Nest defense is a fundamental behavior, which functions to increase reproductive success in bird species vulnerable to predation. By such behavior, parents can enhance the survival of their offspring via predator deterrence (Wiklund 1990; Komdeur & Kats 1999), while simultaneously risking injury or death as a cost of that behavior (Brunton 1986). Thus, the intensity of an individuals’ nest defense should represent a balance between potential costs and benefits, and, theoretically, should be adjusted to optimal levels to achieve their maximum lifetime reproductive success (reviewed by Montgomerie & Weatherhead 1988; Caro 2005).

Many ornithologists have considered that observed variations in the intensity of nest defense are affected by offspring age or the stage of the nesting cycle (Montgomerie & Weatherhead 1988; Brunton 1990); offspring number (Greig-Smith 1980; Wallin 1987); parental age (Pugesek 1983; Shields 1984); and type of predator (Greig-Smith 1980; Buitron 1983). Among these factors, the effects of offspring number have attracted the attention of many researchers, since the benefits are straightforward and fitness increases with the number of surviving eggs and chicks (Montgomerie & Weatherhead 1988). Some studies found evidence for the hypothesis that parents should increase the intensity of nest defense with offspring number (Wallin 1987; Forbes et al. 1994; Albrecht & Klaña 2004), while others did not (Breitwisch 1988; Rytkönen et al. 1995; Galeotti et al. 2000). Therefore, controversy concerning this subject remains unresolved (Caro 2005).

Part of the inconsistency observed in previous studies may derive from individual and sexual differences in nest defense insensitivity and from faulty experimental design. If parents adjust their clutch size according to their ability to raise all of their offspring, then the effects of clutch size on the intensity of nest defense will not be observable in natural populations.
If a positive relationship between the intensity of nest defense and clutch size is detected in a natural population, it may merely reflect the quality of the parents, i.e., parents producing a larger clutch may be in good body condition and therefore have more time to devote to defense (Lessells 1991). Thus, clutch or brood size manipulation experiments using model predators have been carried out in order to exclude those factors (reviewed by Caro 2005). However, some studies may have suffered from defective experimental design; for example, most previous studies have not controlled for variation in either nesting microhabitat or location (Sandercock 1994; Tolonen & Korpimaki 1995; but see Lambrechts et al. 2000). Nesting microhabitat and location may affect the vulnerability of nests to predation and hence parental nest defense behavior (Brunton 1990; Galeotti et al. 2000). Furthermore, in some studies, too short a time was provided for parents to assess correctly the value of their artificially manipulated clutches, before nest defense was assessed (e.g., Tolonen & Korpimaki 1995).

Black-tailed Gull *Larus crassirostris* nests colonially on gentle slopes of coastal islands, where vegetation or rocks provide moderate cover; they lay clutches of 1–3 eggs (Kazama 2007). Jungle Crows *Corvus macrorhynchos* have been shown to take 30% of Black-tailed Gull eggs under natural conditions at the study colony on Rishiri Island, Hokkaido (Kazama 2007). Parent gulls defend their nests aggressively against the crows by intimidating behavior, including: opening the bill and wings, body striking, or swooping (Watanuki 1983; Kazama 2007). Male gulls demonstrate a higher frequency or duration of nest defense than females (Kazama & Watanuki in press). The long incubation period of this species (25–30 days) may provide sufficient time for parents to assess the value of their artificially manipulated clutches. In this study we examined the effects of clutch size on the intensity of nest defense in Black-tailed Gulls by manipulating clutches; we increased clutches of two eggs by adding four eggs (INCREASED) or reduced them by one (DECREASED). We measured the response of male gulls to a crow decoy in a study plot, where height and density of vegetation and nesting location were controlled to be uniform. We investigated the intensity of defensive behavior before and after clutch size manipulation, and compared these between INCREASED, DECREASED and un-manipulated two-egg clutch groups.

**METHODS**

1) **Study area and period**

The study was conducted on Rishiri Island (45°14′N, 141°09′E), situated in the Sea of Japan/East Sea 40 km west of northern Hokkaido, Japan, from 25 April to 10 July 2007. The island supported >19,000 breeding pairs of Black-tailed Gulls in 2004 (Kosugi et al. 2005). A study area in a sub-colony was established at Oiso, on the gentle, northwestern slope of the island.

All 156 nests within the study area were marked with numbered stakes. Nest contents were checked every one or two days. Black-tailed Gull nests in peripheral areas (<4 m from the edge of the breeding area) and nests located where vegetation is <20 cm high have been shown to be more vulnerable to egg predation by Large-billed Crows than those in the colony center or amongst taller vegetation (Kazama 2007). To control the potential vulnerability of the nests to predation, we established the study area in a small sub-colony where all nests were peripheral, and we also cut back the vegetation around the nests every 1–2 days to maintain its height shorter than 15 cm, so as to control for uniform nest vulnerability to egg predation by natural predators.

All individuals nesting within the study area, were marked with black hair dye (Bigen hair color, containing aminophenol and stearic acid as major ingredients; Hoyu Co., Ltd., Nagoya, Japan) to permit individual identification. During the incubation period, stones or leaves with the dye were placed in the nest cup so that the dye would mark the breast or neck of the gulls when they visited the nest. The sexes of the gulls were determined by observation of body size (Chochi et al. 2002) and mating or copulation behavior.

2) **Model predator experiment and clutch manipulation**

Our simulated predator model was a crow decoy used to examine the response of gulls against predators (a plastic hunting decoy painted to resemble an American Crow *C. brachyrhynchos*; Carry-Lite Inc., Fort Smith, Arkansas). The decoy was placed at ground level 1.5 m from the nest to be examined and covered with a cloth more than 30 minutes before beginning presentation, to permit the gulls to calm down following disturbance by humans. The decoy...
was exposed for two minutes by removing the cloth, which was done by pulling on a line attached to the cloth.

Two repetitions of decoy presentation were conducted for each of 42 males having two egg clutches (the modal clutch size) originally. The first presentation was carried out for males between days 3–19 of initiating incubation. During each trial, the males were incubating and their mates were away from the nests.

Clutch manipulations were done immediately after the first presentation. For 20 males, four dummy eggs (hen Gallus gallus domesticus eggs containing silicon, painted to resemble gull eggs and having the same volume and weight as a gull egg) were added to create clutches of six eggs (“INCREASED males”, Fig. 1). Black-tailed Gulls usually lay 1–3 eggs, but occasionally produce 4–5 eggs under natural condition (K. Kazama personal observation, also see Kosugi et al. 2005). Therefore, in order to ensure that the males would definitely recognize an increased clutch value, we made extremely large clutches with six eggs (INCREASED males; N=20). For seven males, one egg was removed from each clutch so that they would each only have a single egg (DECREASED males; N=7). Eggs removed were transferred into other nests outside the study area. A further fifteen males were “CONTROL males”; their clutches were not manipulated, thus they each retained two eggs in their nests. The second presentation of the decoy was carried out at the same place 9–13 days after the first presentation using the same protocol as in the first presentation. No eggs were lost between the first and the second presentation. All the trials were conducted on clear, calm afternoons (1300–1700). No more than four trials were conducted in a day for nests within 10 m of one another. Fourteen predator presentation trials were carried out in the first trial and 24 in the second.

During each two minute presentation, we counted the number of times each male stopped incubation, gave short alarm calls, flew in circles near the decoy, or directly attacked the decoy (see Shealer & Burger 1992; Stenhouse et al. 2005). We also recorded the time until the first direct attack on the decoy by each male. Based on these records, we assigned each male a responsiveness and aggressiveness score of 0–4, with 0 indicating a non-responsive and non-aggressive male and 4 indicating the most responsive most aggressive male (see Table 1 for details of score as-
This scoring system is similar to those used in other studies of nest defense in gull species (Shealer & Burger 1992; Stenhouse et al. 2005) and in other birds (Hakkarainen et al. 1998; Duckworth 2006). It is known that the intensity of nest defense of individual gulls against the crow decoy matches that against live Jungle Crows (Kazama & Watanuki in press). Furthermore, the intensity of defense (response score) against the decoy does not vary with the number of presentations (Kazama & Watanuki in press). Response scores of one to four nesting male gulls <1.5–2.0 m near the decoy were recorded at a single trial. All counts and records of gull behavior were conducted from a blind placed 5 m from the study area.

No birds abandoned their nests and no pairs stopped incubating their clutches during the study period. No behavioral changes, attributable to color marking, such as increased susceptibility to predation or territorial attack by other birds, were observed. All the males and their mates returned to incubate quickly (within several seconds) after clutch manipulations and other disturbances for the test presentations. The dummy eggs were removed from the nests of INCREASED males immediately after the second presentation.

### 3) Statistical analysis

The dates of first egg-laying by gulls were compared among the type of manipulations using One-way ANOVA. To examine the effects of the manipulation of clutch size on the intensity of nest defense, we fitted a Linear Mixed Model (LMM) to a square root transformed response score using R ver. 2.7.2 (R Development Core Team 2005). “Order of presentation” (before and after clutch manipulations), “type of manipulation” (INCREASED, DECREASED, and CONTROL) and interactions were used as fixed effects. Individual identity was included as a random effect in order to account for pseudo-replication.

To quantify individual consistency in the intensity of nest defense between periods before and after clutch manipulation, repeatability (r) of response score and standard error of (r) were calculated following Lessells and Boag (1987) and Becker (1984), respectively. Where no significant differences in the intensity of nest defense among the types of manipulation were found, the data of all three types of manipulation were included in this calculation (N=42). Response scores were applied to a square root transformation in order to analyze the relationship between the response scores of individuals before clutch manipulation and those after the manipulation.

### RESULTS

The mean egg-laying date (elapsed days from 1 May±SD) of gulls was 4.39±4.01 (N=156). The mean clutch size was 2.25±0.53 (range: 1–4, N=156). The date of first egg-laying (mean elapsed

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<th>Table 2. Intensity of male Black-tailed Gull responses to a crow decoy before and after clutch manipulations.</th>
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<td>Clutch manipulation</td>
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<td>INCREASED (N=20)</td>
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<th>Table 3. Factors affecting the intensity of nest defense by male Black-tailed Gulls against crow decoys. Result of Linear Mixed Model (N=84).</th>
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<td>Factor</td>
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<tr>
<td>Intercept</td>
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<td>Type of manipulation (INCREASED)</td>
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*1 Relative variable if the value of Type of manipulation (CONTROL) was zero.  
*2 Relative variable if the value of Type of manipulation (CONTROL) × The order of presentation was zero.
days from 1 May±SD) did not differ among the three types of manipulation (INCREASED: 6.40±3.30, N=20, DECREASED: 4.43±2.88, N=7, CONTROL: 5.60±4.67, N=15, F2,39=0.837, P>0.05).

The response score before clutch manipulations (mean±SE) was 2.05±0.14 (N=42 males). There was no change in the observed response score among the three types of manipulation, either before or after clutch manipulations (Table 2, Table 3). For all types of clutch manipulation the response score did not differ between before and after clutch manipulations (Table 2, Table 3). Interactions of the order of presentation and the type of treatment (clutch size) were not significant, indicating differences in nest defense intensity levels were not strongly related to the effects of clutch size among the three types of manipulation (Table 3).

The intensity of nest defense of individual birds was very constant and thus significantly repeatable, between before and after clutch manipulations (repeatability: r=0.52±0.11, F41,42=3.17, P<0.001, Fig. 2).

**DISCUSSION**

We believe that we were successful in correcting design flaws in past experiments on clutch manipulation. By controlling nesting positions and habitats, all males were exposed to the same levels of potential nest predation in this study. We found no differences in first egg-laying dates among our experimental groups. Thus variations in body condition or age of individuals linking with egg-laying date (Coulson 2002) among INCREASED, DECREASED and CONTROL groups are thought to be negligible. In previous clutch manipulation studies, parents were allowed only 24h to assess the value of an artificially manipulated clutch or brood (Sandercock 1994; Curio & Onnebrink 1995; Tolonen & Korpimaki 1995). It is suspected that such periods are too short for parents to assess the correct value of their altered clutches (Tolonen & Korpimaki 1995). To compensate, we provided parents 9–13 days to assess the value of their manipulated clutches. Parent birds are able to assess the number of eggs in their own clutch within several days (three or more days) (Lyon 2003). Therefore we assumed that our birds could assess the number of eggs in their manipulated clutches, and we predicted that INCREASED and DECREASED males should increase and decrease the intensity of their nest defense respectively, in response to changes in their clutch size.

The predictions, however, were not supported in our study. It is hypothesized that if predators take one egg from each clutch (a partial reduction) at each predation attempt, the intensity of nest defense will be independent of clutch size (Caro 2005). In our study colony, Jungle Crows took on average 1.58 eggs from each clutch at each predation attempt, thus each pair of Black-tailed Gulls having an average of 1.73 eggs lost almost all of their eggs (Kentaro Kazama, unpublished data). Moreover, if model predators represent too serious a risk of life to parents, rather than just to their offspring, parents should not increase their nest defense effort as clutch size increased (Curio & Onnebrink 1995). Jungle Crows depredate only gull eggs and chicks, not adult gulls (Watanuki 1983; Kazama 2007), thus crow decoys appear to make a suitable threat to derive increased defense activity if Black-tailed Gull parents increase nest defense as clutch size increases.

Why did parent Black-tailed Gulls not adjust the intensity of their nest defense in response to the value of the greater or lesser number of eggs in the manipulated clutch? One possible reason is that parents might not need to adjust their levels of nest defense in relation to the current clutch or brood value, given that they are so long-lived (over 20 years; Narita & Narita 2004). Parent Black-tailed Gulls may have a greater long-term future reproductive opportunity than shorter-lived small passerine birds. Studies
showing positive relationship between clutch or brood size and defense intensity have been carried out in short-lived (approximately <5 years) passerines (Gottfried 1979; Knight & Temple 1986; Rafford & Blakey 2000). In long-lived (>10 years) seabirds, the opportunity for future reproduction should affect the optimum investment in nest defense effort, leading to a lower investment value for the current clutch or brood (Pugesek 1981, 1983; Pearson et al. 2005). As the age of the adults in our study was not known, we could not rule out the effects of the opportunity for future reproduction on our results.

Another possibility is that parents were not able to adjust their levels of nest defense in response to the current clutch value, as a consequence of some genetic and/or physiological constraint. In several bird species, consistent individual variation in aggressiveness has been indicated (e.g. “personality”, reviewed by Groothuis & Carere 2005). The repeatability of response score in our study of the Black-tailed Gull (r=0.52) is the same level as the boldness score relating to the intensity of nest defense and recorded twice in a season in the Great Tit Parus major (r=0.48) (Hollander et al. 2008). Among birds, these consistent individual variations are moderatelyheritable (Drent et al. 2003; van Oers et al. 2005) and often correlate with hormonal levels (Koolhaas et al. 1997; Cockrem 2007; Kralj-Fiser et al. 2007). In gull species it has been demonstrated that androgen (e.g. plasma testosterone) proximately affects individual aggressiveness (Alonso-Alvarez 2001; Alonso-Alvarez & Velando 2001). Thus consistent individual variation in nest defense in Black-tailed Gulls might be the dominant factor, rather than current clutch size, in determining the intensity of nest defense.

Testing directly whether parent Black-tailed Gulls recognize the number of eggs in their nest is difficult. We were only able to assess parental recognition through changes in their behavior. We could not completely rule out the possibility that parents did not recognize that there had been a change in their clutch size. However, this study, apart from the mechanisms of parental recognition of clutch size, clearly indicated, by more rigorous experiments than previously conducted, that parents did not adjust the intensity of their nest defense behavior to the number of eggs in their clutch.

In conclusion, male Black-tailed Gulls did not, or were unable to, adjust the levels of nest defense to the current value of their artificially manipulated clutch size. This was probably because the opportunity for future reproduction, or genetic and/or physiological based consistent individual variation, dominated the nest defense behavior.

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Effect of clutch size on nest defense

163–166.


