Gill Morphometrics of the Thresher Sharks (Genus *Alopias*): Correlation of Gill Dimensions with Aerobic Demand and Environmental Oxygen

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**ABSTRACT** Gill morphometrics of the three thresher shark species (genus *Alopias*) were determined to examine how metabolism and habitat correlate with respiratory specialization for increased gas exchange. Thresher sharks have large gill surface areas, short water–blood barrier distances, and thin lamellae. Their large gill areas are derived from long total filament lengths and large lamellae, a morphometric configuration documented for other active elasmobranchs (i.e., lamnid sharks, Lamnidae) that augments respiratory surface area while limiting increases in branchial resistance to ventilatory flow. The bigeye thresher, *Alopias superciliosus*, which can experience prolonged exposure to hypoxia during diel vertical migrations, has the largest gill surface area documented for any elasmobranch species studied to date. The pelagic thresher shark, *A. pelagicus*, a warm-water epipelagic species, has a gill surface area comparable to that of the common thresher shark, *A. vulpinus*, despite the latter’s expected higher aerobic requirements associated with regional endothermy. In addition, *A. vulpinus* has a significantly longer water–blood barrier distance than *A. pelagicus* and *A. superciliosus*, which likely reflects its cold, well-oxygenated habitat relative to the two other *Alopias* species. In fast-swimming fishes (such as *A. vulpinus* and *A. pelagicus*) cranial streamlining may impose morphological constraints on gill size. However, such constraints may be relaxed in hypoxia-dwelling species (such as *A. superciliosus*) that are likely less dependent on streamlining and can therefore accommodate larger branchial chambers and gills. J. Morphol. :1–12, 2015. © 2015 Wiley Periodicals, Inc.

**KEY WORDS:** gill surface area; elasmobranch; hypoxia; aerobic metabolism; regional endothermy; diffusion capacity

**INTRODUCTION** Fish gill morphology correlates with metabolic demand and habitat (Gray, 1954; Hughes, 1966; Hughes and Morgan, 1973; De Jager and Dekkers, 1975; Hughes, 1984a; Palzenberger and Pohla, 1992; Chapman, 2007; Wegner, 2011). Despite the extreme diversity of fishes, noticeable similarities in respiratory structure occur in even distantly related species that inhabit similar environments or have comparable metabolic requirements. As such, in reviews of gill morphology (e.g., Gray, 1954; Hughes, 1984a; Wegner, 2011), fishes are often categorized into morphological ecotypes based on the respiratory dimensions of the gills, namely gill surface area and the thickness of the gill epithelium (the water–blood barrier distance), which both reflect a species’ capacity for oxygen uptake (i.e., the diffusion capacity of the gills). Currently at least six groups are recognized: (1) fast-swimming oceanic species, (2) marine fishes of intermediate activity, (3) sluggish marine species, (4) freshwater fishes, (5) air-breathers, and (6) hypoxia-dwellers (Wegner, 2011).

While these six morphological ecotypes are generally broad, they encompass the breadth of fish diversity and gill morphology based primarily on fish metabolic requirements and the availability of oxygen in their respective habitats. Fast oceanic species have high oxygen demands associated with elevated capacities for aerobic performance and thus possess large gill surface areas and short gill diffusion distances to facilitate oxygen uptake (Muir and Hughes, 1969; Emery and Szczepanski, 1986; Wegner, 2010a, b). Most fishes comprising this group (tunas, Scombridae; lamnid sharks, Lamnidae; billfishes, Istiophoridae and Xiphiidae) are capable of regional endothermy (the ability to conserve metabolically produced heat to warm certain regions of the body), which results in enhanced physiological function (Dickson, 1995;
Brill, 1996; Bernal et al., 2001; Korsmeyer and Dewar, 2001; Dickson and Graham, 2004; Sepulveda et al., 2007). Marine fishes of intermediate activity have relatively standard gill morphologies due to their more “typical” activity levels, lack of endothermy, and generally normoxic habitats (Gray, 1954; Hughes, 1966; Hughes, 1970; Hughes, 1984a). Sluggish marine species, freshwater fishes, and air-breathers all tend to have reduced gill surface areas and thick water–blood barrier distances, although the environmental and physiological factors influencing these dimensions appear to differ between groups. For sluggish marine species, a reduced gill area and thicker epithelium are associated with low metabolic demands (Gray, 1954; Hughes and Gray, 1972; Hughes and Morgan, 1973, Hughes and Iwai, 1978), while most freshwater fishes likely have a generally reduced gill diffusion capacity due to the relatively higher availability of oxygen in freshwater (air-saturated freshwater contains 15–20% more dissolved oxygen than seawater depending on temperature; Palzenberger and Pohla, 1992). For air-breathing fishes, an increased reliance on oxygen absorption from the air results in smaller and thicker gills (Graham, 1997; Graham et al., 2007; Graham and Wegner, 2010). Although they are often sedentary and found in freshwater, hypoxia-dwelling species have large gill surface areas (and likely short water–blood barriers) to absorb sufficient oxygen to meet their metabolic requirements in an oxygen-deficient habitat (Graham, 2006; Chapman, 2007; Mandic et al., 2009; Wegner, 2011).

Thus, while broad, these morphological ecotypes provide insight into the role of oxygen demand and availability in shaping gill morphology. However, because phylogenetic assemblages tend to experience similar evolutionary pressures, closely related species usually fall into the same ecomorphotypic group. As such, conclusions drawn from these broad morphological ecotypes are often made by comparing distantly related fishes. While general trends in gill morphology can be assessed by such comparisons, determining the detailed effects of specific evolutionary pressures (such as metabolism or environmental oxygen level) on gill morphology can be difficult due to the numerous other selective pressures influencing each group’s evolutionary history. Additional insight on the correlation of gill dimensions with oxygen demand and availability can be gained by comparing cases in which closely related species or populations have recently diverged in life-history characteristics and habitat.

The thresher sharks (Alopiidae) comprise a single genus containing three species, which, based on their life-history characteristics, should span three ecological morphotypes: (1) The common thresher, Alopias vulpinus, is a highly active species (fast-swimming oceanic species) capable of elevating the temperature of the slow-twitch aerobic (red) muscle (Bernal and Sepulveda, 2005; Sepulveda et al., 2007) and is thus expected to have short gill diffusion distances and a large gill surface area like lamnid sharks. (2) The pelagic thresher, A. pelagicus, lacks the capacity for red muscle endothermy (Sepulveda et al., 2005, Patterson et al., 2011) and is thus hypothesized to have lower oxygen requirements and hence a more “standard” gill morphology (i.e., marine fish of intermediate activity). (3) The bigeye thresher, A. superciliosus, is a deep-diving shark (Nakano et al., 2003; Weng and Block, 2004; Musyl et al., 2011) that in many parts of its range experiences prolonged exposure to hypoxia in the oceans’ oxygen minimum zones (OMZs) and is thus hypothesized to have a large gill area with short diffusion distances to facilitate oxygen uptake in a low-oxygen environment.

Because the three thresher shark species differ in life history and habitat and appear to fall into three different ecological morphotypes, they comprise an ideal system for testing how gill dimensions correlate with high oxygen demands (as expected for the regionally endothermic A. vulpinus) and low oxygen availability (as expected for the hypoxia-dwelling A. superciliosus) in comparison to more typical conditions (as expected for A. pelagicus). This study thus examines the respiratory dimensions of the gills (i.e., the gill surface area, water–blood barrier distance, and lamellar thickness) in the three closely related species to better understand the specific effects of metabolic demand and dissolved oxygen availability on gill morphology.

MATERIALS AND METHODS
Gill Collection and Preparation
Gills were collected opportunistically from nine A. vulpinus (Bonnerterre, 1788), nine A. superciliosus (Lowe, 1841), and six A. pelagicus (Nakamura, 1935). All A. vulpinus, most A. superciliosus, and one A. pelagicus were caught by hook and line off the coast of southern California, Hawaii, or Mexico during other scientific studies. These sharks were euthanized by severing the spinal cord at its articulation to the chondrocranium according to protocol S00080 of the University of California, San Diego Institutional Animal Care and Use Committee. Three A. superciliosus and five A. pelagicus were purchased whole from drift gillnet and longline fisheries in southern California and Costa Rica. For all sharks, fork length was measured and weights were estimated using length–weight regressions [Kohler et al. (1995) for A. vulpinus and A. superciliosus; Liu et al. (1999) and White (2007) for A. pelagicus].

Because gill samples were obtained opportunistically from various sources, tissue extraction and preservation method varied. Table 1 shows shark size and collection location data as well as the treatment method of each gill sample. These treatments were as follows:

A. When possible, all five gill arches were excised from both sides of the head immediately following euthanasia and fixed in a 10% formalin solution buffered in seawater.

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A. pelagicus (pelagic thresher) and Hughes (1969)

A. vulpinus (bigeye thresher) and Hughes (1969)

A. superciliosus (common thresher) and Hughes (1984b) to estimate total gill surface area, \( A \), calculated by the equation:

\[ A = L_{fil} \times 2n_{lam} \times A_{lam} \]

\((n_{lam} \text{ is doubled in this equation to represent the presence of lamellae on both sides of each gill filament}).

To determine total filament length, the filaments were counted on all five gill arches from one side of the branchial chamber. Beginning at the dorsal margin of each hemibranch and working ventrally, the gill filaments were separated into bins of 20 (as the total number of filaments was rarely divisible by 20, the final bin usually contained less than 20 filaments). The medial filament of each bin (i.e., filament number 10, 30, 50, etc.) was measured and assumed to be representative of the mean length of a filament in its bin (each middle filament was measured from the embedded base, where filaments are partially covered by a fleshy extension of the gill arch tissue commonly called the branchial canopy, to the tip). The length of all filaments in a bin was calculated by multiplying the length of the medial filament by the number of filaments in the bin. Total filament length \((L_{fil})\) was determined by summing all bins from the five arches and doubling this value to account for the gills on the opposite side of the branchial chamber.

Following determination of \( L_{fil} \), each medial filament was excised and, using a dissection microscope (Zeiss, model #47 50 52) fitted with a digital camera (Canon Digital Rebel XT), magnified photographs were taken of one side of the filament base, middle, and tip to determine mean lamellar frequency (the number of lamellae per mm on one side of a filament) for each bin. With a scalpel, individual lamellae were then removed from each of these three locations on the filament, mounted on slides and photographed. The most complete isolated lamella from each location was measured to determine its bilateral surface area and the three measurements were then averaged to determine the mean bilateral surface area of a lamella in each bin. Digital images of lamellae frequency and lamellar surface area were analyzed using NIH Image J software.

**Gill Measurement and Analysis**

Total filament length, \( L_{fil} \) (i.e., the total length of all gill filaments), lamellar frequency, \( n_{lam} \) (i.e., the average number of lamellae per unit length on one side of a filament), and the mean bilateral surface area of a lamella, \( A_{lam} \), were determined according to the procedure outlined in Muir and Hughes (1969) and Hughes (1984b) to estimate total gill surface area, \( A \), calculated by the equation:

\[ A = L_{fil} \times 2n_{lam} \times A_{lam} \]

\((n_{lam} \text{ is doubled in this equation to represent the presence of lamellae on both sides of each gill filament}).

To determine total filament length, the filaments were counted on all five gill arches from one side of the branchial chamber. Beginning at the dorsal margin of each hemibranch and working ventrally, the gill filaments were separated into bins of 20 (as the total number of filaments was rarely divisible by 20, the final bin usually contained less than 20 filaments). The medial filament of each bin (i.e., filament number 10, 30, 50, etc.) was measured and assumed to be representative of the mean length of a filament in its bin (each middle filament was measured from the embedded base, where filaments are partially covered by a fleshy extension of the gill arch tissue commonly called the branchial canopy, to the tip). The length of all filaments in a bin was calculated by multiplying the length of the medial filament by the number of filaments in the bin. Total filament length \((L_{fil})\) was determined by summing all bins from the five arches and doubling this value to account for the gills on the opposite side of the branchial chamber.

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To calculate the number of lamellae in each bin, the mean of the three lamellar frequency measurements was doubled (to account for lamellae on both sides of the filament) and multiplied by the total filament length of that bin. The total surface area in a given bin was estimated by multiplying the number of lamellae per bin by the mean lamellar bilateral surface area from the corresponding representative filament. Total gill surface area was then divided by the total number of lamellae in the gills to estimate average bilateral lamellar area ($A_{\text{alam}}$), and the total number of lamellae was divided by the total gill filament length ($L_{\text{fil}}$) to determine the lamellar frequency ($n_{\text{alam}}$) of all bins.

For the first specimen examined from each species (#5, 10, and 22), $A_{\text{alam}}$ and $n_{\text{alam}}$ were measured on all five arches and the resultant data were used to identify the gill arch for which these dimensions were most representative of the entire gills. For subsequent specimens, $A_{\text{alam}}$ and $n_{\text{alam}}$ were based on this gill arch (fourth arch for *A. vulpinus* and *A. pelagicus*, third arch for *A. superciliosus*).

### Lamellar Thickness and Water–Blood Barrier Distance

Ten sharks (four *A. vulpinus*, three *A. superciliosus*, three *A. pelagicus*) were selected, based on quality of gill preservation, for analysis of lamellar thickness and water–blood barrier distance. For each shark, six regions of the representative gill arch (three from each hemibranch) were identified in which corresponding lamellar area and frequency best represented the mean values. From each site, a section of four gill filaments was excised for examination with scanning electron microscopy (SEM).

Fixed filament sections were rinsed in deionized water and gradually dehydrated in tert-butyl alcohol (20% increments over 24 h). After dehydration at 80% tert-butyl alcohol, the gill filaments were cut along their long axis to provide cross sections of lamellae for measurement. While soaking in 100% tert-butyl alcohol, samples were kept at 32°C to prevent freezing. After a subsequent wash of 100% tert-butyl alcohol, samples were frozen at -4°C and freeze-dried under vacuum to sublime and extract the alcohol.

Dried filaments were mounted such that lamellar cross-sections laid perpendicular to the SEM field of view. Samples were sputter coated with gold–palladium and photographed under the high-vacuum mode of an FEI Quanta 600 SEM (FEI Instruments, Hillsboro, Oregon). Twenty measurements of lamellar thickness and the water–blood barrier distance from each specimen were made from acquired digital images using Image J.

### Statistical Analysis

For each species, power–law regressions were determined for total gill surface area ($A$) and each of its constituent dimensions ($L_{\text{fil}}$, $n_{\text{alam}}$, $A_{\text{alam}}$) versus body mass using least-squares analysis (Figs. 1–4). For each dimension, species-specific regressions were compared using 10,000 bootstrap replications (R v.2.7.0) of the raw data (Wegner et al., 2010a). Statistical significance between two species was determined where less than 5% of the resultant replicate regressions intersected over a shared weight range of the compared species. Because lamellar thickness and the water–blood barrier distance do not appear to show a distinct relationship with body mass (Hughes et al., 1986, Wegner et al., 2010b) species-specific means ± standard deviation were determined for each dimension (Table 2). Species-specific measurements for each dimension were compared between species using a two-level nested ANOVA (dimension measurement nested within individual nested within species) and a post-hoc Tukey test.

### RESULTS

#### Gill Surface Area

Gill surface area to body mass regressions determined in this study for the three *Alopias* species are shown in comparison to data for *A. vulpinus* from Emery and Szczepanski (1986) in Figure 1. *A. superciliosus* has a significantly larger gill

![Fig. 1. Power–law regressions of total gill surface area (cm²) to body mass (g) for the three *Alopias* species examined in this study (A) and for *A. vulpinus* from Emery and Szczepanski (1986) (B).](image-url)
surface area than *A. vulpinus* for most of their shared body mass range (48.83–81.20 kg). Although the gill surface area regression for *A. pelagicus* lies below that of *A. superciliosus* and above that of *A. vulpinus*, these differences are not statistically significant.

The scaling exponents for gill surface area in relation to body mass for the three species range from 0.78 to 1.03 (Fig. 1) and fall within the range of those determined for other fishes (Hughes, 1972; Palzenberger and Pohla, 1992; Wegner, 2011). Although the scaling exponent for *A. vulpinus* gill area to body mass in this study (1.03) is much larger than that measured by Emery and Szczepanski (1986) (0.41), no significant difference was evident in the gill surface areas determined in

![Graph 1](image1.png)

**Fig. 2.** Power–law regressions of total filament length (cm) in relation to body mass (g) for the three *Alopias* species examined in this study1 and for *A. vulpinus* from Emery and Szczepanski (1986)2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

![Graph 2](image2.png)

**Fig. 3.** Power–law regressions of lamellar frequency (mm⁻¹) to body mass (g) for the three *Alopias* species examined in this study1 and for *A. vulpinus* from Emery and Szczepanski (1986)2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
the two studies over the shared body mass range. The low scaling exponent of *A. vulpinus* in Emery and Szczepanski (1986) lies outside of the range documented for most fishes (Palzenberger and Pohla, 1992) and likely results from the limited body size range sampled. The 95% confidence intervals of the 1.03 scaling exponent of *A. vulpinus* in this study (0.833–1.23) are above the expected scaling exponent of gill surface area to body mass assuming isometric growth of the gills (0.67; the sum of the scaling exponents of its constituent dimensions, total gill filament length (0.33), lamellar frequency (−0.33), and lamellar area (0.67)).

Log–log plots for total gill filament length in relation to body mass for the three thresher species are shown in Figure 2. Total filament length in *A. superciliosus* is significantly greater than in the other *Alopias* species and contributes to the larger gill surface area of *A. superciliosus* in comparison to *A. vulpinus*. High total filament length in *A. superciliosus* results from significantly longer filaments and significantly more filaments than in *A. vulpinus* and *A. pelagicus*. *A. pelagicus* has a significantly longer total filament length than *A. vulpinus* over most overlapping body masses (11.82–64.90 kg). This results from more filaments in *A. pelagicus*; the mean filament length does not differ significantly between these species.

The relationship between lamellar frequency and body mass are plotted in Figure 3. *A. vulpinus* has a significantly lower lamellar frequency than the other *Alopias* species examined in this study and *A. vulpinus* from Emery and Szczepanski (1986). [Color figure can be viewed in the online issue, which is available at wileyonline-library.com.]

### TABLE 2. Lamellar dimensions (means ± standard deviation) of the three thresher shark species

<table>
<thead>
<tr>
<th>Species</th>
<th>Fork length (cm)</th>
<th>Mass (kg)</th>
<th>Lamellar thickness (μm)</th>
<th>Water–blood barrier thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. pelagicus</em></td>
<td>70.0</td>
<td>11.82</td>
<td>12.22 ± 1.47</td>
<td>1.53 ± 0.36</td>
</tr>
<tr>
<td><em>A. pelagicus</em></td>
<td>91.5</td>
<td>21.16</td>
<td>13.12 ± 1.44</td>
<td>1.47 ± 0.23</td>
</tr>
<tr>
<td><em>A. superciliosus</em></td>
<td>163.0</td>
<td>77.77</td>
<td>12.18 ± 1.28</td>
<td>1.83 ± 0.52</td>
</tr>
<tr>
<td><em>A. pelagicus</em></td>
<td></td>
<td></td>
<td>12.51 ± 0.53</td>
<td>1.61 ± 0.19</td>
</tr>
<tr>
<td><em>A. superciliosus</em></td>
<td>163.0</td>
<td>59.34</td>
<td>11.49 ± 0.97</td>
<td>1.54 ± 0.27</td>
</tr>
<tr>
<td><em>A. pelagicus</em></td>
<td>173.0</td>
<td>71.29</td>
<td>15.44 ± 1.29</td>
<td>1.69 ± 0.39</td>
</tr>
<tr>
<td><em>A. superciliosus</em></td>
<td>192.0</td>
<td>98.26</td>
<td>10.56 ± 1.14</td>
<td>1.57 ± 0.27</td>
</tr>
<tr>
<td><em>A. pelagicus</em></td>
<td></td>
<td></td>
<td>12.50 ± 2.59</td>
<td>1.69 ± 0.08</td>
</tr>
<tr>
<td><em>A. superciliosus</em></td>
<td>146.0</td>
<td>53.24</td>
<td>13.56 ± 1.61</td>
<td>2.25 ± 0.37</td>
</tr>
<tr>
<td><em>A. pelagicus</em></td>
<td>181.0</td>
<td>91.47</td>
<td>15.67 ± 1.22</td>
<td>2.86 ± 0.51</td>
</tr>
<tr>
<td><em>A. superciliosus</em></td>
<td>146.0</td>
<td>53.24</td>
<td>13.87 ± 1.27</td>
<td>2.67 ± 0.52</td>
</tr>
<tr>
<td><em>A. pelagicus</em></td>
<td></td>
<td></td>
<td>14.06 ± 1.48</td>
<td>2.41 ± 0.56</td>
</tr>
<tr>
<td><em>A. superciliosus</em></td>
<td>181.0</td>
<td>91.47</td>
<td>14.06 ± 1.48</td>
<td>2.41 ± 0.56</td>
</tr>
</tbody>
</table>

*Significantly different from *A. pelagicus* and *A. superciliosus*
both *A. superciliosus* (48.83–83.15 kg) and *A. pelagicus* (entire comparable mass range). Lamellar frequency does not differ significantly between *A. superciliosus* and *A. pelagicus*. The scaling relationships of mean lamellar bilateral surface area to body mass plotted in Figure 4 do not differ significantly between the three species.

**Lamellar Thickness and Water–Blood Barrier Distance**

The lamellar thickness and water–blood barrier distance measured for four *A. vulpinus*, three *A. superciliosus*, and three *A. pelagicus*, as well as the mean for each species are given in Table 2. Lamellar thickness did not differ significantly between species. However, *A. vulpinus* has a significantly thicker water–blood barrier (2.55 ± 0.27 μm) than both *A. superciliosus* (1.60 ± 0.08 μm) and *A. pelagicus* (1.61 ± 0.19 μm; \( P < 0.0001 \)).

**DISCUSSION**

This study shows that all three *Alopias* species possess respiratory specializations for increased gas exchange, including large gill surface areas and short diffusion distances. *Alopias* gill surface areas are among the highest measured for any elasmobranch group, rivaling those of the regionally endothermic lamnid sharks (e.g., shortfin mako, *Isurus oxyrinchus*, and white shark, *Carcharodon carcharias*) and are greater than both those of pelagic carcharhiniform sharks (which have gill surface areas consistent with fishes of intermediate activity), such as the blue shark, *Prionace glauca*, and less-active benthic elasmobranch species (Fig. 5). Although the water–blood barrier distances in *Alopias* (range: 1.60–2.55 μm) are not as short as those of the shortfin mako (1.15 μm), they are similar to the blue shark (1.65 μm; Wegner, 2010b) and are much less than those of less-active species (*Scylliorhinus*, *Squalus*, *Galeorhinus*, and *Raja*; range: 4.85–11.27 μm; Hughes and Wright, 1970). Finally, *Alopias* lamellae are also relatively thin (12.50–14.29 μm) resulting in narrow vascular channels that likely force red blood cells close to the water–blood barrier.

Like lamnid sharks, the large gill surface areas in *Alopias* result from high total filament lengths and large lamellae. This morphometric configuration adheres to the model proposed by Hughes (1966) for augmenting gill surface area while minimizing increases in gill resistance to ventilatory flow. In contrast to highly active teleosts such as tunas and billfishes that couple high total filament lengths with high lamellar frequencies to increase gill area (Muir and Hughes, 1969; Wegner et al., 2010a), lamellar frequencies in *Alopias* and lamnids are not greater than those of less-active elasmobranchs. This likely reflects the presence of interbranchial septa in elasmobranch gills that inherently increase gill resistance as water is...
forced through septal canals (Wegner et al., 2012). Because having a higher lamellar frequency would further increase gill resistance, the presence of interbranchial septa in elasmobranchs, likely causes alopids and lamnids to augment gill surface area differently than high-performance teleosts.

Due to the general lack of concordance in the phylogenetic relationship of Alopidae and Lamnidae within the Lamniformes (Naylor et al., 1997, 2012; Shimada, 2005) and the paucity of information available on the gills of other members of the group, it is difficult to assess if lamnids and alopids separately evolved large gill surface areas as a result of high activity or if the common ancestor to these, and some other Lamniform groups (the various proposed phylogenies do not consider the alopids and lamnids as sister groups) had large gills. Given the general plasticity of gill morphology (Chapman, 2007; Wegner, 2011) and the less-streamlined body forms (and presumably lower activity levels) of the other Lamniform groups, it is probable that the large gill areas of the two groups are independently derived. However, additional studies on the gills of other Lamniform species are needed to further address such phylogenetic questions.

**Alopias Interspecific Comparisons**

*A. superciliosus* has the largest gill surface area and one of the highest total filament lengths documented for any elasmobranch studied to date. This is likely associated with its ability to tolerate prolonged exposure to hypoxia. In many parts of its range, including the collection sites in this study, the daytime depth preference of *A. superciliosus* is 200–500 m (Nakano et al., 2003; Weng and Block, 2004; Musyl et al., 2011), which coincides with a midwater OMZ where dissolved oxygen can be below 1.5 ml O$_2$ l$^{-1}$ (<30% saturation; Garcia et al., 2010). *A. superciliosus* is thus similar to other OMZ organisms in having a larger respiratory surface area in comparison to closely related species living in normoxic waters (Yang et al., 1992; Childress and Seibel, 1998; Levin, 2003; Wegner, 2010a). In addition, the short water–blood barrier distance (1.60 ± 0.08 μm) and thin lamellae (12.50 ± 2.59 μm) of *A. superciliosus* should facilitate O$_2$ absorption within the OMZ and are likely required for this species to meet its metabolic demands while foraging at depth.

At a mass of 50 kg, *A. superciliosus* has a 26% larger gill surface area than *A. vulpinus*, which does not frequent the OMZ (Cartamil et al., 2010, 2011). This results from a 28% higher total filament length in *A. superciliosus* and corresponds to larger branchial chambers in this species (Fig. 6). The laterally expanded branchial chambers of *A. superciliosus* extend dorsally to meet the epaxial musculature and result in the distinct “helmeted contour” which lines the dorsal surface of the head (Fig. 6; Compagno, 2001; Smith et al., 2008). These nuchal grooves are commonly used to identify *A. superciliosus* and are exaggerated by the large size of its branchial chambers relative to *A. vulpinus* (Fig. 6) and other sharks. Augmentation of gill surface area through extension of total filament length is usually limited by the volume of the branchial chambers. However, in *A. superciliosus*, it appears the branchial cavities have become enlarged to house longer filaments that ultimately increase gill surface area at the cost of cranial streamlining. This reduced streamlining may not have much impact on *A. superciliosus*, which appears to feed heavily on the deep scattering layer (Preti et al., 2008), where prey are likely relatively slow-moving at depth in the OMZ (Seibel and Drazen, 2007).

For the more surface-oriented *A. vulpinus* and *A. pelagicus*, which have to catch faster and more active prey, streamlining is likely much more important. Correspondingly, the total filament length in both *A. vulpinus* and *A. pelagicus* is significantly lower than that of *A. superciliosus*, and for *A. vulpinus* this results in a significantly smaller gill surface area. Despite a smaller gill area than *A. superciliosus*, *A. vulpinus* has gills that are larger than those of most elasmobranchs and are comparable to other regionally endothermic sharks (Fig. 5). Although its diffusion distances are also short in comparison to less active elasmobranchs (Hughes and Wright, 1970), *A. vulpinus* has a thicker water–blood barrier (2.55 ± 0.27 vs. 1.15 ± 0.22 μm) and thicker lamellae (14.29 ± 0.94 vs. 11.38 ± 1.61 μm) than the shortfin mako (Wegner et al., 2010b). Bernal and Sepulveda (2005) showed that while *A. vulpinus* is capable of red muscle endothermy, it does not appear able to increase the temperature of the eye and brain region nor the viscera, and that the elevation of red muscle temperature above ambient is less than that of the mako and other lamnids. Patterson et al. (2011) further showed that *A. vulpinus* possesses smaller, less complex vascular retia perfusing the red muscle than lamnids. In particular, the red muscle rete of *A. vulpinus* has a lower arterial–venous contact surface area, thus indicating a lower ability to transfer heat from the venous to arterial blood and ultimately a lower capacity for heat retention. A less well-developed capacity for red-muscle endothermy would result in lower metabolic demands directly via a Q$_{10}$ effect as well as a reduction in the energetic costs associated with the maintenance of tissue specialization needed for high performance (e.g., large heart, high hematocrits and blood volume, increased capillary densities, and high enzyme activities; Dickson, 1995; Bernal et al., 2001). The longer diffusion distances in the gills of *A. vulpinus* in comparison to lamnids...
may thus correspond to lower metabolic requirements associated with a reduced capacity for regional endothermy.

Despite a generally lower capacity for regional endothermy, *A. vulpinus* has a higher scaling exponent for gill surface area in relation to body mass (1.03) than most other fishes, and this may be related to an increased ability for heat retention and aerobic performance with size. Isometric scaling of the gills predicts a geometric regression coefficient of 0.67 for gill area to body mass (area/volume); however, for most fishes, this scaling exponent is closer to 0.80, which is thought to correlate with the mean regression coefficient for fish standard metabolic rate with body mass (=0.81) (Palzenberger and Pohla, 1992; Wegner, 2011). The high scaling exponent of *A. vulpinus* gill area with body mass suggests that its metabolic demands may increase disproportionately with size, possibly associated with an increased capacity for endothermy. As these sharks grow, the body's surface-area-to-volume ratio decreases and the medial red muscle becomes more insulated from ambient water, likely reducing the fraction of

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Fig. 6. Lateral- and dorsal-view renderings of the cranial region of *A. superciliosus* (top) and *A. vulpinus* (bottom) showing the relative size of the branchial chambers. Also shown are comparative images of the gill arches (left) and scanning electron micrograph cross-sections through the lamellae (right) for the two species. Excised gill arches are from a 59.34 kg *A. superciliosus* and 63.93 kg *A. vulpinus*; lamellar cross-sections are from a 71.29 kg *A. superciliosus* and 14.45 kg *A. vulpinus*. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
metabolic heat lost by convection across the surface of the body. Despite the high metabolic costs presumably associated with regional endothermy in A. vulpinus, this species does not have a significantly greater gill surface area than that of A. pelagicus, which lacks the ability to retain body heat and was thus hypothesized to have smaller gills. Because A. pelagicus is a tropical species that inhabits warm waters (Compagno, 2001; Smith et al., 2008), much of its body is likely warmer than that of A. vulpinus which is found in cooler, temperate waters (Hanlan et al., 1993; Compagno, 2001; Smith et al., 2008) and only regionally warms the red muscle. Thus the aerobic requirements (and performance) of A. pelagicus may equal or exceed that of A. vulpinus, resulting in comparable gill areas. The shorter water–blood barrier distance of A. pelagicus in comparison to A. vulpinus may be required to meet similar metabolic demands in warm tropical water which, due to a reduced solubility coefficient at warmer temperatures, has less oxygen.

Alopias Species Relationships and Hypotheses On Gill Evolution

The phylogenetic relationship of the three thresher shark species within the genus Alopias remains unresolved (Naylor et al., 1997; Shimada, 2005; Naylor et al., 2012). The results of this study lead us to hypothesize that the Alopias ancestor was likely an active, ectothermic, pelagic, warm-water shark that had a large gill surface area and short diffusion distances consistent with traits exhibited by A. pelagicus. The subsequent evolution of regional endothermy in A. vulpinus (possibly as a result of its range expansion into colder water) would have used a large gill surface area to maintain high levels of aerobic performance, while selection for a thin water–blood barrier may have become slightly relaxed in more oxygen-rich temperate waters. For A. superciliosus, expansion into deeper water and exploitation of slow-moving prey in the OMZ would have likely relaxed selection for fast swimming and cranial streamlining thus allowing for a further enlargement of the gills for increased hypoxia tolerance, while also maintaining short diffusion distances.

Influence of Aerobic Demands and Environmental Oxygen on Gill Dimensions

Like previous studies on gill morphology, the respiratory surface areas and diffusion distances of the three thresher shark species appear to correlate with both oxygen demand and availability. In this case, exploitation of a hypoxic environment by A. superciliosus correlates with the largest gill surface area of any elasmobranch reported to date. While the more active A. vulpinus and A. pelagicus have relatively large gill areas, their gill dimensions and ultimately metabolic scope appear constrained by the same streamlining that allows for their fast and continuous swimming. Thus, with fewer morphological constraints, hypoxia-dwelling fishes may, in general, be able to achieve greater gill diffusion capacities than more active species that, by definition, require cranial streamlining.

This study also emphasizes the effect of temperature on metabolism and gill morphology. Although A. pelagicus was expected to have lower metabolic demands at a given temperature than A. vulpinus, the warm temperature of its environment (which increases its oxygen demands and decreases environmental oxygen availability) correlates with gill dimensions comparable to those of temperate dwelling, regionally endothermic sharks. Thus, in general, the diffusion capacity of the gills in warm-water fishes is likely to exceed that of cold-water fishes of similar activity level.

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