1	Inter-ecosystem variation in the food-collection behaviour in
2	climbing perch Anabas testudineus, a freshwater fish
3	
4	
5	V. V. Binoy ^{*1} V. B. Rakesh ² and Anindya Sinha ¹
6	
7 8	¹ National Institute of Advanced Studies, Indian Institute of Science Campus, Bangalore 560012, India
9	² School of Environmental Sciences, Cochin University of Science and Technology, Main
10	Campus, Trikakkara, Kochi 682022, India
11	
12	
13	*Corresponding author
14	
15	V. V. Binoy
16	National Institute of Advanced Studies,
17	Indian Institute of Science Campus,
18	Bangalore 560012, India
19	Tel: +91-7829496778
20	
21	Email: vvbinoy@gmail.com
22	
23	Running title: Cross ecosystem variation in food collection
24	
25	
26	
27	
28	
29	
30	
31	

bioRxiv preprint doi: https://doi.org/10.1101/573600; this version posted March 12, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

32 Abstract

33 A unique piscine behaviour—collection and temporary storage of food materials inside 34 the mouth during times of availability and particularly in response to starvation—has 35 been reported in a single species, the climbing perch Anabas testudineus. In this study, 36 we documented a significant variation in the amount of food collected by populations of 37 climbing perch inhabiting different ecological regimes, kole paddy fields, canals and 38 water channels in coconut plantations, after experiencing starvation for two different 39 periods of 24 and 48 h. Our results revealed a significant flexibility in this unique 40 behaviour, depending on the ecological conditions and hunger experienced by the 41 individuals.

42

43 Keywords Feeding behaviour . Foraging . Inter-population variation . Hunger .
44 Behavioural flexibility . Food stocking

45

46 Introduction

47 The collection and storage of food for later consumption is an effective strategy displayed 48 by different species for the successful exploitation of transitory food resources and to out-49 compete potential competitors while foraging in groups [1, 2]. Additionally, the ability to 50 move food materials out of predation-prone feeding grounds to safer areas for 51 consumption enhances the personal safety of the individual [3]. This effective foraging 52 strategy of collection and stocking of food for either short or long durations of time was 53 traditionally considered the domain of only some species of birds and mammals [2, 4] 54 until Binoy and Thomas [5] added a freshwater fish, the climbing perch Anabas 55 *testudineus*, to the list. This fish species, the only one known so far to display this unique 56 behaviour, collects food materials in its mouth before ingestion, in a fashion possibly 57 analogous to the storage of food in the cheek-pouches of cercopithecine primates [4]. 58 Interestingly, as reported in various mammalian species, the climbing perch also 59 enhances food collection over food consumption after experiencing food deprivation [5]. 60 We would, however, like to point out that although this behaviour, as shown by the 61 climbing perch, was described as 'food stocking' by Binoy and Thomas [5], such a term 62 better applies to the relatively more prolonged periods of food storage shown by birds 63 and mammals [2]. The term 'food collection' may be more appropriately used to denote 64 this behaviour, as the climbing perch collects and stores food materials in its mouth for a 65 few minutes alone [5].

66 The climbing perch (Anabas testudineus Bloch) is a shoal-living fish, inhabiting 67 different kinds of lentic and lotic freshwater ecosystems in India and several southeast 68 Asian countries [6]. Being equipped with the labyrinthine organ to breathe atmospheric 69 air, this fish has been reported from waterbodies such as ponds, rivers, marshes, sewage 70 canals, irrigation canals, *kole* paddy fields and areas of saline intrusion, all of which differ 71 significantly in their ecological characteristics [7]. In natural habitats, this fish consumes 72 a wide range of food items including crustaceans, worms, molluscs, insects, algae, soft 73 parts of aquatic plants and organic debris.

It is a well-known fact that the tracing of modulations that characterise the vital components of the foraging behaviour of a species in relation to the changes happening in the biotic and abiotic components of its environment is crucial for a comprehensive understanding of the evolution of its foraging behaviour [8]. The climbing perch thus offers an excellent model system to study this vital topic in a piscine species due to its tremendous capacity to adapt to starkly contrasting ecological conditions [7].

Furthermore, such information will also be useful in optimising food consumption behaviour and hence, enhancing the survival and growth of this economically important fish in artificial environments in which it is cultivated extensively. Unfortunately, till date, very few studies have explored foraging behaviour of this species in natural or artificial environments [7, 9, 10], no information is available on the food collection behaviour of climbing perch populations surviving in contrasting ecological conditions or on the factors influencing this behaviour.

The current paper examines variation in food-collection behaviour of climbing perch individuals from three different ecosystems—*kole* paddy fields, shallow water channels in coconut plantations and canals—and deprived of food for two different durations, 24 and 48 h.

91

92 Materials and Methods

93 Subject fish and husbandry

94 Climbing perch were collected from the following three ecosystems, located in different
95 parts of Thrissur district of Kerala state, southern India.

96 Water channels: These shallow channels are characteristic of coconut and banana 97 plantations of central Kerala [7]. The climbing perch were collected from channels of a 98 coconut plantation, located at Edathirinji (10.33°N, 76.18°E). This particular water body 99 (mean water depth \pm SD of 60 \pm 20 cm and breadth, 50 \pm 15 cm), situated in between the 100 rows of coconut plants, was turbid, foul-smelling, due to decaying plant materials, and 101 occupied by various aquatic and semi-aquatic plants. Although poor-quality water and 102 reduced availability of space in this shallow ecosystem supported very few piscine 103 species, the climbing perch survived in this extreme environment due to its capacity to

breathe atmospheric air and pliantly available food materials, insect larvae and decayingplant materials [7].

106 *Canals*: This lotic ecosystem, from which the fish were collected, was located at 107 Irinjalakuda (10.34°N, 76.19°E) and was contaminated by sewage from houses 108 present on its banks. The climbing perch population living in this ecosystem had to 109 face considerable harshness of water flow, transitory food materials as well as the 110 reduced availability and diversity of food items [7].

Kole paddy fields: Kole is a unique agro-wetland ecosystem, located in the central region of Kerala state. Being positioned in the basins of two major rivers, this paddy field resembles a large shallow lake during the monsoons and is utilised for cultivating paddy for the rest of the year [11]. The *kole* paddy fields are typically extensive in distribution, hold good-quality water, rich in spatial complexity and exhibit higher levels of primary and secondary productivity [12], as compared to the irrigation canals and channels.

The fish were collected from the Irinjalakuda (10.35°N, 76.21°E) region of the *kole* and other two focal ecosystems with the help of expert fishermen and transferred to the laboratory. Only fish of standard length (mean \pm SD of 62.4 \pm 26.0 mm) were used in the experiments. Due to the lack of sexual dimorphism in this species [13], however, the subject fish could not be sexed.

Individuals from each ecosystem were kept isolated in aquaria ($45 \times 22 \times 22$ cm) and these aquaria themselves were used for the subsequent experiments involving the subject fish. Water temperature was maintained at $25 \pm 1^{\circ}$ C and light hours at 12L: 12D. Three sides of all aquaria were covered with black paper while steel grids, placed on the top, prevented the fish from jumping out. Twenty-five food pellets were dropped together at a specific site in the aquarium once a day (between 09:00 and 10:30) to acclimatise the fish with a specific feeding schedule [5]. No hesitation was shown by the subject fish in consuming the commercial food pellets from the first day of isolation itself. Unused pellets were siphoned out 30 min after their addition to the aquarium.

131 After giving five days for acclimating with the laboratory environment, the food-132 collection behaviour was tested as follows. In order to standardise their hunger state, the 133 subject fish were deprived of food materials for 24 h before the experiment. Food pellets 134 of length (mean \pm SE) of 3.58 \pm 0.14 mm and mass 0.01 \pm 0.003 g were dropped, one by 135 one, at intervals of 1 s at the same site in the aquarium. The subject fish continuously 136 collected the pellets in their mouth (see also Binoy and Thomas 2008 and Supplementary 137 Material S1). Pellets falling out of the mouth of the fish due to overfilling or the 138 individual not gathering food granules for a continuous period of 120 s were marked the 139 end points of the experiment. The same fish were re-tested to evaluate the impact of 140 prolongation of the starvation period to 48 h, six days after the first experiment, by 141 following the same protocol⁵. The availability of climbing perch was much less in the 142 water channel and canal ecosystems, in comparison to the *kole* paddy fields and hence, 16 143 individuals were tested from each of the first two ecosystems and 20 fish from the kole 144 paddy field.

145 The parametric tests ANOVA, Tukey's test and paired *t*-test were used for 146 analysis as the data was established to follow normal distribution (Kolmogorov-Smirnov 147 Test).

148

149 **Results**

150 The study climbing perch populations, collected from different ecological conditions,151 differed significantly in their ability to collect food materials inside their mouths after

experiencing food deprivation for 24 h (ANOVA, $F_{2,49} = 4.63$, p < 0.05; Fig. 1). The fishes from the canal ecosystem collected significantly greater number of pellets than did their counterparts from the water channels (Tukey's test, *tij* = -3.13, p < 0.01). However, no significant difference was observed in the number of pellets collected by the members of the canal and *kole* paddy field (*tij* = -1.93, p > 0.05) or between the water channel and *kole* paddy field populations (*tij* = 0.95, p > 0.05).

158 Only individual climbing perches from the kole paddy field exhibited significant 159 modification in the food collection behaviour (ANOVA, $F_{2.57} = 9.26$, p < 0.001) when the 160 duration of food deprivation was increased from 24 to 48 h. These fish collected 161 significantly more food material in the mouth in response to prolonged starvation than did 162 their counterparts from the canals (tij = 2.55, p < 0.05) or from water channels (tij = 4.79, 163 p < 0.001). However, channel and canal populations were not significantly different from 164 one another in the performance of this unique behaviour when the duration of food 165 derivation was doubled (tij = -2.12, p > 0.05).

Differences in the amount of food pellets collected by the individuals from each of the three focal populations, after experiencing starvation for the two different periods, was also analysed. The climbing perch from *kole* paddy fields almost doubled the amount of food collected (paired *t*-test, $t_{19} = -4.17$, p < 0.001; Fig. 1) when the food deprivation was increased from 24 to 48 h but no such behavioural modification was observed in the individuals from irrigation canals ($t_{15} = 2.12$, p > 0.05) or from water channels ($t_{15} = 1.74$, p > 0.05).

173 **Discussion**

The marked differences in the food-collection behaviour of the climbing perch,collected from three different ecological conditions, could be the result of variation in the

176 food availability, inter- and intra-specific competition and predation pressures 177 experienced by the individual fishes in their respective habitats [1,5]. The climbing perch 178 living in the canal, possibly the harshest amongst the three ecosystems studied, have to 179 survive on the very little food material available in their surroundings. The prevalent 180 turbidity and water flow could also increase the difficulty of food acquisition manifold 181 for this visually orienting species [14] in the canals. According to Binoy and Prasanth [7], 182 the only food present in the gut of the climbing perch, collected from this ecosystem, 183 were insect larvae (mainly *Chironomus* spp.) and debris, and the studied individuals had 184 rather low amounts of food in their digestive tracts. Hence, the climbing perch from such an ecosystem could be collecting the maximum number of food pellets it could, at the 185 186 time of availability, which reflected as significant deviation from those taken by the fish 187 from the water channel and *kole* paddy field populations.

188 In contrast to the canal and water channel ecosystems, the *kole* paddy field 189 constitutes a geographically widespread habitat covering more than 10 thousand hectares 190 and famous for the diversity of microhabitats and life forms that it harbours [12]. 191 However, the climbing perch inhabiting this ecosystem typically have to face increased 192 levels of both predation pressures and competition for food materials from conspecific as 193 well as heterospecific individuals, in comparison to their counterparts from other two 194 focal habitats. Generally, air-gulping piscine species synchronise their surfacing activity 195 in order to reduce the probability of falling prey to aerial predators [15]. Accordingly, the 196 number of climbing perch observed preforming orchestrated air-gulping activity in each 197 bout was considerably high in the kole paddy fields (pers. obs.).

198 Moreover, an exploration of the catch of fishermen revealed that the number of 199 individuals of the major piscine predators of the subject species, the snakeheads (*Channa*

200 striatus and C. marulius), were much less prevalent in the water channel and canal 201 ecosystems (pers. obs.). Enhancing levels of food collection, therefore, could be a 202 behavioural adaptation exhibited by hungry climbing perches living in the *kole* paddy 203 field ecosystem, to out-compete their conspecifics or heterospecifics by acquiring more 204 food during times of availability. The climbing perch investigated in a previous study [5] 205 were captured from a large pond, which resembled a kole paddy field in its ecological 206 properties, and showed a similar enhancement in their food collection strategy. 207 Furthermore, a recent study by Zworykin [10] has proven that the presence of conspecific 208 individuals could influence the feeding behaviour of climbing perch; this study reported a 209 significant variation in the foraging behaviour of individuals when tested in isolation and 210 in presence of the conspecifics. It may also be noted here that a similar analogous 211 enhancement of food stocking in the cheek pouch to out-compete competitive 212 conspecifics during intensive feeding bouts has also been reported in troops of different 213 cercopithecine primate species [1, 5].

214 In conclusion, therefore, individuals of species such as the climbing perch could 215 vary their ability to utilise food resources due to their adaptation to the food web in which 216 they are embedded [16, 17]. Additionally, however, the species that we studied is famous 217 not only for its specific ability to survive under a wide range of ecological conditions but 218 also for migration over land and consequently changing habitats during the monsoons 219 [18]. An earlier study by our group [7] revealed that climbing perch populations living in 220 contrasting ecological conditions display great disparity in the nature and quantity of food 221 items consumed. A detailed analysis of the adaptive modifications of foraging behaviour 222 by this species in dissimilar ecosystems could thus reveal the interplay between 223 physiological, social and environmental factors in determining the unique food-collection

224	1 1 •	1. 1	11 .1 .	•
224	behaviou	r disnlavec	1 hv this	snectes
	00lla v 10ul	r displayed	a Oy unis	species.

225	Acknowledgements VVB is grateful to the Science and Engineering Research Board
226	(SERB), Department of Science and Technology, Government of India, for a Young
227	Scientist (Fast Track) Grant (SB/FT/LS-155/2012) that enabled this study. The
228	experiments reported in this paper comply with the current relevant laws of India.
229	
230	References
231	
232	1. Smith LW, Link A, Cords M (2008) Cheek pouch use, predation risk, and feeding
233	competition in blue monkeys (Cercopithecus mitis stuhlmanni). Am J Phys
234	Anthropol 137: 334–341
235	2. Pravosudov VV, Roth II TC (2013) Cognitive ecology of food hoarding: the
236	evolution of spatial memory and the hippocampus. Ann Rev Ecol Evol Syst
237	44: 173–193
238	3. Valone TJ, Lima SL (1987) Carrying food items to cover for consumption: The
239	behavior of ten bird species feeding under the risk of predation. Oecologia 71:
240	286–294
241	4. Lambert JE (2005) Competition, predation and evolutionary significance of
242	cercopithecine cheek pouch: The case of Ceropithecus and Lophocebus. Am J
243	Phys Anthropol 126: 183–192
244	5. Binoy VV, Thomas KJ (2008) Influence of hunger on the food stocking behaviour
245	of climbing perch. J Fish Biol. 73: 1053–1057
246	6. Talwar PK, Jhingran AG (1991) Inland fishes of India and adjacent countries.
247	Oxford and I B H Publishing Company, New Delhi

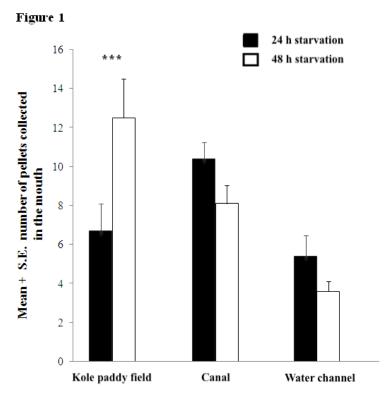
248	7.	Binoy VV, Prasanth PS (2016) Diet variation in climbing perch populations
249		inhabiting eight different types of ecosystems. ArXiv Preprint, arXiv:1610.03668.
250	8.	Quispe R, Villavicencio CP, Cortés A, Vásquez RA (2009) Inter-population
251		variation in hoarding behaviour in degus, Octodon degus. Ethology 115:
252		465–474
253	9.	Binoy VV (2015) Food patch choice in climbing perch Anabas testudineus
254		(Bloch, 1792). I J Fish 62: 149–151
255	10	. Zworykin DD (2018) The behaviour of climbing perch, Anabas testudineus, with
256		novel food in individual and social conditions. J Ichthyol 58: 260-264
257	11	Johnkutty I, Venugopal VK (1993) Kole-Lands of Kerala. Kerala Agricultural
258		University, Trissur, India
259	12	. Thomas JK, Sreekumar S, Cheriyan J (2003) Muriyad wetlands: Ecological
260		changes and human consequences. Project report submitted to Kerala Research
261		Programme on Local Development, Centre for Developmental Studies,
262		Thiruvananthapuram, India
263	13	Zworykin DD (2012) Reproduction and spawning behaviour of the climbing
264		perch Anabas testudineus (Perciformes, Anabantidae) in an aquarium. J Ichthyol
265		52: 379–388

266	14. Binoy VV, Kasturirangan R, Sinha A (2015) Sensory cues employed for the
267	acquisition of familiarity-dependent recognition of a shoal of conspecifics by
268	climbing perch (Anabas testudineus Bloch). J Biosci 40: 225–232
269	15. Killen SS, Esbaugh AJ, F Martins N, Tadeu Rantin F, McKenzie D J (2018)
270	Aggression supersedes individual oxygen demand to drive group air-breathing in
271	a social catfish. J Anim Ecol 87: 223–234
272	16. Bolnick DI, Amarasekare P, Araújo MS, Bürger R, Levine JM, Novak M, Rudolf
273	VH, Schreiber SJ, Urban MC, Vasseur DA (2011) Why intraspecific trait
274	variation matters in community ecology. Trends Ecol Evol 26: 183–192
275	17. Nifong JC, Layman CA, Silliman BR (2015) Size, sex and individual-level
276	behaviour drive intra-population variation in cross-ecosystem foraging of a top-
277	predator. J Anim Ecol 84: 35–48
278	18. Sokheng C, Chhea CK, Viravong S, Bouakhamvongsa K, Suntomratana U,
279	Yoorong, N, Tung NT, Bao TQ, Poulsen AF, Jorgensen JV (1999) Fish
280	migrations and spawning habits in the Mekong mainstream: a survey using local
281	knowledge (basin-wide). Assessment of Mekong Fisheries: Fish Migrations and
282	Spawning and the Impact of Water Management Project (AMFC). AMFP Report
283	2, Vientiane, Lao PDR
284	
285	
286	
287	
288	

bioRxiv preprint doi: https://doi.org/10.1101/573600; this version posted March 12, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

290 Legend to Figure 1

- 292 Mean number of food pellets collected in the mouth by climbing perch living in different
- 293 ecosystems, after experiencing food deprivation for 24 and 48 h.



bioRxiv preprint doi: https://doi.org/10.1101/573600; this version posted March 12, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

306 Legend to the Electronic Supplementary Material S1

- 308 The study species, climbing perch, collecting food pellets for storage in its mouth.