

# Eating Eels to Extinction A Review of Restaurant Consumption in Hong Kong

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Suggested Citation: Chan J. 2021. *Eating Eels to Extinction: A Review of Restaurant Consumption in Hong Kong*. WWF-Hong Kong, Hong Kong SAR.

### **Executive Summary**

Eels are among the most threatened group of vertebrates on the planet. Six freshwater eel species are considered threatened and most have declined rapidly since the 1970s. Eel consumption in East Asia, which includes southern China and Hong Kong, drives much of the illegal trade in endangered eels. To implement effective conservation and recovery actions for threatened species, it is important to know which species of eel are commonly sold in restaurants across Hong Kong. Through DNA analysis on eel products from 80 licensed sushi restaurants, nearly 90% of samples were identified as critically endangered or endangered eel species. Nearly half (36) of 80 samples were identified as European eel (Anguilla anguilla), which is critically endangered and requires Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permits for international trade. However, ongoing and numerous eel seizures at airports and borders indicate that Hong Kong is a major trafficking hub of eels from Europe and North Africa to Asia. High demand for eel and loose labelling regulations and monitoring systems provide opportunities for smugglers to launder illegally harvested eel into the supply chain. This study highlights the prevalence of A. anquilla in restaurants and indicates that potentially illegal species are a common feature in Hong Kong's eel supply chains. Businesses must stop selling endangered eels and should take on the responsibility of monitoring and ensuring the legality and traceability of eels in their supply chains. The Hong Kong government must enhance commodity coding systems to collect accurate figures on the eel trade. Eel smuggling is large-scale, run by organized entities, including crime syndicates. For this reason, the Hong Kong government should include wildlife crime under the Organized and Serious Crime Ordinance to empower officials with stronger investigative powers to prosecute transnational criminal groups trafficking eels to or through Hong Kong.

Eels are disappearing around the world. Intensive eel fishing combined with rapidly growing demand, changing climates, and the damming and pollution of freshwater streams they reproduce in are driving many species towards extinction. This study spotlights Hong Kong's major role in the global eel trade with the aim of providing practical actions that the seafood sector can take to stop serving endangered eels, avoid risks to their business, and improve the traceability of eels in their supply chains. We provide evidence for the widespread,

potentially illegal <sup>1</sup> use of endangered eels in the Hong Kong catering sector, including European eel (*Anguilla anguilla*), American eel (*Anguilla rostrata*), and Japanese eel (*Anguilla japonica*).

Of the 16 species of freshwater eels in the genus *Anguilla*, one is Critically Endangered (the European eel) and another 6 are Threatened, 4 Near Threatened, 4 Data Deficient, and only 2 considered of Least Concern¹ according to the International Union for Conservation of Nature Red List of Threatened Species (IUCN). Globally, the most common traded eel species have seen an average decline of 92% between 1970 and 2016². More than 60% of these eel populations are considered threatened². Some eel species have been given formal protection, including the Critically Endangered European eel which now features on the list of protected species in Appendix II of CITES. American eel and Japanese eel are classified as Endangered by the IUCN. Eels need real conservation action now if they are to survive.

Different life stages of most *Anguilla* species, such as eel fry and mature eels, are harvested and traded globally for consumption<sup>2</sup>. Hong Kong is a major consumer and global trading hub for eels<sup>2</sup>, including some critically endangered and endangered species<sup>3</sup>. Responding to strong demand for eel in East Asia, glass eels and juvenile eels (known as elvers) are caught, many in the Atlantic Ocean, and commonly transported through Hong Kong to aquacultures in Asia to be raised to maturity<sup>4</sup>. Some glass eels are consumed live in restaurants as well.

Globally, more than 90% of all *Anguilla* commercial production is from aquaculture<sup>5</sup>. Japanese eel farming operations have occurred in East Asia since the 1970s. Due to the dramatic decrease of wild catches from 1990s<sup>5</sup>, those eel farms have shifted to using European eel glass eels to stock their farms. While there has been some recent success in eel breeding, whether

i Algeria: prohibition of trade in glass eels and elvers, 30 cm minimum catch size. Morocco: annual quotas of glass eel and >30 cm eel export, restrictions on fishing season & gear. Tunisia: minimum market size of 30 cm, restrictions on fishing gear, and quotas. These 3 countries have significant trade in European eel (CITES AC30 Doc. 12.2)

ii Kindai University has artificially incubated endangered 30 eels in 2019: "Kindai's research into the artificial production of fry has only just begun. Since 2019, we've been working towards full-cycle aquaculture but have only succeeded in egg collection, hatching and the early rearing stage of larvae. Broodstock maturation, egg collection, physical properties of larvae feed, feed ingredients, palatability, and the rearing of large numbers of fry all remain difficult. There are still big hurdles to overcome". High production cost, the technology is still a big issue for mass production.

farmed glass eels can be produced on a commercial scale<sup>5</sup> remains uncertain as the majority of glass eel continue to be wild<sup>ii</sup> caught.

# Background

Seafood is a popular and healthy food choice for many Hong Kong people. With an average of 71.8 kg seafood consumed per year, Hong Kong ranks second in Asia in consumption and is the world's seventh- largest seafood consumer<sup>6</sup>. Most of our seafood is imported, so our seafood choices have a large and enduring influence on the fisheries of source countries and seas. However, most of us purchase seafood that does not provide sufficient and accurate information about the products to inform consumers about sustainability. Seafood mislabeling in Hong Kong leads to food safety alerts, violates the Trade Descriptions Ordinance of Hong Kong, and is used to abet overcharging.

Information about species, country of origin and production methods is crucial for consumers wishing to check the sustainability of the seafood they purchase. WWF-Hong Kong has urged supermarkets to list sufficient and accurate product information on their product labels. In order to provide evidence for the need to adjust labelling practices, WWF Hong Kong performed DNA analyses of prepacked seafood sold in Hong Kong's supermarket groups in 2016<sup>3</sup> and 2018<sup>4</sup>., We found that most of the samples collected had mislabelling issues. The results were presented to supermarkets selling mislabelled seafood products and several supermarkets have made some improvements in labelling based on this data. In 2018 academics from University of Hong Kong, Division of Ecology and Biodiversity carried out another DNA analysis specifically focusing on eel products. Mislabelling was found to be prevalent, similar to our prior work on seafood labelling. The mislabelled packaging of eel products facilitates illegal eel species being sold in supermarkets. This University of Hong Kong study identified nearly 50% of retail eel products in two major supermarket groups as European eel <sup>7</sup>. European eel is listed on Appendix II of the Convention on International Trade in Endangered Species (CITES), requiring export permits and inspection upon arrival in Hong Kong, under the Protection of Endangered Species of Animals and Plants Ordinance. These

<sup>3</sup> Major Hong Kong Supermarket Groups Fail to Provide Sufficient and Accurate Information on Seafood Products Consumers are being misled and overcharged for mislabelled seafood

<sup>&</sup>lt;sup>4</sup> New study finds continued seafood product mislabelling at local supermarkets

results are alarming and show that nearly half of all eel sales could be illegal. To get an overview picture of the eel species sold in Hong Kong, WWF-Hong Kong has extended the DNA analysis to the catering sector to investigate the percentage of endangered species being consumed in Hong Kong.

If Hong Kong is to support sustainable fisheries and stated goals of conserving threatened species and accurate product labelling, then the government and eel vendors must take dramatic action to improve transparency in the trade chain and stop selling threatened species.

# Hong Kong's role in the eel trade

The Sustainable Eel Group has described the eel trade as the "greatest wildlife crime on the planet" with an estimated worth of about US\$4 billion annually<sup>6</sup>. According to the report "Runway to Extinction" from C4ADS<sup>7</sup>, European eel trafficking seizures have been dramatically increasing. For example, in Hong Kong the volume of seizures of illegal European eels in 2019 was 25.3 tons, (frozen, no water, one seizure) compared with 206 kg in 2018 (seven seizures, including water) and 116 kg in 2017 (five seizures, including water<sup>8</sup>).

Hong Kong has a major role providing live eel fry<sup>9</sup> for aquaculture in mainland China and other Asian countries. Records from Hong Kong's Agriculture, Fisheries and Conservation Department (AFCD) show 3.4 tons of live eel were legally imported in 2019. Since there are no glass eel fisheries or eel farms in Hong Kong (pers. comm. AFCD and retailers, March 2020), most imported glass eel are re-exported to aquacultures in other Asian countries.

In addition to Hong Kong's role as a global hub for regional glass eel aquaculture, there is considerable local consumer demand. In 2016, WWF-Hong Kong found all nine major supermarket chains in Hong Kong were selling endangered eel products in their outlets<sup>10</sup>. Recently, researchers at the University of Hong Kong found European eels commonly sold in two major supermarket chains across the city<sup>11</sup>. According to the Population Health Survey 2014/15 conducted by the Department of Health, over 80% of persons aged 15 or above reported eating out (including breakfast, lunch and dinner as a whole) at least once a week<sup>12</sup>. And local demand for sushi, of which eel is a popular dish, has grown significantly since 2000<sup>13</sup>.

This report focuses on the local trade in eel products for Hong Kong consumers with a look at the types of eel species available on the market. We will use this information for engaging with eel suppliers to understand their supply chains and discuss ways to improve traceability and sustainability.

#### Method

# Sampling

Eel products, including roasted eel and sushi, were collected from 80 licensed sushi restaurants from 12 May to 22 June 2020. These restaurants were randomly selected from the *List of Licensed Restaurants and Factory Canteens* on the website of the Food and Environmental Hygiene Department<sup>14</sup>. A total of 1,439 restaurants (dated 6 May 2020) are approved to sell sushi. At least 5% of the caterers were visited in order to collect samples. For chain restaurants with numerous branches, samples were collected from one branch randomly selected across Hong Kong depending on product availability. All samples were examined at The University of Hong Kong for DNA analysis by the School of Biological Sciences, DM Baker lab.

#### DNA extraction & polymerase chain reaction

DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen). The CO1 barcoding region was amplified using Fish CO1 barcoding primers. Sequences were submitted to BGI for Sanger sequencing and results were aligned and inspected using Geneious, then BLASTed against the Barcode of Life Database (BOLD) for identification and the species ID was reported. BOLD results are reliable as the accessions are verified and provides a solid species ID rather than a table of hits with similarities.

## **Key Findings**

Among the 80 eel products subjected to the DNA tests, 36 samples were confirmed to be European eel. Twenty-nine samples were identified as American eel, 5 samples, 4 samples and 1 sample were determined to be Japanese eel, Conger eel (*Conger myriaster*), and marbled eel (*Anguilla marmorata*), respectively. Three samples could not be identified in the

DNA analysis, possibly due to contamination from sauces or marinades of those samples. All failed samples were the same dish (eel don) so it is possible that some element in the food preparation impaired DNA extraction. Two samples were possibly tropical shortfin eel (*Anguilla bicolor*), but these could not be confirmed. It is possible that the availability of reference sequences for the tropical shortfin eel family is much lower than the American and European eel. Due to a lack of reference data, some eel species may be difficult to identify.

Nearly 50% of the surveyed eel products were identified as European eel and almost 90% of the samples were determined to be critically endangered or endangered eel species, implying that large numbers of European eel are being imported into<sup>5</sup> and farmed in Asia and, subsequently, consumed in Hong Kong.

# A Way Forward

Conservation Actions Recommended to Reduce the Use of Illegal Eel Species in Hong Kong

WWF has been in discussion with retailers and chefs on the reasons they use endangered eel in their operation to better understand their perspective on the benefits and drawbacks of selling eel. Most know about the conservation status of European and Japanese eel. Nearly all of them assumed the eel are farmed in Southeast Asia, which is not incorrect, but the pervasive use of wild-caught glass eel to stock farms is not well understood. From their perspective, farmed eel is a silver bullet to tackle the drastic drop of the species. However, eel farms are still having major negative impacts on wild eel populations as their glass-eel stock is primarily wild-caught. Retailers are also influenced by the high local demand for eel products and are unlikely willing to lose out financially by stopping sales of eel products. Hence, few are open to bold action to ban uncertified eel products.

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<sup>&</sup>lt;sup>5</sup> European eel can be legally imported from North Africa, e.g. Morocco, Algeria, Tunisia. However, there are national regulations to export European eel in these countries. Morocco has set quotas to export glass eels. Algeria exports adult eels only. Glass eels cannot be captured for commercial use in Tunisia, so the legal export of eels should only be adults. However, the CITES record can only identify live, meat, bodies, and fingerlings. It is difficult to tell if European eel trade records from the CITES trade database are for mature eels and legally sourced from the origins.

This study reconfirmed that Hong Kong's seafood market is flooded with European eel, most likely smuggled from Europe. As such, we recommend a series of actions that can, over time, reduce the demand for and importation of endangered eel species.

# Stop selling endangered species

Three species of eel are being sold as grilled eel products in Hong Kong—the American eel, Japanese eel, and European eel. All three are endangered or critically endangered species on the IUCN Red List of Threatened Species<sup>15, 16, 17</sup>. The International trade of European eel has been regulated under Appendix II of CITES since 2009<sup>18</sup>. To strengthen European eel protection, the European Union has banned exports of European eel since 2011<sup>19</sup>. However, European eel can still be legally imported from North African countries. Despite these protections, this critically endangered species is still widely consumed as delicacy. Eel importers, wholesalers, supermarkets, small seafood vendors and restaurants must commit to stop serving endangered eel species, no matter the source.

# Proposed alternatives to replace endangered eel species

To avoid the depletion of endangered eels, relevant businesses need to explore other non-threatened (species not classified as Critically Endangered, Endangered, or Vulnerable by IUCN) and edible eel species as alternatives to replace threatened ones on menus or shelves. Substitutes can be freshwater eel like marbled eel (*Anguilla marmorata*), Australian longfin eel (*Anguilla reinhardtii*) which is listed as Least Concern. Another option is non-threatened conger eel (*Conger myriaster*) which is similar in shape but differs in texture<sup>20</sup>. In Japan, the Japanese eel is called *unagi*, while the conger is known as *anago*. Due to its high price and a general eel shortage, some Japanese companies are using non-eel species, such as farmed pangasius (*Pangasianodon hypophthalmus*) to imitate the taste of grilled eel<sup>21</sup>.

#### Proper labeling of eel species on product

Information on species, country of origin, and production methods are needed for sellers and consumers to ensure eel products they purchase are not illegally sourced. However, the general practice on food labeling of all prepackaged food under Cap. 132W<sup>22</sup> does not require prepacked eel products to list their scientific name. Most eel products are simply listed as "eel" or "Japanese eel" in Hong Kong. The use of generic local names for eel or the Japanese

unagi by caterers does not inform consumers about the eel species they are purchasing. Under these circumstances, smuggled or illegal harvested eels can be easily laundered into the supply chain. Requiring species-specific names on importation certificates and permits and available at the point of sale, along with the production method, and geographic origin, will enable consumers to make more informed seafood choices.

# Random forensics test on eel products

Species identification can identify fraud and prevent illegal eel products reaching the market. Because the European eel is currently listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), any trade of the species requires official permits to identify traded specimens that were legally caught and traceable through the supply chain. Responsible businesses, caterers, retailers, and suppliers can take proactive measures by conducting random forensics tests on eel products to mitigate chances of selling smuggled European eel. Such tests should be required and monitored by the government.

# Regular monitoring on eel farms

European eel found in Asian aquaculture are all sourced from wild caught glass-eels. If these farms are unable to provide valid CITES documents, chances are that the eels were smuggled from Europe or North Africa<sup>623</sup>. Eel farms can adopt the Environmental DNA (eDNA<sup>24</sup>) testing of eel aquaculture water to ensure there is no European eel DNA in samples. Responsible businesses and government could require such testing in farms where eels are sourced.

#### Collaborations with other offices and stakeholders

In the eel supply chain, Hong Kong is a major re-exporter and end user of eel species. To enhance its transparency, collaboration with other relevant countries or territories is necessary. WWF-Hong Kong plans to collaborate with eel farms, mainly in China, Taiwan, Japan, to improve transparency in the chain of custody of glass eel catch and trade.

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<sup>&</sup>lt;sup>6</sup> European eel is listed as CITES Appendix II. There is no national ban in North Africa nations, but there are regulations on size of eels, fishing gear restrictions, life stage restrictions, and quotas to manage European eel fisheries at national levels. The Wildlife Trade Portal of Traffic records several seizures from Morocco in 2018. Despite regulations, European eel sourced from North Africa are potentially illegal.

# Enhance commodity coding system on eel

The Hong Kong Harmonized System (HKHS) codes used for the eel trade are typically too general and only record products as *Anguilla* species commodities (Table 1). However, those codes do not differentiate between the various *Anguilla* species and their life stages. Thorough, specific, and accurate trade data are crucial to facilitate and implement conservation action and policies for eel species. Apart from CITES-regulated European eels, some Asian countries, for example, the Philippines, Indonesia, Taiwan, have bans on the eel fry trade<sup>25</sup>. The customs codes have a major role to play in gathering this data with regards to eel legality, traceability, and sustainability issues.

Table 1. Customs codes and descriptions of all *Anguilla* commodities (live, fresh, frozen, smoked, and prepared) in Hong Kong's import and export records<sup>26</sup>

HKHS Code (2020)	Goods Description (2020)
03019210	EEL FRY (ANGUILLA SPP.), LIVE
03019290	EELS (ANGUILLA SPP.), OTHER THAN FRY, LIVE
03027400	EELS (ANGUILLA SPP.), FRESH OR CHILLED, EXCLUDING FILLETS AND OTHER FISH MEAT, LIVERS, ROES, MILT, FISH FINS, HEADS, TAILS, MAWS AND OTHER EDIBLE FISH OFFAL
03032600	EELS (ANGUILLA SPP.), FROZEN, EXCLUDING FILLETS AND OTHER FISH MEAT, LIVERS, ROES, MILT, FISH FINS, HEADS, TAILS, MAWS AND OTHER EDIBLE FISH OFFAL
03043900	FILLETS OF CARP (CYPRINUS SPP., CARASSIUS SPP., CTENOPHARYNGODON IDELLUS, HYPOPHTHALMICHTHYS SPP., CIRRHINUS SPP., MYLOPHARYNGODON PICEUS, CATLA CATLA, LABEO SPP., OSTEOCHILUS HASSELTI, LEPTOBARBUS HOEVENI, MEGALOBRAMA SPP.), EELS (ANGUILLA SPP.) AND SNAKEHEADS (CHANNA SPP.), FRESH OR CHILLED
03045100	MEAT OTHER THAN FILLETS, OF TILAPIAS ( <i>OREOCHROMIS</i> SPP.), CATFISH ( <i>PANGASIUS</i> SPP., <i>SILURUS</i> SPP., <i>CLARIAS</i> SPP., <i>ICTALURUS</i> SPP.), CARP ( <i>CYPRINUS</i> SPP., <i>CARASSIUS</i> SPP., <i>CTENOPHARYNGODON IDELLUS</i> , <i>HYPOPHTHALMICHTHYS</i> SPP., <i>CIRRHINUS</i> SPP., <i>MYLOPHARYNGODON PICEUS</i> , <i>CATLA CATLA</i> , <i>LABEO</i> SPP., <i>OSTEOCHILUS HASSELTI</i> , <i>LEPTOBARBUS HOEVENI</i> , <i>MEGALOBRAMA</i> SPP.), EELS ( <i>ANGUILLA</i> SPP.), NILE PERCH ( <i>LATES NILOTICUS</i> ) AND SNAKEHEADS ( <i>CHANNA</i> SPP.), FRESH OR CHILLED

03046900	FILLETS OF CARP (CYPRINUS SPP., CARASSIUS SPP., CTENOPHARYNGODON
	IDELLUS, HYPOPHTHALMICHTHYS SPP., CIRRHINUS SPP.,
	MYLOPHARYNGODON PICEUS, CATLA CATLA, LABEO SPP., OSTEOCHILUS
	HASSELTI, LEPTOBARBUS HOEVENI, MEGALOBRAMA SPP.), EELS (ANGUILLA
	SPP.) AND SNAKEHEADS ( <i>CHANNA</i> SPP.), FROZEN
03049300	MEAT OTHER THAN FILLETS, OF TILAPIAS (OREOCHROMIS SPP.), CATFISH
	(PANGASIUS SPP., SILURUS SPP., CLARIAS SPP., ICTALURUS SPP.), CARP
	(CYPRINUS SPP., CARASSIUS SPP., CTENOPHARYNGODON IDELLUS,
	HYPOPHTHALMICHTHYS SPP., CIRRHINUS SPP., MYLOPHARYNGODON PICEUS,
	CATLA CATLA, LABEO SPP., OSTEOCHILUS HASSELTI, LEPTOBARBUS HOEVENI,
	MEGALOBRAMA SPP.), EELS (ANGUILLA SPP.), NILE PERCH (LATES NILOTICUS)
	AND SNAKEHEADS ( <i>CHANNA</i> SPP.), FROZEN
03053100	FILLETS OF TILAPIAS (OREOCHROMIS SPP.), CATFISH (PANGASIUS SPP.,
	SILURUS SPP., CLARIAS SPP., ICTALURUS SPP.), CARP (CYPRINUS SPP.,
	CARASSIUS SPP., CTENOPHARYNGODON IDELLUS, HYPOPHTHALMICHTHYS
	SPP., CIRRHINUS SPP., MYLOPHARYNGODON PICEUS, CATLA CATLA, LABEO
	SPP., OSTEOCHILUS HASSELTI, LEPTOBARBUS HOEVENI, MEGALOBRAMA SPP.),
	EELS (ANGUILLA SPP.), NILE PERCH (LATES NILOTICUS) AND SNAKEHEADS
02054400	(CHANNA SPP.), DRIED, SALTED OR IN BRINE, BUT NOT SMOKED
03054400	TILAPIAS (OREOCHROMIS SPP.), CATFISH (PANGASIUS SPP., SILURUS SPP.,
	CLARIAS SPP., ICTALURUS SPP.), CARP (CYPRINUS SPP., CARASSIUS SPP.,
	CTENOPHARYNGODON IDELLUS, HYPOPHTHALMICHTHYS SPP., CIRRHINUS
	SPP., MYLOPHARYNGODON PICEUS, CATLA CATLA, LABEO SPP., OSTEOCHILUS HASSELTI, LEPTOBARBUS HOEVENI, MEGALOBRAMA SPP.), EELS (ANGUILLA
	SPP.), NILE PERCH (LATES NILOTICUS) AND SNAKEHEADS (CHANNA SPP.),
	SMOKED, INCLUDING FILLETS, OTHER THAN EDIBLE FISH OFFAL
03056490	CATFISH (PANGASIUS SPP., SILURUS SPP., CLARIAS SPP., ICTALURUS SPP.),
03030490	CARP (CYPRINUS SPP., CARASSIUS SPP., CTENOPHARYNGODON IDELLUS,
	HYPOPHTHALMICHTHYS SPP., CIRRHINUS SPP., MYLOPHARYNGODON PICEUS,
	CATLA CATLA, LABEO SPP., OSTEOCHILUS HASSELTI, LEPTOBARBUS HOEVENI,
	MEGALOBRAMA SPP.), EELS (ANGUILLA SPP.), NILE PERCH (LATES NILOTICUS)
	AND SNAKEHEADS (CHANNA SPP.), IN BRINE OR SALTED BUT NOT DRIED OR
	SMOKED, OTHER THAN EDIBLE FISH OFFAL

# Include Cap. 586 under the Organized and Serious Crime Ordinance

During the 2018-2019 fishing season, European law enforcement seized 5,789 kg of smuggled glass eels with an estimated value of HK\$18,000 per kilo<sup>27</sup>. In early 2020, a seafood salesman with links to organized crime syndicates, smuggled an estimated 5.3 million critically endangered glass-eel (post-larval stage) of European eels to East Asia via the United Kingdom<sup>28</sup>

To effectively eradicate the illegal trade route and networks in endangered eel species, CITES must be strictly implemented to ensure that the relevant regulations are effectively and comprehensively enforced at port and business level. Hong Kong's wildlife crime offences should be included under Schedule 1 of the Organized and Serious Crimes Ordinance (OSCO), which would make them subject to greater fines, deeper levels of investigation, and intelligence-sharing amongst agencies to further deter transnational criminal groups who use Hong Kong to smuggle eel species.

Currently, Cap. 586 ordinance does not provide investigative powers to the wildlife authority (Agriculture, Fisheries, and Conservation Department [AFCD]) to prosecute only the smugglers in eel smuggling cases<sup>29</sup>. While the United Nations Office on Drugs and Crime<sup>30</sup> and Interpol endorse a 'follow the money' approach to target the syndicates behind wildlife smuggling, Hong Kong still focuses on the small smugglers in the supply chain. Unless the Hong Kong government confers investigative power to tackle illegal wildlife trade by uplisting Cap. 586 under OSCO, the organised networks and the kingpins of eel smuggling will continue to drive wild eels to extinction without repercussion.

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#### FORENSIC REPORT

Sample Collection Date: May-June 2020

DNA Study on Eel in Hong Kong Restaurants

# Methodology

Approximately 5mg of fish was excised from each sample provided by WWF and incubated in  $180\mu l$  lysis buffer and  $20\mu l$  proteinase K at  $56^{\circ}$ C for an hour or until dissolved. DNA extraction was performed using the DNeasy Blood and Tissue Kit (Qiagen) following manufacturer's instructions. A negative control was prepared and underwent the identical procedure outlined above.

Target sequence: 655bp fragment of mitochondrial COI barcode region

Primers: FishF1-5' TCA ACC AAC CAC AAA GAC ATT GGC AC -3' (26 bp) FishR1-5' TAG ACT TCT GGG TGG CCA AAG AAT CA -3' (26 bp)

A 655- bp fragment from the region of the mitochondrial COI gene was amplified using the primers FishF1 and FishR1. PCR amplification were performed in 20  $\mu$ l volume containing 6  $\mu$ l ddH2O, 8  $\mu$ l 10x Taq PCR MasterMix (Qiagen), 2  $\mu$ l of each primer (10  $\mu$ M), and 2  $\mu$ l of template DNA. Thermal cycling was performed on a Veriti 96 Well Thermal Cycler at 95°C for 1 min, followed by 35 cycles of 15 seconds at 95°C, 15 seconds at 48°C, and 30 seconds at 72°C, with a final extension at 72°C for 7 min.

PCR products were visualized on a 1% agarose gel. PCR products were sent for sequencing by Beijing Genomics Institute (BGI). Three samples failed to amplify, most likely due to inhibiting factors present in the sauces and preparation of the food that negatively influenced DNA extraction and amplification.

All successfully amplified sequences were aligned with Geneious 10.2.5 and trimmed and edited by eye. Consensus sequences were submitted to the Barcode of Life Database, which contains validated reference sequences for COI barcoding for identification.

Item no.	Name of restaurant	Product		BOLD ID	Groups	Follow- up action
1	<u>井家</u> (Don Ya)	Eel hand roll	JC02	Anguilla anguilla		
2	Jun Sushi	Eel sushi	JC01	Anguilla rostrata		
3	Nakayo Shokudou (Aeon Style)	Eel Don	KZ01	Anguilla rostrata		
4	Genki Sushi	Eel sushi	KZ02	Anguilla anguilla	Maxim's	
5	丼丼屋 Dondonya	Eel Don	MH01	Anguilla anguilla	Maxim's	

42	Ramen Noodle Ltd.	Eel sushi	JC17	Anguilla rostrata		
41	RED LEAF FUSION (no website)	Eel sushi	JC16	Anguilla anguilla		
40	ANA Sushi	Eel sushi	JC15	Anguilla anguilla	Lubuds	
39	GyokYou Sushi	Eel hand roll	JC14	Anguilla anguilla		
38	Sushi express	Eel Don	JC13	Anguilla anguilla	Sushi express	
37	Umegaoka Sushi No Midori Souhonten	Eel sushi	JC12	Anguilla anguilla	Well Core Holding Ltd.	
36	Big Bang Don	Eel sushi	JC11	Anguilla rostrata		
35	Lo Ku Ma Ru Japanese Casual Restaurant	Eel sushi	JC10	Anguilla anguilla		
34	Sushi Sooshi	Eel sushi	MH15	Anguilla rostrata		
33	MOSHI MOSHI	Eel Don	MH16	Anguilla anguilla		
32	Yummy Delight	Eel Don	MH14	Anguilla rostrata		
31	ENJOY SASHIMI	Eel Don	MH13	Anguilla rostrata		
30	Harvest House Sushi	Eel sushi	MH12	Conger myriaster		
29	Hokahoka	Eel sushi	MH11	Anguilla rostrata		
28	和居酒屋( No Eng name)	Eel sushi	MH10	Anguilla anguilla		Shut down
27	Yoneko	Eel Don	MH09	Anguilla rostrata		
26	Dream Maker Restaurant	Eel sushi	MH08	Anguilla rostrata	rvhiess	
25	Teishoku 8	Eel Don	MH07	Anguilla anguilla	Sushi Express	
24	3hreesixty	Grilled eel	JC09	Anguilla rostrata		
23	Sen-ryo (千両 )	Eel sushi	JC08	Anguilla japonica		
22	Maruju Japanese Shokudo	Eel Don	MH06	Anguilla rostrata		
21	Katiga Japanese Food Shop	Eel sushi	KZ09	Anguilla anguilla		
20	Owarima	Eel Don	KZ08	Anguilla rostrata		
19	( <b>俊</b> の <b>テ</b> ) Ichimoto Japanese Restaurant	Eel sushi	KZ07	Anguilla anguilla		
18	Sakura Pavilion and Japanese Cuisine (櫻の亭)	Eel sushi	KZ06	Anguilla rostrata		
17	Kago Shima Japanese Food	Eel & Egg Don	KZ05	Conger myriaster		
16	Yamada Dining	Eel & Egg Don	KZ04	Anguilla anguilla		
15	Dai Jou Bu Sho Ku Do	Eel Don	KZ03	Anguilla anguilla		
14	Inoue Japanese Restaurant	Eel Don	JC07	Anguilla anguilla		
13	三上日本料理(No Eng name)	Eel Don	JC06	Anguilla rostrata		
12	Izumisake Japanese Restaurant	Eel Don	JC04 JC05	Fail		
11	千之壽料理店(No o Eng name)	Eel sushi	JC03	Anguilla rostrata		
10	立哥麵家(No Eng name)	Eel Don	JC03	Anguilla rostrata		
9	Naruto Uo Ichiba Izakaya	Eel sushi	MH05	Anguilla rostrata		
7 8	Edomae Japanese Cuisine	Grilled eel	MH04	Anguilla rostrata  Anguilla anguilla		
	Mi-Ne Sushi	Eel sushi	MH03	Anguilla roctrata		İ

43	Rock Salt Japanese Restaurant	Eel sushi	KZ10	Anguilla anguilla	<b>↓</b> =□	
44		Eel Don			大喜屋	
	Maruju Aburi Farm 仨島日本料 SA Shima		KZ11	Anguilla rostrata		
45		Eel sushi	MH17	Anguilla rostrata		
46	MAKOTOAJI	Eel hand roll	MH18	Anguilla rostrata		
47	Heart Sushi	Eel roll	MH19	Anguilla anguilla		
48	Matsuzaka Japanese Restaurant	Eel sushi	MH20	Anguilla anguilla		
49	Toriyamana	Eel sushi	MH21	Anguilla anguilla		
50	瀛燒居酒屋(No Eng name)	Eel Don	MH22	Anguilla rostrata		
51	琥酌日本料理(No Eng name)	Eel Don	JC18	Anguilla rostrata		
52	Sapporo Japanese Restaurant	Eel Don	MH23	Anguilla anguilla		
53	Roppongi Japanese Food	Eel Don	MH24	Anguilla rostrata		
54	IKKYU Japanese Casual Restaurant	Eel sushi	MH25	Anguilla anguilla		
55	Yakawa Sushi	Eel sushi	MH26	Anguilla anguilla		
56	<u>Uoshin Japanese Restaurant (魚心)</u>	Eel Roll	KZ12	Anguilla anguilla		
57	<u>Ippudo</u>	Eel Don	KZ13	Anguilla anguilla	Maxim's	
58	<u>Oishi Sushi</u>	Eel roll	KZ14	Anguilla anguilla		
59	Bin Song Ting Japanese Restaurant	Eel sushi	KZ15	Anguilla japonica		
60	KAIYO	Eel roll	KZ16	Anguilla anguilla	Dishtag	
61	Genki Ippai	Eel roll	KZ17	Anguilla anguilla		
62	Sushi Kyushu Ichiba	Eel Don	JC19	Anguilla rostrata		
63	Kokonoi Sushi Restaurant	Eel sushi	JC20	Anguilla japonica		
64	Nippon Sushi Dining	Eel roll	MH27	Anguilla japonica		
65	The Little Devil	Eel Sushi	KZ18	Conger myriaster		
66	Isakichi	Eel Don	KZ19	Conger myriaster		
67	<u>元福魚市場</u> (Genfuku Japanese Restaurant)	Eel Don	KZ20	Anguilla anguilla		
68	Shota Japanese Restaurant	Eel Don	JC21			
	( <u>將太日本料理</u> )	Lei Doll	JCZI	Anguilla anguilla		
69	Tanaka Japanese Restaurant (田中 日本料理)	Eel sushi	JC22	Anguilla anguilla		
70	RYORIBAN NO GINJI	Eel sushi	JC23	Anguilla japonica		
71	YUMMY (YOHO)	Eel don	JC24	Anguilla rostrata		
72	ICHIMOKU JAPANESE RESTAURANT	Eel egg roll	JC25	Anguilla rostrata		
73	Akita Japanese Restaurant (秋田日 本料理)	Eel fried rice	JC26	Anguilla marmorata		
74	Shodoshima (小豆島日本料理)	Eel sushi	JC27	Anguilla japonica		
75	ITACHO SUSHI	Eel hand roll	JC28	Anguilla anguilla	板長	
76	<u>Kazutoyo 一豊</u>	eel roll	JC29	Anguilla anguilla		
77	<u>Ten Tori</u>	Eel roll	JC30	Anguilla rostrata		
78	圍屋和食料理	Eel roll	JC31	Anguilla japonica		
79	Zushi Ana	Eel Don	JC32	Fail		
80	Sushi Zushi	Eel Don	KZ21	Fail		





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