Testis function of invasive male Pallas's squirrels (*Callosciurus erythraeus*) does not change seasonally in a Japanese temperate zone

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Abstract

The invasive Pallas's squirrel (*Callosciurus erythraeus*) is now established in Japan, and it is abundantly fertile, which results in a constant increase in the population size and distribution. The reproductive characteristic of this invasive squirrel is continuous breeding, although native squirrels demonstrate seasonal breeding. The reproductive seasonality of this invasive squirrel seems to be less affected by seasonal changes in food availability, which regulate reproductive seasonality in native species. In the present study, we postulated that testis size index, an indicator of male reproductive function, does not differ between seasons with high (June–November) and low (December–May) food availability, and that it positively correlates with the body condition index throughout the year due to energy allocation to the testis. We therefore assessed seasonal changes in testis size index in 284 euthanized Pallas's squirrels, and seasonal associations between testis size and body condition indices. Testis size index did not differ between seasons. Furthermore, testis size and body condition were positively and significantly associated. Our findings suggest that whereas male reproductive effort in this invasive squirrel consumes energy like native species, testis function maintains stable the whole year. This could be a characteristic of continuous breeding that might be associated with the robust reproductive activity of this squirrel.

Keywords: invasive species, testis, food availability, temperate zone, energy allocation.

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Short title

Testis function in invasive Pallas's squirrels

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Introduction

Invasive species are major problems because they are key drivers of biodiversity loss through interspecific competition, predation, and the transmission of infectious diseases (Daszak et al. 2000; García-Berthou and Padilla 2004; Gurnell et al. 2004; Cox and Lima 2006). In invasive species, high reproductive outputs may advantageously promote the population growth (mammals, Capellini et al. 2015; reptiles and amphibians, Allen et al. 2017). Furthermore, some invasive mammals develop different reproductive traits in new environments (Proaktor et al. 2008). For example, the invasive grey squirrels in Italy have higher litter size than that of native areas (Maranesi et al. 2020). Thus, understanding the reproductive traits of invasive mammals is important.

The Pallas's squirrel (*Callosciurus erythraeus*) is an invasive species in Japan, Hong Kong, Argentina, France and the Netherlands (Bertolino and Lurz 2013), and it is native to East and Southeast Asian countries (Lurz et al. 2013). In Japan, this squirrel originated from Taiwan has become established since its initial arrival during 1935 despite continuous nuisance control (Oshida et al. 2007; Bertolino and Lurz 2013). This invasive squirrel is abundantly fertile, which results in a concomitant increase in the population and distribution (Tamura 2004).

One of the difference in the reproductive characteristics between native and invasive squirrels is a reproductive seasonality. Japanese native squirrels demonstrate seasonal breeding (Kawamichi 2010). For example, mating season of Japanese squirrel (*Sciurus lis*) is only from early spring to autumn (Sasaki et al. 2005; Kataoka et al. 2010). The reproductive seasonality of native species is regulated by the seasonal changes in food availability, to maximize reproductive success (Bronson 2009). On the other hand, the Pallas squirrel in Japan is a continuous breeder, although the pregnancy rate decreases in winter. However, the food availability of this invasive squirrel in Japan also changes seasonally; it is abundant between June and November than at any other time of the year (Tamura et al. 1989). Thus, the reproductive activity in this invasive squirrel seems less susceptible to the seasonal change of food availability, although this relationship is still unrevealed.

In the present study, we aimed to assess if testis function of this invasive squirrel may be independent of the seasonal change of food availability. Testis growth is the result of activated spermatogenesis (Klonish et al. 2005), and testis size is associated with reproductive success in some mammals (Preston et al. 2003). Thus, testis size is an indicator of male reproductive function (Schulte-Hostedde et al. 2005; Sarasa et al. 2010; Liao et al. 2013; Sugianto et al. 2018). Testis size in some native species is regressed when food availability is low (Gockel and Ruf 2001), and it is also true for Pallas's squirrel in the native population (Yo et al. 1992). However, if the male reproductive activity in this invasive squirrel is less susceptible to food availability, we speculated that the testis size of the males would be stable in a whole year. Moreover, testis size positively correlates with the body condition index (BCI) (Schulte-Hostedde et al. 2005; Sarasa et al. 2010; Liao et al. 2013). This result is interpreted as a result of energy consuming reproductive activity (Schulte-Hostedde et al. 2005; Sarasa et al. 2010; Liao et al. 2013). The positive correlation in mole-shrews is only found around the breeding season, and not in winter (Liao et al. 2013). Liao et al. (2013) notified that the non-positive correlation in winter might be due to the fact that males do not invest their energy in testis in this season. So, if this invasive squirrel maintains testis function in a low food availability season in addition to high food availability season, we expected that this squirrel would demonstrate the positive correlation in all seasons.

In this work, we explored seasonal changes in testis size in Pallas's squirrels harvested as a nuisance control in Japan, and the seasonal relationship between BCI and testis size. Here, we predicted that testis size would not differ between seasons with high (June–November) and low (December–May) food availability, and that testis size would positively correlate with BCI throughout the year.

Materials and methods

Study area and animals

All experimental procedures in the present study followed the relevant laws and Guidelines Concerning Animal Experimentation of the Nippon Veterinary and Life Science University and the Mammal Society of Japan. This Animal Experiment Committee at the Nippon Veterinary and Life Science University approved the study (No. 2020S-2).

Between October 2017 and November 2020, 284 Pallas's squirrels were euthanized for eradication control in 32 areas (35°21′–35°44′ N, 139°58′–139°74′ E; elevation 24–176 m) within the adjacent cities of Yokohama and Yokosuka in Kanagawa Prefecture, central Honshu, Japan (Table 1). Mean daily temperatures range from 6.1°C in January, to 27.0°C in August, and the annual precipitation is 1,730.8 mm in the study area. We classified the seasons according to food availability as high (June–November) and low (December–May) as described by Tamura et al. (1989). Although our research areas are different from those of Tamura et al (1989), research areas are close (Kamakura city, Tamura's research area, is adjacent to Yokohama), and vegetation of both areas is similar. It is consistent with the similarity of main food diets of this squirrel between them (Ozaki et al. 1986; Takahata et al. 2020). For this reason, we and city officer, responsible for this squirrel, observed the same seasonal trends of food availability in our study area, although we do not have a quantitative data.

The body weight (> 320 g), hair color (pigmentation of scrotum), and testicular palpation *via* the scrotum were assessed in adult males as described by Tamura and Terauchi (1994), Tang and Alexander (1979) and Yo et al. (1992). The length and weight of the squirrels were measured by the same individual. The length and width of both lightly squeezed testes were measured in each squirrel using a digital caliper (Mitutoyo, Kanagawa, Japan) (Figure 1). The geometric mean of the measurements was taken as the testis size index (TSI), and this positively correlated with testis mass (Supplemental data, $n = 43$, $P = 0.01$), indicating the validity of the methodology. The BCI was determined using residuals from the regression of body size on body mass (Schulte-Hostedde et al. 2005).

Statistical analysis

We pooled all data regardless of the source area (Yokohama and Yokosuka) and year (2017-2020) of sample collection, and separated them into seasons with high and low food availability. To assess the factors associated with TSI, we used a liner model with TSI as a response variable, and season, BCI and an interaction of BCI×season as explanatory variables. The normality was confirmed visually with quantile–quantile plots. We added the interaction of BCI×season, to assess if the relationship between TSI and BCI differs between seasons. Values in all statistical analyses with $P < 0.05$ were considered significant different. Data were statistically analyzed using R version 3.6.1 (R core team 2019).

Results

Tables 1 and 2 show details of the 284 squirrels. The TSI did not significantly differ between high and low food availability seasons (Season effect 0.11 ± 0.17, $p = 0.51$; Figure 2), and there was no BCI by season interaction (estimate -0.96 ± 6.20, $p = 0.88$). The TSI during the high food availability season varies from 17.48 to 25.15 (mean ± standard error 21.7±1.6), and that during the low food availability season varies from 17.30 to 24.90 (mean ± standard error 21.58 ± 1.26). The TSI and BCI were positively and significantly associated (estimate 15.4 ± 4.5, $t = 3.38$, $p < 0.05$; Figure 3).

Discussion

The TSI did not differ between high and low food availability seasons, suggesting that male reproductive activity may be stable even in low food availability season. Because the TSI in the original population of Pallas's squirrels decreases in winter (Yo et al. 1992), this invasive males may have longer reproductive activity compared with the original population. Although pregnancy rates decrease in the Japanese population especially in winter, 24% of females still become pregnant (Tamura et al. 1999). Thus, male squirrels might need to maintain testis function prepared for the potential mating opportunity and male-male competition even in the low food availability season (Tamura 1995). Our results also indicate that the cause of the decreased pregnancy rates of the Japanese population when food availability low is not associated with male factors.

To our knowledge, this is the first study to determine a relationship between the TSI and BCI in invasive mammals. The TSI and BCI were positively and significantly associated, and the interaction of BCI×season was not significantly associated with the TSI. This finding suggested that male reproductive effort consumes energy like native seasonal breeders (Saraza et al. 2010; Liao et al. 2013), and that energy allocation to the testes in this invasive squirrel does not differ between seasons. This is consistent with our findings that TSI was not seasonally affected. Thus, maintaining testis function throughout the year is considered a characteristic of continuous breeding, and it might be involved with the robust reproductive activity of this invasive squirrel.

Our study had two limitations. We do not have data regarding the relationship between testis size and spermatogenesis, and it is difficult to identify the TSI level, which is biologically meaningful. To verify our findings, future studies should check the spermatogenesis by histological analysis as well as measuring the testis size. Furthermore, snapshot sampling from euthanized samples was conducted in this work. Thus, to check the direct relationship between the energetic condition and testis functions, the same individuals should be monitored repeatedly in each season.

In summary, our results suggest that testis function of this invasive squirrel is activated throughout the year. Moreover, our results may support the possibility that the reproductive activity in this invasive squirrel may be less susceptible to the seasonal change of food availability. Further study is needed to clarify the physiological mechanism(s) through which Pallas's squirrels maintain reproductive activity throughout the year.

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Table 1. Numbers of male squirrels collected between October 2017 and November 2020.

	All (n)	High food availability						Low food availability					
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Yokohama	65	1	8	25	6	8	6	5	2	1	3	0	0
Yokosuka	219	28	31	0	0	0	30	39	23	27	25	3	13
All	284	143						141					

Table 2. Characteristics of Pallas squirrels during low and high food availability.

	High food availability (n = 143)	Low food availability (n = 141)
Length (cm)	239.48 ± 10.44	237.91 ± 8.38
Weight (g)	349.02 ± 23.18	347.16 ± 23.41
Body condition index	0.00 ± 0.03	0.00 ± 0.03
Testis size index	21.70 ± 1.64	21.58 ± 1.26

Data are shown as means ± standard deviation.

Figure legends

Figure 1. Determination of testis size index. Dorsal view of scrotum in male Pallas's squirrels. Length and width of testes were measured by slight squeezing.

Figure 2. Seasonal changes in testis size index in Pallas's squirrels. Values are expressed as means ± SD. Short lines show individual values. Grey and white bars, high (June–November) and low (December–May) food availability, respectively.

Figure 3. Relationship between testis size and body condition indexes in Pallas's squirrels. Grey and white squares, high (June–November) and low (December–May) food availability, respectively. Body condition index positively and significantly correlates with testis size index ($P < 0.05$).

Fig. 1.

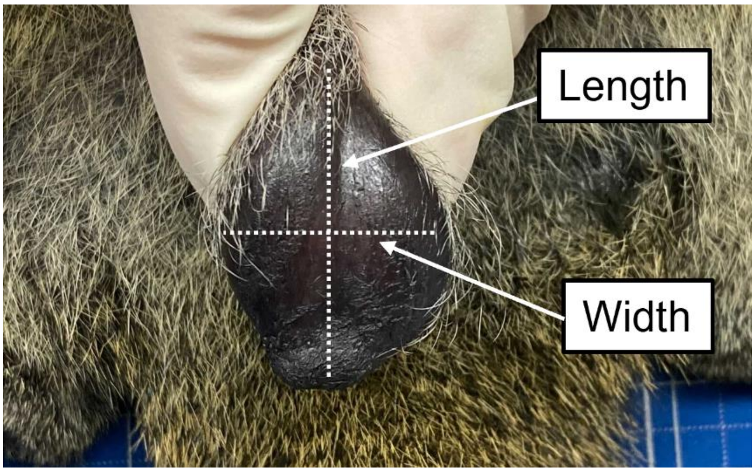

Length
Width

Fig. 2.

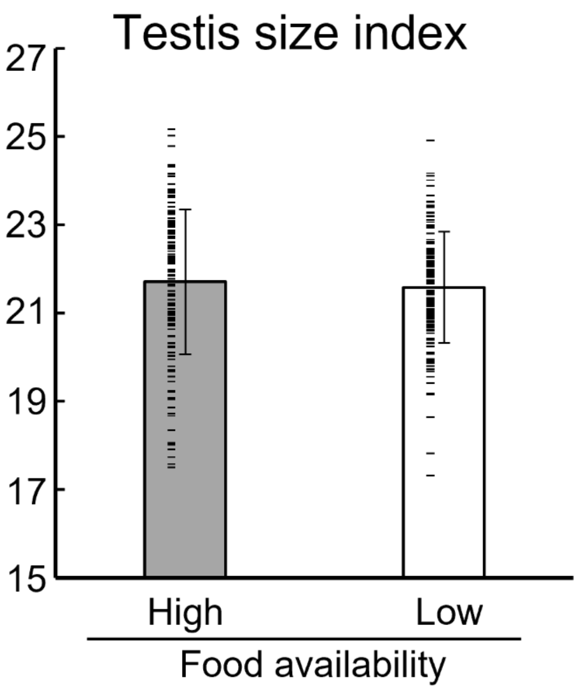
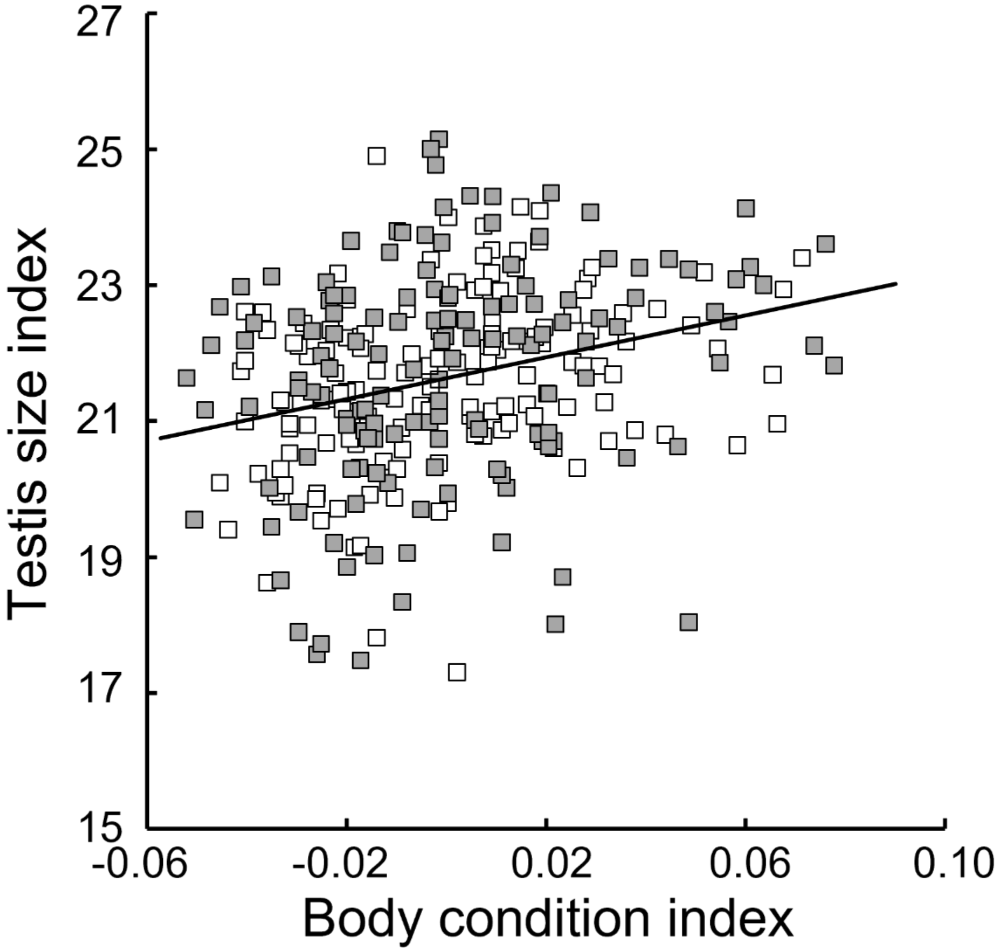

Testis size index
27
25
23
21
19
17
15
High
Low
Food availability

Fig. 3.


27
25
23
21
19
17
15
-0.06
-0.02
0.02
0.06
0.10
Testis size index
Body condition index