Marine Biodiversity Hub

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Conservation of handfishes and their habitats Final Report 2020

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Project A10 – Conservation of handfishes and their habitats

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2. Red and Spotted handfish morphometric study (lead: J. Stuart-Smith)
3. Spotted handfish population dynamics, conservation, and ASH plants (lead: T. Lynch)
4. Environmentally Friendly Moorings servicing (lead: T. Lynch)

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EXECUTIVE SUMMARY

Red handfish head-starting and post-release survival

In late 2018, the National Handfish Recovery Team implemented a Red handfish (*Thymichthys politus*) re-population strategy ('head-starting' program) to bolster wild population numbers. We successfully collected Red handfish eggs, hatched and raised them in captivity at Seahorse World in 2018, and at the Institute for Marine and Antarctic Studies (IMAS) Taroona aquaculture facility in 2019. For the first ever, planned release of captive-reared Red handfish juveniles occurred back into the wild for population-bolstering purposes, which meant that we had two cohorts to return (one- and two-year old individuals).

Prior to release we implemented several essential components not outlined in the original project plan – habitat restoration (removal of barrens-causing urchins at the two known populations), tagging of some of the juveniles prior to release (to enable us to validate spot pattern recognition in young individuals), and pre-release conditioning to familiarise juveniles with their natural ecosystem prior to release (habitat, fishes, and invertebrates they are likely to encounter in the wild).

Forty-two juvenile Red handfish were successfully released into the wild across two existing populations in south-eastern Tasmania. This first-ever release of captive-hatched Red handfish was undertaken in 2020, and dive surveys re-sighted released juveniles at 1-, 3-, and 5-days post-release (across both sites).

Demonstration of head-starting as a successful strategy via post-release monitoring means that this approach should now be scaled-up to continue bolstering of wild populations – before the existing population size dwindles further. This work needs to be actioned in combination with increased effort in habitat assessment, restoration and management. Based on the success in integrating captive-raised juveniles back into the wild, we should now look to resourcing captive-breeding trials, which have greater capacity to replenish populations than head-starting and crucially would also serve as an insurance population. The feasibility of translocating individuals from wild and captively bred stocks to sites where they do not currently occur should be investigated as a potential extinction-avoidance tool for Red handfish under the aim of creating more populations in less threatened habitats.

Red and Spotted handfish morphometric analysis

An important requirement for captive breeding and any reproductive research on threatened species is being able to distinguish males and females using non-invasive techniques.

Adult and sub-adult *T. politus* and *Brachionichthys hirsutus* preserved specimens and underwater images were used for analysing morphometrics (comprising of specimens from CSIRO National Fish Collection as well as photographs collected as part of other research, e.g. T. Bessell's PhD). Individuals were measured for the morphological traits using electronic callipers for preserved specimens or using Image J software for photos.

We were unable to discern any clear differences between males and females in Red or Spotted handfish using external morphometric analyses, although there was some potential intraspecies variation, such as proportion of upper nostril size, inter-dorsal length proportion, dorsal height in Red handfish, and upper nostril proportional size in Spotted handfish – there were no clear groupings. The level of interspecific variation observed may possibly relate to gender differences, although more data are needed. Measurements were difficult from images, however, and thus the utility of using morphometrics for evaluating sex ratios in the wild may be limited. Exploration of other means of differentiating gender is recommended.

Spotted handfish population dynamics and conservation management

We developed a database of Spotted handfish surveys (80 surveys) across 9 sites within the Derwent estuary from 1997-2019. With this new, integrated, longitudinal database we conducted preliminary modelling of Spotted handfish population dynamics with a generalised liner mixed model (GLMM).

Local populations of handfish can be highly dynamic over both time and in relation to each other. There has been an overall decline in the Derwent estuary's Spotted handfish population since 1997, but this decline had stabilised by 2014. Declines in local populations can occur very quickly, especially declines occurring in high density areas. There are also examples of increases in local densities.

Within the Derwent estuary, both genomics and population dynamics suggest a well-structured population, with local populations acting in isolation from each other, or at best, as small groups. For practical conservation management of Spotted handfish there are seven functional conservation areas: 1) Howrah and Bellerive Beach, 2) Battery Point, 3) Sandy Bay, 4) Ralphs Bay and Tranmere, 5) MaryAnn Bay, Opossum Bay and Halfmoon Bay, 6) the mouth of the Huon River and 7) Storm Bay.

Due to the observed rapidity of declines in local populations, annual, rather than bi-annual or decadal monitoring is the best temporal scale to provide any opportunity to intervene in conserving local populations.

Direct interventions can include monitoring ascidian density as a proxy for natural spawning habitat and then planting artificial spawning habitats (ASH) when ascidian numbers decline. Replacement of swing moorings with environmentally friendly moorings (EFMs) is another practical conservation action. Removal of these chain moorings between Battery Point and Sandy Bay may provide opportunities for connection between these two currently isolated populations.

Removal or suppression of northern Pacific seastars would benefit Spotted handfish and many other species. However, due to the seastars ubiquitousness and fecundity, a population-wide approach, such as a species-specific pathogen or genetic control such as through a CRISPR mediated gene drive, would be required as a control method.

Re-introductions to locations where Spotted handfish have historically gone extinct is another action that could be considered. A prime candidate is Primrose Sands, which is adjacent to a known population of the Spotted handfish's close relative the Red handfish (*T. politus*). A re-introduction here would allow for a better understanding of ecological niche separation between these two closely related species and would also provide administrative efficiencies for the conservation of multiple species of handfish.

As handfish are extremely local in their distributions they are a prime candidate for spatial management. Formal consideration of handfish locations by both planning and natural resource management authorities is another important practical conservation method.

Environmentally Friendly Mooring servicing notes

No EFM moorings failed during the 15-16-month trial period and vessels were subject to a wide variety of weather conditions, including extreme wind events.

At our first service we learnt many lessons around potential CSIRO EFM failure modes, handling and materials. Failure modes for EFMs may differ from standard chain moorings and these need to be explained to both owners, mooring contractors and regulators. We provide a list of recommendations to improve the survivorship of CSIRO's EFMs.

We initially used nylon in our EFMs for the headline from the yacht down to the hardware set, as this material provided additional and important shock absorption within our mooring model. The downside to nylon is that it is not as hard wearing or UV stable as polypropylene line. We observed some chaffing of the Nylon on the two larger yachts in the trial, one through her bow roller and another through her port fairlead and replaced the top-most section with polypropylene, while retaining nylon mostly below the waterline.

A new, potential failure mode for EFMs is reduction in strength through galvanic corrosion of the top hardware set that connects the nylon component to the rubberised strop. We observed galvanic corrosion across all mooring hardware sets, though this was mild except for the Clansman 30's mooring, which had severe corrosion.

We addressed the corrosion issue with the fitting of sacrificial anodes onto the hardware set and, where possible, insulation of the mousing wire. All moorings fitted with anodes showed no further electrolysis of the hardware. All installed anodes can be inspected by manually hauling the mooring line from the owner's vessel and do not require diving or a mooring barge.

The bottom connection to chain leading to the anchor, which is a common point of failure for chain moorings, does not appear to have suffered much wear on the EFMs.

Our only issues with the strops were handling problems. When hauling the Maple Leaf 42's mooring we degloved the rubberised covering off one of our original strops. This occurred even though we were using the manufacturers recommended hauling technique. This may have been due to excessive anchoring and suction of the bottom sediments. We replaced strops with a new type that has hardened hauling points. Sub-surface floats appear to be important for keeping strops off the bottom and this may be especially important for strops that have hauling points.

1. RED HANDFISH HEAD-STARTING AND RELEASE

1.1 Background – red handfish

The Red handfish (*Thymichthys politus*) is a critically endangered marine fish, endemic to Tasmania. The estimated population size of Red handfish is thought to be fewer than 100 mature individuals in the wild, and it is listed under Australia's Environment Protection and Biodiversity Conservation Act 1999 as Critically Endangered (i.e., protected in Tasmania). The International Union for Conservation of Nature's Red List of Threatened Species was updated in 2020 and now recognises Red handfish as Critically Endangered (i.e. considered to be facing extremely high risk of extinction in the wild). Previously widespread, it is now only known to exist on two, small patches of rocky reef in Frederick Henry Bay, south-eastern Tasmania. At the known locations, critical habitat of healthy seaweed and seagrass has declined dramatically, and the remaining plants are under intense grazing pressure from (native) sea urchins.

Unlike many marine species, handfishes recruit directly onto the benthos at the point of spawning - they have no planktonic larval stage, which excludes the potential for wide dispersal, and when combined with a restricted range and sedentary benthic lifestyle, this makes handfish local populations vulnerable to disturbance and makes re-establishment of locally extinct local populations unlikely without intervention. Additionally, handfishes do not possess a swim bladder and prefer to walk on modified pectoral fins rather than swim – further limiting dispersal capability.

Low population numbers and fragmented populations combined with threats that include local anthropogenic impacts (close vicinity to urban areas increasing likelihood of run-off, pollution, and sedimentation which degrade habitat), as well as direct disturbance, warming seas, and (indirect) impacts of fishing all serve to increase the risk of extinction for the species. In late 2018, the National Handfish Recovery Team implemented a Red handfish re-population strategy (head-starting program) to bolster wild population numbers. Basic biological knowledge is largely lacking for this species, which reduced the conservation options available – so this project was comprised of a post-release survival study for captive-reared juveniles as part of a 'head-starting' conservation strategy.

1.1.1 Population restoration strategies

Population restoration is a conservation strategy outlined by the IUCN Guidelines for Reintroductions and Other Conservation Translocations (IUCN/SSC, 2013). This strategy aims to bolster populations within the species' indigenous range, via 'reinforcement' – i.e., the deliberate movement and release of an organism into an existing population of conspecifics. Reinforcement aims to enhance population viability, for instance by increasing population size, genetic diversity, or the representation of specific demographic groups or stages.

‘Head-starting’ is an applied conservation intervention technique that can be incorporated as part of a population restoration strategy (see Armstrong and Seddon 2008; Burke 2015; Crane and Mathis 2011; Pritchard 1979). Head-starting is a restocking strategy that involves the captive rearing of juveniles, to limit high natural mortality in early life-stages, prior to release. Its primary goal is to bolster declining wild populations. This strategy involves captive-bred or reared juveniles being kept in captivity during their initial life stages to increase their likelihood of survival during these early, vulnerable life stages – where they are protected from predators and adverse conditions and provided food *ad libitum* – therefore giving them a ‘head-start’ on life (before returning them to the wild). In consultation with the National Handfish Recovery Team and relevant research scientists, a head-starting strategy was implemented for Red handfish, with the recovery action components detailed in Table 1-1-1.

1.2 Head-starting Red handfish

The first release of two cohorts of captive-hatched and head-started Red handfish (from project A10 in 2018, 2019) was undertaken in 2020, and dive surveys used to assess whether juveniles could be resighted post-release (at 1 day, 3 days, and 7 days post-release). The post release survival study was implemented to evaluate the success of the head-starting program – to determine whether juveniles would survive the transition from an extended period in captivity before being released into the wild, and therefore whether this was an effective conservation tool for Red handfish population bolstering. The program was centred around several key species ‘recovery action’ components (detailed below, Table 1-1) which combined to increase the likelihood of survival in the juveniles or improve the assessment of this program.

Table 1-1 Recovery action components for Red handfish: population restoration via head-starting and release into the wild

#	**Recovery action component for Red handfish**
1	Collect egg masses from wild population and hatch in captivity
2	Rear juvenile handfish in captivity
3	Implement habitat management strategies for sites
4	Tagging before release
5	Pre-release conditioning
6	Coordinate translocation of juveniles into known populations
7	Monitor release success
8	Implement education/ engagement initiatives to improve awareness

1.2.1 Recovery action components

1. Collect egg masses from wild population and hatch in captivity.

Two egg masses were collected from Site 2 (Frederick Henry Bay) in November 2019 by Institute for Marine and Antarctic Studies (IMAS) divers. Female handfish associated with egg masses were collected at the same time (to facilitate egg guarding/cleaning), but later released at exact point of capture (neither females performed egg-guarding within the tanks while in captivity).

2. Rear juvenile handfish in captivity.

Eggs began hatching within a day of collection. Handfish were approximately 3-4 mm in length upon hatching. Some initial egg mortality occurred (exact number of eggs was difficult to count since they could not be disturbed before the remaining viable eggs had hatched). From the CSIRO egg clutch collected (also from Site 2) in 2018 (approximately 50+ eggs), 16 survived and were transported to Seahorse World after 3 days post-hatching. Of these, 11 survived. From the IMAS egg clutches collected in 2019, 71 juveniles hatched successfully from eggs (Figure 1-1). Juveniles were fed Instar I *Artemia*, then on-grown *Artemia*. Over the first 4 months, 52 juveniles survived. Most mortality causes were unknown but included some outbreaks of ciliates (that infest the gills) which were treated with consultation by the UTAS veterinarian. At approximately 4 months of age, juveniles transitioned onto eating live amphipods (collected from the Derwent estuary).

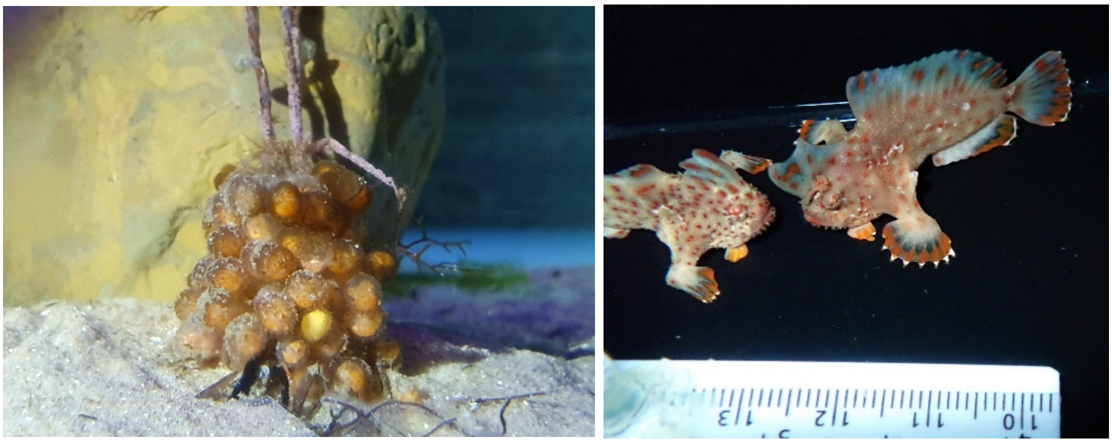

Figure 1-1 Eggs (left) collected and hatched in captivity at IMAS Taroona, and juveniles (right, approximately 7 months old in captivity; images J. Stuart-Smith).

3. Implement habitat restoration strategies for Red handfish sites.

Increases in native urchins (*Heliocidaris erythrogramma*) have occurred at one of the Red handfish sites (Figure 1-2). In late 2018, IMAS divers, working under the direction of a scientific team and the Department of Primary Industries, Parks, Water and Environment (DPIPWE), removed approximately 6,000 urchins from this site. In August 2020, a collaborative effort between the Tasmanian Commercial Divers Association (TCDA), led by President Renison Bell, and IMAS (through J. Stuart-Smith), worked alongside DPIPWE to tackle the increase in urchins at both Red handfish sites. Tasmanian Commercial Divers Association removed 1276 kg (almost 17,000 native urchins) from the over-grazed areas immediately adjacent to both sites (Figure 1-2). This component was not originally identified as part of the release strategy project but was considered an essential component and undertaken with in kind support from TCDA and IMAS.

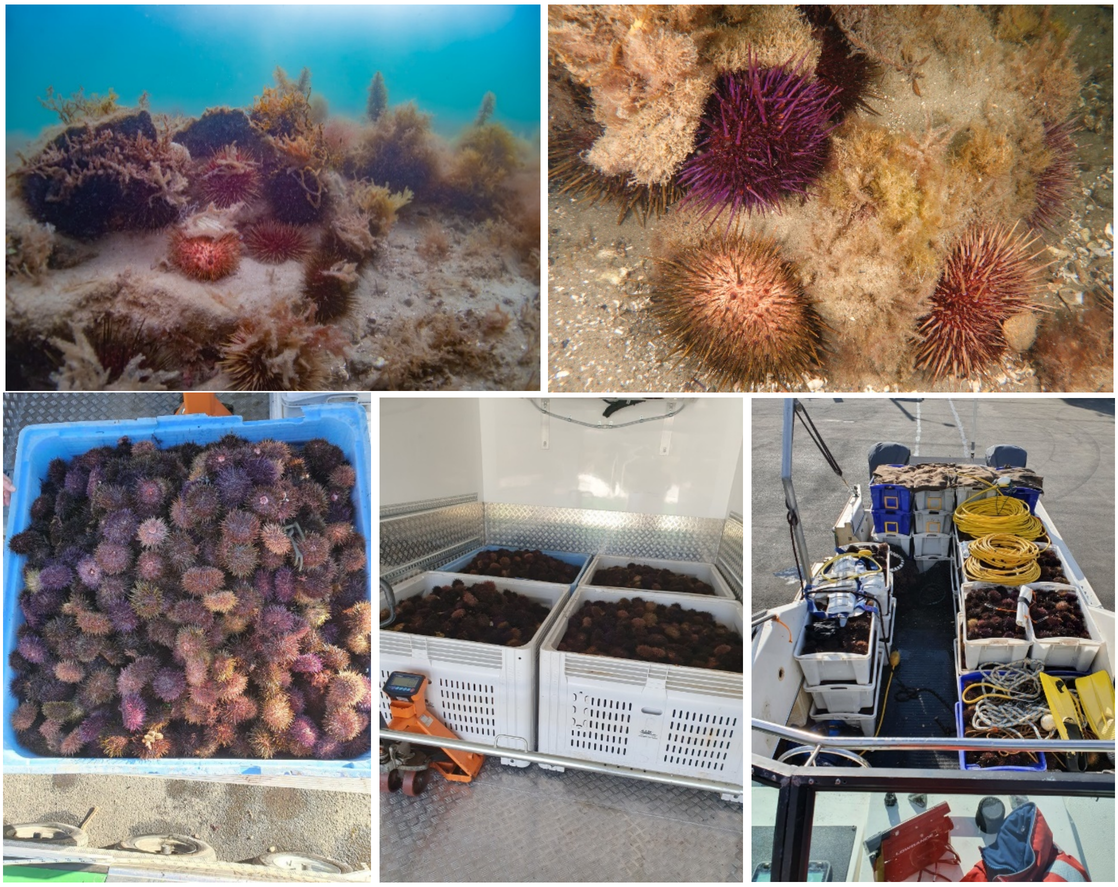

Figure 1-2 Top, Urchins (*Heliocidaris erythrogramma*) grazing Red handfish habitat (images Scott Ling and Tyson Bessell) and bottom, urchin removals via the Tasmanian Commercial Divers Association (images: Renison Bell).

4. Tagging Red handfish before release.

We tagged a subset of juveniles from both Seahorse World (n=6) and IMAS (n=13) during September 2020 – allowing time for recovery prior to conditioning and preparing fish for release. Fish tagging involved sedating fish (using 2-phenoxyethanol 0.4 ml in 1L filtered seawater) until fish failed to react to touch stimulus, usually after ~3 minutes of immersion, a procedure which had been trialled previously on Spotted handfish at CSIRO. Red handfish juveniles were tagged using a visible implant elastomer (VIE) from Northwest Marine Technology (Inc.). Fish were then placed into a recovery aquarium filled with filtered seawater. On occasion it was necessary to move the fish through the water to flush the anaesthetic from the gill cavity before respiration would resume. Tagged fish were returned to the nursery aquaria to allow the insertion wound to heal. Seahorse World juveniles were tagged using pink elastomer on the RHS of the dorsal fin. IMAS juveniles were tagged using yellow elastomer applied beside the dorsal fin (on the LHS for clutch A, and the RHS for clutch B). All fish recovered fully from the anaesthetic and no adverse effects were noted from the tagging procedure.

The day prior to release each fish was photographed (both lateral sides, and a dorsal image with ruler to determine length) so that photographs could be used for identifying individuals (as well as tags, Figure 1-3, 1-4). This component was not originally identified as part of the release strategy but was considered an essential component in informing the effectiveness of the head-starting strategy and was therefore coordinated as an additional activity.

Figure 1-3 Juvenile Red handfish tagged in captivity, and re-sighted in wild (arrow marks yellow tag, which fluoresces under blue light, images J. Stuart-Smith).

5. Pre-release conditioning.

Conditioning of captive-bred/reared animals prior to release into the wild is thought to significantly increase their chance of survival (Lyles and May 1987). It is well known that foraging (Reiriz et al. 1998), anti-predator (Yamaoka et al. 1994) and migratory behaviour (Dittman and Quinn 1996; Dodson 1988) are to a large extent acquired through learning throughout a fish’s lifetime (Brown and Laland 2001). Individuals must be able to procure food and shelter, develop anti-predator skills, interact properly with conspecifics, and orient (navigate, migrate, and/or disperse) in a structurally complex environment (Biggins et al. 1999). Fish reared in complex habitats have enhanced foraging performance (compared to those reared in simple habitats) and therefore increased likelihood of survival (Brown et al. 2003).

Behavioural traits that may influence reintroduction success include locomotion skills (e.g., moving in complex environments, constructing home sites like dens and nests, and movement patterns), predator avoidance (recognition and evasion), foraging (including finding, identifying, acquiring, and handling food), interacting in social groups (including courtship, mating, and raising and training young), and habitat selection.

Given the need to be prepared for the complex reef habitats and mixed macroalgal communities present at the Red handfish release sites, an important component of the Red handfish release and re-stocking strategy included conditioning/acclimatising and exposure to complex habitats and other species prior to release. This component of the release was not considered in the original project proposal but was considered as an essential component and added to the program.

To condition fish, the following occurred:

Seahorse World fish:

- 8 fish (~2 years old) were transported from Seahorse World in Beauty Point to CSIRO, Hobart. Fish were placed in a single aquarium which contained sand substrate, rocks, seaweed and some species they are likely to encounter in the wild (e.g., fishes: *Trachinops caudimaculatus*, *Forsterygion varium*, *Neoodax balteatus*, and invertebrates including pistol shrimp, nudibranch). Fish were maintained in these conditions from Oct 15 – 28/29 October) and fed live amphipods during this time.

IMAS Taroona fish:

- 34 fish for release (~1 year old) were placed in conditioning tanks, rotated through for 3-4 days at a time at IMAS Taroona. Conditioning tanks contained fine rock substrate, rocks, seaweed and some species they are likely to encounter in the wild (e.g., fishes: *Trachinops caudimaculatus, Forsterygion varium*, and invertebrates including hermit crabs, oysters). Fish were also presented amphipods and other crustaceans (e.g., porcelain crab, *Petrolisthes elongatus*) – which they fed on immediately.

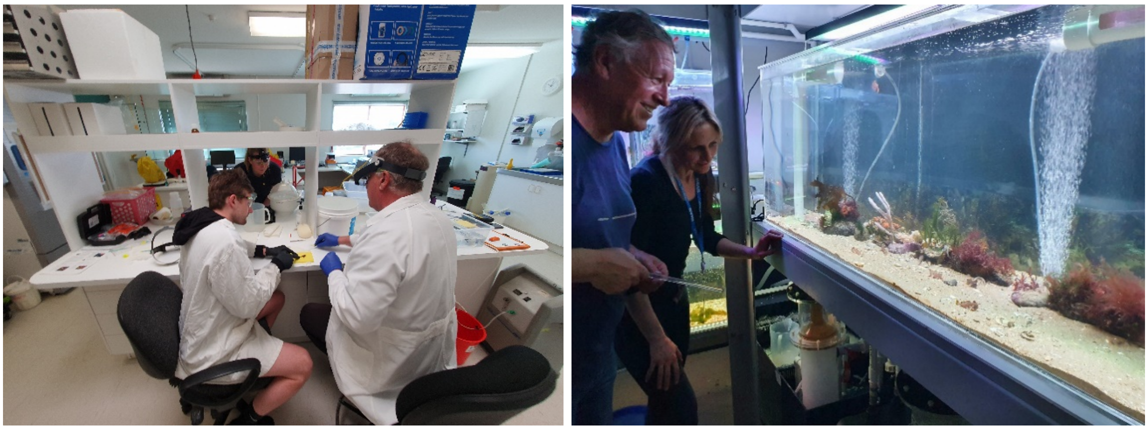

Figure 1-4 Tagging of juvenile Red handfish at IMAS Taroona (left) and conditioning of at CSIRO (image Vanessa Mann).

6. **Coordinate translocation of Red handfish juveniles into known populations.**
   Release of juveniles to known populations (Primrose Sands and Site 2) was conducted over two days on October 28-29, 2020. On October 28, 28 fish were released at 6 sub-surface buoys within the Primrose Sands site. This was comprised of 5 fish raised at Seahorse World (~2 years of age, from a single clutch) and 23 fish raised at IMAS Taroona (~1 year of age, from two different clutches). On October 29, 14 fish were released at 3 sub-surface buoys at Site 2. This was comprised of 3 fish from Seahorse World (~2 years of age, from a single clutch) and 11 fish raised at IMAS Taroona (~1 year of age, from two different clutches).

8. Monitoring release success.

Release points were surveyed in the week following. This included a 3 m diameter search of the seafloor by experienced Red handfish divers from IMAS and Reef Life Survey teams over several days. Some released fish were sighted on every occasion, as detailed in the Table 1-2, Figure 1-5.

Figure 1-5 Release of Red handfish at Site 2 (left, image Antony Cave) and resighting of released individual (right, image Rick Stuart-Smith).

Table 1-2 Post-release surveys and resightings of released juvenile Red handfish.

Site	Release date (# released)	Survey date (# re-sighted)		
Primrose	28-10-20 (28)	30-11-20 (5)	1-11-20 (2)	3-11-20 (2)
Site 2	29-10-20 (14)	30-11-20 (6)	1-11-20 (5)	3-11-20 (3)

9. Implement education/ engagement initiatives to improve awareness of Red handfish

We aimed to improve the profile of the species and work through the Handfish Conservation Project (handsfish.org.au) to increase awareness for the threatened handfish species which will also facilitate conservation policy and management actions, and to generate additional support through fundraising. In addition to this, these components demonstrate impact of our work outside of academic outputs (e.g. Ravenscroft et al. 2017).

The list of media/outreach in the last year alone includes the following (full list of media in Appendix 1, with some notable mentions below):

- Notice of Motion presented in Senate by Senator Peter Whish-Wilson and Senator Nick McKim (December 2020) to note the listing of smooth handfish as extinct and the release of Red handfish back into the wild, and to acknowledge the work of the IMAS/CSIRO/Handfish Conservation Project. The motion was agreed to without any dissent and Senator Whish-Wilson highlighted it as a win for cross-parties working together to get proper backing for the science community in conservation.
- High profile media: National Geographic (https://on.natgeo.com/3lWKcxl), Ocean Geographic (article and online presentation), Scientific American, The Australian (https://bit.ly/31ODnG5), ABC feature on Tasmanian threatened species, Hank Green video blog (https://bit.ly/2ziGDNX).

- Community presentations: Tasmanian Mermaids (dive club) on International Women's Dive Day, Friends of Tasmanian Museum and Art Gallery, UTAS Threatened Species Day online forum.
- The Handfish Conservation Project website and social media (handfish.org.au)
- 12+ local radio interviews, including a children's radio activity segment during COVID lock-down (via Leon Compton ABC) to encourage locals to learn about Tasmanian marine life.
- Blackmans Bay Primary School handfish presentation (Grade 2 and 6), Mt Nelson Primary School (Grade 2), Margate Primary School (Grade 2,3,6).
- Sorell community presentation which included Sorell Mayor and councillors (J. Stuart-Smith and T. Bessell).
- UTAS Animal Ethics Committee presentation (J. Stuart-Smith).

The benefits to the community have gone beyond the protection of our natural heritage, and have also significantly reached the arts community and schools, enriching our community in ways most scientific endeavours do not. This has included:

- supported a funding proposal for a local artist to purchase paint and develop stencils that resulted in the creation of a public Red handfish wall mural in North Hobart.
- supplied imagery and information to artist Melanie Stranger to assist her drawing of Red handfish (with artwork sold in 2021 and a portion of profits donated to the Handfish Conservation Project).
- set up a handfish exhibition at the Kingston Library that involved ten local artists
- has collaborated with other artists working on handfish projects (including Wilderness Bling, Melanie Stranger, Kat Richardson Creative, Poco People, Manning Polymer Clay, Tim Fountain, Jane Bamford).
- facilitated sessions at two Archaica Schola 'Paint n Sip' events for the public to feature the plight of handfish through art and engagement.
- Assisted with local author children's book which has now been published: (https://katherinerichardson.net/index.php/product-category/books/) and is being used as prescribed text in the Charles Sturt University Australian Children's Literature class in 2021.

Fostering public support is considered essential for developing long-term, sustainable management of handfish populations, improving our ability to properly manage these species and their habitats.

1.3 Discussion

Reintroduction of captive-reared juveniles has not previously been trialled for Red handfish, and it was the first-time juveniles had been hatched and maintained in captivity for long periods (up to 2 years). It was also the first time that Red handfish had been tagged, conditioned, and released back into the wild. With an estimated 100 adult fish thought to remain in the wild, releasing 42 heathy juveniles was considered a significant boost to the wild population and species survival. In post-release monitoring of released juveniles, we recorded re-sightings on all three occasions – demonstrating initial transition of juveniles to the wild had been successful and implying that head-starting likely provides a viable and effective option for population bolstering.

While the early post-release monitoring or Red handfish indicates early success of the head-starting program, further work is needed to understand long term survival of released juveniles by implementing re-capture surveys over greater time periods to determine the success of juveniles reaching maturity (in combination with field studies that assess wild juvenile survival in wild populations). Improved biological information on habitat use and requirements, movement, diurnal activity patterns, and breeding cues are needed to better inform future reintroduction programs, and in particular, to better inform conditioning of juveniles before release into the wild. Release survival may be improved by pre-release exposure to key biotic (e.g. predator avoidance training, specific habitat acclimatisation) and abiotic environmental cues, and the implementation of targeted spatial and temporal release strategies (e.g. Alberts 2007) to improve behavioural competency – which has not been assessed for Red handfish.

Future focus

Continuation of the head-starting program is essential to the immediate continuation of the species at the two known Red handfish populations, and must be conducted in combination with habitat monitoring, restoration and management. Translocation (assisted colonisation) – moving individuals to populate to new areas - should now be considered as a viable option for Red handfish given that: populations are highly fragmented, human-driven impacts have reduced habitat quality and connectivity, and re-introduction of captive-reared juveniles has been demonstrated a viable option (see translocation feasibility framework in Hoegh-Guldberg et al. 2008 Fig 1-6). This will require determination of appropriate sites which meet the biophysical needs of the species and assessments of risks and feasibility (discussed in Butt et al. 2021; Hoegh-Guldberg et al. 2008; Hunter 2007; IUCN/SSC 2013; McLachlan et al. 2007). Assessment of the potential for *in situ* release-site pens for minimizing stress and facilitating gradual acclimatization should be considered if feasible.

Implementation of captive-breeding studies which may also double as insurance populations should also be considered an important conservation measure given the success of the captive-rearing, head-starting and reintroduction program.

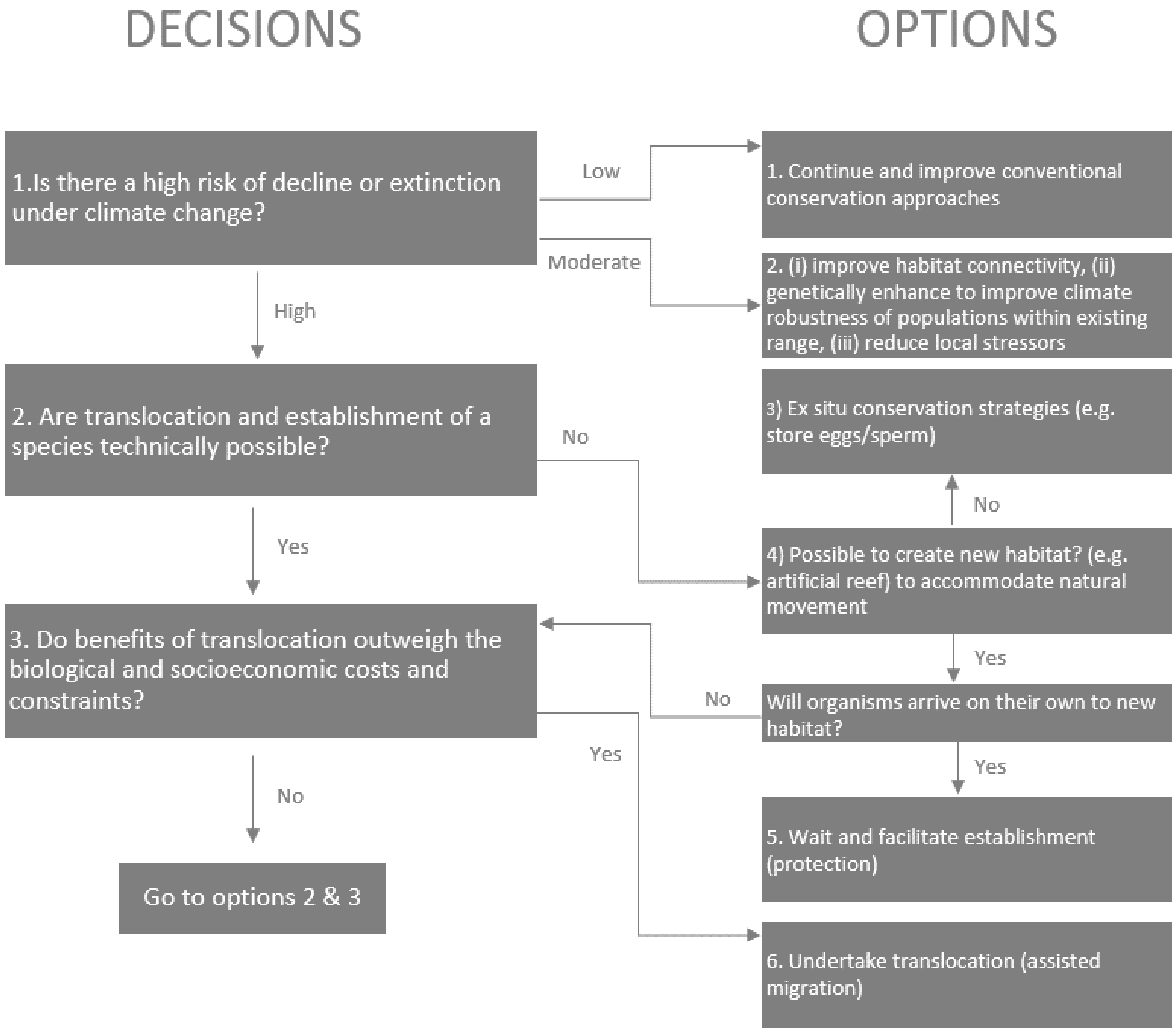

Figure 1-6 Decision framework for assessing possible species translocation for extinction prevention (adapted from Hoegh-Guldberg et al. 2008)

With population size critically low, continued habitat decline, and unknown impacts of climate change ahead, Red handfish conservation requires immediate and sustained action. Fostering political and public support is paramount to implementing important conservation strategies which may require protection or reduction of disturbance to Red handfish habitats. Delays in policy formation and lack of on-ground action and continued intervention will likely see this species go extinct.

2. RED AND SPOTTED HANDFISH MORPHOMETRIC STUDY

2.1 Background

Establishing a full captive rearing/breeding and release program will take several years and require more research on aspects such as cues required for maturation, mating, spawning, and optimal conditions needed for egg development, juvenile growth, and maximising survival prior to release. This needs to be coupled with assessment of released juveniles through to maturity in the wild (i.e., the ultimate success of released juveniles). Determination of gender is important to establishing a captive breeding program. This study aims to assess whether sex determination of individuals is possible from external morphometric characteristics. Any non-invasive method would expediate implementation of captive-breeding trials, lower risk to broodstock, and provide a rapid means to understand sex ratios in the wild (which are currently unknown).

Sexual dimorphism is common in fishes, and morphometrics studies have been widely used to distinguish sexes in birds, reptiles, mammals and crustaceans. A literature review was undertaken first to explore the range of measurements and common sources of sexual dimorphism observed in fishes, with particular attention to measurements associated with the closely related and better-studied angler fishes (also known as frogfishes). This review guided the characters assessed for each individual handfish in this study. Some examples are shown in Figure 2-1, and provided in Falahatkar and Poursaeid (2014) and Oliveira and Almada (1995). There have also been anecdotal reports of larger nostril size in male Red handfish, perhaps for use of scent to find females (example of differences in nostril size shown in Figure 2-1 – sex unknown). The number and arrangement of the multiple sets of nostrils may also differ between sexes, as well as various other body traits.

Figure 2-1 Examples of Red handfish with small (left) and large (right) nostril size (images T. Bessell, R. Stuart-Smith)

Literature

Sexual size dimorphism (SSD), or morphological differentiation between males and females, is prevalent in the animal kingdom (Andersson 1994; Fairbairn et al. 2007; Fairbairn 1997; Shine 1990). It can be so extreme that the sexes of a single species were originally thought separate species, or non-existent and difficult to tell the sexes apart.

The ultimate causes of SSD are sexual selection (fecundity or mate selection) and niche divergence hypothesis (to allow gender differences in ecological niches – sexual segregation) (Parker 1992; Shine 1990). SSD is linked to growth, behaviour, ecology, physiology, demography, and evolution. There are often complex and intertwined functions and often reflect trade-offs in fecundity, mate selection strategies, and habitat utilisation. The proximate causes or the mechanisms behind SSD evolution include ontogenetic, physiological and physiological mechanisms (e.g., stage at occurrence, differing post maturity growth, age at maturity, different endocrine mechanisms). Variation in the degree of SSD among species and taxa is generally considered to arise from differences in the relative intensity of each of these selective forces (Horne et al. 2020). As well as body size, sexually selected traits or secondary sexual characters (ornaments and armaments) are often common in the animal kingdom – an ornament is a trait that enhances appearance for mate attraction, an armament a trait used in protection or competition (Berglund et al. 1996; Hill 2014).On top of this, geographic (e.g. latitudinal) variation in the degree of SSD is known to exist for many groups, e.g. fishes (Estlander et al. 2017; Tamate and Maekawa 2006), birds (Friedman and Remeš 2016), and reptiles (Agha et al. 2018).

In fishes, males are often the smaller sex (Parker 1992). Social organisation, mating system, and mode of reproduction (e.g., mouth brooders) also have implications for SSD. In protogynous systems where males mate with many females, male reproductive success is strongly linked to body size, so they tend to be larger (McCormick et al. 2010). Deep-sea Anglerfish (suborder Ceratioidei) exhibit one of the most extreme occurrences of SSD. This presents in the form of sexual parasitism where males are dwarfed and attach themselves (either temporarily or permanently) to bodies of relatively gigantic females (Pietsch 1976, 2005) which can be over 60 times as long and half a million times as heavy as the males. But in other species, male-male interactions can drive trait evolution, such as dorsal fin size in *Xiphophorus birchmanni* (Robinson et al. 2011) when these are correlated with (direct or indirect) benefits that females receive from those males (Robinson et al. 2011).

Specific sexual size dimorphism in other fishes can include multiple trait differences. In cichlids (Oliveira and Almada 1995), males have distinctive and conspicuous breeding colours, are generally bigger than females and in some species have enlarged jaws and unicuspid teeth when mature; other males have a tassel-like appendage on the genital papilla, dorsal and anal fins pointed in mature males and rounded in females [*Sarotherodon galilaeus* and *Oreochromis aureus*, (Chervinski 1965), pelvic fins reaching or passing the anus in males but not in females [*Tilapia zillii* (Gervais), *S. galilaeus* and *O. aureus*, Chervinski, 1983], males with one urogenital opening and females with two [*T. zillii*, *S. galilaeus* and *O. aureus*, Chervinski, 1983; *O. mossambicus*, (Datta and Roy 1984) there is a thicker and continuous dorsal fin in mature males and notched dorsal fin in females (O. aureus, Fishelson 1966), and thicker lip in upper jaw in mature males (O. mossambicus, Seitz, 1949 in Oliveira and Almada 1995). In cardinalfishes, cichlids, jawfishes, which are all mouth brooders (Hoey et al. 2012) – generally mouth size is larger in males (larger buccal cavity), and in anglerfishes (e.g. Pietsch 1976, 2005) overall body size (larger in females) and nostril size are known sexually dimorphic traits in some species.

Being unable to differentiate between sexes by external morphology (i.e., non-destructively) – which is as well as being important for understanding life history strategy (also time at maturation, habitat use, behaviour etc), is also severely limiting the development of these essential captive breeding programs. The caveats to this are that we do not have any data on size at maturity for handfishes, and we are unable to destructively sample many known specimens to be able to validate trait measurements (although for some, sex has been confirmed by dissection or x-ray).

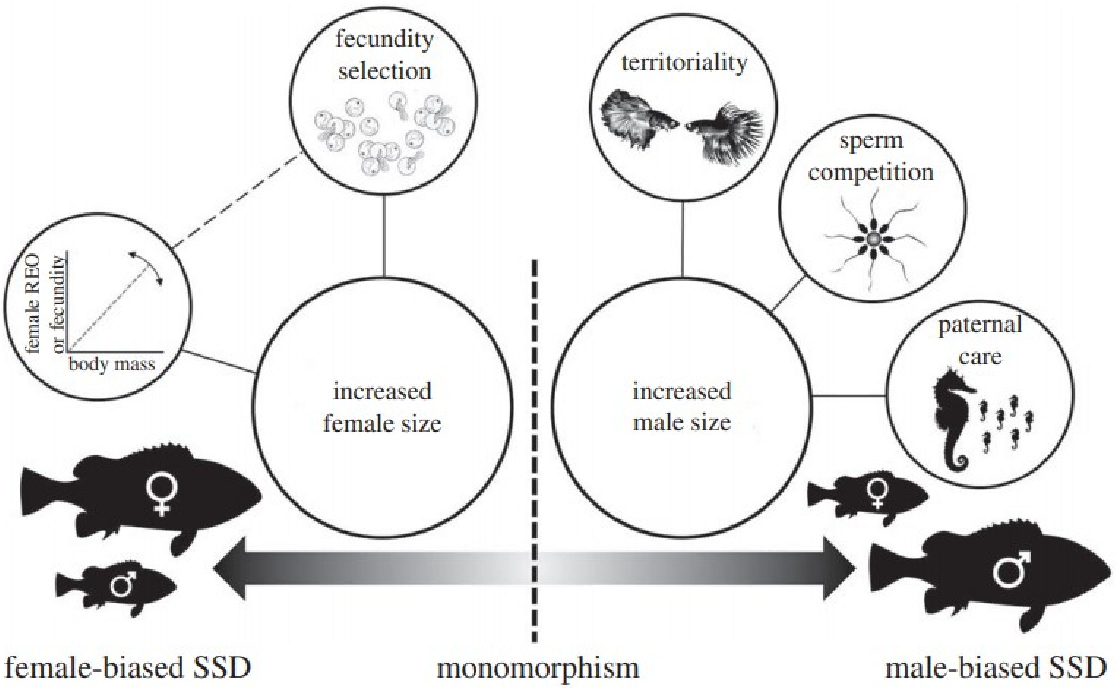

Figure 2-2 A summary of the key life-history characteristics that might select for increased body size in females versus in males (taken from Horne et al. 2020).

Note that female-biased SSD is usually attributed to fecundity, and the variation dependent on the allometric scaling of reproductive energy output (REO) or fecundity in females (i.e., the extent that larger females reproduce is proportionately more than smaller individuals). Selection for increased male size may be associated with territoriality, sperm competition, and/or paternal care.

2.2 Study species

Red handfish (*Thymichthys politus)* and Spotted handfish *(Brachionichthys hirsutus*, Figure 2-3) are small, demersal marine fish that are found only in south-eastern Tasmania (Bruce et al. 1999; Last and Gledhill 2009). They are members of the Order Lophiiformes (Anglerfishes), and in the Family Brachionichthyidae. They lack swim bladders and prefer to walk on the sea floor using modified pectoral fins that resemble human hands rather than swim. Red handfish are known from only two small populations in Frederick Henry Bay, and are found in shallow rocky reef habitat, usually amongst seaweed or seagrass. Spotted handfish are found only in the Derwent estuary, and are found in open sand/silt habitat. Spotted Handfish are the most well-studied of all 14 handfish species (due to work on captive breeding trials at the CSIRO).

Information is lacking on biological information for both Red and Spotted handfish. Both species are listed as Critically Endangered on the IUCN Red List of Threatened Species and the Environment Protection and Biodiversity Conservation Act 1999, and conservation strategies are turning towards aspects including captive rearing and reintroduction programs. The rationale was that any morphological variation substantial enough to allow future usage of visual inspection for determining sex would show up with two distinct groups for any given measurement or set of measurements.

Figure 2-3 Spotted handfish (left, *Brachionichthys hirsutus*) and Red handfish (right, *Thymichthys politus*); images: R. Stuart-Smith.

2.3 Morphological measurements

Adult and sub-adult *T. politus* and *B. hirsutus* preserved specimens and underwater images were used for analysing morphometrics (comprising of specimens from the CSIRO Australian National Fish Collection and underwater images were used for analysing morphometrics (Table 2-1). Individuals were measured for the morphological traits using electronic callipers (±0.1 mm) for preserved specimens (e.g. Figure 2-4) and using Image J software for digital records (Schneider et al. 2012). Note digital image size calibration occurred using a ruler in images or from size taken *in situ*. Before data analysis, all measurements were expressed as a proportion of the standard length to ensure quantitative comparison could be made between individuals of different overall size. Two sets of data analysis were then undertaken; the first exploring the distribution of values from each of the individual measurements (i.e., a univariate approach) and the second exploring the similarity between individuals based on the entire set of measurements. The univariate analysis involved plotting the density distributions of each measurement to contrast differences in body shapes between species and look for any signs of bimodality in the distribution of a measurement within species. The multivariate analysis was done using Principal Coordinates analysis using the vegan package in *R* and based on Manhattan distance of standardised data. This analysis could only be undertaken for the subset included which laboratory specimens on which all measurements could be taken (i.e. complete cases). Not all measurements were possible from most of the photographed individuals from the wild, and so these were unable to be analysed in the multivariate analysis. This analysis aimed to see whether any natural groupings emerged among individuals with similar sets of measurements within species, and so red and spotted handfish were considered separately.

Trait measurements were divided by standard length, so they were proportional to body size. For left and right traits, we took an average of measurements from both sides before including them in analyses.

Table 2-1 Morphometric measurements taken from images and specimens of *B. hirsutus* and *T. politus.*

#	Region	Term	Abbr.	Measurement
1	Body	Total length	TL	Anterior edge of the upper lip to the tip of middle rays of caudal fin
2		Standard length	SL	Anterior edge of the upper lip to the base of the caudal fin
3		Snout-vent length	SVL	Anterior edge of the upper lip to anterior insertion of the first anal fin
4		Body depth (anal fin origin)	BDa	Maximum depth
5		Body depth (2nd dorsal origin)	BDd	Maximum depth
6		Maximum body width	MB	Maximum width
7		Pre-dorsal length	PDL	Anterior edge of the upper lip to anterior insertion of the 1st dorsal fin
8		Pre-pelvic length	PPL	Anterior edge of the upper lip to the base of the pelvic fins
9	Head	Illicium length	IL	To the base of the esca
10		Esca length	EL	From illicium insertion
11		Pre-illicial distance	PID	from the snout tip to the anterior articulation of the illicium
12		Head height	HH	Maximum height between the dorsal and ventral contour of the head
13		Head width	HW	Widest point before operculum
14		Head length	HL	Anterior edge of the upper lip to the most posterior part of the gill pore (anterior-most edge of the opercular opening)
15	Eye	Eye size	ES	Horizontal diameter of the iris
16		Interorbital width	IW	Least width of the skull between the eyes
17	Nostrils	Nostril size (upper)	NSU	Horizontal diameter
18		Nostril size (lower)	NSL	Horizontal diameter
19		Upper dist apart	NU	Shortest distance between upper nostrils
20		Lower dist apart	NL	Shortest distance between lower nostrils
21		Dist between upper & lower	NDl NDr	Height between upper and lower nostrils (both sides – l & r)
22	Mouth	Premaxilla width	PMW	Maximum width of the upper lip
23		Mandible width	MW	Maximum width of the lower lip
24		Premaxilla length	PML	Anterior edge of the upper lip to the posterior edge of the jaw
25	Dorsal fins	Height (1st)	1DH	Length of the largest fin ray of the dorsal fin
26		Length (1st)	1DL	Length between insertion
27		Height (2nd	2DH	Length of the largest fin ray of the dorsal fin
28		Length (2nd)	2DL	Length between insertion
29		Interdorsal length	IDL	Dist from base of 3rd dorsal spine to origin of second dorsal fin
30	Caudal fin	Height	CH	Length of the largest fin ray
31		Length	CL	Length between insertion
32		Caudal peduncle height	PDH	Maximum height between dorsal & ventral contour of caudal peduncle
33	Pelvic fins	Length	PL	Length of the largest fin ray
34		Width	PW	Width at widest part
35		Inter-pelvic distance	IPL	Distance between pelvic fins
36	Pect fins	Length	PecL	Length of the largest fin ray
37		Width	PecW	Width at widest part
38		Pectoral fin rays	PFR	Number/count
39	Anal fin	Height	AH	Length of the largest fin ray
40		Length	AL	Length between insertion points

Figure 2-4 Red and Spotted handfish images based on original specimens (*Thymichthys politus* – registered H 782-01, 89.5mm TL, and H797-1 *Brachionichthys hirsutus* H 4118-01, 88.7 mm TL), photos: Australian National Fish Collection, CSIRO.

2.4 Results

We identified 40 morphometric trait variables (Table 2-1) to be measured for preserved specimens, although not all measurements could be attained for all specimens (due to preservation issues or damaged specimens), and a sub-set of these measurements could be attained from images (depending on whether fins were clearly displayed, etc).

The univariate analysis showed a wide variation in values of most measurements, for both Spotted and Red handfish, and limited evidence of clear intraspecific splits (Figure 2-5). For Red handfish, snout-vent length (top left), body width (top row, 2nd from left), upper nostril size (3rd row, 2nd from left), showed signs of possible bimodality, but are not convincing. Spotted handfish showed even fewer signs of variation in any measurement, with a pattern in upper nostril size tending towards that seen in red handfish. Despite limited intra-specific differentiation among the measurements, clear differences exist between red and spotted handfish in many of the measurements.

In the multivariate analysis, the first 3 axes of the PCO describe 58% of the variation among individual Red handfish in overall body shape (i.e. across all measurements), yet when plotting the first two axes (which collectively represent 44% and 36% of the variation for Red and Spotted handfishes, respectively), no groupings among individual are evident (Fig 2-6). The primary axis of variation (PCO1) is heavily influenced by two outliers, and the second axis (PCO2) distinguishes individuals on a continuous gradient of head-related measurements (e.g. interdorsal and second dorsal length in Spotted handfish, esca length in Red handfish). The first three axes of the spotted handfish PCO explained 46% of the variation among individuals, and also showed no signs of groupings. No single measurements or set of measurements appeared to dominate in describing variation in the direction of either of the first two axes presented in Figure 2-6.

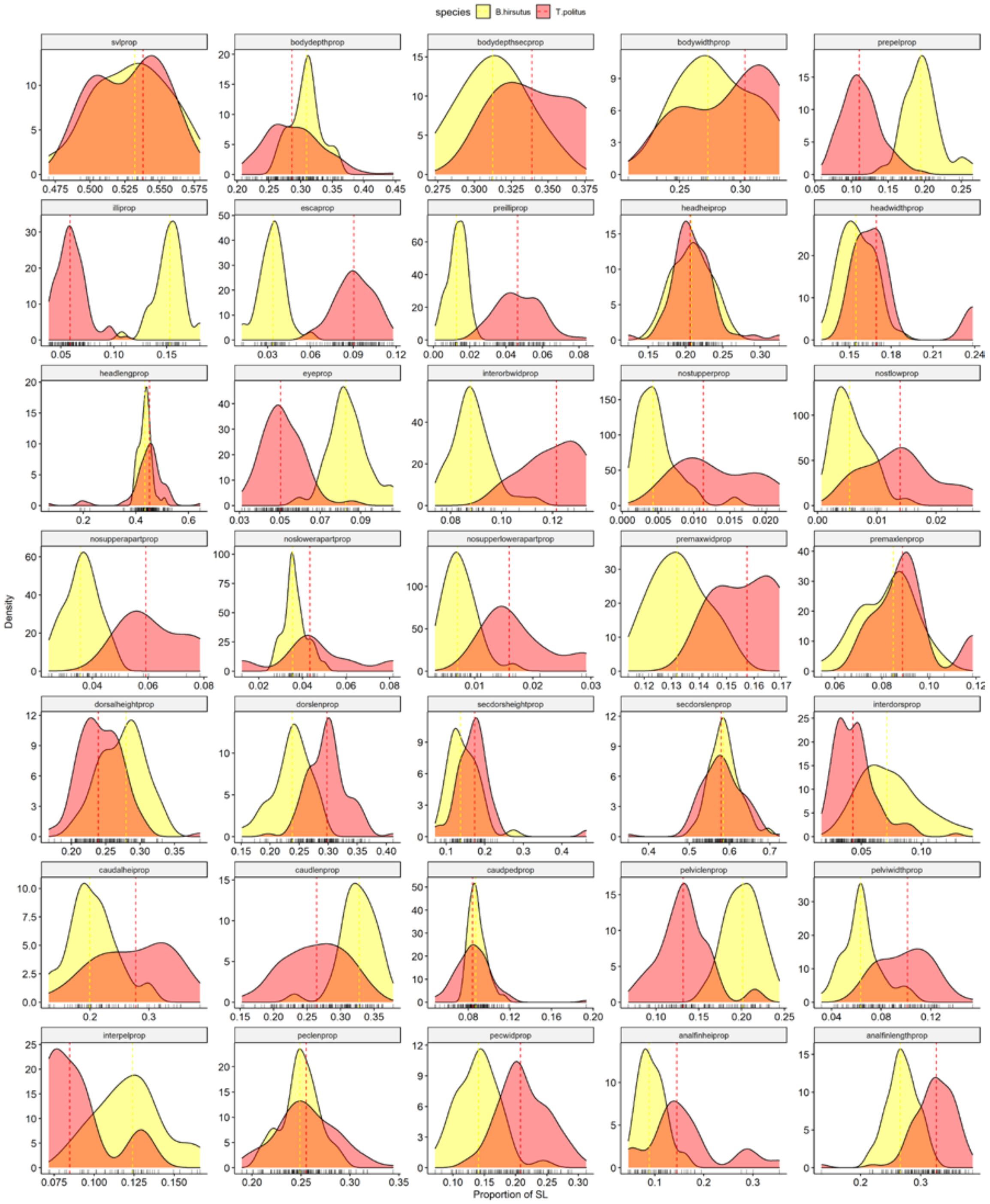

Figure 2-5 Density plots showing the distribution of values for each measurement for Spotted handfish (yellow, n=38) and Red handfish (pink, n=114). Dashed vertical lines represent the median for each species, and individual data points are shown as a rug chart on the x axis.

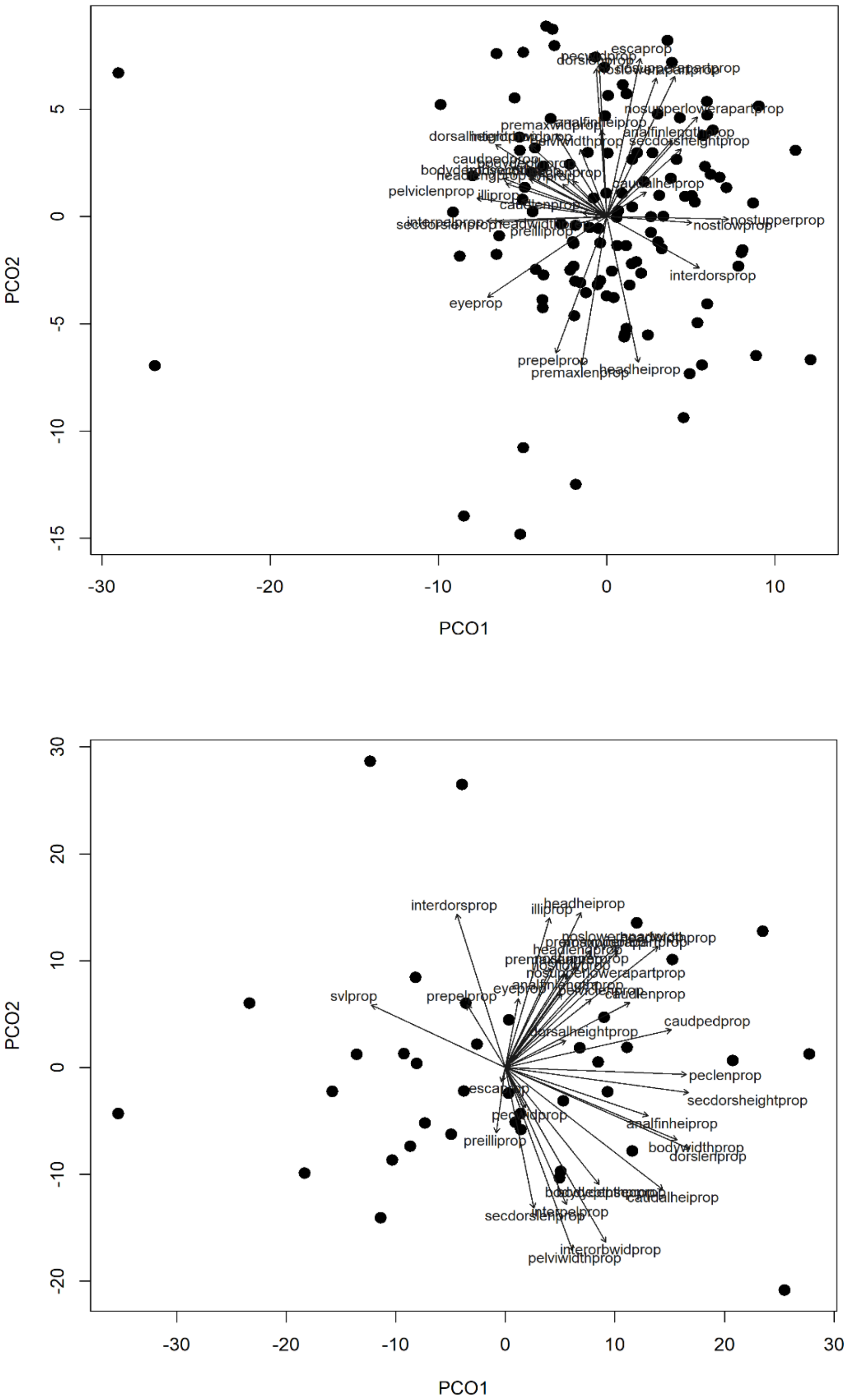

Figure 2-6 Principle Coordinates Analyses of proportional morphometric traits for Red (top) and Spotted (bottom) handfishes. The first two axes shown describe 44% and 36% of variation in body shape amongst individuals of each of these two species respectively. Individual measurements are overlaid as vectors with their length related to their correlation with the first two axes displayed.

2.5 Discussion

We examined whether there were any possible sex-related (or other) proportional size differences between Red and Spotted handfish adults. These data also allowed us to investigate differences in these proportional trait sizes between Red and Spotted handfish. We were unable to discern any clear differences between male and female handfish using external morphometric analyses. Density plots (Figure 2-5) indicated some potential intraspecies variation, such as upper nostril size, inter-dorsal length, dorsal height in Red handfish, and upper nostril size in Spotted handfish – but there were no clear groupings. For some of these traits it is possible that some distinctions could emerge from analysis of a much larger dataset, yet variation so subtle may be of limited value for ascribing gender to individuals based on visual inspection, so may not serve the desired application here.

The density plots showed some very clear separation *between* species, however. Notable differences between species included relative included body width, pre pelvic length, illicium length, esca length, pre-illicial distance, eye size, interorbital width nostril size (upper and lower), pre-maxilla width, dorsal fin length, inter-dorsal length, caudal and pelvic/pectoral fin size and anal fin length. These differences include many of the characteristics used to distinguish the species taxonomically (see below) but may be useful in elucidating different life history strategies between species, including aspects of habitat use and behaviour. This would be an avenue for further research.

The illicium structures of handfishes were compared in Last and Gledhill (2009), and Spotted handfish have a longer, slender illicium and relatively small esca, compared to Red handfish, which have a smaller, thicker illicium and fluffy esca. The pre-illicial distance was greater for Red handfish (distance between tip of the snout to anterior base of the illicium). Although this structure has been hypothesised to be associated with feeding (perhaps inferred from the role of the same structure in anglerfishes), limited observation on illicium use has been undertaken for either species, and it is thus even less clear why these differences between the species have evolved.

Although the family Brachionichthyidae possesses several characteristics that the literature suggests should select for larger female size, such as being oviparous and having females guarding eggs, they do also have external fertilization and are demersal, which in theory may select for larger male size. The data provided here were not able to determine any clear differences in body size (or proportional trait sizes) within species.

Limitations

Inability to properly evaluate the features hypothesised to differ between sexes (nostril size) was apparent due to: (1) Too few preserved specimens (especially where sex could be confirmed), and (2) A lack of purpose-collected images that would support more accurate and complete measurement-taking (i.e., high resolution front-on images with scalebar). In addition, we were unable to dissect any of the preserved specimens in the CSIRO Australian National Fish Collection. This meant that neither hypotheses built on observation of individuals of known sex, nor validation of any implied differences were possible. In addition, Spotted handfish morphometrics were based on preserved specimens, while Red handfish measurements were a mix of preserved and images. Preserving techniques may influence size and shape of traits.

Further work

Lack of clear separation between males and females based on morphological trait analysis indicates that future work should be focused on other methods for non-destructive sex determination, such as the use of hormones and genetic signals (sex-determining genes). In addition, five Spotted handfish adults held in captivity at Sea Life Melbourne Aquarium underwent ultrasound (under the guidance of Dr. Shane Simpson from the Unusual Pet Vets) to confirm the sexes of these fish. A 18MHz Linear Probe was used. The ultrasound was performed with the fish contained in a plastic bag in water, no sedation was required (Figure 2-7). Clear sonogram images and results were produced using this probe and method (see Appendix 2). As such, this represents a viable option for determining sex in individuals in captivity but is limited to those with mature gonads.

Figure 2-7 Photos of Spotted handfish ultrasound performed at Sea Life Aquarium Melbourne (image courtesy of The Aquarium Vet and Paul Hale)

3. PRELIMINARY MODELLING OF SPOTTED HANDFISH POPULATIONS

3.1 Data preparation and estimates of handfish density over the time-series

We have developed a 22-year longitudinal database of 80 surveys for Spotted handfish (Table 3-1) at 9 locations in the Derwent estuary. These surveys were undertaken between 1997 and 2019. Other surveys have been taken at various sites outside of the Derwent estuary (e.g. Primrose Sands, Simpsons Point, Flathead Bay), but these nine locations represent the best longitudinal time-series. Surveys were each composed of multiple underwater visual census transects by SCUBA divers, though the number of transects varied between study periods as did the method for parameterising the length of the transects.

Table 3-1 Numbers and totals of surveys taken by location across the NESP sampling period (2015-2019) and for all previous samples (1997-2014).

Location	1997-2014	2015-2019	Total
Battery Point	5	5	10
Bellerive	1	5	6
Halfmoon Bay	5	5	10
Howrah Beach	1	5	6
Mary-Ann Bay	2	5	7
Opossum Bay	9	5	14
Ralphs Bay	3	5	8
Sandy Bay	6	5	11
Tranmere	3	5	8
	35	45	80

3.2 Survey methods 1997-2019

In 1997, various survey methods were trialled at Opossum Bay to determine the best approach for population studies (Green and Bruce 1998), with randomised reel transects determined as the most suitable approach.

Transects all originated from a 6 m depth baseline transect of 500 m length that ran parallel to the shoreline. Start positions along the baseline transect was randomly selected from 51 points which were spaced 10 m apart. Initially two 100 m transects reels were joined to make a 200 m transect, with searching terminated once 10 m depth was reached. This technique was then slightly modified (1998 onwards) with a single 100 m transect reel, which ran from the baseline transect to depths of 10-12 m dependent on the location. From experience it was concluded that divers could only successfully observe Spotted handfish across a search width of 1.5 m, and with a buddy pair of SCUBA divers this resulted in 3 m x 100 m search swaths. This swath width has remained consistent across all years in the database.

Surveys of 42 transects were conducted at each location and the total length of encountered fish was measured with vernier callipers, a left-side photo of the fish was taken and the presence/absence of handfish egg masses was also noted. Numbers of the introduced northern pacific seastar *Asterias amurensis* and the stalked ascidian *Sycozoa* sp. were also counted along the transects.

Field work continued in 1998-2001 to facilitate the implementation of the first Spotted Handfish Recovery plan (Bruce and Green 1998) at two locations, Opossum and Halfmoon Bay in the lower Derwent estuary in 1998-2000. In 1999, repeat surveys in spring and autumn were taken (83 surveys for each location), and in 2000, 12 transects were undertaken at Mary Ann Bay. Only Opossum Bay was surveyed in 2001.

Population surveys were again conducted for the second Spotted Handfish Recovery Plan 2002–2006 (Green and Bruce 2002). This included resurveying Opossum and Half Moon Bay in 2002 and 2005. Two additional sites, Bellerive and Ralphs Bay were also surveyed in 2005 (Green 2005).

The Spotted Handfish Recovery Plan fieldwork for 2006/07 involved surveying many potential sites in the Derwent estuary from November 2006 to April 2007. In 2008, testing was carried out at a single site (Tranmere) to assist power modelling to determine how many transects/sampling occasions were required to detect robust numbers of handfish for density estimates. Standard monitoring surveys were conducted at the two existing sites, Opossum and Halfmoon Bay (Green 2009).

Monitoring for handfish populations in the Derwent estuary continued in 2011 to 2014 with community and contractor dive surveys (Green et al. 2012a). This resulted in a change of method, with 2 weighted 100 m transect lines joined to make 200 m length with surface buoys at 0 m, 100 m and 200 m along the line. Each end of the line was held in situ with a 1.5 kg diver’s weight and marked by a surface buoy during deployment.

Transects were laid diagonally against the depth gradient in 5 – 12 m depth, with transect lines set and reset parallel over the survey area using the surface floats as guides. Divers each searched a 1.5 m width area swimming from deep to shallow depths along the transect. The target was 40 transects at a location during a survey period. In addition, two 50 m transects were searched by divers surveying a width of 2 m to count the introduced northern Pacific seastar (*Asterias amurensis)* and the stalked ascidian (*Sycozoa* sp).

From 2014 diver surveys have used the survey method described in Lynch et al. (2015). This new method used longer (200-300 m) GPS parameterised transects (divers tow a GPS on a surface float). Transect would start at one depth, move in a randomised (coin toss) direction parallel to the shoreline and then at 30 min the divers would swim towards the shore to a depth at least >1 m shallower and start a new transect along the back bearing. Fish observed on the transit between transects were measured but not included in estimates of densities.

The longer transect lengths were modelled to be a more powerful method for detection of handfish compared to 100 m reel transects, both suppressing zero counts in the dataset and increasing area searched per dive by eliminating reel handling time. Dive bottom time was limited to 60 min per transect (30 minutes each direction) but to avoid short transects an additional 3 min was added to each dive if a fish was observed, to account for non-searching fish processing time. Dive bottom time was capped at 70 min, regardless of the number of fish observed.

Transect start locations and directions were determined using spatially balanced randomisations (Foster et al. 2017), within location boundaries developed by Mark Green across 1997-2014. Power modelling to determine the most effective number of transects to undertake before additional transects added very little precision were found to be between 8-10 per site (Wong et al. 2018). The increase in efficiency and power from this GPS method allowed for many more sites to be monitored per year, with a functionally orthogonal dataset produced for nine sites between 2015-2019. The COVID-19 pandemic resulted in the 2020 surveys being abandoned.

Data from both periods 1997-2014 and 2015-2019 were combined into a single dataset and analysed to produce a time-series of average density estimates with standard errors across 9 sites (Figure 3.1).

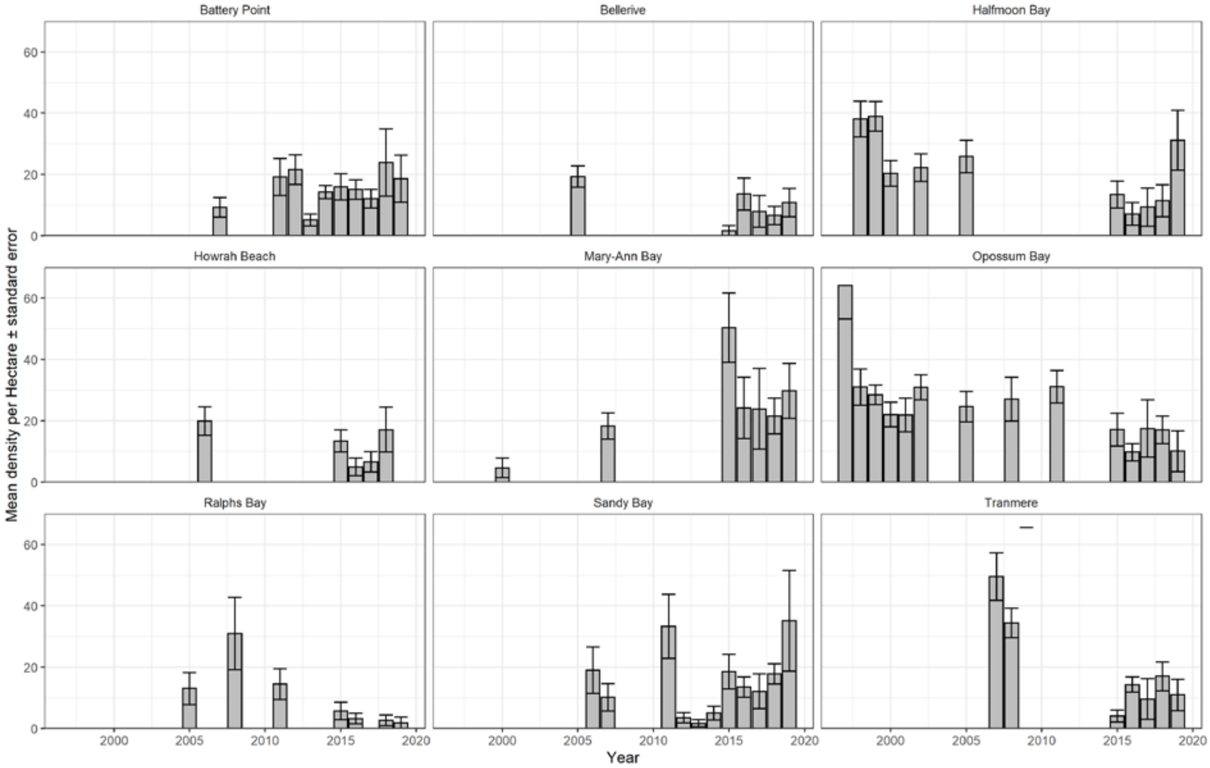

Figure 3-1 Densities of Spotted handfish over time for 9 locations in the Derwent estuary

3.3 Modelling

We conducted preliminary modelling of the handfish population dynamics with a generalised liner mixed model (GLMM) using R (RStudio 2020). Our longitudinal dataset of handfish count data, which extended from 1997 – 2019, was fitted to a random regression model (Laird and Ware 1982), with sample year transformed into a scaled, numeric, fixed effect and location as a factorial random effect. Counts of handfish were transformed into densities for the model with a log link function based on swath area searched by divers per transect.

Three model runs were conducted to explore the data. These were: a) 1997-2019, which represented the entire database, b) 2015-2019, which is the most modern data and was strongly orthogonal, with all locations surveyed each year with similar effort, and c) 1997-2014, which is a sparser dataset, with numerous data gaps for years and locations.

The initial model run of the entire dataset (1997-2019) (Figure 3-2) suggested a significant overall decline in the total population of handfish ($z = -2.288$, $p = 0.0221$). There was also high variance between sites (location variance = 0.1239) and a negative correlation between locations and year (-0.51) suggested that densely populated locations decreased more rapidly than less densely populated sites.

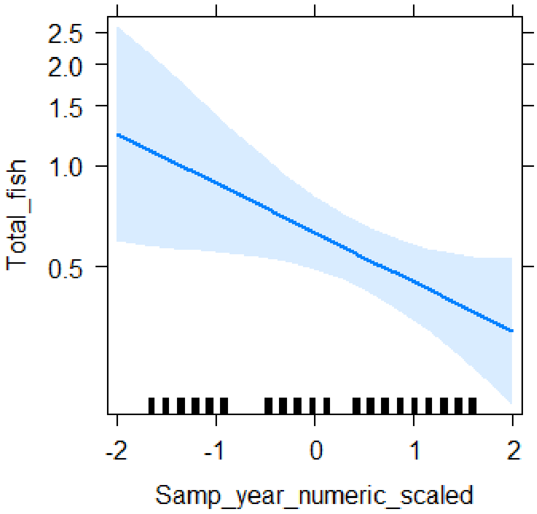

Figure 3-2 Modelled trend of handfish density between 1997-2019

The second model run from the data sub-set of the more recent observations (2015-2019) (Figure 3-3), suggested that the modern population was stable with, perhaps, the suggestion of positive increase, though this was not significant (z = 1.357, p = 0.175).

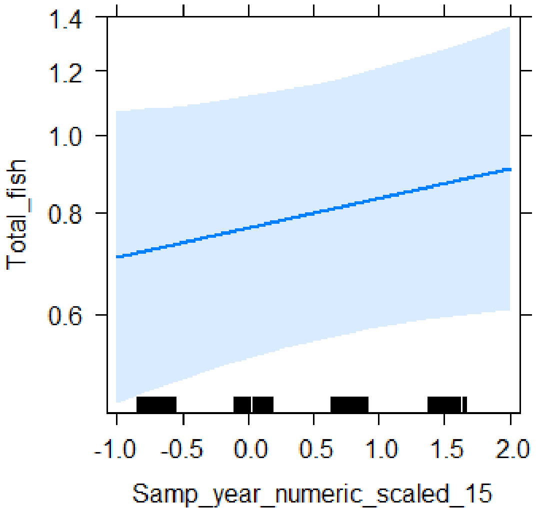

Figure 3-3 Modelled trend of handfish density between 2015-2019

Variance between locations was even higher in this model run (location variance = 0.2955) when compared to the entire dataset, and there was again a strong negative correlation between location and year (-0.70) suggesting that dense local populations of fish can decline quickly.

The third model run was for the sub-set of the historic data (1997-2014) (Figure 3-4). Like the modern data sub-set, this historic sub-set showed no significant difference in population over time (z = 0.01, p = 0.992). The model displayed a “butterfly” shape for the random location effect over time. This suggest an inflection point halfway through the time period (2005-2006) where local populations that were declining and those that were rising passed each other in the model space.

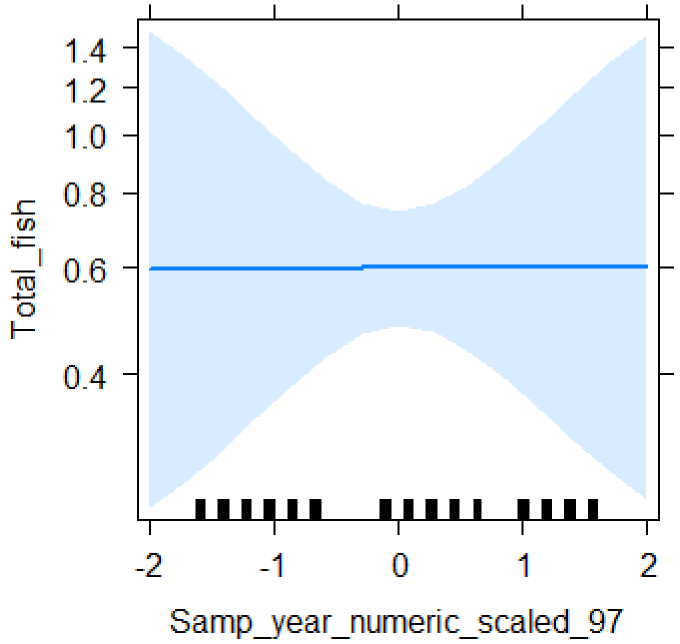

Figure 3-4 Modelled trend of handfish density between 1997-2014

3.4 Data and model limitations

Care should be taken in interpreting the entire dataset (1997-2019) due to low levels of replication and large gaps in observations by year between 1997-2014 compared to 2015-2019. In the case of Mary-Ann Bay and Ralphs Bay there is only one pre-2015 survey and for Sandy Bay and Tranmere there are only two survey years. There is also a decade gap in surveys at Halfmoon Bay, similar gaps occur across most sites except for Opossum Bay.

Our modelling is also at a very preliminary stage for understanding handfish population dynamics and there are various limitations to drawing inference from our results. First, our model was simple and does not include terms that consider seasonality (i.e. date transects/surveys were taken within year), the planting of artificial spawning habitats (ASH), or time from planting of ASH, interannual environmental factors (e.g. climate), stochastic site-based impacts, changes in method (transect reel vs GPS underwater visual census) or observers. Though changes in method and personnel were somewhat accounted for by normalising counts of handfish by the swath areas searched (1.5 m per person and known transect distances), strong overlaps by the principal investigators (M Green, L Wong and T Lynch) and surveys of the same locations.

Although our GLMM uses a Poisson distribution and log offset, which may allow for a better fit for some non-normal responses, it is essentially a regression. This means curvilinear, bimodal or other more complex data distributions may be poorly fitted. For example, the distribution of handfish at Sandy Bay shows four modes in 2006, 2011, 2015 and 2019.

Analysis using alternative models such as generalized additive mix models (GAMMs) might provide better fits to these complex local population dynamics over time.

3.5 Handfish population dynamics

While acknowledging the limitations of our longitudinal dataset and modelling, there are a number of insights into Spotted handfish populations dynamics that can be gleaned with some confidence. As research on Spotted handfish has been used as a proxy for other handfish species (Stuart-Smith et al. 2020), these results may also be of importance as a model for understanding the population dynamics of the wider family.

First, local populations of handfish can be highly dynamic over time and in relation to each other. The high variances within the model between sites and the inflection point in Figure 3-4 demonstrate how some local populations can increase, others decrease, and some remain stable.

Second, there appears to have been an overall decline in the total density of the Derwent estuary handfish population since 1997, but the overall population has stabilised since 2014. Most noticeably, declines have occurred at Ralphs Bay and Tranmere, which are adjacent to each other. A smaller decline appears to have also occurred at Opossum Bay. The GLMM regression may have also considered Bellerive, Howrah Beach and Halfmoon Bay as declines, though due to the limited number of surveys at these locations prior to 2015, this may be an artefact of the analysis.

Third, declines can occur very quickly, especially declines from high densities. In particular, the Ralphs Bay and Tranmere local populations, which are adjacent to each other, have suffered large, recent declines. Conversely, locations with lower densities of fish tend to be more stable in their population dynamics and populations can also persist at low densities.

Finally, across local populations there are examples of booms in densities of handfish. These booms occurred within both the historic and modern parts of the dataset. Our new understanding of handfish genomics (Lynch et al. 2020) allows us to discount either adult or juvenile emigration, dispersal or migration to explain these dynamics. Highly variable inter-annual dynamics in these local populations hence suggest both rapid increases in abundance after successful recruitment events but also declines following recruitment failure as cohorts rapidly age and are removed through natural mortality. These population dynamics correspond well with what we know about Spotted handfish life history strategies.

Spotted handfish appear to be relatively short lived, with a maximum age of 10 years, and 90% of the observed population ≤ 5 years of age (Bessell 2018). As they do not reach adulthood until they are 2 years old, this only leaves a short window for reproduction. If spawning fails, then population declines may occur over short periods of time as cohorts quickly pass through the (hypothesized) limited 2-3 year window for breeding and die out.

Spotted handfish do, however, have a range of traits that could make them resilient to declines and responsive to management intervention. Fish appear to be able to persist in low densities (<3 fish per hectare) and find each other for breeding and courtship either as pairs or as groups (Lynch pers obs). They also have booms in population numbers, probably from successful breeding events and high juvenile survivorship. As recruitment is direct following parental guarding (Bruce et al. 1998), the common mass juvenile mortality of marine species during the planktonic phase is avoided. Juveniles recruit at 4 mm (Green and Bruce 1999) but they then are only again observed at ~30 mm (Bessell 2018). Prior to this they appear to be highly cryptic, hiding within shell hash or within stands of marine vegetation such as seagrasses (Lynch pers obs).

3.6 Stochastic event and chronic pressures

Periodic rapid collapses in local populations occurs in our time-series, we now hypothesize that declines in handfish population dynamics can occur both from stochastic events and chronic pressures. Chronic ecological pressures include introduced marine pests like the northern Pacific seastar (*Asterias amurensis*) consuming natural spawning habitats, such as stalked ascidians (Ross et al. 2003) and vessel moorings that remove habitat (Lynch et al. 2015; Wong et al. 2018).

Specific examples of stochastic declines include the Primrose Sands local population, which was robust in 1999 but had disappeared by 2005 (Green 2007) and not returned to this site when sampled again in 2017 (Wong and Lynch 2017). It was hypothesis that a storm event removed the habitat (Green pers comm), the habitat had recovered by 2017.

Ralphs Bay (Figure 3-1) was able to be tracked at multiple points through time and this local population displayed a tight exponential decline from 53.3 fish per hectare in 2005 to only 3.2 by 2016, and no fish in 2017. The Ralphs Bay site was observed to be covered in a blanket of filamentous algae in both 2012 (Green et al. 2012a) and 2015 (Lynch et al. 2016), though the cover had lifted by 2016. This algal bloom was followed by high numbers of introduced pie crust crabs (*Metacarcinus novaezelandia*) (Lynch pers obs 2017) though these had disappeared in 2018-19 and small numbers of Spotted handfish were observed again.

Recruitment in the 1998 spawning season was extremely low at the South Arm locations. There were two environmental events that may have contributed towards poor recruitment at Opossum Bay. First, there was a decrease in the abundance of stalked ascidians at the location and second there was a very high abundance of the bivalve (*Electroma papilionacea*) in the lower Derwent estuary during the spring 1998 surveys. This rapidly growing species attached to handfish egg masses and settled onto the seafloor in dense mats. This may have affected the recruitment of the handfish by smothering egg masses, hindering development and hatching of fish or by smothering the sediments and restricting access of juvenile fish to benthic prey. It was noted on the autumn survey that the death and subsequent decay of the oysters turned the surface sediments anoxic which may have also had a negative effect on the survival of very young handfish (Green and Bruce 2000).

These pressures may lead to failed breeding for cohorts, and when combined with Spotted handfish's highly restricted opportunities for either adult or juvenile dispersal and relatively short lifespan, fragmentation and local extinctions of populations appears to have occurred. Once fish become locally extinct, for example following a stochastic event, while the habitat may recovery the handfish will not. Most habitat specialists have planktonic recruitment or adult dispersal but direct recruiters, such as handfish, can only recruit into new areas following gradual expansion of ranges from the small individual movements of adults.

3.7 Practical conservation management

To maintain the global population of Spotted handfish each local or at best adjacent populations should be viewed as independent conservation units that need to be individually monitored and conserved. Seven functional conservations groupings could be: 1) Howrah and Bellerive Beach, 2) Battery Point, 3) Sandy Bay, 4) Ralphs Bay and Tranmere, 5) MaryAnn Bay, Opossum Bay and Halfmoon Bay, 6) the mouth of the Huon River and 7) Storm Bay. Other unknown locations may also persist with local Spotted handfish populations.

Due to the observed rapidity of declines in local populations, annual, rather than bi-annual or decadal monitoring is the best temporal scale to assist in the management of local populations. This rate of monitoring will provide opportunities to intervene in local populations as required.

Direct intervention, such as monitoring ascidian density as a proxy for natural spawning habitat and then planting artificial spawning habitats (ASH, see Appendix 3) if ascidian numbers decline, is one way to support local populations. Fish will only use ASH to any great rate if natural spawning habitat is not available (Lynch et al. 2018).

Also critical to their survival are mitigating site specific threats, such as replacing destructive chain moorings with more environmentally friendly mooring (EFM) designs. Removal of these chain moorings between Battery Point and Sandy Bay may provide connection between these genomically isolated populations.

Removal or suppression of northern Pacific seastars would benefit Spotted handfish and many other species. However, due to the seastars ubiquitousness and fecundity, a population-wide approach, such as a species-specific pathogen or genetic control such as a CRISPR mediated gene drive, would be required as a control method. On a local level, seastars may be able to be controlled, in the short term, through collection by divers. Intensive trapping is discouraged as this may attract large numbers of seastars to the area (Andrews et al. 1996)

The recently extinct local populations at Primrose Sands and Simpsons Point may provide useful test beds for reintroductions to expand the global population. The Primrose Sands location is adjacent to a known population of the Spotted handfishes close relative the Red handfish (*Thymichthys politus*) (they occupy different benthic habitats). A reintroduction at this site would not only expand the known global population into a coastal bay somewhat removed from other populations that previously had an extant population but would also allow for a better understanding of ecological niche separation between these two closely related species. The site would also provide administrative efficiencies for spatial management for conservation of multiple species of handfish. Another potential re-introduction site could be Simpsons Point which would provide a local population in the D’Entrecasteaux channel between the Derwent estuary, Huon estuary and Storm Bay populations.

As handfish are extremely local in their distributions they are a prime candidate for spatial management. Consideration of handfish locations by planning authorities is hence another important practical conservation method. In water developments, such as jetties, marinas, seawalls, groynes and aquaculture may impact handfish and their habitats. Other infrastructure such as pipelines or cables may provide a barrier to movements and further fragment local populations. Changes to catchment processes, such as increased sediment and nutrient loads should also be considered as these may produce macroalgal blooms that smother habitat.

4. ENVIRONMENTALLY FRIENDLY MOORINGS SERVICING NOTES

4.1.1 Dates of servicing and mooring locations and vessels

Three of the four CSIRO Environmentally Friendly Moorings (EFMs) were hauled and given their first service by the mooring contractor between 11th - 18th August 2020. The final mooring for the Adams 12 was serviced on the 28th September 2020. The rebuilt mooring for the Maple Leaf 42 was replaced by a dive team on the 18th September 2020. The moorings (Table 4.1) had been deployed between 2nd – 8th May 2019 so this represented 16 months of service. Servicing was meant to occur at 12 months but there were various delays due to COVID-19 and contractor availability.

We also responded to concerns by the owner of the Maple Leaf 42 over chaffing of the headline rope on the 21st July 2019. Following the full service and a range of modifications we conducted another inspection via dive survey and small boat on 26 November 2020.

Table 4-1 Moorings converted from chain to ES.

Mooring #	Vessel	Location	Vessel Length (m)	Depth (m)	East	North
10150	Maple Leaf 42	Sandy Bay	12.8	8	527943	5249750
11150	Clansman 30	Sandy Bay	9.14	6	527920	5249639
3636	Roberts 36	NW Bay	11.2	8	522324	5234255
10674	Adams 12	NW Bay	13.1	6	521826	5231207

4.1.2 Initial mooring designs

The moorings were all the same basic design (Figure 4-1). Yachts were hitched to the mooring with nylon headline, which had chaffing protection. This nylon line travels down through a large, pink, plastic float to a plastic booted Sampson Nylite spool connected to a high tensile steel, bolted and moused D-shackle. Sampson spools are a wheel around which a line can be run and then spiced as a connection point. The Sampson spool and shackle assembly was connected to a steel swivel, which then attached to a moused, screwed D-shackle (Maple Leaf 42, Clansman 30, Adams 12) or a bow D-shackle with bolt (Roberts 36). These attached to the top eyelet tether of the strop.

The bottom eyelet of the strop was joined to 2 m of heavy chain (38 mm) with a screw-in moused D-shackle. The heavy chain was looped through railway wheels which acted as the anchor and was secured with a screwed-in and moused D-shackle. The strops for the moorings in Sandy Bay and North West Bay were of slightly different designs. For the North West Bay moorings, an eyelet attachment point was built into the strop halfway down the line. This allowed for a sub-surface float to be attached, which produced an inverse catenary profile mooring line when at rest in mild wind and current conditions (Figure 4-2).

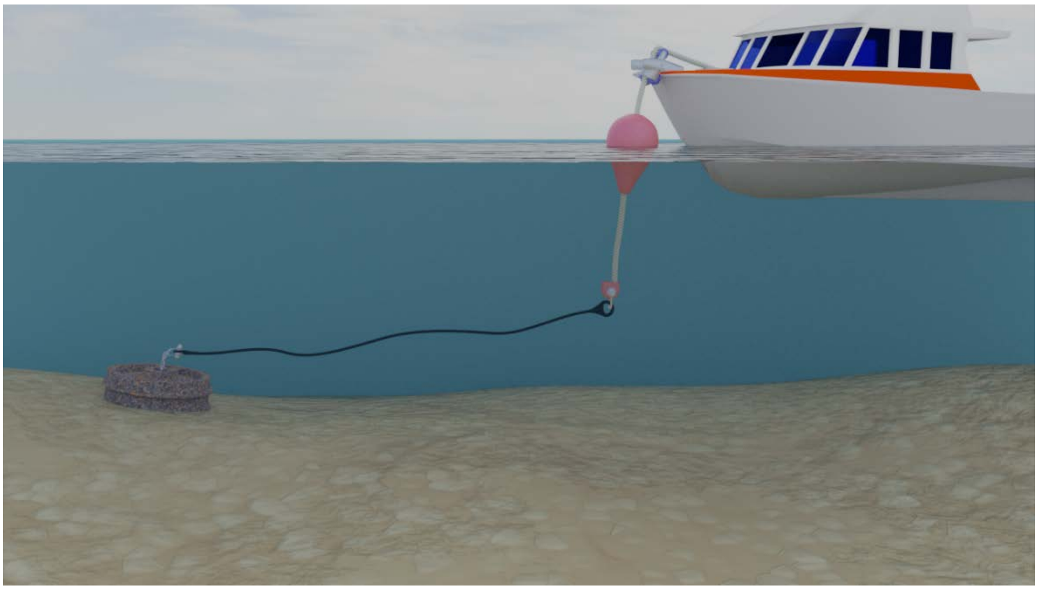

Figure 4-1 Simple schematic of the CSIRO EFM

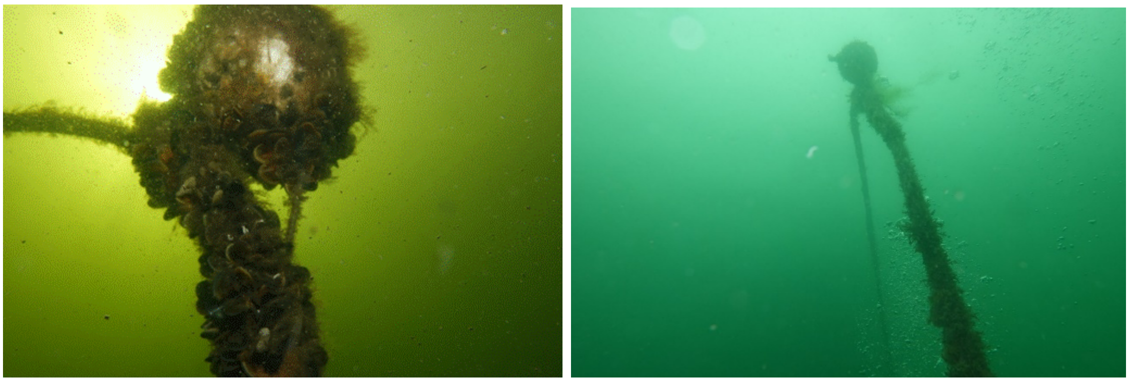

Figure 4-2 Left) Sub-surface float on the mooring line after 15 months of service and Right) view from down the strop

The sub-surface float appeared to work well, with recovery of stalked ascidians within the mooring scar previously created by the traditional chain mooring,under the Adams 12 (Figure 4-3). We noticed some marks on the bottom around the Maple Leaf 42, which may be due to the mooring strop touching the sediments in very calm conditions.

Figure 4-3 Recovery of stalked ascidians in the old mooring scar of the Adams 12 in NW Bay

4.2 Assessments of mooring components at first service

No moorings failed during the 15–16-month trial period and vessels were subject to a wide variety of weather conditions, including extreme wind events. At servicing all components were visually inspected by the mooring contractor, after a high-pressure washing with a water jet. We asked the contractor to rank components as poor, fair or good from their inspection (Table 4.2). Components that were ranked poor or fair were replaced.

Generally, we think that the moorings performed well but we found variable levels of wear of the nylon component that acted as the headline attachment point to the vessel and also electrolysis in the hardware group of the Sampson, swivel and D-shackle that connected the Nylon to the strop. While all moorings will fail eventually without maintenance, how they fail i.e., their 'failure mode' appear to have both similarities and differences between traditional chain moorings and EFMs.

Table 4-2 Ranked wear on mooring components

Mooring component	Maple Leaf 42	Clansman 30	Roberts 36	Adams 12
Nylon	Fair	Good	Poor	Good
Sampson	Good	Good	Good	Good
Swivel	Fair	Poor	Good	Fair
D-shackle top	Good	Poor	Good	Good
Strop	Good	Good	Good	Good
Sub-surface float	*N/A*	*N/A*	Fair	Fair
D shackle bottom	Good	Fair	Good	Good
Chain	Good	Good	Good	Good
D shackle chain	Good	Good	Fair*	Good

*this component had already been used on the previous chain mooring
As this was the first scheduled service for this new type of EFM we also had to develop methods for handling the new materials in the mooring line with the existing mooring barge.

We learnt a variety of lessons around potential CSIRO EFM failure modes, handling and materials and describe these in subsections based on the functional units of headlines, hardware sets, strops and anchors.

4.2.1 Headlines – nylons, and chaffing protection

Chaffing of the headline is a common issue with all moored vessels. It is difficult to solve through standard designs due to the wide variety of methods for attachment and the potential for user error as the mooring is released and re-attached repeatedly over the service period. This liability problem has been addressed by other EFM programs by making it clear that contractors do not have responsibility for this section of the mooring (Rachel Nasplezes pers comm).

The worst chaffing occurred on the Roberts 36 (Figure 4-4a). The headline was switched from the bow roller to the port fairlead when the owner fitted a larger anchor and the chaffing protection no longer protected the line (Figure 4-4b).

Following advice from another volunteer, on the 21st July 2019 we also replaced the nylon headline on the Maple Leaf 42, which was suffering from mild chaffing. For both the Maple Leaf 42 and Roberts 36 we replaced the nylon that attached to the vessel with a short length of polypropylene (PPE) line (Figure 4-5a). We also modified the chaffing protection for the Maple Leaf 42 with a clear plastic tube (Figure 4-5b). The headlines on both the Clansman 30 and Adams 12 were in good condition and were left in place.

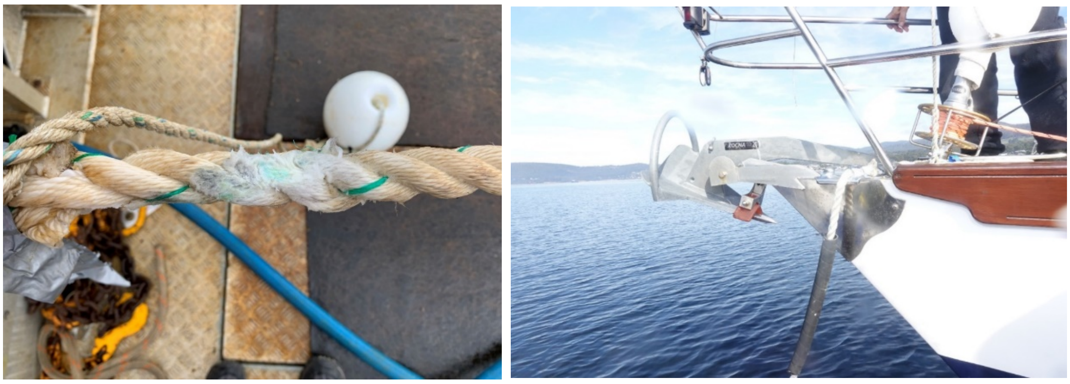

Figure 4-4 a) Chaffing of the headline on the Roberts 36 following b) loss of chaffing protection

Figure 4-5 a) replacement of the Nylon with polypropylene b) new chaffing protection solution. Mr Bob Kelly (left) and Mr Jeff Whyman from Channel Mooring Maintenance.

We initially used nylon as the headline down to the hardware set to provide additional elasticity and negative buoyancy as suggested by our mooring engineering model. The downside to nylon is that it is not as hard wearing or UV stable (Figure 4-6) as PPE.

Figure 4-6 The Nylon headline on the Adams 12

By adding a short section of PPE rope into the mooring line as well as modifications to the chaffing protection we have tried to solve the chaffing problem. This removes the nylon from the area of wear and also predominantly from direct UV exposure. Another potential modification could be to the surface float, which is currently a run-through design with passage of the line through the middle of the float. A different design, with hard attachment points on either side of the float, would further separate the nylon component from UV radiation.

4.2.2 The Hardware set and galvanic corrosion

A new potential failure mode for environmentally friendly mooring (EFMs) is degradation through galvanic corrosion of the top hardware set that connects the nylon component to the strop. We observed corrosion across all mooring hardware sets, though this was mild (Figure 4-7) except for the Clansman 30's mooring, which had severe corrosion (Figure 4-8). This included pitting, gouging from the mousing wire and dissolving of the D-shackle thread.

The hardware set is made up of dissimilar metals with included the hard, high tensile steel of the Sampson's D-shackle, the milder steel of the swivel and D-shackles and the stainless steel of the mousing wire. In a traditional chain mooring, this set is connected to the light thrash chain, where the entire length of the chain acts as a large anode and the effects are distributed and thus far less on each individual component. As EFMs generally replace this light chain component with non-metal materials, this failure mode may be of more general interest for servicing most variants of EFM moorings.

We do not know why the Clansman 30's mooring suffered so severely from corrosion, but we made several observations. Unlike the other vessels the Clansman 30 did not have any sacrificial anodes on the yacht itself, it was also suffering from electrical problems and was in shallow water near a seawall. Both vessel electro-magnetic factors and increased wave rebound may have placed greater strain onto the hardware set.

Figure 4-5 Mild galvanic corrosion of the hardware set on the Roberts 36 following cleaning

Figure 4-6 Severe corrosion from the Clansmann 30's hardware set

We addressed the corrosion issue with the fitting of sacrificial anodes onto the hardware set and, where possible, insulation of the mousing wire (Figure 4-9). We used two types of sacrificial anodes, a ball and a donut types based on availability and fit as the different moorings had slightly different hardware. We fitted the sacrificial anodes after the mooring contactor servicing, by hauling the hardware set to the surface and onto the deck of our small (5.7 m) dive boat.

Figure 4-7 Ball and Donut sacrificial anodes fitted to the hardware sets a) Ball anode on the Maple Leaf 42 and b) replacement donut anode on the Clansman 30

4.2.3 Strops, floats and bottom mooring hardware

The strops were all in good condition (Figure 4-10), though the foam floats disintegrate under the pressure washer and we replaced these with small, hard, plastic trawler floats.

Figure 4-8 . Cleaned mid-point strop tether and float of Roberts 36 mooring the NW Bay strop. Note the mooring badge's hauling clip attached to the tether eyelet, a method that has since been revised by the manufacture.

The eyelet tether point midway along the strop eliminated the need for choking off the strop for hauling. With the tether the entire mooring assembly could now be winched onboard in a straight line, with stopping off to reattach from the top, middle and then end tether point of the strop.

Our only issues with the strops was the handling problem. For instance, strops with mid tether points needed to be orientated correctly in the mooring line. When we attempted to service the Adams 12's mooring we found that the strop had been deployed upside down. If we had hauled the mid tether point, we would have delaminated the tether off the strop. This delayed the mooring servicing as we had to attach a haul up line to the anchor with a dive team.

While we used these eyelet attachment points for hauling this service, further communications with the manufacture (Black Snake) suggested that this was no longer considered ideal and they had developed a new solution for hauling strops with hardened node anchor points.

The original strops, placed onto the vessels in Sandy Bay, differed to the those deployed in NW bay as they lacked the tether point midway along the line. When hauling the Maple Leaf 42's mooring we degloved the rubberised covering off one of these original strops (Figure 4-11). This occurred even though we were using the manufactures recommended technique of choking the strop with a running knot and hauling from this point.

Figure 4-9 Degloved strop of the Maple 42 mooring during servicing.

The Maple Leaf 42 was the largest vessel in our study and hence had the heaviest anchors of multiple train wheels. As part of our agreement with MAST we had also been required to add additional train wheels to our EFM moorings. The Sandy Bay site, where the vessel was moored, also has fine sediments, into which the anchor deeply embedded following deployment of the EFM. This meant that the hauling of the anchor had to overcome both the mass of the train wheels and the suction from the bottom sediments. All these factors may have contributed to excessive load being placed onto the strop leading to it being degloved.

We rebuilt this mooring with the new strop that includes the hardened nodes (Figure 4-12) and reattached it to the anchor via the dive team in the following month. Generally, the bottom attachment of the strop to the short heavy chain that binds the anchor's train wheels together showed little wear or corrosion (Figure 4-12). We did replace one of these D-shackles on the Roberts 36's mooring, but this component already had a long service life prior to the EFM deployment, having been used on the previous chain mooring.

Figure 4-10 Rebuilt mooring for the Maple Leaf 42. Note the bulbous hardened node midway along the black strop. Mr Tim Fountain (CSIRO Mooring technician) is in the foreground and Mr Andrew Martini (CSIRO Engineer) is mid shot and b) attachment to the anchor and chain.

4.3 Interim inspection of moorings

After our initial service we conducted an interim inspection of the moorings on the 27th and 30th of November. This was primarily to gauge the corrosion rate of the sacrificial anodes but also to consider all other aspects of the gear.

The sacrificial anodes appear to be a workable solution to the galvanic corrosion in the EFMs caused by the dissimilar metals in the hardware set of Sampson, swivel and D-shackle. For all the moorings fitted with anodes we found no evidence of corrosion of the hardware, which were all still in good condition. We added an anode to the Robarts 36's mooring set (NW Bay mooring) as this was not previously equipped. This hardware set showed mild corrosion but was still in good condition.

One benefit of this EFM system is that rapid visual inspection can be performed without a winch or crane by lifting the top section of the strop and hardware set, which appear to be the parts of the EFM that are the most likely to fail. This is unlike traditional chain moorings, where a common failure mode is at the thrash chain, which needs to be dived upon or hauled to insect due to the significant weight of the chain. During this interim inspection we hauled these components onto a 5.7m RHIB manually in mild conditions for the two smaller yachts (Clansman 30 and Adams 12) even when the yachts was still attached to the mooring. We did not do this with the Maple Leaf 42 however, as the wind had increased to 15 knots and the yacht is large. The Roberts 36 was not attached to its mooring, which made hauling to the hardware set even easier.

On the Adams 12’s mooring (NW Bay) we observed a decrease in anode mass by ~1/6. The existing anode was deemed operational and was left deployed though it did need to be tightened. On the Clansman 30’s mooring (Sandy Bay) we replaced the smaller donut anode that had decrease by ~1/4 in mass. This anode had been cracked when it was initially fitted, and we were concerned it may fall off. We dove on the Maple Leaf 42’s mooring and observed that the anode had decayed by ~ 1/3 but did not replace due to the available anode not being able to be fitted.

The nylon headlines on both the Adams 12’s and Clansman 30’s moorings were beginning to show early signs of deterioration but looked in good condition and the chaffing protection was effective. The Maple Leaf 42’s PPE line was in good condition and the clear plastic chaffing protection looks like the best solution with good coverage and the ability to inspect the line. The Roberts 36’s PPE line showed mild chaffing but was still in good condition with no severed strands. It looks like the chaffing protection needed to be readjusted.

When we dove on the Maple Leaf 42 we observed markings on the bottom that looks like the strop was touching the bottom in mild conditions. This was not as aggressively as the chain but we think the new strop with the hardened nodes may drag the gear onto the bottom in very calm conditions. These EFMs now all probably need a sub-surface float to fulfil their purpose of conserving the bottom biodiversity and sediments.

4.4 Recommendations and improvements to CSIRO EFMs

Generally, we were pleased with the performance of the CSIRO designed EFMs but also gained considerable insights into potential methods of failure and handling. Servicing an array of 4 moorings was a suitable intermediate step to any larger roll out, as our improvements will provide greater confidence of survivability.

We suggest the following improvements:

1. Failure modes for EFMs may be different to standard chain moorings and these need to be explained to both owners, mooring contractors and regulators.
2. Reuse the headline of the prior mooring setup down to the surface float and install the CSIRO EFM from the anchor to the surface float only, with the headline being the owner’s responsibility.
3. If vessel owners request CSIRO to replace their headline, then 24 mm PPE rope (comparable in strength to the nylon rope of the EFM) with clear vinyl tubing for wear protection through the bow roller shall be used and should be made as similar to the previous headline as possible. The clear vinyl tubing also provides easy inspection.
4. As nylon can UV degrade, replace rope-through buoys with hard attachment buoys with the Nylon below and polypropylene above the water surface.
5. Standardise the mooring hardware set (D-bolt shackle, Swivels, Sampson Nylite spools, mousing wire, anodes) to minimise corrosion.

6. The position of the hardware set allows for inspection via haul up. This can be done without a barge and potentially by owners between services.
7. A sub-surface float is required to stop scrapping of the bottom by the strop. These floats need to be properly depth rated (foam floats not acceptable).
8. Moorings are systems and the temptation to over engineer individual components should be avoided. For instance, anchors should size appropriately to minimise mooring handling issues.

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APPENDIX 1

Table 5-1 Handfish Conservation Project media 2018-2020

Date	Media	Presenter	Topic/Title	Link	Country
24/01/2018	ABC Radio	Rick Stuart-Smith	Tassie scientists discover rare Red Handfish	https://www.abc.net.au/radio/hobart/programs/mornings/red-handfish/9361020	Australia
24/01/2018	ABC news online		Red handfish 'needle in haystack' population found at second Tasmanian location	https://www.abc.net.au/news/2018-01-24/rare-red-handfish-population-found/9358794	Australia
24/01/2018	ABC Radio	Rick Stuart-Smith	Tassie scientists discover rare Red Handfish	https://www.abc.net.au/radio/hobart/programs/mornings/red-handfish/9361020?smid=abchobart-Twitter_Organic&WT.tsrc=Twitter_Organic&sf180196368=1	Australia
24/01/2018	National Geographic	Rick Stuart-Smith Antonia Cooper	RARE FISH WITH HANDS SPOTTED IN TASMANIA	https://www.nationalgeographic.com.au/australia/rare-fish-with-hands-spotted-in-tasmania.aspx	International
24/01/2018	Sky News Australia	Rick Stuart-Smith Antonia Cooper	Rare walking handfish found in Tasmania	https://www.skynews.com.au/details/_5718092826001	Australia
29/03/2019	Handfish Conservation Project website	Danny Rumsby	Wildlife Wizards	https://handfish.org.au/wildlife-wizards/	Australia
2/07/2019	Handfish Conservation Project website	Tyson Bessell	New student working on red handfish	https://handfish.org.au/meet-the-new-phd-student-working-on-red-handfish/	Australia
4/07/2019	ABC Radio	Tyson Bessell	The Red Handfish is even rarer than its more famous cousin	https://www.abc.net.au/radio/hobart/programs/breakfast/the-red-handfish-is-even-rarer-than-its-more-famous-cousin/11277132	Australia
15/07/2019	Handfish Conservation Project website	Lincoln Wong	Research on boat moorings and spotted handfish	https://handfish.org.au/research-on-boat-moorings-and-spotted-handfish/	Australia
19/08/2019	Handfish Conservation Project website	Alex Hormann	Spawning behaviour of spotted handfish	https://handfish.org.au/spawning-behaviour-of-spotted-handfish/	Australia
22/08/2019	Marine Biodiversity Hub		One patch two patch: last stand for the Red Handfish?	https://www.nespmarine.edu.au/news/one-patch-two-patch-last-stand-red-handfish?fbclid=IwAR1bc780-8HNLo4ZO-Eli1flDprW0Cf5jReqflVrWRRzDat55AVvNJjdiSY	Australia

23/09/2019	Handfish Conservation Project website	Tyson Bessell and Sharon Appleyard	Handfish DNA	https://handfish.org.au/handfish-dna/	Australia
2/10/2019	The Canberra Times	Rick Stuart-Smith	Public call to name rare Tasmanian fish	https://www.canberratimes.com.au/story/6418780/public-call-to-name-rare-tasmanian-fish/	Australia
3/10/2019	ABC news online	Rick Stuart-Smith	Want to name a handfish? Now's your chance	https://www.abc.net.au/news/2019-10-03/rare-handfish-up-for-adoption-bullying-training-for-councillors/11569014	Australia
11/10/2019	Studio Ten Online	Sheree Marris	One Of The World's Rarest Fish Calls Australia Home And Walks On Its ‘Hands’	https://10daily.com.au/news/australia/a191010hmjzt/one-of-worlds-rarest-fish-calls-australia-home-and-walks-on-its-hands-20191011	Australia
4/12/2019	Seven News	Jemina Stuart-Smith Tim Lynch	Red handfish hatchlings give species hope	https://7news.com.au/news/environment/red-handfish-hatchlings-give-species-hope-c-589182	Australia
4/12/2019	The Canberra Times	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.canberratimes.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/?cs=14231	Australia
4/12/2019	The Examiner	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.examiner.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/?cs=9397	Australia
4/12/2019	The Courier	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.thecourier.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	The Northern Daily Leader	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.northerndailyleader.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/?cs=9397	Australia
4/12/2019	Wellington Times	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.wellingtontimes.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	The Advocate	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.theadvocate.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Daily Liberal	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.dailyliberal.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	The Singleton Argus	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.singletonargus.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/?cs=7	Australia

4/12/2019	The Ararat Advertiser	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.araratadvertiser.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Western Advocate	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.westernadvocate.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	The Standard	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.standard.net.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	The Wollondilly Advertiser	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.wollondillyadvertiser.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/?cs=3674	Australia
4/12/2019	Glen Innes Examiner	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.gleninnesexaminer.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	The Stawell Times - News	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.stawelltimes.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Glouster Advocate	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.gloucesteradvocate.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Bombala Times	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.bombalatimes.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Border Chronicle	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.borderchronicle.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/?cs=9397	Australia
4/12/2019	Parkes - Champion Post	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.parkeschampionpost.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/?cs=9397	Australia
4/12/2019	Newcastle Star	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.newcastlestar.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	The Daily Advertiser	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.dailyadvertiser.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	The Bellingen Shire Courier	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.bellingencourier.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia

4/12/2019	South Coast Register	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.southcoastregister.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Great Lakes Advocate	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.greatlakesadvocate.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Nyngan Observer	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.nynganobserver.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Beaudersert Times	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.beaudeserttimes.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Barossa Herald	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.barossaherald.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	The Naracoote Herald	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.naracoorteherald.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Port Stephens Examiner	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.portstephensexaminer.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/?cs=7	Australia
4/12/2019	Yass Tribune	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.yasstribune.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/?cs=9397	Australia
4/12/2019	Eyre Peninsula Tribune	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.eyretribune.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Musswellbrook Chronicle	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.muswellbrookchronicle.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/?cs=7	Australia
4/12/2019	The Macleay Argus	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.macleayargus.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	The Inverell Times	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.inverelltimes.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/?cs=7	Australia
4/12/2019	St George & Sutherland Shire The Leader	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.theleader.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia

4/12/2019	West Coast Sentinel	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.westcoastsentinel.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Goodiwindi Argus	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.goondiwindiargus.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/?cs=9397	Australia
4/12/2019	Narromine News	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.narrominenewsonline.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Cessnock Advertiser	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.cessnockadvertiser.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Cowra Guardian	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.cowraguardian.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Boorowa News	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.boorowanewsonline.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/?cs=9397	Australia
4/12/2019	The Transcontinental Port Augusta	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.transcontinental.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Port Lincoln Times	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.portlincolntimes.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Wingham Chronicle	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.winghamchronicle.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	The Rural	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.therural.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	The Camden-Narellan Advertiser	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.camdenadvertiser.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	Bay Post Moruya Examiner	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.batemansbaypost.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	China.org.au	Jemina Stuart-Smith	New hatchlings give hope for "world's rarest fish"	http://www.china.org.cn/world/Off_the_Wire/2019-12/04/content_75476491.htm	China

4/12/2019	ecns.cn	Jemina Stuart-Smith	New hatchlings give hope for "world's rarest fish"	http://www.ecns.cn/news/sci-tech/2019-12-05/detail-ifzrnvym4351856.shtml	China
4/12/2019	Junee Southern Cross	Jemina Stuart-Smith	Red handfish hatchlings give species hope	https://www.juneesoutherncross.com.au/story/6527113/red-handfish-hatchlings-give-species-hope/	Australia
4/12/2019	IMAS media release	Jemina Stuart-Smith	TINY RED HANDFISH HATCHLINGS A LIFELINE FOR WORLD'S RAREST FISH	https://www.imas.utas.edu.au/news/news-items/tiny-red-handfish-hatchlings-a-lifeline-for-worlds-rarest-fish	Australia
5/12/2019	ABC news online	Jemina Stuart-Smith	Kid-gloves treatment for baby handfish	https://www.abc.net.au/news/2019-12-05/news-briefing-5-december/11766326	Australia
5/12/2019	XinhuaNet	Jemina Stuart-Smith	New hatchlings in Australia give hope for "world's rarest fish"	http://www.xinhuanet.com/english/2019-12/05/c_138607136.htm	China
16/01/2020	Handfish Conservation Project website	Helen O'Neill	Handfish monitoring with Helen O'Neill	https://handfish.org.au/handfish-monitoring-with-helen-oneill-csiro/	Australia
30/01/2020	ABC Radio	Jemina Stuart-Smith	Red handfish surveys IMAS/RLS survey weekend)		Australia
6/04/2020	Peter Underwood Centre	Jemina Stuart-Smith	The Wonder Weekly (UTAS children's paper)	https://www.utas.edu.au/__data/assets/pdf_file/0003/1317981/The-Wonder-Weekly-April-6.pdf	Australia
17/04/2020	Hank Green video	Hank Green	The Rarest Fish in the World is an Anglerfish (American video blogger)	https://bit.ly/2ziGDNX	USA
21/04/2020	ABC Radio	Jemina Stuart-Smith	(ABC Radio Play segment for kids - draw a handfish)	https://ab.co/3eIer7h	Australia
30/04/2020	Peter Underwood Centre	Jemina Stuart-Smith	Handfish overview	https://bit.ly/2xLtVXO	Australia
1/05/2020	Handfish Conservation Project website	Katherine Richardson	Writing about handfish with Katherine Richardson	https://handfish.org.au/writing-about-handfish-with-katherine-richardson/	Australia
4/05/2020	Handfish Conservation Project website	Jemina Stuart-Smith	The search for Ziebell's and Red handfish	https://handfish.org.au/the-search-for-red-and-ziebells-handfish/	Australia

6/05/2020	Kat Richardson Creative	Jemina Stuart-Smith	Interview with Dr Jemina Stuart-Smith	https://katherinerichardson.net/index.php/2020/05/06/interview-with-dr-jemina-stuart-smith/	Australia
11/05/2020	Your Afternoon with Helen Shield for ABC Hobart (radio)	Karen Lyttle	Handfish art in Hobart		Australia
26/06/2020	Mongabay (online article)	Elizabeth Claire Alberts	The first modern-day marine fish has officially gone extinct. More may follow	https://news.mongabay.com/2020/06/the-first-modern-day-marine-fish-has-officially-gone-extinct-more-may-follow/	USA
29/06/2020	Ocean Geographic (live online presentation)	Jemina Stuart-Smith	Saving the world's rarest fish	https://www.facebook.com/OceanGeographic/	USA
1/07/2020	Scientific American	David Shiffman	Smooth handfish extinction marks a sad milestone	https://www.scientificamerican.com/article/smooth-handfish-extinction-marks-a-sad-milestone/	Australia
1/07/2020	Australian National Maritime Museum ('Signals' magazine)	Tim Lynch & Jane Bamford	Tasmania's spotted handfish (Ceramics & science help save a species)	https://www.sea.museum/discover/publications/signals-magazine	International
3/07/2020	Phys.org (online article)	Daniel Steadman, Fauna & Flora International	Farewell smooth handfish: What can we learn from the world's first marine fish extinction?	https://phys.org/news/2020-07-farewell-smooth-handfish-world-marine.html?fbclid=IwAR3KnbbDh22FblvndvXHUGdLc-8MHIiyR2gQblvr4kgOyhlE8xfqazEwVIs	Australia
14/07/2020	ABC news online (tv - national)	Jemina Stuart-Smith	Endangered Tasmanian species		Australia
17/07/2020	ABC radio	Rick Stuart-Smith	Smooth handfish extinction		Australia
17/07/2020	Ocean Geographic (magazine)	Jemina Stuart-Smith	Saving the last handfish	https://handfish.org.au/ocean-geographic-feature-on-red-handfish/	International
22/07/2020	Handfish Conservation Project website	Carlie Devine	Finding spotted handfish with Carlie Devine	https://handfish.org.au/finding-spotted-handfish-with-carlie-devine/	Australia

24/07/2020	The guardian	First Dog on the Moon	Meet' the now officially extinct smooth handfish	https://www.theguardian.com/commentisfree/2020/jul/24/meet-the-now-officially-extinct-smooth-handfish	Australia
28/08/2020	National Geographic	Douglas Main	A fish that walks on the seafloor has gone extinct. Can its cousins be saved?	https://www.nationalgeographic.com/animals/2020/08/smooth-handfish-extinct-other-handfishes-threatened/	International
7/09/2020	UTAS webinar (155 participants)	Jemina Stuart-Smith	Devil in the detail: public forum (Threatened Species Day)	https://www.youtube.com/channel/UCtHM5hf5uUBSi5ZaNidrssQ.	Australia
26/09/2020	Tasmanian Times (online article)	Geoffrey Swan (JSS & CD)	Red Handfish on the Brink of Extinction	https://tasmaniantimes.com/2020/09/red-handfish-on-the-brink-of-extinction/	Australia
7/07/2020	The Mercury newspaper	Peter Whish-Wilson	Talking Point: Smooth handfish gone forever	https://www.themercury.com.au/news/opinion/talking-point-smooth-handfish-gone-forever/news-story/7d497fcd09594a59cfc4bb0d63fdadc7	Australia
21/10/2020	The Guardian	Zoe Kean	Why the death of a small, punk-like fish rocked the marine world	https://www.theguardian.com/environment/2020/oct/21/why-the-death-of-a-small-punk-like-fish-rocked-the-marine-world-aoe	Australia
11/11/2020	UTAS media release	Jemina Stuart-Smith, Tim Lynch, Andrew Trotter	Red handfish juveniles released to boost endangered wild population	https://www.utas.edu.au/communications/general-news/all-news/red-handfish-juveniles-released-to-boost-endangered-wild-population	Australia
11/11/2020	7 news	Jemina Stuart-Smith	Rare red handfish given boost in Tasmania	https://7news.com.au/news/wildlife/rare-red-handfish-given-boost-in-tasmania-c-1559999	Australia
11/11/2020	The Examiner	Jemina Stuart-Smith	Tasmanian red handfish bred in captivity released	https://www.examiner.com.au/story/7008608/watch-a-red-handfish-in-its-natural-habitat/	Australia

Table 5-2 Handfish Conservation Project presentations

Date	What/where	Presenter	Title	Link/details
19/11/2019	Antarctic Experience (CSIRO)	J. Stuart-Smith	Handfish Conservation	School students
18/07/2020	Tasmanian Mermaids event (international women's dive day)	J. Stuart-Smith	Handfish conservation	(public presentation)
29/06/2020	Ocean Geographic (live online presentation)	J. Stuart-Smith	Saving the world's rarest fish	https://www.facebook.com/OceanGeographic/
7/09/2020	UTAS Threatened species Day	J. Stuart-Smith	Handfish Conservation	
23/09/2020	Mt Nelson Primary School	J. Stuart-Smith	Handfish	(Grade 1-2)
11/09/2020	Blackmans Bay Primary School	J. Stuart-Smith	Handfish	(Grade 2 & 5)
July 2020	Tasmanian Museum & Art Gallery (Friends of TMAG)	T. Lynch and Jane Bamford	Spotted handfish art & science	
August 2020	Kingston Library handfish exhibition			Month-long public exhibition
Dec 2020	Margate Primary School	J. Stuart-Smith	Handfish	(Grade 2-6)
Dec 2020	UTAS Animal Ethics committee meeting	J. Stuart-Smith	Red handfish head-starting	Forum for AEC
Dec 2020	Sorell community presentation	J. Stuart-Smith & T. Bessell	Red handfish conservation	Attended by community members, Sorell mayor and councillors

APPENDIX 2

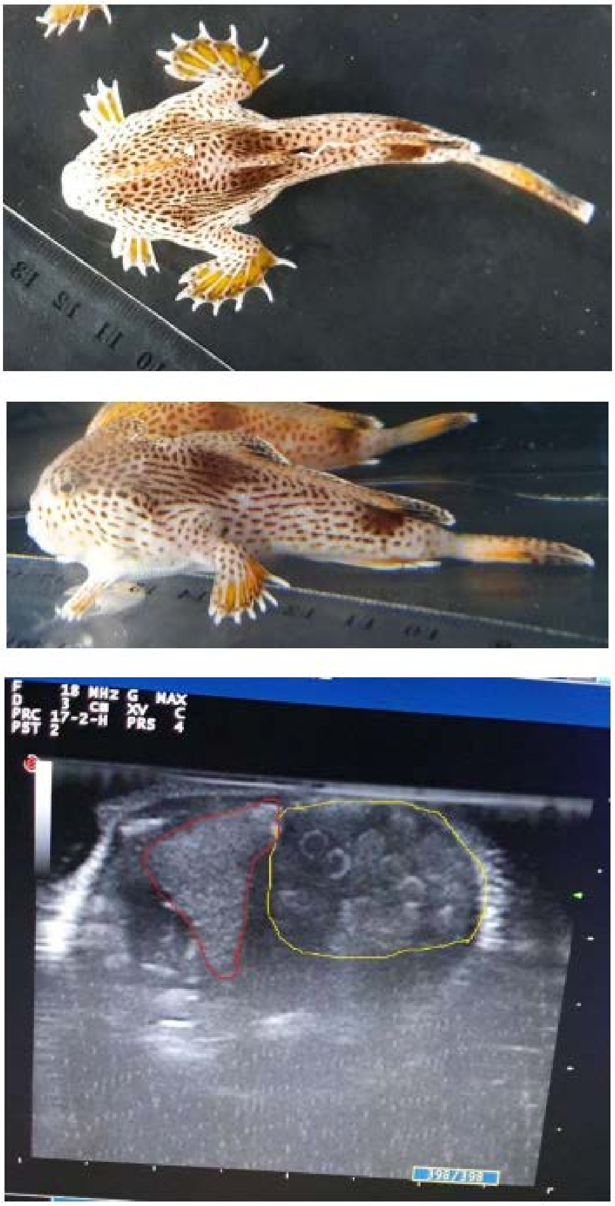
Ultrasound Examination for Sex Determination of the Spotted handfish (*Brachionichthys hirsutus)* at Sea Life Melbourne Aquarium.

The five Spotted handfish (*Brachionichthys hirsutus*) were ultrasounded on October 22, 2020 with Dr. Shane Simpson from the Unusual Pet Vets to confirm the sexes. A 18MHz Linear Probe was used. The ultrasound was performed with the fish contained in a plastic bag in seawater, no sedation was required. Excellent sonogram images were produced with this probe and method. Sexes of all five handfish were confirmed. Liver evaluation of all five fish revealed homogenous echogenicity and appeared similar in between sexes.

FISH 1. “Rose” BP-002 – Female
FISH 2. “Handfish McHandfish Face” BR-002 - Female
FISH 3. “Hands Christian Anderson” MAB-004 - Female
FISH 4. “Handjolena” MAB-002 – Female
FISH 5. “Hand Solo” TR-001 – Male

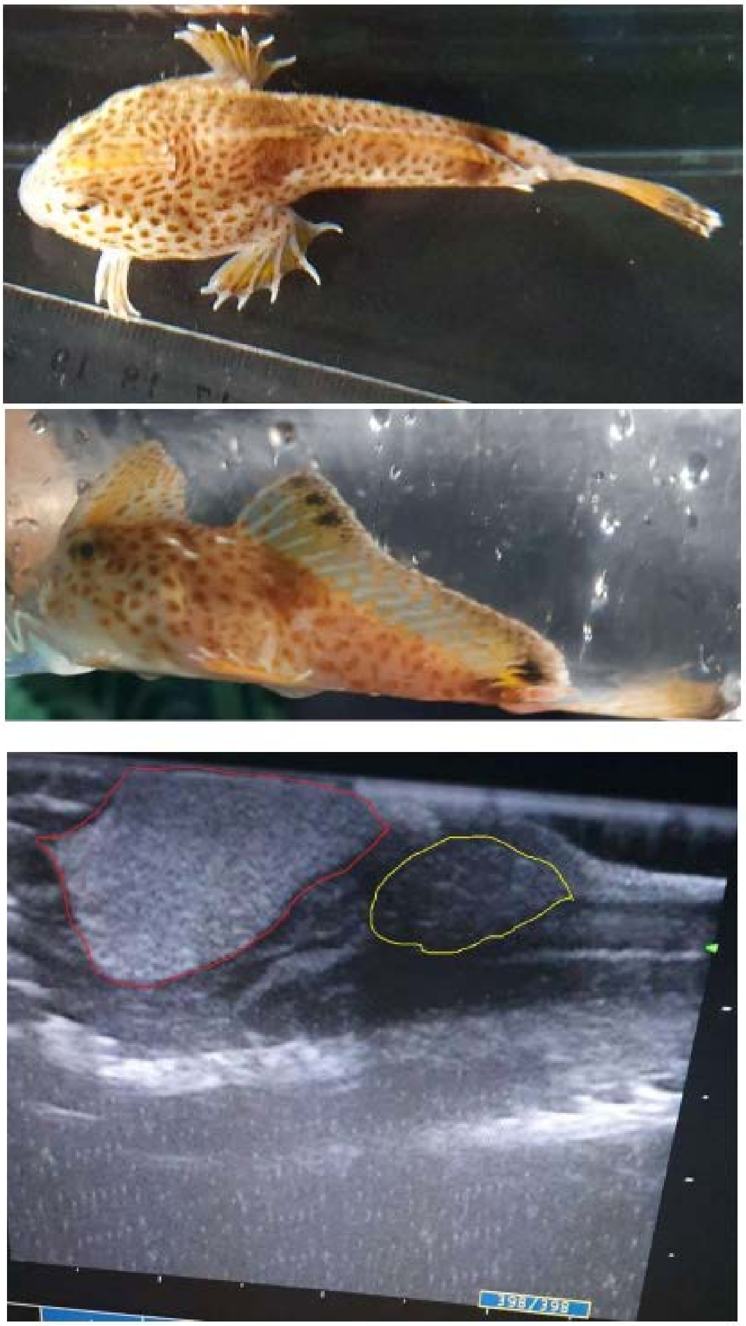
Liver outlined in red (left) and ovary outlined in yellow (right).

Figure 5-1 FISH 1. “Rose” BP-002 Sex: Female A 18MHz Linear Probe was used. The ultrasound was performed with the fish contained in a plastic bag; no sedation was required.



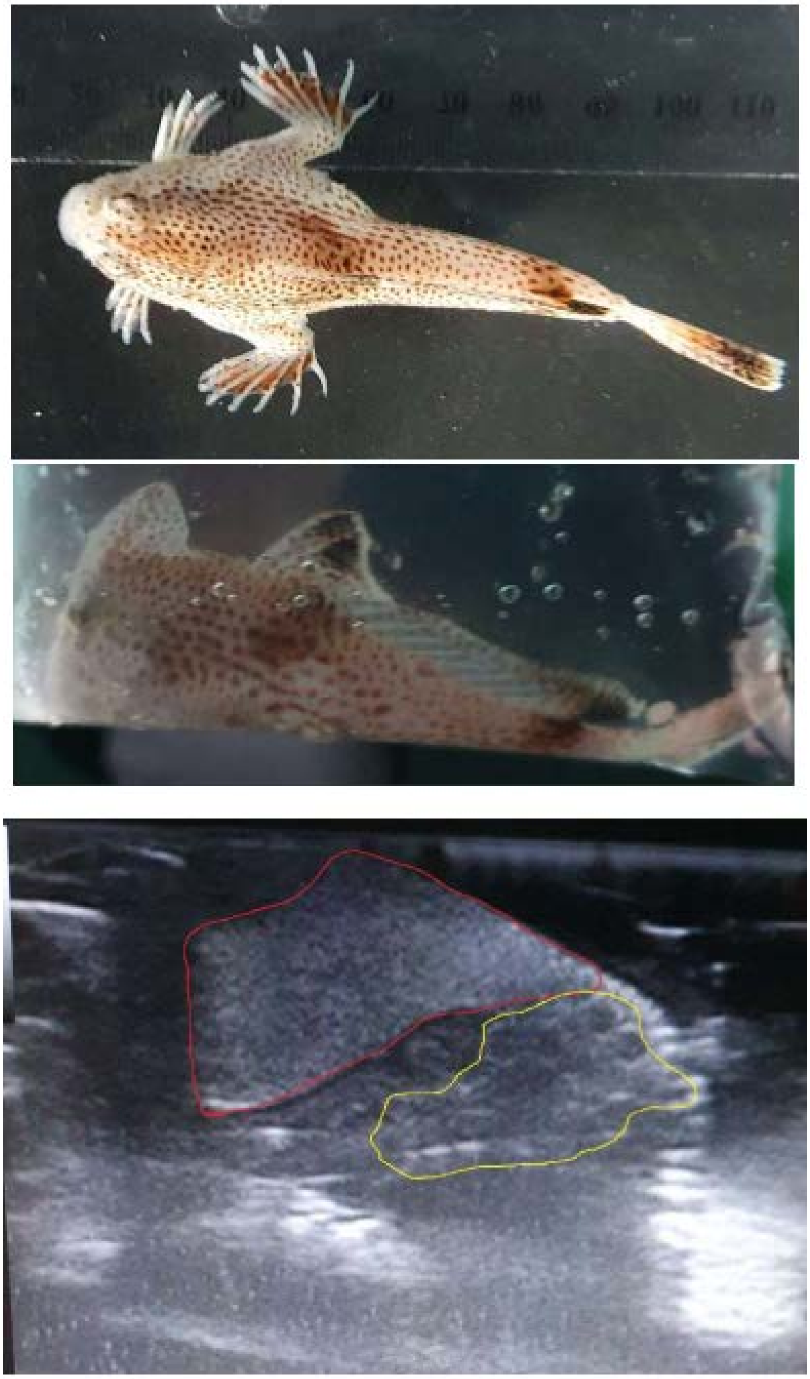
Liver outlined in red (left) and ovary outlined in yellow (right).

Figure 5-2 FISH 2. “Handfish McHandfish Face” BR-002 Sex: Female A 18MHz Linear Probe was used. Ultrasound was performed with fish contained in a plastic bag; no sedation required



Liver outlined in red (left) and ovary outlined in yellow (right).

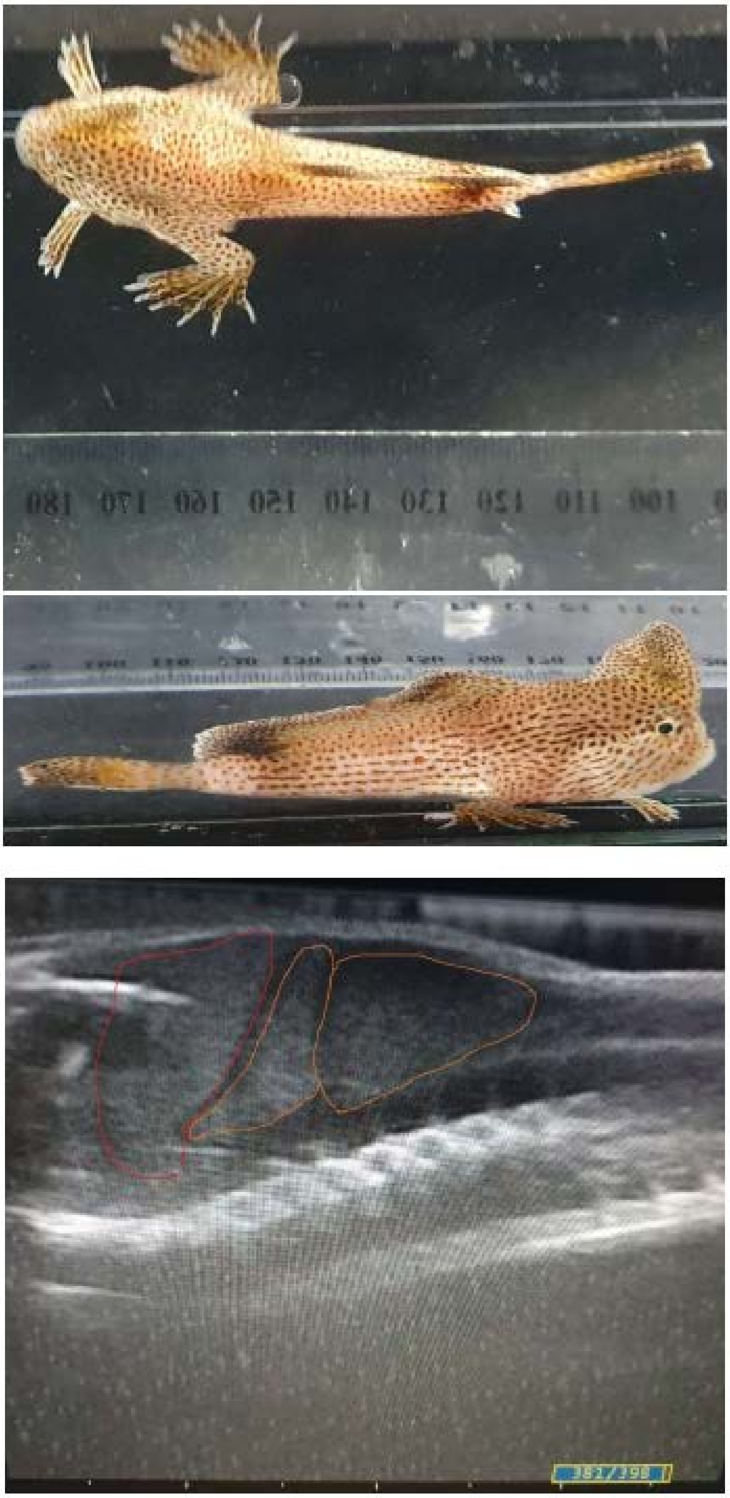
Figure 5-3 FISH 3. “Hands Christian Anderson” MAB-004 Sex: Female A 18MHz Linear Probe was used. The ultrasound was performed with the fish contained in a plastic bag; no sedation was required.



Liver outlined in red (left) and ovary outlined in yellow (right).

Figure 5-4 ISH

Fish 4. “Handjolena” MAB-002 Sex: Female A 18MHz Linear Probe was used. The ultrasound was performed with the fish contained in a plastic bag; no sedation was required.



Liver outlined in red (left) and testes outlined in orange (right).

Figure 5-5 FISH 5. “Hand Solo” TR-001 Sex: Male

APPENDIX 3

Artificial spawning habitat planting

03/9/2020
Howrah beach.
Planted 125 ASH total in two lines interspersed 9mm and 11mm. This was abandoned as the line became too rocky to plant. Waypoint is from the east and then 125 m to the west.

03/9/2020
Howrah Beach. Planted 500 ASH total into two lines interspersed 9mm and 11mm. Placed a Starpicket ½ way along and at both ends. This provided 4 transects. Waypoint is from the east and then ASH runs 250m to the west.

09/09/2020
Bellerive Beach. Plant 500 ASH total into two lines interspersed 9mm and 11mm. Placed a Starpicket ½ way along and at both ends. This provided 4 transects. Waypoint is from the east and then 250m to the west.

Artificial spawning habitat check

Figure 5-6 Number of ASH of various thickness (mm) planted and survived (Surv) at Bellerive (BR) and Howrah Beach sites by transect.

Site	Transect	Plant 11mm	Plant 9mm	Surv 11mm	Surv 9mm
BR	1	62	63	57	53
BR	2	63	62	61	54
BR	3	62	63	56	54
BR	4	63	62	59	50
HB	1	62	63	62	63
HB	2	63	62	61	51
HB	3	62	63	54	63
HB	4	63	62	60	51

Table 5-3 Use of ASH by site, transect, ASH thickness and whether this included fish guarding eggs, just fish or just eggs/remnants.

Site	Transect	Fish with eggs 11mm	Fish with eggs 9mm	Fish 11mm	Fish 9mm
BR	1	0	0	0	0
BR	2	0	0	0	0
BR	3	0	0	0	0
BR	4	0	0	0	0
HB	1	1	1	0	0
HB	2	0	0	1	1
HB	3	0	0	1	0
HB	4	0	0	0	0

Site	Transect	Eggs 11mm	Eggs 9mm
BR	1	0	0
BR	2	0	0
BR	3	0	0
BR	4	0	0
HB	1	0	0
HB	2	0	0
HB	3	0	0
HB	4	0	0

We planted ASH at 5-6 m at Bellerive, which was parallel and closer to the shore of the previous ASH plant at Bellerive in 2019. In 2019 ASH was used by fish at Bellerive but not in 2020. We hypothesis that this may have been due to the ASH being planted too shallow. There also may have been a surplus of natural spawning habitat at this site or the fish were using the old deeper lines.

Table 5-4 Some notes on historic ASH deployments timeline from records.

Year	Location	comment	
1996	OB/PS	fish locations marked with stakes during survey	Some fish noted with egg masses on these at later dives?
1998	OB	ASH planted in August (12 quadrats with 20 ASH in each), checked in Oct	many lost, 1 quadrat lost all, 4 quadrats only with 1-5 ASS, only 2 egg masses found
	HMB	ASH planted in August, checked in Oct (12 quadrats with 20 ASH in each)	52 egg masses on ASS Subsequent spike in Spring 1999 survey
1999	OB	550 ASH deployed September, inserted into sediments deeper	Spike in recruitment followed

2000	OB	ASH from 1999 left in place, checked in Nov 2000 (420 left)	11 had egg masses
2002	HMB	400 ASH deployed	not sure of time of year - check
2002	PS	Habitat rehabilitation trial - Caulerpa transplantation pilot	
2004	OB?	Habitat rehabilitation trial - Caulerpa transplantation	lacking details
2008	OB	Counting of transplanted Caulerpa from 3 years previously found some had appeared to spread and establishment has failed in other areas (died or been dislodged)	

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