1

2   DR SHANE G. DUBAY (Orcid ID : 0000-0002-7221-7546)

3   PROFESSOR FUMIN LEI (Orcid ID : 0000-0003-2759-3435)

4

5

6   Article type   : Research Article

7

8

11   **TITLE**: Life history predicts flight muscle phenotype and function in birds

12

13   **AUTHORS:** Shane G. DuBay[1,2]*[†], Yongjie Wu[3]*, Graham R. Scott[4], Yanhua Qu[5], Qiao Liu[6], Joel H. Smith[7],

14   Chao Xin[8], Andrew Hart Reeve[9], Chen Juncheng[3], Dylan Meyer[7], Jing Wang[5,10], Jacob Johnson[7], Zachary A.

15   Cheviron[11], Fumin Lei[5,10], John Bates[2]

16

17   **AFFILIATIONS**

18   [1] Committee on Evolutionary Biology, University of Chicago, Chicago, USA

19   [2] Integrative Research Center, Field Museum of Natural History, Chicago, USA

20   [3] Key Laboratory of Bio-resources and Eco-environment of Ministry of Education, College of Life Sciences,

21   Sichuan University, Chengdu, China

22   [4] Department of Biology, McMaster University, Hamilton, Canada

23   [5] Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of

24   Sciences, Beijing, China

25   [6] Institute of Mountain Hazards and Environment, Chinese Academy of Sciences, China

26   [7] Department of Ecology and Evolution, University of Chicago, Chicago, USA

27   [8] Laboratory of Molecular Evolution and Molecular Phylogeny, College of Life Sciences, Shannxi Normal

28   University, Xi'an, China

29   [9] Biosystematics Section, Natural History Museum of Denmark, University of Copenhagen, Copenhagen,

30   Denmark

31   [10] University of Chinese Academy of Sciences, Beijing, China

32   [11] Division of Biological Sciences, University of Montana, Missoula, USA

33   [†] Present address: Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor,

34   USA

35   *** for correspondence**: dubaysg@umich.edu (SGD), wuyongjie@scu.edu.cn (YW)

36

37

ABSTRACT

1. Functional traits are the essential phenotypes that underlie an organism's life history and ecology. Although biologists have long recognized that intraspecific variation is consequential to an animals' ecology, studies of functional variation are often restricted to species-level comparisons, ignoring critical variation within species. In birds, interspecific comparisons have been foundational in connecting flight muscle phenotypes to species-level ecology, but intraspecific variation has remained largely unexplored.

2. We asked how age- and sex-dependent demands on flight muscle function are reconciled in birds. The flight muscle is an essential multifunctional organ, mediating a large range of functions associated with powered flight and thermoregulation. These functions must be balanced over an individual's lifetime.

3. We leveraged within- and between-species comparisons in a clade of small passerines (*Tarsiger* bush-robins) from the eastern edge of the Qinghai-Tibet Plateau. We integrated measurements of flight muscle physiology, morphology, behavior, phenology, and environmental data, analyzing trait data within a context of three widespread, adaptive life-history strategies – sexual dichromatism, age and sex-structured migration, and delayed plumage maturation. This approach provides a framework of the selective forces that shape functional variation within and between species.

4. We found more variation in flight muscle traits within species than has been previously described between species of birds under 20 g. This variation was associated with the discovery of mixed muscle fiber types (i.e. both fast glycolytic and fast oxidative fibers), which differ markedly in their physiological and functional attributes. This result is surprising given that the flight muscles of small birds are generally thought to contain only fast oxidative fibers, suggesting a novel ecological context for glycolytic muscle fibers in small birds. Within each species, flight muscle phenotypes varied by age and sex, reflecting the functional demands at different life-history stages and the pressures that individuals face as a result of their multi-class identity (i.e. species, age, and sex).

5. Our findings reveal new links between avian physiology, ecology, behavior, and life history, while demonstrating the importance of demographic-dependent selection in shaping functional phenotypic variation.

70   seasonal migration

71

72   **1. INTRODUCTION**

73   Functional traits are the essential phenotypes (physiology, morphology, phenology, etc.) that impact

74   survival, growth, and reproduction (Violle et al. 2007), and underlie the life history and ecology of an

75   individual. Although single traits can be critical to an organism's fitness (Grant and Grant 2002, Jones et al.

76   2018, Storz 2018), they do not evolve in isolation – they are parts of complex systems that must balance

77   multiple functional demands. These demands, however, can vary markedly by species, age, and sex, which

78   together impact the ways in which traits evolve and co-vary in populations (Bolnick et al. 2011). While we

79   have a general understanding of how age and sex impact explicitly reproductive traits in animals, we know

80   little about how these demographic axes shape other essential traits that are more broadly associated with an

81   organism's behavior and ecology. In this study, we leveraged within- and between-species comparisons in a

82   clade of small passerines to test how age- and sex-dependent demands on function are reconciled in the avian

83   flight muscle, an essential multifunctional organ in birds.

84

85   **1.1 Functional demands on the avian flight muscle**

86   The avian flight muscle has a pervasive impact on life history and ecology in birds, modulating

87   diverse sets of other functional traits that span morphology, physiology, behavior, and phenology (Rayner

88   1988, Scott et al. 2009, Dakin et al. 2018). The flight muscle facilitates a large range of functions associated

89   with powered flight and thermoregulation, as birds generate endogenous heat through muscle shivering (Marsh

90   and Dawson 1989). As the dominant muscle associated with flight, the pectoralis major is responsible for

91   generating lift and thrust, and it accounts for the majority of muscle mass in birds (Biewener et al. 1992). The

92   pectoralis is thus inextricably linked to life history and essential ecological tasks, like seasonal movements,

93   foraging/prey capture, predator avoidance, social interactions, and thermoregulation. The pectoralis must

94   consequently balance the diverse functional demands on powered flight and thermoregulation (Rosser and

95   George 1986, Scott et al. 2009, Segre et al. 2015, Dakin et al. 2018).

96   Comparative methods have uncovered striking variation in flight muscle form and function that reflect

97   taxon-specific selection on flight performance (Rosser and George 1986, Dakin et al. 2018). For example, bar-

98   headed goose (*Anser indicus*) populations that migrate over the Himalayas have accumulated changes in their

99   pectoralis phenotype that seem to enhance aerobic capacity and tissue oxygen supply to support prolonged

100   flight under extreme hypoxia (Scott et al. 2009, Hawkes et al. 2011). In hummingbirds, variation in flight

101   muscle size contributes to functional differences in flight performance and maneuverability (Segre et al. 2015,

102   Dakin et al. 2018). In comparison, island birds, which often face reduced predation pressure, have evolved

103   smaller pectoralis muscles than continental relatives. This could reflect a cost or a need for regular use to

104   maintain flight muscles, and it underscores the functional role of flight muscles in predator avoidance (Wright

105   et al. 2016). Studies comparing species with contrasting ecologies, such as these, have been foundational in

106 connecting flight muscle phenotypes to species-level ecology, but critical variation that exists within species
107 remains largely unexplored. Without considering selection on flight muscle function along intraspecific axes,
108 like age and sex, we are limited in our ability to address fundamental questions about the processes that shape
109 phenotypes over the evolutionary history of a lineage and over the lifetime of an individual.

110   By accounting for variation along intraspecific axes of age and sex, we can test how multiple shifting
111 demands on flight muscle function are reconciled across the lifetime of an individual and between sexes. When
112 used in concert with interspecific comparisons, this approach can reveal how flight muscle phenotypes are
113 differentially optimized by age, sex, and species to meet and balance identity-dependent demands on function.
114 Male birds, for example, have a general tendency in migratory species to arrive earlier to breeding grounds in
115 the spring to establish and defend territories (Morbey and Ydenberg 2001), which might increase selective
116 pressures to invest resources in flight muscle traits that enhance competitive ability and cold tolerance. Female
117 birds, however, may benefit from maximizing direct investment in reproductive traits, like egg formation,
118 which could potentially draw from protein stores in the pectoralis (Raveling 1979). If demands on the flight
119 muscle differ between males and females, then we would expect to find associated variation in their flight
120 muscle phenotypes. Similarly, within a sex, social dynamics are often structured by age class, with older
121 individuals being socially dominant (Rohwer 1982, Lyon and Montgomerie 1986, Hawkins et al. 2012). If
122 older birds are competitively dominant, then we would expect these individuals to have flight muscle traits that
123 enhance competitive ability. Under these hypotheses, we expect to find age- and sex-structured variation in
124 flight muscle phenotypes that reflects the distinct demands on each demographic class.
125
126 **1.2 Muscle response to functional demands**
127   The diverse functional demands on the pectoralis muscle can be met in part by modifying underlying
128 muscle components, such as muscle fiber composition (Scott et al. 2009). The pectoralis of most volant birds is
129 composed exclusively of the two fast-twitch skeletal muscle fiber types, fast oxidative (FO) fibers and fast
130 glycolytic (FG) fibers, and considerable variation exists across species in the relative abundance of these fibers
131 in the pectoralis (Rosser and George 1986, Welch and Altshuler 2009). For instance, the pectoralis of small-
132 bodied birds is often exclusively comprised of FO fibers, whereas the pectoralis of larger birds contains a
133 much greater proportion of FG fibers (Rosser and George 1986, Welch and Altshuler 2009). Only non-volant
134 and soaring species typically incorporate the third main skeletal muscle fiber type, slow oxidative fibers,
135 underscoring the demand on fast-type fibers for powered flight (Rosser et al. 1994, Meyers and Stakebake
136 2005). Fiber types differ markedly in their physiological and functional attributes (Peter et al. 1972), and their
137 relative abundance within the pectoralis should fundamentally impact an individual's capacity for powered
138 flight and thermogenesis. FO fibers are aerobic and can sustain muscle contractions over longer periods of time
139 (Peters et al. 1972). By increasing these fatigue-resistant FO fibers, the pectoralis can enhance functions
140 associated with sustained aerobic performance, like shivering thermogenesis and powered flight. In contrast,
141 FG fibers contain few mitochondria and are primarily anaerobic, but they can generate high mechanical power

142 in short bursts (Peters et al. 1972). FG fibers lack endurance and are not typically recruited during sustained

143 shivering thermogenesis, but are often associated with short-burst flight capacity, like predator avoidance,

144 takeoff, and other rapid flight maneuvers (Dial et al. 1987, Askew et al. 2001). Through variation in underlying

145 flight muscle fiber composition, species have achieved remarkably diverse ecologies, and while variation in

146 fiber composition within species might also underlie age and sex differences in behavior and ecology, this

147 remains untested.

148

149 **1. 3 Study system: Himalayan Bush-robins**

150   We studied three closely related species of *Tarsiger* bush-robins (*Tarsiger indicus*, *T. chrysae*us, and

151 *T. rufilatus*) that show species-specific ecologies associated with life along seasonal and elevational gradients

152 in the temperate mountains of Asia. Bush-robins are small passerine birds (12-16 g) that are sexually

153 dichromatic and socially monogamous (Morimoto et al 2006). All three species show delayed plumage

154 maturation, in which first-year males (hereafter referred to as subadults) retain female-like plumage despite

155 being fully capable of breeding. Delayed plumage maturation is a widespread, adaptive life-history strategy in

156 which subadult males signal their inferior competitive status to avoid the costs of direct competition against

157 socially dominant adult males (Hawkins et al. 2012). All three species also engage in altitudinal migration, an

158 understudied but widespread behavior in montane animals (Boyle 2017, Hsiung et al. 2018). Bush-robin

159 species, however, show striking differences in their winter elevational distributions – the three focal species

160 breed in sympatry above 3000 m in the Himalayas and Hengduan mountains of Asia, but migrate downslope to

161 different elevations for the winter (Fig. 1). Species thus experience different environmental pressures over the

162 course of a year, particularly during the harsh montane winter. Furthermore, we show here that each species

163 also exhibits age- and sex-structured differences in the timing of migration, which is another widespread,

164 adaptive life-history strategy in which differences in migratory timing have evolved between sexes and

165 between age classes to optimize lifetime reproduction (Morbey and Ydenberg 2001).

166   We leveraged within- and between-species comparisons in *Tarsiger* bush-robins, integrating

167 measurements of flight muscle physiology, morphology, behavior, phenology, and environmental data to ask

168 how age- and sex-dependent demands on flight muscle function are reconciled in this essential multifunctional

169 organ. We analyze and interpret flight muscle phenotypes within the context of the three classic life-history

170 strategies described above – sexual dimorphism, delayed plumage maturation, and age- and sex-structured

171 migration (Morbey and Ydenberg 2001, Hawkins et al. 2012). This approach provides the necessary

172 framework to interpret our results by evaluating the different selective forces acting on age and sex classes. By

173 extending beyond species-level comparisons, we discovered age- and sex-structured variation in flight muscle

174 size and fiber composition that appears to reflect discrete life-history stages and the distinct selective pressures

175 that individuals face as a result of their multi-class identity. Our findings suggest new links between avian

176 physiology, ecology, behavior, and life history, highlighting the importance of age-and sex-dependent

177 selection in shaping functional phenotypic variation.

178

2. MATERIALS AND METHODS

2.1 Study site, temperature data, and sampling

181   We caught bush-robins (n = 329) over three consecutive spring seasons as they migrated upslope to

182 breeding elevations in Hailuogou Valley, Sichuan, China (29.576096°N, 101.998624°E), on the eastern edge

183 of the Qinghai-Tibet Plateau (Fig. 1). In this region, the three species are each other's closest relatives,

184 breeding in sympatry above 3000 m elevation (Fig. 1). Birds were caught at 3000 m elevation using mist-nets

185 between the dates of April 5th–April 22nd in 2014, March 24th–April 26th in 2015, and March 24th–May 10th in

186 2016. On these days, 8-13 12-meter mist-nets were open from sun-up (+/- 1hr) to sundown (+/- 1hr). All

187 individuals were caught within one kilometer of a local weather station in subalpine forest as they moved

188 between elevations (Fig. 1), either executing annual spring migration or short-term elevational movements in

189 response to acute early-spring cold weather events. We recorded maximum and minimum temperatures for

190 each individual from the day they were caught, which we collected from the weather station at 3000 m run by

191 the Institute of Mountain Hazards and Environment, Chinese Academy of Sciences. All individuals used in

192 this study were binned into one of three demographic classes: adult male, subadult male, and female. We

193 treated females as a single group because female age classes are indistinguishable by plumage.

194   Mass was recorded for individuals upon capture, after which birds were either sacrificed for

195 characterization of pectoralis phenotype (n = 129), kept in captivity for other research (n = 36), or immediately

196 released back into the environment at 3000 m (n = 164). In 2014, 11 individuals were target netted, rather than

197 being passively caught. These 11 individuals were thus excluded from arrival time and temperature analyses.

198 Data for each individual can be found in the associated dataset. For all released birds, we uniquely trimmed

199 one to two tail feathers (<1cm of the tip) to identify if they were recaptured within a given year. For example,

200 one individual might have rectrix right 6 trimmed, while another individual might have right 6 and left 1

201 trimmed. Bush-robins annually molt tail feathers, so it is unknown if individuals were recaptured across years.

202 However, within a given year recapture rates were relatively low – only 21 released individuals were caught

203 more than once (over the 3 years: 1 subadult male *T. indicus*, 1 adult male *T. chrysaeus*, 3 adult male *T.*

204 *rufilatus*, 9 subadult male *T. rufilatus*, and 7 female *T. rufilatus*). For individuals that were caught more than

205 once in a year, we report body mass from the day the individual was first caught. We report temperature values,

206 however, from the coldest day an individual was caught (determined from the minimum daily temperature) to

207 specifically test hypotheses associated with cold sensitivity. Because the sex of bush-robins with female-like

208 plumage cannot be determined visually, all released birds with female-like plumage were genetically sexed

209 from blood samples following methods described by Griffiths et al. (1996). All other birds were sexed by

210 examining gonads during dissection. Birds were netted and collected with authorization from Gongga Shan

211 National Nature Reserve and Hailuogou Forestry Bureau in compliance with the National Wildlife

212 Conservation Law of China, and birds were handled in accordance with the Institutional Animal Care and Use

213 Committee at the University of Chicago.

214

2.2 Pectoralis mass and histology

215

216   Over the three sampling years, a subset of individuals of each species (*T. indicus* n = 34, *T. chrysaeus*

217   n = 31, *T. rufilatus* n = 64, total n = 129) were euthanized using isoflurane anesthesia and thoracic compression

218   on the day of capture. For this subset, we measured pectoralis muscle phenotypes. We weighed pectoralis

219   muscles and quantified muscle fiber type. We dissected out the right flight muscle within minutes of each

220   individual being euthanized. The pectoralis major was then separated from the supracoracoideus and the entire

221   right pectoralis was weighed. We report total organism pectoralis mass as the right pectoralis multiplied by

222   two. We then dissected out the middle third of the right pectoralis (in relation to the length of the keel), coating

223   this muscle section in O.C.T. embedding medium, which was then flash-frozen in liquid nitrogen-cooled

224   isopentane. Embedded muscle was then sealed in aluminum foil and stored in liquid nitrogen until being

225   transferred into a -80 °C freezer, where they were stored until being sectioned.

226   Muscle fiber types were characterized in the pectoralis using methods described by Deveci et al.

227   (2001). Pectoralis muscles were sectioned (10 μm) in a cryostat at -20 °C, transverse to the length of the

228   muscle fibers. Sections were then stained for myosin-ATPase (preincubation at pH 4.4) and succinate

229   dehydrogenase (SDH) activity to distinguish muscle fiber types (Deveci et al. 2001). We imaged the stained

230   sections with a light microscope and used stereological methods described by Eggington (1990) to make

231   unbiased measurements of numerical fiber density, areal fiber density, and average transverse area of each

232   fiber type. For each sample, we analyzed 12 images to account for fiber heterogeneity within the muscle,

233   which we determined in preliminary measurements to be a sufficient number of images to achieve a stable

234   mean value for an individual bird. The mean areal densities of each fiber type were used as an estimate of the

235   proportion of muscle volume composed of that fiber type. This approach allowed us to multiply mean areal

236   density by pectoralis mass to calculate the total mass of each fiber type in the pectoralis for each individual.

237   This calculation assumes that each fiber type had the same tissue density (mass per volume), and therefore

238   ignores the small potential differences in tissue density that could arise from differences in lipid content

239   between fiber types. However, intracellular lipid volume density differs by <5% between FO and FG fibers in

240   bird flight muscle (Scott et al. 2009), so the differences in tissue density between fiber types may be even

241   smaller. Fiber type was preferentially assessed using myosin-ATPase stained sections unless these sections

242   were unavailable (due to tissue damage during staining procedure). In these cases, we quantified fiber type

243   from SDH stained sections. We confirmed that fiber-typing outcomes were consistent between the two staining

244   methods by comparing a subset of individuals in which both staining methods were analyzed (Fig. S1).

245

2.3 Vouchered specimens and morphometric measurements

246

247   All birds collected for pectoralis phenotyping are vouchered as specimens at the National Zoological

248   Museum of the Institute of Zoology (Chinese Academy of Sciences) or at the Nature Museum of Sichuan

249   University, except for five individuals that were stolen by a Siberian weasel (*Mustela sibirica*) at our study site

250 in 2015. The list of vouchered specimens can be found in the associated dataset. With these specimens, we
251 measured tarsus length and Kipp's index as proxies for ecological differences among species (Kipp 1959).
252 Tarsus length and Kipp's index (i.e. wing shape) are standard morphological measurements that are strongly
253 linked to ecology (Lockwood et al. 1998). We report Kipp's index on a size-independent scale (0-1) from short,
254 rounded wings to long narrow wings (Kipp 1959). Kipp's index was calculated as: (length of longest primary
255 feather – length of first secondary feather) / length of longest primary feather.
256
257 **2.4 Statistical analyses**
258   We tested for statistical differences among species, between sexes, and between male age classes with
259 analysis of variance tests. We report means and standard errors for each trait by species, age, and sex class. We
260 analyzed all flight muscle traits (i.e. pectoralis mass, FG fiber mass, and FO fiber mass) as raw values and
261 relative values (calculated as mass of the given trait divided by body mass), but the interpretations of results
262 remain the same with and without controlling for differences in body mass. We tested for differences among
263 species in each trait using ANOVAs (type III sums of squares) with accounting for class identity (i.e. adult
264 male, subadult male, female) and year (2014, 2015, 2016) as additional predictor variables. We then performed
265 a Tukey's post-hoc test to determine which pairwise species comparisons differed significantly for each trait.
266 Within each species, we then tested for sex differences in each trait with ANOVAs (type III sums of squares),
267 accounting for year as a predictor variable. For these analyses, adult males and subadult males were analyzed
268 together relative to females. Analyses of sex differences were conducted for each species independently. We
269 then tested for age differences in males for each trait with ANOVAs (type III sums of squares), also accounting
270 for year as a predictor variable. Analyses of age differences were conducted for each species independently.
271 Differences were treated as significant if p-values were < 0.05, and effect sizes were calculated from ANOVAs
272 as partial eta squared (η^2).
273
274 **3. RESULTS**
275 **3.1 Variation in flight muscle phenotype**
276   Pectoralis mass and body mass varied foremost by species and sex class – pectoralis muscles and body
277 mass were larger in *T. indicus* and *T. rufilatus* than in *T. chrysaeus*, and larger in males than in females (Fig. 2;
278 Tables 1 and 2). Despite the small body size of bush-robins (12-16 g), we discovered the presence of both fast-
279 type muscle fibers (FO and FG) in the pectoralis of all three species (a representative image of a bush-robin
280 pectoralis with mixed fiber types can be seen in Fig. 3). We found no evidence of slow oxidative fibers in the
281 pectoralis of bush-robins. The relative proportions of FO and FG fibers that we observed within the pectoralis
282 of bush-robins varied consistently within species by age and sex (Fig. 4). This intraspecific variation was
283 greater within species than has been previously described between species under 20 g (Rosser and George
284 1986; Lundgren and Kiessling 1988).
285   When we considered the relative contributions of each fiber type to total pectoralis mass, the total

masses of both FO fibers and FG fibers – calculated as the product of muscle mass and the areal density of each fiber type – were larger in males than females (Fig. 4; Table 2; see Fig. S2 for areal density, numerical density, and average traverse area for FG and FO fibers by species, age, and sex class, as well as average fiber size, which combines FG and FO fibers). FO fiber mass did not differ between male age classes, but FG fiber masses were consistently larger in adult males than subadult males (Fig. 4; Table 3), reflecting increased investment by adult males in FG fibers. Among species, *T. rufilatus* had the largest FO fiber mass (Fig. 4a,b), which is consistent with a more flighted ecology as suggested by its longer wings and shorter legs (Fig. S3) (Lockwood et al. 1998). *T. indicus* and *T. chrysaeus*, however, have similar wing shapes and tarsus lengths (Fig. S3), and had strikingly similar FO fiber mass, despite *T. indicus* having larger pectoralis muscles (Fig. 4a,c; Table 1). The differences in pectoralis mass between *T. indic*us and *T. chrysaeus* reflect increases in FG fibers in *T. indicus*, rather than changes in FO fiber mass.

3.2 Variation in timing of arrival to breeding elevations

Bush-robins differed within and among species in their timing of migration to breeding elevations. Arrival order among species was associated with wintering elevation and seasonal temperature – species that wintered higher arrived earlier to breeding elevations (Fig. 5a; Fig. S4), and birds that arrived earlier experienced colder temperatures (Fig. 5b; Fig. S5; Tables 1-3). *T. indicus* arrived first, followed by *T. rufilatus* and then *T. chrysaeus*. Within each species, we observed the same qualitative patterns of age- and sex-structured migration, in which adult males arrived before subadult males, which in turn arrived before females (Fig. 5b). The one exception to this pattern is with *T. chrysaeus* females, which arrived between adult and subadult males. This exception might reflect low sample sizes for this group or terminating sampling before the completion of female migration for *T. chrysaeus*. In each sampling year, some males of *T. indicus* were already singing above 3000 m on the breeding grounds when we began sampling, indicating that these individuals had already passed through our sampling area. This observation implies that we are conservatively estimating the arrival time of *T. indicus* males as well as the disparity in arrival time and experienced temperature between *T. indicus* age and sex classes.

3.3 Association of flight muscle phenotypes with arrival and seasonal temperature

Species, age, and sex classes caught earlier in spring, when temperatures were colder, generally had larger body mass, as well as larger raw and relative pectoralis masses (Fig. 6a,b; Fig. S6a; and Fig. S7a,b,e; Tables 1-3). The association between pectoralis mass and temperature appears to be driven by differences in FG fiber mass among and within species. That is, species, age, and sex classes that were caught earlier, when temperatures were colder, have increased FG fiber mass in their pectoralis (Fig. 6c; Fig. S6b; Fig. S7c,f). This result is counterintuitive, as the other fiber type (FO fibers) is believed to contribute to the majority of sustained thermogenesis, while FG fibers contribute very little (Marsh and Dawson 1989), yet individuals that experience colder temperatures are investing resources in FG fibers, which could be otherwise invested in FO

322 fibers. The larger FO fiber mass in males than females was generally associated with earlier arrival and colder

323 temperatures, but differences among species in FO fiber mass were not (Fig. 6d, Fig. S6c; Fig. S7d,g).

324

325 **3.4 Variation in sensitivity to cold stress and extreme weather events**

326   We found that bush-robins vacate breeding territories in response to acute cold weather events in early

327 spring. In response to these events, bush-robins shifted downslope or halted migration upslope, returning to

328 breeding elevations as soon as temperatures warmed, even when snow still covered the ground (Fig. S8). The

329 propensity for birds to vacate breeding elevations, however, varied by species, age, and sex in association with

330 wintering elevation and arrival order. That is, species that winter higher were more likely to persist at breeding

331 elevations through acute cold weather events, as were adult males (Fig. S8). This behavioral flexibility

332 confounds and introduces noise into our arrival time data because not all captures at 3000 m reflect an

333 individual's first arrival for the given season. Nevertheless, despite these events, we recovered species-, age-,

334 and sex-structured differences in occurrence patterns at 3000 m elevation (the lower elevational breeding limit)

335 that are associated with wintering elevations and temperature.

336

337 **4. DISCUSSION**

338 **4.1 Evidence for a new ecological context of fast glycolytic fibers**

339   The discovery of mixed fiber types in the pectoralis of bush-robins is striking given their small body

340 size (12-16 g) because the pectoralis of most birds under 20 g had previously been found to contain only FO

341 fibers (Rosser and George 1986, Welch and Altshuler 2009). Given that FO fibers alone are sufficient to meet

342 all functional demands on the pectoralis in other small-bodied birds (Dakin et al. 2018, Welch and Altshuler

343 2009), the presence of mixed fibers in bush-robins suggests a new functional context for FG fibers. In larger

344 birds, FG fibers are essential for short but high intensity bursts of muscle contraction, like those needed for

345 takeoff, because high mechanical power is required to lift larger birds into the air (Dial et al. 1987). Smaller

346 birds, however, are less constrained by mass-specific demands on fiber type for takeoff, and FO fibers are

347 generally thought to meet all of their functional demands of powered flight (Welch and Altshuler 2009). To

348 date, FG fibers have been observed in the pectoralis of only two other species under 20 g, the Ovenbird

349 (*Seiurus aurocapilla*; Rosser and George 1986) and the European Robin (*Erithacus rubecula*; Lundgren and

350 Kiessling 1988), a close relative of *Tarsiger* bush-robins (Sangster et al. 2010). It is possible, however, that

351 these fibers may be more taxonomically widespread since fiber composition has been characterized in less than

352 5% of bird species (which we estimated from a literature search).

353   If FG fibers are not necessary for takeoff in small birds, then their presence in robins might be

354 associated with alternative forms of high-intensity flight, such as burst flights associated with competitive

355 interactions and/or predator avoidance. The high mechanical power of FG fibers should enhance short-burst

356 flight capacity, improving for example an individual's ability to secure and defend breeding territories and

357 mates (Altshuler 2005). Under this hypothesis, we would expect to find increases in FG fibers in socially

358   dominant individuals, which we found support for in all three bush-robin species (Fig. 4b). Males had more FG

359   fibers than females, and adult males had more FG fibers than subadult males. Alternatively, in sexually

360   dichromatic species, like bush-robins, adult males might experience increased predation risk due to bright,

361   conspicuous plumage or reduced vigilance during competitive interactions (Götmark 1993, Jakobsson et al.

362   1995). Under this hypothesis, adult males would benefit from FG fibers and the associated increases in short-

363   burst flight capacity to avoid predation. These two hypotheses (i.e. competition and predation) are not mutually

364   exclusive, but require further testing to tease apart. Nevertheless, the presence of FG fibers in bush-robins

365   represents a unique functional context in which variation in muscle phenotypes in small birds appears to be

366   tied to life-history trade-offs and social demands.

367

368   **4.2 Sex-dependent selection on flight muscle phenotype**

369   We found that male bush-robins generally arrived to breeding grounds before females (Fig. 5b), which

370   is a common migratory strategy in birds (Morbey and Ydenberg 2001). This life-history strategy is assumed to

371   arise through sex-dependent selection that increases reproductive success in one or both sexes (Morbey and

372   Ydenberg 2001). A number of hypotheses have been proposed to explain the adaptive significance of sex-

373   structured migration. For example, territorial males may be under selection to arrive earlier to breeding

374   grounds to establish territories (Myers 1981). This hypothesis is supported by a preponderance of work

375   showing early-arriving males achieve higher reproductive success (Perrins 1970, Lozano et al. 1996). In

376   addition, females may be under stronger selection to arrive later to avoid unfavorable environmental conditions

377   in early spring (Ketterson and Nolan 1983). This hypothesis is particularly notable in our temperate, high-

378   elevation context because female bush-robins should disproportionately benefit from investing resources

379   directly into reproductive traits, such as egg formation, rather than investing in muscle changes that enhance

380   cold tolerance (Wedell et al. 2006). Although the fitness consequences of sex-structured migration in bush-

381   robins remain unknown, this adaptive life-history strategy exposes males and females to different

382   environmental and social pressures (Fig. 5b). Male bush-robins should thus benefit from morphological and

383   physiological traits that enhance thermogenic capacity and competitive ability, like increases in both FO and

384   FG fibers, facilitating early arrival and territoriality, while females should benefit from later arrival to

385   breeding grounds when conditions are more favorable, allowing them to maximize direct investment in

386   reproductive traits, like gametes and parental care (Wedell et al. 2006). Despite a conservative estimate of

387   male arrival for *T. indicus* (due to the fact that males were already singing at breeding elevation when we

388   began sampling), we still recovered signals of sex- and age-structured variation in experienced temperature.

389   The variation we observed between sexes in flight muscle phenotypes is consistent with the sex-

390   dependent selection associated with sex-structured migration. Compared to females, male bush-robins showed

391   increases in the total mass of fatigue-resistant FO fibers in the pectoralis, which is expected to enhance the

392   capacity for shivering thermogenesis (Swanson et al. 2013, Petit and Vézina 2014). Males also showed greater

393   total mass of the more powerful FG fibers, which should enhance short-burst flight capacity, which supports

394   the hypothesis that FG fibers may play a role in social dynamics and competitive interactions in bush-robins.

395   Increases in both muscle fiber types appear to have evolved in association with environmental and social

396   demands on males, suggesting that functional trade-offs at the fiber level might be overcome by making the

397   whole flight muscle bigger. In contrast, females show reduced investment in the pectoralis in association with

398   their later arrival to breeding elevations. This reduced investment was generally associated with reduced

399   investment in both fiber types (Fig. 4), but the lower proportional abundance of FG fibers in females (Fig. S2)

400   suggests that females reduce investment in this fiber type by a greater magnitude. By arriving when spring

401   temperatures are warmer, and after males have established territories, females should experience reduced

402   pressure to allocate resources into flight muscles that increase cold tolerance and competitive traits. Instead,

403   females can invest resources in reproductive traits outside of the flight muscle. These results underscore how

404   the tensions shaping flight muscle phenotypes, and functional optima, differ between sexes.

405

406   **4.3 Age differences in social dominance and flight muscle phenotype**

407   The variation in FG fiber mass between male age classes further supports the hypothesis that FG

408   fibers are important in social dynamics and competitive interactions. Adult males showed increased FG fiber

409   mass compared to subadult males (Fig. 4b), and given what we know about the physiological and functional

410   differences between muscle fiber types (Peters et al. 1972), this age-structured variation in muscle morphology

411   should contribute to greater competitive abilities in adult males. If males are under selection to arrive early to

412   secure breeding territories, then traits that enhance territory defense and competitive ability, like FG fibers,

413   should also be under selection to increase. The lower number of FG fibers in subadult males likely contributes

414   to their reduced competitive ability, which is further supported by their migratory behavior and plumage

415   phenotype. If subadult males are competitively inferior to adult males because they have less capacity for burst

416   muscle performance (i.e. they have fewer FG fibers), then subadult males should benefit from avoiding costly

417   interactions, like fighting, with dominant males. Subadult males appear to avoid these costly interactions by

418   arriving later to breeding elevations, and by retaining female-like plumage through delayed plumage

419   maturation. The life-history strategy of delayed plumage maturation allows subadult males to honestly and

420   effectively signal their inferior competitive ability to avoid costly aggressive interactions (Hawkins et al. 2012).

421   Our results imply that it is not only experience in breeding and fighting that makes subadult males

422   inferior (Arcese and Smith 1985), but that they have invested less in various flight muscle traits, which may

423   render them less competitively able than their adult counterparts. At this time, the proximate causes of this

424   reduced investment in FG fibers are unknown, but in subadult males, they may simply need more time to grow

425   and develop the phenotype typical of adult males. The proximate causes might also include carryover effects

426   from winter in which traits that impact breeding success, such as body condition or the timing of migration, are

427   linked to wintering habitat quality (Harrison et al. 2011, Studds and Marra 2005). If subadult-male bush-robins

428   occupy lower quality wintering habitats, this would likely have downstream consequences on their ability to

429   allocate resources to competitive traits, such as FG fibers. Irrespective of the potential causes, however, our

430 findings provide some of the first evidence to suggest that variation in functional traits might be associated
431 with delayed plumage maturation and the competitive inferiority of subadult males.
432
433 **4.4 Species differences in ecology and flight muscle phenotype**
434   We found that species that arrive earlier in spring have larger pectoralis muscles than later arrivals,
435 which is consistent with past work showing that cold tolerance increases with flight muscle size (Swanson et al.
436 2013, Petit and Vézina 2014). The relationship between flight muscle mass and cold tolerance, however, has
437 been previously defined in species whose pectoralis muscles contain only FO fibers (e.g. the American
438 Goldfinch [*Spinus tristis*] and Black-capped Chickadee [*Poecile atricapillus*]; Rosser and George 1986,
439 Swanson et al. 2013, Petit and Vézina 2014). In bush-robins, when we consider the relative contributions of
440 fiber type to overall pectoralis mass and function, *T. indicus* and *T. chrysaeus* have strikingly similar FO fiber
441 mass (Fig. 4a,c), despite experiencing dramatically different thermal environments in a given year. The
442 differences in pectoralis mass between *T. indicus* and *T. chrysaeus* are thus not due to changes in FO fiber
443 mass, but rather differences in FG fiber mass. The predominant expectation is that FG fibers could contribute
444 to short bursts of high intensity shivering, supported by anaerobic metabolism, but the low mitochondrial
445 content of FG fibers makes them unlikely to contribute to long periods of sustained thermogenesis (Marsh and
446 Dawson 1989). These results underscore the need to account for fiber-type variation when interpreting
447 differences in flight muscle size, while also highlighting the need to better understand the phylogenetic extent
448 of mixed fibers in the pectoralis.
449   Among species, the staggered pattern of migration suggests a connection between seasonal
450 distributions, migratory distance, phenological events, and cold tolerance. These data suggest that *T. indicus* is
451 more cold tolerant than *T. rufilatus*, which is more cold tolerant than *T. chrysaeus*. Under thermogenic
452 predictions alone, we would expect *T. indicus* to have pectoralis phenotypes that confer adaptive increases in
453 cold tolerance, such as increases in the mass of FO fibers. *T. rufilatus*, however, had the highest FO fiber mass.
454 This discrepancy can be reconciled when we consider species-specific ecology, migratory distance, and that a
455 system of interconnected traits within the flight muscle and organism work in concert to define whole-
456 organism metabolism and capacities for thermogenic and locomotory performance (McClelland and Scott
457 2019; Storz and Scott 2019). First, *T. rufilatus* appears to have a more flighted ecology than the other two
458 species, as suggested by morphometric data (Fig. S3), which might drive increased FO fiber mass in *T.
459 rufilatus*. *T. rufilatus* and *T. chrysaeus* also migrate further than *T. indicus*, which may require higher
460 proportional FO fiber content to aid in these seasonal movements (Dawson et al. 1983) (Fig. S2). This FO fiber
461 requirement may be especially true for *T. chrysaeus*, which migrates the furthest despite being the least
462 flighted and most terrestrial (as inferred from wing and leg data; Fig. S3; de Hoyo et al. 2005).
463   The predicted differences in cold tolerance between species might be achieved through variation in
464 other traits that work synergistically with muscle fibers to impact metabolism and heat loss at upper elevations.
465 Given that high-elevation environments are hypoxic, species differences in thermogenic capacity could arise

466 from variation in the ability to supply oxygen to muscle mitochondria, independent of the variation in muscle

467 size and fiber composition (McClelland and Scott 2019; Storz and Scott 2019). The mechanisms underlying

468 such differences could include variation in hemoglobin oxygen-binding affinity and muscle oxygen diffusion

469 (Zhu et al. 2018), enzyme activity in metabolic pathways (Stager et al 2015), and the position of mitochondria

470 within muscle fibers (Scott et al. 2009). Species differences in cold tolerance could also result from variation in

471 feather insulation (Herreid and Kessel 1967). Variation in these other traits might compensate for variation in

472 fiber-type composition that does not serve to improve cold tolerance, allowing fiber type to evolve along other

473 functional axes. Modifications in these other traits could also vary within species along demographic axes, thus

474 offering promising directions for future research.

475

476 **5. CONCLUSIONS**

477   By integrating temperature data with physiological and behavioral data across demographic axes, we

478 discovered age- and sex-structured variation in flight muscle phenotypes that is associated with discrete life-

479 history stages. This functional variation can be understood within a context of the selective pressures imposed

480 on each demographic class. By analyzing flight muscle phenotypes within the context of well-studied, adaptive

481 life-history strategies, like sexual dichromatism, age- and sex-structured migration, and delayed plumage

482 maturation, we can better understand the selective environments under which variation in flight muscle

483 phenotypes arises and is maintained, which in turn provides insight into the mechanisms underlying these life-

484 history strategies. While it is unclear at the moment whether the observed phenotypic variation is genetic,

485 environmentally induced, or seasonally plastic, this variation should nevertheless have downstream

486 consequences on muscle performance across age and sex classes. Our findings demonstrate how flight muscle

487 phenotypes are shaped at the nexus of life history and multi-class identity (i.e. species, age, and sex),

488 highlighting the importance of intraspecific selection in shaping functional phenotypic variation within animal

489 populations.

490

491

492

493

494

495

502

503 from the National Natural Science Foundation of China (31501851) and the International Visiting Program for

504 Excellent Young Scholars of Sichuan University; and by grants to FL from the Strategic Priority Research

505 Program, Chinese Academy of Sciences (XDB13020300) and the State Key Program of NSFC (31630069).

506 We thank Jake Allgeier, Susanna Campbell, Yalin Cheng, Katie Collins, Stewart Edie, Valentina Gómez-

507 Bahamón, Eric Gulson-Castillo, David Jablonski, Dallas Krentzel, Marcus Kronforst, Daniela Palmer, Teresa

508 Pegan, Trevor Price, Mariah Scott, Gang Song, Tom Stewart, Supriya, David Swanson, Alex White, Brian

509 Weeks, Benjamin Winger, and Xiaojia Zhu for their support and thoughtful comments on previous versions of

510 this manuscript.

511

512 **AUTHORS' CONTRIBUTIONS**

513 S.G.D., Y.W., G.R.S, Y.Q., Z.A.C., F.L., and J.B. designed research; S.G.D., Y.W., J.H.S., C.X., A.H.R., C.J.,

514 D.M., and J.W. performed research; S.G.D. and J.J. analyzed data; Y.W. and Q.L. coordinated data provision;

515 and S.G.D. wrote the paper.

516

517 **DATA AVAILABILITY**

518 Data for this article are deposited on Dryad Public Repository (https://doi.org/10.5061/dryad.jsxksn05s)

519

520 **REFERENCES**

521 Altshuler, D.L., 2005. Flight performance and competitive displacement of hummingbirds across elevational

522 gradients. *The American Naturalist*, *167*(2), pp.216-229.

523 Arcese, P. and Smith, J.N.M., 1985. Phenotypic correlates and ecological consequences of dominance in song

524 sparrows. *The Journal of Animal Ecology*, pp.817-830.

525 Biewener, A.A., Dial, K.P., Goslow, G.E., 1992. Pectoralis muscle force and power output during flight in the

526 starling. *Journal of Experimental Biology*, *164*(1), pp.1-18.

527 Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., Rudolf, V.H., Schreiber,

528 S.J., Urban, M.C. and Vasseur, D.A., 2011. Why intraspecific trait variation matters in community

529 ecology. *Trends in ecology & evolution*, *26*(4), pp.183-192.

530 Boyle, W.A., 2017. Altitudinal bird migration in North America. The Auk: Ornithological Advances, 134(2),

531 pp.443-465.

532 Dakin, R., Segre, P.S., Straw, A.D. and Altshuler, D.L., 2018. Morphology, muscle capacity, skill, and

533 maneuvering ability in hummingbirds. Science, 359(6376), pp.653-657.

534 Dawson, W.R., Marsh, R.L. and Yacoe, M.E., 1983. Metabolic adjustments of small passerine birds for

535 migration and cold. *American Journal of Physiology-Regulatory, Integrative and Comparative*

536 *Physiology*, *245*(6), pp.R755-R767.

537 del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. & de Juana, E. (2005). Handbook of the Birds of the World

538   Alive. Lynx Edicions, Barcelona.

539   Deveci, D., Marshall, J.M. and Egginton, S., 2001. Relationship between capillary angiogenesis, fiber type,

540   and fiber size in chronic systemic hypoxia. American Journal of Physiology-Heart and Circulatory

541   Physiology, 281(1), pp.H241-H252.

542   Dial, K.P., Kaplan, S.R., Goslow, G.E. and Jenkins, F.A., 1987. Structure and neural control of the pectoralis

543   in pigeons: implications for flight mechanics. The Anatomical Record, 218(3), pp.284-287.

544   Egginton, S., 1990. Morphometric analysis of tissue capillary supply. In Vertebrate Gas Exchange (pp. 73-141).

545   Springer, Berlin, Heidelberg.

546   Götmark, F., 1993. Conspicuous coloration in male birds is favoured by predation in some species and

547   disfavoured in others. *Proceedings of the Royal Society of London. Series B: Biological*

548   *Sciences*, *253*(1337), pp.143-146.

549   Grant, P.R. and Grant, B.R., 2002. Unpredictable evolution in a 30-year study of Darwin's finches. science,

550   296(5568), pp.707-711.

551   Griffiths, R., Daan, S. and Dijkstra, C., 1996. Sex identification in birds using two CHD genes. Proc. R. Soc.

552   Lond. B, 263(1374), pp.1251-1256.

553   Harrison, X.A., Blount, J.D., Inger, R., Norris, D.R. and Bearhop, S., 2011. Carry‐over effects as drivers of

554   fitness differences in animals. Journal of Animal Ecology, 80(1), pp.4-18.

555   Hawkes, L.A., Balachandran, S., Batbayar, N., Butler, P.J., Frappell, P.B., Milsom, W.K., Tseveenmyadag, N.,

556   Newman, S.H., Scott, G.R., Sathiyaselvam, P. and Takekawa, J.Y., 2011. The trans-Himalayan flights of

557   bar-headed geese (Anser indicus). *Proceedings of the National Academy of Sciences*, *108*(23), pp.9516-

558   9519.

559   Hawkins, G.L., Hill, G.E. and Mercadante, A., 2012. Delayed plumage maturation and delayed reproductive

560   investment in birds. Biological Reviews, 87(2), pp.257-274.

561   Herreid II, C.F. and Kessel, B., 1967. Thermal conductance in birds and mammals. Comparative Biochemistry

562   and Physiology, 21(2), pp.405-414.

563   Hsiung, A.C., Boyle, W.A., Cooper, R.J. and Chandler, R.B., 2018. Altitudinal migration: ecological drivers,

564   knowledge gaps, and conservation implications. *Biological Reviews*, *93*(4), pp.2049-2070.

565   Jakobsson, S., Brick, O. and Kullberg, C., 1995. Escalated fighting behaviour incurs increased predation

566   risk. *Animal Behaviour*, *49*(1), pp.235-239.

567   Jones, M.R., Mills, L.S., Alves, P.C., Callahan, C.M., Alves, J.M., Lafferty, D.J., Jiggins, F.M., Jensen, J.D.,

568   Melo-Ferreira, J. and Good, J.M., 2018. Adaptive introgression underlies polymorphic seasonal

569   camouflage in snowshoe hares. Science, 360(6395), pp.1355-1358.

570   Ketterson, E.D. and Nolan, V.A.L., 1983. The evolution of differential bird migration. In Current ornithology

571   (pp. 357-402). Springer, Boston, MA.

572   Kipp, F.A., 1959. Der Handflügel-Index als flugbiologisches Maß. Vogelwarte, 20(2), pp.77-86.

573   Lockwood, R., Swaddle, J.P. and Rayner, J.M., 1998. Avian wingtip shape reconsidered: wingtip shape indices
574   and morphological adaptations to migration. Journal of Avian Biology, pp.273-292.

575   Lozano, G.A., Perreault, S. and Lemon, R.E., 1996. Age, arrival date and reproductive success of male
576   American redstarts Setophaga ruticilla. *Journal of Avian Biology*, pp.164-170.

577   Lundgren, B.O. and Kiessling, K.H., 1988. Comparative aspects of fibre types, areas, and capillary supply in
578   the pectoralis muscle of some passerine birds with differing migratory behaviour. Journal of Comparative
579   Physiology B, 158(2), pp.165-173.

580   Lyon, B.E. and Montgomerie, R.D., 1986. Delayed plumage maturation in passerine birds: Reliable signaling
581   by subordinate males?. Evolution, 40(3), pp.605-615.

582   Marsh, R.L. and Dawson, W.R., 1989. Avian adjustments to cold. In Animal Adaptation to Cold (pp. 205-253).
583   Springer, Berlin, Heidelberg.

584   McClelland, G.B. and G.R. Scott. 2019. Evolved mechanisms of aerobic performance and hypoxia resistance
585   in high-altitude natives. Annu. Rev. Physiol. 81, 561-583.

586   Morbey, Y.E. and Ydenberg, R.C., 2001. Protandrous arrival timing to breeding areas: a review. Ecology
587   letters, 4(6), pp.663-673.

588   Morimoto, G., Yamaguchi, N. and Ueda, K., 2006. Plumage color as a status signal in male–male interaction
589   in the red-flanked bushrobin, Tarsiger cyanurus. Journal of ethology, 24(3), pp.261-266.

590   Myers, J.P., 1981. A test of three hypotheses for latitudinal segregation of the sexes in wintering birds.
591   Canadian Journal of Zoology, 59(8), pp.1527-1534.

592   Perrins, C.M., 1970. The timing of birds 'breeding seasons. Ibis, 112(2), pp.242-255.

593   Peter, J.B., Barnard, R.J., Edgerton, V.R., Gillespie, C.A. and Stempel, K.E., 1972. Metabolic profiles of three
594   fiber types of skeletal muscle in guinea pigs and rabbits. Biochemistry, 11(14), pp.2627-2633.

595   Petit, M. and Vézina, F., 2014. Phenotype manipulations confirm the role of pectoral muscles and haematocrit
596   in avian maximal thermogenic capacity. Journal of Experimental Biology, 217(6), pp.824-830.

597   Raveling, D.G., 1979. The annual cycle of body composition of Canada Geese with special reference to control
598   of reproduction. The Auk, pp.234-252.

599   Rayner, J.M., 1988. Form and function in avian flight. In *Current ornithology* (pp. 1-66). Springer, Boston,
600   MA.

601   Rohwer, S., 1982. The evolution of reliable and unreliable badges of fighting ability. American Zoologist,
602   22(3), pp.531-546.

603   Rosser, B.W. and George, J.C., 1986. The avian pectoralis: histochemical characterization and distribution of
604   muscle fiber types. Canadian Journal of Zoology, 64(5), pp.1174-1185.

605   Sangster, G., Alström, P., Forsmark, E. and Olsson, U., 2010. Multi-locus phylogenetic analysis of Old World
606   chats and flycatchers reveals extensive paraphyly at family, subfamily and genus level (Aves:
607   Muscicapidae). Molecular Phylogenetics and Evolution, 57(1), pp.380-392.

608   Schwabl, H., 1992. Winter and breeding territorial behaviour and levels of reproductive hormones of migratory

609   European robins. Ornis Scandinavica, pp.271-276.

610 Scott, G.R., Egginton, S., Richards, J.G. and Milsom, W.K., 2009. Evolution of muscle phenotype for extreme

611   high altitude flight in the bar-headed goose. Proceedings of the Royal Society of London B: Biological

612   Sciences, p.rspb20090947.

613 Segre, P.S., Dakin, R., Zordan, V.B., Dickinson, M.H., Straw, A.D. and Altshuler, D.L., 2015. Burst muscle

614   performance predicts the speed, acceleration, and turning performance of Anna's hummingbirds. ELife, 4.

615 Stager, M., Swanson, D.L. and Cheviron, Z.A., 2015. Regulatory mechanisms of metabolic flexibility in the

616   dark-eyed junco (Junco hyemalis). Journal of Experimental Biology, pp.jeb-113472.

617 Storz, J.F., 2018. Hemoglobin: Insights Into Protein Structure, Function, and Evolution. Oxford University

618   Press, USA.

619 Storz, J.F. and Scott, G.R., 2019. Life Ascending: Mechanism and Process in Physiological Adaptation to

620   High-Altitude Hypoxia. *Annual Review of Ecology, Evolution, and Systematics*, *50*.

621 Studds, C.E. and Marra, P.P., 2005. Nonbreeding habitat occupancy and population processes: an upgrade

622   experiment with a migratory bird. Ecology, 86(9), pp.2380-2385.

623 Swanson, D.L., Zhang, Y. and King, M.O., 2013. Individual variation in thermogenic capacity is correlated

624   with flight muscle size but not cellular metabolic capacity in American goldfinches (Spinus tristis).

625   Physiological and Biochemical Zoology, 86(4), pp.421-431.

626 Violle, C., Navas, M.L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. and Garnier, E., 2007. Let the concept

627   of trait be functional!. Oikos, 116(5), pp.882-892.

628 Wedell, N., Kvarnemo, C. and Tregenza, T., 2006. Sexual conflict and life histories. *Animal Behaviour*, *71*(5),

629   pp.999-1011.

630 Welch Jr, K.C. and Altshuler, D.L., 2009. Fiber type homogeneity of the flight musculature in small birds.

631   Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 152(4), pp.324-

632   331.

633 Wingfield, J.C., Ball, G.F., Dufty, A.M., Hegner, R.E. and Ramenofsky, M., 1987. Testosterone and

634   aggression in birds. American Scientist, 75(6), pp.602-608.

635 Wright NA, Steadman DW, Witt CC. Predictable evolution toward flightlessness in volant island birds.

636   Proceedings of the National Academy of Sciences. 2016 Apr 6:201522931.

637 Zhu, X., Guan, Y., Signore, A.V., Natarajan, C., DuBay, S.G., Cheng, Y., Han, N., Song, G., Qu, Y.,

638   Moriyama, H. and Hoffmann, F.G., et al. 2018. Divergent and parallel routes of biochemical adaptation in

639   high-altitude passerine birds from the Qinghai-Tibet Plateau. Proceedings of the National Academy of

640   Sciences, 115(8), pp.1865-1870.

Table 1. Species differences in body mass, flight muscle phenotypes, experienced temperature, and arrival time, analyzed with an analysis of variance (ANOVA, type III sums of squares) with accounting for class identity (i.e. adult male, subadult male, female) and the year an individual was caught in the model as additional predictor variables. Means, standard errors, and sample sizes for each trait are reported for each species. Comparisons were performed using Tukey's post-hoc tests for each trait. Superscript letters denote pairwise statistical differences for a given trait in which species with different letters are significantly different at p<0.05, while species with similar letters are not statistically different from one another. The effect size of "species" is reported as partial eta squared (η^2) for each trait. "FO" = fast oxidative. "FG" = fast glycolytic. "raw" = mass of the specified trait uncorrected for body mass. "relative" = mass of the specified trait analyzed as a proportion of body mass.

Table 1

Trait	*T. indicus*	*T. chrysaeus*	*T. rufilatus*	η^2
Body mass (g)	14.26 ± 0.10 [A]	13.81 ± 0.09 [B]	13.83 ± 0.06 [B]	0.10
N =	69	76	181	
Pectoralis mass (g) (raw)	1.53 ± 0.03 [A]	1.37 ± 0.02 [B]	1.79 ± 0.01 [C]	0.60

N =	34	31	64	
Pectoralis mass (%) (relative)	10.77 ± 0.15 [A]	10.31 ± 0.10 [B]	12.95 ± 0.08 [C]	0.64
N =	34	30	63	
FO fiber mass (g) (raw)	1.26 ± 0.02 [A]	1.27 ± 0.01 [A]	1.51 ± 0.01 [B]	0.42
N =	29	26	56	
FO fiber mass (%) (relative)	8.83 ± 0.11 [A]	9.53 ± 0.10 [A]	10.99 ± 0.07 [B]	0.50
N =	29	26	55	
FG fiber mass (g) (raw)	0.30 ± 0.02 [A]	0.10 ± 0.01 [B]	0.29 ± 0.01 [A]	0.45
N =	29	26	56	
FG fiber mass (%) (relative)	2.08 ± 0.13 [A]	0.76 ± 0.07 [B]	2.04 ± 0.05 [B]	0.45
N =	29	26	55	
Maximum daily temperature (C)	9.03 ± 0.52 [A]	11.46 ± 0.67 [B]	9.61 ± 0.32 [A]	0.09
N =	65	71	182	
Minimum daily temperature (C)	0.22 ± 0.24 [A]	1.54 ± 0.25 [B]	1.06 ± 0.14 [B]	0.11
N =	65	71	182	
Arrival date (Julian date)	102.46 ± 1.07 [A]	113.08 ± 0.73 [B]	107.72 ± 0.54 [C]	0.21
N =	65	71	182	

Table 2. Sex differences in body mass, flight muscle phenotypes, experienced temperature, and arrival time, analyzed with an analysis of variance (ANOVA, type III sums of squares). The year an individual was caught was included in the models as a predictor variable to account for any interannual variation. Means, standard errors, and sample sizes for each trait are reported by sex for each species. P-values are bolded when p<0.05. Partial eta squared effect sizes (η^2) are reported by species for each trait. "FO" = fast oxidative. "FG" = fast glycolytic. "raw" = mass of the specified trait uncorrected for body mass. "relative" = mass of the specified trait analyzed as a proportion of body mass.
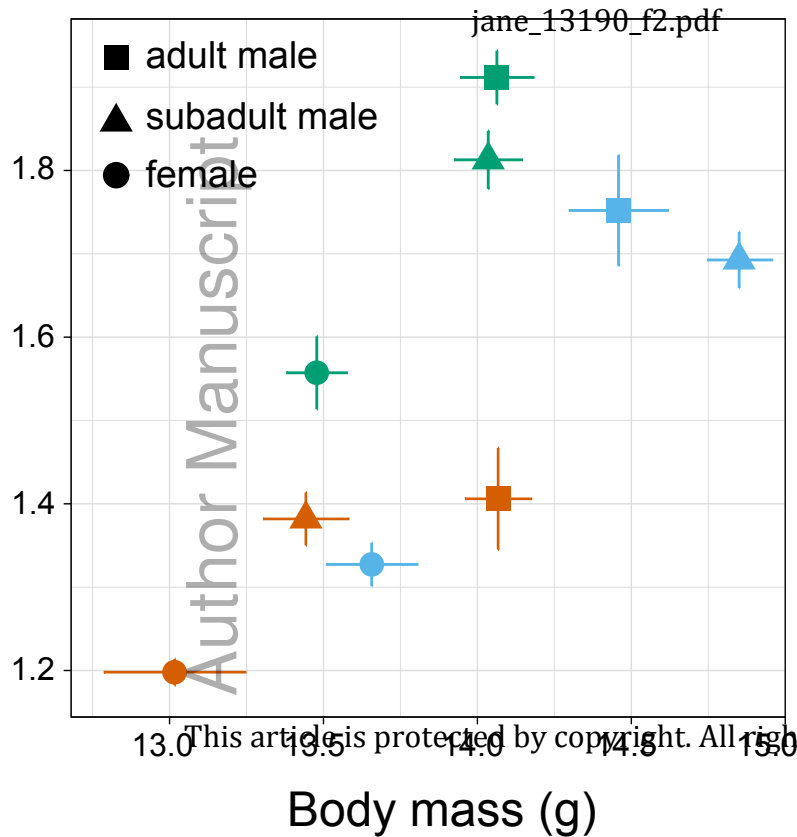
Trait	T. indicus female	T. indicus male	F	P	η2	T. chrysaeus female	T. chrysaeus male	F	P	η2	T. rufilatus female	T. rufilatus male	F	P	η2
Body mass (g)	13.66 ± 0.15	14.73 ± 0.09	39.92	**2.72E-08**	0.38	13.02 ± 0.23	13.88 ± 0.09	9.08	**0.0036**	0.11	13.48 ± 0.10	14.05 ± 0.08	25.67	**1.01E-06**	0.13
N =	30	39				6	70				69	112			
Pectoralis mass (g) (raw)	1.33 ± 0.02	1.71 ± 0.02	91.94	**1.21E-10**	0.75	1.20 ± 0.01	1.39 ± 0.02	6.45	**0.0172**	0.19	1.56 ± 0.02	1.86 ± 0.02	40.60	**2.92E-08**	0.40
N =	16	18				4	27				16	48			
Pectoralis mass (%) (relative)	9.75 ± 0.14	11.67 ± 0.14	53.08	**4.11E-08**	0.64	9.25 ± 0.07	10.48 ± 0.09	7.83	**0.0096**	0.23	11.83 ± 0.12	13.34 ± 0.09	25.83	**4.04E-06**	0.30
N =	16	18				4	26				16	47			
FO fiber mass (g) (raw)	1.18 ± 0.03	1.32 ± 0.03	5.41	**0.0284**	0.18	1.15 ± 0.02	1.29 ± 0.02	2.82	0.1073	0.11	1.38 ± 0.02	1.55 ± 0.01	15.58	**0.0002**	0.23
N =	13	16				4	22				14	42			
FO fiber mass (%) (relative)	8.71 ± 0.18	8.93 ± 0.15	0.36	0.5538	0.01	8.89 ± 0.12	9.64 ± 0.10	2.21	0.1510	0.09	10.43 ± 0.10	11.18 ± 0.09	5.79	**0.0198**	0.10
N =	13	16				4	22				14	41			
FG fiber mass (g) (raw)	0.16 ± 0.02	0.41 ± 0.02	48.07	**2.88E-07**	0.66	0.05 ± 0.02	0.11 ± 0.01	0.90	0.3531	0.04	0.19 ± 0.01	0.32 ± 0.01	14.15	**0.0004**	0.21
N =	13	16				4	22				14	42			
FG fiber mass (%) (relative)	1.22 ± 0.13	2.77 ± 0.13	38.63	**1.68E-06**	0.61	0.36 ± 0.17	0.83 ± 0.08	0.88	0.3580	0.04	1.44 ± 0.06	2.24 ± 0.07	12.96	**0.0007**	0.20
N =	13	16				4	22				14	41			
Maximum daily temperature (C)	10.37 ± 0.78	8.01 ± 0.68	4.45	**0.0390**	0.07	11.07 ± 1.98	11.50 ± 0.71	0.00	0.9783	0.00	8.67 ± 0.54	10.19 ± 0.42	0.81	0.3693	0.00
N =	28	37				6	65				69	113			
Minimum daily temperature (C)	1.24 ± 0.42	−0.56 ± 0.24	21.40	**1.99E-05**	0.26	1.75 ± 1.22	1.52 ± 0.26	0.16	0.6919	0.00	0.91 ± 0.22	1.15 ± 0.19	0.03	0.8553	0.00
N =	28	37				6	65				69	113			
Arrival date (Julian date)	104.04 ± 1.59	101.22 ± 1.46	1.67	0.2012	0.03	114 ± 1.88	113 ± 0.78	0.02	0.8881	0.00	111.65 ± 0.73	105.32 ± 0.70	23.94	**2.21E-06**	0.12
N =	28	37				6	65				69	113			

Table 3. Male age class differences in body mass, flight muscle phenotypes, experienced temperature, and arrival time, analyzed with an analysis of variance (ANOVA, type III sums of squares). The year an individual was caught was included in the models as a predictor variable to account for any interannual variation. Means, standard errors, and sample sizes for each trait are reported age class for each species. P-values are bolded when p<0.05. Partial eta squared effect sizes (η^2) are reported by species for each trait. "FO" = fast oxidative. "FG" = fast glycolytic. "raw" = mass of the specified trait uncorrected for body mass. "relative" = mass of the specified trait analyzed as a proportion of body mass.

Trait	*T. indicus* adult male	*T. indicus* subadult male	F	P	$\eta2$	*T. chrysaeus* adult male	*T. chrysaeus* subadult male	F	P	$\eta2$	*T. rufilatus* adult male	*T. rufilatus* subadult male	F	P	$\eta2$
Body mass (g)	14.46 ± 0.16	14.85 ± 0.11	3.11	0.0864	0.08	14.07 ± 0.10	13.44 ± 0.14	12.28	**0.0008**	0.16	14.06 ± 0.12	14.04 ± 0.11	0.29	0.5886	0.00
N =	12	27				49	21				49	63			
Pectoralis mass (g) (raw)	1.75 ± 0.04	1.69 ± 0.02	0.33	0.5744	0.02	1.41 ± 0.03	1.38 ± 0.03	2.22	0.1501	0.09	1.91 ± 0.02	1.81 ± 0.02	5.67	**0.0217**	0.11
N =	5	13				10	17				25	23			
Pectoralis mass (%) (relative)	12.27 ± 0.26	11.44 ± 0.14	2.01	0.1780	0.13	10.69 ± 0.14	10.36 ± .15	2.29	0.1446	0.09	13.68 ± 0.17	12.98 ± 0.08	6.70	**0.0130**	0.13
N =	5	13				9	17				24	23			
FO fiber mass (g) (raw)	1.28± 0.04	1.33 ± 0.03	0.07	0.7898	0.01	1.29 ± 0.02	1.29 ± 0.03	1.08	0.3123	0.06	1.56 ± 0.02	1.53 ± 0.02	0.90	0.3483	0.02
N =	5	11				8	14				24	18			
FO fiber mass (%) (relative)	8.94 ± 0.22	8.93 ± 0.20	0.05	0.8318	0.00	9.52 ± 0.14	9.72 ± 0.18	0.06	0.8169	0.00	11.26 ± 0.14	11.06 ± 0.10	0.32	0.5754	0.01
N =	5	11				8	14				23	18			
FG fiber mass (g) (raw)	0.47 ± 0.04	0.37 ± 0.02	1.43	0.2552	0.11	0.19 ± 0.01	0.07 ± 0.01	6.82	**0.0177**	0.27	0.36 ± 0.02	0.27 ± 0.01	5.91	**0.0198**	0.13
N =	5	11				8	14				24	18			
FG fiber mass (%) (relative)	3.33 ± 0.26	2.52 ± 0.13	2.06	0.1769	0.15	1.39 ± 0.09	0.52 ± 0.10	6.16	**0.0231**	0.26	2.48 ± 0.12	1.95 ± 0.05	5.13	**0.0295**	0.12
N =	5	11				8	14				23	18			
Maximum daily temperature (C)	5.62 ± 0.98	8.90 ± 0.82	5.24	**0.0286**	0.14	10.79 ± 0.84	13.22 ± 1.30	2.80	0.0994	0.04	9.11 ± 0.60	11.04 ± 0.56	13.04	**0.0005**	0.11
N =	10	27				46	19				50	63			
Minimum daily temperature (C)	−1.34 ± 0.30	−0.27 ± 0.30	5.02	**0.0319**	0.13	1.20 ± 0.28	2.30 ± 0.52	4.08	**0.0478**	0.06	0.81 ± 0.30	1.42 ± 0.47	4.94	**0.0283**	0.04
N =	10	27				46	19				50	63			
Arrival date (Julian date)	100.2 ± 2.14	101.59 ± 1.90	0.16	0.6956	0.00	112.24 ± 0.80	114.84 ± 1.83	1.62	0.2083	0.03	104.3 ± 1.10	106.13 ± 1.64	0.59	0.4449	0.01
N =	10	27				46	19				50	63			
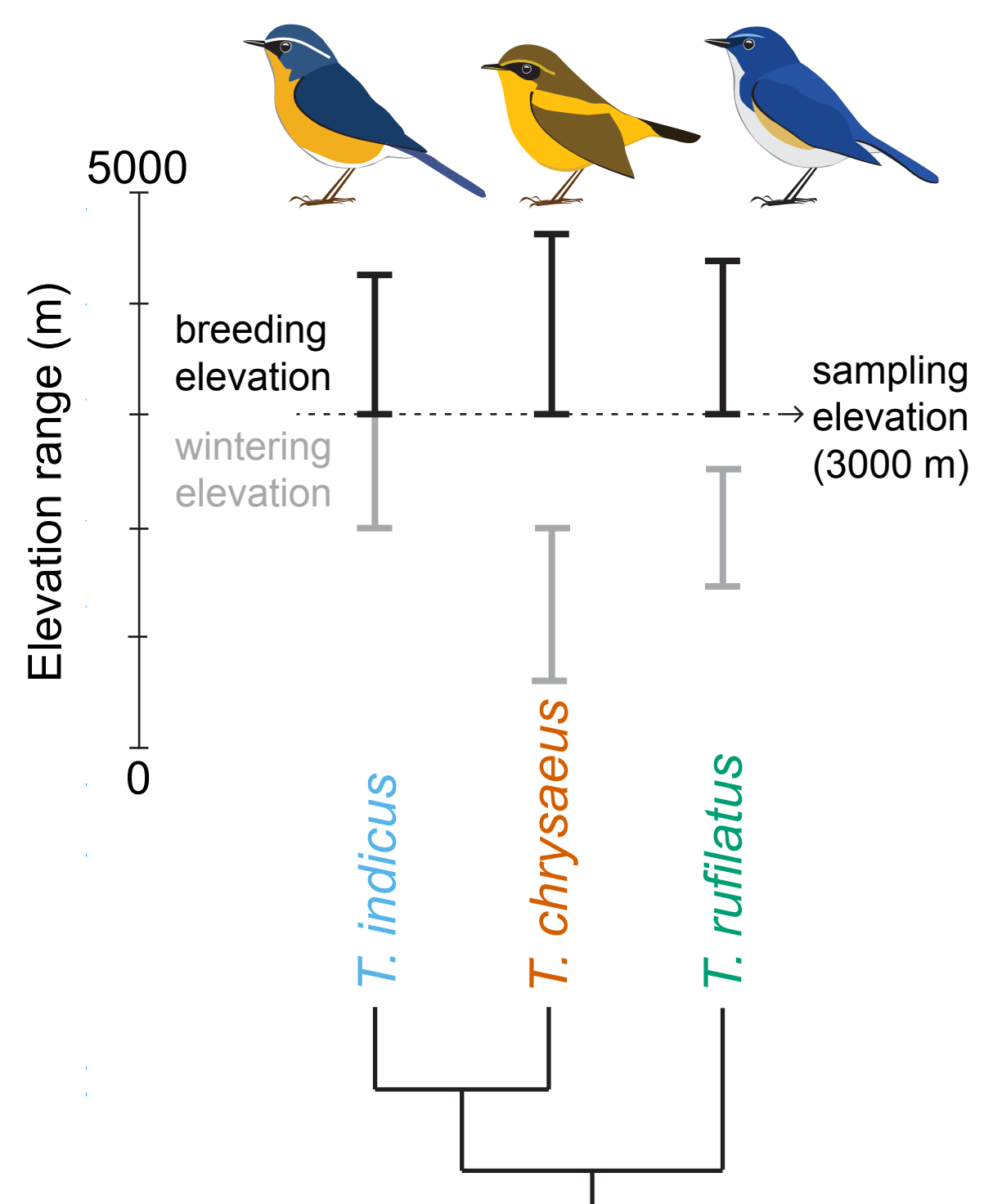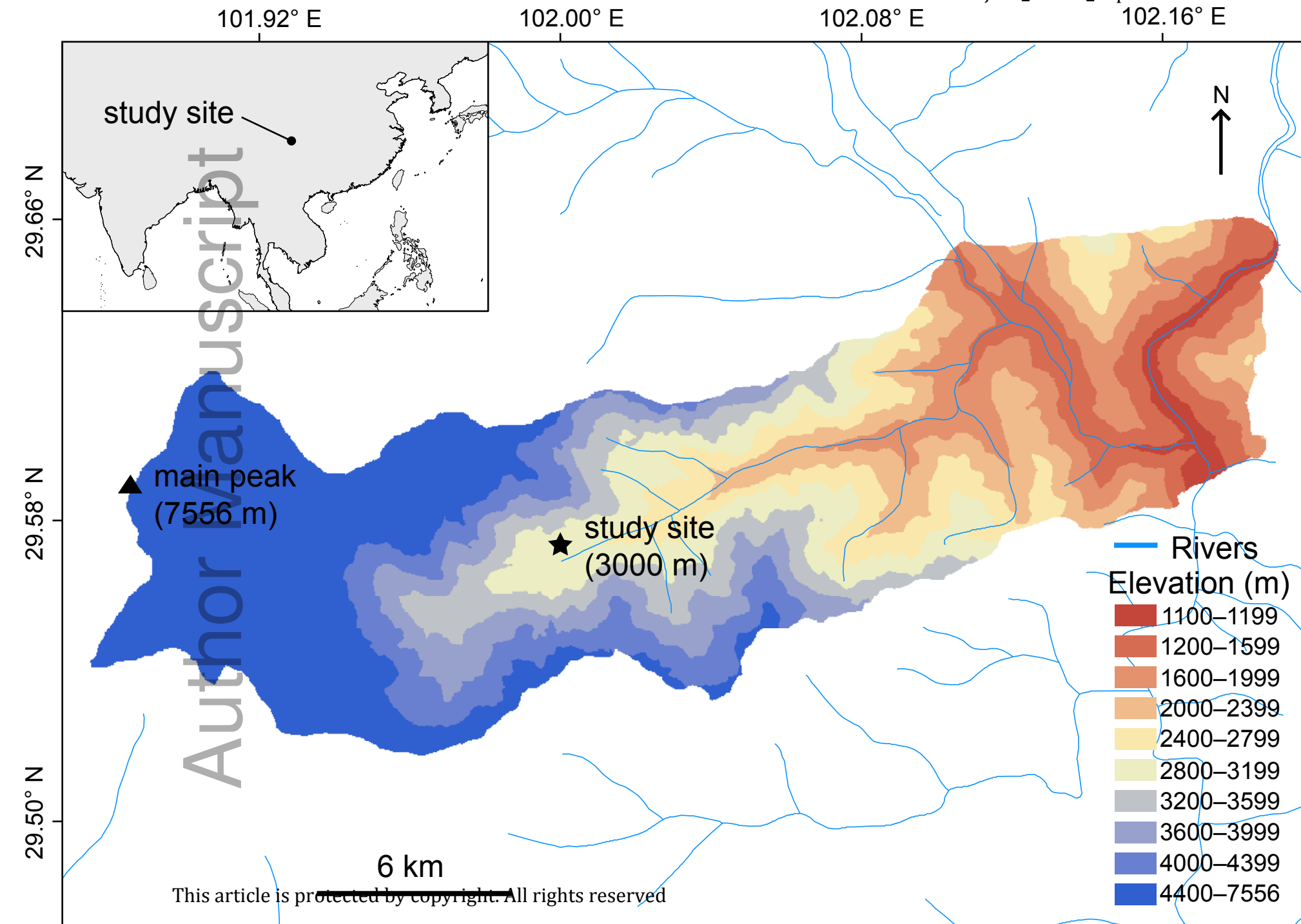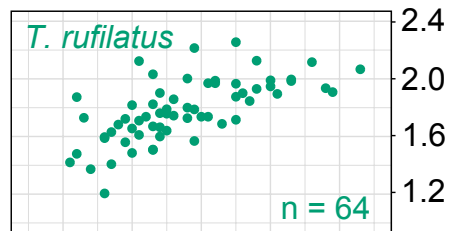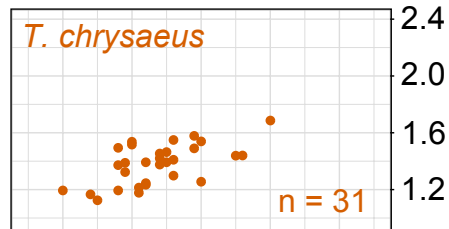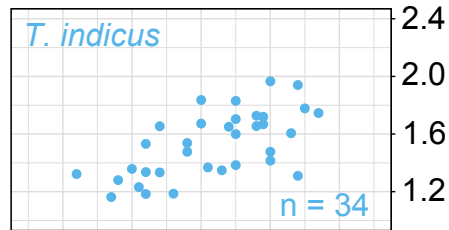
jane_13190_f2.pdf

FG and FO fibers   # FO fibers only

200 μm

A — Winter mid-point elevation (m) vs. Arrival date in 2016

- *T. indicus* (n = 20)
- *T. rufilatus* (n = 97)
- *T. chrysaeus* (n = 30)

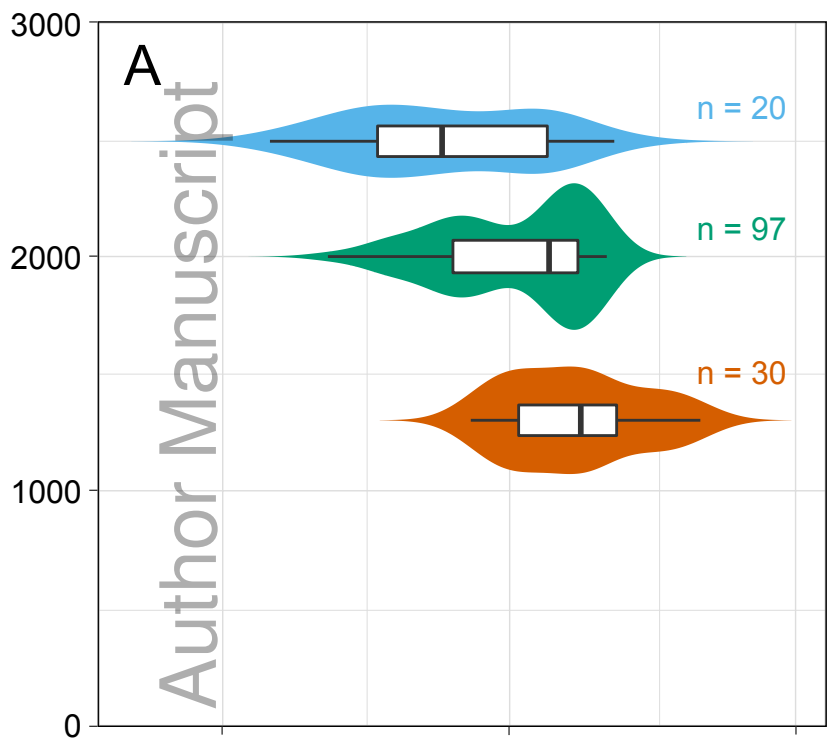**B** — Minimum daily temperature (C) vs. Arrival date (3 year composite)
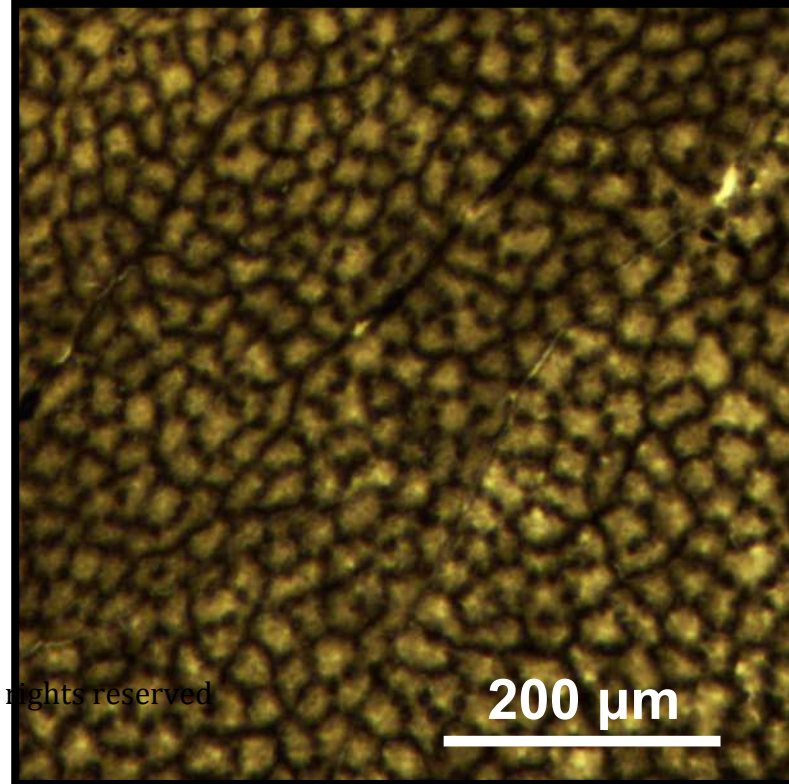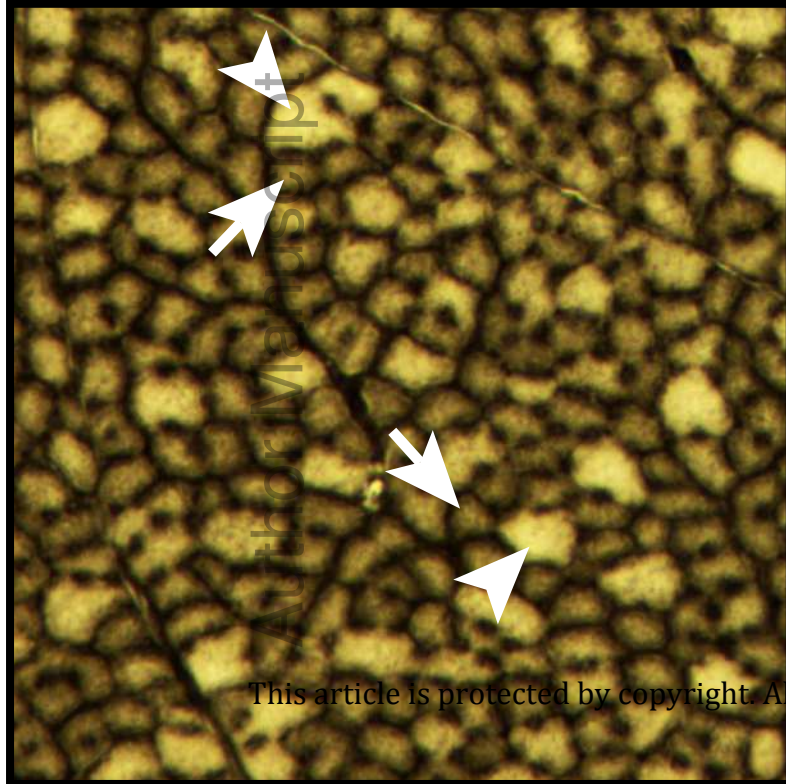
Symbols: ▲ adult male, ■ subadult male, ● female
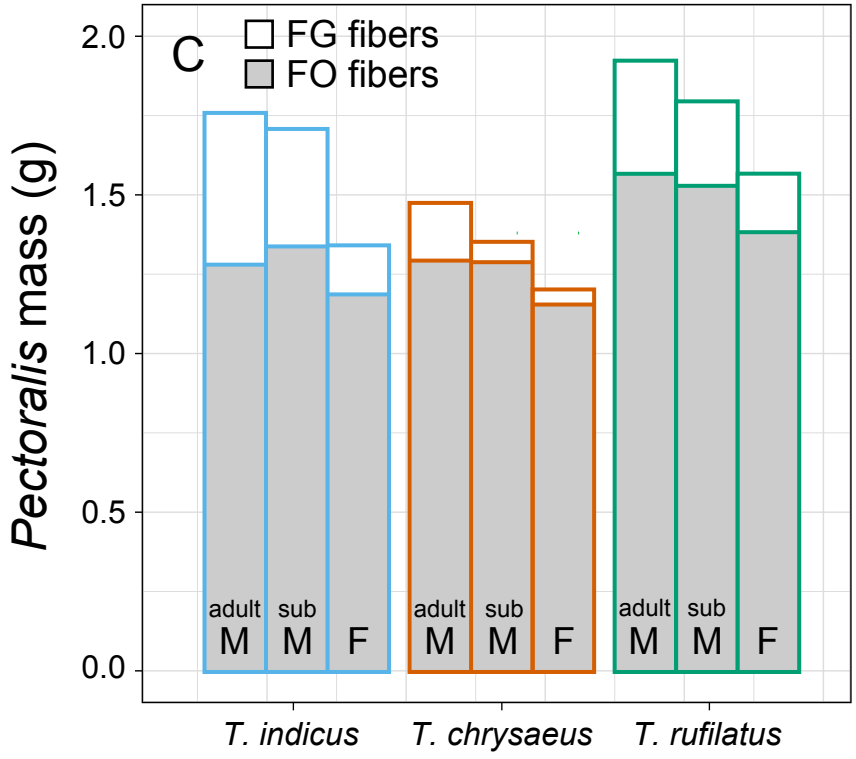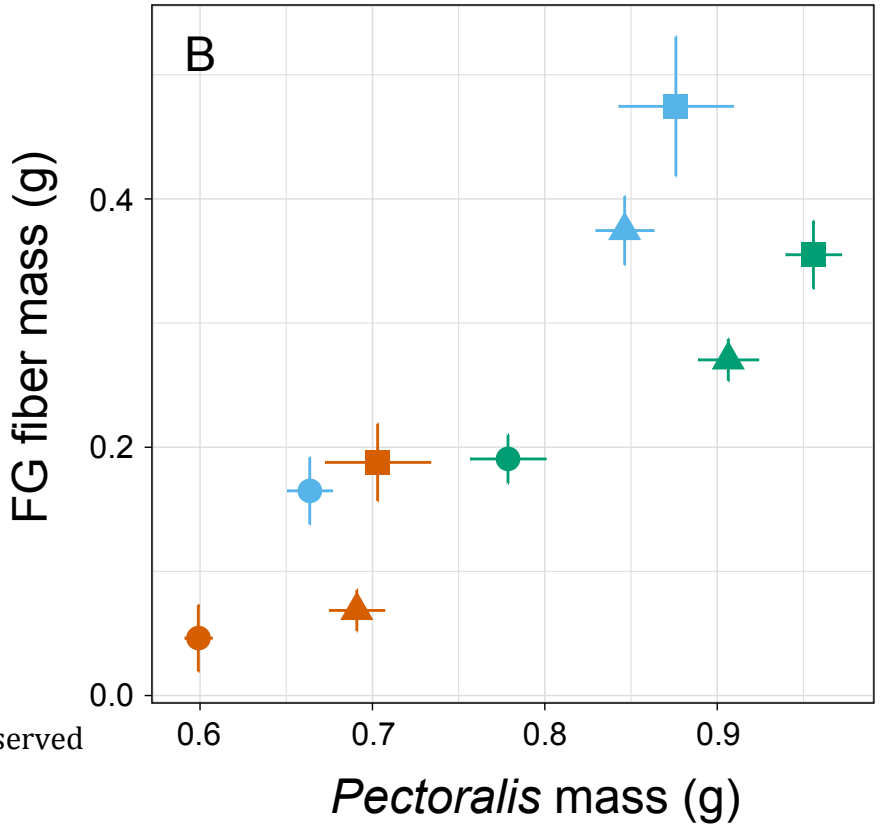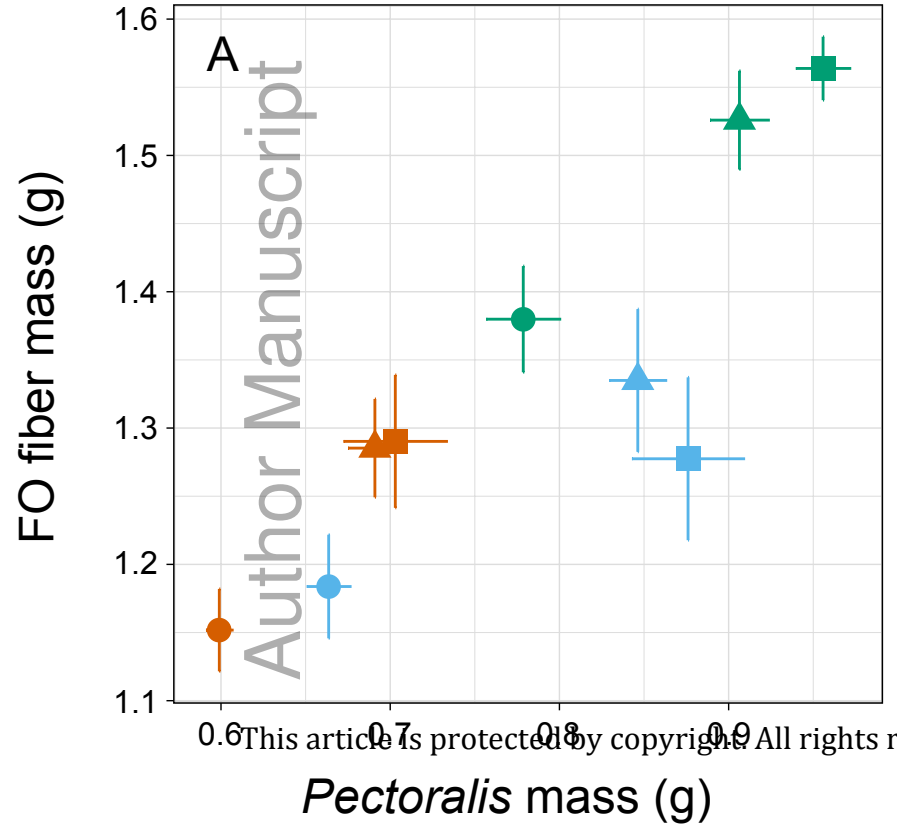
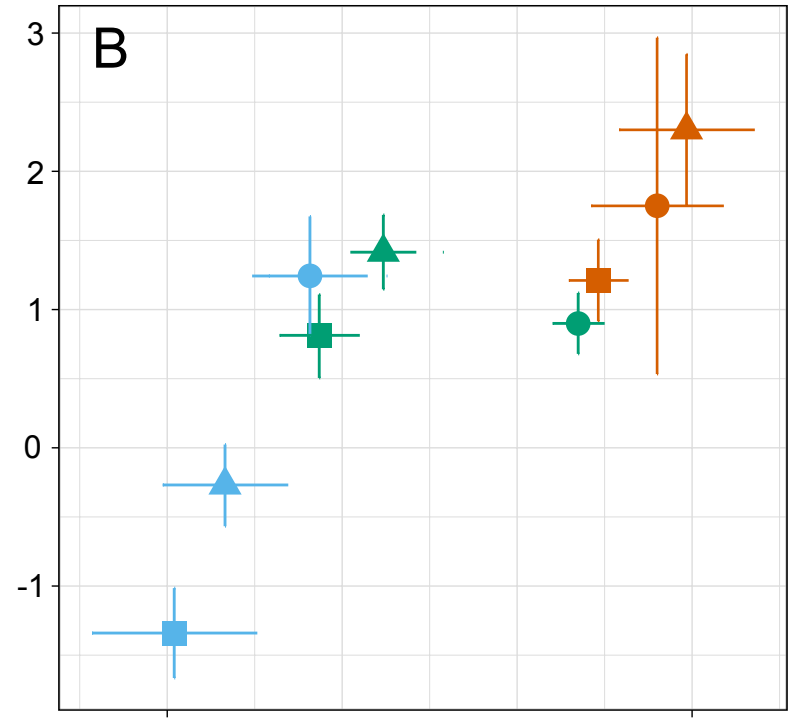Legend: — *T. indicus* — *T. rufilatus* — *T. chrysaeus* ■ adult male ▲ subadult male ● female

Y-axes: A: Body mass (g); B: *Pectoralis* mass (g); C: FG fiber mass (g); D: FO fiber mass (g)

X-axis: Minimum daily temperature (C)