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2 The Growth Effect of a Commercial Diet versus Calcium-Dusted
3 *Blaptica dubia* and *Carica papaya* on Juvenile *Correlophus ciliatus*

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27 Abstract.-

28 **Background:** Reptiles and amphibians are increasing in households across the United States. Pet
29 owners are struggling to maintain their well-being due to lack of proper husbandry and nutrition,
30 resulting in a poor quality of life and a reduced lifespan. This study was conducted to provide
31 more information on nutrition to reptile owners, breeders, herpetologists, veterinary professionals
32 and more. The goal is to provide dietary information for increased growth in *Correlophus*
33 *ciliatus*, the crested gecko.

34 **Materials and Methods:** A food trial was conducted for 5 weeks with 60 juvenile crested
35 geckos, 30 of which were fed a diet of calcium-dusted cockroaches and fresh papaya and the
36 remaining 30 were fed Pangea © crested gecko diet. Their environments remained controlled and
37 measurements were taken once a week, on their fourth feeding day. IACUC Approved: 19.006

38 **Results:** Before and after the food trial, the geckos had the same behavior, activity and appeared
39 healthy, therefore their welfare was not compromised. Upon conducting a two-way repeated
40 measures ANOVA and a paired t-test examining weight and length, the geckos of the insect/fruit
41 diet group on average gained more weight and showed increased growth compared to the
42 commercial diet group. The data analysis on length did not demonstrate any significant
43 difference. Statistically, from Week 1 to 5, the insect/fruit diet and commercial diet groups had a
44 total body percent increase of 24.13% and 13.07%, respectively.

45 **Conclusions:** Based on the study conducted, the juvenile crested geckos tested exhibited
46 increased growth on a diet comprised of fruit and insect rather than a commercial diet.

47 **Clinical relevance:** With this data and analysis, crested gecko nutrition is better understood and
48 statistically available for establishing better nutritional standards and procedures.

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50 *Key Words:* Gecko; Dubia; Pangea; Papaya; Reptile

51 Introduction.-

52 Pets are a crucial part of society. Consequently, reptiles and amphibians are moving up
53 the list of common household pets. According to the American Pet Product Manufacturers
54 Association, more than 3.9 million households in the United States contained one or more pet
55 reptiles or amphibians in 2000. This is a 44 percent increase since 1998. According to wildlife
56 biologist and medical scientist Clifford Warwick, 75 percent of reptiles die during their first year
57 in a domestic home environment [1]. Unfortunately, there is not a plethora of research supporting
58 their biological needs, compared to canines for example. All reptile species demand a complete
59 and balanced diet including calcium, vitamin D3, vitamin A and fiber. Yet it is known that a
60 common factor of the high mortality rates in households is poor nutrition [2].

61 This study is conducted to help enhance the welfare for household reptiles. With the
62 volume of food products in the market and brands that do not have research showing successful
63 growth, development and lifespan with the feed, it is difficult as a pet-owner to decide what
64 brand to feed their reptile or what type of diet to choose. To better understand reptile nutrition
65 and development, we tested the effects of a commercial, Pangea© crested gecko diet, on juvenile
66 crested geckos- *Correlophus ciliatus*- versus a fruit/insect diet consisting of calcium-dusted
67 juvenile cockroaches and pureed papaya. These two options for feed were chosen specifically, as
68 they are a reasonable replication of what is expected to a natural diet for the species in the wild
69 [3]. This species is particularly prone to developing Metabolic Bone Disease (MBD) due to
70 lacking minerals, such as calcium, in their diet. Vitamin D deficiency is a leading cause of MBD,
71 as Vitamin D is the necessary agent for calcium absorption [4]. By having results of growth in
72 groups treated with a homemade diet and a commercial diet, consumers of all backgrounds can

73 better understand the demands of a reptile and invest in feeding a diet that will prolong their
74 lifespan and enhance their well-being. Prior to beginning the 5-week food trail, we hypothesized
75 that the commercial diet fed group will have grown more than the insect/fruit diet fed group. We
76 hypothesized that a commercial formula is more complex and contains more essential nutrients
77 and minerals than a basic insect/fruit diet. For example, the commercial diet contains riboflavin,
78 folic acid, vitamin B12, vitamin A, folic acid and sulfur, all considered essential in gecko
79 nutrition [5]. Therefore, the commercially treated group will ideally show the best growth
80 results.

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92 Materials and Methods.-

93 The crested geckos used for this food trial experiment were obtained by the permission of
94 one breeder and the experiment the use of the animals was approved by the Institutional Animal
95 Care and Use Committee (19.006). The crested geckos used for this food trial experiment ranged
96 from 3-4 months at the start of the food trial period. Exactly 60 geckos were used for this
97 experiment, 30 in the insect/fruit diet fed group and 30 in the commercial diet fed group. The
98 geckos were genetically related but were randomly categorized into the two groups for the
99 experiment. Prior to beginning the trial, they were all on Pangea©. A humidifier was set in the
100 room at all times, with humidity set to remain between 50-90%. The room remained between 70
101 and 73 Fahrenheit at all times. The humidity and temperature monitor brand was Govee Home©,
102 purchased from Amazon©.

103 Each crested gecko resided individually in a labelled, hole-punched individual plastic
104 container, measuring about 33 cm in length, 20 cm in width and 15 cm in height. The container
105 brand was Rubbermaid© and they were purchased from WalMart© and Target©. Each container
106 was furnished with a paper towel base, plastic green plant, egg carton piece, plastic food dish and
107 plastic water dish. The paper towel brand was Bounty© and they were purchased from
108 WalMart© and Target©. The plastic plants were purchased from Pangea© Reptile, manufactured
109 by Zoo Med©. The egg cartons were obtained from Uline© and cut into 10 cm by 10 cm
110 sections. The scale used for all measurements of feed weight and gecko weight was AC Pro-200
111 (200 g x 0.01 g) ©, purchased from Amazon©. The geckos were fed 4 times a week, therefore
112 every other day and one consecutive day. Data was collected once a week, every Sunday. On
113 feeding days, each gecko had a clean water dish replaced, a new paper towel and misting of its
114 habitat and itself.

115 Prior to beginning the food trial, the three feed samples (cockroaches, papaya and
116 Pangea©) were submitted to a nutrient analysis laboratory according to their sample size
117 guidelines and shipping instructions. This tool was utilized to provide more information on the
118 macronutrient content, testing for percent fat, protein, moisture, ash and fiber. The laboratory
119 used was Cumberland Valley Analytical Services, Inc.

120 The commercial feed used was Pangea© Fruit Mix with Insects Complete Gecko Diet (as
121 shown on the manufacturers website). The package itself calls it Pangea© Gecko Diet “With
122 Insects.” This was originally in powder formula, therefore each week a new paste was
123 reconstituted with water, placed in a wide-mouth dispensing bottle and refrigerated. The paste
124 was reconstituted following the instructions on the commercial diet “Directions” Label. A 1:2
125 ratio of paste to water, respectively, was stated, therefore we used 100 milliliters of paste and 200
126 milliliters of water. The mixture was added into the dispensing bottle immediately and shaken for
127 30 seconds. The geckos in this food group were given 2 grams paste, on an inverted clear plastic
128 cup that measured 2 cm height and 2 cm diameter.

129 The insect/fruit group was fed a diet of roaches and papaya. The roaches used were
130 juvenile cockroaches, *Blaptica dubia*, obtained from www.dubiaroaches.com. The roaches were
131 gutloaded with Pangea© Superpig and Nature Zone© Total Bites. Approximately 2-3 roaches
132 were fed to each gecko in the commercial diet group. The total weight of roaches fed to each
133 gecko was 1 gram and sizes of each individual roach was decided based on size of the respective
134 gecko. The protocol used was crude examination of the head of the gecko: roaches were given
135 that were smaller than the size of the respective gecko head. The roaches were placed in a clear
136 plastic cup (2 cm height and 2 cm diameter) and dusted with 0.1 gram of calcium powder. The
137 calcium powder brand used was ReptiCalcium© with D3, manufactured by Zoo Med©. The

138 ingredients were precipitated calcium bicarbonate and cholecalciferol (source of vitamin D3).
139 The papayas were purchased from Sprouts Market© and the peels and kernels were discarded
140 with each use. The papaya used was blended and placed in a wide mouth dispensing bottle, then
141 refrigerated. The papaya was used up within the week and replaced with new blended papaya at
142 the end of the week for optimal freshness. Each gecko was given 1 gram of the papaya, placed on
143 an inverted clear plastic cup (2 cm height and 2 cm diameter).

144 On data collection days, a protocol of minimal handling was set in place, to prevent gecko
145 stress and loss of tail. The gecko was removed from its habitat, and first placed on a scale and
146 weight in grams was measured. Following this, the gecko was placed on a laminated grid paper,
147 parallel to the lines and a photo was taken directly above the gecko, 90 degrees to the table. The
148 grid paper used had the following measurements: 5 squares=1 centimeter. This photo was then
149 used to manually count and measure the total length of the gecko in centimeters.

150 At each data collection date, the geckos were physically evaluated for soundness and
151 health. The following signs were looked out for as indications of poor health: jaundice
152 (yellowing of eyes, mucous membranes or scales), dryness of scales, appearance of spine, loss of
153 tail and reduced motility.

154 For data, the values used were body weight and total length. To test the variation in the
155 data for the values collected, a t-test, paired t-test and a two-way repeated measures ANOVA
156 were used.

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160 Results.-

161 Upon physical examination from Week 1 to Week 5, there were no evident negative
162 health differences in the geckos from both groups. No tails were lost and all geckos appeared
163 healthy based on physical examination findings and lack of poor health signs stated above.

164 A two-way repeated measures ANOVA (one factor repetition) was conducted comparing
165 live weight from week 1 to week 5. The data failed the normality test (ShapiroWilk) and equal
166 variance test (Brown-Forsythe). There is a statistically significant interaction between diet and
167 week ($P = <0.001$).

168 A two-way repeated measures ANOVA (one factor repetition) was conducted comparing
169 live weight from week 2 to week 5. The data failed the normality test (ShapiroWilk) and passed
170 the equal variance test (Brown-Forsythe). There is a statistically significant interaction between
171 diet and week ($P = <0.001$).

172 An all pairwise multiple comparison procedures test (Bonferroni t-test) was conducted
173 comparing insect/fruit diet versus commercial diet. By week 5, there was a significant weight
174 gain ($P < 0.050$) in the insect/fruit diet group compared to the commercial diet group.

175 A t-test conducted on change in weight and percent change in weight of the two groups
176 by week 5 demonstrated a statistically significant difference ($P = 0.016$). A t-test conducted on
177 change in live weight demonstrated statistical insignificance.

178 Time was a significant factor and showed growth over time in lengths. Figure 1 shows
179 that the crested geckos started at approximately the same mean weight at Week 1 and at
180 approximately Week 2-3, the commercial diet group plateaued while the insect/fruit diet group
181 continued to increase. After Week 3, the commercial diet group resumed a steady growth pattern.

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183 According to Table 3, by Week 5, there was growth exhibited in both groups, but the
184 insect/fruit diet group averaged 4.055 grams and the commercial diet group averaged 3.684
185 grams. Table 2 demonstrates that the average weight gain in the insect/fruit diet group was 0.813
186 and in the commercial group the average weight gain was 0.4263. The percent increase for the
187 insect/fruit diet group and the commercial diet group was 24.35% and 13.07%, respectively.
188 Figure 2, comparing mean length of the groups from Week 1 to Week 5 demonstrated growth
189 and change but no significant difference between the two groups.

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201 Discussion.-

202 Based on the results from the t-test and the ANOVA, we can conclude that the ANOVA
203 results are more significant and credible. The insect/fruit diet group had a significant increase in
204 weight compared to the commercial diet group. We can reject our hypothesis and state that the
205 insect/fruit diet group, over a five-week span, has shown greater average weight gain, therefore
206 greater average growth, than the commercial diet treatment group.

207 Upon consideration of the nutrient content of each feed, it is evident as to why the
208 insect/fruit diet group exhibited more growth and higher weights. The fat percentage of the
209 cockroaches and papaya combined were 8.6%, compared to the commercial diet value of 4.6%,
210 shown in Table 1. Furthermore, the protein percentages were 30.4% and 25.5% for the natural
211 diet combination and the commercial diet, respectively. There is significance in considering dry
212 matter, mineral content and prey size, as it alters digestibility per individual [6]. The roaches
213 were dusted in calcium and were kept at a small size, possibly increasing the overall
214 digestibility in the insect/fruit diet group.

215 The sex of the geckos was not determined during the study, as they cannot be
216 distinguished until they have reached sexual maturity. For the nature of this experiment, the sex
217 was deemed insignificant, considering the sample size was 30 per group and randomized.

218 The length of the geckos showed growth over time but they did not demonstrate a
219 difference in groups, therefore it is not a significant factor when evaluating growth on a dietary
220 basis. Measurement of body composition and width or girth to see what tissues are accounting
221 for the increase in weight will also be a goal, to help understand the change in body composition
222 and have more solid results.

223 A future direction would be for only one individual to conduct data collection and
224 statistical analysis for less percent error and greater consistency.

225 For future studies, we would like to conduct the food trial for over 5 weeks. It would be
226 ideal to monitor growth until the geckos were no longer juvenile and became sexually mature.
227 This way the geckos can also be sexed and data can be collected comparing males and females as
228 adults.

229 As part of the nutrient analysis, it would have been more complete to have specific
230 mineral content percentages. For example, phosphorus, calcium and magnesium, as these are
231 essential in any reptile diet to prevent Metabolic Bone Disease and to ensure healthy growth and
232 development. For future studies, a micronutrient analysis will be conducted along with a
233 macronutrient analysis.

234 Testing the fecal output to compare dry matter versus wet matter would also further
235 conclude the data and provide stronger support. Another crucial part for any feed study would be
236 recording the amount consumed quantified by deducting feed refusals. For example, we know
237 that 2 grams of commercial diet was offered, but we did not quantify how much was consumed.
238 For future projects, this is essential information to account in statistical analysis. It is possible
239 that there is difference in taste of the diets and each gecko has difference in palatability which is
240 a factor to consider [7].

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251 laboratory for conducting nutrient analysis and advising on what profile would best fit the needs
252 of the study. We acknowledge Cumberland Valley Analytical Services, Inc. for conducting
253 nutrient analysis on the commercial diet and the papaya. IACUC Approval- 19.006

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322 *Figure Captions.* –

323 Fig. 1. Average group weight per week from week 1 through week 5. The average weights of the
324 insect/fruit diet and the commercial diet groups were approximately the same in the beginning of
325 the study. At week 2 a deviation was seen, where the commercial diet group plateaued while the
326 insect/fruit diet group continued weight gain. After week 3, the commercial diet group resumed
327 growth and by week 5, the insect/fruit diet group exhibited increased weight gain compared to
328 the commercial diet group.

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330 Fig. 2. Average group length from week 1 through week 5. The insect/fruit diet group averaged
331 with greater lengths throughout the food trial compared to the commercial diet group. The
332 lengths gradually increased in the insect/fruit group. Whereas in the commercial group, between
333 week 1 and week 2 a slight decrease in length was exhibited. The same pattern was seen from
334 week 3 to week 4. There was no significant difference between the groups was exhibited by
335 week 5.

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356 Table 1:

Nutrient Analysis of <i>Blaptica dubia</i> , <i>Carica papaya</i> and Pangea© Diet					
Feed (Fresh)	Fat (%)	Moisture (%)	Protein (%)	Ash (%)	Fiber (%)
<i>Blaptica dubia</i> (as fed)	6.1	71.5	21.4	1.3	2.6
<i>Carica papaya</i> (as fed)	2.5	92.7	9.0	0.6	15.8
Pangea© (dry matter)	4.6	7.4	25.5	7.4	1.9

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358 Table 2:
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Comparison of Crested Geckos' Mean Starting Weight, Mean Weight Gain and Totally Body Percent Increase in Insect/Fruit Diet vs. Commercial Diet Groups				
Group	N	Mean Starting Weight (g)	Mean Weight Gain (g)	Total Body Percent Increase (%)
Insect/Fruit Diet	30	3.24	0.81	24.35
Commercial Diet	30	3.26	0.43	13.07

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362 Table 3:

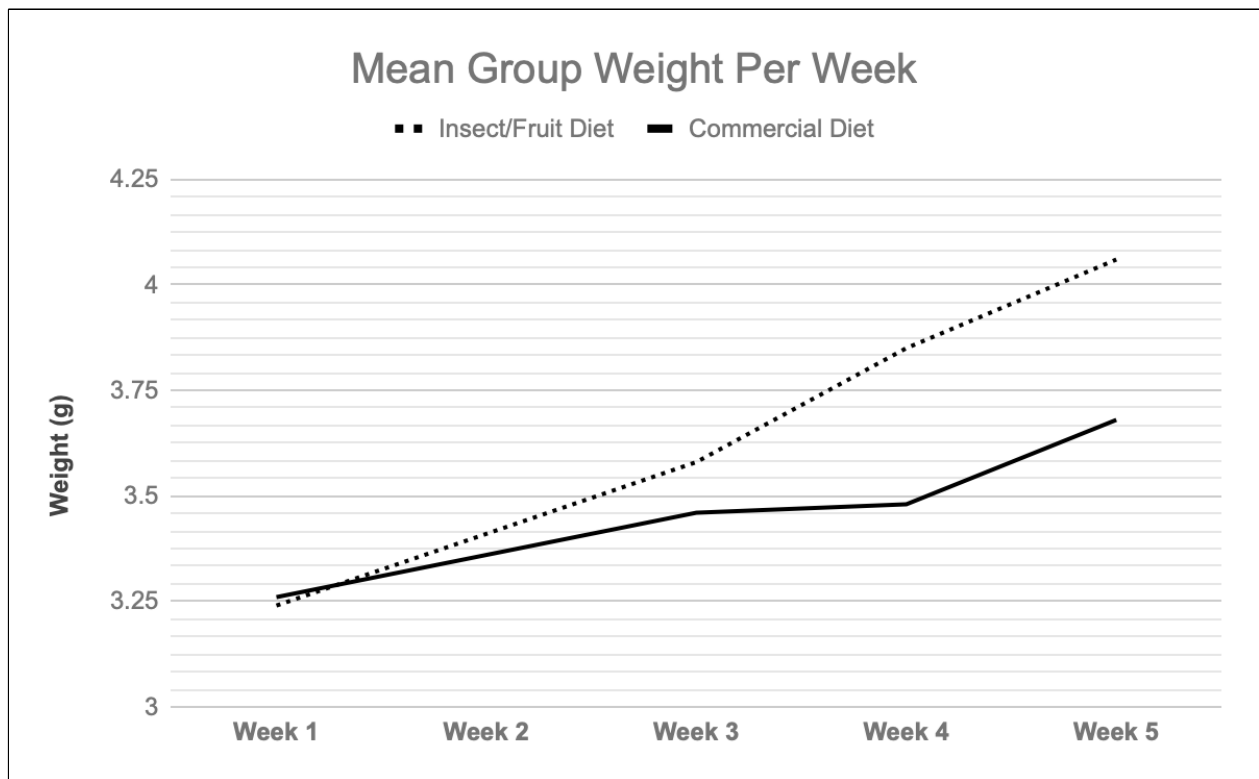
Comparison of Crested Geckos' Mean Group Weight per Week in Insect/Fruit vs. Commercial Diet Groups		
	Insect/Fruit Diet (g)	Commercial Diet (g)
Week 1	3.24	3.26
Week 2	3.41	3.36
Week 3	3.58	3.46
Week 4	3.85	3.48
Week 5	4.06	3.68

363 Table 4:

Comparison of Crested Geckos' Mean Group Length per Week in Insect/Fruit vs. Commercial Diet Groups		
	Insect/Fruit Diet (cm)	Commercial Diet (cm)
Week 1	10.25	10.16
Week 2	10.61	10.15
Week 3	10.78	10.77
Week 4	10.9	10.72
Week 5	10.98	10.84

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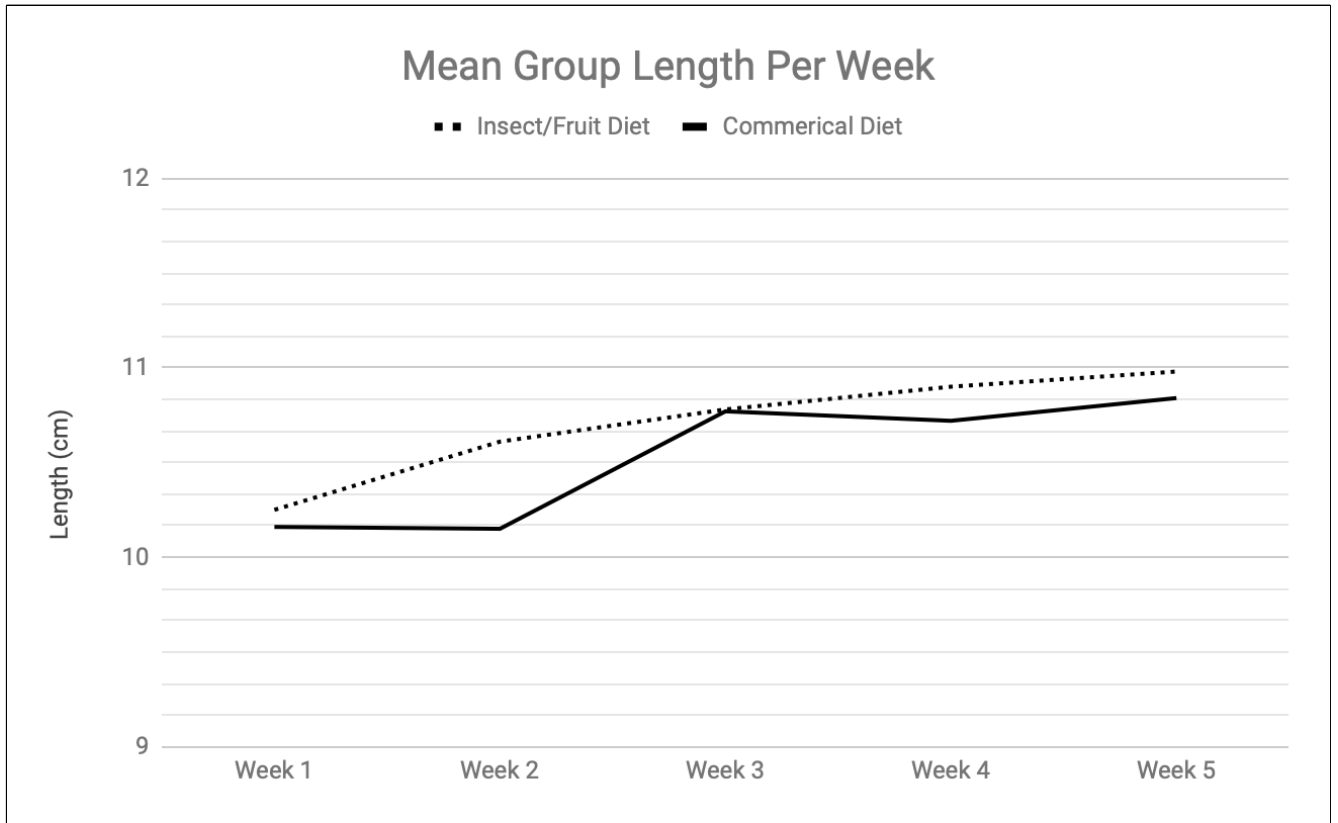
365 Figure 1:



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369 Figure 2:



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