This is a pre-copyedited, author-produced version of an article accepted for publication, following peer review.

Published version: https://dx.doi.org/10.1111/1365-2664.14220

NIOZ Repository: http://imis.nioz.nl/imis.php?module=ref&refid=352101

[Article begins on next page]

1 **Evaluating staging habitat quality to advance the conservation of a declining**

2 **migratory shorebird, Red Knot *Calidris canutus***

3 Tong Mu[1]*, Shangxiao Cai[2], Hebo Peng[3,4,5], Chris J. Hassell[6], Adrian Boyle[6],

4 Zhengwang Zhang[7], Theunis Piersma[3,4,5,6], David S. Wilcove[1,8]

5

6 [1] Department of Ecology and Evolutionary Biology, Princeton University, Princeton,

7 NJ 08544, USA

8 [2] Department of Biology, Lund University, Sölvegatan 37, Lund, Sweden

9 [3] Rudi Drent Chair in Global Flyway Ecology, Conservation Ecology Group,

10 Groningen Institute for Evolutionary Life Sciences (GELIFES), University of

11 Groningen, PO Box 11103, Groningen, 9700 CC, The Netherlands

12 [4] Department of Coastal Systems, NIOZ Royal Netherlands Institute for Sea Research,

13 PO Box 59, Texel, The Netherlands

14 [5] Center for East Asian–Australasian Flyway Studies, School of Ecology and Nature

15 Conservation, Beijing Forestry University, Qinghua East Road 35, Beijing, 100083,

16 China

17 [6] Global Flyway Network, PO Box 3089, Broome, WA, 6725, Australia

18 [7] Ministry of Education Key Laboratory for Biodiversity Science and Ecological

19 Engineering, College of Life Sciences, Beijing Normal University, Beijing 100875,

20 China

21 [8] Princeton School of Public and International Affairs, Princeton University,

22 Princeton, NJ 08544, USA

23 * Corresponding author: mutongpku@gmail.com

**Abstract**

1. Identifying where and when population "bottlenecks" occur is critical to the conservation of migratory species, many of which are declining precipitously worldwide. Especially challenging is the evaluation of changes to staging sites. These sites are indispensable links in the migratory cycle but are typically used only briefly.

2. We devised a field-based approach to assess the quality and carrying capacity of a critical staging site in Nanpu, China, for the declining, migratory Red Knot (*Calidris canutus rogersi & C. c. piersmai*) during northward migration. The Nanpu tidal flat supports 50,000-100,000 Red Knots annually, and while there, the knots feed almost exclusively on the bivalve *Potamocorbula laevis*. We simultaneously monitored changes in the abundance of Red Knots and bivalves across the entire staging site in spring 2018.

3. After taking into account potential competition with other shorebird species, we estimated that the Nanpu tidal flat was capable of supporting approximately 1.46-1.70 times the current observed level of usage of the site by Red Knots, and therefore is operating below, but close, to carrying capacity with respect to food resources for Red Knots. This result suggests that any further development of this site could harm the EAAF Red Knot population.

4. *Synthesis and applications*: Quantitative monitoring and evaluation of habitat quality of staging sites are essential to successfully conserve declining migratory species. In particular, researchers and conservation practitioners should incorporate both population size and staging duration in order to more accurately

47    assess the importance of different sites and to quantify how changes in staging

48    habitat quality may translate into changes in migrant population sizes at both local

49    and global scales.

50

51

52    **摘要**

53    1. 识别影响候鸟种群数量变化的关键栖息地和生命阶段，对于保护这些快速减少

54    的迁徙物种来说十分重要。尽管每年利用的时间较短，中停地对于候鸟的迁徙和生

55    存有着至关重要的作用，然而如何量化和评估中停地的栖息地质量及其对于候鸟种

56    群的影响却颇具挑战。

57    2. 本研究设计了一套基于野外调查的中停地栖息地质量及环境承载力评估方案，

58    并利用每年春季北迁期间在河北南堡滩涂停歇的红腹滨鹬 (*Calidris canutus*

59    *rogersi* & *C. c. piersmai*) 测试了该方案的可行性。每年春天约有 5-10 万只红腹

60    滨鹬到访南堡，并主要取食滩涂中的光滑河篮蛤 (*Potamocorbula laevis*) 进行能

61    量补给。我们在 2018 年春天对整个南堡滩涂的红腹滨鹬和光滑河篮蛤数量变化进

62    行了持续监测。

63    3. 将其他种类鸻鹬的种间竞争影响考虑在内，南堡滩涂的红腹滨鹬环境承载力约

64    为当年实际种群利用强度的 1.46-1.70 倍。综合分析表明，南堡滩涂的红腹滨鹬利

65    用强度十分接近其环境承载力，而当地栖息地质量的变化将会极大地影响整个东亚

66    -澳大利西亚迁飞区的红腹滨鹬种群数量。

67    4. 总结及实际应用：针对重要中停地的栖息地质量监测和评估对于保护那些数量

68    快速下降的候鸟种群具有十分重要的意义。在研究和保护中，人们尤其需要同时考

69    虑中停地的候鸟数量及其停留时长，以便更为准确地评估不同中停地对于候鸟的重

70    要性，进而定量化地研究和预测栖息地质量变化将如何影响候鸟种群数量变化。

71

72

73    **Key words**: carrying capacity, staging site, habitat quality, Red Knot, *Calidris*

74    *canutus*, conservation, migration

75

76

## Introduction

Migratory species are crucial for the functioning of ecosystems (Bauer & Hoye, 2014), yet their populations worldwide are declining precipitously due to anthropogenic threats (Wilcove & Wikelski, 2008). To conserve migratory species, it is imperative to identify where and when population "bottlenecks" occur – i.e., to know which sites and life stages are exerting a significant impact on overall population growth (Crouse *et al.*, 1987; McGowan *et al.*, 2011; Liu *et al.*, 2022). This task is challenging due to the many sites and habitats migratory animals utilize annually, the myriad threats they face, and the scarcity of information on migratory connectivity (Webster *et al.*, 2002). Especially challenging is evaluating the threats or changes to staging sites. The loss or degradation of staging sites has been linked directly to local and global population declines (McGowan *et al.*, 2011; Moores *et al.*, 2016; Piersma *et al.*, 2016), yet, it remains particularly challenging to answer the seemingly simple question, "how much staging habitat does a migratory species need?" (Kraan *et al.*, 2009).

Coastal shorebirds represent an excellent group for exploring questions related to staging sites, because, in many species, a large fraction of the population crowds into a discrete number of sites, illustrating a migration pattern consisting of two distinct phases: prolonged staging periods and long-distance migratory flights. Moreover, coastal shorebird populations are declining precipitously worldwide (Delany *et al.*, 2009; Studds *et al.*, 2017; Rosenberg *et al.*, 2019). Along the East Asian–Australasian Flyway (EAAF), shorebird declines have been largely attributed to the loss of critical

100      staging habitats, especially Yellow Sea tidal flats, due to coastal development

101      (Piersma *et al.*, 2016; Studds *et al.*, 2017). This raises the important issue of how

102      much of the remaining intertidal habitat needs to be protected to stabilize current

103      populations or, ideally, sustain larger populations of EAAF shorebirds if other

104      threats can be abated (Yang *et al.*, 2011).

105

106      Here, we devise a field-based approach to assess the quality and carrying capacity of

107      Nanpu tidal flat, the best known staging site for Red Knots (*Calidris canutus piersmai*

108      and *C. c. rogersi*) migrating along the EAAF, by simultaneously monitoring changes

109      in food abundance and numbers of foraging birds across the entire site. EAAF Red

110      Knot population has decreased at an average rate of -4.4% $yr^{-1}$ over the past three

111      decades (Studds *et al.*, 2017) driven primarily by the loss of staging habitat (Yang *et*

112      *al.*, 2011; Piersma *et al.*, 2016). During northward migration, at least 45% and likely

113      close to 100% of the EAAF Red Knot funnel into Bohai Bay, primarily to the Nanpu

114      tidal flat and adjacent areas (Rogers *et al.*, 2010; Tomkovich *et al.*, 2013; Lok *et al.*,

115      2019; Piersma *et al.*, 2021).

116

117      Previous research at Nanpu by Yang *et al.* (2013) found that Red Knots feed almost

118      exclusively on a small bivalve, *Potamocorbula laevis*, which constituted 92.7% (in

119      ash-free dry mass, AFDM) of the knots' diet reconstructed from fecal samples. *P.*

120      *laevis* is mostly sedentary and does not reproduce during the northward migration

121      (Yang *et al.*, 2013). Continuous monitoring of land cover, Red Knots, and benthic

122      invertebrates at Nanpu showed no major changes to the intertidal habitat, its bird

123    community (including Red Knots), the foraging behavior of Red Knots, or the

124    benthic invertebrate community from 2013 to 2018, the year when we conducted

125    fieldwork (Yang *et al.*, 2016; Lok *et al.*, 2019; Hebo Peng unpublished data). Thus,

126    there is no evidence to suggest that the importance of *P. laevis* or Nanpu to Red

127    Knots has changed. Accordingly, the Nanpu tidal flat offers a rare opportunity to

128    observe most or all of the flyway population of a declining migratory shorebird at a

129    single critical staging site where its diet is largely restricted to a single food resource

130    that can be measured readily. This is similar to the situation of Red Knots (*C. c. rufa*)

131    staging in Delaware Bay, United States, which rely on the eggs of Atlantic Horseshoe

132    Crab (*Limulus polyphemus*) (Baker *et al.*, 2004; McGowan *et al.*, 2011).

133

134    We first sample the distribution of food resources on the Nanpu tidal flat. Combining

135    data on food resources with bird monitoring data, we ascertain whether changes in

136    food distribution and abundance during the main staging period of Red Knots arise

137    mainly from foraging by Red Knots. We then estimate the carrying capacity of this

138    critical staging area for Red Knots. Our approach is particularly useful in the absence

139    of information on functional response (a key component of depletion models that

140    are often used to estimate carrying capacities (Sutherland & Anderson, 1993)), or

141    giving-up densities (GUDs, the prey density below which birds will stop foraging at a

142    given spot) (van Gils *et al.*, 2004).

143
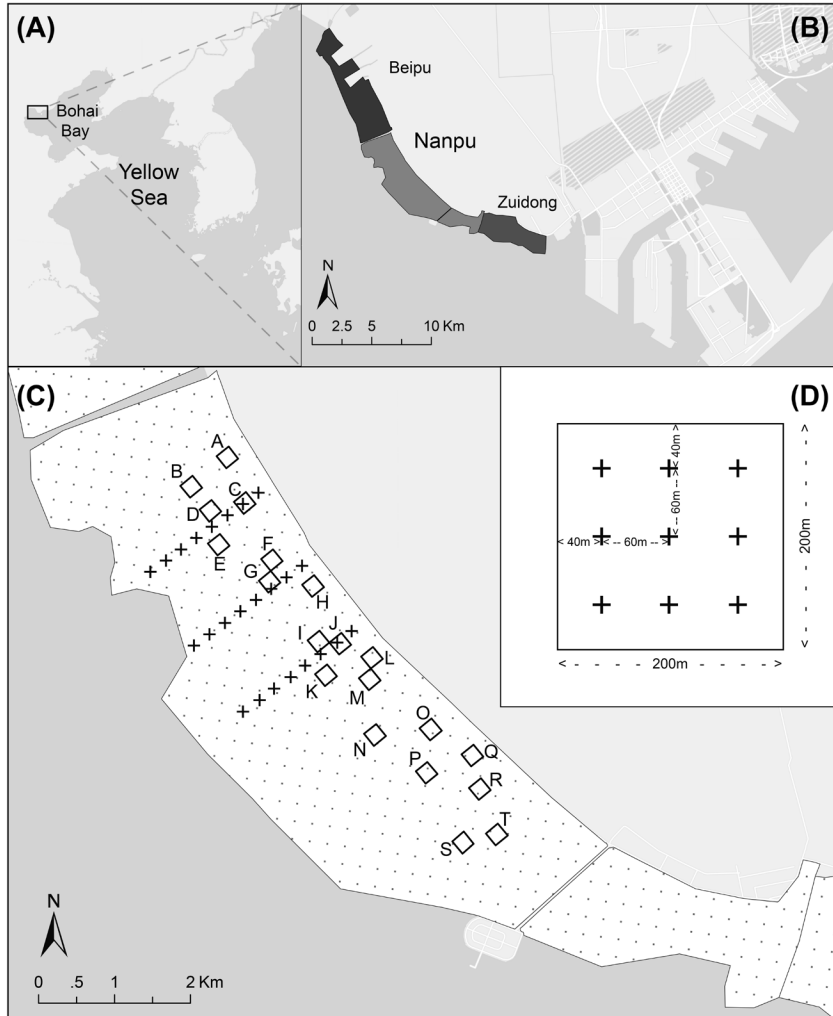
144

145　**Figure 1**. Study site and the layout of sampling plots and transects on the Nanpu

146　tidal flat. (a) Location of Nanpu in Bohai Bay. (b) The tidal flats at Nanpu (study site),

147　Beipu, and Zuidong constitute the area commonly referred to as the Luannan coast

148　(Rogers *et al.*, 2010). (c) The layout of sampling plots (letter-marked squares, with

149　the layout of benthic sampling points in each plot shown in (d)) and transects on the

150　Nanpu tidal flat. Crosses in (c-d) denote the location or arrangement of benthic

151　sampling points. In all figures, lighter grey areas represent land, grey represents sea

152　or other water body, and darker grey areas in (b) or stippled areas in (c) represent

153　tidal flats.

154

**Materials and Methods**

*Study site*

Fieldwork was conducted primarily on the Nanpu tidal flat in Bohai Bay, China (39.1

N, 118.2 E) from April to June 2018 (Figure 1). Although the two subspecies of Red

Knot of EAAF differ slightly in their migration schedule and staging duration, they

overlap extensively in their seasonal use of this staging site and exhibit identical

foraging behavior (Tomkovich *et al.*, 2013; Piersma *et al.*, 2021); accordingly, we did

not distinguish between them in our fieldwork, which encompassed the northward

migration period of both subspecies.

*Mapping the distribution of food resources*

To measure the distribution and abundance of food resources for Red Knots, we

collected data on benthic invertebrates from two sets of sampling points on the tidal

flat. First, we randomly selected 20 200m × 200m plots within 1km from the seawall

(squares in Figure 1c), the main area where knots forage (Yang *et al.*, 2016; Mu &

Wilcove, 2020). In each plot, we systematically selected nine sampling points in a

3×3 array, 60m apart from each other and 40m away from the plot boundary

(Figure 1d). To cover the entire elevational gradient of the tidal flat, we also set up

three transects running perpendicular to the seawall, with 8 sampling points, 250m

apart, along each transect (Figure 1c). This gave us a total of 204 benthic sampling

points arrayed in plots (20 plots × 9 points each) and transects (3 transects × 8

points each) across the tidal flat.

177

178 We conducted two rounds of benthic sampling, coinciding with the beginning and

179 end of Red Knot staging period. The first round took place on May 1–2 (transects)

180 and 2–5 (plots), and the second round took place on May 24, 30 (transects) and 24–

181 27 (plots). One benthic core sample (5cm deep, 15.5cm in diameter) was taken at

182 each sampling points during each round of sampling, where 5cm represents the

183 maximum depth that foraging knots may reach (Yang *et al.*, 2013). Core samples

184 were sieved through a 0.5mm mesh in the field and food items remaining on the

185 mesh were placed immediately into 75% ethanol (polychaetes and other soft-

186 bodied items) or plastic bags (mollusks, crabs, etc.), and then stored at -20℃.

187 Because bivalves constitute almost the entirety of the Red Knots' diet at Nanpu

188 (Yang *et al.*, 2013), only bivalve species were further processed in the laboratory.

189 Each individual bivalve was identified to species. Shell length of all bivalves

190 collected from plot sampling points plus a randomly selected subsample of

191 individuals from each transect sampling points were measured to the nearest 0.01

192 mm under a dissecting microscope.

193

194 Since *P. laevis* constitutes 92.7% of the Red Knot's diet (Yang *et al.*, 2013) and >94%

195 of all bivalve individuals sampled at this study site, we used the abundance of *P.*

196 *laevis* with a shell length <8mm (>99.99% of all *P. laevis* sampled) to represent the

197 potential food resources for Red Knots. To obtain the biomass of food available to

198 the knots, we transformed the shell lengths of *P. laevis* into ash-free dry mass (AFDM,

199 g), using the allometric relationship derived by Yang *et al.* (2013). To map the

200  spatial distribution of *P. laevis*, we spatially interpolated the density (log-

201  transformed) and AFDM of *P. laevis* with kriging (Cressie, 1993) using package *fields*

202  (Nychka *et al.*, 2017) in R (v. 3.6, R Core Team).

203
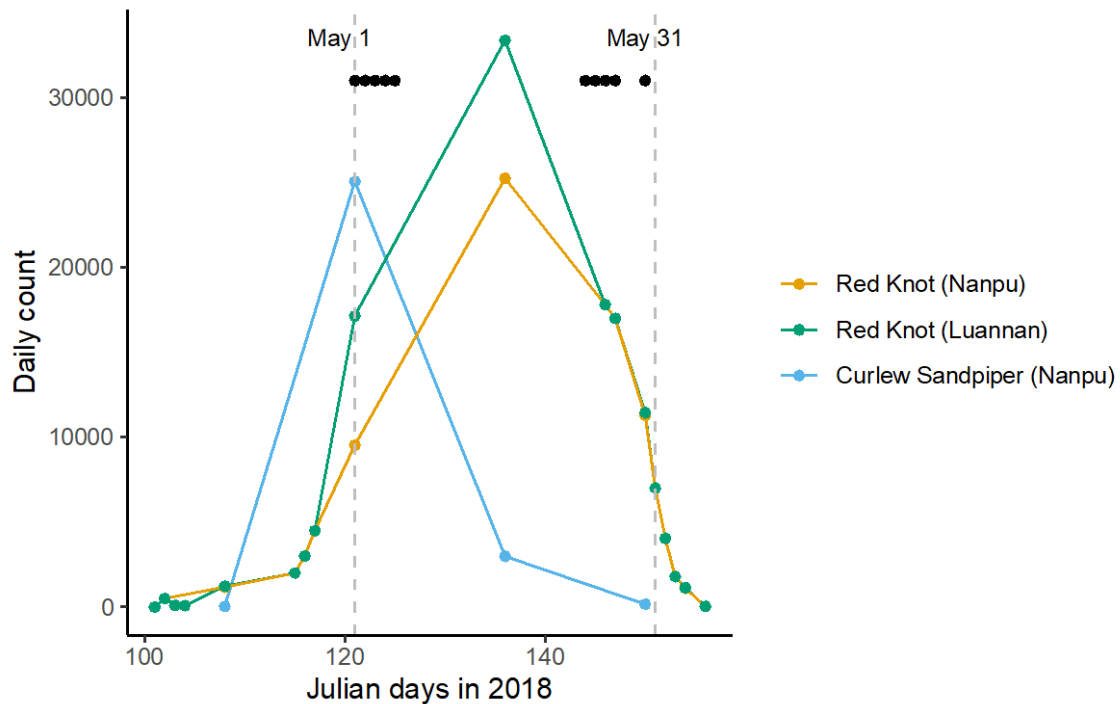


204

205  **Figure 2**. Daily counts of Red Knots at Nanpu and Luannan (the larger staging area

206  that includes Nanpu; see Figure 1), compared to Curlew Sandpiper numbers at

207  Nanpu. Colored dots represent bird counting data. Black dots denote the dates of

208  benthic sampling.

209

210

211  *Verifying the impacts of Red Knot foraging on* P. laevis *abundance*

212  A key yet infrequently tested assumption in estimating carrying capacity is that the

213  consumption of prey by the focal species is the main driver of observed reductions

214    in prey abundance (Bijleveld *et al.*, 2015). While the diet of Red Knots at Nanpu has

215    been well studied (Yang *et al.*, 2013), comparable data do not exist for most other

216    shorebird species there. Based on observed foraging behaviors and distribution

217    patterns of shorebird species at Nanpu, we identified Curlew Sandpiper (*C.*

218    *ferruginea*) as the only likely competitor of Red Knots. Similar to Red Knots, the

219    EAAF Curlew Sandpiper population has been declining at ~7.5% annually (Studds *et*

220    *al.*, 2017). Nanpu is also the most important staging site for Curlew Sandpipers

221    along the EAAF and regularly holds >10%, and up to 69% of the species' total flyway

222    population during northward migration (Yang *et al.*, 2011; Lei *et al.*, 2018). Curlew

223    Sandpipers utilize both saltpans and tidal flat for foraging at Nanpu (Lei *et al.*, 2021).

224    While on the tidal flat, Curlew Sandpipers usually forage with or near Red Knots in

225    the upper zone (Mu & Wilcove, 2020) and consume *P. laevis* at least occasionally

226    (Lei *et al.*, 2021; Weipan Lei unpublished data), but the contribution of this bivalve

227    to Curlew Sandpipers' diet is unknown. Curlew Sandpipers at Nanpu peak

228    approximately two weeks ahead of Red Knots, but there is a period of overlap when

229    the two species co-occur in sizeable numbers (Figure 2). To estimate the degree to

230    which Curlew Sandpipers could have competed with Red Knots and contributed to

231    the changes in *P. laevis* abundance, we monitored the abundance of Curlew

232    Sandpiper in the study area once every two weeks, and assumed varying levels of *P.*

233    *laevis* intake by them, ranging from 0 (no take of *P. laevis*) to 1 (taking *P. laevis* at the

234    same rate as do Red Knot individuals, Table S1). We define this relative rate of *P.*

235    *laevis* consumption by Curlew Sandpipers as the "impact factor" in the following

236    analyses.

237

238 We compared the decline in *P. laevis* abundance and the total cumulative foraging

239 time of Red Knots between the two rounds of benthic sampling in each of the 20

240 benthic sampling plots, and estimated the intake rate of individual Red Knot

241 consuming *P. laevis*, while taking into account the potential competition from

242 Curlew Sandpipers. If changes in *P. laevis* abundance in the sampling plots are

243 proportional to the total cumulative foraging time of Red Knots recorded within the

244 plots, and the estimated intake rate of Red Knot is comparable to that estimated

245 from an earlier study (90±24 bivalve individuals $min^{-1}$) that focused on the same

246 study system but used a different approach (Yang *et al.*, 2013), we would then

247 conclude that the observed changes in *P. laevis* abundance were primarily due to

248 depredation by Red Knots (and potentially Curlew Sandpipers) rather than natural

249 death or movement of *P. laevis*.

250

251 Specifically, to estimate the total cumulative foraging time of Red Knots, we

252 monitored the numbers of foraging Red Knots in each plot at average intervals of

253 28±33 min (mean±SD) during daytime tidal cycles. We first calculated the

254 cumulative foraging time of Red Knots for each plot during each monitored tidal

255 cycle, in bird-minute(s), by plotting changes in the numbers of foraging knots

256 against the time during the tidal cycle. The area under this curve is the cumulative

257 foraging time of Red Knots, which provides a quantitative measurement of Red

258 Knots' foraging intensity than using just peak or average numbers of foraging

259 individuals. We then multiplied the cumulative foraging time of the daytime tidal

260    cycle by 2 to get the daily cumulative foraging time; we did this because there are

261    two tidal cycles per 24-hour day, and Red Knots forage by probing, which allow

262    them to feed with equal intensity during both day and night (Piersma *et al.*, 1998;

263    van Gils & Piersma, 1999). As we did not measure the daily cumulative foraging time

264    during all of the days between the two rounds of benthic sampling, the total

265    cumulative foraging time of Red Knots in each plot was calculated by multiplying the

266    average daily cumulative foraging time (from the days with measurements) by the

267    total number of days between two rounds of benthic sampling.

268

269    To evaluate the potential effect of Curlew Sandpipers on changes in *P. laevis*

270    abundance and on our estimation of the intake rate of Red Knot consuming *P. laevis*,

271    we first estimated the total cumulative foraging time of Curlew Sandpipers on *P.*

272    *laevis* in each plot by multiplying the total cumulative foraging time of Red Knots in

273    each plot by the average ratio of Curlew Sandpipers bird-days to Red Knot bird-days

274    across the entire tidal flat (see below), and multiplying again by the impact factor

275    (0-1; see above). We used this approach because we only counted the Curlew

276    Sandpipers every two weeks across the entire tidal flat and did not monitor this

277    species daily in each plot as we did for Red Knots. We then summed up the total

278    cumulative foraging time of Curlew Sandpipers and Red Knots as the adjusted total

279    cumulative foraging time of both species combined, and estimated the intake rate of

280    Red Knot consuming *P. laevis* using linear regression. Detailed steps for these

281    calculations are included in Supporting Information.

282

283    *Estimating the current carrying capacity*

284    Due to the large variation in the available daily foraging time (i.e., duration of

285    exposure time) across the tidal flat, we could not estimate the average daily *P. laevis*

286    consumption rate using the instantaneous intake rate estimated above. Instead, we

287    estimated the carrying capacity of Nanpu (i.e., the maximum number of bird-days

288    that Nanpu may support) by estimating the average daily *P. laevis* consumption rate

289    from the relationship between observed level of Red Knot usage and the decline of

290    *P. laevis* abundance accessible to Red Knot across the entire tidal flat. The observed

291    level of Red Knot usage, in bird-days, was estimated by counting the total number of

292    Red Knots using the entire tidal flat throughout the staging period and then

293    calculating the area under the curve of the number of Red Knot numbers against the

294    date in Julian days. The bird-day value of Curlew Sandpiper was derived using the

295    same method.

296

297    To estimate the abundance of *P. laevis* accessible to Red Knots, we approximated the

298    giving-up density (GUD) of Red Knots consuming *P. laevis* by finding the lowest *P.*

299    *laevis* density at sampling points (5th percentile) that have experienced at least a

300    moderate level of Red Knot foraging (showing a decline of >1000 *P. laevis*

301    individuals m$^{-2}$) during the second round of benthic sampling. The sensitivity of GUD

302    estimates to the arbitrary selection criteria used above was shown in Table S2. We

303    used GUD here as an operational threshold because it is unrealistic to assume that

304    birds could take all the bivalves in a given area of mud (van Gils *et al.*, 2004).

305    Because the Red Knots may not have fully depleted *P. laevis* in the area with the

306    lowest observed *P. laevis* density during the second round of benthic sampling, the

307    GUD estimate we derived was probably overestimated. To account for the

308    uncertainty around GUD estimates and our analyses based on GUD, we also

309    conducted a sensitivity analysis to demonstrate how the variation in GUD estimates

310    might affect the carrying capacity estimates for Red Knots (Table S3).

311

312    Taking the GUD into consideration, we then calculated the decline in the abundance

313    of accessible *P. laevis* (i.e., the portion above the estimated GUD) between the two

314    rounds of benthic sampling. Assuming a constant daily *P. laevis* consumption rate by

315    Red Knots throughout the staging period and taking into account the potential effect

316    of Curlew Sandpipers, we estimated the maximum bird-days that could be

317    supported by the food resources at the beginning of the staging period, i.e., the

318    carrying capacity of the Nanpu tidal flat for Red Knots. Detailed steps for these

319    calculations are included in Supporting Information.

320

321    **Results**

322    The bivalve *P. laevis*, the main prey of Red Knots at Nanpu, is concentrated on the

323    upper portion of the tidal flat, achieving a density of >50,000 individuals m$^{-2}$ at the

324    beginning of Red Knot staging period (Figure 3a). After three weeks, the density

325    declined drastically, with areas of higher initial density showing a larger

326    proportional decline (linear regression $F_{1,18}$ = 38.07; $p<0.01$; Figure S1), leading to

327    a more homogenized distribution with lower peaks (Figure 3b). The biomass (AFDM)

328    of *P. laevis* showed a similar but smaller reduction (Figure 3c-d), likely resulting

329   from the natural growth in size of *P. laevis* individuals between two sampling

330   periods (Figure S2).

331

332   It is worth noting that the tidal flat very close to seawall still held relatively high

333   densities of *P. laevis* towards the end of the staging period (Figure 3b, d). Although

334   we found evidence suggesting that Red Knots spent less time foraging close to the

335   seawall, proximity to the seawall did not lead to statistically detectable decreases in

336   the decline of *P. laevis* abundance, after controlling for the effect of *P. laevis* initial

337   density (Table 1).

338

339   The decline of *P. laevis* abundance in sampling plots was proportional to the total

340   cumulative foraging time of Red Knots in the same plot, and the slope suggested that

341   Red Knots fed on *P. laevis* at an estimated intake rate of 206±48 individual bivalves

342   min$^{-1}$ (F1, 18 = 18.69; p<0.01; r$^2$=0.509) (Figure 4). This intake rate estimate would

343   be lower, down to as few as 148±34 individual bivalves min$^{-1}$, after correcting for

344   the potential foraging by Curlew Sandpipers (Table S1).

345

346   Between the two rounds of benthic sampling, the total abundance of *P. laevis*

347   decreased by 45.5% across all sampling plots. With a giving-up density (GUD) of

348   2750 *P. laevis* individuals m$^{-2}$ estimated for Red Knots (Figure S3, Table S2), the

349   decline in the abundance of accessible *P. laevis* was 54.3% across all sampling plots,

350   and on average 49.0%±21.9% for individual sampling plots (Table S3), which,

351   unlike the decline in total *P. laevis* abundance (Figure S1), did not correlate with the

352    initial *P. laevis* density (linear regression F1,16 = 3.335; p=0.087; Figure S4). This

353    decline in *P. laevis* abundance during the study period supported an estimated

354    $4.40×10^5$ bird-days of Red Knots staging at Nanpu (Figure 2). Extrapolating to the

355    entire staging period, the maximum bird-days of Red Knots that could be supported

356    by *P. laevis* at the beginning of the migration season in 2018 was estimated at

357    $8.88×10^5$ bird-days, or 1.46× the level of the actual observed Red Knot usage on the

358    Nanpu tidal flat in 2018 ($6.06×10^5$ bird-days, Figure 2).

359

360    If Curlew Sandpipers depredate *P. laevis* at the same rate as do Red Knots, the

361    estimated carrying capacity of Nanpu tidal flat for supporting Red Knot could reach

362    $1.03×10^6$ bird-days (Table S1). This increase in the carrying capacity estimate of Red

363    Knots  when Curlew Sandpipers are taken into account is due to the lower estimated

364    daily *P. laevis* consumption rate by Red Knots, resulting from a larger number of

365    individual shorebirds (with the addition of Curlew Sandpipers) contributing to the

366    observed decline in *P. laevis* abundance, which, in turn, leads to higher estimated

367    number of Red Knot bird-days that can be supported by the bivalves that remaining

368    in the tidal flat at the end of the staging period.

369
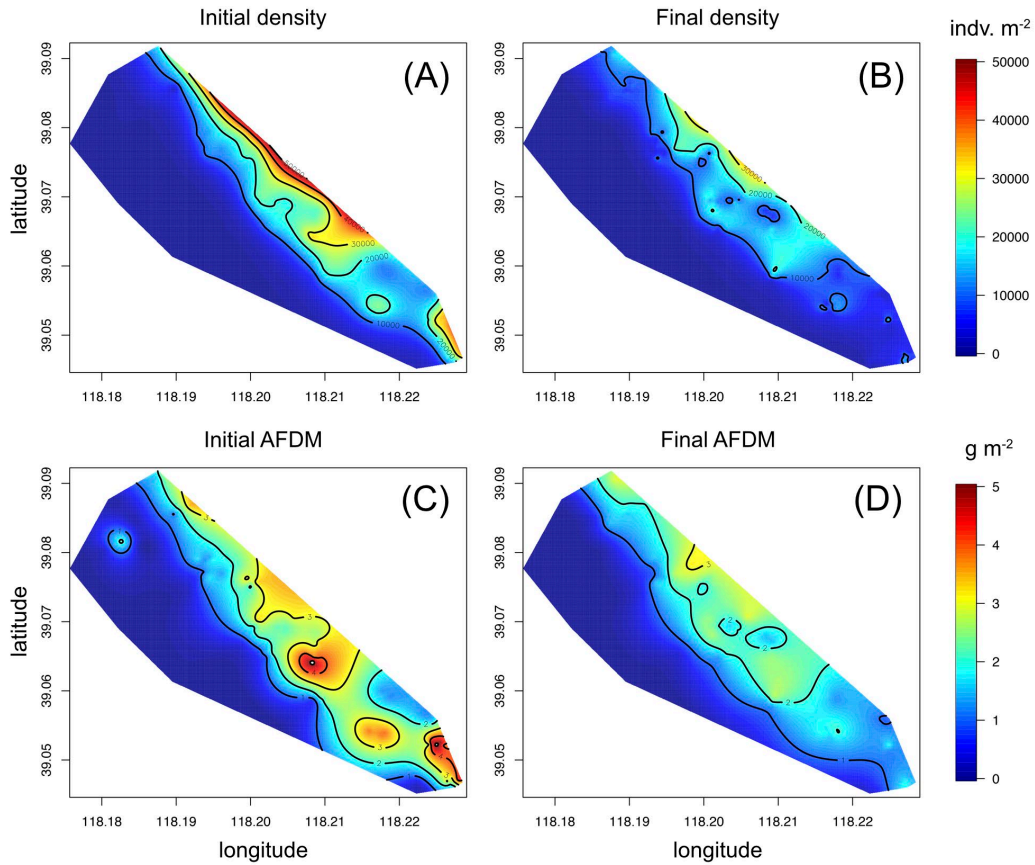
**Figure 3**. Distribution and changes in the density (a-b) and biomass (c-d) of *P. laevis*

at Nanpu during the first (a, c) and second rounds (b, d) of benthic sampling.

**Table 1**. Model selection results about the effect of proximity to the seawall on Red

Knot foraging and *P. laevis* density.

| Full model: total cumulative foraging time ~ distance to seawall + initial *P. laevis* density + decline in *P. laevis* density + proportion of decline in *P. laevis* density | | | |
|---|---|---|---|
| Top 6 models | df | AIC$_C$ | $\triangle$AIC |
| Foraging time ~ distance + initial density* | 4 | 571.97 | - |
| Foraging time ~ initial density | 3 | 572.64 | 0.68 |

| | | | |
|---|---|---|---|
| Foraging time ~ decline in density | 3 | 573.22 | 1.25 |
| Foraging time ~ initial density + decline in density | 4 | 574.93 | 2.97 |
| Foraging time ~ distance + initial density + decline in density* | 5 | 575.33 | 3.37 |
| Foraging time ~ distance + decline in density* | 4 | 575.36 | 3.39 |

375 * The coefficients estimated for "distance to seawall" were all positive, indicating that Red

376 Knots showed reduced foraging intensity close to the seawall.

377

Full model: proportion of decline in *P. laevis* density ~ distance to seawall + initial *P. laevis*

density + total cumulative foraging time

| Top 4 models | df | AIC$_C$ | $\triangle$AIC |
|---|---|---|---|
| Proportion of decline ~ initial density | 3 | -7.17 | - |
| Proportion of decline ~ distance + initial density* | 4 | -6.76 | 0.42 |
| Proportion of decline ~ initial density + foraging time | 4 | -4.17 | 3.01 |
| Proportion of decline ~ distance + initial density + foraging time* | 5 | -3.24 | 3.93 |

378 * The coefficients estimated for "distance to seawall" were all negative, indicating that the *P.*

379 *laevis* density showed greater decline in plots closer to the seawall, and the effect of seawall

380 was opposite from what we would expect if Red Knots showed reduced foraging intensity

381 close to the seawall.

382
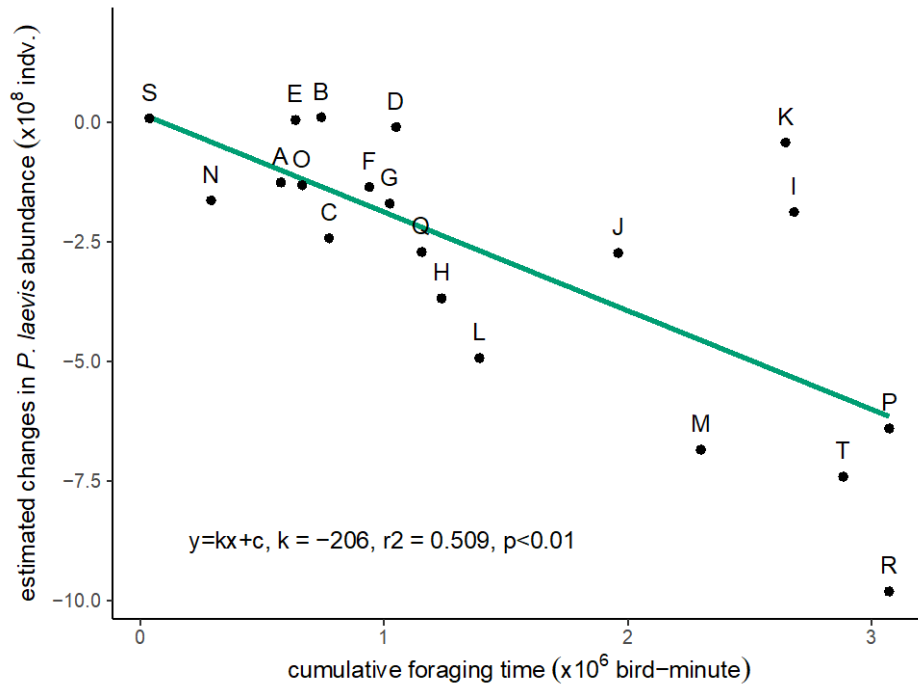
**Figure 4**. The relationship between the decline in *P. laevis* abundance and the total cumulative foraging time in sampling plots, and the estimated intake rate of Red Knot consuming *P. laevis* based on linear regression.

**Discussion**

Staging sites are indispensable links in the annual movement cycles of migratory species, and an understanding of the location, usage, quality and carrying capacity of these sites is crucial for the conservation of migratory species (McGowan *et al.*, 2011; Moores *et al.*, 2016; Piersma *et al.*, 2016). However, these temporarily used sites are probably the least understood aspect of the habitat requirements of migratory species, especially with respect to quality and carrying capacity. In 2018 spring, we tested a field-based approach to quantify the habitat quality and to estimate the current carrying capacity for Red Knots at Nanpu in Bohai Bay, China,

397    the best-known staging site for this species along the EAAF (Rogers *et al.*, 2010;

398    Piersma *et al.*, 2021). We estimated that, in 2018, Nanpu tidal flat was capable of

399    supporting roughly 1.46-1.70 times the actual level of Red Knot usage, operating

400    below but close to its carrying capacity with respect to food availability. Because our

401    data reflect only a single year at this critically important site, we recognize that this

402    conclusion is only tentative and awaits confirmation with additional years of data.

403    Fortunately, our approach can be applied readily over multiple years, yielding long-

404    term data on the carrying capacities of this or other staging sites for Red Knots.

405

406    *Evaluation of staging habitat quality*

407    Verifying that foraging by the focal species is the main driver of the reduction in

408    food resources is a key (yet infrequently taken) step in estimating carrying capacity

409    (Bijleveld *et al.*, 2015), whether with model-based (Sutherland & Anderson, 1993)

410    or field-based approaches. We identified Curlew Sandpiper as the only likely

411    competitor of Red Knots for *P. laevis* at Nanpu during our study period, based on the

412    abundance and distribution patterns of all shorebird species on the tidal flat (Mu &

413    Wilcove, 2020). Nonetheless, because detailed information on the diet of Curlew

414    Sandpipers in Bohai Bay is incomplete, we assumed varying levels of Curlew

415    Sandpiper's relative *P. laevis* intake rate (the "impact factor"), to quantify the

416    potential impact of Curlew Sandpipers on *P. laevis* abundance and, therefore,

417    carrying capacity estimates for Red Knots.

418

419  Our estimated intake rate of *P. laevis* by individual Red Knots (206±48 bivalves min$^{-1}$)

420  is similar to the rate derived in an earlier study in Nanpu: Yang *et al.* (2013)

421  estimated an intake rate of 90±24 individuals min$^{-1}$ for Red Knots consuming *P.*

422  *laevis*, based on data collected during northward migration on the defecation

423  intervals, the sizes of *P. laevis* found in Red Knot droppings, and the relationship

424  between *P. laevis* shell fragments found in droppings and Red Knots' actual *P. laevis*

425  consumption through a digestion experiment using individual birds captured at

426  Nanpu. The overlapping 95% confidence intervals between Yang *et al.*'s (2013)

427  estimate of intake rate and our own, combined with an intercept close to zero

428  suggesting a proportional relationship between the intensity of Red Knot foraging

429  and reduction in food abundance (Figure 4), indicated that Red Knot foraging is the

430  principle contributor to the reduction in *P. laevis* abundance we observed. In their

431  estimate, Yang *et al.* (2013) used a larger average *P. laevis* shell length (3mm) than

432  we did (2.18±0.49mm and 2.65±0.51mm, Figure S2), which we believe most likely

433  reflects a difference in the resolution of measurements between the two studies

434  rather than a true biological difference. Adjusting the average shell length used by

435  Yang *et al.* (2013) to the smaller value we observed, or taking into account the

436  potential impact of Curlew Sandpiper on *P. laevis* abundance (Table S1), further

437  narrows the gap between the two estimates of intake rates.

438

439  Food density and distribution are not the only habitat attribute that affects carrying

440  capacity. We also tested for the potential effects of predation danger and human

441  disturbance on carrying capacity estimates (van Gils *et al.*, 2004), as indicated by

442   proximity to the seawall, on Red Knot foraging and *P. laevis* density (Table 1). In the

443   absence of a significant statistical effect of distance to seawall on the reduction of *P.*

444   *laevis* densities, we cannot conclude whether the high *P. laevis* densities close to the

445   seawall during the second round of benthic sampling (Figure 3b) were solely the

446   result of high initial *P. laevis* densities (Figure 3a), or whether Red Knots avoided

447   foraging in areas close to obstructions as a predator- or disturbance-avoidance

448   strategy (Piersma *et al.*, 1993).

449

450   In recent years, the Luannan Coast as a whole (encompassing our study area, Nanpu,

451   where most Red Knots stage, and the adjacent Beipu and Zuidong tidal flats, Figure

452   1c) has supported 50,000–100,000 Red Knots each spring, with an average staging

453   duration of 5–9 days between May 10-30 (Lok *et al.*, 2019). This translates into an

454   average of $4.5–5.0×10^5$ bird-days, similar to the $5.0×10^5$ bird-days we estimated for

455   Luannan in 2018 during the same period (with most individuals staging in Nanpu,

456   Figure 2). In addition, given that *P. laevis* densities on the Nanpu tidal flats have

457   been relatively stable in recent years, and 2018 did not appear to be an unusually

458   good or bad year for this bivalve (Yang *et al.*, 2016, Hebo Peng unpublished data),

459   we are cautiously confident that our conclusions apply beyond the single year of our

460   study. However, both identifying and continuously monitoring the full set of key

461   staging sites along the EAAF are needed to understand how the relative changes in

462   carrying capacity and actual bird usage at each site may alter Red Knots' migration

463   strategies, migratory routes, and flyway populations (Piersma *et al.*, 2021), using

464   this and other approaches.

465

466     *Applications to conservation*

467     Our results lead to several recommendations for the conservation of EAAF Red

468     Knots and of migratory birds in general. First, had our calculations indicated that

469     Nanpu was operating at or above its carrying capacity for Red Knots, it would

470     suggest that this major staging site is potentially acting as a population bottleneck

471     that is driving the ongoing decline of EAAF Red Knots (Piersma *et al.*, 2016).

472     Fortunately, that does not seem to be the case. Nonetheless, because our results

473     indicate the site is operating close to carrying capacity, it is possible that some Red

474     Knots may have already been displaced to use other staging sites of lower quality

475     (Ntiamoa-Baidu *et al.*, 2014), thus cautioning against any further development of

476     Nanpu tidal flat. To quantify how migratory species have been and will be

477     threatened by habitat loss and degradation, it is imperative to establish more

478     nuanced and mechanistic relationships between changes in habitat quality and

479     migrant population sizes at both local and global scales (Liu *et al.*, 2022).

480

481     Second, the spatial heterogeneity of the Red Knot's food resources reaffirms the idea

482     that the tidal flats constitute a heterogeneous habitat, thus the loss of high-quality

483     sections may result in disproportionately large declines in the overall habitat quality

484     (Mu & Wilcove, 2020). The subtle heterogeneity of the Nanpu tidal flat, with

485     different shorebird species foraging in different zones of the tidal flat (Mu & Wilcove,

486     2020), and with densities of a key prey species varying markedly within and

487     between intertidal zones (Figure 3), underscores the importance of considering the

488    quality, and not just the quantity, of tidal flats as shorebird staging habitat (Zhang *et*

489    *al.*, 2018). Research and long-term monitoring are needed to understand how prey

490    distribution and heterogeneity could be affected by land-use change or sea-level rise

491    to inform more effective habitat conservation and restoration decisions.

492

493    Third, a measurement of bird-days, which incorporates both the number of birds

494    present at a staging site and the cumulative time they spend there, provides a more

495    accurate representation of how migratory species actually use a site and therefore

496    the site's relative importance, than does the much more popular metric of maximum

497    peak count of birds (Figure 2). Moreover, the bird-day measurement is less sensitive

498    to differences in the migration schedules of each population, whereas factors

499    including staging duration, intraspecific variation in migration timing, and survey

500    frequency greatly affect peak count numbers and, consequently, assessments of site

501    importance based on peak counts (Frederiksen *et al.*, 2001; Lok *et al.*, 2019). To

502    facilitate more informative evaluation and comparison of the importance of staging

503    sites to migratory species, population monitoring and surveys need to be conducted

504    frequently across the entire migration season, and should include metrices that

505    incorporate staging duration (e.g., bird-days).

506

507    Furthermore, designation of sites as either Wetlands of International Importance

508    (Ramsar Sites) or as Important Bird and Biodiversity Areas includes a criterion

509    based on supporting ≥1% of the population of a species or subspecies (BirdLife

510    International, 2021; Ramsar Convention Secretariat, 2021). This criterion is usually

511   evaluated using peak counts and does not consider staging duration. We suggest

512   that additional key sites for migratory birds might be identified if the designations

513   include measurements that take staging duration into consideration, because peak

514   counts usually underestimate the total number of birds using a particular site

515   (Frederiksen *et al.*, 2001; Lok *et al.*, 2019).

516

517   Finally, we were able to evaluate Nanpu's carrying capacity for Red Knots because

518   the diet, foraging behavior, staging duration, and habitat use of Red Knots have been

519   thoroughly studied (Piersma *et al.*, 1998; Yang *et al.*, 2013; Lok *et al.*, 2019; Mu &

520   Wilcove, 2020). Such information, however, is typically lacking or incomplete for

521   most migratory species, including many shorebird species and sites along the much-

522   threatened EAAF (Choi *et al.*, 2017; Zhang *et al.*, 2018; Lei *et al.*, 2021). Estimating

523   the long-term carrying capacity of a site would further require detailed information

524   on the productivity, recruitment, and growth of prey species, as well as how these

525   parameters are affected by both biotic and abiotic factors (Yang *et al.*, 2016; Zhang

526   *et al.*, 2018). This lack of knowledge greatly hampers the development and

527   evaluation of conservation efforts for these species: Whether staging sites are

528   operating above or below carrying capacities will greatly affect how migrant

529   populations respond to habitat changes, and inform us on how the remaining sites

530   must be protected to secure current populations or even reverse ongoing declines

531   (Yang *et al.*, 2011; Zhang *et al.*, 2018; Liu *et al.*, 2022). While obtaining similar

532   estimates of carrying capacities for other migratory species at other sites will be

533   more challenging than was the case for Red Knots at Nanpu, it is increasingly

534     important to do so as populations of migratory species around the world continue to

535     decline (Wilcove & Wikelski, 2008).

536

537     **Data Accessibility Statement**

538     Data associated with this manuscript will be archived in the Dryad Data Repository.

539

540     **Authors' contributions**

541     TM and DSW conceived the study and designed the methodology; TM, SC, HP, CJH

542     and AB collected the data with supports from ZZ; TM and SC analyzed the data with

543     inputs from HP and TP; TM and DSW led the writing of the manuscript. All authors

544     contributed critically to the drafts and gave final approval for publication.

545

552

553     **References**

554     Baker, A.J., Gonzalez, P.M., Piersma, T., Niles, L.J., do Nascimento Ide, L., Atkinson,

555         P.W., Clark, N.A., Minton, C.D., Peck, M.K. & Aarts, G. (2004) Rapid population

556       decline in red knots: fitness consequences of decreased refuelling rates and

557       late arrival in Delaware Bay. *Proc Biol Sci,* **271,** 875-882.

558    Bauer, S. & Hoye, B.J. (2014) Migratory animals couple biodiversity and ecosystem

559       functioning worldwide. *Science,* **344,** 1242552.

560    Bijleveld, A.I., Twietmeyer, S., Piechocki, J., van Gils, J.A. & Piersma, T. (2015) Natural

561       selection by pulsed predation: survival of the thickest. *Ecology,* **96,** 1943-

562       1956.

563    BirdLife International (2021) Global IBA Criteria. URL:

564       http://datazone.birdlife.org/site/ibacritglob. Accessed May 16, 2021.

565    Choi, C.-Y., Battley, P.F., Potter, M.A., Ma, Z., Melville, D.S. & Sukkaewmanee, P. (2017)

566       How migratory shorebirds selectively exploit prey at a staging site

567       dominated by a single prey species. *The Auk,* **134,** 76-91.

568    Cressie, N.A.C. (1993) *Statistics for spatial data*. John Wiley & Sons, New York, USA.

569    Crouse, D.T., Crowder, L.B. & Caswell, H. (1987) A Stage-Based Population Model for

570       Loggerhead Sea Turtles and Implications for Conservation. *Ecology,* **68,**

571       1412-1423.

572    Delany, S., Scott, D., Dodman, T. & Stroud, D. (2009) *An atlas of wader populations in*

573       *Africa and Western Eurasia*. Wetlands International, Wageningen, the

574       Netherlands.

575    Frederiksen, M., Fox, A.D., Madsen, J. & Colhoun, K. (2001) Estimating the Total

576       Number of Birds Using a Staging Site. *The Journal of Wildlife Management,* **65**.

577    Kraan, C., van Gils, J.A., Spaans, B., Dekinga, A., Bijleveld, A.I., van Roomen, M.,

578       Kleefstra, R. & Piersma, T. (2009) Landscape-scale experiment demonstrates

579        that Wadden Sea intertidal flats are used to capacity by molluscivore migrant

580        shorebirds. *Journal of Animal Ecology,* **78,** 1259-1268.

581    Lei, W., Masero, J.A., Dingle, C., Liu, Y., Chai, Z., Zhu, B., Peng, H., Zhang, Z. & Piersma,

582        T. (2021) The value of coastal saltpans for migratory shorebirds:

583        conservation insights from a stable isotope approach based on feeding guild

584        and body size. *Animal Conservation,* **24,** 1071-1083.

585    Lei, W., Masero, J.A., Piersma, T., Zhu, B., Yang, H.-Y. & Zhang, Z. (2018) Alternative

586        habitat: the importance of the Nanpu Saltpans for migratory waterbirds in

587        the Chinese Yellow Sea. *Bird Conservation International,* **28,** 549-566.

588    Liu, J., Lei, W., Mo, X., Hassell, C.J., Zhang, Z. & Coulson, T. (2022) Unravelling

589        processes between phenotypic plasticity and population dynamics in

590        migratory birds. *J Anim Ecol.*

591    Lok, T., Hassell, C.J., Piersma, T., Pradel, R. & Gimenez, O. (2019) Accounting for

592        heterogeneity when estimating stopover duration, timing and population

593        size of red knots along the Luannan Coast of Bohai Bay, China. *Ecology and*

594        *Evolution,* **9,** 6176-6188.

595    McGowan, C.P., Hines, J.E., Nichols, J.D., Lyons, J.E., Smith, D.R., Kalasz, K.S., Niles, L.J.,

596        Dey, A.D., Clark, N.A., Atkinson, P.W., Minton, C.D.T. & Kendall, W. (2011)

597        Demographic consequences of migratory stopover: linking red knot survival

598        to horseshoe crab spawning abundance. *Ecosphere,* **2**.

599    Moores, N., Rogers, D.I., Rogers, K. & Hansbro, P.M. (2016) Reclamation of tidal flats

600        and shorebird declines in Saemangeum and elsewhere in the Republic of

601        Korea. *Emu - Austral Ornithology,* **116,** 136-146.

602    Mu, T. & Wilcove, D.S. (2020) Upper tidal flats are disproportionately important for

603        the conservation of migratory shorebirds. *Proceedings of the Royal Society B:*

604        *Biological Sciences,* **287,** 20200278.

605    Ntiamoa-Baidu, Y., Nuoh, A.A., Reneerkens, J. & Piersma, T. (2014) Population

606        Increases in Non-Breeding Sanderlings in Ghana Indicate Site Preference.

607        *Ardea,* **102,** 131-138.

608    Nychka, D., Furrer, R., Paige, J. & Sain, S. (2017) fields: Tools for spatial data. R

609        package version 11.6, <URL: https://github.com/NCAR/Fields>.

610    Piersma, T., Aelst, R.v., Kurk, K., Berkhoudt, H. & Maas, L.R.M. (1998) A new pressure

611        sensory mechanism for prey detection in birds: the use of principles of

612        seabed dynamics? *Proceedings of the Royal Society of London. Series B:*

613        *Biological Sciences,* **265,** 1377-1383.

614    Piersma, T., Hoekstra, R., Dekinga, A., Koolhaas, A., Wolf, P., Battley, P. & Wiersma, P.

615        (1993) Scale and intensity of intertidal habitat use by knots *Calidris canutus*

616        in the Western Wadden Sea in relation to food, friends and foes. *Netherlands*

617        *Journal of Sea Research,* **31,** 331-357.

618    Piersma, T., Kok, E.M.A., Hassell, C.J., Peng, H.B., Verkuil, Y.I., Lei, G., Karagicheva, J.,

619        Rakhimberdiev, E., Howey, P.W., Tibbitts, T.L. & Chan, Y.C. (2021) When a

620        typical jumper skips: itineraries and staging habitats used by Red Knots

621        (*Calidris canutus piersmai*) migrating between northwest Australia and the

622        New Siberian Islands. *Ibis,* **163,** 1235-1251.

623    Piersma, T., Lok, T., Chen, Y., Hassell, C.J., Yang, H.-Y., Boyle, A., Slaymaker, M., Chan,

624        Y.-C., Melville, D.S., Zhang, Z.-W., Ma, Z. & Fuller, R. (2016) Simultaneous

625        declines in summer survival of three shorebird species signals a flyway at

626        risk. *Journal of Applied Ecology,* **53,** 479-490.

627    Ramsar Convention Secretariat (2021) Designating Ramsar Sites. URL:

628        http://www.ramsar.org/sites-countries/designating-ramsar-sites. Accessed

629        May 16, 2021.

630    Rogers, D.I., Yang, H.-Y., Hassell, C.J., Boyle, A.N., Rogers, K.G., Chen, B., Zhang, Z.-W. &

631        Piersma, T. (2010) Red Knots (*Calidris canutus piersmai* and *C. c. rogersi*)

632        depend on a small threatened staging area in Bohai Bay, China. *Emu - Austral*

633        *Ornithology,* **110,** 307-315.

634    Rosenberg, K.V., Dokter, A.M., Blancher, P.J., Sauer, J.R., Smith, A.C., Smith, P.A.,

635        Stanton, J.C., Panjabi, A., Helft, L., Parr, M. & Marra, P.P. (2019) Decline of the

636        North American avifauna. *Science,* **366,** 120-124.

637    Studds, C.E., Kendall, B.E., Murray, N.J., Wilson, H.B., Rogers, D.I., Clemens, R.S.,

638        Gosbell, K., Hassell, C.J., Jessop, R., Melville, D.S., Milton, D.A., Minton, C.D.,

639        Possingham, H.P., Riegen, A.C., Straw, P., Woehler, E.J. & Fuller, R.A. (2017)

640        Rapid population decline in migratory shorebirds relying on Yellow Sea tidal

641        mudflats as stopover sites. *Nat Commun,* **8,** 14895.

642    Sutherland, W.J. & Anderson, C.W. (1993) Predicting the Distribution of Individuals

643        and the Consequences of Habitat Loss: The Role of Prey Depletion. *Journal of*

644        *Theoretical Biology,* **160,** 223-230.

645    Tomkovich, P.S., Porter, R.R., Loktionov, E.Y. & Niles, L.J. (2013) Pathways and

646        staging areas of Red Knots *Calidris canutus rogersi* breeding in southern

647        Chukotka, Far Eastern Russia. *Wader Study Group Bulletin,* **120,** 181-193.

648 van Gils, J. & Piersma, T. (1999) Day and nightime movements of radiomarked Red

649        Knots staging in the western Wadden Sea in July-August 1995. *Wader Study*

650        *Group Bulletin,* **89,** 36-44.

651 van Gils, J.A., Edelaar, P., Escudero, G. & Piersma, T. (2004) Carrying capacity models

652        should not use fixed prey density thresholds: a plea for using more tools of

653        behavioural ecology. *Oikos,* **104,** 197-204.

654 Webster, M.S., Marra, P.P., Haig, S.M., Bensch, S. & Holmes, R.T. (2002) Links

655        between worlds: unraveling migratory connectivity. *Trends in Ecology &*

656        *Evolution,* **17,** 76-83.

657 Wilcove, D.S. & Wikelski, M. (2008) Going, going, gone: is animal migration

658        disappearing. *PLoS Biol,* **6,** e188.

659 Yang, H.-Y., Chen, B., Barter, M., Piersma, T., Zhou, C.-F., Li, F.-S. & Zhang, Z.-W.

660        (2011) Impacts of tidal land reclamation in Bohai Bay, China: ongoing losses

661        of critical Yellow Sea waterbird staging and wintering sites. *Bird Conservation*

662        *International,* **21,** 241-259.

663 Yang, H.-Y., Chen, B., Piersma, T., Zhang, Z. & Ding, C. (2016) Molluscs of an intertidal

664        soft-sediment area in China: Does overfishing explain a high density but low

665        diversity community that benefits staging shorebirds? *Journal of Sea*

666        *Research,* **109,** 20-28.

667 Yang, H.Y., Chen, B., Ma, Z.J., Hua, N., van Gils, J.A., Zhang, Z.W. & Piersma, T. (2013)

668        Economic design in a long-distance migrating molluscivore: how fast-fuelling

669        red knots in Bohai Bay, China, get away with small gizzards. *J Exp Biol,* **216,**

670        3627-3636.

671     Zhang, S.-D., Ma, Z., Choi, C.-Y., Peng, H.-B., Bai, Q.-Q., Liu, W.-L., Tan, K.U.N., Melville,

672         D.S., He, P., Chan, Y.-C., Van Gils, J.A. & Piersma, T. (2018) Persistent use of a

673         shorebird staging site in the Yellow Sea despite severe declines in food

674         resources implies a lack of alternatives. *Bird Conservation International,* **28,**

675         534-548.

676