Sandpipers Select Red Brine Shrimps Rich in Both Carotenoids and Parasites

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Abstract

Cestode parasites using brine shrimps *Artemia* as intermediate hosts and waterbirds as final hosts are known to change the colour of shrimps from pale pink to bright red owing to an increase in carotenoid pigments. In order to assess whether this coloration increases the chances of shrimp predation by the final host, we conducted an experiment in which 10 *Calidris* sandpipers were given a choice of 20 bright red and 20 pale pink shrimps. A strong preference for red, infected shrimps was recorded, suggesting that red colour is a manipulation by cestodes to increase transmission to its final hosts. The factors regulating access and functions of carotenoids in birds are under intense debate. For the first time, we show that access to carotenoid-rich foods in birds can have a cost derived from an increased exposure to parasites. To our knowledge, this is the first time that avian hosts have been shown to exert a colour-based preference for parasitized prey.

Introduction

Trophically transmitted parasites often manipulate the behaviour of their intermediate host, which is assumed to increase the vulnerability to predation by the final host (for a review, see Moore 2002). However, the adaptive value of behavioural changes associated with infections is difficult to demonstrate. Increased predation by the final host is the main criterion supporting adaptive manipulation by parasites (Poulin 1994), yet this has rarely been shown because most studies of trophically transmitted parasites concentrate on their interactions with intermediate hosts.

Old World brine shrimps *Artemia* spp. (Crustacea, Branchiopoda, Anostraca) are intermediate hosts for several species of avian cestodes whose transmission depends on predation by shorebirds (*Anomotaenia tringae*, *A. microphallos*, *Euricestus avoceti*), flamingos (*Flamingolepis liguloides*, *F. flamingo*, *Gynandrotaenia stammeri*), grebes (*Confluaria podicipina*) and gulls (*Wardium stellorae*) (Georgiev et al. 2005). Natural populations of *Artemia* show a continuous distribution of coloration ranging from very pale individuals to intensely red coloured ones. *Artemia* recorded near the water surface, where birds feed, tend to be red and infected with cestodes, whereas those close to the bottom tend to be pale pink and parasite free (Gabrion et al. 1982; Sánchez et al. 2007a). Red colour is likely to be an indirect consequence of parasite-induced surface behaviour (Gabrion et al. 1982; Sánchez et al. 2006). To avoid damage by solar radiation, *Artemia* possess pigments with photoprotective functions (all-*trans*-canthaxanthins) (Nelis et al. 1988), which confer a pink coloration at high concentrations. Thus red coloration in *Artemia* is carotenoid based, and infected individuals are especially rich in carotenes and lipids (Amat et al. 1991; Sánchez et al. 2006, 2008).

Many shorebirds are visual predators that regularly consume *Artemia* (e.g. Verkuil et al. 2003; Sánchez et al. 2007b). Red *Artemia* are a main source of carotenoids which are essential pigments for birds (Hill & McGraw 2006). Strong preference for orange,

yellow and red food items have been showed in frugivorous birds, suggesting a preference for carotenoid-indicating colours (Borgia & Keagy 2006). Thus, shorebirds may prefer red *Artemia*.

Alternatively, because red shrimps are parasitized, birds may avoid them. Antiparasite behaviour has previously been reported in oystercatchers, which avoid larger cockles that offer both increased energy intake and increased risk of parasitism (Norris 1999). The aim of this study was to test whether (1) sandpipers select red *Artemia* rich in carotenoids (Lozano 1994), or (2) birds avoid red individuals in order to avoid parasitism (Lozano 1991), using a prey choice experiment. We consider the implications of our results for tri-trophic predator–prey–parasite interactions and the value of carotenoids to birds.

Methods

The prey choice experiment was carried out during the daytime with 10 sandpipers (*Calidris* spp.) which use visual cues for feeding during the day (Robert & McNeil 1989) and regularly consume *Artemia* (Masero 2003; Verkuil et al. 2003). Four dunlin *C. alpina* were captured using mist nets on 12 Nov. 2003 and three *C. alpina*, two curlew sandpipers *C. ferruginea* and one little stint *C. minuta* on 21 Sept. 2004 in the El Pilar salt pans (Cádiz Bay, SW Spain, 36°23′N, 6°8′W). Prior to the choice experiment, the birds were taken to Jerez Zoo where they were held for 5 d in individual cages, and fed *ad libitum* on *Nereis diversicolor* and *Artemia*. Cylindrical cages (1.5 m diameter, 1 m high) were radially divided into four compartments (one per bird), and covered by a 5 mm mesh net, allowing birds to maintain visual contact.

Parthenogenetic *Artemia* were collected from salt pans in the Odiel marshes (Huelva, SW Spain, 37°17′N, 6°55′W), where the prevalence of cestodes is high and has been intensively studied (Georgiev et al. 2005, 2007). In autumn, *Artemia* population is integrated by 38.5% of red, 41% of pale pink and 20.5% of intermediate colour individuals (M. I. Sanchez, B. B. Georgiev, P. N. Nikolov, G. P. Vasileva & A. J. Green, unpublished data). Because we required live *Artemia* for our experiments, we could not quantify the abundance of cestodes in the individuals used, because the parasites can only be counted after killing the specimen (Georgiev et al. 2005). However, the three species of dilepididae cestodes parasitizing shorebirds in our study area (*A. tringae*, *A. microphallos* and *E. avoceti*) are disproportionately represented in red compared with pale pink *Artemia*

(Sánchez et al. 2006). In a similar sample collected from the Odiel salt pans in Nov. 2003, 38% of red *Artemia* contained cestodes specifically parasitizing shorebirds, compared to only 12% of pale pink *Artemia* (data reanalysed from Sánchez et al. 2007a). Likewise, in a similar sample collected in Sept. 2002, 46% of red *Artemia* contained cestodes parasitizing shorebirds, compared to only 17% of pale pink *Artemia* (data reanalysed from Sánchez et al. 2006). The intensity of infection (i.e. number of cestodes per infected *Artemia*) was also higher in red *Artemia* (Sánchez et al. 2006, 2007a).

We eliminated the confounding effect of parasites on *Artemia* behaviour by placing *Artemia* in very shallow water (1 cm) to avoid spatial segregation of infected and uninfected individuals in the water column (see Sánchez et al. 2007a). A brown tray was used to imitate the colour of the bottom of salt ponds under natural conditions. We offered a set of 20 red and 20 pale pink *Artemia* (see Fig. 1 for examples of the colour differences between both categories) of the same size to each bird within the individual cages described above. Before the experiment, each shrimp was measured from the naupliar eye to the end of the

Fig. 1: Colour differences between infected (red) and uninfected brine shrimp (pale pink).

Table 1: Number of red and pale pink *Artemia* (out of 20) ingested by *Calidris* spp. in the prey choice experiment

Wader species	Date of experiment	Ingested *Artemia*	
		Red	Pale pink
Calidris alpina	19 Nov. 2003	17	9
Calidris alpina	19 Nov. 2003	11	8
Calidris alpina	19 Nov. 2003	20	0
Calidris alpina	19 Nov. 2003	16	3
Calidris alpina	28 Sept. 2004	6	3
Calidris alpina	28 Sept. 2004	11	5
Calidris alpina	28 Sept. 2004	16	9
Calidris ferruginea	28 Sept. 2004	17	11
Calidris ferruginea	28 Sept. 2004	13	5
Calidris minuta	28 Sept. 2004	10	6

abdomen to the nearest 10 μm using an image analyser. Each bird was then allowed to pick in the tray approx. 20 times before we stopped the experiment, counting the number of red and pale pink *Artemia* remaining. Each bird was used only once.

To compare the size of red and pale pink shrimps we used a one-way ANOVA, with feeding trial as a random block. We compared the number of red and pale shrimps predated with a Wilcoxon matched-pair test.

Licenses and permits to catch the birds and to conduct the experiments were provided by Junta de Andalucia, Consejeria de Medio Ambiente, Direccion General de Gestion del Medio Ambiente (14 Jul. 2003 and 21 Jul. 2004). The experimental design and procedures were carried out in order to minimize the stress to the captive birds. Individual cages of equal size (70 × 70 × 100 cm) contained only one bird. Cages were placed outside, so birds were exposed to normal photoperiod. The birds were kept for a total of 6 d, and released at the capture site the day following the experiments.

Results

Differences in size between red ($\bar{x} \pm$ SE, 7.48 $\pm$ 0.06, n = 200) and pale ($\bar{x} \pm$ SE, 7.44 $\pm$ 0.06, n = 200) *Artemia* were not significant ($F_{1,389} = 0.59$, p = 0.44). All 10 birds showed a preference for red over pale pink *Artemia* (Table 1). One individual consumed all 20 red shrimps and none of the pale ones. Red *Artemia* were ingested significantly more often than pale ones ($Z = 2.80$, p = 0.005).

Discussion

Our choice experiment strongly suggests that the red coloration increases parasite fitness by improving

transmission success, while decreasing shrimp fitness via increased risk of mortality. On the basis of the data from Sánchez et al. (2006, 2007a), shorebirds ingesting a red *Artemia* will have a probability of contact with a cestode parasite (*A. tringae*, *A. microphallos* and/or *E. avoceti*) three times higher than those consuming pale pink individuals (43.3% vs. 15.3% respectively). Shorebirds (*Calidris* spp.) feeding on *Artemia* have very high pecking rates, with a mean of up to 64 pecks/min registered by Verkuil et al. (2003). The strength of the choice expressed by waders was underestimated because predated, red individuals were not replaced and became steadily scarcer as the experiment proceeded. In natural conditions, the selection of red, infected shrimps would probably be even stronger, as colour change is also accompanied by behavioural changes that increase accessibility to shorebirds (Sánchez et al. 2007a). Shorebirds generally pick *Artemia* from the water surface and the behavioural and colour change induced by cestodes appears well matched to the final host foraging ecology.

Excluding any possibility of behavioural effect on prey choice would require the manipulation of colour, which is problematic in this system. Removing the colour cue from the sandpiper's perception (for example under coloured lights or with coloured lenses applied to the birds' eyes) should be considered in future choice tests.

To our knowledge, this is the first study showing selective colour-based predation of infected prey by avian final hosts. Our study is also novel in that the colour modification not only has a cost for the predator (increasing parasite transmission) but also a potential benefit, since red shrimps provide a major source of carotenoids considered to be highly beneficial for birds (Alonso-Alvarez et al. 2007). We can not tell whether birds actively select red shrimps because of their higher visibility or because they actively select carotenoid-rich prey, but either way the result is that, by preying on red *Artemia*, birds are increasing their intake or carotenoids. Carotenoids have important immunological functions (Lozano 1994), seem to play an important role in the control of oxidative stress (Alonso-Alvarez et al. 2007; Peters 2007) and are responsible for a range of yellow to red coloration in vertebrate tissues known to play a major role in sexual selection (Lozano 1994). As birds can not synthesize these pigments (Goodwin 1984) and they are a scarce resource in nature (Endler 1980; Grether et al. 1999), a trade-off in allocation of carotenoids between its different functions is likely to occur (Lozano 1991; Von Schantz et al. 1999).

The factors regulating access and functions of carotenoids in birds are the focus of recent research (Alonso-Alvarez et al. 2007; Peters 2007; Constantini & Moller 2008). Although none of the *Calidris* species used in our experiment displays carotenoid-derived coloration, our results add a new dimension to the study of the implications of carotenoids in health and sexual selection by showing that access to carotenoid-rich foods in birds can have costs derived from an increased exposure to parasites. This cost may be especially significant to a species such as the redshank *Tringa totanus*, a shorebird that feeds heavily on parasitized *Artemia* (Sánchez et al. 2005) and which has red carotenoid pigmentation in its legs.

With few exceptions, the pathological effects of cestode parasites on birds are unknown, partly because mixed parasite infection is the general law in natural populations. High parasite load may contribute to the general debility of birds, especially when associated with factors like malnutrition (Ballweber 2004) and, exceptionally, cestodes can cause death (Kellogg et al. 1971; Price 1985). Non-lethal effects may include a reduction in the development of breeding plumage in bar-tailed godwit that may result in reduced breeding output (Piersma et al. 2001). The absence of an antiparasitic behavioural response in *Calidris* when exposed to cestode parasites may be explained if the benefit of feeding on infected prey is higher than the energetic cost of parasitism (Anderson & May 1979; Lafferty 1992). However, additional information on infective doses and costs of parasitism, including artificial infection and field data, is essential to evaluate the strength of the potential trade-off between parasite infection and carotenoid ingestion, and the lack of evolution of antiparasite mechanisms.

Acknowledgements

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