Clutch size determination in shorebirds: revisiting incubation limitation in the pied avocet (*Recurvirostra avosetta*)

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Summary

1. Traits strongly related to fitness, such as offspring number, are expected to show intraspecific variation among individuals. However, offspring number is invariant in several reptiles, birds, and mammals. Most shorebirds (210+ species), for example, have an invariant clutch size of four eggs, which is unexpected in such an ecologically, behaviourally and socially diverse group.

2. The incubation-limitation hypothesis (ILH) suggests that shorebird clutch size is limited by the inability of adults to incubate clutches larger than four eggs. Several recent studies reported no overall costs of incubating experimentally enlarged clutches and concluded no support for the traditional ILH. However, most studies have not measured all potential costs, and none has quantified costs beyond egg hatching. We conducted a clutch-enlargement experiment and measured potential costs both during incubation and chick rearing in pied avocets (*Recurvirostra avosetta* L.).

3. Hatching was more asynchronous and egg hatchability was marginally lower in enlarged clutches than in controls. Nonetheless, more young hatched from enlarged clutches (mean: $4 \cdot 2 \pm 0 \cdot 17$ SE) than from controls ($3 \cdot 4 \pm 0 \cdot 09$), and the two groups did not differ in incubation period, complete or partial clutch failure, or hatchling body size, apparently refuting the ILH.

4. However, pairs incubating enlarged clutches occupied poorer feeding territories during chick rearing, experienced higher chick mortality, and eventually raised fewer young to independence (mean adjusted for season: $0 \cdot 7 \pm 0 \cdot 16$ SE juveniles) than did control pairs ($1 \cdot 2 \pm 0 \cdot 13$). Chick survival was primarily associated with prey availability, and predation risks were not higher in larger broods.

5. Our results provide evidence that incubating unusually large clutches can affect post-hatching performance and lead to lower annual reproductive success in shorebirds. This study, therefore, supports the ILH and points to the importance of monitoring reproductive success beyond the hatching of the chicks.

Key-words: clutch manipulation, cost of reproduction, life history, optimal clutch size, precocial birds

Introduction

Life-history theory suggests that reproductive traits strongly related to fitness, such as the number of offspring, should vary among individuals according to intraspecific variation in individual quality, body size, and/or condition. Paradoxically, invariant offspring numbers are found in several taxa, including reptiles, birds, and mammals (Johnsgard 1983; Vitt 1986;

Elgar & Heaphy 1989; Read & Harvey 1989; Shine & Greer 1991). The invariant clutch size of shorebirds (superfamilies Charadroidea and Scolopacoidea, over 210 species) has long intrigued ecologists and evolutionary biologists. Shorebirds range from the tropics to arctic areas, from coastal to alpine habitats, from open semi-deserts to closed boreal woodlands (Johnsgard 1981), have highly diverse mating systems (Reynolds & Székely 1997) and parental care patterns (Székely, Webb & Cuthill 2000), yet most species lay an invariant clutch of four eggs, and some species lay an invariant clutch of two or three eggs (Maclean 1972). In birds, optimal clutch size is limited below the maximum number of eggs that

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can be produced by females by the energetic costs of producing eggs, incubating eggs, and provisioning young during chick rearing (Godfray, Partridge & Harvey 1991; Vander-Werf 1992; Monaghan & Nager 1997). Egg removal experiments show that shorebirds are capable of laying many more eggs than four, indicating that the costs of producing eggs are not exceptionally high (Kennedy 1991; Haywood 1993; Grønstøl, Blomqvist & Wagner 2006). Furthermore, parental provisioning of young is unlikely to be a strong selective force on clutch size as most shorebirds do not feed their precocial young and parental care is less costly than that in altricial birds (Winkler & Walters 1983; Monaghan & Nager 1997). Therefore, most attention regarding clutch-size limitation in shorebirds has turned to the costs of incubation.

Lack (1947) was the first to hypothesize that clutch size in shorebirds is limited by the ability of parents to incubate large clutches successfully. The incubation-limitation hypothesis (ILH) has been experimentally tested in at least eight clutch-size manipulations. Two experiments fully supported the hypothesis by demonstrating lower reproductive success for pairs incubating enlarged clutches compared to those incubating non-manipulated controls (Hills 1983; Delehanty & Oring 1993). Other experiments reported non-definitive costs associated with larger-than-normal clutches and concluded partial support (Shipley 1984; Székely, Karsai & Williams 1994; Yogev, Ar & Yom-Tov 1996). Costs of incubating larger-than-normal clutches included more frequent clutch abandonment, longer incubation and hatching periods, longer exposure to predation, disproportionate egg loss to predation and reduced egg hatchability. After quantifying these potential costs in two *Calidris* sandpipers, Sandercock (1997) concluded that clutch size is unlikely to be limited by the incubation ability of the parents. Two more recent studies also found that shorebirds are capable of successfully incubating five-egg clutches without incurring any measurable cost until hatching (Wallander & Andersson 2002; Larsen, Lislevand & Byrkjedal 2003).

In a review of six earlier experiments, Arnold (1999) warned that the lack of a substantial overall cost, such as lower reproductive success for parents incubating enlarged clutches, cannot be used to argue against the ILH because several subtle costs also may synergistically limit clutch size. Furthermore, several potential costs have gone undetected in earlier manipulations (Sandercock 1997; Arnold 1999). For example, none of the eight experimental studies quantified reproductive success after the hatching of eggs, and we have no information on how incubation costs influence chick survival and annual reproductive success.

We manipulated the costs of incubation by adding extra eggs to clutches of pied avocets, and measured the effect of clutch enlargement both during incubation and chick-rearing. The pied avocet is a medium-sized, colonially breeding and ground-nesting shorebird with a modal clutch size of four eggs, which are incubated by both adults. Clutches containing five or six eggs, presumably produced by two females, regularly occur in dense colonies (Gibson 1971; Hötker 2000), indicating no strong selection against larger clutch sizes.

In addition, a clutch manipulation in the closely related American avocet (*Recurvirosta americana*; Shipley 1984) found no major costs of incubating experimentally enlarged clutches, suggesting that there may be selection for improved incubation ability in avocets. Based also on more recent experiments (Sandercock 1997; Wallander & Andersson 2002; Larsen *et al*. 2003), we expected that the incubation of extra eggs will not result in an overall cost during incubation. Such an overall cost was even less likely during chick-rearing. Parental care in precocial birds benefits all young equally (Lazarus & Inglis 1986) and increases little with the number of young (Lessells 1987; Seddon & Nudds 1994). In the studied population of pied avocets, the number of young is positively related to prey abundance on the chick-rearing territory and chicks survive better in larger broods (Lengyel 2007), indicating that the costs of parental care are probably small relative to the benefits accruing from larger broods. Based on these considerations, we expected that pairs incubating enlarged clutches and producing more young should occupy better territories and raise more young than should control pairs. Our findings, however, showed that pairs incubating enlarged clutches and producing more hatchlings occupied poorer territories, and raised fewer young to fledging compared to control pairs. Overall, these patterns provide evidence that extra costs during incubation may contribute to poorer post-hatching performance and lower annual reproductive success in avocets.

Methods

FIELD METHODS

The experiment was carried out at Kelemen-szék, a 600-ha alkali lake in Kiskunság National Park (Hungary) in May–June 2000. The breeding population was 260 pairs and the experiment involved 142 pairs nesting in six colonies. Avocet breeding biology and general field methods are given in Lengyel (2006, 2007). Nesting colonies were found by observing nest-building birds, and nests were mapped soon after colony initiation. The date of laying of the first egg was determined either by counting back from the number of eggs (in clutches with < 4 eggs; average laying interval: 1·3 days, Cramp & Simmons 1983) or by estimating the average stage of incubation by egg-flotation (in clutches found with four eggs; see below). Nests were checked once every 4 days and daily before the expected date of hatching. Most chicks were marked individually by colour and metal rings and broods were identified based on marked chicks.

CLUTCH SIZE ENLARGEMENT

For clutch enlargements, we used extra eggs rescued by national park authorities from clutches laid in temporarily drained fishponds and threatened by flooding after the re-filling of the ponds. Source fishponds were located 87 km southeast (*n* = 30 eggs, manipulation date: May 8) and 180 km east (*n* = 35 eggs, May 18) from the study site. Eggs were kept warm (35 °C) during transport in thermoboxes and were put into the nests < 6 h after they had been collected. All eggs were inspected for intactness and viability. Four damaged eggs were rejected and 61 eggs were used in the experiment. Eggs were floated in water to determine their incubation stage (Nol & Blokpoel

Table 1. Basic reproductive parameters (means ± SDs) in experimental groups

Group[a]	Date of laying of first egg	Egg volume, (cm^3)	Natural clutch size	Experimental clutch size	Incubation cost (total egg-days)
Enlarged	May 2 ± 9·3d	31·6 ± 1·78 (51)	3·96 ± 0·19	5·1 ± 0·38	109 ± 13·1 (50)
Control	May 3 ± 8·5d	31·4 ± 2·00 (66)	3·92 ± 0·35		94 ± 10·8 (72)
t (df)	0·60 (140)	0·71 (115)	0·98 (140)	18·59 (140)	6·89 (120)
P	0·552	0·479	0·391	< 0·001	< 0·001

[a]sample sizes are 55 (enlarged) and 87 (control), except where shown in parentheses.

1983). We estimated incubation stage based on a scheme developed by repeated measurements of the same clutches during incubation in American avocets (Alberico 1995) and in pied avocets (40 nests, 1998–99, Szabolcs Lengyel, unpublished data). Our estimates were accurate to ±1 day, therefore, mismatches in timing between extra eggs and natural eggs were small. The incubation stage of extra eggs did not influence whether they hatched or not (logistic regression, Wald $\chi^2 = 1·58$, d.f. = 1, $P = 0·209$). There was no difference in the proportion of eggs hatching between the two sources/dates (63%, $n = 24$ vs. 69%, $n = 32$, respectively; Yates-corrected $\chi^2_1 = 0·04$, $P = 0·839$; five eggs taken by predators excluded), therefore, we pooled data from the two groups.

We randomly selected clutches for enlargement among 142 nests that were at the same incubation stage as the extra eggs. We added one egg to 49 clutches and two eggs to six clutches. Two eggs were added when only two same-aged clutches were available, and one was selected for enlargement and one for control. Response variables (see below) did not differ between five-egg and six-egg enlarged clutches, and the results were qualitatively similar when six-egg clutches were included or excluded for all response variables (not shown), therefore, we present results from all enlarged clutches. Clutches that were not selected for enlargement ($n = 87$) were designated as controls, which were visited and handled the same way as enlarged clutches, except that no egg was added to them.

Clutches containing five or six eggs laid by two females are regularly incubated in avocets (Hötker 2000). In our study, clutch enlargement did not lead to changes in incubation behaviour, and incubation resumed normally. None of the enlarged clutches were abandoned, and extra eggs were not removed from the nest by the adults. Enlarged clutches had more eggs, and were exposed to higher incubation costs ('egg-days', see below) than controls, but they did not differ from controls in egg-laying date or egg volume (Table 1), indicating little difference in quality between the experimental groups.

VARIABLES

Response variables in the incubation phase corresponded to the costs reported or hypothesised in previous studies (Arnold 1999). (i) Incubation period was determined as the number of days between the observed (not estimated) date of laying of the last egg and the hatching of the first chick. (ii) Duration of hatching was the number of days between hatching of the first chick and hatching of the last chick. (iii) Hatching success was measured by the proportion of clutches hatching at least one egg and by the number of young hatched. Complete clutch failure was inferred when all eggs were lost too early before the expected date of hatching, or when signs of hatching (e.g. eggshell fragments, Mabee 1997) were not found. Partial clutch failure was when single eggs disappeared from nests due to predation (judged by direct signs of predators or simultaneous

disappearance of eggs from neighbouring nests) or removal by parents. For analysis, we used apparent nest success, because the existence and location of all study nests were known during egg laying. However, we also report Mayfield's (1975) daily survival rate and nest success to facilitate comparison with previous studies. (iv) Egg hatchability was the proportion of eggs hatching relative to all eggs present in the nest-cup (not taken by predators) at the end of incubation. (v) Chick body size variables (tarsus length, culmen length and body mass) were measured for most hatchlings < 24-h old. Body size was estimated by factor scores from a principal component analysis of these three variables. Body condition was estimated as the residuals from an ordinary least squares regression of body mass on tarsus length ($B = 0·35 ± 0·068$ SE, $F_{1,249} = 26·50$, $P < 0·001$). Body size variables were averaged per brood for analysis.

Response variables in the chick-rearing phase included (i) the number or percentage of young surviving to fledging, and (ii) prey abundance on the feeding territory. Fledging occurs at age 35 days, when avocet young begin to fly (Cramp & Simmons 1983). The number of fledglings was determined from brood monitoring, during which the location and composition of every brood was recorded once every three days until chicks died or survived to fledging. We assumed that chicks died if they were not seen on three consecutive monitoring observations or any time later. Resighting probability was high because the study lake had sparse vegetation and was surrounded by agricultural fields, and broods did not leave the lake. For example, only one brood (0·5% of $n = 187$ broods hatching at the study lake in 1998–2000) was found to fledge without being resighted during chick-rearing. Chick survival probabilities were not used because the number of fledged young was known for 93% of the broods ($n = 122$) and it was considered a better measure of adult reproductive success. Chick adoption is known to influence chick survival under high predation in artificial habitats (Lengyel 2007), but was not likely to play a role in natural habitats where the experiment was conducted, and where predation is lower than in artificial habitats (Lengyel 2006).

Prey abundance on the feeding territory was estimated by sampling macroscopic aquatic invertebrates available as potential prey for the chicks (Oligochaeta; Chironomidae; Odonata; Heteroptera: Corixidae, Notonectidae, Gerridae; Coleoptera: Dytiscidae, Gyrinidae). We sampled territories only if they were occupied for more than 3 days by the same < 2-week-old brood to avoid sampling transient territories defended by pairs during brood movements, and to focus on the first two weeks post-hatch, when most chicks die (Lengyel 2006). Prey were sampled by collecting all macroinvertebrates from a water column enclosed by a plastic cylinder (diameter 45 cm) placed in the centre of the territory, where water depth varied between 2 cm and 13 cm, using a sweepnet (diameter 15 cm, mesh size 0·2 mm). Aquatic macroinvertebrates were sorted to major taxa, counted, and released. Sampling was restricted to one occasion for

Table 2. Partial clutch failure in enlarged ($n = 50$) and control ($n = 72$) clutches

| | Eggs lost during incubation | | | | Non-hatched eggs | |
| | Predation | | Parental removal | | Remained in nest | |
Group	Nests	Eggs	Nests	Eggs	Nests	Eggs
Enlarged	7	10	2	2	19	29
Control	8	11	2	2	11	14

each brood to reduce disturbance, that may force pairs to leave their territory. Other studies (Kiss *et al.* 2001; Bela Kiss and Szabolcs Lengyel, unpublished data) showed that variation in prey abundance was small within territories (few 10 m of shoreline) relative to larger spatial scales (> 100 m, e.g. between areas used vs. not used for chick-rearing), suggesting that the rapid assessment used here adequately estimated prey abundance. Prey abundance was estimated as the number of prey per litre water on the feeding territories of 38 broods (17 from enlarged and 21 from control clutches).

DATA ANALYSIS

Clutches were enlarged at different stages of incubation. Most clutches were enlarged during egg laying ($n = 14$) or early in incubation (16 during week 1, 11 in week 2), and 14 clutches were enlarged later (incubation period: 23 days). To study the effect of the timing of clutch enlargement on response variables, we estimated energy expenditure by the parents during incubation using 'egg-days' (one egg-day = one egg incubated for 1 day). We summed egg-days to obtain total egg-days per clutch (e.g. four eggs incubated for 23 days gives 92 total egg-days), and used this measure as an independent variable in linear regression models for each response variable.

We also used linear regression to test whether response variables were influenced by season (egg-laying date or hatching date). Season significantly influenced the number of young fledged per brood, therefore, we used an analysis of covariance (ANCOVA) to statistically control for the effect of season in analyses of fledging success. Season did not affect any of the other response variables; therefore, we tested differences in means between experimental groups using *t*-tests.

To evaluate the effect of non-independence of clutches arising from colonial nesting, we performed regressions and mean comparisons also by using 'colony identity' as a random factor in generalised linear mixed-effects models (function 'lme' in R, http://www.r-project.org). The random colony effect was not significant in any of the analyses (intercept SD << residual SD), therefore, for simplicity, we present results without the random effect.

We applied parametric tests only when the assumptions of such tests were met. Prey abundance data were log-transformed to obtain homoscedasticity. To analyse 2×2 contingency tables, we used χ^2-tests with Yates' correction for continuity. Means ± SDs are given, except where indicated, and all probabilities ($\alpha = 0.05$) are two tailed.

Results

INCUBATION PHASE

Extra incubation costs imposed on pairs averaged 15·6 ± 9·4 egg-days (range: 1–31, $n = 50$ enlarged clutches), which
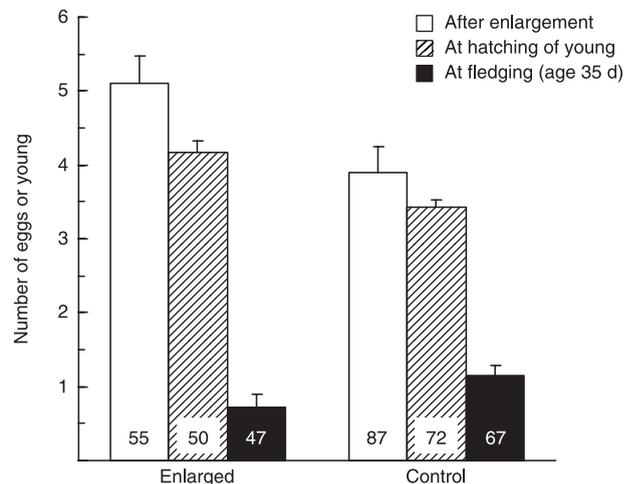

Fig. 1. Mean ± SE clutch size, number of hatchlings and fledglings in enlarged and control clutches of pied avocets. Means are adjusted for season by ANCOVA for number of fledglings. Sample sizes (number of clutches or broods) are shown at the base of bars.

corresponded to incubating one extra egg for (on average) 69% of the length of the incubation period (22·6 ± 1·1 days in controls). Total egg-days did not influence any of the response variables (not shown), indicating that the timing of clutch enlargement had little influence on our results.

Incubation period did not differ between enlarged clutches (22·8 ± 1·70 days, $n = 30$) and controls (22·6 ± 1·10, $n = 24$; $t_{52} = 0.62$, $P = 0.537$), but there was a significant difference in the duration of hatching because enlarged clutches took longer to hatch (2·2 ± 0·79 days, $n = 50$) than controls (1·9 ± 0·69, $n = 72$) ($t_{120} = 2.26$, $P = 0.026$).

The proportion of clutches in which at least one young hatched was similar in enlarged clutches (91%, $n = 55$) and controls (83%, $n = 87$; Yates-corrected $\chi^2_1 = 1.24$, $P = 0.266$). Complete clutch failure occurred in 20 nests (five enlarged and 14 control clutches taken by mammalian predators, one control clutch abandoned by the pair). Partial clutch failure was similarly rare in enlarged and control clutches (Table 2).

Significantly more young hatched from enlarged clutches than from control clutches (Fig. 1, $t_{120} = 4.02$, $P < 0.001$), indicating that avocets were able to successfully incubate more than four eggs. However, the difference was, on average, 0·6 hatchlings, or half of the difference in the number of eggs after enlargement (1·2 eggs, Table 1), suggesting lower egg
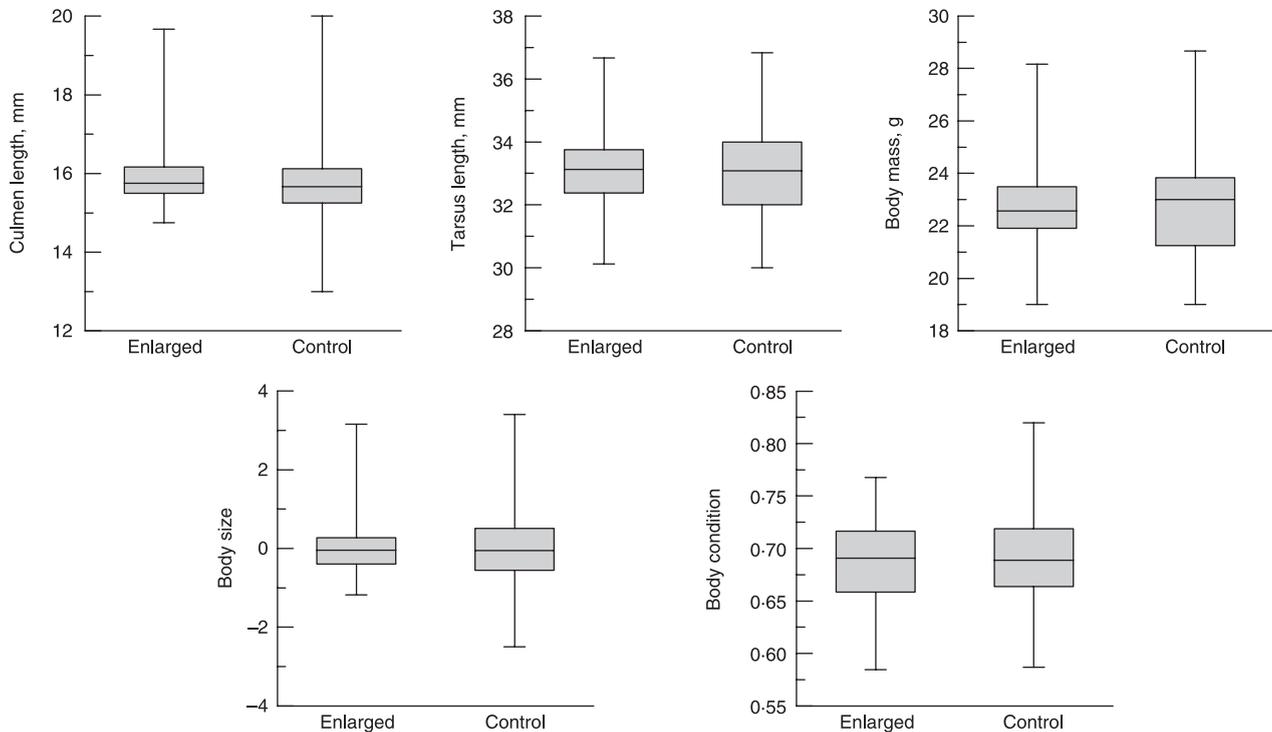
Fig. 2. Body size variables of chicks hatching from enlarged and control clutches. Box-plots show the median and lower and upper quartiles (box) and minimum and maximum values (whiskers). Data from chicks are averaged for broods from $n = 42$ enlarged and $n = 56$ control clutches.

hatchability in enlarged clutches. Egg hatchability, calculated based upon single eggs as in previous studies (Arnold 1999), was slightly lower in enlarged clutches (86%, $n = 242$) than in controls (91%, $n = 270$). Mean egg hatchability per clutch was non-significantly lower in enlarged clutches (86 ± 21·2% of all eggs hatched, $n = 50$ clutches) than in controls (92 ± 16·7%, $n = 72$; $t_{120} = 1·74$, $P = 0·085$). Within enlarged clutches, extra eggs had lower hatchability (66%, $n = 56$) than did natural eggs (92%, $n = 186$; Yates $\chi^2_1 = 21·75$, $P < 0·001$). The extra egg hatched synchronously with natural eggs in all but four enlarged clutches. In two cases, the extra egg hatched 2 days early and chicks were adopted in non-experimental broods. In two other cases, the extra egg hatched 1 day after natural eggs hatched; these chicks disappeared. These four chicks were not used in analyses of chick survival.

Chicks hatching from enlarged clutches were not smaller and did not weigh less than chicks from control clutches as neither bill length, tarsus length, body mass, nor body size or body condition differed between the two groups (Fig. 2). When the mean mass of eggs in the clutch was included as a covariate in an ANCOVA (Larsen *et al.* 2003), the mean body mass of chicks hatching from enlarged and control clutches also did not differ (adjusted means ± SEs: enlarged, 22·9 ± 0·28 g; control, 22·8 ± 0·27 g; $F_{1,85} = 0·02$, $P = 0·889$), while the effect of egg mass was significant ($F_{1,85} = 22·45$, $P < 0·001$). When the above analyses were repeated using only clutches enlarged during egg laying ($n = 14$), the differences remained nonsignificant for each body size variable (not shown).

CHICK-REARING PHASE

Soon after the young hatched, pairs moved their self-feeding young to nearby chick-rearing areas and established territories. Prey abundance on the feeding territory was lower for pairs incubating enlarged clutches than for pairs incubating control clutches (Fig. 3; $t_{36} = 2·16$, $P = 0·037$). Season did not influence this pattern (see Methods) and the date of sampling was similar for experimental and control pairs (enlarged: June 10 ± 14·1 days, $n = 17$; control: June 14 ± 15·2, $n = 21$; $t_{36} = 0·84$, $P = 0·407$).

The number of young surviving to fledging decreased with season (hatching date; $B = −0·05 ± 0·11$ SE, $R^2 = 0·40$, $F_{1,121} = 22·29$, $P < 0·001$). An ANCOVA, controlling for the effect of season, showed that fewer young survived to fledging in broods from enlarged clutches than in broods from control clutches (Fig. 1; enlarged vs. control $F_{1,111} = 4·19$, $P = 0·043$; hatching date $F_{1,111} = 22·26$, $P < 0·001$; data missing from three enlarged and five control broods). The number of young surviving to fledging was positively related to prey abundance on the feeding territory in controls ($r_S = 0·53$, $n = 21$, $P = 0·013$), whereas no such relationship was found in broods from enlarged clutches ($r_S = −0·17$, $n = 17$, $P = 0·516$).

Chick adoption did not affect differences in chick survival. Pairs with broods from enlarged and control clutches were similarly likely to adopt chicks (12%, $n = 50$ vs. 15%, $n = 72$, respectively, Yates-corrected $\chi^2 = 0·06$, $P = 0·804$), and whether pairs adopted chicks or not did not influence the number of young fledged (ANCOVA refitted, adoption status $F_{1,110} = 0·27$,
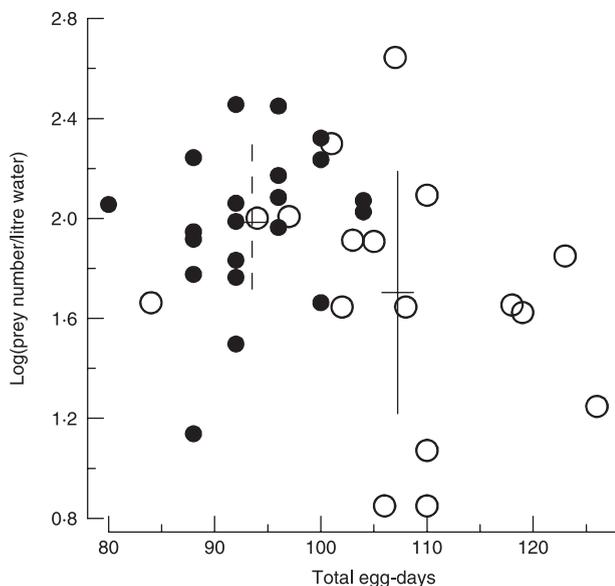
Fig. 3. Prey abundance on feeding territories of pairs incubating enlarged clutches (open symbols) and control clutches (closed symbols, broken line) as a function of incubation cost (total egg-days). Mean ± SD prey abundance is shown at mean incubation cost in each group. Linear regression, enlarged: $b = -0.01$, $n = 17$, $P = 0.325$; control: $b = 0.01$, $n = 21$, $P = 0.271$.

$P = 0.607$), while the effects of clutch enlargement ($F_{1,110} = 3.99$, $P = 0.048$) and hatching date ($F_{1,110} = 20.10$, $P < 0.001$) remained significant.

Brood size also was not likely to affect response variables during chick rearing. Neither prey abundance on the feeding territory, nor the number of young fledged differed by whether the extra egg hatched (i.e., brood size increased relative to natural clutch size) or not (brood size equalled natural clutch size) in enlarged clutches (prey abundance, $t_{15} = 0.21$, $P = 0.839$; number of fledged young, $t_{48} = 0.10$, $P = 0.923$).

Lower chick survival in broods from enlarged clutches could not be explained solely by predation. The proportion of entire broods disappearing (most likely taken by predators) was similar for the two groups (enlarged: 55%, $n = 47$ vs. control: 46%, $n = 67$, Yates-corrected $\chi_1^2 = 0.58$, $P = 0.447$). Furthermore, the difference in chick mortality was not significant during week 1 after hatching, when broods move to feeding areas and mortality due to predation is highest (Lengyel 2006) (ANCOVA, season-adjusted mean ± SE percentage of chicks surviving until day 7: enlarged, 35 ± 5·5%; control, 46 ± 4·6%; $F_{1,111} = 2.67$, $P = 0.105$). However, mortality differences grew to be significant by the end of week 2, when broods had already spent a week or more in the feeding territory (percentage of chicks surviving until day 14: enlarged, 27 ± 5·2%; control, 42 ± 4·3%; $F_{1,111} = 4.97$, $P = 0.028$). In the end, pairs incubating enlarged clutches reared 21 ± 4·7% of their young to fledging and pairs incubating control clutches reared 35 ± 3·9% of their young to fledging ($F_{1,111} = 4.65$, $P = 0.033$). These results suggest that fewer prey on the feeding territory could also play a role in lower chick survival in broods from enlarged clutches.

Discussion

INCUBATION-LIMITATION HYPOTHESIS

Our results support the hypothesis that clutch size in shorebirds can be limited by the incubation ability of the parents. We found both a substantial overall cost (fewer young raised to fledging) and several subtle costs (higher hatching asynchrony, marginally lower egg hatchability, fewer prey on the feeding territories) for pairs incubating enlarged clutches. Our data show that the subtle costs may not add up to an overall cost by the end of incubation, because more young hatched from enlarged clutches than from control clutches. However, results from the chick-rearing phase suggest that extra incubation costs may have an effect later, and that the subtle costs may interact synergistically towards the overall cost (lower annual reproductive success) as suggested by Arnold (1999).

This study is the first experimental test of the ILH in which the annual reproductive success of pairs until the fledging of young was measured. Previous studies that did not monitor broods from manipulated clutches could not possibly detect the longer-term, more 'cryptic' costs found here. Reliable estimates of incubation costs to be used in life-history theory require equal attention to the incubation phase as well as the chick-rearing phase (Heaney & Monaghan 1996). In passerine birds, for example, clutch size is often limited by how many young parents can rear to fledging ('Lack' clutch size; Godfray *et al.* 1991; VanderWerf 1992), suggesting that costs during brood-rearing can lead to adaptations limiting clutch size. Our results, therefore, clearly support the ILH and reflect to previous calls (Sandercock 1997; Arnold 1999; Wallander & Andersson 2002) for more thought on clutch size limitation in shorebirds.

Whether the extra costs of rearing more chicks hatching from enlarged clutches contributed to our results remains an open question. However, such a scenario is unlikely. First, pairs incubating enlarged clutches had poorer feeding territories and fledged fewer young regardless of whether they cared for more chicks (when the extra egg hatched) or for the number of chicks corresponding to their natural clutch size (when the extra egg did not hatch). Second, both prey abundance on the feeding territory and the number of young surviving to fledging increased with the number of young hatched from non-experimental nests (Lengyel 2006, 2007), and pairs adopted alien chicks frequently (Lengyel 2002), which are not expected if parental care is costly. Therefore, the costs of rearing extra chicks are likely to be low in pied avocets, and those costs should be greatly exceeded by the benefits of a larger brood size. The observation that pairs with broods from enlarged clutches, which produced more hatchlings, could not exploit these benefits further reinforces the idea that the costs of extra incubation had a prolonged cryptic effect on the pairs.

COSTS OF EXTRA EGGS DURING INCUBATION

Avocets appeared to be less affected by the costs of incubating enlarged clutches during nesting. The estimated number of young hatched (cumulative reproductive value, Arnold 1999)

Table 3. Nesting parameters in enlarged and control clutches. Daily survival rate and nest success are calculated as in Mayfield (1975). The cumulative reproductive value (Arnold 1999) is given as R = C × N × P × H. Data from clutches naturally containing four eggs are used only

| Clutch size (C) | Nesting period, day | | | | Daily survival rate | Nest success (N) | Partial survival (P) | Hatchability (H) | R |
	Laying	Incubation	Hatching	Total					
Five	6·5	22·8	2·26	31·56	0·995	0·865	0·998	0·770	3·33
Four	5·2	22·6	1·97	29·77	0·993	0·800	0·998	0·893	2·85

was 17% higher for enlarged clutches than for controls (Table 3). This value was +13% in the closely related American avocet, +9% in the spur-winged plover (*Vanellus spinosus*), and ranged between −55% and +1% in seven other shorebirds (Arnold 1999). The positive values show no major costs of incubating enlarged clutches until hatching and suggest that such birds would benefit from laying one extra egg. We found evidence for only two types of costs during the incubation phase. First, hatching asynchrony increased in enlarged clutches compared to controls, which was also found previously in calidridine sandpipers (Hills 1983; Sandercock 1997). Second, statistically nonsignificant lower hatchability of eggs in enlarged clutches has been found in all but one previous experiment (Hills 1983; Shipley 1984; Delehanty & Oring 1993; Székely *et al*. 1994; Yogev *et al*. 1996; Sandercock 1997; Larsen *et al*. 2003, but see Wallander & Andersson 2002). Lower egg hatchability is most often explained by the inability of incubating parents to adequately warm all five eggs (Hills 1983). In our study, lower hatchability in enlarged clutches was largely due to extra eggs, which hatched in smaller proportions than did the natural eggs. Although increased failure of extra eggs can be related to our transport and handling, this seemed unlikely because most (15 of 19) of the extra eggs that failed were collected as fresh (before or during week 1 of incubation), at which stage the eggs are normally less sensitive to handling. A developed embryo was found in all six extra eggs that were opened after the natural eggs had hatched, which indicated that embryo development continued for some time after transport (the remaining 13 eggs were not opened). These embryos most likely died during incubation as a result of inefficient heat transfer (Hills 1983), which may occur, for example, if parents discriminate between an extra egg and their own natural eggs. Nevertheless, whether the extra egg hatched or not did not affect response variables during chick rearing, therefore, lower egg hatchability in enlarged clutches was not likely to affect our major conclusions.

We did not find evidence for other costs reported in previous studies. There was no evidence of higher rates of abandonment for enlarged clutches (found in Hills 1983; Delehanty & Oring 1993; Székely *et al*. 1994). Incubation period did not increase in enlarged clutches, which has been reported in most other shorebirds (Hills 1983; Székely *et al*. 1994; Yogev *et al*. 1996; Sandercock 1997; Wallander & Andersson 2002; Larsen *et al*. 2003). Enlarged clutches did not suffer from higher predation rates than did controls, in

contrast to other studies that reported more frequent losses of single eggs (Hills 1983; Shipley 1984; Delehanty & Oring 1993; Sandercock 1997). Finally, chicks hatching in enlarged clutches were not smaller or did not weigh less than chicks hatching from control clutches. Lower chick body condition, if accompanied by lower chick survival, was suggested as a potential cost of five-egg clutches in northern lapwings (*Vanellus vanellus*) (Larsen *et al*. 2003).

COSTS OF EXTRA EGGS DURING CHICK-REARING

Increased hatching asynchrony and lower egg hatchability did not result in an overall decrease in the number of young hatched from enlarged clutches. Similar patterns were found in American avocets (Shipley 1984) and some other shorebirds of medium to large body size (Wallander & Andersson 2002; Larsen *et al*. 2003), where more chicks hatched from enlarged clutches than from control clutches. Pairs with supernormal clutches parasitized by other females also hatched more young in another study of pied avocets (Hötker 2000). However, this study shows that the number of chicks hatched may not appropriately reflect the costs of extra incubation.

Incubating larger-than-normal clutches requires higher energy expenditure from the parents due to the larger total egg volume that needs to be kept warm (Piersma & Morrison 1994; Thomson, Monaghan & Furness 1998). Our results suggest that the extra energy expenditure during incubation contributes to lower performance of adults during chick rearing. For example, extra energy expenditure may have resulted in poorer body condition or lower success in competing for territories for pairs incubating enlarged clutches. Behavioural time budgets measured when pairs settled in feeding territories showed that pairs incubating enlarged clutches spent more time (28%, $n = 4$ pairs) feeding than did pairs incubating control clutches (13%, $n = 12$); however, the results were inconclusive because feeding occurred in only 4 of 12 time budgets of pairs incubating enlarged clutches and there were no differences in other behaviour types between experimental groups (Lengyel 2001). Our results do show, however, that less food was available for chicks hatching from enlarged clutches than for chicks from controls. Two results suggest that food availability may have influenced the survival of chicks. First, prey abundance was positively related to chick survival in controls. Second, the increased mortality of chicks from enlarged clutches occurred

after broods spent some time in their feeding territories of lower-than-average prey abundance (by the end of week 2), and not when chick mortality due to predation is most intense (during week 1, Lengyel 2006).

Alternative mechanisms may also explain lower chick survival in broods from enlarged clutches. First, increased mortality may be partly caused by the incubation environment as suggested by Larsen *et al.* (2003) and Gorman & Nager (2004). The altered microclimate in enlarged clutches is known to reduce hatching success without any apparent direct costs to the incubating parent (Reid, Monaghan & Ruxton 2000). To separate the effects of incubation costs to parents and embryonic developmental costs to chicks would require cross-fostering, when chicks hatching from unmanipulated clutches are raised together with chicks from enlarged clutches, allowing an intra-brood comparison of chick survival. Second, intra-brood competition for food among chicks may also be higher in larger broods. However, in our study population, there is a positive correlation between brood size and prey abundance on the territory, and chick survival is higher in larger than in smaller broods (Lengyel 2007). Moreover, the experimental addition of an extra chick to broods in which parents incubated four eggs did not influence fledging success at the study site (Lengyel 2007), indicating that the overall effect of intra-brood competition on chick survival is likely to be small in pied avocets. Third, a seasonal decline in food resources is also possible, and may be related to why the number of fledglings depended on season in this study. However, there were no seasonal differences among the experimental groups (Table 1), therefore, seasonal effects do not directly explain lower chick survival in broods from enlarged clutches. Finally, it is possible that food resources are depleted more rapidly on territories of broods from enlarged clutches than on territories of control pairs. Our results do not rule out the possibility that food depletion and subtle seasonal differences may contribute to lower food availability and lead to fewer fledglings in broods from enlarged clutches in avocets. In other shorebirds, one or more of the alternative mechanisms during chick-rearing may also be important in limiting clutch size.

Our results suggesting a relationship between food availability and chick survival refine the general view that increased predation risks in larger, more conspicuous broods is primarily important in limiting brood and clutch size in shorebirds. Safriel's (1975) early work in semi-palmated sandpipers (*Calidris pusilla*), a small-sized, arctic-nesting shorebird with uniparental care showed that chick survival was significantly lower in broods enlarged to five chicks ($n = 27$ broods in 2 years) than in four-chick control broods ($n = 39$). He proposed that a greater foraging effort by food-stressed chicks in large broods makes them more conspicuous to predators, which results in their lower survival. Although broods larger than four chicks might be more prone to predation in the studied population of avocets as well, our previous findings showed that more chicks survived to fledging in larger broods, and that increasing brood size by natural adoption was almost a pre-requisite to rear filial young to fledging in

high-predation areas (Lengyel 2007). Our previous and current findings indicate that greater vulnerability to predation resulting from higher chick activity in large broods may not directly affect chick survival in avocets. Furthermore, no other experiment in precocial birds has ever reported greater mortality of chicks in larger broods (Rohwer 1985; Lessells 1986; Milonoff & Paananen 1993; Sandercock 1994; Williams, Loonen & Cooke 1994), and one study (Loonen *et al.* 1999) found that adult Barnacle Geese (*Branta leucopsis*) with broods enlarged after hatching occupied better feeding territories and fledged more young than did adults with control or experimentally reduced broods. These studies suggest that a mechanism other than greater predation on large broods is necessary to explain clutch size limitation in shorebirds. Our results point to poor body condition or reduced competitive ability of parents and lower food abundance on territories of pairs incubating enlarged clutches as a likely explanation.

Two aspects of this study need to be mentioned for the correct interpretation of the results. First, pairs were allocated lower extra incubation costs in our study than in previous ones. Clutches in most previous experiments were enlarged at egg laying and experimental eggs were present throughout incubation. In contrast, several rescued eggs used here were already some days into incubation, preventing the allocation of full costs to all pairs in this study. It appears plausible that had the full costs been allocated to all pairs, other costs would have been found during the nesting phase or the costs of incubating enlarged clutches detected would have even been larger. Second, we did not exchange eggs in control clutches. A study in which eggs are swapped among nests but clutch size is kept constant would have been a more effective control for the manipulation during clutch enlargement. Egg swapping has been studied previously and concluded to have no effect on egg hatchability in one of the eight previous shorebird clutch manipulations (Wallander & Andersson 2002). We did not use such a control because it was essential to collect baseline information on annual reproductive success in a reliably large sample of unmanipulated nests that may allow for frequent clutch failure and low chick survival (50, 31%, respectively, Lengyel 2006). Moreover, conducting egg-swapping in a meaningful sample size would have presented considerable logistical challenges and would have increased disturbance to nesting colonies, potentially leading to artefacts such as increased rates of clutch abandonment. Because eggs were not swapped, we cannot conclusively exclude the possibility that transport and handling led to lower hatchability of extra eggs. However, this possibility seemed small relative to the differences found. If the extra eggs ($n = 56$) had had similar hatchability as the natural eggs (92%), 15 chicks would have hatched in addition to those that did hatch ($n = 37$), which, assuming chick survival at 21%, corresponds to three additional fledglings or to an increase from 0·7 to 0·8 in the mean number of fledglings in broods from enlarged clutches ($n = 47$). This value (0·8) is still considerably smaller than that in broods from control clutches (1·2), indicating that lower hatchability of extra eggs was not likely to contribute much to the lower overall success for pairs incubating enlarged clutches.

In conclusion, our results support the hypothesis that the incubation of extra eggs causes an energetic cost to parents and that this cost manifests at a later time, during chick rearing. The exhaustion of parents due to the extra incubation costs may prevent pairs from occupying better territories and fledging more young. Extra incubation costs are related to an overall decrease in chick survival, and thus, in the parents' annual reproductive success. Therefore, our study provides evidence that the invariant clutch size of shorebirds can be explained by limitations in the incubation ability of parents (Lack 1947). Other costs, detectable on longer time-scales (e.g. juvenile survival to breeding, recruitment rate, adult survival and future reproductive success) will also need to be measured for a full understanding of clutch size evolution in shorebirds.

Acknowledgements

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