SUPPORTING INFORMATION

Section 1. Methods

1.1 Electronic tagging

Archival tag data for Pacific bluefin tuna *Thunnus orientalis* (PBFT), yellowfin tuna *T. albacares* (YFT), and albacore tuna *T. alalunga* (ALB) collected between 2002 and 2009 as part of the Tagging of Pacific Predators (TOPP) project (Block *et al.*) (1) were analyzed to identify periods of spatial and temporal overlap between these three tuna species. We identified overlap as periods when all three tuna species occurred within a 0.5×0.5 degree area during a seven day period. We used a sensitivity analysis for combinations of grid size $(0.25, 0.5,$ and 1 degree grids) and time periods $(1 - 14 \text{ days})$ to determine this window. Combinations of lower grid size and/or short timeframes resulted in too few data for analysis, while largest grid size and/or timeframes resulted in maximum data points but inclusion of data from days that were likely not representative of actual overlap. A 0.5 degree grid and 7 day time period maintained a reasonable sample size while keeping spatial resolution fine enough to ensure that all three species were overlapping (Figure S1). We restricted our analysis to months for which we had stomach content data (July – October). Because ALB were primarily tagged during 2004 and 2005, those are the years during which there were periods of overlap in all three species. Hence results presented in this paper are from this period, however further analysis of the entire dataset (2002-2009) demonstrated that the relative patterns of depth and temperature distribution between these species during the 2004-2005 time period was consistent across the entire tag dataset.

For each week of data, we searched for all grid cells where all three species occurred. Each cell where overlap occurred became the core of an overlap area. The maximum and minimum possible latitude and longitude of fish within that cell was estimated to account for geolocation error $(\pm 1.9^{\circ} \text{ latitude}, \pm 0.8^{\circ} \text{ longitude})$ (1). The grid area encompassed by this maximum and minimum latitude and longitude was used to identify all fish that could have also overlapped with fish in the core overlap cell given geolocation error. All data from fish within this overlap area were compiled and used to quantify the extent to which these three species vertically and thermally partitioned their environment during periods of overlap.

To describe each species' vertical and thermal distribution during periods of overlap, we calculated proportion of time spent at depth (TAD) and time spent at temperature (TAT) by time of day in fifteen minute intervals as described in Carlisle *et al.* (2) for each fish selected for analysis. Mean TAD and TAT figures were then generated for each species (Fig. S3). To directly assess which particular species used particular depths or temperatures to the greatest extent, we determined which species spent the greatest proportion of time in each time and depth or temperature bin. To validate that fish were indeed occurring in the same (or similar) bodies of water with the same thermal characteristics during periods of overlap, we calculated the temperaturedepth profile for each day of data and plotted mean $(\pm SD)$ temperature depth profiles for each species(Fig. S2). For each day we also estimated the depth of the isothermal layer, which is a proxy for mixed layer depth, using the method described by Kara *et al.* (3).

To test for inter-specific differences in TAD and TAT, for each individual fish we calculated total proportion of time spent in each bin, using 10 m depth bins and 1 and 5°C temperature bins (see Table S3) for PBFT $(n = 43)$, YFT $(n = 31)$, and ALB $(n = 13)$. Proportions of TAD and TAT were arcsine-transformed and compared using 1-way ANOVA for each depth and temperature bin, using individual fish as replicates for a given species. Tukey's honest significant difference criterion was used for the multiple comparison test. Significant difference are reported for $\alpha = 0.05$ and $\alpha = 0.1$ (table S3). We only reported differences when one species spent significantly more time in a given bin than the other two species.

1.2 Cardiac gene expression

Pacific bluefin tuna *Thunnus orientalis* (PBFT; *n* = 7), yellowfin tuna *T. albacares* (YFT; $n = 5$) and albacore tuna *T. alalunga* (ALB; $n = 8$) individuals were caught in the month of June 2008 off the coast of Baja California by hook and line fishing on board the F/V Shogun. Immediately following capture the individuals were euthanized and cardiac tissue was excised and placed in RNAlater® (Applied Biosystems, Foster City, CA, USA) at 4° C followed by freezing at -80 $^{\circ}$ C for long term storage. Curved fork length of the specimens ranged from 80 to 95 cm.

Cardiac ventricle tissue samples were homogenized with a TissueLyser II and stainless steel beads (Qiagen, Valencia, CA, USA). Total RNA was purified from cardiac samples using TRIZOL® reagent as recommended by the manufacturer (Invitrogen Life Technologies, Carlsbad, CA, USA). Concentration and purity of the RNA were determined using a spectrophotometer (NanoDrop® ND-1000, NanoDrop Technologies Inc., Wilmington, DE, USA) with 230, 260, and 280 nm readings. RNA quality was assessed for all samples by visualization on a denaturing formaldehyde RNA gel (protocol recommended by Qiagen, Valencia, CA, USA) and ethidium bromide staining. Total RNA was DNase treated with the TURBO DNA-free™ kit (Ambion Life Technologies, Carlsbad, CA, USA) as per the manufacturer's protocol to remove any remaining genomic DNA from the samples.

qPCR was used to determine relative transcript abundance in cardiac ventricle tissue of two gene targets from three different species of tuna. First-strand cDNA was synthesized using 1 μg of total RNA and iScript[™] reverse transcript supermix, according to the manufacturer's instructions (BIO-RAD. CA, USA). Assays was performed on the CFX Connect™, using SsoAdvanced™ SYBR® Green supermix (BIO-RAD. CA, USA). A total reaction volume of 10 μl was used. The reaction mix included a final cDNA template concentration of 0.5 ng, $150 - 200$ nM for each primer, 5 µl SsoAdvancedTM SYBR® Green supermix with the remaining reaction volume of 10 μl consisting of RNA-free water. The qPCR assays were performed using the following conditions: 95°C for 3 min followed by 40 cycles of 95°C for 10 s and 55°C for 30 s, then 95°C for 10s and 55 - 95°C for 5s in 0.5°C increments. Dissociation curves were analyzed on all samples for presence of non-specific amplification. No-template controls and no-reversetranscriptase controls were also performed to determine the presence of reagent and genomic contamination respectively. Standard dilution curves with a minimum of 4 points were generated for each assay using pooled cDNA from all the samples of the appropriate species. Amplification efficiencies for all assays ranged between $90 - 110$ %. qPCR assay specifics for each assay are indicated in Supplementary Table 1. Relative expression values for each gene were calculated using a standard curve method. To compare gene expression values across species, gene expression values calculated above for SERCA2 and RYR2 were normalized to beta-actin expression values in each species. Beta-actin normalized gene expression data followed a Gaussian distribution and was analyzed based on ordinary one-way ANOVA followed by Tukey's test to correct for multiple comparisons. Multiplicity adjusted *P*-values are reported.

1.3 Diet analysis

Stomachs were collected from PBFT, YFT, and ALB from the fishing vessel R/V Shogun during the months of July-October in the years 2008-2010. Stomachs were also collected from anglers returning from day trips during the same time period. Stomachs were collected from each species only during periods when all three species were being caught in the same general area, according to information from recreational boat captains. Only one stomach for each tuna species was analyzed per sampling site to eliminate pseudoreplication of a single day's feeding conditions. Stomachs were immediately frozen at - 20°C prior to analysis.

Stomachs were thawed, opened, and the contents were removed by rinsing into a sieve. All identifiable prey items including whole prey, hard parts (otoliths, cephalopod beaks, vertebrae) and other organic material was removed and transferred to 95% ethanol. Contents were identified visually utilizing fish vertebrae (4), cephalopod beaks (5, 6), and otolith structure following Glaser *et al.* (7)*.* Some prey were unidentifiable; these largely consisted of eyes (cephalopods) and vertebrae and otoliths (fishes) that were too degraded to be identified with confidence. We generated cumulative prey curves based on methods in Bizzarro *et al.* (8) (Fig. S4) to assess whether sample size of analyzed stomachs for each tuna species was adequate to properly represent diet. For each tuna species, cumulative prey curves neared asymptotic values within the range of the number of stomachs sampled (Fig. S4), indicating that our sample size for each tuna species was adequate to represent overall diet in the region of spatiotemporal overlap. Length and mass of identifiable prey were calculated using published algorithms for each species (9- 12), when available. Energetic value of prey was then calculated using prey-specific energy densities (ED; $kJ g^{-1}$) reported in Glaser *et al.* (7) for prey of the California Current.

A modified index of relative importance (IRI), using % prey energetic content in place of % volume was calculated for the top four items in each predator diet. The standard equation for IRI from Pinkas (5) is

$$
IRI = (\% N + \% V) (\% F) \tag{1}
$$

where $N =$ number, $V =$ volume, and $F =$ frequency of occurrence. However, we were interested in the energetic contribution of prey to tuna diet. Therefore we used the modified equation:

$$
IRI = (% N + % kJ) (% F)
$$
 (2)

where KJ = the estimated energetic content of the given prey based on the mass of that prey item. IRI values were highest in all species for a few prevalent prey items, then decreased rapidly (Supplementary Tables 4-6). Therefore we report the IRIs for only the four most prevalent items in the diet of each tuna species.

The Shannon-Weiner Information Measure (*H*) was also calculated for each tuna species. This index is a measure of diet diversity, and is calculated according to the equation from Wilson & Bossert (13):

$$
H = -\sum_{i=1}^{S} (p_i) \ln(p_i)
$$
 (3)

where p_i is the proportion of a given food item, and can be relative number, mass, etc. We used relative kJ values (% kJ) for p_i , with $S = 4$ (the four most prominent items by energetic content in each tuna species' diet.

We calculated the prey-specific abundance (PSA) for the top four prey items in each tuna species. PSA is a measure of specialization on a particular prey item, and is defined as the percentage a prey taxon comprised of all prey items in only those stomachs in which that actual prey occurred (14). It is calculated following the equation from Amundsen *et al.* (14):

$$
PSA_i = (\sum S_i / \sum S_t)
$$
 (4)

where PSA is the prey-specific abundance of prey i , S_i is the stomach content comprised of prey *i*, and *S^t* is the total stomach content only in predators with prey *i* in their stomach (14). A PSA value > 0.5 suggests specialization on that prey, with a value of 1 representing full specialization (14). We only found a $PSA > 0.5$ for one prey species (sardine *Sardinops sagax*) in one tuna species (PBFT). We thus calculated PSA for sardine in all three tuna species to compare relative specialization on this prey resource across PBFT, YFT, and ALB.

We calculated mean kJ stomach⁻¹ for each tuna species. Mean kJ stomach⁻¹ and sardine PSA values were compared across tuna species using the non-parametric Kruskal Wallis test, with significance at $\alpha = 0.05$. Non-normality of PSA and kJ stomach⁻¹ data necessitated this non-parametric test.

Prey of high importance to all bluefin species (Atlantic bluefin *Thunnus thynnus,* Southern bluefin *T. maccoyii*, and PBFT *T. orientalis*) in various regions was assessed from the literature (Supplementary Table 7). We included any studies that concluded the dominance of one or a few prey in diets of three bluefin tuna species diets, in various ocean regions. Energy densities $(kJ g^{-1})$ for these prey were reported in the literature (Supplementary Table 6).

Figure S1. All geolocation positions, estimated from archival tag data, of Pacific bluefin tuna *Thunnus orientalis* (*n* = 725 days), yellowfin tuna *T. albacares* (583 d), and albacore tuna *T. alalunga* (210 d) during periods of overlap. Circles are daily geolocation positions, colored for each species: Pacific bluefin tuna (PBFT; blue), yellowfin tuna (YFT; yellow), and albacore tuna (ALB; grey).

Figure S2. Reconstructed water column profiles utilizing archival tag data from all fish during periods of overlap. Solid lines are mean temperatures at depth for Pacific bluefin *Thunnus orientalis* (PBFT, blue), yellowfin *T. albacares* (YFT, yellow) and albacore *T. alalunga* (ALB, grey) and dotted lines the 95% CIs.

Figure S3. Time-at-depth (TAD, % and log %) and time-at-temperature (TAT, % and log %) by time of day for Pacific bluefin *Thunnus orientalis*, yellowfin *T. albacares* and albacore *T. alalunga* tuna during periods of overlap. Note that maximum values in the color bar represent values \geq that value.

Figure S4. Mean cumulative prey curves for Pacific bluefin *Thunnus orientalis* (PBFT), yellowfin *T. albacares* (YFT), and albacore *T. alalunga* (ALB). Error bars show standard deviation (see Supplementary Tables 3-5 for specific diet information).

Figure S5. Histograms showing frequency distributions for estimates of kJ/stomach for Pacific bluefin *Thunnus orientalis* (blue), yellowfin *T. albacares* (yellow), and albacore *T. alalunga* (grey). Empty stomachs were not included.

Table S2. Numbers of archival tag datasets from Pacific bluefin *Thunnus orientalis* (PBFT), yellowfin *T. albacares* (YFT), and albacore *T. alalunga* (ALB), and numbers and percentages, by month, of days for each species that were used in data analysis.

Table S3. Depth and temperature bins where proportion of time spent by Pacific bluefin *Thunnus orientalis* (PBFT; $n = 43$), yellowfin *T. albacares* (YFT; $n = 31$), or albacore *T. alalunga* (ALB; $n = 13$) were statistically higher than other two species Rows are color coded to indicate the species that spent the most time in a given bin (blue: PBFT, gray: ALB, yellow: YFT). Proportional data were arcsine-transformed and compared using 1 way ANOVA. Significance reported at $\alpha = 0.05$ and $\alpha = 0.1$. 'ns': not significant (no species spent significantly more time in that bin than the other two); n/a: no data or sample size too low for comparison.

Depth	α		Temp	α		Temp	α	
(m)	0.05	0.1	$({}^{\circ}C)$	0.05	0.1	$({}^{\circ}C)$	0.05	0.1
10	$\rm ns$	ns	26	PBFT	PBFT	25	ns	$\rm ns$
20	ALB	ALB	25	PBFT	PBFT	20	YFT	YFT
30	ALB	${\bf ALB}$	24	$\rm ns$	$\rm ns$	15	ALB	ALB
40	ALB	ALB	23	ns	ns	10	ns	$\bf ns$
50	ALB	ALB	22	$\rm ns$	$\rm ns$	\mathfrak{S}	$\rm ns$	PBFT
60	ALB	ALB	21	ns	ns	$\boldsymbol{0}$	ns	ns
70	ALB	ALB	20	ns	ns			
80	ALB	ALB	19	ns	ns			
90	ALB	ALB	18	ns	ns			
100	$\,ns$	ns	17	ALB	ALB			
110	ns	ns	16	ALB	ALB			
120	ns	ns	15	ALB	ALB			
130	ns	ns	14	ALB	ALB			
140	ns	ns	13	ALB	ALB			
150	ns	ns	12	ALB	ALB			
160	ns	ns	11	$\rm ns$	ALB			
170	ns	$\rm ns$	10	ns	ns			
180	ns	ns	9	ns	ns			
190	ns	PBFT	8	ns	PBFT			
200	ns	$\rm ns$	$\boldsymbol{7}$	ns	PBFT			
210	ns	ns	6	PBFT	PBFT			
220 230	ns	PBFT	5 $\overline{4}$	n/a	n/a			
240	ns	ns	3	n/a n/a	n/a n/a			
250	$\,ns$	ns	\overline{c}	n/a	n/a			
260	ns $\,ns$	ns ns	$\mathbf{1}$	n/a	n/a			
270	ns	ns						
280	ns	ns						
290	PBFT	PBFT						
300	PBFT	PBFT						
310	ns	ns						
320	ns	ns						
330	$\rm ns$	PBFT						
340	ns	ns						
350	ns	$\rm ns$						
360	ns	ns						
370	ns	ns						
380	ns	ns						
390	$\rm ns$	$\rm ns$						
400	ns	ns						
410	PBFT	PBFT						
420	PBFT	PBFT						
430	PBFT	PBFT						
440 450	PBFT	PBFT						
	n/a	n/a						

450 n/a n/a
460 n/a n/a n/a n/a n/a n/a n/a

Table S4. Metrics showing prey species found in 82 Pacific bluefin (*Thunnus orientalis*) stomachs in 2008, 2009, and 2010. Metrics are as follows: N is abundance, %N is relative abundance, FO is frequency of occurrence by stomach, %FO is % of stomachs containing that food item. Total mass is mass of prey items estimated from length, otolith size (fish), or beak metrics (cephalopods), and total kJ is estimated from mass using energy density values from Glaser *et al.(7).* IRI is shown for the 4 most prevalent prey items. A hyphen (–) indicates that metric(s) could not be calculated because species were unidentified; 'nd' ('no data') indicates that conversion algorithms were not available for mass and kJ for that species.

Table S5. Metrics showing prey species found in 86 albacore (*Thunnus alalunga*) stomachs in 2008, 2009, and 2010. Metrics are as follows: N is abundance, %N is relative abundance, FO is frequency of occurrence by stomach, %FO is % of stomachs containing that food item. Total mass is mass of prey items estimated from length, otolith size (fish), or beak metrics (cephalopods), and total kJ is estimated from mass using energy density values from Glaser *et al.(7).* IRI is shown for the 4 most prevalent prey items. A hyphen (–) indicates that metric(s) could not be calculated because species were unidentified; 'nd' ('no data') indicates that conversion algorithms were not available for mass and kJ for that species.

Table S6. Metrics showing prey species found in 75 yellowfin (*Thunnus albacares*) stomachs in 2008, 2009, and 2010. Metrics are as follows: N is abundance, % N is relative abundance, FO is frequency of occurrence by stomach, % FO is % of stomachs containing that food item. Total mass is mass of prey items estimated from length, otolith size (fish), or beak metrics (cephalopods), and total kJ is estimated from mass using energy density values from Glaser *et al.(7).* IRI is shown for the 4 most prevalent prey items. A hyphen (–) indicates that metric(s) could not be calculated because species were unidentified; 'nd' (no data') indicates that conversion algorithms were not available for mass and kJ for that species.

Table S7. Dominant prey for three bluefin tuna species: Atlantic (*Thunnus thynnus*), Pacific (*T. orientalis*), and southern (*T. maccoyii*) bluefin tuna. Prey energy density (ED; kJ g -1) are shown. For comparison, major prey items of yellowfin tuna (*T. albacares*) and albacore (*T. alalunga*) and associated prey ED are also shown.

Tuna species	Region	Preferred prey	ED	Reference
T. orientalis	E. Pacific	Sardinops sagax	7.3(7)	this study
		Engraulis mordax	6.6(7)	Pinkas (5)
T. orientalis	W. Pacific	Etrumeus teres	7.5(15)	Shimose et al. (16)
		Engraulis japonicas	6.6(7)	Shimose et al. (16); Yamanaka et al. (17)
		Sardinops melanostictus	7.3(7)	Shimose et al. (16)
T. thynnus	W. Atlantic	Clupea harengus	12.6(18)	Chase (21) ; Estrada et al. (22)
		Ammodytes americanus	7.3(19)	Logan et al. (23); Chase (21);
				Estrada et al. (22)
		Scomber scombrus	10.3(17)	Chase (21)
		Engraulis encrasicolus	$6-8.4(20)$	Logan et al. (23)
T. thynnus	E. Atlantic	Engraulis encrasicolus	$6-8.4(20)$	Ortiz de Zarate & Cort (24)
T. thynnus	Med	Engraulis encrasicolus	$6-8.4(20)$	Collette and Nauen (26)
		Sardina pilchardus	$4-14.2(25)$	Sanz Brau (27); Orsini Relini et al. (28)
		Myctophid spp.	7.1(7)	Karakulak et al. (29)
		Trachurus spp.	6.4(7)	Karakulak et al. (29)
T. thynnus	W. Atlantic	Brevoortia tyrannus	6.2(30)	Butler (31)
	(N. Carolina)			
T. maccoyii	Australia	Sardinops neopilchardus	5.6(32)	Ward et. al. (34)
		Engraulis australis	5.2(33)	Ward et. al. (34)
		Scomber australasicus		Ward et. al. (34)
T. maccoyii	E. Tasmania	Sardinops neopilchardus	5.6(32)	Fitzgibbon et al. (32)
T. alalunga	E. Pacific	Euphausiid spp.	3.1(7)	this study; Madigan et al. (35)
T. alalunga	E. Pacific	Trachurus symmetricus	6.4(7)	this study
T. alalunga	E. Pacific	squids	4.4(7)	this study
T. alalunga	E. Pacific	amphipods	2.5(7)	Pinkas (5)
T. alalunga	S. Pacific	squids	4.4(7)	Young et al. (36)
T. alalunga	E. Pacific,	Engraulis mordax	6.6(7)	Pinkas (5); Bernard et al. (37); Watanabe et al.
	NPTZ			(38)
T. albacares	E. Pacific	Sardinops sagax	7.3(7)	this study
T. albacares	E. Pacific	Trachurus symmetricus	6.4(7)	this study
T. albacares	CPO	Malacostracans	3.2(7)	Graham et al. (39)
T. albacares	CPO	squids	4.4(7)	Graham et al. (39)
T. albacares	W. Atlantic	squids	4.4(7)	Rudershausen et al. (40)
T. albacares	E. Pacific	pelagic red crab	3.0(7)	Olson et al. (41)

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