I have used the storage compound information from this field data, in combination with a large outdoor exploratory experiment, to investigate storage dynamics of a range of species with very different growth strategies. This experiment comprises eight species and two treatments of one or two full defoliations in a randomised block design. I have measured under-ground/above-ground biomass and storage compounds after six harvests. I show that, by the end of the growth season, defoliated plants have significantly less storage biomass available for spring re-growth and that biomass in defoliated and control plants varies depending on species and growth rates. I suggest that the costs of fast growth after defoliation treatments include reduced fecundity and the 'ultimate cost', which is death of the organism.

In conclusion, the growth–survival trade-off is a major and ubiquitous trade-off, which explains much of the ecological and life-history variation between species. It is exciting to begin to discern the mechanistic basis of this trade-off, especially due to recent advances in metabolic profiling.

YSAS.2
Beyond buoyancy and vision: The potential for the Root effect to deliver oxygen to tissues other than the swim bladder and eye

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Teleost fish possess a unique, pH-sensitive hemoglobin (Hb) that, in the presence of an acidosis, substantially reduces the affinity and carrying capacity for O_2 (Root effect). To date, this efficient O_2 delivery mechanism is only known for filling a swim bladder (SB) against huge pressure gradients (>50 atm) associated with depth and for oxygenating the metabolically active, yet avascular retinal tissue of the eye. In spite of the clear benefits to O_2 delivery for buoyancy and vision, no study has been conducted to determine whether the Root effect may be important in optimizing O_2 delivery to other tissues such as muscle, which is the focus of this research.

During environmental or exercise-induced stress, blood pH may fall; however, some fish regulate red blood cell (RBC) intracellular pH (pH_i) by releasing catecholamines that activate the sodium/proton (Na^+/H^+) exchanger (βNHE) on the RBC membrane. The βNHE removes H^+s from the RBC resulting in an intracellular alkalosis, an increase in Hb–O_2 affinity, and O_2 uptake at the respiratory surfaces is safe-guarded, which is the ultimate goal of this mechanism. In our proposed model, when adrenergically stimulated blood encounters plasma-accessible carbonic anhydrase (CA), an enzyme found in the RBC but also membrane-bound and potentially plasma-accessible in select locations, it will catalyze H^+s removed from the RBC to form CO_2. This CO_2 will back-diffuse into the RBC creating an intracellular acidosis (extracellular alkalosis), reducing Hb–O_2 affinity, and ulti-mately elevating PO_2 via the Root effect. We created an *in vitro* closed system using rainbow trout (*Oncorhynchus mykiss*) blood where we can (1) simulate an acid-induced Root effect, (2) adrenergically stimulate the RBCs, and finally (3) short-circuit the βNHE via CA (CA-mediated Root effect), all of which can be monitored in real-time (Fig. 1). Data generated currently support our *Hypothesis: adrenergic RBC pH regulation can be short-circuited in the presence of plasma-accessible CA, therefore generating a Root effect increase in PO$_2$*. In fact, if this scenario also occurs in the tissues of *O. mykiss*, CA-mediated short-circuiting of adrenergic pH regulation can facilitate an increase in PO_2 over 30 times that which would be generated in vertebrates possessing only a Bohr shift! We are ready to test our model *in vivo* by implanting fiber-optic O_2 sensors in *O. mykiss* muscle while simulating environmental and exercise stress with and without CA blockers. Furthermore, even though CA is not found in general circulation, there are membrane-bound and potentially plasma-accessible isoforms in muscle endothelia, and research is underway to localize this enzyme to understand the relationship between location and function of the short-circuiting.

Teleost fish, which are more numerous than all other vertebrates combined (terrestrial and aquatic), have evolved an extraordinary O_2 delivery mechanism, the Root effect, that allows O_2 delivery to the eye and to the SB, thus allowing efficient buoyancy regulation, which may be one of the most important factors responsible for the extensive adaptive radiation in teleost fishes. Therefore, it is particularly interesting that the Root effect has not yet been investigated for general O_2 delivery. If the Root effect can also facilitate *general* O_2
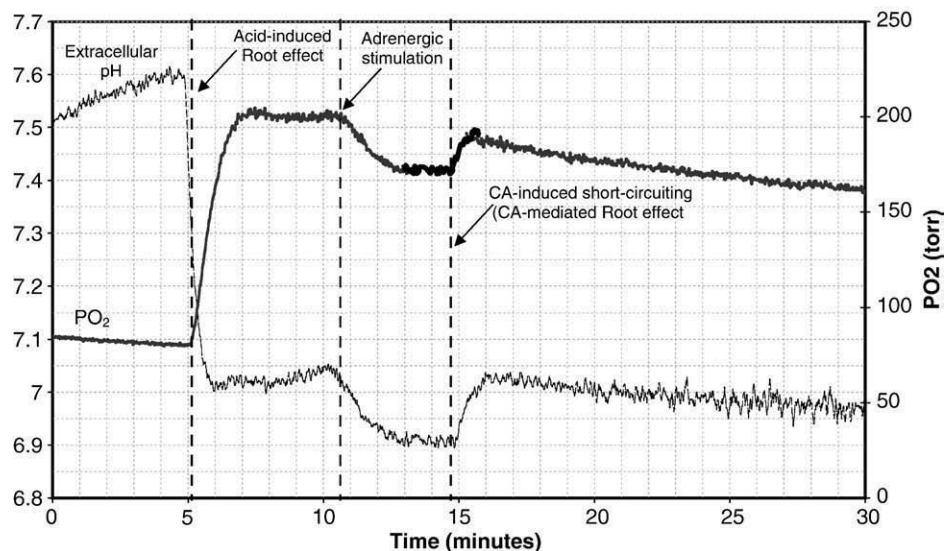

Fig. 1. Representative trace depicting real-time changes in extracellular pH (light gray, y-axis on the left) and PO_2 (black, y-axis on the right) upon an acid induced Root effect (vertical dashed line furthest to the left), adrenergic stimulation (middle vertical dashed line), and carbonic anhydrase (CA) induced short-circuiting (vertical line on the right) over the course of time (minutes).

delivery *in vivo*, which our data currently support, this would help shed insight into how the Root effect was selected for prior to the evolution of the βNHE, choroid gland and retia of the eye, and the gas gland and *rete mirabile* associated with the SB.

YSAS.3
Matching O₂ supply and demand in the high flying bar-headed goose

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The bar-headed goose flies over the Himalayan mountains twice a year on its migratory route between southern and central Asia. This species must therefore fly over the highest mountains in the world, at altitudes of up to 9000 m where oxygen levels are 1/5 of those at sea level (Javed et al., 2000). The ability of bar-headed geese to maintain O_2 supply in these conditions is especially impressive when considering that O_2 consumption rates increase 10–20-fold above resting levels during flight (Ward et al, 2002). The haemoglobin of bar-headed geese has an inherently higher O_2 affinity (Petschow et al., 1977), which should facilitate O_2 loading into the blood during flight in severe hypoxia, and thus help match O_2 supply to O_2 demand. Until recently, however, the presence of additional high altitude adaptations along the oxygen transport pathway of this species was uncertain.

From the results of our recent theoretical study (Scott and Milsom, 2006), we predicted that a heightened ability to increase breathing should enhance O_2 loading into the blood and therefore be adaptive for high altitude flight. We tested this prediction with a series of *in vivo* experiments where we exposed waterfowl to hypoxia, and measured numerous cardiorespiratory variables to understand how breathing and metabolism are controlled. We found that ventilation was nearly 2-fold higher in bar-headed geese than in greylag geese and pekin ducks during severe poikilocapnic (uncontrolled CO_2) hypoxia (Scott and Milsom, 2007). This was largely caused by their greater tidal volumes, and increased blood O_2 loading by 40%. Two mechanisms probably caused these differences in bar-headed geese (Scott and Milsom, 2007): (i) a reduced sensitivity to the decreases in CO_2 that occur during hypoxia; and (ii) a blunted metabolic depression response. We subsequently found that body temperature depression and peripheral heat loss, which normally facilitate metabolic depression, are also reduced in bar-headed geese during hypoxia (Scott et al., in press).

Our theoretical study (Scott and Milsom, 2006) and those of others (Hochachka and Burelle, 2004) predict that enhancing O_2 supply and utilization within exercising muscle should also be adaptive at high altitude. We tested this prediction by exploring the control of mitochondrial regulation *in situ* in permeabilized flight muscle fibers of waterfowl using high-resolution respirometry. While respiratory capacities were similar between species, bar-headed geese muscle had more characteristics of fatigue-resistance than muscle from greylag geese, barnacle geese, or mallard ducks. Sensitivity of mitochondrial respiration to ADP was reduced (2-fold higher Km) while creatine sensitivity was enhanced in this species compared to low altitude waterfowl. These properties may better coordinate ATP supply and demand in the flight muscle, which should correspondingly improve the balance between cellular O_2 supply and demand in bar-headed geese during hypoxia.

Our data suggest that a suite of adaptations have evolved in bar-headed geese to better match O_2 supply and demand during flight at high altitude. By sustaining metabolism and ATP turnover, during hypoxia so severe that many animals can barely function, bar-headed geese have an exceptional ability to fly high.

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YSAS.4
Deformation and aerodynamic performance of a flapping artificial butterfly wing in free flight

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A butterfly's large wing dynamically deforms during flapping motion. The deformation of the wing accompanied by a change of aerodynamic performance is passively controlled by wing veins. Therefore, it is thought that a design of a vein structure ranging from a base to outer edge is optimized mechanically and aerodynamically in the course of evolution. The effect of the vein structure on flight, however, is still unknown, since it is impossible to compare flights with wings of different veins, that is, we cannot exchange living butterfly's wings experimentally. Moreover, it is very difficult to distinguish a motion by wing deformation from that by active control such as change of flapping amplitude or frequency.

As a new tool making it possible to experiment with arbitral vein structures at constant flapping motion, we have developed a MEMS (Micro Electro Mechanical Systems) butterfly wing mounted on an ultra-light rubber powered ornithopter (Fig. 1). The artificial butterfly wing consists of a thin polymer membrane supported by plastic veins of rectangular cross-section of which thickness and width can be changed as designed. Cross-sectional sizes of the veins are a few hundred micrometers like an actual butterfly's veins. Its planar shape and venation is the same as those of a swallowtail butterfly, *Papilio karna*. Other major parameters are in the same range of an actual butterfly; a total mass of the ornithopter including the wings is 0.39 g, and a flapping frequency is around 10 Hz. Unlike an experiment with an actual butterfly, we can extract only the effect of veins on flight by exchanging wings for different ones, since the flapping motion of the ornithopter is a mechanically constant.
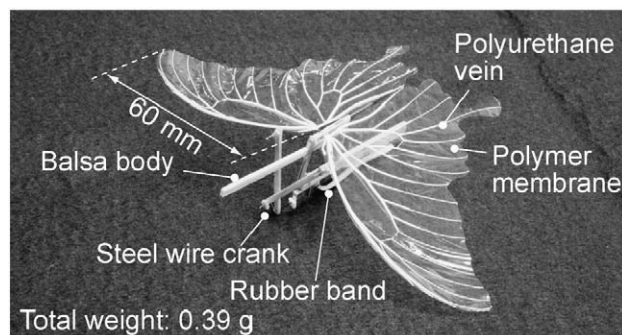

Fig. 1. Rubber powered ornithopter with artificial butterfly wings.