DNA Damage Analysis of Fish Tissue Samples Collected in the St. Lucie and Jupiter Inlets of South Florida June 2001

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1.0 INTRODUCTION

In recent studies the occurrence of tumors, lesions and other histological disorders were observed in fish collected in St. Lucie Estuary, Florida. Resident fish species including Irish pompano (*Trachinotus carolinus*), mangrove snapper (*Lutjanus griseus*), spottail pinfish (*Diplodus holbrooki*), and sheepshead (*Archosargus probatocephalus*) were found to display a variety of abnormalities, including fin rot, dorsal fin ray deformation, "chromatophore clusters", scale disorientation, and hemorrhaging. These findings have prompted the design of follow-up studies which will attempt to further characterize the frequency of these and other abnormalities in multiple fish species at numerous sites within the estuary to determine the extent of this phenomena.

Tissues from fish collected at stations in St Lucie estuary and at a reference station were analyzed using the SCG/Comet assay to determine whether the fish exposed to the water and sediment in the selected areas had a higher incidence of cytotoxic and genotoxic DNA damage above that of fish from the reference station.

The null hypothesis of the study is that no significant increase in DNA damage will be observed in tissues of fish from the St Lucie estuary collection sites.

2.0 METHODS

From June 26-29th fish were collected by hook & line at numerous sites within St. Lucie Inlet and Jupiter Inlet. The stations were located at 5 NOS (NOAA National Ocean Service) sampling areas which loosely correspond to stations previously used for the collection of fish health data by the NMFS. NOS station 32 corresponds with NMFS station #4 near the ocean mouth of the St. Lucie estuary, NOS station 47, corresponds with Jupiter Inlet stations, NMFS designations have not been received for these stations other than #42, NOS station 40, corresponds with mid-estuary NMFS sites #21 & 38, and two NOS stations in the lower estuary, NOS # 33, corresponds to NMFS sites # 18 and 35, and NOS # 34 was adjacent to NMFS site #22.

Tissue samples were collected at NOS 32 on 6/26 and 6/29, NOS 47 on 6/27, and NOS 40, 33 & 34 on 6/28. NOS 32 (NMFS #4), was sampled twice due to the interruption of efforts by a thunderstorm on 6/26. Up until 6/26 the Stuart, FL area had received little if any rainfall for a number of weeks. The fish collected at NOS 32 on 6/26 (NOS 32A) had therefore not been exposed to storm runoff, whereas all subsequent stations would be expected to have been, including fish collected at NOS 32 on 6/29 (NOS 32B). Three target species were collected, Irish pompano (*Trachinotus carolinus*), mangrove snapper (*Lutjanus griseus*), and spottail pinfish (*Diplodus holbrooki*). Only mangrove snapper were caught at all 5 NOS stations (see Table 1). Tissues from other species were collected in case they were collected at subsequent stations. In addition to the tissue samples collected for SCG/Comet analysis, skin and liver samples were collected and preserved for histopathology analysis. None of the fish sampled or caught showed signs of saddleback, scale disorientation, skin discoloration or lesions.

Table 1. Occurrence of Fish Species at Collection Stations

NOS #/FH Station	Species:(species/number collected) P	M	S	Sum
NOS 32/FH 4 (6/26)	+/7	+/3		2
NOS 32/FH 4 (6/29)	+/3	+/5	+/5	3
NOS 47/(trestle)			+/5	1
NOS47/(lighthouse)			+/5	1
NOS 47/(US 1 bridge)				
NOS 47/FH 42		+/5		1
NOS 40/FH 38	+/1	+/5		2
NOS 40/FH 21	+/1	+/5		2
NOS 33/FH 18	+/1	+/5		2
NOS 33/FH 35	+/1			1
NOS 34/FH 22		+/5		1
Sum=	6	7	3	

P= Irish pompano (*Trachinotus carolinus*)
M= mangrove snapper (*Lutjanus griseus)*
S= spottail pinfish (*Diplodus holbrooki*)

DNA Damage Analysis Protocols

Samples of blood, liver and gonadal tissue were collected and preserved in the field June 26-29, 2001. Tissue samples were collected immediately after the fish were caught and brought onboard. Blood was preserved by gently mixing and freezing a small volume (<100 ul) in 1 ml of ice cold cryopreservation solution, phosphate buffered saline/10% DMSO. Small sections of liver and gonad (collected from both male & female fish with well developed gonads) were placed in 1 ml of ice cold cryopreservation solution. Within 20 minutes all samples were frozen in liquid nitrogen. Samples were transported to the CSC Biomarker Laboratory and transferred to a –80^{o}C freezer. To prepare samples for DNA damage analysis cryopreserved samples were thawed on ice; 10-100 µl of blood was added to 140 µl ice cold PBS; liver was homogenized using dissection scissors and 25 µl of suspended cells added to 100 µl ice cold PBS; and 10 µl cryopreservation solution from a gonad sample vial added to 140 µl ice cold PBS, in a clean 1.5 ml centrifuge tube and the remaining sample re-frozen. Cells were pelleted at 600 x g for 2 min., supernatant was discarded and the pellet resuspended in 50-600 µl 0.7% low melting temperature agarose (FisherBiotech, low melting DNA grade agarose) in PBS at 30^{o}C (PBS/LMA). Twenty-five microliters of the the resuspended cells were then transferred onto GelBond slides and the cell/agarose suspension allowed to solidify on an ice chilled stainless steel tray, and covered with a top-coat of 25 µl PBS/LMA. After solidifying the slides were placed in 4 ^{o}C lysing solution (LS), 2.5 M NaCl, 10 mM Tris, 0.1 M EDTA, 1% Triton X-100, and 10% DMSO, pH 10.0 (LS) in polycarbonate trays and incubated at 4^{o}C for at least 1 hr.

Standard human cell samples of known damage were run along with each batch of samples in order to track batch to batch variability, all values were within the normal range. Slides were then transferred from LS to trays filled with distilled water, the water

replaced with fresh 2X over a 10 min. period then placed in a submarine gel electrophoresis chamber filled with 300 mM NaOH, 1 mM EDTA, and the DNA denatured under alkaline conditions for 15 min. After unwinding electrophoresis was performed at 300 mA, 25 V for 4V-hours. The slides were then neutralized with two 3 min. rinses in 0.4 M Tris, removed, excess solution blotted away, and placed in ice cold ethanol for 5 min. The slides fixed slides were dried in an oven at 37^{o}C for 20 min. and transferred to slide boxes.

For analysis the DNA was stained with 15 µl of a 20 µg/ml solution of ethidium bromide in distilled water (EtBr), covered with a coverslip. Stained slides were analyzed by viewing at 200X with an epifluorescent microscope (excitation filter 510-560 nm green light, barrier filter 590 nm) with an attached CCD camera and image analysis software (Komet image analysis system, Kinetic Imaging, Ltd U.K.). The fluorescent "head" or nucleus diameter and the length (µm) of any accompanying trailing DNA "tails" resulting from strand breakage were measured for each nucleus analyzed. Measurements were made in five sectors on each slide, counting 5 nuclei in each sector randomly positioning the lens above each sector and counting left to right from the upper left-hand corner of the field of view. Overlapping nuclei or tails were not counted. An effort has been made to standardize SCG/Comet assay procedures worldwide, in an effort to comply with these protocols duplicate runs were made of the mangrove snapper samples, 25 cells were scored from each duplicate and the values of all 50 combined for the calculation of the sample mean.

The image system used for scoring SCG/Comet slides calculates a large number of quantitative parameters for each nuclei the most important being the total intensity of each comet (comet optical intensity), the % DNA in the tail, and the tail moment, which is the product of the %DNA in the tail X the tail length/100. Comet results were compared statistically by single-classification analysis of variance, ANOVA. Since ANOVA assumes that populations have equal variances comparisons were not performed unless this assumption was examined using Bartlett's test. Comparisons where made using a Tukey-Kramer test. Comparison of station NOS 32 A & B data was performed using a one-tailed unpaired t-test. All statistical comparisons were performed using InStat® (GraphPad™, San Diego, CA) statistics software.

3.0 RESULTS & DISCUSSION

Table 2 is the sample label code key, Table 3 is a summary of the samples collected, and a summary of the Comet results is in Table 4. Because the mangrove snapper was the most abundant species caught at all 5 NOS stations statistical comparisons were made using the Comet results from this species comparing tail moment (TM) and %DNA in Comet tail (%DNA) results for NOS and NMFS station comparisons. For NOS comparisons NOS 32A represents the results from samples collected at NMFS station 4 on 6/26, and NOS 32B represents the results from the NMFS station 4 6/29 collection. In addition, a temporal comparison of samples collected at NOS 32 on 6/26 and 6/29 was performed.

NOS station TM or %DNA means were calculated for each tissue, combining the values from NMFS stations in the proximity of NOS sampling sites. Gonad sample numbers were not adequate for statistical comparisons. In most cases gonad samples yielded germ cell (sperm) nuclei with some easily distinguishable somatic cells mixed in. It was found that the gonad samples from female fish contained somatic cells exclusively. Gonad samples were prepared very gently, with no disruption of the tissue. Sperm dislodge

from the tissue in great numbers without effort in mature fish that are not spent. Gonad samples from female fish were collected for comparison and in all cases yielded somatic cell nuclei.
NOS station results are in Figure 1 comparing TM values & Figure 2 comparing %DNA. Liver TM and %DNA were significantly elevated at NOS 40 compared to NOS 32A and 34, $P<0.05$. Station to station comparisons of NMFS stations 4A,4B, 42, 38, 21, 18, and 22 were performed. Blood cell damage was not significantly increased at any station. Liver damage at station 21 was greater than stations 4A, 42, and 22 for both TM (figure 3) and %DNA (figure 4). Both the NOS and NMFS station comparisons indicate that mangrove snapper in the mid estuary area of St. Lucie inlet had greater than expected levels of DNA damage in their livers.
NMFS station 4 was sampled on 6/26 (4A) and 64 hrs. later on 6/29 (4B). Station 4A blood and liver damage was statistically lower than 4B, $P<0.05$ and $P<0.01$ respectively. A similar relationship was found when comparing %DNA results though only liver damage was found to be significantly elevated, $P<0.01$. Thunderstorms persisted from the evening of the 26th until the evening of the 28th , so all stations were exposed to stormwater run-off with the exception of the 4A sampling, and damage at 4A was lower than any other station. Fish collected at NMFS stations 42 and 22 were exposed to stormwater run-off and still had damage levels significantly lower than station 21 samples. Therefore it would seem that stormwater exposure alone does not result in significant increases in DNA damage. It may be that contaminants or other components associated with stormwater at that station may cause increased DNA strand damage.

The other target fish species were in lower abundance and spatial extent, results from pompano and spottail pinfish samples are included in figures 5 & 6. Pompano (figure 5), numbers only allowed statistical comparisons between samples gathered at NOS 32 A & B (NMFS station 4A & 4B). Unlike the snappers no significant difference in DNA strand break levels was observed. The presumed influence of stormwater run-off was not evident in this species. Spottail results (figure 6) were similar to the snapper, samples from NOS station 32B and the Jupiter Inlet trestle and lighthouse collection sites (NOS 47) all had similar levels of strand breakage. This would support snapper results which showed similar levels of damage in samples collected at NOS 32 on 6/29 and Jupiter Inlet station NMFS 42.

Since no fish with abnormalities were observed it was not possible to determine whether such fish had significantly elevated DNA damage compared to apparently normal fish.

This lab has a large DNA damage database for various fish species. How do the baseline levels of DNA damage in other species compare to those found in the south Florida mangrove snapper? Table 5 is a summary of the damage levels (TM) found in the tissues of various flatfish species collected off the coast of southern California along with the low and high end levels found in the mangrove snapper during this study. In the case of the hornyhead turbot significantly elevated values resulting from moderate chronic exposure to urban contaminants are included as well. Snapper DNA damage in both blood and liver appears to be quite similar to that found in tissues from English sole and bigmouth sole.
More indepth interpretation of these data will be possible once the chemistry and histopathology results are received.

Table 2. Codes Legend

Species:	Genus species	Code
Irish pompano	*Trachinotus carolinus*	P
Mangrove snapper	*Lutjanus griseus*	M
Spottail pinfish	*Diplodus holbrooki*	S
Lane snapper	*Lutjanus synagris*	L
White grunt	*Haemulon plumieri*	W
Tissues:		**Code**
Blood		B
Liver		L
Gonad		G

Table 3. St. Lucie Fish Sampling Datasheet

NMFS#	NOS#	Date	Species	#	(cm) Length	Sex	Tissues
4	32	6/26/01	m	1	17.5	?	B,L
4	32	6/26/01	w	1	19	?	B,L,G
4	32	6/26/01	m	2	21.5	?	B,L,G
4	32	6/26/01	m	3	12.5	?	B,L,G
4	32	6/26/01	p	1	29	F	B,L,G
4	32	6/26/01	p	2	19.5	M	B,L,G
4	32	6/26/01	p	3	17	M	B,L,G
4	32	6/26/01	p	4	21	M	B,L,G
4	32	6/26/01	p	5	17.5	M	B,L,G
4	32	6/26/01	p	6	19	M	B,L,G
4	32	6/26/01	p	7	19	F	B,L,G
4	32	6/26/01					
W.trestle	47	6/27/01	s	1	16	?	B,L,G
W.trestle	47	6/27/01	s	2	16	?	B,L,G
W.trestle	47	6/27/01	s	3	15	?	B,L,G
W.trestle	47	6/27/01	s	4	15.5	?	B,L,G
Trestle	47	6/27/01	s	5	14.5	?	B,L
Trestle	47	6/27/01	s	6	14	?	B,L,G
Trestle	47	6/27/01	s	7	14	?	B,L,G
Trestle	47	6/27/01	s	8	13.5	?	B,L,G
Trestle	47	6/27/01	s	9	13.5	?	B,L
Trestle	47	6/27/01	s	10	15	?	B,L
Bridge	47	6/27/01	l	1	14.5	?	B,L
Bridge	47	6/27/01	l	2	16	?	B,L
Bridge	47	6/27/01	l	3	13	?	B,L
Bridge	47	6/27/01	l	4	14.5	?	B,L
Bridge	47	6/27/01	l	5	13	?	B,L
#42	47	6/27/01	m	1	16	?	B,L
#42	47	6/27/01	m	2	15.5	?	B,L
#42	47	6/27/01	m	3	18	M	B,L,G

#42	47	6/27/01	m	4	28.5	M	B,L,G
#42	47	6/27/01	m	5	10.5		B,L
38	40	6/28/01	p	1	26	F	B,L,G
38	40	6/28/01	m	1	25	M	B,L,G
38	40	6/28/01	m	2	13.5	?	B,L
38	40	6/28/01	m	3	16	?	B,L
38	40	6/28/01	m	4	18	?	B,L
38	40	6/28/01	m	5	9.5	?	B,L
21	40	6/28/01	p	2	26	F	B,L
21	40	6/28/01	m	6	20	M	B,L
21	40	6/28/01	m	7	15	?	B,L
21	40	6/28/01	m	8	14	?	B,L
21	40	6/28/01	m	9	20	?	B,L,G
21	40	6/28/01	m	10	15	M	B,L,G
18	33	6/28/01	p	1	20	M	B,L,G
18	33	6/28/01	m	1	18	?	B,L
18	33	6/28/01	m	2	15	?	B,L,G
18	33	6/28/01	m	3	17.5	?	B,L
18	33	6/28/01	m	4	30	F	B,L,G
18	33	6/28/01	m	5	17	?	B,L
35	33	6/28/01	p	2	20	F	B,L,G
22	34	6/28/01	m	6	13.5	?	B,L
22	34	6/28/01	m	7	20	?	B,L
22	34	6/28/01	m	8	13	?	B,L
22	34	6/28/01	m	9	20	?	B,L
22	34	6/28/01	m	10	15.5	?	B,L
4	32	6/29/01	p	1	20	M	B,L,G
4	32	6/29/01	p	2	20	M	B,L,G
4	32	6/29/01	p	3	22	F	B,L
4	32	6/29/01	m	1	15	?	B,L
4	32	6/29/01	m	2	17	?	B,L
4	32	6/29/01	m	3	18	?	B,L
4	32	6/29/01	m	4	20	?	B,L
4	32	6/29/01	s	1	17.5	?	B,L
4	32	6/29/01	m	5	19	?	B,L
4	32	6/29/01	s	2	17	?	B,L
4	32	6/29/01	s	3	11	?	B,L
4	32	6/29/01	s	4	15.5	?	B,L
4	32	6/29/01	s	5	18	?	B,L

Table 4. DNA Damage Analysis Results-

Date	NMFS#	NOS#	Species	#	Tissue	TM	Station TM	sem		% DNA in Tail	Mean	sem
6/26/01	4	32	M	1	B	3.8	**5.4**	**1.2**		17.0	**21.2**	**2.6**
6/26/01	4	32	M	2	B	4.6				20.5		
6/26/01	4	32	M	3	B	7.7				26.1		
6/26/01	4	32	M	2	G	22.1	**15.9**			59.4	**45.1**	
6/26/01	4	32	M	3	G	9.7				30.7		
6/26/01	4	32	M	1	L	12.7	**8.9**	**2.0**		37.0	**29.5**	**3.8**
6/26/01	4	32	M	2	L	5.9				24.5		
6/26/01	4	32	M	3	L	8.1				27.0		
6/26/01	4	32	P	1	B	3.5	**4.3**	**1.1**		15.5	**17.6**	**3.1**
6/26/01	4	32	P	2	B	0.6				5.7		
6/26/01	4	32	P	3	B	4.0				16.6		
6/26/01	4	32	P	4	B	7.9				29.1		
6/26/01	4	32	P	5	B	8.1				27.1		
6/26/01	4	32	P	6	B	1.9				11.0		
6/26/01	4	32	P	7	B	3.9				18.2		
6/26/01	4	32	P	1	G*	0.1	**1.6**	**0.2**		3.9	**7.6**	**0.6**
6/26/01	4	32	P	2	G	0.4				3.3		
6/26/01	4	32	P	2	G	0.4				4.5		
6/26/01	4	32	P	3	G	0.9				7.2		
6/26/01	4	32	P	4	G	0.3				3.7		
6/26/01	4	32	P	5	G	0.3				4.7		
6/26/01	4	32	P	6	G	1.6				6.5		
6/26/01	4	32	P	7	G*	8.8				27.3		
6/26/01	4	32	P	1	L	15.5	**9.2**	**1.7**		42.5	**27.0**	**3.9**
6/26/01	4	32	P	2	L	9.7				27.1		
6/26/01	4	32	P	3	L	6.9				22.1		
6/26/01	4	32	P	4	L	3.9				14.8		
6/26/01	4	32	P	5	L	14.9				39.1		
6/26/01	4	32	P	6	L	6.3				18.6		
6/26/01	4	32	P	7	L	7.6				24.7		
6/29/01	4	32	M	1	B	9.2	**9.0**	**1.0**		27.9	**28.1**	**2.8**
6/29/01	4	32	M	2	B	7.0				25.8		

6/29/01	4	32	M	3	B	10.3				30.3		
6/29/01	4	32	M	4	B	6.4				19.5		
6/29/01	4	32	M	5	B	12.1				36.9		
6/29/01	4	32	M	1	L	13.2	**14.7**	**0.7**		35.9	**39.7**	**1.3**
6/29/01	4	32	M	2	L	16.9				43.5		
6/29/01	4	32	M	3	L	14.2				38.9		
6/29/01	4	32	M	4	L	13.7				38.5		
6/29/01	4	32	M	5	L	15.5				41.7		
6/29/01	4	32	P	1	B	3.0	**6.2**	**1.8**		13.8	**22.1**	**4.7**
6/29/01	4	32	P	2	B	6.2				22.3		
6/29/01	4	32	P	3	B	9.3				30.1		
6/29/01	4	32	P	3	G	5.2				17.3		
6/29/01	4	32	P	1	L	5.8	**8.0**	**3.1**		18.1	**24.3**	**8.6**
6/29/01	4	32	P	2	L	4.2				13.5		
6/29/01	4	32	P	3	L	14.1				41.2		
6/29/01	4	32	S	1	B	6.8	**5.1**	**0.5**		23.9	**19.8**	**1.1**
6/29/01	4	32	S	2	B	5.7				20.0		
6/29/01	4	32	S	3	B	4.8				18.0		
6/29/01	4	32	S	4	B	4.0				17.9		
6/29/01	4	32	S	5	B	4.1				19.1		
6/29/01	4	32	S	1	L	6.7	**9.5**	**1.8**		22.0	**28.4**	**4.6**
6/29/01	4	32	S	2	L	12.5				35.2		
6/29/01	4	32	S	3	L	11.5				33.8		
6/29/01	4	32	S	4	L	3.8				13.3		
6/29/01	4	32	S	5	L	13.1				37.5		
6/27/01	Bridge	47	L	1	B	8.0	**6.5**	**0.9**		28.2	**21.9**	**2.6**
6/27/01	Bridge	47	L	2	B	4.1				15.8		
6/27/01	Bridge	47	L	3	B	5.1				16.7		
6/27/01	Bridge	47	L	4	B	8.9				27.3		
6/27/01	Bridge	47	L	5	B	6.3				21.3		
6/27/01	Bridge	47	L	1	L	13.0	**15.6**	**1.2**		36.7	**42.4**	**2.2**
6/27/01	Bridge	47	L	2	L	13.9				40.9		
6/27/01	Bridge	47	L	4	L	18.0				46.6		
6/27/01	Bridge	47	L	5	L	17.4				45.3		
6/27/01	42	47	M	1	B	15.9	**11.1**	**2.5**		43.0	**32.5**	**5.3**

6/27/01	42	47	M	2	B	14.7				40.0		
6/27/01	42	47	M	3	B	15.0				40.3		
6/27/01	42	47	M	4	B	5.5				20.5		
6/27/01	42	47	M	5	B	4.6				18.5		
6/27/01	42	47	M	3	G	3.1	**5.3**	**1.7**		11.8	**16.4**	**4.4**
6/27/01	42	47	M	4	G	7.6				21.0		
6/27/01	42	47	M	1	L	8.6	**13.6**	**1.8**		27.1	**37.1**	**3.6**
6/27/01	42	47	M	2	L	19.2				46.9		
6/27/01	42	47	M	3	L	11.3				31.3		
6/27/01	42	47	M	4	L	14.3				42.8		
6/27/01	42	47	M	5	L	14.4				37.5		
6/27/01	W. Trestle	47	S	1	B	4.5	**8.5**	**2.1**		20.5	**26.9**	**5.3**
6/27/01	W. Trestle	47	S	2	B	12.7				40.0		
6/27/01	W. Trestle	47	S	3	B	4.4				16.7		
6/27/01	W. Trestle	47	S	4	B	10.0				31.4		
6/27/01	W. Trestle	47	S	3	G	0.5	**7.4**			4.2	**21.5**	
6/27/01	W. Trestle	47	S	4	G	14.3				38.8		
6/27/01	W. Trestle	47	S	1	L	5.7	**9.6**	**2.4**		16.1	**29.4**	**6.1**
6/27/01	W. Trestle	47	S	2	L	8.3				29.3		
6/27/01	W. Trestle	47	S	3	L	7.9				26.6		
6/27/01	W. Trestle	47	S	4	L	16.5				45.7		
6/27/01	Trestle	47	S	5	B	5.6	**7.3**	**2.0**		19.0	**24.1**	**4.6**
6/27/01	Trestle	47	S	6	B	4.9				19.5		
6/27/01	Trestle	47	S	7	B	17.4				46.5		
6/27/01	Trestle	47	S	8	B	5.9				18.4		
6/27/01	Trestle	47	S	9	B	6.1				24.3		
6/27/01	Trestle	47	S	10	B	4.1				17.0		
6/27/01	Trestle	47	S	6	G	11.2	**10.0**			32.6	**29.8**	
6/27/01	Trestle	47	S	8	G	8.9				27.1		
6/27/01	Trestle	47	S	5	L	5.6	**10.2**	**1.0**		17.8	**30.2**	**2.7**
6/27/01	Trestle	47	S	6	L	10.8				32.6		
6/27/01	Trestle	47	S	7	L	9.3				28.7		
6/27/01	Trestle	47	S	8	L	13.1				37.3		
6/27/01	Trestle	47	S	9	L	11.6				33.4		
6/27/01	Trestle	47	S	10	L	10.7				31.7		

6/28/01	38	40	M	1	B	11.1	**10.3**	**0.7**		33.8	**31.7**	**2.0**
6/28/01	38	40	M	2	B	10.9				33.6		
6/28/01	38	40	M	3	B	12.4				36.8		
6/28/01	38	40	M	4	B	8.5				26.7		
6/28/01	38	40	M	5	B	8.9				27.4		
6/28/01	38	40	M	1	G	0.2				2.9		
6/28/01	38	40	M	1	L	15.9	**18.0**	**2.7**		41.2	**46.4**	**6.1**
6/28/01	38	40	M	2	L	18.1				43.9		
6/28/01	38	40	M	3	L	25.0				63.0		
6/28/01	38	40	M	4	L	21.5				56.0		
6/28/01	38	40	M	5	L	9.3				27.9		
6/28/01	38	40	P	1	B	11.3				31.1		
6/28/01	38	40	P	1	G	6.9				24.0		
6/28/01	38	40	P	1	L	8.7				26.9		
6/28/01	21	40	M	6	B	12.8	**10.0**	**1.1**		38.0	**31.8**	**2.8**
6/28/01	21	40	M	7	B	7.6				26.0		
6/28/01	21	40	M	8	B	8.3				25.8		
6/28/01	21	40	M	9	B	12.8				38.6		
6/28/01	21	40	M	10	B	8.8				30.6		
6/28/01	21	40	M	6	G	9.3	**14.3**	**2.8**		25.3	**38.1**	**7.5**
6/28/01	21	40	M	9	G	14.6				38.0		
6/28/01	21	40	M	10	G	19.0				51.1		
6/28/01	21	40	M	6	L	16.7	**22.2**	**3.0**		44.1	**56.0**	**6.3**
6/28/01	21	40	M	7	L	13.9				39.4		
6/28/01	21	40	M	8	L	28.7				68.9		
6/28/01	21	40	M	9	L	28.0				70.0		
6/28/01	21	40	M	10	L	23.7				57.8		
6/28/01	21	40	P	2	B	3.7				14.0		
6/28/01	21	40	P	2	G	8.4				26.2		
6/28/01	21	40	P	2	L	6.0				20.3		
6/28/01	18	33	M	1	B	9.3	**9.5**	**1.1**		29.8	**30.4**	**2.9**
6/28/01	18	33	M	2	B	13.6				40.8		
6/28/01	18	33	M	3	B	8.8				29.6		
6/28/01	18	33	M	4	B	8.9				28.9		

6/28/01	18	33	M	5	B	6.6				22.8		
6/28/01	18	33	M	4	G*	15.8				46.6		
6/28/01	18	33	M	1	L	14.1	**16.3**	**1.3**		40.3	**44.3**	**3.7**
6/28/01	18	33	M	2	L	19.1				52.4		
6/28/01	18	33	M	3	L	19.4				51.8		
6/28/01	18	33	M	4	L	15.9				44.5		
6/28/01	18	33	M	5	L	12.8				32.7		
6/28/01	18	33	P	1	B	5.0				18.0		
6/28/01	18	33	P	1	G	0.3				2.6		
6/28/01	18	33	P	1	L	10.0				31.5		
6/28/01	35	33	P	2	B	6.2				19.1		
6/28/01	35	33	P	2	G*	14.2				39.1		
6/28/01	35	33	P	2	L	9.2				29.1		
6/28/01	22	34	M	6	B	14.7	**9.8**	**1.5**		43.6	**30.5**	**4.0**
6/28/01	22	34	M	7	B	6.7				24.4		
6/28/01	22	34	M	8	B	11.8				35.8		
6/28/01	22	34	M	9	B	7.4				22.4		
6/28/01	22	34	M	10	B	8.3				26.5		
6/28/01	22	34	M	6	L	12.7	**12.3**	**1.4**		31.5	**33.4**	**2.8**
6/28/01	22	34	M	7	L	11.7				33.9		
6/28/01	22	34	M	8	L	7.7				23.7		
6/28/01	22	34	M	9	L	16.0				39.4		
6/28/01	22	34	M	10	L	13.6				38.5		

L=Lane snapperG=not germ cells, somatic cells.

Table 5. Species DNA Damage Comparison

Species	Common name	Baseline TM Blood	Liver	Elevated TM Blood	Liver
Lutjanus griseus	Mangrove snapper	5.4 ± 1.2	8.9 ± 2.0	10.1 ± 1.1	22.2 ± 3.0
Pleuronichthys verticalis	Hornyhead turbot	0.34 ± 0.05	1.9 ± 0.7	1.46 ± 0.55	6.2 ± 0.93
Pleuronectes vetulus	English sole	4.1 ± 1.6	8.3 ± 1.6		
Hippoglossina stomata	Bigmouth sole	3.1 ± 0.4	5.3 ± 1.2		

Figure 1.

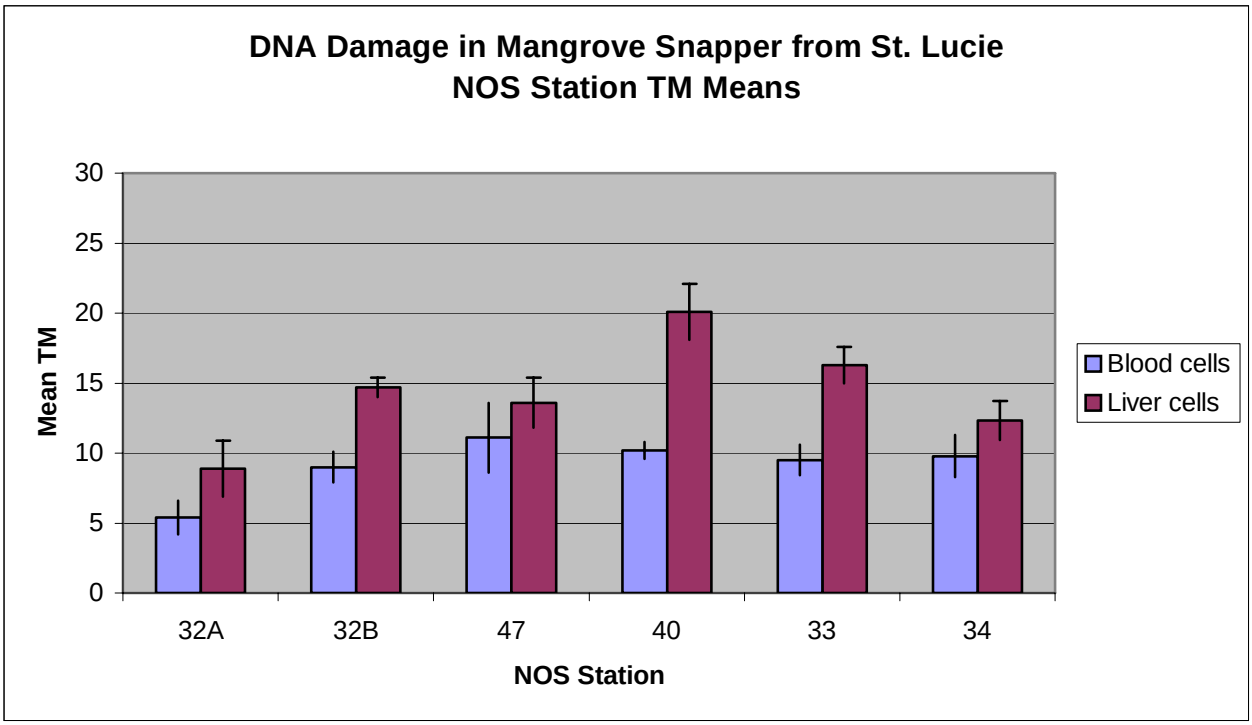


Figure 2.

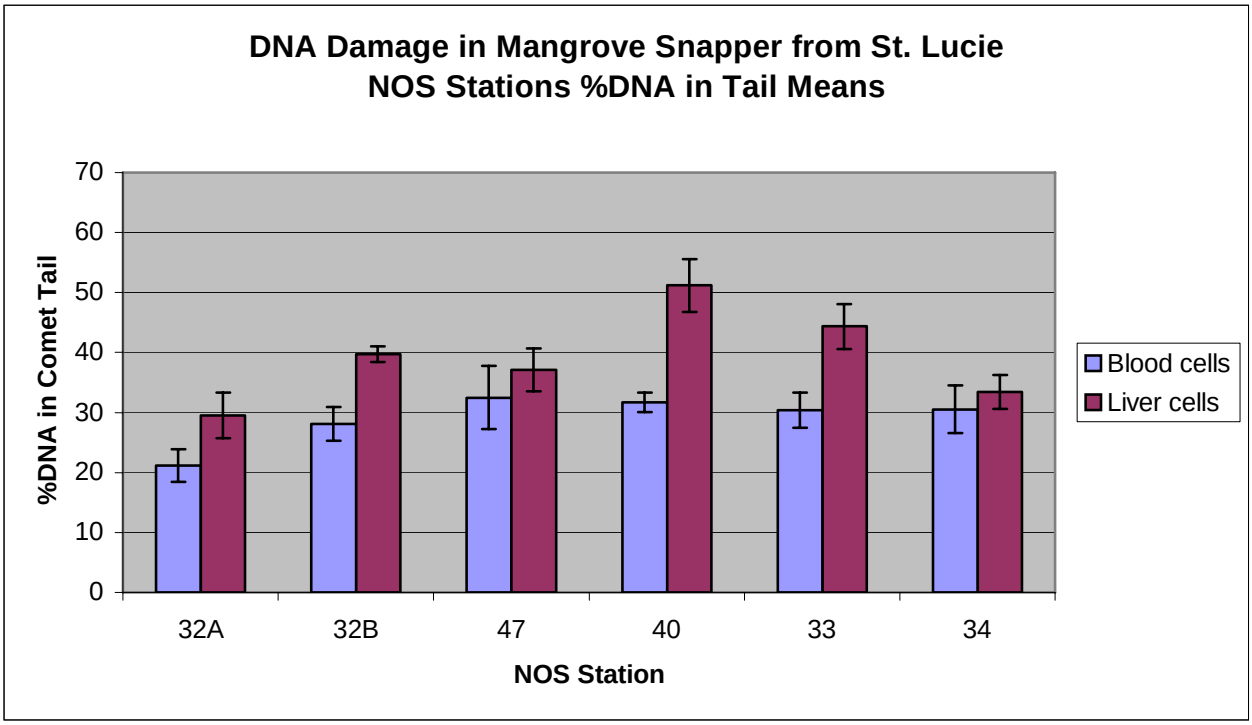


Figure 3.

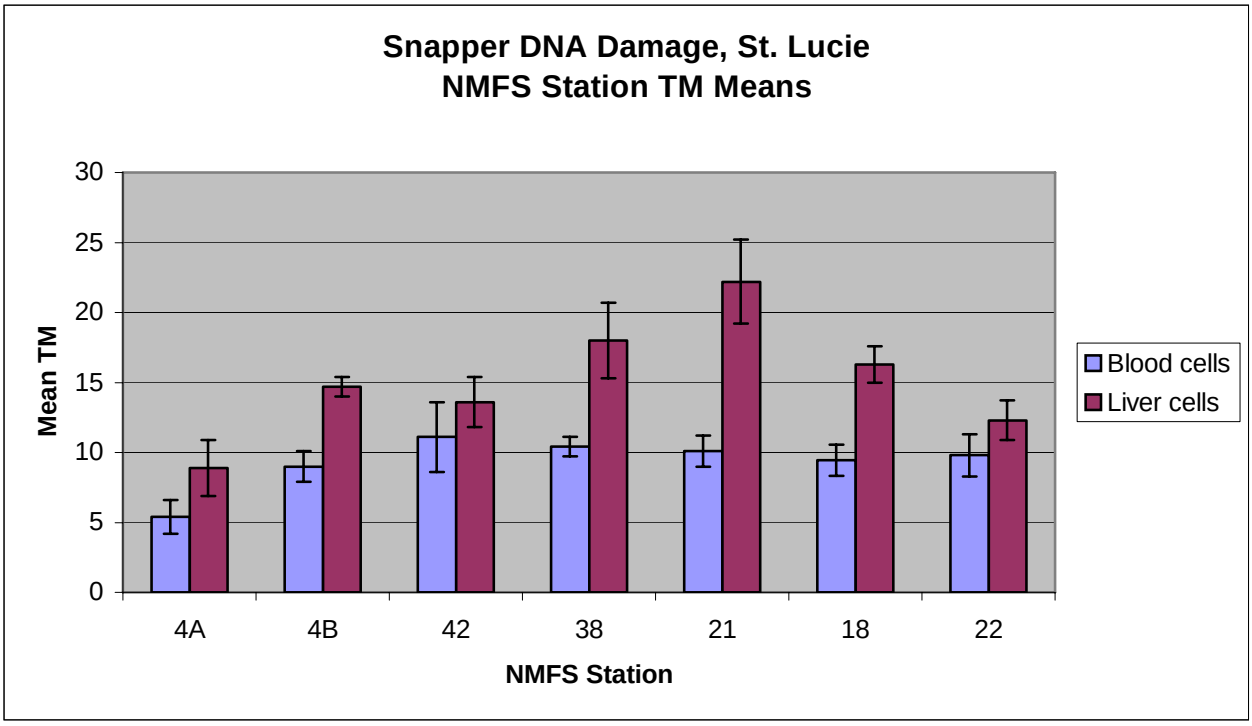


Figure 4.

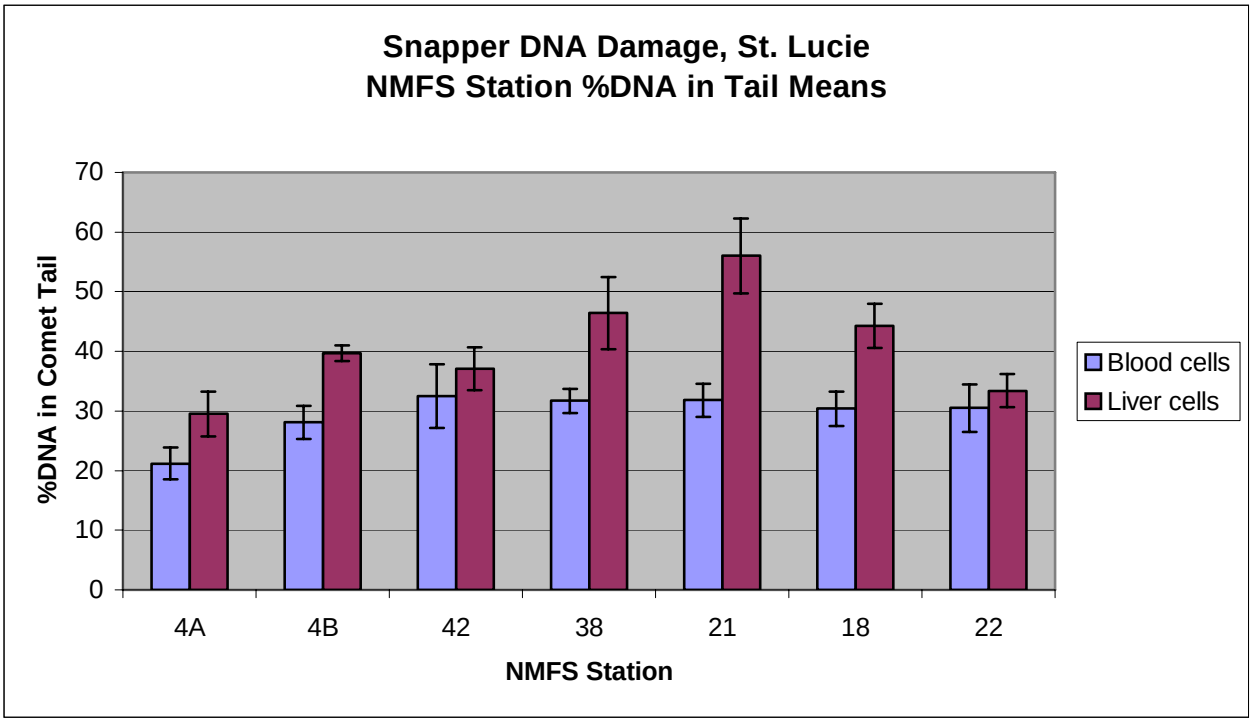


Figure 5.

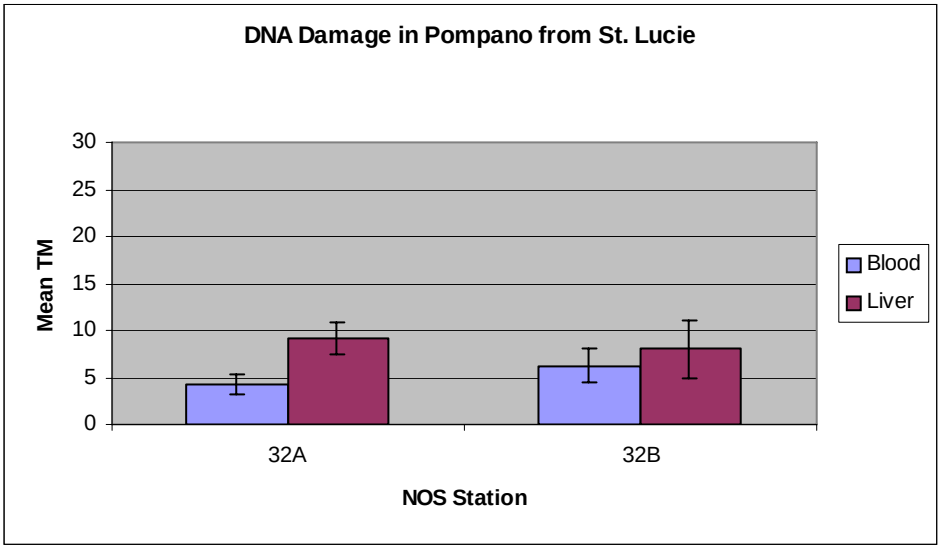
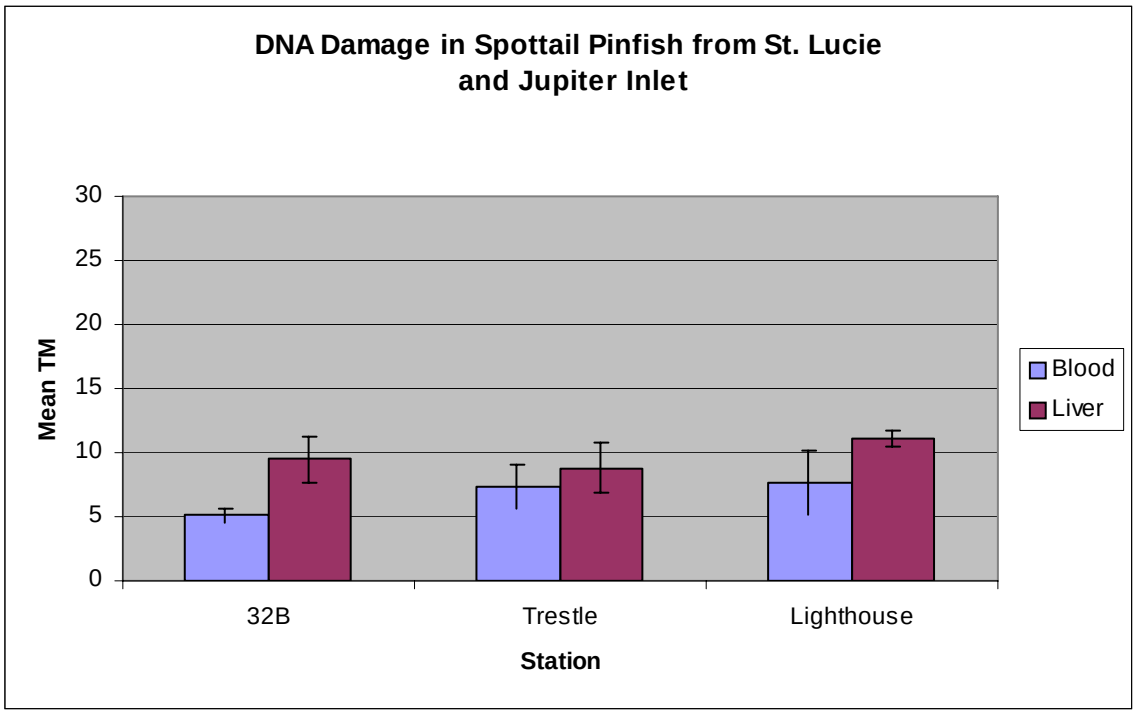


Figure 6.


DNA Damage in Spottail Pinfish from St. Lucie and Jupiter Inlet
Mean TM
30
25
20
15
10
5
0
32B
Trestle
Lighthouse
Station
Blood
Liver