Ruddy turnstones *Arenaria interpres* rapidly build pectoral muscle after raptor scares

Piet J. van den Hout, Theunis Piersma, Anne Dekinga, Suzanne K. Lubbe and G. Henk Visser


To cope with changes in the environment, organisms not only show behavioural but also phenotypic adjustments. This is well established for the digestive tract. Here we present a first case of birds adjusting their flight machinery in response to predation risk. In an indoor experiment, ruddy turnstones *Arenaria interpres* were subjected to an unpredictable daily appearance of either a raptor or a small gull (as a control). Ruddy turnstones experiencing threat induced by a flying raptor model, longer than after similar passage by the gull model, refrained from feeding after this disturbance. Pectoral muscle mass, but not lean mass, responded in a course of a few days to changes in the perceived threat of predation. Pectoral muscle mass increased after raptor scares. Taking the small increases in body mass into account, pectoral muscle mass was 3.6% higher than aerodynamically predicted for constant flight performance. This demonstrates that perceived risk factors may directly affect organ size.

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It is becoming evident that evolutionary design processes that affect behaviour (general activity, habitat use, daily routines), also affect physiological processes, including the size of functional organs, and that they do so over similar time scales (Piersma and Drent 2003). In every organism where it has been studied, the digestive tract responds rapidly and in highly integrated ways to variations in food quality and energy demand (e.g. Starck and Rahmaan 2003, Van Gils et al. 2003, Pennisi 2005, Starck 2005, Van Gils et al. 2005). Free-living organisms are affected in major ways also by the danger of predation (Lima and Dill 1990, Cresswell 1994, Cresswell and Whitfield 1994, Caro 2005). For example, incorporating risk management into the suite of selective pressures, Relyea and Auld (2004) found that tadpoles of the wood frog *Rana sylvatica* that grew up in pools with predators showed slower growth because they invested in tails (for swimming speed) rather than guts (for food processing).

Building on the knowledge that the main flight organ of birds, the pectoral muscle complex, demonstrates size variability in a seasonal context (Marsh 1984, Evans et al. 1992, Jehl 1997, Piersma et al. 1999), Lind (2001) suggested that moulting tree sparrows *Passer montanus* could increase pectoral muscle size to reduce risk of predation by airborne raptors (see also Lind and Jakobsson 2001). Here we test whether a perceived threat of predation may directly affect organ size by exposing ruddy turnstones *Arenaria interpres* to one sudden disturbance per day of a model predator and noninvasively measuring the size of the flight machinery, the pectoral muscle complex. We assumed that an increase in pectoral muscle size would enhance escape flight performance. Ruddy turnstones, as a rule, take flight when disturbed by a raptor (Whitfield 1988, Lima 1993). Moreover, Dietz et al. (unpubl. data), for a similar type of shorebird, showed that birds with relatively larger pectoral muscles had greater manoeuvrability in flight.
Methods

Ruddy turnstones are medium-sized shorebirds that feed on small prey in nearshore habitats in small flocks, where the risk of predation by surprise-attacks may be relatively high (Metcalfe and Furness 1984, Whitfield 1990). Thirty birds were captured on a high tide roost in the Wadden Sea, The Netherlands, in November 2002. They were kept in two separate flocks of 15 birds in indoor aviaries at the Royal Netherlands Institute for Sea Research (NIOZ).

The experiment took place from March to June 2003 in an 8 × 7 m wide and 3 m high indoor aviary in which a mudflat system was created. Three food trays supplying ad libitum food (Trouvit fishmeal pellets, Trouw Nutrition, The Netherlands) were placed evenly across the mudflat. A roosting area of 2 × 1 m was constructed in a corner of the mudflat. A constant light and tidal regime were implemented, with high tides in darkness (‘moonlight’ illumination available; food trays on high tide roost) between 18.00 and 08.00 h.

For general habituation all birds were kept in the experimental aviary for a week. Thereafter birds were kept in the two smaller aviaries of 3.85 × 1.85 × 2.40 m, and five birds were randomly selected for each trial. After the experiments the birds were moved to outdoor aviaries where they were kept until release as one flock in June 2003.

To control for the effects of disturbance rather than the threat of a raptor per se, we chose to use a gliding raptor model against a model of an innocuous gull (both models in flying posture are depicted in Fig. 2). A threat of predation was induced by gliding the raptor along a line along one wall of the aviary, once a day but at unpredictable times between 09.00 and 17.00 h. Initially we used a peregrine falcon Falco peregrinus model. From the fourth trial onwards, we were able to replace the falcon by a sparrowhawk Accipiter nisus, a species known to catch shorebirds on the ground and thus a predator for which escape flights rather than crouching would represent the proper response (Cresswell 1996). From the third trial onwards we played shorebird alarm calls recorded at a roost as the ‘predator’ approached. The control treatment throughout consisted of passage by a black-headed gull Larus ridibundus model, with gull contact calls played from the fourth trial onwards. All sounds were played on a Sony CD walkman, supplied with a Sony SRS-A21 speaker system, tuning volume to 5.5–7 dB.

We carried out six trials, each consisting of two treatment periods. Four trials started with the control disturbance and two with the raptor disturbance. The first three trials lasted two weeks, with treatment periods of seven days. After three trials we concluded that we missed time for habituation to the new environment. In order to keep the overall experimental duration constant, we subsequently used the first week for habituation. This necessarily restricted the treatment periods to 3.5 days each. All variations in experimental procedures are presented in Table 1; the statistical effects were accounted for in the analysis (see Table 2).

During the experiments we kept a continuous record of the birds’ activities. Upon appearance of the model intruder, the turnstones usually froze. As a behavioural measure of disturbance caused by the two types of models, we recorded, by direct observation, the time between the glide of the model and the moment that the birds resumed their normal activities, usually foraging (see Dill and Gillett 1991, Gotceitas and Godin 1991, Moore 1994 for studies focused on interrupted feeding).

Morphological changes were measured at the onset and at the end of each treatment period. Body mass was measured to the nearest 0.1 g on a Sartorius balance, type 3862. Pectoral muscle size (muscle thickness, to the nearest 0.1 mm) was measured by AD using a Pie 200 ultrasound apparatus with a 7.5 MHz linear probe (Pie Medical Benelux BV, Maastricht, The Netherlands; for further details, see Dietz et al. 1999). To prevent observational bias, these measurements were performed without knowledge of either the history of the measured individual or the outcomes of the measurements (double-blind). Dietz et al. (1999) found a repeatability (intra-class correlation; Lessells and Boag 1987) of 0.83 for the measurements of pectoral muscle. We computed pectoral muscle mass (PMM; g) from muscle thickness (MT; mm) using the predictive equation derived from a calibration exercise on similarly dimensioned red knots Calidris canutus: PMM =

### Table 1. Overview of the experimental set-up.

<table>
<thead>
<tr>
<th>Trial</th>
<th>No. of birds</th>
<th>Habituation period</th>
<th>Experimental period (days)</th>
<th>Calls</th>
<th>Predator ID</th>
<th>Control ID</th>
<th>Predator or control first?</th>
<th>Successful pectoral muscle measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>0</td>
<td>14</td>
<td>−</td>
<td>Falcon</td>
<td>Gull</td>
<td>C</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0</td>
<td>14</td>
<td>−</td>
<td>Falcon</td>
<td>Gull</td>
<td>C</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0</td>
<td>14</td>
<td>+</td>
<td>Falcon</td>
<td>Gull</td>
<td>P</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>+</td>
<td>Hawk</td>
<td>Gull</td>
<td>C</td>
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<tr>
<td>5</td>
<td>5</td>
<td>7</td>
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<td>+</td>
<td>Hawk</td>
<td>Gull</td>
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<td>+</td>
<td>Hawk</td>
<td>Gull</td>
<td>C</td>
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</tr>
</tbody>
</table>

The heterogeneity due to the length of habituation and trial period and presence of calls was statistically accounted for by examining the effect of trial number (= factor ‘group’; see Methods and Table 2).
– 10.93 + 31.73 × MT (r² = 0.797, P < 0.001; Dietz et al. 1999) (note that the absolute mass estimate is not important, but the relative mass change is). During the last experiment no pectoral muscle data were obtained because of instrument failure. We thus limited the comparisons involving body mass components to the first five trials.

To estimate lean (fat-free) mass (and, by subtraction from body mass, the fat mass), at the start, after control and after raptor treatments, we determined the size of the body water pool by stable isotope dilution. Procedures are presented in detail by Visser et al. (2000). Briefly, birds were injected subcutaneously above the pectoral muscle with 0.25 g isotope enriched water (2H₂O). The exact amount injected was determined by weighing the syringe (in g) to the fourth decimal before and after the injection. To obtain background values of 2H (deuterium), prior to this treatment blood samples were gathered from three birds (out of five). An hour after deuterium was injected, blood samples were taken for investigation of changes in the body water pool. All blood samples were taken by puncturing the brachial vein and collecting four samples of 15 ml of blood in micro-capillary tubes. The tubes were immediately sealed in a flame and stored by 4°C until analyses. All injections were made by AD.

Isotope enrichments of blood samples were determined in triplicate at the Centre for Isotope Research, University of Groningen, The Netherlands. Using an infrared mass spectrometer ²H equilibration concentrations in the blood samples were determined (values were recalculated to ppm). Based on doses of ²H injected, the ²H dilution space was then calculated (in g; Visser et al. 2000). As the ²H dilution space is assumed to over-estimate the actual amount of body water by 5%, calculations of total body water were corrected for this. Finally, lean wet mass (g) was determined assuming a constant water content of 69% (Piersma and van Brederode 1990). For the first trial start values could not be obtained, which limited sample sizes for lean and fat mass to four trials (20 birds).

Statistically, trials are comprised of the successive treatments each carried out with a different ‘group’. To statistically control for the effects of treatment ‘order’ within a trial (control or predator first) of the different ‘groups’ and of the ‘individual’ on the variables of interest (i.e. pectoral muscle size, body mass, lean mass and fat mass), we performed a staggered factorial nested ANOVA (Montgomery 2001) using the GLM module in SYSTAT. At the highest level the random factor ‘group’ was nested within the fixed factor ‘order’. By subsequently nesting the random factor ‘individual’ within ‘group’ and ‘order’, we obtained a repeated measures design, whereby each individual served as its own control. To remove body size related variation among individual birds, all mass variables were standardised, by dividing them by the values at the start of the trial. Effects of the factor order were tested against the random factor group, effects of group were tested against the factor individual; all other terms were tested against the error term (Montgomery 2001). Post-encounter disturbance interval was measured on group level. Assumptions of normality and homoscedasticity were verified by visual inspection of probability plots. Pectoral muscle and body mass values are presented as least square means ± SE. Two birds drowned during the experiment, so one experimental group contained three rather than five birds (see Table 1).

**Results**

Exposure to the avian models usually produced a behavioural response from the turnstones, which froze for some time, stayed put, or crouched down on their heels. The effect of a simulated raptor scare was significantly larger than of a more neutral (gull) scare: the disturbance intervals after exposure (i.e. the time interval between disturbance and restart of activities;
Fig. 1) were much longer for the raptor treatment (7.3 ± 1.5 min) than for the control treatment (2.0 ± 1.4 min; P = 0.014; Table 2A). In this case, and for all the phenotypic variables discussed next, the heterogeneity in the details of the experimental design (as controlled for by the factor ‘group’) did not affect body mass, lean mass and fat mass (P > 0.05), but did explain some of the heterogeneity in pectoral muscle size (P = 0.033, Table 2B).

Body composition data indicate that while both bird models elicited some physiological responses in the turnstones, the raptor threat resulted in the largest responses. This is evident in comparisons of trials where the order in which the models were presented was reversed. When the raptor was presented first, pectoral muscle thickness increased by 3.8% from an average of 1.27 ± 0.01 cm to 1.32 ± 0.02 cm. When subsequently the same birds were exposed to the control model, pectoral muscle size dropped with 7.2% to 1.22 ± 0.02 cm. In contrast, while the gull-first treatment resulted in an initial increase in pectoral muscle size (to 1.35 ± 0.02 cm), exposure to the raptor model caused a further 1.8% increase in muscle size (to 1.37 ± 0.02 cm). This interaction between treatment and order was significant for the pectoral muscle (P = 0.038; Table 2B), but was not found for body mass (P = 0.475).

Overall, regardless of treatment order, exposure to the raptor model resulted in a change in body composition and mass. Average pectoral muscle thickness at the start of the experiment was 1.29 ± 0.02 cm. Birds that were exposed to the raptor model for 3–7 days increased pectoral muscle size by 4.1% while the same birds decreased pectoral muscle size by 0.5% during periods with a daily intrusion by the gull model (start = 1.29 ± 0.02 cm, raptor treatment = 1.34 ± 0.01 cm, control treatment = 1.28 ± 0.01 cm; P = 0.001; Table 2, Fig. 2). Lean mass, determined with smaller error dilution space (Visser et al. 2000) than pectoral muscle thickness (Dietz et al. 1999), rather surprisingly, did not differ among treatments, the average being 83.3 ± 0.8 g (P = 0.312).

Discussion

We found that captive ruddy turnstones were capable of distinguishing between the apparent threat of an avian predator and a similar-sized bird that posed no real threat. Behaviourally, turnstones showed a longer interruption to their regular routines when exposed to a raptor rather than a gull. Furthermore, disturbances by model birds elicited a response in the form of changes in the flight apparatus that were greater when birds were exposed to the threat of raptor predation. The limited space of the aviary may explain why, contrary to the natural situation, birds tended to freeze instead of fly after a raptor disturbance (Cresswell 1996).

When the ‘innocuous’ gull model was the first to be presented, turnstones initially increased pectoral muscle size. This indicates that in the context of a ‘safe’ indoor aviary, even a neutral object can be perceived as a risk. If so, then an increase in pectoral muscle size in birds exposed to the raptor model, and the subsequent decrease when exposed to the control model, could be interpreted as birds becoming accustomed to novel
intrusions. However, when the reverse order of the model was presented gull first followed by the raptor, the secondary response was a further increase in muscle mass, clearly indicating that turnstomes were responding differentially to the control and predator treatments. Furthermore, regardless of the experimental order, raptor scares resulted in overall increases in pectoral muscle size and mass whereas the control treatment did not.

Although body mass and pectoral muscle size changed in the same direction after disturbances with a raptor (a peregrine falcon or a sparrowhawk) rather than a control (gull), the change was more than twice as large in pectoral muscle. Apparently, in this context, pectoral muscle size was not a constant fraction of body mass as suggested by Biebach (1998), Piersma et al. (1999) and Lindström et al. (2000), but was effectively decoupled from it (Lind et al. 2001). This may reflect a strategic choice for an increase in escape capability (Lind et al. 2001, Kullberg et al. 2005).

Using Dietz et al. (1999; see Methods), the overall difference in muscle thickness (start = 1.29 ± 0.02 cm, raptor treatment = 1.34 ± 0.01 cm, control treatment = 1.28 ± 0.01 cm) translates into pectoral muscle masses of 30.0 ± 0.65 g before the disturbances, 31.7 ± 0.27 g after the raptor scares and 29.8 ± 0.27 g after the gull intrusions (5.6% increase versus 0.6% decrease). Body mass increased by 1.4% in the raptor treatment, but decreased by 0.7% in the control treatment (start = 111.5 ± 1.8 g, raptor model = 113.0 ± 0.67 g, gull model = 110.7 ± 0.67 g; P = 0.020). Subtraction of the estimated pectoral muscle mass values from the estimates of lean mass suggests that increases in pectoral muscle mass must have come at the expense of other lean tissue. Calculated pectoral-free lean mass changed from 52.6 g at the start of trials, to 50.7 g after raptor scares and 51.3 g after the control intrusions by the gull model.

Based on aerodynamic considerations, Dietz et al. (unpubl. data) predicted that for flight performance to remain constant, pectoral muscle mass should allometrically scale with body mass to the power 1.25. With an average body mass of 110.7 g and a pectoral muscle of 29.8 g after the control treatment, for a constant flight performance turnstomes (with an average body mass of 113.0 g) exposed to raptors are predicted to have a pectoral muscle mass of 30.6 g. The actual estimate of 31.7 g is 3.6% above this prediction. We thus suggest that turnstomes exposed to raptors were prepared to boost flight performance by an additional investment in pectoral muscle size. At this point we remain ignorant about the causal factors translating fear into bigger muscles.

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References


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