UNIVERSITY OF CALIFORNIA, SAN DIEGO

# Gill morphometrics of thresher sharks (genus *Alopias*): An investigation of the evolutionary pressures influencing gill morphology

A thesis submitted in partial satisfaction of the requirements for the degree
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Biology

by

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2011

The Thesis of Thomas Paul Wootton is approved and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

2011

This Thesis is for my family.

I owe them everything.

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ABSTRACT OF THE THESIS

Gill morphometrics of thresher sharks (genus *Alopias*): An investigation of the evolutionary pressures influencing gill morphology

by

Thomas Paul Wootton

Master of Science in Biology

University of California, San Diego, 2011

Professor Jeffrey Graham, Chair
Professor Eric Allen, Co-Chair

This study reports gill morphometrics for the three thresher shark species (genus *Alopias*) to determine how metabolism and habitat influence respiratory specializations for increased gill diffusion capacity. Thresher sharks have high gill surface areas, short water-blood barrier distances (1.60-2.55 μm) and thin lamellae (12.50-14.29 μm). Their high gill surface areas are derived from long total filament lengths and large bilateral lamellar areas, a morphometric configuration documented in other active elasmobranches (i.e., Lamnidae), which optimally augments respiratory surface area while limiting

branchial resistance to ventilatory flow. The bigeye thresher, *A. superciliosus*, has the largest gill surface area documented in any elasmobranch species to date, which may be associated with prolonged exposure to hypoxia during diel vertical migrations. The common thresher shark, *A. vulpinus*, a red muscle endotherm, has gill surface areas comparable to *A. pelagicus*, despite expected higher aerobic requirements required for the conservation of metabolic heat. Additionally, *A. vulpinus* has a significantly longer water-blood barrier distance (2.55 ± 0.48 μm) and thicker lamellae (14.29 ± 1.39 μm) than *A. pelagicus* (1.61 ± 0.37; 12.51 ± 1.40 μm) and *A. superciliosus* (1.60 ± 0.31; 12.50 ± 1.14 μm). This likely reflects the cold, well-oxygenated habitat of *A. vulpinus* relative to the other *Alopias* species.

# INTRODUCTION

Fish gill morphology correlates with metabolic demand and habitat (Gray, 1954; Muir and Hughes, 1969; Hughes and Morgan, 1973; De Jager and Dekkers, 1975; Palzenberger and Pohla, 1992; Chapman, 2007; Wegner, 2011), and exhibits extreme diversity associated with the extensive radiation of fishes into a variety of ecological niches. Nonetheless, distantly related taxa display remarkable convergence in respiratory dimensions, likely due to similar evolutionary pressures affecting the gills. In particular, total gill surface areas and water-blood barrier distances (i.e., the thickness of the gill epithelium) have been used to categorize fishes into six morphological ecotypes, based on their respiratory adaptations. These groups include: (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).

The gill adaptations (and thus categorization) of a species are influenced by two main factors--its aerobic demand (associated with activity and metabolic rate), and the dissolved oxygen content of its habitat. Fast oceanic species have high metabolic demands resulting from high levels of activity and/or regional endothermy (i.e., the ability to warm certain regions of their bodies to enhance physiological function) (Brill, 1996; Korsmeyer *et al.*, 1996; Bernal *et al.*, 2001; Dickson and Graham, 2004; Sepulveda *et al.*, 2007). Thus, these fishes generally have gill adaptations, such as large gill surface areas and short water-blood barriers that facilitate oxygen absorption (Muir and Hughes, 1969; Emery and Szczepansky, 1986; Wegner, 2010a,b; Wegner, 2011). Marine fishes of intermediate activity have relatively standard gill morphologies due to their more

"typical" activity levels and their generally normoxic habitats (Hughes, 1970; Hughes, 1984; Wegner 2011). Sluggish marine species, freshwater fishes and air-breathers all have relatively small gill surface areas, although the factors contributing to this differ: For sluggish marine species, small gills are due to low metabolic demands associated with relative inactivity (Hughes and Iwai, 1978; Wegner, 2011); freshwater fishes have low gill surface areas due to high oxygen availability (air-saturated freshwater contains 15-20% more dissolved oxygen than seawater, for a given temperature) (Palzenberger and Pohla, 1992); and air-breathing fishes, have reduced gills resulting from an increased reliance on oxygen absorption from the air (Graham, 1997, 2006). In contrast, hypoxia-dwelling species have large gill surface areas (and likely have short water-blood barriers) associated with their oxygen-deficient habitats (Graham, 2006; Wegner, 2011).

Categorization based on these groups allows for some general conclusions about the factors sculpting gill dimensions. However, because related species tend to experience similar evolutionary stresses affecting gill morphology (i.e., similar aerobic demands and oxygen availabilities), they tend to fall into the same ecomorphotypic groups. This limits the conclusions that can be drawn from species level comparisons. However, the three thresher shark species comprising the genus *Alopias* [the bigeye thresher shark (*Alopias superciliosus*), the common thresher shark (*Alopias vulpinus*) and the pelagic thresher shark (*Alopias pelagicus*)] have remarkable differences in energetic demands and habitats, thus making them ideal candidates for a detailed investigation of the specific influences imposed by oxygen demand and availability on gill morphology.

*A. superciliosus* has a diel diving pattern unique among the *Alopias* species. In particular, the daytime depth preference of this species (300-500m) is much deeper than

that of *A. vulpinus* and *A. pelagicus*. In many areas of its range, this coincides with prolonged exposure to hypoxia in oxygen minimum zones (OMZs) (Weng and Block, 2004; Nakano *et al.* 2003; World Oceans Atlas, 2009). The relatively high activity of this species in this oxygen-deficient environment (it exploits the OMZ to forage on fishes in the deep scattering layer) likely requires respiratory adaptations typical of hypoxia dwelling species (Yang *et al*., 1992; Chapman *et al*., 2002; Chapman, 2007; Wegner, 2011).

*A. vulpinus*, in contrast to *A. superciliosus* and *A. pelagicus*, is an aerobic red muscle (RM) endotherm (Bernal and Sepulveda, 2005). Because of the proportional relationship between ion diffusion rates and temperature, regional endothermy accelerates the rate of physiological processes in corresponding tissues (Block, 1994; Dickson and Graham, 2004). RM endothermy allows for increased muscular performance (i.e., higher contraction frequency and increased muscular output) (Altringham and Block, 1997) and thus, *A. vulpinus* likely has higher aerobic demands than the other two *Alopias* species.

*A. pelagicus* lacks the circulatory, muscular and physiological specializations for RM endothermy (Patterson *et al*., 2011; Sepulveda et al., 2005). Additionally, its consistent presence near the surface throughout the day (Oliver *et al*., 2011) suggests a limited diving capacity (and as a result, limited exposure to hypoxia).

Despite their relatedness, interspecies differences in the three *Alopias* species thus suggest that they belong to three separate morphological ecotypes. *A. superciliosus* is a hypoxia dweller, *A. vulpinus* is a fast-swimming oceanic species, and *A. pelagicus* is a marine fish of intermediate activity. This study examines the gill respiratory dimensions (i.e., gill surface area, water-blood barrier distance and lamellar thickness) in the three

thresher shark species in order to investigate the specific effects of metabolic demand and dissolved oxygen availability on gill morphology.

# METHODS AND MATERIALS

## Gill collection and preparation:

Gills were collected opportunistically from nine *A. vulpinus*, nine *A. superciliosus*, and six *A. pelagicus*. All *A. vulpinus* and most *A. superciliosus* were caught by hook and line off the coast of southern California and Hawaii 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 all *A. pelagicus* and 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 thus shows shark size and collection location data as well as the treatment method of each gill sample. These treatments are as follows:

1. 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.

2. When formalin was not available, gills were frozen and stored until they could be fixed. (All specimens purchased from fisherman were frozen.)

3. In order to verify that formalin fixation or freezing did not substantially alter gill

dimensions, gills of two sharks (4 and 11) were perfused with a microvascular casting solution (Mercox, Ladd Research, Williston, VT) using procedures outlined in Wegner *et al*. (2010a,b). Following euthanasia, these sharks were placed on a V-shaped cradle with their ventral side up while the gills were irrigated with aerated seawater. The heart was exposed by midline incision and canulated. Sharks were perfused with heparinized shark saline for 2-3 minutes, followed by microvascular casting solution. Perfusions were performed at 75-90 mmHg, which is consistent with the aortic systolic pressures determined for active sharks (Lai *et al*. 1997). After full polymerization (<15 min) the gills were excised and bathed in several washes of 15% KOH solution until all gill tissue was digested. The vascular casts were subsequently rinsed and air-dried.

4. Two sharks (no. 19 and 20) were mistakenly fixed in a 25% formalin solution. In order to assess the potential tissue shrinkage associated with the higher formalin concentration, the five gill arches from one side of a third shark (no. 15) were fixed in 25% formalin while the gills of the other side were fixed in the normal 10%. By comparing the two, the percent difference in each gill dimension was calculated and used to correct measurements for sharks 19 and 20.

## 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 of 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 (1984) in order to estimate total gill surface area, $A$, using the equation:

$$A = L_{fil} \bullet 2n_{lam} \bullet A_{lam}$$

Lamellar frequency is doubled in this equation to represent the presence of lamellae on both sides of each gill filament.

To determine the total filament length of a shark, 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 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 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 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 base, middle and tip of each filament to determine lamellar frequency. (In particularly short filaments only one or two photographs could be taken.) With a scalpel, individual lamellae were then removed from each of these sections, mounted on slides and photographed. The most complete isolated lamella was measured to determine $A_{lam}$. Digital images of lamellar frequency and lamellar surface area were analyzed using NIH Image J software.

The three lamellar frequency measurements from each medial filament were averaged, doubled (to account to lamellae on both sides of the filament) and multiplied by the length of all filaments in its bin to calculate the number of lamellae in the 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 measurements from the corresponding representative filament. Total gill surface area was determined by summing the surface area of all bins, then doubling this value to account for the gills on the opposite side of the branchial chamber. Total surface area was then divided by the total number of lamellae in the gills to estimate average bilateral lamellar area ($A_{lam}$), and the total number of lamellae was divided by the total gill filament length ($L_{fil}$) to determine lamellar frequency of all bins ($n_{lam}$).

For the first shark examined from each species (no. 8, 14, and 20), $A_{lam}$ and $n_{lam}$ were measured for all five arches. The resultant data were used to identify the gill arch for each species in which these dimensions were most representative of the entire gills. For subsequent specimens, $A_{lam}$ and $n_{lam}$ 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 thickness:

Ten sharks (four *A. vulpinus*, three *A. superciliosus*, three *A. pelagicus*) were selected, based upon 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 in order 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 were made from acquired digital images using Image J.

**Statistical Analysis:**

Regressions for total gill surface area and its constituent dimensions were determined using least-squares analysis and compared to one another using 10,000 bootstrap replications (R v.2.7.0) of the raw data. Statistical significance between species was determined where less than 5% of the resultant, replicate regressions intersected over a shared weight range of the compared species. Lamellar thickness and water-blood barrier thickness were determined as means ± standard of deviation, and were compared using a one-way ANOVA and post-hoc Tukey test.

*Table 1. Shark fork length (FL), body mass (kg), collection location and preservation method*

| Species (common name) | Specimen # | FL (cm) | Mass (kg) | Collection location | Preservation method* |
|---|---|---|---|---|---|
| *A. pelagicus* | 1 | 70 | 11.82 | Costa Rica | 2 |
| (pelagic thresher) | 2 | 91.5 | 21.16 | Costa Rica | 2 |
| | 3 | 111 | 33.61 | Costa Rica | 4 |
| | 4 | 135 | 51.22 | Costa Rica | 4 |
| | 5 | 163 | 77.77 | Mexico | 2 |
| | 6 | 163 | 78.16 | Costa Rica | 2 |
| *A. superciliosus* | 7 | 153 | 48.83 | Southern California | 2 |
| (bigeye thresher) | 8 | 161.5 | 57.67 | Hawaii | 3 |
| | 9 | 162 | 58.23 | Southern California | 2 |
| | 10 | 163 | 59.34 | Southern California | 2 |
| | 11 | 173 | 71.29 | Southern California | 2 |
| | 12 | 175 | 73.85 | Southern California | 2 |
| | 13 | 192 | 98.26 | Southern California | 1 |
| | 14 | 198 | 108.37 | Southern California | 2 & 4 |
| | 15 | 209 | 127.27 | Southern California | 2 |
| *A. vulpinus* | 16 | 69 | 7.91 | Southern California | 1 |
| (common thresher) | 17 | 76 | 10.28 | Southern California | 2 |
| | 18 | 82 | 12.45 | Southern California | 2 |
| | 19 | 87 | 14.45 | Southern California | 1 |
| | 20 | 104 | 22.65 | Southern California | 3 |
| | 21 | 116 | 29.83 | Southern California | 1 |
| | 22 | 146 | 53.24 | Southern California | 1 |
| | 23 | 157 | 63.93 | Southern California | 1 |
| | 24 | 181 | 91.47 | Southern California | 1 |

* *1.) Fixed in 10% formalin solution immediately following euthanasia.*
*2.) Frozen, then fixed in 10% formalin solution.*
*3.) Perfused with microvascular casting solution.*
*4.) Fixed in 25% formalin solution.*

# RESULTS

## Gill Surface Area

Figure 1 shows gill surface area to body mass regressions determined for the three *Alopias* species in this study and data for *A. vulpinus* from Emery and Szczepansky (1986). Data determined in this study shows that *A. superciliosus* has a significantly larger gill surface area than *A. vulpinus* for most of the shared weight range (48.83-81.20 kg). Although the *A. superciliosus* regression lies above that of *A. pelagicus* over their entire shared weight range, the difference in gill surface area values between these species is not significant. Similarly, the gill surface areas of *A. pelagicus* and *A. vulpinus* do not differ significantly.

The gill surface area scaling exponents for the three species range from 0.78 to 1.03, and fall within the range of those of other fishes (Hughes, 1972a,b; Palzenberger and Pohla, 1992; Wegner, 2011). The 95% confidence intervals of the scaling exponent of *A. vulpinus* (0.833-1.23) fall above the expected scaling exponent of gill surface area to body mass assuming isometric growth of the gills [0.67: the sum of its constituent dimensions, total gill filament length (0.33), lamellar frequency (-0.33) and lamellar area (0.67)]. Although the *A. vulpinus* regression of the present study has a much larger scaling exponent (1.03) than that of Emery and Szczepansky (1986) (0.41), no significant difference was evident in their gill surface areas over the shared body mass range. The low scaling exponent of *A. vulpinus* in Emery and Szczepansky (1986) lies outside of the range documented for most fishes (Palzenberger and Pohla, 1992) and likely results from the limited weight range sampled. The relatively lower confidence interval of the *A.*

*superciliosus* regression ($R^2$ = 0.7373) in the present study may also be due to a limited weight range, resulting from a lack of juveniles (< 48.83 kg) available.

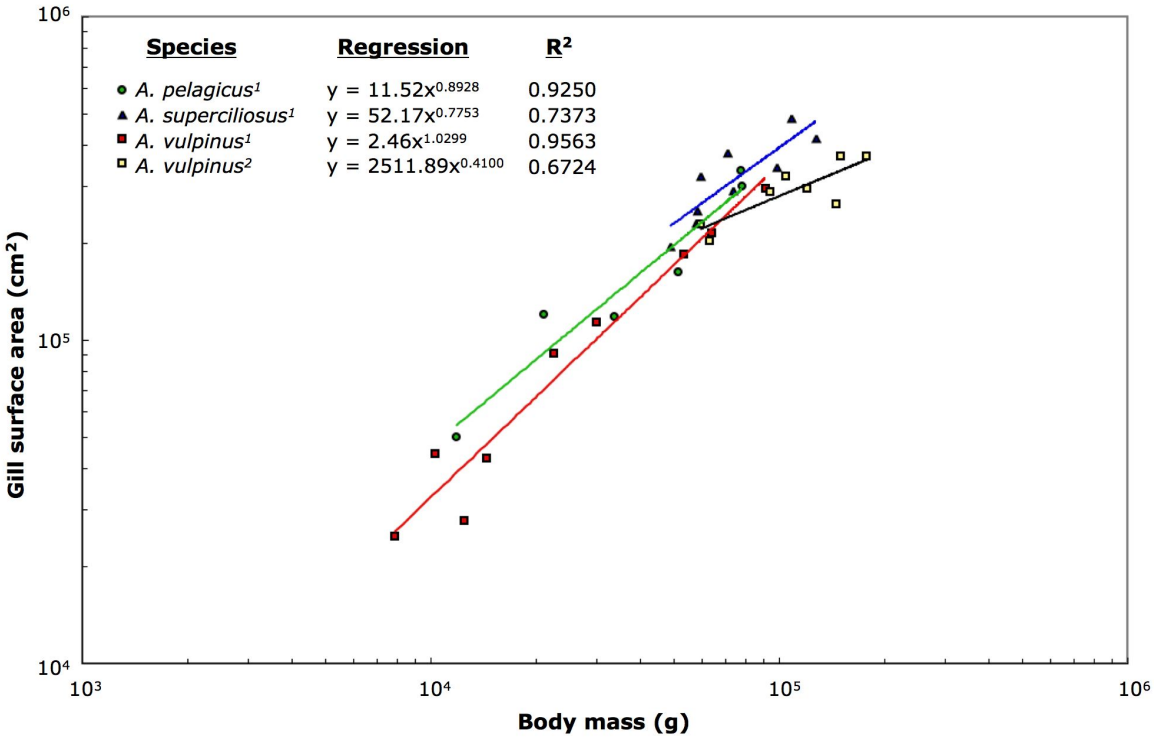


*Fig. 1. Linear regressions of total gill surface area ($cm^2$) to body mass (g) for the three* Alopias *species examined in this study*[1] *and for* A. vulpinus *from Emery and Szczepansky (1986)*[2].

## Total Filament Length

Figure 2A shows log-log plots for total gill filament length to body mass for the three species examined in this study. Total filament length in *A. superciliosus* is significantly greater than the other *Alopias* species and contributes to the larger gill surface area in *A. superciliosus* than *A. vulpinus*. High total filament length in *A. superciliosus* results from significantly longer filaments (Fig. 2B) and significantly more

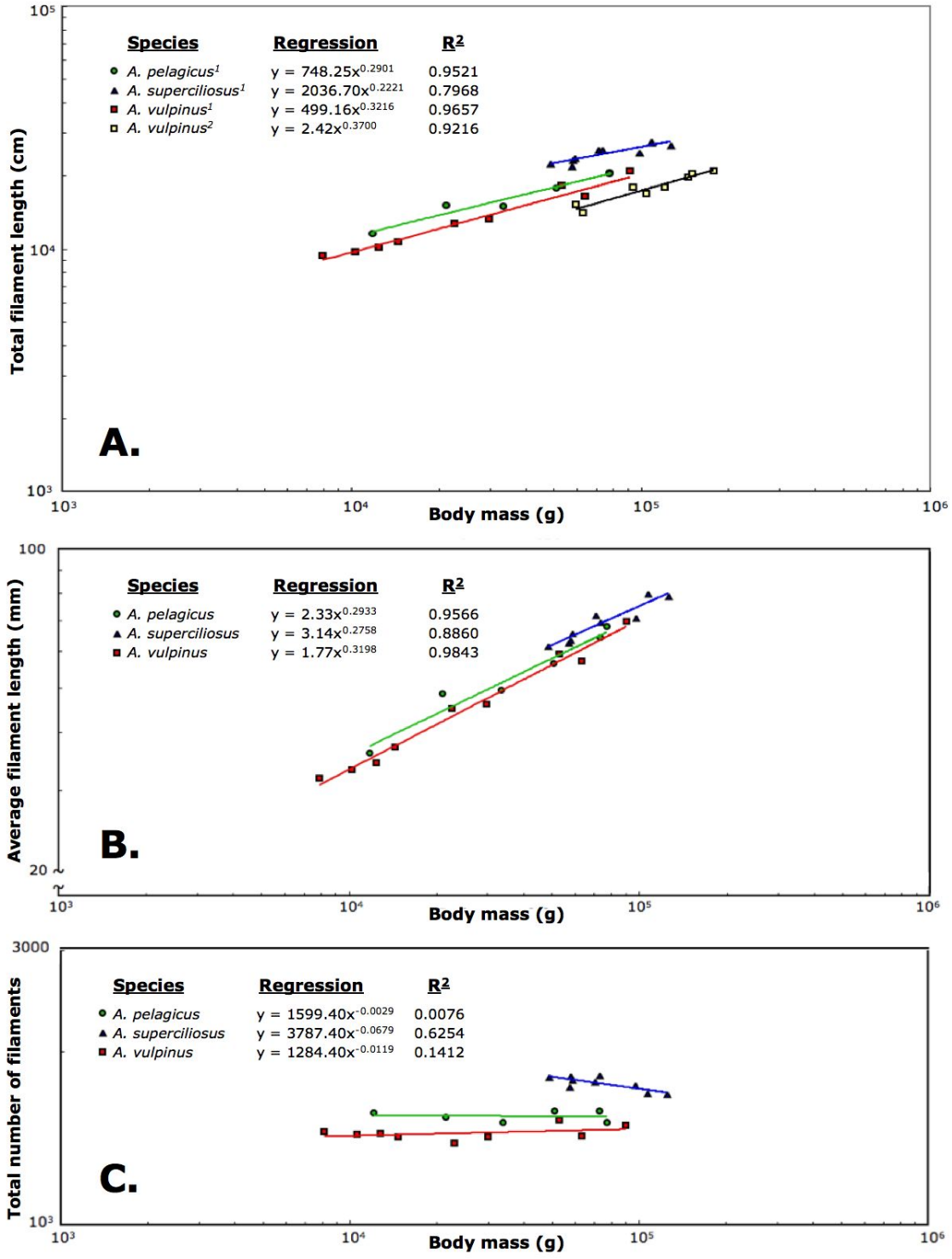


*Fig. 2. Linear regressions for (A) total total filament length (cm), (B) mean filament length (mm), and (C) total number of filaments in relation to body mass (g) for the* Alopias *species examined in this study[1] and for* A. vulpinus *from Emery and Szczepansky (1986)[2].*

filaments (Fig. 2C) than *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 than *A. vulpinus*, although the mean filament length between these species does not differ significantly. Total filament length of *A. vulpinus* from Emery and Szczepansky was significantly shorter than all regressions determined in the present study.

## Lamellar area and lamellar frequency

Regressions of lamellar frequency to body mass are plotted in Figure 3. Both *A. superciliosus* and *A. pelagicus* have higher lamellar frequencies than *A. vulpinus*. For *A. pelagicus*, this trend exists for all comparable body masses, and for *A. superciliosus,* it extends over the majority of the shared weight range (48.83-83.15 kg). There are no significant differences in lamellar frequency between *A. superciliosus* and *A. pelagicus*. Lamellar frequency of *A. vulpinus* from Emery and Szczepansky is significantly higher than that of *A. vulpinus* in this study for the entire overlapping range of body masses and than that of *A. pelagicus* and *A. superciliosus* for most of the comparable weight ranges (59.42-77.18 kg and 59.42-94.32 kg, respectively). Regressions of mean lamellar bilateral surface area are plotted in Figure 4. These did not differ significantly between species.

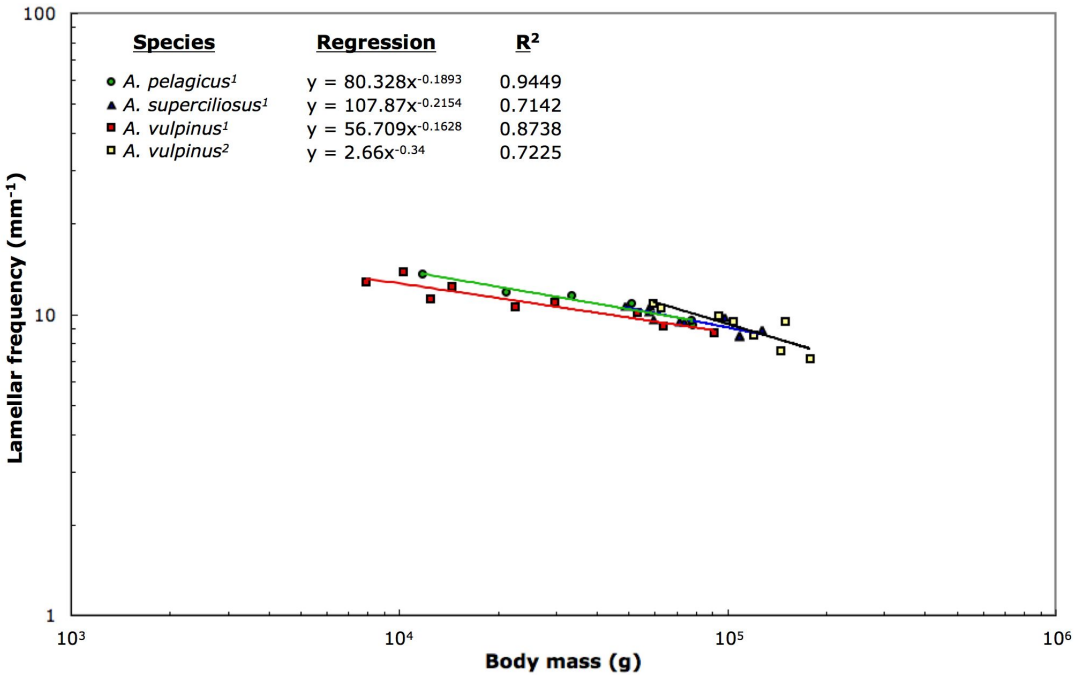


*Fig. 3. Linear regressions of lamellar frequency ($mm^{-1}$) to body mass (g) for the three* Alopias *species examined in this study[1] and for* A. vulpinus *from Emery and Szczepansky (1986)[2].*

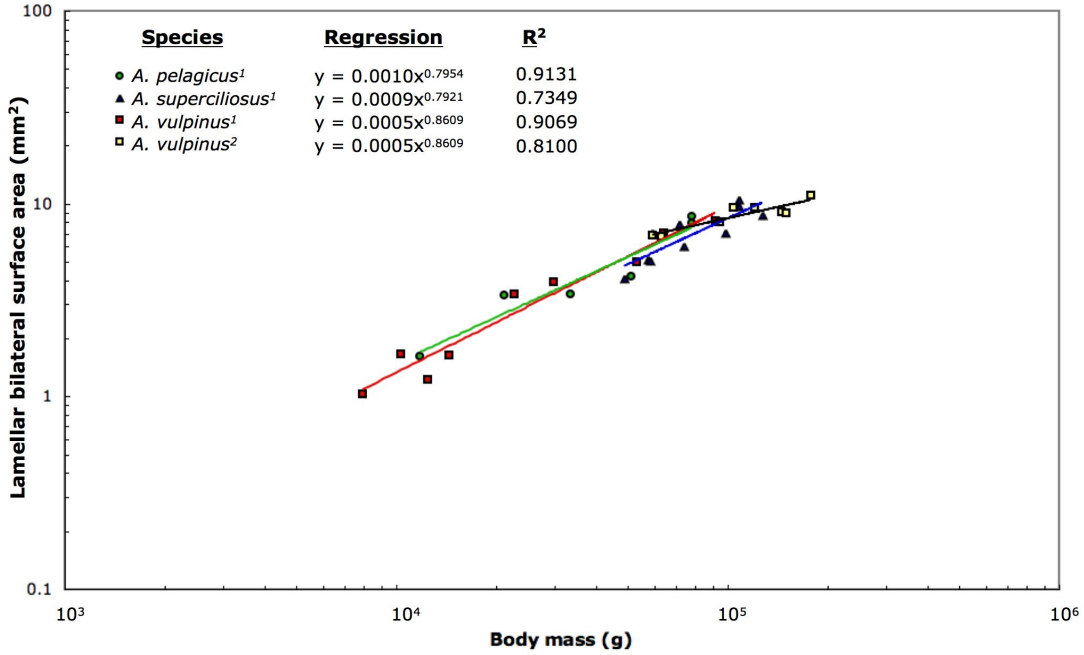


*Fig. 4. Linear regressions of lamellar bilateral surface area ($mm^2$) to body mass (g) for the three* Alopias *species examined in this study[1] and for* A. vulpinus *from Emery and Szczepansky (1986)[2].*

## Lamellar Dimensions

Table 2 shows the lamellar thickness and the water-blood barrier distance measured for four *A. vulpinus*, three *A. superciliosus*, and three *A. pelagicus*, as well as the mean for each species. *A. vulpinus* has significantly thicker lamellae (14.29 ± 1.39) than *A. superciliosus* (12.50 ± 1.14) and *A. pelagicus* (12.51 ± 1.40) ($P < 0.0001$) which is associated with a significantly thicker water-blood barrier (2.55 ± 0.48) than the other two species (*A. superciliosus*, 1.60 ± 0.31; *A. pelagicus*, 1.61 ± 0.37) ($P < 0.0001$). There are no significant differences in these lamellar dimensions between *A. superciliosus* and *A. pelagicus*.

*Table 2. Lamellar dimensions for the three* Alopias *species examined (means ± standard deviation)*

| Species | Fork Length (cm) | Mass (kg) | Lamellar thickness (μm) | Water-blood barrier thickness (μm) |
|---|---|---|---|---|
| *A. pelagicus* | 70 | 11.82 | 12.22 ± 1.47 | 1.53 ± 0.36 |
| *A. pelagicus* | 91.5 | 21.16 | 13.12 ± 1.44 | 1.47 ± 0.23 |
| *A. pelagicus* | 163 | 77.77 | 12.18 ± 1.28 | 1.83 ± 0.52 |
| $\bar{x}$ | | | **12.51 ± 1.40** | **1.61 ± 0.37** |
| *A. superciliosus* | 163 | 59.34 | 11.49 ± 0.97 | 1.54 ± 0.27 |
| *A. superciliosus* | 173 | 71.29 | 15.44 ± 1.29 | 1.69 ± 0.39 |
| *A. superciliosus* | 192 | 98.26 | 10.56 ± 1.14 | 1.57 ± 0.27 |
| $\bar{x}$ | | | **12.50 ± 1.14** | **1.60 ± 0.31** |
| *A. vulpinus* | 87 | 14.45 | 13.56 ± 1.61 | 2.25 ± 0.37 |
| *A. vulpinus* | 116 | 29.83 | 15.67 ± 1.22 | 2.86 ± 0.51 |
| *A. vulpinus* | 146 | 53.24 | 13.87 ± 1.27 | 2.67 ± 0.52 |
| *A. vulpinus* | 181 | 91.47 | 14.06 ± 1.48 | 2.41 ± 0.56 |
| $\bar{x}$ | | | **14.29 ± 1.39** | **2.55 ± 0.48** |

## DISCUSSION

This study documents respiratory specializations for increased gas exchange in the three *Alopias* species, which include large gill surface areas, short water-blood barrier distances, and thin lamellae. *Alopias* gill surface areas are among the highest measured for any elasmobranch, and rival those of the regionally endothermic shark family, Lamnidae (Emery and Sczcepansky, 1986; Wegner, 2010b). The water-blood barrier distances in *Alopias* ranges from 1.60 to 2.55 μm, which is also comparable to other active elasmobranchs (e.g., shortfin mako, *Isurus oxyrinchus* and blue shark, *Prionace glauca*; range = 1.15-1.65 μm) (Wegner, 2010b) and is less than that of less active species (*Scyliorhinus*, *Squalus*, *Galeorhinus*, and *Raia*; range = 4.85-11.27 μm) (Hughes and Wright, 1970). *Alopias* lamellae are also thin (12.50-14.29 μm) resulting in narrow vascular channels that force red blood cells close to the water-blood barrier. The combination of large gill areas and short diffusion distances increases the gill diffusion capacity of *Alopias* above that of most other elasmobranchs.

Because gill dimensions correlate with activity, metabolic demand and the dissolved oxygen content of the environment (Gray, 1954; Hughes, 1966, 1970; Hughes and Morgan, 1973; Palzenberger and Pohla, 1992; Wegner, 2011), variations in gill morphology between elasmobranchs likely reflect fundamental differences in their ecological niche, physiology and habitat. Figure 5 shows that lamniform (Alopiidae, Lamnidae) gill surface areas are larger than those determined for other elasmobranch orders, such as the carcharhiniforms and torpediniforms. Large gill areas are likely

required to support greater oxygen demands associated with their higher levels of activity. The marbled electric ray (*Torpedo marmorata*) and nursehound (*Scyliorhinus stellaris*) are relatively sedentary, benthic species (Hughes, 1978; Hughes and Johnston, 1978; Sims *et al.*, 2005) and have correspondingly low gill surface areas.

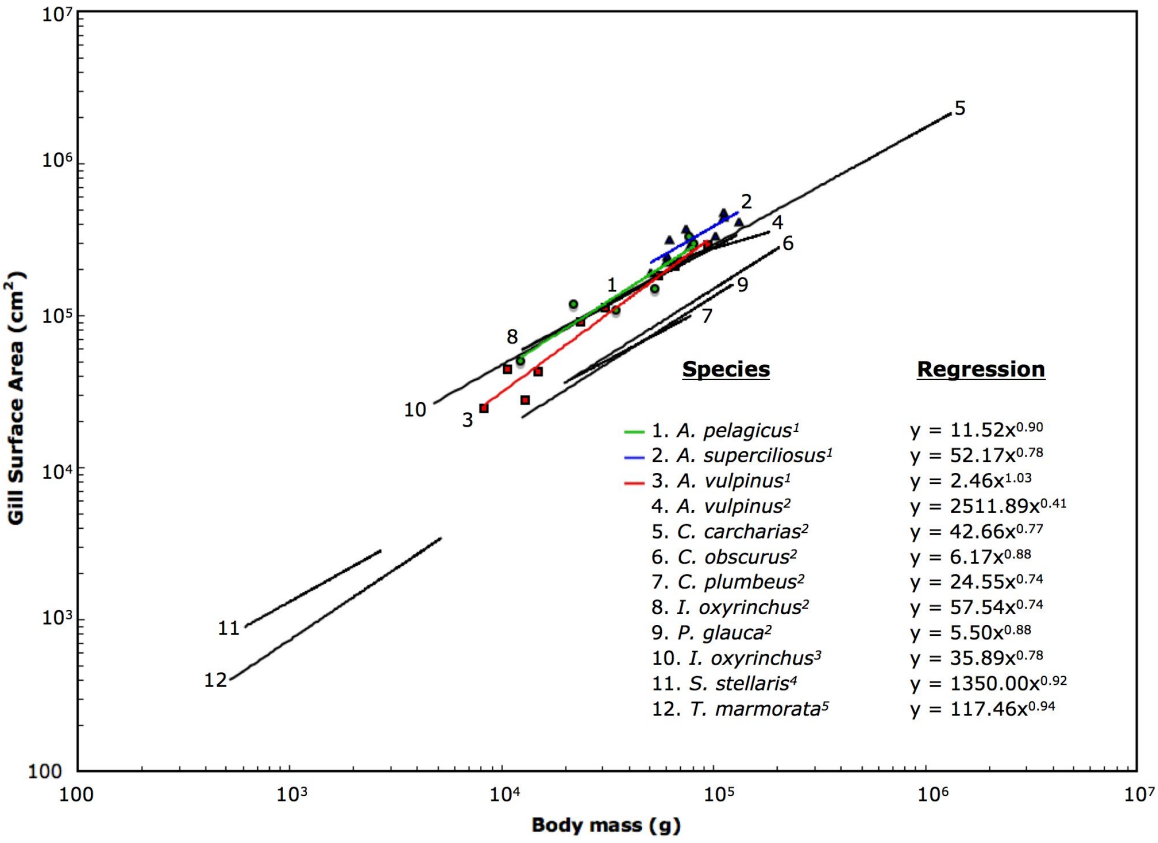


*Fig. 5. Linear regressions of total gill surface area ($cm^2$) to body mass (g) for the three* Alopias *species examined in this study alongside regressions for other elasmobranchs. Sources: [1]Present study, [2]Emery and Szcerpansky (1986), [3]Wegner et al. (2010), [4]Hughes (1978) and [5]Hughes et al. (1986).*

Large gill surface areas in *Alopias* are convergent with those of lamnids and result from a consistent set of morphometric trends (i.e., high total filament lengths and large bilateral lamellar surface areas). These trends follow the model proposed by Hughes

(1966) for increasing gill surface area without increasing gill resistance to ventilatory flow. Although highly active teleosts also recruit high lamellar frequencies to increase gill area, lamellar frequencies in *Alopias* and lamnids are not greater than those of less-active species. This likely reflects the presence of interbranchial septa in the elasmobranch gills, which inherently increase gill resistance as water is forced through septal canals (Grigg and Read, 1971; Wegner *et al*., *in press*). Because having a higher lamellar frequency would further increase gill resistance, the presence of interbranchial septa likely forces alopiids and lamnids to augment gill surface area differently than high-performance teleosts.

### *Alopias superciliosus*

*A. superciliosus* has the largest gill surface area and highest total filament length documented for any elasmobranch studied to date. This is likely associated with its ability to tolerate prolonged exposure to hypoxia. In many parts of the range, including the collection sites in this study, the daytime depth preferences of *A. superciliosus* (300-500 m) (Nakano *et al*., 2003; Weng and Block, 2004) coincide with a midwater oxygen minimum zone (OMZ) (Fig. 6). *A. superciliosus* is thus similar to other hypoxia-dwelling organisms in having a large respiratory surface area to increase gas exchange (Yang *et al*., 1992; Chapman *et al*., 2002; Levin, 2003; Wegner, 2011). In addition, the short water-blood barrier distance ($1.60 \pm 0.31$) and thin lamellae ($12.50 \pm 1.14$) of *A. superciliosus* would also facilitate $O_2$ absorption within the OMZ and are likely required for this species to meet relatively high metabolic demands while foraging at depth.

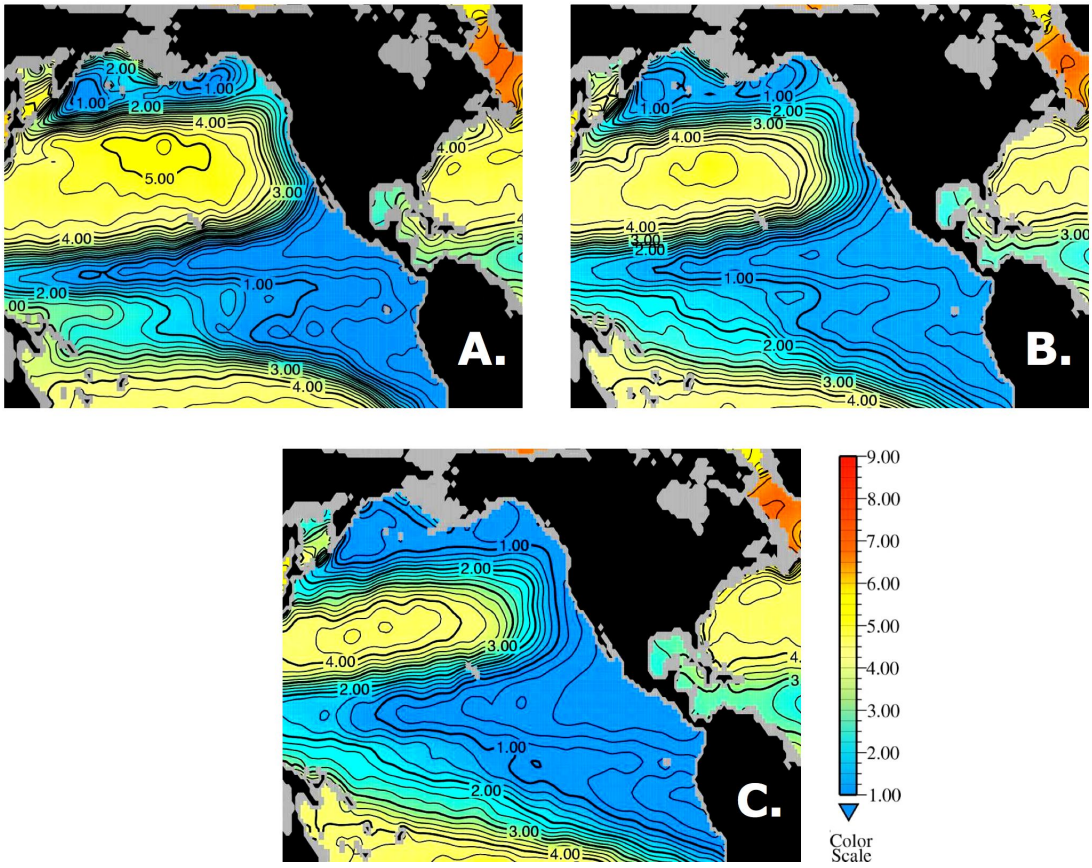


*Fig. 6. Annual dissolved oxygen levels at 300m (A.), 400m (B.) and 500m (C.), illustrating the oxygen minimum zone along the eastern boundary of the Pacific Ocean. Values are given in (milliliters $O^2$ / liter seawater). Source: World Ocean Atlas (2009).*

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, 2010, 2011). This results from a 28% higher total filament length in *A. superciliosus*, and corresponds to larger branchial chambers in this species. Figure 7 compares the branchial chambers of *A. vulpinus* and *A. superciliosus.* As the gills of *A. supreciliosus* extend dorsally they meet the epaxial musculature and result in a distinct "helmeted contour" which lines the dorsal surface of the head (Compagno, 2001). These nuchal grooves are commonly used to identify *A.*

*Fig. 7. Five gill arches removed from the left side of the branchial chamber of a 63.93 kg* A. vulpinus *(top) with those of a 59.34 kg* A. superciliosus *(bottom).*

*superciliosus* and may be exaggerated by the immense size of the branchial chambers in this species relative to *superciliosus* and may be exaggerated by the immense size of the branchial chambers in this species relative to other sharks. Augmentation of gill surface area through extension of the total filament length is usually limited by the volume available for expansion of these branchial chambers (Hughes, 1966, 1972b). The unique cranial morphology of *A. superciliosus* may provide this species with a way to circumvent these limitations.

The relationship between the gill surface area and habitat use in *A. superciliosus* and *A. vulpinus*, is similar to that in two billfish species, the swordfish, *Xiphias gladius*

and striped marlin, *Kajikia audax*. Like *A. superciliosus*, *X. gladius* has a deep (often below 400 m) diel vertical migration (DVM) pattern, whereas *K. audax* is generally located closer to the surface (Carey and Robison, 1981; Carey, 1990; Block *et al.*, 1992; Brill *et al.*, 1993; Dewar *et al.*, 2011). Correspondingly, *X. gladius* has a larger gill surface area than *K. audax* (Wegner *et al.*, 2010a).

### *Alopias vulpinus*

*A. vulpinus* is capable of aerobic red muscle (RM) endothermy (Bernal and Sepulveda, 2005) and has a correspondingly large gill surface area comparable to two other regional endotherms, the white shark, *Carcharadon carcharias* and *I. oxyrinchus* (Emery and Szczepansky, 1986). However, *A. vulpinus* gills are not larger than those of *A. pelagicus*, and are smaller than those of *A. superciliosus*. Additionally, although its diffusion distances are short, in comparison to most elasmobranchs (Hughes and Wright, 1970; Wegner, 2011), *A. vulpinus* has the longest water-blood barrier distance (2.55 ± 0.48 μm) and thickest lamellae (14.29 ± 1.39 μm) of the three *Alopias* species. *A. vulpinus* is generally found in temperate climates (Hanan *et al.*, 1993; Compagno, 2001), which are characterized by higher dissolved oxygen concentrations than the tropics (due to the higher solubility of $O_2$ at lower temperatures). The slightly thicker diffusion distances in this species may thus reflect the well-oxygenated surface waters it inhabits.

The scaling exponent for gill surface area in *A. vulpinus* is 1.03. This is significantly higher than the scaling exponent expected for isometric gill growth with body mass, which would be 0.67--the sum of the scaling exponents of its constituent dimensions [$L_{fil}$ (0.33), $A_{lam}$ (0.67), and $L_{freq}$ (-0.33)]. Most fishes, including *A. superciliosus* and *A.*

*pelagicus*, have scaling exponents (mean of all fishes 0.80) that appear to correlate with those of standard metabolic rate (mean of all fishes 0.81) (Wegner *et al*., 2011). A significantly higher scaling exponent in *A. vulpinus* could imply a variety of other physiological factors influencing gill growth.

One possible explanation is a disproportionate increase in metabolic rate with respect to body mass, due to increased endothermic capacity with age. RM temperature measurements made with a large size range of *A. vulpinus* support this hypothesis (Bernal and Sepulveda, unpublished). Juvenile sharks, because of their small size, have a higher surface area to volume ratio, which likely results in a higher proportion of heat lost to ambient seawater through convection. While juvenile lamnids appear to possess the endothermic efficiency to overcome this heat loss (Sepulveda *et al*., 2004, 2007; Wegner *et al*., 2010b), *A. vulpinus* may not. Although *A. vulpinus* possesses the necessary physiological adaptations for RM endothermy (i.e., lateral circulation, muscular *retia*, and medial/anterior RM position in the body), Patterson *et al*., 2011 showed that it had smaller, less complex muscular *retia* than regionally endothermic lamnids. In particular, the *retia* of *A. vulpinus* has lower arterial-venous contact surface area, implying less heat transfer from venous to arterial blood, and a lower capacity for heat retention. As these sharks grow, the volume to surface area ratio increases, distancing the medial RM from ambient water and reducing the fraction of metabolic heat lost by convection across the surface of the body. Increased ability to heat RM may correspond with the habitat shift seen in this species from shallow coastal waters to offshore environments in which increases in dive frequency and depth occur with age (Cartamil *et al.*, 2010, 2011).

### *Alopias pelagicus*

*A. pelagicus* has the most limited distribution of the three *Alopias* species and is generally confined to tropical latitudes (Compagno, 2001). The high temperatures and low dissolved oxygen concentrations associated with this habitat (Fig. 8) may have influenced the intermediate gill surface area (relative to *A. superciliosus* and *A. vulpinus*) and short water-blood barrier distances in this species.

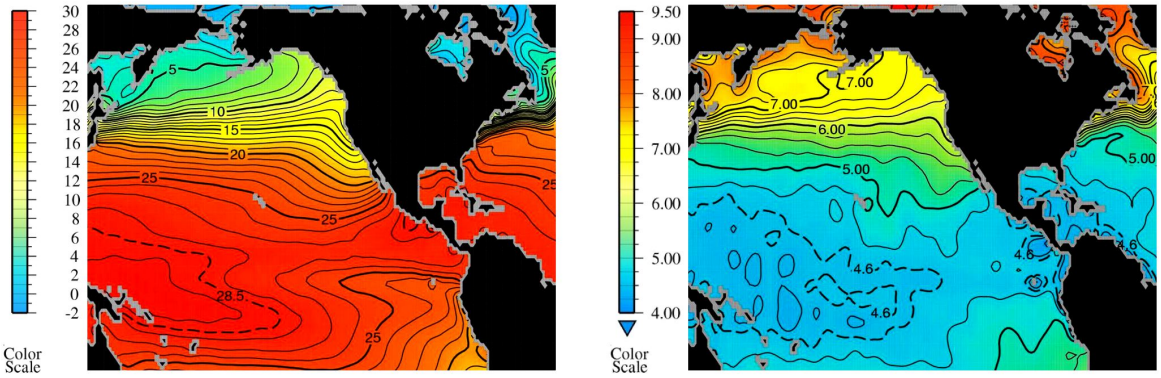


*Fig. 8. Temperature (left) and dissolved oxygen content (right) in the epipelagic (10m depth) Pacific Ocean. Values are given in °C and ml/l $O_2$, respectively.*

Like *A. superciliosus*, *A. pelagicus* lacks the physiological specializations to support RM endothermy, and likely experiences body temperatures comparable to ambient seawater. However, *A. pelagicus* generally inhabits warm water (often above 25ºC), which may result in internal temperatures comparable to *A. vulpinus*, regardless of endothermic capacity. By extension, *A. pelagicus* likely has similar aerobic requirements to *A. vulpinus*. Due to the lower oxygen content in the tropics however (Fig. 9), *A. pelagicus* may require thinner lamellae and a shorter water-blood barrier distance to meet similar metabolic demands.

*A. pelagicus* is the least studied species in its genus and little is known about their

life history and movement patterns. Investigation of these characteristics could further our understanding of the evolutionary pressures affecting gill morphology.

## Emery and Szczepansky (1986)

The gill dimensions measured for *A. vulpinus* in this study differ somewhat from those determined by Emery and Szczepansky for the same species. Although no significant differences were observed in gill surface area, *A. vulpinus* examined in this study have a different morphometric configuration than those examined in Emery and Szczepansky (1986). Specifically, *A. vulpinus* in this study have significantly higher total filament lengths, and lower lamellar frequencies. This emphasizes the potential for plasticity in respiratory morphometric characteristics between subpopulations experiencing different environmental conditions (Chapman *et al*., 2002; Chapman, 2007). Additionally, although Emery and Szczepansky (1986) focus on the effect of regional endothermy on gill dimensions, this study emphasizes that oxygen availability is also a primary contributor to respiratory morphology.

## Effects of oxygen availability and metabolic demands on gill morphometrics

This study documents the specific effects of environmental conditions and of aerobic requirements on respiratory morphology through investigation of three species belonging to the same genus--*Alopias*. The high gill surface areas, short diffusion distances and thin lamellae documented in these species are convergent with those of lamnid sharks and suggest that highly active elasmobranchs recruit a distinct set of respiratory adaptations that allow for high gill diffusion capacities. Additionally, this study suggests that large gill surface areas in these species are achieved through a

consistent morphometric configuration (i.e., high total filament lengths and large lamellae).

This Thesis, in full, is currently being prepared for submission for publication as Wootton, T. P., Sepulveda, C. A., Wegner, N. C., and Graham, J. B. Gill morphometrics of thresher sharks (genus *Alopias*): An investigation of the evolutionary pressures influencing gill morphology. The Thesis author is the primary investigator and author of this paper.

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