





## RESEARCH ARTICLE

## Ecological lifestyles and the scaling of shark gill surface area

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**Abstract**

Fish gill surface area varies across species and with respect to ecological lifestyles. The majority of previous studies only qualitatively describe gill surface area in relation to ecology and focus primarily on teleosts. Here, we quantitatively examined the relationship of gill surface area with respect to specific ecological lifestyle traits in elasmobranchs, which offer an independent evaluation of observed patterns in teleosts. As gill surface area increases ontogenetically with body mass, examination of how gill surface area varies with ecological lifestyle traits must be assessed in the context of its allometry (scaling). Thus, we examined how the relationship of gill surface area and body mass across 11 shark species from the literature and one species for which we made measurements, the Gray Smoothhound *Mustelus californicus*, varied with three ecological lifestyle traits: activity level, habitat, and maximum body size. Relative gill surface area (gill surface area at a specified body mass; here we used 5,000g, termed the 'standardized intercept') ranged from 4,724.98 to 35,694.39 cm<sup>2</sup> (mean and standard error: 17,796.65 ± 2,948.61 cm<sup>2</sup>) and varied across species and the ecological lifestyle traits examined. Specifically, larger-bodied, active, oceanic species had greater relative gill surface area than smaller-bodied, less active, coastal species. In contrast, the rate at which gill surface area scaled with body mass (slope) was generally consistent across species (0.85 ± 0.02) and did not differ statistically with activity level, habitat, or maximum body size. Our results suggest that ecology may influence relative gill surface area, rather than the rate at which gill surface area scales with body mass. Future comparisons of gill surface area and ecological lifestyle traits using the quantitative techniques applied in this study can provide further insight into patterns dictating the relationship between gill surface area, metabolism, and ecological lifestyle traits.

**KEYWORDS**

allometry, ecomorphology, gill surface area, metabolism, scaling

## 1 | INTRODUCTION

In most fishes, gills function as the primary site of oxygen uptake used to support aerobic metabolism, resulting in an intimate relationship between gill surface area and metabolic rate (Hughes, 1966; Hughes & Morgan, 1973; Wegner, 2011). The diffusive flux of oxygen across the gills is dependent on their surface area, such that an increase in gill surface area augments oxygen uptake (Hughes, 1970; Hughes, 1984a; Hughes & Morgan, 1973). Fishes with higher metabolic demands thus have greater gill surface areas, with active species in oceanic habitats typically having greater gill surface areas than less active species in coastal, benthic habitats (Gray, 1954; Hughes, 1966; Hughes, 1984a).

These patterns have led several reviews of gill morphology to categorize fishes into ecological lifestyle groupings (i.e., groups of species that have similar habitats and activity) based primarily on their gill surface area.

Such categorizations of gill surface area, activity, and habitat began with Gray (1954), who descriptively categorized 31 teleost species into three ecological groups based on relative gill surface area (i.e., gill surface area at a specified body mass). These groups included (a) active, pelagic species with the greatest relative gill surface areas, (b) fishes of 'moderate' activity with 'intermediate' relative gill surface areas, and (c) 'sluggish', benthic species with the lowest relative gill surface areas. Since then, subsequent reviews have further elaborated

upon and attempted to define these groups (Hughes, 1984a; Palzenberger & Pohla, 1992; Wegner, 2011). However, such comparisons of gill surface area across large species groups in relation to ecological lifestyle have mostly been descriptive or qualitative in nature, rather than analyzed quantitatively.

The quantitative assessment of how gill surface area varies across species and with respect to ecological lifestyle requires a thorough understanding of how gill surface area scales ontogenetically with body growth, or the allometry of gill surface area. This allows for both an understanding of the relative gill surface area (gill surface area at a specified mass, or the intercept of the allometric relationship) and the rate at which gill surface area scales with body mass (slope of the allometric relationship). For many species, gill surface area has not been examined for a sufficient size range of individuals to establish such relationships. For those species with sufficient gill surface area data across a size range of individuals, it is standard practice to estimate and report the regression equation for this scaling relationship (Hughes, 1984b; Emery & Szczepanski, 1986; Palzenberger & Pohla, 1992). However, comparisons of gill surface area across species or with respect to ecological lifestyle are generally discussed in descriptive or qualitative terms (Emery & Szczepanski, 1986; Palzenberger & Pohla, 1992; Wegner, 2011). Thus, it remains largely untested if observed differences in gill surface area across species with diverse ecological lifestyles are statistically significant, and if the intercept, the slope, or both allometric regression coefficients vary with specific ecological lifestyle traits.

This study thus seeks to quantitatively assess how the allometry of gill surface area varies with specific ecological lifestyle traits. We focused our efforts on elasmobranch fishes as the majority of previous studies examining gill surface area across species and ecological lifestyles focus primarily on teleost fishes (De Jager & Dekkers, 1975; Palzenberger & Pohla, 1992; Satora & Wegner, 2012). Chondrichthyans, and specifically elasmobranchs, offer an opportunity to evaluate the generality of gill surface area patterns as they are one of three taxonomic classes of fishes and have evolved separately for over 420 million years (Heinicke, Naylor, & Hedges, 2009; Stein et al., 2018). Additionally, the elasmobranch gill differs from that of teleosts in their evolutionary retention of the plate-like interbranchial septum that gave rise to their name, 'elasmobranch', which translates into 'plate-gill' (Wegner, 2016; Wilson & Laurent, 2002). This structure, which is largely absent from the teleost gill, has important consequences for gill function and morphology (Wegner, 2016; Wegner, Lai, Bull, & Graham, 2012; Wegner, Sepulveda, Olson, Hyndman, & Graham, 2010).

Here, we examine if specific ecological lifestyle traits are quantitatively related to shark gill surface area, and if so, ask if these traits are related to the relative gill surface area (standardized intercept), the rate at which gill surface area scales with body mass (slope), or both. First, we estimated gill surface area allometries for 11 shark species from the literature, and one species for which we made measurements, the Gray Smoothhound *Mustelus californicus*. We then assessed if the allometric regression coefficients (standardized intercept and slope) were related to the ecological lifestyle traits of activity level, habitat type, or maximum body size, all of which likely influence gill surface area.

## 2 | MATERIALS AND METHODS

### 2.1 | Gill surface area measurement and statistical analysis of the Gray Smoothhound

Eight Gray Smoothhound (*Mustelus californicus*; Gill, 1864) specimens were collected opportunistically off the coast of southern California from anchored benthic gillnet surveys for other scientific studies. For each specimen, mass (kg), total length (TL, cm), and fork length (FL, cm) were measured, and the gills were fixed in 10% formalin buffered in seawater for later processing. Only limited tissue shrinkage is associated with fixation and storage in 10% buffered formalin (Wootton et al. 2015).

Total gill surface area ( $A$ ) of each specimen was estimated following Muir and Hughes (1969) and Hughes (1984c):

$$A = L_{\text{fil}} \times 2n_{\text{lam}} \times A_{\text{lam}},$$

where  $L_{\text{fil}}$  is the total length of all the gill filaments,  $n_{\text{lam}}$  is the average number of lamellae per unit length on one side of the filament (lamellar frequency), and  $A_{\text{lam}}$  is the mean bilateral surface area of a lamella. This method of estimating gill surface area was chosen so our results were comparable to the other elasmobranch gill surface area estimates.

First, total filament length was estimated. All filaments on each of the nine hemibranchs from the right side of the branchial chamber were counted using a dissecting scope (Zeiss Stemi 2000-C) fitted with a digital camera (Lumenera INFINITYLite). Filaments were then binned into groups of approximately 8–10 filaments, beginning at the dorsal margin and moving ventrally along the arch. Consistent with previous work, the medial filament of each bin was assumed to be representative of all filaments in that bin (Muir & Hughes, 1969; Wegner, 2011). A magnified photograph was taken of each medial filament, and image-processing software (ImageJ, NIH) was used to measure the length of the filament from its base, embedded under the branchial canopy, to the tip. The total length of all filaments in each bin was estimated by multiplying the length of the medial filament by the number of filaments in the bin. Total filament lengths for each bin were then summed to estimate the total filament length of each hemibranch. To determine the total filament length of the entire fish, the total filament lengths for each hemibranch were summed, and then doubled to account for the filaments from hemibranchs on the other side of the branchial chamber.

Second, we determined average lamellar frequency and the mean bilateral surface area of a lamella from the most representative hemibranch. This was the hemibranch with the smallest difference in average filament length compared to the average filament length for all hemibranchs. To estimate average lamellar frequency, the medial filament of each bin on the representative hemibranch was removed from the interbranchial septum and dissected into two sections, a base half and tip half. Magnified photographs were taken of one side of the filament at approximately the midpoint of the base section and midpoint of the tip section. The number of lamellae per millimeter at both locations were then counted using ImageJ and averaged to obtain a mean lamellar frequency for each medial filament. The mean lamellar frequency of each medial filament was multiplied by the total

filament length of its respective bin, and each bin was then summed. This number was then divided by the total filament length of the representative hemibranch to estimate the average lamellar frequency for the entire hemibranch and gills.

To estimate the mean bilateral surface area of an individual lamella, cross-sections were made at the midpoint of the base section and midpoint of the tip section of each medial filament on the representative hemibranch. These cross-sections were then laid flat to expose the lamellae, which were photographed under magnification. The surface area of one side of these base and tip lamella were measured using ImageJ, averaged, and doubled to obtain the mean bilateral lamellar surface area of a lamella in that bin. Each mean bilateral lamellar surface area from each medial filament was then multiplied by the total number of lamellae within its respective bin, and these measurements were summed to estimate the total bilateral lamellar surface area of all lamellae for the entire hemibranch. This was then divided by the total number of lamellae on the representative hemibranch to determine the mean bilateral surface area for the entire gills.

The relationships of gill surface area and associated dimensions in the Gray Smoothhound (total filament length, average lamellar frequency, and mean bilateral lamellar surface area) in relation to body mass were determined by Ordinary Least Squares Regression using the `lm` function in R v. 3.3.2 (R Core Team, 2016). To linearize the expected power law relationship, body mass, gill surface area, total filament length, average lamellar frequency, and the mean bilateral lamellar surface area were  $\log_{10}$ -transformed.

## 2.2 | Comparative gill surface area analyses

### 2.2.1 | Data

Gill surface area and body mass data for the 11 other shark species were compiled from previously published studies (Table 1). We conducted a literature search using Google Scholar and Web of Science

with combinations of the following keywords: 'shark', 'elasmobranch', 'gill surface area', 'respiratory surface area', 'gill surface area allometry', 'respiratory surface area allometry', 'gill morphometrics', and 'gill dimensions'. Three species (Common Thresher Shark *Alopias vulpinus*, Shortfin Mako *Isurus oxyrinchus*, and Sandbar Shark *Carcharhinus plumbeus*) had more than one study reporting gill surface area and body mass data, and for these species, data were combined resulting in one data set per species. Raw data were obtained from Wegner et al. (2010a) and Wootton, Sepulveda, & Wegner (2015) for four species (Pelagic Thresher *Alopias pelagicus*, Bigeye Thresher *Alopias superciliosus*, Common Thresher Shark, and Shortfin Mako). When raw gill surface area and body mass data were not available from the remaining studies, image-digitizing software was used to extract these data points from published graphs (Plot Digitizer: <http://plotdigitizer.sourceforge.net/>). Including the Gray Smoothhound (Table 1), we know of sufficient data to estimate gill surface area allometric regressions for 12 shark species (Bigman et al. 2018 figshare). In three other shark species for which published gill surface area data exist (Scalloped Hammerhead *Sphyrna lewini*, Blacktip Shark *Carcharhinus limbatus*, and Spiny Dogfish *Squalus acanthias*; Boylan & Lockwood, 1962; Emery & Szczepanski, 1986; Hata, 1993), the sample sizes were too low (i.e., three or fewer individual estimates) to compute reliable regression coefficients. Rays (superorder Batoidea) were not included in this study as there are only three species that have published gill surface area data from more than a few individuals, and only one species where this data covers a range of body masses (Wegner, 2016).

### 2.2.2 | Estimation of regression coefficients

Both linear and nonlinear regression frameworks are commonly used to fit power law relationships, such as those between body mass and morphological traits, e.g., gill surface area. Linear regression on log-transformed data applies a model with additive error on the transformed scale and multiplicative error when back-transformed to the

**TABLE 1** Gill surface area allometric regression coefficients and three ecological lifestyle traits (caudal fin aspect ratio, habitat type, maximum body mass) for 12 shark species

Species	Common name	Standardized intercept	Slope	Caudal fin aspect ratio	Habitat type	Max. body mass (kg)	Source for gill area data
<i>Alopias superciliosus</i>	Bigeye Thresher	35,694.39	0.8061	4.67	Oceanic	363.8	Wootton et al., 2015
<i>Carcharodon carcharias</i>	White Shark	30,040.00	0.7715	3.12	Oceanic	2,080.4	Emery and Szczepanski, 1986
<i>Isurus oxyrinchus</i>	Shortfin Mako	29,248.26	0.7590	2.52	Oceanic	505.8	Emery and Szczepanski, 1986, Wegner et al. 2010
<i>Alopias pelagicus</i>	Pelagic Thresher	24,547.09	0.8946	5.63	Oceanic	127.7	Wootton et al., 2015
<i>Galeocerdo cuvier</i>	Tiger Shark	20,141.88	0.9136	3.19	Oceanic	807.4	Hata, 1993
<i>Alopias vulpinus</i>	Common Thresher Shark	19,404.39	0.8918	5.54	Oceanic	348.0	Emery and Szczepanski, 1986, Wootton et al., 2015
<i>Carcharhinus obscurus</i>	Dusky Shark	12,336.73	0.8761	3.18	Coastal	346.5	Emery and Szczepanski, 1986
<i>Carcharhinus plumbeus</i>	Sandbar Shark	11,040.79	0.9012	3.17	Coastal	117.9	Emery and Szczepanski, 1986, Hata, 1993
<i>Prionace glauca</i>	Blue Shark	9,667.18	0.8820	3.48	Oceanic	205.9	Emery and Szczepanski, 1986
<i>Scyliorhinus canicula</i>	Lesser Spotted Dogfish	9,423.24	0.9555	1.63	Coastal	1.3	Hughes, 1972
<i>Mustelus californicus</i>	Gray Smoothhound	7,297.94	0.7840	2.14	Coastal	4.8	This study
<i>Scyliorhinus stellaris</i>	Nursehound	4,724.98	0.7783	1.63 <sup>a</sup>	Coastal	2.6	Hughes, Perry, & Piiper, 1986

Coefficients were re-estimated from  $\log_{10}$ -transformed gill surface area and  $\log_{10}$ -transformed and centered body mass data. Intercepts are back-transformed and represent the gill surface area ( $\text{cm}^2$ ) at 5000 g.

<sup>a</sup> The caudal fin aspect ratio of *S. canicula* was used for *S. stellaris*.

original scale (White & Kearney, 2014). In contrast, nonlinear regression applies a model with additive error on the untransformed or original scale (White & Kearney, 2014). To identify whether a linear or nonlinear regression was most appropriate for our particular dataset we compared error structures of a linear regression on  $\log_{10}$ -transformed data and a nonlinear regression on raw data following Xiao, White, Hooten, and Durham (2011). We concluded that the additive error structure on a transformed scale (i.e., using linear regression on  $\log_{10}$ -transformed data) provided a better fit to our comparative dataset (Burnham & Anderson, 2002; AICc for linear regression = -73.2, AICc for nonlinear regression = 3,550.4). Nonlinear regression was performed using the `nls` function in R and linear regressions were performed using the `lm` function (R Core Team, 2016). All statistical analyses were performed in R v 3.2.2 (R Core Team, 2016).

### 2.2.3 | Comparison of coefficients across species

Allometric regressions on the logarithmic scale estimate the intercept at 1 gram (g) of body mass, but for most species, particularly elasmobranchs, 1 g lies far outside the range of body masses of the actual specimens measured. Hence, intercepts and slopes are often correlated and centering the data can help reduce this correlation (Quinn & Keough, 2002). We thus estimated a meaningful intercept of gill surface area at 5,000 g, which we termed the 'standardized intercept'. The body mass of 5,000 g was chosen as it is approximately the midpoint of the range of body masses for all shark specimens compared in this study and thus, the  $\log_{10}$  of 5,000 g was subtracted from all individual body mass estimates for all species. To compare slopes and intercepts across species, the R-language formula notation, ' $\log_{10}(\text{gill surface area}) \sim \log_{10}(\text{body mass}) * \text{species}$ ' was used, where the response variable was  $\log_{10}$ -transformed gill surface area and the explanatory variables were  $\log_{10}$ -transformed and centered body mass (i.e., centered around 5,000 g), species identity (as a factor), and the interaction term of  $\log_{10}$ -transformed and centered body mass and species. The inclusion of this interaction term allowed us to estimate standardized intercepts and slopes for each species. Species-specific coefficients were assessed to be significantly different if  $p < .05$ . For further comparison, regression coefficients were bootstrapped to estimate the distribution of slopes and standardized intercepts for each species; this provided a better idea of the uncertainty for each coefficient. To do this, the coefficients and corresponding covariance for each species were extracted from the linear models, values were drawn from a multivariate normal distribution, and coefficients were bootstrapped 500 times.

### 2.2.4 | Comparison of coefficients across ecological traits

Standardized intercepts and slopes of gill surface area allometries were compared across three ecological lifestyle traits: activity level, habitat type, and maximum body size (Table 1). These three traits were chosen based on data availability and their inclusion in studies examining differences in gill surface area with respect to ecological lifestyles. Due to data limitations in terms of not only gill surface area but also metabolic rate, swimming speed, ventilation strategy, and so forth, we were limited in our ability to assess other ecological lifestyle

traits as well as how these traits act in concert to shape gill surface area.

We used caudal fin aspect ratio as a quantitative metric for activity level as this has been shown to relate to swimming speed (Sambilay, 1990; Thomson & Simanek, 1977), daily ration (Palomares & Pauly, 1989), and metabolic rate (Campos, Val, & Almeida-Val, 2018; Killen et al., 2016). Caudal fin aspect ratio (A) was calculated for each species as  $A = h^2/s$ , where  $h$  is the height and  $s$  is the surface area of the caudal fin (Palomares & Pauly, 1989; Sambilay, 1990) (Table 1). As fresh caudal fins are difficult to obtain, caudal fin aspect ratios are often calculated from anatomically correct drawings (Palomares & Pauly, 1989; Sambilay, 1990; Campos et al., 2018). Here, we calculated caudal fin aspect ratios from anatomically correct drawings published in *Sharks of the World* (Ebert, Fowler, & Compagno, 2016). Although we recognize there are shortcomings with this approach (e.g., there is potential for modest changes to tail shape with growth; caudal fin morphology in the thresher sharks also represents specialization to aid in feeding), using caudal fin aspect ratio as a quantitative metric to infer activity level improves the rigor of analyses regarding the relationship of gill surface area and activity, as most previous gill surface area studies have only examined broad, descriptive categories of activity level (e.g., 'sluggish', 'moderate activity') based on the perceived activity of each species rather than a quantitative metric.

For each species, habitat type was assigned based on methodology in Dulvy et al. (2014), where species were categorized as coastal and continental shelf, pelagic, or deepwater, based on a species-specific depth distribution and to a lesser extent, position in the water column (Table 1). The 12 species examined in this study did not include any representatives from the deepwater habitat type, so only the two habitat types of (a) coastal and continental shelf and (b) pelagic were included. To simplify, we used the term 'coastal' for the coastal and continental shelf habitat type and the term 'oceanic' for the pelagic habitat type. The maximum body size (mass) for each species (not to be confused with the largest individual for which gill surface was determined) was obtained from Fishbase (Table 1; Froese & Pauly, 2000). As maximum body mass reported for the Gray Smoothhound was larger in Castro (2010), this estimate was used in favor of the Fishbase estimate. The body mass of the largest individual Nursehound, *Scyliorhinus stellaris*, specimen examined in Hughes et al. (1986) was greater than the maximum body mass reported for this species in Fishbase, and it was used in favor of the Fishbase estimate.

To assess if standardized intercepts and slopes differed with respect to ecological lifestyle traits, mixed-effects models were performed using the `lme` function in the `nlme` package (Pinheiro & Bates, 2000). Separate models were performed for each ecological lifestyle trait following R-language formula notation, ' $\log_{10}(\text{gill surface area}) \sim \log_{10}(\text{body mass}) * \text{ecological lifestyle trait} + (\text{ecological lifestyle trait} | \text{species})$ ', where the response variable was  $\log_{10}$ -transformed gill surface area and the explanatory variables were the fixed effects of  $\log_{10}$ -transformed and centered body mass, the ecological lifestyle trait (e.g., caudal fin aspect ratio, habitat type, or maximum body size), and the interaction between the two. We also included a random effect of '(ecological lifestyle trait | species)', which allowed a separate

slope and standardized intercept to be estimated for each species, yet, the effect of the ecological lifestyle trait on the coefficients was the same. Coefficients were assessed to be significantly different if  $p < .05$ .

### 3 | RESULTS

#### 3.1 | Gray Smoothhound gill surface area

Gill surface area for the eight Gray Smoothhounds examined in this study ranged from 1,103.68 to 4,762.70 cm<sup>2</sup> over the body mass range of 560 to 2,600 g. The standardized intercept, or gill surface area at 5,000 g, was 7,297.94 cm<sup>2</sup> and the slope of the relationship of gill surface area and body mass was 0.7840 (95% CI = 0.4784 to 1.0896; Table 1, Figure 1a). For purposes of comparison with previous studies, the allometric slopes for the gill dimensions of total filament length, average lamellar frequency, and mean bilateral lamellar surface area were 0.2567 (95% CI = 0.1396 to 0.3738), -0.1808 (95% CI = -0.2891 to -0.0724), and 0.6983 (95% CI = 0.4795 to 0.9171), respectively (Figure 1b–d). Complete regression equations for gill surface area and associated dimensions are reported in Figure 1a–d.

#### 3.2 | Comparison of coefficients across species

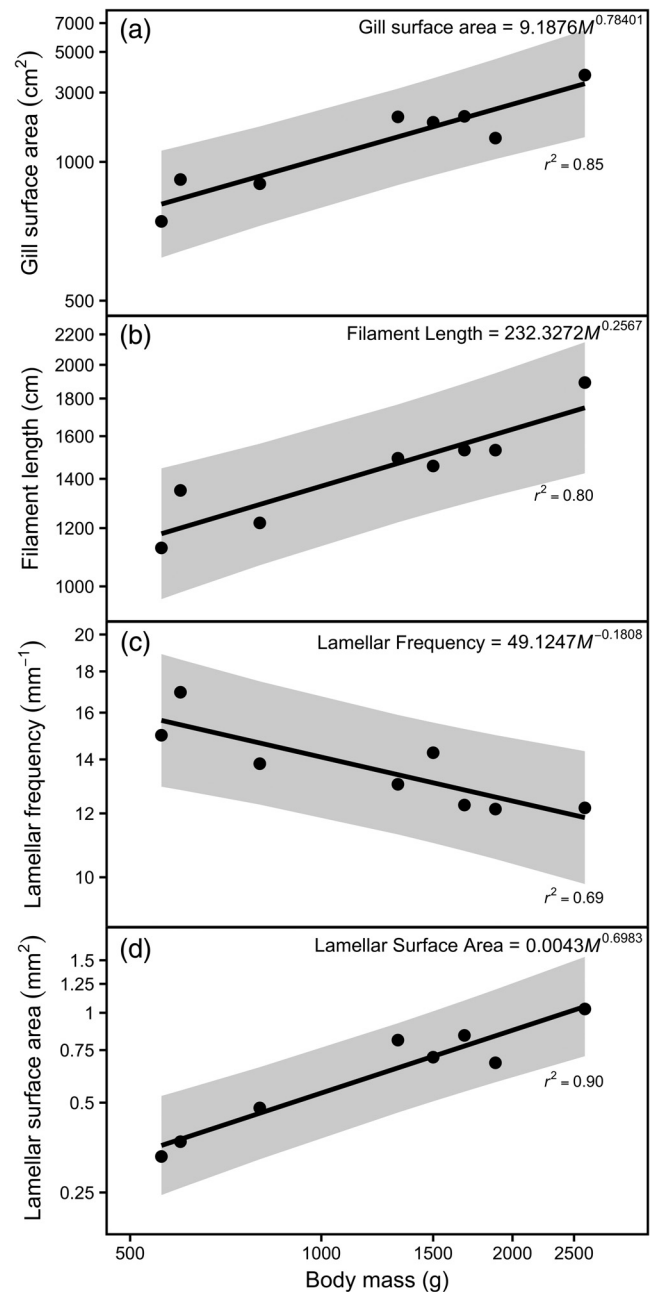
The standardized intercepts varied considerably across species and ranged from 4,724.98 cm<sup>2</sup> in the Nursehound to 35,694.39 cm<sup>2</sup> in the Bigeye Thresher, with a mean and standard error of 17,796.65 ± 2,948.61 cm<sup>2</sup> (Table 1, Figure 2). The slopes of gill surface area allometries were fairly consistent across species with all species ranging between 0.7590 in the Shortfin Mako to 0.9555 in the Lesser Spotted Dogfish *Scyliorhinus canicula*, with a mean and standard error of 0.8512 ± 0.0193 (Table 1, Figure 2).

#### 3.3 | Comparison of coefficients across ecological lifestyle traits

Standardized intercepts differed with respect to all three ecological lifestyle traits (Figure 3a,c,e). More active species with higher caudal fin aspect ratios had significantly greater gill surface area at 5,000 g than less active species ( $t = 2.54$ ,  $df = 10$ ,  $p = .03$ ; Figure 3a). Oceanic species exhibited a significantly greater gill surface area at 5,000g than coastal species ( $t = 4.00$ ,  $df = 10$ ,  $p = .003$ ; Figure 3c). Lastly, larger-bodied species had significantly greater gill surface area at 5,000g than smaller-bodied species ( $t = 3.95$ ,  $df = 126$ ,  $p = .001$ ; Figure 3e). Slopes of gill surface area allometries did not differ across the three ecological lifestyle traits assessed (Figure 3b,d,f). Specifically, slopes did not differ with respect to caudal fin aspect ratio ( $t = .14$ ,  $df = 127$ ,  $p = .89$ ; Figure 3b), habitat type ( $t = -1.07$ ,  $df = 127$ ,  $p = .29$ ; Figure 3d), or maximum body size ( $t = -.44$ ,  $df = 126$ ,  $p = .66$ ; Figure 3f).

### 4 | DISCUSSION

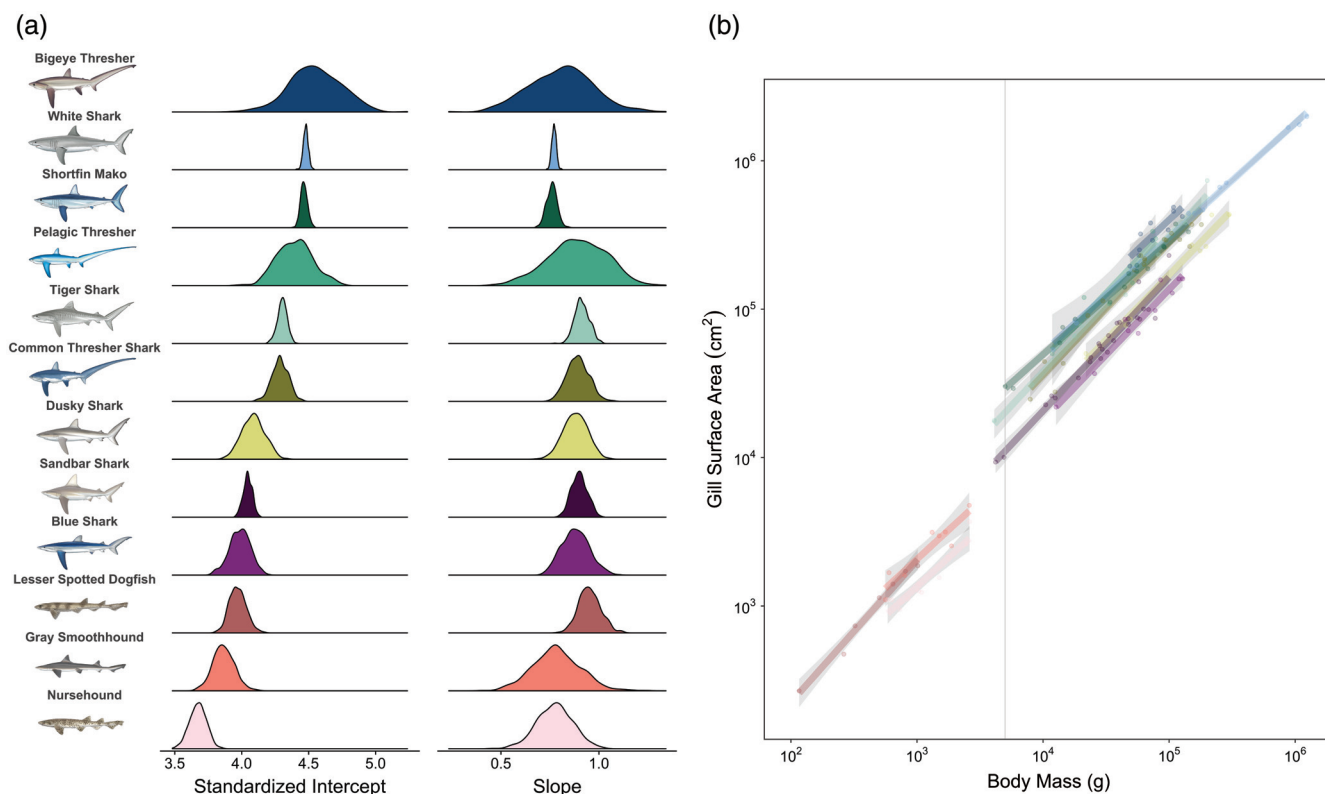
Our results quantitatively confirm that gill surface area varies with ecological lifestyle traits. Specifically, we found that relative gill



**FIGURE 1** The relationship of (a) gill surface area (cm<sup>2</sup>), (b) total filament length (cm), (c) average lamellar frequency (mm<sup>-1</sup>), and (d) mean bilateral lamellar surface area (mm<sup>2</sup>) and body mass (g) for eight Gray Smoothhounds, *Mustelus californicus*. The fitted regression lines and equations are from linear models of log<sub>10</sub>-transformed gill surface area data as functions of log<sub>10</sub>-transformed body mass. The shaded grey region indicates the 95% prediction interval

surface area (i.e., the gill surface area at a specified mass; here we used 5,000g and termed this the 'standardized intercept') varied with activity level, habitat type, and maximum body size. Larger-bodied, oceanic, active species had greater relative gill surface area than smaller-bodied, coastal, less active species. However, the rate at which gill surface area scaled with body mass (slope) did not differ with respect to the same ecological lifestyle traits. These results suggest that relative gill surface area, as opposed to the rate at which gill surface area scales with body mass, is influenced by the ecology and environment of a species. First, we compare the relative gill surface





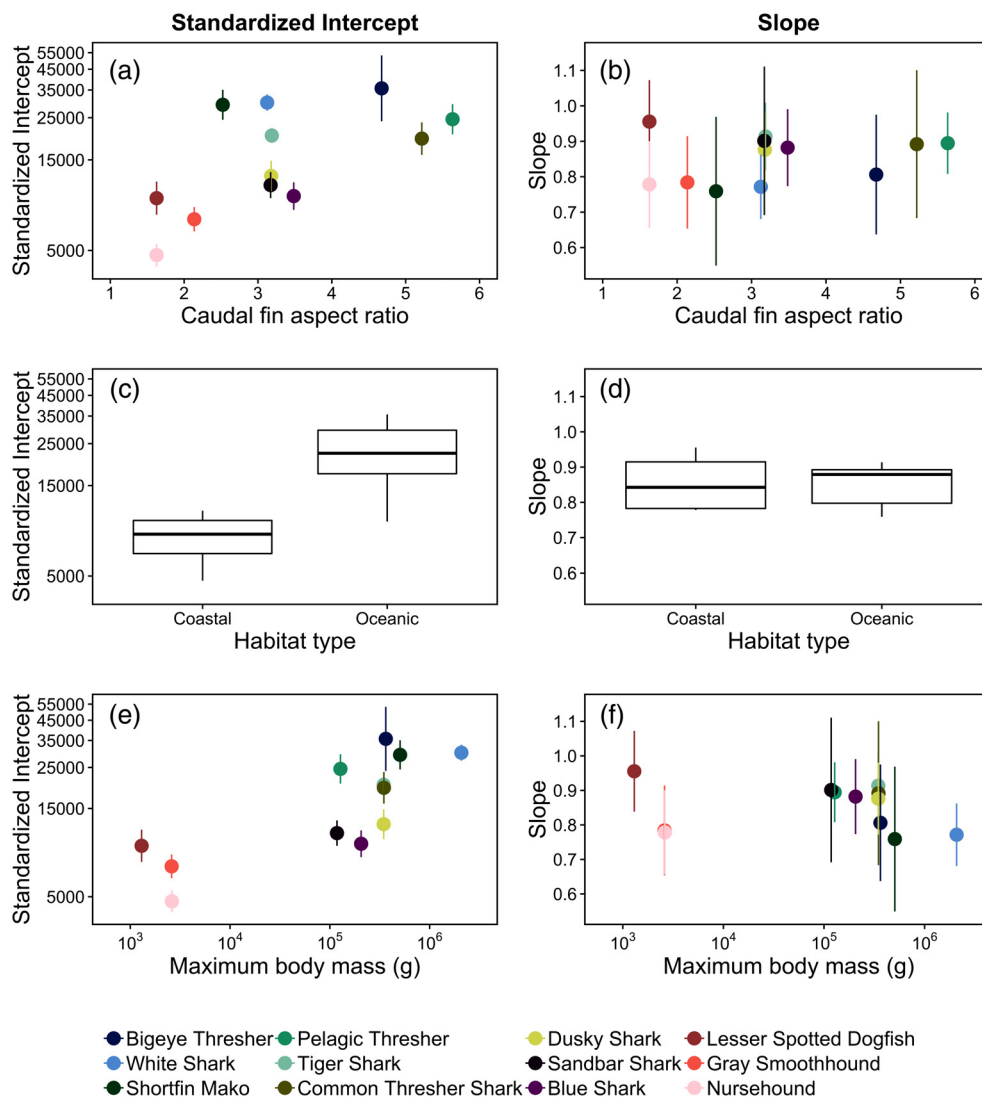
**FIGURE 2** The distribution of regression coefficients and gill surface area allometries for 12 shark species showing highly variable standardized intercepts (i.e., gill surface area at 5,000 g) yet consistent slopes. (a) The distribution of regression coefficients for the allometry of gill surface area in 12 shark species, as estimated by bootstrapping standardized intercepts and slopes from species-specific linear regressions computed with  $\log_{10}$ -transformed gill surface area and  $\log_{10}$ -transformed and centered body mass data at 5,000 g. (b) The relationship of gill surface area ( $\text{cm}^2$ ) and body mass (g) for 12 species of sharks. The fitted regression lines are from a linear model of  $\log_{10}$ -transformed gill surface area as a function of  $\log_{10}$ -transformed and centered body mass for each species. The vertical grey line represents a body mass of 5,000 g. The shaded grey region indicates the 95% confidence interval

area across shark species and ecological lifestyle traits, and then discuss these results in the context of other fishes. Second, we consider the consistency of slope values across species and ecological lifestyle traits and note exceptions among fishes. We then discuss the allometry of gill surface area of the species for which we made new measurements, the Gray Smoothhound. Finally, we highlight future questions to consider once more gill surface area data are available.

Relative gill surface area ranged about an order of magnitude across the 12 shark species (Table 1). On average, we found that oceanic species had approximately 2.6 times greater relative gill surface area than coastal species, more active species had 1.3 times greater relative gill surface area than less active species, and larger-bodied species had 1.6 times greater relative gill surface area than smaller-bodied species. The Bigeye Thresher had the largest relative gill surface area out of the 12 species examined. In addition to being an active and oceanic shark, this species also spends considerable time diving to depth where exposure to subsurface hypoxia may also provide selective pressure for increased gill surface area (Wootton et al., 2015). The Nursehound had the lowest relative gill surface area, which reflects its less active lifestyle, coastal and benthic habitat, and small maximum body size.

The order of magnitude difference in relative gill surface area observed in this study across the 12 shark species is considerably less than the two orders of magnitude range in relative gill surface area documented in teleost fishes (De Jager & Dekkers, 1975;

Palzenberger & Pohla, 1992; Wegner, 2011). This appears to partially reflect the more diverse ecological roles observed in teleost fishes. For example, the lowest relative gill surface areas for teleost fishes are found in “sluggish” freshwater or estuarine species that have developed facultative or even obligate air-breathing capacities, and thus are not solely dependent on the gills for respiration (Graham, 1997; Graham, Lee, & Wegner, 2007; Palzenberger & Pohla, 1992). In addition to differences in ecological radiations, fundamental differences in the gill morphology may also play a role in the more limited range of relative gill surface areas observed for sharks. Despite the similarity in ecological lifestyles between regionally endothermic teleosts (tunas) and regionally endothermic sharks (lamnid sharks) in terms of their high activity, oceanic habitat, and streamlined, fusiform body types, relative gill surface areas are two to three times greater in tunas compared to lamnid sharks (Muir & Hughes, 1969; Wegner, Sepulveda, Bull, & Graham, 2010). This apparent upper limit to elasmobranch gill surface area has been suggested to reflect constraints on water flow imposed by the elasmobranch interbranchial septum that appear to affect lamellar spacing and ultimately, limit gill surface area (Wegner, 2016; Wegner, Sepulveda, Olson, et al., 2010; Wegner et al., 2012). Finally, the more limited range of gill surface areas observed in sharks compared to teleosts may also reflect the much smaller number of shark species for which gill surface area data have been acquired to-date. The addition of gill surface area measurements for new



**FIGURE 3** Gill surface area allometric standardized intercepts (a, c, e) and slopes (b, d, f) for 12 shark species in relation to three ecological lifestyle traits: caudal fin aspect ratio as a measure of activity level, habitat type, and maximum body mass

elasmobranch species from more diverse habitats (e.g., freshwater and estuarine species) and activity levels may increase the range of relative gill surface areas observed for this group.

The rate at which gill surface area scaled with body mass (slope) did not differ across shark species or ecological lifestyle traits examined in this study, and as such, were consistent across species that differed in activity level, habitat type, and maximum body size. The range of slopes observed in this study (0.76 to 0.96) was relatively small and fell within the 0.33 to over 1.00 range exhibited by freshwater and marine teleost fishes (De Jager & Dekkers, 1975; Palzenberger & Pohla, 1992; Wegner, 2011). It has long been noted that the slope of the gill surface area and body mass relationship mirrors that of the scaling relationship of metabolic rate and body mass (Hughes & Morgan, 1973; Palzenberger & Pohla, 1992; Wegner, 2011). Accordingly, the mean slope of the relationship of gill surface area and body mass determined in this study ( $0.85 \pm 0.02$ ) is strikingly close to the mean slope of the scaling relationship of metabolic rate ( $0.84 \pm 0.05$ ), which was estimated for a limited number of elasmobranchs with metabolic rate data (six total; four batoids and two sharks; Wegner, 2016). This similarity in the allometry of metabolic rate and the allometry of

gill surface area is consistent with the idea that gill surface area and oxygen diffusion capacity (i.e., rate of gas transfer across the respiratory surface per unit of gas partial pressure) have evolved to match metabolic demand (Gillooly, Gomez, Mavrodiev, Rong, & McLamore, 2016; Lefevre, McKenzie, & Nilsson, 2017; Wegner, 2011). Alternatively, it has been suggested that this intimate scaling relationship may reflect a constraint of metabolic rate based on geometric constraints of gill surface area growth within the space-limited opercular/parabranchial cavities (Pauly, 2010; Pauly & Cheung, 2018), although this hypothesis has been highly contested (see Lefevre et al., 2017; Norin & Gamperl, 2017).

While our results did not show any relationship between the ecological lifestyle traits examined and the slope of the gill surface area allometries for the 12 sharks in this study, there are clear examples from the teleost literature in which the slope does appear to be affected by other underlying physiological and ecological stressors. For example, the Blackfin icefish *Chaenocephalus aceratus* (a hemoglobin-lacking species) has a high gill surface area allometric slope of 1.09, which is thought to reflect the need for a disproportionately large respiratory surface area to help mitigate the effects of a

greatly reduced blood-oxygen carrying capacity, which becomes increasingly problematic with growth (Holeton, 1976; Nilsson, 2010). On the other end of the spectrum, low gill surface area allometric slope values of less than 0.33 have been observed in some air-breathing fishes that reflect their increased capacity for breathing air and thus reduced reliance on the gills for oxygen uptake as they grow (Hakim, Munshi, & Hughes, 1978; Santos, Fernandes, & Severi, 1994; Perna & Fernandes, 1996). Thus, while the narrow bounds of the gill surface area allometric slope that we found in this study as well as those seen in other studies are likely explained by the relationship between gill surface area and metabolic rate, there are clear exceptions.

Gill surface area data determined in this study for the Gray Smoothhound were similar to those of the Nursehound and Lesser Spotted Dogfish from the literature (Hughes, 1972; Hughes et al., 1986). Despite the broad ecological similarity of these three species being coastal, smaller-bodied, and less active, the Gray Smoothhound had the highest estimated activity level based on caudal fin aspect ratio, as well as the largest maximum body size. While the Gray Smoothhound had 1.5 times greater relative gill surface area than the Nursehound, its relative gill surface area was 0.77 times lower than that of the Lesser Spotted Dogfish. Gill surface area data for the Lesser Spotted Dogfish were from a more limited size range than for both the Gray Smoothhound and the Nursehound, and this may have affected the estimates of regression coefficients (Hughes, 1972; Hughes et al., 1986). For many species examined in this and other gill surface area allometry studies, the sample sizes are small and the body mass ranges do not fully represent the size range of the species. Future work should thus focus not only on adding additional species that have diverse ecological lifestyles but also ideally incorporate gill surface area measurements for the entire size range of the species to provide the most accurate comparative data possible.

Overall, our findings indicate that ecological lifestyle differences among species are reflected in the relationships of gill surface area and body mass in sharks. Specifically, we found that activity level, habitat type, and maximum body size may all act to help shape gill surface area. However, such ecological and environmental influences appear to primarily affect the gill surface area at a given body mass (intercept), rather than the rate at which gill surface area scales with body mass (slope). The rate at which gill surface area scaled with body mass was narrowly-bounded in the 12 shark species examined, likely reflecting its tight relationship with metabolic rate. Due to the nature of only having 12 species with sufficient gill surface area data, we were limited in our ability to test other hypotheses and ask additional questions. For example, we could not tease apart the influence of ecological lifestyle and evolutionary relatedness on gill surface area. Phylogenetic analyses are needed to examine if any of the differences in the relative gill surface area or lack of differences in the rate at which gill surface area increases with body mass are related to shared evolutionary history, but such analyses will only be meaningful once more gill surface area data are available from additional species. Additional gill surface area data, including from more ecologically diverse shark and batoid species (e.g., those inhabiting estuarine environments, or additional species dwelling in chronic hypoxia) as well as larger body size ranges within species, should allow for a more thorough

understanding of how elasmobranch gill surface area allometries relate to other fish groups. Additionally, these data could inform how ecological lifestyle traits act in concert to shape gill surface area.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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