

## How does agonistic behaviour in albino and pigmented fish differ?

Ondřej Slavík, Pavel Horky, Marie Wackermann

In addition to hypopigmentation of the skin and red iris colouration, albino animals also display distinct physiological and behavioural syndromes. However, information on the social interactions of albino animals is rare and has mostly been limited to specially bred strains of albino rodents. Differentiating between the effects of albinism and domestication on behaviour in rodents can be difficult, and the agonistic behaviours of albino offspring of pigmented parents have yet to be described. In this study, we characterized two types of aggressive behaviour – aggressive displays (further subdivided into frontal and lateral displays) and mobile displays (further subdivided into chasing and biting) – in albino and pigmented juvenile *Silurus glanis* catfish. We found that the total number of aggressive interactions was lower in albinos compared with pigmented catfish. In addition, in the albino group, probability of chasing decreased over time, whereas the probability of lateral display did not show any significant trend. In the pigmented group, probability of chasing also decreased over time, whereas the probability of lateral display sharply increased. In neither group did the probability of frontal display or biting vary significantly over time. Distance between conspecifics was also analysed, and albinos showed a tendency towards greater separation from their same-coloured conspecifics compared with pigmented catfish. These results demonstrate that albinism can be associated with lower aggressiveness and a tendency towards greater separation from conspecifics.

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## 6   **Abstract**

7   In addition to hypopigmentation of the skin and red iris colouration, albino animals also display  
8   distinct physiological and behavioural syndromes. However, information on the social  
9   interactions of albino animals is rare and has mostly been limited to specially bred strains of  
10   albino rodents. Differentiating between the effects of albinism and domestication on behaviour in  
11   rodents can be difficult, and the agonistic behaviours of albino offspring of pigmented parents  
12   have yet to be described. In this study, we characterized two types of aggressive behaviour –  
13   aggressive displays (further subdivided into frontal and lateral displays) and mobile displays  
14   (further subdivided into chasing and biting) – in albino and pigmented juvenile *Silurus glanis*  
15   catfish. We found that the total number of aggressive interactions was lower in albinos compared  
16   with pigmented catfish. In addition, in the albino group, probability of chasing decreased over  
17   time, whereas the probability of lateral display did not show any significant trend. In the  
18   pigmented group, probability of chasing also decreased over time, whereas the probability of  
19   lateral display sharply increased. In neither group did the probability of frontal display or biting  
20   vary significantly over time. Distance between conspecifics was also analysed, and albinos  
21   showed a tendency towards greater separation from their same-coloured conspecifics compared

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22 with pigmented catfish. These results demonstrate that albinism can be associated with lower  
23 aggressiveness and a tendency towards greater separation from conspecifics.

24 Running title: Aggressive behaviour in catfish

## 25 **Key words**

26 Albinism, pleiotropic effect, aggressive and mobile display, catfish, Siluriformes

27

## 28 **Introduction**

29 Humans have long been fascinated by the rarity of albino animals, and the white colour of such  
30 animals has historically been associated with ideas of nobility (e.g., the white whale, hart, ox,  
31 etc.). By contrast, albinos in human populations are generally stigmatized, often considered by  
32 local religions to be a punishment for ancestral sins (Hong, Zeeb & Repacholi, 2006) or  
33 portrayed in Hollywood films as murderous villains (Gross, 2008; Brown, 2003). Albinism is  
34 generally the result of combinations of homozygous recessive mutations from pigmented parents,  
35 and in particular, albinos are often unable to synthesize tyrosine and melatonin hormones  
36 (Carden et al., 1998). This disability is not only associated with red irises and light skin  
37 colouring (oculocutaneous albinism, OCA; Carden et al., 1998) but also with physiological,  
38 behavioural and social limitations.

39 Information on the prevalence of albino animals in the wild is primarily based on reports, and  
40 information on social interactions between albinos is mostly limited to studies of specially bred  
41 strains of albino rats, whose behaviour is strongly influenced by domestication (Himmler et al.,  
42 2014). The eyes of albino rodents show reduced adaptation to light, often leading to

43 photoreceptor degradation (Prusky et al., 2002; Refinetti, 2007; Marc et al., 2008), which in turn  
44 can cause loss of vision (Buhusi, Perera & Meck, 2005) and movement perception (Hupfeld &  
45 Hoffmann, 2006), eventually leading to acrophobia and/or photophobia (Abeelen & Kroes, 1967;  
46 Owen, Thiessen & Lindzey, 1970). Albino rodents have a poorer sense of smell (Keeler, 1942;  
47 Sachs, 1996) and display lower activity levels compared with pigmented conspecifics (Fuller,  
48 1967; DeFries, 1969). In particular, their activity is low during the day and increases during the  
49 night (Stryjek et al., 2013). Albino rats also spend longer periods in deep sleep (rapid eye  
50 movement, REM), especially during the dark phase (Benca, Gilliland & Obermeyer, 1998), and  
51 during the night, they sleep more often out of the nets relative to pigmented conspecifics (Stryjek  
52 et al., 2013). Albino rats are slower to inhibit the fear response and explore new objects (Pisula et  
53 al., 2012), and they are less effective in completing spatial tasks (Harker & Whislaw, 2002). For  
54 example, albino rats displayed higher hoarding activity (Rebouças & Schmidek, 1997), and they  
55 burrowed slower and constructed less complex systems of tunnels compared with wild  
56 conspecifics (Stryjek, Modlińska & Pisula, 2012). Finally, albinism is also associated with lower  
57 reproductive success, due to fewer and less interested partners (Sachs, 1996).

58 According to Protas et al. (2006), blind albino tetra *Astyanax mexicanus* living in isolated albino  
59 populations in caves can be more aggressive compared with their pigmented and sighted  
60 conspecifics living in surface waters. Slavík, Horký & Maciak (2015) found spatial separation of  
61 albino catfish *Silurus glanis* (L.) originating from a non-isolated population of pigmented  
62 conspecifics, although they did not record any data on the agonistic behaviours of these animals.  
63 Under laboratory conditions, we have observed a number of aggressive interactions in differently  
64 pigmented groups of juvenile catfish, with pale hues often being associated with aggression and  
65 dominance (Beeching, 1995; O'Connor, Metcalfe & Taylor, 1999; Suter & Huntingford, 2002;

66 Miyai et al., 2011). Moreover, environmental light intensity strengthens aggressive behaviour  
67 (Valdimarson & Metcalfe, 2001; Pauers et al., 2012; Carvalho et al., 2013), as do pale substrata  
68 (Höglund, Balm & Winberg, 2002; Merighe et al. 2004). Finally, albino catfish are not able to  
69 darken their body colour to avoid the aggression of dominant conspecifics, which is observed in  
70 salmonids (O'Connor, Metcalfe & Taylor, 1999; Höglund, Balm & Winberg, 2000; Larsson et  
71 al., 2004) and cichlids (Volpato et al., 2003; Myiai et al., 2011). Hence, we hypothesized that  
72 higher levels of aggression would be observed in albino catfish compared with pigmented  
73 conspecifics, which we then set out to test.

74

## 75 **Materials & Methods**

76 Albinism in catfish has been commonly recorded in the wild (Dingerkus, Seret & Guilbert,  
77 1991), where catfish usually occur in groups (Boulêtreau et al., 2011; Cucherousset et al., 2012).  
78 Only juvenile catfish were used to reflect the behaviour of wild fish spatially separated from  
79 adults (Slavík et al., 2007), and moreover they showed complex social behaviours under the  
80 experimental conditions (Slavík, Maciak & Horký, 2012; Slavík et al.,).

## 81 **Experimental animals**

82 The fish used in this experiment were hatchery-reared juvenile catfish. One shoal of pigmented  
83 and one shoal of albino catfish that were unfamiliar to each other were obtained from local fish  
84 suppliers (Czech Fishery Ltd., Rybářství Hluboká and Rybářství Třeboň, Czech Republic,  
85 respectively). A total of 400 approximately equally sized fish (200 from each shoal) were  
86 transported from the hatcheries to the laboratory at 4 months of age. The fish were transported  
87 under stable conditions in oxygenated tanks in an air-conditioned loading space. Transport lasted

88 approximately 2 hours, and there were no observable effects on the health or mortality of the  
89 fish.

90 The fish were maintained in 2 separate holding tanks (380 L each, initial density  $1.9 \text{ kg m}^{-3}$ ;  
91 one shoal or 200 individuals per tank) for 8 weeks prior to the start of the experiment. The fish  
92 were fed food pellets ad libitum (Biomar Group, Denmark, [www.biomar.com](http://www.biomar.com)) that were  
93 distributed throughout the entire tank, providing free access to food to all individuals twice a  
94 day. The fish were maintained under a natural photoperiod, which was the same regime they  
95 had become accustomed to in the hatchery. The water was purified using biological filters with  
96 an integrated UV sterilizer (Pressure-Flo 5000, Rolf C. Hagen Inc., [www.lagunaponds.com](http://www.lagunaponds.com)). The  
97 water temperature and dissolved oxygen were controlled automatically (HOBO data logger;  
98 Onset Computer Corporation, Bourne, Massachusetts, USA). Fish were measured (mean 103  
99 mm; range 90 - 117 mm) and weighed (mean 10 g, range 6 - 15 g) at the end of the experiment  
100 and removed to separate tanks to prevent mixing with unused conspecifics.

101 All experimental fish (400 individuals) survived. After the experiment, the fish were released  
102 under the control of the Fish Management Authorities into fish ponds with extensive production  
103 management.

#### 104 **Experimental design**

105 The experiment was conducted in the laboratory between December 2013 and January 2014. A  
106 pair of randomly selected individuals of the same colour was placed into a rectangular plastic  
107 experimental arena (36 cm long, 18 cm wide, 20 cm high) at the beginning of each trial. The  
108 arena was separated by a partition into two equal parts, and the individuals were placed on  
109 opposite sides of the arena. After an acclimation period of 1 minute, the partition was removed  
110 and the behaviour of the fish was recorded for 5 minutes using a digital camera (GoPro Hero,

111 GoPro, Inc., San Matteo, CA, USA). The arena was flushed out and filled with clean water after  
112 every trial. In total, 40 trials (20 pairs of albinos and 20 pairs of pigmented individuals) were  
113 conducted.

#### 114 **Data analysis**

115 In the laboratory experiment, we tested two levels of aggressive interactions among juvenile  
116 catfish, designated as aggressive or mobile displays (Lehtonen, 2014). Aggressive displays were  
117 further subdivided into frontal and lateral displays, and mobile displays were further subdivided  
118 into chasing and biting displays (Hsu & Wolf, 1999; Dijkstra et al., 2009). The sum of aggressive  
119 interactions, referred to as ‘total aggression,’ was used in further analyses (Pauers et al., 2012).  
120 The bottom of the experimental arena was divided into six equally sized squares ( $9 \times 12$  cm) that  
121 were used in the analyses of ‘mutual distance.’ Mutual distance was set as a three level class  
122 variable. A mutual distance equal to 1 meant that the individuals were in the ‘same zone,’  
123 meaning that both conspecifics were in the same square. A mutual distance equal to 2 meant that  
124 the individuals were in ‘adjacent zones,’ meaning that conspecifics were in adjacent squares. A  
125 mutual distance equal to 3 meant that the individuals were in the ‘farthest zones,’ meaning that  
126 there was one square between conspecifics. Variable ‘size difference’ was defined as the  
127 difference between the weights of two interacting conspecifics in an experimental arena (mean  
128 1.9 g; range 0 - 6 g). For the purpose of analysing the probability of occurrence of different types  
129 of agonistic behaviour over time, a ‘time grid’ of 5 seconds was set. For every time grid value,  
130 the probability of occurrence of a particular agonistic behaviour was recorded as 1 (behaviour  
131 occurred) or 0 (behaviour did not occur). The EthoLog software program  
132 (<http://www.ip.usp.br/docentes/ebottoni/EthoLog/ethohome.html>) was used to assign the number

133 of particular agonistic behaviours as well as the ‘duration’ (in seconds) that conspecifics spent at  
134 particular mutual distances.

### 135 **Statistical analysis**

136 Statistical analyses were performed using the SAS software package (version 9.2; SAS Institute  
137 Inc., Cary, NC, USA). When necessary, the data were square root transformed to meet normality  
138 requirements.

139 Total aggression and duration were analysed using mixed models with random factors (PROC  
140 GLIMMIX with Poisson distribution for total aggression and PROC MIXED with normal  
141 distribution for duration). Random factors were used to account for repeated measures collected  
142 for the same experimental units (pair of conspecifics) across the duration of the experiment. The  
143 significance of each exploratory variable (i.e., fixed effects, including their interactions) in the  
144 particular model was assessed using an F-test in which we sequentially dropped the least  
145 significant effect, beginning with the full model (backward selection procedure). Least-squares  
146 means (LSM), henceforth referred to as “adjusted means,” were computed for class variables.  
147 The differences between the classes were tested using a t-test, and a Tukey–Kramer adjustment  
148 was used for multiple comparisons. The degrees of freedom were calculated using the Kenward–  
149 Roger method (Kenward & Roger, 1997).

150 The probabilities of occurrence for particular agonistic behaviours were analysed using the  
151 generalized estimating equation (GEE) approach (Liang & Zeger, 1986) for categorical, repeated  
152 measurements using the GENMOD procedure with binomial distributions. This approach is an  
153 extension of generalized linear models that provides a semi-parametric approach to longitudinal  
154 data analysis. In this study, four separate GENMOD procedures were designed to estimate the

155 probability of occurrence of particular agonistic behaviours (i.e., chasing, biting, frontal and  
156 lateral displays) across the duration of the experiment.

### 157 **Ethics statement**

158 All of the laboratory experimental procedures were in compliance with valid legislative  
159 regulations (law no. 246/1992, §19, art. 1, letter c). The permit was granted to O. Slavík,  
160 according to Law no. 246/1992, §17, art. 1; permit no. CZ00167. All laboratory samplings were  
161 conducted with the permission of the Departmental Expert Committee for Authorization of  
162 Experimental Projects of the Ministry of Education, Youth and Sports of the Czech Republic  
163 (permit no. MSMT-31220/2014-6). This study did not involve endangered or protected species.

164

### 165 **Results**

166 In total, we observed 1208 aggressive interactions, 68% of which were classified as lateral  
167 displays, 16% as frontal displays, 11% as chasing displays and 5% as biting. The total number of  
168 aggressive interactions was lower in the albino group ( $F_{1, 110.6}=14.51$ ,  $P<0.0002$ ; Fig. 1). In  
169 addition, the probability of chasing ( $\chi^2=6.64$ , d.f.=2;  $P<0.0362$ ; Fig. 2a) and lateral display  
170 ( $\chi^2=6.04$ , d.f.=2;  $P<0.0488$ ; Fig. 2b) changed over time and differed between groups. In the  
171 albino group, the probability of chasing decreased over time, whereas the probability of lateral  
172 display did not show any significant trend. In the pigmented group, the probability of chasing  
173 also decreased over time, whereas the probability of lateral display sharply increased. In neither  
174 group did the probability of frontal display or biting vary significantly over time. The results  
175 indicated that albinos were less aggressive compared with their pigmented conspecifics, which  
176 was primarily due to a higher probability of lateral display behaviours in the pigmented group.

177 Furthermore, the time that conspecifics spent at a particular distance from other conspecifics  
178 varied in both groups ( $F_{5, 111}=29.43$ ,  $P<0.0001$ ; Fig. 3). While the catfish (both albino and  
179 pigmented) generally spent the least amount of time in the farthest zones (time spent in the  
180 farthest zones did not differ between albino and pigmented catfish), albinos spent more time in  
181 adjacent zones and less time in the same zones than pigmented conspecifics (Adj.  $P<0.05$ ).  
182 Taken together, we found that albino catfish showed a higher tendency to be spatially separated  
183 from conspecifics, whereas pigmented catfish showed a tendency towards close contact.

184

## 185 **Discussion**

186 The assumption that albinism is associated with different levels of aggression is supported by  
187 this study. In particular, our results support the theory that species with different levels of  
188 colouration should display different levels of aggression (Pryke & Griffith, 2006) and that the  
189 level of aggression should correspond to different colour morphs (Pryke, 2009; Dijkstra et al.,  
190 2009). Indeed, one colour morph is often predicted to be more aggressive than others (Dijkstra et  
191 al., 2010). Aggression tends to be directed towards individuals that were the same colour as the  
192 aggressor (Dijkstra et al., 2007; Pauers et al., 2008; Lehtonen, 2014). Thus, albinos might have  
193 been expected to display higher aggressiveness towards other uniformly coloured albino  
194 conspecifics compared with pigmented catfish, which are highly variable in their colour and  
195 pattern. However, contrary to our assumption, the albinos were less aggressive compared with  
196 their pigmented counterparts.

197 It is possible that the lower aggression of the albinos is due to pleiotropic effects of the genes  
198 responsible for albinism. Pleiotropic effects of albinism have been reported previously in albino

199 mice, and albinism has been correlated with lower movement activity, acrophobia, photophobia  
200 and lower visual acuity (Fuller, 1967; Abeelen & Kroes, 1967; DeFries, 1969; Owen, Thiessen &  
201 Lindzey, 1970; Prusky et al., 2002; Buhusi, Perera & Meck, 2005). Our results are consistent  
202 with recent findings showing that albinism has pleiotropic effects that are mediated through  
203 hormones that can affect both skin pigmentation and aggressive behaviour (Gonzales, Varizi &  
204 Wilson, 1996; Ducrest, Keller & Roulin, 2008). As reported by Horth (2003), increases in the  
205 aggression of the melanic form of mosquitofish correlate with changes in melanin synthesis.  
206 Therefore, albinism, in contrast to melanism, may be generally associated with lower aggression  
207 due to shared genetic control mechanisms.

208 Aggression is also associated with social position or rank (Mazur & Booth, 1998; Staffan,  
209 Magnhagen & Alanära, 2002). For example, an albino female vampire bat *Desmodus rotundus*  
210 breed with pigmented individuals had the lowest social position (Uieda, 2001). Hence, albinism  
211 may not only be associated with lower aggressiveness but also with lower dominance, as these  
212 characteristics are often correlated (Dijkstra, Seehausen & Groothuis, 2005; Pryke & Griffith,  
213 2006). Indeed, consistent with this idea, ostracism of albino catfish by a group of pigmented  
214 conspecifics has been previously described (Slavík, Horký & Maciak 2015). Albino humans not  
215 only suffer from impaired visual acuity (Buhusi, Perera & Meck, 2005), photophobia, extreme  
216 sensitivity to the sun and skin cancer, but they also suffer from the social consequences of being  
217 albino, although these effects have only been described in humans during ontogenesis (Javangwe  
218 & Mukondyo, 2012). These effects are often manifested through religious stigmatization and  
219 community ostracism (Hong, Zeeb & Repacholi, 2006). Furthermore, lower participation in  
220 social relationships, such as friendships, marriages and career opportunities (Hong, Zeeb &  
221 Repacholi, 2006; Estrada-Hernández & Harper, 2007), has been recorded among albino people.

222 Finally, African albino children displayed less aggressive play behaviours compared with other  
223 children (Javangwe & Mukondyo, 2012), and there is also evidence that albino people tend to be  
224 more shy within the greater community.

225 Some of our behavioural findings are also consistent with those of albino rodents. For example,  
226 specially bred strains of albino rats (Sprague-Dawley) displayed a higher probability of playful  
227 attacks compared with wild-type pigmented strains (Himmler et al., 2014). The behaviour of this  
228 Sprague-Dawley strain, however, was also different from other albino strains. Interestingly, the  
229 authors attributed these differences to differing levels of domestication in each of the strains.  
230 Playful attacks are associated not only with domestication (see review Himmler et al., 2014) but  
231 also with reduced aggression, as albino rats are less aggressive than their wild-type pigmented  
232 counterparts (Barnett, Dickson & Hocking, 1979; Barnett 1981). Although a direct comparison  
233 between aggressiveness in catfish and rats is not possible, we speculate that albinism may be  
234 generally associated with lower aggression compared with normally pigmented conspecifics.

235 Albino catfish also showed a greater tendency towards spatial separation compared with their  
236 pigmented conspecifics, which preferred to be nearer to one another. This finding differs from  
237 what was observed in domesticated albino rats, which were found to be more tolerant of  
238 conspecifics compared with wild-type strains (Himmler et al., 2013; 2014). Indeed, colour-  
239 assortative shoaling is often observed (McRobert & Bradner, 1998; Spence & Smith, 2006;  
240 Gómez-Laplaza, 2009; Rodgers, Kelley & Morell, 2010). Considering the fact that albino catfish  
241 are unable to darken their body colour to avoid aggressive interactions with dominant  
242 conspecifics (O'Connor et al., 1999; Höglund, Balm & Winberg, 2000), spatial separation may  
243 be a strategy for avoiding the escalation of aggressive behaviours. Thus, spatial separation may  
244 be another explanation for the decreased aggressive behaviour observed in albino catfish. Large

245 numbers of albinos existing together in the wild has only been reported in insects (Hoste, 2003),  
246 and whether albinos are mutually attracted to each other and form larger groups in nature  
247 remains unknown.

248

## 249 **Conclusions**

250 The assumption that pale colouration is associated with aggressive behaviours in the wild is  
251 common but not well supported. Lack of pigmentation can dramatically affect social interactions  
252 and despite the fact that low colour variability is generally correlated with higher levels of  
253 aggression among conspecifics, albino catfish were significantly less aggressive than their  
254 pigmented conspecifics. In addition, albinos maintained larger distances between themselves  
255 compared with pigmented individuals, inconsistent with the phenomenon of colour-assortative  
256 shoaling that is commonly reported in fish and the playful, non-aggressive behaviours of albino  
257 rodents. Therefore, colour may not only be linked to aggression in albinos but may also have  
258 other pleiotropic effects, as described previously. Based on available information concerning the  
259 behaviour of albino individuals, including humans, we conclude that albinism is most often  
260 associated with lower aggressiveness, lower dominance and social exclusion.

261

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445

#### 446 **Figure legends**

447 Fig. 1: Total number of aggressive interactions (adjusted means  $\pm$  SE) across albino and  
448 pigmented treatments.

449 Fig. 2: Probability of chasing (a) and lateral display (b) as a function of time across the two  
450 treatments. Dotted line = albino catfish; black line = pigmented catfish.

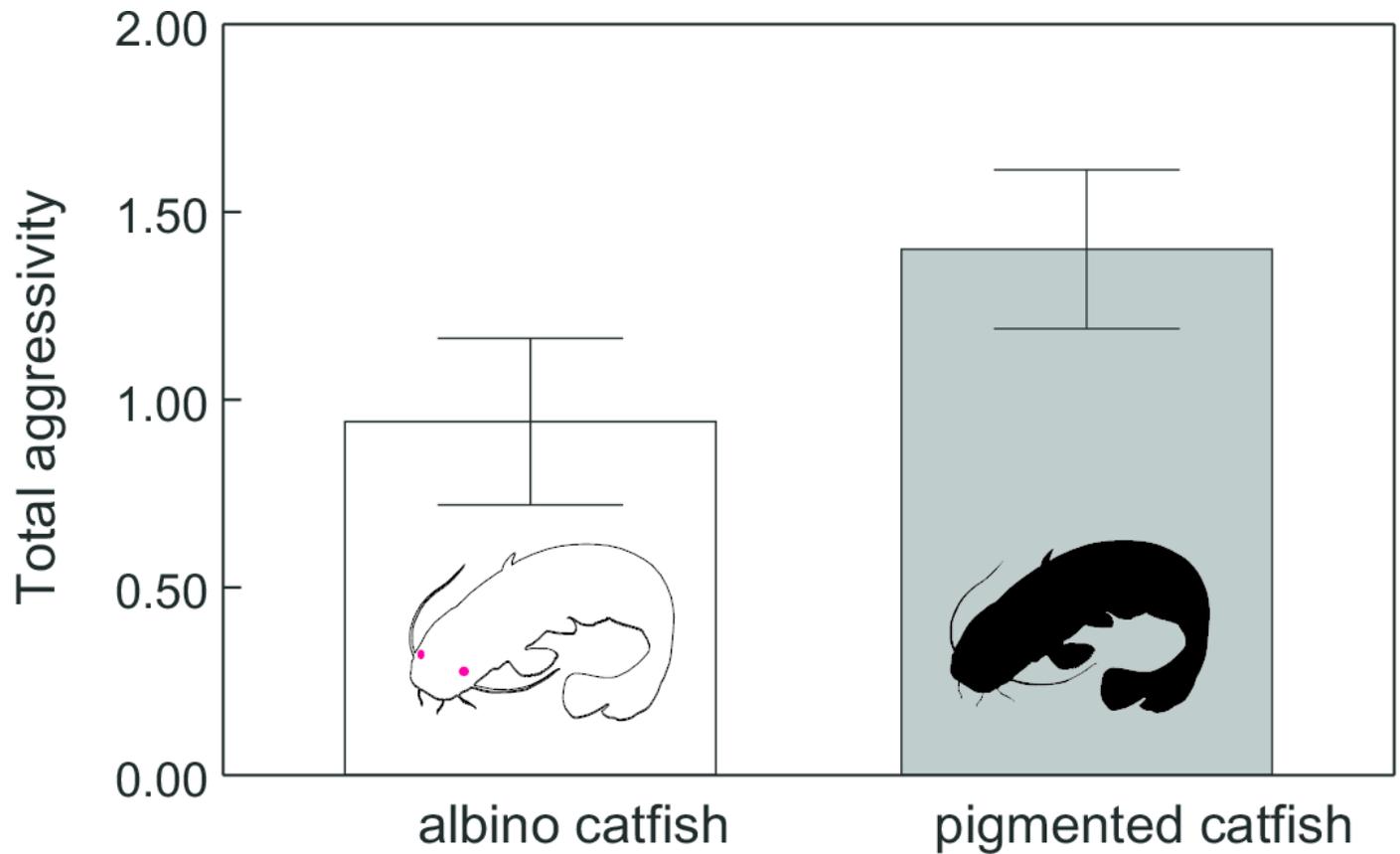
451 Fig. 3: Duration (adjusted means  $\pm$  SE of square root data) of time that conspecifics spent at  
452 particular mutual distances across albino and pigmented treatments.

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## 1

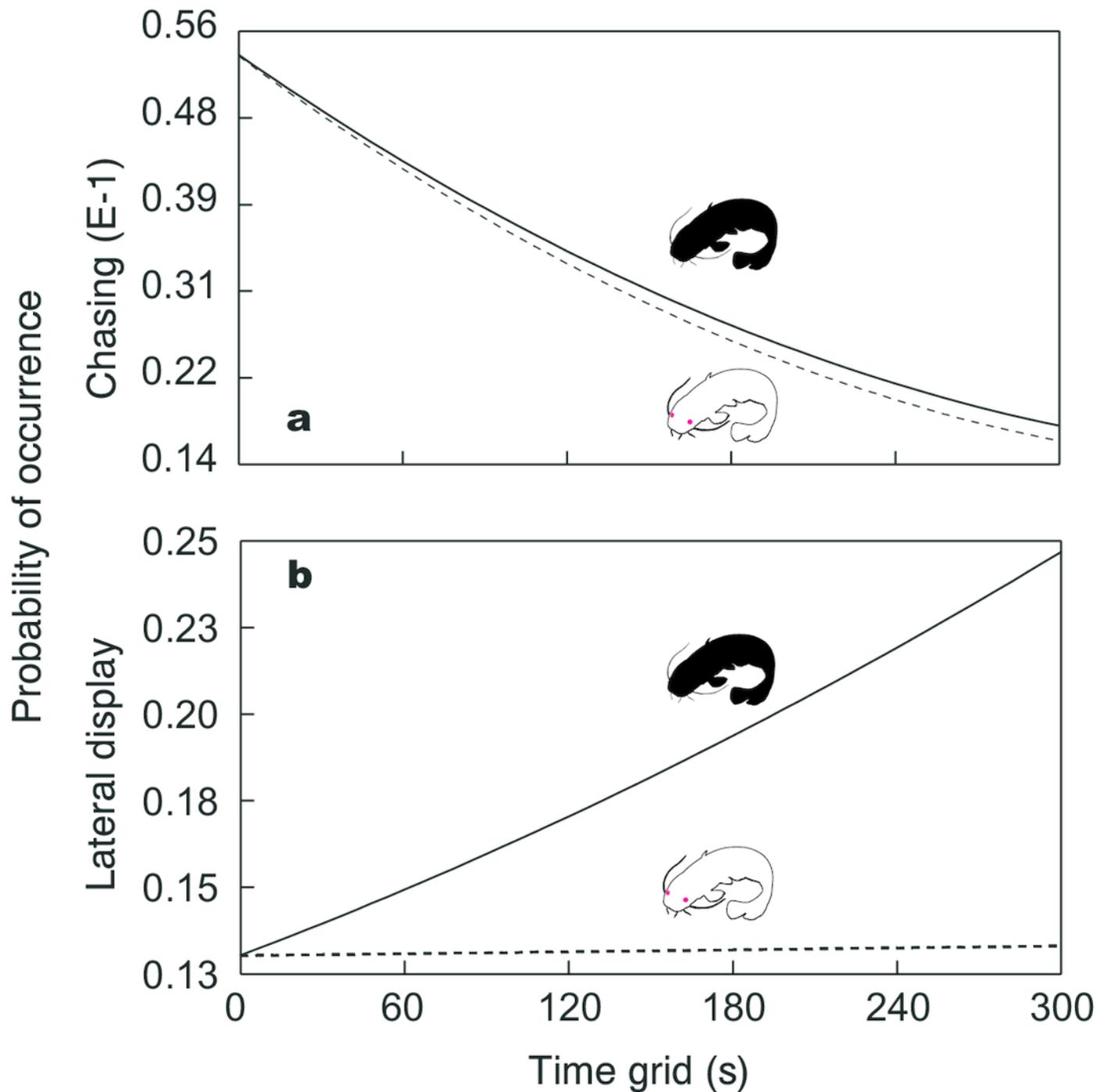
Total number of aggressive interactions (adjusted means  $\pm$  SE) across albino and pigmented treatments.



## 2

Probability of chasing (a) and lateral display (b) as a function of time across the two treatments.

Dotted line = albino catfish; black line = pigmented catfish.



## 3

Duration (adjusted means  $\pm$  SE of square root data) of time that conspecifics spent at particular mutual distances across albino and pigmented treatments.

