



Faculty of Applied Ecology and Agricultural Sciences

Christian Holte Heia Fagertun

Master thesis

A comparison of body height in Crucian carp (*Carassius carassius*) in lakes with- and without predators



Master in Applied Ecology

2020

Date

Place

Signature

I agree that this thesis is for loan in the library

YES NO

I agree that this thesis is open accessible in Brage

YES NO

Table of Contents

Abstract	4
Introduction	5
Material and methods	8
Sampling design and study areas.....	8
Sampling procedures	9
Laboratory work	11
Environmental measurements	11
Morphological analyzes	12
Statistical analyzes	13
Results	16
AIC selection results for both models	16
Model for PC1	17
Model for Relative body height.....	19
Discussion	21
Conclusion.....	24
Acknowledgements	25
References	26

Abstract

Induced morphometrical defenses have been reported in crucian carp (*Carassius carassius*), and these defenses are presumed to be induced by predators. Crucian carp was sampled during 2018 and 2019 in 12 lakes in southeast Norway. In three of these lakes there were no piscivorous predators, while in the remaining nine lakes there were top predators such as trout (*Salmo trutta*), perch (*Perca flavescens*) and pike (*Esox lucius*). I observed how crucian carp develop a greater body height in the presence of predators and other abiotic variables. Results confirm what other studies have found, that crucian carp grow a higher body with the presence of predators, but that also the abiotic factor lake size have a major impact on the growth. The size of the lake has been poorly reported in the literature as a factor that can drive changes in body height. A larger lake can compromise of a much more complex biological system than a small lake, which maybe can explain the growth in body height.

Key words: *Carassius carassius*, morphology, predation, inducible defenses, phenotypic plasticity, *Esox lucius*.

Introduction

In ecological systems, predation is an important mortality factor (Nilsson, Brönmark & Petterson, 1995; Brönmark, Paszkowski, Tonn & Hargeby, 1995), which may drive evolutionary change. In 1973 Leigh Van Valen presented the red queen hypothesis, that species must adapt and evolve to survive the battle against competitors and predators in an evolutionary arms race; but also that evolution would continue even in the absence of abiotic disturbances (Nordbotten & Stenseth, 2016). Prey adapt in order to reduce predation risk in different ways, where for example marine and freshwater invertebrates can develop spines, keels and helmets as a response to cues from their predator (Gilbert, 1966; Harvell, 1984; Harvell, 1990, Hulthén, Chapman, Nilsson, Hollander & Brönmark, 2014). Such adaptations are either constitutive or inducible, this depends on whether or not prey need environmental stimuli for activation (Harvell, 1990; Brönmark & Miner, 1992). Morphological defenses can be inducible through phenotypic modification activated by cues from predators (Harvell, 1990; Brönmark & Miner, 1992). Phenotypic plasticity is the ability of an organism to express different phenotypes in response to distinct biotic or abiotic environments (Trussell & Smith, 2000; Andersson, Johansson & Söderlund, 2006; Weber, Roundst & Brown, 2011). This allows prey organisms to express defenses and reduce vulnerability to predators when needed but may also save energy when predators are present by e.g. reduce swimming activity (Harvell, 1990; Hulthén, et.al, 2014).

Studying the relationship between the morphology of an organism and its environment is a central concepts in biology (Gaston & Lauer, 2015), and are divided into biotic (Agrawal, 2001) and abiotic factors (Fulton, Binning, Wainwright & Bellwood, 2013). As such, morphological studies have a long history also in ichthyology (Sidlauskas et. al., 2010), with regard to illuminating putative adaptation of different species as well as in setting taxonomic names. For instance, several studies have revealed that predation risk is linked to the predator's mouth gape (Brönmark & Miner 1992; Nilsson et. al., 1995; Magnhagen & Heibo, 2001). Further, Webb (1984) proposed the theory of the “morphological triangle” revealing that differences in fish body form influence how specialized a fish is at i.e. cruising, accelerating and maneuvering. Fish species needs to cope with both intra- and interspecific competition as well as intra- and interspecific predation in their living environments. This

suggest that an optimal trade-off exist between anti-predator defence strategies and optimal foraging so it will survive until it can reproduce.

Crucian carp (*Carassius carassius*) is a widespread fish species in Eurasia which are commonly found in lakes and ponds (Vøllestad, Varreng & Polèo, 2004). Crucian carp is adapted to both of these environments as it can live in well-oxygenated lakes as well as in anoxic and solid frozen lakes (Fagernes et. al. 2017). Crucian carp is described as two different forms: an abundant shallow-bodied “dwarf” form and a considerably larger “deep-bodied form” (Polèo et. al., 1995; Holopainen, Tonn & Paszkowski, 1997). This difference in shape is linked to the system where it lives, if it is an allopatric system (i.e. only Crucian carp) or a sympatric system with piscivore predators (e.g. Polèo et. al., 1995). Crucian carp living in allopatry often achieve high numbers and are shallow bodied, whereas in sympatry with piscivorous fish crucian carp is usually less abundant and are deep-bodied (Brönmark & Miner, 1992; Polèo et.al., 1995; Nilsson et.al., 1995; Vøllestad et. al., 2004). Diet differences exist among the different sized fish, where shallow bodied crucian carp tend to feed on zooplankton whereas the deep bodied crucian carp feed on benthos (Tonn, Holopainen & Paszkowski, 1994).

Predator induced morphological defense is an evolutionary paradigm where the existence of a predator induces an adaptive change in prey morphology that reduces predation risk (Bordeau & Johansson, 2012). The presence of predator fish can change prey fish behavior, leading to a decrease in activity, resulting in increase or decrease in prey growth (Bordeau & Johansson, 2012). In 1992, Brönmark & Miner experimentally tested in a laboratory how high and low food availability and presence of pike affected the body shape of crucian carp. They found that the presence of pike (*Esox Lucius*) was a contributor to a deeper body in the crucian carp. They further hypothesized that the change in body morphology could be explained by 1. selective predation, 2. an increase in resource availability, or 3. a predator-induced phenotypic modification of body shape (Brönmark & Miner, 1992). They concluded, and with later support, that a deeper body shape directly reduces predation risk, because a deeper bodied prey is less preferred and thus provide a refugium against a gape-limited piscivore (Brönmark & Miner, 1992; Tonn et.al., 1994; Nilsson et.al., 1995). In young walleye (*Sander vitreus*), a fusiform prey was more often preferred over deep-bodied prey (Einfalt & Wahl, 1997), as handling time in general is often less with a fusiform body shape (Nilsson et. al., 1995; Einfalt & Wahl, 1997; Weber, Dettmers, Wahl & Czesny, 2010).

The main aim of my study was to test if the body height and body shape of crucian carp differ in the presence of piscivorous fish (sympatry) compared to systems only harboring crucian carp (allopatry). This was accomplished by comparing a set of three different predation assemblages (i.e. comprising three types of top predators) against allopatry of crucian carps. Further, a set of three lake replicates within each of the four categories was used to reach a more general evaluation.

General hypothesis:

1. Body height in crucian carp living with the presence of predators will have higher bodies than crucian carp living without predators.
2. The differences in body height is only associated with predators
3. Predators with the biggest mouth area will have the biggest effect on growth in body height.

Material and methods

Sampling design and study areas

In this study a set of 12 lakes were chosen: three without predators and nine with predators (Table 1). The nine lakes with predators present were divided into groups according to the top predator species present in the lake: three with brown trout (*Salmo trutta*), three with perch (*Perca fluviatilis*) and three with pike as the top predator.

Lake sampling localities were selected and situated in southeast Norway in forest, urban and farmland areas. Vegetation surrounding the forest and urban lakes primarily consisted of birch (*Betula spp.*) and pine (*Pinus sylvestris*). Urban lakes primarily were situated close to parks, roads, and birch and pine forests. Farmland lakes was situated in cultivated areas. In most of the lakes, peat, grass and water plants were also present.

Table 1. The twelve study lakes, arranged according to top predators

Lake	Top predator	Surroundings	TotN (µg/L)	TotP (µg/L)	TOC (µg/L)	Surface (ha)	Max depth (m)	Secchi depth (m)	Vegetation (%)
Forkerudtjennet	No predator	Urban/Farmland	1985	82	23.4	1.24	2.2	0.2-0.7	30-50
Langmyrtjern	No predator	Forest	702	20	14.2	0.3	5	1	20
Motjennet	No predator	Forest	688	23	11.2	0.94	11.3	3	30-50
Karussputten	Trout	Forest	361	9	5.4	0.25	4.6	1.9	30
Posttjennet	Trout	Forest	312	8	9.7	1.7	11	2.5	15
Småvanna	Trout	Urban/Forest	616	14	10.1	0.5	3	1.8	25
Svartkulp	Perch	Forest	550	13	9.9	6	10	1.2	20
Bjørnmyrdammen	Perch	Urban/Forest	672	26	6.5	2	3.1	0.4	50-80
Øvresetertjern	Perch	Urban/Forest	446	13	6.6	3.5	3.5	1.7	10
Bugårdsdammen	Pike	Urban	980	54	9.5	3.8	2	1.5	40
Nustjennet	Pike	Farmland	1090	164	16.4	13	1	0.4	50-60
Stomperudtjennet	Pike	Farmland	1660	146	18.4	3.7	1	0.4	50-80

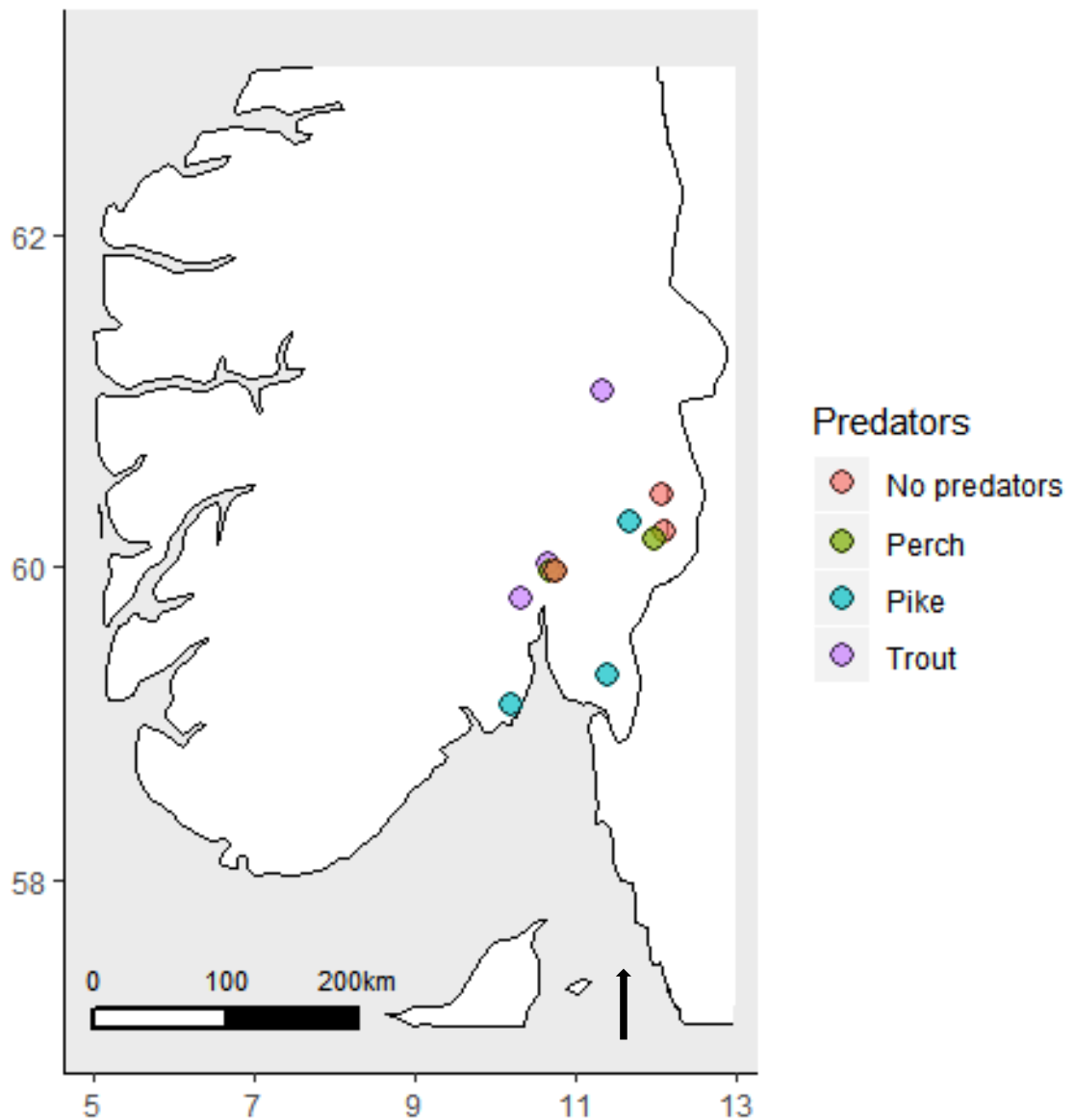


Figure 1. The geographic location of the study lakes in south east Norway (Map gotten from Ilaria de Meo).

Sampling procedures

Fieldwork was conducted from May to August 2018 and 2019. For representative sampling of the fish community, we used Nordic multimesh gillnets, i.e. each net with 12 panels of mesh sizes 5-55 mm according to EU/Norwegian standard (Appelberg et. al., 1995). The number of Nordic multimesh gillnets put in the lakes differed, but each lake was sampled with minimum

on five nets. This permits calculation of catch per unit effort (CPUE) for each species in each lake. In addition, in each lake standard floating nets (mesh size 25mm and 50mm, knot to knot) was used to increase the catch. Drum net fish traps was also used to capture as much fish as possible. Nordic multimesh was set 90 degrees from shore as described in the Norwegian standard protocol (Appelberg, et. al., 1995). Floating gillnets were set in both the littoral and the pelagic zone, covering most parts of the lake and both habitats. Traps were baited with white bread and put in the littoral zone and close to the shoreline in vegetated areas. Sampling were conducted for 2 to 8 nights at different time periods for every lake, depending on how fast we reached the minimum aim of 30 crucian carps for every lake. All fish species were identified in the field and stored separately in a freezer at the university.

A clean bottle was used to collect water samples from the upper layer of the water body approximately in the middle of the lake.

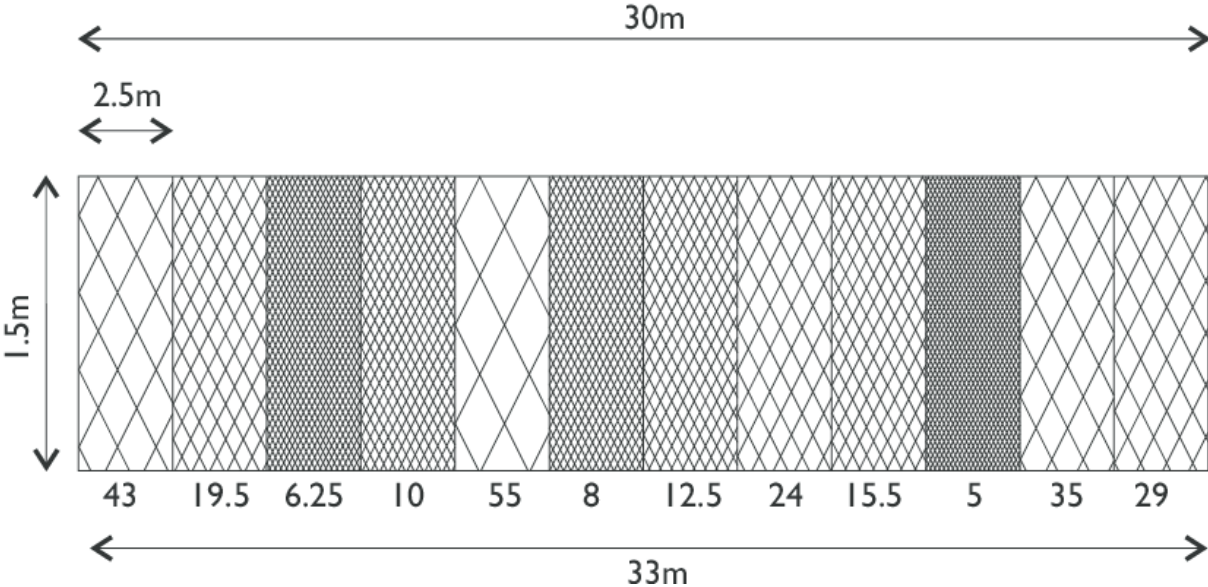


Figure 2: Illustration of nordic multimesh gillnet (Eira, Olofsson, Ville & Sundström, 2008).

Table 2: Visualization of number of nights fished in every lake with nets and traps.

Lake	Fishing effort (#nights)	Lake type	# crucian carp caught	# other species caught	Mean crucian carp CPUE (ind/N/h)	Mean crucian carp CPUE (g/N/h)	Mean predator CPUE (ind/N/h)
Forkerudtjennet	3	No predator	263	0	10.270	212.848	0
Langmyrtjern	3	No predator	198	1	1.383	13.054	0
Motjennet	2	No predator	229	0	6.243	115.971	0
Karussputten	3	Predator	112	1	0.793	43.039	0.127
Posttjennet	7	Predator	47	2	0.129	26.865	36.022
Småvanna	2	Predator	96	2	0.770	44.517	0.228
Svartkulp	4	Predator	125	3	0.296	39.530	1.075
Bjørnmyrdammen	3	Predator	229	2	2.11	103.953	0.409
Øvresetertjern	3	Predator	55	2	0.276	143.957	1.639
Bugårdsdammen	3	Predator	32	2	0.103	83.028	0.781
Nusttjennet	8	Predator	100	4	0.337	279.222	0.116
Stomperudtjennet	9	Predator	127	6	0.290	150.183	0.139

Laboratory work

In the laboratory, each captured crucian carp specimen was measured with respect to: weight (g), total length (mm), and body height (mm), measured with a caliper from the front of the pelvic fin and to the front of the dorsal fin). All captured predator specimens were measured with respect to weight (g) and total length (cm). In addition, mouth height (mm) and mouth width (mm) were measured by placing the caliper into the fish mouth and stretching it until the mouth does not open anymore. Afterwards, all fish were dissected, and gonads were used to determine the fish sex. Water samples were sent to Lammi station in Finland for analyses, where water chemistry with respect to total nitrates, total phosphates and total organic carbon was analysed.

Environmental measurements

Max depth (m) of the lakes was measured using the handheld depth-finder (see sampling procedures). Altitude (m.a.s.l.) and the surface for each lake was obtained from public information (norgeskart.no). Secchi depth (m) was measured using a secchi disk, when the secchi disk is no longer visible in the water, the depth is measured. For each lake, a map with

a line drawn around the areas where there were plants was used to calculate the percentage of emergent macrophytes in each lake. Littoral area (%) was estimated from measurements done in field for each lake, using the handheld depth-finder. The area was estimated from 0% to 100 %, and the limit of littoral area was set to maximum 3 meters depth. Specific conductivity was measured using a handheld conductivimeter (see sampling procedures). Mean air temperature was obtained from yr.no and was calculated from 2010-2019 for June to September, this period was used to have a more accurate mean air temperature. Land use was scored in categories from one to three, forest lakes have value one, urban lakes value two and farmland lakes value three. Number of species is the number of fish species caught in each lake.

Morphological analyzes

In the lab, pictures of all crucian carp specimens for morphometrical analyzes was taken with a Nikon D5300 camera, on a tripod. The camera lens was set to a specific level of zoom, so every picture was captured at the same distance every time the camera was set up. Crucian carp was placed in a box painted black on the inside and layered with a 2 cm thick styrofoam plate covered with black plastic at the bottom. On top of the Styrofoam there was a wooden frame with a mesh. The mesh was not stapled to tight to the frame, in order to ensure that each fish would lay as flat as possible. A ruler was also included in the photographs to facilitate the calibration of size in the landmark program tpsUtil32 (Rohlf, 2019) during analyzes of the images (Vinterstare, Hegeman, Nilsson, Hulthèn & Brönmark, 2018). Each crucian carp was placed in the box laying on its right side, in order to take a lateral photo of the left side of each fish. The dorsal, tail, anal and pelvic fins were spread and fixed in position to the styrofoam with needles. This was important for setting the landmarks around the fins. A set of 23 landmarks were then digitalized, and their x and y coordinates were captured. These 23 landmarks were selected to cover most of the fish body.

The data collected were from 30 individual crucian carps from each of the 12 lakes (Fig.1, Table, 1), i.e. 360 individuals in total. Individuals were selected based on the length distribution in catches trying to cover the whole size distribution in the lake. Landmark-based geometric morphometrics were used analyzing the body shape. Landmark-based geometric morphometrics summarizes the shape in terms of how the landmarks are configurated (Webster & Sheets, 2010). Here, tpsUtil32 (Rohlf, 2019) was used to add 23 landmarks on the left side of the fish (Fig. 3). A transversal line was drawn from the mouth to the middle of the tail fin using tpsUtil32 (Rohlf, 2019). On 90-degree angles of this line, two lines were drawn,

one through the center of the eye and one along the anterior edge of the operculum respectively (Fig. 3). This was done to standardize where to put landmark 2, 4, 13 and 15 (Fig. 3)

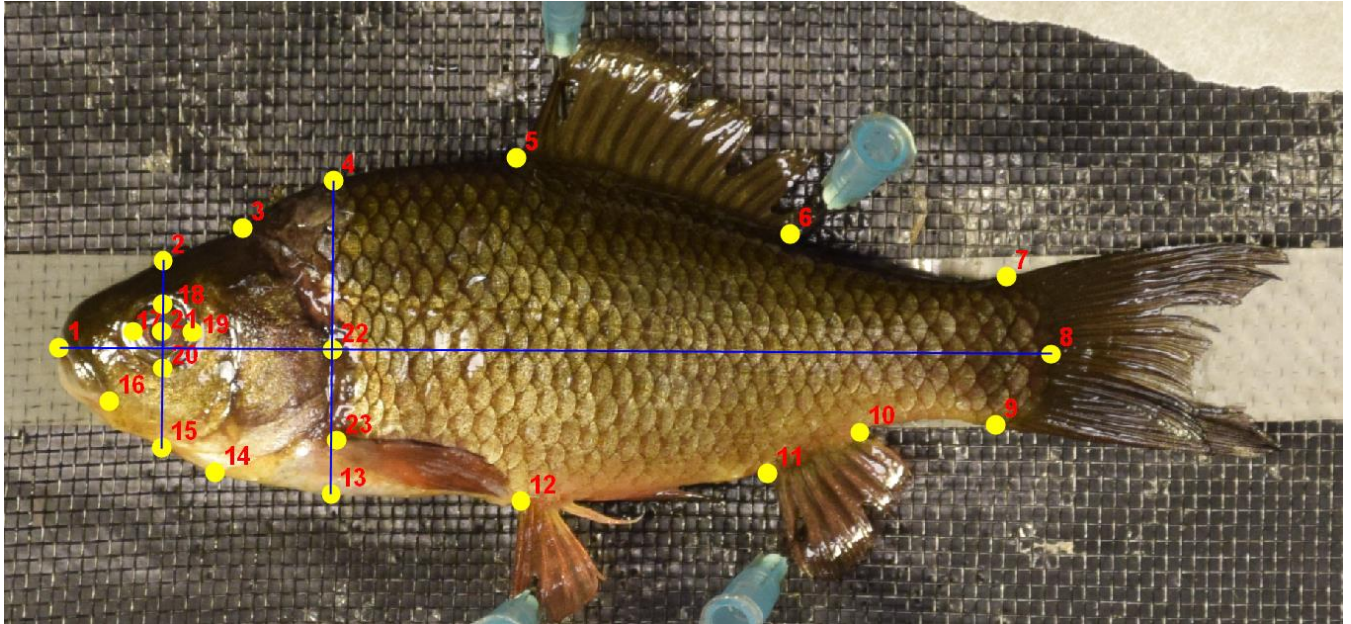


Figure 3: The 23 landmarks that were the basis for morphological measurements. Landmark 1 at the mouth, then going over the back and around the tail fin and stop at the maxillary. Then the eye is landmarked, then from the operculum and down to the pectoral fin.

Statistical analyzes

The software MorphoJ version 1.07a. (Klingenberg, 2011) was used to analyze the morphological variables derived from the digitized landmarks (Fig. 3) and used to characterize the shape of the fish. Landmark configurations were standardized using a generalized Procrustes superimposition, which scales and rotates all the individuals, so they line up as close as possible (Andersson et. al., 2006). This gives a unison body shape for all the individuals. Principal component analyses (PCA) was run with MorphoJ, which are widely used on morphometrical data. These analyses are not only visualizing but also reduce the dimensionality of landmarks to match statistical degrees of freedom after variation in size, position, and orientation are removed by Procrustes superimposition (Du, 2019). Principal component one (PC1), was captured for every fish, explaining the body shape primarily the body height of each crucian carp.

All statistical analyses were conducted in the statistical computing package Rstudio version 1.2.5033 (R Core Team, 2019). Packages used was: ggplot2 (Wickham, 2016), GGally (Schloerke et. al., 2020), lme4 (Bates, Maechler, Bolker & Walker. 2015), lmerTest (Kuznetsova, Brockhoff & Christensen, 2017), sjPlot (Lüdecke, 2020a), ggsignif (Eltze, 2019), sjstats (Lüdecke, 2020b). The use of these statistical packages is described in more detail below for each of the analyses.

In order to describe the relation between body shape and environmental variables, I used a linear mixed effect regression model with the packages lme4 and lmerTest, where lake was used as random variable. Linear mixed effect regression models were performed on relative body height and PC1 which represent body height as response variables. The starting model for both relative body height and PC1 was sex + CPUE for crucian carp + CPUE for predators + predator mouth area + total nitrates + lake surface + lake code as a random effect. By using ggcorr from GGally package a correlation matrix was created, and I checked correlation between all the variables. Variables that had a Pearson's correlation coefficient >0.7 was dropped from the model (Fig, 4). Models were developed using backwards stepwise modeling method using the function step function (Perazzo et.al., 2018). This function uses Akaike's information criterion (AIC) as a predictor for selecting the best model (Table, 3). The function r2 from the sjstats package was used to find the conditional R² values for the different models (Table, 3).

The function ggplot from the package ggplot2 was used to visualize the regressions and boxplots. For the boxplot the package ggsignif was used to visualize the significance between the two variables.

Catch per unit effort (CPUE) is a statistical method to calculate the number of fish caught per unit of effort (Harley, Myers & Dunn, 2001). This was done to provide information of how much fish there is in each lake. The formula by Appelman (2015) was used here:

$CPUE = ind/N/h$. *ind* is the number of fish caught for each species, *N* is number of Nordic nets (minimum five in each lake) and *h* are the hours the nets were in the water. This was calculated for crucian carp and predators.

Relative body height (*RBH*) of the crucian carp was calculated by dividing body height by total length. Total length was not used in the models as this is already body size corrected for in both *RBH* and the PCA analyses.

Predator mouth area was calculated using the highest mouth gape and multiplying it with the mouth width, this was done for each lake.

11 fish were dropped from the statistical analyses as for these fish it was not possible to identify the sex. These fish were distributed throughout the different lakes, and thus do not affect one particular lake more than other lakes with regard to the reduction in numbers finally analysed.

Results

Correlation matrix showing correlation between the variables (Fig, 4). There are several variables that are highly correlated, therefore these were dropped from the model.

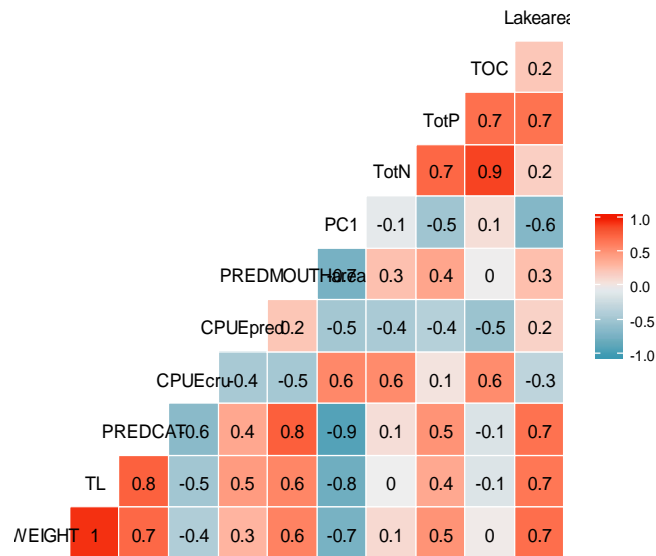


Figure 4: Correlation matrix with weight, total length, predation category, CPUE for crucian carp, CPUE for predators, predator mouth area, PC1, total nitrates, total phosphates, total organic carbon and lake surface.

AIC selection results for both models

In the AIC model selection results show that for relative body height the final model of the AIC score decreased by 67.431 (Table, 3), and for PC1 the final model the AIC score decreased by 36.376 (Table, 3) compared to the first model. Since the step function in R start with the whole model, the output is the best model, and in between models are not shown. This gives that the best model for PC1 was sex + predator mouth area + lake surface + lake code as a random effect. Whereas for relative body height the best model was predator mouth area + lake surface + lake code as a random effect.

Table 3: Show AIC values and the conditional R² values for the two different models before and after backwards selection using the step function

Response variable	AIC first model	AIC final model	Conditional R ² first model	Conditional R ² final model
Relative body height	-1673.142	-1740.573	0.889	0.882
PC1	-2198.995	-2235.371	0.943	0.943

Model for PC1

Crucian carp living with predators had a lower PC1 score than fish living without predators. For the PC1 values the lower the number is, the larger the crucian carp is. Therefore, it looks like e.g. the predator mouth area is negatively correlated with PC1, when they indeed are positively correlated. The results also show that males were significantly (Fig, 7) larger than females, and that the bigger lakes have bigger crucian carp. The model for PC1 body height had a conditional R^2 value of 0.941.

Table 4: Results of a linear mixed effect regression on PC1 body height with the variables sex (male and female) , predator mouth area and lake surface (ha). Confidence intervals 2.5% and 97.5%.

Predictors	Estimates	CI (2.5%-97.5%)	p-values	t-values	df
Intercept	0.03	0.019 – 0.045	0.001	4.675	8.869
Sex (male)	0	-0.004 - -0.000	0.010	-2.582	336.2
Predator mouth area	0	-0.000 - -0.000	0.004	-3.759	8.781
Lake surface	-0.52	-0.765 - -0.243	0.005	-3.594	9.166
Observations	349				
Marginal R2 / Conditional R2		0.715 / 0.941			

The mouth area of the predator significantly increased the PC1 body height for crucian carp, so the bigger the mouth area of the predator the higher body the crucian carp showed

($p=0.004$, $ci= -0.000 - -0.000$, Table 4).

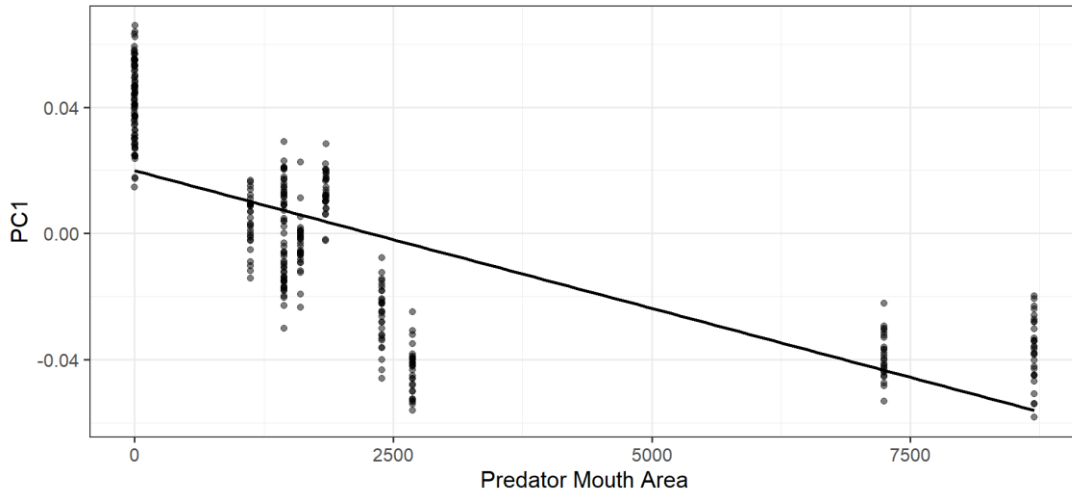


Figure 5: The relationship between PC1 body height and predator mouth area for all 12 lakes. The three predator free lakes are at 0 on the x-axis, and the three pike lakes to the right, the two trout lakes Posttjernet and Karussputten have the same predator mouth area and are therefore together.

Larger lakes have a significant effect on the PC1 body height of crucian carp, when the lake increase in surface area the crucian carp grows a higher body height ($p = 0.005$, $ci = -0.765 - -0.243$, Table 4).

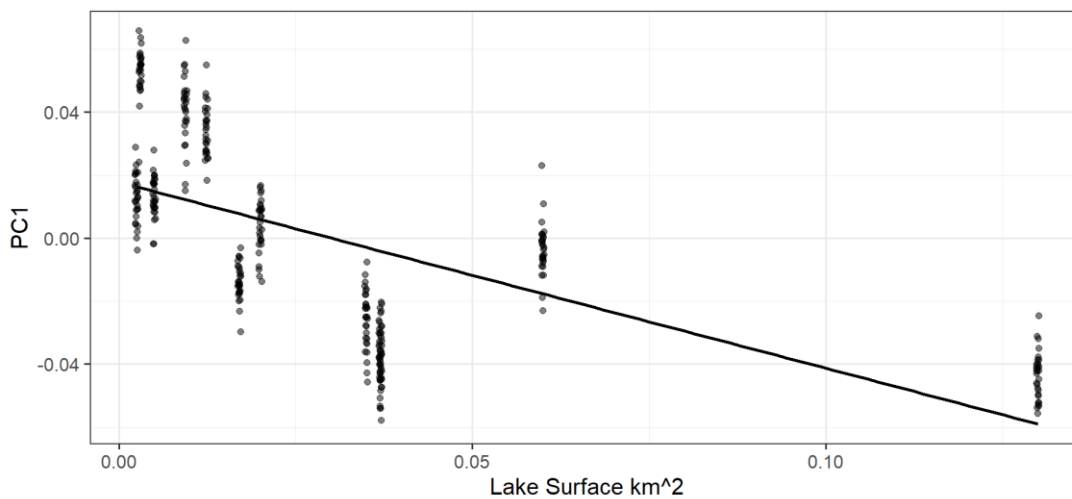


Figure 6: The relationship between PC1 body height and lake surface for all 12 lakes.

Males ($p = 0.010$, $ci = -0.004 - -0.000$) have a higher PC1 body height than females ($p = 0.001$, $ci = 0.019 - 0.045$). (Table, 4. Fig, 7). But as the box plot show, there is not a big difference between the two sexes.

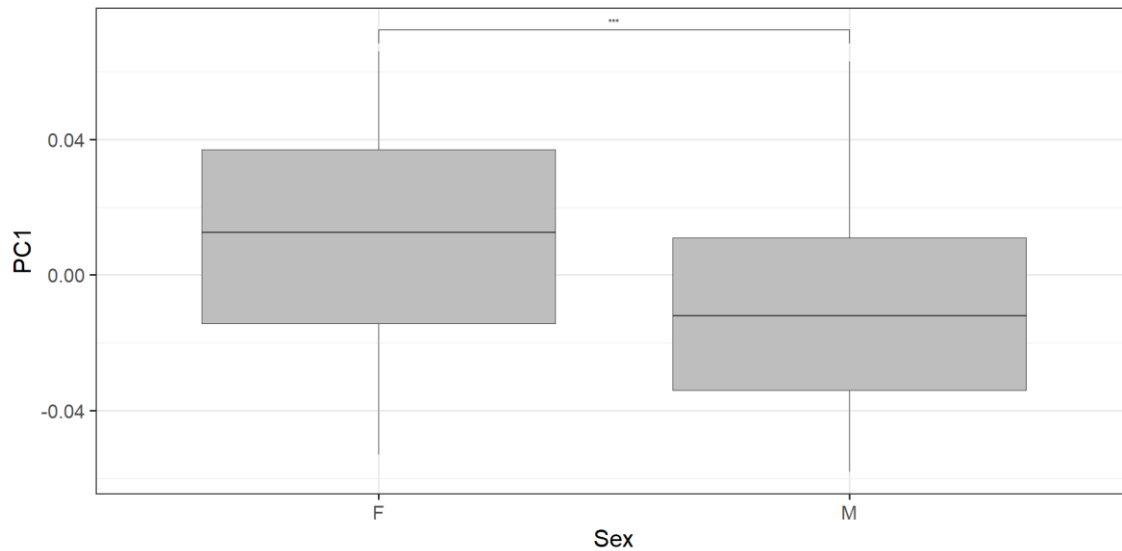


Figure 7: Comparison between sex, male and female with regard to PC1 body height for all the 12 lakes.

Model for Relative body height

The model for relative body height was a simpler model than the one for PC1. But as with the results for PC1, the presence of predators gives a larger relative body height. The surface of the lake was also significant stating that bigger lakes have the highest bodied crucian carp.

For relative body height predator mouth area and lake surface are the two most important factors both with a positive p value of $p < 0.001$ (Table, 5). Predator mouth size had a significance of ($p < 0.001$, $ci = 0.00 - 0.00$), and lake surface had a significance of ($p < 0.001$, $ci = 0.30 - 1.05$). The model for relative body height had a conditional R^2 value of 0.882.

Table 5: Results of a linear mixed effect regression on relative body height with the variables predator mouth area and lake surface (ha). Confidence intervals 2.5% and 97.5%.

Predictors	Estimates	CI (2.5%-97.5%)	p-values	t-values	df
Intercept	0.29	0.270 – 0.310	<0.001	30.409	9.063
Predator mouth area	0	0.000 – 0.000	<0.001	4.621	9.053
Lake surface	0.673	0.300 – 1.050	<0.001	3.496	9.200
Observations	349				
Marginal R^2 / Conditional R^2	0.701 / 0.882				

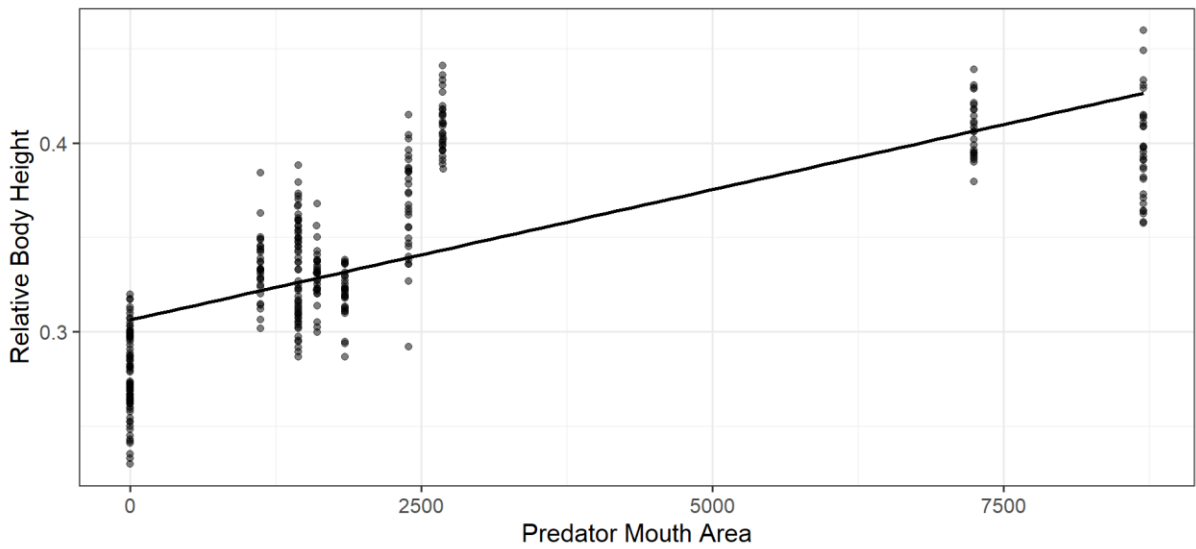


Figure 8: Relationship between predator mouth area and relative body height in the 12 lakes. The three predator free lakes are at 0 on the x-axis, and the three pike lakes to the right, the two trout lakes Posttjernet and Karussputten have the same predator mouth.

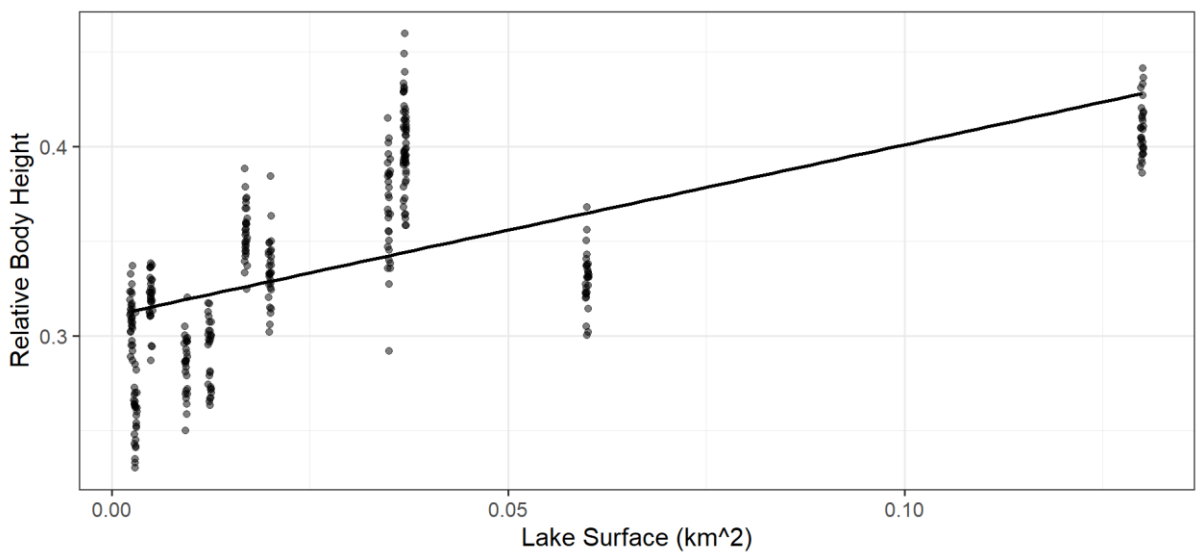


Figure 9: Relationship between relative body height and lake surface, for all the 12 lakes.

Discussion

In this study I found that the two most important factors considering the growth of crucian carps body height is the predators mouth area and the size of the lake. The bigger the mouth area of the predator the crucian carp grows a higher body height, compared to lakes where piscivore predators have either smaller mouth sizes or lakes without predators. In lakes with a larger surface area a higher body height is shown. Sex are also a factor that contribute to a higher a body height for males.

My first hypothesis is valid as the results show that crucian carp living with predators have a higher body shape than crucian carp living without predators. This is supported by studies done by several authors (e.g. Poléo et. al., 1995; Johansson & Andersson, 2009). It is believed that prey detect cues from predators, and that e.g. smell is one of the cues that drives this change in body height (Nilsson, et.al., 1995). Higher bodied crucian carp also have the advantage that even though the absolute size refugium have not been reached, a higher body increase the probability of escape as they have more strike power in their caudal fin (Nilsson, et.al., 1995). However relative body height and PC1 is two different ways of calculating the body height of crucian carp. I mean this gives additional support that predators induce this change in crucian carp, since both methods showed that crucian carp got a deeper body when living with predators.

In this study I demonstrated that there are not only biotic factors driving the increase in body height in crucian carp but also an abiotic factor. The lake surface is an important factor regarding the growth of crucian carp. When the surface of the lake increases the body height of crucian carp follows and grows higher bodies than in the smaller lakes. Why this is the case I can only speculate in, but one explanation is that when the lake surface increase the more complex the lake system gets, as there is more space for e.g. other fish species, benthic macro invertebrates etc. Larger lakes also consist of more space for shelter against predators, which can lead to energy saving and increased growth (Brönmark et.al., 1995). In nature high growth rates are shown when food availability is high (Holopainen, Vornanen & Huuskonen, 1996), and a larger lake may hold more food than a smaller lake. There is strong support that lakes with a larger surface (e.g. it is a much more complex system, than smaller lakes) induce higher bodies in crucian carp since both the results for PC1 and relative body height is positively correlated to lake surface.

Pike is the predator counting for the greatest increase in body height and is the predator which is studied the most regarding crucian carp morphology (Brönmark & Miner, 1992; Brönmark

& Pettersson, 1994; Magnhagen & Heibo, 2001). Pike being considered the top predator in boreal water systems bordering to the subalpine region (Byström et.al., 2007), it impacts the lake community more than trout and perch. In a study by Ölund, et.al. (2019) they showed that pike was the key driver of ecological speciation in European whitefish (*Coregonus lavaretus*), inducing both small pelagic “dwarfs” and big benthic “giants” fish in the same lake. While pike induce the highest body height for crucian carp, both trout and perch induce a change in the body height of crucian carp. In this study the mouth area of both trout and perch is quite similar, so it is hard to say which of them is inducing more change than the other. However, pike is considered being an aggressive species from very small sizes (Morrow & Miller, 1998), which maybe accelerates the growth of crucian carp as they need to outgrow the predation window fast. Moreover Nilsson, et.al. (1995) experimentally tested how crucian carp morphology effected pike behavior, showing that there was no effect on behavior (e.g. activity, search, follow, observing and capture success), when crucian carp had a deeper body. For crucian carp living in the absence of pike, but later introduced to pike, showed a decrease in swimming speed compared to crucian carp that had coexisted with pike (Pettersson et. al., 2000).

Why sex is a part of the PC1 model but not for the relative body height model could be that the landmarks also capture more of the general body shape and not only the height and length as for the relative body height. In one study female crucian carp lost their behavior response towards predator during the final stages of maturation, i.e. to prevent interruption of spawning (Lastein, Höglund, Mayer, Øverli & Døving, 2008). Since crucian carps can spawn up to three times from May to August (e.g. Holopainen & Pitkänen, 1985; Laurila, Piironen & Holopainen, 1987), this can make females more prone to predation, and that all energy is used on spawning and not growing. Taking this into consideration females can be more concerned about spawning than avoid predation in the summer months. This is also in the period when we caught crucian carp, which maybe can explain why females have shallower body than males in the summer months.

Since this study is done on fish caught in different habitats and not experimentally tested in a laboratory. It is hard to say what the exact driving factors are. Others e.g. Brönmark & Pettersson (1994), Andersson et. al., (2006), tested this experimentally. They experimentally tested if predation would be a driving factor changing the morphology of crucian carp.

I did not statistically analyze what the crucian carps have been feeding on. It has been demonstrated in studies that crucian carp feeding on benthic prey have a deeper body than crucian carp feeding on plankton prey (Tonn et.al, 1994). This can maybe be due to that lakes with larger crucian carps, often live in more complex systems, with more competition. Pike for most part tend to be living and hunting in the littoral zones of a lake (Vøllestad, Skurdal & Qvenlid, 1986), which may can give the bigger benthic feeding crucian carp an advantage as it may not tend to come across pike as often as the shallower bodied plankton eating crucian carp. Moreover, the study done by Brönmark & Miner (1992) gut content showed that there was no difference in diet for crucian carp in aquariums with pike and pike less sections.

Methods used for data collection are well known methods and have been used for a very long time. One issue I can think of when analysing the data, is that I did not collect the whole range of size classes. The use of multi mesh nets are size selective in the sense they have many different mesh sizes, but some fish sizes will still not be captured.

My results can be used in management, as a study by Gu et. al. (2016) showed that small crucian carp can negatively affect the growth of macrophytes. Managers can then in restoration ecology, where a fishless pond is overgrown with macrophytes, introduce small “dwarf” crucian carps to reduce the macrophytes.

I suggest further studies should be concentrated on the lake and its community as a whole. Because body height and body shape have been well studied, but to my knowledge there is not done much research on how the lake size and its community can induce changes in body height of crucian carp. Since there also are contradictory studies on diet, I suggest to study this so there will be stronger evidence if the diet between the shallow bodied and deep bodied crucian carp are different or not. Generally, very little is known about the crucian carp, especially the two morphs, is it genetic? Is it immigration? There are still many questions of crucian carp yet to explore, especially on the genetics.

Conclusion

In conclusion, this study supports previous studies done explicitly on crucian carp and predator interaction. But also raises questions on how the lake size, where bigger lakes may be more complex systems than smaller lakes can contribute to crucian carp's ability to grow higher bodies.

Acknowledgements

Thanks to my supervisors Kjartan Østbye & Toni Polèo, for giving me the opportunity to do this work. And a special thanks to Ilaria de Meo, for all the good times in field and in the lab, and a lot of help and discussions. I also would like to thank Jan Heggenes & Kimmo K. Kahilainen for reading and giving very good feedback. And thanks to Morten Dæhlen for last comments. Thanks to Olivier Devineau for statistical discussions. And thanks to fieldworkers who have helped us catch enough crucians.

References

- Andersson, J., Johansson, F. & Söderlund, T. (2006). Interactions between predator- and diet-induced phenotypic changes in body shape of crucian carp. *Proceedings of the Royal Society* 273, 431–437doi:10.1098/rspb.2005.3343
- Appelberg, M., Berger, H. M., Hesthagen, T., Kleiven, E., Kurkilahti, M., Raitaniemi, J. & Rask, M. (1995). Development and intercalibration of methods in nordic freshwater fish monitoring. *Water, Air and Soil pollution*. 85: 401-406. DOI: 10.1007/BF00476862
- Appelman, M. (2015). *A Catch Per Unit Effort (CPUE) Spatial Metric with Respect to the Western North Atlantic Pelagic Longline Fishery*. (Master thesis, Nova Southeastern University Florida). Retrieved from: https://nsuworks.nova.edu/occ_stueta/36/
- Agrawal, A. A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science*. Vol. 294. No. 5541. pp. 321-326.
- Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015). Fitting linear mixed-effect models using lme4. *Journal of statistical software*. 67(1), 1-48. doi:10.18637/jss.v067.i01.
- Bordeau, P. E. & Johansson, F. (2012). Predator-induced morphological defenses as by-products of prey behavior: a review and prospectus. *Oikos*. 000: 001–016, 2012. doi: 10.1111/j.1600-0706.2012. 20235.x
- Brönmark, C. & Miner, J. G. (1992). Predator-Induced Phenotypical Change in Body Morphology in Crucian Carp. *Science*. Vol. 258. 1348-1350.
- Brönmark, C. & Petterson, L. B. (1994). Chemical cues from piscivores induce a change in morphology in crucian carp. *Oikos*. Vol. 70, No. 3, pp. 364-402
- Brönmark, C., Paszkowski, C. A., Tonn, W. M. & Hargeby, A. (1995). Predation as a determinant of size structure in populations of crucian carp (*Carassius carassius*) and tench (*Tinca tinca*). *Ecology of freshwater fish*. 4:85-92. DOI: 10.1111/j.1600-0633.1995.tb00121.x

- Byström, P., Karlsson, J., Nilsson, P., Kooten, T. V., Ask, J. & Olofsson, F. (2007). Substitution of top predators: effect of pike invasion in a subarctic lake. *Freshwater biology*. 52, 1271-1280. doi:10.1111/j.1365-2427.2007.01763.x
- Du, T. Y. (2019). Dimensionality reduction techniques for visualizing morphometric data: comparing principal component analysis to nonlinear methods. *Evolutionary biology*. 46:106-121. <https://doi.org/10.1007/s11692-018-9464-9>
- Einfalt, L. M. & Wahl, D. H. (1997). Prey selection by juvenile walleye as influenced by prey morphology and behavior. *Canadian journal of fisheries and aquatic sciences*. 54: 2618–2626 (1997)
- Eira, L., Olofsson, P., Ville, H. & Sundström, B. (2008). *TRIWA II Management of an International River Basin District – Torne River*. Helsinki: Helsinki University Print. ISBN 978-952-11-3042-7
- Eltze, C. A. (2019). *ggsignif: Significance Brackets for 'ggplot2'*. <https://CRAN.R-project.org/package=ggsignif>
- Fagernes, C. E., Stensløkken, K. O., Røhr, Å. K., Berenbrink, M., Ellefsen, S. & Nilsson, G. E. (2017). Extreme anoxia tolerance in crucian carp and goldfish through neofunctionalization of duplicated genes creating a new ethanol-producing pyruvate decarboxylase pathway. *Sci Rep* 7, 7884 (2017). <https://doi.org/10.1038/s41598-017-07385-4>
- Fulton, C. J., Binning, S. A., Wainwright, P. C. & Bellwood, D. R. (2013). Wave-induced abiotic stress shapes phenotypic diversity in a coral reef fish across a geographical cline. *Coral Reefs* 32:685-689. doi: 10.1007/s00338-013-1039-8
- Gaston, K. A. & Lauer, T. E. (2015). Morphometric variation in bluegill *Lepomis macrochirus* and green sunfish *Lepomis cyanellus* in lentic and lotic systems. *Journal of Fish Biology*. (2015) 86, 317–332 doi:10.1111/jfb.12581
- Gilbert, J. T. (1966). Rotifer Ecology and Embryological Induction. *Science*. Vol. 151, No. 3715, pp. 1234-1237.
- Gu, J., Jin, H., He, H., Ning, X., Yu, J., Tan, B., Jeppesen, E. & Li, K.
- Harley, S. J., Myers, R. A. & Dubb, A. (2001). Is catch-per-unit-effort proportional to abundance? *Can. J. Fish. Aquat. Sci.* 58: 1760–1772 (2001). DOI: 10.1139/cjfas-58-9-1760

- Harvell, C. D. (1984) Predator-Induced Defence in Marine Bryozoan. *Science*. Vol. 224, No. 4566, pp.1357-1359.
- Harvell, C. D. (1990). The ecology and evolution of inducible defences. *The quarterly review of biology*. Vol. 65, No.3, pp. 323-340
- Holopainen, I. J. & Pitkänen, A. K. (1985). Population size and structure of crucian carp (*Carassius carassius* (L.)) in two small, natural ponds in Eastern Finland. *Ann. Zool. Fennici* 22:397-406
- Holopainen, I. J., Vornanen, J. A. & Huuskonen, H. (1996). Phenotypic plasticity and predator effects on morphology and physiology of crucian carp in nature and in the laboratory. *Journal of fish biology*. 50, 781-798.
- Holopainen, I. J., Tonn, W. M. & Paszkowski, C. A. (1997). Tales of two fish: the dichotomous biology of crucian carp (*Carassius carassius*(L.)) in northern Europe
- Hulthén, K., Chapman, B. B., Nilsson, P. A., Hollander, J. & Brönmark, C. (2014). Express yourself: bold individuals induce enhanced morphological defences. *Proceedings of the Royal Society B*. Vol. 281, No. 1776, pp. 1-8. <http://dx.doi.org/10.1098/rspb.2013.2703>
- Johansson, F. & Andersson, J. (2009). Scared fish get lazy, and lazy fish get fat. *Journal of animal ecology*. 78, 772-777. doi: 10.1111/j.1365-2656.2009.01530.x
- Klingenberg, C. P. (2011). MorphoJ: An integrated software package for geometric morphometrics. *Molecular Ecology Resources*, 11(2), 353-357. <https://doi.org/10.1111/j.1755-0998.2010.02924.x>
- Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, 82(13), pp. 1-26. doi:10.18637/jss.v082.i13
- Laurila, S., Piironen, J. & Holopainen, I. J. (1987). Notes on egg development and larval and juvenile growth of crucian carp (*Carassius carassius* (L.)). *Ann. Zool. Fennici* 24:315-321.
- Leigh, V. V. (1973). A new evolutionary law. *Department of biology the university of Chicago*. Got from: <https://www.mn.uio.no/cees/english/services/van-valen/evolutionary-theory/volume-1/vol-1-no-1-pages-1-30-1-van-valen-a-new-evolutionary-law.pdf>

Lüdecke, D. (2020a). *sjPlot: Data Visualization for Statistics in Social Science*. doi: 10.5281/zenodo.1308157

Lüdecke, D. (2020b). *sjstats: Statistical Functions for Regression Models*. doi:10.5281/zenodo.1284472

Magnhagen, C. & Heibo, E. (2001). Gape size allometry in pike reflects variation between lakes in prey availability and relative body height. *Functional ecology*. 15, 754-762.

Morrow, J. V. & Miller, G. L. (1998). Size of larval northern pike emigrating from spawning wetlands. *Journal of freshwater ecology*. Vol. 13, Num. 3, pp:343-349. Doi: <https://doi.org/10.1080/02705060.1998.9663627>

Nilsson, P. A., Brönmark, C. & Pettersson, L. B. (1995). Benefits of a predator-induced morphology in crucian carp. *Oecologia (1995)*. Vol. 104, No. 3 (1995), pp. 291-296

Nordbotten, J., & Stenseth, N. (2016). Asymmetric ecological conditions favor Red-Queen type of continued evolution over stasis. *Proceedings of the National Academy of Sciences of the United States of America*, 113(7), 1847-1852.

Öhlund, G., Bodin, M., Nilsson, K. A., Öhlund, S. O., Mobley, K. B., Hudson, A. G., Peedu, M., Brännström, Å., Bartels, P., Præbel, K., Hein, C. L., Johansson, P. & Englund, G. (2019). Ecological speciation in European whitefish is driven by a large-gaped predator. *Evolution letters*. Doi: <https://doi.org/10.1101/543744>

Perazzo, G. X., Corrèa, F., Calviño, P., Alonso, F., Salzburger, W. & Gava, A. (2018). Shape and size variation of *Jenynsia lineata* (Jenyns 1842) (Cyprinodontiformes: Anablepidae) from different coastal environments. *Hydrobiologia*. <https://doi.org/10.1007/s10750-018-3794-6>(012

Polèo, A. B. S., Øxnevad, S. A., Østbye, K., Heibo, E., Andersen, R. A., Vøllestad, L. A. (1995). Body morphology of crucian carp *Carassius carassius* in lakes with or without piscivorous fish. *ECOGRAPHY* 18: 225-229.

- Pettersson, L. B., Nilsson, P. A. & Brönmark, C. (2000). Predator recognition and defence strategies in crucian carp, *Carassius carassius*. *Oikos*. 88: 200-212.
- RStudio Team (2019). RStudio: Integrated Development for R. *RStudio, Inc., Boston, MA* URL <http://www.rstudio.com/>.
- Schloerke, B., Crowley, J., Cook, D., Briatte, F., Marbach, M., Thoen, E., Elberg, A. & Lar marange, J. (2020). *GGally: Extension to 'ggplot2'. R package version 1.5.0.* <https://CRAN.R-project.org/package=GGally>
- Rohlf, F. J. (2019). TpsUtil32 version 1.79. Free at <https://life.bio.sunysb.edu/morph/software/tpsutil.html>
- Sidlauskas, B. L., Mol, J. H. & Vari, R. P. (2010). Dealing with allometry in linear and geometric morphometrics: a taxonomic case study in the *Leporinus cylindriformis* group (Characiformes: Anostomidae) with description of a new species from Suriname. *Zoological Journal of the Linnean Society*, 62, 103–130. doi: 10.1111/j.1096-3642.2010.00677.x
- Tonn, W. M., Holopainen, I. J. & Paszkowski, C. A. (1994). Density-dependent effects and the regulation of crucian carp populations in single-species ponds. *Ecology*. Vol. 75, No. 3, pp. 824-834
- Trussell, G. C. & Smith, L. D. (2000). Induced defences in response to an invading crab predator: An explanation of historical and geographical phenotypic change. *PNAS*. Vol. 97, No. 5, pp. 2123-2127.
- Vinterstare, J., Hegemann, A., Nilsson, P. A., Hultèn, K. & Brönmark, C. (2018). Defence versus defence: Are crucian carp trading off immune function against predator-induced morphology? *Journal of Animal Ecology*. 2019, 88: 1510–1521. DOI: 10.1111/1365-2656.13047.
- Vøllestad, L. A., Skurdal, J. & Qvenlid, T. (1986). Habitat use, growth, and feeding of pike (*Esox lucius* L.) in four Norwegian lakes. *Archiv fur Hydrobiologie*. Vol. 108, pp. 107-117.

- Vøllestad, L. A., Varreng, K. & Polèo, A. B. S. (2004). Body depth variation in crucian carp *Carassius carassius*: an experimental individual-based study. *Ecology of Freshwater Fish*. 2004: 13: 197–202
- Webb, P. W. (1984). Form and Function in Fish Swimming. *Scientific American*. Vol.251, No.1. pp. 72-83.
- Weber, M. J., Dettmers, J. M., Wahl, D. H. & Czesny, S. J. (2010). Effects of predator-prey interaction and benthic habitat complexity on selectivity of a foraging generalist. *Transactions of the American Fisheries society*. 139:1004-1013. Doi: 10.1577/T09-100.1
- Weber, M. J., Rounds, K. D. & Brown, M. L. (2011). Phenotypic variation and associated predation risk of juvenile common carp *Cyprinus carpio*. *Journal of Fish Biology*. (2012) 80, 49–60
doi:10.1111/j.1095-8649.2011.03140.x
- Webster, M. & Sheets, H. D. (2010). A practical introduction to landmark-based geometric morphometrics. *The paleontological society papers*. Vol 16. Pp-163:188.
- Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. *Springer-Verlag New York*.