

1 **Diet preferences of captive reared green turtles (*Chelonia mydas*) in a multiple**  
2 **choice feeding experiment**

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4 Maria Koivisto<sup>1,2\*</sup>, Anu Riihimäki<sup>2</sup>

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6 <sup>1</sup>Department of Biological and Environmental Sciences, Aquatic Sciences, Helsinki, Finland

7 University, E-mail: maria.koivisto@helsinki.fi, tel. +66856192847, +358-445208305

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9 <sup>2</sup>Phuket Marine Biological Center, Endangered Species Unit, 51 Moo 8 Sakdidat Rd, 83000

10 Muang Phuket, Thailand

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13 \* Corresponding author. E-mail: maria.koivisto@helsinki.fi

14 **ABSTRACT**

15 The feeding behavior of juvenile sea turtles has been poorly studied despite its conservational  
16 and management value. Sea turtles in head-starting programs are often fed diets that produce  
17 maximal growth and survival, but virtually nothing is known about the effects that this  
18 procedure might have on the turtle's subsequent feeding behavior. In this study, the feeding  
19 preferences of eight head-started juvenile green turtles equivalent to the size of pelagic stage  
20 juvenile turtles were studied in a multiple choice feeding experiment. Food items included  
21 four animal prey items and one plant item commonly found in the turtles' area of release. We  
22 tested the null-hypothesis that no food imprinting exists in these turtles due to early feeding  
23 experiences and that there are no differences in diet preferences among turtles. Based on our  
24 results, no food imprinting exists in head-started green turtles and they rapidly adjust to new  
25 food items. Seagrass was the least preferred choice of all study turtles except one. There were  
26 also some differences in the individual diet preferences among turtles. Results suggest that  
27 head-started turtles can adapt to natural foods which they encounter in the wild.

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29

30 **Keywords:**

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32 Sea turtles, *Chelonia mydas*, multiple choice feeding experiment, juvenile turtles, head-  
33 starting

34

35 Feeding behavior and food preferences of sea turtles have been poorly studied in spite of their  
36 relevance for understanding ecological requirements and management strategies. While  
37 extensive research on the feeding behavior of adult sea turtles has been carried out (e.g.  
38 Garnett et al., 1985; Lopez-Mendilaharsu et al., 2005), very little is known about the early  
39 feeding behavior and food preference development of juvenile sea turtles (Whiting et al.,  
40 2008). According to classical belief, green turtles in their early, pelagic stages are omnivores  
41 with a strong tendency to carnivory (Bjorndal, 1985). When they become older and move to  
42 coastal areas they start feeding on plant and algal matter but occasionally consume animal  
43 matter (Bjorndal, 1997; Seminoff et al., 2002), mangrove vegetation and fruits (Limpus and  
44 Limpus, 2002). Geographical differences in food choice linked to food availability and  
45 selective feeding are also apparent (e.g. Read and Limpus, 2002; Amorocho and Reina, 2007;  
46 Bjorndahl, 1980). However, significant gaps still remain in our understanding of the foraging  
47 ecology of sea turtles and for some species, little progress regarding diet and feeding habitats  
48 has been made during the last 20 years (Bjorndal, 1985; Mortimer, 1982). To be able to  
49 understand the role of sea turtles in marine ecosystems and to elucidate how nutrition acts as a  
50 regulating mechanism of sea turtle populations, more studies about diet selection is needed.

51

52 Increasing our understanding of early feeding behavior in sea turtles is especially important  
53 because of the intense conservation efforts involving these threatened animals. Although a  
54 highly debated praxis, head-starting programs of sea turtles still exist in many countries (e.g.  
55 Shimizu et al., 2006; Win and Lwin 2009). In these programs, sea turtle hatchlings are raised  
56 in captivity for up to 1.5 years prior to release. The purpose of the procedure is to circumvent  
57 the suspected high period of predation during the animals' early life history. Turtles are often  
58 fed diets that produce maximal growth and survival, but virtually nothing is known about the

59 effects this procedure might have on the turtle's subsequent feeding behavior. Food  
60 preferences as based on early feeding experiences with reversible effects have been shown to  
61 exist in loggerhead sea turtles, *Caretta caretta* (Grassman and Owens, 1982) but no such  
62 records exist for green turtles.

63

64 In this experiment, we tested the diet preference of nine months old captive bred juvenile  
65 green turtles equivalent to the age of pelagic stage natural sea turtles (9 months, < 25 cm  
66 SCL) and tested the null-hypothesis that food imprinting does not exist in green turtles.  
67 Specifically, we were interested in studying the following: 1) Do juvenile green turtles have  
68 diet preferences when subjected to different food items, 2) do they show a preference for diet  
69 that they are accustomed to and finally, 3) are there differences between turtles? To be able to  
70 control and quantify negative effects of human activities on turtle foraging behavior such as  
71 the degradation of foraging habitats, turtle diet quality and availability, a rapid understanding  
72 about diet selectivity in all life stages is needed as wildlife managers are expected to make  
73 important decisions on which habitats to protect.

74

## 75 **METHODS**

### 76 **Study site and study animals**

77 The study was conducted in the Phuket Marine Biological Center, Thailand (7°48'N, 98°24'E).  
78 In the center, juvenile green turtles are head-started with the authorization of the  
79 governmental Department of Fisheries and Marine Coastal Resources as a part of a sea turtle  
80 conservation program. These head-started green turtles are collected as eggs in the Similan  
81 area and hatched in the Phuket Marine Biological Center. Each year, about 50 green turtles  
82 from the center return to the Andaman Sea.

83

84 In our experiment, the feeding behavior of eight juvenile green turtles equivalent to the size of  
 85 pelagic stage juvenile green turtles was studied. Study turtles were chosen randomly from  
 86 the same size class and all turtles were equally old (9 months). The turtles measured 18.5-24.5  
 87 cm in CCL length and weighed 0.79-1.73 kg (Table 1). Turtles were studied in two separate  
 88 groups to be able to observe each individual feeding behavior.

89

90 **Table 1.** CCL length, weight and study group (1, 2) of the study turtles.

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92 <b>Turtle</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>
93 <b>CCL (cm)</b>	21	20.5	19.6	18.5	23	23.7	24.5	22.5
94 <b>Weight (kg)</b>	1.09	1.03	0.9	0.79	1.27	1.4	1.73	1.25
95 <b>Study group</b>	1	1	1	1	2	2	2	2

96

97 **Food items**

98 In this study, we tested for preferences of five different food items; fish (*Clupeidae* sp.),  
 99 shrimp (*Litopenaeus stylirostris*), jellyfish (*Cassiopeia* sp.), squid (*Loligo formosana*) and sea  
 100 grass (*Cymococcea rotundata*) (Table 2). These items were selected since they are relatively  
 101 common in the study area and since we were especially interested in distinguishing  
 102 preferences among animal prey items due to the omnivorous nature of these turtles (Bjorndal,  
 103 1985). Nutrient contents of prey items were roughly estimated according to the US  
 104 Department of Agriculture 2009. Fish and squid were used to study whether head-started  
 105 turtles show food imprinting due to early feeding experiences since they are custom food to  
 106 the turtles in the center whereas shrimp and jelly functioned as novel food items. Penaeid  
 107 shrimps are common in the pelagic area of the Andaman Sea and are therefore a highly  
 108 potential prey item of juvenile sea turtles (Tangrok-Olan et. al., 2007). Also *Cassiopeia* sp.  
 109 jellyfish is commonly found in the area on sandy mud-flats of shallow waters (up to 5 m

110 depth) where it attaches to sandy bottoms (George et al., 1979). As plant matter we chose sea  
 111 grass *Cymococea rotundata* which is commonly used as a food source by green turtles  
 112 (Kongkiat Kittiwattanawong, personal communication, 2009). All food items were whole and  
 113 frozen except the jellyfish which was alive. Because of the highly liquid texture of jellyfish it  
 114 was not frozen. Food items were melted under running water before given to turtles.

115

116 **Table 2.** Weight (mean±SD) and nutritional values of prey items used (ARS 2009).

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	Fish	Shrimp	Jelly	Squid	Seagrass
119 <b>Weight (g±SD)</b>	25±4.3	20±2.1	20±3.2	20±2.1	15±3.0
120 <b>Nutrient content</b>	158	106	36	92	
121 <b>(Kcal/100 g)</b>					

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123

#### 124 **Experimental design**

125 Each study turtle (N = 4) was assigned to an individual study pool containing 700 liter sea  
 126 water. Turtles were kept in the pool for a four days adaptation period before starting the  
 127 experiment and fed with fish according to the centre's feeding routines. In the experiment,  
 128 food items were placed at the bottom of each pool side by side so that the turtle had visual  
 129 stimuli of each food item at the same time. We used simultaneous multiple food choices to  
 130 comply with the natural situation in the sea. The turtles' feeding behavior was then observed  
 131 for one hour which was sufficient time for the turtles to consume all offered items. The  
 132 experiment was repeated twice with a one month gap between study groups. The studies were  
 133 conducted during January- February 2009.

134

### 135 **Statistical analysis**

136 To explore differences in the diet preferences of the turtles, univariate analyses were done on  
137 the preference orders of the turtles using food items as a dependent variable. ANOVAs were  
138 followed by *a posteriori* Tukey HSD tests to identify means that differed significantly at  
139  $\alpha=0.05$ . All preference data met assumptions for parametric testing (normality; Kolmogorov-  
140 Smirnov test for homogeneity of variance with Levene's test). Tests were carried out with  
141 SPSS 15.0.

142

143 To test whether the food preferences of the turtles differ, the food preferences of all turtles  
144 were subjected to a non-metric multidimensional scaling (nMDS) ordination based on Bray-  
145 Curtis dissimilarity with the PRIMER-6 package (Clarke, 2006). The effect of study animal  
146 on food choice was tested on all treatments using analysis of similarity (ANOSIM) based on  
147 Bray-Curtis dissimilarity data. ANOSIM uses rank-similarity Bray-Curtis matrices and  
148 randomized permutations to calculate the statistic *R* and a significance level for differences in  
149 the composition of sample groups. *R* lies between 0 and 1, with 0 indicating sample similarity  
150 and 1 indicating dissimilarity (Clarke 1993).

151

## 152 **RESULTS**

### 153 **Diet and foraging behavior**

154 The results in this study showed clear discrimination between food items and some individual  
155 food preferences between the studied turtles. All turtles except one preferred carnivorous  
156 items and had a tendency to avoid the offered seagrass or consume it as the least preferred  
157 choice (Table 3; Table 4). There were also clear behavioral differences between the two turtle  
158 replicate groups. Study group 1 clearly preferred food items that they were used to (fish and

159 squid) and only one turtle fed on the live jellyfish. On the contrary, the other turtles avoided  
 160 contact with the jellyfish. All turtles in study group 1 usually waited about 5 minutes before  
 161 they started feeding and took long pauses between prey items. In study group 2, all turtles had  
 162 a tendency to favor prey items that were easily swallowed and instantly fed on all available  
 163 prey items except seagrass. Seagrass was the least preferred food item in group 2 among all  
 164 turtles. Noteworthy was also that fish, which had the longest handling time of the offered food  
 165 items, tended to be preferred as the forth choice after the other prey items in group 2.

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167 **Table 3.** Number of times each of the five food stimuli (fish, shrimp, jelly, squid, seagrass)  
 168 were chosen as the turtles' first, second, third, forth, last choice or not eaten at all.

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**Food item choices of turtles in study group 1**

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	<b>First</b>	<b>Second</b>	<b>Third</b>	<b>Forth</b>	<b>Fifth</b>	<b>Not eaten</b>
Fish	<b>9</b>	7	7	1	0	8
Shrimp	<b>9</b>	<b>9</b>	2	3	0	9
Jelly	1	2	2	0	1	<b>26</b>
Squid	7	<b>10</b>	9	5	0	5
Sea grass	6	3	7	<b>11</b>	0	5

180 **Table 4.** Number of times each of the five food stimuli (fish, shrimp, jelly, squid, seagrass)  
 181 were chosen as the turtles' first, second, third, forth, last choice or not eaten at all.

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**Food item choices of turtles in study group 2**

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	<b>First</b>	<b>Second</b>	<b>Third</b>	<b>Forth</b>	<b>Fifth</b>	<b>Not eaten</b>
Fish	4	5	8	<b>14</b>	0	1
Shrimp	6	7	<b>10</b>	7	0	2
Jelly	7	<b>11</b>	5	6	1	2
Squid	<b>13</b>	5	7	3	0	0
Sea grass	0	0	0	2	<b>24</b>	6

192

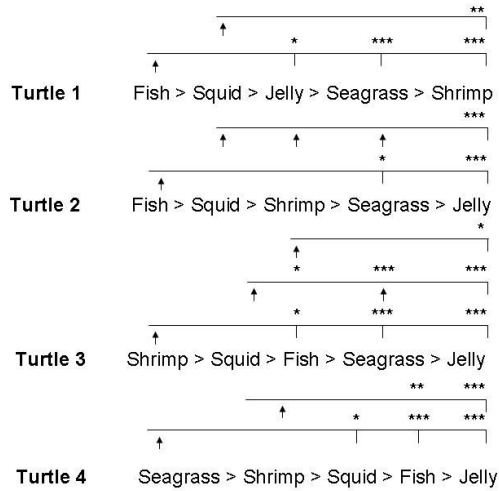
193 Of the offered food items, squid and jelly had the shortest handling time and they were  
 194 usually eaten in a few bites or even swallowed whole. Shrimp was also eaten rapidly (> 10 s.)  
 195 but usually required some chewing. Fish were eaten in the time of a few minutes to 15

196 minutes depending on turtle. Two out of the studied eight turtles did not consume the fish  
197 head and parts of the spine. The food preferences for the turtles in group 1 changed somewhat  
198 over the course of the experiment. During the first few days, all turtles except one chose fish  
199 as their first food choice but switched to other items (jelly, squid, shrimp) after 2-3 days.

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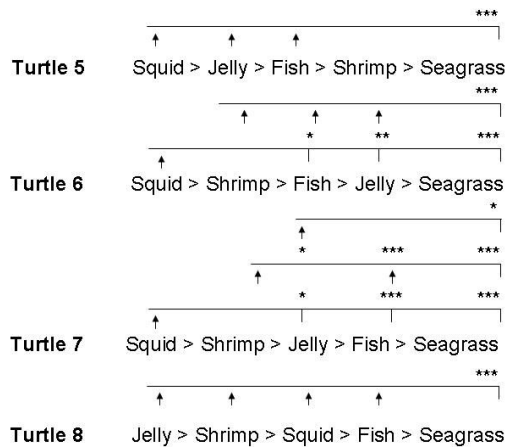
### 201 **Individual diet preferences of subjects**

202 There were clear individual differences of food item preference in study group 1 (Fig. 1; Fig.  
203 2). In general, jellyfish and seagrass belonged to the least favored food items whereas fish,  
204 squid and shrimp belonged to the preferred items. However, one turtle also preferred seagrass.  
205 In study group 2 there were no individual differences between turtles but seagrass was the  
206 least preferred food item of all turtles. Notable is also that no turtle in study group 2 chose  
207 fish as their first food item choice. When all diet preferences were subjected to an nMDS-  
208 analysis, the differing individual preferences of the turtles in study group 1 became apparent  
209 as they were clearly grouped differently whereas the diet preferences of the turtles in study  
210 group 2 showed partial overlaps (Fig. 3).



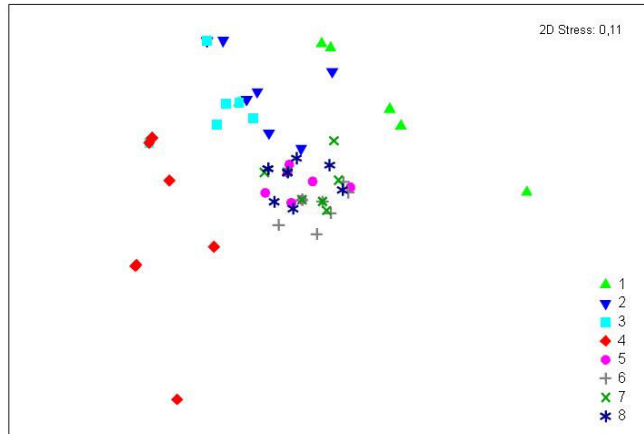
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213 **Fig. 1** Average individual food preferences of turtles in study group 1 as listed in preference  
 214 order. Lines above food items indicate significant differences between items. Arrows above  
 215 bars indicate treatments with significantly higher values as shown by the Tukey post hoc.  
 216  $p = * \leq 0.05, ** \leq 0.01, *** \leq 0.001$ .



217

218 **Fig. 2** Average individual food preferences of turtles in study group 2 as listed in preference  
 219 order. Lines above food items indicate significant differences between items. Arrows above  
 220 bars indicate treatments with significantly higher values as shown by the Tukey post hoc.  
 221  $p = * \leq 0.05, ** \leq 0.01, *** \leq 0.001$ .



222

223 **Fig. 3** MDS-ordination showing the individual food preferences of turtles 1-8. Each dot  
 224 represents a one day sample consisting of the food choice order of a certain turtle. Analysis  
 225 based on Bray-Curtis dissimilarity (raw data).

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227

228 **DISCUSSION**

229 Based on our results, there are no clear food item preferences among juvenile turtles (< 25 cm  
 230 CCL) between small crustaceans, cnidarians, fish and mollusks. However, seagrass seems to  
 231 be the least favored item which may be the result of the age of the study turtles. Juvenile  
 232 turtles usually return to littoral areas and start feeding on plant matter when they exceed a 25  
 233 cm CCL (Bjorndal, 1985). Some preference for fish existed in group 1 but the preference  
 234 changed over the course of the experiment and in group 2, fish was usually preferred as the  
 235 forth choice (Fig. 1-2). The hypothesis that food imprinting due to early feeding experiences  
 236 would exist in head started turtles can therefore be rejected.

237

238 Previous behavioral studies on green turtles have found that they remember specific food  
239 items (Angermeier & Hidalgo 1996). Similar preferences for original food items were found  
240 for green turtles that had been brought into captivity as large juveniles from their inshore  
241 foraging grounds (Swimmer and Mailloux, 2003). These turtles clearly preferred food items  
242 that were eaten first while in captivity. In our study, however, turtles rapidly switched to less  
243 time-consuming prey items and even chose the prey item that they were accustomed to as the  
244 least preferred animal prey item.

245

246 There were big differences in the behavior of the two study groups. Turtles in study group 2  
247 clearly preferred prey items with a short handling time whereas turtles in study group 1  
248 preferred mostly fish and squid. Optimal foraging strategies state that the profitability of a  
249 prey item is the ratio of the item's energy content ( $E$ ) to the time required for handling the  
250 item ( $h$ ) (Krebs and McCleery, 1984). However, in the absence of search and handling time  
251 (and presented with prey in equal densities as in this experiment), the diet choice should be  
252 the items that maximize energy intake. In this experiment, shrimp and squid probably had the  
253 highest profitability since the handling time for fish was much longer. In both study groups,  
254 shrimps and squid were preferred among the first diet choices but did not significantly differ  
255 from other animal prey items. However, accurate nutrient content analysis and exact time of  
256 food handling would have been needed to estimate the most profitable prey in this study.

257

258 Our results suggest that juvenile green turtles thrive on an opportunistic feeding strategy and  
259 that there are some individual food preferences among turtles (Fig. 3). This may be an  
260 adaptation to a dynamic environment that allows them to exploit unpredictable food  
261 resources. Based on our results, head-started turtles would be capable of adapting to natural  
262 foods which they encounter in the wild. Our results are supported by satellite data and mark-

263 recapture experiments from head-starting programs which indicate that sea turtles survive  
264 well in the natural environment and tend to migrate long distances (Caillouet et al., 1995;  
265 Nagelkerken et al., 2003). Head-started green turtles and Kemp's ridley turtles have also been  
266 found nesting (Shaver, 1996) but there is still limited evidence about similar projects having  
267 produced mature adults capable of contributing to local breeding populations at the point of  
268 release. Additional monitoring and research is needed to ascertain if more recently released  
269 cohorts of head-started turtles contribute to local populations and if head-started individuals  
270 have reproductive success comparable with wild turtles. Our results suggest that juvenile  
271 turtles in head-starting programs can be fed with any animal prey items with no significant  
272 effects on the feeding behavior after their release to natural environments. However, caution  
273 should be taken while extrapolating our results since the items fed to the turtles in this study  
274 consisted of dead matter. More studies about the foraging tactics including energy cost and  
275 benefit parameters are needed to assess the feeding behavior of juvenile sea turtles.  
276

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