**Seasonal feeding habits of coypu (Myocastor coypus) in South Korea**

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**Abstract**

Since their introduction in 1985, coypus (Myocastor coypus) have spread widely throughout South Korea and are now considered an invasive species, with negative impacts on both agriculture and native biodiversity. Management of the species began in 2005, and related research has focused on factors influencing population control. Cold weather may cause significant population declines but the basis of that susceptibility has yet to be identified. Therefore, based on the analysis of 28 coypus trapped on Eulsuk Island in the Nakdong River over a 12-month period, we sought to: (1) investigate coypu diet and body condition using the relationships between the δ13C and δ15N stable isotope values of coypu liver and hind-leg muscle tissues, mean temperature, and body condition index (log weight/log body length); (2) clarify the relative use of aquatic and terrestrial food plants, and (3) determine seasonal variations in coypu diet. Carbon and Nitrogen isotope values both differed seasonally and, in winter, between adults and juveniles. Carbon, but not Nitrogen, isotope values were influenced by temperature in the weeks before sampling. The δ15N values of liver tissues were influenced by sex and life stage at low temperatures; otherwise, with regard to diet, isotope ratios suggested that coypu primarily fed on aquatic vegetation. Coypus appear to make more use of the heavier nitrogen isotope in hind-leg muscle during winter, presumably associated with muscle tissue metabolism contributing to weight loss. During winter, these higher metabolic requirements together with the decreased availability of aquatic vegetation suggest that baiting near waterways in winter could be an effective method to control invasive coypu populations.

**Keywords:** Myocastor coypus, invasive species, diet analysis, stable isotope, trapping period

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**Introduction**

Coypus (Myocastor coypus) are native to tropical regions of South America but have been introduced to a variety of other climatic zones and ecosystems across North America, Europe, Asia, and Africa, primarily for exploitation of their fur and meat (Carter and Leonard, 2002). The escape and subsequent establishment and spread of farmed coypus into the wild has resulted in significant impacts, particularly the disturbance of native ecosystems, damage to crops, and flooding that results from their burrowing activities (Carter et al., 1999; Lowe et al., 2000; Baroch and Hafner, 2002; Panzacchi et al., 2007; Angelici et al., 2012). However, owing to the tropical origins of coypu, their mortality is often high during winter months in the colder parts of their invaded range (Gosling, 1981a; Battisti et al., 2015). Therefore, we investigated coypu diet, feeding, and body condition, particularly focusing on seasonal changes and how those might be exploited to improve coypu management.

In South Korea, coypus have spread widely throughout the Nakdong River basin since the late 1990’s; however, we previously found that coypu cannot survive in areas subject to prolonged periods of temperatures below −4°C (Hong et al., 2014; Battisti et al., 2015). Although coypu feeding habits have been well studied in their native habitat and in other countries where they have been introduced, little is known about the feeding habits of coypus in South Korea, especially regarding their relative use of aquatic and terrestrial plants (Abbas, 1991; Borgia et al., 2000; Guichón et al., 2003; Prigioni et al., 2005; Corriale et al., 2006; Panzacchi et al., 2007). In the present study, we used δ13C and δ15N values to investigate coypus feeding habits, since stable isotopes can be used to evaluate the relative use of aquatic and terrestrial plants (Kielland, 2001). In this methodology, higher isotope ratios indicate a higher assimilation on animal tissues and, hence, greater food intake (Fry, 2006). In addition, different tissues of animals have different isotope turnover rates, with liver tissues having the fastest rate (DeNiro and Epstein, 1981). We hypothesized that seasonal changes in temperature would be correlated with the δ15N values of liver and hind-leg muscle tissues, since coypu feeding activity is reduced by cold temperatures (Gosling, 1981a), and we expected that a coypu body condition index (log weight/log body length) would be correlated with the δ15N values of hind-leg muscle tissue. The turnover rate of liver tissue is relatively higher than that of hind-leg muscle (DeNiro and Epstein, 1981).

Specifically, we sought to: (1) investigate coypu diet and body condition using the relationships between the δ13C and δ15N stable isotope values of coypu liver and hind-leg muscle tissues, mean temperature, and body condition index (log weight/log body length); (2) clarify the relative use of aquatic and terrestrial food plants, and (3) determine seasonal variations in coypu diet.

**Materials and methods**

**Study area**

The area (35°6'25.30" N, 128°57'10.94" S) used for trapping coypus was located around a small pond (6.94 ha) with three key trap locations (Fig. 1). The area was located on Eulsuk Island in the Nakdong River estuary, and is part of a nationally-designated refuge for migratory birds (Lee et al., 2010). The island covers ≈336 ha and has several wetlands and ponds that have been colonized by coypus. There was a sewage treatment facility on the island from 1975 to 1993, and the island was used as a landfill from 1993 to 1997. In 2005, the island was designated as a National Wild Life Refuge.
as a migratory bird park (Williams et al. 2006; http://wetland.busan.go.kr).


**Figure 1** Study area on Eulsuk Island (336 ha) in the southern Nakdong River estuary of South Korea. Coypu trapping was conducted in the area surrounding the pond (6.84 ha). The filled circles are the key sites for trapping.

### Tissue collection
Coypus were captured from January 2013 to January 2014 using unbaited live animal traps (28 × 30 × 90 cm, self-made) to avoid influencing isotope ratios by bait. The traps were set continuously and checked twice a week, and trapped animals were euthanized. Since the coypu is classified as an invasive species in South Korea, no approval was required to trap the animals. For each trapped coypu, recorded data included sex (female, male), body length (cm), and weight (kg), with animals weighing <1.25 kg considered juveniles (Brown, 1975). In addition, tissue samples (liver and hind-leg muscle) were taken from each coypu killed and samples were stored at −20 °C until analysed (4–6 weeks). For analysis, the tissues were dried at 60 °C to constant weight and then soaked in a solution of methanol, chloroform, and water (2:1:0.8, volume) for 24 h to remove lipids. After three washes with distilled water, the tissue samples were re-dried at 60 °C for 2 d and ground to a fine powder, using a porcelain mortar and pestle (Choi et al., 2014).

### Stable isotope analysis
To obtain δ¹³C and δ¹⁵N values, 0.4–0.6 mg dried tissue samples were individually placed in tin capsules (4 × 6 mm) and analysed by continuous-flow isotope ratio mass spectrometry (CF-IRMS; Micro-mass IsoPrime, Centre for Research Facilities, Pusan National University). The isotope ratios were then determined from the following relationship:

\[
δ \times \% = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000 \%
\]

where \(X\) is \(δ\)¹³C or \(δ\)¹⁵N and \(R\) is the corresponding \(δ\)¹⁵N/\(δ\)¹⁴N or \(δ\)¹³C/\(δ\)¹²C value. Pee Dee Belemnite (from the PeeDee Formation, South Carolina, USA) and \(N_2\) gas were used as standards for \(δ\)¹³C and \(δ\)¹⁵N analysis, respectively. The ratios are reported as means ± standard error (SE) (Fry, 2006).

### Estimating the effects of low temperatures on the \(δ\)¹⁵N values of coypu tissues
To investigate the relationship between temperature and body condition, we used mean temperatures over time periods that corresponded to the isotope turnover rates for each tissue (liver tissue, 3 weeks before capture; hind-leg muscle tissue, 3 months before capture; Major et al., 2007). In order to assess the relative importance of low temperatures, we analysed the relationship between the \(δ\)¹⁵N values of the liver and hind-leg muscle tissues, body condition index, and mean temperature, using multiple regression analysis (as implemented in SPSS 18, IBM Inc., Chicago, IL, USA) for coypu groups and life stages. We log-transformed the \(δ\)¹⁵N values of the liver tissues and all values were normalized to remove bias (Zar, 1999).

#### Food plant analysis
We defined plants that did not occur in the pond as terrestrial plants and the others as aquatic plants. The sampling area was restricted to within 100 m of the water’s edge, since coypu feeding is limited to that area (Abbas, 1991). Because isotopic values of soils are likely to have influenced the plant isotopic values, plants from around the pond were chosen randomly, with a distance of at least 30 m between specimens from the same species (Corriale et al., 2006), and the plants were identified using the taxonomic keys in Lee (2006). In order to assess any preference of coypu for particular plant parts, the plants were separated into above- and below-ground parts. Vegetation sampling was limited during winter months since the aboveground plant parts were dead. Plant parts were dried at 60 °C for 2 d and ground separately, except for floating plants which were dried and ground in their entirety since coypu eat them whole (Gosling, 1981a; Abbas, 1991; Wilsey et al., 1991; Marini et al., 2013). The vegetation components were then soaked in 1 mol/L HCl for 24 h to remove organic carbon, after which they were re-dried at 60 °C for 2 d (Choi et al., 2014).

#### Statistical analysis
An index of body condition was calculated using log weight per log body length (Anderson and Neumann, 1983), and for data analysis, winter was defined as the period from December to February, spring from March to May, summer as from June to August, and autumn as from September to November (Abbas, 1991; Hong et al., 2014; Battisti et al., 2015).

If the elapsed time between coypu captures exceeded 3 months, we assumed that the coypu population had been depleted and that a new group had colonized. Therefore, we compared the body condition indices and isotope ratios of the coypu groups (all or adults only), as well as the body condition indices and isotope ratios of adult and juvenile coypus, using the independent t-test for normally distributed data or the Mann-Whitney test for variables that did not exhibit normal type error distributions. In addition, we also compared the sex ratio between the groups, using chi-square tests (contingency table analysis), and the relationship between sex and body condition, using independent t-tests. In order to assess the influence of season, sex, and life stage on the isotopic ratios and the occurrence of interactive effects, we conducted two-way multivariate analysis of variance (MANOVA; Johnson and Wichern, 1988) as implemented in SPSS 18 (IBM Inc., Chicago, IL, USA).

Subsequently, the isotope ratios of the aquatic and terrestrial plants and coypus were separately analysed, using SigmaPlot 10.0 (Systat Software, San Jose, CA, USA). We used t-tests for normally distributed variables and the Mann-Whitney test for variables that did not exhibit normal type error distributions. Seasonally divided isotope ratios of plants and coypus were plotted against the C/N ratio in multiple scatter plots and were compared using t-tests.

### Results
#### Sample data
During the 12-month trapping period, we captured a total of 28 coypus, with one group (n=13) trapped during winter to spring and a second group (n=15) trapped during autumn to winter (Fig. 2). Juveniles (n=4) were only trapped in the winter of 2013 (Tab. 2). The sex ratio of the two groups was similar (χ²=3.49, df=1, \(p>0.05\)), and, excluding juveniles, the mean body condition index of the two groups was also similar (t=–1.132, df=22; Fig. 2, \(p>0.05\)).

We found 6 aquatic, 10 terrestrial, and 4 unknown plant species in the study area (Tab. 3). As the mean temperature increased, the occurrence of floating and submerged vegetation also increased.

Excluding juveniles from the analysis, the \(δ\)¹³C values of the two groups differed significantly, for both liver and hind-leg muscle tissue (liver: \(t=2.83, df=16, p<0.05\); hind-leg muscle: \(t=4.183, df=21, \(p<0.05\)).
Relationship between the $\delta^{15}N$ values of coypu tissues and other factors

The $\delta^{15}N$ values of hind-leg muscle were more highly correlated with body condition ($\beta=0.42, p<0.05$) than with mean temperature of the 3 months before capture ($\beta=0.24, p>0.05$; $r^2=0.47, p<0.01$). For adults (n=24), the $\delta^{15}N$ values of hind-leg muscle were more highly correlated with body condition index ($\beta=0.35, p>0.05$) than mean temperature ($\beta=0.19, p>0.05$; $r^2=0.33, p<0.05$). Whereas for the juveniles (n=4), the $\delta^{15}N$ values of hind-leg muscle were more highly correlated with mean temperature ($\beta=0.98, p<0.05$) than body condition index ($\beta=0.16, p>0.05$; $r^2=1.00, p<0.05$).

Isotope ratios of food plants

The isotope ratios of terrestrial and aquatic plants differed significantly for both above- and below-ground tissues ($\delta^{13}C$: $z=-3.30, p<0.01$; $\delta^{15}N$: $z=-6.09, p<0.01$). No differences were observed in the isotope ratios of above- and below-ground parts for either aquatic plants ($\delta^{13}C$: $z=-1.25, p>0.05$; $\delta^{15}N$: t=-0.21, df=33, p>0.05) or terrestrial plants ($\delta^{13}C$: t=-0.31, df=37, p=0.05; $\delta^{15}N$: $t=0.31, df=37, p>0.05$). However, the isotope ratios of aquatic plants clustered closer to those of coypus than to those of terrestrial plants (Fig. 4).

The $\delta^{15}N$ values of aquatic plants differed significantly from those of terrestrial plants, except during the first winter sampling period, when analysis was limited by low sample sizes (winter: $p<0.05$, $z=1.464$; spring: $p<0.01$, $t=5.33$, df=12.68; autumn: $p<0.01$, $t=5.07$, df=9; winter: $p<0.01$, $t=3.00$, df=19). In addition, the $\delta^{15}N$ values of roots and stems from aquatic plants differed significantly ($\delta^{15}N$: $t=2.85$, df=7, $p<0.05$). In general, the isotope ratios of the coypu were more similar to those of the aquatic plants than the terrestrial plants. During the first winter, coypu diets included little, if any, aquatic plants. During spring, the $\delta^{15}N$ values of aquatic plants were closer to those of the coypu tissues. By autumn, the floating plants had emerged. The nitrogen isotope ratios of the underground parts of aquatic plants and coypu tissues were related. The aboveground portions of the aquatic plants yielded comparatively higher nitrogen isotope ratios than the coypu tissues. During early winter, the overall $\delta^{15}N$ values of any aquatic plants and coypus decreased, and the values of livers and hind-leg muscles also decreased. Similarly, the values of aquatic plants were closely related to the values of coypus (Fig. 4).
that between isotope assimilation by hind-leg muscle tissue and mean temperature. Coypus would be expected to exhibit higher rates of metabolism at lower temperatures than at higher temperatures (Dixon et al., 1979; Fry, 2006). The $\delta^{15}N$ values of the hind-leg muscle tissues were correlated with the relatively long-term mean temperature (i.e., 3-month average), since the turnover rate of the hind-leg muscle tissue was relatively slow, owing to the lower metabolic rate of muscle tissue at lower temperatures. However, the isotope ratios in hind-leg muscle tissues exhibited a stronger relationship to the body condition index than to mean temperature, and the body condition indices of trapped coypus decreased after winter (Tab. 1). Therefore, coypus appear to make more use of the heavier nitrogen isotope in hind-leg muscle during winter, presumably associated with muscle tissue metabolism contributing to weight loss. In addition, this pattern was even more evident in juveniles, perhaps a reflection of their lower fat reserves and higher surface area to volume ratios than adults. This may explain the higher rate of mortality in juveniles than in adults (Aliev, 1973; Doncaster and Micel, 1990).

Seasonal variation in isotope ratios enables has been used to identify changes in the relative importance of vegetation types in animal diets (Owen-Smith, 1994; Kiellland, 2001). During the winter, the $\delta^{15}N$ values of aquatic plants decreased at the study site, but the C/N ratios of the coypu tissues did not follow the changes in vegetation isotope ratios, likely because the coypus fed on the live submerged parts of some aquatic plants, thus, maintaining similar $\delta^{15}N$ values. In addition, as mentioned previously, the isotopic values could be maintained by the metabolism such as use of the heavier nitrogen isotope in hind-leg muscle. Therefore, the $\delta^{15}N$ liver values could be higher than hind-leg muscle values. During spring, floating plants were not included in the analysis since they had not emerged yet, so the aquatic plants are fully featured in the diet, and coypus appeared to eat both above- and below-ground plant parts (Tab. 2). As floating plants emerged, the $\delta^{15}N$ hind-leg values increased, and reflected with those of coypu tissues. At our study site, there were few floating plants, so we were unable to determine food preferences within aquatic plants, although coypus are known to prefer floating plants (Lemna spp.) and plants in the Pontederiaceae (Wilsey et al., 1991; Guichón et al., 2003). Although we could not determine preferences for specific plants, it was
Table 3 – Plants collected from Eulsuk Island, South Korea and their carbon and nitrogen isotope levels in different seasons (n = sample size).

<table>
<thead>
<tr>
<th>Aquatic plants</th>
<th>Emergent</th>
<th>Poaceae</th>
<th>Winter n</th>
<th>Winter $\delta^{13}$C</th>
<th>Winter $\delta^{15}$N</th>
<th>Spring n</th>
<th>Spring $\delta^{13}$C</th>
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<td>6.33±2.71</td>
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<td>Phragmites communis</td>
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<td>-28.71±0.62</td>
<td>6.46±0.24</td>
<td>2</td>
<td>-27.71±1.02</td>
<td>5.54±0.86</td>
<td>5</td>
<td>-27.48±0.63</td>
<td>10.79±1.04</td>
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<td>-26.51±0.18</td>
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<td>4.49±0.98</td>
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<td>3</td>
<td>-30.87±0.53</td>
<td>3.95±1.40</td>
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<td><strong>Cannabaceae</strong></td>
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<tr>
<td>Humulus japonicus</td>
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<td>-29.05</td>
<td>2.17</td>
<td>1</td>
<td>-28.97</td>
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<td>Lamiaceae</td>
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<tr>
<td>Salvia plebeia</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>-26.60±2.64</td>
<td>2.08±0.75</td>
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<tr>
<td><strong>Unknown species</strong></td>
<td>4</td>
<td>-30.06±0.96</td>
<td>-0.76±3.0</td>
<td>2</td>
<td>-28.97</td>
<td>4.61</td>
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clear that the diet of coypus was largely derived from the aquatic system, as has been reported previously (Borgnia et al., 2000; Guichón et al., 2003; Corriale et al., 2006; Battisti et al., 2015).

The low temperatures of winter influence thermoregulation, so that more energy is required for maintenance and activity (Moinard et al., 1992), and both food availability and coypu feeding activity are reduced during winter months (Gosling, 1979). Therefore, coypus appear to survive winter by sacrificing body mass and using various strategies to maintain their body temperature, including the avoidance of heat loss, controlling the rate at which food passes through the pyloric sphincter (Gosling, 1979), burrowing in dens, and huddling in groups (Gosling et al., 1980; Moinard et al., 1992). Furthermore, although not observed in the present study, coypus may also invade crops and farm buildings (Panzacchi et al., 2007; Battisti et al., 2015). Thus, the winter food shortage can be exploited, by baiting for coypus during that period.

The coypu population in the lower Nakdong River has previously been affected by cold weather (Hong et al., 2014), but the high productivity of the survivors has enabled rapid population recovery (Ehrlich, 1966; Doncaster and Micol, 1989). Therefore, annual control during winter may be the best approach for sustained coypu population reduction (Hong et al., 2014).

References


