ENERGETIC CONSEQUENCES OF COORDINATING WINGBEAT AND RESPIRATORY RHYTHMS IN BIRDS

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Summary

The coordination of ventilatory and locomotor rhythms has been documented in many birds and mammals. It has been suggested that the physiological significance of such coordination is a reduction in the cost of ventilation which confers an energetic advantage to the animal. We tested this hypothesis by measuring the external work required to ventilate birds mechanically during simulated flight. Patterns of wing motion and breathing were produced in which the relationship between wing motion and breathing was in phase and out of phase with the relationship seen during normal flight. Differences between the energetic costs of in-phase *versus* out-of-phase synchronization were particularly large (26 %) in instances where locomotion and respiration frequency were synchronized at one breath per wingbeat. The saving (9 %) obtained from in-phase *versus* out-of-phase coordination at the 3:1 coordination ratio seen normally in free-flying Canada geese was smaller but still supported the hypothesis that there is a significant net saving obtained from reducing the mechanical interference between locomotion and ventilation by locomotor–respiratory coupling.

Key words: *Branta canadensis*, Canada goose, simulated flight, ventilation, work of breathing, entrainment, locomotor–respiratory coupling.

Introduction

During galloping, quadrupeds from the gerbil to the rhinoceros take one breath per stride (Bramble and Carrier, 1983; Young *et al.* 1992), the breath always occurring at the same part of the locomotor cycle. Similarly, in some birds during flight, inspiration is synchronized with the upstroke of the wings (Tomlinson, 1957; Hart and Roy, 1966; Butler *et al.* 1977; Berger *et al.* 1970). Such coordination could reduce the work of breathing in two ways: (i) by reducing the mechanical interference between locomotion and respiration (both require movements of the thorax and abdomen); (ii) by transferring the work of producing ventilation, at least in part, from the normal respiratory muscles to the movements produced by locomotion. For example, by acting as a bellows, the action of bending and extending the trunk during galloping in the horse may completely relieve the work of the diaphragm (Young *et al.* 1992). Furthermore, inertial oscillations of the viscera in hopping wallabies (Baudinette *et al.* 1987) and trotting dogs (Bramble and Jenkins, 1993) may act as a piston mechanism (Alexander, 1993) assisting in producing respiratory airflow. It should be noted, however, that such transfer of work will only reduce the net work of breathing if work is transferred to muscles that are more efficient.

The hypothesis that the work of breathing is reduced by reducing the mechanical interference between locomotion and respiration appears reasonable, especially for cases where the locomotor and respiratory rhythms show 1:1 coordination. Coordination ratios other than 1:1, however, are frequently observed in bipeds and quadrupeds during overground locomotion (Bramble and Carrier, 1983) and in birds during flight (Berger *et al.* 1970; Butler and Woakes, 1980; Funk *et al.* 1993). For example, many birds exhibit 3:1 coordination between wingbeats and respiration (three wingbeats to each breath) (Butler and Woakes, 1980; Funk *et al.* 1993). In this case, there are two downstrokes and one upstroke associated with inspiration and two upstrokes and one downstroke associated with expiration. The mechanical and energetic consequences of this coupling are not immediately obvious.

Despite its appeal, the hypothesis that locomotor–respiratory coupling reduces the work of breathing has not been tested. In no instance has an energy saving associated with locomotor–respiratory coordination been measured, largely because of the difficulty of uncoupling locomotion and respiration in freely behaving animals. Recently, we developed a technique for artificially flapping the wings of decerebrate, paralyzed Canada geese (*Branta canadensis*) which produced locomotor–respiratory coordination similar to that seen in conscious, flying animals (Funk *et al.* 1992*a*). In the present study, we employed this technique together with artificial

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ventilation of animals at wingbeat to ventilation ratios of 1:1 and 3:1 with the relationship between respiration and wingbeat both in phase and out of phase with that seen in free flight. This should mimic the best- and worst-case conditions, respectively, if the above hypothesis is correct. By measuring the effects of altering the phase relationship on the mechanical work required to ventilate the lungs, we were able to provide a crude test of this hypothesis.

Materials and methods

Experiments were performed on three Canada geese (*Branta canadensis* Alison, 3.4±0.2 kg body mass; mean ± S.E.M.). The surgical procedures used were identical to those described previously (Funk *et al.* 1992*a*). Briefly, surgery was performed under halothane/nitrous oxide anesthesia. The trachea was cannulated to permit artificial ventilation and to monitor ventilation *via* pneumotachography. Body temperature was monitored *via* a thermistor placed down the esophagus and maintained at 41 °C by the use of a heat lamp and a copper, water-perfused heat exchanger inserted through the anus into the intestine.

The birds were supported in a sling, their heads were placed in a stereotaxic head holder, and the animals were decerebrated. The body was then suspended and stabilized from the dorsal surface by two bone clamps, one attached to the pelvic girdle and the other to the dorsal vertebral spines just caudal to the brachial plexus. This was required to prevent compression of the thorax onto the sling during the downstroke of the wing flapper.

Each wing was fastened to a bar that held it in the approximate position seen during free flight (Funk *et al.* 1992*a*). Each wing bar was attached to a support rod that overlay an eccentric adjustable cam. The anchored end of the support rod acted as a pivot. A 186 W direct-current motor rotated the cam and generated the passive wing movements. Wing flapping frequency (fw) was adjusted *via* a rheostat attached to the motor. Wingbeat amplitude was adjusted to approximate the amplitude observed during free flight at 50–60 km h^{-1} (Funk *et al.* 1992*a*) by adjusting the diameter of the eccentric cam.

In the present study, we also electrically stimulated the pectoralis major muscles, since they have the largest impact on the mechanics of the thorax. They form the largest part of the wing musculature (unlike any other tetrapod muscle, they may constitute up to 35 % of body mass) and are the main muscles driving the power stroke (the downstroke) of the wing cycle. Although precise activation and coordination of many muscle groups is required to produce wing motion during free flight, the energetic consequences of activating additional wing musculature were not examined.

The electrical stimulus was applied in a manner that resembled the activity pattern of the pectoralis electromyogram (EMG) activity in free-flying birds (Dial *et al.* 1988). The stimulus parameters used were 10–15 V, 0.3–0.6 ms and 50 Hz, and the stimulus was applied between 0.90 and 0.40 of the wing cycle period, where 0.0 represents the top of the upstroke, 0.45 represents the bottom of the downstroke and 1.00

Table 1. *Cost of ventilation at rest and during simulated flight*

	1:1 coordination	N	3:1 coordination	N
Total work × 10⁶ (J breath⁻¹)				
No flapping	448±120	19	333±83‡	18
In phase	407±73*	14	359±82	10
Out of phase	522±204*,†	14	393±69*	13
Resistive work × 10⁶ (J breath⁻¹)				
No flapping	140±49	36	79±31‡	36
In phase	88±22	14	64±23	10
Out of phase	165±88–	14	93±22‡	13
Elastic work × 10⁶ (J breath⁻¹)				
No flapping	310±76	36	252±63	36
In phase	313±86	14	294±61	9
Out of phase	333±90	16	301±61	13

Total, resistive and elastic work per breath required to ventilate the lungs were measured under control conditions (no flapping) and under conditions in which the lungs were inflated in phase and out of phase with the wing movements at ratios of one and three wingbeats per breath (1:1 and 3:1, respectively).

Values are reported as means ± S.D. *N* represents the total number of breaths analyzed from the three birds.

*Significant difference from control values; †Significant difference from in phase values; ‡Significant difference from 1:1 values.

represents completion of the cycle. Thus, the stimulus was applied from just before the top of the upstroke until just before the completion of the downstroke, closely resembling the activity pattern of pectoralis EMG activity in free-flying pigeons *Columba livia* (Dial *et al.* 1988). The electrodes were sewn into the muscle and these stimulus parameters were supramaximal, suggesting that the entire muscle was recruited.

Following surgery, anesthesia was discontinued and animals were paralyzed using gallamine triethiodide and artificially ventilated using a positive pressure pump (Harvard Apparatus dual-phase respirator, model 665).

We used this technique while mechanically ventilating the lungs of the geese at rest and during artificial wing flapping at 1:1 and 3:1 ratios of wing flapping frequency (fw) to ventilation frequency (fv). Ventilation frequencies and volumes were initially set to equal the respiratory frequencies and volumes recorded in intact animals at rest and were appropriate for maintaining blood gases at constant levels (Funk *et al.* 1989). For 3:1 coordination, we selected a fw (0.60–0.70 Hz) that was three times greater than resting respiratory frequency (fv=0.20–0.23 Hz). We then increased fv to match fw to produce 1:1 coordination. This was performed where: (i) the phase relationship between respiration and wingbeat frequency observed in free-flying birds (Tomlinson, 1957; Hart and Roy, 1966; Butler *et al.* 1977; Berger *et al.* 1970; Butler and Woakes, 1980; Funk *et al.* 1993) was reproduced (in-phase activity; for 3:1 entrainment this was two downstrokes and one upstroke associated with each inspiration and two upstrokes

and one downstroke associated with each expiration; for 1:1 entrainment, the upstroke was produced during inspiration and the downstroke during expiration); (ii) the phase relationship between wingbeat and respiration frequency was 180° out of phase with that observed during free flight.

Under all conditions, we measured the mechanical work of ventilation (Otis, 1964). Pressure–volume loops, constructed from tracheal pressure (monitored using a strain-gauge pressure transducer attached to the tracheal cannula) and tidal volume (monitored *via* pneumotachography) data were used to calculate the total, resistive and elastic work required to produce each breath using the method described by Otis (1964). Differences in calculated work levels were compared for the different conditions using an analysis of variance (ANOVA), and Bonferroni simultaneous confidence intervals for multiple comparisons (RS/PROBE, BBN Laboratories) were used to test for differences between means (*P*<0.05 was assumed to be significant).

Results

With the wing flapping device turned off, the work required

to ventilate the lungs of these birds at 12–14 breaths min^{-1} (equivalent to the ventilatory rate used at a 3:1 ratio) was $333 \times 10^{-6} \pm 83 \times 10^{-6}$ J breath^{-1}, and this increased to $448 \times 10^{-6} \pm 120 \times 10^{-6}$ J breath^{-1} when the ventilation frequency was increased to 36–42 min^{-1} (equivalent to the ventilatory rate at a 1:1 ratio) (Table 1). This was due to a 1.23-fold increase (not significant) in the work required to overcome elastic forces and a 1.77-fold increase in the work required to overcome resistive forces. These values are not surprising given that tidal volume remained constant while only breathing frequency was increased.

Fig. 1A shows examples of the pressure, flow and volume traces obtained during 1:1 coordination (synchronization) of wingbeat and respiration, both when the upstroke was coincident with inspiration, as occurs in birds which exhibit this ratio in nature ('in phase', Table 1) (Tomlinson, 1957; Hart and Roy, 1966; Butler *et al.* 1977; Berger *et al.* 1970) and when the phase relationship was shifted by 180° so that inspiration occurred during the downstroke ('out of phase', Table 1). Fig. 1B,C shows the pressure–volume loops associated with the traces illustrated in Fig. 1A. Note the much larger pressure excursions associated with artificial ventilation at a constant
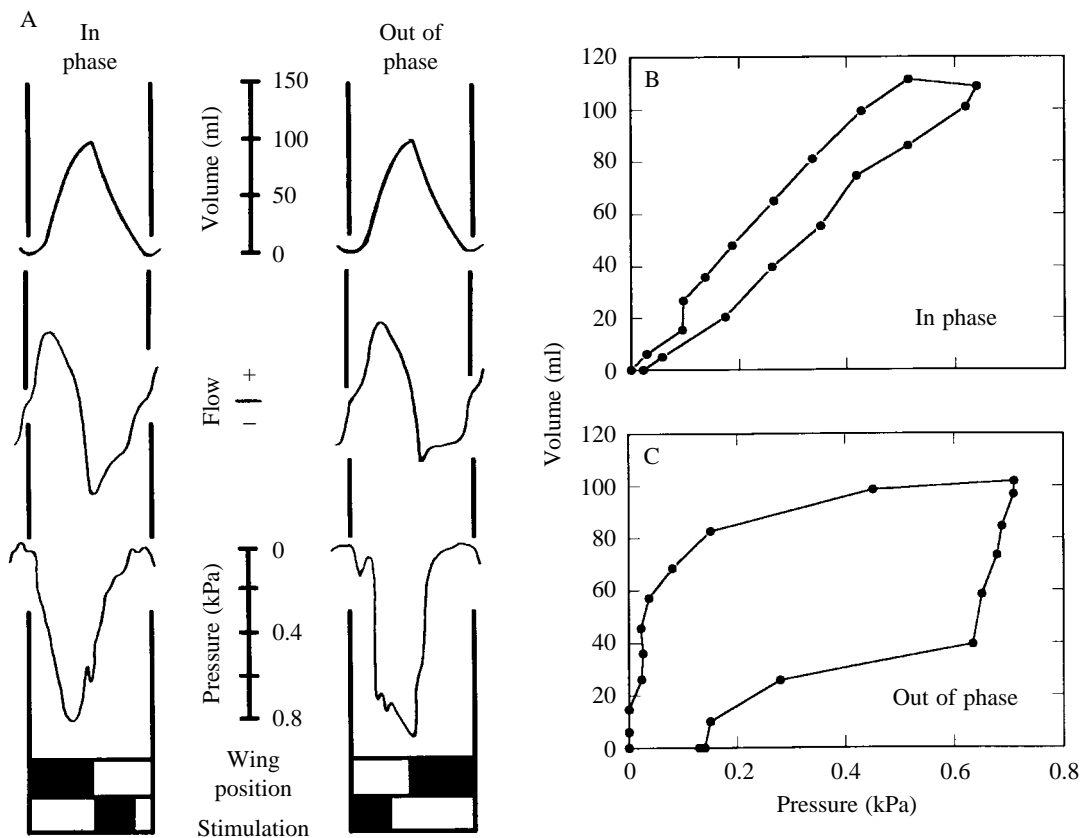

Fig. 1. (A) Volume, airflow and intrapulmonary pressure traces from an individual Canada goose during artificial ventilation. Ventilation was coupled 1:1 with artificial wing flapping such that inspiration occurred during the upstroke (in phase) or during the downstroke (out of phase). The upper horizontal bar below the traces indicates when the wings were rising (upstroke, black band) and falling (downstroke, white band). The black band in the lower horizontal bar indicates when electrical stimulation of the pectoralis muscles occurred. (B,C) Pressure–volume relationships obtained from the traces shown in A. Measurements of the work required to overcome elastic and resistive forces to produce each breath were obtained from these relationships as described in the text.
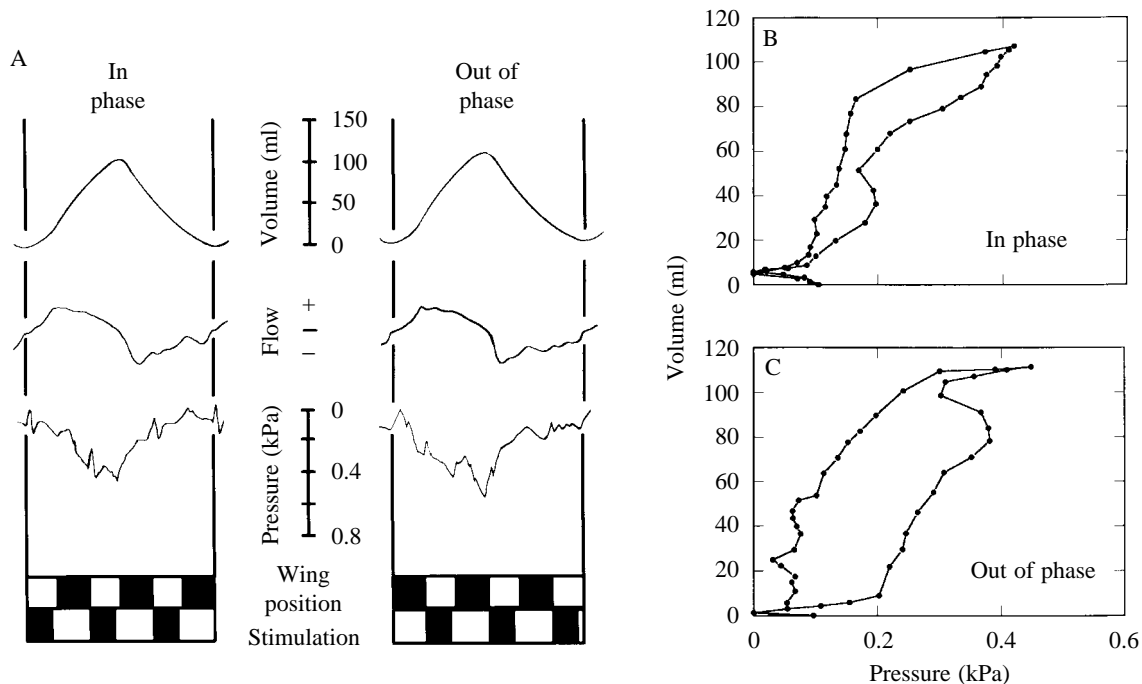
Fig. 2. (A) Volume, airflow and intrapulmonary pressure traces from an individual Canada goose during artificial ventilation. Ventilation was coupled 3:1 with artificial wing flapping such that two downstrokes and one upstroke occurred during inspiration (in phase) or two upstrokes and one downstroke occurred during inspiration (out of phase). The upper horizontal bar below the traces indicates when the wings were rising (upstroke, black band) and falling (downstroke, white band). The black band in the lower horizontal bar indicates when electrical stimulation of the pectoralis muscles occurred. (B,C) Pressure–volume relationships obtained from the traces shown in A. Measurements of the work required to overcome elastic and resistive forces to produce each breath were obtained from these relationships as described in the text.

tidal volume when inspiration occurs during the downstroke (out of phase, Fig. 1C) than when it occurs during the upstroke (in phase, Fig. 1B). The total mechanical work required to ventilate the lungs decreased significantly (9 %) from control levels (no flapping, Table 1) with the onset of in-phase wing flapping. When the phase relationship was shifted by 180° so that inspiration occurred during the downstroke ('out of phase', Table 1), the cost of ventilating the lungs increased by 17 % relative to control values. Thus, the reduction in the cost of ventilation during 'in-phase' flapping relative to 'out-of-phase' flapping was 26 % (Table 1). The difference between the cost of ventilation during 'in-phase' *versus* 'out-of-phase' flapping was primarily due to a 54 % decrease in the work required to overcome flow resistive forces (resistive work, Table 1) between these two conditions.

Wingbeat and respiration frequency are primarily coupled at a 3:1 ratio during free flight in Canada geese, with inspiration beginning at the top of the upstroke and ending at the bottom of the downstroke of the following cycle (i.e. two downstrokes and one upstroke for each inspiratory event) (Funk *et al.* 1993). Fig. 2A shows examples of the pressure, flow and volume traces obtained during 3:1 coordination of wingbeat and respiration, both when synchronization occurred in phase with the relationship seen in nature (Tomlinson, 1957; Hart and Roy, 1966; Butler *et al.* 1977; Berger *et al.* 1970) and when the phase relationship was shifted by 180° so that two upstrokes and one downstroke occurred during inspiration

('out of phase'). Fig. 2B,C shows the pressure–volume loops associated with the traces illustrated in Fig. 2A. Again, note the much larger pressure excursions associated with artificial ventilation at a constant tidal volume when the inspiration is produced out of phase than when it is produced in phase. Under these simulated 'in-phase' conditions, the total mechanical work required to ventilate the lungs was not significantly greater than that required to ventilate the lungs under control conditions (Table 1). When the phase relationship between wingbeat and respiratory movements was shifted to 180° out of phase with the relationship seen during free flight, the mechanical cost of ventilating the lungs increased significantly (17 %) relative to that required to ventilate the lungs under control conditions. Under these conditions, both flow resistive work and the work required to overcome elastic recoil forces (Table 1) increased, although these increases were not significant. The net savings of 'in-phase' *versus* 'out-of-phase' coordination were much less for 3:1 than for 1:1 coordination (9 % *versus* 26 %). While the total work associated with in-phase flapping for 3:1 coordination was not significantly greater than that associated with 'no flapping' conditions, the total work associated with out-of-phase coordination was significantly elevated above the latter value.

Discussion

The measurements obtained in the present study provide

only an indication of the mechanical work required to artificially ventilate the lungs of the birds under our experimental conditions, as opposed to the biological work required for the bird to ventilate its lungs under natural conditions. We believe that these values will underestimate both the total costs of ventilation and the energetic benefits of natural locomotor–respiratory coordination. Resting ventilation was produced in birds with reduced postural tone and hence a relaxed thorax and abdomen, and the artificial activation of the flight muscles during wing flapping was confined to the pectoralis muscles alone. During free flight, postural tone would be high, and precise activation and coordination of many muscle groups is required to produce wing motion. Activation of other muscles is likely to increase chest stiffness and increase elastic work, which should further increase the difference between in-phase and out-of-phase flapping. Furthermore, the wingbeat and ventilatory frequencies used in the present study ($f_W=0.6$–0.7 Hz; $f_V=0.2$–0.7 Hz) were much lower than those recorded in free flight ($f_W=3.9$–4.5 Hz; $f_V=1.3$–1.5 Hz; Funk *et al.* 1993) owing to the technical difficulties of flapping the wings at such high frequencies. Our use of low values for f_W and f_V would probably reduce the differences in work between in-phase and out-of-phase coordination since the mechanical costs of ventilation increase with increasing f_V (Table 1).

Despite these likely underestimates, there is a significant saving between in-phase and out-of-phase coordination of wingbeat and ventilation frequency that supports the hypothesis that synchronization of locomotion and respiration serves to reduce the cost of breathing in flying birds. This saving was larger for 1:1 coordination than for 3:1 coordination.

Although 1:1 coordination of wingbeat and respiration frequency in Canada geese has only been observed under laboratory conditions where 'flight' was induced by electrical stimulation of specific brainstem locomotor command centers (Funk *et al.* 1992*b*; Valenzuela *et al.* 1990), 1:1 coordination does occur during free flight in pigeons and crows (Tomlinson, 1957; Hart and Roy, 1966; Butler *et al.* 1977; Berger *et al.* 1970). Our data suggest that the energetic savings from locomotor–respiratory coupling may be greatest in these species.

Wingbeat and respiration frequencies are coupled primarily at a 3:1 ratio during free flight in Canada geese, with inspiration beginning at the top of the upstroke and ending at the bottom of the downstroke of the following cycle (i.e. there are two downstrokes and one upstroke for each inspiratory event) (Funk *et al.* 1993). Although 3:1 coordination of f_W and f_V is the common pattern during free flight in Canada geese, this relationship is not fixed. Brief transitions from 3:1 to 2:1 coordination are occasionally observed (Funk *et al.* 1993). 3:1 coordination is also the preferred pattern in Barnacle geese (*Branta leucopsis*), although patterns of 2:1 and 4:1 coordination have been observed (Butler and Woakes, 1980). Under the simulated 'in-phase' conditions of the present study, the total mechanical work required to ventilate the lungs in a

3:1 ratio was not significantly greater than that required to ventilate the lungs under control conditions (Table 1). When the phase relationship between wingbeat and respiratory movements was shifted by 180° to be out-of-phase relative to the relationship seen during free flight ('out of phase' then representing two upstrokes and one downstroke for each inspiratory event), the mechanical cost of ventilating the lungs increased significantly (17 %) relative to that required to ventilate the lungs under control conditions. Under these conditions, both flow resistive work and the work required to overcome elastic recoil forces (elastic work, Table 1) showed a tendency to increase, although this was not significant. The net saving from 'in-phase' *versus* 'out-of-phase' coordination, was much less for 3:1 than for 1:1 coordination (9 % *versus* 26 %). In both instances, the increase in total work associated with out-of-phase breathing was due primarily to muscle activation since differences in work associated with passive flapping with no electrical stimulation were only 1 % and 7 % for 3:1 and 1:1 coordination, respectively (results not shown).

Thus, the results from the 3:1 coordination experiments were also consistent with the hypothesis that coordination reduces ventilatory costs. Since synchronizing inspiration to the upstroke of the wings was energetically favorable for 1:1 coordination, we anticipated that the pattern of coupling which maximized the coincidence of upstroke and inspiration during 3:1 coupling (two upstrokes and one downstroke) would also be energetically favorable. This was not the case. The 'in-phase' pattern of two downstrokes and one upstroke used by geese during free flight (Butler and Woakes, 1980; Funk *et al.* 1993) was less expensive and suggests that the benefits of such coordination are derived more from a reduction in mechanical interference than from a transfer of the work of breathing from the respiratory muscles to locomotor activity. D. F. Boggs, F. A. Jenkins and K. P. Dial (in preparation) have shown that inspiratory flow in black-billed magpies (*Pica pica*) is impeded by the downstroke but assisted by the upstroke (and *vice versa* for expiratory flow), which more strongly supports this conclusion.

In summary, these results suggest that locomotor–respiratory coordination significantly reduces the mechanical costs associated with breathing, especially under conditions in which the coupling ratio is 1:1. Reductions in biological work are predicted to be larger than the reductions in mechanical work reported here.

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