


Red operculum spots, body size, maturation and evidence for a satellite male phenotype in non-native European populations of pumpkinseed *Lepomis gibbosus*

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Abstract

Carotenoid-based pigmentation is a striking feature of many taxa, yet the function, if any, of colour traits is often unclear. Pumpkinseed *Lepomis gibbosus*, a widely introduced freshwater sunfish that exhibits alternative male mating strategies, express a striking, red operculum spot. To investigate the potential function of this red spot as a signal in this species' mating system, we determined the presence and measured the size of red operculum spots in fish collected from 12 populations in five European countries in which pumpkinseed is an established non-native species. We subsequently related the presence and size of the red spot to body size and mating strategy, based on an analysis of relative gonad size (gonado-somatic index, GSI), using a mixed modelling approach. The study demonstrated that the presence of a red operculum spot in pumpkinseed is associated with sexual maturation, with GSI frequency distributions suggesting that cuckolders in some non-native populations comprised both sneaker and satellite males, the latter not having previously been reported for this species. Further, the size of red spot correlated strongly with body size in parental and cuckolder males, although there was no difference in the presence or size of the red operculum spot between male mating strategies. The function of a red

operculum spot in females is not clear but may be partly mediated by pleiotropic genetic mechanisms. Red operculum spots appear to function as signals of male maturation and body size in pumpkinseed, irrespective of mating strategy.

KEYWORDS

alternative mating strategy, carotenoid, centrarchidae, GLMM, sexual selection, visual signal

1 | INTRODUCTION

Visual signals play a key role in the environmental biology of teleost fishes (Marshall, 2000; Wootton, 1998). Pigmentation is an important component of visual signals in fishes, both within and among species. The expression of colour, which often comprises multicomponent signals, involves pigment-based and structural colours (Price, Weadick, Shim, & Rodd, 2008; Wootton & Smith, 2015). Carotenoid-based colouration, which typically appears as yellow, orange or red, has been of particular interest because these are costly to produce and thereby serve as a reliable indication of individual condition and foraging ability (Olson & Owens, 1998), with a potential role in mating system evolution (Wootton & Smith, 2015). Different carotenoid pigments are responsible for conveying different information to potential mates or rivals (McLennan, 2007). Colour traits can function in sexual selection, which acts on differences in fitness among individuals that arise due to the number and identity of their mates. Because sexual selection tends to act more strongly on males than females (Andersson, 1994), it is males that usually express the brightest and most elaborate visual traits, including nuptial colouration. Although a broad simplification, there is a tendency in many taxa for a preference to mate with more colourful and ornamented individuals (Andersson, 1994; Price et al., 2008). Understanding the adaptive value of mate choice (sensu Kokko, Brooks, Jennions, & Morley, 2003) based on visual signals is a controversial field of research, but may arise through fitness benefits associated with mate choice, or an arbitrary mechanism (Andersson, 1994; Wootton & Smith, 2015).

Pigmentation in North American sunfishes (Centrarchidae), in common with most other teleosts, is more extensive in males than in females (Scott & Crossman, 1973). In the pumpkinseed *Lepomis gibbosus*, males have a striking, red operculum spot that is associated with male aggression (Stacey & Chiszar, 1978). In aquarium studies, breeding males were shown to attack fish models that intruded into their nesting area, and models with red colour on the operculum spot or iris received more attacks and aggressive displays than those without colour (Stacey & Chiszar, 1978). It is recognised that dominance relationships among sunfishes are related to colouration, with dominant, brightly coloured parental (territorial) pumpkinseed males building and defending nests, and performing courtship behaviour to attract females to their nest for spawning. Male sunfishes also adopt alternative mating strategies (sensu Wootton, 1984), whereby subordinate “cuckolder” males attempt to intrude into the nest of a parental male that is in the act of spawning (Gross, 1979, 1982). Cuckolder males are early maturing, do not perform courtship and

avoid fitness costs associated with parental care (Smith & Wootton, 1995). Small cuckolders, which typically possess disproportionately large testes, are referred to as “sneakers” due to their rapid entry into the nest of a spawning territorial male to release sperm at the moment of spawning. Large cuckolders, which have testes of intermediate size relative to parental and sneaker males, are termed “satellites” and participate in spawning by mimicking female characteristics and entering the nest of a territorial male as a potential mate (Garner & Neff, 2013; Gross, 1982). Despite aggressive defence of their nest by parental males, cuckolder males frequently intrude successfully during spawning events (Rios-Cardenas, 2003). In the close congener, bluegill *Lepomis macrochirus*, a model taxon for research on alternative reproductive strategies (Taborsky, 2008), Gross (1982) characterised two cuckolder phenotypes, based on a population in Lake Opinicon, Canada. Sneaker bluegill are small and have relatively large testes relative to parental males, with a mean gonado-somatic index (GSI) of 4.6 (contrasting with parental males with a mean GSI of 1.1). In contrast, satellite male bluegill are larger than sneakers, with GSI values (mean 3.3) that are intermediate between those of parental males and sneakers. In applying these criteria to pumpkinseed from the same lake, Gross (1982) identified the sneaker phenotype, but not that of the satellite. In a subsequent paternity study, Neff and Clare (2008) noted that the satellite strategy had yet to be reported for pumpkinseed. In a subsequent genetic study of bluegill–pumpkinseed hybridisation, Garner and Neff (2013) did not distinguish between satellites and sneakers, referring to them collectively as cuckolders. Thus, there are no reports that the satellite phenotype (sensu Gross, 1982) is expressed in pumpkinseed, and hereafter, we use the term “cuckolder” to refer to the alternative male mating strategy in pumpkinseed to the parental strategy.

The approach, onset and end of the reproductive period in fishes can often be identified by variation in GSI, the relationship between gonad weight and somatic weight (Wootton, 1998). Male mating polymorphisms, particularly the cuckolder strategy, are associated with sperm competition, which occurs when the spermatozoa of different males compete for the same eggs (Parker, 1970). Males expressing alternative mating strategies tend to experience a higher “risk” of sperm competition, that is an elevated probability that their sperm will compete with those of other males, because they usually mate in the presence of at least one competitor, typically the territorial male and often other cuckolder males (Wedell, Gage, & Parker, 2002; Wootton & Smith, 2015). An outcome is that cuckolders are predicted to show adaptations for sperm competition, including relatively (or even absolutely) larger testis size, larger ejaculates and

faster swimming spermatozoa (Montgomerie & Fitzpatrick, 2009). Consequently, individual GSI can be used to identify male mating strategy.

Pumpkinseed have been widely established outside their native North American range. Most introductions have been in Europe, where the species was introduced in the late 19th and early 20th centuries, establishing populations in at least 28 countries (Copp & Fox, 2007). Most of the research undertaken on pumpkinseed in Europe has focused on female growth and life-history traits (reviewed in Fox & Copp, 2014), with relatively few studies on males (e.g. Almeida, Tobes, Miranda, & Copp, 2012; Valente et al., 2016). In its native range, Neff and Clare (2008) observed temporal variability in the patterns of pumpkinseed paternity and suggested that these reflect temporal variation in mating opportunities, parental male defence ability or cuckold densities. Given this temporal variability, spatial variations are also feasible, possibly extending to visual signals, such as the operculum spot, which is assumed to be important for males for attracting mates (Stacey & Chiszar, 1978).

The aim of this study was to determine whether the presence and size of the red operculum spot serve as a signal of sex and/or mating strategy in pumpkinseed. To do this, we used estimates of GSI to categorise males as nesters or cuckolders and fitted models to test whether spot presence and size varied between the sexes and between males adopting different reproductive strategies. We predicted that parental males, which experience intrasexual contests and intersexual mate choice, would be more likely than cuckold males and females to express a red operculum spot and that these males would also express larger red spots.

2 | METHODS

Previously unpublished data for 1514 pumpkinseed were obtained from established non-native populations in Belgium, England, France, the Netherlands and Poland (Table 1), with all data elements being available for 924 specimens of which 465 were males, 387 were

TABLE 1 Country, population, location (WGS84 latitude and longitude coordinates) and capture method for male pumpkinseed *Lepomis gibbosus*, including number of specimens (*n*), mean and *SD* of standard length (mm) and the mean, minimum (Min) and maximum (Max) of the gonado-somatic index (GSI) for each population by water body. ANG, angling; ELF, continuous electrofishing; NET, small seine netting; TRC, minnow traps, catch-per-unit-effort (see Fox et al., 2011).

Country	Waterbody name	Latitude	Longitude	Method	<i>n</i>	SL	SD of SL	GSI		
								mean	Min	Max
Belgium	Webbekomsbroek Pond	50.975833N	5.076111E	ELF	76	61.2	15.7	2.4	0.2	5.3
France	Briere Marsh	47.366111N	2.312500W	ELF	63	90.9	16.4	1.9	0.5	4.6
	Grand-Lieu Lake	47.083333N	1.650000W	ELF	51	69.4	21.2	3.1	0.2	8.3
	Berg-sur-Moselle River	49.430556N	6.307222E	ELF	11	97.1	30.7	1.0	0.3	1.4
	Mirgenbach Reservoir	49.421667N	6.236111E	ELF	27	60.8	21.2	2.2	0.2	9.5
	Richardmesnil Gravel Pit	48.590000N	6.160833E	ELF	54	82.7	15.1	1.5	0.3	4.5
	Uckange (Moselle River)	49.298611N	6.169722E	ELF	6	74.3	22.8	0.6	0.3	1.4
Netherlands	Schoapedobbe Pond	52.951944N	6.258333E	NET	30	124.2	16.6	2.0	0.7	5.6
	Meeuwven Pond	51.391111N	5.499722E	NET	58	67.3	3.8	3.5	1.3	10.7
Poland	Brodowski Pond	53.450342N	14.565856E	ELF	24	77.1	11.1	2.0	0.2	5.2
	Power Plant discharge channel	53.213983N	14.468975E	ELF	233	95.6	16.9	1.2	0.1	10.2
England	Cottesmore Pond (Lower)	51.089167N	0.219444W	TRC	31	64.5	12.5	2.3	0.4	9.5
	Cottesmore Pond (Upper)	51.088056N	0.219444W	TRC	82	64.9	12.3	2.6	0.8	8.3
	Cottesmore Pond (School)	51.087222N	0.219444W	TRC	56	64.4	16.2	1.8	0.2	2.9
	Batts Stream at Cackle Street	51.018611N	0.074444W	ELF	28	75.2	12.4	1.7	0.7	6.4
	Tanyard Specimen Lake 3	51.019843N	0.012377W	ANG	39	92.7	16.4	2.4	0.9	12.5

females and 266 were juveniles (Table 2). The fish were collected by a variety of sampling gear (Table 1), depending upon location characteristics and gear availability. Note that sampling of the two Netherlands populations was incorrectly reported as by funnel trap in Cucherousset et al. (2009). Where possible, the sampling aimed to obtain ≈ 100 specimens for growth and life-history trait analysis (e.g. Cucherousset et al., 2009; Villeneuve, Copp, Fox, & Stakénas, 2005) and so was not quantitative. In the three English sites, sampling was by catch-per-unit-effort (CPUE) with minnow traps (see Fox, Villeneuve, & Copp, 2011). The data on female growth and life-history traits, but not operculum spot occurrence or size, have been previously published for all these populations (Cucherousset et al., 2009; Fox et al., 2011; Valente et al., 2016; Villeneuve et al., 2005), except the two populations from north-west Poland (Table 1): (i) a former clay pit (Brodowski Pond) of ≈ 0.9 ha (maximum depth of 7 m) situated in Szczecin with a water temperature that ranges from 4°C under thick ice cover in winter to 25°C in summer; and (ii) an artificial channel (4 km long, mean depth of 4.5 m), situated on the lower River Oder near the city of Gryfino that receives heated water effluent from a power plant, resulting in daily water temperature fluctuations of 5–15°C, with maximum temperatures of 30°C in summer and 15°C in winter. There is currently no published study on the North American source populations of pumpkinseed in Europe.

For all populations, pumpkinseed were collected at the beginning of the spawning season (between May and June), immediately killed

with a lethal dose of anaesthetic following licensed procedures in the country of collection and cooled to freezing for subsequent processing in the laboratory. After thawing, specimens were photographed (left side only) using a Nikon Coolpix 5000 camera for morphological analysis. The presence of operculum spots (denoted as present or absent) was recorded, including the rare occurrence of deviations from red (i.e. yellow or orange, or reddish shades thereof). Note that the reflectance spectra, hue or intensity of operculum spots was not measured, nor was the spectral sensitivity of pumpkinseed in the red region of the light spectrum known. The presence of an operculum spot was recorded for both sides of each specimen assessed, but only the left side of the fish (the standard for fish measurements) was used for quantifying the size of the red operculum spot, which was estimated as the surface area of the spot (ImageJ, 1.48v). Fish were measured for standard length (SL, nearest 1 mm) and mass (nearest 1 g) and dissected to determine sex and gonad mass (in mg) when present. GSI was calculated as: $100 \times \text{gonad mass}/\text{total body mass}$ (Wootton & Smith, 2015).

2.1 | Statistical analysis

To categorise males as parentals or cuckolders, a frequency distributions of male GSI were plotted by country. The resulting multimodal GSI frequency distributions were decomposed to normal distributions using the Bhattacharya method (Bhattacharya, 1967), with a

TABLE 2 Mean (SD) pumpkinseed body mass, standard length (SL) and gonado-somatic index (GSI; n/a, not available) among countries of origin and reproductive status. Male reproductive strategy (either cuckolder or parental) was identified by separation of GSI frequency distributions by Bhattacharya's (1967) method.

Country of origin	Reproductive status	n	Mass (g)	SL (mm)	GSI
Belgium	Female	61	8.33 (9.89)	61.3 (16.0)	n/a
	Juvenile	10	3.85 (1.63)	52.5 (6.5)	n/a
	Cuckolder	21	4.81 (2.59)	55.9 (7.3)	3.60 (0.86)
	Parental	24	14.86 (10.22)	75.2 (16.4)	1.26 (0.59)
France	Female	66	17.81 (11.68)	81.1 (18.5)	n/a
	Juvenile	16	1.28 (0.75)	38.9 (6.1)	n/a
	Cuckolder	15	5.03 (2.95)	55.6 (8.9)	4.90 (1.11)
	Parental	91	22.58 (14.21)	88.2 (17.1)	2.00 (0.83)
Netherlands	Female	62	23.77 (23.99)	86.7 (30.2)	n/a
	Juvenile	1	7.00	66.6	n/a
	Cuckolder	33	7.25 (1.16)	66.3 (4.5)	4.25 (1.32)
	Parental	51	36.35 (26.58)	101.3 (30.4)	2.10 (0.63)
Poland	Female	18	20.25 (11.76)	76.4 (14.5)	n/a
	Juvenile	0	n/a	n/a	n/a
	Cuckolder	10	11.01 (7.24)	64.2 (10.3)	4.19 (1.05)
	Parental	39	23.79 (12.42)	80.4 (14.4)	1.26 (0.62)
England	Female	180	14.46 (14.64)	76.5 (19.9)	n/a
	Juvenile	45	2.56 (3.14)	46.2 (13.4)	n/a
	Cuckolder	16	5.69 (7.08)	59.4 (14.4)	6.20 (2.23)
	Parental	165	12.29 (10.04)	76.1 (16.2)	n/a

meaningful separation of normal distributions identified with a separation index (SI) > 2 (Gayanilo, Soriano, & Pauly, 1989). GSI values below the threshold value were categorised as parental and those equal to or above the threshold value were categorised as cuckolders. Data were pooled by country, rather than population, to maximise the precision with which threshold values could be identified.

To test whether parental males were more likely to express a red operculum spot than other males and females, the presence of a red spot in pumpkinseed was modelled using a Bernoulli generalised linear mixed model (GLMM). Fish SL and mass were collinear, and mass was subsequently dropped from the analysis. Only a single fish that was classified as a juvenile expressed a red spot, and juveniles were subsequently dropped from the analysis. The distribution for the model response variable, link function and predictor function was specified as:

$$\begin{aligned} \text{Spot}_{ij} &\sim \text{Binomial}(\pi_{ij}, n_{ij}) \\ E(\text{Spot}_{ij}) &\sim n_{ij} \times \pi_{ij} \text{ and } \text{var}(\text{Spot}_{ij}) = n_{ij} \times \pi_{ij} \times (1 - \pi_{ij}) \\ \eta_{ij} &= \beta_1 + \beta_2 \times \text{SL}_{ij} + \beta_3 \times \text{strategy}_{ij} + \text{population}_i \\ \text{logit}(\pi_{ij}) &= \eta_{ij} \\ \text{population}_j &N(0, \sigma_{\text{population}}^2) \end{aligned}$$

where Spot_{ij} is the probability of fish i in population j having a red operculum spot in n_{ij} independent individuals, which was assumed to follow a binomial distribution with an expected probability (E) of expressing a spot of mean $\pi_i N_i$ and variance $\pi_i \times (1 - \pi_i)$, with a logit link function. The logit function ensures the fitted probability of a red spot falls between 0 and 1. The variable strategy_{ij} is a categorical covariate with three levels, corresponding with fish mating strategy; female, territorial or cuckolder. The model also contained a linear effect for fish SL (SL_{ij}). Exploratory analyses demonstrated differences among populations in the size and expression of red operculum spots. To accommodate this effect in the model, the random intercept population_j was included to introduce a correlation structure between observations for different fish from the same population, with variance $\sigma_{\text{population}}$ distributed normally and equal to 0.

To test whether parental males expressed larger red operculum spots than other males and females, the size of red spots was modelled on a zero-truncated subset of the data using a Gaussian GLMM, specified as:

$$\begin{aligned} \log_{10} \text{Spotsize}_{ij} &\sim N(\mu_{ij}, \sigma^2) \\ E(\log_{10} \text{Spotsize}_{ij}) &= \mu_{ij} \text{ and } \text{var}(\log_{10} \text{Spotsize}_{ij}) = \sigma^2 \\ \mu_{ij} &= \beta_1 + \beta_2 \times \log_{10} \text{SL}_{ij} + \beta_3 \times \text{strategy}_{ij} + \text{population}_i \\ \text{population}_j &N(0, \sigma_{\text{population}}^2) \end{aligned}$$

where $\log_{10} \text{Spotsize}_{ij}$ is the \log_{10} size of red spot on fish i in population j and $\log_{10} \text{SL}_{ij}$ is the \log_{10} standard length of fish i in population j . Expected $\log_{10} \text{Spotsize}_{ij}$ is μ_{ij} with variance σ^2 . Analysis was conducted using the *lme4* library (Bates, Maechler, Bolker, & Walker, 2014) in R (R Core Development Team, 2017). Model residuals were examined to ensure assumptions were met and that effects were adequately accounted for by the model.

3 | RESULTS

Male GSI distributions were multimodal within countries (Figure 1). With the exception of data from the Netherlands, the overlap of normally distributed GSI groups was low; SI was always >2 and thereby provided meaningful separation of modal groups, even for the Netherlands specimens (separation index = 2.68). Decomposition of GSI distributions showed the threshold GSI value that distinguished parental and cuckolder males varied among countries, from 2.3 (Poland) to 4.2 (England) (Table 2, Figure 1). In Belgian, French and Polish populations, separation of male strategies was ambiguous, with Bhattacharya decomposition revealing two thresholds within these data (Figure 1). In each case, the higher threshold was used as the cut-off between male strategies.

The probability of expressing a red operculum spot was predicted by male SL, with a significantly greater likelihood of a red spot in larger males (Table 3; Figure 2). The probability of a red operculum spot was also predicted by sex, with both cuckolder and territorial males significantly more likely to express a red spot than females (Table 3; Figure 2). Among pumpkinseed that expressed a red spot, there was a significantly stronger interaction between SL and spot size in males than in females (Table 4; Figure 3), although there was no difference between male strategies classified by GSI (Figure 3). Two specimens were observed to possess a yellow operculum spot, a mature female from Schoapedobbe Pond and a male from Brière Marsh (Table 1).

4 | DISCUSSION

Our analysis of introduced pumpkinseed populations across Europe demonstrated that the expression of a red operculum spot was more likely in mature males than females (Table 3), whereas only a single sexually immature juvenile expressed a red spot. Further, a significantly stronger interaction was observed (Table 4) between red spot size and body size in parental and cuckolder males than in females. Taken together, these findings indicate that in pumpkinseed, the presence and size of a red operculum spot signal sexual maturity and correlate strongly with body size in males. This signal, however, does not distinguish between the male mating strategies of parental and cuckolder.

Colour signals can have multiple functions, potentially conveying information to rivals, mates or both. In pumpkinseed, the function of the red operculum spot is equivocal. The present findings are consistent with previous studies in demonstrating that a red spot has a potential role in male–male interactions by eliciting an aggressive response by males (Stacey & Chiszar, 1978). Notably, during aggressive intrasexual contests, male sunfishes flare their opercula (Colgan & Gross, 1977), displaying the red spot to a rival facing them head-on. Our results support a role for the red operculum spot in male contests, with the expression of a red spot primarily associated with sexually mature males and correlating with body size. However, this finding contradicts our prediction that parental males, which

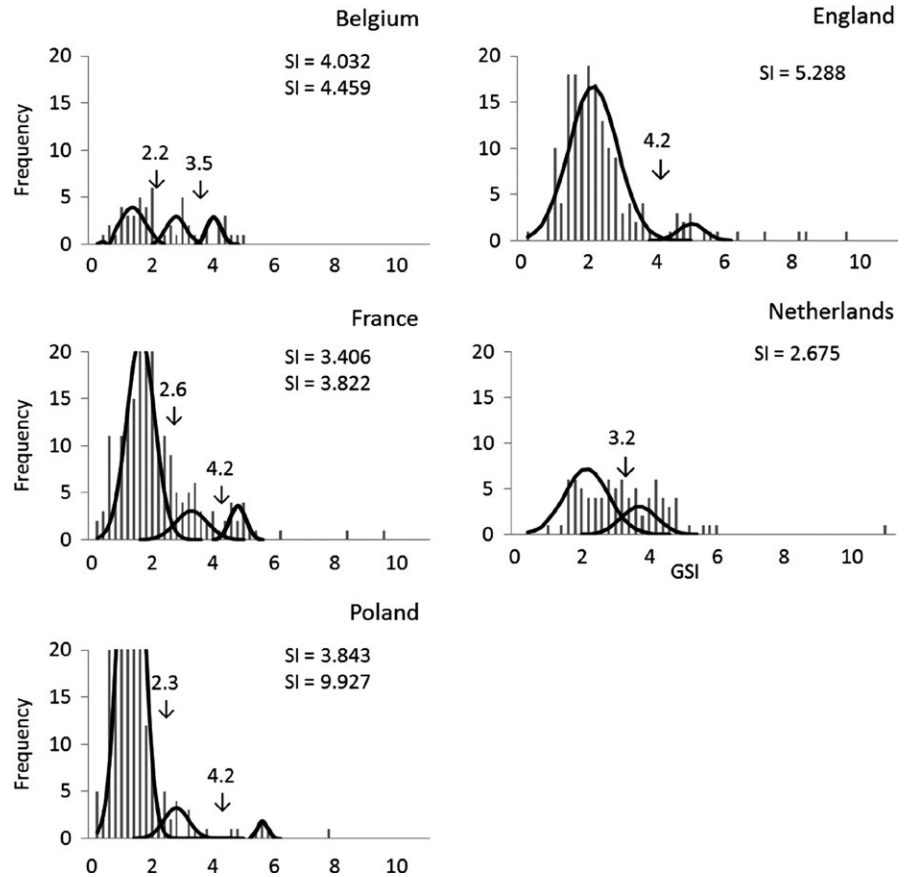


FIGURE 1 Observed gonadosomatic index (GSI): frequency plots for pumpkinseed among country of origin with Gaussian distribution curves and separation indices (SI), generated using Bhattacharyya's (1967) method. Threshold GSI values for separating frequency distributions for male mating strategies are indicated with arrows

TABLE 3 Summary of Bernoulli GLMM to model the probability of pumpkinseed expressing a red operculum spot. SL, standard length; Fish from different populations were fitted as random intercepts, with standard deviation of 1.47. $N_{\text{obs}} = 897$.

Model parameter	Estimate	SE	<i>p</i>
Intercept _(female)	-8.02	0.80	<.001
SL	0.08	0.01	<.001
Strategy _(cuckolder)	2.30	0.34	<.001
Strategy _(territorial)	2.35	0.23	<.001

experience intrasexual contests and intersexual mate choice, should be more likely than cuckolder males to express a red operculum spot.

The expression of a red carotenoid-based colour signal may convey information about male condition, which could be functional in the context of male–male contests. Carotenoids are acquired solely in the diet by vertebrates and these compounds have a number of critical physiological functions, making carotenoid-based signals a potentially reliable indication of individual condition and fighting ability (Olson & Owens, 1998). Whether additional information is conveyed by the size, reflectance spectra and intensity of the red colour spot, or whether the red operculum spot comprises one component of a multimodal signal in pumpkinseed, remains to be investigated. Experimental studies would be particularly effective in differentiating these aspects of signal evolution in pumpkinseed.

Surprisingly, we detected no significant difference between parental and cuckolder males, either in the probability of expressing a red spot or in the strength of the relationship between red spot size and male size. Alternative mating strategies in fish, which are exclusively shown by males in teleost fishes, are reflected most strikingly in reproductive behaviour, but also in external appearance and physiology. In contrast to Gross' (1982) interpretation of his sunfish model to a single native population of pumpkinseed, our analysis of non-native populations in Europe suggests that, while a clear distinction can be seen between the parental and cuckolder male reproductive strategies in relative testes size, the GSI value threshold that distinguishes parental and cuckolder males varies among populations, ranging from 2.3 (Poland) to 4.2 (England).

A possible alternative explanation for the observed range of GSI values may relate to variation in pumpkinseed population density, which has consequences for the reproductive success of cuckolder males. In water bodies where the populations occurred at high density, traits associated with sperm competition, including large testes size, are favoured, thereby potentially selecting for relatively larger gonads (high GSI) in both parental and cuckolder males, with a consequent upward shift in the threshold between them. In the three ponds in England for which quantitative density data were available (Fox et al., 2011), pumpkinseed was virtually the only species present in the two ponds where the species have been observed in high density and males in these ponds had higher mean and maximum male GSI values than observed in the

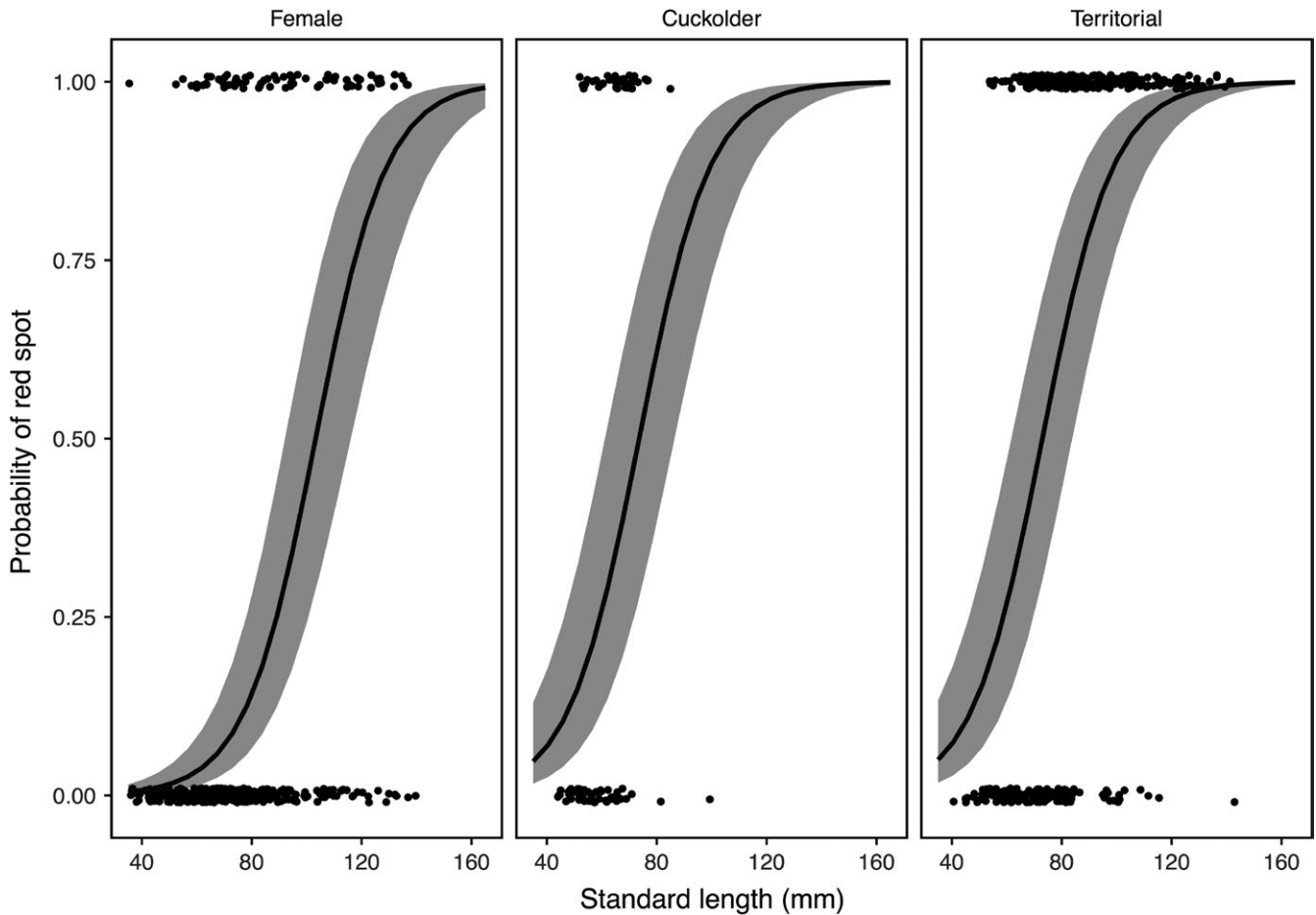


FIGURE 2 Mean fitted probability (solid line) of pumpkinseed expressing a red operculum spot as a function of standard length (SL in mm) with 95% confidence intervals (shaded area) for females, cuckolder males and parental males. Data were modelled with a Bernoulli GLMM. Fish from different populations were fitted as random intercepts in the model

low-density pond. In water bodies where populations occur at low densities, where the risk of sperm competition for parental males is low, relative testes size is predicted to be smaller and the GSI threshold between parental and cuckolder males lower (Wootton & Smith, 2015).

A caveat to these conclusions was the finding that many females also expressed red spots in their opercula, albeit with lower probability than males (Figure 2). The size of red operculum spots in females also conveyed less information about size than in males (Figure 3). If red operculum spots function primarily in male–male contests, then it is unclear why females would display this trait. For example, the red operculum spot may have multiple functions, potentially signalling male size and fighting ability, but also undergoing intersexual selection in the context of mate choice. If the red operculum spot is selected through intersexual selection, then it is possible that females express the trait for the same reason as males. Female ornamentation is surprisingly common in teleosts, and in the context of the pumpkinseed mating system, in which males are the sole providers of parental care, the evolution of male mate choice and female ornamentation is possible (Wootton & Smith, 2015).

TABLE 4 Summary of a Gaussian GLMM to model the size of pumpkinseed red operculum spots. Fish from different populations were fitted as random intercepts, with standard deviation of 0.24. $N_{\text{obs}} = 344$

Model parameter	Estimate	SE	<i>p</i>
Intercept _(female)	-7.43	0.99	<.001
Log ₁₀ SL	1.82	0.22	<.001
Strategy _(cuckolder)	-5.31	2.77	.055
Strategy _(territorial)	-4.38	1.06	<.001
Log ₁₀ SL × Strategy _(cuckolder)	1.40	0.66	.033
Log ₁₀ SL × Strategy _(territorial)	1.18	0.24	<.001

SL, standard length.

The expression of red operculum spots in males is adaptive solely through intrasexual selection, whereas in females, the expression of red spots may be through a genetic correlated response. In threespine stickleback (*Gasterosteus aculeatus*), red throat colouration is known to function in intrasexual and intersexual selection on males and is

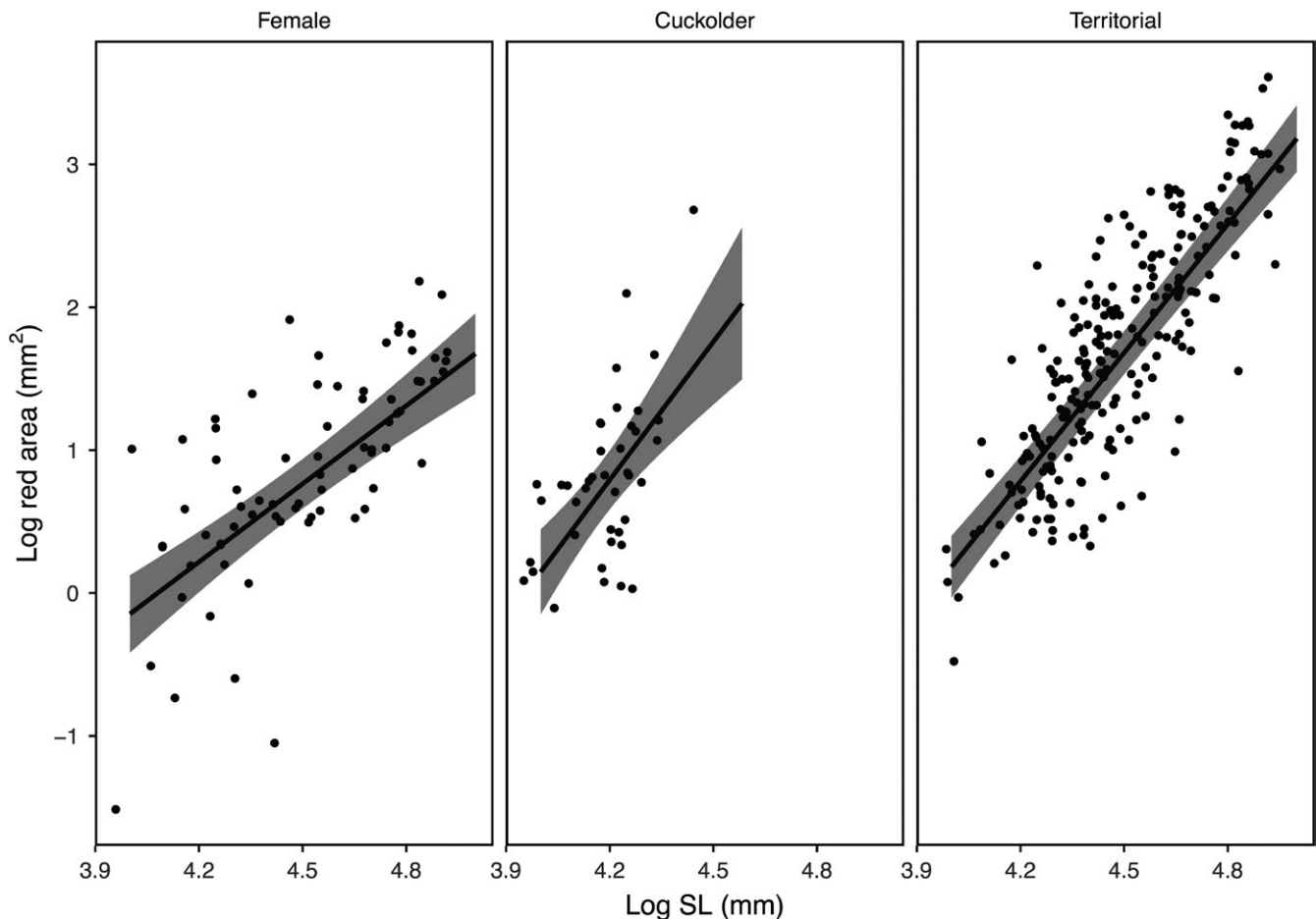


FIGURE 3 Mean fitted Log_{10} area (mm^2) of red operculum spot (solid line) of pumpkinseed as a function of log_{10} standard length (SL in mm) with 95% confidence intervals (shaded area) for females, cuckolder males and parental males fitted with a Gaussian GLMM. Fish from different populations were fitted as random intercepts in the model

a reliable indicator of body condition and parasite resistance (Barber, Arnott, Braithwaite, Andrew, & Huntingford, 2001; Milinski & Bakker, 1990). The red ornament, which has a strong genetic component, is also found in females and its expression in females is consistent with pleiotropy (Yong, Peichel, & McKinnon, 2016). Pleiotropy may also mediate the expression of a red operculum spot in female pumpkinseed, and possibly also in cuckolder males, although this conclusion relies on there being: (i) a shared genetic basis to red spots in males and females and (ii) no adaptive value in expressing a red spot in females and cuckolders. These assumptions remain to be tested.

The presence of two male GSI thresholds in pumpkinseed populations in Belgium, France and Poland provides evidence that, contrary to Gross (1982), both the satellite and sneaker strategies may be present in some non-native European pumpkinseed populations (Figure 1). Alternatively, these findings may simply reflect the greater heterogeneity in age at maturity within some European populations (Copp & Fox, 2007; Fox & Copp, 2014), which is a possible outcome of multiple sources of introduction or adaptation to conditions outside the natural range of the species. Behavioural studies, complemented with morphological analyses, are needed to clarify the existence of a satellite strategy in European pumpkinseed populations. Similarly, variation in predation

among populations has the capacity to modify the selective landscape for red operculum spots, as sexual selection for visual signals can be limited through natural selection by predators in teleosts (Endler, 1980; Wootton & Smith, 2015). Although outside the scope of the present study, the effect of selective predation on pumpkinseed in response to the expression of the red operculum spot is not known and warrants further research.

In conclusion, the present study demonstrates that the presence of a red operculum spot in pumpkinseed is associated with sexual maturation, primarily in males. The size of red spot correlates with body size in parental and cuckolder males, although there was no difference in the presence or relative size of the red operculum spot between male mating strategies. The observed GSI distributions suggest the possible existence of the satellite mating strategy in some European populations, but alternatively, this may be due to variations in pumpkinseed population density or to selective predation. The function, if any, of a red operculum spot in females is not clear but is consistent with pleiotropy.

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AUTHOR CONTRIBUTION

The study was conceived by GHC and MGF. Specimens were collected and processed by GHC, GZ, MGF, SY, EZ, GM, JC, HV and HHvK. Data were analysed by GHC, SY, GZ, MP and CS. The initial draft of the manuscript was prepared by SY, EZ, GZ and GHC, and then extensively revised by GZ, CS and MP, with co-author contributions.

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