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Supporting Online Material for

Satellite Tagging and Cardiac Physiology Reveal Niche Expansion in Salmon Sharks

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Materials and Methods

The movements of salmon sharks (*Lamna ditropis*) were monitored using SPOT and PAT tags (Wildlife Computers, Redmond, WA, USA). SPOT tags provided Argos geopositions and PAT tags recorded pressure, ambient temperature and light. Several hardware versions of the tags were used during the course of the experiments. In 1999 we tagged two salmon sharks with PAT1 tags. In 2002 we used the SPOT2 and PAT2 tags; in 2003 we used SPOT2, SPOT3 and PAT3 tags; and in 2004 we used SPOT4 and PAT4 tags. Accuracy and resolution of electronic tag sensors is available from the manufacturer's website (www.wildlifecomputers.com). All study animals are detailed in Table S1. We tagged 51 individual salmon sharks in Prince William Sound (PWS), Alaska. Twenty-one were double-tagged with SPOT and PAT tags; 17 with SPOT tags only; 10 with PAT tags only; and three with tags that failed to report data. A total of six tagged sharks were recaptured in PWS, three after more than a year, confirming fidelity to the region after long-distance migrations. Shark #37374 was recaptured during our 2003 research cruise after 396 days at liberty, within one km of the previous year's tagging location. The animal was re-tagged with a new SPOT tag and released. Sharks #41669 and #41667 were recaptured near Valdez, AK after 688 days and 748 days at liberty, respectively. Sharks #52136, #52143 and #52150 were recaptured after 46, 28 and 27 days at liberty, respectively, and PAT tags were recovered with complete archival records.

Salmon sharks tagged in this study averaged 233 ± 9 cm (mean ± SD) straight total length and were mature based on size at maturity estimated for salmon sharks in the eastern North Pacific (*S1*). All tagged salmon sharks were female. In 2003, sharks were measured for curved fork length (CFL) and precaudal length (PCL) and these results were used to generate a CFL-PCL conversion equation (PCL = 0.88111 x CFL - 0.3; $r^2 = 0.95$). In other years, only CFL was measured, and the CFL-PCL conversion equation was used to estimate PCL. Total length (TL) was estimated from PCL using the equation of Nagasawa (*S2*).

Sharks were captured from the R/V Montague and R/V Solstice in PWS, Alaska using handlines comprising a 10 mm rope, a 1.8 mm stainless steel leader covered with plastic tubing, and a circle hook. Hooks were baited with salmon, sardine or squid. Handlines were deployed directly from the research vessel or attached to polypropylene floats to control the depth of the bait. Sharks were guided into a hoist and lifted out of the water on a stretcher deployed from the ship's crane. A soft moist cloth containing artificial anti-bacterial fish slime was placed over the eye to calm the animal and a saltwater hose was placed in the shark's mouth to irrigate the gills. The shark was secured with straps during the period on board the vessel.

SPOT tags were attached to the dorsal fin such that the antenna and conductivity switch of the tag would be exposed in air when the animal swam at the surface with the dorsal fin exposed. Tags were placed on the leading edge of the fin and as high up as possible while retaining support from the fin structure. Attachments were made using small stainless steel bolts (3 mm x 40 mm) coated with plastic shrink-wrap so that no metal was in contact with the tissues of the animal. The ventral surface of the SPOT tag and the bolts and shrink-wrap were all soaked or dipped in Betadine microbicide (Purdue Pharma L.P., Stamford, CT, USA) prior to attachment. Each PAT tag was attached to a titanium dart (59 mm x 13 mm) with a 15 cm segment of 136 kg monofilament line (300 lb test Extra-hard Hi-catch, Momoi Manufacturing, Japan). The titanium dart was dipped in Betadine and inserted into the dorsal musculature of the shark at the base of the first dorsal fin. A plastic loop secured the PAT tag in position and prevented interaction with the body of the shark.

For comparative purposes we tagged 31 blue sharks (*Prionace glauca*) with SPOT and PAT tags using the same methods described for salmon sharks, and obtained records for 27 individuals. In some cases nylon bolts were used to attach the tag. SPOT tags on five blue sharks failed to report data; in two such instances, PAT records showed the shark remaining stationary on the sea floor, indicating mortality directly after the tagging event. Blue sharks were captured from the R/V David Starr Jordan using a longlines in the Southern California Bight during 2002-2004, and averaged 197 ± 23 cm (mean ± SD) straight total length (Table S3).

SPOT tags transmitted to Argos satellites when the antennas were exposed to air, allowing the position of the animal to be calculated by the Argos satellite system. Argos provides error estimates for location events, which they term location classes (*S3*). Location classes 3, 2 and 1 are given error estimates, while classes 0, A, B and Z are not rated (Table S2). In order to remove erroneous locations we filtered out those implying an unreasonable speed for the animal. To determine the threshold for reasonable speed, we estimated the maximum speed of sharks using only location classes 3, 2 and 1. For animals tracked in this study, the distribution of maximum speed as a function of elapsed time between locations is approximately level at high elapsed times, and becomes highly non-linear at elapsed times below 0.1 days. Therefore, we discarded all locations that were less than 0.1 days apart for the purposes of estimating the threshold speed. The calculated speed threshold (1.75 m/s for salmon sharks and 1.25 m/s for blue sharks) was used to filter the entire dataset in a species-specific algorithm, including all locations regardless of the elapsed time between them. All locations of class Z and those on land were also discarded.

The *in situ* sea surface temperature (SST) for each salmon shark location was determined using eight-day averaged Pathfinder AVHRR satellite data obtained via the TOPP live access server, courtesy of NOAA/NESDIS/NODC. To illustrate the geographic variation in temperature experienced by blue sharks we created an average SST field for the eastern North Pacific for the duration of the tracking period. We used a 0.1 degree by 0.1 degree geographic grid and calculated the arithmetic mean temperature for each point, based on 8-day average Pathfinder AVHRR data provided by NOAA/NESDIS/NODC through December 31, 2004, and a comparable near real time data set from NOAA/NESDIS/OSDPD for the remainder of the period.

The habitat occupancy of salmon sharks during each meteorological season was analyzed with the kernel density method (ArcMap version 9, ESRI Inc., Redlands, CA, USA). The temporal frequency of geopositions obtained from SPOT tags varied between animals and locations, due to the coverage of the Argos system and the behavior of salmon sharks. Variations in temporal frequency of positions cause bias in the kernel density method, because locations where positions are obtained at high frequency are weighted more than those where positions are obtained at low frequency, even if the subject spent equal time in each location. To address these biases, we filtered tracks to one position per 24 hours. To account for spatial variation in the number of animals, we divided each value by the number of animals represented at that location. The resulting values were used to calculate kernel density with a smoothing radius of one degree.

PAT tags collected data at 1-minute intervals, summarized it into 6- 12- or 24-hour bins and transmitted summary data to Argos satellites (PAT tag software version 1 in 1999; 2.08e in 2002; 3.01d in 2003; 4.01e in 2004; Wildlife Computers, Redmond, WA, USA). The time occupancy in each of twelve designated depth bins and twelve temperature ranges was calculated on-board the tag to provide vertical and thermal distributions of habitat preference. These discrete values were used to make contour plots of time-at-depth and time-at-temperature using MatLab (The MathWorks, Natick, MA, USA). Thermal profiles of the water column were constructed by measuring the minimum and maximum temperature at the surface, maximum depth, and six intermediate depths, for the deepest dive in each time interval. These profiles were used to create a time-series slice of the ocean environment along the track of the animal. Transmitted PAT tag records yielded a total of 42,240 *in situ* measurements. The recovery of three PAT tags with 1-

minute archival records yielded 101 days of data containing 145,440 *in situ* measurements of depth and temperature, such that the total number of *in situ* measurements was 187,680.

Subarctic and subtropical watermass designations were based upon the physical oceanography of the North Pacific, after Roden (*S4*): subarctic waters were defined as those north of the Subarctic Front where no thermocline existed, stability was low and surface temperatures were under 8°C; and subtropical waters were south of the Subtropical Front where surface and mixed layer waters exceeded 18°C, waters were thermally stratified and stability was high. The continental shelf of North America was denoted by the 1000 m contour on Figures 1 and S3.

Surface chlorophyll-a concentration was determined by comparing Argos positions for SPOT-tagged sharks with eight-day averages of chlorophyll concentration measured by the SeaWiFS sensor. Data was obtained via the TOPP live access server, courtesy of NASA/GSFC/DAAC and Orbimage Inc. Chlorophyll-a concentration was compared between three zones: the subArctic gyre (defined as waters north of 45°N), the transition zone (defined as waters between 28 and 45°N) and the subtropical gyre (defined as waters south of 28°N).

Six salmon sharks and four blue sharks were euthanized during 2002 and 2003 and samples of heart tissues were obtained. Atria and ventricles were sliced into thin pieces, freeze-clamped in liquid nitrogen, and stored at –80°C. Fifteen adult Wistar rats (~300g) of both sexes were euthanized, their hearts were removed and the ventricles immediately freeze-clamped in liquid nitrogen and stored at -80°C. To compare SERCA2 and RyR protein expression across species (Fig. S5), we obtained heart samples from mako shark (*Isurus oxyrinchus*) and white shark (*Carcharodon carcharias*). Heart tissues were homogenized and microsomal fractions isolated by centrifugation (*S5*). SR Ca^{2+}-ATPase (SERCA2) activity was analyzed according to the methods of Landeira et al. (*S5*). The Ca^{2+} sensitive dye (fura-2) was used to measure microsomal Ca^{2+} uptake using a spectrofluorophotometer (Shimadzu, Japan). In addition, SR microsomal fractions were resolved on 3-12% SDS polyacrylamide gels for ryanodine receptor analysis or on 4-20% pre-cast Tris-Hepes-SDS polyacrylamide mini-gels (Pierce Biotechnology Inc, Rockford, IL, USA) for SERCA2 analysis. Gels were silver stained or blotted onto PVDF membranes and probed with a polyclonal antibody specific to either SERCA2 (*S6*) or ryanodine receptor (*S7*). Densitometry was performed using NIH Image (National Institutes of Health, MD, USA).

Table S1. Salmon Sharks Tagged with SPOT and PAT Satellite Tags

	Length[1] (cm)	Tagging Date	SPOT Tag	SPOT Days	Track Distance (km)	PAT Tag	Popup Date	Lat	Lon	PAT days
1	240	25-Jul-99				99-044	23-Oct-99	60.51	60.63	90
2	230	24-Jul-99				99-047	26-Sep-99	-146.90	-146.42	64
3	232	17-Jul-02	37374[2]	453	5672	03-163	DNR[3]			
4	236	16-Jul-02	37375	486	15929	00-1002	13-Jan-03	57.32	-133.67	181
5	214	14-Jul-02	37376	248	12649	00-885	14-Nov-02	41.65	-124.92	123
6	219	17-Jul-02	37377	1162	14237					
7	234	15-Jul-02	37378	723	10940	00-1004	13-Jan-03	60.79	-146.69	182
8	230	16-Jul-02	37379	220	4196					
9	236	17-Jul-02	37380	640	18220	01-040	DNR			
10	225	14-Jul-02	37381	184	9123	00-775	15-Sep-02	41.66	-124.91	63
11	244	17-Jul-02	37382	620	14691					
12	226	15-Jul-02	37383	338	10282	00-931	DNR			
13	232	17-Jul-02				00-767	15-Sep-02	60.49	-147.00	60
14	227	18-Aug-03	41663	355	9396					
15	219	15-Aug-03	41664	30	2047	03-164	21-Nov-03	28.62	-149.17	98
16	236	16-Aug-03	41665	685	11485					
17	231	17-Aug-03	41666	351	11363					
18	234	15-Aug-03	41667[4]	286	6957	03-165	16-Feb-04	35.48	-135.77	185
19	238	17-Aug-03	41669[4]	35	1861	03-168	20-May-04	59.60	-144.66	277
20	226	18-Aug-03	41670	279	10411	03-186	20-Feb-04	58.47	-147.13	186
21	247	19-Aug-03	41671	98	3881					
22	223	17-Aug-03	41672	293	4453					

	Length[1] (cm)	Tagging Date	SPOT Tag	SPOT Days	Track Distance (km)	PAT Tag	Popup Date	Lat	Lon	PAT days
23	247	19-Aug-03	41673	31	1503					
24	235	19-Aug-03	41674	67	3982	03-179	DNR			
25	237	18-Aug-03	41675	319	10471	03-171	20-Feb-04	22.27	-160.24	186
26	236	19-Aug-03	41677	687	11107	03-176	20-Feb-04	31.53	-142.75	185
27	234	18-Aug-03	41679	289	9738	03-174	20-Feb-04	43.05	-134.25	186
28	225	13-Jul-04	52136[4]	DNR		04-097	28-Aug-04	60.72	-146.07	46
29	230	11-Jul-04	52137	DNR		04-099	8-Nov-04	59.941	-148.00	120
30	235	14-Jul-04	52138	DNR						
31	206	12-Jul-04	52139	441	14325	04-105	9-Jan-05	57.194	-152.77	181
32	242	12-Jul-04	52140	DNR						
33	241	13-Jul-04	52141	425	9246	04-108	9-Jan-05	57.9	-154.13	180
34	233	13-Jul-04	52142	383	11930	04-109	9-Jan-05	56.899	-153.10	180
35	236	11-Jul-04	52143[4]	DNR		04-110	8-Aug-04	60.69	-146.59	28
36	242	13-Jul-04	52144	252	2791	04-111	9-Jan-05	59.29	-147.12	180
37	234	13-Jul-04	52145	38	1690	04-113	DNR			
38	236	14-Jul-04	52146	423	9158	04-115	12-Mar-05	30.14	-134.63	241
39	247	14-Jul-04	52147	58	1703	04-101	DNR			
40	229	13-Jul-04	52148	36	1651	04-107	DNR			
41	220	13-Jul-04	52149	369	11804	04-118	10-Jan-05	34.16	-123.38	181
42	232	12-Jul-04	52150[4]	DNR		04-119	8-Aug-04	60.60	-146.60	27
43	239	12-Jul-04	52151	433	11549	04-125	9-Apr-05	41.14	-145.36	271
44	232	12-Jul-04	52152	300	5413	04-126	9-Apr-05	60.60	-147.29	271
45	230	15-Jul-04	52153	418	13181	04-114	DNR			

	Length[1] (cm)	Tagging Date	SPOT Tag	SPOT Days	Track Distance (km)	PAT Tag	Popup Date	Lat	Lon	PAT days
46	237	11-Jul-04	52154	442	11123	04-128	10-Apr-05	42.45	-124.75	273
47	240	11-Jul-04	52155	438	11045	04-129	12-Apr-05	33.96	-120.81	275
48	228	14-Jul-04				04-130	2-Mar-05	59.11	-152.62	231
49	228	14-Jul-04				04-132	11-Nov-04	57.97	-152.21	120
50	242	13-Jul-04				04-134	DNR			
51	226	15-Jul-04				04-136	8-Jan-05	45.63	-164.41	177

[1] Straight Total Length.

[2] This individual was tagged in 2002, then recaptured and re-tagged in 2003, so the tracks were combined.

[3] Did not report.

[4] Recaptured.

Table S2. Composition of Argos Location Classes Obtained from SPOT Tags on Salmon Sharks and Blue Sharks for Filtered Positions

Argos LC	Salmon shark % of positions	Blue shark % of positions	Error within 1 SD
3	7	5	<350 m
2	23	16	<500 m
1	29	25	<1000 m
0	7	9	not reported
A	14	17	not reported
B	20	27	not reported

Table S3. Blue Sharks Tagged with SPOT and PAT Satellite Tags

	Length[1] (cm)	Sex	Tagging Date	SPOT Tag	SPOT Days	Track Distance (km)	PAT tag	Popup Date	Lat	Lon	PAT days
1	199	M	01-Jul-02	36894	132	2218	00-695	26-Aug-02	33.00	-118.38	56
2	220	M	29-Jun-02	36895	148	4756	00-719	25-Oct-02	32.68	-117.48	118
3	225	M	24-Jun-02	37097	178	5735	00-762	26-Aug-02	33.58	-117.45	63
4	222	M	05-Jul-02	37098	18	769					
5	225	M	06-Jul-02	37099	142	3520	00-733	7-Dec-02	32.98	-118.30	154
6	260	M	01-Jul-03	37606	195	6342					
7	225	M	28-Jun-03	37607	DNR[3]		02-652	DNR			
8	215	M	24-Jun-03	37608	161	7595	02-660	27-Dec-03	33.52	-119.28	186
9	193	M	26-Jun-03	37609	110	5168	02-653	DNR			
10	185	F	19-Jun-04	41678	DNR		03-395	n/a[2]			
11	220	M	21-Jun-04	41680	7	189	03-294	DNR			
12	187	M	01-Jul-04	52127[4]	93	3703	03-282	DNR			
13	175	F	17-Nov-04	52130[4]	106	2218	04-247	15-Mar-05	27.13	-114.30	118
14	212	F	11-Oct-04	52216	287	10756	04-359	DNR			
15	208	M	13-Nov-04	52217	DNR		04-147	DNR			
16	179	F	13-Nov-04	53791	112	4886					
17	197	F	12-Nov-04	53792	91	3595	04-148	9-Feb-05	36.82	-122.00	89
18	199	F	15-Nov-04	53793	244	5120	04-246	DNR			
19	183	F	16-Nov-04	53794	87	3422	04-248	DNR			
20	174	F	16-Nov-04	53795	85	3130	04-116	DNR			
21	188	F	09-Nov-04	54579[4]	39	1552	04-149	DNR			
22	164	F	08-Nov-04	54580	44	1602	04-146	27-Dec-04	35.13	-121.83	49

	Length[1] (cm)	Sex	Tagging Date	SPOT Tag	SPOT Days	Track Distance (km)	PAT tag	Popup Date	Lat	Lon	PAT days
23	173	F	16-Nov-04	54581	32	1016	04-143	24-Dec-04	35.63	-121.32	38
24	189	F	16-Nov-04	54582	58	1470	04-152	4-Mar-05	5.74	-119.09	108
25	187	F	11-Nov-04	54583	139	5578	04-363	12-Feb-05	36.82	-122.00	93
26	186	F	12-Nov-04	54584	DNR		04-151	23-Nov-04	36.40	-122.19	11
27	195	F	09-Nov-04	54585	41	1647	04-362	25-Dec-04	35.40	-121.08	46
28	190	F	08-Nov-04	54586	242	8133	04-173	DNR			
29	188	F	10-Nov-04	54587	91	2315	04-150	5-May-05	7.28	-124.03	176
30	187	F	11-Nov-04	54588	88	3546	04-145	25-Dec-04	12.55	-121.15	44
31	142	F	24-Jun-05	54590	DNR		04-534	n/a[2]			

[1] Straight Total Length.

[2] Not applicable, mortality occurred immediately after tagging

[3] Did not report.

[4] Recaptured.

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Supplement Figure Legends

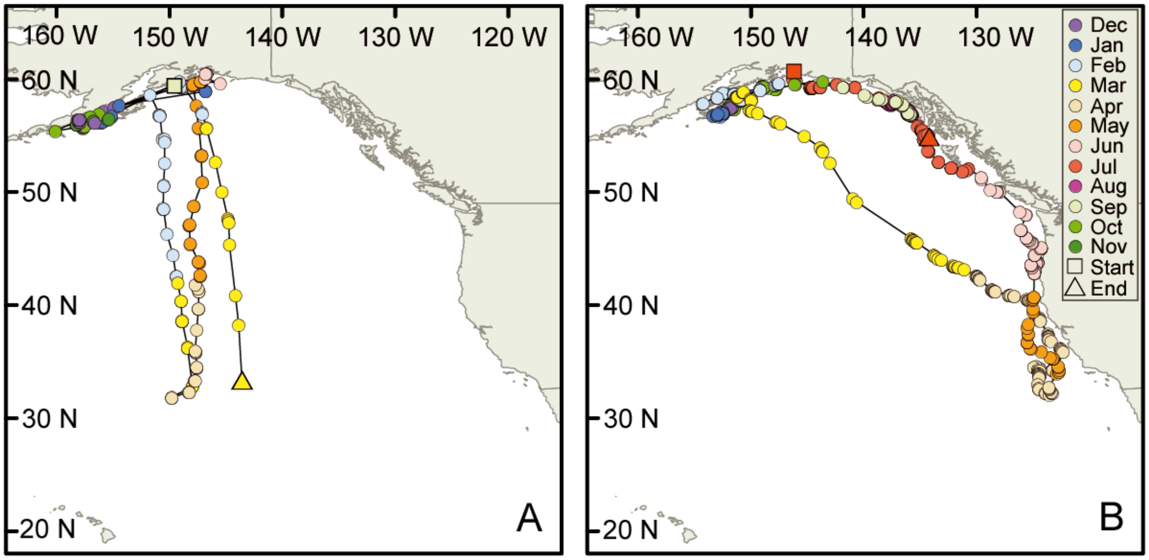
Figure S1. Seasonal Migrations of Salmon Sharks from Subarctic to Subtropical Waters. (A) Shark #37382 moved from waters of Prince William Sound and the Gulf of Alaska into the pelagic eastern North Pacific during two consecutive annual migrations, utilizing similar pelagic regions in both years. Southward movements occurred during February and March and the animal returned to the Gulf of Alaska in May. (B) Shark #52139 departed Gulf of Alaska waters in March and traveled to waters off California, arriving in April. It remained in the California Current System through September as it moved north along the margin of the North American continent. Argos positions (circles) are colored according to month with squares denoting the beginning of tracks and triangles denoting the end.

Figure S2. Low Productivity of Southern Habitat of Salmon Sharks. The oligotrophic waters visited by salmon sharks during spring suggest a non-foraging purpose for the migration, consistent with movements to pupping grounds. Surface chlorophyll-a concentration was significantly lower at the southern destinations of salmon sharks (subtropical gyre, south of 28°N) than in their northern habitat (subarctic gyre, north of 45°N) (Kruskal-Wallis test, $H = 1540.96$, $DF = 2$, $P = 0.000$). Inset: chlorophyll-a concentration decreases as salmon shark #37383 migrates to the south. Chlorophyll data courtesy of NASA/GSFC/DAAC and Orbimage Inc.

Figure S3. Movements of Blue Sharks in the Eastern North Pacific. SPOT tags mounted on the first dorsal fins of blue sharks successfully recorded movements across a broad area of the temperate and tropical eastern North Pacific from 104-157°W and 4-37°N. Sharks were tagged in the Southern California Bight in June 2002 (black), June 2003 (grey) and November 2004 (white). Blue sharks aggregated in the California Current upwelling system off California and Baja, but individual animals made long distance movements into oligotrophic waters to the south and west. SST satellite data is averaged for the tracking period, courtesy of NASA/JPL/PODAAC (California Institute of Technology) and NOAA/NESDIS/OSDPD.

Figure S4. Temperate and Tropical Habitats of a Blue Shark. (A) Depth-temperature profiles along the track of a blue shark (#53792) in waters off California and Mexico show a 16-27°C mixed layer and thermocline waters cooling to 8°C. (B) Contour plots made from discrete measurements show that the thermal habitat of the blue shark is predominantly from 14-27°C in the mixed layer and upper thermocline, with only brief periods in waters cooler than 10°C. In comparison to salmon sharks, blue sharks inhabited waters with warmer surface temperatures, and spent less time in cold waters beneath the upper mixed layer.

Figure S5. SERCA2 and RyR Protein Expression in the Hearts of Lamnid Sharks. Western blot analysis of atrial (A) and ventricular (V) microsomal preparations from salmon shark, mako shark and white shark, with blue shark and rat ventricle shown for comparison. (Upper panel): SERCA2- specific antibody labels a ~110 kDa band revealing high expression of the Ca^{2+}ATPase among the Lamnidae family. (Lower panel): RyR-specific antibody labels a ~565 kDa band representative of the SR Ca^{2+} release channel protein.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30 N
20 N
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B
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Jan
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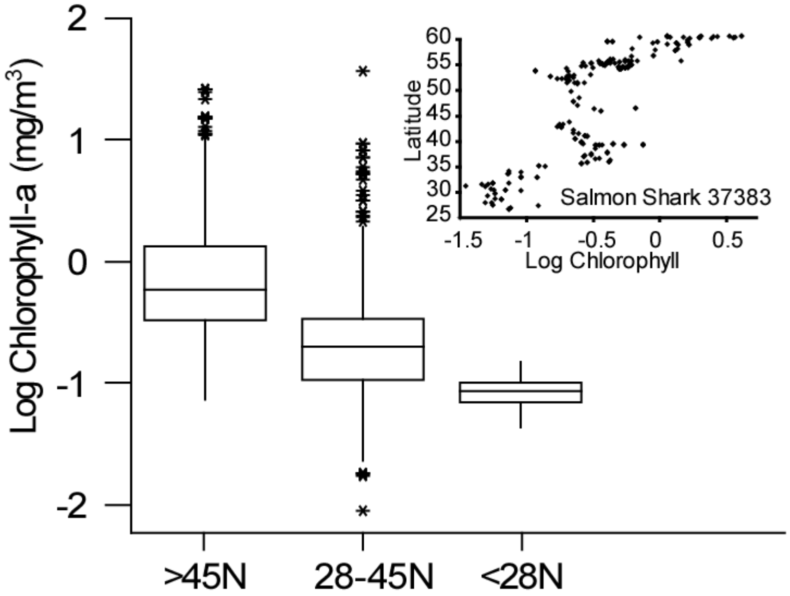
Jun
Jul
Aug
Sep
Oct
Nov
Start
End

Figure S1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<28N
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Latitude
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Log Chlorophyll
Salmon Shark 37383

Figure S2

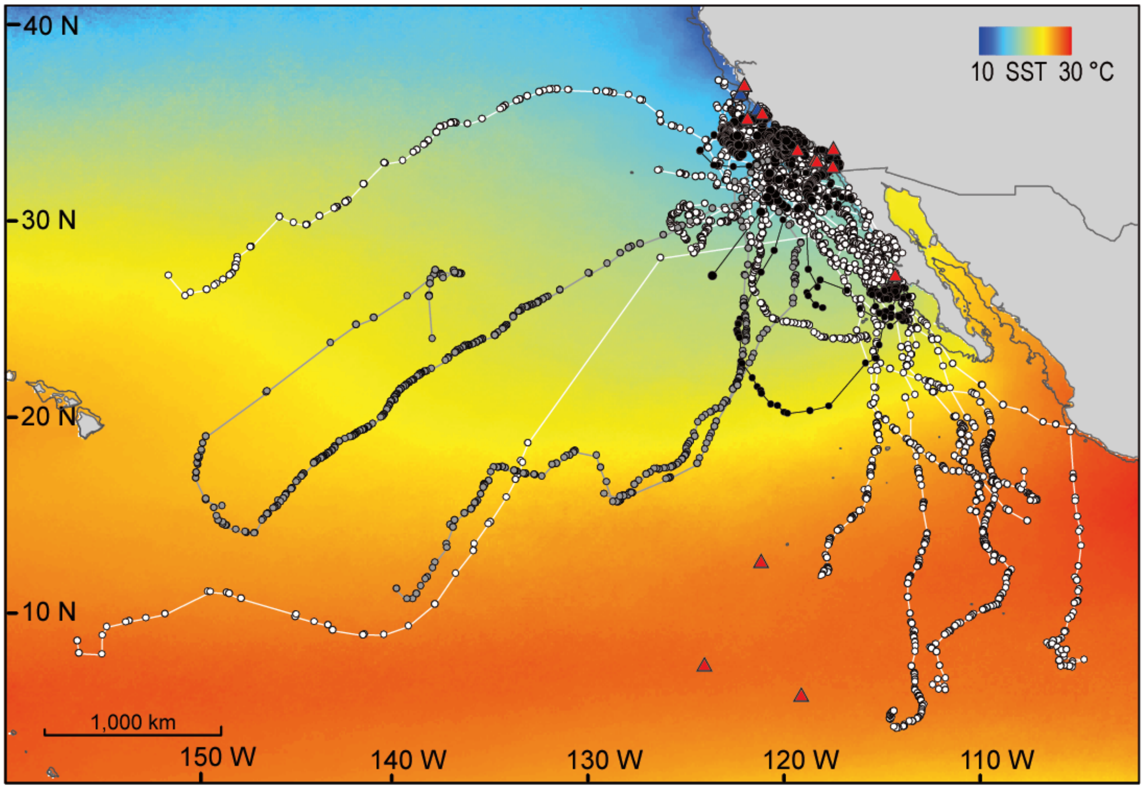
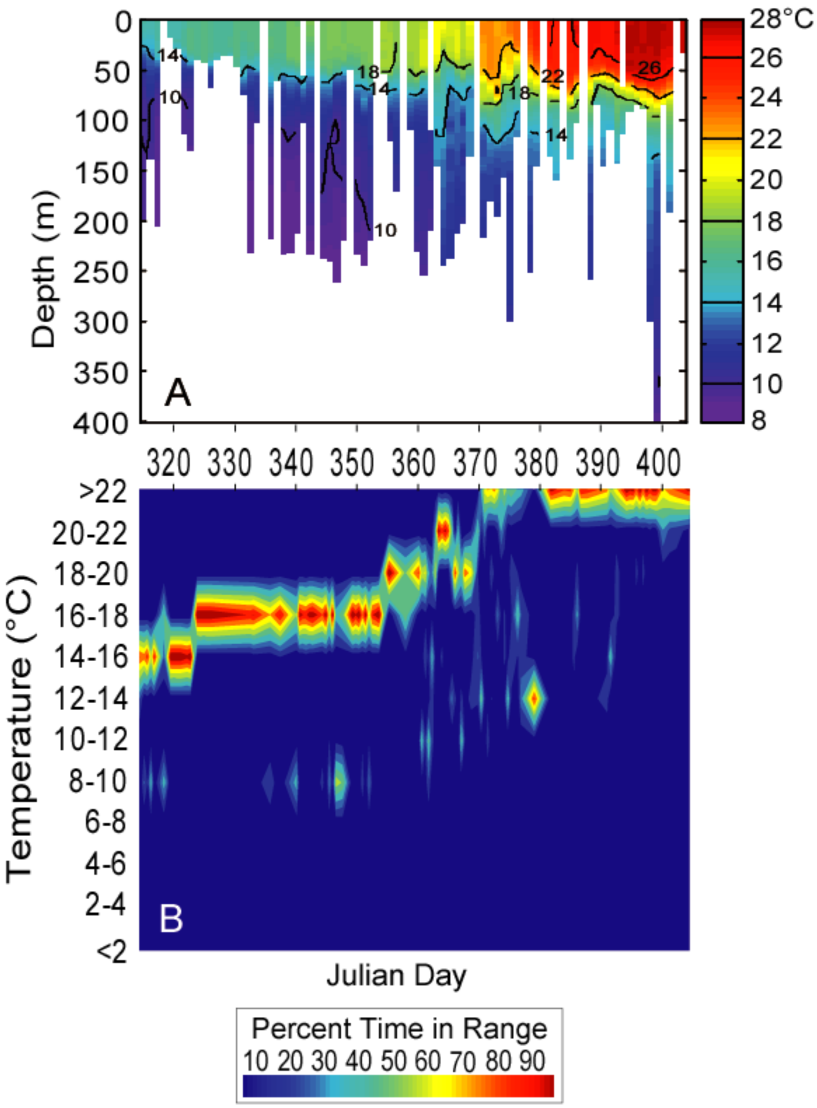


Figure S3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350
400
A
320 330 340 350 360 370 380 390 400
Temperature (°C)
>22
20-22
18-20
16-18
14-16
12-14
10-12
8-10
6-8
4-6
2-4
<2
B
Julian Day
Percent Time in Range
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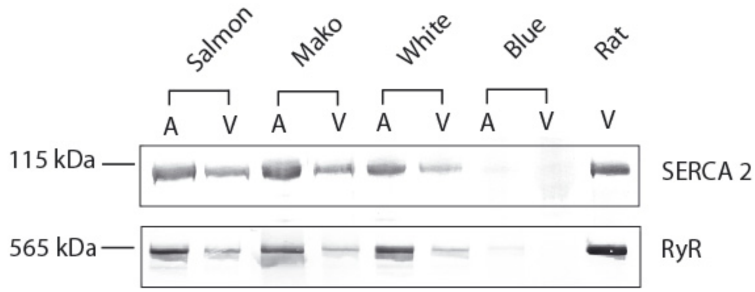
Figure S4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Mako
White
Blue
Rat
A
V
A
V
A
V
A
V
V
115 kDa
565 kDa
SERCA 2
RyR

Figure S5