Methods of evaluating the spermatogenic ability of male raccoons
(Procyon lotor)

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Abstract. Feral raccoons (Procyon lotor) have been growing in number in Japan, and they are becoming a problematic invasive species. Consequently, they are commonly captured and killed in pest control programs. For effective population control of feral raccoons, it is necessary to understand their reproductive physiology and ecology. Although the reproductive traits of female raccoons are well known, those of the males are not well understood because specialized knowledge and facilities are required to study them. In this study, we first used a simple evaluation method to assess spermatogenesis and presence of spermatozoa in the tail of the epididymis of feral male raccoons by histologically examining the testis and epididymis. We then evaluated the possibility of using 7 variables—body weight, body length, body mass index, testicular weight, epididymal weight, testicular size and gonadosomatic index (GSI)—to estimate spermatogenesis and presence of spermatozoa in the tail of the epididymis. GSI and body weight were chosen as criteria for spermatogenesis, and GSI was chosen as the criterion for presence of spermatozoa in the tail of the epididymis. Because GSI is calculated from body weight and testicular weight, this model should be able to be used to estimate the reproductive state of male raccoons regardless of season and age when just these two parameters are known. In this study, GSI was demonstrated to be an index of reproductive state in male raccoons. To our knowledge, this is the first report of such a use for GSI in a member of the Carnivora.

Key words: Gonadosomatic index, Procyon lotor, Raccoon, Reproduction, Spermatogenesis

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The raccoon (Procyon lotor), an omnivorous medium-sized member of the Carnivora, has a wide native distribution ranging from the southern part of Canada to Central America. In Japan, however, feral raccoons have been growing in number to such an extent that they are becoming a problematic invasive species and therefore are being commonly captured. Feralization of raccoons in Kanagawa Prefecture, Japan, was first recognized in Kamakura [1], where over a thousand feral raccoons are now captured every year. Although the density of feral raccoons has decreased in some areas of Kanagawa, their distribution has expanded. In order to decrease the population of feral raccoons through an eradication program, it is essential to elucidate their reproductive traits, which are strongly related to their population dynamics.

The reproductive traits of raccoons have been studied mainly in North America. Although the mating season of raccoons is generally in the spring in North America [2], regional differences in reproductive season have been confirmed. The mating seasons of raccoons in southern Texas are in spring and autumn [3]. Although raccoons may reproduce throughout the year in areas with warm climates such as South Carolina, Florida and Alabama [2], the breeding season of female raccoons may be delayed due to delayed estrus in areas at higher latitudes with severe winters, such as Illinois [4]. In addition, geographical variation in litter sizes of raccoons has been observed; that is, litter size tends to be larger in northern latitudes [5]. Furthermore, the sexual maturity of raccoons is delayed in areas with severe winters, such as Illinois and North Dakota, because of a delayed delivery season [4, 6]. There are regional differences in breeding season even between comparably northern areas in North America; in Illinois, yearling male raccoons mate in their first breeding season in summer after adult male raccoons mate in spring [4], but in North Dakota, yearling male raccoons mate first [6]. As mentioned above, this is because the raccoon dynamically changes its reproductive parameters according to habitat status.

The parturition periods of raccoons have also been estimated for several areas in Japan, and they also indicated regional differences. The parturition period of raccoons in Hokkaido has one peak in spring, the same as in North America [7]. The parturition period of the Kamakura raccoon population, however, is thought to be from February to October and shows a bimodal distribution, with parturition gradually increasing until May, dropping in June and peaking again to a lesser extent between July and September; this pattern is also observed for populations in southern Texas [8]. In Hokkaido, it has been proposed that feral raccoons could be effectively captured by shifting capture efforts to spring, which is when the animals are caring for their offspring. However, in Kanagawa, capture efforts cannot be concentrated because of the raccoons’ long parturition period. Thus, it has instead been proposed that capture throughout the year and monitoring and analyzing captive raccoons are more important than paying attention to the season for capture. Therefore, local management plans should be formulated with an understanding...
of local raccoon reproductive traits in mind.

The reproductive traits of female raccoons have only been studied by macroscopically observing nipples and the uterus to provide useful information including litter size [4–6, 8, 9], pregnancy rate [4–6, 8, 9] and reproductive season [3–6, 8, 9]. Hence, such observation methods of female raccoons have allowed for direct evaluation of reproductivity for use in management plans for female raccoons. In male raccoons, although a few studies have performed macroscopic observations, such as of baculat growth [10–13] and seasonal testis size [14–16], spermatogenic ability has never been evaluated without using histological methods [14–16]. At present, male spermatogenic ability is not discussed in management plans, unlike female reproductivity, because the histological method to evaluate it is both more difficult and more expensive than macroscopic observation.

We histologically examined the testis and the tail of the epididymis for two reasons. First, male raccoons are reported to retain the ability to ejaculate fertile sperm for up to 4 weeks after castration [4]. Therefore, fertile sperm may exist in the tail of the epididymis even if spermatogenesis is not recognized, such as when testicular function is reduced in summer. Second, spermatozoa may not have matured in the tail of the epididymis even if spermatogenesis is not recognized. Therefore, male raccoons having reproductive capability and those capable of spermatogenesis are not necessarily equivalent. In this study, the spermatogenesis of feral male raccoons was evaluated by histologically examining the testis and the tail of the epididymis to establish a simple method of estimating spermatogenesis from external measurements.

Materials and Methods

Animals

All permanent teeth of raccoons erupt at 5 months of age [9, 17], and male raccoons are considered to reach sexual maturity at about 1 year old [9, 16, 18]; thus, male raccoons without eruption of all permanent teeth were assumed to be sexually immature. Carcasses of feral male raccoons in which all the permanent teeth had erupted (n = 182) were collected through the pest control activities in Kamakura, Kanagawa (N35°, E139°), Japan, from March 2005 to September 2008. All 182 animals were captured using box traps (Havahart Model 1089; Woodstream, PA, USA; or traps made by trappers) around houses invaded by raccoons. Raccoons were euthanized by trappers with CO2 gas, in accordance with the Guidelines for the Management of Invasive Alien Species of the Japan Veterinary Medical Association [19]. Raccoon carcasses were weighed, total length (TL) and length of tail vertebrae (LTV) were measured, and then the skinned head, both testes and both epididymides were removed. The testes were weighed, and the long diameter (LD), short diameter (SD) and thickness (TN) were measured; the epididymides were only weighed. Skinned heads were frozen at −25°C, and reproductive organs were fixed in 10% neutral buffered formalin for later examination. Subsequently, the skinned heads were boiled, and the canines of the maxilla were removed for histological age determination.

Age determination

The ages of the raccoons were determined by the number of cementum annuli in canines according to the method of Grau et al. [20]. Raccoons were classified into 4 age classes (AC) as follows: Class 1, age 0 years; Class 2, age 1 year; Class 3, age 2 years; and Class 4, age 3 years or older (Table 1).

Histology

The left testis and the tail of the epididymis of individual raccoons were dehydrated in an ethanol series, embedded in paraffin wax, sectioned at a thickness of 4 μm, and stained with periodic acid Schiff–hematoxylin (Fig. 1). Testicular samples were examined under a light microscope at ×100 magnification to evaluate spermatogenesis. Twenty seminiferous tubules from each sample were chosen randomly, and the absence of spermatozoa was considered to indicate a lack of spermatogenesis. The samples of the tail of the epididymis were examined under a light microscope at ×400 magnification to confirm the presence of spermatozoa. Twenty ducts from each samples of the tail of the epididymis were chosen randomly, and the absence of spermatozoa was considered to indicate a lack of spermatogenesis.

Study variables

General external measurements (7 variables) were determined from the raccoon carcasses: body weight (BW, g), body length (BL, mm), body mass index (BMI), testicular weight (TW, g), epididymal weight (EW, g), testicular size (TS, mm) and gonadosomatic index (GSI). We examined BW and BL, which was calculated according to the equation BL (mm) = TL (mm) – LTV (mm), to evaluate the spermatogenic ability of raccoons. BMI was calculated according to the equation BMI = BW (kg) / BL (m)2 and was used as an index of nutritional status because it was a suitable complementary index for assessing the relative body fat of raccoons [21]. TW and EW were taken as the means of the right and left organs. TS was taken as the mean of the right and left organs and was calculated according to the equation TS = [LD (mm) × SD (mm) × TN (mm)]. GSI, which is frequently used in fish research to monitor breeding activities, was calculated according to the equation GSI = gonad weight (g) / BW (g) × 102 [22–24]. In this study, TW was used as the gonad weight.

Statistical methods

Classification and regression trees (CART) [25] were chosen as statistical models. CART was fitted by binary recursive partitioning, whereby a data set was successively split into increasingly homogeneous subsets until it was infeasible to continue [26]. The advantages to using CART in this study included: the flexibility to handle a broad range of response types, including numeric, categorical, ratings and survival data; invariance to monotonic transformations of the explanatory variables; ease and robustness of construction; ease of interpretation; and the ability to handle missing values in both response and explanatory variables [27]. The Mann-Whitney U test, distributed using CART, was used for comparisons between groups. Statistical analyses were performed using the tree function of the R statistical package [28]. P < 0.05 was considered to indicate statistical significance.

Results

Histological analysis revealed that 84 raccoons had spermatogenesis and 76 had spermatozoa in the tail of the epididymis; these raccoons...
SPERMATOGENIC ABILITY OF MALE RACCOONS

Table 1. Numbers of feral male raccoons (in total and showing spermatogenesis) captured in pest control activities in Kamakura, Kanagawa, from March 2005 to September 2008, by month

| Age class | Month     | Total |
|-----------|-----------|-------|
|           | Jan  | Feb  | Mar  | Apr  | May  | Jun  | Jul  | Aug  | Sep  | Oct  | Nov  | Dec  | Total |
| Class 1   | 10 (5) | 5    | 13 (1) | 8    | 5    | 8    | 2    | 2 (1) | 7    | 9    | 10 (1) | 10 (2) | 89 (10) |
| Class 2   | 4 (4)  | 5 (5) | 6 (5) | 5 (5) | 4 (3) | 1 (1) | 6 (3) | 5 (2) | 4 (3) | 4 (3) | 2 (2) | 2 (2) | 48 (38) |
| Class 3   | 0      | 1 (1) | 4 (4) | 3 (3) | 4 (4) | 2 (2) | 3 (1) | 4 (2) | 0    | 1 (1) | 2 (2) | 0    | 24 (20) |
| Class 4   | 1 (1)  | 2 (2) | 1 (1) | 1 (1) | 5 (5) | 1 (1) | 1 (1) | 3 (2) | 3    | 1    | 1 (1) | 1 (1) | 21 (16) |
| Total     | 15 (10) | 13 (8) | 24 (11) | 17 (9) | 18 (12) | 12 (4) | 12 (5) | 14 (7) | 14 (3) | 15 (4) | 15 (6) | 13 (5) | 182 (84) |

The number of individuals with spermatogenesis is shown in parentheses. Raccoons were aged by the number of cementum annuli of canines and classified into 4 age classes as follows: Class 1, age 0 years; Class 2, age 1 year; Class 3, age 2 years; and Class 4, age 3 years or older.

Discussion

It has been reported that in North America [4] and Japan [14, 15], the greatest percentage of adult male raccoons are sexually inactive from July through October and that individual males had periods averaging 3–4 months in which they were incapable of breeding. In this study, class 3–4 raccoons were confirmed to exhibit no spermatogenesis in the period of July to October; however, in this period class 1–2 raccoons, which had likely just reached sexual maturity, did exhibit spermatogenesis. The parturition periods of

were found throughout the year (Table 2). However, in the class 3–4 raccoons, all members of which had presumably reached sexual maturity, the number of raccoons with spermatogenesis decreased from July to October, and no raccoons (n = 3) had spermatogenesis in September (Table 1). There were 8 raccoons with spermatogenesis that did not have spermatozoa in the tail of the epididymis, and there were no raccoons with spermatozoa in the tail of the epididymis but no spermatogenesis. All raccoons with spermatogenesis but not spermatozoa in the tail of the epididymis were less than 1 year old.

The model for spermatogenesis is shown in Fig. 2. According to this model, raccoons with a GSI value above 48.72 × 10⁻³ had spermatogenesis, and those with values below 34.40 × 10⁻³ did not. For raccoons with a GSI value between 34.40 × 10⁻³ and 48.72 × 10⁻³, those with a BW lighter than 5250 g had spermatogenesis, and those with a heavier BW did not. For raccoons with a GSI value between 34.40 × 10⁻³ and 48.72 × 10⁻³ (n = 18), there were significant differences between those with a BW lighter than 5250 g (n = 5) and those with a heavier BW (n = 13) in AC (U = 8.50, P = 0.01), BMI (U = 4.00, P < 0.01) and TW (U = 6.00, P < 0.01), indicating that raccoons with a BW lighter than 5250 g were younger, were more malnourished and had a lighter TW than those with a heavier BW. Misclassification occurred for 5 of the 182 raccoons (misclassification rate, 0.03), and there were no apparent commonalities.

The model for presence of spermatozoa in the tail of the epididymis is shown in Fig. 3. According to this model, raccoons with a GSI value higher than 48.72 × 10⁻³ had spermatozoa in the tail of the epididymis and those with a lower GSI value did not. Misclassification occurred for 4 of the 182 raccoons (misclassification rate, 0.02), and there were no apparent commonalities.

The number of races shown in parentheses. Raccoons were aged by the number of cementum annuli of canines and classified into 4 age classes as follows: Class 1, age 0 years; Class 2, age 1 year; Class 3, age 2 years; and Class 4, age 3 years or older.

Discussion

It has been reported that in North America [4] and Japan [14, 15], the greatest percentage of adult male raccoons are sexually inactive from July through October and that individual males had periods averaging 3–4 months in which they were incapable of breeding. In this study, class 3–4 raccoons were confirmed to exhibit no spermatogenesis in the period of July to October; however, in this period class 1–2 raccoons, which had likely just reached sexual maturity, did exhibit spermatogenesis. The parturition periods of
raccoons in Kamakura have a bimodal distribution from February to October [8], and our results suggest that the second peak in this distribution was due to the activity of male raccoons that had just reached sexual maturity in the latter half of the breeding season. This result is the same as that seen in Illinois [4].

In this study, GSI and BW were chosen as criteria of spermatogenesis, and GSI was chosen as the criterion for presence of spermatozoa in the tail of the epididymis. Because GSI is calculated from BW and TW, this suggests that spermatogenesis and presence of spermatozoa in the tail of the epididymis of male raccoons can be estimated by having sufficient knowledge of BW and TW. GSI is frequently used to monitor breeding activities in fish [22–24] and is also studied in reptiles such as lizards [29] and the red-sided garter snake (Thamnophis sirtalis parietalis) [30] and in mammals such as the red-bellied tree squirrel (Callosciurus erythraeus) [31], greater cane rat (Thryonomys swinderianus) [32] and rhesus macaque (Macaca mulatta) [33]. Cyclic changes in GSI and the relationship between GSI and spermatogenesis have been studied in red-bellied tree squirrels in particular, and a positive relationship between a GSI, corrected ten times (× 10), greater than 8.0 and sexual activity was reported for adults [31]. In contrast, in other members of the Carnivora, there have been numerical report of GSI (testes/body weight, as a percentage) [34, 35], but these values have not been used as an index of reproductive state because they are low; for example, the mean GSI of domestic cats (Felis catus) was reported to be 0.078 [35].

In the present study, GSI was shown to be an index of the reproductive state of male raccoons. To our knowledge, this is the first report of such a use for GSI in the Carnivora.

For raccoons with a GSI value between 34.40 × 10–3 and 48.72 × 10–3, those with a BW lighter than 5250 g exhibited spermatogenesis. Raccoons with a BW lighter than 5250 g were younger, were more malnourished and had a lighter TW than those with a heavier BW. Therefore, it was thought that even when raccoons with a BW lighter than 5250 g had spermatogenesis, TW remained low, thereby

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**Table 2.** External measurements of feral male raccoons captured in pest control activities in Kamakura, Kanagawa, from March 2005 to September 2008 distributed according to spermatogenetic ability

| Status      | Spermatogenesis | Spermatozoa |
|-------------|-----------------|-------------|
| Sample size | 76              | 8           |
| BW (g)      | 6329.8 ± 973.5  | 5306.3 ± 1002.0 | 5004.3 ± 1189.0 |
| BL (mm)     | 566.3 ± 27.4    | 554.3 ± 16.9 | 528.9 ± 39.0 |
| BMI         | 19.71 ± 2.47    | 17.26 ± 2.98 | 17.71 ± 2.86 |
| TW (g)      | 4.71 ± 0.99     | 2.53 ± 0.44  | 0.97 ± 0.84 |
| TS (mm)     | 20.23 ± 1.43    | 16.47 ± 0.87 | 11.27 ± 2.83 |
| GSI         | 75.48 × 10–3    | 48.92 × 10–3 | 17.86 × 10–3 |
| EW (g)      | 1.06 ± 0.21     | 0.68 ± 0.09  | 0.41 ± 0.21 |

GW, body weight, g; BL, body length, mm; BMI, body mass index; TW, the mean of the right and left testicular weight, g; TS, the mean of the right and left testicular sizes, mm; GSI, gonadosomatic index; and EW, the mean of the right and left epididymal weights, g. All values are shown as means ± SD (range). Values were calculated according to the following formulas: BL (mm) = total length (mm) – length of tail vertebrae (mm), BMI = BW (kg) / BL (m)², testicular size (mm) = long diameter (mm) × short diameter (mm) × thickness (mm), GSI = TW (g) / BW (g) × 10².

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**Fig. 2.** Tree-based model of spermatogenesis in feral male raccoons. BW, body weight, g; GSI, gonadosomatic index. GSI = mean of the right and left testicular weights (g) / BW (g) × 10².

**Fig. 3.** Tree-based model for the presence of spermatozoa in the tail of the epididymis in feral male raccoons. BW, body weight, g; GSI, gonadosomatic index. GSI = mean of the right and left testicular weights (g) / BW (g) × 10².
lowering their GSI values.

Our results suggest that the model developed in this study could be used to estimate the reproductive state of male raccoons in Kamakura regardless of season and age based on knowledge of only BW and TW for each captured raccoon. Therefore, in future studies, the reproductive state of male raccoons can be estimated in a cheaper and easier manner, without the need for histological analysis. However, our model may not have the flexibility to be applied to other regions because of the dynamic changes in reproductive parameters that raccoons make to adapt to their habitat. Though the finding of GSI as an index of reproductive state in this study was limited to male raccoons, the ideas and concepts presented here may be useful for further research.

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