

**Guppy getaway:
A study of vulnerability to fishing through size and shape using
the Trinidad guppy (*Poecilia reticulata*) in laboratory fishing
experiments**

Endre Åbø Hallingstad

Master in Science of Biology – Biodiversity, Evolution and Ecology

Supervisors:

Beatriz Diaz Pauli, Department of Biological Sciences, University of Bergen

Mikko P. Heino, Department of Biological Sciences, University of Bergen



Department of Biological Sciences

University of Bergen, Norway

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Endre Åbø Hallingstad

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Abstract

Human-induced evolution has led to large changes and evolution in many species. The some of the most well-known are household animals like the dog and sheep or plants like the tomato. Others are fish like the Atlantic salmon (*Salmo salar*). The selection done on such animals have resulted in evolution of phenotypes that might not be beneficial in the wild but are instead sought after by humans.

Fisheries-induced evolution is a form of human-induced evolution caused by commercial and recreational fishing through the selection of fish through the use of fishing gear. This is mainly a size selective venture, but also selects on other traits like boldness and mouth size depending on the gear.

Due to the complexity of fisheries-induced evolution, it can be hard to find the exact causes of these changes and if they stem from evolutionary changes or plastic responses in life history traits exhibited by the species of interest. Therefore, size-selective laboratory experiments and other applications of model organisms such as the Trinidadian guppy (*Poecilia reticulata*) in the lab can lead to better understanding the selective process that happen during harvesting of wild fish and the life-history changes that happen because of it.

The use of length, size and shape of the guppy and hand fishing with an aquarium net show that the size and shape of fish from different size-selective lines can influence the vulnerability to being caught during group fishing and that there can be substantial differences in shape and size because of size-selective treatment over time. Mainly the differences in shape resulting in variable vulnerability to fishing was found in females.

Key words:

Fisheries-induced evolution, evolution, selection, fishing, human-induced evolution, size-selection, morphology, shape changes.

Contents

Acknowledgements	I
Abstract	III
Contents	V
List of figures	VII
List of tables	IX
1 Introduction	1
1.1 Human-induced evolution.....	1
1.2 Experiments to study fisheries-induced evolution.....	4
1.3 Objective.....	5
2 Methods	7
2.1 Model species: The guppy (<i>Poecilia reticulata</i>).....	7
2.2 Predator selection on size.....	8
2.3 Size-selective harvest.....	9
2.4 Fish used in the present study.....	10
2.5 Marking and individual ID.....	11
2.6 Vulnerability to capture.....	13
2.6.1 Group fishing.....	14
2.6.2 Individual fishing.....	14
2.6.3 Replicates and resting period.....	15
2.7 Morphology.....	15
2.7.1 Photography.....	15
2.7.2 Landmarks.....	17
2.8 Statistical analyses.....	19
2.8.1 Vulnerability.....	19
2.8.2 Morphology.....	21
3 Results	23
3.1 Size-selective fishing experiment.....	23
3.1.1 Group fishing.....	24
3.1.2 Individual fishing.....	28
3.2 Morphology.....	30
3.2.1 Shape differences between sexes and among size-selective treatments.....	30

3.2.2 Effect of shape on vulnerability to fishing.....	35
4 Discussion	37
4.1 Summary of results	37
4.2 Group vulnerability.....	37
4.3 Individual vulnerability	38
4.4 Sex and selection on shape	38
4.5 Individual vs group vulnerability.....	40
4.6 Effect of shape on vulnerability to fishing	41
4.7 Size-selective harvests	42
4.8 Relevance.....	42
5 Literature	44

List of figures

Figure 2.1 Settings used for the camera during photography.

Figure 2.2 Picture of the male guppy ID-ed as 1MRY (Pop. 1, male, right-side mark, colour yellow) with a scale for length and colour and an ID mark. Fins and body adjusted to preferred angles.

Figure 2.3 1MRY with the ten defined landmarks used to digitise the shape of the fish.

Figure 2.4 1MRY fitted with the wireframe used in graphics produced by MorphoJ.

Figure 3.1 Overall length distribution of the fish, in millimetres, grouped by size-selective treatment. Density plot with vertical lines noting the group wise mean length.

Figure 3.2 Length distribution of the female fish, in millimetres, grouped by size-selective treatment. Density plot with vertical lines noting the group wise mean length.

Figure 3.3 Length distribution of the male fish, in millimetres, grouped by size-selective treatment. Density plot with vertical lines noting the group wise mean length.

Figure 3.4 Time until a fish was caught in group fishing plotted against the length of the fish. Mean times were 242.10, 252.43 and 193.19 seconds for negative, positive, and random size-selection respectively.

Figure 3.5 Time until a fish was caught in group fishing plotted against the length of the fish. Females only. Mean times were 276.24, 296.66 and 209.64 seconds for negative, positive, and random size-selection respectively.

Figure 3.6 Time until a fish was caught in group fishing plotted against the length of the fish. Males only. Mean times were 207.96, 209.67 and 176.73 seconds for negative, positive, and random size-selection respectively.

Figure 3.7 Replicate 1 for time until capture for individual fishing in a 3 L tank plotted against the length of the fish. Mean times were 5.74, 6.16 and 6.52 seconds for negative, positive, and random size-selection respectively.

Figure 3.8 Replicate 2 for time until capture for individual fishing in a 3 L tank plotted against the length of the fish. Mean times were 5.45, 4.73 and 5.96 seconds for negative, positive, and random size-selection respectively.

Figure 3.9 Shape changes between male (dark blue) and female (light blue). All fish included. Scale of changes in shape set to 5.

Figure 3.10 Distinct groups for male and female fish along the plotted variate.

Figure 3.11 Shape differences as illustrated by wireframe along the plotted variates (light blue to dark blue) showing the groupings for the three size-selective treatments for females only. Scale of changes in shape set to 5.

Figure 3.12 Shape differences as illustrated by wireframe along the plotted variates (light blue to dark blue) showing the groupings for the three size-selective treatments for males only. Scale of changes in shape set to 5.

Figure 3.13 Centroid size and shape changes for females along the regression for time until caught for group fishing. Light blue is the start and dark blue is the end. Scale of changes set to 5.

List of tables

Table 2.1: Examples of marking treatment and ID.

Table 2.2 Definition of the used landmarks.

Table 2.3 Repeatability and P-value as given by rptR.

Table 3.1 Linear mixed effects model results for both male and female and the sexes separate with time until caught for group fishing. Both refers to the sexes together in the same model, whereas the other two contain only female and male fish respectively. Mean length = 18.9 (± 2.5) mm.

Table 3.2 Differences in total time to catch a population.

Table 3.3 Linear mixed effects model results for time until capture for individual fishing. Replicate refers to the two times the time variable was assessed. The estimate and standard error (SE) are natural log transformed. Mean length = 18.9 (± 2.5) mm.

Table 3.4 Shape differences in Procrustes (absolute distance) and Mahalanobis distance (relative to within-group variation) between male and female. Result id from a CVA where the data was grouped by sex.

Table 3.5 Pairwise comparison of shape differences in Procrustes (absolute distance) and Mahalanobis distance (relative withing-group variation) for female fish between the size-selective treatments and their P-values.

Table 3.6 Pairwise comparison of shape differences in Procrustes (absolute distance) and Mahalanobis distance (relative withing-group variation) for male fish between the size-selective treatments and their P-values.

Table 3.7 Regression results for the three time variables separately as independent variables and plotted against centroid size, the overall size of the fish.

1 Introduction

1.1 Human-induced evolution

Human induced evolution happens as humans introduce selective pressure in the part of nature they interact with (Herron and Freeman, 2015a). Evolution itself is based on changes of the genetic material we know as DNA, and can be as small as the change of one single base pair (Herron and Freeman, 2015b). However, such small changes do not have to enact actual changes in phenotypes, the expression of genes. Such changes can be the result of random mutations. In sexual species, genetic changes could also occur by the mixing of genetic material from two parents (recombination). What drives evolution by natural selection, however, is that the environment the phenotypes are exposed to drives selective pressures making some phenotypes have higher fitness (survival and reproduction). For the most part, no two individuals are exactly the same and therefore, a population can contain many different alleles, gene variants, that can be passed down to the next generation through reproduction. If some individuals are favoured or selected over others in the environment (i.e., they have higher fitness), these can pass down their genes to the next generation, i.e., the selected traits are heritable. At the core of evolution by natural selection, there must be phenotypic variation in traits, environmental selection that results in differential fitness among the traits, and trait heritability. For a whole population or species, this results in a change in total genetic content and the new generation will be different to the previous one. Potentially, new mutations may have come into play or the frequency of the alleles already present has changed. Over, time the population will change and adapt to new selective pressures, resulting in evolution.

The rate of human-induced evolution is not constant, it changes according to the selective pressure and type of selective pressure. One of the clearest examples of human-induced evolution is domestication. As people started to tame animals, most of the evolution for these captive species was due to breeding animal species such as the wolf, by selecting for docile traits which, became the many types of dogs (Løberg; Eikeseth and Roig, 2020). Sheep in many cases have been bred to make pure white wool instead of the grey or brown wool that would more easily camouflage them in the wild (Blix and Vangen, 2023). Therefore, human-induced selection is opposite to natural selection. Other examples of domestication are the tomato and the pigeon. The cultivated varieties of tomato often are selected for sizes larger than the wild variety and as a consequence have a fw2.2 gene that is less active than in smaller domesticated

or wild varieties (Frary *et al.*, 2000). For pigeons, they were selected for several purposes, one of them being their visual appearance and colours (Herron and Freeman, 2015a). Here, the individuals with the traits deemed most desirable were paired up for reproduction in the hope that their offspring would be even more desirable than their parents. This type of artificial selection is, however, not the only type of selective pressure humans exert upon nature.

Several aquatic organisms have also been domesticated and kept as food sources. Some of them are even kept as farm animals such as the Atlantic salmon (*Salmo salar*) (Norris; Bradley and Cunningham, 1999). The selection of salmon used in farming has even resulted in genetic differences to such a degree that analysis of DNA microsatellites can provide information as to the origin of the individual fish and if it is wild or farmed. Furthermore, individuals that can be considered to originate from farm stocks have adaptations that would be disadvantageous in the wild (Diserud *et al.*, 2022). This is because the salmon harvested from farms are selectively bred to cope with the high density of fish in the nets or tanks they are kept in, as well as selected for other traits such as fast growth to maximise food production. Again, giving evidence that human-induced selection might counteract natural selection.

Far from all fish commonly used as food are kept as farm animals. Some are instead harvested from the wild, such as Atlantic cod (*Gadus morhua*), which is seasonally harvested by fishermen in countries such as Norway both commercially and recreationally using different forms of passive and active fishing with several types of equipment (Jørgensen; Ernande and Fiksen, 2009). This enacts selective pressure on the fish stocks and results in changes in phenotypes due to plasticity and genetic changes in the overall population. Therefore, fishing selection could lead to human induced evolution, commonly termed as fisheries-induced evolution. Fishing most commonly leads to selection on size, but different fishing gears may also lead to different selective pressures.

Passive fishing with gill nets, for example, selectively capture fish with a head that closely matches the mesh size in the net, and lets smaller fish to go through the net, while it acts as an obstacle for fish that are too big for the net (Jørgensen; Ernande and Fiksen, 2009). Therefore, gillnets select for intermediate size fish, which results in disruptive selection and higher fitness

for fish of smaller and larger sizes. Trawls on the other hand, a form of active fishing, are less size selective, and harvest everything over a certain size that ends up within the net, leading to directional selection for small sizes. Such harvesting selection on size can lead fish to adapt to size ranges outside the harvest range. In addition, changes in size may lead to changes in life history traits such as growing slower and reallocating the energy that would have been used for growth over to maturation and reproduction (Miller, 1957; Law and Grey, 1989). On top of large scale fishing with nets and trawl, smaller scale fishing with rod and reel or angling, also has an effect on life history traits of the populations harvested (Cooke *et al.*, 2019). However, angling acts by catching the interest of the fish that are caught, either by resembling their prey using flies or other lures, presenting something shiny or by giving the fish an easy meal. This form of fishing, therefore, selects more on behaviour than physical traits, like size (Arlinghaus *et al.*, 2017). This includes inquisitiveness, boldness, and hunger. In addition, such fishing can result in the harvest of a wider size range and result in a more evenly spread harvest size-wise (Kleiven *et al.*, 2016).

Different levels of harvesting result in differing amounts of selective pressure and, therefore, differing rates of change. Commercial harvesting of wildlife is often higher than that of recreational harvest, like the 2022 data from the Norwegian Directorate of Fisheries show (Fiskeridirektoratet, 2022a; Fiskeridirektoratet, 2022b). This can result in commercial strategies for harvest having a larger impact on the populations targeted for harvest, especially those that are less used by recreational fishermen. In some cases, where the fishing grounds are easily available to recreational fishing, the many types of gear that the recreational fishermen implement can result in higher cumulative harvest than that of commercial activity and therefore, higher selective pressure (Kleiven *et al.*, 2016).

Even though it is predicted that fishing-induced selection of size affects size and other life history traits, many issues are not clear, like how fast the process is or if size is the only trait affected (Audzijonyte; Kuparinen and Fulton, 2013). If any, what other traits are affected? Are the changes a result of genetic change, making it evolution, or are they a result of plasticity or learning? This is where laboratory experiments, using specific animal species as model organisms and maintaining controlled environments, can yield information about what happens to wild populations. Laboratory experiments create parallels of what happens in the wild, but

under more controlled environments. Using model organisms, which commonly take up less space and have a shorter generation time than the original species of interest, predictions of what will happen to harvested populations can be obtained.

1.2 Experiments to study fisheries-induced evolution

In general, experiments have a significant chance of revealing data and results that are obscured by unknown variables in the wild (Diaz Pauli and Heino, 2014). This happens because of the sheer complexity of an ecosystem and the ecology in or around the studied system or organism. By reducing the number of variables and therefore simplifying the studied system, experiments can reveal previously unknown interactions or changes in populations or areas under study. The experimental study of fisheries-induced evolution lends insight into the effects of selective fishing (Diaz Pauli, 2012; Diaz Pauli and Heino, 2014). Using artificial selection in the lab or in regulated ponds, simplified parallels to what happens in the wild can be established and be used as proxies for what happens to populations that are under size-selective pressure from fisheries.

Experiments on fisheries-induced evolution have also uncovered changes in overall behaviour among fish because of fishing (Diaz Pauli and Heino, 2014). For instance, fishing-induced selection of larger individuals led to reduced feeding rate, reduced willingness to forage after a predator attack (reduced boldness) in silversides (*Menidia menidia*) (Walsh *et al.*, 2006), while in zebrafish (*Danio rerio*) it led to less exploration and bold behaviour (Uusi-Heikkilä *et al.*, 2015), as well as, more individual vigilance but reduced attention to social cues and hence reduced shoal cohesion (Sbragaglia *et al.*, 2022). This reduction of cohesion was predicted by a model to reduce vulnerability to fishing, but increase vulnerability to a natural predator because the reduced cohesion and hence reduced confusion effect was a disadvantage in front of a natural predator and not fishing gear (Sbragaglia *et al.*, 2022).

In some cases, escaping from nets can be seen as a proxy to escaping from predators because the netting simulates the casing of a predator (Li; Li and Zhang, 2019), but it will never be the same as predators are live creatures that give of other signals such as smell and has a behaviour of its own. Nets, on the other hand, are inanimate objects that the fish must observe in order to

know are present. In addition, shoaling behaviour is anti-predator behaviour, as individuals in large groups are less vulnerable to predator attack, but shoaling are more vulnerable to fishing.

1.3 Objective

This study aims to find differences in escape behaviour among the selection lines of Trinidadian guppies (*Poecilia reticulata*) at the University of Bergen. These populations have been selected for size in three different ways, positive size-selection, where the larger individuals are harvested; negative size-selection where the smaller individuals are harvested; and random selection where a mixture of both large and small individuals is harvested. These lines resemble differing forms of size-selective mortality that can be expected from predators or humans. Mostly, humans select for larger fish to acquire as much food as possible, whereas predatory fish can have strategies leading them to focus their efforts on small-sized, medium-sized, or large-sized fish, or even a mixture according to species of predator and prey and their survival strategies (Reznick and Endler, 1982; Jørgensen; Ernande and Fiksen, 2009). Individuals exposed to positive size-selection are smaller in size, have faster life histories (Diaz Pauli, 2012; Bartuseviciute *et al.*, 2022), and had higher sociability and less boldness (lower inspection of natural predators) than negative and random size-selected fish (Diaz Pauli *et al.*, 2014)

Therefore, this study aims to identify differences in how effective the fish from three different selection lines are in their escape behaviour using an aquarium net, while also considering the body shape differences between the selection lines after generations of size-selective harvest.

The predicted outcome is that the larger fish from the negatively selected populations used in this study will be able to avoid the net for longer than those from the positively and randomly selected populations (Reznick and Endler, 1982; O'Steen; Cullum and Bennett, 2002; Cano-Barbacil *et al.*, 2020).

This study is focused on behaviour and morphology linked to vulnerability to capture, rather than the size of the fish. The fishing in this study is a form of active fishing using a small net and entails visually finding the fish before catching them in the net. This means that the

experiment measures net avoidance behaviour and is linked to swimming speed, acceleration, and agility further connected to fish size and shape (Webb, 1994; Cano-Barbacil *et al.*, 2020).

2. Methods

2.1 Model species: The guppy (*Poecilia reticulata*)

The model species for the experiment is the guppy from Trinidad (Tjernshaugen, 2021). The guppy is a freshwater fish originally found in the rivers and streams in the northernmost parts of South America. The species has its vernacular name from Robert John Lechmere Guppy, whom, as a British citizen that sent sample individuals from Trinidad to the Natural History Museum in London.

The phylogeny places the species within the live-bearing tooth-carps or Poeciliidae along with other fish such as the molly. The guppy, therefore, does not lay eggs, but perform live births (David, 2011; Tjernshaugen, 2021). The species also has significant sexual dimorphism with the male being much smaller than the female at maximum length of 3.5 and 6 cm respectively. In addition, the male is brightly coloured with orange and dark pigments and have refractive scales that can give of colours such as silver or gold. The female, on the other hand, are grey to olive-green.

As the guppy is a small fish it can be kept in a relatively small volume of water compared to larger species of fish. In addition, generation time is only 2-3 months, and therefore, react quickly to selective pressures (David, 2011; Tjernshaugen, 2021). As a result of these traits, the guppy has not just been used as a model organism in research but is also a pet animal with many colourful breeding varieties.

One of the more prominent researchers that have used this guppy as an experimental organism was John A. Endler (1980), looking into the evolution of colour patterns in *P. reticulata*. Specifically, the effect of predation and sexual selection. These experiments showed that the guppy is highly polymorphic in terms of colour patterns, but also that the patterning in different populations can be quite similar if the physical environment and the level of predation is the same.

2.2 Predator selection on size

Reznick and Endler (1982) looked more broadly into the impact of predation, focusing on life history traits, and showing that the fish can adapt to selective pressures in many traits. They also demonstrate that the overall size of the fish within distinct populations differ according to the predation they experience. Within some of the streams where *Anablespsoides hartii* (previously *Rivulus hartii*), a predator that mainly preyed on smaller guppies of smaller sizes. Other streams contained other predators, like the *Crenicichla alta* or *Hoplias malabaricus*, that like to feed upon larger prey. With predation on opposite sides of the size spectrum, over time, the fish started to differ in size between the streams, resulting in larger fish that invested more energy in growth in the streams containing *A. hartii* and smaller fish, investing more energy in reproduction in streams containing larger predators. This is a result of larger individuals surviving better in streams with *A. hartii*, and smaller individuals having a greater chance of surviving in the streams with *C. alta* and *H. malabaricus*.

Predator avoidance is a big part of the survival strategies the small guppies have, and they have several strategies (Magurran and Seghers, 1990; Magurran *et al.*, 1992; Bleakley; Martell and Brodie, 2006). One of them is schooling, which, according to Magurran *et al.* (1992), increases significantly in wild populations where predation is a significant threat compared to populations with low predation. The same can be said for inspection behaviour, where fish from low predation areas approach closer to the predator model than those from high predation areas. On top of this, fish familiar with a predator, or that lived in a high predation area was more weary of the head of the predator and generally stayed further away from the predator itself and the attack cone of said predator (Magurran and Seghers, 1990). Guppies also display the ability to change behaviour according to stimuli as Bleakley; Martell and Brodie (2006) show that the fish spend more time frozen or in a state of agitation after presented with a predator.

The morphology of the guppy is also influenced by the remaining biotic and abiotic factors in the local environment as well as predation. One such example is the difference in tail depth between some wild and laboratory populations (Burns; Di Nardo and Rodd, 2009). For wild females living in running water their tails were larger, or deeper, and more powerful tails than the tails of fish kept in captivity. This is a result of the flow of water in the guppies' natural habitat and the lack thereof in laboratory aquariums. This difference was not as pronounced in

male guppies as they naturally reside in calmer waters at the edges of the streams. As for the effect of predation, more heavily predated populations of fish develop stronger tails that allow them to more easily escape their predator, much like when the current in the stream is strong (Hendry *et al.*, 2006).

2.3 Size-selective harvest

The fish used in this study had undergone a size-selective harvest experiment where the fish originally were collected from the Yarra river in Trinidad. All populations were kept in a dedicated fish-lab since April of 2009 in tanks of the same size, 400 litres, and given plastic netting, simulating weed, floating on the surface of the water for fish to hide in. They were all given the same food, Artemia (Brine shrimp). The research group responsible for the fish lab and the harvest protocol is EvoFish at the University of Bergen.

The size-selective harvest experiment consists of nine populations that had undergone size-selective harvest for over a decade, which occurred every 6 weeks from 2010 to 2019 and every 12 weeks after that.

The three selection treatments all serve a purpose. The positive size-selection mimics the selection that fisheries perform. Fish are selected according to size through the mesh size in nets and trawls, as well as entrances and escape holes in traps or the size of a hook. Like commercial or hobby fishing there is a minimum size regulation, either on the equipment used or for the size of the fish itself, for landing and euthanizing fish (Norwegian Ministry of Trade, 2021). The negative size-selection on the other hand is the opposite of this and more closely resembles natural mortality where smaller, younger fish have a larger chance of dying (Jørgensen; Ernande and Fiksen, 2009). The third treatment, random, is used as a control on size-selection, as fish are fished out independently on size. On top of this, the random size-selection subjects the fish to increased mortality like the other two selection lines. This increased mortality also ensures that the individual populations do not suffer from overcrowding.

The nine separate populations are selected after one of three selection treatments replicated three times:

- 1) Random size-selection:
An equal portion of both large and small fish are harvested from the population.
- 2) Positive size-selection:
A large portion of the larger fish are harvested from the population.
- 3) Negative size-selection:
A larger portion of the smaller fish are harvested from the population.

In all three of the selection lines the fish were separated into two buckets of fish below 16 mm length and over 16 mm in length. The measurement of length used was standard length, the length from the tip of the snout to the base of the caudal peduncle. The buckets were then sorted so that an appropriate fraction of the fish was selected for euthanasia. In the case of positive and negative size-selection $\frac{3}{4}$ of the fish of appropriate size were selected for euthanasia. As for the random size-selection, $\frac{3}{8}$ of each group, both smaller and larger than 16 mm were selected adding up to the same fraction as for the other selective treatments. The chosen method of euthanasia was an overdose of the sedative MS222 (Tricaine methane sulfonate), often used for sedation of fish.

2.4 Fish used in the present study.

The fish used in this study (N = 180 individuals; 20 from each population) were sampled from the populations between the 26th and the 29th of September 2022, during size-selective harvest number 68.

In total, 288 fish, 32 fish from each population were separated from their parent population and set aside as possible participants in the study as well as backups in case of death during procedures or other unfortunate events. All fish were adults, and 16 mm or longer. This was done to homogenise the size and the life stage of the focal fish.

The individual fish were selected at random from within the bucket. This was done by the usage of a small aquarium net and the visual selection of a random fish in the selected bucket. This was then repeated until a total of 32 fish, 16 males and 16 females, were selected from each

population. A total of 20 fish, 10 females and 10 males, from each population were then randomly picked for marking.

If possible, the selected fish were taken from the buckets selected for euthanasia during the size-selective harvest, leaving as many as possible of the fish supposed to go back into their parent population. This applied to the populations under a positive or random selection regime. As for the negative selection regime, the fish were taken from buckets supposed to go back into the main population. The reason for this choice is based in the thought that a loss of life is to be avoided as well as minimising the potential effect the removal of additional fish can have on the already running size-selective experiment.

2.5 Marking and individual ID.

Marking of the fish was done to give each fish a recognisable mark and a unique ID and was performed using elastomer injections under the skin of the fish with 1 mL syringes (Visible Elastomer tags or VIE tags;(Northwest Marine Technology). The tail was selected as the site of injection because the skin in this area was lighter in colour. The fish were marked on the left or right side of the tail to account for differences in marking and escape time caused by the marking procedure and potential damage the injection can have caused. The selected colours were red, blue, yellow, pink, and green, giving a total of ten combinations when all five colours were used on both the left and right side of the fish. As a total of ten individuals per sex were selected, every fish would then be given a unique ID including population number, sex, the side the elastomer was injected into, and the colour injected. Some of the elastomers were also luminescent and gave of a shine with the use of a UV-light, making it easier to spot of the mark had weakened. The marking treatment as seen by examples in **Table 2.1**, was the result with five selected colours.

Population	Sex	Side	Colour	ID
1	Female	Left	Red	1FLR
1	Female	Right	Red	1FRR
1	Male	Left	Red	1MLR
1	Male	Right	Red	1MRR
2	Female	Left	Pink	2FLP
2	Female	Right	Green	2FRG

In preparation of the marking procedure an appropriate solution of the sedative MS222 (mixture of 0.030 g MS222 and 0.020 g bicarbonate to 100 mL of water from the system) was mixed. As the mixture grew less effective with dilution through extra water with transfer of the fish, new batches were mixed if necessary. In addition, a platform with good lighting and a soft and moistened sponge, was prepared for the marking procedure.

During marking, two people worked in unison: one sedating the fish one by one and recording length in millimetres and weight in grams before handing over to person number two for marking. The fish were dabbed dry with a paper towel before weighing to get an accurate value from the scale.

During the marking itself, the fish was placed onto a wet sponge under good lighting with the head facing to the left of the person executing the marking procedure (right-handed). The needle with the polymer was then inserted into the tail of the fish at a sharp angle from the tail towards the head and adjusted after the skin broke so that the pigment would be as close to the surface of the skin as possible to make the pigmented polymer more visible. Placement of the pigment mark had to be adjusted from time to time if the fish had a slender tail or other, natural pigments such as the males. In this case, the pigment was placed higher or lower in the tail to have the individual fish identifiable also by the placement of the mark and so that the pigment was not obscured by dark spots (areas high in melanin) on the skin of the fish.

After marking the required fish, the individual populations were placed in small (3 L) tanks, with circulation of water, overnight to ensure that every fish survived through the recovery period. If any fish died, they were identified by their mark and a new fish was selected from the

remaining redundancies and given the same marking. The required measurements were done for the new fish and overwrote those of the previous one.

The marking was performed on the 3rd of October 2022. The fish were then checked after the recovery period, on the 4th of October. A few fish were then found dead, and were replaced, before being rechecked on the 5th.

After the required 180 fish survived recovery, they were placed in larger tanks (80 L) and left to acclimatise to their new surroundings. These same tanks were used for the “Group fishing”-part of the experiment.

2.6 Vulnerability to capture.

This study used size-selective harvested populations as the origin of our fish, and the fishing method used in the present study are different from the size-selective harvesting performed on the parent populations. In this study, fishing is equivalent to removal from the population and short-term transfer to new accommodations for the group fishing and catch and release for individual fishing. No fish were intentionally hurt or euthanised during the fishing experiment. While the size-selective harvests focused on size alone, this study is focused on behaviour and morphology linked to vulnerability to capture, rather than the size of the fish. The fishing in this study is a form of active fishing using a small net and entails visually finding the fish before catching them in the net. This means that the experiment measures net avoidance behaviour and is linked to swimming speed, acceleration, and agility.

As the fish were raised in a lab for several years, the aquarium-nets are the only possible equivalent to predators the fish have had contact with for several generations. However, avoidance behaviour is in many ways the same between wild and captive populations (Magurran and Seghers, 1990). This means that the net avoidance being measured can be used to speculate around predator avoidance in the wild.

2.6.1 *Group fishing*

The focal populations (20 individuals, sex ratio 1:1) were housed in 80 L tanks. The tanks were arranged in two levels with five tanks in each level.

The fishing itself was performed with a small aquarium net, a hand-net, elongated with a plastic stick so that the net could reach the bottom of the tank. Each catch of one or more fish was then placed in small tanks with numbered sections to maintain the order in which the fish were caught. The timing of the individual catch was also noted using a stopwatch and the data was transferred to a datasheet. Therefore, the vulnerability to capture within a group was measured as both time until capture and order in the capture sequence. In addition, the total time used to catch the whole population was recorded.

This procedure was performed for three populations per day for three days within the same week, Monday to Wednesday. The group fishing was performed twice for each population.

As the separation between the tanks were glass, the fish could see through to other tanks on their level and the decision was made to not fish two adjacent populations on the same day to minimise the stress on each individual and any potential learning from the experience of the adjacent population.

2.6.2 *Individual fishing*

Immediately following the *Group fishing*, the fish were separated into individual tanks (3 L) and left to acclimatise overnight in preparation for the individual fishing. The following morning paper or cardboard was placed between the tanks for the fish not to see the procedure being performed on a neighbouring fish and reducing the amount of stress on them. After the paper was placed between the tanks there would be an acclimatisation period of 10 minutes before the fishing experiment was performed to enable the fish to settle after the disturbance (O'Neill *et al.*, 2018). The reason for not separating the fish visually at an earlier stage is that the guppy is a social species and spend a significant amount of time socialising (Wilson *et al.*, 2014).

The individual fishing was performed in the 3 L tanks with a small, round sieve. The choice of the sieve was made to give a handicap to the fisher during fishing procedure so that the fish would have more opportunity to escape than a square net would provide, given the size and shape of the smaller tanks.

Time was measured from the time the sieve touched the water until the fish was safely in the sieve and out of water for 2 seconds. If the fish jumped out of the sieve immediately, the fishing continued as if the target fish was never caught. The time it took to catch each fish was recorded in a datasheet. This time variable is referred to as time until capture in individual fishing.

2.6.3 Replicates and resting period

In total the complete fishing experiment, including both group- and individual- fishing was performed twice before the next stage of the experiment. The populations were given a resting period of a minimum 10 days.

2.7 Morphology

Morphological data for the study was gathered using photographs of each fish, presenting the opportunity to use geometric morphometrics to study the shape of the fish through 10 chosen landmarks.

2.7.1 Photography

The fish were sedated using MS222 (same mixture as used during marking), one at a time and measured for length before being dabbed dry with a paper towel and weighed. Afterwards, the fish was placed on a plastic plate with a white background, millimetre-paper for scale, and a colour scale along with a note for ID on the side of the picture. The plate was well lit, and the camera was placed on a rail above the platform. A Canon DS126231 fitted with a Tamron SP 60 mm F/2 macro lens, was chosen for the photography session. The settings for the camera can be seen in **Figure 2.1**.

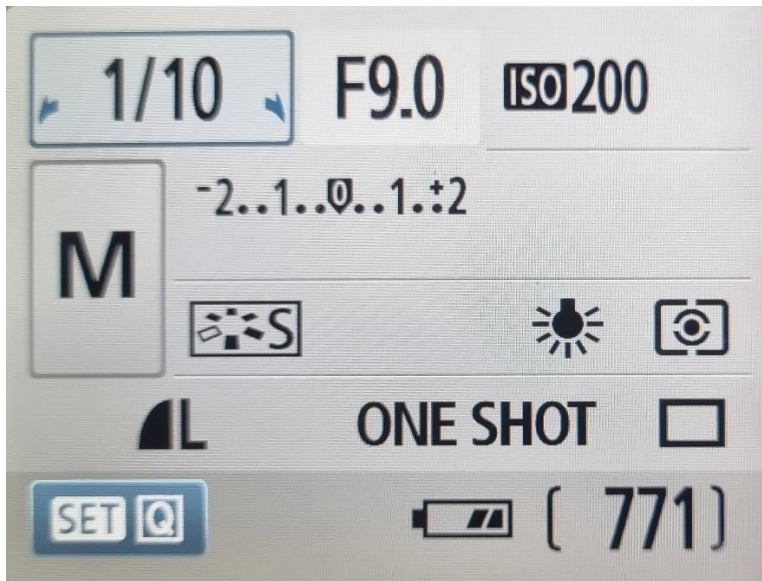


Figure 2.1 Settings used for the camera during photography.

Before the photo could be taken, the fish was adjusted using a small, moistened paintbrush so that the body was straight, and the fins were separated from the body as shown in **Figure 2.2**. The focus on the camera was then adjusted and the picture was taken. This procedure was repeated for every fish.

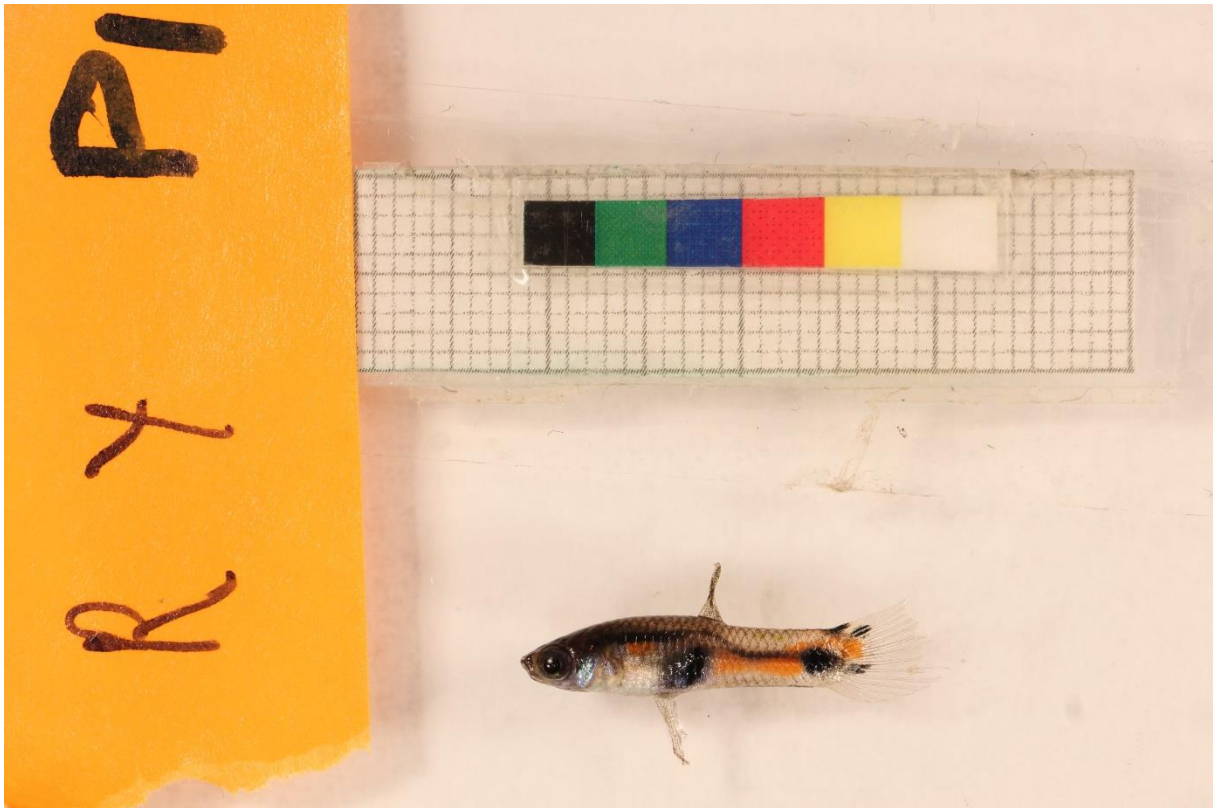


Figure 2.2 Picture of the male guppy ID-ed as 1MRY (Pop. 1, male, right-side mark, colour yellow) with a scale for length and colour and an ID mark. Fins and body adjusted to preferred angles.

2.7.2 Landmarks

Ten homologous landmarks were selected to characterise the lateral body shape of adult guppies (**Table 2.2** and **Figure 2.3**) following earlier studies (Jayawickrama, 2013; Klingenberg, 2013; Ahmed Idris, 2016). Landmarks were digitised from pictures using the software tpsUtil (V. 1.82) to create a “.tps” text file, and tpsDig2 (V. 2.32) to place the landmarks, and a size scale, which was defined using every image as explained by Crampton (2021) in in her video tutorials for morphometrics.

Table 2.2 Definition of the used landmarks.	
Landmark nr.	Anatomical position
1	Tip of the snout
2	Centre of the eye
3	Anterior extent of the dorsal fin insertion
4	Posterior extent of the dorsal fin insertion
5	Dorsal extent of the caudal insertion
6	Posterior tip of the caudal peduncle
7	Ventral extent of the caudal fin insertion
8	End of the caudal fin
9	Posterior extent of the anal fin insertion
10	Anterior extent of the anal fin insertion



Figure 2.3 1MRY with the ten defined landmarks used to digitise the shape of the fish.

The landmarks data was imported to the software MorphoJ (V. 1.07a) (Klingenberg, 2011) which was used to extract the shape data from the landmarks (as a covariance matrix or Procrustes fit) through Procrustes superimposition by removing the information on orientation, position and scale. In addition, a wireframe was created (**Figure 2.4**) to approximate the shape of the fish into graphics, which was later used to visualise the data and analysis. The outer connections between the landmarks are for the general shape, whereas the line from landmark

1 to landmark 2 is to note the position of the eye and the connection between landmark 4 and landmark 9 is to indicate the approximate start of the tail on the fish.

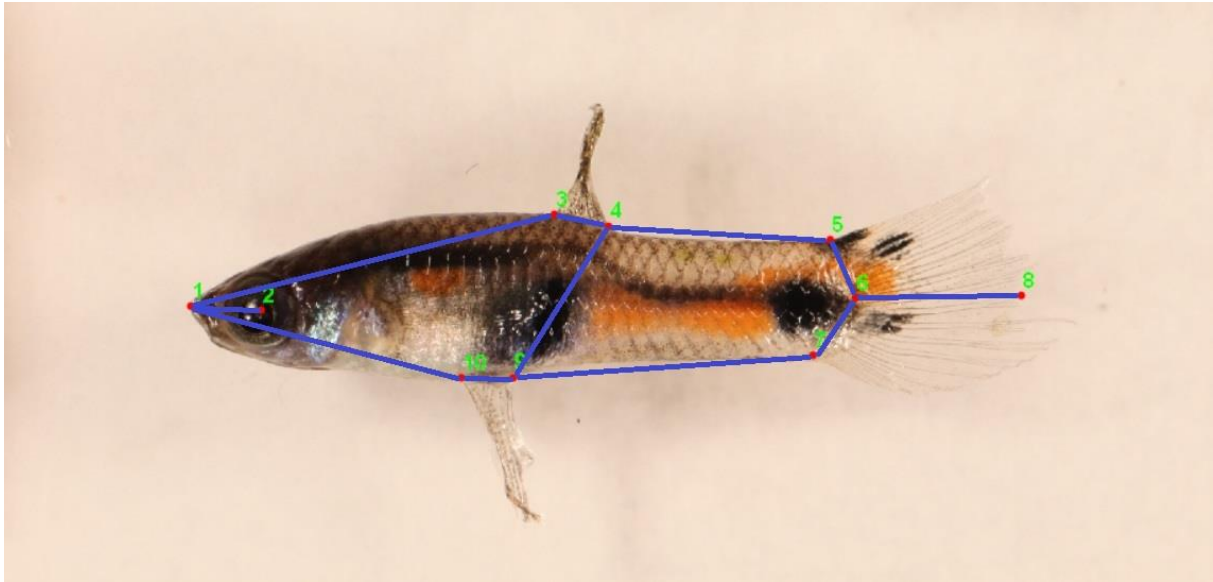


Figure 2.4 1MRY fitted with the wireframe used in graphics produced by MorphoJ.

2.8 Statistical analyses

2.8.1 Vulnerability

Vulnerability to fishing was represented by three time variables in this study: 1) time until capture during individual fishing, 2) time until capture during group fishing, 3) total time used to catch the whole population during group fishing. In addition, we recorded sex, population, harvest size-selection treatment, length of the fish in millimetres, and the ID of the fish as potential explanatory variables. These response time variables were then analysed in R (V. 4.1.1) through RStudio (V. 2022.07.2+576) (RStudio Team, 2022).

Firstly the time variables were checked for repeatability (**Table 2.3**) using the package rptR (Stoffel; Nakagawa and Schielzeth, 2017). Repeatability is a quantification of variability between replicates of the same experiment and for measurements performed by the same operator (Zanobini *et al.*, 2016). Repeatability is therefore a value that indicates the accuracy of the gathered data. In addition, trait repeatability is a measure of how stable the trait is over time within individuals. This is important when considering the effects of selection, as for

selection to occur the traits should be important for fitness and heritable. Trait repeatability has been suggested to set the upper limit for trait heritability (Killen *et al.*, 2016). The variable for total time to catch a whole population gave the highest repeatability but represents the whole population vulnerability rather than individual vulnerability. Using this time variable as an estimate of individual vulnerability would be pseudo replication if used to compare with the individual values of each fish. Therefore, time until capture from group fishing started was used to represent individual vulnerability in a group setting. This variable was selected over order in the capture sequence because repeatability of the former was higher and both variables were highly correlated (corr. = 0.89). Pairwise correlation between variables was assessed using the package Performance Analytics (Peterson and Peter, 2020) by making a correlation matrix with absolute values for Pearson correlation. All time variables were log-transformed before analysis.

Table 2.3 Repeatability and P-value as given by rptR.		
Tested variable	Repeatability	P-value (LRT/Permutations)
Time until capture during individual fishing	0.14	0.111 (LRT)
		0.103 (Permutation)
Order in the capture sequence (group fishing)	0.15	0.025
		0.024
Time until capture from start of group fishing	0.17	0.015
		0.009
Total time used to catch the whole population (group fishing)	0.31	<0.001
		0.010

The three time variables representing vulnerability to fishing were tested if they were affected by the size-selective harvest regimes and the length of the fish. All three response variables were log-transformed. This was performed separately for each sex using one of the time variables as a dependent variable, giving in total six models.

The full model setup used was based on linear mixed effects models from the R package lme4 (Bates *et al.*, 2015), using the intercept as a random effect on the population without interaction with the other variables. The other independent variables were set to 1) the length of the fish centered to the mean length (18.9 mm) and scaled by the standard deviation of length (2.5 mm), and 2) the selection regime from the size-selective harvests. The reasoning for doing it this way, and not including more variables, was that the initial model was extremely complex. Using the drop1-function, AIC-scores were compared between the potential models. And the model with the lowest AIC was retained as the final model.

The final touch was to control that the models performed well and satisfied all requirements through the use of DHARMA (Hartig, 2022) by checking for deviation in residuals. One change made from the original model was to separate the group fishing from the individual fishing because the Levene test for homogeneity of variance was not satisfied. In addition, the packages tidyverse (Wickham *et al.*, 2019) and ggplot2 (Wickham, 2016) was the two packages used for cleaning the data and visualising data and models.

2.8.2 Morphology

All analyses using morphological data were performed with the intent of finding differences between the size-selection treatments used on the origin populations, the populations that the focal fish were selected from during the size-selective harvest. In addition, the effect of the shape on vulnerability to fishing was tested.

Using MorphoJ (Klingenberg, 2011), selection treatments were tested for differences in shape or morphology in the three size-selective treatments. Three different types of analysis were performed on the shape data. Some variables from the fishing experiment were also used in regressions. 1) Canonical Variant Analysis (CVA) used to confirm sexual dimorphism between the sexes by using the Procrustes coordinates together with sex as a classifier. 2) After sexual dimorphism was confirmed in the Procrustes coordinates, the dataset was split into female and male, and the CVA was redone for the separate sexes with selection as a classifier. 3) Regression analyses were performed separately for two time variables: time until capture in individual fishing (replicate 1 and 2) and the time until a fish was caught in group fishing. The vulnerability

time variables were the response variable while the centroid size for the fish was the independent variable. The total time to catch an entire population ($N = 20$) was not used because it would imply pseudo replication. The time variables were also transformed into logarithmic values before use in the analyses. Permutation tests were performed in all analyses to test for significant differences. For all tests, the effect of size allometry on shape was accounted for.

3 Results

3.1 Size-selective fishing experiment

One of the first variables registered was the length of the fish. The overall distribution is shown in **Figure 3.1**, showing that all three size-selective treatments have two peaks, indicating groupings within the treatments. This is further confirmed by plotting female (**Figure 3.2**) and male (**Figure 3.3**) separately and confirming that the sexes are different in length. These data were further used in the analysis of the time variables recorded during the fishing experiment. Note that these differences in body length among size-selective treatments were not statistically tested. Populations are known to differ in standard length (Diaz Pauli, 2012; Bartuseviciute *et al.*, 2022).

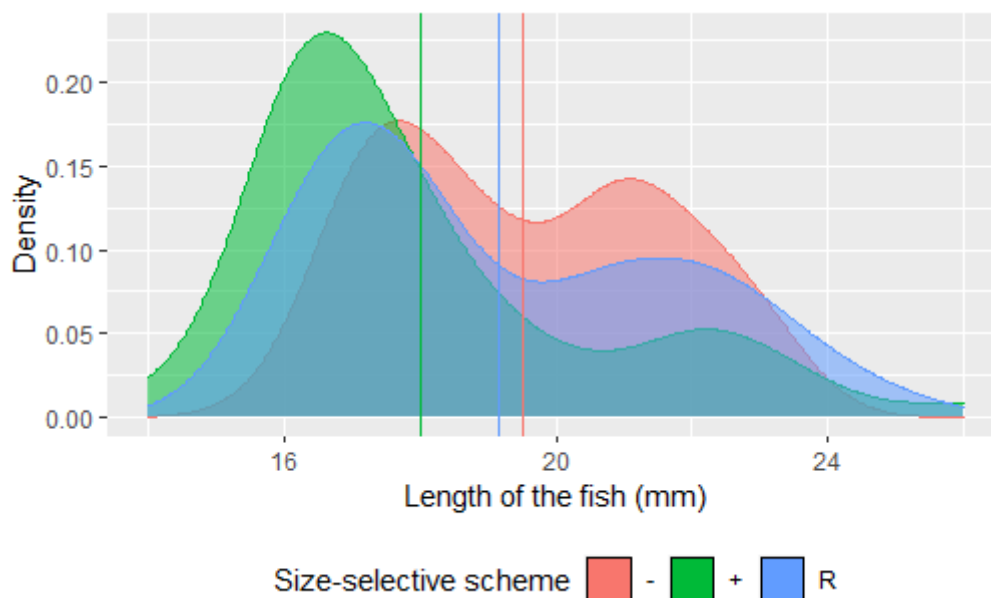


Figure 3.1 Overall length distribution of the fish, in millimetres, grouped by size-selective treatment. Density plot with vertical lines noting the group wise mean length.

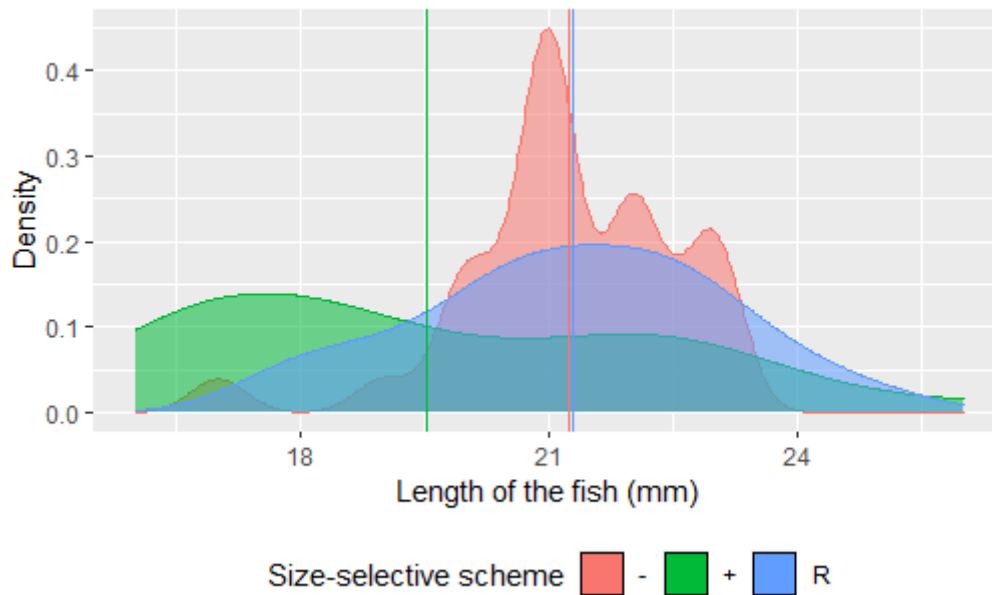


Figure 3.2 Length distribution of the female fish, in millimetres, grouped by size-selective treatment. Density plot with vertical lines noting the group wise mean length.

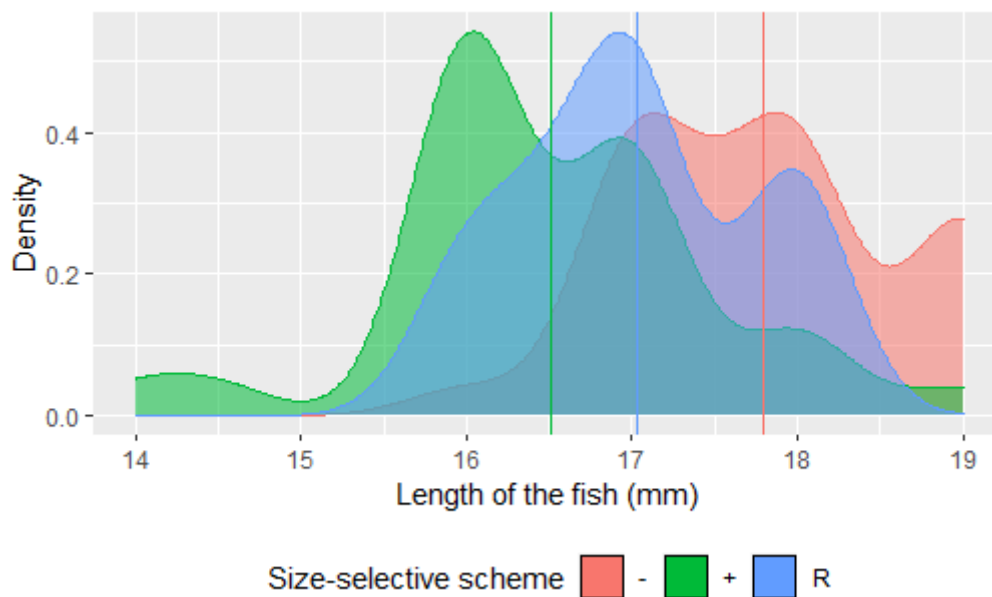


Figure 3.3 Length distribution of the male fish, in millimetres, grouped by size-selective treatment. Density plot with vertical lines noting the group wise mean length.

3.1.1 Group fishing

Time to capture for group fishing did not show a significant correlation with fish length (**Table 3.1; Figure 3.4**), as opposed to replicate 1 of time until capture in individual fishing. The lack

of relationship between time to capture in group fishing is the same for female fish (**Figure 3.5**) and male fish (**Figure 3.6**). In addition, the linear mixed effects models show that there is no significant difference between the size-selection treatments. This means that neither length or size-selection treatment had any effect on time until capture in group fishing.

Table 3.1 Linear mixed effects model results for both male and female and the sexes separate with time until caught (in seconds, log-transformed) for group fishing. “Both” refers to the sexes together in the same model, whereas the other two contain only female and male fish respectively. Mean length = 18.9 (± 2.5) mm.

Sex	Variable / Interaction	Estimate	SE	t-values	P
Both	Negative selection	5.22027	0.32522	16.05	<2e-16
	Length	0.16591	0.29665	0.56	0.577
	Positive selection	0.17774	0.36641	0.49	0.631
	Random selection	-0.55535	0.41972	-1.32	0.192
	Males	-0.03870	0.41620	-0.09	0.926
	Length : Positive selection	0.03692	0.33212	0.11	0.912
	Length : Random selection	0.25788	0.36698	0.70	0.483
	Length : Males	0.29410	0.56022	0.53	0.600
	Positive selection : Males	-0.03855	0.61415	-0.06	0.950
	Random selection : Males	0.39156	0.66513	0.59	0.557
Females	Length : Positive selection : Males	-0.10743	0.70173	-0.15	0.879
	Length : Random selection : Males	-0.61131	0.81905	-0.75	0.456
	Negative selection	5.33424	0.18509	28.82	2.11e-06
	Length	0.17109	0.24145	0.71	0.481
	Positive selection	0.19693	0.26764	0.74	0.499
	Random selection	-0.36020	0.25917	-1.39	0.230
Males	Length : Positive selection	-0.01562	0.27750	-0.06	0.955
	Length : Random selection	0.19591	0.29954	0.65	0.515
	Negative selection	4.851478	0.220037	22.05	<2e-16
	Length	0.182156	0.206135	0.88	0.379
	Positive selection	0.201381	0.295679	0.68	0.498
	Random selection	0.089000	0.277812	0.32	0.749
	Length : Positive selection	-0.002196	0.267888	-0.01	0.993
	Length : Random selection	-0.142242	0.317252	-0.45	0.655

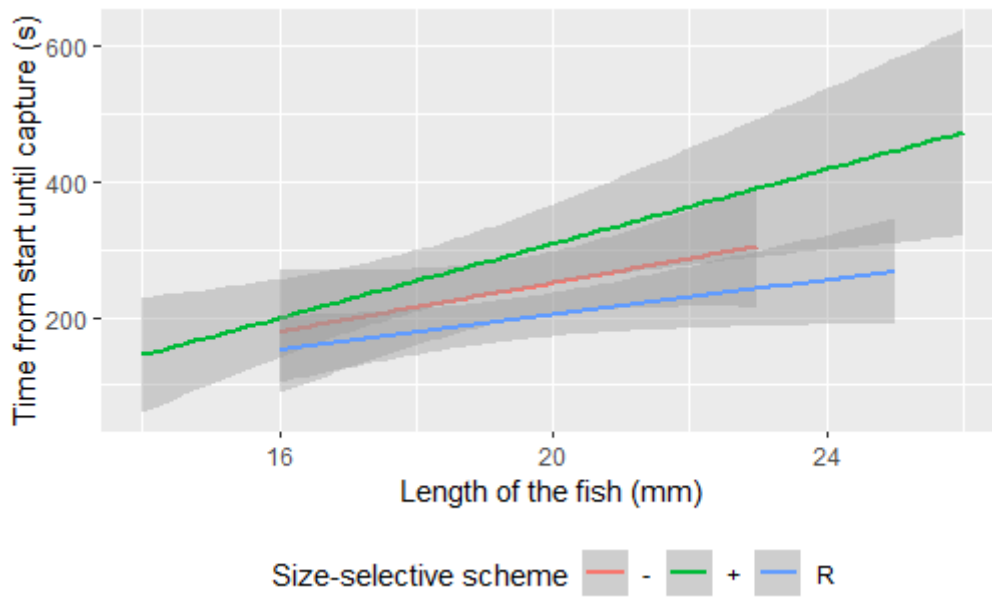


Figure 3.4 Time until a fish was caught in group fishing plotted against the length of the fish. Mean times were 242.10, 252.43 and 193.19 seconds for negative, positive, and random size-selection respectively.

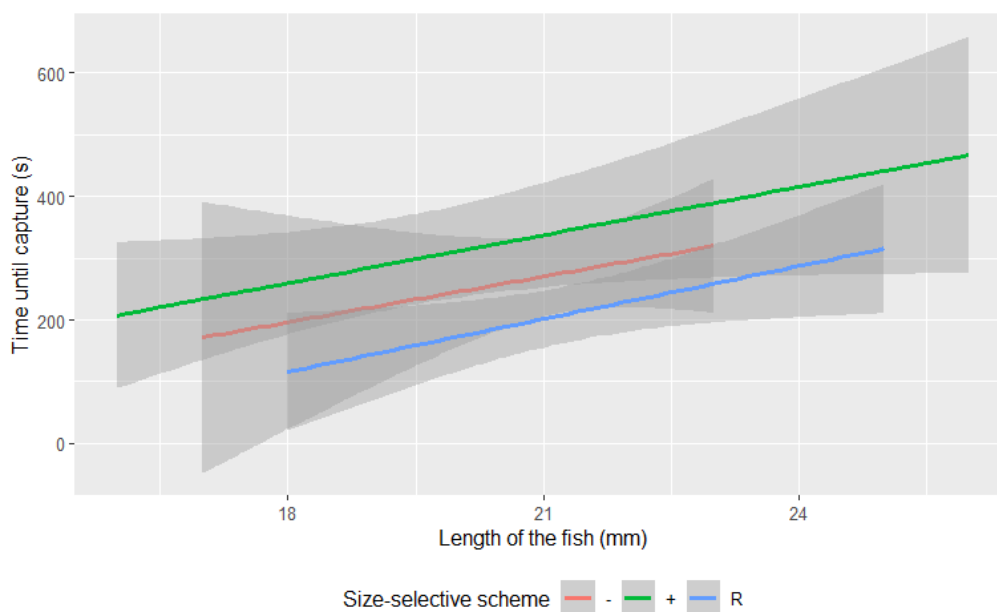


Figure 3.5 Time until a fish was caught in group fishing plotted against the length of the fish. Females only. Mean times were 276.24, 296.66 and 209.64 seconds for negative, positive, and random size-selection respectively.

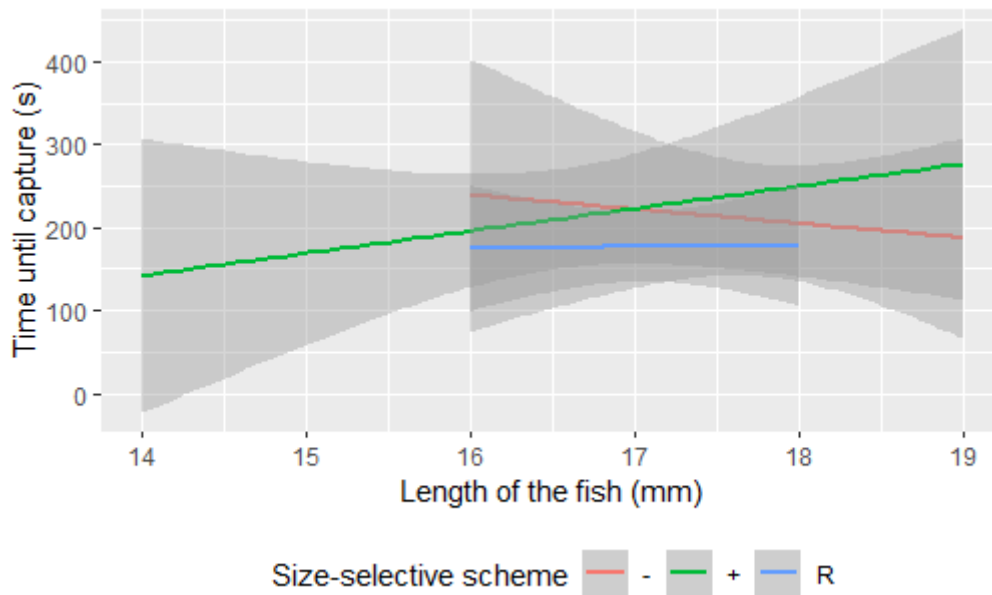


Figure 3.6 Time until a fish was caught in group fishing plotted against the length of the fish. Males only. Mean times were 207.96, 209.67 and 176.73 seconds for negative, positive, and random size-selection respectively.

For the total time to catch a whole population (20 fish, 9 populations), there are no clear results as to differences between the size-selection treatments, except for an indication that negatively size-selected populations took longer to catch as a whole compared to the random selection treatment ($P = 0.0511$, **Table 3.2**). Positive size-selection treatment presents no difference from the negatively selected.

Table 3.2 Differences in total time to catch a population.				
Variable	Estimate (log(s))	SE	t-values	P
Negative selection	6.42923	0.08	81.23	<2e-16
Positive selection	-0.03545	0.11	-0.32	0.7558
Random selection	-0.23732	0.11	-2.12	0.0511

3.1.2 Individual fishing

In the first replicate negatively size-selected fish of mean length have a time until caught for individual fish of 4.53 seconds (**Table 3.3**). An increase in length of 1 SD (Standard Deviation) to mean length the time to capture becomes 5.53 seconds, meaning that length has a negative effect (**Table 3.3; Figure 3.7**). Looking then at the rest of the output, especially the interactions between length and the remaining two selection treatments, there is no significant difference in the trend here compared to the other size-selective treatments (**Table 3.3**). Therefore, the three size-selective treatments have equal time until capture for individual fishing and the same negative trend with increasing length (**Figure 3.7**).

The second replicate of time until caught for single fish tells a slightly different story. Here there is no longer the negative relationship of time until capture for individual fishing with increased length (**Table 3.3**). But there is a significant difference between the negative size-selection lines and the positive size-selection treatment, showing that fish of mean length exposed to positive selection has a time until caught that is 0.85 seconds shorter than that of the negative size-selection (**Table.3.3; Figure 3.8**). This resulting in fish exposed to positive size-selection being more vulnerable to capture in individual fishing in the second replicate. The remaining variables and interactions do not account for any significant difference from the negatively selected fish in either a negative or positive trend.

Even though the models present significant results, it should be remembered that the time variable was not stable. The first and second replicates of time to capture an individual fish were unrepeatable. Explaining the different results obtained for replicate 1 and 2.

Table 3.3 Linear mixed effects model results for time until capture for individual fishing. Replicate refers to the two times the time variable was assessed. The estimate and standard error (SE) are natural log transformed. Mean length = 18.9 (± 2.5) mm.

Replicate	Variable / interaction	Estimate (log(s))	SE	t-values	P
1	Negative selection	1.71	0.11	16.03	$6.87 \cdot 10^{-6}$
	Length	-0.20	0.06	-3.26	0.001
	Positive selection	-0.01	0.15	-0.09	0.935
	Random selection	0.06	0.15	0.39	0.713
	Length : positive selection	0.16	0.09	1.77	0.079
	Length : Random selection	0.05	0.08	0.66	0.509
	2	Negative selection	1.62	0.06	28.75
Length		-0.07	0.07	-1.06	0.291
Positive selection		-0.17	0.08	-2.07	0.039
Random selection		0.04	0.08	0.45	0.652
Length : positive selection		0.01	0.08	0.15	0.881
Length : Random selection		0.07	0.08	0.85	0.396

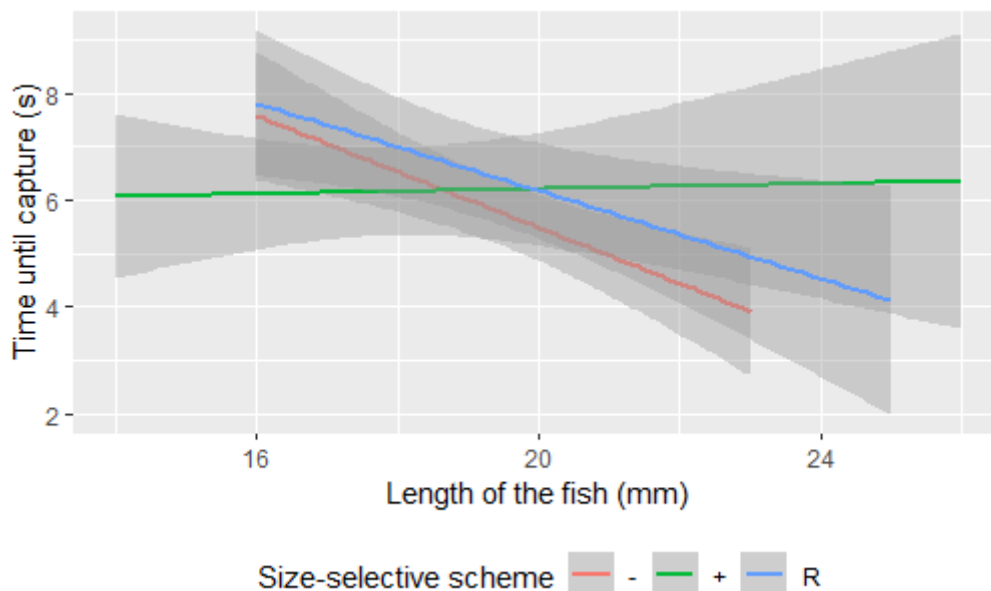


Figure 3.7 Replicate 1 for time until capture for individual fishing in a 3 L tank plotted against the length of the fish. Mean times were 5.74, 6.16 and 6.52 seconds for negative, positive, and random size-selection respectively.

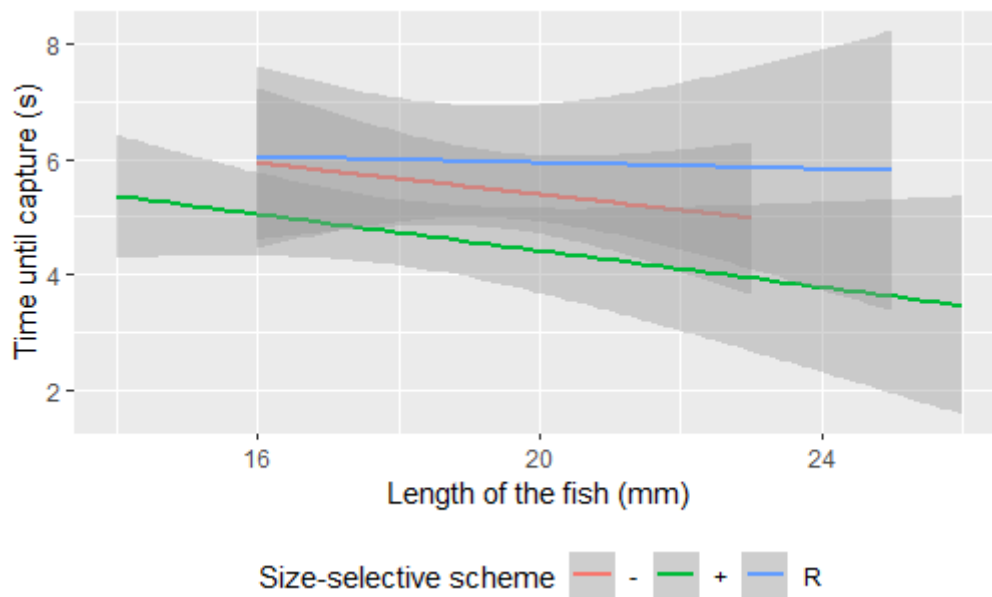


Figure 3.8 Replicate 2 for time until capture for individual fishing in a 3 L tank plotted against the length of the fish. Mean times were 5.45, 4.73 and 5.96 seconds for negative, positive, and random size-selection respectively.

3.2 Morphology

3.2.1 Shape differences between sexes and among size-selective treatments

The shape differences between male and female are significant. Males and females' shape differed in 17 standard deviations (Mahalanobis distance; $P = <0.001$, **Figure 3.9**, **Table 3.4**). The wireframe shows that females (light blue, **Figure 3.9**) are somewhat leaner, have a slightly shorter caudal fin, and have an anal fin that is both wider and placed further back compared to their male counterpart. The separate grouping for male and female fish can also be seen in **Figure 3.10**.

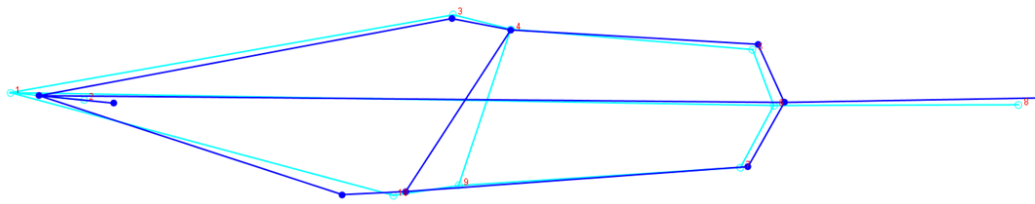


Figure 3.9 Shape changes between male (dark blue) and female (light blue). All fish included. Scale of changes in shape set to 5.

Table 3.4 Shape differences in Procrustes (absolute distance) and Mahalanobis distance (relative to within-group variation) between male and female. Result id from a CVA where the data was grouped by sex.

	Mahalanobis distance	Procrustes distance
Value	17.4794	0.1631
P-value	<0.001	<0.001

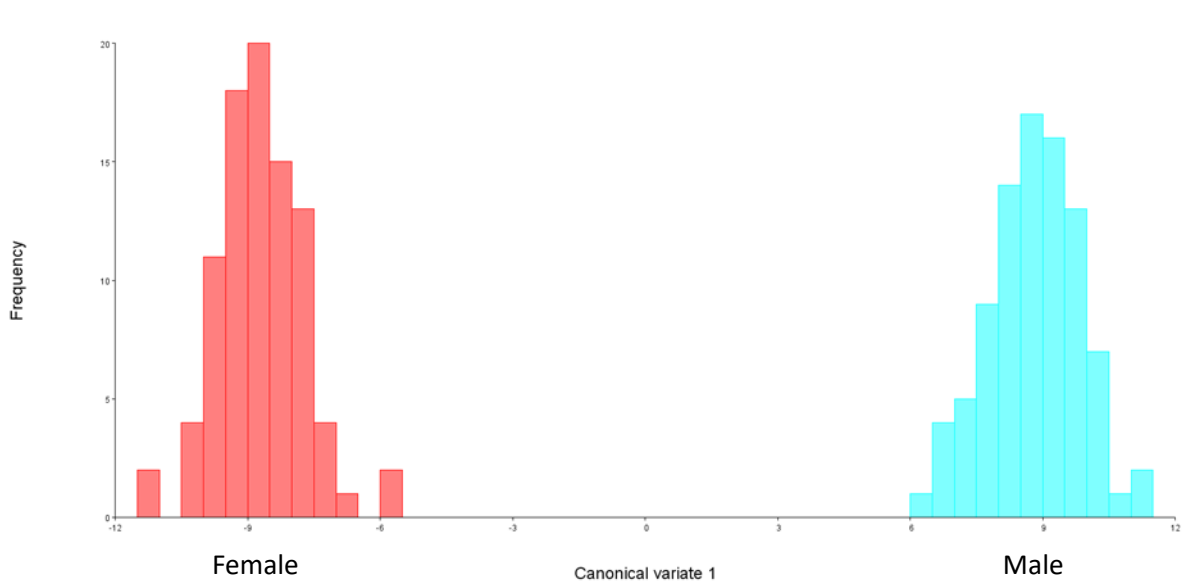


Figure 3.10 Distinct groups for male and female fish along the plotted variate.

Moving on to comparisons of size-selective treatments within sexes, the differences between the groups are smaller compared to between sexes and in addition, not all Canonical Variate Analysis gave significant P-values. CV1: Eigenvalue = 77.24, Variance = 100 %.

For female fish, all three size-selection treatments were different from each other **Table 3.5**. This is illustrated in **Figure 3.11** where the positively size-selected female fish are significantly shorter and leaner than their counterparts subjected to random or negative size-selection. Furthermore, negative size-selection has a more robust tail than the other two size-selection treatments, at least towards the end of the tail and the base of the tail fin.

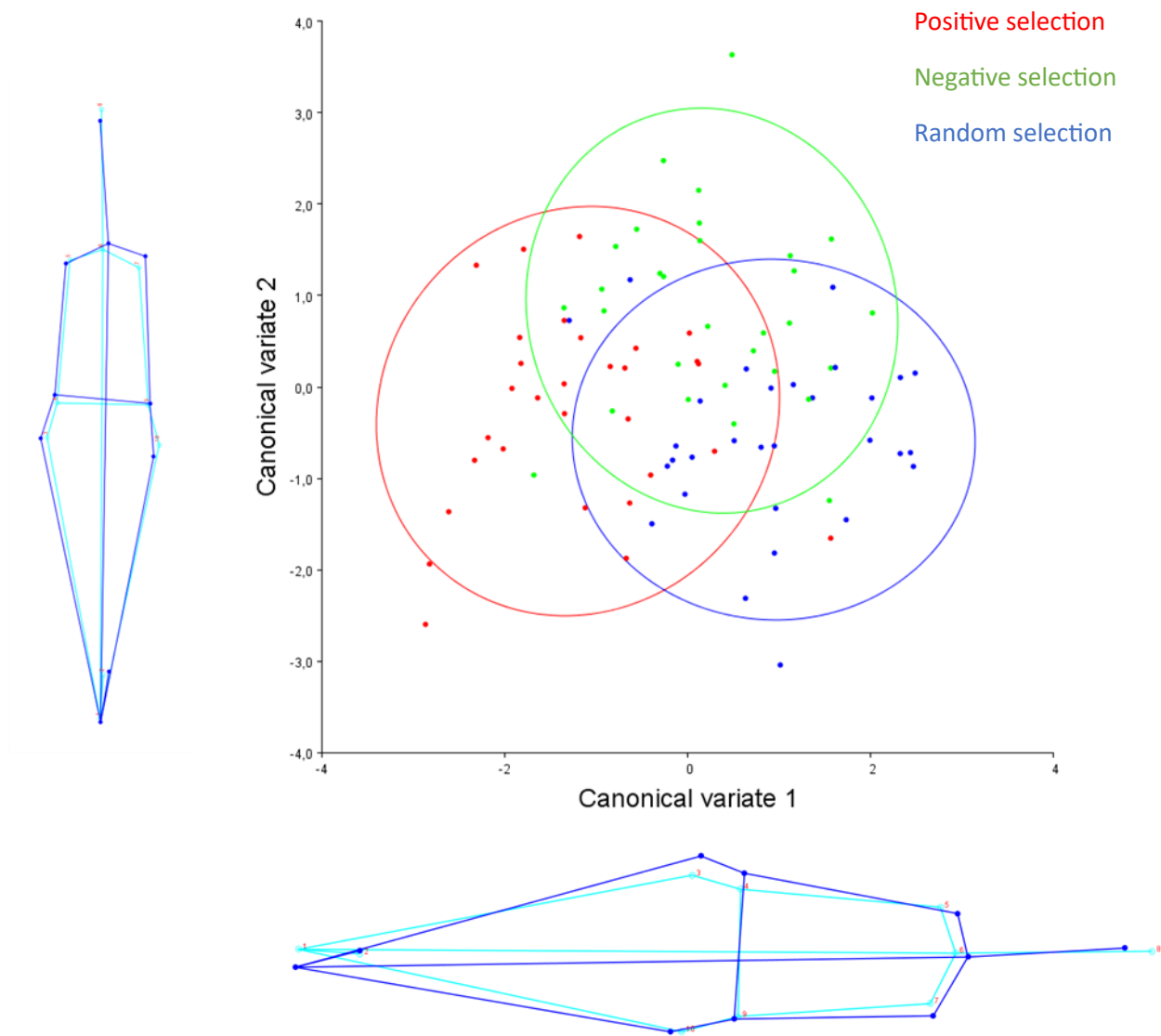


Figure 3.11 Shape differences as illustrated by wireframe along the plotted variates (light blue to dark blue) showing the groupings for the three size-selective treatments for females only. Scale of changes in shape set to 5.

Table 3.5 Pairwise comparison of shape differences in Procrustes (absolute distance) and Mahalanobis distance (relative within-group variation) for female fish between the size-selective treatments and their P-values.				
Selection comparison	Mahalanobis distance	P	Procrustes distance	P
Positive:Negative	1.83	<0.0001	0.01	0.0096
Positive:Random	2.16	<0.0001	0.01	0.0072
Negative:Random	1.56	0.0010	0.01	0.7928

For the male fish much the same as for the females when looking at the positively size-selected fish (**Figure 3.12, Table 3.6**), shorter and leaner. The positively size-selected fish are shorter and leaner than their counterparts from the other two treatments, but also longer than the randomly selected fish that seem to be more compact. The negatively selected fish are overall the longest fish. For females the largest shape difference was between positive and random size-selection, while for males the largest differences occurred between positive and negative size-selection (**Table 3.5 and Table 3.6**).

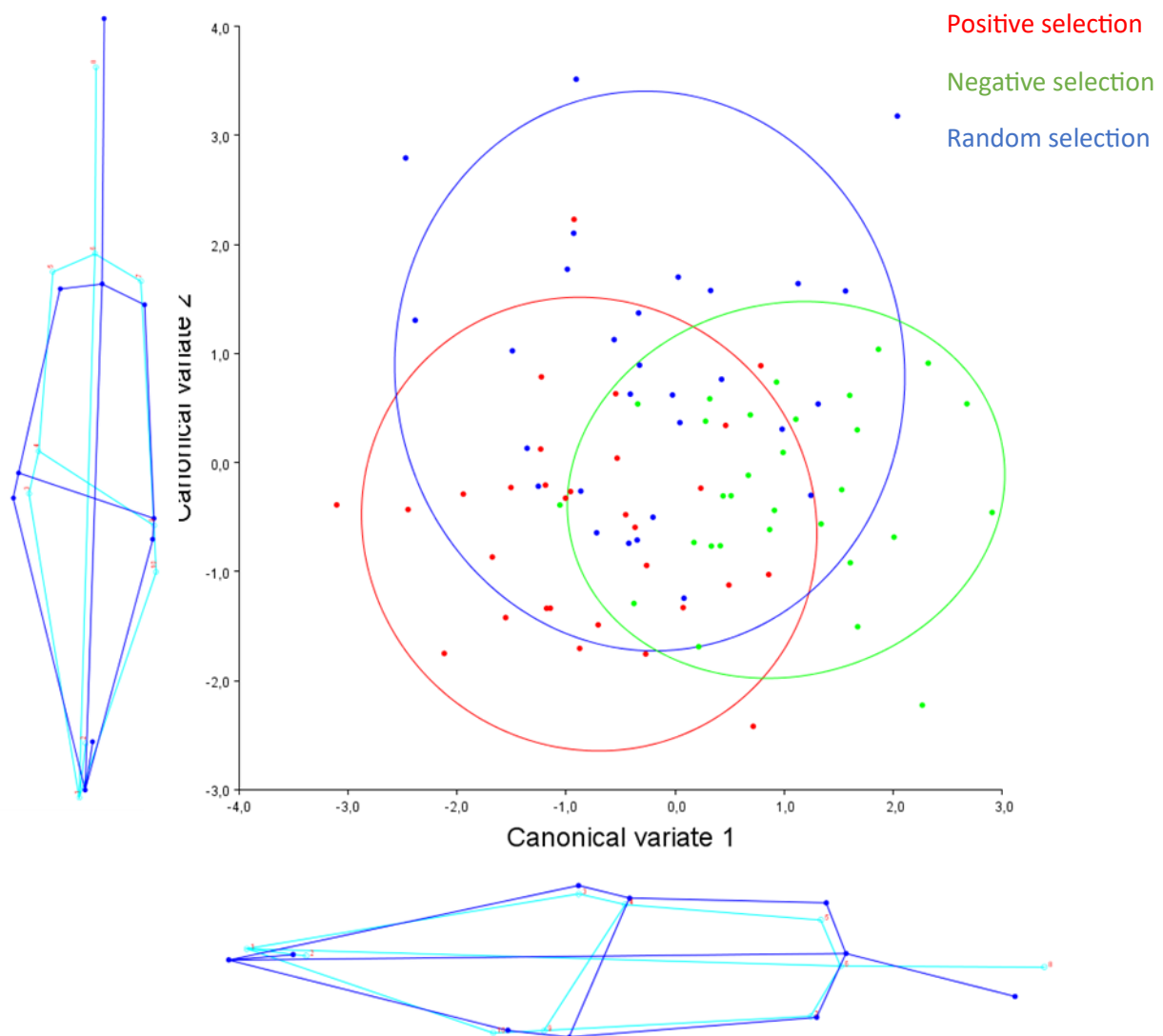


Figure 3.12 Shape differences as illustrated by wireframe along the plotted variates (light blue to dark blue) showing the groupings for the three size-selective treatments for males only. Scale of changes in shape set to 5.

Table 3.6 Pairwise comparison of shape differences in Procrustes (absolute distance) and Mahalanobis distance (relative within-group variation) for male fish between the size-selective treatments and their P-values.

Selection comparison	Mahalanobis distance	P	Procrustes distance	P
Positive:Negative	1.83	< 0.0001	0.01	0.0474
Positive:Random	1.51	0.0035	0.02	0.0574
Negative:Random	1.66	0.0001	0.02	0.0158

3.2.2 Effect of shape on vulnerability to fishing

For the time until catch for individual fish (in both replicas) in a 3 L tank, there were no significant effect of centroid size and the morphology of the fish explained very little of the variance (<1.2%, not significant, **Table 3.7**). However, time until capture for group fishing was affected by the centroid size, at least for females (**Table 3.7**). Here the centroid size and shape of the fish accounts for almost 6% of the variance seen for female fish ($P = 0.025$). But a similar shape effect is not seen for male fish. The size effect for females was positive (0.55, $P = 0.025$, **Table 3.7**), meaning that a larger size will lead to longer time until caught as can be seen by the shape change in **Figure 3.13**, where the female fish have longer caudal peduncle along the CV1 for the regression. Longer caudal peduncle can therefore be indirectly linked to longer times until caught during group fishing. The depth of the fish are also larger.

Table 3.7 Regression results for the three time variables separately as independent variables and plotted against centroid size, the overall size of the fish.

Time variable	Sex	% prediction	Regression coefficient	P
Time until caught for individual fishing 1	Female	<0.01	<-0.01	0.993
	Male	0.16	-0.08	0.703
Time until caught for individual fishing 2	Female	0.63	-0.09	0.456
	Male	1.12	0.21	0.326
Time until caught for group fishing	Female	5.73	0.55	0.025
	Male	1.41	0.47	0.272

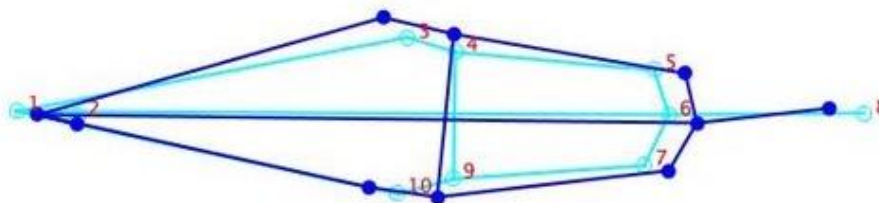


Figure 3.13 Centroid size and shape changes for females along the regression for time until caught for group fishing. Light blue is the start and dark blue is the end. Scale of changes set to 5.

4. Discussion

4.1 Summary of results

Overall, there are no clear and consistent differences among size-selection in vulnerability to fishing. For time until capture for individual fishing is impacted by length in replicate 1 and positive selection in replicate 2. In both cases the difference is negative compared to the negatively selected fish. However, time to capture for group fishing was not affected by either length or size-selection. For the total time it took to fish a whole population, there are little speak of although there is an inkling that the randomly size-selected fish are easier to catch than the negatively size-selected fish.

As expected from the literature and the original measurements of length, there are significant differences in the shape of the fish between the sexes. Furthermore, the positively size-selected fish of both sexes are shorter and leaner than the randomly and negatively size-selected fish. For females, the tail of the negatively size-selected fish is somewhat longer and more robust than the other two. The randomly size-selected females are the most robust and have the deeper body. For males, the negatively size-selected fish are slightly longer and more robust than positive and random, whereas positively and randomly size-selected fish are not significantly different, although there is an indication that random is the shorter of the two. The morphology had no significant effect on time until caught for males in group fishing nor for males and females in individual fishing. Centroid size explains almost 6% of the variation shown in time until caught for females in group fishing.

4.2 Group vulnerability

In general fish body length did not affect vulnerability to group fishing. Killen; Nati and Suski (2015) also found that the length of the fish does not have significance for the vulnerability to be captured. Instead, they point to the metabolism of the fish and adaptations for anaerobic metabolism. This is because burst swimming, which utilises the anaerobic capacity of the animal, is often used by fish to avoid predators or to catch prey. In addition, size-selected lines did not differ in their vulnerability to fishing when accounting for differences in body-length.

For the total time to catch the whole population (20 fish, 9 populations), there is no significant difference between the size-selective treatments. However, the randomly size-selected may be easier to catch than the negatively size-selected line. This may suggest that the longer fish of the negatively size-selected populations are better at avoiding the net, possibly through a higher maximum speed (Cano-Barbacid *et al.*, 2020). But when vulnerability to fishing was tested for each fish length, no significant result was found. Thus, this difference in vulnerability between random and negative size-selected lines might be due to other factors not accounted for in this study.

4.3 Individual vulnerability

The results for vulnerability to individual fishing show a negative correlation between time until caught and the length of the fish (replicate 1). This means that the shorter fish had a better chance of avoiding being caught when alone. When considering replicate 2, positively size-selected fish are more vulnerable to fishing relative to negative size-selected fish. Therefore, the populations where the larger fish had been selected out (positive size-selection) also had a negative effect on time until caught for individual fish and was more vulnerable to being caught. This, to some degree nullifies the result from replicate 1. If size-selected lines only differed in size, the result from replicates 1 and 2 would indicate opposite effect of length on vulnerability to individual fishing. However, the size-selected lines differ in other traits and thus the vulnerability of fishing might be due to other factors (Diaz Pauli, 2012). The time until capture in individual fishing was also unstable (not consistent over time), and the results should not be trusted outright, as both time variables might measure different things, as habituation or learning might have occurred.

4.4 Sex and selection on shape

Sex plays a major part in the size differences in the fish. The differences are large enough to create two distinct groups in **Figure 3.10**. This is, however, expected as the sexual selection is evident in the several conspicuous ways. Colouration, for one, is an energy expenditure that the females do not have. On top of this the males have the specialised gonopodium, adding to the differences between the sexes. Although pure shape differences are not as visible to the naked eye as colouration and size, they are large enough that the results were muddled by using both

sexes in the same analysis. The clearest difference between the sexes shape wise is the position of the anal fin, being placed further forward in the male fish and that it has a narrower attachment to the fish.

When concerning the size-selective treatments, the sexes were split, and the differences between the groups became more obvious. For the females, positive size-selection resulted in leaner fish and negative and random resulted in more robust fish. For the males the positive selection resulted in leaner fish, like it did for females. However, in this case randomly size-selected fish are now different from negative and like positively size-selected. This means that the randomly selected fish were affected oppositely between sexes in the randomly selected populations. These results are different from previous studies made on the same selection lines performed by Ahmed Idris (2016) and Jayawickrama (2013) who found no significant differences between the size-selective lines. The reason for this change in results may be because of the fish still adapting to aquarium tanks without water currents like Burns; Di Nardo and Rodd (2009) found. Further selection and evolution may therefore have proceeded in a slightly different direction than in these earlier studies. The selection lines may also have experienced genetic drift or changed through a plasticity response before, and selection can have taken a firmer grip over time by eliminating some of the gene pool and leading to more directional evolution for the positive and negative size-selection over time.

Although fishing with a handheld net is not the same as rod and lure like Alós *et al.* (2014) derived their data from. Fishing equipment can affect the morphology of fish. In the case of wild fish being selected by rod and lure, the fish with larger mouths are being selected as they can swallow the lure more easily and driving the selection towards smaller mouths on future generations in the populations.

Again, the shape differences between the size-selective treatments were as follows: For females, positive size-selection resulted in leaner fish and the other two resulted in more robust fish. For the males the positive selection resulted in leaner fish, like it did for females. However, randomly size-selected fish are now different from negative and like positively selected. This means that the randomly selected fish were affected oppositely between sexes in the randomly selected populations.

Shape differences being linked to vulnerability to capture can be found in other studies as well. Allendorf and Hard (2009) for example found that populations of Sockeye salmon (*Oncorhynchus nerka*) that are under pressure from fishing evolve smaller and more slender bodies less attractive to fishermen as the fish are smaller in weight. This can be compared to the negatively size-selected line where the smaller fish are harvested leading to larger and more robust fish compared to the positive size-selective line. Similarities in change caused by selection can also be found in a study by Alós *et al.* (2014) where recreational fishing with rod and reel result in harvesting the fish with larger mouths and more streamlined bodies. In other words, fish that have an easier time swallowing the lure and can glide through the water at a faster velocity. Fenberg and Roy (2008) also found that the selection of larger individuals has large effects on the targeted species resulting in more than changes in size and shape, but also transferring effects over to other life-history traits. This may help explain the lack of significance in the case of length for the lines. Differences between the seize-selective lines found in other traits not explored in this study can be evening out the differences between the selection lines when looking at length.

4.5 Individual vs group vulnerability

In natural conditions, within a shoal an individual fish gets muddled and it is hard to target by a predator, in addition, there are better opportunities to forage when fish shoal as the individual spends less time looking out for predators (Thambithurai *et al.*, 2018). In nature active fishing such as trawling by larger fishing vessels will find and follow large shoals of fish using sonar to achieve larger catches, large shoals are more easily seen and thus more vulnerable to fishing. Thambithurai *et al.* (2018) also suggest that passive and active fishing gear select for opposite behaviour patterns as larger shoals, normally consisting of more timid fish are more likely to be caught by a trap than smaller shoals of bolder fish and that trawling may target bolder fish that are more willing to approach an unknown object. The use of an aquarium net to catch fish is not the same as setting a trap and can therefore not be compared directly. Lone fish have to be much more vigilant towards predators in the wild, and therefore, have much less time to forage for food (Thambithurai *et al.*, 2018). This suggests that the fish are more sensitive to changes and may react more intensely to a predator than a single individual in a shoal.

Although direct comparison between individual and group fishing were not possible, there are indications that length can impact time until capture for both groups and individual fish. For the groups this is from the total time until capture for the whole population, randomly size-selected fish may be easier to catch relative to the other two selection treatments, suggesting a positive relationship between vulnerability and length, whereas the vulnerability individual fishing may indicate is a negative relationship between length and time until caught.

Can the negative trend with length be contributed to the confined space the fish was in? Considering the tanks were as small as 3 L, they were smaller, relatively than the group tanks with a volume of 80 L (4 L per fish). On top of that the sieve that was used, although smaller than the net used for the group fishing and with rounded edges was also relatively large for the size of the tank. These inconsistencies in the design of the experiment may have contributed to the group fishing and individual fishing being so different that the model could not compare them. As a result, the model became too complex, and any possible significance muddled.

In addition to the inconsistencies in the choice of instruments, there is a large possibility that the fisherman in this study became better at manoeuvring the aquarium net and the sieve during the fishing experiment, affecting the time variables collected. This can have led to shorter times between replicate 1 and replicate 2 as well as between populations as they were consecutively fished. The marking procedure may also have affected the populations differently as it proceeded. This is because one of the people performing the marking had limited experience beforehand, and can have improved significantly over time, leading to fish marked early potentially being more affected by the injection because of unnecessary amounts of elastomer injected into the fish or a bad angle of the syringe leading to unnecessary injury.

4.6 Effect of shape on vulnerability to fishing

There was little of note for the effect of shape on the vulnerability to being caught in both individual and group fishing. This is with the exception of time from group fishing started until a fish was caught for female fish. Here the shape of the fish explained 5.73 % ($P = 0.025$) of the variability seen in the time until caught. This is with a positive coefficient, meaning that larger fish can avoid being caught for longer.

Furthermore, Hendry *et al.* (2006) found that fish are affected by higher predation may end up with a more robust tail, meaning that the fish adapting to new predators are selected for avoidance through a more powerful tail potentially resulting in faster bursts when avoiding predators. This is, however, not the same as pure size-selective treatment, but shows that a more powerful tail can help the fish in the effort of avoidance.

4.7 Size-selective harvests

The main selective treatment performed on the fish used in this study has been size-selective (Diaz Pauli, 2012; Jayawickrama, 2013; Ahmed Idris, 2016). This is apart from the random selection process performed during the last harvest where the fish for the current study was separated from the origin populations. This means that it is the size-selective treatment that has resulted in shape and size differences between the three size-selection treatments and therefore also been linked to vulnerability to capture. This treatment may also have affected other traits not addressed here, and therefore, would need further research to investigate if the indicative results are correct.

4.8 Relevance

The results of this study can be related to the effects of trawling in some way, even though the fishing activity is somewhat different in the reaction to the movement of the fish. Nonetheless, it implies that selective treatment can lead to differences in vulnerability to capture from fishing and that the differences in shape and length can affect overall vulnerability.

This coincides with what Fenberg and Roy (2008) found, where many traits are affected by the size-selective harvest of wild fish for consumption. Among them are a reduction in body size and reproductive investment. Further research, looking at experiments like this study has used also found that understanding of fisheries-induced evolution can be obtained (Diaz Pauli, 2012). And that such research can make it easier to build programs for conservation (Cooke *et al.*, 2019). The native environment of a species is a vastly more complex system than the lab and can contain many variables that can influence selection (Killen *et al.*, 2016). Therefore, laboratory experiments are vital in the acquisition of knowledge.

This study contributes to that, but more research is needed, and the experiment needs alteration and more stringent guidelines to produce better results.

5. Literature

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