Positional and ontogenetic variation in vertebral centra morphology in five batoid species

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Running head: Morphology of batoid vertebral columns

Abstract

An increasing number of studies on elasmobranchs have shown that band pair counts in vertebral centra do not accurately reflect age. Research in sharks indicates that the number of band pairs vary with body size and centrum morphology is related to structural needs. A study of this kind has not been undertaken on batoids, thus we examined the relationship between band pair deposition and morphology of centra along the vertebral column, and ontogenetically, for five batoid species (little skate, *Leucoraja erinacea*, winter skate, *Leucoraja ocellata*, barndoor skate, *Dipturus laevis*, Atlantic stingray, *Dasyatis sabina*, and round ray, *Urobatis halleri*). Centrum morphology and band pair count varied along the vertebral column in all individuals of all species except in young of the year. Variation in band pair counts among centra within individuals supports the hypothesis that band pair formation is related to somatic growth and body shape rather than to an annual cycle.

Additional keywords: band pair counts, skates and rays, somatic growth, age

Introduction

The vertebral centra of elasmobranchs have characteristic alternating opaque and translucent bands (termed a band pair) that have been assumed to represent one year of growth and used to estimate age (Ridewood 1921; Haskell 1948; Ishiyama 1951; Cailliet *et al*. 2006). Annual band pair formation was assumed because more band pairs were found in the centra of larger individuals (Ridewood 1921). Subsequent studies demonstrated positive relationships between increasing body size and both centrum size and number of band pairs across individuals

(Ishiyama 1951; Jones and Geen 1977; Cailliet and Goldman 2004) supporting the use of vertebral bands pairs for age estimates.

Over time issues have become apparent in the use of vertebral band pairs for age estimates. Several species do not deposit band pairs annually, instead, depositing them relative to somatic growth (Natanson and Cailliet 1990; Tanaka 1990; Natanson *et al.* 2008). Other studies demonstrate decreased band pair deposition in older and larger individuals showing band pair counts underestimate age (see review by Harry 2018). Identifying these instances is critical to avoid inaccurate age estimates.

Another issue relates to the positive relationship between the number of band pairs and the size of the centrum. This positive relationship is true among individuals where larger individuals have larger centra with more band pairs (Cailliet and Goldman 2004), but it is also true within an individual; where centrum size and number of band pairs varies along the vertebral column (Natanson *et al.* 2018). Several studies have shown that small (young of the year [YOY]) individuals often have the same number of band pairs throughout their vertebral column and similar centrum sizes however, band pair counts and centra size increasingly vary along the vertebral column in larger, maturing individuals of the same species (Natanson and Cailliet 1990; Natanson *et al.* 2008; Huveneers *et al.* 2013; Natanson *et al.* 2018). This has been shown in several species belonging to different families. If band pair deposition varies along the vertebral column with centrum size then band pair count cannot be related to age. Natanson *et al.* (2018) concluded that the number of band pairs vary along the column in direct correlation with the girth of the fish where the centra were taken and any relationship with time is not causative, but correlative through the somatic growth rate.

A majority of the research on elasmobranch vertebral column morphology and frequency of band pair deposition have been conducted on sharks, not batoids (Harry 2018; Natanson *et al.* 2018). Ageing of batoids is subject to the same assumptions (Ishiyama 1951; Cailliet *et al*. 2006), but there are only three confirmed batoid species demonstrating age underestimation (Natanson 1993; McPhie and Campana 2009; Pierce and Bennett 2009; James 2020). This is likely due to fewer validation studies on batoids (Harry 2018) rather than the absence of age underestimation.

The goal of the present study was to investigate whether vertebral band pairs are related to somatic growth and/or ontogeny in batoids. We measured centrum dimensions and counted the band pairs in individual centra along the columns of various-sized individuals of five batoid species (little skate, *Leucoraja erinacea*, winter skate, *Leucoraja ocellata*, barndoor skate, *Dipturus laevis*, Atlantic stingray, *Dasyatis sabina*, and round ray, *Urobatis helleri*; James 2018). We then related centrum morphology to the number of band pairs of each centrum along the vertebral column and examined these relationships by size, sex, and species. Variation of band pair counts along the vertebral column of an individual calls into question the assumption that band pair formation is directly related to time, and raises concerns for using band pair counts for age estimates.

Methods

Skates were obtained opportunistically from commercial fishermen off the coast of Rhode Island and Massachusetts, USA. Atlantic stingrays were obtained from Seal Beach, California, and round rays from Indian River, Florida. Total length (TL; straight-line distance from snout tip to tail tip), and disc width (DW; straight-line distance from wing tip to wing tip) were measured to the nearest 0.1 cm on all individuals. By convention, TL is used in analyses for skates, while DW is used in analyses for stingrays (Francis 2006). Sex and maturity status were determined by visually inspecting gonad condition (Ebert 2005).

Forty-two little skates (14 immature [small], 15 near size-at-maturity [medium], and 13 mature [large]), six winter skates and six barndoor skates (two small, two medium, and two large of each), nine Atlantic stingrays (three small, three medium, and three large), and ten round rays (two small, four medium, and four large) were collected and measured for analysis of centrum morphology (Table 1; James 2018). Band pair counts were conducted along the vertebral columns of a subset of nine little skates, and six each of Atlantic stingray and round rays (Table 1). The subset for each species included small, medium, and large individuals. All winter skates and barndoor skates were analyzed for the number of band pairs (Table 1). Males and females were evenly represented where possible (Table 1).

Centrum Morphology

The vertebral column was extracted from each fish starting with the first vertebra behind the synarcual cartilage and ending at the 80th vertebra. One round ray had sustained a tail injury and vertebrae were only available to the 48th centrum.

Each vertebral centrum was measured to the nearest 0.1 mm in three dimensions: dorso-ventral diameter (DVD), lateral diameter (LD) and rostro-caudal length (LEN), using Vernier calipers following Natanson *et al.* (2018) (Figure 1). Each measurement was divided by TL for the skate species and DW for the stingray species to standardize data across sizes for direct comparison. Standardized data were plotted against centrum number for each individual, noting the centrum number at the transition from abdominal cavity to tail.

Multiple generalized additive models (GAMs) were fit to each species using mgcv package in R (Wood 2011; R Core Team 2017) to assess whether each centrum measurement (DVD, LD, and LEN) was similar within a species, by sex, by size, or by individual (James 2018). Four GAM variations were run for each measurement for each species: all data pooled, data grouped by sex, size class, and individual. Three GAM iterations were run: different intercepts only, different smoothing functions only, and different intercepts and smoothing functions for each sex, size class, and individual scenario. For each GAM variation the number of knots was specified to be larger than the estimated degrees of freedom using the gam.check function of mgcv in R. Model fit was assessed with Akaike's Information Criterion (AIC) (Haddon 2001).

Band Pair Counts

To determine if band pair number varied along the vertebral column of an individual, every fifth centrum was processed histologically to visually enhance the band pairs (as per Natanson *et al.* 2007). Centrum sections were viewed under a dissecting microscope (Nikon SMZ1500®, Melville, NY, USA[1]) using reflected light and images were captured with a digital camera (Nikon DSR12, Tokyo, Japan) and image processing software (NIS Elements, v. 4.40, Nikon, Tokyo, Japan). Two band pair counts were made for each individual by a primary (KCJ) and a secondary reader using editing software (Adobe Photoshop CC (Adobe Systems, San Jose, CA, USA)). The birth band was identified as the first fully-formed band beyond the focus and was associated with an angle change in the corpus calcareum of the centrum (Casey *et al.* 1985; Cailliet and Goldman 2004). Each sample was assigned a unique ID number so that the reader

[1] Use of Trade Names does not imply endorsement from the NMFS.

had no knowledge of the size, sex, or location along the vertebral column. Coefficient of variation (CV) was calculated within and between readers to assess repeatability of counts and precision (Chang 1982). Values <10% were considered acceptable. Bias, as a result of either systematic or random error, was assessed using the Evans-Hoenig's (1998) test of symmetry. Band pair counts of zero are excluded from precision and bias analyses. Intra-reader precision and bias were compared between the first and second count of each reader while inter-reader precision and bias was compared between the second band pair counts. If the second band pair count differed by three or more band pairs, the centrum was examined together and a consensus count was reached. Final band pair counts were assigned from the primary reader's second count or the consensus count.

Final band pair count was plotted by centrum number for each individual. The mean band pair count and 95% confidence interval (CI) of the mean was calculated for each individual to test if band-pair count varied significantly among centra along the vertebral column. If more than 5% of the band pair counts fell outside of the 95% CI then band pair counts were significantly different within an individual. A mixed-effects model was used to determine if there was a correlation between band pair count and the three centrum measurements for each species with individual included as a random effect.

Results

Centrum Morphology

Centrum morphology varied along the vertebral column in all species (Figure 2-6; James 2018). The transition between abdominal and caudal centra occurred at the 24^{th} to the 47^{th} centrum depending on the species (Table 1). For little and winter skates DVD and LD increased from the head and peaked at the level of the mid-abdominal cavity (approximately in line with the pectoral fin tips) then decreased through the transition from abdominal to caudal centra and continued to decrease in the caudal centra (Figures 2 and 3). Abdominal centra were wider than they were tall (LD > DVD; ovoid), while caudal centra were circular (LD = DVD; Figures 2 and 3). Rostro-caudal length in little and winter skates increased from the head to the transition from abdominal to caudal centra where the LEN decreased sharply; LEN was constant among the caudal centra (Figures 2 and 3).

While trends in size of DVD and LD along the vertebral column in the barndoor skate were similar as in winter and little skates, the centra shape was different along the column. Barndoor skate centra were circular along the entire length of the column (Figure 4). In the barndoor skate, LEN followed a similar trend as in little and winter skates until approximately the 45th centrum, where LEN was greater than DVD and LD (Figure 4).

Atlantic stingray and round ray had similar centra morphologies, which differed from the skate species. In the rays, DVD and LD increased from the head, were constant along the abdominal cavity, and decreased in the caudal centra (Figures 5 and 6). Atlantic stingray and round ray centra were slightly ovoid along the abdominal cavity. Rostro-caudal length increased from the head until the transition from abdominal to caudal vertebrae after which LEN quickly decreased, but the decrease was less dramatic than that seen in the skate species. In both ray species, LEN was constant along the tail.

For all species studied, the centrum morphology along the vertebral column was best described by individual variation (Figure 7). The best-fit GAMs modeled each individual with its own intercept and smoothing function for all species and measurements rather than by sex or species (Supplemental Table 1; James 2018). The only exception was the LEN measurements in

Atlantic stingrays, which was best modeled by each individual with its own intercept, but the same smoothing function for all individuals (Supplemental Table 1). However, all models applied to the DVD and LD measurements of little skate, winter skate, barndoor skate, and round ray fit the data well with adjusted $r^2 > 0.79$ (Supplemental Table 1). The LEN measurement for these species and all measurements for Atlantic stingray had lower adjusted r^2 values ranging from 0.28 to 0.91 (Supplemental Table 1).

Band Pair Counts

The number of centra counted per batoid ranged from 11-17 (Table 1). Intra-reader CV ranged between 6.9 - 14.9% for primary reader and 6.4 - 12.5% for the secondary reader (Table 2), while the inter-reader CV ranged from 10.1 - 21.4% (Table 2) depending on species. Of 478 samples, 13.6% were read by consensus (Table 2). Intra-reader bias was detected for barndoor skate (primary reader) and for Atlantic stingray (secondary reader) using the Evans-Hoenig (1998) test of symmetry. Inter-reader bias was detected only for barndoor skate (Table 2).

Significant differences in band pair counts were found along the column of all individuals except in YOY. Excluding YOY, 17.6 – 100% of the band pair counts fell outside of the 95% CI (Figures 2-6; James 2018). The band pair counts for the three skate species were roughly correlated with the pattern of the DVD and LD measurements (Figures 2-4). Band pair counts for the Atlantic stingray and the round ray did not exhibit a trend along the vertebral column, but still showed significant differences among different centra within an individual (Figures 5 and 6). The largest range in band pair counts within an individual was seven band pairs for little skate, eight band pairs for winter skate, 11 band pairs in barndoor skate, five band pairs in Atlantic stingray, and six band pairs for round ray. Abdominal centra typically had higher band pair counts than caudal centra. The two smallest Atlantic stingray specimens examined were YOY and did not have band pairs (Figure 5).

The number of band pairs was related to centrum morphology in all species, except winter skate. Dorso-ventral diameter, LD, and LEN were significantly correlated with the band pair counts of little skate, barndoor skate, and round ray (Table 3; James 2018). Atlantic stingray were significantly correlated with DVD and LD, but not with LEN. Winter skate did not have any significant correlations.

Discussion

Variable band pair counts among centra within an individual has now been observed in 15 species representing 9 elasmobranch families (Natanson and Cailliet 1990; Natanson *et al.* 2008; Huveneers *et al.* 2013; Natanson *et al.* 2018; current study). The presence of this variation suggests that the mechanism that regulates the formation of band pairs is not related to time. When differences in band pair counts between more anterior and more posterior centra were detected in previous studies, it was suggested that band pairs in smaller, caudal centra were more difficult to interpret (Brown and Gruber 1988; Officer *et al.* 1996; Natanson *et al.* 2006; Piercy *et al.* 2006). In this study we did not find it difficult to interpret band pairs in smaller centra, and we confirmed that the number of band pairs within an individual varies along the vertebral column in batoids. Band pair counts that vary among centra along the vertebral column of an individual cannot accurately reflect a single age estimate (Natanson *et al.* 2018). The positive relationship observed between band pair count and centra morphology for four of the five batoid species examined in this study makes band pair counts unreliable as a tool for ageing in these species.

The hypothesis that structural needs of the individual may regulate the formation of band pairs is suggested on the basis that mineralization of the centra enhances skeletal strength and mechanical support (Kemp and Westrin 1979; Clement 1992; Porter *et al.* 2006; 2007). Regardless of species, larger centra have more band pairs indicating that band pair deposition is a structural requirement of the individual and not related to time (Natanson *et al.* 2018). The positive correlation of body girth measurements to centrum size led Natanson *et al.* (2018) to suggest that differences in deposition patterns are correlated with body type and swimming mode among species. This was supported by Thomson and Simanek's (1977) five categories of body and tail type, in which species of similar body shapes and swimming styles also had similar centrum morphology and Ingle *et al.* (2018) who found that larger centra with more band pairs had lower toughness and stiffness than smaller centra with fewer band pairs. More band pairs (present in abdominal vertebrae) may support body mass, while fewer band pairs (present in anterior and posterior vertebrae) with higher toughness and stiffness allow absorption of more energy and elastic recoil to facilitate swimming (Ingle *et al.* 2018; Natanson *et al.* 2018). Batoids possess vastly different swimming styles than many sharks, however the Atlantic angel shark uses a swimming mode that is an intermediate between caudal fin propulsion and paired fin propulsion (Wilga and Lauder 2004). These dorso-ventrally flattened sharks also demonstrate a relationship between body shape and centrum morphology (Natanson *et al.* 2018), so it is reasonable to extend this relationship to batoids. The body girth measurements used by Natanson *et al.* (2018) did not translate to a batoid body plan (James, unpub. data) so a different approach will have to be used to investigate the relationship between body shape and centrum morphology.

Centrum morphologies in these five batoid species were roughly similar to the centrum morphology of sharks (Natanson *et al.* 2018). The Atlantic angel shark had the most similar centrum morphology to the batoids with the largest centrum in the middle of the abdominal cavity (approximately in line with the tips of the pectoral fins), while the largest centrum for the other shark species was at the end of the abdominal cavity (Natanson *et al.* 2018). The Atlantic angel shark also had ovoid centra (Natanson *et al.* 2018) similar to the abdominal centra of batoids. In carcharhinids and lamnids, centra were circular, except for the abdominal centra of very large lamnids (i.e. shortfin mako; Natanson *et al.* 2018). We suggest that ovoid centra are characteristic of dorso-ventrally compressed elasmobranchs and may be related to undulation of pectoral fins as a swimming strategy, a benthic lifestyle, or the ability to bend dorso-ventrally, not just laterally as in most sharks.

Similarities in centrum morphology exist across and within species. Individual variation was the best descriptor of centrum morphology for batoids, however most GAMs fit the data well (Supplemental Table 1). The good fit of these models may be in part due to the uniformity of morphology of caudal vertebrae (e.g. Figure 7). In contrast, abdominal vertebrae display high individual variation (e.g. Figure 7) suggesting that the conditions an individual experiences influences growth. In Atlantic salmon, *Salmo salar* Linnaeus 1758, centra within an individual grew at different rates depending on the photoperiod regime (Fjelldal *et al.* 2005). Based on our results, we suggest that factors affecting individual body growth (food availability, temperature, population density, and genetics [McDowall 1994]) may also affect individual centrum morphology in batoids.

The paradigm of annual band pair deposition within centra of elasmobranchs has been disproven in many species (Natanson and Cailliet 1990; Tanaka 1990; Francis *et al.* 2007;

Huveneers *et al.* 2013; Harry 2018; Natanson *et al.* 2018). Here we add to that body of literature with five batoids species, supporting the idea that band pair number is related to somatic growth and/or the structural needs of the individual in elasmobranchs as a group (Natanson and Cailliet 1990; Tanaka 1990; Natanson *et al.* 2008; Natanson *et al.* 2018). We also reinforce the call for caution when using band pair counts as a proxy of age without validation (Beamish and MacFarlane 1983). Future work investigating the impact of inaccurate ages on stock-assessment model results and determining an alternate method to age elasmobranchs should be at the forefront of elasmobranch ageing.

Conflicts of Interest

The authors declare no conflicts of interest.

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Table and Figure Captions

Table 1. Individuals of five batoid species used in this study. For little skate *Leucoraja erinacea*, the first nine individuals were analyzed for band pair counts. For winter skate *Leucoraja ocellata*, barndoor skate *Dipturus laevis*, Altantic stingray *Dasyatis sabina*, and round ray *Urobatis halleri*, the first six individuals were analyzed for band pair counts. All individuals were measured for centrum morphology. For brevity, only condensed data are presented for the additional 33 little skates used for centrum morphology. TL is total length, DW is disc width, L is large, M is medium in the Size column and Male in the Sex column, S is for small, F is for female.

Table 2. Bias and precision of band-pair counts within and between readers. Asterisk indicates significant bias.

Table 3. Linear mixed-effects model comparing band-pair counts with the three centrum measurements with individual included in the model as a random effect. Asterisk indicates significant correlation.

Figure 1. Diagram of a vertebral centrum and the three measurements: dorso-ventral diameter (DVD), lateral diameter (LD), and rostro-caudal length (LEN). R is rostral and C is caudal.

Figure 2. Band-pair count and standardized centrum measurements along the vertebral column for nine little skates analyzed for band pairs.

Figure 3. Band-pair count and standardized centrum measurements along the vertebral column for the six winter skates analyzed for band pairs.

Figure 4. Band-pair count and standardized centrum measurements along the vertebral column for six barndoor skates analyzed for band pairs.

Figure 5. Band-pair count and standardized centrum measurements along the vertebral column for six Atlantic stingrays analyzed for band pairs.

Figure 6. Band-pair count and standardized centrum measurements along the vertebral column for six round rays analyzed for band pairs.

Figure 7. Example of generalized additive model variations with (a) all data pooled, data pooled (b) by sex, (c) by size class, and (d) by individual fit to barndoor skate data.

Supplemental Table 1. Generalized additive model results for 10 different models to best describe centrum morphology along the vertebral column for five batoid species. k is the number of knots used in each model.

Table 1. Individuals of five batoid species used in this study. For little skate *Leucoraja erinacea*, the first nine individuals were analyzed for band pair counts. For winter skate *Leucoraja ocellata*, barndoor skate *Dipturus laevis*, Altantic stingray *Dasyatis sabina*, and round ray *Urobatis halleri*, the first six individuals were analyzed for band pair counts. All individuals were measured for centrum morphology. For brevity, only condensed data are presented for the additional 33 little skates used for centrum morphology. TL is total length, DW is disc width, L is large, M is medium in the Size column and Male in the Sex column, S is for small, F is for female.

Species	TL (cm)	DW (cm)	Size	Sex	Maturity	Transition[a]	Centra Counted[b]	Individual ID
Little Skate	49.0	29.0	L	F	Mature	22	13	LE03
	48.5	29.5	L	M	Mature	22	12	LE01
	48.0	27.0	L	F	Mature	24	17	LE07
	42.8	25.2	M	F	Immature	27	17	LE06
	41.5	23.8	M	M	Mature	26	17	LE05
	39.6	24.0	M	F	Immature	27	17	LE19
	26.1	14.5	S	M	Immature	26	17	LE04
	25.6	15.5	S	F	Immature	21	14	LE02
	23.4	13.7	S	F	Immature	22	15	LE20
	43.0-47.5	24.6-27.3	L	5 F, 5 M	1 Immature, 9 Mature	22-25	N/A	N/A
	38.3-42.4	22.8-25.4	M	5 F, 7 M	11 Immature, 2 Mature	23-26	N/A	N/A
	25.1-32.6	15.2-19.8	S	5 F, 6 M	Immature	21-25	N/A	N/A
Winter Skate	80.0	49.9	L	F	Mature	27	17	LO04
	75.3	47.8	L	M	Mature	30	17	LO07
	63.3	39.1	M	F	Immature	26	17	LO08
	61.7	40.5	M	M	Immature	27	16	LO09
	43.2	26.9	S	M	Immature	31	17	LO06
	37.1	22.0	S	F	Immature	29	16	LO05
Barndoor Skate	130.0	92.6[c]	L	M	Mature	25	17	DL04

	117.4	80.9	L	F	Mature	25	17	DL16
	107.5	76.7[c]	M	F	Mature	27	14	DL01
	90.0	64.3[c]	M	M	Immature	27	16	DL03
	52.3	37.6	S	F	Immature	31	17	DL05
	49.0	36.3	S	M	Immature	24	17	DL06
Atlantic Stingray	48.9	26.5	L	F	Mature		15	DS24
	44.8	26.9	L	F	Mature	36	17	DS26
	40.0	18.0	M	F	Immature	49	16	DS32
	36.6	17.8	M	F	Immature	46	17	DS30
	34.2	13.4	S	F	Immature	46	16	DS27
	25.0	13.3	S	F	Immature	47	15	DS33
	59.8	30.3	L	F	Mature	31	N/A	DS31
	42.6	16.0	M	F	Immature	46	N/A	DS29
	30.0	11.0	S	M	Immature	46	N/A	DS25
Round Ray	36.7	20.4	L	M	Mature	31	17	UH01
	21.3	20.0	L	F	Mature	32	11	UH08
	30.5	16.7	M	F	Mature	32	17	UH05
	30.0	16.5	M	M	Mature	34	16	UH10
	24.0	13.3	S	M	Immature	34	17	UH09
	22.8	13.1	S	F	Immature	41	17	UH07
	34.2	19.8	L	M	Mature	34	N/A	UH02
	33.4	18.6	L	M	Mature	30	N/A	UH03
	29.9	17.8	M	F	Mature	31	N/A	UH04
	27.2	16.1	M	F	Mature	31	N/A	UH06

[a]Vertebral number where the transition between abdominal and caudal vertebrae occurs.

[b]Number of centra counted for each individual. N/A means band-pair counts were not determined.

[c]Disc Width was estimated using Total Length to Disc Width relationship from Gedamke 2006.

Table 2. Bias and precision of band pair counts within and between readers for each of the five batoid species. n is the total number of centra counted.

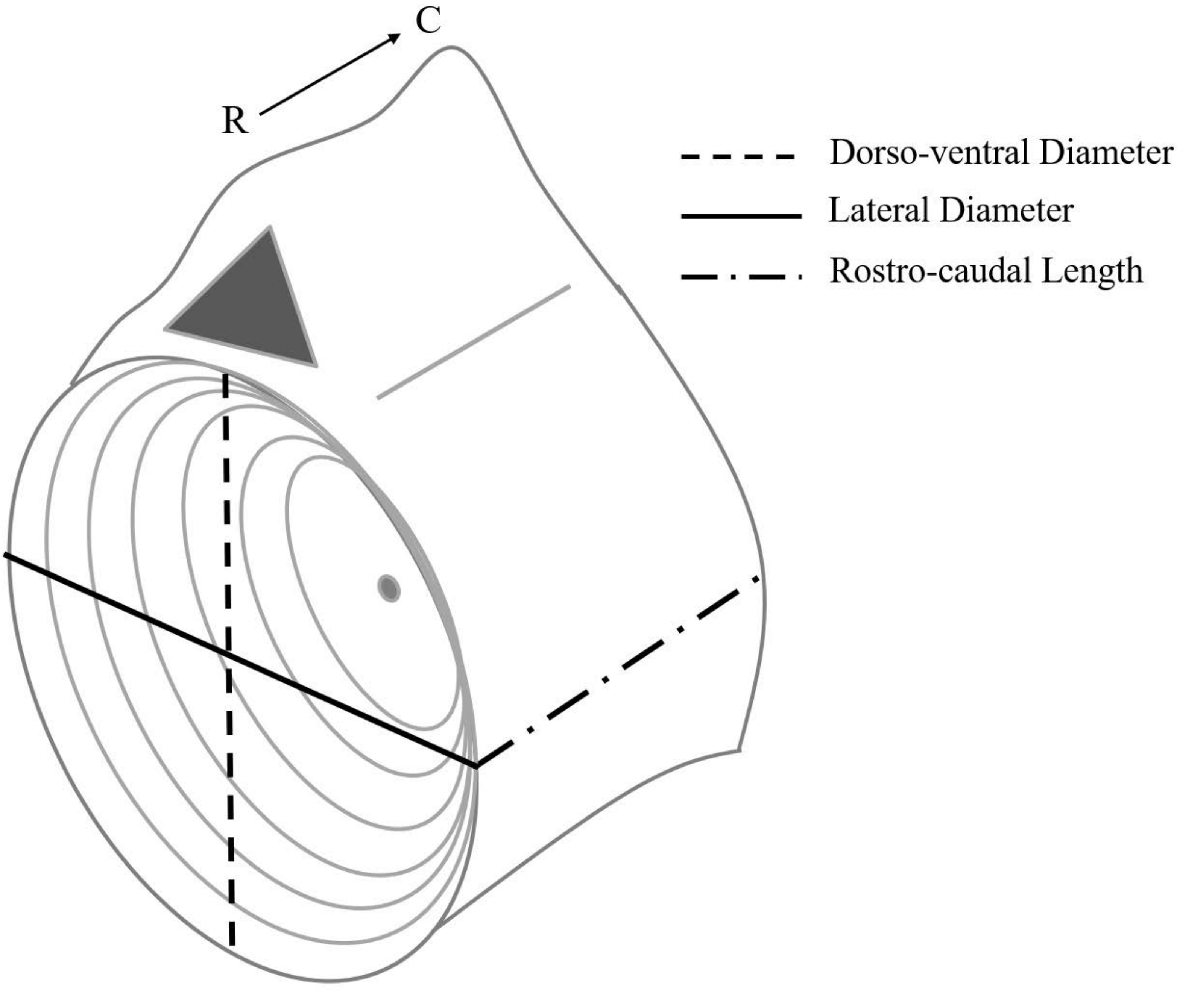
Species				**Evans-Hoenig (1998) Bias test**				
		n[a]	**df**	**χ^2**	**p**		**CV**	**Consensus[b]**
Little Skate	Primary reader		4	8.71	0.069		9.08	
	Second reader		3	2.38	0.497		11.89	
	Inter-reader	130	7	11.09	0.135		16.77	16
Winter Skate	Primary reader		6	11.30	0.800		6.86	
	Second reader		5	7.69	0.174		8.80	
	Inter-reader	99	6	7.22	0.301		10.08	11
Barndoor Skate	Primary reader		5	11.49	0.042	*	9.35	
	Second reader		4	3.42	0.490		6.37	
	Inter-reader	95	5	12.63	0.027	*	16.61	14
Atlantic Stingray	Primary reader		3	2.50	0.475		14.88	
	Second reader		2	9.76	0.008	*	12.54	
	Inter-reader	63	4	8.43	0.077		21.35	9
Round Ray	Primary reader		4	4.06	0.398		9.81	
	Second reader		3	2.44	0.486		9.93	
	Inter-reader	91	4	3.31	0.507		11.83	15

[a]Band pair counts of zero are excluded from bias and precision analyses

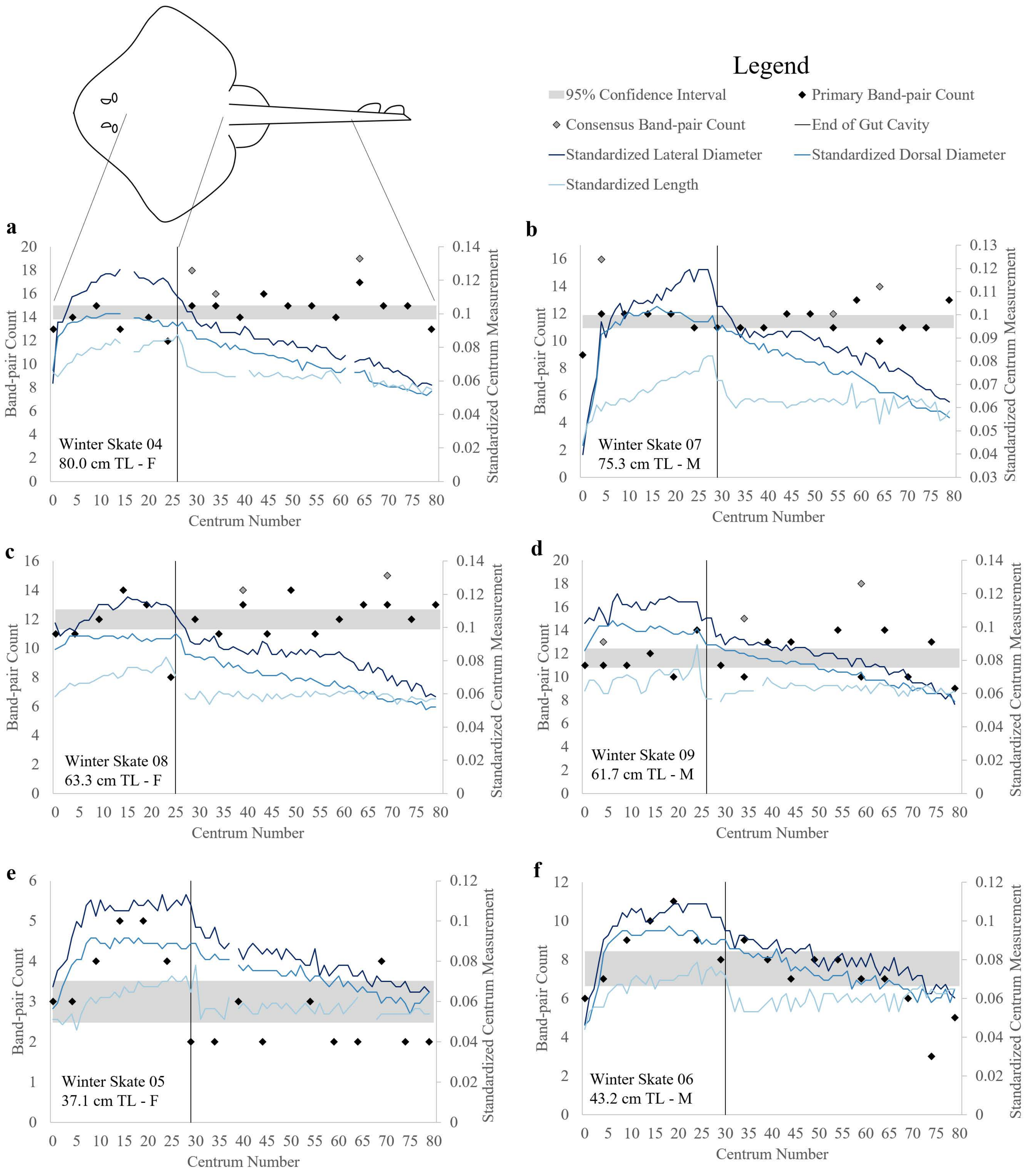
[b]Number of centra counted by both readers together for consensus.

Table 3. Linear mixed-effects model comparing band pair counts with the three centrum measurements with individual included the model as a random effect. Asterisk indicates significant correlation.

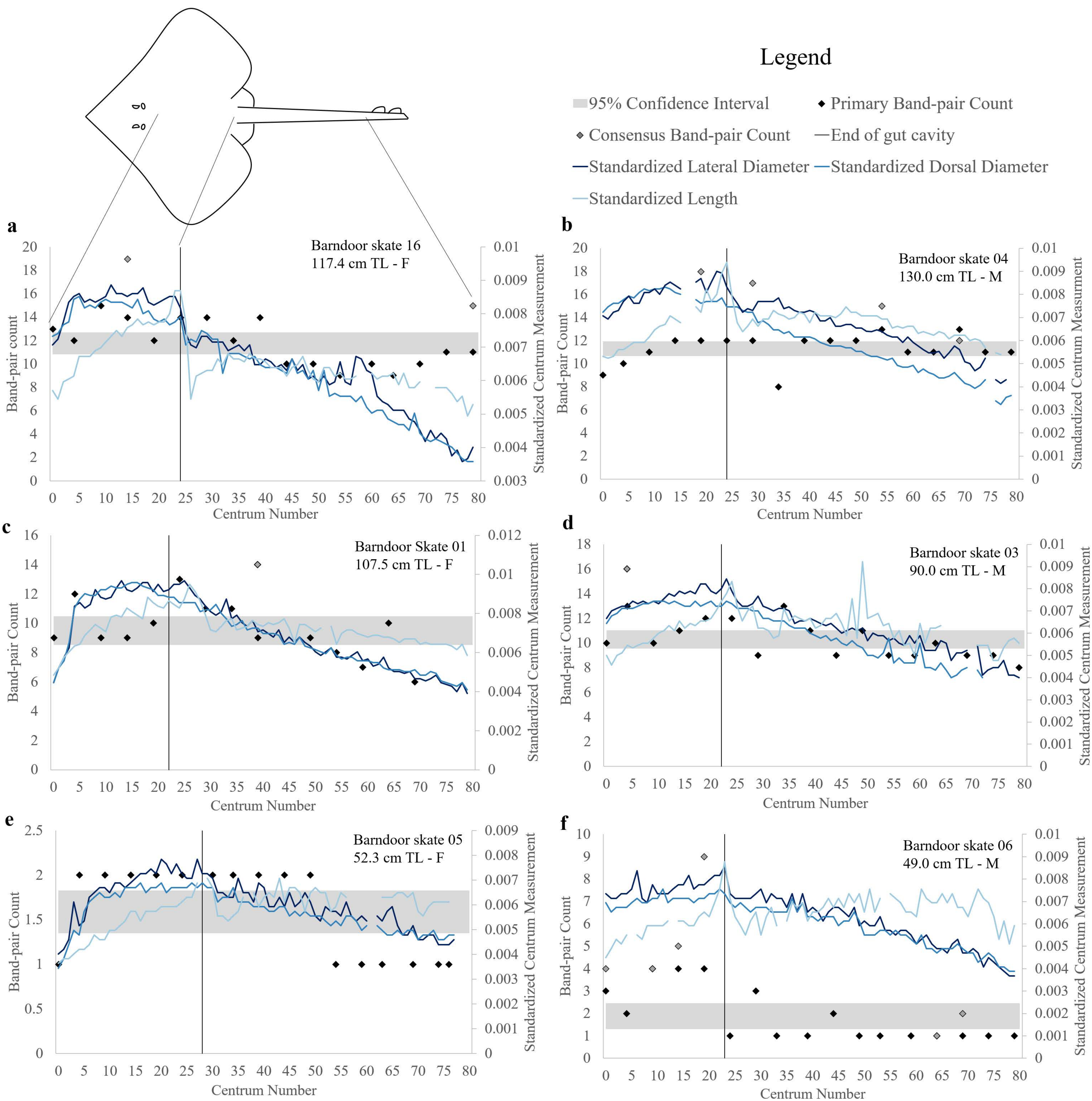
Species	**Measurement**	**Estimate**	**S.E.**	**df**	**t-value**	**p-value**	
Little Skate	Dorsal Diameter	0.591	0.218	129	2.716	0.0075	*
	Lateral Diameter	0.435	0.140	129	3.101	0.0024	*
	Length	0.624	0.300	127	2.078	0.0398	*
Winter Skate	Dorsal Diameter	0.123	0.184	93	0.667	0.5066	
	Lateral Diameter	0.092	0.146	93	0.628	0.5316	
	Length	-0.325	0.364	92	-0.894	0.3737	
Barndoor Skate	Dorsal Diameter	0.572	0.148	91	3.864	0.0002	*
	Lateral Diameter	0.646	0.154	91	4.205	0.0001	*
	Length	0.666	0.244	90	2.732	0.0076	*
Atlantic Stingray	Dorsal Diameter	0.865	0.326	88	2.649	0.0096	*
	Lateral Diameter	0.961	0.210	88	4.574	0.0000	*
	Length	0.915	0.499	87	1.833	0.0703	
Round Ray	Dorsal Diameter	0.807	0.278	88	2.909	0.0046	*
	Lateral Diameter	0.625	0.264	88	2.371	0.0199	*
	Length	0.900	0.431	86	2.085	0.0400	*


C
R
Dorso-ventral Diameter
Lateral Diameter
Rostro-caudal Length

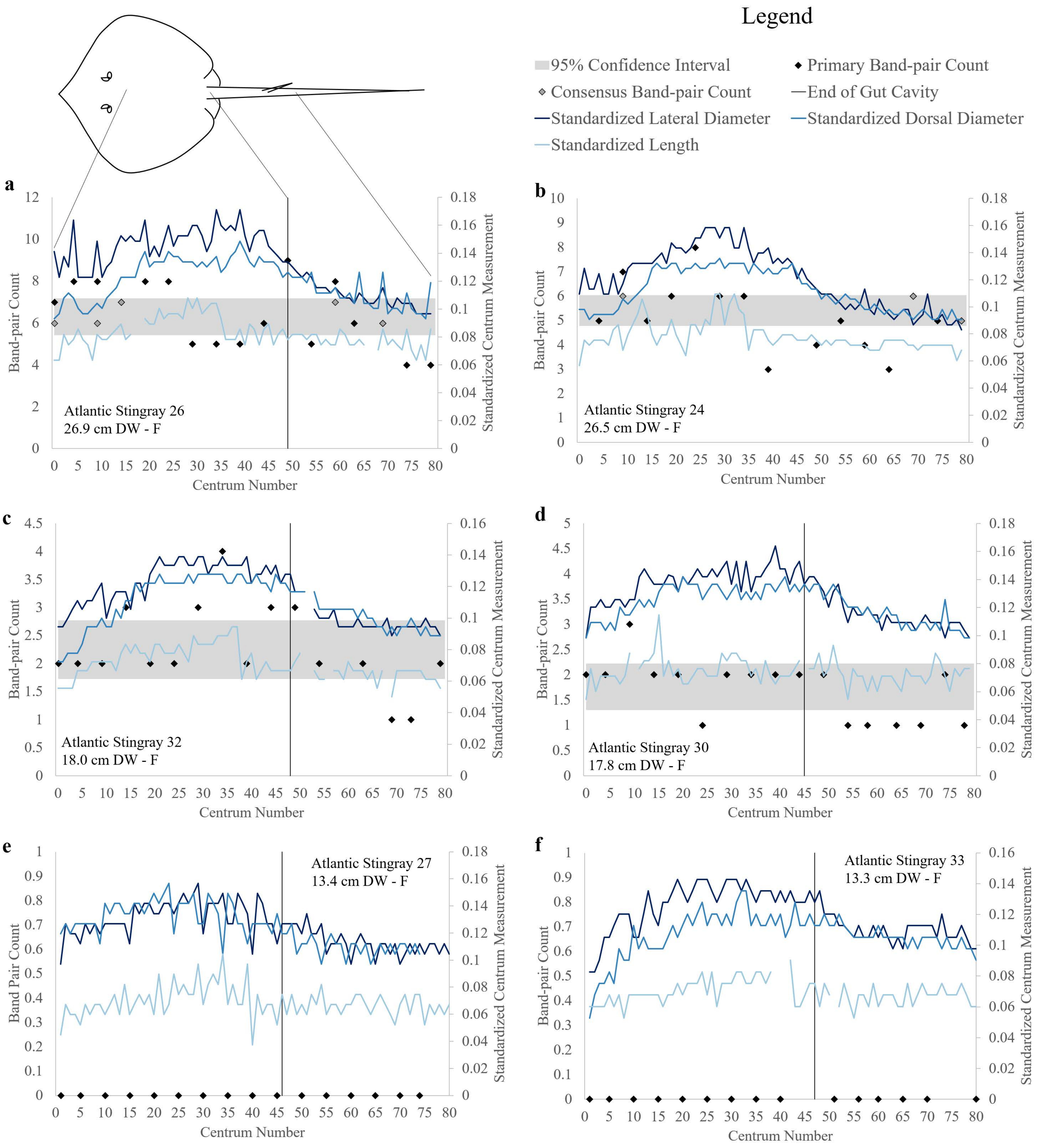

Legend
95% Confidence Interval
Primary Band-pair Count
Consensus Band-pair Count
End of Gut Cavity
Standardized Lateral Diameter
Standardized Dorsal Diameter
Standardized Length
a
Little Skate 07
48.0 cm TL - F
Band Pair Count
Standardized Centrum Measurement
Centrum Number
b
Little Skate 03
49.0 cm TL - F
Band-pair Count
Standardized Centrum Measurement
Centrum Position
c
Little Skate 01
48.5 cm TL - M
Band-pair Count
Standardized Centrum Measurement
Centrum Number
d
Little Skate 06
42.8 cm TL - F
Band-pair Count
Standardized Centrum Measurement
Centrum Number
e
Little Skate 19
39.6 cm TL - F
Band-pair Count
Standardized Centrum Measurement
Centrum Number
f
Little Skate 05
41.5 cm TL - M
Band-pair Count
Standardized Centrum Measurement
Centrum Number
g
Little Skate 02
25.6 cm TL - F
Band-pair Count
Standardized Centrum Measurement
Centrum Number
h
Little Skate 20
23.4 cm TL - F
Band-pair Count
Standardized Centrum Measurement
Centrum Number
i
Little Skate 04
26.1 cm TL - M
Band-pair Count
Standardized Centrum Measurement
Centrum Number



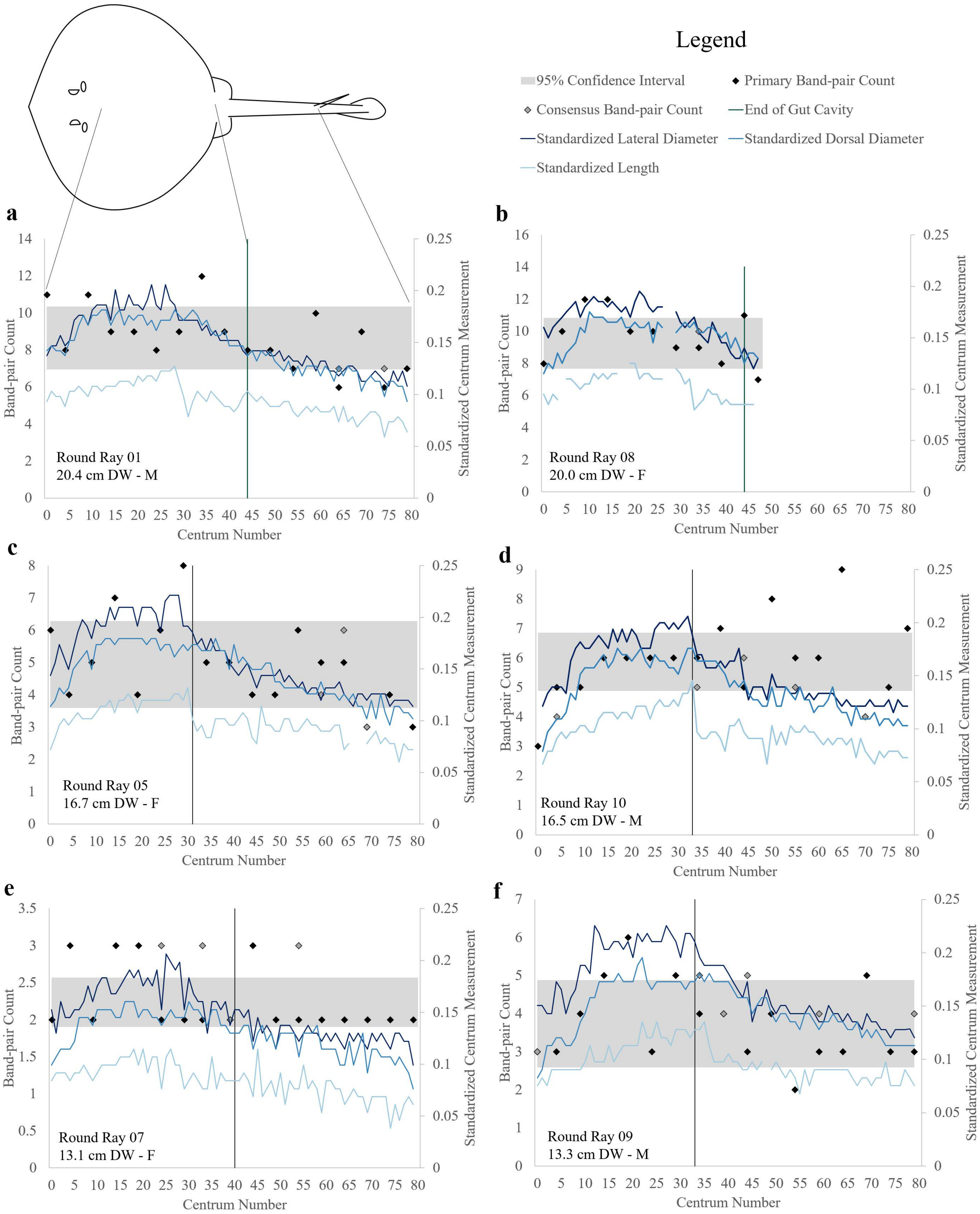
Legend
95% Confidence Interval
Primary Band-pair Count
Consensus Band-pair Count
End of Gut Cavity
Standardized Lateral Diameter
Standardized Dorsal Diameter
Standardized Length
a
Winter Skate 04
80.0 cm TL - F
b
Winter Skate 07
75.3 cm TL - M
c
Winter Skate 08
63.3 cm TL - F
d
Winter Skate 09
61.7 cm TL - M
e
Winter Skate 05
37.1 cm TL - F
f
Winter Skate 06
43.2 cm TL - M
Band-pair Count
Standardized Centrum Measurement
Centrum Number



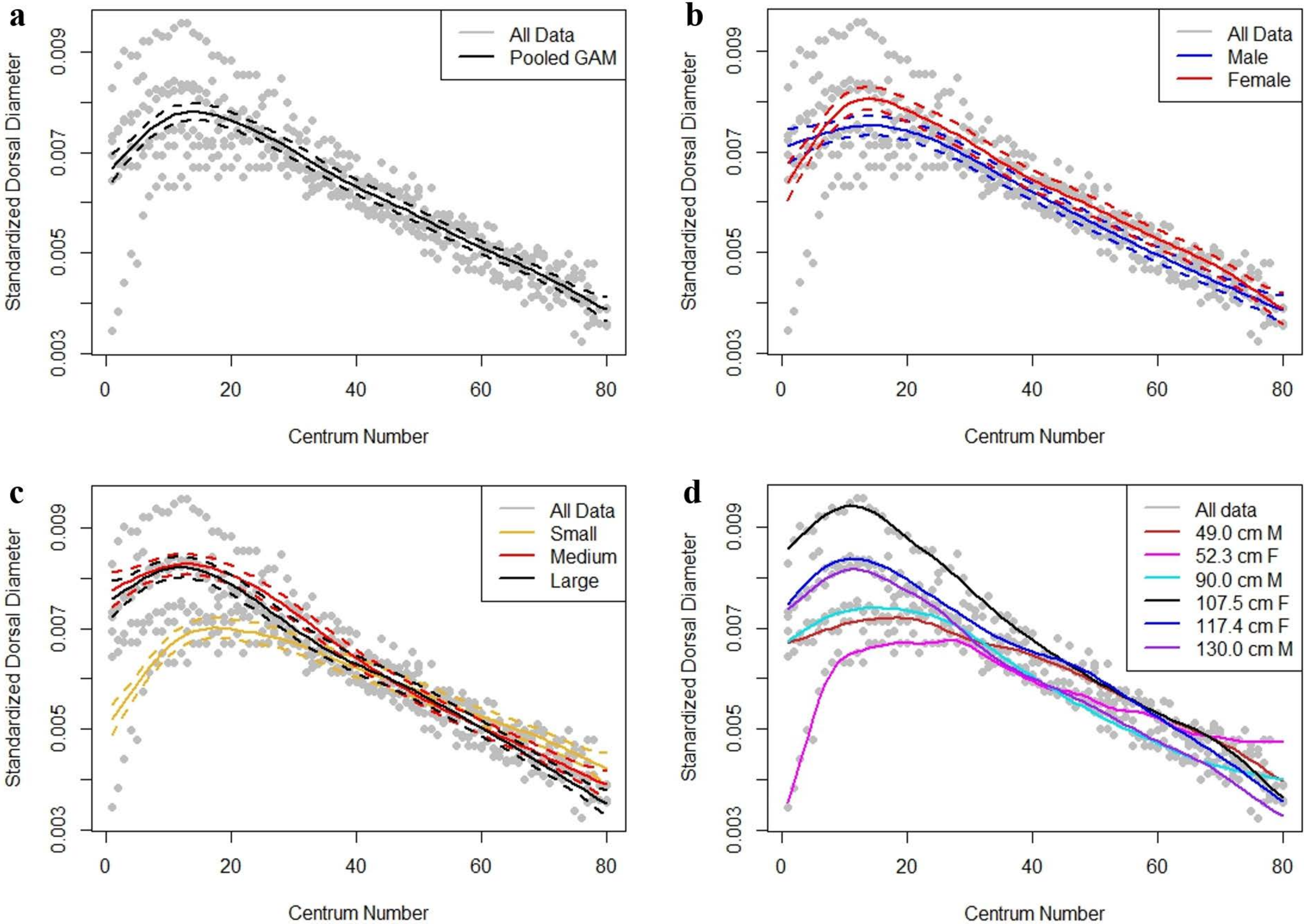
Legend
95% Confidence Interval
Primary Band-pair Count
Consensus Band-pair Count
End of gut cavity
Standardized Lateral Diameter
Standardized Dorsal Diameter
Standardized Length
a
Barndoor skate 16
117.4 cm TL - F
b
Barndoor skate 04
130.0 cm TL - M
c
Barndoor Skate 01
107.5 cm TL - F
d
Barndoor skate 03
90.0 cm TL - M
e
Barndoor skate 05
52.3 cm TL - F
f
Barndoor skate 06
49.0 cm TL - M
Band-pair count
Band-pair Count
Centrum Number
Standardized Centrum Measurement



Legend
95% Confidence Interval
Primary Band-pair Count
Consensus Band-pair Count
End of Gut Cavity
Standardized Lateral Diameter
Standardized Dorsal Diameter
Standardized Length
a
Atlantic Stingray 26
26.9 cm DW - F
b
Atlantic Stingray 24
26.5 cm DW - F
c
Atlantic Stingray 32
18.0 cm DW - F
d
Atlantic Stingray 30
17.8 cm DW - F
e
Atlantic Stingray 27
13.4 cm DW - F
f
Atlantic Stingray 33
13.3 cm DW - F
Band-pair Count
Band Pair Count
Standardized Centrum Measurement
Centrum Number



Legend
95% Confidence Interval
Primary Band-pair Count
Consensus Band-pair Count
End of Gut Cavity
Standardized Lateral Diameter
Standardized Dorsal Diameter
Standardized Length
a
Round Ray 01
20.4 cm DW - M
b
Round Ray 08
20.0 cm DW - F
c
Round Ray 05
16.7 cm DW - F
d
Round Ray 10
16.5 cm DW - M
e
Round Ray 07
13.1 cm DW - F
f
Round Ray 09
13.3 cm DW - M
Band-pair Count
Standardized Centrum Measurement
Centrum Number



a
All Data
Pooled GAM
b
All Data
Male
Female
c
All Data
Small
Medium
Large
d
All data
49.0 cm M
52.3 cm F
90.0 cm M
107.5 cm F
117.4 cm F
130.0 cm M
Standardized Dorsal Diameter
Stanardized Dorsal Diameter
Centrum Number
0.003
0.005
0.007
0.009
0
20
40
60
80

Supplementary Table 1. Generalized additive model results for 10 different models to best describe centrum morphology along the vertebral column for five batoid species. k is the number of knots used in each model.

			Dorso-ventral Diameter					Lateral Diameter					Rostro-caudal Length				
Species	**Model**		**Deviance**	**k**	**Adjusted r^2**	**AIC**	**ΔAIC**	**Deviance**	**k**	**Adjusted r^2**	**AIC**	**ΔAIC**	**Deviance**	**k**	**Adjusted r^2**	**AIC**	**ΔAIC**
Little Skate	Pooled		3277.71	15	0.84	-25275.12	4449.48	3273.56	18	0.88	-23443.01	4433.9	3088.55	15	0.45	-23522.05	2241.74
	BySex	Different Smoother	3265.75	18	0.84	-25263.06	4461.54	3261.56	18	0.88	-23453.14	4423.8	3072.59	18	0.47	-23590.06	2173.73
		Different Intercept	3275.28	18	0.84	-25276.17	4448.43	3272.56	18	0.88	-23443.65	4433.3	3085.32	18	0.46	-23576.90	2186.89
		Different Smoother and Intercept	3264.76	18	0.84	-25261.29	4463.31	3260.57	18	0.88	-23454.93	4422	3071.55	18	0.48	-23637.57	2126.22
	BySize	Different Smoother	3256.68	18	0.85	-25330.62	4393.98	3250.89	18	0.89	-23733.30	4143.6	3060.25	18	0.52	-23877.46	1886.33
		Different Intercept	3274.29	18	0.84	-25278.37	4446.23	3271.6	18	0.88	-23440.82	4436.1	3084.36	18	0.47	-23575.90	2187.89
		Different Smoother and Intercept	3254.69	18	0.85	-25329.92	4394.68	3248.95	18	0.89	-23734.11	4142.8	3058.12	18	0.53	-23926.75	1837.04
	ByIndividual	Different Smoother	3034.36	18	0.9	-26446.93	3277.67	2963.43	18	0.93	-24876.66	3000.3	2756.94	18	0.65	-24496.00	1267.79
		Different Intercept	3234.89	18	0.9	-26696.52	3028.08	3232.78	18	0.92	-24667.08	3209.9	3045.69	18	0.58	-24288.32	1475.47
		Different Smoother and Intercept	2901.33	18	0.96	-29724.60	0	2813.94	18	0.97	-27876.94	0	2644.62	18	0.78	-25763.79	0
Winter Skate	Pooled		462.05	15	0.86	-3614.77	926.251	462.03	16	0.86	-3451.75	691.53	454.63	13	0.52	-3589.57	414.821
	BySex	Different Smoother	452.01	18	0.87	-3650.95	890.077	451.25	18	0.88	-3489.35	653.93	449.49	10	0.54	-3602.86	401.532
		Different Intercept	459.59	20	0.87	-3663.15	877.869	460.36	18	0.86	-3449.98	693.3	450.41	18	0.86	-3372.12	632.271
		Different Smoother and Intercept	450.31	18	0.89	-3708.53	832.493	450.26	18	0.88	-3487.91	655.37	440.36	18	0.88	-3408.80	595.59
	BySize	Different Smoother	443.47	15	0.93	-3896.50	644.521	439.29	17	0.92	-3677.35	465.93	441.87	10	0.58	-3633.88	370.514
		Different Intercept	459.99	16	0.87	-3638.81	902.213	459.56	17	0.88	-3481.86	661.42	451.96	14	0.6	-3677.31	327.083
		Different Smoother and Intercept	442.44	14	0.93	-3925.36	615.662	434.22	20	0.93	-3724.76	418.52	439.14	10	0.66	-3735.08	269.31
	ByIndividual	Different Smoother	412.76	17	0.95	-4084.52	456.506	397.4	20	0.96	-3999.54	143.74	426.11	12	0.65	-3696.49	307.90
		Different Intercept	456.29	17	0.89	-3701.02	840.003	456.59	17	0.88	-3479.35	663.93	451.37	10	0.64	-3717.61	286.781
		Different Smoother and Intercept	378.1	23	0.98	-4541.02	0	385	20	0.97	-4143.28	0	394.28	17	0.83	-4004.39	0
Barndoor Skate	Pooled		459.00	10	0.79	-5574.04	1085.87	462.9	15	0.83	-5725.93	670.57	442.45	10	0.32	-5212.10	561.733
	BySex	Different Smoother	453.69	18	0.79	-5574.10	1085.81	458.24	10	0.83	-5731.21	665.29	437.25	10	0.33	-5209.29	564.541
		Different Intercept	457.93	10	0.8	-5594.87	1065.04	462.8	10	0.83	-5723.23	673.28	441.47	10	0.32	-5211.42	562.41
		Different Smoother and Intercept	453.24	10	0.8	-5596.25	1063.66	457.26	10	0.83	-5730.57	665.94	436.28	10	0.33	-5208.82	565.017
	BySize	Different Smoother	448.64	10	0.87	-5799.38	860.529	452.42	10	0.87	-5840.35	556.15	432.06	10	0.47	-5319.94	453.889
		Different Intercept	457.05	10	0.82	-5632.87	1027.04	461.75	15	0.86	-5808.95	587.56	440.27	10	0.4	-5263.24	510.597
		Different Smoother and Intercept	446.04	10	0.89	-5852.63	807.275	449.73	10	0.89	-5922.32	474.19	429.11	10	0.55	-5389.19	384.644
	ByIndividual	Different Smoother	433.82	10	0.92	-5955.65	704.26	434.07	10	0.93	-6090.14	306.37	418.69	10	0.52	-5348.10	425.73
		Different Intercept	453.72	10	0.89	-5807.76	852.147	457.46	10	0.89	-5875.56	520.94	437	10	0.57	-5417.61	356.227
		Different Smoother and Intercept	406.41	18	0.98	-6659.91	0	416.74	15	0.96	-6396.50	0	388.06	18	0.82	-5773.83	0
Atlantic Stingray	Pooled		695.09	10	0.59	-4384.39	950.027	694.26	15	0.69	-4296.73	606.96	676.88	10	0.29	-4428.66	255.101
	BySex	Different Smoother	691.18	15	0.61	-4420.81	913.605	690.54	10	0.69	-4295.07	608.62	673.52	10	0.69	-4193.63	490.136
		Different Intercept	693.28	15	0.59	-4382.33	952.085	694.03	10	0.69	-4307.25	596.44	677.05	10	0.69	-4206.18	477.585
		Different Smoother and Intercept	690.2	15	0.61	-4418.96	915.457	689.51	10	0.69	-4307.12	596.57	672.5	10	0.69	-4206.19	477.577
	BySize	Different Smoother	681.98	15	0.64	-4465.12	869.296	683.35	10	0.72	-4375.49	528.2	666.12	15	0.3	-4414.27	269.488
		Different Intercept	691.94	15	0.68	-4538.10	796.308	692.29	10	0.69	-4304.36	599.33	670.6	18	0.47	-4620.88	62.883
		Different Smoother and Intercept	678.48	15	0.72	-4634.37	700.044	681.32	10	0.73	-4379.01	524.68	660.07	15	0.46	-4587.61	96.157
	ByIndividual	Different Smoother	658.12	10	0.66	-4487.95	846.458	654.97	10	0.74	-4386.82	516.87	648.52	10	0.28	-4378.91	304.85
		Different Intercept	686.68	10	0.81	-4891.77	442.645	686.89	15	0.82	-4661.20	242.49	665.7	15	0.52	-4683.76	0
		Different Smoother and Intercept	622.79	15	0.91	-5334.41	0	631	15	0.88	-4903.69	0	629.71	15	0.52	-4620.90	62.858
Round Ray	Pooled		750.84	16	0.81	-4794.71	538.898	751.51	15	0.84	-4662.83	468.25	743.36	15	0.66	-4860.46	236.788
	BySex	Different Smoother	745.95	10	0.81	-4806.81	526.799	742.96	15	0.85	-4710.33	420.76	736.55	15	0.67	-4891.93	205.319
		Different Intercept	752.88	10	0.82	-4831.30	502.313	749.58	18	0.85	-4680.77	450.32	742.35	15	0.66	-4869.58	227.665
		Different Smoother and Intercept	744.91	10	0.82	-4843.31	490.301	741.88	15	0.86	-4732.16	398.92	735.51	15	0.68	-4904.28	192.967
	BySize	Different Smoother	734.47	15	0.82	-4824.02	509.591	736.98	15	0.85	-4685.57	445.52	730.4	15	0.66	-4853.07	244.178
		Different Intercept	749.22	15	0.81	-4808.90	524.709	749.47	15	0.85	-4698.94	432.14	741.32	15	0.67	-4888.59	208.66
		Different Smoother and Intercept	732.75	14	0.82	-4841.70	491.916	738.3	10	0.86	-4720.63	410.46	728.5	15	0.67	-4880.24	217.012
	ByIndividual	Different Smoother	685.33	17	0.86	-4924.13	409.486	698.01	11	0.87	-4763.74	367.35	695.76	15	0.7	-4904.17	193.08
		Different Intercept	741.4	16	0.87	-5037.33	296.281	742.03	15	0.89	-4937.57	193.52	733.99	15	0.72	-5009.08	88.165
		Different Smoother and Intercept	656.47	23	0.92	-5333.61	0	680.26	12	0.92	-5131.09	0	681.04	15	0.77	-5097.25	0