ADVANCES IN CICHLID RESEARCH V

Inbreeding affects juvenile shoaling behaviour in sibling groups of a cichlid fish

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Abstract Whilst the effects of inbreeding on growth and survival have been well studied, knowledge on the impact of inbreeding on the social behaviour is scarce. Animal groups are often composed of relatives, which can facilitate cooperation (due to kin selection) and improve group performance accordingly. Therefore, increased genetic relatedness in inbred kin groups could increase group performance, whilst the reduced genetic diversity could have negative effects (inbreeding depression). We compared the juvenile shoaling behaviour of inbred and outbred sibling groups in *Pelvicachromis taeniatus* (Boulenger 1901), a West-African cichlid fish with kin mating preferences. Activity (travelled distance), shoal density (inter-individual distance) and recovery from a disturbance (freezing time) in a novel environment were analysed using tracking software. Inbred individuals travelled shorter distances compared to outbred individuals, which may decrease conspicuousness and thus predation risk. Furthermore, inbred sibling groups recovered faster after the disturbance compared to outbred sibling groups suggesting improved coordination. In conclusion, our study revealed differences in shoaling between inbred and outbred sibling groups. The higher relatedness in inbred groups may have positive effects in the form of a reduced conspicuousness. The reduced activity may impair foraging success under natural conditions which might, however, be compensated by faster recovery from disturbances.

Keywords *Pelvicachromis taeniatus* · Social behaviour · Sociality · Kin selection

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Introduction

Group living is widespread in the animal kingdom and especially in fishes most species spent at least a part of their life in groups. Grouping has numerous advantages, e.g. reduced predation risk (Herbert-Read et al., 2017) or improved foraging (Ward & Hart, 2005). Accordingly, the presence of predators affects shoaling decisions in mosquitofish (*Gambusia affinis* Baird & Girad 1835) with naïve focal fish preferring predator-experienced individuals (McGhee, 2019). Predation risk can even promote mixed-species shoaling in tropical reef fishes (Paijmans et al., 2020). Next to shoaling decisions, the collective behaviour within shoals (shoal performance) is also affected by predation. The organisation and the transfer of information

within shoaling fishes is based on the collective capacity for information acquisition and processing followed by collective decision-making (Ioannou et al., 2011). Predation risk has a high impact on shoaling in fishes and local information about a threat can be propagated rapidly. In detail, reactions of individuals to predators can transmit escape behaviour through the entire shoal (Ioannou et al., 2011). In guppies (*Poecilia reticulata* Peters 1859), the attraction–repulsion dynamics as well as individuals' acceleration and deceleration responses are affected by predation risk and individuals originating from high-predation environments form larger and more cohesive shoals (Herbert-Read et al., 2017). Because predation represents a major selection factor in prey animals (Ioannou, 2021), shoaling performance should be adjusted to predation pressure. Thus, adaptive plasticity in antipredator behaviour can increase the survival when predators are present (e.g. Hasenjager & Dugatkin, 2017; James et al., 2018).

In addition to environmental factors, the composition of shoals, for example, the sex ratio (Rystrom et al., 2018) or physiological traits of shoal members (reviewed in Killen et al., 2017), can affect shoal performance. An important factor in terms of shoal composition is the relatedness between group members. The inclusive fitness theory by Hamilton (1964) predicts that the genetic relatedness between group members is important for grouping and plays a central role in the evolution of cooperation and group living (Bourke, 2011). Individuals showing altruistic behaviour directed to related conspecifics are expected to be favoured by natural selection as this can increase their indirect fitness (reviewed in Ward & Webster, 2016). In shoaling contexts, relatedness has frequently been associated with shoaling decisions (reviewed in Ward & Hart, 2003) and for three-spined sticklebacks (*Gasterosteus aculeatus* Linnaeus 1758) it was shown that individuals prefer to shoal with kin over non-kin (Frommen et al., 2007).

Inbreeding, that is, mating between genetically related individuals leads to increased relatedness in inbred siblings compared to outbred siblings, due to the higher proportion of alleles that individuals share with each other (reviewed in Szulkin et al., 2013). Higher relatedness in inbred offspring compared to outbred offspring may enhance cooperation due to kin selection. In contrast, inbreeding results in higher homozygosity and reduced genetic variability

in inbred offspring, which can lead to inbreeding depression, as deleterious recessive alleles are more likely to be expressed (Charlesworth & Willis, 2009). Because of inbreeding depression, inbreeding has long been supposed to be generally avoided in the animal kingdom (reviewed in Pusey & Wolf, 1996). However, a recent meta-analysis showed that inbreeding avoidance is rare in animals (de Boer et al., 2021). Theory predicts benefits from inbreeding as it helps to spread genes identical by descent (Kokko & Ots, 2006). When benefits of inbreeding exceed the costs, this can result in inbreeding tolerance or even preference (reviewed in Szulkin et al., 2013).

In the present study, we examined the impact of inbreeding on shoal performance in *Pelvicachromis taeniatus*, a monogamous, cichlid fish with biparental brood care from West and Central Africa. In detail, we compared shoals consisting of inbred and outbred siblings in their shoaling performance as well as their response to an abruptly occurring, mechanical disturbance cue. *P. taeniatus* shows a preference for closely related conspecifics in mate choice, that is, active inbreeding (Thünken et al., 2007a, b, 2012) and so far, no signs of inbreeding depression. After having left their parents, juvenile *P. taeniatus* live in shoals (Hesse & Thünken, 2014) and prefer to shoal with groups consisting of related individuals, which is associated with fitness benefits in the form of better growth (Thünken et al., 2016). In addition, Hesse & Thünken (2014) showed that siblings form denser shoals and that kinship promotes cooperative behaviour during predator inspection (Hesse et al., 2015a). However, increased perceived competition decreases kin preference during shoaling (Thünken et al., 2020).

We compared baseline shoaling behaviour and the change in shoaling performance of shoals consisting of inbred full siblings and shoals consisting of outbred full siblings following a mechanical disturbance cue in two generations. The disturbance cue was created by a single pendulum stroke on one of the outer sides of the tanks (see Meuthen et al., 2016). We applied computer-based, automatic tracking methods to track unmarked *P. taeniatus* in groups. Higher relatedness can promote cooperative behaviour between shoal members which may result in improved coordination and a more adequate, that is, less stressed shoaling performance. Abrupt disturbance should evoke startle responses. Recovery from the disturbance may depend on coordination within

the group and, thus, might be positively or negatively affected by inbreeding.

Materials and methods

Experimental animals

Juvenile F2- and F3-generation offspring of wild caught *P. taeniatus* (also described as *Pelvicachromis kribensis*, which is a revalidated species name for several *P. taeniatus* populations including the one used in this study; see Lamboj et al. 2014) from the Moliwe River population in Cameroon (near Limbe, West Cameroon 04°040 N/09°160E) were used as experimental fish. Inbred families were generated by mating full siblings and outbred families by matings between unrelated individuals. Families were bred under standardised laboratory conditions at the Institute of Evolutionary Biology and Ecology of the University of Bonn (see Thünken et al., 2007a) and had an age of 94 ± 3 days in the F2 and 137 ± 4 in the F3-generation (mean $\pm$ SD) when being used in the experiment. Individuals measured on average 2.15 ± 0.02 cm in the F2 and 2.31 ± 0.17 cm in the F3-generation (mean $\pm$ SD). Clutches were separated from the parents at the egg stage and transferred to a small plastic box (Karlie Smart Keeper, $18.5 \times 11.5 \times 13.5$ cm, length $\times$ width $\times$ height) equipped with an air stone to provide oxygen supply. Water was replaced by one-day aged temperate tap water 6 days a week during egg and non-swimming larvae (wriggler) stages. From the free-swimming juvenile stage on (approximately 7-day post-hatching), groups were fed 6 days a week with freshly hatched *Artemia* nauplii. Water was replaced approximately 30 min after feeding by 1-day aged temperate tap water. Clutches consisting of 10 or more individuals were uniformly split into two groups at the age of 3 months and transferred to glass tanks, measuring $30 \times 20 \times 20$ cm (length $\times$ width $\times$ height), equipped with a filter (BIOCLEAR, Europet Bernina) and sand as substrate. About 30% of the water volume was replaced by temperate tap water every 2 weeks and food was switched from *Artemia* nauplii to a mixture of defrosted Chironomidae larvae and adult *Artemia* which was provided five times a week *in excess*. The light/dark cycle was 12:12 h and the water temperature was kept constantly at 25 ± 1 °C. Group sizes ranged from 5 to 15 in the

F2-generation and 4 to 33 in the F3-generation. In total, for the F2-generation, 26 trials were conducted (17 with inbred and 9 with outbred shoals from 8 inbred and 3 outbred families), using 130 fish from 26 clutches. In the F3-generation, 34 trials were conducted (18 with inbred and 16 with outbred shoals from 13 inbred and 8 outbred families), using 136 fish from 21 clutches. Family-group size was not significantly different between inbred and outbred families in both generations (F2-generation: lme, $N_{inbred} = 17$, $N_{outbred} = 9$, $F = 1.917$, $P = 0.197$; F3-generation: Wilcoxon rank-sum test, $N_{inbred} = 18$, $N_{outbred} = 16$, $W = 160$, $P = 0.590$) and was not significantly different between generations (Wilcoxon rank-sum test, $N_{F2} = 36$, $N_{F3} = 34$, $W = 359.5$, $P = 0.488$). Mean standard length ($\pm$ SD) did not differ significantly between shoals consisting of inbred and outbred individuals in the F2- and F3-generation (F2-generation: inbred: 2.103 ± 0.064 cm, outbred: 2.233 ± 0.055 cm; F3-generation: inbred: 2.310 ± 0.030 cm, outbred 2.313 ± 0.045 cm; Table 2). The variance in standard length within shoals also did not differ significantly between the inbred and outbred groups in the F2-generation, but was significantly higher in F3-generation shoals consisting of inbred individuals compared to shoals consisting of outbred individuals (Table 2).

Experimental setup and procedure

A plastic tank measuring $38.5 \times 30 \times 24.5$ cm (length $\times$ width $\times$ height) was used as the experimental tank. All sides, including the bottom, were covered by opaque, white adhesive foil to prevent disturbance from outside and maximise contrast between experimental fish and background for the analyses. Before each trial, the tank was rinsed with tap water and filled to a height of 5 cm with 1-day aged tap water with a temperature of 25 ± 1 °C. The water level was chosen to be sufficient to enable natural swimming behaviour but minimise vertical swimming as this could not be measured with the used setup.

In shoal experiments using the F2-generation, shoals consisted of five individuals. In experiments using the F3-generation, four individuals were used. Experimental fish were obtained from group tanks, including full siblings. Individuals were visually size matched to avoid extremes in size because homogeneity can affect shoaling in fishes (e.g. Cattelan & Griggio, 2018). Meuthen et al. (2016) showed that in *P.*

taeniatus, the relationship between homogeneity and density of shoals was altered by previous predation risk in the form of a negative correlation in groups exposed to alarm cues and a positive correlation in the control group. Large shoal heterogeneity may affect shoaling as it might impair hydrodynamic properties (Belyayev & Zuyev, 1969; Weihs, 1973), competition for food resources (Ranta et al., 1994) as well as predation risk (Conradt & Roper, 2000). Thus, in the present study, the variance in standard length within groups was included as covariate in the linear models. The experimental fish were carefully transferred from their holding tank to the experimental tank using a dip net and a small plastic box (Karlie Smart Keeper, 18.5×11.5×13.5 cm, length×width×height), filled with water from their original tank to a height of 4 cm.

Video recordings of shoals were taken using a camera, fixed centrally at a distance of 40 cm above the water surface and the software VirtualDub (version 1.10.4) installed on a laptop (Fujitsu Lifebook S Series SH531). Shoals from the F2-generation were filmed using a Logitech QuickCam Pro 9000 and a resolution of 640×480 pixels at 30 frames per second. The F3-generation was recorded using a high-definition webcam (Logitech HD Pro C920) and the resolution was adjusted to 1920×1080 pixels at a frame rate of 30 frames per second, which allowed the usage of a software-based tracking method.

Directly after recordings were started, all fish of one shoal were carefully poured out of the box into the centre of the test tank. The recording time was 35 min, of which the first five minutes were defined as acclimatisation time. After 20 min, a standardised hit against one side wall of the test tank functioned as a mechanical disturbance cue. This was achieved by letting a laboratory bottle (11×6×5 cm, filled with 125 ml of water) hit one side wall of the tank. The bottle was fixed to the ceiling of the room with a string and hit the middle of the tank wall from 67 cm distance with an angle of 28° (see Schons et al., 2021). After the trials, the standard lengths of the fish were measured to the nearest millimetre using scale paper.

Manual tracking (F2-generation)

From the recorded videos, one frame every 15 s was extracted using the software VirtualDub (version 1.10.4) and imported into the software ImageJ (version 1.52a). Using the "multi-point tool" in ImageJ, a marking was placed at the middle of the head of each fish. Coordinates of each fish within a frame were exported to an excel sheet. Inter-individual distances (IID; the distance from the middle of the head of each fish to the middle of the head of every other fish) were calculated as distances between the exported coordinates and subsequently converted to metric units according to the known dimensions of the experimental tank.

Automated tracking (F3-generation)

The videos were analysed using the tracking software idTracker (Pérez-Escudero et al., 2014). The software automatically identifies each fish in each frame using reference images and an algorithm for estimations of positions of crossing or occluded individuals (for details see Pérez-Escudero et al., 2014). The following settings were applied in idTracker: the number of individuals was set to 4, the intensity threshold to 0.75, the minimum size to 250 pixels and the number of frames for references to 6000. The values in the generated dataset, with coordinates for each fish in each video frame, were changed from pixels into centimetres using a conversion factor, which was determined using the software ImageJ (version 1.52a) based on the dimensions of the experimental tank.

Analysis

For both generations, the exact time point in each video recording at which the mechanical disturbance occurred was identified visually. After disturbance cue exposure, fish stopped swimming immediately (freezing). We measured the time until the whole shoal started swimming again (freezing time). Data were then split into two subsets, one consisting of all frames before the stimulus was given (pre-disturbance), for analysing baseline shoaling performance and another one including all frames afterwards (post-disturbance). For the automated analysis (F3-generation), based on the coordinates provided by the idTracker, the distance travelled and IID were calculated as average values for each shoal (Table 1).

For each variable, an average value for each shoal and each minute was calculated. All variables were calculated for both datasets, pre-disturbance and

Table 1 The variables calculated to score the behaviour of shoals

Variable	Formula	References
Distance travelled between two video frames	$D(t) = \sqrt{(x(t) - x(t-1))^2 + (y(t) - y(t-1))^2}$	Audira et al. ([2018](#))
Distance travelled (distance)	Average distance travelled by all shoal members per minute	
Inter-individual distance (IID)	$\text{IID}(t) = \sqrt{(x_1(t) - x_2(t))^2 + (y_1(t) - y_2(t))^2}$	Fu ([2016](#))
	Average inter-individual distance (cm) per minute	

$x(t)$ and $y(t)$ are the coordinates of the fish at time t and d is the length of the time interval between two frames, i.e. 1/30 s

post-disturbance. For the F2-generation, the change in IID (IID_{index}) and for the F3-generation, IID_{index} and travelled distance (distance_{index}) following the mechanical disturbance cue were calculated by subtracting values of the dataset post-disturbance from pre-disturbance divided by the sum. For the post-disturbance period, variables were calculated from the minute in which all individuals started swimming after the mechanical disturbance cue was given. Consequently, the time period included in the analyses differed between shoals as it was determined by the last individual of one shoal starting to swim again. Thus, relative variables (average values for one minute) were calculated for distance and IID for 15 one-minute periods pre-stimulus and for post-stimulus analyses on average for 8 ± 0.5 SE (mean $\pm$ SE) minutes (shoals consisting of inbred individuals: 9 ± 0.8 min; shoals consisting of outbred individuals: 8 ± 0.7; mean $\pm$ SE).

Statistical analyses

Statistical analyses were performed using R 3.6.0 (R Core Team, 2019). Data for the F2- and the F3-generation were analysed separately. Linear mixed-effects models (lme) were performed using the lme4-package (Bates et al., 2014). For both generations, the mean standard length within each shoal (SL mean), the variance in standard length within shoals (SL variance), the freezing time, the IID and the change in IID (IID_{index}) were used as dependent variable and for the F3-generation additionally the travelled distance and the change in travelled distance (distance_{index}). As explanatory variables, the treatment groups (inbreeding and outbreeding), as well as the variance in standard length were included in each linear mixed-effects model. As the variance in standard length within shoals of the F3-generation was significantly

different between shoals consisting of inbred and outbred individuals, this variable was included in every linear model regarding the F3-generation as fixed factor. The family was included as random factor to account for shoals originating from clutches of the same parents.

Backward stepwise model reduction of the linear mixed-effects models was performed using the "step" function from the "lmerTest" package (Kuznetsova et al., 2017). Non-significant explanatory variables were removed from the models in order of their statistical relevance. Tests of significance were F-tests based on Satterthwaite's or Kenward–Roger approximations. The normality of the residuals of all initial models was confirmed using the "check normality" function and the homogeneity of variances was confirmed using the "check homogeneity" function included in the "performance" package (Lüdecke et al., 2021). The residuals of the model including family-group size of the F2-generation, i.e. the group in which the fish grew up prior to the experimental phase, were not normally distributed and the response variable failed to respond to transformation. Therefore, a nonparametric Wilcoxon rank-sum tests was used to compare family-group sizes between inbred and outbred families of the F2-generation. The residuals of the model including the IID of the F3-generation were not normally distributed. After a Box–Cox transformation (Box & Cox, 1964) of the response variable, the normality of the residuals could be confirmed.

In the F3-generation, three shoals from the inbred group did not start to swim within the 15-min post-stimulus phase and, thus, were excluded from post-disturbance analyses, including the analysis of freezing time. In total, 34 shoals (18 shoals consisting of inbred and 16 shoals consisting of outbred individuals) were used for analyses regarding the phase

Fig. 1 Differences of **a** inter-individual distance (IID) and **b** freezing time between shoals consisting of inbred (grey) and outbred (white) individuals for the phase preceding the mechanical disturbance. Given are mean values ± SE. *$P < 0.05$; ns = $P > 0.05$
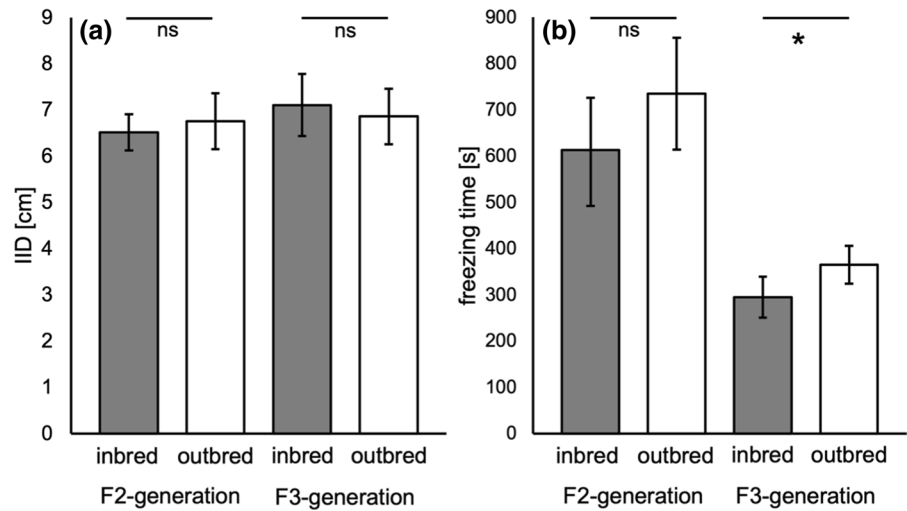
Table 2 Results of linear mixed-effects models calculated for the phase preceding the disturbance cue (pre-disturbance) and the change followed by the disturbance cue (index)

Category	Dependent variable	Explanatory variable	F	P
F2-generation				
Size	SL mean	Inbred–outbred	0.749	0.406
	SL variance	Inbred–outbred	4.437	*0.060*
Response	Freezing time	Inbred–outbred	0.873	0.382
		SL variance	1.472	0.239
Pre-disturbance	IID	SL variance	0.052	0.821
		Inbred–outbred	0.082	0.777
Post-disturbance	IID_{index}	Inbred–outbred	0.008	0.932
		SL variance	1.367	0.255
F3-generation				
Size	SL mean	Inbred–outbred	0.006	0.938
	SL variance	Inbred–outbred	5.305	**0.021**
Response	Freezing time	SL variance	7.993	**0.008**
		Inbred–outbred	4.453	**0.043**
Pre-disturbance	Travelled distance	SL variance	4.191	*0.053*
		Inbred–outbred	5.270	**0.032**
	IID	Inbred–outbred	0.031	0.861
		SL variance	0.727	0.340
Post-disturbance				
Size	$Distance_{index}$	Inbred–outbred	2.608	0.116
		SL variance	0.002	0.965
	IID_{index}	Inbred–outbred	0.100	0.756
		SL variance	0.042	0.839

Family was included as random factor in each model. During stepwise model reduction, degrees of freedom always differed by one. Tendencies ($P < 0.1$) are printed in italics and significant results are printed in bold ($P < 0.05$). In the models regarding pre-disturbance and change variables, the variance in standard length (SL variance) was included as fixed factor and not removed during model reduction

preceding the disturbance cue and 31 shoals (15 shoals consisting of inbred and 16 shoals consisting of outbred individuals) for analyses regarding the response to the disturbance cue were included in the statistical analyses.
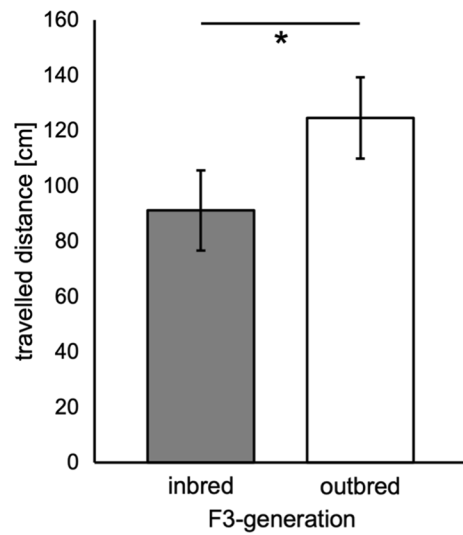
Results

In the F2-generation, freezing time, IID and the change in IID (IID_{index}) did not differ significantly between shoals consisting of inbred or outbred

individuals (Figs. 1a and 3a, Table 2) and were not significantly correlated with variance of standard length (Table 2). Furthermore, the IID_{index} did neither change significantly (deviation from zero) in both groups together nor within the inbred and outbred group (Table 3).

In the F3-generation, travelled distance was significantly lower in shoals consisting of inbred individuals (Fig. 2, Table 2). The IID was not significantly different between the in- and outbred group and not correlated with variance in standard length (Fig. 1a, Table 2). Freezing time was significantly lower in inbred groups (Fig. 1b, Table 2) and was positively correlated with variance in standard length. The changes in travelled distance ($distance_{index}$) and IID (IID_{index}) were not significantly different between shoals consisting of inbred and outbred individuals and not significantly correlated with the variance in standard length (Table 2).

In the F3-generation, the deviation of the IID from no change was neither significant in both groups together nor in the in- and outbred group, separately

Fig. 2 Difference of the travelled distance between shoals consisting of inbred (grey) and outbred (white) individuals for the phase preceding the mechanical disturbance. Given are mean values ± SE. *$P < 0.05$

Table 3 Results of intercept models conducted to analyse the deviation from no change (intercept) followed by the disturbance cues, separately for shoals consisting of inbred and outbred individuals as well as for both groups together

Category	Dependent variable	F	t	P
F2-generation				
IID_{index}				
Inbred and outbred	SL variance	1.367		0.255
	Intercept		− 1.638	0.133
Inbred	SL variance	0.358		0.562
	Intercept		− 1.005	0.345
Outbred	SL variance	1.016		0.340
	Intercept		− 1.947	*0.083*
F3-generation				
$Distance_{index}$				
Inbred and outbred	SL variance	0.002		0.965
	Intercept		− 5.854	**< 0.001**
Inbred	SL variance	0.006		0.941
	Intercept		− 2.720	**0.016**
Outbred	SL variance	2.794		0.114
	Intercept		− 6.469	**< 0.001**
IID_{index}				
Inbred and outbred	SL variance	0.042		0.839
	Intercept		− 0.657	0.519
Inbred	SL variance	0.055		0.822
	Intercept		− 0.269	0.793
Outbred	SL variance	0.031		0.864
	Intercept		− 0.649	0.535
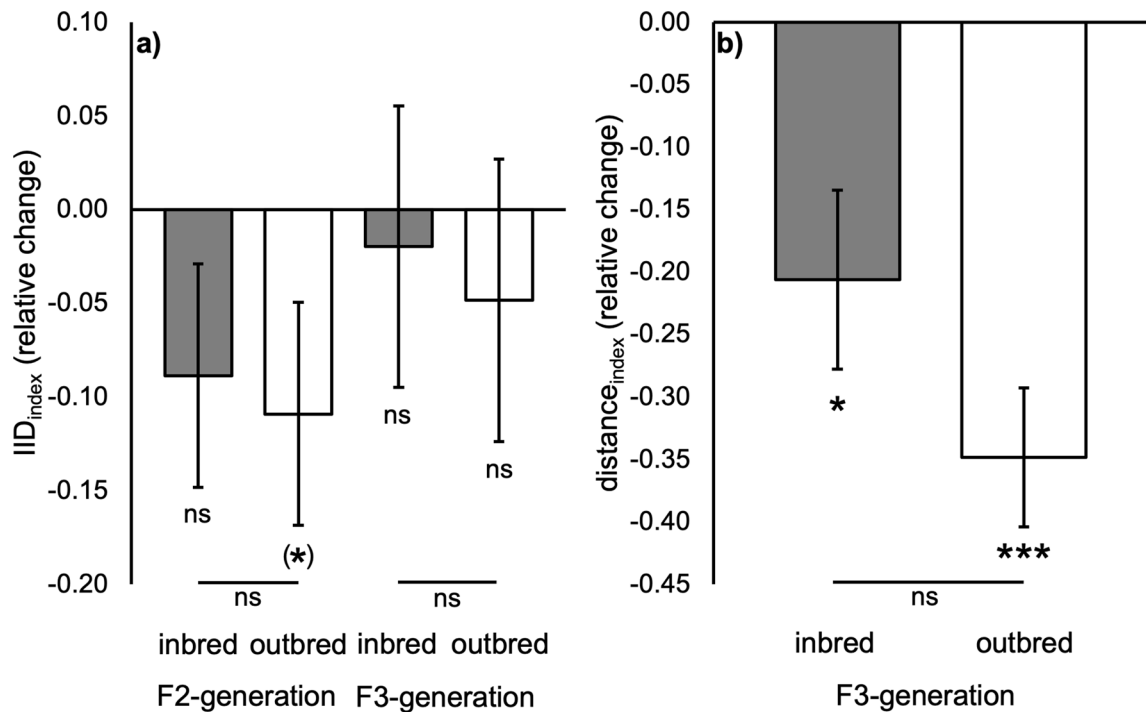
Fig. 3 Relative changes (indices) of **a** the inter-individual distance (IID_{index}) and **b** the distance travelled ($distance_{index}$) for shoals consisting of inbred (grey) and outbred (white) individuals. Shown are deviations from zero (no change) below each bar as well as differences between both groups. Given are mean values $\pm$ SE. ***$P < 0.001$; *$P < 0.05$; (*)$P < 0.1$; ns $= P > 0.05$

(Fig. 3a, Table 3). The travelled distance was significantly reduced following the mechanical disturbance cue when analysing inbred and outbred shoals together as well as within the inbred and outbred groups separately (Fig. 3b, Table 3).

Discussion

Software-based tracking in the F3-generation revealed that the travelled distance preceding the mechanical disturbance was significantly lower in shoals consisting of inbred individuals compared to shoals consisting of outbred *P. taeniatus*. Furthermore, the time the shoal was inactive following the disturbance was significantly shorter in shoals consisting of inbred individuals. Inbreeding did not affect the IID preceding the disturbance cue and the change in IID in both generations of *P. taeniatus*.

Shoals of the F3-generation consisting of inbred individuals covered less distance than those consisting of outbred shoal members preceding the

disturbance cue. The experimental tank was not structured and therefore there was not much information to gain about the environment and there were no resources to discover. According to optimal foraging theory, foragers should not only maximise gain but also minimise predation risk (Townsend & Winfield, 1985) and, thus, should adjust their decisions about when to forage or when to reduce activity according to their environment. Shorter travelled distance of inbred individuals may represent a less stressed behaviour and may reduce predation risk under natural conditions. A similar effect has been shown recently in *P. taeniatus* with reduced shoal activity followed by longer existence of groups, that is, intensified group member familiarity (Schons et al., 2021). Although, the optimal level between activity and inactivity depends on a trade-off because reduced activity lowers encounter rates with predators but also with food resources. Alternatively, inbred fish might be less mobile or mobility comes at a greater energetic cost due to potential inbreeding depression. Whether such effects still occur under natural

conditions, when resources as well as predation risk are highly variable in time and space, remains open.

The time of collective inactivity of a shoal (freezing time) can be assumed to be dependent on interactions amongst shoal members. Thus, the reduced freezing time, i.e. the faster recovery from the disturbance, of shoals consisting of inbred individuals, which was significantly lower in the F3-generation, may represent a more coordinated response to the mechanical disturbance cue in comparison to the shoals consisting of outbred *P. taeniatus*. The used mechanical disturbance cue resulted in the intended fright reaction, i.e. freezing, of all shoals. However, the simulated threat was of short duration and did not come along with additional sensory stimuli, e.g. visual or olfactory cues. Consequently, a short-termed reaction and a prompt resumption of shoaling expressed by the inbred individuals might represent an appropriate response to such a disturbance and could be a result of improved cooperative behaviour within shoals consisting of inbred individuals in terms of accurate shared decision-making. Within-group conflicts of interest, e.g. shoal members avoiding to restart moving first (being most conspicuous when moving solitarily), might be more pronounced in the outbred group and may reduce decision-making accuracy compared to shoals of inbred individuals. Relatedness promoting cooperation in *P. taeniatus* has been observed before in terms of predator inspection behaviour (Hesse et al., 2015b) and shoaling (Hesse & Thünken, 2014). Also inbreeding promoting cooperative behaviour of *P. taeniatus* during parental care has been shown before (Thünken et al., 2007a). Nevertheless, as we cannot evaluate the functional costs and benefits of the observed differences, further studies are needed to draw a final conclusion because faster recovery from a disturbance might also be disadvantageous in a risky environment.

The IID is a common measure of shoaling performance in groups of fishes and was also used to study shoaling in *P. taeniatus* before (Meuthen et al., 2016). Abiotic environmental factors were shown to affect IID in fishes. In brown trout fry (*Salmo trutta* Linnaeus 1758) and delta smelt (*Hypomesus transpacificus* McAllister 1963), the IID increases with elevated temperatures (Colchen et al., 2016; Davis et al., 2019). Biotic factors seem to have less pronounced impact on the IID. In three-spined sticklebacks, an infection with the cestode *Schistocephalus*

solidus (Müller 1776) did not significantly affect the IID (Demandt et al., 2021). The risk of predation, mediated by alarm cues, was also shown to affect the shoal density in fishes. Banded killifish (*Fundulus diaphanus* Lesueur 1817) form larger groups in the presence of an alarm cue and larger groups were shown to have greater density/decreased neighbour distances (Hoare et al., 2004). Similar effects were described for the x-ray tetra (*Pristella maxillaris* Ulrey 1894) with more tightly clustered shoals as a response to alarm cues (Schaerf et al., 2017). A study on *Pelvicachromis pulcher* (Boulenger 1901), a closely related species to *P. taeniatus*, did not find significant effects of resource heterogeneity or inbreeding on the IID (Schons et al., 2022). Also studies on *P. taeniatus* in which the IID was used to quantify shoaling cohesion showed no significant effects on the IID regarding environmental habituation and sexual composition (Schons et al., 2021) or perceived predation risk, i.e. being raised under conspecific alarm cues, which signal predation risk (Meuthen et al., 2016). In the present study, the IID was not significantly different between shoals consisting of inbred and outbred individuals. Inbreeding seems to have no significant effect on shoal cohesion, i.e. IID, in *P. taeniatus*. However, the IID was reduced, but not significantly, after the disturbance and tended to deviate from zero (no change) in the outbred group of the F2-generation. Absent effects on IID might be caused by the setup which consisted of an unstructured tank in which all members of the shoal could monitor their surroundings. Thus, a more structured setup, including areas hidden from view, may have made shoal cohesion more important. Increased relatedness due to inbreeding might affect cooperation within shoals moving through structured habitats and, thus, may influence cohesion under natural conditions. Whether social organisation of free-ranging *P. taeniatus* is affected by inbreeding remains open. Generally, little is known about the size, composition and dynamics of free-ranging groups of fishes (Krause et al., 2000; but see Ward et al., 2017). The changes in distance travelled, swimming speed and IID following the mechanical disturbance cue were not significantly different between shoals consisting of inbred and outbred *P. taeniatus*. Both inbred and outbred shoals responded to the disturbance by decreasing activity, but they did not differ significantly in this respect.

In the F3-generation, variance of standard length within shoals tended to affect the travelled distance preceding and significantly affected time freezing after the mechanical disturbance cue was applied. Many benefits of shoaling, e.g. the predator confusion effect, are maximised by homogeneity in appearance (e.g. Krause et al., 1996). Consequently, homogeneity is an important aspect in shoaling which has been shown to affect shoaling decisions in fishes (reviewed in Krause et al., 2000). Homogeneity of shoals also affects shoaling behaviour within *P. taeniatus* shoals with high homogeneity causing tighter shoals (Meuthen et al., 2016). In this study, there was a positive correlation between variance of standard length and the travelled distance as well as time freezing following the disturbance cue. As described above, less active shoaling seems to be a beneficial behaviour in the experimental setup, that is, an unstructured environment which may reduce predation risk under natural conditions. Here, heterogeneity (higher variances in standard length within shoals) may impair shoaling performance and lead to higher activity before and longer inactivity after the disturbance cue.

In summary, this study shows effects of inbreeding on shoaling performance in juvenile cichlid fish. Reduced travelled distance and less time inactive following a mechanical disturbance cue in shoals consisting of inbred *P. taeniatus* compared to those consisting of outbred individuals is likely caused by improved coordination within shoals which leads to more adequate and calmer shoaling performance. In a limited and unstructured environment without any resources, higher activity levels are wasteful and may attract predators. Under natural conditions—including a spacious and structured environment, predation risk as well as competition for food resources—the positive effects of inbreeding resulting from kin selection can be expected to be more pronounced and may affect individuals' survival.

Declarations

Conflict of interest We declare that we have no competing interests.

Ethical approval The present study follows the Animal Behaviour Society guidelines for the use of animals in research, as well as the legal requirements of Germany and was conducted in accordance with German laws for animal experiments. Experiments were approved by the regional office for nature, environment and consumer protection North Rhine–Westphalia (LANUV NRW, 237 reference no. 84e02.04.2015.A580).

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