

**Title:** Blood volumes following pre-season heat versus altitude: a case study of Australian Footballers

**Submission type:** Brief report – case study

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**Running head:** Comparison of pre-season heat vs altitude

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**Abstract word count:** 249

**Text only word count:** 1,738

**Number of figures and tables:** 3

This manuscript has been read and approved by all the listed co-authors and the authors declare no potential conflicts of interest.

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14 potential conflicts of interest.

15 **ABSTRACT**

16 **Purpose:** There is debate as to which environmental intervention produces most benefit for team sport  
17 athletes, particularly comparing heat and altitude. This quasi-experimental study aimed to compare  
18 blood volume (BV) responses to heat and altitude training camps in Australian Footballers. **Methods:**  
19 The BV of seven professional Australian Footballers ( $91.8 \pm 10.5$  kg,  $191.8 \pm 10.1$  cm) was measured  
20 throughout three consecutive spring/summer pre-seasons. During each pre-season, players participated  
21 in altitude (*year 1 and year 2*) and heat (*year 3*) environmental training camps. *Year 1 and year 2*  
22 altitude camps were in November/December in the USA, while the *year 3* heat camp was in  
23 February/March in Australia after a full exposure to summer heat. BV, red cell volume (RCV) and  
24 plasma volume (PV) were measured at least three times during each pre-season. **Results:** RCV  
25 increased substantially following altitude in both *year 1* ( $d=0.67$ ) and *year 2* ( $d=1.03$ ), before returning  
26 to baseline four weeks post-altitude. Immediately following altitude, concurrent decreases in PV were  
27 observed during *year 1* ( $d=-0.40$ ) and *year 2* ( $d=-0.98$ ). With spring/summer training in *year 3*, BV  
28 and PV were substantially higher in January than temporally matched post-altitude measurements  
29 during *year 1* (BV  $d=-0.93$ , PV  $d=-1.07$ ) and *year 2* (BV  $d=-1.99$ , PV  $d=-2.25$ ), with *year 3* total BV,  
30 RCV and PV not changing further despite the 6-day heat intervention. **Conclusions:** We found greater  
31 BV after training throughout spring/summer conditions, compared with interrupting spring/summer  
32 exposure to train at altitude in the cold, with no additional benefits observed from a heat camp  
33 following spring/summer training.

34 **Keywords:** hypoxia, environmental, plasma volume, red cell volume

35 **INTRODUCTION**

36 Many high-intensity, intermittent team sports undertake prolonged pre-season training, which are  
37 important for developing physical capacities essential for competitive success. Environmental stimuli  
38 such as altitude<sup>1</sup> or heat<sup>2</sup> have been applied during these periods, in an attempt to enhance players'  
39 physiology and performance. Indeed, improvements in team-sport athletes' running performance has  
40 been shown following heat,<sup>2</sup> altitude<sup>1</sup> and a combination of heat and hypoxic exposures.<sup>3</sup>

41

42 High-intensity training leads to increases in plasma volume (PV)<sup>4</sup> which occur within 48 hours of the  
43 first training bout and may be further augmented by hot environments.<sup>5</sup> Red cell volume (RCV) is less  
44 influenced by training alone, but may be augmented by living/training at altitude for more than two  
45 weeks.<sup>6</sup> While these environmental interventions (i.e. altitude/hypoxic or hot environments) offer  
46 haematological<sup>6</sup> and non-haematological benefits,<sup>7</sup> changes in PV (heat<sup>4</sup>) and RCV (altitude/hypoxia<sup>6</sup>)  
47 are thought to be the primary adaptations contributing to improved exercise capacity following such  
48 interventions. The time course of these haematological responses is also important to consider as PV  
49 adaptations can dissipate in as short as 10 days with detraining and RCV usually normalizes by four  
50 weeks of returning to sea-level.<sup>8</sup> However, some evidence suggests that environmentally induced  
51 increases in both PV and RCV may be maintained for up to four weeks in team sport athletes continuing  
52 with pre-season training programs.<sup>3</sup>

53

54 There has recently been debate as to which environmental interventions produce the most beneficial  
55 changes in physiology and performance, particularly for team sports.<sup>5</sup> However, there is a paucity of  
56 data comparing physiological and/or performance responses to different environmental interventions  
57 in team sport athletes. Therefore, the aim of this case study was to compare hematological responses  
58 to altitude and heat interventions in elite Australian Football (AF) players.

59 **METHODS**

60 Forty-three AF players were tracked over three pre-seasons (November-March 2011-2014). Twenty-  
61 five players were on the clubs list for all three seasons and individuals were eliminated if data were  
62 missing from any measurements (see Figure 1) due to not participating in all camps/training because  
63 of injury (n=8) or team planning (n=8), with two players unavailable on specific testing days. This  
64 resulted in a final cohort of seven players (mean±SD: 91.8±10.5 kg, 191.8±10.1 cm). All subjects  
65 provided written informed consent and this study was approved by the Human Research Ethics  
66 Committee at Australian Catholic University. Results from *year 1*<sup>1</sup> and *year 2*<sup>9</sup> have previously been  
67 reported.

68

69 The primary training location in every year was Melbourne, Australia, with the following addition of  
70 off-site training camps:

71 *Year 1*–21-day ‘altitude’ camp, Flagstaff, Arizona, USA

72 *Year 2*–19-day ‘altitude’ camp, Park City, Utah, USA

73 *Year 3*–6-day ‘heat’ camp, Gold Coast, Queensland, Australia

74

75 INSERT FIGURE 1 ABOUT HERE

76

77

78 Timeline of hematological measures:

79 *Year 1*–15<sup>th</sup> November, 16<sup>th</sup> December, 10<sup>th</sup> January

80 *Year 2*–27<sup>th</sup> November, 5<sup>th</sup> December, 19<sup>th</sup> December, 16<sup>th</sup> January

81 *Year 3*–14<sup>th</sup> January, 24<sup>th</sup> February, 7<sup>th</sup> March

82

83 Blood volume (BV), PV and RCV were assessed via venous and capillary blood samples in  
84 combination with carbon monoxide (CO) rebreathing to calculate total haemoglobin mass (Hb<sub>mass</sub>).  
85 Preceding venous blood-sample collection, subjects’ were seated for 15 minutes. The same  
86 hemoximeter (OSM3, Radiometer, Copenhagen, Denmark) was used for *year 1* and *2* measurements,  
87 while a different hemoximeter (ABL80, Radiometer, Copenhagen, Denmark) was used during *year 3*.

88

89 Venous blood samples collected in Melbourne (i.e. all samples excluding *year 2* measures at altitude)  
90 were analyzed using a Sysmex XE-5000 Automated Hematology Analyzer (Roche Diagnostics, Castle  
91 Hill, Australia) at St Vincent's Hospital Pathology (Fitzroy, Australia). Altitude samples (i.e. during  
92 *year 2* camp) were analyzed using a Sysmex XT-4000i Automated Hematology Analyzer (Sysmex,  
93 Lincolnshire, USA) at Park City Medical Centre (Park City, USA). Mean corpuscular haemoglobin  
94 concentration (MCHC), RCV, BV and PV were calculated as follows:

95  $MCHC = \text{venous haematocrit (\%)} / \text{venous haemoglobin concentration (g/dl)}$

96  $RCV \text{ (mL)} = (\text{Hb}_{\text{mass}} \text{ (g)} / MCHC) \times 100$

97  $BV \text{ (mL)} = \text{Hb}_{\text{mass}} \times 100 / \text{haemoglobin concentration (g/dL)} / 0.91$

98  $PV \text{ (mL)} = BV \text{ (mL)} - RCV \text{ (mL)}$

99 Our typical error for these measures were calculated using those data without any intervention; that is,  
100 January and February data from *year 3* on all players with duplicate measures who participated that  
101 year ( $n=20$ ). The typical errors for MCHC, RCV, BV, PV and  $\text{Hb}_{\text{mass}}$  were 2.0, 3.9, 3.2, 3.8 and 2.8 %,   
102 respectively.

103

104 Weather data were recalled from the Australian Government Bureau of Meteorology [Melbourne  
105 (37.81°S, 144.97°E), Gold Coast (27.94°S, 153.43°E)] and National Oceanic and Atmospheric  
106 Administration [Flagstaff, Arizona (35.15°N, 111.68°W), Park City, Utah (40.62°N, 111.53°W)]  
107 databases.

108

### 109 ***Statistical analysis***

110 A magnitude-based approach<sup>10</sup> was used to detect effects of practical importance. Changes were  
111 assessed relative to the smallest worthwhile change (SWC), set to a small effect size ( $d=0.2 \times$  the  
112 between-participant SD) and reported as mean change/difference  $\pm 90\%$  confidence limits. Changes  
113 were deemed 'substantial' if there was  $>75\%$  likelihood of the difference exceeding the SWC.

114 **RESULTS**

115 During *year 1* ( $d=0.67$ ) and *year 2* ( $d=1.03$ ) there was a substantial increase in RCV following altitude,  
116 that returned to baseline in January (see Figure 2). Post-altitude RCV during *year 1* ( $d=0.74$ ) and *year*  
117 *2* ( $d=1.57$ ) was substantially higher than RCV in January ( $d=0.74$ ) of *year 3*.

118

119 In *year 1*, PV was highest during November, before substantially decreasing in December ( $d=-0.40$ )  
120 and January ( $d=-0.74$ ). During *year 2*, PV decreased from November to December ( $d=0.98$ ) and  
121 January, ( $d=0.47$ ). All *year 3* PVs were substantially higher than December and January values during  
122 *year 1* (but not November) and all *year 2* measurements.

123

124 *Year 1* BV substantially decreased from November to January ( $d=-0.59$ ). In *year 2*, BV increased from  
125 November to December ( $d=0.81$ ), returning to baseline in January. All *year 3* BV measurements were  
126 substantially higher than January BV during *year 1* and *year 2*.

127

128 INSERT TABLE 1 ABOUT HERE

129 INSERT FIGURE 2 ABOUT HERE

130 **DISCUSSION**

131 Our main finding is that total BV and PV after 8 weeks of warm-weather (spring/summer), pre-season  
132 training was higher than the preceding two years, when warm-weather training was interrupted for 3  
133 weeks to complete altitude training camps in cold conditions. These findings are particularly  
134 meaningful as they are taken from real world data, thereby producing ecological validity and relevance  
135 to practitioners. Immediately post-altitude in *years 1* and *2*, BVs were similar to *year 3* values, due to  
136 altitude-induced increases in RCV. But 4 weeks after altitude, RCV returned to baseline without a  
137 compensatory increase in PV. This erythropoietic timeline following altitude exposure is consistent  
138 with previous results in team sport athletes.<sup>1,9</sup>

139

140 Plasma volume increases following high intensity training<sup>4</sup> and environmental heat may further  
141 augment PV expansion.<sup>2</sup> During January in *year 1* and *2*, PVs were relatively low considering the  
142 athletes' training status (i.e. 2-3 months of pre-season training) and the warm spring/summer conditions  
143 of Melbourne. In contrast, January PV in *year 3* was higher, which was likely affected by warm training  
144 environments throughout November-December and suggests that these athletes had capacity to  
145 increase PV which was not realized in *year 1* and *2*. During *years 1* and *2*, athletes experienced regular  
146 temperatures below freezing while in the northern hemisphere winter. As previous longitudinal data  
147 shows that PV is suppressed during colder months,<sup>11</sup> this cold exposure may have contributed to lower  
148 PV.

149

150 In addition to cold environments, positive heat adaptations can be blunted when heat training  
151 interventions are combined with living in hypoxia.<sup>12</sup> McCleave et al showed heat-induced increases in  
152 PV and BV do not occur when the same heat exposure is combined with living in hypoxia (13 h·d<sup>-1</sup>,  
153 FiO<sub>2</sub> = 14.4%).<sup>12</sup> Following stays at altitude, a complex hormonal cascade initiates active red blood  
154 cell destruction, a process known as neocytolysis.<sup>13</sup> This hormonal cascade is stimulated by lower  
155 erythropoietin levels, and may also influence PV. In this case study, relatively low PVs during *year 1*  
156 and *2* persisted at least one month after returning to sea-level, despite hot ( $\sim 28 \pm 6$  °C) living/training  
157 conditions during this time. As RCV normalizes during the same period, our work showed lower BVs  
158 (driven by low PV) following winter altitude training camps, when compared with exclusively training  
159 in the Australian summer. While specific detail of training content is not available, this should not be  
160 overlooked when interpreting these data as training prescription can also mediate BV responses.  
161 Indeed, the overall periodization varied slightly during *year 3* in this case study, as eliminating the  
162 altitude camp, and associated international travel, allowed for increased training frequency (and  
163 therefore overall volume) during November/December.



164 *Year 3* also describes selected heat adaptations during an AF pre-season. Initial *year 3* measurements  
165 were taken after eight weeks of warm-weather pre-season training in Melbourne and show higher PV  
166 and BV than almost all *year 1* and *2* measurements. Initial *year 3* BVs and PVs were higher than those  
167 previously reported after a ‘successful’ heat training intervention in professional AF (PV–57.0 vs 54.6  
168 ml/kg; BV–90.9 vs 86.1 ml/kg)<sup>3</sup> and there were no additional increases following the 6-day ‘heat’  
169 camp undertaken during March. This suggests that players had maximized PV adaptations prior to the  
170 first measurement in *year 3* and could be approaching a physiological ‘ceiling’ over this length training  
171 cycle (i.e. 3-4 months). These data suggest that warm temperatures in the Melbourne [home base for  
172 9/18 Australian Football League (AFL) teams] summer are sufficient to maximize heat-related  
173 physiological adaptations.

174

### 175 **Limitations**

176 While this work adds to the understanding of physiological responses to environmental stimuli, it must  
177 be acknowledged that performance data are not included, and these observations do not constitute a  
178 causal link to performance changes. Detailed information on training content is also not available,  
179 which may impact performance and physiological variables. The order of interventions should also be  
180 considered as all subjects undertook the heat intervention in *year 3*, following two years of additional  
181 training (including altitude camps). While this work does not represent a controlled experimental study,  
182 these real world data with repeat measures in the same elite team sport athletes across multiple years  
183 has a high degree of ecological validity.

184

### 185 **Conclusion**

186 More optimal BV profiles (i.e. higher PV and BV) are evident in AF players after completing a full  
187 pre-season training in warm environmental conditions, compared with the inclusion of cold, altitude  
188 camps within the corresponding period. There appears to be no additional haematological benefit for  
189 southern-based (e.g. Melbourne) AF teams traveling north to complete ‘heat’ training camps during  
190 the pre-season. This may inform planning of pre-season training camps/locations, as physiological  
191 benefits are often cited as a driving factor in these decisions.<sup>5</sup>

192 **PRACTICAL APPLICATIONS**

- 193 • Training in the heat should be chosen over cold, altitude environments, if the goal is to  
194 optimize athletes' haematological profile
- 195 • Heat training adaptations are evident in Melbourne during pre-season, spring/summer months.  
196 Therefore, AF teams (and other Australian winter team sports) may not achieve additional  
197 benefits traveling to warmer climates during this period

198 **ACKNOWLEDGEMENTS**

199 The authors would like to thank the players and staff at Collingwood Football Club for their support  
200 of this research project. No additional external funding was received to complete this work and the  
201 authors declare no conflicts of interest.

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235

237 **Table 1.** Melbourne and training camp temperatures during *years 1-3* during for each month, separated  
 238 into total days in that month and training days only. Training camp locations; *year 1* Flagstaff  
 239 (elevation ~2100 m), USA, *year 2* Park City (elevation ~2000 m), USA and *year 3* Gold Coast  
 240 (elevation < 20 m), Australia. Values are mean temperature (°C) ± standard deviation, with number of  
 241 days spent in each condition represented in brackets. No temperatures are shown after the last day of  
 242 data collection in each year and ‘rest period’ days (see Figure 1) are not included.

		Melbourne ‘year 1’	Melbourne ‘year 2’	Melbourne ‘year 3’
Nov	Temperature	24.7 ± 4.7 °C <sup>*#</sup>	23.3 ± 5.2 °C	22.0 ± 4.9
	(total days)	(21 days)	(30 days)	(30 days)
Nov	Temperature	26.4 ± 4.2 °C <sup>*#</sup>	25.3 ± 5.0 °C <sup>#</sup>	23.7 ± 5.9
	(training days)	(11 days)	(7 days)	(15 days)
Dec	Temperature	25.3 ± 4.5 °C	25.8 ± 5.2 °C	24.7 ± 5.6
	(total days)	(20 days)	(13 days)	(30 days)
Dec	Temperature	25.5 ± 3.8 °C	NA	23.5 ± 4.6 °C
	(training days)	(4 days)	(0 days)	(13 days)
Jan	Temperature	27.7 ± 6.1 °C	27.8 ± 6.4 °C	28.6 ± 7.5 °C
	(total days)	(10 days)	(14 days)	(31 days)
Jan	Temperature	24.6 ± 3.2 °C	28.0 ± 6.2 °C	29.5 ± 8.3 °C
	(training days)	(2 days)	(7 days)	(17 days)
Feb	Temperature	-	-	29.0 ± 6.0 °C
	(total days)	-	-	(28 days)
Feb	Temperature	-	-	28.6 ± 5.5 °C
	(training days)	-	-	(17 days)
		Flagstaff ‘year 1’	Park City ‘year 2’	Gold Coast ‘year 3’
Camp	Temperature	7.7 ± 6.3 °C <sup>*</sup>	-3.0 ± 7.3 °C	29.3 ± 0.3 °C <sup>^*</sup>
	(total days)	(19 days)	(18 days)	(6 days)
Camp	Temperature	7.9 ± 7.5 °C <sup>*</sup>	-2.9 ± 7.4 °C	29.3 ± 0.3 °C <sup>^*</sup>
	(training days)	(17 days)	(16 days)	(5 days)

243

244 <sup>^</sup> Substantially higher than *year 1* <sup>\*</sup> substantially higher than *year 2* <sup>#</sup> substantially higher than *year 3*.

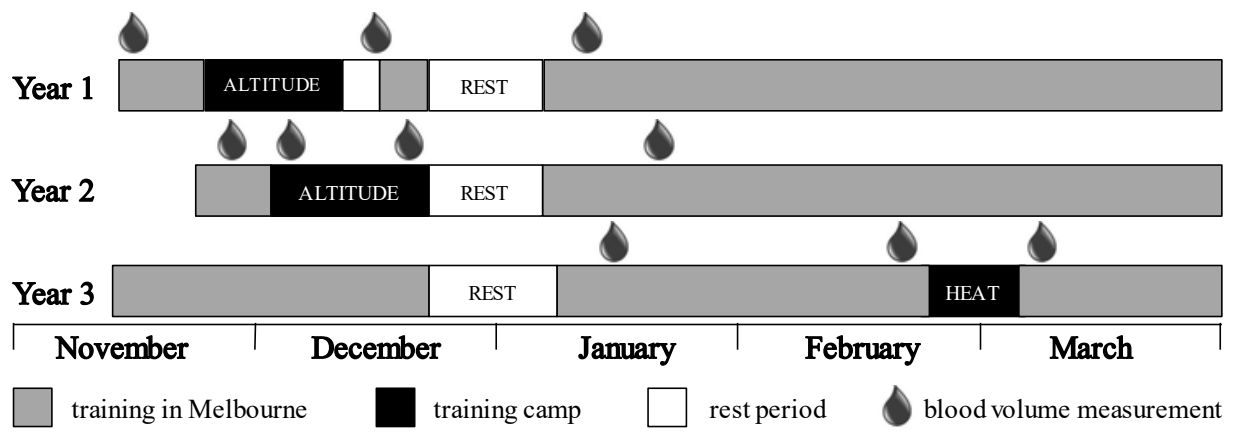
245 NA=not applicable.

246 **FIGURE LEGENDS**

247 **Figure 1.** Timeline of BV measurements over years 1-3.

248

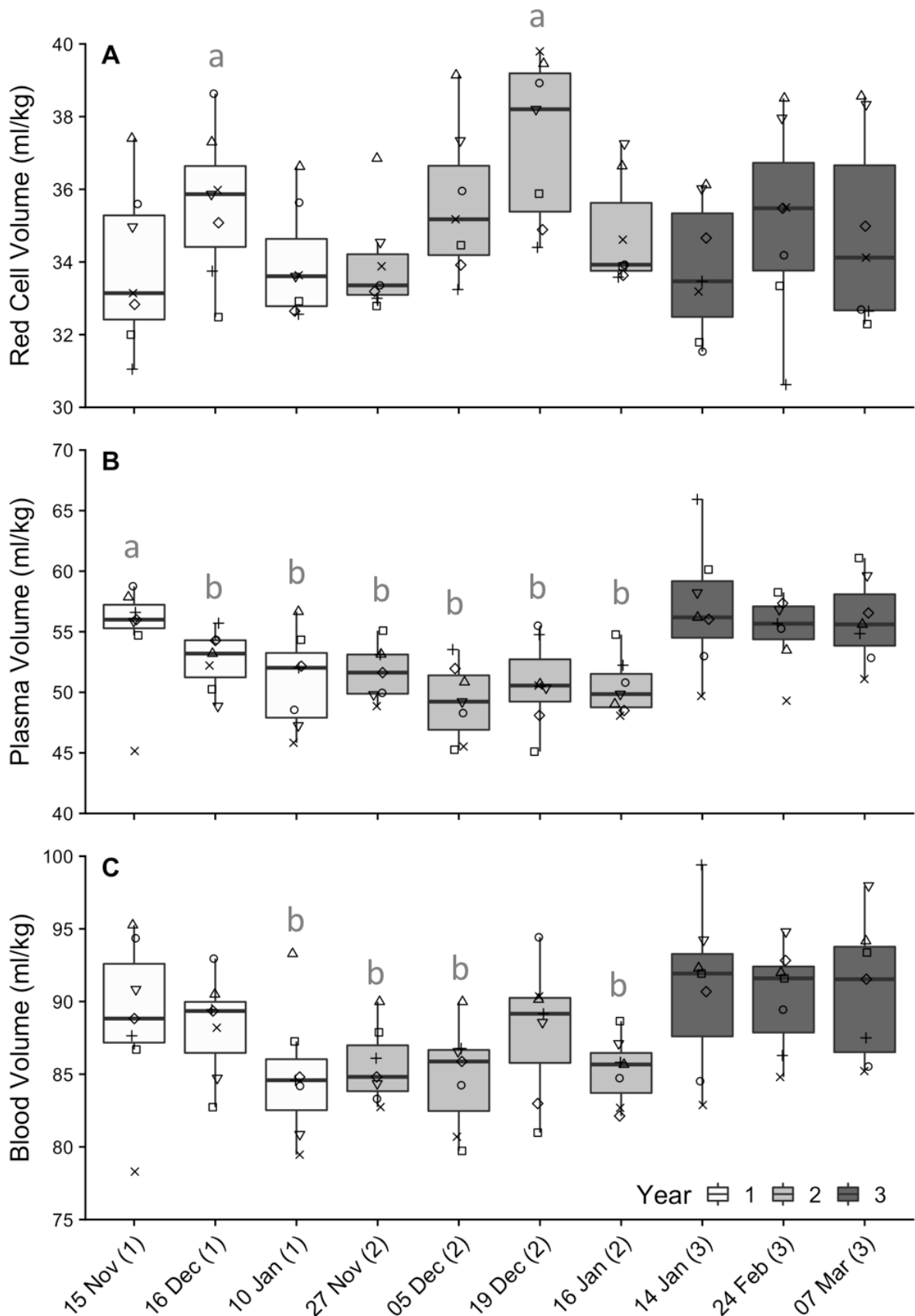
249 **Figure 2.** Relative A) Red Cell Volume, B) Plasma Volume, and C) Total Blood Volume during years  
250 1-3 (data is for same seven players during each year). Year listed in brackets next to testing date. <sup>a</sup>  
251 Substantially higher than all other measurements during that year; <sup>b</sup> substantially lower than all *year 3*  
252 measurements for that variable. Individual data points defined by symbol shape.



253

254 **Figure 1.** Timeline of blood volume measurements over *years 1-3*.





255

256 **Figure 2.** Relative A) Red Cell Volume, B) Plasma Volume, and C) Total Blood Volume during years  
 257 1-3 (data is for same seven players during each year). Year listed in brackets next to testing date.<sup>a</sup>  
 258 Substantially higher than all other measurements during that year; <sup>b</sup> substantially lower than all year 3  
 259 measurements for that variable. Individual data points defined by symbol shape.