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Trophic control of the *Daphnia longispina/galeata* complex in lakes containing the invasive European whitefish (*Coregonus lavaretus*)

Master's thesis in Natural Resources Management
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Norwegian University of
Science and Technology

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Acknowledgement

Abstract

Freshwater ecosystems are changing rapidly in a devastating direction due to unsustainable human activity, and of special concern is the ongoing biodiversity loss. Freshwater species are going extinct at an alarming rate, with one of the main drivers being the introduction of invasive alien species. If an alien species successfully invade a lake, it may have detrimental effects on all organizational levels of the ecosystem, ultimately deteriorating the natural resources that are essential for human civilization. Modification of native trophic networks is one of the possible impacts induced by an alien species, and trophic control has a major structuring effect on freshwater ecosystems. For example, the introduction of a top predator might cause major changes in a lake's food web by increasing the strength of the top-down control. In this study, I examined whether the introduction of a highly efficient zooplanktivorous fish would cause shifts in the trophic control of the zooplankton community in East-Central Norway by comparing the morphology of defence structures and fecundity in *Daphnia longispina/galeata* in lakes with introduced, invasive European whitefish (*Coregonus lavaretus*) to lakes with only native brown trout. However, the prediction of a shift towards top-down control in lakes with introduced whitefish was not supported by the results. First, *Daphnia* of mean size in lakes with introduced whitefish were found to have equally long or slightly shorter defence structures (helmet and tail spines) than *Daphnia* in lakes with only native brown trout. This could be explained by helmet and tail spines not being an efficient defence mechanism against whitefish predation combined with a higher level of predation by invertebrates in the trout lakes. Second, fecundity was higher in *Daphnia* of mean size early in the season in lakes with introduced whitefish, but there was also pronounced seasonal variation in fecundity, with a substantial reduction during summer and autumn. Higher fecundity early in the season is often associated with high fish predation, however the seasonal variation in fecundity suggested an effect of resource limitation and hence bottom-up control of *Daphnia* even in the presence of introduced whitefish.

Sammendrag

Miljøtilstanden i ferskvannsøkosystemer endrer seg raskt i en negativ retning. Hovedårsaken er menneskelig aktivitet, og spesielt bekymringsverdig er den pågående nedgangen i biodiversitet. Ferskvannsarter dør ut i økende tempo, og en av hovedårsakene er introduksjonen av invasive fremmede arter. En fremmed art kan innføre store ødeleggende effekter på alle organisasjonsnivåer dersom den lykkes i å invadere en innsjø. Dette vil igjen svekke de naturressursene som er essensielle for vår menneskelige sivilisasjon. En av de mulig innvirkningene en fremmed art kan påføre et ferskvannsøkosystem er modifikasjoner av det naturlige trofiske nettverket i innsjøen, og trofisk kontroll har en sterkt strukturerende effekt i ferskvannsøkosystemer. For eksempel, introduksjonen av en topp-predator kan forårsake store endringer i næringsnettet i en innsjø ved å øke styrken på «ovenfra-og-ned»-kontrollen. I denne studien undersøkte jeg om introduksjonen av en effektiv zooplanktivor fisk ville forårsake endringer i den trofiske kontrollen av zooplanktonsamfunnet i østsentrale Norge ved å sammenligne morfologien av forsvarsstrukturer og fekunditet i *Daphnia longispina/galeata* i innsjøer med introdusert, invasiv sik (*Coregonus lavaretus*) og innsjøer med kun ørret. Prediksjonen om et skifte mot «ovenfra-og-ned»-kontroll i innsjøer med introdusert sik ble ikke støttet av resultatene. For det første, *Daphnia* av gjennomsnittsstørrelse i innsjøer med introdusert sik hadde like lange eller litt kortere forsvarsstrukturer (hjelm og halepigg) enn *Daphnia* i innsjøer med kun ørret. Dette kan forklares av at hjelm og halepigg ikke er en effektiv forsvarsmekanisme mot predasjon fra sik, samt at det trolig var sterkere predasjon fra evertebrater i innsjøer med kun ørret. For det andre, fekunditeten var høyere i *Daphnia* av gjennomsnittsstørrelse tidlig i sesongen i innsjøer med introdusert sik, men det var også en distinkt sesongbasert variasjon i fekunditet med en betraktelig reduksjon under sommer og høst. Høyere fekunditet tidlig i sesongen er ofte assosiert med høy predasjon fra fisk, men den sesongbaserte variasjonen tyder på en effekt av ressursbegrensning og dermed «nedenfra-og-opp»-kontroll av *Daphnia* selv i nærværet av introdusert, invasiv sik.

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Introduction

Freshwater is an essential natural resource for human civilization. It provides food, drinking water, flood control, transportation routes, electricity, and industry, and the need for freshwater will only grow stronger with the ever increasing human population. Despite its pertinence, freshwater is a resource we have misused for centuries (Brönmark & Hansson, 2018). The world's freshwater ecosystems and the many valuable resources they provide are changing rapidly in a devastating direction, all caused by unsustainable human activity (United Nations, 2021). Of special concern is the ongoing biodiversity loss in lakes and ponds (Brönmark & Hansson, 2018). Freshwater species are now going extinct at an alarming rate; a rate suggested to be higher than in both marine and terrestrial ecosystems (Jenkins, 2003). In Norway, as of 2021, 325 species of plants and animals with freshwater as their main habitat is considered to be threatened with extinction (Artsdatabanken, 2021). Exact global numbers are not known as the monitoring is limited or non-existent, however they may be expected to be large (Brönmark & Hansson, 2018). Gaining knowledge to improve sustainable management of freshwater ecosystems is therefore of utmost importance for a sustainable future (United Nations, 2021).

The introduction of invasive alien species (IAS) is, together with land use change, one of the major drivers for biodiversity loss in lakes (Fischer et al., 2018). Ponds and lakes can be seen as freshwater islands, and are therefore particularly vulnerable to invasive species (Simberloff, 2001). Two factors further increase their vulnerability: 1) dispersion between freshwater systems is mainly facilitated by human activity, and 2) dispersal within a freshwater system is rapid and often reliant of passive transportation (Brönmark & Hansson, 2018). If an alien species successfully invade a lake, it may have detrimental effects on all native organizational levels of the ecosystem. Changes include, among other things, habitat shifts, shifts in size and age structure, and changes in nutrient dynamics (Parker et al., 1999). All depends on which species is being introduced and the trophic level of it, as well as which native species that are already present in the host system (Strayer, 2010). Alien species may also cause huge economic losses by affecting ecosystem services such as drinking water, food sources and recreation. Identifying potential invasive species and their effects on lake ecosystems is therefore critical for both ecological and economic needs in the society (Brönmark & Hansson, 2018).

Modification of native trophic networks is one of the possible impacts induced by an introduced alien species (Pysek et al., 2020), and trophic control has a major structuring effect on freshwater ecosystems (Brett & Goldman, 1996). *Daphnia* is a key genus acting as a main food source for many lake fish larvae and juveniles (Karus et al., 2014), and given its central placement in the food web, daphnids (as well as other members of the zooplankton community) may be controlled top-down, by predation, or bottom-up, by nutrient availability (McQueen et al., 1986; Polishchuk et al., 2012). Introducing an efficient zooplanktivorous fish species might therefore cause major changes in a lake's food web by altering the strength of the top-down control of the zooplankton community. Top-down and bottom-up control are not mutually exclusive processes (Tessier, 1986; Threlkeld, 1985) – it is rather a question about when, where and the strength of the control (Brönmark & Hansson, 2018). This is all part of a seasonal succession pattern controlled by the abiotic frame and biotic interactions.

According to the PEG (Plankton Ecology Group) model by Sommer et al. (1986), zooplankton in lakes without an efficient zooplanktivore often experience regular seasonal succession patterns in bottom-up control by phytoplankton (Brönmark & Hansson, 2018). Increasing temperature and light availability in early spring allow for rapid growth of phytoplankton. Consequently, the abundance of herbivorous zooplankton will also increase rapidly, up until the point when grazing rates exceeds phytoplankton growth rates in the spring clear water phase. Zooplankton abundance then decreases as a result of high competition for resources and reduced fecundity. Becoming released from grazing, the phytoplankton may experience a second bloom, which again may be followed by a second peak in zooplankton abundance. When autumn arrives, temperatures drop and light conditions worsen, leading to decreases in both phyto- and zooplankton abundances (Sommer et al., 1986).

In lakes containing an efficient zooplanktivorous fish, the zooplankton will additionally experience top-down control by the predator. Increasing temperatures and the arrival of the young-of-the-year fish in the spring might exert a high predation pressure on the zooplankton, keeping zooplankton biomass relatively low (Carpenter et al., 1985). Also, predation by zooplanktivorous fish is size-specific, as they usually select large, ovigerous females carrying larger clutches (Zaret, 1980). As a result, the grazing pressure on phytoplankton is reduced, and phytoplankton biomass may remain sufficiently high to reduce the potential for resource competition in the zooplankton. This, in turn, leads to a higher and temporally less variable zooplankton fecundity (Leibold, 1991). When autumn arrives, the

predation pressure declines with decreasing temperatures, allowing for an increase in zooplankton abundance if the temperatures are not too low (Gliwicz & Pijanowska, 1989).

Several factors influence the potential for an invasive predator to cause a shift from bottom-up to top down control of zooplankton. For example, the efficiency of the invasive predator will depend on the availability of deep and dark waters serving as refugia during diel vertical migration of the zooplankton (Lampert, 1989). Furthermore, ecological interactions between the zooplankton prey, the invasive predator, and other taxonomic groups (such as vertebrate predators) already present in the system may require an understanding of the complete food web and the strength of pairwise interactions to predicting the response of the invasive species (David et al., 2017). Thus, determining whether the invasive predator causes a shift in trophic control a priori will in most cases be challenging. For trophic control of zooplankton, two approaches may aid in inferring such shifts. First, planktonic prey organisms are known to develop plastic morphological defence structures in response to the presence of a predator (Lass & Spaak, 2003). For example, in several *Daphnia* species, such defence mechanisms in response to fish predation include elongated helmet and tail spines that make them harder to ingest (Barnhisel, 1991; Spaak & Boersma, 1997). Second, zooplankton fecundity is limited by food availability and quality (Kilham et al., 1997; Wu & Culver, 1994), thus functioning as an indicator of the resource competition and the strength of bottom-up control. Analysing morphology of defence structures and fecundity in zooplankton may be used as an indicator for the relative strengths of the top-down or bottom-up control exerted upon them by an introduced predator (Polishckuk et al., 2012).

Here I will explore the consequences of introducing a highly efficient zooplanktivorous fish with respect to changes in trophic control of zooplankton in East-Central Norway. Specifically, I studied the seasonal changes in morphological defence structures (helmet and tail spine lengths) and fecundity (clutch size and egg size) in the *Daphnia longispina/galeata* complex, and compared lakes with introduced invasive European whitefish (*Coregonus lavaretus*) to lakes that only had native brown trout (*Salmo trutta*). Given that whitefish is a highly efficient predator of *Daphnia* (Berg et al., 1994; Sotton et al., 2014), I hypothesise that *Daphnia* in lakes with this invasive species experience a stronger degree of top-down control than they do in lakes with only native brown trout, and hence that daphnids display longer helmet and tail spines (relative to body length) (Barnhisel, 1991; Spaak & Boersma, 1997) (Fig. 1A). I also predict seasonal variation in defence structure lengths, with longer helmets and tails in early spring, as the predation pressure presumably

would be stronger during the emergence of the young-of-the-year whitefish (Laforsch & Tollrian, 2004). Additionally, given that food availability is a limiting factor for fecundity, I predict a higher and less variable *Daphnia* fecundity in lakes with whitefish as top-down control will maintain a low level of competition for resources within the *Daphnia* population (Leibold, 1991; Sommer et al., 1986) (Fig. 1B). Given that optimal egg size is affected by size-selective predation (Macháček, 1991; De Meester et al., 1999) and resource limitation (Brambilla, 1982; Green, 1956), I expect *Daphnia* in whitefish lakes to have smaller egg sizes throughout the season, whereas *Daphnia* in trout lakes are expected to have larger and more variable egg sizes following the seasonal patterns in available resources.
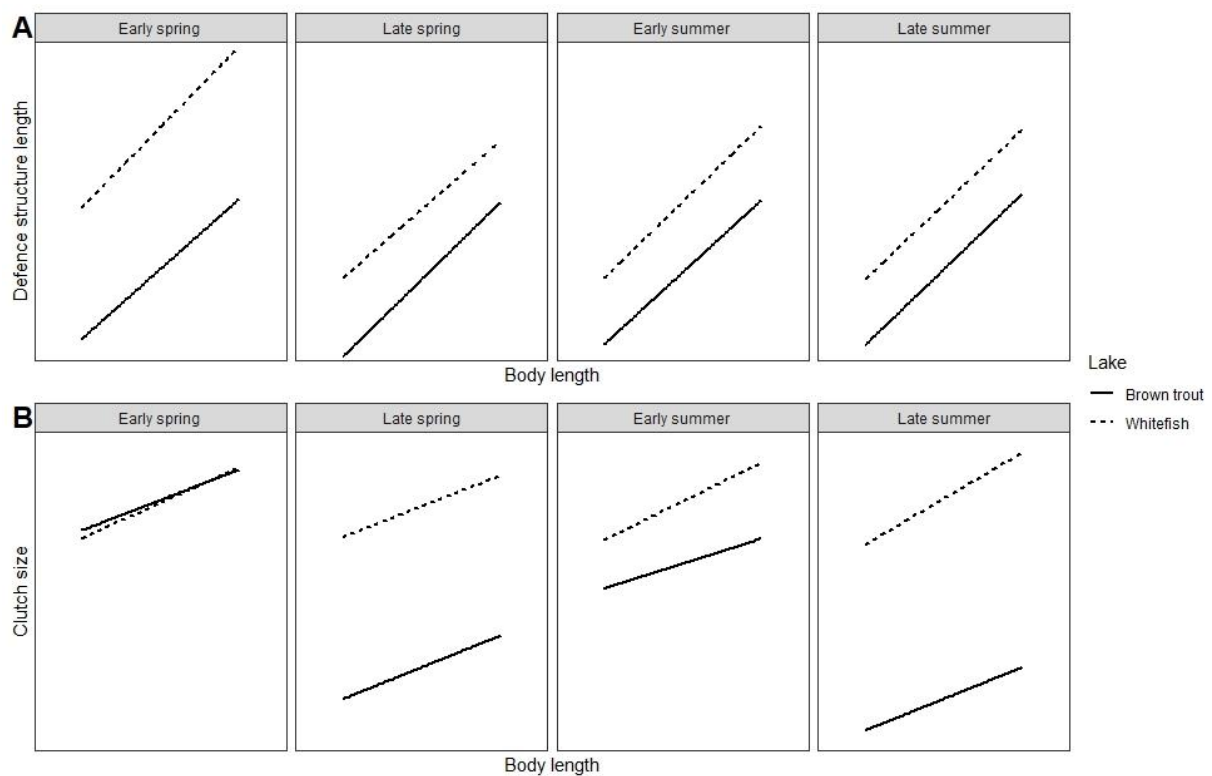
Fig. 1: Predicted responses in (A) morphological defence structure (helmet and tail spine, here combined into "defence structure length"), and (B) fecundity (clutch size) in *Daphnia longispina/galeata* to introduced invasive European whitefish. Solid lines mark lakes with native brown trout and dashed lines mark lakes with introduced whitefish. Longer defence structure lengths (Barnhisel (1991); Spaak & Boersma (1997); Laforsch & Tollrian (2004)), and a higher and less variable fecundity (Leibold, 1991; Sommer et al., 1986) is expected in lakes with introduced invasive whitefish.

Material and methods

Study area

The study area originally consisted of 12 lakes located in Røros and Holtålen municipalities in Trøndelag, Norway (Fig. 2, Table 1). The lakes were grouped into two types: one with European whitefish (*Coregonus lavaretus*) (hereafter referred to as whitefish lakes) and one with only native brown trout (*Salmo trutta*) (hereafter referred to as brown trout lakes). Two of these lakes (L. Åbbårtjønna and L. Olaloken), had very few daphnids and were therefore excluded from the rest of the analyses because of missing data. This resulted in a dataset with five lakes in each lake type group. The regionally invasive European whitefish was originally only native to the nearby L. Femunden, but became introduced to nearby lakes during the 18th century unintentionally by log flumes, and also intentionally for recreational purposes (Huitfeldt-Kaas, 1918; Sandlund et al., 2013). It is a highly efficient zooplanktivore, hence often constituting a strong competitor to other pelagic fish species such as the native Arctic char (*Salvelinus alpinus*) (Artsdatabanken, 2015; Pethon & Vøllestad, 2022).

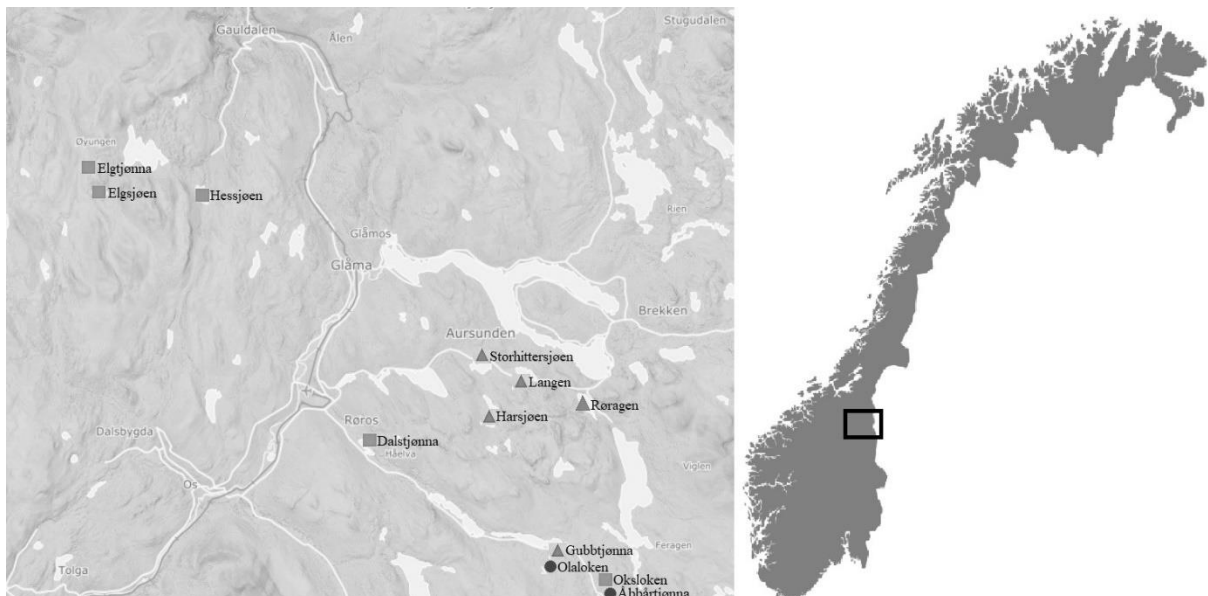Fig. 2: Map of study area separating lakes with introduced European whitefish (▲) and only native brown trout (■). Two lakes were excluded from analyses due to missing data (●). The map was obtained from Norgeskart (https://norgeskart.no, accessed 11th of November 2022) and Pngwing (https://vemaps.com/uploads/img/large/no-03.jpg, accessed 4th of May 2023), and edited in Microsoft Paint (version 21H2).

Table. 1: Information about the sampled lakes. Coordinates from lake centre (lat/long), lake area (km^2) and lake ID were gathered from the Norwegian Water Resources and Energy Directorate's lake database (https://temakart.nve.no/tema/innsjodatabase, accessed 11th of November 2022).

Lake ID	Whitefish	Fish species	Coordinates	Area (km^2)	Lake ID
Gubbtjønna	Yes	Brown trout, burbot, perch, whitefish	62.4692° N 11.7918° E	0.0517	35445
Harsjøen	Yes	Arctic char, brown trout, burbot, whitefish	62.5735° N 11.6565° E	1.4298	247
Langen	Yes	Arctic char, brown trout, burbot, perch, minnow, grayling, whitefish	62.5996° N 11.7169° E	0.7734	35338
Røragen	Yes	Brown trout, burbot, minnow, perch, pike, whitefish	62.5818° N 11.8056° E	1.3448	177
Storhittersjøen	Yes	Arctic char, burbot, minnow, grayling, whitefish	62.6054° N 11.6364° E	1.1449	246
Dalstjønna	No	Brown trout	62.5295° N 11.4757° E	0.1754	35396
Elgsjøen	No	Brown trout	62.7228° N 10.9849° E	0.5815	35203
Elgtjønna	No	Brown trout	62.7444° N 10.9772° E	0.1329	35150
Hessjøen	No	Brown trout	62.7253° N 11.1534° E	1.0337	884
Oksloken	No	Pike	62.4606° N 11.8134° E	0.0087	140001
Olaloken*	No	Brown trout	62.4674° N 11.7945° E	0.0123	139978
Åbbårtjønna*	No	Perch	62.4258° N 11.8646° E	0.0218	140171

* L. Olaloken and L. Åbbårtjønna was excluded from analyses due to missing data.

Sampling

Zooplankton samples were gathered during the summer/autumn of 2021. Lakes were sampled six times with three-week intervals, starting in early June and ending in late September. The deepest part of each lake was identified with an echosounder, and each sample was taken from 1 m above the bottom at the deepest part up to the surface using a zooplankton net. The net had a diameter of 30 cm, a mesh size of 95 µm, and was towed at approx. 0.5 m/sec. The content of each haul was transferred to separate 50 ml tubes and added 0.5 ml Lugol's solution for preservation. In the lab, individuals of *Daphnia longispina/galeata* were collected from these samples and used for analysing morphology of defence structures (helmet and tail spines).

Samples from 2021 could not be used to obtain data on fecundity due to egg loss during fixation. Thus, I conducted a second series of sampling in 2022. The 10 lakes (excluding L. Åbbårtjønna and L. Olaloken due to absence of daphnids) were sampled three times: medio June, medio August, and medio September. Based on observed water temperatures, the first sampling event was likely closely related to the hatching of whitefish eggs (Larsen, 2022; Næsje et al., 1986; Price, 1940). Samples were obtained by horizontal or vertical net hauls; throwing the net from land or hauled from a boat, depending on the abundance of daphnids. The net-type was the same as used for net hauls described above. The contents were transferred to a white tray, and then the 30 largest *Daphnia spp.* were sampled and stored individually in Eppendorf tubes with 96% ethanol.

Lab

From the zooplankton samples of 2021, 20 of the largest female *Daphnia longispina/galeata* and 10 smaller individuals were haphazardly chosen from each lake and sampling round. Due to a limited number of daphnids in some samples, the total sample size was 1423 individuals. Each daphnid was photographed individually using a Leica MZ95 stereomicroscope and Leica Application Suite X (version 3.7.4.23463). The length of the body, helmet and tail spine was measured using ImageJ (version 1.53m) (Fig. 3).
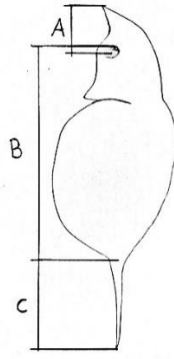
Fig. 3: Length measurements of morphological defence structures of *Daphnia longispina/galeata.* A: helmet length, from the middle of the eye to the top of the helmet. B: body length, from the top of the eye to the base of the tail spine. C: tail spine length, from the base to the tip of the tail spine.

The complete contents of the Eppendorf tubes containing individually stored daphnids from the 2022 samples were transferred to a petri dish and placed under a stereomicroscope where they were photographed and measured for body length with the same method as for the morphology measurements. Eggs, including those that had been lost from the brood pouch during fixation, were counted. For individuals that carried early development stages of eggs (i.e. spherical eggs), egg size was measured as the average egg diameter of up to three eggs per individual. The total sample size was 674 individuals for clutch size and 213 individuals for egg size. However, it should be noted that egg diameter might not have been the most accurate measurement of egg size when taking measurements from eggs still inside the brood pouch. Removing and measuring eggs one by one (Trubetskova & Lampert, 1995) or using dry weight (Berberovic et sl., 1990; Guisande & Gliwicz, 1992) could have been a better method for measuring egg size.

Statistical analysis

All statistical analyses were done using R, version 4.2.3 (R Core Team, 2023) within RStudio, version 4.2.0 (Posit team, 2023).

Prior to analyses of morphological defence structures in *Daphnia*, body length was centred relative to the mean body length (1.22 mm) of all lakes and sampling rounds in 2021.

Linear Mixed Effects Models (LME) were fitted (using maximum likelihood), including all two- and three-way interactions between the three explanatory variables centred body length, sampling round, and lake type, and a random effect of lake ID. This was done using the function *lme* from the *nlme* package (v3.1-162, Pinheiro & Bates, 2023). The best fitting model was chosen based on AICc values found with the function *dredge* in the *MuMIn* package (Table A1) (v1.47.5, Bartón, 2022). All models with ΔAIC < 2 were refitted with restricted maximum likelihood (REML) and inspected for homoscedasticity, linearity and normal distribution of residuals (Fig. A1-A2). A pattern was observed in the residuals vs. fitted plot for both helmet and tail spine, and this was resolved by $\log_e$-transforming tail spine length prior to model fitting. For plotting model results, the function *predict* from the *stats* package was used to obtain predicted values (v4.2.3, R Core Team, 2023). A regression line based on these predicted values were then added to scatter plots of helmet/tail spine and centred body length using *geom_line* in the *ggplot2* package (v3.4.1, Wickham et al., 2022). Estimates of body length and lake type effects for each sampling round were obtained by releveling the data frame and rerunning the final model.

Prior to analyses of *Daphnia* fecundity and reproductive traits, body length was centred relative to the mean body length (1.43 mm) of all lakes and sampling rounds in 2022. I used a Generalised Linear Mixed Model (GLMM) from the *glmmTMB* package (v1.1.6, Brooks et al., 2022) to model clutch size. To deal with overdispersion and zero-inflation, I fitted a total of six full models including all two- and three-way interactions between the three explanatory variables centred body length, sampling round and lake type: Poisson GLMM, two Negative Binomial GLMMs (negbinom 1 with variance = $\varphi\mu$, negbinom 2 with variance = $\mu(1 + \mu/k)$), Zero-Inflated Poisson GLMM and two Zero-Inflated Negative Binomial GLMMs. For egg size, an LME was fitted including all two- and three-way interactions between the three explanatory variables. All models had lake ID as a random effect. The best combination of fixed effects (and type of zero-inflation in clutch size) were found based on AICc values from the function *dredge* (Tables B1, C1). The models with the lowest AICc was refitted with REML and inspected for homoscedasticity, linearity and normal distribution of residuals (Figs. B1, C1). A pattern was observed in the residuals vs. fitted plot for egg size, and this was resolved by $\log_e$-transforming egg size prior to model fitting. For plotting model results, the function *predict* from the *stats* package was used to obtain predicted values. A regression line based on these predicted values were then added to scatter plots of clutch size/egg size and centred body length using *geom_line* in the *ggplot2* package. Estimates of

body length and lake type effects for each sampling round were obtained by releveling the data frame and rerunning the final model.

Results

Morphological defence structures

For helmet length variation, models excluding or including the three-way interaction between centred body length, lake type and sampling round had similar support in terms of AICc values (ΔAICc < 2) (Table A1). The simplest model with the second lowest AICc value was chosen for further analyses. There was no evidence for an effect of the whitefish lake type on the helmet length for a *Daphnia* of mean size (Fig. 4). There was also considerable variation with regard to helmet lengths within lake types (Fig. 5). However, there was evidence for an increasing positive effect of body length on helmet length throughout the season (Figs. 4, 5, Table A2).

Fig. 4: Model (LME) estimates of $\log_e$ helmet length (mm) in *Daphnia* as a function of lake type and centred body length releveled for each sampling round. The estimate for lakes with introduced whitefish is a contrast to the lakes with native brown trout, with both being given for a *Daphnia* of mean size. Sampling times (all in 2021): A: early June, B: late June, C: medio July, D: early August, E: early September, F: late September.

Fig. 5: Seasonal variation in the relationship between log$_e$ helmet length (mm) and centred body length (mm) of *Daphnia* in lakes with introduced whitefish (dotted lines) or native brown trout (solid lines). Regression lines are fitted values derived from the LME model. Sampling times (all in 2021): A: early June, B: late June, C: medio July, D: early August, E: early September, F: late September.

For *Daphnia* tail spine length variation, the model with the lowest AICc score included the three-way interaction between centred body length, lake type and sampling round (Table A1). *Daphnia* of mean size had approximately equal tail spine lengths in the two lake types in sampling round 1, but it became progressively shorter in the whitefish lakes relative to in the trout lakes as the season progressed (Fig. 6). The effect of body length on tail spine length was stronger in whitefish lakes than in trout lakes, particularly during sampling rounds 1-5. Thus, the main difference was found among smaller individuals, where *Daphnia* in trout lakes had longer tail spines than those in whitefish lakes (Fig. 7, Table A3).
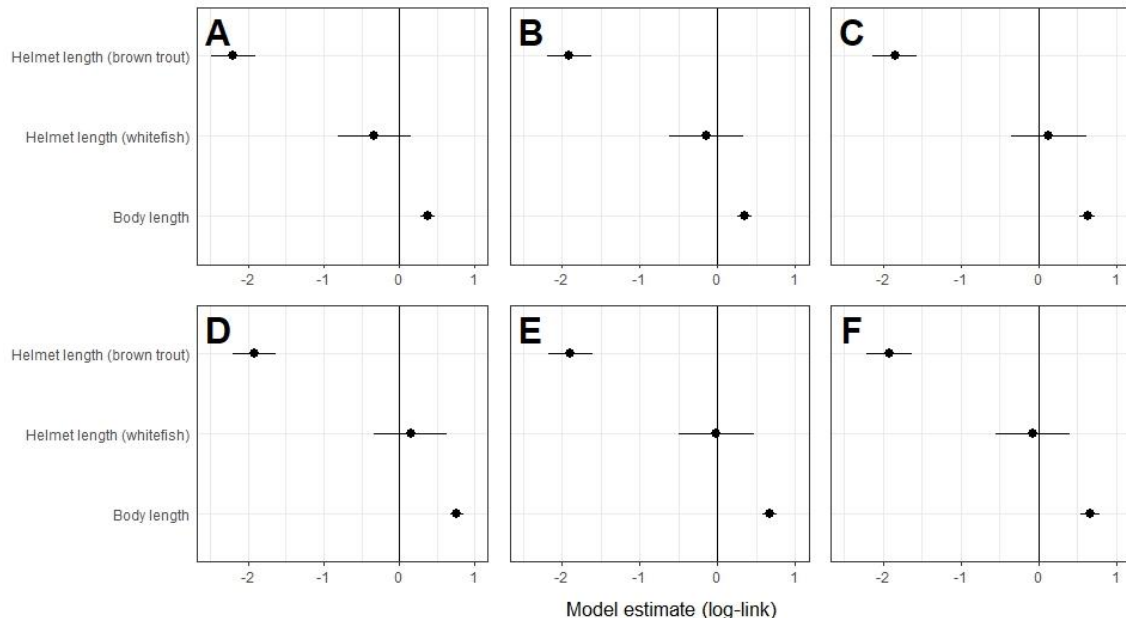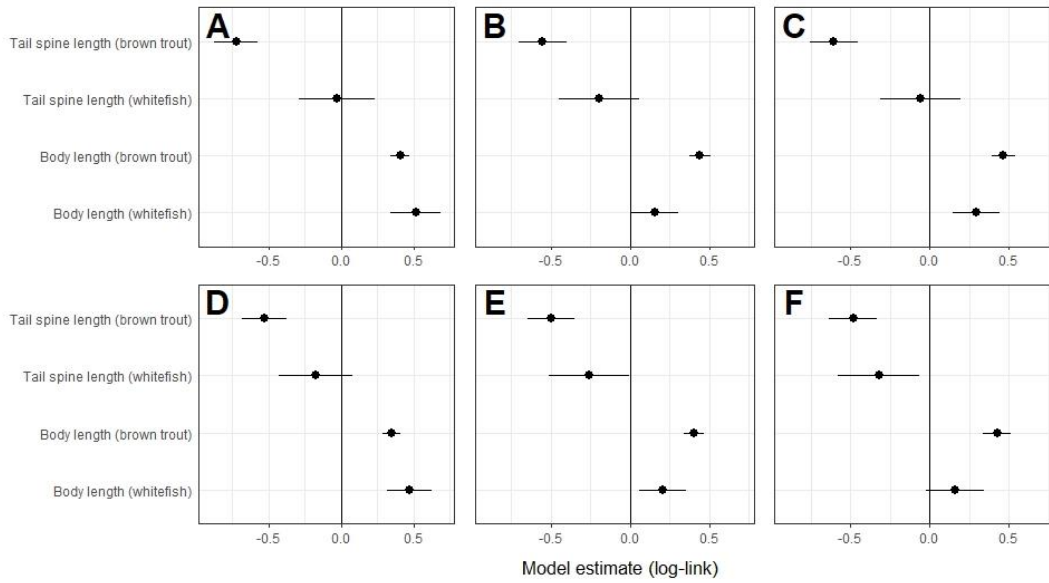
Fig. 6: Model (LME) estimates of $\log_e$ tail spine length (mm) in *Daphnia* as a function of lake type and centred body length releveled for each sampling round. The estimate for lakes with introduced whitefish is a contrast to the lakes with native brown trout, with both being given for a *Daphnia* of mean size. Sampling times (all in 2021): A: early June, B: late June, C: medio July, D: early August, E: early September, F: late September.
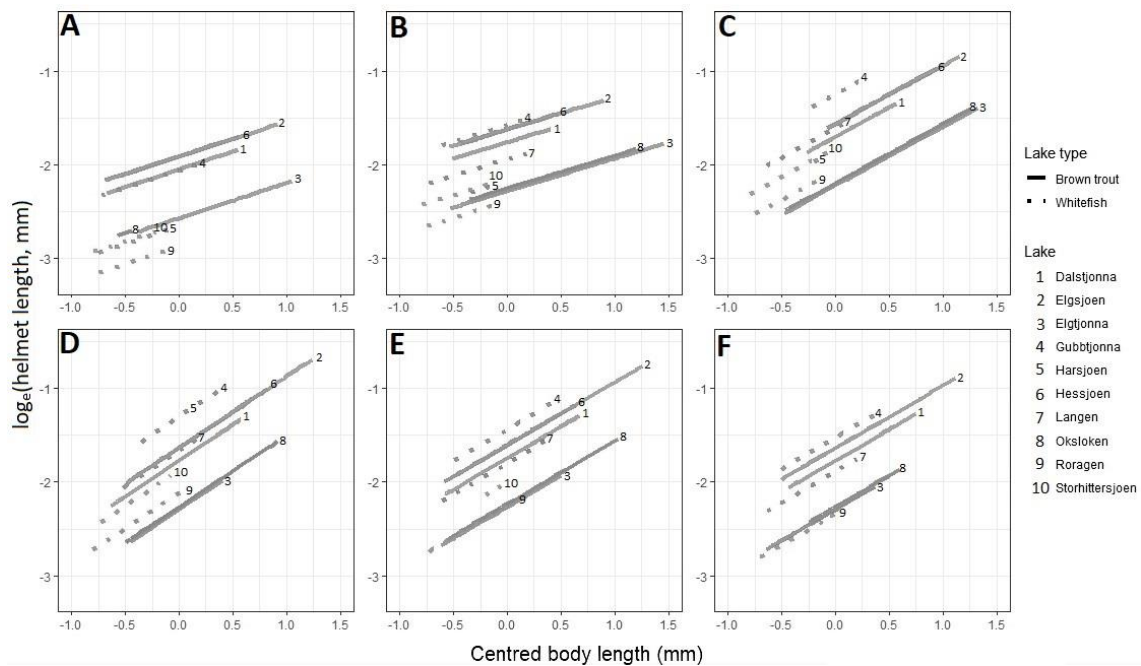

Fig. 7: Seasonal variation in the relationship between $\log_e$ tail spine length (mm) and centred body length (mm) of *Daphnia* in lakes with introduced whitefish (dotted lines) or native brown trout (solid lines). Regression lines are fitted values derived from LME model. Sampling times (all in 2021): A: early June, B: late June, C: medio July, D: early August, E: early September, F: late September.

Fecundity
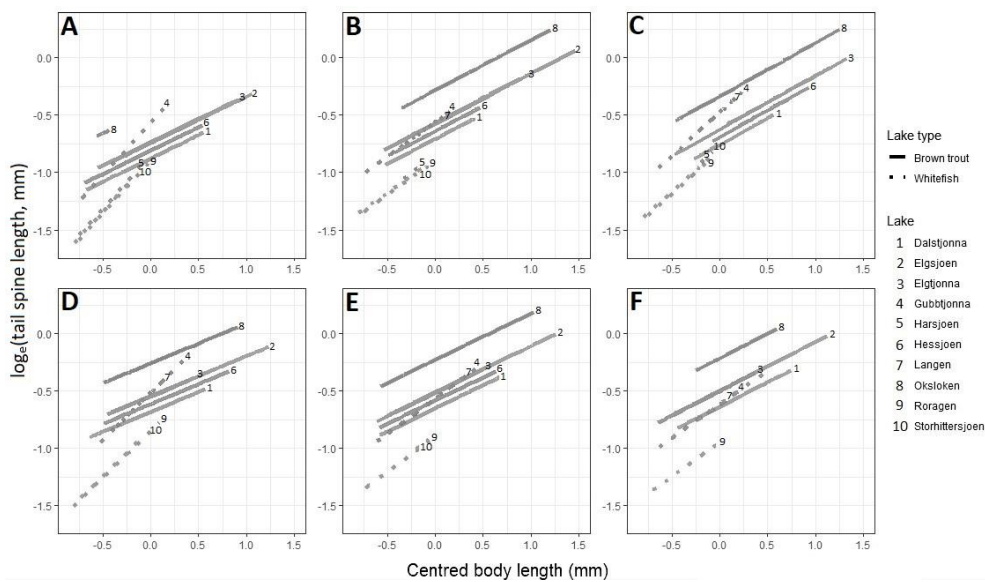
When comparing the different models of variation in clutch size, a model with Zero-Inflated Poisson GLMM (ZIPGLMM) had the lowest AICc score with ΔAICc = 2.0 compared to the next best model (Zero-Inflated Negative Binomial 2 GLMM) (Table B1). This model allowed the conditional mean to depend on a three-way interaction between centred body length, sampling round and lake type, and to vary randomly by lake ID. Also, it allowed the number of extra (i.e. structural) zeroes to depend on the same variables (but not in a three-way interaction). The conditional component of the model describes the effect of the explanatory variables (i.e. sampling round, centred body length and lake type) on the clutch size in the data that adhere to a regular Poisson distribution, while the zero-inflated component of the model describes the probability of observing an extra zero that is not generated by the conditional model (Brooks et al., 2017). Inspection of residuals indicated that the models sufficiently met normality assumptions (Fig. B1).

When comparing *Daphnia* clutch size across sampling rounds, daphnids in whitefish lakes had larger clutches in round 1 (medio June), and then smaller and similar clutches in round 2 (medio August) and 3 (medio September) (Fig. 8). In trout lakes, clutch size decreased in round 2, before increasing again in round 3.

There was evidence for higher clutch size in *Daphnia* of mean size in whitefish lakes than in trout lakes in sampling round 1 and 2 (Figs. 8A, 8B, Table B2). Whereas *Daphnia* in trout lakes had an increased probability for extra zeroes in sampling round 1, the opposite was true for those in whitefish lakes (Fig. 9A). This was displayed as *Daphnia* under mean size in trout lakes having approximately zero eggs (Fig. 8A). No evidence was found for differences in fecundity between the two lake types in sampling round 3, and small individuals in both lake types rarely carried eggs during this sampling (Figs. 8C, 9C). There was also considerable variation in clutch size and the effect of body length within the trout lake type.

There was a positive effect of body length on clutch size in both lake types and all sampling rounds, but this effect was stronger in whitefish lakes compared to trout lakes in sampling round 2 (Figs. 8B, 9B). There was a negative effect of body size on the probability of zeroes, but this was less pronounced in whitefish lakes than in trout lakes (Fig. 9).

Fig. 8: Seasonal variation in the relationship between *Daphnia* clutch size and centred body length in lakes with introduced whitefish (dotted lines) and native brown trout (solid lines). Regression lines are based on predicted values derived from the ZIPGLMM model, and takes into account the combined effect of the zero-inflation and the conditional model components.. Sampling times (all in 2022): A: medio June, B: medio August, C: medio September. Note the difference in y-axis scales across panels.
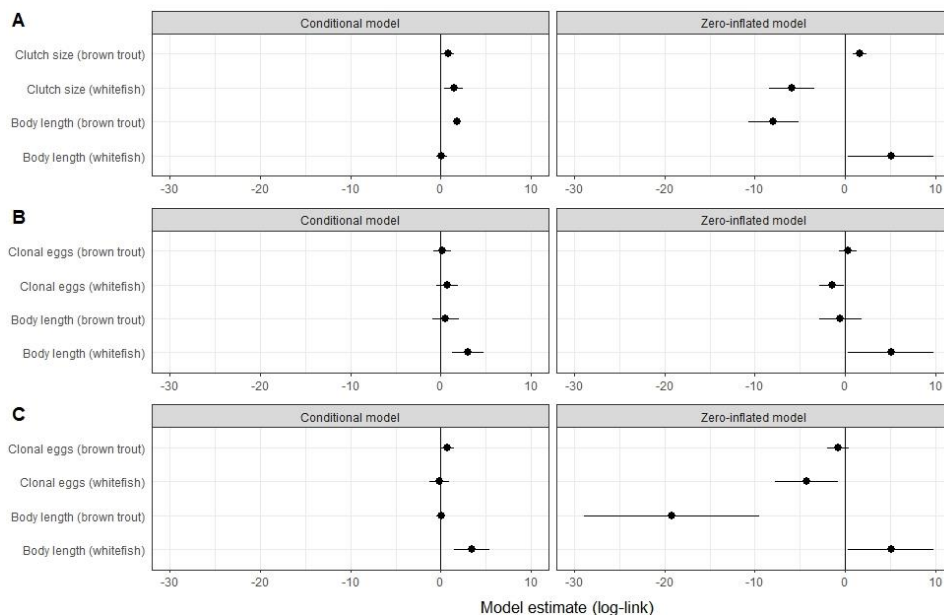
Fig. 9: Model (GLMM) estimates of *Daphnia* clutch size as a function of centred body length, lake type and sampling round, releveled for each sampling round. Clutch size was measured as the number of clonal eggs in a female *Daphnia* of mean size. Estimates for lakes with introduced whitefish are presented as contrasts to lakes with native brown trout. Sampling times (all in 2022): A: medio June, B: medio August, C: medio September.

Reproductive traits

For *Daphnia* egg size variation, the model with the lowest AICc score included an interaction between centred body length and lake type, as well as an interaction between lake type and sampling round (Table C1). There was no significant evidence for an effect of the whitefish lake type on egg size for a *Daphnia* of mean size, but daphnids in whitefish lakes had slightly smaller eggs in round 1 and slightly larger eggs in round 3 compared to daphnids in tout lakes (Fig. 10). Two more pronounced pattern were that egg size was somewhat smaller in sampling round 1 than in rounds 2 and 3, particularly in whitefish lakes (Fig. 11), and that whereas the effect of body length on egg size was slightly negative in whitefish lakes, it was strongly positive in trout lakes (Figs. 10, 11, Table C2).

Fig. 10: Model (LME) estimates of *Daphnia* egg size as a function of centred body length, lake type and sampling round, releveled for each sampling round. Egg size was measured as the average egg diameter of up to three eggs per individual. Estimates for lakes with introduced whitefish are presented as contrasts to lakes with native brown trout. Sampling times (all in 2022): A: medio June, B: medio August, C: medio September.
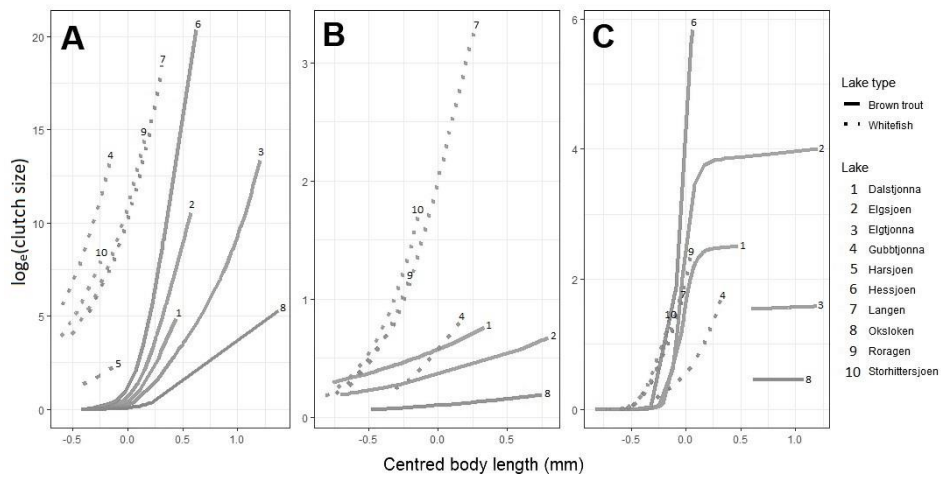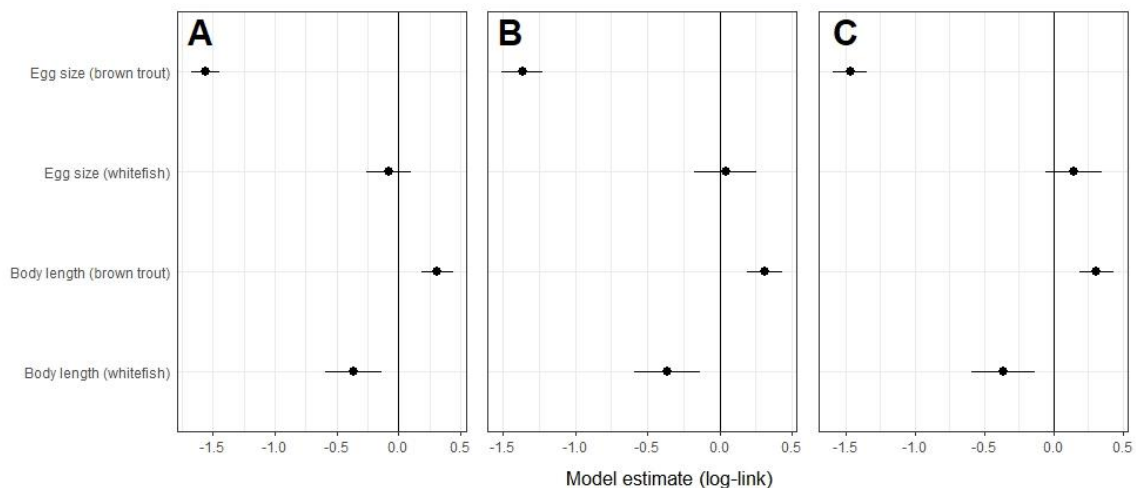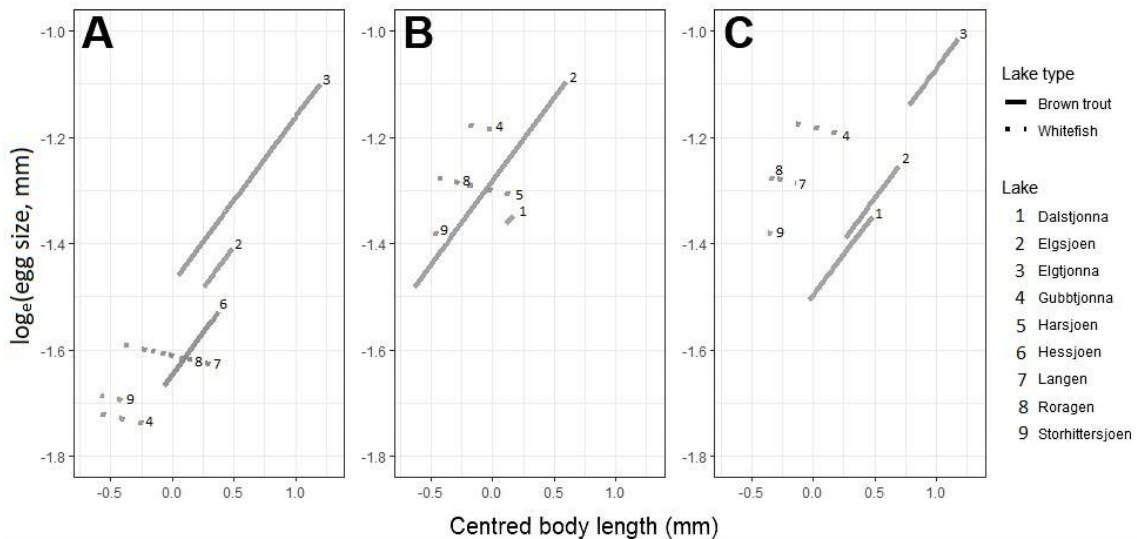
Fig. 11: Seasonal variation in the relationship between *Daphnia* egg size and centred body length in lakes with introduced whitefish (dotted lines) and native brown trout (solid lines). Regression lines are based on predicted values derived from the LME model. Sampling times (all in 2022): A: medio June, B: medio August, C: medio September.

Discussion

The seasonal dynamics of *Daphnia* morphology and fecundity was found to differ between lakes with introduced whitefish and those that only contained native brown trout. First, no significant differences were found in helmet length between lake types. Second, relative tail spine lengths for *Daphnia* of mean size were slightly shorter in lakes with whitefish, and particularly so for the smallest individuals. Finally, fecundity was higher in *Daphnia* of mean size in whitefish lakes during spring and summer, but there was also pronounced seasonal variation in fecundity, with substantial reduction in clutch size during summer and autumn. In addition, the effect of body length on clutch size was stronger in whitefish lakes during spring and summer. Difference in egg size between lake types were small and inconsistent throughout the season, but egg sizes were smaller in spring compared to summer and autumn, and particularly so in the whitefish lakes. The observed patterns were not consistently in agreement with the predictions based on the hypothesis that introducing an efficient zooplanktivore into the system would cause a shift from bottom-up to top-down control of the zooplankton population. The expectations of longer relative helmet and tail spines, higher and less variable fecundity, and smaller and less variable egg sizes in the

whitefish lakes compared to the trout lakes were not supported. Ultimately, the seasonal patterns in fecundity and egg size seem to suggest an effect of resource limitation and hence bottom-up control even in the presence of whitefish.

The finding of equally long helmets and slightly shorter tail spines in *Daphnia* of mean size in whitefish lakes contrasts the prediction of a shift towards top-down control when introducing whitefish. However, one possible explanation of the findings in this study that may explain the deviations from the predicted elongated defence structures might be that helmet and tail spines are not efficient defence mechanisms against whitefish predation. This discrepancy could be explained by the considerable size difference between whitefish and *Daphnia*. The results should also be viewed in the light of a scarce body of empirical evidence on the relationship between whitefish and elongated helmet and tail spines. Most studies on these defence mechanisms regard invertebrate predators or smaller/juvenile fish (Laforsch & Tollrian, 2004; Pijanowska, 1990: Swaffar & O'Brien, 1996). In light of the results and previous studies, comparing morphology of helmet and tail spine lengths in the *D. longispina/galeata* complex appears to be an unfitting indicator for predation pressure (and thus also top-down control) from European whitefish in this system.

Small *Daphnia* in whitefish lakes had shorter tail spines than small *Daphnia* in trout lakes, but there was no detectable difference in tail spine length in the larger *Daphnia* between lake types. A possible explanation for this at first glance contra intuitive results, could be differences in size-selective predation pressure between lakes with or without whitefish. One might speculate that tail spines are primarily regarded to be an effective defence mechanism against invertebrate predators (Sih, 1987; Dodson, 1989). Invertebrate zooplanktivores primarily prey on small *Daphnia* (Zaret, 1978), and these should represent a bigger threat in trout lakes compared to whitefish lakes since they themselves are vulnerable to predation from zooplanktivorous fish. Former master student Larsen (2022) recorded the presence of predatory zooplankton in all studied lakes. The copepods *Cyclops scutifer* and *Heterocope saliens/appendiculata*, as well as the cladocerans *Bythotrephes longimanus*, *Polyphemus pediculus*, and *Leptodora kindtii* were present in both lake types, but potential differences in abundance between lake types are still unknown. Previous studies show that all of these predators may indeed efficiently prey on small *Daphnia* (Branstrator & Lehman, 1991; Lehman & Cácares, 1993; Sandøy & Nilsen, 1987; Strickler & Bal, 1973; Young & Taylor, 1988). One might speculate that the abundance of invertebrate predators is higher in the studied trout lakes as whitefish is known to prey on several of them (Pothoven et al., 2001;

Pothoven, 2005; Sotton et al., 2014), ultimately making it beneficial for *Daphnia* in trout lakes to allocate energy to producing longer tail spines at smaller sizes.

Daphnia in trout lakes showed seasonal variation in fecundity as predicted based on the PEG-model (Sommer et al., 1986), with one peak in spring and one in early autumn (Scott et al., 1999; Swar & Fernando, 1979). The main difference in fecundity between lake types occurred early in the season, when *Daphnia* fecundity was higher in whitefish lakes than in trout lakes. However, there was no apparent difference in the late season samples. The difference early in the season was most apparent in the smallest individuals. Visually hunting fish predators, like whitefish, are known to selectively prey on large, ovigerous *Daphnia* females carrying large clutches (Manca et al., 2008). Therefore, allocating energy to reproduction early in the season at small body sizes should be beneficial in whitefish lakes. This result also coincides with previous findings by Leibold (1991), Reznick & Endler (1982), and Reznick et al. (1990), showing selection for smaller neonate and primiparous size and larger reproductive effort when mortality increases with body size. The observed higher fecundity in small sizes early in the season indicates that whitefish indeed may be an important predator of *Daphnia*. However, the reduction in fecundity throughout the season also suggests that this predation is not sufficiently strong to release the *Daphnia* from resource limitation during summer and autumn (Leibold, 1991). An increasing role of resource limitation throughout the season in whitefish lakes is also consistent with the observed seasonal increase in egg size. An increase in egg size have previously been observed under low food conditions (Brambilla, 1982; Green, 1956), as larger egg size leads to larger neonates at hatching which survive longer under starvation (Guisande & Gliwicz, 1992). The observed seasonal pattern in fecundity and egg size therefore supports the presence of resource competition and hence bottom-up control of the *Daphnia* population even in the presence of introduced whitefish.

The negative effect of increasing body length on the probability of zeroes differed between the two lake types. Specifically, this effect was weaker in whitefish lakes than in trout lakes, such that larger individuals maintained a higher rate of zeroes in whitefish lakes. Thus, for large individuals, it appeared that *Daphnia* in whitefish lakes have longer interclutch intervals (zero-inflation model), but that the clutches that they do produce are larger (conditional model). Daphnids without eggs are probably less vulnerable to predation by visually hunting predators like whitefish as they are less conspicuous (Bernatowicz & Pijanowska, 2011). Spending a longer time accumulating energetic reserves between clutches,

and then producing larger clutches once eggs are oviposited into the brood pouch may then represent a strategy that reduces the total amount of time spent carrying eggs and thus the probability of being eaten by whitefish, while still maintaining a high reproductive output. However, this remains speculative, as determining the fitness consequences of such a strategy requires quantitative information on the risk of predation and how that risk depends on reproductive status.

One factor making it challenging to measure the effects of introducing whitefish in this study is spatio-temporal distribution of *Daphnia*. Many species of *Daphnia* are known to execute both diel vertical (Ringelberg, 1999) and diel horizontal migration (Burks et al., 2011) in response to fish predation. Also worth mentioning is that Green (1967) found spatial variation in helmet lengths of *D. lumholtzi* in Lake Albert, East Africa, with longer helmets being more common along the margins of the lake where zooplanktivorous fish were more common. The samples I used for morphology of defence structures should account for differences in vertical distribution, as all depths from 1 m above the lake floor and up to the surface were sampled. However, while sampling for fecundity, there seemed to be a notable difference in the spatial distribution of *Daphnia* in several lakes, as a much greater abundance was discovered when doing vertical hauls at greater depths compared to horizontal hauls from the shore. This spatial distribution of *Daphnia* within a single lake also differed substantially across sampling rounds. As the efficiency of introduced whitefish as a *Daphnia* predator would depend on lake depth (Lampert, 1989; Giske & Salvanes, 1995), further research on this topic could therefore benefit from investigating this possible spatio-temporal distribution in *Daphnia* as there was some variability in lake depths of the studied lakes (ranging from 6.1 to 16.3 m). Additionally, samplings for *Daphnia* fecundity could benefit from more and more frequent sampling events to better track the seasonal variation in fecundity. Examining how *Daphnia* fecundity matches nutrient availability throughout the season could also be of interest.

It should be noted that all whitefish lakes in this study additionally contained other the other zooplanktivorous fish species European perch (*Perca fluviatilis*) and Arctic char (*Salvelinus alpinus*). It is therefore possible that my study design was not testing for an effect of introduced whitefish in particular, but rather for the effect of an efficient zooplanktivore in general. The lack of general effects in my study may therefore be a result of the effect of an introduced species varying depending upon the specific species composition of the community into which it is released (David et al., 2017). Whitefish lakes L. Langen, L.

Harsjøen, and L. Storhittersjøen all contained Arctic char. Whether Arctic char was present prior to the introduction of whitefish is unknown, but if so, the *Daphnia* population could have already been adapted to fish predation. Ideally, yet probably difficult in the wild, the whitefish lake type group should have been lakes with only native brown trout and introduced whitefish in order to capture the effect of this introduction of an alien species more precisely.

Conclusion

This study looked at what effects introducing a highly efficient zooplanktivorous fish might have on the trophic control of the zooplankton community by comparing morphology of defence structures and fecundity in *Daphnia longispina/galeata* in lakes with introduced, invasive European whitefish to lakes with only native brown trout. However, the predicted shift towards top-down control in lakes with introduced whitefish was not supported. The length of the defence structures (helmet and tail spines) were found to be equally long or slightly shorter in lakes with introduced whitefish, which could be explained by helmet and tail spines not being an efficient defence mechanism against whitefish predation, combined with a higher level of predation by invertebrates in lakes with only native brown trout. Fecundity was higher in *Daphnia* of mean size during spring and summer in lakes with whitefish – a trait often associated with high fish predation. However, there was also pronounced seasonal variation in fecundity and egg size, with a substantial reduction in fecundity and increase in egg size during summer and autumn, which suggests an effect of resource limitation and hence bottom-up control even in the presence of introduced whitefish.

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Appendix A – Morphological defence structures

Table A1: Morphology model selection based on AICc scores using the function *dredge*. All models had lake ID as random effect. CBL: centred body length, LT: lake type, SR: sampling round, df: degrees of freedom, W_i: weight.

| Model	Variables	df	AICc	ΔAIC	W_i
Helmet length					
1	CBL+LT+SR + CBL*LT + CBL*SR + LT*SR	21	608.6	0.00	0.586
2	CBL+LT+SR + CBL*SR + LT*SR	20	610.5	1.93	0.223
3	CBL+LT+SR + CBL*LT*SR	36	610.8	2.24	0.191
4	CBL+LT+SR + CBL*LT + LT*SR	16	659.8	61.15	0.000
5	CBL+LT+SR + LR*SR	15	662.0	53.40	0.000
Tail spine length					
1	CBL+LT+SR + CBL*LT*SR	26	-714.4	0.00	0.882
2	CBL+LT+SR + CBL*Lt + LT*SR	16	-710.2	4.23	0.106
3	CBL+LT+SR + CBL*LT + CBL*SR + LT*SR	21	-705.8	8.60	0.012
4	CBL+LT+SR + CBL*LT	11	-673.1	41.25	0.000
5	CBL+LT+SR + CBL*LT + CBL*SR	16	-666.5	47.92	0.000
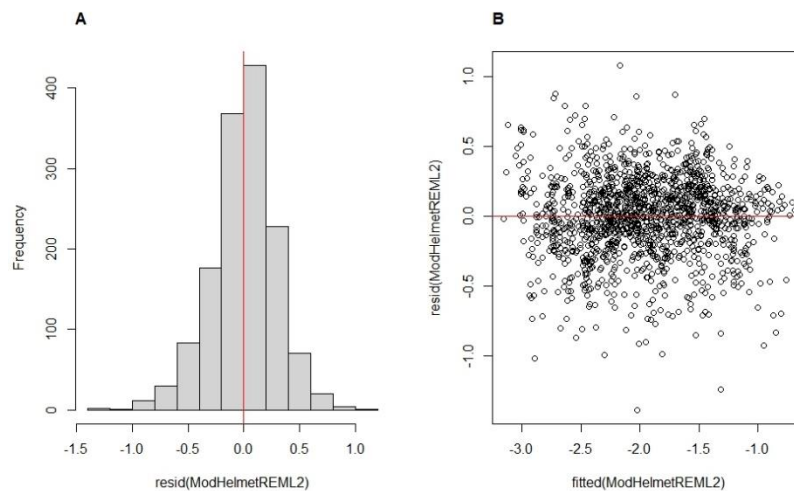
Fig. A1: Residuals (A) and residuals vs. fitted (B) for Linear Mixed Effects Model of *Daphnia* helmet length.
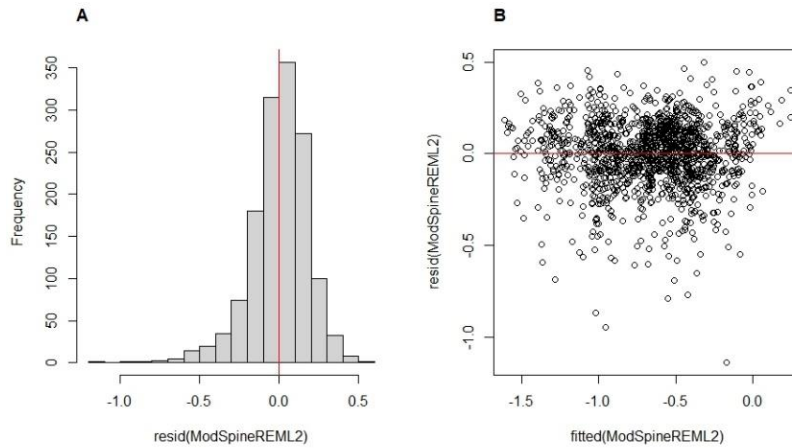
Fig. A2: Residuals (A) and residuals vs. fitted (B) for Linear Mixed Effects Model of *Daphnia* tail spine length.

Table A2: Model estimates for the best fitting model of helmet length, with interaction between centred body length and sampling round, and lake type and sampling round. Estimates are on $\log_e$ scale. (Intercept): helmet length for a daphnia of mean size in a lake with native brown trout in sampling round 1. DF: degrees of freedom, CBL: centred body length, SR: sampling round, LTw: lake type whitefish.

Variable	Estimate	Std. error	df	t-value	p-value
(Intercept)	-2.19881	0.14615	1396	-15.04542	0.0000
CBL	0.37642	0.04823	1396	7.80425	0.0000
SR2	0.28989	0.03695	1396	7.84596	0.0000
SR3	0.34761	0.04073	1396	8.53401	0.0000
SR4	0.27871	0.03677	1396	7.57999	0.0000
SR5	0.30839	0.03617	1396	8.52529	0.0000
SR6	0.28001	0.04007	1396	6.98850	0.0000
LTw	-0.33067	0.20829	8	-1.58756	0.1510
CBL*SR2	-0.02699	0.06777	1396	-0.39816	0.6906
CBL*SR3	0.25153	0.06968	1396	3.61005	0.0003
CBL*SR4	0.38888	0.06551	1396	5.93652	0.0000
CBL*SR5	0.29523	0.06705	1396	4.40327	0.0000
CBL*SR6	0.29064	0.07980	1396	3.64223	0.0003
SR2*LTw	0.18959	0.06143	1396	3.08627	0.0021
SR3*LTw	0.46348	0.06416	1396	7.22384	0.0000
SR4*LTw	0.47995	0.06198	1396	7.74395	0.0000
SR5*LTw	0.31600	0.05947	1396	5.31332	0.0000
SR6*LTw	0.25635	0.06642	1396	3.85955	0.0001

Table A3: Model estimates for the best fitting model of tail spine length, with three-ways interaction between centred body length, lake type and sampling round. Estimates are on $\log_e$ scale. (Intercept): tail spine length for a daphnia of mean size in a lake with native brown trout in sampling round 1. DF: degrees of freedom, CBL: centred body length, SR: sampling round, LTw: lake type whitefish.

Variable	Estimate	Std. error	df	t-value	p-value
(Intercept)	-0.72367	0.07680	1385	-9.42226	0.0000
CBL	0.40693	0.03267	1385	12.45724	0.0000
LTw	-0.02919	0.11353	8	-0.25710	0.8036
SR2	0.16976	0.02343	1385	7.24594	0.0000
SR3	0.11986	0.02652	1385	4.52036	0.0000
SR4	0.19429	0.02321	1385	8.37255	0.0000
SR5	0.22490	0.02273	1385	9.89546	0.0000
SR6	0.23972	0.02522	1385	9.50393	0.0000
CBL*LTw	0.51306	0.08639	1385	5.93859	0.0000
CBL*SR2	0.03131	0.04681	1385	0.66880	0.5037
CBL*SR3	0.05658	0.04904	1385	1.15375	0.2488
CBL*SR4	-0.05968	0.04466	1385	-1.33635	0.1817
CBL*SR5	-0.00297	0.04591	1385	-0.06461	0.9485
CBL*SR6	0.01795	0.05617	1385	0.31957	0.7493
LTw*SR2	-0.16621	0.04931	1385	-3.37050	0.0008
LTw*SR3	-0.03019	0.04873	1385	-0.61963	0.5356
LTw*SR4	-0.14649	0.04899	1385	-2.99001	0.0028
LTw*SR5	-0.22766	0.04708	1385	-4.83543	0.0000
LTw*SR6	-0.29109	0.05059	1385	-5.75434	0.0000
CBL*LTw*SR2	-0.35535	0.11296	1385	-3.14597	0.0017
CBL*LTw*SR3	-0.22176	0.11156	1385	-1.98782	0.0470
CBL*LTw*SR4	-0.04338	0.11366	1385	-0.38170	0.7027
CBL*LTw*SR5	-0.30675	0.11457	1385	-2.67736	0.0075
CBL*LTw*SR6	-0.35151	0.12626	1385	-2.78407	0.0054

Appendix B – Fecundity

Table B1: Clutch size model selection using ΔAICc. All models had lake ID as random effect. ZI: zero-inflated, P: Poisson, NB: negative binomial, CBL: centred body length, LT: lake type, SR: sampling round, df: degrees of freedom.

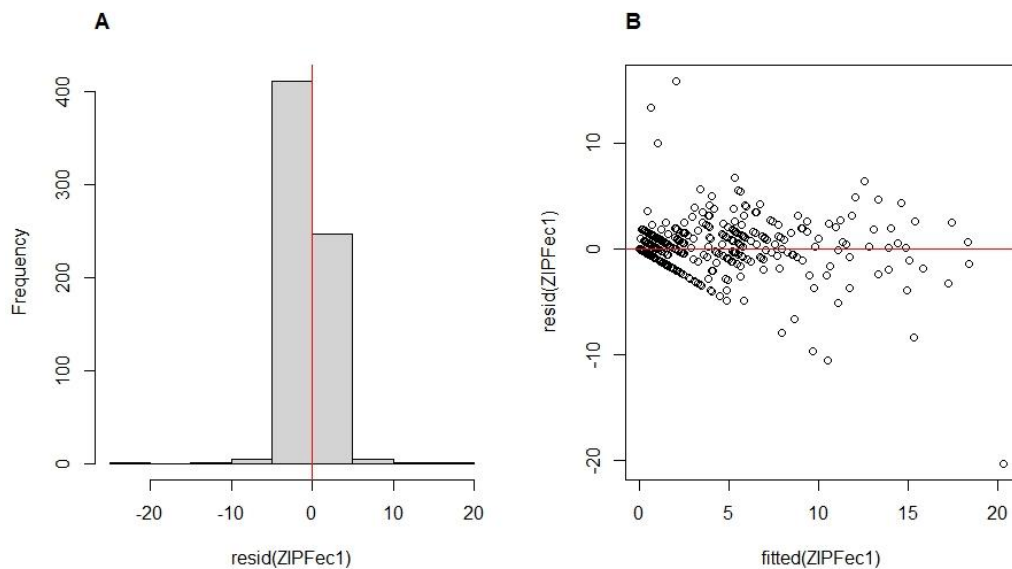| Model	Variables		df	AICc	ΔAICc
	Conditional model	**Zero-Inflated model**			
ZI-P	CBL*LT*SR	CBL*LT + CBL*SR + LT*SR	23	1866.7	0.0
ZI-NB2	CBL*LT*SR	CBL*LT + CBL*SR + LT*SR	24	1868.9	2.2
ZI-NB1	CBL*LT*SR	CBL*SR + LT*SR	23	1871.0	4.3
NB1	CBL*LT*SR	-	14	2107.3	240.6
NB2	CBL*LT*SR	-	14	2145.9	279.2
P	CBL*LT*SR	-	13	2193.9	327.2

Fig. B1: Residuals (A) and residuals vs. fitted (B) for Zero-Inflated Poisson Generalized Linear Mixed Effects Model of *Daphnia* clutch size.

Table B2: Model estimates from the best fitting model (ZIPGLMM) for *Daphnia* clutch size. (Intercept): clutch size of a female of mean size in a lake with native brown trout in sampling round 1. Conditional estimates are on log scale and zero-inflated estimates are on logit scale. CBL: centred body length, LTw: lake type whitefish, SR: sampling round.

	Variable	Estimate	Std. error	z value	Pr(>|z|)
Conditional model	(Intercept)	0.79020	0.38279	2.064	0.03899
	CBL	1.77356	0.21239	8.351	< 2e-16
	LTw	1.46319	0.52753	2.774	0.00554
	SR2	-0.63433	0.38564	-1.645	0.10000
	SR3	-0.05621	0.19360	-0.291	0.77143
	CBL*LTw	0.08347	0.30780	0.271	0.78625
	CBL*SR2	-1.23457	0.80164	-1.540	0.12355
	CBL*SR3	-1.72809	0.26281	-6.576	4.85e-11
	LTw*SR2	-0.73052	0.40702	-1.795	0.07269
	LTw*SR3	-1.61791	0.27085	-5.973	2.32e-09
	CBL*LTw*SR2	2.93990	0.92385	3.182	0.00146
	CBL*LTw*SR3	3.34163	1.03632	3.225	0.00126
Zero-inflated model	(Intercept)	1.6153	0.3985	4.053	5.05e-05
	CBL	-7.9265	1.3929	-5.691	1.27e-08
	LTw	-5.8947	1.2818	-4.563	5.04e-06
	SR2	-1.2840	0.6516	-1.971	0.04877
	SR3	-2.3606	0.7244	-3.259	0.00112
	CBL*LTw	5.0772	2.4255	2.093	0.03633
	CBL*SR2	7.3978	1.7754	4.167	3.09e-05
	CBL*SR3	-11.3082	5.0310	-2.248	0.02459
	LTw*SR2	4.4310	1.5243	2.907	0.00365
	LTw*SR3	1.6218	2.0889	0.776	0.43753

Appendix C – Reproductive traits

Table C1: Egg size model selection using ΔAICc. All models had lake ID as random effect. CBL: centred body length, LT: lake type, SR: sampling round, df: degrees of freedom, W_i: weight.

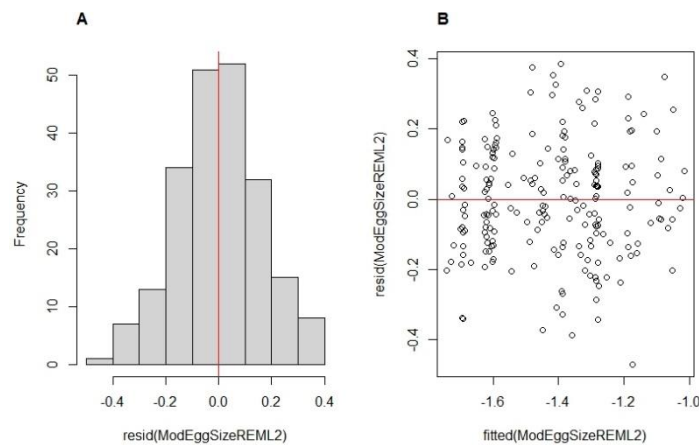| Model	Variables	df	AICc	ΔAICc	W_i
1	CBL+LT+SR + CBL*LT + LT*SR	10	-129.5	0.00	0.393
2	CBL+LT+SR + CBL*LT + CBL*SR + LT*SR	12	-129.3	0.16	0.364
3	CBL+LT+SR + CBL*LT*SR	14	-127.1	2.32	0.123
4	CBL+LT+SR + CBL+SR + LT+SR	11	-126.8	2.63	0.106
5	CBL+LT+SR + LT*SR	9	-122.3	7.19	0.011

Fig. C1: Residuals (A) and residuals vs. fitted (B) for Linear Mixed Effects Model of *Daphnia* egg size.

Table C2: Model estimates from the best fitting model (LME) for *Daphnia* egg size. (Intercept): $\log_e$ egg size of a female of mean size in a lake with native brown trout in sampling round 1. CBL: centred body length, LTw: lake type whitefish, SR: sampling round.

Variable	Estimate	Std. error	DF	t-value	p-value
(Intercept)	-1.55855	0.05983	197	-26.05019	0.0000
CBL	0.31233	0.06346	197	4.92172	0.0000
LTw	-0.07873	0.07962	8	-0.99013	0.3511
SR2	0.19138	0.07515	197	2.54653	0.0116
SR3	0.09061	0.04500	197	2.01373	0.0454
CBL*LTw	-0.36414	0.11631	197	-3.13088	0.0020
LTw*SR2	0.11924	0.08357	197	1.42681	0.1552
LTw*SR3	0.22621	0.06016	197	3.76025	0.0002