Table 39

Fluorescent aromatic compounds in bile of English sole as an indicator of aromatic hydrocarbon metabolites.

Investigators: Dr. Sylvester Spencer and Ms. Carla Stehr

Fish ID	Site	BaP ng/g BaP equivalents	NpH ng/g NpH equivalents	PhN ng/g PhN equivalents	protein mg/ml	BaP/protein micrograms Bap equiv./ g protein	NpH/protein micrograms NpH equiv./ g protein	PhN/protein micrograms PhN equiv./ g protein
990018	T49	43	67984	17219	5.78	7	11800	3000
990021	T49	103	63747	18063	1.21	85	52700	14900
990023	T49	17	39975	7737	1.45	12	27600	5300
990024	T49	0	46381	10788	0.73	0	63500	14800
990025	T49	2	34963	6756	0.74	3	47200	9100
990026	T49	126	92543	20100	3.82	33	24200	5300
990027	T49	146	65597	18872	4.45	33	14700	4200
990028	T49	68	60419	18387	1.22	56	49500	15100
990029	T49	8	47221	10798	2.61	3	18100	4100
990030	T49	34	58343	12366	3.62	9	16100	3400
990046	T11B	39	32035	8237	1.26	31	25400	6500
990047	T11B	65	25186	7841	2.69	24	9400	2900
990048	T11B	59	15953	3260	0.37	159	43100	8800
990050	T11B	61	36938	10084	0.78	78	47400	12900
990051	T11B	12	24338	4197	0.59	20	41300	7100
990056	T11B	103	52419	13400	0.80	129	65500	16800
990057	T11B	264	77572	23551	2.36	112	32900	10000
990058	T11B	193	37488	10262	0.89	217	42100	11500
990059	T11B	66	43457	8768	2.97	22	14600	3000
990060	T11B	24	31106	7506	0.76	32	40900	9900
990076	T38	145	50537	17149	0.39	372	129600	44000
990077	T38	640	113577	39821	3.29	195	34500	12100
990078	T38	653	157332	48605	1.41	463	111600	34500
990079	T38	264	61821	21043	0.98	269	63100	21500
990080	T38	542	109260	35919	0.82	661	133200	43800
990081	T38	317	78125	27623	1.00	317	78100	27600
990082	T38	613	109489	40691	0.65	943	168400	62600
990083	T38	187	66444	22008	0.67	279	99200	32800
990084	T38	871	208133	69907	1.03	846	202100	67900
990085	T38	1286	196675	78234	1.39	925	141500	56300
990106	T48	354	93895	34511	1.62	219	58000	21300
990107	T48	264	115193	28954	0.69	383	166900	42000
990108	T48	202	90608	28082	0.82	246	110500	34200
990109	T48	154	89655	22214	1.05	147	85400	21200
990110	T48	149	117084	28480	0.61	244	191900	46700
990111	T48	442	278040	66008	9.43	47	29500	7000
990112	T48	187	67919	17871	0.98	191	69300	18200
990113	T48	76	57373	17320	0.47	162	122100	36900
990114	T48	233	95952	25810	0.67	348	143200	38500
990115	T48	172	76422	22175	0.91	189	84000	24400
990136	T50	13	50016	13443	1.07	12	46700	12600
990137	T50	11	64390	16542	3.19	3	20200	5200
990138	T50	12	56892	14763	2.54	5	22400	5800
990139	T50	12	48196	10765	1.70	7	28400	6300
990140	T50	72	83530	23289	2.66	27	31400	8800
990141	T50	8	44505	9109	0.98	8	45400	9300
990142	T50	7	32261	8624	1.14	6	28300	7600
990143	T50	14	42137	9852	2.14	7	19700	4600
990144	T50	54	76427	19346	5.70	9	13400	3400
990145	T50	32	47542	14708	1.29	25	36900	11400

BaP = Benzo[a]pyrene wavelength equivalents
NpH = naphthalene wavelength equivalents
PhN = Phenanthrene wavelength equivalents

Ten fish from each site were individually analyzed for biliary metabolites.

Table 40

Quality assurance data for fluorescent aromatic compounds in bile of English sole.

Investigators: Dr. Sylvester Spencer and Ms. Carla Stehr

Quality Control number	Control Name	BaP ng/g BaP equivalents	NpH ng/g NpH equivalents	PhN ng/g PhN equivalents	Replicate number
B48031	Blank	0	0	0	1
B48001	Initial Calibration	98	14460	5941	1
B48002	Initial Calibration	98	15512	5929	2
B48003	Continuing Calibration	99	16059	6055	1
B48004	Continuing Calibration	100	16328	6042	2
B48010	Continuing Calibration	98	16021	6024	3
B48017	Continuing Calibration	98	15772	6111	4
B48024	Continuing Calibration	103	16294	5781	5
B48038	Continuing Calibration	103	16013	6059	6
B48005	Bile Reference Material	353	97483	47222	1
B48040	Bile Reference Material	495	100475	55983	2
B47902	Blank	1	129	31	1
B47931	Blank	4	0	0	2
B47901	Initial Calibration	111	15095	5653	1
B47903	Initial Calibration	105	16790	6344	2
B47904	Continuing Calibration	111	17181	6718	1
B47910	Continuing Calibration	105	16879	5863	2
B47917	Continuing Calibration	88	15203	5210	3
B47924	Continuing Calibration	89	15840	6045	4
B47938	Continuing Calibration	91	15011	6166	5
B47905	Bile Reference Material	466	107127	57562	1
B47940	Bile Reference Material	398	91295	49358	2

BaP = Benzo[a]pyrene wavelength equivalents
NpH = naphthalene wavelength equivalents
PhN = Phenanthrene wavelength equivalents

Table 41

Cytochrome P4501A activity and protein levels in liver microsomes of English sole.

Investigator: Dr. Stelvio Bandiera

FishID	Site	CYP Conc. (nmol/mL)	Protein Conc. (mg/mL)	Specific Content (nmol/mg protein)	EROD Activity A (pmol/min/nmol total CYP)	EROD Activity B (pmol/min/mg protein)	CYP1A Level A (ROD/pmol total CYP)	CYP1A Level B (ROD/mg protein)
990001-03	T49	5.94	24.9	0.24	4830	1152	0.26	62.16
990004	T49	4.18	13.5	0.31	2699	837	0.21	65.10
990005	T49	5.61	23.5	0.24	8636	2065	0.35	84.84
990006	T49	7.04	24.3	0.29	2983	863	0.18	51.77
990007	T49	3.14	10.1	0.31	3578	1108	0.38	118.73
990008	T49	4.62	24.4	0.19	7049	1335	0.18	34.87
990009	T49	3.03	14.0	0.22	4886	1058	0.14	30.03
990010	T49	6.27	16.5	0.38	5114	1946	0.18	68.59
990031	T11B	1.98	10.4	0.19	2691	511	0.28	53.20
990032	T11B	1.76	10.9	0.16	3337	540	0.27	42.48
990033	T11B	5.94	13.9	0.43	4034	1726	0.35	150.29
990034	T11B	10.56	23.2	0.45	4830	2195	0.27	119.93
990035	T11B	4.9	14.4	0.34	5483	1863	0.20	67.32
990036	T11B	4.29	15.4	0.28	3153	879	0.15	42.56
990037	T11B	4.29	11.0	0.39	13011	5065	0.29	111.15
990038	T11B	7.48	18.0	0.41	5273	2188	0.27	109.06
990039	T11B	0.99	6.6	0.15	946	142	0.21	31.88
990040	T11B	1.98	6.8	0.29	1767	511	0.44	126.88
990061	T38	3.85	12.0	0.32	6026	1932	0.63	202.40
990062	T38		26.0			3011		301.90
990063	T38	7.04	20.6	0.34	8908	3040	0.83	282.37
990064	T38	2.31	8.6	0.27	11476	3068	0.81	219.24
990065	T38	6.82	14.1	0.48	8282	4006	0.80	382.08
990066	T38	5.94	16.1	0.37	10318	3807	1.01	373.33
990067	T38	4.73	10.4	0.45	11931	5426	1.03	462.38
990068	T38	9.02	22.3	0.40	14947	6051	0.51	203.80
990069	T38	9.46	21.1	0.45	13383	5994	0.48	217.80

Table 41

Cytochrome P4501A activity and protein levels in liver microsomes of English sole.

Investigator: Dr. Stelvio Bandiera

FishID	Site	CYP Conc. (nmol/mL)	Protein Conc. (mg/mL)	Specific Content (nmol/mg protein)	EROD Activity A (pmol/min/nmol total CYP)	EROD Activity B (pmol/min/mg protein)	CYP1A Level A (ROD/pmol total CYP)	CYP1A Level B (ROD/mg protein)
990070	T38	7.26	18.9	0.38	14215	5455	0.54	205.01
990071	T38	6.86	24.1	0.28	19047	5426	0.62	173.60
990072	T38	7.26	20.9	0.35	11881	4119	1.11	386.58
990091	T48	5.94	15.5	0.38	7853	3011	1.25	474.43
990092	T48	7.26	18.5	0.39	5707	2244	1.04	404.82
990093	T48	9.24	21.3	0.43	8113	3523	0.86	371.09
990094	T48	5.5	15.0	0.37	4959	1818	0.67	247.53
990095	T48	5.5	15.7	0.35	6569	2301	0.80	281.23
990096	T48	3.63	8.1	0.45	6379	2869	0.74	331.43
990097	T48	7.48	18.2	0.41	10685	4403	0.51	210.13
990098	T48	6.16	13.7	0.45	3026	1364	0.33	148.50
990099	T48	2.53	6.3	0.40	8560	3438	0.73	290.80
990100	T48	3.96	15.0	0.26	7425	1960	0.54	139.36
990101	T48	0.77	5.8	0.13	1495	199	0.21	26.59
990102	T48	7.26	14.9	0.49	5597	2727	0.48	232.75
990121	T50	3.74	9.6	0.39	16619	6488	0.75	292.11
990122	T50	7.7	20.0	0.38	15710	6042	0.91	344.47
990123	T50	3.3	8.6	0.38	22102	8452	0.70	266.19
990124	T50	2.6	10.8	0.24	10689	2585	1.40	334.80
990125	T50	8.8	19.7	0.45	9375	4198	0.92	415.13
990126	T50	3.43	5.3	0.65	8626	5625	0.58	377.98
990127	T50	7.26	13.5	0.54	3722	2000	0.62	332.37
990128	T50	9.68	18.0	0.54	5994	3225	0.89	481.95
990129	T50	5.83	11.4	0.51	13097	6715	0.66	336.09
990130	T50	1.91	6.3	0.30	7753	2358	1.28	384.30
990131	T50	6.71	13.9	0.48	14773	7152	1.37	656.88
990132	T50	5.35	12.3	0.44	13295	5807	1.37	601.48

Table 41

Cytochrome P4501A activity and protein levels in liver microsomes of English sole.

Investigator: Dr. Stelvio Bandiera

FishID	Site	CYP Conc. (nmol/mL)	Protein Conc. (mg/mL)	Specific Content (nmol/mg protein)	EROD Activity A (pmol/min/nmol total CYP)	EROD Activity B (pmol/min/mg protein)	CYP1A Level A (ROD/pmol total CYP)	CYP1A Level B (ROD/mg protein)
990133	T50	5.28	9.2	0.57	14574	8373	1.45	828.78
990134	T50	5.06	9.9	0.51	15682	7999	1.03	524.28
990151	T49	3.96	14.6	0.27	7933	2159	0.27	71.82
990152	T49	4.4	15.7	0.28	6906	1932	0.22	61.74
990153	T49	3.3	12.2	0.27	6849	1847	0.29	78.17
990154	T49	7.26	14.9	0.49	8915	4347	0.31	149.45
990155	T49	5.5	10.8	0.51	3950	2017	0.15	74.97
990156	T49	3.96	12.8	0.31	6046	1875	0.21	65.72
990157	T49	5.39	15.7	0.34	4971	1705	0.22	73.95
990158	T49	1.98	8.7	0.23	2884	653	0.16	35.65
990159	T49	3.52	14.7	0.24	2840	682	0.25	60.96
990160	T49	2.2	11.6	0.19	1499	284	0.12	22.14
990161	T49	2.97	15.5	0.19	5054	966	0.20	38.38
990162	T49	4.29	17.3	0.25	6625	1648	0.28	69.50

CYP1A = cytochrome P4501A
EROD = ethoxyresorufin *O* -deethylase activity
ROD = relative optical density
CYP Conc. = Microsomal cytochrome P450 concentration
Protein Conc. = Microsomal protein concentration
CYP = total Cytochrome P450 enzymes, (includes CYP1A and other Cytochrome P450 enzymes)

Ten or more fish were indivdiually analyzed from each site.
A composite sample, where equal amounts of liver from three fish were combined (Fish ID numbers 990001, 990002, and 990003), was also analyzed.

Table 42
Cytochrome P4501A activity and protein levels in treated English sole.
Investigator: Dr. Stelvio Bandiera

Treatment Fish ID/ Treatment	CYP Conc. (nmol/mL)	Protein Conc. (mg/mL)	Specific Content (nmol/mg protein)	EROD Activity A (pmol/min/nmol total CYP)	EROD Activity B (pmol/min/mg protein)	CYP1A Level A (ROD/pmol total CYP)	CYP1A Level B (ROD/mg protein)
BNF1	3.41	7.6	0.45	18807	8494	0.95	428.18
BNF2	3.41	6.1	0.56	18693	10382	0.82	457.24
BNF3	2.68	9.1	0.30	14403	4256	0.75	225.15
BNF4	8.8	10.2	0.86	14972	12904	1.07	915.90
BNF5	10.56	13.8	0.77	16619	12736	1.05	807.73
BNF6	3.74	6.2	0.60	13097	7900	1.01	608.10
BNF7	2.53	4.8	0.53	14907	7841	0.92	485.22
BNF8	1.21	2.8	0.43	17810	7642	0.86	370.66
BNF9	4.18	11.3	0.37	13750	5068	0.72	267.33
BNF10	3.52	6.3	0.56	18153	10111	0.56	313.60
CornOil1	2.53	13.8	0.18	4063	748	0.12	22.23
CornOil2	5.65	20.5	0.28	1619	446	0.13	35.84
CornOil3	6.16	17.7	0.35	313	109	0.02	8.05
CornOil4	2.97	10.2	0.29	795	233	0.08	23.20
CornOil5	1.1	6.5	0.17	833	142	0.11	18.28
CornOil6	4.18	14.5	0.29	4688	1354	0.21	60.61
CornOil7	3.89	11.6	0.33	3409	1141	0.20	66.66
CornOil8		23.2			142		6.05
CornOil9	5.5	15.0	0.37	881	323	0.26	97.31
CornOil10	3.08	8.8	0.35	739	259	0.09	31.04

CYP1A = cytochrome P4501A
EROD = ethoxyresorufin*O* -deethylase activity
ROD = relative optical density
CYP Conc. = microsomal cytochrome P450 concentration
Protein Conc. = microsomal protein concentration
CYP = total Cytochrome P450 enzymes, (includes CYP1A and other Cytochrome P450 enzymes)

Table 43

Vitellogenin in blood plasma from English sole (ng/ml plasma).

Investigators. Mr. Dan Lomax, Dr. Munetaka Shimizu

Fish ID	Site	Sex	Vitellogenin	Investigator
990001	T49	male	ND	Shimizu
990002	T49	female	ND	Shimizu
990003	T49	male	ND	Shimizu
990004	T49	male	ND	Shimizu
990005	T49	male	ND	Shimizu
990006	T49	male	ND	Shimizu
990007	T49	male	ND	Shimizu
990008	T49	male	ND	Shimizu
990008	T49	male	ND	Lomax
990009	T49	male	ND	Shimizu
990009	T49	male	ND	Lomax
990010	T49	male	79*	Shimizu
990010	T49	male	ND	Lomax
990011	T49	male	ND	Lomax
990016	T49	male	ND	Lomax
990017	T49	male	ND	Lomax
990018	T49	male	ND	Lomax
990019	T49	male	ND	Lomax
990020	T49	male	ND	Lomax
990031	T11B	male	ND	Shimizu
990031	T11B	male	ND	Lomax
990032	T11B	male	ND	Shimizu
990032	T11B	male	ND	Lomax
990033	T11B	female	ND	Shimizu
990034	T11B	female	ND	Shimizu
990035	T11B	female	ND	Shimizu
990038	T11B	female	ND	Shimizu
990039	T11B	male	ND	Shimizu
990039	T11B	male	ND	Lomax
990040	T11B	male	ND	Shimizu
990040	T11B	male	ND	Lomax
990047	T11B	male	ND	Shimizu
990047	T11B	male	ND	Lomax
990048	T11B	male	ND	Shimizu
990048	T11B	male	ND	Lomax

ND = not detected

*value is very close to the non-detect limit.

The method used by Dr. Shimizu was an enzyme-linked immunosorbent assay for carp vitellogenin.

The method used by Mr. Lomax was an enzyme-linked immunosorbent assay for English sole vitellogenin.

Table 44
Imposex measurements in gastropods from Victoria and Mission Point.
Investigator: Dr. Zhengyan Li

Site	Sample	Species	Shell Height (mm)	Aperture Length (mm)	Sex	Penis Length (mm)	VDS
Ogden Point	1	*Nucella emarginata*	26	17.2	F	2.2	2
Ogden Point	2	*Nucella emarginata*	24	17.5	F	2.5	2
Ogden Point	3	*Nucella emarginata*	26	17.1	F	1.7	2
Ogden Point	4	*Nucella emarginata*	23.3	16	F	2.5	2
Ogden Point	5	*Nucella emarginata*	22.4	16.2	F	1.9	2
Ogden Point	6	*Nucella emarginata*	21	15.2	M	5.2	
Ogden Point	7	*Nucella emarginata*	23.5	16	M	4.9	
Ogden Point	8	*Nucella emarginata*	22	14.2	F	1.5	2
Ogden Point	9	*Nucella emarginata*	23.3	19.4	M	6.8	
Ogden Point	10	*Nucella emarginata*	21.1	18.7	F	2.5	2
Ogden Point	11	*Nucella emarginata*	25.5	18	F	2	2
Ogden Point	12	*Nucella emarginata*	25.5	16	F	1.9	2
Ogden Point	13	*Nucella emarginata*	25.8	17	F	2.8	2
Ogden Point	14	*Nucella emarginata*	20.3	14	F	1.8	2
Ogden Point	15	*Nucella emarginata*	20.3	14.5	M	5.5	
Ogden Point	16	*Nucella emarginata*	21.6	15.6	F	2.5	2
Ogden Point	17	*Nucella emarginata*	26.3	18.8	F	2.1	2
Ogden Point	18	*Nucella emarginata*	23.7	16.3	M	6.5	
Ogden Point	19	*Nucella emarginata*	22	16.1	F	1.4	2
Ogden Point	20	*Nucella emarginata*	23.1	16	F	2	2
Ogden Point	21	*Nucella emarginata*	21.1	14.6	F	2.2	4
Ogden Point	22	*Nucella emarginata*	22	15.7	F	1.2	2
Ogden Point	23	*Nucella emarginata*	22.2	15	F	2	2
Ogden Point	24	*Nucella emarginata*	23.5	16	F	1	2
Ogden Point	25	*Nucella emarginata*	22.8	16	F	2.2	2
Ogden Point	26	*Nucella emarginata*	23.5	15.6	F	2.1	2
Ogden Point	27	*Nucella emarginata*	22	15.4	F	2	2
Ogden Point	28	*Nucella emarginata*	21.5	15	F	1.2	2
Ogden Point	29	*Nucella emarginata*	21.5	14.9	F	1.5	2
Ogden Point	30	*Nucella emarginata*	21.1	14.1	F	1.9	2
Ten Mile Point	1	*Nucella emarginata*	22	14	F	0.9	2
Ten Mile Point	2	*Nucella emarginata*	21.5	13	F	1.2	2
Ten Mile Point	3	*Nucella emarginata*	22	13.9	M	6	
Ten Mile Point	4	*Nucella emarginata*	20	13.5	F	0.5	2
Ten Mile Point	5	*Nucella emarginata*	16.4	10.1	F	0.6	2
Ten Mile Point	1	*Nucella lamellosa*	39.6	22.9	M	5.8	
Ten Mile Point	2	*Nucella lamellosa*	37.1	23.9	M	4.2	
Ten Mile Point	3	*Nucella lamellosa*	42	27.8	F	0	0
Ten Mile Point	4	*Nucella lamellosa*	36	23.2	F	0	0
Ten Mile Point	5	*Nucella lamellosa*	40.2	26	F	0	0
Ten Mile Point	6	*Nucella lamellosa*	39.8	25	F	0	0
Ten Mile Point	7	*Nucella lamellosa*	41.8	26.8	F	0	0
Ten Mile Point	8	*Nucella lamellosa*	39.2	25.9	F	0	0
Ten Mile Point	9	*Nucella lamellosa*	36.7	22.8	F	0	0

Table 44
Imposex measurements in gastropods from Victoria and Mission Point.
Investigator: Dr. Zhengyan Li

Site	Sample	Species	Shell Height (mm)	Aperture Length (mm)	Sex	Penis Length (mm)	VDS
Ten Mile Point	10	*Nucella lamellosa*	43.8	27.5	M	6.2	
Ten Mile Point	11	*Nucella lamellosa*	35.5	23.5	M	5	
Ten Mile Point	12	*Nucella lamellosa*	38.1	24.2	M	5.5	
Ten Mile Point	13	*Nucella lamellosa*	35.3	20.2	M	5.3	
Ten Mile Point	14	*Nucella lamellosa*	42	26	F	0	0
Ten Mile Point	15	*Nucella lamellosa*	39	25	F	0.3	2
Ten Mile Point	16	*Nucella lamellosa*	35	22	M	7	
Ten Mile Point	17	*Nucella lamellosa*	33.5	22.1	M	5.6	
Ten Mile Point	18	*Nucella lamellosa*	33.2	20.8	F	0	0
Ten Mile Point	19	*Nucella lamellosa*	36	22.6	M	6	
Ten Mile Point	20	*Nucella lamellosa*	44	27	M	7.6	
Ten Mile Point	21	*Nucella lamellosa*	45	27.2	F	0.8	2
Ten Mile Point	22	*Nucella lamellosa*	37.5	23.2	F	0.4	2
Ten Mile Point	23	*Nucella lamellosa*	31.9	20.8	M	5.8	
Ten Mile Point	24	*Nucella lamellosa*	32.2	20.8	M	5.6	
Ten Mile Point	25	*Nucella lamellosa*	34	22.8	M	7	
Ten Mile Point	26	*Nucella lamellosa*	39.6	22.1	F	0	0
Ten Mile Point	27	*Nucella lamellosa*	42.5	26.9	F	0	0
Ten Mile Point	28	*Nucella lamellosa*	39	25.1	F	0	0
Ten Mile Point	29	*Nucella lamellosa*	41	27	F	0	0
Ten Mile Point	30	*Nucella lamellosa*	36	21.1	M	7.2	
Mission Point	1	*Nucella lamellosa*	43	26	M	7.1	
Mission Point	2	*Nucella lamellosa*	40.1	24.9	F	0.5	2
Mission Point	3	*Nucella lamellosa*	47.2	26.9	F	2	2
Mission Point	4	*Nucella lamellosa*	43	27.5	F	1.2	2
Mission Point	5	*Nucella lamellosa*	35.9	22.5	M	7.2	
Mission Point	6	*Nucella lamellosa*	39.9	24.1	F	1.2	2
Mission Point	7	*Nucella lamellosa*	36.3	22.2	M	6.5	
Mission Point	8	*Nucella lamellosa*	37.6	22.4	M	5	
Mission Point	9	*Nucella lamellosa*	36.6	23.2	M	5.3	
Mission Point	10	*Nucella lamellosa*	34.7	21.6	M	7.5	
Mission Point	11	*Nucella lamellosa*	35.3	21.3	M	6	
Mission Point	12	*Nucella lamellosa*	41.1	23.6	F	1.2	4
Mission Point	13	*Nucella lamellosa*	35.3	23	M	7	
Mission Point	14	*Nucella lamellosa*	34.1	20.3	M	7	
Mission Point	15	*Nucella lamellosa*	35	21.1	F	1.3	2
Mission Point	16	*Nucella lamellosa*	37.8	23.1	F	2.2	2
Mission Point	17	*Nucella lamellosa*	36.8	23	F	1	2
Mission Point	18	*Nucella lamellosa*	37	23.3	F	1	2
Mission Point	19	*Nucella lamellosa*	36.9	22.1	M	5.5	
Mission Point	20	*Nucella lamellosa*	41.6	23.9	F	1.5	3
Mission Point	21	*Nucella lamellosa*	43.1	22.9	F	0.8	3
Mission Point	22	*Nucella lamellosa*	43.9	22.7	F	1.3	4
Mission Point	23	*Nucella lamellosa*	14.2	9.6	M	1.5	

Table 44
Imposex measurements in gastropods from Victoria and Mission Point.
Investigator: Dr. Zhengyan Li

Site	Sample	Species	Shell Height (mm)	Aperture Length (mm)	Sex	Penis Length (mm)	VDS
Mission Point	24	*Nucella lamellosa*	14.6	11	M	1.6	
Ogden Point	1	*Nucella canaliculata*	32.5	21.1	F	1.5	2
Ogden Point	2	*Nucella canaliculata*	30	20.3	F	1.3	2
Ogden Point	3	*Nucella canaliculata*	33.1	21	M	8.6	
Ogden Point	4	*Nucella canaliculata*	34.1	21.1	F	1.2	2
Ogden Point	5	*Nucella canaliculata*	35	21.8	F	3.3	4
Ogden Point	6	*Nucella canaliculata*	33.5	22.1	M	8	
Ogden Point	7	*Nucella canaliculata*	36.3	24	F	1.2	2
Ogden Point	8	*Nucella canaliculata*	35	21.1	F	1.1	2
Ogden Point	9	*Nucella canaliculata*	31	20	M	10	
Ogden Point	10	*Nucella canaliculata*	32.3	21	F	1	2
Ogden Point	11	*Nucella canaliculata*	31.5	20.1	F	1	2
Ogden Point	12	*Nucella canaliculata*	33.1	21.5	M	10	
Ogden Point	13	*Nucella canaliculata*	29.6	20.9	M	8.2	
Ogden Point	14	*Nucella canaliculata*	30	20	F	0	0
Ogden Point	15	*Nucella canaliculata*	30.6	20	F	1	2
Ogden Point	16	*Nucella canaliculata*	31.3	19	M	7.1	
Ogden Point	17	*Nucella canaliculata*	29	18.3	F	2.7	2
Ogden Point	18	*Nucella canaliculata*	28.8	18.3	F	0	0
Ogden Point	19	*Nucella canaliculata*	29.1	19	F	1.2	2
Ogden Point	20	*Nucella canaliculata*	30.3	19.2	F	0.8	2
Ogden Point	21	*Nucella canaliculata*	27.1	18	F	1.5	2
Ogden Point	22	*Nucella canaliculata*	26.8	17.5	M	8.1	
Ogden Point	23	*Nucella canaliculata*	16.5	12	M	4.8	
Ogden Point	24	*Nucella canaliculata*	20.8	15.1	M	5.1	
Ogden Point	25	*Nucella canaliculata*	20.5	14.3	F	0	2
Ogden Point	26	*Nucella canaliculata*	25.7	17	F	0.5	2
Ogden Point	27	*Nucella canaliculata*	19	13.2	M	6.5	
Ogden Point	28	*Nucella canaliculata*	30.1	18.9	F	1.8	2
Ogden Point	29	*Nucella canaliculata*	25.5	17.1	M	7.2	
Ogden Point	30	*Nucella canaliculata*	21.8	15	F	0	1
Clover Point	1	*Nucella canaliculata*	28	17.5	M	5.2	
Clover Point	2	*Nucella canaliculata*	31.2	19.3	M	9	
Clover Point	3	*Nucella canaliculata*	33.6	20.1	M	6	
Clover Point	4	*Nucella canaliculata*	26.6	18.1	F	0	2
Clover Point	5	*Nucella canaliculata*	26	17.4	F	0.6	2
Clover Point	6	*Nucella canaliculata*	28.8	17.6	M	7.3	
Clover Point	7	*Nucella canaliculata*	32	19.9	F	1.2	2
Clover Point	8	*Nucella canaliculata*	28	17.5	F	0.5	2
Clover Point	9	*Nucella canaliculata*	25.9	17.1	M	7.8	
Clover Point	10	*Nucella canaliculata*	33.1	20.9	M	7.5	
Clover Point	11	*Nucella canaliculata*	29.5	19	F	0.5	2
Clover Point	12	*Nucella canaliculata*	28.5	19	F	0	0
Clover Point	13	*Nucella canaliculata*	29	18.7	M	8	

Table 44
Imposex measurements in gastropods from Victoria and Mission Point.
Investigator: Dr. Zhengyan Li

Site	Sample	Species	Shell Height (mm)	Aperture Length (mm)	Sex	Penis Length (mm)	VDS
Clover Point	14	*Nucella canaliculata*	28.4	17.6	F	0.9	2
Clover Point	15	*Nucella canaliculata*	30.9	18.8	F	0.5	2
Clover Point	16	*Nucella canaliculata*	30	18.1	M	4	
Clover Point	17	*Nucella canaliculata*	29.7	18.8	F	0.8	2
Clover Point	18	*Nucella canaliculata*	29.1	18	F	0.8	2
Clover Point	19	*Nucella canaliculata*	30.2	18.6	F	0	1
Clover Point	20	*Nucella canaliculata*	29	18	M	6.1	
Clover Point	21	*Nucella canaliculata*	28.5	18	M	6.3	
Clover Point	22	*Nucella canaliculata*	28	18	M	5.2	
Clover Point	23	*Nucella canaliculata*	28	18.5	M	5.5	
Clover Point	24	*Nucella canaliculata*	29.6	18	F	1.2	2
Clover Point	25	*Nucella canaliculata*	28.6	18	F	0	0
Clover Point	26	*Nucella canaliculata*	29	17.2	M	6.2	
Clover Point	27	*Nucella canaliculata*	25.3	16	M	7	
Clover Point	28	*Nucella canaliculata*	29.2	17	M	3	
Clover Point	29	*Nucella canaliculata*	33	20.7	M	6.1	
Clover Point	30	*Nucella canaliculata*	30	18.9	F	0	0
Ten Mile Point	1	*Nucella canaliculata*	28.1	18.8	F	0	0
Ten Mile Point	2	*Nucella canaliculata*	25.9	16.3	F	0	0
Ten Mile Point	3	*Nucella canaliculata*	26	15.2	F	0	0
Ten Mile Point	4	*Nucella canaliculata*	28.5	17.2	F	0	1
Ten Mile Point	5	*Nucella canaliculata*	25.4	17	F	0	0
Ten Mile Point	6	*Nucella canaliculata*	30.9	19	F	0	0
Ten Mile Point	7	*Nucella canaliculata*	29.9	19	F	0	0
Ten Mile Point	8	*Nucella canaliculata*	24	14.9	F	0	1
Ten Mile Point	9	*Nucella canaliculata*	26	15.6	M	8	
Ten Mile Point	10	*Nucella canaliculata*	26	16	F	0.5	1
Ten Mile Point	11	*Nucella canaliculata*	25.5	16	M	8.1	
Ten Mile Point	12	*Nucella canaliculata*	24.2	15.8	M	9	
Ten Mile Point	13	*Nucella canaliculata*	25.2	16	F	0	0
Ten Mile Point	14	*Nucella canaliculata*	26.9	16.1	F	0	0
Ten Mile Point	15	*Nucella canaliculata*	27.5	17	M	8.8	
Ten Mile Point	16	*Nucella canaliculata*	27.1	16	M	6.2	
Ten Mile Point	17	*Nucella canaliculata*	26	15.1	M	7.5	
Ten Mile Point	18	*Nucella canaliculata*	25.6	15.9	F	0	0
Ten Mile Point	19	*Nucella canaliculata*	25.9	15.6	F	0	0
Ten Mile Point	20	*Nucella canaliculata*	22.8	13.8	F	0	0
Ten Mile Point	21	*Nucella canaliculata*	24.5	16	F	0	0
Ten Mile Point	22	*Nucella canaliculata*	23	15	M	4.8	
Ten Mile Point	23	*Nucella canaliculata*	20	12.5	F	0	0
Ten Mile Point	24	*Nucella canaliculata*	25	15.2	M	7.5	
Ten Mile Point	25	*Nucella canaliculata*	22.5	14	M	7.1	
Ten Mile Point	26	*Nucella canaliculata*	23	13.5	F	0.3	2
Ten Mile Point	27	*Nucella canaliculata*	24	15.1	F	0	0

Table 44
Imposex measurements in gastropods from Victoria and Mission Point.
Investigator: Dr. Zhengyan Li

Site	Sample	Species	Shell Height (mm)	Aperture Length (mm)	Sex	Penis Length (mm)	VDS
Ten Mile Point	28	*Nucella canaliculata*	22.2	15	M	7.2	
Ten Mile Point	29	*Nucella canaliculata*	24	15	M	7.4	
Ten Mile Point	30	*Nucella canaliculata*	22	13.5	F	0	0
Clover Point	1	*Searlesia dira*	38.5	18.7	M	8.9	
Clover Point	2	*Searlesia dira*	39	21	M	12.5	
Clover Point	3	*Searlesia dira*	36	17	F	0	0
Clover Point	4	*Searlesia dira*	33.5	18	M	10	
Clover Point	5	*Searlesia dira*	33.6	18	M	8	
Clover Point	6	*Searlesia dira*	31	17.5	F	0	0
Clover Point	7	*Searlesia dira*	30.9	17.1	M	9	
Clover Point	8	*Searlesia dira*	27.6	15.9	F	0	0
Clover Point	9	*Searlesia dira*	26.8	14.5	M	5	
Clover Point	10	*Searlesia dira*	23.9	13.1	F	0	0

VDS = vas deferens sequence index; stages are based on Gibbs et al., 1987. J. Mar. Biol. Ass. U.K. 67:507-523.
M=male; F=female

Table 45

Imposex measurements in gastropods from Victoria and Mission Point.

Investigator: Dr. Toshihiro Horiguchi

Sample Date: 990531 **Location:** Victoria, Odgen Point **Species:** *Nucella lima*

Specimen No.	Shell height (mm)	Shell width (mm)	Shell weight (g)	Soft tissue weight (g)	First sex determination	Penis present?	Penis length curved (mm)	Penis length straight (mm)	VDS Index	Opening of vulva blocked?	Second sex determination
1	33.9	20.4	6.6	1.6	F	Y	3		3	N	imposex
2	31.8	19.9	4.7	1.1	F	Y	2		3	N	imposex
3	32.7	20.0	5.5	1.2	M	Y	10				M
4	32.3	20.2	5.2	1.2	F	Y	1		3	N	imposex
5	30.7	19.1	4.8	1.0	M	Y	11				M
6	31.7	19.6	6.0	1.2	M	Y	13				M
7	34.5	20.0	6.0	1.6	F	Y	2		3	N	imposex
8	32.0	20.2	5.1	1.2	F	Y	1.5		3	N	imposex
9	32.2	18.8	4.8	1.3	M	Y	11				M
10	33.3	19.8	5.7	1.5	F	Y	2		3	N	imposex
11	36.8	22.0	8.9	2.1	M	Y	8.5				M
12	30.0	18.1	4.4	1.2	M	Y	12				M
13	29.4	17.7	3.9	1.0	M	Y	10				M
14	31.7	20.5	5.3	1.6	F	Y	2		3	N	imposex
15	37.3	21.9	7.1	2.0	F	Y	1		3	N	imposex
16	29.5	19.2	4.4	1.0	M	Y	8				M
17	29.2	18.8	3.9	1.0	F	Y	1		3	N	imposex
18	27.8	17.0	3.5	0.7	M	Y	8				M
19	33.9	21.0	6.9	1.7	M	Y	11				M
20	32.0	20.6	4.8	1.2	M	Y	11				M
21	38.1	23.9	8.38	2.68	F	Y	3	2.7	3	N	imposex
22	41.2	24.8	11.66	3.50	F	Y	4	3	4	N	imposex
23	39.0	23.2	8.24	2.42	F	N			1	N	imposex
24	35.8	22.7	8.79	2.49	F	Y	2	2	3	N	imposex
25	39.8	22.4	8.11	2.14	F	Y	1.5	1.4	3	N	imposex
26	35.5	20.8	6.28	1.99	F	Y	2	2.2	3	N	imposex
27	35.4	21.5	7.71	1.88	M	Y	6	5.7			M
28	35.3	21.6	6.60	1.97	F	Y	2	2	3	N	imposex
29	34.7	21.9	6.64	1.58	F	Y	2	2	3	N	imposex
30	34.7	21.2	7.54	1.67	F	Y	2	2.1	3	N	imposex

Table 45

Imposex measurements in gastropods from Victoria and Mission Point.

Investigator: Dr. Toshihiro Horiguchi

Sampling Date: 990531 **Location:** Victoria, Clover Point **Species:** *Nucella lima*

Specimen No.	Shell height (mm)	Shell width (mm)	Shell weight (g)	Soft tissue weight (g)	First sex determination	Penis present?	Penis length curved (mm)	Penis length straight (mm)	VDS Index	Opening of vulva blocked?	Second sex determination
1	33.9	19.0	5.2	1.4	F	Y	1		3	N	imposex
2	30.4	19.4	4.2	1.4	F	Y	1		2	N	imposex
3	33.0	19.4	5.3	1.7	F	Y	1.5		3	N	imposex
4	30.2	17.5	4.0	1.1	F	Y	1.5		2	N	imposex
5	25.5	17.5	3.5	0.8	F	Y	2.5		3	N	imposex
6	27.0	16.6	3.0	1.0	F	Y	1.5		2	N	imposex
7	28.8	17.5	3.2	0.9	M	Y	7.5				M
8	29.8	17.5	3.6	1.0	F	Y	2		3	N	imposex
9	29.8	17.8	3.9	1.2	F	Y	1		3	N	imposex
10	27.7	17.1	3.2	0.9	M	Y	11				M
11	31.8	18.5	4.53	1.46	F	Y	1.5	1	3	N	imposex
12	31.0	18.6	4.48	1.30	F	Y	2	1.4	3	N	imposex
13	30.7	18.0	4.17	1.29	F	N			0	N	F
14	32.4	19.7	4.95	1.58	F	N			0	N	F
15	33.0	19.0	5.08	1.30	F	Y	1.5	0.9	3	N	imposex
16	29.4	17.9	4.11	1.14	F	Y	1.2	1	4	N	imposex
17	29.4	17.6	3.88	1.11	F	Y	1	1	3	N	imposex
18	29.5	17.1	3.47	1.12	F	N			0	N	F
19	29.6	16.5	3.48	0.94	F	N			0	N	F
20	27.5	17.0	3.25	0.79	F	N			0	N	F

Table 45

Imposex measurements in gastropods from Victoria and Mission Point.

Investigator: Dr. Toshihiro Horiguchi

Sampling Date: 990531 **Location:** Victoria, Ten-mile Point **Species:** *Nucella lima*

Specimen No.	Shell height (mm)	Shell width (mm)	Shell weight (g)	Soft tissue weight (g)	First sex determination	Penis present?	Penis length curved (mm)	Penis length straight (mm)	VDS Index	Opening of vulva blocked?	Second sex determination
1	29.0	17.4		1.2	F	Y	<1	0.5	2	N	imposex
2	28.0	15.6		0.7	M	Y	10				M
3	26.0	15.3		0.8	F	N	0			N	F
4	26.0	16.9		1.0	F	N	0			N	F
5	26.0	14.4		0.7	F	N	0		1	N	imposex
6	30.5	17.6		1.0	F	Y	<1	0.5	2	N	imposex
7	29.0	16.3		0.9	M	Y	10.5				M
8	29.3	17.1		1.0	F	Y	1		2	N	imposex
9	27.1	15.2		0.8	F	N	0		1	N	imposex
10	31.3	17.3		1.0	M	Y	6.5				M
11	37.1	22.7	10.40	1.45	F	N			1	N	imposex
12	44.4	25.6	12.55	2.61	M	Y	7	5.8			M
13	37.2	22.4	9.61	1.31	F	Y	<0.5	0.6	2	N	imposex
14	39.6	24.4	8.66	1.84	M	Y	7	7			M
15	40.9	25.4	9.43	2.05	F	Y	1	1	3	N	imposex
16	41.4	23.9	9.60	2.43	F	Y	1	0.9	2	N	imposex
17	35.4	22.2	7.20	1.48	F	N			1	N	imposex
18	35.4	22.7	7.73	1.42	M	Y	8	5.9			M
19	36.8	21.5	6.49	1.56	M	Y	7	6.6			M
20	34.9	21.4	6.49	1.50	F	Y	1	0.8	2	N	imposex

Table 45

Imposex measurements in gastropods from Victoria and Mission Point.

Investigator: Dr. Toshihiro Horiguchi

Sampling Date: 990602 **Location:** Mission Point (Wilson Creek) **Species:** *Nucella lamellosa*

Specimen No.	Shell height (mm)	Shell width (mm)	Shell weight (g)	Soft tissue weight (g)	First sex determination	Penis present?	Penis length curved (mm)	Penis length straight (mm)	VDS Index	Opening of vulva blocked?	Second sex determination
1	38.7	23.6	9.84	1.95	F	Y	2	1.6	3	N	imposex
2	41.2	25.2	11.41	2.36	F	Y	2	1.5	3	N	imposex
3	37.5	22.0	8.01	1.59	M	Y	9	7			M
4	38.8	22.4	9.32	1.74	M	Y	8	7			M
5	38.2	22.3	8.00	1.91	F	Y	1.5	1	3	N	imposex
6	35.8	20.4	7.05	1.43	M	Y	8.5	5.8			M
7	36.0	20.0	5.71		M	Y	6.5	5.9			M
8	33.3	20.1	5.36		M	Y	9	7			M
9	34.2	18.8	5.81	0.89	M	Y	7.5	6.6			M
10	32.7	20.2	6.13	1.36	M	Y	6	4.8			M

VDS Index = Vas Deferens Sequence Index. This is based on stages described by Gibbs et al., 1987. J. Mar. Biol. Ass. U.K. 67:507-523.

M = Male; F = Female; Y = Yes; N = No

A gastropod penis is curved. Two methods were used to measure its length:
The curved length was measured with thread. This measurement was used to calculate the indicies used in Table 46.
The straight length was measured from the bottom to the tip of the penis.

imposex = females with penis development, and/or vas deferens development

Table 46

Imposex indicies and tributyltin (ng/g wet weight) in gastropods from Victoria and Mission Point.

Investigators: Dr. Toshihiro Horiguchi

Site	Species	Tributyltin*	RPL Index	RPS Index	VDS Index	N**
Clover Pt.	*Nucella lima*	9.6	11.8	0.2	2.1	14f/16m
Ogden Pt.	*Nucella lima*	2.4	19	0.7	2.9	19f/11m
Ten-Mile Pt.	*Nucella lima*	7.3	3.3	0.004	1.1	19f/11m
Ten-Mile Pt.	*Nucella lamellosa*	8.7	8.2	0.1	1	16f/14m
Mission Pt.	*Nucella lamellosa*	21.9	23.1	1.2	1	12f/12m

*each value is the analysis of one sample containing 6-18 female gastropods of the same species.
** = number of females (f) and males (m) measured to obtain the indicies. Measurements of individual animals are listed in Dr. Horiguchi's imposex measurements data (Table 45) of this report.

RPL Index = Relative Penis length [(mean penis length in females)/ (mean penis length in males)]*100

RPS Index = Relative Penis size Index = [(mean penis length in females)3/(mean penis length in males)3]*100.

VDS Index = Vas Deferens sequence index. Stages based on Gibbs et al., 1987. J. Mar. Biol Ass. U.K. 67:507-523.

Table 47

Histopathology of liver, kidney, gonad and spleen in English sole from Vancouver Harbour.

Investigators: Mr. Mark Myers and Ms. Carla Stehr

Site	Fish ID	length (mm)	weight (g)	Gross Sex	Histo Sex	Age (yr)	Liver Necrosis	Liver Apoptosis	Liver Hemosid	Liver NP/MH SDN	Liver RegenProlif Prolif	Liver Cholfibrosis Prolif	Liver TotProlif
T49	990001	262	131	M	M	8	0	0	0	0	0	0	0
T49	990002	263	152	F	F	8	0	0	0	0	0	0	0
T49	990003	204	63	M	M	3	0	0	0	0	0	0	0
T49	990004	200	63	M	M	3	0	0	0	0	0	0	0
T49	990005	240	112	M	M	10	0	0	0	0	0	0	0
T49	990006	311	261	M	F	9	0	0	0	0	0	0	0
T49	990007	215	80	M	M	9	0	0	0	0	0	0	0
T49	990008	250	130	M	M	6	0	0	0	0	0	0	0
T49	990009	223	98	M	M	5	0	0	0	0	0	0	0
T49	990010	218	90	M	M	5	0	0	0	0	0	0	0
T49	990011	207	78	M	M	6	0	0	0	0	0	0	0
T49	990012	261	158	M	M	2	0	0	0	0	0	0	0
T49	990013	243	115	M	M	5	0	0	0	0	0	0	0
T49	990014	274	165	F	F	6	0	0	0	0	0	0	0
T49	990015	290	220	F	F	9	0	0	0	0	0	0	0
T49	990016	251	137	M	M	9	0	0	0	0	0	0	0
T49	990017	257	158	M	M	8	0	0	0	0	0	0	0
T49	990018	230	103	M	M	8	0	0	0	0	0	0	0
T49	990019	227	109	M	M	8	0	0	0	0	0	0	0
T49	990020	227	110	M	M	5	0	0	0	0	0	0	0
T49	990021	295	193	F	F	7	0	0	0	0	0	0	0
T49	990022	263	157	F	F	6	0	0	0	0	0	0	0
T49	990023	265	161	F	F	5	0	0	0	0	0	0	0
T49	990024	275	160	F	F	4	1	0	0	0	0	0	0
T49	990025	260	140	F	F	4	0	0	0	0	0	0	0
T49	990026	259	156	F	F	6	0	0	0	0	0	0	0
T49	990027	245	133	F	M	10	0	0	0	0	0	0	0
T49	990028	265	174	F	F	7	0	0	0	0	0	0	0
T49	990029	255	150	F	F	6	0	0	0	0	0	0	0
T49	990030	278	171	F	F	6	0	0	0	0	0	0	0
T11B	990031	223	90	M	M	8	0	0	0	0	0	0	0
T11B	990032	218	87	M	M	7	0	0	0	0	0	0	0
T11B	990033	262	148	F	F	6	0	0	0	0	0	0	0
T11B	990034	311	284	F	F	11	0	0	0	0	0	0	0

Table 47

Histopathology of liver, kidney, gonad and spleen in English sole from Vancouver Harbour.

Investigators: Mr. Mark Myers and Ms. Carla Stehr

Site	Fish ID	Liver EosinFocus Preneo	Liver BasoFocus Preneo	Liver CCFocus Preneo	Liver TotPreneo	Liver CholCarc Neoplasm	Liver HepAdenoma Neoplasm	Liver TotNeoplasm	Liver AnyToxLes	Kidney MesScl	Kidney MesLysis	Testis Stage	Ovary Stage	Ovary Atresia	Spleen MAFreq
T49	990001	0	0	0	0	0	0	0	0	1	0	6			5
T49	990002	0	0	0	0	0	0	0	0	0	0		3	1	5
T49	990003	0	0	0	0	0	0	0	0			2			5
T49	990004	0	0	0	0	0	0	0	0	0	0	5			5
T49	990005	0	0	0	0	0	0	0	0	0	0	6			4
T49	990006	0	0	0	0	0	0	0	0	0	0		3	1	5
T49	990007	0	0	0	0	0	0	0	0	0	0	6			7
T49	990008	0	0	0	0	0	0	0	0	0	0	5			4
T49	990009	0	0	0	0	0	0	0	0	0	0	4			5
T49	990010	0	0	0	0	0	0	0	0	0	0	6			4
T49	990011	0	0	0	0	0	0	0	0	0	0	6			4
T49	990012	0	0	0	0	0	0	0	0	0	0	4			3
T49	990013	0	0	0	0	0	0	0	0	0	0	6			6
T49	990014	0	0	0	0	0	0	0	0	0	0		2	1	5
T49	990015	0	0	0	0	0	0	0	0	0	0		2	1	5
T49	990016	0	0	0	0	0	0	0	0	0	0	6			5
T49	990017	0	0	0	0	0	0	0	0	0	0	4			5
T49	990018	0	0	0	0	0	0	0	0	0	0	4			5
T49	990019	0	0	0	0	0	0	0	0	0	0	6			5
T49	990020	0	0	0	0	0	0	0	0	0	0	2			5
T49	990021	0	0	0	0	0	0	0	0	0	0		2	1	6
T49	990022	0	0	0	0	0	0	0	0	0	0		3	1	4
T49	990023	0	0	0	0	0	0	0	0	0	0		2	1	5
T49	990024	0	0	0	0	0	0	0	0	0	0		2	1	4
T49	990025	0	0	0	0	0	0	0	0	0	0		3	0	5
T49	990026	0	0	0	0	0	0	0	0	0	0		3	0	5
T49	990027	0	0	0	0	0	0	0	0	0	0	6			5
T49	990028	0	0	0	0	0	0	0	0	0	0		3	0	5
T49	990029	0	0	0	0	0	0	0	0	0	0		2	1	4
T49	990030	0	0	0	0	0	0	0	0	0	0		2	1	6
T11B	990031	0	0	0	0	0	0	0	0	0	0	6			
T11B	990032	0	0	0	0	0	0	0	0	0	0	6			5
T11B	990033	0	0	0	0	0	0	0	0	0	0		2	1	5
T11B	990034	0	0	0	0	0	0	0	0	0	0		2	0	5

Table 47

Histopathology of liver, kidney, gonad and spleen in English sole from Vancouver Harbour.

Investigators: Mr. Mark Myers and Ms. Carla Stehr

Site	Fish ID	length (mm)	weight (g)	Gross Sex	Histo Sex	Age (yr)	Liver Necrosis	Liver Apoptosis	Liver Hemosid	Liver NP/MH SDN	Liver RegenProlif Prolif	Liver Cholfibrosis Prolif	Liver TotProlif
T11B	990035	272	188	F	F	9	0	0	0	0	0	0	0
T11B	990036	325	276	F	F	10	0	0	0	0	0	0	0
T11B	990037	254	135	F	F	5	0	0	0	0	0	0	0
T11B	990038	256	148	F	F	7	0	0	0	0	0	0	0
T11B	990039	232	100	M	M	7	0	0	0	0	0	0	0
T11B	990040	243	120	M	M	5	0	0	0	0	0	0	0
T11B	990041	273	172	F	F	9	0	0	0	0	0	0	0
T11B	990042	229	97	F	F	5	0	0	0	0	0	0	0
T11B	990043	216	81	F	M	5	0	0	0	0	0	0	0
T11B	990044	235	116	F	F	5	0	1	0	0	0	0	0
T11B	990045	247	148	F	F	7	0	0	0	0	0	0	0
T11B	990046	240	122	F	F	9	0	0	0	0	0	0	0
T11B	990047	228	108	M	M	7	0	0	0	0	0	0	0
T11B	990048	227	91	M	M	6	0	0	0	0	0	0	0
T11B	990049	217	88	F	M	6	0	0	0	0	0	0	0
T11B	990050	217	86	F	F	6	0	0	0	0	1	0	1
T11B	990051	258	145	F	F	7	0	0	0	0	0	0	0
T11B	990052	237	112	F	F	7	0	0	0	0	0	0	0
T11B	990053	241	121	F	F	8	0	0	0	0	0	0	0
T11B	990054	227	104	M	F	4	0	0	0	0	0	0	0
T11B	990055	236	111	F	F	4	1	0	0	0	0	0	0
T11B	990056	228	98	F	F	5	0	0	0	1	0	0	0
T11B	990057	226	89	F	F	4	0	0	0	0	0	0	0
T11B	990058	228	95	F	F	4	0	0	0	0	0	0	0
T11B	990059	230	103	F	F	4	0	0	0	0	0	0	0
T11B	990060	218	89	F	F	4	0	0	0	0	0	0	0
T38	990061	257	129	F	F	7	0	0	0	0	0	0	0
T38	990062	322	265	F	F	8	0	0	0	0	0	0	0
T38	990063	290	184	F	F	7	0	0	0	0	0	0	0
T38	990064	285	160	M	M	11	0	0	0	0	0	0	0
T38	990065	342	298	F	F	10	0	0	0	0	0	0	0
T38	990066	336	312	F	F	8	0	0	0	0	0	0	0
T38	990067	289	170	M	M	15	0	0	0	0	0	0	0
T38	990068	332	275	F	F	12	0	0	0	0	0	0	0

Table 47

Histopathology of liver, kidney, gonad and spleen in English sole from Vancouver Harbour.

Investigators: Mr. Mark Myers and Ms. Carla Stehr

Site	Fish ID	Liver EosinFocus Preneo	Liver BasoFocus Preneo	Liver CCFocus Preneo	Liver TotPreneo	Liver CholCarc Neoplasm	Liver HepAdenoma Neoplasm	Liver TotNeoplasm	Liver AnyToxLes	Kidney MesScl	Kidney MesLysis	Testis Stage	Ovary Stage	Ovary Atresia	Spleen MAFreq
T11B	990035	0	0	0	0	0	0	0	0	0	0		2	1	5
T11B	990036	0	0	0	0	0	0	0	0	0	0		3	1	4
T11B	990037	0	0	0	0	0	0	0	0	0	0		2	1	4
T11B	990038	0	0	0	0	0	0	0	0	0	0		2	1	6
T11B	990039	0	0	0	0	0	0	0	0	0	0	4			5
T11B	990040	0	0	0	0	0	0	0	0	0	0	6			4
T11B	990041	0	0	0	0	0	0	0	0	0	0		2	1	6
T11B	990042	0	0	0	0	0	0	0	0	0	0		2	1	6
T11B	990043	0	0	0	0	0	0	0	0	0	0	6			5
T11B	990044	1	0	0	1	0	0	0	1	0	0		2	1	5
T11B	990045	1	1	0	1	0	0	0	1	0	0		3	1	5
T11B	990046	0	0	0	0	0	0	0	0	0	0		2	1	6
T11B	990047	1	0	0	1	0	0	0	1	0	0	6			5
T11B	990048	0	0	0	0	0	0	0	0	0	0	6			5
T11B	990049	0	0	0	0	0	0	0	0	0	0	6			5
T11B	990050	0	0	0	0	0	0	0	1	0	0		2	1	4
T11B	990051	0	0	0	0	0	0	0	0	0	0		2	1	5
T11B	990052	0	0	0	0	0	0	0	0	0	0		3	0	5
T11B	990053	1	0	0	1	0	0	0	1	0	0		2	1	4
T11B	990054	0	0	0	0	0	0	0	0	0	0		2	1	5
T11B	990055	0	0	0	0	0	0	0	0	0	0		2	1	4
T11B	990056	0	0	0	0	0	0	0	1	0	0		2	1	4
T11B	990057	0	0	0	0	0	0	0	0	0	0		2	1	4
T11B	990058	0	0	0	0	0	0	0	0	0	0		2	1	4
T11B	990059	0	0	0	0	0	0	0	0	0	0		2	0	5
T11B	990060	0	0	0	0	0	0	0	0	0	0		2	1	5
T38	990061	0	0	0	0	0	0	0	0	0	0		2	1	4
T38	990062	0	0	0	0	0	0	0	0				4	1	5
T38	990063	0	0	0	0	0	0	0	0	0	0		2	1	6
T38	990064	0	0	0	0	0	0	0	0	0	0	6			7
T38	990065	0	0	0	0	0	0	0	0	0	0		2	0	5
T38	990066	1	0	0	1	1	0	1	1	0	0		2	1	6
T38	990067	0	0	0	0	0	0	0	0	0	0	6			6
T38	990068	0	0	0	0	0	0	0	0	0	1		2	0	7

Table 47

Histopathology of liver, kidney, gonad and spleen in English sole from Vancouver Harbour.

Investigators: Mr. Mark Myers and Ms. Carla Stehr

Site	Fish ID	length (mm)	weight (g)	Gross Sex	Histo Sex	Age (yr)	Liver Necrosis	Liver Apoptosis	Liver Hemosid	Liver NP/MH SDN	Liver RegenProlif Prolif	Liver Cholfibrosis Prolif	Liver TotProlif
T38	990069	332	301	F	F	9	0	0	0	0	0	0	0
T38	990070	315	242	F	F	11	0	0	0	1	0	0	0
T38	990071	344	297	F	F	11	0	0	0	0	0	0	0
T38	990072	332	313	F	F	15	0	0	0	0	1	1	1
T38	990073	338	350	F	F	9	0	0	0	0	0	0	0
T38	990074	321	266	F	F	8	0	0	0	0	0	0	0
T38	990075	304	204	F	F	8	0	0	1	0	0	0	0
T38	990076	306	220	F	F	10	0	0	0	0	0	0	0
T38	990077	297	221	F	F	11	0	0	0	0	0	0	0
T38	990078	290	168	M	M	15	0	0	0	0	0	0	0
T38	990079	295	201	F	F	6	0	0	0	0	0	0	0
T38	990080	284	170	F	F	6	0	0	0	0	0	0	0
T38	990081	262	137	F	F	7	0	0	0	0	0	0	0
T38	990082	283	183	F	F	8	0	0	0	0	0	0	0
T38	990083	280	165	M	M	14	0	0	0	0	0	0	0
T38	990084	300	220	M	M	10	0	0	0	0	0	0	0
T38	990085	271	152	M	M	10	0	0	0	0	0	0	0
T38	990086	284	153	M	M	10	1	0	0	0	0	0	0
T38	990087	258	139	F	F	5	0	0	0	0	0	0	0
T38	990088	258	137	F	F	6	0	0	0	0	0	0	0
T38	990089	265	141	F	F	6	0	0	0	0	0	0	0
T38	990090	282	202	F	F	7	0	0	0	0	0	0	0
T48	990091	302	192	F	F	10	0	0	0	0	0	0	0
T48	990092	287	192	F	F	6	0	0	0	0	0	0	0
T48	990093	290	190	F	F	9	0	0	0	0	0	0	0
T48	990094	271	152	F	F	6	0	0	0	0	0	0	0
T48	990095	255	160	F	F	9	0	0	0	0	0	0	0
T48	990096	247	125	F	F	6	0	0	0	0	0	0	0
T48	990097	286	188	F	F	7	1	0	0	0	1	0	1
T48	990098	278	178	F	F	7	0	0	0	0	0	0	0
T48	990099	230	94	M	M	8	0	0	0	0	0	0	0
T48	990100	266	40	M	M	11	0	0	0	0	0	1	1
T48	990101	256	120	M	M	5	0	0	0	0	0	0	0
T48	990102	271	176	M	M	13	0	0	0	0	0	0	0

Table 47

Histopathology of liver, kidney, gonad and spleen in English sole from Vancouver Harbour.

Investigators: Mr. Mark Myers and Ms. Carla Stehr

Site	Fish ID	Liver EosinFocus Preneo	Liver BasoFocus Preneo	Liver CCFocus Preneo	Liver TotPreneo	Liver CholCarc Neoplasm	Liver HepAdenoma Neoplasm	Liver TotNeoplasm	Liver AnyToxLes	Kidney MesScl	Kidney MesLysis	Testis Stage	Ovary Stage	Ovary Atresia	Spleen MAFreq
T38	990069	0	0	1	1	0	1	1	1	0	0		2	1	5
T38	990070	1	0	0	1	0	0	0	1	0	0		2	0	5
T38	990071	0	0	0	0	0	0	0	0	1	0		3	1	6
T38	990072	0	0	0	0	0	1	1	1	0	0		2	0	
T38	990073	0	0	0	0	0	0	0	0	0	0		2	1	
T38	990074	0	0	0	0	0	0	0	0	0	0		4	0	5
T38	990075	1	1	0	1	0	0	0	1	0	0		2	1	7
T38	990076	0	0	0	0	0	0	0	0	0	0		2	1	6
T38	990077	0	0	0	0	0	0	0	0	0	0		2	1	6
T38	990078	1	0	0	1	0	0	0	1	0	0	6			6
T38	990079	0	0	0	0	0	0	0	0	0	0		2	0	5
T38	990080	0	0	0	0	0	0	0	0	0	0		3	1	5
T38	990081	0	0	0	0	0	0	0	0	0	0		2	1	6
T38	990082	0	0	0	0	0	0	0	0	1	0		2	1	5
T38	990083	0	0	0	0	0	0	0	0	0	0	6			7
T38	990084	0	0	0	0	0	0	0	0	0	0		2	1	6
T38	990085	0	0	0	0	0	0	0	0	0	0	6			7
T38	990086	0	0	0	0	0	0	0	0	0	0	6			7
T38	990087	0	0	0	0	0	0	0	0	0	0		2	0	5
T38	990088	0	0	0	0	0	0	0	0	0	0		2	1	6
T38	990089	0	0	0	0	0	0	0	0	0	0		2	1	5
T38	990090	1	0	0	1	0	0	0	1	0	0		3	0	7
T48	990091	0	0	0	0	0	0	0	0	0	0		2	1	5
T48	990092	0	0	0	0	0	0	0	0	0	0		2	1	5
T48	990093	1	0	0	1	0	0	0	1	0	0		2	1	5
T48	990094	0	0	0	0	0	0	0	0	0	0		2	1	5
T48	990095	0	0	0	0	0	0	0	0	0	0		2	1	6
T48	990096	0	0	0	0	0	0	0	0	0	0		2	1	4
T48	990097	0	0	0	0	0	0	0	1	0	0		3	1	5
T48	990098	0	0	0	0	0	0	0	0	0	0		3	1	4
T48	990099	0	0	0	0	0	0	0	0	0	0	6			5
T48	990100	1	1	0	1	0	1	1	1	0	0				6
T48	990101	1	1	0	1	0	0	0	1	0	0	6			7
T48	990102	0	0	0	0	0	0	0	0	0	0	4			6

Table 47

Histopathology of liver, kidney, gonad and spleen in English sole from Vancouver Harbour.

Investigators: Mr. Mark Myers and Ms. Carla Stehr

Site	Fish ID	length (mm)	weight (g)	Gross Sex	Histo Sex	Age (yr)	Liver Necrosis	Liver Apoptosis	Liver Hemosid	Liver NP/MH SDN	Liver RegenProlif Prolif	Liver Cholfibrosis Prolif	Liver TotProlif
T48	990103	285	176	F	F	10	0	0	0	0	0	0	0
T48	990104	318	283	F	F	10	0	0	0	0	0	0	0
T48	990105	302	235	F	F	8	0	0	0	0	0	0	0
T48	990106	275	168	F	F	5	0	0	0	0	0	0	0
T48	990107	262	150	F	F	5	0	0	0	0	0	0	0
T48	990108	248	126	F	F	4	0	0	0	0	0	0	0
T48	990109	255	135	F	F	5	0	0	0	0	0	0	0
T48	990110	274	155	F	F	6	0	0	0	0	0	0	0
T48	990111	247	126	M	M	9	0	0	0	0	0	0	0
T48	990112	252	132	F	F	7	0	0	0	0	0	0	0
T48	990113	250	134	F	F	6	0	0	0	0	0	0	0
T48	990114	265	146	F	F	6	0	0	0	0	0	0	0
T48	990115	297	228	F	F	6	0	0	0	0	0	0	0
T48	990116	262	153	F	F	6	0	0	0	0	0	0	0
T48	990117	274	177	F	F	9	0	0	0	0	0	0	0
T48	990118	274	168	F	F	8	0	0	0	0	0	0	0
T48	990119	256	143	F	F	4	0	0	0	0	0	0	0
T48	990120		265	F	F	11	0	0	0	0	0	0	0
T50	990121	246	113	F	F	6	0	0	0	0	0	0	0
T50	990122	295	204	F	F	12	0	0	0	0	0	0	0
T50	990123		125	F	F	6	0	0	0	0	0	0	0
T50	990124	265	124	M	M	13	0	0	0	0	0	0	0
T50	990125	280	166	F	F	6	0	0	0	0	0	0	0
T50	990126		95	F	F	6	0	0	0	0	0	0	0
T50	990127	235	91	M	M	8	0	0	0	0	0	0	0
T50	990128	245	112	F	F	7	0	0	0	0	0	0	0
T50	990129	246	120	F	F	6	0	0	0	0	0	0	0
T50	990130	243	107	F	F	4	0	0	0	0	0	0	0
T50	990131	234	90	M	M	8	0	0	0	0	0	0	0
T50	990132	269	142	F	F	9	0	0	0	0	0	0	0
T50	990133	228	97	M	M	8	0	0	0	0	0	0	0
T50	990134	230	101	M	M	9	0	0	0	0	0	0	0
T50	990135	240	105	M	M	6							
T50	990136	233	96	M	M	10	0	0	0	0	0	0	0

Table 47

Histopathology of liver, kidney, gonad and spleen in English sole from Vancouver Harbour.

Investigators: Mr. Mark Myers and Ms. Carla Stehr

Site	Fish ID	Liver EosinFocus Preneo	Liver BasoFocus Preneo	Liver CCFocus Preneo	Liver TotPreneo	Liver CholCarc Neoplasm	Liver HepAdenoma Neoplasm	Liver TotNeoplasm	Liver AnyToxLes	Kidney MesScl	Kidney MesLysis	Testis Stage	Ovary Stage	Ovary Atresia	Spleen MAFreq
T48	990103	0	0	0	0	0	0	0	0	0	0		2	1	5
T48	990104	0	0	0	0	0	1	1	1	0	0		3	0	5
T48	990105	0	0	0	0	0	0	0	0	0	0		2	0	5
T48	990106	0	0	0	0	0	0	0	0	0	0		3	0	4
T48	990107	0	0	0	0	0	0	0	0	0	0		3	1	5
T48	990108	0	0	0	0	0	0	0	0	0	0		2	1	5
T48	990109	0	0	0	0	0	0	0	0	0	0		2	1	6
T48	990110	0	0	0	0	0	0	0	0	0	0		2	1	5
T48	990111	0	0	0	0	0	0	0	0	0	0	6			6
T48	990112	0	0	0	0	0	0	0	0	0	0		2	0	4
T48	990113	1	0	0	1	0	0	0	1	0	0		2	1	5
T48	990114	0	0	0	0	0	0	0	0	0	0		2	1	4
T48	990115	0	0	0	0	0	0	0	0	0	0		3	0	5
T48	990116	0	0	0	0	0	0	0	0	0	0		2	1	6
T48	990117	0	0	0	0	0	0	0	0	0	0		2	1	7
T48	990118	1	0	0	1	0	0	0	1	0	0		2	1	5
T48	990119	0	0	0	0	0	0	0	0	1	1		2	1	4
T48	990120	0	0	0	0	0	0	0	0	0	0		2	1	5
T50	990121	0	0	0	0	0	0	0	0	0	0		2	0	5
T50	990122	0	0	0	0	0	0	0	0	0	0		2	1	6
T50	990123	0	0	0	0	0	0	0	0	0	0		2	0	5
T50	990124	0	0	0	0	0	0	0	0	0	0	5			7
T50	990125	0	0	0	0	0	0	0	0	0	0		2	1	6
T50	990126	0	0	0	0	0	0	0	0	0	0		2	0	5
T50	990127	0	0	0	0	0	0	0	0	0	0	4			5
T50	990128	0	0	0	0	0	0	0	0	0	0		2	1	5
T50	990129	0	0	0	0	0	0	0	0	0	0		2	1	4
T50	990130	0	0	0	0	0	0	0	0	0	0		2	1	4
T50	990131	0	0	0	0	0	0	0	0	0	0	6			6
T50	990132	0	0	0	0	0	0	0	0	0	0		3	0	5
T50	990133	0	0	0	0	0	0	0	0	0	0	5			6
T50	990134	0	0	0	0	0	0	0	0	0	0	6			6
T50	990135														
T50	990136	0	0	0	0	0	0	0	0	0	0	6			7

Table 47

Histopathology of liver, kidney, gonad and spleen in English sole from Vancouver Harbour.

Investigators: Mr. Mark Myers and Ms. Carla Stehr

Site	Fish ID	length (mm)	weight (g)	Gross Sex	Histo Sex	Age (yr)	Liver Necrosis	Liver Apoptosis	Liver Hemosid	Liver NP/MH SDN	Liver RegenProlif Prolif	Liver Cholfibrosis Prolif	Liver TotProlif
T50	990137	229	99	F	F	7	0	0	0	0	0	0	0
T50	990138	235	94	M	M	9	0	0	0	0	0	0	0
T50	990139	224	92	F	F	7	0	0	0	0	0	0	0
T50	990140	239	89	M	M	3	0	0	0	0	0	0	0
T50	990141	246	93	F	F	3	0	0	0	0	0	0	0
T50	990142	234	92	M	M	11	0	0	0	0	0	0	0
T50	990143	225	79	M	M	8	0	0	0	0	0	0	0
T50	990144	216	61	M	M	6	0	0	0	0	0	0	0
T50	990145	237	96	J	M	7	0	0	0	0	0	0	0
T50	990146	235	92	M	M	6	0	0	0	0	0	0	0
T50	990147	234	99	F	F	6	0	0	0	0	0	0	0
T50	990148	230	85	M	M	9	0	0	0	0	0	0	0
T50	990149	225	80	F	M	6	0	0	0	0	0	0	0
T50	990150	229	85	M	M	9	0	0	0	0	0	0	0
T49	990151	285	172	F		3							
T49	990152	260	135	M		6							
T49	990153	290	178	F		6							
T49	990154	292	193	F		5							
T49	990155	265	141	F		6							
T49	990156	254	145	F		9							
T49	990157	290	213	M		6							
T49	990158	234	103	M		7							
T49	990159	265	155	M		7							
T49	990160	227	108	M		6							
T49	990161	237	107	F		4							
T49	990162	255	132	M		6							

Fish numbers 990150 - 990162 did not have any tissues collected for histopathological examination.

Thanks to Dr. Colin Levings, Dept. Fish and Oceans, Canada for the age data.

BasoFocus = basophilic focus

CCFocus = clear cell focus

CholCarc = cholangiocellular carcinoma

Cholfibrosis =cholangiofibrosis

EosinFocus = eosinophilic focus

Gross Sex = sex determined by visual observation at the time of necropsy

Hemosid = Hemosiderosis

HepAdenoma = Hepatocellular adenoma

Histo = Histology

Histo sex = sex determined by histology

Length = total length (head to tail) reported in millimeters

AnyToxLes = indicates a fish that has one or more lesions considered to be toxicopathic, including neoplasms, preneoplasms, SDN and proliferative lesions.

MAFreq= Macrophage aggregate frequency

MesLysis = Mesangial lysis

MesScl = Mesangial sclorosis

MH = Megalocytic hepatosis

NP = Nuclear Pleomorphism

Table 47

Histopathology of liver, kidney, gonad and spleen in English sole from Vancouver Harbour.

Investigators: Mr. Mark Myers and Ms. Carla Stehr

Site	Fish ID	Liver EosinFocus Preneo	Liver BasoFocus Preneo	Liver CCFocus Preneo	Liver TotPreneo	Liver CholCarc Neoplasm	Liver HepAdenoma Neoplasm	Liver TotNeoplasm	Liver AnyToxLes	Kidney MesScl	Kidney MesLysis	Testis Stage	Ovary Stage	Ovary Atresia	Spleen MAFreq
T50	990137	0	0	0	0	0	0	0	0	0	0		3	0	5
T50	990138	0	0	0	0	0	0	0	0	0	0	6			5
T50	990139	0	0	0	0	0	0	0	0	0	0		3	0	4
T50	990140	0	0	0	0	0	0	0	0	0	0	6			4
T50	990141	0	0	0	0	0	0	0	0	0	0		2	0	3
T50	990142	0	0	0	0	0	0	0	0	0	0	5			5
T50	990143	0	0	0	0	0	0	0	0	0	0	6			5
T50	990144	0	0	0	0	0	0	0	0	0	0	6			5
T50	990145	0	0	0	0	0	0	0	0	0	0	6			5
T50	990146	0	0	0	0	0	0	0	0	0	0	6			5
T50	990147	0	0	0	0	0	0	0	0	0	0		3	1	5
T50	990148	0	0	0	0	0	0	0	0	0	0	6			6
T50	990149	0	0	0	0	0	0	0	0	0	0	6			5
T50	990150	0	0	0	0	0	0	0	0	0	0	6			6
T49	990151														
T49	990152														
T49	990153														
T49	990154														
T49	990155														
T49	990156														
T49	990157														
T49	990158														
T49	990159														
T49	990160														
T49	990161														
T49	990162														

Preneo = preneoplasm (includes Basophilic focus, clear cell focus, and eosinophilic focus)

Prolif = Proliferative lesion

RegenProlif = Regenerative Proliferation

SDN = Specific Degeneration/Necrosis (includes megalocytic hepatosis and nuclear pleomorphism)

TotProl = indicates fish having one or more types of proliferative lesions

TotPreneo = indicates fish having one or more types of preneoplastic lesions.

TotNeoplasm = indicates fish having one or more types of neoplastic lesions.

A note on sex determination: Gross sex was determined by visual observations of gonads at the time of necropsy. Smaller fish have undeveloped ovaries, so it is sometimes difficult to determine sex in younger fish. Therefore, sex determination by histology is more accurate than gross sex.

Testis stages:

1=regressed; spermatogonia and primary spermatocytes

2= early recrudescence; secondary spermatocytes

3= late recrudescence; secondary spermatocytes to spermatids

4= early spermiogenesis or sperm production

5= late spermiogenesis; spawning

6= spawned out; few mature sperm remaining

M=Male, F = Female, J=Juvenile, sex undetermined

Macrophage Aggregate Frequency Ratings

0= none

1= minimal, very few

2= minimal-mild

3= mild, few

4= mild-moderate number

5= moderate number

6= moderate-severe number

7= Severe, numerous

Ovary stages:

1= regressed; oogonia and primary oocytes

2= late regressed; secondary oocytes

3= previtellogenic; vacuolated secondary oocytes

4= vitellogenic

5= some hydrated oocytes; no post-ovulatory follicles (POFs)

6= spawning; hydrated oocytes with POFs

7= spawned out