JIMAR, PFRP ANNUAL PROGRESS REPORT
FY 2003

P.I. Name: MOYES, Christopher D

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

Funding Agency: PFRP

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 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.

2. Progress during FY 2003. Provide a thorough discussion of accomplishments <u>and</u> problems.

MILESTONES

We have almost completed the analyses of the shark data (Appendix 2). I am preparing a manuscript based on these analyses in conjunction with mortality data obtained from tagging studies.

Problems
There have been no significant technical problems to date. Unfortunately, there seems to be a rather high rate of premature release, likely due to nuptial bites.

Accomplishments

4/2002. **Cruise:** 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.

7/2002. **Conference**: I presented our preliminary results at American Fisheries Society meeting in Vancouver (Abstract attached as Appendix 1).

11/2002. **Cruise**. Technician N. Fragoso worked on a two week cruise tagging sharks and billfish.

12/2002. PI meeting. Fortunately, I was able to attend the December 2002 PFRP meetings, which normally collide with my teaching schedule.

Throughout the entire fiscal year we are actively analyzing samples that have been collected from a total of 3 cruises (4/ 2001, 4/2002, 12/2002). The analyses continue and I expect to have a paper submitted on the biochemistry and tagging work by June.

RESULTS TO DATE

1. PSATS:

Complete information on the status of the PSATs can be found in the progress report from Musyl and Brill. The PSAT approach has provided good data on survival of released sharks for at least a short period. The data suggest that the majority of released sharks suffer little delayed mortality.

2. Plasma analyses:

Plasma analyses have been performed on more than 50 sharks. The data are summarized in Appendix 2.

***3. Molecular analyses*:**

We are very excited about the results we are obtaining with the analyses of heat shock proteins. 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 and 2002 cruises. An example of our northern analyses is shown in Figure 1, illustrating the variation in HSP70 mRNA in several sharks (Note the darker bands in shark 102, 117, 118).

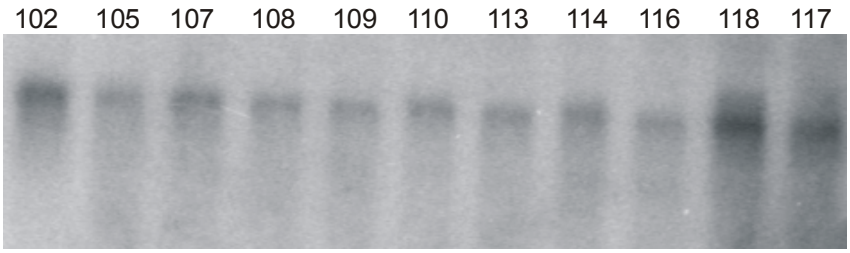

FIGURE 1: HSP70 mRNA levels in blue shark blood.

3. Plans for the current fiscal year.

Almost all of the molecular data are analyzed and I am in the process of writing up the study for publication.

4. List of papers published in refereed journals during FY 2003.

Gillis TE, **CD Moyes** & GF Tibbits. Sequence mutations in teleost cardiac troponin C that are permissive of high Ca^{2+} affinity of site II. Am. J. Physiol. in press 2003.

Moyes CD & DL Hood. Origins and consequences of mitochondrial variation. Ann. Rev. Physiol. in press 2003.

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. 1591 11-20, 2002

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 Heart 283: H540-H548, 2002

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. 47: 1873-1885, 2002.

Lund SC, P Dyment, 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. 172: 1467-1476, 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

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

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

PI meeting: Fortunately, Moyes was able to attend the December 2002 PFRP meetings, which normally collide with his teaching schedule.

American Fisheries Society: Moyes attended the American Fisheries Society meeting in Vancouver, presenting the analyses of the blue shark tagging study (Poster attached as Appendix III)

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

None.

7. For multi-year projects, provide budget for the next year on a separate page.

Not applicable

Appendix 1. American Fisheries Society Conference Proceedings

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

Chris Moyes[1], Nuno Fragoso[1], Mike Musyl[2] and Rich Brill[3]
[1]Department of Biology, Queen's University, Kingston, Canada,
[2] JIMAR, Honolulu, Hawaii
[3]NMFS, 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 cost 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 bout[1]. 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 cells[2]. 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 fragmentation [3].

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.

Appendix IA. Analyses of plasma proteins in blue sharks.

				Plasma enzymes (U/L)					Glucose	Lactate	Protein	Albumin	HCT (%)
#	Status	Sex	cm	ALT	AST	CK	ALP	LDH	mM	mM	g/L	g/L	%
normal range				*7-40*	*12-45*	*55-197*	56-1119	*94-250*	3.5-10	0.5-2.2		28-44	
38	**3**	F	162	<5	4	10	4	<5	3.2	29.1	14	3	12
28	**0**	F	196	<5	<4	10	<3	<5	5.2	2.9	9	2	17
34	**2**	M	140	<5	<4	10	4	<5	4.5	0.9	13	2	20
33	**0**	F	200	<5	<4	10	4	<5	4.0	0.5	14	3	21
31	**1**	M	158	10	<4	10	4	<5	5.0	1.9	14	3	24
2	**1**	F	170	<5	16	10		118	1.2	27.3			
41	**2**	M	186	<5	1	11	3	1	5.4	4.2	15	5	16
37	**2**	F	200	10	5	15	4	<5	5.6	3.9	18	4	22
22	**3**	M	102	<5	<4	16	<3	<5	7.1	8.8	10	2	13
29	**5**	M	148	<5	<4	21	5	<5	6.1	2.2	14	2	27
44	**3**	F	160	1	5	27	2	3	5.5	37.3	9	3	15
35	**2**	M	60	<5	<4	32	3	<5	4.4	8.7	10	4	11
39	**2**	F	160	<5	<4	32	3	<5	5.1	2.0	16	6	22
26	5	F	>200	<5	<4	32	4	<5	3.6	2.9	14	<2	14
45	**3**	F	156	<5	25	34	3	8	3.8	44.3	11	3	
40	**3**		213	<5	6	35	2	4	5.1	28.6	15	4	22
36	**2**	M	150	<5	<4	42	5	<5	3.7	4.7	9	3	16
19	**5**	F	175	12	<4	44	4	13	3.8	1.8	14	3	18
5	**1**	F	180	<5	8	48		<5	5.2	4.8			
32	**2**	M	130	<5	<4	48	4	<5	5.1	1.5	15	3	22
25	**0**	M	120	<5	<4	55	4	<5	4.8	1.5	12	<2	18
27	**2**			4	<4	61	<3	<5	4.7	3.1	15	3	21
30	**1**	M	180	6	<4	62	4	<5	5.3	4.4	14	2	20
43	**3**	F	179	1	29	64	3	10	0.8	28.7	9	3	15
1	**3**			7	58	91		46	6.6	8.2			
13	**1**	F	150	<5	<4	92		<5	7.4	4.0			
42	**3**	F	176	3	8	98	5	4	2.4	36.8	12	3	19
23	**1**	F	>200	12	4	230	<5	58	3.5	5.9	17	3	21
4	**0**	F	**150**	100	8	370		<3	4.0	27.5			
11	**0**	M	140	<5	<4	396		40	6.0	1.4			
20	**0**	M	140	8	14	804	3	237	5.5	8.6	12	2	17
14	**0**			<5	19	871		369	5.9	30.8			
17	**0**	M	131	<5	21	909	3	482	8.0	25.1	14	2	23
16	**1**	F	186	<5	7	1120	6	209	3.5	35.0	14	3	16
21	**0**	M	180	8	21	1396	<3	356	5.0	3.8	13	4	21
9	**1**	F		<5	24	1588		302	3.6	0.6			
12	**0**	F	160	<5	28	2116		536	5.6	1.4			
3	**2**			<5	12	2649		631	4.6	3.9			
6	**1**	F	140	8	74	4192		592	6.2	3.0			
15	**3**			<5	103	4457		976	3.0	27.7			
18	**0**	F	145	14	75	5047	3	1400	5.3	8.7	20	4	23
10	**1**	M	204	<5	36	5136		1094	5.0	1.6			
24	**0**	M	130	12	65	5789	4	798	4.4	1.2	18	4	23
8	**0**	F		<5	70	7588		1560	5.2	28.8			
7	**1**	F	150	8	252	25642		5184	4.4				

Appendix IB. Analyses of plasma ions in blue sharks

#	Creatine uM	Cl mM	Ca mM	Mg mM	K mM	Na mM	Urea mM	Osm mOsM	Pi mM	Fe uM
normal range	***<106***	***95-107***	***2.15-2.65***	***0.8-1.0***	**3.5-5.2**	**133-145**	**3.0-7.0**	**281-297**	**0.8-1.5**	**10-28**
38	<10	238	3.17	1.39	5.6	245	296	1119	4.11	4
28	<10	216	3.24	0.86	4.1	243	346	1049	2.18	3
34	<10	260	3.10	0.87	3.5	253	338	1048	2.35	5
33	<10	257	3.00	1.01	4.3	257	344	1041	1.66	4
31	<10	241	3.37	1.00	3.5	250	366	1045	2.70	3
2	<10	232	3.14	1.20	7.2	272	358			
41	5	230	3.63	1.06	4.2	250	360	1047	1.62	3
37	<10	259	3.23	0.89	3.6	265	334	1074	2.16	4
22	<10	257	3.40	1.53	5.5	266	368	1053	2.22	1
29	<10	255	3.26	0.98	4.1	262	355	1031	1.92	4
44	8	225	3.82	1.18	6.6	280	356	1065	2.38	3
35	<10	286	3.50	1.25	4.3	290	297	1059	2.50	2
39	<10	257	3.19	0.73	3.3	257	318	1102	1.96	3
26	<10	251	3.07	0.87	3.9	264	359	1033	1.82	20
45	9	235	4.26	1.82	6.2	255	351	1075	2.94	3
40	8	248	3.65	1.27	5	270	334	1052	2.15	4
36	<10	239	2.93	1.27	3.7	240	331	1068	2.61	1
19	<10	255	3.30	0.98	3.8	263	376	1055	1.35	3
5	<10	228	3.04	0.80	4.2	258	366			
32	<10	259	3.07	1.00	4.6	256	323	1010	2.60	10
25	<10	254	2.89	0.80	5.1	274	336	1017	2.39	30
27	<10	251	3.17	1.36	4.3	259	357	1054	1.93	4
30	<10	225	3.26	1.13	4.5	246	345	1056	2.14	2
43	15	230	3.99	1.92	9.5	265	348	1061	1.98	4
1		>140	3.01	1.70	6	>180				
13	<10	238	3.22	1.20	4.8	266	360			
42	15	220	4.08	1.56	10.8	265	362	1082	3.01	3
23	31	257	3.49	0.87	4.8	270	358	1067	1.35	4
4	<10	232	3.40	1.00	5.6	280	330			
11	<10	232	2.94	0.80	4.6	260	362			
20	11	234	3.28	1.05	4.5	267	345	1064	2.79	3
14		>140	3.09	1.00	5	>180	400	1189		
17	<10	271	3.90	1.64	5.3	303	359	1088	3.21	3
16	<8	232	3.74	1.32	5.7	271	354	1009	1.92	6
21	<10	234	3.21	1.13	4.7	255	344	1076	2.01	2
9	<10	234	2.82	1.00	5.6	258	350			
12	<10	232	3.18	0.80	5	254	360			
3		>140	2.82	1.10	5.8	>180	436	1167		
6	<10	228	3.02	0.80	5.2	256	364			
15		>140	3.93	1.75	7.9	>180	404	1206		
18	<10	255	3.49	1.20	4.4	269	329	1060	2.00	5
10	<10	234	3.14	1.00	5.2	260	362			
24	<10	252	3.15	1.06	4.6	256	353	1044	1.89	2
8	<10	236	3.10	1.00	5	282	324			
7	<10	226	2.30	1.00	9	264	328			