Is “sustainable” seafood labelled accurately?

*An assessment of fresh and frozen seafood products in SE Queensland using DNA barcoding*

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**Summary**

A major threat to marine biodiversity is the commercial harvesting of wild species. An important aspect of making the seafood industry more sustainable is to improve product transparency with accurate species labels throughout the supply chain. Unfortunately, the seafood industry has weak regulations and poor product traceability. These complications lead to seafood fraud, which is misleading information about a seafood product, occurring at an average of 30% globally with significant impacts on our society and environment.

With a DNA-based methodology, my study aims to answer the question: are sustainable fresh or frozen seafood products available in South East Queensland accurately labelled? Species will be identified by sequencing DNA extracted from seafood products sold in SEQ. Thus, I expect to detect which species, if any, are suffering seafood fraud. With just two studies done in Australia in very specific regions, more evidence is urgently needed to adequately quantify seafood fraud in other highly populated areas like SE Queensland. The outcomes of my work will support conservation efforts against endangered species, assess better fish stocks and support labelling regulations. Finally, this study will provide a baseline with more evidence for further research in Australia to reduce significantly reduce seafood fraud impacts.

1. **Background**
   1. **Sustainable seafood**

Annually, people consume approximately more than 20 kg of seafood worldwide, being one of the most traded products globally (Béné et al. 2015). Over time, consumers wanted to know more about the identity of the products and often they are willing to pay more for those that have sustainability or health certifications on their labels (Kelly et al. 2005). That consumer desire indirectly encourages producers to invest in these certification schemes (Menozzi et al. 2020).

For instance, The Marine Stewardship Council (MSC) is an internationally recognised non-profit organisation that provides a label certification to sustainably managed wild-caught seafood to raise consumer confidence and diminish seafood fraud. Specifically in Australia, 20 fisheries are certified by MSC (Council 2014, 2021). Also, the Australian Marine Conservation Society (AMCS) created the “Sustainable Seafood Guide” as an online tool to encourage Australians to choose sustainable products and avoid those with high ecological negative impacts (AMCS 2019).



Figure 1. Left, the sustainable certification of Marine Stewardship Council (MSC). Right, the logo of the Sustainable Seafood guide by the Australian Marine Conservation Society (AMCS).

Sustainability is a concept where the definition may fluctuate depending on each person’s perspective. Often, authors do not define their view of sustainability and its polysemy might lead to methodological errors (Salas‐Zapata & Ortiz‐Muñoz 2019). A broadly used definition from 1987 by the Brundtland Commission is: “the capacity of present generations to meet their necessities without compromising this same capacity for future generations” (Brundtland & Khalid 1987). This project will adopt Brundtland’s sustainability concept as a general principle. But also I will consider the more specific description of sustainable fisheries done by Hilborn in 2015 where to be considered sustainable, fisheries need to target specific species and fish stocks, reducing bycatch and destructive fishing techniques that will not compromise these resources for coming generations (Hilborn et al. 2015).

On the other hand, a major challenge to manage sustainability in seafood is the lack of traceability of products or activities occurring on the sea. Traceability refers to the capability to follow the materials, parts or processes used in production, that ensures food quality and increases food safety (Leal et al. 2015). Missing or wrong information of a product can be exchanged at every step in the seafood supply chain, from wholesalers or transporters to packers and retailers (Moore et al. 2012). Besides, this traceability is even lower when a product is processed in one country or region and sold in another going through different retailers before the consumer (Food & Nations 2020). This issue leaves a window open for illegal, unreported and unregulated (IUU) products to reach the consumers (Tickler et al. 2018).

* 1. **Seafood fraud**

Seafood fraud takes advantage of low traceability and high numbers of IUU. Fraudulent products are traded under a different identity from their true one including any practice that distorts their identification at any step of the supply chain. (Spencer & Bruno 2019). Seafood fraud can be represented in different forms like different species labelled, aquaculture products labelled as wild-caught and misreporting the origin (Luque & Donlan 2019). Examples of species that are commonly substituted are swordfish by mako shark, Pacific salmon by Atlantic salmon, and red snapper by rockfish (Buck 2007). Nevertheless, mislabelling can be unintentional by an incorrect assessment from a common vernacular name or because of the missing information of the product during the steps of the supply chain (Cohen et al. 2009).

Globally, last advances in technology have enabled access to more accurate techniques on research facilitating the report of seafood fraud. Only in 2015, 51 studies were published against just 4 in 2004 (Luque & Donlan 2019). A literature review of recent 51 peer-reviewed studies determined an average of 30% of seafood fraud worldwide (Pardo et al. 2016). However, the number of these studies are not equally distributed across countries. For instance, the US, Spain and Italy contain more than 10 studies each. Whereas just four have been done in China and none in Japan despite these being high seafood producing countries. Nevertheless, mislabelling rates of seafood products across countries appear to be similar (Luque & Donlan 2019).

Depending on the focal species, the mislabelling rate varies significantly. A study done in Italy regarding shark products found an average of 80% of labelling fraud (Barbuto et al. 2010) as well as other work which found 73% of the total mislabelled samples were from bluefin tuna (*Thunnus thynnus*) (Gordoa et al. 2017). However, a work that looked at a wide range of different fish sold in restaurants in the US reported a seafood fraud rate of 16.3% (Khaksar et al. 2015). Furthermore, depending on the type of establishment sampled, the mislabelling rate appears to be different as (Cawthorn et al. 2012)found 9% of seafood fraud from suppliers and distributors and 31% from direct retailers and restaurants**.**

In Australia, only two studies have been done, one in 2015 and another more recent in 2019. The first aimed to quantify mislabelling seafood in Tasmania. They analysed a total of 38 products of different fishes from fishmongers and seafood markets and none was found to be mislabelled (Lamendin et al. 2015). The second was established as a citizen science project involving high school students which reported 10% mislabelled species with a total sample of 68 from fish sourced from just two retailers in Sydney (Mitchell et al. 2019). Despite the positive results of these two studies, there is still a wide gap of evidence on this topic in Australia that needs to be urgently filled with more complex and systematic studies.

Therefore, this project aims for a broader sampling effort collecting a much larger sample size with a wide range of different samples of fish fillets from different types of establishments such as restaurants, fish shops and seafood markets. Products with different origins and nationalities will be included for a wider analysis as they are also an important part of Australian consumers.

I will assess mislabelled species substitution with a genetic approach that might also provide information on the origin (such as ocean basin). However, the discrimination between farmed and wild-caught will be restricted to the labelled information provided, as it is very difficult to select reliable markers for different populations with this approach. To identify this component in a further investigation, instead of a DNA-based method, near-infrared spectroscopy (NIRS) has been observed to be successful for this judgement (Ottavian et al. 2012).

* 1. **Seafood mislabelling impacts**

One of the major impacts of this problem falls under conservation. Having IUU products and seafood mislabelling, fish stocks cannot be managed accurately on time and so species might enter a detrimental state leading to an irreversible extinction. Also, species that are widely available on the markets might create a false impression that there is a plentiful abundance of that species on the sea (Marko et al. 2004). More importantly, having incorrect information under these products can be used to sell illegally endangered species. Indeed, a study that took place in Brazil, found that 55% of the samples analysed were substituted with largetooth sawfish (*Pristis perotteti*) which is a critically endangered species by the IUCN (Palmeira et al. 2013). Thus, a labelling approach that is clear and appropriate is critical to help endangered species and to allow fisheries regulations to assess fish stocks correctly (Naaum et al. 2016).

Generally, if a product is mislabelled, the substituted species is of lower price and often with lower nutritional benefits than the one that is labelled. For instance, it has been found that some species that can produce health problems for certain consumers like escolar (*Lepidocybium flavobrunneum*) or species living in polluted waters like Nile tilapia and Nile perch (*Oreochromis niloticus* and *Lates niloticus* respectively) have been used as a substituted species (Cawthorn et al. 2012; Hu et al. 2018). Also, in a recent study, 12 different products were substituted by striped catfish (*Pangasius spp*.) which if handled inappropriately can produce food poisoning(Kroetz et al. 2020).

Besides the health human risks, misrepresentation of species might have social impacts. The success of sustainable certification programs such as the MSC relies on correct label information and traceability. So, misinformation on the label can lead to a false certification and hence people might buy the product thinking that is certainly a sustainably produced fish supporting unintentionally this issue (Willette et al. 2017).

Species with high mislabelling rates should be prioritised in research as they are the most prone to be highly affected by this issue (Luque & Donlan 2019). Notwithstanding, focusing only on species with high mislabelling rates might provide impractical or insufficient results for management. Thus, species of high consumption rates also should be considered (Kroetz et al. 2020). However, the mentioned gaps in evidence and traceability make harder the efforts to describe and quantify accurately seafood mislabelling impacts (Hofherr et al. 2016). Furthermore, most of the studies have insufficient data to obtain significant results or the sampling effort is too low to have strong power to provide a realistic representation of this issue (Luque & Donlan 2019).

* 1. **Seafood labelling regulations in Australia**

Australia shares with New Zealand the Food Standards Code which determines the principles of food regulations and their correct approach when labelling products. These principles are different depending on the type of product, packaging and establishment sold. The code aims to ensure food safety and provide useful information on the label for consumers. For instance, it is mandatory for retailers who sell non-cooked products to show where the seafood comes from. However, this standard is not compulsory for cooked products sold in service food venues like restaurants. (Australian Government 2021a).

Besides, there is the added problem of species with multiple common sold names. Different establishments may use different names to refer to the same species complicating a standardisation for labelling (Luque & Donlan 2019). In 2007 the Australian Fish Names Standard (AFNS) was founded as an alternative guide to standardise the labelling of seafood products and progressively more retailers will make use of it (Blakeney 2016).

This study will focus on raw or frozen products as they will have labelled their species, origin and production method. Besides, without being cooked or processed, the products will conserve DNA of good quality to enable identification. I will use AFNS to standardise and record the names of each seafood product collected

Other regulatory bodies that are relevant is the Department of Agriculture, Water and Environment. The department reviews environmental impacts such as bycatch rates, assess fish stocks on Commonwealth Waters and issues with marine protected species. It collaborates closely with another organisation, the Australian Fisheries Management Authority (AFMA), to ensure that environmentally sustainable principles are being applied (Australian Government 2021b). So, a proper and strict labelling system is key to support these regulatory frameworks and assess efficiently fisheries and their products.

* 1. **Seafood identification using DNA-based methods**

Morphology was one of the first approaches used in species identification but it often requires expert training and with processed food products, this method is likely to be impractical. Nowadays, DNA-based approaches are the most useful and broadly applied in species identification as they are more affordable, accurate and openly accessible (Naaum & Hanner 2016).

In 2003, a DNA-based methodology was proposed to be the core of the taxonomic systems as a single gene sequence is enough to distinguish most of the species (Tautz et al. 2003). The gene cytochrome oxidase subunit I (COI or cox1) was suggested to be used in worldwide bio identification schemes where each species will be linked to a “barcode” corresponding to their sequence for this gene creating the DNA barcoding concept (Hebert et al. 2003).

DNA barcoding relies upon sequences between close taxonomic species being more similar than those among distant taxonomic individuals. As an example, inside the genus *Thunnus* (tunas), a study reported an average of K2P distance, a measure of genetic sequence differentiation, of just 0.11% among 5 different tuna species (Ward et al. 2005). Also, this method needs a database with references to screen or match the sequence found such as the Barcode of Life Database (Ratnasingham & Hebert 2007; Hellberg & Morrissey 2011). But overall, barcoding is highly accurate, quick and affordable. Thus, DNA barcoding could be a vital approach to answer the vital question of what, how much and where seafood fraud is happening in Australia.

* 1. **Objectives and aims**

With only two previous DNA based studies done in Australia of seafood identification, more robust evidence is needed to appropriately quantify this issue to support endangered species, fisheries regulations and consumer’s decisions. Therefore, this project aims to:

1. Analyse and sequence different fresh and frozen sustainable seafood products of fishes and invertebrates from South East Queensland (SEQ). I will collect products considered sustainable by the good fish guide (AMCS 2019). With a DNA-based barcoding approach, I will collect products considered sustainable by the good fish guide (AMCS 2019) to identify the real species and country of origin if possible. Hence, I would be able to quantify how many and which sustainable species are accurately labelled or not in SEQ.
2. Following the same methodology, I will collect additional samples categorised as less (eat less) or non-sustainable (say no) by the good fish guide (AMCS 2019) do a comparative analysis among different sustainable categories. Effects of seafood fraud might also affect non-sustainable products so they need to be considered.
3. I will compare observed outcomes in mislabelled species against global published studies to find similar trends and results. Hence, I can give suggestions for future conservation management and fisheries regulations to reduce this problem.
4. Finally, I will quantify the accuracy of the DNA barcoding approach as a tool for species identification on different fishes sold in SEQ so further research can adopt the same approach or use the results as a baseline for a new study.
5. **Research Plan and Methods**
   1. **Collecting Samples**

Firstly, a wide range of diverse fresh raw or frozen products will be collected from different types of establishments such as restaurants and seafood markets. The location for sampling will be SEQ. Specifically between the areas of Brisbane and Gold Coast with their surrounding suburbs. Special sampling efforts will be focused on raw fish fillets as the primers used in PCR are designed especially for fish (Ward et al. 2005) and is how seafood fraud can be hidden easier. However, I will collect some samples from other groups of animals such as cephalopods or molluscs to test the success of this approach as these are also common products sold in SEQ.

Samples will be collected from all the different sustainable categories by the AMCS on the good fish guide. However, a stronger sampling effort will be employed for those products considered sustainable (named “better choice” in the guide) (AMCS 2019).

The collection will consist of asking the establishment to collaborate voluntarily or otherwise buy anonymously the product. Preferably a small part of the muscle tissue will be collected. From each sample, I will collect its origin, species labelled, production method, type of establishment sold and any sustainability certification it might have.

Due to the timeframe limitation, there is a trade-off between collect a high number of individuals from a few seafood products or a few individuals from a high number of seafood products. For this study, I have considered that is more significant to assess a higher number of different products with a few individuals of each. Thus, leaving scope for further research to focus on the products or species with high mislabelling rates, if any, collecting a high number of individuals of each for stronger statistical power.

I expect to collect around 200 samples from at least 40 different products within the SEQ in total. From those, I predict that around 100 would be classified as sustainable by the AMCS. Multiple samples of each product from different sources will be collected when possible to add statistical power as suggested in a similar study (Ward et al. 2005). When collected, to preserve their DNA properties, samples will be stored in a fridge or freezer with ethanol 100% until processed at the laboratory as other studies have done (Hajibabaei et al. 2005; Rodriguez-Ezpeleta et al. 2013).

The information collected from the sample collection will look something like the table below:

Table 1. Representation of the data collected from each sample

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Unique Identifier (UID) | Type of establishment | Form | Reported catch method | REPORTED Origin (if known) | Australian State (if known) | Labelled species | Sustainability certification by AMCS |
| 1 | Seafood Market | Fresh | Wild-caught | Australia | SA | Whiting | Better choice (sustainable) |
| 2 | Restaurant | Frozen | Farmed | Vietnam | N/A | King Prawn | N/A |
| 3 | Fish and Chips | Fresh | Wild-caught | Australia | QLD | Snapper | Eat less (less sustainable) |

An Honours student, Tia Vella, has a closely related project that is analysing the accessibility of sustainable seafood in SEQ (Vella 2021) and she has extensively surveyed establishments in SEQ but has not undertaken any DNA based work. Part of the data of this project will be used as support when choosing the product or establishment and to discuss future results.

In this study, samples collected are from dead animals not killed for scientific purposes. The UQ ethics committee has assessed the project and we received an exemption from animal ethics. Also, samples will be collected under the establishment consent or directly bought and the names of establishments will not be published under any conditions to avoid conflicts of interest.

* 1. **Processing samples**

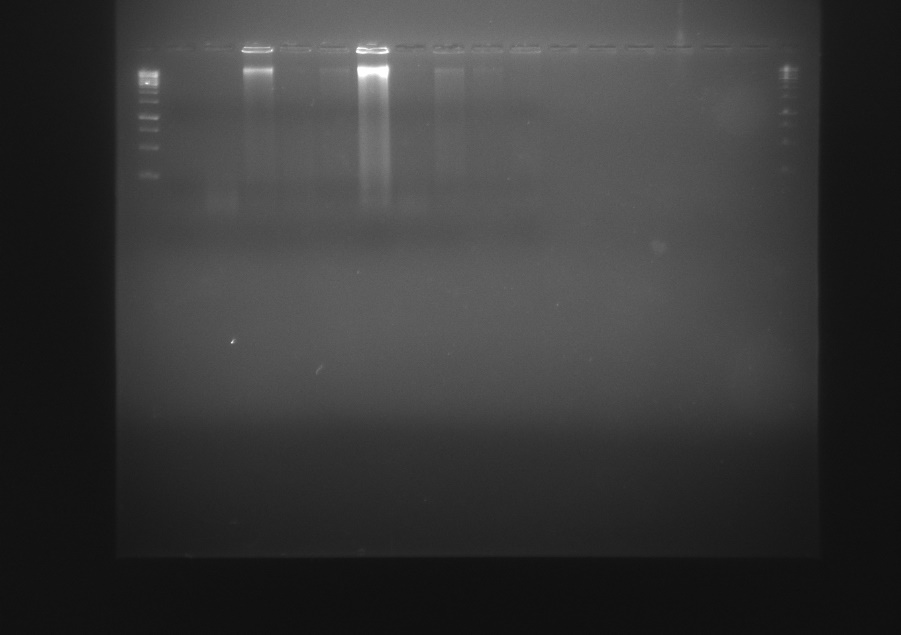
Once at the laboratory, a small piece of tissue will be used for DNA extraction with the salting-out method as is quick, safe and affordable for a large number of samples (MWer et al. 1988) followed by DNA visualisation via gel electrophoresis used as quality control. Then, for the DNA barcode, PCR will be needed. Here, I will amplify a segment of 655 bp of the gene COI from mitochondrial DNA as is a popular marker used in barcoding species as it is well conserved through evolution and able to discriminate between species (Yearsley et al. 1999; Hellberg & Morrissey 2011). We will use the commercial primers FishF1, FishR1, FishF2 and FishR2 designed by (Ward et al. 2005) that have been widely used before in seafood identification studies (Ivanova et al. 2007; Naaum & Hanner 2016).

Figure 2. Image of the bands in a gel electrophoresis resulting from DNA extracted following Salting-out method on fish tissue by Sergio Garcia.

Finally, we will perform another gel electrophoresis to determine if the PCR was successful and store the produced sampled in the fridge until submitted for sequencing. Groups of 96 samples (including 2 controls) will be sent to sequence the specific marker gene COI to Macrogen Company in South Korea. This methodology will be strictly followed and compared with a well-cited similar study which can be consulted for further details (Ward et al. 2005)

* 1. **Expected analysis**

Once obtained the results, the observed sequence can be compared with known species in databases. One that has been widely used and contains most Australian fish species is the Barcode of Life Data System (BOLD) (Ratnasingham & Hebert 2007). The biostatistical software Geneious will enable me to visualise and manually adjust the electropherograms representing DNA sequences (Kearse et al. 2012). Subsequently, I will undertake a blast local alignment search tool (BLAST) against the BOLD database to discriminate potentially related sequences (species) based on their value of similarity. I expect to have a clear resolution to discriminate between most of the species and so the more similar are the sequences the more related are taxonomically the species.

* 1. **Expected outcomes, implications and limitations**

This study will represent the first attempt to quantify how much, if any, seafood fraud of sustainable products occurs in SEQ enabling comparison across other categories, regions, countries or food industries. I expect to have positive effects on conservation efforts protecting endangered species and marine habitats since the outcomes will improve the knowledge and awareness for mislabelled species. Then, stakeholders, decision-makers and producers can reduce the environmental impacts of the industry. Also, human health can be improved from the outcomes as consumers can do more informed decisions to avoid those products that might contain undesired substances or pollutants.

On the other hand, depending on the species evolution and distribution, might not be possible to discriminate at species resolution just with COI marker since biogeography and geographical distance will cause genetic differentiation among populations (Riginos et al. 2011; Hellberg et al. 2016). To support this limitation, biogeography data like ocean basins will be combined for those products that COI could not completely identify. For instance, for tuna species, biogeography publications might be combined with barcoding as happened in another project before (Viñas de Puig & Tudela 2009).

Finally, the outcomes of this study will be used to suggest where to put further research efforts to significantly reduce seafood fraud impacts. For instance, future studies can assess a specific type of products or species, a particular type of establishment, collect a higher number of individuals to add power to the analysis or try a different methodology. Also, this study is focused on the ecological and environmental aspects of seafood fraud from the retailing point. But it would be interesting to assess at what step of the supply chain the mislabelling has happened and evaluate legally and morally who is responsible for it and the penalties involved.

* 1. **Expected timeline**



Table . Representation of the timeline expected of the project. In orange is when the activity will occur.

* 1. **Acknowledgements**

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