Exposure to pesticides impairs the expression of fish ornaments reducing the availability of attractive males

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Ornament magnitude often reflects a local balance between sexual selection and other sources of natural selection opposing their elaboration. Human activity may disrupt this balance if it modifies the costs of producing, maintaining or displaying the ornaments. When costs are increased, a shortage of acceptable partners may ensue, with consequences commensurate with how stringent (and effective) the process of mate choice is. Here, we show that the expression of ornaments in the viviparous amarillo fish (*Girardinichthys multiradiatus*) is influenced by embryonic exposure to low concentrations of an organophosphorus insecticide. Male ornamental fin size, dimorphic yellow coloration and display rates were all compromised in exposed fish, but unaffected in their paternal half-sibling controls and in their sisters (morphology and colour). Exposure resulted in smaller fish of both sexes, thus the differential effect by sex was restricted to attributes such as fin size only above the naturally selected magnitude shown by females. Father phenotype predicted offspring morphology of controls, but not of exposed males, which were discriminated against by both control and exposed females. Since stringent female mate choice can result in females refusing to mate with suboptimal mates, this sub-lethal developmental effect can reduce the effective population size of amarillo fish populations.

Keywords: cost of ornaments; sexual selection; handicap; insecticides; viviparous fish

1. INTRODUCTION

Implicit in the word ornaments is the idea that epigamic or secondary sexual characters are costly to produce, maintain or display. Their existence, which seemed to be at odds with the economy of design expected under the action of natural selection, led Darwin (1859) to propose the concept of sexual selection to explain exaggeration of traits by the action of (typically female) mate preferences. While it is still debated whether ornaments evolve because they are costly (Zahavi 1975), or they become costly because their magnitude is exaggerated as a result of mate choice (Fisher 1958), there seems to be little doubt that ornaments are expensive to produce and/or maintain (reviewed by Jennions *et al.* 2001). Indeed, ornaments are readily recognized because they are attributes that depart from the naturally selected optimum in one or both sexes, depending on whether the intensity of sexual selection is symmetrical or not. As inferred from the fundamental theorem of natural selection (Fisher 1958), small departures near the optimum trait value are of little selective consequence compared with similar departures when the attribute is already far from its optimum value (Fisher 1958). This means that large ornaments are expected to be much more costly than small ones, and a certain level of plasticity in the production of ornaments may be selectively advantageous. If a developing male can adjust its investment in epigamic characters in response to stress, then their final size will reflect the past condition of the bearer (i.e. it will be condition dependent; Andersson 1982).

Condition dependence of the ornament's expression is one possible reason why females base their mate choice on their magnitude. This would lead them to mate with healthy partners either with superior genetic constitution (indirect benefits to be passed to the offspring; after Zahavi 1975) or capable of providing superior resources to the female or to her offspring (direct benefits; see Kokko *et al.* 2002). Another reason to prefer ornamented males would be the exploitation of sensory biases by the males' ornaments (Ryan & Keddy-Hector 1992; Endler & Basolo 1998; Macías García & Ramirez 2005). If they are costly to produce, maintain or display, the ornament's expression can be opposed by other selective forces such as predation (Endler 1980), which may skew the availability of males towards less-preferred morphs (Macías García *et al.* 1998). Owing to the several physiological effects of agrochemicals, we hypothesized that ornament expression would also be affected by the presence of insecticides. We addressed this question experimentally using the expression of ornaments in a viviparous, sexually dimorphic fish.

Sexual selection has been implicated in the origin of large (and yellow) male fins (Macías García *et al.* 1994) and on the flamboyant male courtship display (Macías García & Saborío 2004) of the amarillo fish (*Girardinichthys multiradiatus*), a goodeid which also uses fin colour (Macías García 1991) and UV colour patterns during mate choice (Burt de Perera & Macías García 2003). There is evidence that developing large fins (assessed from correlated body shape) increases the risk of being captured by aquatic specialist garter snakes (*Thamnophis melanogaster*; Macías García *et al.* 1994, 1998), and several sources of evidence

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suggest that courtship behaviour is also a liability in this context (Macías García 1994; C. Berea & C. Macías García 1998, unpublished data). Sexual selection, as evidenced by pre-mating isolation among populations, appears to have led to population differentiation in which male, but not female, morphology differs statistically between populations (González Zuarth & Macías García 2006), suggesting that either female preferences vary arbitrarily between localities or that the development of male ornaments is influenced by the local environment. Females can effectively refuse mating with suboptimal males, and, in aquaria, this often results in a large proportion (41–76%) failing to breed altogether (Macías García *et al.* 1998; Macías García & Saborío 2004; González Zuarth & Macías García 2006).

We used the fact that a widespread insecticide is often found in the habitat of the amarillo in low concentrations (De La Vega Salazar *et al.* 1997), to test whether this novel challenge impairs ornament development. Methyl parathion (MeP) is bio-concentrated in embryos, but minimally in the mother's tissues (De La Vega Salazar *et al.* 1997), which may be due to the fact that some metabolic pathways (e.g. the cytochromes of the group P450) needed to combat the toxicity of this organophosphorus compound are not fully functional in the embryos. Preliminary work (O. Arellano-Aguilar & C. Macías García) demonstrates that the concentrations found in the field are sub-lethal, but still produce some toxic effects on the embryos and to a lesser extent on their mothers. We used one such concentration to evaluate the consequences of embryonic intoxication on adult sexual performance (ornament and courtship expression and attractiveness to females).

2. MATERIAL AND METHODS

(a) *Sample*

Fish were the laboratory-born progeny of fish collected at San Juanico in 2001 and kept in outdoor ponds (described in Macías García *et al.* 1998). We used 11 males: as the number of females available was limited to 32, six males were presented with one matched pair of similarly sized non-pregnant females each, and five were presented with two matched pairs of females each. Goodeid females do not store sperm between broods, thus by using only females who had recently given birth we guaranteed that the progeny would be sired by the male we provided. This procedure also meant that the matched broods would be born with very few days of difference, thus simplifying our experimental protocol. Females were introduced to the male home tank and kept there for 10 days, after which they were placed in isolation. A total of 32 females were used, but most failed to become pregnant (normally less than 60% of females become pregnant when presented only one male; e.g. Macías García *et al.* 1998; González Zuarth & Macías García 2006). Each female of those paired to the same male was arbitrarily assigned to either the treatment (exposure to MeP) or the control group (sham exposure; see below). Females were kept in individual 31 aquaria for 50 days (gestation in laboratory 53 ± 7.9 days, $n=13$; E. Saborío & C. Macías García 2002, unpublished data), and both female and offspring survival were recorded. Each newborn brood was transferred to a MeP-free 40 l aquarium fitted with an air-powered foam filter, fed *ad libitum* on commercial fish food flakes and

maintained at 12/12 hours photoperiod and 25°C until they reached 90 days of age.

A dilution of 99.4% purity MeP (TECROM, Mexico) was added to the commercial food flakes (Sera Vipan, Italy) using HPLC-grade acetonitrile (TECSIQUIM, Mexico) as carrier so that the concentration of MeP in the food was $0.005 \mu\text{g g}^{-1}$. This is twice the concentration measured in water from their locality of origin, and one order of magnitude lower than field concentrations elsewhere. Individual 0.005 g doses wrapped in tin foil to protect the photolabile insecticide were given to the fish twice a day (note that the MeP concentration in the water of the aquaria would then have been well below field measurements). Control females were fed the same food soaked (and subsequently allowed to dry) in acetonitrile, but without the insecticide.

Fish in each brood were separated by sex from approximately 60 days of age, when sexes can already be distinguished, and raised in isolation in cylindrical compartments within single-sex aquaria. Ornaments were measured when fish were fully adult, following behavioural trials that began at the age of approximately 90 days.

(b) *Morphology*

The day after the completion of the trials (see §2d), fish were anaesthetized with a dilution of 1 g benzocaine per 100 ml acetone, placed next to a ruler on a photographic board and photographed with a digital camera. The images were stored on a computer and subsequently measured using Image Tool shareware. We followed the protocol used by González Zuarth & Macías García (2006), and measured six attributes historically associated with female mate choice and four not associated with it so far (see fig. 2 of González Zuarth & Macías García 2006). The averages of three measurements of each attribute of males and females mate from all the broods were entered into a principal components analysis (PCA). An independent PCA was performed with only the males that fathered both exposed and control broods.

(c) *Colour*

The colour of anaesthetized males (but not of their parents) was assessed using a Minolta CM-2600D hand-held spectrophotometer with pulsed xenon lamps (spectral range was from $\lambda=360$ to 740 nm), which was calibrated with the manufacturer's white calibration plate. We obtained the reflectance spectrum of one point in the operculum (known to be reflective in the UV), one point in the flank and one in each of the median fins (dorsal, caudal and anal). We measured the same in females, except that the small size of their dorsal and anal fins (which are not coloured) precluded measuring their reflectance. From the spectra, we calculated total chroma saturation, yellow chroma saturation, red chroma saturation and UV chroma saturation (see §3).

(d) *Behaviour*

(i) *Courtship performance*

Once fish from one matched brood reached their 90th day of age, each male was individually transferred to an observation tank divided into two compartments by an opaque partition, and left in the male compartment to habituate for 24 hours. One of three standard females from our stocks was then introduced into the female compartment within a transparent plastic bag. After 5 min of habituation to temperature and light conditions the female was presented, always within her bag, to the male. Fish were allowed to settle for a further

Table 1. Eleven males were given the opportunity to mate with one or two pairs of matched females, but only five males (A–E) had broods with two matched females. (Exposed females were fed commercial flakes with the organophosphorus MeP, and their broods experienced substantial mortality and some fish were malformed. Only seemingly normal fish were used. Broods were trimmed to the size of their matched brood.)

father	mother	group	date ^a	born	adult males	adult females	abnormal	males used	females used
A	1	C	11	10	4	6		2	2
	2	E	16	10	2	3	1	2	2
B	3	C	20	12	4	8		2	2
	4	E	20	31	2	4	2	2	2
C	5	C	20	3	1	2		1	0
	6	E	20	13	1	0		1	0
D	7	C	20	6	3	3		1	2
	8	E	20	5	1	2		1	2
E	9	C	19	5	2	2		2	2
	10	E	16	6	2	4		2	2

^a All broods were born in May 2005.

5 min and a 15 min recording session commenced, when we recorded the frequency and duration of courtship fin-folding, lateral fin display, frontal fin display and flagging, and the frequency of figure-of-eight dance and of copulation attempts (see González Zuarth & Macías García 2006). Observations were conducted by two observers who were kept ignorant of the identity of the fish. Trials took place between 11.30 and 15.30, exposed fish and their half-sibs were observed alternatively, and the procedure was repeated the following day until the behaviour of every fish has been recorded in the presence of the three standard females. We performed a PCA with the behavioural data from all males. Mean male scores of the first two components (PC1 and PC2) were analysed using a mixed ANOVA with father as a random covariate, and family (mother) and treatment (exposed or control) as treatments.

(ii) Female preferences

We formed eight pairs of one exposed and one control male with the condition that they were not half-siblings. Three females were transferred on day 1 to the central compartments of three observation 40 l tanks. These were illuminated with 20 W General Electric Daylight fluorescent tubes, which provided a peak of near-UV light below 400 nm; acetate covers with 80% transmittance between 380 and 650 nm ensured that near-UV light was available during trials (but wavelengths below 360 nm would have been absent). After 24 hours, the males of one pair were individually introduced to the lateral compartments of each tank. Partitions were transparent (as the acetate covers mentioned above), and after 5 min of habituation we recorded for 20 min the frequency and duration of visits (to within 3 cm of a male compartment, using as reference a line marked on the outside of the tank) and the frequency of copulation attempts (which under this condition have to be initiated by the female assuming the mating position next to the courting male). All fish were returned to their enclosures, and the next three (or two) females were transferred to the observation tanks for their 24 hours habituation. We repeated this procedure alternating the position of the males between left and right, until all 16 females had been exposed to all eight male pairs ($n=8$ trials per female). Records were conducted by the two naive observers. We analysed the data with one repeated measures ANOVA per variable, where we looked for the effects of female origin (exposed or control), male origin (exposed or control) and of the interaction.

3. RESULTS

(a) Sample

Information on broods is detailed in table 1. Broods sired by the same male were born within 5 days (range 0–5, $\bar{x} \pm \text{s.d.} = 1.6 \pm 2.3$ days) and all broods were born within a fortnight. Only five males had broods with the two females of a matched pair. Exposed broods experienced a high mortality (68%) before reaching adulthood and three fish were malformed. These (all females) became adult and were excluded from analyses, as we were not concerned with teratological consequences of early exposure to MeP. In order to keep as balanced a design as possible, we used all the available males and females from the smaller (normally the exposed) brood, and arbitrarily picked their matches among their half-siblings. All these resulted in a reduced sample of eight males and eight females in each of the exposed and control groups.

(b) Morphology

As has been the case in the past (González Zuarth & Macías García 2006), the first two components of the PCA separated size (PC1; 47.6% variance explained) and sexually dimorphic shape (PC2; 26.7%). In particular, the second component gave very large loadings to three of the four measurements of the dimorphic fins: the base of both the dorsal and anal fins, and the length of the latter. Exposed fish, both male and female, were significantly smaller than their counterparts ($F_{1,21} = 13.2$, $p = 0.0015$; figure 1). This was true for both the sexes (treatment $\times$ sex, $F_{1,21} = 0.42$, $p = 0.52$), but the effect was more pronounced in males ($F_{1,21} = 9.2$, $p = 0.0062$) than in females ($F_{1,21} = 4.5$, $p = 0.046$). The normal difference in size between the sexes (where size-related fecundity selects for larger females) was somewhat blurred ($F_{1,23,2} = 3.25$, $p = 0.085$). As expected, males had larger loadings on the second PC, which measures the dimorphic fin size and body shape ($F_{1,25,7} = 178.6$, $p < 0.0001$), but the treatment was not quite significant ($F_{1,24,1} = 3.2$, $p = 0.086$). Yet, the interaction between sex and treatment was significant ($F_{1,24,1} = 8.0$, $p = 0.009$). This was caused by the exposed males ($F_{1,24,1} = 10.65$, $p = 0.003$), but not by the females ($F_{1,24,1} = 0.54$, $p = 0.47$), having reduced fins (figure 1). Thus, trait size was only affected in the sex where it has evolved into an ornament.

Increased cost of ornaments with ornament size was evidenced by independently regressing the score of sexual

Figure 1. The first principal component gave large loadings to the measurements reflecting overall size, and significantly ($p=0.003$) separated exposed (to $0.005 \mu\text{g g}^{-1}$, filled symbols) and control fish (open symbols). Interestingly, owing to the reduced size of exposed females, the reversed sexual size dimorphism common in viviparous fishes is not significant here. The second PC separated fish by sex ($p<0.001$: ♀, females; ♂, males), giving large loadings to the dimorphic fin and body size and shape. Prenatally exposed males, but not females, developed smaller median fins, which when fully grown act as male ornaments in this fish.

dimorphism of experimental and control males against their father's score. This was calculated using only males, yet it again separated size (PC1; 63% of variance explained) and sexually dimorphic shape (PC2; 16.6%), giving high loadings to body height (a correlate of fin size; see Macías García *et al.* 1994) and to the length of both the dorsal and anal fins. We used the scores in the second principal component as a measure of parental ornamentation, and regressed on it the filial PC2 scores separately for exposed and control males (figure 2). The ornament development of control ($F_{1,3}=34.4$, $p=0.01$), but not of experimental males ($F_{1,3}=1.01$, $p=0.39$), was a function of paternal ornamentation (figure 2). More importantly, the slope of the exposed males' regression, which was not different from zero, was significantly lower than that of the controls (interaction from an ANOVA with father and treatment as factors: $F_{1,6}=7.25$, $p=0.036$). This confirms that attempting to develop a large ornament is substantially costlier than attempting to develop a modest one (offspring scores converge in the ornament size of the less-ornamented parents; figure 2), a pattern consistent with the idea that the exaggerated male fins of the amarillo are condition-dependent ornaments.

(c) Colour

There were several differences in colour between treatment and between the sexes, mostly in agreement with our hypothesis that early exposure to insecticides should hamper ornament expression; the results are shown in table 2. We did not anticipate sexual differences in the colour of the operculum or the fish flank, although both reflect in the UV (Macías García & Burt de Perera 2002), and females use UV reflection to select among males. Yet we found that females reflect significantly more UV in both the operculum and the flank than males, but this was unaffected by early exposure to MeP. Conversely, male operculum and flank were more saturated in the red region than those of females (see below), which again was unaffected by the treatment. Total chroma saturation was also higher in males on both the flanks and the

Figure 2. Ornament development (PC2) of control males (filled circles) was a function of paternal ornamentation. Offspring values of singletons or mean values for litters with two males (table 1) were regressed on parental (father) values (Falconer & Mackay 1996). Heritability of male ornamentation was calculated as $h^2 = 2 m = 1.13 \pm 0.096$ s.e., a value which may indicate a degree of sex linkage, as has been suggested for male ornaments in *Poecilia reticulata* (Hughes *et al.* 2005). The regression was not significant for exposed males (open circles), and it is traced as a dotted line only to aid comparison.

opercula, and control males had more saturated chroma on the operculum than exposed fish (but the interaction was not significant). Yellow chroma was also higher in the opercula of control than in those of exposed fish of both the sexes (table 2).

We obtained the reflectance of the three median fins of males (dorsal, anal and caudal) and of the caudal fin of females. The caudal fin is normally not dimorphic in colour (to us), yet we found that male caudal fins had higher red chroma saturation than female fins. Yellow chroma was also more saturated in control than in exposed fish, and the total chroma saturation was much higher in control fish, particularly because control males had very high chroma saturation (table 2). Dorsal and anal fins of control males had higher yellow chroma saturation than those of their exposed siblings, and their anal fins were also of higher red chroma saturation than those of exposed males. It is apparently puzzling that UV chroma was higher in the dorsal fins of exposed than in those of control fish (see §4c).

(d) Behaviour

(i) Courtship performance

In order to capture as much as possible of the variance in courtship behaviour in our analyses, we performed the PCA before calculating the average of the three trials of each male (rather than calculating the average and then performing the PCA). The first two principal components explained 71% of the variance in our data. The first principal component (49% of the variance explained) gave high positive loadings to active courtship behaviours (frequency and duration of courtship fin-folding and flagging, and frequency of figure-of-eight dance), whereas the second one gave even higher, but negative loadings to passive courtship behaviours (frequency and duration of static fin displays). Control males performed more active courtship than their exposed half-siblings; as we were testing a directional prediction, we used a one-tailed test

Table 2. Comparisons of colour ($\bar{x} \pm \text{s.e.}$, rounded to two decimals) between amarillo fish exposed to MeP during gestation (E) and their half-sibling controls (C). (Aspects of chroma were defined as follows: spectral saturation, $Ch = (R_{\max} - R_{\min})/B_M$; red chroma, $C_R = R_{[630-740]}/R_{[360-740]}$; yellow chroma, $C_Y = R_{[500-630]}/R_{[360-740]}$; UV chroma, $C_{UV} = R_{[360-400]}/R_{[360-740]}$. R =reflectance (%) in the specified wavelength (λ) and B_M (mean brightness) = $R_{[360-740]}/n_w$, where n_w is the number of wavelength measured. Reflectance of female dorsal and anal fins was not measured. Significant contrasts are given in italics.)

structure	chroma	group	males	females	sex		treatment	
					$F_{1,27}$	p	$F_{1,27}$	p
operculum	Ch	E	1.804 ± 0.100	1.650 ± 0.095	12.82^a	0.002	15.48	$0.001^{b,c}$
		C	2.292 ± 0.095	1.833 ± 0.095				
	C_R	E	0.337 ± 0.016	0.293 ± 0.015	17.95^d	0.0003	0.24^e	0.63
		C	0.341 ± 0.015	0.277 ± 0.015				
	C_Y	E	0.396 ± 0.008	0.395 ± 0.008	0.22	0.65	6.70	0.02
		C	0.418 ± 0.008	0.412 ± 0.008				
flank	C_{UV}	E	0.095 ± 0.005	0.103 ± 0.005	8.37	0.008	1.16	0.29
		C	0.083 ± 0.005	0.104 ± 0.005				
	Ch	E	1.872 ± 0.169	1.364 ± 0.158	6.18	0.019	0.00	0.95
		C	1.754 ± 0.158	1.461 ± 0.158				
	C_R	E	0.371 ± 0.013	0.322 ± 0.013	19.78^f	0.0002	0.37^g	0.55
		C	0.364 ± 0.013	0.316 ± 0.013				
caudal fin	C_Y	E	0.360 ± 0.005	0.347 ± 0.005	2.66	0.11	0.01	0.92
		C	0.355 ± 0.000	0.353 ± 0.005				
	C_{UV}	E	0.107 ± 0.008	0.133 ± 0.007	16.43^h	0.0004	2.06^f	0.16
		C	0.113 ± 0.007	0.147 ± 0.007				
	Ch	E	8.058 ± 1.001	7.814 ± 0.936	2.12	0.16	4.90	0.04
		C	11.309 ± 0.936	8.781 ± 0.936				
sexually dimorphic fins	C_R	E	0.419 ± 0.004	0.407 ± 0.004	4.42^a	0.046	0.10^i	0.75
		C	0.413 ± 0.004	0.410 ± 0.004				
	C_Y	E	0.406 ± 0.002	0.406 ± 0.002	0.57	0.46	7.42	0.01
		C	0.412 ± 0.002	0.412 ± 0.002				
	C_{UV}	E	0.035 ± 0.002	0.038 ± 0.002	3.03	0.09	2.09	0.16
		C	0.032 ± 0.002	0.036 ± 0.002				
			exposed	control	F	d.f.	p	
dorsal	Ch	C > E ^j	7.14 ± 0.767	7.85 ± 0.715	0.56	1,10.5	0.470	
	C_R		0.42 ± 0.006	0.43 ± 0.005	2.11	1,9.4	0.178	
	C_Y		0.40 ± 0.002	0.41 ± 0.002	14.25	1,10.3	0.003	
	C_{UV}		0.04 ± 0.002	0.03 ± 0.001	9.23	1,10.3	0.012	
anal	Ch	C > E	8.24 ± 0.917	7.94 ± 0.855	0.08	1,10.6	0.780	
	C_R		0.42 ± 0.005	0.44 ± 0.003	21.84	1,9.5	0.001	
	C_Y		0.40 ± 0.002	0.41 ± 0.002	5.62	1,10.2	0.039	
	C_{UV}		0.04 ± 0.002	0.03 ± 0.001	0.95	1,10.4	0.350	

^a d.f. = 1,24.5. ^b d.f. = 1,24.4. ^c Sex $\times$ treatment, $F_{1,24.4} = 3.21$, $p = 0.086$. ^d d.f. = 1,24.3. ^e d.f. = 1,24.1. ^f d.f. = 1,24.2. ^g d.f. = 1,24. ^h d.f. = 1,24.7. ⁱ d.f. = 1,23.9. ^j Direction of significant contrasts.

here ($F_{1,14} = 3.21$, p (one-tailed) = 0.048). We conclude that embryonic exposure to MeP impairs courtship performance in adult life.

(ii) Female preferences

Exposed and control males received a similar number of visits from both types of female (exposed females' visits to exposed males, $\bar{x} \pm \text{s.e.} = 19.9 \pm 2.2$; to control males, $\pm \text{s.e.} = 24 \pm 2.2$; control females' visits to exposed males, $\pm \text{s.e.} = 25.8 \pm 2.2$; to control males, $\pm \text{s.e.} = 22.2 \pm 2.2$; male contrast, $F_{1,28} = 0.02$, $p = 0.89$; female contrast, $F_{1,28} = 0.087$, $p = 0.36$). Duration of visits, on the other hand, revealed discrimination against exposed males, which received shorter visits from the control females (313.6 ± 24.9 s) than their non-exposed half-siblings (323.5 ± 24.9 s), and also received shorter visits by the exposed females (258 ± 24.9 s) than the controls (356.6 ± 24.9 s); the difference between males being significant ($F_{1,28} = 4.7$, $p = 0.038$). There was no

difference between the two female groups ($F_{1,28} = 0.2$, $p = 0.66$), but exposed females appear to be somewhat more discriminating (male $\times$ female interaction, $F_{1,28} = 3.2$, $p = 0.086$). Exposed males also received fewer copulation attempts (the female moved next to the enclosed male and shared in the attempt to embrace) than control males ($F_{1,28} = 4.74$, $p = 0.038$). This again was unaffected by female origin ($F_{1,28} = 0.027$, $p = 0.87$).

4. DISCUSSION

(a) Sample

It is common for goodeid females to successfully avoid mating when confined with a single male, a condition in which normally no more than approximately 60% become pregnant (Macías García & Saborío 2004; González Zuarth & Macías García 2006), which is one reason why sexual selection is thought to be strong in this fish (Ritchie *et al.* 2005). This determined the number of broods to be

used in our experiments, whereas the number of fish was determined by mortality in the exposed brood. This might potentially bias our results in a conservative direction, since presumably only the best fish would have survived and developed seemingly normal phenotypes after exposure to MeP during their early development, superior exposed fish may thus obscure the hypothesized effect. As we saw this was not the case.

(b) Morphology

From the PCA, it may appear that the size differences between exposed and control fish were very large, raising the possibility that exposed fish were abnormal, teratological cases (figure 1). In fact, the magnitude of the difference in standard length between exposed and control fish was small (approx. 1–3 mm; $\bar{x} \pm \text{s.e.}$; control females 32.0 ± 0.4 mm, exposed females 31.4 ± 2.0 mm, control males 27.9 ± 1.3 mm and exposed males 25.3 ± 0.6 mm), and the males' size was within the lower quartile of that reached by laboratory-born individuals measured when they were twice as old (at the age of six months; Macías García *et al.* 1998). In viviparous fishes (including the amarillo; Macías García & Saborío 2004), fecundity is a function of size, thus the reduction in female size due to embryonic exposure to MeP may have negative fitness consequences, although probably not very large given the rather small effect size.

Males exposed to MeP during gestation grew smaller, but also developed smaller ornaments. The differential effect by sex was evident only on the extent of the sexually dimorphic dorsal and anal fins, and the differences in median fin size between paternal half-sibs were proportional to the attractiveness of the father. This means that the final magnitude of these ornaments is determined by the paternal contribution as well as by the conditions experienced during development. Therefore, the sexually dimorphic dorsal and anal fins of *G. multiradiatus* are ornaments that reflect the past condition of the bearer and may thus be used in adaptive mate choice by females (Andersson 1982; Bonduriansky 2007; see §4c).

(c) Colour

This fish is known as amarillo, which is Spanish for yellow, owing to the colour of the males' median fins, thus we expected colour differences between the sexes but also between the treatments. There were in fact several colour differences between the sexes and between exposed and control fish. Most notably, yellow chroma saturation was higher in control fish in four of the five areas measured, including the sexually dimorphic fins whose yellow colour in the males is a sexually selected trait (Macías García 1991). It is unlikely that any traces of MeP remained in the adult fish when colour (presumably carotenoids) was being deposited on the fins. It is thus also unlikely that the low yellow chroma saturation of exposed fish is the result of carotenoids being used for the removal of free radicals produced during the detoxification of MeP (Krinsky 2001). Instead, it appears that the insecticide caused some long-lasting damage to the physiology of the embryos, and that some of those effects persist into adulthood, including a reduced capability to process/deposit ornamental carotenoids or to combat oxidative stress: in fact, we know that exposure to MeP can lead to

permanent damage to the nervous system, as well as to the liver (see a review of effects by García *et al.* 2003). If any such indirect process can cause a reduction in the level of signalling, this raises the possibility that carotenoid coloration may be used by animals to reflect past, and not only current, condition; a possibility that deserves future investigation.

There were also differences in UV chroma saturation between exposed and control males (dorsal fins), and between the sexes (operculum and flank). In both cases, the relevant structures had higher spectral or yellow chromatic saturation in fish with lower UV chroma. It is thus possible that UV reflectance is structural, and becomes increasingly evident when pigments (which confer chromatic saturation) such as carotenoids are scarce. If any such mechanism is at work, it should imply that UV marks are produced cheaply or that the cost of producing them is different from that involved in producing equivalent yellow marks. This may suggest that the UV-based female mating preference in the amarillo is not related to quality assessment. Because the main predators of *G. multiradiatus*, the snakes of the genus *Thamnophis*, can perceive UV light (Sillman *et al.* 1997), this relative preponderance of UV markings in experimental fish cannot be interpreted as a mechanism used by weak or vulnerable fish to exploit a private communication channel (e.g. Cummings *et al.* 2003).

(d) Behaviour

(i) Courtship performance

Male–female interactions in the amarillo normally follow a pattern in which the male approaches the female and raises its dorsal and anal fins (fin display), then remains rather static for a few seconds. This can lead to escalated aggression or escalated courtship (Valero *et al.* 2005), and appears to be a relatively inexpensive behaviour. The propensity to escalate into the more energetic flagging or figure-of-eight dance varies between populations (González Zuarth & Macías García 2006), and fish not escalating tend to prolong their interactions for much longer than fish which escalate (C. Berea, C. Dominguez, J. Núñez-Farfán & C. Macías García 1998, unpublished data). Here we found that prenatal exposure to MeP produced differences in courtship style between paternal half-sibs as notorious as those found between populations of the amarillo. Compared with the controls, exposed fish had a more parsimonious courtship style, only rarely performing the seemingly more energetic dances that characterize courtship escalation in this species. This was the case even when experimental fish also had smaller ornamental fins than controls, a trait which can hamper swimming performance (Ryan 1988; Nicoletto 1991). Subdued courtship may reflect a generally depressed condition due to metabolic deficiency, but may also betray permanent damage to the central nervous system: both may have similar fitness consequences and further work should aim at assessing the pathway between prenatal exposure to MeP and adult courtship behaviour.

(ii) Female preferences

Apart from reduced survival, the most damaging consequence of a pesticide must be its capability to reduce reproductive output, through compromising the production

of gametes. We did not examine this possibility, but found instead a significant reduction in attractiveness that was consistent across groups of females. Male courtship behaviour was not facilitated during the female-choice trials, although females might have been able to assess the general level of activity of the males. Yet morphology and colour (although not distant UV wavelengths) were available, and the concordance among experimental female groups in their preference for control fish over their exposed counterparts shows that the latter were unattractive as reproductive partners (and that the male assessment capabilities of females were not obviously impaired by early exposure to MeP). Female preferences as measured here correlate with mating probability, and, in aquaria, the probability that the female *G. multiradiatus* remain unmated increases from 44% (with attractive males) to 76% when housed with suboptimal males (González Zuarth & Macías García 2006). Thus, besides any reduction in female fecundity via a decrease in size, such an effect on male attractiveness would reduce the overall fitness of populations exposed to MeP, thereby posing a threat to their survival that is directly linked to sexual selection. This assumes that the extreme female selectivity observed in laboratory also takes place in the field, yet it is conceivable that given enough time and genetic variability, female mating preferences would adjust to local male availability, and male ornament expression would increase as insecticide resistance is selected for. Still, the speed at which populations have to adapt to anthropogenic intervention and the erosion of heritable variation (e.g. Wilson *et al.* 2006) may preclude adaptation. We suggest that our results support the idea that sexual selection can compromise population survival, and that anthropogenic factors may thus disproportionately affect species in which sexual selection is intense.

We adhered to the ethical guidelines for the use of animals in research published jointly by the Association for the Study of Animal Behaviour–Animal Behaviour Society. Licence to collect fish was granted by CONAPESCA.

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NOTICE OF CORRECTION

The caption for [figure 2](#) is now presented in the correct form.

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