# Biochemical identification of shark fins and fillets from the coastal fisheries in New Zealand

**Peter J. Smith**
**Peter G. Benson**

National Institute of Water and Atmospheric Research Ltd
301 Evans Bay Parade
Wellington, New Zealand
E-mail address (for P. J. Smith): p.smith@niwa.cri.nz

**Abstract**—The protein fingerprints of shark fillets and fins taken from commercial landings in northern New Zealand waters were compared with the protein fingerprints from control samples of ten species of coastal sharks. Isoelectric focusing (IEF) in agarose gels revealed species-specific protein profiles in the ten control species. The fillets and fins were identified as school shark (*Galeorhinus galeus*), rig (*Mustelus lenticulatus*), hammerhead shark (*Sphyrna zygaena*), and bronze whaler (*Carcharhinus brachyurus*). Around 40% of fillets from cartons labelled as lemon fish (*M. lenticulatus*) were from other species. Shark fins were identified from four species, two of which are prohibited target species in northern New Zealand. The large number of mislabelled shark products necessitates the use of a simple biochemical technique for identification of shark species in commercial shark products. With IEF, around 100 specimens can be identified by a laboratory technician each working day from small amounts (<0.5 g) of white muscle.

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Several shark species are common in New Zealand coastal waters and form the basis of fisheries producing fillets for the domestic market and fins for the Asian market. Like most sharks, the New Zealand species are vulnerable to over exploitation owing to their slow growth rate, large size at maturity and low fecundity (Castro et al., 1999). Three shark species are currently managed under the New Zealand Quota Management System (QMS): school shark (*Galeorhinus galeus*), rig (*Mustelus lenticulatus*), and the Callorhinchidae elephant fish (*Callorhinchus milii*) (Annala et al.[1]). Quotas were introduced for these species in 1986 following dramatic increases in catches. Two species, *G. galeus* and *M. lenticulatus*, are caught in set-net fisheries that target coastal sharks, and since the introduction of the QMS, the fisheries have stabilized at around 5000 tons per annum (Annala et al.[1]). A competitive quota has been established for spiny dogfish (*Squalus acanthias*), but other species of shark, either because of low abundance or assumed low value, have been excluded from the QMS. Some of these shark species, such as bronze whaler (*Carcharinus brachyurus*), hammerhead shark (*Sphyrna zygaena*), and blue shark (*Prionace glauca* ), are taken as bycatch in other fisheries but are prohibited as target species.

The shark species in the New Zealand coastal fisheries are large, and whole fish are readily identified by external characteristics. However most sharks are processed at sea: specimens are "trunked"—the head, guts, and fins are removed—and the product chilled or frozen. Further processing may take place on land and the product is sold as either trunks or fillets and fins. It is difficult to identify fillets and fins to the species level and landed catches may include several species. Management and enforcement of multispecies shark fisheries require that landed products can be correctly identified to the species level. Thus a robust test is needed that will allow identification of fillets and fins.

Several molecular genetic methods have been applied to fisheries-related taxonomic problems to identify and distinguish closely related species. Allozymes have been the primary tool for taxonomic problems in fishes (e.g. Dayton et al., 1994; Lacson and Bassler, 1992) and have revealed cryptic species in teleosts (e.g. Lacson, 1994; Smith et al., 1996). More sophisticated and expensive molecular methods, based on DNA extraction, followed by sequencing or restriction enzyme digestion, are increasingly used for similar identification problems (Bartlett and Davidson 1992), including shark species (e.g. Martin, 1993; Heist and Gold, 1998). Isoelectric focusing (IEF) of muscle proteins has been the preferred method for identification of teleost fillets and products (Lundstrom, 1980; Rehbein, 1990); the method is used in legal cases to identify mislabeled fish products. Polyacrylamide IEF (Lundstrom, 1977, 1980) has been adopted by the U.S. Food and Drug Administration for iden-

[1] Annala, J. H., K. J. Sullivan, C. J. O'Brien, and S .D. Iball. 1998. Report from the Fishery Assessment Plenary, May 1998: stock assessments and yield estimates, 409 p. Unpublished report held in NIWA Library, 301 Evans Bay Parade, Wellington, New Zealand.

**Table 1**
Common and scientific names of shark control samples used in the isoelectric focusing analyses.

| Common name | Other common names | Scientific name |
|---|---|---|
| Rig | gummy shark, smoothhound, spotted dogfish, spotted smoothhound, lemon fish, pioke | *Mustelus lenticulatus* |
| School shark | grey shark, tope, flake, makohuarau, tupere | *Galeorhinus galeus* |
| Spiny dogfish | southern spiny dogfish, spurdog, spineback, spikey dog | *Squalus acanthias* |
| Northern spiny dogfish | grey spiny dogfish, shortspine spurdog, green-eyed dogfish | *Squalus mitsukurii* |
| Hammerhead shark | hammerhead | *Sphyrna zygaena* |
| Bronze whaler | whaler | *Carcharhinus brachyurus* |
| Blue shark | blue pointer, blue whaler | *Prionace glauca* |
| Porbeagle | mackerel shark | *Lamna nasus* |
| Mako | mackerel shark | *Isurus oxyrinchus* |
| Carpet shark | swell shark, cat shark | *Cephaloscyllium isabellum* |

tification of fish product (Tenge et al., 1993) and applied to shark species (Weaver et al., 1999); it provides a finer separation of proteins than conventional starch and cellulose acetate electrophoresis, and the muscle proteins exhibit little intraspecific variation. In addition, muscle proteins are stable, withstanding repeated freezing and thawing, and provide a species profile in one gel, unlike allozyme methods, where several gels are required in order to identify a range of species. However, some closely related species of teleosts share protein profiles (Bartlett and Davidson, 1991; Smith et al., 1994; Smith et al., 1996) and thus IEF is not always amenable for distinguishing closely related species. Our study was undertaken to evaluate agarose IEF (Lundstrom, 1981, 1983) of shark muscle proteins in order to provide a quick and robust biochemical method to identify shark fins and fillets from commercial vessels and the market place.

## Materials and methods

### Samples

Control samples were taken from shark specimens caught on research and commercial vessels around New Zealand (Table 1). The specimens were frozen whole at sea and returned to the laboratory where the identity of the species was confirmed through the use of identification keys (Paulin et al., 1989). Samples of white muscle were removed from up to ten specimens of each species. For *Mustelus lenticulatus* and *Galeorhinus galeus*, ten specimens were tested from the Bay of Plenty, North Island, and ten specimens from the east coast South Island. Muscle tissue samples were stored in separate, labelled bags at –70°C. In addition, samples of body muscle from the head, mid, and tail regions were compared with samples of muscle from the base of the pectoral and dorsal fins taken from the same specimens for both *M. lenticulatus* and *Sphyrna zygaena*.

*Mustelus lenticulatus* and *G. galeus* are in the same family of smoothhounds (Triakidae) and lack the characteristic dorsal spine of the spiny dogfishes. Whole specimens of *M. lenticulatus* are distinguished from *G. galeus* by the size of the second dorsal fin, which is nearly as large as the first dorsal fin in *M. lenticulatus*, but much smaller in *G. galeus*, and by their teeth; there are small teeth or grinding plates in *M. lenticulatus* and distinctive large triangular teeth in *G. galeus* (Paulin et al., 1989). Both species are widely distributed in temperate coastal waters and total recorded landings in New Zealand are around 3200 tons for school shark and 1800 tons for *M. lenticulatus* (Annala et al.[1]). Two species of spiny dogfish (Squalidae) are common in New Zealand waters, the shortspine dogfish (*Squalus mitsukurii*) in northern waters and the spiny dogfish (*Squalus acanthias*) in southern waters. The hammerhead shark (*Sphyrna zygaena*, family Sphyrinidae) was included because small specimens are caught in northern waters of New Zealand. The species has a characteristic black margin to the fins, but we have noted that juvenile, but not adult, *G. galeus* also have a black margin on the dorsal fin. Two species of requiem sharks (family Carcharhinidae), *Carcharhinus brachyurus* and *Prionace glauca*, are also common in coastal waters but are prohibited as target species in northern New Zealand. *Lamna nasus*, *Isurus oxyrinchus*, and *Cephaloscyllium isabellum*, which are caught in northern New Zealand, were also included as controls (Table 1).

Three-hundred and eighty shark fins were supplied by Ministry of Fisheries staff from commercial shark fisheries in the Bay of Plenty in northern New Zealand. In the laboratory, a small piece of muscle tissue was removed from each fin and stored at –70°C. The color and shape of each fin was noted and the maximum length between the flesh area and the fin tip was recorded; fins were stored frozen at –20°C. Eight 10-kg cartons of shark fillets, all labelled as lemon fish (=*M. lenticulatus*), were tested. A small piece of muscle tissue was removed from each individual fillet, labelled, and stored at –70°C prior to isoelectric focusing.

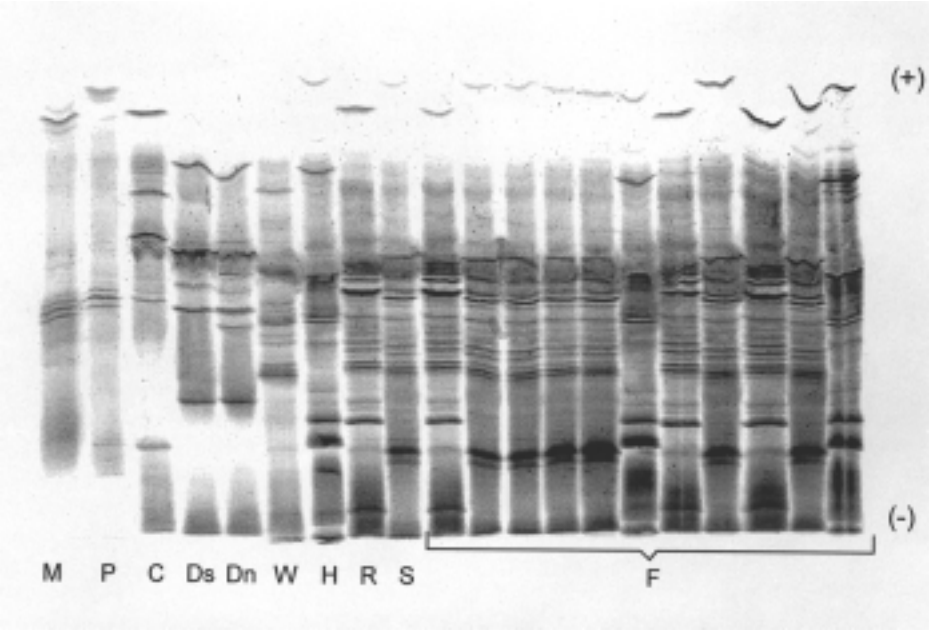


**Figure 1**

Isoelectric focusing plate of muscle protein fingerprints of nine species of New Zealand sharks and the protein fingerprints of some suspect fillets, all labeled as lemon fish (*M. lenticulatus*). M = *Isurus oxyrinchus*, P = *Lamna nasus*, C = *Cephaloscyllium isabellum*, Ds = *Squalus acanthias*, Dn = *Squalus mitsukurii*, W = *Carcharhinus brachyurus*, H = *Sphyrna zygaena*, R = *Mustelus lenticulatus*, S = *Galeorhinus galeus*, F = fillets labelled as *M. lenticulatus*.

## Isoelectric focusing

Small samples (about 0.5 g) of white muscle were removed from each of the tissue samples taken from the fins and fillets and from control samples taken from known specimens of New Zealand shark species. The muscle samples were homogenized individually in two volumes of cold (4°C) deionised water and centrifuged at 12,000 g for five minutes at 4°C. The clear supernatants were placed on filter paper wicks that were placed directly onto agarose IEF gels (Amersham Pharmacia Biotech, Uppsala) on a flat bed IEF system (Amersham Pharmacia Biotech, Uppsala). The 1-mm 1% agarose gels were made up in wide range pharmalyte, pH 3–10 (Amersham Pharmacia Biotech, Uppsala), and focused at 1500 volts for 90 minutes. After focusing, the proteins were fixed, washed, stained with coomassie brilliant blue (BDH Laboratory Supplies, Poole), destained, and dried (Benson and Smith, 1989). An initial gel was run with control samples only to ensure that each species produced a unique protein fingerprint. In addition, samples of body muscle from the head, mid, and tail regions were compared with samples of muscle from the base of pectoral and dorsal fins taken from the same specimens of *M. lenticulatus* and *S. zygaena*. Twenty-five suspect and seven control samples were run on subsequent gels. The same control samples were used in each IEF gel to avoid mismatches between gels.

## Results

The muscle tissue samples from the control specimens produced different protein fingerprint patterns in each species (Fig. 1). Tests of samples of body muscle and fin muscle from the same specimen produced the same IEF pattern, demonstrating that the muscle control samples were suitable for identification of fillet or fin samples. Samples of *M. lenticulatus* and *G. galeus* from the Bay of Plenty and east coast South Island showed no intraspecific variation in protein profiles.

The fins could be grouped into three types based on shape: dorsal, pectoral, and caudal (or tail). There was a wide range of sizes from 7 to 28 cm. It is possible that some small dorsal fins may have been anal fins or second dorsal fins. Most fins were of a pale gray color, but some were a darker gray color and had a narrow black margin; most of these latter fins had a narrower shape than the pale gray dorsal fins. A few pectoral fins were dark gray on one surface and light gray on the other surface. None of the fins had a spine or showed sign of a spine having been cut out.

The protein fingerprints of most of the suspect shark fins matched with one of the fingerprint patterns of *M. lenticulatus*, *G. galeus*, *S. zygaena*, or *C. brachyurus* (Table 2). Four of the 392 fins produced a weak and indistinct fingerprint pattern that could not be matched to any of the control samples. Around 10% of the fins were from two spe-

**Table 2**

Number of shark fins and fillets identified to species level with isoelectric focusing of muscle proteins. All of the fillets were from commercial cartons labeled as lemonfish (*Mustelus lenticulatus).* No ID = not identified because of a weak protein fingerprint.

| Fin or fillet | *M. lenticulatus* | *G. galeus* | *S. zygaena* | *C. brachyurus* | No ID |
|---|---|---|---|---|---|
| Pectoral fin | 127 | 69 | 0 | 7 | 0 |
| Dorsal fin | 81 | 46 | 25 | 3 | 3 |
| Tail fin | 0 | 6 | 22 | 1 | 1 |
| Unidentified | 1 | 0 | 0 | 0 | 0 |
| Fillet | 113 | 27 | 28 | 26 | 1 |

cies that are prohibited as target species, *S. zygaena* and *C. brachyurus* (Table 2). None of the protein fingerprints of the fin samples matched with the control samples from *S. mitsukurii*, *S. acanthias*, *P. glauca*, *L. nasus*, *I. oxyrinchus*, or *C. isabellum*.

Protein fingerprints of fillets from cartons labelled as lemon fish (*M. lenticulatus*) showed that the fillets were from four shark species, two of which are prohibited as target species, *S. zygaena* and *C. brachyurus* (Table 2), and that 41.8% of the fillets were not the species shown on the carton labels.

## Discussion

To identify the suspect shark fillets and fins, it is essential to use a test that can distinguish closely related species of sharks. Occasionally closely related pairs of species are found with very similar protein fingerprints, e.g. the teleosts tarakihi and king tarakihi, in which case additional biochemical tests are sought to identify specimens (Smith et al., 1996). The muscle tissue samples from the shark control specimens produced different protein fingerprint patterns in each species (Fig 1.). Samples of body muscle and fin muscle from the same specimen produced the same IEF pattern. In addition, samples of *M. lenticulatus* and *G. galeus* from the Bay of Plenty in the North Island and east coast South Island showed no intraspecific variation in protein profiles. These observations demonstrate that muscle protein profiles are an appropriate tool for the identification of shark fillets or fins taken in the New Zealand coastal fishery.

It is not possible to quantify the results and state how many fish specimens have been sampled in the fins and fillets. Each fish may yield two pectoral, two dorsal, one caudal, and one anal fin, but fishermen may discard small or damaged fins. Likewise with fillets, each fish may yield a minimum of two fillets, but four or more fillets may be taken from large specimens. Nevertheless the results indicate that both quota and nonquota species are being landed for the fillet and fin markets, and that the domestic market has cartons of mislabelled species. Around 40% of the fillets tested in our study were not the species on the label—*M. lenticulatus* (Table 2). Such observations demonstrate that shark landings recorded in New Zealand waters may be inaccurate, which will not only confound catch statistics but may compromise assessments upon which regulatory decisions are made. The mislabeled fillets identified in our study suggest that effort is targeting non-ITQ species or that prohibited target species such as *Carcharhinus brachyurus* (prohibited in area 1), and *Sphyrna zygaena* (prohibited in all areas) are being landed.

Unlike other biochemical techniques, such as allozyme and DNA markers, the protein fingerprints revealed by IEF show little intraspecific variation (Lundstrom, 1981). Most individuals from the same species have identical protein fingerprints. When protein fingerprints vary among individuals from the same species, the differences are restricted to the presence or absence of one or a few of the protein bands; the majority of bands are shared among all individuals.

Isoelectric focusing is a relatively quick and cheap identification technique (Lundstrom, 1981) compared with DNA-based extraction methods. One operator is able to process and identify up to 100 specimens in one working day. The IEF technique works well with fresh and frozen material and produces clear protein profiles. However, some proteins denature when they are heat-treated (Keenan and Shaklee, 1985). Therefore, shark products, such as sun-dried fins, may require alternative methods, such as polyacrylamide gel electrophoresis of parvalbumins (Keenan and Shaklee, 1985) or DNA-based methods (Martin, 1993; Heist and Gold, 1998), for species identification.

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