

Anatomy and Histochemistry of Flight Muscles in a Wing-Propelled Diving Bird, the Atlantic Puffin, *Fratercula arctica*

Christopher E. Kovacs¹ and Ron A. Meyers^{2*}

¹Department of Ecology and Evolutionary Biology, Brown University, Providence, Rhode Island

²Department of Zoology, Weber State University, Ogden, Utah

ABSTRACT Twenty-three species within the avian family Alcidae are capable of wing-propelled flight in the air and underwater. Alcids have been viewed as Northern Hemisphere parallels to penguins, and have often been studied to see if their underwater flight comes at a cost, compromising their aerial flying ability. We examined the anatomy and histochemistry of select wing muscles (Mm. pectoralis, supracoracoideus, latissimus dorsi caudalis, coracobrachialis caudalis, triceps scapularis, and scapulo-humeralis caudalis) from Atlantic puffins (*Fratercula arctica*) to assess if the muscle fiber types reveal the existence of a compromise associated with “dual-medium” flight. Pectoralis was found to be proportional in size with that of

nondiving species, although the supracoracoideus was proportionally larger in puffins. Muscle fiber types were largely aerobic in both muscles, with two distinct fast-twitch types demonstrable: a smaller, aerobic, moderately glycolytic population (FOg), and a larger, moderately aerobic, glycolytic population (FoG). The presence of these two fiber types in the primary flight muscles of puffins suggests that aerial and underwater flight necessitate a largely aerobic fiber complement. We suggest that alcids do not represent an adaptive compromise, but a stable adaptation for wing-propelled locomotion both in the air and underwater. *J. Morphol.* 244:109–125, 2000.

© 2000 Wiley-Liss, Inc.

Of the 9,700-plus species of birds, 32 are capable of flight under water in addition to “typical” (aerial) flight. These birds fall into three unrelated groups: alcids, diving petrels, and dippers. Alcids (which include puffins, murres, and auklets) inhabit the Northern Hemisphere and have often been compared to penguins, their Southern Hemisphere counterparts. Clearly, these two groups are “counterparts” in the loosest sense, as alcids are capable of both aerial and aquatic flight, whereas penguins are restricted to “flight” in the aquatic medium only.

Storer (1960) proposed that the capacity for flight in air and water in alcids and diving petrels must represent a compromise stage between birds well suited for flight in air and those suited only to aquatic propulsion such as penguins. He suggested that compromise adaptations are reflected in the maximum and minimum body sizes attainable in wing-propelled divers. Larger birds require proportionately larger wings that are less effective underwater; he concluded that this restricts the maximum body size possible for alcids. The concept of alcids as compromise species between nondiving birds and penguins has led many researchers to probe the differences between alcids, penguins, and nondiving birds in an effort to identify adaptations specific to dual-medium fliers. Stettenheim (1959) compared the skeletal and muscular anatomy of a common murre with two nondiving species in the genera *Larus* and *Limosa*, and provided many anatomical

examples which he believed important for wing-propelled diving. Raikow et al. (1988) examined forelimb joint mobility in alcids and noted that in contrast to penguins, alcids possess intrinsic wing muscles and do not show wing rigidity. Raikow and colleagues suggest that the presence of intrinsic wing muscles in alcids constrains the evolution of a flipper-like wing because subtle wing movements are required for aerial flight. These two important studies, along with more recent flight and physiological research, provide a foundation for our discussion of the adaptations in alcids.

There have been a few accounts of the wing and shoulder anatomy of alcids and their involvement in underwater locomotion (Stettenheim, 1959; Storer, 1960; Spring, 1971), but to date, there has been no comprehensive study of the histochemical characteristics of muscles in alcids. For avian species limited to aerial flight, the pectoralis and supracoracoideus muscles are specialized to accommodate a variety of flight styles, from high frequency flapping to pro-

Contract grant sponsors: Department of Ecology and Evolutionary Biology at Brown University, the Company of Biologists Ltd., the National Science Foundation; Contract grant number: IBN 9220097.

*Correspondence to: Ron A. Meyers, Department of Zoology, Weber State University, Ogden, UT 84408-2505.
E-mail: RMEYERS@WEBER.EDU

longed gliding and soaring (e.g., Rosser and George, 1986a,b; Rosser et al., 1994). In penguins, these muscles are highly aerobic and contribute entirely to thrust generation and not body support (Bannasch, 1994). We predict that the flight muscles of puffins will contain attributes found in both aerial fliers and penguins and that their histochemical composition will reflect this.

In this report, we have two major objectives. First, we describe the anatomical features of the wing and shoulder apparatus in the Atlantic puffin (*Fratercula arctica*) we believe to be important for wing propulsion underwater. The anatomical arrangement of the pectoral girdle musculature in puffins will be compared with that of other alcid and diving and nondiving birds. Second, we will document the histochemical profiles of the primary wing muscles. The correlation between muscle fiber histochemistry and muscle function has been described in mammals (Burke et al., 1971; Armstrong et al., 1982; Hermanson and Hurley, 1990; Hermanson et al., 1993) and birds (Welsford et al., 1991; Meyers, 1992a; Sokoloff et al., 1998), and we will use this foundation for determining any specializations associated with the Atlantic puffin's ability for aerial and underwater flight. We hope to determine if alcids represent an intermediate in the evolution of wing-propelled divers such as penguins, or if they are an adaptive compromise for dual-medium flying. Additionally, we will analyze 1) interindividual differences between individual puffins, and 2) the variation in mean muscle fiber diameter between multiple birds in six different muscles.

MATERIALS AND METHODS

Twenty Atlantic puffins (*Fratercula arctica*) were purchased from local hunters in Heimaey, Iceland. Muscles from eight birds were collected for histochemical analysis and the remaining birds were dissected both fresh and preserved for anatomical studies. All muscles for histochemistry were excised within 10 h of death. Samples were collected and all material exported with permission of the Icelandic Ministry of the Environment. No birds were killed for the sole purpose of this project. Muscle samples were collected during July 1995 and 1996 at the University of Iceland's Fisheries Research Unit in Heimaey, Iceland.

Anatomical Study

Dissections were made of five specimens, fixed in formalin and stored in 2% phenoxyethanol. An iodine solution (Bock and Shear, 1972) was used to help contrast muscle from connective tissue. Anatomical nomenclature is from *Nomina Anatomica Avium* (Baumel et al., 1993). Names of processes and fossae to which muscles attach are given de-

scriptively and are then referred to by their NAA name.

Figures were prepared by tracing the dissections with a holbein camera lucida. The outline was then enlarged to cover a surface of approximately 20 × 30 cm. This drawing was compared to the original dissection specimen, then corrected and details added. Final drawings were traced in ink and labeled.

Muscle Histochemistry

The following muscles were removed and weighed: pectoralis pars thoracicus, supracoracoideus, latissimus dorsi caudalis, triceps scapularis, coracobrachialis caudalis, and scapulohumeralis caudalis. Samples (<1 cm³) from each muscle were mounted on wooden tongue depressors using Tissue-Tek embedding medium (Sakura Finetek, Torrance, CA) and rapidly frozen in isopentane cooled to about -150°C in liquid nitrogen. Samples were stored in liquid nitrogen until being transferred to dry-ice for transport back to Brown University, where they were then stored at -70°C. Transverse serial sections (12 μm) were cut in a cryostat (Leica, Jung Frigocut 2800N) at -20°C. Sections were transferred to glass cover slips and air-dried for 30 to 120 min. For identification of fiber types, serial sections were stained for the presence of myofibrillar adenosine triphosphatase (mATPase) following either alkaline (pH 10.4) or acidic (pH 4.3–4.6 in 0.1 increments) preincubation (Padykula and Herman, 1955; Guth and Samaha, 1969, 1970; Green et al., 1982). Samples were incubated in an ATP buffer solution (pH 9.4) for 30 min at 37°C and rinsed in calcium chloride (1%), cobalt chloride (2%), and sodium barbital (0.01 M). Immersion in ammonium sulfide (1%) formed a precipitate identifying the stable mATPase. Sections were dehydrated in successive grades of ethanol and mounted on microscope slides using xylene based Permount (Fisher Scientific, Fair Lawn, NJ).

Two additional sections from each block were stained for a glycolytic enzyme (α -glycerophosphate dehydrogenase, α -GPD) and an oxidative enzyme (nicotinamide adenine dinucleotide diaphorase, NADH-D) following the protocols of Novikoff et al. (1961) and Meijer (1968), respectively. Sections were dehydrated and mounted onto microscope slides as above.

Slides were viewed under a Nikon microscope and digital (8 bit gray scale) images of each section were acquired at 200X magnification using a CCD camera, NIH Image software (W. Rasband, National Institutes of Health) and a Macintosh Centris 650 computer. The area of each fiber was measured using the alkaline preincubation section as the fiber boundaries following this reaction were most clearly defined.

Nomenclature

George and Naik (1959) identified muscle fiber types in mammals as either red, white, or intermediate. These fiber type descriptors were expanded to include physiological parameters; red became fast-twitch red, white became fast-twitch white, and intermediate fibers became slow-twitch intermediate. Peter et al. (1972) argued that fibers of the same color are composed of different quantities of metabolic enzymes and renamed fibers based on three criteria: 1) contraction time relative to other fibers in the muscle, 2) glycolytic capacity, and 3) oxidative capacity. Thus, fast-twitch white fibers were termed fast-twitch glycolytic fibers (FG); fast-twitch red fibers became fast-twitch oxidative-glycolytic (FOG) and slow-twitch intermediate fibers were more appropriately named slow-twitch-oxidative fibers (SO). In addition to these three categories of twitch fibers, tonic fibers have been identified in several species of birds (Rosser and George, 1986a,b; Rosser et al., 1987; Meyers, 1992a). Slow tonic fibers have different physiological and morphological characteristics that distinguish them from twitch fibers (Barnard et al., 1982). Tonic fibers are multiply innervated and do not propagate an action potential (Moore et al., 1983; Johnston, 1985); they also have substantially slower contraction times than twitch fibers. Mammalian fast-twitch-glycolytic fibers can have twitch times of 17.8-129 msec, and fast-twitch-oxidative-glycolytic fibers, 58-193 msec (Henneman and Olson, 1965). In contrast, avian slow tonic fibers take several seconds to fully contract and relax (Barnard et al., 1982).

Rosser and George (1986b) showed that many avian flight muscles contain only fast-twitch glycolytic, fast-twitch oxidative-glycolytic, and tonic fibers. However, Barnard et al. (1982) described an avian equivalent to the mammalian slow-oxidative fiber in chickens. These fibers have characteristics that include moderate mATPase staining at low pH and moderate NADH-D staining. Morphologically, these avian "SO" fibers are smaller in diameter than FG or FOG fibers and have a high mitochondrial concentration. Slow-oxidative fibers have been further documented in the flight muscles of other avian species (Meyers, 1997; Meyers and Mathias, 1997).

Throughout this study, we will use the convention of Peter et al. (1972) to describe the muscle fiber types present in six flight muscles of the Atlantic puffin that are thought to be important for underwater and aerial propulsion. Prior work of many researchers provides a strong foundation for comparing the muscle fiber types of an alcid to nondiving, foot-propelled diving birds, and penguins (Hikida and Bock, 1974; Rosser and George, 1984, 1986a,b; Rosser et al., 1987, 1994; Hikida, 1987; Meyers, 1992a, 1997; Meyers and Mathias, 1997; Ponganis et al., 1997).

Fiber Type Comparisons

Distinguishing between FG and FOG fibers was not as obvious using mATPase activity, glycolytic, or oxidative enzyme activity as reported for other species (Rosser and George, 1986a; Sokoloff et al., 1998). Several authors (Peter et al., 1972; Gollnick and Hodgson, 1986) cite a continuous staining intensity of metabolic enzymes as a compelling reason for not using oxidative enzymes exclusively for classifying fibers. According to Gollnick and Hodgson (1986), the subdivisions using oxidative enzymes "may be more artificial than real." A preferred method is to separate fibers using the glycolytic staining intensity of α -GPD which correlates well with the glycolytic potential of fibers (Meijer, 1968). For these reasons, after the area of an individual fiber was measured using the mATPase stain (alkaline preincubation), the corresponding serial section stained for α -GPD was observed for final fiber typing. The vast majority of fibers were identified as fast-twitch based on the stability of mATPase following alkaline and acidic preincubation, and then evaluated on the basis of intensity of oxidative and glycolytic activity (Peter et al., 1972). When the criteria for slow-twitch-oxidative or slow-tonic fibers were satisfied, such fibers were separated from the fast-twitch fibers and analyzed separately.

For this aspect of the study, all six muscles were examined in two birds. Between 476 and 593 fibers were classified and measured in each muscle. Using the statistical program JMP (SAS Institute, Cary, NC) an analysis of variance (ANOVA) was performed comparing the cross-sectional areas of fast fibers for each muscle among the birds.

Fiber Size Comparisons

To compare fiber sizes, the areas of 100 to 2,000 muscle fibers were measured in each of the six muscles. Eight birds were used for the comparison of the pectoralis and supracoracoideus muscles. Three birds were used for comparison of the remaining four muscles. While the entire muscle cross-section could be sampled in the smallest muscles, the supracoracoideus and pectoralis muscles had to be divided into three and four individual blocks, respectively, and those blocks sampled. Thus, in these two largest muscles not every fiber could be sampled. The regions sampled in the pectoralis and supracoracoideus muscles are shown in Figure 1.

The statistical program JMP (SAS Institute) was used to compare the different regions of the pectoralis and supracoracoideus among the different birds. ANOVA was performed which included two factors, the area of the fibers (response) and the regions sampled (predictor).

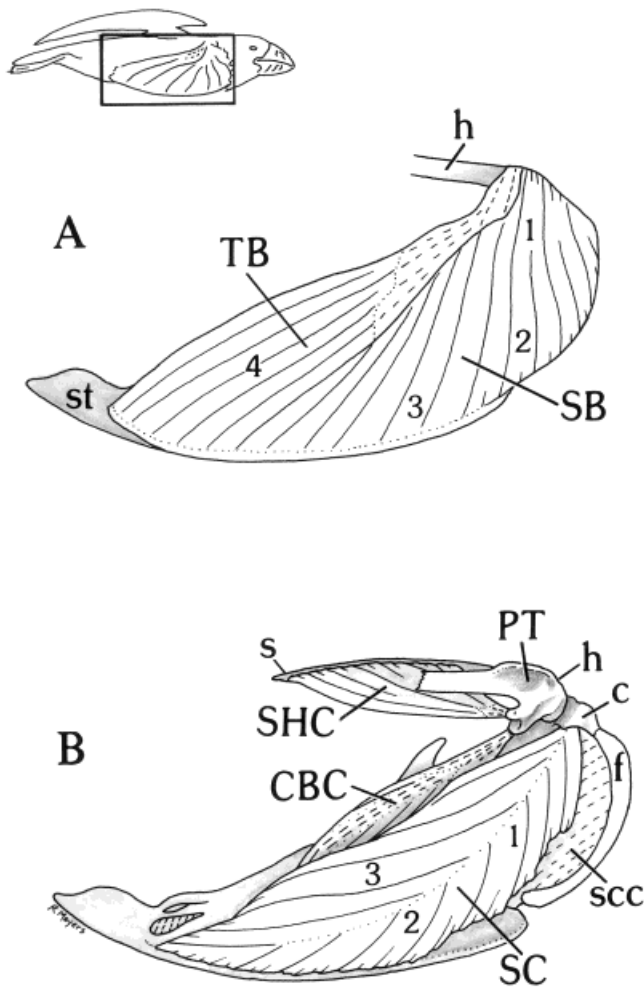


Fig. 1. *Fratercula arctica*. Lateral view of the right pectoral region of the Atlantic puffin. **A**: Superficial view showing thoracobrachialis (TB) and sternobrachialis (SB) regions of *M. pectoralis*. **B**: Deep view showing *Mm.* supracoracoideus, coracobrachialis caudalis, and scapulohumeralis caudalis. Numbers indicate muscle regions sampled (see text). CBC, coracobrachialis caudalis; c, coracoid; f, furcula; h, humerus; PT, insertion of pectoralis onto humerus; s, scapula; SB, sternobrachialis region of pectoralis; SC, supracoracoideus; sc, sterno-coraco-clavicular membrane; SH, scapulohumeralis caudalis; st, sternum; TB, thoracobrachialis region of pectoralis.

RESULTS

Various morphological features measured in the Atlantic puffin are listed in Table 1, along with similar measurements for other alcids and nondiving birds. The average body mass of our Atlantic puffins was 433 g, a value comparable with those reported by Pennycuik (1987) and Hansen (1995). Wing span (0.568 m), wing area (0.0376 m²) and wing-loading (114.9 Nm⁻²) are also consistent with values reported by these authors.

Anatomy — Introduction

Muscles were selected based on their presumptive function in flight. *Mm.* pectoralis and supracoracoideus

are the primary downstroke and upstroke muscles, respectively. Additional muscles, *Mm.* latissimus dorsi pars caudalis, and scapulohumeralis caudalis, coracobrachialis caudalis, and triceps scapularis, were also selected due to their anatomical orientation. Muscles are described relative to the avian anatomical position (see Raikow, 1985; Baumel et al., 1993), in which the wing is in an extended position. Thus, the wing can be divided into dorsal and ventral aspects, with the “leading edge” cranial and the “trailing edge” caudal.

The anatomical description of each muscle includes a brief orientation of the muscle, its relationship to surrounding structures, and a description of the origin and insertion. A statement of possible function, based on muscle attachment, is also included (see Raikow, 1985). Comparisons, when appropriate, are made with muscles described in Stettenheim (1959) for murrets (*Uria*), Hudson et al. (1969) for alcids, McKittrick (1991) for diving petrels (*Pelecanoides*), and Schreiweis (1982) for penguins.

Three different muscle fiber types were identified in the puffin: fast twitch, slow twitch, and slow tonic. All fast fibers reacted positively for both oxidative and glycolytic enzymes, but differed in intensity. Based on the level of glycolytic staining (see above), two distinct populations could be identified. We could categorize fibers as either fast twitch, highly oxidative, and moderately glycolytic, or fast twitch, moderately oxidative, and highly glycolytic. We will use the abbreviations FOg and FoG, respectively, to refer to these two fiber populations. No fast twitch glycolytic fibers (FG) as described in the pigeon or chicken pectoralis were observed in *Fratercula*.

Musculature

***M. latissimus dorsi pars caudalis* (LD).** The latissimus dorsi pars caudalis forms a large triangular-shaped sheet with the pars cranialis, and forms the most superficial muscle layer of the back. It is triangular and extends from the vertebral column to the humerus. LD arises from the caudal half of the notarium and also from the fascia overlying the *M. iliотrochantericus caudalis*. It tapers greatly as it passes over the scapula toward the humerus. LD inserts onto the humeral shaft (margo caudalis) just caudal to the deltoideus major, and dorsal to the triceps humeralis (Fig. 2). At the insertion, it lies ventral to the fascicles of pars cranialis, but the fasciae of both muscles are fused. LD is in a position to retract the humerus. Both Stettenheim (1959) and Hudson et al. (1969) ascribed its relatively large size in alcids to a functional role in underwater flight.

LD is comprised of 52% FoG fibers and 48% FOg. There are no slow fibers in the puffin LD, a finding consistent with most avian species (Bock and Hikida, 1968).

TABLE 1. Morphometrics of alcids and other birds

Species	Mass (kg)	Wing span (m)	Wing area (m ²)	Wing load (Nm ⁻²)	Source
Alcidae					
Least auklet (<i>Aethia pusilla</i>)	0.104	0.325	0.0144	72.2	Spear and Ainley (1997)
Dovekie (<i>Alle alle</i>)	0.153	0.320	0.0146	104.8	Pennycook (1987)
	0.096		0.0146	65.8	Poole (1938)
Cassin's auklet (<i>Ptychoramphus aleuticus</i>)	0.190	0.441	0.0262	72.5	Spear and Ainley (1997)
Marbled murrelet (<i>Brachyramphus marmoratus</i>)	0.226	0.443	0.0240	94.2	Spear and Ainley (1997)
Parakeet auklet (<i>Cyclorhynchus psittacula</i>)	0.282	0.502	0.0334	84.4	Spear and Ainley (1997)
Atlantic puffin (<i>Fratercula arctica</i>)	0.433	0.568	0.0370	117.0	This study
	0.398	0.549	0.0369	107.9	Pennycook (1987)
Female	0.424	0.571	0.0348	122.0	Hansen (1995)
Male	0.474	0.593	0.0367	129.3	Hansen (1995)
Pigeon guillemot (<i>Cepphus columba</i>)	0.470	0.578	0.0422	111.4	Spear and Ainley (1997)
Rhinoceros auklet (<i>Cerorhinca monocerata</i>)	0.560	0.615	0.0440	127.3	Spear and Ainley (1997)
Razorbill (<i>Alca torda</i>)	0.620	0.661	0.0462	134.2	Pennycook (1987)
	0.74				Piatt and Nettleship (1985)
Horned puffin (<i>Fratercula corniculata</i>)	0.663	0.607	0.0434	152.8	Spear and Ainley (1997)
Tufted puffin (<i>Fratercula cirrhata</i>)	0.799	0.662	0.0512	156.1	Spear and Ainley (1997)
Common murre (<i>Uria aalge</i>)	0.950	0.707	0.0544	174.6	Pennycook (1987)
	0.93				Piatt and Nettleship (1985)
Thick-billed murre (<i>Uria lomvia</i>)	1.033	0.727	0.0560	184.5	Spear and Ainley (1997)
Great auk (<i>Pinguinus impennis</i>)	5		0.0150	2200	Livezey (1988)
Laridae					
Laughing gull (<i>Larus atricilla</i>)	0.299	0.590	0.1014	29.5	Hartman (1961)
Herring gull (<i>Larus argentatus</i>)	0.940	1.310	0.1810	51.9	Pennycook (1987)
Spheniscidae					
Little penguin (<i>Eudyptula minor</i>)	1.200				Montague (1985)
Emperor penguin (<i>Aptenodytes forsteri</i>)	22				Kooyman et al. (1971)
Gaviidae					
Black-throated loon (<i>Gavia arctica</i>)	2.425		0.1358	178.6	Poole (1938)
Pelecanoididae					
Common diving petrel (<i>Pelecanoides urinatrix</i>)	0.137	0.393	0.0220	62.3	Pennycook (1982)
	0.150	0.425	0.0221	67.9	Spear and Ainley (1997)
S. Georgia diving petrel (<i>Pelecanoides georgicus</i>)	0.114	0.381	0.0200	57.0	Spear and Ainley (1997)
Columbidae					
Pigeon (<i>Columba livia</i>)	0.314		0.0576	54.5	Poole (1938)

M. pectoralis pars thoracicus (PT). The pectoralis muscle, the largest in birds, is elongate in alcids. It is a broad, complex muscle that covers the entire ventral surface of the chest region. PT arises from the distal edge of the sternal keel, from the lateral and caudal surfaces of the sternal body, from the lateral surface of the furcula, and from adjacent membranes. PT is divided into distinct sternobrachialis (SB) and thoracobrachialis (TB) portions by an internal sheet of connective tissue (Fig. 1). These portions have discrete fascicle arrangements. Those SB fascicles originating from the furcula are aligned transversely (due to the cranial "bowing" of this bone) and can function in wing protraction. The TB is arranged longitudinally, arising from the caudal surface of the sternum and adjacent membranes, and is in a position to retract the wing. PT mainly inserts onto the ventral surface of the pectoral crest of the humerus, but also has a connective tissue connection to the bicipital crest of the humerus (see also George and Berger, 1966; Meyers, 1992b).

The pectoralis muscle is approximately 15% of the total body mass and 70–75% of the flight muscle mass (Fig. 3). Eighty-seven percent of the muscle

fibers in the pectoralis of the Atlantic puffin are FOg fibers (Fig. 4) and have a mean area of approximately $751 \pm 294 \mu\text{m}^2$ (30–33 μm diameter) (Table 2). The remaining 13% of the fibers are FoG and are significantly larger than the FOg fibers, possessing a mean cross-sectional area and diameter of $1,195 \pm 393 \mu\text{m}^2$ and 40–41 μm , respectively. Fibers in the TB portion (region 4 in Fig. 1) were significantly larger ($895 \pm 292 \mu\text{m}^2$; $P < 0.05$) than those of the regions 1, 2, and 3 in the SB portion ($768 \pm 253 \mu\text{m}^2$, $790 \pm 275 \mu\text{m}^2$ and $764 \pm 239 \mu\text{m}^2$, respectively).

M. supracoracoideus (SC). The supracoracoideus muscle is a fusiform-shaped muscle, lying deep to the pectoralis on the ventral surface of the body. SC arises from the sternal body and keel (Fig. 1) and from the sterno-coraco-clavicular membrane. The thick tendon emerges from the triosseal foramen onto the dorsal surface of the wing, deep to M. deltoideus minor (Fig. 2). The insertion of SC onto the tuberculum dorsalis of the humerus is very broad, and consists of two parts. The main tendon attaches along a depression on the caudal surface of the humerus. A smaller tendon attaches cranially to it, but lies more proximal, adjacent (and proximal) to the

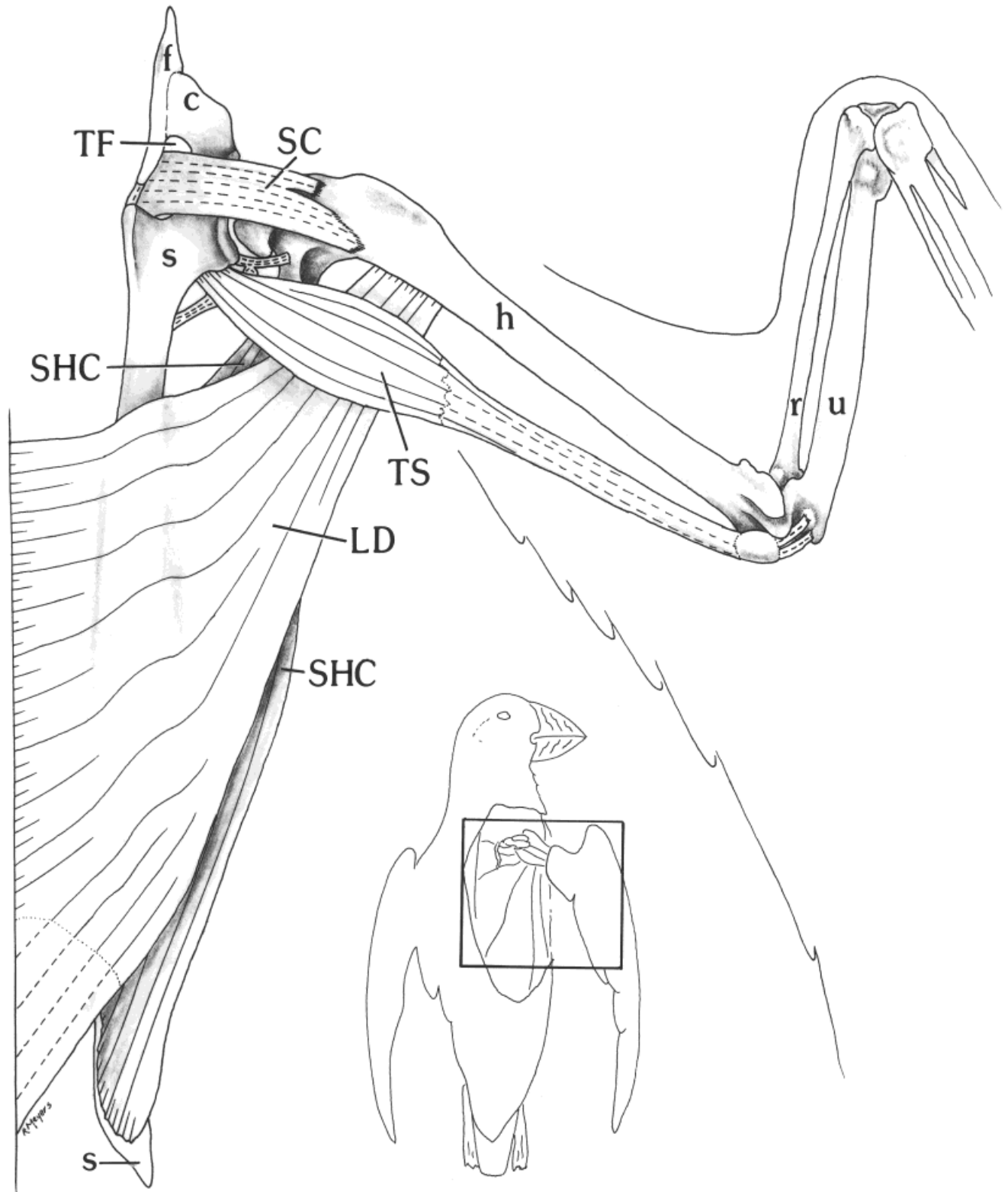


Fig. 2. *Fratercula arctica*. Dorsal view of the right forelimb of the Atlantic puffin illustrating muscle examined in this study. c, coracoid; f, furcula; h, humerus; LD, latissimus dorsi pars caudalis; r, radius; s, scapula; SC, supracoracoideus tendon; SH, scapulohumeralis caudalis; st, sternum; TF, triosseal foramen; TS, triceps scapularis; u, ulna.

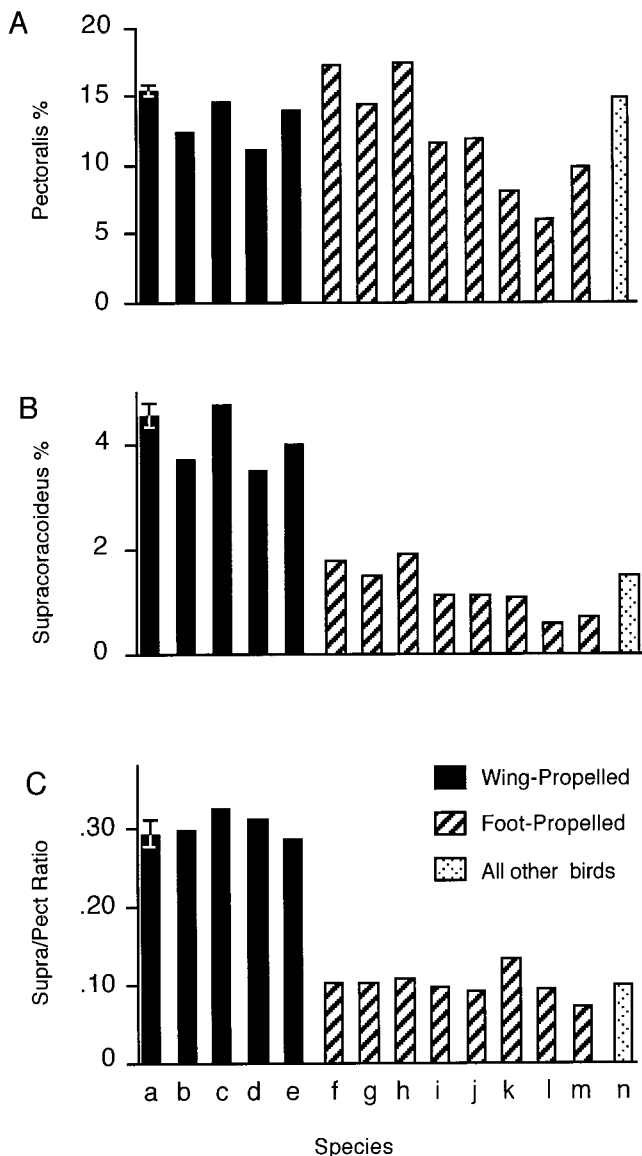


Fig. 3. Comparison of pectoralis and supracoracoideus muscle masses across a variety of wing-propelled and foot-propelled diving birds. **A:** Pectoralis muscle mass as a percent of body mass. No significant difference between wing-propelled and foot-propelled divers. **B:** Supracoracoideus muscle mass as a percent of body mass. A significant difference ($P < 0.001$) was found between wing-propelled and foot-propelled divers. **C:** Ratio of supracoracoideus mass to pectoralis mass. For wing-propelled divers, the mass of the supracoracoideus is approximately 30% of the pectoralis mass, a value much greater than that for foot-propelled divers. Key: a, Atlantic puffin, *Fratercula arctica* (this study); b, razorbill, *Alca torda*; c, common murre, *Uria aalge*; d, Atlantic puffin; e, dovekie, *Alle alle*; f, red-breasted merganser, *Mergus serrator*; g, common merganser, *M. merganser*; h, smew, *Mergellus albellus*; i, great crested grebe, *Podiceps cristatus*; j, red-necked grebe, *P. grisegena*; k, little grebe, *Tachybaptus rufolavatus*; l, red-throated loon, *Gavia stellata*; m, common loon, *G. immer*; n, all other birds. All values are single data points from Greenwalt (1962) except "a" which is a mean (\pm SD) from eight individuals.

insertion of the deltoid minor on the dorsal deltopectoral crest (Fig. 2). In alcids the insertion is neither on the proximal nor the leading edge of the humerus as in many aerial species (George and Berger, 1966), but is situated more caudally, and somewhat distally. McKittrick (1991) described a deep layer of SC in *Pelecanoides*; however, this layer (also present in *Fratercula*) is more accurately defined as a deep head of the M. deltoideus minor due to its innervation (see also George and Berger, 1966; Baumel et al., 1993).

The supracoracoideus muscle comprises 4–5% of the total body mass, 20–22% of the flight muscle mass (Fig. 3), and is approximately 30% of the pectoralis muscle mass. Seventy-two percent of the muscle fibers of the SC are highly aerobic FOg and are significantly smaller in area ($762 \pm 259 \mu\text{m}^2$) than the 28% FoG fibers ($1,230 \pm 392 \mu\text{m}^2$) (Fig. 5; Table 2). There was no significant difference in fiber area between any of the three regions sampled.

M. scapulohumeralis caudalis (SHC). The scapulohumeralis caudalis lies on the dorsal surface of the body, beneath the latissimus dorsi complex (Fig. 2). It is a thick, triangular muscle, extending from the scapula to the proximal humerus. SHC arises from the dorsal surface of the scapula, caudal to M. subscapularis caput laterale. The muscle fascicles taper to a short, stout tendon and insert onto the distal process of the tuberculum ventrale of the proximal humerus (Fig. 1), adjacent to the origin of the triceps humeralis. SHC is in a position to retract and adduct the humerus (Stettenheim, 1959).

The scapulohumeralis caudalis is made up of a majority (61%) of FoG fibers which are significantly larger ($P < 0.05$) in cross-sectional area than the FOg fibers (Table 2). Approximately 4% of the fibers sampled were stable at pH 4.3 for mATPase (Fig. 4). These fibers satisfy the criteria for mammalian slow-twitch-oxidative fibers (Barnard et al., 1982), and are reported as such.

M. triceps scapularis (scapulotriceps) (TS). The triceps scapularis lies on the caudal surface of the brachium, and extends from the scapula to the ulna. TS arises via a complex of fleshy and tendinous tissue from the caudal border of the glenoid, adjacent scapular shaft, and the dorsal scapulohumeral ligament (Fig. 2). At about the level of the insertion of the latissimus dorsi pars cranialis, a humeral anchor connects TS to the humeral shaft. TS becomes tendinous about halfway down the length of the humerus. The tendon of TS lies cranial to that of M. triceps humeralis, to which it fuses at the distal humerus. Two sesamoid bones are present in the conjoined tendons near the elbow joint (Fig. 1). In the resting folded wing position, these sesamoid bones lie between the humerus and the ulna. When the forearm is extended to approximately 80° , the sesamoid bones slide cranially and lie within the fossae at the distal end of the humerus (sulcus scapulotricipitalis and sulcus humerotricipitalis).

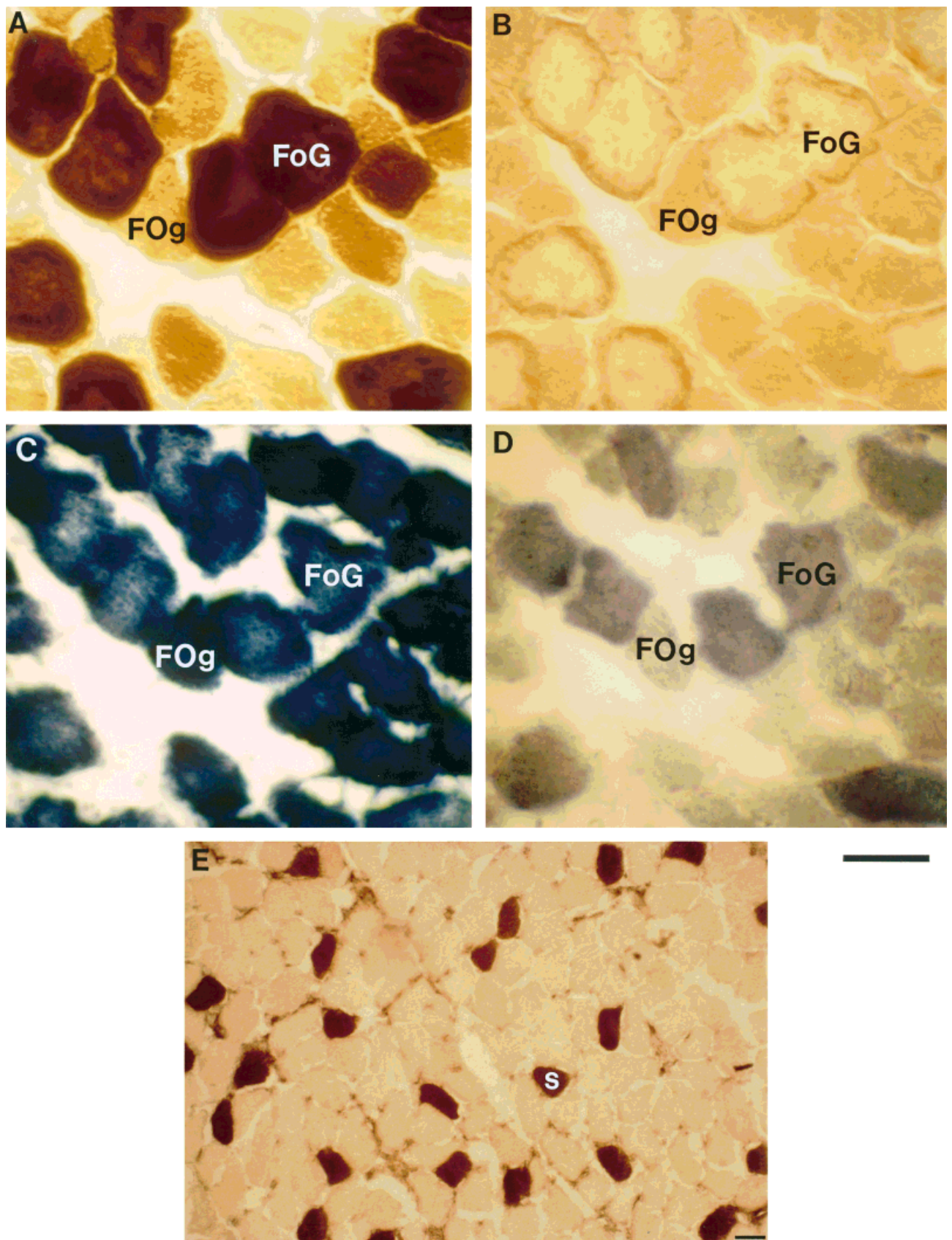


Fig. 4. *Fratercula arctica*. Serial sections of histochemistry of *M. pectoralis* (A-D) and *M. scapulohumeralis caudalis* (E). A: Alkaline preincubation (pH 10.4). B: Acidic preincubation (pH 4.6). C: NADH. D: GPD. E: Acidic preincubation (pH 4.4) showing avian slow-twitch fibers, S. Note that the FOg fibers react moderately for NADH, and weakly for GPD, whereas the FoG fibers react weakly for NADH and strongly for GPD. Scale = 25 μ m.

TABLE 2. Muscle fiber type comparisons

Muscle	Mean fiber area (mm ²) (standard deviation)			% of total fibers sampled			Weighted cross- sectional area (%)		
	FoG	FOg	Slow	FoG	FOg	Slow	FoG	FOg	Slow
Latissimus dorsi caudalis	799 (385)	799 (336)	—	52%	48%	—	52%	48%	—
Pectoralis	1,195 ^a (396)	751 (294)	—	13%	87%	—	19%	81%	—
Supracoracoideus	1,230 ^a (392)	762 (260)	—	28%	72%	—	39%	61%	—
Scapulohumeralis caudalis	1,152 ^a (270)	851 (221)	777 ^c (177)	35%	61%	4%	42%	54%	3%
Triceps scapularis	1,100 (199)	988 (253)	688 ^{b,d} (173)	21%	78%	1%	23%	76%	1%
Coracobrachialis caudalis	1,257 (511)	931 (361)	480 ^{b,c} (83)	25%	68%	7%	32%	65%	3%

^aSignificantly larger than FOg fibers ($p < 0.05$).

^bSignificantly smaller than FoG and FOg fibers ($p < 0.05$).

^cSlow-twitch.

^dSlow-tonic.

These sesamoid bones are thought to contribute to the stiffening of the wing for underwater propulsion in penguins (Bannasch, 1994). They may function to lock or otherwise assist in the maintenance of the flexed wing of puffins during the downstroke in underwater flight. Neither Stettenheim (1959) nor McKittrick (1991) reported any ossification in either of the triceps tendons in murrets or diving petrels, respectively. From the united tendons, a thin tendon passes cranially to insert onto the impressio scapulotriceps of the ulna (Fig. 2). TS plays an important role in aerial flight (Dial et al., 1991; Dial, 1992).

Triceps scapularis is composed mostly (79%) of FOg fibers. Of the 480 fibers sampled, four (1.4%) were classified as slow-tonic fibers (ST). These ST fibers were smaller in area (Table 2) than either of the FoG or FOg populations and stained moderately for both α -GPD and NADH-D. Slow-tonic fibers stained lightly for mATPase at all pH levels, while surrounding fast-twitch fibers were alkali-stable and acid-labile. It is doubtful that these slow fibers could contribute much force generation. No tonic fibers, but a few slow twitch fibers were found in the triceps scapularis of one individual of the gull, *Larus californicus* (Meyers and Mathias, 1997). No slow fibers of any kind were reported in this muscle of the gull *L. cachinnans* (Torella et al., 1998)

M. coracobrachialis caudalis (CBC). The coracobrachialis caudalis arises from the lateral margin of the sternum along the costo-sternal joint, and from the lateral surface of the linea intermuscularis ventralis of the coracoid and adjacent membranes. It ascends dorso-laterally and inserts on the tuberculum ventrale of the ventral surface of the humerus along with the subcoracoideus muscle (Fig. 1). This bipinnate muscle is divided by its internal tendon into lateral and medial regions, which have distinct muscle fiber types and different fiber morphologies.

The lateral region of the coracobrachialis caudalis muscle is composed primarily of FOg fibers with very few FoG fibers. Slow fibers are entirely absent in this region. The medial region is predominantly made up of FoG fibers with SO fibers comprising only 9% (Fig. 5). The SO fibers are significantly

smaller ($P < 0.05$) in area than FoG and FOg fibers (Table 2). The proportion and distribution of SO fibers in the Atlantic puffin is similar to that observed in the California gull (Meyers and Mathias, 1997).

Comparisons of Fiber Types

A typical series of micrographs from the muscles examined is represented by the pectoralis sections in Figure 4. Individual fibers are labeled as FoG or FOg. The FoG fibers are not only larger than the FOg fibers but they tend to have more stable mATPase following alkaline preincubation and are also most stable at low pH. Peter et al. (1972) noted similar findings for FG fibers at low pH. The full range of fiber type percentages can be found in Table 2. ANOVA was conducted to compare the cross-sectional areas of the FoG and FOg fibers. The mean area of FoG fibers is significantly larger than that of FOg fibers in the pectoralis, supracoracoideus, and scapulohumeralis caudalis muscles, and tends to be larger, but not significantly, in the coracobrachialis caudalis, latissimus dorsi caudalis, and the triceps scapularis muscles. An effect of the variation between individual birds exists in three muscles, the pectoralis, supracoracoideus, and scapulohumeralis caudalis.

FoG muscle fibers represented between 13% and 52% of the fibers sampled in the six muscles (Table 2). The pectoralis had the lowest percentage of FoG fibers while latissimus dorsi had the highest. Slow-tonic fibers comprised less than 1.4% of the fibers in the triceps scapularis, and slow-twitch oxidative muscle fibers comprised 7% and 4% of the fiber population of the coracobrachialis caudalis and scapulohumeralis caudalis muscles, respectively (Table 2).

Comparisons of Fiber Sizes

One goal of this study was to assess the inter- and intramuscular variation in mean muscle fiber area within the pectoralis and supracoracoideus muscles. In the pectoralis, fibers in regions 1, 2, and 3 (Fig. 1) have similar mean fiber areas, 768.5, 789.4, and

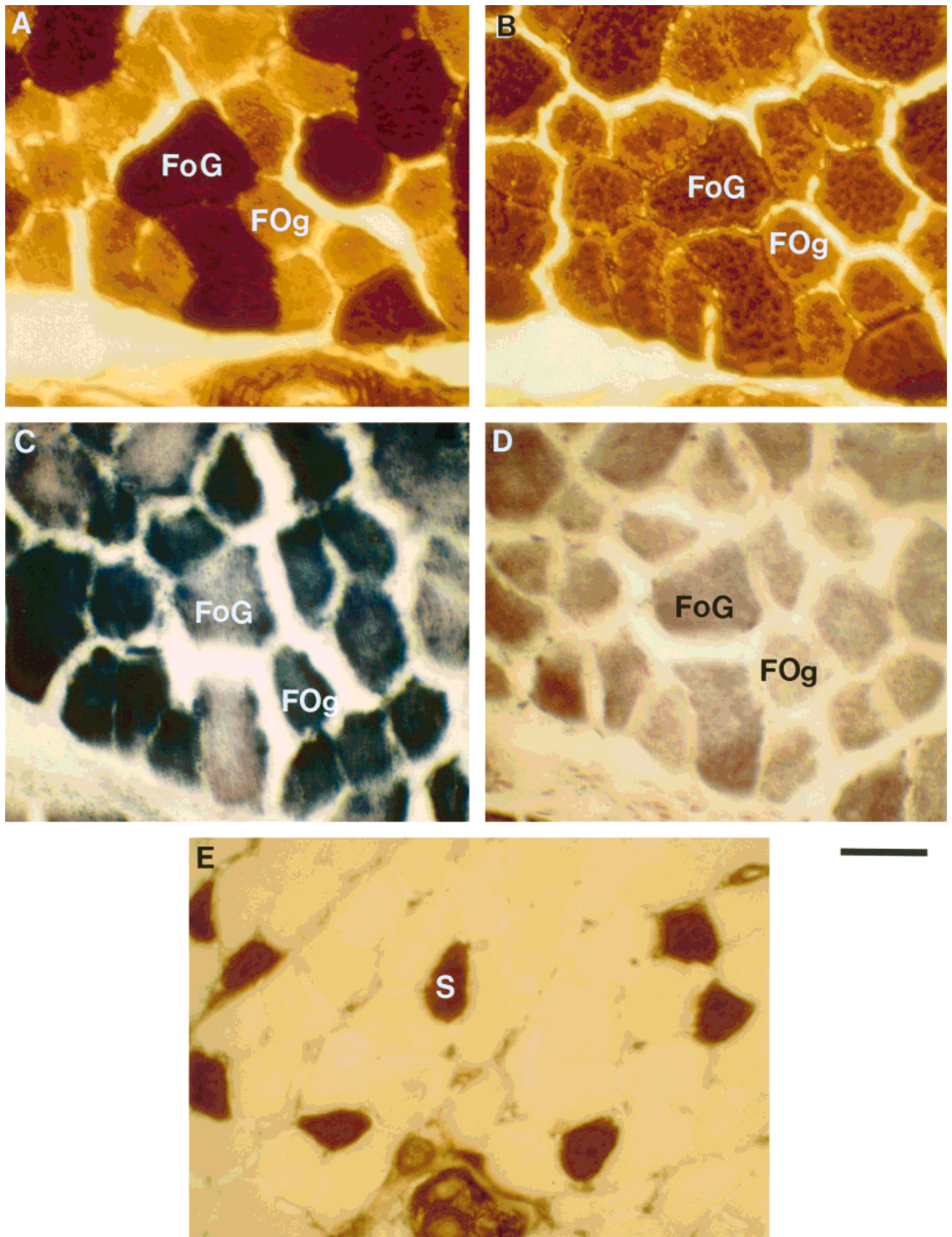


Fig. 5. *Fratercula arctica*. Serial sections of histochemistry of *M. supracoracoideus* (A-D) and *M. coracobrachialis caudalis* (E). **A:** Alkaline preincubation (pH 10.4). **B:** Acidic preincubation (pH 4.5). **C:** NADH. **D:** GPD. **E:** Acidic preincubation (pH 4.4) showing avian slow-twitch fibers, S. Note that the FOg fibers react moderately for NADH, and weakly for GPD, whereas the FoG fibers react weakly for NADH and strongly for GPD. Scale = 25 μ m.

764.1 μm^2 , respectively, while fibers from region 4 are significantly larger, 895 μm^2 (unpaired, two-sided *t*-test, $P < 0.05$). Region 4 is located in the thoracobrachialis portion of the pectoralis, and regions 1, 2, and 3 are from the sternobrachialis portion. Variation in muscle fiber cross-sectional area is also present in the supracoracoideus muscle. Fibers from region 3 tend to be smaller, although not significantly (unpaired, two-sided *t*-test, $P > 0.05$), than those from regions 1 and 2 (941.3 μm^2 vs. 998.8 μm^2 and 991.9 μm^2 , respectively).

Considerable variation exists among individuals in each of the regions sampled in the pectoralis and supracoracoideus muscles. At least one bird among those examined exhibited a significant difference in mean muscle fiber area within a given region. Mean muscle fiber area also correlated highly with muscle mass and body mass ($P < 0.001$), an important result when evaluating the differences in muscle fiber area between different individuals. The largest birds tended to have the largest fibers; therefore, a comparison of any region among different birds will be affected by the mass of the sampled muscle.

Of the remaining four muscles, only the coracobrachialis caudalis was tested for regional differences in fiber area. The medial region of the coracobrachialis caudalis had much larger fibers than the lateral region ($P < 0.05$). For the latissimus dorsi caudalis, scapulohumeralis caudalis, and triceps scapularis, there were significant differences in mean muscle fiber cross-sectional areas among the different birds tested.

DISCUSSION

Wing-propelled diving is rare among birds, yet this form of locomotion is a characteristic of 23 species of alcids, four species of diving petrels, and five species of dippers (Monroe and Sibley, 1993). In contrast to both foot-propelled diving birds (loons, grebes, diving ducks) and penguins, which use a single locomotor system, alcids employ the wings in air and water, media of vastly different physical properties. The effects of gravity are negligible underwater and parasite drag (drag of the body) restricts forward motion. As a result, the ratio of wing area to disk area (maximum cross-sectional area of the trunk) strongly impacts aquatic flight performance (Storer, 1960; Pennycuick, 1987; Bannasch, 1994; Thompson et al., 1998). Body mass and the effects of gravity on it are critically important for aerial flight, and thus strictly volant birds are under selection pressure to maximize wing area while minimizing body mass. Alcids are subjected to conflicting selection pressures and have balanced the requirements of both aerial flight and aquatic flight through morphological and behavioral modifications (Storer, 1960; Raikow et al., 1988).

Pennycuick (1987) compared alcids to a "standard seabird" he defined as having the mass, wing area,

and wing span that fall in the center of an allometric series of nonwing-propelled diving members of the order Procellariiformes. Compared to this "standard seabird," alcids have a reduced wing area and span yet maintain an aspect ratio similar to the "standard seabird." This area reduction occurs in the most distal skeletal element of the wings, the carpometacarpus (Middleton and Gatesy, 1997) and in the remiges (primary flight feathers). Pennycuick (1987) viewed the alcid wing as an intermediate form between nondiving birds and penguins, and suggested that the reduced wing area is a prerequisite to wing-propelled diving. Lovvorn and Jones (1994), however, argue that reduced wing area (and thus high wing-loading) is not necessarily a requirement for wing-propelled diving because foot-propelled divers (loons, grebes, diving ducks) also have reduced wing area and high wing-loading. In fact, wing-loading in alcids is indistinguishable from that of foot-propelled divers (Greenwalt, 1962). Lovvorn and Jones suggest that high wing-loading is an adaptation for high speed flight, an asset for birds that must traverse large expanses of ocean in search of prey. Murres can travel up to 168 km each way from breeding to foraging territories (Benvenuti et al., 1998). The concurrent lack of maneuverability in high wing-loaded birds (Rayner, 1986), a seemingly necessary corollary, may not be a detriment, however. To achieve take-off, for example, seabirds can run along the water surface, and a poorly executed landing on water is more forgiving than on land (Lovvorn and Jones, 1994). In addition, there are few obstacles disrupting the level terrain of the ocean. The small wings of alcids may not be an adaptation for wing-propelled diving as proposed by Pennycuick (1987) but rather a result of selection pressures for high-speed wings. Penguin wings are optimally designed for underwater propulsion, but penguins are not under the same selection pressure as alcids to additionally maintain aerial flight, a demand that restricts the minimum wing area in alcids. Alcids, however, are able to reduce the effective wing surface area underwater by flexing the elbow and wrist joints to reduce wing span by approximately 50% (pers. obs.).

In the present study, we identified possible specializations for wing-propelled diving in only two muscles, the pectoralis and the supracoracoideus. The remaining muscles examined are unremarkable with respect to wing-propelled diving and will be discussed collectively.

M. pectoralis

The pectoralis muscle, the largest muscle in volant birds (Hartman, 1961), is the primary downstroke muscle responsible for generating the power required for flight (Biewener et al., 1992; Dial and Biewener, 1993; Dial et al., 1997).

In alcids, PT is relatively elongated, producing an increase in the fascicle length while decreasing the overall thickness of the muscle (Stettenheim, 1959; Spring, 1971). This muscle was described as having two layers in *Pelecanoides* (McKittrick, 1991), but this is probably not related to underwater flight since diving petrels are related to gannets, petrels, and albatrosses, all of which possess a deep and superficial layer (Kuroda, 1961; Pennycuik, 1982). Despite the relative increase in length of the pectoralis in alcids, the relative mass has not changed. In the Atlantic puffin, the pectoralis is 15.4% of the total body mass. Greenwalt (1962) predicted the pectoralis muscle of volant birds should be 15.5% of total body mass. Therefore, despite the structural rearrangement of the fascicles, the pectoralis muscle in the Atlantic puffin does not exhibit the same relative hypertrophy seen in the supracoracoideus muscle.

The fiber type composition of the pectoralis in the Atlantic puffin is somewhat different from that of nondiving birds. The only previous study of pectoralis histochemistry from an alcid (Cassin's Auklets, *Ptychoramphus aleuticus*) observed mostly FOG fibers (Meyers et al., 1992). In the present study, we identified two populations of fast, oxidative, and glycolytic muscle fibers that could be separated on the basis of enzyme staining intensity and fiber diameter. Eighty-seven percent of the muscle fibers in the pectoralis of the Atlantic puffin are FOG fibers and have a mean diameter of 30–33 μm . The remaining fibers (13%) are FoG and are significantly larger in diameter (40–41 μm) than the FOG fibers. This small proportion of FoG fibers may play a significant role in power production. Laidlaw et al. (1995) calculated the weighted cross-sectional area, or the percentage of the total cross-sectional area, occupied by a single fiber type of several hindlimb muscles in the turtle. They determined that a small proportion of relatively large fibers can contribute significantly to the total cross-sectional area of the muscle and therefore to force output. Thus, while our FoG fibers make up 13% of fibers by percentage, they make up 19% by weighted cross-sectional area (Table 2). Sokoloff et al. (1998) showed similar results from a small percentage of FG fibers in the pigeon. They concluded that a pectoralis comprised of 10% FG fibers could contribute 36% of the muscle's total power. This is because FG fibers in pigeons have a 5.5-fold greater area than the FOG fibers (Kaplan and Goslow, 1989).

In the Atlantic puffin, FOG fibers have similar areas to those reported for FOG fibers by Kaplan and Goslow (1989) in pigeons, and by Talesara and Goldspink (1978) for pigeons, gulls, and sparrows. However, puffin FoG fibers are considerably smaller than FG fibers in these other birds (mean diameters of 40 μm in the puffin vs. 78 μm in the pigeon; Kaplan and Goslow, 1989). Oxidative fibers are typically smaller in diameter than those of lower oxida-

tive capacity and have higher diffusion rates of oxygen (Burke, 1981; Hermanson and Hurley, 1990). The relatively small size and proportion of FoG fibers in the Atlantic puffin may not provide the same functional qualities (i.e., force production) of the FG fibers observed in pigeons; however, these fibers may be important as they can produce more force than the smaller FOG fibers, and sustain this aerobically due to their greater oxidative capacity. Thus, the highly aerobic pectoralis of the puffin is capable of producing rapid, sustained wing beats in air and can store large quantities of oxygen for increasing the aerobic dive capacity during wing-propelled diving (Davis and Guderley, 1987).

Based on the anatomy of the pectoralis muscle and corresponding histochemistry, we conclude that there are few modifications of this muscle specific to wing-propelled diving. The relative muscle mass is similar to nondiving birds (and penguins), and the presence of oxidative enzymes in all of the muscle fibers may be an adaptation to limit fatigue of these motor units in both air and water. Davis and Guderley (1990) found similar oxidative capacities between murrets and puffins and pigeons (though more myoglobin in alcids) and suggested that evolving a diving physiology does not require much modification from a prior aerobic system. Diving, in general, seems to require a highly aerobic locomotor system, as evidenced by penguins (Ponganis et al., 1997), as well as seals (Kanatous et al., 1999).

M. supracoracoideus

The avian supracoracoideus has a bipinnate architecture, robust tendon and broad attachment to the delto-pectoral crest that reflect the high stresses imparted by this muscle during the upstroke. Stettenheim (1959) proposed that elongation of the supracoracoideus in alcids does not translate into greater shortening distance, but rather greater force production. He also suggested that SC can elevate the wing with the elbow and humerus adducted with more power in the murre than in gulls or godwits due to the longer in-lever of its relatively distal insertion. Poore et al. (1997) reported the supracoracoideus in pigeons and European starlings is capable of producing 39.4 ± 6.2 N and 6.5 ± 1.2 N, respectively. In these species and most other nondiving birds, the supracoracoideus is only 1.6% of the total body mass and 10% of the pectoralis mass (Greenwalt, 1962; Poore et al., 1997). In contrast, the supracoracoideus muscle of the Atlantic puffin is 4–5% of total body mass and 28–30% of the pectoralis mass, figures in agreement with Greenwalt (1962) (Fig. 3).

Poore et al. (1997) illustrated that the primary role of the supracoracoideus is to produce a high velocity, forceful rotation about the long axis of the humerus which repositions the wing for the subsequent downstroke. High wing-loading in birds is

compensated for by increasing wing beat frequency (Pennycuik, 1987). Therefore, it might be suggested that the supracoracoideus muscle is specialized for rapid elevation of the wing. However, foot-propelled diving birds also have high wing-loading and an increased wing beat frequency—without any proportional increase in supracoracoideus size (Hartman, 1961; Greenwalt, 1962). In contrast, penguins have a relatively large supracoracoideus muscle which is 10–12% of total body mass and nearly 50% of the pectoralis muscle mass. Thus, rapid wing beats and high wing-loading do not necessitate a large supracoracoideus. Rather, the relatively large supracoracoideus in alcids and other wing-propelled diving birds most likely evolved to raise the wing against the resistive drag of water.

Seventy-two percent of the muscle fibers of the supracoracoideus of the Atlantic puffin are FOG. These fibers resemble the FOG fibers of the pigeon and may possess similar physiological properties. FoG fibers make up only 28% percent of the muscle by number, but 39% by weighted cross-sectional area (Table 2). Davis and Guderley (1987) reported that the pectoralis and supracoracoideus of common murre and Atlantic puffins possess similar oxidative capacities to the pigeon, and concluded that they relied on aerobic metabolism to support diving. In addition, the content of myoglobin within muscles is highly correlated with their oxygen storage capacity (Baldwin, 1988). For the supracoracoideus muscle, myoglobin is highest in penguins (Mill and Baldwin, 1983; Ponganis et al., 1997), intermediate in alcids, and lowest in pigeons (Davis and Guderley, 1990). Davis and Guderley (1987) propose that high concentrations of mitochondrial enzymes are required to support aerial flight and limit the amount of myoglobin which can be maintained.

In alcids, the relative size of the supracoracoideus muscle is significantly larger than that of nondiving, closely related species. A similar pattern is observed in dippers (Goode, 1957). Any additional hypertrophy of the supracoracoideus in alcids might increase body mass (and hence wing-loading) beyond the capacity for aerial flight; hence, among diving birds greater supracoracoideus size is seen only in “aerially flightless” penguins.

Mm. coracobrachialis caudalis, latissimus dorsi caudalis, scapulohumeralis caudalis, and triceps scapularis

Stettenheim (1959) observed that the latissimus dorsi caudalis in murre inserts more distally on the humerus than in the gull; this increases the mechanical advantage of this muscle for retracting the humerus. In murre and Atlantic puffins (and penguins), the latissimus dorsi caudalis is more robust than in birds restricted to aerial flight. During the downstroke, forward thrust is generated by the downstroke, which tends to draw the wings forward. The

scapulohumeralis caudalis and the latissimus dorsi caudalis are in a position to retract the wing, or pull the body forward with respect to the wings. The increased force output of these muscles may be important for wing-propelled species as the relative drag on the body is greater underwater than in air when contrasted with the drag on the wings.

The scapulohumeralis caudalis is also in a position to assist in retracting or adducting the wing during the downstroke in air and underwater (Stettenheim, 1959; Torrella et al., 1998). This muscle is similar in alcids and gulls (Stettenheim, 1959), and is largely fast and oxidative, with a small number (<4%) of slow-twitch oxidative fibers. These SO fibers may be involved in maintaining a folded wing posture (Meyers, 1992a).

The coracobrachialis caudalis is a bipinnate muscle capable of exerting an isometric force of 8.9 N at its point of attachment on the humerus in pigeons (Woolley and Goslow, 1998). Force is greatest when the humerus is abducted. In pigeons, when the coracobrachialis caudalis is activated, the humerus is rotated to raise the leading edge of the wing (positive pitch), an action counter to the rotation of the wing (negative pitch) produced by the pectoralis. The morphology of this muscle in alcids is not significantly different from other seabird species (Stettenheim, 1959; Meyers and Mathias, 1997). Stettenheim states that the development of this muscle is important for mobility of the coraco-sternal joint. The coracobrachialis caudalis is relatively large in gulls, as is the mobility of their coraco-sternal joint. Its two regions, lateral and medial, possess different populations of fibers types which may represent a functional partitioning of this muscle. The histochemical profile of coracobrachialis caudalis in the puffin is similar to that found in gulls (Meyers and Mathias, 1997).

During level, cruising flight, the coracobrachialis caudalis has a biphasic electromyogram in both pigeons (Woolley and Goslow, 1998) and magpies (Tobalske et al., 1997), but is monophasic in starlings (Dial et al., 1997). The biphasic pattern is characterized by activation during late upstroke and a second burst of activity during mid-downstroke. The pattern of activity in puffins has yet to be determined and interpreted.

The triceps scapularis is least developed in the murre compared to gulls (Stettenheim, 1959; Torrella et al., 1998). It also appears somewhat reduced in size in Atlantic puffins. In contrast, Goode (1957) reported that in dippers the triceps scapularis was *larger* in comparison to that of related, aerial flyers. Stettenheim (1959) proposed that reduced development of the triceps brachii (scapular and humeral heads) and biceps brachii are adaptations to reduce the thickness of the wing, thus reducing the drag of the wing underwater. He supported this by noting the humerus, radius, and ulna are all dorsoventrally flattened in alcids; these elements are also ex-

tremely flat and broad in penguins (Bannasch, 1994).

Another interesting anatomical feature of the triceps scapularis is the presence of two sesamoid bones in its tendon. These sesamoid bones, which slip into fossae on the humerus when the elbow joint is extended to approximately 80°, may be a passive locking mechanism that maintains the wing in a fixed position. This angle corresponds to the angle of the joint as seen during the downstroke in wing-propelled diving. Bannasch (1994) described large sesamoids in the elbow of penguins and proposed that these bones stiffen the wing and reduce joint mobility to increase the flipper-like properties of the penguin wing. Hummingbirds also possess well-developed sesamoids in this tendon (Zusi and Bentz, 1984); they too fly with a stiff elbow joint. The functional role of these sesamoid bones needs to be evaluated further; they may be an important contribution to wing-propelled diving in alcids. Stettenheim (1959) reported no ossification in either of the triceps tendons, but suggested that the deep tricipital groove in the thin humerus (accommodating a thick triceps tendon) was, in fact, an adaptation to underwater flight.

Variation of Fiber Sizes

One outcome of this study significant for future research is that of the variability of fiber sizes. Namely, that variation may exist within a muscle of an individual bird and also between individuals for a given muscle. The puffin pectoralis showed significant differences in fiber area by region (TB>SB). This indicates that for larger muscles, either some sampling approach must be used to remove the bias of a particular region (see Laidlaw et al., 1995) or multiple regions must be examined and compared (this study; see also Rivero et al., 1993).

These regional differences may have important functional correlates. In addition, we found a correlation of fiber size (area) with body size, in that larger individuals tended to have larger fiber diameters. This can be significant for studies examining species that undergo seasonal weight changes over the course of a year (breeding, migration, etc.).

CONCLUSIONS

Northern Hemisphere alcids have often been described as being convergent with penguins of the Southern Hemisphere (Storer, 1960; Freethy, 1987). Storer's figure (his number 1) showing the adaptive stages in the evolution of wing propelled divers illustrates Northern and Southern Hemisphere parallels with gulls and petrels as the aerial flyers (his stage A), alcids and diving petrels as the bimodal flyers (his stage B), and the great auk (*Pinguinis impennis*) and penguins as the aquatic-only flyers (his stage C).

We believe that, although alcids may be useful models of an evolutionary intermediate, they represent a stable adaptation in their ability to fly in the air and underwater. The discovery of structural compromises (which make aquatic flight possible at the expense of aerial flight) has been the goal of a number of studies. Some of these "compromises," such as a dorsoventral flattening of the wing bones and a reduction in the relative size of intrinsic wing muscles (Stettenheim, 1959) suggest a similarity to the penguin flipper and a "reduction" of aerial flying ability. Alcids are often described as possessing little agility in the air (Rayner, 1986), yet fly rapidly and can travel great distances by air.

Based on our studies of several flight muscles of the shoulder and the histochemical properties of their fibers, we conclude there are several specific specializations for wing-propelled diving present in the Atlantic puffin (*Fratercula arctica*). The most prominent skeletal feature unique to wing-propelled diving is an elongate sternum (also observed by Stettenheim, 1959) that accommodates a relatively elongated, but not proportionately larger pectoralis muscle. The supracoracoideus muscle, however, is relatively enlarged. Wing-propelled divers must be able to raise the wing against water, which is 800 times as dense as air (Pennycuik, 1987). Clark and Bemis (1979) demonstrated that the upstroke in penguins develops forward thrust. Rayner (1995) described vortex rings created in the wake of murres as indicative of a nonthrust-producing upstroke. Although the upstroke in Atlantic puffins may not produce forward thrust, it may be important to prevent the bird from floating upwards during the recovery phase of the wing beat cycle. Of the remaining flight muscles examined in this study, the latissimus dorsi caudalis and scapulohumeralis caudalis are relatively robust in alcids and may be involved in the retraction of the wing underwater.

The overall nature of the flight muscles examined was that of fast twitch muscle fibers with present, though variable, quantities of oxidative and glycolytic enzymes. Two fiber populations can be discerned based on the degree of oxidative and glycolytic capacities; these possess discrete cross-sectional areas. The FOG fibers were similar in area and oxidative capabilities to "typical" FOG fibers of other birds such as pigeons. The fast-twitch, oxidative, and highly glycolytic (FoG) fibers were larger in area than the FOG (thus more force production; see Welsford et al., (1991)), yet still capable of aerobic metabolism. These fibers may in fact be similar to mammalian type IIX fibers, which react similarly for glycolytic and oxidative enzymes (in rats; Latorre et al., 1993) and appear to lie in between IIa and IIb on the continuum of enzymatic activity (nomenclature of Brooke and Kaiser, 1970; see Pette and Staron, 1990, for discussion of compatibility of schemes). We can therefore view the flight muscles of puffins as "intermediate" between aerial species and penguins

with respect to these fiber populations. In aerial species, the flight muscles are largely aerobic; a number of species do possess populations of FG fibers lacking aerobic capacity (Rosser and George, 1986a; Kaplan and Goslow, 1989). Puffins maintain two separate, identifiable oxidative fiber types, in contrast to a single oxidative fiber type in penguins (Ponganis et al., 1997). Along the histochemical gradients studied here, however, these two fiber types are more similar to each other than the FG and FOG fiber types in nondiving birds. The moderate staining for oxidative enzymes in the FoG fibers of the Atlantic puffin may be an adaptation for wing-propelled diving. Thus, as birds evolve from purely aerial to dual-medium to obligate swimming, the proportion of large, rapidly contracting FG fibers decreases and the level of oxidative capacity in all fibers increases. In addition, the levels of myoglobin in the pectoralis and supracoracoideus muscles in alcids lie in the mid-range between that of nondiving birds and penguins (Davis and Guderley, 1990).

The several adaptations described for dual-medium flight in alcids are considered by many to be compromises between the demands of aerial flight and swimming. We suggest, however, that alcids are not a collection of compromise adaptations, but rather unique organisms adapted to a dual-medium lifestyle.

ACKNOWLEDGMENTS

The authors thank G.E. Goslow Jr. for his constant support and encouragement. Drs. G.E. Goslow and A. J. Sokoloff made helpful suggestions on the manuscript. Trips to Iceland were financially supported by the Department of Ecology and Evolutionary Biology at Brown University, and the Company of Biologists Ltd. for a grant awarded to C.E.K. Laboratory space and transportation to Atlantic puffin colonies in Iceland were made possible by Páll Marvín Jónsson of the University of Iceland's Fisheries Research Unit and Erpur Hansen of the University of Missouri, St. Louis. This study was completed in partial fulfillment of C.E.K.'s Master's Degree in the Program in Ecology and Evolutionary Biology at Brown University.

LITERATURE CITED

- Armstrong RB, Saubert CW IV, Seeherman HJ, Taylor CR. 1982. Distribution of fiber types in locomotory muscles of dogs. *Am J Anat* 163:872-898.
- Baldwin J. 1988. Predicting the swimming and diving behavior of penguins from muscle biochemistry. *Hydrobiologia* 165:255-261.
- Bannasch R. 1994. Functional anatomy of the 'flight' apparatus in penguins. In: Maddock L, Bone Q, Rayner JMV, editors. *Mechanics and physiology of animal swimming*. Cambridge, UK: Cambridge University Press. p 163-192.
- Barnard EA, Lyles JM, Pizzey JA. 1982. Fibre types in chicken skeletal muscles and their changes in muscular dystrophy. *J Physiol* 331:333-354.
- Baumel JJ, King AS, Breazile JE, Evans HE, Vanden Berge JC. 1993. *Handbook of avian anatomy: Nomina Anatomica Avium*, 2nd ed. Cambridge, MA: Nuttall Ornithol Club.
- Benvenuti S, Bonadonna F, Dall'Antonia L, Gudmundsson GA. 1998. Foraging flights of breeding thick-billed murres (*Uria lomvia*) as revealed by bird-borne direction recorders. *Auk* 115: 57-75.
- Biewener AA, Dial KP, Goslow GE Jr. 1992. Pectoralis muscle force and power output during flight in the starling. *J Exp Biol* 164:1-18.
- Bock WJ, Hikida RS. 1968. An analysis of twitch and tonus fibers in the hatching muscle. *Condor* 70:211-222.
- Bock WJ, Shear CR. 1972. A staining method for gross dissection of vertebrate muscles. *Anat Anz* 130:222-227.
- Brooke MH, Kaiser KK. 1970. Muscle fiber types: how many and what kind? *Arch Neurol* 23:369-379.
- Burke RE. 1981. Motor units: anatomy, physiology, and functional organization. In: Brooks VB, editor. *Handbook of physiology*, vol. 2: motor control, part 2. Bethesda, MD: American Physiological Society. p 345-422.
- Burke RE, Levine DN, Zajac FE III. 1971. Mammalian motor units: physiological-histochemical correlation in three types in cat gastrocnemius. *Science* 174:709-712.
- Clark BD, Bemis W. 1979. Kinematics of swimming of penguins at the Detroit Zoo. *J Zool* 188:411-428.
- Davis MB, Guderley H. 1987. Energy metabolism in the locomotor muscles of the common murre (*Uria aalge*) and the Atlantic puffin (*Fratercula arctica*). *Auk* 104:733-739.
- Davis MB, Guderley H. 1990. Biochemical adaptations to diving in the common murre, *Uria aalge*, and the Atlantic puffin, *Fratercula arctica*. *J Exp Zool* 253:235-244.
- Dial KP. 1992. Activity patterns of the wing muscles of the pigeon (*Columba livia*) during different modes of flight. *J Exp Biol* 262:357-373.
- Dial KP, Biewener AA. 1993. Pectoralis muscle force and power output during different modes of flight in pigeons (*Columba livia*). *J Exp Biol* 176:31-54.
- Dial KP, Goslow GE Jr, Jenkins FA Jr. 1991. Functional anatomy of the shoulder in the European starling. *J Morphol* 207:327-344.
- Dial KP, Biewener AA, Tobalske BW, Warrick DR. 1997. Mechanical power output of bird flight. *Nature* 390:67-70.
- Freethy R. 1987. *Auks: an ornithologists guide*. New York: Facts on File.
- George JC, Berger AJ. 1966. *Avian myology*. New York: Academic Press.
- George JC, Naik RM. 1959. Studies of the structure and physiology of the flight muscles of birds. 4. Observations on the fiber architecture of the pectoralis major muscle of the pigeon. *Biol Bull* 116:239-247.
- Gollnick PD, Hodgson DR. 1986. The identification of fiber types in skeletal muscle: a continual dilemma. *Exerc Sport Sci Rev* 14:81-104.
- Goode WR. 1957. *Structural and functional adaptations for aquatic life in the dipper (Cinclus mexicanus)*. PhD Thesis, Seattle: University of Washington.
- Green HJ, Reichmann H, Pette D. 1982. A comparison of two ATPase based schemes for histochemical muscle fibre typing in various mammals. *Histochemistry* 76:21-31.
- Greenwalt CH. 1962. Dimensional relationships for flying animals. *Smithson Misc Coll* 144:1-46.
- Guth L, Samaha FJ. 1969. Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. *Exp Neurol* 25:138-152.
- Guth L, Samaha FJ. 1970. Procedure for the histochemical demonstration of actomyosin ATPase. *Exp Neurol* 28:365-367.
- Hansen ES. 1995. *Habitat selection of the Atlantic puffin (Fratercula arctica): in the perspective of a functional constraint by aerodynamical "take-off" capacity and the geometrical aspects of burrowing*. Fourth Year Research Thesis, Department of Biology, University of Iceland.
- Hartman FA. 1961. Locomotor mechanisms of birds. *Smithson Misc Coll* 143:1-91.

- Henneman E, Olson CB. 1965. Relations between structure and function in the design of skeletal muscles. *J Neurophysiol* 28: 581–598.
- Hermanson JW, Hurley KJ. 1990. Architectural and histochemical analysis of the biceps brachii muscle of the horse. *Acta Anat* 137:146–156.
- Hermanson JW, Cobb MA, Schutt WA, Muradali F, Ryan JM. 1993. Histochemical and myosin composition of vampire bat (*Desmodus rotundus*) pectoralis muscle targets a unique locomotory niche. *J Morphol* 217:347–356.
- Hikida RS. 1987. Quantitative ultrastructure of histochemically identified avian skeletal muscle fiber types. *Anat Rec* 218:128–135.
- Hikida RS, Bock WJ. 1974. Analysis of fiber types in the pigeon's metapatagialis muscle. I. Histochemistry, end plates and ultrastructure. *Tissue Cell* 6:411–430.
- Hudson GE, Hoff KM, Vanden Berge J, Trivette EC. 1969. A numerical study of the wing and leg muscles of Lari and Alcae. *Ibis* 111:459–523.
- Johnston IA. 1985. Sustained force development: specializations and variation among vertebrates. *J Exp Biol* 115:239–251.
- Kanatous, SB, DiMichele, LD, Cowan, DF, Davis, RW. 1999. High aerobic capacities in the skeletal muscles of pinnipeds: adaptations to diving hypoxia. *J Appl Physiol* 86:1247–1256.
- Kaplan SR, Goslow GE Jr. 1989. Neuromuscular organization of the pectoralis (pars thoracicus) of the pigeon (*Columba livia*): implications for motor control. *Anat Rec* 224:426–430.
- Kooyman GI, Drabek CM, Elsner R, Campbell WR. 1971. Diving behavior of the emperor penguin, *Aptenodytes forsteri*. *Auk* 88:775–795.
- Kuroda N. 1961. Analysis of three adaptive body forms in the Steganopodes, with notes on pectoral muscles. *Misc Rep Yamashina Inst Ornith Zool* 3:54–66.
- Laidlaw DH, Callister RJ, Stuart DG. 1995. Fiber-type composition of hindlimb muscles in the turtle, *Pseudemys (Trachemys) scripta elegans*. *J Morphol* 225:193–211.
- Latorre R, Gil F, Vazquez JM, Moreno F, Mascarello F, Ramirez G. 1993. Skeletal muscle fiber types in the dog. *J Anat* 182:329–337.
- Livezey BC. 1988. Morphometrics of flightlessness in Alcidae. *Auk* 105:681–698.
- Lovvorn JR, Jones DR. 1994. Biomechanical conflicts between adaptations for diving and aerial flight in estuarine birds. *Estuaries* 17:75–82.
- McKittrick MC. 1991. Forelimb myology of loons (Gaviiformes), with comments on the relationships of loons and tubenoses (Procellariiformes). *Zool J Linn Soc* 102:115–152.
- Meijer AEFH. 1968 Improved histochemical method for the demonstration of the activity of α -glucan phosphorylase. *Histochemie* 12:244–252.
- Meyers RA. 1992a. The morphological basis of folded-wing posture in the American kestrel, *Falco sparverius*. *Anat Rec* 232: 493–498.
- Meyers RA. 1992b. Morphology of the shoulder musculature of the American kestrel, *Falco sparverius*, with implications for gliding flight. *Zoomorphology* 112:91–103.
- Meyers RA. 1997. Anatomy and histochemistry of spread-wing posture in birds. 1. Wing drying posture in the double-crested cormorant (*Phalacrocorax auritus*). *J Morphol* 233:67–76.
- Meyers RA, Mathias E. 1997. Anatomy and histochemistry of spread-wing posture in birds. 2. Gliding flight in the California gull, *Larus californicus*: a paradox of fast fibers and posture. *J Morphol* 233:237–247.
- Meyers RA, Fisher K, Goslow L, Goslow GE Jr. 1992. Underwater locomotion and musculoskeletal organization in some wing-propelled diving birds. *Am Zool* 32:157A.
- Middleton KM, Gatesy SM. 1997. The evolution of theropod forelimb design and function. *Am Zool* 37:58A.
- Mill GK, Baldwin J. 1983. Biochemical correlates of swimming and diving behavior in the little blue penguin *Eudyptula minor*. *Physiol Zool* 56:242–254.
- Monroe BL Jr, Sibley CG. 1993. A world checklist of birds. New Haven: Yale University Press.
- Montague TL. 1985. A maximum dive recorder for the little penguin. *Emu* 85:264–267.
- Moore G, Johnston IA, Goldspink G. 1983. The pCa-tension characteristics of single skinned fibres isolated from the anterior and posterior latissimus dorsi muscle of the chicken. *J Exp Biol* 105:411–416.
- Novikoff AB, Shin WY, Drucker J. 1961. Mitochondrial localization of oxidative enzymes: staining results with two tetrazolium salts. *J Biophys Biochem Cytol* 9:47–61.
- Padykula HA, Herman E. 1955. The specificity of the histochemical method for adenosine triphosphate. *J Histochem Cytochem* 3:170–183.
- Pennycuik CJ. 1982. The flight in petrels and albatrosses (Procellariiformes) observed in South Georgia and its vicinity. *Philos Trans R Soc Lond (Biol)* 300:75–106.
- Pennycuik CJ. 1987. Flight of auks (Alcidae) and other northern seabirds compared with southern Procellariiformes: ornithological observations. *J Exp Biol* 128:335–347.
- Peter JB, Barnard RJ, Edgerton VR, Gillespie CA, Stempel KE. 1972. Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. *Biochemistry* 11:2627–2633.
- Pette D, Staron RS. 1990. Cellular and molecular diversities of mammalian skeletal muscle fibers. *Rev Physiol Biochem Pharmacol* 116:1–76.
- Piatt JF, Nettleship DN. 1985. Diving depths of four alcids. *Auk* 102:293–297.
- Ponganis PJ, Costello ML, Starke LN, Mathieu-Costello O, Kooyman GL. 1997. Structural and biochemical characteristics of locomotory muscles of emperor penguins, *Aptenodytes forsteri*. *Resp Physiol* 109:73–80.
- Poole EL. 1938. Weights and wing areas of North American birds. *Auk* 55:511–517.
- Poore SO, Ashcroft A, Sánchez-Haiman A, Goslow GE Jr. 1997. The contractile properties of the m. supracoracoideus in the pigeon and starling: a case for long-axis rotation of the humerus. *J Exp Biol* 200:2987–3002.
- Raikow RJ. 1985. Locomotor system. In: King AS, McLelland J, editors. Form and function in birds, vol. 3. London: Academic Press. p 57–147.
- Raikow RJ, Bicanovsky L, Bledsoe AH. 1988. Forelimb joint mobility and the evolution of wing-propelled diving birds. *Auk* 105:446–451.
- Rayner JMV. 1986. Pleuston: animals which move in water and air. *Endeavour* 10:58–64.
- Rayner JMV. 1995. Dynamics of the vortex wakes of flying and swimming vertebrates. *Symp Soc Exp Biol* 49:131–155.
- Rivero JLL, Serrano AL, Diz AM, Morales JL. 1993. Changes in cross-sectional area and capillary supply of the muscle fiber population in equine gluteus medius muscle as a function of sampling depth. *Am J Vet Res* 54:32–37.
- Rosser BWC, George JC. 1984. Some histochemical properties of the fiber types in the pectoralis muscle of an emu (*Dromaius novaehollandiae*). *Anat Rec* 209:301–305.
- Rosser BWC, George JC. 1986a. The avian pectoralis: histochemical characterization and distribution of fiber types. *Can J Zool* 64:1174–1185.
- Rosser BWC, George JC. 1986b. Slow muscle fibers in the pectoralis of the turkey vulture (*Cathartes aura*): an adaptation for soaring flight. *Zool Anz* 217:252–258.
- Rosser BWC, Davis MB, Brocklebank JR, George JC. 1987. On the histochemical characterization and distribution of fast and slow muscle fibers in certain avian skeletal muscles. *Acta Histochem* 81:85–93.
- Rosser BWC, Waldbillig DM, Wick M, Bandman E. 1994. Muscle fiber types in the pectoralis of the white pelican, a soaring bird. *Acta Zool* 75:329–336.
- Schreiwies DO. 1982. A comparative study of the appendicular musculature of penguins (Aves: Sphenisciformes). *Smithson Contrib Zool* 341:1–46.
- Sokoloff AJ, Ryan JM, Valerie E, Wilson DS, Goslow GE Jr. 1998. Neuromuscular organization of avian flight muscle: morphology and contractile properties of motor units in the pectoralis (pars thoracicus) of pigeon (*Columba livia*). *J Morphol* 236:179–208.

- Spear LB, Ainley DG. 1997. Flight behaviour of seabirds in relation to wind direction and wing morphology. *Ibis* 139:221–233.
- Spring L. 1971. A comparison of function and morphological adaptations in the common murre (*Uria aalge*) and thick-billed murre (*Uria lomvia*). *Condor* 73:1–27.
- Stettenheim P. 1959. Adaptations for underwater swimming in the common murre (*Uria aalge*). PhD thesis, Ann Arbor: University of Michigan.
- Storer RW. 1960. Evolution in the diving birds. *Int Ornith Congr* 12:694–707.
- Talesara GL, Goldspink G. 1978. A combined histochemical and biochemical study of myofibrillar ATPase in pectoral, leg and cardiac muscle of several species of bird. *Histochem J* 10:695–709.
- Thompson CW, Wilson ML, Melvin EF, Pierce DJ. 1998. An unusual sequence of flight-feather molt in common murres and its evolutionary implications. *Auk* 115:653–669.
- Tobalske BW, Olson NE, Dial KP. 1997. Flight style of the black-billed magpie: variation in wing kinematics, neuromuscular control, and muscle composition. *J Exp Zool* 279:313–329.
- Torrella JR, Fouces V, Palomeque J, Viscor G. 1998. Capillarity and fibre types in locomotory muscles of wild yellow-legged gulls. *Physiol Zool* 71:425–434.
- Welsford IG, Meyers RA, Wilson DS, Satterlie RA, Goslow GE Jr. 1991. Neuromuscular organization for “wing” control in a mollusc (*Clione limacina*) and a bird (*Columba livia*): parallels in design. *Am Zool* 31:670–679.
- Woolley JD, Goslow GE Jr. 1998. The functional characteristics of an avian flight muscle: the coracobrachialis posterior. *Am Zool* 38:34A.
- Zusi RL, Bentz GD. 1984. Myology of the purple-throated carib (*Eulampis jugularis*) and other hummingbirds (Aves: Trochilidae). *Smithson Contrib Zool* 385:1–70.