RESEARCH ARTICLE

Ways to be different: foraging adaptations that facilitate higher intake rates in a northerly wintering shorebird compared with a low-latitude conspecific

Daniel R. Ruthrauff1,2,* Anne Dekinga2, Robert E. Gill, Jr1, Jan A. van Gils2 and Theunis Piersma2,3

ABSTRACT

At what phenotypic level do closely related subspecies that live in different environments differ with respect to food detection, ingestion and processing? This question motivated an experimental study on rock sandpipers (Calidris ptilocnemis). The species’ nonbreeding range spans 20 deg of latitude, the extremes of which are inhabited by two subspecies: C. p. ptilocnemis that winters primarily in upper Cook Inlet, Alaska (61°N) and C. p. tschuktschorum that overlaps slightly with C. p. ptilocnemis but whose range extends much farther south (∼40°N). In view of the strongly contrasting energetic demands of their distinct nonbreeding distributions, we conducted experiments to assess the behavioral, physiological and sensory aspects of foraging and used the bivalve Macoma balthica for all trials. C. p. ptilocnemis consumed a wider range of prey sizes, had higher maximum rates of energy intake, processed shell waste at higher maximum rates and handled prey more quickly. Notably, however, the two subspecies did not differ in their abilities to find buried prey. The subspecies were similar in size and had equally sized gizzards, but the more northern ptilocnemis individuals were 10–14% heavier than their same-sex tschuktschorum counterparts. The higher body mass in ptilocnemis probably resulted from hypertrophy of digestive organs (e.g. intestine, liver) related to digestion and nutrient assimilation. Given the previously established equality of the metabolic capacities of the two subspecies, we propose that the high-latitude nonbreeding range of ptilocnemis rock sandpipers is primarily facilitated by digestive (i.e. physiological) aspects of their foraging ecology rather than behavioral or sensory aspects.

KEY WORDS: Intake rate, Foraging ecology, Functional response, Nonbreeding distribution, Subspecific differences

INTRODUCTION

The ways in which animals satisfy their daily energy requirements ultimately influence nearly every aspect of their ecology (Piersma and van Gils, 2011; Stephens and Krebs, 1986). Given the imperative to remain in energy and nutrient balance, an animal’s foraging ecology will be subject to strong selection pressure that can reflect an optimization of behavioral, environmental and physiological processes (Perry and Pianka, 1997). The differential phenotypic expression of these processes with respect to an animal’s life history forms a rich basis for many ecological studies and such inquiry has demonstrated the evolutionary significance of seemingly minute differences in foraging adaptations between closely related organisms, describing patterns and traits that help drive speciation (Grant, 1999; Schluter, 1995). As a result of their relative ease of observation and diversity of foraging strategies, shorebirds (Charadriiformes) are common subjects of foraging studies (Colwell, 2010; Goss-Custard et al., 2006; van de Kam et al., 2004). During the nonbreeding season, shorebirds experience high energetic demands (Kersten and Piersma, 1987; Wiersma and Piersma, 1994), a natural history trait that also makes shorebirds ideal study subjects of the interplay between an organism’s foraging ecology and its energetic requirements (Kvist and Lindström, 2003; van Gils et al., 2005a; Yang et al., 2013). Previous studies of intake rates in shorebirds have demonstrated that they rapidly increase with prey density, but quickly reach an asymptote beyond which intake rates stabilize. The asymptote defines a constraint to ever-increasing rates of prey intake (Jeschke et al., 2002), constraints that, in shorebirds, are typically caused by prey handling (Zwarts and Esselink, 1989) or digestive (van Gils et al., 2003b; Zwarts and Dirksen, 1990) limitations. Such observations conform to the more general patterns first derived by Holling (1959) and elucidated in shorebirds by others (e.g. Duijns et al., 2014; Lourenço et al., 2010; Piersma et al., 1995). In its simplest form, observations are modeled by the equation:

\[
N = \frac{aD}{1 + \frac{a}{T_0}D}
\]

In this model, the number of prey consumed (N) over total time (T) is described as a function of a predator’s instantaneous area of discovery (a; cm² s⁻¹; also termed search efficiency; Hassell, 1982; van Gils et al., 2005c), prey density (D; m⁻²) and handling time per prey item (T₀; s).

For molluscivorous shorebirds that must crush their hard-shelled prey in their muscular gizzard, the physical act of crushing and processing prey shell waste is the digestive bottleneck that limits intake rate (van Gils et al., 2003b, 2005b; Wanink and Zwarts, 1985). Because molluscivorous shorebirds efficiently exploit prey even at relatively low densities (Piersma et al., 1998), their energy intake rates are not typically limited by their ability to find or handle prey but instead by the interaction between the size of their gizzard and the quality [i.e. energy per unit shell mass (kJ g⁻¹)] of the prey itself (Yang et al., 2013; Zwarts and Blomert, 1992). The interaction of these factors provides a fruitful experimental context to explore the life-history consequences of these traits within and among species (Dekinga et al., 2001; Piersma et al., 2003; Quaintenne et al., 2010; van Gils et al., 2003a, 2005a).

Most previous studies comparing the foraging ecologies of closely related subjects examined differences in the context of sympatric...
niche differentiation (Benkman, 1993; Huey and Pianka, 1981; Kawamori and Matsushima, 2012; Labropoulou and Eleftheriou, 1997; Pulliam, 1985). Here, we compare two subspecies of the rock sandpiper: Calidris p. ptilocnemis Coues 1873 (hereafter ptilocnemis) and Calidris p. tschuktschorum Portenko 1937 (hereafter tschuktschorum). These subspecies are equipped with nearly identical foraging ‘tools’ (i.e. body size, bill morphology, diets, foraging behaviors), but endure strongly contrasting environmental conditions across their largely allopatric nonbreeding ranges (e.g. table 1 in Ruthrauff et al., 2013a). We conducted experimental foraging trials on captive individuals of both subspecies maintained under identical conditions to determine whether their distinct nonbreeding life histories are reflected by inherent differences in foraging ecologies. First, we offered birds differently sized unburied prey (the bivalve Macoma balthica) to determine size preferences when choice was an option. We predicted that both subspecies would maximize intake rates by selecting the highest quality prey when given a choice (van Gils et al., 2005b). Next, we conducted a second trial where choice was not an option, wherein birds were offered ad libitum quantities of unburied Macoma of just one size. These trials enabled us to estimate maximum rates of energy and shell intake as a function of prey size. Under such conditions, these rates are defined by physiological aspects of digestive capacity. In molluscivorous shorebirds, digestive capacity is a function of both a bird’s ability to crush hard-shelled mollusks in its gizzards and its ability to assimilate nutrients and excrete wastes (Battley and Piersma, 2005). Because the size of a shorebird’s gizzard is directly related to its ability to crush prey (Piersma et al., 1993; van Gils et al., 2005c), these dual processes can be partially disentangled via the non-invasive measurement of gizzard size (e.g. Dietz et al., 1999). Given their consistently higher winter metabolic demands and near-complete reliance on Macoma as prey in upper Cook Inlet, Alaska, we predicted that ptilocnemis would achieve higher maximum rates of energy intake by processing shell waste more quickly than tschuktschorum. Finally, we conducted a third trial involving buried Macoma of different sizes and densities to determine each subspecies’ intrinsic ability to find and handle prey (i.e. functional response), responses measured by estimating the parameters $a$, $T_s$ and $T_f$ [search time per prey item (s)]. Because ptilocnemis uses primarily mudflat habitats whereas tschuktschorum uses primarily rocky intertidal habitats, we predicted that ptilocnemis would more efficiently find prey buried in soft sediments [i.e. they would have a lower $T_s$ and a higher instantaneous area of discovery, $a$ (Piersma et al., 1995)] and handle and swallow prey more quickly than tschuktschorum (lower $T_f$). Differences between the subspecies in these three experiments would provide measures of the importance of behavioral, physiological and sensory aspects of rock sandpiper foraging ecology relative to the species’ biogeography.

The study system

Rock sandpipers are the shorebird species with the most northerly nonbreeding distribution in the Pacific Basin, common at locations along the eastern Pacific coast from 61°N (Ruthrauff et al., 2013b) to ~40°N (Gabrielson and Lincoln, 1959; Paulson, 1993). There are four recognized subspecies of rock sandpiper (American Ornithologists’ Union, 1957; Conover, 1944) and the extremes of the species’ nonbreeding distribution are occupied by ptilocnemis to the north and tschuktschorum to the south (Gill et al., 2002). This wide latitudinal range exposes these two subspecies to starkly contrasting environmental conditions and is reflected by predicted mid-winter maintenance metabolic rates over 30% higher in ptilocnemis compared with tschuktschorum (Ruthrauff et al., 2013a). Despite these predicted differences in site-specific metabolic rates, the basic metabolic capacities of these two subspecies do not differ. Ruthrauff et al. (2013a) determined that the basal metabolic rates, metabolic responses to cold and thermal conductance values did not differ between the two subspecies maintained under identical laboratory conditions. It was posited that, under natural settings, the two subspecies acclimated to their respective environmental conditions, a phenotypically flexible response that enables increased metabolic capacities at lower temperatures (Ruthrauff et al., 2013a; Vézina et al., 2011). Because the two subspecies do not differ in their intrinsic metabolic capacities, we hypothesized that the consistently higher energetic demands of ptilocnemis during winter compared with tschuktschorum would be supported by innate differences in foraging ecologies.

Although the winter (October–April) nonbreeding ranges and habitat affinities of ptilocnemis and tschuktschorum have received little formal study (Gill et al., 2002; Ruthrauff et al., 2013a), observations suggest broad contrasts between the subspecies in these traits. Ptilocnemis is distributed primarily on mudflat habitats in upper Cook Inlet, Alaska (61°N, 151°W) during winter (Gill et al., 2002; Ruthrauff et al., 2013b). The average daily temperature in upper Cook Inlet is ≤0°C for nearly half the year, making this the coldest site regularly used by shorebirds (Ruthrauff et al., 2013c). Tschuktschorum, in contrast, is distributed as far south as northern California, primarily on rocky intertidal habitats (~40°N; Paulson, 1993; Gill et al., 2002). The subspecies exhibit contrasting phenotypic responses that reflect the distinct environmental conditions of their respective nonbreeding ranges. Ptilocnemis carries high fat stores and augments the size of digestive organs during winter in upper Cook Inlet, whereas tschuktschorum carries low fat stores and maintains smaller digestive organs at more southerly sites (Ruthrauff et al., 2013c). The two subspecies co-occur in small numbers where the southern limit of the ptilocnemis range overlaps the northern limit of the tschuktschorum range, but their winter distributions and habitat affinities are largely distinct. The small bivalve Macoma balthica essentially constitutes the entirety of the ptilocnemis diet on the mudflats of upper Cook Inlet (Gill et al., 2002; Ruthrauff et al., 2013b), while tschuktschorum consumes invertebrates associated with rocky intertidal habitats [e.g. mollusks (Mytilus sp., Littorina sp.) and crustaceans (barnacles, isopods); Gill et al., 2002]. Differences in diet and habitat affinities may naturally predispose the two subspecies to different foraging ecologies, but the subspecies co-occur at migratory stopover sites where both consume Macoma (D.R.R. and R.E.G., unpublished results).

RESULTS

Size dimorphism between birds included in the experiments followed the sex-specific and subspecific patterns described by Gill et al. (2002). The average length of exposed culmen was 34.2±1.1 mm and 29.4±0.8 mm for female and male ptilocnemis, respectively, and 34.0±0.2 mm and 27.3±0.6 mm for female and male tschuktschorum, respectively. Average body mass at the end of all experiments was 82.7±9.9 g for ptilocnemis females, 75.1±2.5 g for ptilocnemis males, 74.4±1.1 g for tschuktschorum females and 64.8±3.2 g for tschuktschorum males. Prior to commencing the experiments, the height and width of the birds’ gizzards did not differ when individuals were maintained on diets of soft fish chow (all comparisons between sexes and subspecies $P>0.53$, $t≤0.66$) and the height and width of experimental birds’ gizzards increased

The Journal of Experimental Biology
the gizzard sizes of the subspecies did not differ overall (P = 0.91 for width, t = 3.26 for height, and values represent means±s.e.m. Birds from 2010 (diet of soft fish chow; Cerastoderma edulis) and 2011 (diet of Mytilus edulis) and values represent means±s.e.m. Birds from 2010 (diet of Mytilus edulis) and 2011 (diet of Cerastoderma edule and Mya arenaria) combined. Before (soft fish chow; N=14) and after (hard-shelled mollusks; N=16) measures derive from 12 individual birds, four of which were measured in both years and whose two measures were treated as independent samples.

Fig. 1. Differences in height and width of rock sandpiper gizzards when birds were switched from a diet of soft fish chow to hard-shelled mollusks. Measures were made using ultrasonography (see Materials and methods) and values represent means±s.e.m. Birds from 2010 (diet of Mytilus edulis) and 2011 (diet of Cerastoderma edule and Mya arenaria) combined. Before (soft fish chow; N=14) and after (hard-shelled mollusks; N=16) measures derive from 12 individual birds, four of which were measured in both years and whose two measures were treated as independent samples.

an average of 35±8% and 27±6%, respectively, when their diets were switched to hard-shelled prey (Fig. 1). When maintained on hard-shelled prey, the gizzards of females were larger than males (P<0.01, r=3.26 for height, P=0.05, r=2.93 for width), but the gizzard sizes of the subspecies did not differ overall (P=0.79, r=0.27 for height, P=0.91, r=0.12 for width).

Experiment I: prey choice

Prey quality was highest in the smallest Macoma size class (size 1; 2.83 kJ g⁻¹ shell) and slightly lower in size 2 Macoma (2.58 kJ g⁻¹ shell). The larger size classes were progressively lower in quality: 2.21 kJ g⁻¹ shell for size 3 and 2.01 kJ g⁻¹ shell for size 4 (Fig. 2). In experiment I, the two smallest size classes of Macoma were overwhelmingly consumed in preference to the two larger size classes. Across the 14 trials in which Macoma were consumed, only four (2.9%) Macoma of the largest size class (size 4) were ingested; 20 (14.3%) Macoma of the second largest size class (size 3) were consumed and most of the two smallest sizes were consumed [114 (81.4%) and 125 (89.3%) for sizes 2 and 1, respectively]. However, small within-group sample sizes precluded statistical comparison and we show graphical summaries of the selection trials in Fig. 3. In general, pilocnemis consumed more Macoma across a wider range of sizes than tschuktschorum (Fig. 3).

Experiment II: maximum intake rate of exposed prey

Experiment II demonstrated that maximum intake rates were higher for pilocnemis compared with tschuktschorum and that birds of both subspecies increased these rates when consuming smaller prey. The sum of model weights (Σwᵢ) for models including Macoma size was 1.0 for analyses with both ash-free dry mass (AFDM) and shell ballast as response variables and models containing subspecies also exhibited strong support (Σwᵢ=0.78 and 0.79 for AFDM and shell ballast, respectively). The effect of sex (Σwᵢ=0.27 and 0.21 for AFDM and shell ballast, respectively) on maximum intake rates received little support. Accordingly, the only model-averaged parameter estimates with 95% confidence intervals that did not overlap zero were those for prey size and subspecies (Table 1). Model-averaged predictions indicated that the maximum intake rate of both AFDM and shell ballast were lower for tschuktschorum than pilocnemis across all size classes (Fig. 4). The model-averaged point estimates of AFDM and shell ballast were higher in pilocnemis females than males, which were, in turn, higher than tschuktschorum females; tschuktschorum males had the lowest estimated maximum intake rates. Within each subspecies, the 95% confidence intervals on these estimates overlapped between females and males within each size class, but many estimates differed between pilocnemis and tschuktschorum (Fig. 4). Maximum intake rates were higher for the two smaller size classes of Macoma than the two larger sizes; maximum rates of ballast intake were achieved for all birds at prey size class 2, but AFDM intake rates were highest at size class 1. However, there was broad overlap between size classes 1 and 2 within each sex/subspecies group (Fig. 4).

Fig. 2. Shell ballast and ash-free dry mass as a function of shell length for Macoma balthica. Shell ballast (open circles) and ash-free dry mass (closed circles) are shown in mg and shell length is shown in mm; all are plotted on a log₁₀ scale. Relationship calculated from Macoma collected at Baie de Somme, France and used in trials to determine maximum intake rates of pilocnemis and tschuktschorum rock sandpipers. The solid line (+95% confidence interval) describes the relationship log₁₀(AFDM)=−2.182+ 3.095×log₁₀(shell length) and the dotted line (+95% confidence interval) describes log₁₀(shell ballast)=−1.902+ 3.681×log₁₀(shell length). Back-transformed estimates of the ratio AFDM: shell ballast were multiplied by metabolizable energy content to calculate Macoma quality (kJ g⁻¹ shell; see Materials and methods), represented on the right-hand axis by the dashed and dotted line. Numbers 1–4 denote quality estimates for Macoma size classes used in the prey choice and maximum intake trials.
Experiment III: functional response to buried prey

The model selection process of search time yielded strong support for the influence of two-way interactions ($\Sigma_{w}=0.99$). Model-averaged parameter estimates indicated a strong interaction between sex and Macoma size, with males requiring more time to find larger prey (Table 2). Tschuktschorum required more search time to discover Macoma than pilocnemis and all birds required more time to find larger Macoma (size 2; Table 2). For small Macoma (size 1), model-averaged predictions of search time for females and males of both subspecies were similar and decreased as prey densities increased (Fig. 5B). For large Macoma, however, males of both subspecies (but especially tschuktschorum) required more time than females to find prey (Fig. 5A). Because of the interaction between sex and prey size in search times, we calculated the instantaneous area of discovery (a) only for small Macoma (size 1). Instantaneous area of discovery is inversely related to search time as a function of density (see Materials and methods). Because search time decreased as density increased at a rate slightly less than $-1$ (Table 2, Fig. 5), this indicated that instantaneous area of discovery likewise declined as Macoma density increased. This decline was reflected by decreasing point estimates for $a$ as densities increased, but the 95% confidence intervals on these estimates overlapped broadly across the range of densities in our trials. The confidence intervals on these estimates also overlapped across sex/subspecies groups. At densities of 208 Macoma per m$^2$, estimates of $a$ (cm$^2$ s$^{-1}$) were 22.3 (95% confidence interval: 13.7–30.9) for pilocnemis females, 18.2 (13.7–22.6) for pilocnemis males, 17.1 (11.0–23.2) for tschuktschorum females and 24.1 (13.2–35.0) for tschuktschorum males.

After bringing a prey item to the surface of the sand, sandpipers required more time to handle large prey than small prey and these times did not vary by prey density (Fig. 6). The model selection process yielded strong support for an effect of Macoma size ($\Sigma_{w}=1$) on handling time and limited support for differences between the two subspecies ($\Sigma_{w}=0.5$). Accordingly, prey size class and subspecies were the only variables in the handling time analysis with 95% confidence intervals of parameter estimates that did not overlap zero. Parameter estimates indicated that large Macoma required more handling time and that tschuktschorum handled Macoma longer than pilocnemis (Table 2). The point estimates for handling time per swallowed prey item were lower for pilocnemis than for tschuktschorum (Fig. 6), but confidence intervals on these estimates overlapped across groups. Prey handling times averaged about five times longer for large Macoma (2.2–3.2 s) compared with small Macoma (0.4–0.6 s) and the 95% confidence intervals did not overlap between the two size classes (Fig. 6).

**DISCUSSION**

Compared with other closely related shorebirds, pilocnemis and tschuktschorum rock sandpipers are unusual in that they possess nonbreeding habitat affinities (mudflat versus rocky intertidal) that should seemingly favor disparate foraging modes (probing versus visual). Despite the differences in foraging habitat preferences, we found no parallel differences in the ability of the two subspecies to discover buried prey via probing. We detected no differences between the two subspecies in their instantaneous area of discovery, $a$, the functional-response parameter that describes an organism’s effective search area per unit time. We did, however, observe clear differences in other aspects of the foraging ecologies of pilocnemis and tschuktschorum rock sandpipers. Pilocnemis had higher shell processing capacities than tschuktschorum, which led to higher maximum AFDM intake rates (Table 1, Fig. 4). Pilocnemis were also more effective at handling prey ($T_h$; Table 2, Fig. 6) and could consume larger prey than tschuktschorum (Fig. 3). Taken together, these differences reflect the greater

---

**Table 1. Model-averaged parameter estimates from linear mixed-effect models used to assess factors influencing maximum intake rates for pilocnemis and tschuktschorum rock sandpipers consuming Macoma balthica**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Maximum intake rate model set</th>
<th>Ash-free dry mass</th>
<th>Shell ballast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macoma size 3$^a$</td>
<td>$-0.073 (-0.087$ to $-0.058)$</td>
<td>$-0.285 (-0.398$ to $-0.172)$</td>
<td></td>
</tr>
<tr>
<td>Macoma size 4$^b$</td>
<td>$-0.111 (-0.129$ to $-0.094)$</td>
<td>$-0.537 (-0.672$ to $-0.402)$</td>
<td></td>
</tr>
<tr>
<td>Subspecies$^c$</td>
<td>$-0.028 (-0.049$ to $-0.006)$</td>
<td>$-0.207 (-0.364$ to $-0.049)$</td>
<td></td>
</tr>
</tbody>
</table>

Biologically relevant combinations of body mass, Macoma size [classes 1 (smallest) to 4 (largest); see Fig. 2], sex and subspecies as fixed effects and individual birds as random effects in model sets. Only parameters with confidence limits that do not overlap zero are shown; units for parameters are mg s$^{-1}$; 95% confidence intervals are shown in parentheses.

$^a$Macoma size 1 is the reference level.

$^b$Calidris p. pilocnemis is the reference level.
importance of high sustained rates of energy intake for *ptilocnemis* compared with *tschuktschorum*.

The lack of obvious difference in instantaneous area of discovery between the subspecies, however, is more difficult to interpret than differences in processing capacities. Such similarity may represent a relatively low importance of habitat-specific foraging adaptations (i.e. tactile versus visual cues) in rock sandpipers. For example, with prey densities in upper Cook Inlet exceeding 400 *Macoma* per m² (Ruthrauff et al., 2013b), detection of prey by probing may not be subject to strong selection pressure. Alternatively, given the reliance of *tschuktschorum* on probe-feeding during migratory staging periods in spring and autumn, the similar subspecific values for instantaneous area of discovery may instead reflect the shared importance of this trait between the subspecies. Affirming these distinct interpretations requires additional study.

Although differences in prey size preferences probably relate to physical limitations of smaller birds compared with larger birds (e.g. smaller gape and esophagus), other differences between the two subspecies do not obviously correlate with structural size. For digestively constrained foragers such as rock sandpipers, maximum intake rates are determined primarily by the physical capacity of a bird’s digestive ‘machinery’ and reflect physiological aspects of their foraging ecology (Battley and Piersma, 2005; McWilliams and Karasov, 2001). For example, van Gils et al. (2005a, 2005b) determined that red knots (*Calidris canutus*) selected foraging patches based on the density and diversity of the benthic prey community and that these choices reflected the size, and hence processing capacity, of their gizzards. In contrast, prey handling potentially represents a mix of behavioral (e.g. learned aspects related to orientation and mandibulation of prey items) and structural (e.g. intrinsic aspects of prey handling related to bill length or size of gape) adaptations. Whereas within-sex differences between *ptilocnemis* and *tschuktschorum* in bill length and gizzard size were small, *ptilocnemis* females and males were ~10–14% heavier than their same-sex *tschuktschorum* counterparts. Such differences in body mass suggest that physiological processes unrelated to structural size influence differences in maximum intake rates. As indicated by ultrasound measurements (Fig. 1), gizzards may have reached an upper (and equal) size limit in both subspecies and differences in body mass may reflect subspecific differences in other digestive organs that facilitate higher intake rates in *ptilocnemis* (e.g. Battley and Piersma, 2005; Diamond, 2002; Dykstra and Karasov, 1992). We did not sacrifice the birds at the end of the trials to compare the morphologies of relevant digestive organs, but given the similarity between the subspecies in sex-specific structural and gizzard sizes, we propose that differences in body mass between the trial birds reflect a hypertrophy of digestive organs that facilitate higher intake rates in *ptilocnemis*. In a similar comparison of nonbreeding populations of the closely related purple sandpiper (*Calidris maritima*), Summers et al. (1998) detected no difference in stomach mass (composed primarily of gizzard) between individuals from Norway and Scotland, but birds from Norway had significantly heavier livers and heavier and longer intestines than birds from Scotland. These differences were interpreted as a flexible phenotypic response to the higher rates of food intake necessary to satisfy the higher energetic demands of wintering in Norway (Summers et al., 1998). Such phenotypic changes in gut morphology and function are well documented in

![Fig. 4. Predicted intake rates of shell ballast and ash-free dry mass for female and male *p*tilocnemis* and *tschuktschorum* rock sandpipers. Shell ballast is represented by top four symbols where values are >0.20 mg s⁻¹ and ash-free dry mass by bottom four symbols with values <0.20 mg s⁻¹ for each size class. Size 1 *Macoma balthica* are the smallest and size 4 the largest (see Materials and methods and Fig. 2). Values represent model-averaged predictions ±95% confidence intervals. Predictions derive from analysis of 29 trials involving four *p*tilocnemis* birds [two females (seven trials each) and two males (seven and eight trials)] and 17 trials involving three *tschuktschorum* birds [one female (seven trials) and two males (four and six trials)].](fig4)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Functional-response model set</th>
<th>Handling time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male X large <em>Macoma</em></td>
<td>0.355 (0.141 to 0.570)</td>
<td>n.a.</td>
</tr>
<tr>
<td>log₁₀ Macrona density</td>
<td>−0.715 (−0.953 to −0.476)</td>
<td>n.a.</td>
</tr>
<tr>
<td>Subspeciesᵃ</td>
<td>0.162 (0.021 to 0.302)</td>
<td>0.206 (0.034 to 0.377)</td>
</tr>
<tr>
<td><em>Macoma</em> sizeᵇ</td>
<td>0.301 (0.201 to 0.401)</td>
<td>0.698 (0.650 to 0.746)</td>
</tr>
<tr>
<td>Intercept</td>
<td>2.517 (1.994 to 3.04)</td>
<td>−0.454 (−0.787 to −0.121)</td>
</tr>
</tbody>
</table>

Biologically relevant combinations of *Macoma* density, *Macoma* size [classes 1 (small) and 2 (large)], sex and subspecies as fixed effects and individual birds as random effects in model sets; search time (s per *Macoma*) and handling time (s per *Macoma*) were the response variables. Search time models included interaction terms, but handling time model did not. Only parameters with confidence limits that do not overlap zero are shown; units for response variables are on the log₁₀ scale (see Materials and methods); 95% confidence intervals are shown in parentheses. n.a., not applicable.

ᵃ*Calidris p.* *p*tilocnemis* is the reference level.

ᵇSmall *Macoma* (size 1) is the reference level.

**Table 2. Model-averaged parameter estimates from linear mixed-effect models used to assess factors influencing the functional response of *p*tilocnemis* and *tschuktschorum* rock sandpipers to buried *Macoma balthica***
many species in response to a variety of environmental and life-history stimuli (Clissold et al., 2013; Dykstra and Karasov, 1992; Price et al., 2013; Starck, 1999). However, given the identical holding conditions of our experimental setup, differences between the subspecies noted here probably represent intrinsic adaptations rather than phenotypic responses.

We noted apparent differences between the subspecies in the time necessary to find buried Macoma (Table 2, Fig. 5). It was counterintuitive, however, that larger prey items with a greater cross-sectional area should seemingly have been more difficult to find by substrate-probing shorebirds. Upon closer examination of trial videos, it was evident that longer search times resulted from underlying differences in prey size preferences. When buried prey were encountered during these trials, birds would widen the gape of their bill, cease probing and reposition their head and feet to more easily extract the Macoma from the sand. For trials involving large Macoma (size 2), however, birds would often assess the size of the Macoma while the prey remained below the surface of the sand, reject it in place and resume their search for additional (smaller) prey items. Because birds did not bring these large prey items to the surface of the sand where they were visible to us, we could not be certain that they had in fact encountered a prey item. Hence, such behaviors inflated the amount of time that these birds searched before apparently ‘finding’ a prey item (i.e. raised the item to the surface). Smaller rock sandpipers (especially tschuktschorum males) appeared to reject large buried Macoma more often than did larger birds, which was reflected by an increase in search time (Table 2, Fig. 5) and by the observed interaction between sex and Macoma size (i.e. longest search times for large Macoma in males; Table 2). These findings were meaningful in the context of prey-size thresholds, but obscured unbiased assessment of the instantaneous area of discovery. To avoid such biases, we parsed the dataset to focus only on trials with small Macoma, which were never rejected by any birds during the trials, to calculate $a$. Contrary to our prediction based on nonbreeding habitat preferences, we found no evidence of a difference between the subspecies in their intrinsic search efficiencies. Thus, although the two subspecies have different intake rates, evidence suggests that this derives from differences in digestive capacities and not sensory differences related to their ability to find buried prey.

For animals facing potential bottlenecks in prey intake, it is instructive to view prey intake both as a function of its profitability (energy intake as a function of searching and handling time) and its quality (energy intake as a function of shell ballast; both definitions sensu van Gils et al., 2005c). Model results indicate that a female pilocnemis exploiting Macoma at a density of 208 individuals per m² requires about 3.5 s more time to find, handle and swallow large Macoma compared with small Macoma. Although more costly with respect to foraging time, such a strategy yields higher profitability in terms of energy intake (1.29 mg AFDM per second for large Macoma compared with 0.77 mg AFDM per second for small Macoma). For digestively constrained foragers such as rock sandpipers, however, energy intake rates over longer durations are better predicted as a function of prey quality (Quaïntenne et al., 2010; van Gils et al., 2005b), a relationship that maximizes the ratio of energy to shell ballast. Rates of ballast intake for the same bird consuming large Macoma are nearly double those compared with small Macoma (10.54 mg shell ballast per second compared with 5.30 mg shell ballast per second). Thus, for digestively constrained foragers, prey selection on the basis of energy per unit shell ballast maximizes energy intake by prolonging the duration over which consumers can forage.
Inlet feed on rock sandpipers in upper Cook Inlet (table 2 in Ruthrauff et al., 2013c). Because molluscivorous shorebirds reach digestive bottlenecks at relatively low prey densities (Goss-Custard et al., 2006; van Gils et al., 2005b), we predict that *ptilocnemis* rock sandpipers in upper Cook Inlet feed on *Macoma* of high quality (i.e. small sizes) such that net energy intake is maximized as a function of shell waste. Given the lack of inherent differences in metabolic rates between *ptilocnemis* and *tschuktschorum* (Ruthrauff et al., 2013a), we posit that intrinsic physiological differences of the digestive system related to assimilation and excretion, but not the physical crushing of food, play the largest role in facilitating the unique nonbreeding distribution of *ptilocnemis* rock sandpipers.

Studies of other organisms have demonstrated that such traits can evolve over a matter of generations (Hendry and Kinnison, 1999; Schluter, 2000) and it is instructive to explore the timescale of these aspects of *ptilocnemis* foraging ecology. The rock sandpiper is among several polymorphic Beringian endemic species (e.g. Abbott and Brochmann, 2003; Cook et al., 2005; Dawson et al., 2014; Pruett and Winker, 2005) whose polymorphy has been shaped by rapid, dynamic geologic processes throughout the region (Hopkins, 1959, 1973). The final formation of Cook Inlet as a geographic feature is believed to have occurred ~14,000 years ago (Reger et al., 2007; Schmoll et al., 1999) and fossil evidence indicates immediate colonization of the region thereafter by *Macoma* (Schmoll et al., 1972). Cook Inlet is the most northerly site in the region with abundant benthic food supplies that occur in the absence of permanent sea or shore-fast ice during winter (Ruthrauff et al., 2013b). It may be that rapid climate warming within the last century (Hinzman et al., 2005; Moritz et al., 2002; Sereze et al., 2000) only recently established ice-free mudflats and sufficiently relaxed energetic demands to permit the winter occupancy of Cook Inlet by *ptilocnemis*. Regardless of their inception, such apparently intrinsic differences in foraging ecologies reflect the discrete processes by which environmental conditions lead to adaptive differences between closely related organisms (Reznick and Ghalambor, 2001; Schluter, 1996) and underscore the many aspects of foraging performance that can promote adaptive radiations (Grant and Grant, 1993; Liem, 1980; MacArthur, 1958; Schluter, 1993).

**MATERIALS AND METHODS**

**Experimental animals and maintenance**

We captured 30 adult rock sandpipers on 28 August 2009 at a post-breeding site on the Yukon Delta National Wildlife Refuge, Alaska (61.3°N, 165.8°W) and transported them to the Royal Netherlands Institute for Sea Research (NIOZ), Texel, The Netherlands, on 21 September 2009. Transport of the birds to The Netherlands was authorized by the United States Fish and Wildlife Service (permit MB 789738) and followed United States Geological Survey animal care and use permit 2008-22. We determined the subspecific identity of birds based on diagnostic plumage characteristics of the wing and mantle (Gill et al., 2002) and sex from blood samples via standard PCR techniques (Grifiths et al., 1996). Female rock sandpipers are larger than males (2–3% greater in wing length and tarsus, ~13% in bill length; supplementary material appendix 2 in Gill et al., 2002) and *ptilocnemis* individuals are slightly larger than *tschuktschorum* individuals (5–8% greater in the same measures; supplementary material appendix 2 in Gill et al., 2002).

In 2010, we conducted experiments in outdoor aviaries. The mean temperature (±s.e.m.) over the experimental period in 2010 was 4.1±0.5°C in February, 8.4±0.6°C in March and 9.8±0.6°C in April. In 2011, we conducted experiments in indoor aviaries maintained at 14°C, conditions under which the birds were also maintained. See Vézina et al. (2006) for aviary details. When not subject to experimental trials, all rock sandpipers were fed commercial fish chow (47% protein; manufactured by Skretting, Fontaine-les-Vervins, France). Soft diets cause gizzards to atrophy (Piersma et al., 1993) and in order to rebuild and maintain the gizzards of rock sandpipers, we slowly and permanently switched the diet of experimental birds from fish chow to hard-shelled bivalves. *Macoma balthica* is a preferred bivalve prey of rock sandpipers (Gill et al., 2002) and we used only *Macoma* as prey during all experimental trials. We harvested *Macoma* at the Baie de Somme estuary, France (50.2°N, 1.6°E), for trials conducted in 2010 and near the mouth of the Kasilof River, Alaska (60.4°N, 151.3°W).
for trials conducted in 2011. *Macoma* were maintained at 8°C in large saltwater aquaria at NIOZ. We were unable to collect enough *Macoma* to sustain birds throughout the trial periods and instead provided *Mytilus edulis* (2010) and a mix of *Cerastoderma edule* and *Mya arenaria* (2011) collected near the island of Texel, The Netherlands. To determine the quality of the *Macoma* prey, we calculated the relationship of shell length to AFDM and shell mass (i.e. ballast) using standard techniques (van Gils et al., 2005b; Zwarts, 1991). To satisfy underlying model assumptions, we calculated these relationships after transforming AFDM, shell ballast and shell length using log_{10} transformations (Fig. 2). We back-transformed these estimates to yield outputs in mg. To link intake to metabolizable energy, we converted estimates of shell ballast intake into their energetic equivalent (kJ g^{-1} shell ballast) assuming an energy density of 22 kJ g^{-1} AFDM *Macoma* flesh (van Gils et al., 2005b; Zwarts and Wannink, 1993) and an assimilation efficiency of 0.8 (Yang et al., 2013).

We measured the response of experimental birds to their diet switch by measuring their gizzards using ultrasound techniques outlined by Dietz et al. (1999). We measured the height and width of the gizzards of all birds immediately prior to switching diets and again upon completion of foraging trials. All measurements were collected by A.D. and birds were measured using a system that ensured that A.D. was ignorant of the identity of each bird as it was measured. Care and handling of the birds and all experimental procedures complied with the Dutch Law on Experimental Welfare and the animal welfare guidelines of the Royal Netherlands Academy of Arts and Sciences (DEC permit NIOZ 09.01).

**Experiments**

We randomly assigned individuals to experimental trials based on subspecies and sex, selecting two members of each subspecies of each sex for all experimental trials (eight individuals total). Birds required up to 4 weeks to permanently switch diets from fish chow to hard-shelled bivalves, but some individuals had difficulty switching diets and could not maintain healthy body mass. These birds were replaced with new individuals in the experimental trials until we could maintain the body mass of eight rock sandpipers on a bivalve diet for all trials. In 2010, we were only able to maintain one *tschuktschorum* female on a bivalve diet and we included a third *ptilocnemis* male in these trials. For all trials, we removed food from the aviaries at 08:00 h to ensure that birds were hungry and foraged in a motivated manner. Trials commenced at 14:00 h and trials were ended after 10 min or once five prey were consumed. On no occasions were birds able to consume all the *Macoma* provided during a trial and the average±s.e.m. number of *Macoma* of size 1, 2, 3 and 4 consumed per trial was 133.5±5.9, 78.9±4.5, 19.3±2.3 and 7.4±1.9, respectively.

**Experiment I: prey choice**

We sorted *Macoma* into four size classes for trials in 2010, using a sieve to speed separation of the two smallest size classes and hand sorting the two larger size classes. This method created slight overlap between adjacent size classes (mean±s.e.m. lengths 7.5±0.1 mm, 8.8±0.1 mm, 11.4±0.1 mm and 13.5±0.1 mm for size classes 1–4, respectively). We conducted trials from 24–27 March 2010 to determine the prey size preferences of rock sandpipers. We presented each bird 10 *Macoma* of each size class in four identical Petri dishes simultaneously and we randomized the placement of dishes with respect to each other in each trial. Trials lasted 15 min and we counted the number of each size class that was consumed upon completion of each trial. We performed one trial per bird per day across three consecutive days. Despite conducting initial unrecorded ‘training’ exercises, these first trials were characterized by an unwillingness to feed. No *Macoma* were consumed in 14 of 28 prey size selection trials, but such reluctance dropped as birds acclimated to experimental conditions.

**Experiment II: maximum intake rate of exposed prey**

Using the same group of eight birds, we conducted trials from 30 March–6 April 2010 to determine the long-term maximum intake rate (mg AFDM *Macoma* per second and mg *Macoma* shell per second) of rock sandpipers. For these trials, birds were presented a Petri dish containing *Macoma* of just one size class. We provided *Macoma* *ad libitum* quantities to ensure that a bird could not consume all the prey during a 45 min trial. We conducted two trials per size class for each bird and performed one trial per bird per day across eight consecutive days. We recorded each trial using digital video and abutted a clear plastic barrier against the side of the Petri dish facing the video camera to orient the birds such that we could clearly observe all prey consumptions.

One *ptilocnemis* male never consumed any *Macoma* in the eight maximum intake trials in which it was involved. There were eight other trials in which no prey were consumed, one involving size 3 *Macoma* and seven involving size 4 *Macoma*. Five of these eight instances occurred during trials with the two male *tschuktschorum* birds, which never consumed any size 4 *Macoma*. Thus, no prey were consumed in 16 of 64 maximum intake trials. In another trial involving a *tschuktschorum* male, the bird consumed only eight size 2 prey items and spent most of the trial roosting; this trial was also excluded from analysis. Thus, we analyzed video from 47 of the 64 maximum intake trials. Exceptions aside, birds fed in a motivated manner during the 45-min-long trials. On no occasions were birds able to consume all the *Macoma* provided during a trial and the average±s.e.m. maximum intake per size class (mean±s.e.m. lengths 7.5±0.1 mm, 8.8±0.1 mm, 11.4±0.1 mm and 13.5±0.1 mm) by hand. We buried all *Macoma* in their natural orientation at 2 cm depth immediately prior to the start of each trial and randomly distributed the *Macoma* in the tubs based on coordinates across a 1 cm² grid. We measured the functional response of rock sandpipers to two size classes (see above) of buried prey across three prey densities: 67, 133 and 208 *Macoma* per m² (16, 32 and 50 *Macoma* per tray, respectively). We conducted three trials per bird at each of the six combinations of *Macoma* size and density and recorded trials using digital video. We placed a small mirror against the back of the tub containing the *Macoma* to ensure that we could clearly observe foraging behaviors regardless of a bird’s orientation to the video camera. We observed the trials through a two-way mirror and trials ended after 10 min or once five *Macoma* had been consumed, whichever was first. We performed one trial per bird per day.

Birds consumed no prey in 3 of 144 functional-response trials. In an additional 16 trials, birds consumed 1–4 *Macoma* in the 10 min trial period. Most (N=11) of these trials involved male *tschuktschorum* subjects; these birds could typically only swallow two or three size 2 prey items before requiring a digestive pause. Every size 1 *Macoma* (8–10 mm) that was brought to the surface of the sand was consumed, but 94 size 2 *Macoma* (11–13 mm) that were brought to the surface of the sand were rejected across 31 trials. *Macoma* were rejected by females and males of both subspecies and the average length of the rejected *Macoma* was 12.2±0.1 mm.

**Video analysis and statistical analyses**

Across all three experimental trials, we removed observations in which no *Macoma* were consumed from all subsequent analyses. Video observation conditions were excellent during trials and before/after counts of *Macoma* corroborated our video observations. We replayed the video of each feeding trial at slow speed and recorded the number and duration of relevant behaviors using JWatcher software (Blumstein and Daniel, 2007). In the maximum intake rate trials, we divided (number of *Macoma* consumed−1) by the duration between the consumption of the first and last *Macoma* in each trial. We applied the results of our *Macoma* prey quality assessments to the maximum intake rate trials to estimate the AFDM (mg) and shell ballast (mg) for each size class. We applied these estimates to calculate the intake rate of *Macoma* flesh (mg AFDM *Macoma* per second) and shell (mg shell per second). For the functional-response trials, we calculated the time each bird spent searching [total time in sand-filled tub−(time loafing+time in digestive pause+time handling discovered prey)] and the handling time for each *Macoma* discovered and brought to the...
surface of the sand. We sieved each tub following each trial to determine how many *Macoma* were discovered but not consumed and how many were discovered and consumed. We synthesized these data to calculate the average search time per *Macoma* discovered (\(T_s\) in seconds per *Macoma*) and the handling time per *Macoma* swallowed (\(T_h\), in seconds per *Macoma*) per trial. We calculated the instantaneous area of discovery \(a\) using the formula:

\[
a = \frac{1}{T_s D^2},
\]

following Loureiro et al. (2010) and van Gils et al. (2005b). We determined \(T_s\) for each *Macoma* that was consumed and accounted for the depletion of prey when integrating density \((D)\) in our estimates of \(a\). Search time and handling time were the response variables in the functional-response trials.

We fitted generalized linear mixed-effects models to assess the effect of relevant biological parameters on the foraging behaviors of rock sandpipers. We followed the multi-model information-theoretic analytical approach outlined in Burnham and Anderson (2002) to examine support for our hypotheses about factors affecting the foraging ecology of rock sandpipers. For each analysis, we included biologically relevant combinations of the explanatory variables. For the maximum intake rate trials, these included sex, subspecies and *Macoma* size. We also included body mass as a covariate in all maximum intake trials to control for potential size-related differences in metabolic rates (e.g. McKechnie and Wolf, 2004; West et al., 2002) that might affect intake rates. For the functional-response trials we included sex, subspecies, *Macoma* size and *Macoma* density as explanatory variables.

Exploratory plots indicated potential interactions between sex, subspecies and *Macoma* size with respect to search time and so we included models in our analysis of search time to account for these patterns. To better fit underlying model assumptions, we transformed search time, handling time and *Macoma* density using log_{10} transformations. We gauged support for each model based on Akaake’s Information Criterion adjusted for small sample size (AICc) and based model inference on Akaake weights (\(w_i\); Burnham and Anderson, 2002). We calculated model-averaged parameter estimates using Akaake weights and considered parameters to be biologically meaningful if their model-averaged 95% confidence intervals did not overlap zero. We conducted all analyses in R version 3.1.0 (R Development Core Team, 2014), fit mixed-effects models using the lme4 package (Bates et al., 2014) and averaged model outputs using the AICmodavg package (Mazerolle, 2014). Estimates are presented as means ± s.e.m.

Acknowledgements
Allert Bijleveld, Maarten Brugge, Ysbrand Galama, Sander Holthuijsen, Rim Lucassen, Andy Reeves and John Terenzi were instrumental in helping to collect, sort and maintain the bivalves used in the trials. Dick Visser assisted with figures and Anneke Bol provided genetics-based sexing results. Colleen Handel, Robert Rickfels and three anonymous reviewers provided comments that greatly improved the manuscript. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

Competing interests
The authors declare no competing or financial interests.

Author contributions
All authors contributed to the conceptual approach of the study; D.R.R., A.D. and R.E.G. collected data and D.R.R. wrote the manuscript with important input from R.E.G., J.A.V.G. and T.P.

Funding
This research was funded by the US Geological Survey’s Ecosystems Mission Area and by the Marine Ecology Department at NIOZ Royal Netherlands Institute for Sea Research. J.A.V.G. was supported by an NWO VIDI grant (864.09.002).

References


