

Capillarity and Fibre Types in Locomotory Muscles of Wild Yellow-Legged Gulls (*Larus cachinnans*)

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ABSTRACT

This study analyzes the capillarity and fibre-type distribution of six locomotory muscles of gulls. The morphological basis and the oxygen supply characteristics of the skeletal muscle of a species with a marked pattern of gliding flight are established, thus contributing to a better understanding of the physiology of a kind of flight with low energetic requirements. The four wing muscles studied (scapulotriceps, pectoralis, scapulohumeralis, and extensor metacarpi) exhibited higher percentages of fast oxidative glycolytic fibres (>70%) and lower percentages of slow oxidative fibres (<16%) than the muscles involved in nonflight locomotion (gastrocnemius and iliotibialis). Capillary densities ranged from 816 to 1,233 capillaries mm^{-2} , having the highest value in the pectoralis. In this muscle, the fast oxidative glycolytic fibres had moderate staining for succinate dehydrogenase and relatively large fibre sizes, as deduced from the low fibre densities (589–665 fibres mm^{-2}). All these findings are seen as an adaptive response for gliding, when the wing is held outstretched by isometric contractions. The leg muscles studied included a considerable population of slow oxidative fibres (>14% in many regions), which suggests that they are adapted to postural activities. Regional variations in the relative distributions of fibre types in muscle gastrocnemius may reflect different functional demands placed on this muscle during terrestrial and aquatic locomotion. The predominance of oxidative fibres and capillary densities under 1,000 capillaries mm^{-2} in leg muscles is probably a consequence of an adaptation for slow swimming and maintenance of the posture on land rather than for other locomotory capabilities, such as endurance or sprint activities.

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Introduction

The varied lifestyles of vertebrates, and the wide range of locomotory modes by which they move, are reflected in considerable structural diversity of muscles. In birds, most studies of locomotion have focused on wing muscles, in which fibre-type composition, capillary supply, and architecture reflect the high metabolic needs associated with flight (see, e.g., Rosser and George 1986; Lundgren and Kiessling 1988; Tobalske 1996). The energetic demands required by flapping and gliding, the two major types of bird flight, are very different (Baudinette and Schmidt-Nielsen 1974; Goldspink et al. 1978; Butler and Woakes 1980; Meyers 1993). In order to cope with the higher oxidative demands of flapping flight, and to improve the oxygen arrival to mitochondria, the pectoralis muscle of birds that exhibit a large component of flapping flight have high capillary densities and small fibre cross-sectional areas (Suarez 1992; Mathieu-Costello et al. 1994).

In addition to flight, birds also use many other forms of locomotion, including walking, running, diving, and different ways of swimming (see Butler 1991). These locomotory behaviours also have an important role in shaping the structural characteristics of avian muscles, especially those of the hindlimbs. In spite of the importance of these forms of locomotion, only a few studies dealing with muscles other than those involved in flight are available (see, e.g., Suzuki et al. 1985; Boesiger 1986; Patak and Baldwin 1993).

To supplement the knowledge of bird muscle structure and its ecophysiological implications, we analyzed the fibre types and capillarization of several wing and leg muscles of a wild species of bird with a pattern of gliding flight, the yellow-legged gull (*Larus cachinnans*).

Material and Methods

Animals and Muscles

A total of six yellow-legged gulls (*Larus cachinnans* Pallas) of both sexes with a mean body weight (\pm SEM) of $1,062 \pm 63$ g were used for this study. Wild animals were obtained from the Medes Islands (Girona, Spain) in April 1993, during a campaign for the control of the population of this species undertaken by wildlife management technicians of the Departament d'Agricultura, Ramaderia i Pesca de la Generalitat de Catalunya.

The following six muscles, according to the nomenclature of Vanden Berge (1979), were selected for this study: pectoralis (wing depressor), scapulohumeralis caudalis (humerus retractor), scapulotriceps (elbow extensor), extensor metacarpi radialis (wrist extensor), iliotalibialis cranialis (femur protractor), and gastrocnemius lateralis pars externa (tarsometatarsus extensor). Figure 1 shows the exact position of each muscle.

The whole muscles were completely excised from each gull, except for the samples from pectoralis, which were selected from the midbelly of the muscle, taking special care to dissect them out entirely from the superficial to the deep part. Muscles were marked before excising in order to determine sample orientation when processing. After their removal, muscles were cleaned of excess connective tissues and left in a resting position on a flat surface, frozen in 2-methylbutane cooled to -160°C , and stored in liquid nitrogen until sectioning.

Histochemical Analysis

Transverse serial sections from the muscle equatorial zone and longitudinal sections of 14–20 μm thick were cut in a cryostat (Reichert, Jung) at -20°C and mounted on 2% gelatinized slides. Sections were subsequently incubated for 5 min in a buffered fixative (Viscor et al. 1992) in order to prevent shrinkage or wrinkling. This fixation procedure does not influence the fibre typing, because it does not alter the activities of the enzymes used in histochemical procedures (Rosenblatt et al. 1987; Viscor et al. 1992). Thereafter, the following histochemical assays were performed according to the methods referenced: succinate dehydrogenase (Nachlas et al. 1957), α -glycerophosphate dehydrogenase (Wattenberg and Leong 1960), myofibrillar adenosine triphosphatase (ATPase; Brooke and Kaiser 1970), muscle capillary identification by the ATPase method

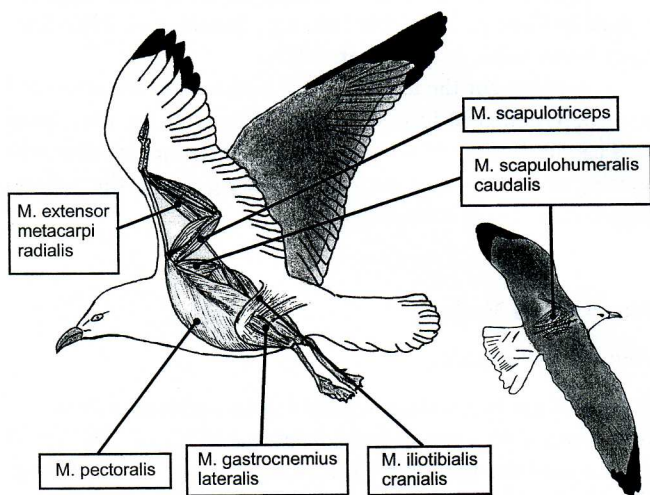


Figure 1. Anatomical location of the muscles studied. Pectoralis, extensor metacarpi radialis, scapulotriceps, iliotalibialis cranialis, scapulohumeralis, and gastrocnemius lateralis from a lateral view; scapulohumeralis also from a dorsal view.

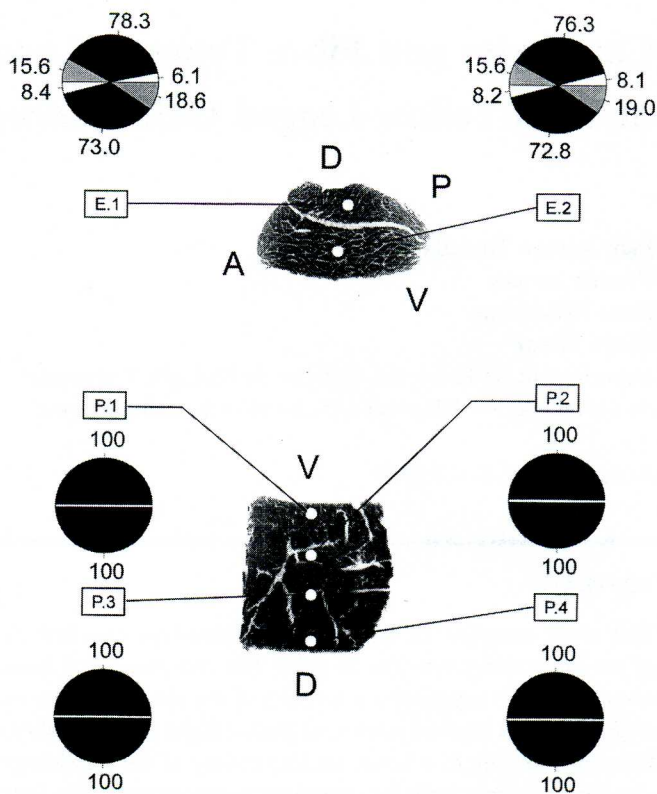


Figure 2. Transverse sections of muscle extensor metacarpi radialis (above) and muscle pectoralis (below). Each section displays the fields used for fibre-type determination and capillarization measurements. Numbers in each circle are the means for the six animals of the percentages of fibre types by number (upper semicircles) and the percentages of fibre types by area (lower semicircles). Anatomical location: A, anterior; D, dorsal; P, posterior; V, ventral. Sector code colour: grey, fast glycolytic; black, fast oxidative glycolytic; white, slow oxidative.

(Fouces et al. 1993), Sudan black B (Chiffelle and Putt 1951), and nerve-ending identification by the combined myofibrillar ATPase and acetylcholinesterase technique (Torrella et al. 1993), developed in both longitudinal and transverse sections.

Sampling Procedure and Measurements

We designed a sampling protocol for each muscle to allow regional description of fibre-type composition and capillarization. Since this protocol has been extensively explained elsewhere (Torrella et al. 1996), only a brief description follows. First, we determined the major axis (i.e., the longest diameter) of the transverse section from each muscle and divided it into several regular intervals. Thereafter, secondary orthogonal axes that transected the divisions were drawn as lines, the total length of which was also divided into several regular intervals. This procedure yielded a grid on each sample from which we selected the zones or "fields" for obtaining data. Thus, depending on the muscle studied, from two to seven fields were sampled (see Figs. 2–4 for the the exact position of fields).

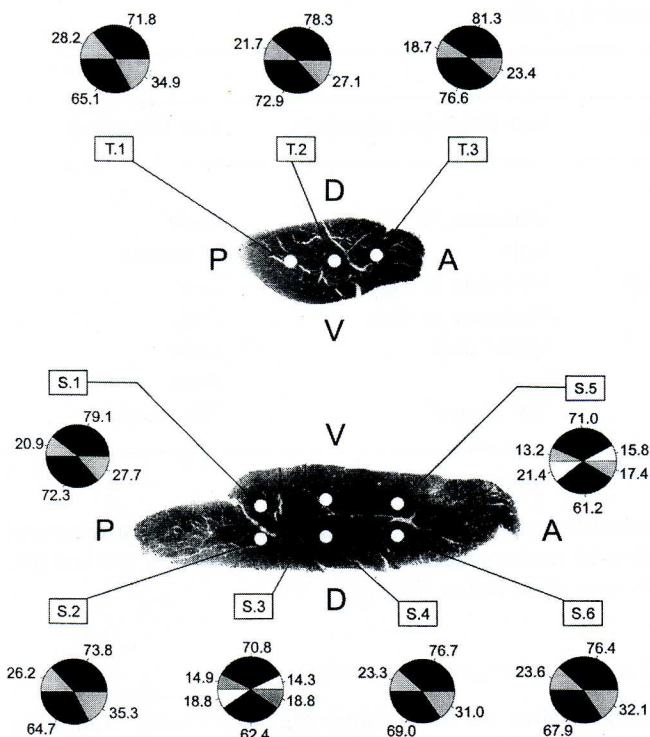


Figure 3. Transverse sections of muscle scapulotriceps (*above*) and muscle scapulohumeralis caudalis (*below*). Each section displays the fields used for fibre-type determination and capillarization measurements. Numbers in each circle are the means for the six animals of the percentages of fibre types by number (*upper semicircles*) and the percentages of fibre types by area (*lower semicircles*). Anatomical location: A, anterior; D, dorsal; P, posterior; V, ventral. Sector code colour: *grey*, fast glycolytic; *black*, fast oxidative glycolytic; *white*, slow oxidative.

Fibre types were classified according to the basic scheme of Peter et al. (1972), with the histochemical assays mentioned above as descriptive criteria. Measurements were made from photomicrographs taken at a magnification of 80× and 200× with a light microscope (Dialux, Leitz, Wetzlar, Germany) equipped with a camera (Wild, MPS51, Heerburg, Switzerland). Fibre-type frequencies were obtained by counting all muscle fibres (100–200) of the field and were expressed as percentages (number of fibres of a particular type/number of all fibres in field). The contribution of each fibre type to the total cross-sectional area of the muscle field (percentage of fibre by area) was calculated by multiplying the percentage of a fibre of a particular type by the mean cross-sectional area of that fibre type and dividing the result by the total area of all the fibres of the field. All fibre measurements were carried out by means of a digitizer tablet (Calcomp 23180-4, Anaheim, Calif.) connected to a personal computer using suitable software (Sigma-Scan version 1.20, Jandel Scientific, Erkrath, Germany). Capillary density, fibre density, and capillary-to-fibre ratio were determined from $2 \times 10^5 \mu\text{m}^2$ areas of tissue in each field and corrected to express them as capillaries and fibres per square millimeter.

Statistics

Data from all variables are expressed as sample means with 95% confidence limits. The capillary and fibre densities, capillary-to-fibre ratio, and percentage of oxidative fibres by number and by area (considering slow and fast oxidative fibres together) were analyzed with a two-way ANOVA for each muscle, taking “field” (regional variability) and “animal” (individual variability) as factors. A multiple comparison test using Scheffé’s procedure was performed in order to determine differences in sample means between all pairs of fields from the same muscle.

Results

Fibre Types

Table 1 and Figure 5 show the fibre types found in the six muscles studied and their descriptive characteristics. It is inter-

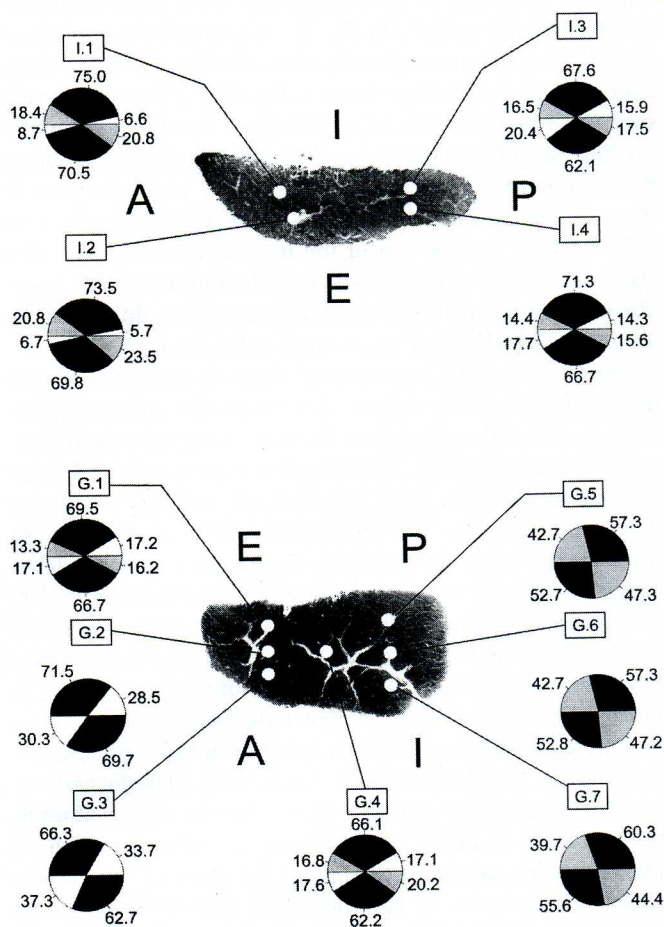


Figure 4. Transverse sections of the muscles iliotibialis cranialis (*above*) and muscle gastrocnemius lateralis (*below*). Each section displays the fields used for fibre-type determination and capillarization measurements. Numbers in each circle are the means for the six animals of the percentages of fibre types by number (*upper semicircles*) and the percentages of fibre types by area (*lower semicircles*). Anatomical localization: A, anterior; E, external; I, internal; P, posterior. Sector code colour: *grey*, fast glycolytic; *black*, fast oxidative glycolytic; *white*, slow oxidative.

Table 1: Skeletal muscle fibre types based on their histochemical profile

	Fibre Type		
	Slow Oxidative	Fast Oxidative Glycolytic	Fast Glycolytic
Myofibrillar ATPase:			
Alkali preincubation	Light	Moderate, dark ^a	Dark
Acid preincubation	Dark	Light	Moderate
Succinate dehydrogenase	Moderate to high	Moderate to high	Low
α -glycerophosphate dehydrogenase	Low	Moderate to high	High
Sudan black B	Moderate	Light, ^a dark	Light
Innervation pattern	Multiple	Focal	Focal
Neuromuscular junction	Small knobs	"En plaque"	"En plaque"

^a Only present in some muscle pectoralis areas (fields P.1, P.2, and P.3 in Fig. 2).

esting to note that in most pectoralis areas sampled (fields labeled P.1, P.2, and P.3 in Fig. 2), two myofibrillar ATPase-staining intensities were found in fast oxidative glycolytic fibres after alkali preincubation (Table 1; Fig. 5H). The predominant subtype of fast oxidative glycolytic fibres had moderate alkali myofibrillar ATPase stability, a dark staining pattern of Sudan B reaction, and a distribution throughout pectoralis muscle from 77.0% in field P.1 to 80.2% in field P.2 and 85.6% in field P.3 (Fig. 2). The rest of the muscle was composed of a fast oxidative glycolytic subtype that showed dark alkali myofibrillar ATPase and light Sudan B staining. No differences in the other histochemical assays were noted between them.

Figures 2–4 show transverse sections of each muscle with the proportion of the different fibre types for each field sampled. Fast oxidative glycolytic fibres were the dominant fibre type in all muscle fields. Their proportion by number was over 70% of all fibres in 19 of the 26 fields studied, which was especially evident for the wing muscles (Figs. 2, 3). It is also noteworthy that in the pectoralis muscle, the succinate dehydrogenase staining of fast oxidative glycolytic fibres was moderate compared with the other muscles, such as the gastrocnemius (Fig. 5A and C), that had high activities. Fast glycolytic fibres were the second most common fibre type. Although in much lower proportion than fast oxidative glycolytic fibres, they were present in all fields sampled except in the pectoralis muscle (Fig. 2) and in fields G.2 and G.3 (Fig. 4) of the gastrocnemius muscle. Slow oxidative fibres were not found either in the pectoralis or in the scapulothorax but were found in all other muscles, generally occupying the parts of the muscle closest to the bone. In the iliotibialis, slow oxidative fibres made up about 15% in both posterior fields (fields I.3 and I.4 in Fig. 4) but only 5% in the anterior parts of this muscle (fields I.1 and I.2 in Fig. 4). In contrast, in the gastrocnemius, slow oxidative fibres composed 17%–34% of all muscle fibres. In this muscle, fibres were present in anterior and medial fields but were completely lacking in posterior fields (Fig. 4). The only wing muscle with a marked presence of slow oxidative fibres was scapulothorax, which had in its anatomically deepest and most anterior part (fields S.3 and S.5 in Fig. 3) a

numerical proportion of almost 15%. The extensor metacarpi showed similar amounts of slow oxidative fibres, 6% and 8%, in both of its bellies (Fig. 2).

Tissue Morphological Parameters

Table 2 shows the tissue morphological parameters studied for each field. In all cases in which fast glycolytic fibres were present, the values of the percentage of oxidative fibres by area were from 1% to 9% lower than the percentages by number, as a consequence of the greater relative size of fast glycolytic versus oxidative fibres. Table 2 also shows the capillary density values, which had a low range of variation (from 816 to 1,233 capillaries mm^{-2}) among the different muscle regions. Fields from iliotibialis and pectoralis had the highest fibre densities (over 600 fibres mm^{-2}). Capillary and fibre densities in the pectoralis muscle of different bird species are compiled in Table 3.

Regional Muscle Variability

Table 4 shows the significance of the differences between fields and animals, from a two-way ANOVA test for each parameter and muscle. Two findings are noteworthy. First, there is great individual variability among wild yellow-legged gulls in all the parameters studied. Second, with the exception of gastrocnemius and scapulothorax, there are no significant differences between the sampled fields for most parameters when the same muscle fields are compared. If a conservative multiple comparison test, such as Scheffé's procedure, is applied, no significant differences are evident between fields, with the exception of the percentage of oxidative fibres by area in gastrocnemius (Table 5). A clear difference ($P \leq 0.001$) for this parameter was evident between the aerobically anterior (fields G.2–G.3, Fig. 4) and anaerobically posterior parts (fields G.5–G.7, Fig. 4) of the gastrocnemius. Smaller differences ($0.05 \geq P > 0.01$ or $0.01 \geq P > 0.001$) between other pairs of fields indicate the presence of a gradient of percentage of oxidative fibres by area throughout the major axis of this muscle.

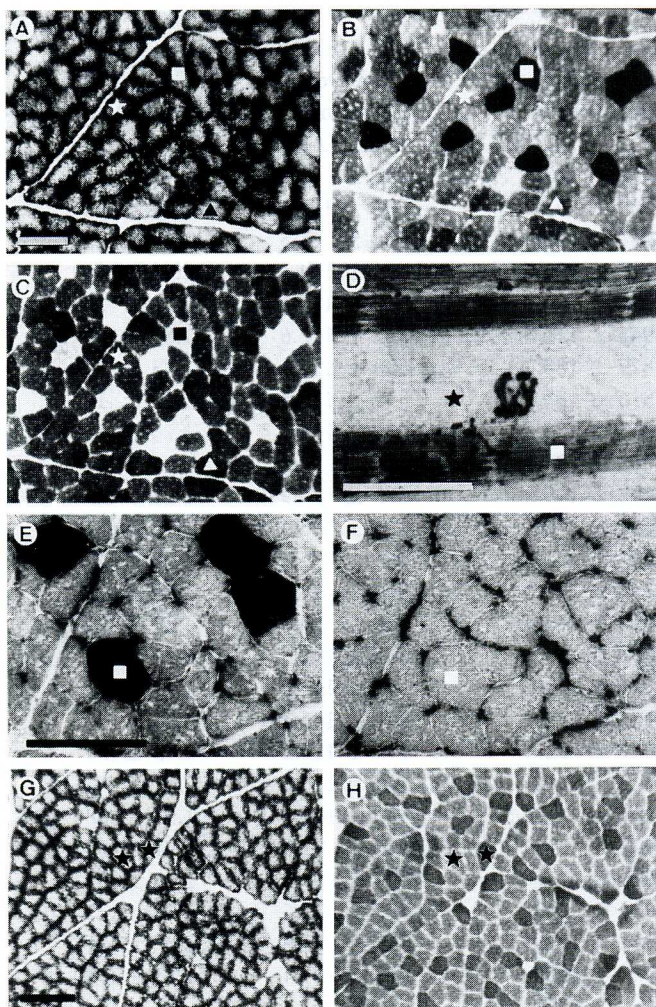


Figure 5. Transverse sections of different gull muscles. (A, B, C, gastrocnemius; E, F, iliotibialis; G, H, pectoralis) processed for myofibrillar ATPase (C and H preincubated at pH 11.0; B and E preincubated at pH 4.2), succinate dehydrogenase assay (A and G), and ATPase in order to reveal muscle capillaries (F). Micrograph D is a longitudinal section of gastrocnemius processed for the combined myofibrillar ATPase (preincubated at pH 4.2) and acetylcholinesterase method. Different fibre types are identified on the micrographs. Fibre-type codes: *squares*, slow oxidative; *triangles*, fast glycolytic; *stars*, fast oxidative glycolytic. Bars represent 100 μm .

Discussion

Fibre Types and Functional Implications

Leg Muscles. The presence of slow oxidative fibres in leg muscles of birds is well documented, and it is widely accepted that the slow oxidative fibres of leg muscles are recruited mainly for postural activities (see Butler 1991). Since yellow-legged gulls spend much time loafing and standing (Cramp and Simmons 1985), the presence of high amounts of slow oxidative fibres in this species is not surprising. In the present study, slow oxidative fibres were found to compose 20%–40% of some muscle fields (Fig. 4), which suggests that gull leg musculature

is well adapted to postural activities. It is interesting to note that, in some regions of gull leg muscles, fast oxidative glycolytic together with slow oxidative fibres represent 100% of fibres (Table 2). This exclusive aerobic fibre-type presence contrasts with that found in leg muscles of ducks, which possess a large percentage of fast glycolytic fibres (Turner and Butler 1988; Torrella et al. 1996). Differences in fibre types between these species may be related to locomotor behaviour; whereas a wide range of swimming and terrestrial behaviours are observed in ducks, gulls prefer buoyant swimming and walk infrequently (Cramp and Simmons 1985).

Wing Muscles. Most wing-muscle fields sampled in the present study were devoid of slow oxidative fibres. This is in accordance with the results reported by Maier (1983), who found no slow oxidative fibres in most forearm muscles of the pigeon. The distribution of slow oxidative fibres in the present study suggests that, of the wing muscles, the deep scapulohumeralis (fields S.3 and S.5, Fig. 3) is the most adapted to a postural role, perhaps assisting in wing folding by pulling the humerus to the body. In extensor metacarpi, slow oxidative fibres, composing less than 10% of all fibres (Fig. 2), may play a role in maintaining extension of the wing and stabilizing it during gliding flight.

In the yellow-legged gull, the pectoralis was composed solely of fast oxidative glycolytic fibres, a finding in agreement with previous studies on the pectoralis of other gull species (Rosser and George 1986; Viscor et al. 1991; Caldow and Furness 1993). This contrasts with the pectoralis muscle of the pigeon or the duck, where two markedly different fibre-type populations (fast glycolytic and fast oxidative glycolytic) have been found (for reviews, see George and Berger [1966] and Butler [1991]). These two populations are proposed to support different functional roles. Fast oxidative glycolytic fibres are thought to be mainly involved in sustained activities during flight, whereas fast glycolytic fibres could be preferentially active during take-off, landing, or sudden changes in direction (Rosser and George 1986; Welsford et al. 1991). Despite possessing a single fibre type, fibre populations of differing functional roles may, however, be present in the pectoralis of the yellow-legged gull. In the present study, two populations of fast oxidative glycolytic fibres differing in alkali stability and lipid densities were observed. This finding is similar to that reported by Caldow and Furness (1993) in the herring gull (*Larus argentatus*). Since differences in shortening velocity between fast fibre types have been demonstrated in other muscles (see Pette and Staron 1990), fast oxidative glycolytic fibres of dark myofibrillar ATPase and light Sudan B staining, in the gull pectoralis, might be recruited during burst activity, whereas the lipid-rich fast oxidative glycolytic fibre population might be preferentially recruited during long glides.

Yellow-legged gulls have a low capacity for manoeuvrability in tight spaces (Cramp and Simmons 1985), performing minimal phases of burst activity, and they glide even when preparing

Table 2: Tissue morphological parameters for each field

Field	Oxidative Fibres by Number (%)	Oxidative Fibres by Area (%)	Capillary Density (capillaries mm ⁻²)	Fibre Density (fibres mm ⁻²)	Capillary-to- Fibre Ratio
E.1	84.4 ± 10.3	81.4 ± 12.2	1,182 ± 57.7	497 ± 81.9	2.42 ± .33
E.2	84.4 ± 7.2	81.0 ± 8.5	1,156 ± 179.5	520 ± 123.8	2.24 ± .62
G.1	86.7 ± 7.5	83.8 ± 8.8	940 ± 78.4	456 ± 61.5	2.08 ± .22
G.2	100.0 ± .0	100.0 ± .0	1,066 ± 199.6	534 ± 164.1	2.06 ± .33
G.3	100.0 ± .0	100.0 ± .0	1,017 ± 294.2	510 ± 175.0	2.04 ± .29
G.4	83.2 ± 7.4	79.8 ± 8.8	945 ± 114.3	522 ± 118.6	1.85 ± .29
G.5	57.3 ± 20.4	52.7 ± 22.5	879 ± 188.2	500 ± 122.4	1.78 ± .20
G.6	57.3 ± 18.5	52.8 ± 19.3	816 ± 88.1	532 ± 113.4	1.56 ± .19
G.7	60.3 ± 17.5	55.6 ± 17.3	865 ± 102.5	510 ± 146.4	1.76 ± .33
I.1	81.6 ± 6.1	79.2 ± 6.8	1,062 ± 104.1	646 ± 59.3	1.65 ± .18
I.2	79.2 ± 8.9	76.5 ± 9.4	1,032 ± 100.1	636 ± 77.9	1.63 ± .23
I.3	83.5 ± 4.6	82.5 ± 4.8	1,094 ± 182.8	626 ± 93.1	1.75 ± .11
I.4	85.6 ± 3.3	84.4 ± 3.1	1,137 ± 201.9	643 ± 75.0	1.77 ± .21
P.1	100.0 ± .0	100.0 ± .0	1,233 ± 113.2	650 ± 94.5	1.91 ± .17
P.2	100.0 ± .0	100.0 ± .0	1,210 ± 143.0	665 ± 75.8	1.83 ± .21
P.3	100.0 ± .0	100.0 ± .0	1,087 ± 98.4	614 ± 106.3	1.80 ± .28
P.4	100.0 ± .0	100.0 ± .0	1,126 ± 131.6	589 ± 105.2	1.95 ± .32
T.1	71.8 ± 9.9	65.1 ± 13.7	1,119 ± 270.8	554 ± 146.7	2.05 ± .47
T.2	78.3 ± 9.0	72.9 ± 12.0	1,039 ± 132.8	497 ± 116.1	2.13 ± .39
T.3	81.3 ± 6.9	76.6 ± 8.2	1,046 ± 201.6	504 ± 130.3	2.11 ± .42
S.1	79.1 ± 6.4	72.3 ± 7.9	931 ± 122.4	491 ± 100.9	1.93 ± .28
S.2	73.8 ± 7.7	64.7 ± 8.5	929 ± 57.2	489 ± 91.8	1.94 ± .28
S.3	85.1 ± 9.3	81.2 ± 11.5	1,056 ± 117.0	474 ± 99.8	2.29 ± .45
S.4	76.7 ± 8.0	69.0 ± 9.6	924 ± 94.8	519 ± 75.6	1.80 ± .22
S.5	86.8 ± 8.8	82.6 ± 11.9	994 ± 97.8	505 ± 71.8	2.00 ± .31
S.6	76.4 ± 8.7	67.9 ± 11.3	980 ± 105.2	537 ± 55.4	1.83 ± .14

Note. Values are sample means with 95% confidence limits. Fields are named by the first letter of the muscle followed by the number of the field (see Figs. 2-4 for their locations). Muscles code: E, extensor metacarpi radialis; G, gastrocnemius lateralis; I, iliobtibialis cranialis; P, pectoralis; T, scapulotriceps; S, scapulohumeralis caudalis.

to alight on the water (Pennycuik 1987). The differences in the use of different modes of locomotion between birds such as pigeons or ducks and gulls may be reflected in differences in the histochemical organization of the pectoralis of these species. Flapping flight represents a five- to eightfold increase in oxygen consumption from resting levels (Tucker 1972), and this high energy consumption is reflected in the high levels of succinate dehydrogenase activity found in flapping-flight birds (Suarez 1992; León-Velarde et al. 1993). Gliding, in contrast, requires only a twofold increase in oxygen consumption from resting levels (Baudinette and Schmidt-Nielsen 1974) and involves, in the gull, less activity in the pectoralis than does flapping flight (Goldspink et al. 1978). The lower energy demands of this flight mode may be reflected in the moderate levels of succinate dehydrogenase staining found in the pectoralis in the present study (Fig. 5G). This suggestion derives some support from studies of several species of woodpeckers, where fast oxidative glycolytic fibres with moderate staining

for oxidative enzymes have also been reported (Tobalske 1996). These birds perform intermittent flight, periods of flapping alternated with periods of gliding, which also requires less energy than flapping (Rayner 1985).

In addition to pectoralis, the other wing and shoulder muscles studied stand out for their high proportion of fast oxidative glycolytic fibres; all 11 fields studied have percentages higher than 70%. The small muscle cross-sectional area occupied by fast glycolytic fibres in wing and shoulder muscles of yellow-legged gulls may reflect the only occasional need for nonsteady flapping flight in this species. Likewise, the presence of a large proportion of fast oxidative glycolytic fibres may reflect a need for endurance fibres during the long periods of time that these birds remain in flight (see Carrera et al. 1981, 1993).

Capillarization and Behavioural Associations

Leg Muscles. There are two possible explanations for the low range of variation found in the capillary density values within

Table 3: Capillary and fibre densities of muscle pectoralis of different bird species

Bird Species	Capillary Density (capillaries mm ⁻²)	Fibre Density (fibres mm ⁻²)	Reference
Hummingbird <i>Selasphorus rufus</i> (n = 4)	6,237–11,788	3,465–7,059 ^a	Mathieu-Costello et al. 1992
Andean coot, <i>Fulica americana peruviana</i>	2,477	1,283 ^a	León-Velarde et al. 1993
Pigeon <i>Columba livia</i> :			
Domestic breeds (n = 12)	1,491–5,680	766–2,367 ^a	Mathieu-Costello 1991
Urban	2,362	970	Fouces et al. 1993
Urban and homing	2,374–2,808	1,020–1,104	Viscor et al. 1992
Urban (flying-restricted)	2,075–2,429	1,646–1,967	Rakušan et al. 1971
Wild	4,387	2,194 ^a	Mathieu-Costello et al. 1994
Tufted duck, <i>Aythya fuligula</i>	3,361	1,530 ^a	Turner and Butler 1988
Mallard duck, <i>Anas platyrhynchos</i>	1,339	930	Torrella et al. 1996
Various passerines (15 species)	1,400–2,500	. . .	Lundgren and Kiessling 1988
Black-headed gull, <i>Larus ridibundus</i>	1,280	695	Viscor et al. 1991
Yellow-legged gull, <i>Larus cachinnans</i>	1,164 ^b	630 ^b	This study

Note. The range of capillary and fibre density values is presented when authors did not calculate a mean of the sample.

^a Calculated from capillary density and capillary-to-fibre ratio.

^b Mean of the four fields sampled (for range values, see Table 2).

the gull gastrocnemius (816–1,066 capillaries mm⁻²) and iliotibialis (1,032–1,137 capillaries mm⁻²; Table 2). First, a high percentage of slow oxidative fibres is found in oxidative zones of both gull leg muscles (Fig. 4). Slow fibres have lower energy turnover than fast fibres, since they hydrolyze ATP at a slower

rate (Goldspink 1981). This may result in reduced oxygen and nutrient supply by capillaries to the working muscles, a task that could be affordable by having relatively low capillary density, that is, barely over 1,000 capillaries mm⁻². Second, though the buoyant swimming performed by gulls is a high-endurance

Table 4: Results of a two-way ANOVA test showing the significance of the differences between muscle fields or animals

Muscle	% Oxidative Fibres by Number	% Oxidative Fibres by Area	Capillary Density	Fibre Density	Capillary-to- Fibre Ratio
Extensor metacarpi:					
Field	NS	NS	NS	NS	NS
Animal	NS	NS	NS	*	NS
Gastrocnemius:					
Field	**	***	**	NS	***
Animal	**	**	***	***	***
Iliotibialis:					
Field	NS	NS	NS	NS	NS
Animal	NS	NS	**	*	*
Pectoralis:					
Field			**	*	NS
Animal			***	***	**
Scapulotriceps:					
Field	***	**	NS	NS	NS
Animal	***	***	**	***	**
Scapulohumeralis:					
Field	**	***	*	NS	**
Animal	**	**	**	***	***

Note. No results are shown for % oxidative fibres by number or % oxidative fibres by area in pectoralis because this muscle had exclusively oxidative fibres, making the test irrelevant. NS, not significant; *, 0.05 ≥ P > 0.01; **, 0.01 ≥ P > 0.001; ***, P ≤ 0.001.

Table 5: Results of multiple comparison tests by Scheffé's procedure in gastrocnemius lateralis muscle

Fields	% Oxidative Fibres by Area						
	G.1	G.2	G.3	G.4	G.5	G.6	G.7
G.1	**	**	NS	*	*	*
G.2	NS	**	***	***	***
G.3	**	***	***	***
G.4	NS	NS	NS
G.5	NS	NS
G.6	NS

Note. Since the comparisons were not significant in all pairs for capillary density, fibre density, and capillary-to-fibre ratio, here we show only the results of the test for the percentage of oxidative fibres by area. The results of the comparison test between all pairs of fields are given inside of the cells. Field locations are identified in Figure 4. NS, not significant; *, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $P \leq 0.001$.

activity, the recruitment of fast fibres during this type of swimming may not be as high-energy demanding as the sustained or short-burst swimming activities described in other bird species such as ducks (Aigeldinger and Fish 1995). In fact, mallard ducks have greater regionalization of leg muscles, with higher capillary densities in the aerobic zones and lower densities in the anaerobic parts (Turner and Butler 1988; Torrella et al. 1996). This regionalization might reflect a wider range of locomotory activities. In gulls, less variation is present between the anterior and posterior parts of the gastrocnemius (Table 5). This slight regionalization suggests that gastrocnemius is not as functionally specialized as wing muscles, possibly owing to the use of the gastrocnemius in both aquatic locomotion and terrestrial maintenance of posture.

Wing Muscles. Capillary density in the pectoralis muscle of yellow-legged gulls is the lowest reported for the pectoralis of all the bird species listed in Table 3. This table shows that species performing hovering or flapping flight, such as hummingbirds or pigeons, have higher capillary densities in their pectoralis than the two species of gulls, who have a marked component of gliding flight. Moreover, fibre density values found in both gull species are the lowest reported in the literature for birds (Table 3). Since high capillary density and small fibre sizes are known to be related to the high oxidative capacity of muscles (Schmidt-Nielsen and Pennycuik 1961; Romanul 1965), these data may reflect the low oxygen demands imposed on the pectoralis by gliding flight. Even in those fields of the other wing muscles that have oxidative proportions greater than 70%, lower capillary density values were found ($924-1,182$ capillaries mm^{-2}) as compared to the values shown in Table 3. This could be a consequence of the involvement of wing muscles in maintaining the extension of the humerus and the wrist during gliding, pulling the tendons by means of isometric contraction, which is less costly than isotonic con-

traction (Goldspink 1981). These values agree with some cardiovascular adaptations reported in relation to flight activity in birds. Viscor et al. (1985) found that gulls have lower blood volume per unit of body weight, hematocrit, and hemoglobin concentration than pigeons. Butler and Woakes (1980) showed that, in the barnacle goose (*Branta leucopsis*), the heart rate during soaring is 50% less than during flapping flight, indicating a significant reduction in oxygen uptake.

In all the wing muscles studied, the lack of significant differences found between muscle regions for all the parameters (Scheffé's multiple comparison test) indicates that none of the wing muscles have great regional variations in capillarization or in percentage of oxidative fibres by area. This morphology might be a consequence of the wing muscles' specialization for gliding as a result of an adaptive constraint imposed by the need to be airborne for long periods of time.

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Literature Cited

- Aigeldinger T.L. and F.E. Fish. 1995. Hydroplaning by ducklings: overcoming limitations to swimming at the water surface. *J. Exp. Biol.* 198:1567–1574.
- Baudinette R.V. and K. Schmidt-Nielsen. 1974. Energy cost of gliding flight in herring gulls. *Nature* 248:83–84.
- Boesiger B. 1986. Histologie, histochemie et innervation comparées des fibres musculaires striées du muscle peroneus longus. *Acta Anat.* 126:103–109.
- Brooke M.H. and K.K. Kaiser. 1970. Muscle fiber types: how many and what kind? *Arch. Neurol.* 23:369–379.
- Butler P.J. 1991. Exercise in birds. *J. Exp. Biol.* 160:233–262.
- Butler P.J. and A.J. Woakes. 1980. Heart rate, respiratory frequency and wing beat frequency of free flying barnacle geese, *Branta leucopsis*. *J. Exp. Biol.* 85:213–226.
- Caldow R.W.G. and R.W. Furness. 1993. A histochemical comparison of fibre types in the M. pectoralis and M. supracoracoideus of the great skua *Catharacta skua* and the herring gull *Larus argentatus* with reference to kleptoparasitic capabilities. *J. Zool. (Lond.)* 229:91–103.
- Carrera E., X. Monbailliu, and A. Torre. 1993. Ringing recoveries of yellow-legged gulls in northern Europe. Pp. 181–194 in J.S. Aguilar, X. Monbailliu, and A.M. Paterson, eds. Status and Conservation of Seabirds. Ecogeography and Mediterranean Action Plan. Sociedad Española de Ornitología, Madrid.
- Carrera E., M.R. Nebot, and F.X. Vilagrassa. 1981. Comentaris sobre els desplaçaments erràtics de la població catalana de gavià argentat (*L. argentatus michahellis*). *Butlletí de l'Institut Català d'Història Natural (Secció Zoologia, 4)* 47:143–153.
- Chiffelle T.L. and F.A. Putt. 1951. Propylene and ethylene glycol as solvents for Sudan IV and Sudan Black B. *Stain Technol.* 26:51–56.
- Cramp S. and K.E.L. Simmons, eds. 1985. Handbook of the Birds of Europe, the Middle East and North Africa: The Birds of the Western Palearctic. Vol. 3. Waders to Gulls. Oxford University Press, Oxford.
- Fouces V., J.R. Torrella, J. Palomeque, and G. Viscor. 1993. A histochemical ATPase method for the demonstration of the muscle capillary network. *J. Histochem. Cytochem.* 41:283–289.
- George J.C. and A.J. Berger. 1966. Avian Myology. Academic Press, New York and London.
- Goldspink G. 1981. The use of muscles during flying, swimming, and running from the point of view of energy saving. *Symp. Zool. Soc. Lond.* 48:219–238.
- Goldspink G., C. Mills, and K. Schmidt-Nielsen. 1978. Electrical activity of the pectoral muscles during gliding and flapping flight in the herring gull (*Larus argentatus*). *Experientia* 34:862–865.
- León-Velarde F., J. Sanchez, A.X. Bigard, A. Brunet, C. Lesty, and C. Monge. 1993. High altitude tissue adaptation in Andean coots: capillarity, fibre area, fibre type and enzymatic activities of skeletal muscle. *J. Comp. Physiol.* 163B:52–58.
- Lundgren B.O. and K.H. Kiessling. 1988. Comparative aspects of fibre types, areas, and capillary supply in the pectoralis muscle of some passerine birds with differing migratory behaviour. *J. Comp. Physiol.* 158B:165–173.
- Maier A. 1983. Differences in muscle spindle structure between pigeon muscles used in aerial and terrestrial locomotion. *Am. J. Anat.* 168:27–36.
- Mathieu-Costello O. 1991. Morphometric analysis of capillary geometry in pigeon pectoralis muscle. *Am. J. Anat.* 191:74–84.
- Mathieu-Costello O., P.J. Agey, R.B. Logemann, M. Florez-Duquet, and M.H. Bernstein. 1994. Effect of flying activity on capillary-fiber geometry in pigeon flight muscle. *Tissue Cell* 26:57–73.
- Mathieu-Costello O., R.K. Suarez, and P.W. Hochachka. 1992. Capillary-to-fiber geometry and mitochondrial density in hummingbird flight muscle. *Respir. Physiol.* 89:113–132.
- Meyers R.A. 1993. Gliding flight in the American kestrel (*Falco sparverius*): an electromyographic study. *J. Morphol.* 215:213–224.
- Nachlas M.M., K.C. Tsou, E. De Souza, C.S. Cheng, and A.M. Seligman. 1957. Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. *J. Histochem. Cytochem.* 5:420–436.
- Patak A. and J. Baldwin. 1993. Structural and metabolic characterization of the muscles used to power running in the emu (*Dromaius novaehollandiae*), a giant flightless bird. *J. Exp. Biol.* 175:233–249.
- Pennycuik C.J. 1987. Flight of auks (Alcidae) and other northern seabirds compared with southern Procelariiformes: ornithodolite observations. *J. Exp. Biol.* 128:335–347.
- Peter J.B., R.J. Barnard, V.R. Edgerton, C.A. Gillespie, and K.E. Stempel. 1972. Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. *Biochemistry* 11:2627–2633.
- Pette D. and R.S. Staron. 1990. Cellular and molecular diversities of mammalian skeletal muscle fibers. *Rev. Physiol. Biochem. Pharmacol.* 116:1–76.
- Rakušan K., B. Ošťádal, and M. Wachtlová. 1971. The influence of muscular work on the capillary density in the heart and skeletal muscle of pigeon (*Columba livia dom.*). *Can. J. Physiol. Pharmacol.* 49:167–170.
- Rayner J.M.V. 1985. Bounding and undulating flight in birds. *J. Theor. Biol.* 117:47–77.
- Romanul F.C.A. 1965. Capillary supply and metabolism of muscle fibers. *Arch. Neurol.* 12:497–509.
- Rosenblatt J.D., W.M. Kuzon, M.J. Plyley, B.R. Pynn, and N.H. McKnee. 1987. A histochemical method for the simultaneous demonstration of capillaries and fiber type in skeletal muscle. *Stain Technol.* 62:85–92.
- Rosser B.W.C. and J.C. George. 1986. The avian pectoralis:

- histochemical characterization and distribution of muscle fiber types. *Can. J. Zool.* 64:1174–1185.
- Schmidt-Nielsen K. and P. Pennycuik. 1961. Capillary density in mammals in relation to body size and oxygen consumption. *Am. J. Physiol.* 200:746–750.
- Suarez R.K. 1992. Hummingbird flight: sustaining the highest mass-specific metabolic rates among vertebrates. *Experientia* 48:565–570.
- Suzuki A., T. Tsuchiya, S. Ohwada, and H. Tamate. 1985. Distribution of myofiber types in thigh muscles of chickens. *J. Morphol.* 185:145–154.
- Tobalske B.W. 1996. Scaling of muscle composition, wing morphology, and intermittent flight behavior in woodpeckers. *Auk* 113:151–177.
- Torrella J.R., V. Fouces, J. Palomeque, and G. Viscor. 1993. A combined myosin ATPase and acetylcholinesterase histochemical method for the demonstration of fibre types and their innervation pattern in skeletal muscle. *Histochemistry* 99:369–372.
- . 1996. Capillarity and fibre types in locomotory muscles of wild mallard ducks (*Anas platyrhynchos*). *J. Comp. Physiol.* 166B:164–177.
- Tucker V.A. 1972. Metabolism during flight in the laughing gull, *Larus atricilla*. *Am. J. Physiol.* 222:237–245.
- Turner D.L. and P.J. Butler. 1988. The aerobic capacity of locomotory muscles in the tufted duck, *Aythya fuligula*. *J. Exp. Biol.* 135:445–460.
- Vanden Berge J.C. 1979. Myologia. Pp. 175–219 in J.J. Baumel, A.S. King, A.M. Lucas, J.E. Breazile, and H.E. Evans, eds. *Nomina Anatomica Avium: An Annotated Anatomical Dictionary of Birds*. Academic Press, New York.
- Viscor G., M.S. Marqués, and J. Palomeque. 1985. Cardiovascular and organ weight adaptations as related to flight activity in birds. *Comp. Biochem. Physiol.* 82A:597–599.
- Viscor G., J.R. Torrella, V. Fouces, and J. Palomeque. 1991. Capillary supply in skeletal muscles of birds. *Pfluegers Archiv* 418:R191. (Abstr.)
- . 1992. Skeletal muscle capillarization and fiber types in urban and homing pigeons (*Columba livia*). *Comp. Biochem. Physiol.* 101A:751–757.
- Wattenberg L.W. and J.L. Leong. 1960. Effects of coenzyme Q₁₀ and menadione on succinic dehydrogenase activity as measured by tetrazolium salt reduction. *J. Histochem. Cytochem.* 8:296–303.
- Welsford I.G., R.A. Meyers, D.S. Wilson, R.A. Satterlie, and G.E. Goslow. 1991. Neuromuscular organization for “wing” control in a mollusc (*Clione limacina*) and a bird (*Columba livia*): parallels in design. *Am. Zool.* 31:670–679.