Individuals from the 13 spotted handfish collections (see Figure 14).

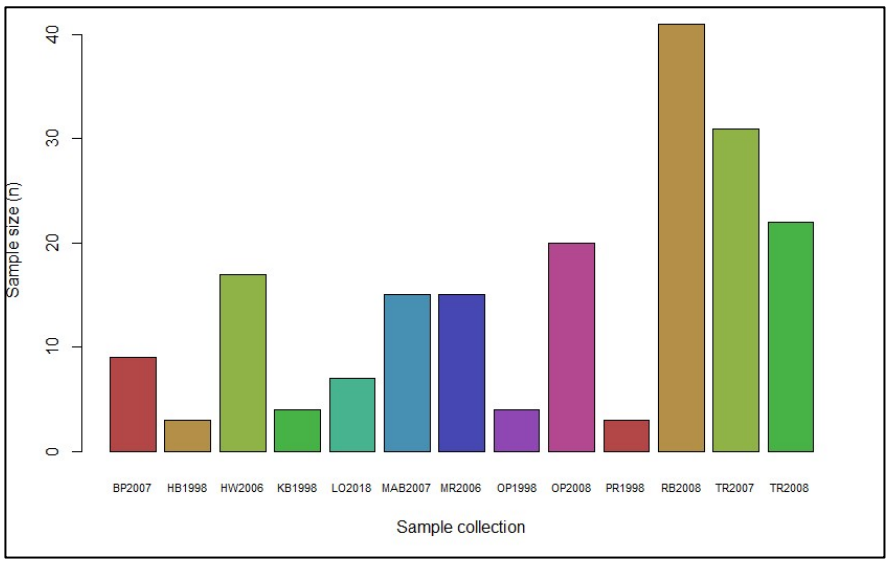

Figure 13 *B. hirsutus* samples analysed for SNPs following initial filtering steps

There were several collections where the number of individuals post filtering were less than five (i.e. N < 5 in Table 6). It is difficult (and often non-sensical) to compare population diversity estimates and pair-wise comparisons when samples sizes are small. Additionally, the much lower number of individuals in these collections resulted in lower number of alleles and genetic diversity estimates such as H_O. Additionally, one of the collections (PR1998) is presumed lost, therefore this, and collections with less than five individuals, were not considered further in the genomics analyses. These collections were:

Table 6 Summary of sample size (N) and the number of alleles observed per population across the samples (A), in spotted handfish (*B. hirsutus*) collections removed from further downstream analyses

Collection	N	A	Ar
HB1998	2	5895	1.17
KB1998	1	5059	1.21
BPM2018	2	5870	1.18
OB1998	2	5735	1.13
PR1998	2	5241	1.07

N = number of individuals per collection genotyped per locus;
A = total number of alleles observed per collection; Ar = allelic richness per locus

4.5.1 Genetic diversity in *B. hirsutus* collections from River Derwent

Genetic diversity, measured as the percentage of polymorphic SNP loci and heterozygosity, varied across the remaining eight collections. As Table 7 outlines, the percentage of polymorphic loci ranged from 71% in BP2007 and HW2006 to > 90% in OP2008, RB2008 and TR2007. The number of individuals that were genotyped per collection also varied with the largest collection in this study being that from Ralphs Bay, sampled in 2008. It should be noted here, that there are no current (i.e. 2018 or 2019) samples or collections in the genomic connectivity analyses, and only two instances of temporal collection sampling (TR2007 and TR2008).

Table 7 Summary of genomic diversity (averages given here) based on 4 172 SNPs screened in spotted handfish (*B. hirsutus*) collections from the River Derwent

Collection	N	A	%poly loci	Ar	H_O	H_E
HW2006	11	7165	71.7	1.14	0.251	0.290
MR2006	14	7271	74.3	1.16	0.272	0.286
BP2007	8	7145	71.2	1.16	0.282	0.310
MAB2007	15	7848	88.1	1.19	0.253	0.275
TR2007	28	7945	90.4	1.17	0.232	0.250
OP2008	19	7965	90.9	1.20	0.248	0.263
RB2008	40	7974	91.1	1.17	0.221	0.235
TR2008	18	7738	85.5	1.17	0.241	0.261

N = number of individuals per collection genotyped per locus; A = total number of alleles observed per collection, where SNP alleles = 8 344; % = percentage of polymorphic loci; Ar = allelic richness per locus; H_O = observed heterozygosity per locus; H_E = expected heterozygosity per locus

The average gene diversity (based on the number and the abundance/evenness of alleles) within the collections was H_S = 0.224, with mean observed heterozygosity in the spotted handfish collections being 0.250. Observed and expected heterozygosity were similar among the collections, with observed heterozygosity in each of the collections varying slightly; with H_O (in the largest collection, RB2008) the smallest in all collections. The overall global F_{IS} estimate (as per Weir and Cockerham 1984) was 0.073, which as a reflection of the proportion of variance in the sub-collections contained in individuals was not high, and therefore inbreeding in the collections/sites was not considered problematic.

Analysis revealed genetic differentiation across all loci and *B. hirsutus* collections. The global differentiation estimate was G''_{ST} = 0.092. Genetic distance (based on overall F_{ST}) across the eight collections was also significant at 0.043 (P = 0.001). On further inspection, pair-wise F_{ST} values between collections (Table 8) also demonstrated significant genetic differentiation between most collections aside from:

- BP2007 & HW2006 (F_{ST} = 0.024, P > 0.002);
- the spatially close OP2008 and MAB2007 (F_{ST} = 0.004, P > 0.002);
- and the two temporal samplings at Tranmere (F_{ST} = 0.0004, P > 0.002)

While the F_{ST} values in Table 8 are small and relative to each other, the F_{ST} values are well informed, being based on over 4 000 SNP loci. For example, the low and non-significant F_{ST} value between OP2008 and MAB2007 (which are two spatially and temporally independent collections) was a magnitude larger than that observed between the two temporal collections at Tranmere (cf. 0.004 and 0.0004). The largest pair-wise F_{ST} value was observed between MR2006 and RB2008 (F_{ST} = 0.070, P < 0.002). Wright (1978) suggested an F_{ST} of 0.0 – 0.05 indicates little genetic variation, while a value between 0.05 – 0.15 (i.e. 54% of the *B. hirsutus* F_{ST} comparisons) indicates moderate genetic differentiation. The differentiation detected between individuals at nearby Manning Reef (Sandy Bay) and Battery Point was therefore just moderate (F_{ST} = 0.052) while estimates between spatially close, Tranmere and Ralphs Bay collections (TR2007, TR2008 & RB2008) were significant but small (cf 0.040 and 0.039).

Table 8 *B. hirsutus* pair-wise SNP genetic differentiation (F_{ST}*) comparisons among collections where N > 5. Significant (based on 10 000 permutations) F_{ST} values are shown in bold (significance was calculated following sequential Bonferroni correction).

Collections	HW2006	MR2006	BP2007	MAB2007	TR2007	OB2008	RB2008	TR2008
HW2006	*****							
MR2006	**0.056**	*****						
BP2007	0.024	**0.052**	*****					
MAB2007	**0.051**	**0.056**	**0.047**	*****				
TR2007	**0.053**	**0.060**	**0.052**	**0.039**	*****			
OB2008	**0.044**	**0.050**	**0.043**	0.004	**0.036**	*****		
RB2008	**0.059**	**0.070**	**0.058**	**0.053**	**0.039**	**0.045**	*****	
TR2008	**0.057**	**0.062**	**0.053**	**0.042**	0.0004	**0.037**	**0.040**	*****

**F*-statistics or genetic fixation indices describe the expected level of heterozygosity in a population. Hartl and Clark (1997) stated F_{ST} <0.050 represented little genetic differentiation; 0.050-0.150 = moderate genetic differentiation and an F_{ST} = 1 indicates different species. F_{ST} values which are bolded represent significant differences in SNP allele frequencies between collections

AMOVA (which considers overall variance among all individuals and collections) indicated a similar significant differentiation based on Euclidean distances and frequency covariances (see Φ_{ST} = 0.045, across all collections and sampling periods). Hierarchical AMOVA demonstrated significant differentiation (i.e. structure) existed amongst the various spotted handfish collections (both spatially and temporally). While the spatial effect is credible based on the sampling, the temporal effect is most probably an artefact of the statistical analysis, with years being confounded by site and not replicated with the exception of TR. The lack of any difference between TR2007 and TR2008 supports this relegation of the temporal difference as an artefact (Table 9).

Table 9 *B. hirsutus* SNP hierarchical AMOVA testing, with significance tested following 10 000 permutations.

Tested groupings	Φ-statistics*		
B. hirsutus (testing panmixia)	Φ_{ST} = 0.045, *P* = 0.000		
B. hirsutus (temporal - 2006 v 2007 v 2008)	Φ_{ST} = 0.045, *P* = 0.000	Φ_{SC} = 0.044, *P* = 0.000	Φ_{CT} = 0.000, *P* = 0.411
B. hirsutus (spatial - MR2006 & BP2007 v HW2006 v RB2008, TR2007, TR2008 v MAB2007 & OP2008)	Φ_{ST} = 0.051, *P* = 0.000	Φ_{SC} = 0.028, *P* = 0.000	Φ_{CT} = 0.023, *P* = 0.010

*Φ_{ST} = the variance among sub-collections relative to the total variance; Φ_{SC} = the variance among sub-collections within groups; Φ_{CT} = the variance among groups relative to the total variance

While K = 2 was chosen by the Evanno method as the most likely number of *B. hirsutus* genetic clusters in the River Derwent (see Figure 15), additional sub-structuring was detected by the STRUCTURE analyses as the K=3 plot in Figure 16 highlights.

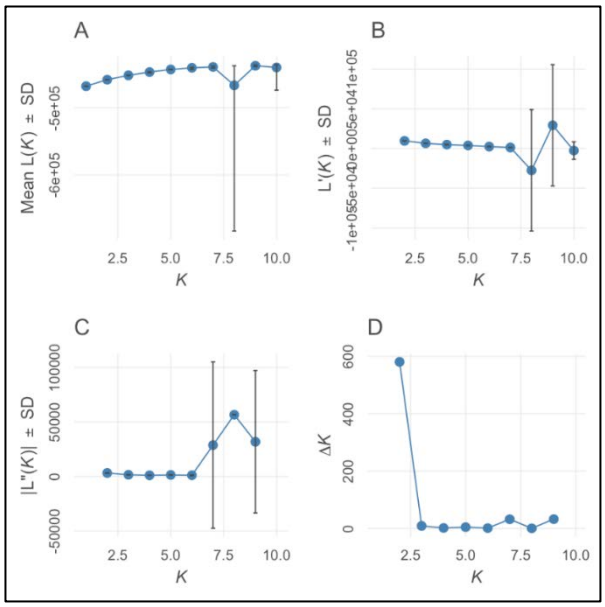

Figure 14 Evanno output from Structure Harvester, analyses based on SNPs in *B. hirsutus* collections. Delta K is shown in panel D.

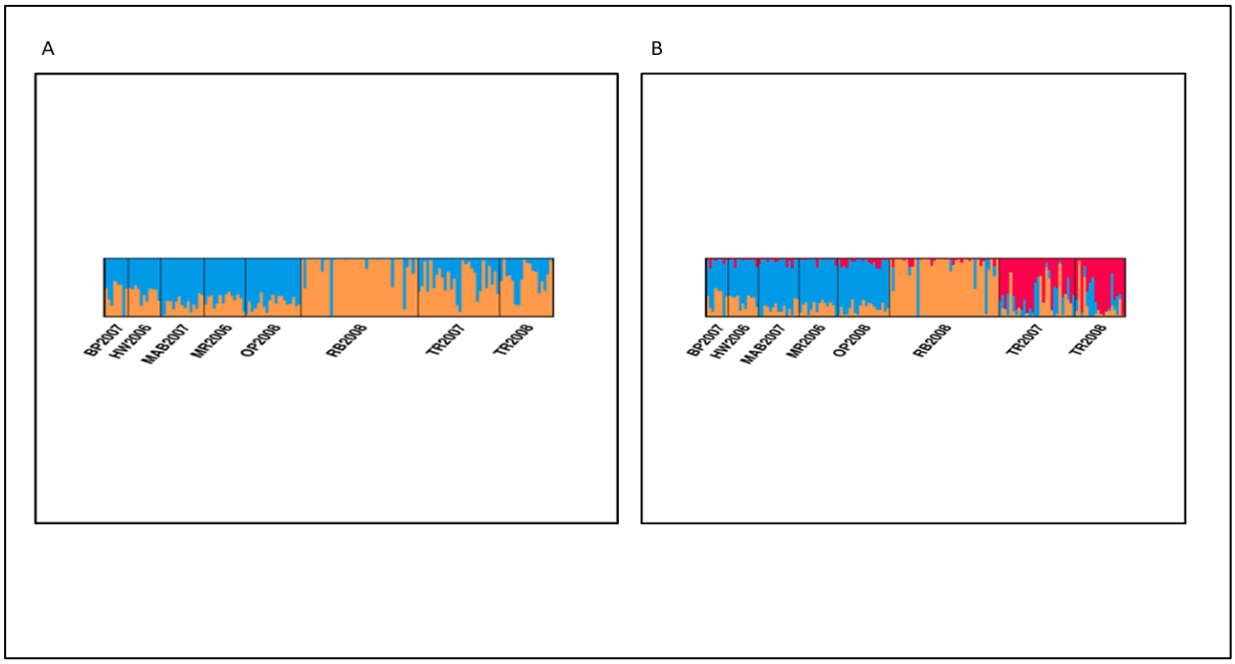

Figure 15 Population clustering for *B. hirsutus* based on STRUCTURE outputs for SNPs. Colours represent different clusters as defined by K values, populations are as labelled. A) K = 2; B) K = 3.

The PCA revealed clustering across the eight collections – Figure 6 below shows the SNP diversity represented in two complimentary ways with individuals that were more genetically different being placed further apart in space, and different colours representing genetic differences. Individuals were separated (see Figure 17), with 3.61% of the variation explained by PC1 in a large cluster at the top of the plot, while PC2 and PC3 explained 2.74% and 2.68% of the variation respectively. Individuals that were sampled from Tranmere in 2007 and 2008 are clustered together in the second half of the plot.

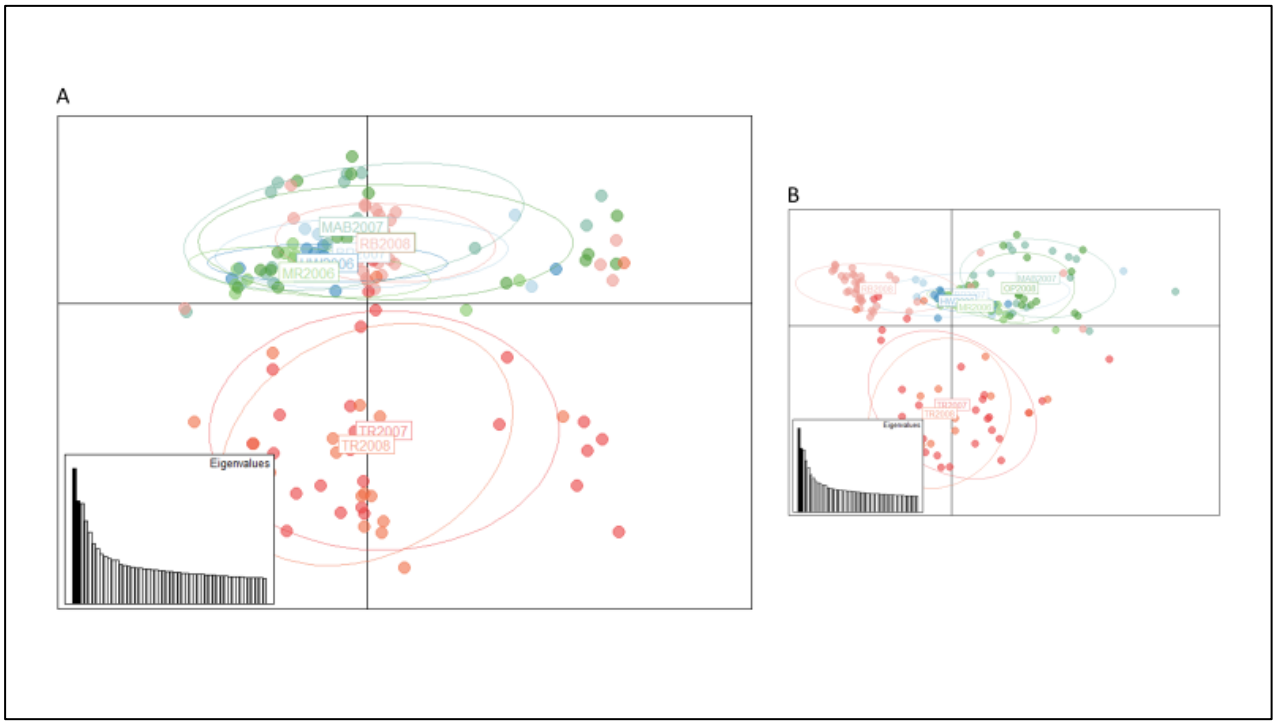

Figure 16 Principal component analysis of SNP frequencies. A) separation on the PC1 axis; B) separation on the PC2 axis. Each dot represents one sample and is coloured according to the sampling collection.

The non-model DAPC highlighted a defined pattern of genetic structuring across the sampling sites (Figure 18) with separation observed across three main groupings – samples from HW2006, MR2006 and BP2007 formed one group of genetic proximal individuals; a second strong/overlapping group of individuals from TR2007, TR2008 and RB2008 and a third proximal grouping of individuals (again overlapping) from MAB2007 and OP2008 in the lower half of the plot. The Battery Point individuals, while part of the proximal genetic grouping in the top left of the plot, were more separated in space from the Manning Reef (Sandy Bay) individuals – likely reflecting the small but significant F_{ST} value between these two collections. The spatial placement of the *B. hirsutus* groups in the DAPC somewhat mimics that of the locations in the River Derwent, with the Battery Point, Sandy Bay and Howrah Beach locations being northerly to those of Tranmere, Ralphs Bay and the more southerly locations of Mary-Ann Bay and Opossum Bay. The strength of the separation among the clusters is reflected in the pair-wise F_{ST} values.

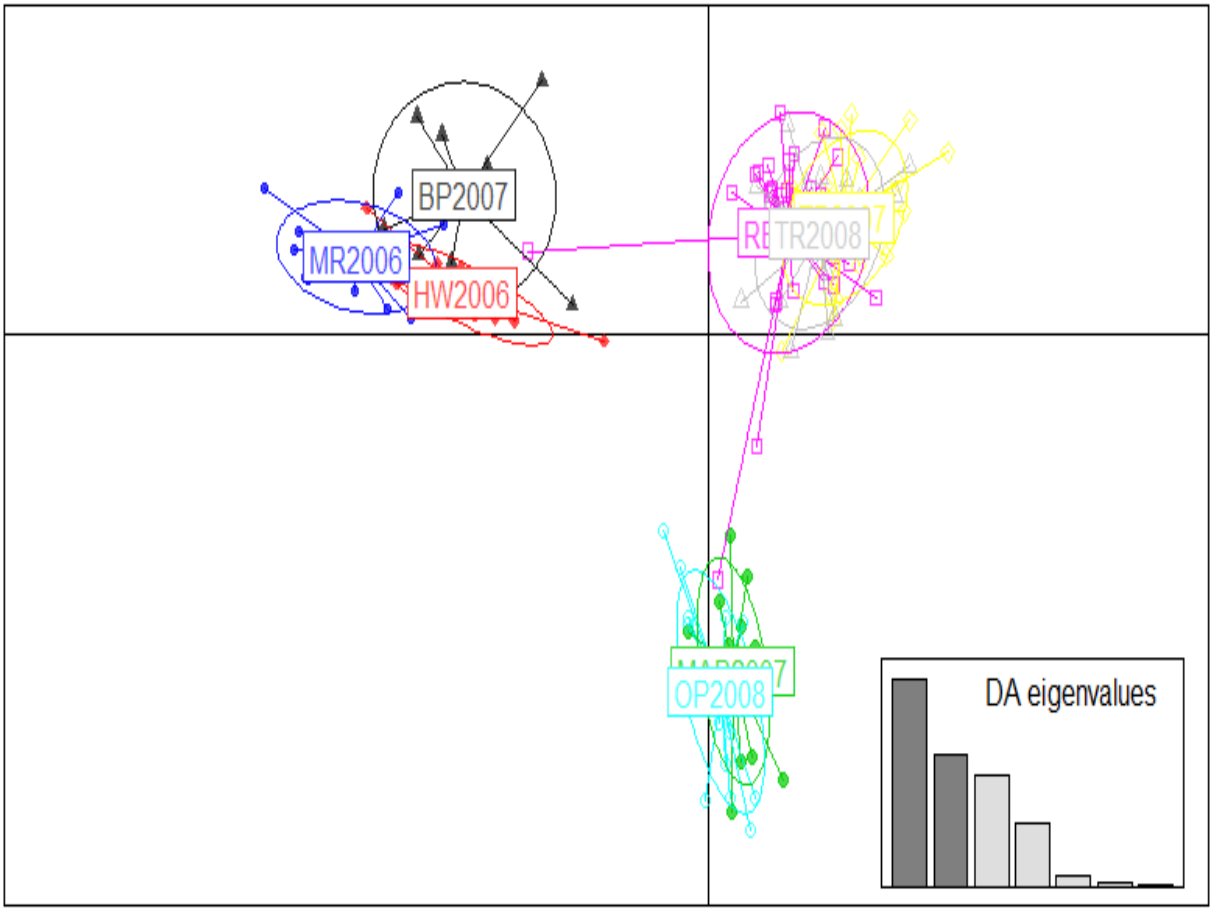

Figure 17 Discriminant analysis of principal components (DAPC) with priori grouping corresponding to the sample collections of *B. hirsutus*. Scatter plot of DAPC based on 4 172 nuclear SNPs. While labels are partly obscured in the figure, the three clusters are also geographically clustered. In the top left are collection taken from the upper estuary (around Sandy Bay/Manning Reef, Howrah and Battery Point), the top right are collections from the mid estuary, (Ralphs Bay and Tranmere) and in the bottom left are collections from the local population in the lower estuary (Opossum Bay and Mary-Anne Bay).

In comparison, Appendix B shows the DAPC of all *B. hirsutus* genotyped individuals (from 1998 - 2018). In this DAPC, KB1998 clusters with the Howrah Beach and Beauty Point individuals with the Manning Reef (Sandy Bay) individuals slightly to the left of the overlap; PR1998 and HMB1998 clusters with the Opossum Bay and Mary-Ann Bay collections and the BPM2018 (shown as LO2018 – 'local') individuals did not cluster with any particular group but were most genetically similar to individuals in the southern locations and the upper Derwent collections.

4.6 Discussion

This is the first population genomics study on any handfish species. Here, the genetic diversity (in 13 sampling collections) and connectivity/structure (across 8 sampling collections) of *B. hirsutus* in the River Derwent (up to the late 2000's) was examined with over 4 100 SNPs verifying the existence of biologically meaningful conservation 'units'. Additionally, the study proved that small, non-destructively sampled fin clips provided suitable high-quality genomic DNA for SNP genotyping in hand. The genomics results do not include any microsatellite loci genotyping; however, if funds were made available, the *in-silico B. hirsutus* microsatellite

library could be used in future studies to develop loci suitable for parentage and sibship analyses for captive breeding programs for spotted handfish. Additionally, it is likely that any primers developed from the *in-silico* microsatellite library may amplify in other closely related handfish species, thereby enabling cross species amplification and genotyping without the need for development of additional handfish microsatellite loci.

Sample sizes in five collections were small (i.e. N < 5 individuals); these collections were not considered in the connectivity analyses. Subsequently, seven spatial and two temporal samplings (with the most recent samplings from 2008) indicated significant *B. hirsutus* structuring within the river.

Genetic diversity and structure across the genome wide SNPs suggest *B. hirsutus* individuals from spatially differentiated locations in the River Derwent should not be considered panmictic (i.e. mating is not random across all locations; all *B. hirsutus* individuals are not potential partners, as mating depends on spatial location and distance between). The genetic variation among locations and spatial and temporal groups was larger than variation observed within individuals of locations. This is indictive of restricted connectivity across the range of the species (Ovenden 2013), driven likely by limited spatial dispersion. Restricted movement and contribution of spotted handfish individuals among locations is suggested, with reproduction and replenishment of locations reliant mostly on recruitment within locations.

Genomic analysis detected relative structure in *B. hirsutus* through the frequency of SNP alleles differing among individuals in different locations in the River Derwent. If fish were moving and interbreeding between the spatial locations, collections would have the same genes at the same frequencies (Ovenden 2013). The SNP data and principle co-ordinate spatial analyses suggests genetically differentiated *B. hirsutus* collections/groups were present in the River Derwent, at least until 2008. The reduced gene flow, represented by genetic heterogeneity, implied that individuals in the north and south of the river did not undertake longer distance migrations followed by reproduction, or that eggs are not dispersed widely throughout the estuary.

The genetic results give strong support to the 'hotspot' location concept for spotted handfish in the River Derwent. While *B. hirsutus* individuals may exist between the sites throughout the river (albeit in much lower densities) (Bessell et al., 2019), the genetic results indicate the lack of genetic contribution from one hotspot to another. While all significant, the pair-wise F_{ST} values were smallest (indicating more gene flow) among individuals from spatial locations more geographically close (e.g. between Mary-Ann Bay and Tranmere; Tranmere and Ralphs Bay; Tranmere and Opossum Bay) than those among collections further apart in the river (e.g. Manning Reef/Sandy Bay and Ralphs Bay). The smallest (and non-significant) F_{ST} values were between the two Tranmere temporal samplings and between geographically close Mary-Ann Bay and Opossum Bay. The Manning Reef/Sandy Bay individuals were shown to be somewhat genetically different to other *B. hirsutus* individuals; even to those at nearby Battery Point. There is a marina located between Battery Point and Sandy Bay and this mooring infrastructure may act as a physical barrier between the two geographically close sites (T. Bessell pers.comm.). However, this is speculative and requires further testing of contemporary individuals from these locations to confirm or refute.

Contemporary (i.e. 2018/2019) collections were not available for this study, however deceased individuals from the current captive breeding program (i.e. samples from BPM2018) were genotyped but not included in collection analyses. The current activity did not have experimental design over the sampling; individuals had been fin clipped previously and the samples were archival. It is important to note that low sample sizes can impact a population genomics study, with a failure to invest in robust sampling wasting the investment in genotyping (Meirmans 2015). However, more robust or meaningful sampling of this TEPS (as outlined in Meirmans 2015) could be difficult for this rare species.

Spotted handfish are known to have restricted dispersal capacity with no planktonic larval stage; adult females lay eggs (60-250 eggs) in masses around ascidian stalks (Bruce et al., 1998) (and other similar structures), and adults move by 'walking' on their fins rather than swimming, except for short bursts to avoid predators, as they lack swim bladders (Bruce et al., 1998; Last et al., 2007). The maximum recorded distance moved by a spotted handfish to date is approximately 570 m (Bessell et al., 2019), with a single individual taking 585 days to move this distance (Bessell et al., 2019). Spotted handfish have been recorded moving within multiple sites (such as within Battery Point and Mary-Anne Bay), however no movement between sites in the River Derwent have yet been observed (Bessell et al., 2019). Relatively low densities and limited wider movements (available data suggest individuals only undertake small within site movements over long-time frames, Bessell et al., 2019) are likely due to *B. hirsutus*'s biology, ecology and microhabitat preferences (Wong et al., 2018). These species-specific aspects of *B. hirsutus* will impact breeding opportunities among locations. Although undetected migration of individuals among locations may occur (Bessell et al., 2019) thereby contributing to demographic connectivity, genetic connectivity (resulting in gene flow) relies on individuals reproducing with others in these locations.

In context, where genetic connectivity was indicated with continued gene flow, F_{ST} values that were smaller by a magnitude to *B. hirsutus* were observed in an African lake-based sprat (*Stolothrissa tanganicae*, global F_{ST} = 0.0068) (De Keyzer et al., 2019); mesopelagic *Maurolicus muelleri* from the Bay of Biscay (pair-wise F_{ST} ranging from 0.000 – 0.011 across eight collections) (Rodriguez-Expeleta et al., 2017) and in two anadromous alosine fishes (of conservation concern) along the Atlantic coast of North America (F_{ST} ranged from 0.006 to 0.140 in *Alosa pseudoharengus* and 0.004 to 0.150 in *A. aestivalis*) (Baetscher et al., 2017).

The genetic differentiation observed between discrete *B. hirsutus* collections and locations confirms that generally individuals do not move widely between locations, rather once hatchlings settle on the benthos, individuals stay in these areas (Bruce et al., 1998). Habitat degradation, risk of predator interactions and ongoing urbanisation may result in *B. hirsutus* relying on specific areas and habitats (i.e. restricted demographic connectivity) and hence results in reduced genetic connectivity.

In 2015, Lynch et al. documented *B. hirsutus* populations in the Derwent Estuary as 1.58 to 43.0 fishes per hectare (seven years post the most recent tissue collection in this study). It is not known if the populations in 2015 or currently (in 2019) would show the same levels of genetic diversity and reduced connectivity as documented from samples collected in 2008. However, the major decline in *B. hirsutus* population numbers occurred in the 1980's and 1990's (pre this current study). With a generation time for *B. hirsutus* estimated at 8-10 years (Bessell et al., 2019), and as significant conservation efforts for spotted handfish have been

undertaken in the last 15 years, it is likely that contemporary genomic variation would be similar to that observed in this study.

The continued differentiation of individuals at these locations in 2019 cannot be verified as there were no collections undertaken in this time frame; albeit surveys in recent years have documented similar *B. hirsutus* densities (see Wong et al., 2018). It seems reasonable to suggest that the location differentiation presumably persists. Wong et al. (2018) examined the benthic microhabitat features in the River Derwent, identifying 13 different features ranging from unconsolidated sand flats to benthic vegetation cover. Known *B. hirsutus* sites in the River Derwent (of which seven sites provided samples for the genetics study) have different microhabitat features (Wong et al., 2018). Battery Point, Half Moon Bay, Mary-Ann Bay and Tranmere are dominated by sandy flats while Howrah Beach and Opossum Bay are dominated by unconsolidated sand ripple, Manning Reef (Sandy Bay) is characterised by sand flats with empty depressions, and Ralphs Bay is dominated by vegetation (Wong et al., 2018). While the observed genetic connectivity was not tested alongside microhabitat type, the distances among these habitats, lack of contemporary continuous connecting habitat in the river and local preferred complex habitats rather than simple open sand flats (Wong et al., 2018) are likely to impact both demographic and genetic connectivity in the species.

These are important considerations when broodstock are sourced for the current captive breeding program and when captively bred juveniles are released back into the River Derwent. In 2008, individuals from the southerly most collections were genetically distinct from those in the northern reaches of the River, while the genetic analysis of individuals at the two temporal samplings at Tranmere (in 2007 and 2008) indicated the genetic homogeneity between the two years.

The results presented here were based on a large set of SNPs. *B. hirsutus* individuals showed a reasonable level of genetic diversity with no strong indications of troublesome inbreeding. The genomic data does not indicate a complete lack of connectivity among spatially differentiated individuals in the river (this would be indicated by an F_{ST} of 1.000); rather gene flow is restricted/reduced among some locations, thereby resulting in closer genetic proximity of individuals that are more geographically close. These findings represent a significant development for this threatened, endangered and protected aquatic species. Integrated data analyses on spotted handfish, including these genomic findings, suggest that spatial locations should continue to be managed or considered as separate conservation units, even within the River Derwent.

4.7 Acknowledgements

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5. A PATHWAY TO DEPLOYMENT OF ENVIRONMENTALLY SENSITIVE (ES) MOORING INTO EXISTING FIELDS VIA MODELS OF WORKFLOW, GOVERNANCE, ENGINEERING AND SPATIAL DISTRIBUTIONS

5.1 Summary

Traditional block and chain swing moorings are highly destructive to the seafloor through removal of sediments by the ongoing mechanical damage from both their heavy ground and lighter thrash chains. Environmentally Sensitive (ES) moorings provide a technological solution to this problem and a number of designs have been available for many years. Up-take of ES moorings, however, has been limited, potentially due to concerns about their engineering integrity, cost, ease of deployment and servicing and their potential to cause collisions in mix fields due to differing behaviours between mooring designs in response to wind and currents. We describe a pathway leading to deployment of ES moorings to secure a range of vessels owned by private individuals. We were guided by a multi-disciplinary working group which allowed for ongoing consultation between the local maritime authority, the mooring contractor, the environmental arm of government, engineers and ecologists. We also recruited, liaised directly with and contracted private vessel owners to participate in the study. For each vessel, engineering models were developed to test loads in extreme and mild conditions for a variety of ES moorings as well as for the traditional chain moorings design. A spatial model was also developed of a mooring field to identify those vessels where a switch to ES moorings would not result in any unforeseen negative interactions with neighbouring vessels and we used this model to seek approval from the maritime authority for replacement of chain moorings with our ES moorings. Our model documented the possible shock loading experienced by traditional chain mooring once it reached a semi-taut configuration in extreme condition, where it can negatively affect the performance of the mooring. The chains low elasticity when fully elongated results in cyclical shock loading across the mooring, which exceeded the safe working load (SWL). In contrast, certain ES mooring designs perform better in the models than chain moorings in extreme conditions, with the peak loads being 39% to 57% lower than their chain mooring counterpart. Our spatial model suggests that a sequential approach to change over would be required to avoid any negative interactions between mooring types. Our approach should be widely transferable to allow for conversion of chain moorings to ES moorings in sheltered and shallow coastal ecosystems. In addition to environmental benefits, ES moorings may also provide a safer solution for securing vessels exposed to extreme conditions, which may locally increase in frequency and intensity as a result of climate change.

5.2 Introduction

Globally, the coastal zone supports a large proportion of the human population, with a predicted trend of increased habitation (Cohen and Small 1998; Neumann et al. 2015; Small and Nicholls 2003). This disproportional distribution of human settlement along the coast is probably due to the abundance and easy access to resources that supporting cultural, recreational and economic values; this makes the coasts one of the most utilised but also and

one of the most degraded ecosystem worldwide (Airoldi and Beck 2007; Dugan et al. 2011; Little et al. 2017; Lotze et al. 2006). Specific major threats to the coastal zone include: climate change, physical alteration and degradation of habitat, over-exploitation of resources, pollution/contamination, modification of the hydrological cycle and the introduction of invasive species (Halpern et al. 2007; Kennish 2002; Little et al. 2017).

Growing urbanisation and alteration of waterways through infrastructure development and modifications pose a significant threat to the health of the coastal zone (Bishop et al. 2017; Bulleri and Chapman 2010; Dafforn et al. 2015; Dugan et al. 2011; Kennish 2002; Kienker et al. 2018; Marzinelli et al. 2012). Structures such as seawalls and marinas can form physical barriers (Bishop et al. 2017) which can alter the hydrodynamics (Floerl and Inglis 2003). Connectivity from boats facilitate the establishment of invasive species (Airoldi et al. 2015; Glasby et al. 2007) and their moorings and propeller wash destroy sensitive shallow benthic ecosystems (Sagerman et al. 2019).

One area of growth in the use of coastal resources is recreational boating (Burgin and Hardiman 2011; NMMA 2012). With this growth comes an increased demand for infrastructure such as moorings. Standard block and chain swing moorings (hereafter called chain mooring) commonly consist of an anchor followed by a length of heavy and then lighter thrash chain. The anchor's holding power is a combination of both its weight and the suction force created by being buried in the sediment, which can be up to four times its weight (Bradney, 1987). The heavy chain acts to damp the forces from wind and wave action to secure the vessel.

While chain moorings are commonly used worldwide, they have a range of undesirable impacts. Most obviously, these include destruction of the biodiversity of the impacted benthic communities (Herbert et al. 2009; Serrano et al. 2016), which include plant communities such as seagrass meadows (Collins et al. 2010; Glasby and West 2018; Unsworth et al. 2017). These habitats have been identified as nursey grounds for species important for both commercial and recreational fisheries (Nordlund et al. 2018). Shallow coastal ecosystems, and in particular seagrasses, also have a high potential to be productive carbon sinks (Howard et al. 2017) but this function is compromised by chain moorings, which not only remove the biology but also continuously remove the sediments. Chain moorings have also been identified as a key threating process for various endangered species (Demers et al. 2013; Lynch et al. 2015a; Short et al. 2011). For example, within the Derwent Estuary in Tasmania, mooring impacts are an identified threat to the conservation of the critically endangered spotted handfish (*Brachionichthys hirsutus*) (DoE 2015). The large existing mooring arrays and projected increase in boating infrastructure demand will place increased pressures on a wide variety of coastal soft sediment ecosystems and values.

The observed impacts of chain moorings have led to the development of various environmentally sensitive (ES) mooring designs (Halas 1997). ES mooring is a generic term covering any mooring design that eliminates or minimises benthic disturbance. Despite the history of ES mooring designs dating back to the 1990's (Halas 1997), their adoption remains limited and intermittent. With the exception of some feasibility trials and various grey literature (Batton and Derbyshire 2011; Bowman 2008; Demers et al. 2013) there is limited documentation of large-scale deployment of ES moorings.

An important step in conserving near shore biodiversity, important habitats such as seagrass and endangered species within urbanised waterways would be the conversion of chain to ES moorings. However, due to the common legislative framework for mooring management, achieving this objective will require a wider adoption of ES mooring technology and public support. One potential cause of the low uptake of ES moorings by boat owners may be related to perceived safety concerns due to limited engineering models of designs. Interestingly, the design dynamics of chain moorings is also poorly represented in the literature; the development of their designs appears to be heuristic rather than based on engineered calculations. For such a ubiquitous piece of coastal infrastructure there are generally very limited published studies on moorings, which are mostly about ecological effects (Sagerman et al. 2019) such as on seagrass (Demers et al. 2013) and fish assemblages (Lanham et al. 2018).

In Tasmania, like many other parts of Australia and around the globe, there is a high demand for moorings, which occur as fields in many of the suitable sheltered locations around the coast. In particular, mooring fields within metropolitan regions have reached their full capacity in regards to safe anchorages, with few new mooring leases allocated (MAST 2016a). Any conversions of chain to ES mooring would likely occur across the existing leases, and this would result in a mixed field of mooring designs. It was speculated by the maritime authority that due to the removal of the chain component, the ES moorings may have fundamentally different swing and watch circle behavioural characteristics to chain moorings across varied wind conditions.

Mooring scope (i.e. the total length of the mooring setup) can affects the size of the area required for the mooring as well as the movement behaviour of the vessel in relation to the anchor throughout the moored period. In some ES mooring designs a taut configuration is used with a shorter scope, which is based on the elastic or hydraulic dampening action of the system. When paired with an embedded anchor, some ES mooring designs can have the capability to shorten the overall scope of the setup. This differs from the chain mooring's design which utilises the catenary advantage of the heavy and thrash chains to secure the vessel and dampen forces. Chain moorings however have traditionally had at least a 3:1 scope to be less sensitive to provide an effective design to secure a vessel (Bradney 1987). If the watch circles between moorings overlap (e.g. within a high-density mooring field), as the wind shifts direction, the longer scope of chain mooring could result in collisions if not enough clearance is provided with the more restricted scope of the ES-mooring. Even when the scope is similar between chain and ES mooring designs, in light wind conditions, external forces may not be sufficient to fully extend the chain towards a downwind position at the same speed as ES moorings. ES mooring strops, which replace the chain components, are generally lighter and commonly neutrally buoyant, in light wind conditions the vessel will quickly drift to the downwind position of its watch circle, extending the mooring strop to its full length. Thus, without proper planning, in mooring fields that are at or near capacity there may be an increased risk of collisions between vessels secured by differing mooring systems.

Due to these physical and engineering considerations, successful deployment of ES-moorings at larger scales will require a comprehensive design procedure. In addition, perceived safety concerns of any change from the traditional heuristic chain designs mean that modelling will also need to be conducted to convince both owners and permitting bodies that ES mooring designs will have enough holding power to safely secure the vessel.

Understanding and ameliorating the impact of chain moorings on coastal ecosystems is increasingly important for sustainable coastal development. Our aim is to demonstrate our case study methodology of ES mooring deployments that could be used as a pathway to apply more generally. As part of a larger scale project to examine the ecological effectiveness and conservation usefulness of ES moorings, a series of ES moorings were deployed in south-east Tasmania. We detail the pathway we developed, including consultation, contracting and modelling to deployment. We also provide the engineering modelling results for various ES mooring configurations and how these compare in performance and behaviour to existing chain moorings for a variety of vessels. In addition, at one of the mooring fields we construct a spatial model to investigate mix field interactions if wide scale deployment of ES moorings occurs.

5.3 Methodology:

5.3.1 Development of governance process

In 2016 investigations commenced with two taut ES mooring designs that used an Eco-Mooring bungee component and were deployed on a courtesy mooring for two local yacht clubs in Tasmania. Following consultation with the clubs we chose a gravity anchor consisted of railway wheels rather than a screw anchor to secure our moorings. This design can be more easily deployed and serviced by existing mooring contractors on barges by hauling the gear to the surface rather than by commercial dive teams, which have a much higher cost (~10:1) (MAST pers comms).

Following deployment, we contacted the local maritime safety authority – Marine and Safety Tasmania (MAST) – responsible for leasing mooring sites to boat owners and who are primarily concerned with safety. While MAST does not provide any standards for mooring design, they raised a number of concerns over the unknown engineering integrity of the ES moorings and their potential to cause collisions in mix fields due to differing behaviours between ES and swing mooring designs – particularly for ES moorings with a shorter scope. An ES Mooring Taskforce was established to allow for regular liaison between the regulator, our mooring contractor, the Tasmanian state governments body responsible for local environmental (Derwent Estuary Program), the University of Tasmania and CSIRO. We also expanded our disciplinary reach beyond our conservation and biological expertise by including CSIRO engineering in the work. The Taskforce met quarterly and has provided a steering committee for the research project.

Guided by the committee we developed several research and governance pathways that needed to be followed to ameliorate concerns (Figure 19). These included a) an engineering cycle of design and modelling to remain within safe working loads (SWL) and hence avoid breaking points for ES moorings attached to specific vessels in extreme but expected weather conditions; b) the development of an ES mooring design that was not taut and would behave in a more similar way to traditional swing moorings in mixed fields; and c) an approval role by MAST for the locations of any deployments which also allowed MAST to database the ES moorings.

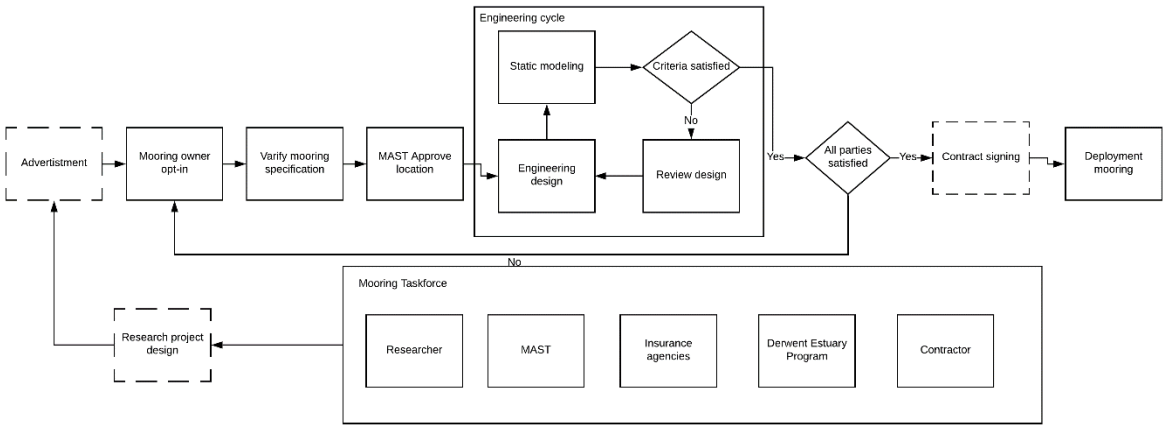


Figure 18 Governance flow diagram of the process used to deploy ES moorings.

We also wanted to move away from the common model of government or institutional owned courtesy moorings being converted to ES moorings as most chain moorings are privately-owned. This meant we needed to recruit owners and determine if their boats fulfilled all our approval and selection criteria (see following section). As this exposed CSIRO to risk if the moorings failed, aa contract was developed between CSIRO and any potential private mooring owners who wished to participate in our study (Appendix A). The contract outlined the risks, the diligence that we had undertaken in design and defined that the moorings upon deployment become the property of the mooring owner. CSIRO also agreed to pay for the gear and deployment, two years of servicing and to also store the previous moorings tackle. The owner agreed to insure the vessel on the mooring.

5.3.2 Selection criteria for field trial moorings

Over the course of a year between 2018 and 2019, private mooring owners located in South East Tasmania were engaged regarding this replacement trial from swing to ES moorings. Potential participants were contacted through various channels, including personal recommendation, yacht club newsletters, and newspaper advertisements. Owners with moorings located in two mooring fields, Sandy Bay/ Battery Point, and North-West Bay were selected. To standardise the moorings used for this study, all potential mooring locations need to fulfil the following selection criteria: 1) the mooring owner agreed to join the study (opt in), 2) the mooring was located at a depth between 5-15 m, 3) the mooring must be actively used, with the mooring being the primary storage method for the vessel, 4) the vessel moored must be insured including for 3rd party liability, 5) the vessel length must be between 8 m – 13 m, 6) the owners must sign the contract, and 7) the location of the mooring must be approved by (MAST).

Those moorings that matched all the above selection criteria were used in the engineering modelling. Technical specifications were recorded for the suitable moorings and vessels from participants and included: 1) mooring location, 2) mooring depth, 3) vessel length, 4) vessel brand/model, and 5) vessel displacement. A total of four moorings were identified as suitable for replacement in Sandy Bay and North West Bay (Table 10 Figure 20, 21).

Table 10 The vessels' model, type, length, displacement and moored depths used in the ES mooring trail.

Model	Type	Length (m)	Displacement (t)	Depth (m)
Maple Leaf 42	Fin with rudder on skeg	12.8	10.9	8
Clansman 30	Fin keel	9.1	4.1	6
Roberts 36	Fin keel	11.2	8.2	8
Adams 12	Centreboard keel	13.1	7.3	6

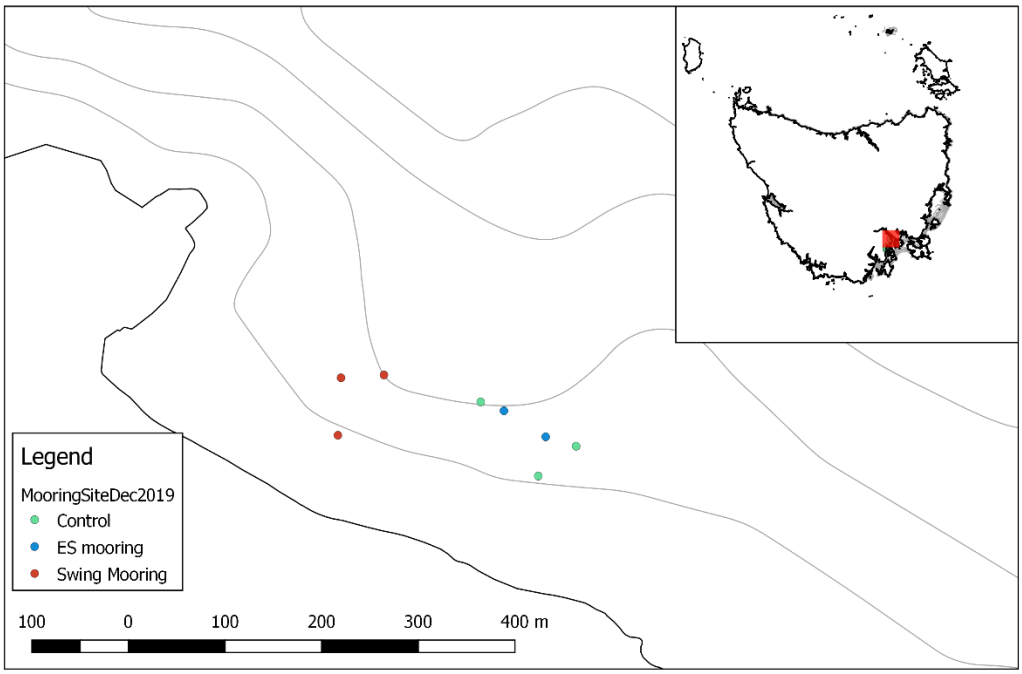


Figure 19 The Sandy Bay / Battery Point study sites.

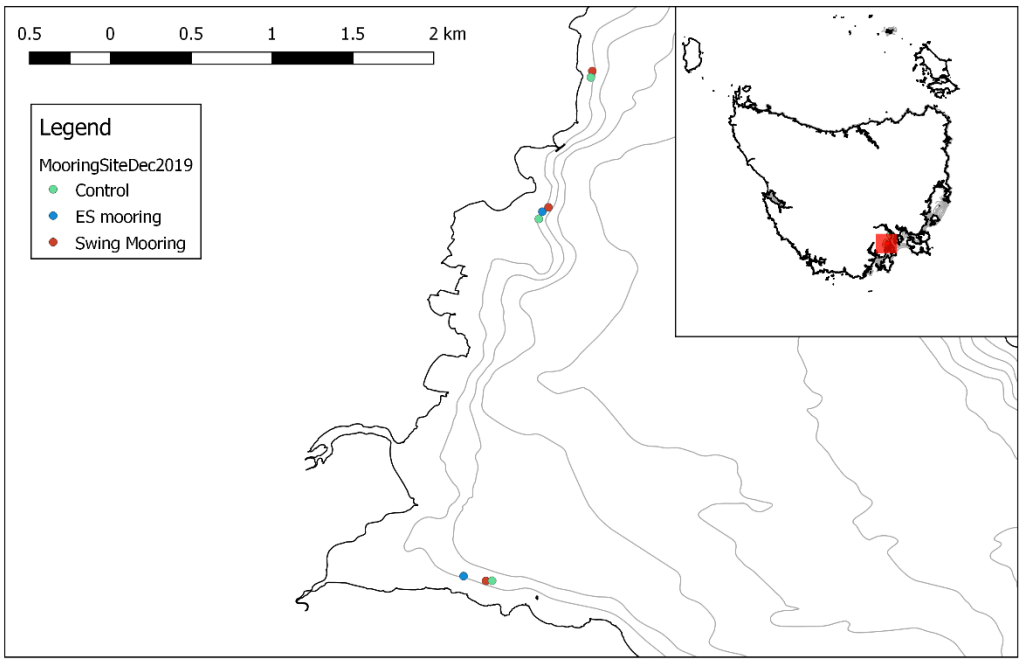


Figure 20 The North West Bay study sites.

5.3.3 Mooring model engineering parameters

We liaised with local mooring contractors and mooring component manufacturers to identify potential designs common within the region and their engineering constraints. Most chain mooring designs utilise a gravity anchor. In Tasmania, this is often a bundle of used cast iron railway wheels connected by 36mm chain, though various other heavy objects are also used (e.g. concrete block, casted iron gear). The total length of the mooring hardware is usually set at three times the water depth (*d*) of the moored vessel. The mooring hardware running from the anchor then consists of 1/3 heavy chain (commonly Ø24mm), followed by 1/3 of lighter thrash chain (commonly Ø 16mm) and 1/3 polypropylene (PPE) rope.

To avoid collision issues due to differing scopes between mooring designs identified by MAST, we matched the common three times water depth (*d*) scope for our ES mooring designs to the traditional chain moorings. In addition, the ES moorings need to be either economically advantageous or similar in cost to the existing chain moorings not only for capital costs but also for deployment and servicing costs. This ruled out some ES mooring solutions that require a screw anchor, which needs to be installed by a commercial dive team and is relatively expensive compared to traditional moorings with clump weight anchors which are serviced without the need for divers.

Safety concerns also meant we changed the design away from using a high energy/ bungee component due to the potential for high energy fly back in catastrophic failure events when either deploying the mooring from the barge or retrieving it for servicing. Based on the design criteria, we selected a synthetic core, vulcanised rubber sleeve strop (Black Snake) from Sealite (https://www.sealite.com/) as the key component to replace the chain in our initial ES mooring modelling assessment.

The Black Snake strop utilises a nylon 6-6 core wrapped in a vulcanised rubber outer sleeve. The strop is sold as neutrally buoyant with integrated eyelets and has the capability to be used with clump weight anchors. The strop has a restricted elongation rate, with the material stretching up to 20% when under load. Due to the restricted elongation, the strop can be handled similarly to traditional chain moorings during hauling and lifting operations for deployment and servicing. There are multiple weight classes of strops commercially marketed, of which two weight classes, 8 t and 12 t were selected for analysis. The 8 t strop is lower rated in SWL than the 'traditional' components but it was assessed in our model as it reduces the overall load on the mooring line.

We collected manufacturer specified engineering specifications for all components within a mooring setup to construct our models (Table 11). This included the component's maximum breaking stress (MBS) and safe working load (SWL). The chain that we used has a SWL but the manufacturer does not publish its MBS, and only says not to load it above the SWL value. Conversely, the synthetic components of our study only have MBS values. A design factor is required to specify a SWL that these components should not exceed. A common safety factor of 4 was used on all synthetic components. This value was also recommended by Sealite for the Black Snake strops.

The elasticity or elastic axial stiffness (EA) of each component in each mooring setup was also included for the model. This combines Young's Modulus (E), a value that quantifies the

stress/strain relationship of the material in the elastic region, with the cross-sectional area of the material (A). This gives a relationship for how the specific component will behave whilst being stretched in the elastic region (i.e. before it starts to deform) in Newtons (N) of force. This is the required force to stretch the component to double its length. The chain takes 239,000kN (kilonewtons) of force to stretch, whilst the 24mm nylon rope only requires 179kN, hence the chain is ~ 1334 times stiffer than nylon rope. For the synthetic components, where the EA value is more sensitive and has a higher impact on the behaviour of the mooring, a test program was conducted to calculate all EA values.

Table 11 Specifications of mooring components used in the engineering models. Size, Maximum Breaking Strain (MBS), Safe working Limit (SWL) and Elastic Axial Stiffness (EA).

Component	Size	MBS (kg)	SWL (kg)	EA (N)
PWB Proof Coil Chain	Ø24mm	N/A	5,340	239,000,000
PWB Proof Coil Chain	Ø 16mm	N/A	2,310	228,600,000
Duraline PPE Rope	Ø 24mm	9,950	2,488	494,000
Black Snake Strop 12t		12,000	3,000	440,000
Black Snake Strop 8t		8,000	2,000	293,333
TMD Nylon Rope	Ø 24mm	12,000	3,000	179,000

5.3.4 Mooring environmental parameters

All mooring designs were modelled under two environmental conditions, categorised into extreme but expected or mild for the study sites based on the *Guidelines for design of marinas* (AS 3963-2001) and the *Structural design actions: Wind actions* (AS/NZS 1170.2-2011) (Table 12). Both sites had similar environmental characteristics.

Table 12 Environmental condition parameters for the study sites.

	Extreme case	Mild case
Wind speed	30.4 ms^{-1}/109.4 kmh^{-1}	12.0 ms^{-1}/43.2 kmh^{-1}
Current	1.0 ms^{-1}/3.6 kmh^{-1}	0.5 ms^{-1}/1.8 kmh^{-1}
Swell	1.5m @ 10s	1.0m @ 10s

Wind data was also validated using historic records from the Bureau of Meteorology (BoM) for both sites an example for Battery Point is given in Fig 22 which shows that over the time period (Jan 2010 - Apr 2018) winds in the extreme category (105-115 kmh^{-1}) occurred at this site.

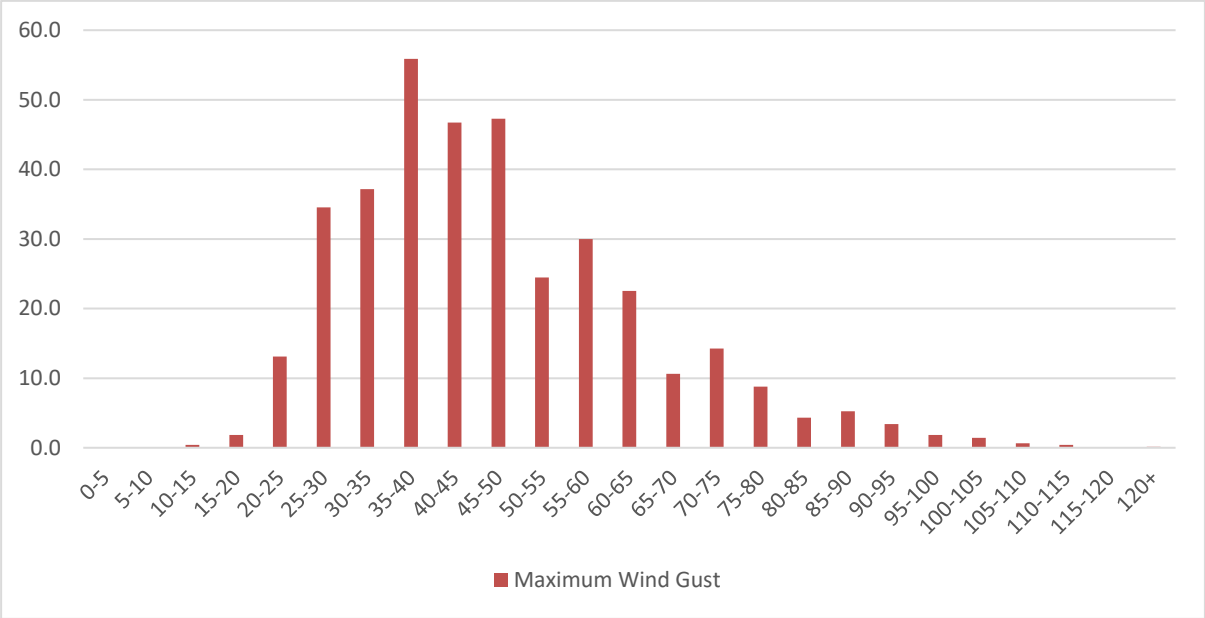


Figure 21 Percentage distribution of maximum wind gusts observations in kilometres per hour (kmh^{-1}) recorded in Battery Point between January 2010 and April 2018.

5.3.5 Mooring designs (2019)

We modelled five mooring design configurations in three categories (Table 13). The first category was a "business-as-usual" case, where the common chain mooring was modelled (Chain). The second category was a direct, like-for-like replacements of the existing chain component for an ES mooring strop of identical length either in an 8 t or 12 t strop configuration (ES Strop). Based on pilot testing, the strop did not behave effectively as a shock absorber for safe anchorage, thus a third category was considered for modelling, which used either the 8 t and 12 t strop plus a nylon rope component (ES Strop + Nylon). All designs were modelled using a clump weight anchor. For all ES mooring designs an additional train wheel was added to the clump weight anchors, which increased the mass of the anchor by either 30 or 50%.

Table 13 Mooring designs used in the model. In each case the water depth (*d*) was used to determine the length of sections within the designs. All Environmentally Sensitive (ES) moorings were modelled with both the 8t and 12t strops.

	Chain	ES Strop	ES Strop + Nylon
Strops	N/A	8t or 12t Strop	8t or 12t Strop
Surface termination	Float	Float	Float
Section 1	1*d* PPE rope	3*d* Strop	1*d* Nylon rope
Connection	D shackle		Swivel and D shackle
Section 2	1*d* light chain (16mm)		2*d* (less 2m) Strop
Connection	D shackle	Swivel and D shackle	D shackle
Section 3	1*d* heavy chain (24mm)		2m heavy chain (24mm)
Bottom termination	Clump weight	Clump weight + 30-50%	Clump weight + 30-50%

In addition, discussions with MAST during the design phase also specified that all ES mooring designs should have a 2 m section of heavy chain from the anchor to avoid any interaction

between the strop and the clump weight and the seafloor. The basic template for each ES + Nylon mooring design is illustrated in Fig 23.

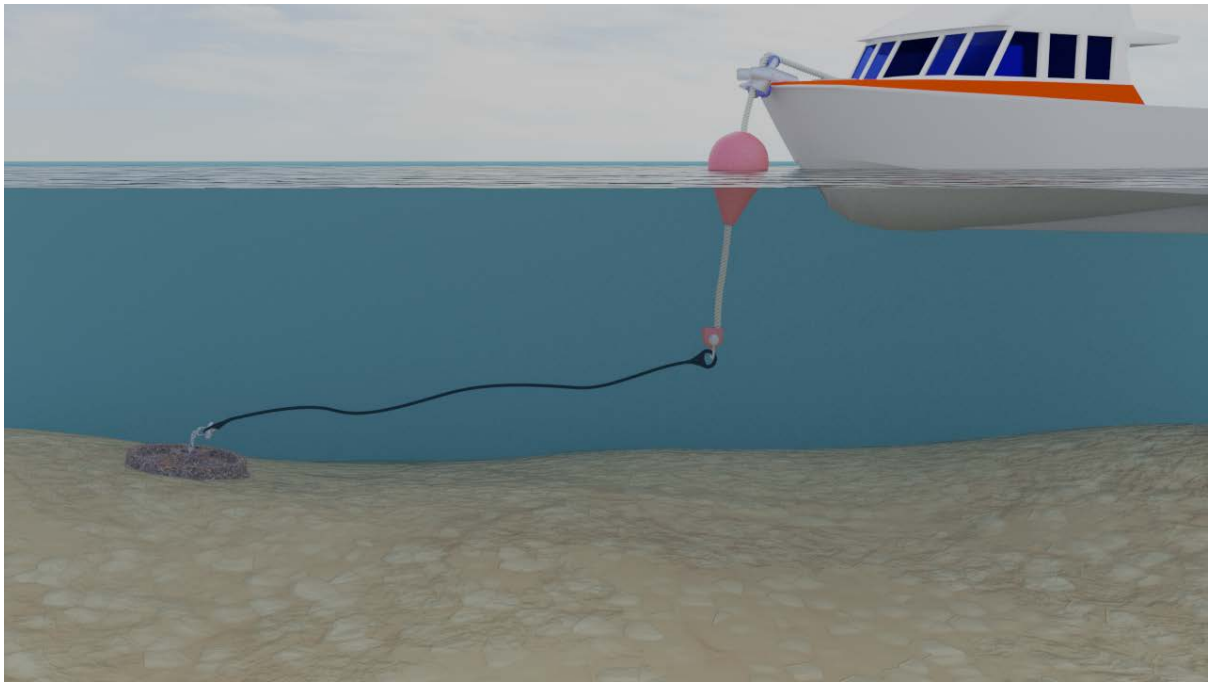


Figure 22 Schematic of the ES mooring plus nylon design. Components included railway wheels clump weight, ES mooring strop and nylon section leading to the surface float and the vessel. (credit: D. Fulton, CSIRO).

5.3.6 Mooring engineering model

We modelled all five design configurations for our four vessels under both the extreme and mild environmental conditions. To do this we used Woods Hole Oceanographic Institution (WHOI) Cable (Gobat and Grosenbaugh 2000) to model the mooring behaviour, line tension and horizontal movement of the mooring setups over time. A 2D dynamic solution was plotted for each of the five mooring designs.

As WHOI Cable was initially designed to target oceanographic instrumentation moorings, it has reduced capacity to model the unique geometry of each vessel. An idealised shape was constructed for each vessel that accounted for its mass, displacement and drag profiles (wind and current) that are critical in mooring behaviour.

In order to understand the behaviour of the mooring line, high-resolution model was used, with sample nodes uniformly distributed across the modelled structure. Pilot testing indicated a spacing of 100 mm between sample nodes provided optimal result for this trial, and thus was adopted. An increased density of every 50 mm was required in the touch down point of the mooring (i.e. wherever chain was lying on the seafloor). Each model simulation was conducted for a duration of 300 seconds, with a solution solved at 0.05 second intervals.

Environmental variables including current and wind were set to constant based on the defined environmental case (Table 12). The simulated wave action was set at an interval of 1.5 m at

10 s and 1.0 m at 10 s for our extreme and mild case respectively, with the wave height randomised throughout the model. Additional variables regarding the sediment characteristic, including bottom stiffness and bottom damping were set to 1000 Nm^{-1} and 1 respectively, which are both on the conservative end of the scale recommended by the simulation manual.

5.3.7 Neighbour mooring spatial clearance model

Due to the potential difference in the watch circle and behaviours of ES and chain moorings, the clearance between potential trial sites and neighbouring moorings were also examined. The modelling case assumes a scenario where a wind direction changes of 180° occurred, and the ES mooring repositions to the downwind position, yet the nearby mooring maintains its original position due to inertia from the chain mooring system, with only the vessel repositioned.

The location of the ES moorings and all neighbouring moorings were extracted using the MAST mooring database (https://maps.mast.tas.gov.au/). Based on the location of the proposed trial mooring, the distance to their corresponding closest neighbouring mooring was measured and selected for the clearance analysis. The watch circle for chain moorings were estimated using equation 1. A 5 m error margin was also introduced to ensure the conservativeness of the model.

Equation 1 Neighbour mooring clearance model.

$$C = D - (HD_{con} + VL_{ES} + HD_{ES} + \varepsilon)$$

C = Clearance
D = Euclidian distance between neighbour mooring
HD = maximum horizontal distance from mooring anchor to buoy
VL = vessel length
ε = error margin

5.3.8 Mooring field spatial clearance extrapolation

The moorings field between Battery Point/ Sandy Bay is in an urban setting and is at near full capacity, with few safe anchorages left while still maintaining clearance for vessels. As a typical dense urban mooring field which we were using for our ES mooring trials, we selected this location for a case study to extrapolate our neighbour mooring clearance model over a larger spatial scale. This model identified which chain moorings within the entire field which could be replaced initially to an ES moorings, specifically the ES strop + Nylon design safely, with no risk of moored vessels contacting due to the changes in movement behaviour of ES mooring as weather condition changes.

This model was constructed based on the assumption all moorings within the specified mooring field are available to be replaced with an ES+N mooring setup, each mooring within the targeted mooring field is to be replaced, and calculate the clearance with their corresponding closet neighbour for each targeted mooring. We extracted from the MAST mooring database the Battery Point/Sandy Bay subset of moorings' position data and the

approved vessel lengths for each mooring lease. The coordinates for all the moorings were projected, and the distance between all mooring pairs calculated. The distance matrix between mooring pairs were sorted to identify the closest neighbour for each individual mooring.

Due to the limited data on water depth of the moorings from the MAST database, a field verification of moored depth for all moorings in the selected mooring field was then conducted. A course was plotted using the mooring positions for surveying the entire field. As the vessel moved between all visible moorings the vessel was held alongside each mooring and using the vessel's onboard depth sounder (Raymarine A series), the water depth was recorded. We inspected each mooring buoy for mandatory mooring identification marks and a photograph was taken at each position to allow for geotagging and positioning with a tracking GPS (Holux GPSport 245). For moorings without visible identification markings, the GPS track and photo were used to compare against coordinates on record from the MAST mooring dataset to cross-validate potential moorings and the vessels with which they were associated.

For moorings that did not have an obvious surface float but are present in the MAST database, the position from the mooring database was approached and a depth measurement was taken. For these moorings that we were not able to validate in the field, we assumed in the model that these moorings are present, and the depth was taken as the deeper end of each 5 m depth contour. These assumptions provided a "worst case" distribution and depth of moorings for potential interactions with neighbouring vessels in our model. Data from local tide tables were also extracted and used to standardise all mooring depths to a high tide estimate. The clearance formula (Equation 1) was then applied to all the individual mooring pairs within the region.

Two variations of the model were generated, the first assumed the ES mooring line had a fully taut profile, so it was stretch out in response to extreme wind. In this case the horizontal distance of the mooring line is 3 times water depth (*3d*). The second variation assumed the top section sits vertically, due to its negative buoyancy property, thus creating a profile where the horizontal distance is equal to two times the water depth (*2d*). This secondary comparison assumed the ES mooring scope was similar to chain moorings. Based on these assumptions, the number of chain moorings suitable for immediate replacement to ES moorings within the targeted field with no chance of any negative interactions with nearby vessels was determined.

5.4 Results

5.4.1 Mooring model engineering outputs

Generally, the behaviour of the mooring designs was consistent between vessels. In extreme weather the ES + Nylon moorings placed the least load on the mooring and surface terminations and the highest loads recorded were on the traditional chain moorings. However, across the model parameters and designs there was variation between vessels (Table 14a).

When modelled with an ES + N design, a comparison between the two variants of ES strop showed that 8 t strops translate a lower maximum load then 12 t strops between all corresponding pairs except for the Adams 12. Between the two ES mooring designs (ES & ES + N), in all cases, again except for the Adams 12, the ES + N design place a lower load on the terminations in extreme conditions. In the case of the Adams 12, the simple 8t strops design

placed a slightly lower load than the 8 t + Nylon, though the 12t + N design placed the least load of all for this vessel. When comparing the 12 t ES+N design to the traditional chain mooring, all cases consistently demonstrated a lower vessel load in extreme weather. The recorded maximum load decreased between 39% and 57% compared to a chain mooring setup.

Table 14 Maximum loads in kilograms (kg) on vessel termination of modelled vessel for the five modelled mooring designs (refer to Table 4) under extreme (a) and mild (b) weather condition.

a) Extreme weather

Vessel	Chain	ES 8t	ES 8t + N	ES 12t	ES 12t + N
Adams 12	7159	3598	3642	6046	3065
Clansman 30	5375	2779	1737	4227	3144
Maple Leaf 42	3056	2327	925	2981	1842
Robert 36	4514	2996	1870	4389	2225

b) Mild weather

Vessel	Chain	ES 8t	ES 8t + N	ES 12t	ES 12T + N
Adams 12	156	1674	1038	2488	942
Clansman 30	127	1179	895	1173	1002
Maple Leaf 42	140	1313	445	1384	614
Robert 36	129	1214	817	1074	1662

When simulated under a mild weather case, chain moorings consistently showed the lowest maximum load across all tested vessels, with tension ranging from 127 kg to 156 kg. Results for the ES mooring designs is mixed; all ES mooring designs show a higher maximum load in mild weather conditions, due to the damping effect of the chain catenary shape.

Comparing the model results to the safe working load (SWL) of each system using a 4:1 standard and under extreme weather conditions, the maximum vessel tension for all vessels on chain moorings exceeded the SWL (Fig 24). Using an ES strop only, the maximum load for all but 1 case (ES 12t, Maple Leaf 42) exceeded the SWL. When modelled under an ES+N configuration, one the vessel (Adams 12) exceeded the SWL with an 8 t strop, while two cases- Adams 12, and Clansman 30- exceeded the SWL when modelled with a 12 t strop (Fig 23). Despite at least one modelled case exceeding the SWL for each mooring solution, the ES+N 12t design only exceed the limit by a small margin, by 2.2% and 4.8% (65 kg – 144 kg) for the Adams 12 and Clansman 30. In contrast, the traditional chain mooring exceeded the SWL by 32% - 210% (746 kg – 4849 kg). By choosing the most appropriate ES mooring design vessels can be moored even extreme conditions without maximum loads exceeding the SWL of the system.

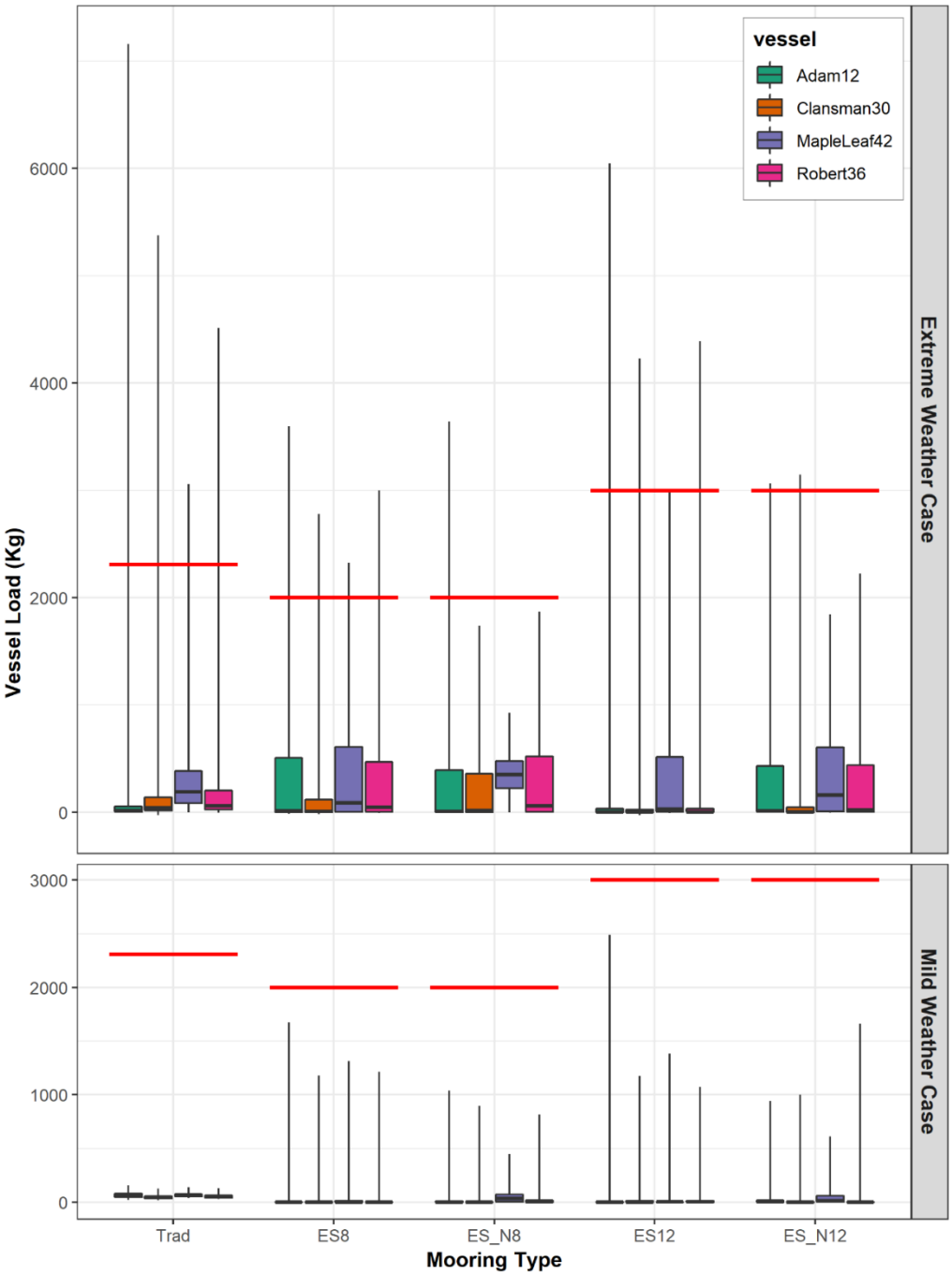


Figure 23 Summary of tension loading (Kg) at vessel modelled under extreme and mild weather conditions for the five tested mooring designs, traditional chain mooring (Trad), 8t and 12t variants of the ES only design (ES8 & ES12), and 8t and 12t variants of ES strop plus nylon top section design (ES_N8 & ES_N12) for all four vessels. The box illustrates the interquartile range (25th percentile to 75th percentile) with the median denoted by the horizontal line. The vertical bar illustrates the full load range with the absolute maximum/ minimum value for each case. The red line indicates the safe working load (SWL).

When the model's load tension output over time was examined more closely in extreme weather conditions (Fig 25), the loading comes on very fast for both chain moorings and the ES 12 t + N design, indicating a shock loading on the mooring components. However, not only is the peak loading less severe for the ES 12 t + N design, the load also ramps up and down

over a slightly longer timeframe, showing how the nylon component acts as a shock absorber to lower the peak loading.

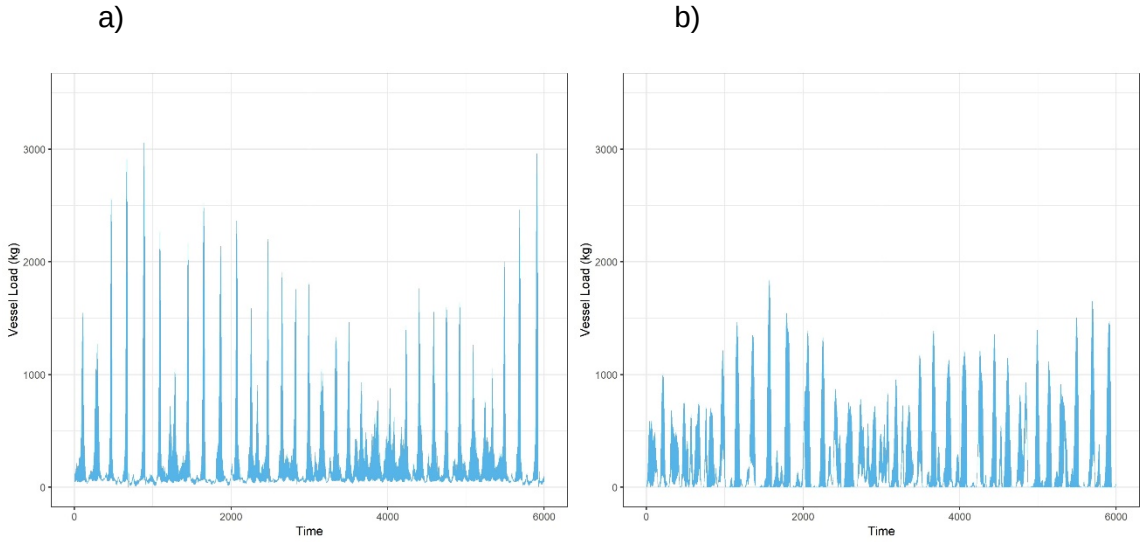


Figure 24 Model outputs or tension on the surface termination over time for the Maple Leaf 42 vessel for a) traditional swing moorings and b) ES 12 t + N mooring.

Under extreme conditions, the ES 8 and 12 t strop moorings showed a consistent trend of the largest horizontal movement across all modelled moorings (Figure 26), ranging from 6.3 m to 14.6 m. Conversely, externally induced movement were the smallest for all modelled cases when using an ES+N setup, which were very similar in behaviour to the traditional chain moorings in extreme conditions.

In mild weather conditions, traditional chain moorings showed almost no movement from their nominal position, with a difference between 0.3 m and 0.5 m across all vessels. Performance across the various ES solutions was mixed.

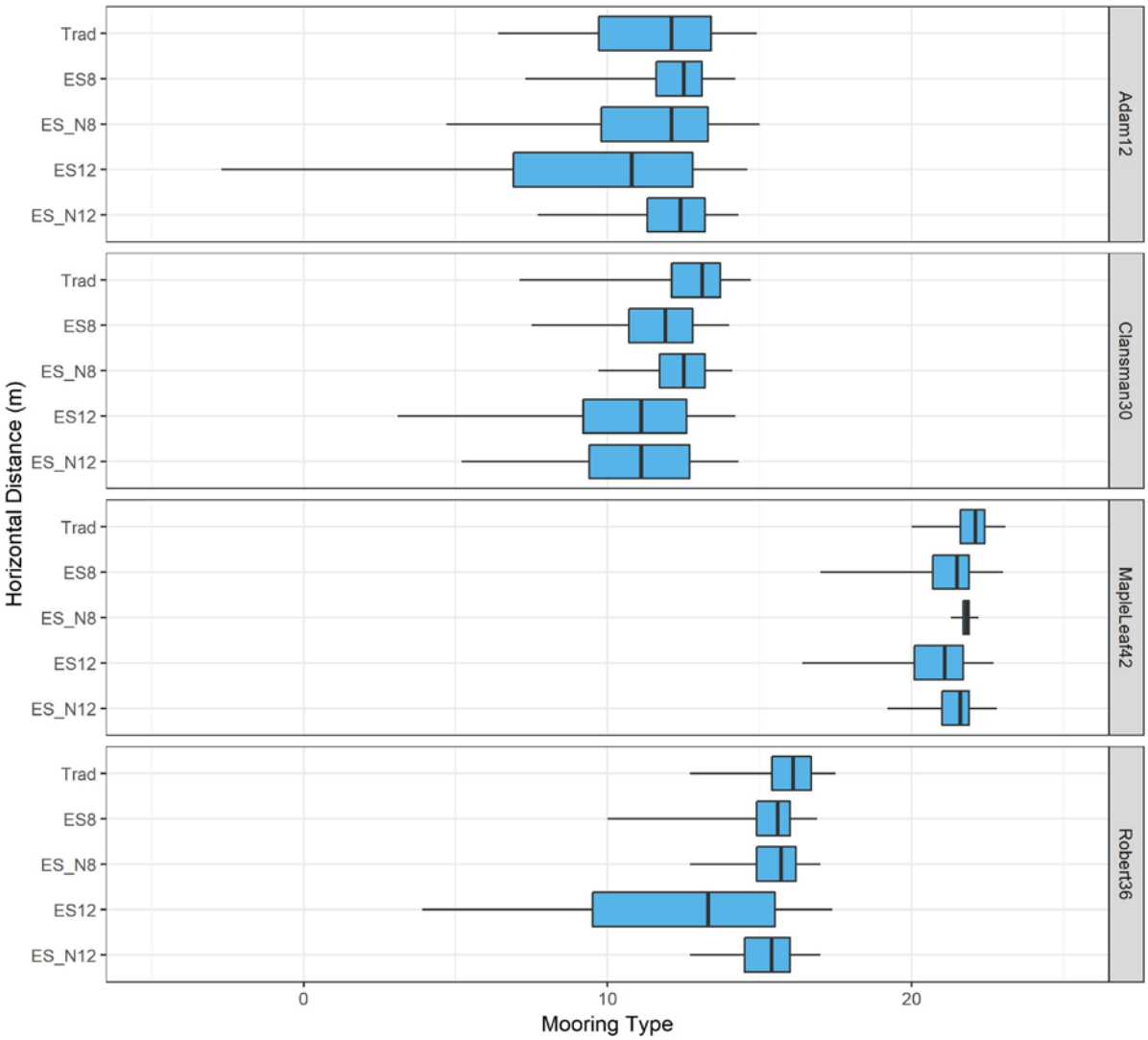
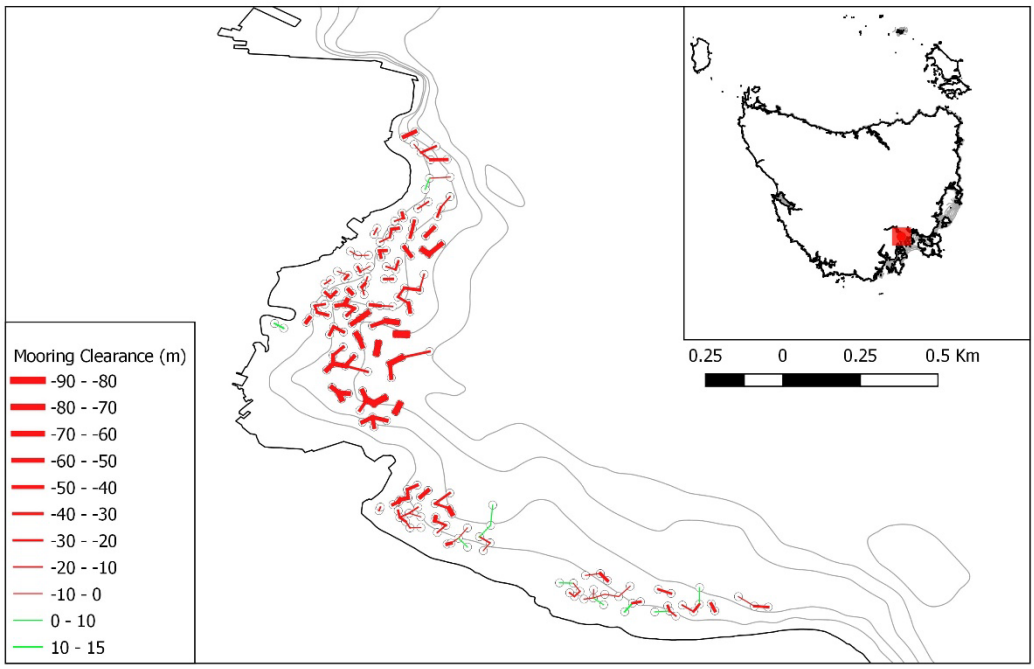


Figure 25 Movements of mooring designs laterally in response to extreme weather.

5.4.2 Mooring field spatial clearance extrapolation

Based on MAST's mooring dataset, there are 195 moorings in the Battery Point/ Sandy Bay region with moorings located between 1.6 m and 21.1 m depth. In total, 30 moorings (15%) from the MAST database were missing or unverifiable from field observations and we applied our 'worst-case' depth scenario for the depth at the location listed on the database. Spatial extrapolation of respective closest neighbour's moorings to each other indicated that initially 19 moorings or 9.7% of the field have enough clearance for safe replacement to an ES mooring setup when a *taut* position for the ES mooring is assumed (Fig 27a). The average gap between these mooring pair was 7.11m ± 1.13 m and the average depth was 5.72 m.

a)


Mooring Clearance (m)
-90 - -80
-80 - -70
-70 - -60
-60 - -50
-50 - -40
-40 - -30
-30 - -20
-20 - -10
-10 - 0
0 - 10
10 - 15
0.25 0 0.25 0.5 Km

b)

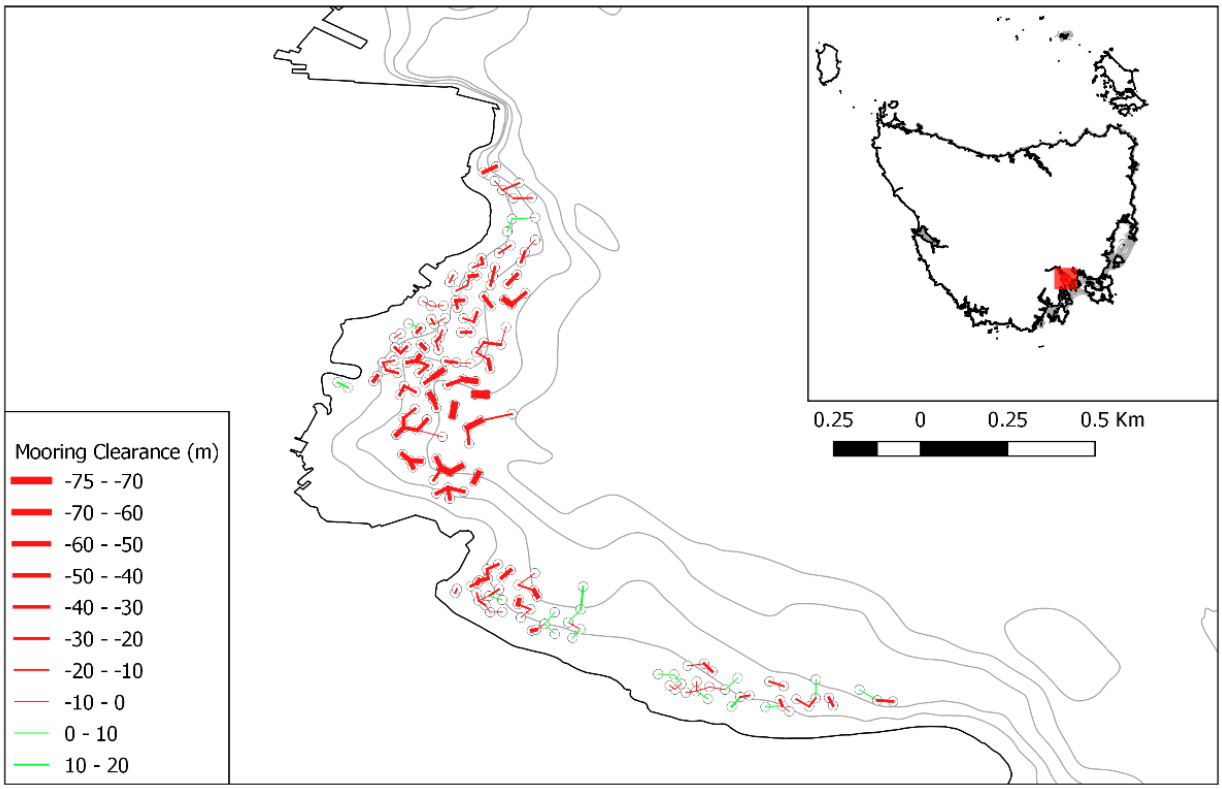

Mooring Clearance (m)
-75 - -70
-70 - -60
-60 - -50
-50 - -40
-40 - -30
-30 - -20
-20 - -10
-10 - 0
0 - 10
10 - 20
0.25 0 0.25 0.5 Km

Figure 26 Model of vessel clearance to identify those moorings that would be suitable for replacement with ES moorings with little chance of collisions with neighbouring traditional swing moorings. a) for a taut ES mooring line and b) for 2 times water depth (2*d*). Red lines indicated a negative clearance between the targeted mooring and its neighbour and green lines indicated no risk of collision when both boats are securely moored.

Our second comparison, where we assumed the ES mooring scope was more like chain moorings, where the horizontal distance 2 times water depth (2*d*) (Figure 27b) allowed up to 35 mooring's or (17.9%) of the field to be initially safely replaced within the existing mooring field configuration.

5.5 Discussion

Replacing existing traditional chain mooring fields with ES moorings may be an effective ecosystem scale restoration action for coastal waterways, but achieving this with a large number of mooring holders using a well-established design, will require considerable development. In our study, we have summarised the process we followed for replacing existing, privately owned, chain moorings with ES moorings in both a dense and sparse mooring field. In doing this we identified and addressed problems through a consultative approach with multiple stakeholders and an engineering workflow. This involved a design phase for the ES mooring and then both engineering modelling to ensure the designs were safe and clearance modelling to avoid collisions between vessels in mixed fields of ES and chain moorings.

5.5.1 Engineering load model

Initially, we modelled a straightforward 'like-for-like' replacement of the chain components of the traditional design with an ES mooring strop. However, despite the strop fulfilling various technical specifications, namely the breaking strength and the restricted stretch, the strop by itself didn't provide enough elasticity for effective force dissipation. This was evident with the modelled vessel loading, where in all but one case, the maximum load exceeded the components SWL. Upon evaluation we added to the design a section of more flexible nylon rope, which has a low axial stiffness to increase the elasticity. When we re-ran the model the new ES+Nylon (ES+N) mooring design provided increased elasticity and improved the mooring's performance to avoid, in most cases, exceeding of the SWL. One downside of Nylon is that it does not have the same resistance to chaffing and UV stabilisation as polypropylene (PPE) rope. In the field we compensated for this by adding a sleave to wear points and highlighting with the mooring contractor the need to inspect the rope for UV damage during servicing.

Our engineering models showed the fundamental mechanical differences between the catenary chain moorings and our ES moorings, which are a stiffness based semi-taut design. With a traditional chain mooring design, during extreme weather events, when the external force exceeds the weight of the ground chain it un-trenches from its buried position in the seafloor. The mooring then extends from its catenary shape into a semi-taut configuration. At this point the axial stiffness of the chain component becomes the dominating force (Johanning et al., 2007). The lack of elasticity in the chain component creates rapidly cycling spikes in tension, which are transferred through the mooring line and onto the terminations of the vessel's deck fittings. When the force reduces, the chain drops back to the ground, before

again returning to the semi-taut configuration until the weather condition subside. The time base model of vessel loading illustrates this cyclical high shock loading produced by traditional chain moorings.

While a similar cyclical pattern was observed across all the designs, tensions spikes in ES+N design are both slower and lower than chain moorings under the same extreme modelled weather condition. By design ES+N mooring strops are positioned along the water column, and combined with material with lower axial stiffness, this design has a lower energy threshold to reach their semi-taut configuration. Under extreme weather conditions the low axial stiffness of ES+N allowed for better shock absorption compare to chain mooring in a semi-taut configuration, as reflected by the lower peak load in our model.

Our models indicated traditional chain moorings still worked better than our ES moorings in mild conditions, when the heavy chain remains on the seafloor, providing an inertia force to hold the vessel in place. During the design phase of this project we underwent two iterations of the engineering cycles highlighted in our conceptual pathway, which led to the final design, incorporating a nylon top section to the ES mooring strop. The initial design parameter focused on safety of the system and the need to match the holding power to the existing system under extreme condition. Further iterations could be developed to improve ES mooring behaviour in mild conditions, focusing on better dampening forces to improve ride.

We did, however, see at least one case in each design category that exceeded the SWL under extreme condition. Even for the final ES+N mooring designs, two of our modelled vessels using the ES+N 12t mooring exceeded their SWL, though to a much smaller extent than chain mooring. This suggests that while ES moorings provide a superior solution in extreme conditions, different vessel types moored in various depths will likely require specific design configurations, until further work to monitor real-world responses is completed.

While we only had a small number of vessels to model, the length and displacement of modelled vessel does not appear to simply corelate with the maximum load translated to the system. Rather, the moored depth appears to have a major influence on the load and overall effectiveness of the mooring. Engineering optimisation will require mooring line components to balance elasticity and strength with the vessel's characteristics and moored depth. Further verification of the model with instruments such as accelerometers deployed onto vessels moored to various designs, an expansion of vessels types as well as moored depths would be ideal.

The output from our extreme weather model, where the high axial stiffness of traditional mooring chains produced shock loading, may explain common mooring failure modes. The first of these is when vessels break free at the surface termination when excessive load is applied to the deck fittings/ fairleads. The second is failure of the lighter thrash chain component. While this section of traditional moorings has a relatively high SWL when new, the chain is constantly working metal on metal in seawater, while being covered in abrasive sediments. This both erodes and corrodes the chain link diameters over relatively short time periods, resulting in declines in this components SWL. In our model we did not consider the SWL of deck fixtures that secure vessels to moorings, as they will be unique to each vessel, or the rate of decline in strength of thrash chains but these components will be placed under

large, cyclical loads in extreme weather conditions, especially when the boat is attached to a traditional chain mooring.

Our parameters used to model the moorings behaviour in extreme environmental conditions are based on the Australian Standard AS/NZS 1170.2-2011. Our model treated wind as a constant, though in reality the wind would be expected to be more variable over time which may influence model outputs. These extreme wind speeds are, however, realistic, having been observed multiple times at our study sites in the last decade. Over the longer term, for certain areas of the world including SE Australia, a consequence of climate change is increased occurrence and intensity of extreme wind events (McInnes et al. 2011), which will increase the risk of damage to vulnerable infrastructure (Stewart et al. 2018). Traditional chain mooring designs across our case study vessels already exceed SWL in current extreme weather conditions. Without adaptation of technology in a future scenario of rapid climate change there may be an increased general risk of mooring failure.

5.5.2 Engineering lateral movement and mooring clearance models

Throughout the consultation period, input from our stakeholders emphasised the need to account for the scope of moorings to allow adequate clearance between vessels. An important consideration is always to mitigate the risk of collisions between moored vessels in dense fields. Our engineering and clearance models showed the potential for collisions is an issue to consider if fields are to be safely switch from chain to ES moorings. This potential or collision was due to the movement range of all variants of the ES mooring setup were greater than their traditional chain mooring counterparts, due to the lack of inertia from the chains in the ES designs. As such, vessels on ES moorings will move more quickly to the downwind position as the wind conditions changes.

Overlapping scopes between neighbouring moorings was particularly high toward the centre of the field. The low number of available mooring for initial replacement highlighted the need for a sequential approach starting from the edge of the field when creating mixed fields. In our model we only ran the initial iteration of time of replacement. Following replacement of this first iteration of candidate moorings, or even after the first replacement, the model could be run again, and more moorings would become available for replacement.

When moorings have the same scope the risk of vessels collisions between designs was negatively correlated with wind speed, being greatest during mild conditions in a mixed field setup. A third weather condition, an extreme calm (wind speeds less than 0.1m/s) condition was also considered for the model. However, due to the extreme value, a solution could not be produced with a suitable level of confidence and this was discarded from our study. Our spatial clearance model was, however, extremely conservative. We assumed: a) no movement when the wind changed direction by the nearest neighbour vessel that was attached to the traditional chain mooring, b) an instantaneous movement by the ES moored vessel to its furthest, taut, extent, and c) it moved in a direct vector to the neighbouring vessel.

Subsequent field observations since the deployment of ES mooring at the trial site suggests they follow a similar profile to chain moorings. Due to the negatively buoyant nylon section, which produces a similar catenary shape to the chain mooring in mild conditions, the actual watch circle may be similar between mooring designs. When we modelled a smaller watch

circle (*2d*) which seem to be closer to our real-world observations, we expanded the initial number of potential chain moorings that could be switched to ES moorings without risk of collision.

Further testing of the spatial clearance model using GPS data will provide additional data on the movement behaviour of the ES + N moorings compared to traditional designs. This could be coupled with three-dimensional numerical modelling to provide a higher resolution model for refining the current engineering outputs.

5.5.3 Management implications

Chain mooring have significant local impact on the seafloor (Sagerman et al. 2019) through mechanical disturbance causing fragmentation of the habitat (Serrano et al. 2016; Unsworth et al. 2017). Depending on the habitat type this can decrease seagrass productivity (TANNER 2005), as well as cause declines in fish communities (Lanham et al. 2018) and invertebrate communities (Herbert et al. 2009). Certainly in Tasmania (MAST 2016b), and probably in a global context, most vessel moorings are privately leased and of a traditional chain mooring design.

To achieve a high level of habitat recovery a complete cessation of disturbance is often required (Duarte et al. 2015), though areas with historic impact may require longer to recover (Hiddink et al., 2017). Depending on the local resilience and connectivity of estuary soft sediment eco-systems and species (Thrush et al. 2008), replacing existing fields of chain moorings with ES moorings may be an effective ecosystem scale restoration technique. However, implementing this will require considerable research and development. For instance, ES moorings may need to be optimised for different depths and vessel displacements and there is a potential issue with collisions between mooring of different designs in a mixed field. Due to the private leasing nature of moorings, support from the mooring community is also required for large scale replacement to occur.

We identified and address the problems of private owner up-take of ES moorings with our mooring task force. Meetings by the task force provided insight into a deployment pathway, including engineering and permitting issues. Advice from task force members, including the permitting authority (MAST) and the mooring contractor allow us to frame our questions and guiding our research. The inclusion of an engineer, into the initially conservation biology focused project team, was also important for building trust within the group as well as allowing for a greater understanding of the physics of moorings. With the taskforce we developed a workflow for managing the transition from chain to ES moorings which should be widely transferable. This multi-disciplinary and consultative approach may be an important management consideration for success of conservation or climate change projects that require adaptation of public or private infrastructure.

Due to the experimental nature of this study, the engagement with mooring owners and subsequent engineering modelling was conducted on a case-by-case basis. Mooring owners were engaged directly to join or “opt-in” prior to the commencement of modelling. We did this to ensure locations are suitable for replacement. Across our advertising phase we received many more offers from owners to join the program than those that were eventually accepted. In these rejected cases the criteria of our workflow were not reached for safe deployments,

primarily where their mooring did not have enough clearance with neighbouring mooring to allow for a safe replacement. The strong response from members of the public that were interested in joining the program suggests that, if a suitable, simple, safe and cost-effective solution is available many mooring lease owners will adopt new mooring designs.

The clearance model also identified the potential for developing a replacement schedule for switching entire fields to ES moorings. Replacements could be undertaken sequentially, working first with the moorings on the edge of the field with suitable clearance and then conducting a re-analysis of the spatial model to identify the next round of moorings available for change. The model could also be used to consider clearance requirements between moorings of different scopes, including alternative ES mooring designs with scope shorter than the current 3 times water depth scope. As mooring standards are often not set by the local authority but rather are at the discretion of private operators and contractors deploying their own designs, our simple clearance model may provide guidance to avoid collisions.

With the capital cost of ES+N moorings being similar to traditional moorings and potentially placing less strain on vessels in extreme conditions, they may hold some economic advantage over chain moorings. The current insurance and legislative requirement for moorings are for annual or bi-annual servicing. It is suggested from the supplier that the strop component of ES moorings can have a relatively long-life expectancy compared to chain. This may provide more incentive for private mooring owners to replace existing moorings to ES moorings in the future.

Our comparative models highlighted the fundamental differences between traditional chain moorings and several ES mooring design, that vessels differ in their response within the model, and we need to better understand the effect of depth on design performance. For mass adoption of ES moorings, vessel model (or size class of vessel) and depth specific engineering design tables may be necessary. To reduce separation of vessels from moorings in extreme conditions these sorts of tabulations would also be useful for traditional swing designs.

For tabulations into engineering design tables, many more model simulations across a comprehensive range of depths, designs and vessels is required. As current run times for the model were approximately 4 hrs, these simulations would need to be automated. An adequate, representative sample of the simulations would also need to be verified from field observations. The simulated and field data could then be used to conduct analysis to predict the proportional effects of different parameters on the model's outputs.

5.6 Conclusions

Despite the long history of ES moorings, there is a lack of a conceptual framework for deployment. We proposed a deployment workflow, incorporating engineering which can be adopted by management agency for replacing existing mooring fields to ES moorings. Once a target site was selected a spatial analysis should occur to identify clearances with closest neighbour. Following that, contracting should occur and then the engineering modelling cycle is commenced to set the appropriate design and deploy the mooring. This workflow should be overseen by a multi-disciplinary mooring taskforce as workflows and model parameters will be dependent on local conditions. To succeed in removing mooring disturbance to recover marine habitat, replacement of tradition chain with ES mooring needs to first focus on waterway safety.

Our model highlighted that under extreme weather conditions, traditional chain moorings, once they reached a semi-taut configuration perform poorly compare to ES moorings. But in mild conditions chain moorings still place lower loads on mooring components and further refinement of the ES mooring design will be needed to allow for dampening of low forces.

While these results were encouraging, our case study would require expansion to provide more generalised results. More extensive modelling and testing of model outputs from observations, inspection of ES moorings during servicing and tabulation into design standards may all be useful next steps towards mass adoption.

5.7 Acknowledgements

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6. HANDFISH CONSERVATION PROJECT

6.1 Website

Individual red handfish can be identified by unique markings/colouration (pattern recognition), which means that a mark-recapture study can be implemented to estimate population size and other demographics/parameters – such as fine-scale movement of individuals, growth over known periods, and timing of events (e.g. mating, reproduction) – all essential information for the conservation of the species.

The Handfish Conservation Project website (handfish.org.au) included development of a custom photo-identification library for storage, collation, and display of individual red handfish sighting information from ongoing monitoring efforts of both known populations (found here: https://handfish.org.au/meet-the-fish/). The web pages act as both a central image/information storage bank (i.e. photo management tool), and as an interactive public sighting display portal.

The photo-library has been populated with the sighting images and information that has been collected (approximately 162 observations to date, of which less than 100 are adults) and will be used to record and display recapture information (see Fig 28).

6.2 Fundraising and awareness

Fundraising for the Project was promoted via a donation's portal (https://handfish.org.au/donate/). From 18 December 2018 – 02 October 2019 donations totalled $23,540.50. Fundraising has been promoted via the Facebook and Twitter pages set up specifically for the Project.

The Project launched a 'Name a Red Handfish' fundraising and awareness campaign on October 2nd, 2019. The campaign invites members of the public, groups, organisations and businesses to select an individual red handfish via the 'Meet the Fish' page, and by donating $1000, they are able to officially 'Name' that individual. Within the first 2 weeks, 12 individuals had been named (see Fig 28), and there are now 20 named in total. Naming donors receive naming rights, recognition via the website, mention in the annual report, and a thank you letter, printed photo of their individual Red Handfish, postcard, and certificate (Figs 29 - 31).

Figure 27 Table of sighting images, named fish and donor acknowledgement available on the website.

Gabrielle		Red handfish (Thymichthys politus)	NAMED	Gabrielle Beer
Ginger Ninja		Red handfish (Thymichthys politus)	NAMED	Sarajayne Lada
Hippocrates		Red handfish (Thymichthys politus)	NAMED	Dr Ian Payne City Doctors and Travel
Irwin		Red handfish (Thymichthys politus)	NAMED	Megan and Patrick Alessandrini
Optimist		Red handfish (Thymichthys politus)	NAMED	Anonymous

Figure 28 Individual Red Handfish page (includes re-sighting and naming supporter information).

Fish ID: Red_14 Fish Name: Ginger Ninja Named by: Sarajayne Lada

Species: Red handfish (Thymichthys politus)
Life Stage: Juvenile
Sex: Unknown

NAMED

Comments

Red handfish are one of the rarest fish on the planet. They are critically endangered, and currently known in only two locations in Tasmania. Key threats include a small and fragmented population, and degradation of the seaweed habitat that provides shelter. Other processes that affect their habitat (i.e. pollution, nutrients, or interactions associated with sea urchins and their predators) also represent additional threats. Collection for the aquarium trade and introduced species are also potentially important, while warming seas may have contributed to long-term declines. Researchers and community members are working tirelessly to protect the species from extinction. Your support is critical in supporting this research. Find out more about here: handfish.org.au/projects/

<< Sighting information for this fish >>

Date	Size (mm)	Depth (m)	Comments	Images	Observer
2018-11-10	20mm	m			

Showing 1 to 1 of 1 entries

Figure 29 Red Handfish Naming campaign products for donors.

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8. APPENDIX A – DNA SAMPLE NUMBERS

Table 15 Location and sample numbers of spotted handfish (*B. hirsutus*) extracted for DNA.

Location/collection	Sample size	Sampling data
Primrose Sands	3	1998
Half Moon Bay	4	1998
Kangaroo Bay	4	1998
Opossum Bay	5	1998
Howrah Beach	17	2006
Manning Reef (Sandy Bay)	15	2006
Battery Point	9	2007
Mary-Ann Bay	15	2007
Tranmere	31	2007
Opossum Bay	20	2008
Ralphs Bay	58	2008
Tranmere	75	2008
Breeding Program morts	7	LO2018
Total	262	

9. APPENDIX B – EXTENDED DAPC PLOT

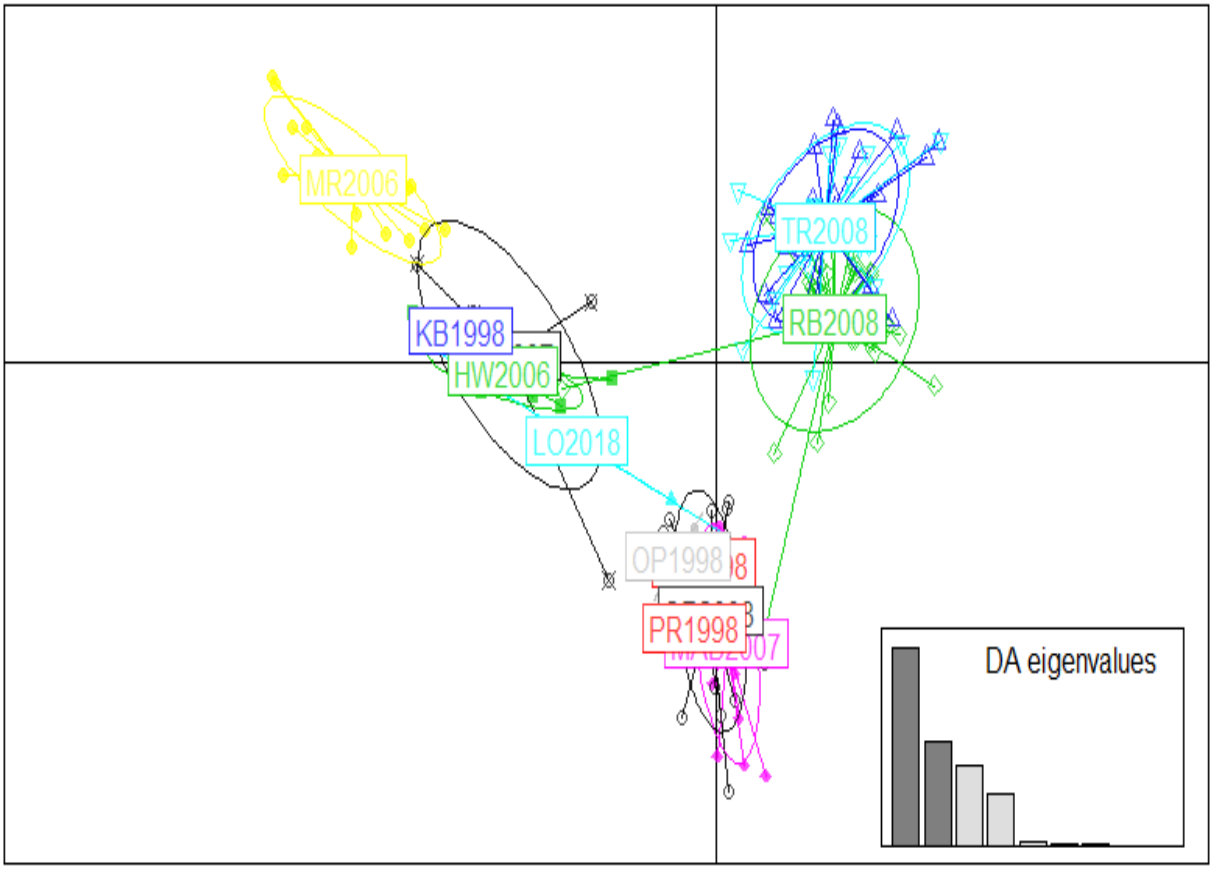

Figure 30Discriminant analysis of principal components (DAPC) with priori grouping corresponding to the thirteen sample collections of *B. hirsutus* as outlined in Appendix A. Scatter plot of DAPC based on 4 172 nuclear SNPs. Local populations are grouped by upper estuary (top left), mid estuary (top right) and lower estuary (bottom middle).

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