



Evolutionary implications of size-selective mortality on the ontogenetic development of shoal cohesion: a neurochemical approach using a zebrafish, *Danio rerio*, harvest selection experiment

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Abstract

Size-selective mortality may evolutionarily alter life-history as well as individual behavioral and physiological traits. Moreover, size-selective mortality can affect group behavioral traits, such as shoaling and collective properties (e.g., shoal cohesion), which are relevant for finding food and reducing risk of predation. Here, we present experimental evidence using selection lines of zebrafish (*Danio rerio*) that were exposed to positive (large-harvested), negative (small-harvested), and random (control) size-selective mortality for five generations, followed by eight generations during which harvesting was halted to remove maternal effects and to study evolutionarily fixed outcomes. We investigated changes in shoal cohesion and turnover in monoamines in zebrafish through ontogeny. To that end, we repeatedly measured inter-individual distance in groups of eight fish and the turnovers of dopamine and serotonin in brains of fish from juvenile to the adult stage at 40-day intervals. We, firstly, found that shoal cohesion was overall consistent through ontogeny at group levels suggesting the presence of collective personality. Secondly, we found a decrease in shoal cohesion through ontogeny in the small-harvested and control lines, while the large-harvested line did not show any ontogenetic change. Thirdly, the selection lines did not differ among each other in shoal cohesion at any ontogenetic stage. Fourthly, dopamine turnover increased through ontogeny in a similar way for all lines while the serotonin turnover decreased in the large-harvested and control lines, but not in the small-harvested line. The large-harvested line also had higher serotonin turnover than controls at specific time periods. In conclusion, intensive size-selective mortality left an evolutionary legacy of asymmetric selection responses in the ontogeny of shoal cohesion and the underlying physiological mechanisms in experimentally harvested zebrafish in the laboratory.

Significant statement

The evolution of animal behavior can be affected by human activities both at behavioral and physiological levels, but causal evidence is scarce and mostly focusing on single life-stages. We studied whether and to what extent size-selective harvesting, a common selection pattern in fisheries, can be an evolutionary driver of the development of shoal cohesion during ontogeny. We used a multi-generation experiment with zebrafish to study cause-and-effects of opposing size-selection patterns. We quantified shoal cohesion, and serotonin and dopamine turnover in the brain. We found that shoal cohesion emerged as a collective personality trait and that behavioral and physiological responses were asymmetrical with respect to the opposing selection patterns.

Keywords Fisheries-induced evolution · Shoaling · Collective personality · Dopamine · Serotonin · Zebrafish

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Introduction

Size-selective mortality is a common evolutionary driver across most fish species (Sogard 1997; Lorenzen 2000). Under natural patterns of size-selective mortality, e.g., gape-limited predation, smaller individuals often suffer higher

mortality than larger ones (Sogard 1997; Urban 2007). However, in small-bodied fish species, it is also possible that larger individuals may suffer higher mortality than smaller ones within a cohort because they provide larger absolute amount of energy per predation event and larger body size may not offer safety from predation (Johansson et al. 2004; Weitz and Levin 2006).

Predator and fishing preferences for specific fish sizes can be a strong driver of predator–prey coevolution (Mougi 2012; Edeline and Loeuille 2021). Anthropogenic factors like harvesting, where larger fish typically experience greater mortality rates than smaller individuals, can interact with natural selection and modify patterns of size-selective mortality expected from natural selection alone (Olsen et al. 2012; Heino et al. 2015; Czorlich et al. 2022). For example, harvest and natural size selection may have similar strength but opposing directions in fish (Monk et al. 2021). Specific harvest regulations or fishing gear functioning may also result in smaller individuals experiencing greater harvesting rates than larger ones, e.g., in response to maximum length limits or harvest slot regulations where intermediate fish are harvested (Ahrens et al. 2020). Both size-selective mortality patterns (selective mortality acting on either small or large fish as is common in natural predation or fisheries, respectively) may evolutionarily alter life-history traits in exploited populations (Conover and Munch 2002; Uusi-Heikkilä et al. 2015; Renneville et al. 2020; Czorlich et al. 2022). Changes in life-history can, in turn, be associated with changes in individual behavioral traits, as suggested by experimental (Diaz Pauli et al. 2019; Sbragaglia et al. 2019a; Monk et al. 2021) and theoretical studies (Andersen et al. 2018; Claireaux et al. 2018). Moreover, size-selective mortality can affect individual physiological traits (Hollins et al. 2018; Koeck et al. 2018; Renneville et al. 2020; Sbragaglia et al. 2021b), as well as group behavioral traits, such as shoaling (Sbragaglia et al. 2022). Shoaling plays a paramount role in group-living species in obtaining food and escaping predators (Pitcher 1986; Krause and Ruxton 2002). Shoaling is also one of the key drivers in the capture process of many fishing gears, such as trawls and seines (Parrish 1999; Hollins et al. 2019). Therefore, changes in shoaling behavior in response to size-selective mortality can play a relevant role in population, food web, and fisheries dynamics (Sbragaglia et al. 2021a).

Size-selective mortality can affect shoaling through a range of mechanisms, for example, by modifying individual behavioral traits (Sbragaglia et al. 2021a), which alter the interaction rules among individuals (Couzin and Krause 2003). Size-selective mortality can impact individual vigilance and consequently change shoal cohesion, as reported in zebrafish, *Danio rerio* (Sbragaglia et al. 2022). Phenotypic changes in shoal cohesion can have an evolutionary underpinning as shown by rapid evolution in

response to artificial selection in guppies *Poecilia reticulata* (Kotrschal et al. 2020). Despite accumulating evidence on the potential evolutionary mechanisms governing changes in shoaling behavior in response to predation (Huizinga et al. 2009; Herbert-Read et al. 2017) and to selective harvesting (Arlinghaus et al. 2017; Diaz Pauli and Sih 2017; Sbragaglia et al. 2021a), there is one crucial aspect that has been largely overlooked and that is how animal behavior changes through ontogeny (Stamps and Groothuis 2010; Cabrera et al. 2021). Most research on the effects of size-selective mortality is restricted to characterize behavioral responses at specific life-stages (but see how ontogeny can influence the effects of size-selective mortality on risk-taking behavior; Roy and Arlinghaus 2022) and this may not reveal the full picture of changes induced by size-selective mortality. For example, selection for small body size in zebrafish increased individual boldness in juveniles (Uusi-Heikkilä et al. 2015), but decreased boldness in adult females (Sbragaglia et al. 2019a). Considering that individual behavior often changes through ontogeny (Stamps and Groothuis 2010; Cabrera et al. 2021) and that the heterogeneity of individual traits can affect group behavior (Jolles et al. 2020), it is conceivable that size-selective mortality could affect the ontogeny of group behavior. A recent study showed that group risk-taking behavior increased in adult zebrafish when small fish were selectively harvested (Sbragaglia et al. 2021b) and this change has been found to be consistent through ontogeny (Roy and Arlinghaus 2022). Understanding how size-selective mortality affects the ontogeny of shoaling behavior and how it can impact population and food web dynamics, is poorly understood.

Shoaling behavior in fish may change through ontogeny (Magurran 1986; Masuda and Tsukamoto 1998; Fukuda et al. 2010) meaning that juveniles and adults may not aggregate in a similar way. For example, in situ observations in sardines *Sardina pilchardus*, an obligate schooling species, indicated that fish form more cohesive shoals as juveniles than adults (Tsagarakis et al. 2012). Laboratory studies in zebrafish and medaka, *Oryzias latipes*, revealed that shoaling towards conspecifics increased with increasing ontogenetic age (Buske and Gerlai 2011; Isoe et al. 2016), while in three-spined sticklebacks, *Gasterosteus aculeatus*, shoal cohesion decreased with ontogenetic age (MacGregor and Ioannou 2021). These studies indicated species-specific responses in shoal cohesion through ontogeny.

Most behavioral processes have a physiological underpinning (Laland et al. 2011; Sih et al. 2015). For example, brain serotonergic and dopaminergic systems have been suggested as key modulators of several behavioral traits (Coppens et al. 2010; Winberg and Thörnqvist 2016; Soares et al. 2018). Indeed, changes in shoal cohesion in zebrafish have been linked to changes in the turnover of dopamine and serotonin in the brain (Buske and Gerlai 2012; Oliveira 2013).

Specifically, an increase in shoal cohesion across ontogeny has been found to be accompanied by an increase in dopamine and its metabolite, while serotonin and its metabolite followed an inverted U trajectory, i.e., an initial increase followed by a decrease across ontogeny (Mahabir et al. 2013). Similarly, dopamine receptors (*drd2a* and *drd2b*) have a higher expression in bold than shy zebrafish (Thörnqvist et al. 2019), and serotonin has been related to differences in aggressive behavior (Backström and Winberg 2017). The brain serotonergic system seems to have effects that, at least to some extent, are opposite to those of the dopaminergic system (Winberg and Thörnqvist 2016; Backström and Winberg 2017). Given this documented link between shoal cohesion and monoamine turnover in the brain, it is possible that alteration of shoal cohesion due to size-selective mortality could be associated with changes in the dopaminergic and serotonergic system.

In this study, we asked if size-selective mortality evolutionarily fosters change in shoal cohesion through ontogeny and whether this is associated with changes in the dopaminergic and serotonergic turnover in the brain. We addressed this question by taking advantage of three size-selected lines that were generated in a multi-generation selection experiment (large-harvested line: mimicking most commercial and recreational fisheries; small-harvested line: representing specific fisheries or gape-limited natural predation; random-harvested line with respect to size as control; Uusi-Heikkilä et al. 2015). Previous studies with these selection lines showed that shoaling and group risk taking was altered by size-selective harvesting (Sbragaglia et al. 2021b, 2022). In groups, the small-harvested line took significantly more risks (Sbragaglia et al. 2021b) and formed more cohesive groups as adults while the large-harvested line formed less cohesive groups than the controls (Sbragaglia et al. 2022). These studies were limited to the adult life stage. Any possible impact of size-selective harvesting on the ontogenetic change in group risk-taking behavior has only been studied by Roy and Arlinghaus (2022) revealing that the small-harvested line were consistently bolder while the large-harvested line did not differ in boldness from the controls across juvenile (22 days post-fertilization; DPF) to adult (190 DPF) life-stages.

Here we investigate the change in shoal cohesion in fish from juvenile (30 DPF) to adult (190 DPF) stages using the abovementioned zebrafish selection lines. Because group functioning with respect to exploration and foraging is influenced by coordination and shoal cohesion (Jolles et al. 2017; Wilson et al. 2019), changes in group risk-taking during feeding could be related to changes in shoaling behavior. Following Roy and Arlinghaus (2022), we expected that shoal cohesion, like group risk-taking, will emerge as a collective personality trait through ontogeny in zebrafish across all selection lines. Knowing that larval and juvenile zebrafish

tend to shoal less than the adults (Buske and Gerlai 2011), we expected that shoal cohesion will increase with ontogenetic age among all zebrafish lines. We further expected that size-selective harvesting will impact the change in shoal cohesion through ontogeny and that the small-harvested line will form less cohesive shoals while the large-harvested line will not differ in shoal cohesion compared to the control line. As changes in shoal cohesion are associated with change in monoamine expression in zebrafish brains (Mahabir et al. 2013), we also examined if the turnover of dopamine and serotonin changed among fish across selection lines from age 50 to 210 DPF. We expected that the turnover of monoamines would increase through ontogeny among all lines and that the small-harvested line would show a lower expression and the large-harvested line no difference in turnover compared to the control line.

Materials and methods

Zebrafish selection lines

We used the F_{13} of three zebrafish selection lines (Fig. 1A–B), each with a replicate (Uusi-Heikkilä et al. 2015). Briefly, a wild-caught zebrafish population was subjected to a strong directional harvest (a 75% per-generation harvest rate) acting on the large (generating the large-harvested line mimicking most commercial and recreational fisheries), small (generating the small-harvested line representing specific fisheries or gape-limited natural predation), and random (control) fish for five consecutive generations (Uusi-Heikkilä et al. 2015), followed by a relaxation of selection for eight generations to remove maternal effects (Mousseau and Fox 1998). Each selection line was replicated two times, which adds up to six selection lines. After the selection was halted, the lines differed not only in life-history, behavioral and physiological traits (Uusi-Heikkilä et al. 2015, 2016; Sbragaglia et al. 2019a, b, 2022; Roy et al. 2021), but also in gene expression and allele frequencies (Uusi-Heikkilä et al. 2015, 2017; Sbragaglia et al. 2021b). In the present study, we examined the evolutionary outcomes eight generations after the selection was stopped to test for adaptive changes that became fixed after five generations of intensive size-selection. The six selection lines were reared in six different tanks in a common garden set-up under the following conditions: water temperature at 26 ± 0.5 °C; photoperiod of 12:12-h LD; and ad libitum feeding (TetraMin, Tetra). At F_{12} , we randomly selected groups composed of two females and four males to create the next generation used in the experiments here. Spawning has been carried out as previously described for these selection lines (Uusi-Heikkilä et al. 2018; Sbragaglia et al. 2019b). After hatching, we maintained the larval fish in 3 l boxes and fed them

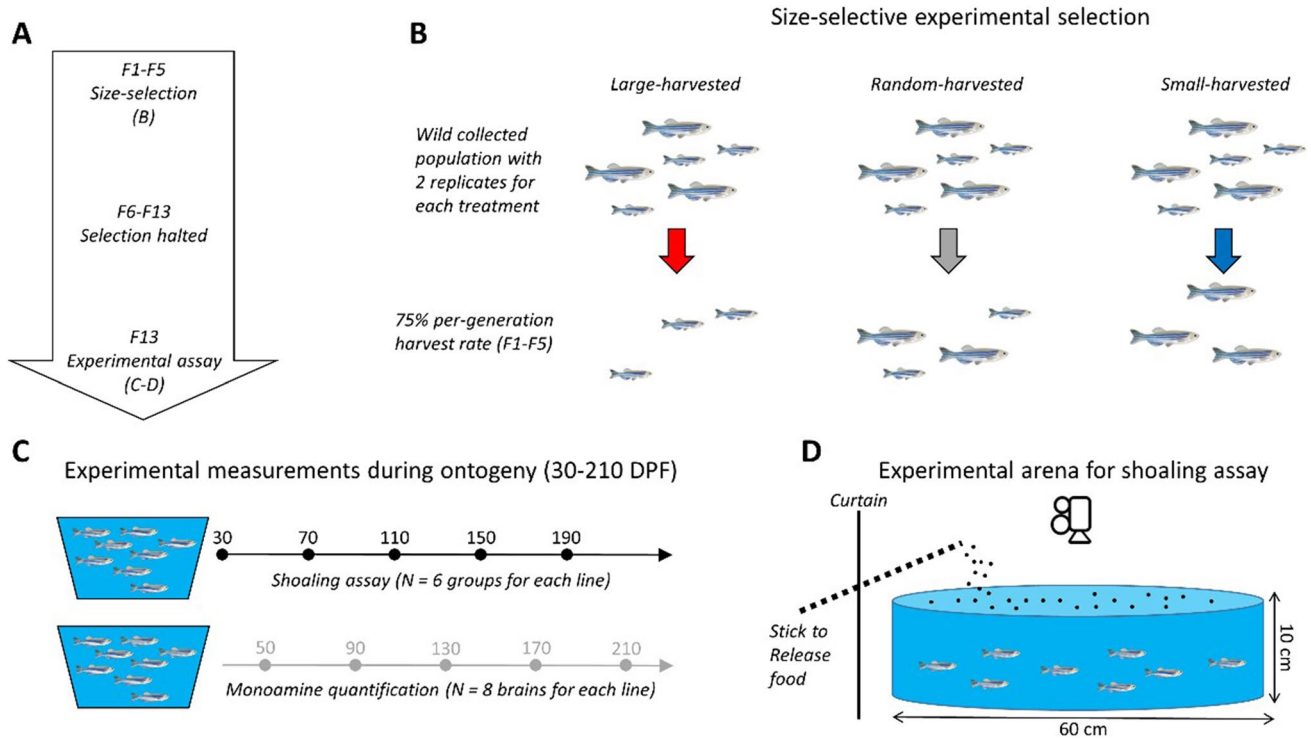


Fig. 1 Panel **A** shows a schematic representation of the experimental approach presented in this study. Panel **B** shows the process of size-selective harvesting (Uusi-Heikkilä et al. 2015). Panel **C** shows the experimental timeline. Assays were conducted at F_{13} across ontogeny

(shoal cohesion: 30–190 DPF, $N = 6$ per line; monoamines quantification: 50–210 DPF, $N = 8$ per line). Panel **D** shows the experimental setup for the shoaling assay. Zebrafish image: <https://commons.wikimedia.org>

with dry food (TetraMin, Tetra) and *Artemia*. At 30 DPF, we randomly sorted zebrafish from F_{13} into experimental groups. We used separate fish for testing shoal cohesion and monoamines levels (see next section).

Experiments

We randomly stocked eight fish (age 30 DPF) into 3-l rearing boxes, using 36 groups (six replicates per each of the six selection lines; 12 groups per treatment) to test the development of shoal cohesion through ontogeny, and 30 groups (five replicates per each of the six selection lines; 10 groups per treatment) to test for ontogenetic change in monoamines in the zebrafish brains. The fish were fed ad libitum with dry food (TetraMin, Tetra) three times per day throughout the period of experiments and maintained under the same conditions reported previously. We conducted the experimental trials every 40 days, testing shoal cohesion from 30 to 190 DPF and measuring the monoamines turnover from 50 to 210 DPF (Fig. 1C).

Behavioral assay

We measured inter-individual distance among the fish groups in a white circular arena (diameter 60 cm) with

10 cm water (Fig. 1D) like in previous studies (Miller and Gerlai 2012; Miller et al. 2013). We introduced a group of zebrafish into the experimental arena by gently pouring them along with water from the holding box and allowed them to acclimate for 30 min. We then added food on the surface of water from a distance and video recorded the behavior of fish for 5 min using an overhead webcam placed 95 cm above the circular arena (Logitech C920; resolution: 1920×1080 pixels; frame rate: 30 fps) connected to a computer (Fig. 1D). We transferred the fish back to the respective holding box after the experiment. We randomized the order through which the groups were tested in the experimental arena. The experimenter who run the behavioral assays also analyzed the video recordings using EthoVision XT 9, (Noldus Information Technologies Inc.; www.noldus.com). To minimize observer bias, blinded methods were used when behavioral data were analyzed. In particular, to reduce observer bias in setting the automated tracking parameters, all the videos related to each one of the 5 time points (control and treatments together; $N = 36$) were analyzed with the same EthoVision configuration that was set on a random video from both control and treatments (three videos in total). Inter-individual distances of the groups were extracted from the automated tracking of videos at a frequency of 10 positions per second (i.e., 3000 positions for

each fish in each behavioral assay). EthoVision tracked the position of the eight fish within the group with an error rate of (mean \pm standard deviation) $7.37 \pm 1.77\%$ (i.e., frames in which EthoVision did not locate one or more of the fish). The error rate was not correlated with the inter-individual distance ($r_{178} = 0.02$; $p = 0.722$), and it did not significantly differ: (i) across ontogeny ($F_{4,165} = 0.539$; $p = 0.707$); (ii) overall among the selection lines ($F_{2,165} = 0.012$; $p = 0.987$); (iii) among the selection lines within each ontogenetic time and, vice versa, across ontogeny within the same selection line ($F_{8,165} = 1.464$; $p = 0.174$).

Monoamines quantification

We measured monoamines in the different selection lines at each time point by lethally sampling eight fish brains per line every 40 days from 50 to 210 DPF (16 brains per treatment; Fig. 1C). We euthanized fish using clove oil, dissected the brain out using micro-scissors under a stereomicroscope, immediately transferred it into liquid nitrogen and stored it at $-80\text{ }^{\circ}\text{C}$. Brain levels of norepinephrine (NE), serotonin (5-HT), 5-HT metabolite 5-hydroxyindole-3-acetic acid (5-HIAA), dopamine (DA), and the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were analyzed using high-performance liquid chromatography with electrochemical detection (HPLC-EC) as described by Øverli et al. (1999) with some modifications. Briefly, we homogenized the frozen brains in 0.25 ml ice-cold acetate buffer (pH 5.0) containing 10 ng ml⁻¹ 3,4-dihydroxybenzylamine (DHBA, as internal standard) using a Sonifier cell disruptor B-30 (Branson Ultrasonics, Danbury, CT, USA) and centrifuged at 21,000 g for 10 min at 4 °C. We used the supernatant to quantify the amounts of monoamines in the samples using HPLC-EC. The HPLC-EC system consisted of a solvent delivery system model 582 (ESA, Bedford, MA, USA), an autoinjector Midas type 830 (Spark Holland, Emmen, The Netherlands), a reverse phase column (Reposil-Pur C18-AQ 5 μm , 150 \times 4 mm column, Dr Maisch HPLC GmbH, Ammerbuch-Entringen, Germany) kept at 40 °C, and an ESA 5200 Coulochem II EC detector (ESA, Bedford, MA, USA) with two electrodes at reducing and oxidizing potentials of -40 and $+320$ mV. A guarding electrode with a potential of $+450$ mV was employed before the analytical electrodes to oxidize any contaminants. The mobile phase consisted of 75 mmol l⁻¹ sodium phosphate, 1.4 mmol l⁻¹ sodium octylsulphate, and 10 $\mu\text{mol l}^{-1}$ EDTA in deionized water containing 7% acetonitrile brought to pH 3.1 with phosphoric acid. Samples were quantified by comparison with standard solutions purchased from Sigma (MERCK; NE: A9512; 5-HT: H7752; 5-HIAA: H8876; DA: H8502; DOPAC: 850217; HVA: H1252) with a concentration of 10 ng/ml. DHBA (Sigma MERCK: 858781) was used as the internal standard to correct for recovery using

HPLC software Clarity™ (DataApex Ltd., Prague, Czech Republic). We used the protein content in the pellet after centrifugation for normalization of brain monoamine levels. We resuspended the pellets in 100 μl of 20 mM Tris buffer pH 7.5, centrifuged at 2000 g for 1 min, and the amount of brain proteins in the supernatant were measured in a Qubit Fluorometer (Invitrogen) using Qubit protein assay kit Q33212. Finally, turnover was calculated as the ratio between monoamines and their metabolites (DOPAC/DA and 5-HIAA/5HT).

Statistical analysis

We applied an exponential lambda transformation to obtain response variables with a normal distribution (mean inter-individual distance and concentrations of monoamines). We first estimated the adjusted repeatability following Nakagawa and Schielzeth (2010) across two consecutive time points (age) for all selection lines together, considering selection line as fixed effect. Then, we estimated the overall repeatability across all time points for each selection line considering age as the fixed effect. We included selection line replicate as random intercept in the models. The estimation of behavioral repeatability and consistency across ontogeny informed if shoal cohesion emerged as a collective personality trait like group risk-taking behavior (Roy and Arlinghaus 2022; Sbraglia et al. 2021b).

We used linear mixed-effects models to test if selection lines differed in shoal cohesion and monoamine expression in the brain through ontogeny. Specifically, we used the transformed measures of inter-individual distance and monoamines as dependent variables and interaction of selection lines and ontogenetic age as fixed effect. We used group IDs nested within line replicate as random effects for inter-individual distance, and only line replicates as random effect for monoamines. We conducted Tukey's post hoc tests for multiple comparisons. We examined model fitting by checking the normality of residuals and plotting theoretical quantiles vs. standardized residuals. We used the "rcompanion" package for power transformation (Mangiafico and Mangiafico 2017), "rptR" for calculating repeatability (Stoffel et al. 2017), "lme4" package for constructing linear mixed models (Bates et al. 2012), and "lmerTest" for obtaining p -values (Kuznetsova et al. 2017). All analyses were conducted in R 3.5.0 (R Development Core Team 2018).

Results

We found a significant repeatability ($p < 0.001$) of shoal cohesion between consecutive ages for all selection lines ($R = 0.50$ to 0.62; Table 1; Fig. 2A–D). When testing consistency in shoal cohesion within each line, we did not find a significant

Table 1 Variance partitioning (V_g = among-group variance; V_r = residual or within-group variance) after linear mixed-effects models for inter-individual distance (shoal cohesion) and the repeatability estimates. Repeatability estimates are shown along with the confidence intervals (R [CI]) and p values. Significant results are in bold and marginal non-significance is indicated with “†.” *LH*, large-harvested line; *RH*, random-harvested line; *SH*, small-harvested line

Age	N	Inter-individual distance			
		V_g	V_r	R [CI]	p
30, 70	36	0.00190	0.00179	0.50 [0.21–0.72]	< 0.01
70, 110	36	0.00163	0.00110	0.58 [0.29–0.77]	< 0.01
110, 150	36	0.00114	0.00072	0.56 [0.30–0.77]	< 0.01
150, 190	36	0.00089	0.00051	0.62 [0.35–0.80]	< 0.01
Lines	N	V_g	V_r	R [CI]	P
LH (30–190)	12	0.00014	0.00128	0.02 [0–0.27]	0.17
RH (30–190)	12	0.00023	0.00107	0.11 [0–0.37]	0.06†
SH (30–190)	12	0.00051	0.00117	0.30 [0.01–0.56]	< 0.01

repeatability for inter-individual distance in the large-harvested line ($R=0.02$, $p=0.17$; Table 1; Fig. 2E), marginally non-significant repeatability in the control line ($R=0.11$, $p=0.06$; Table 1; Fig. 2F) and significant repeatability in the small-harvested line ($R=0.3$, $p<0.01$; Table 1; Fig. 2G).

We found a significant interaction effect of ontogenetic age and selection line on shoal cohesion ($\chi^2_{8,132} = 16.25$; $p<0.05$; Table 2). Specifically, ontogenetic differences in shoal cohesion were detected in the small-harvested and control lines, but not in the large-harvested line (Fig. 3). Zebrafish groups of the control line formed significantly less cohesive groups as adults at 110 ($p<0.05$) and 150 ($p<0.01$) DPF compared to juveniles at 30 DPF (Fig. 3). Similarly, zebrafish groups of the small-harvested

line formed significantly less cohesive groups as adults at 110 ($p<0.05$) DPF compared to juveniles at 30 DPF (Fig. 3). We did not detect significant differences in shoal cohesion among the selection lines within each ontogenetic age (Fig. 3).

We found a significant interaction effect of age and selection line on both dopamine ($\chi^2_{8,132} = 20.50$; $p<0.01$) and serotonin ($\chi^2_{8,132} = 20.62$; $p<0.01$) turnover in the zebrafish’s brains (Table 2). Fish across all lines had significantly lower turnover as subadults at 50 DPF ($p<0.01$) compared to adults at ontogenetic ages 90–210 DPF (Fig. 4). The control line had also significantly lower ($p<0.05$) dopamine turnover at 90 and 170 DPF compared to 130 and 210 DPF (Fig. 4). We did not detect significant differences in dopamine turnover among the selection lines through ontogeny (Table 2; Fig. 4). We further found that the serotonin turnover in the large-harvested line was significantly lower ($p<0.05$) at 170 and 210 DPF compared to 130 DPF (Fig. 5). The turnover in the control line closely showed an inverted U-shaped pattern where it was significantly lower at 50 DPF compared to 130 DPF ($p<0.05$) and at 210 DPF compared to other ontogenetic ages ($p<0.01$; Table 2; Fig. 5). The small-harvested line did not show significant differences in serotonin turnover across ontogeny (Fig. 5). As adults at 210 DPF, the large-harvested line had a significantly higher serotonin turnover ($p<0.05$) than the control line (Fig. 5).

Discussion

Our results demonstrated that intensive (75%) size-selective mortality caused ontogenetic changes in shoal cohesion and monoamines turnover in zebrafish. However, the changes were asymmetric with respect to the selection

Fig. 2 Inter-individual distance across ontogeny in groups of 8 zebrafish across three selection lines; large-harvested (LH in red color), random-harvested (RH in gray color), and small-harvested (SH in blue color). The top panels show transition in behavior across consecutive time points through ontogeny together for all selection lines ($N=36$) and the bottom panels show change in inter-individual distance across five time points through ontogeny for each selection line ($N=12$)

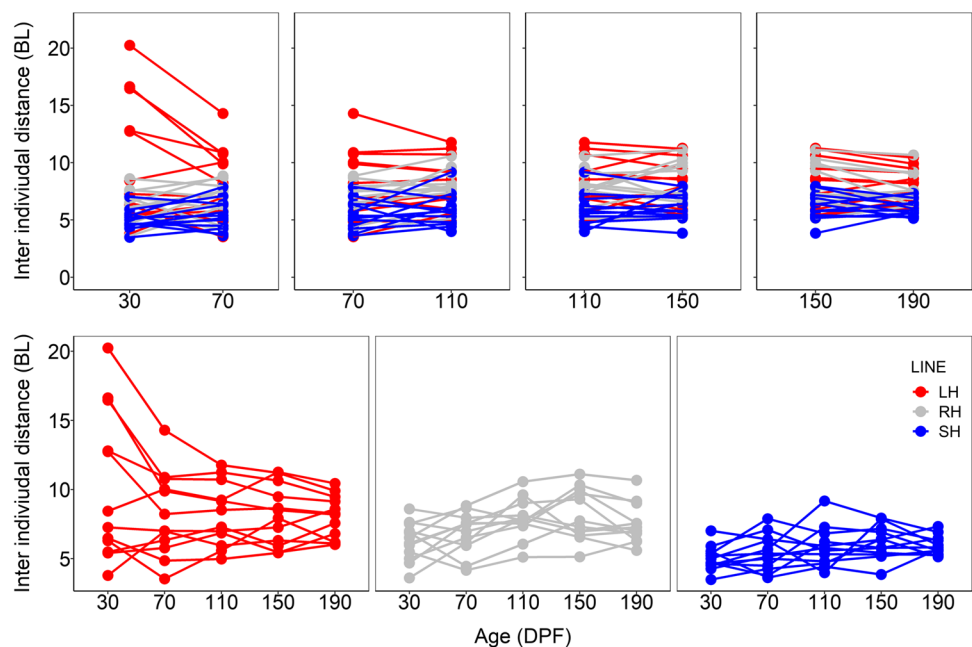


Table 2 Model estimates and *p* values from linear mixed-effects models constructed for testing change in turnover of monoamines (DOPAC/DA and 5HIAA/5HT) across ontogeny. Significant results are in bold

Response variable	Fixed effect	df	X ²	<i>p</i>
Inter-individual distance	Age	4	2.57	0.63
	Line	2	5.38	0.07
	Age × line	8	16.25	< 0.05
Turn over dopamine	Age	4	34.39	< 0.01
	Line	2	1.53	0.46
	Age × line	8	20.50	< 0.01
Turn over serotonin	Age	4	14.76	< 0.01
	Line	2	0.65	0.72
	Age × line	8	20.62	< 0.01

treatments: shoal cohesion changed across ontogeny only in the small-harvested line relative to the control; dopamine turnover changed in the same directions for both size-selective treatments; and serotonin turnover changed only in the large-harvested line with respect to control. Asymmetrical selection responses in size-selection experiments is a recurrent finding in similar experimental harvesting systems based on small-bodied model fish, indicating the complexity involved in artificial evolutionary experiments in terms of responses to selection drivers (Le Rouzic et al. 2020; Renneville et al. 2020; Crespel et al. 2021b; Bartuseviciute et al. 2022). We discuss four major results and stress

the significance of this study to advance the understanding of evolutionary processes involved in size selection.

We found group consistency in shoal cohesion through ontogeny, indicating shoaling to be a collective personality trait in zebrafish, similar to other species (Bengston and Jandt 2014; Jolles et al. 2018). The finding implied that intergroup differences in shoal cohesion were present within life-stage and that consistent intergroup differences were maintained through ontogeny, much like it was reported for three-spined sticklebacks, *Gasterosteus aculeatus* (MacGregor and Ioannou 2021). Using the same zebrafish selection lines, Sbragaglia et al. (2022) previously reported that adult zebrafish (150 and 190 days post-fertilization) showed consistency in shoal cohesion between consecutive time points under the same experimental conditions but without the addition of food. This shows that consistency of shoal cohesion in the zebrafish lines used here is not affected by a feeding context. Another recent study with the same zebrafish selection lines showed that group risk-taking is also a repeatable behavior throughout ontogeny, though not in the larval stage (Roy and Arlinghaus 2022). The result that consistency in shoal cohesion was found though ontogeny are thus in broad alignment with previous work in zebrafish focusing on other behavioral traits. Indeed, individual personality in zebrafish tends to be stable through ontogeny (Roy et al. 2017; Roy and Bhat 2018). Consistency in shoal cohesion through ontogeny could be related to the fact that individuals tend to remain in

Fig. 3 Comparison of inter-individual distance (shoal cohesion) in groups of 8 zebrafish from 30 to 210 days post-fertilization (DPF) in the selection lines (LH: large-harvested; RH: random-harvested; SH: small-harvested). Inter-individual distance is standardized to average body length of the fish group. Data points are shown for each fish group (*N*=12) together with boxplots (the horizontal line represents the median, the box corresponds to the first and third quartiles, and the whiskers represent the largest and smallest values excluding outliers). Letters represent the output of the Tukey post hoc test (*a* < *b*)

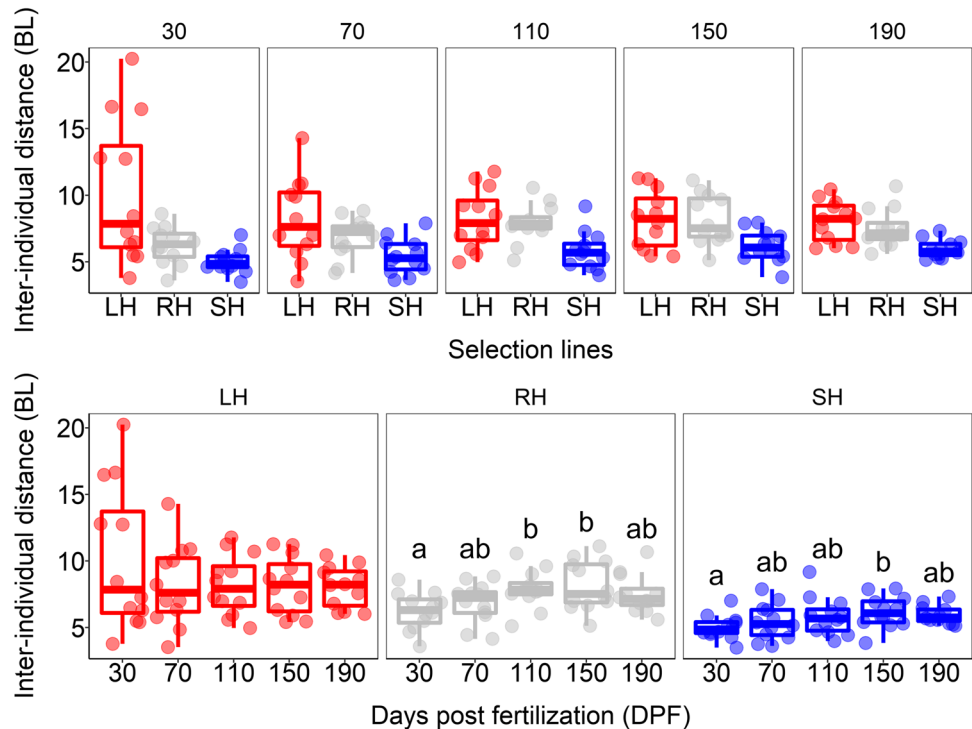


Fig. 4 Comparison of dopamine (DOPAC/DA) turnover in zebrafish brain across selection lines at each time point during ontogeny from 50 to 210 DPF (upper panel; LH: large-harvested; RH: random-harvested; SH: small-harvested), and pairwise comparisons within each line through ontogeny (lower panel). Data points are shown for each individual brain sampled ($N=16$) together with boxplots (the horizontal line represents the median, the box corresponds to the first and third quartiles, and the whiskers represent the largest and smallest values excluding outliers). Letters represent the output of the Tukey post hoc test ($a < b < c$)

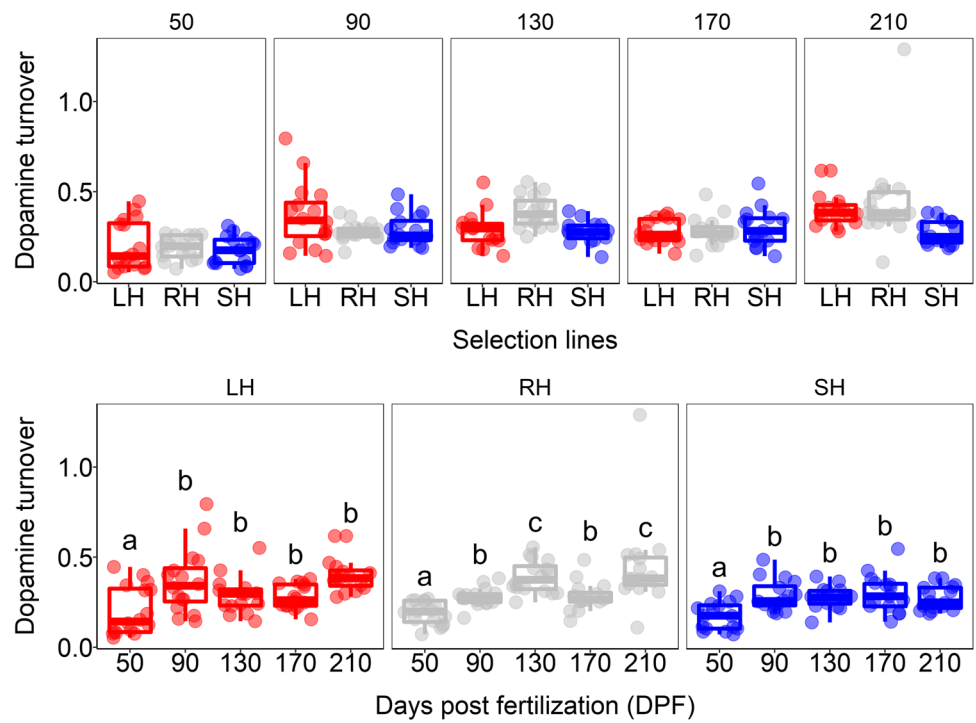
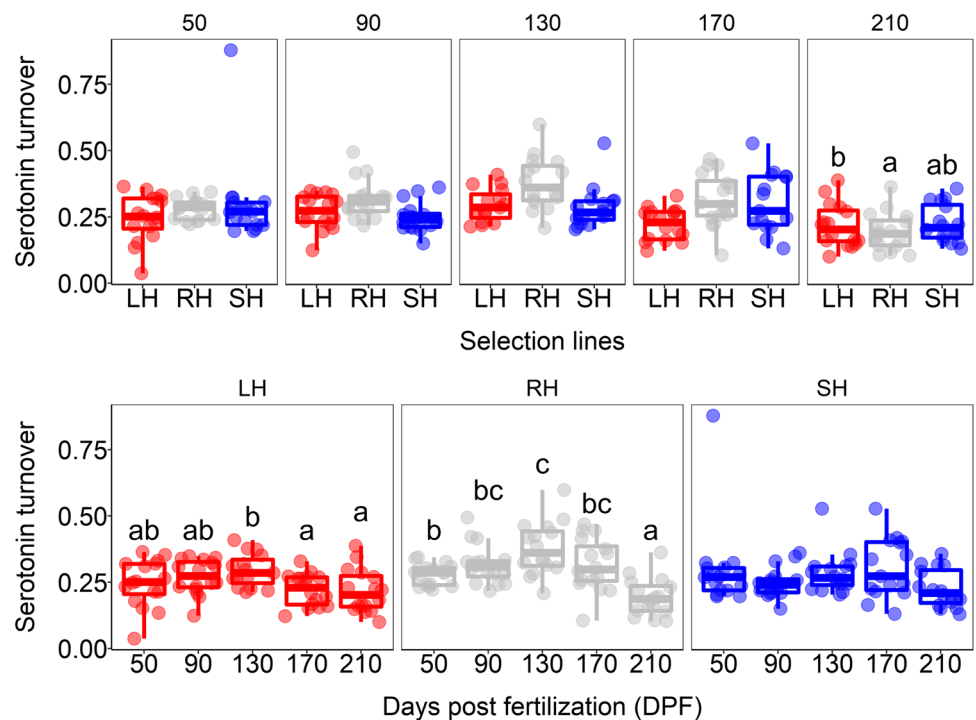


Fig. 5 Comparison of serotonin (5HIAA/HT) turnover in zebrafish brain across selection lines at each time point during ontogeny from 50 to 210 DPF (upper panel; LH: large-harvested; RH: random-harvested; SH: small-harvested) and pairwise comparisons within each line through ontogeny (lower panel). Data points are shown for each individual brain sampled ($N=16$) together with boxplots (the horizontal line represents the median, the box corresponds to the first and third quartiles, and the whiskers represent the largest and smallest values excluding outliers). Letters represent the output of the Tukey post hoc test ($a < b < c$)



close association with others or be dispersive in a similar way when measured across ontogeny (Hinz and de Polavieja 2017).

When we examined group consistency through ontogeny within selection lines, we found a significant repeatability of shoal cohesion only in the small-harvested line. These findings reveal that size-selective harvesting can

modify collective personality through ontogeny. Groups of the small-harvested line consistently take more risks during feeding through ontogeny (Roy and Arlinghaus 2022); therefore, it is conceivable that consistency in risk-taking behavior translates to consistency in shoal cohesion, thereby indicating behavioral syndrome between these collective personality traits (i.e., correlations among different

personality traits; Sih et al. 2004). However, the lack of repeatability in shoal cohesion in the large-harvested line did not align with the group risk-taking behavior reported by Roy and Arlinghaus (2022), where the large-harvested line showed significant and larger repeatability in risk-taking behavior during feeding compared to the other two lines. A possible interpretation is that the large-harvested zebrafish line has evolved a fast life-history and smaller body size compared to the control line as adults (Uusi-Heikkilä et al. 2015; Sbragaglia et al. 2019b; Roy and Arlinghaus 2022; see also Fig. S1 for ontogenetic trajectories of growth). The internal energetic trade-off between maintaining behaviors that lead to energy acquisition through foraging to accumulate resources for early and high investment into gonads (needed for fast life history), while avoiding predation due to relatively smaller body size as adults (Roy et al. 2021) could be responsible for breaking a possible behavioral syndrome between group risk-taking behavior and shoal cohesion through ontogeny. For example, shoal cohesion is probably a more complex group emergent behavior than risk-taking behavior, and the evolution of shoal cohesion could be consequently driven by individual behavioral and physiological traits (Jolles et al. 2020). Moreover, the absence of a real predation cue during the recording of shoal cohesion could have affected the expression of collective personality traits. Finally, within- and among-group variances in shoal cohesion across selection lines were lower in magnitude in the present work than what has been found by Roy and Arlinghaus (2022) for group risk-taking behavior. This suggests that shoal cohesion might be also less variable and less plastic as a group measurement than group risk-taking behavior.

We found that shoal cohesion of the small-harvested line decreased in adults compared to the juveniles (i.e., inter-individual distance increases), and this ontogenetic trajectory was similar to that of the control line. Such pattern of decrease in shoal cohesion through ontogeny does not agree with previous studies that showed that shoal cohesion increased through ontogeny in zebrafish (Buske and Gerlai 2011, 2012; Mahabir et al. 2013). A possible explanation is that such studies used well-established laboratory zebrafish strains (lines AB and TU), while we used a wild-caught zebrafish population exposed to size selection. Considering that collective behavior can rapidly evolve in response to artificial selection (Kotrschal et al. 2020), it is possible that shoal cohesion has evolved and developed differently in the different strains/subpopulations. However, our results agree with a recent study in three-spined sticklebacks where groups of fish became less social showing reduced shoal cohesion, polarization, speed and information transfer over shorter, and longer timescales (MacGregor and Ioannou 2021). Maintaining high sociality in fish while moving collectively is energetically expensive (Di Santo et al. 2017). Therefore, fish may reduce sociality when repeatedly exposed to the same experimental context

over time due to acclimatization to the low-risk environments (Miller and Gerlai 2012; MacGregor and Ioannou 2021). In terms of differences among selection line, the fact that the large-harvested line did not show a decrease of shoal cohesion through ontogeny could be related to the fact that this line has evolved a fast life-history and smaller body size compared to the control (Uusi-Heikkilä et al. 2015; Sbragaglia et al. 2019b; Roy and Arlinghaus 2022; see also Fig. S1). Indeed, although it is widely accepted that juvenile fish are at a higher risk of predation than adults (Sogard 1997), in small-bodied species such as zebrafish, adults may be at higher risk of predation than juveniles because they provide larger absolute amount of energy per predation event as showed in guppies, *Poecilia reticulata* (Johansson et al. 2004). Therefore, from an evolutionary perspective, the fact that shoal cohesion did not decrease in the large-harvested line through ontogeny with respect to control could be related to the fact that life-history evolution is linked to changes in shoal cohesion through ontogeny and consequently can be modified through size-selective harvesting. Moreover, differences in risk-taking behavior, and in particular individual vigilance at the adult stage, could be responsible for the differences observed in shoal cohesion, as recently demonstrated with the same selection lines (Sbragaglia et al. 2022).

Despite the ontogenetic changes, we did not find significant differences in shoal cohesion among selection lines at any ontogenetic stage. A previous study using these same selection lines showed that adults of the large- and small-harvested lines formed less and more cohesive shoals, respectively, compared to the controls when tested in the absence of food (Sbragaglia et al. 2022). We measured shoal cohesion in the presence of food after several hours of starvation. Zebrafish adults combine individual and social information to achieve optimal foraging efficiency and income equality in groups when food is present (Harpaz and Schneidman 2020). Consequently, zebrafish across all lines could have reduced their evolved differences in shoal cohesion to prioritize feeding. Moreover, it should be noted that although we did not find significant differences among selection lines in terms of shoal cohesion, the observed trends of shoal cohesion among the selection lines were qualitatively the same relative to our previous work, i.e., the large-harvested line being less cohesive and the small-harvest line more cohesive than the controls as adults (Sbragaglia et al. 2022). Finally, although Roy and Arlinghaus (2022) found significant differences in group risk-taking behavior during feeding through ontogeny, it should be considered that they measured time spent at the surface while we measured 2D distances among individuals. Since zebrafish shoal in 3D (Suriyampola et al. 2016), a 3D-tracking of shoaling behavior (Maaswinkel et al. 2013) might have captured the differences in shoaling among selection lines more prominently.

We found that the turnover of dopamine increased through ontogeny in a similar way across all lines. The link between the dopaminergic system and shoal cohesion is not straightforward because we found shoal cohesion to decrease across ontogeny in the small-harvested line but we found an increase in dopamine turnover across all lines. This is in contrast with the study by Buske and Gerlai (2012) that showed a simultaneous increase in both shoal cohesion and dopamine turnover in zebrafish across development. Previous studies on this topic used laboratory strains (AB and TU strains; Scerbina et al. 2012; Mahabir et al. 2013) while the size selection applied to wild-caught fish in our work could have caused the dopaminergic system to evolve differently in our study. Importantly, the levels of endogenous dopamine showed a steep increase at 210 DPF in the small-harvested line compared to the control (Table S1; Fig. S2, S3), which may be related to changes in risk-taking behavior. Indeed, previous studies with the same zebrafish selection lines at F_{13} showed that adults (230–240 DPF) of the small-harvested line took more risks than the control line (Sbragaglia et al. 2021b), and other studies in zebrafish showed that bold zebrafish have a higher expression of dopamine receptors in the brain (Thörnqvist et al. 2019). Unlike dopamine, we found that serotonin turnover decreased in the large-harvested line, while the small-harvested line did not show any change in serotonin through ontogeny compared to the control. Serotonin turnover in the control line nearly followed an inverted U-trajectory as reported in a previous study in zebrafish (Mahabir et al. 2013). Importantly, serotonin turnover reached the highest levels at 130 DPF age (adult stage) and then declined, which contrasts with Mahabir et al. (2013) where the highest levels were reached at 70 DPF (subadult stage). This suggests that artificial selection probably delays the time of peak turnover of serotonin, although strain differences (laboratory strains vs experimentally evolved lines) might equally explain the different findings. Domestication to laboratory conditions is known to affect serotonin turnover (Lepage et al. 2000), with obvious repercussion for copying styles and consequently for behavior (Puglisi-Allegra and Andolina 2015). Moreover, at 210 DPF, the large-harvested line had higher serotonin turnover than the controls, which could be linked to the evolution of life histories. As explained above, the large size-selected line evolved a fast life-history (Uusi-Heikkilä et al. 2015; Sbragaglia et al. 2019b; Roy and Arlinghaus 2022; see also Fig. S1), which can be evolutionarily linked to a low hypothalamic–pituitary–adrenal axis reactivity (Réale et al. 2010), and serotonin is known to be a regulator of hypothalamic–pituitary activity in teleost fish (Winberg et al. 1997; Winberg and Thörnqvist 2016), which could explain the changes in the serotonergic system of the

large-harvested line. Like the dopaminergic system, the link between the serotonergic system and shoal cohesion is not straightforward. Both the large- and small-harvested lines showed higher levels of endogenous serotonin than the control at 130 DPF (Table S1; Fig. S2, S3), which may be linked to aggressive behavior. Previous studies with the same zebrafish selection lines at F_{11} indicated that males of the large- and small-harvested lines at about 130 DPF were more aggressive than controls during spawning trials (Sbragaglia et al. 2019b), and serotonin expression is known to be correlated with aggressive behavior (Backström and Winberg 2017). However, differences in endogenous serotonin were not constant across ontogeny, and therefore, our interpretation is speculative. In summary, our results of the effects of size-selective mortality on the link between shoal cohesion and monoamines turnover in the brain remain explorative and inconsistent and they suggest complex evolutionary links between behavior and physiology.

Our experimental approach has limitations that are important to consider for future studies. Firstly, shoaling behavior can be characterized by different variables describing the collective motion of group members while we only focused on the inter-individual distance measured in two dimensions as an indicator of shoal cohesion. However, we recently demonstrated that in adults of the selection lines, inter-individual distance is strongly correlated to nearest-neighbor distance (i.e., changes in inter-individual distance were not related to a split of the main group into subgroups), and that there were no differences in polarization of individual movements (Sbragaglia et al. 2022). Therefore, we consider inter-individual distance as a good indicator of the evolution of shoaling behavior in the zebrafish selection lines in two dimensions, but we cannot exclude that other important features of shoaling could be captured using three-dimension tracking. Secondly, evolved differences in metabolic needs of the selection lines could possibly explain the observed differences in shoaling and monoamines. However, previous results showed that juveniles of the zebrafish selection lines did not show differences in standard metabolic rate (Uusi-Heikkilä et al. 2015), and the circadian molecular clockwork of genes related to energy balance, growth, and lipid metabolism did not differ among the selection lines (Sbragaglia et al. 2021b). Nevertheless, we cannot rule out that our results could be possibly explained by ontogenetic differences in metabolism. Finally, it is possible that the patterns we documented here could have been different in previous generations closer to when selection was halted as genetic drift and domestication could have eroded evolved differences among lines (Lande 1976; Price 1999), and uncontrolled density variations in the holding tanks could

also have contributed to partially mask the effects reported here (Bouffet-Halle et al. 2021; Crespel et al. 2021a).

Conclusions

Our results showed that intensive size-selective mortality leads to complex, multivariate, and asymmetric responses that vary with the type of size-selection operating on fish, a pattern also reported in other recent studies (Claireaux et al. 2018; Le Rouzic et al. 2020; Renneville et al. 2020; Crespel et al. 2021b; Bartuseviciute et al. 2022). However, we can conclude that size-selective mortality left an evolutionary impact on the ontogenetic changes of shoal cohesion and also impacted the physiological basis of behavior. Because our experiment was conducted in the laboratory, the transferability of our findings to the wild should be done with great care as laboratory and experimental conditions, species, and strain-specific effects likely have a strong impact on which traits evolve in response to size selection. With that in mind, we can tentatively expect that size selection, both in predator–prey and fisheries scenarios, may evolutionary alter ontogeny of shoal cohesion and affect the dopaminergic and serotonergic systems, which have a range of knock-on impacts on ecological and evolutionary processes. In particular, fisheries-induced evolution can alter many more traits than the classical morphological or life-history traits largely discussed in the literature (Heino et al. 2015).

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Author contribution V. S., S. W., and R. A. conceived the study. V. S. conducted the experiments with inputs from J. F. L. P. T. and S. W. quantified the monoamines. V. S. analyzed the data with inputs from T. R. V. S. and T. R. wrote the manuscript with inputs from all authors.

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Data availability Data are available as supplementary material together with the R codes for statistics. All original video recordings of experimental trials are available upon request.

Declarations

Ethics approval All applicable international (2010/63/EU), national, and/or institutional guidelines for the use of animals were followed.

The experimental protocols were approved by a committee on animal welfare (reference number A13191003).

Conflict of interest The authors declare no competing interests.

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