

CEFAS FISHERIES LABORATORY, LOWESTOFT, SUFFOLK, ENGLAND.

2001 RESEARCH VESSEL, PROGRAMME.

REPORT: RV *CIROLANA*; CRUISE 5B/01.

STAFF: J Thain (SIC)
K Thomas
J Jones from 24th June
M Kirby from 4th July
W Reynolds
M McHugh from 24th June
S Feist (Weymouth) to 23rd June
G Stentford (Weymouth) to 29th June
G Jones (Weymouth)
J Bignall (Weymouth) from 29th June
B Lyons (Lowestoft) to 4th July
P Leonard (DEFRA CSG) to 23rd June
K Coorman (SFD Belgium) to 23rd June
O De Cock (SFD Belgium) to 23rd June
A Fevery (SFD Belgium) to 23rd June
I McFazden (Plymouth) to 4th July
J Wedderburn (Plymouth) to 4th July
B Zaldibar (Bilbao) to 23rd June
T Laing (Germany) 18th & 19th June

All staff from Burnham Laboratory unless indicated otherwise.

DURATION:
16th June to 16th July, 2001.

LOCATION:
North Sea, Irish Sea, Celtic Sea English Channel.

AIMS:

1. To collect samples of demersal fish for chemical analysis from the North Sea, Eastern English Channel and Irish Sea in support of UK National Marine Monitoring Programme (NMMP) and EC Fish Products Directive Work.
2. To collect water samples for NMMP biological effects studies and to deploy fractionation/bioassay techniques on water samples from offshore and near shore/estuarine locations.
3. To collect sediment samples for biological effects studies (whole sediment) at NMMP sites and from additional selected sites as appropriate.

4. To collect fish samples at NMMP sites, for fish disease and genetic toxicological analysis (e.g. DNA adducts).
5. To further investigate the use of appropriate biochemical, cytochemical and other biological techniques in support of NMMP.
6. To sample representative offshore NMMP locations using grab, core, and trawl for trace metal contaminants, PAHs and other organic contaminants (including nonylphenols, flame retardants and HCs) and the benthic fauna.
7. To collect NMMP summer water samples for nutrients, salinity and chlorophyll analysis.
8. To collect fish cages, water and biota samples as part of the ICES / IOC BECPELAG workshop and in support of studies to investigate the significance of offshore chemicals as endocrine disrupters in fish.
9. To assess the potential for UV-light to increase the toxicity and/or genotoxicity of seawater samples to invertebrate bioassay organisms.

ADDITIONAL AIMS:

10. To carry out sampling at a selection of stations previously worked as part of the ICES 1986 North Sea Benthos Survey.
11. To record items of litter collected in all deployments of the Granton trawl.

NARRATIVE:

RV CIROLANA sailed from Lowestoft at approximately 1900 hr on the evening tide of 16 June 2001. On passage to Helgoland, water samples were taken off Rotterdam close to the main shipping lanes. These samples were taken at 0500 on the morning of the 17 June, using the ships *Searider*: sub-surface bulk water was taken at 0.5m and sea surface microlayer (SMIC) using a *Garrat* Screen and glass rotating drum (*Pedalo*). The sea was mirror calm, providing optimum conditions for this type of sampling. A 2-m beam was deployed twice to sample whelks and hermit crabs but none were caught. *CIROLANA* arrived at Helgoland in the German Bight at 0900 h on the 18 June and berthed courtesy of the Fisheries Institute at Helgoland. Three scientists from Belgium, one from Germany and one from Spain, scientific equipment and samples in liquid nitrogen were taken onboard. At approximately 1100 h *CIROLANA* sailed to a position 10 miles south of Helgoland to retrieve one of five fish cages (German Bight 1: GB1) deployed seven weeks earlier under the ICES/IOC BECPELAG workshop (aim 8). On arrival the marker buoy was clearly visible. The buoy was easily retrieved and secured to the ship but the mooring rope parted before the

cage was retrieved. This was a result of an insufficient length of rope between the marker lifting buoy and the mooring. This was designed at 4m but the freeboard of the vessel was approximately 5 m. Sea conditions were also poor with a swell of approximately 3 m. After consultation with the fishing skipper the Granton trawl was deployed and towed over the position and the cage retrieved (Plate 1). Fish were kept alive and material processed for aims under the BECPELAG workshop (aim 8).

On the morning of the 19 June *CIROLANA* had steamed to a position 5 miles to the north of Helgoland to retrieve fish cage GB2. This was successfully retrieved by lassoing the marker buoy and winching the cage up the fish ramp using the net drum. This station was completed by early afternoon and the scientist from Germany was put ashore on the Searider before steaming north-west towards the Dogger Bank. *CIROLANA* arrived at the GB3 position at 0400 h on the morning of 20 June. No marker buoy was visible, verifying the report by the *RV Belgica* from Belgium (also involved in the BECPELAG workshop) the previous week. *CIROLANA* then deployed the Granton trawl and successfully retrieved the cage. All the cod were processed and in addition, mussels, SPMDs and DGTs. This work was completed by midday and *CIROLANA* steamed for the Dogger Bank to collect fish cages GB4 and GB5. The cage at GB5 was retrieved successfully. The buoy at GB4 was missing, as had been reported by the *RV Belgica* the previous week. Several attempts were made to trawl for the cage but this was not successful.

At 2000 h *CIROLANA* steamed overnight to a position just north of the Hospital Ground on the Dogger Bank. Fishing commenced on the morning of the 21 June and fish were obtained for aims 1, 4 and 5. In addition, 60 l bulk water samples were taken using a churn sampler and sediment samples using a Day grab. This station was completed by late morning and *CIROLANA* steamed for Stavanger and *en route* completed a further fishing, sediment and water station on the north-east Dogger Tail End.

CIROLANA arrived at Stavanger on the morning of the 23 June. Five scientists disembarked *CIROLANA* at Stavanger and three scientists from the Burnham Laboratory joined the ship. Equipment pertaining to the workshop and several hundred samples (fish, mussels, SPMDs and DGTs) in liquid nitrogen were off-loaded for transport to the Norwegian Institute for Water Research, Oslo. Two hundred litres of liquid nitrogen was taken on board to replenish that used for the BECPELAG workshop and needed for the remainder of the cruise.

CIROLANA departed Stavanger at midday on the 24 June and steamed to the Statfjord reference station where bulk water samples and sediment samples were collected using the churn sampler and Day grab, respectively (aim 8). SMIC samples were also collected from the ship's searider (aim 9) before proceeding to the Statfjord oil platform to trawl for cod for plasma VTG studies (aim 8). The Granton trawl was deployed at several sites in the vicinity of the oil platform but no cod were caught and substantial damage was incurred to the

trawl, requiring a new trawl to be fitted. Permission was sought and subsequently granted for the ship's Searider to enter the 500 m exclusion zone around the oil platform to collect sub-surface bulk water at 0.5 m and sea surface microlayer (SMIC) samples using the pedalo (Plate 3) and Garrat Screen (Plate 4) (aim 9).

After completing work at Statfjord on the evening of the 25 June, *CIROLANA* steamed to NW Hutton to collect water and sediment samples (aim 8) before heading for the Irish Sea, arriving at Dundrum Bay on the morning of the 28 June. The Granton trawl was deployed at the inner site under aims 1, 4 and 5 but only sufficient numbers of dab were caught to complete the biomarker studies, no fish disease studies were carried out. Sediment and water samples were collected at the mid-tow position (aims 2 and 3). A Sextant grid of 5 Day grabs was then completed for sediment contaminants and benthos (aim 6), after which *CIROLANA* proceeded to the outer Dundrum Bay station. The Granton trawl was deployed but no dabs were caught. Benthos was collected from the Sextant benthos position using the 2-m wooden beam. The ship then steamed to SE Isle of Man and deployed the scallop dredge in the late evening collecting queens and sea urchins for bioassay work.

Early on the morning of 29 June, the Granton trawl was deployed at NMMP 805, SE Isle of Man, to collect fish under aims 1, 4 and 5. In gradually deteriorating weather, water and sediment samples were also collected for aims 2 and 3 and the ship's Searider was then deployed to collect sub surface bulk water, and surface microlayer (SMIC) using the Garrat screen and pedalo (aim 9). The station was completed by 1100 hrs and *CIROLANA* headed for Douglas, Isle of Man, for a staff change over, in what was now a south westerly force 7, berthing at 1400 hrs. In addition to changing staff it was necessary to purchase fuel for use on the Searider, purchase wood to repair the fish sorting table and to take on-board boxes of 500 ml glass jars to replace those broken on-board during the earlier part of the voyage.

CIROLANA sailed out of Douglas at 1830 hrs. With little improvement in the weather and a bad weather forecast, the NMMP SE Isle of Man sextant station was abandoned (aim 6) and the ship headed for Red Wharf Bay to shelter in the lee of the North Welsh Coast and trawl first thing the following morning.

The Granton trawl was deployed at 0630 hrs on the 30 June. Sufficient dab were obtained to carry out a full CEMP suite of fish disease, contaminant analysis and integrated biomarker studies (aims 1, 4 and 5) and samples of whelks were obtained for imposex/TBT analysis. Sediment and water samples were collected from the mid-tow position (aims 2 and 3), *CIROLANA* then proceeded to NMMP 715 Liverpool Bay. Only small numbers of fish were obtained from this site, insufficient to complete the full fish disease and biomarker studies, so the ship proceeded to the Liverpool Bay Trend site where dab were caught in sufficient numbers to complete the CEMP suite studies and length stratified groups of plaice and dab were collected for time trends (aims 1,

4 and 5). Sediment and water samples were also collected from the mid-tow position (aims 2 and 3).

The Granton trawl was deployed again at 0630 hrs on the 1 July at the Burbo Bight site and sediment and water samples were also collected. The station was successfully completed by 1100 hrs, after which the Searider was deployed for SMIC sampling in the River Dee. *CIROLANA* then steamed back to NMMP 715 where a Sextant grid of 10 Day grabs was completed for trace metal contaminants and the beam trawl deployed for benthos, for flame retardant analysis (aim 6). By midnight, the Off Morecambe Bay NMMP site was also successfully completed for full fish disease and biomarkers and *CIROLANA* steamed overnight to Outer Cardigan Bay (NMMP 665).

Only limited numbers of dab were obtained at NMMP 665. Full biomarker studies were completed but no fish disease studies were carried out or chemical contaminant samples collected. Sampling at the Inner Cardigan Bay site was slightly better enabling limited fish disease studies along side the biomarker work. During the evening, the 2-m beam was deployed and a Sextant grid of 10 Day grabs was completed. *CIROLANA* then steamed overnight to the Celtic Deep.

The Granton trawl was deployed at the Celtic Deep site on the morning of the 3 July. No dab were caught and the fish studies were aborted. Samples of whiting and gurnard were kept for flame retardant analysis. In the afternoon, a Sextant grid was successfully completed using the Day grab and the 2-m beam was deployed. The multicorer was then deployed for meiofauna samples. There were numerous problems with this sampling device, but these were eventually overcome with persistence, and advice from the Fishing Skipper and the station was successfully completed at 2230 hrs after which *CIROLANA* sailed for Plymouth.

CIROLANA arrived at Plymouth at 1045 am on the morning of the 4 July and anchored in the Sound. Four scientists were put ashore and 3 new scientists joined the ship. *CIROLANA* departed Plymouth at 1400 hrs and steamed to Rye Bay, arriving at 1130 am on the 5 July. The Granton trawl was deployed at the Outer station but insufficient dab were obtained to carry out biomarker or fish disease studies, so the ship proceeded to the Inner station where dab were caught in sufficient numbers to complete the station in full. *CIROLANA* left Rye Bay at 1500 hrs and steamed to the Thames calling at Varne on route to collect NMMP water and sediment samples. The ship arrived at Shimmering Sands around 2 am on the morning of the 6 July. Three anchorages were trawled with the 2-m steel beam for whelks, hermit crabs and starfish for TBT and flame retardants (aims 5 and 6). Samples were successfully collected from anchorage 3 only. Sediment and bulk water samples were also collected from anchorage 3.

After leaving the Thames *CIROLANA* headed north and on the morning of the 7 July trawled with the Granton trawl at the Outer Gabbard (NMMP 475). Four

tows were required to obtain sufficient fish for aims (1, 4 and 5). Water and sediment were collected from the mid tow position with the Churn and Day grab respectively. On the evening of the 7 July the Granton trawl was again deployed to collect fish at the Smiths Knoll/Brown Ridge Trend station. Length stratified samples of dab, whiting and plaice were collected.

Overnight *CIROLANA* steamed northwards towards the Off Humber NMMP station (345), close to the Silver Pit. Throughout the cruise no cod had been caught for aim 5. Therefore *en route* to the Silver Pit two additional sites were trawled for cod, at the Indefatigable Bank and Markhams Hole. No cod were caught but there were large numbers of dab at both sites. At the Indefatigable Bank the dab were very large (in excess of 30 cm) and since this was rare the opportunity was taken to conduct a full fish disease record. On the evening of the 8 July, *CIROLANA* successfully trawled for fish at the Off Humber NMMP station and also took water and sediment samples. In addition to dab approximately 30 cod were caught and samples of plasma taken for VTG analysis.

On the morning of the 9 July *CIROLANA* had arrived at the Wash NMMP station (385). The Granton trawl was deployed, six dab were caught and the net was heavily laden with weed. No further tows were made at this position. A sediment and water sample was taken before *CIROLANA* moved to the Lower Humber NMMP (376) station. Samples of dab, water and sediment were collected and the station completed by 1700 h. With the 3-4 day weather forecast for bad weather, *CIROLANA* steamed out overnight to the West Dogger NMMP station (285). The Granton trawl was deployed and dab collected for aims 1,4 and 5. A further station was successfully fished for aims 1, 4 and 5 at the north Dogger and was completed by 1700 h. *CIROLANA* then steamed approximately 30 miles to the North West to Lisborgs Revile to start Day grabbing on a grid of stations for the ICES Benthos Survey. Four grabs were successfully taken at the first station but *en route* to the second station the weather deteriorated rapidly. In view of the poor weather 3-day forecast *CIROLANA* headed for the NE coast arriving off Blyth in the early morning of the 11 July.

With the wind at force 6 and off the land from the north-west *CIROLANA* was able to trawl at the Tyne NMMP station (Off Amble). Three tows were completed by midday and sufficient dab collected for aims 1, 4 and 5. In addition, 20 cod were sampled for plasma and VTG analysis. Sediment and water samples were taken from the mid-tow position using the Day grab and Churn sampler respectively. The station was completed by midday and by then the weather had deteriorated to a westerly gale 8. With the forecast poor *CIROLANA* steamed inshore and completed several beam trawl tows from the Tyne to the Tees to collect whelks and hermit crabs for TBT analysis in relation to anchorages and the Tyne spoil ground. In the late evening *CIROLANA* steamed and anchored off the Tees to commence work the following morning on the Tees NMMP station (295).

On the morning of the 12 July, the wind remained at force 6-7, occasionally 8, from the north-west. *CIROLANA* trawled the Tees NMMP station using the Granton trawl. Two tows were completed before local fishing boats shot drift nets across the tow. The station was abandoned, with the aim of fishing the position at a later time. By midday the wind had eased and *CIROLANA* headed south to the Off Flamborough fishing position, arriving there at 1700 h. An unusually small number of dabs were obtained from this site, with the majority of the samples being small, i.e. under 20 cm, making it difficult to complete a full disease record. Sufficient samples were obtained to complete the biomarker studies, contaminant analysis and histology records (aims 1 and 4). Sediment and water samples were collected mid-tow (aims 2 and 3). *CIROLANA* proceeded to the Humber anchorage, where the 2-m steel beam was deployed in the late evening/early morning of the 13 July. Whelks, hermit crabs and starfish were obtained for TBT and flame retardant studies (aim 6). *CIROLANA* then headed back north to re sample the Tees NMMP site abandoned on the previous day. Unfortunately drift nets were still positioned across the tow making it unworkable. The site was abandoned and the ship steamed to Farne Deep to fish for cod under aim 8. Twenty-one cod were obtained from 3 tows. Blood samples were taken for VTG analysis back at the Laboratory and all samples were examined for disease. A large number of *Neptunia* were obtained and these were examined for imposex and samples retained for TBT analysis. After completing the station *CIROLANA* proceeded northward to begin work on a group of 7 North Sea Benthos Survey sites (additional aim), reaching the first at 2030 hrs. The sites were worked consecutively in shifts over the following 24 hrs completing the last at 2315 hrs on the night of the 14 July after which *CIROLANA* steamed back to the abandoned Tees NMMP site to complete the final tow. The Granton trawl was successfully deployed at 0600 hrs on the morning of the 15 July and sufficient dab were obtained to complete the station. *CIROLANA* then steamed for Lowestoft docking on the early morning tide, 0600 hrs on the 16 July.

RESULTS

Aim 1. Seventeen samples of whole dab and fourteen samples of bulk dab livers were collected from NMMP sites where biomarker and fish disease studies were carried out. Six samples of plaice, whiting and dab in five consecutive length stratified groups were collected for trend purposes from Liverpool Bay and Smiths Knoll. All samples were frozen and stored at -20°C for analysis at the Burnham Laboratory.

Aim 2. Twenty-one water samples were taken from NMMP stations and at sites sampled in previous cruises and extracted on-board using C2/env+ solid phase cartridges for chemical analysis of organics (fractionation processing) and biological effects studies at Burnham. Five very large (200 l) samples of seawater were taken at the NW Hutton oil platform, a transect away from the Statfjord oil platform and a reference site. Each sample was extracted on-board

for analysis of organics and biological effects studies in relation to the BECPELAG workshop and investigations into the effects of offshore endocrine disrupting chemicals emanating from oil platforms.

Aim 3. Sediment samples were collected from 17 NMMP stations using a Day grab and frozen on board. These samples will be used if necessary to confirm and validate sediment bioassay results from previous cruises.

Aim 4. In excess of 3,700 dab from 18 sites were examined and recorded for diseases according to ICES recommended protocols for standard disease monitoring. In addition to ICES recommended diseases, incidence of external and internal parasites was also recorded. Bile retention in the liver (Plate 6) was frequently noted at several sites and was often found to be associated with a swollen liver and spleen, indicative of an inflammatory response. Aetiology of this condition will be investigated by histopathology and the data analysed to assess the significance, if any, of this condition. The microsporidian glugea was also noted and, in severe cases, where the lower intestine was packed with cysts, appeared to be associated with emaciation; this will also be investigated further.

The 50 macroscopic liver nodules (Plate 5) detected during the sampling programme have been preserved for histopathological confirmatory diagnosis. Otoliths from the fish were sampled to allow for age correlation. Several tumours were also sampled for mutation analysis. In addition, 520 fish were sampled for histopathological screening for pre-neoplastic hepatic lesions and general assessment of kidney, spleen and gonad. Among these samples a further 23 liver nodules were detected.

One hundred and fifty samples of a range of normal and diseased tissues were taken for the registry of aquatic pathology.

Aim 5. Fish tissue was collected from dab at 19 locations (including NMMP) for biological effects investigations using biomarkers. Sections of dab liver were stored in liquid nitrogen for measurement of EROD activity and DNA adducts: accompanying bile was taken and frozen for PAH analysis. Liver samples were also taken from male and female dab, stored in liquid nitrogen for metallothionein analysis at the Burnham laboratory. Dab muscle was taken and stored in liquid nitrogen for Ache activity. Bulk liver samples were taken for supporting chemical analysis.

Whelks were collected from 16 sites for the identification of imposex and TBT analysis. In support of this work, samples of hermit crabs and sediments were taken for chemical analysis. Initial observations show a higher prevalence of imposex at sites close to anchorage's and shipping lanes. The samples will be evaluated fully at the Burnham Laboratory.

Aim 6. A grid of 10 Day grabs within a 200 m bullring was successfully collected at each of the following sites, Dundrum Bay, SE Isle of Man, Cardigan Bay and Celtic Deep. The sediments were sieved on-board and all benthos preserved for identification and enumeration on return to the Burnham Laboratory. Sediment samples were also taken for particle size analysis, meiofauna, and chemical analyses of metals and organics. The 2-m beam trawl was successfully deployed at each of the above sites and macro benthos preserved for examination on return to the Burnham laboratory. A grid of five multicorer samples was taken at the Celtic Deep site for meiofauna investigations.

Aim 7. Sea surface water samples were taken at NMMP sites or at least once each day when *CIROLANA* was in UK waters for the determination of salinity, chlorophyll and nutrient. All samples were preserved on-board for analysis at the Lowestoft Laboratory.

Aim 8. Four of the five ICES BECPELAG workshop cages were successfully retrieved from the German Bight. There were problems associated with the retrieval of these cages: the length of the mooring was insufficient in relation to the sea surface to deck height of *CIROLANA*, there was chaffing on the mooring rope: sea conditions were poor for the retrieval of the first two cages: and two of the five cages had lost their marker buoys. In the latter instance *CIROLANA* was able to retrieve one of the cages by deploying the Granton trawl. The success of this work owes much to the skills of the fishing skipper and deck crew. Cod were sampled from each of the four cages for the workshop aims: the tissues collected were liver, kidney, bile, blood, muscle, and urine all in liquid nitrogen. Tissue samples were also taken for histological examination and chemical analysis. Mussels were also sampled from the third cage and tissues from individual mussels were stored in liquid nitrogen. All of the samples were off-loaded in Stavanger, Norway and transported to NIVA for distribution to the workshop participants. A Norwegian vessel as part of this BECPELAG programme sampled corresponding fish cages the week before. Five scientists from Belgium, Germany and Spain participated in this part of the cruise programme

Aim 9. Sea surface micro-layer (SMIC) layer samples were taken using a *Garra*t screen (wire mesh) and a glass, rotating drum mounted on a pedalo (on loan from the Plymouth Marine Laboratory). Bulk water samples (0.5m below the surface) were taken wherever SMIC samples were taken. A total of fourteen stations were sampled: from the German Bight (4 stations), Stevanger harbour, Statfjord oil field (6 stations) and the Dee estuary (3 stations). Samples were either tested onboard (oyster embryo bioassays) or frozen down for bioassay analysis at the Lowestoft Laboratory (*Tisbe* and genotox bioassays). Parallel samples (Bulk and SMIC) from the BECPELAG sites were also collected on behalf of Dr Scott Carr (USA).

Aim 10. A set of four Day grabs was taken at eight locations in the North Sea, off the north-east coast out towards the Dogger Bank. The samples were taken

for particle size analysis, analysis of metals and organic contaminants, meiofauna and benthos. The sampling and data work up contributes towards the ICES North Sea Benthos Survey.

Aim 11. The Granton trawl was deployed at 27 locations during the voyage. A total of 68 tows were made each tow for a distance of c. 2 miles. All items of litter were recorded in terms of type, size etc. Litter was most abundant at Off Cardigan and Off Morecombe on the west coast of the UK and Rye Bay, Outer Gabbard, Markhams Hole and Tees Bay on the south and east coasts of the UK.

Cetaceans and other sightings

26 June 0600 hrs: 6 miles off Sumburgh Head	>100 dolphins
27 June 1900 hrs: position 56° 14.6 N 07° 14.0 W	6 Basking sharks
2 July 1800 hrs: Cardigan Bay	Dolphins
3 July 0700 hrs: 51° 28.5 N 05° 51.3 W	>100 dolphins
4 July 1900 hrs: 50° 12.9 N 03° 16.0 W	~20 dolphins

Acknowledgements: I would like to acknowledge the active support and help of the ship's officers and crew in completing the above programme of work. Of particular note was the skill of the fishing skipper and deck crew in retrieving the BECP ELAG fish cages on the Dogger Bank and German Bight.

John Thain (**Scientist In Charge**)
16th July 2001

SEEN IN DRAFT: Captain J de Barr

A Lincoln (Fishing Skipper)

INITIALLED: Dr M Waldock (CEFAS EQ Science Area Head EQ and Chief Executives Office)

DISTRIBUTION:

Basic list: +

J Thain (SIC)
S Fiest

K Thomas
J Jones
M Kirby
W Reynolds
M McHugh
G Stentford (Weymouth)
G Jones (Weymouth)
J Bignall (Weymouth)
B Lyons (Lowestoft)
P Leonard (DEFRA CSG)
K Coorman (SFD Belgium)
O De Cock (SFD Belgium)
A Fevery (SFD Belgium)
I McFazden (Plymouth)
J Wedderburn (Plymouth)
B Zaldibar (Bilbao)
T Laing (Germany)
A Franklin



Plate 1. Retrieval of BECPCLAG cages from the German Bight

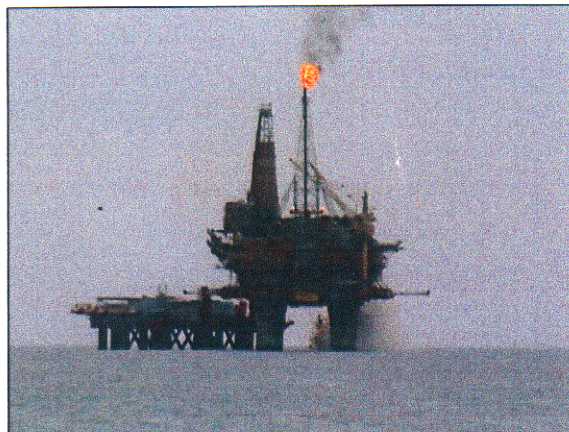


Plate 2. Statfjord B oil platform



Plate 3. Glass Rotating Drum sampling of sea surface microlayer.



Plate 4. Garrat Screen sampling of sea surface microlayer.

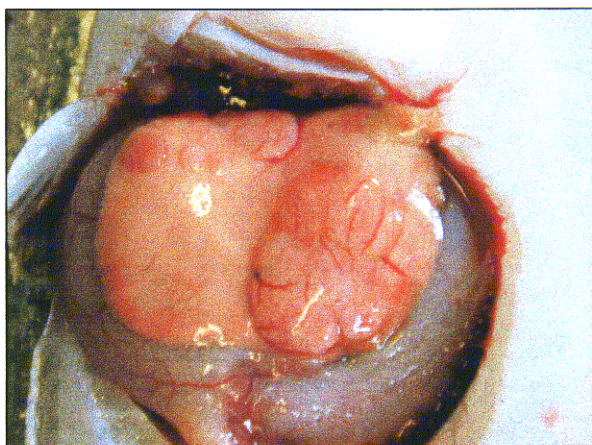


Plate 5. Dab liver showing nodules.

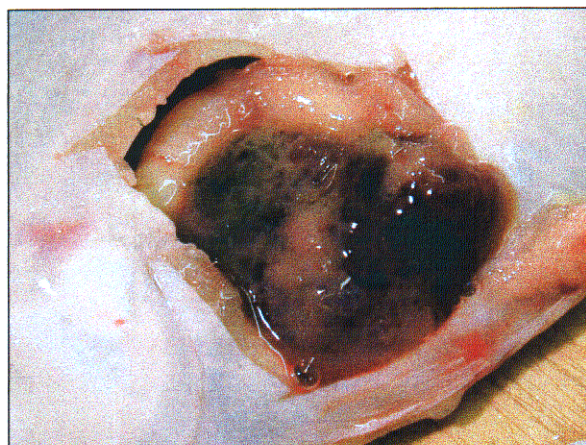


Plate 6. Dab liver showing necrosis, nodules and bile retention.