

1 **Differential muscular myosin heavy chain expression of the pectoral and pelvic girdles**
2 **during early growth in the king penguin (*Aptenodytes patagonicus*) chick**

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20 running title: Muscular maturation in the king penguin

21

22 **Summary**

23 Continuous growth, associated with a steady parental food supply, is a general pattern
24 in offspring development. Growth is usually marked by a fast maturation of muscles during
25 which different myosin heavy chain (MyHC) isoforms are expressed, permitting young chicks
26 to acquire their locomotor autonomy. However, because of seasonal changes in food supply,
27 energy allocation between tissue maturation and other energy demanding processes may be
28 conflicting. To address this trade-off we investigated muscular maturation in both the pectoral
29 and pelvic girdles of the king penguin chick. This species has an exceptionally long growing
30 period (1 year) which is prolonged when parental food provisioning is drastically reduced
31 during the sub-antarctic winter. After approximately one month post hatching, chicks acquire
32 a functional pedestrian locomotion whereas muscles of the pectoral girdle will be required for
33 swimming one year later. We therefore tested the hypothesis that leg muscles reach a mature
34 state in their muscular myosin heavy chain (MyHC) content before pectoral muscles. The
35 composition of MyHCs in leg muscles changed with the progressive acquisition of pedestrian
36 locomotion while in pectoral muscles, fibres reached their mature MyHC profile as early as
37 hatching. Contrary to our predictions, the acquisition of the adult profile in pectoral muscles
38 could be related to an early maturation of the contractile muscular proteins, presumably
39 associated with early thermoregulatory capacities of chicks, necessary for survival in their
40 cold environment. This differential maturation appears to reconcile both the locomotor and
41 environmental constraints of king penguin chicks during growth.

42

43 **Keywords:** development, bird, king penguin, skeletal muscle, myosin heavy chain

44 INTRODUCTION

45 During growth, individuals have to develop capacities such as thermoregulation or
46 locomotion in order to rapidly acquire their independence. A fast post-embryonic
47 development is necessary, linked to the maturation of muscles and skeleton (Ricklefs, 1979;
48 Olson, 2001; de Margerie et al., 2004). However in birds, muscle growth rates differ between
49 altricial and precocial species (Hohtola and Visser, 1998), in relation to variation in the degree
50 of tissue maturation at hatching (Nice, 1962; Ricklefs 1979; Starck and Ricklefs 1998). In
51 precocial species, the early development of the pelvic girdle and the delayed maturation of
52 pectoral muscles allow young birds to perform pedestrian locomotion quickly after hatching
53 and before acquiring flight capability (Starck and Ricklefs 1998; Phillips and Hamer 2000;
54 Bennett 2008). On the contrary, offspring of altricial species are totally reliant on parental
55 care and present a low maturation of leg and pectoral muscles at hatching; they acquire
56 terrestrial and aerial locomotion before fledging (Olson, 2001). As development and
57 maturation of different organs and tissues are energetically costly, growth is generally
58 associated with a steady parental food supply (Ricklefs, 1979). However, when the rearing
59 period is long, parental food supply may show seasonal fluctuation (Cherel et al., 1993) and
60 become a growth limiting factor (Heath and Randall, 1985). Hence, during growth chicks may
61 face periods of low food availability that can delay their development (Schew and Ricklefs
62 1998). In this context, the specific strategies of energy allocation that are developed to ensure
63 future survival and fledging of the offspring are not fully understood.

64 We addressed this question in the king penguin (*Aptenodytes patagonicus*) chick, a
65 semi-altricial species showing an exceptionally long rearing cycle (c.a. one year) in the sub-
66 antarctic and interrupted by a period of severe food restriction during the 2-3 winter months
67 (Stonehouse 1960; Barrat 1976). At hatching in summer, the chick totally depends on its
68 parents for warmth and food. When one month old, thermogenic processes become
69 sufficiently mature and chicks are then able to walk. Parents continue to forage intensively at
70 sea to allow them to store sufficient amounts of body reserves before winter. From May to
71 August, chicks mainly rely on their fat stores as an energy source and can lose half of their
72 body mass and stop growing (Stonehouse 1960; Barré 1978). From September onwards,
73 parental feeding rate increases allowing chicks to grow and moult before departing to sea and
74 becoming independent (Barrat, 1976). The locomotor capacity and activity of the king
75 penguin chick continuously changes throughout its growth until its independence. During the
76 first weeks after hatching, chicks remain hidden in the parental brood patch and show limited
77 locomotor activity. At one month old they use exclusively pedestrian locomotion. Thereafter,

78 one year later, king penguin chicks switch from an exclusively terrestrial locomotion to a
79 mixed terrestrial and aquatic locomotion. During their aquatic life, muscles of the pectoral
80 girdle power the flippers, which allow king penguins to forage and dive to depths of 200-300
81 meters (Barrat, 1976). In this way, the king penguin chick with this distinctive growth cycle is
82 a useful model to investigate the impact of environmental conditions on skeletal muscle
83 development.

84 In birds, each mode of locomotion (flapping flight, aquatic propulsion or terrestrial
85 locomotion) and postural maintenance corresponds to different metabolic and contractile
86 properties of muscles that are determined by their fibre types (Torrella et al., 1998). Fast-
87 twitch oxidative-glycolytic (FOG) fibres permit a sustained rapid contraction while fast-
88 twitch glycolytic (FG) fibres contract more powerfully and fatigue rapidly (Sokoloff et al.,
89 1998). In contrast, slow fibres are adapted for slow sustained contraction and are therefore
90 numerous in postural muscles (Meyers and Mathias, 1997). In addition to the activities of
91 their energy-generating enzymes, contractile properties of the muscle fibres also depend on
92 their myosin heavy chain (MyHC) isoforms (Rosser et al., 1996). During muscular growth, a
93 sequence of different MyHC isoforms is expressed in each avian fibre type (Bandman and
94 Rosser, 2000) as shown in the FG fibres of gallineous birds such as the chicken (*Gallus*
95 *gallus*, Hofmann et al., 1988; Tidyman et al., 1997), the turkey (*Meleagris gallopavo*,
96 Maruyama et al., 1993) or the Japanese quail (*Coturnix japonica*, Merrifield et al., 1989).
97 Moreover, a differential expression of MyHCs in the FOG and FG fibres during the
98 development of the pectoral muscle has been demonstrated in the domestic pigeon (*Columba*
99 *livia*, Rosser et al., 1998). Effective muscular contractions need an optimal myofibrillar
100 ATPase activity which has been related to myosin heavy chain composition (Rivero et al.,
101 1996). Moreover, recent studies on king penguin chicks indicate that the developmental
102 pattern of muscles and bones in the pectoral and pelvic limb are markedly different during the
103 first weeks after hatching. High rates of periosteal bone tissue growth (de Margerie et al.,
104 2004) and protein accretion (Erbrech et al., 2008) in the pelvic muscles allow nestlings to
105 rapidly acquire an effective pedestrian locomotion that is essential for their survival.
106 Conversely, the development of the pectoral girdle, that is required for aquatic locomotion, is
107 delayed. Together with the acquisition of locomotion, muscular development has also been
108 shown to be of a major importance in the ontogeny of thermogenic processes in penguin
109 chicks (Duchamp et al., 2002).

110 In the present work, we therefore focused on muscular development during the first
111 two months of growth of king penguin chicks, i.e. from hatching to the period when

112 pedestrian locomotion and thermoregulation capacities are sufficiently developed. To validate
113 the MyHC composition in king penguin muscles we first compared MyHC isoforms with the
114 domestic chicken (*Gallus gallus*). Then, we tested the hypothesis that muscles of the pelvic
115 girdle (*Gastrocnemius lateralis* and *Iliotibialis cranialis*) reach a mature state in their MyHC
116 content before the pectoral girdle (*Pectoralis major*), given that chicks acquire terrestrial
117 locomotion far in advance of aquatic locomotion.

118 MATERIAL AND METHOD

119 *Study area and specimens*

120 Field work was conducted in the colony of the Baie du Marin, Possession Island,
121 Crozet Archipelago (46°26'S, 51°52'E; Indian Ocean) during the sub-antarctic summer, from
122 January to April 2006. About 25,000 breeding pairs of king penguins are habituated to human
123 presence due to its proximity to the Alfred Faure Scientific Station. The study protocol was
124 approved by the French Ethic Committee of the Institut Polaire Paul-Emile Victor (IPEV) and
125 by the Polar Environment Committee of the Terres Australes et Antarctiques Françaises
126 (TAAF).

127 During daily surveys in the breeding colony, eggs with embryos close to hatching
128 (n=4), chicks (n=25) and adult (n=5) king penguins (*Aptenodytes patagonicus*) were collected
129 immediately after being stolen and/or killed by predators (subantarctic skuas *Catharacta*
130 *lonnbergi* for eggs and chicks or giant petrels *Macronectes* sp. for chicks and adults). In a
131 shelter close to the colony, embryos and birds were weighed to the nearest 0.1g or 1g,
132 depending on their absolute body mass. Within minutes after death muscle samples from the
133 pelvic and pectoral girdles were excised (c.a. 200mg) and kept in crushed ice (less than 4
134 hours) until myosin extraction. The length (accuracy \pm 0.5mm) of beak, foot, and flipper was
135 measured according to Stonehouse (1960) and the age of the embryo was determined from
136 flipper length (Stonehouse, 1960). Chick age was estimated according to down appearance,
137 body mass and behaviour (Verrier, 2003). In addition to adults, six developmental stages were
138 considered for chicks (Table 1). To limit the impact of the experiments on the predator
139 populations, the remaining parts of the carcasses were returned to the colony.

140 *Muscle sampling*

141 Three muscles were selected for the study: *Pectoralis major* (PM) from the pectoral
142 girdle, which is involved in aquatic locomotion, *Gastrocnemius lateralis* (GL) and *Iliotibialis*
143 *cranialis* (ITC) from the pelvic girdle that is essential for pedestrian locomotion. PM inserts
144 on the deltoid crest of the humerus (George and Berger, 1966) and is recruited for the
145 movement of flippers, allowing propulsion underwater. ITC arises from the anterior iliac crest
146 and inserts on the patellar ligament (George and Berger, 1966). It is recruited for the hip
147 flexion and knee extension (Smith et al., 2006). GL takes its origin on the proximal surface of
148 the fibular condyle of the femur and ends on the most lateral part of the *tendo achilis* (George
149 and Berger, 1966). This muscle is mainly recruited for ankle extension and knee flexion
150 (Smith et al., 2006).

151 *Myosin extraction and electrophoresis of MyHC analysis*

152 Each muscle sample was weighed ($\pm 0.1\text{mg}$) and myosin was extracted in a specific
153 high ionic strength buffer according to D'Albis et al. (1979). The extracts were kept at -80°C
154 until analysis. Protein concentrations in the extracts were determined using the method
155 described by Bradford (1976). Isoform separations were performed according to the method
156 of Talmadge and Roy (1993). The stacking and separating gels were respectively composed
157 of 4 and 8% acrylamide-N,N'-methylene-bis-acrylamide (bis) (50:1). Mini-gels (0.75mm
158 thickness) were used in the Bio-Rad Mini-protean II Dual Slab Cell. Electrophoreses were
159 carried out at a constant 70V voltage for 28 hours in a cold room ($+ 4^{\circ}\text{C}$). The amount of
160 protein run on the gel was of approximately $5\mu\text{g}$ of total protein per lane. The gels were
161 stained with Coomassie blue R-250. The relative amounts of the different MyHCs were
162 measured using an integration densitometer Bio-Rad GS-800, and analysed with the Quantity
163 one 4.2.1 Program. Only bands representing more than 1% of total MyHCs were taken into
164 account. Moreover, muscle samples obtained from an adult chicken (*Gallus gallus*) were used
165 to compare the composition in MyHC isoforms of this species with the one of adult king
166 penguins and to validate our extraction procedure.

167 168 *Statistical analysis*

169 Means \pm s.e.m are provided. Multiple comparisons were made using non-parametric
170 Kruskal-Wallis ANOVAs, followed by Dunn's post-hoc tests. Relative percentages were
171 analysed after transformation to arcsin square roots. All statistical analyses were carried out
172 using Statview 5.0. Statistical significance was set at $P<0.05$.

173 **RESULTS**

174 ***Validation of the extraction procedure (Fig.1)***

175 *Pectoralis major (PM)*

176 A single MyHC isoform could be observed in the PM for both the domestic chicken
177 and the adult king penguin. However, the PM isoform content was of a lower mobility in the
178 king penguin than in the domestic chicken.

179 *Gastrocnemius lateralis (GL)*

180 GL of the adult king penguin contained two MyHCs (bands 1 and 4), whereas three
181 isoforms could be observed in the domestic chicken. In the two species, the slowest isoform
182 presented a similar electrophoretic mobility whereas other MyHCs displayed a different
183 mobility.

184 *Iliotibialis cranialis (ITC)*

185 Two MyHC isoforms were delineated in the adult king penguin (bands 1 and 4) and in
186 the domestic chicken. The slowest migrating isoform presented also a similar electrophoretic
187 mobility in both species, but the second band detected in the chicken was slower than band 4
188 in the king penguin.

189 These results of isoform separations in PM and GL muscles in the domestic chicken
190 are in accordance with previous studies which used immunoblot and gene expression analyses
191 (Hofmann et al., 1988; Tidyman et al., 1997), therefore validating our extraction protocol.

193 ***Differential foot and flipper growth in king penguin chicks (Fig. 2)***

194 The foot and flipper lengths of king penguin chicks significantly increased during the
195 first two months of post-hatching life ($P \leq 0.001$). From stages A to F (within the first week of
196 growth), they increased from 44.1 ± 1.9 and 49.4 ± 0.4 mm to 117.5 ± 4.5 and 117.6 ± 7.8 mm,
197 respectively. In emancipated chicks (stage F), feet and flippers had reached 76.2 % and
198 43.1 % of their adult size, respectively.

200 ***Developmental expression of MyHCs in the pelvic and pectoral girdles of king penguin*** 201 ***chicks***

202 A total of six different MyHC isoforms could be detected (Fig.3). They were noted
203 band 1 to 6 according to their electrophoretic mobilities: bands 1 and 6 were respectively the
204 slowest and the fastest migrating isoforms. However, only four MyHC isoforms (bands 1, 3, 4
205 and 6), representing more than 1 % of total MyHC, could be detected by the densitometric
206 analysis. We therefore focused on these four isoforms (Fig.3 and Table 2).

207 *Developmental expression of MyHCs in Pectoralis major*

208 Band 1 could be observed in PM at the growth stages A to F and in adults (Fig. 3).
 209 Band 3 was only detected (5.6 ± 1.7 %) at stage E (3-4 weeks old chicks; table 2).

210 *Developmental expression of MyHCs in Gastrocnemius lateralis*

211 From stage A to D (up to 2 weeks old chicks), the expression of three isoforms (bands
 212 1, 4 and 6) was observed in the GL muscle (Fig. 3). Band 1 was the most predominant
 213 isoform and its relative percentage (67-76 %) did not vary significantly within this period
 214 ($P=0.100$; Table 2). The relative percentages of bands 4 and 6 were lower than band 1, and
 215 did not change significantly from stage A to C ($P=0.087$ and 0.350 , respectively). At stage D
 216 (1-2 weeks old chicks), the expression of band 4 was similar to stage A, while band 6
 217 decreased significantly ($P=0.001$; Fig. 3, Table 2).

218 At stages E and F (3-4 weeks and 1-2 months old chicks), only bands 1 and 4 were
 219 detected. Their relative percentages were similar to those of the adult group ($P>0.195$).
 220 However, when the chicks were 1-2 months old, band 1 expression increased and reached its
 221 highest contribution among chick groups (92.9 ± 2.5 %), while the variations of band 4
 222 expression were not significantly different.

223 *Developmental expression of MyHCs in Iliotibialis cranialis*

224 At stages A and B (1 week before hatching and 3 days post-hatching), three MyHC
 225 isoforms (bands 1, 4 and 6) were detected (Fig. 3). Band 1 was the most predominant isoform
 226 (about 72 %) while the relative proportions of bands 4 and 6 were lower (12-16%, Table 2).

227 At stage C (6-7 days old), band 3 was detected (16.2 ± 4.7 %; Fig. 3, Table 2). The
 228 relative contribution of bands 1 and 4 were not significantly different from the previous two
 229 stages ($P>0.118$), but band 6 decreased significantly by 1.6 fold in stage C compared with
 230 stages A and B ($P=0.002$) (Table 2).

231 From stages D to F (1 week to 2 months old), the expression of band 1 and 3 did not
 232 change significantly ($P>0.359$) whereas bands 4 and 6 disappeared (Table 2). In the adult
 233 group, the contribution of band 1 did not vary significantly compared with the chick groups
 234 ($P=0.380$). Band 4 was however observed, but its expression (16.9 ± 0.7 %) was not
 235 significantly different compared to stages A, B and C (up to 1 week old chicks; Table 2).

236

237 **DISCUSSION**

238 During the early life of king penguin chicks, their feet and flippers increased in size
239 and reached respectively 76.2 % and 43.1 % of their average adult size at two months of age.
240 At the end of the first period of growth, feet practically reached their adult size, while on the
241 contrary flippers reach their adult length later, at the end of the fledging period (Cherel et al.,
242 2004). In parallel, de Margerie et al. (2004) showed that the pelvic girdle of king penguin
243 chicks possessed the highest bone tissue growth rate during the first month after hatching. The
244 fast structural development of the lower limb thus allows chicks to rapidly acquire a bipedal
245 posture and pedestrian locomotion (Verrier, 2003). The survival of chicks at the beginning of,
246 full emancipation, is indeed linked to their ability to escape predators and to chase their
247 parents for food when they return from foraging trips.

248 Considering the pectoral muscle (PM), two isoforms could be detected from hatching
249 to the full emancipation of king penguin chicks. Band 1 was the most predominant isoform at
250 each stage, while band 3 only appeared with a low relative percentage for chicks aged 3-4
251 weeks old. These results diverge from those found in the domestic chicken, for which the
252 expression of five MyHCs has been detected in the developing pectoral muscle (Tidyman et
253 al., 1997): three embryonic MyHC isoforms are supplanted after hatching by a neonatal
254 isoform that is in turn replaced by an adult isoform. In the domestic chicken, PM has been
255 reported to contain almost FG fibres (Rosser et al., 1996); whereas only FOG fibres were
256 found in king penguin chicks PM (Erbrech et al., unpublished data). As these two fibre types
257 are linked to the expression of different MyHC isoforms during development (Rosser et al.,
258 1996), the variation in MyHC content between chicken and king penguins could result from
259 differences in the composition of the fibre types in this muscle. Surprisingly, muscle fibres of
260 the pectoral muscle of king penguin chicks expressed a MyHC profile similar to adults as
261 early as hatching. This result reveals an early maturation of the pectoral muscle contractile
262 proteins, although in king penguin chicks of 3-4 weeks old, the cross-sectional area of the
263 fast-twitch fibres is 30 times lower than in adults (Erbrech et al., 2008). These results contrast
264 with those found in the domestic pigeon (Rosser et al., 1998), where PM fibres reach their
265 adult MyHC composition and adult size after fledging. As fibre size is a major determinant
266 for the production of the mechanical force essential to locomotor activity (Olson, 2001), we
267 suggest that despite king penguin chicks PM expressed a mature MyHC profile, its muscle
268 fibres had not yet acquired the morphological characteristics necessary for the development of
269 an efficient locomotor activity. Thus, in king penguin chicks, the delayed development of PM

270 fibres compared to that found in leg muscles (Erbrech et al., 2008) is not linked to the MyHC
271 isoform type content.

272 Considering the pelvic girdle, we revealed a MyHC polymorphism in the GL and ITC
273 muscles of king penguin chicks, from hatching to about two weeks of age. Composition of
274 MyHC isoforms differentially changed in GL and ITC with the progressive acquisition of
275 terrestrial locomotion, in agreement with the fact that contractile activity is essential for the
276 maturation of avian skeletal muscle fibres (Bandman and Rosser, 2000). Changes in the
277 activity of muscle fibres are indeed linked to the expression of myosin isoforms, which can be
278 delayed or induced as a function of the intensity of muscle solicitation (Salmons and Sreter,
279 1976; Brown et al., 1983; Cerny and Bandman, 1987a). In the ITC muscle, bands 4 and 6
280 disappeared and were replaced by band 3 when the chick was 7-15 days old, corresponding to
281 the time when chicks are emerging from the brood patch and are standing in front of their
282 parents (Stonehouse, 1960; Barrat, 1976; Verrier, 2003; personal observations). In the GL
283 muscle, the proportion of band 6 decreased slightly until it disappeared at c.a. three weeks of
284 age, when chicks are starting to walk actively. As the principal function of ITC is to protract
285 the femur (Torrella et al., 1998), this muscle should be recruited early to maintain a bipedal
286 posture and to support the chick's body mass. In contrast, the GL muscle, involved in ankle
287 extension and knee flexion, is essentially recruited for bipedal locomotion (Smith et al.,
288 2006). At the time of emancipation (3-4 weeks), the GL muscle appeared to be already
289 mature, containing similar MyHC isoforms to adults. On the contrary, the ITC muscle had not
290 reached this state at the end of the brooding period: band 3, present in the emancipated chick,
291 was replaced by band 4 in adults. This switch can possibly be explained by the less intensive
292 use of this muscle in adults, in relation to their marine life. During the chick-rearing period,
293 young birds possess an exclusively terrestrial locomotion and therefore use more intensively
294 ITC muscles than adults that spend 75% of their time at sea, alternating travels at sea and
295 sojourns on land to moult and breed (Stonehouse, 1960; Barrat, 1976). However, to test this
296 hypothesis, it would be necessary to evaluate the MyHC isoform composition in older chicks,
297 particularly at the end of the second growth period, at approx. 1 year of age.

298 In addition to these hypotheses considering mechanical factors to explain changes in
299 myosin profiles, hormonal and thermal factors are likely to be involved. Thyroid hormones
300 (T3 and T4) in particular have been shown to induce changes in MyHCs during the
301 development of muscle fibres (Maruyama et al., 1993; Gardahaut et al., 1992). In the
302 domestic turkey, Maruyama et al. (1993) showed that the increase in thyroxine plasmatic
303 level (T4) could support the transition from embryonic to neonatal MyHCs during the

304 development of the breast muscle. Thus, in king penguin chicks, the progressive
305 disappearance of band 6 in the GL muscle, the disappearance of bands 6 and 4 followed by
306 the appearance of band 3 in the ITC muscle, together with the detection of band 3 in the PM
307 muscle, may be related to the increase in T4 plasma level also observed by Cherel et al.
308 (2004) in the same species during early growth. Moreover, at hatching, king penguin chicks
309 are essentially heterothermic, while the rapid improvement of thermoregulatory processes and
310 thermal insulation during the first two to three weeks of life allows them to acquire thermal
311 emancipation (Duchamp et al., 2002). Changes in plasma T4 levels (the major thermogenesis
312 regulating hormone) also correspond to the period when chicks gain independent
313 thermoregulation. Thermoregulatory capacity is critical for chicks survival during the sub-
314 antarctic winter when weather conditions deteriorate and parental food supply is restricted.
315 Studies undertaken by Duchamp et al. (2002) on GL and PM muscles indicate that muscular
316 shivering is the main thermogenic mechanism in growing chicks. Production of heat by
317 repetitive muscular contraction therefore requires a rapid maturation of skeletal muscles. The
318 development of endothermy in young birds requires the maturation of the neuromuscular
319 system, an increased muscular oxidative capacity, as well as the development of myofibrillar
320 ATPase in muscle fibres (Hohtola and Visser, 1998). Moreover, myofibrillar ATPase activity
321 is linked to myosin heavy chain composition (Rivero et al., 1996). In the pectoral muscle of
322 the domestic chicken, embryonic fast MyHCs were shown to possess less contraction velocity
323 than the neonatal isoform (Lowey et al., 1993a). In this context, changes in MyHC content
324 occurring in king penguin chick leg muscles could be related to the progressive acquisition of
325 homeothermy from their second week of life. Moreover, Duchamp et al. (2002) showed that
326 shivering in king penguin chicks PM, assessed by integrated electromyographic activity, was
327 revealed immediately after hatching, even though thermal insulation was not fully developed.
328 The MyHC adult profile in PM of young chicks could therefore indicate an early maturation
329 of the contractile muscular proteins that would allow shivering as early as hatching.
330 Furthermore, this muscle is known to be the major source of shivering and non-shivering
331 thermogenesis in adults (Duchamp et al., 1989).

332

333 In order to rapidly acquire an effective pedestrian locomotion essential to their
334 survival, chicks during early growth invest energy in the development of the pelvic girdle at
335 the expense of the pectoral girdle (de Margerie et al., 2004; Erbrech et al., 2008). Considering
336 the immature fibres size (Erbrech et al., 2008) and the mature MyHC content (this study) of
337 PM in young chicks, we also suggest that pectoral muscles are essential for thermoregulatory

338 functions at this stage of development while their locomotor function will be developed
339 several months later before departing to sea. Together, these results from king penguin chicks
340 illustrate the trade-off between muscle growth rate and functional capacity, as suggested for
341 several bird species (reviewed in Krijgsveld et al., 2001). At the end of these first weeks of
342 growth, king penguin chicks have acquired a functional pedestrian locomotion and are
343 thermally emancipated. However, their growth will still last several months where they have
344 to face a period of severe and prolonged under-nutrition during the sub-antarctic winter,
345 before finally departing to sea where they can forage independently. Throughout their winter
346 fast, together with their locomotor and thermoregulatory functions, skeletal muscles may act
347 as important protein reserves. One major task in future studies should therefore be to
348 investigate the effect of this winter energy restriction on muscle development (fibre size and
349 types, MyHC content) in both the pelvic and pectoral girdles when chicks are fully grown.

350

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477 Table 1: Muscle sampling schedule of king penguin chicks in relation to age, thermoregulatory capacity, locomotor activity and plumage
 478 (following Barrat, 1976; Stonehouse, 1960; Verrier, 2003).

479

Abbreviations	Age	Body mass range	Thermoregulatory capacity	Locomotor activity	Plumage
<i>Chicks</i>					
Stage A	Embryonic < 1 week before hatching	125-160g			Unfeathered
Stage B	Post hatching 1 – 3 days	< 300g	Heterotherm	Brooding phase: no locomotor activity	Unfeathered
Stage C	6 – 7 days	300-500g	Acquisition of homeothermy	Brooding phase: no locomotor activity	Brown down
Stage D	7 – 15 days	0.5-1kg	Homeotherm	Chicks sitting upright in front of their parent	Brown down
Stage E	3 – 4 weeks	1-2kg	Homeotherm	End of brooding phase: chicks begin to move away from adults	Brown down
Stage F	1 – 2 months	2-3kg	Homeotherm	Terrestrial locomotion: emancipated chicks wander alone on the colony	Brown down
<i>Adults</i>	> 4 years	10-12kg	Homeotherm	Aquatic locomotion: adults forage at sea and come on land to moult and breed	Feathers

480 Table 2: Distribution of myosin heavy chain (MyHC) isoforms in three muscles (*Pectoralis*
 481 *major*, *Gastrocnemius lateralis*, *Iliotibialis cranialis*) of king penguin chicks from hatching to
 482 emancipation (A: < 1 week before hatching, B: 1-3 days, C: 6-7 days, D: 7-15 days, E: 3-4
 483 weeks, F: 1-2 months) and adults.

484 N.D: not detected.

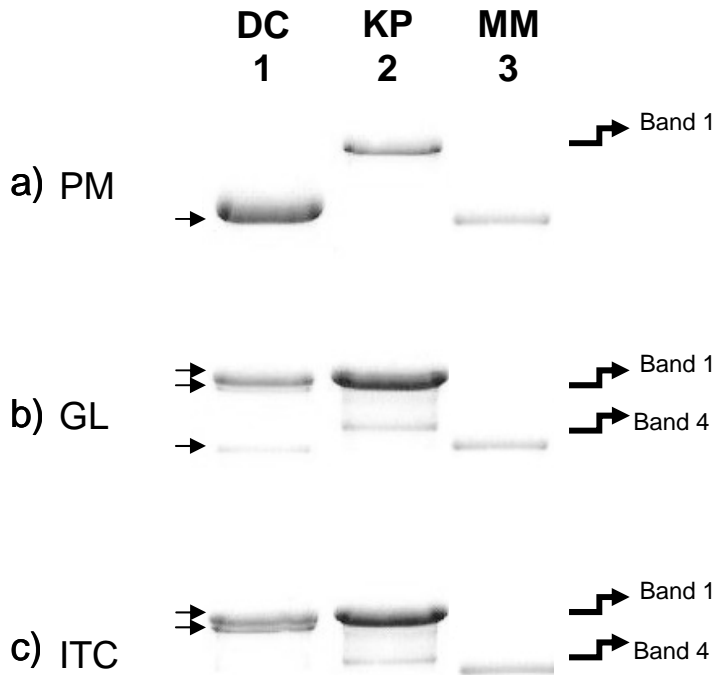
485 Ped. locomotion: pedestrian locomotion

486 Values are mean percentages of total myosin heavy chain \pm s.e.m

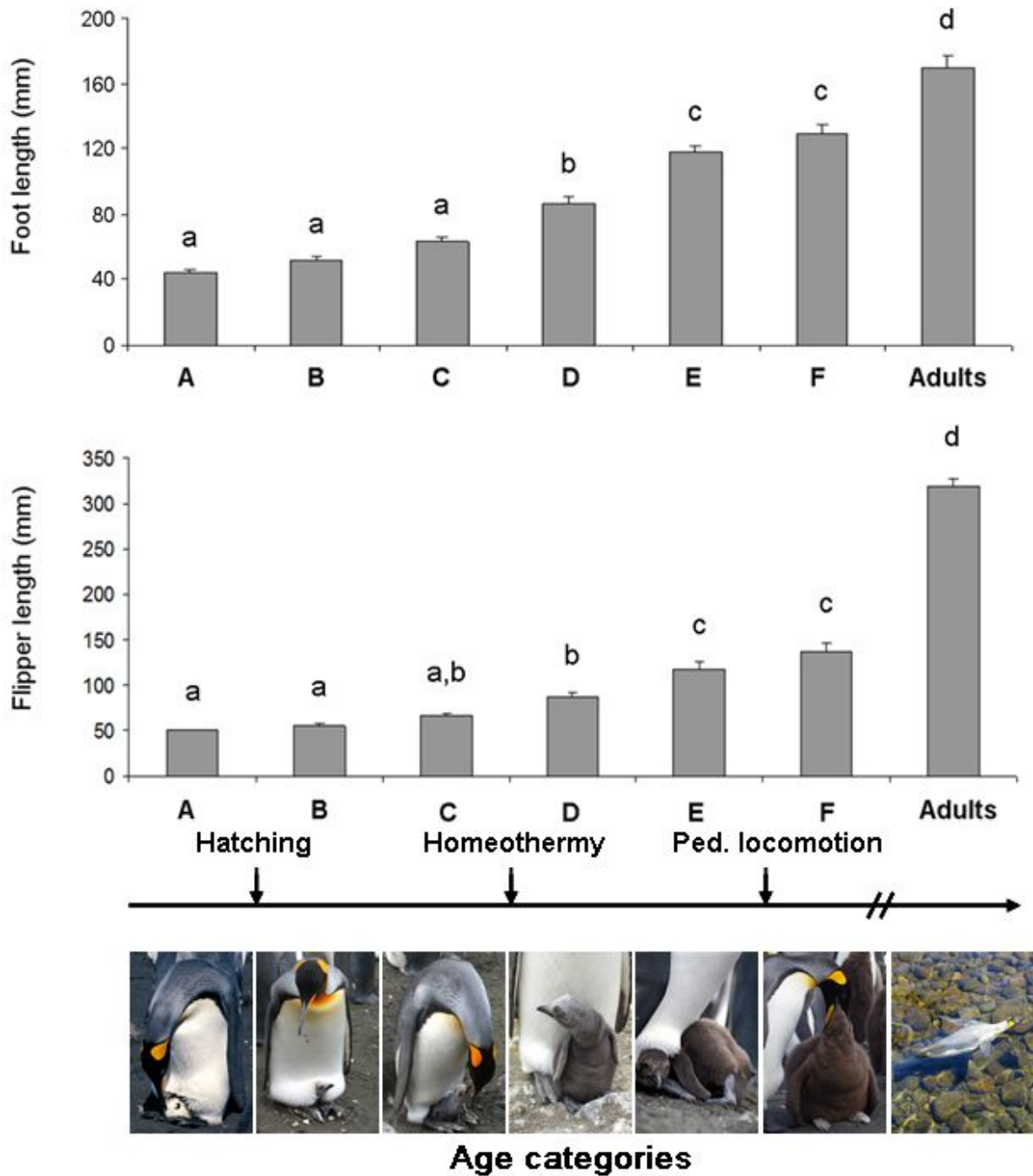
487 For a given muscle and MyHC band, different letters indicate significant differences between
 488 groups ($P < 0.05$).

	Groups	MyHC				n
		Band 1	Band 3	Band 4	Band 6	
<i>Pectoralis major</i>						
	Stage A	100.0	N.D	N.D	N.D	4
	Stage B	100.0	N.D	N.D	N.D	5
	Stage C	100.0	N.D	N.D	N.D	2
Homeothermy →	Stage D	100.0	N.D	N.D	N.D	7
	Stage E	94.4 \pm 1.7	5.6 \pm 1.7	N.D	N.D	6
Ped. locomotion →	Stage F	100.0	N.D	N.D	N.D	4
	Adults	100.0	N.D	N.D	N.D	5
<i>Gastrocnemius lateralis</i>						
	Stage A	67.0 \pm 3.5 ^a	N.D	15.9 \pm 1.5 ^a	17.1 \pm 2.1 ^a	4
	Stage B	72.5 \pm 1.5 ^{a,b}	N.D	14.0 \pm 0.7 ^a	13.5 \pm 1.1 ^{a,b}	5
	Stage C	67.1 \pm 1.9 ^a	N.D	18.3 \pm 1.0 ^a	14.6 \pm 1.2 ^{a,b}	3
Homeothermy →	Stage D	75.6 \pm 2.6 ^{a,b}	N.D	17.4 \pm 1.1 ^a	7.1 \pm 1.8 ^b	7
	Stage E	84.9 \pm 2.4 ^{b,c}	N.D	15.1 \pm 2.4 ^a	N.D	5
Ped. locomotion →	Stage F	92.9 \pm 2.5 ^c	N.D	7.1 \pm 2.5 ^a	N.D	4
	Adults	90.0 \pm 4.2 ^c	N.D	10.0 \pm 4.2 ^a	N.D	5
<i>Iliotibialis cranialis</i>						
	Stage A	71.9 \pm 5.1 ^a	N.D	12.4 \pm 4.4 ^a	15.7 \pm 1.3 ^a	4
	Stage B	72.5 \pm 1.6 ^a	N.D	15.0 \pm 1.0 ^a	12.5 \pm 0.7 ^a	4
	Stage C	69.5 \pm 2.1 ^a	16.2 \pm 4.7 ^a	6.4 \pm 3.2 ^a	7.9 \pm 0.3 ^b	3
Homeothermy →	Stage D	69.9 \pm 6.1 ^a	30.1 \pm 6.1 ^a	N.D	N.D	7
	Stage E	77.5 \pm 3.5 ^a	22.5 \pm 3.5 ^a	N.D	N.D	6
Ped. locomotion →	Stage F	76.4 \pm 1.8 ^a	23.6 \pm 1.8 ^a	N.D	N.D	4
	Adults	83.1 \pm 0.7 ^a	N.D	16.9 \pm 0.7 ^a	N.D	4

489



490
 491 Figure 1: Electrophoretic mobilities of myosin heavy chain (MyHC) isoforms found in
 492 *Pectoralis major* (a, PM), *Gastrocnemius lateralis* (b, GL) and *Iliotibialis cranialis* (c, ITC)
 493 in the domestic chicken (DC, lane 1) and the king penguin (KP, lane 2). Line 3 (MM)
 494 indicates the mobility of porcine myosin heavy chain of a standard molecular mass (200kDa;
 495 Laemmli 1970).



496

497 Figure 2: Mean lengths (mm, \pm s.e.m.) of the foot and flipper of chicks for the different age
 498 categories (A: < 1 week before hatching, B: 1-3 days, C: 6-7 days, D: 7-15 days, E: 3-4
 499 weeks, F: 1-2 months), and of adults. Different letters indicate significant differences between
 500 categories ($P < 0.05$).

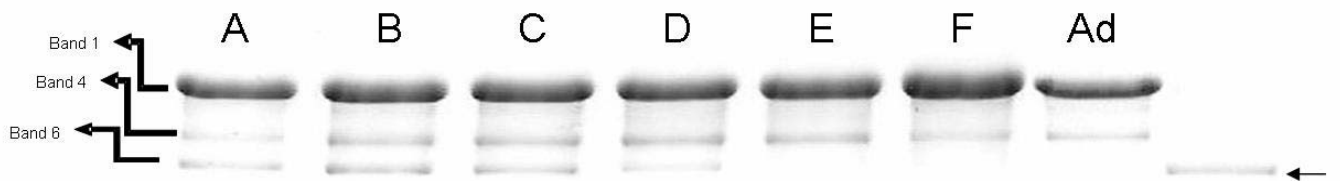
501 a) *Pectoralis major*



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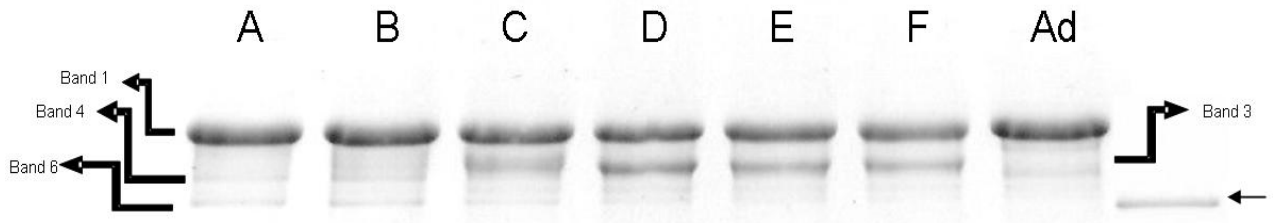
504 b) *Gastrocnemius lateralis*



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506

507 c) *Iliotibialis cranialis*



508

509

510 Figure 3: Myosin heavy chain isoforms of three different muscles (a, b, c) of king penguin
511 chicks at different ages and of adults revealed by SDS-PAGE electrophoresis. A, < 1 week
512 before hatching; B, 1-3 days; C, 6-7 days; D, 7-15 days; E, 3-4 weeks; F, 1-2 months; Ad,
513 adults. Small arrows represent porcine myosin heavy chain of a standard molecular mass
514 (200kDa; Laemmli 1970).

515