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# DNA identification of Pacific bluefin tuna (*Thunnus orientalis*) in the New Zealand fishery

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**Abstract** Muscle samples were collected from 69 specimens identified as Pacific bluefin tuna (*Thunnus orientalis*) (Temminck and Schlegel, 1844) in the New Zealand Exclusive Economic Zone (EEZ) between 1990 and 2000. Identifications before 1996 were based on body size and colour of the caudal keel; later identifications were mostly based on the shape of abdominal cavity. The tissue samples were tested with a diagnostic mitochondrial DNA marker that distinguishes southern bluefin *Thunnus maccoyii* (Castelnau, 1872) and Pacific bluefin tuna *T. orientalis*; 59 specimens were confirmed as *T. orientalis* and 10 as *T. maccoyii*. Specimens recorded as Pacific bluefin tuna by the shape of the abdominal cavity were correctly identified as *T. orientalis*, and this character can be used to identify large specimens landed on tuna vessels. Some specimens recorded as Pacific bluefin tuna on the basis of colour and size were *T. maccoyii*; and early records of *T. orientalis* in New Zealand waters, based on these characters, are unreliable. Unusual colour patterns were reported in some specimens of *T. orientalis* but not *T. maccoyii*. The Pacific bluefin tuna *T. orientalis* accounted for less than 0.3% of the bluefin tuna catch in the New Zealand EEZ during the 1990s.

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## INTRODUCTION

Southern bluefin tuna *Thunnus maccoyii* (Castelnau, 1872) are widely distributed in all oceans south of c. 30°S. Commercial fishing of southern bluefin tuna started in the 1950s and was initially focused on the spawning grounds south of Indonesia. The fishery expanded rapidly during the 1960s and 1970s, moving into Australian waters and eventually into New Zealand waters. The stock is now in a depleted status at only 5–8% of the 1960 parental biomass (Anon. 1998). Around New Zealand bluefin tuna are caught by domestic and charter vessels by longlining and handling, and occasionally trolling. This is a small fishery of c. 420 t/annum, but with an export value of nearly NZ$19 million in the 1999–2000 fishing year. The highest price paid for a fish from the New Zealand domestic fishery was NZ$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 maccoyi*; and includes the fish with the scientific name *Thunnus thynnus*" (Ministry of Fisheries 2000). Each year a number of bluefin tuna caught in the New Zealand fishery are recorded as Pacific bluefin tuna *Thunnus orientalis* (Temminck and Schlegel, 1844) by Ministry of Fisheries observers and skippers on both domestic and charter vessels. The New Zealand southern bluefin tuna fishing regulations dictate that Pacific bluefin tuna be recorded against the southern bluefin tuna quota, and that the fishery be managed as a single species fishery, with no allowance for the presence of a second, closely related species. Pacific and southern bluefin tuna are separate species, originally distinguished by 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 (Gibbs & Collette 1966; Collette & Nauen 1983), although the colour of the

caudal keel was not reliable in large specimens (Gibbs & Collette 1966). In the northern hemisphere two subspecies of bluefin tuna *Thunnus thynnus thynnus*, in the Atlantic and Indian Oceans and the Mediterranean Sea, and *T. t. orientalis*, in the Pacific Ocean, were recognised, but now are considered as full species, Atlantic bluefin tuna *T. thynnus* and Pacific bluefin tuna *T. orientalis*, based on morphological and molecular data (Collette 1999). Given the high unit value of the New Zealand fishery and the limited quota, it is important that individual fish, in particular specimens of *T. orientalis*, are correctly identified.

The bluefin tuna species have different distributions in the Pacific Ocean with *T. maccoyii* solely in the southern hemisphere and *T. orientalis* primarily 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). Different characters have been used to identify specimens as Pacific bluefin tuna in the New Zealand fishery. Before 1996 most specimens recorded as Pacific bluefin tuna, by New Zealand observers, were identified by large size and colour of the caudal keel.

Use of external characters to distinguish bluefin tuna has been questioned (Anon. 1994) and genetic tests, based on allozymes, indicated that size and body colour alone are unreliable for correct species' identification (Smith et al. 1994). Similarly DNA tests on Australian specimens of Pacific bluefin tuna have shown that some specimens have been incorrectly identified (Ward et al. 1995). Japanese fishing skippers have identified Pacific 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). Diagnostic DNA markers have been developed for the identification of Pacific and southern bluefin tuna and more than 200 specimens have been tested for variation in the mitochondrial genome (Chow & Inoue 1993; Chow & Kishino 1995). Here we use the DNA markers that have been developed to distinguish the major commercial tuna species (Chow & Inoue 1993).

The aim of this project was to apply a diagnostic DNA marker to identify specimens reported as Pacific bluefin tuna from New Zealand waters, and to determine if there are reliable field characters for identification of Pacific bluefin tuna on commercial fishing vessels.

## METHODS

### Sample collection

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 (Japan with black caudal keel for *T. orientalis* and New Zealand with yellow caudal keel for *T. maccoyii* (Collette & Nauen 1983)). Muscle samples were collected from 69 fish in the New Zealand Exclusive Economic Zone (EEZ) between 1990 and 2000 that had been recorded as northern (= Pacific) bluefin tuna, and from a further three specimens recorded as southern bluefin tuna with black caudal keels (Table 2). Early samples (pre-1994) of Pacific bluefin tuna were identified by New Zealand

**Table 1** Summary of the number of suspect Pacific bluefin tuna (NTU) from the New Zealand Exclusive Economic Zone identified as *Thunnus orientalis* with a diagnostic DNA marker.

| Year | No. suspect NTU | No. *T. orientalis* 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 |

Ministry of Fisheries observers on the basis of large size and a dark caudal keel. Samples collected from Pacific bluefin tuna specimens between 1990 and 1992 were the same as those used by Smith et al. (1994) for allozyme identification. Most specimens recorded as Pacific bluefin tuna from 1996 onwards were identified by the shape of the dorsal wall of the body cavity.

For each specimen recorded as Pacific bluefin tuna, and for control samples, a small piece (c.10 g) of muscle tissue was removed and frozen in individual plastic bags –60 to –70°C at sea.

**Table 2** Summary of identification characters recorded by observers, and DNA identification results, for bluefin tuna *Thunnus orientalis* and *Thunnus maccoyii* in the New Zealand Exclusive Economic Zone. (B, black caudal keel; D, dark body colour; E, elongated body shape; M, ventral mottling; N, Pacific northern bluefin tuna; P, prominent body wall; R, reduced body wall; S, southern bluefin tuna; S*, southern bluefin tuna with black caudal keels; Se, relatively small eye; Sp, speckled body patterns; Y, yellow caudal keel). A space in the table indicates no information recorded by observer.

| Year | No. of fish | Obs. ID | DNA | Keel colour | Body colour | Body shape | Eye size | Abdominal wall shape |
|---|---|---|---|---|---|---|---|---|
| **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 | Se | 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 | | E | | R |
| | 8 | N | N | B | | E | Se | |
| | 1 | N | N | B | M | | | |
| | 1 | N | N | B | | | Se | R |
| | 1 | N | N | B | M | | Se | R |
| | 1 | N | N | B | D | | | R |
| | 3 | N | N | B | | | | R |
| | 2 | N | N | | | | | |
| **1998** | 1 | N | N | B | D | | Se | |
| | 1 | N | N | B | D,M | | Se | |
| | 2 | N | N | B | M | | Se | |
| | 3 | N | N | B | M | | | R |
| | 3 | N | N | B | D,M | E | Se | R |
| | 1 | N | N | | | | | R |
| | 2 | N | N | | D | E | | R |
| | 1 | N | N | B | Sp | | | R |
| | 1 | N | N | B | | | | R |
| | 2 | N | N | | | | | R |
| | 4 | N | N | | | | | |
| | 1 | N | S | | | | | |
| | 1 | S* | S | B | | | | |
| Control | 10 | S | S | | | | | P |
| **1999** | 1 | N | N | | | | | |
| | 1 | N | N | B | M | | | R |
| | 1 | N | N | B | M | E | Se | R |
| | 2 | S* | S | B | | | | |
| **2000** | 1 | N | N | | | E | Se | R |
| | 1 | N | N | B | Sp | E | | R |
| | 1 | N | N | B | | | | |
| | 1 | N | N | | | | | |

Tissue samples were stored at –70°C in the laboratory.

### DNA amplification and digestion

DNA was extracted from muscle tissue of *T. maccoyii* and *T. orientalis* and the suspect Pacific bluefin tuna with a proteinase K extraction, followed by chloroform-isoamyl alcohol clean-up and ethanol precipitation after Chow & Inoue (1993). The DNA pellet was air dried and resuspended in 40 µl sterile water.

The primer pair flanking the region between the mitochondrial ATPase and cytochrome oxidae subunit III genes, designated ATCO (Chow & Inoue 1993), was used to amplify DNA in all bluefin tuna specimens. Amplifications were carried out in a final volume of 20 µl of polymerase chain reaction (PCR) reaction mixture as described in Chow & Inoue (1993). PCR-RFLP (restriction fragment length polymorphism) analysis of mtDNA has been used in population (Cronin et al. 1993; Chow & Ushiama 1995) and taxonomic (Chow et al. 1993) fisheries studies, including identification of tuna species (Chow & Inoue 1993). All *Thunnus* species could be identified from species-specific restriction profiles by the restriction enzymes *Alu* I, *Mse* I and *Hinf* I or *Hinc* II (Chow & Inoue 1993), and these specific restriction profiles have been substantiated by nucleotide sequence and RFLP analyses with a large number of individuals (Takeyama et al. 2001). Two restriction enzymes, *Alu* I and *Mse* I, produced diagnostic restriction profiles in 82 specimens of *T. maccoyii* and 122 specimens of *T. orientalis*, although *T. thunnus* and *T. maccoyii* shared *Mse* I restriction profiles (Takeyama et al. 2001). As specimens used in this study were either *T. maccoyii* or *T. orientalis*, the two diagnostic restriction enzymes, *Alu* I and *Mse* I, were used to digest the amplified product. Digested PCR products were separated in 1.4% agarose gels in a TBE buffer (25 m*M* Tris, 0.5 m*M* EDTA, and 25 m*M* boric acid) and stained with ethidium bromide. DNA fragments were viewed under an ultraviolet (UV) light source and photographed.

### Field characters

Observer records on all tuna identified as northern (= Pacific) bluefin tuna were extracted from the New Zealand Ministry of Fisheries database, and the records matched with the DNA results. The length-weight relationships of *T. orientalis* and *T. maccoyii* were tested in fish of comparable size, because fishery observers had reported that *T. orientalis* appeared to be more elongated than *T. maccoyii*. Likewise the size frequencies of *T. orientalis*, and specimens identified as *T. maccoyii* by the diagnostic DNA marker, were plotted, because fishery observers reported that Pacific bluefin tuna were larger than *T. maccoyii*.

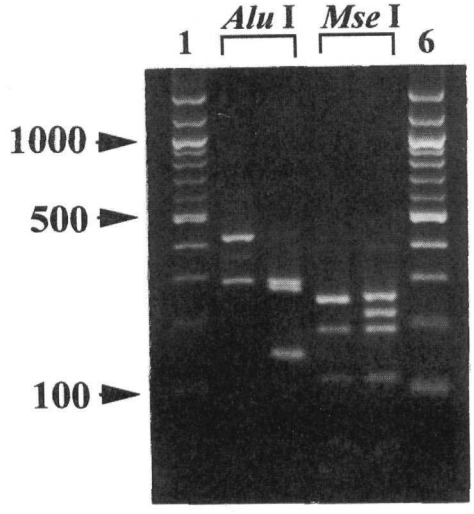


**Fig. 1** Restriction profiles of the mitochondrial DNA ATCO segment of *Thunnus orientalis* (lanes 2 and 4) and *T. maccoyii* (lanes 3 and 5) digested with the diagnostic restriction enzymes *Alu* I (lanes 2 and 3) and *Mse* I (lanes 4 and 5). Lanes 1 and 6 are molecular size markers (100 bp DNA ladder, New England BioLabs), and sizes "in base pairs" are indicated along the left margin.

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" (Gibbs & Collette 1966). 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 Pacific bluefin tuna by New Zealand observers since 1996 have been checked for the absence or reduced size of the dorsal bulge.

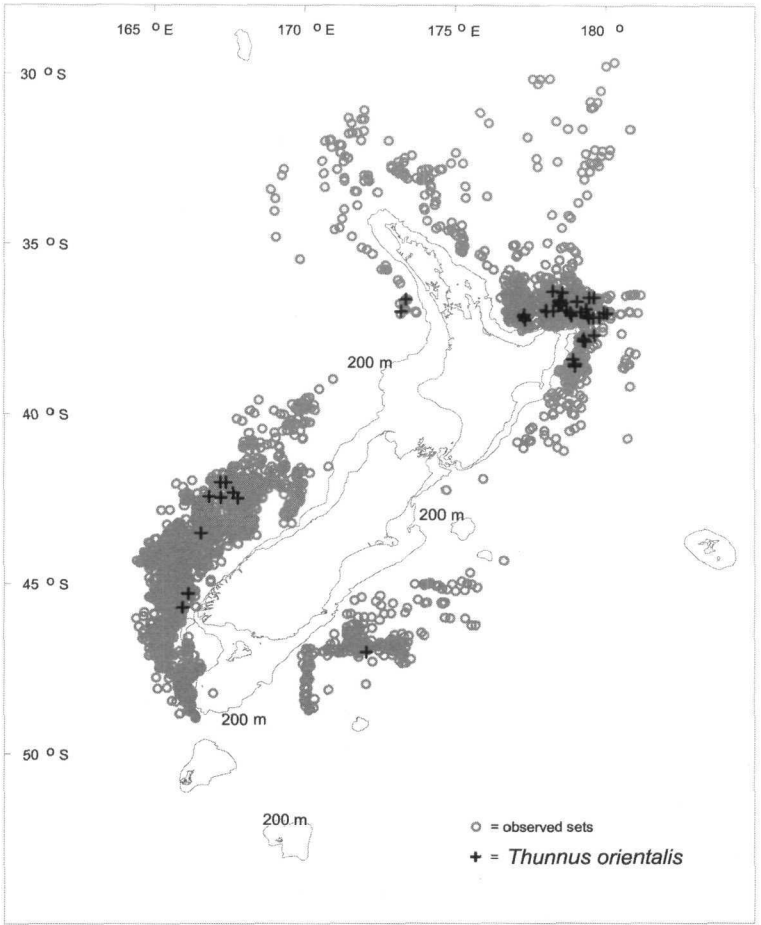


**Fig. 2** Distribution of bluefin tuna specimens identified as *Thunnus orientalis* by DNA markers, and the observed bluefin tuna sets, in the New Zealand Exclusive Economic Zone from 1990 to 2000.

## RESULTS

### DNA analyses

DNA, extracted from bluefin tuna muscle tissue samples collected between 1990 and 2000, was successfully amplified for the ATCO region of mitochondrial DNA. The resultant DNA fragments, cut with the restriction enzyme *Alu* I or *Mse* I, revealed diagnostic restriction profiles in the control samples from *T. orientalis* and *T. maccoyii* (Fig. 1). The suspect Pacific 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 Pacific bluefin tuna were tested and 59 were identified as *T. orientalis*. Three specimens landed in 1998 and 1999, and recorded as southern bluefin tuna (*T. maccoyii*) with black caudal keels, were identified as *T. maccoyii* by the diagnostic DNA marker (Table 2). The locations of *T. orientalis* specimens caught in the New Zealand EEZ, and identified by DNA markers, are given in Fig. 2, along with the positions of observed tuna longline sets.

### Field characters

The field characters recorded by Ministry of Fisheries observers and the DNA results are summarised in Table 2. Most of the specimens recorded as northern (= Pacific) bluefin tuna in New Zealand waters before 1994 were *T. maccoyii*. These specimens had been identified by large body size and dark colour of the caudal keel, although the observer records are incomplete (Table 2). Most of the specimens recorded as Pacific bluefin tuna since 1996 had been correctly identified (Table 1). All specimens of Pacific bluefin tuna identified by the absence/reduced

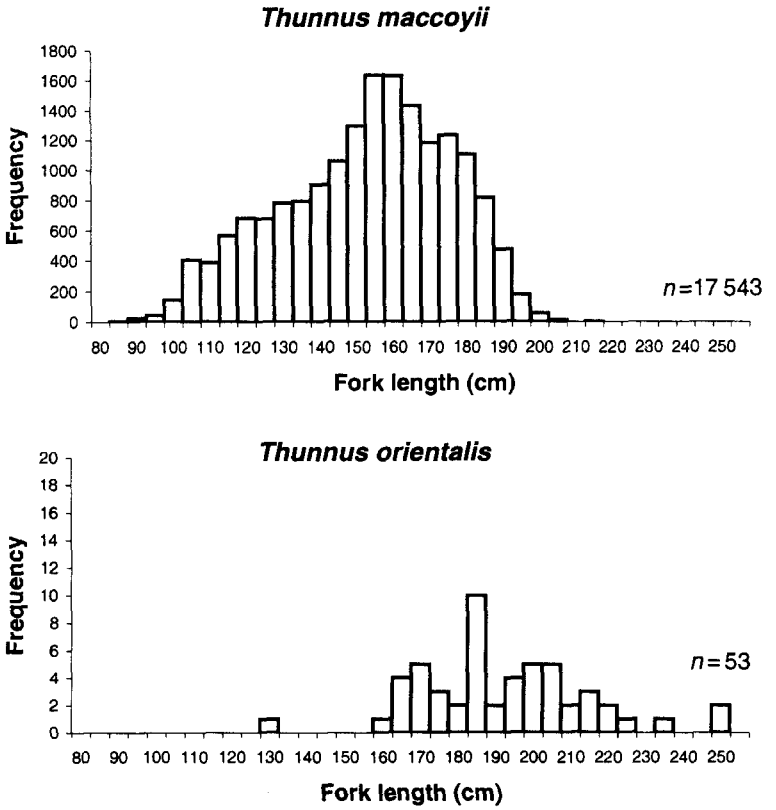


**Fig. 3** Length frequency distributions of *Thunnus orientalis* and *T. maccoyii* caught on observed vessels in the New Zealand Exclusive Economic Zone.

size of the dorsal bulge were confirmed as *T. orientalis* by the DNA test (33 specimens, Table 2).

Some observers noted specimens with unusual colour patterns with blue flecks or speckling above the pectoral fins and sometimes on the head (Table 2). Other unusual colour patterns were a bluish mottled pattern, posterior to the anal fin, and 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). Bluefin tuna specimens that displayed the speckling or mottling colour pattern were always *T. orientalis* when tested for DNA. Observers recorded four specimens with blue flecks or speckling, and 13 with the ventral mottling pattern; for several other specimens the colour patterns were not recorded (Table 2). In addition, observers recorded 10 specimens with darker body coloration, all of which were confirmed as *T. orientalis* with the DNA marker (Table 2).

The length-weight relationship of *T. maccoyii* was compared with that of *T. orientalis*, using fish of comparable size (127 cm fork length (FL) or larger, 36 *T. orientalis* and 14 458 *T. maccoyii*). A *t*-test of equivalent slopes showed a significant difference ($P < 0.05$, d.f. = 8, $n$ = 14 494), confirming that there is a quantifiable difference in the length-weight relationship of the two species, that is consistent with the observers' descriptions of elongated shape in *T. orientalis*. Fig. 3 shows the length frequency distributions for *T. maccoyii* and *T. orientalis*. Fork lengths were recorded for 17 543 *T. maccoyii* from observed tuna longline vessels between 1987 and 1999, and from 53 of the confirmed *T. orientalis*. The *T. orientalis* measured by observers in the New Zealand EEZ are generally larger (mean FL 190 cm, range 127–250 cm) than *T. maccoyii* (mean FL 151 cm, range 82–215 cm). Ninety-percent of *T. maccoyii* were less than 180 cm, whereas 67% of *T. orientalis* were greater than 180 cm in FL.

*Thunnus orientalis* are found throughout the New Zealand EEZ (Fig. 2) but appear to be more common around the North Island with 44 out of 54 records from the North Island, despite c. two-thirds of the observed sets around the South Island.

## DISCUSSION

Specimens of Pacific bluefin tuna *T. orientalis* from Japan and southern bluefin tuna *T. maccoyii* from New Zealand have different mitochondrial DNA haplotypes; amplified fragments of the ATCO region produce species-specific fragments when cut with the diagnostic restriction enzyme *Alu* I or *Mse* I (Chow & Inoue 1993; Chow & Kishino 1995).

Most of the specimens recorded as northern (= Pacific) bluefin tuna in New Zealand waters before 1994 were *T. maccoyii*. These specimens had been identified by large body size and dark colour of the caudal keel. The four specimens identified as *T. orientalis* with the DNA restriction enzyme digests were the same specimens identified as *T. orientalis* with allozyme markers (Smith et al. 1994). The allozyme data (Smith et al. 1994) and the DNA data presented here indicate that most specimens recorded as Pacific bluefin tuna before 1994 had been misidentified and that *T. orientalis* are rare in New Zealand waters. The external characters of caudal keel colour and body colour alone are unreliable field characters for identifying *T. orientalis* in New Zealand waters. Large specimens with a yellow caudal keel are *T. maccoyii* (Gibbs & Collette 1966), but large fish with a dark caudal keel can be either *T. maccoyii* or *T. orientalis*.

Field identification based on the presence of the muscular dorsal bulge in the body cavity is a reliable character in large fish. All 33 specimens identified by this character from 1996 onwards were confirmed by the diagnostic DNA marker (Table 2). However, differences in the shape of the dorsal wall of the body cavity may not be apparent in specimens less than c. 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 Pacific bluefin tuna (127 cm FL) 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.

Dark body coloration was only recorded in specimens of *T. orientalis*. Japanese crew on tuna longline vessels refer to both *T. orientalis* and *T. thynnus* as kuro maguro (= black tuna). Speckled or mottled colour patterns were also restricted to *T. orientalis*. Specimens of bluefin tuna with speckled or mottled colour patterns have not been reported in the scientific literature (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. Any specimen of bluefin tuna in New Zealand waters with unusual coloration (dark body colour, speckled or mottled pattern) and/or dark caudal keels should be considered as a possible *T. orientalis*. Identity can be confirmed by the presence/absence of the dorsal bulge in large (>130 cm) specimens. A small piece of muscle tissue should be fixed in ethanol or frozen for DNA confirmation of small specimens. Recently, Takeyama et al. (2000) developed species-specific luminescent DNA probes, for the ATCO region of mitochondrial DNA that differs in just four nucleotides between *T. thynnus* and *T. orientalis*. Future application of this technology will provide a tool for the rapid identification of large numbers of tuna specimens.

The definition of southern bluefin tuna as "fish with the scientific name *Thunnus maccoyi*; and includes the fish with the scientific name *Thunnus thynnus*" that appears in the southern bluefin tuna quota regulations (Ministry of Fisheries 2000) is incorrect. The definition of southern bluefin tuna should be modified to exclude Atlantic bluefin tuna *Thunnus thynnus*.

*T. orientalis* occur in New Zealand waters but are uncommon. In the New Zealand fishery more than 17 500 specimens of *T. maccoyii* were measured between 1990 and 2000. Over the same time period only 59 *T. orientalis* were confirmed, and thus account for less than 0.3% of all bluefin tuna measured in New Zealand waters.

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