

**JIMAR, PFRP ANNUAL PROGRESS REPORT
FY 2002**

P.I. Name: Christopher D. Moyes

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

Funding Agency: PFRP

Project Purpose 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 are being developed in combination with pop-off satellite archival tag (PSAT) data to establish predictors of survival.

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.

Project Activities and Progress During FY 2002:

Milestones

Problems

There have been no significant technical problems to date.

Accomplishments

7/2001. Upon notice of successful renewal of PRFP grant, I was able to renew the contract of Nuno Fragoso for sample collection and analyses.

8/2001. At this point we were informed that early PSAT data was available for a couple fish. Analyses of their tracking data suggest these tags were released prematurely. The remainder of tagged sharks had survived more than 3 months following release.

9/2001. A new MSc student (Anne Dalziel) begins her research focused on analysis of archived samples of sharks and billfish for stress analyses.

7/2001-3/2002. Nuno Fragoso continues analysis of shark samples obtained on the 4/2001 Townsend cruise, focusing on analyses of stress proteins

3/2002. Fragoso travels to Hawaii for scientific cruise of RV Townsend (TC-01-03).

4/2002. During the twenty-nine days at sea, nineteen long-line sets were made, with over 9,000 hooks set. A total of 207 fish and sharks were caught. Forty-seven percent of the animals snared on the long line were blue sharks (98 individuals) and twenty-six of these animals were landed. Of the twenty-six sharks landed, two sharks were dead while the rest were tagged with PSATs (18 individuals) or conventional tags (5 individuals) before release. Blood samples were collected from sixteen of the eighteen PSAT tagged sharks, as well as all the conventionally tagged animals and the two dead animals. Thus blood samples were collected from a total of twenty-four blue sharks. Blood was also collected from three oceanic white tip sharks that were tagged with PSATs and released. Samples were processed at Kewalo Basin and prepared for transport to Queen's University for analyses.

5/2002. We prepared an article for the PFRP Newsletter based upon our biochemical analyses.

7/2002. I will present the preliminary results from the PFRP work at American Fisheries Society meeting in Vancouver (Abstract attached as Appendix I).

Results to Date

I. PSATS:

Complete information on the status of the PSATs can be found in the progress report from Musyl and Brill.

2001: All of the PSATs put out in the first year have reported back. None of the tagged sharks showed signs of delayed mortality, suggesting premature release of tags. The remaining PSATs were released at their pre selected time.

2002: To date, none of the PSAT tags placed on blue sharks during this April's cruise (TC-02-02) have reported back.

Conclusions: Clearly, the PSAT approach provides excellent data on survival of released sharks. The data suggest that the majority of released sharks suffer little delayed mortality.

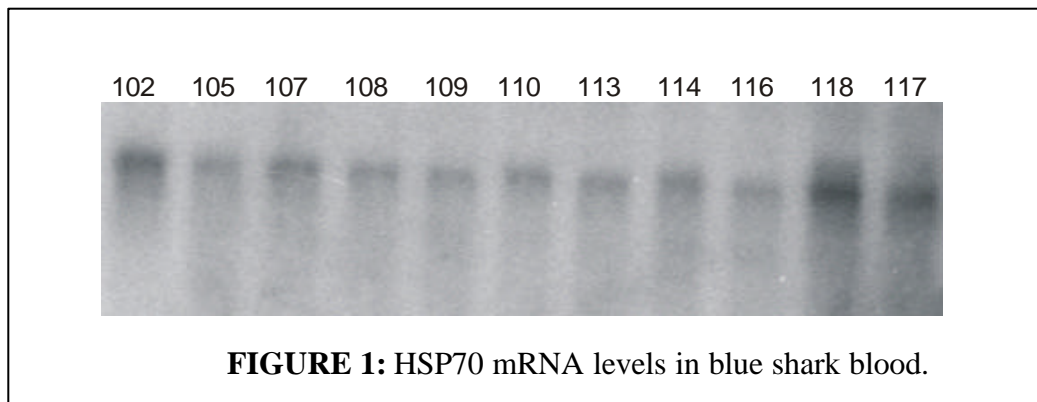
2. Plasma analyses:

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 collected in 2001 and 2002 (Appendix 1; Table 1). Several sharks had significantly elevated creatine phosphokinase (CK; 105, 105-113, 115, 117, 118, 201-203, 205, 206, 209) and lactate dehydrogenase (LDH; 105, 109-111, 113, 115, 118, 203, 209) levels, which are indicative of severe muscle damage. Eleven sharks (102, 103, 107, 111, 117, 118, 201-203, 205, 207) had signs of elevated lactate levels, which would be indicative of exhaustive exercise. Shark number two was dead upon landing and may have suffered a fatal heart attack based on elevated levels of troponin T.

3. Molecular analyses:

The levels of hsp mRNA and protein are sensitive indices of oxidative stress in a spectrum of tissues and species. Using comparative genetic analyses, we developed a set of blue shark-specific HSP70 primers suitable for amplification of a cDNA probe. This allow us to measure levels of HSP70 mRNA levels. RNA was purified from blue shark erythrocytes from the 2001 cruise. Northern analyses (Figure 1) showed elevated levels of HSP70 mRNA in several sharks (Note the darker bands in shark 102, 117, 118). We are currently purifying erythrocyte RNA from sharks sample in the 2002 cruise.



Planned Project Activities for FY 2003:

1. *Molecular analyses:* Blood samples from this year's cruise will be subjected to RNA purification. Northern blot analyses will be conducted to assess the levels of blood stress proteins such as HSP70.

2. *Cooperation with other fisheries 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. We have been approached by several fisheries researchers to incorporate our PFRP approach into their programs.

A) I was listed as a collaborator on a Seda Grant proposal submitted from Scripps Institute of Oceanography by Drs. Diego Bernal and Jeff Graham. Although their grant, focusing on mako sharks was unsuccessful, Dr. Bernal is planning to pursue funds this year to continue work on pelagics.

B) An Alaskan fisheries group who would like to use our approach in assessing the stress conditions of salmon sharks has also approached us. They will be submitting a grant to NOAA to assess the effects of sports fishing practices on stress indices of salmon sharks.

3. Publications

By late summer, the shark PSAT data will tell us which sharks have survived for more than 4 months. At this point we will prepare a manuscript based upon the blood analyses of the shark samples from years 1 and 2.

Papers Published in Journals During FY 2002:

Leary SC, BC Hill, CN Lyons, CG Carlson, D Michaud, CS Kraft, K Ko, DM Glerum & CD Moyes. Chronic treatment with azide *in situ* leads to an irreversible loss of cytochrome *c* oxidase activity via holoenzyme dissociation. *J. Biol. Chem.* 277: 11321-11328, 2002

Moyes CD & DL Hood. Origins and consequences of mitochondrial variation. *Ann. Rev. Physiol* in press.

Wu BW, CD Moyes, KM Thompson, VK Walker & RM Robertson. Anoxia induces thermotolerance in locust flight system. *J. Exp. Biol.* 205:815-827, 2002.

Moyes CD, ML Sharma, C Lyons, SC Leary, M Leon, A. Petri, S Lund & B Tufts. Origins and consequences of mitochondrial decline in nucleated erythrocytes. *Biochim. Biophys. Acta.* in press

Leary SC, D Michaud, C Lyons, T Hale, TL Bushfield, MA Adams & CD Moyes. Bioenergetic remodeling during treatment of spontaneously hypertensive rats with enalapril. *Am J Physiol* in press.

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. *Dig. Dis. Sci.* in press.

Lund SC, P Dymont, MR Gervais, CD Moyes & BL Tufts. Characterization of erythrocyte carbonic anhydrase in an ancient fish, the longnose gar (*Lepisosteus osseus*). Carbonic anhydrase in an ancient fish. *J Comp Physiol B.* in press.

Other Papers, Reports, and Presentations During FY 2002:

A) *PI meetings*: Unfortunately, the dates for the December PFRP meetings frequently collide with my teaching schedule. My courses end in the first week of December and exams begin shortly thereafter. At present, I think my teaching is arranged in a way that will allow me to present our results from years 1 and 2 at the 2002 PI meeting in Honolulu.

B) *PFRP Newsletter*. An article was submitted to Chris Anderson, Editor of the PFRP Newsletter. The article was based upon our biochemical analyses from Year 1.

C) *Other meetings*. This month I will present results from our study at the American Fisheries Society meeting in Vancouver (Abstract attached as Appendix II).

Graduating Students with M.S. or Ph.D. Degrees During FY 2002:

S. Leary (Ph.D. September 2001) *Interactions between bioenergetics and cytochrome c oxidase levels in striated muscles.*

Budget: N/A

Appendix I.

Table 1: Ion, metabolite and protein analyses of plasma from tagged, released sharks (Year 01) and from 16 of year 02 sharks

Shark	Status	HCT	Na ²⁺	K ⁺	Cl ⁻	Iron	Creatine	Glucose	Ca ²⁺	PO ₄ ³⁻	Mg ²⁺	Lactate	Osmolality	CK	AST	ALT
		%	mM	mM	mM	uM	uM	mM	mM	mM	mM	mM	mmol/kg	U/L	U/L	U/L
102	morbid	-	>180	6.0	>140	-	-	6.6	3.01	-	1.70	8.2	NSQ	91	58	7
103	tagged	-	272	7.2	232	-	<10	1.2	3.14	-	1.20	18.2	-	<10	16	<4
105	euthanized	-	>180	5.8	>140	-	-	4.6	2.82	-	1.10	3.9	1167	2649	12	<4
107	tagged	-	280	5.6	232	-	<10	4.0	3.40	-	1.00	23.6	-	370	8	100
108	tagged	-	258	4.2	228	-	<9	5.2	3.04	-	0.80	4.8	-	48	8	<4
109	tagged	-	256	5.2	228	-	<9	6.2	3.02	-	0.80	3	-	4192	74	8
110	tagged	-	264	9.0	226	-	<10	4.4	2.30	-	1.00	NSQ	-	25642	252	8
111	tagged	-	282	5.0	236	-	<9	5.2	3.10	-	1.00	17.6	-	7588	70	<4
112	tagged	-	258	5.6	234	-	<9	3.6	2.82	-	1.00	0.6	-	1588	24	<4
113	tagged	-	260	5.2	234	-	<10	5.0	3.14	-	1.00	1.6	-	5136	36	<4
114	tagged	-	260	4.6	232	-	<10	6.0	2.94	-	0.80	1.4	-	396	<4	<4
115	tagged	-	254	5.0	232	-	<10	5.6	3.18	-	0.80	1.4	-	2116	28	<4
116	tagged	-	266	4.8	238	-	<10	7.4	3.22	-	1.20	4	-	92	<4	<5
117	euthanized	-	>180	5.0	>140	-	-	5.9	3.09	-	1.00	18.9	1189	871	19	<4
118	morbid	-	>180	7.9	>140	-	-	3.0	3.93	-	1.75	18.4	1206	4457	103	18
201	tagged	16	271	5.7	232	6	<8	3.5	3.74	1.92	1.32	33.1	1009	1120	7	<4
202	tagged	23	303	5.3	271	3	<9	8.0	3.90	3.21	1.64	22.6	1088	909	21	<4
203	tagged	23	269	4.4	255	5	<9	5.3	3.49	2.00	1.20	8.7	1060	5047	75	14
204	tagged	18	263	3.8	255	3	<9	3.8	3.30	1.35	0.98	1.8	1055	44	<4	12
205	tagged	17	267	4.5	234	3	11	5.5	3.28	2.79	1.05	8.6	1064	804	14	8
206	tagged	21	255	4.7	234	2	<9	5.0	3.21	2.01	1.13	3.8	1076	1396	21	8
207	euthanized	13	266	5.5	257	1	<9	7.1	3.40	2.22	1.53	8.8	1053	16	<4	<4
208	tagged	21	270	4.8	257	4	31	3.5	3.49	1.35	0.87	5.9	1067	230	4	12
209	tagged	23	256	4.6	252	2	<9	4.4	3.15	1.89	1.06	1.2	1044	5789	65	12
210	tagged	18	274	5.1	254	30	<9	4.8	2.89	2.39	0.80	1.5	1017	55	<4	<4
211	tagged	14	264	3.9	251	20	<9	3.6	3.07	1.82	0.87	2.9	1033	32	<4	<4
212	tagged	21	259	4.3	251	4	<9	4.7	3.17	1.93	1.36	3.1	1054	61	<4	4
213	tagged	17	243	4.1	216	3	<9	5.2	3.24	2.18	0.86	2.9	1049	8	<4	<4
214	tagged	27	262	4.1	255	4	<9	6.1	3.26	1.92	0.98	2.2	1031	21	<4	<4
215	tagged	20	246	4.5	225	2	<9	5.3	3.26	2.14	1.13	4.4	1056	62	<4	6
216	tagged	24	250	3.5	241	3	<9	5.0	3.37	2.70	1.00	1.9	1045	10	<4	10

MEAN 19.9 264 5.1 240.4 6 21 5.0 3.21 2.11 1.09 8.0 1072 2361.333 46 16
MIN 13.1 243 3.5 216 1 11 1.2 2.30 1.35 0.80 0.6 1009 8 4 4
MAX 27.3 303 9.0 271 30 31 8.0 3.93 3.21 1.75 33.1 1206 25642 252 100

Appendix II. American Fisheries Society Conference Proceedings

Developing physiological and biochemical indices of survival in released blue sharks.

Chris Moyes¹, Nuno Fragoso¹, Mike Musyl² and Rich Brill³

¹Department of Biology, Queen's University, Kingston, Canada,

²JIMAR, Honolulu, Hawaii

³NMFS, Kewalo Research Facility, Honolulu Hawaii

EXTENDED ABSTRACT ONLY: DO NOT CITE

Successful management strategies in both sports fisheries and commercial fisheries require information about long-term survival of released fish. Catch-and-release sports fishing and non-retention of commercially caught fish are justifiable management options only if there is a reasonable likelihood that released fish will survive for long periods. All recreational anglers and commercial fisherman who practice catch-and-release fishing hope that the released fish will survive. While it is safe to say that 100% of retained fish will die, it is not known what proportion of released fish will survive. Tag-and-release studies, which have been used broadly within fisheries management, frequently find significant post-release mortality, often days or weeks after release. These tagging programs are vital tools to assessing post-release survival, but they can be difficult and expensive to implement. Conclusions from tag-and-release studies are often difficult to extrapolate to other species. Many factors, such as fish size, water temperature, fight time and fishing gear, could influence survival.

Using funds provided by the Pelagic Fisheries Research Program (PFRP), we have been developing tools that we hope will reduce the need for tagging studies. Whereas tagging studies assess how many fish survive, we are trying to understand *why fish die*. We are developing a set of diagnostic tools to assess the biochemical and physiological status of fish captured on various gear. Our application of these tools is integrated into a comprehensive pop-up archival satellite tag (PSAT) program.

We focused first on the post-release survival of blue sharks, which are frequently by-catch of Pacific long-liners. Using the NMFS vessel *Townsend Cromwell*, we captured blue sharks on scientific long-line gear off the coast of Hawaii. Blood samples were collected from sharks that were fitted with PSATs. The information from the PSAT establishes how long the shark survives. Analysis of the blood sample allows us to evaluate the physiological condition of the shark when it was released. Our goal is to develop predictors of survival based upon analysis of a single blood sample taken just prior to release. Although we focused first on blue sharks, we are anxious to apply this approach broadly to other commercial and recreational fisheries.

Analysis of blood samples

When a fish is caught, it experiences many different physiological challenges that can affect its long-term survival. Our analysis is similar to that used by doctors examining a patient in an emergency room. A lot of information about animal health can be obtained from a blood sample. Every few seconds each red blood cell passes through the heart, along blood vessels that penetrate the tissues, then back to the heart. When the blood passes through the body, it is changed in many ways that reflect the state of the tissues.

Hooked fish may lose significant amounts of blood. If too much blood is lost, the fish may no longer be able to provide adequate oxygen to its tissues. Blood loss is assessed by

measuring *hematocrit*, which reflects the level of blood cells in the circulation. When fish are captured on fishing gear, the vigorous swimming activity can deplete its energy stores. When any animal undergoes extreme exercise, muscle produces high levels of *lactic acid*, which is released into the circulation. Blood lactic acid levels therefore reflect the amount of exercise the animal has experienced. If fish have used too much of its metabolic fuel deposits, it may be unable to recover from the exercise bout¹. Retaining an ability to swim after exhaustive exercise allows fish to avoid predators, but is critical in large pelagics that ram-ventilate.

Strenuous exercise also results in muscle damage. Other forms of tissue damage, such as heart attacks, kidney failure, liver or brain damage could conceivably arise as a result of capture. These damaged tissues release their intracellular contents into the circulation. Since many cells possess unique cellular markers, the presence of these molecules in the blood can be used as an index of tissue damage. By characterizing the profile of blood proteins, we are able to assess the degree of tissue damage. For example, if you were to suffer a heart attack, heart cells release the proteins creatine kinase and troponin I into the plasma. Acute liver cell damage results in the release of the proteins alanine aminotransferase and aspartate aminotransferase into the plasma.

We are also using the properties of blood cells themselves to assess the extent of tissue damage. When fish blood experiences hazardous conditions such as high temperature or oxidative stress, it can activate a line of defence that minimizes the damage to the blood cell. This “stress response” is recognized by stimulation of genes that lead to production of a suite of protective proteins called heat shock proteins. Our analysis of heat shock protein synthesis can be used to categorize the extent of the stress experienced by the blood cells². In many cases, the damage to red blood cell can cause them to trigger programmed cell death, as indicated by elevated levels of caspases, DNA fragmentation³.

At this point we have completed analysis of the first year of our study. A second set of sharks were sampled and tagged in April.

Outcome of tagging studies

In recent years, sharks represent about 45% of the catch on long-line research cruises from the Townsend Cromwell. The dominant shark species caught has been blue sharks. In the past 2 years, we have collected blood samples from 46 blue sharks, 31 of which were fitted with PSATs. These tags will provide them with environmental and behavioral details about these animals. They will also provide a record of survival upon release. Blood samples were also collected from blue sharks that were morbid upon capture (4 sharks) or released without tags.

The PSAT data from the blue sharks tagged in 2001 has shown that blue sharks are remarkably resilient animals. Although none of the first 16 sharks we sampled died within a week of release, many of the sharks had clear signs of stress. Six sharks had elevated creatine kinase levels and lactate dehydrogenase levels, which are indicative of muscle damage. Five sharks had very high levels of lactic acid, which is indicative of exhaustive exercise. One shark that was dead when captured showed the signs of experiencing a fatal heart attack. Its blood had high levels of the heart protein troponin I. All the sharks showed some signs of oxidative stress as demonstrated by hsp mRNA blotting.

Following the analysis of blood samples collected in April from 24 blue sharks, we will expand our analysis of stress indicators. These studies will help us understand the factors that cause delayed mortality of sharks and other large pelagics upon release. We believe these studies can be a valuable tool for fisheries managers. We are anxious to apply the technology to other fisheries.

References

1. Moyes CD & TG West. 1995. Exercise metabolism of fish. *In* Biochemistry and Molecular Biology of Fishes. PW Hochachka & TP Mommsen (eds.), Elsevier Press, pp. 367-392.
2. Currie S, B Tufts & CD Moyes. 1999. Influence of bioenergetic stress on heat shock protein gene expression in nucleated red blood cells of fish. *American Journal of Physiology*. 276: R990-R996.
3. Moyes CD, ML Sharma, C Lyons, SC Leary, M Leon, A Petrie, S Lund and BL Tufts. 2002. Origins and consequences of mitochondrial decline in nucleated erythrocytes. *Biochim. Biophys. Acta* in press.