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RESEARCH ARTICLE

Intestinal digestive enzyme modulation in house sparrow nestlings occurs within 24 h of a change in diet composition

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ABSTRACT

Nestling house sparrows near fledging age (12 days) were previously found to reversibly modulate the activity of their intestinal digestive enzymes in response to changes in diet composition. However, it is not known how quickly nestlings can adjust to new diets with different substrate compositions, nor is it known how early in life nestlings can modulate their enzyme activity in response to changes in diet. In the present study, 3-day-old nestlings were captured from the wild and fed and switched among contrasting diets – one high in protein and low in carbohydrate and another higher in carbohydrate and with lower, but adequate, protein – in order to determine (1) how quickly house sparrow nestlings could adjust to changes in diet composition, (2) how early in life nestlings could modulate their digestive enzyme activity in response to these changes and (3) which digestive enzymes could be modulated in house sparrow nestlings earlier in life. We found that house sparrow nestlings as young as 3 days post-hatch were capable of modulating their intestinal disaccharidase activity within 24 h of a change in diet composition, and nestlings gained the ability to modulate aminopeptidase-N by 6 or 7 days of age. To our knowledge, this is the first evidence of digestive enzyme modulation completed within 24 h of a change in diet in an avian species and the first study to show intestinal digestive enzyme modulation in response to changes in diet composition in any animal this early in development.

KEY WORDS: Digestive physiology, Development, Phenotypic flexibility, Invasion biology, Feeding ecology, *Passer domesticus*

INTRODUCTION

Of all vertebrates, birds have the highest daily energy requirements and feeding rates (Nagy, 2001, 2005). Despite this, birds have smaller small intestines than nonflying mammals of equal size, likely because of selective pressure to reduce the masses of organs that are not required for flight (Price et al., 2015). Owing to mass constraints and short digesta retention times that limit spare digestive capacity of the avian gastrointestinal tract (McWhorter et al., 2009), birds may rely on phenotypic flexibility – reversible variations of traits within an individual (Piersma and Drent, 2003) – to adjust to changes in environment, such as those that occur because of migration, extreme temperatures or food shortages (Dykstra and Karasov, 1992; Piersma and van Gils, 2011). When necessary, birds can drastically increase the digestive and absorptive capacity of their intestines by increasing their digestive organ sizes (Battley and Piersma, 2005; Dykstra and Karasov, 1992; McWilliams and Karasov, 2001), which accommodates higher feeding rate. Omnivorous species may also use phenotypic flexibility to respond to seasonal or unexpected changes in food availability and types (Sabat et al., 1998). Varying diets are often composed of different levels of substrates, and over a dozen avian species have been found to be able to increase the activity of intestinal digestive enzymes when switched to diets higher in their respective substrates (McWhorter et al., 2009).

House sparrows [*Passer domesticus* (Linnaeus 1758)] – the focal species of the present research – have been found to increase their intestinal hydrolytic activity following increases in corresponding dietary substrates. Although adult house sparrows have fixed carbohydrase activities (Caviedes-Vidal et al., 2000), nestlings modulate carbohydrase activity in response to changes in substrate levels (Brzęk et al., 2009). This ability to modulate is beneficial to nestlings, because house sparrows undergo a natural diet switch as nestlings. In their natural diet switch, nestlings transition from a diet of mainly arthropods, which are high in protein and low in carbohydrates, to a diet of mainly seeds, many of which are high in carbohydrates and low in protein (Anderson, 2006). Nestling house sparrows not only can modulate their intestinal carbohydrase activity to adapt to increasing carbohydrate in the diet (Brzęk et al., 2013b), but can also revert back to a diet containing less carbohydrate, showing phenotypic flexibility of nestling digestive physiology (Brzęk et al., 2011). However, previous studies on house sparrow nestlings have primarily focused on nestlings 12 days post-hatch and older, which is around the age at which they fledge. It is not known whether much younger nestlings are capable of modulating their hydrolytic activity, and – if they can – how quickly younger nestlings can modulate their enzyme activity in response to dietary changes.

In this study, we performed multiple experiments to determine (1) whether younger house sparrow nestlings were capable of modulating their intestinal digestive enzyme activity, (2) how quickly nestlings could modulate their enzyme activity and (3) which enzymes could be modulated in younger nestlings. The intestinal peptidase aminopeptidase-N and the intestinal carbohydrases responsible for maltasic and sucrasic activities were investigated along the length of the intestine because much has already been discovered about these intestinal brush-border enzymes in the house sparrow (Brzęk et al., 2011, 2013b). Although it is known that maltase and sucrase activities can be modulated in older nestlings, previous research has failed to show significant modulation of aminopeptidase-N in nestlings in response to changes in protein content in the diet (Brzęk et al., 2009, 2011, 2013b). However, this may be due to previous experimental diets having

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only a relatively small variation in the proportion of protein in the diet. It is possible that greater changes in protein content would induce aminopeptidase-N modulation, especially because aminopeptidase-N modulation occurred in adult house sparrows switched from a diet that was 10% protein to a diet that was 57.5% protein (Caviedes-Vidal et al., 2000). Therefore, the present study used larger protein differences between treatment diets to determine whether aminopeptidase-N activity could be modulated. Because adult rats can modulate their peptidase activity within 24 h (Raul et al., 1987), we predicted that aminopeptidase-N as well as maltase and sucrase activity would change within 24–48 h of a dietary increase in their respective substrates. Also, because even very young house sparrow nestlings undergo a natural diet switch (Anderson, 2006), we predicted that very young nestlings would exhibit digestive enzyme flexibility.

MATERIALS AND METHODS

Most of our methods were developed during previous experiments with nestling house sparrows (Brzęk et al., 2009; Caviedes-Vidal and Karasov, 2001; Lepczyk et al., 1998).

Study site and bird collection

Nestling house sparrows were collected from nests between mid-May and early August 2014 and 2015. During these months, average daily minimum temperatures range from 8 to 16°C and average daily maxima range from 20 to 28°C (www.usclimatedata.com/madison/wisconsin). Natural and artificial nests were located in dairy barns and a parking ramp in close proximity to the Department of Forest and Wildlife Ecology on the University of Wisconsin–Madison campus. Beginning in early April, nests were surveyed once per week to determine the onset of egg-laying and incubation. Active nests were checked daily between 08:30 and 10:30 h CST around the time of hatching to ensure consistent and accurate aging of nestlings. Three days post-hatch, nestlings (N=133 over the course of two consecutive summers) were collected from their nests and housed individually in an environmental chamber in our laboratory under constant conditions of 35°C chamber temperature (because they still have limited thermoregulatory ability; Ovadia et al., 2002), 50–55% relative humidity and 15 h:9 h light:dark photoperiod (lights on at 06:00 h). All experimental procedures were approved by the University of Wisconsin–Madison ethics committee (permit no. RARC A-0570-0-03-14).

Experimental diets and feedings

Two synthetic liquefied diets were used in the present study. Both diets were composed of the same ingredients and only differed in their amounts of corn starch (carbohydrate) and casein (protein) (Table 1). The high protein (HP) diet was 59.5% casein and 5% corn starch, and it was designed to represent a natural diet composed of mainly arthropods that wild house sparrow nestlings consume during the first few days post-hatch (Bell, 1990). The high carbohydrate (HC) diet was 26.5% casein and 38% corn starch, and it was designed to represent the combination of arthropods and seeds that is consumed by wild fledgling house sparrows. Although the HC diet had approximately half the protein of the HP diet, it still contained sufficient levels of essential amino acids and 3–5% more protein than required to support growth in birds (Klasing, 1998). Because of the thickening effect that casein had on the consistency of the liquefied diet, different ratios of dry diet:water had to be used for each liquefied diet so that the nestlings could feed without difficulties. The HP diet was prepared in a mass ratio of 1:4 (dry diet:water), while the HC diet was prepared in a ratio of 1:2.5.

Table 1. Composition of diets used in all experiments

	HC (% of dry mass)	HP (% of dry mass)
Corn starch	38	5
Casein (protein)	26.5	59.5
Corn oil	8	8
Alphacel non-nutritive bulk+silica sand	17	17
Amino acids, vitamins, mineral salt, etc.[a]	10.5	10.5
kJ g^{-1} dry mass based on composition	14.8	15.1

HC, high carbohydrate; HP, high protein. The HC diet was mixed with filtered water in a ratio of 1:2.5 (dry diet:water), and the HP diet was mixed with filtered water in a ratio of 1:4.
[a]Content as described by Lepczyk et al. (1998).

Nestlings in the laboratory were syringe-fed hourly 15 times per day. Meal sizes were calculated using age-specific energy requirements and the diets' dry diet:water ratios. Meal sizes for nestlings consuming the HP diet were 0.64, 0.88, 1.04, 1.20, 1.36, 1.60 and 2.00 ml for nestlings aged 3–9 days post-hatch, respectively. Meal sizes for nestlings consuming the HC diet were 0.42, 0.58, 0.68, 0.79, 0.89, 1.05 and 1.31 ml for nestlings aged 3–9 days post-hatch, respectively. To verify that nestlings on both diets were consuming the same quantities of gross energy per day (ranging from 25 kJ day^{-1} for 3-day-old nestlings to 79 kJ day^{-1} for 9-day-old nestlings), all meal sizes were recorded and diet samples were dried to verify correct dry diet:water ratios daily.

Experimental schedule

The results of three different experiments testing for rapid induction of intestinal brush-border digestive enzymes are reported in this paper.

The goal of the first experiment (switch to high carbohydrate diet), conducted July–August 2014, was primarily to study the time course of induction of carbohydrases in 6- to 9-day-old nestling house sparrows switched from the HP diet to the HC diet. However, aminopeptidase-N was also included in the first experiment (1) to determine whether the decrease of protein in the diet would result in a reduction of the activity of the peptidase and (2) because the goal of a subsequent experiment was primarily to study the induction of this enzyme.

In the second experiment (switch to high protein diet), which was conducted May–July 2015, 6- to 9-day-old nestlings were switched from the HC diet to the HP diet. Although investigating the induction of aminopeptidase-N was the primary focus of the experiment, maltase and sucrase activity were also examined for reasons similar to those described for the inclusion of aminopeptidase-N in the first experiment.

The third experiment (test for modulation in 3- to 4-day-old nestlings), conducted July–August 2015, examined the ability of nestlings only 3–4 days old to modulate the activities of maltase, sucrase and aminopeptidase-N within 24 h. The complete schedule for each of these experiments is discussed further below.

All nestlings, regardless of the experiment in which they were involved, were dissected in the same manner. At the time of dissection, nestlings were weighed, euthanized with CO_2, and dissected to remove and weigh the intestines, pancreas and liver. The whole intestine was perfused with ice-cold avian Ringer solution before the proximal, medial and distal portions were dissevered and preserved in liquid nitrogen. Sections of the intestine were collected and stored separately because (1) it is possible that modulation could have occurred only in certain regions of the small intestine, (2) enzymatic activity differs along the length of the

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intestine and (3) the portion of the intestine with the highest activity is dependent on the enzyme in question (Afik et al., 1995; Caviedes-Vidal and Karasov, 2001; Caviedes-Vidal et al., 2000). Tarsometatarsus length was also measured on dissected birds as an indicator of skeletal growth.

Experiment 1: switch to high carbohydrate diet

Once the nestlings were brought into the laboratory, all nestlings spent 3 days on the HP diet. After spending 3 days on the HP diet, some of the birds were dissected (nestling age=6 days). Another group of nestlings was kept on the HP diet for an additional 3 days before being dissected (nestling age=9 days). All other nestlings were switched to the HC diet after spending the first 3 days on the HP diet. Birds that were switched to the HC diet spent 24, 48 or 72 h on that diet before they were also dissected (nestling ages=7, 8 and 9 days, respectively). The two treatment groups that only consumed the HP diet serve as a baseline for comparison with treatment groups that were switched to the HC diet.

Experiment 2: switch to high protein diet

All nestlings spent the first 3 days in the laboratory on the HC diet. After spending 3 days on the HC diet, some of the birds were dissected (nestling age=6 days). Another group of nestlings was kept on the HC diet for an additional 3 days before being dissected (nestling age=9 days). All other nestlings were switched to the HP diet after spending the first 3 days on the HC diet. Birds that were switched to the HP diet spent 24, 48 or 72 h on that diet before they were also dissected (nestling ages=7, 8 and 9 days, respectively). The two treatment groups that only consumed the HC diet serve as a baseline for comparison with treatment groups that were switched to the HP diet.

Experiment 3: test for modulation in 3- to 4-day-old nestlings

Two nestlings were collected from each nest when they were 3 days old (3 days post-hatch). One nestling from each nest was randomly assigned to consume the HP diet for 24 h while the other nestling was assigned to consume the HC diet for 24 h. After 24 h, the nestlings were dissected (nestling age=4 days).

Intestinal enzyme assays

Assays of the activities of carbohydrases maltase and sucrase and the peptidase aminopeptidase-N were performed as described in Brzęk et al. (2009) with the adjustments implemented in later studies (Brzęk et al., 2011, 2013b). Summed enzyme activities, which are indicative of total hydrolytic capacity, were calculated by (1) determining the total enzyme activity in each section of the intestine (i.e. proximal, medial and distal) in units of micromoles of substrate hydrolyzed per minute and (2) adding the total enzyme activities of the individual sections together to obtain the total enzyme activity over the entire intestine (summed enzyme activity). Mass-specific enzyme activities for each intestinal section were calculated by dividing the total enzyme activities by their respective intestine masses.

Data analysis

In the two experiments involving 6- to 9-day-old birds, data were analyzed using one-way ANOVAs with each combination of diet and time after diet switch representing a treatment group; hence five

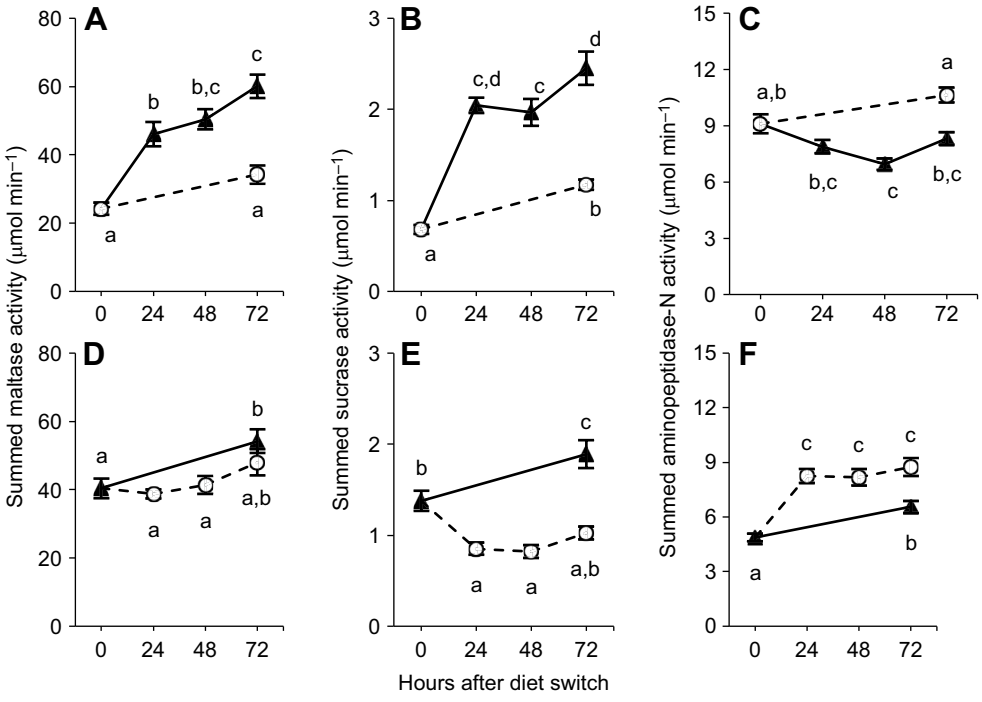

Fig. 1. Summed enzyme activities of maltase, sucrase and aminopeptidase-N of 6- to 9-day-old nestling house sparrows 0, 24, 48 and 72 h after a diet switch. Summed activities of nestlings that switched from the high protein (HP) diet to the high carbohydrate (HC) diet are shown in the first row (A–C), while summed activities of nestlings that switched from the HC diet to the HP diet are shown in the second row (D–F). In A–C, circles and dashed lines indicate groups only fed the HP diet, and triangles and solid lines indicate groups switched to the HC diet and thus eating only the HC diet for the number of hours indicated on the abscissa. In D–F, triangles and solid lines indicate groups only fed the HC diet, and circles and dashed lines indicate groups switched to the HP diet and thus eating only the HP diet for the number of hours indicated on the abscissa. The diet switch occurred at 6 days of age and final sampling was 72 h later at 9 days of age. Values are means±s.e.m. (*N*=10–13 birds in each treatment group). Different letters denote significant differences in summed enzyme activity between treatment groups (one-way ANOVA, followed by Tukey HSD, across the 5 treatment groups, each enzyme tested independently).

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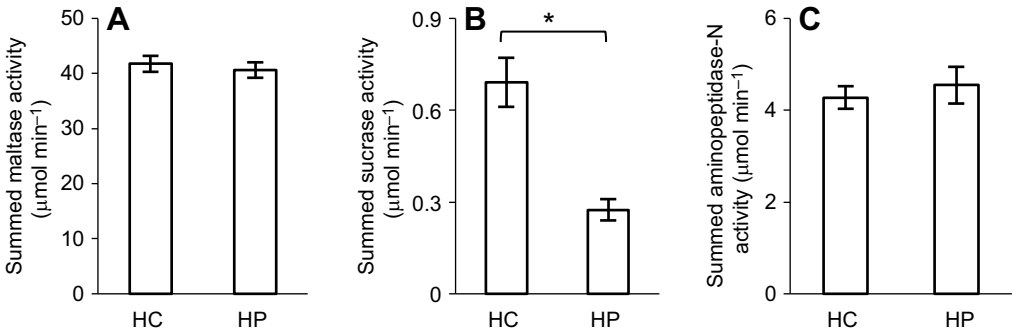

Fig. 2. Summed enzyme activities of 4-day-old nestling house sparrows after ingesting either the HC diet or the HP diet for 24 h. (A) Maltase, (B) sucrase and (C) aminopeptidase-N. Values are means±s.e.m. (*N*=9 birds in each treatment group). Diet had a significant effect on the summed enzyme activity of sucrase (paired *t*-test with sibling nestlings paired by nests; **P*<0.05), but it had no effect on maltase or aminopeptidase-N.

treatment groups in each experiment were compared. Tukey's HSD tests supplemented with the results of Tukey-adjusted least-squares means procedures allowed for comparisons of pairs of treatment group means. Data from the experiment with 3- to 4-day-old birds was analyzed through use of paired *t*-tests with nestlings paired by nest. In all tests, which were performed using SAS software (SAS Institute, Cary, NC, USA), the significance level was set at $\alpha<0.05$. *F*-values of ANOVAs and *t*-values of *t*-tests are provided in the relevant tables with pertinent degrees of freedom as subscripts.

RESULTS

Summed enzyme activities

Experiment 1: switch to high carbohydrate diet

Diet had a significant effect on the summed maltasic, sucrasic and aminopeptidase-N activities ($F_{4,53}\geq11.93$ for all three enzymes, $P<0.0001$ for all three enzymes; Fig. 1A–C). For maltase and sucrase activities, summed carbohydrase activity was significantly increased within 24 h of the switch from the HP diet to the HC diet (Tukey's HSD, $P<0.0001$ for maltase and sucrase activity). For aminopeptidase-N, summed enzyme activity significantly decreased within 24–48 h of the diet switch (Tukey's HSD, $P=0.002$).

For nestlings that remained on the HP diet their entire time in the laboratory, the summed sucrase activity increased with age (Tukey's HSD, $P=0.02$), but summed maltase and aminopeptidase-N activities did not significantly change between nestlings 6 and 9 days old on the HP diet (Tukey's HSD, $P=0.12$ and $P=0.054$, respectively).

Experiment 2: switch to high protein diet

When 6-day-old nestlings were switched from the HC diet to the HP diet for 24–72 h, the change in diet composition had a significant effect on the summed activities of sucrase and aminopeptidase-N ($F_{4,52}\geq17.60$, $P<0.0001$; Fig. 1E–F). For aminopeptidase-N, summed enzyme activity significantly increased within 24 h of the diet switch (Tukey's HSD, $P<0.0001$) and did not change further with additional time. In contrast, sucrase summed enzyme activity significantly decreased within 24 h of the switch from the HC diet to the HP diet (Tukey's HSD, $P=0.003$). Summed maltase activity was also decreased in birds 24–48 h after the switch to the

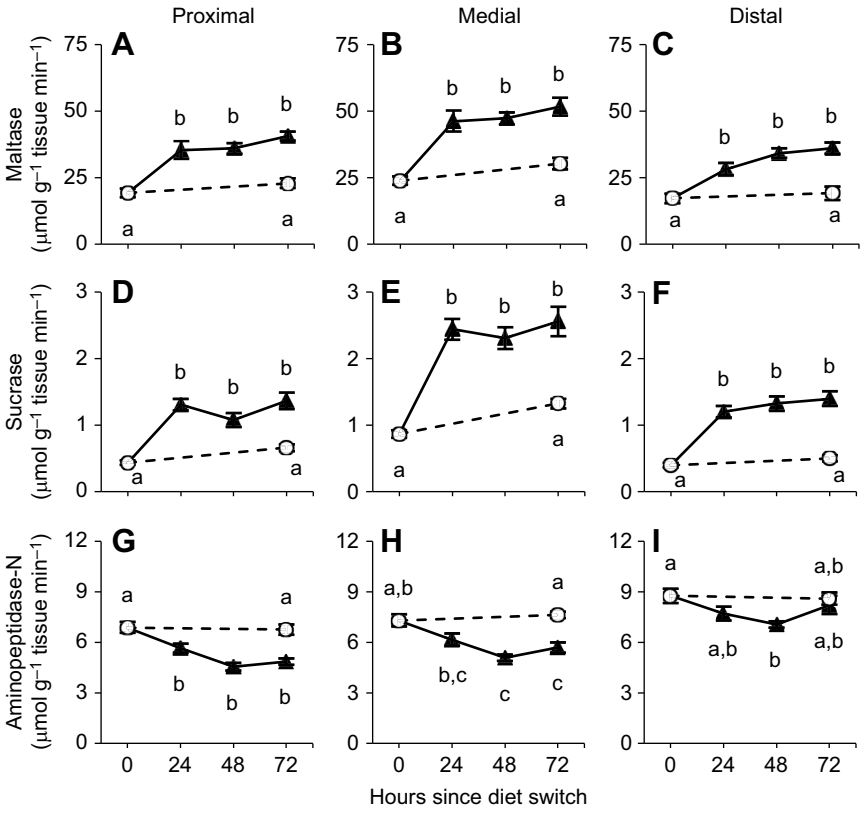

Fig. 3. Mass-specific enzyme activities in each intestinal position in 6- to 9-day-old nestling house sparrows 0, 24, 48 and 72 h after a switch from the HP diet to the HC diet. The activities of (A–C) maltase, (D–F) sucrase and (G–I) aminopeptidase-N are expressed per gram wet tissue in three intestinal positions: proximal, medial and distal. Circles and dashed lines indicate groups only fed the HP diet, and triangles and solid lines indicate groups switched to the HC diet and thus eating only the HC diet for the number of hours indicated on the abscissa. The diet switch occurred at 6 days of age and final sampling was 72 h later at 9 days of age. Values are means±s.e.m. (*N*=11–13 birds in each treatment group). Different letters denote significant differences between treatment groups within each intestinal section (one-way ANOVA, followed by Tukey HSD, across the 5 treatment groups, each enzyme tested independently).

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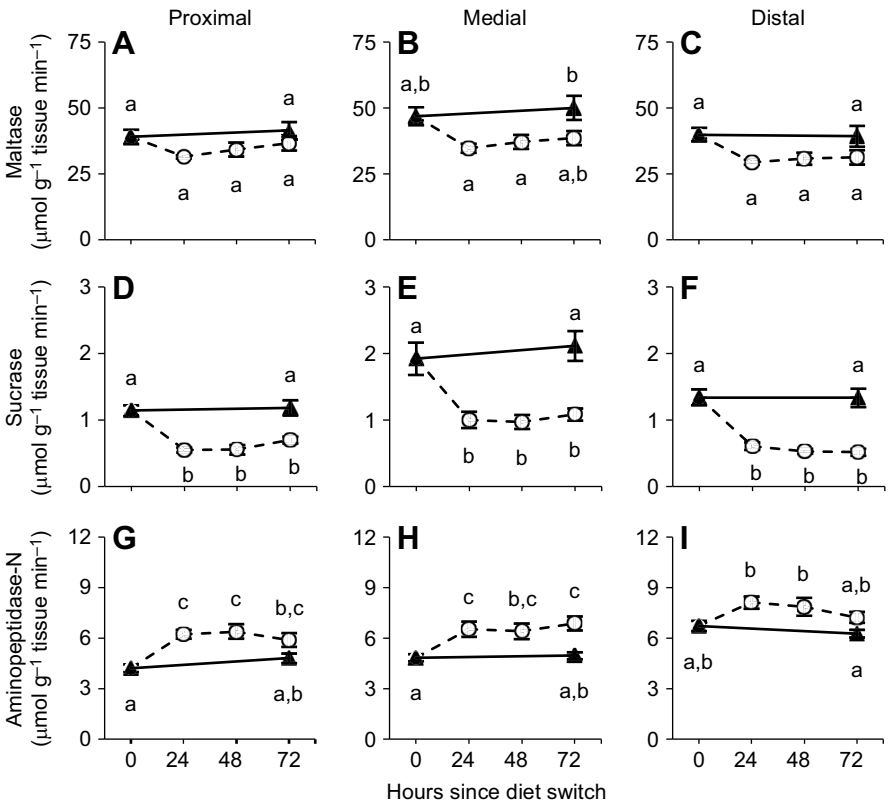

Fig. 4. Mass-specific enzyme activities in each intestinal position in 6- to 9-day-old nestling house sparrows 0, 24, 48 and 72 h after a switch from the HC diet to the HP diet. The activities of (A–C) maltase, (D–F) sucrase and (G–I) aminopeptidase-N are expressed per gram wet tissue in three intestinal positions: proximal, medial and distal. Triangles and solid lines indicate groups only fed the HC diet, and circles and dashed lines indicate groups switched to the HP diet and thus eating only the HP diet for the number of hours indicated on the abscissa. The diet switch occurred at 6 days of age and final sampling was 72 h later at 9 days of age. Values are means±s.e.m. (*N*=11–13 birds in each treatment group). Different letters denote significant differences between treatment groups within each intestinal section (one-way ANOVA, followed by Tukey HSD, across the 5 treatment groups, each enzyme tested independently).

HP diet ($F_{4,52}$=4.79, P=0.002; Fig. 1D), but the differences between the two diets after 72 h were not significant (Tukey's HSD, P=0.58).

For nestlings that remained on the HC diet their entire time in the laboratory, the summed enzyme activities of maltase, sucrase and aminopeptidase-N significantly increased between nestlings 6 and 9 days old (Tukey's HSD, P=0.01, 0.005 and 0.02, respectively).

Experiment 3: test for modulation in 3- to 4-day-old nestlings

When younger nestlings spent only 24 h on either the HP or the HC diet, diet had a significant effect only on the summed activity of sucrase ($|t_8|$=5.97, P=0.0003; Fig. 2B). Maltase and aminopeptidase-N activity were unaffected (($|t_8|\leq$0.74 for maltase and aminopeptidase-N, P=0.5 for maltase and aminopeptidase-N; Fig. 2A,C).

Mass-specific enzyme activities across intestinal regions

The aforementioned changes in summed activity in the three experiments correspond to marked changes in mass-specific activity in all three sections of the intestine and do not correspond to overall changes in intestinal mass (see 'Body measurements'). In the experiment in which 6-day-old nestlings were switched to the HC diet, the mass-specific activities of all three enzymes studied were significantly affected by the change in diet in all three sections of the intestine ($F_{4,53}\geq$2.90 in all cases, $P\leq$0.03 in all cases; Fig. 3). In the complementary experiment in which 6-day-old nestlings were switched to the HP diet, mass-specific sucrase and aminopeptidase-N activities both changed significantly in all three sections of the intestine in response to the switch to the HP diet ($F_{4,52}\geq$4.57, $P\leq$0.003 in all sections for both enzymes; Fig. 4D–I). Although mass-specific maltase activity also appeared to change when

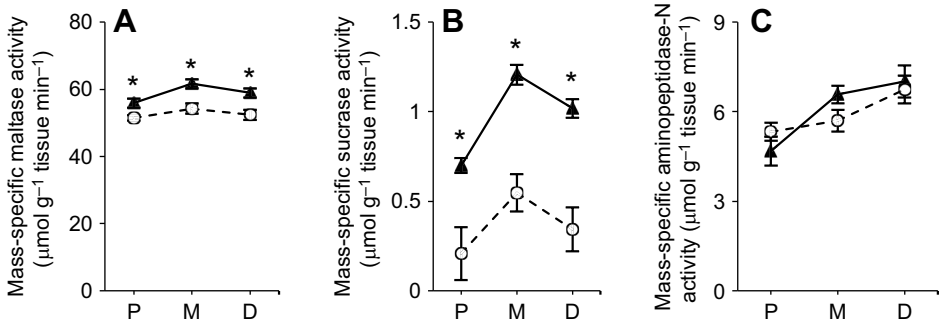

Fig. 5. Mass-specific enzyme activities as a function of intestinal position in 4-day-old nestling house sparrows after ingesting either the HC diet or the HP diet for 24 h. The activities of (A) maltase, (B) sucrase and (C) aminopeptidase-N are expressed per gram wet tissue in three intestinal positions: proximal (P), medial (M) and distal (D). Triangles and solid lines indicate the group fed the HC diet, and circles and dashed lines indicate the group fed the HP diet. Values are means±s.e.m. (*N*=9 birds in each treatment group). Diet had a significant effect on the mass-specific activities of maltase and sucrase at all intestinal positions (paired *t*-test with sibling nestlings paired by nests, **P*<0.05), but did not significantly affect aminopeptidase-N activity at any intestinal position.

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nestlings were switched to the HP diet, the differences in mass-specific maltase activity were not always significant ($F_{4,52}$≥2.47, P≤0.056 in all sections; Fig. 4A–C). In the experiment in which 3-day-old nestlings were placed on either the HC diet or the HP diet for 24 h, mass-specific maltase and sucrase activities were significantly higher in nestlings fed the HC diet ($|t_8|$≥3.10, P≤0.015 in all sections for both enzymes; Fig. 5A,B) while mass-specific aminopeptidase-N activity was unaffected by diet ($|t_8|$≤1.78, P≥0.11 in all sections; Fig. 5C).

Body measurements

In the studies of induction of carbohydrases and aminopeptidase-N in nestlings 6 to 9 days old, all body and organ sizes tended to increase with age owing to natural nestling growth (Figs 6, 7). Body masses for treatment groups with 6- to 9-day-old nestlings, when analyzed by repeated-measures ANOVA, did not differ with diet (data not shown). Likewise, tarsometatarsus length did not show diet effects in any of the experiments. Diet effects on specific organs are reported below for each experiment.

Experiment 1: switch to high carbohydrate diet

Intestine mass and length did not differ with diet 72 h after the diet switch (Tukey's HSD, P=1 and 1, respectively; Fig. 6C,D). Pancreas mass and liver mass appeared to be lower in nestlings switched to the HC diet (Fig. 6E,F), but the differences were not significant at 72 h (Tukey's HSD, P=0.3 for both organs).

Experiment 2: switch to high protein diet

Intestine mass and length did not differ with diet 72 h after the diet switch (Tukey's HSD, P=0.5 and 0.15, respectively; Fig. 7C,D). Pancreas mass and liver mass significantly increased within 24 h of the switch to the HP diet (Tukey's HSD, P<0.0001 for both organs; Fig. 7E,F), and they remained significantly higher than in nestlings that were kept on the HC diet after 72 h (Tukey's HSD, P=0.0002 and 0.002, respectively; Fig. 7E,F).

Experiment 3: test for modulation in 3- to 4-day-old nestlings

In younger nestlings that only spent 24 h on either diet, final body mass, intestine length, pancreas mass and liver mass were all significantly higher in nestlings fed the HP diet ($|t_8|$≥2.63, P≤0.03; Fig. 8). Intestinal mass was also slightly higher in nestlings fed the HP diet, but the difference was not significant ($|t_8|$=1.99, P=0.08).

DISCUSSION

Overall, intestinal digestive enzyme modulation was complete within 24 h, more quickly and completely than we expected. For all three

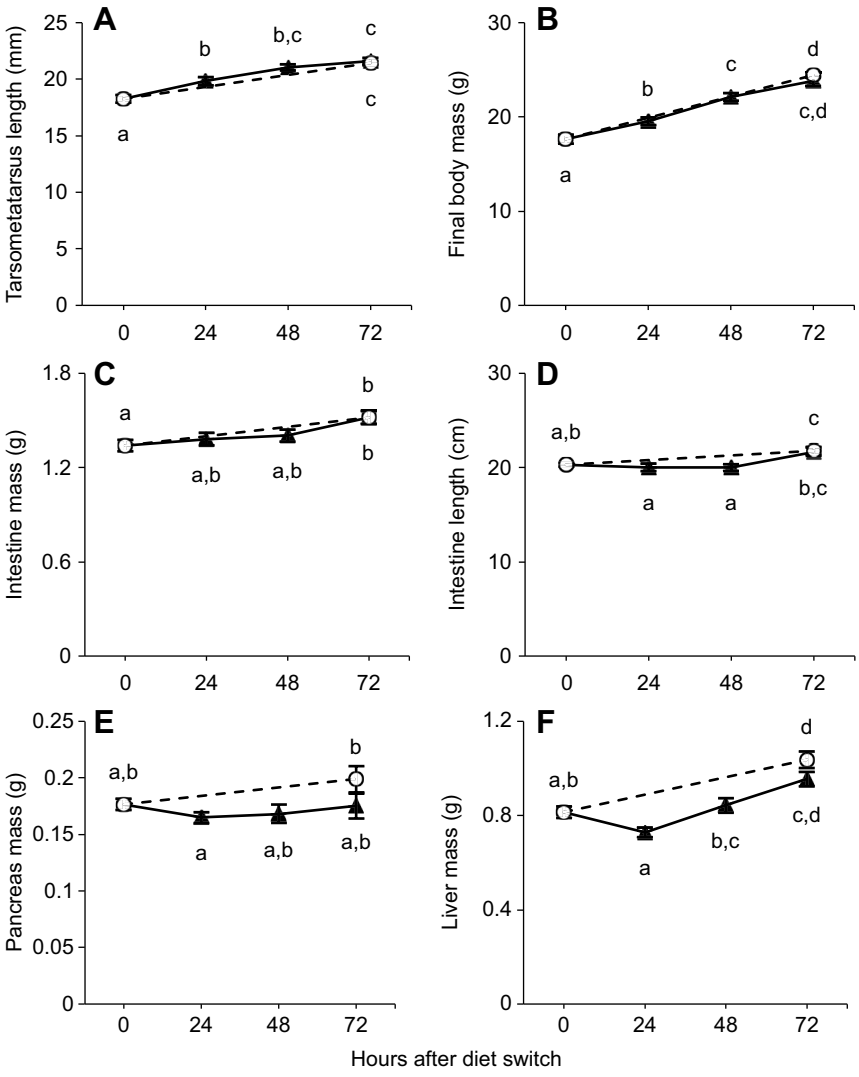

Fig. 6. Body measurements of 6- to 9-day-old nestling house sparrows 0, 24, 48 and 72 h after a diet switch from the HP diet to the HC diet. (A) Tarsometatarsus length, (B) final body mass, (C) intestine mass, (D) intestine length, (E) pancreas mass and (F) liver mass. Circles and dashed lines indicate groups only fed the HP diet, and triangles and solid lines indicate groups switched to the HC diet and thus eating only the HC diet for the number of hours indicated on the abscissa. The diet switch occurred at 6 days of age and final sampling was 72 h later at 9 days of age. Values are means±s.e.m. (N=11–13 birds in each treatment group). Although not statistically significant, nestlings fed the HC diet tended to have smaller organs. The overall increasing trend with hours after diet switch reflects growth. Different lowercase letters denote significant differences between treatment groups (one-way ANOVA, followed by Tukey HSD, across the 5 treatment groups, each measure tested independently).

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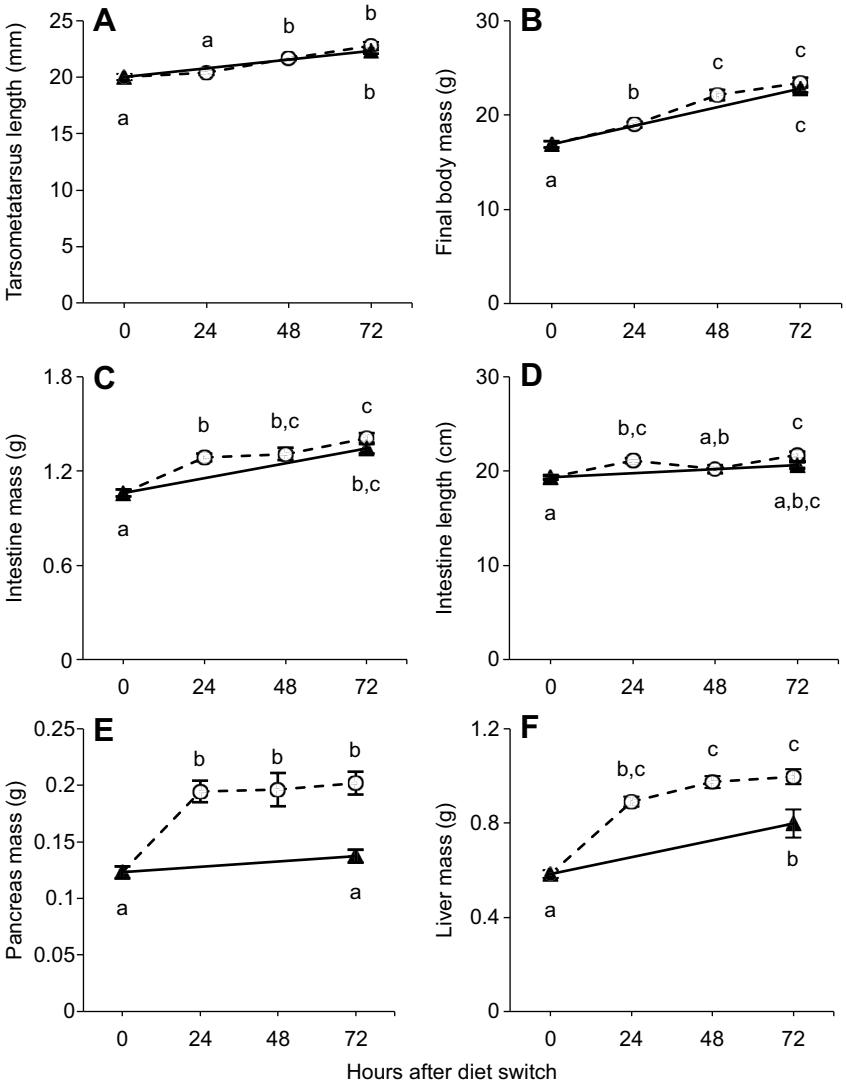

Fig. 7. Body measurements of 6- to 9-day-old nestling house sparrows 0, 24, 48 and 72 h after a diet switch from the HC diet to the HP diet. (A) Tarsometatarsus length, (B) final body mass, (C) intestine mass, (D) intestine length, (E) pancreas mass and (F) liver mass. Triangles and solid lines indicate groups only fed the HC diet, and circles and dashed lines indicate groups switched to the HP diet and thus eating only the HP diet for the number of hours indicated on the abscissa. The diet switch occurred at 6 days of age and final sampling was 72 h later at 9 days of age. Values are means±s.e.m. (*N*=10–12 birds in each treatment group). Pancreas and liver mass were significantly larger in nestlings fed the HP diet. The overall increasing trend with hours after diet switch reflects growth. Different lowercase letters denote significant differences between treatment groups (one-way ANOVA, followed by Tukey HSD, across the 5 treatment groups, each measure tested independently).

enzyme activities studied – maltase, sucrase and aminopeptidase-N – the general pattern was a significant change in enzymatic activity within 24 h of being put on a new diet with little to no significant change in the 2 days thereafter. Interestingly, complementary changes in pancreas and liver masses also occurred. The results of our experiments clearly indicate that house sparrow nestlings as young as 3 days post-hatch are capable of modulating their digestive enzyme activity within 24 h of a change in diet composition. To our knowledge, this is the first evidence of digestive enzyme modulation being completed within 24 h of a change in diet in an avian species and the first study to show intestinal digestive enzyme modulation in response to changes in diet composition in any animal this early in development. These results have both ecological and physiological implications.

The ecology of digestive enzyme flexibility

Although nestlings as young as 4 days of age were able to modulate their enzyme activity to adjust to changes in diet composition, their ability to modulate was much more limited than in birds only a few days older. For instance, 4-day-old nestlings significantly modulated only sucrase activity and barely modulated maltase activity, whereas nestlings 3 days older significantly modulated sucrase, maltase and aminopeptidase-N activity. The absence of an observed ability to modulate aminopeptidase-N at 4 days of age may be related to the reliance of nestlings on insects and therefore high protein content for sustenance during their first few days post-hatch (Anderson, 2006), and the ability to modulate aminopeptidase-N – an enzyme necessary for hydrolysis of protein – may be more costly than beneficial at this age. Although we designed our diets based on current knowledge about nestling protein requirements (Klasing, 1998), another likely indication of the importance of protein during the first few days post-hatch is that body mass was significantly lower in 4-day-old nestlings that had consumed the HC diet for 24 h, whereas older nestlings did not exhibit any diet effects on body mass.

In contrast to 4-day-old nestlings, by 7 days of age, nestlings were able to modulate the activities of aminopeptidase-N, maltase and sucrase (e.g. Fig. 1). This increased ability to modulate the activity of all three of these enzymes within 24 h could be related to the wide range of diet compositions and the amount of dietary change experienced around this time in development, as exhibited by house sparrow nestlings in different populations (Anderson, 2006). Habitat, geographic region and the season in which breeding occurs all affect the food that is available in a particular area, and some populations transition their young to seeds much more quickly than others (Anderson, 2006). Because of the range in diet compositions

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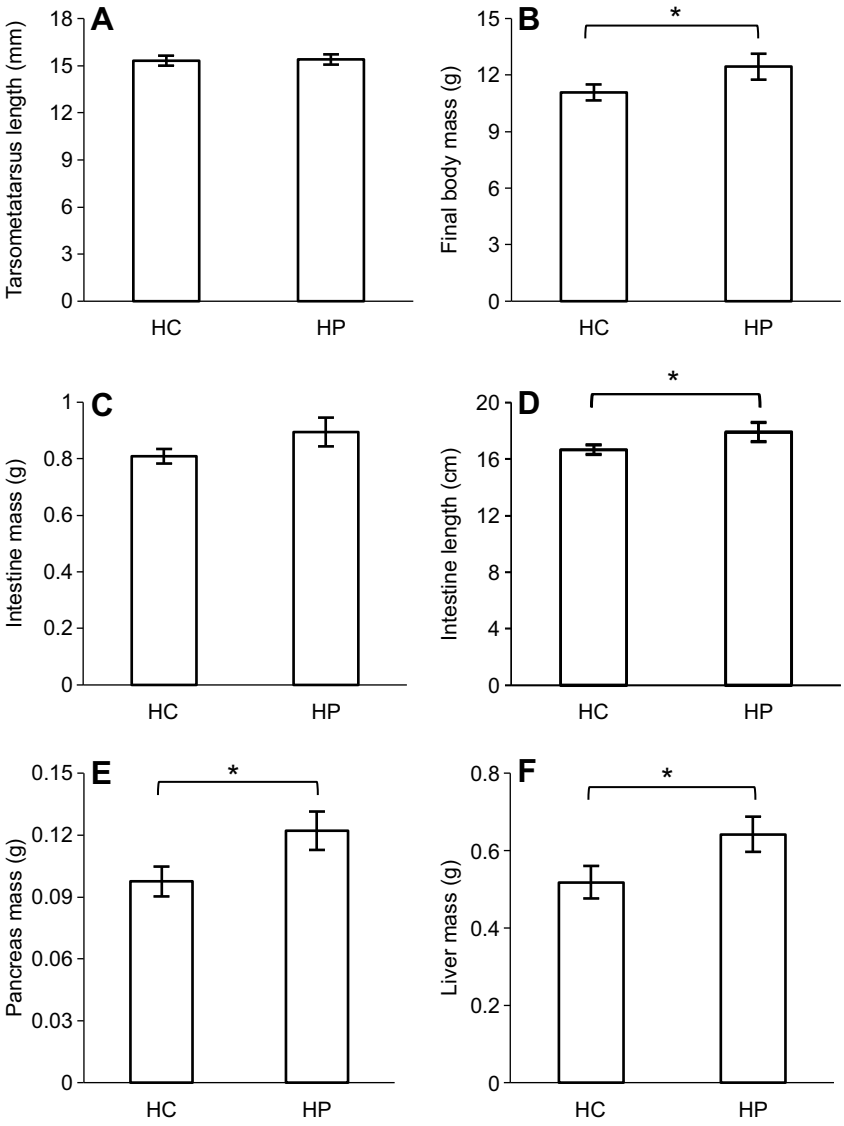

Fig. 8. Body measurements of 4-day-old nestling house sparrows after ingesting either the HC or the HP diet for 24 h. (A) Tarsometatarsus length, (B) final body mass, (C) intestine mass, (D) intestine length, (E) pancreas mass and (F) liver mass. Values are means±s.e.m. (*N*=9 birds in each treatment group). Body mass, intestine length, pancreas mass and liver mass were all significantly larger in nestlings fed the HP diet (paired *t*-test with sibling nestlings paired by nests, **P*<0.05). Intestine mass was slightly higher in birds fed the HP diet, but the difference was only borderline significant (*P*=0.08). Tarsometatarsus length did not differ between birds fed the different diets.

consumed by house sparrow nestlings in different populations, it would be maladaptive to lack the ability to adjust to different diets.

This rapid modulation also allows wild house sparrow nestlings to adjust to sporadic resource fluctuations, such as a sudden scarcity of arthropods owing to cold or inclement weather. Adult house sparrows could and likely do feed their nestlings seeds instead of insects during periods of arthropod scarceness, and the ability of nestlings to adjust quickly to the atypical diet should increase their survival. Furthermore, the nestlings could switch back to insects later to satisfy their protein requirements for proper growth (Klasing, 1998), because digestive enzyme modulation in nestlings is fully reversible (Brzęk et al., 2011). The ability to reversibly adjust their digestive enzyme activity within 24 h to complement a new diet would allow nestlings to develop fairly normally, despite the shortage of their typical food. Therefore, rapid digestive enzyme modulation may allow for enhanced survival of house sparrows during development, as compared with species that are not able to thrive on diets different from ones they typically consume (Brzęk et al., 2010).

For instance, the zebra finch (*Taeniopygia guttata*) – a diet specialist that eats mainly grass seeds throughout development and adulthood (Zann, 1996) – cannot survive during development on a diet lacking carbohydrate, partly because of its inability to modulate its digestive enzyme activity (Brzęk et al., 2010). If seeds were to somehow become unobtainable during development, zebra finch nestling survival would likely decrease. However, given that zebra finches have low digestive flexibility, and selection against this trait must have occurred over time, it is likely that the costs of maintaining the machinery that allows for digestive flexibility outweigh the benefits to this diet specialist (Karasov et al., 2011).

High phenotypic plasticity and flexibility of traits – both behavioral and physiological – have been found to be an important determinant of invasion success in invasive species, such as the house sparrow (Coccia et al., 2013; Davidson et al., 2011; Martin and Liebl, 2014; Sol et al., 2002). Although many native species also tend to have phenotypic plasticity that allows them to adjust to changes in their native environment (Chown et al., 2007), invasive species often have a higher level of plasticity (Davidson et al., 2011) that may help them colonize new areas and outcompete native species (Coccia et al., 2013; Martin and Liebl, 2014). The house sparrow, which is the second most widely distributed bird species in the world (Anderson, 2006), is an exemplary model of invasion success. House sparrows have an arsenal of mechanisms – both behavioral and physiological – that

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have allowed them to establish populations and prosper globally. These mechanisms include an attraction to novel foods and objects, the tendency to disperse more frequently and greater distances than native species, dampened immune responses, and stress hormone regulatory flexibility (Lee et al., 2005; Martin and Fitzgerald, 2005; Martin and Liebl, 2014; Skjelseth et al., 2007). Conceivably, it is the aggregation and perhaps synergism of all of the species' various plastic traits rather than a single one or few that has allowed house sparrows to become so prolific worldwide. Recently, it was suggested that future research on phenotypic plasticity focus on whole organism plasticity rather than the plasticity of individual traits (Forsman, 2015). Although this is an interesting concept, there is currently no method of calculating and comparing whole organism plasticity (Forsman, 2015). Considering the amount of research that has already been done on traits exhibiting plasticity in the house sparrow, including digestive flexibility, researchers interested in quantifying whole organism plasticity may consider the house sparrow as their model species.

The physiology of digestive enzyme flexibility

In this study, we measured the mass-specific enzyme activity in each section of the intestine and found that, for the three enzymes examined, modulation of enzyme activity occurs similarly in all intestinal sections. In retrospect, we spent extra time and effort quantifying and analyzing the sectional mass-specific enzyme activity when we could have simply performed our analyses on whole intestinal tissue. Future studies on house sparrow nestlings may find it more practical to focus solely on the summed and mass-specific enzyme activities in the entire intestine rather than partitioning the intestine and analyzing the sections separately. However, the fact that positional trends and possible significant interactions may be missed must be acknowledged prior to deciding not to partition the intestine.

We must also acknowledge that, although the changes in carbohydrase activity observed in this study were significant, we only measured enzyme activities based on the amounts of substrates broken down into their respective products. Therefore, we cannot ascertain the specific carbohydrase that hydrolyzed the bonds in the substrates from the assays that were performed. For instance, maltase activity actually indexes the sum of the amount of maltose hydrolyzed by both subunits of the complexes maltase–glucoamylase and sucrase–isomaltase (Semenza et al., 2001). The sucrase subunit of the sucrase–isomaltase complex is also responsible for breaking the alpha-1,2 linkages in sucrose (Semenza et al., 2001). The fact that sucrase–isomaltase breaks down multiple substrates coupled with the fact that maltose is hydrolyzed by multiple enzymes accentuates the difficulties in assigning measured hydrolytic activity to specific enzyme complexes. Future studies examining changes in enzyme activity could also perform western blotting and RT-qPCR in order to differentiate the causes of apparent changes in enzyme activity.

Although we have determined that digestive enzyme activity in house sparrows is modulated within 24 h, more research must be done to determine the specific time at which modulation of enzyme activity begins. Furthermore, more research is needed on the molecular mechanisms responsible for this modulation in activity and the time course of these preceding steps. A recent study on 12-day-old house sparrows (close to fledging) found that an induction of maltase activity following an increase in dietary carbohydrate was correlated with an increase in mRNA of an alpha-glucosidase maltase–glucoamylase-like complex, which would support the hypothesis that modulation of these enzymes is under transcriptional control (Gatica-Sosa et al., 2015). More research is needed to determine the underlying gene expression regulatory mechanisms for the disaccharidases and aminopeptidase-N, for which the regulatory mechanisms are unknown in birds (but see Sonoyama et al., 1994 for rats). A better understanding of these mechanisms would give us insight into how organisms – invasive and otherwise – adjust to changes in their environment.

Another possible method of adjusting to environmental changes is by modulating the sizes of organs responsible for enzyme synthesis and nutrient processing. Although unexpected, this study found significant modulation of pancreas and liver masses within 24 h of a change in diet composition, with nestlings consuming the HP diet having larger pancreases and livers than nestlings consuming the HC diet. In the experiment testing for induction of aminopeptidase-N, there was a 58% increase in pancreas mass and 53% increase in liver mass within 24 h of the switch from the HC diet to the HP diet. Pancreas and liver masses also decreased following a switch from the HP diet to the HC diet. Although this remarkable diet effect was unexpected, changes in the sizes of these organs following a shift in diet composition have been noted previously (Brzęk et al., 2009). Furthermore, the liver is usually larger in insectivores (animals that typically consume diets high in protein) than granivores (animals that typically consume diets lower in protein and higher in carbohydrates) (Klasing, 1998). A possible reason that livers are larger in animals consuming diets higher in protein is that much greater post-absorptive processing capacity may be required when greater amounts of amino acids are absorbed from the intestinal lumen. Increasing liver size would effectively increase the capacity to process excess amino acids produced by the hydrolytic activity of intestinal peptidases, such as aminopeptidase-N. Similarly, increasing pancreas size should effectively increase the capacity to produce proteases such as trypsin and chymotrypsin, which are necessary for the breakdown of protein into peptides. Given that the mass-specific activities of pancreatic enzymes are generally not adaptively modulated in birds (Brzęk et al., 2013a), it would be advantageous to possess the ability to modulate enzyme activity by modulating organ size during development. However, we did not measure the activities of pancreatic proteases in this study and therefore can only speculate as to why the organ masses were larger in nestlings consuming the diet higher in protein. More research is needed to determine the reason for observed differences in pancreas and liver masses and the benefit of modulating organ sizes rather than or in addition to mass-specific enzyme activities.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: K.H.R., E.C., W.H.K.; Methodology: K.H.R., E.C., W.H.K.; Validation: E.C., W.H.K.; Formal analysis: K.H.R.; Investigation: K.H.R.; Resources: W.H.K.; Writing - original draft: K.H.R.; Writing - review & editing: K.H.R., E.C., W.H.K.; Supervision: K.H.R., E.C., W.H.K.; Project administration: K.H.R., W.H.K.; Funding acquisition: W.H.K.

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References

Afik, D., Caviedes-Vidal, E., Martínez del Rio, C. and Karasov, W. H. (1995). Dietary modulation of intestinal hydrolytic enzymes in yellow-rumped warblers. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **269**, R413-R420.

Anderson, T. (2006). *Biology of the Ubiquitous House Sparrow: From Genes to Populations*. Oxford: Oxford University Press.

Battley, P. F. and Piersma, T. (2005). Adaptive interplay between feeding ecology and features of the digestive tract in birds. In *Physiological and Ecological Adaptations to Feeding in Vertebrates* (ed. J. M. Starck and T. Wang), pp. 201-228. Enfield: Science Publishers, Inc.

Bell, G. P. (1990). Birds and mammals on an insect diet: a primer on diet composition analysis in relation to ecological energetics. *Stud. Avian Biol.* **13**, 416-422.

Brzęk, P., Kohl, K. D., Caviedes-Vidal, E. and Karasov, W. H. (2009). Developmental adjustments of house sparrow (*Passer domesticus*) nestlings to diet composition. *J. Exp. Biol.* **212**, 1284-1293.

Brzęk, P., Lessner, K. M., Caviedes-Vidal, E. and Karasov, W. H. (2010). Low plasticity in digestive physiology constrains feeding ecology in diet specialist, zebra finch (*Taeniopygia guttata*). *J. Exp. Biol.* **213**, 798-807.

Brzęk, P., Kohl, K. D., Caviedes-Vidal, E. and Karasov, W. H. (2011). Fully reversible phenotypic plasticity of digestive physiology in young house sparrows: lack of long-term effect of early diet composition. *J. Exp. Biol.* **214**, 2755-2760.

Brzęk, P., Ciminari, M. E., Kohl, K. D., Lessner, K., Karasov, W. H. and Caviedes-Vidal, E. (2013a). Effect of age and diet composition on activity of pancreatic enzymes in birds. *J. Comp. Physiol. B* **183**, 685-697.

Brzęk, P., Kohl, K. D., Caviedes-Vidal, E. and Karasov, W. H. (2013b). Activity of intestinal carbohydrases responds to multiple dietary signals in nestling house sparrows. *J. Exp. Biol.* **216**, 3981-3987.

Caviedes-Vidal, E., Afik, D., Martínez del Rio, C. and Karasov, W. H. (2000). Dietary modulation of intestinal enzymes of the house sparrow (*Passer domesticus*): testing an adaptive hypothesis. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **125**, 11-24.

Caviedes-Vidal, E. and Karasov, W. H. (2001). Developmental changes in digestive physiology of nestling house sparrows, *Passer domesticus*. *Physiol. Biochem. Zool.* **74**, 769-782.

Chown, S. L., Slabber, S., McGeoch, M. A., Janion, C. and Leinaas, H. P. (2007). Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. *Proc. R. Soc. B Biol. Sci.* **274**, 2531-2537.

Coccia, C., Calosi, P., Boyero, L., Green, A. J. and Bilton, D. T. (2013). Does ecophysiology determine invasion success? a comparison between the invasive boatman *Trichocorixa verticalis verticalis* and the native *Sigara lateralis* (Hemiptera, Corixidae) in south-west Spain. *PloS One* **8**, e63105.

Davidson, A. M., Jennions, M. and Nicotra, A. B. (2011). Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecol. Lett.* **14**, 419-431.

Dykstra, C. R. and Karasov, W. H. (1992). Changes in gut structure and function of house wrens (*Troglodytes aedon*) in response to increased energy demands. *Physiol. Zool.* **65**, 422-442.

Forsman, A. (2015). Rethinking phenotypic plasticity and its consequences for individuals, populations and species. *Heredity* **115**, 276-284.

Gatica-Sosa, C., Brzęk, P., Chediack, J. G., Cid, F. D., Karasov, W. H. and Caviedes-Vidal, E. (2015). Differential transcriptional responses underlie dietary induction of intestinal carbohydrase activities in house sparrow nestlings. *J. Anim. Physiol. Anim. Nutr.* **100**, 236-242.

Karasov, W. H., Martínez del Rio, C. and Caviedes-Vidal, E. (2011). Ecological physiology of diet and digestive systems. *Annu. Rev. Physiol.* **73**, 69-93.

Klasing, K. C. (1998). *Comparative Avian Nutrition*. New York, NY: Cab International.

Lee, K. A., Martin, L. B. and Wikelski, M. C. (2005). Responding to inflammatory challenges is less costly for a successful avian invader, the house sparrow (*Passer domesticus*), than its less-invasive congener. *Oecologia* **145**, 243-250.

Lepczyk, C. A., Karasov, W. H. and Caviedes-Vidal, E. (1998). Digestive responses during food restriction and realimentation in nestling house sparrows (*Passer domesticus*). *Physiol. Biochem. Zool.* **71**, 561-573.

Martin, L. B. and Fitzgerald, L. (2005). A taste for novelty in invading house sparrows, *Passer domesticus*. *Behav. Ecol.* **16**, 702-707.

Martin, L. B. and Liebl, A. L. (2014). Physiological flexibility in an avian range expansion. *Gen. Comp. Endocrinol.* **206**, 227-234.

McWhorter, T. J., Caviedes-Vidal, E. and Karasov, W. H. (2009). The integration of digestion and osmoregulation in the avian gut. *Biol. Rev.* **84**, 533-565.

McWilliams, S. R. and Karasov, W. H. (2001). Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **128**, 579-593.

Nagy, K. A. (2001). Food requirements of wild animals: predictive equations for free-living mammals, reptiles, and birds. *Nutr. Abstr. Rev. Ser. B* **71**, 21R-31R.

Nagy, K. A. (2005). Field metabolic rate and body size. *J. Exp. Biol.* **208**, 1621-1625.

Ovadia, O., Pinshow, B. and Lotem, A. (2002). Thermal imaging of house sparrow nestlings: the effect of begging behavior and nestling rank. *The Condor* **104**, 837-842.

Piersma, T. and Drent, J. (2003). Phenotypic flexibility and the evolution of organismal design. *Trends Ecol. Evol.* **18**, 228-233.

Piersma, T. and van Gils, J. A. (2011). *The Flexible Phenotype: A Body-Centred Integration of Ecology, Physiology, and Behaviour*, 1st edn. New York: Oxford University Press.

Price, E. R., Brun, A., Caviedes-Vidal, E. and Karasov, W. H. (2015). Digestive adaptations of aerial lifestyles. *Physiology* **30**, 69-78.

Raul, F., Goda, T., Gossé, F. and Koldovskỳ, O. (1987). Short-term effect of a high-protein/low-carbohydrate diet on aminopeptidase in adult rat jejunoileum: site of aminopeptidase response. *Biochem. J.* **247**, 401-405.

Sabat, P., Novoa, F. F., Bozinovic, F. and Martinez del Rio, C. (1998). Dietary flexibility and intestinal plasticity in birds: a field and laboratory study. *Physiol. Zool.* **71**, 226-236.

Semenza, G., Auricchio, S. and Mantei, N. (2001). Small-Intestinal Disaccharidases. In *The Metabolic and Molecular Bases of Inherited Disease* (ed. C. R. Scriver, A. L. Beaudet, D. Valle, W. S. Sly, B. Childs, K. W. Kinzler and B. Vogelstein), pp. 1623-1650. New York, NY: The McGraw-Hill Companies, Inc.

Skjelseth, S., Ringsby, T. H., Tufto, J., Jensen, H. and Saether, B.-E. (2007). Dispersal of introduced house sparrows *Passer domesticus*: an experiment. *Proc. R. Soc. B Biol. Sci.* **274**, 1763-1771.

Sol, D., Timmermans, S. and Lefebvre, L. (2002). Behavioural flexibility and invasion success in birds. *Anim. Behav.* **63**, 495-502.

Sonoyama, K., Kiriyama, S. and Niki, R. (1994). Effect of dietary protein level on intestinal aminopeptidase activity and mRNA level in rats. *J. Nutr. Biochem.* **5**, 291-297.

Zann, R. A. (1996). *The Zebra Finch: A Synthesis of Field and Laboratory Studies*. Oxford: Oxford University Press.

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