Annex 5: Manual for ICES Stomach sampling projects in the North Sea and Baltic Sea

The study group on multi species assessment in the North Sea (SGMSNS; ICES, 2006, ICES 2007) analyzed the precision of average diet estimates for North Sea species and linked precision to sampling level. Based on this summary, the group recommends that the predators sampled are restricted to cod in the Baltic Sea and cod, haddock, whiting, saithe, gurnard, horse mackerel, mackerel, starry ray, turbot and brill in the North Sea with John Dory and hake as optional extensions. The group recommends sampling 5 rather than 10 stomachs per 5 cm size group of each predator, with the exception of saithe, mackerel and horse mackerel, where a large proportion of the stomachs are empty. For these species, 15 stomachs should be sampled from each size group. Stomachs could be taken from fish sampled for maturity and age when possible. Depending on the level of information required from the stomach analyses (see below), the samples can either be analysed on board (only level 1) or frozen individually in plastic bags (levels 1 and 2) including a label describing the sampled fish (table 5.1). Prey is recorded using TSN codes with new codes added for species not currently listed.

Further, the group proposes two different levels of stomach sampling

1. Minimum level. Only predators larger than 15 cm are sampled as fish below this size are generally not piscivorous. Stomach samples are analysed individually and fish prey identified to species. Length of fish prey is measured or estimated to nearest cm below (eggs are recorded as having length 0) and digestive stage is recorded. Invertebrates are identified to larger groups (Table 5.1). Total prey weight and weight of individual prey groups is recorded (species for fish).
2. Extended level providing information on invertebrates. Predators down to 5 cm sampled. Stomach samples are frozen individually on board and analysed in the lab. Fish and invertebrate prey is identified to species when possible. Length of fish and invertebrate prey is measured or estimated to nearest cm below. Total prey weight and weight of individual prey groups is recorded (species for both fish and invertebrates).

It is vital for later use of the data that the information recorded in the exchange format and on the labels used for year, quarter, ship and haul are consistent with those used when haul information is uploaded to DATRAS. This assures that further details of the haul can be obtained when necessary.

Selection of stomachs at sea

The fish sampled for stomachs must be selected with care. To assure the random selection within size classes, the group recommends using the fish selected for maturity sampling whenever possible. Among these fish, care must be taken to obtain reliable data:

1. Everted stomachs. Some fish have everted stomachs. Since it not known whether these stomachs contained food or not, such fish must not be used for stomach sampling.
2. Regurgitated stomachs. Some fish have regurgitated all or part of their stomach contents and these fish must not be collected for analyses. However, the number of regurgitated stomachs encountered during the examination must

be recorded to ensure that the proportion of feeding fish in the sample is accurately defined. In practice, it is often difficult to tell whether regurgitation has taken place, but in situations where the stomach is flaccid or distended, but contains little food, experimental work by Robb (Robb 1992) indicates that the size of the gall bladder is a useful practical indicator of the recent feeding history of the fish. A large densely-coloured gall bladder indicates that a stomach has been empty for some time and has not recently lost its content by regurgitation. The criterions are summarized in table A5.1 and should be applied when assessing whether a stomach should be classified as regurgitated or empty.

3. Stomachs of feeding fish showing no signs of regurgitation. These should be collected for analyses. It should be noted that not all feeding fish have grossly distended stomachs, i.e. feeding does not necessarily mean full.
4. Empty stomachs.
5. Stomachs with only indigestible skeletal remains (polychaete brstles, mollusc shells and opercula, fish bones and otoliths etc).

When stomachs are opened at sea, it is possible to distinguish between those which are truly empty and those containing small prey or indigestible remains. Accurate records can be kept and any indigestible materials should be included in the material collected. However, when entire stomachs are collected at sea, their true state cannot be determined until they are opened in the laboratory and in this case the stomachs of apparently non-feeding fish should be collected.

The material collected at sea to meet the sampling targets should originate from feeding fish showing no evidence of regurgitation and from non-feeding fish. The is, the sampling should continue until a total of 5 stomachs (empty+skeletal remains+feeding-regurgitation). The state of the gall bladder should be recorded using the scale in table A5.1 and to adjust for regurgitated stomachs discovered in the laboratory, 6 rather than 5 (20 rather than 15 for mackerel, horse mackerel and saithe) samples should be taken per 5 cm length group when stomachs are not opened at sea.

Sampling strategy at sea

1. For each predator species and 5 cm size group, aim to collect 5 stomachs per 5 cm size group of each predator, with the exception of saithe, mackerel and horse mackerel, where 15 stomachs should be sampled from each 5 cm size group. Take care not to include fish showing evidence of regurgitation. If stomachs are not opened at sea, collect 6 stomachs per 5 cm size group of each predator, with the exception of saithe, mackerel and horse mackerel, where 20 stomachs should be sampled from each size group.
2. If stomachs are opened at sea: Record the number of stomachs regurgitated, containing food and containing skeletal remains and containing food.
3. Preserve stomachs by freezing. Contents can be emptied into plastic bags before freezing.
4. Each sample should contain a label giving all the information listed in able A5.2.
5. Data are recorded using the data exchange format in table A5.3 (fields 1-70).
6. If stomachs are analysed at sea, table A5.3 fields 71-99 should be used.
7. Stomach samples are analysed individually and fish prey identified to species. When possible, length of fish prey is measured (whole prey) or estimated to fresh length (partially digested but original length still recognizable) in nearest

cm below (eggs are recorded as having length 0) and digestive stage is recorded. Invertebrates are identified to larger groups (Table A5.2). Total prey weight and weight of individual prey groups is recorded (species for fish). Questions can be posed to the species coordinator.

8. After stomach contents have been analysed and recorded, results are submitted to the ICES data centre using the exchange format. ICES will then include the data in the current stomach database

Table A5.1. Condition of gall bladder and hind guts used to differentiate between empty and regurgitated stomachs

STAGE	GALL BLADDER	BILE COLOUR	HIND GUT	STATE
1	Shrunken, empty or with small amount of bile	Pale	Contains large amounts of bile and digested food material	Feeding*
2	Elongate	Pale green to light emerald green	Contains some bile and digested food particles	Feeding*
3	Elongate	Dark green	Empty or contains some food particles	Empty
4	Round	Dark blue	Empty	Empty

*If fish satisfying these criteria are found without food in their stomach they should be classified as regurgitated

TableA5.2. Label to be included with each stomach sample

ICES STOMACH SAMPLING PROGRAMME	
Ship	
Haul number	
Date	
Rectangle	
Species	
Size	
Gall bladder Class (whole stomachs only)	
Sample no	

TabelA5.3. Invertebrate groups and corresponding NODC codes

INVERTEBRATE GROUP	NODC CODE IN 1991 SAMPLING MANUAL

Amphipoda	6168000000 and 6169000000
Annellidae	5000000000
Astacidae	6181000000
Anomura	6183000000
bivalvia	5500000000
brachyura	6184000000
caridea	6179000000
cephalopoda	5700000000
Cnidaria and ctenophora	3700000000 and 3800000000
crangonidae	6179220000
crustacea (unidentified)	6100000000
echinodermata	8100000000
Euphasiacea and mysidae	6174000000 and 5153000000
gastropoda	5100000000
Nephrops norvegicus	6181010301
pandalidae	6179180000
other crustacea	
other invertebrates	
plastic	
Saduria enthomon	

Tabel A5.3 Exchange format for stomach data (revised from ICES 1999)

POSITION	NAME	TYPE[1]	RANGE	COMMENTS
1-2	Record type	2A		Fixed value 'SS'
3	Quarter	1N	1-4	
4-6	Country	3A		ICES alpha code
7-10	Ship	4A		ICES alpha code
11-13	Method	3A		See table 5.4
14-17	Square	4AN		ICES statistical rectangle
18-23	Haul number	6AN		Compatible with the haul number available in

				DATRAS assuring that stomach samples can be matched with trawl data
24-27	Sample no	4N	1-9999	ID number of the stomach collected. If all stomachs in the 5 cm size group are empty, the number is 9999.
28-29	Temperature	2N	-2-26	°C, not known 99.
30-31	Year	2N	10-99	
32-33	Month	2N	1-12	
34-35	Day	2N	1-31	
36-45	Predator code	10N		TSN Codes
46	Size group code	1A	E, F	E= 1cm groups, F=1mm groups below 2 cm, 1 cm groups above 2 cm
47-50	Predator size class code	4N	0-9999	Predator length measured to nearest cm below
59-61	Number with food	3N		Total in 5 cm size group of the predator species
62-64	Number regurgitated	3N		Total in 5 cm size group of the predator species
65-67	Number with skeletal remains	3N		Total in 5 cm size group of the predator species
68-70	Number empty	3N		Total in 5 cm size group of the predator species
71-80	Prey species code	10N		TSN Codes for fish. For invertebrates and unidentified items, use TSN codes if available from WGDIM, otherwise use NODC codes
81-84	Prey size	4N		Prey length measured to nearest cm below (size group code E), or to nearest mm below up to 2 cm, then nearest cm below (size group code F). Eggs have size 0.
85-92	Prey weight	8N		Prey can be weighed together if they are of the same species/species group and same length
93-98	Prey number	6N		
99	Stage of digestion	1N	0-2	0= Intact prey, 1= partially digested prey, 2= skeletal material
100	Padding field			

[1]All numeric field (N), all Alpha (A) and mixed alpha numeric (AN).