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SHORT COMMUNICATION

Increases in cortisol production facilitate the transition to exogenous feeding by larval zebrafish

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ABSTRACT

The transition to exogenous feeding is a critical developmental period that is regulated by corticosteroids in mammals. However, mechanistic evidence linking corticosteroids with feeding transitions in non-mammalian vertebrates remains scarce. Here, we pharmacologically suppressed cortisol production during the period preceding exogenous feeding by larval zebrafish (*Danio rerio*) and determined how this influenced feeding rates and transcript levels of digestive enzymes. Water-borne metyrapone (an 11β-hydroxylase inhibitor) from 3–5 days post-fertilization (dpf) blocked the 2-fold rise in cortisol levels observed in control larvae during this period. While whole-animal growth and development were unaffected, cortisol synthesis suppression during this period resulted in lower transcript abundance of digestive enzymes and reduced feeding rates at 5 dpf. However, these differences dissipated following a two-day washout period. Together, our results indicate that cortisol influences feeding transitions in zebrafish, highlight the plasticity of these processes following perturbations, and suggest that this mechanism is conserved across vertebrates.

KEY WORDS: Corticosteroids, Digestion, Food intake, Glucocorticoids, Gut development

INTRODUCTION

The ability to properly identify, acquire and digest food is critical for the survival of all organisms. Accordingly, developmental changes in feeding-related processes are key ontogenetic periods that involve coordinated behavioural and physiological processes (Calhoun and Hayden, 2015; Zeltser, 2018). However, these periods represent substantial bottlenecks for survivorship, as many individuals cannot successfully make these transitions (Earhart et al., 2020; Harboe et al., 1994; Marchetti and Price, 1989; Quesnel et al., 2012). For example, in European shags (*Phalacrocorax aristotelis*), fledgling mortality rates are ∼5 times greater than those of adults, which is thought to reflect, at least in part, reduced foraging efficiency of fledglings (Daunt et al., 2007). Consequently, numerous studies have focused on understanding the physiological mechanisms underlying these transitions, including the roles of different hormone systems.

While corticosteroids are primarily recognized for their role during vertebrate stress responses (Best et al., 2024; Sapolsky et al., 2000), they also play key developmental roles, and alterations in corticosteroid production during early life can impair growth rates, cognitive function and organ development (Crespi et al., 2013; Moisiadis and Matthews, 2014; Oitzl et al., 2010). For instance, increased corticosteroid production prior to parturition (i.e. transitioning from placental nutrients to milk) and weaning (i.e. transitioning from milk to solid foods) in mammals has been linked to developmental changes in gut morphology (e.g. villi length, crypt depth and mucosa thickness) and digestive enzyme production during these periods (Black, 1988; Majumdar and Nielsen, 1985; Sangild et al., 2002; Yeh and Moog, 1977). However, the relative importance of corticosteroids during feeding transitions in other vertebrates – such as fishes – remains unclear.

As in mammals, cortisol production increases prior to the transition to exogenous feeding in fishes (Alsop and Vijayan, 2008; Earhart et al., 2020; Fuzzen et al., 2011; Pérez-Domínguez and Holt, 2006; Tsalafouta et al., 2015; Wilson et al., 2013). Many authors have suggested a link between cortisol production and feeding transitions in fish as several digestive enzymes are cortisol responsive (Faught and Vijayan, 2019; Khangembam et al., 2017; Ma et al., 2004a) and intestinal expression of glucocorticoid receptors in larvae precedes exogenous feeding (Di Bella et al., 2008). However, evidence that increases in cortisol synthesis directly facilitate the transition to exogenous feeding in fish remains scant. Earhart et al. (2020) reported that pharmacological inhibition of cortisol synthesis in larval lake sturgeon (*Acipenser fulvescens*) delayed gastrointestinal development as indicated by anal plug retention. However, fish in this study displayed high mortality rates and developmental abnormalities (e.g. oedemas around the heart and abdominal organs), suggesting that their treatment might have been too severe in dose and/or duration. Therefore, it is still unclear to what extent cortisol contributes to exogenous feeding transitions in fish.

Zebrafish (*Danio rerio*) are a popular model for developmental studies (Meyers, 2018), and previous studies have shown that manipulation of cortisol levels during early life can impair development (Nesan and Vijayan, 2012, 2013; Wilson et al., 2013, 2015). However, no study has directly evaluated the relationship between endogenous cortisol production and feeding transitions by larval zebrafish. During early development, larvae rely on endogenous nutrients stored in their yolk sac for sustenance, but by ∼5 days post-fertilization (dpf) a functional gastrointestinal tract has developed, the yolk sac has mostly been resorbed and larvae begin to feed exogenously (Belanger et al., 2010; Ng et al., 2005; Strähle et al., 2012; Wallace et al., 2005). The period preceding this stage (3–5 dpf) is associated with a 2- to 3-fold increase in cortisol production (Alsop and Vijayan, 2008; Wilson et al., 2013), which has been hypothesized to contribute to the ability of larvae to begin feeding exogenously. To test this hypothesis, we pharmacologically blocked the rise in cortisol synthesis using metyrapone (an 11β-hydroxylase inhibitor) and evaluated how this affected the ability of larvae to transition to exogenous feeding. Specifically, we predicted that digestive capacity would be impaired in metyrapone-

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treated fish, resulting in reduced transcript levels of digestive enzymes and lower food intake.

MATERIALS AND METHODS

Animal housing and care

Adult zebrafish, *Danio rerio* (F. Hamilton 1822), were held in a recirculating multi-tank system (ZebTEC rack; Tecniplast USA, West Chester, PA, USA) within the Hagen Aqualab at the University of Guelph. Each 3.5 l tank contained ∼20 fish of mixed sex (∼2:1 ratio of females versus males), as well as a mesh-bottomed breeding basket with marbles and artificial plants for enrichment. Fish were maintained on a 12 h:12 h light:dark photoperiod with a water temperature of 28°C and were fed to satiation once per day with each of GEMMA micro 300 (Skretting, Vancouver, BC, Canada) and brine shrimp (Hikari USA, Hayward, CA, USA). Water quality parameters and levels of nitrogenous waste products were evaluated weekly. All procedures were carried out in accordance with the Canadian Council on Animal Care guidelines and approved by the University of Guelph Animal Care Committee.

Embryo collection and rearing

To acquire larvae, a cover was placed over the bottom of each breeding basket the evening before collection, allowing eggs to be collected the following morning. Eggs (∼1 h post-fertilization) were briefly washed to remove debris, and all damaged/dead eggs were removed. Eggs from multiple tanks (≥4) were pooled to avoid potential tank effects. Following this, eggs (∼100) were transferred into 100 mm Petri dishes containing 25 ml of egg water medium [60 µg ml^{-1} Instant Ocean Salt (Spectrum Brands, Blacksburg, VA, USA) with 0.04% Methylene Blue] and placed into an incubator at 28°C, unless otherwise indicated. Egg water medium was replaced every 24 h. Larvae were fed either GEMMA Micro 75 (Skretting) twice daily (experiments 1 and 2) or nauplii brine shrimp (*Artemia salina*; experiment 3) starting at 4 dpf until sampling. While larvae are unlikely to feed at 4 dpf, food was introduced so that larvae had the opportunity to practise hunting behaviours, which improves later feeding success (especially on live food; Lagogiannis et al., 2020). At sampling, larvae were terminally anaesthetized using tricaine (MS-222, 250 mg ml^{-1}; Syndel, Nanaimo, BC, Canada).

Pharmacological manipulation of cortisol synthesis

To inhibit cortisol synthesis, metyrapone (1 or 10 µmol l^{-1}; Sigma-Aldrich, Oakville, ON, Canada) was added to egg water medium from 3 to 5 dpf. These doses were selected based on Wilson et al. (2013), which reported that 1 and 10 µmol l^{-1} were sufficient to either partially suppress (1 µmol l^{-1}) or completely abolish (10 µmol l^{-1}) the rise in endogenous cortisol synthesis that occurs between 3 and 5 dpf in larval zebrafish. These authors also reported that this range of metyrapone did not elicit any morphological abnormalities or affect mortality rates (Wilson et al., 2013). A vehicle control group which was exposed to equivalent amounts of ethanol (0.0076%) in the absence of metyrapone was also included. Egg water medium was replaced every 24 h. For sampling points occurring after 5 dpf, treatment solutions were replaced with normal egg water medium.

To confirm that these doses of metyrapone did not affect the growth and development of larvae (see Figs S1 and S2 for more information), we repeatedly evaluated several morphological measurements in the same larvae both during (3 and 5 dpf) and following metyrapone treatment (7, 9 and 11 dpf). Individual embryos (N=7–9 per treatment) were transferred into the wells of a 24-well plate 1 day before the treatment period (at 2 dpf). Embryos were randomly assigned to different treatment groups across each plate, and the appropriate treatment solution was added post-hatch at 3 dpf. On measurement days, fish were anaesthetized by replacing the solution in individual wells with treatment solution containing MS-222 (100 mg ml^{-1}; Syndel). Once anaesthetized, larvae were placed on their side and imaged using a stereomicroscope (SMZ1500; Nikon, Mississauga, ON, Canada). Images were analysed in ImageJ (v1.52t). Each treatment solution was replaced every 24 h until 5 dpf, after which all solutions were replaced with normal egg water until the experiment ended at 11 dpf. Specifically, we evaluated body length, body width, tail width and yolk sac area (see Fig. S1 for more details).

Experiment 1: determination of larvae cortisol levels

To confirm the efficacy of our metyrapone treatment, whole-body cortisol levels were quantified in pools of 15 larvae at 3 (untreated controls), 5 and 7 dpf, using previously described methods (Fuzzen et al., 2010). Briefly, methanol extraction of homogenized larvae was performed twice, and the resulting supernatant was evaporated. The extract was then reconstituted in acetate buffer and purified using a solid-phase extraction column (HyperSep C18 Cartridges; Thermo Scientific, Mississauga, ON, Canada). The eluted purified extract solution was evaporated, after which it was reconstituted in extraction buffer and cortisol levels were measured using a previously validated enzyme-linked immunosorbent assay kit (Neogen, Lexington, KY, USA; cat no. 402710). Final values were adjusted for extraction efficiency (85%), and intra- and inter-assay coefficients variation were 1.6% and 8.9%, respectively.

Experiment 2: effects of cortisol synthesis inhibition on digestive enzyme expression

To evaluate whether the digestive capacity of larvae was affected by cortisol synthesis inhibition, we measured transcript levels of a suite of enzymes involved in carbohydrate (amylase alpha 2A, *amy2a*), lipid (carboxyl ester lipase, *cel1*) and protein catabolism (carboxypeptidase A1, *cpa1*; carboxypeptidase B1, *cpb1*; chymotrypsinogen B, *ctrb1*; serine protease 1, *prss1*). Specifically, these six enzymes were selected because they can be reliably measured in 5 dpf zebrafish larvae (Fang et al., 2014; Faught and Vijayan, 2019; Guerrera et al., 2015) and they have all previously been reported to be cortisol responsive (Faught and Vijayan, 2019; Khangembam et al., 2017; Ma et al., 2004a).

At 5 and 7 dpf, pools of 15 larvae were collected, immediately frozen on dry ice and stored at −80°C. Larvae were then homogenized in TRIzol reagent (Invitrogen, Burlington, ON, Canada) using a Precellys Evolution tissue homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France). Following the manufacturer's protocol, total RNA was extracted, and its quantity and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific). We then treated 1 µg of RNA with DNase (DNase 1; Thermo Fisher Scientific) and reverse-transcribed cDNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Waltham, MA, USA). We performed real-time polymerase chain reaction (qPCR) using a CFX96 system (BioRad, Hercules, CA, USA) with SYBR green (SsoAdvanced Universal; BioRad). All samples were run in duplicate and negative controls (no-template or no-reverse transcriptase) were included. Each reaction contained a total of 20 µl, which consisted of 10 µl of SYBR green, 5 µl of combined forward and reverse primers (0.2 µmol l^{-1} [final]; Table S1) and 5 µl of 10× diluted cDNA. The manufacturer's default cycling parameters and melt curve protocol were used. To account for differences in amplification efficiency, standard curves were constructed for each gene using

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serial dilutions (4 times) of pooled cDNA. Input values for each gene were obtained by fitting the average threshold cycle value to the antilog of the gene-specific standard curve, thereby correcting for differences in primer amplification efficiency. To correct for minor variations in template input and transcriptional efficiency, we normalized our data to the geometric mean of transcript abundance of elongation factor 1α (*ef1α*) and ribosomal protein L13a (*rpl13a*) as reference genes. All data are expressed relative to the mean value of the vehicle-treated group at 5 dpf.

Experiment 3: effects of cortisol synthesis inhibition on feeding rates

Based on the results of the previous experiments, we specifically focused on whether treatment with 10 µmol l^{-1} of metyrapone (which completely inhibited the endogenous rise in cortisol production) affected when larvae began to feed exogenously, as well as how much they ate. At 3 dpf, larvae were individually placed into wells of a 24-well plate containing 500 µl of either vehicle or metyrapone solution. At 5 or 7 dpf, 5 brine shrimp nauplii were distributed into each well, after which larvae were returned to their incubator for 2 h. After this period, larva and nauplii were terminally anaesthetized using MS-222 (250 mg ml^{-1}), and food intake was quantified by counting the number of nauplii that remained.

Statistical analysis

Statistical analyses were performed using GraphPad Prism (v10.4.1; GraphPad Software Inc.) and a significance level (α) of 0.05 was used for all tests. Data from experiments 1 and 2 are presented as means±1 s.e.m., while count data are presented for experiment 3. Outliers were excluded based on a threshold of 2 times the interquartile range. When data did not meet the assumptions of normality and/or equal variance, data were either log or square-root transformed to improve the fit. Differences in cortisol levels and digestive enzyme transcript abundance between treatment groups were evaluated using one-way ANOVA within each time point. Additionally, a one-way ANOVA was also used to evaluate changes in larval cortisol content across time in non-metyrapone-treated groups (i.e. 3 dpf controls versus 5 dpf vehicle treated versus 7 dpf vehicle treated). Growth and development data (i.e. changes in morphological measures between 3 and 11 dpf) were analysed using 2-way repeated-measure ANOVA that included treatment, time and the interaction between treatment and time. When significant differences were detected, *post hoc* Tukey's tests were performed. Data from experiment 3 were analysed using either a Fisher's exact test (binary feeding data) or a *t*-test (number of brine shrimp nauplii eaten) for each time point.

RESULTS AND DISCUSSION

Confirmation of cortisol synthesis inhibition by metyrapone

Cortisol content in larvae which were not treated with metyrapone increased between 3 and 7 dpf (Fig. 1; $P<0.001$). Specifically, cortisol levels at 5 dpf were 105% greater than at 3 dpf ($P=0.01$), while levels at 7 dpf were 230% and 60% greater than at 3 dpf ($P<0.001$) and 5 dpf ($P=0.04$), respectively. However, the application of metyrapone from 3 to 5 dpf resulted in dose-dependent reductions in larval cortisol content at 5 dpf ($P=0.007$). Cortisol levels in the 10 µmol l^{-1} metyrapone group were ∼55% lower than those of vehicle-treated larvae ($P=0.005$) – comparable to levels in untreated 3 dpf larvae – while levels in the 1 µmol l^{-1} metyrapone group were intermediate to the vehicle ($P=0.36$) and 10 µmol l^{-1} metyrapone ($P=0.11$) groups. In contrast, no significant differences between

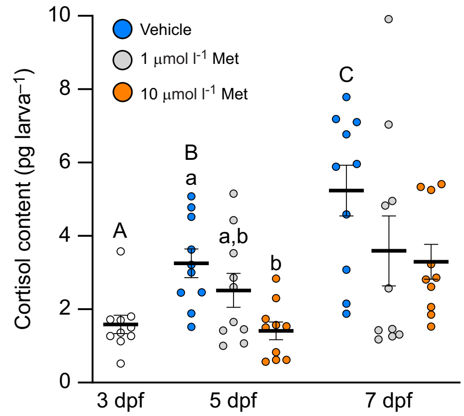

Fig. 1. Metyrapone treatment suppressed cortisol synthesis in 5 dpf zebrafish (*Danio rerio*). Larvae were treated with either vehicle (0.0075% ethanol) or 1 or 10 µmol l^{-1} metyrapone (Met) from 3 to 5 days post-fertilization (dpf). Cortisol levels in each treatment group were measured at 5 and 7 dpf. All larvae were reared in normal egg water from 5 to 7 dpf. Significant differences ($P<0.05$; one-way ANOVA) between treatment groups within a time point are depicted using lowercase letters and differences across time in non-metyrapone-treated larvae are depicted using uppercase letters. Values are presented as means±s.e.m. with individual data points ($N=10$ pools of 15 larvae per group).

groups were detected at 7 dpf ($P=0.15$). Additionally, metyrapone treatment did not affect measures of larvae growth and development (Fig. S2) or survivorship (range of 92.4% to 95.5% survival across all groups during the 3–5 dpf exposure period). While suppression of cortisol synthesis using metyrapone could theoretically cause an accumulation of precursory steroids – especially 11-deoxycortisol, which is converted to cortisol by 11β-hydroxylase (Best et al., 2024) – the affinity of corticosteroid receptors for these precursory steroids is generally much lower than for cortisol (Arterbery et al., 2005; Bury et al., 2003; Sturm et al., 2005). Additionally, as rates of cortisol synthesis were only attenuated (not fully abolished) and levels remained within a physiological range in all groups, it is unlikely that precursory steroids contributed to our results. Overall, these results confirmed that our methods were appropriate to evaluate whether ontogenetic increases in cortisol synthesis facilitate the transition from endogenous to exogenous nutrient sources by larval zebrafish.

Cortisol synthesis inhibition suppresses expression of digestive enzymes

Cortisol is a major regulator of metabolic pathways, including the regulation of many enzymes involved in the catabolism of carbohydrates, lipids and proteins. Indeed, we found that metyrapone treatment reduced transcript abundance of *amy2a* (Fig. 2A; $P=0.02$), *prss1* (Fig. 2B; $P=0.04$) and *ctrb1* (Fig. 2C; $P=0.001$) at 5 dpf, but no differences were observed at 7 dpf (*amy2a*, $P=0.51$; *prss1*, $P=0.91$; *ctrb1*, $P=0.86$). In all cases, levels in the 10 µmol l^{-1} metyrapone group were ∼40% lower than those in the vehicle group (*amy2a*, $P=0.01$; *prss1*, $P=0.04$; *ctrb1*, $P<0.001$), and levels in the 1 µmol l^{-1} metyrapone group were intermediate to those in the vehicle (*amy2a*, $P=0.15$; *prss1*, $P=0.16$; *ctrb1*, $P=0.17$) and 10 µmol l^{-1} metyrapone (*amy2a*, $P=0.50$; *prss1*, $P=0.81$; *ctrb1*, $P=0.08$) groups. Levels of *cpa1* (Fig. 2D; 5 dpf: $P=0.18$, 7 dpf: $P=0.70$), *cpb1* (Fig. 2E; 5 dpf: $P=0.06$, 7 dpf: $P=0.97$) and *cel1* (Fig. 2F; 5 dpf: $P=0.29$, 7 dpf: $P=0.94$) followed a similar pattern, but no significant differences in transcript levels were detected at either time point for any of these genes. These genes have previously been identified as cortisol responsive via a glucocorticoid receptor-

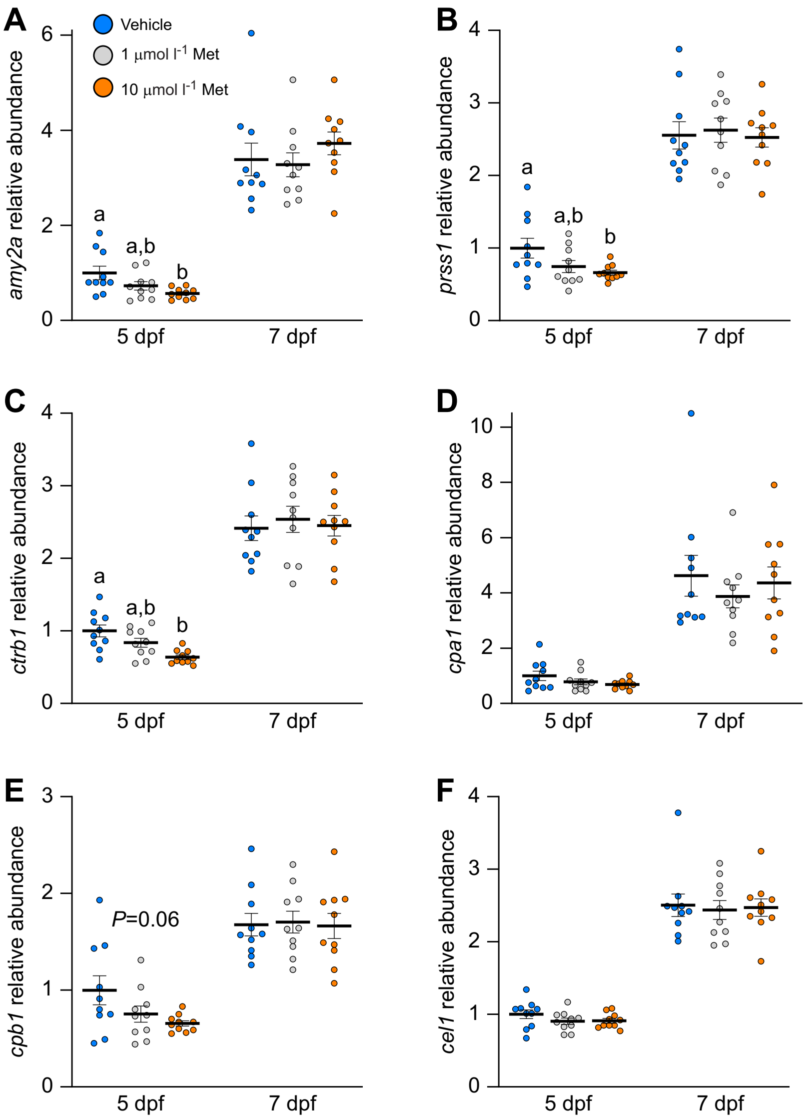

Fig. 2. Metyrapone treatment reduced digestive enzyme expression in 5 dpf zebrafish (*D. rerio*). Larvae were treated with either vehicle (0.0075% ethanol) or 1 or 10 µmol l^{-1} metyrapone (Met) from 3 to 5 dpf. Transcript levels of amylase alpha 2A (*amy2a*; A), serine protease 1 (*prss1*; B), chymotrypsinogen B (*ctrb1*; C), carboxypeptidase A1 (*cpa1*; D), carboxypeptidase B1 (*cpb1*; E) and carboxyl ester lipase (*cel1*; F) were measured at 5 and 7 dpf. All larvae were reared in normal egg water from 5 to 7 dpf. Significant differences (P<0.05; one-way ANOVA) within a time point are depicted using letters. Values are presented as means±s.e.m. with individual data points (N=10 pools of 15 larvae per group). All data are normalized to the geometric mean of elongation factor 1α (*ef1α*) and ribosomal protein L13a (*rpl13a*), and are expressed relative to levels in the vehicle group at 5 dpf. Note that the P-value is reported in E to support the trend for lower levels of *cpb1* in the 10 µmol l^{-1} Met group.

dependent mechanism in teleost fish (Faught and Vijayan, 2019; Ma et al., 2004a,b), suggesting that our results likely reflect direct transcriptional responses to reduced glucocorticoid receptor activity in metyrapone-treated larvae. However, as corticosteroids are important stimulators of intestinal development in mammals (Black, 1988; Majumdar and Nielsen, 1985; Sangild et al., 2002; Yeh and Moog, 1977), it is also possible that general delays in the development of the gastrointestinal tract may have contributed to these transcriptional effects. Indeed, levels of these enzymes increase during the development of the gastrointestinal tract in larval zebrafish (Guerrera et al., 2015), suggesting that a delay in gastrointestinal development may also have contributed to their suppression. Therefore, while our data indicate that developmental increases in cortisol synthesis contribute to the regulation of digestive enzymes, future work should directly evaluate whether dysregulated cortisol levels influence developmental trajectories of the gastrointestinal tract.

Cortisol synthesis inhibition reduces exogenous feeding rates

Feeding rates during our assay were low (<56%) at both 5 and 7 dpf, potentially because fish in our study had not fully depleted their yolk sac reserves (Fig. S2). Regardless, larvae that were treated with 10 µmol l^{-1} of metyrapone were ∼20% less likely to feed than vehicle-treated larvae at 5 dpf (Fig. 3; P=0.01), which is consistent with our predictions. However, no difference was detected at 7 dpf (P=0.12) and the number of brine shrimp that were consumed by vehicle and treatment groups during feeding events was not different at either 5 dpf (1.20±0.13 versus 1.17±0.17; P=0.89) or 7 dpf (1.69±0.24 versus 1.75±0.22; P=0.86). Reductions in feeding at 5 dpf coincided with transcriptional evidence of impaired digestive capacity and it is likely that these impairments contributed, at least in part, to the observed reductions in feeding. However, cortisol can also modulate food intake and feeding behaviours independent of its developmental effects on the gastrointestinal tract. For example, while acute elevations in cortisol do not appear to affect feeding behaviour or intestinal transit time in larval zebrafish (Brady et al., 2017), chronic cortisol exposure reduces food intake via a glucocorticoid receptor-dependent mechanism in adult zebrafish (Nipu et al., 2022). Additionally, previous studies have reported that chronically dysregulated cortisol levels can cause neurological impairments, which might influence feeding. Wilson et al. (2013) found that zebrafish larvae that were treated with metyrapone (10 µmol l^{-1}) from 1 h post-fertilization until 5 dpf were slower and

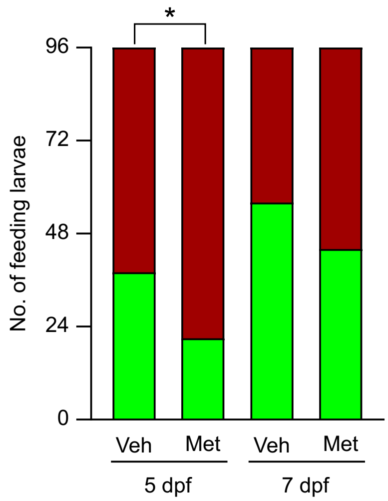

Fig. 3. Suppression of cortisol synthesis reduced feeding rates in 5 dpf zebrafish (*D. rerio*). Larvae were treated with either vehicle (0.0075% ethanol; Veh) or 10 μmol l^{-1} metyrapone (Met) from 3 to 5 dpf. All larvae were reared in normal egg water from 5 to 7 dpf. We tracked whether larvae consumed or did not consume at least one brine shrimp during a 2 h period at 5 or 7 dpf. Significant differences (P<0.05; Fisher's exact test) within a time point are depicted using an asterisk. The number of larvae which either fed (light green) or did not feed (dark red) is depicted (N=96 larvae per group).

less active overall. While their treatment regime was longer than what was used in the current study, it is possible that reductions in food intake by metyrapone-treated larvae reflect an inability to capture live prey owing to locomotor deficits. Similarly, cortisol signalling also contributes to the proper development of visual and olfactory responses in zebrafish (Muto et al., 2013; Nesan and Vijayan, 2013), and hunting behaviour in larval zebrafish is primarily mediated by visual cues (Filosa et al., 2016; Gahtan et al., 2005). Therefore, the effect of altered cortisol synthesis on feeding reported in the current study could also reflect underdeveloped sensory systems. The relationship between cortisol and feeding is further complicated because exogenous feeding itself stimulates cortisol production in larval zebrafish (Alderman and Bernier, 2009; Filosa et al., 2016). As such, there is still much to learn about interactions between cortisol signalling and feeding regulation during teleost development.

Conclusions

Overall, our study provides novel insight into the contribution(s) of cortisol towards the exogenous feeding transition in larval zebrafish. Future work is needed to determine the specific mechanisms underlying this apparent relationship and whether this role is conserved in other teleosts (as well as other non-mammalian vertebrates). However, the current data strongly suggest that corticosteroids have evolutionarily conserved roles during feeding transitions in vertebrates.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: B.M.C., N.J.B.; Data curation: B.M.C.; Formal analysis: B.M.C., E.M., J.A.; Funding acquisition: N.J.B.; Investigation: E.M., J.A.; Methodology: B.M.C., N.J.B.; Project administration: B.M.C.; Resources: N.J.B.; Supervision: B.M.C., N.J.B.; Validation: E.M., J.A.; Visualization: B.M.C.; Writing – original draft: B.M.C.; Writing – review & editing: B.M.C., E.M., J.A., N.J.B.

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Data and resource availability
All data supporting this study are publicly available from the Mendeley Data Repository: https://data.mendeley.com/datasets/hkbf5jxj32/1. Other relevant data and details of resources can be found within the article and its supplementary information.

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