Internship report:

Experimental design: Simulated continuous trawling model

By Jonas Häusler

Freie Universität Berlin

Matrikelnummer: 5308878

The impact of size-selective harvesting on vulnerability towards a continuous trawling-model in zebrafish *Danio rerio*

Supervisors: Prof. Dr. Robert Arlinghaus and Dr. Tamal Roy

Introduction:

Research has shown that intensive size selective harvesting in fisheries is an evolutionary driving force (Heino et al. 2015; Sbragaglia et al. 2022). Fish in exploited populations might be selected for specific traits and therefore, fishing has the potential to alter fish populations (Heino et al. 2015). Commercial fisheries usually target the larger fish thereby sparing the smaller fish that can reproduce. Predators on the other hand might target smaller fish, as they provide an easy access to energy due to lower swimming speed (Sogard 1997). Selection for small body-types (by harvesting the larger individuals) has proven to lead to a fast life history, which is associated with earlier maturation at smaller size, increased reproductive investment and reduced post-maturation growth (Heino et al. 2015). Further, size selective harvesting has the potential to foster changes in behaviour, resulting in exploited populations to show more vigilance and increased timidity, called timidity syndrome (see Arlinghaus et al. 2017).

In this experiment, I studied how large - and small size-selective harvesting can affect vulnerability to trawling using three artificially selected lines of zebrafish, *Danio rerio*. These fish have been selected and housed for several years in the laboratory following the procedure explained in the methods. The large harvested line (i.e., where smaller fish survived) evolved a fast life history characterised by the traits explained above (Uusi-Heikkilä 2015, 2016, 2017). Several experiments carried out on these lines, showed the following tendencies: the small harvested (large) line has been shown to be consistently bolder (Roy and Arlinghaus 2022). The small harvested line has been shown to be more bold (Roy et al. 2023; Sbragaglia et al. 2022) and tends to form more cohesive groups (Sbragaglia et al. 2022). The large harvested line (small) showed tendencies of shy behaviour (Roy and Arlinghaus 2022, Sbragaglia et al. 2021), formed less cohesive shoals (Sbragaglia et al. 2022) and showed slower learning abilities (Roy et al. 2023).

A previous modelling study by Sbragaglia et al. (2022) predicted mortality outcomes for these lines when faced with natural and artificial predators (i.e., fishing gears). The authors predicted that the small line fish will have reduced trawl induced mortality while the large fish will have increased trawl induced mortality compared to the control line. In this study, I tested these model predictions using the three selection lines of zebrafish. Trawling is expected to select on fish behaviour (Crespel et al. 2021). For this experiment a model had to be developed that mimicked commercial continuous trawling. The target species in this case were three lines of size selected zebrafish (*Danio rerio*). The model was designed in a way so that the net was continually chasing the fish, allowing them several escape routes. Fish behaviour during the trawl movement and during an estimated resting phase afterwards and the time taken by every individual to leave the net after it was caught were scored. A vulnerability index (Thambithurai et al. 2022) was estimated and used to evaluate differences in vulnerability of the specific lines towards the trawl.

Based on the results obtained from mathematical models and past experiments mentioned above, I expect (i) the small line to be less vulnerable towards trawling compared to the control, because they tend to form less cohesive shoals (Sbragaglia et al. 2022). Further, I expect (ii) the large line to be more vulnerable towards trawling, because they form more cohesive shoals (Sbragaglia et al. 2022). A change in vulnerability across trials can be expected due to learning, but this would be less in the small line than the large line as the former showed reduced collective learning ability (Roy et al. 2023). And lastly, I expect (iii) a decrease in time taken to escape the net, especially for the large line as they tend to form less cohesive shoals and might find escape routes quicker because they might be more distributed (Sbragaglia et al. 2022).

Methods:

Selection lines:

The parental generation of zebrafish (*Danio rerio*) was wild collected from West Bengal in India. The experimental fish belonged to three selection-lines, each with two replicates (six lines in total). These lines were identified as LS1 (large-selected, replicate 1), LS2 (large-selected, replicate2), SS1 (small-selected, replicate 1), SS2 (small-selected, replicate 2), RS1 (random-selected, replicate 1) and RS2 (random-selected, replicate 2). Size selection was conducted on the parental generation from F_1 to F_5 and halted for the following generations to remove maternal effects (Uusi-Heikkilä et al. 2015). The harvest-rate during selection periods was 75% of the respective line. In the LS lines 75% of the largest (standard length) individuals and for SS lines 75% of the smallest individuals were harvested. The selection in the RS lines happened randomly. The individuals selected for spawning in the following generations (F_6 onwards) were chosen randomly. For detailed information regarding the selection lines consider (Uusi-Heikkilä et al. 2015)

Experimental fish:

The fish in this experiment originated from the F_{19} generation. Thus, selection stopped 14 generations ago. Fish from F_{18} were bred in groups of five comprising three males and two females to produce the experimental fish. 20 experimental fish per replicate line were randomly selected and housed in groups of four, in five 3 litre, transparent boxes. The water temperature was kept consistent at 26 °C and a 12:12 Light:Dark cycle was maintained during holding. All boxes were maintained in the same system with a water exchange-rate of 10 litre/ minute and a total system-water charge of 660 litres. Fish were fed twice a day ad libitum with bloodworms. Before every trial fish were starved to have consistency as these fish were starved for other experiments as well.

Model design:

The trawling model (*Figure 1*) is custom made and consists of an opaque white tub (to reduce disturbances), with a cylinder-like shape but slightly narrower at the bottom compared to the top (60cm diameter at the top, 55cm diameter at the bottom and 35 cm height) with a vertical arbour in the centre supported by a ball bearing. The arbour and ball bearing are encompassed by plastic pipe that was glued to the bottom to prevent water from infiltrating into the system. A studding is attached to the arbour above the water level horizontally. The handle of customary net (20cm x 15 cm) was bent around the studding and was located 2.5 cm above the bottom leaving 2.5 cm of space between the wall and the net and the arbour and the net on the sides. Water was maintained at a level 5 cm lower than the top of the net. The net was held in place by two nuts and two washers. The arbour was powered by a DC switching power supply running at 12.08 Volt, causing the net to travel clockwise in the tank. For adjusting the speed, a regulator was used.

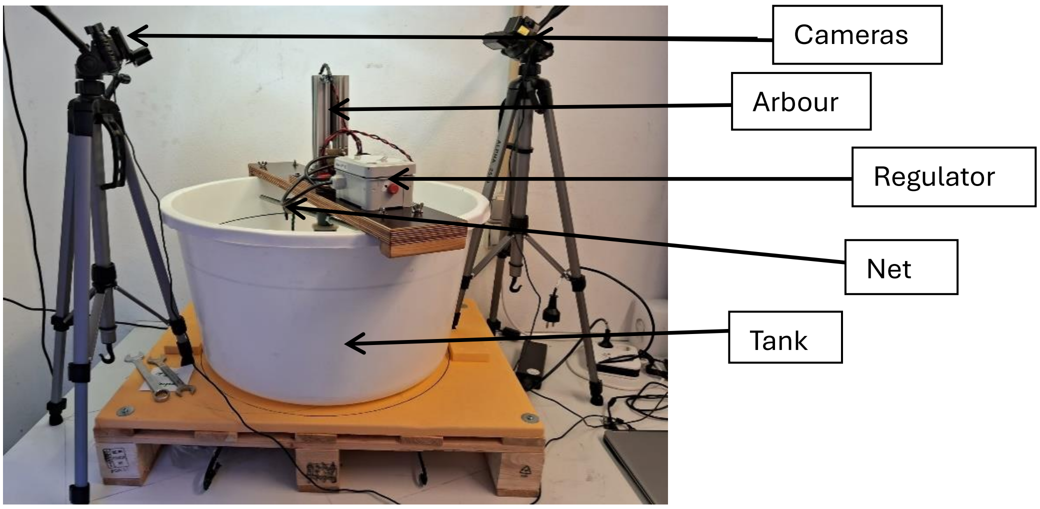

Figure 1 Picture of the continuous-trawling-model

Experimental design:

The experiments were implemented at two different speeds: 108.78 cm/s (referred to as “highs-peed”) and 58.92 cm/s (referred to as “low speed”). The trials were conducted in 5 days from 9:30 am till 4:00 pm for each of the two speeds. In the first week, the tank was filled with water up to the depth of 14 cm from the same system where the zebrafish were housed. Each group was first moved to a transparent cylinder that was positioned at maximum distance from the opening of the trawling-net with the help of a hand net. The fish were allowed to acclimate for two minutes. The according group-ID was stuck to the wall of the tank, so that it is visible under the. After the acclimation time the recording was started using two cameras (Logitech B910) connected to two computers, to capture every angle. Then, the cylinder was removed gently using a string attached to it and the trawl was quickly and steadily accelerated until it was set at the respective speed (marked at the regulator). The trawl moved for two minutes and was then stopped immediately by turning off the regulator instantly. The fish were allowed to rest for three minutes, and the procedure was repeated four times resulting in a total number of five trials. In the second week trials were conducted following the same procedure at the low speed. At the end of the day the tank was drained, the fish were fed and the equipment was cleaned.

Scoring:

The screen of the monitor used for analysing the videos was modified, so that the person could not identify which group is being analysed. Several measures were taken into consideration: during the two minutes trawl, it was scored how many fish escaped in front of the net (E_F), how many fish escaped on the sides (E_S), how many escaped beyond the net (E_B) and how many were caught in the net (C_N) – at maximum and after two minutes. Further, during the 3 minutes rest-phase, we counted how many individuals are caught immediately after the trawl stops, after the water settles and 30 seconds after the water settled. Additionally, the latency of every individual caught to leave the net after the trawl stopped was measured.

Statistical analysis:

To facilitate statistical analysis, a vulnerability index was used based on the study by Thambithurai et al. (2022). Every possible outcome (E_F, E_S, E_B, C_N) was assigned to a specific level of vulnerability, C_N being the most vulnerable, as the fish failed to escape. E_F is the second most vulnerable, E_S the third most vulnerable and E_B the least vulnerable event. The count-data (i.e. number of fish) is multiplied with the penalty accordingly and the sum of all the products provides the vulnerability index. The penalty is provided in the *Table 1* below.

$$\textbf{Vulnerability} = (E_F * \text{Penalty } E_F) + (E_S * \text{Penalty } E_S) + (E_B * \text{Penalty } E_B) + (C_N + \text{Penalty } C_N)$$

Vulnerability index	**Cn+Ef+Es+Eb**
Penalty for C_N	200
Penalty for E_F	150
Penalty for E_S	100
Penalty for E_B	50

Table 1 Vulnerability index and penalties given per possible outcome per trial

All statistical analyses were performed in R 4.1.0, the names of packages used for several models are: "readxl", "ggplot2", "lmerTest", "Rcpp" and "lme4". First, the vulnerability index was calculated for every count-data (i.e. number of fish). Linear mixed effects models were used for all analyses. Fixed effects were population in interaction with trial, and the dependant variables (according to the model) incorporated vulnerability at the end of a trial, maximum vulnerability, vulnerability after the trawl stopped, vulnerability after the water settles, vulnerability 30 seconds after the water settled and mean time taken to leave the net. Random effects included Group ID nested within Replicate. For example, the formula in R for comparing "maximum vulnerability" across lines was:

lmer(Vulnerability_M~Population+Trial+(1|Replicate/GroupID), data = VulM)

Vulnerability_M as a function of Population in interaction with Trial including random effects: GroupID nested in Replicate

The same function was used for fitting the other dependent variables. The variable "mean time taken to leave the net" was log transformed. Then, ANOVA was conducted for every model. The graphs were generated using Excel.

Results:

Low speed:

During trawling

The models showed no significant difference in vulnerability across trials among lines during the two-minute phase of active trawling at low speed. Mean vulnerability of getting caught in trawl did not differ across trials ($F_{1,119}$=1.49, p=0.22) and selection lines of zebrafish ($F_{2,3}$=0.08, p=0.93). Further, the average maximum vulnerability of getting caught in the continuous trawl did not differ across trials ($F_{1,117}$=1.17, p=0.28) and selection lines of zebrafish ($F_{1,119}$=1.17, p=0.28).

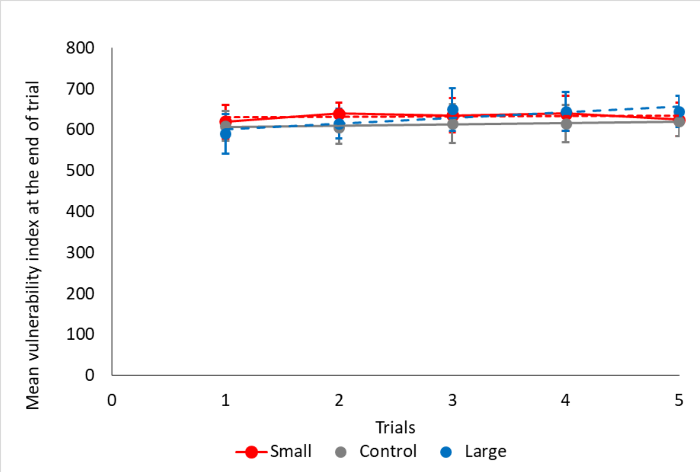

Figure 2. Vulnerability (mean ± SE) of getting caught in the trawl did not differ across trials ($F_{1,119}$=1.49, p=0.22) and selection lines of zebrafish ($F_{2,3}$=0.08, p=0.93).

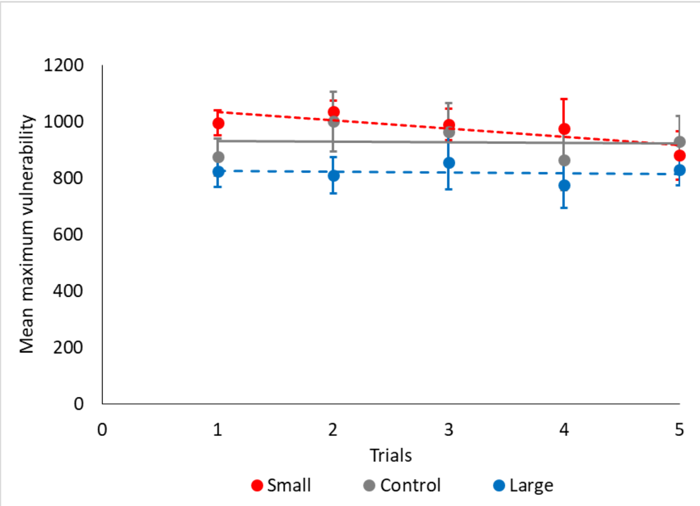

Figure 3. Maximum vulnerability (mean ± SE) of getting caught in the trawl did not differ across trials ($F_{1,119}$=1.17, p=0.28) and selection lines of zebrafish ($F_{2,2.98}$=1.71, p=0.32).

After trawling

During the three-minute rest phase (i.e. after the active trawling) significant differences were observed, mainly across trials. Mean vulnerability of being caught after trawl stops marginally declined across trials in the small line compared to the control (t=-1.81, p=0.07). The mean vulnerability of being caught after water settles at the end of trawl movement did not differ significantly across trials ($F_{1,119}$=0.72, p=0.40) and among selection lines of zebrafish ($F_{2,3}$=0.05, p=0.95). Further, the mean vulnerability of being caught 30 sec after water settles at the end of trawl movement increased significantly across trials in the large line compared to the control line (t=2.19, p=0.03). Regarding the time taken to leave the net after trawl-movement stopped, the mean did not change significantly across trials ($F_{21, 119}$=0.37, p=0.5) and did not differ among selection lines ($F_{2,27}$=0.35, p=0.70).

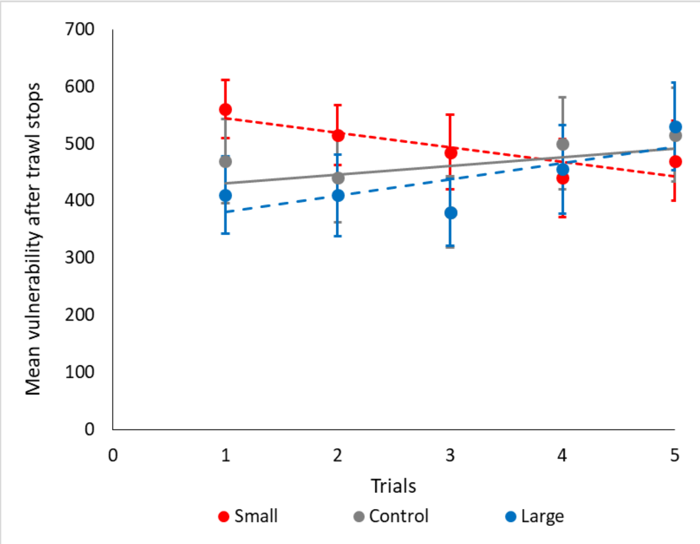

Figure 4. Mean (± SE) vulnerability of being caught after trawl stops marginally declined across trials in the small line compared to the control line (t=-1.81, p=0.07).

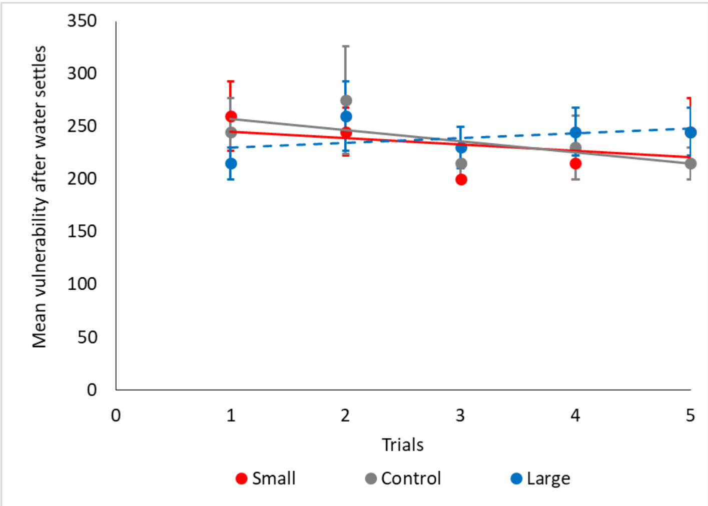

Figure 5. Mean (± SE) vulnerability of being caught after water settles at the end of trawl movement did not differ significantly across trials ($F_{1,119}$=0.72, p=0.40) and among selection lines of zebrafish ($F_{2,3}$=0.05, p=0.95).

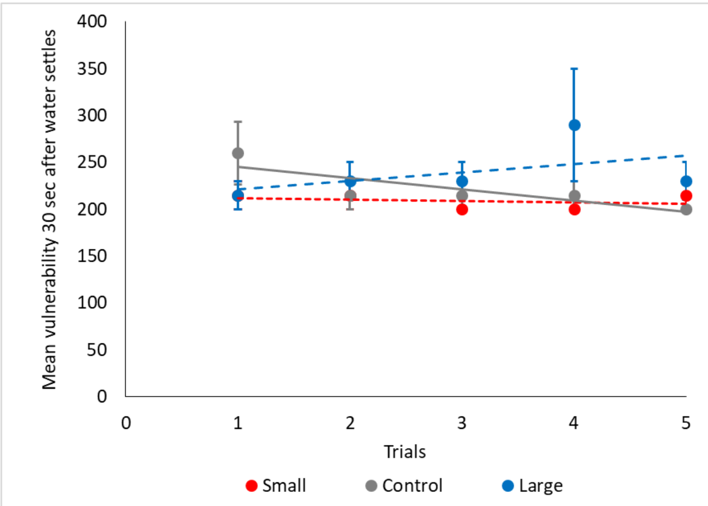

Figure 6. Mean (± SE) vulnerability of being caught 30 sec after water settles at the end of trawl movement increased significantly across trials in the large line compared to the control line (t=2.19, p=0.03).

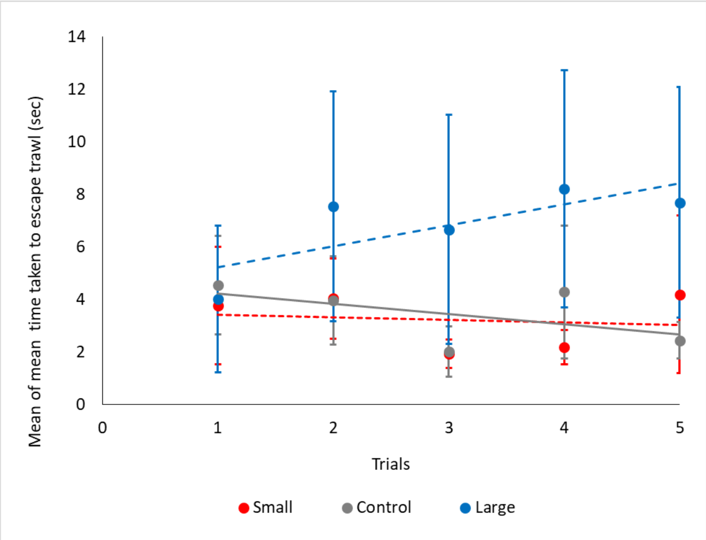

Figure 7. Mean (± SE) latency to escape 30 sec after water settles at the end of trawl movement did not change significantly across trials ($F_{21, 119}$=0.37, p=0.5) and did not differ among selection lines ($F_{2,27}$=0.35, p=0.70).

High speed:

During trawling

Considering the models, the mean vulnerability of getting caught in trawl did significantly differ across trials, meaning the vulnerability decreased significantly across trials (t=-3.26, p<0.01), and was marginally higher in the large line compared to the control line (t=1.75, p=0.09). The average maximum vulnerability of getting caught in the continuous trawl was marginally lower in the small line compared to the control line (t=-2.05, p=0.05).

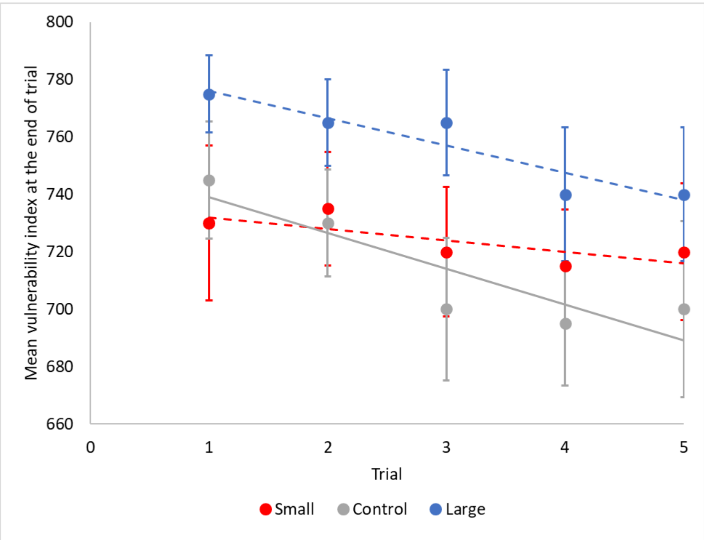

Figure 8. Vulnerability (mean ± SE) of getting caught in the trawl declined significantly across trials (t=-3.26, p<0.01), and was marginally higher in the large line compared to the control line (t=1.75, p=0.09).

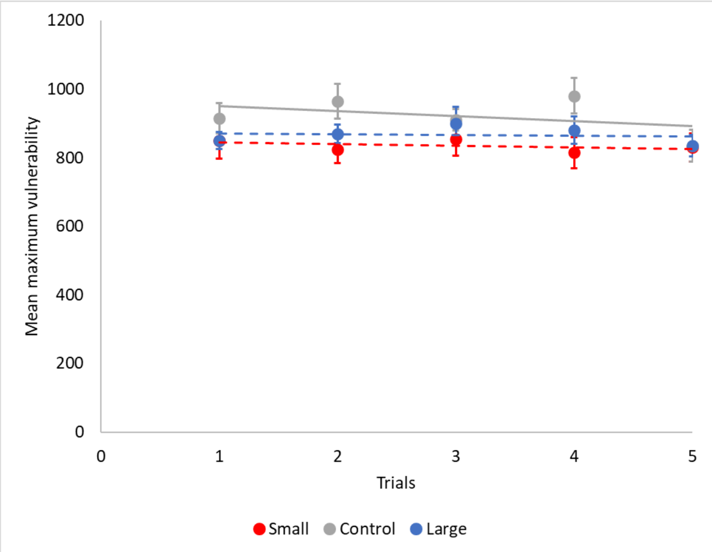

Figure 9. Maximum vulnerability (mean ± SE) of getting caught in the trawl was marginally lower in the small line compared to the control line (t=-2.05, p=0.05).

After trawling

Interestingly, the mean vulnerability of being caught after trawl stops declined significantly across trials (t=-2.48, p=0.01) but did not differ among selection lines of zebrafish ($F_{2,27}$=0.41, p=0.67). Similarly, the mean vulnerability of being caught after water settles at the end of trawl movement declined significantly across trials ($F_{1,117}$=7.39, p=0.01) and did marginally differ among selection lines of zebrafish ($F_{2,111}$=2.89, p=0.06) with decreasing vulnerability for the small line of zebrafish (t=-2.41, p=0.02). The mean vulnerability of being caught 30 sec after water settles at the end of trawl movement did not differ across trials ($F_{1,117}$=2.15, p=0.15), as well as among the selection lines of zebrafish ($F_{2,102}$=0.1, p=0.9). Mean time taken to escape the net after trawl-movement stopped changed significantly across trials ($F_{1,117}$=34.81, p=3.617*e^{-8}), but did not differ among selection lines of zebrafish ($F_{2,72}$=1.62, p=0.21).

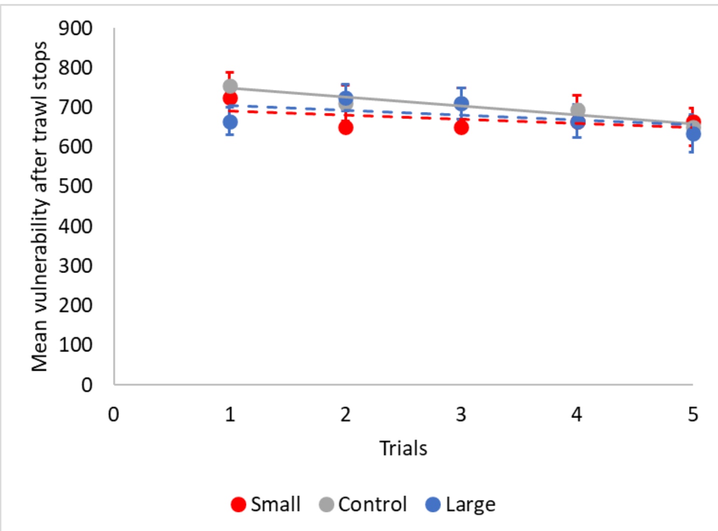

Figure 10. Mean (± SE) vulnerability of being caught after trawl stops declined significantly across trials (t=-2.48, p=0.01) but did not differ among selection lines ($F_{2,27}$=0.41, p=0.67).

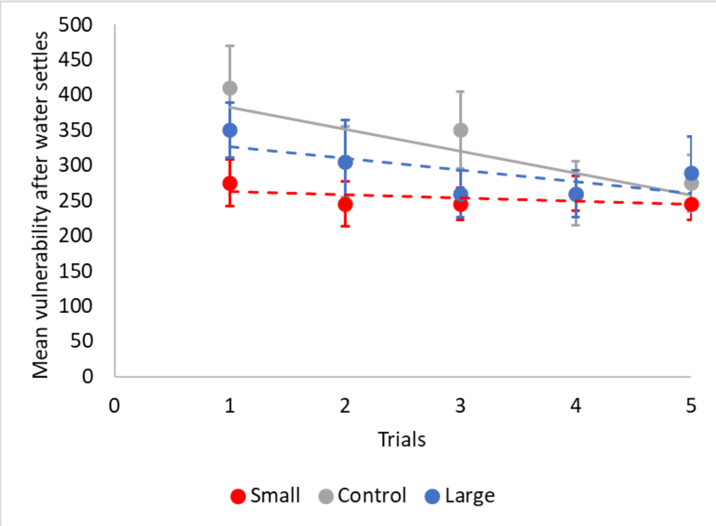

Figure11. Mean (± SE) vulnerability of being caught after water settles at the end of trawl movement declined significantly across trials (t=-2.71, p<0.01) and was marginally lower in the small line than the control line (t=-1.71, p<0.1).

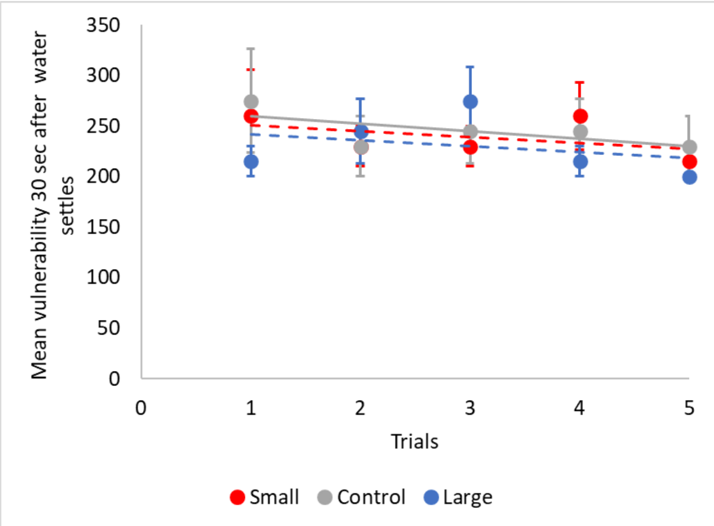

Figure 12. Mean (± SE) vulnerability of being caught 30 sec after water settles at the end of trawl movement did not differ across trials ($F_{1,119}$=2.19, p=0.14) or among selection lines ($F_{2,27}$=0.14, p=0.87).

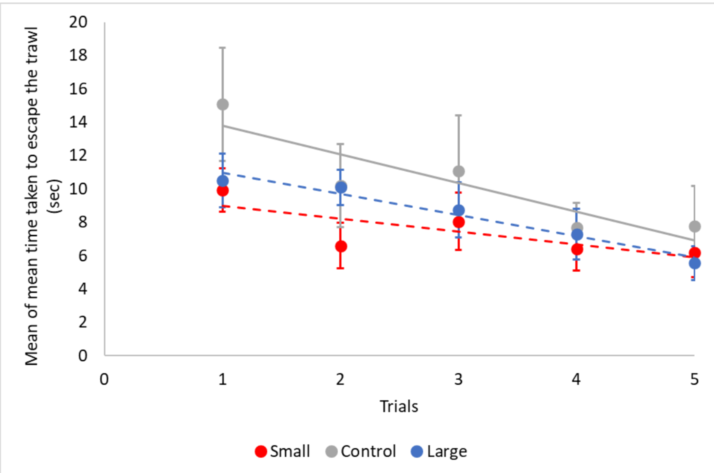

Figure 13. Mean (± SE) latency to escape 30 sec after water settles at the end of trawl movement declined significantly across trials (t=-4.22, p<0.01) and did not differ among selection lines ($F_{2,27}$=0.28, p=0.76).

The main results are summarised in the table below:

S.No.	Variable / Measure	Results Low speed	Results High speed
1	Vulnerability End	No difference among lines and trials	*Declined significantly across trials and was marginally higher in the large line*
2	Vulnerability Maximum	No difference among lines and trials	*Marginally lower in small line*
3	Vulnerability after stop	*Marginally declined across trials in small line*	*Declined significantly across trials*
4	Vulnerability after water settles	No difference among lines and trials	*Declined significantly across trials and was marginally lower in the small line*
5	Vulnerability 30 sec after the water settled	*Increased significantly across trials in the large line*	No difference among lines and trials
6	log mean time taken to leave the net	No difference among lines and trials	*Declined significantly across trials*

Table 2. Summary of significant results, significant results are highlighted in italics.

Conclusion:

Considering these results a marginal decline in the vulnerability after the trawl stops in the small line and significant increase after the water settles in the large line is observable in the low-speed trials, compared to the control. In the high-speed trials at the end of the trawl the vulnerability decreased for all lines and was marginally higher in the large line, compared to the control. Further, the vulnerability after the water settles also declined and was marginally lower for the small line in relation to the control. From this we can conclude that there is a tendency for the small line to be less vulnerable and for the large line to be marginally more vulnerable towards this trawling model in comparison for the control. The hypotheses from the beginning are partly met (although, not as significant as expected).

Also, the time to escape in the low-speed trials did not differ, but it significantly increased in the high-speed trials. Concluding a learning-effect from this result would be speculation, as it does not seem to be repeatable for the low-speed trials. This could be because of shoaling behaviour. Conspecifics outside the net could have motivated the caught fish to leave the net without expecting any harm from it.

Intensive size-selection left a legacy, like different experiments have shown already (Uusi-Heikkilä 2015, 2016, 2017; Roy et al. 2023; Sbragaglia et al. 2022). Although transferring these results onto the wild remains speculative, further evidence is provided that this type of selection might have an influence on exploited populations even if such size selection did not happen in the current generation. Fisheries must learn to adapt to changes.

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Supplementary materials

R-Packages:

- readxl
- ggplot2
- lmerTest
- Rcpp
- lme4