

### SCIENTIFIC COMMITTEE SECOND REGULAR SESSION

7-18 August 2006 Manila, Philippines

# ECOSYSTEM MONITORING AND ANALYSIS: STOMACH SAMPLING OVERVIEW OF THE GEF-SAP PROJECT 2000-2005 AND STOMACH SAMPLING STRATEGY OF THE GEF-OFM PROJECT 2005-2010

WCPFC-SC2-2006/EB IP-6

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# Ecosystem monitoring and analysis: stomach sampling overview of the GEF-SAP project 2000-2005 and stomach sampling strategy of the GEF-OFM project 2005-2010.

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**July 2006** 

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# 1. Introduction

In 2000-2005, the GEF-funded Oceanic Fisheries Management component of the Strategic Action Programme of the Pacific Small Island Developing States (SAP) had the following objective: "*To enable the conservation and sustainable yield of ocean living resources*", with particular reference to oceanic fisheries supported by the Western Pacific Warm Pool Large Marine Ecosystem.

One activity of this project was "Improved scientific advice relating to regional tuna stocks, non-target species and the oceanic ecosystem available to support management decision-making".

In this context, to improve our knowledge on the pelagic ecosystem of the western and central Pacific, and particularly understand the prey-predator relationships, a sampling strategy has been implemented to collect stomach samples and determine the diet of the different components of the pelagic ecosystem.

This report gives an overview of this sampling programme with information on the number of samples collected and their distribution in different strata (species, area, gear, size). Gaps in the dataset are identified.

This overview allows us to design a new sampling strategy that addresses the objectives of the new GEF- funded Pacific Islands Oceanic Fisheries Management Project (OFM), in particular the objective "to improve understanding of the transboundary oceanic fish resources and related features of the western and central Pacific warm pool large marine ecosystem".

# 2. Design of a sampling protocol

Before designing and implementing a sampling strategy, 2 trips at sea on fishing vessels (1 longline and 1 purse seine vessels) were undertaken in 2001 to observe the fishing techniques and design a proper protocol with realistic expectations. From this experience, a sampling protocol has been designed for the collection of stomach (for stomach content examination) and muscle and liver (for stable isotope analysis) for target species (tuna) and bycatch species (sharks, marlins, other predators). The protocol was designed in collaboration with the SPC Observer and Port Samplers coordinators, taking into account comments from different colleagues.

One of the constraints for the design of the protocol was that it needed to be simple and self-explanatory as no specific training was undertaken to train the observers for this sampling programme. The protocol is now explained in the frame of the observer training provided by SPC and FFA.

To try and reduce the extra-work for observers forms already used for observer work were used when possible (longline), however, a new form had to be created for the sampling on purse seine vessels.

The fishing techniques being different 2 protocols were designed, one for longline (both in English and French) (Figure 1) and another one for purse seine (English only).



Figure 1. Extract of the sampling protocol designed for longline caught fish.

# 3. Implementation of the sampling strategy with the observer programmes

Once the sampling protocol was designed, sampling kits with all the necessary equipment were prepared and sent to the different observer programmes involved in this study. Repeated contacts were established with the observer programmes of the western and central Pacific to present the study and the sampling protocol. A presentation was made during the 4<sup>th</sup> Regional Observer Coordinators' Workshop (Honolulu, July 2002) to ask for comments and establish collaboration with the observer programmes. This important meeting was the starting point for the establishment of most of the collaborations, the success of which depended on the degree of organisation of the observer programme and on the facilities available in each country.

<u>Vanuatu.</u> No existing observer programme. In December 2003, a sampling kit was provided to a Vanuatu fishery office staff member and the procedure was explained to him. No follow-up.

<u>Tonga.</u> No existing observer programme. An observer training course was run in October 2003, the sampling protocol was presented and a sampling kit provided. No follow-up. Since end of 2005-beginning of 2006, the observer programme got underway properly and it could be considered introducing sampling in late 2006.

<u>Tuvalu.</u> No existing observer programme. Following the SCTB17 meeting in August 2004, the Tuvalu representative showed interest in the study and offered to collect samples. The protocol was sent and directions given for the organisation of sampling. However no observer programme exists and considering the difficulties to ship equipment and samples to and from Tuvalu, there was no follow-up.

<u>American Samoa</u> (USMLT and FSMA Observer Programmes). First contact was established during the observer coordinators' meeting in July 2002. The idea was to take advantage of the presence of the canneries in Pago and the unloading of US fishing vessels that carry USMLT observers, to collect some samples there. But due to the movements of the fleet away from the western and central Pacific area so that fewer vessels now unload in Pago and due to the lack of space for storage of equipment and samples in the NMFS office the idea was abandoned in October 2005. No follow-up. In 2006 an American Samoa national observer programme has commenced using observes from the Hawaii observer programme to observe aboard longiners fishing in American Samoan waters and in the Northern Cook Islands. It is plausible that these observers may be cooperate with sampling.

<u>Cook Islands.</u> Observer programme with a coordinator, but very few staff members. First contact was established in July 2002 when 4 sampling kits and 2 eskies were provided to the programme. They were very keen to collaborate and 2 sampling trips (29 samples) were undertaken when one of the SPC staff member went there for a training. The samples were transported by staff members as shipment could be problematic.

Unfortunately the programme now has few staff to do the work and no more sampling can be undertaken. Quality of the samples and data collected are good. In 2006 a few trips are about to be undertaken and could offer more opportunities of sampling.

<u>Kiribati</u>. Observer programme. In October 2002, 3 eskies and 3 sampling kits were sent to Christmas Island but soon after that the fishing activity moved to Tarawa so the kits and eskies were transferred there. One freezer was bought by SPC and placed in Hawaii for the storage of Kiribati and Cook Islands samples before sending them to Noumea. The shipment of the samples could be problematic. However, despite the willingness of the coordinator no sampling trip was organised and no samples obtained.

<u>Fiji.</u> Observer programme. 5 sampling kits, 2 eskies and 1 freezer were provided to the observer programme from November 2002. The coordinator of the observer programme showed willingness to implement the sampling but no samples were collected despite significant correspondence with the observer programme. After a new coordinator took charge in 2005 some samples were collected and sent to Noumea.

<u>Marshall Islands.</u> Observer programme with ups and downs. Contacts started in 2001when eskies and sampling kits were provided but nothing really happened until a coordinator took charge of the programme in 2003. Since the beginning of the sampling programme 17 kits were provided as well as 7 eskies and 1 freezer. Samples were collected. Shipment of the samples is sometimes problematic and relies most of the time on SPC staff members travelling there. This observer programme also helps place FSMA observers (see FSMA paragraph). Quality of the samples and data collected are good.

<u>FSMA Observer Programme.</u> Observers from this programme, managed by FFA, participated in the collection of samples using the kits and facilities provided to PNG, Solomon Islands and Marshall Islands programmes. Quality of the samples and data collected are good.

<u>French Polynesia.</u> Observer Programme started in 2002. As soon as a coordinator was recruited for this programme contacts were established, 17 sampling kits and 9 eskies were provided. Direct flight to Noumea facilitated the sending of the samples. Samples have been collected. Quality of the samples and data collected need to be improved. Communication and quality of samples improved once a new coordinator took charge in 2005.

<u>Wallis and Futuna.</u> No tuna fishery, no observer programme. Contacts were established in 2004 for the collection of samples during an exploratory tuna fishing trip planned in 2005. Samples collected are of good quality but for further sampling the observer should be trained.

<u>Solomon Islands.</u> Observer programme with ups and downs, political problems in 2003. Contacts started in 2002 and 16 kits, 16 eskies and 1 freezer were provided to the programme allowing the collection of samples. The samples are sent to Noumea through Vanuatu and the route established works well. The quality of the samples and data are good. <u>Federated States of Micronesia.</u> Observer programme with ups and downs. Contacts started in 2002, 13 kits, 7 eskies and 1 freezer were provided. Samples have been collected. This observer programme also assists place of FSMA observers. The shipment of the samples from FSM is very problematic and some were lost or ruined in the process. Data and sample quality are good.

<u>Papua New Guinea.</u> Observer programme with ups and downs. This programme was first contacted in 2002. 24 kits, 8 eskies and one freezer (in Madang) were provided. Samples were obtained. Problems arose twice with a particular fishing company. Specific instructions were given to the observers following the first problem and after the second problem further discussion and clarification took place with the fishing company. It seems the problem was resolved but this company is now avoided. Good collaboration has been established with the freight agent and the samples transit perfectly through Brisbane. Data and sample quality are good.

<u>New Caledonia.</u> Observer Programme 2002-2005. As the observer programme is based at SPC it is very easy to organise the collection of samples. Kits and eskies were provided and sampling trips were undertaken. No shipment of the samples is needed. The quality of the data and samples are good.

<u>SPC.</u> Samples have been collected by SPC staff members during observer trips.

<u>Ship of opportunity.</u> Volunteers collected predator samples (stomach, muscle, liver) on ships of opportunity, mainly in the New Caledonia EEZ, during scientific cruises, experimental fishing and sport fishing.

The collection of samples is done in collaboration with the national observer programmes of the different countries and territories of the western and central Pacific. It thus relies on the way the observer programmes operate and is dependent on the presence of appropriate staff members (coordinators and observers), on their willingness to collaborate (usually very good), on the fishing activity, on the flight facilities between islands and also on political and economical situations that can be unstable and can induce a slow down or stop to observer programme activities. Success of the implementation of the sampling programme varied amongst countries and territories and changed during the duration of the project.

This important activity which forms the foundation of the laboratory and analytical work is very time consuming. The liaison required to establish good contacts with the observer programmes takes a long time. Constant communication is required with observer programmes, freight agents, quarantine and customs to regularly remind about sampling needs, to ensure correct procedures for sampling are carried out, to make sure that samples are sent optimally and successfully, to find solutions to specific situations that can arise (problem with fishing companies, samples landed in unexpected ports ...), feedback to the coordinators and observers... A visit to the different observer programmes in their countries will probably greatly reinforce the links with SPC and might help to make sure the sampling is organised and properly done.

# 4. Number of samples collected during the SAP project 2000-2005

# 4.1. Number of sampling trips and total number of samples

It is important to keep in mind that the numbers, geographic distribution, species, size and gears described in this report are not representative of the fisheries or of the fish populations, but they illustrate the samples collected during this project.

The sampling programme started at the beginning of 2001 and observers have been asked to stop sampling by the end of 2005.

During the project, 90 sampling trips have been undertaken and 3140 stomachs have been collected by 12 observer programmes (Table 1).

Observer Programme	No of trips	No stomach
Cook Islands	2	29
Fiji	3	48
French Polynesia	22	618
FSM	2	182
FSMA	10	392
Marshall Islands	2	52
New Caledonia	13	527
Papua New Guinea	9	472
Ship of Opportunity	12	71
Solomon Islands	12	357
SPC	2	362
Wallis & Futuna	1	30
Grand Total	90	3140

Table 1. Number of trips undertaken by the different observer programmes and number of samples collected between Jan 2001 and Dec 2005.

Of the 3140 stomachs collected, 2637 have been examined and about 246 samples were lost, rotten or without data. In June 2006 about 284 samples were stored in SPC freezer and needed to be examined.<sup>1</sup>

67 different species have been examined with a number of samples varying between 1 and 559 per species (Table 2). For 38 of these species less than 10 stomachs have been collected; 100 stomachs or more have been collected for only 8 species (WAH, RRU, DOL, ALX, SKJ, ALB, YFT, BET).

<sup>&</sup>lt;sup>1</sup> There is a difference between the number of samples collected and the sum of the numbers of stomachs examined, lost/rotten/no data and stored in the freezer because some prey's stomachs contained into the predators are sometimes subsampled for examination. These subsamples are not considered into the number collected but are considered into the number examined.

Scientific name	Common name	Code	Non- empty	Total	Scientific name	Common name	Code	Non- empty	Total
Thunnus albacares	Yellowfin	YFT	342	559	Gnathanodon speciosus	Golden trevally	GLT	4	6
Katsuwonus pelamis	Skipjack	SKJ	155	506	Makaira indica	Black marlin	BLM	6	6
Thunnus obesus	Bigeye	BET	215	330	Aluterus monoceros	Filefish	ALM	1	5
Thunnus alalunga	Albacore	ALB	164	167	Gempylidae	Snake mackerels & escolars	GEP	1	5
Elagatis bipinnulata	Rainbow runner	RRU	80	147	Scombrolabrax heterolepis	Black mackerel	SXH	1	5
Coryphaena hippurus	Mahi mahi	DOL	93	115	Platax teira	Longfin batfish	BAO	1	4
Acanthocybium solandri	Wahoo	WAH	90	112	Canthidermis maculatus	Ocean triggerfish	CNT	3	3
Alepisaurus ferox	Longsnouted lancetfish	ALX	86	101	Isurus paucus	Long finned mako shark	LMA	3	3
Lampris guttatus	Moonfish / opah	LAG	54	58	Mobula japanica	Manta ray	RMJ	3	3
Balistidae	Oceanic triggerfish	TRI	30	40	Mobulidae	Manta rays	MAN	0	3
Makaira mazara	Blue marlin	BUM	31	37	Caranx sexfasciatus	Bigeye trevally	CXS	1	2
Prionace glauca	Blue shark	BSH	13	36	Chiasmodontidae	Chiasmodontidae	СНМ	2	2
Sphyraena barracuda	Great barracuda	GBA	25	32	Galeocerdo cuvier	Tiger shark	TIG	1	2
Xiphias gladius	Swordfish	SWO	26	30	Promethichthys prometheus	Roudi escolar	PRP	1	2
Carcharhinus falciformis	Silky shark	FAL	19	29	Abudefduf saxatilis	Sargent major	ABU	0	1
Tetrapturus angustirostris	Short-billed spearfish	SSP	27	28	Allothunnus fallai	Slender tuna	SLT	1	1
Tetrapturus audax	Striped marlin	MLS	28	28	Alopias superciliosus	Bigeye thresher shark	BTH	0	1
Dasyatis violacea	Pelagic sting-ray	PLS	21	24	Alopias vulpinus	Thresher shark	ALV	0	1
Lepidocybium flavobrunneum	Escolar	LEC	7	21	Assurger anzac	Razorback scabbardfish	ASZ	1	1
Decapturus macarellus	Mackerel scad	MSD	5	18	Carcharhinus leucas	Bull shark	CCE	1	1
Istiophorus platypterus	Sailfish	SFA	16	18	Desmodema polystictum	Dealfish	DSM	1	1
Auxis thazard	Frigate tuna	FRI	0	14	Elasmobranchii	Sharks	SHK	0	1
Sphyraena spp.	Barracudas	BAR	6	13	Kyphosus cinerascens	Drummer	KYC	0	1
Isurus oxyrhinchus	Short finned mako shark	SMA	6	12	Lobotes surinamensis	Triple-tail	LOB	1	1
Ruvettus pretiosus	Oilfish	OIL	4	12	Lophotus capellei	Crestfish/unicornfish	LOP	1	1
	Unspecified	UNS	8	11	Magnisudis sp.	Barracudina	MUG	1	1
Euthynnus affinis	Kawakawa	KAW	2	11	Omosudis lowei	Omosudid	OMW	1	1
Gempylus serpens	Snake mackerel	GES	3	11	Platax spp	Batfishes	BAT	1	1
Melichthys niger	Black triggerfish	MEN	2	11	Sardina pilchardus	Sardine / pilchard	PIL	0	1
Auxis rochei	Bullet tuna	BLT	0	9	Scopelarchidae	Perleyes nei	PEY	1	1
Carcharhinus longimanus	Oceanic white-tip shark	OCS	2	7	Sphyraena genie	Blackfin barracuda	BAB	1	1
Taractichthys longipinnis	Big-scaled pomfret	TAL	4	7	Sphyrna lewini	Scalloped hammerhead	SPL	1	1
Taractichthys steindachneri	Sickle pomfret	TST	0	7	Sphyrna spp.	Hammerhead sharks	SPN	1	1
Bramidae	Pomfrets	BRZ	4	6			Total	1609	2637

Table 2. Number of stomachs (non-empty and total) examined per species in decreasing order of total stomachs.

# 4.2. Number of samples required to precisely describe the diet of a species

Determining the adequate number of stomachs to precisely describe the diet of a species is very difficult and despite its importance there is very little literature on the subject and this issue is very rarely mentioned in pelagic species diet studies.

A high degree of variation can be observed from one stomach to the other for the same species and it is usually explained by these factors:

- length / age / sex class with frequent observations of differences in the diet of juveniles and adults that can partly be related to different physiological abilities (size of the mouth, speed, ability to dive-swimbladder...),
- temporal (time of the day, season, year) and spatial (vertical and horizontal) distribution of the predators and the preys linked to oceanographic and environmental features as well as species preferences,
- predator individual preferences in the selection of the preys.

The number of stomachs required to properly describe the diet of a species will depend on the magnitude and sources of stomach content variability {Pennington, Bowman, et al. 1981 1694 /id}. The description of the diet of a species population (all size range) over a large area and over a long period of time will require a bigger sample size than for the description of the diet of a particular part of the population (*e.g.* adults) in a small area for a particular season as more variability is expected in the first case than in the second.

Ferry & Cailliet (1996) advocate the use of cumulative prey diversity curves to estimate the sample size sufficiency (Annex 1-BOX 1). They give 3 examples for which sample size sufficiency is reached with about 30 stomachs in one case, about 40 samples in a second example and is not reached in the third example after the examination of 40 stomachs. In the example used by Hoffman (1979), for samples taken from the same habitat on a short period of time (2 weeks), 30 fish were enough for one group, 40 samples did not allow to reach the asymptote for the other group.

Of the 36 studies on the diet of tuna and other large pelagic reviewed and reported in Annex 4, only one study estimated the sample size sufficiency. Markaida & Sosa-Nishizali (1998) applied the method developed by Hoffman (1979) and estimated that 20 to 25 samples were enough to describe the diet of swordfish collected during short periods of time 2-3 weeks in limited areas. In the remaining 35 studies, the sample size sufficiency is not discussed at all and then probably assumed adequate. In these studies, the number of stomachs examined varied from 19 to 1498 stomachs sampled and from 14 to 1064 non-empty stomachs. More than 50% of the studies considered less than 150 stomachs (sampled and non-empty) to describe the diet of the predators (Figure 2).

In our study the sources of variability are multiple with a large magnitude. The collection of samples with different gears (mainly purse seine and longline) will imply the sampling of different size class of the fish population (small *vs.* large fish respectively), at different time of the day (day vs. day and night) and at different depth (surface *vs.* surface and

deep) (cf. paragraph 4.4). Moreover, the sampling over a large area (western and central Pacific) and over a long period of time (2001-2005) all year long introduces a high degree of variability both spatially and temporally. Characterising the diet of the pelagic species at this scale would require a large number of samples to take into account all the potential variability, however, in practise sample size is constrained mainly by the sampling opportunities and possibilities and the time and personnel required for analysing the samples.

Applying the Ferry & Cailliet (1996) method, cumulative curves have been established for the more sampled species and some examples are displayed in Figure 3. These cumulative curves are characterized by a large number of prey species especially for the longline-caught fish (between 58 and 114 prey species). None of the curves reaches the asymptote indicating that the required number of stomachs have been examined to properly describe the diversity of the predator's diet.

A compromise needs to be reached between an ideal number of samples to properly describe the diet of the pelagic species in the western and central Pacific and a reasonable number of samples to collect and analyse. Reaching 100 non-empty stomachs per species per strata (area-gear) appears to be a realistic compromise.



Figure 2. Number of studies on the diet of large pelagics according to the number of stomachs sampled and non-empty stomachs.



Figure 3. Cumulative curves of the number of new preys plotted against the number of stomachs analysed. Average of 10 randomized cumulative curves.

# 4.3. The empty stomach issue

Diet description will be based on analysis of non-empty stomachs (Annex 2) and if they do not provide any information on the list and quantity of preys consumed, empty stomachs inform on the feeding behaviour (*e.g.* a high percentage of empty stomachs may reflect short periods of feeding followed by periods of rapid digestion (Joyce *et al.*, 2002)) and they have to be taken into account to calculate the daily ration.

Among the samples examined in our study, 39% were empty. This percentage varies according to different parameters:

Species: percentage of empty stomachs varies from 0 to 100% but no particular pattern has been observed (Annex 5). Species from the same family or group such as tuna, shark, marlin don't show any similarities in terms of emptiness of the stomachs. It was expected that sharks show a high percentage of empty stomachs due to regurgitation (Annex 3-BOX 2), but it is not the case. Apart from direct observation regurgitation cannot be estimated; it is however a known cause of underestimation of stomach content and potentially of emptiness.

Sampling gear: longline samples show 12% of stomach emptiness while purse seine samples have a percentage of 72.5% with higher values for FAD fishing (*ca.* 77%) than for fishing on free schools (54%) (Table 3). While longline caught fish are actively looking for food, the probability their stomach is not empty is probably higher than for purse-seine fish which are not feeding when under FADs (Annex 3-BOX 5). When caught in free schools, purse seine samples show a lower percentage of empty stomach than under FADs suggesting that they feed while in free schools. The higher value than for longline fish could be linked to the fact that when they are spotted and caught at the surface fish are rarely feeding.

The time of the day: information on the time of the catch is only available for purse seine samples and it will vary according to the fishing technique, FAD fishing happening only in the mornings. Percentage of empty stomachs is high at night and early in the morning (68-90% from 2 to 10h) and tends to decrease from 12h to 16h (55-6%), however the number of samples is low (Figure 4). From this information it is difficult to conclude when the fish eat as their behaviour is particular under FADs and in free schools the number of samples is low; but it seems they are feeding more in the afternoon. More information on feeding time and digestion rate has been gathered in Annex 3-BOX 4 and BOX 3.

Emptiness of a stomach can be due to an artefact, the regurgitation, or can be explained by the fact that, when caught, the fish examined had not fed for a time longer than the time necessary to digest its last meal; it is then a conjunction of when and how often the fish eat (feeding time), how fast it digests (digestion rate) and when and how the fish was caught (fishing gear and fishing strategy).

			Purse Se	ine			Other	
	Longline	Free school	Drifting FAD	Anchored FAD	Troll Line	Handline		
No of samples examined	1449	237	717	212	19	2	1	
% empty stomachs	12.1	54.4	77.0	77.4	31.6	50.0	0.0	

Table 3. Number of samples examined and percentage of empty stomachs per sampling gear type.



**Figure 4.** Percentage of empty stomach according to the time of the day for purse seine samples of different type. Numbers above the bars represent the number of stomachs examined. 2 hour-range: 12h=12h00-13h59.

## 4.4. Impact of the sampling gear on the sample composition

Samples examined have been collected by 5 different gears (Table 3): mainly longline (55% of the samples) and purse seine-drifting FADs (24%), PS-anchored FADs (11%), PS-free school (9%), but also troll line (0.7%), handline (0.1%) and other gear (spearfishing – 0.04%, 1 sample).

Because of the very different gear characteristics/selectivity (Table 4) and fishing strategies, gear will have an impact on the composition of the samples both on the species collected and on the specimens of a particular species (population level).

	Longling	Pur	se seine
	Longine	Free school	Floating object
Setting time	Dawn / morning	Dawn to dusk	Dawn
Hauling time	Dusk / night	Dawn to dusk	Dawn
Fishing depth	50-450 m	0-200/300 m*	
Passive/active	Passive / baited	Active	
Hook / mesh size	Standard Japanese tuna hook	Mesh size about 1	0-25 cm
Areas	Equatorial to temperate	Equatorial	
Main target fish	Albacore, yellowfin, bigeye	Skipjack, yellowf	in
Size of the fish	Large (mainly >80cm)	Small and large (	30 to >120cm)

**Table 4. General characteristics of the fishing gears.** \* depth of the net, however the fish are caught at the surface.

 Table 5. Percentage of the different predators collected per gear type. Only predators with more than 10 samples collected are displayed.

					Purse se	ine
Scientific name	Common name	code	Longline	Free school	Drifting FAD	Anchored FAD
Gempylus serpens	Snake mackerel	GES	100			
Isurus oxyrhinchus	Short finned mako shark	SMA	100			
Ruvettus pretiosus	Oilfish	OIL	100			
Lepidocybium flavobrunneum	Escolar	LEC	100			
Dasyatis violacea	Pelagic sting-ray	PLS	100			
Tetrapturus angustirostris	Short-billed spearfish	SSP	100			
Tetrapturus audax	Striped marlin	MLS	100			
Xiphias gladius	Swordfish	SWO	100			
Prionace glauca	Blue shark	BSH	100			
Lampris guttatus	Moonfish / opah	LAG	100			
Alepisaurus ferox	Longsnouted lancetfish	ALX	100			
Thunnus alalunga	Albacore	ALB	100			
Istiophorus platypterus	Sailfish	SFA	89		11	
Sphyraena barracuda	Great barracuda	GBA	88		9	3
Makaira mazara	Blue marlin	BUM	84	11	5	
Acanthocybium solandri	Wahoo	WAH	82		18	
Coryphaena hippurus	Mahi mahi	DOL	81	2	16	1
Thunnus obesus	Bigeye	BET	62		31	7
Carcharhinus falciformis	Silky shark	FAL	59	3	21	17
Thunnus albacares	Yellowfin	YFT	53	3	31	13
Sphyraena spp.	Barracudas	BAR	46		38	15
Katsuwonus pelamis	Skipjack	SKJ	15	41	34	10
Elagatis bipinnulata	Rainbow runner	RRU	1	1	74	24
Auxis thazard	Frigate tuna	FRI			100	
Decapturus macarellus	Mackerel scad	MSD			100	
Balistidae	Oceanic triggerfish	TRI			100	0
Euthynnus affinis	Kawakawa	KAW		18	55	27
Melichthys niger	Black triggerfish	MEN			36	64

Longline, purse seine on free schools and purse seine on floating objects catch fish at different depths/areas/time with different selectivity (hook *vs.* mesh), they will collect different species: deep and/or large species such as albacore, opah, blue shark or swordfish among others are exclusively sampled with longline, yellowfin, bigeye or barracuda are caught by longline and purse seine while small and/or surface species such as skipjack, triggerfish, frigate tuna, mackerel scad are mainly caught by purse seine (Table 5).

Scientific nome	Common	aada	Fishing		Le	ngth rang	ge (cm)	
Scientific name	name	code	gear / school	<60	60-79	80-99	100-119	>120
Acanthocybium	Wahaa		Longline			3	24	73
solandri	Walloo	WAII	PS-Drifting FAD	5	40	45	5	5
			Longline	6	6	6	29	53
Carcharhinus	Silky chark	EAL	PS-Free					100
falciformis	Sliky Shark	FAL	PS-Drifting FAD		17	33	50	
			PS-Anchored FAD		80	20		
			Longline		1	22	50	27
Coryphaena	Mahi mahi		PS-Free			100		
hippurus	Mani mani	DOL	PS-Drifting FAD	50	44	6		
			PS-Anchored FAD			100		
Istiophorus	Collfich	0.54	Longline				6	94
platypterus	Samsn	SFA	PS-Drifting FAD				50	50
			Longline	10	76	14		
Katsuwonus	Chinia ala		PS-Free	63	37			
pelamis	Бкірјаск	SKJ	PS-Drifting FAD	98	2			
			PS-Anchored FAD	81	19			
			Longline					100
Makaira	Blue marlin	BUM	PS-Free					100
IIIazara			PS-Drifting FAD					100
<u> </u>			Longline	4	7	67	22	
Sphyraena	Great	GBA	PS-Drifting FAD		67			33
Darracuua	Darracuua		PS-Anchored FAD	100				
<u> </u>			Longline			83	17	
Sphyraena	Barracudas	BAR	PS-Drifting FAD	20		20	60	
spp.			PS-Anchored FAD		50	50		
			Longline		3	21	26	50
Thunnus	Valleyfie	VET	PS-Free	29	12	18	6	35
albacares	Yellowfin	YFI	PS-Drifting FAD	76	11	9	3	1
			PS-Anchored FAD	67	24	10		
			Longline	1	5	25	41	27
Thunnus	Dimense	DET	PS-Free			100*		
obesus	ыдеуе	BEI	PS-Drifting FAD	75	15	6	2	2
			PS-Anchored FAD	45	41	14		

 Table 6. Percentage per gear and species of the number of specimens collected per length range.\*

 only one individual.

Mainly because of the hook / mesh selectivity but also due to depth of fishing and vertical distribution of the species, in general specimens from the same species will be larger when sampled with a longline than with purse seine (*e.g.*: mahi mahi, wahoo, skipjack, bigeye) (Table 6, Figure 5). In the case of the yellowfin small, intermediate and large specimens are caught with purse-seine-free school fish while longline catch intermediate and large fish and purse-seine-FADs small fish (Figure 5).



Figure 5. Length distribution of the sampled skipjack, bigeye and yellowfin per gear.

Fish are caught during the day in the purse seine fishery and only early in the morning for FAD fishery, while they are caught day and night by the longline without knowing at what time exactly (Table 4; Figure 4). They are also caught at different depth: the surface for purse seine and from the surface to 400-500 m depth for longline (Table 4). Fish will then be collected at different moment (time and depth) of their feeding cycle.

A strong link also exists between the fishing grounds and the gears: purse seiners are operating in equatorial areas while longline operate from equatorial to temperate areas (paragraph 4.5 and Figure 6).

When analysing the stomach contents of a particular species, samples from the different fishing techniques cannot be mixed, they need to be considered separately as they represent the diet of different parts of the population of the same species, at different time and area. Four fishing techniques will be considered:

-longline, -purse seine-free school, -purse seine-drifting FAD, -purse seine-anchored FAD.

# 4.5. Definition of areas of interests

Samples have been collected in 12 different EEZs and in International Waters (IW). IW samples have been re-allocated to the closest EEZ as they can't constitute a group *per se*. Most of the samples were collected in the EEZ of New Caledonia (24%), Papua New Guinea (17%), French Polynesia (17%) and Marshall Islands (11%) (Table 7).

There is a clear link between areas and sampling gears: samples from New Caledonia, French Polynesia, south of PNG and south of Solomon are exclusively caught with longline while in the equatorial areas where large schools of surface tuna are encountered purse seine is dominant (north of PNG, north of Solomon, Nauru, Gilbert Islands, Marshall Islands) (Figure 6). Purse seine samples are coming from free schools, drifting FADs and anchored FADs. Anchored FADs sampling is concentrated into the Bismark Sea in PNG

		PS-Free	PS-Drifting	PS-Anchored		
EEZ	Longline	school	FAD	FAD	Total	%
Cook Islands	29				29	1.1
Fiji	25				25	0.9
Federated States of						
Micronesia	45		69		114	4.3
Kiribati - Gilbert Islands		22	199		221	8.4
Indonesia			9		9	0.3
Marshall Islands	28	165	95		288	10.9
New Caledonia	640				640	24.3
Nauru		2	162		164	6.2
French Polynesia	435				435	16.5
Papua New Guinea	27	48	164	200	439	16.6
Solomon Islands	211		19	12	242	9.2
Wallis & Futuna	31				31	1.2

Table 7. Number and percentage of samples collected in the EEZ of the region, per gear type.

Grouping samples by EEZ is not appropriate for pelagic species that can move extensively such as the tunas. If diet of the same species is probably different when they are coming from two EEZs far apart such as French Polynesia and New Caledonia, samples coming from adjacent EEZs such as PNG and Solomon Islands might have to be grouped as the diet can be suspected as similar.

It has then been decided to define six areas of interest grouping EEZs (Figure 6):

- French Polynesia (French Polynesia and Cook Islands),
- Fiji-Wallis (Fiji, Wallis & Futuna, Tonga, Samoa, American Samoa),
- New Caledonia,
- Solomon-PNG (Solomon Islands and the south of PNG EEZ),
- Bismark sea in PNG EEZ,

- Micronesia (Nauru, Gilbert Islands, Marshall Islands and FSM, north of Solomon and central and east of PNG).



Figure 6. Geographical distribution of the samples collected per gear type.

## 4.6. Number of samples per strata area/gear

Combining area and gear, the 2 main factors of variation of the sample composition (species-size-depth-time of the day), 8 strata area/gear have been defined.

- French Polynesia / Longline
- Fiji-Wallis / Longline
- New Caledonia / Longline
- Solomon-PNG / Longline
- Bismark / Purse seine Anchored FAD
- Micronesia / Longline
- Micronesia / Purse seine Drifting FAD
- Micronesia / Purse seine Free school.

The number of non-empty samples collected per strata area/gear is detailed by species in Table 8 in decreasing order of the total number of samples per species. The most sampled species, at the top of the table are yellowfin, bigeye, albacore, skipjack, dolphinfish, wahoo, lancetfish rainbow runner. For these species, the more sampled strata are New-Caledonia/Longline, French Polynesia/Longline, Solomon-PNG/Longline and then Micronesia/PS-free school and Micronesia/PS-Drifting FAD.

The objective of 100 non-empty samples per species per strata is only reached for yellowfin in New-Caledonia/Longline; species/strata for which more than 50 samples have been collected are highlighted in Table 8.

# 4.7. Note on the analysis of the samples collected and results on the diet of large pelagics

All the samples collected by the different observer programmes are sent to SPC, New Caledonia where they are processed in the OFP laboratory. A classical procedure is used to examine the stomachs; briefly, preys are sorted by species or group, identified at the lowest taxonomic level, they are counted, weighted and measured. More details are provided in the papers cited downbelow.

Partial analysis have been conducted on the stomach content data and progress have been regularly presented in several SCTB and SC meetings (Allain, 2002; Allain, 2003; Allain, 2004; Allain, 2005).

More detailed analyses will be conducted as the number of samples examined increases.

 Table 8. Number of non-empty stomachs examined per species, per area and per gear type. Dark blue cell: more than 100 samples, light blue cell: more than 50 samples.

 For the last 10 species, only empty stomachs have been collected.

Scientific name	Common name	code	FRENCH POLYNESIA	FIJI- WALLIS	NEW CALEDONIA	SOLOMON -PNG	BISMARK	MICRONESIA	MICRONESIA	MICRONESIA
			Longline	Longline	Longline	Longline	Anchored FAD	Longline	Drifting FAD	Free
Thunnus albacares	Yellowfin	YFT	70	22	109	75	14	15	22	15
Thunnus obesus	Bigeye	BET	56	9	56	67	4	14	8	1
Thunnus alalunga	Albacore	ALB	77	13	52	21		1		
Katsuwonus pelamis	Skipjack	SKJ	18		40	3	1	5	5	83
Coryphaena hippurus	Mahi mahi	DOL	21	2	49	4	1	8	6	2
Acanthocybium solandri	Wahoo	WAH	38	5	29	7		3	8	
Alepisaurus ferox	Longsnouted lancetfish	ALX	11		75					
Elagatis bipinnulata	Rainbow runner	RRU					21	1	57	1
Lampris guttatus	Moonfish / opah	LAG	20		27	7				
Makaira mazara	Blue marlin	BUM	18		9	1		2		1
Balistidae	Oceanic triggerfish	TRI							30	
Tetrapturus audax	Striped marlin	MLS	15		12	1				
Tetrapturus angustirostris	Short-billed spearfish	SSP	10	1	16					
Xiphias gladius	Swordfish	SWO	13		12	1				
Sphyraena barracuda	Great barracuda	GBA	6		11	3	1	2	2	
Dasyatis violacea	Pelagic sting-ray	PLS	7		7	7				
Carcharhinus falciformis	Silky shark	FAL			3	3	4	3	6	
Istiophorus platypterus	Sailfish	SFA		1	8	6		1		
Prionace glauca	Blue shark	BSH			10			3		
	Unspecified	UNS	7		1					
Lepidocybium flavobrunneum	Escolar	LEC	3		3			1		
Isurus oxyrhinchus	Short finned mako shark	SMA	1		5					
Makaira indica	Black marlin	BLM	2		3	1				
Decapturus macarellus	Mackerel scad	MSD							5	
Sphyraena spp.	Barracudas	BAR			2	1			2	
Bramidae	Pomfrets	BRZ				1		2	1	
Gnathanodon speciosus	Golden trevally	GLT							4	
Ruvettus pretiosus	Oilfish	OIL	1			2		1		
Taractichthys longipinnis	Big-scaled pomfret	TAL				4				
Canthidermis maculatus	Ocean triggerfish	CNT							3	
Gempylus serpens	Snake mackerel	GES	1			2				
Isurus paucus	Long finned mako shark	LMA			3					
Mobula japanica	Manta ray	RMJ								3
Carcharhinus longimanus	Oceanic white-tip shark	ocs	1		1					

### Table 8. Continued.

Scientific name	Common name	code	FRENCH POLYNESIA	FIJI- WALLIS	NEW CALEDONIA	SOLOMON -PNG	BISMARK	MICRONESIA	MICRONESIA	MICRONESIA
			Longline	Longline	Longline	Longline	Anchored FAD	Longline	Drifting FAD	Free
Chiasmodontidae	Chiasmodontidae	CHM			1			1		
Euthynnus affinis	Kawakawa	KAW								2
Melichthys niger	Black triggerfish	MEN					1		1	
Allothunnus fallai	Slender tuna	SLT				1				
Aluterus monoceros	Filefish	ALM							1	
Assurger anzac	Razorback scabbardfish	ASZ		1						
Caranx sexfasciatus	Bigeye trevally	CXS							1	
Carcharhinus leucas	Bull shark	CCE			1					
Desmodema polystictum	Dealfish	DSM			1					
Galeocerdo cuvier	Tiger shark	TIG			1					
Gempylidae	Snake mackerels & escolars	GEP	1							
Lobotes surinamensis	Triple-tail	LOB							1	
Lophotus capellei	Crestfish/unicornfish	LOP	1							
Magnisudis sp.	Barracudina	MUG				1				
Omosudis lowei	Omosudid	OMW				1				
Platax spp	Batfishes	BAT							1	
Platax teira	Longfin batfish	BAO							1	
Promethichthys prometheus	Roudi escolar	PRP	1							
Scombrolabrax heterolepis	Black mackerel	SXH	1							
Scopelarchidae	Perleyes nei	PEY				1				
Sphyraena genie	Blackfin barracuda	BAB			1					
Sphyrna lewini	Scalloped hammerhead	SPL			1					
Sphyrna spp.	Hammerhead sharks	SPN			1					
Abudefduf saxatilis	Sargent major	ABU								
Alopias superciliosus	Bigeye thresher shark	BTH								
Alopias vulpinus	Thresher shark	ALV								
Auxis rochei	Bullet tuna	BLT								
Auxis thazard	Frigate tuna	FRI								
Elasmobranchii	Sharks	SHK								
Kyphosus cinerascens	Drummer	KYC								
Mobulidae	Manta rays	MAN								
Sardina pilchardus	Sardine / pilchard	PIL								
Taractichthys steindachneri	Sickle pomfret	TST								
		Total	400	54	550	221	47	63	165	108

# 5. Design of new sampling strategy for the OFM project 2005-2010

# 5.1. Objectives

# **Objective 1:**

The main objective for this new sampling strategy will be to **complete the dataset** already collected in a way of having a valid description of the trophic structure of the pelagic ecosystem in the western and central Pacific.

For each defined strata we should try and collect at least 100 non-empty stomachs.

It is unrealistic to hope collecting a significant number of samples for all species, and we should focus on the more frequent ones, however it is important to maintain the collection of less frequent species to gather data on these poorly known species to include the information, otherwise not available, into ecosystem modelling (*e.g.* Ecopath diet matrix).

# **Objective 2:**

According to the gear used a different part of the tuna population is caught: small fish at the surface during daytime by purse seine and large fish at greater depth during day and night by longline. Hence the picture of the diet given by the samples from the 2 gears is very different.

Because there is very little overlap between the geographical distribution of the 2 gears (Figure 6), when comparing samples from Micronesia to samples from the south Pacific, it won't be possible to determine if it is a geographic or a gear (depth/size/time of the day) effect.

The second objective should be to try and **collect samples from different gears in the same area** in a way to describe the diet of different parts of the tuna population without the area effect.

# **Objective 3:**

Seamount is suspected to have an effect on aggregation of tuna and large pelagic fish in the oceanic areas. The impact of seamounts on the feeding habits of these species is under discussion and has been poorly studied so far.

The third objective should be to try and **collect samples from seamounts** in a way to establish the impact of seamounts on the diet of tuna and other pelagic predators.

# **Objective 4:**

The impact of the very large array of anchored FADs in the Bismarck Sea has been poorly studied so far. As anchored FADs allow to catch small fish, they could represent a threat for the sustainability of the resource and it is important to evaluate their impact.

The fourth objective should be to increase the **collection of samples around anchored FADs in the Bismarck Sea** to determine their impact on the diet of the tunas and other pelagics.

# 5.2. New sampling strategy

# **Objective 1:**

According to Table 8 and mainly because of the high emptiness percentage of purse seine samples, high priority should be put on Micronesia and Bismarck Sea and it will be important to seek the collaboration of PNG, Solomon, FSM and Marshall Island observer programmes.

More samples will also be needed to complete the datasets of longline stomachs; particularly in the Fiji-Wallis area where collaboration only recently began and that also includes Tonga, Samoa and American Samoa; but also in Solomon-PNG, French Polynesia and New Caledonia.

Obviously the species of main interest are the 4 tuna species, but for an overview of the ecosystem more samples of other predators should be collected, particularly lancetfish, mahi mahi, wahoo, rainbow runner, opah, marlins and sharks.

To reach this objective, a strong collaboration with the observer programmes will be needed from the implementation of the new sampling programme.

# **Objective 2:**

Observer programmes from the northern part of PNG, from FSM and Marshall Islands should be encouraged to send observers collecting onboard longline vessels, domestic but also distant-water vessels to have a chance to collect longline samples in the area where purse seine operate.

# **Objective 3:**

The number of potential seamounts in the Pacific Ocean could be around 30,000, but less than 1,000 have been properly identified and (as of 1988) less than 150 had been explored (Smith & Jordan, 1988).

In the context of the GEF OFM project, scientific cruises targeting seamounts will be planned for the collection of samples and it will be a good opportunity to collect stomachs samples.

However only few scientific cruises can be organised and in limited areas, then observer programmes still have an important role to play in this objective as very often fishermen know when they are fishing around seamounts. It should be recommended to the observers to collect samples when they are aware of the presence of a seamount in the vicinity and to identify the samples as such.

Also sampling should be encouraged in areas where seamounts are particularly abundant (Figure 7) (*e.g.* Tonga, Wallis & Futuna, Fiji area).

# **Objective 4:**

Observer programme from the northern part of PNG should be encouraged to collect samples from purse seine sets around FADs.

An important source of samples of Anchored FAD fish will be the tagging programme organised by SPC and scheduled in this area in 2006 and 2007; it should allow the collection of a large set of samples of small size as mainly caught with a pole-and-line vessel.

Support of the observer programme again will be critical, especially from the northern part of PNG to collect Anchored FAD samples of large size fish and of non-tuna species.

*Priorities should be given to sampling in:* 

-Micronesia area for purse seine samples of different school type for skipjack and other tuna including large specimens of yellowfin and bigeye (more than 80 cm), bycatch specimens should also be collected -Micronesia area for longline samples of tuna and bycatch

-Bismarck Sea for purse seine anchored FAD samples of tuna mainly but also bycatch

-Fiji- Wallis area including Tonga, Samoa and American Samoa for longline samples of tuna and bycatch

-Solomon-PNG area for longline samples of tuna and bycatch

More samples from New Caledonia and French Polynesia for longline samples of tuna and bycatch are still necessary to reach the objective of 100 non-empty stomachs per species, but considering the number of samples already available, it is a low priority.

For the bycatch species, focus should be put on marlins, sharks, mahi mahi, opah, wahoo, rainbow runner and lancetfish.

Presence of seamounts on the fishing grounds should be noted and samples should be collected around seamounts in all areas.

Observer programmes will continue to play a very important role in this new sampling strategy as well as the scientific cruises and tagging cruises planned in the region.



Figure 7. Seamounts known in the western and central Pacific which summit is between the surface and 600m depth (based on the list established by (Kitchingman & Lai, 2004) validated with different sources of information).

# 6. Conclusion

In the context of the GEF-SAP project from 2000 to 2005, a sampling protocol has been designed, and collaboration with the western and central Pacific observer programmes has been establish to collect samples for the study of the trophic structure of the pelagic ecosystem.

During the project, 12 observer programmes undertook 90 sampling trips collecting from 67 different species, 3140 stomachs of which 2637 were examined. Examination of the distribution of the number of samples per area, size and gear showed that these 3 factors of variability are related and should be considered as a unique stratum. In this area-gear-size stratum, 8 groups have been defined:

- French Polynesia / Longline
- Fiji-Wallis / Longline
- New Caledonia / Longline
- Solomon-PNG / Longline
- Bismark / Purse seine Anchored FAD
- Micronesia / Longline
- Micronesia / Purse seine Drifting FAD
- Micronesia / Purse seine Free school.

Examination of 100 non-empty stomachs to describe the diet of a species in a stratum has been established as a minimum objective and it is only reached for yellowfin in the New Caledonia/Longline stratum.

For the new GEF-OFM project from 2005 to 2010, 4 objectives have been established:

- complete the dataset of stomach samples to obtain a valid description of the trophic structure of the pelagic ecosystem,

- collect samples from different gears in the same area to describe the diet of different parts of the fish populations,

- collect samples from seamounts to establish their impact on the diet of tuna and other pelagic predators,

- collect samples around anchored FADs in the Bismarck Sea to determine their impact on the diet of tuna and other pelagic predators.

To reach these objectives a strong collaboration with the observer programmes will be necessary and priorities should be given to sampling in:

-Micronesia area for purse seine samples of different school type for skipjack and other tuna including large specimens of yellowfin and bigeye (more than 80 cm), bycatch specimens should also be collected

-Micronesia area for longline samples of tuna and bycatch

-Bismarck Sea for purse seine anchored FAD samples of tuna mainly but also bycatch

-Fiji- Wallis area including Tonga, Samoa and American Samoa for longline samples of tuna and bycatch

-Solomon-PNG area for longline samples of tuna and bycatch.

More samples from French Polynesia and New Caledonia are still required but are of a lower priority and presence of seamounts in the vicinity of sampling should be notified.

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# 8. Annex

#### Annex 1. Complementary information on sample size sufficiency.

# BOX 1

#### Estimating sample size sufficiency

Because of the multiple sources of variation (length, time, area), the preys are not statistically normally distributed among the predators and contents of individual stomachs do not represent the diet of the population. For this reason, classical statistical methods cannot be used to determine the sample size. Hoffman (1979) suggested a method to estimate the number of stomach samples to precisely describe the diet of a predator. It is based on the fact that when increasing sample size, variation tends to decrease. This method also recommended by Ferry & Cailliet (1996) uses the cumulative prey curves created by plotting cumulative diversity of the preys against the cumulative number of stomachs examined. The curve reaches an asymptote as new prey items are being introduced into the diet only rarely. Diversity can be estimated by the number of prev species identified in the diet and the cumulative curve in this case will indicate the sample size to precisely identify all the potential preys of the diet (Ferry & Cailliet, 1996). Hoffman (1979) used Brillouin's diversity index calculated with the number of prey individuals of the different prey species found in the stomachs, and states that the asymptote of the cumulative curve will then indicate the appropriate number of samples to precisely describe the diet of a predator both in number of prey species (qualitative) and in proportions (quantitative) of the different preys in the diet. Morato et al. (2003) used the Shannon-Wiener diversity index which calculation is based on the proportions of individuals of the different prey species. It is important to randomize the order of analysis of the stomachs to prevent bias (Hoffman, 1979; Ferry & Cailliet, 1996). By performing numerous randomizations a mean number of prey items can be determined for each added stomach to see if the curve reaches an asymptote and the variability of the asymptotic region can be assessed by calculating standard deviation of the mean number of prey item (Ferry & Cailliet, 1996). Ferry & Cailliet (1996) also mention that if cumulative curve allow determining the sample size to precisely describe the diet of a predator's population, it does not replace the need to assess sample size sufficiency for performing subsequent diet comparisons between species, area, size class... They recommend the use of a priori power analysis.

# Annex 2. Complementary information on stomach fullness and percentage of unidentified preys in non-empty stomachs.

It has to be noticed that all the non-empty stomachs does not give the same amount of information on the diet; there are 2 factors to take into consideration: the stomach fullness and the percentage of non-identified preys.

When establishing the list of preys consumed, a 100% full stomach will potentially provide more information on the diversity of the preys than a 10% full stomach. Similarly the amount of information extracted from the stomach content will depend on the percentage of preys that are not identifiable, in general because of the degree of digestion of the preys.

These 2 factors are different according to the species and the gear.

There are few samples for Anchored FAD and these values won't be taken into consideration (Table 9). When the stomachs are non-empty, they are fuller in the purse seine – free school sets than in the purse seine – FAD schools and the longline with respectively 41% and 20-29-26% of stomachs half or more full. At the species level it is obvious in the case of skipjack for which 43% are more than half-full in purse seine-free school, while it is only 20% for longline. The fact that longline and purse seine are respectively passive and active gears explain the difference (Annex 3 BOX 5). Fish caught on longline are in an active feeding phase and are hungry enough to bite the bait so with low stomach content; satiated fish would not catch the bait . Purse seine – free school fish are caught during the day when the small surface fish are supposed to be feeding (Annex 3 BOX 4) while under FADs they are suspected not to be feeding.

		Anchor	Anchored FAD			FAD		Free S	Free School			Longline		
Scientific Name	Common Name	No	<1/2	>=1/2	No	<1/2	>=1/2	No	<1/2	>=1/2	No	<1/2	>=1/2	
Makaira mazara	Blue marlin							1	0	100	30	87	13	
Lampris guttatus	Opah										53	92	8	
Elagatis bipinnulata	Rainbow runner	21	62	38	57	63	37	1	100	0	1	100	0	
Acanthocybium solandri	Wahoo				8	75	25				78	91	9	
Coryphaena hippurus	Mahi mahi	1	100	0	6	100	0	2	50	50	83	71	29	
Alepisaurus ferox	Lancetfish										85	66	34	
Thunnus alalunga	Albacore										160	86	14	
Thunnus obesus	Bigeye	4	100	0	8	75	25	1	100	0	200	53	47	
Thunnus albacares	Yellowfin	14	100	0	22	73	27	15	73	27	290	74	26	
Katsuwonus pelamis Skipjack		1	100	0	5	100	0	83	57	43	64	80	20	
Grand Total		41	80	20	106	71	29	103	59	41	1044	74	26	

Table 9. Number of non-empty stomachs (No) and percentages of the number of non-empty stomachs with fullness of the stomach less than half (<1/2) and half and more (>=1/2), for the most frequent predators per sampling gear. Grand Total shows values for all species combined, not only the most frequent.

Concerning the percentage of unidentified preys in the stomach content, there is a large difference between longline and purse seine samples (Table 10) with a percentage of unidentified preys just below 50% for the longline samples while for purse seine the percentage varies from 61% for Anchored FADs to 67% for Drifting FADs and 87% for Free Schools. Again it is linked to the gear; longline fish are feeding and supposedly have been eating fresh preys recently before getting caught while for purse seine, their last meal can have been taken a long time before being caught and is then more digested and more difficult to identify.

	Common		Purse se FAD	eine - /	Anchored	Purse sei FAD	ne - Dri	ifting log/	Purse school	seine	- Free	Longline		
Scientific Name	Name	Code	Number of samples	Mean	StdDev	Number of samples	Mean	StdDev	Number of samples	Mean	StdDev	Number of samples	Mean	StdDev
Makaira mazara	Blue marlin	BUM							1	0		30	38	38
Lampris guttatus	Opah	LAG										53	72	33
Elagatis bipinnulata	Rainbow runner	RRU	22	63	40	56	71	43	1	100		1	72	
Acanthocybium solandri	Wahoo	WAH	2	100	0	6	74	43				82	48	37
Coryphaena hippurus	Mahi mahi	DOL	1	3		6	100	0	2	70	15	81	48	39
Alepisaurus ferox	Lancetfish	ALX										86	7	19
Thunnus alalunga	Albacore	ALB										164	57	28
Thunnus obesus	Bigeye	BET	4	64	44	8	75	46	1	100		202	48	31
Thunnus albacares	Yellowfin	YFT	14	60	48	22	36	49	15	88	23	280	52	32
Katsuwonus pelamis	Skipjack	SKJ	1	0		5	100	0	83	87	30	66	58	36
Grand Total			44	61	43	103	67	45	103	87	30	1045	49	35

Table 10. Percentage (mean and standard deviation) of unidentified preys in the non-empty stomachs of the most frequent predators per sampling gear. Grand Total shows values for all species combined. Unidentified preys are unrecognizable items, unidentified fish, mollusc, cephalopods, Teuthida, crustaceans, invertebrates, cephalopod beaks and gladius.

#### Annex 3. Complementary information on regurgitation, digestion and feeding time.

#### Regurgitation

Fish regurgitating part of their meal when landed onto the deck has sometimes been mentioned by observers at sea. It usually concerns fresh preys that might have been swallowed just prior the catch and might not even have reached the stomach. Partial regurgitation can induce a bias in stomach content data and some authors consider this phenomenon to partly explain low values of stomach fullness (Bard, 2001). Regurgitation has particularly been mentioned for sharks (Morato *et al.*, 2003; Rancurel & Intes, 1982)

Regurgitation is also evoked to explain how the cephalopods beaks are evacuated from the stomach. While the calcareous hard ototlith are dissolved in the acid gastric juices, the chitinous beaks are difficult / impossible to digest. They tend to accumulate in the stomach but at one stage would be expulsed from the stomach by regurgitation (Zavala-Camin, 1987).

Total regurgitation is mentioned by different authors (Grandperrin, 1975; Batts, 1972) to explain the high degree of empty stomachs, but it is actually difficult to prove. Stress during the catch or fight of the fish on the hook could induce regurgitation (Ménard *et al.*, 2000b). No study has been published on the subject, but the examination of the thickness of the stomach wall could provide some information on potential regurgitation. Thickness of the stomach wall vary with the degree of fullness; it will stretch and become very thin when the stomach is full while it will be thick and with circumvolutions on the inner wall when it is empty. This information is not recorded when stomachs are examined but experience shows that the empty stomachs observed have thick stomach wall meaning they have been empty for a while; regurgitation is then improbable even if this hypothesis cannot be excluded.

Tuna landed with everted stomachs due to decompression obviously had empty stomachs but were not sampled; it mainly concerns albacore caught on longlines.

Apart from direct observation on the deck of the boat when the fish is landed onboard, regurgitation is difficult to recognise (Maldeniya, 1996) and cannot be estimated. However it is a known cause of underestimation of stomach content and can potentially introduce a bias into the percentage of empty stomachs; it then needs to be acknowledged.

#### Digestion/evacuation rate

Few studies have been conducted on the digestion rate and gastric evacuation of tuna. Experimental work conducted by Magnusson (1969) and Olson & Boggs (1986) on captive skipjack and yellowfin concluded that these species evacuated food from the stomach faster than most other fishes studied by a factor 2 to 5.

Skipjack stomachs fed with fish were essentially empty within 12 hours after a meal; if fish stopped feeding at sunset, by sunrise their stomachs could be considered empty (Magnuson, 1969). Yellowfin fed with fish will completely digest their meal in a little bit more than 10 hours (Olson & Boggs, 1986). A study based on the examination of albacore stomach contents from caught fish concluded that the digestion of a meal would take between 15 and 27 hours and that a fish of medium size would be digested in about 24 hours (Aloncle & Delaporte, 1973). As a generalisation, a small tuna will completely empty its stomach approximately 5-12h after being fed to satiation (Brill, 1996).

Evacuation rate is significantly affected by the type of prey ingested: high-lipid content preys such as mackerel are digested slower than other fish or squids by captive yellowfin (Olson & Boggs, 1986). Squids are digested fast (except the beaks) followed by fish and crustaceans (Zavala-Camin, 1987). The effect of the meal size on gastric evacuation rate is equivocal and again it could be linked to the type of prey: small meals of mackerel is digested faster then large meals of mackerel while for squid and other fish no effect was detected, the time required to evacuate a meal of squid or other fish remains approximately the same regardless of food volume (Olson & Boggs, 1986).

According to the few studies conducted it can be considered that tuna will completely digest and evacuate a fish meal in 10-12h; it is faster than most other species studied by a factor 2 to 5. Digestion rate will vary according to the prey type: squids are digested faster than fish followed by crustaceans.

BOX 2

# BOX 3

# BOX 4

#### Feeding time

Experiments on captive fish show that skipjack don't wait until their stomach is empty before eating additional food, that they can eat all day long when offered food, but they present more intense feeding periods particularly in the morning (0630-0830) (Magnuson, 1969). Experiments do not reflect what happens in the wild but it gives insight on the capabilities of the fish behaviour.

Determination of the time of feeding on a 24h cycle is difficult and most of the studies trying to establish the feeding cycle are based on partial sampling of the 24h period with sampling gears introducing a bias into the results. There is no consensus among the studies because of inadequate sampling strategies but also probably because of variability in the pattern of feeding as shown in experiments (Magnuson, 1969).

No generalisation is possible, but several studies support the fact that because they are visual predators, the small-size surface tuna and surface species such as yellowfin and skipjack would mainly eat during the day with a peak just after sunrise around 07:00-09:00 am potentially followed by lower peaks later in the day, particularly early in the evening around 18:00 and little feeding all day long (Magnuson, 1969; Nakamura, 1965; Zavala-Camin, 1987; Ortega-Garcia *et al.*, 1992; Dragovich, 1969). It is generally assumed that skipjack do not feed at night because of inadequate illumination (Forsbergh, 1980), however feeding at night by skipjack has been shown in a recent study by Schaefer & Fuller (2005). Feeding at night by the large yellowfin is probable even if it might be less intensive than during the day (Watanabe, 1958; Ménard *et al.*, 2000b). Deeper predators such as bigeye and albacore are thought to feed night and day (Zavala-Camin, 1987; Legand *et al.*, 1972; Calkins, 1980; Pusineri *et al.*, 2005; Aloncle & Delaporte, 1973; Schaefer & Fuller, 2005) with potentially more activity during the night for bigeye (Watanabe, 1958), but less for albacore (Iversen, 1962).

Longlines operate day and night and if bigeye are known to catch the baits at night (Calkins, 1980), it has been concluded from some longline fishing that albacore and yellowfin do not feed at night (Legand *et al.*, 1972). However longline does not provide information on the exact time when the fish was caught. Moreover, it would be more exact to say that they don't bite the bait at night rather than concluding that they do not eat at night. Tuna are believed to be visual hunters and if longline baits are not visible during the night, it might not be the case of forage organisms as many of them have photophores. Even if tuna don't catch the baits at night they might still be able to feed on luminescent forage organisms. Studies on the characteristics of the eye of large pelagic species support the idea that tuna can probably hunt even in low light: bigeye and yellowfin show specific adaptations for vision in dim light (Brill *et al.*, 2005; Fritsches & Warrant, 2001). Feeding time might be different according to the developmental stage. A field study on iuvenile (up to 6 cm

Feeding time might be different according to the developmental stage. A field study on juvenile (up to 6 cm long) skipjack and *Thunnus sp.* showed they were feeding during the daytime with a peak in the afternoon but they do not eat during the night (Tanabe, 2001).

Feeding pattern might be related to the availability of forage organisms (abundance and spatial distribution including their diurnal vertical movements) and to satiation (Nakamura, 1965; Zavala-Camin, 1987).

There is no consensus and no certainty on the feeding time of tuna, but skipjack and yellowfin would mainly feed during the day with a possible peak just after sunrise. Yellowfin probably eat at night also. Bigeye and albacore would eat night and day. So according to the time of the catch during the day the probability to catch fish with empty stomach will vary. Considering a digestion time of 10-12h, a fish that is not feeding during the night would have an empty stomach early in the morning.

### Interaction with fishing gears

Success of baited fishing methods (pole-and-line, longline) depends upon the feeding motivation of the fish that is influenced by the quantity of food in the stomach (satiation). This idea is supported by the fact that small quantity of natural food is usually found in the skipjack stomachs caught by pole-and-line: only hungry fish would bite while satiated individuals would not get caught (Magnuson, 1969). The same observation has been done for longline caught bigeye, albacore and yellowfin, in the study of Bard {Bard 2001 1708 /id/d} and Bertrand *et al.* (2002), and comparison with active gears (purse-seine and gillnet) suggest that only tuna with empty stomachs or low repletion are taken by the longline. However other studies on longline, including the one presented in this report show different results with a low percentage of empty stomachs for longline caught fish and high values for purse seine caught fish: few fish with empty stomachs bite the longline caught fish have lower fullness than purse-seine caught fish: few fish with empty stomachs bite the longline baits, but their stomachs only contain a small amount of food (Annex 2 - Table 9)

Purse-seine is an active gear encircling the fish so satiation won't have any influence. However for this gear operating only during daytime at the surface, the feeding behavior will still have an impact. It is suspected that, at midday, when the forage organisms have a deep distribution some tunas might also dive to follow them and would not be accessible anymore to the surface purse seine fishing (Ortega-Garcia *et al.*, 1992). In their study Ménard *et al.* (2000b) mention that the percentage of empty stomachs of fish caught by purse seine around FADs is very high (85%) compared to purse seine on free schools (25%). It is mainly linked to the fact that setting around FADs occur early in the morning, usually before 08:00, so basically before fish eat. However FADs sets later during the day also show high percentage of empty stomachs and it is believed that small fish do not feed when under FADs. The fish would leave the FAD during the day to form swimming schools to feed actively and they may come back under the FAD they use as a refuge or meeting point (Ménard *et al.*, 2000b; Ménard *et al.*, 2000a). On the other hand, large yellowfin might use the FADs to feed on the smaller fish refuge under the FAD; this idea is supported by the observation of Yesaki (1983) who observed only 5% of empty stomachs for large yellowfin (>112cm) caught by handline around payaos in the Philippines.

When caught on a baited gear (longline) the fish are actively looking for food while when caught with a passive gear they can be on whatever state of satiation; the gear will then have an influence on the percentage of empty stomachs.

BOX 5

Reference	Time	Area	Size range of	Predator	Number	Number of	Number	Number	Number
			the predator	species	of	non-empty	of prey	of prey	of prey
			(cm)	•	stomachs	stomachs	organisms	families	taxa
Pusineri et al. (2005)	Summer 1993	Bay of Biscay 10-25W; 40-	53-93	ALB	78	51	4571	8	12
		50N							
		900,000 sq km							
Roger & Marchal (1994)	Nov 1992	Atlantic Equatorial East 8-	42-51	ALB	38	66			7
		17W; 1-5N	07.70	OVI	20				
	G	290,000 sq km	37-70	SKJ	39	101			2.6
Watanabe <i>et al.</i> (2004)	Sept 2001	Transition region in the	49-76	ALB	132	124			36
	May-Jul 2002	central North Pacific							
		34-45N; 151-1/5E							
L	<b>I</b> 1 N 1079	2,800,000 sq km	52.02	ALD	005	927	24800	40	(5
Iverson (1971)	Jul-Nov 1968	Eastern Pacific 30-34N;12/-	52-93	ALB	905	827	34890	40	65
	Jui-Sept 1969	122W + 5454-50N;121-							
		125  W + 45-47  N; 125-127  W							
$C_{mu}$ by $a_{1}(2002)$									
Glubbs <i>et al</i> . (2002)		Cross Seemount		VET	112	94		15	
		50 sa km		IFI BET	350	04 280		43 78	
		50 Sq Kill		DET	339	280		78	
		4 NOAA weather buoy		YFT	80	40		22	
		200 sq km		BET	162	16		12	
Batts (1972)	Summer 1964,	N-Carolina	26-76	SKJ					
	1965	Hatteras			341	193	1159		53
		Oregon Inlet,			364	124	5198		34
Bernard et al. (1985)	15 Aug - 1 Sept 1983	Southern California	69 +-9SD	ALB	94		6967		7
		ALB 33'59-36'44N; 121'19-	49 +-2SD	SKJ					
		122'30W							
		25,000 sq km			31		194		7
		_							
		SKJ 32'38-33'46N; 117'57-							
		119'16W							
		15,000 sq km							

Annex 4. Number of stomachs collected and number of preys identified in different studies on the diet of large pelagics.

Matthews et al. (1977)		Western North Atlantic 35-75W; 35-45N 5,000,000 sq km	76-166 96-106	YFT ALB	281 48				236 for the 3 tuna grouped
			142-165	BET	14				8 1 1
			48-138	ALX	89				150
Barut (1988)	Nov 1983 - Oct 1984	Philippines, Moro gulf 35,000 sq km	100-147	YFT	620	616	2718	30	43
Ortega-Garcia <i>et al.</i> (1992)		Cortez Sea 300,000sq km	54-132	YFT	402	323			32
Watanabe (1958)	Jul-Aug 1950 Jul-Aug 1951	W equatorial Pacific 3-8N; 132-137E + 3-5N; 140-		YFT	343		8244		46
	Sept-Nov 1953	142E + 149-175E; 1-9N 3,000,000 sq km		BET	147		3478		30
Dragovich (1970)	1965-1966	Atlantic N, S, E and W 1,100,000 sq km	22-81	SKJ	1060	686			159
			40-155	YFT	611	575			174
Iversen (1962)	1950-1957	Central and NE Pacific 24,000,000 sq km	50-120	ALB	348		9285		112
Dragovich & Potthoff (1972)	Feb-Apr 1968 Sept-Nov 1968	Coast of west Africa 680,000 sq km	36-63	SKJ	711	558	22392		137
			52-94	YFT	132	126	6185		129
King & Ikehara (1956)	1950-1953	Central Pacific 180-119W; 17N-14S 25,000,000 sq km	87-172	YFT	439		22764		182
			77-196	BET	166		7414		123
Reintjes & King (1953)	Feb 1950 – Sept 1951	Central Pacific, Line & Phoenix Islands 2,340,000 sq km	50-170	YFT	1097	996	189000		99
Nakamura (1965)	1957-1959	French Polynesia, Marquesas & Tuamotu 90,000 sq km	35-89	SKJ	603	458			52
Ortiz de Zarate (1987)	Jul-Oct 1986	Bay of Biscay – Atlantic 43-47N; 2-18W 570,000 sq km	52-90	AL B	97	84			16

Hida (1973)	Oct-Nov 1969	Eastern Equatorial Pacific 2,700,000 sq km		SKJ BET YFT	268 44 45	132 29 28	198 72 50	17 11 10
	Feb-Apr 1970	Samoa 130,000 sq km		SKJ YFT	205 24	141 14	372 21	24 11
Chase (2002)	Jul-Oct 1988- 1992	Massachusetts, Atlantic 42N; 70W 27,000 sq km	227 +-44SD	BFT*	819	568		32
Young et al. (1997)	May-Jul 1992- 1994	Tasmania 120,000 sq km	40-192	SBF*	1223	1064	15495	92
Pinkas (1971)	Jul-Oct 1968 Jan-Sept 1969	Southern California, Baja California 200,000 sq km	53-136	BFT*	1073	650	23666	41
Oliphant (1971)	Jan 1968 – Sept 196	Southern California, Baja California 200,000 sq km	29-78	BEP*	1498	821	5510	19
Moteki <i>et al.</i> (2001)	Jun 1994 – Sept 1997	Eastern Tropical Pacific 10N-20S; 130-80W 18,500,000 sq km	94-169 70-172 65-146 116-194 35-129 43-142 77-161	YFT BET SWO MLS DOL ALX PTH*	30 42 25 48 38 19 20		204 324 211 347 74 319 196	23 29 22 24 18 9 15
Grandperrin (1975)	1959-1962; 1968; 1971-1974	New Caledonia - Vanuatu 160-170E; 15-25S 1,000,000 sq km	90-160	ALB YFT BET	235 435 28			88 161 23
Massutí <i>et al</i> . (1998)	May 1990-Nov 1991	Majorca W Mediterranean) 5,000 sq km	14-117	DOL	316	229	1187	61
Ribeiro Simões & Pedro Andrade (2000)		Azores, Atlantic 5,000 sq km	86-233	SWO	82	73	551	27
Velasco & Quintans (2000)	Sept-Oct 1998	NE Atlantic off Portugal and off Sahara 160,000 sq km	68-258	SWO	142	91		20
Scott & Tibbo (1974)	Summer-Autumn 1971	NW Atlantic 140,000 sq km		SWO	141			18

Orsi Relini et al. (1994)		Ligurian Sea, Mediterranean	78-192	SWO	129	118	1690		26
		13,000 sq km	49-107	BFT*	67	63	3218		18
Hernández-García	1990-1991	Central East Atlantic	Central East Atlantic 103-201 SWO 75 71						
(1995)		156,000 sq km							
Markaida & Sosa-	Feb 1992 – Jan	Baja California         76-234         SWO         173         159         1818							44
Nishizali (1998)	1993	176,000 sq km							
Salman (2004)	Autumn 1999 –	S Aegean Sea		SWO	108	103	1230		45
	Spring 2000	50,000 sq km							
Rosas-Alayola et al.	Summer-Autumn	Gulf of California	108-199	SFA*	576		12765		78
(2002)	1989-1991	200,000 sq km							
Brock (1984)	Jul 1981 – Aug	Kona, Hawaii	50-330	BUM	108	65	482		56
	1982	10,000 sq km							
Simpfendorfer et al.	Sept 1994 – Jul	W Australia	118-361	TIG*	176	84	163		29
(2001)	1997	60,000 sq km							

\*, PTH: pelagic thresher shark, *Alopias pelagicus*; SFA: sailfish, *Istiophorus platypterus*; TIG: tiger shark, *Galeocerdo cuvier*; BFT: atlantic bluefin tuna, *Thunnus thynnus*; SBF: southern bluefin tuna, *Thunnus maccoyii*; BEP: Pacific bonito, *Sarda chiliensis*.

			Nb of					Nb of	
Scientific Name	Common Name	Code	stomachs examined	% empty stomachs	Scientific Name	Common Name	Code	stomachs examined	% empty stomachs
Allothunnus fallai	Slender tuna	SLT	1	0.0	Taractichthys longipinnis	Big-scaled pomfret	TAL	7	42.9
Assurger anzac	Razorback scabbardfish	ASZ	1	0.0	Carcharhinus longimanus	Oceanic white-tip shark	OCS	7	71.4
Carcharhinus leucas	Bull shark	CCE	1	0.0	Taractichthys steindachneri	Sickle pomfret	TST	7	100.0
Desmodema polystictum	Dealfish	DSM	1	0.0	Auxis rochei	Bullet tuna	BLT	9	100.0
Lobotes surinamensis	Triple-tail	LOB	1	0.0		Unspecified	UNS	11	27.3
Lophotus capellei	Crestfish / Unicornfish	LOP	1	0.0	Euthynnus affinis	Kawakawa	KAW	11	81.8
Magnisudis sp.	Barracudina	MUG	1	0.0	Melichthys niger	Black triggerfish	MEN	11	81.8
Omosudis lowei	Omosudid	OMW	1	0.0	Gempylus serpens	Snake mackerel	GES	11	81.8
Platax spp	Batfishes	BAT	1	0.0	Isurus oxyrhinchus	Short finned mako shark	SMA	12	50.0
Scopelarchidae	Perleyes nei	PEY	1	0.0	Ruvettus pretiosus	Oilfish	OIL	12	66.7
Sphyraena genie	Blackfin barracuda	BAB	1	0.0	Sphyraena spp.	Barracudas	BAR	13	53.8
Sphyrna lewini	Scalloped hammerhead	SPL	1	0.0	Auxis thazard	Frigate tuna	FRI	14	100.0
Sphyrna spp.	Hammerhead sharks	SPN	1	0.0	Istiophorus platypterus	Sailfish	SFA	18	11.1
Abudefduf saxatilis	Sargent major	ABU	1	100.0	Decapturus macarellus	Mackerel scad	MSD	18	72.2
Alopias superciliosus	Bigeye thresher shark	BTH	1	100.0	Lepidocybium flavobrunneum	Escolar	LEC	21	66.7
Alopias vulpinus	Thresher shark	ALV	1	100.0	Dasyatis violacea	Pelagic sting-ray	PLS	24	12.5
Elasmobranchii	Sharks	SHK	1	100.0	Tetrapturus audax	Striped marlin	MLS	28	0.0
Kyphosus cinerascens	Drummer	KYC	1	100.0	Tetrapturus angustirostris	Short-billed spearfish	SSP	28	3.6
Sardina pilchardus	Sardine / Pilchard	PIL	1	100.0	Carcharhinus falciformis	Silky shark	FAL	29	34.5
Chiasmodontidae	Chiasmodontidae	CHM	2	0.0	Xiphias gladius	Swordfish	SWO	30	13.3
Caranx sexfasciatus	Bigeye trevally	CXS	2	50.0	Sphyraena barracuda	Great barracuda	GBA	32	21.9
Galeocerdo cuvier	Tiger shark	TIG	2	50.0	Prionace glauca	Blue shark	BSH	36	63.9
Promethichthys prometheus	Roudi escolar	PRP	2	50.0	Makaira mazara	Blue marlin	BUM	37	16.2
Canthidermis maculatus	Ocean triggerfish	CNT	3	0.0	Balistidae	Oceanic triggerfish	TRI	40	25.0
Isurus paucus	Long finned mako shark	LMA	3	0.0	Lampris guttatus	Moonfish / Opah	LAG	58	6.9
Mobula japanica	Manta ray	RMJ	3	0.0	Alepisaurus ferox	Longsnouted lancetfish	ALX	101	14.9
Mobulidae	Manta rays	MAN	3	100.0	Acanthocybium solandri	Wahoo	WAH	112	19.6
Platax teira	Longfin batfish	BAO	4	75.0	Coryphaena hippurus	Mahi mahi	DOL	115	19.1
Aluterus monoceros	Filefish	ALM	5	80.0	Elagatis bipinnulata	Rainbow runner	RRU	147	45.6
Gempylidae	Snake mackerels & escolars	GEP	5	80.0	Thunnus alalunga	Albacore	ALB	167	1.8
Scombrolabrax heterolepis	Black mackerel	SXH	5	80.0	Thunnus obesus	Bigeye	BET	330	34.8
Makaira indica	Black marlin	BLM	6	0.0	Katsuwonus pelamis	Skipjack	SKJ	506	69.4
Bramidae	Pomfrets	BRZ	6	33.3	Thunnus albacares	Yellowfin	YFT	559	38.8
Gnathanodon speciosus	Golden trevally	GLT	6	33.3	Grand Total			2638	39.0

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