Stock/Management unit determination in the Northern Territory offshore snapper fisheries

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Abbreviations	Full form
AS	Arafura Sea
ASE	Arafura Sea East
ASW	Arafura Sea West
CI	confidence interval
DF	Demersal Fishery
df	degrees of freedom
GOC	Gulf of Carpentaria
ICPMS	inductively coupled plasma mass spectrometry
ITQ	Individual Transferable Quotas
JBG	Joseph Bonaparte Gulf
LA - ICPMS	laser ablation inductively coupled plasma mass spectrometry
LDFA	Linear Discriminant Function Analysis
LOD	limits of detection
MANOVA	multivariate analysis of variance
NIST	National Institute of Standards and Technology
OSF	offshore snapper fisheries
SD	standard deviation

Abbreviations	Full form
SDM	stock differentiation matrix
TL	total length
TRF	Timor Reef Fishery

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1. Executive summary

For fisheries managers to develop contemporary management frameworks, it is critical to have a knowledge of the stock structure of harvested species to inform the appropriate scales of management. The best way to identify a species stock structure is to apply a holistic approach using multiple techniques on the same individuals sampled from selected populations, and combine results across spatial and temporal scales to produce a weight of evidence conclusion.

Saddletail Snapper, Goldband Snapper and Red Emperor are all important species in the Northern Territory offshore snapper fisheries (OSF). The stock structure for these species was investigated to inform the development of contemporary management frameworks for these fisheries, using both otolith microchemistry and parasitology analyses. All species were found to have multiple stocks within the OSF area. Goldband Snapper and Red Emperor showed the most structuring, existing as separate stocks from all sampling regions, while Saddletail Snapper had separate stocks at the eastern and western portions of the fishery, and a large single stock in the centre.

A generic set of management units has been identified to be used to inform the development of contemporary management frameworks for these fisheries. Importantly, the stock structure and associated management units that are suggested do not align with the current boundaries of the fisheries in this region.

2. Background

The OSF include both the Demersal and Timor Reef fisheries (DF and TRF) that operate in waters 15 nautical miles from the coastal baseline to the outer limit of the Australian Fishing Zone. The OSF have operated under a management system based on Individual Transferable Quotas (ITQ) since 2011.

The OSF target a variety of fish species, including Saddletail Snapper, Crimson Snapper, Goldband Snapper and Red Emperor as key target species. Catches of Saddletail and Crimson Snapper have increased rapidly as three additional trawling vessels were introduced. The OSF are currently undergoing a management review to develop a management framework(s) and associated harvest strategies to align the fisheries management with known biological stocks. Consequently, it is critical to know the stock structure of the important species in the OSF to ensure the framework(s) developed and associated harvest strategies operate at the optimum population scale to ensure sustainable harvest.

The species chosen for this project included two of the three most harvested species, Goldband Snapper and Saddletail Snapper, as well as Red Emperor as an important, high-value, secondary species. While these species are abundant in the OSF catch, they also have some of the most highly vulnerable biological characteristics, being long-lived, late-maturing and slow-growing (Newman et al., 2000; Fry and Milton, 2009).

Generally, the two OSF fisheries operate with different fishing gear and in very separate regions. The DF mostly uses trawl gear to target red snapper species and operates in the eastern Arafura Sea, Gulf of Carpentaria and Joseph Bonaparte Gulf. The TRF mainly uses trap gear to target Goldband Snapper, red

snapper species and Red Emperor and operates only within the Timor Sea. However, fish trapping also occurs in the DF in the western Arafura Sea which is directly adjacent to the TRF and both fisheries operate over the same reef habitat (Lloyd and Puig 2009).There is evidence suggesting the OSF have different genetic populations of Goldband Snapper in the Timor Sea compared to the eastern Arafura Sea (Newman et al., 2000). In contrast, genetic information suggests that Saddletail and Crimson Snapper each comprise a single genetic population across northern Australia (Elliot, 1996; Salini et al., 2006).

Genetic studies on Red Emperor stock structure have suggested a panmictic population across their Australian distribution (van Herwerden et al., 2009) but otolith microchemistry has revealed separate populations in Western Australia (Stephenson et al., 2001). This study used multiple methods (otolith microchemistry and parasite incidence analyses) for the three species in a holistic approach to determine stock structure (Welch et al., 2015). The Australian Institute of Marine Science completed further genetic analyses in a project investigating the spatial ecology and genetic connectivity of exploited these species across northern Australia.

This project was developed to address two key needs in the OSF. Firstly, to fill an important information gap on the biology of three key species vulnerable to overfishing to enable development of appropriate species abundance monitoring and management regimes for the OSF. Secondly, the project enabled participation in a collaborative research program between OSF licence holders, the Territory Government's Department of Industry, Tourism and Trade, the Australian Institute of Marine Science, the West Australian Department of Primary Industry and Regional Development, and Santos (formerly ConocoPhillips).

2.1. Objectives

1. Examine the population structure and connectivity at various scales for three commercially important species in the NT; Goldband Snapper, Saddletail Snapper and Red Emperor.
2. Inform fisheries management on the appropriate management spatial units for the OSF, based on knowledge of the biological stocks of the major target species.

3. Methods

3.1. Sample collections

All fish samples were collected from commercial fishers in the OSF. Fish caught were placed on ice or frozen on the vessel and transported to the laboratory in Darwin for processing. The sample locations for all species were:

- Joseph Bonaparte Gulf (JBG)
- Timor Reef Fishery (TRF, Timor Sea)
- Arafura Sea West (ASW)
- Arafura Sea (AS)
- Gulf of Carpentaria (GOC; Figure 1, Table 1).

In the laboratory, total length (TL) and sex of each specimen were recorded. Biological samples were also taken from each specimen for analyses by the respective methods for stock structure determination. For genetic analyses, a clip from the spine of the dorsal fin was retained. Genetic samples were frozen in 95% molecular grade ethanol. For otolith microchemistry analyses, the pair of sagittal otoliths was dissected from each fish, cleaned and rinsed thoroughly, dried and stored in paper envelopes. For parasite

analyses, the gills, pharyngeal teeth plates and internal body organs were removed, placed in a labelled bag and frozen for later examination.

Table 1: Number of each species sampled from each location for each of the separate analyses.

Sample Site	Species	Date	Sample No.
Joseph Bonaparte Gulf	Goldband Snapper	03/08/2015	31
Joseph Bonaparte Gulf	Saddletail Snapper	03/08/2015	30
Joseph Bonaparte Gulf	Red Emperor	03/08/2015	28
Timor Reef Fishery (Timor Sea)	Goldband Snapper	16/09/2015	30
Timor Reef Fishery (Timor Sea)	Saddletail Snapper	16/09/2015	30
Timor Reef Fishery (Timor Sea)	Red Emperor	18/10/2015	22
Timor Reef Fishery (Timor Sea)	Saddletail Snapper	28/10/2016	12
Arafura Sea West	Goldband Snapper	30/03/2016	30
Arafura Sea West	Saddletail Snapper	22/04/2016	19
Arafura Sea West	Red Emperor	30/03/2016	30
Arafura Sea East	Goldband Snapper	10/08/2015	30
Arafura Sea East	Saddletail Snapper	10/08/2015	30
Arafura Sea East	Red Emperor	03/02/2016	40
Gulf of Carpentaria	Goldband Snapper	06/08/2015	30
Gulf of Carpentaria	Saddletail Snapper	12/08/2015	30
Gulf of Carpentaria	Red Emperor	06/08/2015	22

Note: An additional Saddletail Snapper sample was collected from the Timor Sea (Timor Sea B, from approximately the same location as the Timor Sea sample) given that the original samples were all small individuals.

3.2. Otolith chemistry analyses

3.2.1. Otolith preparation

The left sagittal otolith was selected from each individual and embedded into epoxy resin (West System 105 epoxy resin and West System 206 hardener) with the sulcus facing downwards. A Buehler IsoMet® low speed saw was used to cut transverse sections through the primordium of each otolith at approximately 350µm thick. Sections were polished with three grades of 3M diamond lapping film (30, 9 and 3µm), rinsed thoroughly with Milli-Q water and air-dried. Otolith sections were mounted onto microscope slides using epoxy resin. Once dry, the section mounts were triple-rinsed with Milli-Q water and allowed to dry in a laminar flow cabinet.

Figure 1: Locations of sample collections across northern Australia.

3.2.2. Analysis of trace elements

Elemental analysis was performed using the laser ablation-ICP-MS (LA-ICPMS) located at the University of Melbourne, which comprises an Agilent 7700x quadrupole inductively coupled plasma mass spectrometer coupled to a custom-built RESOlution laser ablation system with a HelEx cell. The RESOlution system is constructed around a Compex 110 ArF excimer laser, which was operated using a spot size of 72µm in diameter with laser energy at 2.7 J/cm^2 and a repetition rate of 5Hz.

Laser software (GeoStar v6.14) was used to digitally plot three ablation areas on each individual otolith section. The first area was at the primordium (referred to hereafter as 'core'), the second area was just outside the first opaque zone ~500µm to ventral side of the core ablation ('near core'), and the third at the ventral margin adjacent to the sulcus acusticus ('margin'). The multi-elemental data collected from each ablation position is intended to represent the general locations of the larval dispersal phase (core), post-larval juvenile phase (near core), and the sub-adult/adult phase (margin). Ablations occurred inside a sealed chamber in an atmosphere of pure He with the ablated material being transported to the ICPMS in the Ar carrier gas.

A total of 11 trace elements (^{7}Li, ^{25}Mg, ^{23}Al, ^{49}Ti, ^{53}Cr, ^{55}Mn, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{88}Sr, ^{138}Ba) and the internal standard (^{43}Ca) were analysed from all three ablation zones for each otolith. The laser ablation spot sample consisted of a 20-second blank, followed by an ablation period of 50 seconds, of which the first 5 seconds and the last 1 second were excluded from data integration to allow for signal stabilisation. Data reduction and processing was completed using the trace elements data reduction scheme (Woodhead et al., 2007) of the specialised software package Iolite version 3 (Paton et al., 2011).

Subtraction of background ion counts from otolith counts was followed by the normalisation of each element to ^{43}Ca and the National Institute of Standards and Technology (NIST 612) glass standard was used as the external calibration standard, which was analysed after every 10 otolith samples to correct for any long-term drift in the instrument.

The limits of detection (LOD) were calculated for each sample from the ablation yield equivalent to 3 x standard deviation (SD) of the blank background measurements. Concentrations of ^{23}Al, ^{49}Ti and ^{53}Cr were <LOD and were not included in the analysis. For all elements, the ratio of element isotope intensity to ^{43}Ca intensity was used to estimate the element:^{43}Ca ratio. These ratios were converted to molar ratios and were expressed as element: Ca molar ratios in mmol mol-1 or µmol mol-1. Finally, the ablated otolith sections were digitally photographed using a Leica M80 stereo dissecting with image analysis software (Image-Pro Plus 7.0), and ablation zones checked for accuracy.

3.2.3. Statistical analysis

All multi-elemental otolith data was examined and subsequently $\log_{10}$ transformed to meet assumptions of normality and homogeneity of variance (Quinn and Keough, 2002). Spatial variation in otolith near core and edge chemistry among sample locations were investigated using single-factor multivariate analysis of variance (MANOVA). The Pillai's trace statistic was reported as it is considered the most robust (Scheiner, 1993). Correlation between total length of fish at each of the collection sites and each of the otolith elemental ratios measured were tested using Pearson's parametric correlations. Linear Discriminant Function Analysis (LDFA) was conducted to provide statistical and visual indication of the similarities within the multi-elemental otolith chemical signatures among samples at the regional spatial scale. Standardised coefficients for the discriminant functions were used to measure which elements contributed most to group separation.

Results of the LDFA were plotted as graphs of the first and second discriminant axes, with 95% confidence interval (CI) ellipses established around the centroid. Significant statistical differences between locations occurred when there was no overlap between the 95% CI (confidence interval) ellipses. Classification success for the LDFA was calculated by jack-knife cross-validation matrices. All these analyses were conducted using R (R Core Team 2015).

The otolith jack-knife cross-validation matrices were analysed by means of randomisation tests to determine if the jack-knifed classification estimates were significantly different from random, and were conducted using code supplied in White and Ruttenberg (2007). A script was run in Matlab (version 2013a) to calculate the classification success rates and associated P values (probability of obtaining the observed classification rate due to chance alone) using uniform prior probabilities and 10,000 randomisations of the data (White and Ruttenberg, 2007).

3.3. Parasites

3.3.1. Parasite collection

Once defrosted, gills were removed, separated into individual arches and washed in water (vigorously shaken to dislodge parasites). Gill arches were then examined individually under a dissector microscope and any parasites still attached were removed. The length of the gill arch was opened for examination and any parasites encountered were removed as gently as possible.

The mouth of the fish (that is, pharyngeal teeth plates and the tissue behind them) was also washed before examination under a dissector microscope. Parasites found attached to the pharyngeal teeth plates (such as *Encotyllabe* sp.) were gently grasped with forceps and pulled. The tissue behind the pharyngeal plates was examined. Encysted parasites were easily removed. Philometrid nematodes were dissected from between tissue layers.

The gill and pharyngeal teeth wash was allowed to settle, then the supernatant was poured off, discarded, and the sediment examined under a dissector microscope for parasites that had been removed in the wash.

Examination of the internal organs involved the separation of the stomach and intestinal tract from the mesenteries and associated organs. The liver, swim bladder and spleen were not examined for parasites. The stomach and intestine were each slit along their length and washed (as above) for parasite examination. Philometrid nematodes and didymozoid digeneans in the stomach wall were visible through the tissues and were dissected out as carefully as possible.

The supernatant of the intestinal washings was decanted as above. If there was a large amount of intestinal content (that is, partly digested food), the process was repeated until the remaining sediment was clear enough to be able to find parasites. The mesenteries that connect the internal organs were removed from the organs, washed and examined under a dissector microscope. Encysted parasites were removed from the mesenteries. All encysted parasites were released from their associated cysts for identification prior to fixation. For female fish, ovaries were slit along their length and examined under a dissector microscope for the presence of philometrid nematodes.

Representative samples of parasites from each fish host and collection location were placed directly into 70% ethanol.

3.3.2. Parasite identification

As parasites were collected, they were identified (as far as possible) and counted within those identifications. The identifications and counts were rechecked after all dissections had been completed. Parasites were identified to the lowest possible taxonomic unit. Some were able to be identified to species but many could not be identified beyond family in the time scale of the project. It is acknowledged that the broader taxonomic separation of the parasites into their various groups based on morphological examination will contain new or cryptic species, upon further investigation.

Where a relevant expert in a particular parasite group had been identified and was able to assist with identifications, parasite specimens were sent for examination. For parasites not sent elsewhere, identification was completed as far as possible by Dr D. Barton.

The following techniques were used for examination of these particular parasitic groups.

Monogeneans, digeneans and cestodes

Specimens were stained in Aceto-Carmine, dehydrated in a graded ethanol series, cleared in xylene and mounted in Canada balsam as permanent slides. Some specimens were mounted unstained. Some specimens of monogeneans were mounted in lactophenol, which dissolves the soft tissues of the organism leaving the sclerotised haptoral armature. Coverslips of specimens mounted in lactophenol were ringed with nail varnish to seal the slide and make a permanent mount.

Nematodes

Specimens were mounted as temporary wet mounts in lactophenol or glycerol. Upon completion of the required examination and measurements, specimens were returned to 70% ethanol.

Pentastomes

Specimens were mounted as temporary wet mounts in lactophenol. Some specimens were mounted whole, while other specimens were partly dissected for the removal of the anterior hooks required for identification. Upon completion of the required examination and measurements, whole specimens were returned to 70% ethanol. Coverslips of dissected specimens were ringed with nail varnish to seal the slide and make a permanent mount.

Parasites were identified by morphological examination of whole mounted material. Published records and keys in scientific papers assisted in identification, with distinctive characters used to classify parasites to

family, genus or species. In addition, voucher and type material was borrowed from collections within Australia for comparison with material collected in this study. Drawings of specimens were made with the aid of a camera lucida and measurements were made using an ocular micrometre. Photos were taken using a 9MP Microscope Digital Camera (AmScope Model MU900).

3.3.3. Statistical analysis

Summary statistics were compiled for each location. These included mean abundance (total number of individuals of a particular parasite per sample divided by the total number of hosts examined, including uninfected hosts) and prevalence (number of hosts infected with a particular parasite divided by the number of hosts examined, expressed as a per cent) for each of the parasite species, following the terminology of Bush et al. (1997). Parasites were identified as potential biological markers if they exhibited a prevalence ≥ 10% in at least one sample location component species (Bush et al., 1990), and were relatively easy to find, identify and count. The natural logarithm of the parasite +1 [ln(x+1)] was used to minimise the variance of the abundance data. These transformed data were used throughout the analyses.

Pearson's correlations were used to explore the relationships between the total lengths of fish with individual parasite species. For parasites that showed a significant correlation, parasite abundances were corrected to the mean host TL, as described in Moore et al. (2003). No correction was made if the parasite abundance was zero.

Spatial variation in parasite assemblages among regions and locations within regions was investigated using single-factor MANOVA. As for the otolith chemistry analyses, the Pillai's trace statistic was reported as it is considered the most robust (Scheiner, 1993).

Linear Discriminant Function Analysis (LDFA, R Core Team, 2015) was conducted to provide a visual indication of the similarities of the parasite assemblages among samples. Results of the LDFA were plotted as graphs of the first and second discriminant axes, with 95% CI ellipses established around the centroid. Significant statistical differences occurred when there was no overlap between the 95% CI ellipses. A jack-knife reclassification success matrix indicating overall percentage correct for fish classified to each location, as well as the number of fish classified across the locations examined, is presented.

In accordance with Poulin and Kamiya (2015), comparison of the calculated per cent correct classification (by LDFA) was compared against the 'proportional chance criterion', which is the expected proportion of fish classified correctly based on chance alone. This allows a benchmarking of the performance of the classification.

4. Integration

This study used multiple methods for 3 species in a holistic approach to determine stock structure. To integrate the potentially contrasting results of the different techniques for each species, the study used the stock differentiation matrix (SDM) described by Welch et al. (2015). This enabled clear and simple visualisation of the spatial comparisons made across the different techniques, facilitating interpretation and conclusions about appropriate spatial management units through pooling adjacent non-significantly different sampling locations. If at least one technique showed differences between sampling locations, they were considered separate stocks. If sampling locations showed no differences in any technique, they were considered a joint stock.

This also provided a parsimonious explanation of the spatial structure of the respective species in a potentially more meaningful way to fisheries managers and other stakeholders by taking into account the different spatial and temporal scale that each method informs (Welch et al., 2015).

5. Results

The MANOVA results investigated whether there were significant differences ($p<0.001$) in otolith near core and edge microchemistry and parasite assemblages among the sample collections sites for all 3 species (Table 2).

Table 2: Results of the MANOVA investigating spatial variability in parasite assemblage of all species across all sampling sites. All results are significant at the $p <0.001$ level.

	Goldband Snapper			Saddletail Snapper			Red Emperor		
	Pillai's Trace	df	F	Pillai's Trace	df	F	Pillai's Trace	df	F
Parasitology	2.41	88, 456	7.86	3.2448	120, 630	9.71	2.35	84, 392	6.68
Otolith Near Core	0.78	32, 544	4.12	0.61322	40, 660	2.31	0.56	32, 520	2.67
Otolith Edge	1.20	32, 528	7.09	1.0154	40, 650	4.14	0.99	32, 508	5.22

5.1. Goldband Snapper

The LDFA successfully reclassified a higher percentage of fish back to location of origin in comparison to the proportional chance criterion of 20% (Table 3).

The plots of the scores of the first 2 discriminant functions of the near core otolith microchemistry variations showed separation of JBG from all other sites and then 2 groups, TRF/ASW and ASE/GOC, that overlapped in the 95% confidence ellipses (Figure 2a). The discriminant functions of the edge otolith microchemistry variations showed separation of JBG and ASW from all other sites and then substantial overlap in the 95% confidence ellipses of TRF, ASE and GOC (Figure 2b). The discriminant functions of the parasite assemblage variations showed separation of all sites except TRF and GOC, which overlapped in the 95% confidence ellipses (Figure 2c).

Table 3: Reclassification success of the linear discriminant function analysis (LDFA) for the overall otolith near core and edge microchemistry and parasite assemblage for Goldband Snapper across the offshore snapper fishery. The proportional chance criterion of Poulin & Kamiya (2015) is presented in brackets.

	Near core % correct	Edge % correct	Parasites % correct
JBG	51.7	81.5	78.3
TRF	46.4	55.2	53.6
ASW	43.3	46.7	83.3
ASE	48.3	55.6	69.2
GoC	24.1	32.1	60.0
Overall	42.7 (20)	56.0 (20)	68.6 (20)

5.2. Saddletail Snapper

The LDFA successfully reclassified a higher percentage of fish back to location of origin, in comparison to the proportional chance criterion of 18% (Table 4) for most sample locations. However, the edge microchemistry for TRF-B was very poorly reclassified, likely due to these fish mixing with the ASW fish. The plots of the scores of the first two discriminant functions of the near core otolith microchemistry variations showed separation of JBG from all other sites, and then two groups, TRF-B/ASW and TRF/ASE/GOC, that overlapped in the 95% confidence ellipses (Figure 3a).

The discriminant functions of the edge otolith microchemistry variations showed separation of JBG, and then substantial overlap in the 95% confidence ellipses of all other sites (Figure 3b). The discriminant functions of the parasite assemblage variations showed separation of JBG, TRF and GOC, and then substantial overlap in the 95% confidence ellipses for TRF-B, ASW and ASE (Figure 3c).

Table 4: Reclassification success of the linear discriminant function analysis (LDFA) for the overall otolith near core and edge microchemistry and parasite assemblage for Saddletail Snapper across the offshore snapper fishery. The proportional chance criterion of Poulin & Kamiya (2015) is presented in brackets.

	Near core % correct	Edge % correct	Parasites % correct
JBG	35.7	80.8	93.c
TRF	42.9	66.7	90.0
TRF-B	33.3	8.3	75.0
ASW	18.8	25.0	63.2
ASE	21.4	31.0	80.0
GoC	24.1	55.2	86.7
Overall	29.8 (18)	49.6 (18)	83.4 (18)

5.3. Red Emperor

The LDFA successfully reclassified a higher percentage of fish back to location of origin, in comparison to the proportional chance criterion of 21% for most sample sites (Table 5). However, the near core samples for TRF and GOC and the edge samples for GOC were very poorly reclassified. The plots of the scores of the first two discriminant functions of the near core otolith microchemistry variations showed separation of JBG from all other sites and then substantial overlap in the 95% confidence ellipses for the rest of the sites (Figure 4a).

The discriminant functions of the edge otolith microchemistry variations showed separation of ASW from all other sites, and then substantial overlap in the 95% confidence ellipses for the rest of the sites (Figure 4b). The discriminant functions of the parasite assemblage variations showed separation of JBG, TRF and ASE, and then overlap in the 95% confidence ellipses for ASE and GOC (Figure 4c).

Table 5. Reclassification success of the linear discriminant function analysis (LDFA) for the overall otolith near core and edge microchemistry and parasite assemblage Red Emperor across the OFS. The proportional chance criterion of Poulin & Kamiya (2015) is presented in brackets.

	Near core % correct	Edge % correct	Parasites % correct
JBG	57.1	40.7	75.0
TRF	9.1	40.9	66.7
ASW	50.0	93.3	92.0
ASE	52.6	59.5	76.7
GoC	9.5	10.0	59.1
Overall	39.6 (21)	52.9 (21)	75.0 (21)

Figure 2: Plot of the first two discriminant function scores showing spatial variation in otolith microchemistry in (a) the otolith near core, (b) the otolith edge, and (c) parasite assemblages for Goldband Snapper. Ellipses are 95% CI around the group centroid for each location within each region and data points represent individual fish.

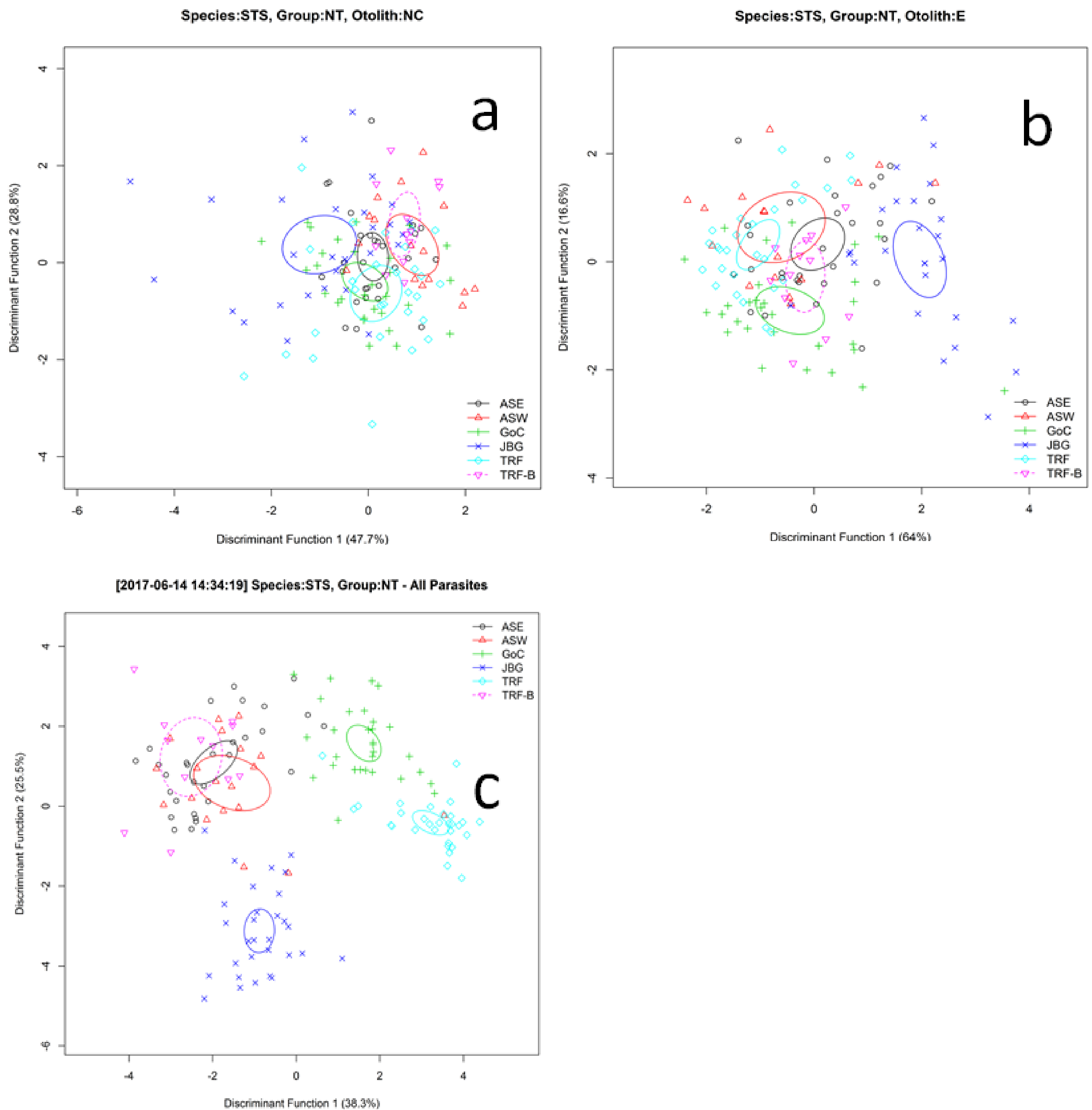


Figure 3: Plot of the first two discriminant function scores showing spatial variation in otolith microchemistry in (a) the otolith near core, (b) the otolith edge, and (c) parasite assemblages for Saddletail Snapper. Ellipses are 95% CI around the group centroid for each location within each region and data points represent individual fish.

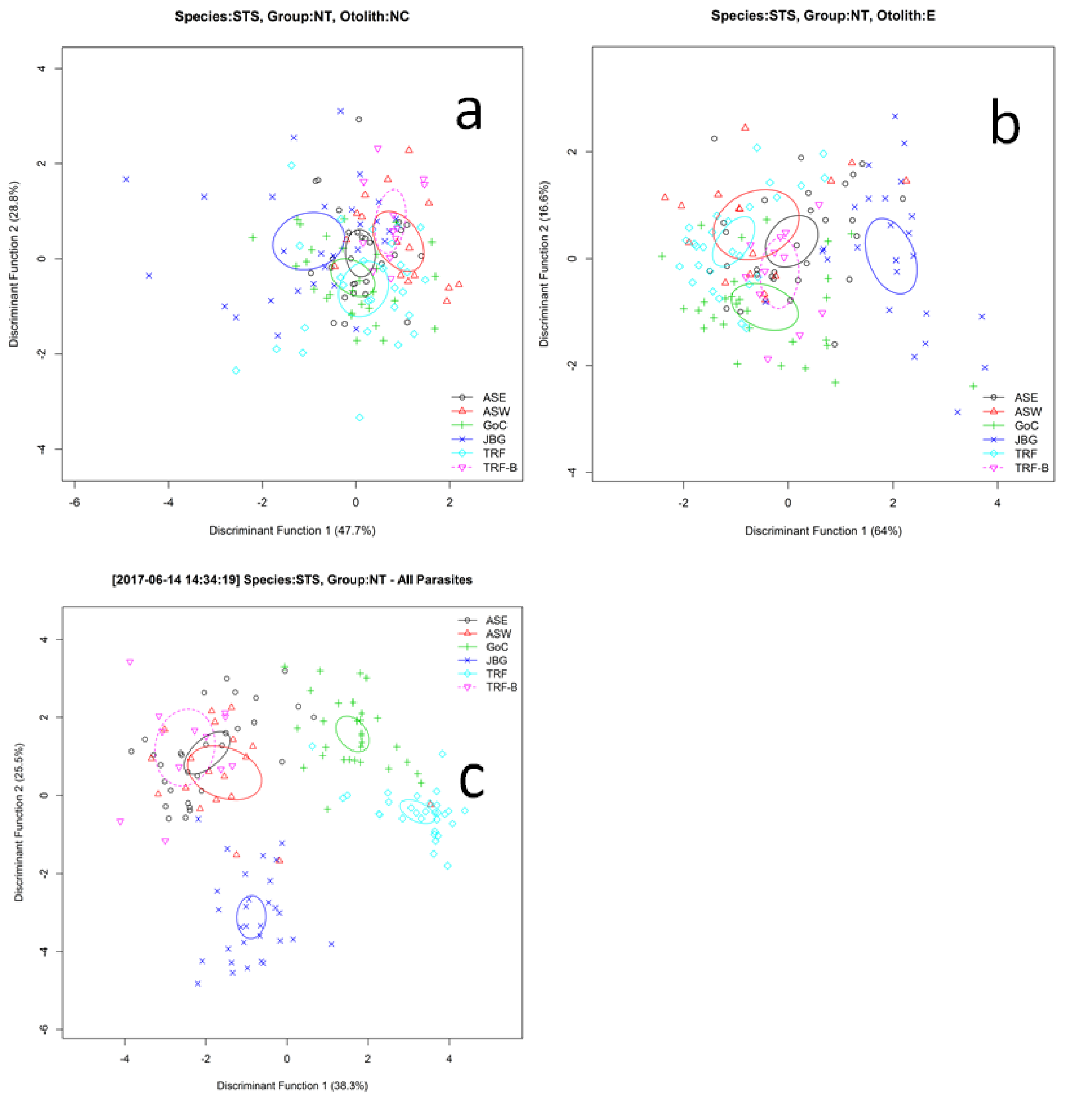
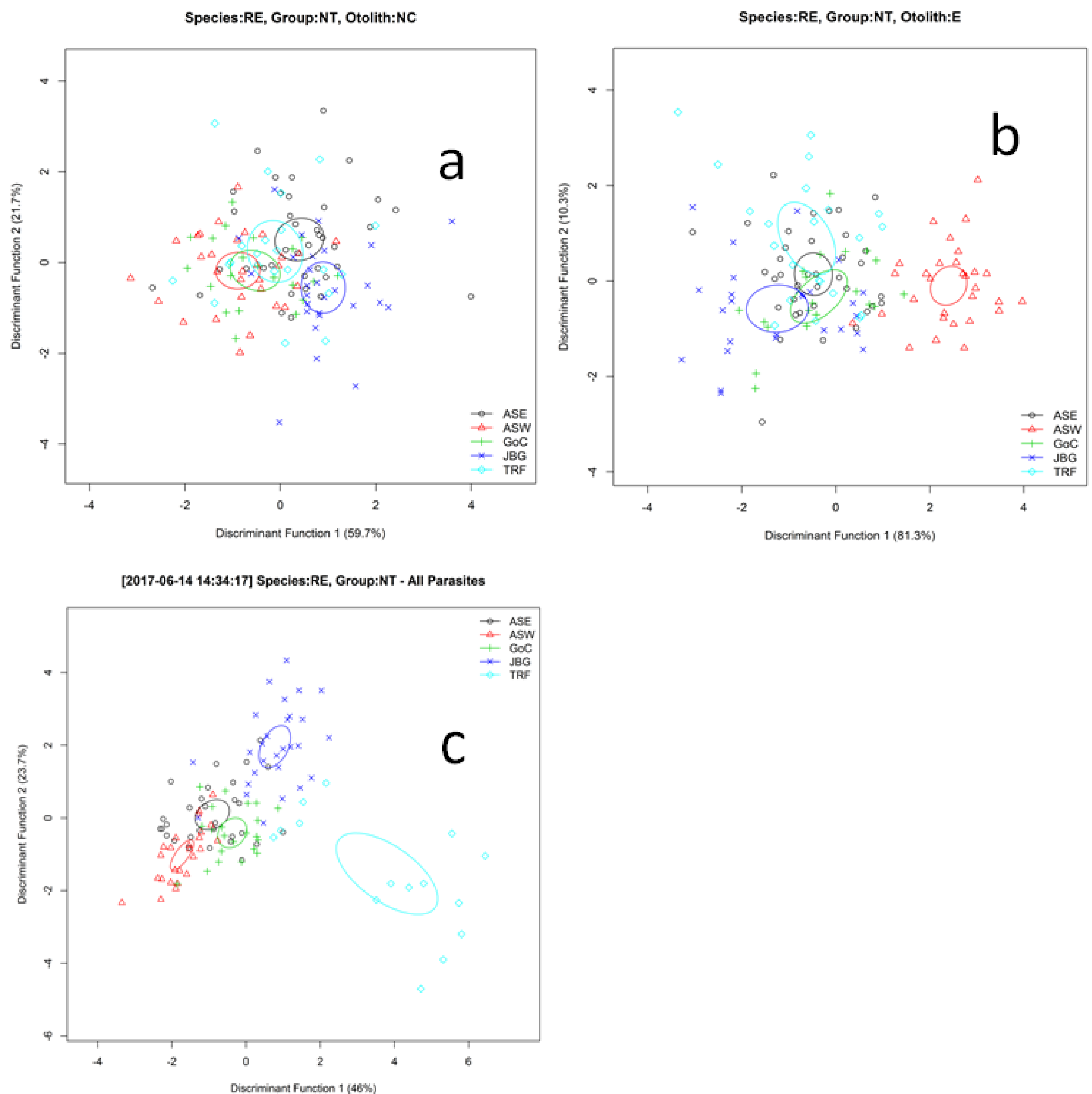


Figure 4: Plot of the first two discriminant function scores showing spatial variation in otolith microchemistry in (a) the otolith near core, (b) the otolith edge, and (c) parasite assemblages for Red Emperor. Ellipses are 95% CI around the group centroid for each location within each region and data points represent individual fish.



5.4. Integration

The integration of results from the otolith chemistry and parasite analyses are presented in a stock differentiation matrix (SDM) for Goldband Snapper (Table 6), Saddletail Snapper (Table 7) and Red Emperor (Table 8).

Table 6: Stock differentiation matrix (SDM) for Goldband Snapper showing the results inferred from the regional pairwise comparisons for the two techniques used in this study. Where significantly different results were found for pairwise comparison of the sampling locations, these are indicated by capital letters: P – parasites O – otolith microchemistry. Non-significant results are indicated by lowercase letters corresponding to the respective techniques. Results for parasites and otolith are from the discriminant function plots in Figure 2. Near core otolith results are used.

Location	JBG	TRF	ASW	ASE	GoC
Joseph Bonaparte Gulf					
Timor Reef Fishery	O, P				
Arafura Sea West	O, P	o, P			
Arafura Sea East	O, P	O, P	O, P		
Gulf of Carpentaria	O, P	O, p	O, P	o, P	

Table 7: SDM for Saddletail Snapper showing the results inferred from the regional pairwise comparisons for the two techniques used in this study. Where significantly different results were found for pairwise comparison of the sampling locations, these are indicated by capital letters: P – parasites, O – otolith microchemistry. Non-significant results are indicated by lowercase letters corresponding to the respective techniques. Results for parasites and otolith are from the discriminant function plots in Figure 3. Near core otolith results are used.

Location	JBG	TRF	TRF-B	ASW	ASE	GoC
Joseph Bonaparte Gulf						
Timor Reef Fishery	O, P					
Timor Reef Fishery B	O, P	O, P				
Arafura Sea West	O, P	O, P	o, p			
Arafura Sea East	O, P	o, P	o, p	O, P		
Gulf of Carpentaria	O, P	o, P	O, P	O, P	o, P	

Table 8: SDM for Red Emperor showing the results inferred from the regional pairwise comparisons for the two techniques used in this study. Where significantly different results were found for pairwise comparison of the sampling locations, these are indicated by capital letters: P – parasites, O – otolith microchemistry. Non-significant results are indicated by lowercase letters corresponding to the respective techniques. Results for parasites and otolith are from the discriminant function plots in Figure 3.
Near core otolith results are used.

Location	JBG	TRF	ASW	ASE	GoC
Joseph Bonaparte Gulf					
Timor Reef Fishery	O, P				
Arafura Sea West	O, P	o, P			
Arafura Sea East	O, P	o, P	o, P		
Gulf of Carpentaria	O, P	o, P	o, P	o, P	

Potential management units were identified in Table 9 based on whether either method showed a significant difference between sample locations (whether there was separation between the 95% confidence intervals around the centroids on the discriminant function graphs) or where there was potential biogeographic connectivity.

For the purposes of the management unit classification, the near core microchemistry results were used rather than those for the edge. This was primarily because edge chemistry tends to represent recent (daily-yearly) connectivity patterns, whereas the near core provides multiple samples of connectivity among different cohorts of juvenile fish so gives information on the longer term (lifetime of the species) connectivity patterns. Additionally, for Saddletail Snapper the TRF samples were not used to determine management units. This was a result of TRF not being comparable to the other sites in the analyses due to the small size of the fish.

The potential management units identified for Goldband Snapper were primarily at the stock scale that existed at the scale samples were collected (Table 9). The exception was a Timor unit that incorporated samples from separate stocks in the Timor Sea and Arafura Sea West. For Saddletail Snapper, the management units matched the stock structure, which was separate stocks from the Joseph Bonaparte and Gulf of Carpentaria samples but a single continuous stock incorporating the samples from the Timor and Arafura seas (Table 9). Red Emperor were found to have the same stock structure as Goldband Snapper and also had the same management units applied to these stocks (Table 9).

Table 9: End-user table that summarises results from all techniques to determine the broadest spatial scale appropriate for management for each species, based on sampling locations in this study.

<table>
<tr><th colspan="3">Goldband Snapper</th></tr>
<tr><th>Otoliths</th><th>Parasites</th><th>Management units</th></tr>
<tr><td>Joseph Bonaparte Gulf</td><td>Joseph Bonaparte Gulf</td><td>Joseph Bonaparte Gulf</td></tr>
<tr><td rowspan="2">Timor</td><td>Timor Sea</td><td rowspan="2">Timor</td></tr>
<tr><td>Arafura Sea West</td></tr>
<tr><td rowspan="2">Arafura/GOC</td><td>Arafura Sea East</td><td>Arafura</td></tr>
<tr><td>Gulf of Carpentaria</td><td>Gulf of Carpentaria</td></tr>
<tr><th colspan="3">Saddletail Snapper</th></tr>
<tr><th>Otoliths</th><th>Parasites</th><th>Management units</th></tr>
<tr><td>Joseph Bonaparte Gulf</td><td>Joseph Bonaparte Gulf</td><td>Joseph Bonaparte Gulf</td></tr>
<tr><td>Timor/Arafura</td><td>Timor/Arafura</td><td>Timor/Arafura</td></tr>
<tr><td>Gulf of Carpentaria</td><td>Gulf of Carpentaria</td><td>Gulf of Carpentaria</td></tr>
<tr><th colspan="3">Red Emperor</th></tr>
<tr><th>Otoliths</th><th>Parasites</th><th>Management units</th></tr>
<tr><td>Joseph Bonaparte Gulf</td><td>Joseph Bonaparte Gulf</td><td>Joseph Bonaparte Gulf</td></tr>
<tr><td rowspan="3">Timor/Arafura/GOC</td><td>Timor Sea</td><td rowspan="2">Timor</td></tr>
<tr><td>Arafura Sea West</td></tr>
<tr><td>Arafura Sea East</td><td>Arafura</td></tr>
<tr><td></td><td>Gulf of Carpentaria</td><td>Gulf of Carpentaria</td></tr>
</table>

6. Discussion

The most powerful way to determine stock structure reliably is through the concurrent use of different techniques. This is referred to as a holistic approach and has been increasingly used and advocated in stock structure studies (for example, Baldwin et al., 2012; Welch et al., 2015). Historically, stock structure studies have employed a single analytical technique to detect differences among populations. However, a major limitation of such approaches is that, when no differences are detected, this may merely reflect the discriminating power of the particular technique. It does not necessarily mean they are from the same stock. Therefore, using a holistic approach greatly increases the likelihood of detecting different stocks where they exist (Begg and Waldman, 1999). A holistic approach provides a 'weight of evidence' to more accurately identify individual fish stocks.

The purpose of establishing the stock structure of exploited species is to inform managers and other stakeholders on the appropriate spatial scale at which management of targeted species should occur. Management at this scale then provides the basis for ensuring biological sustainability. The use of the two

techniques on each species in this study provides information on the connectivity during the actual lifetime of fish whereas genetic information tends to provide historical connectivity over geological time (Begg and Waldman, 1999). Given that fisheries managers generally are interested in management of stocks on time scales relevant to the lifetime of the species in question, the two techniques used are probably more applicable than genetic analysis.

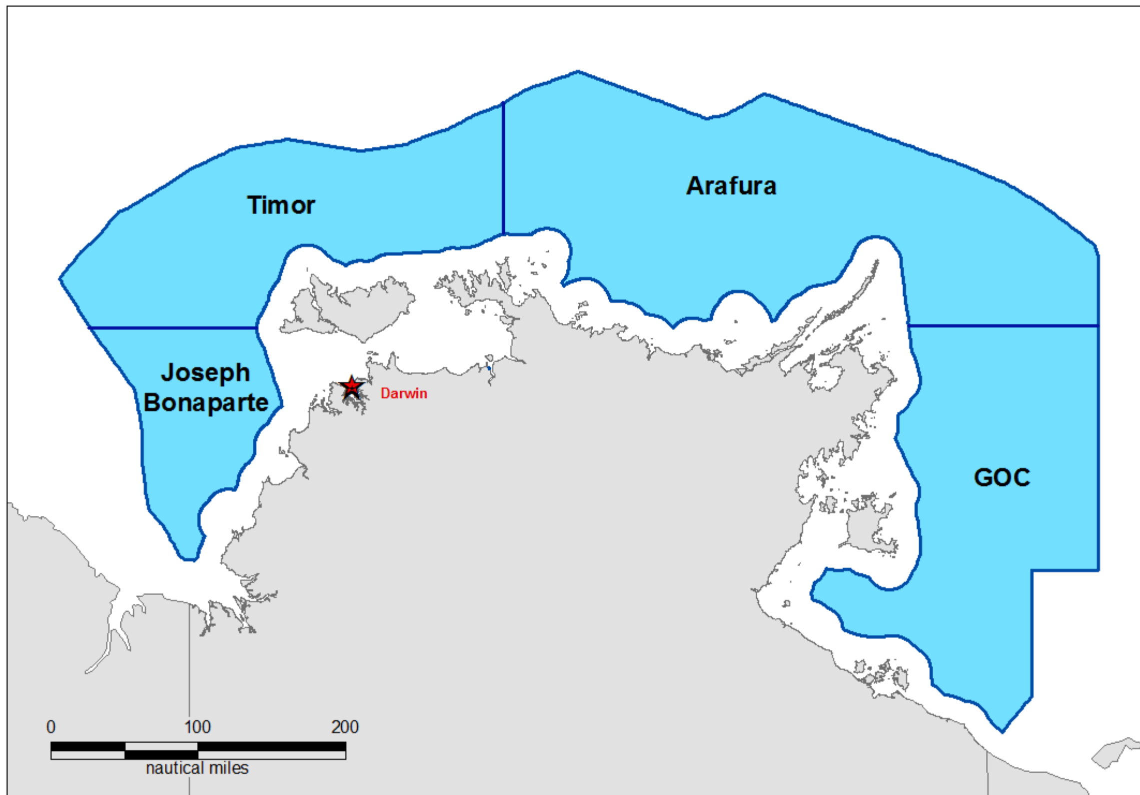
The results of this study demonstrate that all three species examined had separate stocks within the area of the OSF. However, it is important to consider that the ‘stocks’ identified were based on samples that were hundreds of kilometres from the each other. The fact that they were classified as separate stocks does not necessarily mean that a line can be drawn halfway between them as separate management units. In this study, the lines drawn for each management unit were indicative of likely biogeographical features that restricted movements between locations (such as, the Gulf of Carpentaria).

A key finding for all species was that the current fisheries boundaries between the OSF do not align with the stock structure identified. For Goldband Snapper, there were two adjacent stocks in the Timor and Arafura seas that formed into a single management unit due to the lack of likely biogeographic barriers between them. This was probably due to there being a single extensive area of reef between these two areas (Lloyd and Puig, 2009) that is unlikely to restrict movement of this species within this region. For the same reason Red Emperor was considered to form a single management unit between these two stocks as well. Additionally, the formation of these management units is also warranted due to the likely consistent fishing pressure and gear types used in these stocks.

In addition to identifying the appropriate scales of management, the results of this study can be used more specifically to improve the performance of stock status assessments and develop the harvest strategy for the OSF. Where possible, the harvest strategy being developed under the proposed new management framework should be applied at the scale of stocks. However, given the OSF are multispecies fisheries and some species stocks harvested exist at very fine spatial scales (for example, Golden Snapper and Black Jewfish; Saunders et al., 2017), there needs to be a generic set of management units developed that accounts for the different scales at which stocks exist.

Using Goldband Snapper and Saddletail Snapper stock structure as examples, the following management units can be considered: Joseph Bonaparte, Timor, Arafura and Gulf of Carpentaria (Figure 5). In the case of Saddletail Snapper, the stock structure supports two of these units to be joined as a single stock (Timor/Arafura). The other major target species in these fisheries, Crimson Snapper, could also be managed under the same units as Saddletail Snapper, given their very similar patterns in abundance and distribution. Species such as Golden Snapper and Black Jewfish that have finer scale stock structure can then be managed separately within these areas under a harvest strategy.

Figure 5: Suggested management units for the OSF.



7. References

Baldwin, R. E., Banks, M. A. and Jacobson, K. C. (2012). Integrating fish and parasite data as a holistic solution for identifying the elusive stock structure of Pacific sardines (*Sardinops sagax*). *Reviews in Fish Biol and Fisheries*, 22:137–56.

Begg, G. A. and Waldman, J. R. (1999). An holistic approach to fish stock identification. *Fisheries Research*, 43:35–44.

Bush, A. O., Aho, J. M. and Kennedy, C. R. (1990). Ecological versus phylogenetic determinants of helminth parasite community richness. *Evolutionary Ecology*, 4: 1-20.

Bush, A. O., Lafferty, K. D., Lotz, J. M. and Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology*, 83:575-583.

Elliott, N. G. (1996). Allozyme and mitochondrial DNA analysis of the tropical saddle-tail sea perch, Lutjanus malabaricus (Schneider), from Australian waters. *Marine and Freshwater Research*, *47*(7), 869-876.

Fry, G. C., & Milton, D. A. (2009). Age, growth and mortality estimates for populations of red snappers *Lutjanus erythropterus* and *L. malabaricus* from northern Australia and eastern Indonesia. *Fisheries Science*, 75(5), 1219-1229.

Lloyd, J. and Puig, P. (2009). The utilisation of GIS spatial statistical methods to assist in the development of ecosystem-based fishery management strategies, using the Northern Territory Demersal and Timor Reef Fisheries as case studies. Final Report Fisheries Research and Development Corporation project 2005/047. Darwin, Northern Territory Department of Regional Development, Primary Industry, Fisheries and Resources. Fishery Report No 99.

Moore, B., Stapley, J., Allsop, Q., Newman, S., Ballagh, A., Welch, D. and Lester R. (2011). Stock structure of blue threadfin *Eleutheronema tetradactylum* across northern Australia, as indicated by parasites. *Journal of Fish Biology*, 78:923.

Newman, S. J., Steckis, R. A., Edmonds, J. S., & Lloyd, J. (2000). Stock structure of the goldband snapper *Pristipomoides multidens* (Pisces: Lutjanidae) from the waters of northern and western Australia by stable isotope ratio analysis of sagittal otolith carbonate. *Marine Ecology Progress Series*, 198, 239-247.

Paton, C., Hellstrom, J., Paul, B., Woodhead, J. and Hergt, J. (2011). Lolite: Freeware for the visualisation and processing of mass spectrometric data. *Journal of Analytical Atomic Spectrometry*, 26: 12, pp. 2508-18.

Poulin, R. and Kamiya, T. (2015). Parasites as biological tags of fish stocks: a meta-analysis of their discriminatory power. *Parasitology* 142:145-155.

Quinn, G. P. and Keough, M. J. (2002). Experimental design and data analysis for biologists. Cambridge University Press.

R. Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Salini, J. P., Ovenden, J. R., Street, R., & Pendrey, R. (2006). Genetic population structure of red snappers (Lutjanus malabaricus Bloch & Schneider, 1801 and Lutjanus erythropterus Bloch, 1790) in central and eastern Indonesia and northern Australia. *Journal of Fish Biology*, *68*(SB), 217-234.

Saunders, T., Barton, D., Crook, D., Ovenden, J., Newman, S.J., Saunders, R., Taillebois, L., Taylor, J., Travers, M.J., Dudgeon, C., Maher, S. and Welch, D.J. (2016), *Optimising the management of tropical reef fish through the development of Indigenous scientific capability*. Darwin, Northern Territory, Fishery Report No. 117.

Scheiner, S. M. (1993) MANOVA: Multiple response variables and multispecies interactions, p. 94–112. *In*: S. M. Scheiner and J. Gurevitch (Eds.): Design and analysis of ecological experiments. Chapman and Hall.

Stephenson, P. C., Edmons, J. S., Moran, M. J., & Caputi, N. (2001). Analysis of stable isotope ratios to investigate stock structure of red emperor and Rankin cod in northern Western Australia. *Journal of Fish Biology*, *58*(1), 126-144.

van Herwerden, L., Aspden, W. J., Newman, S. J., Pegg, G. G., Briskey, L., & Sinclair, W. (2009). A comparison of the population genetics of *Lethrinus miniatus* and *Lutjanus sebae* from the east and west coasts of Australia: evidence for panmixia and isolation. *Fisheries Research*, *100*(2), 148-155.

Welch, D. J., Newman, S. J., Buckworth, R. C., Ovenden, J. R., Broderick, D., Lester, R. J. G., Gribble, N. A., Ballagh, A. C., Charters, R. A., Stapley, J., Street, R., Garrett, R. N. and Begg, G. A. (2015). Integrating different approaches in the definition of biological stocks: A northern Australian multi-jursidictional fisheries example using grey mackerel, *Scomberomorus semifasciatus*. *Marine Policy*, 55:73-80.

White, J. and Ruttenberg, B. I. (2007). Discriminant function analysis in marine ecology: some oversights and their solutions, *Marine Ecology Progress Series*, 329:301-5.

Woodhead, J.D., Hellstrom, J., Hergt, J.M., Greig, A. and Maas, R., 2007. Isotopic and elemental imaging of geological materials by laser ablation inductively coupled plasma-mass spectrometry. *Geostandards and Geoanalytical Research*, 31(4), pp.331-343.