

Science Policy



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**Identification of northern bluefin tuna in the
New Zealand EEZ**

Peter Smith and Lynda Griggs

**Final Research Report for
Ministry of Fisheries Research Project MOF2000/02B
Objective one**

National Institute of Water and Atmospheric Research

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Final Research Report

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Author	Peter Smith and Lynda Griggs
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7. Executive Summary

Pacific northern bluefin tuna *Thunnus orientalis* are a separate species to southern bluefin tuna *T. maccoyii*, with different distributions and spawning grounds. A range of distinctive external characters have been reported in northern bluefin tuna by observers, researchers, and fishers. These external characters are large body size, black caudal keels, dark body coloration, speckled or mottled patterns on body, elongated body, and small eye. These characters are indicators of species identity and are not reliable when used alone to identify specimens. The key diagnostic character for distinguishing *T. orientalis* and *T. maccoyii* is the shape of the dorsal wall of the body cavity, which can be viewed when gills are removed during onboard processing. However this character has only been reported in large (>130 cm) specimens. DNA based methods, using small pieces of frozen or ethanol fixed muscle tissue, provide a reliable diagnostic tool for discriminating tuna species, including *T. orientalis* and *T. maccoyii* tuna caught in the New Zealand EEZ. From 1990-2000 69 specimens of suspect northern bluefin tuna were tested with a DNA based method. Samples prior to 1994, identified by external characters, contained mostly *T. maccoyii*. Specimens collected since 1996 and identified by the body wall shape were correctly identified as *T. orientalis*.

8. Objectives

Identification of northern bluefin tuna in the New Zealand fishery:

- Collate the information on field characters and DNA markers used to identify the suspect northern bluefin tuna.
- Identify any remaining tissue samples from suspect specimens with the diagnostic DNA marker.
- Prepare a detailed description of key field characters for identification of suspect northern bluefin tuna specimens.

9. Methods

9.1 Background

Southern bluefin tuna *Thunnus maccoyii* (Castelnau, 1872) are widely distributed in all oceans south of about 30° S. Commercial fishing of *T. maccoyii* started in the 1950s and was initially focused in the south-east Indian Ocean, off New South Wales, and south Australia. The fishery expanded rapidly during the early 1960s moving into New Zealand waters. The stock is now in a depleted status at only 5-8% of the 1960 parental biomass (Report 1998). Around New Zealand *T. maccoyii* are caught by domestic and charter longline, handline and trolling vessels. This is a small, but high value fishery of about 420 tonnes per annum, with individual fish selling for thousands of dollars on the Japanese market. The highest price paid for a fish from the New Zealand domestic fishery was \$90 000 in Tokyo in 1999.

The New Zealand southern bluefin tuna fishing regulations define southern bluefin tuna as “fish with the scientific name *Thunnus maccoyii*; and includes the fish with the scientific name *Thunnus thynnus*” (Regulations 2000). These regulations dictate that northern bluefin tuna are recorded against the southern bluefin tuna quota, and the fishery is managed as a single species fishery, with no allowance for the presence of a second closely related species. Each year a number of tuna caught in the New Zealand fishery are recorded as northern (= Pacific) bluefin tuna *Thunnus orientalis* (Temminck and Schlegel, 1844) by observers on both domestic and Japanese vessels. *T. orientalis* and *T. maccoyii* are separate species, the key distinguishing characters being the position of the first ventrally directed parapophysis on the 9th (*T. maccoyii*) and 8th (*T. orientalis*) vertebrae, and the colour of the caudal keel, although the colour of the caudal keel may not be reliable in large specimens (Gibbs & Collette 1966). In the northern hemisphere two subspecies of bluefin tuna have been recognised: *T. thynnus orientalis* in the Pacific Ocean and *T. thynnus thynnus* (Linneaus, 1758) in the Atlantic and Indian Oceans and the Mediterranean Sea (Gibbs & Collette 1966; Collette & Nauen 1983). Recently these subspecies have been considered as full species, *T. thynnus* and *T. orientalis*, based on morphological and molecular data (Collette 1999), and in this report we follow this convention. Given the high unit value of the New Zealand fishery and the limited quota, it is important that individual fish, in particular specimens of northern bluefin tuna, are correctly identified.

The bluefin tuna species have different distributions with *T. maccoyii* in the southern hemisphere and *T. orientalis* generally in the northern hemisphere, although specimens

of *T. orientalis* have been reported from Australia, the Galapagos Islands, and New Zealand (Collette & Smith 1981; Collette & Nauen 1983). Bayliff (1994) reported that northern blue fin tuna are caught by longline vessels east of the Philippines, northeast of Papua New Guinea, southeast of Australia, and especially around New Zealand. The spawning area for *T. orientalis* lies between Japan and the Philippines and in the Sea of Japan.

Different characters have been used to identify specimens as northern bluefin tuna in the New Zealand fishery. Prior to 1996 most specimens recorded as northern bluefin tuna, by New Zealand observers, were identified by large size and the colour of the caudal keel. Fishing masters have also recorded large fish as northern bluefin tuna.

Use of external characters to distinguish bluefin tuna has been questioned (Report 1994) and genetic tests, based on allozymes, indicated that size and body colour are unreliable for correct species identification (Smith et al 1994). Similarly DNA tests on Australian specimens of northern bluefin tuna have shown that some specimens have been incorrectly identified (Ward et al 1995). Japanese fishing masters and some New Zealand tuna skippers have identified northern bluefin tuna by the presence of a "bust", a muscular protrusion in the dorsal abdominal cavity, that is present in *T. maccoyii* and *T. thynnus* but not *T. orientalis* (Iwai et al 1965, Gibbs & Collette 1966).

Molecular techniques are increasingly being used to identify fish product and specimens, and several genetic methods have been applied to the identification of tuna species (Bartlett & Davidson 1991, Chow & Inoue 1993, Smith et al 1994, Ward et al 1995). More than 150 specimens of *T. orientalis* and *T. maccoyii* have been tested for variation in the mitochondrial genome and diagnostic DNA markers developed for the identification of these species (Chow & Inoue 1993, Chow & Kishino 1995).

9.2 DNA methods

Muscle samples were collected from specimens of *T. orientalis* off Japan in 1990 and 1993, and from *T. maccoyii* off New Zealand in 1990, 1998 and 1999 (Table 1). Specimens were identified from geographical location and colour of the caudal keel (black caudal keel for *T. orientalis* and yellow caudal keel for *T. maccoyii*, Collette & Nauen 1983). Muscle samples were collected from 69 fish in the New Zealand EEZ between 1990 and 2000 that had been recorded as northern bluefin tuna, and from a further 3 specimens with some characters of both species. Small pieces of muscle tissue (about 10 g) were removed from tuna specimens at sea and frozen in individual plastic bags. Tissue samples were stored at -70°C in the laboratory. Sample details and identification characters are given in Table 1.

DNA was extracted from muscle tissue of *T. orientalis* and *T. maccoyii* and the suspect northern specimens with a proteinase K extraction, followed by chloroform-isoamyl alcohol clean-up and ethanol precipitation after Chow and Inoue (1993). The DNA pellet was air dried and resuspended in 40 ml sterile water. The primer pair flanking the region between the mitochondrial ATPase and cytochrome oxidase subunit 111 genes, designated ATCO (Chow & Inoue 1993), was used to amplify DNA samples from all tuna specimens. Amplifications were carried out in a final volume of 50 μl of polymerase chain reaction (PCR) reaction mixture after the method of Chow

and Inoue (1993).

PCR-RFLP (restriction fragment length polymorphism) analysis of mtDNA has been used in population (Chow & Ushima 1995, Cronin et al 1993) and taxonomic (Chow et al 1993) fisheries studies, including identification of tuna species (Chow and Inoue 1993). All *Thunnus* species can be identified by species-specific restriction profiles following digestion with the restriction enzymes (Chow and Inoue, 1993). Specimens of *T. orientalis* and *T. maccoyii* have different mitochondrial DNA haplotypes: amplified fragments of the ATCO region of mtDNA produce species specific fragments when cut with the diagnostic restriction enzyme *Alu* I (Chow & Inoue 1993). As specimens used in this study were either *T. orientalis* or *T. maccoyii*, the diagnostic restriction enzyme *Alu* I was used to digest the amplified product. Digested PCR products were separated in 1.4% agarose gels in a TBE buffer (25 mM Tris, 0.5 mM EDTA, and 25 mM boric acid) and stained with ethidium bromide. DNA fragments were viewed under an ultraviolet (UV) light source and photographed.

9.3 Collation of field data

Observer records on all tuna recorded as northern bluefin tuna were extracted from the MFish 1_line database and matched with the DNA data.

9.4 Description of field characters

The scientific literature describing northern and southern bluefin tuna was reviewed and the key diagnostic characters summarized. Field description from New Zealand Ministry of Fisheries Observer records were also collated to find key characters used to identify northern bluefin tuna in the New Zealand fishery.

10. Results

10.1 DNA analysis of tissue samples

DNA, extracted from bluefin tuna muscle tissue samples collected over the past ten years, was successfully amplified for the ATCO region of mitochondrial DNA. The resultant DNA fragments were cut with the restriction enzyme *Alu* I and the control samples from *T. orientalis* and *T. maccoyii* revealed the diagnostic restriction profiles. The suspect northern bluefin tuna had either a *T. orientalis* or *T. maccoyii* restriction profile. Results are summarised by year of collection in Table 1. Sixty nine suspect northern bluefin tuna were tested and 59 were identified as *T. orientalis*. Most of the misidentifications occurred in the early 1990s (Table 1).

Sub samples of *T. maccoyii* and suspect northern bluefin tuna muscle tissue were sent to Prof Chow (National Research Institute of Far Seas Fisheries, Japan) for genetic testing. Results from the two laboratories agree.

A further three specimens had some of the distinguishing characters of northern bluefin tuna (eg black caudal keel and large size), but were recorded as southern bluefin tuna

by observers for no obvious reason. These fish were confirmed as *T. maccoyii* by the DNA test.

Table 1. Summary of the number of suspect northern bluefin tuna from the New Zealand EEZ identified as “true” northern bluefin tuna (*T. orientalis*) with a diagnostic DNA marker.

Year	No. suspect NBT	No. <i>T. orientalis</i> by DNA
1990	10	3
1991	1	0
1993	1	1
1996	10	9
1997	18	18
1998	22	21
1999	3	3
2000	4	4

10.2 Collation of DNA and field data

Table 2 summarises the characters recorded by observers and the DNA results. Most of the specimens recorded as northern bluefin tuna in New Zealand waters in 1990-91 were *T. maccoyii*. These specimens had been identified mainly by large body size and dark colour of the caudal keel, although the observer records are incomplete (Table 2), as the recording system has evolved over the past ten years. For some suspect northern bluefin tuna specimens, the observers have recorded several characters, for other specimens few details are recorded and it is unclear how the specimen was identified as a northern bluefin tuna (Table 2).

10.3 Identification characters for northern bluefin and *T. maccoyii*

A range of characters have been tested and applied to the identification of northern and southern bluefin tuna Gibbs and Collette (1966) provided a good overview of the early literature on identification of *T. orientalis* and *T. maccoyii* and concluded that almost every anatomical, morphometric and meristic character has proved to be similar in all populations of *T. orientalis* and *T. maccoyii*. *T. orientalis* and *T. maccoyii* were best separated on skeletal characters (Gibbs & Collette 1966).

This section provides a brief summary of key characters used in tuna species identification, based on the scientific literature and on observer records. The key characters that have been used to distinguish the three bluefin tuna species are summarised in Table 3.

Table 2: Summary of identification characters recorded by observers, and DNA identification results, for bluefin tuna in the New Zealand EEZ. Character codes: B, black; D, dark; E, elongated; L, large; M, mottled; NP, not present; P, prominent; R, reduced; S, small; Sp, speckled; Y, yellow.

Year	No. Fish	Observer ID	DNA	External characters							Internal character	
				Colour of caudal keels ¹	Body coloration ²			Body size ³	Body shape ⁴	Eye size ⁵		Bodywall protrusion ⁶
					dark	speckled	Mottled					
1990	3	N	N	B								
	7	N	S	B								
(control)	12	S	S	Y								
1991	1	N	S									
1993	1	N	N									
1996	1	N	N	B	D	Sp			E	S	R	
	1	N	N	B		Sp			E		R	
	1	N	N	B	D				E		R	
	6	N	N	B					E		R	
	1	N	S	B								
1997	1	N	N	B				L	E		R	
	8	N	N	B					E	S		
	1	N	N	B			M					
	1	N	N	B	NP	NP	NP			S	R	
	1	N	N	B			M			S	R	
	1	N	N	B	D						R	
	3	N	N	B							R	
	2	N	N									
1998	1	N	N	B	D					S		
	1	N	N	B	D		M	L		S		
	2	N	N	B			M	L		S		
	3	N	N	B			M				R	
	3	N	N	B	D		M		E	S	R	
	1	N	N		NP	NP	NP	L			R	
	2	N	N		D			L	E		R	
	1	N	N	B		Sp		L			R	
	1	N	N	B				L			R	
	2	N	N								R	
	4	N	N									
	1	N	S									
	1	S	S	B	NP	NP	NP			NP		
(control)	10	S	S								P	
1999	1	N	N					L				
	1	N	N	B			M	L			R	
	1	N	N	B			M	L	E	S	R	
	1	S	S	B				L				
	1	S	S	B								
2000	1	N	N					L	E	S	R	
	1	N	N	B		Sp			E		R	
	1	N	N	B				L				
	1	N	N					L				

Table 3. Key field characters in northern bluefin tuna and southern bluefin tuna (from Iwai et al.1965, Gibbs & Collette 1966, Collette & Nauen 1983).

Character	<i>T. thynnus</i> (Northern bluefin)	<i>T. orientalis</i> (Pacific bluefin)	<i>T. maccoyii</i> (Southern bluefin)
Number of gill rakers	34-43	32-40	31-40
Colour of caudal keels	Dark	Dark	Yellow, maybe dark in large specimens
Dorsal body cavity shape (in large specimens >130cm)	Wide bulge with no lateral concavity; narrow lateral trough	Narrow bulge with lateral concavity; wide lateral trough	Wide bulge with no lateral concavity; narrow lateral trough
Distribution	Labrador to Caribbean Sea, and Brazil in western Atlantic; and Norway to South Africa, in the eastern Atlantic and the Mediterranean Sea.	Gulf of Alaska to Baja California in the eastern Pacific; Sea of Okhotsk to Philippines in the western Pacific. Rare reports from southern hemisphere.	Southern oceans south of 30°S; spawning grounds south of Indonesia

Caudal keel colour

The FAO guide (Collette & Nauen 1983) states that median caudal keel is yellow in *T. maccoyii* adults, and dark in *T. orientalis* adults (Table 3). However Gibbs and Collette (1966) note that the caudal keel is yellow in most specimens of *T. maccoyii*, but that this colour is often lost in large adults. One New Zealand observer reported that the black coloration can be scraped away to reveal a pale caudal keel in southern bluefin tuna while the black colour remains in northern bluefin tuna.

We found caudal keel colour to be unreliable as 3 specimens (caught in 1998 and 1999) with dark caudal keels were identified as *T. maccoyii* with the DNA test. The colour of the caudal keel in the pre-1994 specimens was not always recorded.

Body colour

Both *T. orientalis* and *T. maccoyii* have a dark blue dorsal surface with silvery white lower sides and belly (Collette & Nauen 1983). New Zealand observers have noted that *T. orientalis* tend to have a darker dorsal surface than *T. maccoyii* and are referred to as “kuro maguro” (black tuna) by crew on Japanese longline vessels.

Some observers have noted specimens with unusual colour patterns with blue flecks or speckling above the pectoral fins and sometimes on the head, and which have not been reported in *T. maccoyii*. Other unusual colour patterns are a bluish mottled pattern, posterior to the anal fin, or blue-grey flanks. Specimens of *T. orientalis* displayed either the speckling or the mottling pattern, but no specimens were reported with both

colour patterns (Table 2). These unusual colorations have not been reported in the scientific literature (eg Gibbs & Collette 1966, Collette & Nauen 1983). It is possible that these colour patterns fade after death and are lost by the time that frozen specimens are examined in port.

Bluefin tuna that display the speckled or mottled coloration were always *T. orientalis* when tested for DNA. Observers recorded 10 specimens as darker, 4 with blue flecks or speckling, and 13 with the ventral mottling pattern (Table 2). Two suspected northern bluefin tuna did not display these colour patterns; for several other specimens the colour patterns were not recorded and the proportion of *T. orientalis* with unusual coloration is unknown.

Body proportions

Observers have described northern bluefin tuna as being more “elongated” with a head that is smaller in relation to its body size, compared with *T. maccoyii*; some observers described the head as more “wedge-shaped” in northern bluefin tuna. Observers also noted that the eye of northern bluefin tuna is smaller in relation to the size of its head. No formal measurements have been made for head length and eye diameter, and they appear to be subjective characters. The differences in head shape and eye diameter were not reported in early studies (Gibbs & Collette 1966, Collette & Nauen 1983).

The length-weight relationship of *T. maccoyii* was compared with that of *T. orientalis*, using fish of comparable size (127 cm fork length or larger, 36 *T. orientalis* and 14 458 *T. maccoyii*). A t-test of equivalent slopes showed a significant difference ($\alpha = 0.05$, d.f. = 8, n = 14 494), confirming that there is a quantifiable difference in the length-weight relationship of the two species; that is likely to coincide with observers' descriptions of elongated shape in *T. orientalis*.

Pectoral fin

Relative lengths of the pectoral fin have been used as a species character for identification of some species of tuna. The pectoral fin in *T. thynnus* (17-21% fork length) is shorter than in *T. orientalis* (20-23% fork length) and *T. maccoyii* (>23% fork length) in fish of similar size (Gibbs & Collette 1966, Collette & Nauen 1983). There is insufficient differentiation between *T. orientalis* and *T. maccoyii* for this to be a useful field character for species identification.

Body size

T. orientalis caught in the New Zealand EEZ tend to be larger than *T. maccoyii*. Data recorded by observers from 1987-99 show that the average size of *T. maccoyii* is 154 cm fork length, with 99th percentile of 192 cm (maximum 203 cm fork length). Twenty seven per cent of the genuine northern bluefin tunas recorded by observers were greater than the maximum length recorded for *T. maccoyii*. The average fork length for *T. orientalis* is 191 cm, with a range of 127-250 cm. Therefore a bluefin tuna greater than 190 cm fork length is likely to be *T. orientalis*.

The average weight of *T. orientalis* after processing, is 126 kg, compared with 62 kg for *T. maccoyii*.

Internal characters

Gill rakers

Collette and Nauen (1983) reported overlap in gill rakers in *T. maccoyii*, with 31-40 gillrakers on the first arch, and *T. orientalis* tuna with 32-40 (Table 3). This character is only useful for identification of specimens with extreme low (31) counts. Gibbs and Collette (1966) give means of 33.7 for *T. maccoyii* and 35.9 for *T. orientalis*, but do not state numbers of fish tested or standard errors for these estimates.

Vertebrae

Vertebrae numbers (18 precaudal and 21 caudal) are the same in all three species of bluefin tuna (Collette & Nauen 1983). However the three species differ in the height of the parapophysis on the 9th vertebra and the canal height in the 10th vertebra (Gibbs & Collette 1966). These characters are impractical for identification of specimens in a commercial fishery.

Liver appearance

The appearance of the liver, in particular the presence/absence of ventral striations has been used as a species marker in some species of tuna (eg bigeye and yellowfin tuna, Collette & Nauen 1983). The livers of both *T. orientalis* and *T. maccoyii* appear to be similar with three sub equal lobes and ventral striations (Collette & Nauen 1983).

Shape of dorsal wall of abdominal cavity

The shape of the dorsal wall of the body cavity differs among the three species of bluefin tuna (Godsil & Holmberg 1950, Gibbs & Collette 1966). In *T. thynnus* and *T. maccoyii* there is "a wide anterior bulge without lateral concavity, but a deep, narrow trough lateral to the bulge" (Gibbs & Collette 1966). In *T. orientalis* "the anterior bulge is narrow with a lateral concavity, and a wide trough lateral to the bulge" (Table 3). This character has been referred to as the "bust" by Japanese fishers and can be observed as a muscular protrusion in *T. maccoyii* when the gills are removed as part of standard on-board processing. The muscular protrusion is small or absent in *T. orientalis*. Most specimens recorded as northern bluefin tuna by New Zealand observers since 1996 have been identified by the absence or reduced size of the "bust". All specimens of northern bluefin tuna identified by the absence/reduced size of the bust were confirmed as *T. orientalis* by the DNA test.

Differences in the shape of the dorsal wall of the body cavity may not be apparent in specimens less than about 130 cm (Gibbs & Collette 1966), and so this character is only useful for distinguishing large specimens of *T. maccoyii* and *T. orientalis*. In small specimens the colour of the caudal keel may be a more reliable character, but a DNA test would confirm identification. One small northern bluefin tuna (127 cm fork length)

was reported from the New Zealand fishery. The specimen was correctly identified by the crew and confirmed as *T. orientalis* by the DNA test, but unfortunately the identification characters were not recorded.

10.4 Key field characters for identification of suspect northern Pacific bluefin tuna specimens.

A bluefin tuna with any of the following external characters should be examined further as a potential *T. orientalis*:

1. large size, greater than 190 cm fork length;
2. dark caudal keel colour;
3. dark body coloration;
4. speckled or mottled patterns on body;
5. elongated body with small head in relation to body size;
6. relatively small eye.

The above characters are not diagnostic on their own, but have been observed in specimens of *T. orientalis* caught in the New Zealand EEZ. Any tuna with one, or more, of the above external characters should be checked for the shape of the dorsal wall of the abdominal cavity. All bluefin tuna caught on commercial vessels are processed at sea and identification of large specimens (>130 cm) can be readily confirmed by the shape of the dorsal wall of the abdominal cavity. If there is any doubt about identification, a small piece of muscle tissue should be frozen and sealed in an individual plastic bag (or fixed in a tube of ethanol, if available) for DNA confirmation.

11. Conclusions

1. Northern bluefin tuna *T. orientalis* are caught in New Zealand waters. Northern bluefin tuna are a separate species to *T. maccoyii*, with different distributions and spawning grounds. The definition of southern bluefin tuna as “fish with the scientific name *Thunnus maccoyii*; and includes the fish with the scientific name *Thunnus thynnus*” that appears in the southern bluefin tuna Quota Regulations 2000 (New Zealand Ministry of Fisheries 22.6.00) does not reflect the taxonomic status of bluefin tuna. The definition of southern bluefin tuna should be modified and exclude Atlantic bluefin tuna *Thunnus thynnus*, which probably does not occur in the Pacific Ocean.

2. A range of distinctive external characters have been reported in northern bluefin tuna by observers, researchers, and fishers. These external characters are large body size, black caudal keels, dark body coloration, speckled or mottled patterns on body, elongated body, and small eye. These characters are indicators of species identity and are not reliable when used alone to identify specimens.

3. The key diagnostic character for distinguishing *T. orientalis* and *T. maccoyii* is the shape of the dorsal wall of the body cavity, which can be viewed when gills are removed during onboard processing. The muscular bulge or “bust” has only been reported in large (>130 cm) specimens of *T. maccoyii*. Small specimens of *T. maccoyii*

can be distinguished from small specimens of *T. orientalis* by colour of the caudal keel; yellow in *T. maccoyii* and dark in *T. orientalis*.

4. DNA based methods, using small pieces of frozen or ethanol fixed muscle tissue, provide a reliable diagnostic tool for discriminating tuna species, including all specimens of *T. orientalis* and *T. maccoyii* caught in the New Zealand EEZ.

5. From 1990-2000, 69 specimens of suspect northern bluefin tuna were tested with a DNA based method. Samples prior to 1994, identified by external characters, contained mostly *T. maccoyii*. Specimens collected since 1996 and identified by the absence of, or reduced, muscular protrusion were correctly identified as *T. orientalis*.

6. Unusual body coloration patterns were recorded in some specimens of *T. orientalis* which have not been reported in the scientific literature. The Observer bluefin tuna recording form should be revised to record the presence/absence of all the key characters used to identify suspect Pacific bluefin tuna.

12. Publications

Draft manuscript: DNA identification of Pacific bluefin tuna *Thunnus orientalis* in the New Zealand fishery. P.J. Smith, L. Griggs, & C. Chow (to be approved by Ministry of Fisheries).

13. Data Storage

DNA laboratory methods and DNA protocols are stored in hard copy format laboratory records in the genetics laboratory at NIWA Greta Point. Tuna DNA data are stored in an EXCEL file "tunsus" on the H drive at NIWA Greta Point. Fishery data are stored Empress database l_line at NIWA Greta Point.

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References

- Bartlett, S. E.; Davidson, W. S. 1991: Identification of *Thunnus* tuna species by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochrome b genes. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 309-317.
- Bayliff, W.H. 1994: A review of the biology and fisheries for northern bluefin tuna, *Thunnus thynnus*, in the Pacific Ocean. *In Interactions of Pacific tuna fisheries.*

- Edited by S. Shomura, J. Majkowski, and S. Langi. FAO Fisheries Technical Paper 336/2. p244–295.*
- Chow, S.; Inoue, S. 1993: Intra- and interspecific restriction fragment length polymorphism in mitochondrial genes of *Thunnus* tuna species. *Bulletin of the National Research Institute of Far Seas Fisheries* 30: 207-225.
- Chow, S.; Clarke, M.E.; Walsh, P.J. 1993: PCR-RFLP analysis on thirteen western Atlantic snappers (subfamily Lutjaninae): a simple method for species and stock identification. *Fishery Bulletin* 91: 619-627.
- Chow, S.; Kishino, 1995: Phylogenetic relationships between tuna species of the genus *Thunnus* (Scombridae: Teleostei): inconsistent implications from morphology, nuclear and mitochondrial genomes. *Journal of Molecular Evolution* 41: 741-748.
- Chow, S.; Ushiyama, H. 1995: Global population structure of albacore (*Thunnus alalunga*) inferred by RFLP analysis of the mitochondrial ATPase gene. *Marine Biology* 123: 39-45.
- Collette, B.B. 1999: Mackerels, molecules, and morphology. Pp 149-164 In Seret, B & Sire, J.-Y (eds) Proceedings of the 5th Indo-Pacific Fish conference Noumea November 1997, IRD, Paris.
- Collette, B. B.; Nauen, C. E. 1983: FAO Species catalogue. Vol 2 Scombrids of the world. FAO fisheries synopsis 125: 137p.
- Collette, B. B.; Smith, B. R. 1981: Bluefin tuna, *Thynnus thynnus orientalis*, from the Gulf of Papua. *Japanese Journal of Ichthyology* 28: 166-168.
- Cronin, M.A.; Spearman, W.J.; Wilmot, R.L.; Patton, J.C.; Bickman, J.W. 1993: Mitochondrial DNA variation in chinook (*Onchorynchus tshawytscha*) and chum (*O.keta*) detected by restriction enzyme analysis of polymerase chain (PCR) products. *Canadian Journal Fisheries Aquatic Sciences* 50: 708-715.
- Gibbs, R. H.; Collette, B. B. 1966: Anatomy and systematics of tunas. *US Fisheries and Wildlife Service* 66(1): 109–121.
- Godsil, H.C.; Holmberg, E.K. 1950: A comparison of the bluefin tuna genus *Thunnus* from New England, Australia and California. *California Division of Fish & Game Fishery Bulletin* 77 55pp.
- Iwai, T.; Nakamura, I.; Matsubara, K. 1965: Taxonomic study of the tunas. In: Misaki Marine Biological Institute, Kyoto University, Special Report No. 2, February 20, 1965. Pp. 1-51 (in Japanese with English summary).
- Regulations, 2000: Fisheries (T. maccoyii Quota) Regulations 2000. New Zealand Ministry of Fisheries 22.6.00.
- Report, 1994: Report of the thirteenth meeting of Australian, Japanese and New Zealand scientists on T. maccoyii. Unpublished report held at NIWA Greta Point, Wellington.
- Report, 1998: Report of the resumed fourth annual meeting. Commission for the conservation of T. maccoyii, Canberra January 1998.
- Smith, P.J.; Conroy, A.M.; Taylor, P.R. 1994: Biochemical-genetic identification of northern bluefin tuna *Thynnus thynnus* in the New Zealand fishery. *New Zealand Journal of Marine and Freshwater Research* 28: 113-118.
- Ward, R.D.; Elliott, N.G.; Grewe, P.M. 1995: Allozyme and mitochondrial DNA separation of Pacific northern bluefin tuna *Thynnus thynnus orientalis* (Temminck and Schlegel), from T. *Thynnus maccoyii* (Castelnau). *Marine and Freshwater Research* 46: 921-930.

Bluefin Tuna Identification Guide

Pacific bluefin tuna (generally known as “Northern bluefin tuna”), *Thunnus orientalis*, is similar to Southern bluefin tuna, *T. maccoyii*, in external appearance. Identification based on large size of the fish and the colour of the caudal keels has not proved to be reliable, but there are other external differences, outlined below. An internal feature can be used to reliably distinguish the two species. Genetic (DNA) testing is conclusive.

External features:

Black caudal keels (Photos 1. and 2. – over)

The median caudal keel is yellow in adults of Southern bluefin tuna, and black in adults of Pacific (Northern) bluefin tuna, but the yellow coloration of Southern bluefin tuna keels can be lost in larger fish, so this alone is not a reliable indicator. The black coloration can often be scraped away to reveal yellow underneath in Southern bluefin tunas.

Body coloration (Photos 3., 4. and 5. – over)

Pacific bluefin tuna often have distinctive coloration and patterns. They tend to be of darker colour overall (sometimes referred to as “black tuna”). Patterns seen are blue flecks or speckling above the pectoral fins and sometimes on the head (and occasionally over the whole body), or a blueish circular mottling pattern, or reticulation, on the ventral area towards the tail. See over for examples of these patterns. Not all Pacific bluefin tuna display these colour patterns.

Body proportions

Pacific bluefin tuna are more “elongated” with a head that is smaller in relation to its body size, compared with Southern bluefin tuna. The eye of a Pacific bluefin tuna is also relatively smaller in relation to the size of its head, compared with Southern bluefin tuna.

Body size

Pacific bluefin tuna tend to be larger than Southern bluefin tuna. The average size of Pacific bluefin tuna is 191 cm fork length while the average size of Southern bluefin tuna is 154 cm. The average weight of Pacific bluefin tuna after processing, is 126 kg, compared with 62 kg for Southern bluefin tuna. A fish greater than 190 cm fork length is very likely to be a Pacific bluefin tuna. Smaller fish should also be examined as small Pacific tuna are known to occur in New Zealand waters.

The combination of ALL of the above external features i.e. A large fish with the body proportions and colorations described above is likely to be a Pacific bluefin tuna, but identification should be confirmed with the internal feature described below, and/or DNA (genetic) determination.

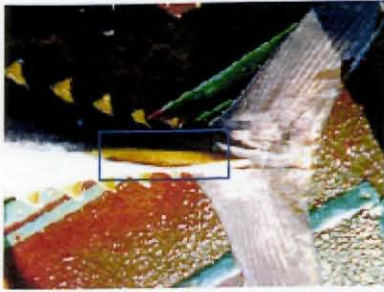
Internal distinguishing feature:

Shape of the dorsal wall of the gut cavity (Photos 6. and 7. – over)

There is an internal fleshy protrusion at the dorsal anterior end of the body cavity, sometimes referred to as a breast plate or “bust”. This feature is pronounced in Southern bluefin tuna and reduced in Pacific bluefin tuna (see over) and can be seen when the gills and guts are removed as part of standard onboard processing. Presence or absence of the dorsal bulge has been found to be a reliable method to distinguish Southern bluefin from Pacific bluefin tuna. This feature may not be apparent in specimens less than about 130 cm. It may be harder to identify smaller *T. orientalis*, but the caudal keels will be black, while small *T. maccoyii* will have yellow caudal keels.

If the identity of a fish is uncertain, this can be confirmed by DNA determination. Freeze a piece of muscle tissue, or place a matchstick strip of muscle in a small tube of ethanol (if available), label it and send it to N.I.W.A. attention Lynda Griggs or Peter Smith, 301 Evans Bay Parade, Greta Pt, Wellington.

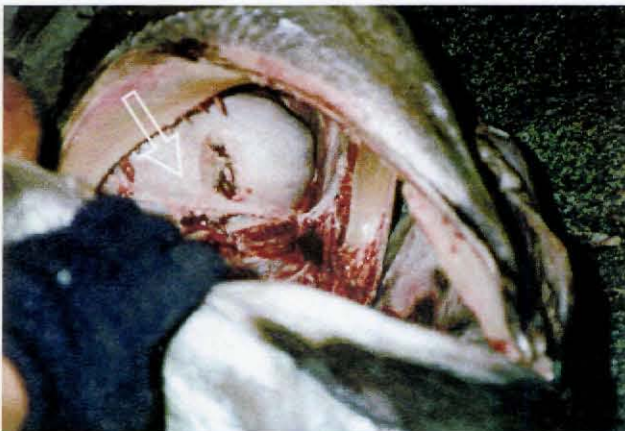
Southern bluefin tuna, *Thunnus maccoyii*



1. Yellow caudal keels
(marked with box)



3. Absence of any speckling or mottling pattern
on the body



6. Prominent bulge
at the anterior end of the gut cavity

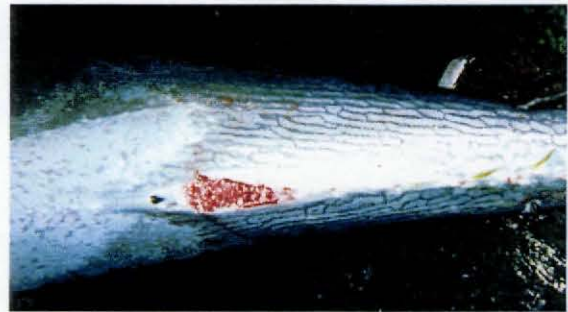
Pacific (Northern) bluefin tuna, *T. orientalis*



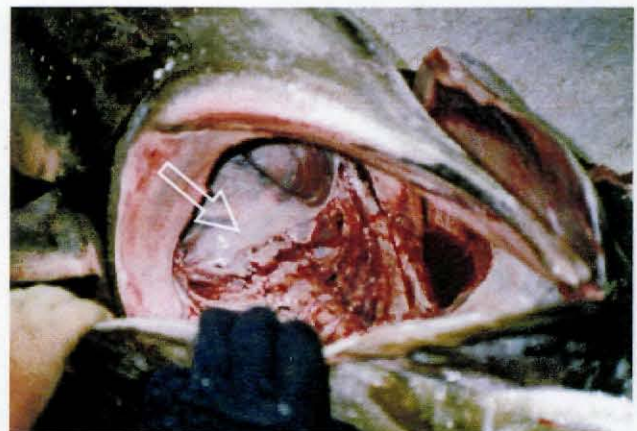
2. Black caudal keels



4. Speckling pattern



5. Ventral mottling pattern



7. Reduced / absent bulge

Photos 6. and 7. show the view into the gut cavity (gills removed). The ventral surface is uppermost and the head is to the bottom right. The operculum is held back to show the prominent bulge in *T. maccoyii* (left) while the feature is much reduced in *T. orientalis* (right). Arrows mark the position of the dorsal bulge.