JIMAR, PFRP ANNUAL PROGRESS REPORT
FY 2001

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Project Proposal Title: Developing Biochemical And Physiological Predictors Of Long Term Survival In Released Blue Sharks

Funding Agency: NOAA, NMFS, JIMAR, Pelagic Fisheries Research Program

1. Purpose of the project and indicative results.

For catch-and-release sports fishing and non-retention of commercially caught fish to be justifiable management options, there must be a reasonable likelihood that released fish will survive long term. At present, there is no scientific basis for making this prediction for any large pelagic fish. Therefore, even when recreational anglers and commercial fisherman practice good catch-and-release fishing, high rates of delayed mortality are a distinct possibility. Tag-and-release programs are important tools to assessing post-release survival, but they can be difficult and expensive to implement. Conclusions from tag-and-release studies are rarely extrapolated to other species because of the many factors (e.g. size, water temperature, fight time and fishing gear) that may influence survivability or mortality. We propose a novel approach to study the basis of post-release mortality. Rather than assessing how many fish survive, we try to understand **why fish die**. We are developing a set of diagnostic tools to assess the biochemical and physiological status of sharks caught by long line on scientific cruises. These tools will be used in combination with pop-off satellite archival tag (PSAT) data to establish correlates of survival or mortality.

We have focused on assessing the extent of tissue damage arising from capture using comprehensive analyses of ions, metabolites and proteins found in the plasma (discussed in detail in our proposal). For example, the damage to myocardial tissue upon a heart attack causes release of proteins such as creatine phosphokinase and troponin I into the plasma. We are also using the properties of blood cells themselves to assess the extent of systemic oxidative damage. Under stressful conditions, a series of genes are induced leading to synthesis of mRNA and protein corresponding to the heat shock proteins (hsp). We have used hsp70 induction in a number of fish models as an index of cellular damage.

2. Progress during FY 2001. Provide a thorough discussion of accomplishments and problems.

There have been no significant technical problems to date. Logistically, we were delayed by mechanical problems with the R/V Townsend Cromwell. Also, I hope to obtain a larger number of shark samples in the next cruise.

12/2000.

- Moyes presents experimental plan at PFRP Principal Investigator Meeting
- Meetings at Kewalo Basin with co-investigators Brill and Musyl to discuss logistics of spring cruise on RV Townsend Cromwell.
- Collected archived samples of tuna and marlin from Brill to initiate DNA collection.
- Sub-contract with U. Hawaii Research Corporation signed, releasing PFRP funds.

1/2001. Nuno Fragoso (M.Sc. Queen's University) hired to conduct collection and analyses of shark samples.

3/2001. Fragoso travels to Hawaii for scientific cruise of RV Townsend (TC-01-03).

4/2001. During the sixteen days at sea, eleven long line sets were made, with approximately 4,000 hooks set. A total of 139 fish and sharks were caught. Twenty percent of the animals snared on the long line were blue sharks and nineteen of these animals were landed. Of the nineteen sharks landed, fourteen were tagged with PSATs and released. Blood samples were collected from eleven of fourteen tagged sharks. The remaining five sharks were dead or seriously injured and sampled for white, red, and heart muscle along with blood. Thus blood samples were collected from a total of fifteen sharks. Samples were processed at Kewalo Basin and prepared for transport to Queen's University for analyses.

Since 5/2001.

- Completion of first set of plasma analyses of ions, metabolites and proteins (see Table 1).
- Development of cDNA probes for northern analyses.

RESULTS TO DATE

1. PSATS: Complete information on the status of the PSATs can be found in the progress report from Musyl and Brill. Although several tags have been released, data from two satellite tags (PSAT #13081 and #13091) have transmitted data back to us. The blue shark tagged with PSAT #13091, showed some apparently normal vertical behavior for the first five days, then expired and sank. The tag automatically jettisoned once the shark reached a depth greater then 3500 ft, floated to the surface and downloaded its data. This confirms that the PSAT systems are working properly. More importantly it allows us to determine the duration of survival.

2. Plasma analyses: Plasma samples were separated from erythrocytes on board the Townsend Cromwell were stored in liquid nitrogen and transported via a dry shipper. Sixteen separate analyses have been performed on the plasma samples (Table 1). Several sharks had significantly elevated creatine phosphokinase (CK; 5, 9, 10, 11, 12, 13, 16 and 18) and lactate dehydrogenase (LDH; 10, 11, 13 and 18) levels, which are indicative of severe muscle damage. Five sharks (2, 3, 7, 11, 17 and 18) had signs of elevated lactate levels, which would be indicative of exhaustive exercise. Shark number two may have

suffered a fatal heart attack as it was dead upon landing and had elevated levels of troponin T.

***3. Molecular analyses*:** RNA and DNA samples have been purified from target tissues. Preparation of shark specific cDNA probes for heat shock protein analyses is underway. Analytical work (e.g. northern analysis) will commence July/2001.

Table 1: Ion, metabolite and protein analyses of plasma from tagged, released sharks.

	Tagged sharks											Untagged sharks			
	8	**16**	**7**	**14**	**12**	**15**	**9**	**13**	**11**	**10**	**3**	**2**	**5**	**17**	**18**
Na^{2+}	258	266	280	260	258	254	256	260	282	264	272	-	-	-	-
K^{+}	4.2	4.8	5.6	4.6	5.6	5	5.2	5.2	5	**9**	**7.2**	6	5.8	5	7.9
Cl^{-}	228	238	232	232	234	232	228	234	236	226	232	-	-	-	-
Glucose	5.2	7.4	4	6	3.6	5.6	6.2	5	5.2	4.4	1.2	6.6	4.6	5.9	2.96
Ca^{2+}	3.04	3.22	3.4	2.94	2.82	3.18	3.02	3.14	3.1	2.3	3.14	3.01	2.82	3.09	3.93
Mg^{2+}	0.8	1.2	1	0.8	1	0.8	0.8	1	1	1	1.2	1.7	1.1	1	1.75
Lactate	4.8	4	**23.6**	1.4	0.6	1.4	3	1.6	**17.6**		**18.2**	8.2	3.9	**18.9**	**18.4**
urea	366	360	330	362	350	360	364	362	324	328	358	-	436	400	404
TnT	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**0.629**	nd	nd	nd
CK-MB	0.4	1.4	0.6	0.8	1	0.4	<0.1	<0.1	<0.1	<0.0	1.2	<0.1	<0.1	<0.1	<0.1
CK	48	92	370	396	**1588**	**2116**	**4192**	**5136**	**7588**	**25642**	<10	91	**2649**	**871**	**4457**
AST	8	<4	8	<4	24	28	74	36	70	**252**	16	58	12	19	103
ALT	<4	<5	100	<4	<4	<4	8	<4	<4	8	<4	7	<4	<4	<4
LDH	<5	<5	<3	40	**302**	**536**	**592**	**1094**	**1560**	**5184**	118	46	631	369	976

(nd) is not detected and (-) is not determined

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3. Plans for the next fiscal year.

1. Molecular analyses: The levels of hsp mRNA and protein are sensitive indices of oxidative stress in a spectrum of tissues and species. We have developed a set of specific HSP70 primers that will allow us to prepare cDNA probes suitable for quantification of HSP70 mRNA levels in a spectrum of species. Once this is completed we hope to compare these levels with HSP70 protein levels via western analysis.

2. Subsequent NMFS cruises: We plan to expand our sampling profile on next springs long-line cruise. Based upon our experience this spring, we plan to expand the complexity of analyses to be conducted on-board.

3. Other programs: A major goal of our work has been to expand the use of appropriate molecular and biochemical approaches to assessing stress in released non-target species. To this end, we will participate in a study proposed by Dr. Jeff Graham at Scripps Institute of Oceanography, pending a successful outcome of his request for funds from Sea Grant funds to study post-release mortality in mako sharks and billfish. We hope to use the tools developed in the PFRP project in his study. His post-doctoral fellow, Diego Bernal, will come to Queen's University to undertake these analyses. The costs of his

travel and analyses of samples are included in the Sea Grant request. We will continue to explore opportunities to expand the use of our approach in this area of fisheries management.

4. List of papers published in refereed journals during FY 2001.

Yang H, GF Tibbits , J. Velema , M Hedrick & **CD Moyes**. Evolutionary and physiological variation in cardiac troponin C in relation to thermal strategies of fish. *Physiol. Biochem. Zool.* 73:841-849, 2000.

Lund SG, M.C. Phillips, **C.D. Moyes** & B.L.Tufts.The effects of cell ageing on protein synthesis in rainbow trout (*Oncorhynchus mykiss*) red blood cells. *J Exp Biol*. 203:2219-2228, 2000.

Phillips M.C.L., **C.D. Moyes** & B.L. Tufts. The effects of cell ageing on metabolism of nucleated red blood cells. *J. Exp. Biol*. 03: 1039-1045, 2000.

CD Moyes, ML Sharma, C Lyons, SC Leary, M Leon, A Petrie, S Lund, BL Tufts, Origins and consequences of mitochondrial decline in nucleated erythrocytes. Submitted to *Am. J. Physiol.* May 2001.

White RJ, GP Morris, **CD Moyes**, MG Blennerhassett, CE Hill, GC Pringle, WG Paterson. Analysis of the muscoal stress response in acid-induced esophagitis in opossum. *Am. J. Physiol*. 2001 (1st revision under review).

5. Other papers, technical reports, meeting presentations, etc.

PFRP PI meeting, Honolulu, Dec 2000

6. Names of students graduating with MS or Ph.D. degrees during FY 2001. Include title of thesis or dissertation.

M. Sharma (M.Sc., September 2000) *Interactions between ageing, oxidative stress and mitochondria in fish erythrocytes.*