JW PROJECT NFS08401

WHITE ROSE BASELINE ADDENDUM 2002 BIOLOGICAL CRUISE

JULY 2003

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PREPARED FOR

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1.0 INTRODUCTION

Husky Energy (Husky) conducted a groundfish/crustacean survey as part of it baseline characteristics program for the White Rose oilfield. Data gaps were identified by the Canada-Newfoundland Offshore Petroleum Board (C-NOPB) in the *White Rose Baseline Characterization Data Report* (Husky 2001). As a result, in June 2002, Husky joined a is a multi-purpose biological survey, with biological samples collected for both the Terra Nova and Hibernia fields (for their respective environmental effects monitoring programs) in addition to the White Rose sampling. The principal purpose of the June 2002 survey was to collect American plaice (*Hippoglossoides platessoides*) (for fish health) and snow crab (*Chionoecetes opilio*) (for body burden and taste panels) samples in the vicinity of the White Rose site and the northwest reference area, primarily to fill the identified data gaps. This Addendum provides an overview of the methods and a reporting of the results (no additional (i.e., statistical) analyses have been conducted) and is meant to serve as an Appendix to the *White Rose Baseline Characterization Data Report* (Husky 2001).

The *White Rose Baseline Characterization Data Report* (Husky 2001) summarized the results of analyses conducted for:

- water chemistry (metals and hydrocarbons) at the White Rose site and Northwest Reference Area;
- sediment chemistry, toxicity and benthic community structure at the White Rose site and Northwest Reference Area;
- body burden (metals and hydrocarbons) in snow crab tissue at the White Rose site;
- body burden (metals and hydrocarbons) in American plaice tissue at the White Rose site and Northwest Reference Area;
- fish health in the White Rose site;
- taste panel results for American plaice between the White Rose site and Northwest Reference Area.

2.0 METHODS

2.1 Biological Survey Methods

This document provides an outline for the fish sampling survey conducted at the White Rose site between June 24 and July 10, 2002.

Sampling was conducted aboard the *Canadian Coast Guard Ship* (*CCGS*) *Wilfred Templeman*. Samples were collected from the White Rose site, as well as from a reference area, approximately 100 km to the northwest of the White Rose site.

Approximately 200 kg snow crab was collected from each of the White Rose study and reference areas. Once ashore, these samples were used in taste tests and tissue chemical analyses. The experimental fishing licence granted by DFO allowed a maximum total catch of 200 kg of snow crab, which were collected using the Campelen 1800 trawl.

Fifty American plaice were sampled from the reference area only since American plaice samples were collected for the White Rose site in 2000. Once ashore, these samples were used in fish health analyses. The experimental fishing license granted by DFO allowed a maximum total catch (all areas combined) of 300 kg of American plaice, which were collected using a Campelen 1800 trawl.

Due to the large area covered during trawling, highly accurate (i.e., 1 m) positioning was not required for the fish sampling survey. Standard positioning equipment on board the *CCGS Wilfred Templeman* was sufficient for the purpose of the sampling program. Each sampling transect was delineated by start and end points of the trawl. These points were entered as way points on the ship's positioning system and the fishing captain navigated using range and bearing to the end point. Each tow duration was 15 minutes. Tow start and end positions are provided in Table 2.1 and illustrated in Figures 2.1 (White Rose site) and 2.2 (Northwest Reference Area).

One-third of the fishing effort for snow crab was focussed at the White Rose site (collected for taste panel comparisons only); two-thirds of the fishing effort was focussed at the northwest reference area (collected for taste panel comparisons and body burden analyses). American plaice were collected at the northwest reference area only; 50 individuals >25 cm in length were required for fish health analyses.

2.2 Sample Handling Quality Assurance/Quality Control

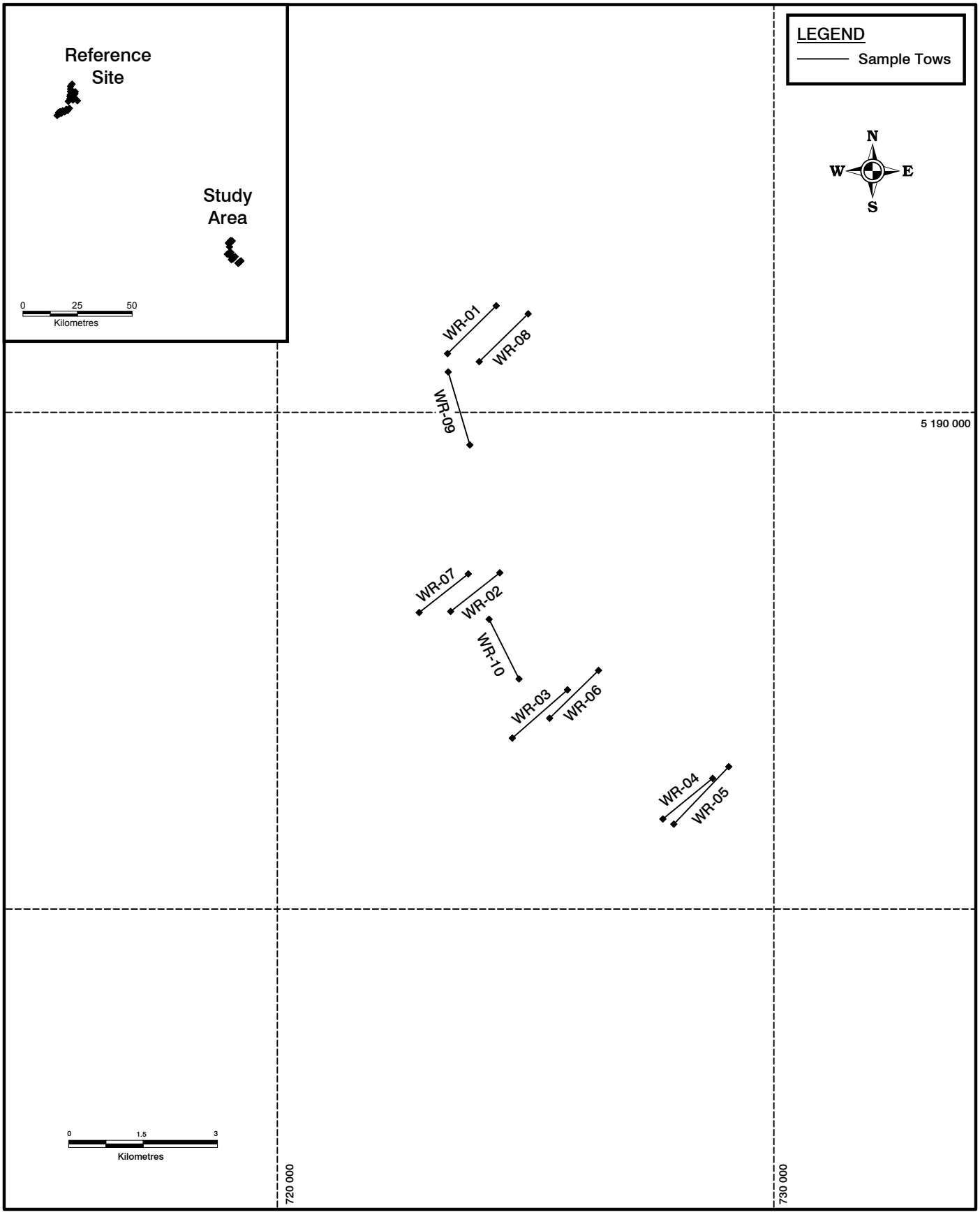
During the execution of the biological survey, the following Quality Assurance/Quality Control (QA/QC) guidelines were adhered to:

- all measuring instruments and work surfaces were washed with mild soap and water then rinsed with distilled water prior to the start of each transect;
- the fishing deck of the survey vessel was washed with degreaser then flushed with sea water. Flushing of the fishing deck and the chute leading to the processing lab was continuous during the entire survey;
- all sampling personnel were supplied with latex gloves to be worn during sample processing then disposed of after each station;
- all samples were transferred to the freezer immediately after processing, within 1 hour after collection; and
- as part of the mobilization, all sample containers were prelabelled and packaged in accordance with JWEL QA/QC protocol.

Table 2.1 Tow Start and End Positions

Tow No.	Tow Start Lat.	Tow Start Long.	Tow End Lat.	Tow End Long.	Direction (°T)	Depth Fished (m)
White Rose Site						
WR-01	46°50.2'N	48°04.2'W	46°50.7'N	48°03.4'W	045	120-122
WR-02	46°47.8'N	48°03.5'W	46°47.4'N	48°04.3'W	240	118-119
WR-03	46°46.0'N	48°03.4'W	46°46.5'N	48°02.5'W	045	119-120
WR-04	46°45.06'N	48°01.07'W	46°45.48'N	48°00.26'W	050	121
WR-05	46°45.6'N	48°00.0'W	46°45.0'N	48°00.9'W	240	120-121
WR-06	46°46.2'N	48°02.8'W	46°46.7'N	48°02.0'W	050	119-121
WR-07	46°47.8'n	48°04.0'w	46°47.4'n	48°04.8'w	230	118-119
WR-08	46°50.1'N	48°03.7'W	46°50.6'N	48°02.9'W	050	121-124
WR-09	46°50.0'N	48°04.2'W	46°49.2'N	48°03.9'W	180	120-121
WR-10	46°47.3'N	48°03.7'W	46°46.64'N	48°03.26'W	170	119-120
Northwest Reference Area						
WR-Ref-01	47°22.9'N	49°04.7'W	47°23.6'N	49°04.1'W	040	110-111
WR-Ref-02	47°23.2'N	49°04.0'W	47°23.8'N	49°03.6'W	030	110-112
WR-Ref-03	47°23.4'N	49°03.1'W	47°24.0'N	49°02.6'W	025	107-108
WR-Ref-04	47°23.71'N	49°01.91'W	47°24.30'N	49°01.35'W	025	105
WR-Ref-05	47°24.1'N	49°00.8'W	47°24.6'N	49°00.2'W	030	104-105
WR-Ref-06	47°28.1'N	48°58.0'W	47°28.6'N	48°57.9'W	030	119-122
WR-Ref-07	47°30.0'N	48°59.6'W	47°30.5'N	48°59.0'W	040	126-129
WR-Ref-08	47°26.9'N	48°59.9'W	47°26.3'N	49°000.5'W	215	112-114
WR-Ref-09	47°28.7'N	48°59.6'W	47°29.29'N	48°59.06'W	035	120-123
WR-Ref-10	47°27.2'N	48°58.3'W	47°26.6'N	48°58.8'W	215	113-116
WR-Ref-11	47°27.1'N	48°58.8'W	47°27.7'N	48°58.6'W	035	113-117
WR-Ref-12*	47°26.4'N	48°57.2'W	nr	nr	150	115-117
WR-Ref-13	47°27.2'N	48°59.2'W	47°27.9'N	48°59.7'W	000	115-117
WR-Ref-14	47°27.5'N	48°59.8'W	47°28.2'N	48°58.8'W	000	116-119
WR-Ref-15	47°27.5'N	48°59.0'W	47°28.2'N	48°59.0'W	000	116-119
WR-Ref-16	47°27.6'N	48°59.1'W	47°28.3'N	48°59.1'W	nr	116-118
WR-Ref-17	47°27.6'N	48°59.3'W	47°28.3'N	48°59.3'W	000	116-119
WR-Ref-18	47°27.5'N	48°59.3'W	47°28.23'N	48°58.81'W	020	115-119

NOTE: Each tow duration 15 minutes
* = 13-minute tow
nr = not recorded



8401-2.WOR 13JAN03 11:50am

Jacques Whitford Environment Limited
Environmental Scientists
Consulting Engineers

FIGURE 2.1

LOCATION OF TOWS
IN THE WHITE ROSE STUDY AREA

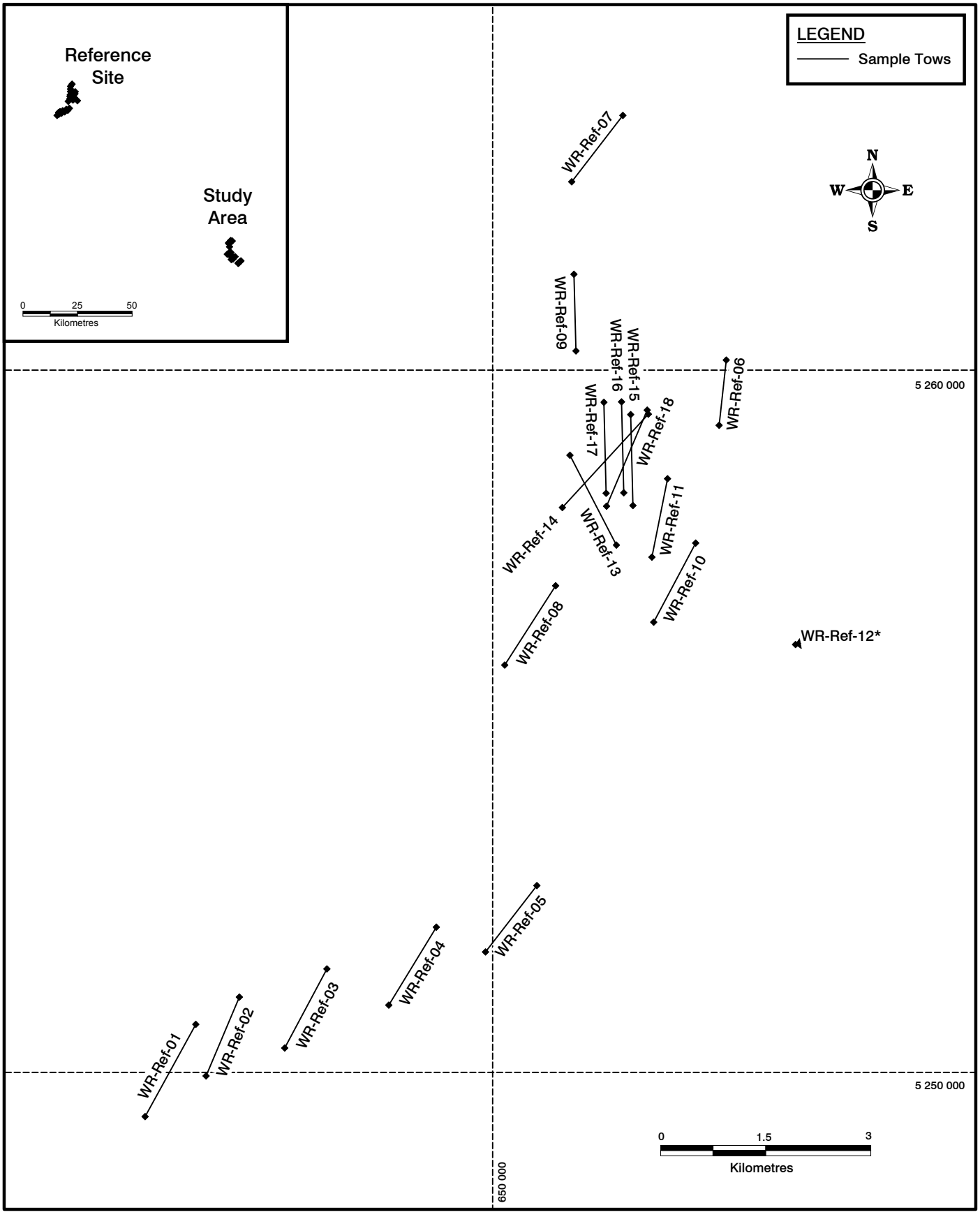


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FIGURE 2.2

LOCATION OF TOWS
IN THE WHITE ROSE REFERENCE SITE

2.3 Sampling

2.3.1 American Plaice

2.3.1.1 Sample Selection

Fishing gear was deployed by the ship's crew at trawl locations defined by the client representative on board. American plaice larger than 25 cm were selected from the catch at the northwest reference area for fish health analyses. Samples were handled in a consistent manner (Section 2.2). All fish retained as samples showed no visible trawl damage pathologies to indicate poor health.

2.3.1.2 Sample Preparation

The liver, top and bottom fillets (with skins removed) and otoliths were retained as individual samples assigned to each fish. Fillets were trimmed so that any fat around the edges or at the base of the dorsal and ventral fins was removed. Incidental observations including incidences of hydrocarbon odours was recorded. The following parameters were recorded for each fish; Fish ID, Total length, Total weight, Gutted weight, Liver weight, Sex, Gonad weight, Maturity, Stomach content. The fillets were stored frozen for future reference, if required.

Upon capture, fish collected by OCEANS Ltd. Personnel were killed by severing the spinal cord, measured to the nearest centimetre for total length and weighed to the nearest gram. Each fish was assessed visually for any external parasites and/or abnormalities and then dissected. Sex and maturity stage were recorded according to procedures used by DFO (Appendix A). Liver and gonad were weighed. Blood, liver, gills, heart, spleen, gonads, kidneys and otoliths were collected and processed for analyses back on shore.

Fish were cleaned by an experienced crewperson dedicated to this task. During sample preparation, a cutter and a technician worked together as a team to efficiently process each sample. The cutter and the technician coordinated with the OCEANS Ltd. technician to provide required fish parts. The OCEANS Ltd. technician provided an OCEANS ID number to the technician so that general recorded parameters for each fish can be matched with OCEANS Ltd. samples at later date. Fish cleaners and technicians wore latex gloves and kept their hands clean with soap and water and kept away from shipboard tasks that may involve the handling of oil products or equipment that may be covered with oil.

Fish were cleaned and tissues extracted on new stainless steel or plastic cutting boards using new stainless steel knives. Boards and knives were scrubbed, rinsed with clean salt water and then with bottled distilled water between fish samples. Fish were cleaned at a rate that allows direct transfer, without creating a backlog, of the samples from the cutting table to the technician for labelling and packaging.

2.3.1.3 Sample Identification

Each fillet, liver or otolith to be saved was packaged individually in ziploc storage bags or paper envelopes and labelled to indicate top fillet (TOP), bottom fillet (BOT), liver (LIV), or otolith (OT) and the Jacques Whitford Trawl Identification number. This numbering system extended across both the study and the reference area samples (for snow crab). The labels were made from water proof paper and completed in pencil. The label information was also marked on the outside of the bag using a waterproof marking pen. The log entry for that fish was completed with the identification of the sample bags. Each fish was processed completely before proceeding to the next so that there was no confusion in identification between fish samples.

2.3.1.4 Sample Storage

Once packaged, all samples were frozen at sea at -20°C in a dedicated freezer. Samples were frozen within one hour of collection. The temperature of the freezer was monitored and logged by the technicians on a Freezer Temperature Log Sheet at regular three-hour intervals throughout the cruise.

2.3.2 Snow Crab

2.3.2.1 Sample Selection

Snow crab were collected by the same Campelen trawl used to collect the American plaice. Once the trawl was on deck, the catch was removed onto a clean deck or clean fish tub and then onto the conveyor belt or carried to the processing area. The deck was to washed between sets to avoid contamination (particularly with oil substances). Samples retained for analysis did not have visible trawl damage or visible pathologies to indicate poor health.

2.3.2.2 Sample Preparation

Each snow crab had the carapace length, width and thickness and total weight (with shell) recorded on the Snow Crab Log Sheet. The crab was euthanized, then the legs were then removed and frozen whole (i.e., tissue was not removed from shell). Incidental observations were recorded, including any incidences of hydrocarbon odour.

Snow crab were prepared by experienced crew dedicated to the task. During sample preparation, a cutter and a biological technician worked together to efficiently process each sample. Cutters and technicians wore latex gloves and kept their hands clean with soap and water and kept away from shipboard tasks that may involve the handling of oil products or equipment that may be covered with oil.

After the legs were removed, they were saved in pre-labelled Ziploc bags. Since samples were chemically analyzed for metals and hydrocarbons and tested by an expert panel for tainting, it was imperative that contamination due to ship-based oils be avoided.

Snow crab were cut on cleaned stainless steel surfaces or clean plastic cutting boards using new stainless steel knives. Boards, knives and gloves were all scrubbed, rinsed with clean salt water and then with bottled distilled water between sets.

2.3.2.3 Sample Identification

Snow crab legs from individual crab were packaged in separate Ziploc bags. The log entry for each snow crab listed a snow crab ID number, the analysis to be conducted and a bulk bag ID number. Each snow crab was processed completely before proceeding to the next set so that there was no confusion in identification between set samples or loss of information.

2.3.2.4 Sample Storage

Once packaged, all samples were frozen at sea at a temperature of -20°C. Samples were frozen within one hour of collection. Temperature of the freezer was monitored and logged by the technicians on a Freezer Temperature Log Sheet at regular three-hour intervals throughout the cruise.

2.4 Taste Test Methods

2.4.1 Sample Preparation

Samples of snow crab legs were delivered frozen to the Fisheries and Marine Institute of Memorial University of Newfoundland for sensory evaluation (taint testing) by triangle test and hedonic scaling. Each panel included 24 untrained panelists who were briefed on the presentation of samples and score sheets (Figures 2.1 and 2.2) prior to the sensory evaluation. Panelists were scheduled, then instructed not to communicate with each other while in the panel room and to leave immediately upon completion of the sensory evaluation.

Half of the frozen samples were thawed overnight at 4ºC, with the remaining half retained frozen for future analyses. Samples were rinsed, enclosed in individual aluminum foil packets (shiny side in), labelled with a predetermined random three-digit code and cooked in a convection oven at 175ºC for 15 minutes. Samples were served at 35ºC.

The triangle test presented the panelists with a three-sample set (triangle) of coded samples wrapped in aluminum foil and the panel was asked to identify the sample which was different from the others. Each panelist was provided a cup of room temperature water for rinsing and a second cup for expectorate. Half the panelists received sets of two coded samples from Treatment A (White Rose study areas) and one coded from Treatment B (reference areas). The other panelists received sets composed of one coded sample from Treatment A (study areas) and two coded from Treatment B (reference areas). Therefore, there were six possible orders in which the coded samples were presented to the panelists (Botta 1994): ABB, AAB, ABA, BAA, BBA, and BAB. Panelists were asked to select the sample that was different and indicate it on the enclosed form (Figure 2.3).

The remaining frozen samples were thawed overnight at 4ºC and presented at a second sensory evaluation for hedonic scaling. Methods for preparing, cooking and presenting the fillets were the same as those for the triangle test. Two coded American plaice samples, wrapped in aluminum foil, were presented to the 24 panelists for the hedonic scaling evaluation. All panelists were presented with one White Rose sample and one northwest reference area sample and were instructed to rate how much they liked or disliked each sample on the form (Figure 2.4).

Figure 2.3 Sample Questionnaire for Sensory Evaluation by Triangle Test

QUESTIONNAIRE FOR TRIANGLE TEST

Name: _____ Date/Time: _____

Product: Snow Crab Legs

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
214	_____
594	_____
733	_____

2. Comments: _____

_____

_____

Figure 2.4 Sample Questionnaire for Sensory Evaluation by Hedonic Scaling

QUESTIONNAIRE FOR HEDONIC SCALING

Name: _____ Date/Time: _____

Product: Snow Crab Legs

1. Taste these samples and check how much you like or dislike each one.

619	835
_____like extremely	_____like extremely
_____like very much	_____like very much
_____like moderately	_____like moderately
_____like slightly	_____like slightly
_____neither like nor dislike	_____neither like nor dislike
_____dislike slightly	_____dislike slightly
_____dislike moderately	_____dislike moderately
_____dislike very much	_____dislike very much
_____dislike extremely	_____dislike extremely

2. Comments: _____

_____

_____

_____

2.4.2 Data Analysis

The triangle test datum is a value which represents the number of correct responses over the number of panelists. This value was compared to values in a standard table to determine the statistical significance of the result. A statistically significant result of the recommended panel size of 24 required 13 correct responses (95 percent significance level).

A nine-point hedonic scale was used, with ratings varying from like extremely to dislike extremely, with neither like or dislike being the middle rating. There are four ratings either side of the middle rating. The panel ratings were assigned numerical values from “like extremely” (9) to “dislike extremely” (1). The data were tabulated and subjected to analysis of variance and presented graphically in a frequency histogram.

3.0 RESULTS

3.1 Snow Crab Tissue Chemistry

A summary of 2000 and 2002 results for variables with results above the estimated quantitation limit (EQL) is provided in Table 3.1. The analytical data from Philip Analytical Services and the summary statistics are provided in Appendix A.

Table 3.1 Metal Concentrations in Snow Crab Leg Samples from White Rose Study Area and Northwest Reference Area

Variable	Units	EQL	Study Area*			Northwest Reference Area**		
			No. Composites	Minimum	Maximum	No. Samples	Minimum	Maximum
Arsenic	mg/kg	0.5	4	4.8	6.8	5	6.1	6.7
Boron	mg/kg	1.5	4	1.7	3.2	5	<EQL	2.8
Cadmium	mg/kg	0.05	4	n/r	n/r	5	0.05	0.16
Copper	mg/kg	0.5	4	3.0	4.2	5	3.5	5.1
Mercury	mg/kg	0.01	4	0.08	0.10	5	0.07	0.10
Selenium	mg/kg	0.5	4	0.5	0.7	5	0.7	0.7
Silver	mg/kg	0.12	4	n/r	n/r	5	0.21	0.36
Strontium	mg/kg	1.5	4	6.2	9.8	5	4.4	120.0
Zinc	mg/kg	0.5	4	24.0	31.0	5	20.0	27.0

* Study Area samples collected in 2000 (Husky 2001).
** Northwest Reference Area samples collected in 2002.
n/r = no concentration above EQL recorded.

The seven variables with results recorded above EQL at the White Rose Study Area are also above EQL at the Northwest Reference Area, with comparable maxima and minima, expect for strontium, which had a maximum of more than 10 times the maximum recorded in the White Rose Study Area. In addition, two additional variables with results above EQL were recorded in the Northwest Reference Area, cadmium and silver.

3.2 Snow Crab Tissue Sensory Evaluations Taste

3.2.1 Triangle Test

The sensory evaluation participants for the triangle test were successful in discriminating 18 out of 24 samples. These results are significant at the 95 percent level, an indicated that taste panellists could detect a difference between the White Rose and Northwest Reference area site (Appendix B). It should be noted that all comments pertained to texture and were not taint related.

3.2.2 Hedonic Scaling

Results from sensory evaluation for hedonic scaling (Appendix C) were assigned numerical values ranging from “like extremely” (9) to “dislike extremely” (1). Data were subjected to ANOVA (Table 3.2) and these ratios are significant, indicating that the taste panellists showed a statistical preference for the White Rose study area. Again, all comments pertain to texture and are not taint related.

Table 3.2 Results of ANOVA Hedonic Scaling

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio	P-value	F critical
Between Groups	20.02083	1	20.02083	7.840724	0.007444	4.051742
Within Groups	117.4583	46	2.553442			
Total	137.4792	47				

3.3 American Plaice Health

Bioindicator studies were carried out on fish sampled at the White Rose study area in 2000 and at the Northwest Reference Area in 2002. Bioindicators have potential to identify adverse health conditions in fish in advance of effects at the population level, and thus provide early warning of potential problems. As such, they can also be a valuable scientific tool for addressing public concerns of a real or perceptual nature about the scope of potential impacts on fish stocks of commercial importance. The bioindicators studied in this post-exploratory baseline survey included external and internal lesions, mixed-function oxygenase (MFO) enzymes, haematology and a variety of liver and gill histological indices.

Thirty-three female and 17 male American plaice were sampled by seven trawl sets in the Northwest Reference Area (July 1, 2002). The results obtained during this cruise were compared to those obtained with American plaice collected in the White Rose study area during the July 2000 cruise (OCEANS Ltd. 2001). The results obtained indicate that the present health status of American plaice collected at the White Rose site is similar to that at more distant sites. Overall, a reasonable level of bioindicator baseline data is now available for comparison with any future studies on fish health once development commences.

With respect to future EEM programs, cross comparisons could be carried out on the various liver pathologies and the more "severe" gill lesions such as epithelial lifting, clubbing with telangiectasis, fusion and severe oedema. However, these have been shown to be essentially absent on the Grand Banks at Reference and Development sites in the case of Terra Nova (where there is three years of data). In comparison to the above, it is not recommended to make inter-year comparisons with the very sensitive and "less severe" gill indices, namely various types of "mild" hyperplasia. The same could be said for the very sensitive MFO response, as well as condition indices that may vary somewhat yearly in relation to feeding, or "unknown" oceanographic conditions. Concerning these more sensitive indices (selected

gill lesions and MFO) and fish condition, it seems best at this time and at least in the near term to look for ‘major" changes at Reference and Development sites without trying to tease out any small inter year differences. For instance, in the case of MFO, induction in the three to five-fold range around rig sites would definitely draw interest but not a two-fold induction. With respect to the major liver and gill histopathologies, if they begin to appear on the Grand Banks, they would obviously be a better fit for temporal trend monitoring.

With respect to plaice mobility, the purpose normally has been to provide information (also as requested by DFO) on potential effects on fish under natural conditions of exposure, in this case on American plaice, a species of major commercial importance and presently under recovery. It is reasonable to suggest that American plaice are resident for periods of weeks to months in specific areas, with any early warning indicators of health such as MFO enzyme induction and selected gill and liver lesions (but probably not carcinomas for instance) being causally linked to those areas. However, fish might move in and out of potential "contaminant" zones very close to study areas, such that exposure is insufficient to result in deleterious effects. If this is the case, then this is equally important, from the viewpoint of evaluating real risk to fish.

However, it is worth noting that information from other areas indicate that fish (as well as crabs) are attracted to rig sites (e.g., through structure attraction, enhanced productivity etc.). Also, fish which are generally considered to be much more mobile than American plaice have demonstrated MFO induction and histopathology around some development sites.

With respect to crabs, much less is known about their sensitivity to various pathologies, etc. in relation to chemical exposures They also lack the sensitive MFO enzyme response, which can be an important index for assessing the biological relevance of contamination by production and displacement waters.

The complete histopathology report (OCEANS 2003) is provided as Appendix D.

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APPENDIX A

Snow Crab Tissue Chemistry Results

	Client ID:			WR-Ref-11	WR-Ref-13	WR-Ref-16	WR-Ref-17	WR-Ref-18
	Project ID:			08401-0004	08401-0004	08401-0004	08401-0004	08401-0004
	PSC Analytical ID:			02-H060764	02-H060765	02-H060766	02-H060767	02-H060768
Parameters	**Method**	**EQL**	**Units**					
>C10-C21 (Fuel Range)	GC/FID	15	mg/kg	< 15	< 15	< 15	< 15	< 15
>C21-C32 (Lube Range)	GC/FID	15	mg/kg	< 15	< 15	< 15	< 15	< 15
TEH (>C10-C32)	Calculated	30	mg/kg	< 30	< 30	< 30	< 30	< 30
TEH Surrogate (IBB)	GC/FID	-	% Rec.	103	102	120	91	117
Naphthalene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Perylene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
1-Methylnaphthalene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
2-Methylnaphthalene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Acenaphthylene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Acenaphthene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Fluorene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Phenanthrene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Anthracene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Fluoranthene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Pyrene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Benz[a]anthracene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Chrysene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Benzo[b]fluoranthene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Benzo[k]fluoranthene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Benzo[a]pyrene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Indeno[1,2,3-cd]pyrene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Dibenz[a,h]anthracene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Benzo[ghi]perylene	GC/MS	0.05	mg/kg	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Aluminum (Biota)	ICP-MS	2.5	mg/kg	< 2.5	< 2.5	< 2.5	< 2.5	< 2.5
Antimony (Biota)	ICP-MS	0.5	mg/kg	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Arsenic (Biota)	ICP-MS	0.5	mg/kg	6.6	6.7	6.1	6.6	6.7
Barium (Biota)	ICP-MS	1.5	mg/kg	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
Beryllium (Biota)	ICP-MS	1.5	mg/Kg	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5
Boron (Biota)	ICP-MS	1.5	mg/Kg	< 1.5	< 1.5	2.8	1.7	2.5
Cadmium (Biota)	ICP-MS	0.05	mg/Kg	0.08	0.06	0.05	0.08	0.16
Chromium (Biota)	ICP-MS	0.5	mg/Kg	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Cobalt (Biota)	ICP-MS	0.2	mg/Kg	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Copper (Biota)	ICP-MS	0.5	mg/Kg	3.8	4.1	3.5	5.1	3.8
Iron (Biota)	ICP-MS	5	mg/Kg	< 5	< 5	< 5	< 5	5
Lead (Biota)	ICP-MS	0.18	mg/Kg	< 0.18	< 0.18	< 0.18	< 0.18	< 0.18
Lithium (Biota)	ICP-MS	0.5	mg/Kg	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Manganese (Biota)	ICP-MS	0.5	mg/Kg	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Mercury - Biota	CVAA	0.01	mg/Kg	0.07	0.09	0.09	0.1	0.09
Molybdenum (Biota)	ICP-MS	0.5	mg/Kg	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Nickel (Biota)	ICP-MS	0.5	mg/Kg	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Selenium (Biota)	ICP-MS	0.5	mg/Kg	0.7	0.7	0.7	0.7	0.7
Silver (Biota)	ICP-MS	0.12	mg/Kg	0.29	0.25	0.21	0.36	0.26
Strontium (Biota)	ICP-MS	1.5	mg/Kg	8	4.4	120	17	14
Thallium (Biota)	ICP-MS	0.02	mg/Kg	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Tin (Biota)	ICP-MS	0.5	mg/Kg	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Uranium (Biota)	ICP-MS	0.02	mg/Kg	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Vanadium (Biota)	ICP-MS	0.5	mg/Kg	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Zinc (Biota)	ICP-MS	0.5	mg/Kg	20	25	27	24	25
Fat, Crude	AOAC922.06	0.5	%(w)	0.5	0.8	0.7	0.8	0.7

Northwest Reference Area Snow Crab Leg Tissue Chemical Analysis Summary Statistics

Variable	Analytical Method	EQL	Unit	No. of Samples	No. > EQL	Mean	SD	Median	Minimum	Maximum
>C10-C21 (Fuel Range)	GC/FID	15	mg/kg	5	0	-	-	-	-	-
>C21-C32 (Lube Range)	GC/FID	15	mg/kg	5	0	-	-	-	-	-
TEH (>C10-C32)	Calculated	30	mg/kg	5	0	-	-	-	-	-
Naphthalene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Perylene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
1-Methylnaphthalene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
2-Methylnaphthalene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Acenaphthylene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Acenaphthene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Fluorene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Phenanthrene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Anthracene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Fluoranthene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Pyrene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Benz[a]anthracene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Chrysene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Benzo[b]fluoranthene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Benzo[k]fluoranthene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Benzo[a]pyrene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Indeno[1,2,3-cd]pyrene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Dibenz[a,h]anthracene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Benzo[ghi]perylene	GC/MS	0.05	mg/kg	5	0	-	-	-	-	-
Aluminum (Biota)	ICP-MS	2.5	mg/kg	5	0	-	-	-	-	-
Antimony (Biota)	ICP-MS	0.5	mg/kg	5	0	-	-	-	-	-
Arsenic (Biota)	ICP-MS	0.5	mg/kg	5	5	6.54	0.25	6.60	6.10	6.70
Barium (Biota)	ICP-MS	1.5	mg/kg	5	0	-	-	-	-	-
Beryllium (Biota)	ICP-MS	1.5	mg/Kg	5	0	-	-	-	-	-
Boron (Biota)	ICP-MS	1.5	mg/Kg	5	3	-	-	1.70	<EQL	2.80
Cadmium (Biota)	ICP-MS	0.05	mg/Kg	5	5	0.09	0.04	0.08	0.05	0.16
Chromium (Biota)	ICP-MS	0.5	mg/Kg	5	0	-	-	-	-	-
Cobalt (Biota)	ICP-MS	0.2	mg/Kg	5	0	-	-	-	-	-
Copper (Biota)	ICP-MS	0.5	mg/Kg	5	5	4.06	0.62	3.80	3.50	5.10
Iron (Biota)	ICP-MS	5	mg/Kg	5	1	-	-	-	<EQL	5.00
Lead (Biota)	ICP-MS	0.18	mg/Kg	5	0	-	-	-	-	-
Lithium (Biota)	ICP-MS	0.5	mg/Kg	5	0	-	-	-	-	-
Manganese (Biota)	ICP-MS	0.5	mg/Kg	5	0	-	-	-	-	-
Mercury - Biota	CVAA	0.01	mg/Kg	5	5	0.09	0.01	0.09	0.07	0.10
Molybdenum (Biota)	ICP-MS	0.5	mg/Kg	5	0	-	-	-	-	-
Nickel (Biota)	ICP-MS	0.5	mg/Kg	5	0	-	-	-	-	-
Selenium (Biota)	ICP-MS	0.5	mg/Kg	5	5	0.70	0.00	0.70	0.70	0.70
Silver (Biota)	ICP-MS	0.12	mg/Kg	5	5	0.27	0.06	0.26	0.21	0.36
Strontium (Biota)	ICP-MS	1.5	mg/Kg	5	5	32.68	49.06	14.00	4.40	120.00
Thallium (Biota)	ICP-MS	0.02	mg/Kg	5	0	-	-	-	-	-
Tin (Biota)	ICP-MS	0.5	mg/Kg	5	0	-	-	-	-	-
Uranium (Biota)	ICP-MS	0.02	mg/Kg	5	0	-	-	-	-	-
Vanadium (Biota)	ICP-MS	0.5	mg/Kg	5	0	-	-	-	-	-
Zinc (Biota)	ICP-MS	0.5	mg/Kg	5	5	24.20	2.59	25.00	20.00	27.00
Fat, Crude	AOAC922.06	0.5	%(w)	5	5	0.70	0.12	0.70	0.50	0.80

APPENDIX B

Snow Crab Tissue Taste Panel Results
Triangle Test Data

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Master Date/Time: Nov. 29/02; 10:30 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
343	Ref
102	Ref
487	T

2. Comments: _____

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Carolyn Dwyer Date/Time: Nov. 29/02, 10:30 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
343	
102	
487	✓

2. Comments: Bland - little flavor.

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Kim Robertson. Date/Time: Nov. 29/02, 10:30 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
343	
102	
487	✓

2. Comments: Tasted better than samples 343 & 102

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Ron Hyde Date/Time: Nov. 29/02, 10:30 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
343	
102	
487	✓

2. Comments: More flavour

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Ann Lynn Holwell Date/Time: Nov. 29/02, 10:30 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
343	
102	
487	✓

2. Comments: _____

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Shawn Fortune Date/Time: Nov. 29/02, 10:30 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
343	
102	✓
487	

2. Comments: 102 was more bland in taste.

QUESTIONNAIRE FOR TRIANGLE TEST

Name: EDWARD HEATON Date/Time: Nov. 29/02, 10:30 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
343	✓
102	
487	

2. Comments: _____

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Master Date/Time: Nov. 29/02; 11:00 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
482	Ref
452	T
223	Ref

2. Comments: _____

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Nancy Smith Date/Time: Nov. 29/02, 11:00 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
482	
452	
223	✓

2. Comments: the third one is not as salty tasting as the otha two but there is not a big difference.

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Angie Bishop Date/Time: Nov. 29/02, 11:00 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
482	✓
452	
223	

2. Comments: _____

_____

_____

_____

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Mary Halliday Date/Time: Nov. 29/02, 11:00 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
482	
452	
223	✓

2. Comments: _____

_____

_____

_____

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Jackie Higdon Date/Time: Nov. 29/02, 11:00am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
482	
452	
223	✓

2. Comments: I found a very slight difference only.

Arlene McDonald

QUESTIONNAIRE FOR TRIANGLE TEST

Name: _____ Date/Time: Nov. 29/02, 11:00am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
482	
452	✓
223	

2. Comments: _____

_____

_____

_____

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Heather Stanup Nuld Date/Time: Nov. 29 02, 11:00 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
482	
452	✓
223	

2. Comments: 452 appeared to have a stronger taste + 282 + 223 were really crunchy... too much shells left!

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Master Date/Time: Nov. 29/02; 11:30 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
583	Ref
187	T
290	T

2. Comments: _____

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Janice Williams Date/Time: Nov. 29/02, 11:30 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
583	✓
187	
290	

2. Comments: It was hard to distinguish between the 3 samples.

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Jason Ralph Date/Time: Nov. 29/02, 11:30 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
583	✓
187	
290	

2. Comments: Sample 583 tasted stronger

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Stephanie Hamlyn Date/Time: Nov. 29/02, 11:30 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
583	✓
187	
290	

2. Comments: The othur 2 were sweeter

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Diana Pike Date/Time: Nov. 29 '02, 11:30 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
583	✓
187	
290	

2. Comments: Not as tasty as the rest #2 ~~too~~ had stronger taste

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Tarah Cunningham Date/Time: Nov. 29/02, 11:30 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
583	✓
187	
290	

2. Comments: _____

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Deanna Lambe Date/Time: Nov. 29/02, 11:30 am

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
583	✓
187	
290	

2. Comments: Sample 583 is rather too salty and had a different flavor.

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Master Date/Time: Nov. 29/02: 12:00 noon

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
204	T
342	Ref
791	T

2. Comments: _____

_____

_____

_____

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Collins Date/Time: Nov. 29/02, 12:00

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
204	
342	✓
791	

2. Comments: Sample 342 has more intense 'sweet' taste.

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Kate Collins Date/Time: Nov. 29/02, 12:00

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
204	
342	✓
791	

2. Comments: 204 - very nice, sweet
342 - slightly fishy - not as sweet
791 - nice, sweet

QUESTIONNAIRE FOR TRIANGLE TEST

Name: AUBREY FREEBORN Date/Time: NOV. 29/02, 12:00

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
204	
342	✓
791	

2. Comments: 342 A BIT DISCOLOURED BUT TASTE THE SAME. DARK COLOUR WILL DEFER PURCHASE.

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Susan Fudge Date/Time: Nov. 29/02, 12:00

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
204	
342	✓
791	

2. Comments: all good but 342 has hard grains & a bit of ~~an aftertaste~~ funny aftertaste

QUESTIONNAIRE FOR TRIANGLE TEST

Name: PAUL RYAN Date/Time: NOV. 29 02, 12:00

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
204	
342	✓
791	

2. Comments: 204, 342, looks same, smells same, taste diff, 791, same as 204

QUESTIONNAIRE FOR TRIANGLE TEST

Name: Wade Hyrock Date/Time: Nov. 29/02, 12:00

Product: Snow Crab

Two of these samples are identical, the third is different.

1. Taste the samples in the order indicated and identify the odd sample. You must choose one of the samples.

Code	Check Odd Sample
204	
342	✓
791	

2. Comments: Stronger flavour almost like something added to the crab ... maybe fish?

APPENDIX C

Snow Crab Tissue Taste Panel Results
Hedonic Scaling Data

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Master Date/Time: Nov. 29/02, 2:30 pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

	234 Ref		382 Test
_____	like extremely	_____	like extremely
_____	like very much	_____	like very much
_____	like moderately	_____	like moderately
_____	like slightly	_____	like slightly
_____	neither like nor dislike	_____	neither like nor dislike
_____	dislike slightly	_____	dislike slightly
_____	dislike moderately	_____	dislike moderately
_____	dislike very much	_____	dislike very much
_____	dislike extremely	_____	dislike extremely

2. Comments: _____

_____

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Joan Murphy Date/Time: Nov 29/02, 2:30pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

234		382	
	like extremely		like extremely
✓	like very much		like very much
✓	like moderately	✓	like moderately
	like slightly		like slightly
	neither like nor dislike		neither like nor dislike
	dislike slightly		dislike slightly
	dislike moderately		dislike moderately
	dislike very much		dislike very much
	dislike extremely		dislike extremely

2. Comments: 234 seemed to be more moister than 382.

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Jenah Helpert Date/Time: Nov. 29/02, 2:30 pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

	234		382
____	like extremely	____	like extremely
✓	like very much	✓	like very much
____	like moderately	____	like moderately
____	like slightly	____	like slightly
____	neither like nor	____	neither like nor
	dislike		dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: Slightly different from each other, but liked both.

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Fred Amstey Date/Time: Nov. 29/02, 2:30pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

234		382
____	like extremely	____
____	like very much	____
____	like moderately	✓
____	like slightly	____
✓	neither like nor dislike	____
____	dislike slightly	____
____	dislike moderately	____
____	dislike very much	____
____	dislike extremely	____

2. Comments: 234 visually less appealing. It also tasted saltier? with a porridge type texture/taste.

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Ray Fitzgerald Date/Time: Nov. 29/02 : 2:30 pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

234		382	
____	like extremely	____	like extremely
✓	like very much	✓	like very much
____	like moderately	____	like moderately
____	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: _____

_____

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Karen Collins Date/Time: Nov. 29/02, 2:30 pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

234		382	
____	like extremely	____	like extremely
____	like very much	✓	like very much
____	like moderately	____	like moderately
____	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
✓	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: 382 had a sweeter flavour + better colour.

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Jean Titford Date/Time: Nov. 29/02, 2:30pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

	234		382
____	like extremely	____	like extremely
____	like very much	____	like very much
____	like moderately	____	like moderately
____	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
____	dislike slightly	✓	dislike slightly
✓	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: Didn't like either.

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Master Date/Time: Nov. 29/02, 3:00 pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

	492 Test		609 Ref
____	like extremely	____	like extremely
____	like very much	____	like very much
____	like moderately	____	like moderately
____	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: _____

_____

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Alistair Struthers Date/Time: Nov. 29/02, 3:00pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

	492		609
____	like extremely	____	like extremely
✓	like very much	✓	like very much
✓	like moderately	____	like moderately
____	like slightly	____	like slightly
____	neither like nor	____	neither like nor
	dislike		dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: 492 too crunchy - shell ?

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Toni Legge. Date/Time: Nov. 29/02, 3:00pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

492		609	
____	like extremely	____	like extremely
____	like very much	____	like very much
✓	like moderately	____	like moderately
____	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	✓	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: 492- a little sweet taste to it

609- bland taste.

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Donna Shapter Date/Time: Nov. 29/02, 3:00pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

492		609	
____	like extremely	____	like extremely
✓	like very much	____	like very much
____	like moderately	____	like moderately
____	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	✓	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: ✓

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Michael Beson Date/Time: Nov. 29/02, 3:00pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

492		609	
____	like extremely	____	like extremely
✓	like very much	____	like very much
____	like moderately	____	like moderately
____	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
____	dislike slightly	✓	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: 609 had a sweet taste but an aftertaste that wasn't as nice. 492 Not as sweet but a nicer taste.

QUESTIONNAIRE FOR HEDONIC SCALING

Name: S. Humphrey Date/Time: Nov. 29 '02, 2:00 pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

492		609	
____	like extremely	____	like extremely
✓	like very much	____	like very much
____	like moderately	✓	like moderately
____	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: 492 sweeter, less "fishy" taste

QUESTIONNAIRE FOR HEDONIC SCALING

Name: ADAM COVERAGE Date/Time: Nov. 29/02, 3:00 pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

	492		609
____	like extremely	____	like extremely
____	like very much	✓	like very much
____	like moderately	____	like moderately
✓	like slightly	____	like slightly
____	neither like nor	____	neither like nor
	dislike		dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: 492 - liked it ^slightly but it was gritty, otherwise good

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Master Date/Time: Nov. 29/02, 3:30 pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

582 Ref		507 Test	
____	like extremely	____	like extremely
____	like very much	____	like very much
____	like moderately	____	like moderately
____	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: _____

_____

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Kevin Carroll Date/Time: Nov. 29/02, 3:30 pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

582		507
____	like extremely	____
____	like very much	✓
____	like moderately	____
✓	like slightly	____
____	neither like nor dislike	____
____	dislike slightly	____
____	dislike moderately	____
____	dislike very much	____
____	dislike extremely	____

2. Comments: 582 Sweeter. however much better flavour characteristic of crab in 507 sample.

QUESTIONNAIRE FOR HEDONIC SCALING

Name: MARK WAREHAM Date/Time: Nov. 29/02, 3:30pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

	582		507
____	like extremely	____	like extremely
____	like very much	____	like very much
✓	like moderately	✓	like moderately
____	like slightly	____	like slightly
____	neither like nor	____	neither like nor
	dislike		dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: 507 may have been a little tastier

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Judy Percy Date/Time: Nov. 29/02, 3:30 pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

582		507	
____	like extremely	____	like extremely
____	like very much	✓	like very much
✓	like moderately	____	like moderately
____	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: Not much difference in taste

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Patricia Cumby Date/Time: Nov. 29/02, 3:30pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

582		507	
____	like extremely	____	like extremely
____	like very much	✓	like very much
____	like moderately	____	like moderately
✓	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: 507 had bits of shell in it other than that was delicious.

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Watts Jantin Date/Time: Nov. 29/02, 3:30pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

	582		507
____	like extremely	____	like extremely
____	like very much	____	like very much
____	like moderately	✓	like moderately
____	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
✓	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: _____

_____

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Geraldine Nash Date/Time: Nov. 29/02, 3:30 pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

582		507	
____	like extremely	✓	like extremely
____	like very much	____	like very much
____	like moderately	____	like moderately
____	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
✓	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: Sample 507 was sweeter tasting. It's texture was more Flaky.
Sample 582 had a fisher (for want of a better word) taste.

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Master Date/Time: Nov. 29/02, 4:00 pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

	381 Test		904 Ref
_____	like extremely	_____	like extremely
_____	like very much	_____	like very much
_____	like moderately	_____	like moderately
_____	like slightly	_____	like slightly
_____	neither like nor dislike	_____	neither like nor dislike
_____	dislike slightly	_____	dislike slightly
_____	dislike moderately	_____	dislike moderately
_____	dislike very much	_____	dislike very much
_____	dislike extremely	_____	dislike extremely

2. Comments: _____

_____

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Lisa Winsor Date/Time: Nov. 29/02, 4:00pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

	381		904
____	like extremely	____	like extremely
____	like very much	✓	like very much
✓	like moderately	____	like moderately
____	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: _____

_____

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Chris Keats Date/Time: Nov. 29/02, 4:00pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

381		904
____	like extremely	____
____	like very much	✓
✓	like moderately	____
____	like slightly	____
____	neither like nor dislike	____
____	dislike slightly	____
____	dislike moderately	____
____	dislike very much	____
____	dislike extremely	____

2. Comments: _____

_____

QUESTIONNAIRE FOR HEDONIC SCALING

Name: MICHELLE CARROLL Date/Time: Nov. 29/02, 4:00pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

	381		904
____	like extremely	____	like extremely
____	like very much	____	like very much
____	like moderately	____	like moderately
✓	like slightly	____	like slightly
____	neither like nor dislike	✓	neither like nor dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: 381 seemed sweeter both were gritty & not favourable because of that.

QUESTIONNAIRE FOR HEDONIC SCALING

Name: D. Francis Date/Time: Nov. 29/02, 4:00pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

	381		904
____	like extremely	____	like extremely
____	like very much	____	like very much
✓	like moderately	✓	like moderately
____	like slightly	____	like slightly
____	neither like nor dislike	____	neither like nor dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: _____

_____

QUESTIONNAIRE FOR HEDONIC SCALING

Name: Debbie SeaJard Date/Time: Nov. 29/02, 4:00pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

	381		904
____	like extremely	____	like extremely
____	like very much	✓	like very much
____	like moderately	____	like moderately
✓	like slightly	____	like slightly
____	neither like nor	____	neither like nor
	dislike		dislike
____	dislike slightly	____	dislike slightly
____	dislike moderately	____	dislike moderately
____	dislike very much	____	dislike very much
____	dislike extremely	____	dislike extremely

2. Comments: _____

_____

QUESTIONNAIRE FOR HEDONIC SCALING

Name: SUZAN TUCKER Date/Time: Nov. 29/02, 4:00 pm

Product: Snow Crab

1. Taste these samples and check how much you like or dislike each one.

381		904
____	like extremely	____
✓	like very much	____
____	like moderately	____
____	like slightly	✓
____	neither like nor dislike	____
____	dislike slightly	____
____	dislike moderately	____
____	dislike very much	____
____	dislike extremely	____

2. Comments: # 381 IS CHOICE

APPENDIX D

Histopathology Report
(OCEANS Ltd. 2003)

WHITE ROSE BASELINE STUDIES:
HEALTH ASSESSMENT OF AMERICAN PLAICE

Prepared for:

Jacques Whitford Environment Ltd.
607 Torbay Road
St. John's, Newfoundland
A1A 4Y6

January 2003

WHITE ROSE BASELINE STUDIES:
HEALTH ASSESSMENT OF AMERICAN PLAICE

Prepared for:

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January 2003

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1.0 SUMMARY

OCEANS Ltd. was contracted by Jacques Whitford Environment Ltd. to carry out a health assessment of American plaice (*Hippoglossoides platessoides*) for the White Rose baseline study. Bioindicator studies were carried out on fish sampled at the Development Area in 2000 as well as at a Reference Area approximately 100 km further Northwest in 2002. Bioindicators have potential to identify adverse health conditions in fish in advance of effects at the population level and thus provide early warning of potential problems. As such, they can also be a valuable scientific tool for addressing public concerns of a real or perceptual nature about the scope of potential impacts on fish stocks of commercial importance. The bioindicators studied in this post-exploratory baseline survey included external and internal lesions, mixed-function oxygenase (MFO) enzymes, haematology and a variety of liver and gill histological indices. The results obtained indicate that the present health status of American plaice collected at the White Rose site is similar to that at more distant sites. However, increased MFO enzyme levels and three cases of megalocytic hepatosis were noted in fish from the Development Area. These bioindicator differences might be due to natural variation, but they are also known to be sensitive early responses to some contaminants. Overall, a reasonable level of bioindicator baseline data is now available for comparison with any future studies on fish health once development commences.

2.0 INTRODUCTION

The effects of environmental contamination can be viewed at different levels of biological organisation, extending from the molecular or biochemical level to effects on organ physiology and histology at the individual animal level and ultimately to the population or community level. Over the past few years, there has been increasing emphasis on use of individual-level indicators of chemical stress to obtain an appreciation of the degree, extent and severity of potential health effects to populations. These indicators are commonly referred to as bioindicators or health effect indicators**.** Use of such indicators at the sub-organismal or organismal level has the potential to identify adverse conditions in advance of responses at the population level and as such can provide an early warning of problem identification and adverse health effects (e.g. Society of Environmental Toxicology and Chemistry (SETAC) Special Publication Series, 1992; Peakall, 1992).

It is important to have background knowledge on selected health effect indicators in fish in order to provide perspective on any future changes which may arise over the life of the White Rose project. In this regard it is also important to note that in relation to concerns about fisheries, which are of major socio-economic importance in Atlantic Canada in general and in Newfoundland in particular, bioindicators can be a powerful tool for "disproving" as well as "proving" whether effects may be occurring.

2.1 Selected Health Effect Indicators

Mixed function oxygenase (MFO) enzymes, gross pathology, haematology and tissue histopathology were studied in American plaice. These bioindicators have been extensively used with various fish species in environmental assessments and studies on MFO enzymes, gross pathology and histopathology have been specifically endorsed by agencies such as the Oslo Paris Commission for use in environmental monitoring and assessment programs (Stagg, 1998). Presence of visible lesions, alterations in blood parameters and especially tissue histological changes are generally viewed as being pathological in nature while induction of MFO is most commonly recognised as an index of chemical exposure. However there is a body of literature associating MFO induction with radical production and mutagenic and carcinogenic processes at the molecular level. Induction in fish has also been specifically linked with effects on reproduction (Spies et al., 1988) as well as effects on the liver at both organ (e.g. Payne et al., 1988) and cellular levels (Au et al., 1999; Au and Wu, 2001). Thus measurement of MFO enzyme status in fish in monitoring programs also has value beyond being an index of chemical exposure.

2.1.1 Mixed Function Oxygenase (MFO) Activity

The MFO system refers to a family of enzymes that transforms the structure of organic chemicals and performs a critical role in detoxification and other physiological processes. The system which has iron-containing hemoproteins, cytochromes P-450, as terminal oxidases is also referred to as cytochrome P-450-dependent MFO. The activity of this

enzyme system commonly increases in animals in the presence of certain classes of organic pollutants. An increase in activity or induction of this enzyme system can therefore be used to monitor exposure to low levels of such pollutants.

Both field and laboratory studies have demonstrated that MFO induction can be a useful index for assessing exposure to various types of mixed organic pollutants such as petroleum hydrocarbons in fish (e.g. Payne et al., 1987). MFO induction is currently used by eight of the contracting parties to the Oslo Paris Commission for monitoring biological effects of contaminants in the North Sea area (Stagg, 1998). With respect to petroleum contamination, field studies have been carried out around oil rigs in the North Sea (Stagg et al., 1995; Stagg and McIntosh, 1996) as well as in connection with major oil spills such as the Exxon Valdez in Alaska (Woodin et al., 1997) and the Braer spill in the Shetland Islands (George et al., 1995).

Variability is common in natural populations for endpoints such as bioindicators in fish or invertebrates and other endpoints such as components of benthic community structure. With respect to MFO, the recognised and adopted practice for many years in monitoring programs is to compare animals of the same sex, within the same size range, taken at similar times of the year (preferably outside the spawning season) at putatively impacted and reference site(s) (Stagg and McIntosh, 1998).

Temporary induction of MFO enzymes for a few days may not be harmful, but prolonged or repeated induction has been associated with a variety of physiological and potential pathological conditions in fish and other animals. This includes decrease in steroid and vitamin levels and potential for reproductive effects, excess formation of free radicals and increased potential for mutagenesis and carcinogenesis (e.g. Stegeman and Hahn, 1993).

The term MFO is a generic expression and a functional definition is often applied, depending on the specific catalytic activity being assayed. One of the more convenient and sensitive assays uses 7-ethoxyresorufin as a substrate with the enzyme activity referred to as 7-ethoxyresorufin O-deethylase (EROD). EROD activity was measured in liver tissues of American plaice in this study.

2.1.2 Gross pathology

Gross pathology refers to the observation and quantification of the presence of visible diseases, lesions and other abnormalities. These external (ulcers of the fins and trunk, lymphocystis, epidermal papilloma, skeletal disorders) and internal (grossly visible organ lesions) pathologies are often natural but may be exacerbated by various stressors, including contaminants. Surveys of gross pathology in marine fish in the United States, the North Sea and coastal and offshore waters of the eastern North Atlantic, have disclosed higher disease prevalences in degraded environments (e.g. Murchelano, 1990). Recently, externally visible fish abnormalities have been recommended as bioindicators for biological monitoring programs at the national or international level by ICES (1999) and are commonly used in survey work by different European countries and in the USA and Canada.

2.1.3 Haematology

Haematological parameters such as change in various types of blood cells can provide important insight into potential effects on immune functions and resistance to disease. As such, haematology is being used more extensively in assessing the health of fish (e.g. Blaxhall, 1972). Numerous environmental pollutants have been shown to interact with components of the immune system (e.g. Dethloff et al., 1998; Khangarot et al., 1999) which can act as early warning bioindicators of the potential harm of environmental toxicants (Weeks et al., 1992).

2.1.4 Tissue Histopathology

Histopathology involves the analysis of tissues for the presence of cellular damage (i.e. lesions and tumours) which can indicate chronic, or long-term, exposure to pollutants. The value of histopathology lies in its ability to reflect the integrated effects of various contaminants over time and as such provides an indication of the potential for more serious health impairment in organisms. For the White Rose baseline survey, both liver and gill tissues were studied.

The liver plays a major role in metabolism, digestion, excretion, and the storage of various substances and can be a key indicator of chemical toxicity. Various field and laboratory studies have shown the relationship between chemical contamination and pathological lesions, such as neoplasia, hydropic vacuolation, megalocytic hepatosis, nuclear pleomorphism and macrophage aggregates in the liver of fish (e.g. Hinton et al., 1992; Myers at al., 1998). Moreover, decrease in prevalence of hepatic lesions has been reported to occur following reduction in input of chemical contaminants, such as PAHs (Baumann and Harshbarger, 1995; Moore et al., 1996). These relationships clearly indicate the utility of these lesions as bioindicators of contaminant-induced effects in marine organisms. Fish liver histopathology has been adopted as one of techniques to be used in biomonitoring programs designed to assess the environmental quality of waters in the North Sea area (Stagg, 1998), in the Pacific Coast (Myers et al., 1994; Stehr et al., 1998) and in the Northeast Coast (Johnson et al., 1993) of USA. Khan (2000) has also carried out some preliminary studies on the Grand Banks of Newfoundland.

Gills are important in respiration, acid-base balance, ionic regulation and excretion, and gill epithelia are quite sensitive to chemical damage (Haensly et al., 1982; Mallatt, 1985; Stoker et al., 1985; Evans, 1987). Several studies have been carried out on the effects of petroleum hydrocarbons showing that gills are damaged upon exposure to relatively low levels (Haensly et al., 1982; Solangi and Overstreet, 1982; Kiceniuk and Khan, 1987). Epithelial hyperplasia, fusion of gill lamellae, and separation of respiratory epithelium from underlying tissue were reported in two species of marine fish exposed to crude oil and its water soluble fractions (Solangi and Overstreet, 1982). A variety of other chemicals have also been shown to produce histological changes in gills of fish (e.g. Evans, 1987).

Confounding issues for some histopathologic bioindicators include distinguishing changes caused by contaminants from those due to infectious diseases and parasites. Generally, the causes of these lesions can be determined histologically by visualisation of the offending organism or the resultant inflammatory response. Seasonal and hormonal changes may also influence some histopathological biomarkers, however, the basic architectural pattern of the organs is not altered and detection of many important lesions is not compromised (e.g. Hinton et al., 1992).

In general, the greater the frequency of different lesions known to be associated with chemical toxicity at a study site, the stronger the case can be made for a direct chemical etiology. However histopathologic studies can also be equally valuable for identifying disease states in tissues which may have arisen from a contaminant mediated increase in disease susceptibility.

2.2 Selected Species

American plaice (*Hippoglossoides platessoides*) is a flatfish of the family Pleuronectidae and inhabits the continental shelves of northeastern North America and northwestern Europe (Scott and Scott, 1988). It is an important commercial species that was abundant before the fisheries moratorium on the northern half of the Grand Bank near Newfoundland (Morgan, 1992). The stock is presently showing signs of recovery. It is generally associated with depths from 80-250 m and cold water temperatures from 1.5 to – 1.8 C, prefers sand-mud bottoms and forages for a variety of invertebrates such as brittle stars, sand dollars and sea urchins, and small fish, mainly capelin, sand lance and mailed sculpin. American plaice are batch spawners (Walsh, 1994) and release batches of eggs every few days during the spawning period which extends on the Grand Bank from late April through early July (Maddock and Burton, 1999). Individuals can attain a maximum length of about 75 cm and age in excess of 25 years, with females growing faster than males (Pitt, 1975). Their long life span is of interest with regard to potential for cumulative effects of a chronic nature.

Studies have indicated that American plaice are relatively sedentary in comparison with other groundfish species. Pitt (1969) in a tagging study with 33 % total return for the Northeast Grand Bank reported that most tagged fish were recovered less than 30 miles from the tagging site seven or eight years after tagging. Morgan (1994) reported more movement of fish tagged on the top of the Bank in 3L but the return rates from this experiment were low (4.2%).

It is reasonable to suggest that American plaice are resident for periods of weeks to months in specific areas with any early warning indicators of health such as MFO enzyme induction and selected gill and liver lesions (but probably not carcinomas for instance) being causally linked to those areas. However, fish might move in and out of potential "contaminant" zones very close to study areas, such that exposure is insufficient to result in deleterious effects. Overall, the presence or absence of health effects in such a commercial species is important from a fisheries and general ecosystem perspective.

3.0 MATERIALS AND METHODS

3.1 Sampling

Fish Collection

The field sampling program was carried out on July 1, 2002 aboard the DFO research vessel Wilfred Templeman using an otter trawl. A total of 50 American plaice were collected by 7 trawl sets in the White Rose Reference Area (~ 100 km Northwest of the Development Area). Table 1 provides information on sampling sets.

Table 1: Information on Fish Sampling Sets

Trawl #	Set #	Sampling Date	Depth (m)	Temp. °C	Fish Sampled
WR-Ref-01	63	July 1, 2002	75	- 0.209	5
WR-Ref-02	64	July 1, 2002	111	- 1.126	4
WR-Ref-03	65	July 1, 2002	102	- 1.163	2
WR-Ref-04	66	July 1, 2002	98	- 1.182	4
WR-Ref-05	67	July 1, 2002	99	- 1.184	3
WR-Ref-06	68	July 1, 2002	115	- 1.335	13
WR-Ref-07	69	July 1, 2002	128	- 1.281	19

Tissue Sample Collection

Upon capture, fish were killed by severing the spinal cord, measured to the nearest centimetre for total length and weighed to the nearest gram. Each fish was assessed visually for any external parasites and/or abnormalities and then dissected. Sex and maturity stage were recorded according to procedures used by DFO (Appendix A). Liver and gonad were weighed. Tissues were processed as follows:

- **Blood** – Blood was drawn from a dorsal vessel near the tail and two blood smears were prepared for each fish according to standard haematological methods (Platt, 1969). Briefly, a tiny drop of blood was spread across a microscope slide to form a uniform thin film. Slides were dried in a chamber and fixed in methanol for assessment.

- **Liver** - The entire liver was excised and bisected. From the right half, a 4-5 mm thick slice was cut and placed in 10% buffered formalin for histological processing and the rest was frozen on dry ice until returning to Port when it was placed in a -80^{0}C freezer for MFO analysis.

- **Gill** - The first gill arch on the right hand side of the fish was removed and placed in 10% buffered formalin for histological processing.

- **Heart, spleen, gonad, and kidney** - Tissue samples of these organs were removed and placed in 10% buffered formalin for histological processing, if required.

- **Otoliths** - A pair of otoliths were removed for ageing.

Throughout the dissection process, any internal parasites and/or abnormal tissues were recorded and preserved in 10% buffered formalin for subsequent identification.

3.2 Sample Analysis

3.2.1 Mixed Function Oxygenase Assay

MFO induction was assessed in liver samples of American plaice as 7-ethoxyresorufin O-deethylase (EROD) activity according to the method of Pohl and Fouts (1980) as modified by Porter et al. (1989).

Sample preparation
Each sample was thawed on ice and homogenised in 4 volumes of 50 mM Tris-sucrose buffer, pH 7.5, (1 gram liver to 4 ml buffer) using at least ten passes of a glass Ten Broek hand homogeniser. The homogenate was centrifuged at 9 000 g for 15 minutes at 4 °C and the supernatant (S9) frozen in triplicate at -80 ^{0}C until assayed. All liver samples were held and processed under the same storage and assay conditions.

EROD assay
The reaction mixture, final volume 1.25 ml, contained 53 nmol Tris-sucrose buffer (50 mM, pH 7.5), 20 µl of S9 liver (diluted 5 times), 2.25 nmol 7-ER (150 µM ethoxyresorufin), and the reaction was started by the addition of 0.16 mg NADPH (1.25 mg/ml). After a 15-minute incubation at 27 ^{0}C in a temperature-controlled water bath, the reaction was terminated by the addition of 2.5 ml of ice-cold methanol. Methanol blanks contained the same components as the sample tubes with methanol being added prior to the addition of NADPH. Assay tubes were vortexed and the protein precipitate removed by centrifugation at 3 600 g for 5 minutes. The fluorescence of resorufin formed in the supernatant was measured in quartz cuvettes (1 cm pathlength) at 585 nm using an excitation wavelength of 550 nm (slit width of 0.5 mm). Enzyme activity was linear with time and protein concentration. Protein concentration of each S9 was determined using the Lowry protein method (Lowry et al., 1951). The rate of enzyme

activity in pmol/min/mg protein was obtained from the regression of fluorescence against standard concentrations of resorufin.

Enzyme activities in liver tissues of fish of the same gender from the two sites were compared by unpaired t-test or Mann-Whitney Rank Sum test when data were not normally distributed. Comparisons having a $p<0.05$ were considered to be statistically significant.

3.2.2 Haematology

Blood smears were stained with Giemsa stain and examined with a Wild Leitz Aristoplan bright field microscope for identifying different types of cells based on previous descriptions (Ellis, 1976). Because blood cells do not disperse randomly on a slide when a smear is made, the standard procedure EBM (Exaggerated Battlement Method) was performed to ensure that cells in one particular area (i.e. the middle or the edges of the slide) are not missed (Lynch et al., 1969).

Size, shape and degree of haemoglobinization of red blood cells were examined and recorded.

A blood cell differential count was performed on lymphocytes, neutrophils and thrombocytes and expressed as a percentage of each type of cells on 200 cells counted on each slide.

3.2.3 Tissue Histopathology

Both liver and gill samples were processed by standard histological methods (Lynch et al., 1969) using an Autotechnicon Tissue Processor. A graded ethyl alcohol series of 70%, 80%, 95%, and two changes of 100%, was used for dehydration of the samples. The organs were then cleared in three changes of chloroform. Finally, the tissues were impregnated with three changes of molten embedding media, Tissue Prep 2 ™. The processed tissues were embedded in steel molds using molten embedding media, and topped with labelled embedding rings. After cooling, the hardened blocks of embedded tissues were removed from their base molds. The blocks were then trimmed of excess wax. Sections were cut at 6µ on a Leitz microtome, floated on a 47 ^{0}C water bath containing gelatin, and then picked up on a labelled microscope slides. After air drying, the slides were fixed at 60 ^{0}C for approximately 2 hours to remove most of the embedding media and allow the sections to adhere properly to the slide. Sections were stained using Mayers Haematoxylin and Eosin method (Luna, 1968). Finally, after coverslips were applied using Entellan ®, the slides were left to air dry and harden overnight.

Liver

All liver samples were assessed microscopically for the presence of different lesions (e.g. Myers et al., 1987; 1991). Among them were:

1. Nonspecific necrosis
2. Nuclear pleomorphism
3. Megalocytic hepatosis
4. Eosinophilic foci
5. Basophilic foci
6. Clear cell foci
7. Hepatocellular carcinoma
8. Cholangioma
9. Cholangiofibrosis
10. Increase in mitotic activity
11. Macrophage aggregates
12. Hydropic vacuolation
13. Hepatocellular vacuolation

Lesions (except macrophage aggregates) were recorded for each fish as not detected (0) or detected (1). The percentage of fish affected by each type of lesions or prevalence of lesion was then calculated.

Macrophage aggregation was recorded on a relative scale from 0 to 7 and prevalence was calculated for fish showing a moderate to high aggregation (3 or higher on the scale). Prevalence of lesions were statistically analysed by the Fisher's exact test. Comparisons between sites having a $p<0.05$ were considered to be statistically significant.

Gill

Gill samples were examined microscopically under low power (63x) to get an overview of the entire section and record the presence of any abnormalities or parasites. Five randomly selected fields were then read for each sample at 250x magnification and examined as follows:

1. Total number of secondary lamellae were counted and recorded.

2. Each lamella was then examined quantitatively for 6 different stages:

- **Stage 1 – Thin lamellae**. Operationally defined here as secondary lamellae having a one-cell thick epithelial layer with the base between two secondary lamellae having a 3-5 cell thick epithelial layer.
- **Stage 2 - Distal Hyperplasia**. Thickening of the epithelium from the basal end and running almost the entire length of secondary lamellae (which may also appear misshapen).
- **Stage 3 - Epithelial lifting**. Separation of the epithelial layer from the basement membrane.
- **Stage 4 - Clubbing**. Swelling of the distal end of secondary lamellae which occurs in two different forms: **a)** ***tip hyperplasia*** - thickening of the epithelium at the very tip of lamellae giving the appearance of a club and **b)** ***telangiectasis*** - a swelling without rupture of the capillary at the distal end of lamellae (i.e. aneurism).

- **Stage 5 - Basal hyperplasia**. Thickening of the epithelium near the base of secondary lamellae where they meet the primary filament.
- **Stage 6 - Fusion**. Fusion of two or more lamellae.

It is important to note that the stages do not follow in any specific order. For example, a Stage 4 does not necessarily proceed to a Stage 5.

Results for each fish were expressed as the percentage of lamellae presenting the stage in relation to the total number of lamellae counted in the fields. Percentages were transformed using arcsin square root before analysis by the unpaired t-test (Sokal and Rohlf, 1981). Comparisons between sites having a $p<0.05$ were considered to be statistically significant.

3. The degree of oedema present, if any, was recorded on a 0-3 relative scale (0-absent, 1-light, 2-moderate and 3-heavy) and compared among sites with the Mann-Whitney Rank Sum test. Comparisons between sites having a $p<0.05$ were considered to be statistically significant.

4.0 RESULTS

Thirty three female and 17 male American plaice were sampled by 7 trawl sets in the White Rose Reference Area (July 1, 2002). The results obtained during this cruise were compared to those obtained with American plaice collected in the White Rose Development Area during the July 2000 cruise (OCEANS Ltd., 2001).

4.1 Physical Characteristics and Condition of Fish

Information on physical characteristics (sex, age, length, and weight) as well as condition of fish is valuable for interpreting results for different bioindicators under study. Fish condition can be defined as a state of physical fitness and assessed by calculating different condition indices (Dutil et al., 1995) such as (a) Condition Index expressed as Fulton's Condition Factor and defined as 100 x body weight/length^3 based on gutted weight (or somatic weight) as well as total weight (b) Hepato-Somatic Index calculated as 100 x liver weight/somatic weight and (c) Gonado-Somatic Index calculated as 100 x gonad weight/somatic weight.

Physical characteristics and condition of male and female American plaice (expressed as mean and standard deviation) from the Reference and Development areas are summarised in Tables 2a and 2b. The complete data set on fish from the 2002 survey is provided in Appendix B.

Table 2a: Physical Characteristics and Condition of Male American Plaice From the 2002 and 2000 White Rose Surveys

	Reference Area 2002	Development Area 2000	p Value [e]
Fish Number	17	6	
Total Body Weight (g)	195 ± 55	246 ± 89	0.118
Gutted Body Weight (g)	181 ± 53	NA [f]	
Length (cm)	29.4 ± 2.3	31.3 ± 3.9	0.159
Liver Weight (g)	2.06 ± 0.56	NA [f]	
Gonad Weight (g)	3.41 ± 2.32	NA [f]	
Age (year)	8.4 ± 1.2	7.5 ± 1.6	0.181
Condition Index (Gutted Weight) [a]	0.695 ± 0.041	NA [f]	
Condition Index (Total Weight) [b]	0.751 ± 0.041	0.762 ± 0.049	0.591
Hepato-Somatic Index [c]	1.178 ± 0.278	NA [f]	
Gonado-Somatic Index [d]	1.817 ± 0.782	NA [f]	

All data are expressed as mean ± standard deviation

[a] Fulton's condition factor: 100 x gutted body weight/length ^{3}

[b] Fulton's condition factor: 100 x total body weight/length ^{3}
[c] Calculated as 100 x liver weight/somatic weight
[d] Calculated as 100 x gonad weight/somatic weight
[e] p Value obtained with Unpaired t-test or Mann-Whitney Rank Sum test
[f] NA = data not available

Table 2b: Physical Characteristics and Condition of Female American Plaice From the 2002 and 2000 White Rose Surveys

	Reference Area 2002	Development Area 2000	p Value [e]
Fish Number	33	27	
Total Body Weight (g)	520 ± 170	363 ± 201	0.002 *
Gutted Body Weight (g)	470 ± 148	NA [f]	
Length (cm)	38.9 ± 3.9	34.3 ± 5.6	< 0.001 *
Liver Weight (g)	5.15 ± 1.58	NA [f]	
Gonad Weight (g)	54.3 ± 55.9	NA [f]	
Age (year)	10.6 ± 1.4	8.8 ± 1.5	< 0.001 *
Condition Index (Gutted Weight) [a]	0.779 ± 0.102	NA [f]	
Condition Index (Total Weight) [b]	0.859 ± 0.114	0.807 ± 0.077	0.047 *
Hepato-Somatic Index [c]	1.129 ± 0.272	NA [f]	
Gonado-Somatic Index [d]	10.530 ± 7.905	NA [f]	

All data are expressed as mean ± standard deviation
[a] Fulton's condition factor: 100 x gutted body weight/length ^{3}
[b] Fulton's condition factor: 100 x total body weight/length ^{3}
[c] Calculated as 100 x liver weight/somatic weight
[d] Calculated as 100 x gonad weight/somatic weight
[e] p Value obtained with Unpaired t-test or Mann-Whitney Rank Sum test
[f] NA = data not available
* Significantly different ($p<0.05$)

Site comparisons were carried out for each sex with the Unpaired t-test or the Mann-Whitney Rank Sum test when the groups were not normally distributed. There were no significant differences ($p \leq 0.05$) for males in either total weight, length, age or Condition Index (based on total weight) between the two areas.

With regard to female fish, total weight, length, age and Condition Index (based on total weight) were significantly different between sites. Fish from the Development Area were smaller and younger.

Information on fish maturity in the two areas is also provided in Table 3a and 3b for general interest. The complete data set on fish from the 2002 survey is provided in Appendix B.

Table 3a: Maturity Stages of Male American Plaice From the 2002 and 2000 White Rose Surveys

	N	Immature [a] (%)	Maturing to spawn [a] (%)	Partly spent [a] (%)	Spent [a] (%)
Reference Area 2002	17	5.8	47.1	47.1	0.0
Development Area 2000	6	33.3	16.7	16.7	33.3
p Value [b]		0.155	0.340	0.340	0.059

[a] Maturity stages were defined according to procedures used by DFO and expressed as percentages.
[b] p Value obtained with Fisher exact test

Table 3b: Maturity Stages of Female American Plaice From the 2002 and 2000 White Rose Surveys

	N	Immature [a] (%)	Maturing to spawn [a] (%)	Partly spent [a] (%)	Spent [a] (%)
Reference Area 2002	33	6.1	27.3	15.1	51.5
Development Area 2000	27	26.0	11.0	7.4	55.6
p Value [b]		0.065	0.195	0.442	0.799

[a] Maturity stages were defined according to procedures used by DFO and expressed as percentages.
[b] p Value obtained with Fisher exact test

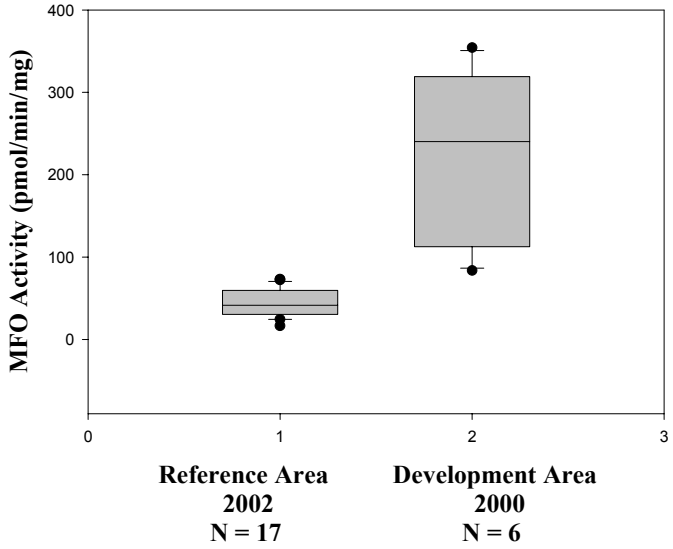
There were no significant differences in maturity stages of either male or female plaice from the Development and Reference areas (Fisher's exact test).

4.2 Mixed Function Oxygenase (MFO) Activity

Results on MFO enzyme activity in male and female fish from the Reference and Development areas are summarised in Figures 1 and 2 as vertical boxes representing the median, 10^{th}, 25^{th}, 75^{th} and 90^{th} percentiles with error bars for total numbers of fish. The complete data set on fish from the 2002 survey is provided in Appendix C.

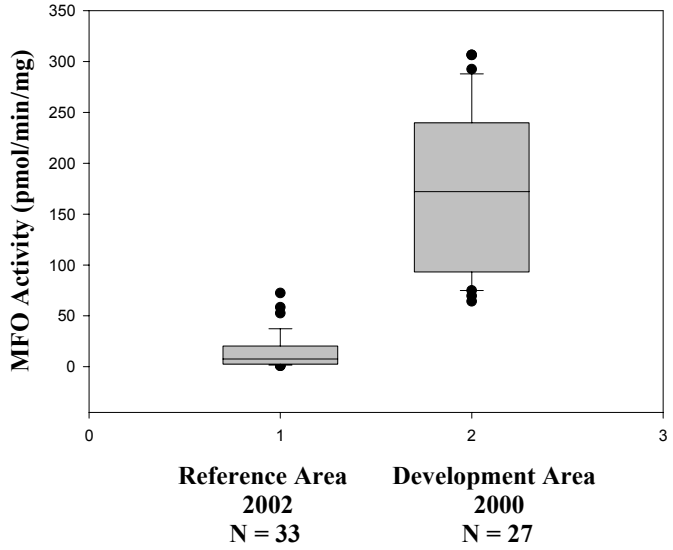
Enzyme activities in both male and female fish from the Development Area were higher than in fish from the Reference Area (Unpaired t-test or Mann-Whitney Rank Sum test; $p<0.001$).

Figure 1: Hepatic MFO Enzyme Activity in Male American Plaice From the 2002 and 2000 White Rose Surveys



Data plotted are median, 10th, 25th, 75th and 90th percentiles as vertical boxes with error bars

Figure 2: Hepatic MFO Enzyme Activity in Female American Plaice From the 2002 and 2000 White Rose Surveys



Data plotted are median, 10th, 25th, 75th and 90th percentiles as vertical boxes with error bars.

4.3 Gross Pathology

One fish from the Reference Area (2002 survey) exhibited a skin nodule. Four fish from the Development Area (2000 survey) showed gill achromasia (or white gill) and another had skin lesions on the ventral surface.

4.4 Haematology

Blood smears from the 50 fish from the Reference Area in the 2002 White Rose Survey were examined for various types of cells. The red blood cells of all fish appeared to be normal in size and shape. Coloration was also similar indicating a similar degree of haemoglobinisation.

A differential cell count of lymphocytes, neutrophils and thrombocytes was carried out. Two hundred cells were counted per fish and the results are expressed as mean percentage ± standard deviation of each cell type (Table 4).

Table 4: Blood Cell Differential Counts in American Plaice From the 2002 White Rose Survey

Blood cell type	Mean percentage ± standard deviation of each cell type
Lymphocytes	76.2 ± 5.0
Neutrophils	5.9 ± 2.4
Thrombocytes	17.9 ± 4.0

A detailed data set on the different cells examined is provided in Appendix D and a representative photograph of the different blood cells (Photo 1) is included in Appendix G.

4.5 Histopathology

4.5.1 Liver Histopathology

No external abnormalities were observed on the livers of fish from either area.

Results of the detailed histopathological studies carried out on liver tissues of American plaice from the Reference and Development areas are summarised in Table 5. The complete data set on fish from the 2002 survey is provided in Appendix E and representative photographs are included in Appendix G with Photo 2 representing a normal liver structure.

The fifty fish from the Reference Area (2002 survey) and 33 fish from the Development Area (2000 survey) did not present any cases of nuclear pleomorphism, eosinophilic foci, basophilic foci, clear cell foci, carcinoma, cholangioma, cholangiofibrosis, increase in mitotic activity or hydropic vacuolation.

Three cases of megalocytic hepatosis were noted from the Development Area.

The frequencies of macrophage aggregates in livers of fish from both areas were very low and only one case of moderate to high aggregation (3 or higher on a 0-7 relative scale) was observed in the Development Area.

Table 5: Number of American Plaice with Specific Types of Hepatic Lesions and Prevalence of Lesions in the 2002 and 2000 White Rose Surveys

	Reference Area - 2002 (N = 50)		Development Area - 2000 (N = 33)	
	Number of fish affected	Prevalence % [a]	Number of fish affected	Prevalence % [a]
Nuclear pleomorphism	0	0	0	0
Megalocytic hepatosis	0	0	3	9.4
Eosinophilic foci	0	0	0	0
Basophilic foci	0	0	0	0
Clear cell foci	0	0	0	0
Carcinoma	0	0	0	0
Cholangioma	0	0	0	0
Cholangiofibrosis	0	0	0	0
Increase in mitotic activity	0	0	0	0
Macrophage aggregation [b]	0	0	1	3.1
Hydropic vacuolation	0	0	0	0
Hepatocellular vacuolation	16	32.0	4	12.1
Parasitic infestation of biliary system	29	58.0	11	33.3

[a] Percentage of fish affected
[b] Moderate to high aggregation (3 or higher rating on a 0-7 relative scale)

Sixteen fish from the Reference Area and 4 fish from the Development Area showed a "patchy distribution" of hepatocellular vacuolation. This type of vacuolation distribution (Appendix G, Photo 3) is likely a reflection of gonadal maturational stage.

An infestation of the biliary system with a myxosporean parasite (Appendix G, Photo 4), possibly *Myxidium sp*., was observed in similar proportions of plaice from the Development and Reference areas.

There were no significant differences (Fisher exact test) in any of the liver lesions between the Reference and Development areas.

4.5.2 Gill Histopathology

Results of the detailed histopathological studies carried out on gill tissues of 50 fish from the White Rose Reference Area and 29 fish from the Development Area are summarised in Table 6. The complete data set on fish from the 2002 survey is provided in Appendix F and representative photographs are included in Appendix G.

Table 6: Occurrence of Different Stages and Oedema Condition in the Gill of American Plaice from the 2002 and 2000 White Rose Surveys

	Reference Area 2002 (50) [a]	Development Area 2000 (29) [a]	p Value [b]
Stage 1: Thin lamellae [c]	50.7 ± 12.3	42.9 ± 19.1	0.050 *
Stage 2: Distal hyperplasia [c]	43.4 ± 12.0	45.2 ± 17.3	0.714
Stage 3: Epithelial lifting [c]	0	0	1.000
Stage 4 a: Clubbing with tip hyperplasia [c]	5.9 ± 5.6	11.8 ± 9.1	0.001 *
Stage 4 b: Clubbing with telangiectasis [c]	0	0	1.000
Stage 5: Basal hyperplasia [c]	28.9 ± 19.4	55.9 ± 19.7	0.001 *
Stage 6: Fusion [c]	0	0.1 ± 0.4	1.000
Oedema condition [d]	1.45 ± 0.70	1.32 ± 0.56	0.723

[a] Total number of fish examined
[b] p Value obtained with the t-test analysis after arcsin square root transformation of percentages
[c] Mean ± standard deviation of percentages of lamellae presenting the stage
[d] Mean ± standard deviation of rating on a relative 0-3 scale
* Significantly different ($p<0.05$)

Epithelial layers of secondary lamellae may vary in thickness. All the fish studied displayed a variable percentage of thin secondary lamellae which is operationally defined here as secondary lamellae having a one cell thick epithelial layer with the base between two secondary lamellae having a 3-5 cell thick layer (Appendix G, Photo 5).

Distal hyperplasia (Appendix G, Photo 6), tip hyperplasia (Appendix G, Photo 7) or basal hyperplasia (Appendix G, Photo 8) of secondary lamellae were observed in most of gill samples, indicating general lamellar thickening putatively of a background nature. Fusion was seen in 2 fish from the Development Area. No cases of epithelial lifting and telangiectasis were observed in either area.

There were significant differences in the percentages of thin lamellae, tip hyperplasia and basal hyperplasia, putatively of a background nature, between fish from the two areas (t-test analysis after arcsin square root transformation of percentages).

The levels of oedema (rated on a 0-3 relative scale) observed were quite low and no significant difference was observed between the Development and Reference areas.

5.0 DISCUSSION

Cellular and sub-cellular measures along with observations on visible lesions on skin and internal organs are valuable monitoring tools for identifying adverse health conditions in animals in advance of population level responses. As such they can provide early warning of potential health effects and aid in identifying their nature and cause (see reviews by Payne et al., 1987; Peakall, 1992; Society of Environmental Toxicology and Chemistry (SETAC) Special Publication Series 1992). They can also be valuable tools for "disproving" as well as "proving" concerns about potential deleterious effects.

Activity of mixed-function oxygenase (MFO) enzymes were determined in liver tissues of male and female American plaice taken in the White Rose Reference Area (100 km Northwest of the Development Area in 2002). Blood smears were examined for changes in red blood cell morphology, staining characteristics and changes in different types of white blood cells. Pathological studies were also carried out and included observations on gross pathology of fish as well as detailed histopathological studies on liver and gill tissues. Histopathological studies included observations on 13 different indices in liver and 7 in gills. A comparison was carried out between fish sampled in the Reference Area in 2002 and fish obtained in the Development Area in the 2000 survey (OCEANS Ltd., 2001).

Induction of MFO enzymes is known to be a sensitive response to selected organic contaminants of environmental concern including petroleum hydrocarbons (e.g. Payne et al., 1987) while haematological changes can provide insight into potential contaminant mediated effects on immune function and resistance to disease (e.g. Weeks et al., 1992). Pathology can provide information on the potentially injurious and cumulative effects of various contaminants, e.g. petroleum hydrocarbons, metals, etc. (e.g. Evans, 1987; Hinton et al., 1992).

Fish Physical Characteristics and Condition Indices

Information on fish physical characteristics and condition indices is valuable for interpreting results of bioindicator studies (Levine et al., 1995). Although a limited number of fish are being analysed from a population perspective (Dutil et al. 1995), such data can also provide a level of information for assessing major effects on animal condition.

Total weight, length, age and Condition Index (based on total weight) were similar in male fish from the Reference and Development areas, whereas they were lower in females from the Development Area. However, the female fish from the Development Area were younger and the differences are likely a reflection of natural spatial variations in populations and feeding.

Mixed Function Oxygenase

A higher level of MFO enzyme activity was found in both male and female plaice from the Development Area. Induction of MFO in fish has been established as a sensitive biological indicator of hydrocarbon pollution of aquatic biotopes (Payne et al., 1987). The higher enzyme levels noted here could be due to exposure of fish to contaminants related to the exploratory drilling activity. However, fish from the Development Area were sampled in 2000, whereas fish from the Reference area were captured in 2002 and caution is warranted in making comparisons between Reference and test fish sampled in different years for such a sensitive response as MFO enzyme induction.

It could also be suggested that the site differences in level of enzyme activity noted in females might be related to size and age differences between the two sites (Whyte et al., 2000). We compared data obtained on female American plaice from surveys carried out on the Grand Banks over the past few years but did not find any significant differences in MFO enzyme activity in relation to fish length.

Haematology

The percentages of lymphocytes, neutrophils and thrombocytes in fish from the Reference Area (no data being available from the Development Area) were in the range of those obtained for American plaice from other surveys carried out in the Grand Banks. The morphology and staining characteristics of red blood cells appeared normal.

Pathology

Gross anatomy was assessed visually in all fish during the necropsies for any external or internal abnormalities or parasites. Two fish, one from the Development Area and one from the Reference Area, showed skin lesions. Four fish from the Development Area displayed gill achromasia (pale gill filaments) and X-cell lesions. A low prevalence of this lesion has been reported in a few other species such as cod (Mellergaard and Lang, 1999) and eelpouts (Desser and Khan, 1982). The aetiology of X-cells is unknown and there is debate on whether they are host cells such as protozoa (Alpers et al., 1977) or cells which have undergone transformation due to pollution or viral infection (Lange and Johannessen, 1977; Peters et al., 1978).

With respect to liver histopathology, the observation of three cases of liver megalocytic hepatosis in fish from the Development Area is of interest since this type of lesion may represent early signs of stress (Myers et al., 1987). A variety of other lesions (nuclear pleomorphism, eosinophilic, basophilic and clear cell foci, carcinoma, cholangioma, cholangiofibrosis, increase in mitotic activity and hydropic vacuolation) associated with chemical toxicity in field and laboratory studies were not detected or were found at a very low frequency in either area. The presence of a myxosporean parasite in the liver (biliary

system) of a relatively large proportion of fish from both areas did not appear to result in other pathological changes in hepatic tissues. The "patchy distribution" of hepatocellular vacuolation observed in fish from both areas was not associated with degenerative changes. Such hepatocellular vacuolation is likely linked to gonadal maturation stage (Timasho, 1981; Bodammer and Murchelano, 1990; Couillard et al., 1997).

Regarding studies on gill microstructures, fish from the Development Area displayed more gill tip hyperplasia and basal hyperplasia than plaice from the Reference Area. However, the hyperplasia was mild in nature and small changes in hyperplasia are likely a normal occurrence in populations. Microstructural changes which could be more pathological in nature such as gill oedema, telangiectasis and lamellar fusion were absent or found at similarly low frequencies between the two areas.

Conclusions

The results obtained on external and internal abnormalities, MFO enzymes in liver, haematology, and liver and gill histopathology provide baseline information on selected health indicators in American plaice in advance of development at the White Rose site. Results of the various bioindicator studies indicate that the present health status of American plaice collected at the White Rose site is similar to that at more distant sites. However, increased MFO enzyme levels and three cases of megalocytic hepatosis were noted in fish from the Development Area. These bioindicator differences might be due to natural variation, but they are also known to be sensitive early responses to some contaminants.

Overall, a reasonable level of bioindicator baseline data is now available for comparison with any future studies on fish health once development commences.

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APPENDIX A

Description of Groundfish Maturity Stages Used by DFO in the Newfoundland Region

Description of Groundfish Maturity Stages Used by DFO in the Newfoundland Region

Male	Female
Immature (100): Testes narrow and translucent, vasa deferentia very narrow and thin walled.	**Immature (500):** Ovary small, grey to pink in colour; membrane thin and translucent; eggs not visible to the naked eye.
Spent L (110): Vasa deferentia wide and opaque, sometimes with residual milt from spawning in previous year; outer edges of testes not pinkish or greyish as in maturing fish; spent in previous (L-last) year.	**Spent L (510):** Ovary thick-walled with no new eggs visible to the naked eye; spent in the previous (L-last) year. **Mat A-P (520):** Eggs visible to naked eye in ovary itself; all eggs opaque; maturing to spawn in present year. **Mat B-P (530):** Opaque and clear eggs present with less than 50% of the volume being clear eggs; maturing to spawn in the present (P) year.
Mat P (140): Testes relatively thick compared with immature, with outer edges pink or grey in early stage and white in later stage; early in the year some testes may show evidence of spawning in a previous year but the edges of the testes indicate recovery; maturing to spawn in present (P) year, i.e. year of capture.	**Mat C-P (540):** 50% or more of the volume are clear eggs; this stage also includes the ripe condition where the ovarian content is almost liquid with clear eggs; to spawn or spawning in the present (P) year.
Partly Spent (150): Some milt extruded in present year, but residual milt in testes and vasa deferentia.	**Partly Spent P (550):** Ovary not full as in Mat C-P; some eggs extruded but many clear eggs remaining.
Spent P (160): Spawning completed in present year; recovery not sufficiently advanced for outer edges of testes to be pinkish or greyish in colour.	**Spent P (560):** Spawning completed in present year but possibly a few clear eggs remaining; no new opaque eggs visible to the naked eye.
Spent P Mat N (170): Spawning completed in present year; outer edges of testes pink or grey or even becoming white in preparation for spawning in the next (N) year; this stage becomes Mat P in January of the next year.	**Spent P Mat N (570):** Spawning completed in present year, and new opaque eggs, for spawning in the next (N) year, visible to the naked eye; this stage becomes Mat A-P in January of the next year.
Mat N (180 or 190): Testes developing from immature stage for spawning in the next (N) year; testes becoming thick, being pink or grey early in this stage and gradually whitening; this stage becomes Mat P in January of the next year.	**Mat A-N (580):** No evidence of previous spawning; but new opaque eggs, for spawning in the next (N) year, visible to the naked eye; this stage becomes Mat A-P in January of the next year.

APPENDIX B

Necropsy Data of American Plaice
from the 2002 White Rose Survey

Necropsy Data of American Plaice from the 2002 White Rose Survey

Fish #	Trawl #	Sex - Mat [a]	Length (cm)	Total Weight (g)	Gutted Weight (g)	Liver Weight (g)	Gonad Weight (g)	Observations	Age (year)	CF t [b]	CF g [c]	HSI [d]	GSI [e]
239	WR-R01	F-560	35	320	288	4	12		10	0.746	0.672	1.389	4.167
240	WR-R01	F-560	37	468	416	6	16		9	0.924	0.821	1.442	3.846
241	WR-R01	M-140	28	166	152	2	4		7	0.756	0.692	1.316	2.632
242	WR-R01	M-140	28	166	140	2	2		9	0.756	0.638	1.429	1.429
243	WR-R01	M-140	27	146	136	2	2		10	0.742	0.691	1.471	1.471
244	WR-R02	F-560	42	600	526	6	28		10	0.810	0.710	1.141	5.323
245	WR-R02	F-540	39	586	454	4	102		10	0.988	0.765	0.881	22.467
246	WR-R02	M-140	28	146	140	2	2		8	0.665	0.638	1.429	1.429
247	WR-R02	M-140	26	132	126	1	2		7	0.751	0.717	0.794	1.587
248	WR-R03	F-560	39	458	386	4	24		12	0.772	0.651	1.036	6.218
249	WR-R03	M-140	32	222	200	2	2		9	0.677	0.610	1.000	1.000
250	WR-R04	F-520	46	1020	840	8	270		15	1.048	0.863	0.952	32.143
251	WR-R04	F-560	42	710	614	6	36	Skin nodule	12	0.958	0.829	0.977	5.863
252	WR-R04	M-140	35	352	330	2	10		9	0.821	0.770	0.606	3.030
253	WR-R04	M-140	29	180	164	2	2		8	0.738	0.672	1.220	1.220
254	WR-R05	F-560	37	490	452	4	98		10	0.967	0.892	0.885	21.681
255	WR-R05	F-560	40	618	576	6	78		10	0.966	0.900	1.042	13.542
256	WR-R05	M-150	32	254	242	2	8		9	0.775	0.739	0.826	3.306
257	WR-R06	F-550	44	756	678	4	134		12	0.887	0.796	0.590	19.764
258	WR-R06	F-560	42	612	560	8	18		11	0.826	0.756	1.429	3.214
259	WR-R06	F-550	33	314	286	4	36		9	0.874	0.796	1.399	12.587
260	WR-R06	F-560	35	368	342	6	12		10	0.858	0.798	1.754	3.509
261	WR-R06	F-560	38	454	420	6	12		9	0.827	0.765	1.429	2.857
262	WR-R06	F-550	35	364	330	4	48		10	0.849	0.770	1.212	14.545
263	WR-R06	F-520	39	588	546	8	72		12	0.991	0.920	1.465	13.187
264	WR-R06	F-530	39	602	550	6	120		9	1.015	0.927	1.091	21.818
265	WR-R06	F-550	44	722	710	6	104		14	0.848	0.833	0.845	14.648
266	WR-R06	F-530	32	268	252	2	44		10	0.818	0.769	0.794	17.460
267	WR-R06	M-150	29	174	162	2	4		7	0.713	0.664	1.235	2.469
268	WR-R06	F-540	36	440	414	4	76		10	0.943	0.887	0.966	18.357
269	WR-R06	M-150	29	188	172	2	4		8	0.771	0.705	1.163	2.326
270	WR-R07	F-500	46	390	360	4	12		11	0.401	0.370	1.111	3.333
271	WR-R07	F-560	39	466	430	4	18		10	0.786	0.725	0.930	4.186
272	WR-R07	F-560	43	714	602	6	28		12	0.898	0.757	0.997	4.651
273	WR-R07	F-520	39	532	504	4	60		9	0.897	0.850	0.794	11.905
274	WR-R07	F-560	42	594	556	6	20		11	0.802	0.750	1.079	3.597
275	WR-R07	F-550	45	816	766	8	154		12	0.895	0.841	1.044	20.104
276	WR-R07	F-560	39	462	428	4	14		11	0.779	0.722	0.935	3.271
277	WR-R07	F-560	42	570	510	8	24		12	0.769	0.688	1.569	4.706
278	WR-R07	F-560	39	498	464	4	14		10	0.840	0.782	0.862	3.017
279	WR-R07	F-530	35	364	338	4	34		9	0.849	0.788	1.183	10.059
280	WR-R07	M-150	29	184	172	2	2		8	0.754	0.705	1.163	1.163

Fish #	Trawl #	Sex - Mat [a]	Length (cm)	Total Weight (g)	Gutted Weight (g)	Liver Weight (g)	Gonad Weight (g)	Observations	Age (year)	CF t [b]	CF g [c]	HSI [d]	GSI [e]
281	WR-R07	F-500	33	280	260	4	4		10	0.779	0.723	1.538	1.538
282	WR-R07	F-520	35	388	366	4	60		10	0.905	0.854	1.093	16.393
283	WR-R07	F-560	34	332	284	4	10		10	0.845	0.723	1.408	3.521
284	WR-R07	M-150	31	222	210	2	2		9	0.745	0.705	0.952	0.952
285	WR-R07	M-150	32	260	246	4	4		9	0.793	0.751	1.626	1.626
286	WR-R07	M-150	27	148	138	2	4		7	0.752	0.701	1.449	2.899
287	WR-R07	M-100	30	220	194	2	2		7	0.815	0.719	1.031	1.031
288	WR-R07	M-150	28	162	152	2	2		11	0.738	0.692	1.316	1.316

[a] Sex (F = Female and M = Male) and maturity stages expressed as a number (see Appendix A for description of the different stages)

[b] Fulton's condition factor expressed as 100 x total body weight/length^3

[c] Fulton's condition factor expressed as 100 x gutted body weight/length^3

[d] Hepato-somatic index expressed as 100 x liver weight/gutted weight (somatic weight)

[e] Gonado-somatic index expressed as 100 x gonad weight/gutted weight (somatic weight)

APPENDIX C

Hepatic MFO Activity in Male and Female American Plaice from the 2002 White Rose Survey

Hepatic MFO Activity in Male and Female American Plaice from the 2002 White Rose Survey

Male	
Fish #	**MFO * pmol/min/mg**
241	72.35
242	63.02
243	24.60
246	40.53
247	48.01
249	24.47
252	16.62
253	59.09
256	37.19
267	26.98
269	51.41
280	33.01
284	41.57
285	31.66
286	57.33
287	60.86
288	73.17

Female	
Fish #	**MFO * pmol/min/mg**
239	22.97
240	58.30
244	2.56
245	0.84
248	33.55
250	0.78
251	1.96
254	2.12
255	1.65
257	7.27
258	23.66
259	9.35
260	12.94
261	19.39
262	7.57
263	2.44
264	4.19
265	1.31
266	1.71
268	6.60
270	17.47
271	23.31
272	25.34
273	3.61
274	16.62
275	5.05
276	6.83
277	11.09
278	12.12
279	16.14
281	72.36
282	4.39
283	52.61

* Mixed Function Oxygenase (MFO) enzyme activity measured as 7-ethoxyresorufin O-deethylase (EROD)

APPENDIX D

Haematological Results of American Plaice
from the 2002 White Rose Survey

Haematological Results of American Plaice from the 2002 White Rose Survey

Fish #	Lymphocytes % *	Neutrophils % *	Thrombocytes % *	Red Blood Cells Appearance
239	81	4	15	Normal
240	71	8	21	Normal
241	75	5	20	Normal
242	81	3	16	Normal
243	69	8	23	Normal
244	73	8	19	Normal
245	79	9	12	Normal
246	71	10	19	Normal
247	79	9	12	Normal
248	80	5	15	Normal
249	78	6	16	Normal
250	81	3	16	Normal
251	77	6	17	Normal
252	69	8	23	Normal
253	79	9	12	Normal
254	80	5	15	Normal
255	78	6	16	Normal
256	73	8	19	Normal
257	72	5	23	Normal
258	81	3	16	Normal
259	76	8	16	Normal
260	70	5	25	Normal
261	71	8	21	Normal
262	86	3	11	Normal
263	73	8	19	Normal
264	78	7	15	Normal
265	73	9	18	Normal
266	80	4	16	Normal
267	77	6	17	Normal
268	70	5	25	Normal
269	72	5	23	Normal
270	77	3	20	Normal
271	79	4	17	Normal

Fish #	Lymphocytes % *	Neutrophils % *	Thrombocytes % *	Red Blood Cells Appearance
272	81	3	16	Normal
273	76	4	20	Normal
274	84	3	13	Normal
275	71	11	18	Normal
276	69	9	22	Normal
277	89	3	8	Normal
278	73	3	24	Normal
279	86	3	11	Normal
280	70	7	23	Normal
281	80	4	16	Normal
282	76	4	20	Normal
283	71	10	19	Normal
284	73	8	19	Normal
285	68	6	26	Normal
286	77	6	17	Normal
287	81	3	16	Normal
288	77	4	19	Normal

* Expressed as a percentage of each type of cells identified on two hundred cells counted.

APPENDIX E

Liver Histopathological Results of American Plaice
from the 2002 White Rose Survey

Liver Histopathological Results of American Plaice from the 2002 White Rose Survey

Fish #	NP [a]	MH [b]	MA [c]	HV [d]	Parasites [e]
239	0	0	0	0	0
240	0	0	0	1	1
241	0	0	0	0	1
242	0	0	0	0	0
243	0	0	0	0	1
244	0	0	0	1	1
245	0	0	0	1	1
246	0	0	0	0	1
247	0	0	0	0	0
248	0	0	0	0	1
249	0	0	0	0	1
250	0	0	0	1	1
251	0	0	0	1	1
252	0	0	0	0	1
253	0	0	0	0	1
254	0	0	0	0	1
255	0	0	1	1	1
256	0	0	0	0	0
257	0	0	1	0	0
258	0	0	0	0	1
259	0	0	0	0	0
260	0	0	0	1	0
261	0	0	0	0	1
262	0	0	0	0	0
263	0	0	0	0	0
264	0	0	0	1	1
265	0	0	0	1	1
266	0	0	0	1	0
267	0	0	1	0	0
268	0	0	0	1	1
269	0	0	0	0	0
270	0	0	0	0	0
271	0	0	0	1	1
272	0	0	0	0	0
273	0	0	0	0	0

Fish #	NP [a]	MH [b]	MA [c]	HV [d]	Parasites [e]
274	0	0	1	0	0
275	0	0	0	1	1
276	0	0	0	1	1
277	0	0	0	0	1
278	0	0	0	1	1
279	0	0	0	1	1
280	1	0	0	0	0
281	0	0	0	0	1
282	0	0	0	0	1
283	0	0	0	0	0
284	0	0	0	0	0
285	0	0	0	0	0
286	0	0	0	0	0
287	0	0	0	0	1
288	0	0	1	0	1

[a] **NP** = Nuclear pleomorphism: 0 (not detected) or 1 (detected)
[b] **MH** = Megalocytic hepatosis: 0 (not detected) or 1 (detected)
[c] **MA** = Macrophage aggregation: Rating on a 1-7 relative scale.
[d] **HV** = Hepatocellular vacuolation: 0 (homogeneous distribution) or 1 ("patchy" distribution)
[e] **Parasites** = Parasitic infestation of biliary system: 0 (not detected) or 1 (detected)

APPENDIX F

Gill Histopathological Results of American Plaice
from the 2002 White Rose Survey

Gill Histopathological Results of American Plaice from the 2002 White Rose Survey

Fish #	Stage 1 [a] %	Stage 2 [a] %	Stage 3 [a] %	Stage 4 a [a] %	Stage 4 b [a] %	Stage 5 [a] %	Stage 6 [a] %	Oedema [b]
239	52.3	39.3	0.0	8.4	0.0	49.5	0.0	0.8
240	31.2	57.0	0.0	11.8	0.0	19.4	0.0	0.8
241	64.3	35.7	0.0	0.0	0.0	25.4	0.0	1.0
242	43.9	53.2	0.0	2.9	0.0	35.3	0.0	1.0
243	58.5	36.6	0.0	4.9	0.0	13.8	0.0	1.4
244	41.5	58.5	0.0	0.0	0.0	48.1	0.0	2.0
245	50.7	38.8	0.0	10.4	0.0	32.8	0.0	2.0
246	40.5	59.5	0.0	0.0	0.0	44.3	0.0	1.4
247	42.0	33.3	0.0	24.6	0.0	31.2	0.0	0.4
248	39.8	53.1	0.0	7.1	0.0	32.7	0.0	2.4
249	52.6	42.2	0.0	5.2	0.0	11.2	0.0	1.0
250	66.0	29.1	0.0	4.9	0.0	32.0	0.0	0.8
251	42.2	51.0	0.0	6.9	0.0	30.4	0.0	2.2
252	48.6	39.1	0.0	12.3	0.0	17.4	0.0	1.0
253	61.7	38.3	0.0	0.0	0.0	17.4	0.0	1.4
254	55.2	37.1	0.0	7.8	0.0	17.2	0.0	1.4
255	38.9	60.2	0.0	0.9	0.0	41.7	0.0	1.0
256	62.8	37.2	0.0	0.0	0.0	34.5	0.0	3.0
257	43.3	51.5	0.0	5.2	0.0	25.8	0.0	2.8
258	44.2	49.2	0.0	6.7	0.0	21.7	0.0	2.2
259	51.5	43.1	0.0	5.4	0.0	21.5	0.0	1.0
260	48.8	51.2	0.0	0.0	0.0	34.7	0.0	0.6
261	49.4	38.8	0.0	12.1	0.0	47.4	0.0	0.8
262	66.9	33.1	0.0	0.0	0.0	1.5	0.0	0.8
263	50.0	37.4	0.0	13.0	0.0	16.5	0.0	1.2
264	54.8	39.3	0.0	5.9	0.0	32.6	0.0	1.0
265	42.0	45.8	0.0	12.2	0.0	0.0	0.0	1.0
266	82.5	17.5	0.0	0.0	0.0	12.4	0.0	1.0
267	68.2	25.3	0.0	6.5	0.0	20.8	0.0	1.0
268	57.2	41.3	0.0	1.5	0.0	0.0	0.0	1.0
269	65.9	34.1	0.0	0.0	0.0	0.0	0.0	1.0
270	46.4	38.2	0.0	15.5	0.0	69.1	0.0	2.4
271	38.6	51.8	0.0	9.6	0.0	72	0.0	0.4

Fish #	Stage 1 [a] %	Stage 2 [a] %	Stage 3 [a] %	Stage 4 a [a] %	Stage 4 b [a] %	Stage 5 [a] %	Stage 6 [a] %	Oedema [b]
272	69.8	30.2	0.0	0	0.0	52.8	0.0	1.4
273	68.9	21.8	0.0	9.2	0.0	9.2	0.0	1.0
274	54.8	31.9	0.0	13.3	0.0	84.4	0.0	1.4
275	42.7	54.5	0.0	2.7	0.0	51.8	0.0	2.4
276	32.5	65.9	0.0	1.6	0.0	46.3	0.0	2.4
277	57.4	33.3	0.0	9.3	0.0	37	0.0	1.0
278	37.2	62.8	0.0	0	0.0	12.4	0.0	0.8
279	43.4	47.8	0.0	8.8	0.0	0	0.0	1.2
280	51.9	46.5	0.0	1.6	0.0	8.5	0.0	0.8
281	38.9	55.6	0.0	5.6	0.0	14.3	0.0	1.8
282	48.1	38.3	0.0	13.5	0.0	52.6	0.0	1.6
283	38.3	46.7	0.0	15	0.0	48.6	0.0	1.4
284	44.3	46.4	0.0	9.3	0.0	35.7	0.0	1.8
285	16.5	81.1	0.0	2.4	0.0	37	0.0	3.0
286	69.4	30.6	0.0	0	0.0	15	0.0	2.4
287	58.5	41.5	0.0	0	0.0	12.7	0.0	2.6
288	60.5	39.5	0.0	0	0.0	16.3	0.0	2.2

[a] Percentage of lamellae presenting the stage in relation to the total number of lamellae counted in five random fields per fish (**Stage 1**: Thin lamellae; **Stage 2**: Distal hyperplasia; **Stage 3**: Epithelial lifting; **Stage 4**: Clubbing **a**: tip hyperplasia and **b**: telangiectasis; **Stage 5**: Basal hyperplasia; **Stage 6**: Fusion).
[b] Mean of rating on a relative 0-3 scale in five randomly selected fields.

APPENDIX G

Representative Histological Photographs
of American Plaice from the 2002 White Rose Survey.

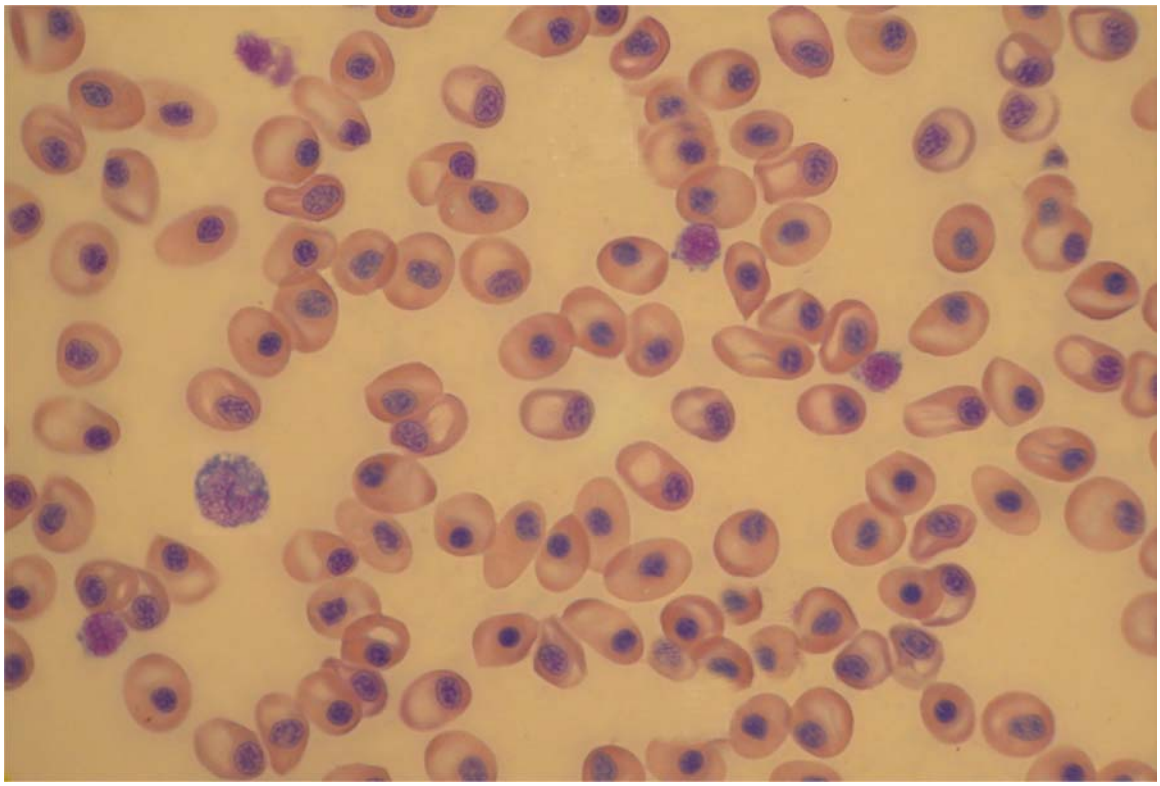

Photo 1: Blood smear of American plaice showing different types of cells (Giemsa x1000).

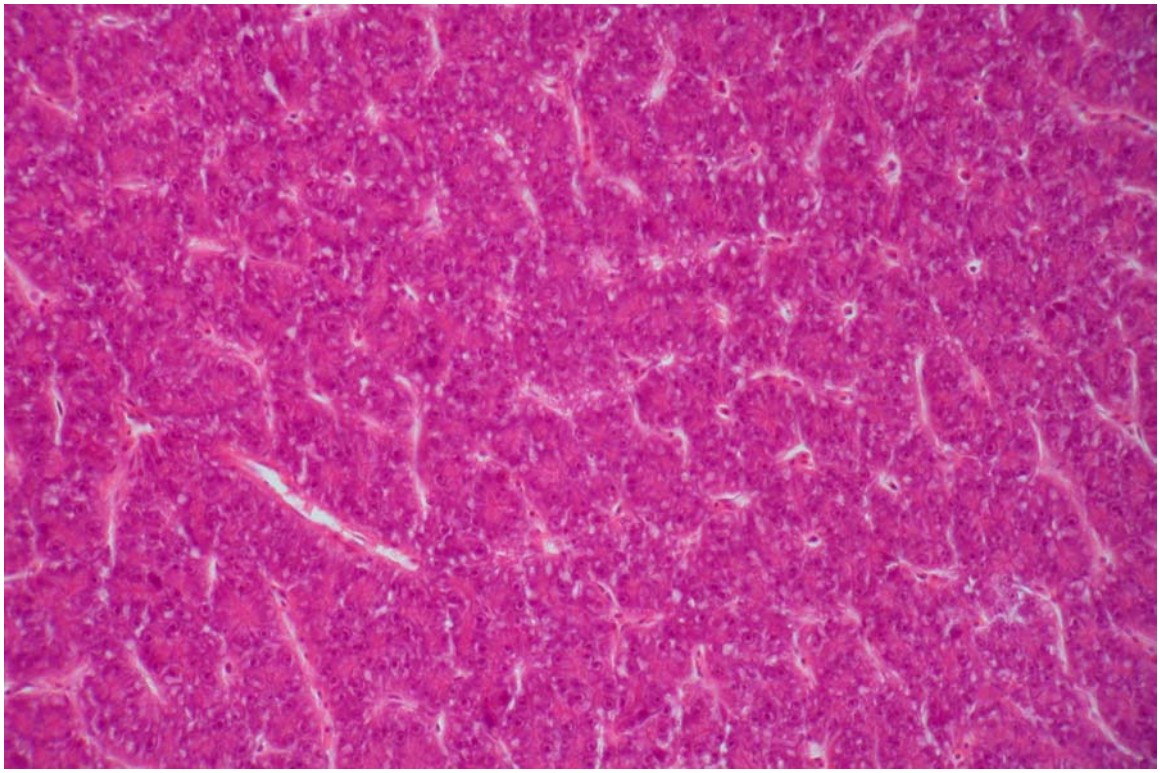

Photo 2: Normal structure of liver of American plaice (H & E x63).

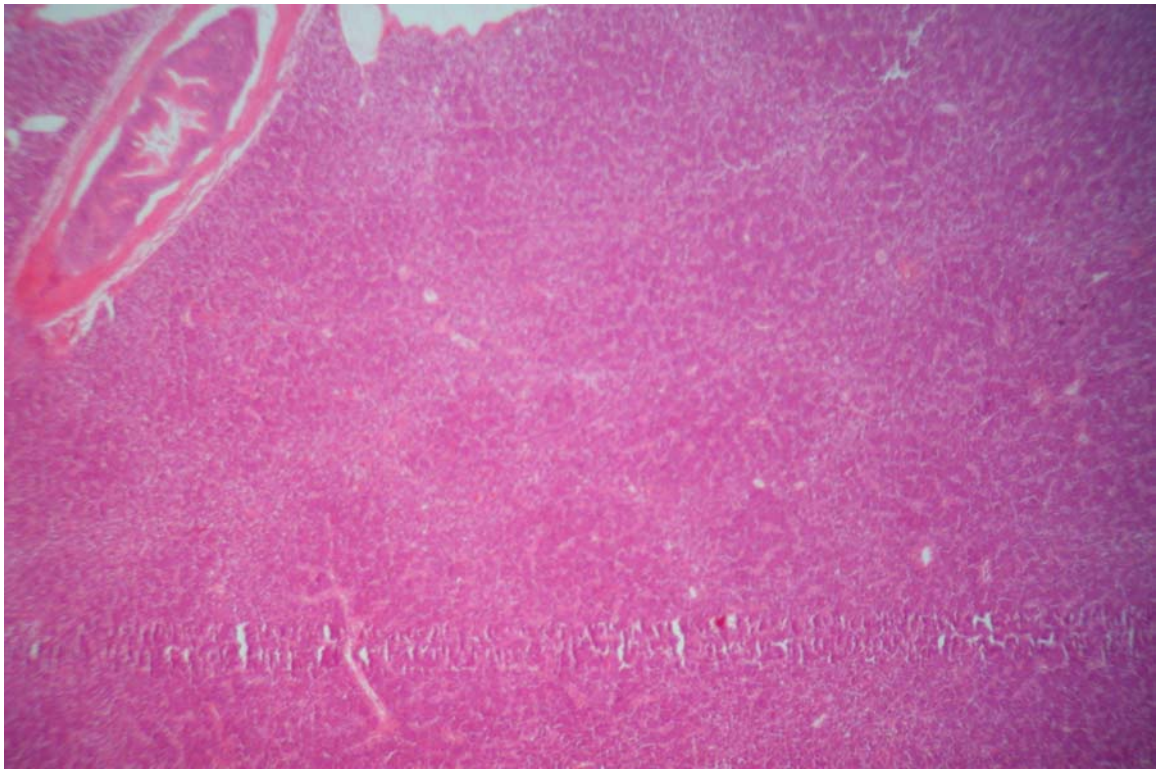

Photo 3: "Patchy" hepatocellular vacuolation in liver of American plaice (H & E x63).

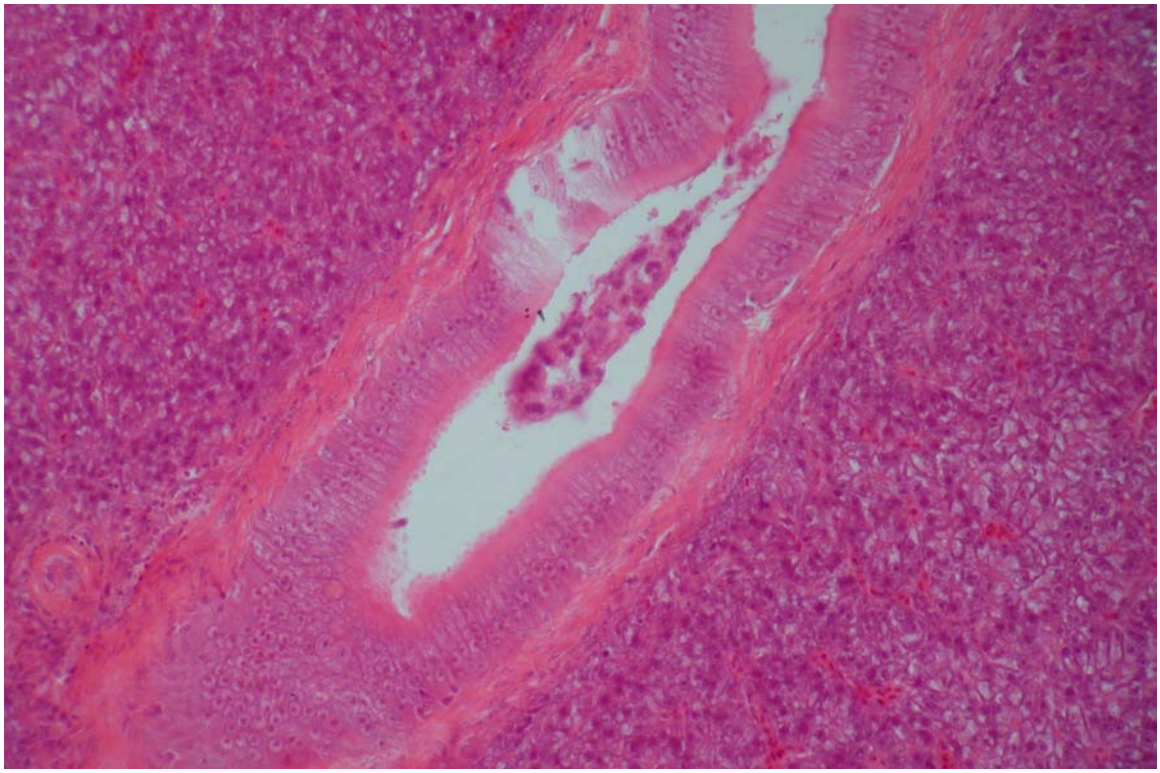

Photo 4: Parasitic infestation in biliary system of American plaice (H & E x250).

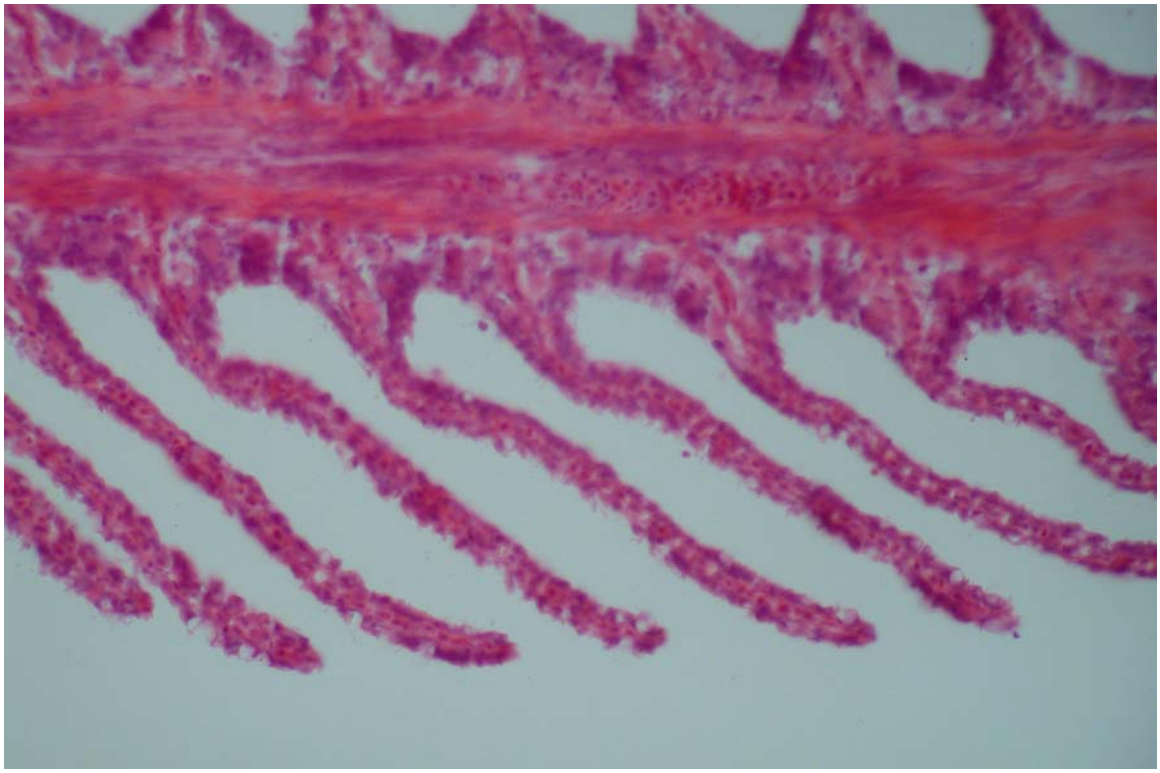

Photo 5: Thin secondary lamellae in gill of American plaice (H & E x250).

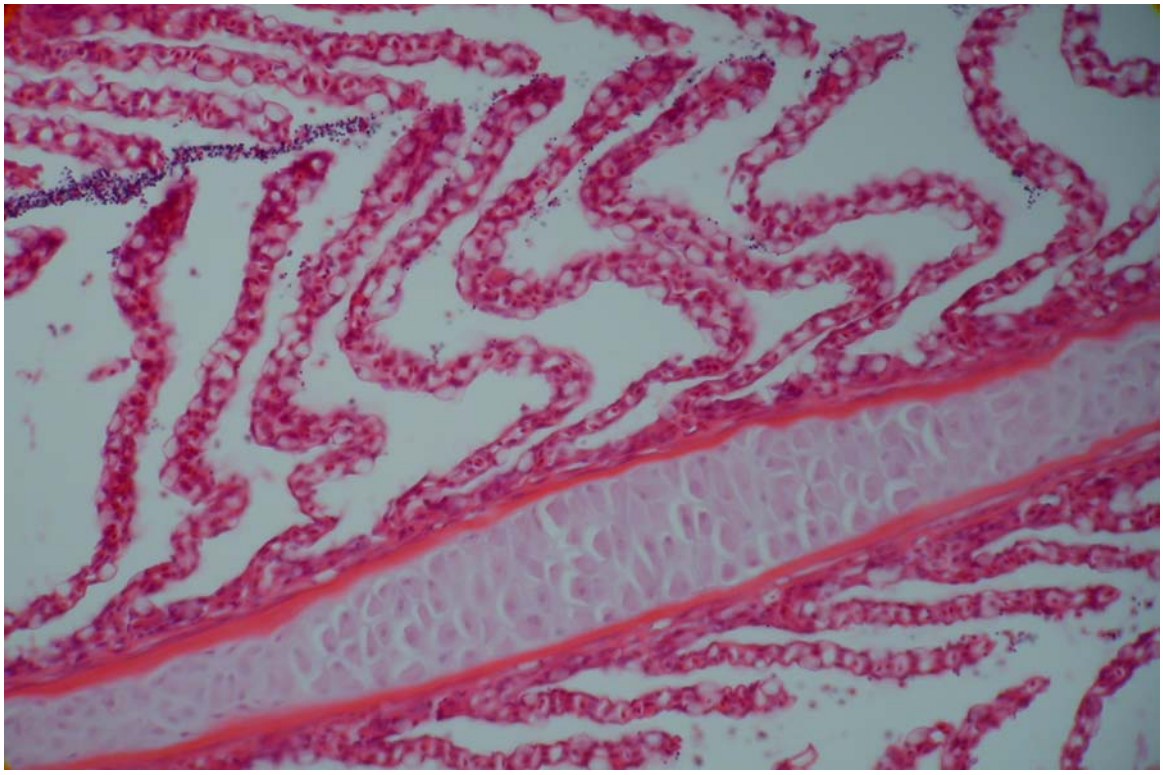

Photo 6: Distal hyperplasia in gill of American plaice (H & E x250).

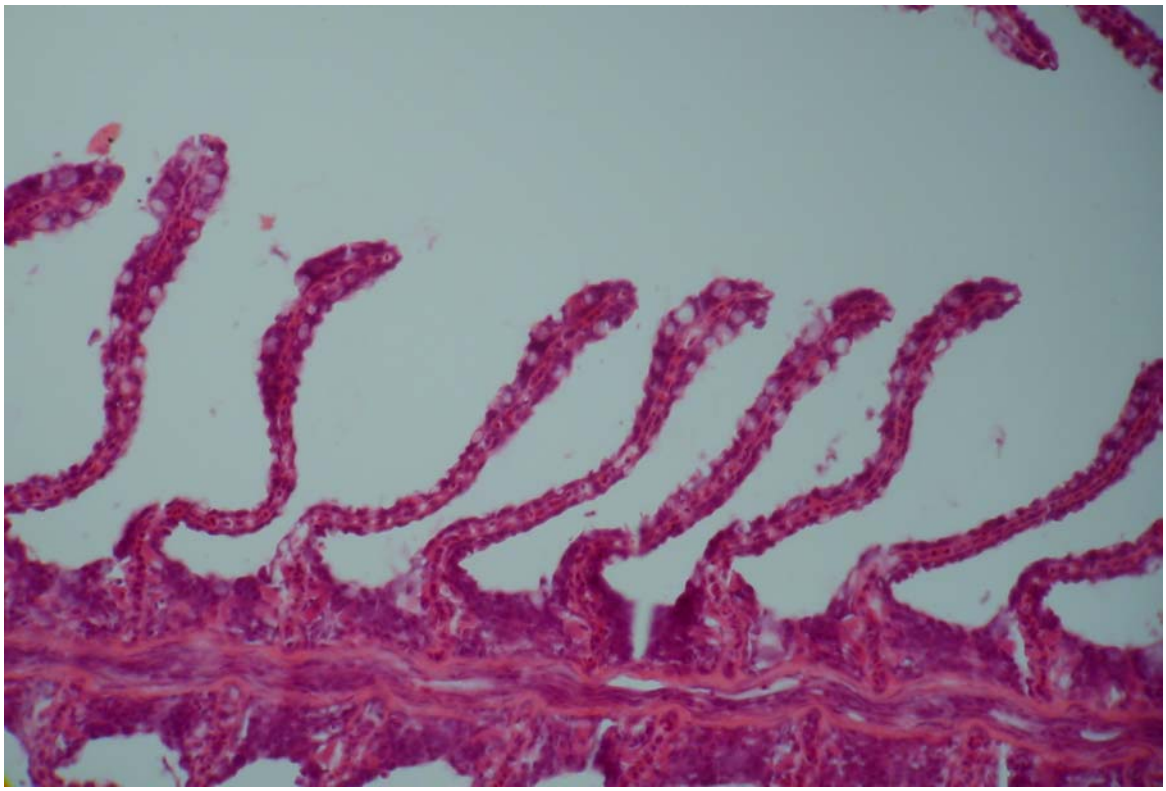

Photo 7: Tip hyperplasia in gill of American plaice (H & E x250).

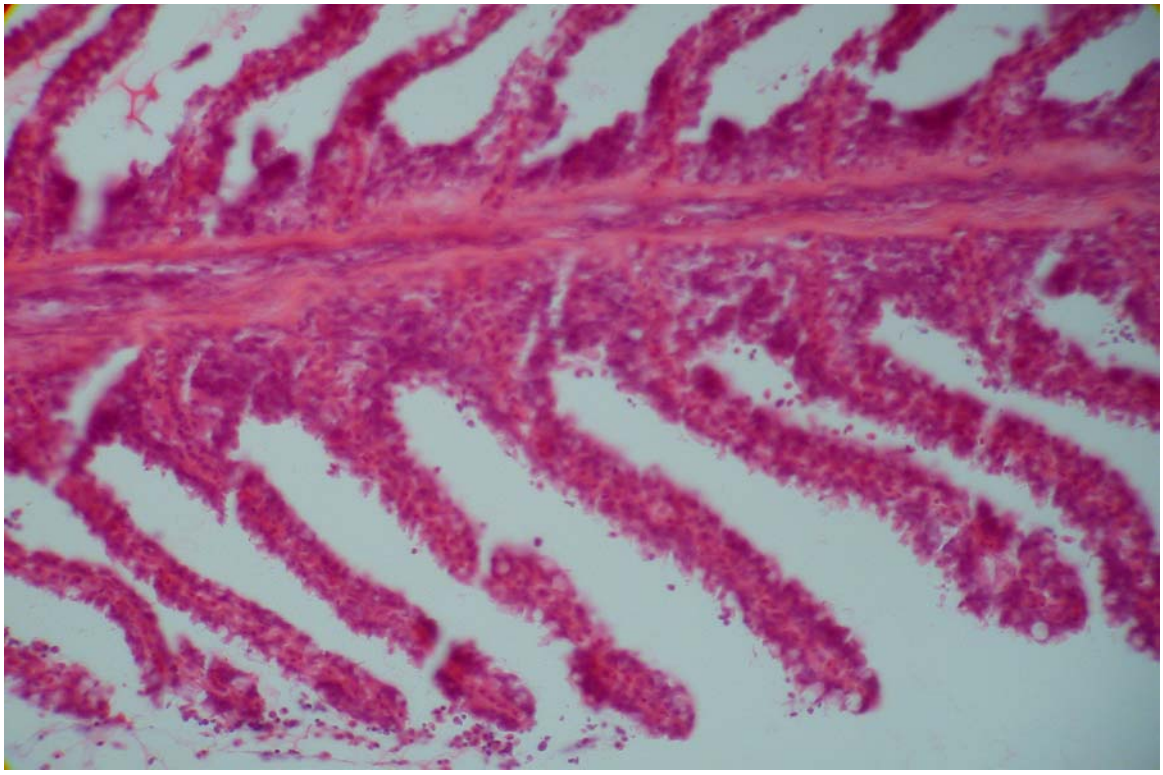

Photo 8: Basal hyperplasia in gill of American plaice (H & E x250).