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Male phenotype and resource type influence nesting behaviour in a fish

Abstract

In many brood-rearing species, suitable nesting resources are needed for nest construction. Here, we used males of a small marine fish, the sand goby, *Pomatoschistus minutus*, to study the associations between the nest owner's phenotype (i.e. body size), the characteristics of the nesting resource used for nest construction (i.e. resource size and shape), and nest building behaviour (i.e. eagerness to build a nest and extent of nest elaboration). We found that male body size was associated with nesting resource size and resource architecture in the field, with the smallest males occupying small flat resources and the biggest males occupying large arched resources. In the laboratory, the type of resource occupied in the field had a limited effect on the level of nest elaboration, but not other nesting behaviours. Large body size, in turn, was associated with preference for larger resources, and, in some circumstances, also the level of nest elaboration. Body size did not affect the eagerness to initiate nest building. Furthermore, males chose arched nesting resources more often than those that were flat, and this preference was also reflected under a "no-choice" scenario, based on the time taken for males to initiate nest building. Overall, the results indicate that the importance of male size in nest building is context-dependent, while nesting behaviours can also be affected by resource size, resource architecture, and, under some circumstances, the nest builder's experience with resource use.

Keywords:

binary choice, body size, context dependence, extended phenotype, male-male competition, nest architecture, nesting behaviour, parental care, resource, sand goby

INTRODUCTION

Many animals rear their offspring in purpose-built nests, the characteristics of which affect their reproductive success (Barber, 2013; Guillette & Healy, 2015). Larger nests, for example, may provide better thermal insulation for the developing brood (Hoi, Earley, & Wolf, 1994), conspicuous nests may improve a nest owners' encounter rate with potential mates (Genner, Young, Haesler, & Joyce, 2008), while nest concealment may reduce the risk of brood predation (Cresswell, 1997; Weidinger, 2002; Fisher & Wiebe, 2006). Indeed, both the choice of nesting location and nest architecture can affect offspring survival in a range of taxa, including fish (Takegaki & Nakazono, 2000; Raventos, 2006), amphibians (Byrne & Keogh, 2009), birds (Burton, 2006; Quader, 2006) and mammals (Bult & Lynch, 1997). With regard to nest characteristics, individuals may need to trade-off between multiple factors to optimise their reproductive output. For example, optimal thermoregulation of eggs in the nest may have to be traded against the need for nest crypticity against would-be predators, as in piping plovers, *Charadrius melodus* (Mayer et al., 2009). Similarly, while possession of a high quality nest can improve mating opportunities for nest holders, it may also increase their likelihood of being challenged and usurped by superior competitors, as in Mediterranean wrasse, *Symphodus ocellatus* (Alonzo, 2004).

The characteristics of the ready-built nest, such as its size and elaboration, may also function as an extended phenotype (Schaedelin & Taborsky, 2009) and play a critical role in mate attraction (Hastings, 1988; Johnson & Searcy, 1993; Danylchuk & Fox, 1996; Östlund-Nilsson, 2001; Takahashi & Kohda, 2002; Eckerle & Thompson, 2006). For this to be the case, one should expect nest structures and their owners to show consistent covariation. For example, in barn swallows, *Hirundo rustica*, the volume of nest material is positively related to immune response (Soler, Martín-Vivaldi, Haussy, & Møller, 2007), while in common gobies, *Pomatoschistus microps*, the amount of sand piled on top of a male's nest is correlated with his body condition (Kvarnemo, Svensson, & Forsgren, 1998). However, the appearance of the constructed nest is not the only important aspect of nest building behaviour that might correlate with builder phenotype. In some taxa (including many species of nest guarding fish), before nest construction can even take place, individuals must first acquire one or multiple suitable resources with which to build their nest (Barber, 2013). Yet, in contrast to the attention given on the relationship between builder phenotype and characteristics of the ready-built nest, much less is known about how a nest builder's phenotype might influence its choice of nesting resource.

The sand goby, *Pomatoschistus minutus,* is an excellent model species for studying the relationship between nest-builder's phenotype and nest-related behaviours. High success in nesting attempts is important because the species typically has only a single, extended breeding season (Healey, 1971). After finding an empty mussel shell or a flat stone (hereafter referred to as 'nesting resource'), a sand goby male builds a nesting chamber underneath the resource and piles sand on top of it (Lindström, 1988; Svensson & Kvarnemo, 2003; Lehtonen, Wong, & Kvarnemo, 2016). The characteristics of the nesting resource, as well as the completed nest, can have a direct impact on male mating success. In particular, nest characteristics (e.g. size and architecture) can physically limit the maximum number of eggs a male is able to acquire (Lindström, 1988), and affect the investment in brood care (Järvi-Laturi, Lehtonen, Pampoulie, & Lindström, 2008), the susceptibility of eggs to predation (Lindström & Ranta, 1992; Lehtonen, Vesakoski, Yli-Rosti, Saarinen, & Lindström, 2018), or the intensity of intra-specific competition (Lindström, 1992; Svensson & Kvarnemo, 2003). Hence, males should be highly selective when choosing nesting resources, with their phenotype, especially body size, potentially being important (Kvarnemo, 1995; Japoshvili et al., 2012; Lehtonen et al., 2016). In this respect, breeding sites occupied by sand gobies vary in terms of availability of nesting resources and, hence, available opportunities for resource choice without first having to evict the prior resident (Forsgren, Kvarnemo, & Lindström, 1996; Lehtonen & Lindström, 2004). Female sand gobies of this population, in turn, do not always prefer large males, but rather males that are matched in size with their nesting resource (Lehtonen, Rintakoski, & Lindström, 2007).

Here, our aim was to use the sand goby to study the associations between the nest owner, the nesting resource, and nest building behaviour. In particular, we assessed whether male nesting resource choice and nest building behaviours are linked to the male's body size, prior resource use experience or the characteristics of nesting resources available to him. To do this, we first conducted a field experiment to test whether males of different phenotype (i.e. body size) occupy nesting resources of different architecture (arched and flat) or size (smaller or larger). We then applied two laboratory experiments to test whether a male's size or his earlier nesting resource use relates to his nesting resource choice under controlled laboratory conditions. In this context, we also investigated how nest building behaviours (eagerness to build a nest and the extent of nest elaboration) may be affected by male size, prior experience (i.e. the nesting resource that the male was using in the field at the time of capture) and type of nesting resources offered in the laboratory. We hypothesised that male size is positively associated with the size of his chosen resource, his nest building effort and his probability of preferring the more conspicuous arched nesting resource type (Figure 1). We also expected that males occupying larger resources in the field might, under laboratory conditions, show a similar preference or be more diligent nest builders.

METHODS

Both the field and laboratory components (see below) of this study were conducted during the sand goby breeding season, between late May and late June, in 2016, at the Tvärminne

Zoological Station of the University of Helsinki (59°50.7´ N; 23°15.0´ E). The field site (Vargskär) has a large area of sandy substrate covered by shallow water (≤ 1.5 m). Suitable nesting resources (such as mussel shells and flat stones) at this site are scarce, resulting in male-male competition over nesting resources and high rates of nest occupancy of the resources that are available (Lindström, 1988; Lehtonen & Lindström, 2004).

Field experiment: Distribution of male phenotypes based on nest architecture and size

To test the relationship between nesting resources and their holders in the wild, we placed unglazed ceramic nesting resources that were either arched (halved terracotta flowerpots) or flat (tiles) across the study site at Vargskär. For both architecture types, arched and flat, two different sizes were deployed. Smaller arched nesting resources had a maximum mouth diameter of 4.5 cm and length of 4.2 cm, while the same measures for larger arched nesting resources were 8.3 cm and 8.1 cm, respectively (Figure 1). The two flat nesting resource sizes were 5.0 cm × 5.0 cm and 9.9 cm × 9.9 cm. Importantly, both the smaller and larger nesting resources of the two types had matching surface areas. Arched (i.e. halved flowerpots) and flat (tiles) nesting resources are similar to the natural nesting resources found in the area (i.e. mussel shells and flat stones) and are readily accepted by nesting sand goby males both in the field (arched nesting resources: Forsgsren et al., 1996; Lindström & Pampoulie, 2005, flat nesting resources: Lindström, 1988; Wong, Lehtonen, & Lindström, 2018) and under laboratory conditions (arched nesting resources: Japoshvili et al., 2012; Lehtonen et al., 2016, flat nesting resources: Lehtonen, Lindström, & Wong, 2013; Flink & Svensson, 2015).

The nesting resources of all types (with respect to architecture and size) were placed in the study area either singly or (due to logistic restrictions) in pairs of the same type, with their locations identified by marks on weighed rope lines that ran along the sandy substrate, each line anchored into the substrate at both ends. When in pairs, the minimum distance between the two nesting resources was 40 cm, while the minimum distance between pairs, as well as nesting resources places without a pair, was 2 metres. A similar number (~20) of each type of nesting resource was placed in the field site at a time. For logistic reasons, ~6 - 10 nesting resources closest to each other were of the same type (i.e. arched or flat and of a certain size). The nesting resources were sampled on 10 different occasions across the field site during the peak breeding season (late May - late June), with 3 - 5 days between each sampling effort. To control for any microhabitat variation, after every other sampling occasion, the locations of the marker lines, and hence nesting resources, were changed, without reusing any previous locations. Nest occupation level was high for all resource types.

The nesting resources were sampled with the aid of a mask and snorkel, on each occasion using a hand net to catch males that had built a nest (with catching success of ~75 - 95% for all resource types, depending on the weather and water conditions during, and the time available for, catching the fish). We then recorded the type of resource the male had been occupying. The males were transported in containers back to the field station, where they were weighed to the nearest 0.01 g on an electronic balance, and their total lengths measured to the nearest 0.5 mm on a measuring board with a grid scale. In total, we measured total lengths of males from the smaller arched (N = 143), smaller flat (N = 135), larger arched (N = 144) and larger flat (N = 137) nesting resources. Most of these males (see below for sample sizes) were later used for the laboratory experiments, as detailed below.

Laboratory experiment 1: Male preference for nest architecture

Back at the field station, males were first kept, typically for a couple of days and always less than a week, in holding aquaria of ~100 litres (with a substrate of fine sand and max 20 individuals in each tank). During that time, the males were fed with live opossum shrimp (*Neomysis integer*) *ad libitum*. The holding and experimental tanks (see below) were located in a non-insulated greenhouse and supplied with a continuous through-flow of sea water, pumped from a nearby bay. This ensured natural water conditions that were typical for the study site at the time of the year (temperature: 10 - 15 °C, salinity: ~5.5 ppt; Merkouriadi & Leppäranta, 2015) and day / night cycle (approx 18.5 hours / 5.5 hours at the time of the experiments) throughout the study.

In the first laboratory experiment, we investigated whether males have a preference towards arched or flat nesting resource (in the absence of rivals), and whether any such preference is related to the type of nesting resource the male was occupying in the field. We also assessed whether eagerness to nest (time to initiate nest building) and the extent of nest elaboration (amount of sand piled on the nest) were affected by these factors. The trials were run in tanks that measured 68 cm × 25 cm × 22 cm (length × width × height of water level) and had a 4 cm layer of fine sand as substrate. Before the onset of a trial, two nesting resources (see below for details) were placed randomly either to the left, centre or right position within the experimental tank (see Lehtonen et al., 2016). Hence, one of the three possible positions where the resource could have been placed within the aquarium was left empty in a randomised fashion. All randomisation for the study was done using random numbers that were generated at https://www.random.org/.

Each focal male was given the option of choosing between an arched and a flat nesting resource. The sizes of the nesting resources were chosen so that the effective area for egg deposition was, as closely as possible, the same for the two types of nesting resources. The two resource types were also similar in colouration, although an earlier study has shown that sand goby males do not discriminate between nesting resources of different colours (Wong et al., 2008). We had three different categories of replicates, with males given the option of the following choices: (i) two small nesting resources (an arched resource with the maximum diameter at mouth and length: 4.2 cm and 4 cm, and a flat resource: 4.3 cm × 4.3 cm), (ii) two medium-sized resources (arched: 6.5 cm and 6 cm, and flat: 6.7 cm × 6.7 cm) and (iii) two large resources (arched: 10.3 cm and 10.0 cm, and flat: 11.7 cm × 11.7 cm). The entrance of the arched nesting resource was facing one of the longer sides of the tank. The tanks were positioned behind blinds to minimise disturbance.

Gobies were allocated to the different treatments in a randomised fashion so that, as far as possible, the same number of individuals occupying each nesting resource type in the field was used in each treatment. A replicate was initiated by placing a sand goby male into the experimental arena (haphazard location with respect to the nesting resources). The male was then given up to 48 hours to initiate nest building. During this time, the experimental tanks were checked ~7 times a day, with the checks distributed as evenly as possible between 08:00 and 23:00. At each check, we recorded male location and any signs of nest building. A male was considered to have initiated nest building when it had started to pile sand on top of, and excavate under, the nesting resource, leaving a single narrow entrance to the nest. In such cases, the time to initiate nest building was recorded as the midway point in time between when the onset of nest building was first observed and when the tank was last checked without any such signs (Lindström & Lehtonen, 2013). After the male was observed to have initiated nest building, it was left in the tank for at least 12 (but no more than 24) hours to finish building a nest, which usually took one hour or less, with additional nest elaboration sometimes taking place within the following few hours. In some of these replicates (~10% over the data-set), both nesting resources showed signs of the male having initiated a nest. In such cases, we employed a criterion used in previously published studies (Japoshvili et al., 2012; Lehtonen et al., 2016), which is to tally up the total number of times that the male was observed using the nests it had built up and determine the 'chosen' option as the nesting resource with which the male had associated with most frequently. Although this does not differentiate between males that were more decisive (i.e. only built up one nest) versus those that were less decisive (built two nests but settled in one), we do not consider such a potential source of noise in the data set to be an issue, given our sample sizes. Our approach also avoids having to define choice using more subjective criteria that would require the exclusion of data. In all cases, time to the onset of nest building was defined by the first signs of nest building.

After completion of the replicate, we measured the amount of sand on top of the chosen nesting resource as a measure of the level of nest elaboration (*sensu* Lehtonen & Wong, 2009). This was done by carefully lifting the nesting resource onto a tray, which collected the sand that the goby had piled on top of his nest. Due to the shape of the arched resources (Figure 1), only the sand placed directly on the ridge of the resource (halved pot) was collected. The weight of this fraction is a good indicator of the total amount of sand that the male had placed on the nest (Lehtonen et al., 2016). For both nest types, the collected sand was later dried in an oven for 36 hours at 60 ˚C, and then weighed on an electric balance (Lehtonen & Wong, 2009, Lehtonen et al., 2016).

In total, nest type preferences of $N = 112$ males were assessed (total length 51.0 ± 0.6 mm [mean ± SE], weight 1.05 ± 0.04 g). Of these, 99 initiated nest building within 48 hours. Each individual was used in only one of the two laboratory experiments.

Laboratory experiment 2: Male preference for nest size

Experiment 2 used replicates that were also included in a complementary study that assessed whether sand gobies make comparative versus absolute resource choice decisions (Lehtonen & Wong, unpublished data). Experiment 2 had the same procedures as experiment 1, except that focal males were given the opportunity to choose between nesting resources that were of the same type but differed in size. In particular, $N = 273$ males (total length 50.4 ± 0.4 mm, weight 0.99 ± 0.02 g, size data missing for three males) were used to investigate the following choice scenarios: (i) a small (S) vs. a medium-sized (M) nesting resource ($N_{\text{arched}} = 39$, $N_{\text{flat}} = 71$), (ii) small (S) vs. large (L) resource ($N_{\text{arched}} = 38$, $N_{\text{flat}} = 43$), and (iii) medium (M) vs. large (L) resource ($N_{\text{arched}} = 36$, $N_{\text{flat}} = 46$). These replicates resulted in $N = 199$ choices, as detailed in the results.

Statistical analyses

All analyses were run using R 3.3.2 software (R Development Core Team). To assess whether different types of nesting resources attracted males of different sizes in the field, we ran an ANOVA with the total length (square-root transformed) as the response variable, and the nest type in the field (small arched / small flat / large arched / large flat) as the explanatory variable. Here, Tukey HSD was used for assessing significance of pairwise differences.

To assess, in experiment 1, whether male size or the nesting resource type he had occupied in the field was associated with his choice of nesting resource architecture in the laboratory, we applied a generalised linear model with a binomial distribution, with the choice (arched / flat) as the response variable and the nesting resource occupied in the field (small arched / small flat / large arched / large flat), the size of the two available nesting resources (small / medium / large) and male body size (total length) as explanatory variables. For the simplicity of interpretation, we only assessed the main effects.

To assess males' choice between two nesting resources of the same type but different size (i.e. laboratory experiment 2), we applied a generalised linear model with a binomial distribution with the choice (smaller / larger of the two nesting resources) as the response variable and the nest type occupied in the field (smaller arched / smaller flat / larger arched / larger flat), the available size options in the different treatments (S vs. M / S vs. L / M vs. L) and male body size (total length) as explanatory variables.

For both laboratory experiments 1 and 2, we also tested whether the time it took for males to start nest building (as a proxy of their eagerness to build a nest) differed depending on the type of nesting resource earlier occupied in the field. For both data sets (experiments 1 and 2), we applied a Cox proportional hazards analysis that included the nesting resource type occupied in the field (smaller arched / smaller flat / larger arched / larger flat) and male total length as variables. In the analysis of experiment 1, the model also included the size of the two available nesting resources (S / M / L) as another explanatory variable. The analysis of experiment 2, in turn, included the 3 nesting resource size choice scenarios (S vs. M / S vs. L / M vs. L) and the type of the offered resources (arched / flat) as additional explanatory variables. Males that did not commence nest building within the allocated 48 h period were 'right censored' (Lagakos 1979) in these analyses.

Finally, in both laboratory experiments, we assessed the relationship between the type of nesting resource occupied in the field and the extent of nest elaboration (i.e. the weight of sand collected from the top of the nest, when necessary squareroot or log transformed for improved normality). For datasets of both experiments, the analyses were conducted separately for the arched (only sand directly on the ridge collected) and flat (all sand piled on top of the object collected) nesting resources. In each case, we used a linear model with the type of nesting resource occupied in the field (smaller arched / smaller flat / larger arched / larger flat) and male total lengths as explanatory variables. In experiment 1, the size of the nesting resources available in the replicate and, in experiment 2, the size of the nesting resource used by the male for nest building, were included as additional explanatory variables.

Ethical note

The field survey and laboratory experiments carried out in this study are non-invasive and are designed to investigate nesting decisions and behaviours that sand gobies would make in the wild. All animals, upon completion of their replicates, were returned to the sea. The study was approved by the Finnish Animal Experiment Board (ESAVI/3915/04.10.07/2016).

RESULTS

Resource type use in the field

In the field, different types of nesting resources attracted males of different body sizes (Anova, $F_{3,555} = 25.27$, $P < 0.001$). In particular, the larger arched and flat nesting resources were occupied by males with the largest mean body size (Figure 2). Compared to these two resource types, males occupying smaller arched nesting resources were significantly smaller, while males occupying smaller flat resources were the smallest (Figure 2).

Male preference for nest architecture in the laboratory

In laboratory experiment 1, when males chose between two nesting resources of the same surface area, but of different architecture (arched vs. flat), neither the type of the resource the focal male had occupied in the field (Generalised linear model, $\chi^2_3 = 0.053$, $P = 1.0$) nor the male's body size ($\chi^2_1 = 0.725$, $P = 0.39$) had a significant effect on the choice outcome. However, the nesting resource size treatment did have an effect ($\chi^2_2 = 34.5$, $P < 0.001$). In particular, while males overall chose the arched nesting resource more often (83 times out of 99), the flat option was chosen more often with increasing size of the available resources, with the flat resource chosen in 0 out of 34, 1 out of 33, and 15 out of 32 replicates with small, medium and large sized resources, respectively.

Male preference for nest size in the laboratory

In laboratory experiment 2, when males were allowed to choose between two resources of the same architecture (either arched or flat) but of different sizes, the nesting resource they had occupied in the field did not have a significant effect on whether the larger option was chosen (Generalised linear model, $\chi^2_3 = 4.924$, $P = 0.18$). The sizes of available choice options did have an effect ($\chi^2_2 = 12.93$, $P = 0.002$), with the larger of the two resources chosen in 59 of 65, 59 of 66 and 47 of 68 cases, when the options were a S vs. M resource, S vs. L resource, and M vs. L resource, respectively. In addition, the probability of the larger resource option being chosen increased with male body size ($\chi^2_1 = 5.550$, $P = 0.018$).

Eagerness to build a nest in the laboratory

In experiment 1, in which the focal males chose between an arched and a flat nesting resource of the same size, neither the nesting resource occupied in the field (Cox proportional hazards test, $\chi^2_3 = 2.884$, $P = 0.41$) nor male total length ($\chi^2_1 = 0.1903$, $P = 0.66$) significantly affected the time taken by males to begin nest building. However, it took a longer time for the focal male to initiate nest building when the two nesting resources were small ($\chi^2_2 = 15.97$, $P < 0.001$; Figure 3).

Similarly, in experiment 2, the nesting resource the male had occupied in the field ($\chi^2_3 = 3.412$, $P = 0.33$) and male total length ($\chi^2_1 = 2.663$, $P = 0.10$) did not significantly affect the time it took for him to initiate nest building. Males took longer to begin building their nests when the males were offered small and medium sized nesting resources than when a large resource option was available ($\chi^2_2 = 19.61$, $P < 0.001$; Figure 4). Nest building was also initiated quicker when the male had two arched nesting resources to choose from than when flat nesting resources were offered ($\chi^2_1 = 20.83$, $P < 0.001$; Figure 5).

Extent of nest elaboration in the laboratory

When males chose to build a nest using an arched nesting resource in experiment 1 ($N = 83$ replicates), the size of the nesting resource had an effect on the amount of sand the male piled on the resource (Linear model, $F_{2,76} = 21.01$, $P < 0.001$), with more sand being piled on larger nests. By contrast, the type of the resource occupied in the field ($F_{3,76} = 1.660$, $P = 0.18$) and male total length ($F_{1,76} = 0.0004$, $P = 0.98$) did not have an effect. When a flat nesting resource was chosen in experiment 1 ($N = 16$), none of these factors had a significant effect on the amount of sand piled on the nest (medium vs. large nest: $F_{1,10} = 0.2208$, $P = 0.65$; nest type in the field: $F_{3,10} = 0.5660$, $P = 0.65$; male total length: $F_{1,10} = 0.2539$, $P = 0.63$).

Regarding the replicates of experiment 2 in which the focal male chose between two arched nesting resources ($N = 100$ choices made), the range of available nesting resource sizes had a significant effect on the amount of sand piled on the nest by the focal male (Linear model, $F_{2,91} = 4.971$, $P = 0.009$), with 5.48 ± 0.48 ($N = 33$), 12.34 ± 1.57 ($N = 33$) and 8.50 ± 1.06 ($N = 34$) g of sand piled on the nest ridge when choosing between S vs. M, S vs. L, and M vs. L resources, respectively. In addition, male size ($F_{1,91} = 13.33$, $P < 0.001$) and the type of nesting resource the male had occupied in the field ($F_{3,91} = 2.881$, $P = 0.040$) had an effect on the amount of sand piled on the nest ridge. In particular, large males piled more sand, as did males that had occupied a larger arched nesting resource in the field, with 8.55 ± 0.90 ($N = 24$), 7.02 ± 1.40 ($N = 26$), 11.5 ± 1.71 ($N = 25$) and 8.07 ± 1.36 ($N = 25$) g of sand piled on the nest ridge by males that had occupied a smaller arched, smaller flat, larger arched and larger flat resource, respectively. When focal males of experiment 2 chose between two flat nesting resources ($N = 99$ choices made), the range of available nesting resources ($F_{2,92} = 0.6217$, $P = 0.54$) and the type of nesting resource the male had occupied in the field ($F_{3,92} = 0.2955$, $P = 0.83$) did not have a significant effect on the amount of sand piled on the nest. However, the amount of sand piled on a male's nest was positively associated with his total length ($F_{1,92} = 5.047$, $P = 0.027$).

DISCUSSION

We found a relationship between nesting resource type and male body size in the field. In particular, the largest sand goby males were occupying nesting resources that were larger (of the two size categories) and arched (rather than flat). This relationship between resource type and male phenotype is likely to result from males of different sizes differing not only in their nesting resource preferences (Kvarnemo, 1995; Lehtonen et al., 2016) but also their resource holding potential (Lindström & Pampoulie, 2005), with a male of a certain size more likely to be replaced by a larger rival when it is occupying a resource type that is under more intense competition. This is consistent with findings reported in other taxa, including other species of nest-building fish, in which male resource holding potential correlates positively with the value of the males' resource (e.g. Takahashi, Kohda, & Yanagisawa, 2001; Kelly, 2008).

Regarding resource choice and nest-related behaviours under laboratory conditions, one of our main goals was to investigate whether the choices and nesting behaviours of sand goby males are related to resource size and type, male phenotype, or the male's prior experience in occupying a nesting resource of a certain type in the field. For nesting resource size, we found that the probability of the larger option being chosen in a laboratory setting increased with male body size. Similar findings have been reported in multiple species of nest-building fish (Bisazza, Marconato, & Marin, 1989; Takahashi et al., 2001; Uglem & Rosenqvist, 2002), including the sand goby (Kvarnemo, 1995; Japoshvili et al., 2012; Lehtonen et al., 2016). Such size-assortative choice is most probably due to large males being better able to meet the energetic and ecological demands of owning a large nesting resource. These demands are associated with, for instance, covering the resource with more sand, circulating larger volumes of water when aerating eggs in the nest, or defending the nest and eggs against usurpation, parasitic egg fertilisations or would-be egg predators (Kvarnemo, 1995; Lindström & Pampoulie, 2005; Olsson, Kvarnemo, & Svensson, 2009; Lehtonen et al., 2018). In addition, sand goby females are known to prefer males whose body size matches with nesting resource size (Lehtonen et al., 2007). In the current study, a male's eagerness to initiate nest building was not associated with his body size. However, male size was positively associated with the level of nest elaboration (i.e. the amount of sand piled on top of the nest) when males were given the opportunity to choose between two resources of different sizes (but of the same architecture; experiment 2) but not when males were given the choice between two nesting resources differing in architecture but not size (experiment 1). Similarly, while some previous studies have found a positive correlation between male size and the extent of nest elaboration (Lehtonen et al., 2016), others have not (Svensson & Kvarnemo, 2005). Earlier findings also suggest that the association between male size and the level of nest elaboration may differ depending on ecological factors, such as water clarity (Lehtonen, Lindström, & Wong, 2015). Hence, together with these earlier findings, the current results suggest that the importance of male body size in nest-related behaviours is context-dependent. More generally, small and large individuals may adopt different strategies when trying to maximise their reproductive payoffs (Gross, 1996; Blanckenhorn, 2000).

Nest architecture also matters: males more often chose an arched nesting resource than a flat resource of the same surface area. Interestingly, the popularity of the two resource types depended on nesting resource, but not male, size, with the flat option becoming more attractive with increasing resource size. We consider three mutually non-exclusive, ecologically relevant hypotheses for why sand goby males prefer arched resources. First, because the rim of an arched nesting resource extends higher above the substrate (Figure 1), it may be more conspicuous (before being covered with sand) and act as a stronger stimulus to the male than a flat resource. However, it is important to point out that a potential argument against this hypothesis is the finding that the popularity of the arched option decreased with resource size. A second potential reason why males prefer arched resources is that the arched shape may allow males to expend less time and energy in the initial phases of nest building. Third, as a marine species, sand gobies have evolved with access to arched mussel shells as nesting resources, whereas the use of flat stones in nesting is probably rare for populations of marine gobies, such as the sand goby, living outside the brackish Baltic Sea.

Notably, our conclusions were the same independent of whether we assessed popularity of an option as an actual choice (binary choice scenario) or as the time it took for a male to initiate nest-building (no-choice scenario). Regarding the latter, when two nesting resources of the same type were offered, males initiated nest building quicker when the two resources were arched compared to when they were flat. Similarly, males not only chose the larger nesting resource of the two more often, but it also took less time for the focal male to initiate nest building (in experiment 2) when at least one large nesting resource was available. Moreover, males also took less time to initiate nest building (in experiment 1) when offered choices between larger nesting resources of the same size (i.e. they were quicker under M vs. M and L vs. L scenarios than under S vs. S). Hence, the results suggest that, with regard to nesting behaviour, binary choice and no-choice scenarios yielded consistent results. Thus, our findings highlight the utility of both methods in the study of choice decisions (see also e.g. Kacelnik & Marsh, 2002; Dougherty & Shuker, 2015).

We found limited evidence that the type of nesting resource occupied in the field affects subsequent nesting behaviour in the laboratory. In particular, prior nesting resource experience did not affect resource choice or eagerness to initiate nest building. We cannot rule out the possibility that the result was driven by the level of competition among males being different under laboratory and field conditions. In the field, each male claimed a nesting resource under a competitive situation and in the absence of a different resource option in close proximity, whereas in the laboratory, each male was alone in the choice arena. Nevertheless, when males chose between two arched nesting resources in the laboratory, the ones that had occupied a larger arched nesting resource in the field piled more sand on their nests (even when accounting for body size) than those that had occupied other types of nesting resources. In a number of species, prior experiences have been found to affect key behaviours, such as aggression and mate choice (Rosenqvist & Houde, 1997; Hsu, Earley, & Wolf, 2006). Our results suggest that this may also be the case in the context of nest elaboration in sand gobies. It is also conceivable that, independent of prior experiences, males that were able to invest more in nest elaboration (by displacing, and piling up, larger amounts of sand) were also more likely than other males to occupy a larger arched nesting resource in the field.

In conclusion, this study highlights the important relationship between male phenotype and nesting resource choice. Interestingly, the importance of male size was found to be context-dependent, varying with respect to the available resources and the specific nesting behaviour being assessed. Indeed, besides uncovering the role of nest architecture, our findings provide methodological insights relevant to studies of resource choice and nesting behaviours, with both no-choice and binary resource choice scenarios yielding consistent results regarding male preferences for resource size and architecture. This was underscored, for instance, by males not only choosing an arched over a flat nesting resource when both types were available, but also initiating nest building quicker when they had access to an arched resource. Overall, our findings show how key reproductive behaviours, resource choice and nesting behaviour, can be affected by both the attributes of the nest-builder (body size) and the resource (size and architecture).

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Figures captions

Figure 1. Diagram of the two nesting resource architecture types used in this study, arched and flat.

Figure 2. Mean (±SE) total length of males occupying different types of nesting resources in the field. Resource types without a letter in common are significantly different (Tukey HSD test, $\alpha = 0.05$).

Figure 3. Percentage of replicates in which the focal male had not initiated nest building (laboratory experiment 1, progress during the first 30 h of 48 h is shown). Sample sizes were 45, 34, 33 for replicates with small, medium and large nesting resources (either arched or flat), respectively.

Figure 4. Percentage of nests not built (per hour) in replicates with different nesting resource size options. Replicates of laboratory experiment 2 with arched and flat resources are combined. The sample sizes are 110, 81, 82 for the combinations of small & medium, small & large and medium & larger nesting resources, respectively.

Figure 5. Progress of nest building (% of nests not built) in laboratory experiment 2 with regard to the two types of nest architecture, with different size choice options combined. Sample sizes were 112, and 158 for arched and flat nesting resources, respectively.

Figure 1

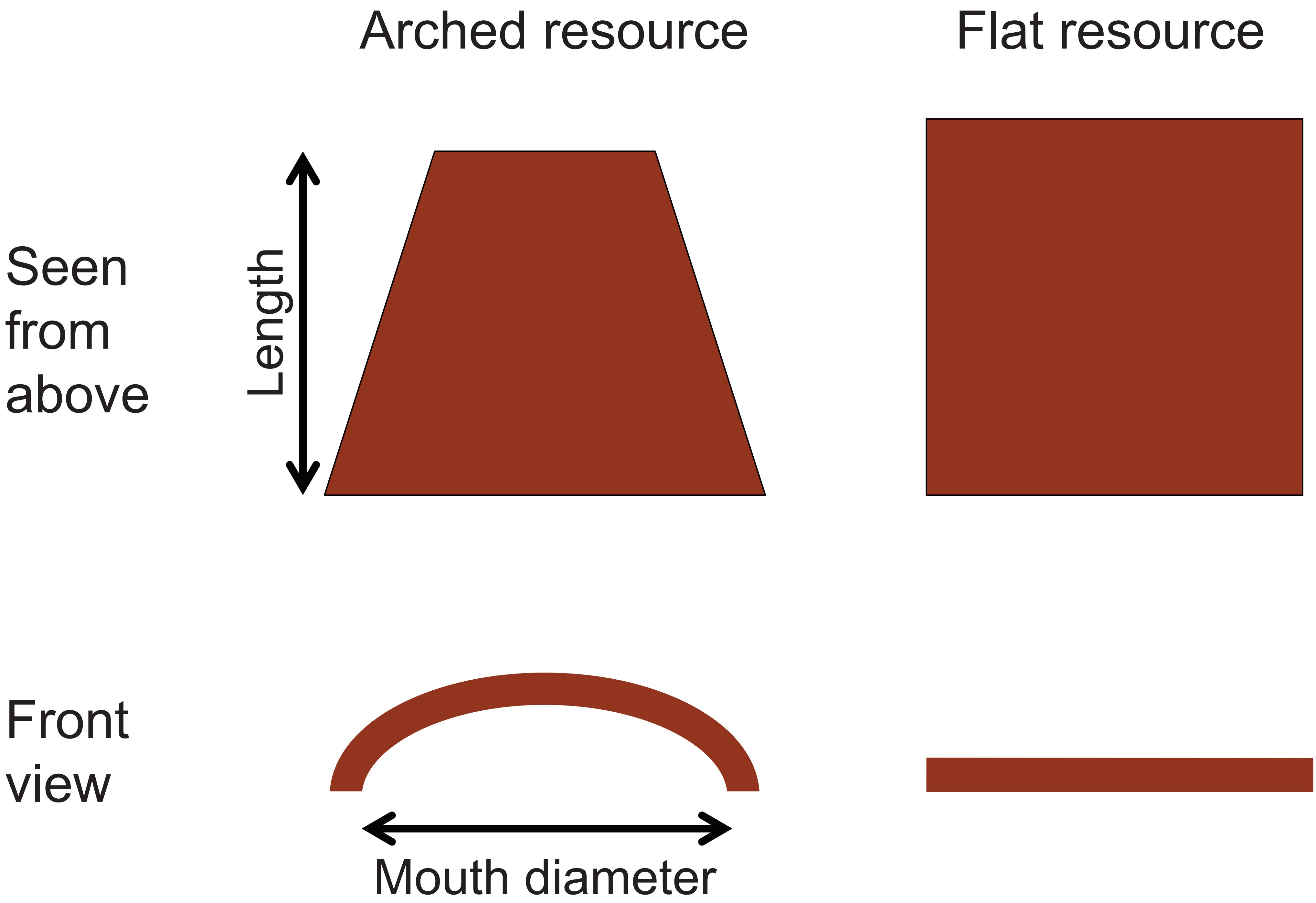

Arched resource
Flat resource
Seen from above
Length
Front view
Mouth diameter

Figure 2

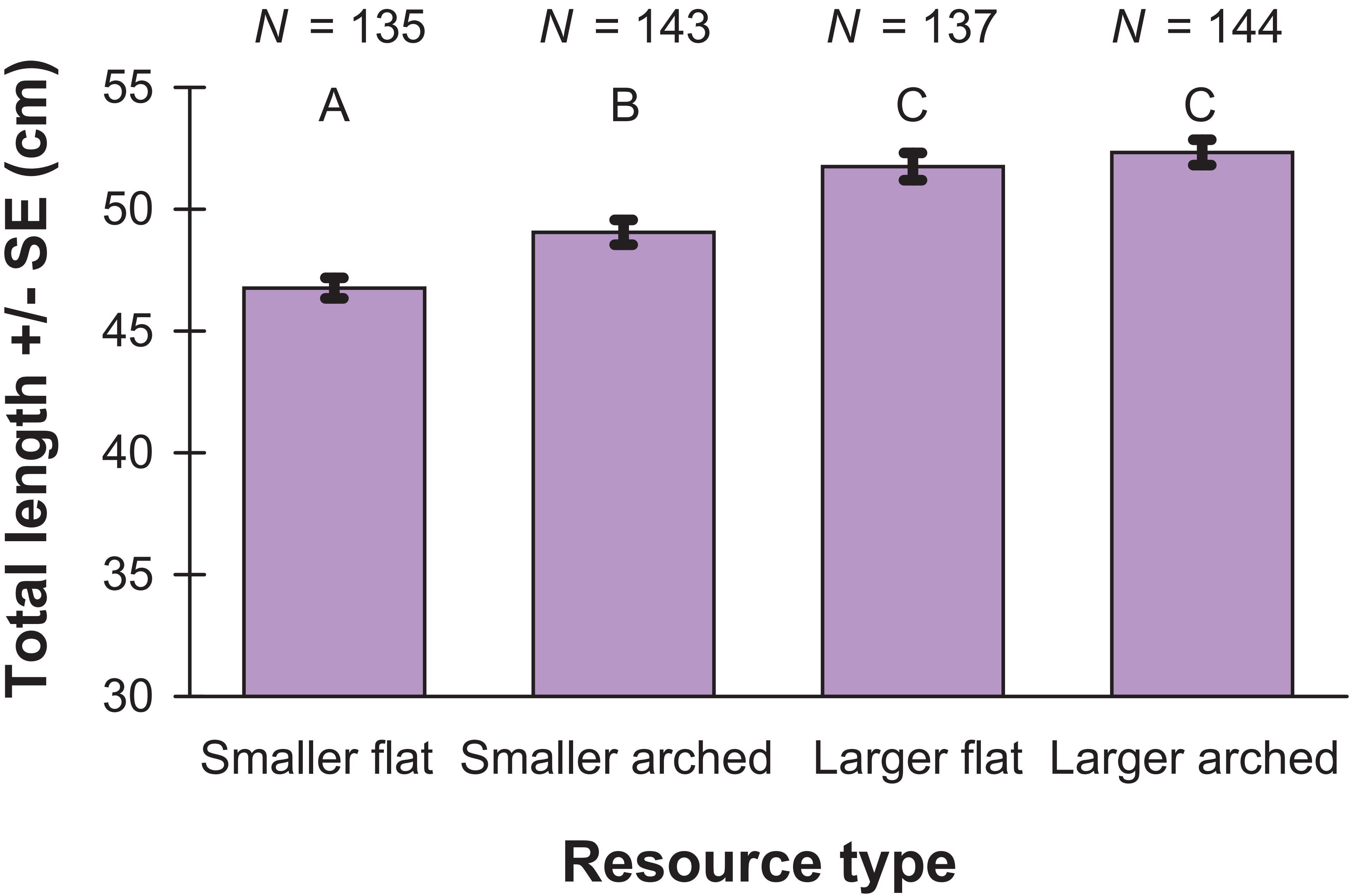
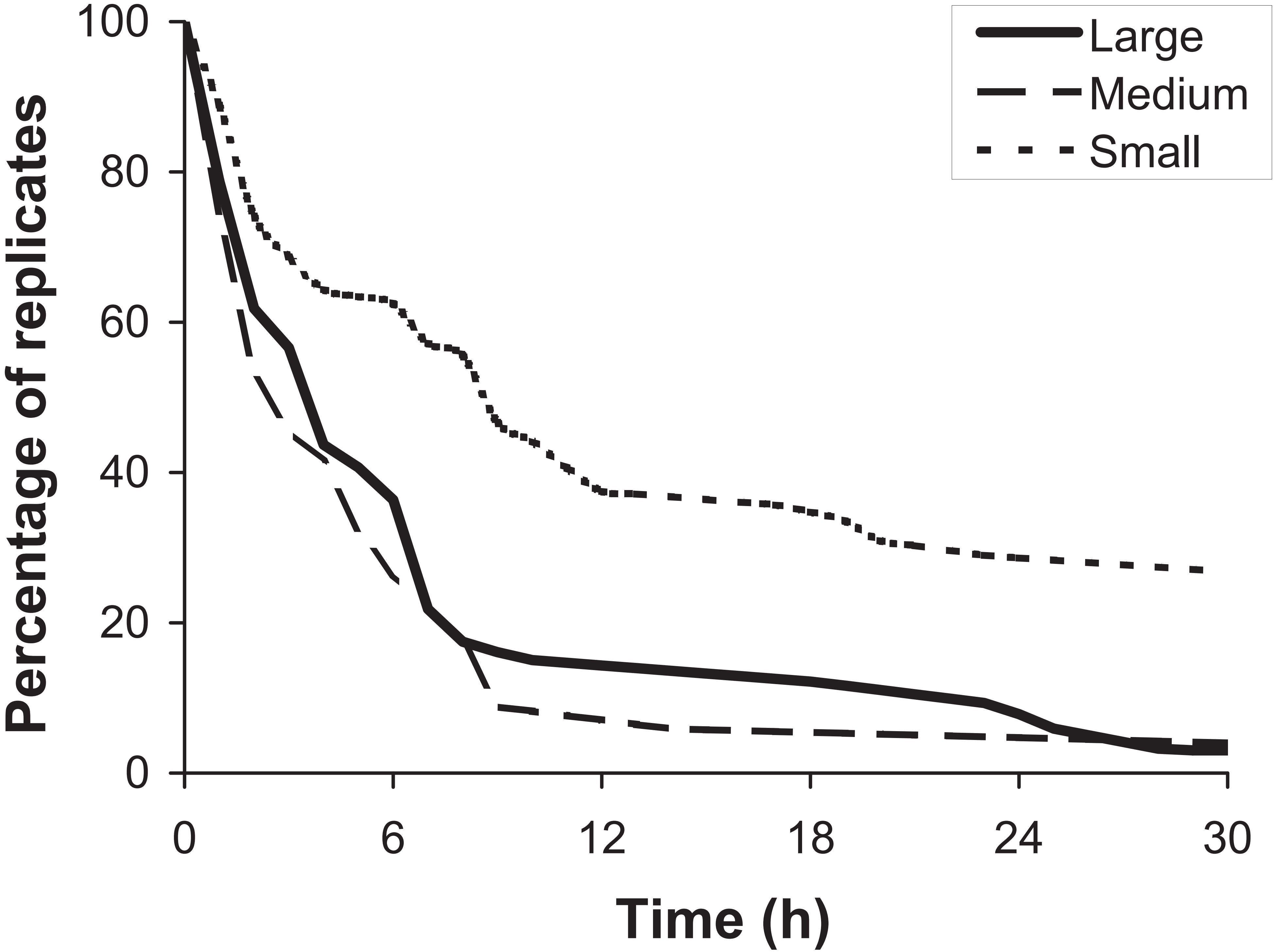

N = 135
N = 143
N = 137
N = 144
A
B
C
C
Total length +/- SE (cm)
55
50
45
40
35
30
Smaller flat
Smaller arched
Larger flat
Larger arched
Resource type

Figure 3



Large
Medium
Small
Percentage of replicates
100
80
60
40
20
0
0
6
12
18
24
30
Time (h)

Figure 4

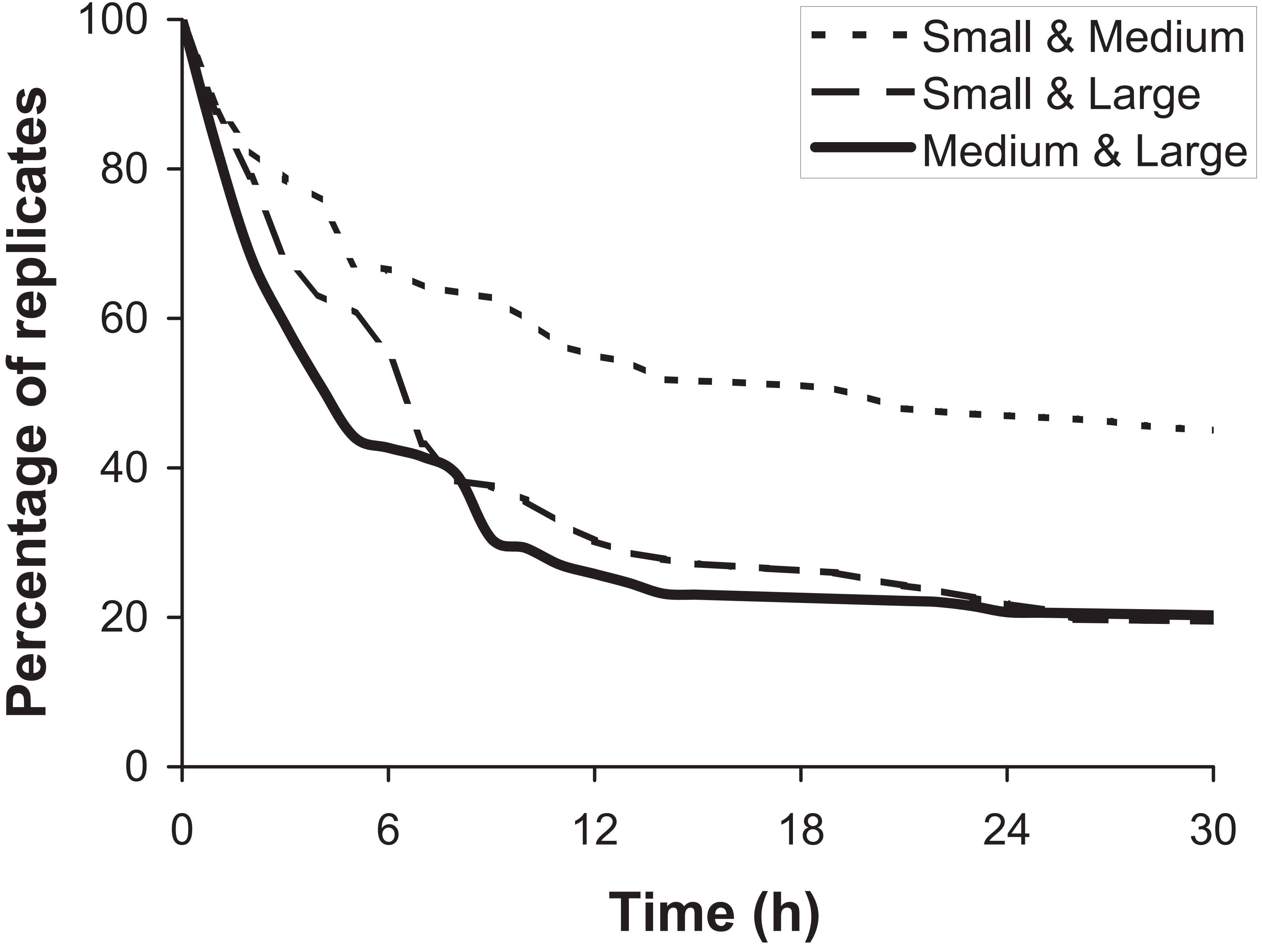
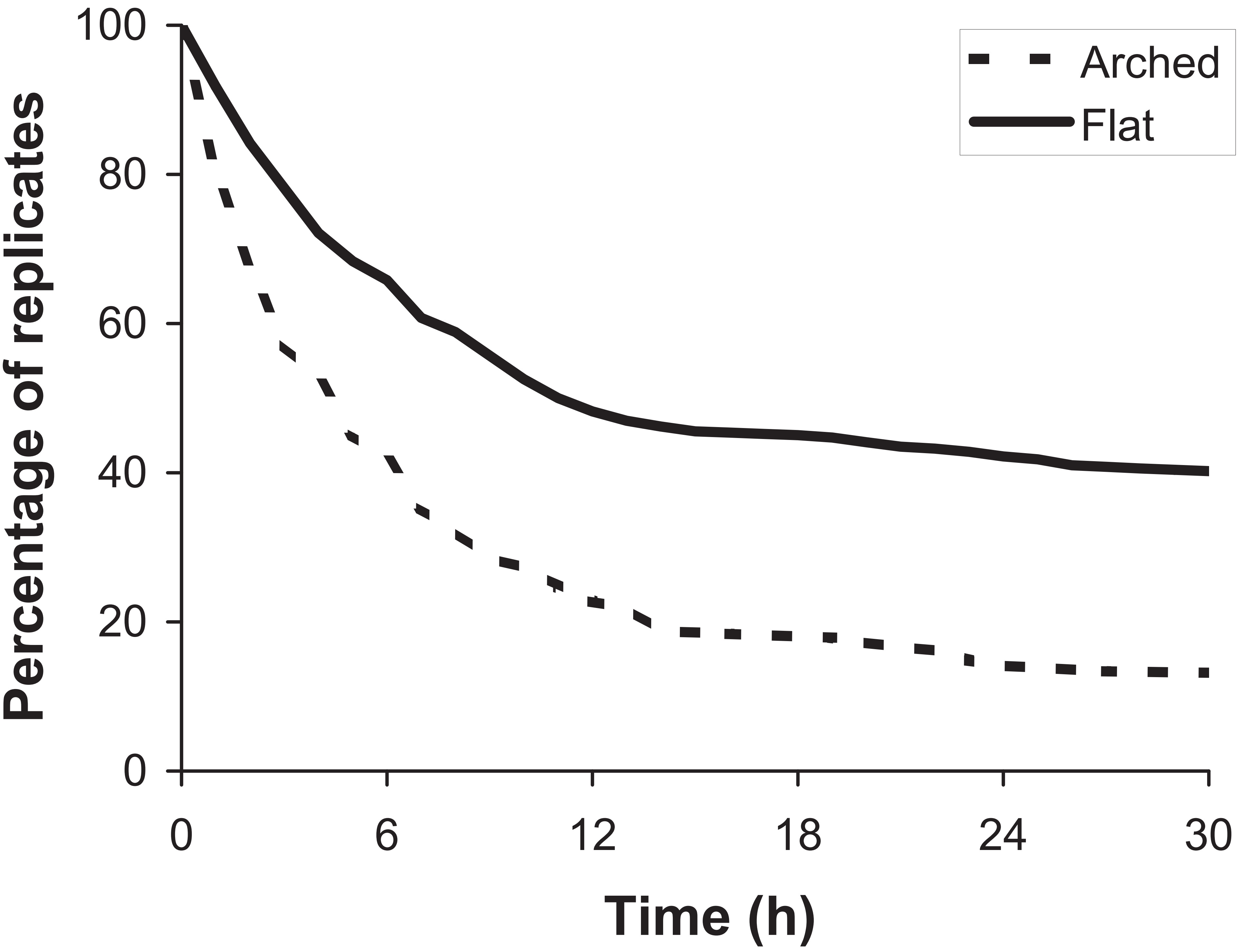

Small & Medium
Small & Large
Medium & Large
Percentage of replicates
100
80
60
40
20
0
0
6
12
18
24
30
Time (h)

Figure 5


Percentage of replicates
100
80
60
40
20
0
0
6
12
18
24
30
Time (h)
Arched
Flat