

## SHORT COMMUNICATION

### A toxic diet: transfer of contaminants to offspring through a parental care mechanism

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#### SUMMARY

The transfer of maternal contaminants to offspring during oogenesis and gestation is documented in many animals, and in mammals, contaminants may pass from mother to offspring during lactation. Although other non-mammalian vertebrates provide parental care in the form of nutritive secretions for offspring to feed from, the potential for toxicant transfer during non-mammalian parental care is rarely considered. The discus fish, *Symphysodon* spp., employs an unusual parental care strategy where fry feed on parental epidermal mucus for several weeks after hatching. This strategy has the potential to act as a method of contaminant transfer. In discus adults, both waterborne and dietary toxicants are sequestered and secreted into their epidermal mucus, the food on which fry depend. To determine whether parents could channel these contaminants directly to offspring, we exposed parents to aqueous cadmium (Cd) and recorded the subsequent feeding behaviour and Cd content of fry. Fry continued to feed normally from contaminated mucus and accumulated significant tissue concentrations of Cd. In conclusion, this parental care mechanism of the discus fish can expose offspring to harmful contaminants during the sensitive early stages of life and highlights that parent to offspring contaminant transfer after birth may be more widespread than previously thought.

Key words: biparental, cadmium, discus, mucus.

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#### INTRODUCTION

The transfer of environmental contaminants from parents to offspring has been reported in a variety of taxa and can occur via a number of routes. The maternal transfer of contaminants from mothers to eggs at various stages of oogenesis occurs throughout the animal kingdom in invertebrates (Tsui and Wang, 2004), fish (Miller, 1993; DeForest et al., 1999; DeForest et al., 2012), amphibians (Hopkins et al., 2006), reptiles (Hopkins et al., 2004), birds (Vandenstein et al., 2009) and mammals (Tilbury et al., 1999). Viviparous mothers may also transfer contaminants to their offspring during gestation (e.g. Wagemann et al., 1988). Post-parturition, both parents could continue to transfer internal contaminants to their young if their reproductive strategies involve provision of nutritional products or secretions to young as they develop. For example, mammalian mothers provide colostrum for their offspring to feed upon after birth and it is well documented that contaminants accumulated by the mother can be partitioned into this lipid-rich milk, resulting in a dietary exposure to the offspring as they feed (Ridgway and Reddy, 1995; Nickerson, 2006). Indeed, of the amounts passed between mother and offspring from *in utero* and lactational exposures, the major portion is considered to come from lactation (Whelton et al., 1993). Such exposures, occurring during the sensitive early stages of development, can impact the fitness and survival of offspring (Bjerke and Peterson, 1994; Anas et al., 2005).

Outside of mammalian reproduction there are examples of other animals that have evolved similar mechanisms to feed their offspring post-parturition (producing nutritional secretions rather than

regurgitation of ingested food). Male and female pigeons, for example *Columba livia*, produce a rich curd-like substance containing nutrients and antibodies in the crop during breeding that is regurgitated and fed to their offspring (Vandeputte-Poma, 1980; Gillespie et al., 2011). Similar crop-feeding of offspring is observed in flamingos, *Phoenicopterus* spp., and male emperor penguins, *Aptenodytes forsteri* (Lang, 1963; Prévost and Vilter, 1963). Parents of the caecilian amphibian *Boulengerula taitanu* modify their skin, which is eaten by their offspring using specialised teeth (Kupfer et al., 2006), and it has been proposed that the fry of mouth-brooding tilapia, e.g. *Oreochromis* spp., ingest mucus containing nutrients from the buccal cavity of their parents (Kishida and Specker, 2000; Iq and Shu-Chien, 2011). Other non-mammalian examples exist where animals are fed a nutritive secretion pre-parturition. In the tsetse fly *Glossina* spp., a single offspring hatches inside the mother's uterus and remains there while it feeds on a milky secretion produced by a highly modified accessory gland (Mattsson et al., 2001). Offspring of the viviparous blenny *Zoarces viviparus* similarly remain in their mother's ovarian cavity feeding on a fluid secreted by the ovary for up to 3 months (Ma and Denlinger, 1974). While the contamination of mammalian milk is well reported, in non-mammalian vertebrates there is a lack of information regarding the transfer of environmental contaminants through parental care.

The discus fish, *Symphysodon* spp. Heckel 1840, is an Amazonian cichlid that provides extended parental care for its offspring by providing an epidermal mucus secretion containing nutritional and immunological components on which offspring obligately feed for a period of around 4 weeks after hatch. Parallels have been drawn

between the reproductive strategy of this species and the provision of lipid-rich colostrum in mammals (Buckley et al., 2010). Adult discus fish can accumulate contaminants in their mucus from both external (aqueous) and internal (dietary) environments (Maunder et al., 2011), providing potential scope for a route of toxicant transfer to offspring in this species. The aim of the present study was to determine whether transfer of contaminants from adults to fry occurs *via* mucus feeding and to assess whether fry show a behavioural aversion when presented with contaminated mucus.

## MATERIALS AND METHODS

### Experimental design

Breeding pairs of discus fish were held in 1001 tanks on a recirculation system at  $29 \pm 1^\circ\text{C}$  on a 12h:12h light:dark photoperiod, with measured water chemistry of (in  $\text{mmol l}^{-1}$ ; mean  $\pm$  s.e.m.)  $0.54 \pm 0.03 \text{ Ca}^{2+}$ ,  $0.40 \pm 0.01 \text{ Na}^+$ ,  $0.036 \pm 0.002 \text{ K}^+$  and  $0.43 \pm 0.02 \text{ Cl}^-$ , and fed *ad libitum* at the University of Plymouth. Husbandry protocols have been previously published (Buckley et al., 2010). Each pair ( $N=4$  pairs) raised two separate clutches of offspring for 3 weeks post-hatch (clutch size =  $65 \pm 15$ ; mean  $\pm$  s.e.m.). During the first 3-week period, adult fish were sequentially removed from the breeding tank and placed into a 50 l treatment tank containing control water ( $0.04 \pm 0.01 \mu\text{g l}^{-1}$  Cd, mean  $\pm$  s.e.m.,  $N=20$ ) for 90 min (based on pilot studies), three times a week (i.e. a total of nine times). During the second 3-week period, when the parents were raising their second clutch of offspring, adults were exposed in the same way to an aqueous Cd concentration [ $52.08 \pm 1.38 \mu\text{g l}^{-1}$  ( $N=49$ ) from  $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ]. This ensured that only parents and not offspring were exposed to the aqueous Cd. The transfer of adults to and from the exposure aquaria was performed in a small container so that the mucosal layer of the fish was undisturbed. Sequential removal of adults maintained parental contact with fry, reducing the chance of clutch rejections. As each parent was returned to the breeding aquarium, the time taken for fry to commence feeding from the returned parent was recorded. The bite rate of the fry feeding on this parent was also assessed. This consisted of counting the number of bites that an individual fry made during a 30 s period, repeated for 10 fry.

Once a week for the 3-week exposure period, a mucus sample was taken from each adult following the 90 min in the treatment tank of control water or aqueous Cd. This was achieved using a polyurethane sponge (Buckley et al., 2010). Mucus samples were eluted, acidified and analysed to confirm Cd content by inductively coupled plasma mass spectrometry (ICP-MS) (XSERIES 2, Thermo Scientific, Leicestershire, UK). Weekly water samples were taken from the breeding aquaria to confirm that there was no leaching of Cd from exposed mucus into the water; levels of Cd in the water remained below the detection limit of the ICP-MS. At the end of the second and third week, groups of 10 fry were pooled from each clutch ( $N=4$ ), terminally anaesthetised (MS-222), digested in  $1 \text{ mol l}^{-1} \text{ HNO}_3$  and analysed for Cd concentration by ICP-MS.

### Ethical statement

All procedures in this study were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 (Project Licence No. 30/2741) and University of Plymouth ethical guidelines.

### Statistical analysis

All statistical analyses were conducted using Minitab 15 (State College, PA, USA). Data sets conformed to normality (Kolmogorov–Smirnov) and equal variance (Levene's) assumptions. For all parameters measured, there was no effect of sex, so this was

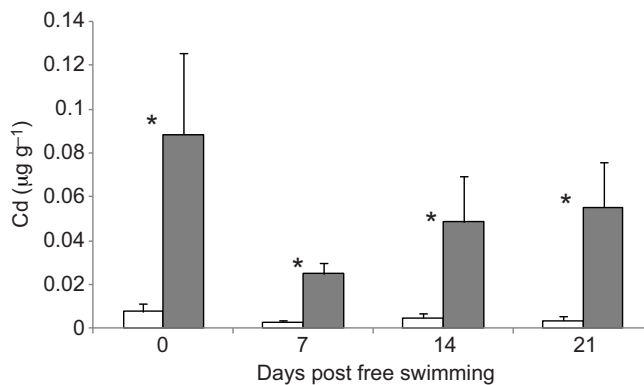


Fig. 1. Epidermal mucus Cd concentrations (mean  $\pm$  s.e.m.,  $N=8$ ,  $\mu\text{g g}^{-1}$ ) from control (white bars) and Cd-exposed (filled bars) *Symphysodon* spp. parents following an aqueous Cd exposure. Asterisks (\*) indicate significant differences to the control within each time point (repeated-measures ANOVA,  $P<0.05$ ). No significant difference between time points was found.

subsequently excluded. Because the same pair was used in both a control and exposed run, comparison between control and experimental mucosal Cd utilised a repeated-measures ANOVA. Differences between treatment groups at individual time points were assessed *post hoc*. Effects of treatment on the fry (whole-body Cd concentrations and behaviour) were determined by two-way ANOVA, with treatment and time during the 3-week period as factors; significant effects were further investigated with *post hoc* tests. Repeated-measures ANOVA was not appropriate for this analysis as although the same parents were involved, we considered the separate clutches of fry to be independent measures as they comprised different individuals.

## RESULTS

Mucus Cd concentrations during the Cd exposure were significantly higher throughout compared with the control exposure ( $F_{1,7}=9.5$ ,  $P=0.006$ ; Fig. 1) confirming accumulation of Cd in parental mucus. There were no significant differences between the control and exposed runs in the time taken for the fry to begin feeding on returned parents ( $F_{1,12}=2.57$ ,  $P=0.12$ ) or the bite rate of fry on the parents ( $F_{1,12}=1.54$ ,  $P=0.22$ ). Cd concentrations in the bodies of fry feeding on Cd-contaminated mucus were significantly higher than in control fry ( $F_{1,3}=144.24$ ,  $P<0.001$ ; Fig. 2), with no difference in accumulation between weeks 2 and 3 ( $P=0.23$ ; Fig. 2). Fry body Cd concentrations at the end of the second and third week closely matched parental mucosal Cd concentrations (Figs 1, 2).

## DISCUSSION

Cd accumulated in parental mucus of *Symphysodon* spp. and was consumed by their fry; no changes in feeding behaviour of fry were observed in response to parental contamination. The adult mucus Cd concentrations measured in the present study were similar to those in the mucus of *Symphysodon* spp. in another study of fish that were exposed for longer (3 weeks), at a lower concentration [ $3 \mu\text{g l}^{-1}$  (Maunder et al., 2011)]. Therefore, while in the present study parents were exposed to a relatively high aqueous concentration for a short duration to avoid clutch rejection, the mucosal Cd concentrations involved remain within realistic environmental predictions. Additionally, Maunder et al. (Maunder et al., 2011) showed that dietary contaminants also rapidly transfer

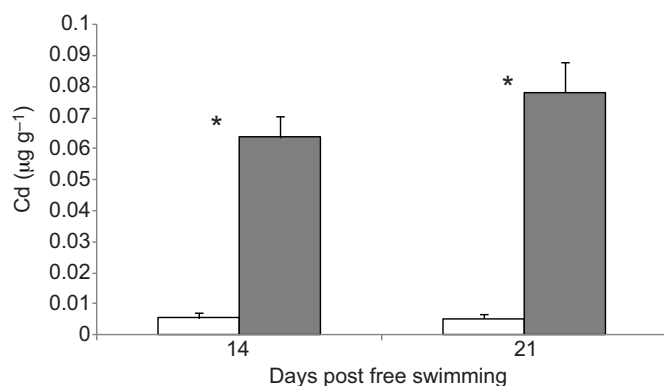


Fig. 2. Whole-body Cd concentrations (mean  $\pm$  s.e.m.,  $N=4$ ,  $\mu\text{g g}^{-1}$ ) of *Symphysodon* spp. fry from control (white bars) and Cd-exposed (filled bars) parents. Asterisks (\*) indicate significant differences to the control within each time point (ANOVA,  $P<0.05$ ). No significant difference between time points was found.

to epidermal mucus in adult *Symphysodon* spp., illustrating another route by which parental contaminants can be channelled to offspring in this species.

In the present study, fry did not alter their feeding pattern in response to contaminated mucus; no significant differences were observed in the time taken to commence feeding or in bite rate frequency. This suggests that fry were either unable to detect the presence of Cd in the mucus or that its presence did not instigate an avoidance response due to unpalatability (e.g. Riddell et al., 2005). Fry were not seen to spit out the mucus, which would be indicative of taste avoidance. Various behavioural modifications have been observed in other fish in response to dietary metals including Cd (Farang et al., 1999; Berntssen et al., 2003; Szczerbik et al., 2006), but after chronic exposures and at higher doses than the Cd in parental mucus here. For example, Szczerbik et al. (Szczerbik et al., 2006) found a decrease in locomotory activity in Prussian carp, *Carassius auratus gibelio*, fed dietary Cd at  $10\text{ mg g}^{-1}$  compared with  $0.08\text{ }\mu\text{g g}^{-1}$  in the mucus diet of the present study. It is well documented that many metal contaminants, including Cd, interfere with chemosensory systems (e.g. Scott et al., 2003) if they come into direct contact with receptors. No Cd was detected leaching from parental mucus into the water. However, the feeding behaviour of the fry could potentially have brought olfactory organs directly into contact with Cd within the mucus, or Cd present at the mucus–water boundary layer; fry bite at the mucus and then twist or shake their body to aid removal (Buckley et al., 2010).

Discus fry feed solely on parental mucus for up to 5 weeks before they begin supplementing their diet with other food items (Hildemann, 1959; Buckley et al., 2010). Whole-body Cd concentrations measured in the 2- and 3-week-old fry were very similar to the Cd concentrations in adult mucus. The frequent feeding performed by fry would result in a continuous dietary exposure to Cd for this period. This exposure period coincides with the early developmental period of these fry and hence is considered a sensitive time of exposure (Brinkman and Hansen, 2007). In fish, the toxicity of dietary contaminants to early life stages (Westin et al., 1985) is not as well studied as the effects of aqueous exposures (Sloman and McNeil, 2012), most likely because of practical reasons of diet manufacture, and certainly warrants further study. While it is usually assumed in fish that exposure to contaminants in the water

causes greater toxicity than dietborne contaminants, there are circumstances where this might not be the case (Erickson et al., 2011). In species that have reproductive strategies involving mucus feeding, accumulation of toxicants in parental mucus from the water over time, or the use of mucus as a depuration route for dietary contaminants by parents, may lead to negative consequences for offspring. Interestingly, it also seems feasible that if fry are continuously removing contaminated mucus from parents, they could potentially increase the effectiveness of this route of depuration for parents.

In the present study we demonstrate that the transfer of parental toxicant load to offspring during parental care is not restricted to mammalian systems and may be more widespread than previously thought. In discus fish, any toxicant sequestered by parental mucus either directly from the water or excreted into the mucus from the diet (Mauder et al., 2011) can potentially be channelled to offspring. This mechanism of toxicant transfer highlights the need to consider whether similar toxicant transfer mechanisms exist in other animals that provide young with nutritional supplements to ensure that this important mechanism of toxicant transfer does not go unnoticed.

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#### AUTHOR CONTRIBUTIONS

All authors were involved in the conception and design of the experiments, and in the interpretation of the findings. R.J.M. and J.B. were involved in the execution of the experiments.

#### COMPETING INTERESTS

No competing interests declared.

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