Marine Biodiversity Hub

National Environmental Science Programme

Conservation of handfish and their habitats – annual report

Tim Lynch, Tyson Bessell, Alexander Hormann, Carlie Devine and Neville Barrett

Project A10 – Conservation of spotted handfish

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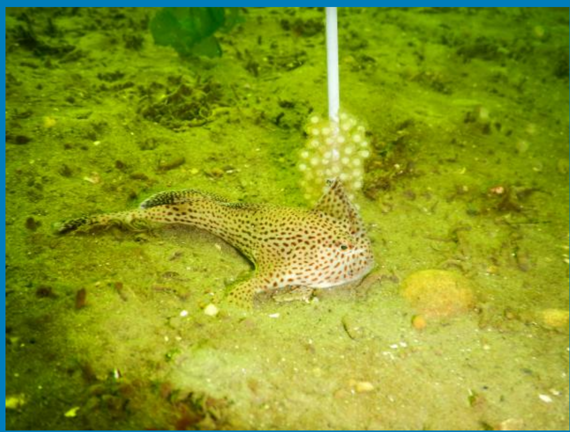

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The project will also report its findings on a semi-annual basis to the National Handfish Recovery Team (NHRT) – see below. This is a governance body that is constituted between the Tasmanian State and the Commonwealth government with other interested parties:

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Contents

List of Figures

List of Tables

1. EXECUTIVE SUMMARY

In 2018 we completed the fourth round of annual performance assessment surveys for spotted handfish across the 9 Derwent Estuary study sites. This provides 62 data points in total, with 36 points from our NESP work building on our 26 data points from previous studies. Two results of interest from this year were the discovery of fish, after a year's absence, at the Ralphs Bay site and a continued decline in numbers at Mary Anne Bay. For the Ralphs Bay site the fish are still in very low numbers, which is consistent with what we have observed since 2015. This suggests that when densities of fish dip below 3-5 fish per hectare then the monitoring program does not have the sensitivity to reliably detect their presence. For the Mary Anne Bay site the recent trend has seen a decline in numbers back to levels that are more commonly seen at numerous other sites. Several student projects this year provided important context to both this results and conservation of the species in general.

The first of these were some preliminary results from Mr Alex Hormanns' UTAS Masters project. This involved the planting of 5000 Artificial Spawning Habitats (ASH) into 5 site based arrays, made up of 50:50 mixes of plastic and ceramic ASH. When we surveyed the arrays numerous eggs masses were being guarded by fish who had spawned onto ASH, with the fish showing a preference for ceramic over plastic ASH. The relative use of ceramic ASH was also higher than the raw numbers suggested as ceramics had a lower survival rate than plastic, with 1964 plastic and 1524 ceramic ASH or 3488 out of the 5000 remaining at the end of the surveys. One variable that seems to explain the amount of ASH used at a particular site was the densities of stalked ascidian which provide natural spawning habitat. Sites that had ascidian densities larger than 0.05 per m^2 saw very low ASH use. The sites that saw the most ASH use had relatively low densities of both ascidians and spotted handfish. We have now incorporated ascidian counts into the monitoring program and will use these data to plan further ASH planting.

The second piece of information was from Mr Tyson Bessell's UTAS honours project (first class), 'Biological Parameters of the Spotted Handfish'. This work demonstrated the accuracy of the I3S autonomous pattern recognition program for identifying spotted handfish – this allowed us to exclude technical issues as a reason for low recapture rates. Tyson then used both recapture data of repeated length measurements of wild fish in combination with a small number of opportunistically collected otoliths to model the age of the fish. The oldest fish in the population appear to be 10 years old, however based on length frequency data only 10% of the fish within the sample population are older than 5 years. Sexual maturity occurs around 2 years of age so most fish will have a 1-3 year window of opportunity to reproduce. Important caveats to this work are that it is based on a limited number of both recaptures (n=13) and otoliths (n = 7) and only one otolith was from a female.

From our new insights into handfish biology and conservation we are starting to be able to develop an understanding of the species local population dynamics. Aspect of our monitoring program that have previously remained un-explained were: trends of declines across years, variability between years and increases in densities between the current and historic data, as well as low recapture rates. These site population dynamics and re-capture rates may now be explainable based on the relatively short lifespan of the species and spawning success related to the availability of natural or artificial spawning habitat. While our monitoring program can provide density estimates, the likelihood of recapturing an individual with is low due to rapid natural mortality (90% dead two years after first capture) when placed in the context of annual sampling. Also, if stalked ascidians density are by nature stochastic then there will be problems with recruitment, especially if stochasticity increases or there is a long-term average decrease in ascidians or other natural spawning habitat due to grazing by introduced marine pests or changes to catchment processes. If spawning fails or is reduced then declines over time periods 1-4 years will occur as cohorts pass through their breeding period and natural mortality cumulatively removes most individuals from local populations.

This relatively short lifespan is also of importance to efforts to captive breed the species. While animals bred in captivity in 2017, none did in 2018. Clearly solutions to enhance breeding will need to be found quite quickly before the brood stock and captive bred fish die.

2. MONITORING

In 2018 we completed the fourth round of annual performance assessment surveys for spotted handfish across the 9 Derwent Estuary study sites (Fig. 1 and Fig. 7). This provides 62 data points in total, with 36 points from our NESP and Threatened Species Commissioner funded work which commenced in 2015. In addition to these we have built on 26 data points from data mostly collected by Mr Mark Green between 1998 and 2012 (Fig. 2). Two points of interest from this year was the discovery of fish, after a year's absence, at the Ralphs Bay site and a continued decline in density of fish at the Mary Anne Bay. For the Ralphs Bay site the fish are still in very low numbers, which is consistent with what we have observed since 2015. This suggests that when densities of fish dip below 3-5 fish per hectare then the monitoring program does not have the sensitivity to reliably detect their presence. For the Mary Anne Bay site the recent trend has been a decline in numbers back to levels, around 20 fish per hectare, that are more commonly seen at numerous other sites such as Battery Point, Sandy Bay and Opossum Bay.

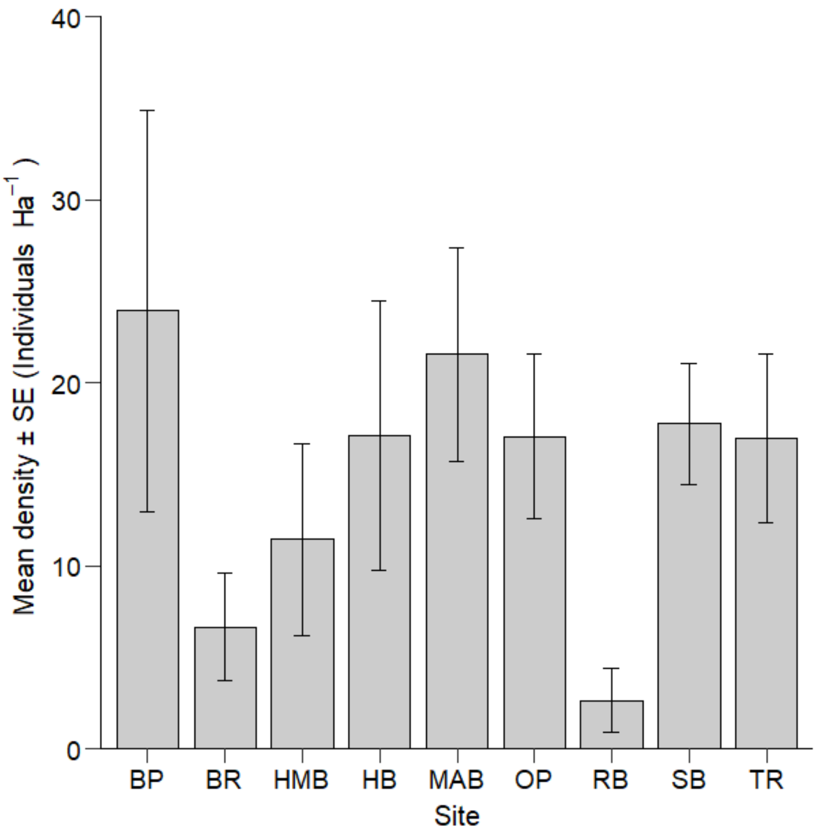

Figure 1 2018 densities of spotted handfish across the 9 Derwent Estuary monitoring sites (BP = Battery Point, BR = Bellerive, HMB = Honeymoon Bay, HB = Howrah Beach, MAB = Mary Anne Bay, OP = Opossum Bay, RB = Ralphs Bay, SB = Sandy Bay, TR = Tranmere)

One aspect of our monitoring program that has remained un-explained is the dynamic temporal variation in densities. This includes both trends of declines across years and more abrupt variations between the current and historic data. While we first considered that this may be an issue with our methods or perhaps migrations of fish, we discounted that as it only occurred at a limited number of sites and for many sites numbers are relatively stable over time. As well re-captures tend to not move far from their initial point of observation. From our new insights into handfish biology and conservation from the student projects conducted this year we now hypothesis that these site population dynamics may be explainable based on the relatively short lifespan of the species and variable breeding success based on the availability of natural or artificial spawning habitat. If spawning fails or is reduced then local population declines over time periods 1-4 years will occur as cohorts quickly pass through a short window for breeding and natural mortality removes them from the populations. Similarly if there is a window of successful breeding, based on either deployment of artificial spawning habitats or good seasons for natural spawning habitats then populations can increase rapidly.

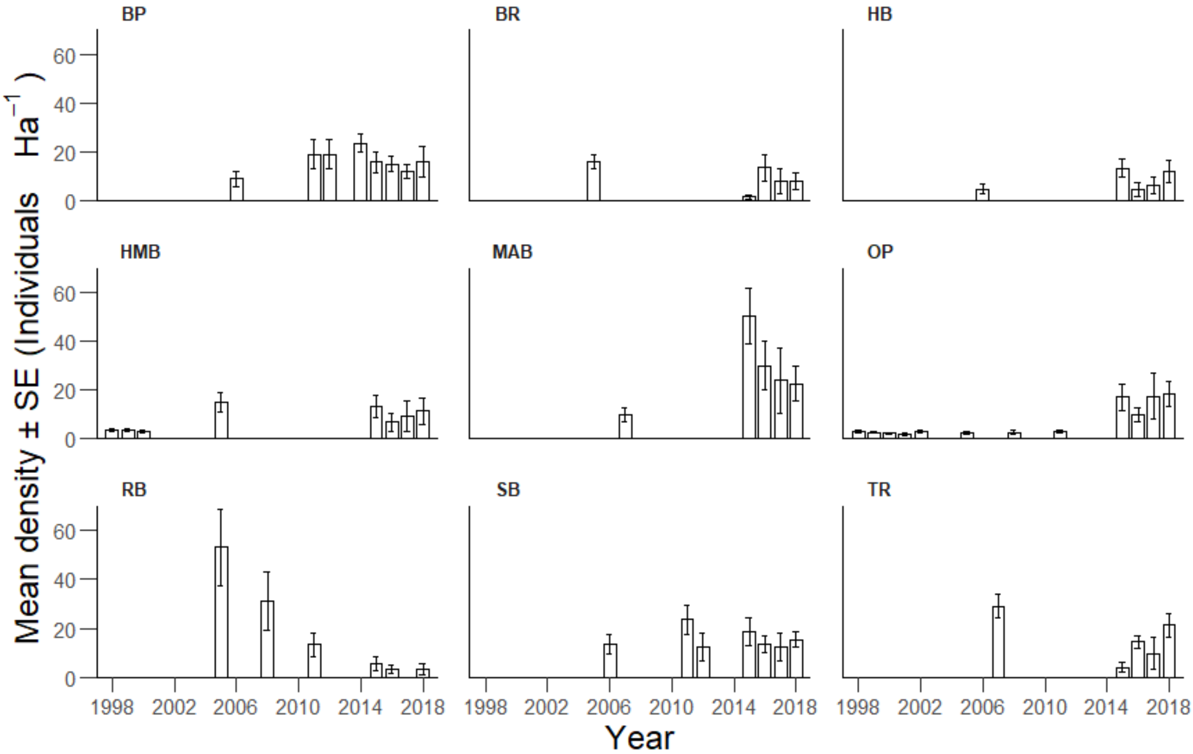

Figure 2 Time-series 1998-2018 of density of spotted handfish at 9 sites in the Derwent Estuary

3. CAPTIVE BREEDING

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

Of the 20 fish captures in 2017 a total of 14 adult fish remain alive as well as approximately 60 juveniles. 1 adult fish was lost at Melbourne Aquarium due to being caught on the filter and 5 fish were lost at Seahorse World through electrocution following an electrical fault with the lighting system in one tank. A small number of juveniles have perished, though in general juvenile fish have continued to survive and grow.

There was no captive breeding events by the adults in the 2018 season. Of the fish captured and lost the two known females from the first year are both still alive but did not become obviously gravid. We have a range of hypothesis why no breeding occurred.

1. Collection bias and non-annual breeding: All the other fish are males and the females' only breed once or every second year. Parsing this hypothesis, we collected two obviously gravid females in 2017 but the other fish may have been all males, perhaps due to an unknown behavioural distribution at the time of collection.

2. Failure of environmental trigger: seasonal temperature, light and perhaps salinity regimes may all be triggers for breeding and we may not have replicated this correctly in the artificial environment

3. Diet and conditioning: we may be missing a dietary component, have a low quality or not sufficient quantity to allow for females to become gravid

4. Behaviour: spotted handfish have known dispersal and aggregating behaviours and our dense tank aggregations of fish do not replicate what is required.

More research is needed to understand what triggers breeding behaviour and/or development of gravid females.

4. ARTIFICIAL SPAWNING HABITAT

The monitoring program (section 2) will continue across all sites and in the future we will attempt to detect changes in densities of adult fish by site based on planting of ASH. We hence developed a block design for assessing ASH effectiveness by first dividing the 9 sites into four sub-regions Western Shore (SB, BP), Eastern Shore (BR, HB), Ralphs Bay (TR and RB) and South Arm (MAB, HMB, OB) and then randomly selected from each block one treatment site: SB, BR, TR, HMB and OP.

We also compared the durability and effectiveness of plastic and ceramic artificial spawning habitats (ASH) as well as natural spawning habitat (stalked ascidians). This included comparing use by fish through observing egg masses on each type as well as the survivability of the ASH over time.

For placement of our ASH arrays, we plotted the GPS spatial distribution of all observations of handfish at sites between 2015 and 2017 and then fitted 250m long lines through the densest clusters and recorded the positions of the start and end points. We then used these co-ordinates to run out two 250m transect reels in the field separated by 8m. We used star pickets with sub-surface floats to mark our start and end points. Along each transect lines we planted two rows of ASH. Rows were evenly interspersed with ceramic and plastic ASH and in total at each site we planted an array of 4 x 250 = 1000 ASH. We completed the array prior to the start of the August 2018 spawning season. Some errors in planting methods occurred in 2 of the 5 sites with the ASH being planted too shallow, thus we replanted fallen ASH during the first round of surveys where we assessed the use and durability of ASH.

The first round of assessment surveys of ASH was completed during the first half of the brooding season (20/09/18 – 08/10/18) so after many fish had laid eggs and were caring for them. Dive teams of 2 swam up the 250m ASH transect along each row and recorded the number of plastic/ceramic ASH still standing, ASH that had fallen/broken, number of stalked ascidians within 3m of the transect lines, number of spotted handfish and the number of egg masses as well as what substrate they were on. Pictures were also taken at 4 angles of the egg masses and the guarding fish so that an egg count could be taken as well as a fish ID so that recaptures could be identified.

The next round of surveys were conducted during the second half of the brooding season (24/10/18 – 12/11/18). The sites surveyed in the same order as the first survey to maintain an even time period between surveys at each site. The same variable were recorded in this round of surveys but no ASH was replanted. Additional surveys were conducted at 2 of the most used sites until all egg masses were hatched in order to track egg survivability. The results of the first survey showed a loss in plastic ASH ranging from 4.4% to 10.8% with the mean being 7.5%. Ceramics on the other hand showed heavier losses from the planting period where the loss of ASH ranged from 16.4% to 65.4% between sites, with the mean being 36.7% (Fig. 3).

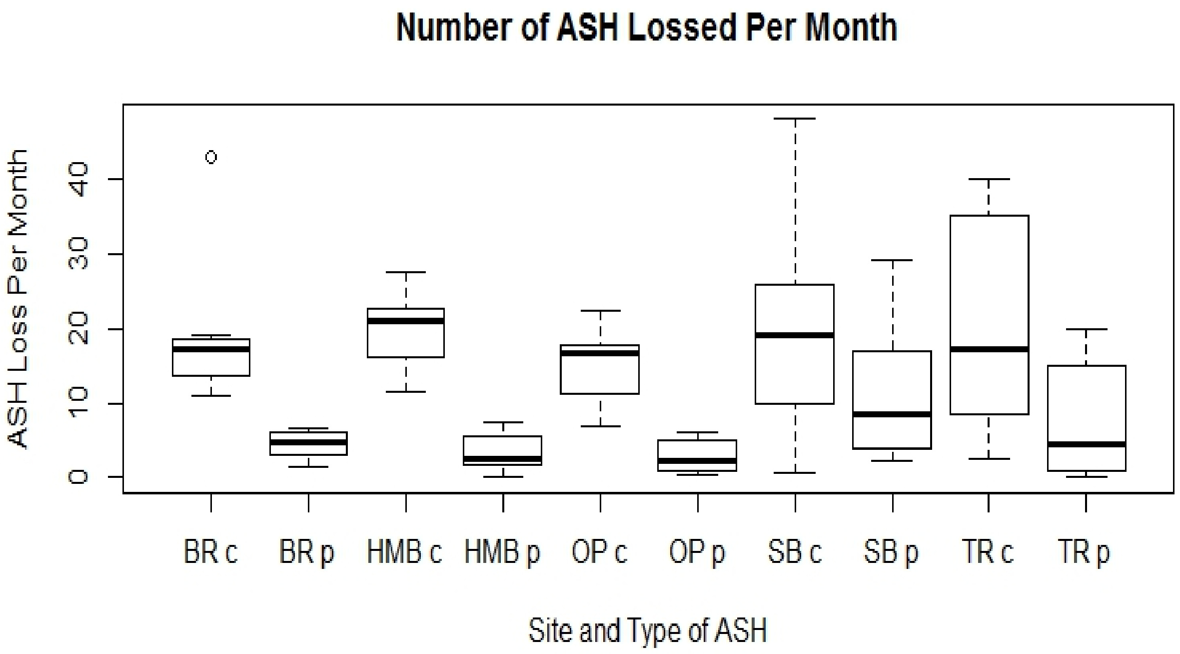

Figure 3 Percentage of ASH lost recorded. These numbers are calculated using the number of ASH remaining over how many were planted (500 of each material per site)

Initially this was thought to be due to the error in the planting method as well as initial losses of ceramics during transporting and handling. However, the second round of surveys produced a loss of 2.5-15.5% with plastics, the mean being 7.7%; and a loss of 13.7-29.8% with ceramic ASH with a mean of 21.9% (Fig. 3). From these results, one could conclude that the ceramic ASH, in its current design, is less durable than the plastic ASH. After the second survey there remains 1964 plastic ASH and 1524 ceramic ASH or 3488 out of the 5000 that were planted. There is also a strong within and between site effects.

Effectiveness of the ASH was measured in two ways, the number of egg masses that were laid on each material, and egg survivability on each material. From the first round of surveys, 16 were found on ceramic, 8 plastic and 5 on natural substrate. The second round of surveys found 14 on ceramic, 6 on plastic and 0 on natural (Fig. 4). Even with less ceramic ASH to lay eggs upon the fish seemed to have a stronger preference for these over plastic ASH, perhaps due to their closer resemblance in colour and thickness to the stalked ascidian or perhaps it is just due to their larger profile and visibility. As for egg survivability, pictures of each egg mass and guarding fish have been taken and are awaiting to be processed in 2019.

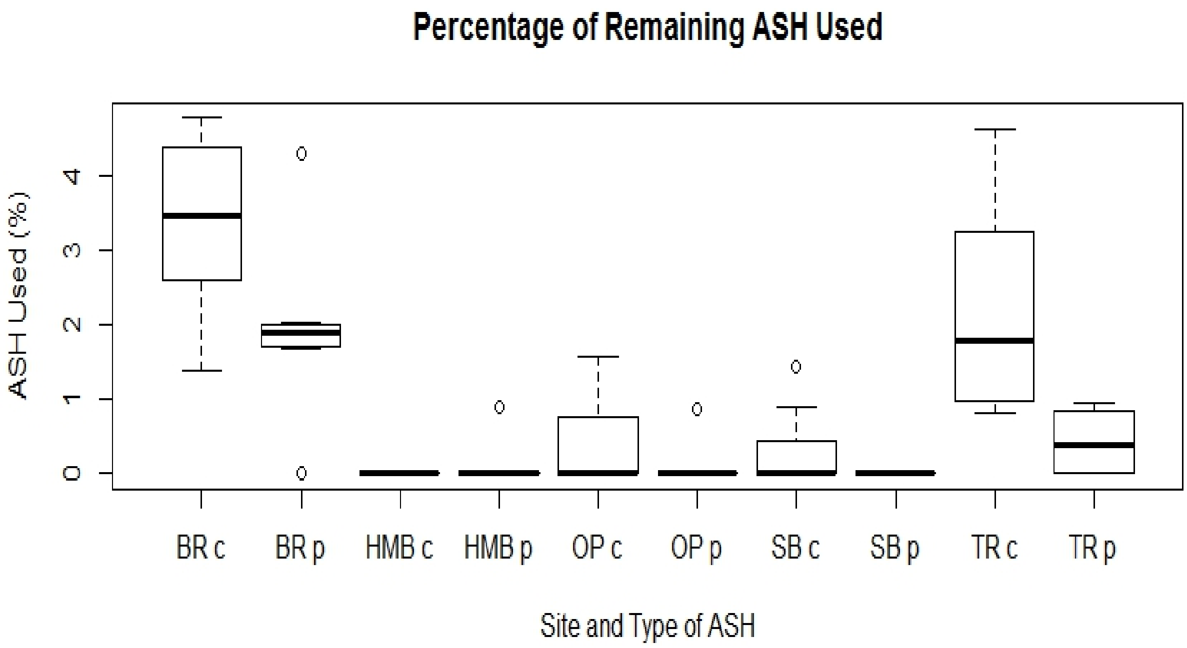

Figure 4 Percentage of ASH used by site and type (ceramic = c and plastic = p).

One variables that seems to determine the amount of ASH used at a particular site is stalked ascidian densities (Fig. 5). Sites that had ascidian densities larger than 0.05 per m^2 saw very low ASH use (less than 3 out of 1000). The two sites that saw the most ASH use had both relatively smaller populations of spotted handfish (Fig. 1) and very low ascidian densities.

Besides assessment of ASH some behaviour work was also completed, video was used from the field and from the captive breeding program to describe specific guarding behaviours, mating behaviour, and interactions between the handfish and other marine life including potential predators. For the mating behaviour, video from the captive breeding program will be used. For all other behaviours, time lapse cameras were deployed on a weekly basis on known egg masses including cameras with red lights that captured night footage. From the field footage, there were 17 instances where the handfish defended their egg masses from various species including 3 successful defences against the North Pacific Sea stars (*Asterias amurensis*).

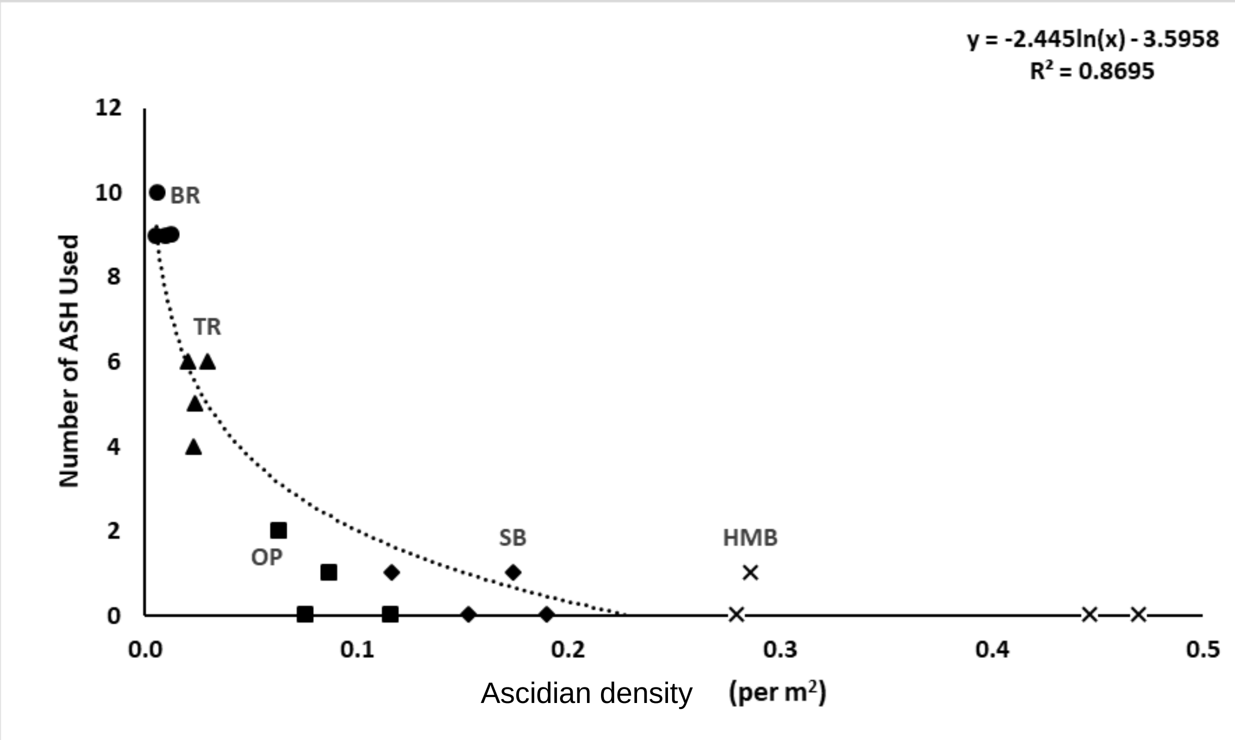

Figure 5 Correlation between ascidian density and ASH use. Ascidian density was calculated using the number of stalked ascidians observed over the 3,000m2 area of transects and averaged between the 2 rounds of surveys.

Other species that the handfish showed guarding behaviour against included several species of crabs, a native species of starfish and small fish including leatherjackets. It seemed that the fish showed different guarding behaviour depending on what species it was guarding against. Additionally, two potential predators were identified; the invasive pie crust crab (*Metacarcinus novaezelandiae*) and sand flathead (*Platycephalus bassensis*), one of which may have eaten a fish that was being videoed. Video also showed that without the parent guarding the nest, it quickly gets preyed upon and is lost within 1 week, which was seen with 3 egg masses. What is believed to be the male fish have also been observed close to the egg masses but did not take part in any egg guarding. In conclusion, we now have a good idea of how the spotted handfish guards its eggs and can assume that as long as the fish is alive guarding the eggs, then few predators would be able to disturb the eggs including the North Pacific Sea star (Fig. 6).

Figure 6 Handfish guarding behaviour against a North Pacific Sea star

5. BIOLOGICAL PARAMETERS OF SPOTTED HANDFISH

5.1 Abstract

Conservation efforts for endangered species often involve determining population estimates, demographic parameters such age and growth and the spatial extent of movements. To estimate these parameters, repeat observations and individual identification is essential. Here we validate a reliable, easy and non-invasive image-based alternative to traditional capture-mark-recapture studies, one particularly applicable to small threatened species. Using this approach, coupled with GPS-parameterised underwater visual census (GUVC) surveys, photo-identification software, and a small number of opportunistically collected otoliths, we assessed age, growth and movement of the critically endangered spotted handfish, *Brachionichthys hirsutus*. *I^{3}S Pattern*, an autonomous photo-identification system, was tested for effectiveness in re-identifying individuals through time, and was found to be highly successful. Von Bertalanffy growth models were developed using annual growth increments in otoliths, which estimated the longevity of *B. hirsutus* to be 10 years. Movements determined from re-sightings ranged from 32 m (13 days) to 567 m (585 days) from the point of first sighting (n = 11). The species was found to have some degree of phenotypic plasticity in individual markings with this being more pronounced in captive populations following capture and re-homing. Despite this, most individuals were able to be reliably re-identified through time. Population estimates based on capture-mark-recapture within populations were explored, however, were found to be unreliable due to the small size of re-sightings in proportion to the overall population. Increased sampling effort is therefore recommended to improve population estimation for rare species such as *B. hirsutus*. This may be significantly assisted by harnessing citizen science, utilising photo-identification techniques.

5.2 Background

Knowledge of basic biological parameters is key to effective management of any species (Caughley and Gunn 1996). For the conservation of species, research efforts often focus on population estimates, spatial distributions and demographic parameters, such as age and growth (Tella et al. 2013). To be able to estimate these parameters, observation is essential (Williams et al. 2002), although for rare, cryptic and elusive species, this can prove to be difficult (Tremblay et al. 2006). For shallow water species, underwater visual census (UVC) surveys are an effective observational technique (Edgar et al. 2004a) even for cryptic species (Edgar et al. 2017), and with the addition of Geographic Positioning Systems (GPS), additional spatial data can be collected (Lynch et al. 2015). However, while UVC is useful for determining presence, diversity, size-frequencies and relative densities, further observations such as the ability to identify individual animals is often required to estimate other population parameters (Wanger et al. 2009) such as age, growth and movement (Taylor and McIlwain 2010).

5.2.1 Individual identification

Methods to identify individuals include physically attaching tags to animals, such as leg bandings (Johnston et al. 2016), implants, such as passive integrated transponder (PIT) tags (Walker et al. 2011), and other physical tags, such as branding, scarring, dyes, and tissue removal. These invasive techniques are widely applied in the aquatic environment both on fish (Edgar et al. 2004b, Hutson et al. 2007) and marine invertebrates (Frusher and Hoenig 2001, Clemente et al. 2007). Advancements in technology over the past few decades (particularly in digital imagery) have facilitated non-invasive options for individual identification. This includes photographic mark-recapture, an alternative to capture-mark-recapture (Bolger et al. 2012), which has been widely applied to many groups of terrestrial and aquatic animals, including cheetahs (Kelly 2001), salamanders (Gamble et al. 2008), African penguins (Sherley et al. 2010), whale sharks (Town et al. 2013), great white sharks (Andreotti et al. 2017), and lionfish (Chaves et al. 2016). Unlike more invasive tagging or branding methods, photographic techniques can either eliminate or minimise handling stress to the animal, and can also reduce the likelihood of altering their natural behaviour (Kelly 2001, Schofield et al. 2008), making non-invasive techniques preferable where possible.

Various computer-assisted programs have been developed to assist with identification of individuals, such as *I^3S* (Van Tienhoven et al. 2007), *Sloop* (Gamble et al. 2008), *Wild-ID* (Bolger et al. 2012), and *APHIS* (Moya et al. 2015). These programs produce digital fingerprints of each individual, allowing searches for previous sightings of that individual within a database of images. Early individual identification software required researchers to manually annotate many natural markings on each animal, resulting in highly laborious post-processing of images. Manual annotation is also a source of inter-observer error as the choice and positioning of annotations is subjective (Correia et al. 2014). To counter this, some individual identification software now has autonomous computer-assisted annotation of features to replace manual processing to more rapidly and independently produce digital fingerprints of individuals with minimal input from the researcher (Van Tienhoven et al. 2007, Moya et al. 2015).

Photographic tagging methods and programs are especially valuable for endangered species, which require added care when subject to research, and are often more highly regulated with permitting for scientific sampling or experimentation. The spotted handfish, *Brachionichthys hirsutus*, is a critically endangered species of anglerfish endemic to Southeast Tasmania (Bruce et al. 1998, Last and Gledhill 2009). The species is related to other critically endangered handfish, such as the red handfish, *Thymichthys politus*, and Ziebell's handfish, *Brachiopsilus ziebelli* (Last and Gledhill 2009). Researching the species is challenging as it is found in cool temperate marine waters and is small (about 130 mm), rare and cryptic (Last et al. 2007). Due to these logistical challenges, much of the species' basic biological, spatial and population parameters are uncertain. *B. hirsutus* have spots covering the majority of the body (Last et al. 2007) with patterns appearing to be unique to each individual and remaining relatively stable throughout their adult life, allowing for individual identification (Bruce and Green 1998). Computer-assisted individual identification from photographs using a manual processing approach has been successfully trialled for this species (Moriarty 2012).

B. hirsutus has experienced a significant population decline since the 1980's (Barrett et al. 1996, Bruce et al. 1997). Causes of this decline may include habitat modification (Edgar et al. 2005, Lynch et al. 2015), pollution (Horwitz and Blake 1992, Macleod and Helidoniotis 2005), historical scallop and oyster fisheries (Barrett et al. 1996, Thrush and Dayton 2002), and the introduced northern Pacific sea star, *Asterias amurensis* (Ross et al. 2003, 2006). As a result of this decline, in 1995 *B. hirsutus* became the first marine fish to be listed under the Federal Endangered Species Protection Act (1992), and the following year the International Union for Conservation of Nature (IUCN) listed the species as Critically Endangered (Green and Bruce 1998). Three recovery plans have been implemented since 1999, with conservation strategies focusing on assisting breeding with deployment of artificial spawning habitats, habitat restoration through environmentally-sensitive moorings, and establishing a captive breeding program (Bruce and Green 1998, Wong and Lynch 2017).

5.2.2 Age and growth

Understanding the longevity of an endangered species such as *B. hirsutus* can be pivotal in successful conservation strategies (Hamidan and Britton 2015). Species with density-dependent mortality have few offspring, mature late and are long-lived (*K*-selected), while *r*-selected species generally have higher rates of reproduction, exhibit little to no parental care and are shorter-lived (Boyce 1984), though these should be considered as end points on a spectrum, with an animal's life history strategy able to exist anywhere between these two extremes (Adams 1980).

Age is arguably the most influential biological parameter in an animal's life history, and it is a fundamental requirement for calculations of demographical parameters, such as growth and survival (Campana 2001). Common fish ageing techniques include analysis of scales (Robillard and Marsden 1996), vertebrae (Stevens 1975), cleithra (Casselman 1990), and opercula (Donald et al. 1992), though the most common age estimator of fish is the otolith (Campana et al. 1995). Typical otolith studies require large sample sizes, although this is unrealistic when working with endangered species due to otolith removal being lethal. Despite this, small samples sizes can provide informative data. For example, Wakefield and Newman (2008), and Norriss and Crisafulli (2010) each utilised only a single otolith to determine the longevity of blue-eye trevalla and Australian snapper respectively. The longevity of *B. hirsutus* is uncertain, with no quantitative ageing study having been conducted, though growth rates of juveniles were recorded during early experimental captive breeding (Bruce et al. 1997, Green and Bruce 2002).

5.2.3 Spatial distribution and movement

While biological parameters, such as age and growth play a key role in the conservation of a species, estimations of spatial parameters are also valuable as they guide on-ground conservation approaches (Tella et al. 2013). For instance, species distribution modelling – a well-developed field of ecology – often relies on species occurrence data and habitat associations (Flowers et al. 2017, Wong et al. 2018). Using such information, models can make predictions of a species' distribution across unsampled locations (Moore et al. 2009). Recently, it was found that *B. hirsutus* appear to be habitat specialists, being found in complex microhabitats consisting of depressions and ripples in the benthos, and avoids sand flats and areas dominated by filamentous algae (Wong et al. 2018).

Species movement patterns can range from sedentary to erratic, with some movement, such as migrations, being predictable. Understanding species movement patterns are critical for effective conservation. For instance, the habitat of sedentary species may be easier to conserve, though their limited distribution can result in them being more prone to extinction from stochastic processes (Sekercioglu 2007). In contrast, a highly mobile species may be difficult to conserve due to movements across juridical boundaries, requiring a broader protection network, with the conservation of one site depending on the condition of other sites (Runge et al. 2014).

There are nine known populations of *B. hirsutus* in the Derwent River, and one additional known population in the D'Entrecasteaux Channel (Barrett et al. 1996, Green 2005, 2007). These populations may be hotspots of suitable habitat, with lower densities of fish existing between each population. Alternatively, these populations may be more fragmented, with sites being too far away to be connected via easy dispersal, with fragmentation probably occurring relatively recently (Lawler 1999). Currently, it is uncertain if movement occurs between sites, although Bruce et al. (1997), Moriarty (2012) and Lynch et al. (2015) all reported that the few re-sighted individuals they observed, over their short-term studies, had not moved far from their initial observations (< 435 m).

5.2.4 Study aims

B. hirsutus are elusive, and critically endangered. Their endangered status prevents extensive collection for research purposes, and invasive tagging of the species is not feasible due to the species being small, and hence at increased risk of harm through handling. Photo-identification is a potential tool for enhancing our knowledge of the species for conservation purposes, particularly when paired with spatial and biological research. This study aims to test the ability of contemporary, autonomous individual identification systems to reliably identify *B. hirsutus*, and, if effective, utilise these to describe movement patterns. In addition, growth information from re-sighted individuals will be cross-validated by comparing with otolith-derived estimates to determine basic growth parameters for this species.

5.3 Materials and methods

5.3.1 Study sites

This study focused on nine well-established monitoring sites for *B. hirsutus* in the Derwent Estuary in southeast Tasmania, Australia (Fig. 7). These sites were selected on the basis of recorded *B. hirsutus* populations (Barrett et al. 1996, Green 2005, 2007). Sites are between 13 ha^{-1} and 33 ha^{-1}, with sampling occurring between the depths of 5 m and 18 m. All sites are dominated by unconsolidated silt and sandy habitat with limited patchy distributions of rocky reef. Some sites, such as Halfmoon Bay, Sandy Bay and Opossum Bay, also contain seagrass beds of varying sizes (Jordan et al. 2001, Lucieer et al. 2007).

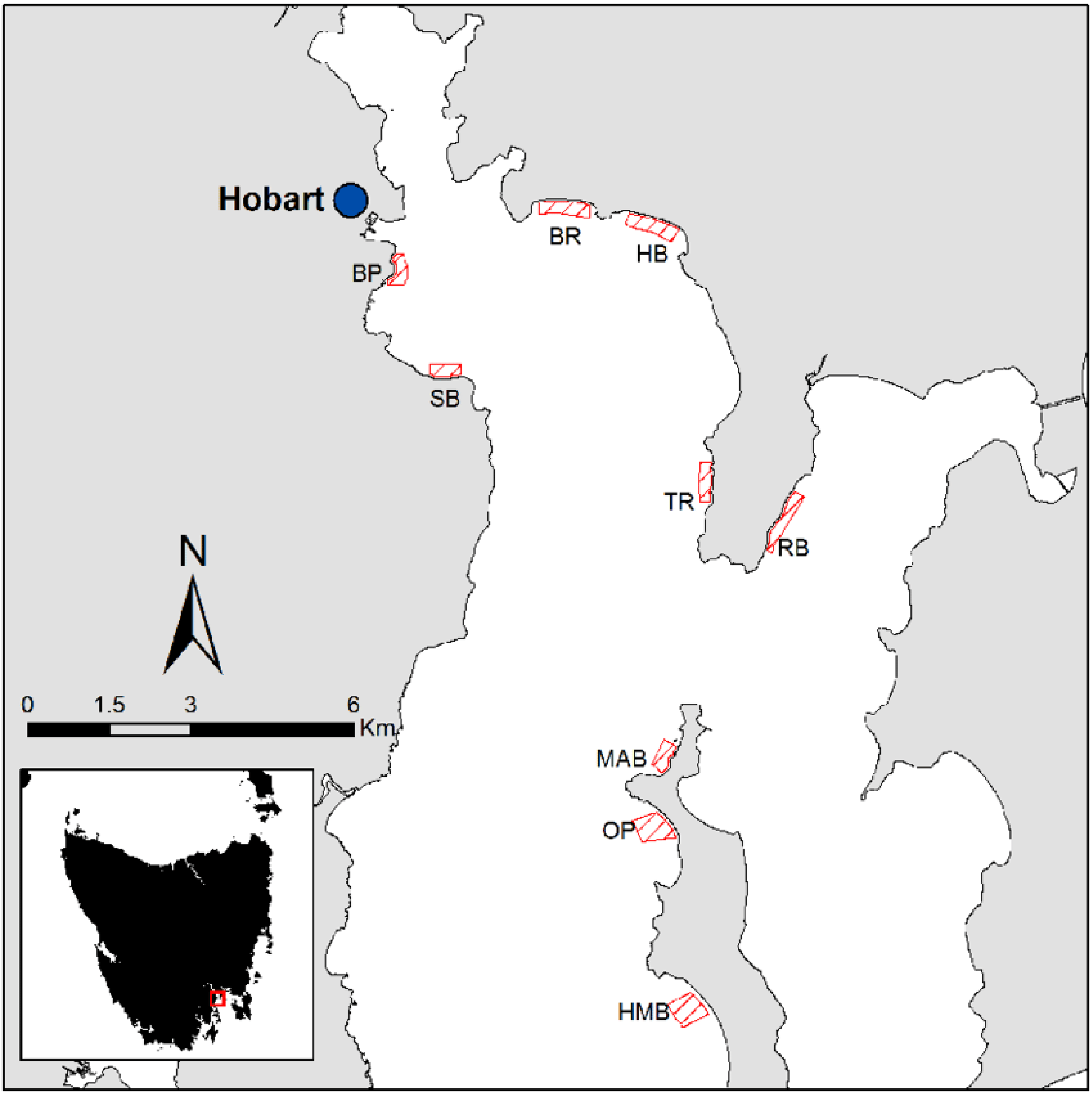

Figure 7 Survey sites. The nine sites (shaded in red) surveyed for B. hirsutus in the Derwent Estuary, southeast Tasmania. Battery Point (BP), Bellerive Beach (BR), Half Moon Bay (HMB), Howrah Beach (HB), Mary-Ann Bay (MAB), Opossum Bay (OP), Ralph's Bay

5.3.2 Data collection

Data has been collected at all sites since 2015 via variable-length SCUBA diver GPS-parameterised underwater visual census (GUVC) transects, as described by Lynch et al. (2015), with additional data collected in 2014 at the Battery Point site. To achieve sufficient power, a minimum of 8 transects each year was conducted at each site (Lynch et al. 2015). Data collection occurred between January and August to correspond with the non-breeding season to avoid potential biases in densities of *B. hirsutus*. Variable-length transects were preferred over traditional fixed-length transects (Green and Bruce 1998, 2002) due to improved statistical power, particularly for cryptic and sparsely distributed species (Haskard 1997, Lynch et al. 2015). Transect start points were selected via a spatially balanced sampling regime that incorporates legacy sites to reduce the probability of sampling a previously sampled location (Foster et al. 2017). During transects, two divers swam for 30 minutes at 0.5 – 1 m above the seabed along a constant depth, searching a 1.5 m-wide transect for *B. hirsutus*. When a fish was sighted, transects were lengthened by 2 minutes to compensate for time lost while photographing the fish. Each dive consisted of two transects separated by a depth increment.

During each survey, divers towed a GPS logger (Holux GPSport 245+) inside a waterproof case attached to a float at the surface to record the tracks of each transect and the position of sighted *B. hirsutus*. If an individual was sighted, they were photographed, and lengths were measured to the nearest millimetre by placing a ruler or pair of callipers in close proximity. Through camera synchronisation with the GPS clock, geolocation of each image was made possible via a timestamped photograph. To reduce positional error, the cable was tightened so the GPS was directly above each sighted handfish (Niedzwiedz and Schories 2013, Lynch et al. 2015). At each sighted *B. hirsutus*, its lateral spot pattern for both sides was photographed (Sony RX100V digital camera) at right-angles to the fish where possible.

GPS tracks of survey transects were uploaded to the program *Holux ezTour for Logger* v3.0 (www.holux.com/dvr). This program displayed transects on an interface that could be used in collaboration with *BR's EXIFextracter* v0.9.14 (www.br-software.com) to geolocate each image taken during surveys in accordance with the alignment of the GPS and camera timestamps. *R* v3.4.4 (www.r-project.org) was used to convert the data into a database-readable format.

5.3.3 Validation of photo-identification

I³S Pattern v4.02 (www.reijns.com/i3s), a freely-available autonomous photo-matching program, was used to validate photo-identification of *B. hirsutus*. The software employs a feature-based algorithm called the Spot Patten Matching (SPM) algorithm to match photos (Van Tienhoven et al. 2007). Following the user's input of three reference points (for this species, in front of the eye, at the base of the first dorsal fin, and the operculum gill opening) and a user defined boundary (Fig. 8), the software created a fingerprint of 45 spots for each *B. hirsutus* based on its unique spot pattern, which are stored in a database for the detection of re-sightings. *I³S Pattern* provides a similarity score based on the closeness of key points of each image, in which the software ranks likely re-sightings. A lower similarity score reflects a better match between images. An image is only classified as a re-sighting when the researcher gives final approval. The software houses a metadata-based filtering system allowing for limiting image comparisons to metadata such as site, or the side of individual images were taken, though when searching for re-sightings in the database the site was not filtered.

Figure 8 I3S Pattern automatically selects spots based on three user-selected reference points (front of the eye, the base of the first dorsal fin, and the operculum gill opening) and a boundary (outlined in green).

To determine the performance of the software in identifying *B. hirsutus* individuals, *I^{3}S Pattern* was used to analyse our large database of images collected since 2014 at the nine survey sites (with a total of 1025 images of 393 different individuals). Preliminary manual and computer-assisted image analysis suggested there were 11 re-sighted individuals within this larger database, which would be used as known re-sightings that success rates could be calculated. Additionally, the performance of the photo-matching software for a small database of images of captive individuals was assessed (with a total of 26 images of 13 different individuals). Images of captive fish were not included in the large database.

The use of *I^{3}S Pattern* on our large image database allowed us to detect for movement that may have occurred between sites and provided re-sightings for use in capture-mark-recapture population estimates. As the database was so large, error matrices were not developed as they were for the above tests, though the rankings of each re-sighting detected by the software was recorded, which also acted as a test of performance of the software.

5.3.4 Age and growth of *B. hirsutus*

Growth in the species was examined in two ways. The first method involved obtaining length measurements of individuals during UVC surveys and then comparing to lengths obtained at re-sighting (n = 13 re-sightings of 11 fish, with 7 re-sightings having times greater than 6 months). All *B. hirsutus* length measurements were examined via a length-frequency plot.

As with all bony fish, *B. hirsutus* have a pair of otoliths. The second method involved developing a growth curve via otolith analysis. To develop a growth model for *B. hirsutus*, otoliths were extracted from opportunistically collected specimens (*e.g.* deceased specimens handed in by the public). Otolith extractions and transverse sections were prepared by Fish Aging Services Pty. Ltd., following the procedures described by Robbins and Choat (2002). During extraction, dissections of specimens determined their sex. The initial age estimated from otolith analysis was used to create a growth model, though had a high degree of uncertainty. Therefore, these estimated ages were adjusted in accordance with the typical egg hatching dates of 1st November (Bruce et al. 1997) by adding approximately one year to the age estimates. Otolith weight was considered when adjusting the age, as were samples with an indication of a translucent edge (suggesting the deposition of the next growth ring). Both initial age estimates and adjusted age estimates are presented in this study. The known size at hatching (age 0 years) is 7 mm (Bruce et al. 1997), which was included in the development of the species' growth curve. Growth in captive individuals was recorded, though not used in this study due to unreliable initial measurements.

Age data derived from otoliths facilitated the development of von Bertalanffy growth equations (Von Bertalanffy 1938) for the species, using equation 1:

$$L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right) \quad (1)$$

Where, L_t is the length at age t, L_∞ is the maximum mean length, K is a growth constant, t is the age of the animal, and t_0 is a modelling artefact that represents the age at which average length was zero.

Von Bertalanffy growth equations for both adjusted and unadjusted ages were developed in *R* v3.4.4 via the package 'FSA'. The 95% confidence intervals for developed growth curves were calculated via bootstrapping based on estimates of L_∞, K, and t_0. Observations of growth identified by GUVC survey re-sightings were compared with the developed growth curves by rearranging the growth equation developed from otolith analysis to estimate age based on length measurements. Age and growth were then superimposed on the von Bertalanffy growth curves.

5.3.5 Movement

Movement of *B. hirsutus* individuals was obtained through plotting the GPS locations of re-sightings identified by the photo-identification software. Each re-sighted fish's positions were plotted in the program *ArcMap* v10.4 (www.esri.com), and distances between points were determined using the measurement tool.

5.4 Results

5.4.1 Validation of photo-identification software

I^3*S Pattern* achieved a high rate of success for photo-identification of *B. hirsutus* (Table 1), with 69.2% of known re-sightings being correctly identified as the highest ranked image. When considering the top 10 rankings, 92.3% of known re-sightings being correctly identified, with all known re-sightings being ranked within the top 20 images. Of the 393 individuals in the database, only 2.8% were re-sightings (13 re-sightings of 11 individuals). These re-sightings were used for growth and movement analysis. The performance of the photo-identification software for the database of captive individuals was similar, with all individuals being identified within the top 20 ranked images, though only 38.5% of images were correctly identified as the top ranked image. The recognition rate greatly increased when considering the top 10 ranked images (92.3%).

Table 1 Photo-identification results for *B. hirsutus* using I3S Pattern.

	Large database	Captive database
No. of images in database	1025	26
No. of individuals in database	393	13
Known no. of re-sightings	13	13

Rankings	Percentage	
Top 1	9/13 = 69.2%	5/13 = 38.5%
Top 5	10/13 = 76.9%	9/13 = 69.2%
Top 10	12/13 = 92.3%	12/13 = 92.3%
Top 20	13/13 = 100%	13/13 = 100%

Results of photo-identification of *B. hirsutus* using *I*^{3}*S Pattern* for both our large database of individuals observed during GUVC surveys and a database of captive individuals showing the number of images and individual within the database. The large database did not include images of captive individuals. Percentages are correctly matched images out of the 13 known re-sightings placed in the top 1, 5, 10 and 20 rankings.

5.4.2 Age and growth of *B. hirsutus*

Total lengths of all observed *B. hirsutus* (*n* = 393) ranged from 28 – 135 mm, with fish 60 to 120 mm long comprising 94.7% of all observed fish (Fig. 9). The length-frequency plot of observed individuals shows signs of selectivity towards individuals larger than 60 mm, with only 3.8% of observed fish being smaller than 60 mm.

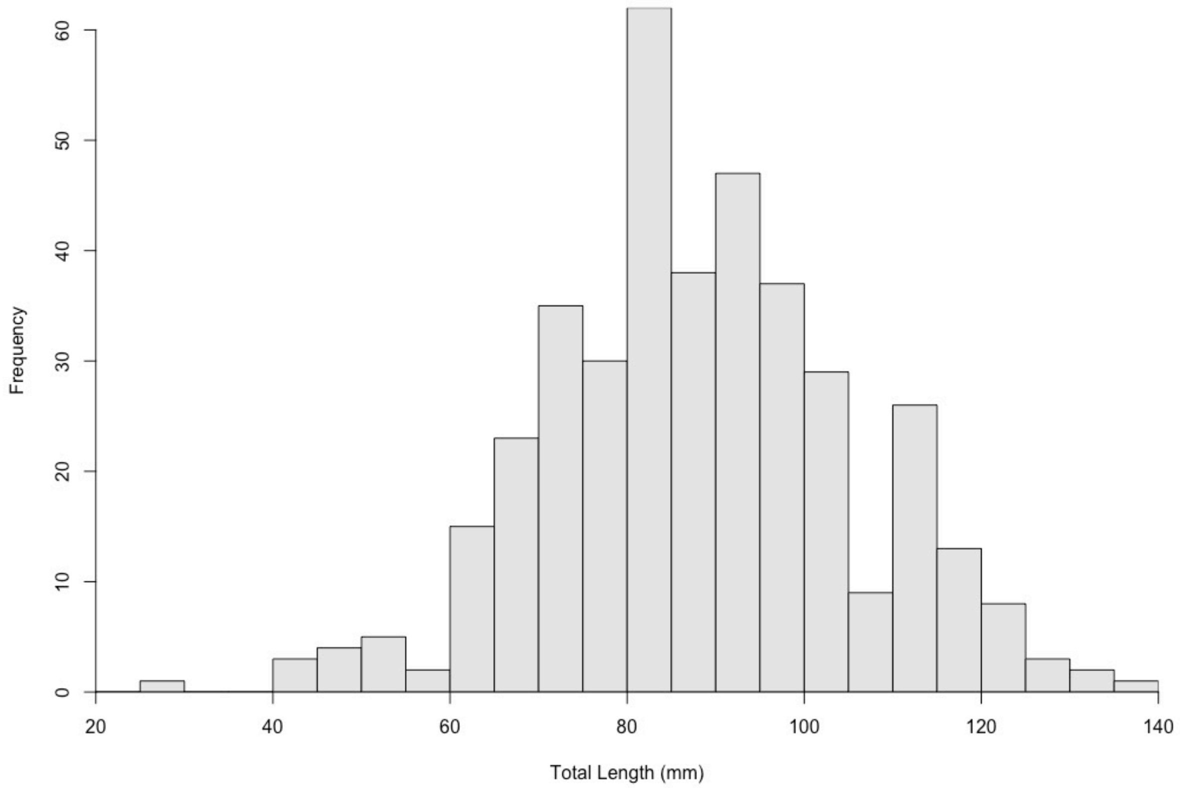

Figure 9 Length-frequency of B. hirsutus observations. Includes observations from all surveyed years and sites (2014 - 2018) in 5 mm bins (N = 393).

Based on estimations of age from otoliths (Table 2), the von Bertalanffy growth equation for *B. hirsutus* using unadjusted ages was:

$$L_t = 118.80(1 - e^{-0.65(t-0.66)}) \quad (4)$$

While the von Bertalanffy growth equation for *B. hirsutus* using adjusted ages was:

$$L_t = 121.02(1 - e^{-0.49(t-0.17)}) \quad (5)$$

Table 2 *B. hirsutus* otolith samples.

Sample	Estimated age (years)	Adjusted age (years)	Length (mm)	Otolith weight (g)	Sex
1	1	2.5	91	0.0019	Female
2	6	6.5	119	0.0068	Male
3	0	1	62	0.0013	Male
4	1	2	74	0.0022	Male
5	1	2	77	0.0012	Male
6	0	1	54	0.0006	Male
7	2	3	91	0.0026	Male

Estimated age, and adjusted age of *B. hirsutus* individuals based on otolith analysis, including length, otolith weight and sex (n = 7). Adjusted age was calculated based on otolith weights and closeness of the date of capture to assumed date of birth (1st November).

The von Bertalanffy growth equation derived from unadjusted ages (equation 4) poorly describes *B. hirsutus* growth (Fig 10A). Estimates of L_∞, K and T_0 had high standard errors, at 22.99, 0.44, and 0.48 respectively. Growth in this model is extremely rapid, with an asymptotic length of 119 mm at *c.* 4 years. It should be noted that mean length at age zero, as described by this model, is 42 mm.

In contrast, the von Bertalanffy growth equation derived from adjusted ages (equation 5) provided a better fit for *B. hirsutus* growth (Fig 10B). Estimates of L_∞, K and T_0 had lower standard errors, at 7.57, 0.08, and 0.11 respectively. Growth, as described by the age-adjusted von Bertalanffy equation, is extremely rapid up to a length of 52 mm in 1 year. Growth remains at a rapid rate until *c.* 2 years, after which growth rate declines and reaches an asymptotic length at 121 mm at *c.*7 years.

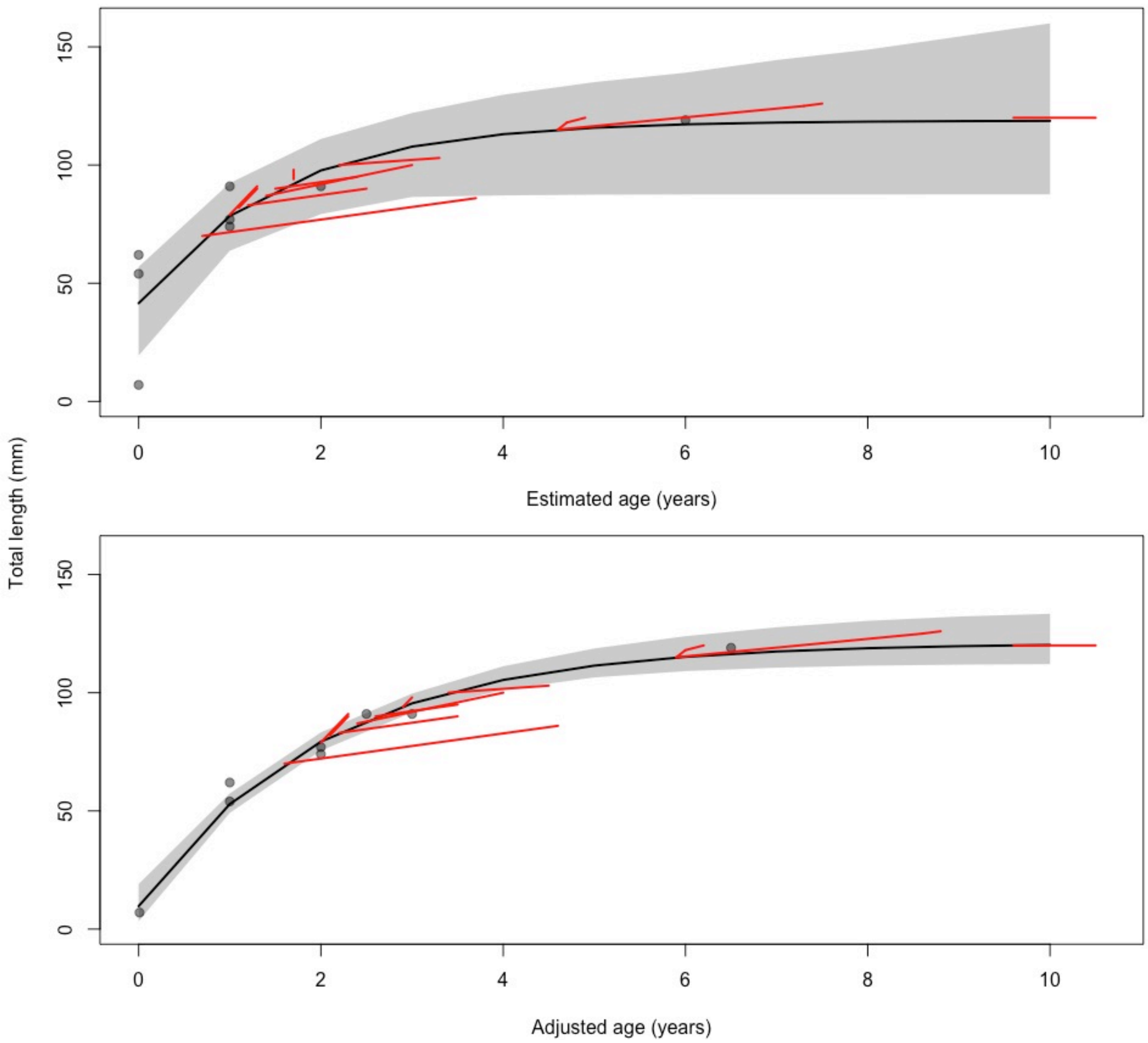

Figure 10 Von Bertalanffy growth functions of B. hirsutus

Length versus estimated age (A) and adjusted age (B) with von Bertalanffy growth functions (solid line) and 95% confidence bands (grey bands) for *B. hirsutus* derived from otolith analysis (*n* = 8, including otolith samples, and observation of length when hatched from eggs). Red lines represent observations of growth from re-sighted individuals from GUVC surveys.

Observations of growth from re-sighted individuals (Table 3) are well represented by the unadjusted age von Bertalanffy growth model, with most observations falling within 95% confidence intervals. In contrast, for the adjusted age von Bertalanffy growth model, multiple growth observations from re-sighted individuals fall outside the 95% confidence intervals, however, this is probably an artefact of the improved confidence intervals of the adjusted age model (equation 5). Both models suggest *B. hirsutus* can live to a maximum of 10 years.

Table 3 Re-sighted *B. hirsutus*

Site	Fish ID	Days between Re-sightings	Initial length (mm)	Total growth (mm)	Distance moved (m)	Rate (m/day)
Battery Point	95	324	90	+5	56	0.17
Battery Point	332	398	100	+3	356	0.89
Battery Point	339	46	115	+3	75	1.63
Battery Point	339	66	118	+2	147	2.23
Battery Point	341	13	94	+4	32	2.46
Battery Point	346	102	79	+12	73	0.72
Battery Point	349	71	82	+8	186	2.62
Howrah Beach	66	987	115	+10	344	0.35
Howrah Beach	66	78	125	+1	110	1.41
Mary-Ann Bay	46	1085	70	+16	321	0.30
Mary-Ann Bay	99	585	87	+13	567	0.97
Mary-Ann Bay	100	457	83	+7	84	0.18
Sandy Bay	199	316	120	0	363	1.15

Time, total growth, movement distance and movement rates of individual *B. hirsutus* between re-sighting periods. Note that fish 66 and 339 were re-sighted twice.

5.4.3 Movement

Of the 393 individuals observed, individual identification software identified a total of 13 re-sightings of 11 individuals over the period of the study (2015 – 2018), with gross individual movement (*i.e.* the distance between the location of initial sighting, and re-sighting) over various periods of time recorded within multiple sites (Battery Point *n* = 7, Howrah Beach *n* = 2, Mary-Anne Bay *n* = 3, and Sandy Bay *n* = 1; see S1 Table), but no movement between sites was observed (Fig. 11, Table 3). The total distances moved between re-sighting periods ranged from 32 m (13 days) to 567 m (585 days), at rates ranging from 0.17 – 2.63 m/day (mean of 1.16 ± 0.86 m/day).

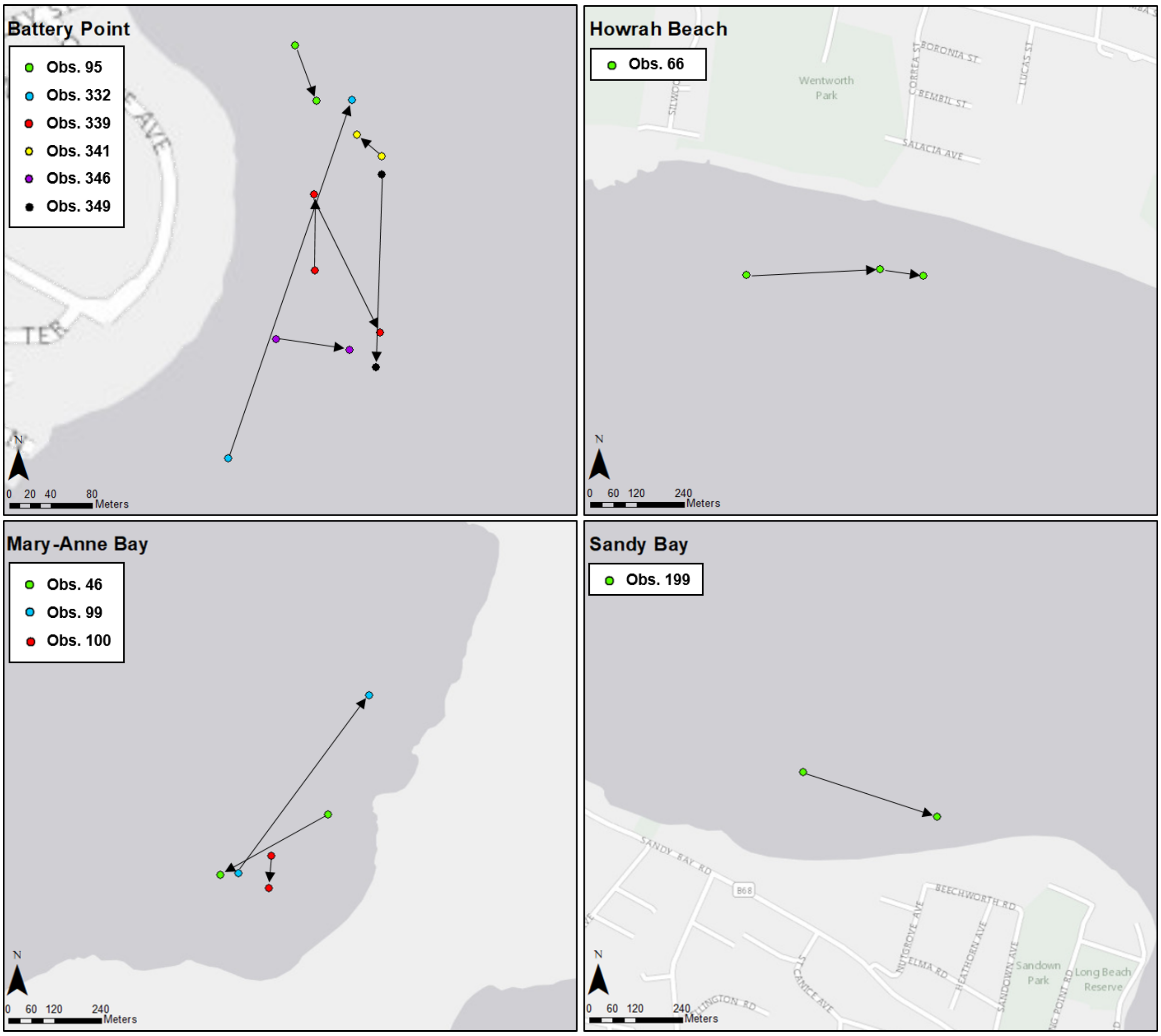

Figure 11 Recorded B. hirsutus movement. Movement of the 11 re-sighted B. hirsutus individuals detected using I3S Pattern. See Table 3 for movement distances and time at large for each individual.

5.4.4 Phenotypic plasticity

In this study, some *B. hirsutus* individuals were observed to alter their spot patterns. Colouration of patches of spots were observed to darken in one wild individual (Fig 12A), while spot patterns in multiple individuals held in captivity changed, most notably in stripes morphing into smaller spots (Fig 6B). The performance of photo-identification systems was not dramatically affected by phenotypic plasticity in this study (Table 1).

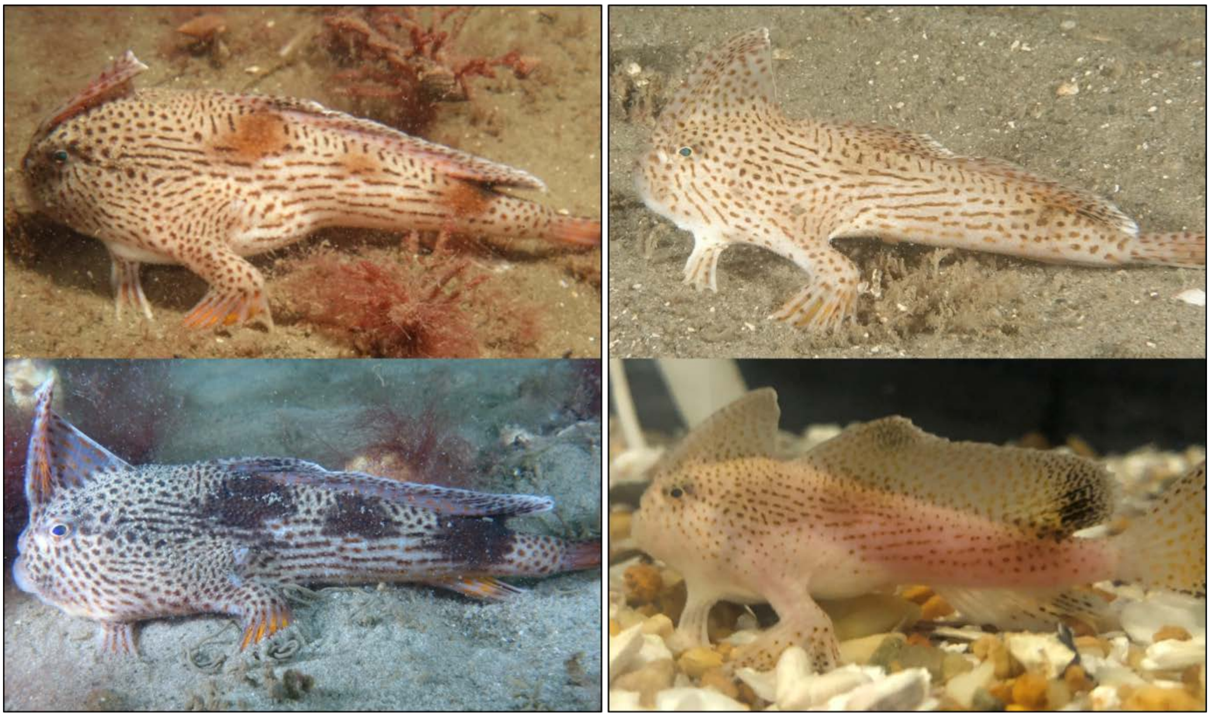

Figure 12 Spot plasticity recorded in B. hirsutus

Changes in *B. hirsutus* spot patterns. (A) Individual observed in the wild (top) and the same individual 987 days later (bottom), with changes most noticeable in the three dark patches across the dorsal surface. (B) Individual in the wild (top) and the same individual after being held in captivity for 204 days (bottom), with a changed spot pattern, particularly below the eye.

5.5 Discussion

5.5.1 Validation of photo-identification software

Working with rare and cryptic species, especially those that require logistically intensive sampling, places conservation researchers into various dilemmas. Of these, low sample size, and consequentially the statistical power to make inferences when combined with the need to consider a high degree of care for animal welfare, mean that the researchers need to address the problems of sample size and data collection from different approaches than normal. We responded to these challenges with photo-identification systems; a non-invasive option for individual identification. The autonomous photo-identification software we trailed proved to be effective in aiding the identification of *B. hirsutus* individuals via photographs of their unique spot pattern. Given the capability of software such as *I³S Pattern* to rapidly and autonomously tag unique spot patterns and compare these against large databases of images, the methods have significant potential as an alternative to both invasive approaches or more labour intensive and subjective manual photo-identification for capture-mark-recapture studies. By testing in both the field and through captive individuals, we are confident that individual identification software can be used as a tool for conservation of *B. hirsutus*, and potentially other critically endangered fish species that also display distinct patterns or markings.

When searching for re-sightings using the software, we can be confident that the individual (given they are indeed a re-sighting) will likely appear in the top 10 ranked images (Table 1). This top 10 recognition rate was greater than the 51% recognition rate recorded using the earlier, manual variant of the software, *I^{3}S Spot*, trialled by Moriarty (2012) on 73 individuals, and is also high compared to recognition rates recorded for other species using photo-based methods (Sreekar et al. 2013, Gardiner et al. 2014, Andreotti et al. 2017, González-Ramos et al. 2017). Compared to manual approaches for individual identification, autonomous image-comparison software provides high success rates, as well as faster, more streamlined comparisons (Matthé et al. 2017). While the software does not completely remove effort by users, requiring input of reference points for each image, and final verification of ranked images, the system is indeed effective and more efficient than manual individual identification systems. This was demonstrated by Matthé et al. (2017) who estimated the processing time for manual image analysis to grow quadratically, compared to computer-assisted analysis, which grows linearly.

We suspect that a major cause of variation in recognition rates based on similarity scores is due to variability in the quality of the image. Reduced image quality was often caused by water turbidity, light attenuation and the angle of the image in relation to the fish; all common issues when using photo-identification software to analyse images taken from the field (Correia et al. 2014). Additionally, the slight differences in recognition rates between the captive fish database and the larger GUVC survey database is most likely attributed to phenotypic plasticity over time.

A potential benefit of autonomous photo-identification systems is the possibility for community involvement through citizen science. This represents a cost-effective way of collecting data on a poorly understood species. Davies et al. (2013) explored this concept with images of whale sharks sourced by tourists and found it to be an effective option, with the technique being employed in research for the species (Araujo et al. 2017), as well as for manta rays (Germanov and Marshall 2014) and sea turtles (Williams et al. 2015). Initiatives such as Reef Life Survey (www.reeflifesurvey.com) have established a database of images of *B. hirsutus*. If citizen-divers photograph *B. hirsutus* correctly (taken at a 90-degree angle to the fish, and sufficiently illuminated) this successful autonomous image analysis may lead the way towards effective management and conservation of the species via more extensive information on movement patterns, growth, and population estimates.

5.5.2 Age and growth

This is the first study using otoliths to estimate age and growth of *B. hirsutus*. Green and Bruce (2002) estimated growth for the species using length measurements and reported that at one year of age, fish were 51 mm, comparing well with the adjusted age model presented in this study (Fig 10B), which estimates length to be 53 mm at one year. Green and Bruce (2002) also reported at two years of age fish are 70 mm, whereas the age adjusted model predicts a length of 79 mm, which lay within the bounds of individual, inter-site and annual variation in growth, suggesting the model has a reasonable degree of accuracy in describing growth for the species. Both estimated age and adjusted age models in this study suggests the longevity of *B. hirsutus* is *c.* 10 years, as is supported by growth observations from re-sighted individuals using photo-identification.

Based on life history factors such as growth, moderate longevity, and a high level of parental care, we suggest *B. hirsutus* is closer along the spectrum to a *K*-selected species than a *r*-selected species (Boyce 1984), but is not extremely long lived. This information should be considered when developing conservation approaches for the species (Tella et al. 2013). Bruce et al. (1997) suggested that individuals reach sexual maturity at two years of age. Based on the distribution of lengths of fish observed in this study (Fig 9), only 10% of fish within the sampled population are older than 5 years of age. The probability of most individuals living longer than 5 years is likely to be low. This would suggest the typical individual has only a few years in which to breed, indicating a heightened risk of species collapse due to recruitment failure over successive years from stochastic disturbances such as seasonal floods, storms and *Asterias amurensis* outbreaks. Therefore, conservation actions to increase breeding success, such as through deployment of artificial spawning habitats (DoE 2015), may be critical to the survival of the species.

While this study has developed two growth models for *B. hirsutus*, they should be interpreted with caution. The estimated age model captured the majority of observed growth seen in re-sighted individuals using photo-identification, however the model does not accurately describe growth for the species, as is highlighted by its broad confidence intervals and highly inaccurate length estimation at age zero. Additionally, our small opportunistic sample of otoliths lacked observations between 4 – 6 years, observations greater than 7 years, and only included one female, and therefore may underrepresent the true age structure of the population. As only one female was aged via otolith analysis, growth differences between males and females could not be determined. Finally, both models were developed from a small sample size, and may not accurately represent the population. Therefore, it is recommended growth and age estates be improved by further field observations aided by capture-mark-recapture approaches. It should be acknowledged that low sample sizes are an unavoidable problem when studying critically endangered species, and while larger sample sizes are desired, it is not practical nor ethical to collect large datasets for this species.

Length frequencies presented by Lynch et al. (2015) showed a range of lengths between 65 and 135 mm, lacking observations of individuals below 65 mm. These are similar to the range in this study (28 – 135 mm; Fig 9), with few observations below 70 mm. This is evidence of selectivity for individuals larger than 70 mm, suggesting fish smaller than this are highly cryptic, and are likely missed by divers during surveys. Alternatively, juvenile fish may be emigrating, though, based on their reproductive biology this is unlikely as *B. hirsutus* lack a pelagic stage and directly recruit to the benthos (Last et al. 2007).

5.5.3 Movement

The autonomous individual identification software tested and proven here, have allowed the movement of re-sighted *B. hirsutus* to be recorded, with five multi-year re-sightings, including the longest time difference between re-sightings documented (1085 days). This provides some indication of long-term movement distances and patterns of *B. hirsutus* individuals. The largest distance moved as recorded by this study was 567 m in 585 days. Previously, the largest recorded distance moved by *B. hirsutus* was 434 m, in 67 days (Moriarty 2012), and prior to that, Bruce et al. (1997) recorded the largest distance moved to be only 85 m in 169 days. These movement data suggest a high degree of residency with relatively small within-site gross movements over long periods. Mean movement rates for the individuals in this study was 1.16 m/day, comparable to means of 0.9 m/day and 3.4 m/day presented by Bruce et al. (1997) and Moriarty (2012) respectively. These are consistently low values, suggesting movement over large distances may take significant amounts of time. For example, at a movement rate of

1 m/day, for a fish to travel between the two closest sites (*c.* 2.5 km), it would take almost all of an individual's life.

It is possible that populations may be fragmented, accounting for lack of detection of inter-site movement. Wong et al. (2018) suggested *B. hirsutus* prefer particular habitat types, which may represent a habitat barrier that prevents movement between sites, though given the highest recorded movement distance is slightly over half a kilometre, some larger-scale movement does occur. Alternatively, movement may occur at a level undetected by the scale of sampling. The *B. hirsutus* survey sites (Fig. 7) have defined boundaries, with no sampling occurring beyond these boundaries during this study. These survey sites may represent population hotspots with a lower density of individuals between sites, with movement of individuals moving in and out of these sites from these lower density areas. Regardless of whether these sites are hotspots or fragmented populations, these may be vulnerable to stresses such as habitat degradation and pest species, highlighting the increased need for conservation through deployment of artificial spawning habitat and transitioning to environmentally-sensitive moorings (Wong and Lynch 2017), as well as general habitat protection.

5.5.4 Phenotypic plasticity

Phenotypic plasticity is well documented in the aquatic environment (Muschick et al. 2011, Abaad et al. 2016), and the photo-identification process trialled here allowed the detection of plasticity in spot patterns of *B. hirsutus* individuals. Interestingly, this was most evident in individuals held in captivity, though there was a case of darkening spots in one fish in the wild (Fig. 12). While spots remain sufficiently stable to allow for individual identification, the phenotypic plasticity observed in this study is contrary to results presented in Moriarty (2012), which detected no alteration in spot pattern during adult life.

A likely cause of this plasticity may be due to changes in substrate colour. *B. hirsutus* inhabit variable, complex soft sediment habitats that are often disturbed by other organisms (Wong et al. 2018) such as skates, rays and flathead. Therefore, it stands to reason that in a variable environment the ability to adapt quickly to minor environmental changes may be essential to increase survival. Darker substrate likely correlates with dark spot patterns, whereas lighter coloured substrate likely correlates with a lighter spot pattern to improve camouflage, though exact causes of this plasticity should be subject to further testing. It should be noted that photo-identification systems in this study were successful in identifying individuals displaying changes in spot pattern, although the final approval must be given by the researcher. Therefore, the implication of this plasticity is that extra care should be taken when identifying individuals to avoid falsely rejecting a re- sighting.

Phenotypic plasticity in the species may have multiple implications on conservation of the species. First, researchers should be aware of the potential for changing of spots for photo-identification. While individual identification software appears to be effective at identifying individuals with slightly changed spot patterns (particularly in captivity), there is the potential for false rejections of re-sightings. Secondly, the species is part of a captive breeding program (Wong and Lynch 2017). It may be necessary to acclimate captive fish intended for release to a substrate of similar colour to the release site to increase probability of survival, and therefore the likelihood of the captive breeding program to be effective. A dedicated study testing the effects of substrate colour on spot pattern plasticity should be explored.

5.5.5 Population estimates

In view of historic decreases in *B. hirsutus* populations (Barrett et al. 1996, Bruce et al. 1997), it is important to assess the effects of conservation strategies. Population estimates allow these strategies to be assessed by comparing previous population estimates with current ones. While population estimates were explored for the species in this study using traditional capture-mark-recapture models, estimates were unreliable due to low numbers of re-sightings (only 2.8% of the 393 observations in this study). The low number of re-sightings is attributed to current sampling design being inadequate, with only a small proportion of seabed at each of the sites being surveyed on any one individual survey (usually < 10% of seabed).

Currently, status of the *B. hirsutus* populations is determined through density estimates (Wong and Lynch 2017), though it would be beneficial to cross validate these estimates with up-to-date populations estimates derived from capture-mark-recapture approaches using photo-identification techniques. Therefore, sampling effort should be increased to improve power. This may be radically assisted by harnessing citizen-science, through SCUBA divers with cameras and GPS devices to build up sampling coverage of both known sites and locations beyond the boundaries of current population locations. The increased autonomy of the photo-identification provides a pipeline process for quick identification of individuals, allowing other image databases to be analysed for re-sightings, such as Reef Life Survey's collection of images. This may also better our understanding of non-resident *B. hirsutus* movement that was not detected in this study, and possibly large-scale movement between survey sites. Furthermore, the number of re-sightings may be increased by increasing sampling effort at one accessible site. This should be initially trailed to validate the technique using the current survey design, and then comparing the effort required to increase re-sightings with the traditional fixed-transect approach to CMR studies.

5.5.6 Conclusion

Photo-identification has significant potential as an alternative to invasive approaches for individual identification. We have validated a reliable, easy and non-invasive method for capture-mark-recapture techniques for a species at risk of extinction. The method is particularly applicable for small, threatened species where other methods are not practical, and may prove useful for other rare and threatened fish species, such as the red handfish, *Thymichthys politus*. Using this method, we have gained valuable biological parameters for ongoing protection of *B. hirsutus*, although further research is required, including better capture-mark-recapture-based estimations and growth estimations. This easy, cost-effective method can be used to empower citizen-based science to improve our coverage and understanding of this critically endangered species.

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