Marine Biodiversity Hub

National Environmental Science Programme

Procedures and methods for establishment of captive breeding populations of spotted handfish

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Project A10 – Conservation of spotted handfish and their habitat

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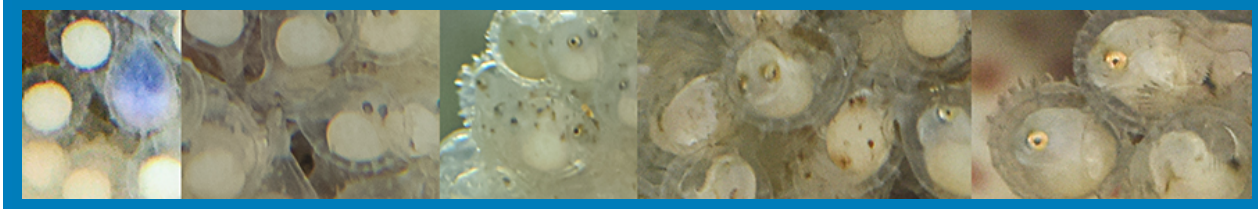
Milestone 10 – Research Plan 3 (2017)



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SEAHORSE WORLD

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Important Disclaimer

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EXECUTIVE SUMMARY

Brood stocks were established at two institutes, with 10 animals each to Seahorse World and SEA LIFE Melbourne Aquarium. Both institutes are known to have at least one fertile pair due to breeding occurring in captivity following collection. The captive population is now 116 fish (30/12/2017) of which 20 are adults or sub-adults and 96 are captive bred juveniles. No fish have died during this process which was unexpected as previously high mortality occurred for juveniles. Our use of marine-tank chiller units, which have become cheaply available since the previous captive breeding work in the mid-1990s, may be responsible for this low mortality.

Knowledge transfer to industry partners (Seahorse World and SEA LIFE Melbourne Aquarium) by CSIRO officers for captive breeding of handfish was made through personal visits, phone calls and the provision of all reports and laboratory notes. A standard operating procedure was developed for data processing and transfer of animals via freight. A stud book was also established - *Spotted handfish ambassador fish program: captive fish studbook*. See References.

Compared to previous years, similar results were obtained for fish densities at the nine long-term monitoring sites, however this year the observed decline at Ralphs Bay continued to its conclusion with no fish observed during 2017.

1. INTRODUCTION

In 2017 we collected brood stock and transferred animals to our institute partners, and continued our performance assessment surveys. Several manuscripts were also prepared and submitted. As major analysis was done in previous reports (Wong and Lynch 2017) this is a more technically focused document made up of a small main body and extensive appendixes.

Our capture-mark-recapture work for spotted handfish only identified one recapture across 2015-2016, based on pattern recognition software. However, as we are only observing between 1-15 fish at each site per year, this is not unexpected even with small populations. More intensive studies (Lynch et al. 2015) at Battery Point in 2014 found 3 recaptures from 38 observations, while another intensive study at Battery Point and Sandy Bay in 2012 provided enough recaptures to estimate populations of 130 and 72 fish per site (Moriarty 2012). If these local population estimates are related to densities, as suggested by Moriarty, then with 5 medium density sites (~100 fish), four low density sites (~50 fish) and one high density site (~ 200 fish), a minimum population across the 10 sites was estimated to be at least 1,000 in 2016. There may be many more fish than this in the wild but, at the moment, our best estimate is that this is the minimum number of fish. Better estimates of total populations may be available with more observations and/or genetic work.

In negotiations with the state government, permitting for sustainable take was limited to less than 5% of the total population. Our industry partners suggested that they would like to receive 10 fish each, which was considered reasonable based on the estimated minimum stock size and genetic diversity. However, they also advised that a death rate not exceeding 50% may occur during establishment of the program. This would mean, as a worst case scenario, that we would take 40 fish in total, which would represent >5% of the suggested minimum population.

As handfish are not sexually dimorphic, taking at least 20 fish increased the likelihood of acquiring both males and females. We also collected in September through to November 2017. This is the breeding season and sex differentiation was possible in some cases as females become heavily gravid, though fish may not breed annually (Green pers comm). We took fish across most sites to minimise disturbance to local populations. Our understanding of connectivity is that any one population has only recently (late 20th century) been fragmented (Lawler 1999), so we hypothesise isolated local populations are similar genetic stock. Again, as with the population estimate, more modern genetic studies could test this hypothesis.

The project aims to provide conservation through the aquarium community sustaining *ex situ* populations of spotted handfish This will be done in conjunction with a well-established program (funded by the National Environmental Science Program and CSIRO), industry partners Seahorse World and SEA LIFE Melbourne Aquarium, as well as through surveys, to establish trends in fish abundance at multiple sites through time. To this end we:

1. captured fish for commercial aquariums, based on estimates of the minimum population

2. settled and conditioned fish for short periods of time before transfer to aquariums

3. facilitated knowledge transfer of CSIRO's previously developed husbandry and breeding techniques to two different institutions, SEA LIFE Melbourne Aquarium and Seahorse World

4. raised the profile of the spotted handfish with the broader community through the ambassador fish program, media, talks, outreach, publications and interpretation materials

5. had facilities on hand to receive and settle captive bred fish for release back into the wild if required

2. SURVEYS

Surveys were planned using the NESP developed spatially balanced sampling procedure (Foster et al.), which took into consideration previous transects. Density surveys were completed prior to the start of the September 2017 breeding season with 8 transects conducted at each of the 9 long term monitoring sites. Data collection and work flows are now well established with an Access data base replacing the previous Excel spreadsheet. A standard operating procedure (SOP) for the data workflow is now also available (Appendix A). Results were similar to previous years (Lynch et al. 2016) for site densities (Fig 1). Consistent with an exponential decline observed in previous years, no fish were observed at Ralphs Bay in 2017.

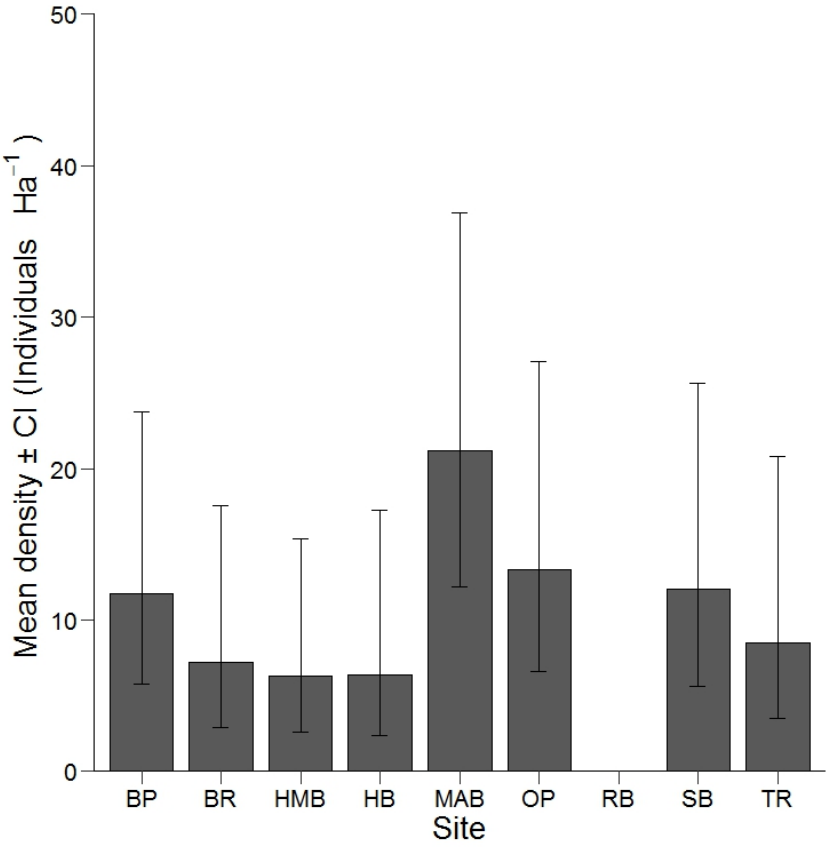


Figure 1 Density estimates for spotted handfish at 9 long term sites in 2017 (BP = Battery Point, BR = Bellerive, HMB = Half Moon Bay, HB = Howrah Beach, MAB = Mary Anne Bay, OP = Opossum Bay, RB = Ralphs Bay, SB = Sandy Bay, TR = Tranmere.

3. BROOD STOCK

As advised by the Handfish Recovery Team (HRT) geneticist, Dr Carolyn Hogg, brood stock were collected from all known sites within the Derwent, with the exception of Ralphs Bay (Table 1). Details and photos of all fish are recorded in the stud book (Appendix B).

Fish were collected by hand, initially using a dip net but then by just opening the perforated collection jar (Fig 2) and gently shuffling the fish into the mouth with the lid. This proved to be much less stressful to the fish. Collection containers and fish were held in a dive bag for the remainder of the dive. At the end of the dive, fish and containers were transferred while underwater into the larger bucket and transported to the surface. This was so fish remained in water when moved from the water into the boat. Once the divers were brought back into the boat we made a fast trip back to CSIRO where another officer would be waiting to swiftly transfer fish into the holding facility. If time permitted we would then travel to a new dive site to collect more fish.

Table 1 Brood stock collection region, site and distribution to institutes

Region	Site	Total number	# Seahorse World	# SEA LIFE
Western	Battery Point	3	1	2
	Sandy Bay	5	3	2
South Arm	Mary-Ann Bay	5	1	4
	Half Moon Bay	1	1	
	Opossum Bay	2	2	
Eastern	Bellerive Beach	2	1	1
	Tranmere	1		1
	Howrah Beach	1	1	
TOTALS		20	10	10

Figure 2 Collection equipment

We collected all fish we saw on collection dives with the exception of fish guarding eggs. This may have resulted in a bias for males but we are certain that each institute has at least one fertile pair as two pairs of fish bred in captivity.

3.1 Aquarium holding facility and captive breeding

Captive husbandry and release of individuals for both red and spotted handfish has occurred in the past (Green and Bruce 2000, 2002). The previous project, however, was mainly for research and methods development. The main difference to the current project is that CSIRO will only be a staging area for fish. They will then transfer the animals to large-scale commercial aquariums. These institutions have provided in-kind commitments to keep fish through to 2020. However, as they do not have business models dependent on short-term competitive granting, but rather will use the fish as a business assets, the captive populations may persist well into the future. We think that this change in approach will allow for fish to become self-sustaining as captive populations with surplus individuals available for re-stocking back into the wild.

A custom built facility was commissioned at CSIRO to hold and condition the fish prior to transfer to the partner institutes (Fig 3).

Figure 3 Mr Tim Fountain and the handfish holding facility during commissioning

The set-up of the facility is:

Large holding tanks (160cm X 58cm X60cm) (~550 litre)

2 x 25 Watt Emperor Aquatics UV sterilisers for water

2 x PPS05 Aquasonic water fractionator to remove wastes and slimes

4 x Tanks 40cm X 40cm X 40cm (~ 60 litre) (conditioning tanks)

2 x Multi 4000 water pumps

2 x HC-300A Hailea Chiller units 1/4hp

2 x ABS25 Bio Filter Sump w/25L Media

Air pumps to work directly off CSIRO building supply

2 x Aqualina multi-channel blue tooth programmable lights to simulate day/night conditions

Once commissioned (Fig 4), the flooring in the holding tank was stocked with natural substrates taken from handfish habitat. We set up this tank to replicate the environment and provide enrichment by using photographs pinned to the tank glass and objects for the fish to hide behind. The programmable light system replicated the diurnal patterns and lux of the natural environment.

The system is designed to remain full of water even if power is cut, through the use of stand pipes in the tanks. Power is monitored 24/7 and alarmed with a call out facility. The main risk is overheating which was mollified by the use of the chillers and a separate air conditioning unit in the small windowless, internal and well insulated room.

Figure 4 Commissioned facility with lights

One pair of fish bred in CSIRO's holding facility (BP-001 and BP-002) soon after collection, as did another pair (OP-001 and OP-002) at Seahorse World soon after transfer (See Stud Book Appendix B). The BP breeding pair were sent to SEA LIFE Melbourne Aquarium.

Fish were fed live food (amphipods) collected 2-3 times per week from the Kingston Beach boat ramp (Fig 5).

Figure 5 Collection apparatus and site for live food.

Water quality parameters for the tanks were taken near daily and were cross checked against natural conditions.

At CSIRO, photographs were taken of the eggs developing (Appendix C). When the eggs began to hatch we quickly transferred 15 juveniles to Seahorse World (within 24 hours) (Fig 6) and then released the remainder back near where the adults were collected at Battery Point. These were later joined by 81 juveniles that hatched at Seahorse World. Unlike previous studies, incubation was longer ~10 weeks compared to 6-8 with few un-hatched eggs. This may be due to better water quality provided by the chiller units and other tank features such as skimmer units.

Figure 6 The 15 captive breed fish from CSIRO after delivery to Seahorse World.

Fish were despatched to institutes in batches of 4-5 fish across 2-3 packing events to minimise the risk of death of brood stock due to a catastrophic transport mishap. Packing and dispatch of fish was a technical process and this is detailed in Appendix D.

No fish were lost during capture, breeding, transfer or since the fish have been accepted by the institutes (30/12/2017). This was unlike the previous study and was unexpected.

APPENDIX A – SOP FOR DATA HANDLING

Handfish survey data entry/processing standard operating procedure

Software requirement

Holux ezTour for data logger
Microsoft Access
R or Rstudio
BR's EXIFextracter

1.0 Introduction

1.1 Outcome

This document outlined the procedure required to extract field data collected during each GUCV search data from GPS and camera's SD card for processing and converting data into a csv file for later analysis.

1.2 Intro

During each handfish search, two set of data are recorded:

1. a GPS track of the dive team's location, recorded using a GPS towed on a surface float; and
2. time stamped photos of handfish or other points of interest captured using an underwater camera which is synchronised with the GPS clock, used for post-hoc georeferencing specific points (e.g. start/end of each search, and location of sighted fish).

As the data were processed, specific information will be extracted, essential for handfish population analysis. Two primary file will be generated, first included metadata of each transect conducted and the second recorded the data of each individual handfish sighting. Data entry and the storage of data were completed with a Microsoft Access database.

2.0 Downloading data

2.1 GPS track

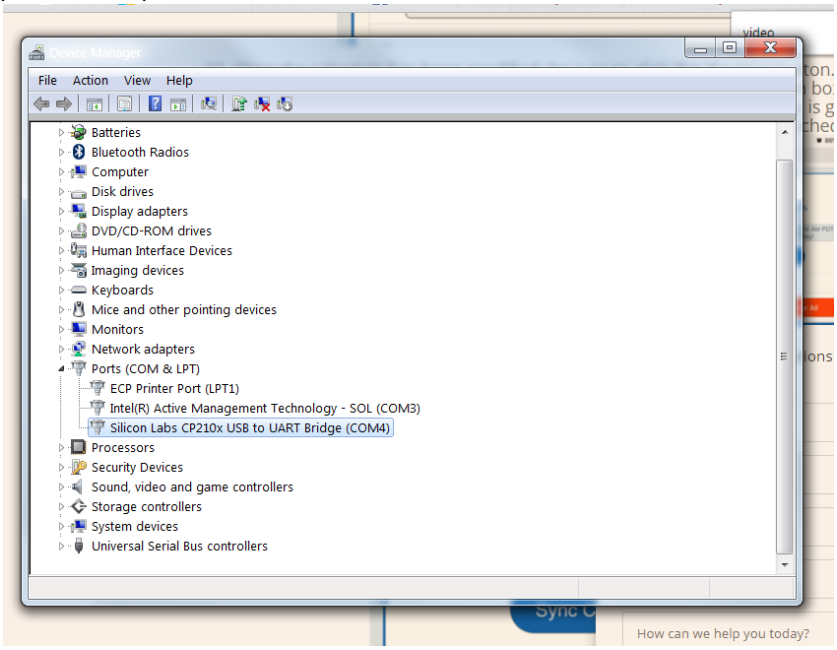
1. Connect Holux GPS to computer via a micro USB 2.0 cable

Box 1

For first time setup, the software will ask for a product identification code and an email address to activate the software. The code is located on the back of the GPS units used for the handfish project.

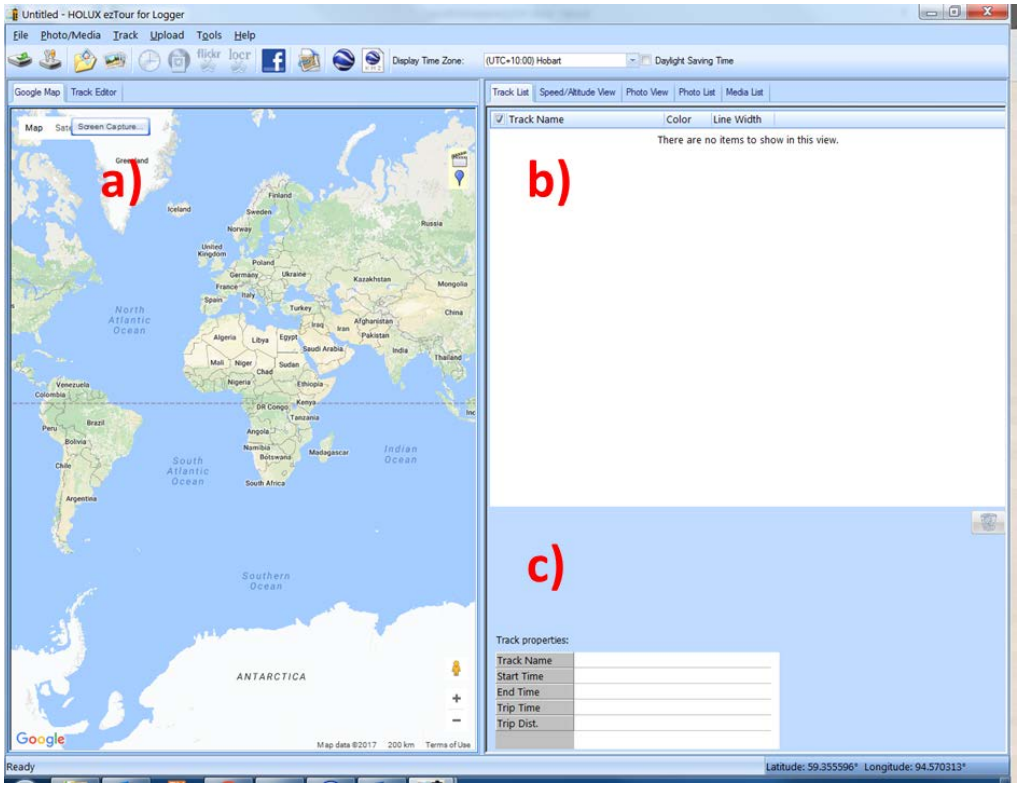
It is possible after connecting the GPS to the computer, the software will indicate that it failed to connect or detect the GPS. This is most likely due to a driver issue. When first setting up the computer for using this Holux ezTour program, ensure that the driver for Silicon Labs CP210x USB to UART Bridge is up to date.

It is possible the computer did not recognise the new driver required (Silicon Labs CP210x USB to UART Bridge) as a COM port, thus failed to connect with the GPS. When first connected the GPS to the computer, open *Device Manager* from the computer (Click start and search for *Device Manager*) and check if "Silicon Labs CP210x USB to UART Bridge" become visible under the Ports (COM & LPT) list



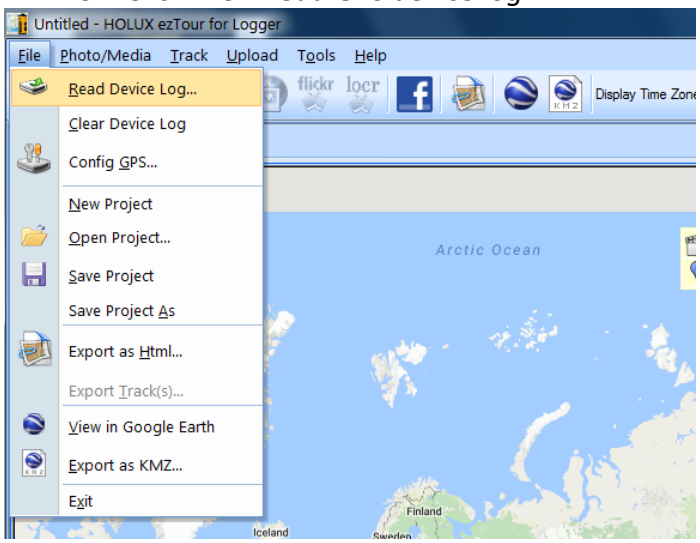
If the driver is not in the list, it is possible the driver was hidden under *Other devices* as an unknown device. Right click on it and select *"Update driver software"*. The system will search for update for the driver automatically and once finished, may require a reboot. Once this step is completed, the driver should be in place and allowed for the GPS to be connected.

2. Open Holux exTour for data logger

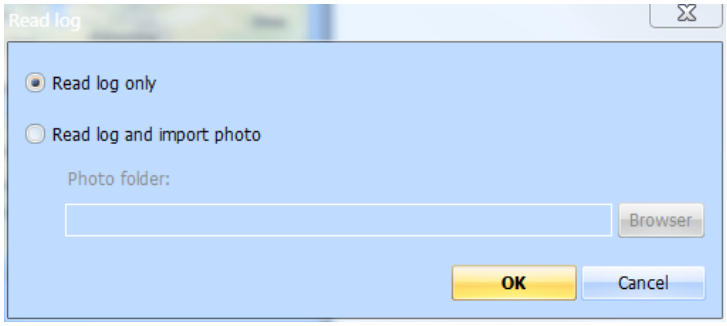


a) Spatial view panel/ track visualisation
b) Track, photo information panel
c) Metadata panel for track/photo

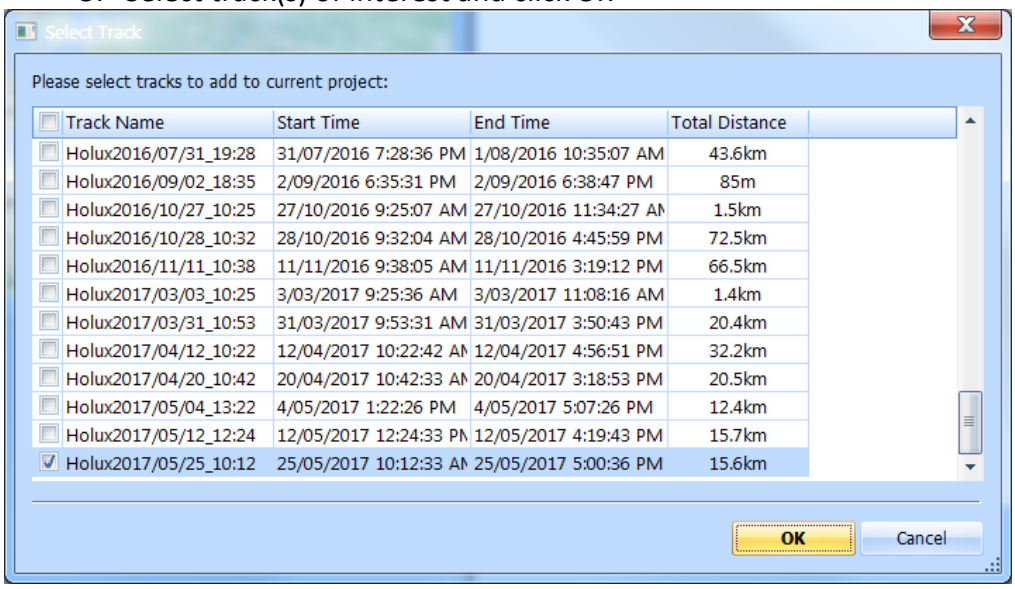
3. Click *File->Read GPS device log*



4. Select *Read log only* and click *OK*



5. Select track(s) of interest and click *OK*



6. Save project

7. as: **Date(yyyymmdd)*_RAW**
   e.g. 20170525_RAW

   at this directory
   My computer
   └ Public (P:)
   └ Tim_Lynch
   └ **HandfishData_year**
   └ **Date (yyyymmdd)**
   └ *

   create new folder when necessary (e.g. new survey year and new survey dates)

2.2 Photo

1. Insert Camera's SD card via card reader
2. Sort photo base on date taken
3. Select photos of interest and copy
4. Save to folder: **Handfish_photos_All_year**
   e.g. *Handfish_photos_All_2017*

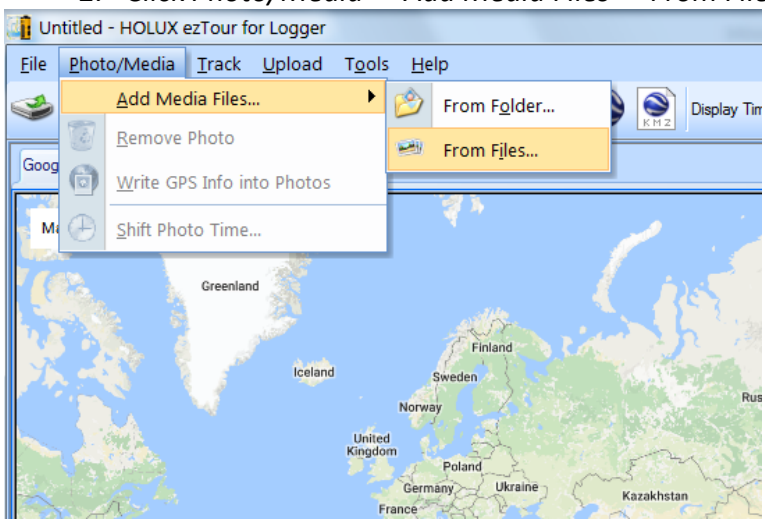
   at this directory
   My computer
   └ Public (P:)
   └ Tim_Lynch
   └ **Handfish_photos_All_year**
   └ *

   create new folder when necessary (e.g. new survey year and new survey dates)

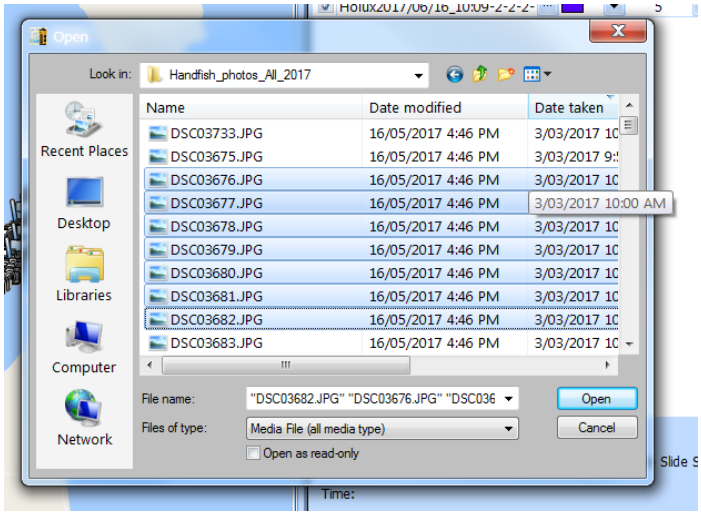
3.0 Extracting transect for GPS track

3.1 Import geotagged photo

1. Open the file Date(yyyymmdd)_Site_RAW
2. Click *Photo/Media -> Add Media Files -> From Files*



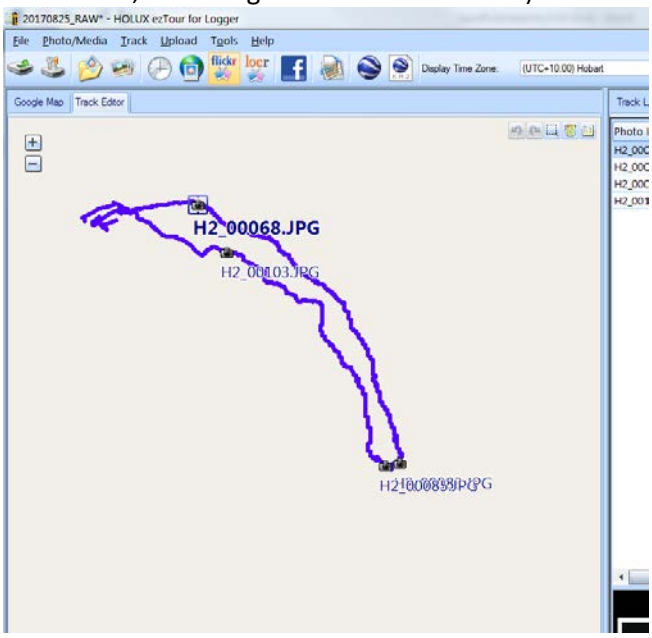
3. Navigate to the photo folder (**Handfish_photos_All_year**)
4. Select photo taken on the date of the transect



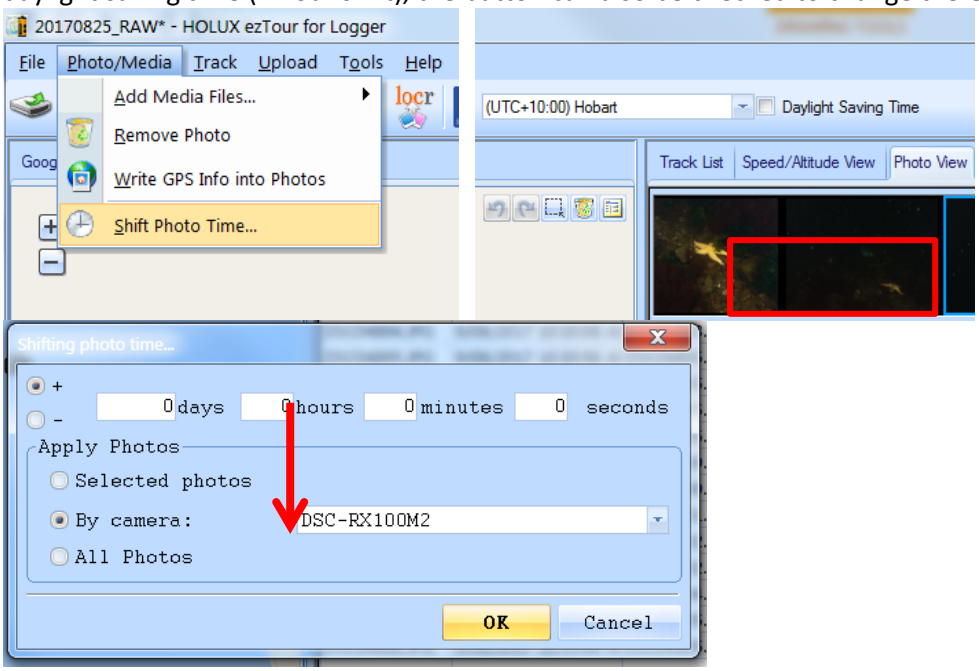
5. Click *Open*
6. Inspect track and location of photos

Box 2

Normally, the section of the GPS track related to the dive will have a "c – shape", with photo present throughout the track. While the actual coordinate of the photo taken can varies due to field limitations, the coordinates of start/end of transect photos ("finger photo") should be distributed in a logical fashion (i.e. close to the corner of the track). The photos should separate the track in to 3 sections (2 long tracks, approximately the same length corresponding to the transect; 1 short track, recording the randomised swim)



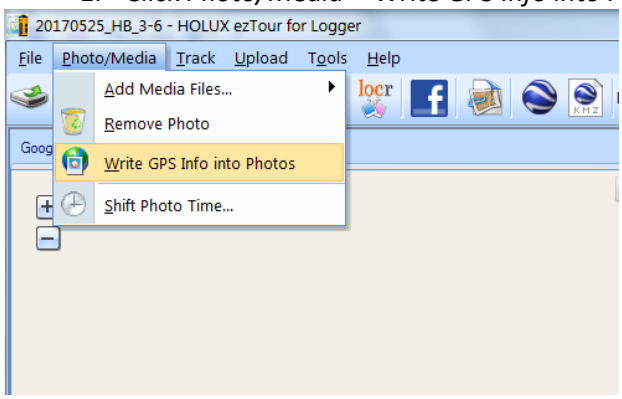
If the start/end transect photo does not line up, it is possible the timestamp on the camera did not line up with the GPS. If required all photos imported into the program can be shifted a set amount of time to line up the GPS track and the captured photos. Alternatively, if the error was caused by daylight saving time (1 hour shift), the button can also be checked to change the GPS time.



7. Inspect the track and photos (camera icon)

3.2 Geotagging photo

1. Click *Photo/Media-> Write GPS Info into Photos*



2. Select *Photo List Panel*
3. Confirm "V" or "✓" present in in the *Geo Tagged column*

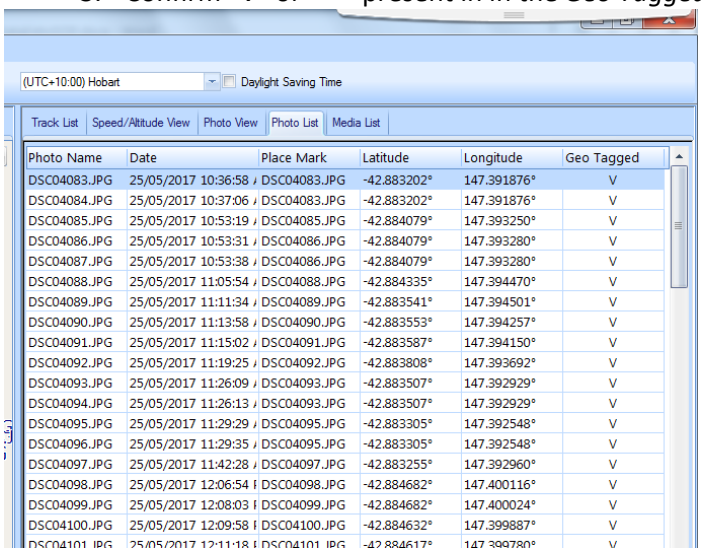


Photo Name	Date	Place Mark	Latitude	Longitude	Geo Tagged
DSC04083.JPG	25/05/2017 10:36:58	DSC04083.JPG	-42.883202°	147.391876°	V
DSC04084.JPG	25/05/2017 10:37:06	DSC04083.JPG	-42.883202°	147.391876°	V
DSC04085.JPG	25/05/2017 10:53:19	DSC04085.JPG	-42.884079°	147.393250°	V
DSC04086.JPG	25/05/2017 10:53:31	DSC04086.JPG	-42.884079°	147.393280°	V
DSC04087.JPG	25/05/2017 10:53:38	DSC04086.JPG	-42.884079°	147.393280°	V
DSC04088.JPG	25/05/2017 11:05:54	DSC04088.JPG	-42.884335°	147.394470°	V
DSC04089.JPG	25/05/2017 11:11:34	DSC04089.JPG	-42.883541°	147.394501°	V
DSC04090.JPG	25/05/2017 11:13:58	DSC04090.JPG	-42.883553°	147.394257°	V
DSC04091.JPG	25/05/2017 11:15:02	DSC04091.JPG	-42.883587°	147.394150°	V
DSC04092.JPG	25/05/2017 11:19:25	DSC04092.JPG	-42.883808°	147.393692°	V
DSC04093.JPG	25/05/2017 11:26:09	DSC04093.JPG	-42.883507°	147.392929°	V
DSC04094.JPG	25/05/2017 11:26:13	DSC04093.JPG	-42.883507°	147.392929°	V
DSC04095.JPG	25/05/2017 11:29:29	DSC04095.JPG	-42.883305°	147.392548°	V
DSC04096.JPG	25/05/2017 11:29:35	DSC04095.JPG	-42.883305°	147.392548°	V
DSC04097.JPG	25/05/2017 11:42:28	DSC04097.JPG	-42.883255°	147.392960°	V
DSC04098.JPG	25/05/2017 12:06:54	DSC04098.JPG	-42.884682°	147.400116°	V
DSC04099.JPG	25/05/2017 12:08:03	DSC04099.JPG	-42.884682°	147.400024°	V
DSC04100.JPG	25/05/2017 12:09:58	DSC04100.JPG	-42.884632°	147.399887°	V
DSC04101.JPG	25/05/2017 12:11:18	DSC04101.JPG	-42.884617°	147.399780°	V

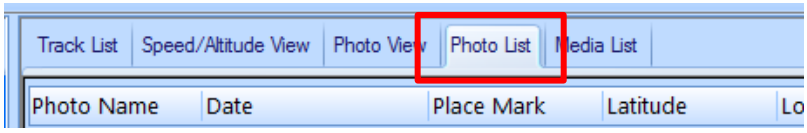
4. Save file as: **Date_Site_DiveNumber**
   e.g. 20170525_HB_D3-6

My computer
└ Public (P:)
└ Tim_Lynch
└ HandfishData_**year**
└ **Date (yyyymmdd)**
└ **Date_Site_DiveNumber**

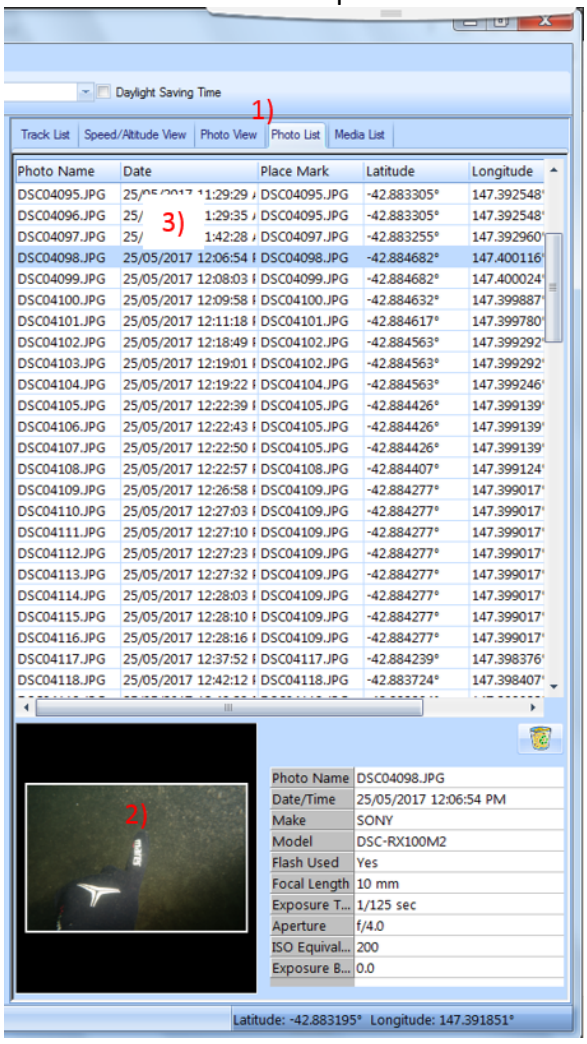
create new folder when necessary (e.g. new survey year and new survey dates)

3.3 Extract transect from GPS track

1. Select the *Photo List panel*



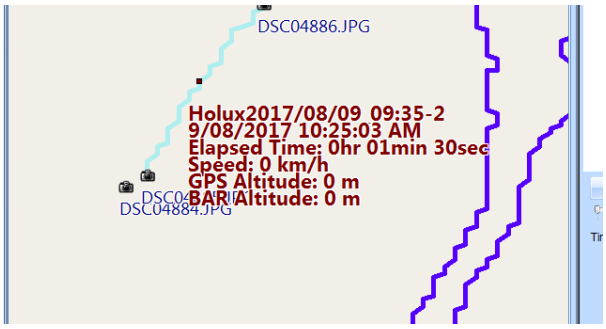
2. Locate photo marking start/end of each transect
3. Make note of capture time on date column



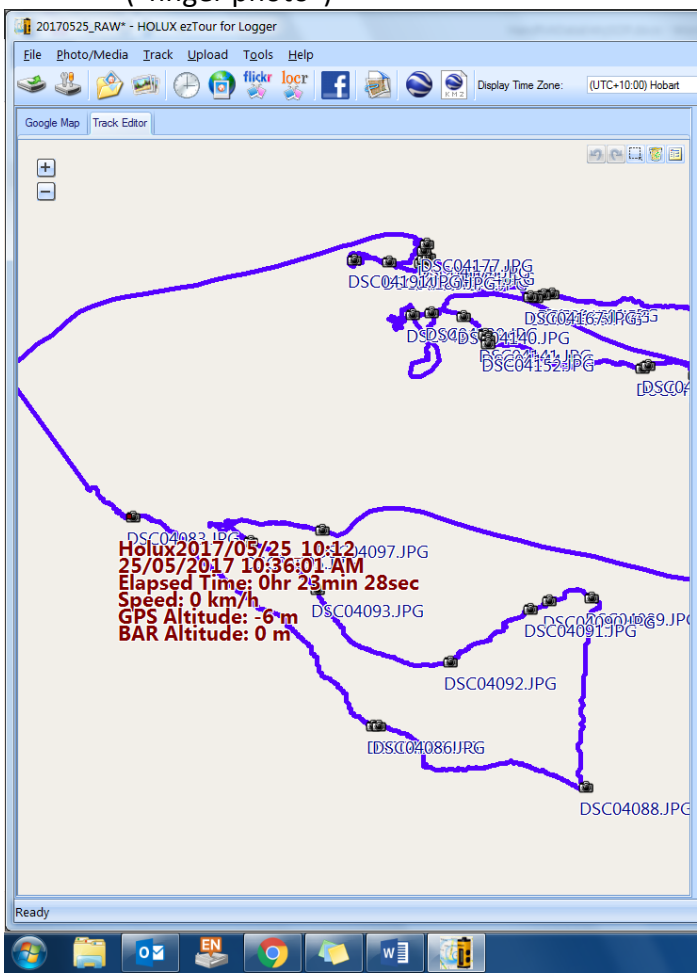
4. Go to *Track Editor*
5. Zoom in to the track (Scroll wheel function disabled) using the zoom (“+”,”-“) buttons

6. Trace along the track and examine the log's detail for the record time

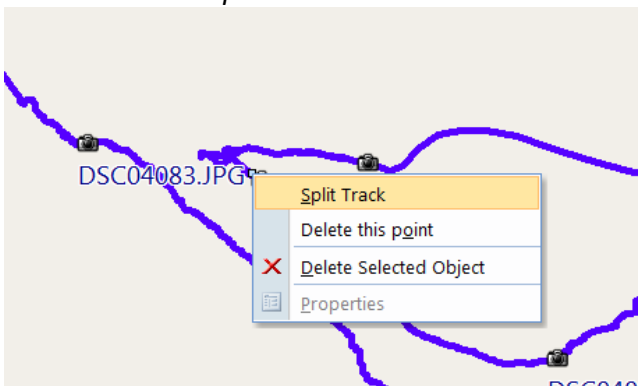
The track is composed of GPS log recorded once every 5 seconds, the details are hidden within the track normally, however when tracing along the track, the cursor will snap to the nearest log and highlight the detail of that specific point, including the captured date and time of the log. Based on the captured time information of transect start/end photos ("finger photos"), and their position along the track, the GPS track can be trimmed at specific times (as close to the capture time) to identify the section of the track which represented a handfish transect.



7. Find the reference point of the track near the capture time of reference photo ("finger photo")



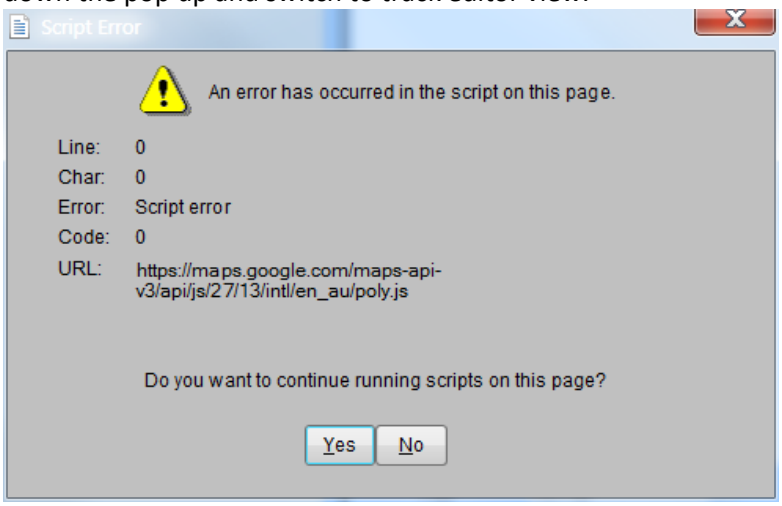
8. Right click on the point
9. Select *split track*



10. Repeat process for all reference photos (4 per dive)

Box 3

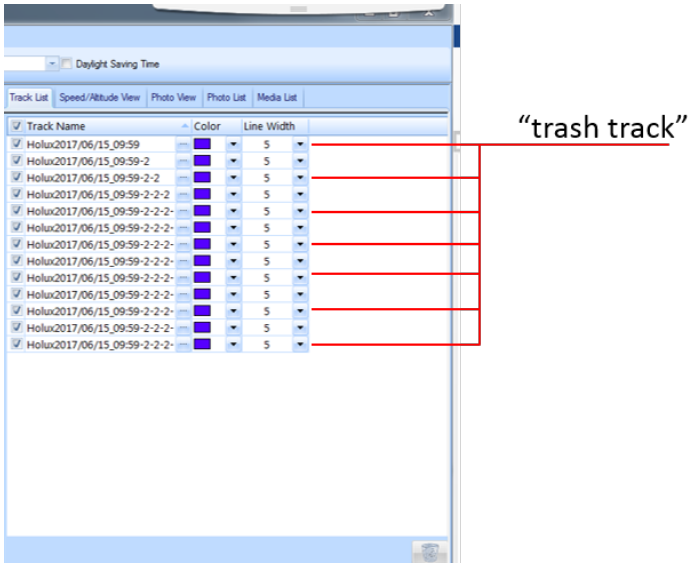
As the Holux ezTour for data logger requires communication with external server from Google Maps, an error message (below) will constantly pop up. To resolve this, press *escape* to close down the pop up and switch to track editor view.



3.4 Delete additional/ trash sections of track

After splitting the track based on the start and end point of each transect, sections of GPS tracks not related (e.g. traveling time between sites and start point) still remain in the file. This section describes the process to remove these tracks.

1. Select *Track List panel*
2. Identify tracks not representing search transect or “trash track”



Usually starting with the first one and every other one
Check the track properties for trip distance, non-transect usually have very long (600m – kms) or very short recorded distance (10m – 30m).

3. Select the non-transect track
   The software only allowed one track to be delete at a time
4. Click *remove*

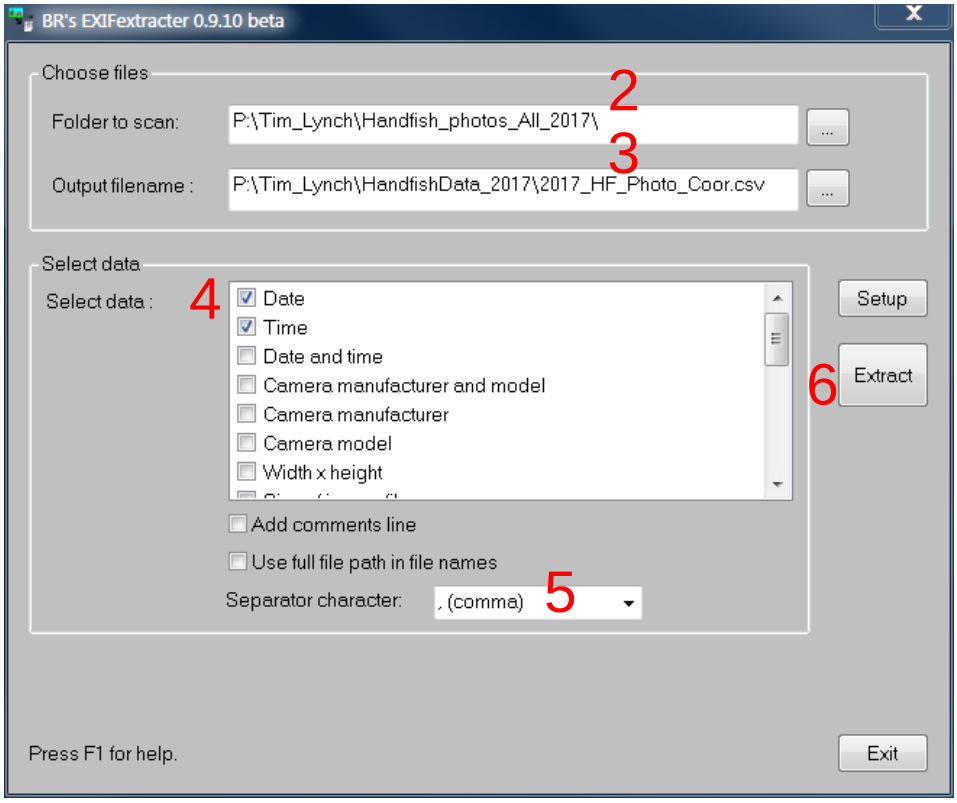
5. Repeat for all non-related section (Surface transverse time, Period between transects)

4.0 Extract photo geolocation

While spatial information is written into each photo after the geotagging process, the program does not allow for such metadata to be extracted in a meaningful way for this project (only allowed for export as KML file or webmap format). Thus a metadata extractor was used to 1) extract data in batch; and 2) extract data in flat file format for storage and further manipulation.

4.1 Extracting metadata

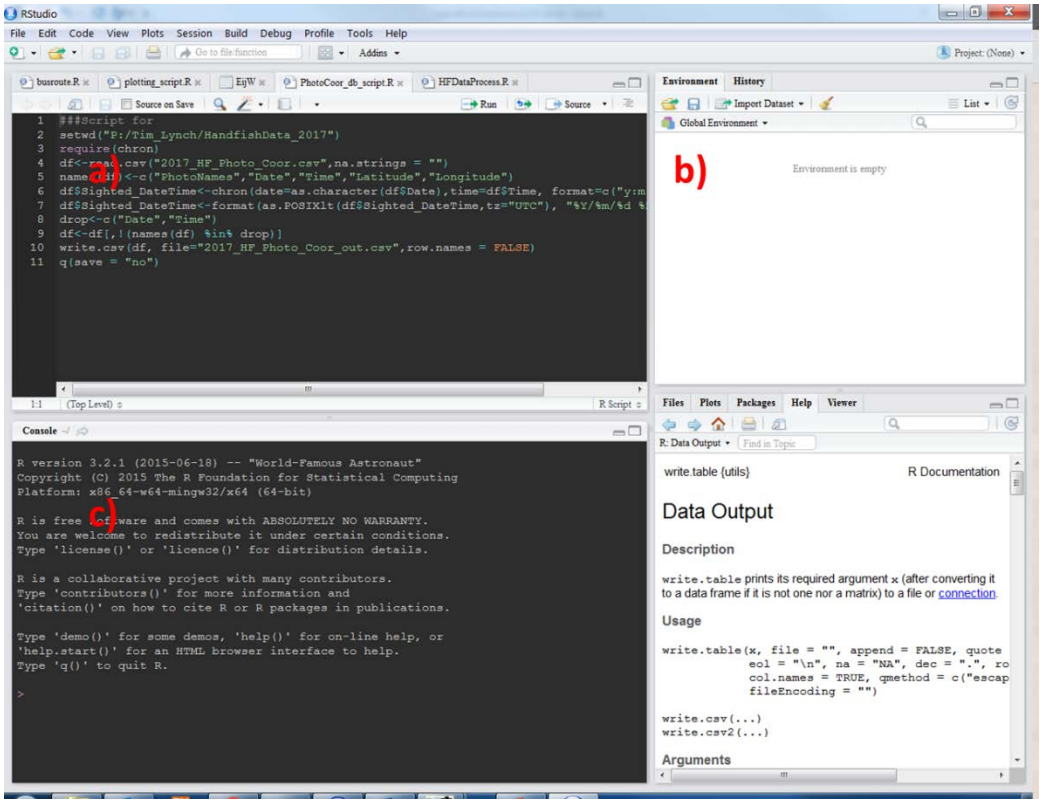
1. Open BR's EXIFextracter
2. Select folder to scan as: P:\Tim_Lynch**Handfish_photos_All_year**\
3. Select Output filename:
   P:\Tim_Lynch*Handfish_Data_analysis**Photo***year_HF_Photo_Coor**
4. Check "Date", "Time", "Latitude", "Longitude" at the Select Data panel
5. Select Separator character as ",(comma)"
6. Click Extract



4.2 Modifying data format

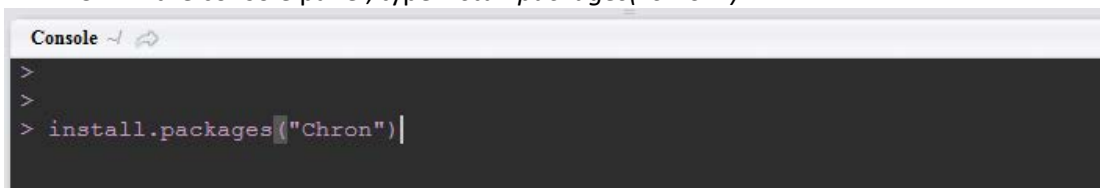
This script will select the extracted file (in csv format) and convert the format that lines up with the Access database in order for the coordinates to be imported into the database.

1. Open R or R studio

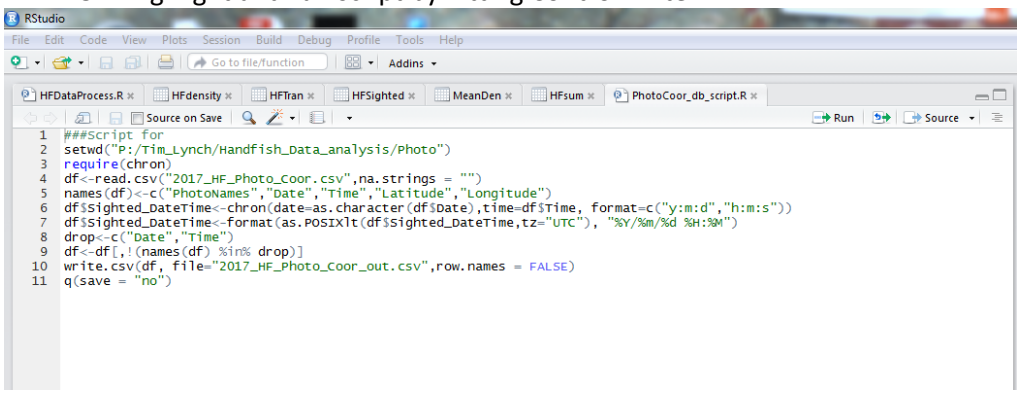


a) Script panel
b) Work directory
c) Console panel

2. When installed the first time, the additional package “chron” needs to be download first
3. In the console panel, type *install.packages(“chron”)*



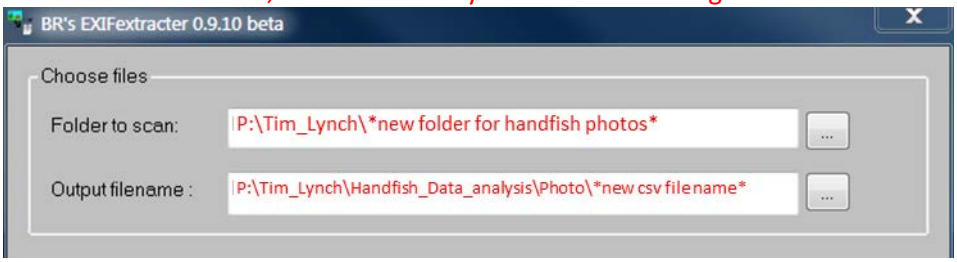
4. Open PhotoCoor_db_script.R at: P:\Tim_Lynch\Handfish_Data_analysis\
5. Highlight and run script by hitting Control+Enter



When the script is finished, it will automatically close RStudio. The new output is called “*Year*_HF_Photo_Coor_out.csv” which is located in the same directory

*Note: for data organisation and reducing processing time, it will be best to separate photos based on survey year. Thus at the beginning of each survey year (or first data processing session each year), the directory of both BR's EXIFextracter and the R script need to be modified.

For BR's EXIPextracter, the file directory needed to be changed as follows:



For the R script, the input needs to be the same as the name of the output from BR's EXIFextracter in order for the code to work. Thus with a new dataset, the script need to change as follows:

```
4   df<-read.csv("2017_HF_Photo_Coor.csv",na.strings = "")
```

New csv file, same as BR's EXIFextracter output

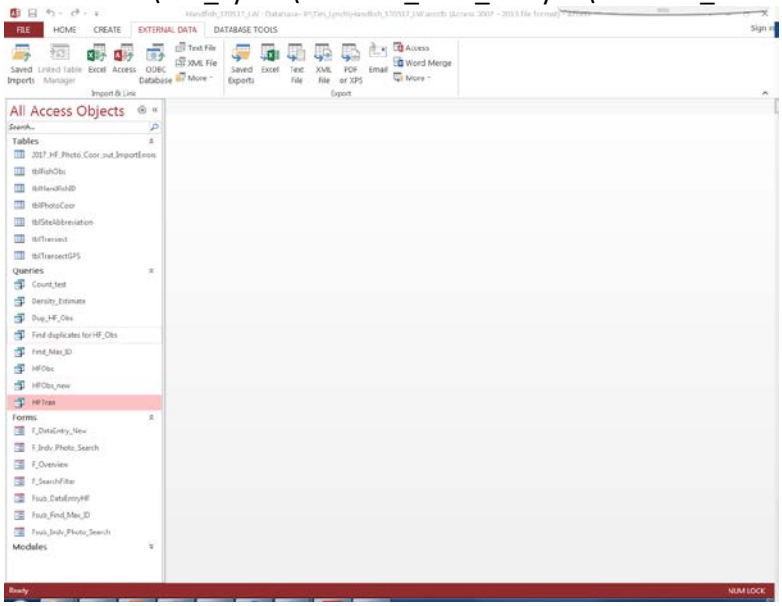
```
10  write.csv(df, file="2017_HF_Photo_Coor_out.csv",row.names = FALSE)
```

Same as new BR's EXIPextracter output with subfix "_out"

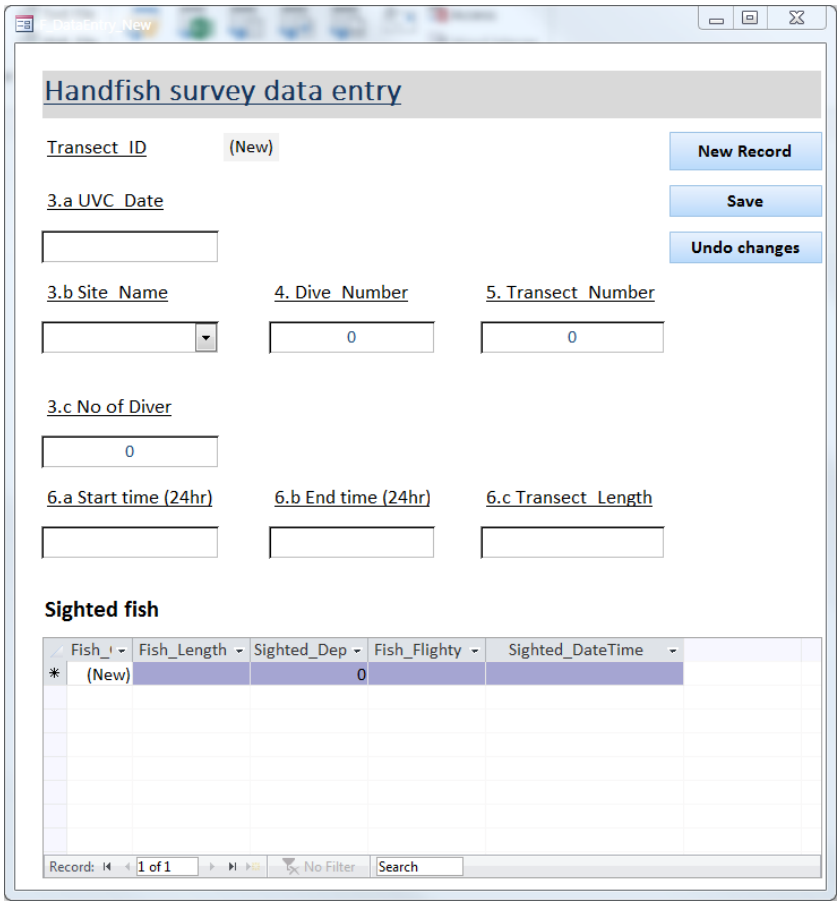
5.0 Input data

5.1 Handfish data

1. Open Handfish Access database at:
   P:\Tim_Lynch\Handfish_Data_analysis\Handfish_150817_LW.accdb



2. When first loading up the access database, click File-> Security-> Enable all content. Double click on the form F_DataEntry_New on the object panel (left) and a new window will pop up



3. Fill in detail of each search
   - 3.a UVC date
   - 3.b Surveyed site
   - 3.c Number of diver
4. Fill in dive number refer to the start point (may not be sequential)
   Dive number correspond to the list of coordinates generated randomly using the spatially balance model. Field survey may not necessarily follow the list sequentially due to dive safety etc. Thus conformation of dive number will be required between field notes and projected coordinates from data

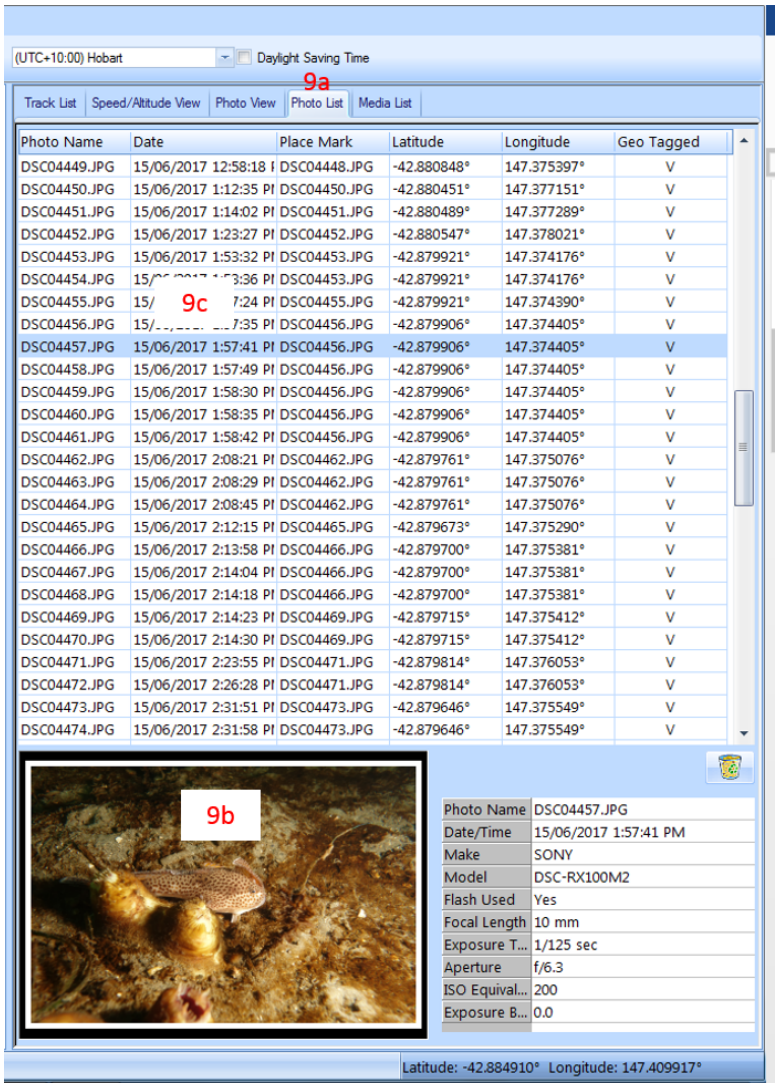
5. Fill in transect number
   *Transect number for first transect of dive = (dive no *2) -1*
   e.g. if dive no = 5
   *1st transect = (5*2)-1 = 9*
   2nd transect = 9+1 = 10

6. Fill in detail of transect, located in processed GPS file (Step 2.4)
   - 6.a Transect start time
   - 6.b Transect end time
   - 6.c Transect length
7. Fill in Sighted fish section

7.a If no fish were sighted, leave Sighted fish section empty
7.b If fish were present, fill in the detail
8. Fill in total length, sighted depth, and stress response
9. Fill in the sighted time of each fish from the Holux file (first photo of the handfish encounter)

A common variable is required to cross reference photos taken for each individual fish to the correct record of the individual fish. Time of sighting was used as it allowed the database to automatically shortlist the photos corresponding to each sighting. While the list only correspond to a portion of photo taken for each fish, it allowed for easier distinguishing of fish from the entire list.

9.a Go to Photo List panel
9.b Find the photos of each sighted fish
9.c Identify the capture time of **first photo**
9.d Do not round up times, ie 12:33:59 = 12:33



10. Fill in the Sighted_DateTime field (format: dd/mm/yyyy hh:mm)
11. Repeat for all fish sighted within the transect
12. Click Save

13. Makes notes if not for density analysis (e.g. collection of fish, ASH survey etc)
14. Click New Record to enter data for next transect
15. Close pop-up when finish data entry

5.2 Importing metadata for geotagged photo

1. Open Handfish Access database at: P:\Tim_Lynch\Handfish_170517_LW.accdb
2. Click *External Data*->Saved Imports
3. Run *PhotoCoor_import*

Common error messages include: key violation (due to NA and overwriting) and failure to run (target files are open). A solution is to close files and remove NA. You can also try deleting data (after saving a copy of the database) from the Access table and then re-importing

6.0 Export data for analysis

1. Click EXTERNAL DATA-> Saved Exports
2. Run *Export_transect_data*
3. Run Export_HFObs

Data analysis

7.0 Processing transect and sighted individuals for density analysis

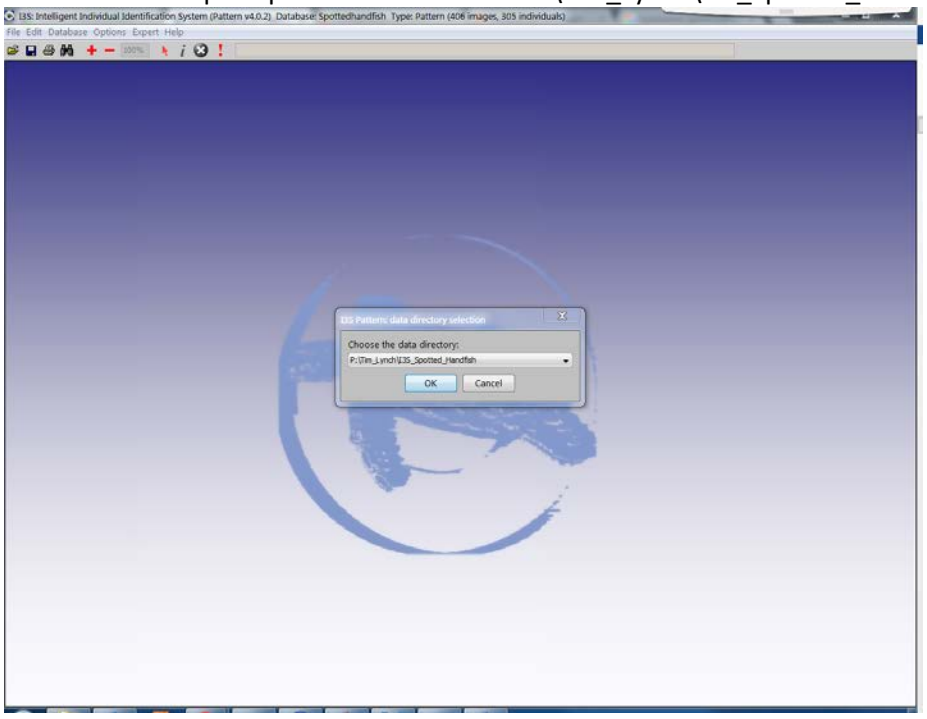
See appendix 2 for R script

8.0 Processing individual photos

The aim of this section is to further separate the data to individual level

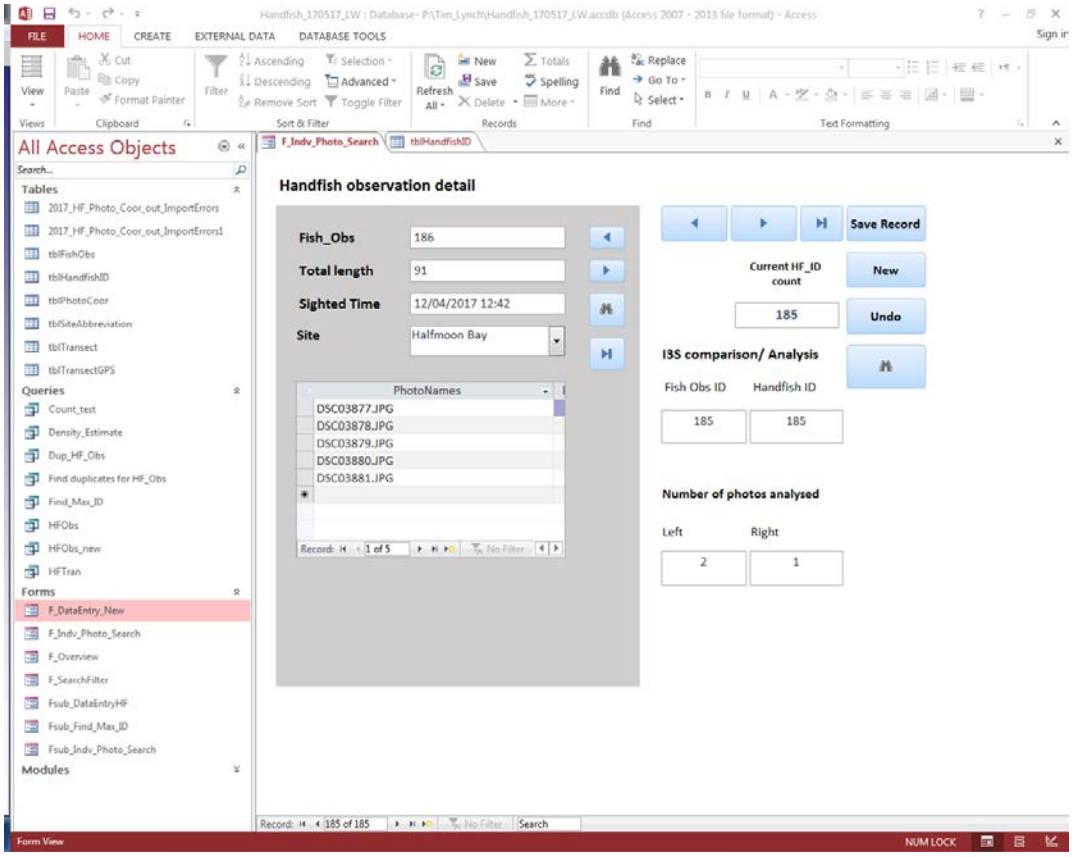
8.1 Setting up I3S pattern

1. Open I3S Pattern
2. When prompted set database to: P:\Tim_Lynch\I3S_Spotted_Handfish



8.2 Setting up Access Database

1. Open Handfish Access database at: P:\Tim_Lynch\Handfish_170517_LW.accdb
2. Open form F_Indv_Photo_Search listed in the object panel (left)



The form F_Indv_Photo_Search fulfilled two primary roles. Firstly, this form provided an interface to select and examine data collected for each individual observation (left). In particular, it provides a list of photos taken during each observation. The highlighted photos represented the first few photos taken of the individual.

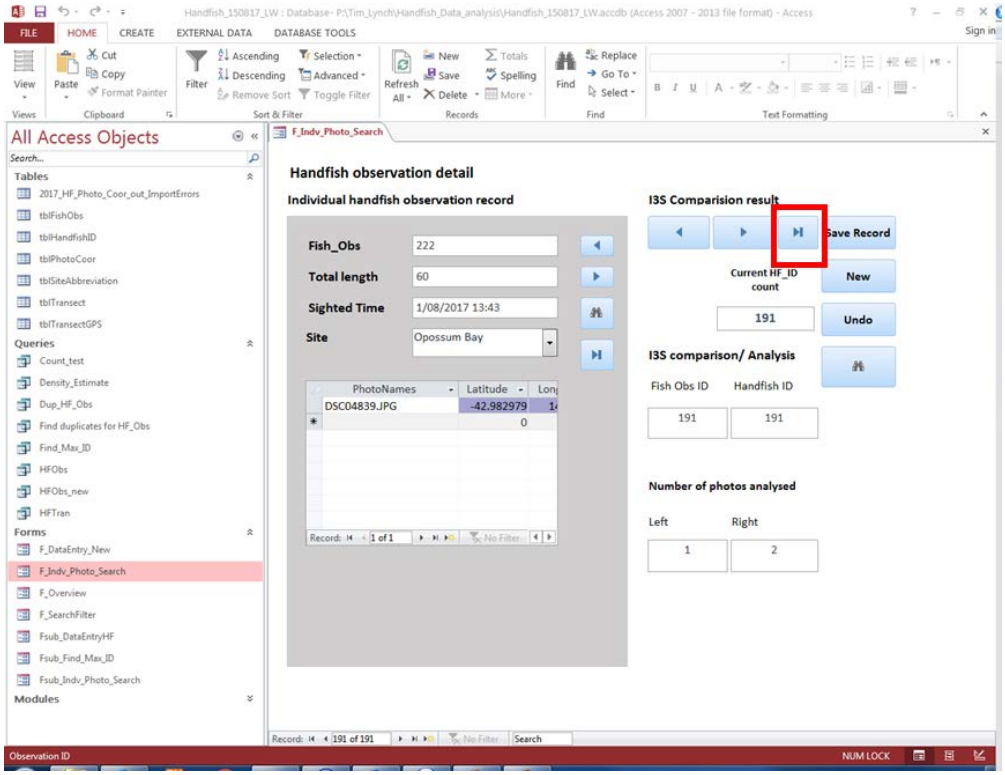
The second role this form is to provide an interface to log the pattern analysis conducted through I3S pattern (Right). This form is linked to a new table (tblHandfishID) which introduced a new variable: HF_ID. This variable is an unique identification number for individual fish sighted.

8.3 Selecting photo for analysis

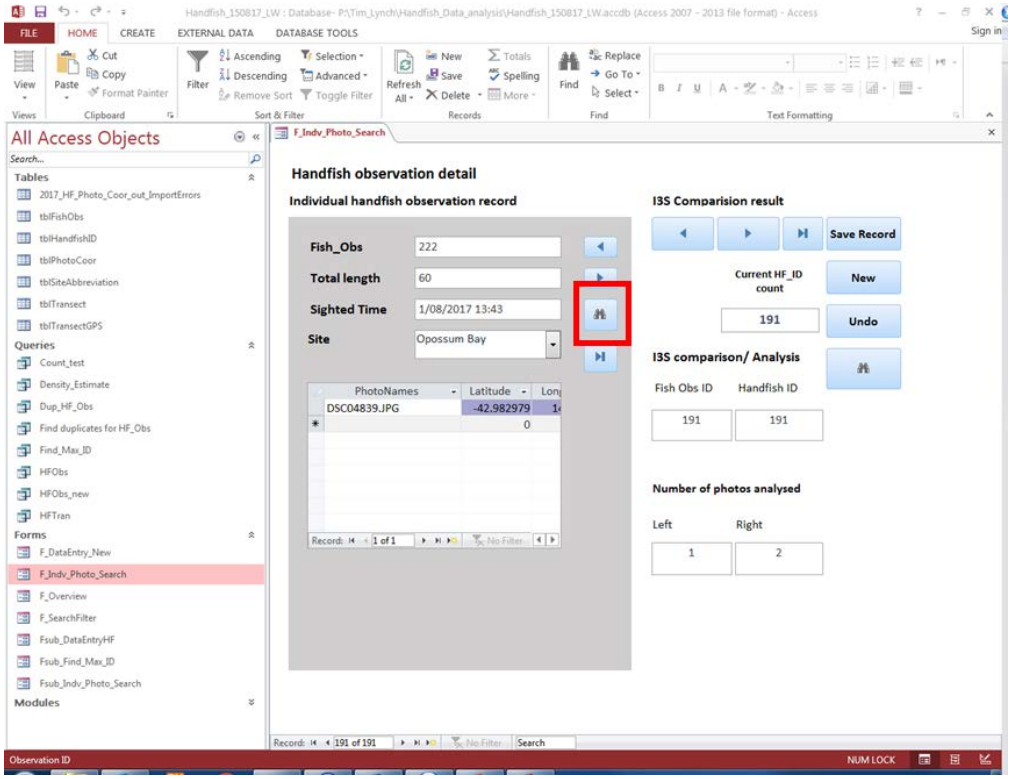
1. Open window explorer to directory for captured photo (*e.g. P:\Tim_Lynch*Handfish_photos_All_2017**)

Preferred setup for this process will be with dual/triple monitor, with I3S, Access, and list of photo showing simultaneously.

2. Click the *Last record* button under the "I3S comparison result" section in Access. This highlighted the last observation that was analysed



3. Click the find button under the “Individual handfish observation record” section

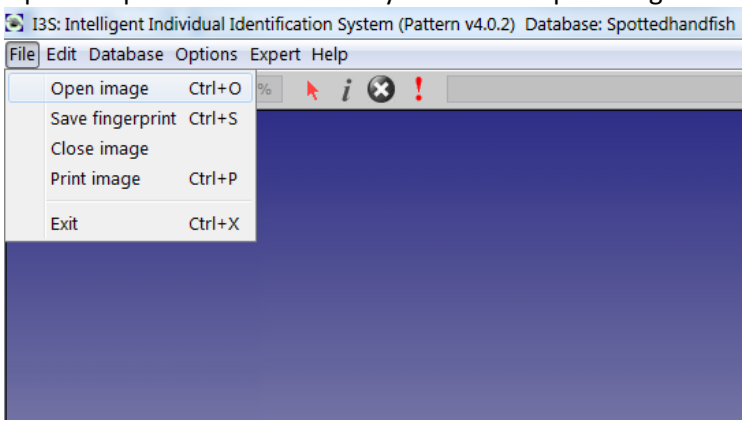


4. In the find what field, type the latest Fish Obs ID (Should be identical to Current HF_ID count)
5. Select the next record in the individual handfish observation record (This will be the next observation that has not been analysed)

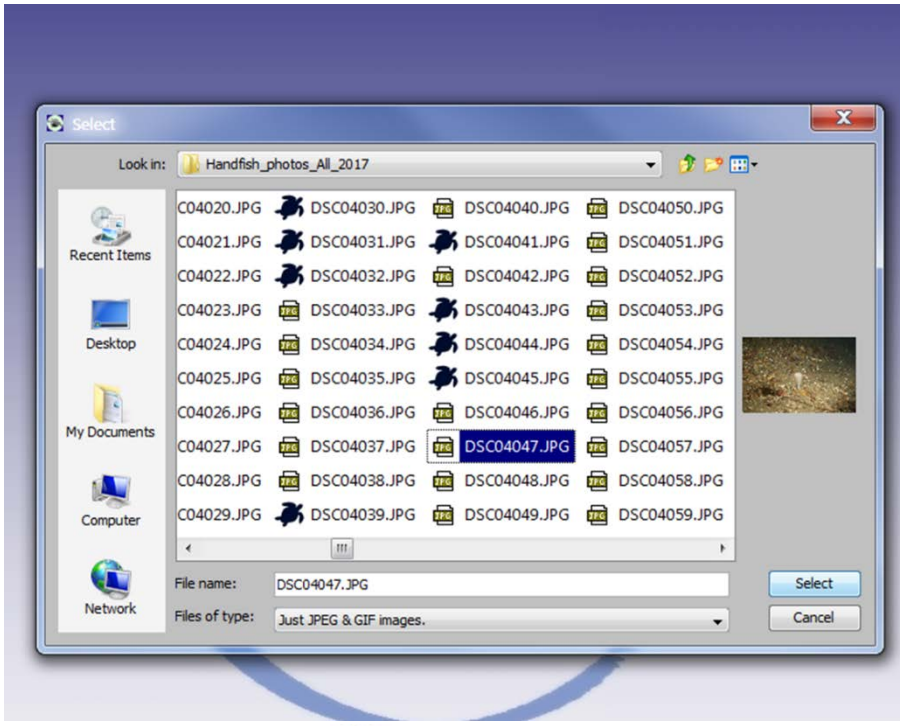
6. Using window explorer of Handfish_Photos_2017, preview the list of photo for the highest quality photos of the handfish observation

7.2 Analysis photo with I3S

1. Open the photo in I3S Pattern by click File-> Open image



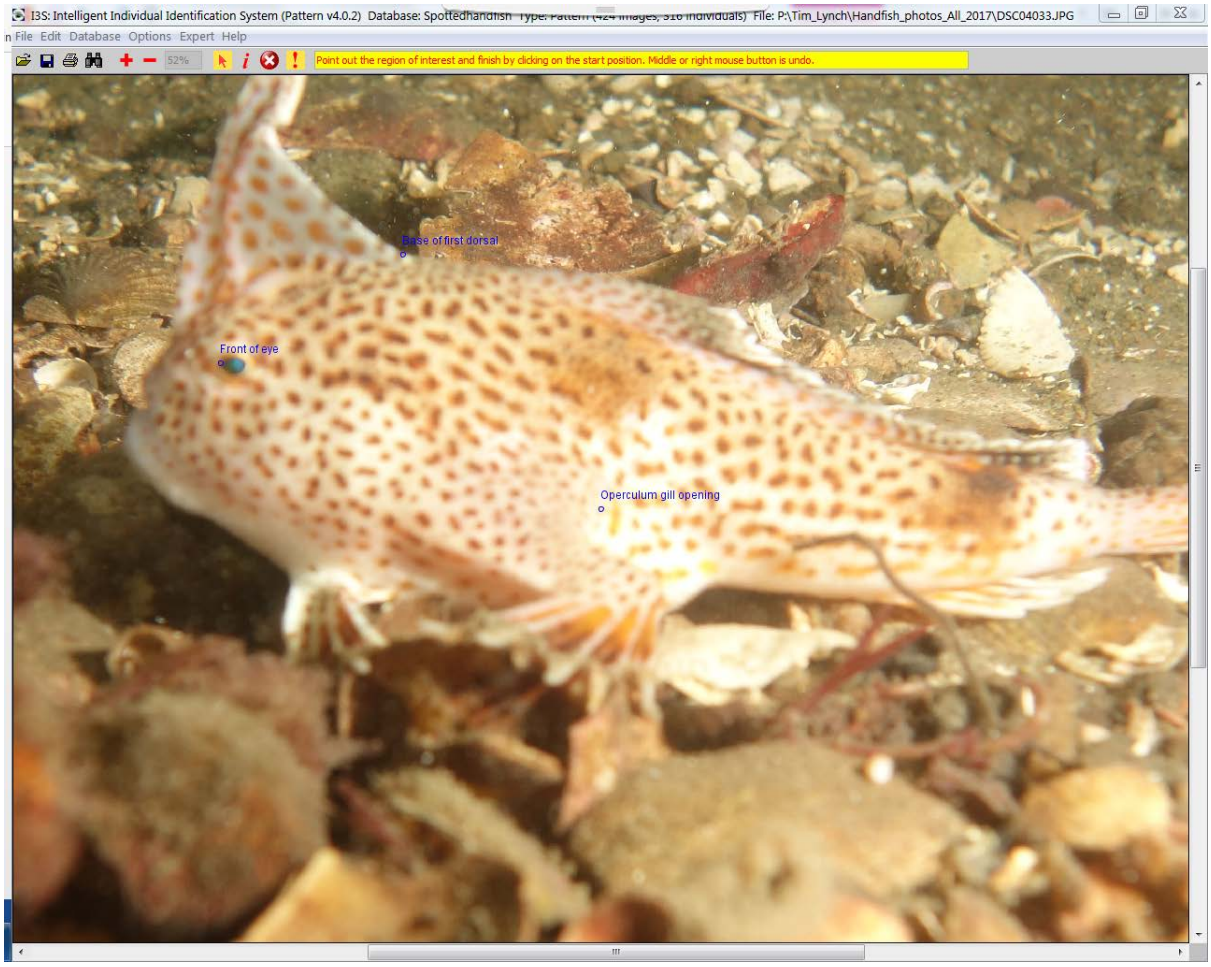
2. Navigate to the directory where all photos were stored (e.g. P:\Tim_Lynch\Handfish_photos_All_2017) and select the photo for analysis



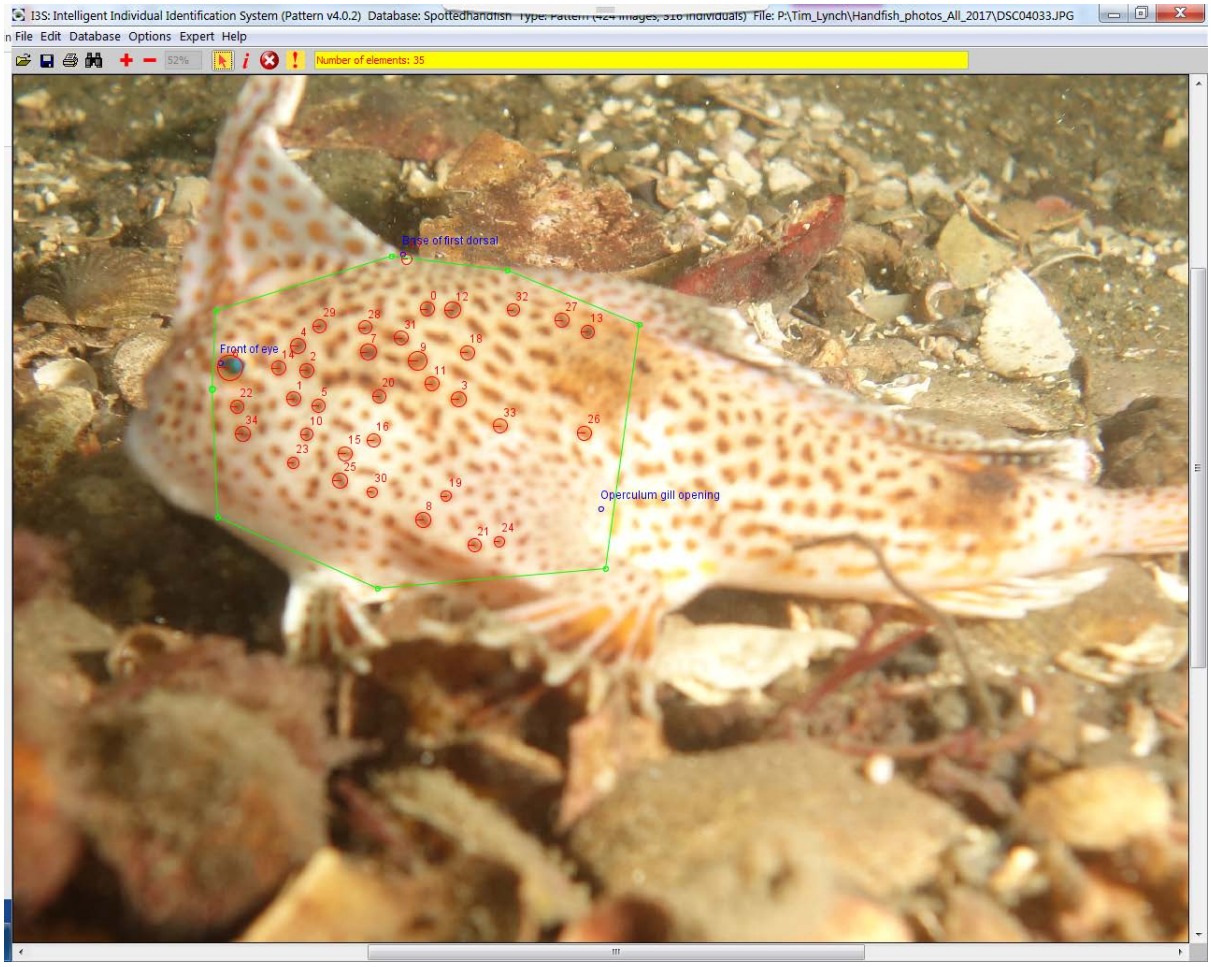
Photos with the turtle icon indicated a photo fingerprint is available, suggesting 1) comparison was completed and individuals was registered in the tblHandfishtable, or 2) a fingerprint was constructed and saved but analysis was not completed.

On the other hand, photos with JPG icon have not been processed with I3S pattern yet.

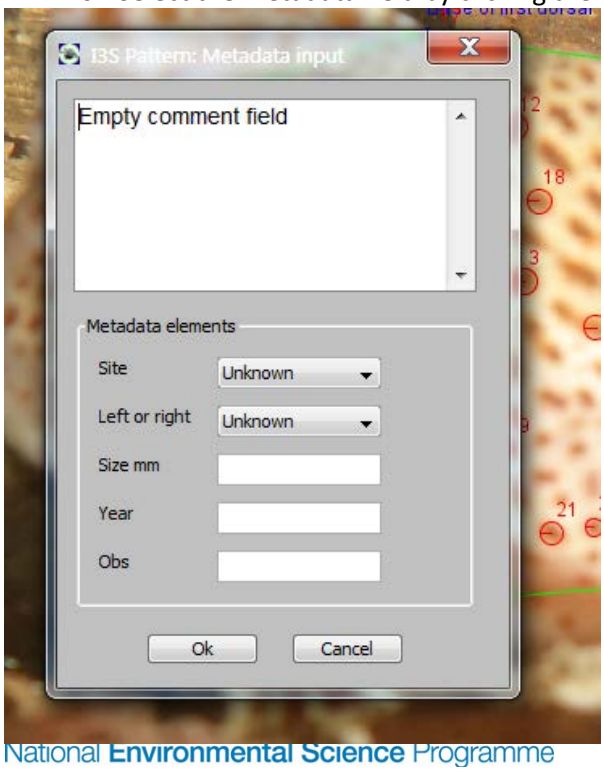
3. Toggle edit mode on by clicking the icon
4. Create fingerprint by first selecting the reference point.



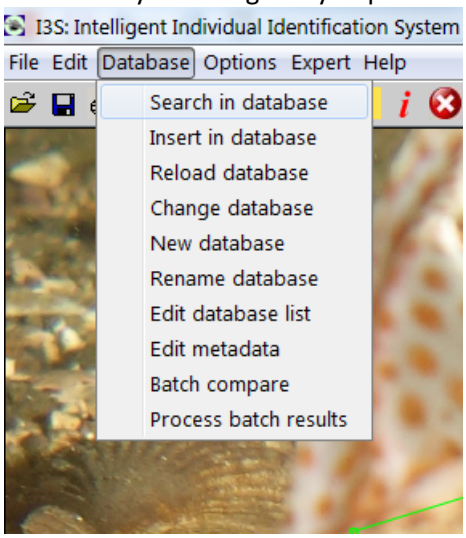
5. Trace along the front of the fish body to highlight the area of interest for I3S to construct the pattern fingerprint automatically

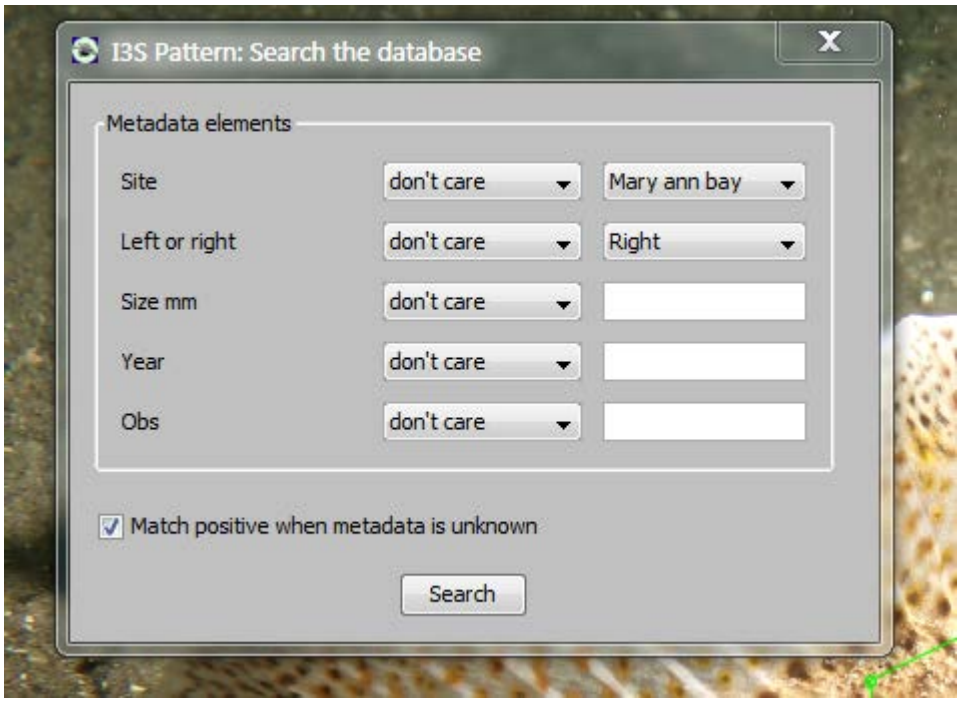


6. Select the metadata field by clicking the  icon

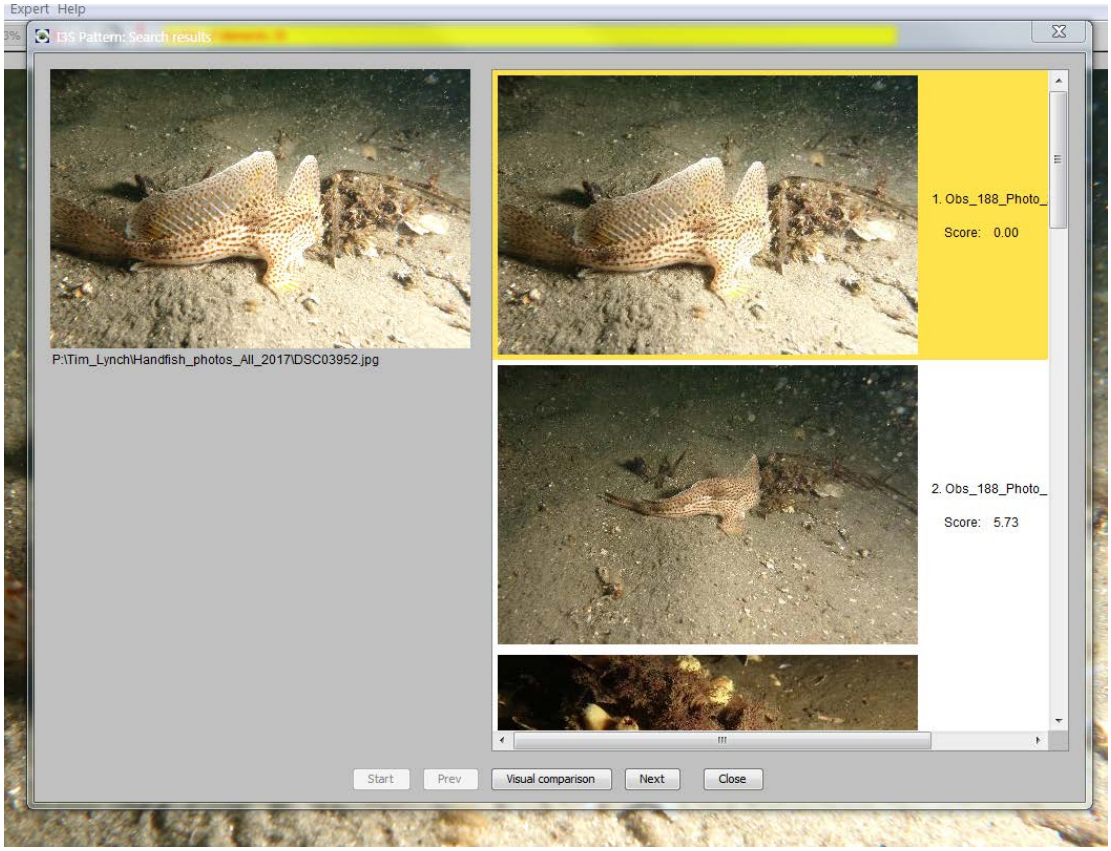


7. Select site information and the side of the fish the photo captured. As all other information is recorded in the database, the other fields can be ignored
8. Select Database-> Search in database to begin comparison with other entries. Refine the selection by selecting entry of photos taken of the same side.

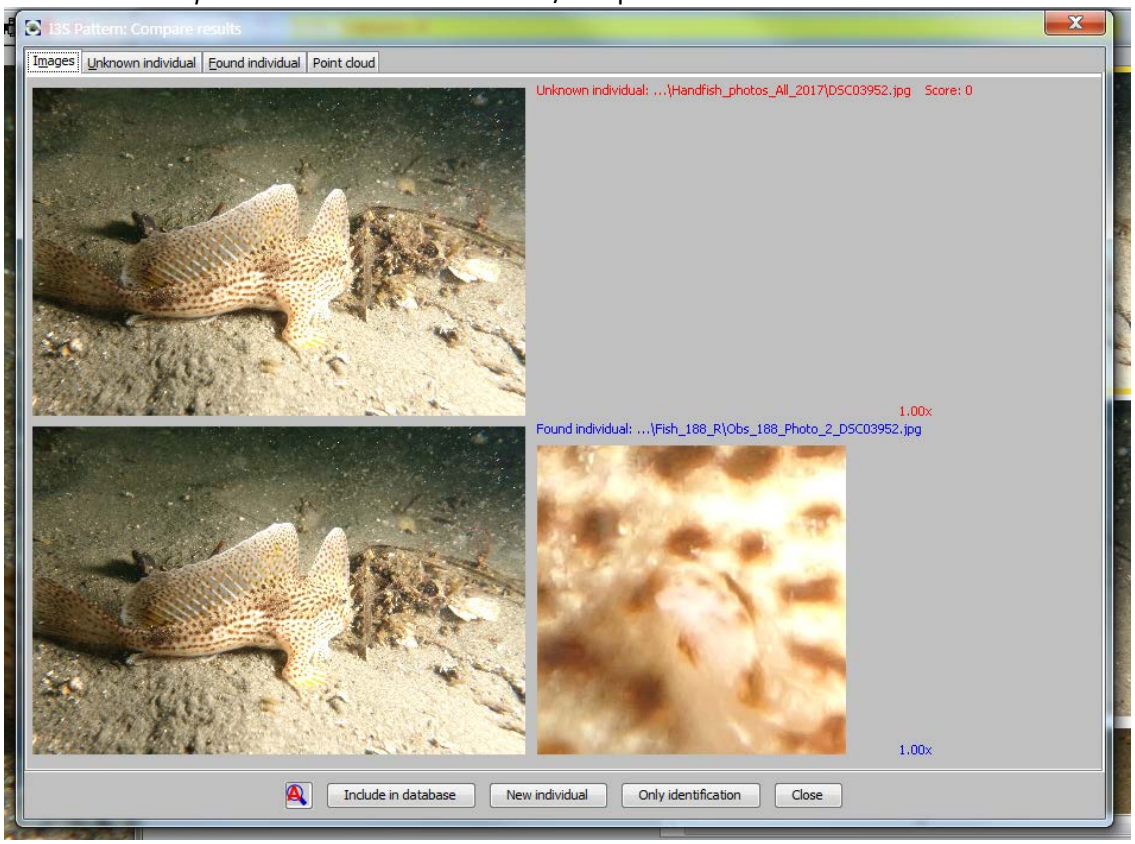




9. I3S Pattern automatically compares the fingerprint of each photo and generates a score based on the similarity of the two and sort the database by level of similarity. The lower the score, the more similar they are.
10. Examine manually to determine if observations were of the same individual for the first 3-4 matches.

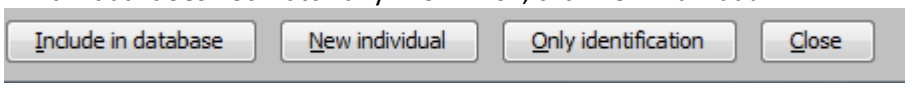


11. Click *Visual comparison* for detail examination/comparison of individual

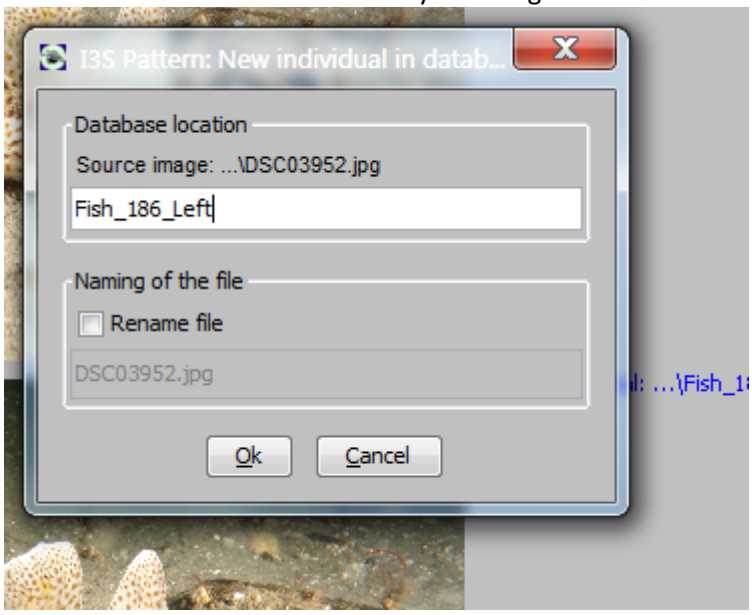


7.3 If observation was a new fish

1. If individual does not match any known fish, click *New individual*

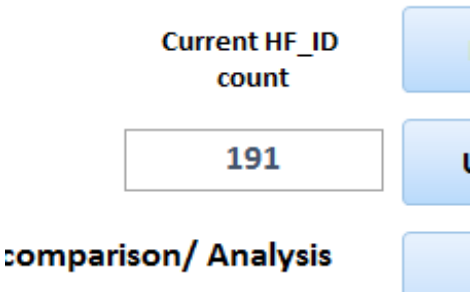


2. Create a new ID for the new fish by inserting a database location.



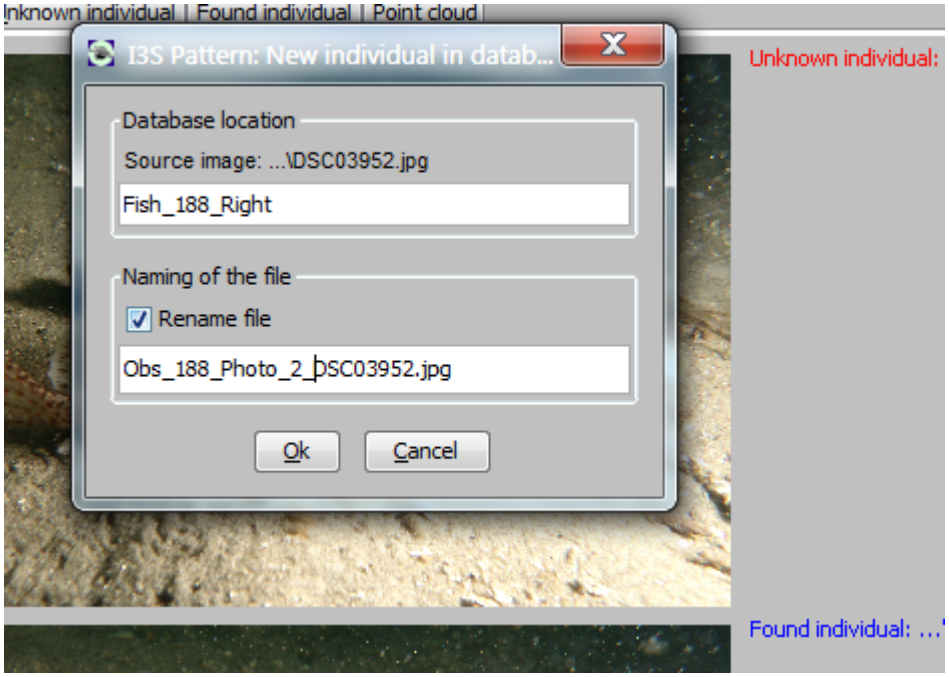
The ID should follow the following naming convention
Fish_newID_photoside
e.g. Fish_36_Left

To ensure the new ID is unique, consult the *Current HF_ID count* text box in the F_Indv_Photo_Search form, which show the highest HF_ID recorded in the system. Thus the new ID should be 1 above the printed number



3. Check rename file in the Naming of the file section
4. Change the name of the record to reflect the observation number
   The name should follow the following naming convention
   Obs_ID_Photo_no_Photoname
   e.g. Obs_169_Photo_1_DSC03952

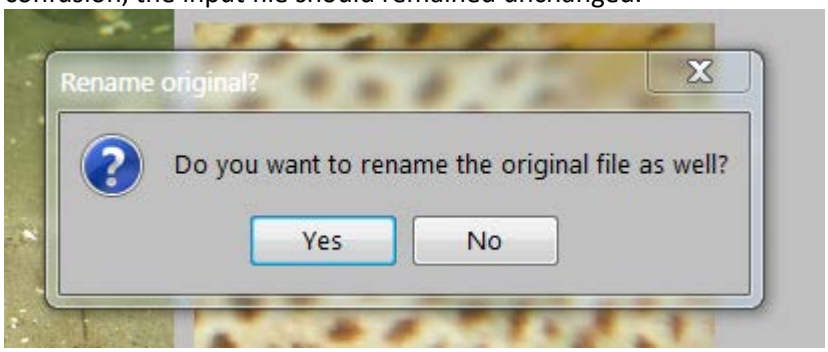
   The observation id identifies which handfish observation this photo was from. The Photo number indicate how many photos of the same observation was analysed. The photo name is the original name of the photo, serves as a unique identifier



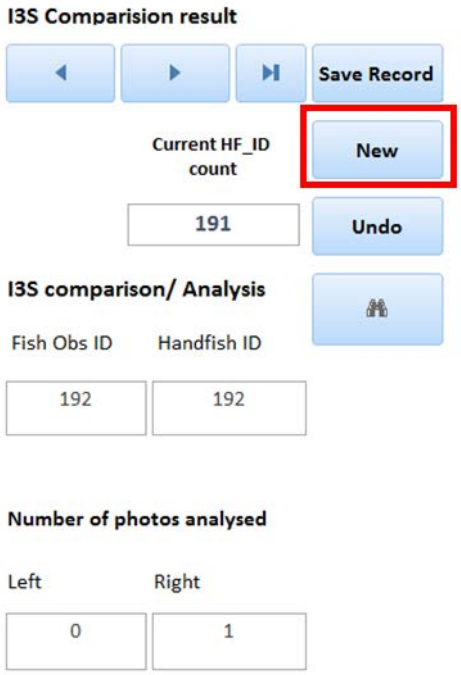
If the fish is a new individual, the Observation ID and HF_ID should be identical

Fish Obs ID	Handfish ID
188	188

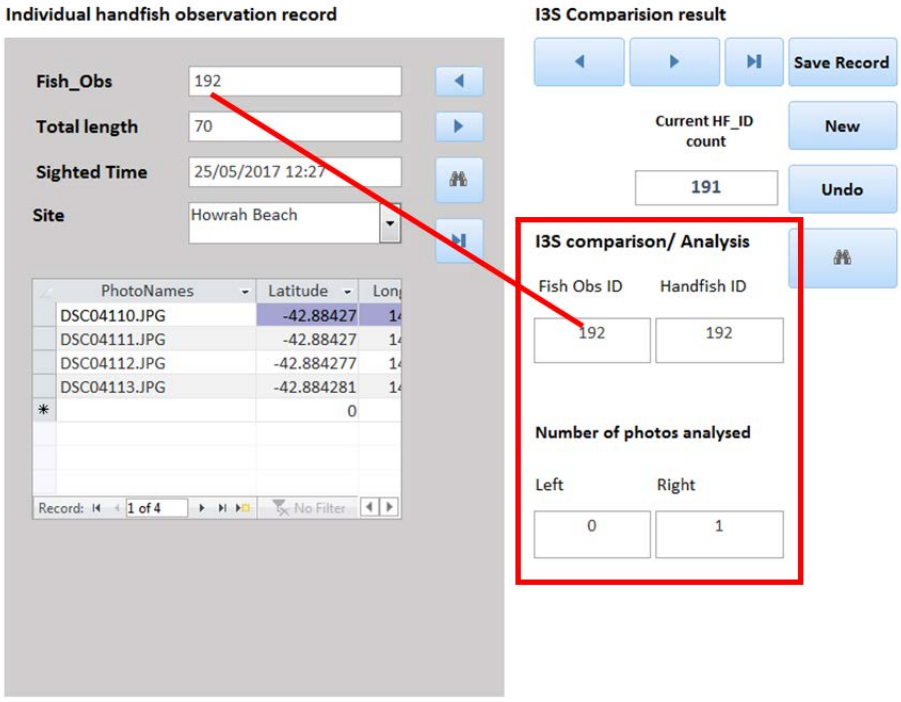
5. Click Ok
6. Select No when prompted if you want to rename the original file as well
   By selecting no, this will not change the name of the input. As we are working directly from the photo storage on the P drive, changing the name of the photo can cause version discrepancy between the Access database and the files (This can be resolved by running the extracting metadata section and importing it to the database again). Thus to minimise confusion, the input file should remained unchanged.



7. Return to the form F_Indv_Photo_Search and click New in the I3S Comparison result section, for each observation analysed. (note: all photos analysed for the fish sighted at the same time are considered as the same observation)



8. Input the observation ID and the newly identified Handfish ID in the corresponding boxes in the I3S Comparison result. Also record the number of photos of each side of the fish analysed

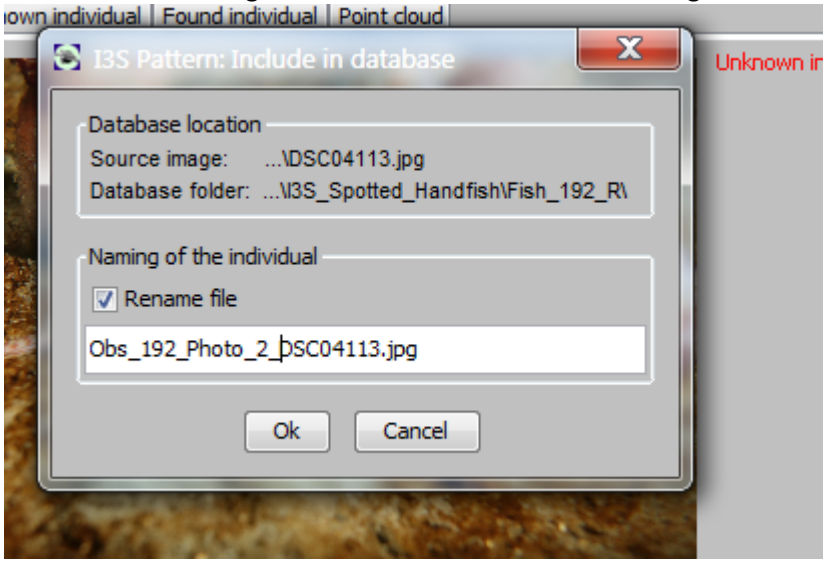


9. Click *Save Record* to save the new entry

7.4 If observation was a recapture (use this procedure if a second photos was analysed for the same fish)

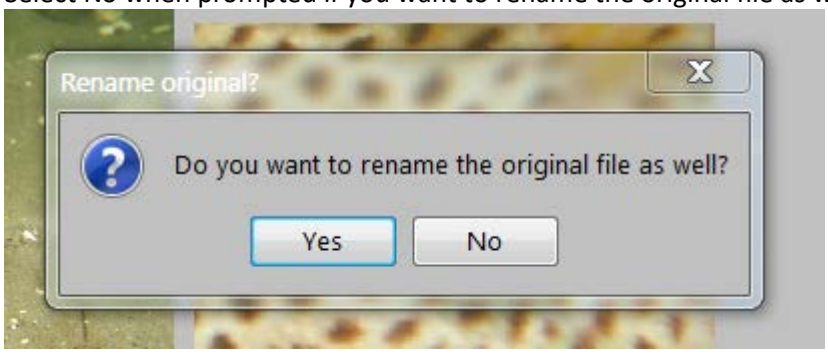
1. After visual inspection, if individual was a recapture, click *Include in database*

2. The new photo will be placed in the same folder as the matched entry in the database. Click rename file an generate a new file name for the image

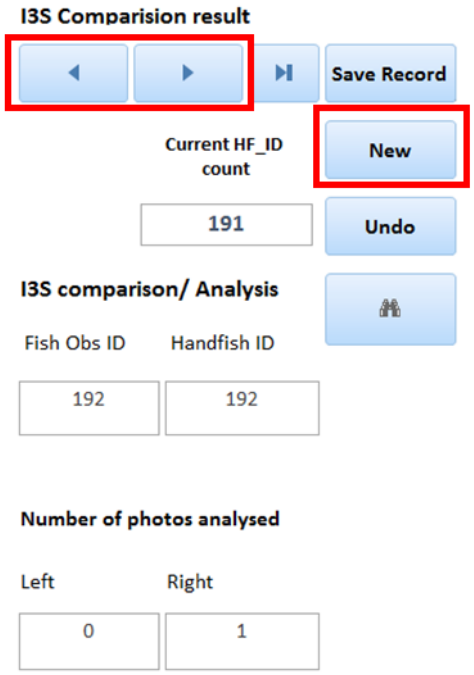


In the file name, identify the observation ID of the photo, the sequence of photo analysed for the observation, and the name of the original file (default).

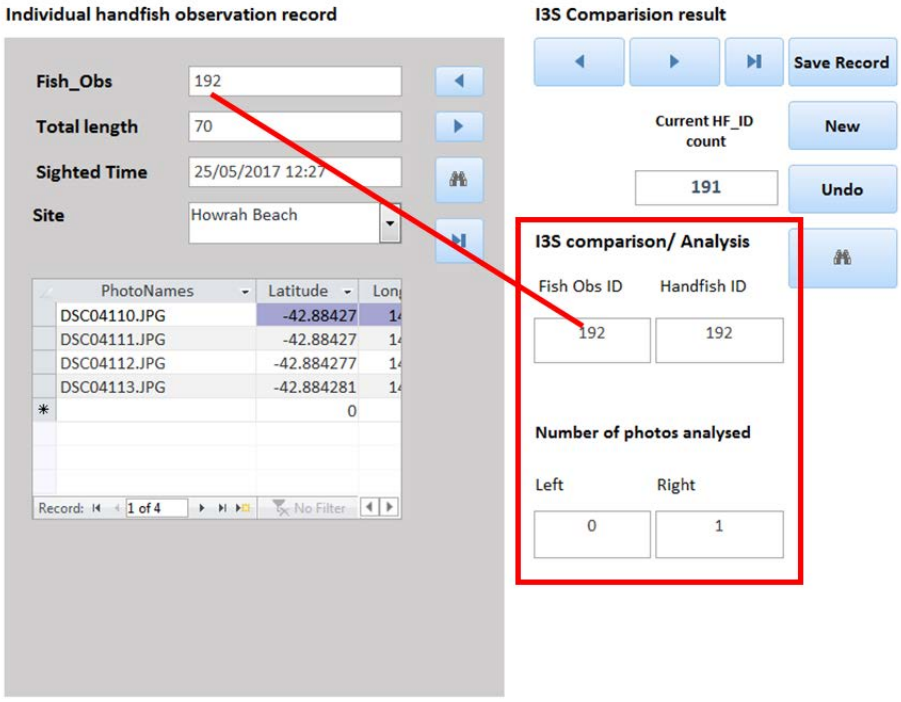
3. Click Ok to save
4. Select No when prompted if you want to rename the original file as well



5. Return to the form F_Indv_Photo_Search and click New in the I3S Comparison result section, for each observation analysed. (If image processed was a second image of the same observation, go back to the original entry using the navigation buttons)



6. Input the observation ID and the newly identified Handfish ID in the corresponding boxes in the I3S Comparison result. Also record the number of photos of each side of the fish analysed



7. Click *Save Record* to save the new entry

SOP Appendix 1:

R script for structuring data

(***remember to change years and directories as required***)

```
setwd("P:/Tim_Lynch/Handfish_Data_analysis/Photo")
require(chron)
df<-read.csv("2017_HF_Photo_Coor.csv",na.strings = "")
names(df)<-c("PhotoNames","Date","Time","Latitude","Longitude")
df$Sighted_DateTime<-chron(date=as.character(df$Date),time=df$Time, format=c("y:m:d","h:m:s"))
df$Sighted_DateTime<-format(as.POSIXlt(df$Sighted_DateTime,tz="UTC"), "%Y/%m/%d %H:%M")
drop<-c("Date","Time")
df<-df[,!(names(df) %in% drop)]
write.csv(df, file="2017_HF_Photo_Coor_out.csv",row.names = FALSE)
q(save = "no")
```

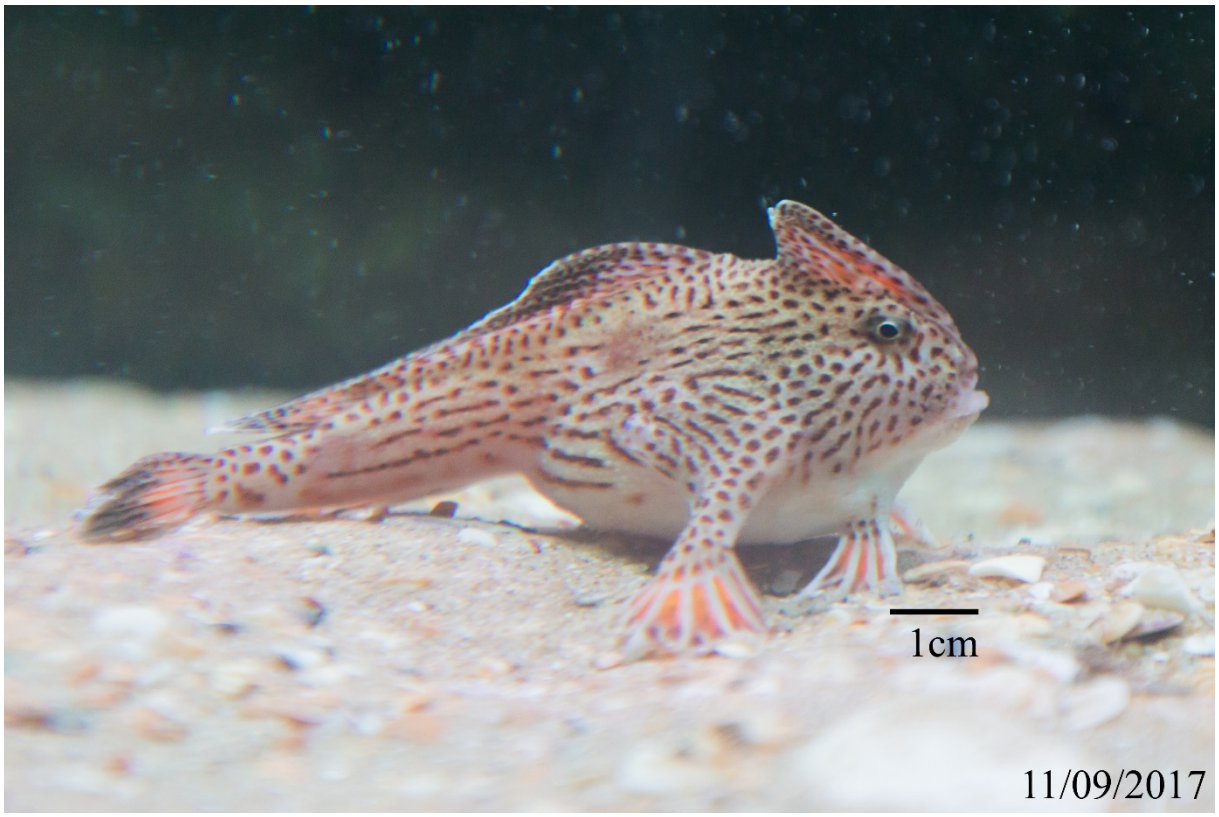
SOP Appendix 2

R script for processing transect and handfish data into density

```
# import library
require(dplyr)   #for count and groupby
require(ggplot2)    #for plotting
require (agricolae)
require(multcomp)       #required for GLM posthoc

setwd("P:/Tim_Lynch/Handfish_Data_analysis")

#Import transect
HFTran<-read.csv("Output/HFTran.csv",na.strings=c("NA",""))
#Import Handfish observation
HFObs<-read.csv("Output/HFObs.csv",na.strings=c("NA",""))
##Discount transects##
HFTran$Dive_no[HFTran$Dive_no == 0]<-NA
HFTran<-HFTran[!is.na(HFTran$Dive_no),]
#Seperate data by year
date<-as.Date(HFTran$Date,"%d/%m/%Y")
date<-format(date, format="%Y")
HFTran<-mutate(HFTran,Year=date)
#count the number of handfish sighted
##Make sure column in both file have the same name
HFSighted<-count(HFObs,Transect_ID)

HFTran$Site_C<-HFTran$Site
levels(HFTran$Site_C)<-c("BP","BR","FB","HMB", "HB", "MAB", "OP", "RB", "SB", "SP","TR")

#Generate density estimate
HFdensity<-merge(x=HFTran,y=HFSighted, by.x = "Transect_ID", all=TRUE)
HFdensity$n[is.na(HFdensity$n)]<-0
HFdensity<-mutate(HFdensity,nHa=(n/Swath.area)*10^4)
HFdensity<-HFdensity[complete.cases(HFdensity["Dive_no"]),]

####ANALYSIS FOR DERWENT ONLY#### (**** first lines remove non Derwent sites SP, FB)
HFdensity<-HFdensity[!HFdensity$Site_C %in% c("SP","FB"),]
HFdensity<-subset(HFdensity, HFdensity$Year == 2016)

##GLM from Spoon
d<-HFdensity

fit <- glm(n ~ Site_C+offset(log(Swath.area)),family=poisson(),data=d)
summary(fit)
opar <- par(mfrow=c(2,2))
plot(fit)
anova(fit,test="Chi")
```

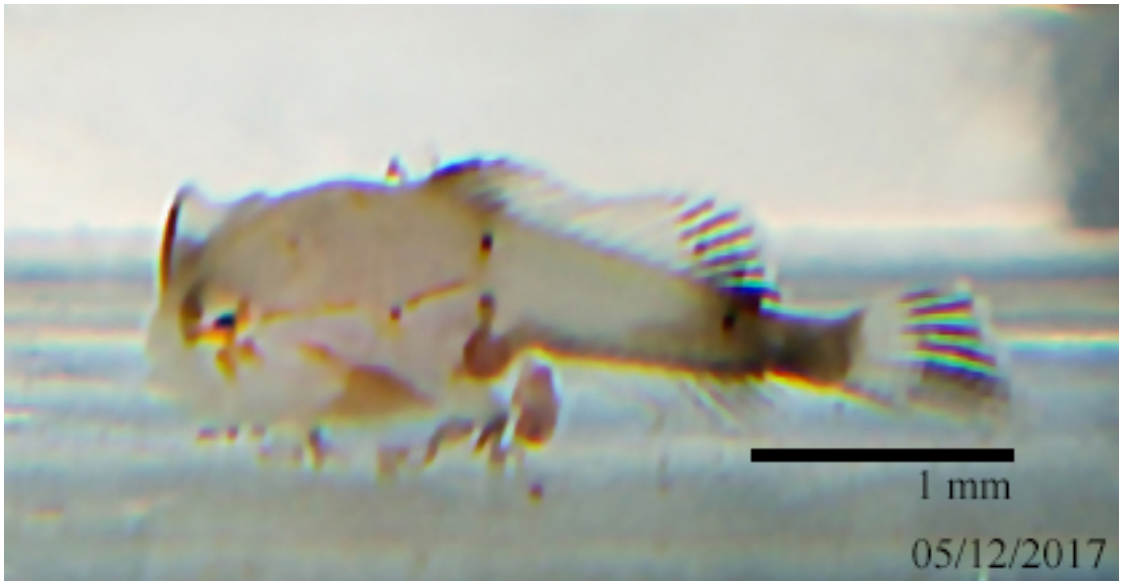
```
mc <- glht(fit,linfct=mcp(Site_C="Tukey"))
summary(mc)
tab <- confint(mc)
tab
exp(tab$confint)

d.pr <- data.frame(Site_C=unique(d$Site_C),Swath.area=1.0E4)
pr <- predict(fit,d.pr,se.fit = T,type="link")
d.pr$Density <- exp(pr$fit)
d.pr$Lower <- exp(pr$fit-2*pr$se.fit)
d.pr$Upper <- exp(pr$fit+2*pr$se.fit)
d.pr

denglm<-ggplot(d.pr,aes(x=Site,y=Density,ymin=Lower,ymax=Upper))+geom_pointrange()
```

APPENDIX B – PHOTOS OF DEVELOPING YOUNG



Rose, gravid with eggs, settling into the tank the day she was captured. Credit: Carlie Devine, CSIRO

Rose laying her eggs to days after capture. Credit: Carlie Devine, CSIRO



3 week old spotted handfish, located at Seahorse World, Beauty Point, Tasmania. Credit: Carlie Devine, CSIRO

3 week old spotted handfish, located at Seahorse World, Beauty Point, Tasmania.
Credit: Carlie Devine, CSIRO

Daily digital capture of the growth of the egg mass began after spotting one egg had turned blue on the 25th September 2017. While the egg mass was clearly visible in the tank the most effect method of monitoring its health was to take a high resolution digital photograph, upload the photograph and zoom into the image at 100 per cent. While it was not possible to see all eggs in one photograph, the high resolution image showed about 50 per cent of the mass. A Nikon D810 digital camera, a Nikon 60mm prime lens and a 128GB San Disk SD card were used for image capture. The Nikon D810 camera has 36 megapixels, a CMOS Sensor, and 7360 pixels on the long side. The large number of pixels allows for tight cropping when the camera can't get close to the subject. The camera's pop up flash was used to brighten the image and freeze the frame. Image capture settings were 1/80th second shutter speed, ISO 800 to bring in more natural light, and f8 for a large depth of field.

The camera lens was placed directly flat onto the glass of the tank and auto focus was used for ease of shooting. The egg mass was approximately 20cm away from the tank glass and the camera. Captured images were uploaded and opened in Adobe Photoshop CC 2017 and were colour balanced, sharpened and cropped for best viewing of the egg mass and then saved as a high resolution tiff file at 300dpi.

A total of 17 days were digitally captured using the Nikon D810, from the 25th September 2017 to the 8th November 2017. It was not possible to take a high resolution digital image every day due Rose being in between the egg mass and the camera, or other field work commitments and weekends. Leaving the camera on a tripod to take a photo automatically was not convenient due to the daily tending of the tank by staff. However, the eggs developed slowly and all changes were digitally captured.

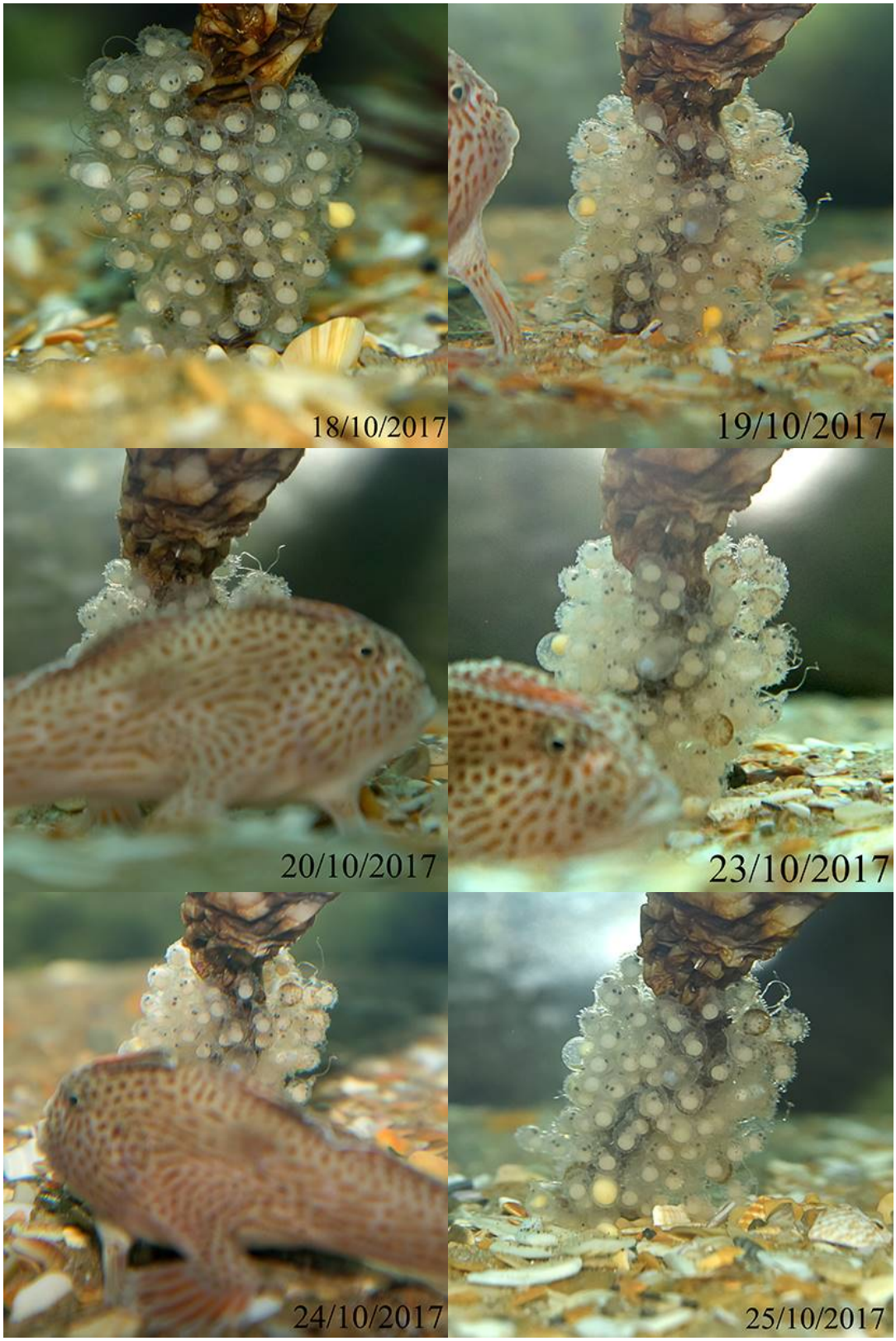
For much of the period between spawning, 13th September 2017, and the 25th September 2017, the day a blue egg was spotted, the eggs did not visually change, they were all white and growing within their individual sacks incrementally. An egg turned blue overnight and there was discussion as to whether it might be possible to surgically removed the egg from the egg mass however, employing high resolution digital capture, it could be determined that it looked to be contained in the egg sack and was no threat to the egg mass.

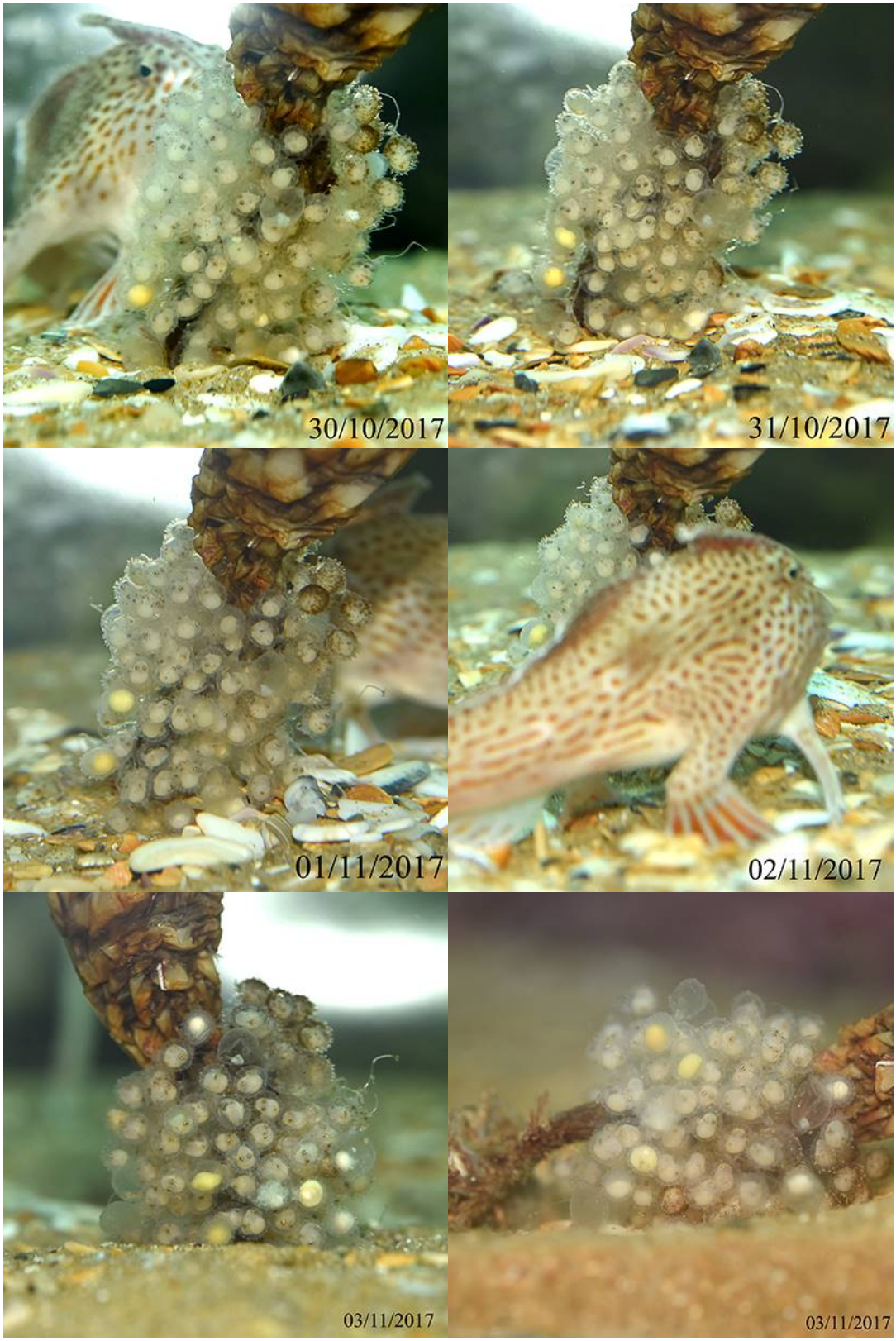
The ascidian came out of the substrate three times and was easily planted back in without disruption to the egg mass. The first time the ascidian was replanted three eggs from the other side were found to be yellow and did not develop. It is assumed these yellow eggs did not survive much past the spawning event.

The first visible growth change was the blacks of the eyes noticed on the 17th October 2017. On the 25th October 2017, a yellow speckle of the eyes appeared and each day from then on the animals would move about the sack from one day to the next. This movement was not visible to the eye but was noticed by comparing one day's image to another's. By the 30th October 2017 it was evident that the bodies were almost fully formed and were popping out of the white of the egg interior. A week before the eggs began to hatch the high resolution capture showed fins, heads, eyes and tails all very clearly.

The use of high resolution digital capture proved successful in capturing the health of the egg mass and the timeline of individual egg growth. Digital photographs are quick, useful tools for

seeing what the eye can't physically see close up, in this case, a moving subject in sea water behind glass. The images also proved useful in garnering public interest and awareness with regular posts on social media of the growing egg mass.


18/10/2017
19/10/2017
20/10/2017
23/10/2017
24/10/2017
25/10/2017


30/10/2017
31/10/2017
01/11/2017
02/11/2017
03/11/2017
03/11/2017


06/11/2017


07/11/2017


08/11/2017

APPENDIX C – TRANSFER PROTOCOLS

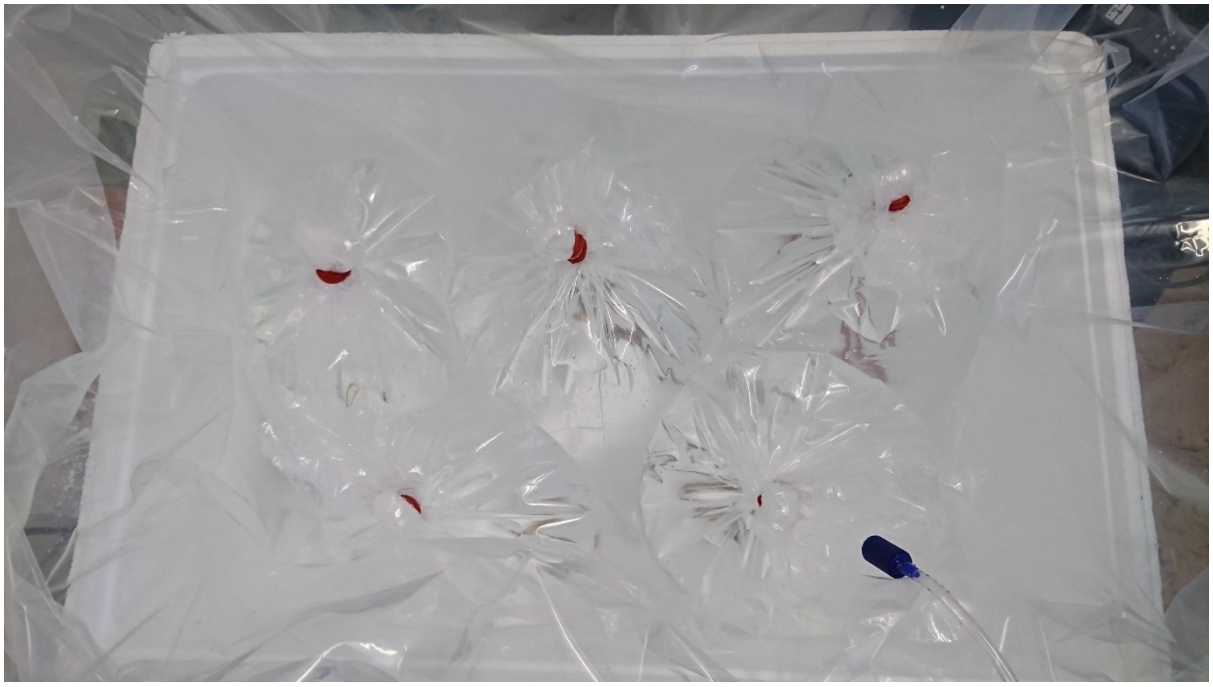
Timing of logistics and packing fish was an important consideration to reduce the risk of mortality. The pack has to be done swiftly and immediately before transport with well organised transfers (car and/or plane and pick-ups).

Thick plastic bag lined polystyrene foam box held the double smaller bags that are to contain the fish. Cool tank water was placed into each bag and oxygen loaded for 1 minute with the help of a bubbler. For the first bag we checked for oxygen saturation with a DO probe with our YSI Professional Plus (Pro Plus) instrument.

Capture of fish from tanks was by dip net and into beakers before transfer, with ample water into the oxygen enriched seawater of the double bags.

Further oxygenation of bags with fish inside the bag then occurred.

Bags were twisted, secured with rubber bands, then secured again.

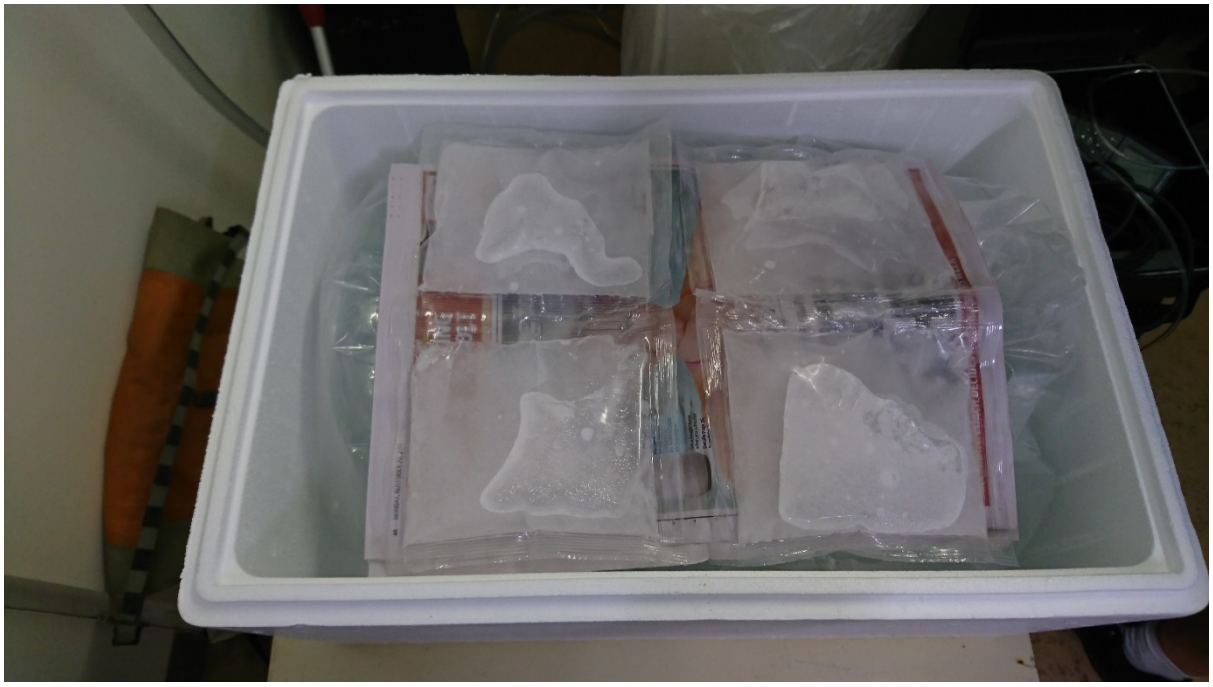


We transported 4-5 fish in each shipment, if required to keep fish snug we added extra bags of water.

Additional oxygen was added to the larger plastic bag.

The larger plastic bag was then secured.

A newspaper added for insulation.



Four thin freezer packs were then added.

The lid was added and taped and, in the case of the transfer to Melbourne paperwork attached (consignment note, export permit, import permit).

Further labelling and weighing at the airport.

Jet pack kept the fish inside the air-conditioned office prior to loading onto the plane and kept us updated on delays.

For all transfers fish arrived alive and well. For transfers to Melbourne parameters were similar to the holding tanks upon unpacking.

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Australian Government | National Environmental Science Programme | Australian Government Australian Institute of Marine Science | CSIRO | Australian Government Geoscience Australia | IMOS Integrated Marine Observing System

Charles Darwin University | University of Tasmania | The University of Western Australia | NSW Government | Museums Victoria

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