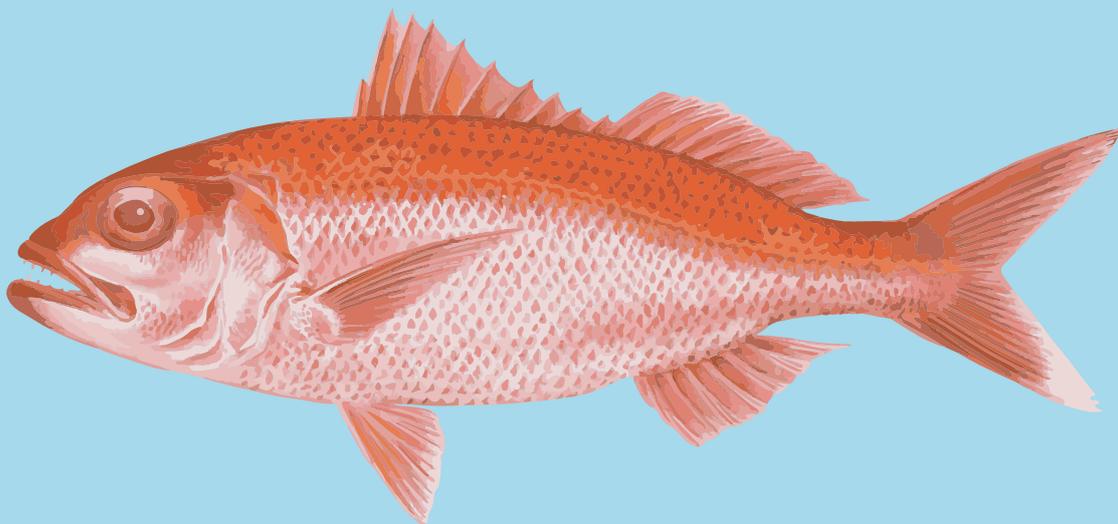




The genetic distribution of three deepwater snappers in the Western Central Pacific Ocean



Introduction

Effective management of fisheries requires information on the stock structure of exploited species. Knowledge of the connectivity and gene-flow of exploited species among regions allows managers to delineate stock boundaries and identify whether stocks are shared with other jurisdictions. There is currently very limited information on the stock structure of deepwater snappers in the Pacific Ocean.

Gomez et al. (2015) suggested that the potential for expansion of existing deepwater snapper fisheries may be limited for many South Pacific countries due to the relatively small area of habitat predicted to be suitable for the major fishery species. The vulnerability of a fishery that relies on limited habitat is high, and there is potential for increased reduction in the gene-pool if mixing of fish among these limited habitats is low. It is, therefore, essential that we understand the diversity and distribution of the gene-pool and the potential mixing that is occurring among fished locations.

Numerous studies now use genetic tools to determine fishery stock units and to assess the level of gene-flow throughout a fishery. Unfortunately, many of these studies are restricted to local areas, or use very small sample sizes from a wide range of local areas, both of which have the potential to under-sample the true genetic variability and to suggest extensive recent mixing when historical mixing of the stock may instead be causing the observed patterns. There is a need to expand on these preliminary studies to examine the stock structure of deepwater snappers.

In this study, we used a large number of samples collected across the Western Central Pacific Ocean. This strategy ensured that samples were representative of the local regions that comprised the total sample. We examined the genetic diversity for three deepwater snapper species that are most important to Pacific Island countries and that are currently being re-assigned as different species: flame tail snapper (*Etelis coruscans*), ruby snapper (*E. sp.*) and the pygmy ruby snapper (*E. carbunculus*). The ruby snapper was previously known as *Etelis carbunculus*, but it is now known to be a different species and is currently being assigned a species name. It is referred to here as an unassigned *Etelis* species (*Etelis sp.*)

Methods



We extracted DNA from 2150 samples (*Etelis coruscans* (1249), *Etelis sp.* (494) and *Etelis carbunculus* (217)) (Table 1). Each sample consisted of approximately 5 mm² of fin or muscle tissue collected during fisher-dependant and fisher-independent trips throughout the Western Central Pacific region. Samples were either frozen or preserved in 70% ethanol depending on on-board or at-port facilities. We extracted DNA from the tissue using Extract-N-Amp™ Tissue PCR kit. We then sequenced a single gene (cytochrome b) from the mitochondrial DNA to investigate the genetic diversity among regions. The quality of the product was tested using standard procedures.

We identified differences among the sequences (variants, also known as haplotypes), and mapped the distribution of the variation through the Western Central Pacific Ocean. In addition, we investigated the relationship between the variants using the number of changes between them and graphed these as a network of changes to show how related the different regions were.

Table 1. Locations where genetic samples were collected from *Etelis coruscans* (1), *Etelis sp.* (2), and *Etelis carbunculus* (3).

Region	Fished location	Species collected
Fiji	Colwyn Ridge	1,2
	Conway Reef	1,3
	Lau Ridg	1,3
	Moore Ridge	1,3
International waters	Monowai Seamount	1,3
New Caledonia	Loyalty Islands	1,2,3
	Hienghene	1,2,3
	Thio	1,2,3
	Touho	1,2,3
Papua New Guinea	Kavieng	1,2,3
Samoa	Field Bank	1,2,3
Tonga	Rochambeau Bank	1,3
	Zephyr Reef	1,3
	Zephyr Reef Seamount	1
	Tonga	1,2,3
Vanuatu	Efate	1,2,3
	Santo	1,2,3
Wallis & Futuna	Arabis Seamount	1,2,3
	Combe Bank	1,2,3
	Foss Bank	2
	Rotuma Shoal	1,2,3
	Siafiafi Bank	1,3
	Lalla Rookh Bank	3

Results

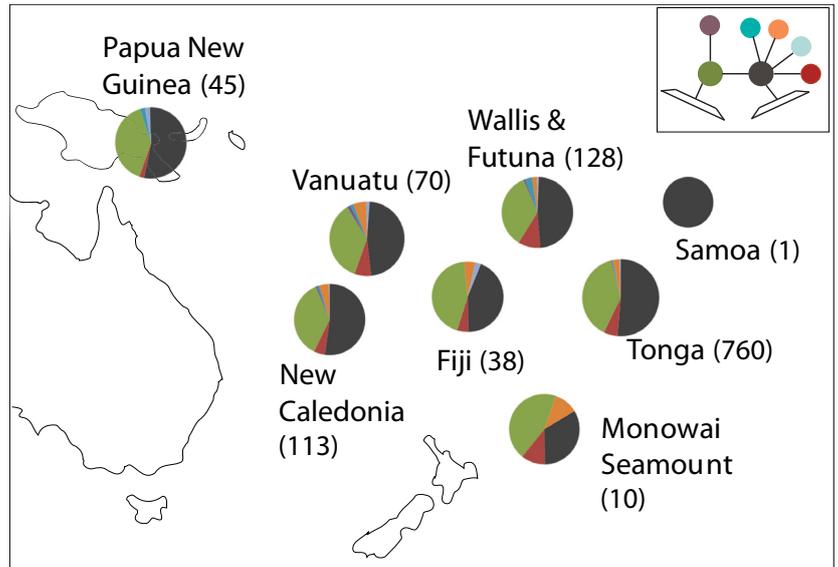
We successfully extracted DNA and sequenced 1670 samples: 1165 samples of *E. coruscans*, 332 samples of *E. sp.*, and 173 samples of *E. carbunculus*. We also identified 209 variations (genetic diversity) in the samples, which were shared across the regions: 134 variations (*E. coruscans*), 53 (*E. sp.*), and 22 (*E. carbunculus*).

The number of variations for each species was similar when you consider the sample sizes used in the study. Similarly, the number of single changes in the sequences creating the variations was low at 1–7 changes (*E. coruscans*), 1–12 changes (*E. sp.*), and 1–5 changes (*E. carbunculus*). This low number of changes was reflected in networks that have very few connections among the variations. The most complex network and the highest change were observed for *E. sp.*

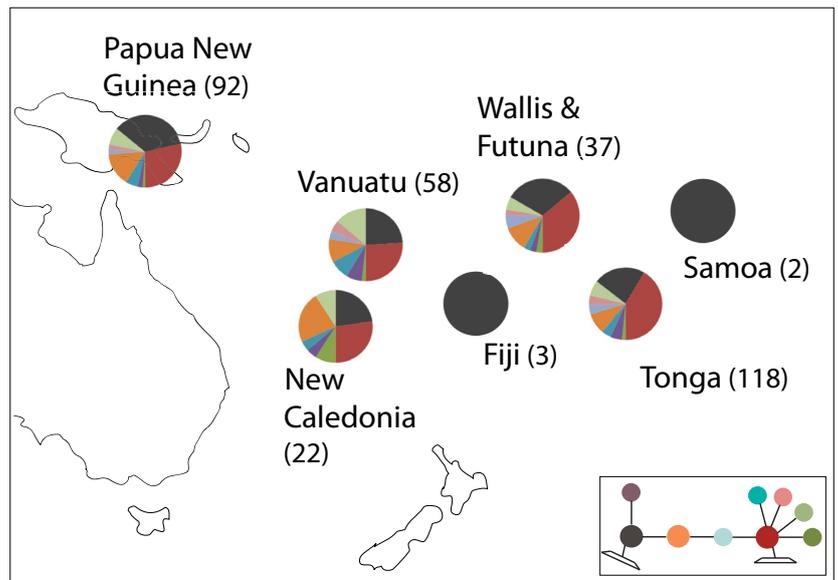
There were no significant differences in the genetic diversity among the regions for any of the species. This means that all regions shared the genetic variation and they cannot be easily distinguished from each other. However, the network suggests that there are closely related groups of variation that occur in many of the regions, but not all, and these warrant further investigation at more local scales (Figure 1a-c inset). The genetic diversity shared among the regions was mostly restricted to one or two core variations that were widespread and occurred in high frequency in all regions. *Etelis coruscans* and *E. sp.* both had more complex networks coming from these cores (Figure 1a-c inset). The distribution of the genetic variation did not show an obvious pattern of isolation for any of the regions, creating a mosaic of genetic variation that varies in the number and frequency of occurrences among regions (Fig. 1a-c). A unique variation was found in Samoa for *E. sp.* and in Papua New Guinea for *E. carbunculus*. These are interesting as they were present in regions where the sample sizes are very small – 3 and 7 respectively – suggesting they may be in high frequency in each of these regions. In comparison, the occurrence of many variations in Tonga was found within a sample size of 760 individuals (*E. coruscans*) representing single individuals.

Figure 1. Distribution of genetic variation for *Etelis coruscans* (a), *Etelis sp.* (b) and *Etelis carbunculus* (c) across the Central West Pacific Ocean, with the sample size (n) displayed beside the region name. Each piece of pie in the pie charts represents one genetic variation and the frequency of the variation in each of the sampled regions. Inset for each species is a network displaying the relationship among genetic variations. Related groups of variation are displayed in the networks to reduce the complexity of the networks. Each coloured circle matches the colour in the pie charts on the maps and each line between groups represents one change between them. The bars represent the numerous singleton variations, all of which are one change different from the core variations.

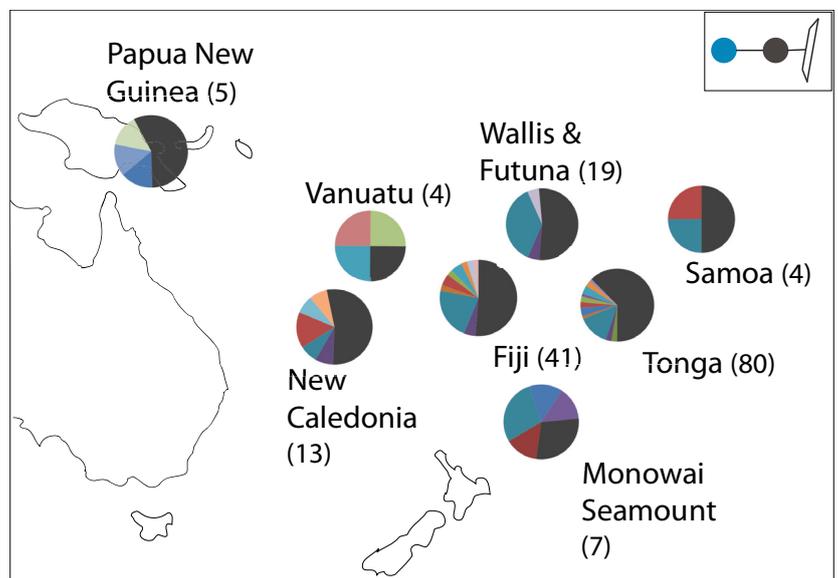
(a) *E. coruscans*



(b) *E. sp.*



(c) *E. carbunculus*



Conclusions

The widespread genetic diversity for all three species suggests widespread mixing and connectivity. However, these results also suggest local patchiness that requires further investigation with additional analyses. A previous study on another deepwater Lutjanid, the crimson jobfish *Pristipomoides filamentosus*, also revealed little to no genetic structuring for this section of DNA across the Indo-Pacific, except when Hawai'i was included (Gaither 2011), and a similar number of changes was observed in that species as observed in our study for the eteline species. Similarly, two recent localised studies on the genetic diversity of *E. coruscans*, *E. sp.* and *E. carbunculus* in New Caledonia (Loeun et al. 2014) and Hawai'i (Andrews et al. 2014) both produced genetic diversity results consistent with those obtained here, although the study in New Caledonia was based on a different section of the DNA (mtDNA control region).

The striking similarity in the networks for *E. coruscans* and *E. carbunculus* from the Western Central Pacific samples and from Hawai'i suggests that unlike *P. filamentosus*, the gene-pool may be more extensively mixed than captured by each

study, and integrating these results should provide deeper understanding of the whole region. Furthermore, to fully appreciate the role of local habitat and fishing pressure on the gene-pool, additional partitioning and more advanced statistical enquiry for the distribution of genetic diversity within each region is an essential next step.

There is likely to be a need to collaborate with other countries to optimise management of these fisheries. Managers should take a precautionary approach to management at the local level and should be aware that the stocks they are fishing likely overlap with other jurisdictions across the Western Central Pacific. If the genetic diversity is being maintained from many different areas, it is essential to keep the full level of diversity by ensuring sustainability of the mixing. Mixing is more likely to occur in large populations where local stocks are not depleted and isolated from other areas. The genetic diversity identified for all three species in this study was low and it is, therefore, important to maintain this level to ensure the species are less vulnerable to change in the future.

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