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SYMP10 Acidification, calcium and avian reproduction

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Acidification has been shown to affect birds through changes in their habitat, effects on their food sources, reduction in calcium availability and increases in biological availability of toxic metals. Despite several studies, our understanding of the effects of acidification and the role of calcium in avian reproduction is limited. Where do birds get sufficient calcium from before egg laying? How is calcium metabolised? What affects calcium metabolism? Are there differences among species and regions and why? Under what circumstances does calcium availability affect reproductive success or survival? This symposium brings together ecologists, physiologists, ecotoxicologists, oologists and experts on avian reproduction to (1) review the current knowledge of the effects of acidification and calcium availability on avian reproduction, (2) try to assess why acid deposition and resulting reduction in calcium availability affect some bird species in certain regions and not others, and (3) to discuss future directions of investigations which will shed more light on the effects of acidification and calcium availability on avian reproduction.

SYMP10-1 Sources of calcium for reproduction of flycatchers in central Europe

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Calcium availability may limit reproductive output in birds under certain conditions. Snail shells are the main source of calcium for many passerine species. This study showed that isopods and millipedes were the main source of calcium for collared (*Ficedula albicollis*) and pied flycatchers (*F. hypoleuca*) in Central Europe. Both isopods and millipedes, found in the nestling diet, contained 3–4 times more calcium than snail shells. Aviary experiments showed that isopods were positively selected from various sources of calcium. Mealworms, isopods, snail shells and pieces of eggshells were peovided ad libitum and feeding behaviour of parents was video-recorded every day from the nest building to the fledging period. Females took isopods mainly during egg laying period, and more often in the evening. During the nestling period, isopods were taken mainly during the period of rapid skeletal growth of chicks. No defective egg was found when isopods were available. However, 47% of eggs lacking eggshells were laid outside the nest when only snail-shells and eggshell fragments were provided. The results confirmed the importance of isopods and millipedes in the diet of reproductive flycatchers. Diet composition should be considered in detail when designing experiments.

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SYMP10-2 Poor calcium availability increases heavy metal toxicity in pied flycatcher (*Ficedula hypoleuca*) nestlings

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Eggshell thickness, egg size, clutch size and hatchability of pied flycatcher eggs are markedly depressed near the copper smelter in Harjavalta, SW Finland. Nestlings have delayed ossification of their leg and wing bones. Of two species studied, migratory flycatchers seemed to be more susceptible to the detrimental effects of heavy metals than resident great tits (*Parus major*). Tits laid relatively normal clutches even in the most polluted areas. Flycatcher nestlings showed markedly lower Ca contents in their faeces than tit nestlings, suggesting differential abilities to acquire Ca between the two species. Nevertheless, heavy metals were more abundant in flycatchers. We estimated calcium availability by counting spilled snail shells in the nests of flycatchers post-fledging. Snail shells were frequently found except at nests closest to the factory where ground layer vegetation was patchy and poor. Snail shell numbers across nests were positively associated with breeding success. Flycatchers demonstrated poor breeding success, especially at territories high in heavy metals and low in snail shells. Snail abundance was highly variable in highly polluted areas, possibly because at some territories birds may obtain snails from artificially limed gardens. Due flexible foraging behaviour, tits breeding in polluted areas may exploit calcium sources unavailable to the less plastic foraging flycatchers. In polluted areas, therefore, perhaps breeding pairs of great tits have relatively better breeding opportunities to gain the necessary calcium than pied flycatchers. This might partly explain the less severe effects of pollutants on eggshell quality in great tits.

SYMP10-3 Calcium and legs: using bone alkaline phosphatase as a marker of delayed skeletal development in great tit nestlings

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Avian ecologists often assess the quality of fledglings on the basis of morphological measurements (e.g. mass and tarsus length). However, achieving full tarsal length is not an indication of its complete ossification. Therefore, it is possible that fledglings with similar tarsus lengths are in different stages of skeletal development. We suggest that a biochemical marker, namely bone-alkaline phosphatase (ALP), may facilitate assessment of the maturity of nestlings at the pre-fledging stage. The activity of bone-ALP is associated with bone calcification processes. Within broods of great tits (*Parus major*), initially smaller siblings (at 8 days of age) had significantly higher activity of bone-ALP in blood plasma at day 15 post-hatch than initially larger siblings. This was despite no significant difference in tarsus lengths at day 15. Furthermore, we demonstrated that calcium supplementation may accelerate skeletal development of great tit nestlings in base-poor environments. While tarsi of the nestlings of control pairs were only slightly (and non-significantly) shorter than those of Ca-provisioned pairs 15 days post-hatch, bone-ALP values were significantly higher in the former indicating delayed skeletal development compared to Ca-provisioned nestlings. Possible biological consequences of delayed development of nestlings and potential applications of this novel marker will be discussed.

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SYMP10-4 A comparative study of the fine structure of the inner eggshell surface of some species of birds

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Eggshell composition and structure are widely published on in the literature. However, few studies have considered them in the broader context of the breeding biology of birds. It has long been recognized that the shell of eggs contributes to successful embryogenesis in many ways, such as in protection, respiration, and water exchange. It is also now well established that eggshell is the major source of calcium for skeletal development in the embryo. Recent studies suggest, moreover, that growth rate may play a fundamental role in the pattern of skeletal development in birds: the faster the growth, the less the skeleton becomes ossified. We predicted, therefore, that fast- and slow-growing bird species should lay eggs encased in shells with different structures adapted for different rates of calcium removal. We tested this prediction by comparing the structural composition of eggshells from birds displaying widely different growth rates and modes of development (e.g. from Passeriformes to Struthioniformes). Using scanning electron microscopy, we examined the fine structure of the inner shell surface (mammillary layer) of both pre- and post-incubated eggs (i.e. before and after embryonic development and accompanying calcium removal). The results were consistent with the prediction, as explained in the talk.

SYMP10-5 Are long-term declines in eggshell thickness of European birds related to acidification?

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Declines of eggshell thickness over 150 years have been demonstrated for thrushes *Turdus* spp., dipper *Cinclus cinclus* and common sandpiper *Actitis hypoleucos* using museum egg collections. The decline started circa 1850 and appears to begin before organochlorine pesticide pollution. Several short-term observational and experimental studies have shown that low calcium availability due to acid rain might reduce eggshell quality. Here I investigate the effects of long-term acid deposition, soil critical load and exceedance on eggshell thickness of several species throughout Europe.

SYMP10-6 Acid rain in North America and widespread declines in the wood thrush: Understanding the processes behind the pattern

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Our earlier work demonstrated a strong decline in the predicted probability of attempted breeding by wood thrushes (*Hylocichla mustelina*) at sites receiving highly acidified rain and a decreased

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probability that a given acidified site will be occupied in multiple years. To explore the processes behind these patterns, we studied the relationships between acid rain, soil properties, calcium-rich food items, and the presence of breeding thrushes at 40 sites in four regions across New York that differed in geological parent material, soil type, and acid deposition regimes. At each site, we collected soil and leaf litter, sampled gastropods and surveyed wood thrushes using playback of territorial vocalizations. Snails were scarce and densities of gastropods, myriapods and isopods varied widely both within and between regions. Soil properties were also heterogeneous. wood thrushes were absent from regions where they had bred commonly some 30 years previously. Based on this work, we developed a gastropod-sampling protocol that will be tested by hundreds of volunteers in the 2003 wood thrush breeding season. Thus, we hope to document the densities of prey species that potentially provide dietary calcium during egg laying, but that also contribute a large proportion of the invertebrate prey available in the leaf litter. Volunteers will provide fine-scale data at the site and landscape levels, but also at a broader level (i.e. across the entire breeding range of the species). This work is currently being carried out in cooperation with the US Environmental Protection Agency.

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