

CORRESPONDENCE

The spleen as an unlikely source of red blood cells during activity in fishes

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We read with interest the paper by Brijs et al. (2020) regarding the ‘blood-boosting’ properties apparently exhibited by the Antarctic notothenioid fish (*Pagothenia borchgrevinki*). Although the data provide additional insights into the physiology of an extreme cold-adapted fish, we believe the authors have drawn erroneous conclusions about the mechanisms involved with this ‘blood-boosting’ phenomenon. The authors conclude that the spleen sequesters enough red blood cells (RBCs) to increase the haematocrit (Hct) and, therefore, blood oxygen carrying capacity in fed and exercise states. Further, the authors posit the spleen holds these RBCs in reserve to reduce blood viscosity until additional oxygen is needed to support increases in metabolic rate. In our view, the increases in Hct in *P. borchgrevinki* can be primarily explained by an alternative mechanism that the authors did not consider in their analysis: elevated blood pressure increases plasma efflux from the vascular to the interstitial space, thus increasing the fraction of RBCs in the vascular space (i.e. Hct).

We present two arguments against a role for the spleen in providing a significant contribution to increased Hct in *P. borchgrevinki*. Our first argument is based on the principle of conservation of mass. Brijs et al. (2020) used uninstrumented fish to examine changes in spleen volume at rest, after feeding and following enforced exercise. The comparisons are partly confounded by significant differences in body mass between groups. We have plotted the authors’ data for unfed and fed animals in resting and exercised states to show the significant relationships between spleen mass and body mass (Fig. 1). If we compare a 74 g fish for both groups, spleen mass for resting fish is 0.365 g and is 0.213 g after exercise (Δ spleen mass=0.152 g). Can this change in spleen mass account for the changes in Hct observed by the authors? If we assume a blood volume of 5% of body mass, for a 74 g fish blood volume is 3.7 g (=3.7 ml, assuming blood has a density of 1 g ml⁻¹). The average Hct was 15.8% and 27.1% for resting and exercised fish, respectively. The mass of RBCs for this blood volume is therefore 0.585 g (rest) and 1.003 g (exercise) with a difference of 0.418 g (ml). It is clear that changes in spleen volume cannot account for the mass of RBCs added to the vascular space during exercise. The changes in spleen mass account for approximately 36% of the change in RBC mass with the remaining 64% of RBC unaccounted for; with unfed fish, Hct changes from 8.6% to 25.1% and the non-splenic contribution is larger (76%). We also note that for resting fish (unfed vs fed), Hct increased from ~9% to ~21%, but absolute spleen mass increased, rather than decreased, a further indication that non-splenic mechanisms account for the increased Hct.

If the spleen volume change cannot fully account for the change in RBC mass and changes in Hct, what mechanistic explanation is responsible for these changes? Our second argument is based on the balance of hydrostatic and oncotic forces at the capillary (i.e. Starling forces). These forces, in conjunction with the capillary filtration coefficient, dictate fluid flux across the capillary. Fish capillaries are highly permeable to protein and have a high transvascular fluid flux (Olson et al., 2003); thus a traditional Starling analysis is inappropriate for fishes. An alternative approach for analyzing the balance of forces for transvascular fluid flux was previously developed for the whole animal and incorporates values for hydrostatic pressures and compliances of the vascular and interstitial spaces, along with a whole-body filtration coefficient (Tanaka, 1979); these relationships can be reduced to:

$$\text{Flux} = F_c(P_{\text{vas}} - P_{\text{int}}), \quad (1)$$

where F_c is the whole-body filtration coefficient, P_{vas} is blood pressure and P_{int} is the hydrostatic pressure of the interstitial space (Tanaka, 1979). Because F_c is high in fish, any increase in P_{vas} relative to P_{int} will result in efflux of plasma from the vascular space. This leads to the prediction that Hct should increase with elevated P_{vas} because plasma efflux is not accompanied by loss of RBCs from the vascular space (see also Hillman et al., 2010). This is precisely what the authors observed in their study: Hct was linearly and significantly related to blood pressure in both splenectomized and sham-operated fish during exercise (see table 1 and fig. 5B in Brijs et al., 2020). This is entirely consistent with plasma efflux from the vascular space causing an increase of Hct. We note that previous work with fishes has also demonstrated that increased Hct can result from potentially three different factors: splenic contraction, RBC swelling and plasma efflux. For example, Pearson and Stevens (1991) showed that RBC swelling and splenic contraction each accounted for about 25% of the increased Hct with exercise and air exposure stress in rainbow trout, with the remaining 50% due to plasma efflux. The relatively small contribution by the spleen is in the same range that we have estimated for *P. borchgrevinki*.

An unknown factor in this analysis is blood volume for *P. borchgrevinki*. Blood volume estimates in fish are highly variable and subject to considerable error as techniques typically use plasma protein labeling (see Olson et al., 2003; Hillman et al., 2010) and lead to overestimates in BV. We have assumed a BV of 5% of body mass (a typical vertebrate value), but for the authors’ conclusion to be correct, BV would have to be approximately 1.5% of body mass. This low value seems unlikely. Increased Hct would increase blood O₂ carrying capacity and contribute to O₂ transport

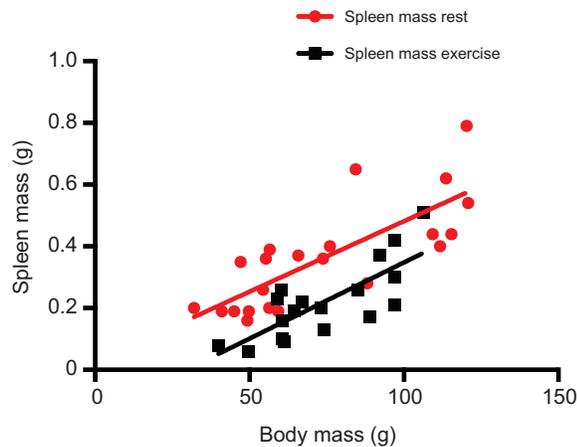


Fig. 1. The relationship between spleen mass (g) and body mass (g) for resting and exercised *P. borchgrevinki* plotted from Brijs et al. (2020). Both resting and exercised animals are combined data from fed and unfed groups. For resting animals, $Y=0.0046+0.03$; $r^2=0.61$; $F_{1,20}=31.4$; for exercised animals, $Y=0.0049-0.15$; $r^2=0.60$; $F_{1,16}=24.2$.

during exercise (Hedrick et al., 2015), but may also be detrimental to O_2 transport with increased viscous resistance and/or limited venous return. What the authors characterize as a unique blood-boosting

role for the spleen as an adaptation to a low temperature environment, we, instead, view the Hct changes as an expected consequence of the vascular properties of fish capillaries in general; the role of the spleen for boosting Hct during elevated metabolic states appears to be significantly overstated.

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CORRESPONDENCE RESPONSE

Response to 'The spleen as an unlikely source of red blood cells during increased activity in fishes'

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We would like to thank Hedrick and colleagues for their thoughtful comments and for the opportunity to further elaborate on the splenic blood-boosting strategy of *Pagothenia borchgrevinki* (Brijs et al., 2020). Hedrick et al. (2020) argue that the reduction in spleen mass observed in our study cannot account for the exercise-induced increase in haematocrit, and that the elevated haematocrit is instead primarily explained by an elevated blood pressure increasing plasma efflux from the vascular to the interstitial space. In our study, we show that the exercise-induced increase in haematocrit is mainly due to the splenic release of erythrocytes, yet, we discussed that cell swelling and haemoconcentration via plasma efflux are also involved. Therefore, we are in agreement with Hedrick et al. regarding the underlying mechanisms and it seems that the disagreement merely concerns the relative contribution of each individual mechanism.

Before we proceed to demonstrate why we have not significantly overstated the role of the spleen for boosting blood oxygen carrying capacity in *P. borchgrevinki*, we would like to clarify that in contrast to what was stated in the correspondence (Hedrick et al., 2020),

none of the comparisons made in our study were confounded by differences in body mass. This is because body mass was included as a covariate in all analyses where it was linearly related to the dependent variable. The correspondence also stated that absolute spleen mass was higher in resting fed fish than in resting unfed fish, which is not the case when corrected for body mass (0.330 versus 0.411 g, respectively, for a 74 g fish).

In our study, we experimentally demonstrated the splenic contribution of erythrocytes during exercise by comparing the exercise-induced increases in haematocrit of fish that were able to eject erythrocytes into circulation from the spleen (sham-operated) to fish that were unable to do so (splenectomised). Haematocrit of sham-operated fish increased from 16.1 to 25.9% in response to exercise, whereas splenectomised fish were only able to elevate haematocrit from 13.1 to 15.3% (Fig. 1A). More importantly, this splenic release of erythrocytes during exercise was associated with substantial metabolic benefits (aerobic scope was ~103% higher in sham-operated fish) and cardiovascular trade-offs [ventral aortic

blood pressure (P_{VA}) and cardiac workload were $\sim 12\%$ and $\sim 30\%$ higher, respectively, in sham-operated fish]. Furthermore, the performance-enhancing benefits of this strategy are demonstrated by the fact that splenectomised fish could not complete an exercise regime and fatigued twice as fast as sham-operated fish (Franklin et al., 1993).

The significant contribution of the spleen in *P. borchgrevinki* is further highlighted by the observed changes in haematocrit and spleen mass of uninstrumented fish following exercise and/or feeding. The exercise-induced increases in haematocrit coincided with decreases in both absolute spleen mass (unfed fish: from 0.411 to 0.214 g, fed fish: from 0.330 to 0.224 g, values corrected for 74 g fish) and relative spleen mass (fig. 3A–C in Brijs et al., 2020). In fact, linear regression analyses revealed that relative spleen mass explained 55.5% and 41.5% of the variation in haematocrit of unfed and fed fish, respectively. Furthermore, relative spleen mass can significantly predict the haematocrit of unfed ($y = -44.798x + 37.290$, $P < 0.001$) and fed *P. borchgrevinki* ($y = -21.256x + 31.469$, $P = 0.001$), where y represents haematocrit and x represents relative spleen mass.

With regards to the argument pertaining to the principle of conservation of mass, the relative contribution of the ejected erythrocytes towards the increase in haematocrit calculated by Hedrick et al. (2020) is incomplete, as they have not taken into account the additional contributions of cell swelling and haemoconcentration via plasma efflux. Interestingly, a previous study by Franklin et al. (1993) attributed $>60\%$ of the exercise-induced increase in haematocrit of *P. borchgrevinki* to the splenic release of erythrocytes, with the remainder mainly attributed to cell swelling (e.g. $>30\%$ decrease in mean corpuscular haemoglobin concentration), as the effects of haemoconcentration via plasma efflux were considered to be relatively minor. The relatively minor contribution of the latter towards the exercise-induced increase in haematocrit is most likely because elevations in P_{VA} of *P. borchgrevinki* during exercise have been shown to coincide with simultaneous decreases in dorsal aortic blood pressure (P_{DA} , Fig. 1B; Axelsson et al., 1994; Sandblom et al., 2008). Thus, the relatively higher level of plasma efflux from the branchial circulation during exercise will be partially counteracted by the

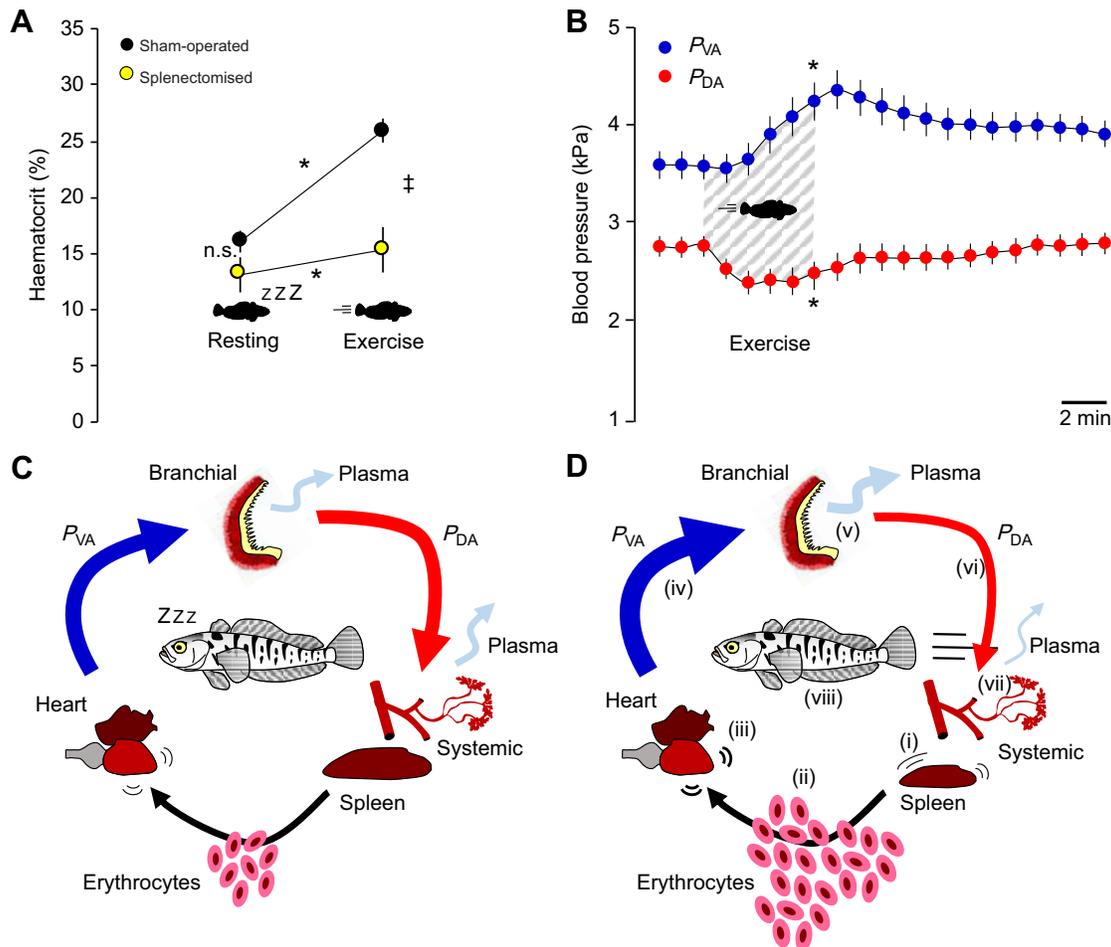


Fig. 1. Exercise-induced changes in haematocrit, ventral aortic (P_{VA}) and dorsal aortic blood pressure (P_{DA}) in *Pagothenia borchgrevinki*, as well as an overview of the splenic blood-boosting strategy of this species. Exercise-induced changes in (A) haematocrit (adapted from table 1 in Brijs et al., 2020) and (B) P_{VA} and P_{DA} of *P. borchgrevinki* (adapted from fig. 2 in Axelsson et al., 1994). Significant differences ($P < 0.05$) within and between groups are represented by * and †, respectively, in A, while differences in ventral and dorsal aortic blood pressure before and after exercise are represented by * in B. (C,D) Overview of the splenic blood-boosting strategy during (C) rest or (D) exercise. In response to exercise, the spleen contracts to eject erythrocytes into circulation (i), which may subsequently swell in size (ii). This increases blood viscosity, which consequently increases cardiac workload (iii) and P_{VA} (iv). This potentially increases plasma efflux at the gills (v). However, because P_{DA} decreases during exercise (vi), plasma efflux may simultaneously be lower in the systemic vasculature (vii). The width of the arrows illustrate the relative differences in P_{VA} (blue arrows), P_{DA} (red arrows) and plasma efflux (light blue arrows) between fish (C) at rest and (D) during exercise.

relatively lower plasma efflux occurring in the systemic circulation (illustrated by changes in the relative thickness of the light blue arrows between resting and exercising fish in Fig. 1C,D). In light of our findings and those referenced in this response, we now summarize the splenic blood-boosting strategy and its consequences for *P. borchgrevinki* (Fig. 1C,D).

In conclusion, *P. borchgrevinki* clearly have a splenic reservoir of erythrocytes that can be released during metabolically demanding situations to substantially elevate blood oxygen carrying capacity. This provides them with an extraordinary facultative aerobic scope that enables an active lifestyle in the extreme Antarctic marine environment, while minimizing the energetic and physiological costs of transporting highly viscous blood during times of reduced energetic demand.

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