DEPARTMENT FOR ENVIRONMENT, FOOD AND RURAL AFFAIRS. CEFAS LOWESTOFT LABORATORY, LOWESTOFT, SUFFOLK, ENGLAND. 2002 RESEARCH VESSEL, PROGRAMME. REPORT: RV CIROLANA; CRUISE 3B/02.

STAFF: J Thain (SIC) J Jones W Reynolds P Nunn B Lyons (Lowestoft) G Stentiford (Weymouth) Thi Bolam 29th June to 10th July

S Feist (Weymouth) 19th June to 29th June M Longshaw (Weymouth) 19th June to 29th June

J Read 19th June to 29th June

G Jones (Weymouth) from 29th June to $10 {\rm th} \, July$

J Bignall (Weymouth) from 29th June to 10th July

K Thomas from 29th June to 10th July

L Johnsey from 29th June to 10th July

I McFazden (Plymouth) 19th June to 29th June

John Wedderburn (Plymouth) from 29th June to 10th July

All staff from Burnham Laboratory unless indicated otherwise.

DURATION:

19th June to 10th July, 2002.

LOCATION:

North Sea, Irish Sea, Celtic Sea and 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).

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.

 To collect sediment samples for biological effects studies (whole sediment and pore water) at NMMP sites and from additional selected sites as appropriate.
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.

8. To assess the potential for UV-light to increase the toxicity and/or genotoxicity of seawater samples to invertebrate bioassay organisms.

9. To collect biota for determination of imposex and TBT analysis, and sediment for TBT analysis from shipping lanes, anchorages and reference sites in support of OSPAR requirements.

Additional aims:

10. To collaborate with staff from Coastal Marine Biotechnologies (CMB), in

support of a DEFRA funded contract to develop cryo-bioassay techniques for biological water quality assessment, for possible use within the NMMP programme

11. To collect sediment and associated benthos at an ICES North Sea benthos station.

12. To make observations on the presence of litter at all NMMP fishing sites and other stations where the Granton trawl is deployed.

NARRATIVE:

RV CIROLANA sailed from Swansea on the evening tide (2230 hr) on the 19th June and steamed overnight to Cardigan Bay. On the morning of the 20th June the Granton trawl was deployed to collect fish for work under aims 1, 4 and 5. In the afternoon a 2-m beam trawl was deployed to collect macrobenthos and a series of Day grabs taken to collect sediment at the NMMP benthos station for aim 6. After completing this work CIROLANA steamed overnight to Red Wharf Bay and trawled for fish for aims 1, 4 and 5. In addition, benthos and sediments were sampled at the anchorage in Red Wharf Bay after which *CIROLANA* moved overnight to Liverpool Bay NMMP 715 to trawl for fish under aims 1, 4 and 5. In addition, to trawling at these two sites water samples were taken with the 50 I churn sampler and sediment samples using a Day grab for aims 2 and 3 respectively. Further trawling took place on the following day, 22nd June, at the Liverpool Bay NMMP stn 705 and TREND station for aims 1, 4 and 5. On the evening of the 22nd June CIROLANA sailed north-west across the Irish Sea arriving at Dundrum Bay on the morning of the 23rd June. Fish were trawled for aims 1, 4 and 5 and a series of Day grabs taken for sediment for macrobenthos and chemical analysis. A 2-m beam was also used to collect macrobenthos. This station was completed by mid afternoon and CIROLANA steamed to Belfast Lough. On the morning of the 24th June, three NMMP stations were sampled for water, sediment and surface micro layer in collaboration with scientists from the DOE NI Office using the RV CAPITELLA for aims 2, 8, and 10. Concurrently, CIROLANA took sediment and water samples at two NMMP stations in Belfast Lough and sampled benthos and sediments on the anchorage off Bangor. During the late afternoon CIROLANA steamed southeast and arrived at St Bees on the morning of 25th June to trawl for fish under aims 1, 4 and 5. In the afternoon further trawling took place using the Granton trawl at the NMMP station 805, SE Isle of Man. A series of Day grabs was also taken at this station and the 2-m wooden beam deployed for benthos and associated chemical analysis (aim 6).

On the morning of the 26th June the Granton trawl was deployed again to sample fish for aims 1, 4 and 5 at the Morecambe Bay NMMP stn 795. This was completed by midday and *CIROLANA* sailed south to trawl for fish for EQS purposes (NMMP 715). On route to this station a series of Day grabs and a 2-m beam was deployed for sediment and benthos, respectively, at the Mersey anchorage. In the evening *CIROLANA* steamed to Cardigan Bay and on the morning of the 27th June trawled for fish in the North of Cardigan Bay and at the outer Cardigan Bay NMMP station 655. On the second tow at the latter station the trawl net was damaged, requiring a complete new net to be fitted. No further fishing was carried out at the outer Cardigan Bay station. *CIROLANA* then sailed south to Falmouth, collecting on route, a sediment sample with the Day grab at the Celtic Deep.

CIROLANA arrived at Falmouth, mid morning on the 28th June. The Searider

was deployed to collect water and surface microlayer samples from Falmouth (aims 2, 8, and 10). Concurrently, *CIROLANA* took sediment and benthos samples at the Falmouth anchorage for aim 9. In the afternoon, *CIROLANA* sailed east towards Weymouth, collecting on route further sediment samples and benthos from the deep-water anchorage in Torbay.

On the morning of the 29th June *CIROLANA* anchored in Weymouth Harbour to change staff. Five scientific staff left the ship and five new scientific staff joined the ship. This was completed by early afternoon and *CIROLANA* sailed to the Solent where the Searider was used to collect water and surface microlayer samples for aims 2, 8, 9 and 10. Overnight *CIROLANA* steamed to Rye Bay and trawled for fish for aims 1, 4 and 5. This was completed by midday. As *CIROLANA* sailed into the southern North Sea sediment samples and benthos were taken at the Thames and Sunk anchorages on the 1 July. Weather conditions deteriorated as *CIROLANA* entered the Thames, but were just acceptable to conduct trawling at the Outer Gabbard NMMP station 475. This station was completed by late afternoon. Current weather conditions and the weather forecast were poor. With strong winds forecast from the West, *CIROLANA* steamed overnight to the Wash.

On the 2nd July, fishing stations were successfully completed using the Granton trawls at the Wash NMMP station 387 and Lower Humber NMMP station 377. Sediment and water samples were also taken from the mid-tow position using the Day grab and churn sampler, respectively. On the evening of the 2nd July sediment samples and benthos were sampled from the Humber anchorage, on route, as *CIROLANA* steamed offshore to the Off Humber NMMP station 346. Weather conditions improved overnight and the Granton trawl was used to obtain fish for aims 1, 4 and 5 at the Off Humber station on the morning of 3 July and at the Off Flamborough NMMP station (344) in the late afternoon.

Overnight *CIROLANA* steamed to the Tees NMMP station (285). The Granton trawl was deployed in the morning and a series of Day grabs was taken over the Tees anchorage, to obtain benthos, using a 2-m steel beam, for aim 6. In the afternoon, the Searider was deployed to obtain sediment and surface microlayer samples from the Tees (aims 2, 8, 9 and 10). After completing this work *CIROLANA* headed north overnight to the Tyne. On the morning of the 5th July the Searider was used to obtain surface microlayer and water samples. Later in the day, Day grabs and benthos were obtained from the Tyne anchorage and in the late afternoon the Granton trawl was deployed to obtain fish for aims 1, 4 and 5 at the Tyne NMMP station off Amble (244).

After completing the station at Amble, *CIROLANA* anchored in the Tyne anchorage ready to commence work on the morning of the 6th July on the Tyne disposal ground. A series of ten stations was sampled using the Reineck Box Corer and on each deployment depth profiles of the sediment were sampled. In addition, four samples were used for pore water extraction for aim 3. In the late afternoon the Granton trawl was deployed at the Farne Deep, north east of Tyne. No dab were obtained but cod were sampled for histopathology, aim 4. On the evening of the 6th July *CIROLANA* headed east towards the Dogger Bank, arriving at the West Dogger NMMP station 286 where the Granton trawl was deployed and fish collected for aims 1, 4 and 5. Water and sediment were taken from the mid-tow position. This station was completed by mid morning and *CIROLANA* headed east to the northeast Dogger. This fishing station was completed by 2300 hr. Overnight, *CIROLANA* moved to the North Dogger and trawled again with the Granton trawl on the morning of the 8th July. This station was completed by late morning and *CIROLANA* headed south towards Lowestoft. On route, two further fishing stations were completed, at the Hospital Ground and Indefatigable Bank, for aims 1, 4 and 5, and a grab station for benthos (aim 11). A third Granton trawl was deployed at Smiths Knoll/Brown Ridge Trend Station for aim 1. *CIROLANA* arrived at Lowestoft on the morning tide of the 10th July, docking at approximately 0900 hr. **RESULTS:**

Weather conditions throughout the voyage were good with the exception of two days as *CIROLANA* entered the Dover Straits and Thames. Weather at that time was poor, but no time was lost due to adverse conditions.

Fishing throughout the voyage was very good. Sufficient fish were obtained at all sites to fulfil the aims and purposes of the cruise. Twenty-six stations were trawled for fish using the Granton trawl. Between one and four deployments of the trawl were needed at each station; on average three deployments were necessary and a total of sixty-eight deployments of the trawl were made during the voyage.

Aim 1. Twenty samples of whole dab and twenty 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. In addition samples of dab, plaice, whiting, sole and codling were collected from Liverpool Bay for the EQS performance indicator. All samples were frozen and stored at -20°C for analysis at the Burnham Laboratory.

Aim 2. Twenty-four water samples were taken from NMMP stations and 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. The cartridges were frozen on-board for subsequent analysis and bioassay investigation at the Burnham Laboratory. Aim 3. Sediment samples were collected from all twenty-six NMMP fishing stations using a Day grab and frozen on board. These samples will be used, if necessary, for chemical analysis to confirm and validate fish disease and biomarker results. Sediment samples were collected from thirty-two sites for reference/background dioxin screening using the DR CALUX assay. Opportunistic pore water samples were also taken in Belfast Lough, the Tees and off the Tyne.

Aim 4. Approximately 5000 dab (*Limanda limanda*) were examined for external disease according to ICES protocols. One hundred and twenty putative preneoplastic and neoplastic toxicopathic liver lesions were detected and sampled for histological confirmation. Furthermore, twenty of the best defined tumours were taken for mutation analysis and comparative proteomics. The majority of tumours were photographed to aid classification and identification in the laboratory. It is envisaged that these images, along with images of the histological features of each tumour will be developed into a training guide for field identification of specific lesion types.

As in previous years, histopathological samples were taken from the 20 fish per site (10 male, 10 female) sampled for biomarkers under aims 4 and 5. In addition, 30 fish from each site were sampled at 14 different sites for the screening of microscopic liver lesions. A total of 830 fish were sampled in this manner. Otoliths were taken from each fish for age determination. Plasma samples were taken from dab captured at the Dogger stations. The profile of proteins within these samples will be compared to that of tumours taken for proteomics (see above). It is envisaged that future analysis of plasma in this way may lead to the development of blood markers for pre-neoplasia and neoplasia. Additional samples were taken from fish exhibiting tumours for the development of assays for tumour promoters.

Bile retention in dab liver was again frequently noted at several sites (particularly at sites within the North Sea). Samples of affected liver, often with gall bladder attached were taken for histopathology in order to elucidate the aetiology of the condition.

In excess of 70 Cod were sampled for histological screening and for apoptosis of their germinal cells (spermatogonia and oogonia). These data will be compared to that collected for BECPELAG during the 2001 cruise. In addition, plasma was collected for vitellogenin quantification.

Commercial species including herring, haddock, sole, brill, turbot, anglerfish, rays and edible crabs were examined for the presence of pathology and parasites. Three hundred and thirty seven miscellaneous samples including horse mussels, sole and examples of external dab diseases were taken for general quality assessment and for addition to the registry of aquatic pathology held at the Weymouth laboratory.

Aim 5. Fish tissue was collected from dab at twenty 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. Bulk liver samples were taken for supporting chemical analysis.

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 Burbo Bight. 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.

Aim 7. No samples were taken for nutrient analysis. This work was programmed but not initiated for this particular cruise.

Aim 8. Samples were collected for studies investigating the potential for ultraviolet (UV) light to modify the toxicity of surface water to embryos of the pacific oyster *Crassostrea gigas*. Sub-surface and sea surface microlayer waters were collected from five estuarine locations (Belfast Lough, Falmouth, Solent, Tees and Tyne) using the sea rider. Sub-surface waters were collected directly into a 2.5L Winchester, whereas sea-surface microlayer samples were obtained using a Garrets screen. Once onboard both sub surface and sea surface microlayer waters were assayed under both room lighting (no UV) and a lighting regime approximating to 10% ambient UV intensity. Parallel batches of water samples were also extracted with pentane in preparation for PAH analysis. At NMMP directly offshore to the five estuaries 50 litre water churns were used to collect water samples for solid phase extraction (SPE).

Aim 9. Whelks were collected from 18 sites for the identification of imposex and TBT analysis. In support of this work, samples of hermit crabs starfish 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.

Additional Aims:

Aim 10. CMB staff attended the NMMP cruise to field validate the use of cryogenically frozen oyster embryos for bioassay testing. Cryogenically frozen oyster eggs, sperm and larvae, (*C. gigas*) were stored on board in dry shippers at -196°C. Environmental samples were collected from Belfast Lough, the Fal Estuary, Southampton water, and the Tyne and Tees estuaries. Three bulk and sea surface microlayer samples were collected at each of these sites. The oyster embryo test was utilised with both fresh and frozen material, enabling a comparison between the two methods to be made. Different preparations of cryogenically preserved material were also tested. Bioassay testing, using fresh and frozen oyster larvae were conducted on environmental samples from all sites. All samples will be scored and the data evaluated on return to the CMB laboratory.

Aim 11. A set of five Day grabs were successfully taken at position 54°15.00 N 02° 30.00 E. The sediments were sieved on-board and benthos preserved for identification at the Burnham Laboratory.

Aim 12. The Granton trawl was deployed at 26 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 North Cardigan Bay and Off Morecambe on the west coast of the UK and Rye Bay and Tees Bay on the south and east coasts of the UK.

Cetacean sightings:

During the ships passage the following cetaceans were sighted.

27th June 2002, 1800 hr, 51° 41.00 N : 05° 49.00 W

Approximately 50 dolphins around the ship for two hours as *CIROLANA* steamed south through the Smalls.

6th July 2002, 1550 hr 55° 28.43 N : 01° 05.59 W

Five common dolphins seen close to the ship for twenty minutes.

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. In particular thanks to the Captain and Fishing Skipper for their advice and contribution to the work programme.

John Thain (Scientist In Charge)

10 July 2002

SEEN IN DRAFT: Capt R. McCurry (Master)

Alex Lincoln (Fishing Skipper)

INITIALLED: Draft seen by Andrew Franklin, contract leader for the NMMP

programme.

DISTRIBUTION:

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