POPULATION GENETICS, ECOLOGY AND SYSTEMATICS OF INDO-AUSTRALIAN SCOMBRID FISHES, WITH PARTICULAR REFERENCE TO SKIPJACK TUNA (*KATSUWONUS PELAMIS*)

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DECLARATION

The research carried out during this study and results presented are, except where acknowledged, my original work.

A.D. Lewis
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Table 2.1

Members of the family Scombridae occurring

TABLE OF CONTENTS

ABSTRACT

CHAPTER 1 INTRODUCTION 1

CHAPTER 2 THE BIOLOGY AND ECOLOGY OF SCOMBRID FISHES 5

2.1 The family Scombridae 5
2.2 The epipelagic habitat 33
2.3 Biology of skipjack tuna 36
2.3.1 Functional morphology 36
2.3.2 Distribution 38
2.3.3 Early life history 40
2.3.4 Environmental correlates of adult distribution 42
2.3.5 Reproduction 46
2.3.6 Schooling behaviour 48
2.3.7 Age, growth and size 49
2.3.8 Nutrition 52
2.3.9 Migration 53

CHAPTER 3 TAGGING EXPERIMENTS WITH SKIPJACK TUNA 55

3.1 Introduction 56
3.1.1 Assumptions 56
3.1.2 Types of movement 57
3.1.3 Previous work 59
3.2 The fisheries 61
3.2.1 The Papua New Guinea Fishery 66
3.3 Releases of tagged skipjack 70
3.3.1 Planning 70
3.3.2 Tagging techniques 72
3.3.3 Releases 74
3.3.4 Size of release 74
3.4 Returns of tagged skipjack 80
3.4.1 Data analysis 80
3.4.2 Total returns 84
3.4.3 Returns by time strata 85

3.4.4 Dispersal 85
3.4.5 Tag loss 89
3.5 Movements within the Bismarck Sea and environs 91
3.5.1 1972 releases 91
3.5.2 Between-year variation in K04 releases 94
3.5.3 Other releases 97
3.5.4 Size-related effects 101
3.5.5 Integrity & composition of schools 105
3.6 Relationships with adjoining areas 106
3.6.1 Emigration 106
3.6.2 Immigration 112
3.7 Discussion 118
3.7.1 Local movements 118
3.7.2 A general hypothesis regarding skipjack movement 118
3.7.3 Gene flow 121
3.7.4 Predictions 124

CHAPTER 4 POPULATION GENETICS OF SKIPJACK TUNA 125

4.1 Introduction 126
4.1.1 The nature of genetic variation 126
4.1.2 Population studies 128
4.1.3 Modes of intraspecific variation 132
4.1.4 Previous studies 134
4.2 Materials and methods 139
4.2.1 Sampling considerations 139
4.2.2 Field collection 141
4.2.3 Material 142
4.2.4 Electrophoretic procedures 143
4.2.5 Statistical procedures 154
4.3 Geographical variation 156
4.3.1 Sources of data 156
4.3.2 Distribution of genes 163
4.3.3 Distribution of genotypes 169
4.3.4 Interpretation of observed variation 173
4.4 Within-area variation 181
4.4.1 Gene & genotype distribution 181
4.4.2 Replicate sampling 186
4.4.3 Size effects 188
4.4.4 Temporal variation 192

	4.4.5	Cohort continuity	200
	4.4.6	Evaluation of predictions from tagging data	201
4.5	Discussion.		204
CHAPTER 5	GENETIC VARIATION WITHIN THE FAMILY SCOMBRIDAE		213
5.1	Introduction		213
	5.1.1	Criteria for establishing levels of variation	213
	5.1.2	Measures of variation	215
5.2	Material		215
5.3	Methods		218
	5.3.1	Electrophoretic methods	218
	5.3.2	The enzymes	219
5.4	Results		224
	5.4.1	Levels of heterozygosity	224
	5.4.2	Correlation with enzyme structure and function	227
	5.4.3	Correlation with species characteristics	229
5.5	Discussion		232
CHAPTER 6	BIOCHEMICAL SYSTEMATICS OF INDO-AUSTRALIAN SCOMBRIDS		239
6.1	Introduction		239
6.2	Material & methods		241
	6.2.1	Material	241
	6.2.2	Electrophoretic techniques	242
6.3	Results		243
	6.3.1	Observed variation & species identity	243
	6.3.2	Relationships inferred from phenetic analysis	247
	6.3.3	Relationships inferred from cladistic analyses	250
6.4	Discussion		254
	6.4.1	Electrophoresis as a taxonomic tool	254
	6.4.2	Evolutionary history of the Scombridae	255
	6.4.3	Phylogenetic relationships between extict species	256
	6.4.4	Zoogeography of the species	260
CHAPTER 7	CONCLUDING REMARKS		265
REFERENCES			268

ABSTRACT

This thesis is essentially concerned with the use of electrophoretically-detected genetic variation, in combination with mark-recapture data, to describe the genetic structure of a high vagility species, the skipjack tuna (*Katsuwonus pelamis*), in the Indo-Australian region. Implications for management of the resource are then considered, and the results related where possible to other members of the family Scombridae.

With a favourable return rate (7.6%) from the 9547 releases of tagged skipjack, it has been possible to discern cyclical movement patterns. These appear to be related to large scale oceanographic events leading to enhanced productivity. An hypothesis to explain these movements recognizes resident (island associated) and nomadic components of the resource. On the basis of these and subsequent tagging experiments, dispersal of adults in quite limited, with < 1% of nett displacements exceeding 1000 nautical miles. Providing dispersal of planktonic phases is of similar scope, the potential for gene flow within one generation is considerably less than in some other scombrids.

Although hampered to some extent by the reliance on variation at one enzyme locus (serum esterase, E^{1}_{SJ}) the electrophoretic studies produced results compatible with the mark-recapture data. The main feature of this variation was a cline in E^{1}_{SJ} frequencies across 12,000 km, matching the longitudinal extent of both the known spawning areas and the distribution of islands. It therefore appears likely that open ocean-island interactions play an important role in skipjack ecology, and in selective action on the E_{SJ} locus. The relative roles of selection and migration in maintaining the cline remain unclear but an isolation-by-distance model may provide the best fit to the available data.

A notable feature of the cline is that variance at any point is considerably greater than expected by chance. Time-series sampling at a site in Papua New Guinea has shown that this is

independent of fish size and other factors and is related in part to episodic influxes of groups of skipjack with atypical gene frequencies. The complexity and continuity of recruitment into the study area did not allow particular cohorts to be monitored genetically.

Heterozygosities at 26 loci in nineteen Indo-Australian scombrids showed considerable inter-locus and inter-specific variation, with maximum values observed in large highly mobile, widely distributed species. The data do not lend themselves to critical test of neutralist and selectionist hypotheses but have been useful in demonstrating that most scombrids harbour more markers on which to base genetic studies than was the case for skipjack.

The electrophoretic data were able to clarify the taxonomic status of two *Grammatorcynus* morphs, which are clearly good species, and were also used for phenetic and cladistic analyses of inter-specific relationships. These showed good agreement with existing schemes based on morphological characters, but indicated that major groups within the Scombridae have long been separated and interpretations of scombrid evolution which regard morpho-physiological specializations as sequential may need some reappraisal. Zoogeographical studies appear likely to benefit from insights provided by electrophoretic comparisons.

CHAPTER 1

INTRODUCTION

Tunas and their relatives, which together comprise the diverse family Scombridae, possess a suite of characteristics of considerable interest to biologists. As a group they have successfully colonized the vast, nutrient-poor epipelagic zone of the world's oceans; they also possess a range of morphological (Kishinouye, 1923; Magnuson, 1973; Collette, 1978) and physiological adaptations (Carey and Teal, 1966; Stevens *et al.*, 1974; Graham, 1975), including efficient hydrodynamic design, warm-bodiedness and high metabolic rate, which make them the most specialized of fishes with regard to sustained high speed locomotory activity. Members of the family are widely distributed throughout tropical, sub-tropical and temperate areas, and form the basis of substantial commercial, subsistence and recreational fisheries.

Natural populations of such widely distributed species are very unlikely to comprise a single panmictic unit (Li, 1976; Wright, 1969), and some genetic differentiation typically occurs. Apart from its intrinsic evolutionary interest and significance, the extent to which genetic differentiation and other forms of population structuring occur has considerable implications for the management of species subject to intensive harvest. Human exploitative activity is rarely distributed evenly across a species' range, particularly where the harvest is global. Such is the case with many tunas, and in this situation, the possibility that a species might be resolved into a series of partially or wholly genetically isolated populations has considerable appeal to managers. Even where harvesting activity is uniformly distributed, some knowledge of population structure and genetic differentiation is desirable. Ricker (1973) for example, has postulated from stock/recruitment models that where groups with even "partial genetic individuality" (in his case the progeny of spawning aggregations of homing salmon) but differing productivity are fished in common, a smaller sustainable yield than that expected from an equivalent level of exploitation of a genetically homogenous group will be obtained. Considerable effort has

therefore been directed towards identifying and defining intra-specific groupings in fishes generally and tunas in particular (Marr, 1957, 1963). With coastal states acquiring extended jurisdiction over marine resources such efforts, particularly under the aegis of international management bodies, can be expected to increase.

In natural populations, intra-specific groupings are not always definable in strictly genetic terms, that is as Mendelian populations (Dobzhansky, 1955) or even in broader terms as "sub-populations" (Marr, 1957). Demographic, sociological and ecological criteria are also employed to define groupings (Harrison and Boyce, 1972). Indeed, eco-geographical groupings or 'stocks' ("the exploitable group of fish existing in a particular area at a particular time" - Anon., 1976) are widely used as a descriptive basic unit of fisheries management. The concern of this study is however directed towards detecting genetic groupings, or more specifically, the degree to which genetic differentiation occurs within natural populations of scombrid species, and in turn, its possible relevance to the management of these highly mobile, widely distributed species.

In recent years, electrophoretic studies in particular have appeared to offer a convenient means of examining genetic differentiation in fish populations (de Ligny, 1969; Kirpichnikov, 1973; Jamieson, 1974; Allendorf and Utter, 1978). In many cases however, results of these studies have been reported in terms of gene and genotype frequencies without relating the conclusions to the species' ecology, and testing the relationships with independent observations on other populations, or on other species with similar ecologies. The relative roles of gene flow and selection in opposing or maintaining genetic differentiation, for example, are subject to continuing debate. The orthodox neo-Darwinian viewpoint (Mayr, 1963; Dobzhansky, 1970) emphasises the homogenizing and integrating effect of gene flow in minimizing differentiation and, as a corollary, the importance of mechanisms interrupting gene flow and isolating populations in maintaining differentiation. This view has been challenged by Ehrlich and Raven (1969) and others, who maintain that gene flow in nature is much more restricted than commonly thought, that populations completely isolated for long periods often show little differentiation, that gene flow may not be random and that populations freely exchanging genes but under different selective regimes may show marked differentiation. The influence of gene flow is thus

determined by the prevailing selective regime and may in fact enhance divergence (Thoday, 1972).

One scombrid species, the warm water cosmopolitan skipjack tuna *(Katsuwonus pelamis)* was chosen for intensive study, using data obtained by applying electrophoretic techniques to detect genetic variation; such variation is referred to as allozyme variation. To encompass the entire range of the species would be clearly beyond the scope of this study and attention was focussed on the Indo-Australian region, with the Papua New Guinea area as its centre. This combination of species and area was chosen for several reasons,

(i) the international harvest of skipjack, currently considered to be an underexploited species, has been steadily increasing, particularly in the western Pacific. This brings with it the need to better understand the population structure of the species at all levels. The extent to which genetic differentiation occurs is potentially an important aspect of this structuring.

(ii) the biology of skipjack tuna is better known than that of many other scombrid species, an important consideration when attempting to interpret allozyme variation.

(iii) fundamental to studies of genetic differentiation, particularly in organisms of high vagility, is a knowledge of dispersal parameters. Suitable data were available from extensive mark-recapture experiments conducted in the Papua New Guinea area during 1971-75.

After a general introduction to the family Scombridae, skipjack biology has been reviewed in some detail, particularly those aspects relevant to population biology (Chapter 2). The mark-recapture data is analysed (Chapter 3) and allozyme variation in skipjack tuna from both a broad geographical and an isotopic time-series viewpoint, has then been discussed with the benefit of this important background information to identify possible management implications (Chapter 4).

Levels of genetic variation in other Indo-Australian scombrid species, which encompass a considerable ecological and biological diversity, were then investigated (Chapter 5). The observed variation

is assessed both in terms of its potential value as markers in studies such as that undertaken with skipjack and in the light of predictions derived from neutralist selectionist hypotheses. It is recognized, however, that such species, which are not amenable to experimental manipulation, are unlikely to provide data for critical tests of these hypotheses.

The electrophoretic data from the species array are then used to attempt to place the members of the scombrid species array in their phylogenetic and zoogeographic context. Such data is increasingly being used to good effect in a systematic role (Avise, 1975) and the present data provides the opportunity not only to clarify taxonomic status in several cases but also to examine inter-species relationships within the family Scombridae from a phenetic and cladistic viewpoint and in so doing, generate information useful in a zoogeographical sense (Chapter 6).

The present study thus can be viewed on two levels - as an attempt to draw together information from several disciplines - ecology, genetics and systematics - to examine how evolutionary forces working at various levels have shaped the extant members of a marine teleost family and, from a more applied viewpoint, as an attempt to evaluate the usefulness of allozyme data, in conjunction with mark-recapture data, to population studies, and ultimately management, of scombrid fishes.

CHAPTER 2

THE BIOLOGY AND ECOLOGY OF SCOMBRID FISHES

2.1 THE FAMILY SCOMBRIDAE

The most recent classification of the Scombridae (Collette & Chao, 1975; Collette, 1978) recognizes 15 genera and about 50 species in two sub-families, the monotypic and aberrant Gasterochismatinae and the Scombrinae. The Scombrinae is composed of four tribes, the mackerels (Scombrini), Spanish mackerels (Scomberomorini), bonitos (Sardini) and tunas (Thunnini). The distribution of genera and species within these tribes and sub-families is shown in Figure 2.1.

Scombrids are members of the large order Perciformes and their closest relatives include the swordfish (Xiphiidae), marlins and spearfish (Istiophoridae) and luvar (Luvaridae). Although commonly regarded as a flourishing "modern" group, they are known in the fossil record from Upper Cretaceous deposits (Shubnikov, 1974), with most palaeontological finds relating to the Eocene and Oligocene (Danil'chenko, 1964).

Morphological evidence (Collette, 1978) indicates a phylogenetic progression from the primitive tribe Scombrini through to the advanced Thunnini. Many of the external diagnostic characters are adaptations associated with continuous high-speed swimming, e.g. the specialized hypural complex enabling increased tail propulsion; the caudal keels accelerating water flow across the tail and reducing turbulence and drag (Fierstine & Walters, 1968); the forked or lunate tail with high aspect ratio; the dorsal and anal finlets to control cross flow and improve tail beat efficiency; squamation patterns (corselets) to reduce form drag (Walters, 1962) and hydrodynamically efficient shape (Alexander, 1967). Internal modifications include high haemoglobin levels (Klawe *et al.*, 1963) high packed cell volumes (Alexander *et al.*, 1980) and gill modifications associated with ram-jet ventilation, namely very high gill area/body weight ratios (Muir & Hughes, 1969) and lamellar and filamentar fusion (Muir & Kendall, 1968).

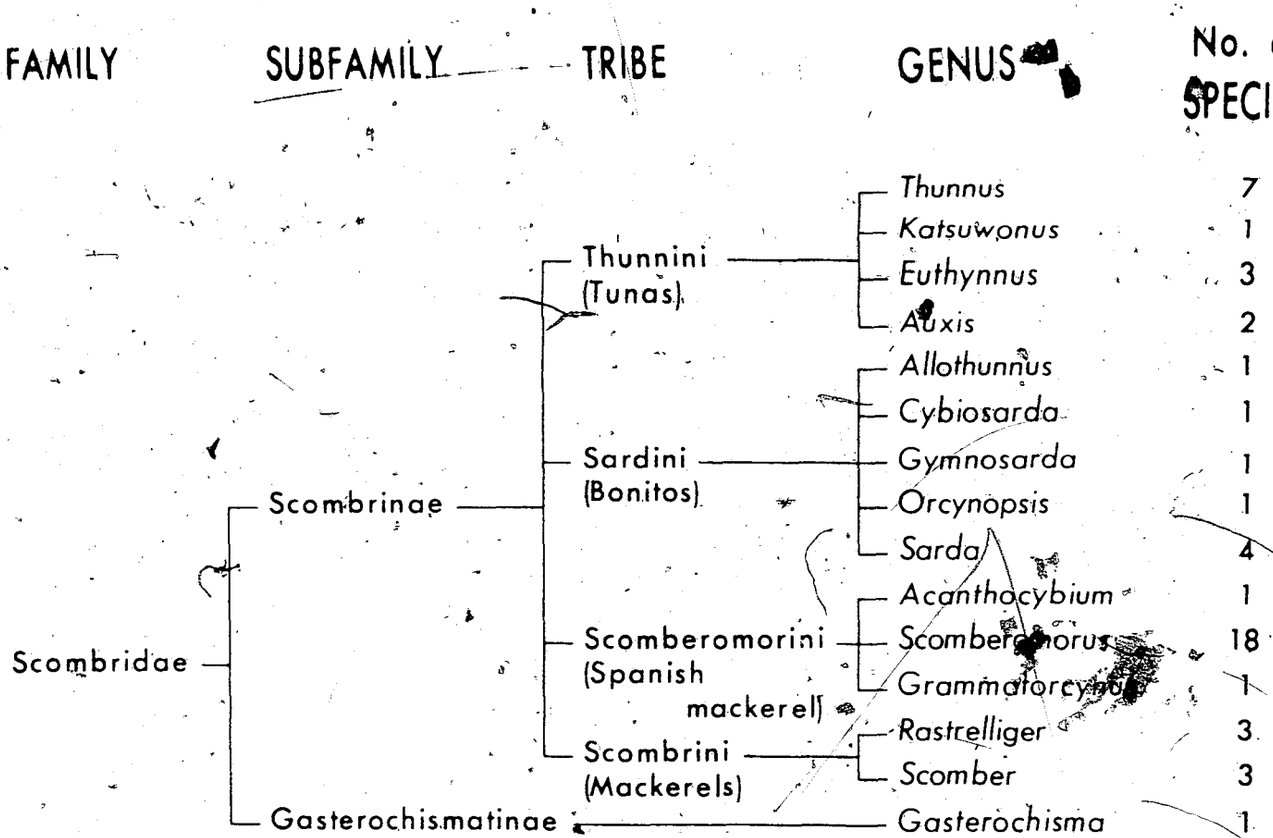


Figure 2.1 The classification of the family Scombridae. Redrawn from Collette (1978).

The most striking developments along this phylogenetic line are seen, however, in:

(a) the increase in proportion of red to white muscle and the degree of internalization of this red muscle (Sharp & Pirages, 1978). Red muscle functions aerobically and provides power for 'cruise' (sustained) swimming, (Rayner & Keenan, 1967), whereas white muscle generally functions anaerobically in providing 'burst' speed;

(b) the development of counter current heat exchangers (retia mirabilia) which conserve metabolic heat and maintain body temperatures warmer than ambient water temperature (Carey *et al.*, 1971; Graham, 1975). This is variously hypothesized to increase muscle power and efficiency, maximum sustained and burst speeds (by increasing red and white muscle temperature respectively), thermal inertia (Stevens and Neill, 1978) and to promote rapid digestion by warming the visceral mass. Some species are also believed to possess thermoregulatory capacity (Dizon & Brill, 1979, and see also Section 2.3.1).

These adaptations, which are considered in detail in Sharp & Dizon (1978), have allowed the advanced scombrids *(Katsuwonus, Thunnus)* to colonize the epipelagic zone where the patchiness of available forage makes the need to minimize time between patches and ability to cover large areas in search of such patches acute. Other fast-swimming groups which have successfully colonized this zone, e.g. the billfishes (Xiphiidae, Istiophoridae) and lamnid sharks, show convergence in some of these adaptations.

The maximum size attained by scombrids varies from less than one kilogram in most mackerels (Scombrini) to more than 700 kilograms in the Atlantic bluefin tuna *(Thunnus thynnus thynnus)*. In the latter case, this is associated with a 10^9 increase in hatching weight (Klawe, 1979). Most species exhibit schooling behaviour to some degree. Dietary opportunism is the rule, although specialized planktivores and active predators of large teleosts are represented. Shubnikov (1974) discusses the origin of ecological groups within the family (neritic, peripheral neritic, neritic-oceanic, and oceanic) relative to their schooling and feeding habits.

Reproduction has not been well studied in the group. Fertilization is external, sexuality normal and mating assumed to be random. Eggs and larvae are invariably pelagic and most species show moderate to high fecundity relative to their size. The fecundity of tunas, for example, approaches 100,000 eggs per kg of body weight. Consistent with their high vagility, many species undertake extensive migrations, either for spawning or feeding.

Thirty species of scombrid fishes, including one recognized as new during the course of this study, occur in the Indo-Australian area, defined here as the waters surrounding Australia, Papua New Guinea and Indonesia. These are listed in Table 2.1. Brief unreferenced descriptions of 23 of these species for which material was collected as part of the present study, follow.

Table 2.1

Members of the family Scombridae occurring in the Indo-Australian region

Common names listed here are used throughout the text

SUB-FAMILY GASTEROCHISMATINAE

Gasterochisma melampus (butterfly mackerel)

SUB-FAMILY SCOMBRINAE

Tribe Scombrini (Mackerels)

Scomber australasicus (slimy mackerel)
Rastrelliger kanagurta (chub mackerel)
R. brachysoma (short-bodied mackerel)
R. faughni (Faughn's mackerel)

Tribe Scomberomorini (Spanish Mackerels)

Grammatorcynus sp. A. (shark mackerel)
Grammatorcynus sp. B. (scad)
Scomberomorus commerson (narrow banded mackerel)
S. queenslandicus (Qld. school mackerel)
S. munroi (spotted mackerel)
S. semifasciatus (grey mackerel)
S. multiradiatus (Papuan mackerel)
S. lineolatus (streaked mackerel)
S. guttatus (Indo-Pacific mackerel)
Acanthocybium solandri (Wahoo)

Tribe Sardini (Bonitos)

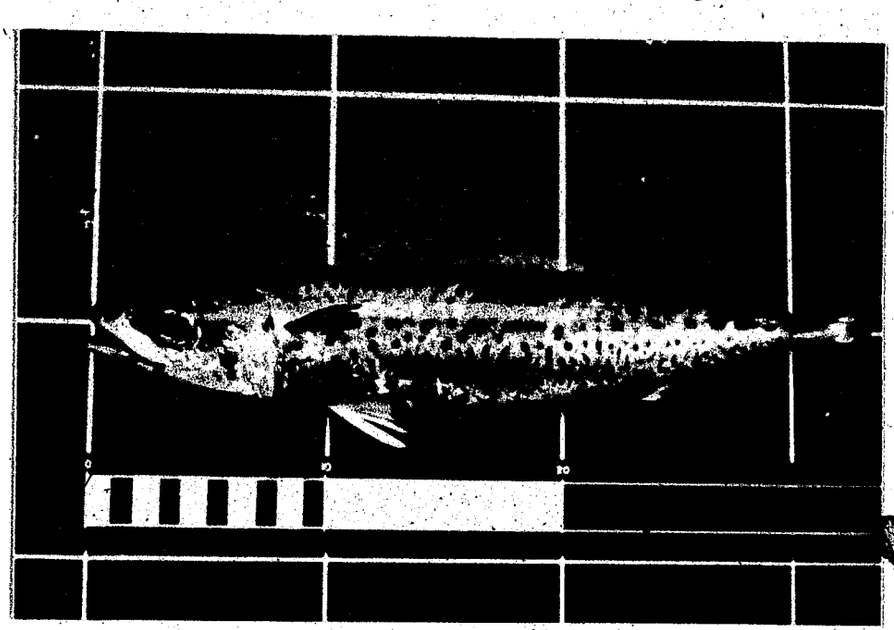
Sarda australis (Australian bonito)
S. orientalis (oriental bonito)
Cybiosarda elegans (leaping bonito)
Gymnosarda unicolor (dogtooth tuna)
Allothunnus fallai (slender tuna)

Tribe Thunnini (Tunas)

Auxis thazard (frigate tuna)
A. rochei (bullet tuna)
Euthynnus affinis (mackerel tuna)
Katsuwonus pelamis (skipjack tuna)
Thunnus albacares (yellowfin tuna)
T. tonggol (longtail tuna)
T. obesus (bigeye tuna)
T. alalunga (albacore)
T. maccoyii (southern bluefin tuna)
T. thynnus orientalis (oriental bluefin tuna)

Scomber australasicus Cuvier, 1831

Slimy or blue mackerel



Distribution: Western Pacific Ocean from Japan to southern Australia, straggling eastwards to some eastern Pacific islands.

Maximum Size: 40cm, common 20-30cm.

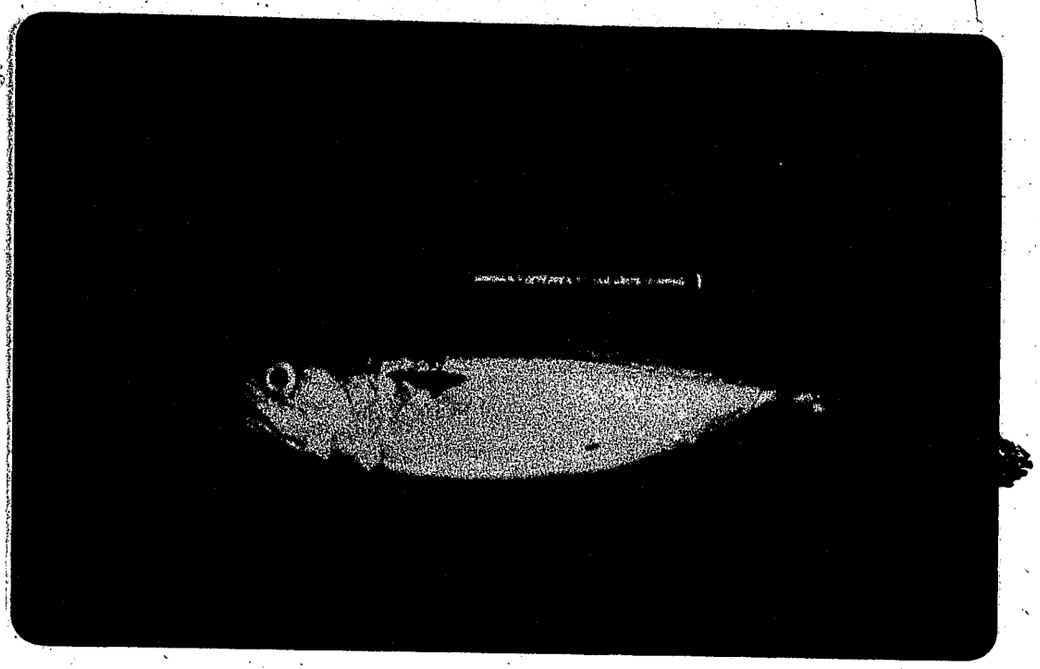
Habitat: Coastal waters, in large schools at varying depths; planktivorous.

Abundance: More abundant in temperate waters where it is commercially exploited.

Comments: S. *japonicus* a polytypic cosmopolitan species, occurs in Pacific waters near Japan and in the eastern Pacific where it is however uncommon in the tropics. S. *australasicus* takes its place in the south-western and tropical western Pacific (uncommon in the latter) and the two species may be mutually exclusive.

Rastrelliger kanagurta (Cuvier, 1817)

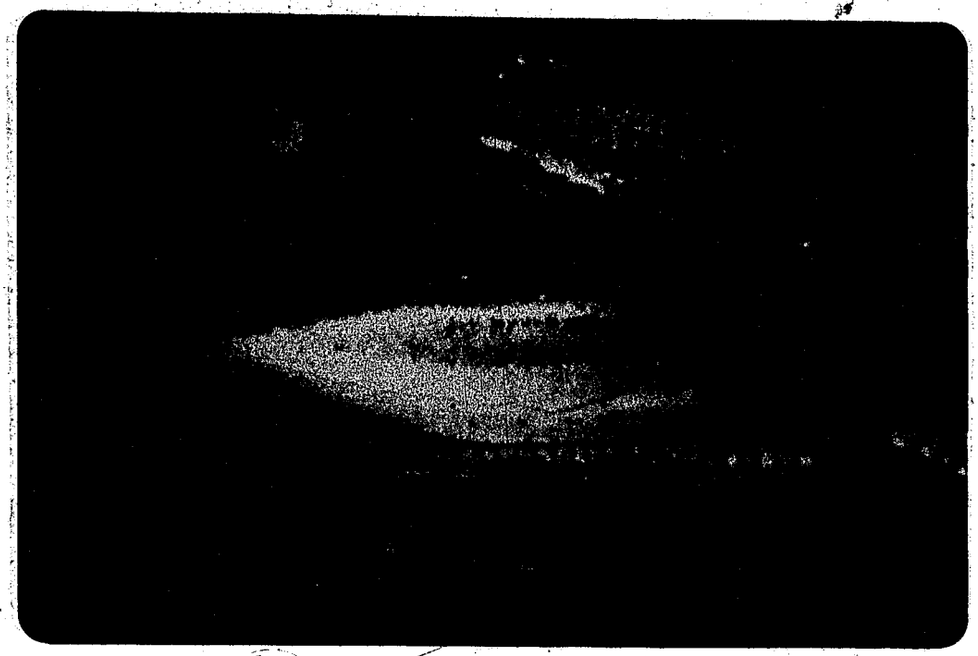
Chub or Indian mackerel



Distribution:	Indo-Pacific coastal waters from east Africa to Micronesia and Melanesia, north to Japan and south to 25°S in Australian waters.
Maximum Size:	35cm, commonly 15-30cm.
Habitat:	Coastal waters, usually occurring in large schools; planktivorous.
Abundance:	Supports very large fisheries in productive S.E. Asian waters.
Comments:	Two less common cogeners have a more restricted range centred on the Indo-Malayan area. R. *brachysoma* is occasionally taken in trawls in northern Australia. The recently described R. *faughni* (Matsui, 1967) has been reported from Papua New Guinea (Lewis *et al.*, 1974) and probably occurs in northern Australia.

Grammatorcynus sp. A.

Shark mackerel



0 25 50cm

Distribution: In Australia, tropical waters with occasional stragglers to 30°S on both east and west coasts. Also known from the Gulf of Papua. Elsewhere, distribution uncertain.

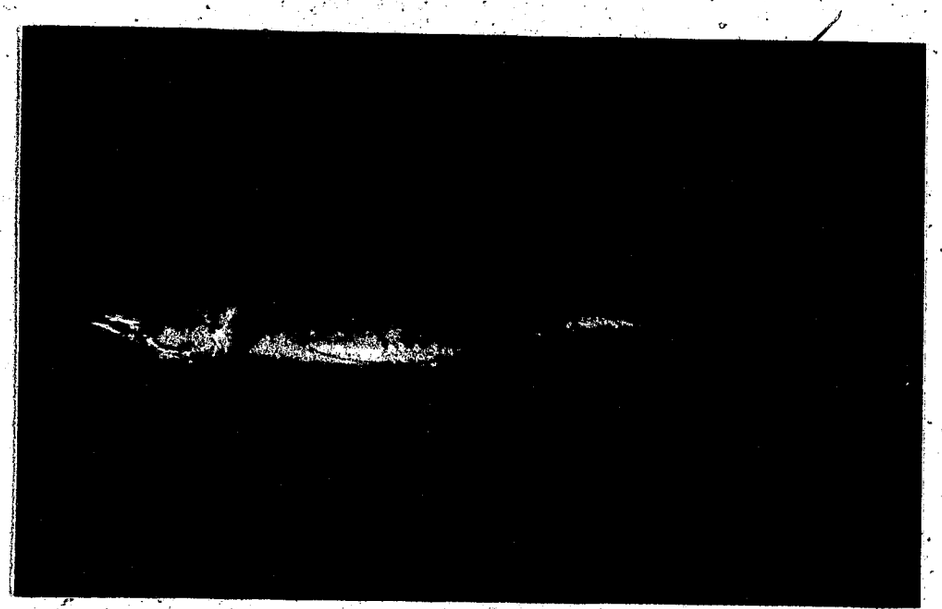
Maximum Size: 13.5 kgs. (110 cm).

Habitat: Usually the lee side of coral reefs; occasionally in more turbid water.

Comments: Two morphs have been recognized in the study and their specific status is examined later. As only one species, *G. bicarinatus*, is currently recognized, details of the distribution and biology of the two morphs will require clarification.

Grammatorcynus sp. B

Scad



0 10 20cm

Distribution: In Australia, probably rare south of 25°S. Occurs throughout Papua New Guinea. Distribution elsewhere uncertain.

Maximum Size: Probably 60cm (<3 kgs).

Habitat: Adjacent to coral reefs and cays; more frequently on the ocean side.

Abundance: Not often seen at the surface, but suspected to be abundant at intermediate depths, on the basis of echo soundings and the abundance of juveniles probably attributable to this species in dip net catches.

Scomberomorus commerson (Lacepede, 1800)

Narrow-banded spanish mackerel

0 10 20

Distribution: Indian Ocean and western Pacific Ocean in coastal waters, from east Africa to Fiji. A recent immigrant into the Mediterranean Sea. Rare south of 32°S (Perth, Kempsey) in Australian waters.

Maximum Size: 230cm (59 kg), commonly 60-120 cm. The largest member of the genus.

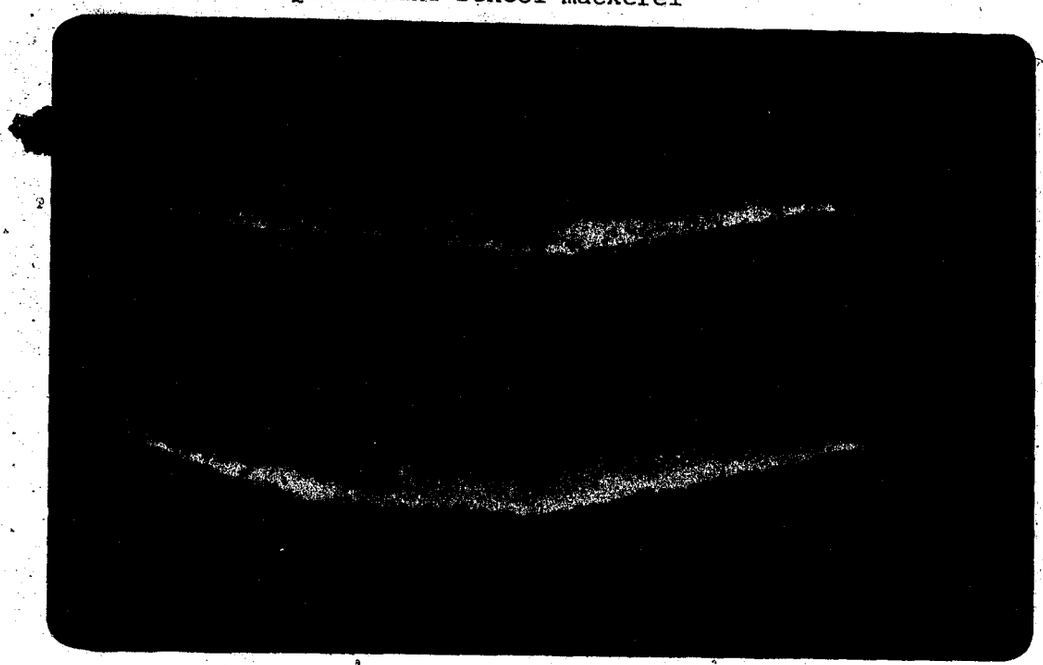
Habitat: Coastal waters at all depths, found in small schools as juveniles, becoming more solitary with age.

Abundance: The most abundant member of the genus and forms the basis of important subsistence and commercial fisheries in many areas.

Comments: Adults frequently undertake lengthy seasonal longshore migrations, as apparently do several of its cogeners viz. *munroi* and *queenslandicus*.

Scomberomorus queenslandicus Munro, 1943.

Queensland school mackerel



0 10cm

Distribution: Northern Australia between northern NSW and Shark Bay (WA); the Gulf of Papua and possibly Fiji.

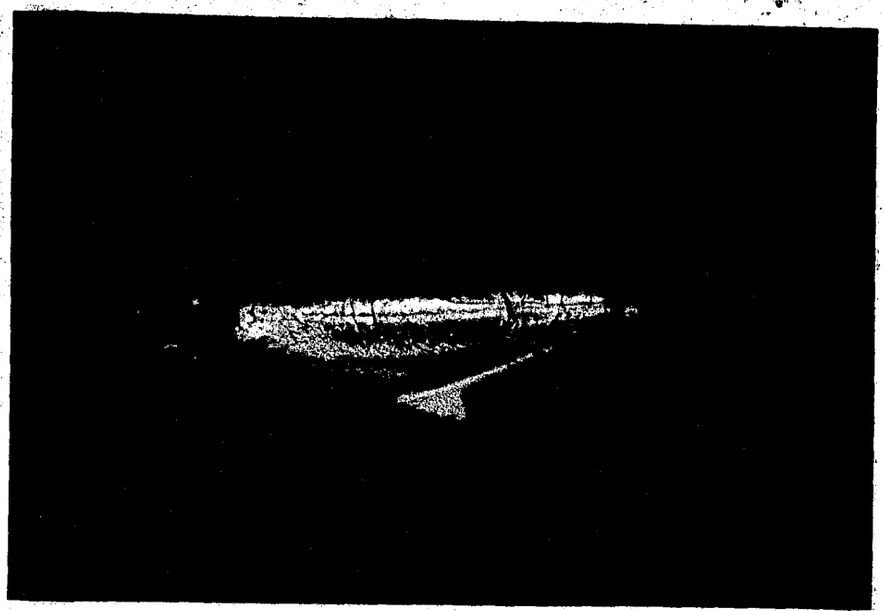
Maximum Size: 100cm (8 kgs), usually 50-80 cm.

Habitat: Inshore coastal waters, generally in small schools.

Abundance: Never as common as *commerson* but frequently found in association with that species, makes an important contribution to commercial catches.

Scomberomorus multiradiatus Munro, 1964

Papuan (spanish) mackerel



0 10cm

Distribution: Turbid waters of the Gulf of Papua.

Maximum Size: Probably 35cm (0.5 kg).

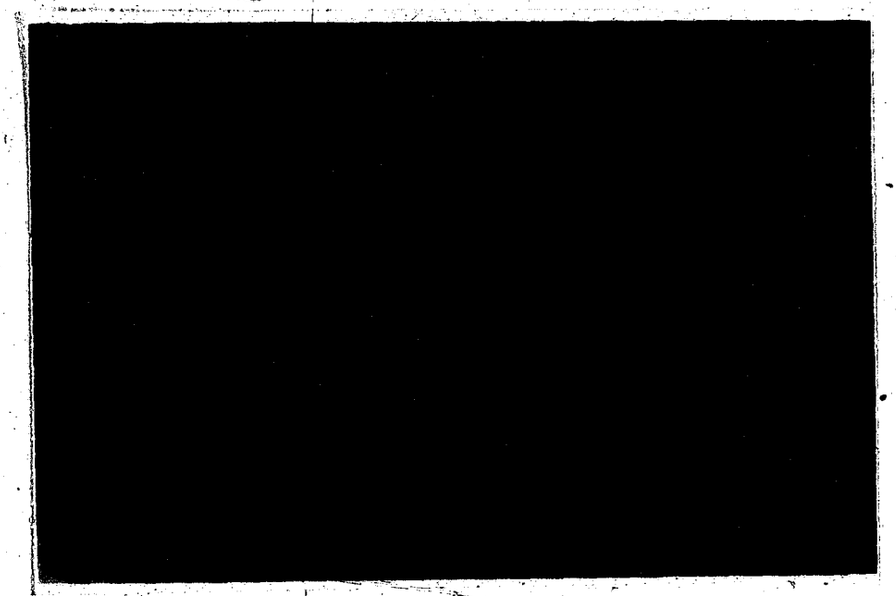
Habitat: Within its very restricted distribution, occurs in shallow near-shore waters only. Schooling and other behaviour unknown;

Abundance: Not uncommon in certain areas within its very limited range.

Comments: The smallest of the 18 known *Scomberomorus* species. Described only recently and biological details virtually unknown, but sexually mature at less than 30 cm.

Scomberomorus semifasciatus (Macleay, 1884)

[illegible]y or broad-barred spanish mackerel



0 10cm

Distribution: Northern Australia between Shark Bay (WA) and northern NSW, and the Gulf of Papua.

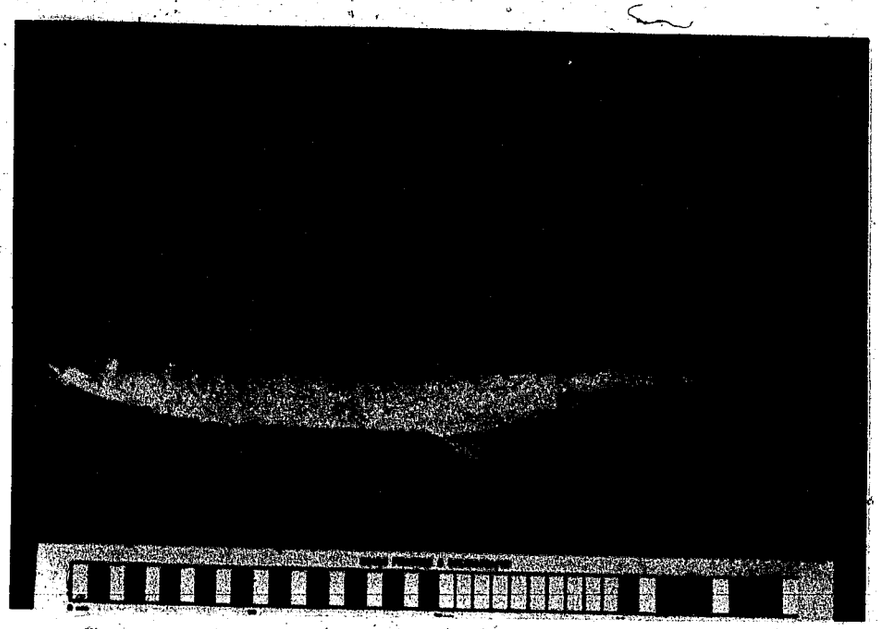
Maximum Size: 120 cm (10 kgs).

Habitat: Coastal waters, but more common in estuarine situations than its cogeners. May form small schools;

Abundance: Nowhere abundant but contribute consistently to commercial catches.

Scomberomorus munroi Collette and Russo, 1980

Spotted spanish mackerel



Distribution: Northern Australia, between Abrolhos Is. (WA) and Kempsey (NSW); and the Gulf of Papua.

Maximum Size: 100 cm (8 kgs), more commonly 50-80 cm.

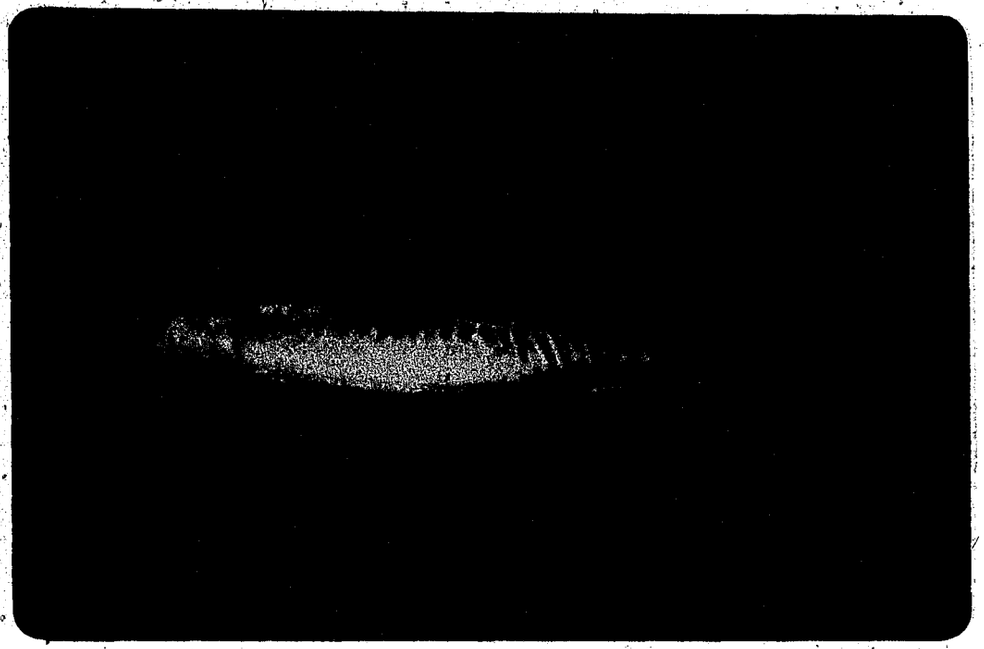
Habitat: Inshore coastal waters - probably in small schools.

Abundance: Nowhere abundant, making incidental contributions to commercial catches.

Comments: Previously grouped with *S. niphonius* a north-western Pacific species, but recently found to be distinct. Its centre of distribution may be subtropical rather than tropical, in contrast to its cogeners.

Acanthocybium solandri (Cuvier, 1831)

Wahoo, ono



20 40cm

Distribution: Cosmopolitan in tropical and sub-tropical waters. Commonly taken seasonally as far south as southern NSW and Perth.

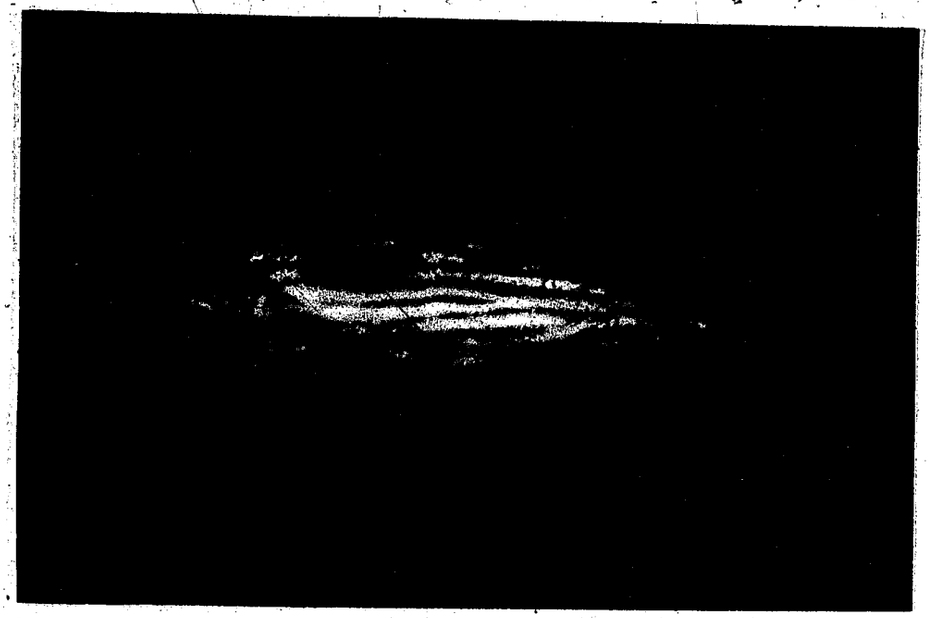
Maximum Size: 70 kgs

Habitat: Oceanic waters, less commonly over continental shelves. Often in association with floating objects and current lines, and generally solitary.

Abundance: Nowhere common. Important as a recreational rather than a commercial species.

Sarda australis (Macleay, 1880)

Australian bonito



Distribution: Restricted to the east coast of Australia between the Tropic of Capricorn and northern Tasmania, and Norfolk Island, with isolated records from New Zealand.

Maximum Size: Probably 8-10 kgs (90 cm).

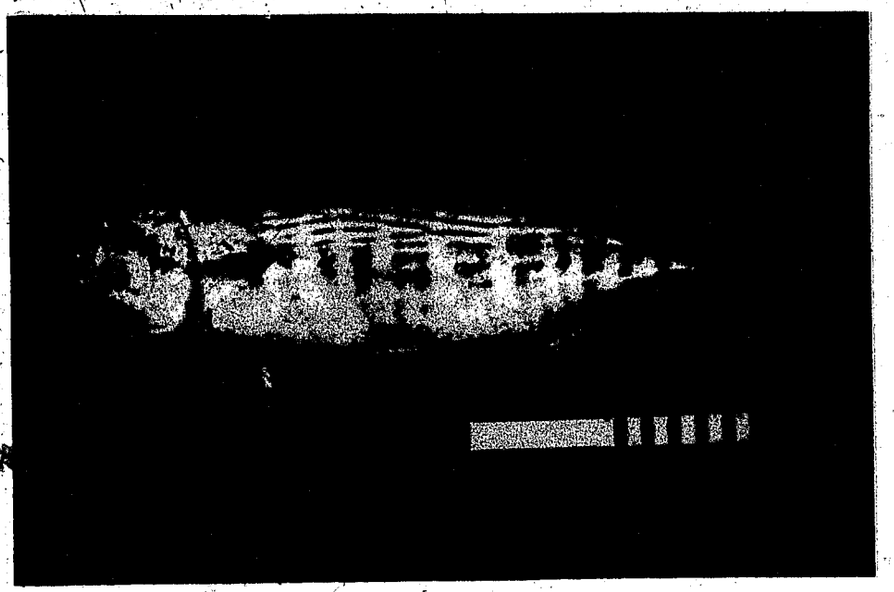
Habitat: Inshore coastal waters, but rarely entering estuaries. Occur in large surface schools.

Abundance: Sporadically abundant; other *Sarda* species support important coastal fisheries elsewhere.

Comments: Little is known of the ecology and life history of any of the bonitos occurring in the area.

Sarda orientalis (Temminck and Schlegel, 1884)

Oriental or Indo-Pacific bonito



Distribution: Indo-Pacific tropical and sub-tropical areas, between South Africa and South America, but with large apparent gaps in distribution. Recorded from Indonesia for the first time during this study.

Maximum Size: 80 cm; unconfirmed reports of larger fish.

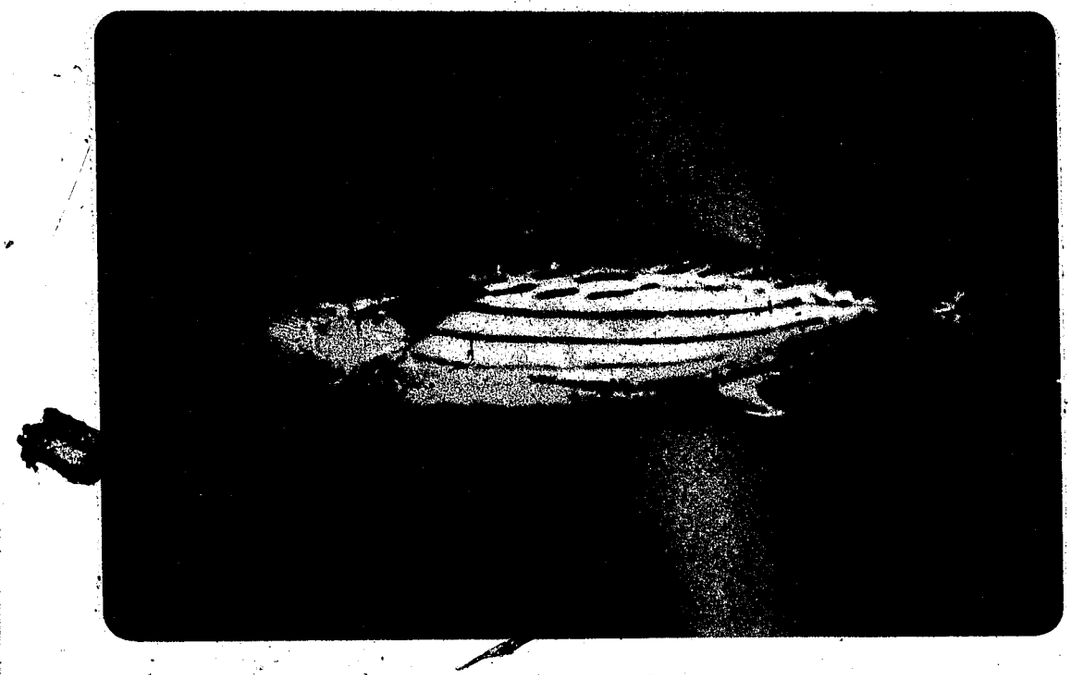
Habitat: Occurs at all depths, commonly below surface and hence less conspicuous than other species; appears to form schools, but large fish probably solitary;

Abundance: Nowhere abundant, local occurences patchy.

Comment: Found only on the west and north coasts in Australian waters. Apparently replaced by the endemic *S. australis* on the east coast.

Cybiosarda elegans (Whitley, 1935)

Leaping bonito



0 10cm

Distribution: Northern Australia, between Sydney and Perth, and southern Papua New Guinea.

Maximum Size: 50 cm (2 kgs).

Habitat: Inshore coastal waters; commonly encountered in small surface schools but not often captured.

Abundance: Apparently more common in tropical areas.

Comments: The smallest of the bonitos and also the only scombriel genus endemic to the area.

Gymnosarda unicolor (Rüppell, 1838)

Dogtooth tuna

0 10 20cm

Distribution: Coral reefs of the tropical Indo-Pacific from the Red Sea to French Polynesia. Unknown south of the Tropic of Capricorn in Australia.

Maximum Size: 70 kgs.

Habitat: A large solitary species inhabiting deep water near reef drop-offs. A lurking rather mobile predator; ecology and life history poorly known.

Abundance: Apparently uncommon, but not a conspicuous species.

Comments: With *Scomberomorus commerson* and wahoo, the largest of the pre-*Thunnus* species.

Allothunnus fallai Serventy, 1948

Slender tuna

Distribution: Cosmopolitan in southern oceans, south of 20°S. Isolated reports elsewhere.

Maximum Size: Possibly 100 cm.

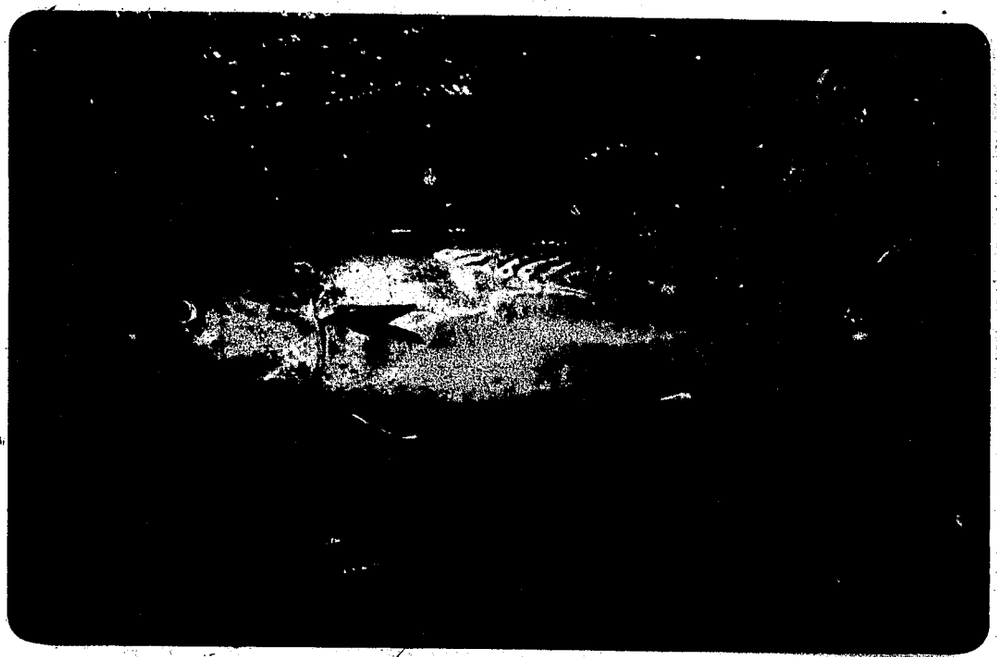
Habitat: Schools in midwater; essentially planktivorous; commonly co-occurs with *Gasterochisma* and *Thunnus maccoyii*

Abundance: Thought to be rare until large catches made off Tasmania in the early 1970's; possibly quite abundant in the West Wind Drift.

Comments: The species was described as recently as 1948 and is intermediate in some features between the Sardini and Thunnini.

Auxis thazard Lacepede, 1800

Frigate tuna



0 10cm

Distribution: Cosmopolitan in warm waters; occurs as far south as Tasmania in Australia.

Maximum Size: 50 cm (3.5 kgs), commonly 25-40 cm.

Habitat: Oceanic and coastal waters in large surface shools; feeds on micronekton.

Abundance: Probably exceeded in standing biomass by only skipjack and the Scombrini, but of limited commercial importance.

Comments: Its cogener *A. rochei* shares a similar distribution but occurs more frequently inshore. Not taken during this study and less well known than *thazard*.

Euthynnus affinis (Cantor, 1849)

Mackerel tuna, kawakawa

0 10cm

Distribution: Warm waters of the Indo-Pacific region, with isolated records from the eastern Pacific. Extends seasonally to southern NSW and Perth in Australian waters.

Maximum Size: 90 cm (14 kgs), commonly 35-60 cm.

Habitat: Coastal waters, occurring as small-medium sized surface schools; feeds on micronekton. Rarely encountered offshore.

Abundance: Common in tropical areas, but subject to large local fluctuations in abundance. Replaced by other species in the eastern Pacific (E. *lineatus*) and Atlantic (E. *alletteratus*).

Katsuwonus pelamis (Linnaeus, 1758)

Skipjack tuna

Distribution: Cosmopolitan in warm waters between 40°N and 40°S.

Maximum Size: 22 kgs (100 cm) with unconfirmed reports of larger individuals.

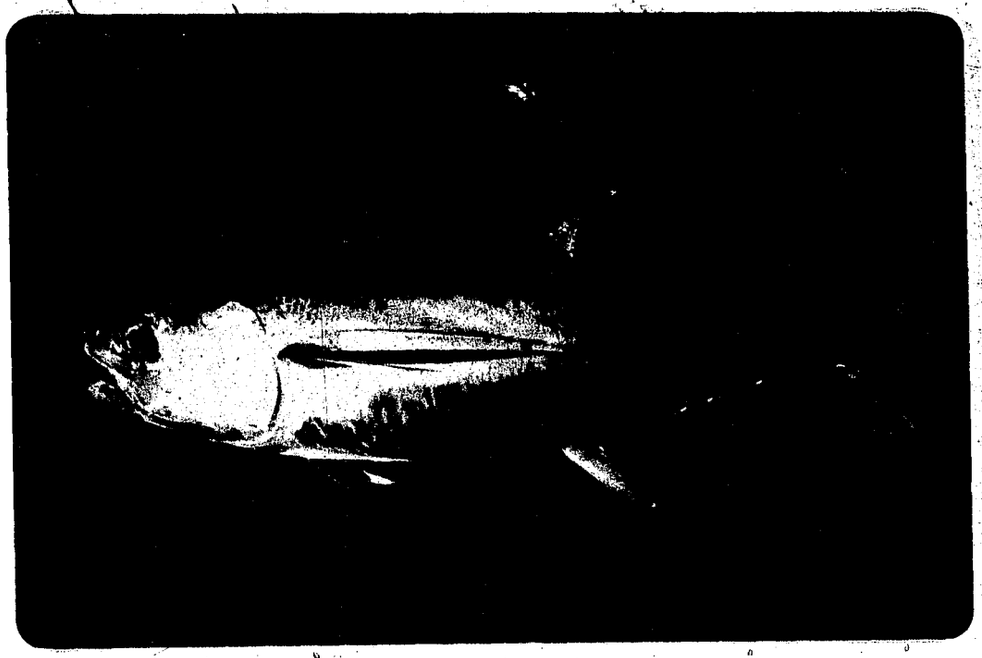
Habitat: The epipelagic zone, but also over continental shelves. Forms very large surface schools; feeds on micronekton, small fishes etc.

Abundance: The most abundant tuna in terms of biomass, but amongst the smallest.

Comments: Skipjack biology is comprehensively reviewed in Section 2.3

Thunnus albacares (Bonnaterre, 1788)

Yellowfin tuna



0 20cm

Distribution: Cosmopolitan in warm waters between 35°N and 35°S.

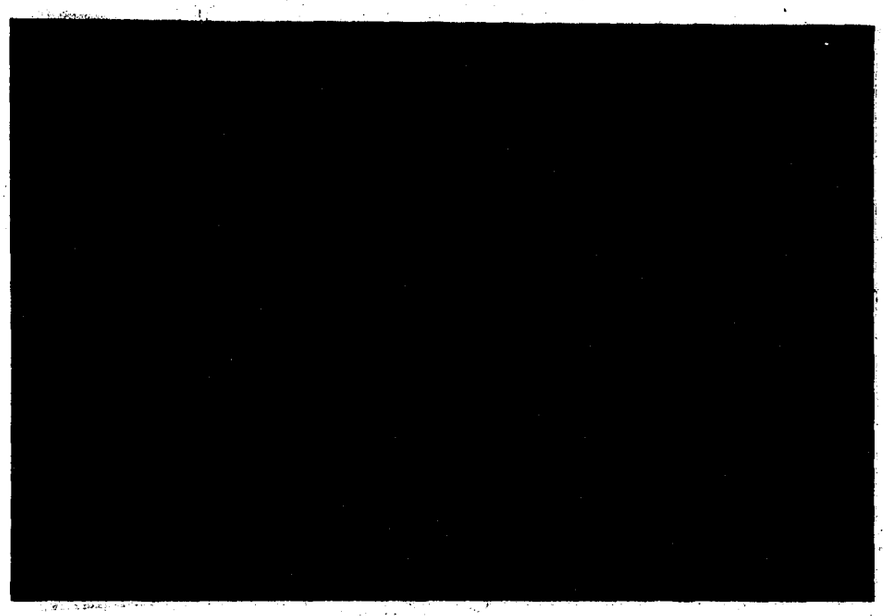
Maximum Size: 180 kgs (195 cm), usually 50-150 cm.

Habitat: Oceanic and near-shore. Occurring in surface schools - frequently in association with skipjack and bigeye as juveniles; spending most time as adults lower down in the vertical profile. Feeds on micronekton, schooling fish etc.

Abundance: Provides the largest commercial catch of tunas after skipjack.

Thunnus tonggol (Bleeker, 1851)

Longtail tuna



0 20cm

Distribution: Warm waters of the Indo Pacific from the Red Sea to Papua New Guinea and southwards to southern NSW.

Maximum Size: Probably 35 kgs.

Habitat: Strictly a neritic species; occurs in small groups rather than large schools;

Abundance: Nowhere common; makes incidental contributions to commercial catches.

Comment: Doubts have been raised during this study about the taxonomy of this "species" which may possibly comprise two allopatric sub-species or species. A similar species (T. *atlanticus*) occurs in the western Atlantic, and these two species are the only exclusively neritic *Thunnus* species.

Thunnus obesus (Lowe, 1839)

Bigeye tuna

0 10cm

Distribution: Cosmopolitan in warm and temperate waters between 45°N - 45°S.

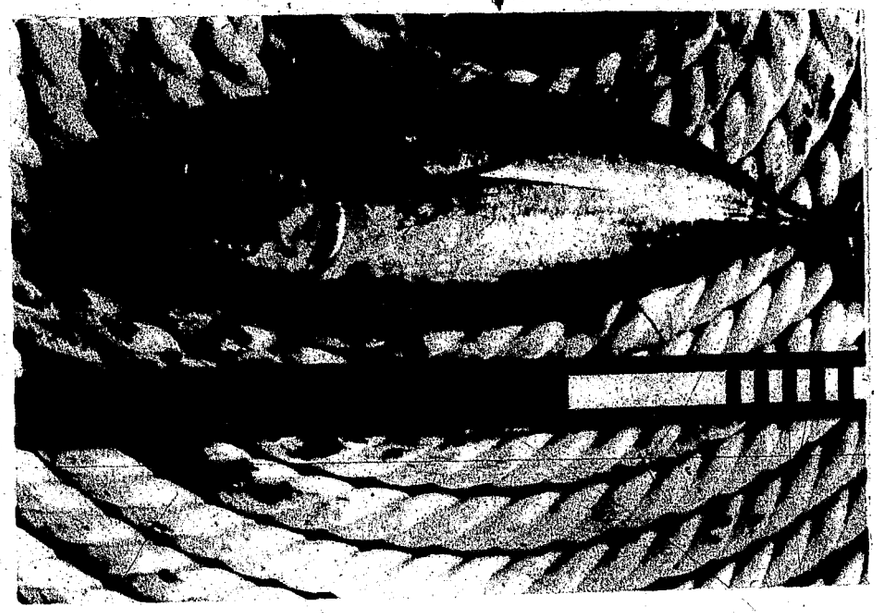
Maximum Size: Approximately 200 kgs.

Habitat: Oceanic, with juveniles schooling at the surface in tropical areas; and adults near or below the thermocline; feeds on micronekton etc.;

Abundance: With the use of gear which fishes deeper, has proved to be more abundant than previously believed.

Thunnus alalunga (Bonnaterre, 1788)

Albacore



Distribution: Cosmopolitan between 50°N and 50°S, although not at the surface in tropical areas, and absent from the eastern tropical Pacific.

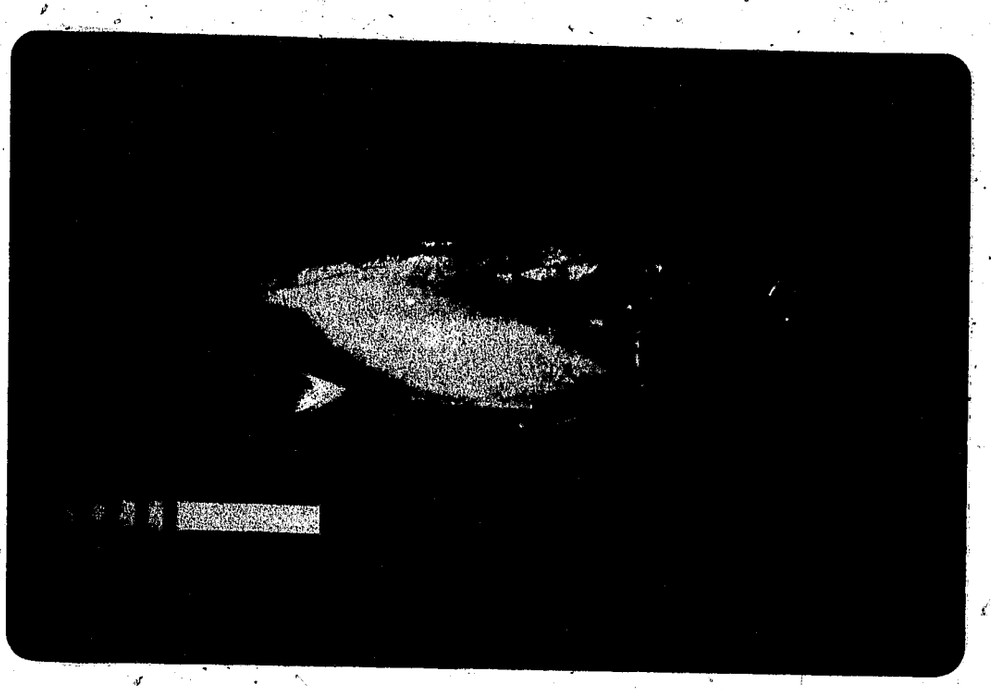
Maximum Size: 42 kgs (140 cm), commonly 40-100 cm.

Habitat: Oceanic, but entering productive coastal areas; forms smaller schools as adults than some other tunas; feeds on micronekton, schooling fishes etc.

Comments: Shown to undertake trans-oceanic migrations; spawns in relatively discrete sub-tropical areas.

Thunnus maccoyii (Castelnau, 1872)

Southern bluefin tuna



Distribution: Cosmopolitan in southern oceans, south of 28°S, except for the eastern Indian Ocean where rather discrete spawning areas extend northwards to 10°S.

Maximum Size: 200 cm (150kgs), commonly 40-160 cm.

Habitat: Oceanic; juveniles at the surface around Australia's southern coastline, adults at depth, returning to a single tropical area in the Indian Ocean to spawn; feed on small fishes, squid etc. May undertake circumpolar movements in the West Wind Drift.

Comments: The oriental bluefin (T. *thynnus orientalis*), a northern Pacific species, has been recorded on a few occasions in the Indo-Australian region (Collette & Smith, MS) and one such individual was sampled for this study (see later).

2.2 THE EPIPELAGIC HABITAT

A universal structural feature of the world's oceans is the change in water temperature with depth. A layer of maximum temperature gradients, the thermocline, occurs within 200m or so of the surface and forms a physical lower boundary to the epipelagic habitat - the illuminated upper transition or mixed layer of the oceans. It is usually considered an independent vertical zone of life, as distinct from deepwater pelagic zones, and has a characteristic ichthyofauna (Parin, 1968).

Briefly, in this habitat,

(a) the thermocline depth varies from area to area within the range 20-250m. In higher latitudes, a thermocline typically develops only in summer, whereas in equatorial and sub-tropical areas it is well expressed at all times. Vertical temperatures within the habitat tend to be uniform, i.e. isothermic.

(b) these temperatures vary zonally, but are also strongly influenced by oceanic circulation and disposition of land masses. Eastern parts of oceans, with currents carrying colder upwelled waters to equatorial areas, tend to be considerably colder than western parts where polewards currents carry warm tropical water into temperate areas.

(c) salinity fluctuates within narrow limits (33-38‰) in the open sea but terrestial run-off may cause local reductions in coastal areas.

(d) dissolved oxygen content usually approaches saturation in surface waters; in some areas, such as the eastern tropical Pacific, low oxygen waters are found immediately below the thermocline.

(e) surface currents arise basically from wind effects, their direction being modified by Coriolis force, coastline orientation and ocean bottom topography. The major currents of the Pacific Ocean, which vary in their spatial constancy are shown in Figure 2.2. Sub-surface currents, i.e. below the thermocline, typically flow in opposite directions to surface currents.

(f) current boundary zones (convergences and divergences) can have important physical and biological effects, such as vertical mixing and increased productivity.

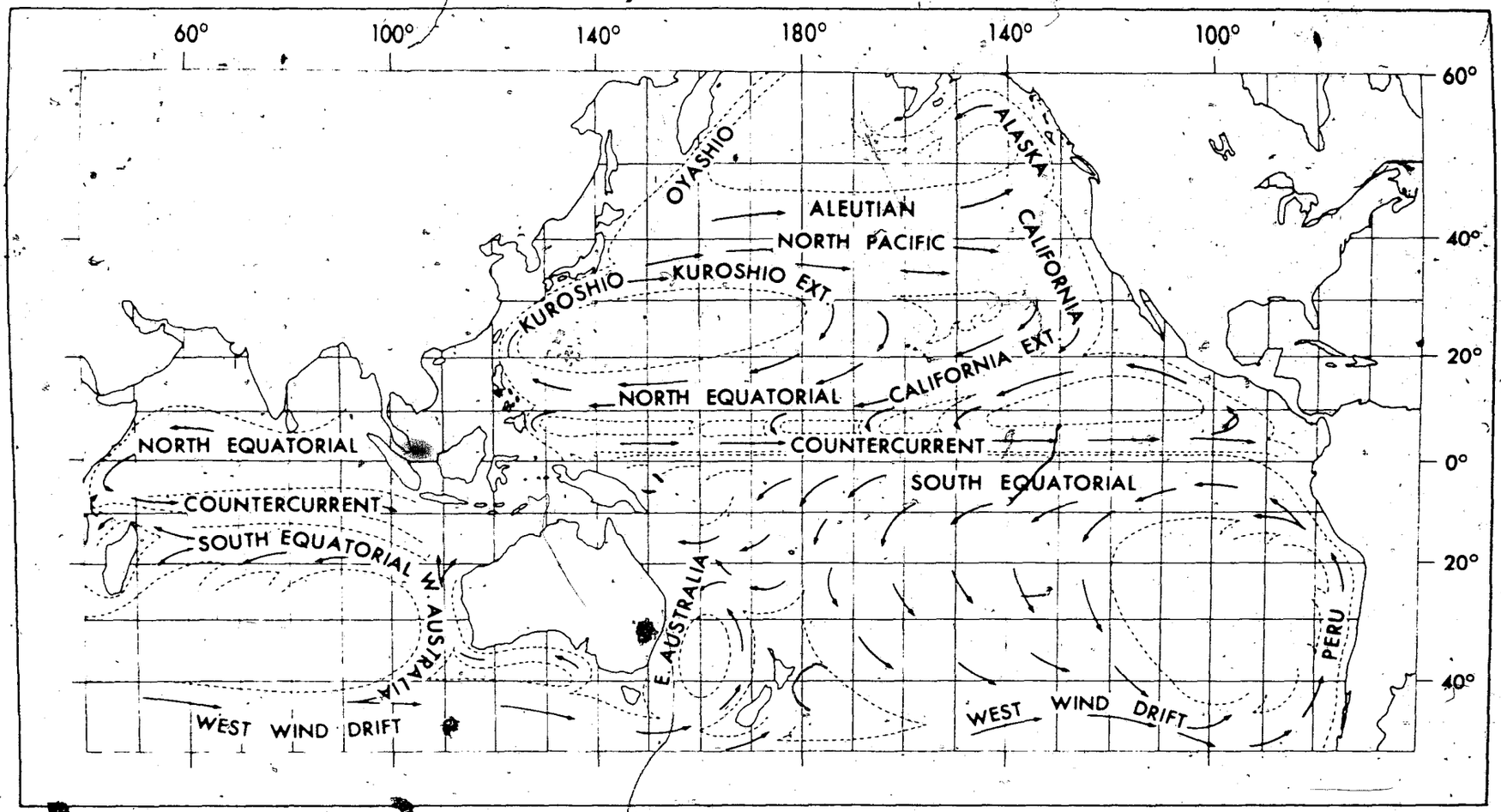


Figure 2.2 The major surface currents of the Pacific and Indian Oceans.

(g) temperature-salinity are often used to characterize water masses which in the epipelagic zone originate from circulation systems. Nakamura (1969) proposed a causal relationship between distribution of water masses and that of various *Thunnus* species.

(h) rates of primary production are highly variable, differing by at least two full orders of magnitude from richest to most impoverished regions. Trophic food chain dynamics also vary widely in response to levels of primary production; this effect may be additive, producing marked patchiness in food resources.

(i) mean productivity of the open ocean relative to coastal areas and upwelling areas, is low (50 g carbon/m^2/yr, cf. 100 to 300) and it has been described as a biological desert (Ryther, 1969). Reid (1962) and Gorshkov (1976) indicate that much of the Pacific within the 40^{o} parallels contains average zooplankton volumes of 25 parts per 10^9 by volume. Assuming adult tuna feed at least one trophic level higher on micronekton, mean forage levels of 2.5 parts per 10^9 must be characteristic of much of the epipelagic zone.

(j) open ocean - island interactions are not well understood, but probably have far-reaching biological effects through, for example, enhanced productivity and increased habitat diversity.

In neritic (coastal or continental shelf) and peripheral neritic (continental slope) areas (cf. oceanic) where many scombrid species spend all or part of their existence, the epipelagic habitat becomes a less distinct zone of life because of the numerous factors promoting mixing e.g. tides, terrestrial run-off, waves, reefs etc. and environment fluctuations become more marked. Relative to other marine habitats, the physical aspects of the epipelagic habitat everywhere still show considerable constancy; biological aspects, in contrast, are likely to show marked patchiness in response to the availability of nutrients, which could best be described as unpredictable rather than cyclic.

2.3 BIOLOGY OF SKIPJACK TUNA

Skipjack biology and ecology has been the subject of several extensive reviews (Waldron, 1963; Jones & Silas, 1963; Postel, 1963; Kawasaki, 1965; Matsumoto & Skillman, M S). The intention here is not to duplicate these efforts, but rather to summarize and highlight aspects relevant to population studies in the Indo-Australian region. These aspects include life history characteristics, reproduction, age and growth, schooling behaviour, environmental correlates of distribution and abundance, and migration.

2.3.1 Functional Morphology

Further to the brief species introduction and figure in Section 2.1, detailed descriptions of external morphology and meristics are given by Postel (1963), Jones and Silas (1963) and Waldron (1963) and of anatomy by Kishnouye (1923), Godsil and Byers (1944) and Godsil (1954). Colour changes associated with specific behaviour patterns are described by Strasburg and Marr (1961).

The far-reaching ecological implications of the specialized internal morphology and physiology warrants their brief description here. In common with most small scombrids, skipjack lack a swim bladder. This adaptation permits rapid vertical movements in the species' near surface habitat - an ascent from 10 m to the surface would result in a 100% increase in the volume of an unrestricted gas bladder and abrupt vertical diving behaviour from the surface to 30-60 m during feeding has been observed (Strasburg, 1961). The absence of a swim bladder however increases the minimum speed required to maintain hydrostatic equilibrium. Skipjack have short pectoral fins which incur little drag but provide very limited lift. As they are also the heaviest scombrid for a given length, it is not surprising in view of these attributes that they have the fastest relative minimum swimming speeds (2.2 body lengths/seconds) observed to date (Magnuson, 1973). Sharp (1978) has calculated that a 50 cm fish swims 60.5 km/day just for hydrodynamic stability and respiration.

The red muscle mass, which functions aerobically to power this sustained basal swimming, comprises 7-8% of the total body mass (Graham & Diener, 1978; Magnuson, 1973) and is completely internalized. Skipjack white muscle is the site of some of the most intense anaerobic glycolysis

known in nature (Hochachka *et al.*, 1978); it also appears to have some aerobic capacity (Guppy and Hochachka, 1978) and powers periods of high speed burst swimming such as pursuit of prey or escape from predators. Maximum burst speeds of over 70 km/hr have been reported for yellowfin tuna and wahoo (Walters and Fierstine, 1964); similar values would be predicted for skipjack. Histological details of muscle fibre types are given by Bone (1978).

The heavy oxygen demand occasioned by the high metabolic rates (Neill *et al.*, 1976) is met by remarkably efficient oxygen removal (90% effective) from seawater during ram-jet gill ventilation (Stevens, 1972). The total gill area is large (Muir & Hughes, 1969) and the extensive fusion of secondary lamellae prevents filaments being forced apart at high flow rates, permitting high oxygen utilization levels. As with other tunas, the heart is extremely large (2% of body weight - Basile *et al.*, 1976), as is the blood volume, and levels of haemoglobin are high (Klawe and Barrett, 1963); other haematological characteristics of the species are given by Alexander *et al.*, 1980.

Retention of metabolic heat generated in the red muscle is accomplished by a central counter current heat exchanger or 'rete mirabile', (Stevens *et al.*, 1974, Graham & Diener, 1978) located in an expanded haemal arch, and two pairs of small lateral sub-cutaneons exchangers. The less advanced genera in the group *(Auxis, Euthynnus)* have large central and less well developed lateral heat exchangers, whereas in the more advanced *Thunnus* species, the central exchanger becomes diminutive and lateral exchangers more prominent. Excess (over ambient) temperatures of 3.1 - 11.1°C, 5.9 - 11.4°C, and 1.9 - 5.6°C have been recorded in red muscle, white muscle and brain respectively by Stevens and Fry (1971). Three thermoregulatory options are theoretically open to tunas (Dizon and Brill, 1979) -

(1) behavioural thermoregulation (by selection of preferred habitat or by reducing activity levels)

(2) passive thermoregulation (water temperature-related and swim velocity-related heat production, thermal inertia and swim velocity-related heat dissipation)

(3) physiological thermoregulation (by the control of the relative contribution of red and white muscle to propulsion -

heat generated in white muscle is dissipated via gills and body surface, whereas heat generated in the red muscle is retained.)

The extent to which some or all of these options, plus acclimatory processes are exercised over the wide range of temperatures (15 - 30°C, Barkley *et al.*, (1978) experienced by the species remains unclear but skipjack do appear to possess limited thermoregulatory capability.

This capability is inadequate to free larger skipjack from problems associated with retention of metabolic heat, a problem which is exacerbated in warm tropical waters with their lower dissolved oxygen concentrations.

Using information gathered on skipjack temperature and dissolved oxygen requirements from tank experiments and a heat balance model developed by Neill *et al.*, (1976), Barkley *et al.*, (1978) have defined hypothetical limits of the skipjack habitat. They suggest that only small (<4 kg.) skipjack can inhabit most surface tropical waters and the habitat of large (>6.5 kb.) skipjack in the tropics is the vicinity of the thermocline, adjacent to cooler water. Where this is poorly oxygenated, large skipjack would be excluded. Gross features of skipjack distribution, including rarity of large skipjack in certain areas (see 2.3.7) appear to broadly fit the hypothesis.

Thus, the capacity for sustained high speed locomotory activity and ability to efficiently exploit a three-dimensional near-surface habitat is attained at the expense of some habitat restriction on larger fish.

2.3.2 Distribution

Skipjack is a holoepipelagic species, in that all stages of the life cycle are spent in the epipelagic zone (Parin, 1968). Adults are cosmopolitan in the world's oceans between 40°N and 40°S, with some expansion of this range in the western Pacific and Atlantic Oceans and compression in the eastern Pacific Ocean. This roughly parallels distribution of the 15°C sea surface isotherm. Seasonal occurrences in eastern Tasmanian waters (approximately 43°S), represent the most southerly regular occurrence of the species (Robins, 1952). Similarly, the species occurs seasonally across the Great Australian Bight, but becomes less

common east of 140°E and has not been recorded from central Bass Strait (Blackburn & Serventy, MS).

The distribution of larvae (Ueyanagi, 1969) and juveniles less than 15cm standard length (Mori, 1972) is more restricted, occurring mostly between the southern and northern limits of the 24°C surface isotherms (roughly 30°N - 30°S); distribution of young skipjack between 15 and 35 cm parallels that of adults.

These definitions of life history stages, as given by Mori (1972), are preferred to the more general definitions of Balon (1975) as more appropriate to the family development characteristics.

Although there is some evidence that larvae (Strasburg, 1960), juveniles (Higgins, 1970) and adults (Yamanaka pers. comm.) do occasionally occur below the thermocline, the species generally seems to be restricted to the surface layers of the ocean. In addition to the expected greater abundance of larvae year-round between 10°N and 10°S, Ueyanagi (1969, 1970) reported a clear westward increase in larval density across the Pacific, whereas Kawasaki (1965) had suggested the centre of abundance of skipjack tuna larvae lay in the central Pacific, (5°N - 4°S, 160°E - 140°W). Matsumoto (1975) attempted to correct available data for diel, latitudinal, seasonal and gear-related variability; of the ten areas he examined, maximum abundance was found in the central Pacific area (10°S - 20°N, 180° - 140°W) corresponding most closely to Kawasaki's centre of abundance. Little progress has since been made resolving this dichotomy of views (Ueyanagi, 1976; Matsumoto, 1976).

The distribution of larvae in relation to environmental factors is not well understood. Most larvae have been taken in 24° - 29°C water, with 22.1°C the apparent lower limit. Tan and Chen (1975) indicated that, in the South China Sea, the optimum temperature range for skipjack larvae was 28 - 29.3°C and Forsbergh (pers. comm.) has shown that larval abundance increases with sea surface temperature, especially above 28°C, although there is presumably an upper limit to this. Wade (1951) and Ueyanagi (1969, 1970) observed diel vertical migrations, with larvae more abundant in surface layers at night. Barkley (1969) suggests that larval distribution is associated with thermohaline circulation, and Ueyanagi

(1976) indicated that higher larval densities in the western Pacific were found in the Equatorial Counter Current (approximately 4° - 8°N). Nakamura and Matsumoto (1966) found no difference in abundance with respect to distance from shore in Marquesan waters (approximately 10°S), whereas Ueyanagi (1976) reports that some studies have revealed higher larval densities near land masses than in offshore areas.

Information currently available on larvae and their distribution is thus of limited value in understanding dispersal and spawning processes.

Although essentially oceanic, adults and young skipjack frequently enter productive neritic and peripheral-neritic areas to feed.

2.3.3 Early Life History

Fertilized eggs are sperical and planktonic, with a single oil droplet and a diameter of 0.8 - 1.20 mm (Brock, 1954; Yoshida, 1966; Ueyanagi *et al.*, (1974). Their similarity to other tuna species makes identification difficult and has hampered studies of egg distribution and abundance. Hatching occurs within 32 hours of fertilization, and the yolk sac is absorbed within two days. (Ueyanagi *et al.*, 1973, 1974).

Beyond morphological (Matsumoto, 1958, 1961) and distributional data (Ueyanagi, 1970; Tan and Chen, 1975; Richards and Simmons, 1971; Gorbunova, 1963), details of larval phases of the life history are minimal. The length of the planktonic phase, the size at which independent mobility is achieved and the degree to which larval development is under the control of endogenous and exogenous factors - all information critical to assessing dispersal in the early life history stages - are unknown. Characteristics of the larval habitat and the extent to which patchiness, on both micro and macro scales (Fasham, 1978) occurs are also poorly understood.

Estimates of larval abundance on a macro scale, as revealed by plankton net tows, have suffered from the low apparent density of larvae in most areas (Miller, 1978) and the difficulty of standardizing results obtained by workers using different size nets and sampling strategies (Matsumoto, 1966).

Information on juvenile and young skipjack has been mostly obtained from examination of stomach contents of apex predators, especially bill-

fishes and larger tunas taken by longline gear (Watanabe, 1960; Yoshida, 1971; Mori, 1972). Their mobility generally precludes net sampling, although high-speed mid-water trawls have been used successfully (Higgins, 1970). Mori (1972) reported that the abundance of juveniles and young was highest in the equatorial western Pacific and decreased gradually to the east. The low year-round abundance in the eastern tropical Pacific supports the belief that little spawning occurs in the area (see later).

The development of a specialized raft-purse seine or payao fishery in the Philippines in the 1970's, has seen commercial exploitation of young skipjack (15 - 35cm.) for the first time. The general rarity of young skipjack at the surface and hence their under-representation in commercial and survey catches, combined with the need for juvenile skipjack to avoid predation by their greatest potential predator, adult skipjack, suggests that they differ from adults in their schooling behaviour and vertical distribution. This has led Kearney (1978) to develop the following hypothesis:

Juveniles aggregate lower in the vertical profile, near the 20°C isotherm, where they feed and grow whilst avoiding much adult predation and migrating polewards to eventually emerge in productive temperate areas at a size of approximately 40 cm one year later. Migration into tropical areas for spawning subsequently occurs.

This hypothesis is supported in part by Higgins' (1970) finding that deeper midwater trawl tows tended to catch larger juvenile skipjack and that juveniles migrated towards the surface at night, when little adult feeding occurs; the predominance of 40 - 45cm skipjack in seasonal temperate fisheries also accords well with this theory. Waldron and King (1963) reported that scombrid juveniles comprised the most important food item by volume for Central Pacific skipjack and Nakamura (1965) found that 31% of skipjack stomachs in French Polynesia contained juvenile tunas of which skipjack were the most common. Although studies in other areas have generally encountered juvenile skipjack in stomach contents less frequently (Hotta and Ogawa, 1955; Dragovich, 1970, Raju, 1964), adult skipjack are potentially very important predators of juveniles, particularly in open ocean situations, and avoidance behaviour as suggested by Kearney may have considerable adaptive value. It may not always be

favourable for juveniles to aggregate near the thermocline, however. The habitat of large skipjack may be the vicinity of the thermocline in certain areas (Barkley *et al.*, 1978) and predation by other deeper swimming tunas and billfishes (King and Ikehara, 1956; Reintjes and King, 1953; Koga, 1960; Watanabe, 1958; Fourmanoir, 1971), also needs to be taken into account.

2.3.4 Environmental Correlates of Adult Distribution

The long list of oceanographic properties and features known to influence adult tuna distribution and abundance include temperature, salinity, dissolved oxygen, thermocline topography, bottom topography, transparency, current systems, water masses, and productivity, (e.g. Forsbergh, 1969; Blackburn, 1965; Brock, 1965; Howard, 1963; Yabe, Yabuta and Ueyanagi, 1963). It is not intended to review the voluminous literature here, as in most cases, deficiencies in the oceanographic and biological data preclude thorough evaluation of the phenomena. Blackburn (1969) concluded that temperature and food supply have the major direct effects, and that other factors usually exert their influence indirectly through them. Turbidity, for example, may affect the efficiency of food search for small transparent prey.

Temperature effects are probably most important in determining species range, although limiting isotherms for skipjack seem to vary from area to area (Blackburn, 1969). Temperature may also influence abundance, particularly in seasonal fisheries which operate near the distributional limits of the species (e.g. Robins, 1952). Towards the centre of the species range, i.e. equatorial areas, temperature seems less likely to exert any direct affect, particularly as the thermal sensitivity of tunas appears inadequate for detecting the weak gradients typical of such areas (Steffel *et al.*, 1976; Dizon *et al.*, 1974). Exceptions to this may be an apparent upper temperature limit for larger skipjack due to heat retention problems - the additive effect of low dissolved O_2 levels ("thermal squeeze" - Neill *et al.*, 1976, and see earlier), and temperature-related survival of larvae (Forsbergh, per. comm.).

The distribution of zooplankton and micronekton would seem to be the more important determinant of distribution and abundance in tropical

and sub-tropical regions. As Blackburn (1965) points out, however, the relationship is frequently difficult to establish.

The abundance of micronekton, several trophic levels above phytoplankton, may be temporally and spatially displaced from the enrichment source because of passive drift. If skipjack show any prey selectivity, total micronekton abundance may be a less reliable guide. Similarly, indices of skipjack abundance as typically estimated from catch data, are subject to numerous errors. Nonetheless, at the present time, the patchy distribution of skipjack finds its best, if imperfect correlation in the distribution of zooplankton and micronekton. This may be particularly true in the sub-tropics, where the standing crop of zooplankton is generally very low (Reid, 1962; Gorshkov, 1976) and productive patches stand out as "oases".

Increased skipjack abundance associated with islands and shoals is a well known occurrence. This may be the result of increased food supply in such areas due to the island mass effect (Doty & Oguri, 1956; Gilmartin & Revelante, 1974) leading to increased primary productivity, concentration of forage by eddies and local current fronts associated with islands (Murphy & Shomura, 1972) and the 'leakage' from coastal ecosystems of nutrients and larval stages. Island points are held to be more productive than the leeward and windward zones (Grandperrin, 1978). In the western Equatorial Pacific, with its abundance of islands, the attendant increased productivity is liable to considerably influence day-to-day nearshore abundance of skipjack.

On a larger scale, the importance of fronts or convergences (Blackburn, 1965) and upwellings (Sette, 1955; Smith, 1968) to skipjack abundance has long been recognized. The latter bring to the surface nutrient-rich water hence enhancing productivity, whereas the former serve as concentrating mechanisms for drifting or weakly swimming biota such as zooplankton.

In the western Equatorial Pacific, a causal relationship between the currents which cross the region (Figure 2.2) and the incidence of high catch rates has been suspected (Kasahara, 1977). Recent work (Donguy *et al.*, 1978) has used the 35‰ surface isohaline as a marker for the convergence between the upwelled, high salinity (>35‰) nutrient rich water in the westward flowing Equatorial Current, and the eastward flowing North and South Equatorial Counter Currents with their nutrient-poor,

lower salinity (<35°/∞) water. These convergences typically occur at about 5°N and 5°S respectively, but do shift latitudinally from month to month (Yamanaka & Yamanaka, 1970).

The equatorial divergence and resultant upwelling is largely induced by winds with an easterly component, the trade winds and their derivatives, blowing at the Equator. With the thermal Equator typically situated north of 0°, the equatorial upwelling is generally strongest during the southern winter, from May to September and is most constant in the central Pacific. Its westward extent and duration fluctuates, probably in response to the strength and duration of the south-east trade winds. This appears to greatly influence skipjack abundance and availability. Donguy *et al.*, (1978) have obtained a good correlation between the position of the 35°/∞ isohaline and the incidence of good catches by the mobile Japanese long range fleet.

When this isohaline reaches westward to the Papua New Guinea area, good catches have generally resulted. Figure 2.3 plots monthly average catch-per-unit effort (CPUE) in the Papua New Guinea fishery for the years 1971-77. As expected, these peak during the April-October period when the south-east trades and the equatorial upwelling should be strongest (Donguy & Henin, 1978), indicating that much of the production might be attributed to this factor. Fluctuations throughout the year are not marked, however, as the timing of such events has a high variance between years. Additionally, another important seasonal enrichment process occurs. During the southern summer from November to March, winds with a westerly component sometimes prevail at the Equator producing an unproductive convergence but at the same time inducing a productive doming at around 10°S. These same winds also produce an upwelling along the north-coast of Papua New Guinea. With the New Guinea Coastal Current setting southeast through the Vitiaz Straits (Yamanaka, 1973; Wyrtki, 1960), maximum zooplankton abundance associated with the north coast upwelling is displaced eastwards, occurring in the Solomon Sea, and leading to increased skipjack abundance there during January to April in some years.

Although the above evidence indicates that enrichment processes and island-associated effects may be the major influences on skipjack availability in the Papua New Guinea area, elsewhere other factors probably need to be considered, particularly in seasonal fisheries outside equatorial regions. Seckel (1972), for example, demonstrated an empirical

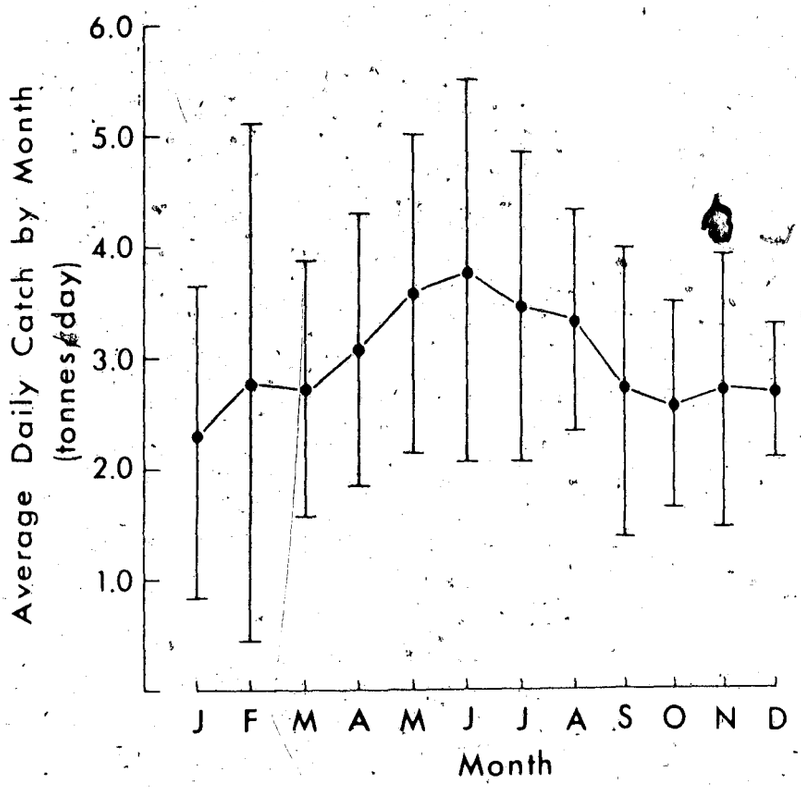


Figure 2.3 Monthly average CPUE in the Papua New Guinea fishery, 1971-1977. Standard deviations about the monthly averages are shown.

relationship between skipjack availability to the Hawaiian fishery and environmental indices (time of warming, salinity) related to particular current systems, rather than seasonal changes in productivity. In most cases, however, there are simply insufficient data on both the animals and their environment to progress beyond description of such associations to experimental testing of hypotheses.

2.3.5 Reproduction

Spawning has been observed neither in nature nor in captivity, although behaviour interpreted as courtship has been observed (Iversen *et al.*, 1970). The species is dioecious (hermaphrodites have occasionally been observed - Uchida, 1961; Raju, 1960), fertilization is external and mating is assumed to be random. Ripe (immediate pre-spawning) individuals are only rarely encountered in commercial catches (Hatai *et al.*, 1941; Brock, 1954; personal observations). This may be associated with some form of avoidance behaviour. Alternatively, recent experience in Hawaii has suggested that final maturation may occur within 7-8 hours of being triggered, in this case by stress associated with capture.

Estimates by various workers of the minimum size of females at first spawning are in good agreement (Brock, 1954; Yoshida, 1966; Raju, 1964a; Simmons, 1969; Batts, 1972c; Stequert, 1976; Nagamuma, 1979). For the purposes of this study, skipjack greater than 45cm in length are therefore referred to as adults.

Spawning seasonality is generally inferred from macro and microscopic examination of gonads (maturity classification, ova diameters, gonad and gonosmatic indices) and distribution of eggs and larvae. A general pattern emerges from the studies carried out to date. In tropical areas, skipjack with ripe or mature ovaries occur year round (Wade, 1950 - Philippines; Stequert, 1976 - Madagascar; Wilson, MS - Papua New Guinea; Nagamuma, 1978 - western Pacific), as do larvae (Ueyanagi, 1970; Nishikawa *et al.*, 1978), although seasonal peaks in the relative proportion of maturing fish may occur. Towards the higher latitudes, spawning is progressively confined more to the summer months (e.g. Yao, 1955 - Japanese waters; Yoshida, 1966 - French Polynesia; Legand, 1971 - New Caledonia) and near the limits of distribution, little or no gonad development is observed (Habib, 1978 - N.Z.; Williams, pers. comm. - southern N.S.W.). An exception to this general pattern is found in the

eastern tropical Pacific, where larval density is low (Matsumoto, 1958; Klawe, 1963), skipjack with mature ovaries are rarely encountered (Schaefer and Orange, 1965; Orange, 1961) and little spawning is thought to occur. Significantly, the few mature fish and larvae recorded have generally been found in the vicinity of offshore islands.

The polymodal configuration of ova diameter frequency polygons typically observed in maturing skipjack ovaries is taken to indicate multiple spawning during the season (Brock, 1954; Bunag, 1950; Raju, 1964a; Stequert, 1976). Estimates of fecundity are generally based on the number of ova in the most advanced mode and this may represent a very conservative estimate of annual fecundity for multiple spawners. Published fecundity estimates (Joseph 1963; Raju 1964b; Simmons, 1969; Batts,1972c and Stequert 1976) for skipjack of various sizes range down from 2 million. Although the fish size - fecundity relationship varies amongst these studies, all indicate that for skipjack below 60 cm. in length, between 100,000 and one million eggs would be released per spawning.

This relatively high fecundity is presumably associated with low survival to reproductive size. In the likely event that survival rates vary widely between individual egg masses, random effects on genetic composition such as genetic drift may need to be taken into account. Information needed to assess this possibility is not currently available.

As spawning has not been observed at all and as ripe female fish are rarely observed, available data on gonad development does not allow spawning areas to be defined more precisely within the wide area where larvae and mature fish are found. As males are in ripe condition for much of the time, the apparent rapid final maturation of ovaries could allow spawning to occur within hours of the appropriate stimulus being received.

Details of how this occurs (eg. the nature of the stimulus, whether or not spawning is roughly co-ordinated within a school etc.) are lacking. Recent studies in Hawaii have shown that the stress of capture is able to induce final maturation, or ovulation within 7-8 hours. A comparable environmental trigger would allow reproduction to occur soon after

arrival in a situation favourable to the survival of larvae, e.g. productive areas near islands etc.

2.3.6 Schooling Behaviour

The propensity to form schools or aggregations varies considerably amongst the scombrids (Shubnikov, 1974) and its adaptive significance to fishes in general has been discussed by many authors (Brock and Riffenburgh, 1960; Olson, 1964; Radakov, 1969; Cushing and Harden Jones, 1968; Weihs, 1973; Breder, 1967).

Skipjack are regarded as a schooling species, at least as adults. Schools tend to be more size-selective than those of other tunas (Brock, 1954; Lewis *et al.*, 1974), and Brock has suggested this selectivity is maintained by differences in maximum and/or basal speeds attained by fish of various sizes. In support of this, Kawasaki (1964) and Iwasaki (1976) examined length frequencies within schools associated with various biotic and abiotic objects and found the less mobile the object, the greater the size range of skipjack associated with it.

Associations occur with other tunas, especially juvenile yellowfin, logs and other flotsam, whale sharks *(Rhincodon typus)*, basking sharks *(Cetorhinus maximus)*, whales (esp. *Balaenoptera borealis*) and in some areas, porpoises *(Stenella* spp). The biological significance of these associations is not fully understood.

Although information is available on the structural aspects of tuna schools (Strasburg & Yuen, 1958; Yuen, 1963; Cahn, 1972), little or no information is available on the integrity of the school as a unit over time. Whitney (1969) and Scott & Flittner (1972) briefly discuss diurnal changes in the structure of tuna schools and Sharp (1978) introduces the concept of the "core" school, where fish within a grouping have a high probability of sharing one parent. Core sizes of around 3 tons were suggested for yellowfin tuna, with larger schools seen as aggregates of individual core or primary schools.

The implications for population genetic studies, for example, of a high degree of sibship within schools, on the one hand, and the school as a random association of unrelated individuals with little continuity in time on the other, are considerable. Data generated by pole-and-line

fisheries are unlikely to be of much use in examining the problem, however, since a variable and unknown proportion of fish are taken from each school.

School sizes have been estimated from purse seine catches-per-set (Orange *et al.*, 1957), pole-and-line catches per fishing station (Broadhead and Orange, 1960) and by experienced aerial observers (West and Wilson, M S); size frequencies generally fit a J-shaped curve and average "sizes" (catch per set or stop) may vary from area to area (Sharp, 1978). School sizes in excess of 200 tonnes have been reported.

Again, the problem arises of having insufficient information to evaluate an important aspect of the species biology i.e. what do schools represent. Large feeding aggregations are probably comprised of numerous "schools" and samples obtained as a consequence of commercial activity probably show bias towards large aggregations and hence greater heterogeneity in biological characteristics.

2.3.7 Age, Growth and Size

Growth estimates for skipjack have been derived from examination of presumed annuli on vertebral centra (Aikawa & Kato, 1938; Chi & Yang, 1973) and first dorsal spines (Shabotiniets, 1968; Batts,1972b; Cayre, 1978), modal progressions in length frequency data (Brock, 1954; Joseph & Calkins, 1969; Wankowski, in press), data from tagging studies (Rothschild, 1967; Joseph & Calkins, 1969; Josse *et al.*, 1979) and more recently, presumed daily rings in otoliths (Uchiyama & Struhsaker,1978; Wild & Foreman, 1979). Results from these studies show wide variation in the average annual increments (length) estimated for fish of various sizes.

Each method has inherent biases or weaknesses. The difficulty of establishing the annual basis of ring formation in the hard parts (centra and spines) of tropical fish species may be exacerbrated in this case by the buffering effect of the species' thermal inertia, the lengthy spawning season and its high vagility allowing movement between habitats to occur. Estimates obtained by Aikawa and Kato (1938), Chi & Yang (1973), Batts (1972) and Cayre (1978) nevertheless agree quite closely with a growth rate of approximately 8 cm per year for 40-60 cm skipjack (Josse *et al.*, 1979).

Analysis of modal progressions, the Peterson method, inevitably involves a considerable amount of subjectivity (Joseph & Calkins, 1969). There may also be difficulties with lack of obvious progression, presumably due to continuous recruitment of fish of similar size (Marcille & Stequert, 1976; personal observation), and inconsistencies in rates of progression between sampling periods. Josse *et al.* (1979), in reviewing the use of the Peterson method, conclude that little reliance can be placed on its application to skipjack length frequency data, where corroboration by other techniques is lacking.

Data generated by tagging experiments are potentially subject to error introduced by inaccuracies in measuring length at release, retardation of growth associated with carriage of the tag and unreliability of recapture data. When efforts are made to minimize these sources of error as was done in the Papua New Guinea tagging experiments (see Chapter 3), consistent results have been achieved.

Using tetracycline labelling of tagged skipjack, Wild and Foreman (1979) demonstrated that, because of periodic growth checks, skipjack otolith increments underestimate time by approximately 24%, whereas yellowfin increments were deposited daily. Previous estimates using the technique (Uchiyama and Strushsaker, 1978) therefore probably over-estimated skipjack growth. Although the method is technically demanding and labour intensive, it appears to hold some promise for short-term estimates of growth, geographical comparisons and possibly much-needed estimates of larval and juvenile growth.

Josse *et al.* (1979) conclude that carefully designed tagging experiments should yield the most reliable estimates of skipjack growth at this time, particularly as migration data produced by them should provide insights into modal progressions. Where possible, otolith studies should be pursued concurrently.

Much interest has been generated by the apparent discrepancies in growth rates obtained for various areas (e.g. Kearney 1975, 1978) particularly the higher growth rates obtained for eastern and central Pacific skipjack (Brock, 1954; Rothschild, 1967; Joseph & Calkins, 1969) relative to western Pacific skipjack (Kearney, 1975; Josse *et al.*, 1979; Wankowski, in press). Critical analysis of all available tagging data by Josse *et al.*

(1979) has shown however that even though calculated growth rates appear to differ slightly, the variance on estimates is so high that they do not differ significantly. Biological sources of this variance may be partitioning of energy budgets between growth or spawning requirements (Kearney, 1978), a suggested difference in growth rates between nearshore and oceanic skipjack (Josse *et al.*, 1979) and migration between areas with different growth regimes. Added to this individual variation, the length of the spawning season in tropical areas makes it biologically meaningless in most cases to arbitrarily assign skipjack of particular size to "age classes".

Recent published studies (Batts, 1972; Marcille & Stequert, 1976; Cayre, 1978; Josse *et al.*, 1979; Wankowski in press) show good agreement in indicating that annual growth increments for 45-60 cm average 6-10 cm. Available estimates of growth during the first year of life are few (Yoshida, 1971; Batts, 1972; Cayre, 1978; Uchiyama and Strushsaker, 1978), and fall in the range 30-45 cm. Preliminary results from recent releases of tagged young skipjack suggest that growth may be more rapid (Kearney, pers. comm.) than these previous estimates suggest, further underlining the doubtful validity of assigning ages from lengths.

Information on size composition by area is restricted to adults (skipjack more than 40-45 cm) since commercial catches are the primary source of such data. There are indications of some size heterogeneity by area. Large skipjack of greater than 65 cm or 6 kgs make a regular and significant contribution to catches only in certain areas (Matsumoto, 1975). These include Hawaii (Rothschild, 1965) and French Polynesia (Doumenge, 1973). Skipjack of this size are comparatively rare in the western Pacific surface (Kearney, 1975; Wankowski, in press) and longline (Murphy & Otsu, 1954) fisheries. The catch in fisheries towards the periphery of the species range e.g. N.E. Japan (Suda, 1971), Baja California (Broadhead & Barrett, 1964) and New Zealand (Habib, 1978) tends to be comprised of small to medium size skipjack, 40-45 cm.

In addition to this regional variation in size composition, examples of some size-specificity within areas are known, e.g. Papua New Guinea (Kearney, 1977 - see 3.5), Japan (Higgins, 1966), eastern tropical Pacific (Broadhead and Barrett, 1964). Recent work in defining the hypothetical habitat of skipjack of various sizes (Barkley *et al.*, 1978) suggests

environmental factors such as dissolved oxygen levels and vertical temperature profiles may be involved.

Kitchell *et al.*, (1978) constructed skipjack energy budgets using data from available field and experimental studies, and suggested small skipjack from 0.6 to 4.0 kg are growth-limited by their ability to consume and process available food. Their calculated expected maximum size agreed closely with the largest skipjack on scientific record, namely 22 kgs (Magnuson, 1973).

In summary, it is clear that assigning ages to skipjack on the basis of length alone is inadvisable, due to possible deficiencies in the methodology, to individual variability and to plasticity in growth rates. It is also likely that skipjack do not distribute randomly by size, and this is important when assessing both tagging and genetic studies.

2.3.8 Nutrition

The many studies of skipjack stomach contents (e.g. Waldron & King, 1963; Nakamura, 1965; Batts,1972a; Raju, 1964) indicate that skipjack show little dietary preference in their consumption of the main forage categories, juvenile fish, crustaceans, and cephalopods. They can best be regarded as facultative filter feeders (Walters, 1966) with the mean gap between gill rakers imposing some selectivity in the minimum size of prey, particularly crustaceans, retained (Magnuson and Heitz, 1971), but not preventing them from ingesting larger prey. The mean gill raker gap for skipjack was the smallest of the species studied by Magnuson and Heitz and only *Allothunnus* amongst scombrids has more gill rakers, emphasizing the species' ability to utilize a wide size range of prey items. A consequence of this trophic opportunism is the ingestion of large numbers of juvenile skipjack in areas where they form a significant component of the nekton (see 2.3.3). The rarity of nictoepipelagic fishes in stomach contents and their more frequent occurrence in yellowfin collected concurrently (e.g. Lewis *et al.*, 1974) confirm that most skipjack feeding occurs in the epipelagic zone.

The rate of digestion was studied by Magnuson (1969) and found to be very rapid relative to other teleosts, with total digestion and absorption achieved within 12 hours. This may be related to the elevated internal

body temperatures. It has the advantage of making energy available relatively rapidly and allowing the stomach to be filled more frequently, thus maximizing the utilization of food patches. Under experimental conditions the equivalent of 15% of the body weight was consumed daily.

Skipjack are thus well adapted to life in a nutrient-poor environment. A wide variety of food can be assimilated and digested quickly, allowing patches to be efficiently exploited and energy rapidly made available. An interesting facet of this broad spectrum foraging strategy is cannibalism of juveniles, which may be a second-order benefit of high fecundity.

2.3.9 Migration

The physiological and morphological adaptations of tunas confer on them great migratory potential. Various attempts to elucidate the extent of skipjack movements have been made from tagging and genetic studies and examination of catch effort data.

Most published information available to date relates to the eastern and central Pacific. Based primarily on evidence that little spawning of skipjack occurs in the eastern tropical Pacific (see 2.3.2) and on a number of tag returns in the central Pacific from Mexican releases, Schaefer (1963) and Rothschild (1965) postulated a central Pacific origin for skipjack exploited in the eastern Pacific fisheries. According to Rothschild, young skipjack migrating from the central Pacific split into northern and southern groups which are recruited at a size of 35-40 cm into the Mexican and Central-South American fisheries respectively. Tagging data (Fink and Bayliff,1970) has subsequently indicated that there is little mixing between these two groups after their arrival in the eastern Pacific. A persistent cell of warm, oxygen-depleted (below 25m) water off central America (Blackburn 1962), representing marginal habitat for skipjack of most sizes (Sharp 1978), probably engenders this split. Williams (1973) proposed three models to account for the migration of young skipjack into the eastern Pacific. These models have yet to be tested although recent releases of young skipjack in the Marquesas Islands will be useful in this regard.

Based on tagging and other data, Kawasaki (1965, and various earlier works) has proposed the existence of resident and migratory groups of skipjack in Japanese waters. Migration into the fishery from

southern areas (10 - 20°N) was postulated to occur along three major routes. South of 10°N, movements detected by tagging experiments appeared random (Kawasaki, 1976).

On a wider scale, Kawasaki (1965) proposed a common central Pacific spawning area (160°E - 140°W) for all Pacific Ocean skipjack. This area was later extended westwards to 120°E, but the common origin was maintained. Genetic studies since then (Fujino 1970, 1972, 1976; Sharp, 1978) have suggested the existence of non-interbreeding sub-populations in this area. Matsumoto (1975) identified fourteen "stocks" within the Pacific, based on shifts in abundance of longline-caught skipjack. These all remain essentially hypotheses and will be examined in greater detail where appropriate in subsequent sections.

Whether or not some of these movements are directed is unknown. Based on ultrasonic tracking experiments, Yuen (1970) suggests that skipjack can navigate over short distances and do have a sense of time. Mechanisms by which this may be achieved can only be guessed at. A photoreceptive function has been ascribed to the pineal organ found in all tunas (Rivas, 1953); examination of a southern bluefin tuna *(Thunnus maccoyii)* obtained by the author and examined by Dr. J. Kirschvink (Princeton University) revealed the presence of about 10^6 single-domain magnetite crystals per cm^3 of tissue, sufficient for the fish to use the geomagnetic field as a navigational aid (Kirschvink, pers. comm.). Since then, an unconditioned magnetic field response in yellowfin to the geomagnetic field has been observed (Dizon, fide Kirschvink) and the magnetite shown to be precisely located in yellowfin, skipjack and kawakawa on the frontal bone and thus well placed to function in a magnetic sensory capacity. As a result, there now seems a distinct possibility that skipjack can navigate in this way.

A further crucial question with far reaching implications remains unanswered - are skipjack capable of directed migrations and by extension, homing?

From this brief review, the following points relevant to the succeeding sections emerge:

(a) skipjack, by virtue of the specialized morphological and physiological adaptations which equip them for sustained high speed locomotion, have developed a distinctive foraging strategy, albeit at high energy cost. These adaptations enable them to locate and utilize productive patches within their generally unproductive habitat.

(b) the potential for dispersal via highly mobile adults and young, as well as planktonic eggs and larvae, is high. The reality has yet to be demonstrated, and the role of stochastic and deterministic factors (e.g. homing) in dispersal is unknown.

(c) the species is iteroparous, with external fertilization, presumed non-assortive mating and high fecundity. Whether spawning activity is continuously distributed across the broad area, is unknown.

(d) details of some important aspects of behaviour and life history, notably larval development and survival, schooling characteristics, age structure and growth rates remain sketchy.

CHAPTER 3

TAGGING EXPERIMENTS WITH SKIPJACK TUNA

3.1 INTRODUCTION

"The main object of a marking experiment is to set up and examine the properties of an 'experimental' population of marked fish in which certain parameters that would be difficult or impossible to estimate in the 'natural' population can be determined with some accuracy". (Beverton and Holt, 1957). In the marine environment, where opportunities for direct observation and experimental manipulation of populations are much reduced, tagging, or mark recapture experiments are widely used to obtain information on fish populations. Parameters commonly estimated include population size, mortality (both natural and fishery-induced), growth, recruitment, rates and extent of movement and geographical range (Paulik, 1963; Ricker, 1956). In this chapter results of tagging experiments are used to investigate the nature of skipjack movements within one area, Papua New Guinea and its surrounds, and then by extrapolation, to assess the potential contribution to gene flow between areas made by these movements.

3.1.1 Assumptions

Central to the use of this technique is the assumption that all animals in the population are equally susceptible to capture. Failure of this assumption may be due to unequal distribution of catchability over the population (i.e. individual heterogeneity) and/or the probability of capture being affected by previous history of capture (contagion - 'trap-shyness' and 'trap addiction') (Cormack, 1968; Carothers, 1980). The former case can only be investigated with a population of known size, clearly not possible with a cosmopolitan oceanic species. The under-representation of sexually mature (ripe) female skipjack in catches by certain gears (Brock, 1954) does indicate the basic assumption may not invariably hold. The latter case has been examined by likelihood ratio (Seber, 1965) and other statistical tests (Leslie *et al.*, 1953; Orians, 1958;

Carothers, 1971), and departures from the assumption have again reported in fish (Beukema, 1970) and other groups (Orians, 1958; Turner, 1960).

Other assumptions regarding retention of tags, unprejudiced survival of tagged fish, correct reporting and return of all tagged fish, equal mixing of tagged and untagged fish and varying rates of immigration and emigration from the study area will affect particular estimates to varying degrees.

The present experiments were undertaken as part of a wider programme accompanying the development of a large scale tuna fishery in Papua New Guinea in the early 1970's (Kearney, 1975), and thus involve commercially exploited populations. In contrast to research on closed populations or populations where data collection following release is under the investigator's control, in this case: "the data are mainly collected as part of a commercial activity whose details are dictated by optimal use of the available resources, and not by the research worker. The re-release of captured individuals is not a part of this process, nor are point samples taken at pre-assigned times. The exploitation is usually continuous, the total catch being divided according to the time interval during which it was taken". (Cormack, 1968). Although commercial activity may be relatively continuous (at least in non-seasonal fisheries), levels of activity typically vary by area and in time, and recaptures must be related to this spatio-temporal distribution of fishing activity. Reliable catch statistics, usually expressed in terms of effort expended and catch by species, area and time strata, are therefore required; where the geographical range of the population is large and recaptures are made by a variety of gears in different countries, difficulties may be experienced with standardizing estimates of effort and abundance and with varying reliability of available catch statistics.

3.1.2 Types of Movement

In a recent comprehensive review of animal migration, Baker (1978) argues for the acceptance of a general term, migration, to define "the act of moving from one spatial unit to another"; this recognizes that a complete spectrum of movement patterns exist. Many other workers (e.g. Heape, 1931; Landsborough-Thompson, 1942; Harden Jones, 1968; Endler, 1977) have found it useful to distinguish between *dispersal*, the roughly random or weakly directed intra-habitat movements made continuously rather

than periodically as a result of daily or seasonable activities, and *migration*, inter-habitat long distance movements made by large numbers of individuals in approximately the same direction at approximately the same time, and usually followed by a regular return movement. These terms have become well established in the literature, the former especially in the genetic literature, and will be used here in that sense.

Fishes provide some of the best-known examples of migration, notably the catadromous migrations of eels (*Anguilla* spp.) and anadromous salmon (*Salmo* spp., *Oncorhynchus* spp.) migrations. In the marine environment, migration can frequently be reduced to a simple triangular relationship (Harden Jones, 1968) - a contranatant migration of adults to a well defined spawning area, denatant movement or drift of young stages to nursery or feeding areas, and recruitment into the adult population. Several tuna species, notably the bluefin tunas (see earlier), with their relatively discrete spawning areas, proven long distance movements to these areas, and pelagic larvae provide pertinent but as yet incompletely documented examples of this generalized pattern.

Migration, however, need not be for spawning purposes (gametic) but can also be climatic (seasonal avoidance of unfavourable conditions), alimental (feeding migrations) (Heape, 1931) or complex combinations of these types. Tagging experiments have been instructive in understanding migration, particular when fish can be marked on spawning grounds. Where several spawning areas exist, fish tagged outside these grounds may produce return patterns which are difficult to interpret unambiguously.

Dispersal is a prerequisite to gene flow between areas, and as gene flow is very difficult to measure, serves in practice to estimate it, subject to various corrections and adjustments. True gametic migration, on the other hand, should tend to maintain genetic continuity.

Where large numbers of animals are marked and representative dispersal patterns documented, the observed dispersal distances, which typically show a leptokurtic distribution, probably over-estimate the extent of gene flow. Dispersal distances tend to be small in most animals but in species of high vagility, where large scale dispersal occurs, the term nomadism (Heape, 1931) is often used. Nomadism is well

known in those terrestrial species whose food resources, like those of tunas, vary strongly between seasons and years within a given area, e.g. birds (Keast, 1968; Ward, 1971), rodent predators (Krebs and Myers, 1974) and desert species (Frith, 1967), and its adaptive significance has been the subject of considerable speculation (e.g. Anderson, 1980; Taylor and Taylor, 1977; Gadgil, 1971). Where it is accompanied by even limited spawning, the potential for gene flow between areas is considerably increased.

As the presence of well defined spawning areas in skipjack has not been demonstrated observed movements are described as dispersal. Similarly, the terms 'emigration' and 'immigration' are used in a general sense to indicate the direction of movement relative to a given area and their use is not necessarily associated with true migration.

3.1.3 Previous work

Successful tagging studies with tunas have a relatively recent history because of the difficulties associated with catching and handling them. Early attempts to tag tunas, including such approaches as the individual stamping of commercial fish hooks, have been discussed by Godsil (1936, 1938) and Rounsefell and Kask (1945). It was not until the development of the loop tag (Wilson, 1953) and its subsequent refinement, the dart tag (Yamashita and Waldron, 1958) in conjunction with improved handling techniques (Marr, 1963; Fink, 1965; Bayliff, 1973) that success was achieved. Tagging experiments have since contributed significantly to present understanding of the population structure of albacore (Otsu and Uchida, 1963), southern bluefin tuna (Chingu, 1965) north Pacific and Atlantic bluefin tuna (Mather, 1963), all of which undertake lengthy and spectacular migrations to relatively discrete spawning areas. Although skipjack (and yellowfin) have been tagged in appreciable numbers, results from tagging experiments with these species have been less than definitive, as suggested in 2.3.9.

Fink and Bayliff (1970), analysing 4381 returns from 90,412 skipjack tagged in the eastern Pacific Ocean, were able to distinguish northern and southern groups which underwent limited mixing. Schaefer (1963), Rothschild (1965) and Kawasaki (1965) had earlier hypothesized

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a central Pacific origin for these eastern Pacific fish. Williams (1973) examined the argument in some detail and proposed three models of migration into the eastern Pacific from the central Pacific. Tagging in Hawaiian waters (13000 fish, over 1300 returns) produced only local Hawaiian returns (Kawasaki, 1965). Prior to 1967, tagging experiments in the long established Japanese fishery had generally been unsuccessful, but returns from over 6,000 releases during 1967-69 (Kasahara *et al.*, 1971) established that the fishery relied in part on seasonal migration from areas to the south and that movements of different groups of fish within the area were complex, varying between and within years.

Results from these experiments, and releases on a small scale in other parts of the Pacific (Bayliff, 1974) whilst contributing to the understanding of movements and stock structure within particular local areas, yielded no information relevant to Papua New Guinea populations, and the present experiments were undertaken without knowing what area returns might be expected to cover. It is useful, therefore, to briefly consider the fisheries of the region and to describe the Papua New Guinea fishery, which could reasonably be expected to account for most short term returns, in some detail.

3.2 THE FISHERIES

Surface fisheries which rely for their efficacy on the schooling behaviour of skipjack account for virtually the entire world skipjack catch. Longline vessels take small quantities incidental to the catch of larger deep swimming tunas and billfish. Predominant amongst the various techniques used are pole and line fishing where live bait is used to enhance the school's feeding response, enabling individuals to be caught using a pole, short line and lure with a barbless hook, and purse seining where the school of fish is encircled by a large net which is pursed below, winched alongside and the catch brailed out. Lesser quantities are caught by trolling and gill netting.

Within the Indian and Pacific oceans, annual nominal catches of skipjack, compiled from FAO statistics (Anon, 1976) and Klawe (1978), are given by country for the years 1971-75 in Table 3.1, and the location of fisheries in Figure 3.1.

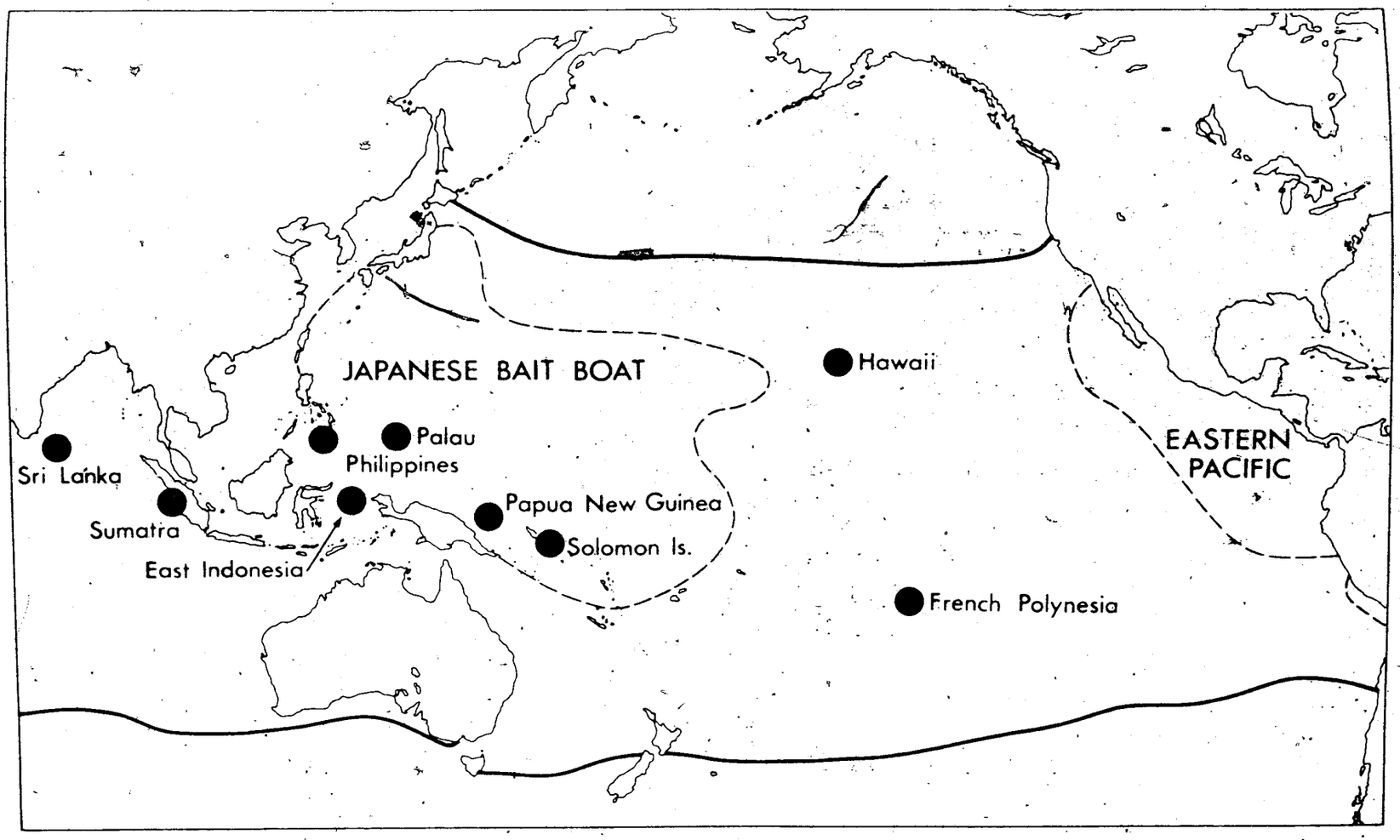
Table 3.1 Annual nominal skipjack catch ('000 tonnes) by country in the Pacific and Indian Oceans, 1971-1975 inclusive

	1971	1972	1973	1974	1975
Western Pacific					
Japan	105.1	156.8	201.3	134.7	136.5
Papua New Guinea	16.9	11.7	27.3	40.2	15.6
Solomon Islands	4.5	6.8	5.8	10.0	7.1
Palau	1.0	0.4	6.2	3.2	4.5
Indonesia	12.4	19.6	22.3	23.6	24.5
Philippines	(0.6)	(0.3)	27.2	17.4	20.0 est
Kiribasi	-	-	-	0.2	0.2
New Zealand	-	-	-	-	0.8
Korea	0.2	0.5	1.7	0.7	4.2
Taiwan	NA	NA	NA	NA	2.2
Australia	-	-	-	0.3	1.4
Eastern Pacific					
U.S.A.	64.7	40.9	36.9	52.0	66.8
Canada	1.3	1.0	3.8	1.7	4.4
Ecuador	13.9	5.5	6.0	8.5	16.9
Mexico	4.5	2.4	2.5	3.9	6.4
Peru	5.7	2.4	4.8	2.3	3.6
Panama	NA	NA	NA	NA	12.8
French Polynesia	1.0	1.0	1.0	1.0	1.2
Indian					
Maldives	28.9	16.0	20.0	24.0	16.0
Indonesia	2.4	3.7	4.1	4.4	4.7
Japan	0.1	0.3	-	-	-
India	NA	NA	NA	NA	6.1
Pakistan	NA	NA	NA	NA	5.8
Sri Lanka	NA	NA	NA	NA	14.0
Australia	-	0.1	-	0.1	0.5

-: Catch <100 tonnes

NA: Figures not available

Fig. 3.1 The location of skipjack fisheries in the Indian and Pacific Oceans. Heavy lines mark the approximate limits of the species distribution.


JAPANESE BAIT BOAT
Hawaii
EASTERN PACIFIC
Palau
Philippines
Sri Lanka
Sumatra
East Indonesia
Papua New Guinea
Solomon Is.
French Polynesia

The Japanese fishery, which dominates the catches, has at least two components, the homewater fishery, north of 20°N, and the southern water, or long range pole-boat fishery south of 20°S. With the advent of larger vessels, the geographical coverage and catches from the latter have increased dramatically since about 1965 (Kasahara, 1977) and the southern water fishery now accounts for over half the Japanese catch each year. Vessels from this fishery first entered the Papua New Guinea area in 1969, and by the end of 1975 operations extended eastwards to 170°W and well south of the Equator (Figure 3.1). Geographical distribution of effort in the southern water fishery is far from stable within the area outlined in Figure 3.1 (Kasahara, 1978; Bour and Galeon, 1979) and given the mobility and opportunism of the fleet, reflects spatial variations in availability as expressed by catch rates. The same is t[illegible]f the fishery next in size, the eastern Pacific fishery, where pur[illegible] seiners account for most of the catch.

The remaining skipjack fisheries are restricted in geographical extent by comparison, and effort within these areas tend to be more uniformly distributed throughout the year. Fisheries in this category within 1,500 km of Papua New Guinea are the Solomon Islands fishery, the Palau fishery and the east Indonesian fishery (Figure 3.1). With the exception of the last named, few if any published data are available; unpublished data have therefore been obtained by approaching the Government agencies concerned.

The quality of statistics from particular fisheries varies. Those from the Japanese fishery are published on an annual basis giving details of species' catch by one degree square (tonnes) for 10 day, monthly and yearly time strata. Effort data by vessels of various size are also provided (Anon. 1975, 1977 a, b, c, d, covering the years 1971-75 inclusive). It is estimated (Kasahara, 1978 a) that 80% of larger (long range) vessels and 70% of small and medium sized vessels are included in these statistics. As the area covered by this fishery is very large, the data in this form (by one degree square) is unwieldy and has recently been summarized in terms of 5° (latitude) x 10° (longitude) quadrangles south of 20°N and six sub-areas north of 20°N (Kasahara, 1978 b). These areas are shown in Figure 3.2.

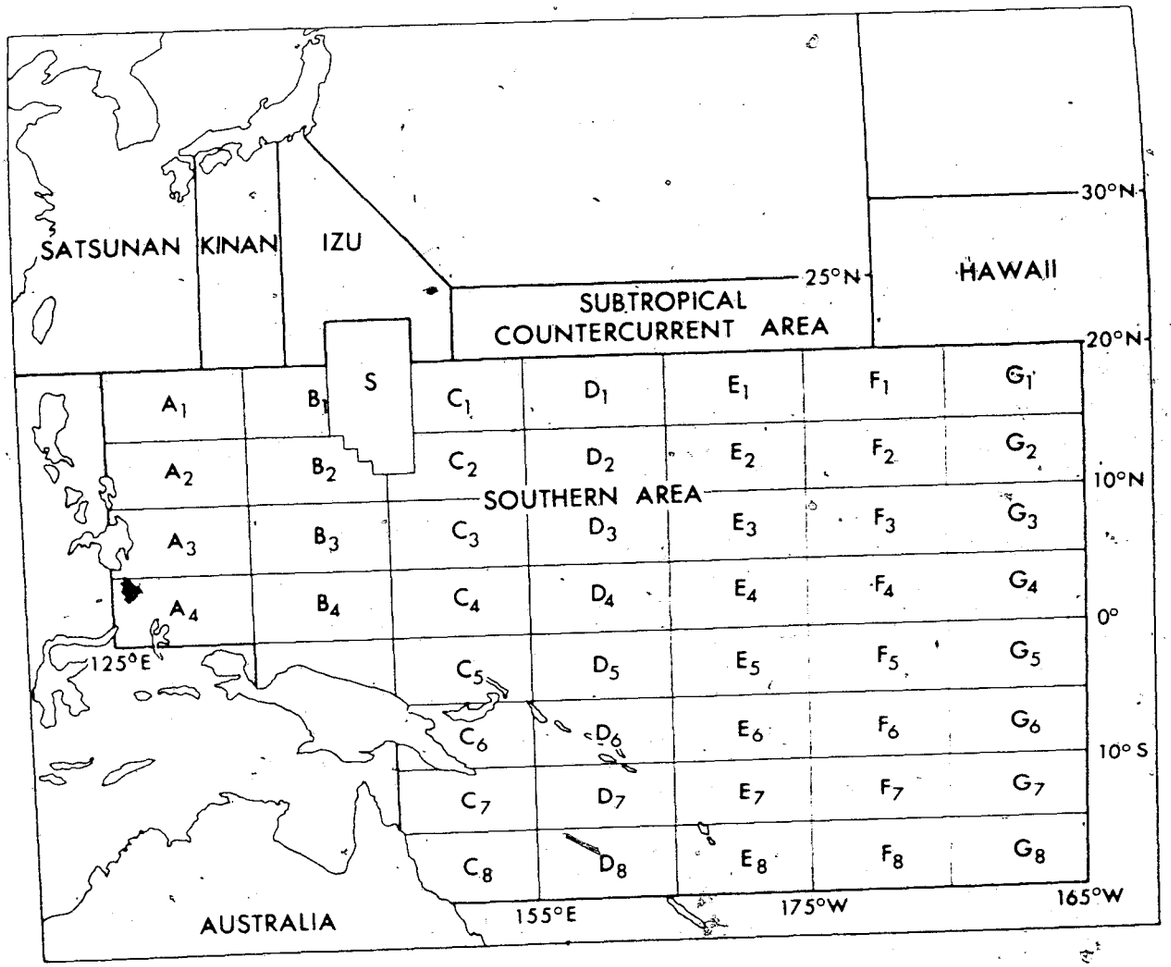


Figure 3.2 Statistical areas of the Japanese pole and line fishery in the western and central Pacific Ocean.

Statistics of the eastern Pacific fishery are collected by the Inter-American Tropical Tuna Commission to assist in the formulation of management policies. They comprise logged catches (in short tons by 1-degree and 5-degree areas, by months, quarters and years, by types of gear (purse seine and bait-boat), and by size classes of vessels), and the corresponding effort, both unstandardized and standardized to particular classes of purse seine and bait-boat vessels. The effort data obtained by the Commission represent about 90% of the total effort and are assumed to have the same distribution by area and time as the total effort. (Bayliff and Rothschild, 1974).

In less developed countries, such as Indonesia and the Philippines, where much of the catch is taken by small vessels for subsistence purposes or domestic consumption, reliable figures are more difficult to obtain. Estimates of total catch are available for most fisheries however, and other than the Japanese and Papua New Guinea fisheries, more detailed information has been sought only when the recapture of tagged fish rendered this necessary.

3.2.1 The Papua New Guinea Fishery

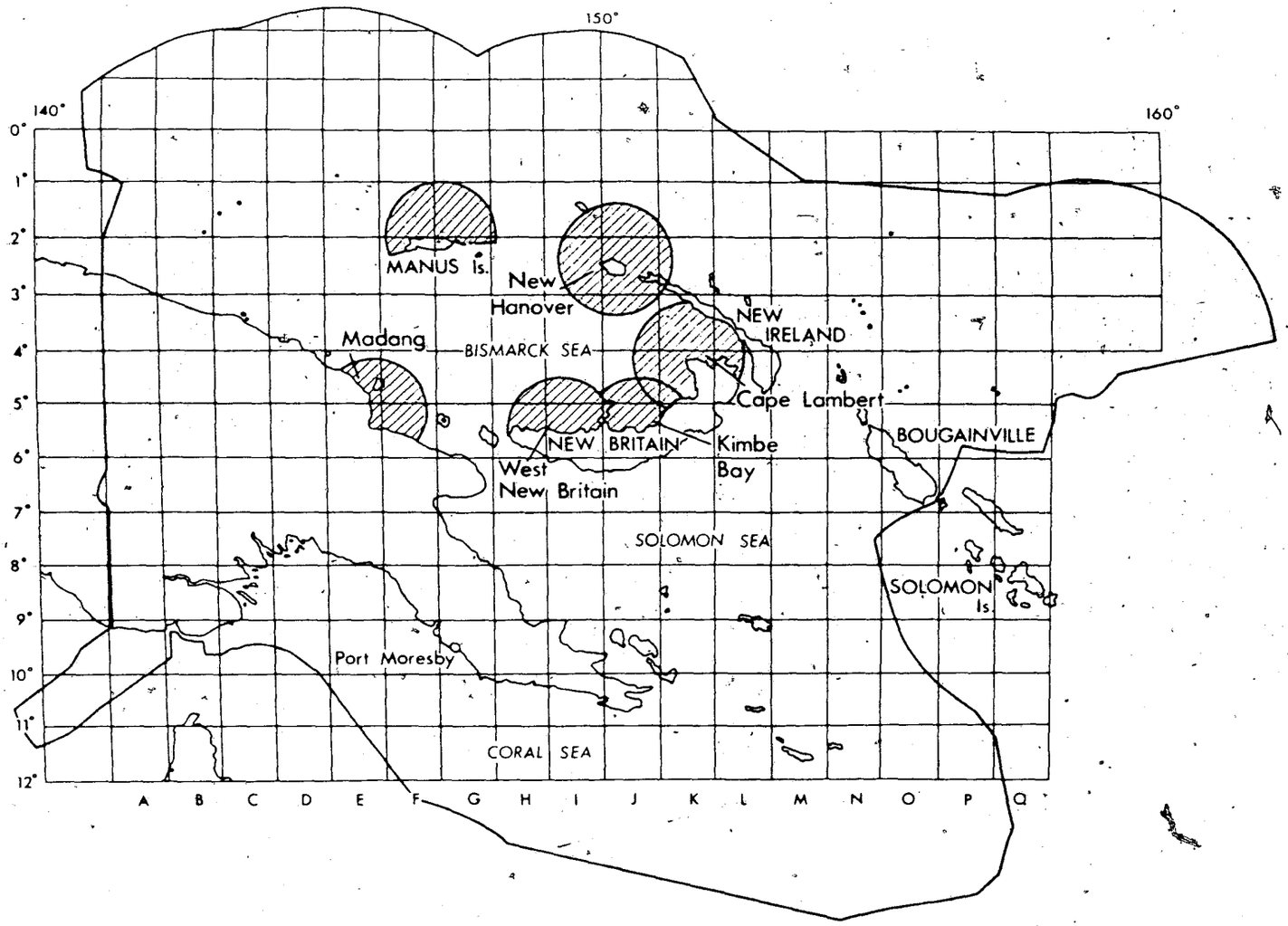
The development of the exclusively pole and line fishery from its beginnings in 1970 is described by Kearney (1975) and features of later years by Lewis (1976) and Lewis and Smith (1977). By 1973, four joint venture companies were operating 33 vessels and by 1974, the catch had reached 40,000 tonnes, placing the fishery amongs the world's largest skipjack fisheries.

Under the conditions of licence, individual vessels were required to provide figures on daily catch by species (tonnes), operating area (one degree grid squares coded as in Figure 3.3), average weight by species and limited environmental data. These data were then processed to provide, on both a per-vessel, per-company and per-one degree square basis, monthly and annual reports on average daily catch, species composition, average weight by species and effort (fishing days i.e. [illegible]bait was carried).

Operations by the company fleets were conducted on a daily basis, with vessels unloading each night to a mother ship. This effectively

Fig. 3.3 The waters of Papua New Guinea

The boundary of the declared Offshore Seas is shown as a solid line and the various sectors of the fishery shaded. One degree grid squares are coded by a combination of a longitudinal letter (A-Q) and a latitudinal number (00-12) as defined below. Sectors are shown as a 100 km radius from the central point of the baiting grounds.


150°
140°
160°
0°
1°
2°
3°
4°
5°
6°
7°
8°
9°
10°
11°
12°
A B C D E F G H I J K L M N O P Q
MANUS Is.
New Hanover
NEW IRELAND
Madang
BISMARCK SEA
Cape Lambert
NEW BRITAIN
Kimbe Bay
West New Britain
BOUGAINVILLE
SOLOMON SEA
SOLOMON Is.
Port Moresby
CORAL SEA

imposed a limit to their operating range which was exacerbrated by the need to secure live bait each night. Suitable fleet baiting areas were relatively few in number and non-contiguous (Lewis, 1977), with the result that the fishery could be resolved into six operational sectors, each described by a 100 km radius from the baiting ground, which showed limited overlap (Figure 3.3). These, with their corresponding one-degree squares, are as follows:-

(1) New Hanover (J01, J02, I01, I02)
(2) Cape Lambert (K03, K04, J04, L03, L04)
(3) Kimbe Bay (J05)
(4) West New Britain (I04, I05, H05)
(5) Madang (E04, E05, F04, F05) and
(6) Manus Is. (F01, G01)

Table 3.2 lists monthly effort (days) and catch per unit effort (tonnes/day) by sector for the years 1972 to 1975 inclusive.

Although Japanese long-range pole boats had operated in the Papua New Guinea area since 1968 (Kasahara, 1977), their area of operations has overlapped to a very limited degree with the local joint venture vessels, reputedly as a matter of policy by vessel owners' associations. The two elements can therefore be treated as separate fisheries in the area, one characterized by continuity of effort in localized areas and the other by patchy distribution of relatively large amounts of effort in both space and time. The catches from both sources for the years 1973-75 are as follows.

	1972	1973	1974	1975
Catch by PNG vessels	11,718	27,234	40,214	15,624
Catch by Japanese vessels*	10,858	22,228	56,595	18,076

* from Kearney, 1979

In 1976, purse seine vessels captured an estimated 3,500 tonnes of tunas, mostly skipjack, within the offshore seas of Papua New Guinea, (unpublished data, Japan Fisheries Agency). Prior to this, however, catches both within Papua New Guinea waters and in adjacent areas had been negligible relative to bait-boat catches and the activities of purse

Table 3.2 Monthly effort (fishing days) and catch per unit effort (tonnes/day) by sector for the years 1972, 1973, 1974 and 1975

A dash indicates no effort, no catch; a number followed by a dash, effort (days) but no catch. The Solomon Sea sector centres on I06 and was developed during 1975.

1972	J	F	M	A	M	J	J	A	S	O	N	D
New Hanover	37(0.3)	71(0.6)	102(1.1)	156(2.6)	267(2.5)	257(2.0)	208(2.5)	162(3.4)	136(0.9)	175(2.7)	170(4.1)	78(2.7)
Cape Lambert	147(2.0)	173(0.6)	322(3.2)	172(2.3)	178(3.3)	101(1.2)	164(2.6)	100(2.2)	48(1.1)	109(4.7)	176(5.4)	242(3.9)
Kimbe Bay	-	-	18(1.0)	31(1.7)	71(1.7)	3(0.4)	3(1.0)	70(1.5)	50(1.5)	86(1.6)	87(2.9)	86(1.5)
West New Britain	-	5(2.9)	1 -	13(0.9)	-	-	-	-	-	-	1(0)	2(5.1)
Madang	201(1.8)	103(0.2)	53(0.3)		65(2.3)	108(0.8)	17(0.4)	-	8(0.2)	20(0.6)	3(0.8)	8(0.1)
Manus	-	-	1 -	9(0.9)		1(0.6)	2(0)	-	-	-	-	-

1973	J	F	M	A	M	J	J	A	S	O	N	D
New Hanover	24(0.4)	8(0.1)	90(1.1)	182(1.4)	195(4.1)	196(6.5)	204(8.4)	208(4.5)	173(6.9)	203(4.6)	I94(5.7)	105(5.6)
Cape Lambert	237(1.2)	116(1.6)	241(1.7)	228(2.0)	479(3.9)	488(2.5)	473(3.3)	408(5.8)	389(4.9)	260(2.3)	15(0.6)	199(6.5)
Kimbe Bay	64(0.9)	4(0.2)	51(0.3)	15(0.1)	1(0.1)	2(1.5)	103(4.0)	171(313)	190(4.4)	151(0.7)	158(1.3)	162(3.7)
West New Britain	-	-	8(1.0)	7(0.5)	1(0.1)	60(5.3)	116(2.1)	145(2.8)	145(4.4)	-	-	11(1.4)
Madang	30(0.2)	126(0.1)	22(0.3)	110(0.8)	135(1.2)	61(1.2)	1(0.5)	-	-	-	2(1.0)	10(1.1)
Manus	3(0)	-		26(0.2)		14(1.9)	-	-	-	12(5.5)	200(5.4)	39(2.9)

1974	J	F	M	A	M	J	J	A	S	O	N	D
New Hanover	-	-	38(8.5)	247(4.4)	386(9.0)	340(8.3)	327(6.4)	282(3.9)	284(3.7)	305(5.6)	310(6.0)	243(4.5)
Cape Lambert	162(6.6)	175(8.3)	276(3.9)	338(4.2)	468(3.2)	438(3.8)	378(3.6)	478(4.6)	450(4.2)	475(3.2)	467(2.1)	292(1.8)
Kimbe Bay	-	-	1(0.7)	16(1.4)	47(2.2)	-	144(3.3)	-	-	-	-	-
West New Britain	59(3.2)	56(6.0)	87(0.8)	18(5.0)	4(3.4)	45(4.9)	138(4.0)	168(3.3)	106(1.0)	12(1.0)	7(0.2)	29(2.3)
Madang	5(0.6)	4(0.3)	66(2.1)	90(5.9)	124(3.4)	121(3.8)	99(4.2)	86(4.6)	103(2.7)	170(2.0)	123(2.2)	89(2.0)
Manus	7(2.2)	-	-	-	-	-	-	-	-	-	-	-

1975	J	F	M	A	M	J	J	A	S	O	N.	D
New Hanover	6(0.8)	9(4.4)	6(5.7)	71(4.2)	224(2.8)	229(3.4)	236(3.7)	224(3.6)	279(3.1)	402(1.7)	226(2.3)	235(1.6)
Cape Lambert	109(2.6)	79(3.4)	130(3.8)	163(4.3)	383(1.6)	467(2.9)	462(1.9)	440(1.7)	265(1.9)	338(2.2)	231(1.0)	313(2.2)
Kimbe Bay	-	-	-	-	-	-	-	-	-	-	-	-
West New Britain	19(0.7)	14(0.5)	23(1.3)	-	1(5.2)	3(1.9)	2(0)	-	-	-	-	-
Madang	60(0.9)	16(0)	6(1.9)	9(1.1)	-	80(3.0)	171(1.1)	-	-	-	-	-
Manus	-	-	-	-	-	-	-	-	-	-	-	-
Solomon Sea	97(2.0)	128(1.6)	130(1.4)	29(1.3)	-	-	-	-	-	-	-	-

seine vessels during the period of the experiments, 1971-75, have been disregarded.

Although traditional fishing activities for near-shore tunas are important in some areas of Papua New Guinea, the main species taken is usually mackerel tuna *Euthynnus affinis*. Subsistence fishing was thus not expected to account for a significant number of recoveries.

In summary, the Papua New Guinea fishery, with its relative continuity of effort, and proximity to the release area, represents the best prospect for detecting cyclical or seasonal components of observed movements, but offers limited geographical coverage. The large Japanese bait-boat fishery, on the other hand, shows little continuity of effort in any given area but provides the only comprehensive coverage of the very large western Pacific region. Statistical data available from both these sources are reliable.

3.3 RELEASES OF TAGGED SKIPJACK

3.3.1 Planning

For the estimation of some parameters from mark-recapture experiments, such as Peterson estimates of population size, it is theoretically possible to calculate the number of fish which have to be tagged and the amount of subsequent sampling needed to obtain estimates within predetermined confidence limits if some knowledge of population size is available (Robson and Regier, 1964). In this case, where the structure of a large mobile population of unknown size was to be studied, such an approach is clearly precluded and optimization of available resources becomes the major consideration. A total of 10,000 skipjack tagged and released in or adjacent to the Papua New Guinea area over a three year period represented a realistic target whilst offering the prospect of building a solid data base if a modest return rate (5% or 500 tags) could be achieved.

A 20 meter vessel was made available for the work, modified and commissioned in mid-1971 (see Kearney *et al.*, 1972 for details). Releases of tagged skipjack then proceeded in three phases:

(1) during the latter half of 1971, appropriate tagging techniques were developed and put into operation;

(2) in the absence of any knowledge of skipjack movements within the area, releases during 1972 were directed at a single centrally located sector of the fishery, the Cape Lambert sector.

(3) releases during 1973 continued in this same sector, providing the basis for between-year comparisons; the geographical coverage of the releases was also extended during 1973 and 1974, and releases made in particular areas to test hypotheses based on early results.

Releases in areas which presented operational problems for the original vessel were facilitated by participation in joint Japan-Papua New Guinea tagging cruises and the charter of a commercial vessel for a limited period.

With the success of the experiments relying heavily on the co-operation of fishermen, processors and other shoreside personnel in returning tags with the required recapture data, a publicity campaign accompanied the establishment of the programme. Posters setting out the aims of the experiments and the recapture information were circulated widely throughout south-east Asia and the Pacific, and a reward of A$2.00* posted for the return of the tag plus associated information. As the local joint-venture companies were expected to account for most early recaptures, personal approaches were made to these companies, with follow-up at intervals, and recapture data sheets (Japanese-English) distributed to each vessel.

Margetts (1963) identifies the four most obvious factors contributing to loss of recapture tags as:

(1) tags not conspicuous enough;

(2) inadequate publicity and instructions to finders as to what to do with tags;

(3) inadequate reward incentive, and

(4) carelessness by tag finders.

* originally 50¢ but increased after a few months.

Use of the bright yellow dart tag protruding 5 cm or more from the fish's dorsal surface was felt to negate factor 1. Factors (2) and (3) were given full consideration and as a result it is hoped that factor (4), largely beyond the investigator's direct control, was minimized.

3.3.2 Tagging Techniques

As noted earlier, skipjack present a particular set of problems for mark-recapture experiments. Their extremely high metabolic rate and susceptibility to rapid physiological damage requires that the time out of water and the amount of handling be minimised; their high basal swimming speeds (Magnuson, 1973) and even higher burst speeds (Yuen, 1966) require that the tag used be securely anchored without causing damage to the fish, yet not interfere with laminar flow.

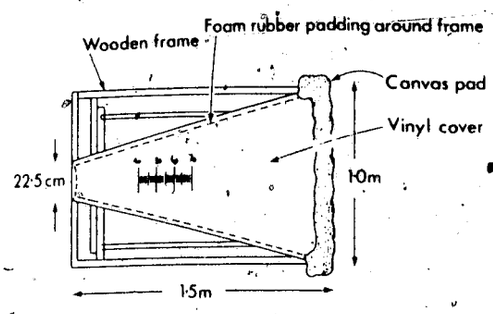
The technique developed was modified slightly from that used for tagging small tunas in other areas (e.g. Yamashita and Waldron, 1958; Fink, 1965; Bayliff, 1973). It involved the use of dart tags and a vinyl-lined cradle (Figure 3.4) and enabled skipjack to be tagged, measured and released within 10 seconds of hookset without suffering apparent damage (Lewis, 1980a).

Poled skipjack were swung towards the gloved hands of an assistant standing at the head of the cradle. The barbless hook was gently shaken loose in the cradle, the fish quickly checked for bleeding, particularly from the gills, and for injuries other than superficial cuts, then was either guided down the sloping cradle towards the tagger or rejected.

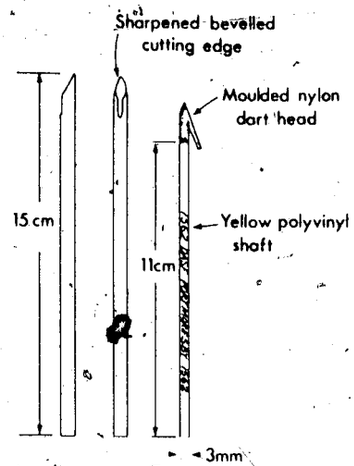
The tag was inserted just behind, or level with, the posterior end of the second dorsal fin at an angle of approximately 45^{o} to the longitudinal axis so as to position the barb securely behind a neural spine or second dorsal fin ray support. The two elements overlap in this region and provide ample anchorage for the tag (Figure 3.4). Care was taken to insert the tag neither too deeply (to decrease risk of damage, particularly to the highly vascularized deep red muscle), nor too superficially in the dorso-lateral musculature (to decrease the possibility of slippage). The applicator was then withdrawn, leaving the tag with about 7 cm of the shaft protruding in medium size (50-60 cm) skipjack. Tagged fish tossed gently from the cradle over the abutting

Figure 3.4 Details of the tagging equipment, the areas of insertion of the tag and a tag in position on a live skipjack prior to release.

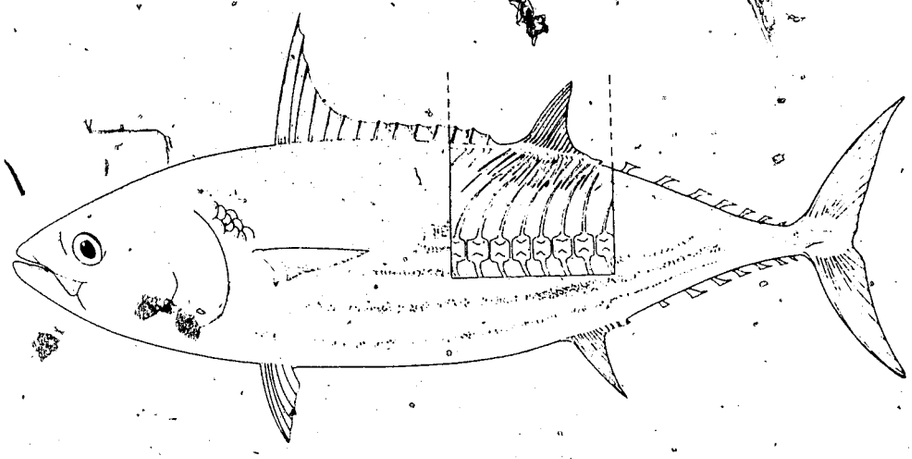
The composite cutaway sketch, based on a series of X-rays, clearly shows the overlap between neutral spines and fin ray supports (dorsal pterygiophores).

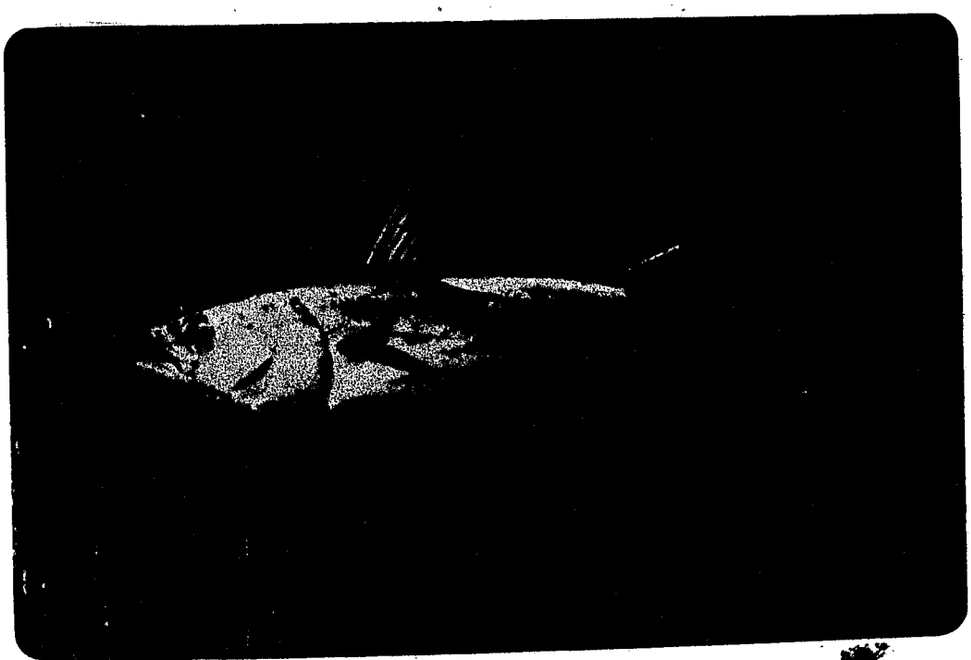


Plan of tagging cradle



Applicator. Tag





gunwale had a short fall of two metres to the sea surface. The vast majority of fish were released within ten seconds of hookset and some within six seconds.

3.3.3 Releases

In general, skipjack tagged during a cruise, i.e. between successive port calls, constitute a release set. Table 3.3 lists locality, dates, numbers of tagged skipjack released and the vessel used for the 9,547 skipjack tagged in 23 release sets between December 1971 and June 1974.

The success of particular cruises as measured by numbers of tags released in a specified area is influenced by a variety of factors, most importantly prevailing weather and fishing conditions. For example, 1972 proved a poor year for the Papua New Guinea fishery and average monthly catch rates exceeding three tonnes/boat/day were experienced only in the October-December period, (Table 3.2). Reflecting this, October and November accounted for 3,012 (87%) of skipjack releases during 1972.

Difficulties at other times were experienced with shortages of suitable bait species, mechanical and logistical problems. Nevertheless, the number and distribution of releases accords well with the original plan. In the Cape Lambert sector, 3,454 and 3,140 skipjack were released in 1972 and 1973 respectively to provide the basis of between-year comparisons, and the remaining 2,953 releases were distributed widely through the Bismarck, Solomon and Coral Seas.

3.3.4 Size at Release

As data from the experiments were to be used to provide estimates of growth (Josse *et al.*, 1979) and as size-related effects on the degree of mobility, or migratory tendency, had been reported (Kawasaki, 1965; Mori, 1974), it was clearly important to obtain measures of size at release.

Length estimates

Until late 1972 no individual size estimates of tagged skipjack were made on the premise that as size uniformity within schools is high,

Table 3.3 Details of tagged skipjack releases between December 1971 and June 1974.

Release set	Dates	Locality	No.	Vessel
1	11-14/12/1971	New Britain south coast I06 (Solomon Sea)	74	FRV Tagula "
2	3-4/4/1972	New Britain south coast J06 (Solomon Sea)	14	" "
3	9-30/5/1972	Open Bay, Cape Lambert K04	143	"
4	2-23/7/1972	" " K04	299	"
5	7-19/10/1972	" " K04	577	"
6	29/10-4/11/1972	" " K04	1488	"
7	9-17/11/1972	" " K04	876	"
8	21-24/11/1972	Offshore, Cape Lambert K03	71	"
9	17-23/4/1973	Solomon Sea I06, J06	34	"
10	5-14/6/1973	Cape Lambert K04	805	"
11	23/6-5/7/1973	" K04	1252	"
12	11-12/7/1973	" L04	400	"
13	5-8/8/1973	New Hanover I01, J02	84	"
14	28-30/8/1973	Cape Lambert L04	471	"
15	5/9/1973	" L04	212	"
16	19-28/9/1973	New Britain - various K04+ K05	388	"
17	21/10-8/11/73	Madang E04, C03	326	"
18	2-12/12/1973	nth Coral Sea I10, G09	300	"
19	16/2-4/4/1973	Port Moresby G09	62	FRV Rossel
20	11-21/10/1973	New Hanover J01, L02, L03	240	RV Fuji Maru
21	29/10/1973	Solomon Sea I06, J07	143	"
22	11/6-28/6/1974	New Hanover J01, J02 I01, I02	1066	MV Daido Maru
23	16/1-18/4/1974	Port Moresby G09	222	FRV Rossel
			9547	

mean school size (obtained from direct measurements of untagged fish on the deck) can be used with confidence as an estimate of size at release for individuals in that school (Rothschild, 1967). This was questioned when aggregations with bimodal length frequency were encountered. In addition, early returns indicated that growth increments in tagged fish were likely to be small, making it desirable to estimate length at release as accurately as possible if growth studies were to be pursued. Consequently, individual lengths were then estimated with reference to prominent lines at 5 cm intervals and smaller lines at 1 cm intervals on the vinyl lining of the cradle. Initial concern that this additional step in the tagging procedure would add considerably to the time taken (Kearney et al., 1972 - p. 109) and so prejudice survival proved unfounded, with an extra second required at most.

For the 1972 releases, individual length estimates (ℓ_1) were available for 508 skipjack only, the mean length within each school ($\bar{\ell}_1$) serving as an estimate of size at release for the remainder (Lewis, 1980a). Individual length estimates (ℓ_1) were available for over 96% of the 1973-74 releases and are summarized by release set in Table 3.4.

Size Composition

Of the skipjack measured during the course of the 1972 releases, 96.4% of individuals were in the 50-60 cm range (Lewis *et al.*, 1974 - Figure 5); 85.7% of the 1973-74 ℓ_1's came into this category with 95.4% of ℓ_1's between 45 and 60 cm (Table 3.4). If 45 cm is accepted as the typical size at first maturity (see 2.3.5), releases consist almost entirely of mature fish. Although estimates of skipjack age and growth vary widely, most recent estimates (see 2.3.7) would place the age of these 45-60 cm fish as 1^+ and 2^+ i.e. in their second and third years of life. The experiments thus provide no information on the movements of immature (0^+) or larger (3^{++}) fish.

As the strategy was to tag and release skipjack of all sizes, the restricted size range of skipjack tagged and released simply reflects availability of particular size classes rather than selectivity imposed by the technique or deliberate avoidance of fish of particular size.

Table 3.4 Frequency of estimated length (ℓ_1) at release for skipjack tagged during 1973-74

ℓ_1	Release set 9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Total	% of total
<40	1										5			2		8	0.1
40	4							1			2			2		9	0.1
41	3									2						5	0.1
42	2										1	2	3			8	0.1
43	2										1	1	5	1		10	0.2
44	1					1						8	6	1		17	0.3
45	3		2	1		5		1			1	9	17	6	1	46	0.8
46	1	1								3	4	4	8	5	9	35	0.6
47		1	1	1	2			4		12	4	8	10	16	31	90	1.6
48	1			3	5	5	1	5		16	3	9	7	18	25	97	1.7
49		5	3	4	7	7		20		23	2	16	9	15	23	134	2.3
50		34	12	5	12	10	1	34		48	4	19	1	25	34	239	4.1
51	3	28	20	7	21	12	1	51		20	3	12	2	24	28	232	4.0
52	3	62	52	57	9	34		44	2	36	4	24	5	15	58	405	7.0
53	2	57	198	108	17	38	6	35	7	30	1	33	2	7	49	590	10.2
54	1	90	154	80	8	49	19	22	8	30	1	17	11	9	70	569	9.8
55	3	113	290	66	0	76	45	19	33	35	1	42	20	9	150	902	15.6
56	1	127	251	9	1	97	46	33	49	12	1	15	10	11	165	868	15.0
57		104	123	7		88	49	13	66	6	1	10	14	7	182	670	11.6
58	1	71	62	4		20	30	23	48	1		4	7	5	114	390	6.7
59		59	11	1		5	9	24	39	2		1	1	1	67	220	3.8
60		25	6			1	2	8	35	2	1		3		38	121	2.1
61		9				1	1	4	19			1			15	50	0.9
62	1	4	2					6	5	2			1		3	24	0.4
63		1						7	7	2					2	19	0.3
64								10	3	1						14	0.2
65		1						6	1	4						12	0.2
66								2		2						4	0.1
67						1		2								3	0.1
68								1		1						2	0.1
69																	
70																	
	33	792	1187	393	82	450	210	375	322	290	40	235	142	179	1064	5794	

Other sources of Data

From March 1972, length frequency data were collected from the fishery to assist in resolving the catch into component size classes and to complement the tagging data. Because of logistical problems, rigorous sampling procedures could not be maintained and these data have not been used. The research vessel data (see later) and the average weight data from the companies remain the only reliable sources of such information during the course of these experiments. Collection of length frequency data from the Papua New Guinea fishery was however resumed during 1977 and has proved a useful adjunct to the genetic studies (see later). These data confirmed that the size composition of stocks exploited by the Papua New Guinea fishery is similar to that of the releases. (Wankowski, in press.)

Modal progression

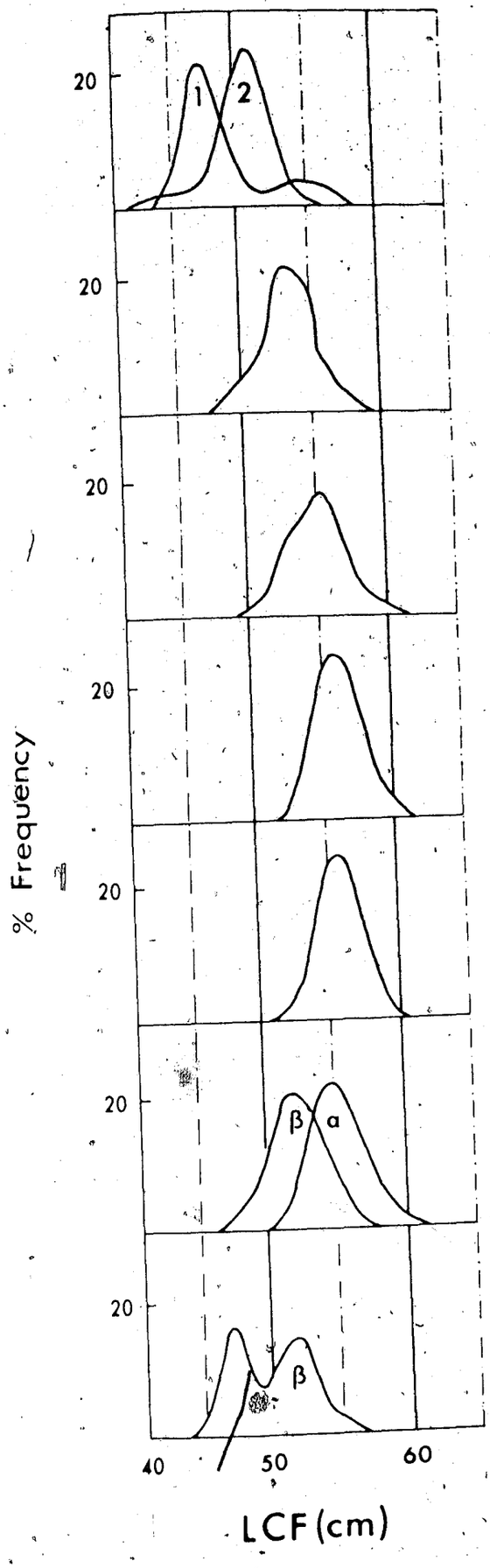
Only releases in the Cape Lambert sector (sets 3-8, 1972; sets 10-12, 14-15, 1973) offer the continuity which might enable modal progressions in length frequencies to be followed amongst release sets.

Some shifts in size distribution were evident in the 1972 data and are taken to reflect changes in stock composition. A slight modal progression in the research vessel length frequency data can be followed through May - October (Figure 3.5, sets 3 - 6). This approximates the growth rates given by Josse *et al.* (1979) for skipjack of this size in the Papua New Guinea area and is consistent with a single group of fish remaining in the release area during the period.

Early in November, however, schools of smaller skipjack appeared and by mid-November these fish had virtually replaced the larger skipjack. Mixing between the two groups was apparently minimal, with mean lengths within schools showing no overlap ($\bar{\ell}_1$ = 50.7 - 53.1 cm cf. $\bar{\ell}_1$ = 54.6 - 56.5), and two distinct elements, α (larger) and β fish have been recognised in the analysis (see set 7, Figure 3.5). The influx of the smaller fish into the fishery was reflected in a dramatic increase in catch rates during October, continuing through November-December, and a decrease in average skipjack size (Table 3.2).

Figure 3.5 Length frequency data from the research vessel catch by release set.

Lengths in one cm groupings have been expressed in terms of percentage frequency and smoothed by a running average of three.



Set 1 11-14/12/71

n = 301

Set 2 3-4/4/72

n = 74

Set 3 9-30/5/72

(a) n = 136

Set 4 2-23/7/72

(a) n = 237

Set 5 7-19/10/72

(a) n = 216

Set 6 29/10-4/11/72

(a) n = 847

Set 7 9-17/11/72

n_α = 134

n_β = 159

Set 8 21-24/11/72

n = 87

In the November data, a third size class, comprising 45-49 cm skipjack, can also be distinguished. Limited numbers of these fish were tagged and released during a brief period of fishing offshore and comprise part of release set 8.

There were thus two major (α,β) and one minor distinct size classes of skipjack tagged and released in the Cape Lambert sector during 1972.

No clear progressions were evident in the 1973 data. As 1973 was a much more productive year for the fishery with over twice the 1972 catch this may reflect a more complex recruitment base. In any event, it was not possible to separate and follow particular size groups between release sets.

3.4 RETURNS OF TAGGED SKIPJACK

3.4.1 Data Analysis

Tag return data

Information potentially available for each fish released was as follows:

- individual identification (the tag number)
- time and date of release
- estimated size at release (mm) - ℓ_1
- mean length of fish in the school or aggregation (mm) - $\bar{\ell}_1$
- position of release to the nearest minute of latitude and longitude.

Persons or agencies returning tagged fish were asked to supply the following:

- date of recapture
- length at recapture (mm) and weight (kg) if possible
- recapture method
- position of recapture (latitude and longitude).

These data were then used to calculate for each return where adequate data were available:

(a) minimum distance moved, great circle distance in nautical miles calculated from the formula -

$$\text{Distance} = \cos^{-1}[\sin(LAT_1)\sin(LAT_2) + \cos(LAT_1)\cos(LAT_2)\cos(LNG_2 - LNG_1)] \times 60$$

where LAT_1 & LNG_1 = latitude and longitude of release

and LAT_2 & LNG_2 = latitude and longitude of recapture.

In the calculations, no allowance could be made for land masses between points of release and recapture. Actual distance is underestimated in such cases, but the number of returns involved was very small.

(b) direction of movement (azimuth)

(c) days at liberty.

To avoid confusion amongst the many terms used in tagging experiments, the terminology adopted by ICNAF (Anon, 1961) and used by Fink and Bayliff, (1970) has been employed throughout:

"releases: the (number of) fish tagged and released;

recaptures: the (number of) tagged fish caught;

recoveries: the (number of) tagged fish detected by fishermen or in any other way;

reports: the (number of) tagged fish concerning which any information reached the tagging organization sufficient to establish that they have been recovered;

returns: the (number of) reported tagged fish or tags which are eventually returned to the tagging organization, or the existence of which is fully authenticated."

Analysis of dispersal

Movement of individual fish can be regarded as the resultant of two components, random and directional movement, and several measures of these components have been developed. Incorporating Skellam's (1951) earlier work, Jones's (1959, 1976) mean square dispersion coefficient (α^2) measures the extent to which individual fish move independently of one another, or "the average amount of deviations from the mean direction of movement" (Bayliff and Rothschild, 1974) and so is a measure of randomness. His mean velocity of dislocation (V) measures the overall displacement of the group, or directional movement. Using Cartesian rather than polar co-ordinates, Bayliff and Rothschild (1974) devised a variance-covariance method. The determinant of the variance-covariance matrix is an index of the dispersion of the fish and as such, is similar to Jones's α^2. The correlation coefficient approaches 0 when movement is random and 1 when most movement is unidirectional or in two opposing directions. It remains far from clear how these measures are affected by schooling behaviour or changes in school integrity and composition, considerations which are important in skipjack (see earlier). Adjustments also need to be made for the distribution of effort.

Here, a more simplified approach has been preferred for the following reasons:

(a) The orientation of the Bismarck Archipelago effectively restricts local movements from releases in the eastern Bismarck Sea (the majority of releases - see later) to the northwest-southwest quadrant (see Figure 3.3). Furthermore, fishing effort to the west (central Bismarck Sea) and east (north Solomon Sea) was low, with no effort by Papua New Guinea vessels and effort by long range vessels either very low or highly seasonal. Local dispersal from the release area could thus be detected along north-west and south-west axes only.

(b) With the restricted mode of operation of vessels based in Papua New Guinea, the relatively small proportion of Papua New Guinea's sea area covered by them and the limited overlap between sectors (Figure 3.3), local returns basically reveal saltatory movements between sectors and as such, are not well suited to the analyses described above. The

continuity of effort in most sectors improve the possibility of detecting cyclical or periodic events, an important consideration when population structure is being investigated.

The number of returns per sector per month for each release set was adjusted by a factor which corrects, albeit imperfectly, for time/area variations in effort over the period of the experiment. This adjusted number of returns (N_{ij}) was calculated as follows, following Bayliff and Rothschild (1974).

$$N_{ij} = \left(\frac{n_{ij}}{f_{ij}}\right) \cdot \left(\frac{\Sigma_i \Sigma_j n_{ij}}{\Sigma_i \Sigma_j \frac{n_{ij}}{f_{ij}}}\right)$$

where n_{ij} = actual number of returns in sector i during month j.

f_{ij} = effort (days) in sector i during month j.

When tabulated, the return data are then in a convenient form for examining the direction and timing of movements between sectors.

Analysis of longer distance returns, specifically, relating to returns to the spatio-temporal distribution of effort, has been attempted on a yearly basis only, because of the relatively small number of returns involved.

Mortality estimates

Estimates of total mortality coefficients, or losses from the tagged population (Z) can be useful in population studies in allowing survival (S), the proportion of tagged fish remaining in an area after a given period, to be calculated from the formula $S = e^{Zt}$. Turnover rates can then be gauged. Such estimates are however subject to numerous sources of error. Using the notation of Bayliff and Mobrand (1972), the number of tags remaining on skipjack after time t is given by:

$$N_t = N_o \pi \rho e^{-Zt}$$

where N_t = no. of tags remaining on skipjack after time t

N_o = no. of tags released

ρ = proportion of skipjack alive after immediate tagging mortality.

π = portion of tags retained after immediate shedding

Z = instantaneous total losses

More specifically, $Z = F + M + G + L + E$

where F = instantaneous fishing mortality

M = instantaneous natural mortality

G = instantaneous tag-induced mortality

L = instantaneous tag shedding

E = instantaneous emigration.

Using the regression method (Fink, 1965, Mather *et al.*, 1974) Z estimates were made for release sets in the Cape Lambert sector during 1973. As dispersal proceeded, effort figures were summed over all sectors from which returns were received, and the estimates thus represent loss rates from the Bismarck Sea. Attempts were made to estimate L and ρ, the instantaneous and immediate coefficients of tag slippage by double tagging experiments, π and G, the immediate and instantaneous coefficients of mortality attributable to tagging, and levels of non-reporting of recaptures (Lewis, 1980b).

3.4.2 Total Returns

A total of 728 returns (7.6%) was received from the 9,547 releases. They originated from the following sources:

	1971-72 releases	1973-74 releases	TOTAL
Papua-New Guinea based vessels	250	351	601
Papua-New Guinea mother ships and shore bases	-	11	11
Joint venture vessels in other fisheries	2	6	8
Long range pole boats	13	45	58
Indigenous fishermen	4	1	5
Canneries (mostly U.S.A.)	4	33	37
Tagging vessel	5	3	8
	278	450	728
% return	7.8	7.5	7.6

A limited number of additional recoveries were reported but these could not be verified and have not been considered. As can be seen from the above, the Papua-New Guinea fishery and the Japanese long range pole boat fishery accounted for most returns, especially as nearly all the cannery returns could be traced to shipments from the Papua New Guinea fishery. The higher proportion of returns from processing facilities (shore bases and canneries) in 1973-74 was probably a function of the larger catches during both years (Table 3.2) when tagged fish were presumably more likely to be overlooked on board the fishing vessels. Return rates, although varying widely between individual release sets (see later), did not differ significantly between 1971-72 and 1973-74 releases.

3.4.3 Returns by time strata

Tables 3.5 and 3.6 give return rates and times at liberty stratified by 50-day intervals for 1971-72 and 1973-74 release sets respectively. Times at liberty ranged from 10 minutes (recaptures by the tagging vessel) to 789 days. 78 (28%) of 1971-72 releases were recovered within 50 days, whereas 235 (52%) of 1973-74 returns were within this period. Conversely, corresponding figures for returns of fish at liberty in excess of 200 days were 142 (51%) and 91 (20%) respectively.

3.4.4 Dispersal

The largest net dispersal recorded from the point of release was 1371 nautical miles and only four other returns beyond 1000 nautical miles were received. The calculated displacements, summed over 100 nautical mile intervals, for all returns where adequate data was available are given in Figure 3.6. As this is biased by the large number of returns immediately following release, the calculated displacement for fish at liberty longer than 100 days is also shown. Approximately 70% of these returns still showed displacement less than 200 miles. The greater number of returns in the 101-200 mile category results from the high return rate in the West New Britain sector during mid-1973 from 1972 releases in the Cape Lambert sector (see later).

The leptokurtic shape of the frequency distribution curve is general in movement studies (e.g. Endler, 1977). Grant (1980) points

Table 3.5. Returns of tagged skipjack from the 1971-72 release sets by 50-day time strata

Days at liberty	Release set 1	2	3	4	5	6	7	8	Total
1-50				6	16	36	18	2	78
51-100			5	17	1		1		24
101-150			9	7	1		3	1	21
151-200			1	1		7	3		12
201-250	1			1	1	6	5		14
251-300				2	10	31	13	1	57
301-350					12	15	5		32
351-400				3	2	2			7
401-450			2	5		1	1		9
451-500				1		2	1		4
501-550									5
551-600						3	2		
601-650					1	5	1		7
651-700					1	1	3		5
701-750						1			1
751-800		1							1
TOTAL	1	1	17	43	[illegible]	110	56	4	277
No. of releases	74	14	143	299	577	1488	876	71	3542
% return	1.4	7.1	11.9	14.4	7.8	7.4	6.4	5.6	7.8

	Cape Lambert					New Hanover			Southern Bismarck Sea		Solomon Sea		Coral Sea			
Release set	10	11	12	14	15	13	20	22	16	17	9	21	18	19	23	Total
1- 50	35	50	42	54	8	4	4	36	2	-		-				235
51-100	26	19	13	1	2	-	-	18	1	1		1				82
101-150	-	3	-	2	-	-	1	11	2	3		2				24
151-200	2	-	1	2	-	1	-	7	3	2						18
201-250	-	1	-	-	-	2	1	1	3	5						13
251-300	2	-	1	1	1	-	-	2	6	4		1				18
301-350	2	-	1	3	4	1	1	2	1	1	1					17
351-400	1	6	2	-	-		1	4	2	2						18
401-450	1	2	3	2	1		-	-	1	1						11
451-500	2	-	1	1			-	1	1							6
501-550	1	-					1							1		3
551-600													1			1
Other								1*			1†		2*			4
TOTAL	72	81	64	66	16	8	9	83	22	19	2	4	3	1	0	450
No. released	805	1252	400	471	212	84	240	1066	388	326	34	143	300	62	222	6005
% return	9.0	6.5	16.0	14.0	7.5	9.5	3.8	7.8	5.6	5.8	5.8	2.8	1.0	1.6	0	7.5

* Incomplete recapture data † 789 days at liberty

Table 3.6 Returns of tagged skipjack from the 1973-74 release sets by 50-day strata. Releases are grouped geographically

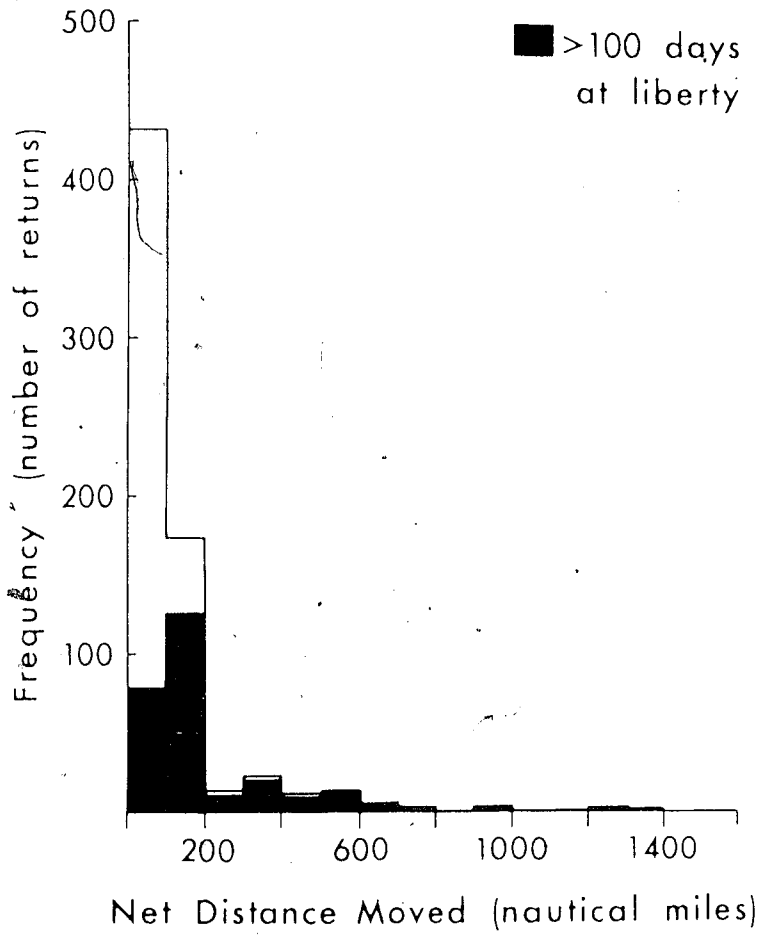


Figure 3.6 Frequency distribution of net distance moved by tagged skipjack.

out that long distance movements are frequently underestimated, and that the tail of the curve needs to be considered when assessing gene flow, particularly over time.

Returns after 100 days accounted for 94% of returns more than 200 miles (320 km) from the release point, suggesting that dispersal proceeds quite slowly. Williams (1972) assumed a figure of 50 miles per day to represent a reasonable distance covered by skipjack during orientated movement (migration). Maximum rates of displacement observed during these experiments fall far short of this figure.

Tag No.	Release details	Recapture details	Distance nm	Days	Mean Miles/day
4425	23/11/1972 3°54'S, 151°44'E	7/3/1973 4°58'N, 139°30"E	906	104	8.7
713	9/11/1972 4°50'S, 151°33'E	22/11/1972 4°16'S, 139°30'E	391	44	8.9
14215	16/6/1974 2°25'S, 149°55'S	20/7/1974 2°48'N, 144°09'E	466	34	13.7

3.4.5 Tag loss

Other than natural mortality, four major sources of tag loss are relevant to these experiments and need to be evaluated. As movement parameters only are being investigated, they become important only if the loss is large and non-random.

(i) Tag shedding (slippage)

From the 358 skipjack double tagged and released, 29 returns were received. In all but two cases, both tags were returned. The two single-tag returns were from outside the Papua New Guinea area in situations which could have resulted in tag loss after capture (a cannery and a long-range pole boat). With only one return from greater than 365 days, the logical division of the data set for the purposes of estimating ρ (portion of tags returned after immediate shedding) and L (instantaneous tag shedding) would be by six monthly periods. With 25 of the 28 returns in the

first 6 month period and 22 within the first 60 days, however, this is clearly of limited value. Inspection of comparable data sets (Laurs *et al.*, 1976; Bayliff & Mobrand, 1971; Lenarz *et al.*, 1973) suggests that values of ρ and L might lie within the approximate ranges of .98-1.00 and .10-.01 respectively. Tag slippage therefore seems a relatively unimportant source of tag loss.

(ii) Mortality attributable to the tagging process

This has immediate (π) and instantaneous (G) components. Two points would suggest that G may approach zero: more than half of the returns from 1972 releases were at liberty more than 200 days, and in the double tagging experiment, return rates from single and double tags did not differ significantly ($\chi_1^2 = 0.42$, $P \simeq 0.5$). Short term or immediate effects are more difficult to gauge - the normal practice of holding the tagged animals for a brief period after capture is clearly not appropriate here. All fish were observed to swim away rapidly and as all steps were taken to reduce the trauma of capture, the proportion surviving may be close to unity.

(iii) Non reporting of tags

The performance of both individual vessels of the same company and companies fishing the same area in returning tags was examined by relating returns received to relative amounts of catch and effort expended (Lewis, 1980b). Although significant between-vessel differences were observed in two of the three cases examined, cannery returns traced to this period could have removed these differences, suggesting that some vessels were less effective in detecting tagged fish on board rather than failing to report recaptures.

It is more difficult to assess non-reporting of recaptures from sources other than Papua New Guinea fishery. The good correlation obtained between levels of effort and number of tags returned by long range pole boats (see later) suggests however levels of any non-reporting were probably constant and random. Non-reporting in other fisheries cannot be estimated. The return of three tags from artisanal fishermen in remote areas suggests however that the publicity programme had been effective.

(iv), Tag Breakdown

The tag in use during the experiments was belatedly found to become brittle and prone to fracture at temperatures less than -20°C. Lower temperatures are common in the holds of long range pole vessels, though not in the holds of Papua New Guinea based vessels. Tags were usually noticed on deck before stowage of the catch, but failing this, tag brittleness could be a source of tag loss. Returns from later releases in the Papua New Guinea area using tags which retain their flexibility at low temperatures has not increased the geographical coverage of returns (see 3.6.1).

In summary, the findings of these experiments do not seem subject to serious bias introduced by variable levels of tag loss.

3.5 MOVEMENTS WITHIN THE BISMARCK SEA AND ENVIRONS

3.5.1 1972 Releases

Figure 3.7 plots the locality of all recaptures within the Papua New Guinea area from 1971-72 releases. The clumping of returns by sector is evident. Table 3.7 lists the adjusted number of returns per sector per month, N_{ij}, calculated as in 3.4.1., for release sets 5, 6 and 7 i.e. Cape Lambert sector releases in October-November, 1972. Returns from earlier releases in this sector (sets 3, 4) have not been included in this analysis because of reporting irregularities in the initial stages of the experiments (Lewis, 1980 a) and resultant changes in the size of reward offered.

The expected exponential decay in the number of returns with time was not observed, with N_{ij}'s in July-August 1973 (i.e. 8 months or more after release) approaching initial N_{ij}'s.

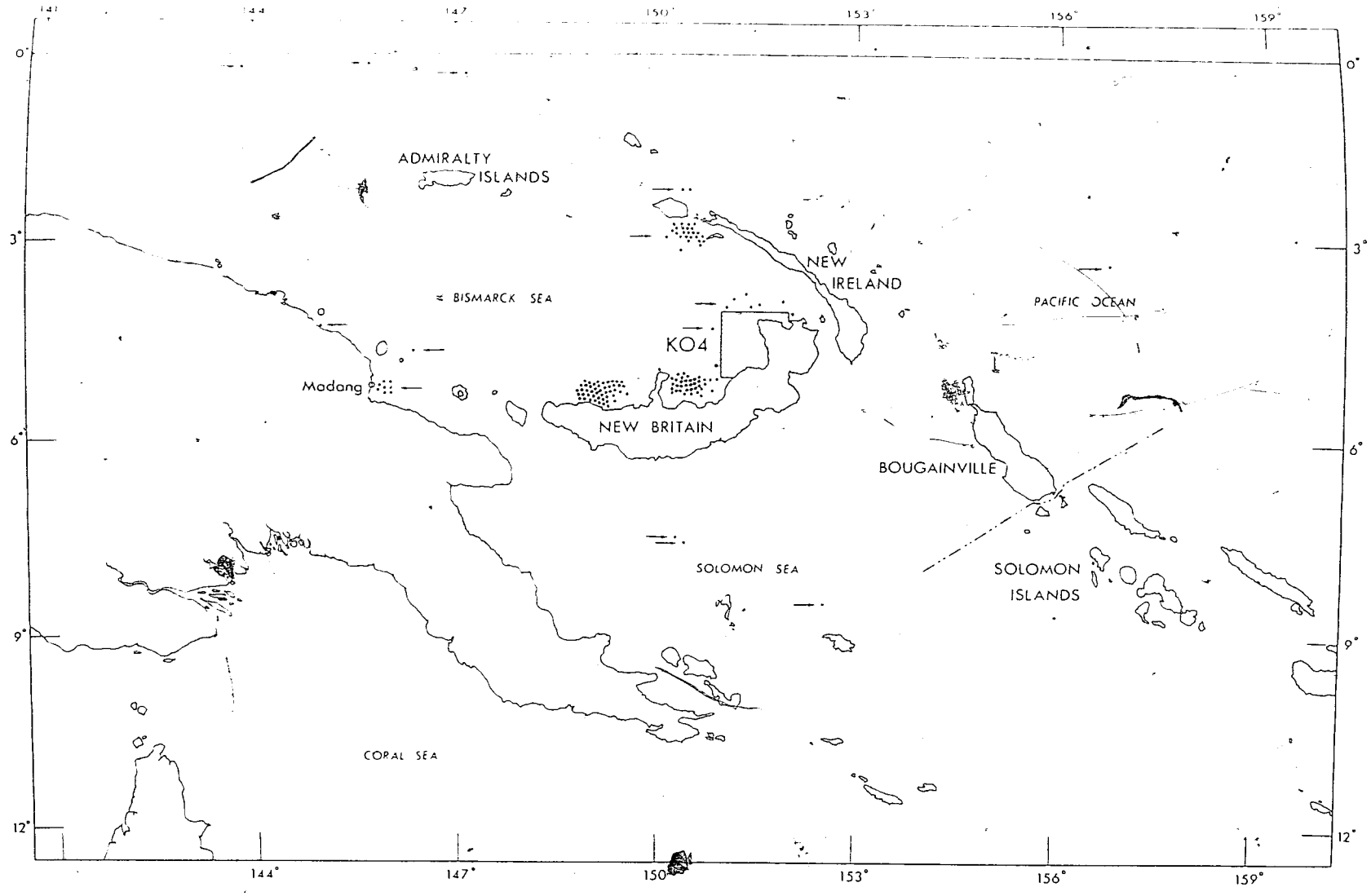
Five features were common to all three sets:-

(1) a strong initial movement to the south-west during November-December which was matched by a corresponding north-west movement in set 7 releases only,

(2) virtual disappearance of tagged fish from the Bismarck Sea sectors, other than isolated recoveries in the Cape Lambert sector (K04) adjacent to the point of release,

Figure 3.7. Location of returns beyond the release sector (KO4) but within offshore seas from skipjack tagged and released during 1971-72.

Arrows highlight the less conspicuous returns.


141°
144°
147°
150°
153°
156°
159°
0°
3°
6°
9°
12°
ADMIRALTY ISLANDS
BISMARCK SEA
NEW IRELAND
PACIFIC OCEAN
KO4
Madang
NEW BRITAIN
BOUGAINVILLE
SOLOMON SEA
SOLOMON ISLANDS
CORAL SEA

SET 5 7-19/10/1972

Month / Sector	1972 O	N	D	1973 J	F	M	A	M	J	J	A	S	O	N	D
New Hanover	–	0.8	–	–	*	–	–	–	–	–	1.9	–	1.3	–	–
Cape Lambert	4.9	(2.7†)	–	–	–	–	–	–	0.3	0.5	0.3	–	–	*	–
Kimbe Bay	–	8.2	4.6	–	*	–	–	*	*	–	–	–	–	–	–
West New Britain	*	*	*	*	*	*	*	*	2.2	8.2	3.7	3.7	*	*	*
Madang	–	*	*	–	–	–	–	–	–	*	*	*	*	*	*

SET 6 29/10-4/11/1972

New Hanover		–	–	–	*	–	–	–	–	–	2.7	1.6	2.0		
Cape Lambert		14.6	1.1	–	–	–	2.5	0.8	0.3	0.6	1.6	–	–	*	–
Kimbe Bay		17.2	4.6	–	*	–	–	*	*	–	0.8	–	–	–	–
West New Britain		*	*	*	*	*	*	*	6.8	16.4	8.4	8.4	*	*	*
Madang		*	*	–	–	–	–	–	–	*	*	*	*	*	*

SET 7 9-17/11/1972

New Hanover		–	7.0+	–	*	–	–	–	–	–	1.4	–	–	–	–
Cape Lambert		7.0‡	1.0‡	–	–	1.1	0.5	0.6	0.8	–	0.3	–	–	*	–
Kimbe Bay		3.0	1.4	–	*	–	–	*	*	–	–	–	0.5	–	–
West New Britain		*	*	*	*	*	*	*	2.2	–	5.6	4.6	*	*	*
Madang		*	*	–	–	–	–	–	–	*	*	*	*	*	*

TABLE 3.7 Adjusted recoveries by month (N_{ij}) during 1972-73 for release sets 5, 6 and 7. All fish were released in the Cape Lambert sector. Recoveries in the New Hanover sector and Kimbe Bay-West New Britain sectors represent north-westerly and south-westerly dispersal respectively

Symbols: * Less than 10 days' fishing effort; † research vessel recaptures; – no returns; + entirely β fish; ‡ entirely α fish.

(3) high recapture rates in the West New Britain sector (I05) as soon as fishing commenced there in June 1973 and continuing until its cessation in September,

(4) restriction of returns for the New Hanover sector (J02) to August-October 1973, possibly indicating movement northwards from the West New Britain sector, and

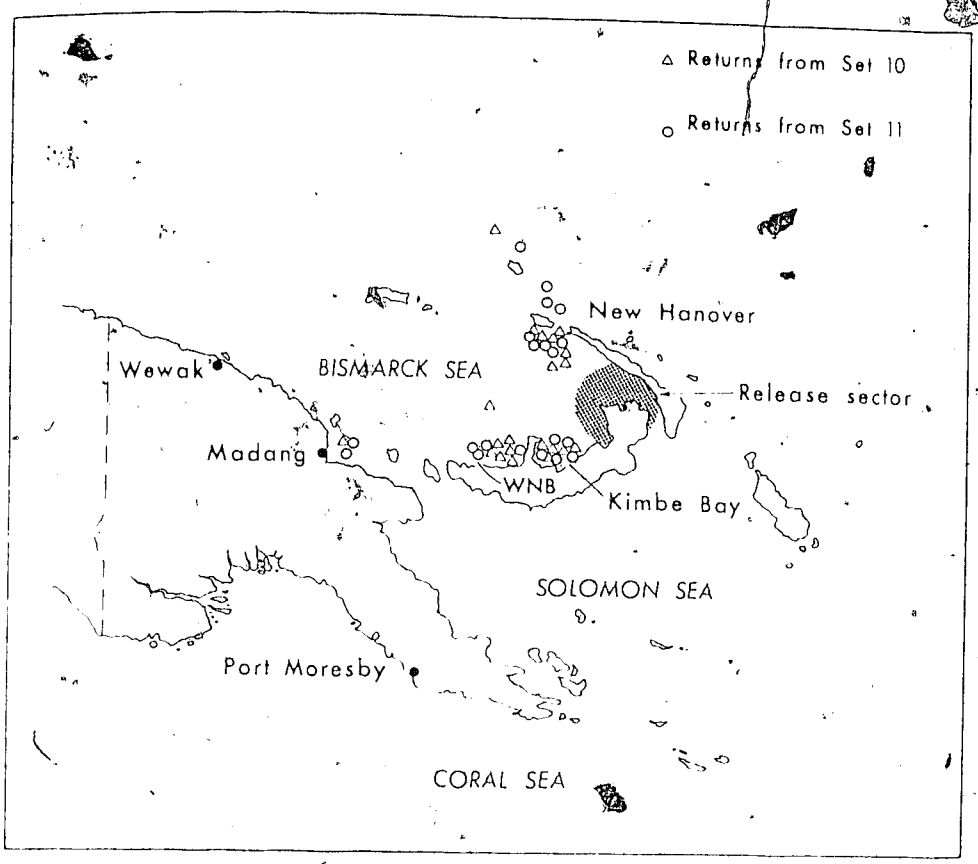
(5) thirteen recaptures during 1974 (not shown on Table 3.7) revealing the presence of tagged skipjack in the Bismarck Sea nearly two years after release. Eight of these were in the Madang sector (F05).

That at least some tagged fish spent the January-March period in the Solomon Sea is indicated by the recapture of three set 6 skipjack by long range vessels in March 1974; there was minimal effort in that area by these vessels in 1973 (Anon., 1977b). Conversely, the only two recaptures from the limited Solomon Sea releases (sets 1, 2) were made in the New Hanover (August 1972) and Cape Lambert (May 1974) sectors.

Returns from sets 3 and 4 releases from October 1972 onwards parallel those described above, and the consistency and synchrony of the return sequence amongst release sets is a feature of the analysis. The following explanation seems to best account for this periodicity. Although some tagged fish underwent little translocation or moved northwards soon after release, most moved to the southwest, apparently to enter the Solomon Sea for some months before reappearing in the southern Bismarck Sea in mid 1973. This pattern of limited northerly movement through the New Hanover sector and oscillation between the Bismarck and Solomon Sea was repeated in approximately the same time frame during the latter half of 1973 and 1974.

3.5.2 Between-year variation in K04 releases

Location of returns from releases in the Cape Lambert sector during June-September 1973 (sets 10, 11, 12, 14 and 15) are shown in Figure 3.8 and the adjusted number of recaptures by month (N_{ij}) in Table 3.8. Although qualitative similarity with 1972 results is evident the 1973 data set differs in several respects:



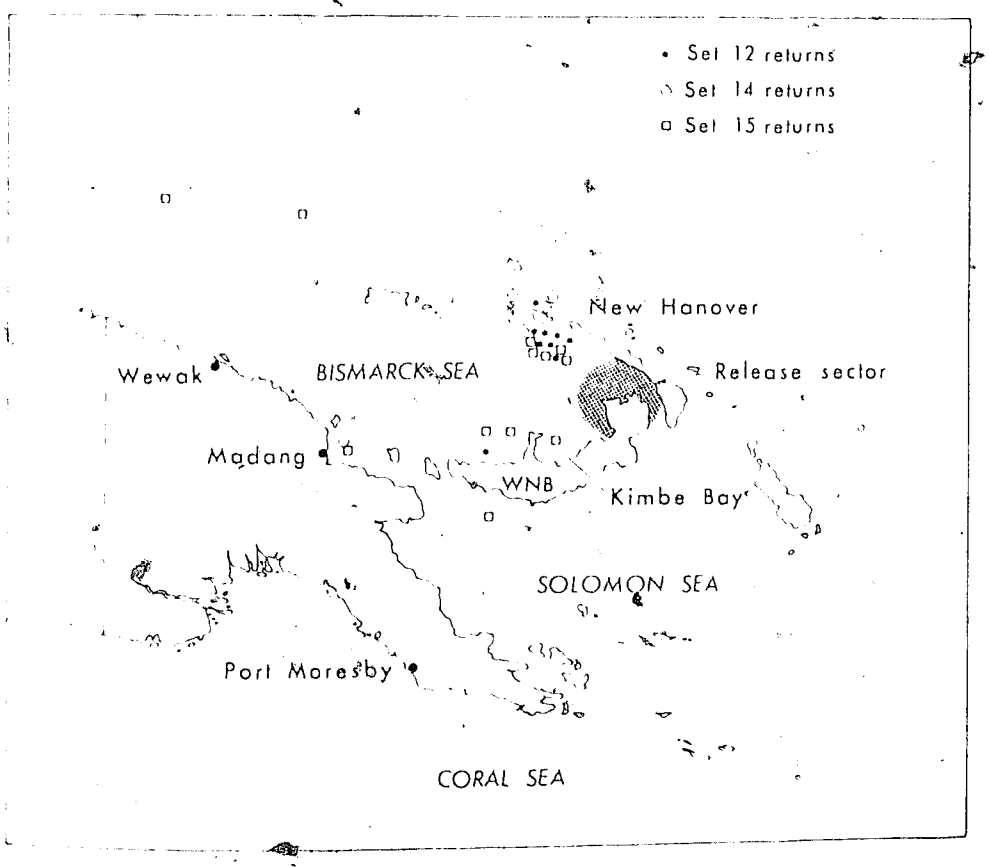


Figure 3.8 Location of returns within the Papua New Guinea area from release sets 10, 11, 12, 14 and 15.

Table 3.8 Adjusted number of returns (Nij) during 1973-74 by month for release sets 10, 11, 12, 14 and 15

All releases were in the Cape Lambert sector

NH: New Hanover; CL: Cape Lambert; KB: Kimbe Bay; WNB: West New Britain; MD: Madang

Symbols: *, <10 days effort; -; no returns

SET 10 5-14/6/1973

	1973							1974			
Month	J	J	A	S	O	N	D	JFM	AMJ	JAS	OND
Sector											
NH	-	-	3.0	6.9	-	-	-	-	0.9	-	-
CL	13.5	7.5	5.1	1.5	-	-	-	-	0.6	-	-
KB	*	6.0	3.6	-	-	-	-	*	-	*	*
WNB	4.8	-	6.3	4.2	*	*	-	-	*	2.2	-
MD	-	*	*	*	*	*	*	-	2.8	3.0	2.4

SET 11 23/6-5/7/1973

NH	-	-	0.3	3.6	4.5	2.7	-	-	-	2.0	-
CL	0.6	13.2	16.1	3.6	-	-	-	-	-	0.7	-
KB	-	5.7	5.4	-	-	-	-	*	-	*	*
WNB	-	-	6.0	2.1	*	*	-	-	-	-	-
MD		*	*	*	*	*	*	-	2.8	3.0	-

SET 12 11-12/7/1973

	-	3.0	6.9	1.8	-	-	-	-	2.0	-
	21.2	8.7	3.9	-	-	-	-	0.6	0.7	0.7
	-	-	-	-	-	-	*	-	*	*
	-	2.1	-	*	*	-	-	-	-	-
	*	*	*	*	*	*	-	-	-	-

SET 14 28-30/8/1973

	-	3.6	4.5	-	-	-	-	-	-
	1.8	34.8	2.4	-	-	-	-	0.7	1.4
	-	-	-	-	-	*	-	*	*
	-	-	*	*	-	-	-	-	-
	*	*	*	*	*	-	-	4.2	-

SET 15 5/9/1973

	-	-	1.5	-	-	-	4.0	-
	5.4	-	-	-	-	0.9	-	-
	-	-	-	-	*	-	*	*
	-	*	*	-	-	-	-	-
	*	*	*	*	-	-	-	2.4

(i) return rates within the release area were higher initially and persisted longer,

(ii) movements out of the release area to the northwest were much more pronounced (in 1972, they were seen in set 7 releases, and to a much lesser extent set 3 and 4 releases). The movements may have continued beyond this sector, resulting in emigration or loss from the Bismarck Sea,

(iii) movements to the southwest were, on the other hand, weaker. In the case of set 14 and 15 releases, only returns in the Madang sector during 1974 suggest this may have occurred. Although returns were received in the West New Britain sector during July-September 1973 from sets 10, 11 and 12, only one return was received in the year after release. This is in marked contrast to the 1972 releases, when return rates from this time-area stratum approached initial levels (Table 3.7).

(iv) Partly as a consequence of (ii) and (iii), the attrition in returns with time was significantly greater for 1973 releases.

Figure 3.9 illustrates these points. With the lack of returns in each case for the April-November period following release regression lines have been filled for two time periods rather than to the total data set.

These results indicate then that what differs between years are the relative proportions of skipjack which move northwards soon after release and are thereafter probably lost to the fishery, and the proportion of skipjack which move southwest and remain for lengthy periods to alternate between the Bismarck and Solomon Seas.

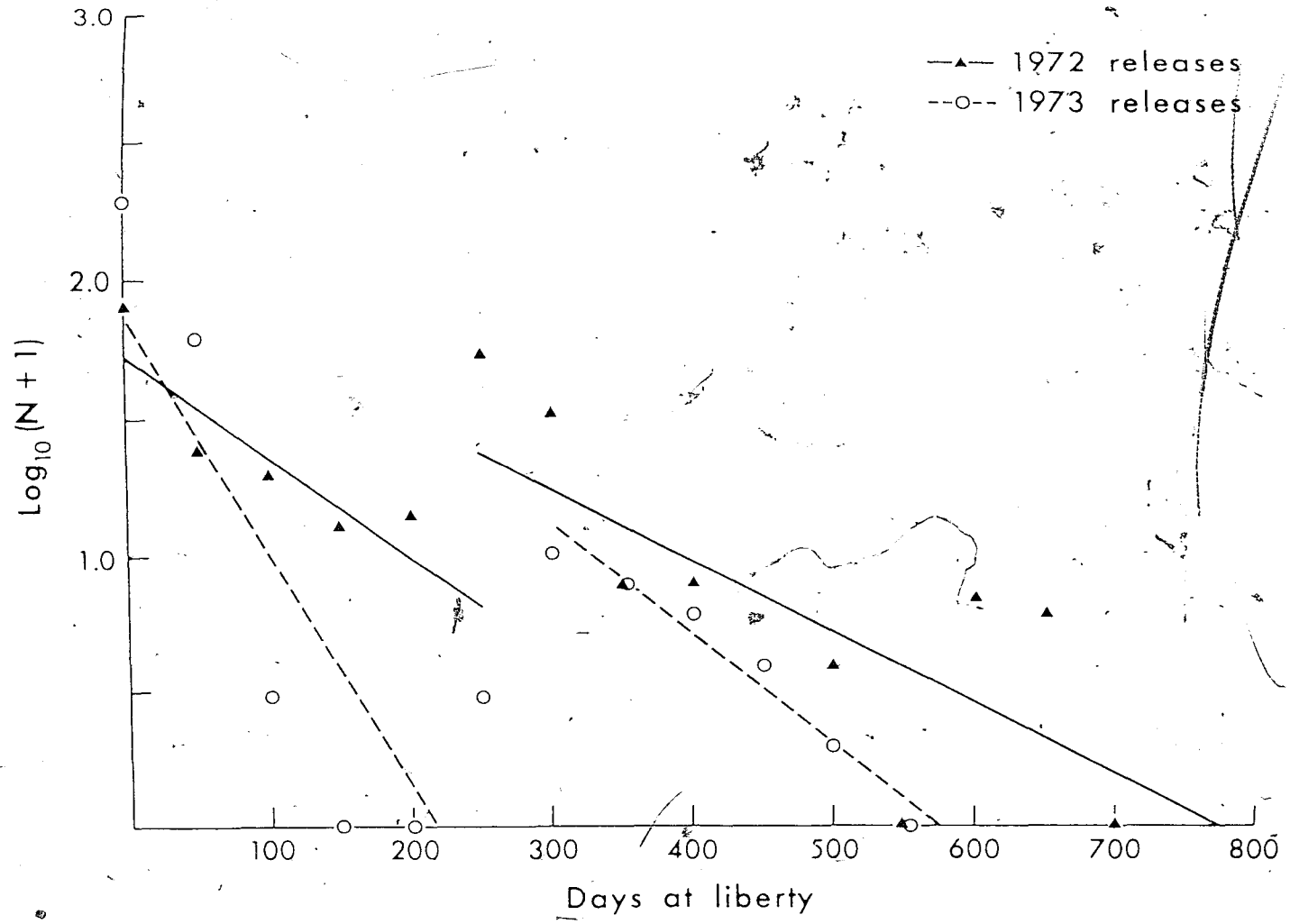
3.5.3 Other releases

New Hanover Sector

Releases in or adjacent to this productive sector were accorded high priority, and were made as follows:

Figure 3.9 The attrition with time in number of local returns received from 1972 and 1973 releases in the Cape Lambert sector.

As the relationship is usually exponential and as no returns were received for some 50-day periods, regressions of log (N + 1), where N = number of returns, against the number of days representing the beginning of each 50-day time period, have been fitted for two time periods in each case. Seasonal movement into the Solomon Sea, with resultant lack of returns, engenders this subdivision (see text).


3.0
2.0
1.0
$Log_{10}(N+1)$
—▲— 1972 releases
--o-- 1973 releases
100
200
300
400
500
600
700
800
Days at liberty

			Returns	(%)
Release set	13	5-8/8/73	84	8	9.5
" "	20	11-21/10/73	240	9	3.8
" "	22	11-28/6/74	1066	83	7.8

Most information therefore derives from release set 22, made from a commercial vessel chartered for a two week period; adjusted numbers of returns (N_{ij}) for these releases are given below and the location of returns from release sets 22, 13 and 20 in Figure 3.10.

	1974							1975		
	J	J	A	S	O	N	D	JFM	AMJ	JAS
New Hanover	7.0	22.3	8.5	4.3	4.0	3.0	-	*	-	-
Cape Lambert	-	-	1.2	0.6	0.6	1.2	-	5.9	1.8	-
Kimbe Bay	*	-	*	*	*	*	*	*	*	*
W. New Britain	-	-	1.8	2.7	-	8	-	-	-	8
Madang	-	-	-	-	-	-	-	-	-	1.8

* <10 days effort
\- no returns

Most returns were made locally in the five months following release, after which time (November 1974) they ceased in the face of continuing effort and good catches (Table 3.2), presumably indicating emigration out of this sector. Returns from the Cape Lambert and West New Britain sectors indicate that some southwards movement occurred, establishing a link between the New Hanover sector and those further south. Returns to the north and northwest, including six within the five months after release, demonstrated that movement out of the Bismarck Sea also occurred. The small release set 13 produced similar results: three returns within the New Hanover sector, one to the south, and the remainder over a wide area to the north. This is analogous to observed movements in the Cape Lambert sector, where northward movement soon after release, and south-westerly movements into other sectors were detected to varying degrees amongst release sets.

Set 20 releases (October 1973) were made both in the New Hanover sector (n = 110) and at several points to the south, off the New Ireland east coast (n = 130). The former produced one local return, one cannery

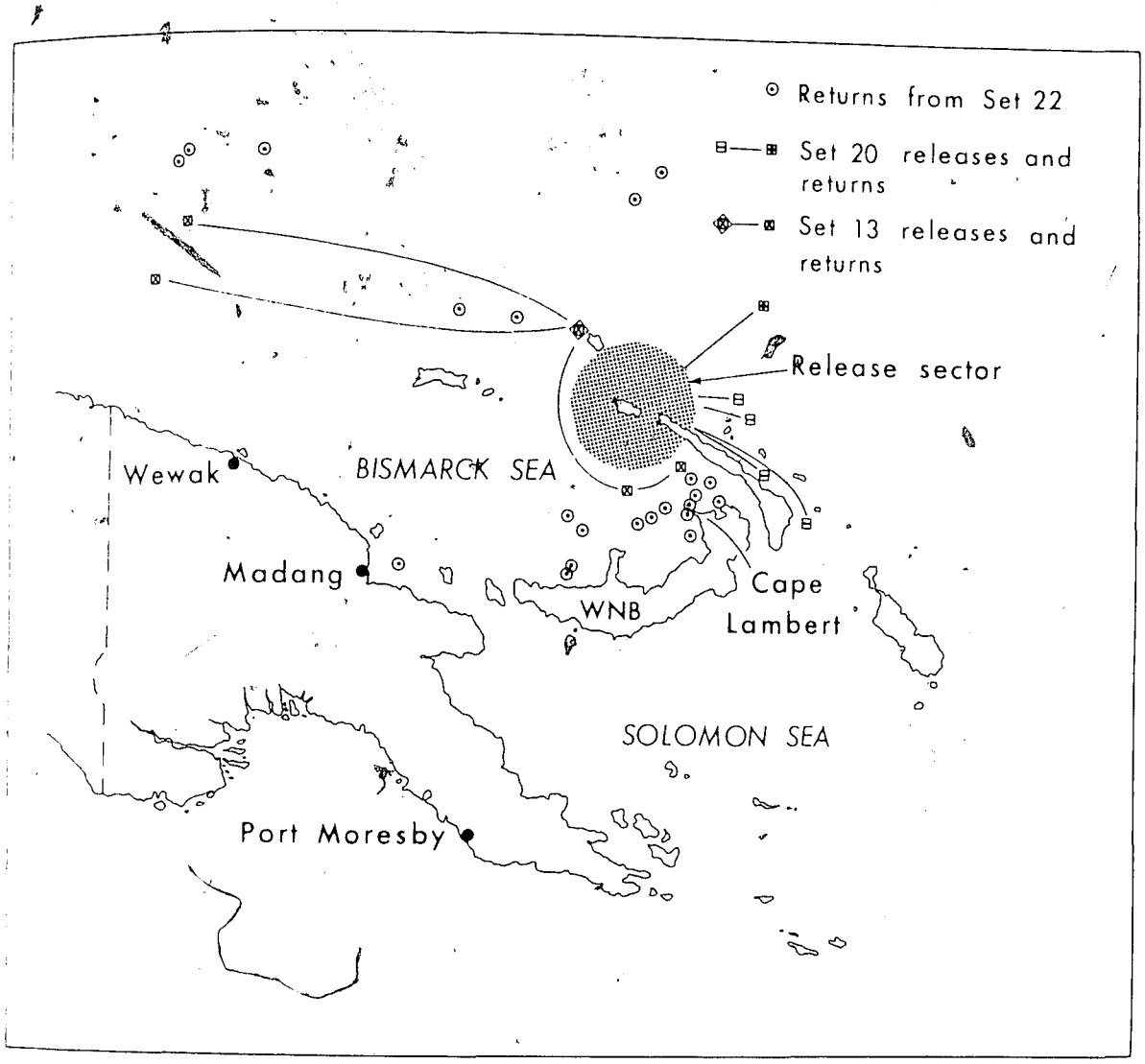


Figure 3.10 The location of returns of release sets 22, 20 and 13.

return likely to have originated from the Cape Lambert sector and two some distance to the north; the latter (n = 130) resulted in three returns soon after in the New Hanover sector, one there during 1975 and one in the Solomon Islands.

Southern and Western Bismarck Sea

Returns from release sets 16 and 17 were able to confirm aspects of the periodic movements inferred in 3.5.1 and 3.5.2 (Figure 3.11).

The set 16 releases, spread over seven one-degree squares along the north coast of New Britain and showing considerable heterogeneity in size, produced returns, on the one hand, west of Wewak, in the Madang sector and in the Solomon Sea, as well as, during the [illegible]-November period, others northwards to the New Hanover sector and beyond.

Returns from set 17 releases, in the Madang sector and near Wewak, extend around the eastern part of the Bismarck Sea and into the Solomon Sea.

Solomon and Coral Sea Releases

There were eight returns from a total of 265 Solomon Sea releases, sets 1 and 2 (1971-72), and sets 9 and 21 (1973) (Figure 3.12). These demonstrate that movement does occur both ways between the Bismarck and Solomon Seas.

Similarly, two returns from 584 releases in the Coral Sea (sets 18 and 23) were received in the Madang sector (Figure 3.12) linking the Coral and Bismarck Seas. This suggests that movement out of the Bismarck Sea and into the Solomon Sea during the November-April period each year may spill over on occasions into the northern Coral Sea.

3.5.4 Size related effects

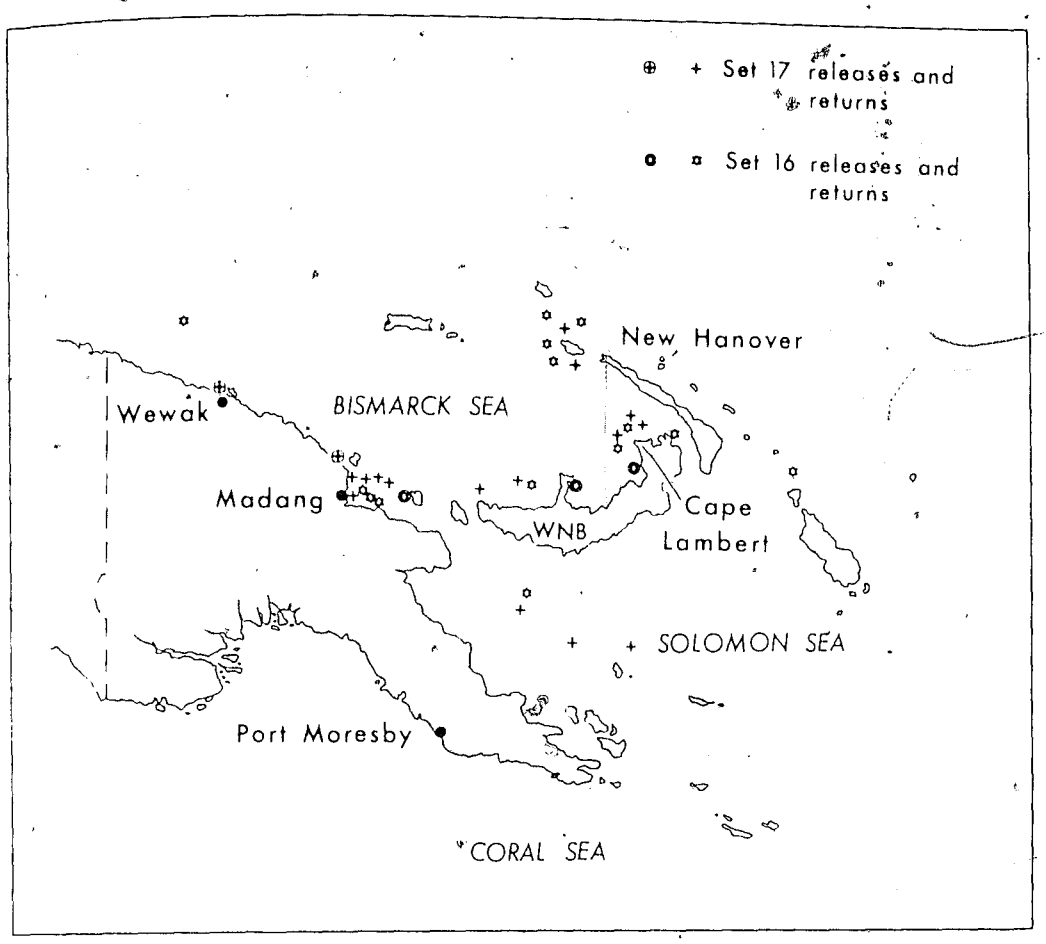
α and β fish, 1972 releases

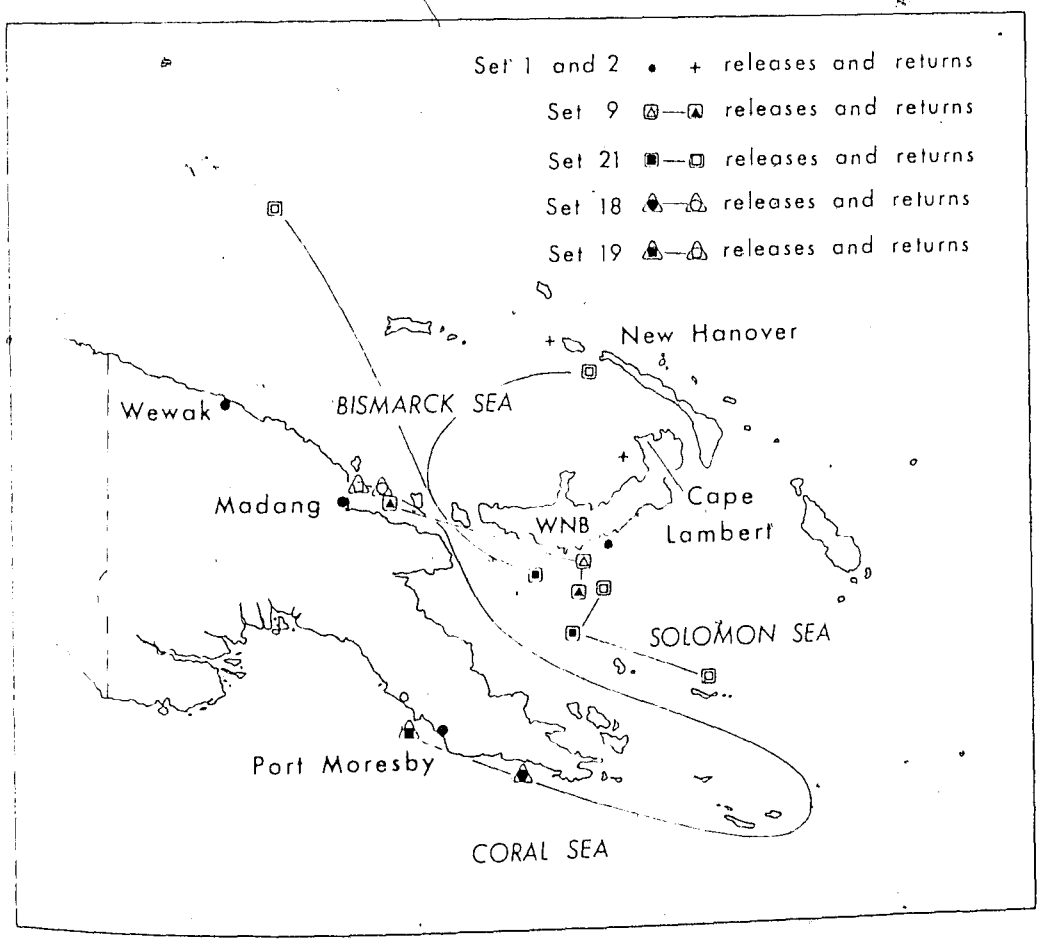
The α and β groups separated in the 1972 Cape Lambert releases on the basis of size differences ($\bar{\ell}_{1_\alpha}$ 54.6 - 56.5, $\bar{\ell}_{1_\beta}$ 50.7 - 53.1 cm - see 3.3.4) yielded Bismarck and Solomon Sea returns in corresponding time area strata through 1973 and 1974. The smaller β skipjack showed stronger initial movement to the northwest and accounted for a significantly greater

Figure 3.11 Location of returns from release sets 16 and 17 in the southern and western Bismarck Sea.

Figure 3.12 Location of returns from release sets 1, 2, 9 and 21 in the Solomon Sea and release sets 18 and 19 in the Coral Sea.

adjacent to the point of release,





number of returns beyond the Papua New Guinea fishery.
($\chi^2_1 = 27.06$, $P < .001$)

	No. released	Size Range (cm)	Returns	Non-local returns
α	2763	53 - 60	227	5
β	740	48 - 54	41	9

A third smaller size class (45-49 cm) comprising part of set 8 releases (41 released, 3 returns, 2 non-local) also seem to account for a disproportionate number of such recoveries.

This suggests that emigratory tendency may be influenced by size, with smaller fish being more mobile. To test this, the total length frequency data for 1973-4 releases (Table 3.4) and the estimated sizes at release for the 69 returns beyond the Papua New Guinea fishery (see later) were subdivided into 4 size groups, <50cm, 51-55cm, 56-60cm and >60cm. Differences in the number of these longer distance recoveries amongst the size classes were not significant (homogeneity $\chi^2_3 = 2.09$, $P > .05$) when combined over all release sets.

	<50cm	51-55cm	56-60cm	>60cm
No. released	686	2664	2247	126
No. of non-local returns	11	34	22	2

As such an analysis ignores the many variables likely to affect movement such as month and year of release, release location, distribution of effort, it is essentially naive, but does suggest either that no simple relationship exists between skipjack size and emigratory tendency, or that a multiplicity of other variables obscures any such effects. It is worth reiterating that these experiments involve only mature fish 45-65 cm in length.

Size specificity by area

Kearney (1977, 1978) has noted the comparative stability in average size of skipjack taken in the various sectors of the Papua New Guinea fishery, particularly in the Madang sector where skipjack >5 kg in size or >60 cm predominate. Figure 3.13, the average weight by month for the Madang, West New Britain and New Hanover sectors during the period 1971-75 illustrates this point.

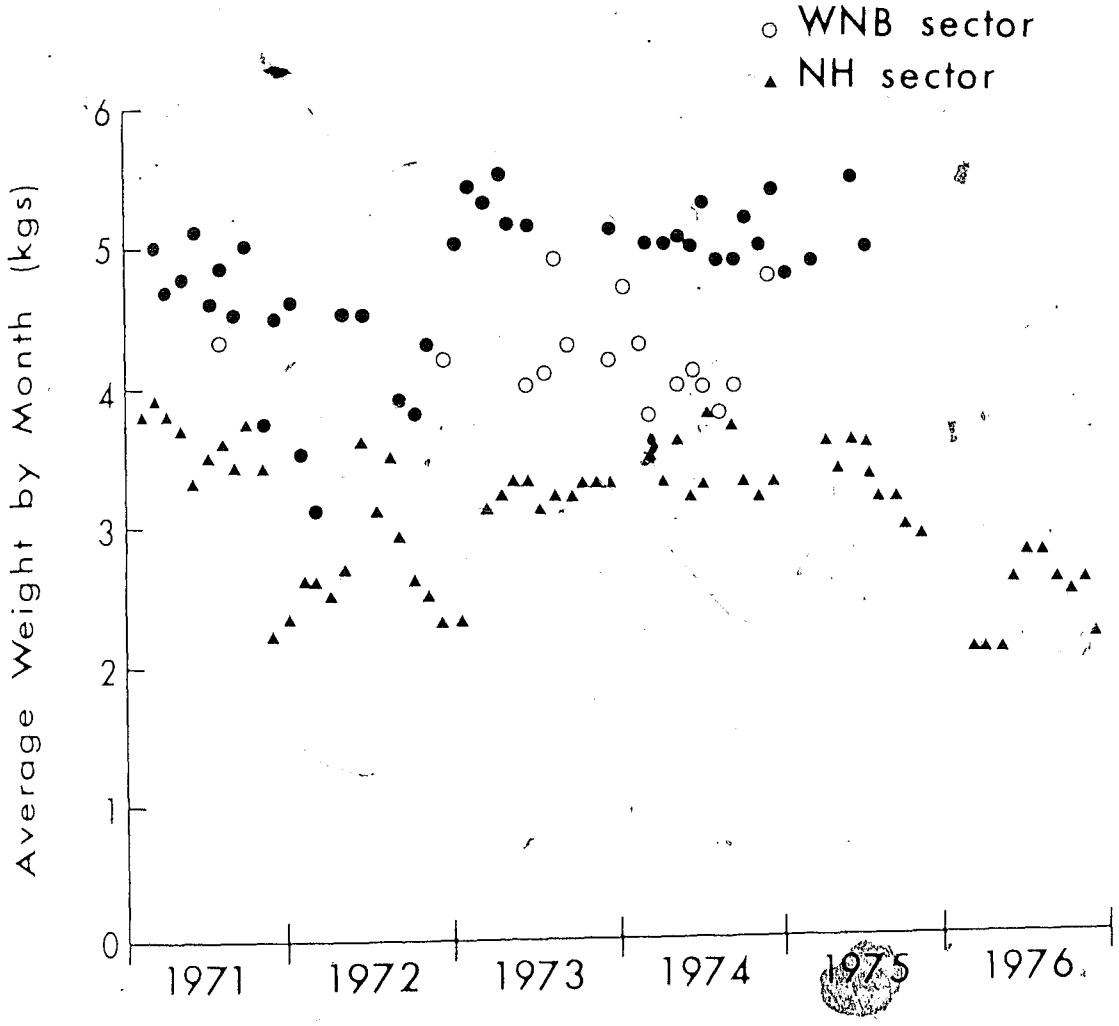


Figure 3.13 Average weight by month for skipjack taken in three sectors of the Papuan New Guinea fishery.

The predilection of large fish for the Madang sector finds confirmation in the tagging data. Of the 23 returns in the Madang sector from releases elsewhere, all but 3 were accompanied with adequate data on size-at-recapture. Mean length of those fish was 62 ± 3 cm at an average weight of 5.2 ± 0.4 kg. Release set 17, in or adjacent to the Madang sector, comprised 326 skipjack with a modal length of 57 cm (~4 kg). Returns from skipjack larger than 58 cm at release were nearly all (7/8) within the Madang sector and nearby Solomon Sea, whereas the majority of those less than 58 cm at release were made in other sectors of the fishery.

Average sizes in other sectors fluctuate more widely, both within and between years. The New Hanover and Cape Lambert sectors almost invariably have the smallest average size skipjack, with West New Britain and Kimbe Bay average weights intermediate. Length frequency data from later years (Wankowski, in press) for the New Hanover and Cape Lambert sectors indicate that a wide range of fish is present but masked by the averages. It is therefore likely that skipjack of between 45 and 65 cm are present in every sector at all times, but in proportions which vary geographically. The underlying basis of this size stability by area remains unknown. Recent work defining the hypothetical habitat of skipjack of various sizes (Barkley *et al.* 1974) suggests that vertical temperature profiles combined with dissolved oxygen levels may be important in defining the habitat of large (>5 kg) skipjack (see 2.3.1).

3.5 Integrity and Composition of Schools

Inspection of the present tagging data provides examples of both concomitant returns of fish released from the same 'school' some distance and/or time after release and returns on the same day isolated by distances clearly incompatible with their belonging to the same unit. Two such examples of each situation are given.

Tag No.	Release Details	Recapture Details	Days Out	Dist. Moved n.m.
2811 2532	30/10/72; 4°51'S 151°37'E	28/4/73; 4°25'S, 151°31'E	180	27
10734 10719	28/8/73; 4°03'S, 152°04'E	8/9/73; 3°57'S, 151°19'E	11	45
11613	28/8/73; 4°03'S, 152°14'E	8/9/73; 2°56'S, 150°43'E	11	113
11615	"	8/9/73; 3°57'S, 151°19'E	11	55
12423	8/11/73; 4°40'S, 145°47'E	6/8/74; 5°09'S, 149°13'E	271	207
12335	"	6/8/74; 2°15'S, 150°10'E	271	300

The genetic analyses (Chapter 4) should provide information on homogeneity of 'schools' at one point in time. Providing uniformity of size is a valid guide, skipjack schools may be less heterogenous than those of other tuna. The following data on size range within schools in the Papua New Guinea area is taken from Lewis *et al.* (1974).

	Maximum range (cm)	Mean range and standard deviation (cm)	No. of Schools
Skipjack	10.9	5.2 ± 2.07	84
Frigate tuna	14.5	6.3 ± 4.56	6
Mackerel tuna	25.4	9.6 ± 6.81	41
Yellowfin tuna	23.3	8.8 ± 6.01	24

3.5.6 Mortality Estimates

Estimates of Z (total mortality) and S (survival) of release sets 10, 11, 12, 13 and 22 were obtained for the period following release (Table 3.9). As it is not possible to account for emigration, Z effectively represents a loss rate from the Bismarck Sea and S an estimate of the proportion of the release set remaining in the area at the end of this initial period. These range between 0.22 and 0.46, and as they they drop further due to movement into the Solomon Sea (sets 10, 11, 12, 13) or increased emigration (set 22), give some indication of the high turnover of skipjack numbers in these two productive sectors.

3.6 RELATIONSHIPS WITH ADJOINING AREAS

Both movement out of the area, or emigration, as revealed by returns from skipjack tagged and released in the Papua New Guinea area and movement into the area, or immigration, as revealed by returns from skipjack tagged and released in other areas, need to be considered.

3.6.1 Emigration

1971-74 releases

A total of 69 returns were received from beyond the various sectors of the Papua New Guinea fishery. There were 37 international returns (outside the offshore seas of Figure 3.3) and these cover a wide area

Table 3.9 Total Mortality Estimates

Legends as follows: R = number of returns;
R^1 = number of returns/100 days' effort;
Zm = calculated monthly total mortality;
Zf = total mortality during months (f) exposed to the fishery

Sectors: CL = Cape Lambert; KB = Kimbe Bay; WNB = West New Britain; NH = New Holland

Month	Sector	Effort (days)	R	R^1	log R^1
Release set 10					
June	CL	488	23	4.71	0.673
July	CL, KB	573	14	2.44	0.387
August	CL, KB, WNB, NH	933	14	1.50	0.176
September	CL, NH, WNB	707	8	1.13	0.054
		Zm = 0.200			
		Zf = 0.800 S = 0.45			
Release Set 11					
July	CL, KB	573	24	4.12	0.622
August	CL, KB, WNB, NH	933	30	3.22	0.507
September	CL, NH, WNB	707	9	1.27	0.104
		Zm = 0.259			
		Zf = 0.777 S = 0.46			
Release Set 12					
July 12-31	CL	295	27	9.15	0.96
August	CL, NH, WNB	762	14	1.6	0.20
		Zm = 0.433			
		Zf = 1.299 S = 0.27			
Release Set 14					
September	CL, KB, NH	756	49	6.4	0.805
October	CL, NH	463	5	1.1	0.04
		Zm = 0.765			
		Zf = 1.530 S = 0.22			
Release Set 22					
July	NH	304	27	8.88	0.948
August	NH, CL	928	11	1.18	0.073
September	NH, CL, WNB	840	8	0.95	$\bar{0}$.021
October	NH, CL	891	5	0.63	$\bar{0}$.199
November	NH, CL	777	5	0.64	$\bar{0}$.191
		Zm = 0.257			
		Zf = 1.285 S = 0.28			

between 10°N and 10°S and 130°E to 175°E (Figure 3.14). The Japanese long range pole boat fishery and joint-venture operations in the neighbouring areas of Solomon Islands and Palau accounted for all but one of these returns. The return data therefore need to be related to the spatio-temporal distribution of effort in these fisheries.

As a first step, 1974 returns (51 out of 69), grouped according to the statistical areas used to analyze the Japanese bait boat fishery, have been compared with corresponding data for each area on unadjusted days effort (fishing days), effective effort and catch ('000 tonnes) during 1974 (Figure 3.15, data from Kasahara, 1978a). Equivalent unadjusted effort figures for the Solomon Islands and Palau fishery were obtained using the long range vessel catch per day in that area during 1974 as a standard daily catch rate and dividing total catch by this figure.

Within the area covered by tag returns (10°N - 10°S, 125° - 175°E), the number of returns in areas north of 5°S (14 quadrangles plus the Palau fishery) shows a reasonable fit to a second degree polynomial in unadjusted effort (Figure 3.16).

In areas south of 5°S (3 quadrangles plus the Solomon Islands fishery) the return rate was relatively much higher, indicating that with the prevailing distribution of effort during 1974, movements south from the Papua New Guinea area were probably underestimated. The number of areas involved is insufficient to quantify the relationship between number of returns and effort, but it appears almost linear.

Examination of effort figures in the adjoining blocks 10° - 20°N, 125° - 175°E and 20°N - 10°S, 175°E - 165°W (Figure 3.15) show effort in only two areas (2/23) to exceed 1,000 days; effort south of 10°S does not exceed 100 days in any square other than the three considered. The absence of returns in these areas could reasonably be attributed to these lower levels of effort.

This is clearly not the case for the western Pacific as a whole however; larger fisheries (the Philippines, Indonesian and Japanese coastwater fisheries - see Table 3.1) adjacent to the area covered by tag returns yielded no returns, suggesting that returns mirror the distribution of effort only within certain geographical limits.

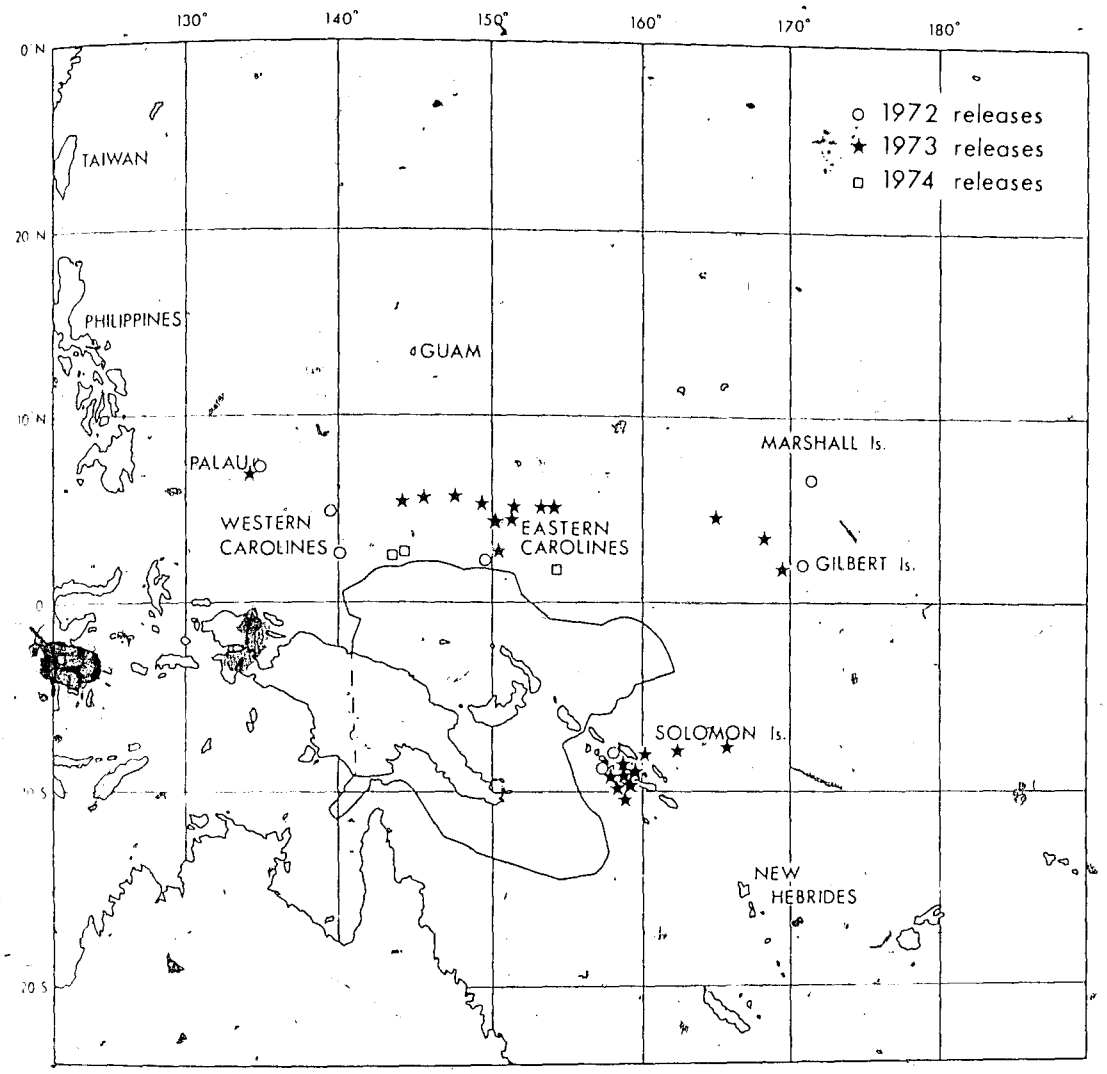


Figure 3.14 Location of returns of tagged skipjack from beyond the offshore seas of Papua New Guinea.

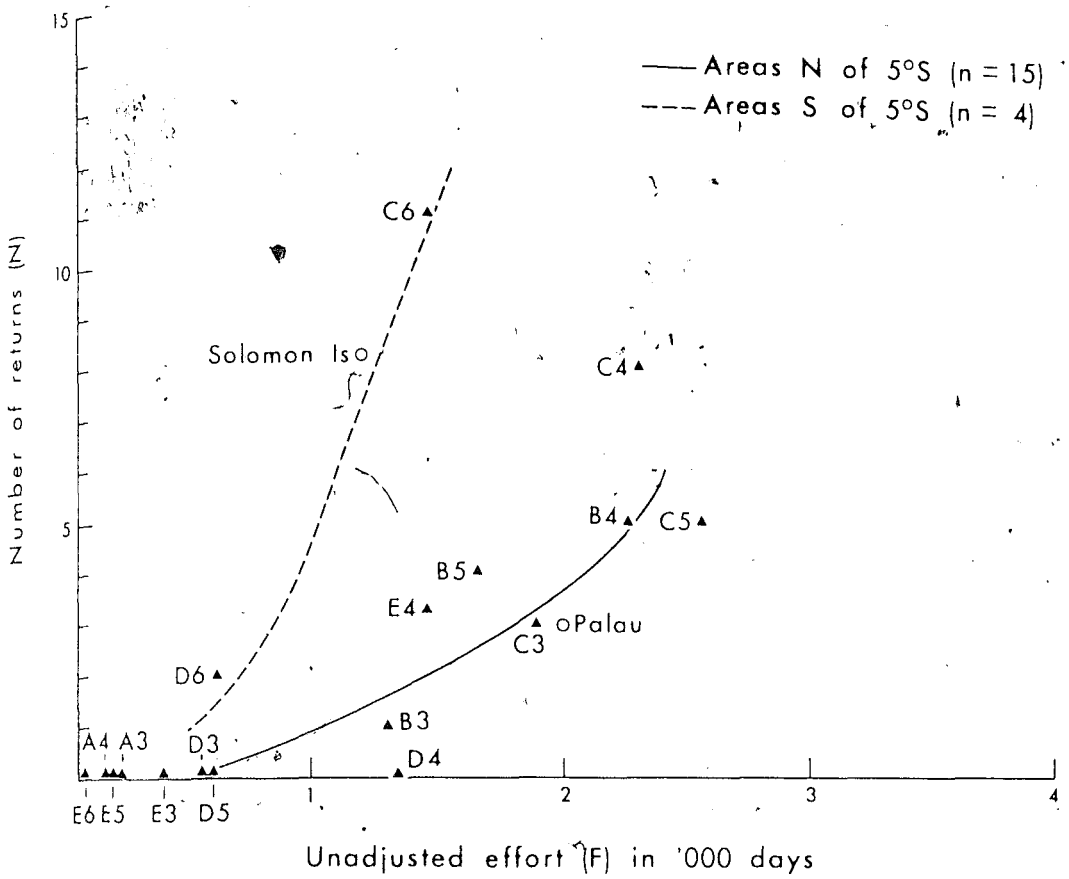
Figure 3.15 Tag returns received during 1974 outside sectors of the Papua New Guinea fishery, relative to distribution of effort and catch.

For each 5° x 10° quadrangle, the number of returns is indicated in bold type, and from top to bottom on the right hand side, unadjusted days effort, effective days effort and catch ('000 tonnes) during 1974. Analogous data for the Solomon Islands and Palau fisheries is also given. Each quadrangle can be coded by longitudinal letter (A-G) and latitudinal number (1-8).

Palau: 2000 est
3 N.A.
10.0 est

Solomon Is: 1205 est
6 N.A.
10.27

	125°E A	B	C	155°E D	165°E E	175°E F	175°W G
20°N	1021	130	1183	146	35	54	9	2
1	0 1378	0 145	0 1354	0 140	0 35	0 41	0 8	0 0
	5.59	0.55	3.68	0.44	0.08	0.23	0.04	0
	704	164		69	50	47	5	6
2	0 914	0 267		0 100	0 43	0 58	0 4	0 6
10°N	4.33	0.45		0.13	0.08	0.14	0.01	0.01
	203	1314	1982	555	410	76	77
3	0 266°	1 1564	3 2576	0 813	0 507	0 88	0 81.6
	1.01	0.45	10.62	2.54	2.52	0.56	0.96
	143	2262	2312	1350	1402	32	3
4	0 166	5 2979	8 2913	0 1648	3 1840	0 34	0 3
0°	0.77	12.697	11.79	6.73	15.66	0.15	0.01
		1673	2543	588	194	2	–
5		4 2028	5 3180	0 698	0 249	0 2	0 –
		13.23	14.26	3.65	1.52	–	–
			1448	603	21	5	–
6			11 1887	2 755	0 23	0 5	0 –
10°S			14.13	5.14	0.09	–	–
			2	22	13	19	–
7			0 2	0 21.4	0 13.7	0 20.5	0 –
			0.01	0.16	0.12	0.17	–
			10	1	1	–	–
8			0 13.5	0 1	0 1	0 –	0 –
20°S			0.01	0.01	0.01	–	–



N of 5°S

(1) $N = -.07316 + .03427\ F + .97996\ F_1^2$ ($r^2 = .77$)

(2) $N = -.21033 + .09434\ F_e + 61917\ F_e^2$ ($r^2 = .81$) where F_e = effective effort.

(3) $N = -1.06978 + 0.5134C - .00781\ C^2$ ($r^2 = .69$) where C = catch in '000 tonnes.

S of 5°S

As only four points were available, the relationship was not quantified.

Figure 3.16 The relationship between number of returns (N) and in unadjusted days effort (F) during 1974 for areas north and south of 5°S respectively.

Returns received during 1973 (13) and 1975 (5) were too few for the above analysis, but show similar trends (Table 3.10).

Releases since 1974

Since the conclusion of the Papua New Guinea experiments in 1974, additional skipjack releases have been made within Papua New Guinea waters as follows:

(a) Japan Papua New Guinea joint research cruises, November-December 1975 with 1600 skipjack released (Anon, 1977e) and November 1976 with 262 skipjack released (Anon, 1978),

(b) commercial vessel charters, November 1976 and March 1979, with 552 skipjack released,

(c) South Pacific Commission Skipjack Survey and Assessment Programme, November-December 1977 with 2347 skipjack released (Kearney and Lewis, 1978), and

(d) as in (c), May-July 1979 with 7683 skipjack tagged and released (Kearney and Hallier, 1979).

The localities of returns other than those made by the Papua New Guinea fishery and available at the time of writing are shown in Figure 3.17. Their distribution closely parallels that of returns from the 1971-74 releases (Figure 3.14). Three returns were made south of 10°S. With the southwards expansion of long range pole boats since 1975 (Bour and Galenon, 1979), this would be predicted from Figure 3.16. The continuing eastwards expansion of this same fishery has however yet to increase the easterly extent of returns.

3.6.2 Immigration

Data on immigration into the Papua New Guinea area come from the following sources:

(1) Releases north of the Equator but south of 20°N by Japanese research organizations

Over 8,000 skipjack were tagged and released by the Tokoku Regional Fisheries Research Laboratory and the Far Seas Fisheries Research

Figure 3.17 Location of returns from releases in Papua New Guinea waters since 1974.

Returns made in the various sectors of the Papua New Guinea fishery are not shown.

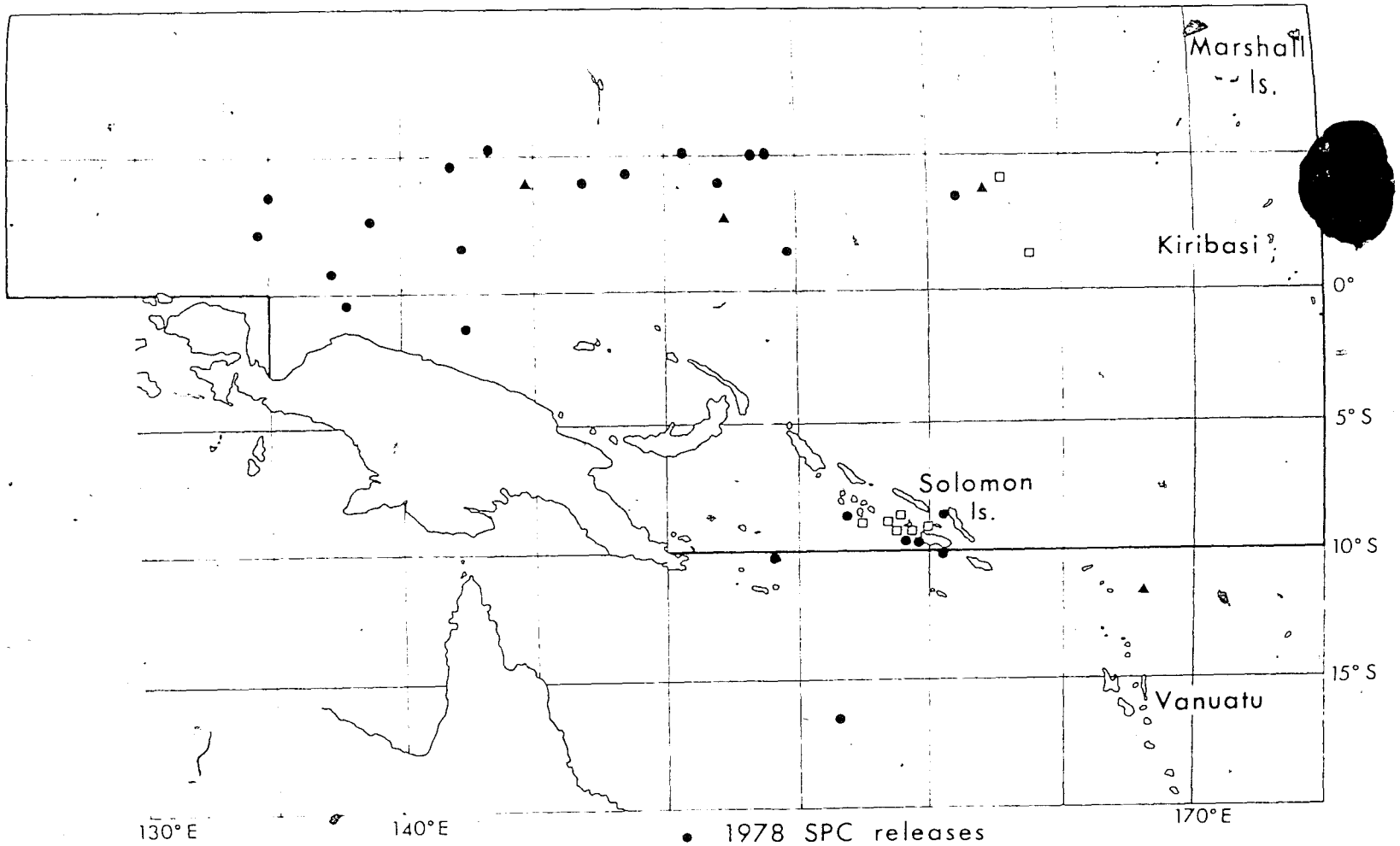

Marshall Is.
Kiribasi
0°
5° S
Solomon Is.
10° S
15° S
Vanuatu
130° E
140° E
170° E
• 1978 SPC releases
□ Joint cruises, 1975-76
▲ Vessel charters, PNG
—— Boundary of return area, 1971-74 releases

Table 3.10 Comparison of number of tags returned, unadjusted effort (days) and catch ('000 tonnes) during 1973 and 1975

	1973			1975		
	Effort (days)	Catch (tonnes)	Tags	Effort (days)	Catch (tonnes)	Tags
North of 5°S						
(A3	340	1.89	-	55	0.13	-
(						
(Palau	(1080)	6.00	-	2960	7.00	-
A4	186	1.49	-	70	0.31	-
B3	2336	13.15	-	548	1.57	-
B4	1453	9.32	2	565	1.88	1
B5	346	2.51	1	256	0.87	-
C3	2063	12.42	1	1846	7.26	-
C4	1495	9.66	-	1077	3.97	1
C5	1007	8.44	3	2772	8.73	1
D3	1939	12.05	-	1529	6.40	-
D4	922	7.49	-	388	12.98	-
D5	272	1.55	1	460	1.70	-
E3	1480	11.71	1	2063	10.69	-
E4	183	1.51	1	507	3.29	-
E5	41	0.16	-	52	0.19	-
South of 5°S						
C6	38	0.17	-	486	2.16	-
(D6	45	0.27	-	1083	5.57	-
(						
(Solomon Is.	(985)	6.00	3		7.00	-
E6	2	0.01	-	31	0.09	1

Laboratory between 1972 and 1976 in the southern water fishery south of 20°N. All were made in an area bounded by 5° to 20°N and 130° to 165°E by a number of different vessels.

Year	Releases	Returns
1972	910	5
1973	4041	50
1974	1619	9
1975	393	6
1976	1040	5
	8003	75 (0.9%)

Very few returns (<1%) were received from these releases, with relevant returns plotted on Figure 3.18. Although six returns crossed the Equator, none were made in the Papua New Guinea fishery. One return was made adjacent to the Madang sector (3°32'S, 149°41'E - 25/3/1974) and another in the Solomon Sea (8°44'S, 152°37'E - 23/3/1974).

The low return rate makes interpretation of this data set difficult. It indicates some movement of fish from north of 5°N, into the Papua New Guinea area does occur but that the fishery probably relies to a minor extent on immigration from this area. Releases in the area 0° - 5°N would clearly have been a useful complement to the above releases.

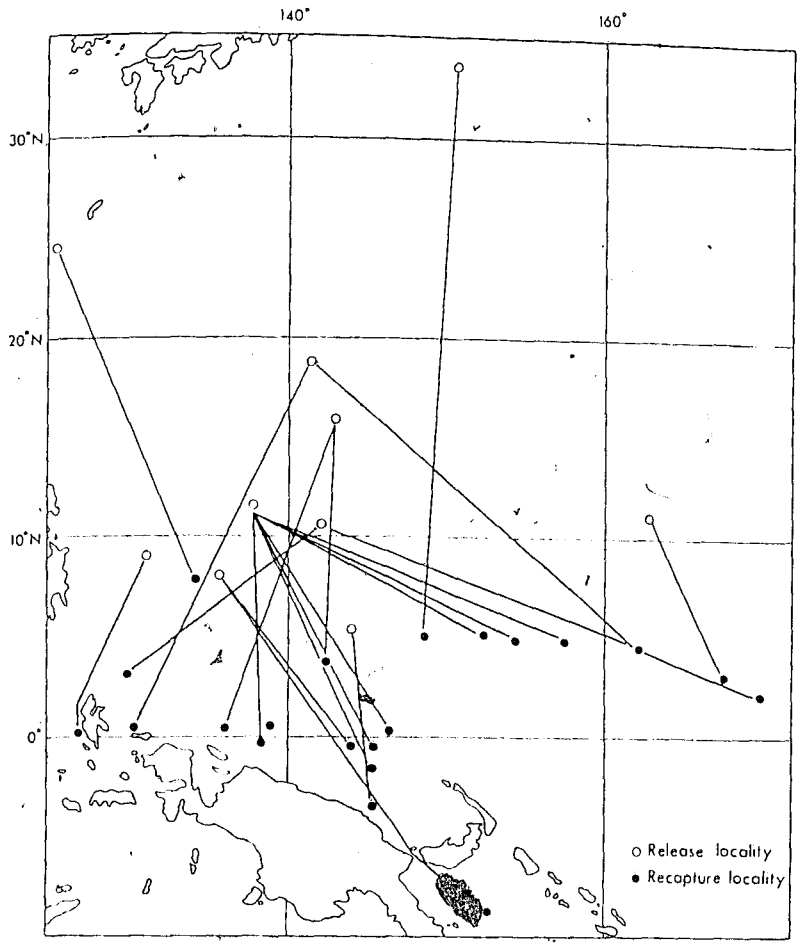
(2) Releases over a wide area of the southern and western Pacific by the South Pacific Commission Skipjack Survey and Assessment Programme during 1978-79

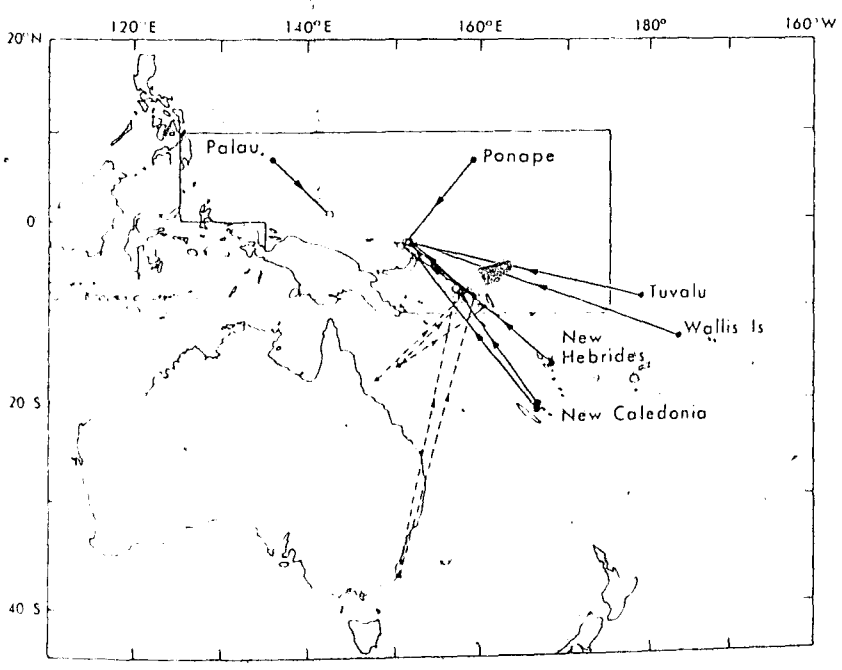
Over 86,000 skipjack were released during the period October 1977 to May 1979. At the time of writing, only 11 of the 4,000 returns from these releases had been made in the Papua New Guinea area (Figure 3.19). This requires some qualification since effort by long range pole vessels has gradually decreased in Papua New Guinea waters since 1974, partly due to a deliberate boycott of these waters by Japanese fishermen following

Figure 3.18 Location of returns from releases north of the Equator by Japanese research organizations.

Figure 3.19 Returns in the Papua New Guinea area from releases elsewhere by the South Pacific Commission Skipjack Survey and Assessment Programme.

12423	8/11/73; 4°40'S, 145°47'E	6/8/74; 5°09'S, 149°13'E	271	207
12335	"	6/8/74; 2°15'S, 150°10'E	271	300





the introduction of licence fees for access to the Papua New Guinea 200 mile fisheries zone (Kearney, 1979). With the greatly reduced effort, such vessels accounted for a single return only.

Returns from releases in the Solomon Islands (4), Palau (1) and Ponape, T.T.P.I. (1) correspond to observed emigrations. Returns from releases further east and south - New Caledonia (2), New Hebrides (1), Wallis Islands (1) and Tuvalu (1), plus returns in the Solomon Islands from Australian releases (Figure 3.19) indicate more interchange with southern Hemisphere areas than norther Hemisphere areas, as the emigration analysis had previously suggested. Unfortunately, no releases by any agencies have been made west of Papua New Guinea in Indonesia or the Philippines, and immigration into the Papua New Guinea area from there cannot be discounted.

Country	Date	No. of Skipjack Released
Papua New Guinea	2/10-1/11/1977 14/5-2/7/79	8,847
Solomon Is.	1/11-4/12/1977	2,225
New Caledonia	13/12/1977-19/1/1978	10,212
New Hebrides	5-13/12/1977 20-23/1/1978	1,256
Fiji	26/1-18/2/78; 28/3-10/4/78	8,497
Tonga	11/4 - 3/5/1978	1,408
Wallis & Futuna Is.	4 - 31/5/78	13,534
Western Samoa	6-14/6/1978	1,768
American Samoa	3/5-5/6/78; 15-21/6/78	74
Tuvalu	25/6 - 4/7/78	2,573
Gilbert Is.	5-25/7/78	4,380
T.T.P.I., Guam	26/7-15/8/78; 2/10-15/11/78	3,715
Tokelau	19-23/11/78	65
French Polynesia	6/12/78 - 3/2/79	8,148
Cook Is.	24/11-5/12/78; 4-11/2/79	1,235
New Zealand	17/2 - 27/3/79	11,614
Australia	1/4 - 13/5/79	6,969
		86,520

Zm = 0.257

Zf = 1.285 S = 0.28

3.7 DISCUSSION

3.7.1 Local Movements

Features of skipjack movements as inferred from the tagging experiments in the Papua New Guinea area can be summarized as follows:

(i) net distance travelled, even after long periods at liberty, was relatively small, and site tenacity was marked. Despite the species' dispersive potential, over 70% of returns recovered after 100 days had moved less than 200 miles (360 km) and only 1% of all returns showed net displacement greater than 1,000 miles (1600 km).

(ii) local movements have a cyclical component, and are clearly not random. Movement into the Solomon Sea and possibly the Coral Sea, with subsequent re-entry into the Bismarck Sea, occurs during the November-April period. Movement northwestwards into the New Hanover sector and beyond is largely restricted to the July-October period. The timing of these movements is consistent between years.

(iii) between-year and between-release set variations occurred in the proportions of skipjack which appeared to emigrate soon after release or remain near the release point for lengthy periods. Periods of greater skipjack abundance seem to be associated with the former.

(iv) no obvious size related effects on movement were observed, although a rough size-specificity by area was apparent.

3.7.2 A General Hypothesis Regarding Skipjack Dispersal

An hypothesis to account for skipjack movements in the Papua New Guinea area would need to integrate these results with relevant ecological and biological data (Chapter 2). Such an hypothesis with possible wider application is developed as follows:

Skipjack in the area are surmised to be composed of residents whose density is related to the average "local" productivity over a preceding time period, and nomads which tend to arrive in synchrony with periods when this local productivity is enhanced by oceanographic events on a larger scale. Nomadic behaviour, combined with the tunas' morpho-physiological adaptations, should promote the efficient utilization of

productivity patchy in terms of both space and time. Several workers, e.g. Sharp and Dizon (1978) and Kawasaki (1965) have touched on the theme but it has not been developed to any degree.

Residents could be sustained in the Bismarck Sea and environs by the generally increased forage levels associated with islands (see earlier). They remain in the area, subject to natural mortality, for lengthy periods, showing limited dispersal and possibly making the major contribution to spawning activity in the area due to the relative temporal stability of favourable conditions. During the southern summer, November to April, many of these residents shift into the Solomon Sea to take advantage of the increased productivity there (2.34), returning with the advent of the south-east trades and resultant disappearance of the enrichment zone.

These residents provide the baseline fisheries production within the area year-round and in 'bad' years, probably make the major contribution. Releases in 1972, for example, produced very few non-local returns and over 50% of returns were made locally after 200 days at liberty, whereas releases in 1973 and 1974, both years of relatively high skipjack abundance, produced many more non-local returns and the majority returns occurred within 50 days of release.

Local environmental characteristics may favour residents of particular size, this sieving effect maintaining some size stability by area. It was suggested that knowledge of vertical temperature profiles and dissolved oxygen levels in particular areas may assist explanation of this phenomenon, particularly with regard to larger (>4 kg) skipjack (2.3.4).

Nomads may represent groups or individuals surplus to an area - specific density determined by that area's average productivity or carrying capacity over a preceding period of time (Taylor and Taylor, 1977, Ricker, 1969). Hence they arise as the result of density-dependent processes. Their continued survival rests on seeking out productive areas (food patches) and accordingly their proportional contribution to an area's catch should be maximal during the most productive periods. This is consistent with the tagging results, which show the proportion of skipjack

which emigrate soon after release is much higher during productive periods.

The 35‰ isohaline which has earlier been linked with productive periods in the Papua New Guinea area (2.3.4) does not appear to extend westward of 150°E and narrows latitudinally west of 160°E. This would generally favour influxes of nomads into the New Hanover and possibly Cape Lambert sectors, which is also consistent with the suggestion from the tagging results that much of the movement into the Bismarck Sea occurs through the New Hanover sector. The results also suggest that limited penetration of the Bismarck Sea occurs and residents may be displaced to the southwest by these influxes of nomads.

Some nomads may remain in the area following the disappearance of broad-scale productive conditions. This process may be an important means of supplementing resident numbers, and at the same time may possibly increase genetic heterogeneity within 'resident' schools.

Tag returns referable to nomads probably include those showing northerly movement into the New Hanover sector (and subsequent loss from the fishery) soon after release, the majority of longer distance returns, and most of the short term returns during periods of good fishing. Although alternative hypotheses to explain observed dispersal patterns can be suggested, e.g. the presence of two sub-populations in the study area (Fujino, 1970), both the results of the tagging studies and observed fluctuations in skipjack distribution and abundance in the Papua New Guinea area are well served by this resident-nomad concept. It also has the advantage of being amenable to test.

Given knowledge of an area's 'background' productivity and timing of oceanographic processes inducing significantly higher productivity, the pattern of tag returns against the distribution of catch and effort in an area should be routinely predictable. Skipjack tagged adjacent to ephemeral mid-ocean zones of enrichment should show considerable two-way dispersal and low site tenacity for example, whereas skipjack tagged in an area highly productive all year round, and with conditions suitable for spawning (e.g. Sulu Sea) should show high site tenacity, some emigration (incipient nomads excess to the areas' carrying capacity) but very little immigration

from surrounding areas. It is significant in this context that neither the Papua New Guinea experiments nor the South Pacific Commission releases in the western Equatorial Pacific have resulted in returns from Indonesia and the Philippines. Skipjack tagged in the seasonally productive temperate areas of New Zealand and S.E. Australia should perhaps be expected to show the widest dispersal of all. Data becoming available from large scale tagging programmes should enable such tests to be made in the near future.

Life histories of most of the larger tunas - albacore (*Thunnus alalunga*), northern bluefin tuna (*Thunnus thynnus orientalis*), Atlantic bluefin tuna (*Thunnus thynnus thynnus*) and southern bluefin tuna (*Thunnus maccoyii*) can be seen as more advanced expressions of this strategy. They typically spend the major parts of their pre-adult life and the inter-reproductive phases of their adult life in productive temperate zones, making extensive migrations and returning to spawn in well defined tropical areas.

Yellowfin tuna (*Thunnus albacares*), considered more primitive than the above species (Collette, 1978), shows greater similarities with skipjack. Its centre of distribution is tropical and larvae are widely distributed between the 26°C isotherms (Ueyanagi, 1969). Analysis of 13590 tag returns (9383 away from the point of release) from eastern Pacific releases (Sharp, MS using data by Bayliff) showed that for returns where distance moved could be caluclated, 50% were recaptured within 80 nautical miles of the release point, 95% within 660 miles and only 1% in excess of 1060 nautical miles. These proportions vary when the data set is subdivided, but the result is remarkably similar to that obtained in these experiments, and suggest adoption of similar resident-nomad strategies.

These differences in expression of resident-nomad strategies can be expected to have important implications for population structure.

3.7.3 Gene Flow

Dispersal does not necessarily lead to gene flow for a variety of reasons (Endler, 1977) and should be regarded as a prerequisite for gene flow (Grant, 1980). The present experiments therefore provide an estimate

of only the *potential* for gene flow via movements of adult skipjack between areas.

The experiments, despite the constraints imposed by the distribution of effort, indicate that dispersal of adult skipjack tagged in the Papua New Guinea area is limited. No returns were received north of 10°N and west of 130°E, despite the presence of large skipjack fisheries. To the east and south, limits are less clear due to low levels of effort, especially south of 10°S, and southwards movements may be quite extensive; eastwards expansion of the long range fleet since 1975 has however produced no corresponding enlargement in the area covered by the tag returns. A similar result is suggested for immigration. Were such movements the primary source of gene flow, partial isolation by distance leading to differentiation across the Pacific Ocean might be predicted; long distance movements do tend to be underestimated in such experiments (Grant, 1980), however, and dispersal over longer time scales of more than one generation need to be taken into account.

In addition, other phases of the life cycle need to be considered. The pelagic egg and larval stages of tunas complicate this issue, as they can be assumed to confer a high additional potential for gene flow. Studies of widely distributed marine species with pelagic larval stages are few, but have produced interesting results. Winans (1980), for example, examined populations of milkfish *(Chanos chanos)*, a euryhaline near-shore marine fish species, across the Pacific and found little genetic differentiation, which he attributed to gene flow via the planktonic larval stages. Similarly, Soule (fide Ehrlich, 1965) found little differentiation in pomacentrid fishes with pelagic larvae across distances of 3,000-5,000 km, and striking differentiation in a species of a related genus showing parental care of young, even between different parts of the Great Barrier Reef.

Information required to evaluate the potential for gene flow afforded by planktonic eggs and larvae includes length of the passive planktonic phase of larval life, geographical distribution of larvae, the role of oceanographic events in transport and/or concentration and factors regulating larval development. For example, *C. chanos*, as an elopoid species has a leptocephalus-like larval stage in which metamorphosis could conceivably be delayed. Passive transport also does not mean random

transport because denatant drift following contranatant adult migration may effectively return larvae to the area normally occupied by adults which spawned them. Once limited mobility is achieved, diel vertical migration, combined with currents in opposing directions at different depths (see earlier), may effectively maintain weakly swimming juveniles in an approximate location.

As we have seen in 2.3.3, details of skipjack larval history are minimal. Ueyanagi *et al.*, (1974) report eggs hatching within 22-27 hours of fertilization at 27°C, and Ueyanagi (pers. comm.) estimates length of the planktonic phase not to normally exceed three weeks. Unidirectional movement in a strong (2 knot) current during this period could result in a displacement of 1,000 nautical miles, with the possibility of further-directed movement once independent mobility was achieved. Alternatively, the limited larval distribution of some *Thunnus* species (Ueyanagi, 1969) and the mechanisms mentioned previously indicate that wide dispersal does not inevitably occur. At present, the role of larval stages in promoting or restricting gene flow in skipjack remains essentially unknown.

The relative contribution made by residents and nomads to spawning is problematical. Kawasaki (1965) recognizes five groups of skipjack in the Japanese fishery, two resident groups which appear to spawn and show slow growth rates and three migratory groups which grow rapidly but do not appear to spawn in the area. Spawning activity in the Papua New Guinea area, as inferred from monthly gonad index values (Lewis *et al.*, 1974; Wilson, MS) is probably continuous throughout the year but is least during the April-September period when nomad numbers would normally be greatest in the north-eastern Bismarck Sea. Influxes into the Solomon Sea during November-April would, on the other hand, coincide with part of the peak spawning period, and have the greater potential to contribute to spawning in that area. Successful spawning, as measured by larval survival, is dependent on depositing eggs in productive patches conducive to larval survival. This should pose few problems for residents. In their extensive movements, nomads may encounter such conditions more frequently but an energy cost which precludes gonadal development. The adaptive value of the nomadic strategy may relate not to some increase in reproductive fitness, but the ability to exploit different resources to residents. Eggs deposited in near island-situations should have several advantages - productive conditions should show some temporal stability;

increasing the chances of survival to a stage where independent mobility is achieved; eddy effects may also serve to maintain larvae in the area.

As noted in 2.3.3, predation by adult skipjack may be an important source of mortality in skipjack juveniles. This leads to the somewhat revolutionary proposition that any nomadic spawning which occurs may be adaptive, because it can result in increased forage production (in the form of skipjack juveniles) in the unpredictable open ocean. Open ocean versus near-island larval densities would then need to be viewed in a different light, as larvae near islands are more liable to survive to adulthood. Such a strategy retains flexibility, and should an area be depleted of residents for any reason, a pool of nomads is available to fill the available niche. If dispersal is limited, as indicated by these experiments, this option is most likely to be exercised by fish from near-by areas.

3.7.4. Predictions relevant to population genetic studies

These genetic data may also provide an independent evaluation of the resident-nomad hypothesis. Predictions, albeit not particularly powerful ones, can be framed as follows.

(i) if there are gene frequencies characteristic of residents, they should be present throughout the year,

(ii) assuming gene frequencies at some loci of at least some nomads will differ from those of residents, the most likely period of their occurrence in the New Hanover sector is April-September. Basal gene frequencies should accordingly be modified during this period.

(iii) if there were gene frequencies characteristic of different geographical areas, nomad frequencies should be representative of the area covered by tag returns.

(iv) genetic heterogeneity at any point in time is liable to be considerable, given the multiplicity of factors promoting it. In particular, the observed gene frequencies should be more variable than that given by random (binomial) sampling of a single frequency.

CHAPTER 4

POPULATION GENETICS OF SKIPJACK TUNA

4.1 INTRODUCTION

Population genetics, in its broadest sense, is the study of the origin and dynamics of genetic variation within and between populations (Lewontin, 1974) and as such, forms an integral part of evolutionary theory. In practice population genetic studies focus on the dynamics of gene frequency change. The concern of this chapter is with a subset of that field, the use of allozymic gene (and genotype) frequencies, in combination with ecological data, to infer the genetic structure of natural populations. It is useful first, however, to briefly review the basic concepts underlying such studies.

4.1.1 The nature of genetic variation

Until relatively recently, a fundamental constraint had been imposed on population genetic studies by the difficulty of interpreting most observed phenotypic variation such as in morphological, meristic and morphometric traits in genetic terms. The mode of inheritance of such characters is frequently polygenic, and the effects of single gene substitution are often small with respect to variation induced by environmental fluctuations. Advances in molecular biology, specifically the development of gel electrophoresis (Smithies, 1955) where proteins are separated in an electric field according to their net charge and molecular size or conformation, and protein-specific histo-chemical staining techniques to visualize these proteins (Hunter & Markert, 1957), have however allowed a proportion of the genetically determined variation at individual structural gene loci, notably those coding for enzymatic proteins, to be identified.

Although it has been argued that only about 30% of single amino-acid substitutions lead to an electrophoretically detectable change in net surface charge (King & Wilson, 1975) and that this value may vary from locus to locus (Johnson, 1974; King, 1973), recent studies (Ramshaw *et al.*,

1979) on completely characterized variants have shown that more sophisticated electrophoretic methods can detect approximately 90% of substitutions. Irrespective of the relationship between phenotypic and genotypic classes the amount of electrophoretic variation detected has generally proved to be large. A variety of animal and plant species has now been screened for electrophoretic variation using standard techniques (Powell, 1975; Nevo, 1978; Brown, 1979) and although estimates vary widely between species, most populations appear to be polymorphic at 25 to 30% of their loci, with individuals heterozygous at 5 to 15% of their loci (Selander, 1976). Where the inheritance of the various electromorphs (King & Ohta, 1975) at a polymorphic locus can be demonstrated to be Mendelian and codominant, they are regarded as direct expressions of underlying alleles and in the case of enzymatic proteins are commonly referred to as allozymes (Prakash *et al.*, 1969).

Although the discovery of this new class of molecular variation has given rise to a wealth of theoretical, experimental and descriptive studies, controversy still surrounds its biological significance and factors responsible for its maintenance: The debate, in its extreme form, embodies two sharply contrasting views - the neutralist (or classical) view (Kimura, 1968; King & Jukes, 1969) which sees most polymorphisms as selectively neutral or near neutral, with mutational input, random extinction and drift contributing to their maintenance, and the selectionist (or balance) view which sees polymorphisms being maintained by some form of balancing selection (Richmond, 1970; Wills, 1973).

The neutralist-selectionist debate remains unresolved (see Spiess (1977) and Roughgarden (1979) for recent reviews) Lewontin, in 1974, listed three principal reasons why this is so, and it appears that little progress has subsequently been made in overcoming these difficulties, namely

(1) neither theory is empirically sufficient - central to both are parameters and combinations of parameters which are not measurable to the degree of accuracy required, e.g. μ (mutation rate per gene per generation), m (migration rate), s (selection coefficient), N_e (effective population size),

(2) in both cases, theory refers to equilibrium conditions, and the knowledge of history necessary to understand equilibria are simply not available and

(3) the theory is not dynamically sufficient in that inter-locus interactions (for example, epistasis, co-adapted gene complexes, linkage, hitchhicking etc.) are not usually taken into account.

Approaches such as that described by Clarke (1975) & Koehn (1978) involving characterization of structural and functional differences between alleles at the molecular level and experimental testing of hypotheses developed, may prove quite powerful for detecting selection on a particular locus, at least in species amenable to experimental manipulation, yet not identify the agents of evolutionary change (Gould & Lewontin, 1979).

The debate has however undergone a subtle shift in direction such that the question asked of neutralist/selectionist theory in explaining observed variation has become "how much of each" rather than "which of the two".

4.1.2 Population Studies

For all the uncertainties surrounding mechanisms involved in its maintenance, electrophoretic variation has proved a very useful tool in several fields. Systematic studies have greatly benefited, particularly as the value of allozymic characters in this context does not rest on selective neutrality or otherwise of alleles (Selander & Johnson, 1973). Avise (1974) details the many advantages of the technique and its limitations, and the topic will be further explored in Chapter 6.

The concern of this chapter is with another use of genetic data generated by electrophoretic studies, namely the elucidation of the population genetic structure of marine fish populations from gene and genotype frequencies in combination with ecological data. Prior to the development of electrophoretic techniques, the basis of fish population genetics studies was, in common with other animal groups, variation detected in morphological, morphometric, meristic and serological characters, supplemented by inferential data, such as apparent discontinuities in distribution, age structure, parasite presence or absence and mark-recapture results (Marr, 1957). Meristic and morphometric characters are continuous variables, typically under polygenic control, and are often subject to environmental influence (Taning, 1952; Barlow, 1961) which may restrict their usefulness as genetic discriminants; technical and

theoretical problems, such as production of specific antisera and relating blood groups to genes, have similarly limited the use of serological and immunological techniques (Utter *et al.*, 1974).

Electrophoretic techniques, on the other hand, allow a proportion of the genetically determined variation at individual structural gene loci to be identified, as discussed earlier, and results can be readily reproduced.

Two basic and somewhat fragile premises underpin studies of genetic differentiation in natural populations.

(a) Significant differences in gene frequency between samples reflect some degree of reproductive isolation and

(b) departures in genotype frequencies from Hardy-Weinberg expectations can be useful in detecting differentiation components such as assortative mating and geographical subdivision.

There is considerable evidence to suggest that these assumptions are not always justified.

The following qualifications are relevant to premise (a):

(1) Populations freely exchanging genes but under different selective regimes may show marked differentiation (Ehrlich & Raven, 1969). An example of this from marine fishes is that of the catadromous American eel (*Anguilla rostrata*). All breeding of the species is presumed to occur in the region of the Sargasso Sea, under conditions of panmixia, yet marked differences in allele frequency are observed between localities along the eastern seaboard (Koehn, 1970; Williams *et al.*, 1973). Although there does exist a slight possibility that micro-differentiation of the spawning area occurs, this example indicates that selection on the loci being used as markers must therefore be taken into account.

(2) Providing a suitable population structure can be postulated, any geographical pattern in allele frequencies at a single locus can be explained by random drift of selectively neutral

alleles (Kimura & Maruyama, 1971). Two or more polymorphic systems should therefore be used, since breeding structure, contrary to selection and drift, affects all loci and alleles uniformly (Christiansen & Frydenberg, 1974; Lewontin & Krakauer, 1973). To ensure that these loci segregate independently, the absence of linkage disequilibrium, or the random association of alleles at two loci should be established.

(3) Non-significant differences in allele frequency between groups indicate only that groups are not necessarily different, and not that they are of similar genetic composition. An allied problem is essentially of a statistical nature - if large enough samples are taken, even very small differences in allele frequency become statistically significant, although the biological significance of the observed difference may be questionable. In practice, some *a priori* decision is usually made about what constitutes a significant difference between subsets (see later).

(4) Population(s) being compared should not have been through recent bottlenecks which increase the probability of allele frequency changes due to random genetic drift (Nei *et al.*, 1975). In practice, such historical information is not readily available, but this possibility cannot be excluded when interpreting allele frequency data.

(5) Although our interest is primarily in deterministic forces which influence gene frequency distributions (notably restrictions on gene flow, selection and recurrent mutation) stochastic effects - drift and founder effects - need to be considered, particularly in small populations or isolates. Aspinwall (1974) provides a good example from studies of the anadromous pink salmon *(Oncorhynchus gorbuscha)* with its unique two year (± 10 days) breeding cycle. Populations in the same stream but breeding in alternate years are effectively isolates, subject to presumably very similar selective regimes; most streams studied showed considerable uniformity within either distantly-spaced odd or even-year

populations, yet in many cases, marked differences were detected between odd and even-year populations in the same stream.

(6) Recent experience has shown that cryptic intra-allelic variation exists (Milkman, 1976; Singh *et al.*, 1975; Johnson, 1977). These alleles have been detected by heat resistance tests (Bernstein *et al.*, 1973), molecular sieving (Johnson, 1976), isoelectric focussing (Singh *et al.*, 1976) or a combination of these (Coyne, 1976). This extra variation may provide increased discriminatory power in studies of genetic differentiation, as well as being of value in establishing the role of selective versus non-selective forces, for example, where clines exist in parallel at some loci but not others (Singh, 1979).

Premise (b), using deviations from Hardy Weinberg expectations to infer aspects of genetic structure, also needs some qualification. An excess of heterozygotes can result in deviations from equilibrium which are commonly attributed to selective advantage or heterosis. However, quite strong selection can occur in two allele-system, on the heterozygote, the alternate homozygotes or any combination without significantly disturbing this equilibrium (Smith, 1970; Leigh-Brown, 1977; Koehn & Williams, 1977). Genotype proportions thus cannot be used in isolation as evidence of selection. Similarly, calculation of nett fitnesses from genotype frequencies has no statistical power for realistic selection values (Lewontin & Cockerham, 1959). A deficiency of heterozygotes (or excess of homozygotes) can result from at least three factors - inbreeding or departures from random mating as observed in small or subdivided populations; mixing of populations with different gene frequencies, the Wahlund effect (Wahlund, 1928) and the presence of null alleles. Distinguishing between the first and second of these factors may be difficult, although comparisons of genotype frequencies at several loci can be informative.

A final problem is again related to sampling. As the Hardy Weinberg Law relates to populations of infinite size, sampling 'accidents' may lead to significant deviations by chance. In fact, the problem is not a serious one since the power of tests for significance are very low

with sample sizes <200 (Sing & Rothman, 1975; Anon, 1980).

A separate set of caveats are of a technical nature, and relate to unambiguously establishing the underlying genetic basis of the systems being used as markers. Phenotypic variation may arise through post-translational modification (Simonarson & Watts, 1969; Uy & Wold, 1977). This can occur in a variety of ways especially during storage (Fairbairn & Roff, 1980), e.g. addition or removal of sialic acid residues and amide groups, but also by the action of modifier loci in the living organism. As mentioned previously, segregation of an inactive or null allele (Harris, 1975; Trippa *et al.*, 1978; Gauldie & Johnson, 1980) can occur, particularly in certain protein classes such as esterases and phosphatases. Detection of these effects relies heavily on the experience and care of the investigator. For understanding possible selective action on a particular locus, it is important to have some understanding of the role of the enzyme being studied. Factors regulating the function of enzymes and the compartmentalization of enzymes within cells is not well understood in the majority of cases.

Finally, it is almost unnecessary to add that detailed knowledge of the species biology and ecology is a prerequisite to the design of experiments which will adequately assess genetic differentiation within that species, and that replicate and time series sampling within a given area are necessary to adequately represent the genetic structure of that area's population(s).

4.1.3 Modes of intraspecific variation

Intraspecific variation in allele or isozyme frequency takes numerous forms, and patterns of variation may differ widely even among closely related species.

Different alleles may predominate in different parts of a species range (Utter *et al.*, 1970) or alleles may be present in one population and absent in another (Payne *et al.*, 1971). In the latter case, some degree of isolation can be assumed, whereas the former would include examples of disruptive selection.

Allele frequencies at polymorphic loci may be similar in all populations studied. (Prakash *et al.*, 1969; Lester, 1979). As this occurs commonly in species with well developed dispersal capabilities, a

logical explanation would be that strong gene flow ensures genetic continuity. This homogeneity would however, need to be confirmed by additional criteria. An alternative selectionist explanation would be that similar selective regimes across the geographical range could produce similar gene frequencies through stabilizing or frequency-dependent selection.

Geographical variation in allele frequency commonly occurs, and can be explained either as a result of drift in isolates, or local adaptation. It has been most intensively studied where changes in allele frequency are directional or clinal (Huxley, 1938), e.g. Frydenberg *et al.*, 1965; O'Gower & Nicol, 1968; Koehn & Rasmussen, 1967. Some of the best evidence for selection in natural populations comes from studies where trends in allele frequency can be related to corresponding trends in environmental variables, e.g. Schopf & Gooch, 1971; Johnson, 1971; Bishop, 1972; Merritt, 1972. A variety of conditions can result in clines (Endler, 1977) and these will be discussed in a later section.

Allele frequencies have been demonstrated to vary in response to factors such as population size (Krebs *et al.*, 1973), and age or year class (Beardmore & Ward, 1977; Chilcote *et al.*, 1980).

It is therefore clear that whilst demonstrating allele frequency differences is an important step in establishing to what degree of a population is genetically differentiated, numerous other factors need to be taken into account before a complete appraisal is possible.

Variation in allele frequency observed between samples collected over a large part of the range of a widely distributed species such as skipjack can be regarded as having three components, geographic variation across the species range, within-area variation due to factors such as seasonal effects, year class differences, and schooling, and variation associated with finite sample sizes.

Previous genetic studies with skipjack tuna are reviewed in the following section (4.2). These have revealed considerable geographical variation in allele frequency at one locus, from which inferences on genetic differentiation have been drawn. The coverage afforded by this sampling, both in space and time relative to the huge area over which skipjack occur, remains grossly inadequate. Because of the attendant logistical difficulties,

it is unlikely that a sampling regime sufficiently rigorous to describe this variation *in toto*, particularly isotopically, can ever be put into effect. In the present study, opportunistic sampling to increase the geographical coverage of the Indo-Australian region was undertaken. This has been integrated with previous work and ongoing studies elsewhere in section 4.4.

Time series sampling within the Papua New Guinea area, from which baseline information in the form of tagging data (Chapter 3) and some ecological data (2.3) were available, was carried out to examine the within-area component of allele frequency variation. This is analyzed in section 4.5. Sampling strategy is considered in section 4.2.1.

4.1.4 Previous Studies

Cushing (1964), de Ligny (1969) and Fujino (1970) review early serological and biochemical studies on tuna populations. Skipjack has been the most extensively studied of all the tunas. Beginning with the work of Cushing (1956), immunological techniques were used to identify genetic variation in C blood group (Sprague and Holloway, 1962), B blood group (Sprague and Holloway, 1962; Fujino, 1967) and Y blood group systems (Fujino and Kazama, 1968). Despite early optimism, those systems have failed to show between-area heterogeneity in the Pacific Ocean when large samples were examined, although both Y and B groups (K_1 positive) phenotypes were of some value in separating Atlantic and Pacific Ocean specimens (Fujino, 1969). Other blood factors have been detected using phytoagglutinins (Sprague and Holloway, 1962; Fujino, unpublished) but no definitive results are known from this work.

The use of electrophoretic techniques has generally proved more useful and convenient. Barrett and Tsuyuki (1967) and Fujino and Kang (1968a) independently studied a three allele serum transferrin polymorphism, the identity of which was verified using Fe^{59} sulphate labelling. No significant heterogeneity within or between areas for skipjack tuna from the Atlantic, western Pacific and central-eastern Pacific was observed. Fujino and Kang (1968a) however presented evidence for differential fertility and viability amongst the phenotypes and their association with fish of different sizes. Mechanisms maintaining this balanced polymorphism in a randomly mating population were discussed. Recent re-analysis of this data (Sharp, MSb) suggested that gene frequencies

were not homogenous with respect to size and the data were possibly not appropriate for such analyses.

Fujino and Kang (1968b) also described a six-phenotype serum esterase (E_{SJ}) system under the control of three co-dominant autosomal alleles, independent of previously described esterase systems and sex and size of fish. Analysis of nearly 15,000 samples in 196 lots ($\bar{n} \simeq 75$) from various areas of the Pacific Ocean (Fujino, 1970b) revealed marked heterogeneity in frequencies of the E^1_{SJ} allele. When subdivided into western Pacific and combined central and eastern Pacific groups, no significant within-area heterogeneity was observed in the E_{SJ} system (nor in serum transferrin and the three blood group systems). Between-area heterogeneity in E^1_{SJ} frequencies was however found to be marked, leading to the suggestion that a skipjack tuna sub-population existed in the western Pacific which was to some extent reproductively isolated from skipjack in the central and eastern Pacific. Skipjack of the western Pacific sub-population were postulated to be present throughout the year in inshore waters off the east coast of Japan and Okinawa, in the Bonin-Marianas area and Palau. The boundary between the two sub-populations was postulated to shift eastwards in the northern summer and westwards in autumn and winter. Collection of additional material in the western Pacific and age composition analysis of commercial catches (Fujino, 1972) led to the suggestion that the western sub-population was comprised of two groups, A and B which spawn in different seasons (northern and southern summers respectively) and show semestral recruitment, but are not genetically isolated (Figure 4:1). Distinct migratory behaviour for each group was implied.

Collection of material (2267 individuals, 61 samples, $\bar{n} \simeq 37$) from the south-western Pacific (Fujino, 1976) enables shifts in the sub-population boundary as postulated for the northern hemisphere (Fujino, 1970b) to be sketched for the southern hemisphere. The boundary was said to exist in the Tasman Sea year round, shifting westwards close to the New South Wales coast in early winter. Rejection limits at the 5% significance levels were recalculated for the two sub-populations giving E^1_{SJ} frequencies of 0.394-0.570 and 0.578-0.758 for the central-eastern and western Pacific sub-populations respectively. The validity of the statistical procedure used, i.e. *a posteriori* separation into two groups, then testing for significant differences, is however doubtful, and the

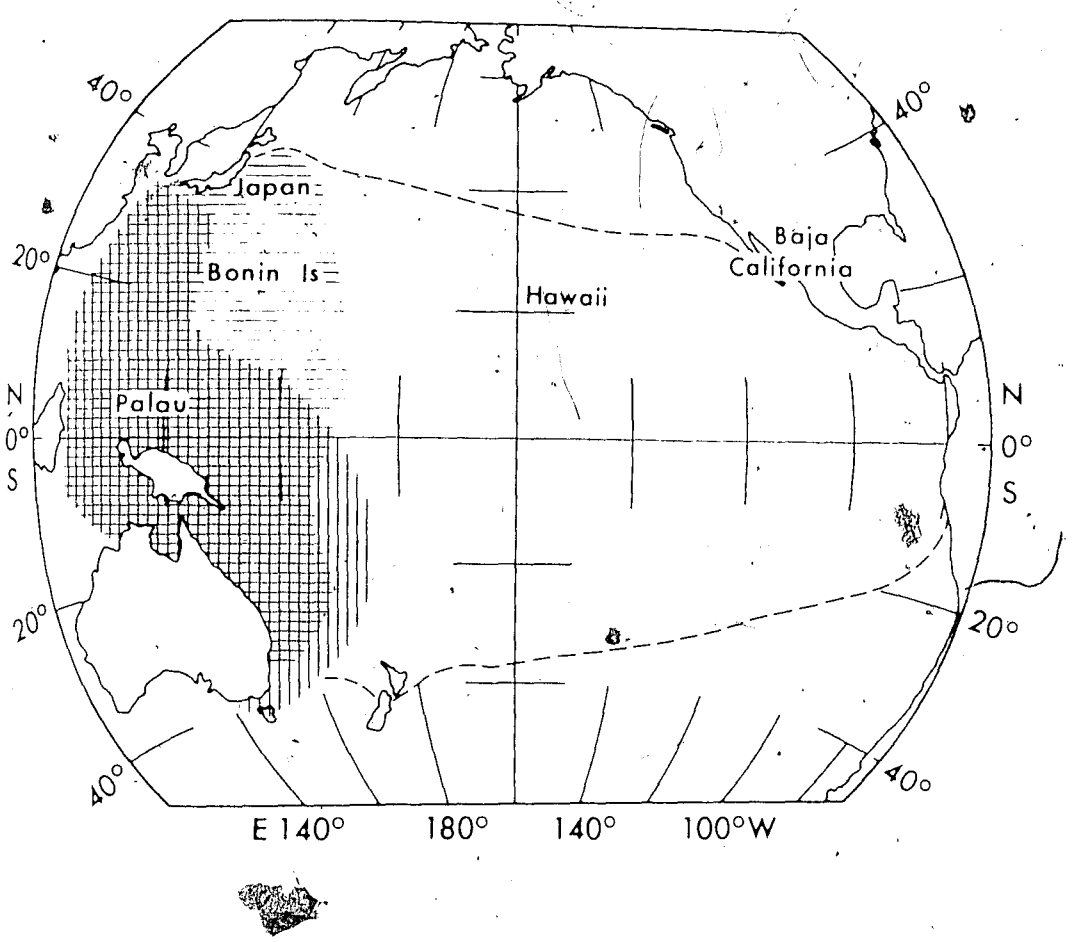


Figure 4.1 Approximate ranges of the hypothesized Pacific Ocean skipjack sub-populations of Fujino (1972).

The geographical range of the two groups in the western sub-population is shown by vertical (southern summer spawners) and horizontal (northern summer spawners) shading respectively.

small sample size detracts from the power of the test (see 3.3.1). Discovery of a second polymorphic esterase in red blood cells (Fujino, 1979) has reportedly allowed genetic separation of the two eastern Pacific sub-groups in Japanese waters.

After working for some years with yellowfin tuna, Sharp (1978 and MSb) organized collections of skipjack material (approximately 7,000 individuals) from Papua New Guinea, New Zealand and the eastern Pacific Ocean during 1975, 1976 and 1977 to evaluate genetic homogeneity of skipjack samples from those fisheries relative to previous results with yellowfin tuna samples, and to independently examine skipjack population genetics. Large sample sizes were collected ($\bar{n} \simeq 170$) and typed for esterase and transferrin phenotypes. Although only 2 of the 40 samples showed significant deviation from Hardy-Weinberg equilibrium, both the esterase and transferrin frequencies were heterogeneous for each of the three areas examined according to the test used. Similarly, re-analysis of Fujino's data including only the larger samples ($n > 80$) showed heterogeneity in sample sets from Hawaii, Palau and Japan. This led Sharp to postulate the existence of at least five 'genetic components' in Pacific Ocean skipjack tuna (Figure 4.2). As had been the case with the earlier Fujino hypothesis, no mechanism by which this situation might be maintained was proposed and the interpretation was essentially subjective. The need for long term localized studies, sampling over a wider area, and additional biological information in critical areas such as reproduction, larval distribution and survival, and the role of open ocean-island interactions in skipjack ecology was emphasized.

Since 1977 further sampling in the south-western Pacific, specifically New Zealand, Papua New Guinea and the Solomon Islands (Richardson, unpublished) has been carried out, and since 1978, collections made during the South Pacific Commission's Skipjack Survey and Assessment Programme have expanded the geographical coverage into many hitherto unsampled south-western and south-central Pacific areas (Richardson, MS).

A major problem remains the reliance of current interpretations on a single genetic system, serum esterase (E^1_{SJ}). The serum transferrin system, although showing considerable heterogeneity within and between areas, does not vary in a consistent or readily interpretable way and explanation of this heterogeneity may lie in the species' reproductive and schooling strategies. The results of a recent search for new genetic systems will be described in a subsequent section (4.2.4).

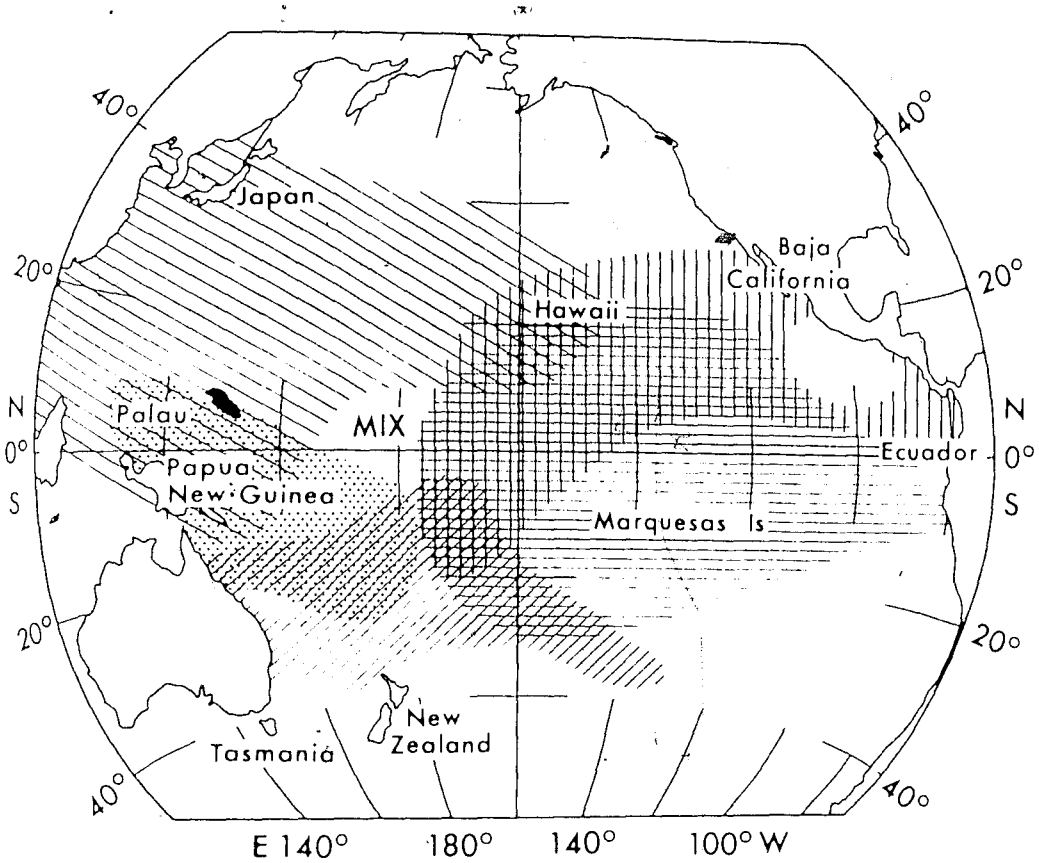


Figure 4.2 Approximate ranges of the hypothesized Pacific Ocean skipjack populations of Sharp (1978).

4.2 MATERIALS AND METHODS

4.2.1 Sampling Considerations

As was stressed in the review of previous studies, the question of appropriate sample size strongly influences some facets of interpretation of genetic data. In practice, the sample size chosen will represent a compromise between a theoretical optimum and a realistic minimum level which is consistently possible to achieve under a variety of sampling conditions; the hypothesis to be tested will also have some bearing on this decision.

In the early stages of population studies, it is frequently necessary to decide whether or not samples are significantly different from one another.

Sharp (in press) points out that under these conditions the choice of optimum size will be influenced by:

- Δ, the minimum difference in allele frequency $p_1 - p_2$ one is willing to accept as being significant between two populations or samples.

- the significance level (α) for rejection of the null hypothesis of no difference between populations. This is traditionally set at $\alpha = 0.05$.

- the desired power of the test ($1 - \beta$), that is, the probability of detecting significance where allele frequency difference is Δ.

- the expected range of values for the allele frequency. The power of the test is lowest near 0.5 and highest near 1.0.

A table of the number of individuals per sample which would permit differentiation of p_1 and p_2 for various values of p, when $\alpha = .05$, $1 - \beta = 0.5$ and $p = \frac{1}{2}(p_1 + p_2)$.

	.95	.90	.80	.70	.55
.35	146	276	492	645	760
.25	50	69	123	162	190
.15	50	25	31	40	48
.05	50	25	13	9	6

A sample size of 200 or more individuals would thus allow discrimination of two samples or populations with $\Delta = 0.10$ over a range of allele frequencies. This may be a typical Δ value; $p_1 + p_2$ for Fujino's (1976) two "subpopulations" were, for example, 0.668 and 0.482, giving $\Delta = 0.186$, and Sharp (MSa) regarded a Δ value of 0.07 as reasonable in his yellowfin tuna studies. The biological significance of such differences is however unclear.

The low power of χ^2 tests to detect deviations from Hardy Weinberg expectations in samples of <200 individuals has been discussed and sample sizes approaching 200 are clearly desirable if the null hypothesis of no difference between populations is to be tested.

Where a certain amount of information on geographical variation in allele frequency already exists, as is the case here, the more common null hypothesis is not $p_1 = p_2$, but whether p_1 differs from a number of other populations. The serious error then becomes not failure to discriminate but incorrect classification. Under these conditions, a sample size of 100 becomes more acceptable. Using the normal distribution as an approximation to the binomial, the 95% confidence interval of the gene frequency estimate is about $\pm$ .065 for a sample of 100 fish, with $\alpha = .05$, and $p = 0.7$, as opposed to $\pm$ .046 for a sample of 200. Simulations have also shown that the probability of misclassifying samples of 100 fish where $p = 0.7$ was less than 5% (Richardson, pers. comm.) as opposed to 25% for samples of 20 fish and 3% for samples of 150 fish.

This result alleviates sampling problems. Experience had shown that whilst sample sizes of 200 from individual "schools" or aggregations could regularly be collected onboard purse seine vessels, such was not the case with smaller pole and line vessels, the major source of samples in the western Pacific (and certainly not the case for non-commercial fishing activity) without sacrificing sample homogeneity by collecting from more than one "school". Sample sizes of 100 individuals could, on the other hand, be obtained relatively more readily.

Furthermore, for calculating the precision of estimates of p, the number of samples becomes relatively more important than individual sample size, since the confidence interval equals $\frac{1}{2}\,\omega/\sqrt{N}$ where ω is the confidence interval width about p and N the number of samples (Anon., 1980).

are simply not available and

Larger sample sizes do however retain the additional advantage of allowing statistical comparison between sample subsets e.g. fish of different size, sex etc.

In addition to the questions of appropriate sample size, the problem of sample homogeneity arises. Sampling on board commercial vessels, the usual and most convenient strategy, introduces a bias towards large feeding aggregations, possibly comprised of more than one "school" in which estimates of genetic heterogeneity may increase. One way in which this may be reflected is increased size ranges within schools. If fish size is likely to have some information on an individual's genetic composition at a given point in space and time, an intuitively reasonable possibility, sampling extensively without regard to modal groupings may reduce the biological significance of any statistical tests subsequently performed. Although this essentially remains beyond the investigator's control, an effort was made to minimize such effects by:

(a) sampling from single schools or aggregations wherever possible.

(b) sampling only the predominant size class in polymodal catches, or where this was not possible, increasing sample size to allow later subdivision by size.

In addition, replicate samples were taken in many cases to further examine this phenomenon.

The final constraint on sampling is the obvious practical one. The problems of co-ordinating the collection and despatch of samples over a wide area such as that covered by the present study are many; facilities taken for granted in one locality are often only marginally adequate in another. Such difficulties are often compounded when large sample sizes ($\geq$200) are involved. In general, however, it proved possible to collect samples of 100 fish both extensively and intensively within the study area according to the guidelines described above.

4.2.2 Field collection

Using disposable plastic syringes and 18-19 gauge needles, 2-5 ml of blood were collected from each fish by cardiac puncture. An equal volume of preservative solution, consisting of 40% glycerol and 60% trisodium citrate

restrict their usefulness as genetic discriminants; technical and

as a 5% solution (Fujino, 1966) was added as soon as convenient. Length of each fish (LCF in mm) was recorded on the barrel of the syringe and in some cases, biological data (sex, gonad maturation) were available for individual fish.

The majority of samples were taken on board pole-and-line vessels soon after capture. Others were taken from purse seine catches, gill net catches, unloading bays at shore bases, market consignments and game fishing tournament catches. In several cases, skipjack which had been captured up to a week previously and subsequently kept chilled were satisfactorily sampled, as were thawed frozen fish on one occasion.

Where possible, a lot of 100 or so fish of similar size from a single school or fishing station were isolated and sampled (see earlier). This degree of sampling rigour could not always be achieved and some samples, particularly those collected in areas not previously sampled, were collected over a period of several days.

Samples were kept iced down or out of direct sunlight until transferral to a freezer could be effected and then freighted (on dry ice when possible) to Canberra for analysis. Care was taken to minimize contamination at all stages, although the use of disposable needles and the syringe itself as the sample container largely circumvents this problem.

Prior to analysis, samples were transferred to labelled one dram glass vials for storage at -10° to -20°C.

4.2.3 Material

During the period January 1978 to August 1980, 108 lots (10,436 individuals, $\bar{n} = 96.6 \pm 20.2$) of skipjack blood samples were collected in the Indo-Australian region bounded by 5°N-45°S, 95° - 160°E. They comprise material gathered opportunistically to extend and complement the geographical coverage afforded by previous studies (54 lots, $\bar{n} = 92.4 \pm 27.2$) and material collected sequentially, the time series sampling in the New Hanover sector of the Papua New Guinea fishery (54 lots, $\bar{n} = 100.8 \pm 6.6$).

For the purposes of this study, the region has been subdivided into four areas (Figure 4.3). The area of most intensive sampling, Papua New Guinea and environs (area 1), has been further subdivided into two sub-

areas. Collections were made for the geographical aspects of the study in the four areas above as follows:-

Area 1a sectors of the Papua New Guinea fishery excluding the New Hanover sector - (12 samples, 1219 individuals) from pole and line catches.

Area 1b between the Equator and 5°N, within or adjacent to Papua New Guinea Offshore Seas - (8 samples, 747 individuals) from purse seine vessels.

Area 2 the east coast of Australia - (25 samples, 2025 individuals), from research vessel, purse seine, gill net and recreational catches.

Area 3 three sites across the Indonesian Archipelago - Ambon (Banda Sea), Pelabuhan Ratu and Padang (Indian Ocean) - (7 samples, 789 individuals) from markets, pole and line catches and troll catches.

Area 4 south-western Australia (Albany, Esperance) - (2 samples, 211 individuals) from pole and line catches.

Sample locations are shown in Figure 4.3 and collection details given in Table 4.1.

Time series samples were collected in the New Hanover sector every three weeks. Initially a single sample was collected, but from early 1979 onwards, replicate samples (2 x 100) were taken on each trip wherever possible. With minimal fishing activity in this sector during the December-March period, some gaps in the sequence of samples have inevitably occurred. A total of 54 samples, collected on 31 occasions, are represented. Collection details are given in Table 4.2. During this period, length frequency data were collected in the same area (Wankowski, in press) with the aim of simultaneously monitoring movement of cohorts or size classes through the New Hanover sector and any changes in gene frequency.

4.4 Electrophoretic procedures

4.4.1 Starch gel electrophoresis

In the first instance, starch was used as the support medium for electrophoresis. Gels containing 32 g of starch (Connaught Medical Research Laboratories, Canada) in 270 ml of buffer were poured into 18.5 cm x 15.5 cm

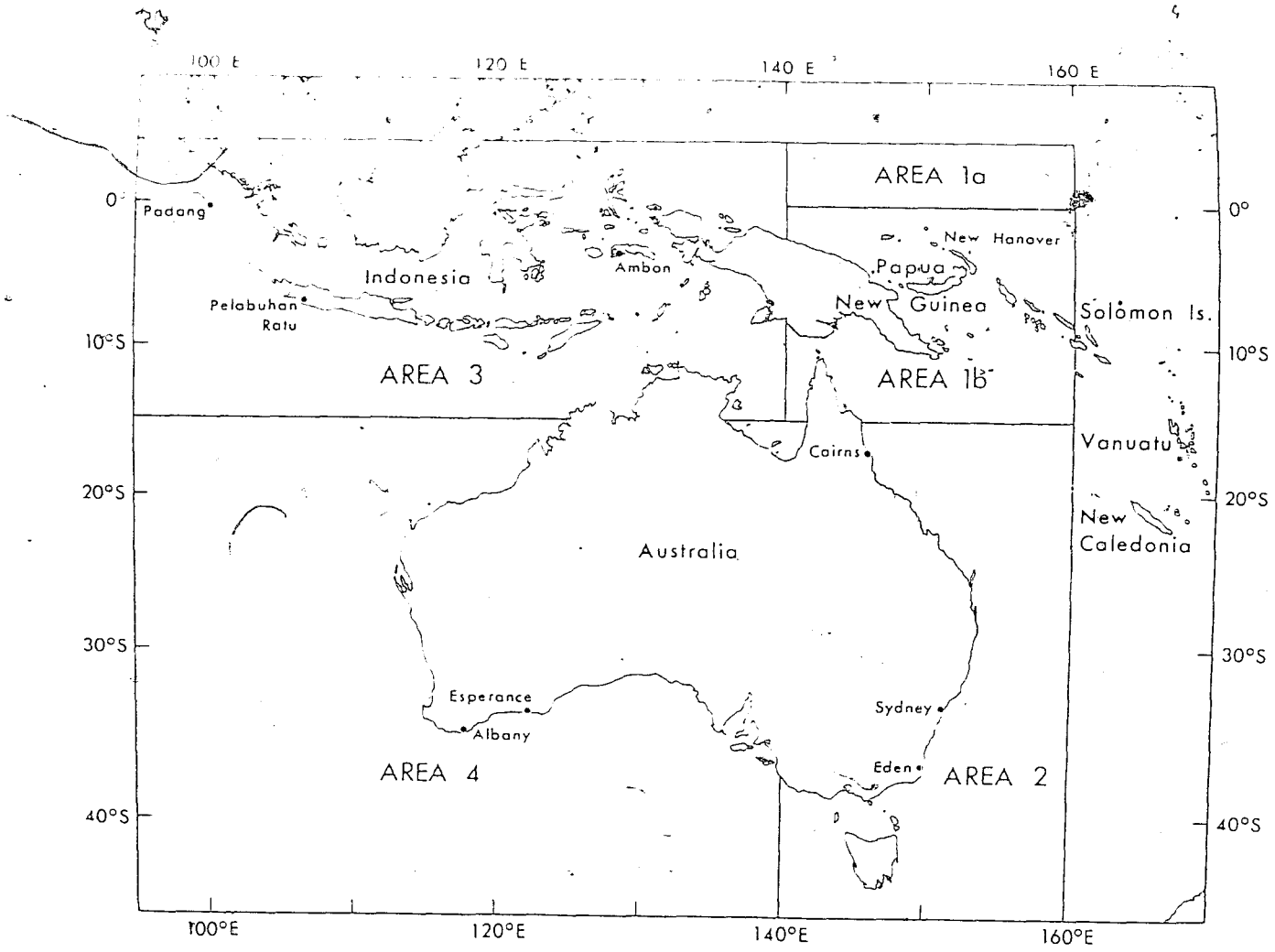


Figure 4.3 Sampling areas within the Indo-Australian region. Sampling localities mentioned in the text are shown.

Table 4.1 Details of skipjack blood samples collected in the Indo-Australian region and analysed by the author, 1978-1980.

Time-series samples are not included.

Key to collectors: PNG - Fisheries Division, Dept. of Primary Industry, Papua New Guinea; LPPL - Marine Fisheries Research Institute, Jakarta; CSIRO - Fisheries & Oceanography Division, Cronulla; SPC - South Pacific Commission Skipjack Survey & Assessment Programme; DPI - Fisheries Division, Dept. of Primary Industry, Canberra; OM - O. Miezitis, Triabunna, Tasmania.

Sample Code	Date	Locality	n	Collector
Papua New Guinea Area (1a)				
PG	19-27/2/78	10°38'S, 146°35'E	114	PNG
PE	18-21/1/78	10°34'S, 148°42'E	52	"
PA	14/4/78	10°38'S, 146°35'E	91	"
SA	20/1/79	6°30'S, 150°35'E	163	Self
SB	21/1/79	6°30'S, 150°30'E	106	"
SD	21/1/79	6°20'S, 150°20'E	114	"
CB	27/1/79	10°25'S, 148°10'E	82	PNG
CB_2	14/2/79	"	97	"
ARA	23/1/79	"	86	"
AM	20/5/79	7°36'S, 149°47'E	107	SPC
AN	3/6/79	4°04'S, 151°01'E	109	"
MAS-A	23/5/79	1°19'S, 146°40'E	105	PNG
Between 0° and 5°N (1b)				
DS	21/10/78	1°44'N, 137°56'E	61	PNG
DT	24/10/78	1°42'N, 136°46'E	102	"

VA	1/1?/78	4°30°N, 139°45'E	95	"
VGB	3/10/78	4°03'N, 140°39'E	99	"
HME	3/12/78	2°51'N, 142°06'E	102	"
WA	6/12/78	00°26'N, 140°12'E	96	"
WAB	11/12/78	00°12'N, 142°33'E	99	"
WAC	14/12/78	00°29'N, 142°44'E	86	"
Indonesia (3)				
BC	15/12/79	3°43'S, 128°50'E	111	Self
BD	17/12/79	3°36'S, 128°22'E	104	"
B MIX	14-17/12/79	3°38'S, 128°00'E	113	"
YY	20/12/79	2°20'S, 99°20'E	113	"
YZ	20/12/79	"	122	"
IA	21/12/79	"	91	"
IF	22-26/4/80	~7°15'S, 107°00'E	149	LPPL
South Western Australia (4)				
IC/IE	11-19/3/80	35°00'S, 152°09'E	139	Self
ID	16-20/3/80	34°00'S, 122°00'E	72	"
Eastern Australia (2)				
CA	21/1/78	34°09'S, 152°09'E	56	CSIRO
CC	23/1/78	35°15'S, 150°57'E	64	"
CD	23/1/78	36°57'S, 150°27'E	68	"
CF	27/1/78	34°45'S, 151°15'E	48	"
CG	2/2/78	33°15'S, 152°27'E	55	"
CH	3/2/78	32°27'S, 152°51'E	71	"

occurs commonly in species with well developed dispersal capabilities, a

		Locality	n	Collector
CI	4/2/78	32°15'S, 152°51'E	94	CSIRO
CJ	9/2/78	32°33'S, 152°51'E	59	"
CK	9/2/78	32°33'S, 152°51'E	127	"
CL	13/2/78	32°33'S, 152°57'E	53	"
CM	13/2/78	32°33'S, 152°45'E	51	"
CO	21/2/78	31°35'S, 159°10'E	64	"
CP	21/2/78	31°35'S, 159°10'E	47	"
CQ	22/2/78	31°35'S, 159°10'E	98	"
AF	5/4/79	36°04'S, 150°24'E	87	SPC
AG	8/4/79	35°06'S, 151°04'E	144	"
AH	9/4/79	34°58'S, 151°05'E	101	"
AJ	1/5/79	17°36'S, 148°22'E	109	"
AK	2/5/79	17°31'S, 148°05'E	98	"
AL	3/5/79	16°22'S, 150°12'E	110	"
NS	8-9/3/79	36°15'S, 150°15'E	47	Self
TA	1-10/5/79	~42°30'S, ~148°15'E	120	OM
SE	21/11/79	~36°25'S; 152°50'E	99	Self
FA	10/12/79	36°19'S, 156°03'E	93	DPI
FB	14/12/79	37°11'S, 155°46'E	70	"
		Total	4991	
		Mean	92.4 ± 27.2	

Table 4.1 cont..

Table 4.2 Details of skipjack time series blood samples collected in the New Hanover Sector of the Papua New Guinea Fishery

All samples were collected by staff of the Fisheries Division, DPI, Papua New Guinea and analysed by the author. Replicate samples have been paired.

Sample code	Date	Location	n
KVA	9/8/78	2° 00'S, 150° 45'E	107
KVB	25/8/78	1° 55'S, 150° 30'E	103
KVC	15/9/78	1° 55'S, 150° 10'E	98
KVD	5/10/78	2° 05'S, 150° 20'E	99
KVE	26/10/78	1° 37's, 149° 45'E	100
KVF	16/11/78	3° 00'S, 151° 40'E	99
KVG	5/12/78	2° 00'S, 151° 05'E	97
KVH	27/2/79	2° 00'S, 150° 50'E	100
KVI			100
KVJ	22/3/79	2° 06'S, 150° 32'E	100
KUK			100
KVL	18/4/79	3° 00'S, 150° 30'E	109
KVM			109
KVN	29/4/79	3° 20'S, 150° 50'E	105
KVO			105
KVP	17/5/79	3° 13'S, 150° 57'E	104
KVQ			104

KVR	7/6/79	2° 17'S, 150° 03'E	104
KVS		2° 17'S, 150° 03'E	105
KVT	3/7/79	3° 16'S, 150° 55'E	104
KVU	23/7/79	1° 45'S, 150° 18'E	106
KVV		1° 45'S, 150° 18'E	107
KVY	4/9/79	2° 50'S, 150° 14'E	107
KVZ		2° 50'S, 150° 14'E	104
KVA-A	22/9/79	3° 12'S, 150° 11'E	104
KVA-C	16/10/79	1° 47'S, 149° 40'E	107
KVA-D		2° 15'S, 150° 10'E	99
KVA-E	6/11/79	1° 55'S, 150° 39'E	105
KVA-F		2° 12'S, 152° 00'E	102
KVA-G	27/11/79	3° 10'S, 150° 40'E	103
KVA-I	17/12/79	3° 10'S, 150° 40'E	99
KVA-J		3° 10'S, 150° 40'E	95
KVA-K	17/12/79	2° 35°S, 149° 35'E	100
KVA-L		2° 35°S, 149° 35'E	99
NHA	2/4/80	3° 20'S, 150° 55'E	101
NHB		3° 20'S, 150° 55'E	104
NNC	17/4/80	2° 15°S, 150° 57'E	81
NHD		2° 10'S, 151° 12'E	106
NHE	8/5/80	3° 19'S, 150° 56'E	101
NHF		3° 19'S, 150° 56'E	79
NHG	29/5/80	2° 11'S, 150° 20'E	108
NHH		2° 11'S, 150° 35'E	104

Sample code	Date	Location	n
NHI	26/6/80	1° 40'S, 150° 10'E	105
NHJ		1° 27'S, 149° 50'E	105
NHK	17/7/80	2° 30'S, 150° 36'E	100
NHL		2° 25'S, 150° 44'E	103
NHM	31/7/80	2° 30'S, 151° 30'E	99
NHN	24/8/80	2° 29'S, 150° 37'E	98
NHO		2° 28'S, 150° 35'E	100
NHP		2° 30'S, 150° 42'E	93
NHQ	11/9/80	2° 28'S, 150° 44'E	82
NHR			83
NHS	16/10/80	1° 50'S, 150° 24'E	101
NHT		2° 11'S, 150° 25'E	103
		Total	5445
		Mean	100.8 ± 6.6

systems will be described in a subsequent section (4.2.4).

moulds, then allowed to set for several hours and usually overnight before use. The unlysed samples were applied to 9 mm x 5 mm filter paper wicks and inserted into ten slots cut into the gel. Horizontal electrophoresis was performed in a cold room (5°C) for 3½-4 hours by maintaining a current of approximately 3.5 mA per cm of gel width. The gel was then sliced horizontally, and stained for esterase and transferrin.

Details of the buffers and stains used, which are modified slightly from Fujino and Kang (1968a, b), are as follows:

- Tank (running) buffer consisting of 11.8 g boric acid/l and 1.5 g lithium hydroxide/l in double distilled water (pH ~ 8.2).

- Gel stock consisting of 1.6 g citric acid/l and 4.8 g tris (tris [hydroxymethyl] aminomethane) in double distilled water. A 3:1 dilution of gel tank buffer comprised the buffer used to prepare the starch gel; two drops of bromophenol blue were added to allow rate of movement along the gel to be monitored.

- Stains

  Esterase

  The freshly made staining solution containing 35 mg α-naphthyl acetate and 50 mg fast blue R.R. dissolved in acetone and 200 ml distilled water added, was poured over the gel and the reaction allowed to proceed in the dark at room temperature. The use of β-naphthyl acetate also gave satisfactory and in some cases, superior resolution of phenotypes.

  Transferrin

  The gel was soaked in a staining solution consisting of 7% (by volume) glacial acetic acid, 25% methanol and 2.5 g/l Coomassie (Brilliant) Blue for 5-15 minutes, depending on the condition of the stain. Destaining in a wash (7% glacial acetic acid, 25% methanol) was allowed to proceed until the bands were clearly visible (usually about 24 hours).

Cellulose acetate electrophoresis

The use of starch is however time consuming, an important consideration when large numbers of samples are to be run, and the methods were gradually

adapted to use with cellulose acetate as the support medium. No loss of resolution resulted and considerable savings in time were achieved. Samples, again unlysed, were applied with an adjustable-gap draughtsman's lining pen to 10 x 7 cm Cellogel (Chemetron, Milan) strips. The gels had previously been soaked in the running buffer for a minimum of 30 minutes. Horizontal electrophoresis was performed in a cold room (5°C) although room temperature also proved satisfactory. Using adjustable-bridge tanks, samples could be applied along the longest axis (25 or more per gel) or on half gels (10 x 8½ cm; up to 14 per gel) as convenient.

Buffers, running conditions and stains for the systems were as follows:

Esterase

Buffer - .05M TEB pH 8.2
6.05 g tris/l
0.363 g EDTA/l
Adjusted to pH 8.2 with boric acid

Running conditions:
25-30 min at 320v and 8-10mA (per two 10 x 7 cm gels).

Stain:
40 mg. Fast Blue RR in 100 ml of 0.05M phosphate buffer (pH 6.5), prepared by mixing X (0.1M Na H_2 PO_4 2 H_2O) and Y (0.1M Na_2HPO_4) stocks in the ratio 68:5:31.5 and adding an equal amount of distilled water; 4 ml of 1% stock of α-naphthyl acetate in 50% acetone was then stirred in, and the mixture poured over the gel which was then incubated at 37°C for 10-20 minutes.

Transferrin

Buffer - .05M TM pH 7.6-7.8
6.05 gm tris/l
0.2 gm magnesium chloride/l
Adjusted to pH 7.8 with Fluka maleic acid.

Running conditions:
1-1½ hr, 20-30 mA per gel.

Stain:
As for starch; stained for 1-5 minutes then destained

in a 50 methanol:50 distilled water:10 glacial acetic acid wash, usually for 1-2 hours.

As a control, 5-10% of samples were routinely retyped, and typings checked after lengthy periods in storage. In neither case were discrepancies discovered.

Phenotypes

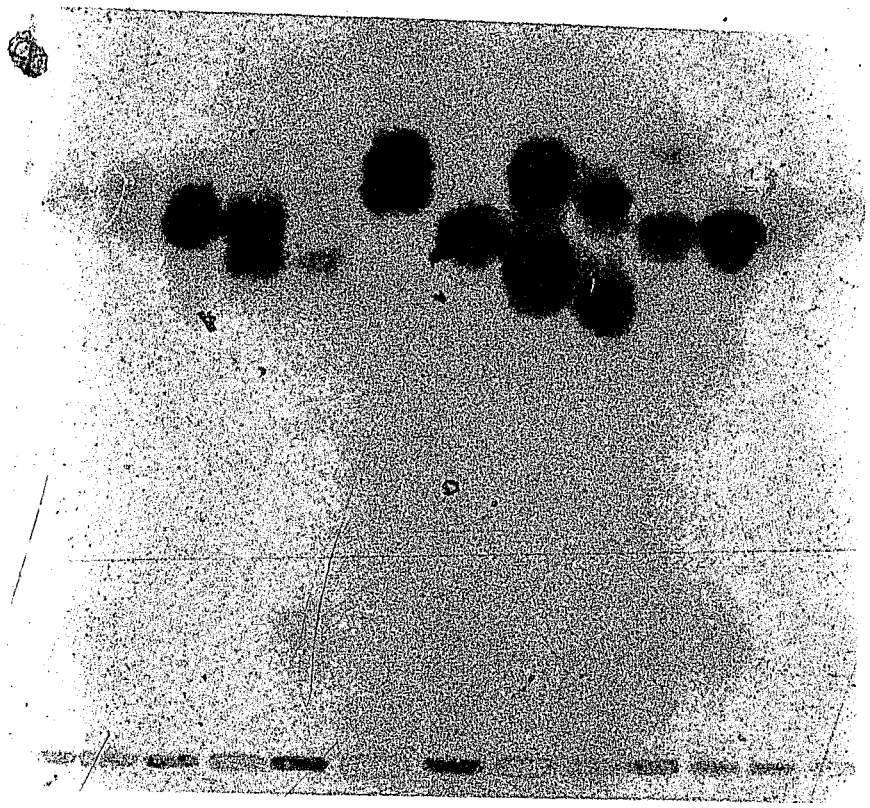
Serum Esterase

In addition to the three alleles and six phenotypes described by Fujino and Kang (1968a), a fourth allele was frequently observed. Although the fastest of the alleles, it has been designated 4. The additional heterozygotes 1-4, 2-4 and 3-4 were all observed. Sharp (MS) also reported this allele and relabelled the alleles 1-4 in order of decreasing anodal mobility. The nomenclature (E_{SJ}) and superscripts used by Fujino are now so well established in the literature that their usage has been retained although his nomenclature does not follow established procedure (Giblett, 1976) and is at variance with the recent practice of assigning proportional mobilities relative to a common allele mobility of 100.

Single examples of faster and slower presumed alleles, designated E_{SJ}^{6} and E_{SJ}^{5} respectively were observed. A selection of observed phenotypes grouped on a single gel is shown in Figure 4.4.

Numbers of individual samples were retyped at random, both immediately following initial typing and after lengthy periods in storage at -10 to -20°C. No discrepancies were observed. Relatively little difficulty was experienced typing material from frozen fish, thawed to enable sampling, and from skipjack kept on ice for several days after capture and hence in fair condition only. Samples from skipjack stored in brine, even for brief periods, not unexpectedly proved very difficult to type on the one occasion this was tried. Both the esterase and transferrin systems however seem robust enough to discount artifacts produced by deterioration during storage.

Null alleles appear to occur relatively often in esterase systems (e.g. Trippa *et al.*, 1978) and the possibility that such an allele occurs at the esterase locus needs to be considered. However whenever there was no esterase activity at all, as opposed to smudging or smearing, in individual



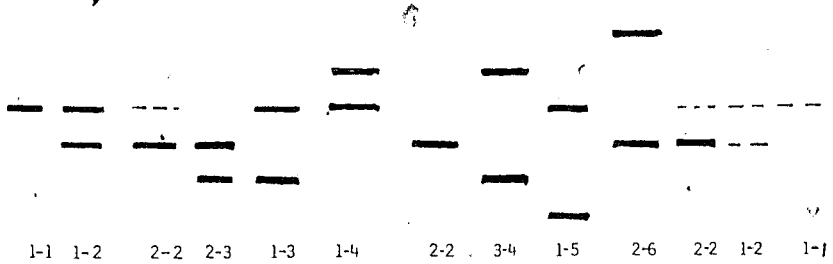


Figure 4.4. Observed skipjack serum esterase phenotypes.

samples, a similar lack of activity was observed in transferrin, which is contrary to expectations if two enzymes are encoded by different loci segregating independently. Such loss of activity can therefore be attributed to sample contamination with other tissue fluids, particularly digestive fluids in stomach, leading to loss of activity. In the absence of breeding data, this and other possibilities (post-translational modifications, switch mechanisms) cannot however be unequivocally excluded. The fact that very few samples were out of Hardy Weinberg equilibrium and that no hint of irregularity was seen in the large number of samples typed (>10,000) supports the assumption that phenotypes at both esterase and transferrin loci segregate as codominant autosomal alleles in simple Mendelian fashion. These observed phenotypes are thus regarded as expressions of underlying alleles and will hereafter be referred to as genotypes.

Both Fujino (1968b) and Richardson (pers. comm.) have established that the two loci associate randomly. They have thus been treated as independent loci in all subsequent analyses.

Serum transferrin

A fourth transferrin allele, in addition to the three reported by Fujino and Kang (1968a) and Barret and Tsuyuki (1967), was observed on one occasion. It is the slowest of the four alleles and has been designated 4.

Additional systems

The screening of nearly 60 presumptive loci in skipjack blood samples (Richardson, MS) revealed genetic polymorphism (common allele frequency <0.95) in four additional loci, ADA (adenosine deaminase, E.C.3.5.4.4.), GPI (glucose phosphate isomerase, E.C.5.3.1.9.), GDA (guanine deaminase, E.C.3.5.4.2.) and PGD (phosphogluconate dehydrogenase (decarboxylating) E.C.1.1.1.44.).

With the exception of GDA, which shows a weak longitudinal cline in the frequency of the GDA_1 allele from east (0.11) to west (0.25) (Richardson, MS), all had common allele frequencies >0.90 over a wide area and showed no consistent geographic variation, as had been the case with transferrin. Independent searches by Walker (pers. comm.) and Sharp (pers. comm.) produced similar findings, the former however reporting a GD (glucose phosphate dehydrogenase; E.C.1.1.1.49) 'polymorphism'. This has yet to be confirmed,

but variable levels of breakdown amongst samples are suspected to be the source of this variation. Turner & Cederbaum (1975) document cases of non-genetic variation in this enzyme.

As mentioned earlier, Fujino (in press) has recently described a red blood cell esterase polymorphism. This system had been partly described in a previous publication, (Fujino and Kang, 1968b). Fujino (in press - Table 2) seems to have been able to type approximately 60% of samples only and similar difficulties were experienced when the system was investigated by the author. It appears considerably less stable than the serum esterase system (E_{SJ}) and its practical application at this stage seems restricted to samples collected and analysed within a few days of capture.

A limited number of polymorphic systems are known for enzyme loci present in other tissues, but showing weak activity or not expressed in blood. McCabe *et al.*, (1970) reported a low frequency polymorphism in GPD (glycerol -3- phosphate dehydrogenase (NAD+) E.C. 1.1.1.8) and PGD and Fujino (1976) has referred to LAP (leucine ammopeptidase, E.C. 3.4.11.2 and SOD (superoxidase dismutase E.C. 1.15.1.1) polymorphisms in liver and pyloric caecae; Clegg & McCabe (*fide* Fujino, 1970) discovered a four-allele dimeric esterase polymorphism in liver, and Fujino (1970) found three phenotypes in skeletal light muscle protein which appeared to be under genetic control. The author (see later) has observed additional low level variation in MPI (mannose phosphate isomerase, E.C.5.3.1.8) ICD (isocitrate dehydrogenase (NADP+), E.C.1.1.1.42), SORDH (sorbitol dehydrogenase E.C.1.1.1.1.3) and GOT (aspartate aminotransferase E.C.2.6.1.1), and higher level variation at a rather unstable ADA locus in liver samples.

In general, the increased difficulties associated with handling tissue other than blood and the practicability of obtaining large samples from the major source, commercial catches (which are generally sold as whole, undamaged fish), have precluded use of any of these systems on the large scale necessary for them to contribute to skipjack population studies; the serum esterase and transferrin systems accordingly remain the basis of the present study.

4.2.5 Statistical procedures

Estimation of statistical differences observed in codominant allele frequencies usually employs a chi-square goodness of fit test in one of its many asymptotically equivalent forms (for example, the Z test of Walpole and

Myers (1972), the G-test or log-likelihood test of Sokal and Rohlf (1969)) rather than Fisher's maximum likelihood method (Spiess, 1977). The tests vary in their applicability to particular problems. As the G-test, for example, compounds gene and genotype frequency effects in one statistic, a simpler step-wise approach examining first allele frequencies then genotype frequencies was adopted. Presumably because of this compounding effect the step-wise approach and G statistics have produced conflicting results when applied to some of the data sets considered in this study (see later).

Individual allele frequencies can be compared by grouping all other alleles to form a single alternative class and testing for homogeneity, using a 2xN contingency table; 3xN tests can also be performed (in the case of E_{SJ}, it is necessary to combine the rare E^3_{SJ} and E^4_{SJ} alleles) to test if one of the common alleles (E^1_{SJ}, - E^2_{SJ} or Tf^2_{sj}, - Tf^3_{SJ}) covary with the rare alleles.

Gauldie and Johnston (1980) have argued that where there are likely to be differences in fitness amongst alleles, it can no longer be assumed that samples are independent and chi square tests are not appropriate. They suggest analysis of variance might be better utilized. As it is not yet feasible to partition skipjack samples by age, area of origin and other factors (see 2.4) to provide a basis for analysis of variance, this approach has not been considered in the present analyses.

The power of tests to estimate, using genotype frequencies, deviations from Hardy Weinberg expectations as individual samples of the size collected during this study (200 > n > 80) is uniformly low for the statistics commonly in use, namely F tests (Ward and Sing, 1970), G tests (Anon., 1980) and Smith's H test (Smith, 1970). Chi square-related tests have the problem of not being able to distinguish between positive and negative deviations i.e. between too many and too few heterozygotes. G statistics, in particular, have the advantage of increased degrees of freedom but the possibility of obtaining significant and possibly spurious deviations may also be increased, as deviations are squared and summed, disregarding sign.

To overcome this and related problems associated with subdivision of samples, Smith (1970) devised the statistic which now bears his name. It is calculated for a simple two allele system from the formula

$$H = \frac{4n^2 pq - (2n-1)Y}{4n(n-1)}$$

where n = sample size, p & q = gene frequencies and Y = observed number of heterozygotes. Where n > 10, the variance of H is approximately $p^2q^2/(n-\frac{1}{2})$. A deviation of twice the standard error i.e. $2\sqrt{p^2q^2/(n-\frac{1}{2})}$ is regarded as equivalent to a 95% confidence limit, and the test statistic is thus judged significant when the 95% confidence interval does not include zero. A further advantage of Smith's H is its amenability to summation in a manner more acceptable both biologically and mathematically than is the case with chi-square tests. A summed $\bar{H}$ with narrow confidence limits (due to increased sample size) is obtained without increasing the Wahlund effect.

A two allele Smith's H (combining E^2_{SJ}-E^3_{SJ}-E^4_{SJ} and Tf^1_{SJ}-Tf^3_{SJ} respectively in one class) has therefore been used to examine deviations from equilibrium both within (H) and across samples ($\bar{H}$).

One problem which potentially arises with three allele systems where two of the alleles covary positively (see later) is that an *excess* of heterozygotes involving the two covarying alleles can result from mixing the Wahlund effect), rather than the deficiency of heterozygotes expected in a two-allele system (Milkman, 1975). With a third allele frequency of < .015, as is the case here with both systems, calculations have shown that this complication does not arise (Richardson & Calaprice, pers. comm.). Mixing should therefore lead to heterozygote deficiency over the range of allele frequencies involved here.

4.3 GEOGRAPHICAL VARIATION

4.3.1 Sources of Data

The logistical problems inherent in obtaining adequate coverage of the vast area in question, the Pacific Ocean between 40°N and 40°S and the eastern Indian Ocean, are formidable. They are exacerbated by the lack of sustained commercial fishing activity, the primary source of material for genetic analysis, over much of this area (see 3.2) and the spatio-temporal fluctuations in the availability and abundance of the target species.

The problems have in part been offset by the extensive cooperation and exchange of material and unpublished information among interested

research groups and individuals. Beginning with co-ordinated sampling in Papua New Guinea, New Zealand and the eastern Tropical Pacific in 1975-76 (Sharp, MSb), this approach has since been fostered by the South Pacific Commission, (SPC) through its Skipjack Survey and Assessment Programme.

Since 1978, during its skipjack tagging cruises, the Programme has collected blood samples in many hitherto unsampled localities throughout its area of concern (Figure 4.5) as well as in the contiguous regions of New Zealand and the Australian east coast. It has funded analysis of this material and organized two workshops to bring together and evaluate all available genetic data on skipjack within the area, in combination with results of its own large scale tagging programme. As of early October 1980, when the second workshop was held, genotype numbers and gene frequencies from nearly 200 lots ($\bar{n} \sim 100$, total $n \sim 20{,}000$) were on file.

Over half of this data set consists of material from the Indo-Australian region collected and analyzed by the author (108 lots, areas 1, 2, 3, 4 - see Tables 4.1, 4.2). This is of limited value *per se* in examining geographical variation throughout the Pacific Ocean and eastern Indian Ocean and must be combined with data from other areas. The reports of the workshops (Anon, 1980, and in prep.), which integrate these data with data from other areas, and analyses of the SPC samples (Richardson, MS), especially those collected outside the Indo-Australian region, will thus be extensively referred to in the discussion which follows.* In the above analyses, the SPC area was arbitrarily divided into four regions, A (130-170°E), B (170°E - 160°W), C (160°W - 125°W) and Temperate (south of 25°S) to facilitate data manipulation and discussion (Figure 4.5). Material collected by the author within the SPC region is from areas "A" and "Temperate".

Detailed information (genotype numbers etc.) has been obtained on three relevant data sets generated prior to 1978: Ecuador_S and PNG_S (Sharp, MSb) and Palau_F (Fujino, unpublished data on file at the National Marine Fisheries Service Laboratory, Honolulu). Other samples, collected in the PNG-Solomon Is. area during 1976-77 have been included in the area A data set of Richardson. Material collected in the south-west Pacific by Fujino (1976) generally involved small lots ($n < 50$) and has accordingly not been used.

It should be pointed out that material collected since 1978 has all been from areas south of 10°N. Consequently attention will initially focus

* The author acknowledges his debt to fellow participants at these workshops for providing invaluable insights into the interpretation and analysis of these data.

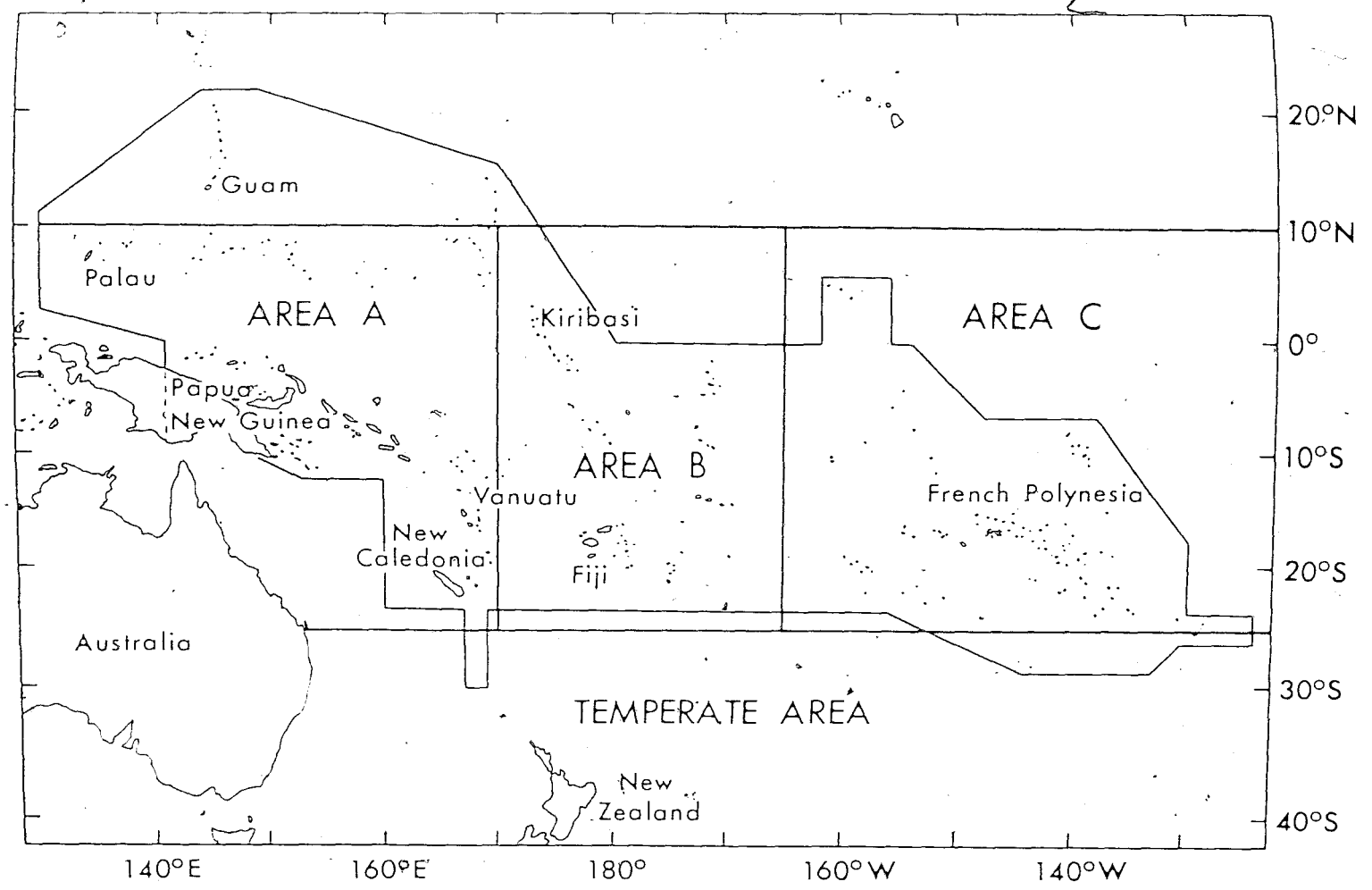


Figure 4.5 Sampling regions within the South Pacific Commission area, the limits of which are clearly marked.

on the equatorial and southern Pacific and the eastern Indian Ocean, still an enormous area of ocean.

In attempting to draw out working hypotheses from the data it has generally proved necessary to group sample lots both within some rather broad areas and across potentially disparate time strata and size classes. Analysis of the time series samples should later provide some indication of whether interpretations derived on this basis need qualification.

For the 54 lots collected in the Indo-Australian area (i.e. excluding the time series samples), sample size, mean length, allele proportions, genotype frequencies and Smith's H values for esterase and transferrin systems are given in Tables 4.3 and 4.4. For material collected by other workers, summaries published or in press rather than raw data have been referred to.

A summary of basic analyses by the various geographical groupings is given in Table 4.5. These groupings are distributed as follows:

Western Tropical Pacific	Area 1a	- PNG archipelagic waters
	1b	- north of PNG
	PNG_S	- general PNG area
		- $10^{\circ}N$ - $25^{\circ}S$,
	A	- $130^{\circ}E$ - $170^{\circ}E$
	$Palau_F$	- $\sim 8^{\circ}N$ $138^{\circ}E$
Western Temperate Pacific	2	- Australian east coast
	Temp.	- S.E. Australia and N.Z.
Tropical West-central Pacific	B	
Tropical Central Pacific	C	
Tropical Eastern Pacific	$Ecuador_S$	
Indonesia & tropical East Indian Ocean	3	
Temperate East Indian Ocean	4	

Subscripts - F - collected and analyzed by K. Fujino
S - collected and analyzed by G. Sharp.

Table 4.3 Serum esterase allele proportions & genotype frequencies in Indo-Australian area samples.

The sample size (n) mean length of skipjack in the sample ($\bar{\ell}$) & two allele Smith's H value are also given.

An asterisk indicates sample significantly out of equilibrium.

			Allele proportions			Genotype frequencies							
Sample	n	$\bar{\ell}$	1	2	3/4	11	12	13	14	22	23	24	33	Smith's H
Area 1a														
PG	114	55	.781	.215	.004	67	43	1		3				-.021
PE	52	56	.817	.183	-	36	13			3				.026
PA	91	49	.813	.187	-	59	30			2				-.012
SA	163	60	.797	.200	.003	106	48			8	1			.015
SB	106	49	.722	.269	.009	54	45			5	2			-.011
SD	114	50	.75	.237	.013	64	41	1	1	6	1			-.003
CB	81	49	.75	.25		43	34		1	3				-.024
CB2	96	51	.755	.234	.01	55	33	2		6				.004
ARA	81	48	.728	.271		43	32			6				.002
AM	107	51	.672	.313	.014	45	41	2	1	8				.031
AN	109	54	.697	.298	.005	55	41	1		12				.019
MAS-A	105	52	.652	.323	.023	44	48	1		18	1	3		-.005
Area 1b														
DS	61	52	.622	.360	.016	24	27	1		8		1		.007
DT	102	53	.622	.358	.019	38	50		1	10		3		.014
VGA	95	53	.654	.31	.005	40	50			4		1		-.046*
VGB	100	53	.69	.30	.01	50	37	1		11		1		.025
HME	102	-	.627	.348	.025	42	41	3		14	1	1		.019
WA	96	52	.614	.359	.026	32	49	3	2	10				-.043
WB	99	52	.661	.323	.015	48	33	2		15		1		.048*
WAC	87	52	.741	.247	.011	47	34	1		4	1			-.008
Area 2														
CA	56	47	.652	.339	.009	23	26	1		6				-.012
CC	64	49	.726	.258	.015	33	26	1	1	3				-.011
CD	68	53	.742	.25	.007	38	24	1		5				.009

CF	48	0	.635	.364		18	25				5				-.027
CG	55	47	.755	.236	.009	30	22	1			2				-.022
CH	71	49	.706	.197	.007	44	24	1			2				.012
CI	94	53	.777	.218	.005	57	31	1			5				.004
CJ	59	51	.780	.203	.016	34	22	1	1		1				-.030
CK	127	53	.795	.204		78	46				3				-.018
CL	53	51	.679	.311	.009	22	28				2	1			-.044
CM	51	-	.774	.225		31	17				3				.010
CO	64	57	.554	.445		16	39				9				-.056
CP	47	(53)	.606	.361	.032	18	20		1		6	2			.018
CQ	98	56	.617	.362	.020	38	43	2			13	2			.008
KAF	87	45	.661	.327	.011	38	39				8	1	1		.001
KAG	144	46	.712	.288		74	57				13				.008
KAH	101	46	.574	.410	.014	33	49	1			16		2		-.002
KAJ	109	64	.692	.293	.014	53	43				10		1		.007
KAK	98	48	.663	.337		42	46				10				-.010
KAL	110	48	.673	.309	.018	53	40	2			13	2			.030
TA	119	47	.613	.382	.004	41	64				13		1		-.030
NS	47	47	.596	.393	.011	16	23	1			7				-.012
SE	99	48	.565	.429	.005	31	49	1			18				-.006
FA	92	50	.668	.315	.016	42	36	2		1	11				.011
FB	64	48	.609	.375	.015	25	27	1			1	1			.021
Area 3															
BC	111	49	.801	.189	.009	70	37	-	1		2		1		-.011
BD	101	48	.767	.212	.020	58	36	1	2		3		1		-.014
B MIX	113	49	.80	.185	.013	72	34	1	2		4				-.004
YY	105	43	.852	.133	.014	75	26	2	1		1				-.012
YZ	119	(42)	.861	.126	.012	88	26	1	2		2				-.002
1A	91	53	.857	.138	.005	67	22				1		1		.002
1F	149	54	.822	.171	.007	103	38	1			6	1			.015
Area 4															
1C	139	(51/54)	.838	.151	.010	97	36	2	1		3				-.004
1D	72	55	.854	.132	.014	52	17		2		1				.007

Table 4.4 Transferrin allele proportions & genotype frequencies.

The sample size (n) mean length of skipjack in the sample ($\bar{\ell}$) & two allele Smith's H value are also given.

An asterisk indicates sample significantly out of equilibrium.

Sample	n	$\bar{\ell}$	Allele proportions 1	2	3	Genotype frequencies 22	23	12	33	13	11	Smith's H
Area 1a												
PG	107	55		.719	.281	56	12		9			.006
PE	43	56		.72	.28	22	18		3			-.006
PA	88	49		.795	.205	55	30		3			-.007
SA	163	60		.678	.322	76	69		18			.007
SB	106	49	.006	.66	.334	45	49		11	1		-.010
SD	113	50		.69	.31	55	46		12			.011
	78	49		.603	.397	29	36		13			.010
	92	51		.717	.283	47	38		7			-.003
ARA	80	48	.012	.663	.325	33	38	2	7			-.025
AM	107	51	.005	.752	.243	61	38	1	7			.005
AN	109	54		.693	.307	52	47		10			-.002
MAS-A	105	52	.005	.714	.281	57	36		11	1		.034
Area 1b												
DS	61	52	.008	.606	.385	19	35	1	6			-.055
DT	101	53	.005	.713	.282	49	45	1	6			-.022
VGA	95	53	.005	.732	.263	51	36	1	7			.003
VGB		53			NO DATA							
HME	102	-	.005	.701	.294	50	43		8	1		0
WA	94	52	.005	.676	.319	44	39		10	1		.013
WB	99	52		.758	.242	58	34		7			.013
WAC	87	52		.695	.305	45	31		11			.035
CA	55	47		.772	.228	32	21		2			-.013
CC	61	49	.008	.680	.311	25	32	1	3			-.051
CD	67	53	.014	.701	.284	32	20	1	4	1		-.013
CF	48			.74	.26	27	17		4			.017
CG	53	47	.018	.66	.321	25	20		6	2		.038

CH	69	(49)		.725	.275	35	30		4		-.016
CI	93	53	.005	.677	.317	41	44		7	1	-.017
CJ	58	51		.776	.224	36	18		4		.020
CK	126	53	.012	.706	.282	60	56	2	7	1	-.022
CL	53	51	.009	.67	.321	23	25		4	1	-.012
CM	50	47/52	.02	.75	.23	29	15	2	4		.019
CO	59	57		.627	.373	24	36		9		.015
CP	46	(53)		.652	.348	19	22		5		-.010
CQ	91	56		.758	.242	48	42		1		-.046*
KAF	88	45		.722	.278	43	41		4		-.031
KAG	139	46	.004	.712	.284	70	57	1	11		-.003
KAH	99	46		.712	.288	45	51		3		-.051*
KAJ	109	64	.010	.665	.325	49	46	1	12	1	.009
KAK	98	48		.633	.367	41	42		15		.019
KAL	108	48	.005	.699	.296	51	48	1	8		-.015
TA	119	47	.004	.743	.252	66	44	1	8		-.002
NS	47	47	.010	.723	.266	26	15	1	5		.032
SE	99	48	.010	.657	.333	41	47	1	9	1	-.015
FA	91	50	.016	.681	.302	40	42	2	6	1	-.023
FB	66	48	.008	.652	.340	27	32		6	1	-.013
Area 3											
BC	110		.009	.709	.282	54	47	1	7	1	-.011
BD	99		.010	.656	.333	41	47	1	9	1	-.015
B MIX	112		.009	.652	.339	50	44	2	16		.022
YY	104			.678	.322	48	45*		11		.003
YZ	118			.699	.301	59	47		12		.012
1A	91		.016	.665	.319	40	40	1	8	2	0
IF	148		.007	.699	.293	75	56	1	15	1	.018
Area 4											
1C	139		.01	.701	.288	71	52	1	13	2	.019
1D	72			.694	.292	37	25	1	8	1	0

Area	E^1_{SJ} $\hat{p}$	Lots	n	Homogeneity	$\bar{H}$	Tf^2_{SJ} $\hat{p}$	Lots	n	Homogeneity	$\bar{H}$
1a	.743	12	1219	$\chi^2_{11} = 32.33^*$	-.0033	.699	12	1191	$\chi^2_{11} = 22.09^*$	.003
1b	.660	8	742	$\chi^2_7 = 10.92$	-.0014	.702	7†	639	$\chi^2_6 = 9.82$	.0004
2	.677	25	2025	$\chi^2_{24} = 99.23^*$	-.005	.699	25	1992	$\chi^2_{24} = 29.3$	-.010
3	.823	7	789	$\chi^2_6 = 10.86$	-.003	.688	7	782	$\chi^2_6 = 6.62$	.005
4	.843	2	211	$\chi^2_1 = 0.18$	-.005	.699	2	211	$\chi^2_1 = 0.02$	
A	.673	32	3249	$\chi^2_{31} = 107.4^*$	.012*	.696	32	2918	$\chi^2_{31} = 30.6$	.0026
B	.547	20	2267	$\chi^2_{19} = 46.5^*$	.0071	.720	20	2040	$\chi^2_{19} = 23.3$	.0056
C	.427	11	1011	$\chi^2_{10} = 10.2$	.0039	.689	11	999	$\chi^2_{10} = 10.2$	.0023
PNG_S	.655	14	2302	$\chi^2_{13} = 30.99^*$	.002	.695	14	2299	$\chi^2_{13} = 18.27$	-.002
$Ecuador_S$	.435	8	1591	$\chi^2_7 = 26.12^*$	.003	.698	8	1585	$\chi^2_7 = 22.28^*$	-.0084*
$Palau_F$	.679	21	1604	$\chi^2_{20} = 27.34$	.0105*	.691	21	1495	$\chi^2_{20} = 19.23$	-.0108

†Many samples within one lot not typeable, so this lot not included.

Table 4.5

Results of basic statistical analyses by area groupings for Esterase and Transferrin systems.

Mean gene frequency ($\hat{p}$), sample numbers (lots & total number), homogeneity test values and mean Smith's H values are shown for each of the areas defined in the text. Significant departures from homogeneity or equilibrium are indicated by an asterisk ($P < .05$)

Stain:

As for starch; stained for 1-5 minutes then destained

4.3.2 Distribution of Genes

Esterase

The E^3_{SJ} and E^4_{SJ} alleles occur in all areas at low frequencies, as can be seen in Table 4.6. Tests of homogeneity, performed on allele numbers in these area groupings rather than between lots because of the low numbers per lot in some genotype classes, show E^3_{SJ} frequencies amongst areas to be homogenous ($\chi^2_{10} = 10.0$, $P \sim 0.40$) and E^4_{SJ} frequencies to be heterogenous ($\chi^2_{10} = 30.1$, $P < .005$). As pointed out previously, there is a danger with such large sample numbers (total n = 32 198) that trivial differences of no biological significance may assume statistical significance. This may be the case here, where E^4_{SJ} frequencies vary between .001 and .007. Another possibility is that 1-4 genotypes have been identified in some cases as 1-1 homozygotes with a stronger than usual forward breakdown band (Figure 4.4). This problem does not arise with the 1-3, 2-3 and 2-4 genotypes. That this occurs to some extent is presumably why Fujino (1970, 1972, 1976, 1979) has not recorded the E^4_{SJ} allele in over 20,000 samples. It thus seems reasonable to assume that the observed heterogeneity in E^4_{SJ} frequencies has no biological significance.

The frequencies of the E^1_{SJ} and E^2_{SJ} alleles covary negatively. As the E^1_{SJ} allele is generally the more common, it will form the basis of discussion. E^1_{SJ} frequencies within individual samples collected in the SPC area range from 0.82 to 0.37, and $\hat{p}$ (combined estimates of the frequency) values within areas A, B and C were respectively .673 (n = 6498), .547 (n = 5434); 427 (n = 2022) (Table 4.5). Without making any *a priori* assumptions about the geographical distribution of E^1_{SJ} frequencies, a linear regression of E^1_{SJ} frequency against longitude (coded as negative degrees west of 180° and positive degrees east of 180°) was plotted as a first step. Frequencies were not transformed, since over the range involved here (.35 - .85), transformation has negligible effect (Cox, 1970). The basic relationship derived from the 98 samples available at that time, namely $E^1_{SJ} = .5576 - .0035(L)$ where L = longitude coded as above, accounted for 61% of mean square deviations from the mean gene frequency (Anon, 1980).

Collection of additional material within this area (130°E - 130°W) has confirmed the fit of the regression ($r^2 = 0.81$) without significantly altering the slope of the line.

Esterase

Area	1a	1b	PNG_S	2	3	4	A	B	C	Temp	$Ecuador_S$	Total
2n	2438	1484	4604	4050	1578	422	6529	4534	2022	1386	3182	32,198
E^3_{SJ} No.	16	12	36	26	8	2	62	42	21	9	32	266
E^3_{SJ} Freq.	.007	.008	.008	.006	.005	.005	.014	.009	.010	.006	.010	.008
E^4_{SJ} No.	3	12	32	11	10	3	22	13	15	7	18	146
E^4_{SJ} Freq.	.001	.008	.007	.003	.006	.007	.003	.003	.007	.005	.006	.005

Transferrin

Area	1a	1b	PNG_S	2	3	4	A	B	C	Temp	$Ecuador_S$	$Palau_F$	Total
2n	2382	1278	4598	3984	1564	422	5835	4080	1998	1422	3170	2990	33,723
Tf^1_{SJ} No.	5	5	42	24	11	5					42	19	
Tf^1_{SJ} Freq.	.002	.004	.009	.006	.007	.012	.008	.009	.008	.002	.013	.006	

Table 4.6 Geographical Distribution of rare alleles.

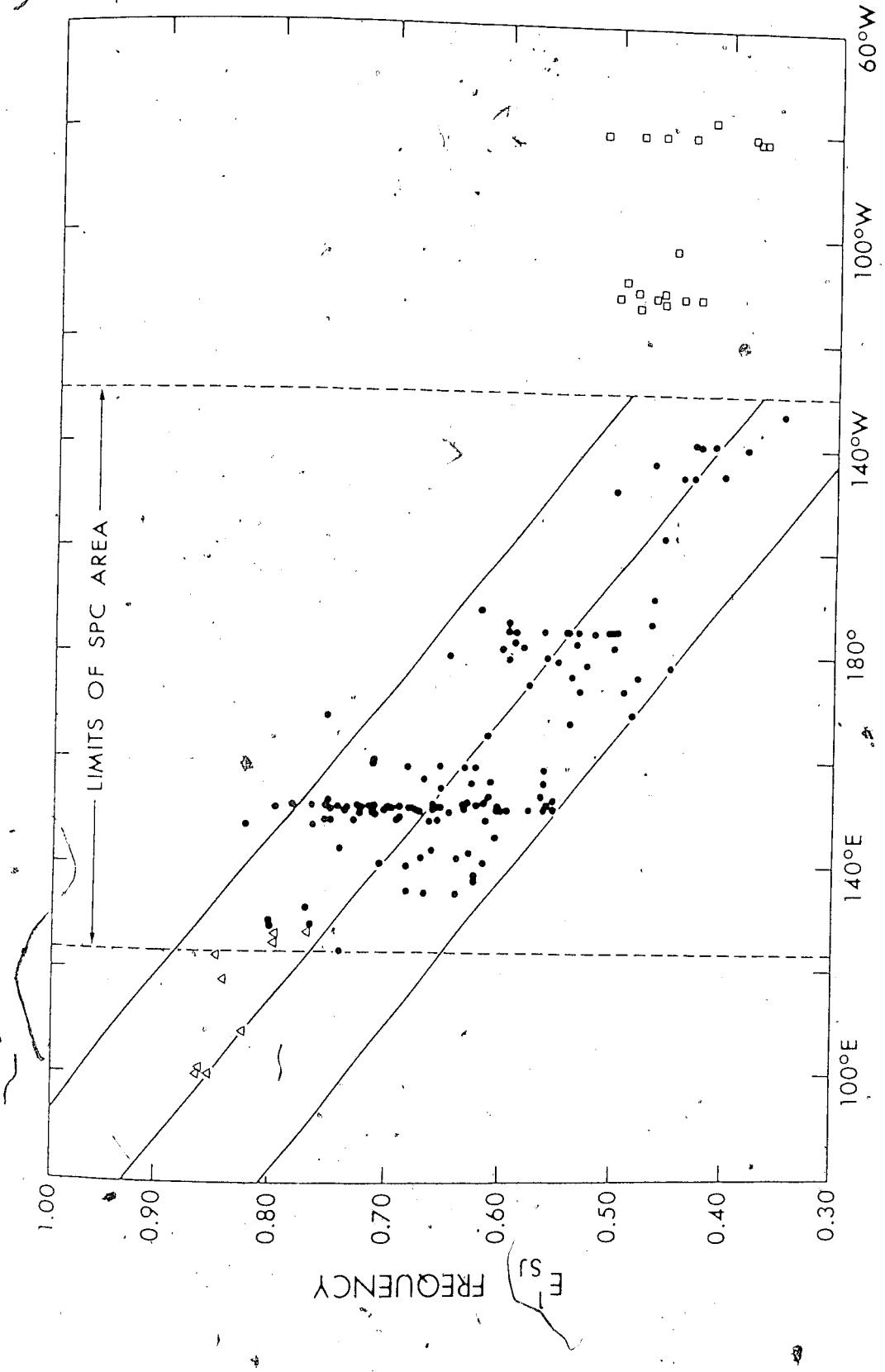
(The data set $Palau_F$ has been excluded for esterase since the E^4_{SJ} allele was not recognized.)

Figure 4.6 Regression of E^1_{SJ} frequency against longitude in the South Pacific Commission area.

This has been extrapolated westwards to include Indonesian and Western Australian material (Δ). Prediction limits (95%) are shown, as are samples collected in the eastern tropical Pacific (□).

This figure is an expansion and modification of a figure compiled at the second SPC workshop (Anon., in prep.).

many asymptotically equivalent forms (for example, the Z test of Walpole and



Material collected to the west of 130°E by the author at three sites in Indonesia (samples BC, BD, Bmix, YY, YZ, 1A and 1F - see Table 4.1 for details) and two sites in Western Australia (1C and 1D) has yielded E^1_{SJ} frequencies (.77 - .86) which lie on the westward extrapolation of the previously fitted regression. These are superimposed on Figure 4.6 and strongly suggest the clinal relationship holds into the eastern Indian Ocean. No material has yet been collected west of 100°E to determine the westward extent of this phenomenon.

Gene frequencies east of the SPC area, i.e. east of 130°W, from the Mexican and Ecuador fisheries (Sharp, MS) are similar to those observed in area C viz $\hat{p} = 0.427$ (n = 2022). This levelling-off of the clinal relationship is consistent with observations that little spawning occurs east of 130°W and might also prove to be consistent with the hypothesis that skipjack exploited in the Mexican and Ecuador fisheries in the eastern tropical Pacific are of central Pacific origin (see 2.3).

Although it would clearly be desirable to have more material, particularly from areas 160°-170°E and 170°W-160°W, the clinal relationship does seem to be a real feature of the data set, with the following attributes:

(1) it appears stable, in time, or at least in a dynamic steady state, over the relatively short period for which data is available. Most data is from the Papua New Guinea area, where mean E^1_{SJ} frequencies have shown no appreciable change between 1975 and 1980. Material collected in Palau (8°N, 135°E) and Tahiti (18°S, 150°W) by Fujino during 1966-67 ($Palau_F$, 21 lots, 1604 fish, $\hat{p} = 0.679$; Tahiti (16 lots, 627 fish, $\hat{p} \sim 0.45$) yielded similar frequencies to those during 1978-79-80 (Palau, 3 lots, 0.639 - 0.684; Area C, 11 lots, $\hat{p} \sim 0.427$).

(2) the variance in E^1_{SJ} frequency at any given longitude is wide. For example, at 150°E the mean E^1_{SJ} frequency (0.66) has 95% confidence limits of ±0.115. The variance expected about this mean with sample sizes of approximately 100 are ±.07, so the observed variance is nearly twice the expected value. Associated with this, χ^2 homogeneity tests (2xN, 3xN) show esterase frequencies within many geographical groupings particularly where the number of lots exceeds 8, to be highly heterogeneous (Table 4.5). The time series sampling

was initiated with the aim of resolving this variance into components and will be considered in Section 4.4.

(3) Latitude effects appear to account for a small amount of the variance observed at a given longitude.
When samples from higher latitudes (> 25°S, where little or no spawning occurs) are removed, the fit of the regression line is only marginally improved. However Richardson (pers. comm.) has shown that subdivision of the data set relative to 5°N, the approximate position of the Thermal Equator and Equatorial Counter Current, enables two clines to be fitted (Figure 4.7). Equivalent slopes are produced, but at any given longitude, the northern cline has a mean E^1_{SJ} frequency approximately 0.06 less than its southern counterpart. The number of samples involved in this northern area is relatively small, further collection is required to verify this interesting preliminary finding.

(4) Inspection of Figure 4.6 suggests that the variance about the regression may decrease eastwards.
The relatively small number of samples taken east of 160°W preclude confirmation of this observation at this stage. Most were taken during the same three month period in two separate years, which may lower the expected variance if there are seasonal effects.

(5) Gaps in the cline at around 165°E and 165°W appear to reflect lack of sampling opportunities rather than discontinuities in skipjack distribution, and there seems little reason to doubt the continuity of the cline between 100°E and 130°W at this stage.

Examination of possible mechanisms maintaining this cline will be deferred until gene and genotype distributions of both variable systems have been described, as will discussion of various hypotheses which might explain the distribution of esterase frequencies.

Transferrin

The Tf^1_{SJ} allele occurred in all areas at low frequencies, typically <.01, as was the case with the E^3_{SJ} and E^4_{SJ} alleles (Table 4.6). A test of homogeneity (2x12) showed that the frequency may be heterogeneous amongst the various geographical groupings. ($\chi^2_{11} = 36.6$, $P < .005$). This is attributable to the relatively high frequency of the allele in samples from

*The author acknowledges his debt to fellow participants at these workshops for providing invaluable insights into the interpretation and analysis of these data.

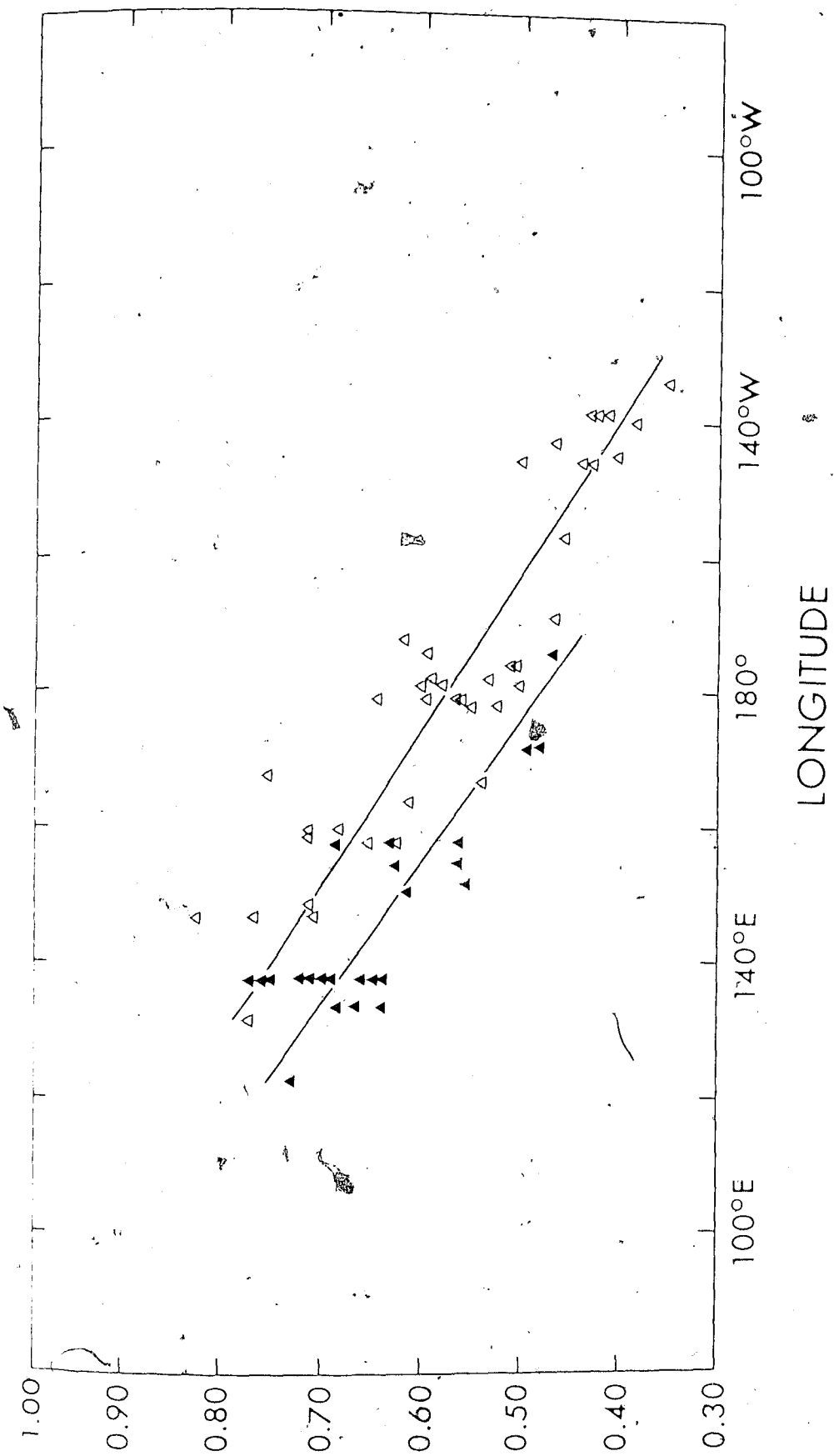


Figure 4.7 Regression of E^1_{SJ} against longitude for samples taken south of 5°N (Δ) and north of 5°N (▲) respectively.

Redrawn from Richardson (MS).

Ecuador and low frequency in groups 1a and Temperate. Again, its significance may be trivial, although the range of within-area frequencies is greater than for E^3_{SJ} and E^4_{SJ} namely .002 - .013 compared with .005 - .014 and .001 - .008.

The frequency of the Tf^2_{SJ} allele did not vary amongst groups ($\chi^2_{11} = 11.7$, $P \sim 0.40$), although two of the groups ($1a_J$, $Ecuador_S$) showed internal heterogeneity. A non-significant regression against longitude shows transferrin gene frequencies to be relatively constant across the area (Anon, 1980).

4.3.3 Distribution of genotypes

Esterase

Of the 158 lots included in this analysis, only four were significantly out of equilibrium on the basis of two allele (E^2_{SJ}, E^3_{SJ} and E^4_{SJ} alleles were combined) Smith's H tests. Three of these deviated in a positive direction and one in a negative direction. Given the size of the individual tests and their number, four significant results are expected by chance alone.

The Smith's H value summed over the total data set, though positive (H = 0.0008) is not significantly out of equilibrium. Two of the geographical groupings, $Palau_F$ and Area A, showed significant positive deviations i.e. a deficiency of heterozygotes (Table 4.5). In the former case ($Palau_F$) this may be partly attributable to non-recognition of the 4 allele by the investigator as noted earlier, leading to an underestimate of heterozygotes, but this effect is likely to be minor.

The large sample numbers involved leave little doubt that the approximation to equilibrium is a general effect, despite the possible action of two forces promoting heterozygote deficiency (positive H), namely potential sibship within schools and the sampling bias towards large feeding aggregations in which genetic heterogeneity may be increased. In addition, lots have been grouped across time-area strata in most cases.

It may be that opposing forces such as heterosis cancel out such effects in most areas. It is interesting to note that the significant deviations observed were generally in the positive direction (3 out of 4 individual schools, and both area groupings).

The area A result, based on a large sample (2n = 6498), may be related to the wider variance in E^1_{SJ} (and E^2_{SJ}) frequencies at the western end of the cline; increased Wahlund effects could be expected to result from mixing of groups associated with this wide spread in gene frequencies. Combining area 1a and 1b results to produce a comparable data set did not, however, produce a similar result ($\bar{H}$ = -.0001 (NS); $\hat{p}$ = .711, 2n = 3922). The area A samples cover a wider area and analysis of the time series data at one locality within these areas may give some indication of how much of the observed significance can be attributed to area-grouping.

Transferrin

Ten lots showed significant deviations from Hardy Weinberg expectations, six in the negative direction (i.e. heterozygote excess) and four positive. As with esterase, the total Smith's H value was not significantly out of equilibrium, but three areas (2, Ecuador$_S$ and Palau$_F$) showed significant heterozygote excess. Although this is generally consistent with Fujino and Kang's (1968a) findings, observed/expected ratios of the 2-3 genotype (around 1.05 in each of these three areas) were lower than those recorded by them in two other areas, the Eastern Pacific 1.24 (n = 175) and Japan 1.11 (n = 401). Their analyses were, however, based on rather small sample sizes. Sharp (MSb) found slight, though not significant heterozygote excess (O/E 1.00 - 1.02) in his samples, in keeping with the ratio observed in the present study.

Fujino and Kang (1968a), assuming that overdominance of the 2-3 genotype was a general phenomenon, postulated that differential fertility and viability of genotypes act to produce this balanced polymorphism, and their study has been widely quoted as one of the few examples of heterozygote advantage known from natural populations. With the benefit of additional sampling, it now appears that the extent to which heterozygote excess occurs has been overestimated and that whatever selective forces are operating, they tend to maintain an equilibrium situation with regard to Hardy Weinberg expectations.

The tendency to overdominance in some areas may be related to the role of transferrin since, associated with its function as an iron-binding protein, transferrin has been implicated in resistance to infectious diseases (Bussman, 1974). Iron enhances the growth and virulence of invading microorganisms, whereas transferrin limits the amount of available endogenous

Temperate East Indian Ocean 4

Subscripts - F - collected and analyzed by K. Fujino
S - collected and analyzed by G. Sharp.

iron by binding it, thus rendering it unavailable for bacterial use (Weinberg, 1974). Suzumoto *et al.* (1977) have recently demonstrated differential resistance to bacterial kidney disease amongst transferrin phenotypes in coho salmon (*Oncorhynchus kisutch*).

It is therefore possible that both the distribution of genes and genotypes essentially reflects the present and recent history of pathogen distribution and abundance

If this interpretation is correct, the Tf_{SJ} system would provide little guide to population subdivision. Selection for a particular heterozygote would also have the effect of flattening the slope of any cline in allele frequency which might develop (Endler, 1977 and see later).

Before leaving discussion of genotype distributions, some further consideration of two points is warranted - inbreeding (or more specifically the degree of sibship within aggregations) and differences in interpretation engendered by the use of different statistical tests.

As seen in section 2.3.6, direct evidence of any temporal continuity within particular aggregations or parts thereof is not available. Using indirect evidence of two kinds, distinctive morphometric variance patterns within some single-aggregation samples and clumping of rare alleles by fish size within certain samples, Sharp (MSb) has concluded that there is good evidence for some degree of temporal cohesion within "schools". It is difficult to comment on the morphometric studies, as a variety of effects, such as allometry and individual measuring bias (the data were collected by various workers) can contribute to such variation. The analysis of rare allele clumping, which involved selecting schools with large numbers of rare alleles, then examining the probability of such an occurrence, can be criticised on statistical grounds. Richardson (MS) has therefore examined the distribution of rare E_{SJ} and Tf_{SJ} alleles within a large number of schools (> 100) with $n \geq 100$, and found their occurrence to fit a Poisson distribution, i.e. the clumping occurred no more frequently than expected for such rare events.

Table 4.7 shows the analysis of two data sets, $Ecuador_S$ and PNG_S, using both

(i) the G statistics (G_p, G_H, G_T; these are as calculated by Sharp and equivalent to his Hardy Weinberg G, heterogeneity G,

Table 4.7 Analysis of heterogeneity with two data sets, using both the step-wise χ^2 - Smith's H approach and G-statistics.

The G-Statistic values are as reported in Sharp (MSb). χ^2 and $\bar{H}$ values were calculated by the author from the raw data with slight modification. His sample ZP-C, collected near the Philippines, was not included in PNG_S, and the ten samples collected from north of the Equator in his "Eastern Pacific Ocean" groupings were removed to generate $Ecuador_S$. An asterisk indicates significant deviation from equilibrium ($P < .05$).

	Esterase		Transferrin	
$Ecuador_S$				
$\chi^2_7 = 26.12$*	$G_H = 43.22(7)$*	$\chi^2_7 = 22.28$*	$G_H = 33.49(7)$*
$\bar{H} = .003$	$G_P = 5.36(8)$	$\bar{H} = -.0084$*	$G_P = 8.08(8)$
	$G_T = 48.58(15)$*		$G_T = 41.57(15)$*
PNG_S			
$\chi^2_{13} = 30.99$*	$G_H = 68.03(12)$*	$\chi^2_7 = 18.27$	$G_H = 32.18(12)$*
$\bar{H} = .002$	$G_P = 12.69$	$\bar{H} = -.002$	$G_P = 25.92(13)$*
	$G_T = 80.72(25)$*		$G_T = 58.10(25)$*

and pooled G respectively) as described in Sokal and Rohlf (1969) and

(ii) the stepwise tests of independence/Smith's H approach. The esterase data show no major discrepancies; the transferrin data, however, offer a different perspective. In both data sets, opposite conclusions are reached with respect to goodness of fit to Hardy Weinberg expectations; in the PNG_S set, the step-wise analysis reveals no heterogeneity in gene or genotype frequency, in contrast to the G tests which reveal heterogeneity at all levels. This problem seems to arise because the G-tests used compound both kinds of deviations, and although providing a sensitive test of "heterogeneity", do not identify the sources of that heterogeneity. This is important, as deviations from Hardy Weinberg expectations and from a $\hat{p}$ value are not necessarily correlated and in fact are independent, biologically meaningful variables.

4.3.4 Interpretation of observed variation

Analysis of the genetic variation observed in transferrin, ADA and GPI systems using standard electrophoretic techniques has revealed no evidence of systematic geographical variation in gene or genotype frequencies. This appears to be true of the rare esterase alleles, E^4_{SJ} and E^3_{SJ}, and the various blood groups (see Section 4.2) as well. As indicated previously, this does not imply an absence of structuring or close genetic relationship but rather that these systems provide little information for detecting such differentiation.

A weak geographical trend was evident in GDA_1/GDA_2 frequencies across areas A, B, C, although frequency differences between areas B and C were not significant (Richardson, MS). Departures from Hardy Weinberg expectations (heterozygote deficiency) were observed in areas A, B and Temperate genotypes but not in area C genotypes. Even though the number of samples involved (n = 4245) is less than for esterase and transferrin, it is nevertheless quite large. That similar events, namely geographical trend in gene frequency and heterozygote deficiency in some geographical groupings, seem to be occurring to some degree at both this and the esterase locus may prove to have some significance.

The most obvious component of observed genetic variation is the cline in E^1_{SJ} (and E^2_{SJ} frequencies) over a distance of 12,000 km (100^{o}E - 130^{o}W) within the latitudes 10^{o}N to 25^{o}S.

It could be argued that the smooth continuous cline, as it stands, is an artifact of the data for several reasons.

(i) the relatively small lot size ($\bar{n} \sim 100$) and opportunistic sampling regime has given rise, by chance, to a pseudo-clinal pattern with wide variance at any given locality. This is doubtful for two reasons. Firstly, collection of additional independent data has both improved the fit of the relationship and extended it geographically. Secondly, whilst the small sample size no doubt leads to a large variance at a given longitude relative to large samples, as expected from the binomial distribution, the mean value ($\hat{p}$) should not be affected, provided a reasonable number of sample lots are available.

(ii) As the data are not distributed evenly across the cline, it may not be continuous and collection of additional data may reveal "steps" in the cline. This remains a real possibility, although with the collection of each new sample lot at longitudes not previously represented, this possibility has become less likely.

It should be reiterated that the cline as proposed does not refer to the entire Pacific and Indian Oceans, but rather the area 100^{o}E - 130^{o}W, 10^{o}N - 25^{o}S. (Data outside that area, particularly north of 10^{o}N, is much more discontinuous, but will be examined in due course.) There seems little reason to doubt the validity of the clinal relationship within this area, particularly as most of the better known and oft-quoted clines in the taxonomic literature are based on considerably less data. Discussion will therefore proceed on the basis that the cline is real and probably in some form of dynamic stability.

Endler (1977) lists four sets of conditions which may favour the development of clines:

(1) chance differentiation among continuous groups of populations by drift, random dispersal effect, recurrent mutations in some localities or combinations of these factors,

(2) secondary contact of populations which have differentiated in isolation, either adaptively or by chance,

(3) adaptive differentiation amongst continuous groups of populations distributed along environmental gradients, and

(4) adaptive differentiation among continuous groups of populations distributed across abrupt spatial changes in environment.

These conditions refer basically to stable clines, but clines can also exist as ephemeral figures. The diffusion of a favourable allele throughout a population (Fisher, 1937) or the formation and subsequent movement of demes within a structured population (Ward and Neel, 1976) provide examples of this. In the absence of long term historical data, there is little point in considering such possibilities; the very limited short term data available point to some measure of stability.

Endler (1977) points out that interpretation of a natural cline is impossible without knowing the geography of absolute survival values and the extent of gene flow. For a species such as skipjack, adequate knowledge of these and other important parameters may never become available and a more constructive approach holds that alternative explanations should be considered and the most likely of these adopted as a working hypothesis which can be subject to specific test and modified or rejected as additional data comes to hand. The available data will thus be related to Endler's four conditions or models.

Condition 1 (chance differentiation)

Although simulations by Endler (1977) have suggested that stochastic influences may produce long lasting clines (but not stable stepped clines), several factors mitigate against such processes being important here.

(a) the species is abundant and widely distributed. With a standing Pacific skipjack biomass of at least 10^6 tonnes, a total N for the Pacific Ocean alone might approach 10^{10}. Regardless of the amount of population structuring, N_es are likely to be large, thus greatly reducing opportunities for drift, which has little effect where N_e [illegible]^4 (Chakraborty and Nei, 1977). In any case, Maynard Smith (1970) has argued that the genetic similarity of populations relative to the extent of differentiation within a population is independent of population size and that hence drift can be ruled out as a cause of differentiation and cline formation.

(b) The slope of the cline is apparently constant over a very large distance (12,000 km) of habitat with some latitudinal but no obvious

longitudinal discontinuity. It is difficult to regard this as being the end result of purely random processes.

The main effect of drift on clines maintained not by stochastic forces but migration and selection may be a reduction in the theoretically expected slope and some variation in location relative to environmental change (Slatkin and Maruyama, 1975; Felsenstein, 1975).

Condition 2 (secondary contact)

This model would see the cline as a contact zone between groups at each end with different frequencies maintained by strong differential selection. Selection in the contact zone would need to be either minimal or show gradation to maintain the cline. The contact zone can also be regarded as a reproductive sink.

Predictions from this model (and their correspondence with available data) would be as follows:

(a) gene frequencies at either end should be fairly constant. This may be true of the eastern end of the cline, where Ecuador$_S$ frequencies do not differ significantly from those in area C (French Polynesia). As little spawning is believed to occur in this area, east of 130°W, it would be necessary that area C be the site of fairly intense spawning activity. This would be in broad agreement with Matsumoto's (1975) views on larval distribution, but at direct odds with those of Ueyanagi (1970, 1976) - see section 2.3. At the western end of the Indonesian archipelago (area 3), available data is limited (7 lots, 789 fish). Within this group, E^1_{SJ} frequencies are homogenous (χ^2_6 = 10.86 (NS) - Table 4.2), a finding common to groupings where the numbers of lots is small (Anon., 1980, and see earlier). Two groups (3 lots each) were collected within a few days of each other at two different sites approximately 30° longitude apart; observed differences in gene frequency between these were consistent and significant (χ^2_1 = 9.78, P < .005), thus strongly suggesting that the cline does not flatten out. It remains a possibility that this occurs westwards of 100°E. This would be important to establish in future studies.

(b) Variances in gene frequencies should be maximal in the contact zone.

Although there is again insufficient data to critically evaluate this possibility, the variance does seem greatest at the western end of the cline, in area A, in contradiction with the prediction.

(c) The cline should be linear for all loci which differ in frequency between the ends of the cline.

It appears that this may not be the case for the GDA locus, although this needs confirmation.

Although it is not possible to definitely exclude this hypothesis, the bulk of available evidence provides little support for its acceptance.

Condition 3 (selection along an environment gradient)

In its extreme form, this model could accommodate panmixia, with fish mixing freely across the spawning zone and strong differential selection producing a cline in allele frequencies, either through fertility or pre-adult viability differences. Such a situation produces a paradox - fish must mix freely before spawning, yet their offspring must remain long enough in the spawning area after selection to be sampled. This paradox persists, even when some realistic qualifications such as limited inbreeding within schools and effective as opposed to instantaneous mixing, are built into the model. Although Ehrlich and Raven (1969) have argued for such differentiation in the face of unrestricted gene flow, Jackson and Pounds (1979) consider that explicit examples are so rare in nature as to raise doubt about the prevalence of this phenomenon.

Assuming that the panmictic form of this model is not acceptable, the alternative forms are variations on an isolation-by-distance model (Wright, 1943, 1946) with the forces of gene flow and selection acting to produce the cline.

Assumptions inherent in this model would be that the probability of fish mating is a decreasing function of the distance between their birth places and that there are no severe restrictions to gene flow across the cline.

It is possible to conceive of several alternate forms of this model consistent with the available genetic data. These must explain not only how the cline is maintained, but also the wider-than-expected variance associated with it. The latter may be attributable to factors such as spatio-temporal variations in the environmental gradient itself. Again, data needed to critically evaluate these models is lacking in several key areas.

(i) the amount and type of gene flow via adults.

Tagging data remains the only available index of potential gene flow. The only data set which offers something approaching intensive longitudinal coverage, that from the SPC Programme, has yet to be analysed in detail. Although it remains difficult to adequately correct for distribution of effort, mortality and other factors, the proportion of longer distance recoveries (say > 1800 km) from other studies and from preliminary results of the SPC study has inevitably been low. No trans-Pacific recoveries are on record, even though tagging of two other tuna species (albacore *Thunnus alalunga*, bluefin tuna *Thunnus thynnus orientalis*) in much smaller numbers has yielded numerous such examples.

Migration/dispersal may also have a selective component, a point critical to evaluating resident/nomad hypothesis.

(ii) larval dispersal

As discussed in seotion 2.3 knowledge of the extent of larval dispersal is completely lacking. This may be an important source of potential gene flow between areas; alternatively if both adult and larval dispersal is restricted, much weaker selection could maintain the cline.

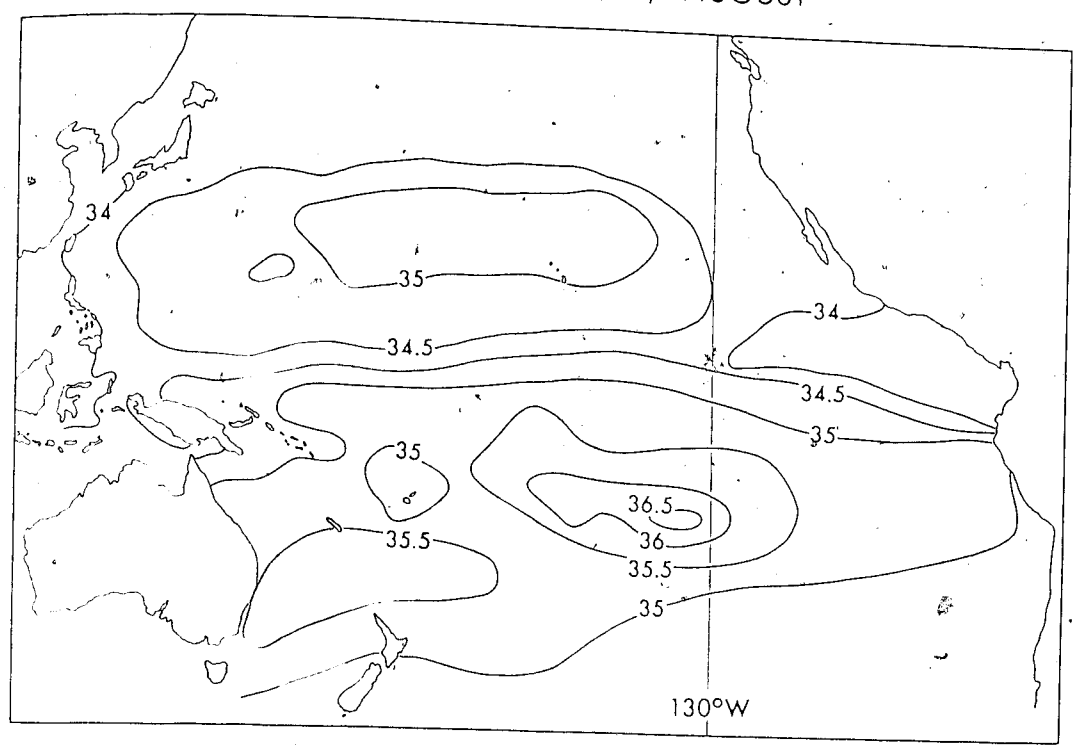
(iii) spawning habits

Knowledge in this area is fragmentary. There is particular need to know what triggers spawning activity, if discrete spawning areas occur within the broad general area, and if any homing to spawning areas occurs. Patterns of tag recaptures would be easier to interpret from the genetic viewpoint if fish in immediate pre-spawning condition could be tagged and released.

(iv) the mode of selection

The function of serum esterases in fishes is unknown. They have been ascribed a detoxifying role in other vertebrate groups but as they are not substrate specific in their action, they may have a variety of roles. Several environmental parameters, notably temperature density and salinity show a weak gradient across the equatorial Pacific (Gorshkov, 1976) (Figure 4.8). Sea surface temperature, in particular, shows a remarkably even decrease of 3^{o} - 4^{o}C across the south equatorial region matching the extent of the cline. These gradients provide a potential basis for selective action of genotypes. Given the huge mortality which must occur in the pre-adult stages, strong selection seems most likely to occur

Sea Surface Salinity (‰): AUGUST



Sea Surface Temperature (°C): DECEMBER

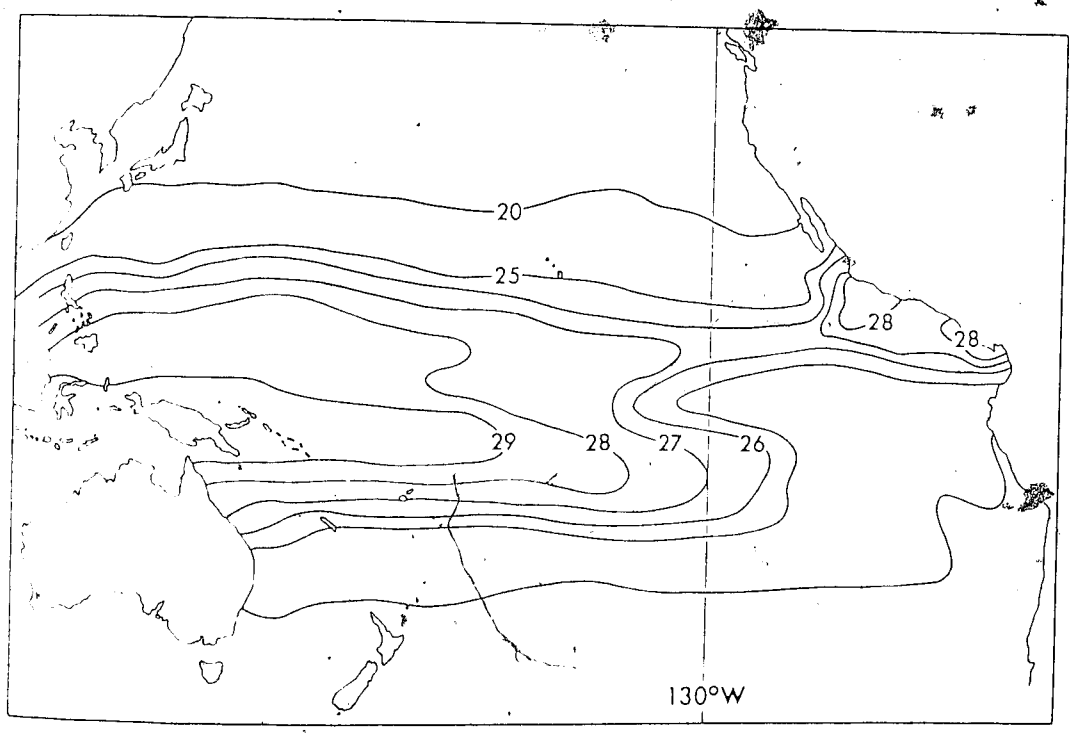


Figure 4.8 Surface isohalines and isotherms in the Pacific during selected months.

during that phase. Indirect evidence of selection on pre-adult stages may come from events at the eastern end of the cline. Adults in the geographically separate area C and Ecuador$_S$ samples share similar E^1_{SJ} frequencies and are hypothesized to have shared pre-adult habitats. If correct, this suggests that migration into a new area has had little or no effect on E^1_{SJ} frequency, implying that these are determined during early life history or reflect parental genotypes.

Predictions which would be made according to isolation by distance models are

(a) variance across the cline should be relatively constant. This generally holds, subject to clarification of area C variance (see earlier).

(b) the range of gene frequencies observed at a given locality should bear some relationship, albeit tenuous, to the distribution of tag returns.

(c) differences in gene frequency between samples should be positively correlated with the longitudinal distance between sampling localities.

The regularity and close fit of the cline attests to this, although in temperate areas, the correlation is less adequate, as will be seen subsequently.

Despite limitations of the data, this selectionist model clearly warrants working hypothesis status at this stage.

Condition 4 (stepped environment or ecotone model)

Under this model either gene flow is restricted along sections of the environmental gradient or selection along the gradient is discontinuous. Where the number of restrictions or discontinuities becomes even moderately large, most data sets could not distinguish between conditions 3 and 4, and the biological distinction would become doubtful. Previously published hypotheses (Fujino, 1970; Sharp, 1978) approach this model most closely.

Endler (1977) points out that stepped clines can evolve in the absence of stepped environments and that stable stepped clines cannot be

produced by stochastic influences. Predictions of the model would be as follows:

(a) if steps represent barriers to gene flow, gene frequency discontinuities should coincide at each locus. This may not be true of selective discontinuities.

(b) the relationship between inter-sample gene frequency differences and longitudinal distance between sampling localities should be stepped.

This model appears to fit the data less adequately than did the previous one, but in view of its prominence in early studies, it will be retained for further scrutiny together with model 3 after examination of the within-area patterns of variation.

4.4 WITHIN-AREA VARIATION

4.4.1 Gene and genotype distribution

Fifty four lots collected sequentially in the New Hanover sector of the Papua New Guinea fishery between August 1978 and October 1980 (Table 4.2) make up the sequential series which will be used to examine within-area variation. Sample size, mean length, allele proportions, genotype frequencies and Smith's H values for esterase and transferrin systems are given in Tables 4.8 and 4.9 respectively. The general sampling locality has been shown on Figure 4.3, while Figure 4.9 shows individual sampling locations within the New Hanover sector.

A total of 5,341 individuals from the 54 samples ($\bar{n}$ = 98.9) were successfully typed for serum esterase, yielding a mean E^1_{SJ} frequency of .699. This is similar to mean values previously listed for area groupings 1a, 1b, PNG_S and A (Table 4.5). A test of independence showed allele frequencies to be heterogenous (χ^2_{53} = 165, P < .005), with eleven of the frequencies (20%) falling outside the 95% confidence limits of .699 ± .07 where n approximates 100. The variation seen at this point source approximates the variance about the cline reported in section 4.3. Figure 4.10 shows the distribution to be strongly skewed, and it is most logically viewed as a normal distribution about 0.73 with approximately a quarter of the samples (14) with lower frequencies of 0.65 or less.

In contrast, the transferrin data (5299 individuals, $\bar{n}$ = 98.1, mean Tf^2_{SJ} = 0.697) was homogenous (χ^2_{53} = 52.9 P ≈ 0.5), with Tf^2_{SJ} frequencies approximating a normal distribution. Only three values (6%)

Table 4.8 Serum esterase allele proportions and genotypic frequencies in the time-series samples

The sample size (n), mean length of skipjack in the sample ($\bar{\ell}$) and two-allele Smith's H value are also given. An asterisk indicates sample significantly out of equilibrium.

Sample	n	$\bar{\ell}$	Allele proportions 1	2	3/4	Genotype frequencies 11	12	13	14	22	23	24	33	Smith's H
KVA	106	53	.613	.382	.005	41	47	1		17				.012
KVB	101	51	.554	.436	.010	30	50	2		19				-.009
KVC	98	53	.576	.408	.015	29	54	1		12		2		-.035
KVD	99	61	.722	.263	.015	55	30	1	2	11				.035
KVE	100	51	.645	.345	.010	38	52	1		8	1			-.035
KVF	99	50	.616	.343	.04	34	50	3	1	7	2	2		-.035
KVG	97	49	.784	.206	.01	61	29	1		5		1		.016
(KVH	100	53	.655	.34	.005	42	46		1	11				-.008
(KVI	100	53	.725	.27	.005	54	37			8		1		.015
(KVJ	99	53	.657	.343		42	46			11				-.006
(KVK	94	53	.697	.303		48	35			11				.026
(KVL	103	54	.68	.31	.01	47	44	2		10				-.005
(KVM	106	54	.632	.358	.009	42	48	2		14				-.002
(KVN	105	55	.652	.324	.023	48	41			11	2	3		.033
(KVO	105	54	.69	.31		53	39			13				.029
(KVP	104	57	.716	.255	.028	54	36	4	1	8		1		.007
(KVQ	104	58	.755	.226	.019	60	34	2	1	6	1			.008
(KVR	104	61	.736	.25	.014	56	38	1	2	7				-.002
(KVS	105	61	.738	.233	.029	59	33	3	1	7	1	1		.018
KVT	101	(55)	.767	.228	.005	61	32		1	7				.016
(KVU	101	49	.604	.376	.02	34	53		1	10	2	1		-.027
(KVV	103	48	.563	.417	.02	32	50		2*	17	2			-.005
(KVY	107	53	.659	.336	.005	45	50			11	1			-.013
(KVZ	103	53	.621	.344	.033	40	41	2	5	15				.003

KVA-A	101	34	.703	.277	.02	50	38	2	2	9			.002
(KVA-C	101	52	.723	.252	.025	54	33	3	2	9			.013
(KVA-D	97	52	.675	.314	.01	43	43	1	1	9			-.011
(KVA-E	102	34	.676	.319	.005	46	46			9	1		-.006
(KVA-F	101	62	.752	.218	.03	57	34	3	1	4	2		-.001
KVA-G	92	(36)	.701	.299		45	39			8			-.001
(KVA-I	98	(55)	.734	.25	.015	52	38	1	1	5	1		-.008
(KVA-J	92	(57)	.723	.266	.011	46	39	1	1	5			-.021
(KVA-K	96	51	.713	.276	.011	46	44		1	4	1		-.029
(KVA-L	98	50	.724	.265	.010	51	38	1	1	7			-.003
(NHA	100	34	.75	.245	.005	57	35	1		7			.008
(NHB	95	37	.710	.279	.011	46	41	2		6			-.020
(NHC	77	35	.682	.299	.019	37	29	2		8	1		.017
(NHD	105	37	.724	.252	.024	57	36	1	1	7	2	1	.020
(NHE	99	46/50	.763	.232	.005	54	42	1		2			-.035
(NHF	78	50	.724	.263	.013	40	33			3	1	1	-.011
(NHG	97	51	.794	.206		61	32			4			-.0004
(NHH	96	51	.76	.229	.011	56	34			4	2		.006
(NHI	105	-	.724	.276		56	40			9			.010
(NHJ	105	59	.643	.324		44	45	1	1	10	2	1	.007
(NHK	100	59	.76	.215	.025	58	32	3	1	5	1		.003
(NHL	103	-	.709	.282	.01	50	44	1	1	7			-.016
NHM	99	51	.687	.278	.035	46	40	3	1	6	1	2	-.006
(NHN	98	56	.755	.224	.02	52	40	4		2			-.039*
(NHO	100	56	.775	.195	.03	61	29	1	3	4	2		.010
(NHP	93	52	.753	.237	.01	56	26	1	1	9			.037
(NHQ	82	50	.683	.305	.012	52	32	1	1	9			.011
(NHR	83	60	.777	.211	.012		30	1		2	1		-.013
(NHS	101	51	.733	.252	.015	55	35	1	2	8			.009
(NHT	103	42	.714	.267	.019	49	47	2		3	1	1	-.033

Table 4.9 Transferrin allele proportions and genotype frequencies in the time-series samples. The sample size (n), mean length of skipjack in the sample ($\bar{\ell}$) and two-allele Smith's H value are also given. An asterisk indicates sample significantly out of equilibrium.

Sample	n	$\bar{\ell}$	Allele proportions 1	2	3	Genotype frequencies 22	23	12	33	13	11	Smith's H
KVA	106	53	.689	.307	.005	49	48		8	1		-.011
KVB	100	51	.72	.265	.015	49	44	2	4	1		-.028
KVC	98	53	.684	.311	.005	46	42		9	1		.003
KVD	97	61	.753	.242	.005	55	36		5	1		.002
KVE	99	51	.657	.339	.005	43	43	1	12	1		.004
KVF	99	50	.702	.293	.005	51	37		10	1		.023
KVG	94	49	.676	.324		44	39		11			.013
(KVH	99	53	.732	.263	.005	51	43		4	1		-.020
(KVI	98	53	.745	.255		52	42		4			-.023
(KVJ	97	53	.711	.289		47	44		6			-.021
(KVK	90	53	.728	.267	.005	46	38		5			-.018
(KVL	99	54	.712	.283	.005	49	43		6	1		-.011
(KVM	105	54	.709	.286	.005	50	49		5	1		-.026
(KVN	104	55	.663	.322	.014	48	60	2	3	1		-.074*
(KVO	105	54	.709	.281	.010	49	49	2	5			-.036
(KVP	104	57	.697	.274	.029	46	47	6	5			-.043*
(KVQ	104	58	.673	.317	.010	45	50		7	2		-.019
(KVR	104	61	.663	.317	.019	48	40	2	12	2		.023
(KVS	104	61	.625	.370	.005	39	52		12	1		-.015
KVT	102	(55)	.716	.275	.009	50	45	1	5	1		-.021
(KVU	100	49	.670	.325	.005	42	50		7	1		.028
(KVV	102	48	.676	.294	.029	50	35	3	11	3		.034

(KVY	107	53	.710	.280	.010	51	48	2	6		-.027
(KVZ	102	53	.720	.270	.010	54	37	2	9		.011
KVA-A	99	34	.697	.283	.020	50	35	3	10	1	.020
(KVA-C	100	52	.720	.270	.010	49	44	2	5		-.028
(KVA-D	96	52	.703	.281	.016	52	29	2	12	1	.049*
(KVA-E	101	34	.718	.282		53	39		9		.011
(KVA-F	102	62	.735	.265		52	46		4		-.030
KVA-G	92	(36)	.745	.250	.005	54	28	1	9		.034
(KVA-I	97	(55)	.706	.284	.103	50	36	1	9	1	.018
(KVA-J	90	(57)	.683	.317		38	47		5		-.044
(KVA-K	96	51	.672	.318		49	29	2	16		.060*
(KVA-L	97	50	.711	.289		48	42		7		-.010
(NHA	100	34	.655	.330	.015	48	35		14	3	.052*
(NHB	93	37	.667	.322	.011	40	44		7	2	-.013
(NHC	80	35	.719	.275	.006	40	34	1	5		-.015
(NHD	105	37	.671	.310	.019	46	48	1	7	3	-.012
(NHE	99	(50)	.682	.313	.005	48	38	1	12		.021
(NHF	77	50	.686	.308	.006	35	36	1	6		-.020
(NHG	97	41	.634	.361	.005	39	45		12	1	.001
(NHH	95	41	.684	.305	.011	44	40	2	9		-.004
(NHI	103	-	.704	.296		50	45		8		-.009
(NHJ	103	59	.694	.296	.010	52	38	1	11	1	.024
(NHK	100	59	.785	.205	.010	60	35	2	3		-.015
(NHL	102	-	.735	.265		53	44		5		-.020
NHM	99	51	.722	.263	.015	51	39	2	6	1	-.005
(NHN	97	56	.696	.299	.005	50	34	1	12		.032
(NHO	99	56	.727	.273		54	36		9		.018
(NHP	92	52	.614	.380	.005	31	51		9		-.039
(NHQ	80	50	.700	.288	.012	37	37	1	4	1	-.026
(NHR	84	60	.673	.298	.030	37	36	3	6	2	-.011
(NHS	101	51	.649	.342	.009	43	44	1	12	1	.006
(NHT	103	42	.680	.320		47	46		10		-.005

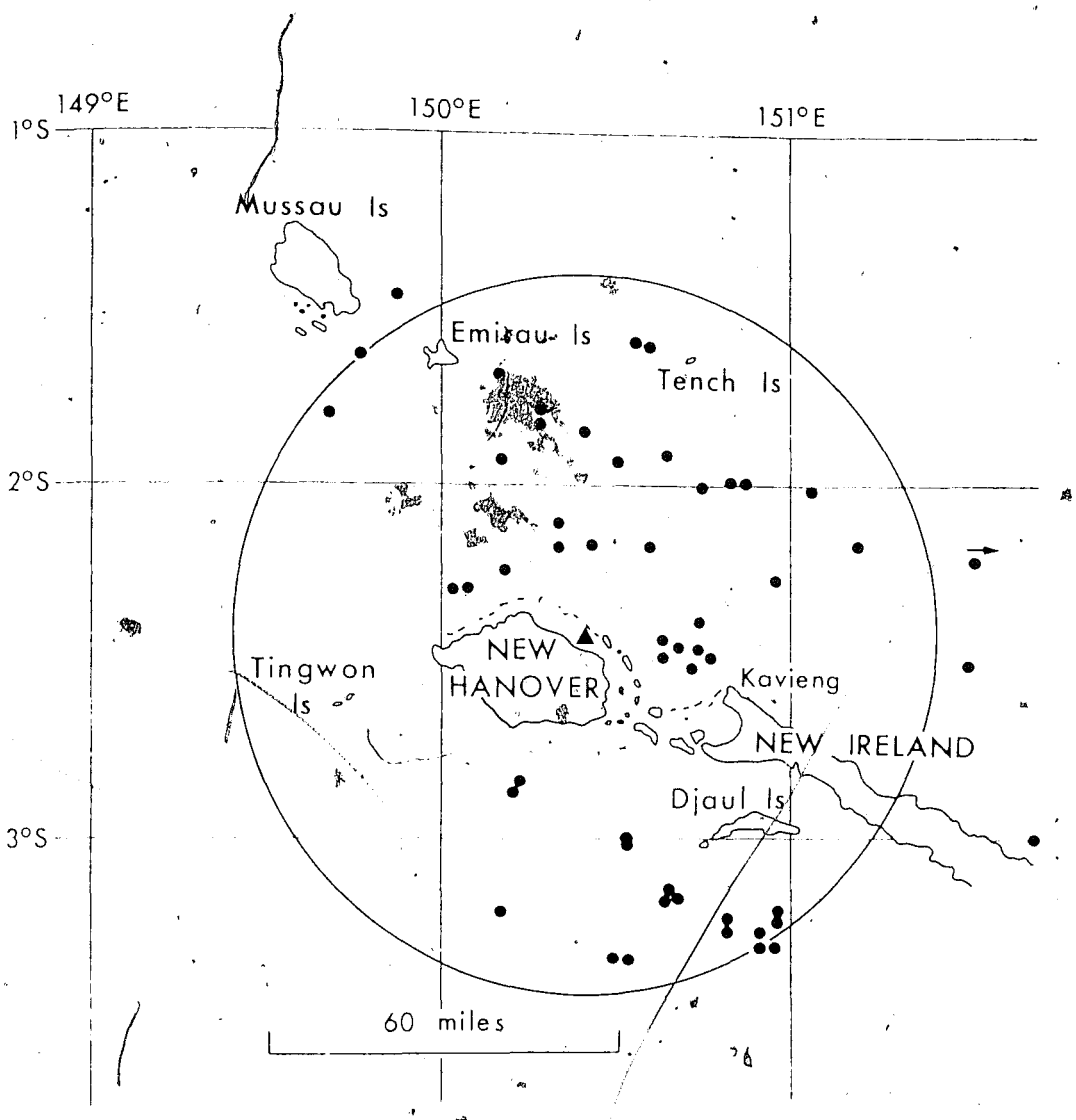
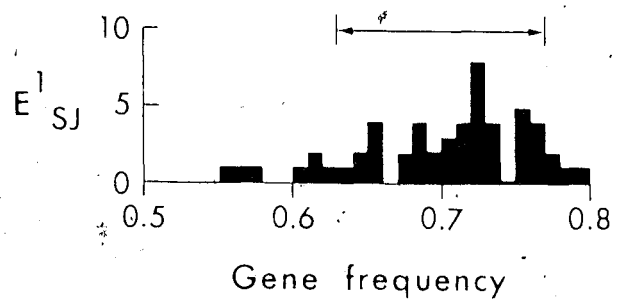


Figure 4.9 Individual sampling locations within the New Hanover sector, defined by a sixty mile radius from the bait fishing grounds (▲).



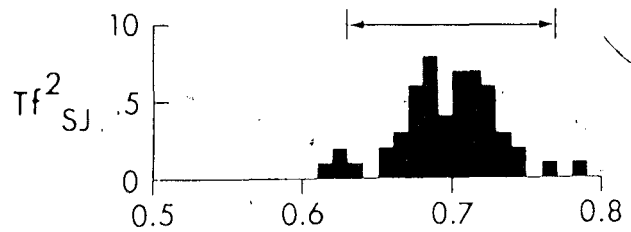


Figure 4.10 Distribution of E^1_{SJ} and Tf^2_{SJ} frequencies in the time series samples.

The 95% confidence limits ($\pm$ .07) about the mean frequency where $\bar{n}$ = 100 are shown.

lay outside the 95% confidence limits, consistent with chance occurrence (Figure 4.10).

The rare alleles E^3_{SJ}, E^4_{SJ}, Tf^1_{SJ} occurred at similar frequencies to those recorded previously in all other areas.

One esterase and five transferrin lots were significantly out of Hardy Weinberg equilibrium on the basis of two allele Smith's H tests. In the case of transferrin, these deviations, although occurring slightly more frequently than expected by chance (9%) were not consistent in direction (3 positive, 2 negative).

The summed Smith's H value for esterase was slightly negative but in equilibrium ($\bar{H} = -.0013$), as were $\bar{H}$ values for areas 1a and 1b (Table 4.5). Area A, which includes these groupings, however showed a significant positive deviation. The $\bar{H}$ value for transferrin was significantly negative ($H = -.0050^*$). The scale of heterozygote excess (O/E = 1.03) is in keeping with that observed in other areas, and its possible significance has been discussed earlier.

As with geographical variation, the main source of within-area variation thus appears to be E^1_{SJ} (and E^2_{SJ}) frequencies.

4.4.2 Replicate sampling

On 21 occasions during the time series sampling, replicate samples were taken on the same day and in the same general area but from different aggregations. These include two replicate sets taken on the same day but in areas approximately 70 nautical miles apart (KVAI - J and KVAK-L) and a set of three samples (NHN-O-P) taken on the same day. In accordance with the sampling strategy laid down, length frequency distributions of replicate members show good correspondence in 18 of the 21 cases (Figure 4.11). Comparison of E^1_{SJ} and Tf^2_{SJ} frequencies between members of these 18 replicates reveal no significant differences, not a surprising result given the power of this test when $n = 100$ (see 4.2.1). Of more relevance is that each frequency lies within the 95% confidence limit (±.07) of each frequency in all but one case for both enzymes (E^1_{SJ} - KVH, KVI, $\Delta = .075$; Tf^2_{SJ} - KVN, KVO, $\Delta = .09$), with mean Δ's for E^1_{SJ} and Tf^2_{SJ} pairs of .035 and .025 respectively.

This result would seem to indicate that skipjack of a given size within a local area at a point in time share a common E^1_{SJ} allele frequency,

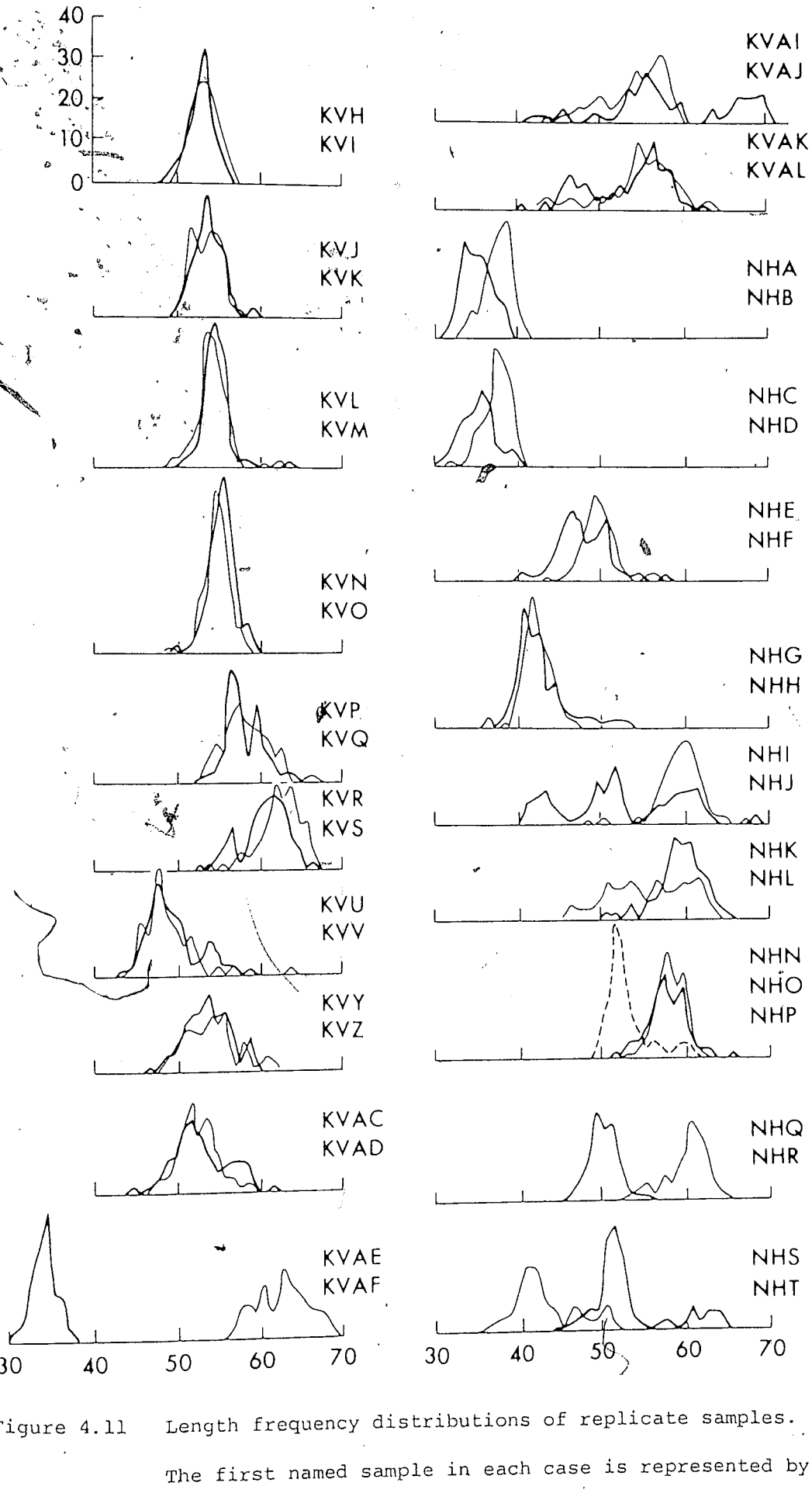


Figure 4.11 Length frequency distributions of replicate samples.

The first named sample in each case is represented by the heavier line.

with the implication that a single sample taken at that time would adequately represent the E^1_{SJ} frequency for fish of that size. The samples collected to examine geographical variation contain several replicates which also bear this out, (see SB-SD & CJ-CK in Table 4.3). A further implication is that a given allele frequency is related to a spatio-temporal dimension rather than to an individual school.

The time scale over which this within-area-by-size constancy holds true is probably highly variable. The available material contains both examples of samples of similar size skipjack collected within a few days of each other showing remarkable consistency (for example, the Indonesian material) and of similar size samples collected a few days apart in the same area differ ing greatly in E^1_{SJ} frequency (e.g. KAF, KAG & KAH, southern N.S.W., 0.574 - 0.712, LCF* ~ 46). The latter may be associated with periodic recruitment events, whereas the former may be the more usual.

The replicate samples, because of their relative size uniformity within sets, provide little information on E^1_{SJ} variability with size at a given point in time and space. That two of the three sets which are grossly dissimilar in size (KVAE-F[illegible] NHQ-R) have Δ's > .07 indicates that extrapolating an allele frequency derived from fish of one size to fish of other sizes even in the same time-area stratum would be inadvisable.

4.4.3 Size effects

The suggestion that fish of different sizes within a given area at one point in time may have different E^1_{SJ} frequencies leads to consideration of size effects on gene and genotype frequencies in general. As sample sizes do not allow examination of size/genotype effects *within* individual aggregations and as grouping across schools requires that numerous assumptions be made, sample mean size has been plotted against E^1_{SJ} and Tf^2_{SJ} frequency for 51 samples with approximately unimodal size distributions (Figure 4.12).

In both cases fitted regressions account for less than 2% of the observed variation. Tf^2_{SJ} frequencies vary uniformly across the size range considered (34-62 cm) whereas E^1_{SJ} frequencies show a slightly different pattern, with aggregations of medium size (45-55 cm) fish exhibiting a wider range of frequencies than either smaller (< 42 cm) or larger (> 55 cm)

*LCF = length to caudal fork

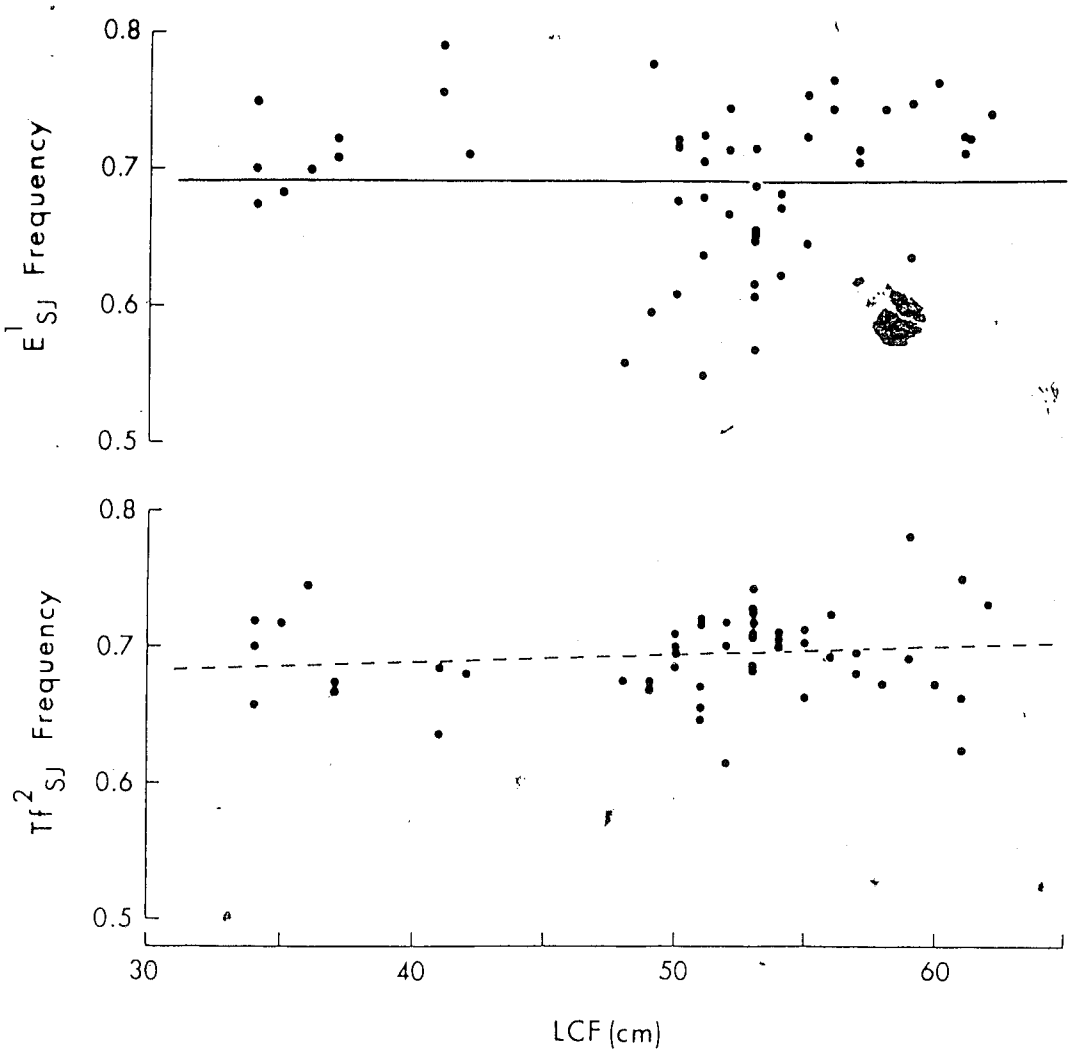


Figure 4.12 Relationship between mean length of skipjack within samples and their E^1_{SJ} and Tf^2_{SJ} frequencies respectively.

fish. Although the numbers of medium size fish are greater, comparison with the Tf^2_{SJ} distribution suggests the effect is real, and that fish of this size make the greatest contribution to the wide variance in E^1_{SJ} frequencies. They also provide the bulk of the fishery's production and form the basis of the tagging experiments considered in Chapter 3. On the basis of results obtained in those experiments, it is tempting to conclude that the smaller and larger fish represent "residents", whereas medium size skipjack include nomadic elements from other parts of the cline with lower gene frequencies. This possibility will be considered later in the section.

The relative scarcity of 40-50 cm fish in samples is not an artifact of sampling and reflects the size composition of the catch. Although probably partly attributable to gear and fisherman selectivity, it provided some of the impetus for the theory developed by Kearney (see earlier) to account for the distribution of skipjack by size.

The relationship between mean school size and Smith's H was examined (Figure 4.13). No obvious trend with greater size towards increased H values (which might be expected if for example, aggregations of older fish are comprised of numerous genetically distinct "core school" remnants), or decreased H values was observed for E^1_{SJ} or Tf^2_{SJ}. In both cases, regressions accounted for very little of the observed variation, viz -

$$H\ (E^1_{SJ}) = -.00927 + .0001714\ (\ell) \quad (r^2 = .005),$$ where ℓ = mean length in cm

$$H\ (Tf^2_{SJ}) = .04389 - .0009464\ (\ell) \quad (r^2 = .074)$$

There was no evidence of a Wahlund effect in the 45-65 cm range where mixing might be anticipated for E^1_{SJ}, and in fact, negative H values were more numerous. The slight decrease in H values for Tf^2_{SJ} is at odds with the Fujino & Kang (1968) model, which has H values increasing with size to approach zero.

It is concluded that size-related effects on gene and genotype frequencies in skipjack > 30 cm are minor. It remains to partition samples or groups of samples according to age, but as has been seen earlier, this is currently not feasible.

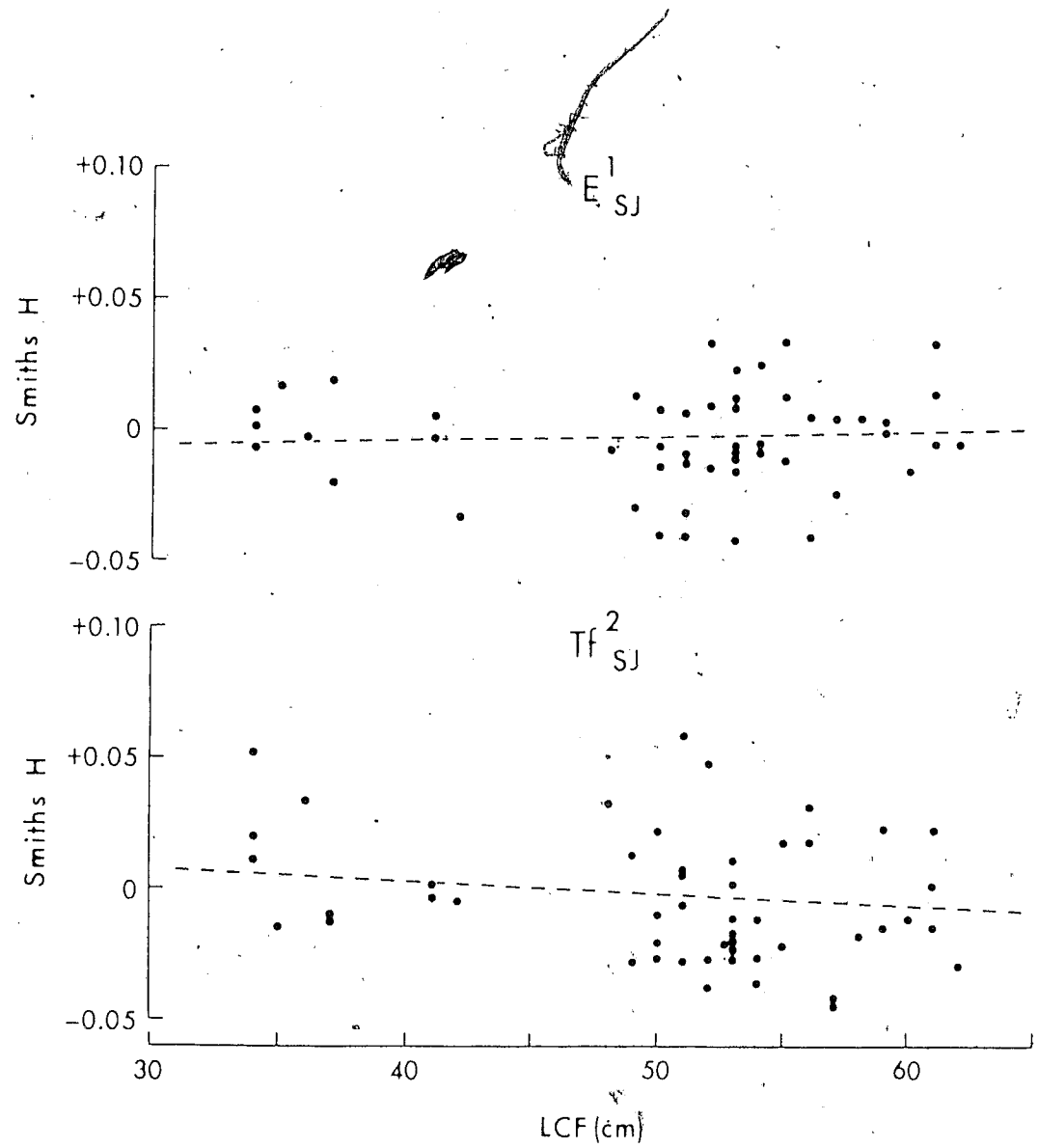


Figure 4.13 Relationship between mean length of skipjack within samples and Smith's H values for E_{SJ} and Tf_{SJ} respectively.

4.4.4 Temporal variation

Replicate sampling has demonstrated that E^1_{SJ} frequencies of comparable size skipjack from the same time area stratum are generally similar; additionally direct size effects on these frequencies are likely to be minor, and temporal variation might therefore be expected to account for some of the observed variation. Figure 4.14 is a chronological plot of the 54 E^1_{SJ} frequencies, with monthly CPUE figures (tonnes/day) arrayed below.

Gaps in sampling during the late December - early March period are the result of cessation in fishing activity in the New Hanover sector over this period with the seasonal onset of unfavourable north-west monsoonal conditions and fishing company holidays. Sampling elsewhere in the Papua New Guinea region during this period, notably in the northern Coral Sea near Port Moresby, has yielded consistently high E^1_{SJ} frequencies, as below.

Sample	Date	Locality	E^1_{SJ}	Tf^2_{SJ}	n
SW*	27-2-77	N. Coral Sea	.823	.692	62
SCX*	"	"	.757	NA	111
PG	19-27-2-78	"	.781	.719	114
PE	18-21-1-78	"	.817	.720	52
ARA	23-1-79	"	.728	.663	81
CB	27-1-79	"	.750	.603	81
CB2	14-2-79	"	.755	.717	96
SA	20-1-79	Solomon Sea	.797	.678	163
SB	21-1-79	"	.722	.660	106
SD	21-1-79	"	.75	.690	114
		TOTAL	.756	.682	930

*from Richardson, MS.

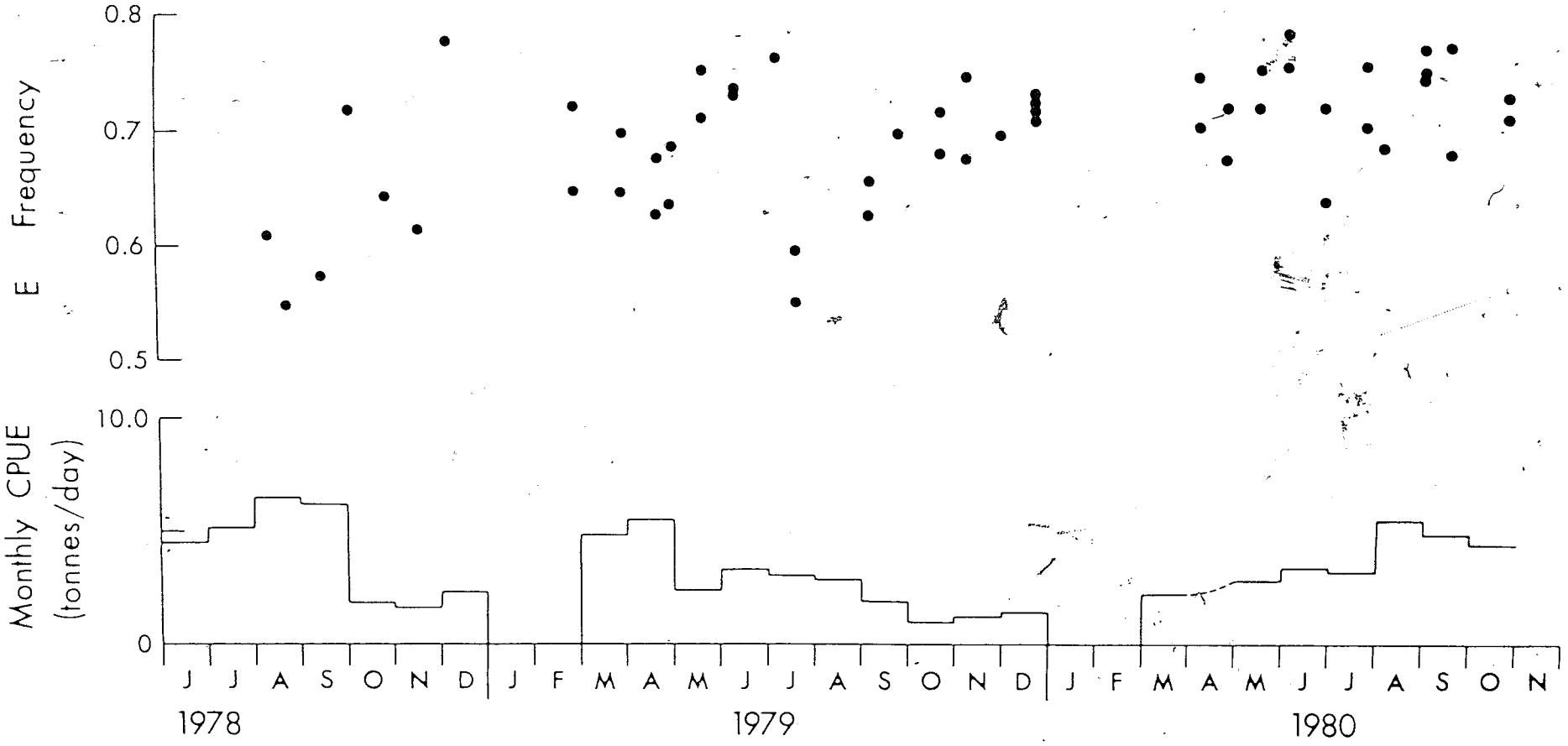


Figure 4.14 Chronological plot of E^1_{SJ} frequencies in the time-series samples, with monthly CPUE figures from the fishery arranged below.

As earlier analysis suggested that in the study area, variation in E^1_{SJ} frequency was associated with medium size rather than smaller or larger size skipjack, the former have been removed and grouped with all other unimodal samples in this size category (42-55 cm mean length) from group 1a and those from PNG_S collected south of the Equator. Analysis of deviance (analysis of variance for proportions) has been performed on this data set, comprising 52 sample lots collected on 38 occasions between November 1975 and October 1980 and including 13 replicates, to enable resolution of the observed variation into temporal and other components.

In the first instance, E^1_{SJ} frequencies, after logit transformation, have been fitted against the following variables

(i) Days - days since the beginning of the year when sampling was initiated (to seek long term trends in the data) i.e. Jan. 1st, 1975

(ii) Season - sinusoidal within-year variation, as expressed by the relationship

$y = a + b \cos t + c \sin t$

where $t = \frac{2\Pi\ (T-\frac{1}{2})}{12}$ i.e. mid-monthly intervals

(T = time in months)

and E^1_{SJ} can be calculated from the transformation $\frac{e^y}{e^y + 1}$

(iii) Sub-Season - more subtle sub-harmonic variation associated with time of the year.

In this case, $y = a + b \cos t + c \sin t + b_2 \cos t + c_2 \sin 2t$

(iv) Occasion - variation within replicates and between occasions can be compared

Source	df	Deviance	Mean Deviance	$F_{*,14}$	$F_{*,32}$
Days	1	6.8	6.8		2.02^{NS}
Season	2	59.7	29.9		8.86^{***}
Sub-Season	2	1.2	0.6		0.18^{NS}
Residual	32	107.8	3.37	4.13^{**}	
Between occasions	37	175.5	4.74		
Within occasions	14	11.4	0.82		
Total	51	186.9			

Clearly, there is little evidence of long term trends or sub-harmonic effects; within-replicate constancy is good, as established previously, and unspecified seasonal effects are highly significant. This can be seen in Figure 4.15, where data from all years is arrayed by time of year and the fitted curve shown. A good deal of the variance however remains unexplained.

In conjunction with season effects, both year-specific effects (year) and seasonal effects specific to a particular year (year-season) were then examined.

Source	df	Deviance	Mean Deviance	$F_{*,14}$	$F_{*,21}$
Season	2	63.0	31.5		15.3***
Year-Seasonal	10	58.9	5.89		2.86*
Year	4	10.32	2.58		1.25^{NS}
Residual	21	43.22	2.06	2.52*	
Between occasions	37	175.5	4.74		
Within occasions	14	11.4	0.82		
Total	51	186.9			

After allowing for different amplitudes and phase differences in each year, the years did not differ in mean gene frequency ($F_{4,21} = 1.25$, NS). However the amplitude and phase differences did differ between years ($F_{12,21} = 2.86$, $P < .05$). This can be seen in the variability of the day on which maximum E^1_{SJ} frequency should occur each year, as calculated from the fitted sine curve for that year (day $= \frac{365}{2\pi} \tan^{-1}(\frac{c}{b})$),

namely
1976 Day 5 (January)
1977 " 40 (February)
1978 " 59 (February)
1979 " 3 (January)
1980 " 191 (July)

(as only 2 samples were collected in 1975, no calculation has been made).

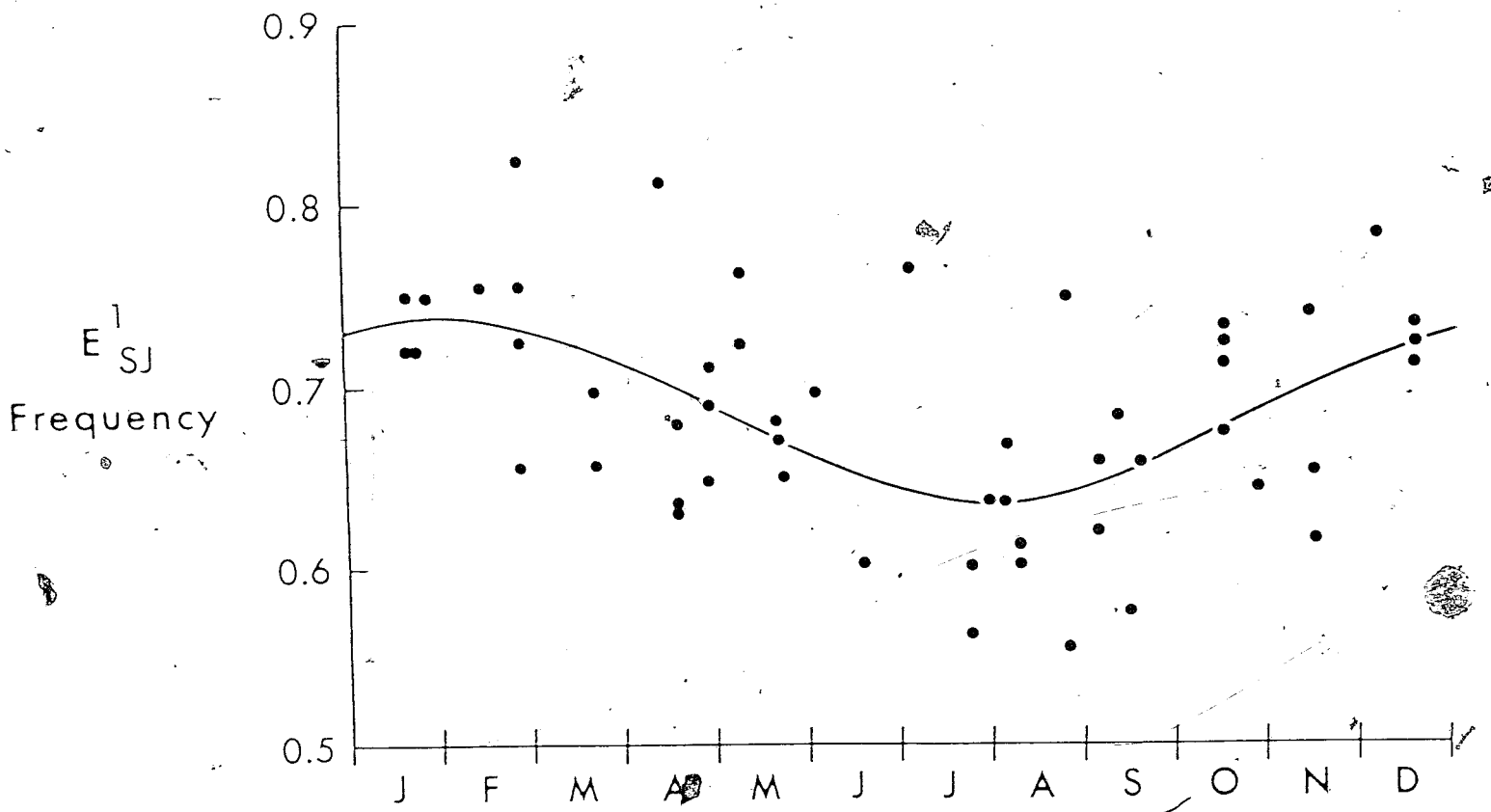


Figure 4.15 Plot of E^1_{SJ} frequency against month of collection, irrespective of year, with the fitted sinusoidal curve shown.

The effect of varying catch levels on E^1_{SJ} frequencies, after seasonal and annual effects had been taken into account, was not significant. Catches do show marked seasonal fluctuation (see earlier) and any effects have probably been already removed.

The residual deviance remains significant ($\chi^2_{21} = 43.2^{**}$, $.001 < p > .01$), indicating that other effects have yet to be accounted for. As has been discussed previously (section 2.3) the main environmental influence on skipjack availability in the time-series sampling area may be the position of the equatorial upwelling as marked by the 35‰ isohaline. Although this most commonly impinges on the area in question during the same June-September period, it does show considerable between-year variation in location and strength. Through the co-operation of ORSTOM, Noumea, it has been possible to obtain information on the position of the 35‰ isohaline during part of the study period (June 1979 - March 1980). Unfortunately data for the entire sampling period is not available precluding its incorporation in the analysis of deviance.

One must expect that chance events will always ensure a considerable amount of residual variance. The 1980 samples, for example, showed little change in E^1_{SJ} frequency during the year. Relatively few of the samples (7/20) were unimodal and within the 42-55 cm mean size range and many were collected closer inshore than usual. These factors alone may be sufficient to obscure general seasonal effects (and increase "year-specific" seasonal effects) when the number of samples involved in relatively small.

Fluctuations in E^1_{SJ} frequency seasonally, plus year-to-year variation in the timing and amplitude of this seasonal fluctuation will obviously explain a considerable amount of the variation seen at a given longitude. By extension, the time of year at which samples were collected should be considered in their interpretation. The various sample groupings provide good examples of this. Over half of the PNG_S samples (8/14) were collected in July-August 1976, giving a lower $\hat{p}$ (0.655) than the geographically comparable Area 1a samples ($\hat{p} = 0.743$) which contain only January-June material. Area C samples from the eastern end of the cline at present show little variance about $\hat{p}$, which has some significance when evaluating alternate explanations of the cline. All 11 samples available have however been collected over a three months period, which may help to explain the reduced variance.

To examine how general seasonal fluctuations in esterase frequency might be, two independent data sets have been considered (Figure 4.16)

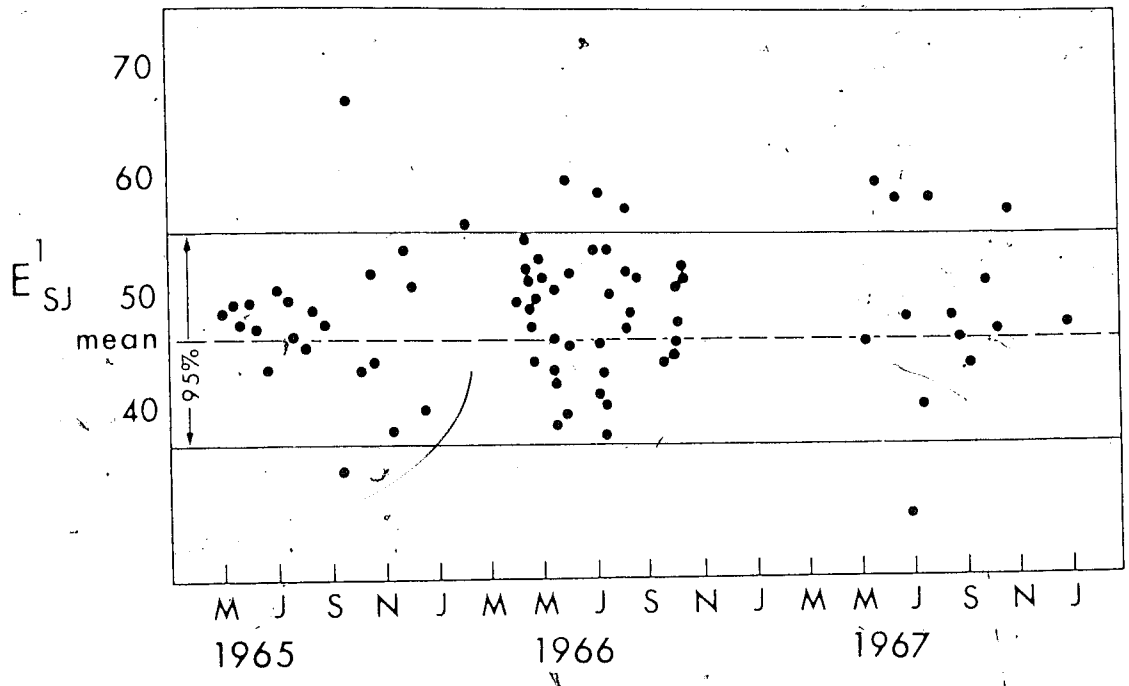
(i) time series samples (78 lots, 7853 individuals) collected and analyzed by Fujino in Hawaii (1965-1967) and summarized by Sharp, (MSb).

(ii) $Palau_F$ samples (21 lots, 1604 individuals) collected in Palau between September 1966 and November 1967.

The Hawaiian material is difficult to compare with the present data as the fishery is seasonal. There are consequently large gaps in the data and no information on fish size was available at the time of writing. No clear fluctuations in gene frequency are evident, but the heterogenous nature of the data set, with numerous values lying outside the 95% confidence limits, shows similarity with the New Hanover data. Eleven samples (14%), occurred outside the 95% confidence limits as calculated by Sharp, mostly (9/11) on the higher side. Little year-to-year variation in $\hat{p}$ appears to occur.

The Palau samples, although limited in number, are more comparable with the PNG samples. The fishery from which they were collected is year round; although 15° further west than the PNG fishery, it shows rough latitudinal symmetry with respect to the thermal equator/Equatorial counter-current (8°N & 2°S, compared with 4°N). It is therefore particularly interesting to note the sharp increase in E^1_{SJ} frequency over the January-March period, as it occurs in phase with the drop in frequencies seen in the PNG time series data,

As the author has no detailed knowledge of this fishery, it is difficult to advance a plausible explanation at this time.



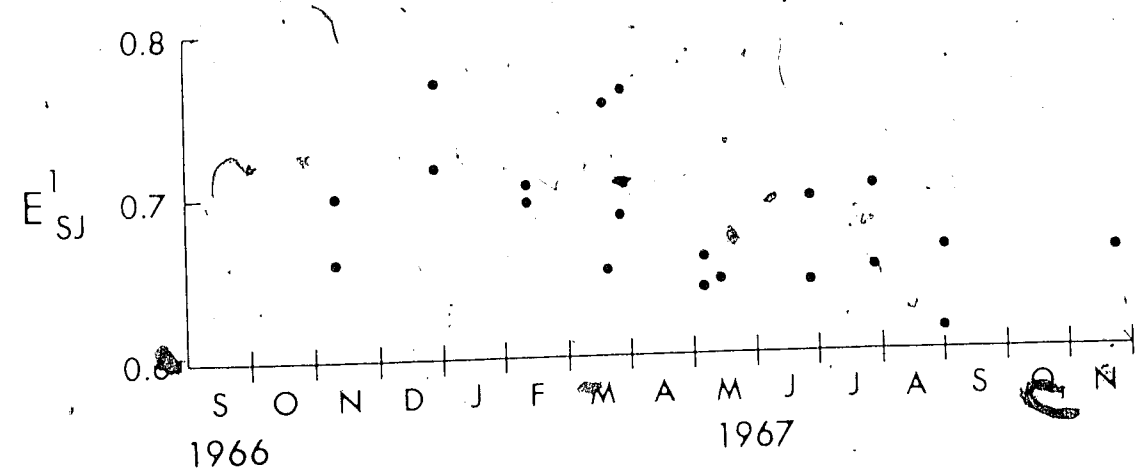


Figure 4.16 Chronological plot of E^1_{SJ} frequencies in time series samples from Hawaii and Palau respectively.

In the first case, 95% confidence limits as calculated by Sharp (MSb) are shown.

4.4.5 Cohort continuity

Another approach to the examination of temporal variation has involved, through the co-operation of the Fisheries Division, Papua New Guinea, collection of length frequency data from the New Hanover sector concomitant with collection of the time series samples. Modal groups were extracted from these data and an attempt made to relate the time-series samples for genetic analysis to particular size groups or cohorts and follow them through time.

Ten skipjack were selected at random from each of the 50 or so vessels operating in the fishery during each daily unloading operation and fork length measured to the nearest cm. Over the period mid-1977 to September 1980, 115,787 skipjack were measured. These data were analyzed on a monthly basis and polymodal distributions resolved into a series of unimodal distributions using the method of successive maxima (Daget & Le Guen, 1975). The modal lengths were plotted against date of collection, and von Bertalanffy growth curves derived from the Papua New Guinea tagging data by Josse *et al.*, (1979) added at six monthly intervals to provide a frame of reference for evaluating modal progression. Finally, the mean lengths of time series samples, with their E^{1}_{SJ} frequencies and 95% confidence limits (± .07), were superimposed on the above, and an attempt made to match these lengths to modal lengths to follow obvious progressions. Because of its complexity, this figure has not been shown.

The analysis was however beset by several problems:

(i) within any month, up to eight size modes could be recognized, with 4 or 5 distinct modes typical. The size structure of the resource therefore appears very complex;

(ii) the stationality and blurring of modal groupings, presumably associated with more-or-less continuous recruitment patterns, lengthy spawning periods and variability in growth sequences, as

reviewed earlier, make it difficult to assess modal continuity; the decision becomes ultimately subjective and the connection between successive modes speculative. Many modal groupings are probably transient, as previous estimates of loss rates from the fishery using the tag data have suggested.

(iii) mean lengths of genetic samples do not always correspond to modal groupings. This may be partly attributable to the departure from unimodality in some samples, but probably also indicates that the composite monthly analysis, in converting a large unwieldy data set into a more usable form, results in the disappearance or incorporation of many minor modal groups.

(iv) individual samples with finite confidence limits on both allele frequency and size, are not unique and within a system of this complexity, affinities and distinctions are virtually impossible to establish.

It is to be expected that similar difficulties would plague other studies in productive tropical areas and it might be more useful to refine the approach first in a seasonal temperate area fishery such as New Zealand. The chances of success might be improved if:

(a) limited tagging of fish, ideally within the same aggregation, could accompany the sampling to supply information on persistence of particular modes within the fishery;

(b) length frequency data could be processed rapidly, enabling samples to be taken from primary modal groupings;

(c) more samples could be collected and analyzed.

Practical limitations would remain severe, however, and given the subjective nature of, and assumptions involved in, length frequency analysis, effort may be better directed to other areas of investigation.

4.4.6 Evaluation of predictions from tagging data

Predictions from the tagging experiments discussed in Chapter 3, couched in terms of the resident-nomads hypothesis, were as follows:-

(i) if there is a gene frequency characteristic of residents, this should be present throughout the year.

(ii) assuming gene frequencies of at least some nomads will differ from those of residents, the most likely period of their occurrence in the New Hanover sector is April-September.

(iii) if there prove to be gene frequencies characteristic of geographical areas, nomad frequencies should be representative of the area covered by tag returns.

(iv) genetic heterogeneity at any point in time is liable to be considerable, given the multiplicity of factors promoting it.

A key assumption prefaces prediction (iii). Replicate sampling has indicated that similar sized fish within an area at a given point in time and space share a similar E^1_{SJ} frequency. The clinal relationship also has predictive value in assessing the probability of encountering a given E^1_{SJ} frequency at a given longitude. On this basis, the E^1_{SJ} frequency of a sample of skipjack can be assumed to broadly reflect affinity of those fish with a given area. This refers only to young and adult skipjack (> 30 cm) and makes no presumptions about how the E^1_{SJ} frequency characteristic of a broad area has arisen, and whether it represents, at one extreme, gene frequency of parents which "home" to or are resident in particular areas, or, at the other extreme, strong area-specific selection within one generation on widely dispersing larvae and juveniles.

Figures 4.12 and 4.15 suggest the first prediction from the tagging data is generally fulfilled because E^1_{SJ} frequencies centering on ~.73 were found in all sizes of fish in all months of the year and may represent a 'resident' gene frequency. Samples from large relatively sedentary fish characteristic of the Madang sector would be a useful further test of this prediction.

The appearance of atypically low E^1_{SJ} frequencies during the July-September period is in agreement with prediction (ii). As skipjack abundance is generally higher at this time, resident frequencies may be "swamped" and not sampled when a small number of samples is taken. Alternatively, 'nomad' gene frequencies could be expected to approximate those of residents in many cases and may swamp abnormal frequencies on other occasions. The test of the prediction is therefore not particularly powerful.

Tag returns have indicated that immigration out of the area and immigration into the area, is unlikely to involve regions north of 10°N a[redacted] of 180°E, with areas south of 10°S likely to be particularly [redacted]nt. According to the confidence limits about the cline, E^1_{SJ} frequencies of 0.55 could be anticipated to occur between 150°E and 145°W. Immigration into the New Hanover sector has been recorded from as far east as Wallis Is. (176°W), the centre of this range, and captures of long range immigrants have generally been made during the predicted period.

Tag No.	Origin		Distance	Recapture Date
SA 1516	New Caledonia	(21°14'S, 166°02'E)	1460 n.m.	29/7/78
SA 5626	"	(20°43'S, 166°18'E)	1142 n.m.	24/9/78
SE 2658	Wallis Is.	(13°34'S, 176°12'W)	2070 n.m.	5/9/78
SK 22715	Tuvalu	(8°59'S, 179°04'E)	1783 n.m.	19/10/79

Emigration as far east as 175°E has been recorded, and there has been an unconfirmed report of a recapture at 5°N, 150°W (Line Islands).

From the occasional presence of very high E^1_{SJ} (> .80) frequencies, particularly in the Coral Sea, recaptures from Indonesian releases would be predicted; no tagging has however been carried out in this area as yet. Returns from north Coral Sea releases have been received in the Madang and New Hanover sectors (Lewis, 1980b; Cooper and Wankowski, 1980) and it is possible that two-way movement along the New Guinean north coast and into the Coral Sea occurs. In accordance with the resident-nomad theory, movement into the Indonesian region with its high year round productivity should be limited, and no returns have been made west of Irian Jaya. Prediction (iii) is therefore generally met by the genetic data, but the tagging data presently available appear to provide a conservative estimate of the occurrence of particular gene frequencies. This may change if greater numbers of fish had been tagged; it may also suggest a possible bias in tagging experiments towards tagging residents rather than nomads.

The final prediction, again not a powerful one, is clearly met, as evidenced by the spread in marker (E^1_{SJ}) amplitude and phase of E^1_{SJ} frequency fluctuations. The time-series analysis has enabled some sources of this heterogeneity to be identified.

4.5 DISCUSSION

Analysis of geographical variation in allozymes has shown clinal variation in esterase allele frequency to be the most significant component of this variation at present. The other polymorphic system considered, transferrin, showed constancy in allele frequency across the Pacific Ocean (and Atlantic Ocean, Fujino, 1969), whilst genotype numbers showed a slight heterozygote excess in some areas; this pattern may be associated with the protein's function. Other systems were either insufficiently polymorphic or subject to severe practical constraints to be of value to the study, although there is some chance that variation at the GDA locus will ultimately prove useful.

The low level of variation may in itself be of significance (see Chapter 5); the reliance on variation at so few loci has undoubtedly increased the difficulty of detecting population sub-division.

The time series data have brought to light several important points relevant to interpretation of the observed variation:

(i) variation in E^1_{SJ} frequency at a point source is representative of the variance about the cline at that longitude and is about five times that expected from binomial sampling.

(ii) in the Papua New Guinea area, much of this extra variation can be traced to the seasonal or intermittent appearance of groups of medium size skipjack with lower E^1_{SJ} frequencies. Tagging results suggest these fish may originate from areas to the east and south-east where E^1_{SJ} frequencies of skipjack are typically lower.

(iii) the appearance of these lower frequencies has a strong seasonal basis but shows considerable between-year variation. The period in question (July-September) is generally associated with high productivity, suggesting nomadic elements may be involved. More importantly, this period is also one of minimal spawning activity and reduced gene flow may accompany these movements (Figure 4.17).

(iv) size effects on gene frequency for fish > 30 cm seem to be minimal.

Providing the Papua New Guinea data can be considered representative of other tropical areas (excluding the eastern tropical Pacific),

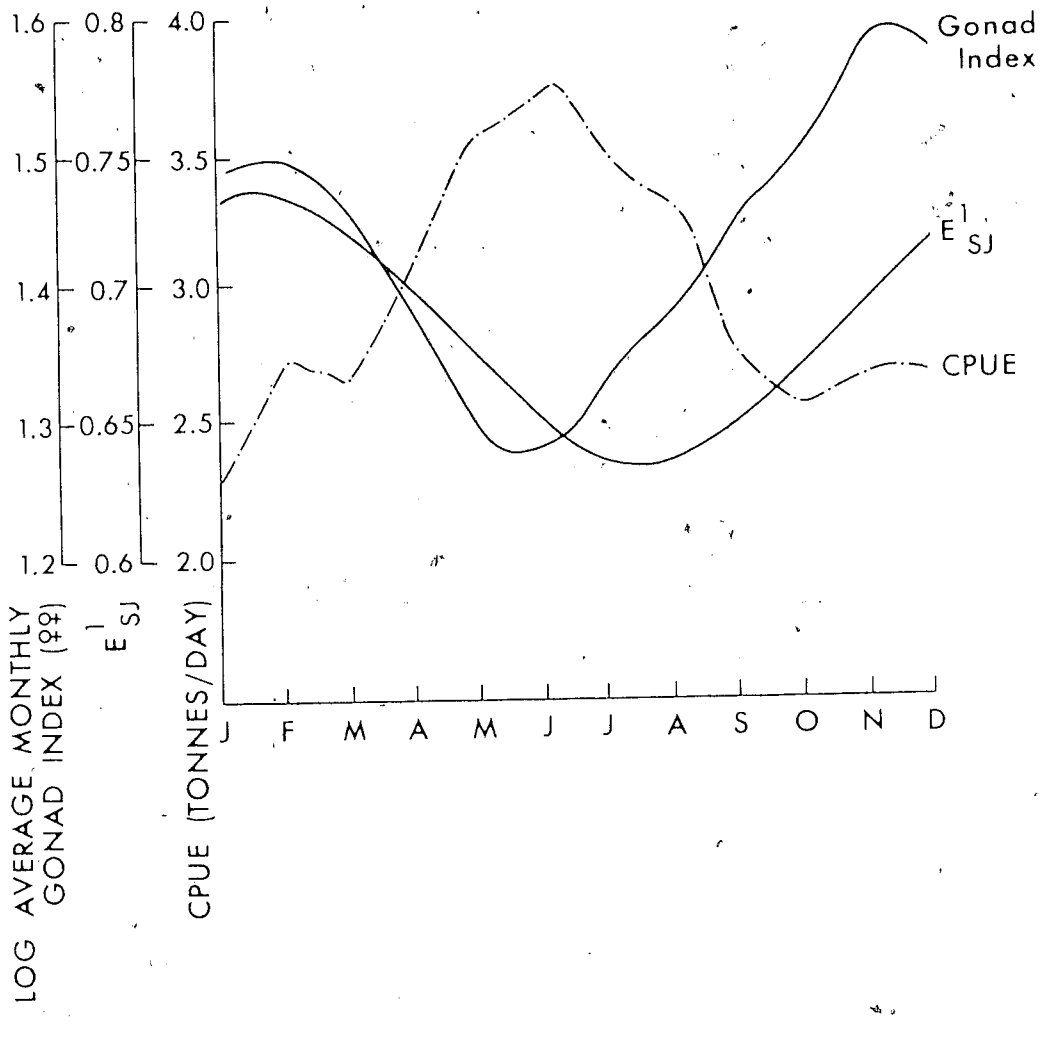


Figure 4.17 Monthly average gonad index and CPUE relative to the sinusoidal relationship derived for E^1_{SJ} frequencies.

alma
hapman
chiyama
illett
alma
mith

these data increase the plausibility of the isolation-by-distance model, with selection along an environmental gradient and some restrictions on gene flow across the area interacting to produce the observed cline in esterase frequency. Slatkin (1973) defines ℓ_c, the characteristic length for the spatial variation in allele frequencies as $\ell_c = \frac{\ell}{s}$, where ℓ is the net mean square gene flow distance and s is the selection strength operating over distance Δ. The population cannot respond to changes in environmental conditions which occur over a distance $< \ell_c$. As the parameters ℓ and s are both poorly known here, Richardson (pers. comm.), following the approach of Endler (1973), has employed simulation techniques to examine the effect of varying migration rates and selection levels on maintenance of the observed continuous cline. Using six populations with 2000 animals in each, (a number of this size was used to overcome drift effects, yet keep computing time reasonable) in a linear array, and taking ten samples of 100 fish at random in the fiftieth generation, the following conclusions were reached.

(i) with a very strong gradient (6%) in selective differentials on the heterozygotes (or either homozygote) across the populations, nearly 50% migration between populations was required to eliminate a cline in allele frequency. A migration rate of 3% produced the closest fit to the observed cline at this level of selection. At lower but still high levels of selection (1%, 0.5%), very restricted migration (< 0.1%) would be necessary for the cline to persist.

(ii) steps in the cline were difficult to induce by varying selection and/or migration.

(iii) no significant divergence from Hardy-Weinberg equilibrium was produced. This is in general agreement with actual observations, where however several cases (area A, $Palau_F$) of heterozygous disadvantage/Wahlund effect were recorded.
Endler (1977) suggests that dominance relationships amongst genotypes may be more important than the effects of gene flow in determining cline steepness. Heterozygous disadvantage, for example, leads to cline steepening.

(iv) with no combination of selection/migration did the heterogeneity induced at one point match that observed in the data. Such hetero-

geneity may be explained if, as suggested by the time series data, much dispersal is not accompanied by gene flow. This again highlights the problem of estimating gene flow from dispersal.

The distance across which gene flow effectively occurs (ℓ) will be less than the dispersal distance estimated from the tagging experiments. In the Papua New Guinea experiments - the only ones analysed in detail so far - not only was dispersal distance limited (even after 100 days, 70% of returns showed less than 200 nautical miles displacement) but the timing of influxes of novel gene frequencies would further reduce possibilities for gene flow. Preliminary results from the SPC programme have yielded similar results, with a very small percentage of returns showing more than one thousand miles absolute displacement, and none more than 4,000 miles; the longitudinal displacement involved is often considerably less. Although the scale of long distance movements is likely to be underestimated (Grant, 1980) a variety of barriers would generally prevent their contributing to effective gene flow (Endler, 1977). Providing the pattern of larval dispersal is not markedly at odds with that described for adults and that additive across-generation effects do not greatly increase ℓ, the apparently quite restricted ℓ may mean that rather modest selective differentials may be sufficient to maintain the cline.

It has been previously noted that selection is most likely to occur on larval and juvenile stages; the finding that E^{1}_{SJ} shows little or no change with size above 30 cm in the Papua New Guinea area lends further support to this. Similarly, the "weak" gradients in several environmental parameters, notably temperature and salinity, may provide a basis for selective action - they are at least within the limits of resolution in field studies, unlike many instances where attempts are made to measure selection. Detailed studies of serum esterase kinetics and function would be a valuable aid to increasing our understanding of selective action on this locus.

The suggestion that residents may make a greater contribution to spawning in tropical areas find support in the predominance of presumed "resident" gene frequencies during the peak spawning season in the Papua New Guinea area. If this proves to hold generally true, island-open ocean interactions may also be critical to understanding the selection process. We have seen, in the area south of the counter-current, that east of 130°W, both a flatening-out of the cline and cessation in spawning activity occurs. It seems highly significant that the myriad of islands and reefs which dot the Pacific south of the Equator also do not extend east of 130°W (see Figure 4.5).

Total 51 186.9

It is useful now to examine available data for the Pacific Ocean north of the counter-current, where, apart from the isolated Hawaiian chain, islands are virtually non existent east of 175°E. Available data consists of $Palau_F$ material (only those with n > 80 (18 lots) have been plotted), Japanese material collected and analysed by Fujino and with n > 80 (11 samples, as in Sharp, 1978), one Philippine sample (Sharp, (MSb)10 samples from Richardson (MS), the Hawaiian time series data and 9 samples collected in the eastern Pacific and analysed by Sharp. The $Ecuador_S$ material is also shown.

A regression line fitted through points from the western Pacific above 5°N has been extrapolated to 175°E, the approximate eastern limit to island groups, then a second horizontal line extrapolated eastwards (Figure 4.18). The fit of available data about these two lines is good. The very wide spread in Hawaiian material suggests movement into the area from as far west as 150°E, and possibly from Ecuador/French Polynesia. Tag returns in Hawaii from releases east of Japan (31°N, 155°E) and conversely a return in the Marshall Islands (12°N, 158°E) from Baja California releases (Sharp, (MSb) provide further evidence that skipjack exploited east of 180°E in the Hawaiian and Eastern tropical Pacific fisheries may originate from spawnings in the vicinity of islands well to the west, with frequencies shaped by selective forces in this area showing little change in adults found east of 175°E. As foreshadowed earlier, island-open ocean interactions may thus play a very important role in skipjack population ecology.

The hypothesis as it now stands appears to explain the available tagging data better than does a discrete sub-population or stepped cline model. If E^1_{SJ} frequencies are however best described by two similar clines i.e. north and south of the Equatorial Counter-Current with inflexion points in different places (175°E, 130°W), the model described by Sharp (1978) from considerably less data, with five overlapping "genetic units", is not too different from the present model. The essential difference between the two can be seen in neutralist-selectionist terms. Whilst the Sharp model appears to implicitly assume that E^1_{SJ} frequencies characteristic of particular groups and which have arisen as a result of stochastic rather than deterministic (selective) forces might eventually be defined, the current explanation sees E^1_{SJ} frequencies as determined by selective forces on larvae and juveniles in island-associated areas and lying on a continuum within the area 100°E to 130°W.

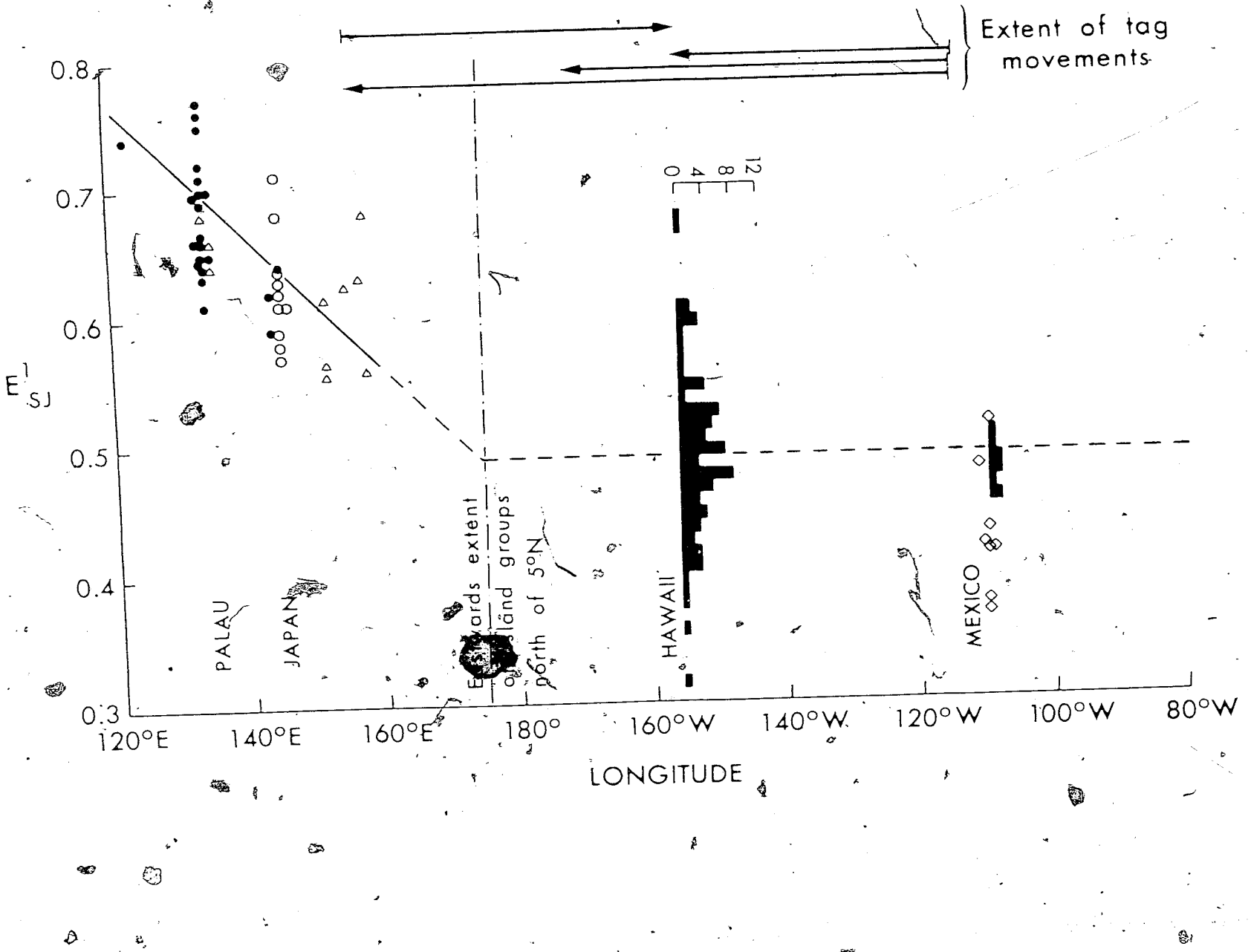
Figure 4.18 Relationship between E^1_{SJ} and longitude across the area north of 5°N.

Both regression lines have been fitted by eye, and the Hawaiian and Mexican frequencies are shown in histogram form. Other symbols are as follows:

- • Fujino Palau samples and one Sharp Philippine sample.
- o Fujino Japan samples.
- Δ SPC samples (Richardson, MS)

Ecuador samples for comparison.

Note that these more closely match area C frequencies rather than Hawaiian frequencies.


Extent of tag movements
0.8
0.7
0.6
0.5
0.4
0.3
E^1_{SJ}
0
4
8
12
PALAU
JAPAN
Eastwards extent of land groups north of 5°N
HAWAII
MEXICO
120°E
140°E
160°E
180°
160°W
140°W
120°W
100°W
80°W
LONGITUDE

The key question from both a population genetic and management viewpoint is - does strong selection act on larvae and juveniles to produce within one generation, an E^{1}_{SJ} frequency characteristic of the area independent of parental E^{1}_{SJ} frequency or do E^{1}_{SJ} frequencies reflect parental frequencies slightly modified by relatively weak selective forces? The extent to which nomads contribute to gene flow and the extent to which they are selected for or against also need to be ascertained. Apparently limited ℓ values suggests that modest selection may be enough to maintain the cline.

If any amount of homing occurs or if residents contribute disproportionally to spawning, the required level of selection could be very modest indeed. Implications for management would then represent a clear challenge to orthodoxy - protection of tropical spawning areas would be of paramount importance, with little need to regulate harvest in either temperate areas or tropical areas inhabited by nomadic non-spawners, such as the eastern Pacific.

As it is not possible to take analysis of the skipjack genetic data any further without further information in key areas of the species biology becoming available, it may be useful to briefly review available data on yellowfin tuna, which shares many similarities with skipjack and has been the best studied tuna after that species.

(i) although attaining a much greater size and possessing more advanced physiology, yellowfin dispersal patterns are strikingly similar to those of skipjack as noted earlier. Less than 1% of returns in extensive eastern Pacific tagging experiments showed displacement > 1060 nm, and tuna tagged near banks and islands show limited dispersal (Schaefer *et al.*, 1961). As with skipjack, tagging experiments may underestimate the extent of long distance movement; yellowfin contaminated as a result of nuclear tests at Bikini Atoll ultimately appeared over much of the Western Pacific (Suzuki *et al.*, 1978). Nonetheless, trans-Pacific migrations as observed in other species have not been recorded and dispersal, and hence potential gene flow, is probably restricted.

(ii) analysis of morphometric data (Royce, 1964) has shown a cline in most characters from the western (130°E) to eastern Pacific, leading Royce (1964), Kamimura and Honma (1963) and Suzuki *et al.*, (1978) to support the concept of "semi-independent subpopulations" or stocks. (No comparable morphometric studies have yet been carried out for skipjack.)

(iii) although yellowfin spawning strategy is liable to be quite different - spawning for example, occurs in the eastern tropical Pacific (cf. skipjack) and samples taken there in offshore areas had a higher percentage of spawners than those in inshore areas (Knudsen, 1978) - the limited genetic data provides some interesting parallels, as the summary below from Sharp and Kane (MS) demonstrates.

Mean Allele Frequency

Area	Est^{1}_{YF}	Tf^{A}_{YF}	GPI^{2}_{YF}	n
Eastern Pacific ($75^{o}W$ - $141^{o}W$)	.969	.727	.340	14,240
Marquesas Is. ($140^{o}W$)	.958	.757	.533	94
Western Pacific (138^{o}-$154^{o}E$)	.975	.726	.674	1,516

Serum esterase showed no useful variation, and transferrin frequencies appear relatively constant at a level similar to Tf^{2}_{SJ}; GPI^{YF}_{2} shows variation of an amplitude (~ 0.35) comparable to that seen in the E^{1}_{SJ} cline over the same region, and may prove to be clinal in a similar but not identical manner to that described for skipjack - no flattening of the cline at its eastern end would be predicted, for example. Finding variation similarly expressed in two enzymes with quite unrelated functions in different but related species is interesting but difficult to interpret. Finding such similarity in several enzymes within the same species would be good evidence that breeding structure of the species and not selection alone, were involved in shaping the clines.

The comparative approach may well provide some insights into population genetics of scombrid fishes and high vagility species in general. As we will see in the following chapter, two tuna species hypothesized to have hemispheric and circumpolar (panmictic) population structure respectively, albacore (T. *alalunga*) and southern bluefin tuna (T. *maccoyii*), possess quite high levels of genetic variation and have been successfully aged. Examination of this variation over a wide area may provide information on the selective process; no difference in allele frequency over wide areas would be predicted in its absence. Carefully designed experiments may further allow selection on particular loci to be assigned to life history stages.

Whilst it is clear that many questions remain unanswered, the characteristics of the species, the paucity of useful known electrophoretic variation and the daunting logistics of high seas sampling also suggest that these questions are not especially tractable ones given existing methodologies. The economic importance of the species does provide considerable incentive for further studies however and it is suggested that these would most usefully focus on the following areas:

(i) biological aspects of open ocean-island interactions, e.g. vertical and horizontal distribution of larvae relative to distance from land; zooplankton distribution, etc.

(ii) distribution of larvae in time and space, complete with details of development. Recent successful spawning of skipjack in aquaria has been an encouraging development in this direction.

(iii) characterizing electrophoretic variation in eggs, larvae and juveniles.

(iv) limited additional sampling of adults to investigate specific hypotheses. This may include collection in the Indian Ocean and possibly time-series collection in other areas, for example, a tropical area intermittently productive (Kirabasi) compared with a tropical area with high year-round productivity (eastern Indonesia).

CHAPTER 5

GENETIC VARIATION WITHIN THE FAMILY SCOMBRIDAE

5.1 INTRODUCTION

The preceding chapter has given some indication of both the potential value of electrophoretically detected variation to population genetics studies, as well as the constraints imposed by having few suitable polymorphic systems on which to base such studies. Apart from recent surveys by Richardson (MS) and Sharp (MS) of enzymes active in skipjack and yellowfin blood respectively, levels of genetic variation in scombrid species have not been adequately screened, although individual enzyme polymorphisms have been described (Fujino, 1970; Serene, 1971; Edmunds and Sammons, 1971). In this chapter, attempts to estimate levels of genetic variation in most Indo-Australian members of the family Scombridae are described.

Genetic data for scombrids should also provide a useful test of some of the hypotheses which have been advanced to explain the levels of variation observed in natural populations (Chapter 4). For example, to determine if selective forces are involved in shaping the amount of variation maintained, one strategy has been to seek correlations between observed variation and ecological/biological characteristics of the organism concerned (Hedrick *et al.*, 1976; Nevo, 1978; Nelson & Hedgecock, 1980). The present large species array within the family Scombridae exhibits considerable diversity in such characteristics as habitat, range, maximum size and physiological adaptations, and appears ideal for this purpose, particularly as it is unnecessary to survey across higher taxa in order to secure an adequate range of ecological and biological characters.

5.1.1 Criteria for establishing levels of variation

According to Lewontin (1974), reasonably reliable estimates of genetic variation in directly sampled natural populations require that four basic criteria be satisfied:

(a) 50 genomes per locus should be sampled;

(b) a large number of loci (ideally 100+) be examined;

(c) particular enzyme functions should not be disproportionately represented;

(d) the loci should be selected without regard to known variability and represent as near as possible, an unbiased sample of the genome.

In practice, sample sizes of 50 individuals are not always easy to obtain. For example in this study whilst only a handful of individuals belonging to the rare species *Scomberomorus multiradiatus* are on record in the scientific literature, 15 individuals were collected for this study and so this was a considerable achievement. Nei and Roychoudhury (1974) also point out that in estimating average heterozygosity per locus, a large number of loci is preferable to a large number of individuals typed per locus. In this study, with 27 loci examined for all species, a sample size of 25 was set as realistic and acceptable. It proved possible to attain this number in 14 species, 13 or more individuals in another 7 species and only one individual of two rarer species. The number of loci screened, whilst falling well short of Lewontin's ideal, exceeds that examined in many published studies (for example, Nevo, 1978). The loci were chosen with regard to ease of resolution of phenotypic classes and cost of analysis, and fortuitiously embody a relatively unbiased sample with respect to enzyme class, enzyme functions and quarterary structure (see later).

Even when the above criteria are fulfilled, there remain some additional qualifications. Firstly, single collections provide estimates of variation within populations rather than species. To adequately characterize variation in widely distributed species, sampling across the species range should be carried out (Nevo, 1978). As the concern here is with the comparative aspects of genetic variation within a single region, the Indo-Australian area, this difficulty is largely avoided. Secondly, only structural loci can be studied using electrophoretic techniques (these may show levels of variation not representative of the entire genome) and, as discussed previously, not all variation at individual structural gene loci can be detected using standard techniques. Examination of the same loci using identical techniques in all species should ensure that valid comparisons can be made.

marketed whole, it was necessary to probe the body cavity through the branchial region and excise liver material without damaging the exterior of the fish. Approximately 10 gm of material were placed in labelled temperature-resistant plastic snap-cap vials and dropped into liquid nitrogen. Where this was not possible, dry ice was carried. Every effort was made to obtain material in premium condition, although experience subsequently showed that preventing temperatures rising above -20°C during storage was at least as important. On return to the laboratory, material was catalogued and stored at -70°C; at this temperature, material stored for 2 years still provided satisfactory results for all enzymes used in this study.

Liver presents more problems with deterioration than other tissue such as muscle and blood, and was chosen because of the range of enzymes available and the strong activity shown by most of these enzymes. Many of the loci coding for liver enzymes are also expressed in blood, the tissue of choice in most population studies with large commercial fish species because of the ability to take samples without damage to the product. It is likely, therefore, that most polymorphisms discovered in this study will also be present in blood and hence valuable for use in population studies.

Table 5.1 lists the sample location, date of collection, size range of individuals, method of collection and collector for all species studied. In nearly all cases, a single locality and sampling occasion is involved, but with several rarer species or species of solitary habitat (e.g. wahoo), it has been necessary to collect on several occasions to obtain a reasonable number of specimens. Sampling localities listed in Table 5.1 are shown in Figure 5.1.

Two other epipelagic species which commonly co-occur with various scombrids were also screened for comparative purposes. These were the black marlin *(Makaira indica,* Family Istiophoridae) a closely related species within the same sub-order Scombroidei, and a more distantly related species, the rainbow runner *(Elegatis bipinnulatus,* Family Carangidae).

5.1.2 Measures of variation

The most widely used measurement of gene variability is the expected frequency of heterozygotes, or heterozygosity (H); this is expressed as either $\bar{H}$, the mean heterozygosity per locus, or $\bar{H}_i$, the mean heterozygosity per individual. Although $\bar{H} = \bar{H}_i$, the variance about $\bar{H}_i$ tends to be normally distributed, while this is not generally expected with $\bar{H}$, and the standard error associated with $\bar{H}_i$ is typically lower than for $\bar{H}$.

A less precise measure is P, the proportion of loci polymorphic at a predetermined level (usually common allele frequency <0.95 or <0.99). Other measures, such as the number of alleles per locus, are less commonly used and rather less satisfactory (Nei, 1975).

After establishing levels of inter-locus and inter-specific variation in Indo-Australian scombrids, it is intended to test predictions from both neutralist and selectionist theory by seeking correlations between heterozygosities and various environmental and biological predictors. Fundamental problems for studies of this type are inevitably posed by the difficulties associated with quantifying variables of interest, e.g. niche breadth, environmental grain, N_e, (effective population size) T (time since divergence), trophic stability and vagility; in practice, resort is made to indicators which can be ordinated, or to pair-wise comparisons between contrasting species.

5.2 MATERIAL

Specimens were collected by a variety of methods, including trolling, pole-fishing, trawling, purse-seining and gill-netting from commercial and research vessels, sampling catches at gamefishing tournaments and purchasing samples at fish markets. The assistance of the many people, who went to considerable trouble not only to collect material in good condition but also to transport bulky containers associated with its preservation and storage often at considerable inconvenience to themselves, has been gratefully acknowledged earlier.

In all cases, liver samples were taken. This could be achieved in most cases by simply opening the body cavity with a mid-ventral incision and excising the sample. In other cases, where the fish were to be

TABLE 5.1 Collection details of material available for screening levels of genetic variation

Only liver material in good condition has been listed

Family Scombridae	No.	Location	Date	Size (LCF)	Method	Collector
Tribe Scombrini (Mackerels)						
Scomber australasicus (slimy mackerel)	32	Port Stephens	24.2.79	19-28 cm	Handline	ADL
Rastrelliger kanagurta (chub mackerel)	30	Ambon (Indonesia)	14.12.79	23-26 cm	Fish market	ADL
Tribe Scomberomorini (Spanish mackerels)						
Grammatorcynus sp. A (shark mackerel)	15	Cairns	19/10-23.1.80	60-86 cm	Trolling	L. Chapman
Grammatorcynus sp. B (scad)	7	Cairns	8.12.79	40-45 cm	Trolling	P. Cooper
Scomberomorus commerson (narrow banded mackerel)	60	Cairns	19-29.10.79	56-114 cm	Trolling	ADL
S. queenslandicus (Qld. school mackerel)	43	Moreton Bay	18-23.4.79	18-33 cm	Trawl	ADL
S. multiradiatus (Papuan mackerel)	18	Gulf of Papua	NA	NA	Trawl	D. Gwyther
S. semifasciatus (grey mackerel)	21	Gulf of Carpentaria	22-23.4.80	60-78 cm	Gill net	G. McPherson
S. munroi (spotted mackerel)	15	South-West Rocks	March 1980	NA	Trolling	D. Mitchell
	(3	Port Stephens	25.2.79	114-128 cm	Game fishing	ADL
Acanthocybium solandri (wahoo)	(4	Narooma	9-11.3.79	113-157 cm	"	ADL
	(6	Moreton Is.	3-4.4.79	122-145 cm	"	J. Pepperell
	(10	Hawaii	5-22.7.79	113-149 cm	Trolling	J. Uchiyama
	(4	Narooma	7-9.3.80	130-163 cm	Game fishing	J. Kalma
	(2	Port Stephens	29.2.80	140-156 cm	"	ADL
Tribe Sardini (Bonitos)						
Sarda australis (Australian bonito)	(11	Port Stephens	19-21.10.79	48-63 cm	"	J. Kalma
S. orientalis (oriental bonito)	(13	Narooma	9-11.3.79	53-76 cm	"	ADL
Cybiosarda elegans (leaping bonito)	25	Albany	10-16.3.80	50-64 cm	Trolling	ADL
Gymnosarda unicolor (dogtooth tuna)	13	Sydney Markets	March, 1980	NA	Seine Net	J. Pepperell
Allothunnus fallai (slender tuna)	1	Sahul Shelf, W.A.	28.6.79	73 cm	Handline	G. West
	1	S.E. Tasmania	July, 1980	85 cm	Trolling	T. Jenkins

Tribe Thunnini (Tunas)						
Auxis thazard (frigate tuna)	(9	Narooma	9-11.3.79	38-41 cm	Game fishing	ADL
	(16	Port Stephens	19-21.10.79	38-43 cm	"	J. Kalma
	(29	Cairns	19.10.79-1.2.80	46-74 cm	Trolled	L. Chapman
Euthynnus affinis (mackerel tuna)	(					
	(49	Hawaii	29.5-1.6.79	38-73 cm	Pole & line	J. Uchiyama
Katsuwonus pelamis (skipjack tuna)	54	Willis Islets	3.5.79	42-52 cm	"	ADL
Thunnus albacares (yellowfin tuna)	~30	Kia Is., Fiji	23.4.80	46-53 cm	"	R. Gillett
T. tonggol (longtail tuna)	29	Moreton Bay	31.1 & 1.2.79	89-119 cm	Handline	ADL
T. obesus (bigeye tuna)	7	Padang, Indonesia	22.12.79	40-50 cm	Pole and line	ADL
T. alalunga (albacore)	(13	Narooma	9-11.3.79	70-82 cm	Game fishing	ADL
	(9	"	7-9.3.80	55-82 cm	"	J. Kalma
T. maccoyii (s. bluefin tuna)	25	Eden	27.11.79	100-105 cm	Purse seine	ADL
T. thynnus orientalis (oriental bluefin tuna)	1	Port Moresby	13.4.79	233 cm	Game fishing	B. Smith
Family Istiophoridae						
Makaira indica (black marlin)	24	Port Stephens	24.2-2.3.80	182-238 cm*	Game fishing	ADL
Tetrapturus audax (striped marlin)	9	"	1-2.3.80	150-281 cm	"	ADL
Family Carangidae						
Elegatis bipinnulatus (rainbow runner)	14	Willis Islets	3.5.79	51-72 cm	Pole and line	ADL

* Total length

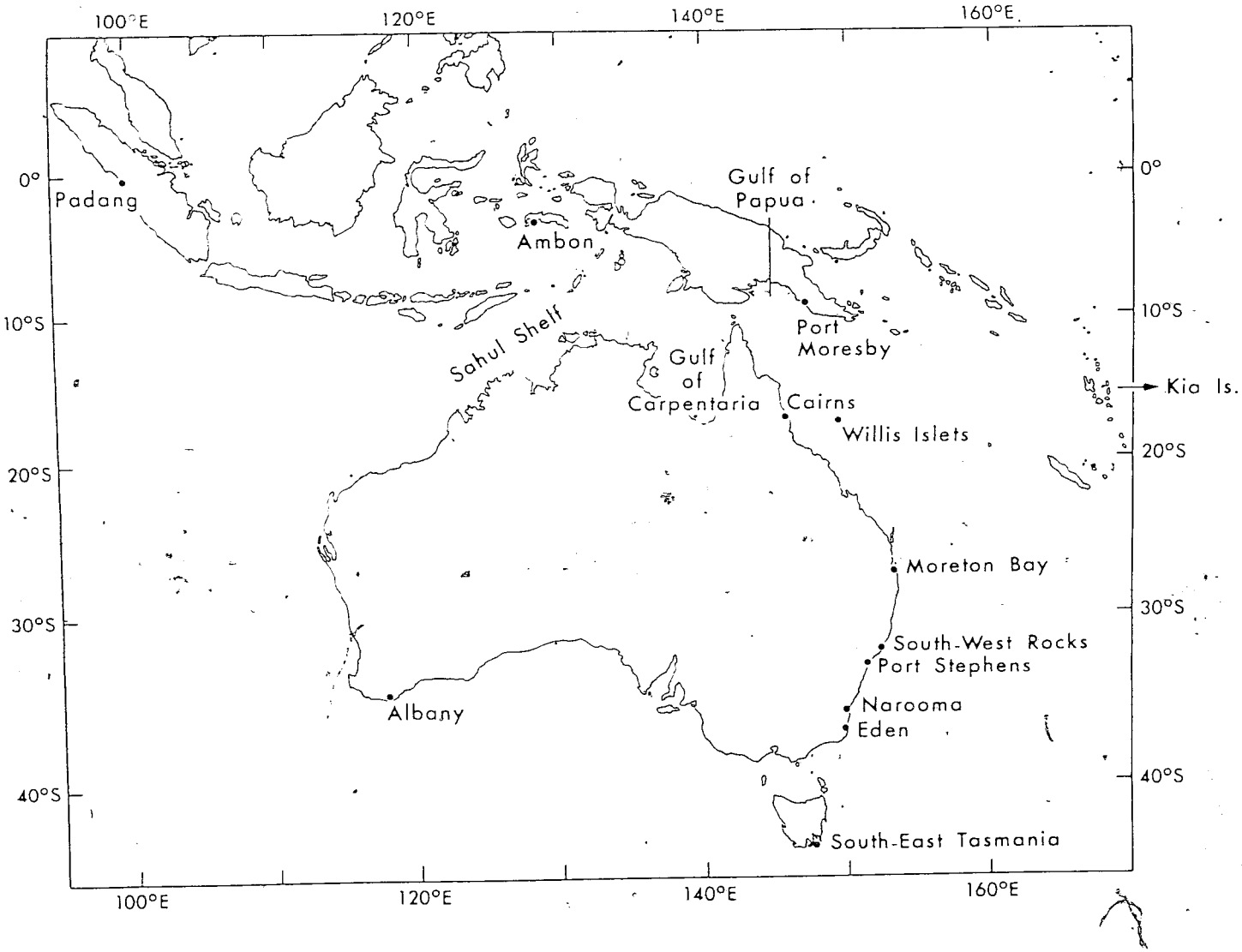


Figure 5.1 Sampling localities listed in Table 5.1.

5.3 METHODS

5.3.1 Electrophoretic procedures

All enzyme systems were separated on cellulose acetate strips as described previously. This contrasts with most previous surveys of genetic variation which have used starch as the medium. Comparisons of known polymorphisms have shown the two media to yield identical results, and cellulose acetate has been preferred because of its convenience. A small piece of liver was cut from the still-frozen liver sample and placed in glass centrifuge vials. An equal volume of lysing solution (0.2% β-mercapto-ethanol in double distilled water) was added, and the material vigorously macerated with a swab stick. The samples were then centrifuged at 3,500 rpm for 10-15 minutes to separate the lysate containing the soluble proteins from the cell debris. Fat globules within the liver cells often coalesced to form a dense surface layer; with care, lysate could be run down the side of the vial without disturbing the fat plug.

Using an adjustable gap draughtsman's lining pen and perspex rule, samples were applied to the gel which had previously been soaked for about 30 minutes in the appropriate buffer. Running times and voltage applied for each enzyme were determined by experience as those necessary to produce optimal resolution, usually involving anodal migration over about 4 to 6 cm of gel. The gel was then allowed to rest in an enzyme-specific histochemical stain mix to visualize the zones of activity. In some cases, these required viewing under ultra-violet light.

Buffers

Varying the molarity, pH and EDTA content of tris maleate, tris borate, phosphate and sodium barbitol solutions produced a variety of buffers for test when working up new systems. It was found however, that for most enzymes, a standard .05M tris-maleate buffer, pH 7.8, provided good resolution. In relatively few cases it was found necessary to develop other buffers. Furthermore, buffers developed for one species invariably proved suitable not only for other species in the family, but also for the other two species screened. This seems to be largely attributable to the excellent condition of most of the material, which appeared to be very tolerant of buffer choice.

Some of the time consuming aspects of such a study were thus considerably reduced and with experience, it proved possible to run up to 25 individuals for the 27 standard loci in a day.

Stains

Stains were modified only slightly from those described by Shaw and Prasad (1970) and Harris and Hopkinson (1977). Details of the buffers and stains used are listed in Table 5.2. As electrophoretic mobility of some enzymes show considerable variation between species, optimal running times will vary accordingly and the times listed in Table 5.2 are approximations only.

5.3.2 The enzymes

As the data from this survey of electrophoretic variation were also to form the basis of biochemical comparisons for systematic studies (Chapter 6), three criteria were stringently applied to the 30 or so presumed enzyme loci initially screened before they were included in the data base.

(i) the locus had to be clearly expressed in all species studied. On this basis, several enzymes were rejected. These included a presumed * slow ADA locus which showed clear variation (dimeric heterozygotes) in some species, whereas in others, resolution was poor and activity low. Activity at second ICD and GPI loci showed a similar pattern. As material was not subjected to sonication, it is probable in some cases that such loci are membrane-bound or mitochondrial, rather than cytoplasmic, with available activity levels varying between species.

(ii) between-species homologies needed to be established with reasonable certainty. An example of an enzyme failing to satisfy this requirement was a peptidase using l-leucine-alanine as a substrate. Two clear zones of activity, both with occasional dimeric heterozygotes, were expressed in some species, and one zone in others. As homologies in this situation would have been difficult to establish, this otherwise satisfactory and variable locus was not included.

* ADA is normally a monome as the fast locus demonstrates. It is likely that activity of another enzyme has been visualized by the stain.

Table 5.2 Running conditions & stains for the enzymes used in the study.

Abbreviations used are as follows: TM - tris maleate; TEB - tris EDTA borate. Molarities are all .05M unless otherwise stated.

Enzyme	Running Conditions	Stain
ADH	TM pH7.8, 250V, 1 hr. 10 mins.	1.0ml. Tris HCl pH8.0, 1 drop Ethanol, 0.1ml. NAD(10mg/ml), 0.1ml. PMS (2mg/ml)
GPD	TM pH7.8 250V, 1 hr. 10 mins.	1.0ml Tris HCl pH8.0, 0.1ml α-glycero phosphate (25mg/ml), 0.1ml NADP (10mg/ml), 0.1ml 0.1M $MgCl_2$, 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml).
SORDH	TM pH7.8 250V, 1 hr. 20 mins.	1.0ml. Tris HCl pH8.0, 0.1ml. Sorbitol (25 mg/ml), 0.1ml. Sodium pyruvate (25mg/ml), 0.1ml. NAD (10mg/ml), 0.1ml.PMS (2mg/ml) 0.1ml MTT (4mg/ml)
LDH	TM pH7.8 250V, 1 hr. 40 mins.	1.0ml 0.1M Tris HCl pH8.0, 0.1ml Lactic acid (25mg/ml), 0.1ml NAD (10mg/ml) 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml).
MDH	TM pH7.8 250V, 1 hr. 10 mins.	0.8ml 0.1M Tris HCl pH8.0, 0.1ml Malic acid (25mg/ml), 0.1ml NAD (10mg/ml), 0.1ml PMS (2mg/ml), 0.1ml MTT (4 mg/ml).
ME	0.03M TM pH7.2 250V, 1 hr.	0.7ml 0.1M Tris HCl pH8.0, 0.1ml Malic acid (25mg/ml), 0.1ml NADP (10mg/ml), 0.1ml 0.1M $MnCl_2$, 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml).
ICD	TM pH7.8 250V, 1 hr. 15 mins.	0.7ml 0.1M Tris HCl pH8.0, 0.1ml Sodium isocitrate (25mg/ml), 0.1ml NADP (10mg/ml), 0.1ml 0.1M $MgCl_2$, 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml).
PGD	TEB pH7.8 300V, 45 mins.	1.0ml 0.1M Tris HCl pH8.6, 0.1ml 6-phospho-gluconate (25mg/ml), $MgCl_2$, 0.1ml NADP (10mg/ml), 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml).
GAPDH	TM pH7.8 250V, 1 hr.	1.0ml Tris HCl pH7.5, 10µl Glyceraldehyde - 3-Phosphoric acid, 0.2ml NAD (10mg/ml), 0.1ml Sodium arsenate (15mg/ml), 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml).
SOD	TEB pH7.8 250V, 45 mins.	1.0ml 0.1M Tris HCl pH8.0, 0.2ml PMS (2mg/ml), 0.2ml MTT (4mg/ml). After incubation white bands appear on a blue background. Best seen on GDA gels.
GOT	TM pH7.8 1 hr. 15 mins.	1.0ml 0.1M Tris HCl pH8.0, 0.1ml Aspartic acid (70mg/ml), 0.1ml αketoglutarate (25mg/ml), 0.1ml Pyridoxal phosphate (5mg/ml), 0.1ml Fast Violet (20mg/ml).
GPT	TM pH7.8 250V, 1 hr. 30 mins	1.0ml 0.1M Tris HCl 0.1ml DL-Alanine (53mg/ml), 0.1ml α-ketoglutarate (25mg/ml), 0.1ml NADH (10mg/ml), 1.0 I.U. Lactate dehydrogenase. View under UV light.
PK	TM pH7.8 250V, 1 hr.	As for AK, but with 0.1ml Phosphoenol pyruvate added.
AK	TM pH7.8 250V, 1 hr.	1ml 0.1M Tris HCl pH8.0, 0.1ml ADP (10mg/ml), 0.1ml 0.1M $MgCl_2$, 0.1ml Glucose (40mg/ml), 0.1ml NADP (10mg/ml), 0.1ml MTT (4mg/ml), 0.1ml PMS (2mg/ml), 2I.U. Glucose-6-phosphate dehydrogenase, 2I.U. Hexokinase.

Table 5.2 cont.

Enzyme	Running Conditions	Stain
PGK	TM pH7.8 250V, 1 hr. 30 mins.	1.0ml 0.5M Tris HCl pH7.8, 0.1ml 3-phosphoglycerate (50mg/ml), 0.2ml ATP (30mg/ml), 0.1ml 0.1M $MgCl_2$, 0.1ml NADH (20mg/ml), 2I.U. Glyceraldehyde-3-phosphate dehydrogenase. Monitor under UV.
PGM	TM pH7.8 250V, 1 hr. 20 mins.	1.0ml Tris HCl pH8.0, 0.1ml Glucose-1-phosphate (25mg/ml + 0.1mg Glucose 1, 6-diphosphate), 0.1ml NADP (10mg/ml), 0.1ml 0.1M $MgCl_2$, 2I.U. Glucose-6-phosphate dehydrogenase, 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml).
PEP	TM pH8.2 250V, 55 mins.	0.5ml 0.1M Tris HCl pH7.4, 0.1ml l-leucyl-glyclglycine (25mg/ml), 0.1ml Amino acid oxidase (5mg/ml), 0.1ml Peroxidase (5mg/ml), 0.1ml O-Dianisidine HCl (25mg/ml)
GDA	TEB pH7.8 45 mins.	1.0ml 0.1M Tris HCl pH7.6, 0.04ml Guanine (25mg/ml. 0.5M NaO4) 0.6I.U. Xanthine oxidase, 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml).
ADA	0.015M TM pH7.2 250V, 1½-2 hrs.	1ml 0.05M Phosphate buffer pH7.5, 0.1ml Adenosine (25mg/ml), 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml), 0.6I.U. Xanthine oxidase, 0.3I.U. Nucleoside phosphorylase.
FUM	TM pH7.8 250V, 1 hr. 15 mins.	1ml 0.05 Phosphate buffer pH7.5, 0.2ml Fumaric acid (neutralized, 25mg/ml), 0.2ml NAD (10mg/ml), 0.2ml PMS (2mg/ml), 0.2ml MTT (4mg/ml), 2I.U. Malate dehydrogenase.
MPI	TM pH7.8 250V, 1 hr. 30 mins.	1.0ml 0.1M Tris HCl pH7.5, 0.1ml Mannose phosphate (25mg/ml), 0.1ml 0.1M $MgCl_2$, 0.1ml NADP (10mg/ml), 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml), 2I.U. Glucose phosphate isomerase, 2I.U. Glucose-6-phosphate dehydrogenase.
GPI	TM pH7.8 250V, 1 hr. 50 mins.	1.0ml Tris HCl pH7.0, 0.1ml Fructose-6-phosphate (25mg/ml), 0.1ml NADP (10mg/ml) 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml), 2I.U. Glucose-6 phosphate-dehydrogenase.

(iii) there be no reason to doubt the underlying genetic basis of the variation. Although breeding experiments represent the ultimate such confirmation, inheritance patterns in all the enzymes studied have been established for humans (Harris and Hopkinson, 1977) and given the conservatism in phenotypic expression across vertebrate groups, have been assumed to apply here unless obvious grounds for doubt existed.

Patterns of variation in three presumed loci, G-6-PD, XO and AK_1, were difficult to interpret unambiguously due to variable breakdown. As a result, these were not included.

An exception was made to criterion (i) with the presumed ADH locus, where expression in more "advanced" forms (Sardini, Thunnini) was consistently weaker and has some potential as a taxonomic character. ADH was included but scored in only 9 of the 19 species.

Application of these criteria left a total of 26 presumed homologous loci common to all species and 27 to 9. Brief description of the phenotypic variation encountered at each of these loci follows (Table 5.3). In all cases where genetic polymorphism was detected, phenotype proportions were consistent with Hardy-Weinberg expectations. A conservative approach was adopted at all times, i.e. clear banding patterns were required to score heterozygotes as such. Consequently, H values are more likely to represent underestimates of actual levels of variation rather than overestimates.

In view of the many esterase loci active in liver samples and the complex interactions between these loci, no esterase has been included in the study. In deference to the widespread use of serum esterase as a marker in population studies however, available blood samples were analyzed and comparisons made with published findings (Table 5.4). Variation at this locus was found to be common among the scrombrid species, but not ubiquitous.

Table 5.3 Phenotypic variation encountered at the 27 presumed loci studied

Enzyme	E.C. No.	No of loci	Variation	Structure	Comments
OXIDOREDUCTASES (12)					
Alcohol dehydrogenase (ADH)	1.1.1.1.	1	P	dimeric	Strongest activity with primary alcohols as substrate; weak activity in most of the Sardini & Thunnini; indistinct faster bands on most gels, possibly corresponding to other alcohol-oxidising enzymes (Chambers *et al.*, 1978)
Glycerol-3-phosphate (GPD) dehydrogenase	1.1.1.8	1	P	dimeric	
Sorbitol dehydrogenase (SORDH)	1.1.1.14	1	P	dimeric	Presumably tetrameric in all species; as the mobility of SOD is almost identical in most species, heterozygote banding patterns were not clear in some cases.
Lactate dehydrogenase (LDH)	1.1.1.27	2	P	tetrameric	Two loci, presumably coding for two polypeptide chains, with a pattern of 3-5 isozymes produced as a result; rare heterozygotes at either locus in the Scombrini.
Malate dehydrogenase (MDH)	1.1.1.37	1-2	M	tetrameric?	Often seen as five-banded phenotypes, possibly hetero-polymers, between an active slower locus & a weaker (mitochondrial) locus, the latter weak or absent in the Thunnini. No variation observed; scored as one locus.
Malic enzyme (ME)	1.1.1.40	2	P	tetrameric	Two loci, probably determining soluble (ME_1) & mitochondrial (ME_2) forms; the faster locus more active, but difficult to obtain sharp resolution.
Isocitrate dehydrogenase (ICD)	1.1.1.42	1		dimeric	A second slower locus, plus heteropolymers, often expressed but not scored.
6-phosphogluconate dehydrogenase (PGD)	1.1.1.44	1	P	dimeric	
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	1.2.1.12	1	M	tetrameric	A weaker faster band of activity not included.
Superoxide dismutase (SOD)	1.15.1.1	1	M	dimeric	Weak, faster band in some species not scored; dimeric heterozygotes reported for T. *thunnus thynnus*; no clear variation observed, but more than one band in some *S.multiradiatus* samples.
TRANSFERASES (9)					
Aspartate aminotransferase (GOT)	2.6.1.1	2	P	dimeric	In S.*commerson* & S.*queenslandicus*, more complex phenotypic patterns, with presumed homozygotes three-banded & heterozygotes nine-banded.
Alanine aminotransferase (GPT)	2.6.1.2	1	M	dimeric	
Pyruvate kinase (PK)	2.7.1.40	2	M	monomeric	Positive stain also visualizes AK, therefore scored by comparison with gel stained for AK only; no clear variation observed, but PK, locus possibly polymorphic in A.*thazard*.
Phosphoglycerate kinase (PGK)	2.7.2.3	1	M	monomeric?	
Adenylate kinase (AK)	2.7.4.3	1	P	monomeric	A faster locus with variable breakdown patterns not scored.
Phosphoglucomutase (PGM)	2.7.5.1	2	P	monomeric	Two zones of activity with a weak intermediate band, possibly a third locus. PGM_2 more active.
HYDROLASES (3)					
Peptidase (PEP)	3.4.1.1	1	P	dimeric	
Guanine deaminase (GDA)	3.5.4.3	1	P	dimeric	Typically with forward breakdown bands.
Adenosine deaminase (ADA)	3.5.4.4	1	P	monomeric	In S.*commerson* & S.*queenslandicus*, as with GOT, complex breakdown bands. Second locus not scored (see text).
LYASES (1)					
Fumarase (FUM)	4.2.1.2	1	P	tetrameric	
ISOMERASES (2)					
Mannose phosphate isomerase (MPI)	5.3.1.8	1	P	monomeric	
Glucose phosphate isomerase (GPI)	5.3.1.9	1	P	dimeric	A second slower locus weakly expressed & not scored, but active in blood of most Thunnini.

Table 5.4 Levels of variation observed in serum esterases of various scombrids, based on published studies & analyses performed by the author.

Species	n	Common allele frequency	No. of alleles	Area	Source
Scomber scombrus	3593	.37 - .75	5+	North Sea	Jamieson *et al.*, 1971
Scomberomorus commerson	288	.91	4	PNG/N.Aust.	Sharp, pers. comm. (confirm. by author)
Acanthocybium solandri	51	.50 - .64	3	Hawaii/Aust.	Author.
Sarda australis	45	.811	3	Aust.	Author.
S. orientalis	50	1.0	1	W. Aust	Author.
Euthynnus affinis	50	(1.0)	1	Hawaii	Author.
Auxis thazard	33	.955	3	Aust.	Author.
Katsuwonus pelamis	>40,000	.3 - .85	4+	All oceans	See Chapter 4.
Thunnus tonggol	127	.84	4	Aust.	Author.
T. albacares	65	.985	3	Hawaii	Fujino & Kang, 1968.
"	46	.957	2	"	Sprague, 1967.
"	>14,000	.952 - .986	4	Pacific	Sharp, MS
T. alalunga	84	.952	3	Atlantic	Fujino & Kang, 1968.
"	175	.954	2	"	Serene, 1971.
"	22	.86	3	E. Aust.	Author.
"	103	.961	2	Pacific	Fujino.
T. obesus	184	.917	3	Hawaii	Sprague, 1967.
"	107	.935	2	"	Fujino & Kang, 1968.
T. maccoyii	70	.809	4	Aust.	Sprague, 1967.

5.4 RESULTS

5.4.1 Levels of heterozygosity

In Table 5.5, heterozygosity values at individual loci are listed for each of the 19 scombrids where acceptable numbers of individuals (>13) were screened. Mean heterozygosities at each locus were obtained by directly averaging these values across the species array. For comparative purposes, heterozygosity values for the two non-scombrid species, plus the two scombrids collected in smaller numbers, are also listed.

Observed polymorphism is clearly not distributed uniformly across loci - 7 of the 27 were monomorphic (i.e. invariant across the species array) and a majority of the overall variation resides at five loci:- GDA (.264), ADA (.224), GPI (.214), PGM_1 (.126) and ADH (.123).

In Table 5.6, $\bar{H}$, $\bar{H}_i$ and %P (at the 95 and 99% levels) have been calculated for all species. $\bar{H}$ (and $\bar{H}_i$) values range from .013 *(Sarda australis)* to .109 *(A. solandri)* and %P (95) values from 0 to 26. Although heterozygosity per individual shows a unimodal distribution within each of the 19 primary species (the frequency of individuals with various numbers of loci heterozygous were plotted for each species to check this), Bartlett's test of homogeneity of variances showed the variance about $\bar{H}_i$ to be significantly heterogenous amongst species ($\chi^2_{18} = 73.6$, $P < .01$). A contingency table, rather than analysis of variance, was therefore used to test the species for homogeneity of H values. A 4 x 19 table (number of individuals with loci heterozygous in four frequency classes 0, 1, 2, 3+ for each species) revealed high heterogeneity ($\chi^2_{54} = 198.59$, $P < .005$).

This heterogeneity is not attributable to any one scombrid tribe as it is also observed within most tribes, *viz. Scomberomorini* ($\chi^2_{18} = 56.12$, $P < .005$), *Thunnini* ($\chi^2_{18} = 72.36$, $P < .005$), *Sardini* (3 x 3 table, since a zero in class 3+ otherwise - $\chi^2_4 = 13.63$, $P < .01$) and *Scombrini* ($\chi^2_3 = 5.8$, $P < .10$). The residual variance obtained by subtracting within-tribe from total χ^2 values indicates that between-tribe variance is considerable but not significant ($F_{11,43} = 1.34$, $P < .05$).

Subsequent analysis clearly therefore needs to consider <u>inter-locus</u> and <u>inter-specific</u> aspects of variation.

Primary spp.	GPD	SORDH	LDH_1	LDH_2	MDH	ME_1	ME_2	ICD	PGD	GAPDH	SOD	GOT_1	GOT_2	GPT	PK_1	PK_2	AK_2	PGK	PGM_1	PGM_2	PEP_2	GDA	ADA	FUM	MPI	GPI	ADH	n	Other loci polymorphic
1	0	.14	0	.03	0	.11	0	.03	.07	0	0	0	0	0	0	0	0	0	.18	0	.57	0	.03	0	.03	.07	28	PEP_1, ADA_2
2	.29	0	.03			.03	0	0	0			0	.03						.11	.03	.07	.07	0	.21	.03	.11	28	PEP_1
3	0	0				0	0	.07	.07			0	0						.07	0	0	0	.07	.40	0	.07	15	
5	0	.04				0	.04	.12	.39			0	0						.15	.04	.08	0	.77	.04	.04	.31	.15	26	ADA_2
6	.32	.08				0	0	0	0			.60	.04						.16	.08	0	.12	0	0	0	.12	.24	25	PEP_1
7	0	0				0	0	.20	0			0	0				.53		.27	0	.07	.13	.20	.13	.07	.07	0	15	PEP_1
8	0	.10				0	0	0	0			0	0						0	0	0	.40	0	0	0	.50	0	20	
9	0	.13				0	.07	.13	0			.13	0						.07	0	0	.07	0	0	0	0	0	15	1
10	.11	.05				0	.32	.05	.05			.05	0						.16	0	0	.53	.53	0	0	.63	.47	19	PEP_1
11	0	0				0	.08	.08	0			0	0						.08	0	0	0	0	0	0	.04		25	ADA_2
12	.04	0				0	.24	.04	.16			.04	0						.24	0	0	.24	0	0	0	.04		25	PEP_1
13	.38	0				.15	0	0	0			0	.23						0	0	0	0	.08	0	.15	0		13	PEP_1
15	.04	0				.60	0	0	0			0	0						0	.04	.08	.12	.12	0	0	.64		25	ADA_2
16	0	0				0	0	.03	.35			0	0						.07	0	0	.35	.14	0	0	.10		29	ADA_2
17	.04	.02				0	0	.04	.04			.02	0						.02	0	0	.38	.12	0	.08	.04		50	ADA_2
18	0	0				.04	0	.04	0			0	0						0	0	.28	.60	.56	0	0	.36		25	ADA_2
19	0	.04				0	0	.08	.04			.08	0						.19	.04	0	.39	.62	.04	.04	.15		26	
21	0	.18				.09	0	0	0			0	0						.05	.59	0	.59	.64	.05	0	.41		22	ADA_2
22	0	.08				0	0	0	.44			0	0						.40	.04	.04	.56	.40	.04	.04	.60		25	
MEAN	.064	.045	.001	.001	0	.054	.039	.048	.085	0	0	.048	.016	0	0	0	.028	0	.126	.045	.055	.269	.224	.021	.054	.214	.123	24	
Other spp.																													
24	0	0	0	0	0	(.12)	0	.04	0	0	0	0	0	0	0	0	0	0	.08	0	0	0	.56	.04	.04	.17	0	24	
25	0	0		(.07)	0	0	0	.07	0	0	0	0	0	0	0	0	0	0	.67	0	.07	0	0	0	0	.14	0	14	
4	0	0	0	0	0	0	0	.14	0	0	0	0	.14	0	0	0	0	0	0	0	0	0	0	.43	0	0	(.14)	7	ADA_2
20	0	.14	0	0	0	0	0	.42	0	0	0	0	0	0	0	0	0	0	0	0	0	.14	0	0	.14	.28	NA	7	ADA_2

Table 5.5 Heterozygosity values per locus for the 19 primary species and four additional species.

Species are coded as below, and other loci known to be polymorphic but not included in the analysis are listed.

1. *Scomber australasicus*
2. *R. kanagurta*
3. *Grammatorcynus* sp. A
4. *Grammatorcynus* sp. B
5. *Scomberomorus commerson*
6. *S. queenslandicus*
7. *S. multiradiatus*
8. *S. semifasciatus*
9. *S. munroi*
10. *Acanthocybium solandri*
11. *Sarda australis*
12. *S. orientalis*
13. *Cybiosarda elegans*
14. *Gymnosarda unicolor*
15. *Auxis thazard*
16. *Euthynnus affinis*
17. *Katsuwonus pelamis*
18. *Thunnus albacares*
19. *T. tonggol*
20. *T. obesus*
21. *T. alalunga*
22. *T. maccoyii*
23. *T. t. orientalis*
24. *Makaira indica*
25. *Elegatis bipinnulatus*

Table 5.6 Mean heterozygosities per locus per individual ($\bar{H}$), mean heterozygosities per individual per locus ($\bar{H}_i$), and percentage of loci polymorphic at two levels, for all species, with confidence limits for the $\bar{H}$ and $\bar{H}_i$ values.

Species	N	$\bar{H}_i$	$\bar{H}$	%P (95)	%P (99)
Scomber australasicus	28	.063 ± .055	.063 ± .170	19	41
Rastrelliger kanagurta	28	.040 ± .043	.040 ± .121	19	44
Grammatorcynus sp.A	15	.028 ± .027	.028 ± .080	4	
(G. sp. B)	(7)	(.032 ± .033)	(.032 ± .033)		
Scomberomorus commerson	26	.080 ± .051	.080 ± .169	22	44
S. queenslandicus	25	.065 ± .052	.065 ± .134	22	33
S. munroi	15	.052 ± .052	.052 ± .074	26	41
S. semifasciatus	20	.037 ± .021	.037 ± .121	11	11
S. multiradiatus	15	.030 ± .021	.030 ± .108	7	15
Acanthocybium solandri	19	.109 ± .049	.109 ± .196	26	41
Sarda australis	25	.011 ± .018	.011 ± .026	0	15
S. orientalis	25	.040 ± .037	.040 ± .083	15	31
Cybiosarda elegans	13	.038 ± .041	.038 ± .090	15	19
Auxis thazard	25	.064 ± .033	.064 ± .165	15	27
Euthynnus affinis	29	.040 ± .034	.040 ± .097	15	23
Katsuwonus pelamis	50	.031 ± .031	.031 ± .076	8	35
Thunnus albacares	25	.072 ± .038	.072 ± .168	15	23
T. tonggol	26	.061 ± .052	.061 ± .140	15	42
(T. obesus)	(7)	(.050 ± .041)	(.050 ± .103)	(19)	(19)
T. alalunga	22	.102 ± .044	.102 ± .196	19	35
T. maccoyii	25	.102 ± .046	.102 ± .188	19	38
MEAN (19 spp.)	24	.056	.056	16	31
BLACK MARLIN	15	.040 ± .022	.040 ± .131	7	22
RAINBOW RUNNER	24	.040 ± .035	.040 ± .112	11	30

5.4.2 Correlation of heterozygosity with enzyme structure and function

Table 5.7 lists, for the 27 loci studied, the following values:

(i) $\bar{H}_1$ and % P (95), obtained from Table 5.6.

(ii) quaternary structure.

Where no heterozygotes were observed (PK_1, PK_2, GPT, PGK), the quarternary structure as given by Harris and Hopkinson (1977) for humans was assumed to apply, as agreement had been found with all other enzymes.

(iii) function I - whether enzymes are glucose-metabolizing (G) or non-glucose metabolizing (NG) (Gillespie and Kojima, 1968).

(iv) function II - whether enzymes are designated by Johnson (1974) as being variable substrate (V), regulatory (R) and non-regulatory (NR). Following his usage, enzymes were classifed as non-regulatory when reported substrate/product ratios (Barman, 1969, 1974) did not deviate by greater than one order of magnitude from equilibrium.

(v) function III - classification of enzymes according to the type of reaction catalyzed (i.e. dehydrogenases, hydrolases, isomerases, and so on - see Table 5.3).

Enzyme quaternary structure

Comparison amongst structural groups show monomers and dimers to be significantly more polymorphic than tetramers in scombrid species (Table 5.8). Monomers were not significantly more variable than dimers and accordingly monomers were no more variable as a group than multimers.

Table 5.8 Relationship between enzyme heterozygosity and quaternary structure

		Mann-Whitney U Test Value
Monomer vs dimer	(6, 12)	36 (NS)
Monomer vs. tetramer	(6, 9)	12 ($P < .05$)
Dimer vs. tetramer	(12, 9)	29 ($P < .01$)
Monomer vs. multimer	(6, 21)	50 (NS)

Table 5.7 Levels of variation observed at the enzyme loci studied and their structural and functional characteristics.

Abbreviations as follows:- G - glucose-metabolizing; NG- non-glucose metabolizing; V - variable substrate; R - regulatory; NR- non-regulatory; O-R - oxido-reductases; T - transferases; H - hydrolases; L - ligases; I - isomerases

Enzyme	$\bar{H}_i$	P(95)	Structure	Function I	Function II	Function III
GPD	.064	21	dimeric	G	R	O-R
SORDH	.045	21	tetrameric	NG	NR	
LDH_1	0	0	tetrameric	G	NR	
LDH_2	.003	0	tetrameric	G	NR	
MDH	0	0	tetrameric	G	NR	
ME_1	.054	16	tetrameric	G	R	
ME_2	.039	11	tetrameric	G	R	
ICD	.048	16	dimeric	G	NR	
PGD	.085	21	dimeric	G	NR	
GAPDH	0	0	tetrameric	G	R	
SOD	0	0	dimeric	NG	NR	
GOT_1	.051	11	dimeric	G	NR	T
GOT_2	.016	5	dimeric	G	NR	
GPT	0	0	dimeric	G	(R)	
PK_1	0	0	tetrameric	G	R	
PK_2	0	0	tetrameric	G	R	
AK_2	.028	0	monomeric	NG	R	
PGK	0	0	monomeric	G	R	
PGM_1	.126	47	monomeric	G	R	
PGM_2	.045	5	monomeric	G	R	
PEP_2	.055	11	dimeric	NG	V	H
GDA	.269	68	dimeric	NG	NR	H
ADA	.224	53	monomeric	NG	R	H
FUM	.021	5	tetrameric	G	NR	L
MPI	.054	16	monomeric	G	NR	I
GPI	.214	53	dimeric	G	R	I
ADH	.12(9)	44	dimeric	NG	R	OR

Because of lack of information, it was not possible to investigate the relationship reported between subunit size and $\bar{H}$ (Harris *et al.*, 1977), and molecular weight and $\bar{H}$ (Koehn and Eanes, 1977; Leigh Brown and Langley, 1979; Nei *et al.*, 1978).

Enzyme function

Two hypotheses maintain that the degree of polymorphism observed in a particular enzyme is related to:

(a) environmental variation in its substrate(s). Gillespie and Kojima (1968) suggest that substrates of non-glucose metabolizing enzymes frequently originate externally and that this may be reflected in greater variability.

(b) involvement in regulatory reactions. Johnson (1974) suggests that enzymes exerting control over flow through metabolic pathways should be most sensitive to the action of selective forces and therefore more variable.

Comparison of overall $\bar{H}$ levels at loci classified as G versus NG and V, R and NR respectively (Table 5.6) provide tests of these hypotheses.

Non-glucose metabolising enzymes showed greater variation at a level verging on significant (one-tailed Mann-Whitney U test, .10 >P>.05; corrected for ties, z = 1.62 and P = .052), in accordance with the first hypothesis. In the latter case, regulatory enzymes (R) were not significantly more variable than non-regulatory ones (NR) (one-tailed U = 78.5, NS). As only one variable substrate (V) enzyme was studied, V-R and V-NR comparisons were not possible.

The 27 enzyme loci were not equally distributed with regard to the type of reaction catalysed (Function III in Table 5.6), reducing the power of the test used. However, it seems likely that some of the variation observed may be related to enzyme class (H^* = 9.02, .10 >P> .05).

5.4.3 Correlation of heterozygosity with species characteristics

Where the biology of species is poorly known, as is frequently the case with scombrid fishes, it is difficult to define characters which

*H = Kruskal - Wallis one-way analysis of variance by ranks statistic.

at once reflect the essence of particular hypotheses as well as being amenable to ordination or quantification across the range of species examined. The following descriptors have been defined for consideration.

(1) *taxon*

The four tribes which show some internal consistency in species ecology and biology (see earlier) suggest themselves as a suitable test of whether or not $\bar{H}$ varies amongst taxa above the species level.

(2) *maximum size*

This is defined in weight rather than length terms, as body shape shows some variation within the family; it should provide a test of Selander and Kaufman's (1973) contention that large mobile animals have lower levels of $\bar{H}$ than small, less mobile animals. Since all scombrids can be considered highly mobile in teleost terms, the test is only a partial one.

(3) *trophic breadth (adults)*

Although available data indicate that the scombrid diet is varied, Magnuson and Heitz (1971) have demonstrated that there is some selectivity associated with gill raker gap which is in turn directly proportional to the number of gill rakers. A mean gill raker number obtained from the taxonomic literature for each species has been used as an index of trophic breadth, as all species take large and small prey items down to the size retained by the minimum gill raker gap. A mobile species with a large number of gill rakers can therefore ingest food of the widest size range. Some correction for fish size might improve the value of this descriptor.

(4) *vagility*

As available data is inadequate to directly assess vagility, an index was devised, based on the sum of two variables:

(i) maximum size on a scale 1-4 (0-5 kgs = 1, 6-25 = 2, 25-50 = 3, >50 = 4).

(ii) degree of internalization of red muscle/development of heat exchangers (and hence capacity for sustained cruising) on a scale 1-4 (1 = primitive lateral wedge *(Scombrini, Scomberomorini)*, 2 = lateral wedge extending between epaxial and hypaxial muscle blocks *(Sardini)*,

3 = internalized red muscle, complete dorsal aorta, lateral and central heat exchangers (*Auxis, Euthynnus, Katsuwonus, T. albacares, T. tonggol*), 4 = deeply internalized red muscle, dorsal aorta vestigial, lateral heat exchangers only (other *Thunnus* spp.)

Although presence or absence of a swim bladder and certain hydrodynamic features might also be incorporated, the indices correlate well with the extent of known migratory abilities.

(5) *geographical range*

Scombrids provide a poor test of any latitudinal gradient in observed variables for several reasons. Firstly, their habitat is three-dimensional, and species with quite different preferred temperatures can exist at the same latitude but at different depths. Nearly all scombrid species spawn in tropical or subtropical areas and accordingly spend some part of their life there. However many pass other life history phases elsewhere. Species range has therefore been expressed on a geographical scale of 1-4, *viz.* 1 = cosmopolitan (all oceans), 2 = tropical and sub-tropical Indo-Pacific, 3 = northern Australia, 4 = localized distribution.

Note that this index relates to the species rather than the population, as population structure remains inadequately known for most species. *Grammatorcynus* sp. A has not been included as its range requires reappraisal in the light of findings discussed in Chapter 6.

(6) *habitat*

Shubnikov (1974) has defined five ecological groups within the Scombridae on the basis of schooling characteristics and feeding strategy. Four of these are applicable to the present species.

1. neritic species feeding on plankton and small schooling fishes and occurring in large schools.

2. neritic predators feeding more on schooling fish and cephalopods, also occurring in larger aggregations.

3. neritic predators feeding on schooling and solitary fishes, cephalopods and crustaceans and forming sparse small schools.

4. neritic-oceanic and oceanic species feeding both on schooling and solitary organisms and forming smaller schools in oceanic areas, but dense aggregations in productive inshore areas.

On the basis of personal observations, several of the species have been reclassified here, with *Cybiosarda* going from Shubnikov's group 3 to 2 and *T. tonggol* from 2 to 3. Contrary to Shubnikov's assertion, wahoo *(A. solandri)* does not sit easily in group 3 (or any other group) and has initially not been included.

(7) *other descriptors*

Whilst it would be desirable to have adequate descriptions of other variables, particularly neutralist terms such as N_e, T etc. but also measures of trophic stability, larval ecology etc., this has not been possible.

Table 5.9 lists $\bar{H}$ and the various indicators for each of the 19 primary species. Observed levels of variation proved to be taxon-independent at the tribe level (Kruskal-Wallis H = 2.28, P > 0.50), as determined previously and unrelated to gill raker number (Spearman Rank Correlation Coefficient (r_s) = .066, t = .272, NS) and habitat (Kruskal-Wallis H = 3.04 P ~0.4). Adding wahoo to habitat category 3 did not alter the last result (H = 3.0, P ~0.4). Heterozygosities were however positively correlated with maximum size (r_s = 0.6, P < .01), vagility (H = 6.5, P < .05) and geographical range (Kruskal-Wallis H = 9.15, P < .05).

These three variables (maximum size, vagility and geographical range) are interdependent to some degree and their relationship with $\bar{H}$ levels demonstrates that large highly mobile species with wide geographical distribution exhibit the highest levels of $\bar{H}$ within the family.

5.5 DISCUSSION

The average $\bar{H}$ observed across the 19 primary scombrid species (.056, Table 5.6) is within the range of average values described for teleosts by other workers (see below).

$\bar{H}$	No. of species/ populations	Loci per species	Source
0.078 ± 0.012	14	21	Selander, 1976
0.058 ± 0.006	31	NA	Powell, 1975
0.051 ± 0.034	51	NA	Nevo, 1978
0.048 ± 0.033	82	NA	Winans, 1980

Table 5.9 Heterozygosity values and descriptors of species character for the 19 primary scombrid species. The results of statistical tests for correlation between H and the various descriptors are indicated.

Species	$\bar{H}$	Taxon	GR	Max.size	Vagility	Range	Habitat
Sc. australasicus	.063	1	37	0.7	2	2	1
R. kanagurta	.040	1	48	0.5	2	2	1
G. sp.A	.028	2	14	11	3	-	3
S. commerson	.080	2	6	60	5	2	3
S. queenslandicus	.065	2	7	8	3	3	3
S. multiradiatus	.030	2	22	0.3	2	4	3
S. munroi	.052	2	12	8	3	3	3
S. semifasciatus	.037	2	10	10	3	3	3
A. solandri	.109	2	0	70	5	1	(3)
Sa. australis	0.11	3	11	9	4	4	2
Sa. orientalis	.040	3	20	9	4	2	2
C. elegans	.038	3	14	2	3	3	(2)
A. thazard	.064	4	40	3.5	4	1	(2)
E. affinis	.040	4	31	14	5	2	2
K. pelamis	.031	4	58	22	5	1	4
T. albacares	.072	4	30	180	7	1	4
T. tonggol	.061	4	23	35	6	2	3
T. alalunga	.102	4	28	42	7	1	4
T. maccoyii	.102	4	35	140	8	1	4
Statistical test result		(NS)	(NS)	*	*	*	(NS)

Ayala and Valentine (1979) have noted that there is a tendency to see the pelagic environment as unusually homogenous, with this reduced spatial heterogeneity having consequences for genetic variability. The $\bar{H}$ values observed across the 19 scombrid species here, plus those observed for the same loci in the two non-scombrid epipelagic species, black marlin ($\bar{H}$ = .040) and rainbow runner ($\bar{H}$ = .040), and the krill species studied by Ayala and Valentine (1979), suggest that neither unusually high or low $\bar{H}$ values are typically associated with the epipelagic zone.

The range of $\bar{H}$ values (.011 - .109) is comparable to the range given by Nevo (1978) for a variety of marine and freshwater teleosts (.006 - .180) and is consistent with his finding that levels of variation may differ as much within taxa as between taxa.

The variation observed was not randomly distributed across loci, with five loci (GDA, ADA, GPI, PGM and ADH) accounting for a disproportionate amount of this variation. Johnson & Mickevich (1976) similarly found GPI, ADA, PGM along with Est to be the most variable enzymes, in that order, across populations of five *Menidia* (Atherinidae: Teleostei) species (GDA and ADH were not studied), suggesting that enzyme characteristics (structure, function) may be implicated.

Examination of $\bar{H}$ values relative to enzyme structure showed that levels of variation observed are likely to be influenced by the relative proportions of monomers, dimers and tetramers examined. Perhaps even more important may be the proportion of multimers which form inter-locus hybrid molecules (Harris *et al.*, 1977), although this could not be tested. Such variation most likely arises independently of environmental influences and is consistent with neutralist theory.

Relating enzyme function to $\bar{H}$ levels proved less satisfactory. Functions have been assigned to enzymes here, particularly those in the R vs. NR categories, on the basis of *in vitro* experiments with other animal groups, and confirmation of the suggested function *in vivo* is generally lacking. Even accepting Johnson's (1971, 1974) classification, which has been subject to criticism (Selander, 1976), regulatory enzymes were found to be no more variable than non-regulatory ones. Non-glucose metabolizing enzymes, whose substrates are more likely to originate externally, were however found to show greater, (though not quite

significant) variability than glucose-metabolizing enzymes, (.10 >P>.05) in accordance with the predictions of Gillespie and Kojima (1968) and Kojima *et al.*, (1970). The external environment may therefore exert some influence in shaping levels of heterozygosity at individual scombrid loci.

Some of the variation observed may also be related to enzyme class (.10 >P .05) but it is not clear why this should be so. An understanding of the kinetics of particular enzymes may ultimately be required to explain the apparent predisposition of certain loci to allelic variation. Richardson (in prep.) has to date, examined three invariant enzymes (LDH, MDH, GAPD) in four scombrid species but similar analysis of the products of polymorphic loci will be required to evaluate the above results.

Enzyme structure and function may therefore influence the amount of variation observed through the choice of loci studied. With the relatively low proportion of NG (7/27) and V (1/27) loci examined, it could be argued for example that the absolute $\bar{H}$ values obtained in this study are underestimates. As a common suite of homologous loci is assumed to have been studied, this source of variation cannot however be expected to explain the considerable inter-specific variation in $\bar{H}$, and it is necessary to consider alternative explanations for this aspect of variation.

Soulé (1976) classifies the many selectionist hypotheses which attempt to relate levels of genetic variation to environmental heterogeneity into three groups:

(a) environmental grain (spatial) (Levene, 1953; Selander and Kaufman 1973)

(b) resource predictability (Valentine, 1971; Ayala and Valentine, 1978; Valentine and Ayala, 1978)

(c) environmental amplitude (temporal) or niche width (Dobzhansky, 1951; Soulé, 1974).

Despite the difficulty in defining suitable environmental and biological descriptors, one general result - (higher $\bar{H}$ values in large

highly mobile species with wide geographical range) was obtained. This, however, stands in direct contrast to predictions from environmental grain theory, i.e. lower $\bar{H}$ in large highly mobile species with greater homeostatic control - these perceive the environment as 'fine grained'. It also contradicts predictions from the related resource predictability theory, as enunciated by Valentine (1976) *viz.* higher $\bar{H}$ in least mobile species, low $\bar{H}$ in large mobile predators, higher $\bar{H}$ in more trophically specialized species and so on. The morpho-physiological specializations of the advanced scombrids are presumably related to the efficient exploitation of patchy, broad-spectrum food (and possibly other) resources. Whilst such adaptations may serve to reduce patchiness, it is difficult to regard the food resources of particularly the nomadic elements of tuna populations as either predictable or dependable and under these conditions low rather than high levels of variation would be predicted by resource stability theory.

The niche width variation models predict higher $\bar{H}$ levels in widespread, vagile, common species ('generalists') as an adaptive strategy for increasing fitness in spatio-temporally heterogenous or uncertain environments. Such predictions are consistent with the results obtained here, and with those of Nevo (1978) who found highest $\bar{H}$ (and P) values in 'habitat generalists'; the problem with this model is that similar predictions would be made under neutralist theory (Soule, 1976) - 'local, sedentary or rare species also have small N_e's, a high probability of bottlenecking, inbreeding or drift and a high probability of a recent origin and founder effects' and hence low $\bar{H}$. Within the Thunnini, the low $\bar{H}$ value for skipjack (.031) relative to albacore (.102) and southern bluefin tuna (.102) might, for example, be readily explained in this way. Albacore and southern bluefin are believed to have hemispheric and panmictic population structure respectively, whereas skipjack populations have been hypothesized to fit an isolation-by-distance model with a series of overlapping partially isolated populations and consequently lower H according to neutralist predictions. On the other hand, the small very abundant Scombrini have very large N_e's relative to other scombrids regardless of the amount of structuring, yet H values are not especially large.

Other neutralist-related hypotheses are equally difficult to evaluate. Although the long evolutionary history of the family Scombridae

is partially known (Danil'chenko, 1964; Shubnikov, 1974; and see Chapter 6), histories of individual species are not, and Soulé's 'time-divergence' hypothesis remains untestable. The loss of variation reported in small isolated populations (e.g. Avise and Selander, 1972) or bottleneck effects could both explain the low $\bar{H}$ values seen in the two Scombrid species with very limited ranges - *S. multiradiatus* and *Sarda australis*. Thus the difficulty of unequivocally evaluating most neutralist predictions remains a major problem, but they seem unlikely to account for all the variation observed in this case.

Nelson and Hedgecock (1980), in a study of enzyme polymorphism and adaptive strategy in 44 species of decapod crustacea similar to this one (26 loci/species, 24 individuals/locus), opted for a hybrid 'environmental heterogeneity - trophic diversity' model to explain observed variation. A specific conclusion of this model was as follows "Large marine vertebrates such as tuna and porpoises are reported to have unusually low heterozygosities .... Hybrid model-explanation: they are trophically specialized homeothermic predators on fish or squid." Basic assumptions and conclusion were both wrong in this case. Tunas in particular, with resident-nomad strategies, broad trophic spectra, life history heterogeneity (pelagic larvae, poikilotherm juveniles, neo-homeotherm adults), wide range and high vagility, appear archetypal generalists and H values as observed in this study, are generally higher than average for teleosts.

We are thus left with a hypothesis to explain observed levels of variation in scombrids which can broadly be interpreted in selectionist (niche width variation) terms but may be equally well accommodated within neutralist theory. It is this very difficulty in unequivocally excluding alternative explanations which has dogged studies such as the present one (Hedrick *et al.*, 1976). Nevo (1978) concluded that genetic polymorphism and heterozygosity are correlated with ecological heterogeneity, and that environmental heterogeneity is a major factor in maintaining and structuring genetic variation in natural populations. This attracts qualified support from the present study.

The present study has also identified and characterized genetic variation in a group attracting increasing attention from population biologists thereby paving the way for future attempts to define one aspect

of population structure of these species using the electrophoretic approach. The large, mobile, widely distributed, commercially important *Thunnus* species are the desirable choices for study, both from a genetic and fisheries management point of view. Ironically it may be that the species which harbour the highest amounts of useful electrophoretic variation, thus enabling subdivision to be detected, will, as a corollary, show the least amount of differentiation.

CHAPTER 6

BIOCHEMICAL SYSTEMATICS OF INDO-AUSTRALIAN SCOMBRIDS

6.1 INTRODUCTION

The challenge presented to classical evolutionary theory by the discovery of large amounts of genetic variation using electrophoretic techniques and the debate as to what proportion of this variation is neutral or selectively maintained (see section 4.1 for a review of these topics) has tended to obscure the value of electrophoresis as a systematic tool for both clarifying taxonomic problems and inferring phylogenetic relationships.

Avise (1975) reviews the advantages of electrophoretic data relative to the morphological and meristic data traditionally used in classical systematic studies. These include its objectivity (relative protein mobilities are scored directly), constancy (scored characters are normally independent of age, size, sex etc.) and precision (single gene products are characterized). A further important advantage relates to sample size. Gorman and Renzi (1979) have determined empirically that genetic distance estimates are hardly affected by sample size and suggest that a single individual may be used to represent a species for inter-specific comparisons, providing a large number of loci is studied. Two to five individuals per species are commonly used in systematic studies.

The electrophoretic technique does, however, have some disadvantages:

(a) Its application is restricted to living organisms;

(b) As evolutionary rates appear to vary amongst loci, the choice of loci has some influence on phylogenetic analyses and to a lesser extent, species delineation;

(c) as cryptic variation can occur within electromorphs (see earlier), identical mobilities may not represent identical amino-acid sequences;

(d) as there are a finite number of mobility states on a gel, chance events lead to identical mobility state and in practice this defines the taxonomic rank below which electrophoretic data is useful for inferring phylogenetic relationships.

In balance, however, the advantages of the technique generally outweigh the disadvantages and electrophoretic data has increasingly been used in systematic studies of a variety of vertebrate, invertebrate and plant groups, including marine teleosts (Johnson, 1975; Utter *et al.*, 1973) freshwater teleosts (Avise & Smith, 1974; Turner, 1973), and marine crustaceans (Mulley & Latter, 1980).

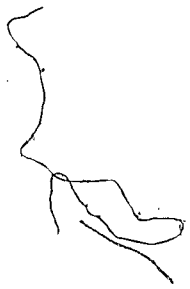
The family Scombridae, although not particularly speciose by comparison with other tropical marine teleost families, has caused taxonomists some problems in the past. Because of the demands of the epipelagic environment and a high speed mode of life, convergence in external characters is marked in this group and elucidation of intra-familiar relationships has to a large extent relied on internal characters (Kishinouye, 1923; Godsil, 1954; Gibbs & Collette, 1967). The widespread distribution of a number of scombrids has resulted in many nominal new species being created on a parochial basis, and a long list of synonomies accompanies most formal species descriptions. The most recent and widely accepted classification of the family Scombridae (Collette & Chao, 1975; Collette, 1978) is depicted in Figure 2.1. With the exception of a few species complexes (see later), species identification presents few problems nowadays and interest in the group has shifted more towards inferring relationships between taxa. Sharp & Pirages (1978) used electrophoretic comparisons of fifteen enzymes in the heart, red and white muscle of eighteen species to construct a biochemical phylogeny. Although directed primarily at the genus *Thunnus* and including a number of species (8/18) not found in the Indo-Australian region, their study did produce a phylogenetic sequence similar to that proposed by Collette (1978) from examination of anatomical characters and provided some indication of the potential of the electrophoretic approach to scombrid evolutionary systematics.

In this chapter, electrophoretic data obtained from the protein products at a presumed 26 genetic loci have been used to examine relationships amongst 23 Indo-Australian scombrids at several levels. Two cases where the discovery of intra-specific variants has challenged the validity of currently accepted species were investigated (section 6.3.1). The data are used to classify species on the basis of similarity (phenetic analysis - section 6.3.2); cladistic methods are then used to infer phylogenetic relationships from the same data (section 6.3.3), and finally the zoogeography of Indo-Australian scombrids is reviewed in the light of these findings.

6.2 MATERIALS AND METHODS

6.2.1 Material

In addition to the species analyzed in Chapter 5, liver samples were collected from another three species, dogtooth tuna *(Gymnosarda unicolor* - one individual), bigeye tuna *(Thunnus obesus* - seven individuals) and oriental bluefin tuna *(Thunnus thynnus orientalis* - one individual). Concerted efforts were also made to obtain material from the two *Grammatorcynus* morphs of unknown specific status (see section 2.1). Both the author and various north Queensland game fishermen had independently noticed differences in maximum size attained, size at first maturity, gross morphology and habitat preference in forms known commonly as "shark mackerel" (sp. A) and "scad" (sp. B). Fifteen and seven individuals respectively of these two morphs were sampled. [Collection details of this material have previously been given in Table 5.1.] Only a single sample in poor condition was obtained for slender tuna *(Allothunnus fallai)*, and it was not possible to type all enzymes for this species. No material was obtained from the primitive aberrant *Gasterochisma melampus*, the only representative of the sub-family Gasterochismatinae, or from several tropical species which are rare in Australian waters, namely *Rastrelliger brachysoma, R. faughni, Scomberomorus lineolatus* and *S. guttatus*. The coverage provided nevertheless remains comprehensive - 23 taxa in 11 nominal genera of the 29 species known from the region (see Table 2.1).



6.2.2 Electrophoretic techniques

As material for the 19 species utilized in Chapter 5 plus that from *T. obesus* and *Grammatorcynus* sp. B had already been typed during the course of examining heterozygosities, it was necessary only to run simultaneous comparisons with one individual of each species whose phenotypes at the 26 loci had already been established. A larger size cellulose acetate strip (10 cm x 20 cm) was used and samples were applied in the order listed in Table 6.1, with skipjack material (species 17) also inserted between species 8 and 9 as an additional control for scoring relative mobilities.

As outlined in the previous chapter, only those enzymes for which homologies could be established with some certainty were used. The slower LDH locus (LDH_2) was finally not included as the homotetramer band to be scored (the slowest of five) was often so weak as to introduce the possibility of error in scoring mobilities. This left 26 loci. As mentioned earlier, ADH activity was very weak in the Sardini and Thunnini, and mobilities were not available for all species.

Because of the large number of species involved, it was not possible to cross-match the mobility of every allele at all loci for all species in the available time. Primary intra-familiar relationships have therefore been based on common allele comparisons, as described by Lakovaara *et al.*, (1972) and others. Although the acquisition of particular alleles may have cladistic significance, it is felt that with the relatively large number of loci used, the amount of information lost has probably been negligible (Nei & Roychoudhury, 1974; Gorman & Renzi, 1979). The *G. unicolor* and *T. t. orientalis* material (one individual in each case) was in fair condition only and some caution has been used in interpreting results for these two species. Since visualized bands are of finite width and subject to slight retardation or advancement due to a variety of effects, for example, binding of sialic acid residues and cofactors and imperfections in the medium, bands showing any overlap and thus of the same nett surface charge, were treated as equivalent when scoring mobilities.

6.3 RESULTS

6.3.1 Observed variation and species identity

Table 6.1 lists common allele mobility states for the 23 taxa, coded alphabetically in order of decreasing mobility. Figure 6.1 illustrates the variation observed in mobility states with gels stained for ICD and GDA and Figure 6.2 for PGD and SORDH.

Loci varied considerably in the number of mobility states exhibited. None showed identical common allele mobility across the range of species but only three positions were observed at the MPI, LDH_1, PGK and AK_2 loci and only four at the ICD locus. As might be expected, the loci with the highest $\bar{H}$ values showed the maximum number of common allele positions - GDA (17), ADA (13), GPI (11), and PGM_1 (11), possibly indicating that these are evolving most rapidly and again emphasizing how the choice of loci needs to be considered when interpreting results from studies of this type.

Although a fixed allelic difference at a single locus is theoretically sufficient to establish separate specific status in sympatric diploid species, most closely related species show allelic differences at 20% - 50% of their loci (Avise, 1975). Baverstock (MS) suggests apparent fixed differences at 15% or more of loci as a rule of thumb for establishing specific status, although recognizing that exceptions do occur. Differences here between species have been expressed in common allele terms. Where loci being compared are not polymorphic such differences are also of course fixed differences, but generally the number of fixed differences between species will be less than the number of common allele differences. All taxa examined here differed in common allele terms at one or more loci. Two species pairs, yellowfin *(T. albacares)* and bigeye tuna *(T. obesus)*, and oriental bluefin *(T. t. orientalis)* and longtail tuna *(T. tonggol)*, showed common allele differences at only two loci (7.5%), and the maximum difference observed amongst the tunas was only 25%. At the other end of the spectrum, slimy mackerel *(S. australasicus)* and frigate tuna *(A. thazard)* shared no common alleles.

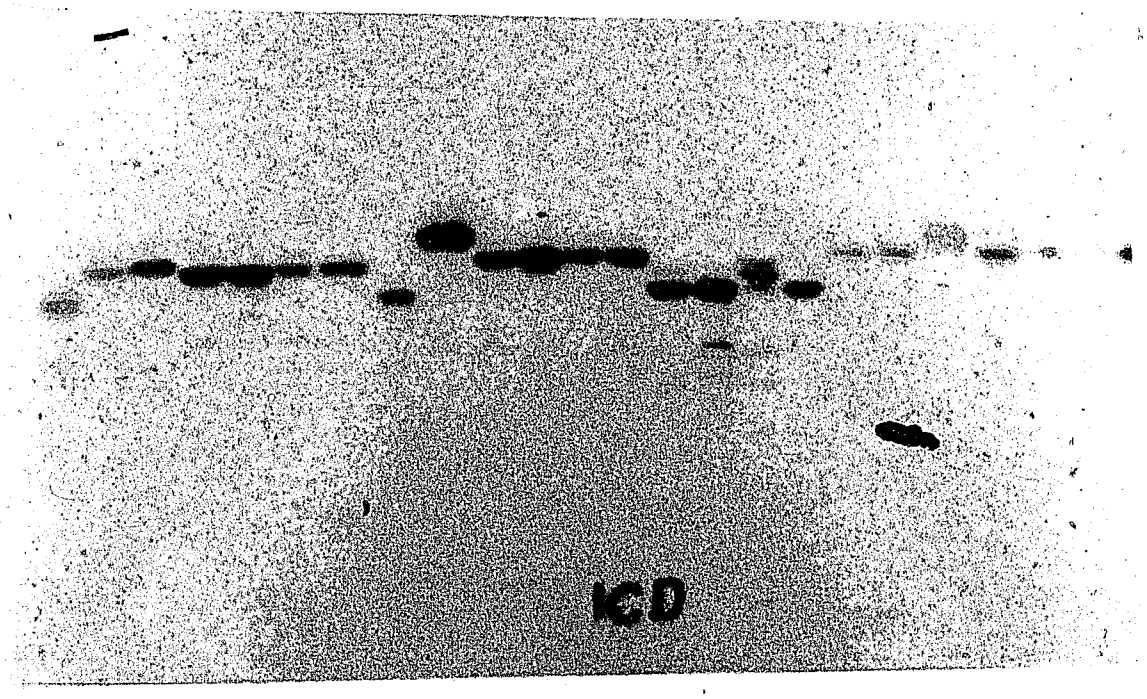
The two *Grammatorcynus* morphs showed fixed differences at six (23%) of the 26 loci (GPD, ADA, ADH, GDA, PK_2, & PGM_1) with apparent common allele frequency differences at two others, (FH, MPI). Fixed differences were also observed at several other loci not used in this study, namely ADA_2 and XO. They are clearly good species, and are now

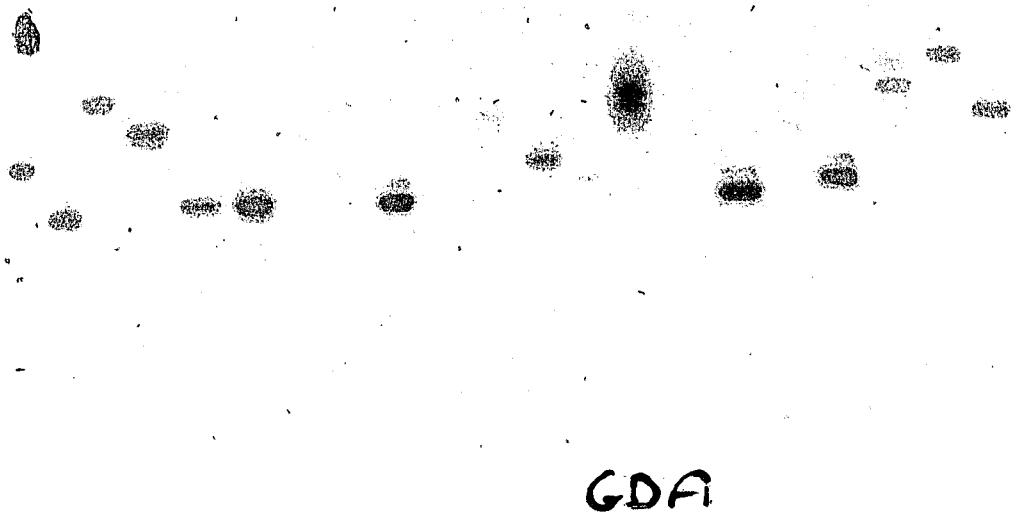
Figure 6.1 Variation in ICD mobility.

Species have been applied in the order used previously, with skipjack (17) inserted as a control between species 8 and 9 on all gels. Dimeric heterozygotes can be seen in species 16 and 20. The species 1 sample failed to stain up before fixation of the gel on this occasion.

Variation in GDA mobility.

Dimeric heterozygotes can be seen in species 10, 13, 18 and 21.


ICD


GDAI

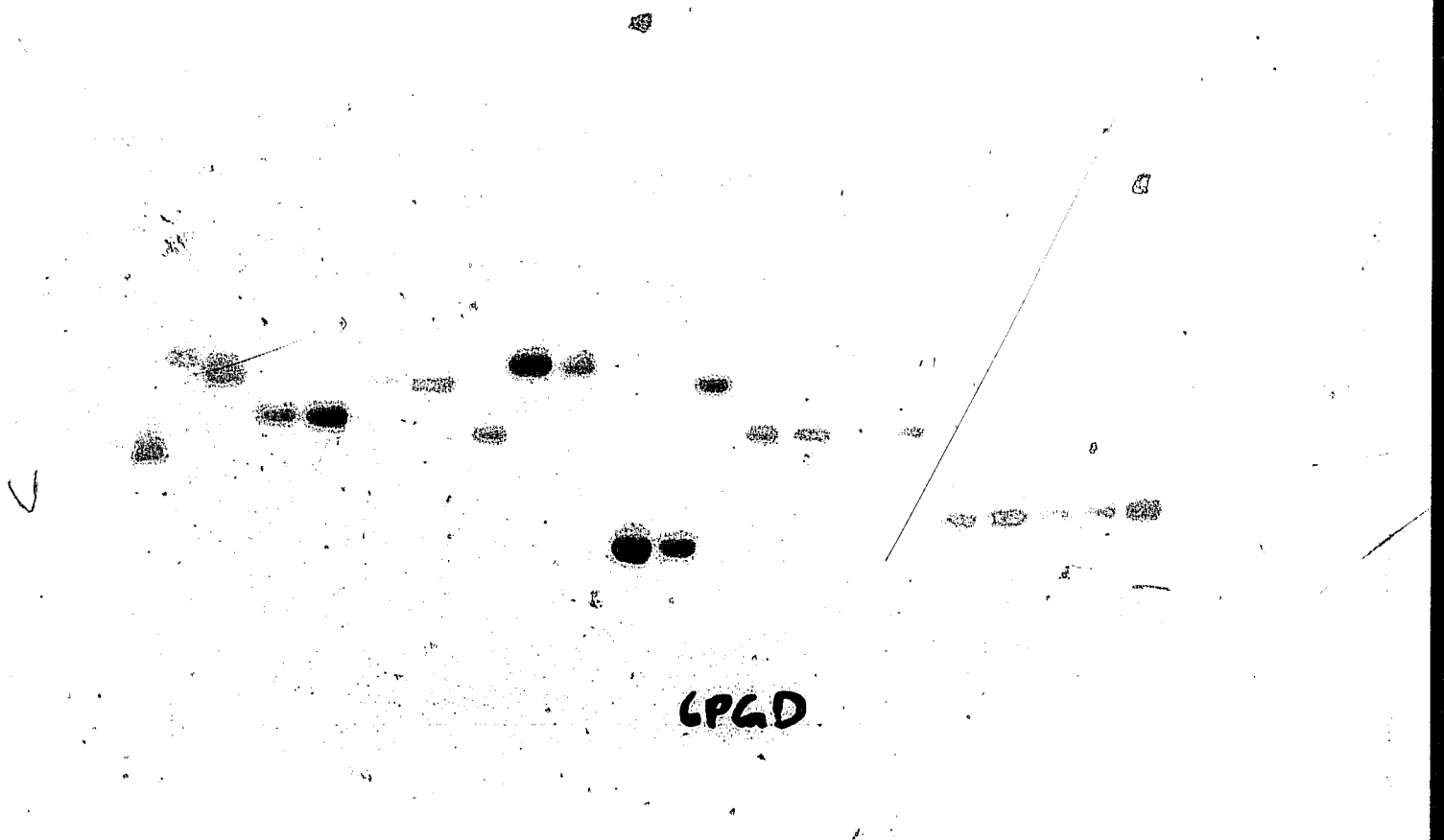
		NA
0.048 ± 0.033	82	

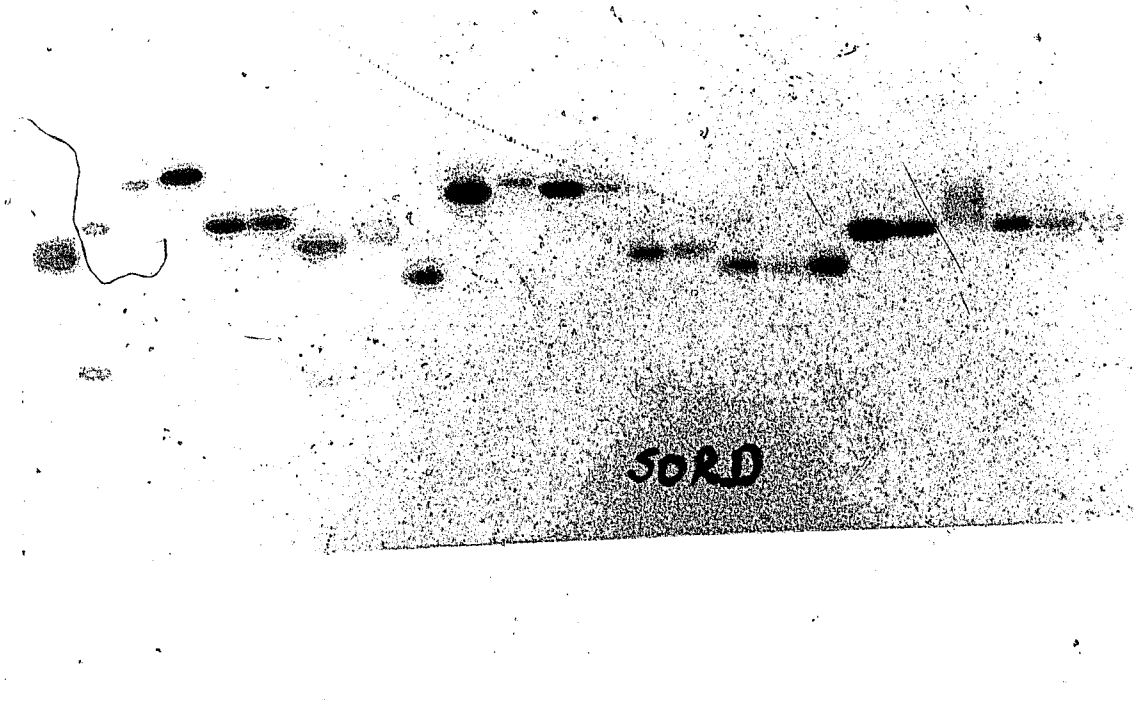
Figure 6.2 Variation in PGD mobility.

Species 23 failed to stain up before fixation on this occasion.

Variation in SORDH mobility.

An indistinct tetrameric heterozygote can be seen in species 20.


6PGD


SORD

	GPD	SORDH	LDH	MDH	ME_1	ME_2	ICD	PGD	GAPDH	SOD	GOT_1	GOT_2	GPT	PK_1	PK_2	AK_2	PGK	PGM_1	PGM_2	PEP_2	GDA	ADA	FUM	MPI	GPI	ADH
1	A	H	A	B	D	B	A	F	B	B	B	C	F	F	E	B	B	I	G	A	H	A	A	C	H	F
2	A	D	C	D	D	B	D	F	B	B	A	E	C	F	G	A	B	K	E	D	P	M	E	A	F	C
3	A	A	A	B	B	B	B	A	B	A	C	C	C	C	B	A	B	G	B	D	B	F	B	B	D	B
4	C	A	A	B	B	B	B	A	B	A	C	C	C	C	A	A	B	D	B	D	D	D	E	B	D	H
5	A	D	C	B	C	B	C	D	C	B	A	C	C	A	D	A	A	D	B	B	O	F	E	B	E	A
6	A	D	C	B	C	B	C	D	G	B	A	C	C	A	B	A	B	D	B	B	O	F	E	B	B	A
7	A	F	C	A	A	B	C	B	D	B	A	C	C	B	B	A	A	B	A	B	L	I	E	B	C	E
8	A	E	C	A	A	B	C	B	E	B	A	C	C	B	B	A	A	B	A	B	K	L	E	B	C	-
9	A	C	C	A	A	B	A	A	G	B	A	C	D	A	B	A	B	D	B	E	L	J	J	B	A	J
10	A	B	B	B	E	B	C	A	G	A	D	E	A	C	B	A	C	G	F	B	E	G	E	B	K	C
11	B	C	C	C	C	B	[illegible]	I	B	E	E	A	E	C	C	B	B	H	C	E	J	G	D	B	I	-
12	B	C	C	E	C	B	[illegible]	I	B	E	E	A	E	C	C	B	B	H	C	C	L	G	D	B	I	-
13	B	I	C	E	E	C	C	C	B	C	E	D	C	D	C	A	B	F	C	E	C	B	D	B	G	E
14	E	I	C	B	C	A	D	E	F	B	C	B	B	C	B	B	C	H	D	C	Q	G	C	B	K	F
15	F	J	C	F	C	C	D	E	A	D	E	D	C	G	F	A	A	J	C	E	N	G	C	B	K	-
16	C	J	C	F	C	B	D	G	B	D	D	D	E	B	A	B	F	C	C	G	H	F	B	J	D	
17	C	J	C	F	C	D	D	E	A	E	E	D	C	E	B	B	A	J	C	F	M	H	G	B	J	G
18	D	G	C	B	B	C	C	H	B	E	E	F	C	H	B	B	B	C	C	E	F	E	I	B	K	I
19	D	G	C	B	B	C	C	H	B	E	E	F	C	H	B	B	B	E	C	E	A	E	I	B	J	(I)
20	D	G	C	B	C	C	C	H	B	E	E	F	C	H	B	B	B	E	C	E	F	E	I	B	K	(I)
21	D	G	C	B	B	C	C	H	B	F	E	F	C	H	B	B	B	C	C	E	I	K	I	B	K	(I)
22	D	G	C	B	B	C	C	H	B	F	E	F	C	H	B	B	B	C	C	E	I	K	I	B	K	(I)
23	D	G	C	B	B	C	C	H	B	E	E	F	C	H	B	B	B	E	C	E	A	E	I	B	K	(I)

Table 6.1 Common allele mobility states for the species studied, coded alphabetically in order of decreasing mobility. Species are coded as previously.

awaiting formal description by Dr. B. Collette, U.S. National Museum of Natural History, in conjunction with the author.

An attempt was also made to examine intra-specific variation in the nominal tuna species *T. tonggol*, as differences similar to those described for the *Grammatorcynus* morphs had been noticed in S.E. Asian individuals compared to northern Australian ones. With much difficulty, a number of blood samples (n = 22) were obtained from Penang (Malaysia) but due to incorrect consignment procedure, these arrived in poor condition. It did however prove possible to compare mobilities at three loci, Est (serum), GPI_1 and GPI_2. The results indicated a fixed allelic difference at the Est locus and allele frequency differences at the GPI_2 locus. Detailed comparisons using fresh liver material are needed to verify this preliminary finding.

It is a comment on the power of the technique that it has led to additions to the complement of known species within a family already studied extensively due to its great commercial importance.

6.3.2 Relationships inferred from phenetic analysis

A variety of phenetic procedures has been used in systematic studies to express, as a two-dimensional dendogram, the relationships between species (Sneath & Sokal, 1973). Electrophoretic data is particularly well suited to such procedures as no weighting of characters is necessary. In this case, a phenogram was constructed from a matrix of similarities derived from Table 6.1, using arithmetic average clustering (Sneath & Sokal, 1973). Generic and tribal status of the 23 species as per Figure 2.1 are indicated on this phenogram (Figure 6.3).

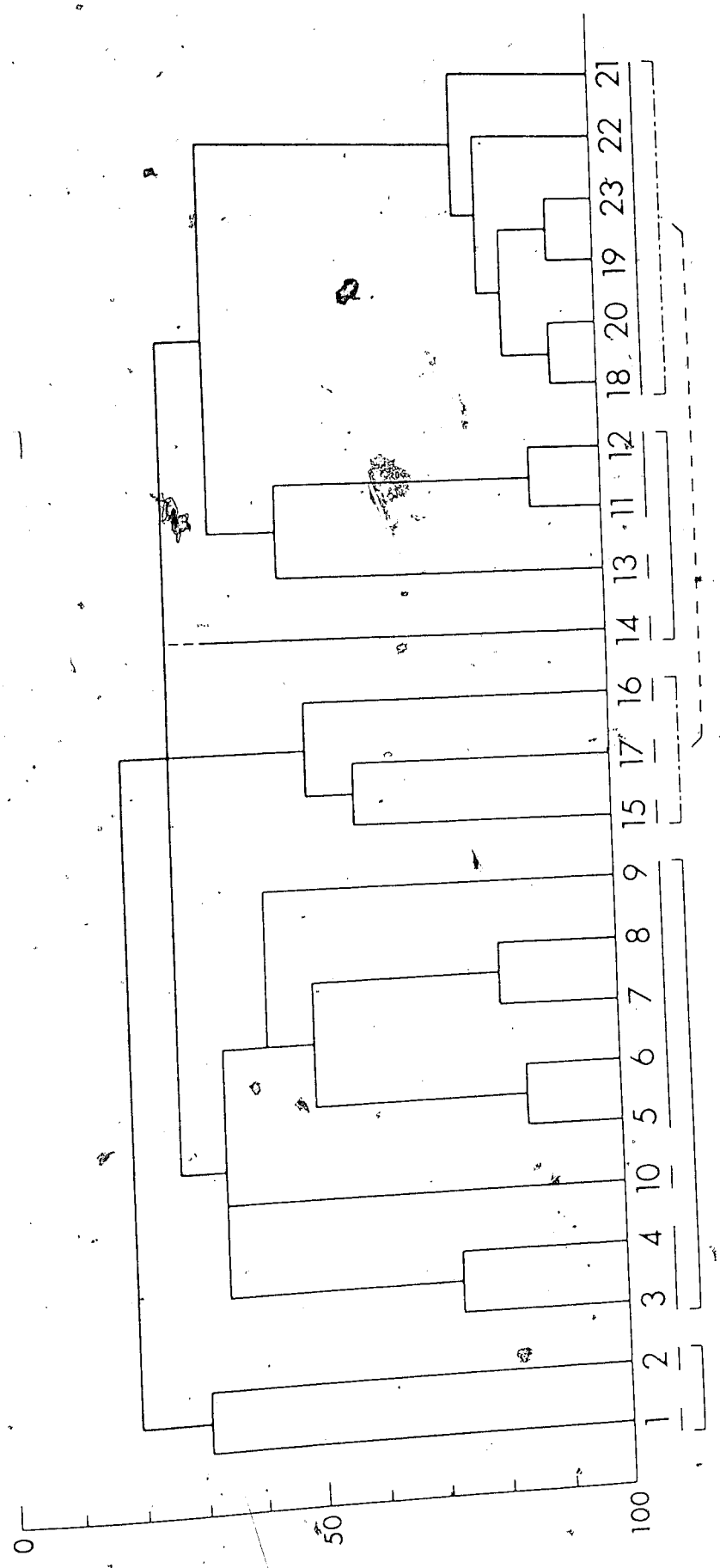
Clearly evident from the comparison of Figures 2.1 and 6.3 is the similarity in species groupings based on anatomical and electrophoretic data respectively. Levels of similarity between closely related species vary markedly.

Four of the 11 genera studied contained more than one species. Species within three of these genera, *Grammatorcynus*, *Sarda* and *Thunnus* are similar at the 70% level or higher. The six *Thunnus* species, in particular, show striking similarity, with the species pairs *T. albacares* (species 18) - *T. obesus* (20) and *T. tonggol* (19) - *T. t. orientalis* (23)

Figure 6.3 Phenogram constructed from common allele mobilities of the 23 scombrid species.

Species comprising a genus are underlined and those comprising a tribe are bracketed. Coding of the species is according to previous usage, i.e.,

1. *Scomber australasicus*
2. *R. kanagurta*
3. *Grammatorcynus sp. A*
4. *Grammatorcynus sp. B*
5. *Scomberomorus commerson*
6. *S. queenslandicus*
7. *S. multiradiatus*
8. *S. semifasciatus*
9. *S. munroi*
10. *Acanthocybium solandri*
11. *Sarda australis*
12. *S. orientalis*
13. *Cybiosarda elegans*
14. *Gymnosarda unicolor*
15. *Auxis thazard*
16. *Euthynnus affinis*
17. *Katsuwonus pelamis*
18. *Thunnus albacares*
19. *T. tonggol*
20. *T. obesus*
21. *T. alalunga*
22. *T. maccoyii*
23. *T. t. orientalis*


21
22
23
19
20
18
12
11
13
14
16
17
15
9
8
7
6
5
10
4
3
2
1
0
50
100
% SIMILARITY

exhibiting alternate common alleles at only two of the 26 loci with no fixed differences. *T. albacares* and *T. tonggol* share the most common alleles, on average, with other *Thunnus* species, and *T. alalunga* the least.

In contrast, the genus *Scomberomorus* appears to be more heterogenous, with *S. munroi* showing only 42% (11/26 loci) similarity with its cogeners. *S. commerson* (5) - *S. queenslandicus* (6) and *S. multiradiatus* (7) - *S. semifasciatus* (8) appear to form closely related species pairs, and the unique breakdown patterns seen at the ADA and GOT_1 loci in *S. commerson* and *S. queenslandicus* (see section 5.3.2) further support the close relationship between these two species. The degree of similarity between the pairs is however low at 50%, and the five Australian members of the genus *Scomberomorus* studied here may represent three relatively distinct lines of evolution.

Supra-generic relationships

Similarities between genera are low; they vary over the range 20-58%, and supra-generic relationships are not readily apparent as a result. The scombrine genera (species 1, 2), are very distinct from one another (30% similarity) but even more distinct from other scombrids and appear to represent a discrete group. The three genera comprising the current Scomberomorini (3-4, 5-9, 10) also form a loose grouping possibly closer to the Sardini and Thunnini than the Scombrini. *Gymnosarda* appears to have no close affinities with any other species studied, including its three fellow Sardini, which otherwise form a close-knit grouping. However, the result obtained here requires confirmation because of the poor condition of the single *Gymnosarda* sample obtained.

The Thunnini appear to be comprised of two quite distinct groups, the genera *Auxis*, *Euthynnus* and *Katsuwonus* (species 15, 16, 17) and the true tunas (*Thunnus* spp). This represents a slight deviation from the existing classifications (e.g. Collette, 1978).

For several reasons, attempts to trace affinities beyond the genus level encounter problems, particularly when comparing genera across existing tribes. The discriminatory power of technique diminishes with increasing divergence to the point where similarities remaining may be

due to chance or secondary convergence. The lack of confidence limits to accompany dendograms, an inherent weakness of numerical taxonomy, further indicates that little weight can be placed on minor differences in % similarities.

The pertinent point is probably that beyond the generic level, relationships are difficult to establish, this amount of divergence indicating a long separation of the major groups.

6.3.3 Relationships inferred from cladistic analyses

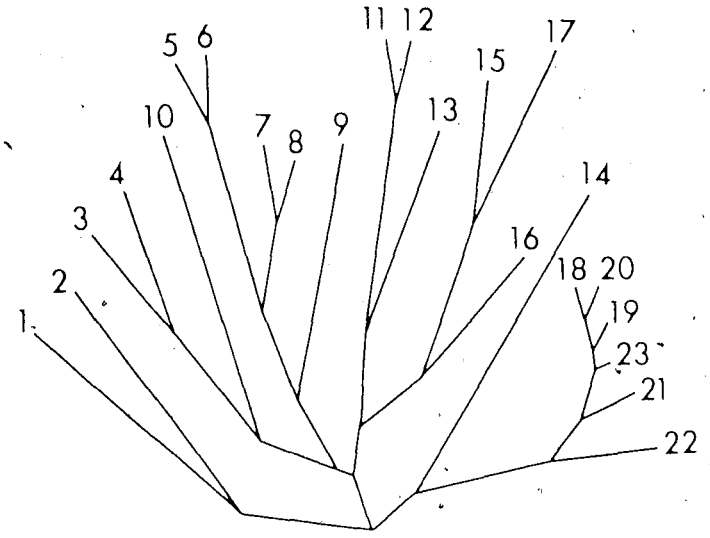
Phenograms are commonly assumed to reflect phylogenetic relationships, as was the case with Sharp & Pirage's (1978) earlier study of scombrids. This is true only if the rates of evolution of the characters (electrophoretic mobilities) are constant between lineages (Mickevich, 1978). Although inevitably based on phenetic data (Sneath & Sokal, 1973), cladistic analyses attempt to reconstruct branching sequences without making any assumptions about rates of evolution. Quite different results are often obtained by the two approaches. Lakovaara *et al.* (1972) and Farris (1974), for example, obtained conflicting results for the *Drosophila obscura* group using phenetic and cladistic analyses respectively, as did Mickevich & Johnson (1976) analysing morphometric and electrophoretic data on the atherinid genus *Menidia*. In addition to the phenetic approach, two cladistic approaches have therefore been considered in this analysis.

(i) Wagner networks (Wagner, 1961; Farris, 1970) were constructed from the data using a BASIC programme on file at the Zoology Department, University of N.S.W. and run on a Cyber 72-76 computer. This method estimates the minimum number of evolutionary steps needed to generate a given set of species characters and thus produces the most parsimonious set of relationships between species, using maximum likelihood estimates. The possibility of reversals, convergences and varying rates of evolution is not exluded (Farris, 1973). Branch lengths on the networks represent patristic distances. Such schemes are unrooted although this can be overcome by using a closely related outgroup or "sister group" to produce a rooted tree. Within the Scombridae, an obvious outgroup would have been *Gasterochisma melampus*, the single member of the sub-family *Gasterochismatinae*, but it was not possible to collect material from this species.

An alternative widely used method was therefore employed. This method arbitrarily locates the root at the midpoint between the most distant points on the tree. It however implicitly assumes approximately equal evolutionary rates per lineage, but as we shall see, this assumption is not of critical importance in the present analysis.

With a large number of species being analysed, as is the case here, the total number of trees which can be fitted to the data becomes impossibly large (Lundberg, 1972). Twenty seven runs, representing 270 trees, were executed, and selected networks converted to dendogram and tree form to facilitate comparison with Figures 2.1 (the classical currently accepted scheme) and 6.1 (the phenogram derived from the same data set). The most parsimonious of these networks involved 308 steps. This was achieved twice, and both networks are shown in Figures 6.4 and 6.5. Note that, in the dendograms, only vertical distances have patristic relevance; horizontal distances provide spacing to assist clarity of presentation.

(ii) The Hennig approach which determines the branching sequence by grouping taxa which share derived as opposed to ancestral character states was also utilized. With the large number of species and character states involved and the low levels of similarity between many species, this approach succeeded only in defining relationships within groups (genera) and was of little value in examining relationships between groups. Consequently, it added little to the Wagner analysis and only the latter has been considered. In the great majority of Wagner tree runs, there was remarkable constancy in the association between species, as exemplified by the two most parsimonious trees shown in Figures 6.4 and 6.5. Species 1 and 2 were markedly divergent but always paired; within the Scomberomorini, species 5 & 6 and 7 & 8 were inevitably paired, whilst species 9 was variously linked to these pairs, and as a result of its divergence from them, occasionally linked to other species, particularly the Scombrini. The *Grammatorcynus* species (3, 4) were always paired and seem to have long diverged from the other Scomberomorini, appearing as a separate entity in most trees. Interestingly, they were most often linked to species 10, *Acanthocybium solandri*. Affinities of this species (10), plus those of *Gymnosarda* (14) (both are considered highly specialized monotypic genera on the basis of morphological and



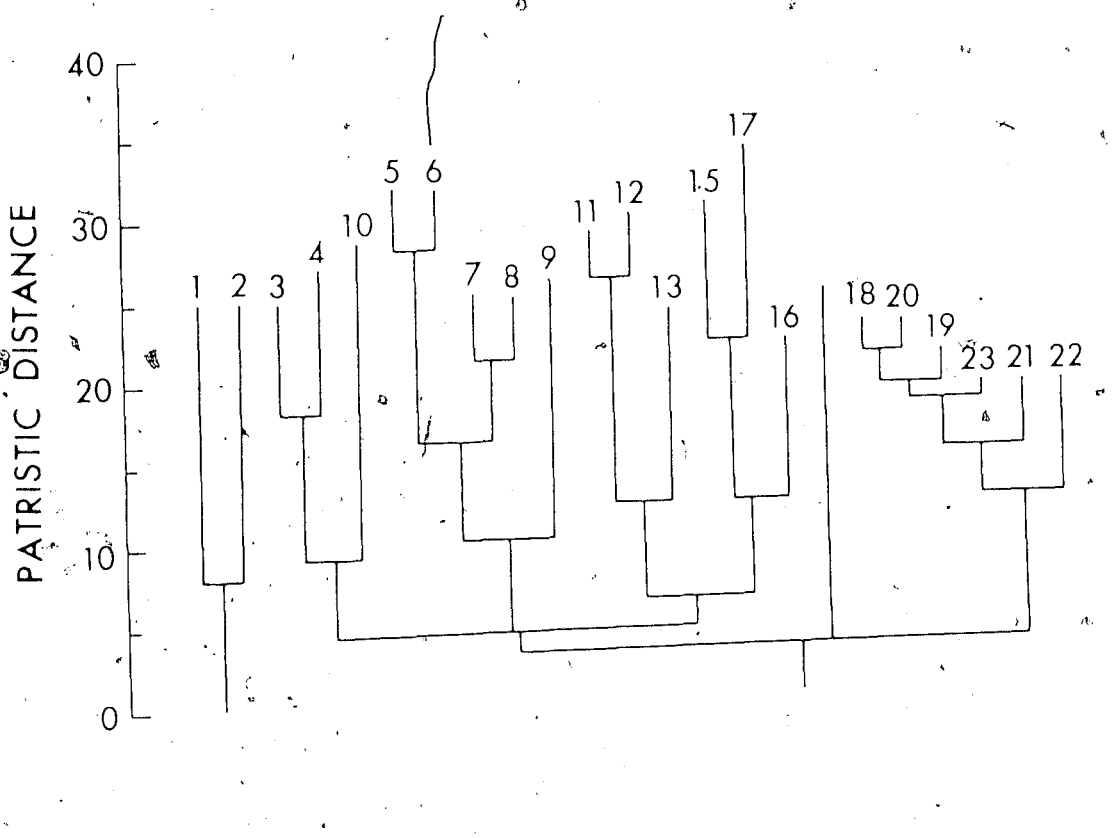
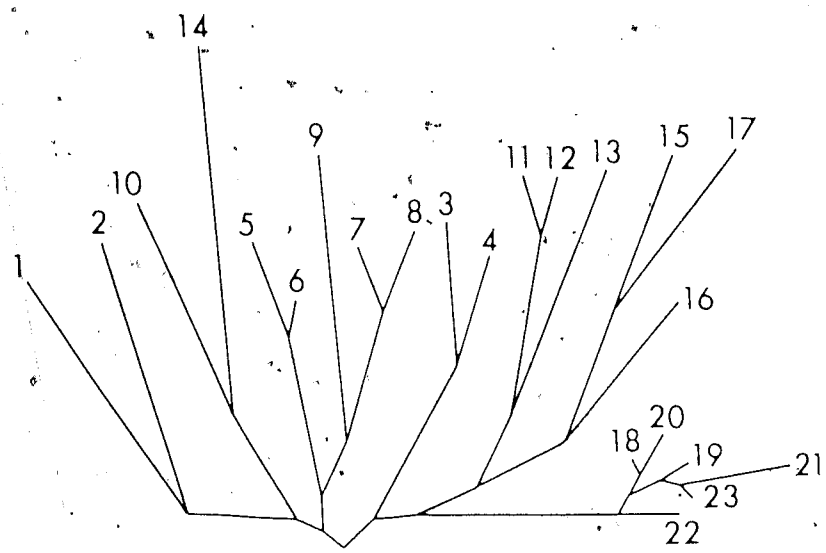


Figure 6.4 One of the two equally parsimonious Wagner networks (308 steps) for the 23 scombrid species, expressed in tree and dendogram form.

Species are coded as previously.



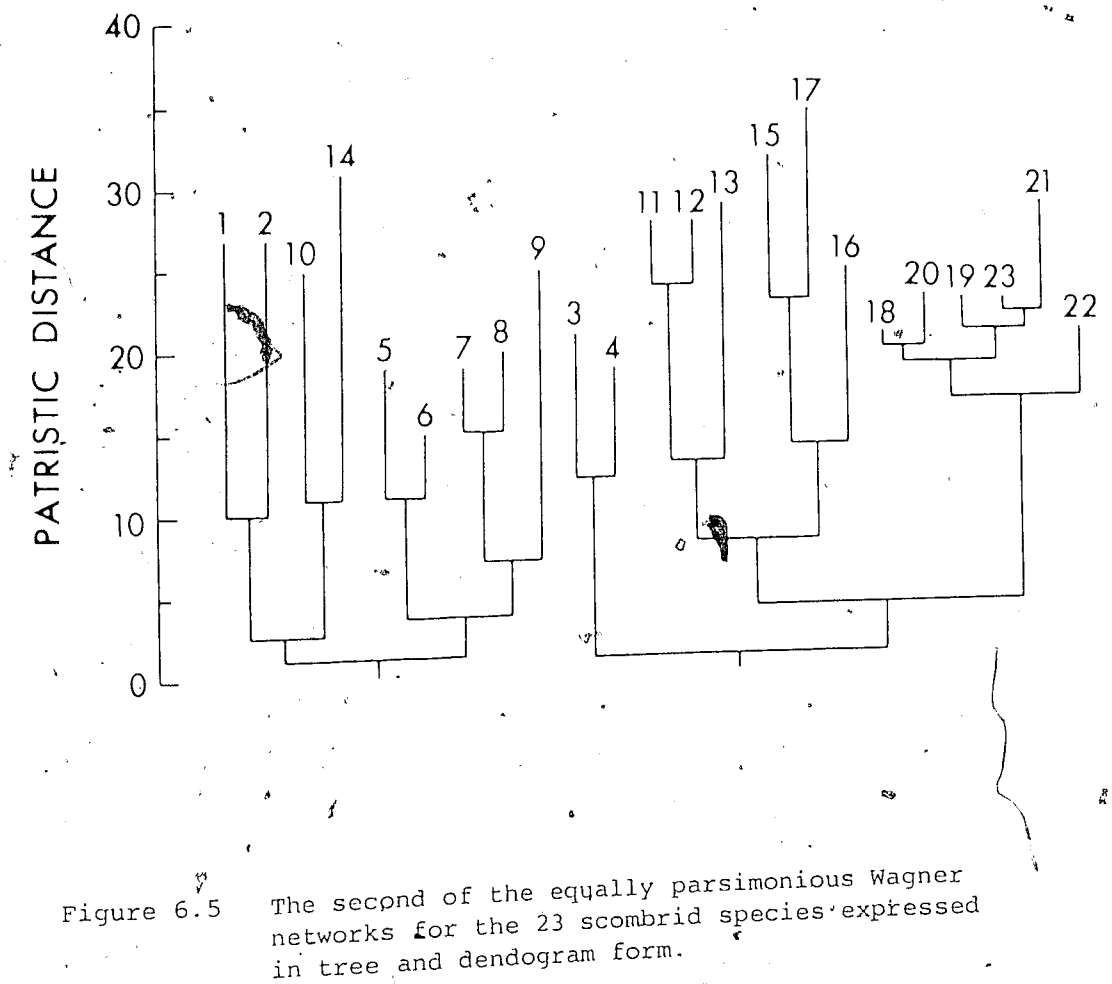


Figure 6.5 The second of the equally parsimonious Wagner networks for the 23 scombrid species expressed in tree and dendogram form.

ecological evidence) vary considerably from tree to tree. Given the large patristic distance to the nearest species in the majority of cases they are best regarded as long divergent independent lineages whose affinities are now difficult to trace.

Of the three remaining Sardini, the two *Sarda* species (11, 12) were invariably paired and more distantly linked with *Cybiosarda*. As in the phenetic analysis, species 15, 16 and 17 were nearly always grouped, but with considerable divergence between them. Species 15 *(Auxis)* and 17 *(Katsuwonus)* were usually linked. The tight grouping within the genus *Thunnus* (species 18-23) was apparent in all trees, with species 18-20 and 19-23 generally forming pairs, and species 21 quite divergent in most cases.

Beyond the generic level, affinities varied considerably. Primary branchings occurred very close to the root in nearly all cases and as with the phenetic analysis, the discriminatory power of the electrophoretic technique is approaching its limits at this level. The most reasonable interpretation is that six major groups (species 1-2, 3-4, 5-6-7-8-9, 11-12-13, 15-16-17 and 18-19-20-21-22-23), plus species 10 and 14, have diverged to the extent that it is no longer possible to trace phylogenetic affinities with any certainty using the available electrophoretic data.

6.4 DISCUSSION

6.4.1 Electrophoresis as a taxonomic tool

A rigorous evaluation of the taxonomy of the Scombridae is neither within the scope of this study nor within the level of competence of the author. Whilst recognizing the difficulty of comparing the two types of dendrograms (Figures 2.1 and 6.3), it is nonetheless encouraging to note the close correspondence, at least at lower levels, between the phenetic relationships inferred here from electrophoretic data and the most recent classifications based on morphological and eco-physiological data (Collete & Chao, 1975; Collette, 1978; Zharov, 1967).

Clear separation of a previously undescribed *Grammatorcynus* species and preliminary separation of two *Thunnus tonggol* morphs illustrates the

usefulness of the electrophoretic approach in resolving problems of specific identity. Whilst the taxonomy of the family presents few difficulties nowadays, the technique could profitably be brought to bear on at least two acknowledged 'problem' species complexes, *Rastrelliger* spp. *(brachysoma-neglectus)* and *Auxis* spp. *(thazard-rochei)*, and on widely distributed species whose discontinuous distribution or polytypic morphology raise questions of species identity (see later).

6.4.2 Evolutionary history of the Scombridae

The use of electrophoretic data to infer the evolutionary history of Indo-Australian members of the family is of interest to this study. Both the cladistic and phenetic analyses suggest that extant species are members of long-separate groups whose affinities are accordingly difficult to establish. Whilst this may be a most significant result, as we shall see later, it needs firstly to be placed in historical perspective. There are two ways of approaching this.

(a) Assuming evolutionary rates at the molecular level are relatively constant across the species array, amino-acid sequencing techniques can be used to estimate time since divergence for species pairs. The concordance of results from the phenetic and cladistic analyses of scombrid species suggests that this assumption is a reasonable one. Material from two species, *Thunnus alalunga* (21) and *Katsuwonus pelamis* (17) has in fact been analysed. Results from cytochrome c sequencing (Nakayama *et al.*, 1971; Kreil, 1965) suggest a time since divergence of approximately 20 million years (Dayhoff, 1972). With only one difference between the respective sequences, the confidence limits on this estimate are probably broad. Published data on insulin sequences were of doubtful reliability and hence not used.

(b) Considerably more useful is the available, but limited, fossil record. The family Scombridae may have originated as early as the Upper Cretaceous approximately 70-80 million years ago. (Nikol'ski, 1971 *fide* Shubnikov, 1974). Its phylogenetic position to obviously related families such as the Xiphiidae, Istiophoridae, Gempylidae and Trichuridae remains unclear. The earliest known scombrid fossil genera, from the upper Eocene (50 million years B.P.) can be placed in the present Scombero-morini (Danil'chenko, 1960). By the late Eocene-Oligocene (30-40 million

years B.P.), species clearly recognizable as *Scomber, Sarda, Scomberomorus* and *Thunnus* are present in the fossil record (Danil'chenko, 1960, 1964), although not as well defined morphologically from one another as they are today. The radiation, from neritic tropical ancestors hypothesized to resemble the extant *Gasterochisma* to a variety of temperate and tropical oceanic and neritic forms, seems to have involved extensive early speciation. Fifteen *Thunnus* forms, compared with 7 today, five *Auxis* (cf. 2), three *Gymnosarda* (cf. 1), ten *Scomber* (cf. 3) and at least ten *Scomberomorus* species (cf. 18) are known from various fossil deposits in the late Eocene, Oligocene and Miocene (5-30 million years B.P.).

Collette (1978) and Sharp & Pirages (1978) on the basis of morphological and biochemical evidence respectively, have argued for a linear evolutionary progression from forms represented by the 'primitive' Scombrini to those represented by the 'advanced' Thunnini. Whilst some fossil forms morphologically intermediate between extant genera, such as *Eothynnus*, are known, the fossil record is too sketchy to evaluate this possibility. The long period of time for which genera have been separated would necessitate that such an 'ascent' occurred relatively rapidly in the late Eocene-early Oligocene, rendering confirmation from the fossil record an unlikely event. Shubnikov (1974) regards scombrid evolution as having proceeded in two main directions from a polymorphous neritic group: towards an exclusively predatory mode of life on the one hand, giving rise to present day *Gymnosarda, Scomberomorus* (and presumably *Sarda*) and to an oceanic mixed planktivorous-predatory mode of life represented by *Thunnus, Euthynnus, Auxis, Katsuwonus* and *Allothunnus* on the other.

An equally plausible alternative to both the above hypotheses and one which gains some support at the molecular level (see later) is the possibility of more-or-less concomitant origin of several independent lineages from a common group of ancestors, with considerable subsequent morphological convergence.

6.4.3 Phylogenetic relationships between extant species

The phylogenies presented here are based on the most parsimonious Wagner trees (Figures 6.4 and 6.5). Mickevich (1978) concluded that such trees produce phylogenetic classifications with the highest internal

stability. Although it would be a relatively simple task to code the available morphological and eco-physiological data to generate a similar tree for comparison, the congruence observed between the current traditional classification (Figure 2.1) and the two schemes based on electrophoretic data (Figures 6.3, 6.4 & 6.5) suggest the above interpretation would not be challenged, at least at lower taxonomic levels. It remains however a worthwhile task for future study.

The Wagner analysis, as well as the phenetic analysis of the same data, have indicated that at least two aspects of current scombrid classificiation need closer examination.

(1) The clear separation of *munroi* from other *Scomberomorus* species requires comment, because on both phenetic and phyletic evidence, it is as divergent from its cogeners as some genera are from each other, e.g. *Auxis-Katsuwonus-Euthynnus* or *Sarda-Cybiosarda*. One can only assume it is a relatively old species within the phylogenetically 'old' genus which has maintained itself in Australian waters in the face of later arrivals of other more modern species. It also highlights the problem of accurately relating lineages of different ages. *Scomberomorus*-like forms, it will be recalled, are the oldest known fossils.

(2) The present study clearly indicates that *Auxis*, *Katsuwonus* and *Euthynnus* share a common lineage, and that they have diverged less than some other genera. This was particularly the case with *Auxis-Katsuwonus*, and is in accordance with the findings of Zharov (1967), Nakamura (1965) and Sharp & Pirages (1978), who have suggested according *Katsuwonus*, *Euthynnus* (and presumably *Auxis*) closer, if not congeneric status. Collette & Chao (1975) however group *Katsuwonus* with *Thunnus* and distinct from *Auxis-Euthynnus*. The present phenetic and cladistic analyses contradict this finding in regarding the *Thunnus* species as no more closely related at the molecular level to the lower Thunnini than they are to the Sardini. This represents the only significant disagreement with previous schemes, and assuming the electrophoretic results are representative of the actual situation, suggests considerable morphological convergence has occurred.

As noted previously, the extent of divergence between groups reflects the long period of time for which most of them have been separate entities. This severely limits the discriminatory power of the electrophoretic data at suprageneric levels.

Phylogenetic relationships within genera/groups appear less problematical and warrant brief discussion.

Tribe Scombrini

Although clearly related, the two genera *Scomber* and *Rastrelliger* appear to have diverged extensively at the biochemical level. However, both adult (Matsui, 1967) and larval morphology (Okiyama & Ueyanagi, 1978) of these species show considerable similarity, presumably due to the conservative retention of symplesiomorphic traits. The long term stability of the morphology of this group can be seen in extant *Scomber* species (*scombrus*, *australasicus* and *japonicus*) which are so similar to each other as to cause identification problems. The group is so little changed from fossil forms that *japonicus* is recorded as such from the Oligocene (Danil'chenko, 1960). Whilst *australasicus* and *scombrus* are known only from the Pacific and Atlantic Oceans respectively, *japonicus* is considered a polytypic world-wide antitropical species (Collette, 1978) and given the morphological conservatism of the group, it would not be surprising if electrophoretic comparisons revealed the existence of more than one species.

Tribe Scomberomorini

The present study shows that the three genera (*Scomberomorus*, *Acanthocybium* and *Grammatorcynus*) which comprise this speciose group are not closely related. Conrad (1938), Mago Leccia (1958) and Devaraj (1975) have all considered *Acanthocybium* to be related to the *S. cavalla* group, which includes *commerson* (5). However, Okiyama & Ueyanagi (1978) found a unique mosaic of larval characters which distinguished *Acanthocybium* from *Scomberomorus* and in fact indicated a closer relationship with *Sarda*. Munro (1943) has suggested that the type of body form seen in *S. commerson* and *Acanthocybium* may be the most suitable for a more oceanic habitat and for extensive migrations and that morphological similarities between *Acanthocybium* and *S. commerson* may therefore be due to convergence. In view of the degree of biochemical divergence between them, this seems likely. The results also give little reason to regard *Grammatorcynus* as either a possible link between the Scombrini and the Scomberomorini, or as being more distantly related to *Scomberomorus* than to *Acanthocybium*, as various authors have suggested.

Within the genus *Scomberomorus*, the three groupings *commerson-queenslandicus*, *semifasciatus-(multiradiatus)*, and *munroi* correspond to Munro's (1943) now disregarded sub-genera *Cybium*, *Indocybium* and *Sawarra* respectively.

Tribe Sardini

Cybiosarda is clearly not recently diverged from *Sarda* but appears closer to that genus than *Gymnosarda*, in contrast to Collette's (1978) suggestion. It is also not congeneric with *Gymnosarda*, as Fraser-Brunner (1950) suggests, nor does it belong within the Scomberomorini, as Munro (1943) suggested.

Despite the morphological affinities of *Gymnosarda* with the Sardini (Collette & Chao, 1975), the results of this study and examination of larval characteristics (Okiyama & Ueyanagi, 1978) indicate that *Gymnosarda* is a highly specialized form not closely related to any other genus.

Allothunnus has been grouped with both the Sardini and the Thunnini by various workers. It is sometimes regarded as a transitional form between these two tribes (Sharp & Pirages, 1978) and would therefore have been an extremely interesting inclusion in the study particularly as a test of the linear evolutionary sequence proposed by those workers. Because of the poor condition of the only sample obtained, this was unfortunately not possible.

Tribe Thunnini

Within the genus *Thunnus*, the great similarity between species is the most significant result. Although the genus is relatively old (35 million years B.P. or more), all the species studied may be of relatively recent origin, having diverged little from a common ancestor. Those species which have successfully colonized the temperate areas (species 21, 22, 23 in particular) are still tied to tropical areas for spawning. This could be further indication of a relatively recent origin. Alternatively, evolutionary rates at the molecular level may have slowed or observed similarities may reflect a high degree of parallel evolution. The genus has been divided into several sub-genera on morphological grounds (Kishinouye, 1923; Godsil & Byers, 1944). Both Gibbs & Collette (1967) and later Le Gall *et al.*, (1976), re-analyzing the same morphological

characters using multivariate techniques, have preferred to recognize two species groups - *atlanticus, tonggol* (19) and *albacares* (18) on the one hand, and *alalunga* (21), *maccoyii* (23) and the *thynnus* complex (22) on the other, with *obesus* (20) an intermediate form. Both Sharp & Pirages (1978) and the present author recognize no clear intrageneric subdivision other than to place *T. alalunga* in a relatively isolated position. At the high levels of similarity observed, grouping particular species is probably strongly influenced by the choice of loci. Sharp & Pirages (1978) found the closest similarity between species 22 and 23 (91%), whereas 92% similarity between species 18-20 and 19-23 was recorded here. Their interesting contention that the two *T. thynnus* subspecies warranted full specific status could not be investigated. Collection of additional material may enable genetic distances to be calculated and intra-generic relationships to be examined with more precision; it is more likely however that the electrophoretic technique has approached the limit of its resolution at this high level of similarity particularly when only the commonest alleles are used in the estimation of similarity coefficients.

6.4.4 Zoogeography of the species

Many of the scombrid species studied here are cosmopolitan rather than Indo-Australian in their distribution. This is consistent with their oceanic or nerito-oceanic habitat and high vagility. Such species, which include wahoo, *(A. solandri)*, frigate tuna *(A. thazard)*, skipjack *(K. pelamis)* and all the *Thunnus* species with the exception of *T. tonggol* (an exclusively continental shelf or neritic species) are accordingly of more interest to the population geneticist than the zoogeographer.

Neritic species, on the other hand, tend to be more localised in their distribution, which logically is reflected in the degree of endemism shown. The table below lists for each genus, the number of cosmopolitan species (C), the number of species occurring only in the Indo-Pacific region (IP) and the number of species endemic to Australia-Papua New Guinea (A).

Genus	Species studied	Distribution category C	IP	A	Comments
Neritic genera					
Scomber	1	–	1	–	
Rastrelliger	1	–	1	–	
Grammatorcynus	2	–	1	1	
Scomberomorus	5	–	1	4	
Sarda	2	–	1	1	
Cybiosarda	1	–	–	1	
Gymnosarda	1	–	1	–	
Euthynnus	1	–	1	–	
	14	–	7	7	
Oceanic/nerito-oceanic genera					
Acanthocybium	1	1	–	–	
Auxis	1	1	–	–	
Katsuwonus	1	1	–	–	
Thunnus	6	5	(1)*	–	* See 6.3.1, *T. tonggol*
	9	8	(1)	–	

Although many of the neritic species are capable of extensive longshore migrations, the level of endemism does suggest that such species are more likely to encounter barriers to distribution, leading in some cases to divergence and ultimately allopatric speciation. Changes in sea level and disposition of land masses may periodically produce such barriers and any localization of spawning activity would further promote this mode of speciation. Present knowledge of both the geological history of the Indo-Australian region and spawning strategy of the great majority of scombrids is however inadequate to evaluate this suggestion.

It is within genera comprised of more than one neritic species that an understanding of phylogenetic relationships can be expected to be most useful in the interpretation of zoogeographical phenomena. Discussion therefore centres on three genera - *Scomberomorus*, *Grammatorcynus* and *Sarda*.

The known distribution of Indo-Australian *Scomberomorus* species provides a distinctive pattern. With the exception of the widespread and abundant *commerson*, there is a complete changeover in *Scomberomorus* species across eastern Indonesia and the Arafura Sea, from Indo-Malayan species (*guttatus*, *lineolatus* and to a lesser degree, *koreanus* and *sinensis*) to Australo-Papuan endemics (*munroi*, *semifasciatus*, *multiradiatus* and *queenslandicus*) (Collette & Russo, 1979). Given the many changes in the disposition of land masses in this area, opportunities for allopatric speciation have probably been greater here than elsewhere in the Indo-Pacific region. Electrophoretic data should in future enable elucidation of evolutionary relationships between these two suites of species, and may throw further light on the affinities of the highly divergent *munroi*. Munro's (1943) sub-generic classification places *guttatus* and *lineolatus* along with *semifasciatus* in the sub-genus *Indocybium* and *koreanus* and *sinensis* in sub-genera with no Australian representatives. This provides an interesting alternative scheme to test with electrophoretic data in future.

It is interesting to note that Atlantic members of the genus similarly comprise one large widely distributed species *cavalla*, closely related morphologically to *commerson*, and four smaller species showing limited or no overlap in their more restricted distributions (*maculatus*, *regalis*, *brasiliensis* and *tr*[illegible] - Collette & Russo, 1978).

Until the specific status of the two *Grammatorcynus* species is formalized, and their respective geographical ranges subsequently defined, little can be said of their zoogeography. In view of the more inshore habit of the larger species (shark mackerel) and the generally smaller size recorded outside Australia for *'G. bicarinatus'* (more consistent with scad than shark mackerel) by various authorities, it would not be surprising if sp. A (shark mackerel) proves to be an Australian endemic. Furthermore, as the known range of *'G. bicarinatus'* shows

a conspicuous gap between the coasts of south-east Asia and the Red Sea, Red Sea forms may similarly prove to be a distinct species. It is perhaps noteworthy that the distribution of this species complex approximately parallels the distribution of well developed coral reef systems within the tropical Indo-Pacific.

Sarda orientalis has an apparently discontinuous distribution across the Indo-Pacific from Malagasy to central America, although this discontinuity is more likely an artifact of its inconspicuous, largely sub-surface habit and propensity to occur in small sparse schools rather than large aggregations. Observations during this study have extended its known range to include Indonesia (Ambon) and Australia's north-western coastline ($19^{o}43'S$, $116^{o}13'E$). There is also a confirmed report from the Gulf of Papua and an unconfirmed one from New Britain, (Papua New Guinea), further reducing distributional gaps. In Australian waters, however, it is definitely restricted to the western and northern coasts. On the east coast, it is replaced by its closely related cogener, *S. australis* which is endemic there although odd vagrants have been taken in New Zealand (James & Habib, 1979). It seems feasible given the limited amount of divergence which has occurred between the two species that *australis* may have been allopatrically derived relatively recently from *orientalis* during one of the periods when the present-day Torres Strait was closed by a land bridge between Australia and Papua New Guinea. It would be interesting to examine, from the genetic viewpoint, relationships between *orientalis* and the eastern Pacific species *S. chiliensis*, and also between the two allopatric sub-species of *chiliensis* currently recognized - *S. c. lineolata* (North America) and *S. c. chilensis* (South America)(Collette & Chao, 1975).

Preliminary comparisons have indicated that some degree of differentiation may have occurred across the Indonesian region within the only neritic *Thunnus* species occurring in the region, *T. tonggol*. This would seem to support the role played by spatial restrictions on gene flow in the differentiation process. With a species of *tonggol's* undoubted) dispersal potential, such differentiation would also be promoted if spawning activity is not widespread but localized. The restricted distribution of juveniles and spawning adults across northern Australia-Papua New Guinea (personal observations) suggests that this may be the case.

In summary, phenetic and cladistic analyses of Indo-Australian scombrids based on electrophoretic data have, with few exceptions, provided strong support for the currently accepted taxonomy of the family Scombridae. These results, together with evidence from the fossil record, suggest that the various groups within the family have evolved independently of one another for a long time, certainly in excess of 30 million years. As a result, it is beyond the power of the electrophoretic technique to elucidate relationships between scombrid taxa above the genus level, in contrast to studies of more recent groups, such as the marsupials, eutherians, and birds. The data do indicate however, that interpretations of scombrid evolution which regard mopho-physiological specializations as sequential may need some reappraisal. Much interesting work remains to be done by extending this study to take in the species complement of adjacent areas. This is particularly true in the zoogeographic context.

CHAPTER 7

CONCLUDING REMARKS

The study of populations of highly mobile oceanic species such as skipjack tuna is not without its inherent difficulties, logistical and biological. Given also the dearth of knowledge in several critical areas, for example the species' reproduction and the ecology of skipjack early life history stages, plus the enforced reliance on the electrophoretic variation at just one locus, there is good reason to feel pleased with the results of the present study. The two independent data sets, the Papua New Guinea mark-recapture results and the allozyme data collected in the same area several years later have, as well as providing a large amount of data useful in a general sense produced complementary findings in two important areas.

Both data sets have provided circumstantial evidence that island-open ocean interactions play an important role in skipjack ecology, especially reproduction and early development. Near-island waters, with their enhanced and perhaps more importantly, relatively stable productivity, may provide a reliable baseline from which it becomes feasible to exploit less certain but periodically productive open ocean situations. Combined with the species' morpho-physiological adaptations enabling individuals to minimize time spent in unproductive situations, the adoption of resident-nomad strategies as outlined here may form the basis of the remarkably successful colonization of the vast and generally unproductive epipelagic zone by skipjack tuna. This remains a fascinating area for critical review and future study.

Despite the species' dispersal potential, there is little evidence that significant long distance movement (> 2000 km) of adults routinely occurs. In the Papua New Guinea area at least, mixing

appears maximal when the probability of genetic exchange between 'residents' and 'nomads' is lowest. Whilst this adds to the plausibility of the isolation-by-distance model which is felt to provide the best fit to the allozyme data, critical evaluation of the model is currently not possible in the absence of information on dispersal patterns of the planktonic eggs and larvae and on the nature of selective or stochastic forces shaping E_{SJ} frequencies. It remains however the most useful working hypothesis.

Both topics require considerable further study, not least because of their far-reaching implications for management. It could be argued, for example, that seasonal fisheries in temperate areas or other fisheries which exploit essentially nomadic components of the resource may be capable of supporting intensive harvest, and hence not requiring regulation, whereas the harvest in island-associated tropical fisheries may warrant careful monitoring. An isolation-by-distance model, as another example, might be used to justify limited consultation between nations in different parts of the Pacific, according to the prevailing political climate. It is therefore clear that these issues require clarification with some urgency.

In the light of the results of the present study, which has been extremely demanding of manpower and resources, what is the prognosis for similar studies on other scombrid fishes, or indeed, larger pelagic species in general? The screening of other scombrids has, in most cases, revealed suitable allozyme variation at more than one locus which should reduce some of the ambiguity of interpretation accompanying the skipjack work. Even with advantage of working with an abundant commercially important species, the difficulties of maintaining a rigorous sampling programme should not however be underestimated, and the crucial intellectual input may be framing precise questions appropriate to the situation. Time-series and replicate sampling clearly have much to offer and should be attempted where appropriate.

A fundamental problem seems likely to remain the difficulty of excluding alternate explanations of the data when dealing with the genetics of natural populations. The present study does however suggest that the concurrent collection of a complementary data set

(not necessarily mark-recapture data) could greatly diminish but never entirely put paid to this objection and as with most management tools, it becomes a question of backing one's judgement, given all available knowledge. In any case, the manager and the geneticist will often be asking different questions of the same data without always realizing it. The electrophoretic approach to problems of 'stock discrimination' or 'population sub-division' can never be the universal panacea as some of its early protagonists have implied, nor should poorly derived conclusions from ill-conceived studies be allowed to completely discredit it. It seems likely and apt that electrophoretic techniques will take their place as one tool amongst others, to be used when the situation warrants. Seen in this context, they have considerable potential.

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