

**JIMAR, PFRP ANNUAL PROGRESS REPORT
FY 2004**

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Project Proposal Title: EVALUATING BIOCHEMICAL AND PHYSIOLOGICAL PREDICTORS OF LONG TERM SURVIVAL IN RELEASED PACIFIC BLUE MARLIN TAGGED WITH POP-UP SATELLITE ARCHIVAL TRANSMITTERS (PSATs)

Funding Agency: NOAA/NMFS

1. Purpose of the project and indicative results.

Our objective is to use biochemical tools to predict the long-term survival of released Pacific blue marlin. We work in close collaboration with the PFRP projects by Musyl and Brill to place pop-up satellite archival tags (PSATs) on these fish and the Moyes, Brill & Musyl project to develop biochemical correlates of delayed mortality in blue shark. We currently focus on assessing the extent of tissue damage arising from capture using comprehensive analyses of ions, metabolites and proteins found in the plasma and muscle. 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.

- Results from our blue shark project have shown that, even in a relatively docile species like blue shark, capture induces elevations in indices of tissue damage to many individuals. Some lost almost 1/3 of their blood volume, as indicated by hematocrit. Others showed signs of heart attacks (elevated cardiac troponin I), liver and kidney damage (plasma enzymes), and most individuals showed severe muscle damage. The most provocative results were found with erythrocyte heat shock protein levels. Heat shock proteins were found to be a strong index of the health of the animal.

2. Progress during FY 2004. Provide a thorough discussion of accomplishments and problems.

Shark study:

We have almost completed the analyses of the shark data (Appendix 2). We are finalizing the manuscript based on these analyses, in conjunction with mortality data obtained from tagging studies.

This study has the ambitious goals of (i) catching viable marlin, (ii) tagging them successfully and (iii) collecting condition data and tissue samples without affecting tagging survival. Our progress in each of these has been exemplary.

(i) Capture success

The project has tagged 38 marlin (36 blue, 1 striped, & 1 black) and blood samples have been collected from another 27 marlin.

Marlin fishing boat captains in the Kona region are to be highly commended for their efforts in helping this project. They have provided ongoing support, including use of their vessels. Captains and crew members are being trained in the deployment of tags and data recording. Crew members on two of the Kona boats have had past veterinary training and have also volunteered to collect blood and tissue samples.

As an enticement to tag the marlin and be involved in the program, both marlin boat captains, and paying fishermen are sent the PSAT results of the fish they have angled. This is proving very popular with the boat captains/owners and charter companies who are posting the marlin movement on their advertising web sites (<http://www.konatournaments.com>).

We have been receiving excellent collaboration with local commercial businessmen and tournament organizers. Marlin fishing tournaments such as: Hawaii International Billfish Tournament (HIBT), (Mr Peter Fithian, Mr Roy Morioka), and Tropicdilla productions (Mr Jody Bright) These tournament organizers have pledged support for the current project, and have included the deployment of PSATs as part of the competition. There has been national television coverage of the PSAT deployments during these tournaments.

(ii) Tagging

Blue marlin (*Makaira nigricans*) are angled from commercial sport-fishing vessels off Kailua Kona. Either a scientist is present to attach the PSAT tag or attachment is performed by a trained crewmember. The PSAT tag is carefully deployed in the upper shoulder of the fish, approximately three inches below the third dorsal spine. The applicator needle is inserted to a depth sufficient that the tag head engages the pterygiophores. The tag heads have been modified with larger stainless-steel floppers for this purpose, and from all reports, they appear to be working well.

(iii) Sampling

If the marlin is small enough (generally not larger than 50 kg) to be safely subdued, the tail is lifted from the water and blood taken from the caudal artery. Accompanying data are also recorded:

- Line class
- Size of fish
- Duration of fight

- Depth of fight (Deep or surface)
- No. of jumps, greyhounds, or tail-walks
- Capture on bait or lure
- Hook position in jaw
- Time at the boat
- Color/ behavior at boat, and at release
- Fish bleeding/ injured etc.
- Time, latitude, longitude

Blood samples have also been taken from moribund or recently killed blue marlin. The same data is recorded as on the fish that have PSATs attached, and the blood samples are processed using the same techniques. These samples provide a significant amount of base line data.

(iv) Tagging Summary and Results

The goals of the study were twofold. Firstly, we wanted to assess the survival rate among marlins released from sports fishing gear with pop-up satellite archival tags (PSATs). Secondly, we wanted to develop a suite of biochemical predictors of survival based upon blood/tissue samples of marlin prior to release. We also wanted to see if factors such as protracted fight times and type of fishing gear (e.g., pound test line, lure v. bait, “J” v. circle hooks) correlated with mortality. We therefore placed PSATs on 38 marlins:

36 blue marlin (average weight approximately 100 kg, range 50-250 kg) tagged near Kona, Hawaii,
 one striped marlin [approximately 60 kg] tagged near Kona, Hawaii,
 and
 one black marlin [approximately 500 kg] tagged in Australia.

Fight times ranged from 5 to 60 min (avg.=15 min) with 16% marlin caught with bait and 84% on lures.

Twenty-nine PSATs have reported, and five of these reach their pre-programmed date. Only two tags failed to transmit data, and seven are assumed to be still attached to fish. For these 29 tags from which data were recovered, days at liberty ranged from 1 to 245 days (avg.=76 days) and we have in aggregate data from 2138 days (ca. 6 years) at liberty.

Vertical data from one blue marlin tag clearly indicate the fish sank and died approximately 4 months after release. The depth data indicate movements were relatively normal until 113 days after tagging. There were no indications in the vertical data prior to sinking that gave any suggestion of abnormal behavior. We are confident that this case represents a mortality because the PSAT worked as designed to detect a mortality (i.e. tag reached a “fail-safe” pressure release depth of around 1136m and jettisoned to the surface to start transmitting data). This fish was captured after a 25 min. fishing bout on live bait (hooked in the mouth). As correlated by a steady rise in SST estimated by the PSAT, the fish moved due south and covered ca. 855 nmi in 113 days. Since this fish apparently died about 4 months after the initial insult (catch-tag-release), we suggest that it would be very difficult to attribute this mortality to the initial insult, and that other factors (e.g., predation, disease, etc.) could have intervened.

In aggregate, the vertical data indicate movements during the day were constrained in the mixed layer with occasional forays beneath the thermocline (i.e., 97%

of time spent from the surface to 150 m). Marlin show diel vertical movement behavior typical of pelagic fishes and sharks (i.e., deeper in the daytime and shallower nighttime), but day/night transitions were not distinct (i.e., 85% of time at night spent from the surface to about 100 m). Day and night, marlin prefer temperatures of at least 27°C (i.e., 90% of time spent at these temperatures) and nighttime diving depth was not significantly correlated with lunar illumination. As measured by either deployment/pop-up locations or Kalman filtered-geolocations, blue marlin exhibited a mixture of 3 horizontal movement patterns: 1) neritic with movements close to the main Hawaiian Islands; 2) eastward movement from Kona (e.g., one tagged blue marlin moved almost due east for 59 days and covered 2,096 nmi, or ca. 40 nmi/day); 3) southern-equatorial movements (e.g., one tagged marlin covered 1,434 nmi in 51 days). These movement patterns corroborate conventional tagging data.

Originally, our intention was to provide a direct correlation between biochemical measures and actual delayed mortality following release, as determined from the PSATs. Unfortunately, due to the unpredictable nature of large and dangerous marlin, we found it very difficult to taken blood samples (or tissue) from restrained fish. Further, we did not want to compound handling stress by restraining fish any longer than necessary whilst attempting to take biochemical samples. Therefore, as we try and develop various devices to sample tissues from restrained marlin that are minimally invasive, tissue and blood samples have been taken from moribund and dead fishes to develop baseline biochemical information for our assays (described below).

Tissue analyses

Moyes received the first shipment of billfish blood samples in early April and analyses are underway. We do not foresee significant technical problems. However, as we move from sharks to marlin, we need to develop several species-specific analytical techniques. Appendix IA and IB summarize the analyses of the shark data from the past 2 years. Appendix II summarizes the billfish data collected to date.

Based upon our experience with the shark study, we have found it advantageous to process and analyze in parallel as many samples as possible. As of April 2004, we have compiled samples from the previous sampling period and are about to begin the analyses. Based upon our experience with the shark work, we expect that analyses of stockpiled samples to require approximately 5 months.

RESULTS TO DATE

3. Plans for the current fiscal year.

Field studies

Blood collection is the hardest aspect of the fieldwork, it is not impossible, but certainly it is the limiting factor. Removing blood samples from live marlin has proven to be a difficult task, so we have adapted by taking tissue samples from live fish, and blood samples from moribund or recently killed fish. The most efficient method of taking blood from moribund or recently killed fish is to insert a needle into the artery located at the base of the pectoral fin. Several prototype tissue samples have been tested. We have designed an instrument that effectively removes a small amount of tissue from dead marlin, which is currently being tested on live fish.

It is expected that additional PSATs to be deployed in 2004/2005 will be provided in-kind through NMFS-PIFSC. Due to the unexpected number of tags deployed, before and during FY 2003 (38 deployments) and the number of tags reporting data (29 out of 31 tags or 94% reporting data which in aggregate represents over 6 years' days-at-liberty [waiting on 7 tags to reach their pop-off date and/or to transmit early]), this new found "wealth" has placed an unexpected burden on the demands of the senior PI. For this reason, the project is requesting ½ salary for Musyl and ½ salary for Lianne M^cNaughton, associate researcher, to assist in the editing, collating, analysis, and presentation of ARGOS, PSAT and GIS data.

Analytical studies

We ironed out the wrinkles in the analytical side of this study during the past two years of shark sample analyses. Comprehensive analyses of archived marlin samples continues. Samples will continue to accumulate in this fiscal year.

4. List of papers published in refereed journals during FY 2002.

Gillis TE, CD Moyes & GF Tibbits. Sequence mutations in teleost cardiac troponin C that are permissive of high Ca²⁺ affinity of site II. *Am. J. Physiol.* 284: C1176-C1184, 2003.

Leary SC, CN Lyons, AG Rosenberger, JS Ballantyne, J Stillman & CD Moyes. Fiber-type differences in muscle mitochondrial profiles. *Am. J. Physiol.* 285: R817-R826, 2003.

McClelland, GB, CS Kraft, D Michaud, JC Russell, CR Mueller & CD Moyes Leptin and the control of respiratory gene expression in muscle. *Biochim. Biophys. Acta*, 1688: 86-93, 2004.

Moyes CD. Controlling muscle mitochondrial content. *J. Exp. Biol.*, 206: 4385-4391. 2003.

Moyes CD & DL Hood. Origins and consequences of mitochondrial variation. *Ann. Rev. Physiol.* 65:177-201, 2003.

Musyl, MK, RW Brill, CH Boggs, DS Curran, MP Seki and TK Kazama. 2003. Vertical movements of bigeye tuna (*Thunnus obesus*) associated with islands, buoys, and seamount of the Hawaiian Archipelago from archival tagging data. *Fisheries Oceanography* **12**, 152-169.

Sibert, JR, MK Musyl and RW Brill. 2003. Horizontal movements of bigeye tuna near Hawaii as determined using archival tags. *Fisheries Oceanography* **12**, 141-152.

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

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

- Brill, R.W. K.A. Bigelow, M.K. Musyl, K.A. Fritsches, and E.J. Warrant. Bigeye tuna behavior and physiology... their relevance to stock assessments and fishery biology. Invited presentation at the Second World Meeting on Bigeye Tuna, Madrid, Spain. March 2004.
- Brill, R.W. K.A. Bigelow, M.K. Musyl, K.A. Fritsches, and E.J. Warrant. Bigeye tuna behavior and physiology... their relevance to stock assessments and fishery biology. ICCAT SCRS Report. (submitted)
- Malte, H., C. Larsen, M.K. Musyl, and R.W. Brill. Differential heating and cooling rates in bigeye tuna (*Thunnus obesus*); a model of non-steady state heat exchange. *American Journal of Physiology*. (submitted).
- Musyl, M., Moyes, C., Brill, R. and West, A. Predicting post-release survival in blue marlin. SSC Meetings, Honolulu, HI, 15 October 2003.
- Swimmer, Y., Arauz, R., Musyl, M., Ballesterro, J., McNaughton, L., and Brill, R. 2004. Survivorship and dive behavior of olive ridley sea turtles after their release from longline fishing gear off Coasta Rica. (manuscript).
- Swimmer, Y., Arauz, R., Musyl, M., Ballesterro, J., McNaughton, L., and Brill, R. 2004. Survivorship and behavior of olive ridley turtles off the coast of Costa Rica following interactions with longline fishing gear". Poster presented at the 24th Annual Symposium on Sea Turtle Conservation and Biology 22 - 29 February 2004, San Jose, Costa Rica..
- Swimmer, Y, Brill, R., Arauz, R., Mailloux, L., Musyl, M., Bigelow, K., Nielsen, A., Sibert, J. 2003. Survivorship and Behaviors of Sea Turtles after their release from Longline Fishing Gear. In: Proceedings of the 54th Annual Tuna Conference. Lake Arrowhead, California, May 13-16. 2003.

Manuscripts currently in preparation:

Dalziel, AC, SE Moore, CD Moyes. Control Of Mitochondrial Enzyme Content In the Muscles of High Performance Fish. Submitted to Am J Physiol 3/2004 (Fish samples were collected during PFRP cruises and PFRP support is acknowledged)

Moyes, CD, Fragoso, N, Musyl, M, Brill, R. Evaluating predictors of post-release survival of large pelagics. In preparation for submission to Science 6/04 (Funded in whole by PFRP)

Musyl, M. and Brill, R. Post release mortality and movements in blue shark identified with PSATs .

Brill, R. and Musyl, M. Movements and habitat preferences of swordfish in the Pacific Ocean.

Bigelow, K., Musyl, M., and Poisson, F. Manuscript detailing the effects of current vectors on predicting catenary depths for over 600 longline sets instrumented with TDRs.

Nielsen, A., Bigelow, K., Sibert, J., Musyl, M. et al. Manuscript detailing results of PSAT-GPS double tagging studies with incorporation of SST into the Kalman filter.

ISC Meetings 26 January - 4 February 2004, Honolulu, Hawaii, USA

Papers:

Robert L. Humphreys, Jr., Michael Musyl and Edward E. DeMartini. SC/04/SWO-WG/02 Biological Research Conducted During 2002-2003 in Support of Swordfish Stock Assessment

Michael Musyl, Chris Moyes, Rich Brill and Andrew West. Evaluating biochemical and physiological predictors of long-term survival in released Pacific Blue Marlin tagged with PSATs. ISC Meeting, Marlin Working Group, Honolulu, Hawaii, 30 January 2004.

Talks:

Michael Musyl and Rich Brill. Results of PSAT attachments to swordfish. ISC Meeting, 29 January 2004, Honolulu, HI, USA.

Michael Musyl, Chris Moyes, Rich Brill and Andrew West. Predicting post-release survival of blue marlin. ISC Meeting, 29 January 2004, Honolulu, HI, USA.

PFRP Principal Investigators Workshop, December 9 - 11, 2003, Imin Conference Center, Asia Room (2nd floor), 1777 East-West Road, University of Hawaii at Manoa.

Yonat Swimmer, Mike Musyl, Lianne McNaughton, Anders Nielson, Richard Brill, and Randall Arauz
Sea Turtles and Longline Fisheries: Impacts and Mitigation Experiments

Christopher Moyes, Michael Musyl, Richard Brill, Queen's University, Canada & NMFS-HL
Physiological Predictors of Blue Shark Survival

Christopher Moyes, Michael Musyl, Richard Brill, Andrew West, Lianne McNaughton
Predicting Post-release Survivability in Blue Marlin using PSATs and Biochemical Assays

Michael Musyl, Richard Brill, NMFS-HL
Movements and Post-release Mortality in Oceanic Sharks tagged with PSATs

Richard Brill, Michael Musyl, NMFS-HL
Fishery Interaction and Movements of Swordfish as Determined with PSATs

JIMAR Review March 4-5, 2004, East-West Centre, Honolulu, Hawaii - Poster Session

Survivorship and Behavior of Olive Ridley Turtles off the Coast of Costa Rica Following Interactions with Longline Fishing Gear.

Yonat Swimmer, Randall Arauz, Michael Musyl, Jorge Ballesterro, Linane McNaughton, and Richard Brill

Pop-up Satellite Archival Tags (PSAT) Studies of Pelagic Fisheries and Turtles in the Pacific Ocean.

Michael Musyl, Yonat Swimmer, Lianne McNaughton, Richard Brill, John Sibert, and Anders Nielsen.

Predicting Post-release Survival in Blue Sharks

Christopher Moyes, Nuno Fragoso, Michael Musyl, and Richard Brill

Predicting Post-Release Survival of Blue Marlin

Michael Musyl, Lianne McNaughton, Richard Brill, John Sibert, Anders Nielsen, and Andrew West

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

Dalziel, A. Control of mitochondrial enzyme content in the muscles of high performance fish.

Kraft, C. Control of mitochondrial gene expression in myogenesis.

Kaushal, V. Role of mitochondria in cobalt-induced cell death.

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

Appendix IA. Analyses of plasma proteins in blue sharks.

#	Status	Sex	cm	Plasma enzymes (U/L)					Glucose mM	Lactate mM	Protein g/L	Albumin g/L	HCT (%) %
				ALT 7-40	AST 12-45	CK 55-197	ALP 56-1119	LDH 94-250					
				normal range					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			

Appendix II. Billfish plasma analyses.

ID	02-1	02-2	03-1	03-2	03-3	03-4		
Species	Sw	Sw	BM	BM	BM	BM		
Status	A	A	D	D	D	D		
Tagged	Y	Y	N	N	N	N		
Sex								
cm	120	136						
Enzymes	U/L	ALT	4	<4	<4	<4	5	
	U/L	AST	90	43	<4	<4	6	20
	U/L	CK	2042	539	14	106	41	199
	U/L	ALP	52	56	275	145	113	56
	U/L	LDH	224	340	228	376	35	170
Metabolism	mM	Glucose	5	10.7	10.6	31.9	2.3	15.3
	mM	Lactate	30	5.3	39.0	37.6	25.8	44.6
	nM	Cortisol			140	104	257	101
Protein		g/L	30	31				
	Albumin	g/L	8	7	9	13	9	13
HCT (%)		%	0.50	0.36				
	uM	Creatine	10	4				
	mM	Cl	210	199	290	187	220	221
	mM	Ca	4.19	3.54	10.98	5.61	4.48	5.22
	mM	Mg	1.8	1.19	18.96	2.91	2.37	5.13
	mM	K	9	6.7	10.4	20.6	9.4	8.8
	mM	Na	246	217	317	228	248	260
	mOsM	Osm	512	451	710	545	516	577
	mM	Pi	3.88	3.13	6.96	6.6	4.31	6.15

Appendix III

PREDICTING POST-RELEASE SURVIVAL IN BLUE SHARKS

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ABSTRACT

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. Whereas tagging studies estimate how many fish survive, we are trying to understand **why fish die**. Using funds provided by the Pelagic Fisheries Research Program (PFRP), we are developing a set of diagnostic tools to assess the biochemical and physiological status of fish captured on various gear. 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, as well as animals that had been captured dead or morbid. After minimal processing on board, blood samples were analyzed to evaluate the physiological condition of the shark when it was released. Samples were subjected to a standardized analytical regime of more than 20 blood parameters. Not surprisingly, many of the dead/morbid sharks showed profound changes in indices of hematocrit, exhaustive exercise, and muscle damage. However, these same indices were elevated in many live sharks, almost all of which survived at least 4 months post-release. However, only dead/morbid sharks showed an increase in the erythrocyte cellular stress response, as indicated by hsp70mRNA levels. We are currently applying this approach to other commercial and recreational fisheries.



INTRODUCTION

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.

Sharks make up a significant percentage of the by-catch in many commercial and recreational fisheries. Until recently, many commercial fishing vessels removed fins to serve the Asia-Pacific market for shark-fin products. This practice was tolerated, in part, because of the widespread belief within the industry that released sharks would not survive release. Our study, funded by the Pelagic Fisheries Research Program, was intended establish if released sharks survived long periods after release. We focused on blue sharks (*Prionace glauca*) caught in the Hawaiian off-shore fishery. This species dominates the by-catch of commercial long-liners, and consequently shows recent signs of significant population decline. Our approach combined pop-up satellite tag (PSAT) technology with biochemical analyses of blood samples.

Our goals were:

1. To assess the survival rate among blue sharks released from (scientific) long-line gear.
2. To develop biochemical predictors of survival based upon blood samples of sharks taken prior to release.

METHODS

Blue sharks were captured using long-line gear deployed from the NOAA Vessel Townsend Cromwell on cruises in March 2001 and April 2002.

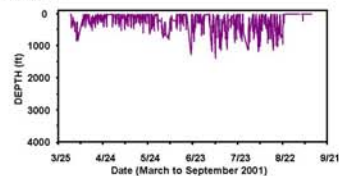
Sharks were fitted with PSATs programmed to detach at a depth of 1000m, or after 4 months. Blood samples were collected from 39 sharks. A total of 26 PSATs were attached.



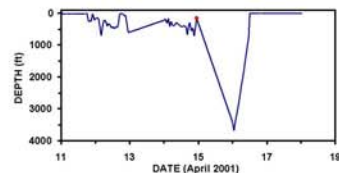
RESULTS

Tagging data

The lower panel is an example of the movements of a blue shark that survived until the tag was programmed to release (144 days). Daily excursions of 500-1200ft depth were recorded.



In contrast to the upper panel, the lower panel is the profile of a blue shark that survived only 4 days post-release. When the shark dies, it sinks with the tag until it reaches approximately 3700 ft. The PSAT was jettisoned by a pressure sensitive mechanism, and returned to the surface.



RESULTS

Survival

Survival of blue sharks post-release was very high. Of all the sharks tagged, only 1 individual died within several months post-release. Unfortunately, no blood sample was obtained from this individual.

Stress indices

Hematocrit

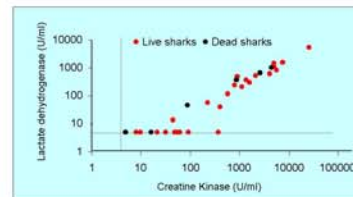
Animals that had suffered blood loss would be expected to show reduced hematocrit. A normal hematocrit is expected to be close to 30-35%, but the highest hematocrit found in these sharks was 27%. Several sharks that were brought aboard dead or morbid showed very low hematocrits (12-13%). However, sharks with hematocrits as low as 14% at time of capture survived more than 4 months. Most sharks fell within the range 18-24%.

Energy metabolism

Exhaustive exercise leads to depletion of tissue glucose/glycogen and buildup of plasma lactate. Exhaustive exercise in tuna, for instance, can lead to blood lactate in excess of 75mM. Blood lactate was less than 5mM in almost half the sharks. Only 1 shark that survived for 4 months had blood lactate greater than 25mM. The dead sharks had much higher lactate levels (mean 16mM, 3.9-36mM). There was little systemic variation in plasma glucose levels.

Muscle damage

Intense exercise also leads to skeletal muscle damage. This results in release of muscle enzymes into the circulation. Many sharks showed signs of extreme muscle damage. Plasma levels of creatine kinase and lactate dehydrogenase in some individuals were elevated by as much as 100-fold. Dead or morbid sharks did not stand out from the survivors.



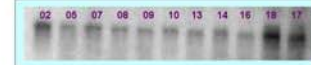
Liver damage

Liver damage can be assessed from plasma samples based upon the presence of enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AP). Although AST levels increased 100-fold in some individuals, neither ALT or AP changed. The changes in AST probably reflect general tissue damage because they parallel the changes in muscle enzymes.

RESULTS

Heat shock protein expression

Various stressful conditions, such as hyperthermia or oxidative stress have the potential to stimulate expression of heat shock proteins. Erythrocyte responses can be used as a barometer of tissue status. Several sharks demonstrated significant expression of hsp70 mRNA, as indicated by the northern blot below. The strongest signals were found in individuals that were dead or morbid when landed (02, 17, 18).



Ion and osmoregulatory disturbances

Animals that had suffered some degree of kidney or gill damage might be expected to show perturbations in ion and osmo regulation. None of the sharks showed significant evidence of such disturbances. Plasma osmolality remained in the range 1009-1205. Plasma urea ranged from 296-404. Narrow ranges were also observed for plasma Mg²⁺ (0.8-1.75) and Ca²⁺ (2.3-3.9). There was no relationship between ionic/osmotic variation and other stress indices.

DISCUSSION

Blue sharks appear to be very robust animals, readily surviving the stresses associated with capture. Almost every animal survived more than 4 months post-release.

Biochemical and molecular indices of "stress" identified several individuals with exceptionally high values, some of which correlated with acute fishing mortality.

Hsp70 mRNA levels were elevated in dead/morbid sharks but not in sharks that survived release.

Many fish that suffered acute fishing mortality also showed signs of muscle damage and high intensity exercise BUT other sharks with equivalent elevations in these parameters survived long-term.

While many sharks were clearly "stressed" based upon biochemical analyses, survival post-release was very high.

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