Anim. Behav., 1996, **52**, 715–726

Effects of photoperiod on memory and food storing in captive marsh tits, *Parus palustris*

NICKY S. CLAYTON & DANIEL A. CRISTOL
Department of Zoology, Oxford University, U.K.

(Received 27 February 1995; initial acceptance 27 June 1995; final acceptance 11 January 1996; MS. number: A7271R)

Abstract. Field observations of food storing in several species of birds indicate that this behaviour pattern varies seasonally. Storing can be experimentally manipulated in captive birds by altering photoperiod and temperature. The objective of this study was to determine whether photoperiod manipulations affected spatial memory in a food-storing bird, the marsh tit, *Parus palustris*. One treatment group received a photoperiod of decreasing daylength (simulated autumn) in August, followed by a period of short days (simulated winter) in September and early October, and a sudden onset of long-day photoperiods (simulated summer) in mid-October. The other group was held on long days until mid-October and then suddenly exposed to short days. Results indicate that differences in photoperiod between treatment groups caused differences in storing behaviour and spatial memory. Birds experiencing short days after an accelerated autumn performed better on a test of spatial memory than those maintained on long days. After the photoperiod regimes were switched between treatment groups, this difference disappeared. On a similar spatial memory test in which subjects could rely only on non-spatial cues (e.g. colour), there were no differences between treatment groups, regardless of photoperiod, indicating that the effects of photoperiod are specific to spatial memory.

Many animals store food and use spatial memory to retrieve caches (reviewed in Sherry 1989; Vander Wall 1990). Selection for enhanced spatial memory appears to have occurred in at least some food-storing species. Several species of birds that rely on stored food have a more accurate and long-lasting spatial memory than closely related species that do not store as much (Balda & Kamil 1989; Olson 1991; Brodbeck 1994; Clayton & Krebs 1994a, b). Among birds and mammals, food-storing species have an enlarged hippocampus (Krebs et al. 1989; Sherry et al. 1989; Jacobs, in press), an area of the telencephalon that plays a role in memory, including that used for retrieval of stored food (Krushinskaya 1966; O'Keefe & Nadel 1978; Sherry & Vaccarino 1989; Bingman 1993). Within at least two families of birds, relative hippocampal volume is approximately correlated with each species' dependence on stored food (Healy & Krebs 1992; Hampton et al. 1995; Basil et al. 1996). In addition to this evolutionary relationship between food storing, spatial memory and hippocampus size, a more proximate relationship may exist between behaviour and brain. Recent studies suggest that hippocampal volume increases in young birds in response to food-storing experience or training on a task requiring spatial memory (Clayton & Krebs 1994c; Clayton 1995a, b).

Field observations indicate that seasonal fluctuations occur in food-storing behaviour, with a peak in autumn and early winter (Odum 1942; Haftorn 1956; Bossema 1979). Shettleworth et al. (1995) showed that food-storing behaviour can be manipulated experimentally by altering photoperiod and temperature to simulate autumn conditions. That study compared black-capped chickadees, *Parus atricapillus*, that were captured in spring and placed on experimental photoperiods and temperature regimes designed to mimic either the passage of summer and autumn or perpetual spring. Birds on the simulated

Correspondence: D. A. Cristol, Department of Biology, College of William & Mary, Williamsburg, VA 23187-8795, U.S.A. (email:dacris@facstaff.wm.edu). CA 95616-8755, N. S. Clayton is now at the Section of Neurobiology, Physiology & Behavior, University of California, Davis, CA 95616, U.S.A.

0003–3472/96/100715+12 $25.00/0

autumn photoperiodic regime stored more seeds in wooden blocks present in their home cages than those housed on a perpetual spring photoperiod.

Some evidence also suggests that the hippocampus changes seasonally along with intensity of food storing. In black-capped chickadees captured at different times of year, hippocampal volume relative to telencephalon volume reached its maximum in October (Smulders et al. 1995). There was also greater neurogenesis in the hippocampal regions of chickadees that were caught in autumn relative to those caught in late summer or midwinter (Barnea & Nottebohm 1994). White-breasted nuthatches, *Sitta carolinensis*, stored more seeds and had larger hippocampal regions in winter than in spring (K. Peterson & D. F. Sherry, unpublished data). Thus, evidence from both field and laboratory suggests that food-storing behaviour increases in autumn, and some evidence suggests neuroanatomical changes in the hippocampus at this time of year in free-living birds.

The control of food-storing behaviour through photoperiod manipulation provides a powerful tool for testing the hypothesis that changes in behaviour are accompanied by changes in brain and memory. Experiments done to date raise several questions about seasonal changes in food-storing behaviour. (1) Are there seasonal changes in spatial memory that correspond with changes in storing behaviour? (2) Can food-storing behaviour be controlled by daylength, even in the absence of appropriate temperature cues? (3) Does intensity of food storing, as measured by counting seeds stored in a wooden block placed in a captive bird's home cage, correlate with amount of food storing observed in a more naturalistic setting? The aim of the present study was to answer these three questions by manipulating photoperiod in two groups of captive marsh tits, *P. palustris*, and then testing them on a battery of memory tests designed to determine accuracy of recall for spatial and non-spatial cues. Aside from the inclusion of memory tests, our study differed from that of Shettleworth et al. (1995) in four respects: (1) we used marsh tits instead of black-capped chickadees, (2) the photoperiod treatments involved a manipulation of daylength but not ambient temperature, (3) we tested food-storing behaviour in an experimental room containing artificial trees in addition to monitoring it with wooden blocks in subjects' home cages, and (4) we moved subjects from natural light onto artificial photoperiods in mid-summer, rather than in spring or autumn.

METHODS

Birds and Housing Conditions

The subjects were 12 marsh tits that had been captured near Oxford, U.K., and kept in outdoor aviaries measuring 2.6 × 4.9 × 1.7 m (unless noted, all measurements are width × length × height) for 4 months. All subjects had been trained on memory tests similar to those described below 2 months prior to the present study. On 15 July 1994 the birds were randomly assigned to individual cages in either of two adjacent rooms. Subjects were maintained on a summer photoperiod of 16:8 h light:dark (lights on 0400 hours) to mimic natural daylength until 8 August. The summer/winter group (SW) was kept on this photoperiod until 16 October and then shifted immediately to 10:14 h L:D (lights on 0700 hours) until the conclusion of the experiment on 17 November. The summer/autumn/winter/summer group (SAWS) was put on a regime of gradually decreasing daylength (morning later and evening earlier by 45 min each week) from 8 August through to 2 September, then maintained on the resulting 10:14 h L:D regime, and shifted back to 16:8 h L:D on 16 October (Fig. 1). During this period we conducted four sets of memory tests and two sets of room-storing tests, as described below (Table I; Fig. 1).

Subjects were housed individually in wire cages measuring 0.44 × 0.77 × 0.44 m in rooms with identical ambient temperatures (recorded at 0700 hours GMT daily). Because the building was poorly insulated, indoor temperature varied somewhat with change in the outdoor temperature (Fig. 1). Food bowls were placed in cages between 1330 and 1430 hours and filled each day with the following: 15 ml insectivorous bird mix (Orlux), 15 ml chopped peanuts, 15 ml chopped sunflower seeds, 25 sesame seeds, three peanuts, three whole sunflower seeds, three dehusked sunflower seeds, three pine seeds and three pumpkin seeds. Food bowls and any uneaten seeds present on the cage floor were removed at 1600 hours. Water was available ad libitum. Subjects also received three large waxmoth larvae at 0700 and 1600 hours, which were usually eaten immediately. In an

natural light onto artificial
-summer, rather than in

THODS

ditions

2 marsh tits that had been
U.K., and kept in outdoor
2.6 × 4.9 × 1.7 m (unless
nts are width × length ×
s. All subjects had been
s similar to those described
o the present study. On 15
ere randomly assigned to
ier of two adjacent rooms.
ned on a summer photo-
lark (lights on 0400 hours)
ength until 8 August. The
(SW) was kept on this
October and then shifted
L:D (lights on 0700 hours)
of the experiment on 17
ner/autumn/winter/summer
t on a regime of gradually
morning later and evening
h week) from 8 August
r, then maintained on the
egime, and shifted back to
ober (Fig. 1). During this
four sets of memory tests
-storing tests, as described

sed individually in wire
× 0.77 × 0.44 m in rooms
temperatures (recorded at
). Because the building was
r temperature varied some-
the outdoor temperature
re placed in cages between
d filled each day with the
ivorous bird mix (Orlux),
, 15 ml chopped sunflower
three peanuts, three whole
dehusked sunflower seeds,
ree pumpkin seeds. Food
seeds present on the cage
1600 hours. Water was
ibjects also received three
at 0700 and 1600 hours,
ten immediately. In an

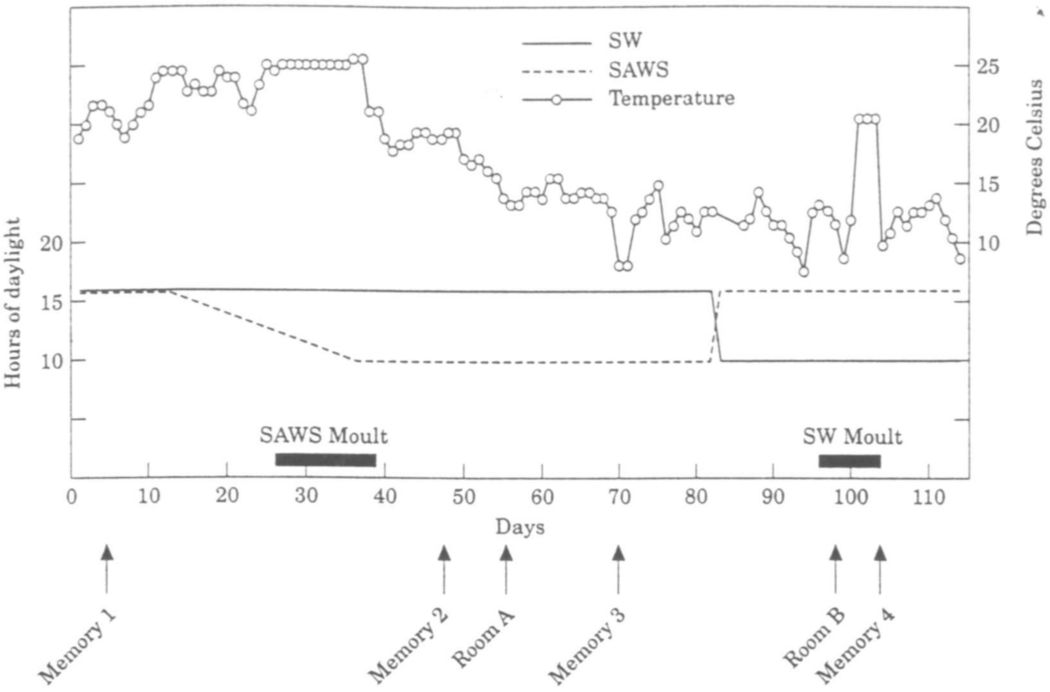

Figure 1. Daylength (left axis) and temperature (right axis) for each treatment group. Also shown are periods when at least five subjects were moulting in either treatment group, and dates of each memory test and room-storing test. SW = summer/winter treatment group; SAWS = summer/autumn/winter/summer treatment group.

Table I. Dates and photoperiods of memory and food-storing tests

Test	Dates	Day number (days since 27 July)	Photoperiod (L:D)	
			SW	SAWS
Memory				
1	1–4 August	5–8	16:8	16:8
2	12–15 September	47–50	16:8	10:14
3a	4–7 October	69–72	16:8	10:14
3b	11–14 October	76–79	16:8	10:14
4a	7–10 November	103–106	10:14	16:8
4b	14–17 November	110–113	10:14	16:8
Storing in room				
A	20–30 September	55–65	16:8	10:14
B	1–4 November	97–100	10:14	16:8
Storing in cage				
1	27 July–4 August	0–9	16:8	16:8
2	6–15 September	41–50	16:8	10:14
3	30 September–7 October	65–72	16:8	10:14
4	3–10 November	99–106	10:4	16:8

SW = summer/winter treatment group; SAWS = summer/autumn/winter/summer treatment group.

attempt to compensate for the extra energetic demands of birds on 16:8 h L:D, these subjects received an additional three pine seeds at 1600 hours, 4 h before the lights went out.

Beginning on 27 July (day 0; Table I), each cage contained two 46 × 9 × 4 cm blocks of wood containing 12 holes in which the birds could store food (hereafter 'storing blocks'). One storing block was placed on the cage floor and the other was hung from the side of the cage. The number of new seeds found in the storing blocks were recorded 4–7 days each week at 1600 hours. The storing blocks were then removed from cages and were replaced intact when food bowls were returned the following day.

Moult

Throughout the experiment we recorded the occurrence of feather moult. Birds were classified as either moulting or not moulting each day by determining whether new feathers or down were present on the floor of each cage at 1600 hours.

Storing Tests in the Room

Storing trials took place in a 3.5 × 2.0 × 2.8-m room, and were observed through a one-way Perspex window. The test room contained four artificial wooden trees (Clayton 1992). Every tree had eight storage sites, each large enough for one seed, which could be covered by a string knot to prevent the bird from using visual cues to relocate stored seeds. Each trial consisted of two phases. In phase 1, the bird received a bowl containing waxmoths, peanuts and pumpkin, pine and sunflower seeds and was allowed to eat or store food for 20 min. Following a retention interval of 1.5 h in the home cage, each bird was allowed back into the room for phase 2, in which they could search for their caches for 10 min. For each bird, we recorded the number of seeds stored and eaten during phase 1. Data on recovery accuracy during phase 2 were not analysed because of methodological concerns. For example, the treatment groups differed substantially in the number of seeds stored, and thus the number of empty cache sites and recoverable seeds available. Birds received four trials of storing; once every other day in room-storing test A (days 55–65), and on consecutive days in room-storing test B (days 97–100). Testing began at 1030 hours and order of subjects was randomized.

Because a variety of food items was available for storing or eating, each with its own caloric content and attractiveness, it was difficult to determine whether there were differences in motivation and hunger between birds during these tests. To test whether hunger levels differed between the treatment groups, birds received another storing trial on the days following room-storing tests A and B, in which only dehusked sunflower seeds were available. On these trials, we recorded the number of seeds eaten during phase 1.

One-trial Associative Memory Tests

The object of these tests was to determine the accuracy with which a subject could return to a previously encountered, baited feeder using information related either to the position of the feeder in the test room (hereafter spatial cues), feeder-specific information (such as colour or pattern of the feeder decorations), or both. We tested birds individually in a 3.5 × 2.0 × 2.8-m test room, and observed them through a one-way Perspex window. The room contained feeders (9 × 4 × 15 cm; Krebs et al. 1990) hung from plastic mesh screens (1 × 2 m) attached to two adjacent walls of the room. A hole in the centre of the face of each feeder could contain a reward of powdered peanut. This hole could be covered by a knot at the end of a short piece of string to prevent the birds from seeing the reward without first removing the knot. Subjects readily learned to pull the string knot away from the hole to inspect the contents on trials when the food was hidden. Each feeder was decorated with trial-unique shapes of adhesive tape in one of 10 possible colours. For each trial, the location of the feeders was chosen at random from 400 possible coordinates on the two mesh-covered walls, with the constraint that the array always included both walls. The redecoration and rearrangement of feeders ensured that both spatial and feeder-specific cues were trial-unique.

Testing began at 1100 hours and was carried out simultaneously by two observers in adjacent rooms. No bird began a test after 1200 hours. Each trial consisted of two phases, separated by a retention interval that varied from 1–2 h, depending on how long it took all birds to complete phase 1. Subjects were tested on phase 2 in the same order as they had received phase 1 to minimize differences in retention interval. In phase 1, one feeder was in the room, the food was clearly visible and the bird was allowed to eat for 10 s after landing on the feeder. In phase 2, the

vere differences in motivation
birds during these tests. To
levels differed between the
rds received another storing
lowing room-storing tests A
y dehusked sunflower seeds
hese trials, we recorded the
n during phase 1.

Memory Tests

e tests was to determine the
a subject could return to a
ed, baited feeder using infor-
to the position of the feeder
reafter spatial cues), feeder-
such as colour or pattern of
s), or both. We tested birds
< 2.0 × 2.8-m test room, and
gh a one-way Perspex win-
ained feeders (9 × 4 × 15 cm;
ng from plastic mesh screens
two adjacent walls of the
centre of the face of each
n a reward of powdered
uld be covered by a knot at
iece of string to prevent the
reward without first remov-
s readily learned to pull the
om the hole to inspect the
n the food was hidden. Each
with trial-unique shapes of
of 10 possible colours. For
n of the feeders was chosen
possible coordinates on the
lls, with the constraint that
cluded both walls. The re-
ngement of feeders ensured
d feeder-specific cues were

100 hours and was carried
y two observers in adjacent
an a test after 1200 hours.
f two phases, separated by a
varied from 1–2 h, depend-
took all birds to complete
re tested on phase 2 in the
had received phase 1 to
in retention interval. In
as in the room, the food was
bird was allowed to eat for
the feeder. In phase 2, the

---

bird was allowed back into the room where the remainder of the reward was hidden by a string knot in the same feeder as in phase 1. Phase 2 differed from phase 1 in two respects: (1) the food was visible in phase 1 but was hidden in phase 2; (2) only one feeder was in the room in phase 1 but there were four feeders (tests 1, 2, 3a, 4a) or seven feeders (tests 3b, 4b) in phase 2. A bird with perfect memory should have returned immediately to the correct feeder in phase 2 to finish eating the reward, but a bird searching at random would have found the baited feeder, on average, after 2.5 or 4 visits, for 4 or 7 feeders, respectively. The total number of visits, excluding revisits (which were very rare), was recorded in phase 2 of each trial. A visit was classified as a subject perching on the feeder and inspecting the contents of the hole by moving the string knot. All individuals had one trial per day and the order of testing on each day was randomized.

There were three types of memory tests, each differing in the cues available to the bird when relocating the reward. In the spatial version of the test, only positional information was available in phase 2, because all feeders were identical and undecorated. The baited feeder, which was uniquely decorated in phase 1, was replaced with an undecorated baited feeder in the same location in phase 2 (Fig. 2). For the feeder-specific version of the test, subjects had to re-locate the original feeder using only feeder-specific cues in phase 2, because none of the feeders were in the same location as in phase 1 (Fig. 2). The feeders in phase 2 were arranged in a circular array, each 50 cm away from the location of the original baited feeder. The baited feeder from phase 1 was placed at a random location in this array. For the compound version of the memory test, subjects could use spatial or feeder-specific cues, or both, because the baited feeder was uniquely marked, and was present in the same location in both phases (Fig. 2).

In each set of tests, subjects received one each of the spatial, feeder-specific and compound tests in random order. Before each block of tests, we familiarized the birds with the test rooms by allowing them into one or the other room for 5 min with a single baited feeder present once daily on three consecutive days. Subjects had 5 such familiarization trials before the first set of tests.

We weighed all birds to the nearest 0.1 g, and determined levels of stored fat by scoring the subcutaneous fat filling the furcula on a subjective scale of 0–5 (0=no visible fat; 5=fat bulging above the ventral surface of the pectoral muscle) at 1500 hours on 16 October (day 81).

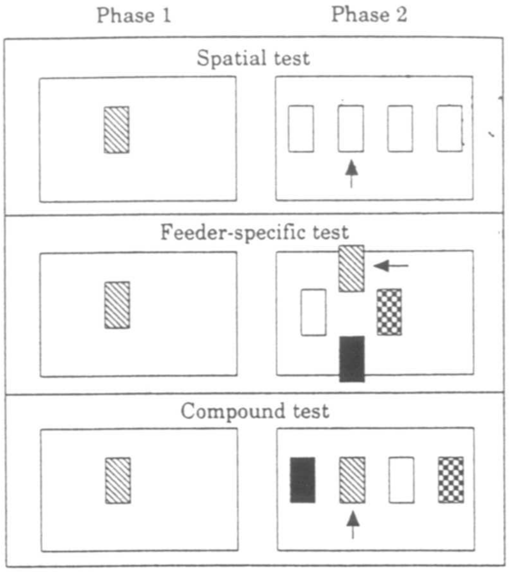

Figure 2. Schematic diagram of memory tests. Subjects were presented with a single, visibly baited feeder in phase 1, followed by multiple feeders in phase 2, including one in which the reward was hidden. Three versions of the memory test were used, differing in whether spatial cues, feeder-specific cues, or both types of cues (compound) could be used to relocate the hidden reward (see text). Arrows indicate rewarded feeder in phase 2. Patterns symbolize feeder-specific decorations.

Data Analysis

Unless otherwise noted, treatment groups were compared using Mann–Whitney *U*-tests. We relied on non-parametric comparisons because of small sample sizes. Parametric tests were used when larger sample sizes made it possible to inspect distributions for deviations from normality, such as when treatment groups were combined for certain analyses. To compare numbers of seeds stored in storing blocks within the home cage by members of each treatment group, we calculated the daily average for four test periods coinciding with the four sets of memory tests. Each storing test period in the cage included the 4 days prior to the four-feeder memory tests (1, 2, 3A, 4A) and the four test days (Table I). A Friedman two-way

Table II. Results of memory tests for all subjects combined

Test	Type	Visits ($\bar{X}$ ± SD)	E	z	P	α_B
1	Spatial	1.7 ± 0.7	2.5	2.3	0.01*	0.017
	Compound	1.7 ± 1.0	2.5	2.3	0.01*	0.025
	Feeder	2.4 ± 1.2	2.5	0.4	0.34	0.05
2	Spatial	1.6 ± 0.7	2.5	2.8	0.002*	0.017
	Compound	1.6 ± 0.8	2.5	2.8	0.002*	0.025
	Feeder	2.0 ± 0.9	2.5	1.5	0.06	0.05
3a	Spatial	2.0 ± 0.8	2.5	1.5	0.06	0.025
	Compound	1.8 ± 1.0	2.5	2.1	0.02	0.017
	Feeder	2.2 ± 1.1	2.5	1.0	0.1	0.05
3b	Spatial	1.9 ± 0.9	4.0	3.3	0.0005*	0.017
	Compound	2.3 ± 1.1	4.0	2.9	0.002*	0.025
	Feeder	2.6 ± 0.8	4.0	2.5	0.007*	0.05
4a	Spatial	1.5 ± 0.7	2.5	3.1	0.001*	0.017
	Compound	1.5 ± 0.7	2.5	3.1	0.001*	0.025
	Feeder	2.2 ± 0.6	2.5	1.0	0.1	0.05
4b	Spatial	2.1 ± 1.1	4.0	3.3	0.0005*	0.025
	Compound	1.7 ± 1.2	4.0	3.9	0.0001*	0.017
	Feeder	2.8 ± 0.8	4.0	2.0	0.02*	0.05

E=expected number of visits for a randomly searching subject. z and P are for Mann–Whitney U-tests comparing performance of all subjects to expectation for random search. α_B=Bonferroni-adjusted α-level for each test. *$P<\alpha_B$.

analysis of variance by ranks test with multiple comparisons (Siegel & Castellan 1988) was used to detect within-group changes across the four cage storing test periods.

Storing behaviour during each storing test in the room was analysed by calculating the average number of seeds stored by each bird during the four trials and comparing treatment groups. The correlation between storing in the home cage and test room was tested for significance with linear regression and ANOVA, using total number of seeds stored by members of both treatment groups in home cages and test rooms during the two periods in which storing tests in the room were administered.

The effect of temperature on food storing was examined separately for birds experiencing 10:14 or 16:8 h L:D photoperiods. We combined data from the two treatment groups for all days when both treatment groups were on 10:14 h light regimes (SW: days 82–113; SAWS: days 37–81), or 16:8 h light regimes (SW: days 37–81; SAWS: days 82–113), and compared them using linear regression and ANOVA.

To determine whether subjects were using memory rather than random searching to relocate the reward in the one-trial associative memory tests, we compared the mean number of visits required to find the baited feeder for all subjects on each test to the expectation for random searching using z-tests. For tests 1 and 2, we used only a four-feeder version. Subjects consistently re-located the reward in fewer visits than the average expected for a randomly searching bird, but we were unable to demonstrate this statistically in every case because of the low expected value for random search. To increase the expected value for random search, we added a seven-feeder version of all tests in test sets 3 and 4.

There were 12 subjects (six in each treatment group) in all analyses, except for the first feeder-specific memory test, in which there were 6 SW subjects and only 5 SAWS subjects. All statistical tests were two-tailed, except for the z-tests for better-than-random performance (described above). An α-level of 0.05 was considered significant, except in cases of multiple, related comparisons, where a sequential Bonferroni adjustment was used (Tables II, III; Rice 1989). All means are presented with standard deviations.

Table III. Results of memory tests for each treatment group

		Visits ($\bar{X}$ ± SD)				
Test	Type	SW	SAWS	z	P	α_B
1	Spatial	1.5 ± 0.8	2.0 ± 0.6	1.3	0.2	0.017
	Compound	1.8 ± 1.2	1.7 ± 0.8	0.1	0.9	0.05
	Feeder	2.2 ± 1.3	2.4 ± 1.2	0.4	0.7	0.025
2	Spatial	1.8 ± 0.7	1.3 ± 0.5	1.3	0.2	0.025
	Compound	1.5 ± 0.8	1.7 ± 0.8	0.4	0.6	0.05
	Feeder	2.5 ± 1.1	1.5 ± 0.5	1.8	0.07	0.017
3a	Spatial	2.7 ± 0.5	1.3 ± 0.5	2.7	0.007*	0.017
	Compound	2.2 ± 1.3	1.5 ± 0.5	0.8	0.4	0.025
	Feeder	2.0 ± 1.1	2.3 ± 1.0	0.7	0.5	0.05
3b	Spatial	2.7 ± 1.0	1.3 ± 0.5	2.4	0.018*	0.025
	Compound	2.2 ± 0.7	1.5 ± 0.8	2.5	0.005*	0.017
	Feeder	2.0 ± 0.8	2.8 ± 0.7	1.0	0.3	0.05
4a	Spatial	1.3 ± 0.5	1.7 ± 0.8	0.7	0.5	0.025
	Compound	1.2 ± 0.4	1.8 ± 0.7	1.7	0.08	0.017
	Feeder	2.2 ± 0.4	2.2 ± 0.7	0.1	0.9	0.05
4b	Spatial	1.8 ± 1.2	2.3 ± 1.0	1.0	0.3	0.017
	Compound	2.0 ± 1.5	1.5 ± 0.5	0.01	0.9	0.05
	Feeder	2.7 ± 0.8	3.0 ± 0.9	0.7	0.5	0.025

SW=summer/winter treatment group, SAWS=summer/autumn/winter/summer treatment group. z and P are for Mann–Whitney U-tests comparing treatment groups. α_B=Bonferroni-adjusted α-level for each test. *$P<\alpha_B$.

RESULTS

Moult and Condition

All subjects moulted body feathers and a few flight feathers shortly after experiencing the onset of decreasing daylength (Fig. 1). The first SAWS bird began moulting on day 24, and the last one terminated moult on day 42. At least five SAWS subjects were moulting every day between days 27 and 39. This intensive moult period began 13 days after the onset of decreasing daylength for these subjects. The first evidence of moult by any SW bird was on day 91. Beginning on day 96, just 10 days after the drastic shortening of their daylength, at least five SW subjects were moulting every day. This intensive moult continued until day 103, and the last SW bird ceased moulting on day 104. There were no significant differences between the treatment groups in mean ± SD mass or fat score (mass: SW=12.4 ± 0.7 g; SAWS=12.8 ± 0.7; z=0.9, P=0.38; fat score: SW=4.8 ± 0.5; SAWS=4.7 ± 0.4; z=0.8, P=0.40).

Storing Tests in the Cage

The number of seeds stored by subjects in the two treatment groups did not differ prior to the first treatment (z=0.25, P=0.80; Fig. 3). At the time of the second test, SAWS subjects had just completed a simulated autumn photoperiod, and stored more seeds in their cages than SW birds (z=2.8, P=0.005). When the third set of tests took place, SW birds were still on summer photoperiod, while SAWS had been on winter photoperiod for more than 1 month and stored more seeds than SW subjects (z=2.9, P=0.004). We reversed the photoperiod treatments before the fourth tests, and SW subjects stored more seeds than SAWS birds (z=2.9, P=0.004).

SAWS birds stored more seeds in the home cage during the third test than the fourth (overall F_r=12.2, P=0.0067; multiple comparison P<0.05). For SW subjects, the number of seeds stored in the home cage also differed across the four testing periods (F_r= 15.0, P=0.0018), with birds storing significantly fewer seeds during the second and third sampling periods than the fourth (both significant in multiple comparison, P<0.05).

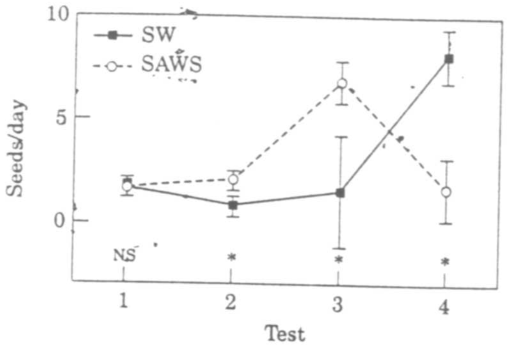

Figure 3. Number of seeds stored by each treatment group in storing blocks in home cages. Values shown are means (± SD) for 8-day periods corresponding with memory tests 1, 2, 3 and 4 (see text). SW=summer/winter treatment group; SAWS=summer/autumn/winter/summer treatment group. Asterisks indicate significant differences between treatment groups.

There was a significant negative correlation between temperature and number of seeds stored in the home cage for birds experiencing 10 h of daylight (regression equation: $Y=69.1+2.4X$; $r^2=0.25$, $F_{1,63}=20.8$, $P=0.0001$), but there was no apparent correlation for birds experiencing 16 h of daylight (regression equation: $Y=12.7+0.44X$; $r^2=0.04$, $F_{1,63}=2.5$, $P=0.12$).

Storing Tests in the Room

SAWS subjects stored, on average, more seeds than SW subjects (SW: $\bar{X}\pm$ SD$=0.6\pm0.8$; SAWS: 6.1 ± 2.1; $z=2.9$, $P=0.004$) when first tested in a room with artificial trees (test A). There was a positive relationship between the number of seeds each bird stored in the room during test A and the number of seeds each stored in its home cage during the same period (regression equation: $Y=6.3+1.6X$; $F_{1,10}=31.9$, $P=0.0002$; Fig. 4). All birds that stored seeds in phase 1 retrieved some seeds during phase 2. Subjects invariably ate several items before beginning to store seeds in the artificial trees. There was no difference in the mean ± SD number of seeds eaten by members of the treatment groups in the sunflower-only trial (SW: 3.7 ± 1.0; SAWS: 3.7 ± 0.8; $z=0.08$, $P=0.93$).

In contrast, SW subjects stored more seeds than SAWS birds on test B, given after the photoperiod treatments had been reversed (SW: 2.8 ± 1.6; SAWS: 1.2 ± 1.0; $z=2.02$, $P=0.043$). The relationship between the number of seeds each bird stored in the room and in its home cage was weaker than for test A, but was still significant (regression equation: $Y=5.9+2.1X$; $F_{1,10}=6.0$, $P=0.03$; Fig. 4). As in test A, there was no significant difference in the mean ± SD number of seeds eaten by members of each treatment group during the sunflower-only trial (SW: 2.9 ± 0.7; SAWS: 3.2 ± 0.7; $z=0.69$, $P=0.49$).

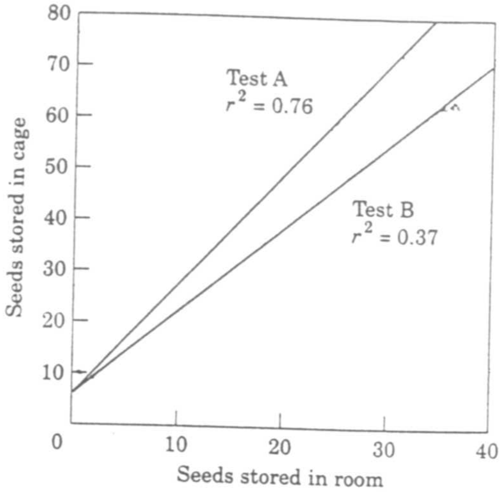

Figure 4. Regression lines for total number of seeds stored in test room and home cage during the period of room-storing tests A and B (see text). r^2 values are shown for each line.

One-trial Associative Memory Tests

Test 1: SAWS and SW on summer photoperiod

During the first 15 days after birds were moved indoors into individual cages, they were maintained on 16:8 h L:D, approximately the natural daylength for late summer. At the end of this period, they were each tested once on each of three types of memory test with four feeders present in phase 2. Birds performed significantly better than expected for randomly searching subjects on the spatial and compound tests, but not on the feeder-specific test (Table II). There were no significant differences in performance between the birds assigned to each treatment group on any test (Table III; Fig. 5).

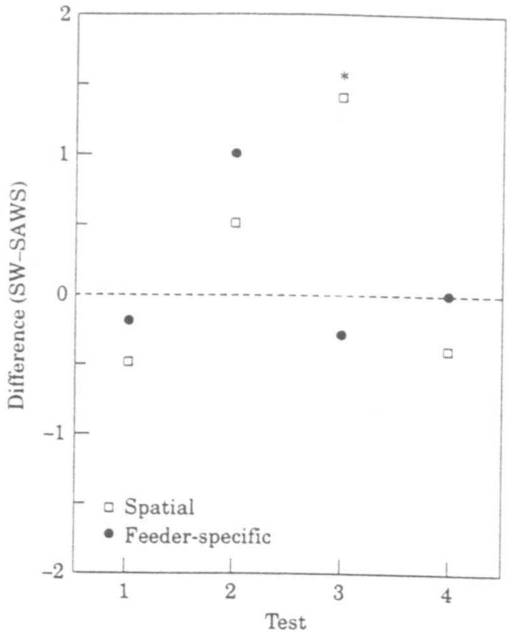

Figure 5. Difference between treatment groups (SW − SAWS) in mean number of visits required to find correct feeder on spatial and feeder-specific memory tests. Positive values indicate better performance (i.e. lower mean number of visits) for SAWS subjects. Asterisk indicates significant difference between treatment groups (Table III). SW = summer/winter treatment group; SAWS = summer/autumn/winter/summer treatment group.

Test 2: SAWS on autumn photoperiod, SW on summer photoperiod

The second set of memory tests began 38 days after the initiation of decreasing daylength for the SAWS group, 22 days after they had begun to moult, and 3 days after their photoperiod had been stabilized at 10:14 h L:D. The SW group had remained on a summer photoperiod of 16:8 h L:D and had not moulted. Birds again performed significantly better than the average level expected for random search on the spatial and compound tests, but not on the feeder-specific test (Table II). As in test 1, there were no significant differences in performance between the birds in each treatment group on any test (Table III; Fig. 5).

Test 3a: SAWS on winter photoperiod, SW on summer photoperiod

When we began this third set of memory tests, SAWS birds had been on 10:14 h L:D for 44 days, and had terminated moult 33 days previously, while SW birds were still on 16:8 h L:D and had not moulted. Birds did not perform significantly better than the level expected for random search (Table II). The performance of SAWS subjects was significantly better than that of SW subjects on the spatial test, but did not differ on the compound or feeder-specific tests (Table III; Fig. 5).

Test 4a: SAWS on summer photoperiod, SW on winter photoperiod

The final set of memory tests began 29 days after SW birds had been shifted abruptly from 16:8 to 10:14 h L:D, and SAWS birds had been shifted from 10:14 back to 16:8 h L:D. SW subjects had completed their moult 7 days before the tests. Subjects performed significantly better than expected for random search on the spatial and compound tests, but not on the feeder-specific test (Table II). There were no significant differences between the treatment groups in performance on any of the memory tests (Table III; Fig. 5).

Tests 3b and 4b: seven-feeder versions of tests 3a and 4a

Because subjects were not consistently performing significantly better than expected for randomly searching birds, we followed tests 3a and 4a with tests 3b and 4b, respectively. These tests were identical except that seven feeders were present during phase 2 instead of four, with a subsequent increase in the number of visits expected for a randomly searching bird. Seven-feeder tests were begun 3 days after completion of four feeder tests (Table I). Subjects performed significantly better than would be expected for random search on all seven feeder tests (Table II). On test 3b the performance of SAWS subjects was significantly better than that of SW subjects on the spatial and compound tests, but SAWS subjects did not differ from SW subjects on the feeder-specific test (Table III). On test 4b there were no significant differences between the treatment groups in performance on any memory test (Table III).

DISCUSSION

Food Storing and Photoperiod

In concurrence with two recent studies on black-capped chickadees (Krebs et al. 1995, Shettleworth et al. 1995), we demonstrated that the amount of food stored by captive marsh tits was affected by photoperiod. Subjects stored very few seeds in mid-summer when first placed into individual cages and housed under long daylengths (16 h of daylight). Birds that experienced a steadily decreasing daylength (to simulate autumn photoperiod) began storing more food after completing a partial moult approximately one month later. Subjects maintained on long days did not moult or increase food storing over the same period. Thus, in two groups of birds housed on the same temperature and dietary regime, only those experiencing shortened daylength moulted and increased food storing.

More than 2 months after the first, gradual photoperiod treatment, all subjects experienced a sudden switch from either long to short days (16 to 10 h of daylight) or vice versa. This treatment, occurring in late October, caused a sharp decline in food storing among the birds suddenly receiving longer days, and a rapid moult and increased storing among the birds belatedly receiving shortened days. The finding that a rapid onset of long days caused a decline of storing contrasts with the results of Shettleworth et al. (1995), in which autumn-captured chickadees continued to store food even after experiencing a premature summer. Our observation that subjects held on a prolonged summer, well into natural autumn, increased seed storing rapidly after experiencing short days, underscores the potency of photoperiod as a behavioural trigger, relative to other possible influences on storing behaviour, such as temperature or endogenous rhythms (Shettleworth et al. 1995).

There was a negative correlation between amount of food stored in the home cage and ambient temperature, but only for birds already triggered to store by a simulated winter photoperiod. Birds held on a prolonged summer photoperiod did not increase storing, even in response to gradually cooling temperatures. In early autumn, photoperiodic change was necessary, but may not have been sufficient, to initiate food storing, because the SAWS birds did not start storing until after temperatures had dropped somewhat. Later in autumn, when the ambient temperature was even lower, photoperiodic change was sufficient to initiate or terminate food storing. We cannot rule out the possibility that after initially being triggered to increase food storing by shortened photoperiod, birds may increase food storing in response to colder temperatures, even in the absence of a photoperiodic shift. An experiment in which birds are housed at constant temperatures while receiving decreasing daylength will be necessary to clarify the interplay between photoperiod and temperature.

Memory and Photoperiod

The regulation of food storing by photoperiod raises the possibility that related aspects of behaviour, such as the ability to remember storage sites, might also be under the influence of photoperiod. We tested the performance of subjects on spatial and non-spatial memory tasks before and after each of two photoperiod treatments. Our most important result was that the ability to relocate a reward using spatial cues differed between birds on different photoperiods. SAWS birds, experiencing short daylengths and storing a lot of food in their home cages, performed better on a test of spatial memory than birds maintained on long days that were storing very few seeds (tests 3a, 3b). Because performance on non-spatial memory tests did not differ between treatment groups, our results suggest a specific effect of the treatment on spatial memory rather than on attention or cognitive abilities in general. After the photoperiod regimes of the treatment groups were reversed there was no longer a difference in spatial memory (tests 4a, 4b), indicating that photoperiod, rather than some uncontrolled variable, was responsible for the observed effect.

In addition to confirming the photoperiodic regulation of food-storing behaviour, and demonstrating that spatial memory abilities differ between birds on summer and winter photoperiods, our results show that the amount of food storing in a bird's home cage correlates with the amount of storing a bird does in a semi-natural aviary setting. Thus, the technique of using storing blocks, suggested by Hampton et al. (1995), offers a means of monitoring individual storing behaviour, and will be useful in continuing to examine the relationship between season, food-storing and spatial memory.

Because they received different photoperiods, subjects in the two treatment groups had different amounts of time to feed, possibly leading to differences in hunger and motivation. All subjects were provided with far more food than they could eat, however, and we found no differences between treatment groups on two measures related to motivation: number of seeds eaten during the room-storing tests and visible fat reserves.

From our results we cannot determine whether photoperiod directly affected subjects' spatial memories, or acted indirectly, for example by increasing the amount of food-storing experience. This question can be answered by altering photoperiod while depriving subjects of the opportunity to store food.

Reports of seasonal changes in the hippocampal regions of food-storing birds on natural photoperiods suggest the intriguing possibility that changes in food-storing behaviour and spatial memory might be related to neuroanatomical modifications (Barnea & Nottebohm 1994; Smulders et al. 1995; K. Peterson & D. F. Sherry, unpublished data). However, experimentally simulated autumn photoperiod failed to bring about changes in the hippocampal volumes of black-capped chickadees (Krebs et al. 1995). In addition, adult willow tits, *P. montanus*, held on constant winter photoperiod and given food-storing experience, did not differ in hippocampal volume from subjects given no opportunity to store food (Cristol, in press). These contrasting results suggest that any relationship between food storing, photoperiod and the hippocampus is complex.

There are few examples of seasonal shifts in behaviour accompanied by cognitive changes. In some birds, the ability to discriminate between conspecific songs is greatest during the breeding season, when the learning of new songs normally occurs (Cynx & Nottebohm 1992; Calhoun et al. 1993). White-breasted nuthatches, which store less food and have smaller hippocampal areas in spring than in winter, underwent no statistically significant change in accuracy of cache retrieval between winter and spring, but there was a trend towards declining accuracy in spring (K. Peterson & D. F. Sherry, unpublished data). In two rodent species, sex differences in spatial learning ability were observed only during the breeding season, simultaneously with the greatest sex differences in spacing behaviour (Galea et al. 1994; Jacobs, in press). Our finding that photoperiod affected recall for spatial cues may be the first demonstration in a non-human animal that daylength manipulations can affect a cognitive ability, and is consistent with the idea that seasonal neural changes in food-storing species are related to memory for cache sites.

ACKNOWLEDGMENTS

Order of authorship was determined by coin toss. This research was supported by a BBSRC University Research Fellowship and BBSRC grant to N.S.C and a NSF-NATO Postdoctoral Fellowship to D.A.C. We are indebted to John Krebs for support and inspiration. We thank Clea Assersohn, Emma Krebs, Nick Lewis and Dave Wilson for help in running the experiments. Sara Shettleworth made valuable comments on an earlier version of the manuscript.

REFERENCES

Balda, R. P. & Kamil, A. C. 1989. A comparative study of cache recovery by three corvid species. *Anim. Behav.*, **38**, 486–495.

Barnea, A. & Nottebohm, F. 1994. Seasonal recruitment of hippocampal neurons in adult free-ranging black-capped chickadees. *Proc. natn. Acad. Sci. U.S.A.*, **91**, 11 217–11 221.

Basil, J. A., Kamil, A. C., Balda, R. P. & Fite, K. V. 1996. Differences in hippocampal volume among food storing corvids. *Brain Behav. Evol.*, **47**, 156–164.

Bingman, V. P. 1993. Vision, cognition, and the avian hippocampus. In: *Vision, Brain and Behavior in Birds* (Ed. by H. P. Zeigler & H.-J. Bischof), pp. 391–408. Cambridge, Massachusetts: MIT Press.

Bossema, I. 1979. Jays and oaks: an eco-ethological study of a symbiosis. *Behaviour*, **70**, 1–117.

Brodbeck, D. R. 1994. Memory for spatial and local cues: a comparison of a storing and a nonstoring species. *Anim. Learn. Behav.*, **22**, 119–133.

Calhoun, S., Hulse, S. H., Braaten, R. F., Page, S. H. & Nelson, R. J. 1993. Responsiveness to conspecific and alien song by canaries (*Serinus canaria*) and European starlings (*Sturnus vulgaris*) as a function of photoperiod. *J. comp. Psychol.*, **107**, 235–241.

Clayton, N. S. 1992. The ontogeny of food-storing and retrieval in marsh tits. *Behaviour*, **122**, 11–25.

Clayton, N. S. 1995a. Development of memory and the hippocampus: comparison of food storing and non-storing birds on a one-trial associative memory task. *J. Neurosci.*, **15**, 2796–2807.

Clayton, N. S. 1995b. The neuroethological development of food-storing memory: a case of use it or lose it! *Behav. Brain Res.*, **70**, 95–101.

Clayton, N. S. & Krebs, J. R. 1994a. One-trial associative memory: comparison of food-storing and non-storing species of birds. *Anim. Learn. Behav.*, **22**, 366–372.

Clayton, N. S. & Krebs, J. R. 1994b. Memory for spatial and object-specific cues in food-storing and non-storing birds. *J. comp. Physiol. A*, **174**, 371–379.

Clayton, N. S. & Krebs, J. R. 1994c. Hippocampal growth and attrition in birds affected by experience. *Proc. natn Acad. Sci. U.S.A.*, **91**, 7410–7414.

Cristol, D. A. In press. Food storing does not affect hippocampal volume in experienced adult willow tits. *Behav. Brain Res.*

Cynx, J. & Nottebohm, F. 1992. Role of gender, season, and familiarity in discrimination of conspecific song by zebra finches (*Taeniopygia guttata*). *Proc. natn Acad. Sci. U.S.A.*, **89**, 1368–1371.

Galea, L. A. M., Kavaliers, M., Ossenkopp, K.-P., Innes, D. & Hargreaves, E. L. 1994. Sexually dimorphic spatial learning varies seasonally in two populations of deer mice. *Brain Res.*, **635**, 18–26.

Haftorn, S. 1956. Contribution to the food biology of tits especially about storing of surplus food. Part III the willow tit (*Parus atricapillus* L.). *K. norske Vidensk. Selsk. Skr.*, **1956**, 1–79.

Hampton, R. R., Sherry, D. F., Shettleworth, S. J., Khurgel, M. & Ivy, G. 1995. Hippocampal volume and food-storing behavior are related in parids. *Brain Behav. Evol.*, **45**, 54–61.

Healy, S. D. & Krebs, J. R. 1992. Food-storing and hippocampus in corvids: amount and volume are correlated. *Proc. R. Soc. Lond. Ser. B*, **248**, 241–245.

Jacobs, L. F. In press. The ecology of spatial cognition: adaptive patterns of hippocampal size and space use in wild rodents. In: *Studies of the Brain in Naturalistic Settings* (Ed. by E. Alleva, A. Fasolo, H.-P. Lipp & L. Nadel). NATO Advanced Studies Institute Series.

Krebs, J. R., Sherry, D. F., Healy, S. D., Perry, V. H. & Vaccarino, A. L. 1989. Hippocampal specialization of food-storing birds. *Proc. natn Acad. Sci. U.S.A.*, **86**, 1388–1392.

Krebs, J. R., Healy, S. D. & Shettleworth, S. J. 1990. Spatial memory of Paridae: comparison of storing and non-storing species *P. ater* and *P. major*. *Anim. Behav.*, **39**, 1127–1138.

Krebs, J. R., Clayton, N. S., Hampton, R. R. & Shettleworth, S. J. 1995. Effects of photoperiod on food-storing and the hippocampus in birds. *Neuro Report*, **6**, 1701–1704.

Krushinskaya, N. L. 1966. Some complex forms of feeding behaviour of nutcracker, *Nucifraga caryocatactes*, after removal of old cortex. *Zh. Evol. Biochim. Fisiol.*, **II**, 563–568.

Odum, E. P. 1942. Annual cycle of black-capped chickadee 3. *Auk*, **59**, 499–531.

Olson, D. J. 1991. Species differences in spatial memory among Clark's nutcrackers, scrub jays, and pigeon. *J. exp. Psychol. Anim. Behav. Proc.*, **17**, 363–376.

O'Keefe, J. & Nadel, L. 1978. *The Hippocampus as a Cognitive Map.* Oxford: Clarendon Press.

Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Siegel, S. & Castellan, N. J., Jr. 1988. *Nonparametric Statistics for the Behavioral Sciences.* New York: McGraw-Hill.

Sherry, D. F. 1989. Food storing in the Paridae. *Wilson Bull.*, **10**, 289–304.

Sherry, D. F. & Vaccarino, A. L. 1989. Hippocampal aspiration disrupts cache recovery in black-capped chickadees. *Behav. Neurosci.*, **103**, 308–318.

Sherry, D. F., Vaccarino, A. L., Buckenham, K. & Herz, R. S. 1989. The hippocampal complex of food storing birds. *Brain Behav. Evol.*, **34**, 308–317

Shettleworth, S. J., Hampton, R. R. & Westwood, R. P. 1995. Effects of season and photoperiod on food-storing by black-capped chickadees, *Parus atracapillus. Anim. Behav.*, **49**, 989–998.

Smulders, T. V. Sasson, A. D. & DeVoogd, T. J. 1995. Seasonal variation in hippocampal volume in a food-storing bird, the black-capped chickadee. *J. Neurobiol.*, **27**, 15–25.

Vander Wall, S. B. 1990. *Food Hoarding in Animals.* Chicago: University of Chicago Press.