

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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Grzegorz Zięba, Carl Smith, Michael Fox, Stan Yavno, Eva Záhorská, et al.. Red operculum spots, body size, maturation and evidence for a satellite male phenotype in non-native European populations of pumpkinseed Lepomis gibbosus. Ecology of Freshwater Fish, 2018, 27 (3), pp.874-883. 10.1111/eff.12399 hal-03210212

HAL Id: hal-03210212 https://hal.univ-lorraine.fr/hal-03210212

Submitted on 29 Apr 2021

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1 Red operculum spots, body size, maturation and evidence for a satellite

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- 3 Lepomis gibbosus
- 4
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- 36 **Running title:** Operculum spot of non-native pumpkinseed
- 37

38 Abstract

39 Carotenoid-based pigmentation is a striking feature of many taxa, yet the function, if any, 40 of colour traits is often unclear. Pumpkinseed Lepomis gibbosus, a widely introduced 41 freshwater sunfish that exhibits alternative male mating strategies, express a striking, red 42 operculum spot. To investigate the potential function of this red spot as a signal in this 43 species' mating system, we determined the presence and measured the size of red 44 operculum spots in fish collected from 12 populations in five European countries in which 45 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 46 47 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 48 49 associated with sexual maturation, with GSI frequency distributions suggesting that 50 cuckolders in some non-native populations comprised both sneaker and satellite males, 51 the latter not having previously been reported for this species. Further, the size of red spot 52 correlated strongly with body size in parental and cuckolder males, though there was no 53 difference in the presence or size of the red operculum spot between male mating 54 strategies. The function of a red operculum spot in females is not clear but may be partly 55 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. 56 57

58 Keywords alternative mating strategy · carotenoid · GLMM · sexual selection · visual
 59 signal · Centrarchidae

60 1 | INTRODUCTION

Visual signals play a key role in the environmental biology of teleost fishes (Marshall, 61 62 2000; Wootton, 1998). Pigmentation is an important component of visual signals in fishes, 63 both within and among species. The expression of colour, which often comprises multicomponent signals, involves pigment-based and structural colours (Price, Weadick, Shim, 64 65 & Rodd, 2008; Wootton & Smith, 2015). Carotenoid-based colouration, which typically 66 appears as yellow, orange or red, has been of particular interest because these are costly 67 to produce and thereby serve as a reliable indication of individual condition and foraging 68 ability (Olson & Owens, 1998), with a potential role in mating system evolution (Wootton 69 & Smith, 2015). Different carotenoid pigments are responsible for conveying different 70 information to potential mates or rivals (McLennan, 2007). Colour traits can function in 71 sexual selection; selection that acts on differences in fitness among individuals that arise 72 due to the number and identity of their mates. Because sexual selection tends to act more 73 strongly on males than females (Andersson, 1994), it is males that usually express the 74 brightest and most elaborate visual traits, including nuptial colouration. Though a broad 75 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). 76 77 Understanding the adaptive value of mate choice (sensu Kokko, Brooks, Jennions & 78 Morley, 2003) based on visual signals is a controversial field of research, but may arise 79 through fitness benefits associated with mate choice, or an arbitrary mechanism 80 (Andersson, 1994; Wootton & Smith, 2015).

81 Pigmentation in North American sunfishes (Centrarchidae), in common with most other 82 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 83 84 associated with male aggression (Stacey & Chiszar, 1978). In aquarium studies, breeding 85 males were shown to attack fish models that intruded into their nesting area, and models 86 with red colour on the operculum spot or iris received more attacks and aggressive 87 displays than those without colour (Stacey & Chiszar, 1978). It is recognised that 88 dominance relationships among sunfishes are related to colouration, with dominant, 89 brightly-coloured parental (territorial) pumpkinseed males building and defending nests. 90 and performing courtship behaviour to attract females to their nest for spawning. Male 91 sunfishes also adopt alternative mating strategies (sensu Wootton, 1984), whereby sub-92 dominant 'cuckolder' males attempt to intrude into the nest of a parental male that is in 93 the act of spawning (Gross, 1979, 1982). Cuckolder males are early-maturing, do not

94 perform courtship and avoid fitness costs associated with parental care (Smith & Wootton, 95 1995). Small cuckolders, typically possessing disproportionately large testes, are referred 96 to as 'sneakers' due to their rapid entry into the nest of a spawning territorial male to 97 release sperm at the moment of spawning. Large cuckolders, which have testes of 98 intermediate size relative to parental and sneaker males, are termed 'satellites' and 99 participate in spawning by mimicking female characteristics and entering the nest of a 100 territorial male as a potential mate (Gross, 1982; Garner & Neff, 2013). Despite aggressive 101 defence of their nest by parental males, cuckolder males frequently intrude successfully 102 during spawning events (Rios-Cardenas, 2003). In the close congener, bluegill Lepomis 103 macrochirus, a model taxon for research on alternative reproductive strategies (Taborsky, 104 2008), Gross (1982) characterised two cuckolder phenotypes, based on a population in 105 Lake Opinicon, Canada. Sneaker bluegill are small and have relatively large testes relative 106 to parental males, with a mean gonado-somatic index (GSI) of 4.6 (contrasting with 107 parental males with a mean GSI of 1.1). In contrast, satellite male bluegill are larger than 108 sneakers, with GSI values (mean 3.3) that are intermediate between those of parental 109 males and sneakers. In applying these criteria to pumpkinseed from the same lake, Gross 110 (1982) identified the sneaker phenotype, but not that of the satellite. In a subsequent 111 paternity study, Neff and Clare (2008) noted that the satellite strategy had yet to be 112 reported for pumpkinseed. In a subsequent genetic study of bluegill-pumpkinseed 113 hybridisation, Garner and Neff (2013) did not distinguish between satellites and sneakers, 114 referring to them collectively as cuckolders. Thus, there are no reports that the satellite 115 phenotype (sensu Gross, 1982) is expressed in pumpkinseed and hereafter we use the 116 term 'cuckolder' to refer to the alternative male mating strategy in pumpkinseed to the 117 parental strategy.

118 The approach, onset and end of the reproductive period in fishes can often be identified 119 by variation in GSI, the relationship between gonad weight and somatic weight (Wootton, 120 1998). Male mating polymorphisms, particularly the cuckolder strategy, are associated 121 with sperm competition, which occurs when the spermatozoa of different males compete 122 for the same eggs (Parker, 1970). Males expressing alternative mating strategies tend to 123 experience a higher "risk" of sperm competition; i.e. an elevated probability that their 124 sperm will compete with the sperm of other males, because they usually mate in the 125 presence of at least one competitor, typically the territorial male and often other cuckolder 126 males (Wedell, Gage, & Parker, 2002; Wootton & Smith, 2015). An outcome is that 127 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.

131 Pumpkinseed have been widely established outside their native North American range. 132 Most introductions have been in Europe, where the species was introduced in the late 19th 133 and early 20th centuries, establishing populations in at least 28 countries (Copp & Fox, 134 2007). Most of the research undertaken on pumpkinseed in Europe has focused on female 135 growth and life-history traits (reviewed in Fox & Copp, 2014), with relatively few studies 136 on males (e.g. Almeida, Tobes, Miranda, & Copp, 2012; Valente et al., 2016). In its native 137 range, Neff & Clare (2008) observed temporal variability in the patterns of pumpkinseed 138 paternity and suggested that these reflect temporal variation in mating opportunities, 139 parental male defence ability, or cuckolder densities. Given this temporal variability, 140 spatial variations are also feasible, possibly extending to visual signals, such as the 141 operculum spot, which is assumed to be important for males for attracting mates (Stacey 142 & Chiszar, 1978).

143 The aim of the present study was to determine whether the presence and size of the 144 red operculum spot serves as a signal of sex and/or mating strategy in pumpkinseed. To 145 do this we used estimates of GSI to categorise males as nesters or cuckolders and fitted 146 models to test whether spot presence and size varied between the sexes and between 147 males adopting different reproductive strategies. We predicted that parental males, which 148 experience intrasexual contests and intersexual mate choice, would be more likely than 149 cuckolder males and females to express a red operculum spot and that these males would 150 also express larger red spots.

151

152 **2** | Methods

153 Previously unpublished data for 1118 pumpkinseed were obtained from established non-154 native populations in Belgium, England, France, the Netherlands and Poland (Table 1), 155 with all data elements being available for 924 specimens of which 465 were males, 387 156 were females, and 266 were juveniles (Table 2). The fish were collected by a variety of 157 sampling gear (Table 1), depending upon location characteristics and gear availability. 158 Note that sampling of the two Netherlands populations was incorrectly reported as by 159 funnel trap in Cucherousset et al. (2009). Where possible, the sampling aimed to obtain \approx 160 100 specimens for growth and life-history trait analysis (e.g. Villeneuve, Copp, Fox, & 161 Stakenas, 2005; Cucherousset et al., 2009) and so was not quantitative. In the three

162 English sites, sampling was by catch-per-unit-effort (CPUE) with minnow traps (see Fox, 163 Villeneuve & Copp, 2011). The data on female growth and life-history traits, but not 164 operculum spot occurrence or size, have been previously published for all these 165 populations (Villeneuve et al., 2005; Cucherousset et al., 2009; Fox et al., 2011; Valente 166 et al., 2016), except the two populations from northwest Poland (Table 1): i) a former clay 167 pit (Brodowski Pond) of ≈ 0.9 ha (maximum depth of 7 m) situated in Szczecin with a water 168 temperature that ranges from 4 °C under thick ice cover in winter to 25 °C in summer; and 169 ii) an artificial channel (4 km long, mean depth of 4.5 m), situated on the lower River Oder 170 near the city of Gryfino that receives heated water effluent from a power plant, resulting 171 in daily water temperature fluctuations of 5–15 °C, with maximum temperatures of 30 °C 172 in summer and 15 °C in winter. There is currently no published study on the North 173 American source populations of pumpkinseed in Europe.

174 For all populations, pumpkinseed were collected at the beginning of the spawning 175 season (between May and June), immediately killed with a lethal dose of anaesthetic 176 following licenced procedures in the country of collection and cooled to freezing for 177 subsequent processing in the laboratory. After thawing, specimens were photographed 178 (left side only) using a Nikon CoolPix 5000 camera for morphological analysis. The 179 presence of operculum spots (denoted as present or absent) was recorded, including the 180 rare occurrence of deviations from red (i.e. yellow or orange, or reddish shades thereof). 181 Note that the reflectance spectra, hue or intensity of operculum spots were not measured, 182 nor was the spectral sensitivity of pumpkinseed in the red region of the light spectrum 183 known. The presence of an operculum spot was recorded for both sides of each specimen 184 assessed, but only the left side of the fish (the standard for fish measurements) was used 185 for quantifying the size of the red operculum spot, which was estimated as the surface 186 area of the spot (ImageJ, 1.48v). Fish were measured for standard length (SL, nearest 1 187 mm) and mass (nearest 1 g) and dissected to determine sex and gonad mass (in mg) 188 when present. GSI was calculated as: 100 × gonad mass ÷ total body mass) (Wootton & 189 Smith, 2015).

190

191 **2.1 | Statistical analysis**

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

207 Spot_{ij} ~ Binomial(π_{ij} , n_{ij})

208 $E(Spot_{ij}) \sim n_{ij} \ge \pi_{ij}$ and $var(Spot_{ij}) = n_{ij} \ge \pi_{ij} \ge (1 - \pi_{ij})$

209
$$\eta_{ij} = \beta_1 + \beta_2 \times SL_{ij} + \beta_3 \times \text{strategy}_{ij} + population_i$$

210 logit
$$(\pi_{ij}) = \eta_{ij}$$

211
$$population_j \sim N(0, \sigma_{population}^2)$$

Where Spot_{ii} is the probability of fish *i* in population *j* having a red operculum spot in n_{ij} 212 213 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 214 logit link function. The logit function ensures the fitted probability of a red spot falls 215 216 between 0 and 1. The variable strategy_{ii} is a categorical covariate with three levels, 217 corresponding with fish mating strategy; female, territorial or cuckolder. The model also 218 contained a linear effect for fish SL (SLii). Exploratory analyses demonstrated differences 219 among populations in the size and expression of red operculum spots. To accommodate 220 this effect in the model, the random intercept *population* was included to introduce a 221 correlation structure between observations for different fish from the same population, with 222 variance $\sigma_{population}$ 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:

226
$$\log_{10}$$
Spotsize_{ij} ~ $N(\mu_{ij}, \sigma^2)$

227
$$E(log_{10}Spotsize_{ij}) = \mu_{ij}$$
 and $var(log_{10}Spotsize_{ij}) = \sigma^2$

- 228 $\mu_{ij} = \beta_1 + \beta_2 \times \log_{10} SL_{ij} \times \beta_3 \times strategy_{ij} + population_i$
- 229 $population_j \sim N(0, \sigma_{population}^2)$

Where $log_{10}Spotsize_{ij}$ is the log_{10} size of red spot on fish *i* in population *j* and $log_{10}SL_{ij}$ is the log_{10} standard length of fish *i* in population *j*. Expected $log_{10}Spotsize_{ij}$ is μ_{ij} with variance σ^2 . Analysis was conducted using the *Ime4* library (Bates, Maechler, Bolker, & Walker, 2014) in R (R Development Core Team, 2017). Model residuals were examined to ensure assumptions were met and that effects were adequately accounted for by the model.

236

237 3 | RESULTS

238 Male GSI distributions were multimodal within countries (Figure 1). With the exception of 239 data from the Netherlands, the overlap of normally-distributed GSI groups was low; SI was 240 always >2 and thereby provided meaningful separation of modal groups, even for the 241 Netherlands specimens (separation index = 2.68). Decomposition of GSI distributions 242 showed the threshold GSI value that distinguished parental and cuckolder males varied 243 among countries, from 2.3 (Poland) to 4.2 (England) (Table 2, Figure 1). In Belgian, French 244 and Polish populations, separation of male strategies was ambiguous, with Bhattacharya 245 decomposition revealing two thresholds within these data (Figure 1). In each case, the 246 higher threshold was used as the cut-off between male strategies.

247 The probability of expressing a red operculum spot was predicted by male SL, with a 248 significantly greater likelihood of a red spot in larger males (Table 3; Figure 2). The 249 probability of a red operculum spot was also predicted by sex, with both cuckolder and 250 territorial males significantly more likely to express a red spot than females (Table 3; 251 Figure 2). Among pumpkinseed that expressed a red spot, there was a significantly 252 stronger interaction between SL and spot size in males than in females (Table 4; Figure 253 3), though there was no difference between male strategies classified by GSI (Figure 3). 254 Two specimens were observed to possess a yellow operculum spot, a mature female from 255 Schoapedobbe Pond and a male from Brière Marsh (Table 1).

256

257 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 signals sexual maturity and correlates 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, 266 267 mates or both. In pumpkinseed, the function of the red operculum spot is equivocal. The 268 present findings are consistent with previous studies in demonstrating that a red spot has 269 a potential role in male-male interactions by eliciting an aggressive response by males 270 (Stacey & Chiszar, 1978). Notably, during aggressive intra-sexual contests, male 271 sunfishes flare their opercula (Colgan & Gross, 1977), displaying the red spot to a rival 272 facing them head-on. Our results support a role for the red operculum spot in male 273 contests, with the expression of a red spot primarily associated with sexually mature males 274 and correlating with body size. However, this finding contradicts our prediction that 275 parental males, which experience intrasexual contests and intersexual mate choice, 276 should be more likely than cuckolder males to express a red operculum spot.

277 The expression of a red carotenoid-based colour signal may convey information about 278 male condition, which could be functional in the context of male-male contests. 279 Carotenoids are acquired solely in the diet by vertebrates and these compounds have a 280 number of critical physiological functions, making carotenoid-based signals a potentially 281 reliable indication of individual condition and fighting ability (Olson & Owens, 1998). 282 Whether additional information is conveyed by the size, reflectance spectra and intensity 283 of the red colour spot, or whether the red operculum spot comprises one component of a 284 multimodal signal in pumpkinseed, remains to be investigated. Experimental studies 285 would be particularly effective in differentiating these aspects of signal evolution in 286 pumpkinseed.

287 Surprisingly, we detected no significant difference between parental and cuckolder 288 males, either in the probability of expressing a red spot, or in the strength of the 289 relationship between red spot size and male size. Alternative mating strategies in fish, 290 which are exclusively shown by males in teleost fishes, are reflected most strikingly in 291 reproductive behaviour, but also in external appearance and physiology. In contrast to 292 Gross' (1982) interpretation of his sunfish model to a single native population of 293 pumpkinseed, our analysis of non-native populations in Europe suggests that, while a 294 clear distinction can be seen between the parental and cuckolder male reproductive 295 strategies in relative testes size, the GSI value threshold that distinguishes parental and 296 cuckolder males varies among populations, ranging from 2.3 (Poland) to 4.2 (England).

297 A possible alternative explanation for the observed range of GSI values may relate to 298 variation in pumpkinseed population density, which has consequences for the 299 reproductive success of cuckolder males. In water bodies where the populations occurred 300 at high density, traits associated with sperm competition, including large testes size, are 301 favoured, thereby potentially selecting for relatively larger gonads (high GSI) in both 302 parental and cuckolder males, with a consequent upward shift in the threshold between 303 them. In the three ponds in England for which quantitative density data were available 304 (Fox et al., 2011), pumpkinseed was virtually the only species present in the two ponds 305 where the species have been observed in high density and males in these ponds had 306 higher mean and maximum male GSI values than observed in the low-density pond. In 307 water bodies where populations occur at low densities, where the risk of sperm 308 competition for parental males is low, relative testes size is predicted to be smaller and 309 the GSI threshold between parental and cuckolder males lower (Wootton & Smith 2015).

310 A caveat to these conclusions was the finding that many females also expressed red 311 spots in their opercula, albeit with lower probability than males (Figure 2). The size of red 312 operculum spots in females also conveyed less information about size than in males 313 (Figure 3). If red operculum spots function primarily in male-male contests, then it is 314 unclear why females would display this trait. For example, the red operculum spot may 315 have multiple functions, potentially signalling male size and fighting ability, but also 316 undergoing intersexual selection in the context of mate choice. If the red operculum spot 317 is selected through intersexual selection, then it is possible that females express the trait 318 for the same reason as males. Female ornamentation is surprisingly common in teleosts, 319 and in the context of the pumpkinseed mating system, in which males are the sole 320 providers of parental care, the evolution of male mate choice and female ornamentation 321 is possible (Wootton & Smith 2015).

322 The expression of red operculum spots in males is adaptive solely through intra-sexual 323 selection, whereas in females the expression of red spots may be through a genetic 324 correlated response. In threespine stickleback (Gasterosteus aculeatus), red throat 325 colouration is known to function in intra- and intersexual selection on males and is a 326 reliable indicator of body condition and parasite resistance (Milinski & Bakker, 1990; 327 Barber, Arnott, Braithwaite, Andrew, & Huntingford, 2001). The red ornament, which has 328 a strong genetic component, is also found in females and its expression in females is 329 consistent with pleiotropy (Yong, Peichel & McKinnon, 2016). Pleiotropy may also mediate 330 the expression of a red operculum spot in female pumpkinseed, and possibly also in

cuckolder males, though this conclusion relies on there being a shared genetic basis to
 red spots in males and females and no adaptive value in expressing a red spot in females
 and cuckolders, and these assumptions remain to be tested.

334 The presence of two male GSI thresholds in pumpkinseed populations in Belgium, 335 France and Poland provides evidence that, contrary to Gross, (1982), both the satellite 336 and sneaker strategies may be present in some non-native European pumpkinseed 337 populations (Figure 1). Alternatively, these findings may simply reflect the greater 338 heterogeneity in age at maturity within some European populations (Copp & Fox, 2007; 339 Fox & Copp, 2014), which is a possible outcome of multiple sources of introduction or 340 adaptation to conditions outside the natural range of the species. Behavioural studies, 341 complemented with morphological analyses, are needed to clarify the existence of a 342 satellite strategy in European pumpkinseed populations. Similarly, variation in predation 343 among populations has the capacity to modify the selective landscape for red operculum 344 spots, since sexual selection for visual signals can be limited through natural selection by 345 predators in teleosts (Endler, 1980; Wootton & Smith, 2015). Although outside the scope 346 of the present study, the effect of selective predation on pumpkinseed in response to the 347 expression of the red operculum spot is not known and warrants further research.

348 In conclusion, the present study demonstrates that the presence of a red operculum 349 spot in pumpkinseed is associated with sexual maturation, primarily in males. The size of 350 red spot correlates with body size in parental and cuckolder males, although there was no 351 difference in the presence or relative size of the red operculum spot between male mating 352 strategies. The observed GSI distributions suggest the possible existence of the satellite 353 mating strategy in some European populations, but alternatively this may be due to 354 variations in pumpkinseed population density or to selective predation. The function, if 355 any, of a red operculum spot in females is not clear but is consistent with pleiotropy.

356

357 ACKNOWLEDGEMENTS

This study, which derives from work initiated as part of a N.A.T.O. Collaborative Linkage Grant (LST-CLG No. 979499), supported by consecutive research contracts (SF0238 and SF0248) from the UK Department of Environment, Food and Rural Affairs (to GHC), which were complemented by a Marie Curie post-doctoral fellowship (PIEF-GA-2008-219707) and a subsequent research grant from the National Science Centre, Poland (decision No DEC-2011/01/D/NZ8/01807) to GZ. We thank two anonymous reviewers for their constructive and perceptive comments. 365

366 Author contribution

367 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,

369 GZ, MP and CS. The initial draft of the manuscript was prepared by SY, EZ, GZ and GHC,

- then extensively revised by GZ, CS and MP, with co-author contributions.
- 371

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373 **REFERENCES**

Almeida, D., Tobes, I., Miranda R., & Copp G. H. (2012). Cuckoldry features of introduced

375 pumpkinseed sunfish (*Lepomis gibbosus*) in contrasting environmental conditions in

- 376 southern Europe. Canadian Journal of Zoology, 90, 1051–1057.
 377 https://doi.org/10.1139/z2012-073
- 378 Andersson, M. (1994). Sexual Selection. Princeton University Press, Princeton.
- Barber, I., Arnott, S. A., Braithwaite, V. A., Andrew, J., & Huntingford, F. A. (2001). Indirect
 fitness consequences of mate choice in sticklebacks: offspring of brighter males grow

381 slowly but resist parasitic infections. *Proceedings of the Royal Society of London Series*

382 *B-Biological Sciences*, 268(1462), 71–76. https://doi.org/10.1098/rspb.2000.1331

- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). Ime4: Linear mixed-effects
 models using Eigen and S4. R package version *1*(7), 1–23.
- Bhattacharya, C. G. (1967). A simple method of resolution of a distribution into Gaussian
 components. *Biometrics*, 23, 115–135. https://doi.org/10.2307/2528285
- Colgan, P. W., & Gross M. R. (1977). Dynamics of aggression in male pumpkinseed
 sunfish (*Lepomis gibbosus*) over the reproductive phase. *Zeitschrift für Tierpsychologie*, 43, 139–151. https://doi.org/10.1111/j.1439-0310.1977.tb00064.x

390 Copp, G. H., & Fox, M. G. (2007). Growth and life history traits of introduced pumpkinseed

391 (Lepomis gibbosus) in Europe, and the relevance to its potential invasiveness. In F.

392 Gherardi (Ed.), *Biological Invaders in Inland Waters: Profiles, Distribution, and Threats*,

393 (pp. 289–306). Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-6029-8_15

Cucherousset, J., Copp, G. H., Fox, M. G., Sterud, E., Van Kleef, H. H., Verreycken, H.,
 & Záhorská, E. (2009). Life-history traits and potential invasiveness of introduced

pumpkinseed *Lepomis gibbosus* populations in northwestern Europe. *Biological Invasions*, *11*, 2171–2180. https://doi.org/10.1007/s10530-009-9493-5

- Endler, J. A. (1980). Natural selection on color pattern in *Poecilia reticulata. Evolution*, 34,
 76–91.
- Fox, M. G., & Copp, G. H. (2014). Old world versus new world: life-history alterations in a
 successful invader introduced across Europe. *Oecologia*, *174*, 435–446.
 https://doi.org/10.1007/s00442-013-2776-7
- Fox, M. G., Villeneuve, F., & Copp, G. H. (2011). Seasonal reproductive allocation, localscale variation and environmental influences on life history traits of introduced
 pumpkinseed (*Lepomis gibbosus*) in Southern England. *Fundamental and Applied Limnology*, *178*, 231–243. https://doi.org/10.1127/1863-9135/2011/0178-0231
- Garner, S. R., & Neff, B. D. (2013). Alternative male reproductive tactics drive
 asymmetrical hybridization between sunfishes (*Lepomis* spp.). *Biology Letters*, 9(6),
 20130658. https://doi.org/10.1098/rsbl.2013.0658
- Gayanilo, F. C., Soriano, M., & Pauly, D. (1989). A draft guide to the Complete ELEFAN.
 International Center for Living Aquatic Resources Management, Manila.
 (www.worldfishcenter.org/content/draft-guide-compleat-elefan).
- Gross, M. R. (1979). Cuckoldry in sunfishes (*Lepomis*: Centrarchidae). *Canadian Journal*of Zoology, 57, 1507–1509. https://doi.org/10.1139/z79-197
- Gross, M. R. (1982). Sneakers, satellites and parentals: polymorphic mating strategies in
 North American sunfishes. *Zeitschrift für Tierpsychologie*, 60, 1–26.
 https://doi.org/10.1111/j.1439-0310.1982.tb01073.x
- Kokko H., Brooks, R., Jennions, M. D., & Morley, J. (2003) The evolution of mate choices
 and mating biases. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 270(1515), 653–664. https://doi.org/10.1098/rspb.2002.2235
- 421 Marshall, N. J. (2000). The visual ecology of reef fish colours. In Y. Espmark, T. Amundsen
- 422 & G. Rosenqvist (Eds.), *Signaling and Signal Design in Animal Communication*, (pp.
 423 83–120). Tapir Academic Press, Trondheim.
- 424 McLennan D. A. (2007) The umwelt of the three-spined stickleback. In S. Ostlund-Nilsson,
- I. Mayer & F. Huntingford F (Eds.), *Biology of the Three-Spined Stickleback* (pp. 179–
 224). CRC Press, Boca Raton.
- Milinski, M., & Bakker, T. C. (1990). Female sticklebacks use male coloration in mate
 choice and hence avoid parasitized males. *Nature*, *344*(6264), 330–333.
 https://doi.org/10.1038/344330a0

- 430 Montgomerie, R., & Fitzpatrick, J. L. (2009). Testes, sperm, and sperm competition. In B.
- 431 G. M. Jamieson (Ed.), *Reproductive Biology and Phylogeny of Fishes (Agnathans and*
- 432 Bony Fishes) (pp. 1–53). Science Publishers, Enfield (NH).
- Morrell, L. J., Backwell, P. R., & Metcalfe, N. B. (2005). Fighting in fiddler crabs *Uca mjoebergi*: what determines duration? *Animal Behaviour*, 70, 653–662.
 https://doi.org/10.1016/j.anbehav.2004.11.014
- 436 Neff, B. D., & Clare, E. L. (2008). Temporal variation in cuckoldry and paternity in two
 437 sunfish species (*Lepomis* spp.) with alternative reproductive tactics. *Canadian Journal*438 of Zoology, 86, 92–98. https://doi.org/10.1139/Z07-121
- Olson, V. A., & Owens, I. P. (1998). Costly sexual signals: are carotenoids rare, risky or
 required? *Trends in Ecology & Evolution*, *13*, 510–514. https://doi.org/10.1016/S01695347(98)01484-0
- 442 Parker, G. A. (1970). Sperm competition and its evolutionary consequences in insects.

443 *Biological Reviews*, 45, 525–567. https://doi.org/10.1111/j.1469-185X.1970.tb01176.x

- 444 Price, A. C., Weadick, C. J., Shim, J., & Rodd, F. H. (2008). Pigments, patterns, and fish
 445 behavior. *Zebrafish*, *5*, 297–307. https://doi.org/10.1089/zeb.2008.0551
- Rios-Cardenas, O. (2003). The use of Molecular Genetic Analyses to Examine Mating
 Patterns a Paternal Care in the Pumpkinseed Sunfish (PhD dissertation). State
 University of New York at Buffalo.
- Scott, W. B., & Crossman, E. J. (1973). Freshwater Fishes of Canada. Fisheries Research
 Board of Canada, Ottawa.
- Smith, C., & Wootton, R. J. (1995). The costs of parental care in teleost fishes. *Reviews in Fish Biology and Fisheries*, 5, 7–22.
- Stacey, P. B., & Chiszar, D. (1978). Body color pattern and the aggressive behavior of
 male pumpkinseed sunfish (*Lepomis gibbosus*) during the reproductive season. *Behaviour*, 64, 271–297. https://doi.org/10.1163/156853978X00062
- 456 Taborsky, M. (2008). Alternative reproductive tactics in fish. In R. F. Oliveira, M. Taborsky
- 457 & H. J. Brockmann (Eds.), *Alternative Reproductive Tactics: An Integrative Approach*458 (pp. 251–299). Cambridge University Press, Cambridge.
- 459 Valente, E., Masson, G., Maul, A., Fox, M. G., Meyer, A., & Pihan J. C. (2016). Seasonal 460 gonadal development and age-related maturity patterns of introduced pumpkinseed 461 (Lepomis gibbosus Linnaeus, 1758) in a heated thermal reservoir and an adjacent river 462 of 60-71. reach. Journal Thermal Biology, 58, 463 https://doi.org/10.1016/j.jtherbio.2016.03.004

- Villeneuve, F., Copp, G. H., Fox, M. G., & Stakenas, S. (2005). Interpopulation variation
 in the growth and life history traits of the introduced sunfish, pumpkinseed *Lepomis gibbosus*, in Southern England. *Journal of Applied Ichthyology*, *21*, 275–281.
 https://doi: 10111/j.1439-0426.2005.00690.x
- Wedell, N., Gage, M. J. G., & Parker, G. A. (2002). Sperm competition, male prudence
 and sperm-limited females. Trends in Ecology and Evolution, *17*, 313-320.
 https://doi.org/10.1016/S0169-5347(02)02533-8
- 471 Wootton R. J. (1984) Introduction: tactics and strategies in fish reproduction. In G. W.
- 472 Potts & R. J. Wootton (Eds.), *Fish Reproduction: Strategies and Tactics* (pp. 1–12).
 473 Academic Press, London.
- 474 Wootton, R. J. (1998). Ecology of Teleost Fishes, 2nd edn. Elsevier, Dordrecht.
- Wootton, R. J., & Smith, C. (2015). Reproductive Biology of Teleost Fishes. WileyBlackwell, Oxford.
- 477 Yong, L., Peichel, C. L., & McKinnon, J. S. (2016). Genetic architecture of conspicuous
- 478 red ornaments in female threespine stickleback. G3-Genes Genomes Genetics, 6(3),
- 479 579–588. https://doi.org/1534/g3.115.024505

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.

							GSI		
Country	Population	Latitude	Longitude	Method	n	SL (SD)	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
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 channe	el53.213983N	14.468975E	ELF	25	77.1 (18.4)	1.8	0.1	5.7
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

⁴⁹⁸ [†]ANG = angling; ELF = continuous electrofishing; NET = small seine netting; TRC = minnow traps, catch-per-unit-effort (see Fox et al. 2011)

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	

503

TABLE 3. Summary of Bernoulli GLMM to model the probability of pumpkinseed 506 expressing a red operculum spot. Fish from different populations were fitted as random 507 intercepts, with standard deviation of 1.47. $N_{obs} = 897$.

Model parameter	Estimate	SE	Р	
Intercept _(female)	-8.02	0.80	<0.001	
SL [†]	0.08	0.01	<0.001	
Strategy _(cuckolder)	2.30	0.34	<0.001	
Strategy _(territorial)	2.35	0.23	<0.001	

508 [†] SL = standard length

TABLE 4. Summary of a Gaussian GLMM to model the size of pumpkinseed red 513 operculum spots. Fish from different populations were fitted as random intercepts, with 514 standard deviation of 0.24. $N_{obs} = 344$.

Model parameter	Estimate	SE	Р			
Intercept _(female)	-7.43	0.99	<0.001			
Log ₁₀ SL [†]	1.82	0.22	<0.001			
Strategy _(cuckolder)	-5.31	2.77	0.055			
Strategy(territorial)	-4.38	1.06	<0.001			
Log ₁₀ SL x Strategy _(cuckolder)	1.40	0.66	0.033			
Log ₁₀ SL x Strategy(territorial)	1.18	0.24	<0.001			
[†] SL = standard length						

518 **Figure legends**

FIGURE 1. Observed gonado-somatic 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.

523 **FIGURE 2.** Mean fitted probability (solid line) of pumpkinseed expressing a red operculum 524 spot as a function of standard length (SL in mm) with 95% confidence intervals (shaded 525 area) for females, cuckolder males and parental males. Data were modelled with a 526 Bernoulli GLMM. Fish from different populations were fitted as random intercepts in the 527 model.

FIGURE 3. Mean fitted Log₁₀ area (mm²) of red operculum spot (solid line) of pumpkinseed as a function of log₁₀ 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.